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# Domestication of Cereals Affected Seed and Rhizosphere Microbiota - microbial composition and diversity analysis of modern and ancient wheat and barley varieties grown at different locations

Cumulative Dissertation
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Doctor of natural sciences (*Dr. rer. nat.*)

submitted by
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# Declaration

I declare that the dissertation here submitted is entirely my own work, written without any illegitimate help by any third party and solely with materials as indicated in the dissertation. I have indicated in the text where I have used texts from already published sources, either word for word or in substance, and where I have made statements based on oral information given to me. At all times during the investigations carried out by me and described in the dissertation, I have followed the principles of good scientific practice as defined in the "Statutes of the Justus Liebig University Gießen for the Safeguarding of Good Scientific Practice".

Gießen, 26. November, 2021 Yulduzkhon Abdullaeva

## List of peer-reviewed publications

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## List of abbreviations

| | |
|---|---|
| **AMF** | Arbuscular mycorrhizal fungi |
| **ASV** | Amplicon sequence variant |
| **GWAS** | Genome-wide association study |
| **PGPR** | Plant growth-promoting rhizobacteria |
| **ITS2** | Internal transcribed spacer 2 |
| **DNA** | Deoxyribonucleic acid |
| **QTL** | Quantitative trait loci |
| **rRNA** | Ribosomal ribonucleic acid |
| **SNP** | Single-nucleotide polymorphism |
| **TCA cycle** | Tricarboxylic acid cycle |
| **VOCs** | Volatile organic compounds |

# Summary

Ever since crop plants were first domesticated, they have undergone enormous genetic changes. The effect of this domestication on the plant microbiome has recently started to be intensively studied with the invention of the "omics" techniques. The effect of domestication on the diversity, assembly, function, inter/intra-kingdom network analysis of root-associated microorganisms is yet to be studied. The ultimate goal of the thesis was to explore how plant domestication affected the root-associated microbiome structure, diversity, co-occurrence and co-evolution of seeds, root endophytes, and the rhizosphere microbiome. Different wheat and barley varieties were chosen as model crop plants in this study because of their long domestication history, nutritional value, and economic importance.

In the first phase of the study, the impact of domestication on the assembly, diversity, and microbial network of seed endophytes of wild and domesticated wheat and barley species was investigated. Subsequently, the phylogenetic resemblance between cereals and their spermosphere as an indication for co-evolution between plants and microbes was examined. The main finding of this study was higher microbial diversity which was found in modern wheat species compared to their corresponding wild progenitors. In contrast, more microbe-microbe interactions were observed in wild species. Furthermore, *Cutibacterium*, known as a human-associated bacteria genus, was found enriched in cultivated cereals as compared to wild cereals. A strong phylogenetic congruence between seed endophytes and host plants was discovered through co-evolutionary analysis.

In the second phase, the effect of plant domestication on the microbial abundance, diversity, microbial network, and the assembly process of endorhiza and rhizosphere microbiome of two couples of genetically connected wheat species (wild diploid *Aegilops tauschii* vs modern hexaploid *Triticum aestivum*; wild tetraploid *T. dicoccoides* vs modern tetraploid *T. durum*) were studied in different environments. For this purpose, a field study was conducted in three locations (experimental farms of Justus Liebig University: Groß-Gerau, Weilburger Grenze, and Rauischholzhausen) in Hessen, Germany. The distinct habitat microbiomes were evaluated using the 16S rRNA gene and fungal ITS2 amplicon sequencing. First, the effect of domestication on the seed-transmitted microbiome to endorhiza and rhizosphere was demonstrated by comparing the proportion of seed ASVs (Amplicon sequence variants) that

transmitted to endorhiza and rhizosphere microbiomes at three locations. The relative proportion of seed-transmitted microbiome was higher, as well as more diverse in the endorhiza and rhizosphere of diploid *A. tauschii* compared to other tetraploid and hexaploid wheat species. Furthermore, a significant location effect on the relative proportion of fungal seed-transmitted microbiome than bacteria was found.

Second, the comparison of differently abundant species revealed that more bacterial genera were differently enriched in the rhizosphere of *A. tauschii* than the other wheat species that were grown in the same site. The differential abundance test showed that the rhizosphere of genetically related couples of wheat species was found enriched with similar bacterial and fungal genera from the bulk soil however, the composition of these enriched microbiomes was different between locations.

The difference in the beta-diversity of bacterial and fungal microbiota between wild and domesticated wheat species was found only in the root endosphere but not in the rhizosphere. However, differential abundance analysis of the rhizosphere microbiome revealed a compositional shift in the rhizosphere of modern wheat species. Furthermore, different domestication effect was observed between two couples of genetically connected wheat species; more drastic changes were found between modern hexaploid *T. aestivum* and its diploid D genome donor diploid *A. tauschii* compared to the other couple. In both modern wheat rhizosphere, the bacterial microbiome was found enriched. As well as the abundance of the fungal microbiome was increased however their diversity was reduced, particularly pathogenic fungi, compared to their wild relatives. Furthermore, less cross-kingdom connectedness was found in the rhizosphere of modern species compared to their ancestors. Besides, the abundance of bacterial genes responsible for the production of proteins involved in nutrient cycling was reduced in the modern wheat species compared to their wild relatives. The correlation of rhizosphere microbiome with functional gene indicated the key microbial species in natural habitats that play a pivotal role in microbial interactions.

By investigating the microbiome of wild plants, we provide insights into the influence of domestication on spermosphere/root endosphere/rhizosphere microbiome composition and function, and this knowledge can be utilized to restore beneficial associations in current cultivars.

# Zusammenfassung

Seit der ersten Domestizierung von Nutzpflanzen haben diese enorme genetische Veränderungen erfahren. Die Auswirkungen dieser Domestizierung auf das pflanzliche Mikrobiom werden seit der Erfindung der "Omics"-Techniken intensiv untersucht. Die Auswirkungen der Domestizierung auf die Diversität, den Aufbau, die Funktion und die Analyse von Netzwerken zwischen und innerhalb der Domänen von wurzelassoziierten Mikroorganismen müssen erst noch untersucht werden. Ziel dieser Arbeit war es, zu untersuchen, wie sich die Domestizierung von Pflanzen auf die Struktur, die Vielfalt, das gemeinsame Vorkommen und die gemeinsame Entwicklung von Samen, Wurzelendophyten und dem Mikrobiom der Rhizosphäre auswirkt. Verschiedene Weizen- und Gerstenarten wurden in dieser Studie aufgrund ihrer langen Domestikationsgeschichte, ihres Nährwerts und ihrer wirtschaftlichen Bedeutung als Modellpflanzen ausgewählt.

In der ersten Phase der Studie wurden die Auswirkungen der Domestizierung auf die mikrobielle Struktur, deren Vielfalt und das mikrobielle Netzwerk von Samenendophyten von wilden und domestizierten Weizen- und Gerstenarten untersucht. Anschließend wurde die phylogenetische Ähnlichkeit zwischen den Getreidearten und ihrer Spermosphäre als Hinweis auf eine Koevolution zwischen Pflanzen und Mikroben untersucht. Das Hauptergebnis dieser Studie war der Befund einer höheren mikrobiellen Diversität von Samenendophyten in modernen Weizenarten im Vergleich zu ihren entsprechenden wilden Vorläufern. Im Gegensatz dazu wurden bei wilden Arten mehr Mikroben-Mikroben-Interaktionen beobachtet. Darüber hinaus wurde festgestellt, dass *Cutibacterium*, eine mit dem Menschen assoziierte Bakteriengattung, in kultivierten Getreidearten im Vergleich zu Wildgetreide angereichert ist. Eine starke phylogenetische Übereinstimmung zwischen Samenendophyten und deren Wirtspflanzen wurde durch koevolutionäre Analysen aufgezeigt.

In der zweiten Phase wurden die Auswirkungen der Pflanzendomestikation auf die Abundanz von Mikroorganismen, deren Diversität, das mikrobielle Netzwerk in der Endorhiza- und Rhizosphäre von zwei Paaren genetisch verwandter Weizenarten (diploider Wildweizen *Aegilops tauschii* vs. moderner hexaploider *Triticum aestivum*; tetraploider Wildweizen *T. dicoccoides* vs. moderner tetraploider *T. durum*) in verschiedenen

Umgebungen untersucht. Dafür wurde eine Feldstudie an drei Standorten (landwirtschaftliche Versuchsstationen der JLU: Groß-Gerau, Weilburger Grenze and Rauischholzhausen) in Hessen, Deutschland, durchgeführt. Die unterschiedlichen Habitat-Mikrobiome wurden durch eine Amplikon-Sequenzierung des 16S rRNA-Genes und der ITS2-Region-untersucht. Zunächst wurde die Auswirkung der Domestikation auf das von den Samen auf die Endorhiza und die Rhizosphäre übertragene Mikrobiom durch den Vergleich des Anteils der Samen-ASVs (Amplicon sequence variant), die auf die Mikrobiome der Endorhiza und der Rhizosphäre übertragen wurden, an den drei Standorten analysiert. Der relative Anteil des durch Samen übertragenen Mikrobioms war in der Endorhiza und Rhizosphäre des diploiden *A. tauschii* höher und vielfältiger als bei anderen tetraploiden und hexaploiden Weizenarten. Außerdem wurde ein signifikanter Standorteffekt auf den relativen Anteil des von Pilzen übertragenen Mikrobioms im Vergleich zu Bakterien festgestellt.

Der Vergleich der unterschiedlich häufig vorkommenden Arten zeigte, dass in der Rhizosphäre von *A. tauschii* mehr Bakteriengattungen signifikant angereichert waren als bei den anderen Weizenarten, die am selben Standort angebaut wurden. Die Untersuchung der Abundanz der einzelnen Bakteriengruppen zeigte, dass die Rhizosphäre genetisch verwandter Paare von Weizenarten mit ähnlichen Bakterien- und Pilzgattungen aus dem Feldboden angereichert war, die Zusammensetzung dieser angereicherten Mikrobiome war jedoch je nach Standort unterschiedlich.

Ein Unterschied in der Beta-Diversität der bakteriellen und pilzlichen Mikrobiota zwischen wilden und domestizierten Weizenarten wurde nur in der Wurzelendosphäre, nicht aber in der Rhizosphäre festgestellt. Die Analyse der Unterschiede in der Abundanz des Mikrobioms in der Rhizosphäre ergab jedoch eine Verschiebung der Zusammensetzung in der Rhizosphäre moderner Weizenarten. Darüber hinaus wurden zwischen zwei Paaren verwandter Weizenarten unterschiedliche Domestizierungseffekte beobachtet; zwischen dem modernen hexaploiden *T. aestivum* und seinem diploiden D-Genomspender *A. tauschii* wurden im Vergleich zu dem anderen Paar drastischere Veränderungen festgestellt. In beiden modernen Weizen-Rhizosphären wurde eine Anreicherung des bakteriellen Mikrobioms festgestellt. Auch die Abundanz des Pilzmikrobioms war erhöht, jedoch war deren Vielfalt, insbesondere die der pathogenen Pilze, im Vergleich zu ihren wilden

Verwandten reduziert. Darüber hinaus wurde in der Rhizosphäre moderner Arten im Vergleich zu ihren Vorfahren eine geringere Interaktion zwischen den Pilzund Bakterienfestgestellt. Außerdem war die Abundanz der bakteriellen Gene, die für die Produktion von Proteinen verantwortlich sind, die am Nährstoffkreislauf beteiligt sind, bei den modernen Weizenarten geringer als bei ihren wilden Verwandten. Die Korrelation zwischen dem Mikrobiom der Rhizosphäre und den funktionellen Genen zeigte diejenigen mikrobiellen Spezies in natürlichen Lebensräumen auf, die eine zentrale Rolle bei mikrobiellen Interaktionen spielen.

Durch die Untersuchung des Mikrobioms von Wildpflanzen erhalten wir Einblicke wie die Domestizierung die Zusammensetzung und Funktion des Mikrobioms der Spermosphäre/Wurzelendosphäre/Rhizosphäre beinflußte und ermöglicht die Nutzung dieses Wissen, um vorteilhafte Assoziationen in aktuellen Kultursorten wiederherzustellen.

# **Chapter 1:** General introduction

### 1.1. Rhizosphere microbiome

The plant microbiome has an important role in overall plant performance. These host-associated microorganisms and the host are called holobiont and the sum of the genetic information of the host and its microbiota is the hologenome (Theis et al., 2016; Zilber-Rosenberg & Rosenberg, 2008) or holobiome (Guerrero et al., 2013). Microbial symbionts can develop close, historic, and/or cooperative relationships with their hosts (Zilber-Rosenberg & Rosenberg, 2008). Most of the interactions take place in the rhizosphere, where plant roots meet the soil. Both prokaryotic (*Bacteria* and *Archaea*) and eukaryotic (Mycorrhizal fungi, protists) macro/microorganisms constitute the rhizosphere microbiome, which continuously interacts with each other as well as with their host plant. The intricate microbe-microbe and microbe-host interactions are frequently the sources of major microbiome impacts on plant fitness (Lemanceau et al., 2017).

As a result of plant breeding and domestication, plants and their associated microbiome have undergone enormous changes (Abbo et al., 2014; Bulgarelli et al., 2012; Pérez-Jaramillo et al., 2016). Plant microbiome and domestication studies of the different plants are essential contributions to sustainable agriculture growth. However, there is a general lack of research on the effect of plant breeding on microbe-microbe, microbe-host interactions, microbial composition, and function. This research aims to identify and evaluate the scale of the domestication effect on root-associated microbiome interaction, structure, and functions employing high-throughput sequencing technology.

#### 1.1.1. Importance of rhizosphere microbiome

Every plant organ harbors a unique microbial consortium and has distinct functions. Overall plant fitness strongly depends on its microbiota and can also be increased by microorganisms (Aschehoug et al., 2014; Newcombe et al., 2009; Redman et al., 2011). These microorganisms constantly interact with their host plant, surrounding environment (air, water, soil etc.), as well as each other, and develop complex interactions, which ultimately have a neutral, beneficial, or negative effect on plant health and survival. The most complex and active interactions take place in the rhizosphere, which is the interface of root and soil, known to have diverse micro/macroorganisms like bacteria, fungi, archaea, protists, nematodes, viruses, earthworms, and others. The physicochemical properties of the rhizosphere are different from the surrounding bulk soil and a hotspot for microorganisms

due to actively released biochemical compounds (low-molecular-weight compounds: amino acids, organic acids, sugars, flavonoids, aliphatic acids, fatty acids, secondary metabolites, and high molecular weight compounds: mucilage, proteins) (Bokhari et al., 1979; Herz et al., 2018; Schurr & Schulze, 1995). Rhizomicrobiome provides important functions for maintaining host plant health through microbial interactions. These benefits include abiotic-biotic stress alleviation, microbe-mediated nutrient acquisition, pathogen suppression, etc. Indeed, rhizobacteria like *Pseudomonas fluorescens, Bacillus amyloliquefaciens* (Nassal et al., 2018), *Rhizobium* (Korir et al., 2017; Montañez et al., 2009) and fungi like arbuscular mycorrhizal fungi (AMF) (Thirkell et al., 2020), can assist plants by in solubilizing inorganic P, fixing nitrogen, and making many other vital micronutrients available for plant uptake. A classic example of the beneficial plant-microbe corporation is the mutualistic symbiosis between nitrogen-fixing rhizobia like *Rhizobium leguminosarum* and legumes where microbes provide nitrogen supply to the plant in exchange for nutrients. Also, suppression of plant pathogens is often found between "*Pseudomonas capeferrum"* and plant*s. "P. capeferrum"* can cooperatively trigger the production of scopoletin*,* an aromatic organic chemical compound releases from the root as a secondary metabolite, which can selectively suppress the soil-borne fungal pathogens *Fusarium oxysporum* and *Verticillium dahliae* (Stringlis et al., 2018).

Most of these beneficial relationships are often triggered by different stress conditions such as drought (Sendek et al., 2019) or pathogen attack (Ardanov et al., 2012), and these surviving mechanisms are conserved in the plant genome (He et al., 2021; Smith et al., 1999). It is believed that the wild ancestors of currently cultivated plants have distinct strategies for dealing with a variety of stressors (Mace et al., 2021; Simon et al., 2021). The mechanisms of employing microorganisms to tolerate those stress conditions and these advantageous traits can profoundly improve plant health and survival during possible increasing climate change. Thus, many wild crop plants showed higher resistance against pathogens and stress tolerance than the cultivated accessions. For example, wild ancestors of Finger millet (*Eleusine coracana* (L.) *Gaertn*. subsp. *coracana*) showed stronger tolerance against blast disease-causing fungus *Magnaporthe grisea* (Dida et al., 2021). Furthermore, wild relatives of eggplants (*Solanum melongena)* (Kouassi et al., 2021), alfalfa (*Medicago sativa* L.) (Humphries et al., 2021), Sorghum (*Sorghum bicolor* L.) (Ochieng et al., 2021) displayed

strong tolerance against drought. Discovering the plant-beneficial effects produced by certain inhabitants of the rhizosphere microbiome is critical for plant health and productivity.

### 1.1.2. Endorhiza and rhizosphere and microbiome assembly driving factors

*Root endosphere microbiome*

Microbial colonization of internal tissues of plants starts from the embryo of seeds (Kuźniar et al., 2020) and is transmitted to seedlings (Johnston-Monje & Raizada, 2011; Lopez-Velasco et al., 2013). Another major source of the root endophytes are soil microorganisms that penetrate (through the root tips, wounds, and stomata) and colonize the plant root endosphere (Compant et al., 2010). However, root endophytes are less diverse than the bulk soil from which they emerged, since they require particular adaptations to colonize the rhizosphere and to gain access to roots (Knights et al., 2021; Schlaeppi et al., 2014). Intimate symbiotic and mutualistic connections with the host might be developed as a result of the long-term co-evolution of hosts with certain microorganisms. A field study in Australia with 470 samples (235 roots and 235 associated bulk soil) from 31 plant species across six plant communities showed that *Bradyrhizobium*, *Rhizobium*, *Burkholderia*, *WPS-2*, *Ellin329*, and *FW68* (uncharacterized lineages) are conserved in the root across plant phyla during plant evolution, and these core root microbiome has evolved with their host plants over million years (Yeoh et al., 2017).

The dominant colonizers of the root endosphere are endophytic AMF, bacteria, archaea, often specific to their host plant. Their interaction with their hosts often benefits the host plant through better uptake of soil nutrients (Knights et al., 2021; B. Wang & Sugiyama, 2020; Yeoh et al., 2017). An example of such beneficial endophytic root colonization is the association of cereals, nonlegume crops, and *Arabidopsi*s with nitrogen-fixing bacteria *Azorhizobium caulinodans* (Cocking, 2003).

Rhizosphere microbiome assembly is a geographical and dynamic process that is triggered by, soil type and root exudates, and plant growth stage.

*Plant genotype and growth stages effect on the rhizosphere microbiome*

Plants can influence the microbiome in their rhizosphere and each plant species fosters a distinct group of rhizosphere microorganisms (Ofek et al., 2014). The evolutionary history of host plants can significantly affect the assembly and composition of their associated

bacterial microbiomes as proved by Bouffaud and colleagues (2014) on maize genotypes and other *Poaceae* with the associated bacterial microbiome. The results showed that the phylogenetic distance between *Poaceae* genotypes significantly correlated with the rhizobacterial microbiome. The effect of plant genotype is better observed when plants are compared with their wild relatives as recently demonstrated by Cordovez and colleagues (2021) in tomato plants. They studied rhizosphere microbiome dynamics over successional cultivation and found an increased dissimilarity in rhizosphere microbiome assembly between wild and domesticated tomatoes.

The host specificity of microbes can be also associated with their genome as Pawlowski et al., (2020) showed that specific trait *loci* identified in the genome of soybean are responsible for making symbiotic interactions with AMF. Their findings are in line with the study by Batstone et al. (2020), where they inoculated five legume genotypes of *Medicago truncatula* with the known ability of selection for effectiveness in N fixation with two, ineffective and effective N-fixing rhizobial isolates of *Ensifer meliloti* which were previously co-cultured for five generations of *Medicago truncatula*. *E. meliloti* quickly adapted to its local host genotype and derived other beneficial microbes when they co-evolved with their host plant (Batstone et al., 2020).

Depending on the plant genotype, the content of root exudate differs as Mönchgesang et al. (2016) discovered a strong variation in root exudate chemistry among *Arabidopsis* accessions. Indeed, plant-specific biochemical compounds released from the root tips attract specific microorganisms in the rhizosphere. Haichar et al. (2008) determined bacterial communities according to preferences of carbon source (root exudates vs soil organic carbon) in the rhizosphere of wheat, maize, rape, and barrel clover, using a stable isotope probing approach. *Sphingomonadales* were found to be specific to monocots wheat and maize, whereas bacteria related to *Enterobacter* and *Rhizobiales* were considered as generalists as they utilized both fresh and ancient carbon. Another similar investigation of the rhizosphere microbiome has revealed that *Bacillaceae* and *Rhizobiaceae* were specifically recruited by multiple tomato genotypes (French et al., 2020).

For certain plant species growing on identical soil conditions, the influence of plant genotype can be larger due to local microbiome selection of genotypes as observed by Wang & Sugiyama, (2020) in the root microbiome of flowering plants. Furthermore, Matus- Acuña et al. (2021) showed maize genotype effect on eukaryotic rhizosphere microbiome of three

maize landraces and one inbred line growing in identical soil. Matus-Acuña and colleagues also found that the maize genotype can shape its rhizosphere eukaryotic microbiome. These results suggest that plant-specific microbes are mostly affected by the plant genotype.
Rhizosphere microbiome establishment and composition constantly change and progress during the whole plant development stages (Chaparro et al., 2013; Cordovez et al., 2021). The microbiome variation in the rhizosphere is mainly related to the changes in root exudate composition (Zhalnina et al., 2018). The interaction of plant exudation traits and microbial substrate consumption result in the patterns of microbiome formation observed in the rhizosphere of an annual grass (Zhalnina et al., 2018). Using a combination of DNA-based community mapping and isolate phenotyping, Hu et al. (2020) proved that by comparing the rhizosphere microbiome of tomato plants (*Lycopersicon esculentum*) in different grow stages (seedling, flowering, and fruiting stages), that the highest stress resistance against abiotic and functional diversity occurs during the flowering stage. Berlanas et al. (2019) found that the composition of fungal and bacterial rhizosphere microbiome changed between old and young grapevine genotypes and also identified distinct microbial taxa (*Bacillus*, *Glomus*) associated with grapevine rootstocks.

Depending on plant genotype or growth stage, root architecture changes, and this is highly correlated with the rhizosphere microbiome. For example, root system architecture significantly changed during domestication due to the selection of specific traits (Maccaferri et al., 2016). A comparative study of root system architecture showed substantial differences between the rhizosphere microbiome of wild and modern maize lines (Szoboszlay et al., 2015). According to this study, potential N-acetylglucosaminidase activity was the main contributor for the found differences in teosinte rhizosphere than other corn species. The results are in line with the results of Pérez-Jaramillo et al. (2017) where they showed the variability in rhizobacterial microbiome assembly between the common bean (*Phaseolus vulgaris*) and its relatives related to changes in root length.

*The effect of root exudates on microbiome composition*

Root exudates (soluble or volatile) are mainly divided into two types. The first one is the primary metabolites containing variable sugars, amino acids, and organic acids that are released from the root apical meristem and rapidly utilized by fast-growing generalists. The

second type is the secondary metabolites, terpenoids, phenolics, alkaloids, nitrogen- and sulfur-containing compounds, which are believed to have more roles in the shaping root microbiome structure (Clocchiatti et al., 2021; Voges et al., 2019). Both primary and secondary root metabolites play an important role in microbiome functioning and assemblies such as plant nutrition enhancement, defense mechanisms against pathogen attack, and abiotic stress mitigation.

Plant nutrition can be improved by secondary metabolite-induced microbe-microbe, host-microbe interactions in the rhizosphere and this includes symbiotic associations with beneficial microbes, such as mycorrhizae, rhizobia, and plant growth-promoting rhizobacteria. For instance, as a strategy to attract nitrogen-fixing rhizobia symbionts legumes produce flavonoids to stimulate bacterial *nod* genes (Varma et al., 2017). The phytohormone strigolactones, a secondary metabolite, initiate the natural colonization of many plant roots by AMF (Varma et al., 2017). Furthermore, secondary metabolites are important in the early colonization of plant growth-promoting bacteria in roots. Using *Arabidopsis thaliana* seedlings, Allard-Massicotte et al. (2016) revealed that *A. thaliana* actively recruited *B. subtilis* via root-secreted chemicals, which are mediated through the chemoreceptors.

Root exudates are also important in plant defense mechanisms. For example, the *Barrassicaceae* plant family can produce sulfur-containing phytoalexins, which can suppress pathogenic fungal growth. The signaling of secondary metabolites, such as jasmonic acid, ethylene, and salicylic acid induces plant resistance (Fan et al., 2017). For instance, *Pseudomonas* sp inoculation can promote the production of benzoic acid and salicylic acid expression against the groundnut stem rot pathogen *Sclerotium rolfsii* (Ankati et al., 2019).

Another group of secondary microbial metabolites is volatile organic compounds (VOCs). VOCs are important signaling molecules within bacterial communities against pathogens. Alkanes, alkenes, alcohols, ketones, terpenoids, and sulfur compounds are some of the chemical classes of microbial VOCs. Schenkel et al. (2015) demonstrated that VOCs are produced by most rhizobacteria. Using isolation, soil bioassays, comparative genomics, and metabolite profiling, Carrión et al., (2018) revealed significant disease-suppressive activity of *Paraburkholderia graminis* against fungal root pathogen *Rhizoctonia solani.* The antifungal

activity of Pa. *graminis* PHS1 was associated with genes that encode the production of sulfurous volatile compounds. *Trichoderma* spp. are also known to produce VOCs, such as heptanal, octanal, and 2-methyl-1-butanol that can inhibit fungal growth (Guo et al., 2019).

Finally, stress alleviation by root-exuded coumarins induces an adaptive reaction of plants against iron deficiency by modulating the rhizosphere microbiome for iron mobilization (Voges et al., 2019). For example, inoculation of pennyroyal (*Mentha pulegium* L.) with *Azotobacter chroococcum*, *Azospirillum brasilense* reduced drought stress significantly by increasing secondary metabolites including flavonoid, phenolic, essential oil contents (Asghari et al., 2020). Another microbial mechanism, phytohormones production by many plant growth-promoting rhizobacteria (PGPR), to address stress management, is the important microbe-mediated mechanism in plant performance.

*Biotic and abiotic factors shaping rhizosphere microbiome*

Soil microbiome can change dramatically across time and geography, resulting in changes in the microbial pool accessible for root/rhizosphere colonization. The primary drivers of differences in soil microbiome composition are often niche-based factors, which consider both abiotic and biotic factors. This implies that a set of attributes can contribute to a variable selection effect on microbial populations, as well as expected and random events, resulting in dynamic changes in microbiome assembly. The dynamic changes in the assembly of bacterial and archaeal soil microbiome were observed by Goss-Souza et al. (2017) under long-term grassland compared to other land management systems (forest and no-till cropping). Furthermore, weather patterns and season, land usage, soil type, and physicochemical properties, agriculture practices such as crop rotation, pesticide/fertilizer inputs, and tillage, were all examined as biotic and abiotic variables impacting the structural and functional diversity of the soil microbiome (Fierer, 2017; Fuka et al., 2008; Lauber et al., 2009; Schmidt et al., 2019; Yin et al., 2017). Soil texture can influence the soil microbiome assembly by affecting biochemical soil reactions. Fuka et al., (2008) revealed the effect of soil texture on the gene abundance encoding protein degrading microbes in the soil. Furthermore, phosphorus (P) mobilization by soil microbes in forest spodosols was affected by soil properties and soil depth (Achat et al., 2012). Additionally, carbon and nutrient content, moisture, and pH-value change the rhizosphere microbiome under different land

use. Vieira et al. (2020) showed that the composition of active rhizosphere bacterial communities in temperate grasslands was influenced by soil characteristics, notably by soil texture, water content, and soil type.

Agricultural intensification drastically changes the environment in which crops are grown and strongly modifies soil microbiome assembly. Fungal communities are altered as a response to tillage. Fungal microbiome and alpha diversity in the rhizosphere and bulk soil were significantly higher under 6 years of zero tillage compared to conventional chisel plow tillage during wheat growth (Wang et al., 2017). The application of fungicides and fertilizers affects the soil microbiome as much as soil agriculture practices. Fungicides significantly change the abundance, diversity, and function of the soil microorganisms (Karas et al., 2018; Monkiedje & Spiteller, 2002).

Environmental stress conditions like salinization, drought, flood, nutrient limitation can significantly alter microbiome composition and can limit microbial activity. However, some of the environmental stress conditions can induce plant-microbe interactions that can mitigate these negative impacts. *Pseudomonas*, *Bacillus*, *Azospirillum*, *Azotobacter*, *Rhizobium*, *Bradyrhizobium*, *Trichoderma*, *Methylobacterium*, *Cyanobacteria,* and other plant growth-promoting bacteria interact with their host for stress mitigation (Jochum et al., 2019). The investigation of genome-wide identification and protein expression analysis revealed that during salt stress rice roots express OsGRAM genes, which induced beneficial interactions with *Bacillus amyloliquefaciens* (SN13) (Tiwari et a., 2020). It was also hypothesized by Cortés & Blair (2018) that wild relatives of currently cultivated plants might be repositories of genes linked to drought resistance.

### 1.1.3. Vertical transmission of microbes and plant domestication

Along with environmental sources, seeds can be considered as one of the important origins of the plant microbiome. Seed microbiota serves as an initial inoculant for plants and plays a vital role in plant development and survival (Bulgarelli et al., 2015; Johnston-Monje & Raizada, 2011). It is known that the seed-endophytes can promote seed germination (Goggin et al., 2015; Li et al., 2017) and benefit seedlings in several ways, including plant growth stimulation by improved nutrient acquisition from soil (Johnston-Monje & Raizada, 2011) and improved disease resistance against pathogens (Díaz Herrera et al., 2016; Khalaf &

Raizada, 2018). Seed-borne microorganisms as the first inhabitants of the rhizosphere promote the establishment of beneficial interactions in the rhizosphere through exuding secondary metabolites and hormones in their immediate environment, attracting microorganisms to inhabit the spermosphere, rhizosphere, and seedling (Truyens et al., 2015; Vignale et al., 2018; Verma et al., 2019). Microbes can be transmitted from seeds to root through two main pathways: i) vertically, where microbes are transmitted from the parent plant through embryo and pericarp, and ii) horizontally, where microbes are derived from the environment (Hardoim et al., 2012; Johnston-Monje & Raizada, 2011). For example, *Alternaria*, *Clostridium*, *Paenibacillus*, *Enterobacter*, *Methylobacteria*, *Pantoea, Erwinia, Rhizobiales, Bacillus, Micrococcus, Acinetobacter*, *Emericella, Stenotrophomonas, Brevundimonas* and *Pseudomonas* species, which are often reported as seed-borne species (Hardoim et al., 2012; Huang et al., 2016; Johnston-Monje & Raizada, 2011; Kuźniar et al., 2020; Ofek-Lalzar et al., 2016; Torres-Cortés et al., 2019).

The majority of vertically transmitted microorganisms seem to have symbiotic, mutualistic connections with their hosts. An isolate *Burkholderia phytofirmans* from the *Zea* landrace seed was tested as a plant promoter and resulted in promoting shoot potato biomass (Johnston-Monje & Raizada, 2011). Using *in vitro* antagonism, Khalaf & Raizada (2018) showed the antagonistic effects of *Lactococcus*, *Pantoea*, *Bacillus,* and *Paenibacillus* endophytes against fungal and oomycete pathogens *Rhizoctonia solani*, *Fusarium graminearum*, *Phytophthora capsici*, *Pythium aphanidermatum* that can threaten the developing seedlings of cucurbit vegetables. A recent study also showed that the heritable symbiont *Epichloë coenophiala* (Sordariomycetes) is a key microbial species of tall fescue (*Schedonorus phoenix*) seed microbiome, which can modulate the fungal endophytic communities (Nissinen et al., 2019). Due to the beneficial interactions between microbes and host plants, many plants have been grown without the use of fungicides for thousands of years. However, whether these beneficial associations are affected or hindered during domestication in modern agriculture, they need a more careful investigation.

The use of high-throughput technology has recently allowed a deep assessment of the seed-associated microbiome of wild and domesticated crop species. According to previous seed endophyte studies, plant breeding significantly shifted the seed microbiome (Leff et al., 2017). Such as, Hassani et al. (2020) found that the vertically transferred bacterial

microbiome of modern hexaploid *Triticum aestivum* was less complex and significantly variable compared to wild emmer *Triticum dicoccoides.* Moreover, fungal seed endophytes of wild *Triticum dicoccoides* and *Aegilops sharonensis* contained more taxonomically diverse fungal endophytes with known beneficial effects than modern bread wheat *T. aestivum* (Ofek-Lalzar et al., 2016). Furthermore, Kim et al. (2020) found that domestication shifted seed microbial communities of wild and domesticated rice with a strong effect on the fungal microbiome. These results suggest that the domestication did not affect beneficial fungal seed endophytes. However, it reduced pathogenic fungi in modern crops as demonstrated by Leff et al (2017) in *Helianthus annuus*. The shift in fungal communities might lead to reduced inter-kingdom networks which are important for plant fitness (Kim et al., 2020).

### 1.2. Domestication of crops

Plants started to be gathered thousand years (50,000) ago by hunter-gatherers; however active domestication of the majority of crops started around 10,000 to 12,000 years ago in different parts of the world. The first domesticated crop plants were different depending on the part of the word e.g. in Near Eastern agriculture for examples emmer wheat, barley, lentil, and pea, and in the Sub-Saharan Africa agriculture plants like sorghum, pearl millet, cowpea, and yam. This area was called the Vavilov center after the Russian scientist Nikolai Vavilov how first identified these centers in 1924 (Abbo et al., 2017).

During the domestication process, farmers used a few progenitor species and only seeds from the best plants were used to create the following generation. This leads to the loss of the progenitor's genetic diversity. Plants carrying favored alleles generated the most offspring to each succeeding generation, while other alleles were removed from the population, resulting in a greater loss of variety in favor of desired characteristics as shown for the maize genome (Wright et al., 2005). For example, comprehensive pan-genome analyses, based on 1,961 cotton lines, revealed that 32,569 and 8,851 non-reference genes were lost from wild *Gossypium hirsutum* and *Gossypium barbadense* reference genomes, respectively, which accounts for 38.2 percent (39,278) and 14.2 percent (11,359) of genes (Li et al., 2021).

The domestication process can be defined as a series of selection that leads to the segregation of desirable traits from parent wild species that are beneficial to agriculture

(Lenser & Theißen, 2013). Improved yield, increased fruit or grain size, regulating plant development, reduced seed shattering, loss of need for vernalization, loss of day length reliance, modification of root architecture, and loss of seed dormancy were among the adaptations that made it possible for humans to cultivate the plants efficiently. The intentional and unintentional selection for agronomic, morphological, and physiological qualities results in genetic alteration and lower genetic variation and allelic diversity of domesticated crops as well as a rise in their vulnerability to environmental challenges.

Quantitative trait loci (QTL) regulate some of the most significant morphological changes that occurred during domestication. For example, the *Q* locus grants free-threshing in hexaploid wheat and is also involved in several other valuable domestication traits, including flowering time, plant height, inflorescence architecture, and encodes a transcription factor from the *AP2* family which plays a crucial role in plant growth, development, and responses to biotic and abiotic stressors (Olsen & Wendel, 2013). QTLs also regulate plant root colonization and symbiotic interactions and are observed on several crop chromosomes. A recent study identified 6 quantitative loci in the genome of soybean linked to the colonization of AMF *Rhizophagus intraradices* (Pawlowski et al., 2020). The same number of QTLs were found in 94 winter wheat genotypes linked to mycorrhizal colonization (Lehnert et al., 2017). A similar result was found in tetraploid wheat genotypes using genome-wide association study (GWAS) and single-nucleotide polymorphism (SNP) array by screening 127 wheat accessions inoculated with the AMF species *Funneliformis mosseae* and *Rhizoglomus irregulare*. GWAS revealed four significant quantitative trait nucleotides involved in mycorrhizal symbiosis, located on chromosomes 1A, 2A, 2B, and 6A (Ganugi et al., 2021). Furthermore, domestication can have an impact on ecological relationships, either through modulating the expression of specific genes linked to tolerance against pathogens or predators or through quantitative trait selection (Chen et al., 2015).

The accumulation of deleterious mutations in the genomes of domesticated crops is a bottleneck of domestication. Lu et al. (2006) discovered that domesticated rice lineages have more non-synonymous replacements, particularly radical amino acid alterations, than wild rice lineages. In comparison to their wild ancestors, domesticated lineages have more deleterious mutations accumulated in their genomes (Moyers et al., 2018).

During the selection of a specific plant trait, genome modification may occur due to amino acid substitution, split-site mutation, regulatory changes, transposable elements, or genome duplication (polyploidy). Many previous comparative studies found more enhanced physical features, simplified morphologies, altered nutritional content, and weakened plant defenses (Arzani & Ashraf, 2017; Roucou et al., 2018) in domesticated crop plants compared to their wild relatives. These morphological changes due to genetic alterations in the host genome are potential sources of hologenomic diversity within the holobiont (Hacquard, 2016). Genetic alterations might have phenotypically neutral, deleterious, or favorable effects on plant holobiont (Rosenberg et al., 2009).

Despite the fact that the genes that underpin domestication are increasingly being identified (Doebley et al., 2006; Olsen & Wendel, 2013; Tang et al., 2010), little is known about how domestication influences the expression of genes that are critical in microbe-host interactions.

### 1.3. Impact of domestication on the plant microbiome

Although domestication improved crop yield and overall performance, plant quantitative traits involved in advantageous plant-microbe interactions could have been lost throughout the domestication process, due to the selective breeding of a few particular traits. The genome modification (gene duplication, accumulation of deleterious mutations, removals, and translocations of QTLs) might have changed or removed the genes involved in microbial symbiosis, root traits which might lead to reduced rhizosphere microbiome interactions. For example, several QTLs were identified in different crops such as maize, soybean, and wheat species linked to mycorrhizal colonization (Lehnert et al., 2017; Pawlowski et al., 2020; Ramírez-Flores et al., 2020) suggesting the possible influence of genetic modification on the beneficial associations between host and microbes.

Furthermore, domestication of crop plants can affect root exudates by changes (regulatory and/or protein modifications in specific genes, structural heterogeneity, transposons, or genome doubling) in the expression of single genes associated with protein production that can modify the molecular structure of precursors (produced in the TCA cycle, or the shikimate pathway) for the synthesis of the secondary metabolites in primary metabolism (He et al., 2003; Jacoby et al., 2021; Ober, 2005). Gene duplication leads to the tremendous expansion of the gene catalog occurring in higher plant evolution that might contribute to

the diversification of secondary metabolites (Gaynor et al., 2020; Hofberger et al., 2013). The variable secondary metabolites lead to increased microbiome diversity in the rhizosphere of modern cultivars as reported by Cardinale et al. (2015) in wild and domesticated lattice rhizosphere.

Furthermore, previous rhizosphere microbiome studies claim that domestication shifted the bacterial composition (Bulgarelli et al., 2015; Pérez-Jaramillo et al., 2017; Schlaeppi et al., 2014) from slow-growing oligotrophic microorganisms that can easily adapt to low-nutrient conditions (*Bacteroidetes*, *Verrucomicrobia*, *Gemmatimonadetes*, fungal phylum *Basidiomycota*) towards fast-growing copiotrophic microbes efficiently utilizing diverse and abundant resources (*Proteobacteria*, *Firmicutes*, *Actinobacteria*, and fungal phylum *Ascomycota*) (Yao et al., 2017). Pérez-Jaramillo et al. (2017) associated the microbiome assembly change in the rhizosphere of *Phaseolus vulgaris* to the genotype and specific root phenotypic traits such as reduced fine hairs, increased exudation of simple sugars of modern crops. They found that *Bacteroidetes* are associated with thin root hairs of wild ancestors of current bean whereas *Actinobacteria* and *Proteobacteria* are related with thick roots of modern varieties. Furthermore, this microbiome composition shift seems to be correlated to increased fertilizer input. A recent study by Terrazas et al. (2019) showed that the nitrogen fertilizer altered the modern barley (*H. vulgare* ssp. *vulgare*) rhizosphere microbiome by planting two genotypes of wild (*H. vulgare* ssp. *spontaneum*) and domesticated (H. *vulgare* ssp. *vulgare*) barley in an agricultural soil supplemented with and without nitrogen (N). Under N-limited conditions, wild barley genotypes had higher nitrogen and sulfur metabolisms than contemporary genotypes, which had richer RNA and cell capsule metabolisms (Terrazas et al., 2019).

A further change in the rhizosphere microbiome is a reduction in pathogenic fungi abundance as well as beneficial fungi in modern crops (Szoboszlay et al., 2015; Leff et al., 2017; Shi et al., 2019; Spor et al., 2020; Tkacz et al., 2020). These results suggest that current crop plant cultivars may have lost some of the functional traits required to attract host-specific root-associated microorganisms which play important role in plant defense. For instance, when tomato rhizospheres were exposed to *Ralstonia solanacearum* pathogen invasions, a stronger correlation was obtained between pathogen invasion and reduced rhizosphere bacterial diversity and abundance, fewer bacterial interactions networks, and loss of several functional genes (Wei et al., 2018). Furthermore, the most effective and

commonly used recessive resistance plant traits against pathogens have been determined in nature. Therefore, the recessive resistance traits can be found in the wild ancestors which are genetically more diverse and adapted to pre-agricultural soils. Such as, wild barley is more resistant against mildew and rust than domesticated barley (Schmalenbach et al., 2008).

Many changes in plant traits occurred in tandem with gradual changes in the environment and management techniques throughout domestication. Reduced fungal rhizosphere microbiome in modern crops implies that agricultural practices against pathogenic fungi might destroy the natural balance of the microbiome where bacteria and fungi interact for mutual benefit. Agricultural methods such as plowing, mono-cropping, or high fertilization rates inhibit hyphal growth and reduce the functioning of the AMF symbiosis (Martín-Robles et al., 2018; Schmidt et al., 2019). Moreover, modern agriculture reduced the dependency of crops on mycorrhizal symbiosis (Spor et al., 2020). For example, Martın-Robles et al. (2018) compared root AMF colonization of 27 different crop species and their wild relatives under varying available P conditions. They found that modern crops only established symbiotic interactions when P was limited whereas wild relatives benefited from AMF regardless of P availability. Besides mycorrhizal fungi, many saprophytic fungi species colonize the rhizosphere and actively consume root exudates. The rhizosphere fungal microbiome may also indirectly boost plant defense and development. However, most of the widely applied synthetic fungicides to suppress plant fungal pathogens are non-target meaning that the chemicals can impact both pathogenic and non-pathogenic fungi (Shao & Zhang, 2017).

Crop domestication also resulted in reduced extracellular enzyme activity such as the total abundance of ammonia monooxygenase gene copies gradually reduced from wild to modern wheat species (Spor et al., 2020). Functional traits of modern crops can be changed during plant domestication as shown by Szoboszlay et al. (2015) that the potential N-acetylglucosaminidase activity patterns were different in the teosinte rhizosphere than the other corn varieties.

The domestication-related changes in the rhizosphere microbiome of modern crops might weaken the microbial network as reported by Kavamura et al. (2020) that fewer connections between the microbiomes of tall cultivars were observed than of semi-dwarf cultivars. Domestication also displaced inter-kingdom connectivity of hub species from fungi to bacteria in rice seed (Kim et al., 2020).

By the use of the newly developed "omics" method, we are just beginning to understand the intricate networks of interactions occurring within natural microbiomes. Next-generation sequencing enabled the study of microbiomes at virtually unknown resolution levels, allowing the identification of hub species in environmental samples. Based on the connection of their frequency patterns, this enabled the examination of potential interactions between microorganisms. Based on the connection of their frequency patterns, we can examine the potential interactions between microorganisms (Alibrandi et al., 2020; Cardinale et al., 2015; Manirajan et al., 2018). Exploring the beneficial interactions in the rhizosphere of wild relatives under natural conditions provides valuable insights about the lost traits during domestication, which could then be re-established using wild crops as a germplasm resource to improve the long-term performance of currently cultivated crops. For example, the *verticillium* wilt disease resistance polygenic trait of wild *Mentha* can be used to develop molecular markers for disease resistance alleles against the soil-borne pathogen *Verticillium* in modern mint species (Vining et al., 2020).

Comparative microbiome analysis of domesticated plants and their wild ancestors allows us to comprehend the consequences of plant domestication at the microbiome level. This knowledge may enable us to enhance crop performance and productivity by identifying the lost key traits that are important for making beneficial microbe-host as well as microbe-microbe interactions.

### 1.4. Cereal domestication and changes in the genome of modern wheat

Cereals are one of the first domesticated staple food crops. The cereals are particularly essential, accounting for more than half of all calories consumed today (http://faostat.fao.com/stats). Most of the currently cultivated wheat are polyploids and developed by hybridization between different species (allopolyploidy). The genome donors of currently cultivated species are wild diploid wheat species: *Triticum urartu* ($A^uA^u$), *Aegilops speltoides* (SS), *Aegilops tauschii* (DD), and *Triticum baeoticum* Boiss ($A^bA^b$) (Valkoun, 2001). For example, the wild emmer *T. turgidum* ssp*. dicoccoides* ($A^uA^uBB$), which is the progenitor of current tetraploid wheat species, originated from the hybridization between wild *Triticum urartu* (AA) and *Aegilops speltoides* lineages (BB) (Valkoun, 2001).

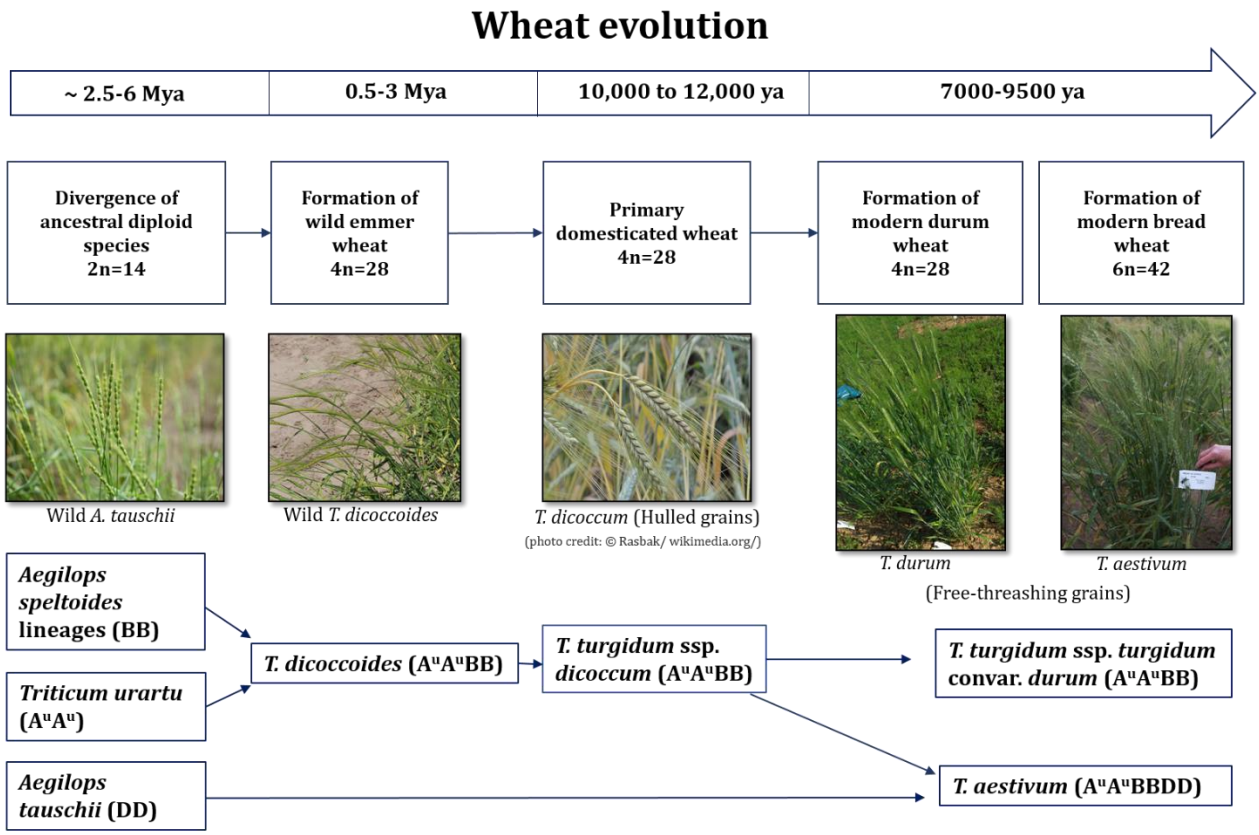


Figure 2. The pathway of domestication, evolution, genome formula, crossing events of current wheat species (Mya, million years ago; ya, years ago).

Around 10,000 to 12,000 years ago, the first cereal crops durum wheat (*Triticum turgidum* L. ssp. *durum* (Desf.)) and emmer (*T. turgidum* ssp. *dicoccum*) from wild emmer (*T. turgidum* ssp. *dicoccoides*) was primarily domesticated in the Fertile Crescent alongside with einkorn, barley (Valkoun, 2001). After that, tetraploid wheat species (*T. turgidum* ssp. *turgidum*) was secondarily bred from the domesticated emmer wheat, followed by the subsequent breeding of hard-grain durum wheat (*T. turgidum* ssp. *turgidum* convar) (Gioia et al., 2015; de Sousa et al, 2021). Hexaploid bread wheat *Triticum aestivum* ($A^uA^uBBDD$) originated from a hexaploidization as a result of hybridization between a descendant of the first tetraploid emmer ($A^uA^uBB$) and the wild diploid *Aegilops tauschii* Coss (DD) (Pont et al., 2019). Diploid einkorn wheat *Triticum monococcum* L. ($A^mA^m$) is another early cultivated wheat that was domesticated from its wild ancestor, *Triticum baeoticum* Boiss ($A^bA^b$) (Valkoun, 2001).

*Hordeinae* subtribes within the *Triticeae* tribe (*Poaceae* family) include another economically important cereal, barley (*Hordeum vulgare* ssp. *vulgare* L.). Barley is a member

of the *Hordeum* genus, which consists of 33 species that carry one of the four diploid genomes known as H, I, Xa, and Xu (Brassac et al., 2012). Annual, diploid wild barley *H. vulgare* ssp. *spontaneum* C. Koch (genome I) is the direct progenitor of cultivated barley (Sakuma et al, 2011).

Cereals, being the most genetically, physiologically, and morphologically transformed agricultural plants throughout domestication, provide an intriguing paradigm for studying the impact of domestication on plant-microbial interactions in the rhizosphere. Wheat has long been a strong choice for yield enhancement, first through domestication and then through selection and breeding due to nutritional and economic importance. However, domestication has resulted in decreased allelic diversity. The most significant gene diversity loss occurred in currently cultivated modern wheat species hexaploid *T. aestivum* and tetraploid *T. durum.* According to previous investigations, the genetic diversity was lost by 69% in hexaploid bread wheat and by 84% in tetraploid durum wheat during domestication (Haudry et al., 2007) as a result of polyploidy. Wheat ploidy resulted in improved crops; however, the polyploidy event is followed by a breeding bottleneck (Doebley et al., 2006), in which the limited number of plants participating in the creation of a new polyploid species limits its early gene diversity. Along with the reduction in gene diversity, genomic alterations occurred in the genome of modern wheat species. For example, the *Hardness* (*Ha*) locus governs grain hardness and leads to soft wheat grains, and is found in the A, B, and D diploid wheat genomes. The currently cultivated pasta wheat was developed by deleting *Ha* locus from both wild and cultivated types of tetraploid (AB genome) wheat (Olsen & Wendel, 2013) during polyploidization. However, subsequent modifications, removals, and translocations of *Ha* in the D genome of hexaploid wheat resulted in variations in hexaploid wheat seed quality, including semi-hard wheat varieties, which involved complicated rearrangements and recombination between genetic elements (Chantret et al., 2005). Another effect of polyploidy includes duplication and subsequent loss of separate paralogs (Olsen & Wendel, 2013). The alterations in the Q gene were caused by a single valine-to-isoleucine amino acid replacement in the A homolog after polyploid formation and the reunion of these now-diverged paralogs into a shared nucleus (Olsen & Wendel, 2013). Lv et al. (2021) discovered that the A-subgenome in tetraploid wheat species had higher levels of histone marker modification than the B-subgenome, and that hexaploid evolution and

domestication had a distinct impact on the epigenetic modifications between the subgenomes compared to tetraploid evolution and domestication.

Reduced allele diversity and gene modifications as a result of quantitative trait selection during cereal domestication have resulted in drastic physiological and morphological changes in plants. For example, a garden experiment with 39 genotypes of tetraploid wheat quantifying the vegetative phenotype of each genotype showed shorter leaf longevity, and a shorter root system, but higher net photosynthetic rate, leaf production rate, and a higher proportion of fine roots in modern cultivars than in wild forms (Roucou et al., 2018).

The significant impacts of domestication and breeding on root exudate composition were found between the rhizospheres of wild and cultivated wheat species (Iannucci et al., 2017). Furthermore, the compositional changes in mineral nutrient concentrations between high-yielding modern wheat seeds and low-yielding wheat cultivars were previously reported (Cakmak et al., 2000; Chatzav et al., 2010). However, how the plant microbiome reacted or adapted to domestication is mostly unknown.

## 1.5. Aim of the study

The overall goal of the thesis was to explore the rhizosphere microbiome of wild relatives of currently cultivated wheat and barley varieties searching for traits that might be involved in the beneficial microbial associations during plant domestication through identifying the root-associated microbiome structure, diversity, microbial network, co-evolution of endophytes with their host plants, root endophytes, and the rhizosphere microbiome.

The main objectives of the studies were:

**1.** To study the impact of plant domestication on the seed endophyte composition, diversity, and co-occurrence by comparing four cereal crops (*Triticum monococcum*, *Triticum aestivum*, *Triticum durum*, and *Hordeum vulgare*) and their wild relatives (*Triticum baeoticum*, *Aegilops tauschii*, *Triticum dicoccoides*, and *Hordeum spontaneum*, respectively), as well as to test the co-evolution patterns by constructing phylogenetic coherence between cereals and their seed microbiota.

**2.** To investigate the impact of domestication on seed-transmitted and soil-originated microbiome of the endorhiza and rhizosphere microbiome. Furthermore, to study the effect of environment and plant genotype on both the root and rhizosphere microbial colonization. Finally, trace the co-evolution, by comparing the relative proportion of microbial seed-transmission and microorganism recruitment of wild and domesticated wheat species.

**3.** To study the abundance, assembly, and inter-kingdom network of the rhizosphere/root endosphere bacterial and fungal microbiome of two genetically related (diploid wild *Aegilops tauschii* vs hexaploid bread wheat *Triticum aestivum*; tetraploid wild *Triticum dicoccoides* vs tetraploid pasta wheat *Triticum durum*) wild and domesticated wheat species. Moreover, to identify key species in the bacterial – fungal networking. Furthermore, to evaluate the effect of domestication on the abundance of microbial genes encoding enzyme involved in N and P-cycles in the rhizosphere.

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# **Chapter 2:** Domestication affects the composition, diversity, and co-occurrence of the cereal seed microbiota

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# Domestication affects the composition, diversity, and co-occurrence of the cereal seed microbiota

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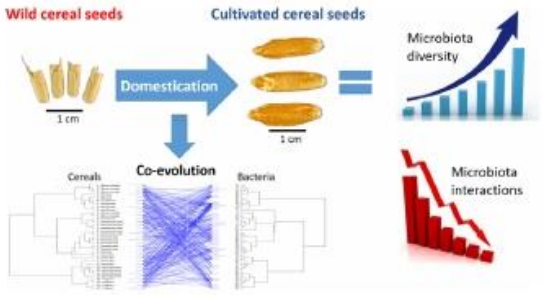
## GRAPHICAL ABSTRACT

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## ABSTRACT

*Introduction:* The seed-associated microbiome has a strong influence on plant ecology, fitness, and productivity. Plant microbiota could be exploited for a more responsible crop management in sustainable agriculture. However, the relationships between seed microbiota and hosts related to the changes from ancestor species to breeded crops still remain poor understood.
*Objectives:* Our aims were i) to understand the effect of cereal domestication on seed endophytes in terms of diversity, structure and co-occurrence, by comparing four cereal crops and the respective ancestor species; ii) to test the phylogenetic coherence between cereals and their seed microbiota (clue of co-evolution).
*Methods:* We investigated the seed microbiota of four cereal crops (*Triticum aestivum*, *Triticum monococcum*, *Triticum durum*, and *Hordeum vulgare*), along with their respective ancestors (*Aegilops tauschii*, *Triticum baeoticum*, *Triticum dicoccoides*, and *Hordeum spontaneum*, respectively) using 16S rRNA gene metabarcoding, Randomly Amplified Polymorphic DNA (RAPD) profiling of host plants and co-evolution analysis.
*Results:* The diversity of seed microbiota was generally higher in cultivated cereals than in wild ancestors, suggesting that domestication lead to a bacterial diversification. On the other hand, more microbe-microbe interactions were detected in wild species, indicating a better-structured, mature community. Typical human-associated taxa, such as *Cutibacterium*, dominated in cultivated cereals,

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suggesting an interkingdom transfers of microbes from human to plants during domestication. Co-evolution analysis revealed a significant phylogenetic congruence between seed endophytes and host plants, indicating clues of co-evolution between hosts and seed-associated microbes during domestication.

*Conclusion:* This study demonstrates a diversification of the seed microbiome as a consequence of domestication, and provides clues of co-evolution between cereals and their seed microbiota. This knowledge is useful to develop effective strategies of microbiome exploitation for sustainable agriculture.



## Introduction

Endophytes are harmless microorganisms living inside plant tissues. Microbial endophytes colonize all plant organs [1], including seeds. The presence of endophytes in the seeds of several plant species has been previously reported, including cereals [2–5]. Microorganisms were hypothesized to have co-evolved with their host plants and animals, and developed symbiotic relationships with the hosts over the years [6]. It has been demonstrated that seed-borne microorganisms facilitate germination by protecting seeds from predation and attack by pathogens [7,8] and by reducing abiotic stresses [9,10]. Moreover, these microbes have a role in plant growth promotion and biocontrol of phytopathogens [4,11,12]. Seed endophytes can later be critical in shaping the root microbiota by 'priority effect' [13] as they are able to colonize very efficiently the rhizosphere [14]. This vertical transmission of seed endophytes was reported for some cereal species, such as maize [2], wheat [15], and barley [3]. However, the underlying evolutionary principles of these interactions remain to be elucidated. This knowledge is necessary to implement effective strategies of microbiome integration into the responsible management of soil and resources, to achieve a more sustainable modern agriculture [16–17].

Wheat and barley are considered as the earliest domesticated crop plants and are, respectively, the first and the fourth most cultivated cereals in the world (FAO - Statistical pocketbook 2018: www.fao.org/3/CA1796EN/ca1796en.pdf). Different varieties of barley and wheat were domesticated from their wild ancestors about 10,000 years ago [18]. Throughout the domestication period, wild plants were transformed into food crops as a result of conscious and unconscious genetic selection of important traits, such as grain size and shape, and seed hull elimination [19]. As the plants evolved, their associated microbiomes are supposed to have undergone substantial changes, too, for instance, because of the loss of the fruit shell [2]. Several studies investigated the influence of plant genotype, crop rotation, fertilizer inputs, fungicide and herbicide application, and cultural practices on the composition of seed endophytes [2,20,21]. Modern plant cultivars may have missed some of the characteristics required to attract beneficial microbes compared to their wild relatives, which are more adapted to pre-agricultural soils [22–24].

Regardless of these evolutionary changes, grains of currently cultivated crops appeared to carry similar microbiota as their wild relatives [2,25]. However, to what extent domestication affected the diversity of seed microbiota and whether these bacterial communities preserve the same traits than in wild forms remains unclear. In this work, to explain the effect of genetic selection and domestication on cereal seed microbiota, wheat and barley were selected because of their historical, economic, and agricultural value. The species analyzed here include three cultivated wheats, *Triticum aestivum* L. ssp. *aestivum* (hereafter "*Triticum aestivum*"), *Triticum monococcum* L. ssp. *monococcum* (hereafter "*Triticum monococcum*"), *Triticum durum* Desf. ssp. *durum* (hereafter "*Triticum durum*"), and the three corresponding wild ancestors, *Aegilops tauschii* Coss. ssp. *tauschii* (hereafter "*Triticum tauschii*"), *Triticum baeoticum* Boiss. ssp. *baeoticum* (hereafter "*Triticum baeoticum*"), and *Triticum dicoccoides* Schweinf. ssp. *dicoccoides* (hereafter "*Triticum dicoccoides*"), as well as the cultivated barley *Hordeum vulgare* L. ssp. *vulgare* (hereafter "*Hordeum vulgare*") and its ancestor *Hordeum vulgare* K.Koch. ssp. *spontaneum* (Coss.) Thell. (hereafter "*Hordeum spontaneum*") (Fig. 1).

In order to confirm genetic bounds with corresponding ancestors of wheat and barley, genetic distances need to be measured. Several molecular methods can be used for assessing plant genetic distances, including Amplified Fragment Length Polymorphism (AFLP), Restriction Fragment Length Polymorphism (RFLP), Random Amplified Polymorphic DNA (RAPD), Inter-Simple Sequence Repeat (ISSR), Simple Sequence Repeat (SSR), among others. RAPD analysis was selected for this study because it is an effective and established method to measure genetic polymorphisms in cereals [26–28].

The objectives of this study were: i) to investigate the effect of cereal domestication on seed endophytes in terms of diversity, structure and co-occurrence, by comparing four crops and the four respective ancestor species; ii) to test the phylogenetic coherence between cereals and their seed microbiota, by comparing the genetic relatedness between cereals with that between the seed-associated bacteria (clue of co-evolution). We hypothesized that: i) a more diverse bacterial microbiota is associated with the seeds of current cultivars of wheat and barley compared to their ancestors, due to an ongoing process of microbiome diversification; ii) the dominant species will be different in the cultivated crops due to the effect of domestication; iii) more correlations (representing potential microbial interactions) will be found in the wild species, evolutionary older and therefore associated to a better-structured microbiota; and iv) cereal evolution has been coupled with a coherent evolution of their associated seed microbiota during the domestication period.

## Materials and methods

### *Seed samples used*

Cultivars of three wheat species, *Triticum aestivum*, *Triticum monococcum*, *Triticum durum*, as well as barley, *Hordeum vulgare*, and their corresponding ancestors (*Aegilops tauschii*, *Triticum baeoticum*, *Triticum turgidum*, and *Hordeum spontaneum*) were used. *Aegilops tauschii* (2n = 2x = 14, DD genomes) is one of the three wild diploid progenitors of the hexaploid bread wheat (*Triticum aestivum*, 2n = 6x = 42, AABBDD genome), which has three sets of homologous chromosomes, AABBDD, where D chromosomes derive from *Aegilops tauschii* and AABB from *Triticum dicoccoides* [29,30]. *Aegilops tauschii* is distributed in eastern Turkey, Azerbaijan, Iran, Syria, and around the Caspian Sea [31].

*Triticum baeoticum* (2n = 2x = 14) is the wild ancestor of the einkorn wheat *Triticum monococcum* (2n = 2x = 14). It occurs in Southeast Europe and Turkey's mountainous regions [32].

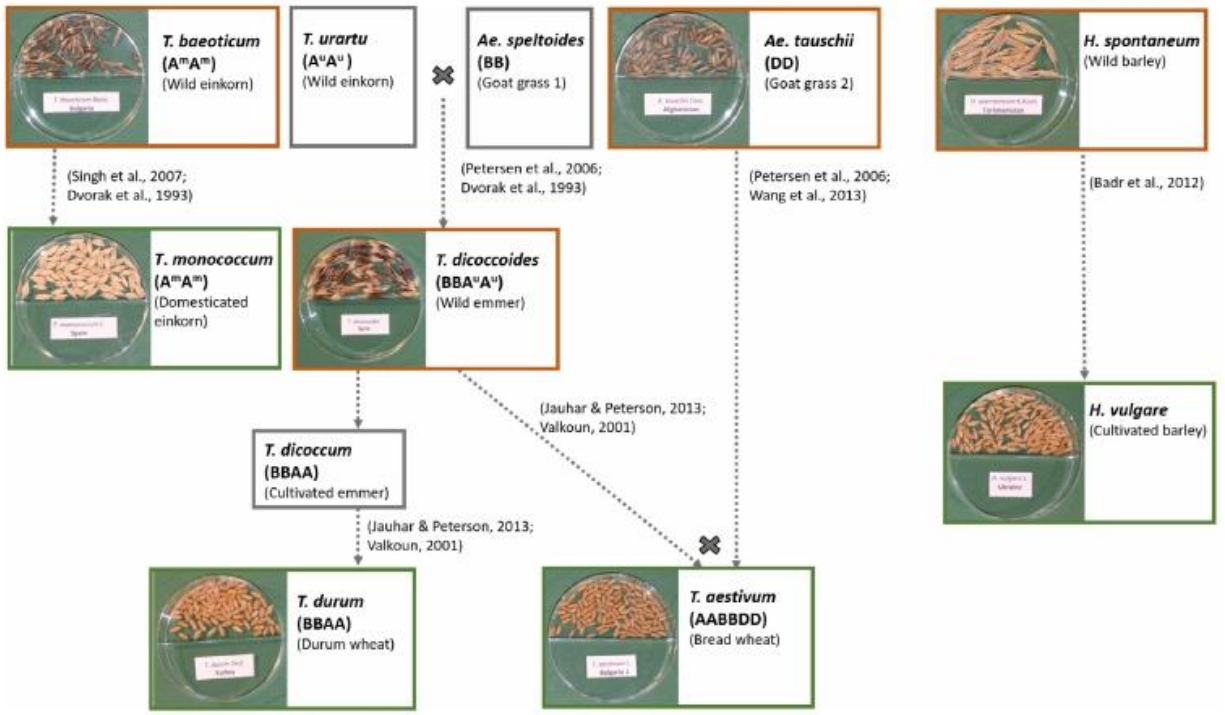


**Fig. 1.** The evolutionary history of wheat and barley crops (green boxes) and their wild relatives, both used (orange boxes) and not used (gray boxes) in this work. Dotted arrows show the parental lines of domesticated forms. Crosses indicate cross-breeding events.

Tetraploid *Triticum dicoccoides* (2n = 4x = 28, AABB genomes), is the ancestor of the durum wheat *Triticum durum* [33]. The A and B chromosomes of the tetraploid *Triticum dicoccoides* derive from an earlier hybridization between *Triticum urartu* [34] and *Aegilops speltoides* [29] (Fig. 1). Durum wheat is predominantly cultivated in the Middle East [35]. *Hordeum spontaneum* is the progenitor of currently cultivated *Hordeum vulgare*, first domesticated in the Israel-Jordan region [36] and predominantly cultivated in temperate areas.

Viable seeds were obtained from the Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Germany. Five different accessions for each plant species were used (Tab. S1; Fig. S1). All seeds were produced, collected, and stored under the same conditions at the IPK. Therefore, the only factor expected to generate differences in the microbiota was the plant genotype. Once arrived at our laboratory, the seeds were stored at 4 °C until analysis.

*Seed surface sterilization and DNA extraction from seeds*

The analysis was performed on two seed subsamples per cereal accession, for two reasons: i) to increase the robustness of the sequencing results and ii) to account for the low number of bacterial sequences usually obtained by metabarcoding of seed microbiomes [5,37]. Prior to the genomic DNA extraction, two aliquots of 10–15 seeds per each wheat or barley accession (total number of samples: 80) were surface-sterilized under room temperature by immersion into 70% ethanol for 2 min and then 2.5% sterilization solution (30 g NaCl, 1.5 g NaOH and 1 g $Na_2CO_3$) for 15 min [38]. Thereafter, seeds were washed with sterile distilled water four times for increasing intervals (5, 15, 25, and 45 min) and under shaking at 100 rpm. Surface sterilized seeds (2 samples per plant accession, 80 samples in total) were grounded using sterile pestle and mortar in liquid nitrogen. The DNA was isolated from 300–500 mg of grounded samples. Initially, each sample was added into a 2 ml screw-cap tube containing 200 µl of sterile glass beads. Then, each sample received 800 µl of extraction buffer (2.5 g $l^{-1}$ SDS, 0.2 M sodium phosphate buffer, 50 mM EDTA and 0.1 M NaCl, pH 8). Cells were disrupted for 2 min at 30 Hz using a cell disrupter MM400 (Retsch GmbH, Haan, Germany) and then centrifuged (Heraeus Fresco, Thermo Fisher Scientific Inc., Waltham, USA) at 12,000 × *g* for 5 min at 4 °C. The supernatant was transferred into a new 2 ml microcentrifuge tube (Laborhaus Scheller GmbH & Co KG, Euerbach, Germany). The cell disruption step was repeated by adding another 700 µl of extraction buffer to the pellet of the same sample. Before moving to the next step, RNA was digested by adding 5 µl RNAse per 1 ml supernatant, and incubated at 37 °C for 30 min. The RNA digestion was followed by 500 µl of phenol/chloroform/isoamyl alcohol (25:24:1) addition. The tube was then centrifuged again at 16,000 × *g* for 5 min at 4 °C and then the upper, aqueous phase was transferred into the new 2 ml tube. Then 500 µl chloroform/isoamyl alcohol (24:1) was added, mixed well by inverting, and centrifuged at 16,000 × *g* for 5 min at 4 °C. Again, the upper, aqueous phase was collected into a new tube. One ml of precipitation buffer [20% polyethylene glycol, 2.5 M NaCl] was added and incubated at room temperature for 30 min and finally centrifuged at 16,000 × *g* for 30 min at 4 °C. The precipitated DNA was washed with 800 µl ice-cold 75% ethanol, dried beside the Bunsen burner flame, and dissolved in 30 µl nuclease-free water. The DNA was quantified by NanoDrop™ 2000 Spectrophotometer (Peqlab, Erlangen, Germany) and then stored at −20 °C until further analysis. This DNA was used for both, the RAPD analysis and the 16S rRNA gene library construction for IonTorrent sequencing.

*Ion Torrent sequencing of prokaryotic 16S rRNA gene libraries*

High-throughput sequencing is a state-of-the-art method to analyze the structure and diversity of microbiomes [39]. Here,

we used the IonTorrent metabarcoding of 16S rRNA gene libraries, using a peptide nucleic acid (PNA) probe to reduce the amplification of plant mitochondrial and plastid DNA [40]. The V4 and V5 regions of the 16S rRNA genes were PCR amplified from the 80 seed samples using the primer 520F and 907 R [41,42]. Fifteen μl of PCR reaction included 10 ng of seed DNA, 1 X KAPAHiFi (KAPA Biosystems, Wodurn, MA) buffer, KAPA dNTP mix 200 μM each, primer 5 pM each, 15 μM of chloroplast-PNA [40] and mitochondrial-PNAII (AAACCAATTCACTTGAGT, designed in this work to replace the mt-PNA of Lundberg *et al.*, [40], which was not suitable due to the different position of the forward primer), and KAPAHiFi polymerase 0.3 units. The PCR was performed using a MycyclerTM (Bio-Rad, USA) for 20 cycles with the initial denaturation for 3 min at 95 °C, cyclic denaturation for 20 sec at 98 °C, PNA annealing for 30 sec at 65 °C, primer annealing for 30 sec at 55 °C, an extension for 30 sec at 72 °C and a final extension at 72 °C for 5 min. The second PCR was prepared with primer 520F and 907 R comp, adapter, and barcodes. The final volume of 50 μl contained 2 μl of the first PCR product, 10 μl of 5X KAPAHiFi buffer, KAPA dNTP mix 600 μM, primer 5 pM and KAPAHiFi polymerase 1 unit. The PCR was performed using MycyclerTM (Bio-Rad, USA) for 8 cycles with the initial denaturation for 3 min at 95 °C, cyclic denaturation for 20 sec at 98 °C, annealing for 30 sec at 55 °C, an extension for 30 sec at 72 °C and a final extension at 72 °C for 7 min.

Final PCR products were eluted and purified from agarose gel using NucleoSpin PCR purification kit (MACHEREY-NAGEL GmbH & Co. KG, Düren, Germany), followed by primer-dimers removal using NucleoMag® beads (NGS clean-up kit, MACHEREY-NAGEL GmbH & Co. KG, Düren, Germany). The concentration of the purified PCR products was quantified using Qubit dsDNA HS assay kit by Qubit® 3.0 fluorometer (Life Technologies, Carlsbad, USA) and then adjusted to 1 μM. Two independent DNA extractions and PCRs were done for each seed accession. The PCR products were then pooled and the final concentration was again adjusted to 26 pM. The pooled product was used for emulsion PCR with Ion One Touch 2 (Ion PGM Hi-Q View OT2 kit, Life Technologies, Carlsbad, USA). The quality of the final product was assessed using Ion Sphere Quality Control Kit (Life Technologies, Carlsbad, USA) and loaded on a 314 or 318 chip for sequencing with an Ion PGM sequencer (Life Technologies, Carlsbad, USA).

### *Analysis of Ion Torrent sequencing data*

Ion Torrent sequencing data were analyzed using QIIME 1.9 [43]. The reads of each two replicate samples per accession were pooled together (number of samples analyzed = 40). The sequences were length (200–500 nucleotides) and quality (threshold: 20) filtered, then chimeric sequences were removed using VSEARCH [44]. OTUs were generated at a sequence similarity level of 97% using the SUMACLUST method [45] and the "SUMACLUST exact" option (a sequence is assigned to the best matching OTU rather than the first OTU passing the similarity threshold). Taxonomy was assigned using the reference sequences of the SILVA 132 database, release: April 2018 [46]. OTUs identified as plastids or mitochondria, as well as singleton OTUs, were removed from the dataset.

Statistical analyses were performed in R using the OTU table generated from QIIME. Taxa summary plots were created using RStudio 1.1.463 [47], package ggplot2 [48]. To compare the alpha diversity indices (Shannon, Simpson, Dominance, and Equitability) and the relative abundances of taxa between wild ancestors and cultivated crops, the Student's *t*-test on a normalized data set (sequencing depth: 1000 reads per sample) was used, after false discovery rate – FDR – adjustment of the p-values (Benjamini-Hochberg method). Beta diversity was calculated based on non-metric multidimensional scaling (NMDS) of weighted Bray-Curtis dissimilarities, calculated on a normalized data set (sequencing depth: 1000 reads per sample); the statistical significance of the factors "cultivation form" and "species" was assessed using the ADONIS test included in the R package 'vegan' [49].

The sequences were submitted to EMBL (www.ebi.ac.uk/ena) under the project number PRJEB36663.

### *Co-occurrence network analysis*

Co-occurrence analysis using high-throughput sequencing data is used to detect potential microbe-microbe interactions as well as to identify hub species [50–52]. Studies on cereal seed endophytes have been carried out focusing on identification, microbial composition, and community structure [15,53] and function [4,5]. However, a complex network of interactions within the seed microbiota and the influence of evolutionary patterns on microbe-microbe interactions were not yet investigated. To investigate the effect of domestication on the microbial interaction network, the co-occurrence analysis was performed with the Co-occurrence Network inference software (CoNet) [54], using not-normalized data as recommended to reduce the compositional effect [55]. Only OTUs occurring in at least 10 samples were considered. Pairwise scores were calculated for four measures: the Bray-Curtis and Kullback–Leibler similarities, and the Pearson and Spearman correlations. For each measure and edge, 100 permutations (with row shuffling re-sampling and re-normalization for correlation measures), as well as the bootstrap scores, were generated. Unstable edges (outside the 2.5–97.5 percentiles of the bootstrap distribution) were deleted. The individual p-values generated by the four measures were merged using Brown's method. Only edges with false discovery rate (FDR)-corrected P-values below 0.05 and supported by at least three measures, were retained. The network layout was generated automatically with the "edge-forced spring embedded" algorithm, which leads to unbiased networks showing interconnected nodes closer to each other and less-linked ones placed in the outside position. Network legends were created with the Cytoscape Add-on "Legend creator" (http://apps.cytoscape.org/apps/legendcreator).

### *Random Amplified Polymorphic DNA (RAPD) analysis*

Twenty RAPD primers (10-mer) (Integrated DNA Technologies, Inc. Coralville, USA) were tested for screening wheat and barley genotypes based on the quantity of the polymorphism they produced. Finally, five of them (OPA-17, OPH-19 [56], OPJ-18 [57], OPO-06 [58] and OPH-13 [59]) were selected for the analysis (Tab. S2).

RAPD assay protocol was adapted from Mantzavinou et al. [58], by further optimizing annealing temperature, using gradient temperature-PCR protocol and $MgCl_2$ concentration for each primer. DNA sample concentration was adjusted at 100 ng $\mu l^{-1}$. PCR was performed using MycyclerTM (Bio-Rad, Hercules, USA) in a reaction volume of 25 μl containing 1 μl DNA template, 1X KAPA-HiFi Buffer (KAPA Biosystems, Wodurn, USA), 0.4 μM each 10-mer primer, 2.5 mM KAPA $MgCl_2$, 200 μM KAPA dNTPs mix and 0.625 units *Taq* DNA polymerase. RAPD was amplified using following thermal profile: 5 min at 94 °C, 40 cycles of 30 sec at 94 °C, 1 min at 30–40 °C (depending on primer, Tab. S2), 1 min at 72 °C, and 10 min at 72 °C for final elongation. The amplification products were separated on 1.5% (w/v) Agarose gel containing 5 μl 100 $ml^{-1}$ DNA dye HDGreen™ (Intas, Göttingen, Germany) in 0.5 X TBE buffer. Both 1 kb and 100 bp DNA ladders (QuickLoad® Purple, New England BioLabs Inc., Ipswich, USA) were used for size comparison. RAPD fragments were illuminated under UV light and images were captured using Gel Doc 2000 (Bio-Rad, Hercules, USA).

Gel images were analyzed using the software GelCompar II version 5.10 (Applied Maths, NV). The five fingerprints for each seed accession were linked to form a composite data set (Fig. S2). The dendrogram was constructed using similarity coefficients based on the number of different bands (optimization: 1%, position tolerance: 1%) with the unweighted paired group method of cluster analysis with arithmetic averages (UPGMA).

*Co-evolution analysis*

To test the co-evolution between cereals and associated seed microbiota, we measured their phylogenetic congruence. A cophylogeny analysis was performed between cereal plants and the corresponding bacterial OTUs, using the host distance matrix obtained from RAPD analysis and the bacterial distance matrix calculated from the high-throughput sequencing. Cophylogeny analysis identifies the effect of evolution on diversification patterns of two or more ecologically associated species [60,61]. To date, cophylogeny studies have been mainly used to study host-parasite relationships or vertically transmitted symbionts [62]. In this study, we established the use of cophylogeny assessment to study the co-evolution of seed microbiota from wild progenitors to modern cultivars of wheat and barley. The various techniques available for cophylogenetic assessment are divided into two categories: event-based and topology-based (global-fit) methods [60]. In this study, we used a global-fit method because it can afford large-scale cophylogenetic analyses and because the quantity of phylogenetic congruence generated by the cophylogenetic assessment can be associated with the significance of co-evolution in the studied scheme [60]. The test was a global goodness-of-fit test performed with 1000 permutations, using the functions cophyloplot and ParaFit in the 'paco' [60] and 'ape' [63] R packages. A tanglegram was created for the visual representation of the shared branching events. ParaFit requires the phylogeny of the host, the phylogeny of bacterial OTUs, and a matrix of connections as input. It compares the observed host and the bacterial distance matrices, and then tests for random associations between the two taxa groups, by randomizing the matrix of association. So, it generates P-values to calculate the contribution of each host-bacteria association to the global statistic testing (ParaFitGlobal) for each random association test between hosts and bacterial OTUs [64]. A global sum of squared residuals, called $m^2_{XY}$, is calculated, which represents the sum of all connection distances in the tanglegram. The observed $m^2_{XY}$ value is statistically compared to the 1000 values generated by random permutations [65], to assess the significance of the phylogenetic congruence: the lower this observed $m^2_{XY}$ value, the higher the statistical significance of the phylogenetic congruence.

## Results

*High-throughput sequencing analysis and taxonomic composition of the bacterial microbiota*

A PCR product was obtained from 78 out of 80 samples; both replicates of one seed accession (*T. durum* TRI_13547, Tab. S1) did not produce a PCR product. A total of 6,595,794 sequence reads were produced and, after filtration of 15,696 sequences, removal of all plant-originated sequences (5,870,289), and singletons (1,744), 708,065 high-quality prokaryotic 16S rRNA gene sequences (1,004 to 93,702 reads per sample) remained. These sequences were grouped into 423 OTUs at 97% similarity level.

*Proteobacteria, Actinobacteria,* and *Firmicutes* were the predominant phyla (Fig. 2A); *Actinobacteria* and *Firmicutes* were found comparatively higher in cultivated species. *Burkholderiaceae, Pseudomonadaceae,* and *Xanthomonadaceae* were the major families. Twenty families were significantly different between wild and cultivated cereals (FDR-adjusted p value < 0.05); the most abundant were *Pseudomonadaceae,* more abundant in wild species, and *Propionibacteriaceae*, more abundant in cultivated crops (Fig. S3). In particular, at the genus level, we found a statistically significant higher abundance of *Pseudomonas* in wild species, while *Cutibacterium* was more abundant in cultivated crops (Fig. 3). When considering individually each couple of wild ancestor and cultivated derivate, *Caulobacteraceae* was found abundant in *Triticum aestivum* compared to *Aegilops tauschii*. *Pseudomonadaceae, Enterobacteriaceae,* and *Xanthomonadaceae* were found abundant in *Triticum dicoccoides*, while *Propionibacteriaceae*, *Burkholderiaceae*, and *Xanthomonadaceae* were more abundant in *Triticum durum*. *Pseudomonadaceae* was found a major abundant family in *Hordeum spontaneum*, while *Xanthomonadaceae, Burkholderiaceae*, and *Propionibacteriaceae* were the major families in *Hordeum vulgare*. Finally, *Pseudomonadaceae* was more abundant in *Triticum baeoticum* compared to *Triticum monococcum* (Fig. 2B).

*Alpha- and beta-diversity, shared taxa and co-occurrence analysis*

All the four calculated alpha diversity indices were significantly different between wild species and cultivar species (*t*-test, $P < 0.05$). Shannon-Weaver, Simpson, and Equitability indices were higher and Dominance was lower in cultivated species compared to wild species (Fig. 4A). We calculated the relative increment % of each cultivated species to the corresponding wild ancestor (Tab. S3). This value was positive for all of them, except for the couple *T. baeoticum/T. monococcum* (but at a lower absolute extent than any other couple). Interestingly, *Tb-Tm* (having a genetic similarity higher than the other couples, see Fig. 1) appears as the most closely related couple also concerning the structure (beta-diversity): in fact, *T.monococcum* and *T.baeoticum* samples are the only ones that largely overlap in the beta-diversity plot (Fig. 4B).

Non-metric multidimensional scaling plot based on weighted Bray-Curtis distances were significantly influenced by factors, cultivation form (ADONIS, $R^2 = 0.078$, $P = 0.003$), plant species (ADONIS, $R^2 = 0.32$, $P < 0.001$) (Fig. 4B), plant varieties (ADONIS, $R^2 = 0.36$, $P < 0.001$), and sets of homologous chromosomes (ADONIS, $R^2 = 0.094$, $P < 0.001$), but not by the factor "country of origin" (ADONIS, $R2 = 0.58$, $P = 0.106$).

The number of exclusive OTUs was higher in cultivated species (43%) than in wild species (24%), which is coherent with the higher alpha-diversity. 33% of the OTUs were shared (Fig. S4A). The ten most abundant OTUs shared between wild and cultivated cereals were *Pseudomonas, Stenotrophomonas, Cutibacterium, Kosakonia cowanii, Burkholderiaceae, Stenotrophomonas, Ralstonia, Pantoea, Delftia,* and *Acinetobacter radioresistens*. OTUs identified as *Pseudomonas, Stenotrophomonas, Kosakonia cowanii,* and *Delftia* were found higher in wild species, while OTUs identified as *Cutibacterium, Burkholderiaceae, Stenotrophomonas, Ralstonia, Pantoea,* and *Acinetobacter radioresistens* were higher in cultivated species (Fig. S4B). OTUs exclusively found in cultivated cereals belonged to several genera, including *Cutibacterium* and *Methylobacterium* (Fig. S5).

Co-occurrence analysis showed that the microbiota of wild species had higher connectivity than cultivated species (Fig. 5). In particular, despite the number of connected nodes was the same, the microbiota of wild cereals had a higher average number of neighbors ("degree") and higher network density and centralization, with respect to the cultivated species (Fig. 5).

*RAPD analysis of genetic distances between cereal species*

Of the 40 initial seed samples, four ones (one of each *T. durum, Ae. tauschii, H. vulgare,* and *H. spontaneum*) did not give bands after

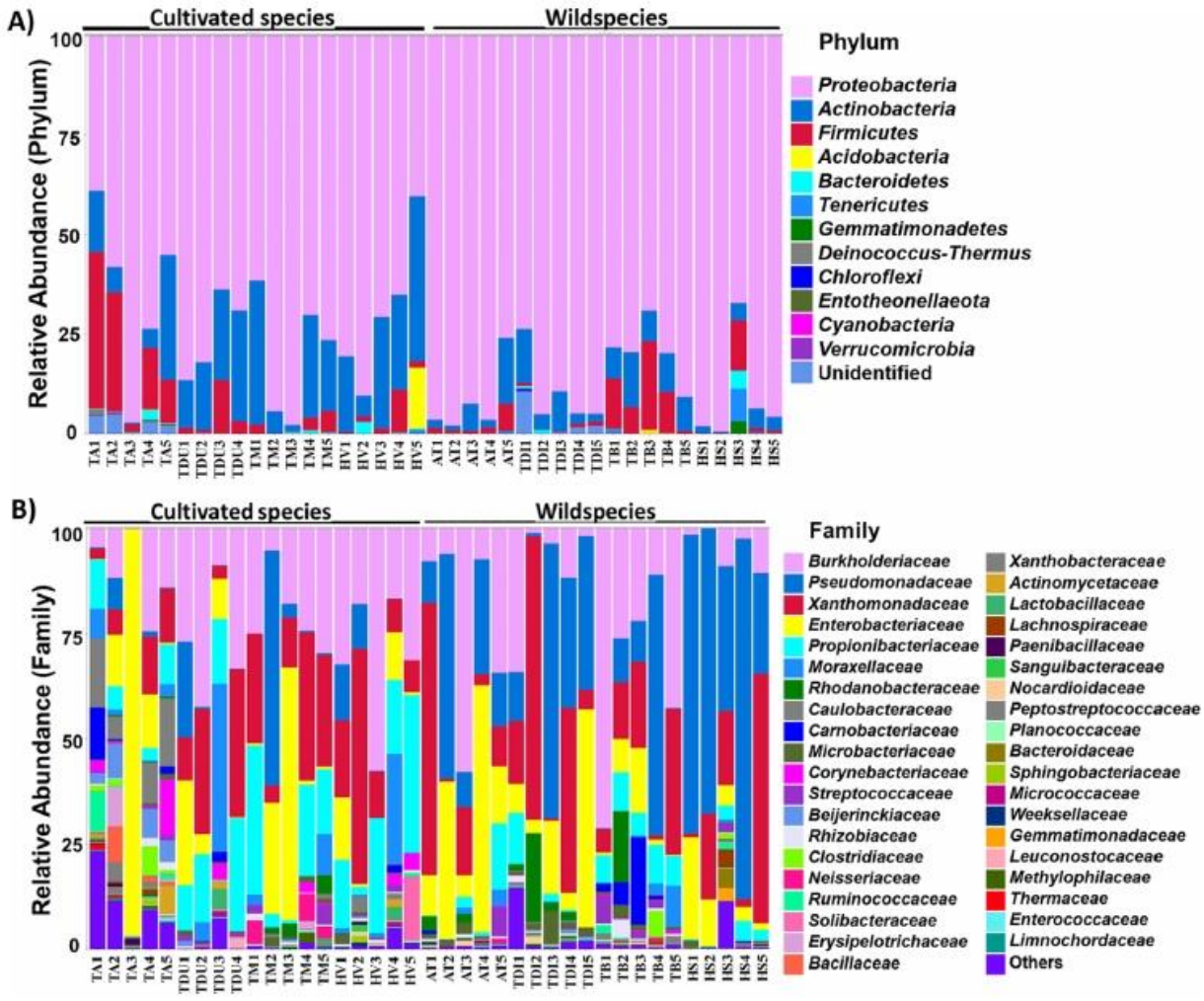


**Fig. 2.** Bacterial taxonomic composition of seed endophytes of wild and cultivated species, at Phylum (A) and Family (B) levels. *Triticum aestivum* (TA), *Triticum durum* (TDU), *Triticum monococcum* (TM), *Aegilops tauschii* (AT), *Triticum diccocoides* (TDI), *Triticum baeoticum* (TB), *Hordeum vulgare* (HV) and *Hordeum spontaneum* (HS). Relative abundance of major taxa only (>1% of total reads) according to 16S rRNA gene metabarcoding.

RAPD PCR, therefore sample number reduced to 36 (Fig. 6, Fig. S2). The UPGMA dendrogram was divided into two main clusters, separating barley and wheat species (Fig. 6, Fig. S2). In the barley cluster, cultivated and wild barley appeared as sister clades. In the wheat cluster, there was a further separation of *Triticum* and *Aegilops* genera. Within the *Triticum* species, *Triticum baeoticum* and *Triticum monococcum* formed a monophyletic group and appeared as sister clades (Fig. 6, Fig. S2). *Triticum durum* and *Triticum dicoccoides* did not cluster together but were mixed with *Triticum aestivum*. However, the accessions of *Triticum aestivum* were placed in the expected position with respect to the ancestor *Aegilops tauschii* (Fig. 6, Fig. S2).

*Co-evolution analysis*

A co-evolution analysis was performed using the host distance matrix obtained by the RAPD analysis and the bacterial OTU distance matrix obtained by metabarcoding analysis. Co-evolution analysis was performed on 35 seed samples since one sample did not give a PCR product for metabarcoding and four ones did not give RAPD profiles. The evolutionary relationships between host species and bacterial OTUs were analyzed by the goodness-of-fit test. The global fit of the regression of bacterial OTUs phylogeny to the host phylogeny was evaluated using $m^2_{XY}$ as a sample statistics, which is determined by a randomization procedure and shows the strength of associations between organisms from different phylogenetic groups. The goodness-of-fit test of phylogenic association between the bacteria and the host species phylogenies revealed a significant topological congruence ($m^2_{XY}$ = 235. 98; P = 0.024; 1000 permutations) (Fig. 7A). 52.5% of the 1000 randomizations had a lower $m^2_{XY}$ than the observed one (Fig. 7B). Here, 62 OTUs (14.6% of all OTUs) significantly contributed to the coherence of the tree topologies (Fig. 7A).

To test whether the unresolved RAPD clustering of the species *T. durum* and *T. dicoccoides* might have affected the co-evolution assessment, we deleted these two species and repeated the analysis: indeed, a more significant topological congruence was obtained ($m^2_{XY}$ = 243.36; P = 0.0054; 1000 Permutations) (Fig. 7C). Only two (0.2%) of the 1000 randomizations resulted in a lower $m^2_{XY}$ than the observed one (Fig. 7D). Here, 160 OTUs (37.8% of all OTUs) significantly contributed to the coherence of the tree topologies (Fig. 7C).

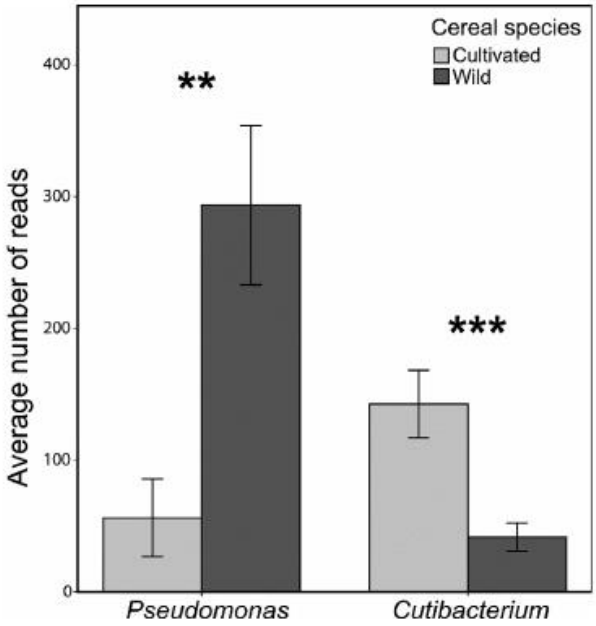


**Fig. 3.** Relative abundance of the genera *Pseudomonas* and *Cutibacterium* in the wild and cultivated cereals, as resulted from metabarcoding analysis, calculated on the rarefied dataset (sequence depth = 1000 reads per sample). ** p = 0.0013; *** p < 0.001.

## Discussion

Domestication and breeding of plants have resulted in productive cultivars, but also in significant changes in plant microbiota with compositional shifts, as already reported for different crops [23,50,66–69]. The idea behind our current study was to analyze the changes from species A to B, from species C to D, E to F, and G to H (four ancestors and their four descendant cultivated cereal species, respectively), and to test whether there were common traits in these changes, which would then suggest a common effect of domestication. The four individual wild species and the four individual cultivated species were treated as "replicates" for the factor "cultivation form" in our experimental design. We therefore intended to go behind the pairwise comparisons between individual species (largely tested in literature) and to assess further potential drivers of the microbiome that could be important at the (co–)evolutionary level, such as domestication.

We found a more diverse microbiota associated to the seeds of modern cereals compared to the wild ancestors. This suggests that cereal breeding lead to a compositional shift in the plant-associated microbiome. This finding is in line with previous studies that showed higher microbial diversity in the rhizosphere of modern crops than wild ancestors [50,66,70]. Suggested drivers of these changes were agricultural soil conditions, crop management methods, and changes in root exudates in wheat [71], since breeding of modern crops resulted in increased root exudation of organic compounds [71,72]. Other factors, such as host genotype [2] and environmental circumstances were indicated as further possible drivers [20–22].

So far, the influence of domestication on bacterial diversity was studied mainly in the root system [22,50,66,70]; a few studies specifically focused on the effect of domestication on seed endophytes and reported minor effects of domestication on community richness [2,69]. Compared to these studies, we observed the effect of domestication in a larger set of species originally derived from areas of different continents (Tab. S1), which can explain the higher microbiota diversification found in our study. The relative increment % of each cultivated species to the corresponding wild ancestor is positive for all of them, except for the couple *T. baeoticum/T. monococcum* (but at a lower absolute extent than any other couple). Coherently, *Tb-Tm* appears as the most closely related couple in Fig. 6, which suggests that perhaps the microbial diversity is

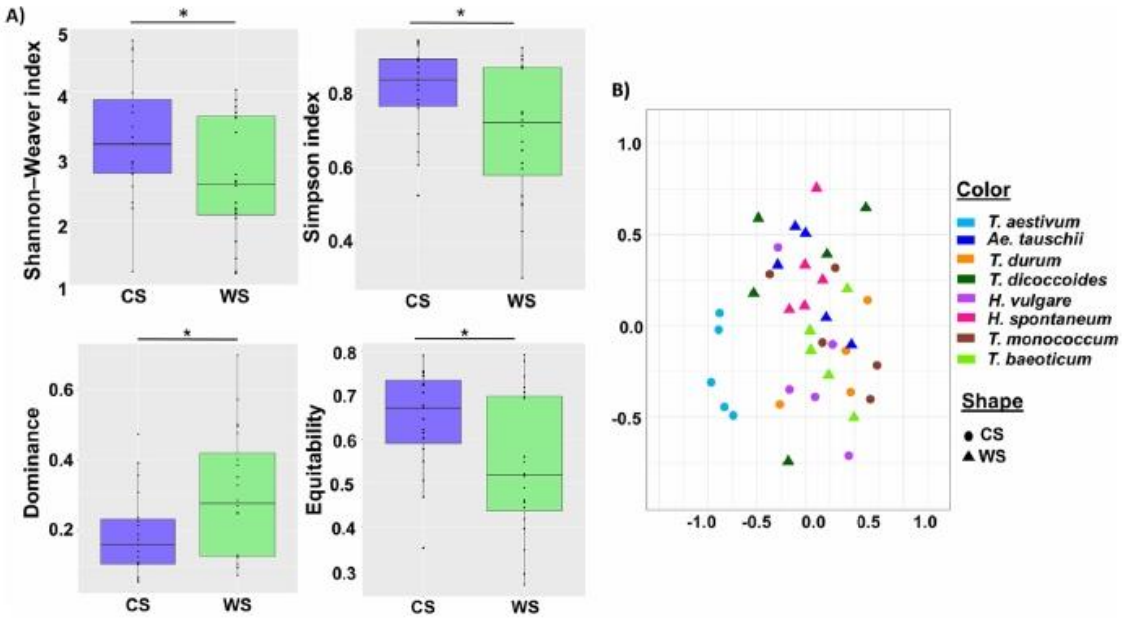


**Fig. 4.** Alpha and beta diversity metrics of seed endophyte microbiota. (A) Shannon–Weaver, Simpson, Dominance and Equitability indices of bacterial microbiota (OTU 97%), grouped by cultivation form (*t*-test, P < 0.05), according to 16S rRNA gene metabarcoding. CS = Cultivated species; WS = Wild species. (B) Non-metric multidimensional scaling plot for bacterial microbiota structure based on weighted Bray-Curtis distances. Samples are colored by plant species and shaped by cultivation form. ADONIS significance test: $R^2$ = 0.078, P = 0.003 for the factor "cultivation form"; $R^2$ = 0.32, P < 0.001 for the factor "species"; stress value: 0.1495.

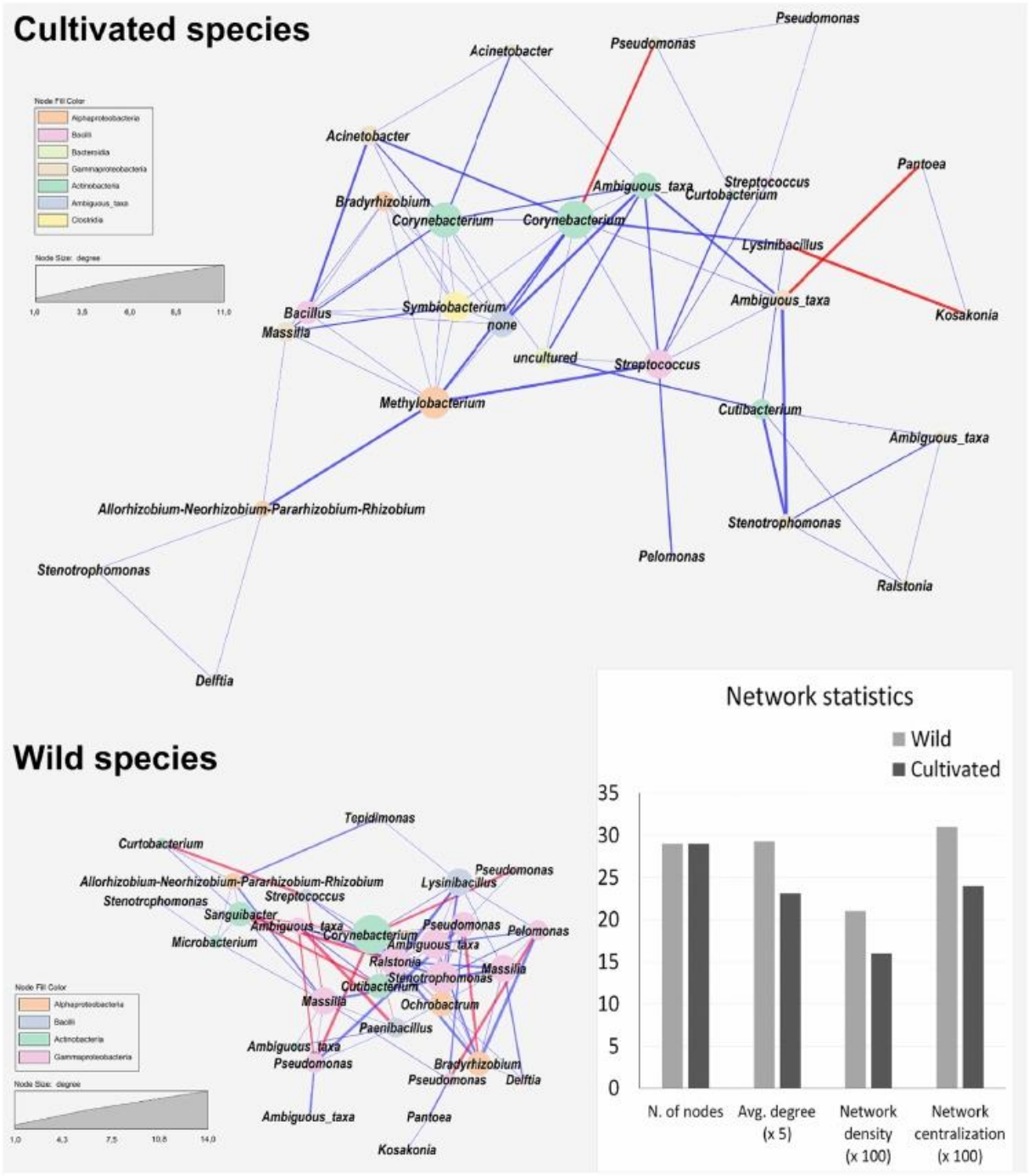


**Fig. 5.** Co-occurrence network of OTUs, calculated for wild and cultivated cereal species separately. Nodes are colored by taxonomy and sized by degree (=n. of connections). Edges are colored by correlation type (blue: positive; red: negative) and the thickness represents merged FDR-corrected P-values (the thicker, the more significant).

not high between them because the genetic similarity between *T. boeticum* and *T. monococcum* is higher than the other couples (Fig. 1). Moreover, this genetic similarity between the plants appears to be reflected not only in the microbial diversity but also in the structure: in fact, *T. monococcum* and *T. baeoticum* samples are the only ones that largely overlap in the beta-diversity plot (Fig. 4B). However, we argue that this observation even supports our conclusion that cereal domestication might lead to a general increase in diversity, which is associated to the genetic distance.

Our beta-diversity analysis supports this idea and shows that domesticated and wild species differ in their microbiota by plant genotype (Fig. 4B). In fact, both factors ("species" and "variety") relate to the host genotype, which is known to be one of the main factors affecting the plant-associated microbiome, and therefore it

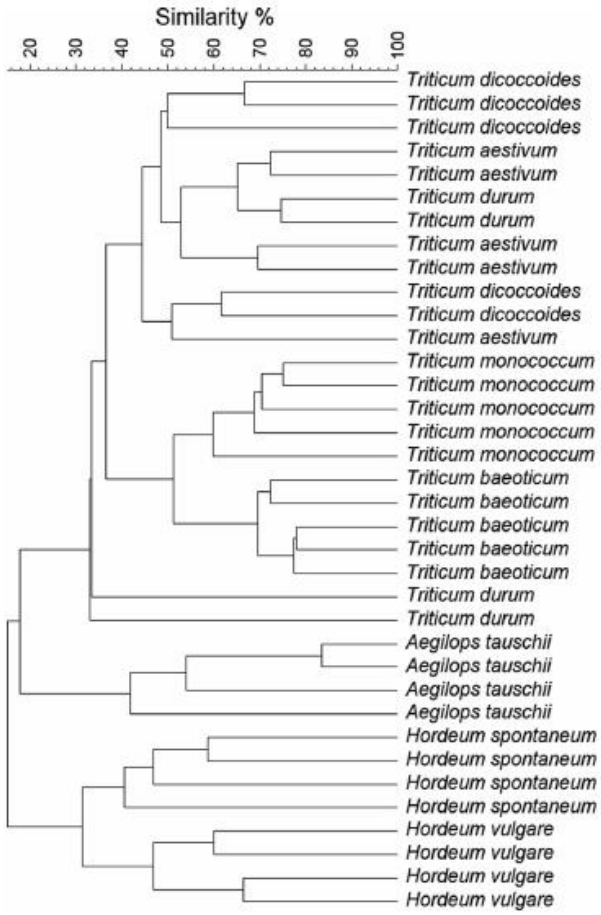


**Fig. 6.** UPGMA dendrogram showing the genetic similarity between cultivars of wheat and barley and their corresponding ancestors, calculated on RAPD data. The dendrogram was constructed using similarity coefficients based on the proportion of different bands.

is not surprising that the effect of the factors "plant species" and "plant varieties" was stronger compared to "cultivation form". Indeed, our aim was not to demonstrate that "cultivation form" (in the sense of "cultivated" or "wild/ancestor") is the most important factor affecting the seed microbiota. Instead, we tested and demonstrated that cereal domestication implied a certain level of a compositional shift in the seed-associated microbiome. This fact is not trivial, since it has important potential implications on crop ecology and plant–microbe interactions in an (co–)evolutionary framework. Moreover, we argue that the common shift from ancestors to cultivated forms can be somehow masked by the strong genotype effect, which drives the microbiome changes in an independent way.

Overall, domestication-related traits, as a factor for plant long-term adaptation, appear to determine a compositional shift in microbiomes of modern crops. Although the seeds of both wild and cultivated plants were dominated by similar bacterial phyla (*Proteobacteria*, *Firmicutes*, and *Actinobacteria*), there was a clear difference between cultivated crops and wild progenitors, which may link to the domestication effect. Among the enriched bacterial taxa in cultivated cereals, we found the genus *Cutibacterium* (family *Propionibacteriaceae*), a dominant member of the human skin microbiota. This could result from the human manipulation of seeds and plants during cereal domestication. It is also possible to assume that the presence of genus *Cutibacterium* (family *Propionibacteriaceae*) in our samples could be a contamination due to sample mishandling. However, recent studies endorse more the idea of an interkingdom exchange of microbes during plant domestication than the possibility of contamination. For example, Kuźniar and colleagues [73] studied eight wheat seeds microbiota in different compartments of seed (the embryo, endosperm, and the seed coat) and they found *Cutibacterium* in all the parts of the studied cultivars. Many other recent studies also found *Propionibacterium* as a member of the core microbiota of cereal seeds such as wheat, barley, maize and rice [20,73,74,75]. Interestingly, Campisano and colleagues [76] found a subspecies of *Propionibacterium acnes* in grapevine (*Vitis vinifera* L.), which evolved from the human-associated strain since a time comparable with the beginning of grapevine domestication. The authors concluded that there was an event of interkingdom exchange between humans and plants during grapevine domestication. Likewise, Yousaf and colleagues [77] investigated the relationships between human and animal pathogens (HAP) with plants. They identified *Propionibacterium* and other HAPs in the grapevine endosphere in both stems and leaves, and concluded that human and animal pathogens can be integrated within plant tissues, adapt to the plants, and finally become plant symbionts, for at least one stage of their life cycle. In our work, we found a similar situation; therefore we suggest that such exchange of microbes from humans to plants (and, perhaps, *vice-versa*) might be an effect of plant domestication more common than currently supposed.

We also found a higher abundance of *Pseudomonas* in the seeds of wild species compared to cultivars. Although some species of this genus are pathogenic to plants, several studies showed that plant-originated *Pseudomonas* ssp. have the ability to promote plant health and productivity by different mechanisms [11,78,79]. Some *Pseudomonas* spp. are also regarded as biocontrol agents against several fungal pathogens [11]. Rahman and colleagues [5] demonstrated that a *Pseudomonas* sp., isolated from barley seeds, has beneficial effects for the host, especially under harsh environmental conditions. This ability to cope with biotic and abiotic stresses of *Pseudomonas* and *Stenotrophomonas* species, which were isolated from wild beetroots, was also documented by Zachow and colleagues [67]. Another dominant OTU found in wild cereals belonged to *Acinetobacter*, which was previously found in rice seeds [80] and was shown to possess nitrogen fixation, siderophore production, and mineral solubilization abilities [81]. This evidences suggest that wild plants, often living under stressed conditions, can be supported by microbes to cope with abiotic and biotic stresses [5,67].

We identified a shared microbiome among seeds of wild and modern cereals from various accessions coming from a range of geographic locations. The presence of a shared microbiome preserved across plant species and geographical locations suggests that the seed-associated microbiome, intimately associated with the host, is in some cases preserved during plant domestication. These observations are consistent with other studies, showing that maize seed-associated endophytic bacteria were preserved from the progenitor species teosinte, growing in different geographical places [2]. The majority of bacterial OTUs of the shared cereal microbiome were related to *Pantoea*, *Pseudomonas*, *Acinetobacter*, *Burkholderiaceae* and *Stenotrophomonas*, which were reported as core microbiota of different plant seeds [80,82]. This suggests that such preserved endophytes are well adapted to the internal seed habitat (high osmotic pressure, low moisture and nutrient deficiency, in mature seeds), and likely resulted from long-term selection and adaptation to the seed microhabitat. However, before being analyzed in our study, all the cereal species were propagated and maintained for several years on the same site. It is therefore possible that some of the shared OTUs are derived from the common soil/site. Nevertheless, in our study, the difference between

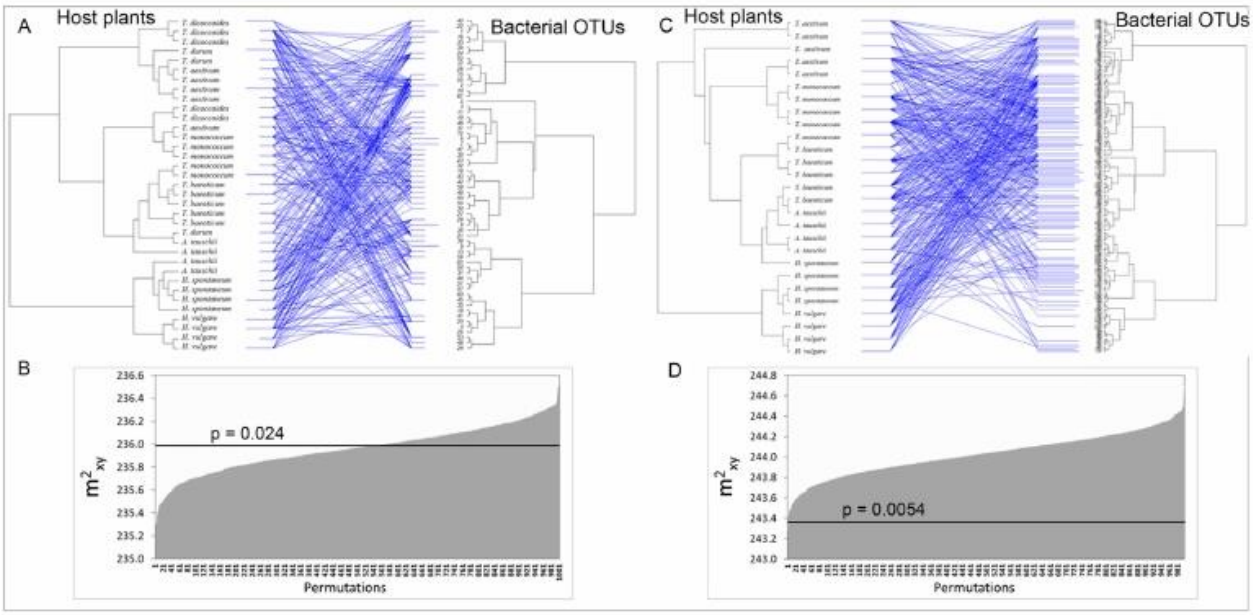


**Fig. 7.** Co-evolution analysis. (A) Tanglegram between the cereal phylogeny, based on RAPD analysis, and the bacterial phylogeny (at OTU level), based on the metabarcoding analysis. Blue lines indicate plant–bacterial associations that were more significant than expected by chance, according to the ParaFitGlobal statistic. (B) Visualization of the global goodness-of-fit statistics over 1000 permutation (P = 0.024); the black line indicates the observed global sum of squared residuals ($m^2_{XY}$) value, while the grey columns indicate the $m^2_{XY}$ value of each of the 1000 randomizations, sorted by ascending values. (C) and (D) Same than (A) and (B), calculated after removal of *T. durum* and *T. dicoccoides* samples.

cereal species, as well as between wild and cultivated cereals, have been identified as significant factors for the variation of the microbiome. Therefore, the existing differences can only be considered as dependent either on the host genotype or the cultivation form.

Interestingly, although the wild cereals harbor a lower bacterial diversity in their seeds, a higher level of connectivity was found by co-occurrence analysis. This means that certain microbial species may have better adapted to the seed habitat and had longer time to develop mutual interactions. This indicates a higher level of "maturity" of the microbiome associated with wild cereals, which suggests co-evolution with the host and vertical transmission across plant generations [5]. In contrast, the microbiota associated with the cultivated cereals did not have enough time yet to establish a solid network of microbial interactions, compared to the wild species.

All the above-discussed evidences strongly suggest a co-evolution of the seed microbiota with the host plants, across the period of cereal domestication. Seed inhabiting microbes are among the most intimate partners of the plant, and they are transmitted to the next plant generations [24]. Therefore, seed endophytes can be regarded as one of the most adapted and specific part of the plant microbiota, if compared to other plant habitats (rhizosphere, phyllosphere, etc.), which are more influenced by external factors and are usually colonized by microbial species recruited from the surrounding environment. Coherently with our conclusion, Wassermann and colleagues [83] found that eight wild plants growing under the same environmental conditions for centuries showed a unique microbiota, and shared just a very small core microbiome, in their seeds. This is surprising, considering that they grew intermixed for decades, and suggests a strong co-evolution. Therefore, we aimed to demonstrate the co-evolution between seed endophytes and cereals by a co-evolution analysis, using RAPD genetic distances of cereals and phylogenetic distances of the associated bacterial OTUs.

The dendrogram based on RAPD profiles showed a clear division of wheat and barley genotype. However, *Triticum aestivum*, *Triticum durum* and *Triticum dicoccoides* were not well discriminated. This phenomenon can be explained by the behavior of the different chromosome sets and polymorphisms of repeated nucleotide sequences, the analysis of which showed close relationships between *Triticum durum* and *Triticum aestivum*. This is likely due to the fact that *Triticum urartu* is the donor of the A genome of these polyploid wheat sorts [48]; indeed, many studies revealed genetic similarity between *Triticum aestivum* and *Triticum durum* [36,84]. Three sets of homologous chromosomes of *Triticum aestivum* derive from the alloploidization of wild DD diploid *Aegilops tauschii* and wild AABB tetraploid *Triticum dicoccoides*, whereas the A and B chromosomes of *Triticum dicoccoides* derive from the wild AA diploid *Triticum urartu* [34] and BB diploid genome donor *Aegilops speltoides* [29]. *Triticum diccocoides* is therefore equally genetically related to both polyploid species of wheat.

The cophylogenetic analysis revealed a significant coherence of phylogenies between seed microbiota and corresponding cereal hosts, from the wild ancestors to the recently cultivated crops, which is a clear clue of co-evolution. This phylogenetic concordance suggests a plant–microbe co-adaptation related to the plant genotype since a stronger effect of the plant genotype on the endophytic bacterial community than on the root-associated bacterial communities was found previously [2,22]. The topology of the cereal tree in the tanglegram (Fig. 7) is not totally coherent with that obtained by the RAPD analysis (Fig. 6); the differences arise from the clustering method applied by the specific R-script for the cophylogeny analysis: this is a principal component analysis, which is not the best method for clustering RAPD profiles. The correct method is UPGMA, like that applied for Fig. 6, where the topology follows well the expected clustering (with the exception of *T. dicoccoides* and *T. durum* that were not discriminated, while for the other species the topology is coherent with the known phylogeny,

at both genus and species level). However, the aim of this kind of cophylogeny analysis is not to get a perfect clustering, but instead to test whether there is significant coherence between the two components (hosts and microbes). We found such significant coherence, which suggests that the two associated components have a certain level of co-evolution. The significance increased drastically when the not resolved species (*T. dicoccoides* and *T. durum*) were removed from the analysis, highlighting the importance to have a well-discriminated analysis of the hosts' genetic distances for performing the co-evolution test.

The approach of identifying interactions and comparing between seed-associated microbial communities and host plants provides the opportunity to move beyond the linear assessments of plant-microbial associations towards a more thorough knowledge of how endophytes are related to host inherited traits. To fully comprehend the processes responsible for these associations, future studies on functional properties and investigations of the impacts of host characteristics on the development of associated microbiomes will be needed.

In this study, we used cereal cultivars and their wild relatives as a model to analyse the effect of plant domestication on bacterial seed endophytes, and to test whether seed endophytes might have co-evolved with their hosts. We are aware that our dataset of four pairs of cereal plants is actually relatively limited; therefore our findings cannot be considered as definitely conclusive, but rather provides: i) indications for a certain level of a compositional shift in the seed-associated microbiome due to domestication, and ii) clues of co-evolution. These intriguing findings, which are in part supported by a limited number of previous studies, need to be tested on further plant species to verify whether they can be generalized or not.

Our understanding of the development of endophytic microbial associations at an evolutionary time scale is presently very restricted. Our work provides new insights into complex microbial interactions and highlights the importance of integrating bacterial seed endophytes into both microbial ecology and applied agricultural microbiology research. From an applied point of view, this knowledge is of paramount importance to develop effective strategies of biofertilization and biocontrol, which are urgently needed to increase sustainability and responsible use of soil resources in modern agriculture.

## Compliance with Ethics Requirements

This article does not contain any study with human or animal subjects.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Acknowledgments

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jare.2020.12.008.

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*Supplementary material to:*

# Domestication affects the composition, diversity, and co-occurrence of the cereal seed microbiota

- **Fig. S1.** Seeds of the 40 accessions of cereals used in this work, 20 cultivated and 20 wild.
- **Fig. S2.** UPGMA dendrogram of RAPD-based gel profiles of 36 cereal accessions. The dendrogram was constructed using similarity coefficients based on the number of different bands (optimization: 1%, position tolerance: 1%).
- **Fig. S3.** Extended-error plot showing bacterial families with significantly different relative abundance between cultivated and wild cereals. Calculations and plot were made with the Stamp software, using the Student's T-test the FDR-corrected p-values.
- **Fig. S4.** Shared and exclusive taxa of cereal seed microbiota. (A) Venn diagram showing the percentage of bacterial OTUs (97% similarity level) shared between cultivated species and wild species. (B) Distribution of 10 biggest shared OTUs between wild species and cultivated species. WF= wild forms; CF= cultivated forms.
- **Figure S5.** Relative abundance of the 15 most abundant OTUs exclusively found in cultivated cereals.
- **Tab. S1.** Seed accessions used in this work.
- **Tab. S2.** Primers used in this study for the Random Amplified Polymorphic DNA (RAPD) analysis.

- **Table S3.** Comparison of alpha-diversity indices between each pair of cultivated cereal and respective ancestor. Values are the average of five independent accessions per species (four only for *Triticum durum*).

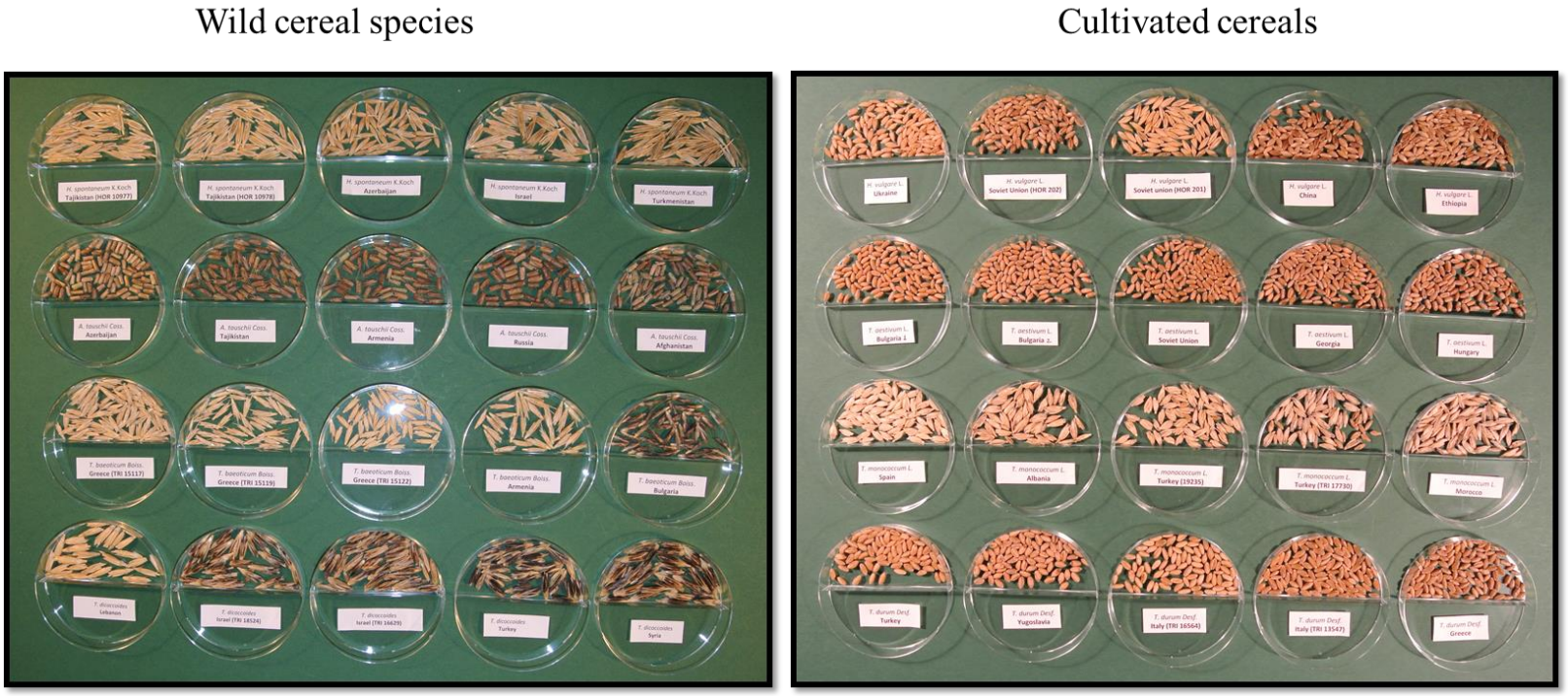


**Figure S1**. Seeds of the 40 accessions of cereals used in this work, 20 cultivated and 20 wild.

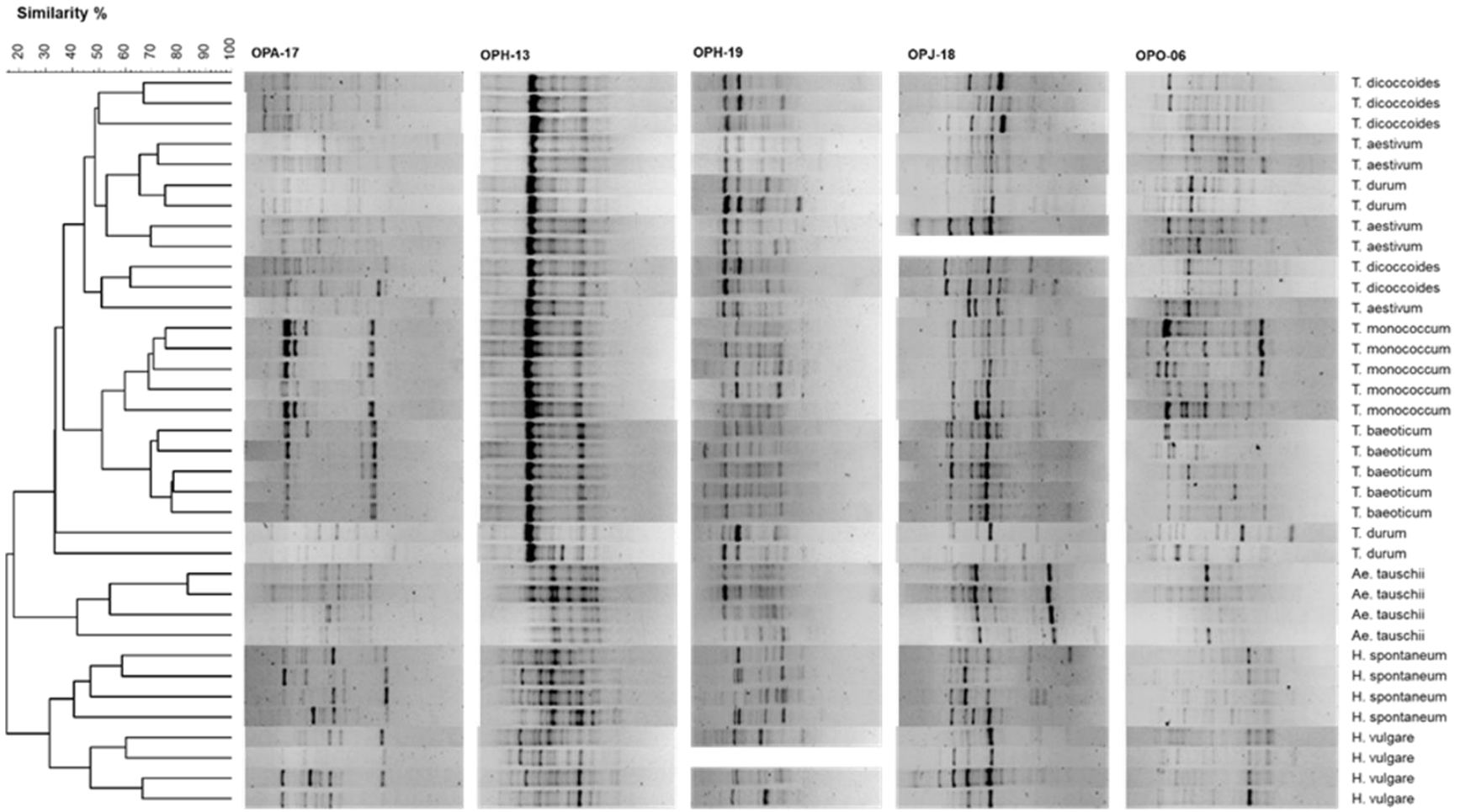


**Figure S2**. UPGMA dendrogram of RAPD-based gel profiles of 36 cereal accessions. The dendrogram was constructed using similarity coefficients based on the number of different bands (optimization: 1%, position tolerance: 1%).

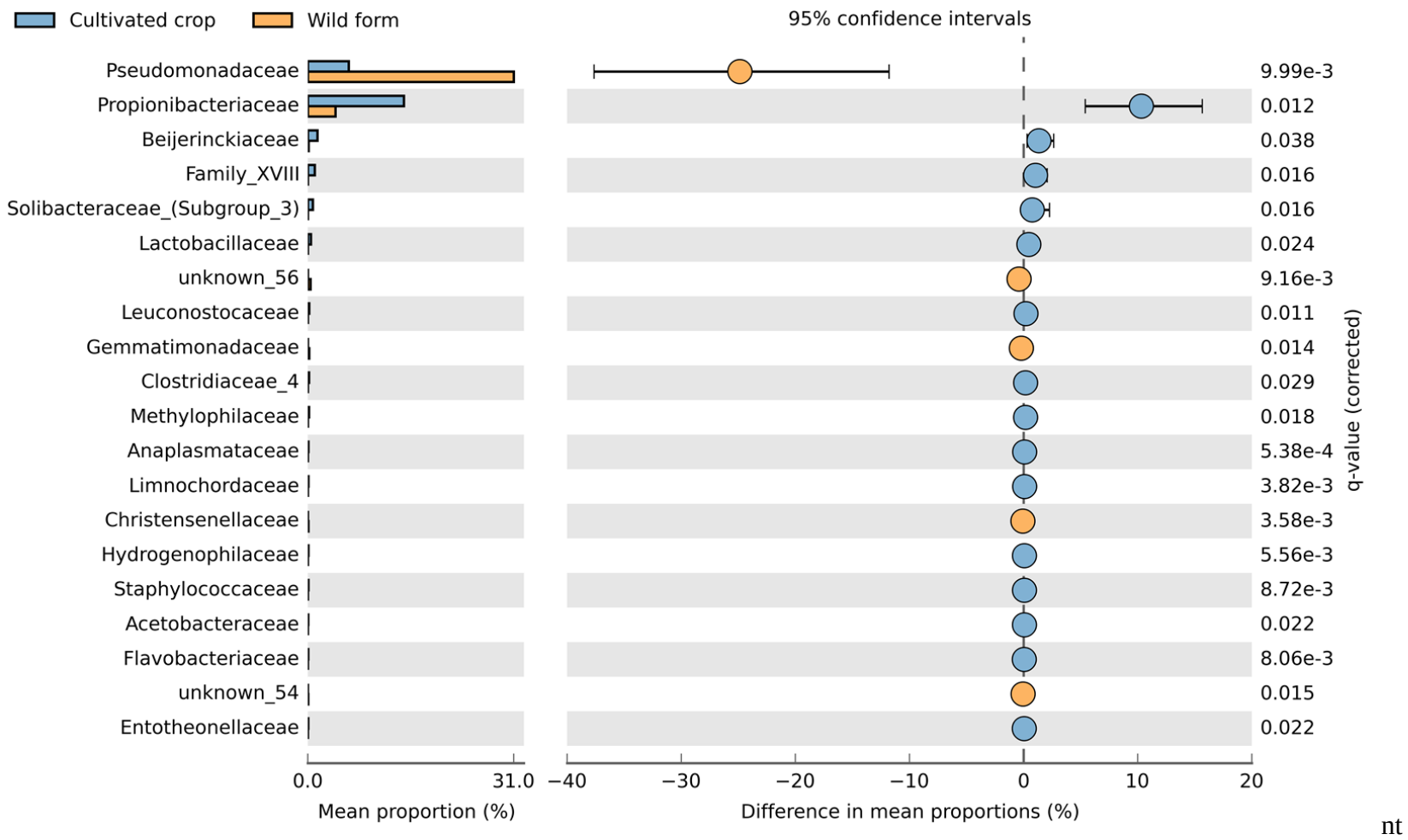


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**Figure S3**. Extended-error plot showing bacterial families with significantly different relative abundance between cultivated and wild cereals. Calculations and plot were made with the Stamp software, using the Student's T-test the FDR-corrected p-values.

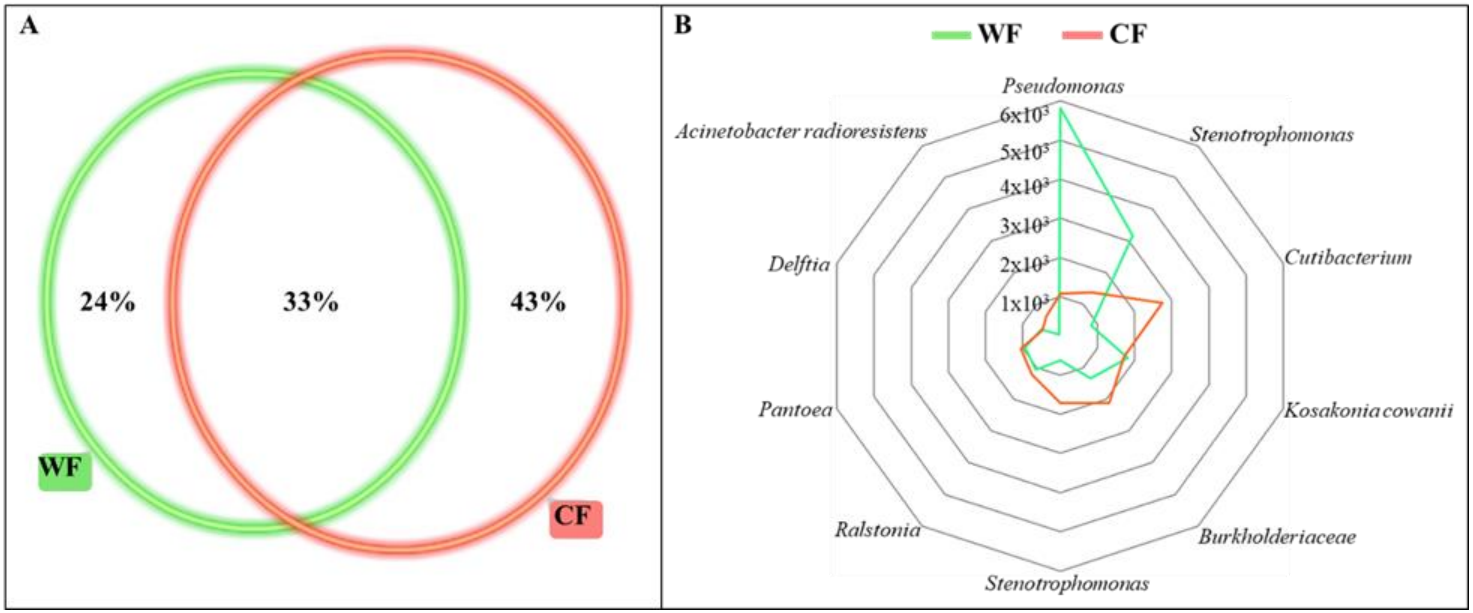


**Figure S4**. Shared and exclusive taxa of cereal seed microbiota. (A) Venn diagram showing the percentage of bacterial OTUs (97% similarity level) shared between cultivated species and wild species. (B) Distribution of 10 biggest shared OTUs between wild species and cultivated species. WF= wild forms; CF= cultivated forms.

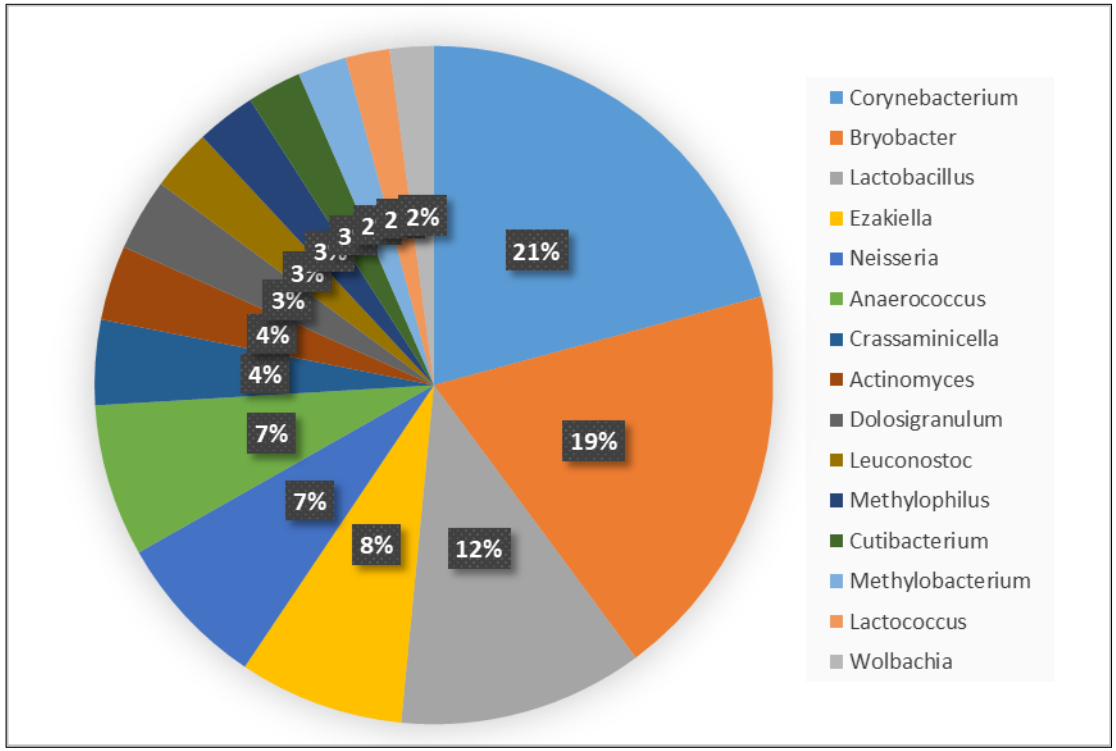


**Figure S5.** Relative abundance of the 15 most abundant OTUs exclusively found in cultivated cereals.

**Table S1**. Seed accessions used in this work.

| Scientific name | Biol. status | IPK Accession number | Country of origin |
|---|---|---|---|
| *Aegilops tauschii* Coss. subsp. *tauschii* var. *meyeri* (Griseb.) Tzvelev | wild | AE 220 | Azerbaijan |
| *Aegilops tauschii* Coss. subsp. *tauschii* var. *meyeri* (Griseb.) Tzvelev | wild | AE 233 | Tajikistan |
| *Aegilops tauschii* Coss. subsp. *tauschii* var. *meyeri* (Griseb.) Tzvelev | wild | AE 235 | Russia |
| *Aegilops tauschii* Coss. subsp. *tauschii* var. *meyeri* (Griseb.) Tzvelev | wild | AE 236 | Armenia |
| *Aegilops tauschii* Coss. subsp. *tauschii* var. *meyeri* (Griseb.) Tzvelev | wild | AE 282 | Afghanistan |
| *Triticum aestivum* L. var. *aestivum* | cultivar | TRI 173 | Hungary |
| *Triticum aestivum* L. var. *aestivum* | cultivar | TRI 365 | Bulgaria |
| *Triticum aestivum* L. var. *aestivum* | cultivar | TRI 368 | Bulgaria |
| *Triticum aestivum* L. var. *aestivum* | cultivar | TRI 7987 | Soviet union |
| *Triticum aestivum* L. var. *aestivum* | cultivar | TRI 13618 | Georgia |
| *Triticum baeoticum* Boiss. subsp. *baeoticum* var. *aznaburticum (*Jakubz.) A.Filat. & Dorof. | wild | TRI 10059 | Bulgaria |
| *Triticum baeoticum* Boiss. subsp. *baeoticum* var. *baeoticum* | wild | TRI 11557 | Armenia |
| *Triticum baeoticum* Boiss. subsp. *baeoticum* var. *baeoticum* | wild | TRI 15117 | Greece |
| *Triticum baeoticum* Boiss. subsp. *baeoticum* var. *baeoticum* | wild | TRI 15119 | Greece |
| *Triticum baeoticum* Boiss. subsp. *baeoticum* var. *baeoticum* | wild | TRI 15122 | Greece |
| *Triticum monococcum* L. var. *monococcum* | cultivar | TRI 17212 | Spain |
| *Triticum monococcum* L. var. *monococcum* | cultivar | TRI 17219 | Albania |
| *Triticum monococcum* L. var. *monococcum* | cultivar | TRI 17730 | Turkey |
| *Triticum monococcum L. var. monococcum* | cultivar | TRI 19235 | Turkey |
| *Triticum monococcum* L. var. *monococcum* | cultivar | TRI 28870 | Morocco |
| *Triticum dicoccoides (*Körn. ex Asch. & Graebn.) Schweinf. convar. *dicoccoides* var.*dicoccoides* | wild | TRI 11501 | Turkey |

| | | | |
|---|---|---|---|
| *Triticum dicoccoides (*Körn. ex Asch. & Graebn.) Schweinf. convar. *dicoccoides* var.*dicoccoides* | wild | TRI 16629 | Israel |
| *Triticum dicoccoides* (Körn. ex Asch. & Graebn.) Schweinf. convar. *dicoccoides* var. *kotschyi Jakubz.* | wild | TRI 18478 | Lebanon |
| *Triticum dicoccoides* (Körn. ex Asch. & Graebn.) Schweinf. convar. *dicoccoides* var.*dicoccoides* | wild | TRI 18504 | Syria |
| *Triticum dicoccoides (*Körn. ex Asch. & Graebn.) Schweinf. convar. *dicoccoides* var.*dicoccoides* | wild | TRI 18524 | Israel |
| *Triticum durum* Desf. subsp. *durum* convar. *durum* subconvar. *durum* var. *hordeiforme* (Host) Körn. | cultivar | TRI 7089 | Turkey |
| *Triticum durum Desf.* subsp. *durum* convar. *durum* subconvar. *durum* var. *affine* Körn. | cultivar | TRI 10715 | Greece |
| *Triticum durum* Desf. subsp. *durum* convar. *durum* subconvar. *durum* var. *affine* Körn. | cultivar | TRI 13547 | Italy |
| *Triticum durum* Desf. subsp. *durum* convar. *durum* subconvar. *durum* var. *affine* Körn. | cultivar | TRI 16564 | Italy |
| *Triticum durum* Desf. subsp. *durum* convar. *durum* subconvar. *durum* var. *affine* Körn. | cultivar | TRI 28771 | Yugoslavia |
| *Hordeum spontaneum* K.Koch var. *ischnatherum* (Coss.) Thell. | wild | HOR 4855 | Turkmenistan |
| *Hordeum spontaneum* K.Koch var. *ischnatherum* (Coss.) Thell. | wild | HOR 4856 | Azerbaijan |
| *Hordeum spontaneum* K.Koch var. *Spontaneum* | wild | HOR 9763 | Israel |
| *Hordeum spontaneum* K.Koch *var. ischnatherum* (Coss.) Thell. | wild | HOR 10977 | Tajikistan |
| *Hordeum spontaneum* K.Koch *var. ischnatherum* (Coss.) Thell. | wild | HOR 10978 | Tajikistan |
| *Hordeum vulgare* L. convar. *vulgare* var. *densum Sér.* | cultivar | HOR 201 | Soviet union |
| *Hordeum vulgare* L. convar. *vulgare* var. *coeleste L* | cultivar | HOR 202 | Soviet union |
| *Hordeum vulgare* L. convar. *vulgare* var. *coeleste L* | cultivar | HOR 211 | Ukraine |
| *Hordeum vulgare* L. convar. *vulgare* var. *coeleste L* | cultivar | HOR 229 | China |
| *Hordeum vulgare* L. convar. *vulgare* var. *coeleste L* | cultivar | HOR 789 | Ethiopia |

**Table S2**. Primers used in this study for the Random Amplified Polymorphic DNA (RAPD) analysis.

| Primer | Sequence 5'—3' | Annealing temperature (°C) | Reference |
|---|---|---|---|
| OPA-17 | GACCGCTTGT | 32 | [56] |
| OPH-19 | CTGACCAGCC | 31 | [56] |
| OPJ-18 | TGGTCGCAGA | 39 | [57] |
| OPO-06 | CCACGGGAAG | 32 | [58] |
| OPH-13 | GACGCCACAC | 38 | [59] |

**Table S3.** Comparison of alpha-diversity indices between each pair of cultivated cereal and respective ancestor. Values are the average of five independent accessions per species (four only for *Triticum durum*).

| **Comparison** | **Alpha-diversity index** | | | |
|---|---|---|---|---|
| | Shannon | Simpson | Equitability | Dominance |
| *Triticum aestivum* (cultivated species) | *3.956* | *0.857* | *0.665* | *0.143* |
| *Aegilops tauschii* (wild ancestor) | *2.836* | *0.745* | *0.576* | *0.255* |
| Relative increment cultivated species/wild ancestor | +39.5 % | +15.0 % | +15.5 % | -43.9 % |
| *Hordeum vulgare* (cultivated species) | *3.115* | *0.802* | *0.635* | *0.198* |
| *Hordeum spontaneum* (wild ancestor) | *1.961* | *0.540* | *0.427* | *0.460* |
| Relative increment cultivated species/wild ancestor | +58.8 % | +48.5 % | +49.0 % | -57.0 % |
| *Triticum monococcum* (cultivated species) | *2.832* | *0.754* | *0.596* | *0.246* |
| *Triticum baeoticum* (wild ancestor) | *3.277* | *0.817* | *0.658* | *0.183* |
| Relative increment cultivated species/wild ancestor | -13.6 % | -7.7 % | -9.4 % | +34.5 % |
| *Triticum durum* (cultivated species) | *3.182* | *0.842* | *0.683* | *0.158* |
| *Triticum dicoccoides* (wild ancestor) | *2.514* | *0.693* | *0.506* | *0.307* |
| Relative increment cultivated species/wild ancestor | +26.6 % | +21.4 % | +34. 9 % | -48.5 % |

# **Chapter 3:** Domestication impacts the cereal rhizosphere colonization by seed- and soil-originated microbiomes

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# Domestication impacts the cereal rhizosphere colonization by seed- and soil-originated microbiomes

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## Abstract

The seed-transmitted microorganisms from the mother plant and the soil microbiome in which the plant grows are major drivers of the rhizosphere microbiome, a crucial component of the plant holobiont. The seed-borne microbiome can be even co-evolved with the host plant as a result of adaptation and vertical transmission over generations. The reduced genome diversity and crossing events during domestication might have influenced plant traits important for root colonization by seed-borne microbes as well as rhizosphere recruitment of microbes from the bulk soil. However, the impact of the breeding on seed-transmitted microbiome composition and the plant ability of microbiome selection from the soil remain unknown. Here, we analysed both endorhiza and rhizosphere microbiome of two couples of genetically related wild and cultivated wheat species (*Aegilops tauschii*/*Triticum aestivum* and *T. dicoccoides*/*T. durum*) grown in three locations, by using 16S rRNA gene and ITS2 metabarcoding, in order to assess the relative contribution of seed-borne and soil-derived microbes to the assemblage of the rhizosphere microbiome.

We found more bacterial and fungal ASVs transmitted from seed to the endosphere of all species compared to the rhizosphere, and these transmitted ASVs were species-specific regardless of location. Only in one location, more microbial seed transmission occurred also in the rhizosphere of *A. tauschii* compared to other species. Concerning soil-derived microbiome, the most distinct microbial genera occurred in the rhizosphere of *A. tauschii* compared to other species in all locations. The rhizosphere of genetically connected wheat species was enriched with similar taxa, differently between locations.

Our results demonstrate that host plant criteria for soil bank's and seed-originated microbiome recruitment depend on both plants' genetic history and availability of microorganisms in a particular environment. This study also provides indications of co-evolution between the host plant and its associated microbiome resulting from the vertical transmission of seed-originated taxa.


Key words: seed microbiome, bulk soil, crop domestication, co-evolution, rhizosphere, endorhiza

## Introduction

Plant domestication significantly altered the plant's physiological, morphological, and genetic characteristics. The targeted and non-targeted selection for specific quality traits results in reduced allelic diversity of domesticated crops (Doebley et al., 2006). However, how the alterations of plant genotype during the domestication influenced the assembly process of the rhizosphere microbiome composition is unknown.

The microbiome inhabiting plant habitats or compartments are known to influence plant health by creating intricate relationships with the host and can play important roles in plant survival (Santos-Medellín et al., 2017). One of the most important microbial habitats for plant health is the rhizosphere (Mendes et al., 2011). The assembly process of the rhizosphere microbiome composition starts immediately after the seed is placed in the soil, and the seed microbiome, the plant genotype and the soil microbiome cooperatively shape the rhizosphere microbiome composition (Tkacz et al., 2020; Walsh et al., 2021). Adequate work demonstrated the role of soil (Berg & Smalla, 2009; Bulgarelli et al., 2012; Lundberg et al., 2012; Schlaeppi et al., 2014) and host plants (Bulgarelli et al., 2012; Edwards et al., 2015; Tkacz, et al., 2020) in determining the structure of the rhizosphere microbiota. However, the dynamics of the seed-transmitted microbiome and plant characteristics that regulate microbial assembly and maintenance remain to be elucidated.

The vertically transmitted seed endophytes play a significant role in plant health, especially in the early stages of plant development (Johnston-Monjee et al., 2011). The colonization of the rhizosphere by seed endophytes might be dependent on the host plant genotype. For example, quantitative trait nucleotides located on plant chromosomes can regulate mycorrhizal rhizosphere colonization as found by Ganugi et al. (2021) in tetraploid wheat genotypes. Moreover, seeds serve as a microbiological habitat for dispersal and dissemination, and this co-existence with the host for several generations eventually leads to plant-microbe co-evolution (Abdullaeva et al., 2021). The symbiotic, mutualistic connections of seed endophytes with their hosts have been previously observed (Nissinen et al., 2019, Johnston-Monjee et al., 2011). Therefore, changes in plant morphology, physiology, gene diversity loss in favor of selected plant traits during domestication such as seed characteristics (hard, soft, big) or root/shoot architecture (Pérez-Jaramillo et al., 2017; Roucou et al., 2018) can influence the seed endophyte assembly (Abdullaeva et al., 2021). It is

possible that the composition or frequency of seed-endophytes that can transmit to the rhizosphere might differ as well as a result of plant traits that facilitate or induce their transmission.

Furthermore, the seed–transmitted microbiome varies depending on the soil in which the plant is grown (Johnston-Monje et al., 2016). The seed endophytes, in contrast, may alter the composition of rhizosphere microbiota as they are initial rhizosphere inhabitants which initiate mutualistic, antagonistic, and symbiotic interactions with other soil microorganisms (Rybakova et al., 2017). The strong effect of soil on the bacterial microbiome assembly of wheat seedlings (*T. aestivum*) was recently showed by characterizing and comparing the bacterial composition of seed and soil on seedling microbiome in a broad range of soils (Walsh et al., 2021). However, the contribution of the seed bacterial and fungal microbiota to the adult plant rhizosphere microbiome, as well as their survival degree in the rhizosphere, were rarely studied in plant holobiont investigations, because it is difficult to trace the transmitted endophytes from seed to rhizosphere during plant development. We could indeed gain knowledge about the significance of seed-originated microbes in shaping the rhizosphere microbiome by glancing into the magnitude of their contribution to the rhizosphere microbiota and how they survive in the rhizosphere across a variety of soil/host systems.



The rhizosphere is densely colonized by a myriad of microorganisms as the result of a major release of organic compounds by the plant roots. The organic carbon like low molecular weight organic acids produced by plants are diverse and can impact the diversity and structure of the rhizosphere microbiome. Through the release of specific secondary metabolites and signaling molecules, plants can selectively recruit different microorganisms from surrounding soil (Bressan et al., 2009; Cotton et al., 2019). This causes changes in microbial diversity and activities around and inside the roots, as well as significantly influences the formation of specific root-inhabiting microbial communities for different plant species/genotypes, even when they grow in the same soil (Ofek-Lalzar et al., 2014). Domestication of crop plants can affect root exudates by changes (regulatory and/or protein modifications in specific genes, structural heterogeneity, transposons, or genome doubling) in the expression of single genes associated with protein production that can modify the molecular structure of precursors (the TCA cycle, or the shikimate pathway) for the synthesis of the secondary metabolites in primary metabolism (Ober, 2005; Jacoby et al., 2021). Ploploidy, (gene duplication) leads to the expansion of the gene catalog occurring in higher plant evolution that might contribute to the diversification of secondary metabolites (Jacoby et al., 2021). The variable secondary

metabolites might lead to increased microbiome diversity in the rhizosphere of modern cultivars as reported by Cardinale and colleagues (2015) in wild and domesticated lettuce rhizosphere. Furthermore, the soil type and physicochemical soil properties have a significant impact on the specificity of the rhizosphere effect. Plants do recruit microorganisms from the soil reservoir, which is likely to differ in composition depending on the soil type. The degree of soil impact on the rhizosphere microbiome is determined by the structure of the soil microbiome due to the variable microbiota of soil able to colonize plant organs (Bulgarelli et al., 2012). Indeed, studies showed that a host plant's rhizosphere effect can differ from one soil type to another (Bulgarelli et al., 2012; Lundberg et al., 2012). However, the question of how the phenotypic and genetic changes in plants impact their ability of microbe selection into the rhizosphere from different soils is left unanswered.

In this study, we investigated the microbiome associated to seed, root endosphere, rhizosphere, bulk soil, and soil before sowing ("seedbed") of wild and domesticated cereals; the latters, as most genetically modified crops, offer perfect scenarios to evaluate the effect of genetic, physiological, and morphological changes caused by domestication on the rhizosphere microbiome selection processes. The rhizosphere microbiome of wheat has been well investigated (Donn et al., 2015; Yin et al., 2017; Schlatter et al., 2019; Schlatter et al., 2020; Tkacz et al., 2020; Zhang et al., 2020). Moreover, a substantial number of studies focused on the characterization of the microbiomes associated with plant seeds (Robinson et al., 2016; Rahman et al., 2018; Kuźniar et al., 2020; Abdullaeva et al., 2021; Alibrandi et al., 2020), roots ( Kavamura et al., 2020; Rossmann et al., 2020; Zhou et al., 2020), and bulk soil and the rhizosphere (Cardinale et al., 2015; Mahoney et al., 2017; Fan et al., 2018; Schlatter et al., 2020) of cereals. However, most of them were conducted under controlled conditions (greenhouse or laboratory), where environmental variability is strictly controlled or at least very limited.

While previous studies have outlined the establishment of rhizosphere microbial communities across plant species, locations, and agro/ecosystems management, the question of whether cereal domestication influence the assembly process of the rhizosphere and, if so, how this effect differs across natural environments, have received far less attention. Here, we characterized the bacterial and fungal microbiota of different soil/plant habitats of four wheat species cultivated in different soils at three locations. Aims of this work were to (1) compare diversity and composition of the bacterial and fungal microbiota in different plant habitats, (2) assess the impact of plant

domestication on the seed-transmitted microbiome and their relative contribution to the endosphere and rhizosphere microbiota, and (3) unravel the effects of changes in plant genotype during domestication and soil/environment on rhizosphere microbiome recruitment. We looked at how the structure of the rhizosphere microbiome of four wheat species shifted as a result of the interaction between plant species and the environment, focusing on the factors affecting the extent of rhizosphere colonization by soil- and seed-derived microbes.

We hypothesized that the relative contribution of the seed-transmitted microbiome to the rhizosphere microbiota of wild cereals will be higher than that of modern cereals. Comparison of seed-borne rhizosphere microbiome of different wheat species grown in different locations allows us to observe co-evolution patterns. We further hypothesized that the enriched bacterial and fungal microbiome in the rhizosphere from the soil will be more diverse in wild relatives than modern species as wild plants are genetically more diverse than modern plants. The outcomes of this study enhance our understanding of how the plant microbiome assembles and thus how the rhizosphere microbiome can be managed and/or manipulated to promote plant growth and health in sustainable agriculture.



## Material and methods

### Plant material

Viable seeds of cereal species, *Triticum aestivum* L. ssp. *aestivum* (hereafter *''T. aestivum''*), *Triticum durum* Desf. ssp. *durum* (hereafter *''T. durum''*), and their corresponding wild ancestors, *Aegilops tauschii* Coss. ssp. *tauschii* (hereafter *''A. tauschii''*), and *Triticum dicoccoides* Schweinf. ssp. *dicoccoides* (hereafter *''T. dicoccoides''*), each from 5 different accessions with a known history, were obtained from the Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Germany (Abdullaeva et al., 2021). All seeds were propagated already for several years at the IPK and collecting/storing were done under the same conditions at the IPK. Once arrived at our laboratory, the seeds were stored in paper bags at 4 °C until analysis.

### Experiment design

Wheat and soil-associated microbiota was evaluated under field conditions, during the season 2018/2019. The experiment was set up with a randomized complete block design (three blocks: a,

b, c) at three research stations of the Justus-Liebig University of Gießen, Germany: Groß Gerau (GG), Weilburger Grenze (WG) and Rauischholzhausen (RH) (Table S1). Wheat species, *A. tauschii*, *T. aestivum*, *T. dicoccoides*, and *T. durum* (Table S2) were planted in each of three blocks in separate rows, randomly arranged to account for minor variations in soil and environmental conditions at small distance scale (Table S1). Prior to sowing, seeds were carefully shelled, cleaned, surface-sterilized in 2.5% sodium hypochlorite for one minute, and pre-soaked in water under sterile conditions for 24 h.

**Harvesting of rhizosphere, bulk soil, root, and seedbed samples.**

Seedbed samples were collected in triplicate from each location before sowing, to determine the primary soil microbial composition. At the plant flowering stage (May to July 2019), root, rhizosphere, and bulk soil samples were collected from all locations to study the microbiota around the plant root system. Plants were manually pulled out, carefully shaken to remove loosely attached soil, cut at the root-shoot boundary, and then placed into plastic bags. Bulk soil samples were collected from soil at the depth of rooting that was not closely adhering to the root. Collected samples were placed in cool boxes and transported to the laboratory, roots were further gently shaken, and then the soil adhering to the roots was collected using a sterile scalp and sieved (2 mm) with a sterile sieve. Clean roots were placed into separate sterile 50 ml screw-cap tubes. Bulk soil and seedbed samples were also sieved and placed in sterile screw-cap tubes. All samples were frozen at −20 °C until DNA extraction.

**Soil analysis**

Soil dry weight, water content, $NH_4^+$, $NO_3^-$, $C_t$, $N_t$, $S_t$, C:N ratio of both rhizosphere and bulk soils (two replicates each) were analyzed. $NH_4^+$, $NO_3^-$, total C, N, S concentrations were measured on air-dried and 2 mm-sieved samples. Approx. two grams of each sample were finely ground using a RETSCH MM 400 Mixer Mill (Retsch GmbH, Haan, Germany) before total C, S, and N analysis using UNICUBE elemental analyzer (Elementar Analysensysteme GmbH, Langenselbold, Germany). Ammonia was determined using the method of Kandeler and Gerber (1988), after extraction with KCl. Nitrate was extracted from the soil as described in Cardinale et al. (2020) and measured with the ion chromatography method (Bak et al., 1991).

**DNA extraction**

196 Total DNA was extracted from the three samples of each set of the root (36) rhizosphere (36), bulk
197 soil (36) and seedbed samples (9), using the PowerSoil® DNA Isolation Kit (MoBio, USA).

198 Before the genomic DNA extraction from roots, these were rinsed several times with sterile water
199 until no further cloudiness was observed in the washing water. Washed roots were then treated with
200 2.5% sodium hypochlorite for 5 min. The samples were then drained and rinsed with autoclaved,
201 deionised water, then incubated in 70% ethanol for 2 min. The ethanol was removed, and samples
202 were rinsed three times with autoclaved, deionised water. Roots were then crushed using sterile
203 pestle and mortar in liquid nitrogen. Grounded roots were decanted into a 2 ml screw-cap tube
204 containing 200 µl of sterile glass beads and then frozen for later DNA extraction. The DNA was
205 isolated from 300–500 mg of grounded samples as described by Abdullaeva et al. (2021).

206 Sequences of bacterial endophytes of seeds were taken from the previous study (Abdullaeva et al.,
207 2021) (accession number: PRJEB36663) and the DNA already available from the previous study
208 was used in this study to determine the fungal diversity by amplification of the ITS2 region. All
209 extracted genomic DNAs were quantified by Nano-DropTM 2000 Spectrophotometer (Peqlab,
210 Erlangen, Germany) then stored at -20 °C until further analysis.

211 **Amplicon library preparation and Ion Torrent sequencing**

212 The V4-V5 regions of the 16S rRNA gene and the ribosomal internal transcribed spacer 2 (ITS2)
213 from the rhizosphere, bulk soil, root, and seedbed were PCR-amplified to characterize the bacterial
214 and fungal microbiota, respectively. The 16S RNA gene was amplified with the primer pair 520 F
215 (5`- AYTGGGYDTAAAGNG-3`) (Claesson et al., 2009) and 907 R (5`-
216 CCGTCAATTCMTTTRAGTTT-3`) (Engelbrektson et al., 2010) in combination with peptide
217 nucleic acid (PNA) clumps, and purified, as described in Abdullaeva et al. (2021). The primer pair
218 for the fungal ITS2 regions was ITS3 KYO2 forward (5'-GATGAAGAACGYAGYRAA-3') and
219 ITS4 reverse (5'-TCCTCCGCTTATTGATATGC-3'); amplification and purification were
220 performed as described in Ambika Manirajan et al. (2018).

221 PCR products were pooled in equimolar concentrations and used for emulsion PCR with Ion One
222 Touch 2 (Ion PGM Hi-Q View OT2 kit, Life Technologies, Carlsbad, USA). The quality of the
223 final product was assessed using Ion Sphere Quality Control Kit (Life Technologies, Carlsbad,
224 USA) and loaded on a 314 or 318 chip for sequencing with an Ion PGM sequencer (Life
225 Technologies, Carlsbad, USA).

226 **Bioinformatic analysis of the 16S rRNA and ITS amplicons**

227 The raw 16S rRNA gene and ITS sequences were processed using the bioinformatic pipeline
228 QIIME2 (version 2020.6) (Bolyen et al., 2019). Fungal ITS and 16S rRNA gene sequences were
229 demultiplexed using Qiime2 *cutadapt* plugin (Martin, 2011). The ITS were then trimmed with ITS
230 express (Rivers et al., 2018) deleting the flanking regions of the rRNA genes to leave only the ITS2
231 region. The QIIME2 plugin DADA2 was used for quality control, filtering, chimera identification,
232 denoising, clustering of the sequences to amplicon sequence variation ASV (99% sequence
233 similarity), and producing the feature table. In the DADA2 step 16S rRNA gene sequences were
234 cut at position 320 bp and the first 15 bp were deleted. ITS2 sequences were already cut in the
235 ITSexpress step but to avoid a large number of ASVs because of the high length variability of the
236 ITS region that may be genetically identical but grouped as separate ASVs the sequences were cut
237 at position 150 bp. Sequences were assigned to taxonomy with the QIIME2 plugin feature-classifier
238 (Bokulich et al., 2018) by pre-trained Naive Bayes classifiers (Pedregosa et al., 2011) trained on
239 the SILVA 138 database (Quast et al., 2013) for the 16S rRNA gene sequences and the UNITE
240 (v8.2) database (Kõljalg et al., 2013) for fungal ITS sequences. Thereafter amplicon sequence
241 variants (ASVs) identified as plastids or mitochondria were removed from the 16S rRNA gene
242 sequences. The sequences were submitted to NCBI database (www.ncbi.nlm.nih.gov/) under the
243 project number (PRJNA773663).

244 **Statistical analysis**

245 Statistical analyses were performed in R-Studio (RStudio PBC, Boston, USA) with R v.4.0.3 (R
246 Core Team, 2020), using the ASV table generated from QIIME2 and were analyzed using the
247 'phyloseq' package (McMurdie & Holmes, 2013).

248 ASVs were previously grouped by genera before diversity assessments. Alpha diversity was
249 estimated using observed richness, Simpson, and Shannon diversity measures, using the mean
250 value from genera tables rarified to even depth. Significant differences between diversity indices
251 between species, sample source, locations, and cultivation form (wild *vs.* cultivated) were
252 determined using the Kruskal-Wallis rank-sum test.

253 Since the number of DNA sequence reads is restricted by the ability of the sequencing machinery,
254 microbiome datasets created by high-throughput sequencing are compositional (Gloor et al., 2016;
255 Tsilimigras & Fodor, 2016). Instead of using regular counts and rarefying, we used a center log-

ratio transformation (CLR) to evaluate the microbial composition of our datasets. Beta diversity was assessed using a distance matrix based on Aitchison distance (Euclidian distance between samples) and variance-based compositional principal component (PCA) plots (Aitchison, 1986; Aitchison & Greenacre, 2002). Significant differences in microbiota composition between groups and experimental factors were detected by permutational multivariate analysis of variance (ADONIS) (Anderson, 2001) using the vegan R package (Oksanen et al., 2017).

We conducted a multivariate homogeneity of groups dispersion test to examine among community similarities between species, sample sources, and locations.

Constrained (canonical) ordination analysis was performed using RDA method to observe variation in the microbial communities between plant compartments, locations, and wheat cultivars by the environmental variables using RStudio with the rhizosphere and bulk soil data.

**The relative proportion of seed-transmitted bacterial and fungal ASVs calculation**

We used ASV counts for the identification of seed-transmitted microbiome proportion to the endorhiza and rhizosphere. The ASV counts for each replicate were manually related to the total seed ASV counts using excel and the median of the relative proportion of replicates was used for graphical analysis. The seed-transmitted genera which were also found in seedbeds have not been considered seed-transmitted. ANOVA (Analysis of Variance) was performed to identify the significant differences among the relative proportion of seed-transmitted microbiomes of wheat species and between locations using RStudio. Followed by Tukey's test to test significant differences or similarities between the specific groups.

**Differential abundance (DA) analysis between species and locations**

We used *ALDEx2* (Fernandes et al., 2013) method to find microbial taxa with significant differential abundances between rhizosphere and bulk soil of each species which enable us to observe the bacterial and fungal microbiota enriched in the rhizosphere of genetically related groups (*A. tauschii*/*T. aestivum* and *T. dicoccoides*/*T. durum*). For evaluation of which genera are significantly enriched in the different treatments, the absolute aldex effect size (> 1 and < -1) was used. For the graphical presentation, only enriched genera in the rhizosphere (> 1) were used.

Furthermore, the compositional difference between the rhizosphere microbiome of wheat species that were grown in the same site (randomly selected) was tested using *ALDEx2* approach.

There are numerous methods for determining which features in these datasets have different relative abundances. They have various characteristics and sometimes produce a large number of false positives, but the compositionally optimal *ALDEx2* is less likely to have these issues (Thorsen et al., 2016; Gloor et al., 2017).

**Table 1**. Geographical coordinates, soil type, and some important physical and chemical properties of studied field soils

| Research stations | Geographical coordinates | Soil type | pH-value | Sand (%) | Silt (%) | Clay (%) | Humus (%) | References |
|---|---|---|---|---|---|---|---|---|
| *Weilburger Grenze (WG)* | 50°60′ N; 8°65′ E 158m a.s.l. | *Fluvic Gleyic Cambisol* [a] | 6.0–6.4 | 6–15 | 40–58 | 36–48 | 2.20 | *Stumpf et al., 2019* |
| *Rauischholzhausen (RH)* | 56°76′ N; 8°88′ E 225 m a.s.l. | *Haplic Luvisol* [a] | 6.9–7.7 | 1.30–3.02 | 64.24 | 32 | 2 | *Macholdt & Honermeier 2018; Wang et al., 2021* |
| *Gross-Gerau (GG)* | 49° 56′ N; 8° 30′ E 90.7 m a.s.l. | *Arenosol* [a] | 6.5 | 85.2 | 9.6 | 5.2 | 1.1-1.5 | *Russo & Honermeir 2016* |

[a]Soil horizons were classified according to the World Reference Base for Soil Resource (WRB, 2014).



## Results

### *16S amplicon sequencing results and taxonomic classification*

The 16S rRNA gene amplicon sequencing yielded 2,690,188 high-quality, nonchimeric sequences across rhizosphere (631,004 sequences), bulk soil (661,618 sequences), root (1,076,002 sequences) and seedbed (321,564 sequences). Bacterial seed sequencing data previously reported (Abdullaeva et al., 2021) and a partial of the bacterial seed sequences (24,204 sequences from seed accsessions AE 220, TRI 368, TRI 18524, and TRI 10715) were used in this study. Two samples of *T. aestivum* from the rhizosphere dataset were removed because of low sequencing quality and number. We

identified 27612 bacterial ASVs from 119 samples in total (34 rhizosphere, 36 bulk soil, 36 root, 9 seedbed, and 4 seed samples).

*ITS amplicon sequencing results*

Sequencing of ITS amplicon library resulted in a total of 904,416 high-quality, nonchimeric sequences across rhizosphere (157,279 sequences), bulk soil (322,386 sequences), root (231,881 sequences), seedbed (176,963 sequences) and seed (15,907 sequences) samples. Two samples of *T. aestivum* from the rhizosphere, one sample of *T. dicoccoides* from the root datasets, and one seedbed sample from Rauischholzhausen were removed because of low sequencing quality and number. We identified 3,136 fungal ASVs from 117 samples in total (34 rhizosphere, 36 bulk soil, 35 root, 8 seedbed, and 4 seed samples).

**Microbial richness and diversity of the different plant and soil compartments of wheat species**

The alpha-diversity indices of bacterial and fungal ASVs were separately tested for significance of the factors location, plant habitat, cultivation form, and species (Table S3). As well as the differences between habitats and species within locations were determined (Table S3). The alpha diversity indices of fungal rhizosphere/endorhiza and bulk soil microbiome significantly changed between locations in contrast to bacterial microbiomes of those habitats except for $\alpha$-diversity indices of root endophytic bacterial microbiome (Fig. S1). Both, fungal and bacterial microbiome $\alpha$-diversities between habitats within locations were significantly different except for the bacterial microbiome in WG (Table S3). Interestingly, the alpha-diversity of the bacterial microbiome in the bulk soil of four species was different in GG and RH (Table S3). The alpha-diversities in the seedbed soil of the three locations and seeds of four wheat species were not different from each other (Table S3). Cultivation forms of wheat species significantly affected the observed richness of fungal communities of bulk and root samples collected from RH.

**Microbiota differences across experimental fields**

Aitchison distances visualized using principal component analysis (PCA) were used to investigate the beta diversity. Both microbial communities were differentiated by the three locations, in particular, bacterial communities of GG were more different than other locations (Fig. 1 A), while fungal communities of all locations were equally dissimilar to each other (Fig. 1 B). The ordination results were further supported by permutational multivariate analysis of variance (ADONIS) based on Euclidian distance. ADONIS test results demonstrated that both bacterial ($R^2 = 0.144$, $p < 0.001$) and fungal ($R^2 = 0.185$, $p < 0.001$) communities were significantly differentiated by locations (Table S4).

**Microbiota differences across plant habitats**

The bacterial communities were also separated by the five plant- and soil-compartments, and those in the seeds were found to retain the most distinguishable bacterial communities (Fig. 1, Fig. S2). The rhizosphere and root endosphere bacterial and fungal microbiome exhibited a community diversity that was more similar to each other than those of the other three compartments (Fig. 1 C, D, Fig. S2). However, the fungal communities were not well differentiated between soil compartments as compared to the bacterial microbiome (Fig. 1 B). The ordination results were further supported by permutational multivariate analysis of variance (ADONIS) based on Euclidian distance, which revealed significant separation of the bacterial ($R^2 = 0.119$, $p < 0.001$) and fungal ($R^2 = 0.140$, $p < 0.001$) communities by compartments and sites (Table S4).

Homogeneity of variance of communities within the same compartments was examined by measuring the distance between the centroid and each sample of the group. Comparison of homogeneity of communities in plant compartments, locations showed significant dissimilarity ($p = 0.001$) among microbial communities of all sample sources (Fig. 1 C, D). The seed and

347 rhizosphere bacterial and fungal communities exhibited the lowest dispersion, while bulk soil and
348 root microbial communities exhibited higher dispersion than other compartments except for
349 seedbed (Fig. 1 C, D). Variations between the dispersion of seedbed bacterial communities and
350 fungal communities were different.

351 **Differences in microbiota across wheat cultivars and cultivation forms**

352 Bacterial microbiota diversity significantly differed across plant compartments (ADONIS, $p > 0.05$
353 in all locations; Table S4). Fungal seed and root community composition were also significantly
354 different between cultivars in all locations ($p > 0.05$). Significant changes in fungal microbiota
355 composition were found in the rhizosphere (only in GG) and bulk soil (only in WG) across wheat
356 species ($p > 0.05$). Fungal rhizosphere and bulk soil community composition did not differ
357 significantly across the wheat species in RH ($p > 0.05$). Overall, bacterial community diversity
358 differed more than fungal communities by the factor of plant species in all compartments. Seed and
359 root microbial community diversity showed the highest variation between plant species as
360 compared to the rhizosphere and the bulk soil.

361 Unconstrained ordination based on Euclidian distance matrices of bacterial and fungal microbiota
362 showed that *T. aestivum* and its wild relative *A. tauschii*, as well as domesticated wheat *T. durum*
363 and its wild relative *T. dicoccoides* were clustered together (Fig. S3, S4). Further tests were carried
364 out to observe the effect of species and cultivation form factors within compartments and locations.
365 ADONIS results showed that the structure of both bacterial and fungal microbiota was significantly
366 changed by the factor "cultivation form" in the root endosphere in all three locations except fungal
367 microbiota in GG (Table S4).

368

**Influence of soil characteristics on the microbial communities of the root-associated microbiome of wheat species**

Preliminary soil physico-chemical characteristics and analysis of the collected rhizosphere and bulk soils provided a wide range of values across the samples (Table 1, Fig. S5). ANOVA results showed that chemical soil properties ($NO_3^-$, $p = 0.000035$, $NH_4^+$, $p = 0.0195$, N, $p = 0.0000009$, C, $p = 0.0632$) with the exception of total carbon significantly differed between locations (n=12) as well as between plant compartments (rhizosphere and bulk soil; Fig. S5). Ammonia was significantly different between compartments only in GG ($p = 0.0024$), nitrate level was significantly different in WG ($p = 0.0018$) and RH ($p = 0.0086$), nitrogen was only different in WG soil ($p = 0.0049$) (Fig. S5) between compartments.

Permutational ANOVA analysis on constrained axes used in ordination showed that the effect on the bacterial community composition of the rhizosphere and bulk soil samples was significantly different depending on the ammonia and moisture in the soil (Fig. 2, Table 2). Whereas, fungal communities changed by the nitrate in GG in both soil compartment and nitrogen content in RH only in the bulk soil (Table 2).

**Table 2.** Permutational ANOVA on constrained axes used in ordination. RDA analysis was used for the bacterial and fungal community composition in the rhizosphere and the bulk soil of each location, and Euclidean distance was calculated for the environmental variables. The numbers indicate the Permutational ANOVA statistic (r) value, with significance as indicated (< 0.001 '***', < 0.01 '**', < 0.05 '*'). Abbreviations: $NH_4^+$, Ammonium; WC-water content; $NO_3^-$, Nitrate; C, total carbon; N, total nitrogen; S, total Sulfur; C:N, carbon-nitrogen ratio.

| | **GG** | | | | **WG** | | | | **RH** | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **Bacteria** | | **Fungi** | | **Bacteria** | | **Fungi** | | **Bacteria** | | **Fungi** | |
| **Environmental variables** | **Rhizo** | **Bulk soil** | **Rhizo** | **Bulk soil** | **Rhizo** | **Bulk soil** | **Rhizo** | **Bulk soil** | **Rhizo** | **Bulk soil** | **Rhizo** | **Bulk soil** |
| **$NH_4^+$** | **0.033*** | 0.505 | **0.041** * | 0.737 | 0.224 | **0.001***** | 0.472 | 0.196 | 0.378 | 0.949 | 0.222 | 0.527 |
| **WC** | 0.116 | **0.047** * | 0.385 | **0.075.** | **0.003**** | **0.018** * | 0.139 | 0.077 | 0.094 | 0.362 | 0.318 | 0.384 |
| **$NO_3^-$** | 0.123 | 0.613 | **0.040*** | **0.046*** | 0.300 | 0.336 | 0.276 | 0.948 | 0.12 | 0.727 | 0.638 | 0.476 |
| **C** | 0.102 | 0.181 | 0.567 | 0.317 | 0.198 | 0.383 | 0.585 | 0.373 | 0.121 | 0.567 | 0.748 | 0.580 |
| **N** | 0.495 | 0.573 | 0.422 | 0.229 | 0.176 | 0.349 | 0.336 | 0.562 | 0.714 | 0.59 | 0.418 | **0.005**** |
| **S** | 0.407 | 0.172 | 0.955 | 0.860 | **0.02*** | 0.357 | 0.600 | 0.546 | **0.**071 | 0.421 | 0.696 | 0.182 |
| **C:N** | 0.272 | 0.198 | 0.604 | **0.**090 | 0.294 | 0.357 | 0.847 | 0.717 | 0.358 | 0.767 | 0.141 | 0.649 |



## The relative proportion of seed–transmitted endorhiza and rhizosphere bacterial ASVs

In general, we observed a higher proportion of seed-derived bacterial and fungal microbiome in the endosphere compared to the rhizosphere. We also found a significantly higher proportion as well as diversity of seed-derived microbiome in the endorhiza and rhizosphere of wild diploid *A. tauschii* than other wheat species. However, this pattern was observed in both, bacteria and fungi, only in one location (bacteria in GG, fungi in WG) (Fig. 3 A, B). We also investigated the effect of location on seed transmission. The relative proportion of bacterial and fungal seed-transmitted rhizosphere microbiome was significantly influenced by location factor whereas, the effect of location has not been observed on the endorhiza bacterial microbiome (Fig. 3 C).

**Seed-originated rhizosphere microbiota**

ASVs belonging to the genera *Verticiella*, *Chryseobacterium*, *Rhodococcus*, *Pseudomonas*, *Stenotrophomonas*, *Plantibacter*, *Methylobacterium-Methylorubrum*, *Luteibacter*, *Aeromicrobium*, *Cutibacterium*, *Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium*, *Nocardioides*, *Massilia, Ulocladium, Alternaria,* were transmitted from seed to rhizosphere of *A. tauschii* (Table S5).

*Pedobacter, Brevundimonas, Ulocladium, Stemphylium, Alternaria* were transmitted from seed to rhizosphere of *T. aestivum* (Table S5).

*Brevundimonas*, *Stenotrophomonas*, *Sphingomonas*, *Pseudomonas*, *Cutibacterium*, *Symbiobacterium*, *Pyrenophora*, *Ulocladium*, *Alternaria*, *Neoascochyta* were transmitted from seed to rhizosphere of *T. dicoccoides* (Table S5).

*Streptococcus*, *Ralstonia*, *Pseudomonas*, *Alternaria* were transmitted from seed to rhizosphere of *T. durum* (Table S5).

**Seed-originated endorhiza microbiota**

Most of the genera found in the endorhiza were similar to the seed-transmitted rhizosphere microbes. *Rhodococcus*, *Enterobacteriaceae*, *Chryseobacterium*, *Verticiella*, *Pseudomonas*, *Stenotrophomonas*, *Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium*, *Nocardioides*, *Luteibacter*, *Duganella*, *Comamonadaceae*, *Methylobacterium-Methylorubrum*, *Plantibacter*, *Cutibacterium*, *Aeromicrobium*, *Massilia,* unknown fungi*, Alternaria* were transmitted from seed to endorhiza of *A. tauschii* (Table S5).

*Brevundimonas*, *Pedobacter*, *Cutibacterium*, *Duganella*, *Massilia*, *Symbiobacterium*, *unknown fungi*, *Alternaria*, *Stemphylium* were transmitted from seed to endorhiza of *T. aestivum* (Table S5).

*Sphingomonas*, *Symbiobacterium*, *Cutibacterium*, *Stenotrophomonas*, *Pseudomonas*, *Neoascochyta*, *Alternaria*, unknown fungi were transmitted from seed to endorhiza of *T. dicoccoides* (Table S5).

*Pseudomonas*, *Streptococcus*, *Methylobacterium-Methylorubrum*, *Cutibacterium*, *Alternaria*, unknown fungi were transmitted from seed to endorhiza of *T. durum* (Table S5).

Among the seed-transmitted fungal genera, unknown fungi were transmitted from seed to endorhiza of all species and this is relevant for all three locations (Table S5).

Some of the above-reported seed-transmitted genera were specific to particular wheat species and found at least in two locations. *Massilia, Methylobacterium-Methylorubrum, Pseudomonas, Plantibacter, Verticiella, Comamonadaceae, Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium, Stenotrophomonas* were specific to the endorhiza of *A. tauschii. Massilia, Methylobacterium-Methylorubrum* were specific to the rhizosphere of *A. tauschii* (Table S5).

*Brevundimonas* was found specific to both endorhiza and rhizosphere of *T. aestivum, Pseudomonas, Streptococcus* to *T. durum,* and *Pseudomonas, Sphingomonas*, fungi *Pyrenophora, Neoascochyta* were specific to *T. dicoccoides* (Table S5).

Most of the fungi transmitted from seed to endorhiza and rhizosphere were specific to a particular location. Such as, bacterial genera, *Symbiobacterium, Cutibacterium, Pedobacter* and fungal genera *Ulocladium, Stemphylium* were found in particular locations (Table S5).

**The enriched rhizosphere microbiota (as compared to the bulk soil)**

The differential abundance test showed that the rhizosphere of genetically connected couples of wheat species differently enriched bacterial and fungal genera from the bulk soil. The rhizosphere of *T. dicoccoides* and *T. durum* grown in the same location were found enriched with similar

445 bacterial and fungal microbiome from the bulk soil (Fig. 4) and the composition of the enriched
446 microbiome was different in three locations. *Abditibacterium*, *Mucilaginibacter*, *Edaphobaculum*,
447 *Lysobacter*, *Aeromicrobium*, *Pedobacter*, *Saccharimonadales*, *Flavobacterium*, *Luteimonas*,
448 *Mesorhizobium*, *Fibrobactereraceae*, *Tepidisphaerales*, *Dokdonella*, *Massillia,* which make up
449 56% of total enriched bacterial genera, were specifically enriched in the rhizosphere of cultivated
450 *T. durum* and its ancestor *T. dicoccoides* in GG (Fig. 4). Similarly, *Lysobacter*, Chloroflexi *KD4-*
451 *96*, Desulfuromonadia *PB19*, Gemmaproteobacteria *R7C24*, Polyangiales *Blrii41*, *Herpetosiphon*,
452 *Sphingobacteriales*, *Pajaroellobacter*, a genus of phylum Candidatus *WS2*, *Luteolibacter*.
453 *Reyranella*, and *Pseudoxanthomonas* (48% of total enriched genera) in WG and *Arenimonas*,
454 *Pseudomonas*, *Polycyclovorans*, *Nocardioides*, *Luteolibacter*, *Luteimonas*, *Reyranella*,
455 *Lysobacter*, *Pajaroellobacter* (50% of total enriched genera) were enriched in RH (Fig. 4).

456 The rhizosphere of modern *T. aestivum* and its wild ancestor *A. tauschii* was found enriched with
457 a less similar bacterial microbiome than the other genetically related group from the corresponding
458 bulk soil. *Reyranella*, *Lysobacter*, *Luteimonas*, *Arenimonas*, *Dokdonella* (20% of total enriched
459 genera) in GG, *Chthoniobacter*, *Marmoricola*, *Pseudoxanthomonas*, *Brevundimonas*,
460 *Pseudomonas*, *Nocardioides*, *Luteolibacter* (24-27% of total enriched genera) in WG, and
461 *Nocardioides*, *Chthoniobacter*, *Pseudomonas* and *Microlunatus* were enriched (36%) were
462 enriched in RH (Fig. 4).

463 Fungal genera that were differentially enriched in the rhizosphere of wheat species were different
464 from each other however, *Microdochium* and *Mortierella* were predominant in almost all
465 rhizospheres (Fig. 5).

466 *Pseudomonas*, *Chthiniobacter*, *Nocardioides,* and one fungal genus *Mortierella* were enriched in
467 the rhizosphere of wild wheat *A. tauschii* repeatedly in all locations (Fig. 4, 5), also

468 *Pajaroellobacter, Reyranella, Lysobacter, Luteolibacter* and *Microdochium* were enriched in the
469 rhizosphere of *T. dicoccoides* in all three fields. *Lysobacter, Mortierella* and *Microdochium* were
470 enriched in the rhizosphere of *T. duru*m and none of the bacterial genera was repeatedly enriched
471 in the rhizosphere of *T. aestivum* compared to the bulk soil (Fig. 4, 5).

472 Further analysis of differential abundance between rhizospheres of different wheat genotypes that
473 were grown in the same site showed the more distinct bacterial rhizosphere microbiome assembly
474 of wild *A. tauschii* from the other wheat genotypes (Fig. 6). The most rhizosphere similarity
475 observed between wild *T. dicoccoides* and modern *T. durum* (Fig. 6) that are genetically connected.
476 However, this result is not the same for the other genetically related couple (Fig. 6). The second
477 most similar rhizosphere microbiome was found between modern wheat species: *T. aestivum* and
478 *T. durum*. The rhizosphere of wild wheat species showed a more diverse however less abundant
479 microbiome in contrast to modern wheat species.

480 The differential abundance between rhizospheres of different wheat genotypes that were grown in
481 the same site showed different fungal rhizosphere microbiome assembly between wild and modern
482 wheat species. The most similar fungal rhizosphere microbiome was found between modern wheat
483 species *T. durum* and *T. aestivum* (Fig. 7). The more different fungal rhizosphere microbiome was
484 found between wild and modern wheat species. However, genetically related wheat species wild,
485 *T. dicoccoides* and modern, *T. durum* showed similar rhizosphere microbiome assembly (Fig. 7).

486

487 ## Discussion

488 Plants have experienced considerable genetic, phenological, and physiological changes as a result
489 of selection for certain quality attributes during domestication. The current study used the 16S

490 rRNA gene and ITS2 regions to determine the impact of plant domestication on main drivers of
491 rhizosphere microbiome assembly, seed-transmitted and soil-originated, of four wheat species
492 grown in different sites. The endorhiza and rhizosphere bacterial and fungal microbiomes were
493 more comparable to one another than the seed microbiome (Fig. 1 C, D), suggesting that the
494 majority of the endorhiza microbiome are originated from the rhizosphere which is consistent with
495 previous studies (Bulgarelli et al., 2012; Leff et al., 2017) whereas seed has a unique environment
496 which has no direct contact with the soil (Hardoim et al., 2012). We further found a significant
497 effect of location (GG, WG, RH) (Fig 1 A, B) with more differentiation of bacterial communities
498 between compartments than fungal microbiome. The results show the stronger effect of location
499 on fungal microbiome than a plant which is similar to the findings of Bonito et al. (2014). Besides,
500 the strong effect of plant genotype (*A. tauschii*, *T. aestivum*, *T. dicoccoides* and *T. durum*) (Fig. S4,
501 S4) on the bacterial and fungal microbiome composition was observed. The findings are in line
502 with the previously reported studies (Bouffaud et al., 2014; Walters et al., 2018; Schlatter et al.,
503 2019). Over the last 20 years, the taxonomic composition of bacterial and fungal microbiomes in
504 different plant habitats across different environments has been extensively studied. In the current
505 study, we wanted to place special emphasis on comprehending the impact of domestication on the
506 seed-borne and soil-originated rhizosphere microbiome, to gain insight into the assembly process
507 of the rhizosphere microbiome, one of the most crucial components of the plant holobiont.

508 Seed-transmitted endorhiza and rhizosphere microbiome of modern wheat species seem to be
509 affected by domestication. We found a higher proportion of seed-borne microbes in the endorhiza
510 and rhizosphere of diploid wild *A. tauschii* than modern wheat species. However, this is not true
511 for another tetraploid wild wheat *T. dicoccoides*. Although, *T. dicoccoides* is wild wheat, its
512 genome size, phenology, morphology is different than diploid *A. tauschii* and more similar to
513 modern wheat species (Luo et al., 2007; Pont et al., 2019) since it has the same genome as *T. durum*

514 and donated two genomes AA to bread wheat *T. aestivum* (Pont et al., 2019). Our results suggest
515 that polyploidy events, even in older polyploid species, influence the transition (or survival) of seed
516 endophytes to the endorhiza and rhizosphere. According to previous investigations, the genetic
517 diversity was lost by 69% in hexaploid bread wheat and by 84% in tetraploid durum wheat during
518 domestication (Haudry et al., 2007) as a result of polyploidy. Moreover, genome duplication also
519 produces gene duplicates inside the same genome known as paralogs, which operate differently
520 from the original gene due to a lack of selection pressure on one copy of the cloned gene (Scannell
521 et al., 2007). The modified function of these redundant genes in the plant genome leads to a change
522 in plant traits such as late flowering time, increased seed number as proved by Guo et al. (2014) in
523 rapeseed, which might cause changes in its associated microbiome. A recent study also showed the
524 effect of ploidy on the composition of the wheat bacterial root and rhizosphere microbiome in a
525 greenhouse experiment however they did not observe the same results in a field experiment (Wipf
526 & Coleman-Derr, 2021). Another similar study by Özkurt et al. (2020) showed that the seed-
527 originated microbiome of roots and leaves of young seedlings were significantly less diverse and
528 inconsistent in domesticated wheat species compared to the wild wheat species.

529 Furthermore, the higher relative proportion of seed-transmitted endosphere microbiome than
530 rhizosphere microbiome indicates the co-evolution of root endophytes with their host plant. The
531 primary factors that lead to co-evolution between wheat species and their endophytes are plant
532 phylogeny (Yeoh et al., 2017) and niche adaptation over many years (Sessitsch et al. 2012; Özkurt
533 et al., 2020). Yeoh et al. (2017) proved the role of plant phylogeny and its co-adapted microbiome
534 in shaping the root-associated microbiome of lycopods, ferns, gymnosperms, and angiosperms.
535 Moreover, in our previous study, we also found phylogenetic congruence between seed endophytes
536 and their host plants (Abdullaeva et al., 2021). One of the interesting findings of this study, where
537 we found different beta-diversity of both, bacterial and fungal microbiome in the endorhiza, not in

538 the rhizosphere, of wild and modern wheat genotypes (Fig. S3, S4) which might exhibit microbe-
539 host co-evolution. These results suggest that domestication can affect co-adaptation and
540 relationships that are established over generations. When seed endophytes colonize plant
541 rhizosphere their proportion gets smaller due to the vast array of microbes attracted from the bulk
542 soil to the rhizosphere and the potential co-evolution factor reduces. Therefore, we did not find any
543 difference in diversity between the rhizosphere of wild and domesticated wheat species due to the
544 strong effect of soil on the rhizosphere microbiome.

545 Our experimental design allowed us to observe the effect of domestication on the seed-transmitted
546 bacterial and fungal rhizosphere microbiome of wheat species in different locations. We found a
547 higher relative proportion of seed-transmitted bacterial endosphere, rhizosphere microbiome of
548 diploid wild wheat in GG, and seed-transmitted fungal rhizosphere microbiome in WG (Fig. 3).
549 The observed differences between locations (Fig. 3 a, b) agree with recent work by Walsh et al.
550 (2021) where the variable proportion of seed endophytes to the wheat seedling microbiome was
551 found between different soils. These authors also showed a strong effect of soil on seedling
552 microbiome assembly where dominant microbes are transmitted from seed (Walsh et al., 2021).
553 Moreover, Özkurt et al (2020) observed the seed-transmitted microbiome of seedlings of cultivated
554 and wild wheat species in two different soils and found a strong effect of soil on the seedling
555 microbiome. The effect of location on the rhizosphere is commonly observed in microbiome
556 studies as the rhizosphere directly contacts the soil. In our study, the proportion of seed-transmitted
557 endorhiza fungi, not bacteria significantly varied between locations (Fig. 3 c). Similarly, we found
558 significant changes in the alpha diversity of the only fungal endorhiza microbiome between
559 locations (Fig. S1). These results suggest that soil origin/environment had a greater impact on the
560 fungal population assemblage in the root endosphere and rhizosphere rather than host species. Our
561 results mirror the results from a field study reported by Bonito et al. (2014) on Populus, Quercus,

562 and Pinus trees sampled in three soils originating from field sites, where soil origin was shown to
563 have larger effects than plant genotype on the structuring of both endosphere and rhizosphere
564 fungal communities.

565 The analyses of environmental variables on the rhizosphere microbiome showed that the bacterial
566 and fungal species were significantly affected depending on the ammonium, nitrate content of soil
567 (Fig. 2, Table 2). Indeed, a low concentration of ammonium and nitrate was determined in the
568 sandy soils of GG compared to loamy clay soils of WG and RH area (Fig. S5). These results
569 indicate that the proportion of seed-transmitted microbiome varies depending on soil
570 characteristics. Furthermore, the proportion of seed-transmitted rhizosphere microbiome of wild *A.*
571 *tauschii* can be higher under lack of nitrogen source. Indeed, most of the seed-transmitted bacteria
572 from seed to rhizosphere of *A. tauschii* were the plant growth-promoting bacteria with the ability
573 to fix $N_2$ and enhance mineralization. For example, *Chryseobacterium* carries nod gene *nifH* and
574 its ability of nitrogen fixation was confirmed when inoculated with groundnut (Dhole et al., 2016).
575 *Rhodococcus* harbors a *nodA* gene (Ampomah & Huss-Danell, 2011). *Methylobacterium-*
576 *Methylorubrum*, *Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium* genus belong to the
577 phylogenetic rhizobial branch which functionally conserves nodulation genes (Renier et al., 2008;
578 Sy et al., 2001). *Plantibacter* (Mayer et al., 2019), *Pseudomonas*, *Burkholderia,* and other non-
579 rhizobial endophytic bacteria were found in nodules and aid in nitrogen fixation in particular stress
580 conditions (Martínez-Hidalgo & Hirsch, 2017). Our findings suggest that modern wheat became
581 less effective in making beneficial interactions with its associated microbes to cope with
582 environmental stressors. As shown in a recent paper, there is a downward trend in making
583 beneficial interactions in terms of N mineralization as wheat domesticated: diploid > tetraploid >
584 hexaploid (Spor et al., 2020). Seed-transmitted microbiome-mediated microbial interaction leads
585 to diverse rhizosphere microbiomes as we found in this study (Fig. 6).

We found that the rhizosphere microbiome of *A. tauschi* is more different than other wheat species by comparing differently enriched genera between the rhizosphere microbiome of four wheat species (Fig. 6). First, domestication related changes in the plant genome, such as gene loss, genomic rearrangements, and gene duplications (Doebley et al., 2006; Pont et al., 2019) significantly influenced plant traits (Szoboszlay et al., 2015; Roucou et al., 2018; Spor et al., 2020) that shape microbiome in different habitats of plants. Such as root exudate content, an important plant trait for assembly prosses of the rhizosphere as Mönchgesang et al. (2016) discovered strong variations in root exudate chemistry among *Arabidopsis* accessions depending on genetic characteristics. Moreover, *T. aestivum* gene diversity significantly reduced as a result of subsequent polyploidy events. Its, D genome was found to conserved more trait *loci* than in the A and B subgenomes (Berkman et al., 2013) however, it was also found that D-subgenome can modify 42.8% of alternative splicing patterns (during gene expression, an alternative splicing process allows a single gene to code for numerous proteins) of the A- and B- subgenomes (Yu et al., 2020) meaning that domestication at the hexaploid level had a greater effect on genetic modifications between subgenomes than same processes at the tetraploid level (Lv et al., 2017). This suggests that subsequent polyploidy events lead to the loss of more genetic information to recruit microorganisms from the bulk soil. These findings are in line with previous work in which the effect of plant domestication on the rhizosphere microbiome of different plant genetic groups of maize (*Zea* mays) and they found that greater similarity of microbiome composition between the rhizosphere microbiome of inbred maize varieties and the teosinte than the hybrid lines (Brisson et al., 2019). Furthermore, plant specifically selects microbes from the bulk soil depending on its genotype. Tkacz and colleagues (2020) found the wheat lines crossed with *A. tauschii*, containing wild D genome, were highly colonized specifically by *Glomeromycetes* and Nematoda by testing several wheat species; wild (*A. tauschii*, *T. dicoccoides*), elite (*T. aestivum*, *T. durum*), and hybrid

610 (SHW) wheat lines. Another study found sex plant qualitative traits in 94 winter wheat genotypes
611 that are responsible for this symbiotic interaction with Arbuscular mycorrhizal fungi (Lehnert et
612 al., 2017).

613 Previous studies reported a significant difference between genomic (Haudry et al., 2007; Peleg et
614 al., 2011), phenotypic diversity (Gioia et al., 2015), and rhizosphere microbiome (Özkurt et al.,
615 2020; Spor et al., 2020) between wild *T. dicoccoides* and *T. durum*. In this study, we observed
616 similar bacterial and fungal taxa enriched the rhizosphere of modern *T. durum* and wild relative *T.*
617 *dicoccoides*. The geographical distribution of the wheat population might explain the similarity of
618 microbiome recruitment of wild emmer with modern wheat species (Luo et al., 2007). Growing in
619 a similar region, the environment can lead to the introgression of domesticated wheat genes into
620 the wild wheat genome (Weide, 2015). Durum wheat was found closely related to progenitor
621 species distributed in the eastern Mediterranean (Israel, Cyprus, Palestine, Greece, Syria Lebanon,
622 Turkey, Jordan, and Egypt). The origin of *T. dicoccoides* (Israel) and *T. durum* (Greece) genotypes
623 that were used in this study was from the same region (Table S2). Furthermore, our previous studies
624 showed strong genetic concordance (UPGMA dendrogram) between *T. dicoccoides*, *T. aestivum*
625 and *T. durum* than *A. tauschii* (Abdullaeva et al., 2021).

626 Furthermore, we found microbes specifically enriched under specific plants in a particular location
627 (Fig. 4). For instance, the rhizosphere microbiome composition of wheat species, specially
628 cultivated *T. durum* and its ancestor *T. dicoccoides* were similar in all three locations (Fig. 4),
629 however, the enriched genera were different in each location. These results also suggest that similar
630 plants do recruit potential rhizosphere colonizing bacterial species available in the soil where they
631 grow and may indicate a strong effect of the growing site on the rhizosphere bacterial assembly but
632 modulated by the plant genotype. Results of constrained ordination analyses also showed a

633 significant effect of growing site and its soil parameters on the rhizosphere microbiota composition
634 (Fig. 2) and the results, further supported by the differential abundance test showed that the genera
635 were significantly affected by the factor location (Fig. 4, Fig. S 6, 7). The rhizosphere bacterial and
636 fungal microbiome abundance of the same plant (*A.tauschii*) that grown in three locations were
637 also different (Fig. S 6, 7). Results imply that soil microbes play a pivotal role in determining the
638 rhizosphere microbiota composition, coherently with previously reported studies (Bulgarelli et al.,
639 2012; Lundberg et al., 2012; Wagner et al., 2014).

640

641 **Conclusions**

642 Our findings indicate that the seed-transmitted microbiome of endorhiza and rhizosphere is
643 impacted by crop domestication. We showed polyploidy effect, by finding less relative seed-
644 transformed microbiome in the endorhiza and rhizosphere of tetraploid and hexaploid wheat
645 species, including wild emmer wheat *T. dicoccoides* than the diploid ancestor *A. tauschii*. We
646 further showed the importance of the seed-transmitted microbiome in shaping the rhizosphere
647 microbiome by identifying the members of these seed-borne microbes. Moreover, we showed the
648 strong effect of the environment on the relative proportion of seed-transmitted microbiome as well
649 as rhizosphere microbial recruitment from the bulk soil. This study also provides some notable
650 clues of co-evolution between the host plant and its microbiome during domestication.

651 Assessing how the plant microbiome altered since plant domestication and how this effect varies
652 across locations and plant species can help us predict how the plant microbiome can be modified
653 or manipulated to improve plant health and crop productivity.

654

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662

663 **References**

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970 **Figure legends:**

971

972 **Figure 1.** Similarity and variation among microbial communities within compartments. Unconstrained ordination
973 based on Euclidian distance matrices of bacterial (A) and fungal (B) communities across rhizosphere, root, bulk soil,
974 and seedbed samples collected from wheat species (*A. tauschii, T. aestivum. T. dicoccoides, and T. durum*) in three
975 locations **GG**-Groß-Gerau, **WG**-Weilburger Grenze and **RH**-Rauischholzhausen) and seeds obtained from the gene
976 bank labeled as **Seed**. Euclidian distance calculated from the data transformed to the centered log-ratio. The colors of
977 the dots denote the compartments of the samples: rhizosphere (forest green), root (light green), bulk soil (brown), seed
978 (yellow), and seedbed (gray). The box plots represent the range of distances from the centroid based on Euclidian
979 distance matrices of bacterial (C) and fungal (D) compositions. The black lines in the box plots correspond to median
980 values, and the dots indicate outliers.

981 **Figure 2.** Constrained (canonical) ordination analyses. The effect of environmental variables on bacterial and fungal
982 species in the rhizosphere and bulk soil samples of cereals.

983 **Figure 3.** The relative proportion of seed-transmitted fungal and bacterial endorhiza and rhizosphere microbiome of
984 three locations. Small letters show the significant differences (ANOVA, $p < 0.05$) between the relative proportion of
985 seed-transmitted rhizosphere microbiome of wheat species. The capital letters show the significant difference between
986 the relative proportion of seed-transmitted endorhiza microbiome of wheat species.

987 **Figure 4.** Bacterial genera that were found differently enriched in the rhizosphere of two genetically connected wheat
988 species (wild *A. tauschii* vs modern *T. aestivum;* wild *T. dicoccoides* vs modern *T. durum*) were grown in three research
989 fields (GG-Groß-Gerau, WG-Weilburger Grenze, RH-Rauischholzhausen) as compared to corresponding bulk soil.
990 The differently abundant genera are considered as significant when absolute aldex affect size is bigger than 1. The
991 dark gray color of bars (n=3) indicates genera found in wild relative and light gray indicates modern wheat species.
992 The orange color shows the genera found in genetically connected wheat species.

993 **Figure 5.** Fungal genera that were found differently enriched in the rhizosphere of two genetically connected wheat
994 species (wild *A. tauschii* vs modern *T. aestivum*; wild *T. dicoccoides* vs modern *T. durum*) were grown in three research
995 fields (GG-Groß-Gerau, WG-Weilburger Grenze, RH-Rauischholzhausen) as compared to corresponding bulk soil.
996 The differently abundant genera are considered as significant when absolute aldex affect size is bigger than 1. The

997 dark gray color of bars (n=3) indicates genera found in wild relative and light gray indicates modern wheat species.
998 The orange color shows the genera found in genetically connected wheat species.

999 **Figure 6.** The rhizosphere bacterial microbiome assembly variation between wheat species (*A. tauschii*, *T. aestivum*,
1000 *T. dicoccoides*, and *T. durum*) grown in the same site (WG). The graph was created based on differential abundance
1001 analysis of core microbiome bacterial genera of rhizosphere soil. The significantly prevalent genera were identified by
1002 looking at aldex effect size table generated by ALDEx2. The differently abundant genera are considered as significant
1003 when absolute aldex affect size bigger than 1 or lower than -1. More bars show higher differences and fewer bars
1004 explain more similarity between two wheat species.

1005 **Figure 7.** The rhizosphere fungal microbiome assembly variation between wheat species (*A. tauschii*, *T. aestivum*, T.
1006 *dicoccoides*, and *T. durum*) grown in the same site (WG). The graph was created on differential abundance analysis of
1007 core microbiome bacterial genera of rhizosphere soil. The significantly prevalent genera were identified by looking at
1008 aldex effect size table generated by ALDEx2. The differently abundant genera are considered as significant when
1009 absolute aldex affect size bigger than 1 or lower than -1.

Figure 1.JPEG

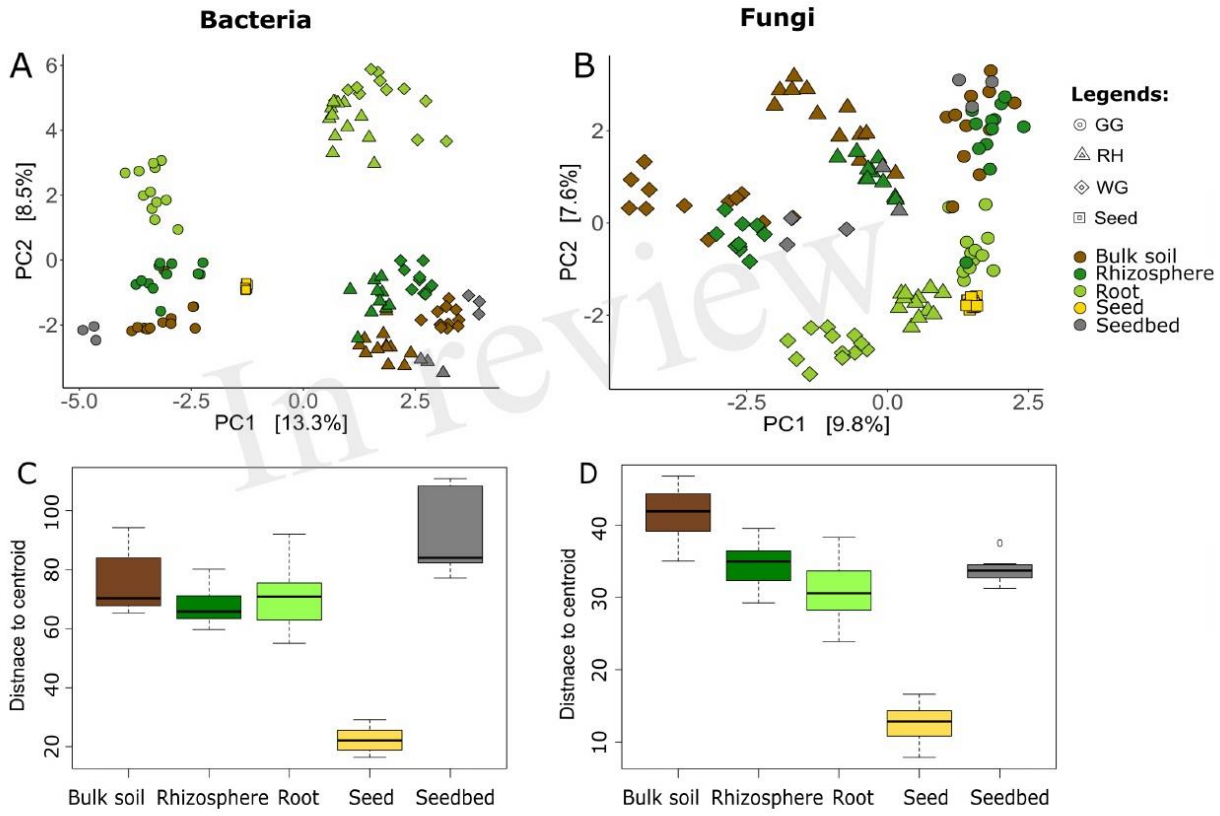


Figure 2.JPEG

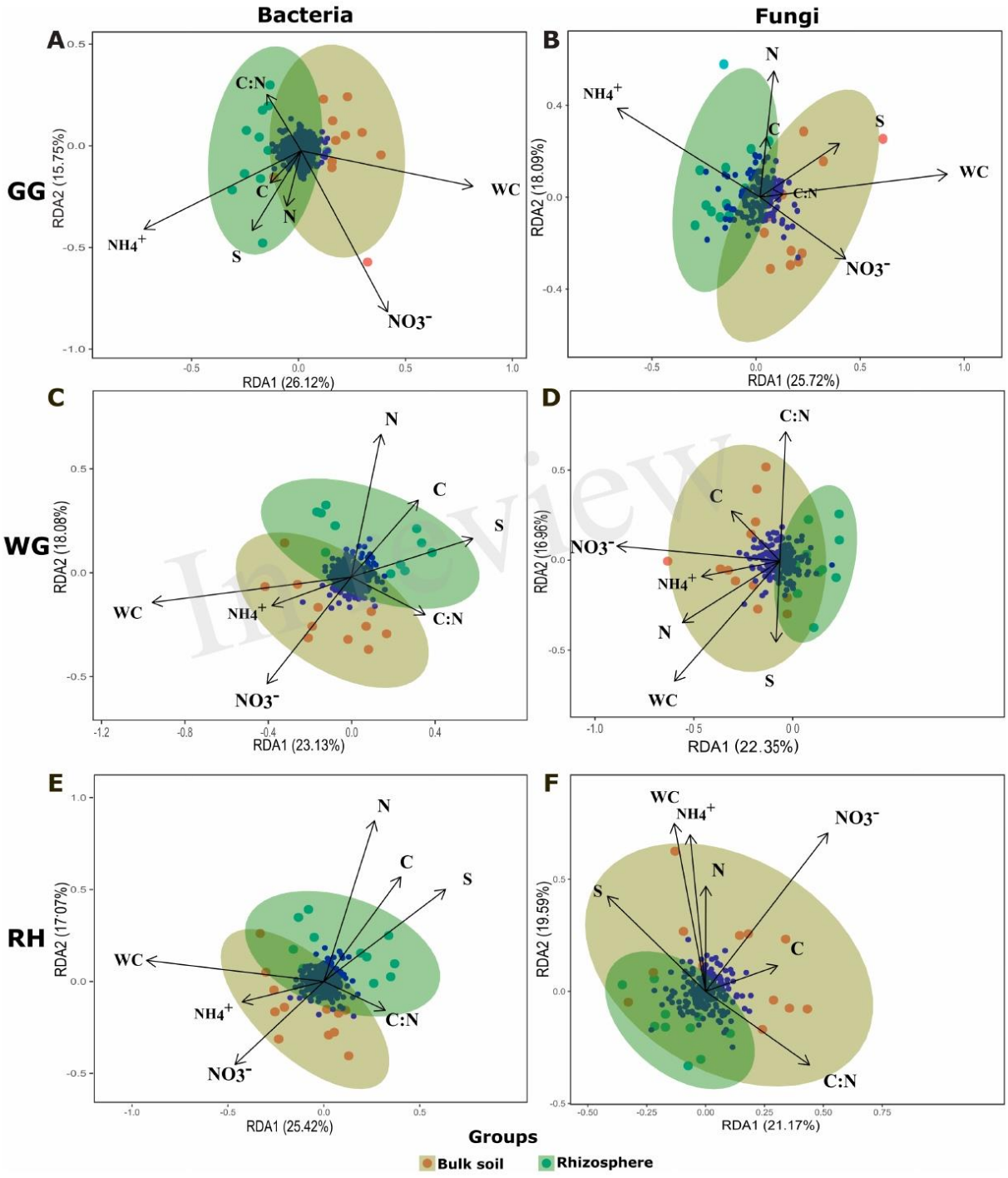
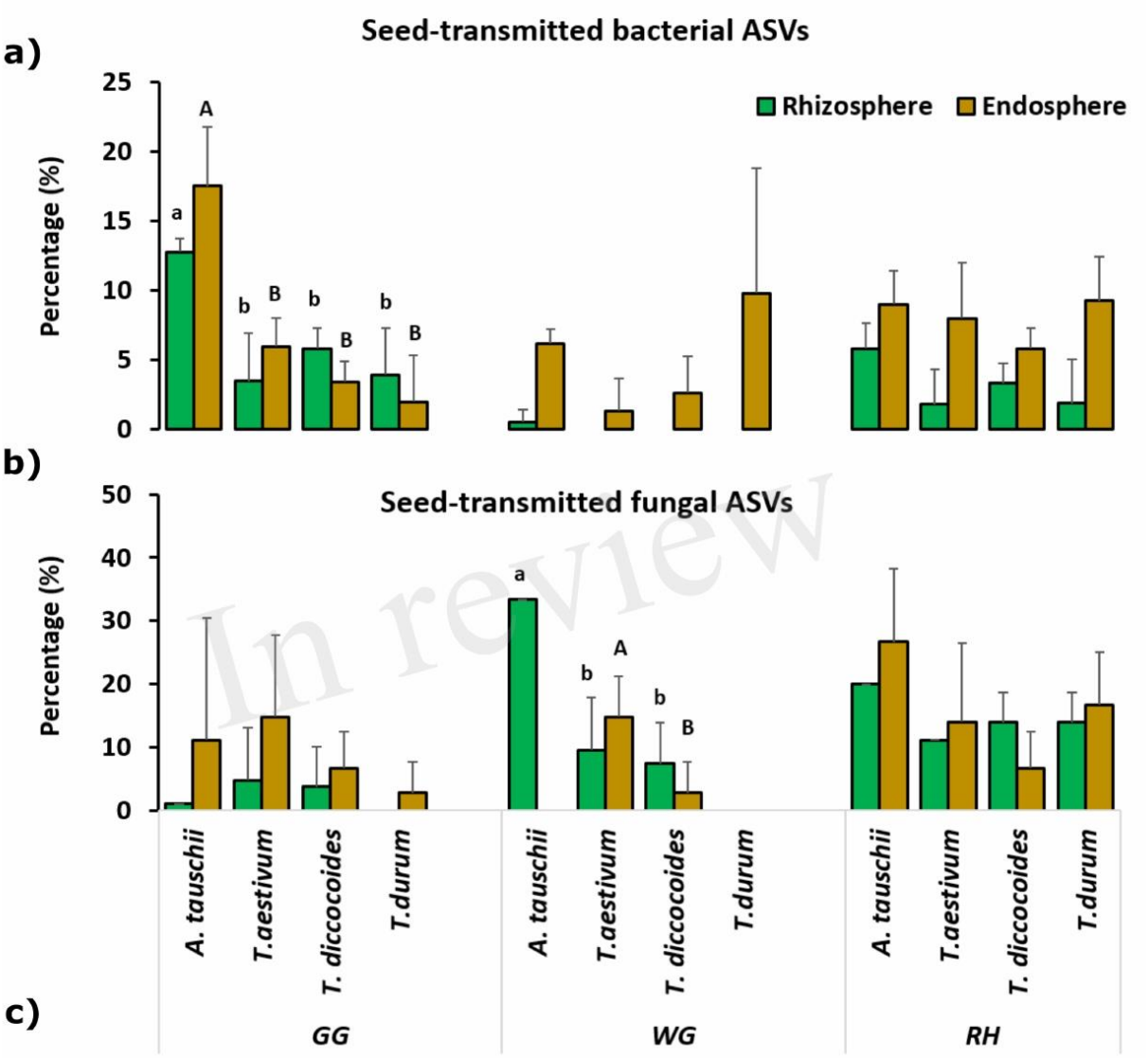


Figure 3.JPEG




c)

| Dataset | | Tukey's HSD test | | | ANOVA |
|---|---|---|---|---|---|
| | | GG | WG | RH | *p* - value |
| Bacteria | Rhizosphere | a | b | c | **5.93e-05 *** ** |
| | Endosphere | a | a | a | 0.361 |
| Fungi | Rhizosphere | b | a | a | **0.00331 ** ** |
| | Endosphere | ab | b | a | **0.028 * ** |

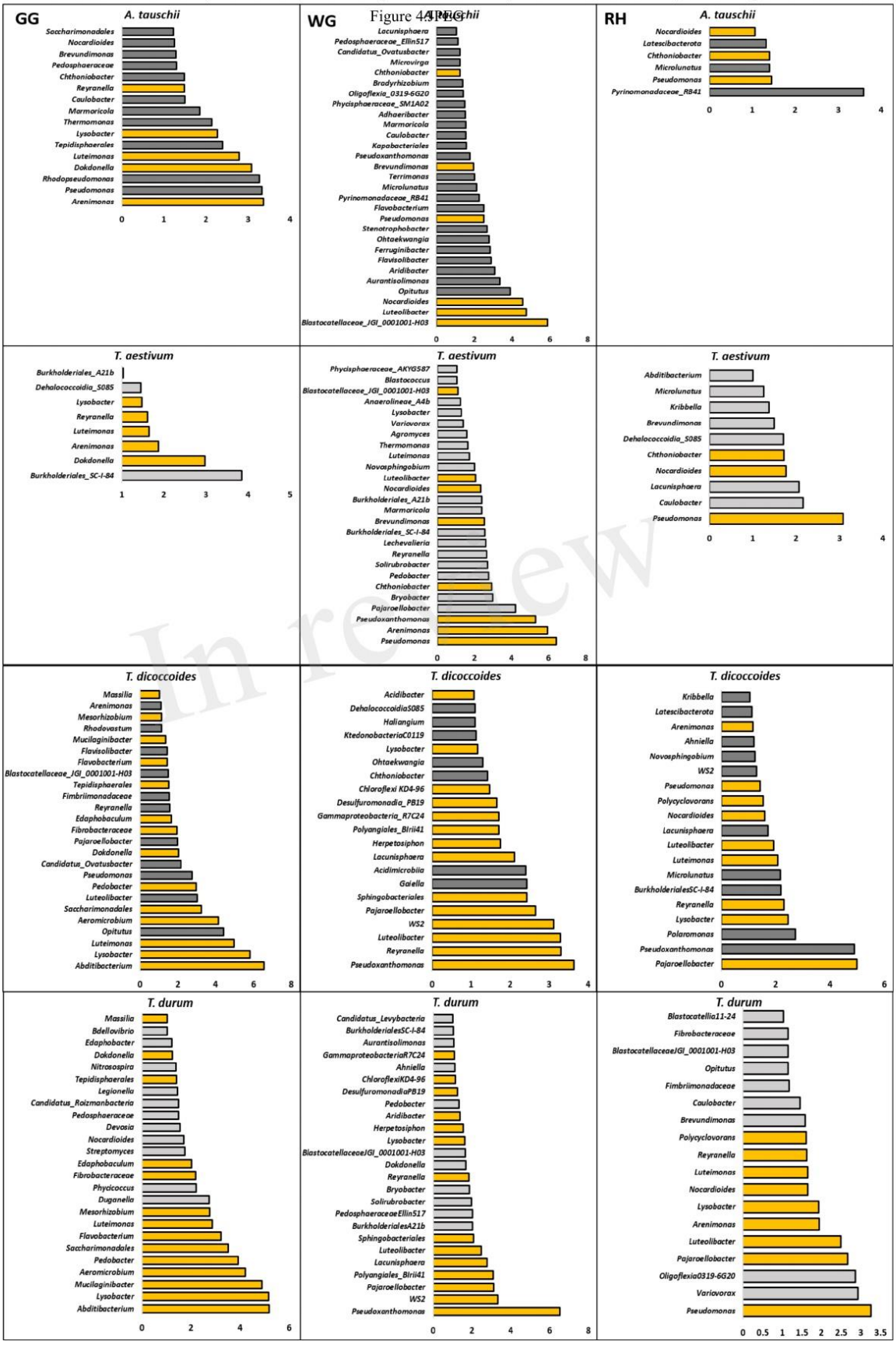



Figure 4.3 HG

Figure 5.JPEG

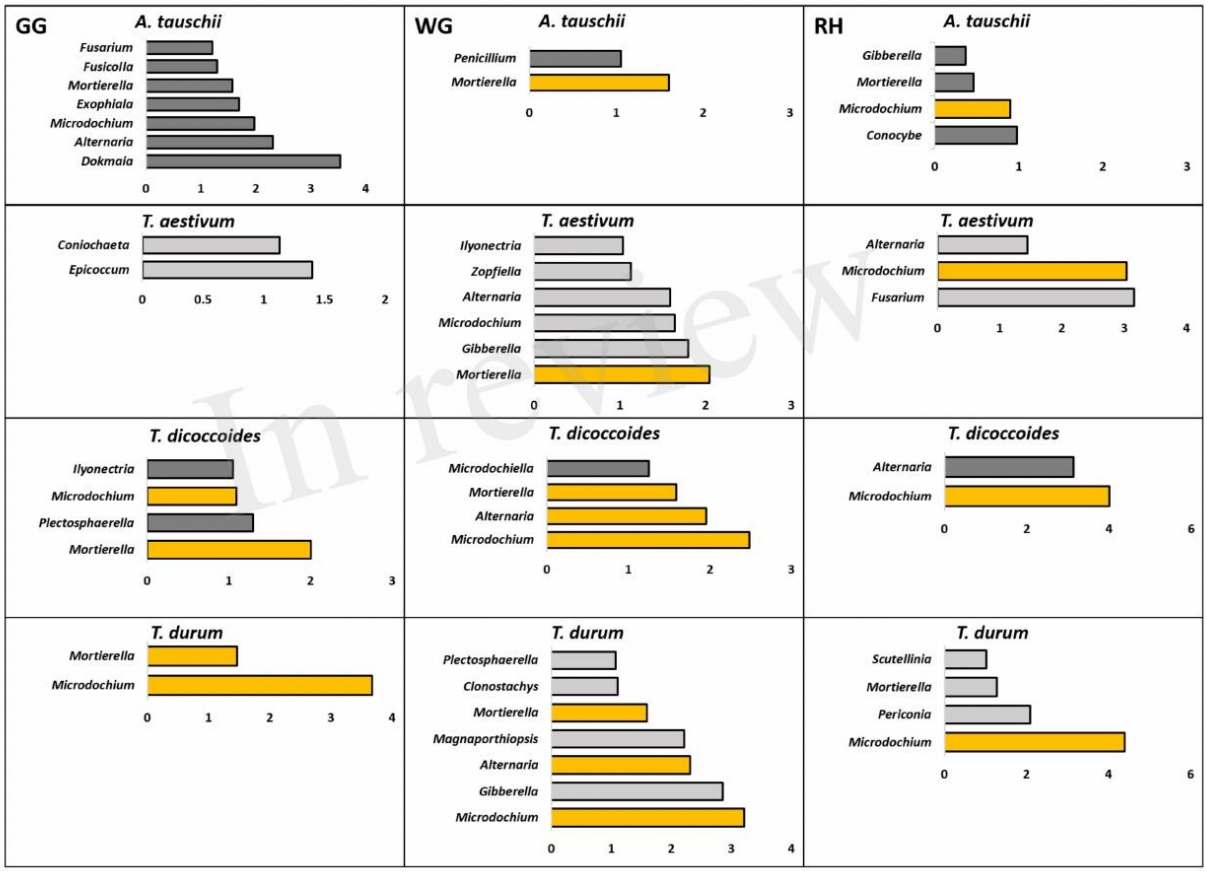


Figure 6. JPEG

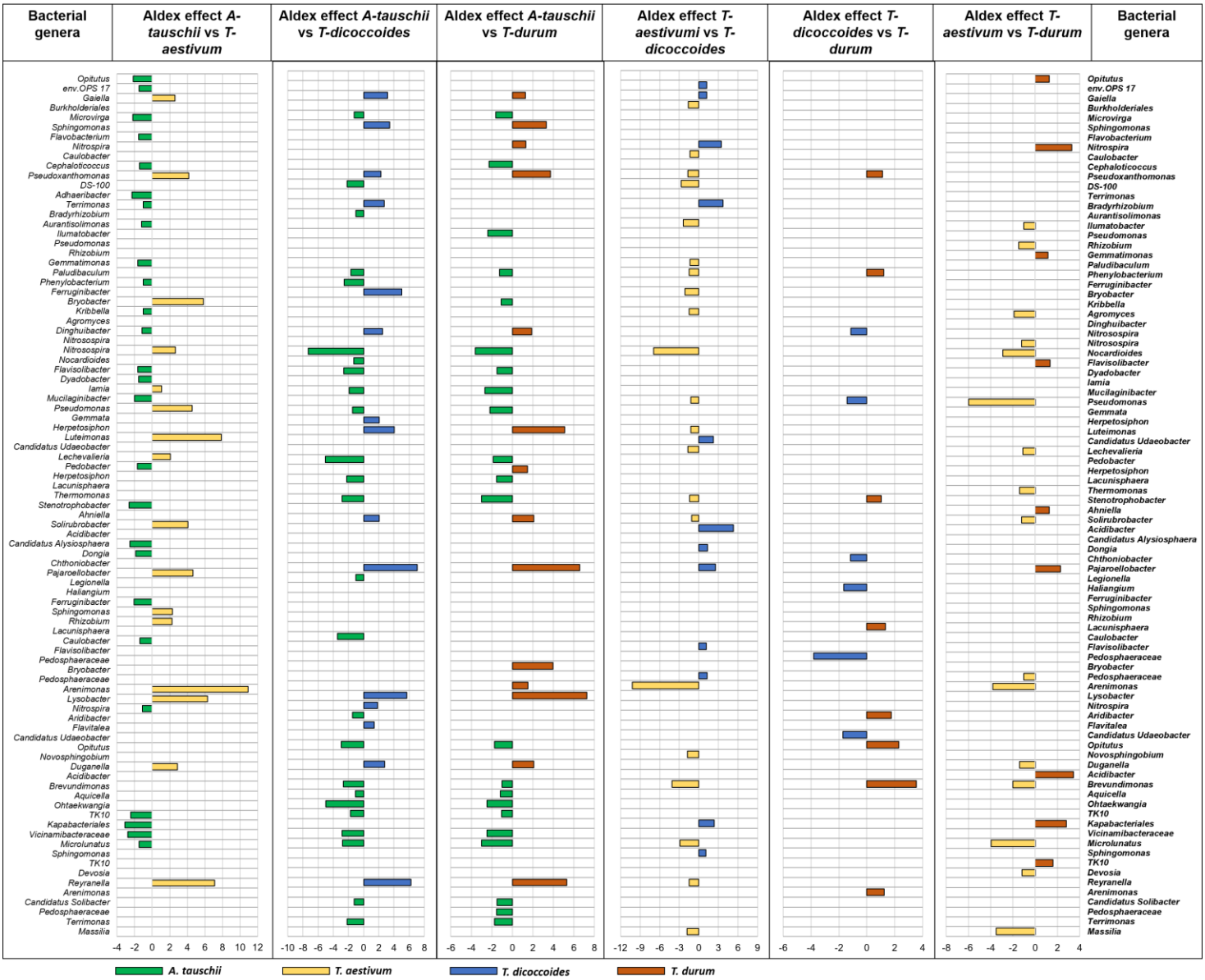
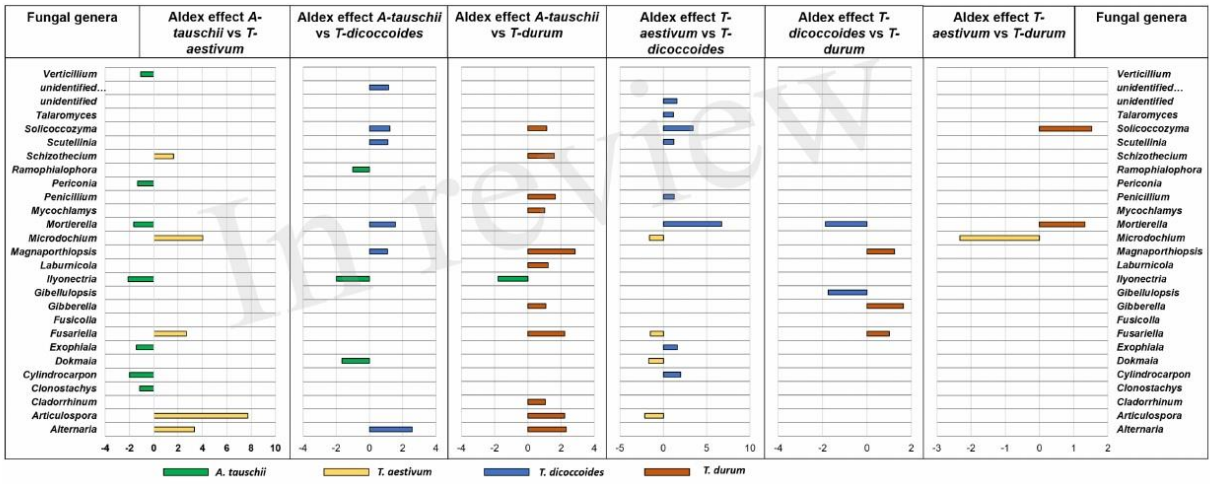


Figure 7.JPEG



*Supplementary material to:*

# Domestication impacts the cereal rhizosphere colonization by seed- and soil-originated microbiomes

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- **Table S1.** Sampling design, sampling locations (GG-Groß-Gerau, WG-Weilburger Grenze, and RH- Rauischholzhausen) and frequency of sampling for different habitats.
- **Table S2.** Origin and biological status of the seed accessions used in this work.
- **Table S3**. Kruskal-Wallis rank-sum test results. Significant differences in microbial communities richness and diversity between groups and experimental factors within datasets were detected
- **Table S4.** Permutational multivariate analysis of variance (ADONIS) test results. The numbers indicate the ADONIS statistic (r) value, with significance as indicated ( < 0.001 '***', < 0.01 '**', < 0.05 '*', < 0.1 '.' ). Significant differences in microbial communities composition between groups and experimental factors within datasets were detected.

- **Table S5.** Composition of seed-transmitted endorhiza and rhizosphere bacterial and fungal in the three locations (GG, WG, RH)
- **Figure S1.** Observed richness and diversity (Shannon's index and Simpson indexes) of bacterial (A) and fungal (B) microbiota of different habitats (root endosphere, rhizosphere, bulk soil) between locations: yellow – Gross-Gerau (GG), green – Rauischholzhausen (RH), and brown – Weilburger Grenze (WG). Box plots show the range of variation in the median values (black lines in the middle), and the dots indicate outliers. Asterisks indicate significant differences between locations ($^{***} = p < 0.001$; $^{**} = p < 0.01$; $^{*} = p < 0.05$).
- **Figure S2.** Bacterial (A) and fungal (B) taxa distribution in different compartments of wheat cultivars grown in three locations. Relative abundance of 95% predominant bacterial and fungal phyla and 99% predominant genera. Each bar represents 9 samples (3xGG, 3xWG, 3xRH).
- **Figure S3.** Bacterial beta diversity in different compartments and locations. Unconstrained ordination based on Euclidian distance matrices of bacterial communities across the root, rhizosphere, and bulk soil samples collected from wild and domesticated wheat species (*A. tauschii, T. aestivum, T. dicoccoides*, and *T. durum*) in three locations (GG-Groß-Gerau, WG-Weilburger Grenze, and RH-Rauischholzhausen). Euclidian distances calculated from the data were transformed to the centered log-ratio.
- **Figure S4.** Fungal beta diversity in different compartments and locations. Unconstrained ordination based on Euclidian distance matrices of bacterial communities across the root, rhizosphere, and bulk soil samples collected from wild and domesticated wheat species (*A.*

*tauschii*, *T. aestivum*, *T. dicoccoides*, and *T. durum*) in three locations (GG-Groß-Gerau, WG-Weilburger Grenze, and RH-Rauischholzhausen). Euclidian distances calculated from the data were transformed to the centered log-ratio.

- **Figure S5.** Soil properties. Ammonium ($NH_4^+$), nitrate ($NO_3^-$), nitrogen (N), and carbon (C) of rhizosphere and bulk soil (n=12). Soil samples collected from three locations Weilburger Grenze (WG), Groß-Gerau (GG), and Rauischholzhausen (RH). One-way ANOVA results. All parameters ($NO_3^-$, $p$ = 0.000035, $NH_4^+$, $p$ = 0.0195, N, $p$ = 0.0000009, C, $p$ = 0.0632) significantly differed between locations (n = 12). The bars represent the mean values of 3 replicates of each wheat species, error bars show standard deviation. Significance values between rhizosphere and bulk soil in each location are represented on the right corner of each barplot.
- **Figure S6.** The bacterial structure variation between locations (GG-Groß-Gerau, WG-Weilburger Grenze, RH-Rauischholzhausen) based on differential abundance analysis of core microbiome bacterial genera of rhizosphere (*A. tauschii*). The significantly prevalent genera were identified by looking at aldex effect size. The differently abundant genera are considered as significant absolute aldex affect size bigger than 1 or lower than -1.
- **Figure S7.** The fungal structure variation between locations (GG-Groß-Gerau, WG-Weilburger Grenze, RH-Rauischholzhausen) based on differential abundance analysis of core microbiome bacterial genera of rhizosphere (*A. tauschii*). The significantly prevalent genera were identified by looking at aldex effect size. The differently abundant genera are considered as significant absolute aldex affect size bigger than 1 or lower than -1.
- **Figure S8.** Taxa distribution of seedbed soil. Relative abundance of 99% predominant bacteria phyla (A) and genera (B) in all samples and 95% predominant fungal phyla (C) and genera (D) (n=9).
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**Table-S1** Sampling design, sampling locations (GG-Groß-Gerau, WG-Weilburger Grenze, and RH- Rauisch-holzhausen) and frequency of sampling for different habitats.

| Species | Locations | Accession number | Roots | Rhizosphere | Bulk soil | Seedbed |
|---|---|---|---|---|---|---|
| ***T. diccocoides*** | GG | TRI 18524 | 3 | 3 | 3 | 3 |
| | WG | TRI 18524 | 3 | 3 | 3 | 3 |
| | RH | TRI 18524 | 3 | 3 | 3 | 3 |
| ***T. durum*** | GG | TRI 10715 | 3 | 3 | 3 | |
| | WG | TRI 10715 | 3 | 3 | 3 | |
| | RH | TRI 10715 | 3 | 3 | 3 | |
| ***T. aestivum*** | GG | TRI 368 | 3 | 3 | 3 | |
| | WG | TRI 368 | 3 | 3 | 3 | |
| | RH | TRI 368 | 3 | 3 | 3 | |
| ***A. tauschii*** | GG | AE 220 | 3 | 3 | 3 | |
| | WG | AE 220 | 3 | 3 | 3 | |
| | RH | AE 220 | 3 | 3 | 3 | |

**Table S2**. Origin and biological status of the seed accessions used in this work.

| Scientific name of the accession | Biological status | IPK* Accession number |
|---|---|---|
| *Aegilops tauschii* Coss. subsp. *tauschii* var. *meyeri* (Griseb.) Tzvelev | wild | AE 220 |
| *Triticum aestivum* L. var. *aestivum* | cultivar | TRI 368 |
| *Triticum dicoccoides (*Körn. ex Asch. & Graebn.) Schweinf. convar. *dicoccoides* var.*dicoccoides* | wild | TRI 18524 |
| *Triticum durum Desf.* subsp. *durum* convar. *durum* subconvar. *durum* var. *affine* Körn. | cultivar | TRI 10715 |

* Leibniz Institute of Plant Genetics and Crop Plant Research (IPK)

| **Table 3** | **Kruskal-Wallis rank-sum test results. Significant differences in microbial communities richness and diversity between groups and experimental factors within datasets were detected** | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | ***Observed richness*** | | | | | | ***Simpson*** | | | | | | ***Shannon*** | | | | | |
| | | ***Bacteria*** | | | ***Fungi*** | | | ***Bacteria*** | | | ***Fungi*** | | | ***Bacteria*** | | | ***Fungi*** | | |
| ***Dataset*** | ***Factor*** | chi-squared | df | *p* value | chi-squared | df | *p* value | chi-squared | df | *p* value | chi-squared | df | *p* value | chi-squared | df | *p* value | chi-squared | df | *p* value |
| **Rhizosphere** | **location** | 2.56 | 2 | 0.2777 | 11.08 | 3 | **0.003914** | 0.618 | 2 | 0.734 | 15.21 | 2 | **0.0005** | 2.31 | 2 | 0.308 | 13.12 | 2 | **0.00141** |
| **Root endophytes** | **location** | 5.8 | 2 | **0.054** | 13.53 | 3 | **0.001154** | 8.14 | 2 | **0.017** | 6.44 | 2 | **0.0399** | 6.18 | 2 | **0.045** | 15.12 | 2 | **0.000519** |
| **Bulk soil** | **location** | 1.35 | 2 | 0.704 | 7.55 | 3 | **0.02286** | 7.58 | 2 | 0.37 | 13.98 | 2 | **0.00092** | 14.7 | 2 | 0.547 | 15.12 | 2 | **0.000519** |
| **Seed** | **species** | 7.43 | 3 | 0.703 | 1.74 | 3 | 0.6264 | 4.04 | 3 | 0.237 | 3.59 | 3 | 0.3087 | 1.85 | 3 | 0.263 | 1.76 | 3 | 0.6224 |
| | **ploidy** | 2.41 | 1 | 0.497 | 0.76 | 1 | 0.3813 | 2.36 | 1 | 0.166 | 0.025 | 1 | 0.87 | 3.26 | 1 | 0.201 | 0.28 | 1 | 0.5967 |
| | **form** | 7.98 | 2 | 0.448 | 1.39 | 3 | 0.49 | 5.33 | 2 | 0.226 | 3.58 | 2 | 0.16 | 1.44 | 2 | 0.173 | 1.53 | 2 | 0.4643 |
| **Seedbed** | **location** | 5.42 | 2 | 0.06 | 6.25 | 3 | **0.043** | 5.42 | 2 | 0.06 | 0.47 | 3 | 0.7897 | 1.18 | 2 | 0.39 | 1.8 | 3 | 0.4054 |
| **Groß-Gerau** | **plant habitat** | 13.58 | 2 | **0.0011** | 13.09 | 3 | **0.001437** | 25.98 | 2 | **$2.27\ 10^{-6}$** | 15.12 | 2 | **0.000256** | 23.87 | 2 | **$6.70\ 10^{-6}$** | 14.15 | 2 | **0.000844** |
| **GG_rhizosphere** | **species** | 2.58 | 3 | 0.45 | 1.65 | 3 | 0.6478 | 4.23 | 1 | 0.23 | 1.106 | 3 | 0.775 | 3.3 | 3 | 0.34 | 1.77 | 3 | 0.62 |
| | **form** | 0.92 | 1 | 0.33 | 0 | 1 | 1 | 0.02 | 1 | 0.87 | 0.533 | 1 | 0.4652 | 0.1 | 1 | 0.74 | 0.133 | 1 | 0.715 |
| **GG_bulk soil** | **species** | 8.74 | 3 | **0.03** | 0.619 | 3 | 0.8919 | 6.84 | 1 | 0.07 | 0.743 | 3 | 0.862 | 9.35 | 3 | **0.02** | 3.307 | 3 | 0.346 |
| | **form** | 0.02 | 1 | 0.87 | 0.104 | 1 | 0.74 | 1.25 | 1 | 0.26 | 0.23 | 1 | 0.631 | 0.41 | 1 | 0.52 | 1.641 | 1 | 0.2002 |
| **GG_root endosphere** | **species** | 5.61 | 3 | 0.13 | 2.28 | 3 | 0.51 | 6.84 | 1 | 0.07 | 5.61 | 3 | 0.131 | 7.82 | 3 | **0.04** | 6.69 | 3 | 0.08238 |
| | **form** | 4.33 | 1 | **0.03** | 0.1 | 1 | 0.7488 | 4.33 | 1 | **0.03** | 1.25 | 1 | 0.2623 | 5.76 | 1 | **0.01** | 1.25 | 1 | 0.2623 |
| **Weilburger Grenze** | **plant habitat** | 1.27 | 2 | 0.52 | 20.91 | 2 | **$2.88\ 10^{-5}$** | 23.55 | 2 | **$7.69\ 10^{-6}$** | 24.69 | 2 | **$4.33\ 10^{-6}$** | 17.69 | 2 | **$1.44\ 10^{-4}$** | 25.03 | 2 | **$3.67\ 10^{-6}$** |
| **WG_rhizosphere** | **species** | 5.28 | 3 | 0.15 | 3.82 | 3 | 0.2813 | 7.95 | 3 | **0.046** | 2.37 | 3 | 0.4976 | 5.4 | 3 | 0.144 | 3.39 | 3 | 0.3348 |
| | **form** | 4.03 | 1 | **0.044** | 0.134 | 1 | 0.7138 | 7.5 | 1 | **0.006** | 2.13 | 1 | 0.1441 | 4.8 | 1 | **0.028** | 0.833 | 1 | 0.3613 |
| **WG_bulk soil** | **species** | 1.86 | 3 | 0.6 | 4.43 | 3 | 0.2181 | 8.07 | 3 | **0.04** | 2.12 | 3 | 0.5462 | 0.74 | 3 | 0.86 | 4.58 | 3 | 0.2044 |
| | **form** | 0.16 | 1 | 0.68 | 0.102 | 1 | 0.7488 | 0.02 | 1 | 0.87 | 0.23 | 1 | 0.631 | 0.41 | 1 | 0.52 | 0.025 | 1 | 0.8728 |
| **WG_root endosphere** | **species** | 6.64 | 3 | 0.084 | 1.99 | 3 | 0.5736 | 7.2 | 3 | 0.065 | 5.35 | 3 | 0.1473 | 6.69 | 3 | 0.08 | 6.38 | 3 | 0.09433 |
| | **form** | 0.64 | 1 | 0.42 | 0.025 | 1 | 0.8726 | 0.92 | 1 | 0.33 | 0.41 | 1 | 0.5218 | 0.025 | 1 | 0.87 | 0.102 | 1 | 0.7488 |
| **Rauischholzhausen** | **plant habitat** | 7.34 | 2 | **0.02** | 21.82 | 2 | **$1.83\ 10^{-5}$** | 24.03 | 2 | **$6.03\ 10^{-6}$** | 17.89 | 2 | **$1.30\ 10^{-4}$** | 23.59 | 2 | **$7.51\ 10^{-6}$** | 22.34 | 2 | **$1.40\ 10^{-5}$** |
| **RH_rhizosphere** | **species** | 1.04 | 3 | 0.79 | 4.09 | 3 | 0.2511 | 1.03 | 3 | 0.79 | 1.28 | 3 | 0.732 | 0.92 | 3 | 0.81 | 0.78 | 3 | 0.8524 |
| | **form** | 0.13 | 1 | 0.7 | 1.64 | 1 | 0.1992 | 0.033 | 1 | 0.85 | 0.833 | 1 | 0.3613 | 0.3 | 1 | 0.58 | 0.533 | 1 | 0.4652 |
| **RH_bulk soil** | **species** | 7.8 | 3 | **0.04** | 7.05 | 3 | 0.07028 | 0.23 | 3 | 0.97 | 1.87 | 3 | 0.5994 | 5.2 | 3 | 0.15 | 5.97 | 3 | 0.1129 |
| | **form** | 1.64 | 1 | 0.2 | 4.33 | 1 | **0.03737** | 0.1 | 1 | 0.74 | 0.02 | 1 | 0.8728 | 0.64 | 1 | 0.42 | 1.641 | 1 | 0.2002 |
| **RH_root endosphere** | **species** | 4.23 | 3 | 0.23 | 7.8 | 3 | **0.05012** | 3.2 | 3 | 0.36 | 3.82 | 3 | 0.2815 | 3.3 | 3 | 0.34 | 5.76 | 3 | 0.1234 |
| | **form** | 2.56 | 1 | 0.1 | 4.34 | 1 | **0.03704** | 1.64 | 1 | 0.2 | 0.923 | 1 | 0.3367 | 1.64 | 1 | 0.2 | 2.56 | 1 | 0.109 |

**Table S4.** Permutational multivariate analysis of variance (ADONIS) test results. The numbers indicate the ADONIS statistic (r) value, with significance as indicated ( < 0.001 '***', < 0.01 '**', < 0.05 '*', < 0.1 '.'). Significant differences in microbial communities composition between groups and experimental factors within datasets were detected.

| Dataset | | **Bacteria** | | | **Fungi** | | |
|---|---|---|---|---|---|---|---|
| **Rhizosphere** | Factor | Df | $R^2$ | Pr(>F) | Df | $R^2$ | Pr(>F) |
| | species | 3 | 0.10017 | 0.173 | 3 | 0.08019 | 0.741 |
| | location | 2 | 0.29548 | **0.001 *** ** | 2 | 0.26305 | **0.001 *** ** |
| | form | 1 | 0.02379 | 0.75 | 1 | 0.02604 | 0.632 |
| **Bulk soil** | | | | | | | |
| | species | 3 | 0.08598 | 0.413 | 3 | 0.08571 | 0.552 |
| | location | 2 | 0.28887 | **0.001 *** ** | 2 | 0.2126 | **0.001 *** ** |
| | form | 1 | 0.02113 | 0.942 | 1 | 0.02762 | 0.542 |
| **Root** | | | | | | | |
| | species | 3 | 0.08722 | 0.385 | 3 | 0.08877 | 0.313 |
| | location | 2 | 0.31587 | **0.001 *** ** | 2 | 0.2216 | **0.001 *** ** |
| | form | 1 | 0.03324 | 0.22 | 1 | 0.02802 | 0.435 |
| **Seed** | | | | | | | |
| | species | 3 | 0.24011 | **0.001 *** ** | 3 | 0.22548 | **0.001 *** ** |
| | form | 1 | 0.09934 | **0.001 *** ** | 1 | 0.06863 | **0.087 .** |
| | genome | 2 | 0.17229 | **0.001 *** ** | 2 | 0.15615 | **0.002 ** ** |
| | varieties | 5 | 0.3918 | **0.007 ** ** | 5 | 0.30893 | **0.046 * ** |
| | | | | | | | |
| **Seedbed** | location | 2 | 0.55212 | **0.005 ** ** | 2 | 0.47079 | **0.008 ** ** |
| **Separately tested by location** | | | | | | | |
| **GG** | species | 3 | 0.11019 | **0.012 * ** | 3 | 0.10794 | **0.019 * ** |
| | form | 1 | 0.02927 | 0.334 | 1 | 0.03086 | 0.288 |
| | sample.source | 2 | 0.17392 | **0.001 *** ** | 2 | 0.1697 | **0.001 *** ** |
| | | | | | | | |
| **GG_rhizosphere** | species | 3 | 0.35878 | **0.003 ** ** | 3 | 0.33509 | **0.01 ** ** |
| | form | 1 | 0.09344 | 0.275 | 1 | 0.10538 | 0.141 |
| | | | | | | | |
| **GG_bulk soil** | species | 3 | 0.31338 | **0.001 *** ** | 3 | 0.31115 | 0.209 |
| | form | 1 | 0.09256 | 0.349 | 1 | 0.10363 | 0.348 |
| | | | | | | | |
| **GG_root endosphere** | species | 3 | 0.34748 | **0.001 *** ** | 3 | 0.33522 | **0.008 ** ** |
| | form | 1 | 0.12361 | **0.021 * ** | 1 | 0.09675 | 0.289 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **WG** | species | 3 | 0.12302 | **0.012 *** | 3 | 0.10233 | **0.065 .** |
| | form | 1 | 0.03252 | 0.201 | 1 | 0.03223 | 0.203 |
| | sample.source | 2 | 0.24235 | **0.001 ***** | 2 | 0.19663 | **0.001 ***** |
| | | | | | | | |
| **WG_rhizosphere** | species | 3 | 0.40588 | **0.001 ***** | 3 | 0.32668 | **0.057 .** |
| | form | 1 | 0.10027 | 0.219 | 1 | 0.10845 | 0.208 |
| | | | | | | | |
| **WG_bulk soil** | species | 3 | 0.37805 | **0.001 ***** | 3 | 0.30812 | **0.006 **** |
| | form | 1 | 0.08784 | 0.484 | 1 | 0.09565 | 0.187 |
| | | | | | | | |
| **WG_root endosphere** | species | 3 | 0.35913 | **0.001 ***** | 3 | 0.3134 | **0.001 ***** |
| | form | 1 | 0.13124 | **0.006 **** | 1 | 0.10804 | **0.032 *** |
| | | | | | | | |
| **RH** | species | 3 | 0.0962 | 0.213 | 3 | 0.0807 | 0.68 |
| | form | 1 | 0.02933 | 0.36 | 1 | 0.03063 | 0.257 |
| | sample.source | 2 | 0.22413 | **0.001 ***** | 2 | 0.18447 | **0.001 ***** |
| | | | | | | | |
| **RH_rhizosphere** | species | 3 | 0.34397 | **0.006 **** | 3 | 0.29179 | **0.065 .** |
| | form | 1 | 0.09643 | 0.533 | 1 | 0.1023 | 0.106 |
| | | | | | | | |
| **RH_bulk soil** | species | 3 | 0.30724 | **0.012 *** | 3 | 0.27236 | 0.518 |
| | form | 1 | 0.0898 | 0.436 | 1 | 0.0999 | **0.092 .** |
| | | | | | | | |
| **RH_root endopshere** | species | 3 | 0.3149 | **0.014 *** | 3 | 0.31134 | **0.007 **** |
| | form | 1 | 0.12608 | **0.01 **** | 1 | 0.10876 | **0.047 *** |

| Table S5 | Composition of seed-transmitted bacterial and fungal genera | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Habitat | location | *A. tauschii* | | *T. aestivum* | | *T. dicoccoides* | | *T. durum* | |
| | | Bacteria | Fungi | Bacteria | Fungi | Bacteria | Fungi | Bacteria | Fungi |
| Rhizosphere | GG | *Verticiella*<br>*Chryseobacterium*<br>*Rhodococcus*<br>*Pseudomonas*<br>*Stenotrophomonas*<br>*Plantibacter*<br>*Methylobacterium-Methylorubrum*<br>*Luteibacter*<br>*Massilia* | | *Pedobacter*<br>*Brevundimonas* | *Stemphylium* | *Brevundimonas*<br>*Stenotrophomonas*<br>*Sphingomonas*<br>*Pseudomonas* | *Pyrenophora*<br>*Neoascochyta* | *Streptococcus*<br>*Ralstonia* | |
| | WG | *Aeromicrobium* | *Ulocladium* | *none* | *Ulocladium* | *none* | *Ulocladium* | *none* | |
| | RH | *Methylobacterium-Methylorubrum*<br>*Cutibacterium*<br>*Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium*<br>*Nocardioides*<br>*Massilia* | *Alternaria* | *Brevundimonas* | *Alternaria* | *Pseudomonas*<br>*Cutibacterium*<br><br><br>*Symbiobacterium* | *Alternaria*<br>*Pyrenophora*<br><br><br>*Neoascochyta* | *Pseudomonas*<br>*Streptococcus* | *Alternaria* |
| Endorhiza | GG | *Rhodococcus*<br><br>*Enterobacteriaceae*<br>*Chryseobacterium*<br>*Verticiella*<br>*Pseudomonas*<br>*Stenotrophomonas*<br>*Massilia*<br>*Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium*<br>*Nocardioides*<br>*Methylobacterium-Methylorubrum*<br>*Luteibacter*<br>*Duganella*<br>*Comamonadaceae* | *unknown fungi* | *Brevundimonas*<br><br>*Pedobacter* | *Stemphylium*<br><br>*unknown fungi* | *Sphingomonas*<br><br>*Pseudomonas* | *unknown fungi* | *Pseudomonas* | *unknown fungi* |
| | WG | *Methylobacterium-Methylorubrum*<br><br>*Plantibacter*<br>*Cutibacterium*<br>*Aeromicrobium*<br>*Stenotrophomonas*<br>*Massilia* | *unknown fungi* | *Cutibacterium* | *unknown fungi* | *Sphingomonas*<br><br>*Cutibacterium* | | *Streptococcus*<br>*Methylobacterium-Methylorubrum*<br>*Cutibacterium* | |
| | RH | *Pseudomonas*<br><br>*Plantibacter*<br>*Verticiella*<br>*Cutibacterium*<br><br><br>*Comamonadaceae*<br>*Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium*<br>*Massilia* | *Alternaria*<br>*unknown fungi* | *Duganella*<br><br>*Massilia*<br>*Brevundimonas*<br>*Cutibacterium*<br><br>*Symbiobacterium* | *Alternaria*<br><br>*unknown fungi* | *Symbiobacterium*<br><br>*Cutibacterium*<br>*Stenotrophomonas*<br>*Pseudomonas*<br><br>*Sphingomonas* | *Neoascochyta*<br><br>*Alternaria* | *Streptococcus*<br><br>*Pseudomonas*<br>*Cutibacterium* | *Alternaria*<br>*unknown fungi* |

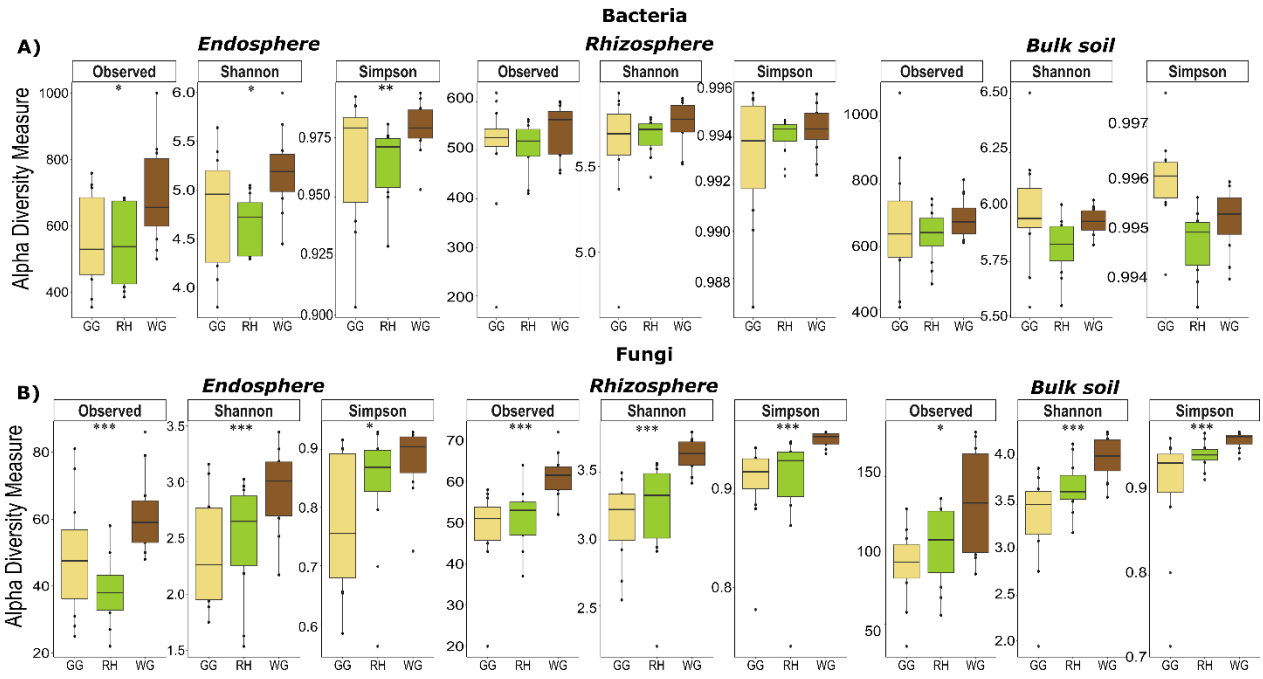


**Figure S1.** Observed richness and diversity (Shannon's index and Simpson indexes) of bacterial (A) and fungal (B) microbiota of different habitats (root endosphere, rhizosphere, bulk soil) between locations: yellow – Gross-Gerau (GG), green – Rauischholzhausen (RH), and brown – Weilburger Grenze (WG). Box plots show the range of variation in the median values (black lines in the middle), and the dots indicate outliers. Asterisks indicate significant differences between locations (*** = $p < 0.001$; ** = $p < 0.01$; * = $p < 0.05$).

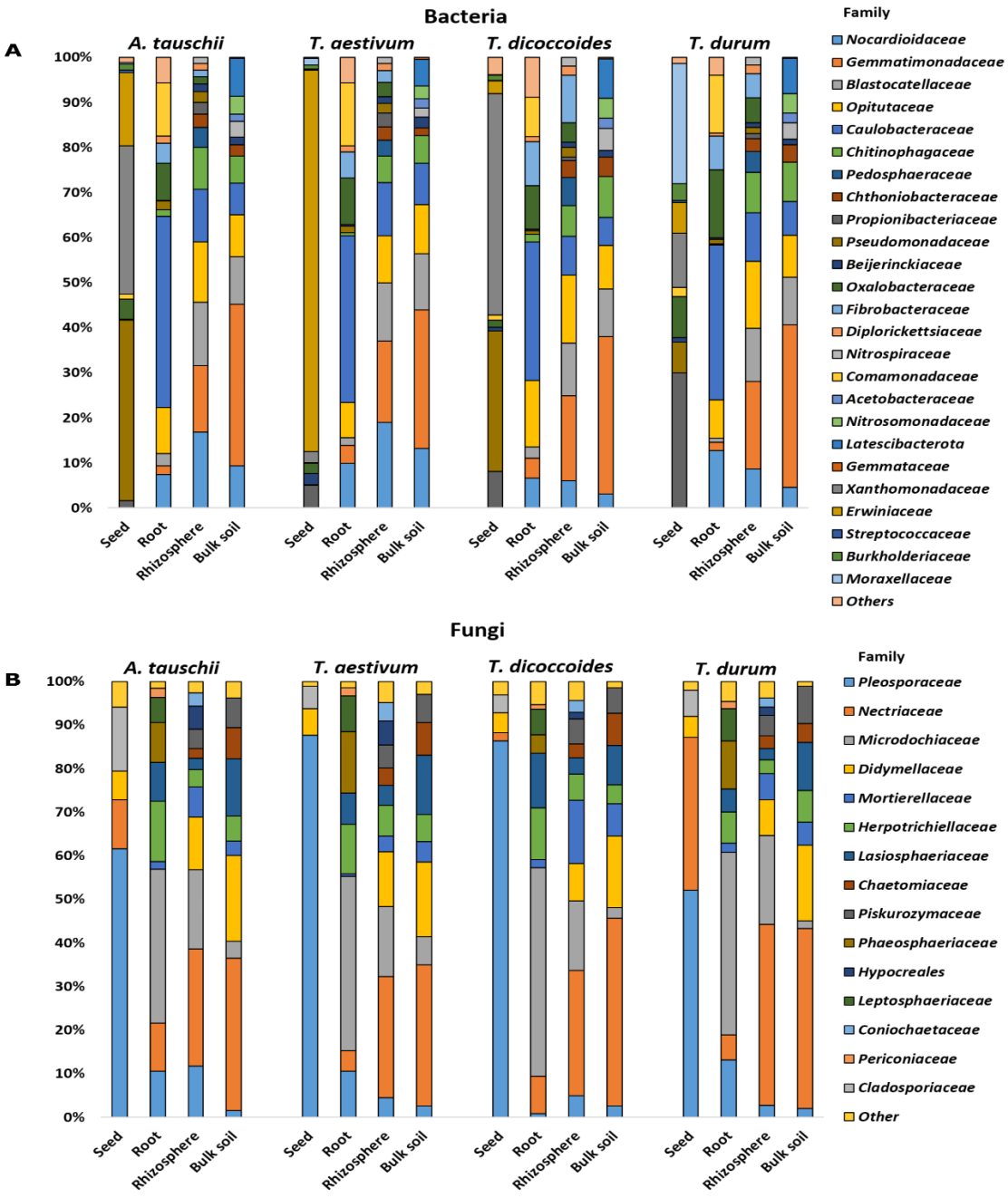


**Figure S2.** Bacterial (A) and fungal (B) taxa distribution in different compartments of wheat cultivars grown in three locations (GG, WG, RH). Relative abundance of 95% predominant bacterial and fungal phyla and 99% predominant genera. Each bar is the average of the data from nine samples, three per location.

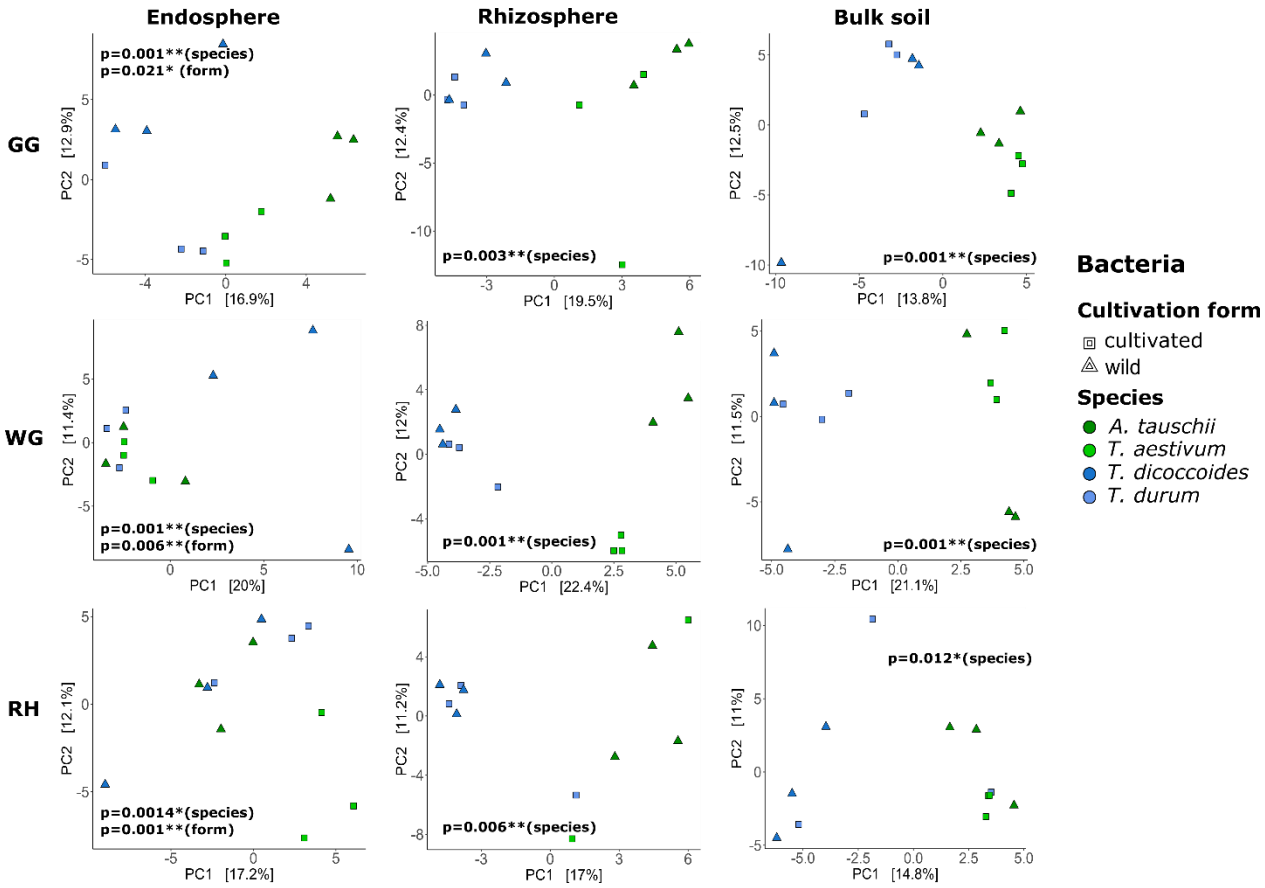


**Figure S3.** Bacterial beta diversity in different compartments and locations. Unconstrained ordination based on Euclidian distance matrices of bacterial communities across the root, rhizosphere, and bulk soil samples collected from wild and domesticated wheat species (*A. tauschii*, *T. aestivum*. *T. dicoccoides*, and *T. durum*) in three locations (GG-Groß-Gerau, WG-Weilburger Grenze, and RH-Rauischholzhausen). Euclidian distances calculated from the data were transformed to the centered log-ratio.

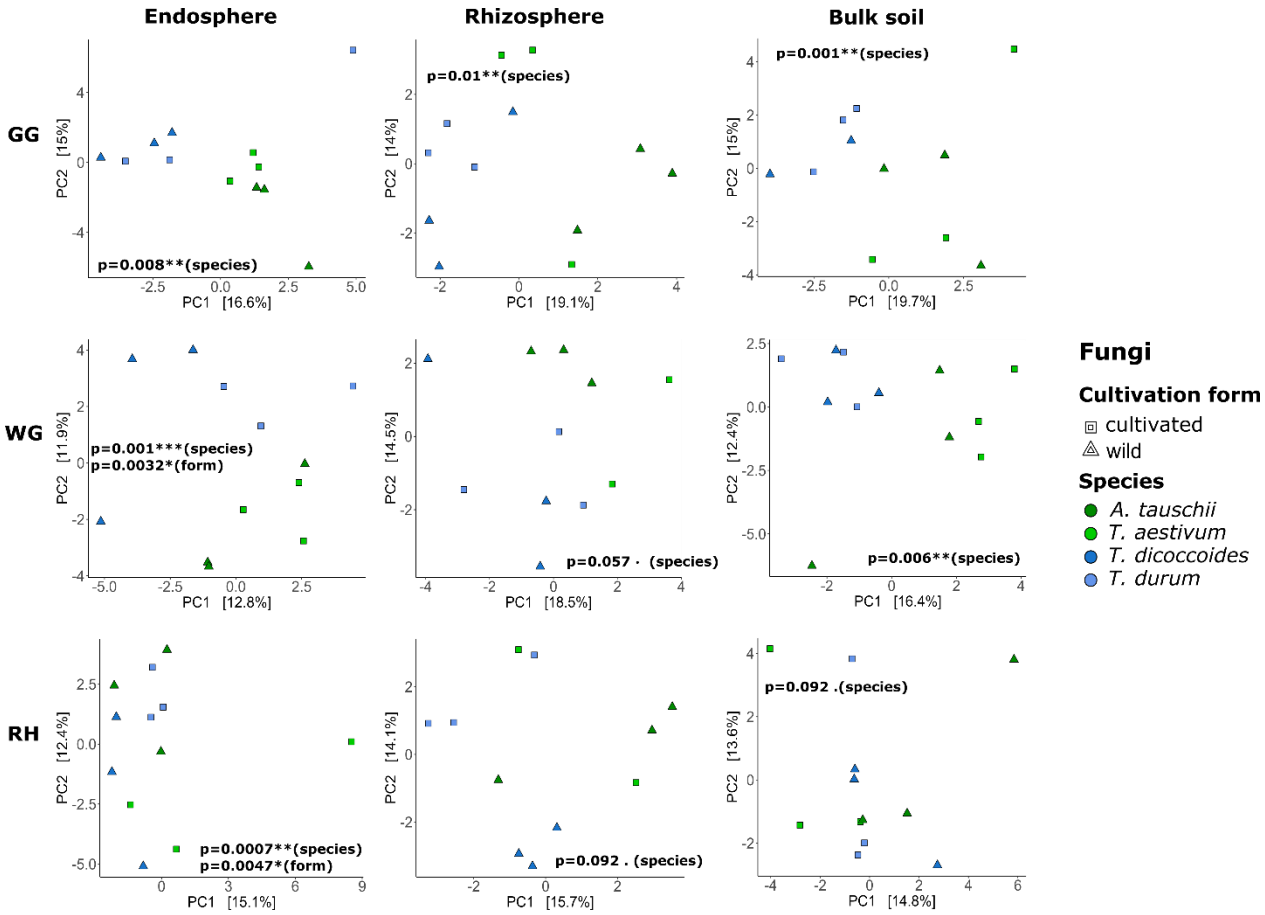


**Figure S4.** Fungal beta diversity in different compartments and locations. Bacterial beta diversity in different compartments and locations. Unconstrained ordination based on Euclidian distance matrices of bacterial communities across the root, rhizosphere, and bulk soil samples collected from wild and domesticated wheat species (*A. tauschii*, *T. aestivum*, *T. dicoccoides*, and *T. durum*) in three locations (GG-Groß-Gerau, WG-Weilburger Grenze, and RH-Rauischholzhausen). Euclidian distances calculated from the data were transformed to the centered log-ratio.

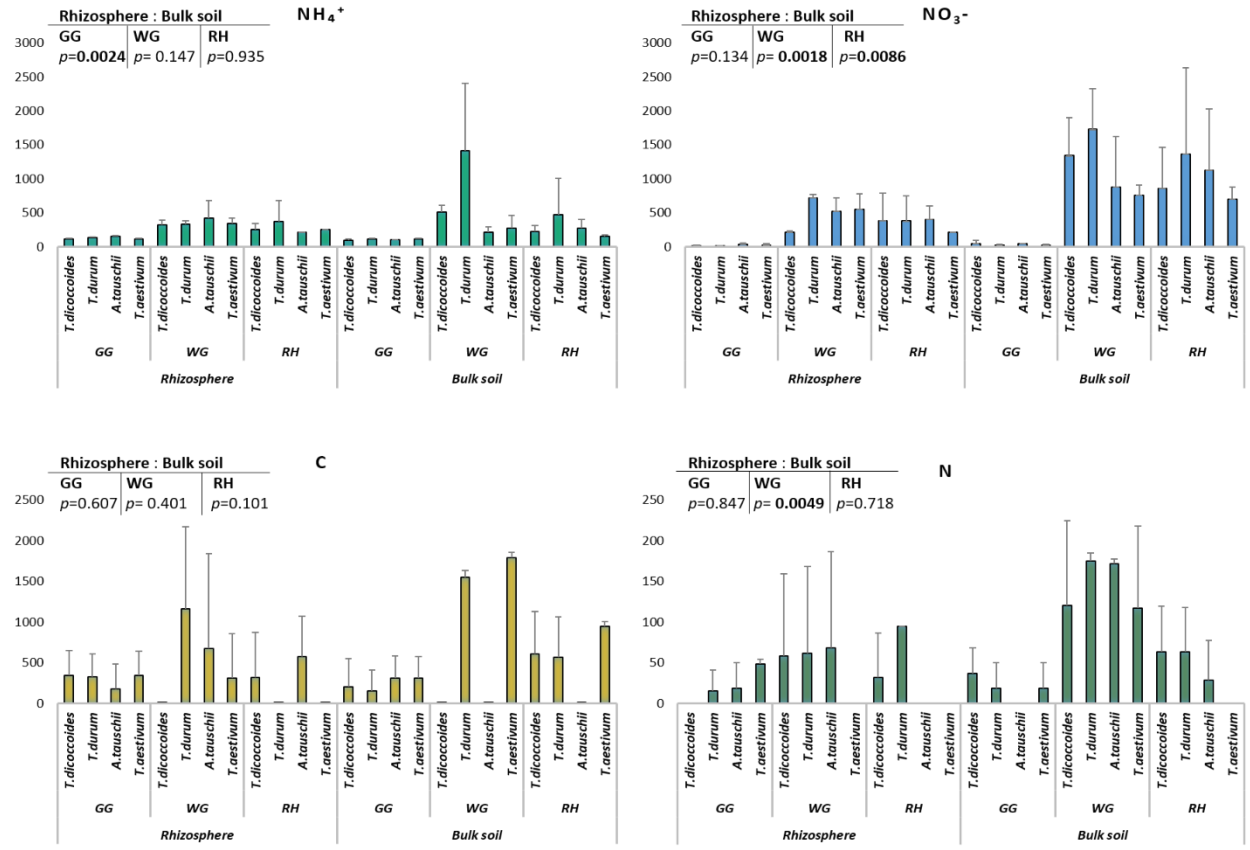


**Figure S5.** Soil properties. Ammonium ($NH_4^+$), nitrate ($NO_3^-$), nitrogen (N), and carbon (C) of rhizosphere and bulk soil (n=12). Soil samples collected from three locations Weilburger Grenze (WG), Groß-Gerau (GG), and Rauischholzhausen (RH). One-way ANOVA results. All parameters ($NO_3^-$, $p < 0.001$, $NH_4^+$, $p = 0.0195$, N, $p < 0.001$) except Carbon ($p = 0.0632$), significantly differed between locations (n=12). The bars represent the mean values of 3 replicates of each wheat species, error bars show standard deviation. Significance values between rhizosphere and bulk soil in each location are represented on the left corner of each barplot.

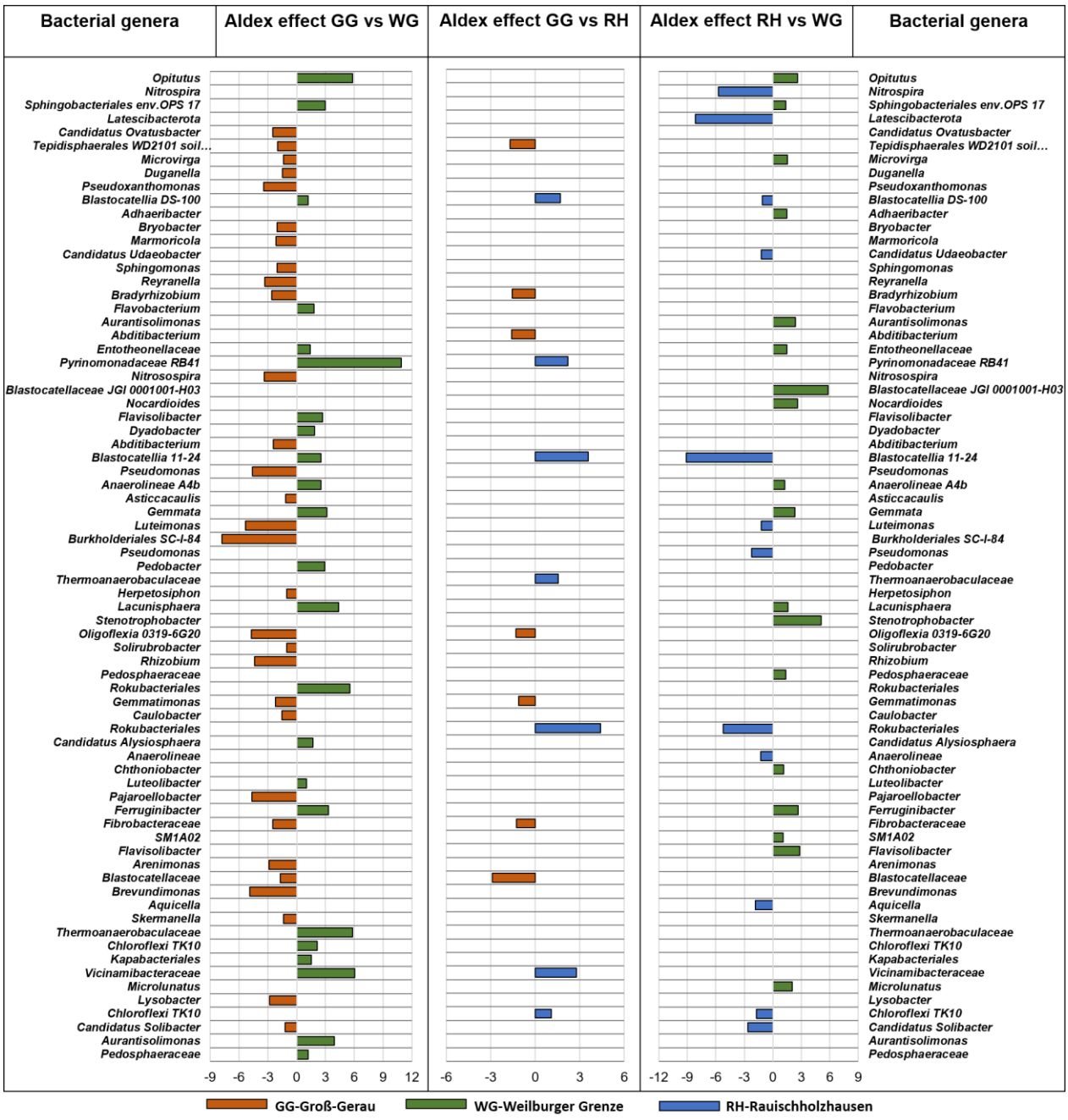


**Figure S6.** The bacterial structure variation between locations (GG-Groß-Gerau, WG-Weilburger Grenze, RH-Rauischholzhausen) based on differential abundance analysis of core microbiome bacterial genera of rhizosphere (*A. tauschii*). The significantly prevalent genera were identified by looking at aldex effect size. The differently abundant genera are considered as significant absolute aldex affect size bigger than 1 or lower than -1.

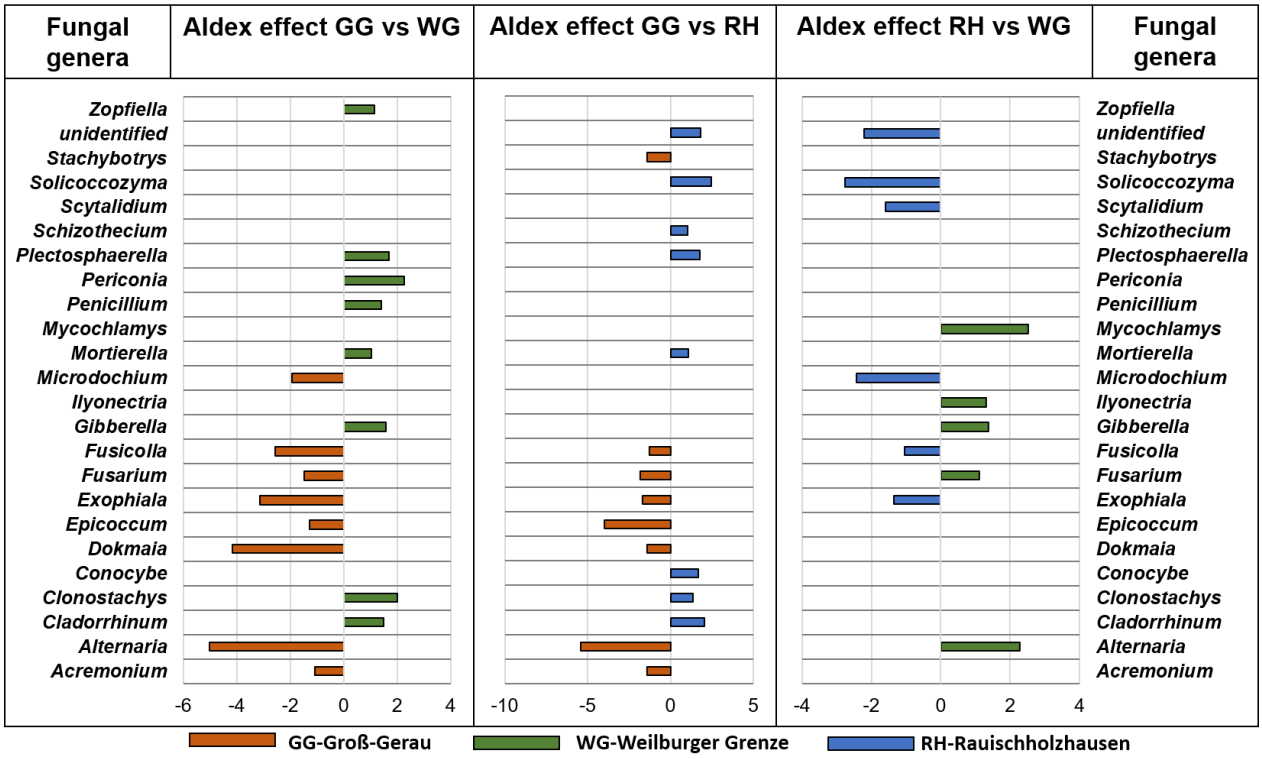


**Figure S7.** The fungal structure variation between locations (GG-Groß-Gerau, WG-Weilburger Grenze, RH-Rauischholzhausen) based on differential abundance analysis of core microbiome bacterial genera of rhizosphere (*A. tauschii*). The significantly prevalent genera were identified by looking at aldex effect size. The differently abundant genera are considered as significant absolute aldex affect size bigger than 1 or lower than -1.

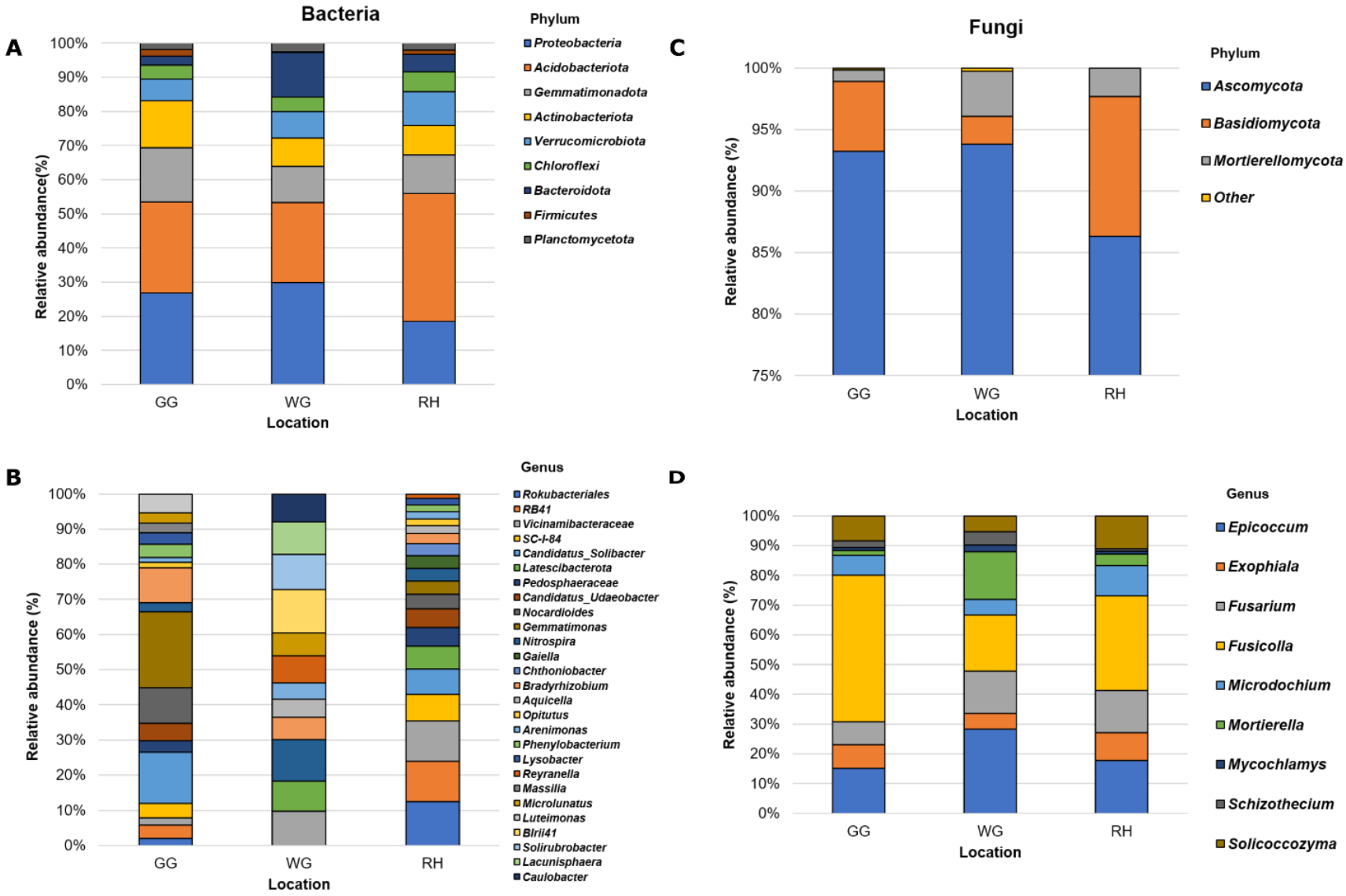


**Figure S8.** Taxa distribution of seedbed soil. Relative abundance of 99% predominant bacteria phyla (A) and genera (B) in all samples and 95% predominant fungal phyla (C) and genera (D) (n=9).

# **Chapter 4:** Host-dependent shifts of the inter-kingdom interactions in the wheat root microbiota during plant domestication

# Host-dependent shifts of the inter-kingdom interactions in the wheat rhizosphere microbiota during plant domestication

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**Abstract**

Modern crops might have lost some of their functional traits, required for interacting with beneficial microbes, as a result of modifications in their genome during domestication. Here, we studied the microbial taxonomic composition, inter-kingdom beneficial interactions using next-generation sequencing of 16S rRNA gene and ITS2 as well as, the abundance of bacterial genes encoding extracellular enzymes involved in N- and P-cycling in the rhizosphere of wild and currently cultivated wheat species.

We found a higher abundance of bacterial and fungal families in modern wheat endorhiza and rhizosphere however, fungal families were less diverse in modern wheat species. Co-occurrence network analysis showed that wild species have more bacterial–fungal interactions than cultivated species. There were more plant pathogenic fungi among the most connected fungal genera in the cross-domain network of wild crops than in those of modern cultivars, in contrast, a higher number of genera used as biocontrol agents became more connected genera in the inter-kingdom network of domesticated species. The abundance of bacterial genes responsible for the production of proteins involved in nutrient cycling were almost the same in wild and modern wheat species except for the *nirS* gene involved in denitrifying which was reduced in the modern wheat rhizosphere. Moreover, potential urease activity was different between the rhizosphere of wild and domesticated wheat species.

Our results indicate a microbiome shift as a result of changes (environment, genotype) caused by plant domestication and reduced the inter-kingdom interactions as well as denitrifying gene abundance.

**Keywords:** co-occurrence analysis, bacteria, fungi, functional gene, qPCR, domestication

## Introduction

Plants are known to host microbial communities that help them to maintain health and fitness, and the relationships between them have evolved over millions of years (Bakker et al., 2014). Studies have shown that plant species, such as maize, tomato, cucumber, wheat, tobacco, and rice (Ofek et al. 2014; Tkacz et al. 2015; Saleem et al., 2016), can selectively recruit and promote the colonization of a unique array of microbes on their roots suggesting the long co-evolution between plant and its microbes. It is assumed that certain microbes have adapted to their hosts and the community has shifted by ecological and anthropological interactions since wild plants were being domesticated.

Domestication is the rapid anthropological selection of crops based on genetic diversity and desirable traits (Abbo et al., 2014). The selection of fertilizer-responsive and disease-resistant cultivars has expedited the domestication process in modern breeding (Sakuma et al., 2011). Many other studies showed cultivated varieties of some crops, e.g. wheat (Hassani et al., 2020; Spor et al., 2020), barley (Bulgarelli et al., 2015) maize (Szoboszlay et al., 2015; Johnston-Monje et al., 2016), rice (Tian et al., 2018), and beets (Zachow et al. 2014), sunflower (Leff et al., 2017), bean (Pérez-jaramillo et al., 2019), and their wild progenitors substantially varied in rhizosphere microbiota structure. A decrease in the relative abundance of *Bacteroidetes*, *Acidobacteria*, and an increase in the relative abundance of *Actinobacteria* and *Proteobacteria* was observed through a transect of evolution from plant ancestors over landraces to modern cultivars (Vanessa N. Kavamura et al., 2020; Kinnunen-Grubb et al., 2020; Pérez-Jaramillo et al., 2017) which implies that domesticated plant's rhizosphere microbiota shifted from oligotrophy microbes towards readily available nutrient favoring copiotroph communities. In addition, domestication has reduced the interaction between plant and rhizosphere microbes that are potentially important for plant nutrient availability, as evidenced by the depletion of genes from the genome of domesticated wheat cultivars involved in N-cycling and symbiotic fungi compared to their progenitor species (Spor et al., 2020).

Wheat (*Triticum spp*.) is one of the first cultivated and economically significant staple crops which were domesticated in the Near-Eastern Fertile Crescent some 10,500 years ago, along with other cereals (Zohary and Hopf 2000). Wheat now covers over 16% of worldwide cropland and 0.7 billion tons are produced each year and accounts for a major portion (one-fifth) of all food consumed by people (http://faostat.fao.org). Most of the wheat grown globally is bread wheat

(*Triticum aestivum*); it has a hexaploid genome made up of three subgenomes (AABBDD) that resulted from hybridization events between *T. urartu* (AA genome) and a close relative of *Aegilops speltoides* (BB genome), as well as a later hybridization with the wild diploid *Aegilops tauschii* (DD genome). Among the other wheat species, the most important in terms of spread and economic impact is durum wheat (*Triticum turgidum* ssp. *turgidum* convar. *durum*; genomes AABB) and its tetraploid progenitor *T. turgidum* spp. *dicoccoides* (AABB). Their domestication has resulted in a variety of genetic changes which lead to diversity and compositional shift of the root-associated bacterial and fungal microbiota. As Hassani and colleagues (2020) found, wild wheat *T. urartu* harbors more diverse microbiota especially in the rhizosphere and phyllosphere than modern cultivar *T. aestivum* which may indicate that breeding selection resulted in a reduced ability to recruit specific microbes in the rhizosphere. This can also be seen from the reduced network of microbe-microbe interactions in the rhizosphere microbiome of several crop species (wheat landraces, lettuce) (Cardinale et al., 2015; Rossmann et al., 2020).

Several plant traits, such as root architecture (Kavamura et al., 2020) leaf area, longevity, production rate (Roucou et al., 2018), primary and secondary metabolite exudation have changed dramatically during domestication (Iannucci et al., 2017). This may have had a significant impact on the composition of the root and rhizosphere microbiome, functions, and interactions as evidenced by a study of changes in root traits and on the assembly of rhizosphere bacterial microbiota from tall wheat cultivars that were distinct from those associated with semi-dwarf cultivars (Kavamura et al. 2020). Additionally, modern cultivars enriched pathogenic fungal taxa in their rhizosphere microbiome compared to their wild ancestors (Leff et al., 2017; Shi et al., 2019). It has also been demonstrated that modern wheat has lost the ability to interact with plant growth-promoting rhizobacteria than ancient wheat species (Valente et al., 2020). A recent study about the effect of ploidy level on the assembly of the wheat bacterial microbiome indicated that host ploidy level and domestication may have an influence on the microbial assembly and explains the variation in alpha and beta diversity for rhizosphere and endosphere microbiomes (Wipf & Coleman-Derr, 2021). Many studies focused on the mycobiota of wild and domesticated wheat root systems (Ofek-Lalzar et al., 2016; Sun et al., 2020; Tkacz et al., 2020) and found higher numbers of differentially prevalent fungal taxa and higher co-occurrence networks among a small number of fungal taxa in the roots of wild wheat species (Sun et al., 2020). Furthermore, another study showed that domestication significantly affects the fungal microbiome (enriched in beneficial *Glomeromycetes* fungi in modern wheat cultivars) than the bacterial microbiome of the

wheat rhizosphere (Spor et al., 2020; Tkacz et al., 2020). Similar results were also recorded by Kim et al. (2020) in the rice seed microbiome. However, so far, entire bacterial and fungal microbiota and their interactions in different plant compartments of wheat species, both wild and domesticated, under different environmental conditions have not been studied simultaneously using metabarcoding.

Here, we explored the bacterial and fungal microbiome composition and co-occurrence patterns in the root system of wild and cultivated wheat species using high-throughput sequencing of the 16S rRNA gene and fungal internal transcribed spacer 2 (ITS2) region. We hypothesized that (i) wheat domestication weakened the association between host plant and ist microbiome (bacteria, fungi) which might result in reduced microbial functional gene abundance in the rhizosphere of modern wheat species. We further hypothesize that (ii) host-associated microbiomes (bacteria, fungi) of cultivated species have a lower network than wild relatives, due to decreased need for abiotic and biotic stress mitigation by microbes.

To test our hypotheses, we assessed both the bacterial and the fungal microbiome of the two wheat genetic groups (*A. tauschii*/*T. aestivum* and *T. dicoccoides*/*T. durum*) in different plant habitats (endosphere, rhizosphere). Examining the root-associated bacterial and fungal microbiota of wild species, comparing them to those of closely related crops, and determining what part of the diversity might be missing in related crops, will contribute to a better evaluation of the true potential of wild species root microbiome for modern crop development, in the context of a more sustainable, ecosystem-based agriculture.

## Methods

### *Site description and sample collection*

The sampling was performed at the three research stations of Justus-Liebig University of Gießen located in the Hessen Province (Germany): Groß-Gerau (GG, 50°60′ N; 8°65′ E 158m a.s.l.), Weilburger Grenze (WG, 49° 56′ N; 8° 30′ E 90.7 m a.s.l.), and Rauischholzhausen (RH, 56°76′ N; 8°88′ E 225 m a.s.l.). Samples were collected at the plant flowering stage, from May to July 2019 (Tab. S1). Experimental setup, soil characteristics, and detailed sample collection procedure is reported in our previous study (Abdullaeva et al., 2021.). Briefly, a total of 36 rhizospheres, 36 roots, and 36 bulk soil samples from four different species [*Aegilops tauschii* (wild goatgrass),

*Triticum aestivum* (bread wheat), *Triticum dicoccoides* (wild emmer), and *Triticum durum* (durum wheat)] were collected (Fig. S1).

The experimental factors considered were: plant species (4, see above), cultivation form (2, wild and cultivated), genetic relationship (*A. tauschii* & *T. aestivum*; *T. dicoccoides* & *T. durum*), habitat (rhizosphere, root, bulk soil), and location (GG, WG, RH).

Total genome DNA extraction from soil and plant tissue, amplicon library construction, bioinformatics analysis of sequences, sequencing data, statistical analysis for alpha and beta diversity, and results were described in our previous work (Abdullaeva et al., 2021) and the same sequencing data were used for this paper.

**Real-time PCR**

The quantification of functional genes involved in the nitrogen and phosphorus cycles together with total bacterial (16S rRNA) and fungal (ITS) abundance was performed on rhizosphere samples only. The same DNA extracts as used in this study were used. Real-time PCR experiments were conducted in a Rotor-Gene Q (Qiagen, Hilden, Germany) and with Q-Rex software version 2.0. qPCR was performed in a reaction volume of 10 µl containing by using Absolute qPCR SYBR Green Mix (Thermo Fisher Scientific). The cycling program adjusted for each gene and primer combination (Tab. S2). After the amplification, a melting curve analysis with a temperature gradient of ±0.3 °C 30 $s^{-1}$ from 65 to 95 °C was performed to confirm that only the specific products were amplified. Together with the samples, quantitative negative and positive controls were amplified.

gBlocks™ Gene Fragments of the *phoX* gene were ordered from Integrated DNA Technologies for standard preparation of this gene. qPCR assay protocol was adapted from Ragot et al. (2017).

The standards for 16S rRNA gene of bacterial and fungal ITS fragments as well as *nirS*, *nosZ*, bacterial *amoA* genes were prepared from environmental clones or pure cultures according to Kampmann et al. (2012). A 10-fold serial dilution of the standard, ranging from $10^2$ to $10^8$ copies•$\mu l^{-1}$, was used to construct the calibration curves. Copy numbers obtained by qPCR were related to dry soil mass. The relative abundance of gene copy numbers was then related to the total gene abundance of 16S RNA gene copies for visual observations.

Statistical comparisons were done with ANOVA and Tukey HSD Test, using RStudio version 4.0. The model included the “plant species” factor and was conducted for each location separately.

**Potential activity of extracellular enzymes**

Phosphomonoesterase activity was determined by measuring p-nitrophenol released after incubating rhizosphere soil with p-nitrophenyl phosphate for 1 h at 37 °C according to Tabatabai (1994). We used a modified universal buffer (MUB) with pH 6.5 for the assay of acid phosphatase (hereafter acid PA) and pH 11 for the determination of alkaline phosphatase (hereafter alkaline PA).

Urease activity was identified using Kandeler and Gerber (1988) method by estimating ammonia released after the incubation of 5 g of rhizosphere soil with urea for 2 h at 37 °C.

**Statistical analysis**

**Differential abundance (DA) analysis**

The *ALDEx2* pipeline (Fernandes et al., 2013) was used for differential abundance analysis (performed on the core species, dominant ASVs found in 99 % of bacteria and 95% fungal dataset from three locations for all sequenced samples, and collapsing taxa annotations to phylum, family, and genera levels) to identify microbial taxa with significant differences in relative abundance between cultivation forms (wild/domesticated). Significance test (Wilcoxon test) was performed on each taxon in the vector of centered log-ratio (clr) transformed values. The median difference in values (*diff.btw*) between the microbiota of domesticated and wild wheat species was used to represent the factor effect. Each resulting p-value was corrected using the Benjamini–Hochberg procedure (Benjamini & Hochberg, 1995).

**Correlation**

The correlation analysis between core bacterial genera of the rhizosphere and rhizosphere soil biochemical parameters (potential enzyme activity, the abundance of genes encoding enzymes involve N/P turnover) was performed using “aldex.corr” function of ALDEx2 pipeline.

Pearson’s correlation coefficient was used to identify significantly correlated genera and *p* values were corrected using the Benjamini–Hochberg.

**Core microbiota** of the rhizosphere (n=9), root (n=9), bulk soil (n=9), samples of each cereal species were collapsed to genus level, and the core microbiome (Bacteria, prevalence = 0.85, fungi, prevalence = 0.75) was identified using the R “microbiome” package (Lahti 2017).

**Inter-kingdom network analysis**

After observing the results only rhizosphere and root microbial interkingdom networks of wild and core microbiomes of each cultivar species were analyzed using the SPIEC-EASI package ( v0.1.4) in Rstudio. SPIEC-EASI (SParse InversE Covariance Estimation for Ecological ASsociation) is a step forward from existing methods for inferring microbial ecological networks from microbiome composition datasets. SPIEC-EASI transforms ASV data based on compositional data analysis. As next SPIEC-EASI uses one of two approaches to estimate the interaction graph from the transformed data: neighborhood selection method (also called *MB*) or sparse inverse covariance selection (also called *glasso*). In our study, we used the MB method for neighborhood selection (Meinshausen & Bühlmann 2006) which involves solving *p* regularized linear regression problems that deal with graph reasoning and producing local scale-free structure predictions for each node (Kurtz et al., 2015). Edge weights were obtained from the optimal beta matrix and symmetrize and selected from MB neighborhood selection with SPIEC-EASI package functions "getOptBeta" and "symBeta". Based on the node and edge attribute table created by SPIEC-EASI networks were visualized in Cytoscape software version 3.8.2 (Shannon et al., 2003) and analyzed using "Analyse Network" tool. The "Group Attributes Layout" tool was used to create the network layout. The hub taxa were defined by weighing edge counts against betweenness centrality and closeness centrality (Agler et al., 2016).

## Results

### Taxonomic structure of the different plant habitats of wild and domesticated wheat microbiota with differential abundance test

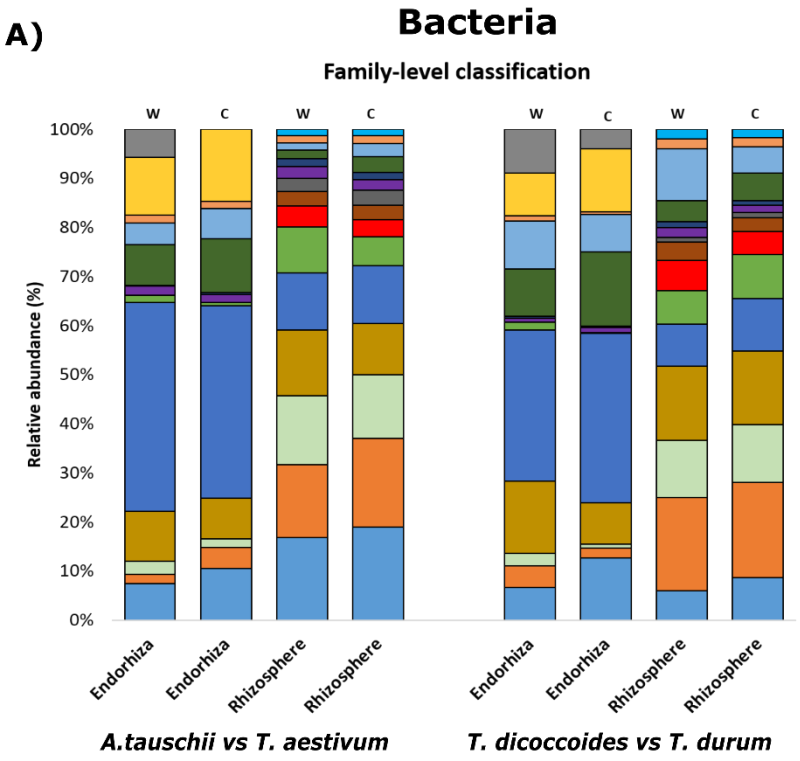
The bacterial and fungal microbiota in different plant habitats was highly variable in the taxonomic structure, reflecting the wide range of habitats from which they were collected (Fig. 1, Fig. S1, S2). In the endosphere, we observed significantly different structures of bacterial and fungal microbiome between wild and domesticated wheat species (Fig. 1). Especially, several bacterial families enriched in the endosphere of cultivated *T. durum* as compared to its dominator *T. dicoccoides*. Similarly, the rhizosphere of modern wheat *T. aestivum* has diverse microbial families significantly enriched as compared to its wild genome donor *A. tauschii* (Fig. 1 A). In contrast, more fungal families enriched in the endosphere (*A. tauschii*) and the rhizosphere of wild wheat (*T. dicoccoides*) species than the modern ones (Fig. 1 B). Although fewer fungal families were dominant in the modern wheat species, the abundance of fungal families were significantly higher than the wild wheat species (Fig. 1 B).

**In the endosphere**

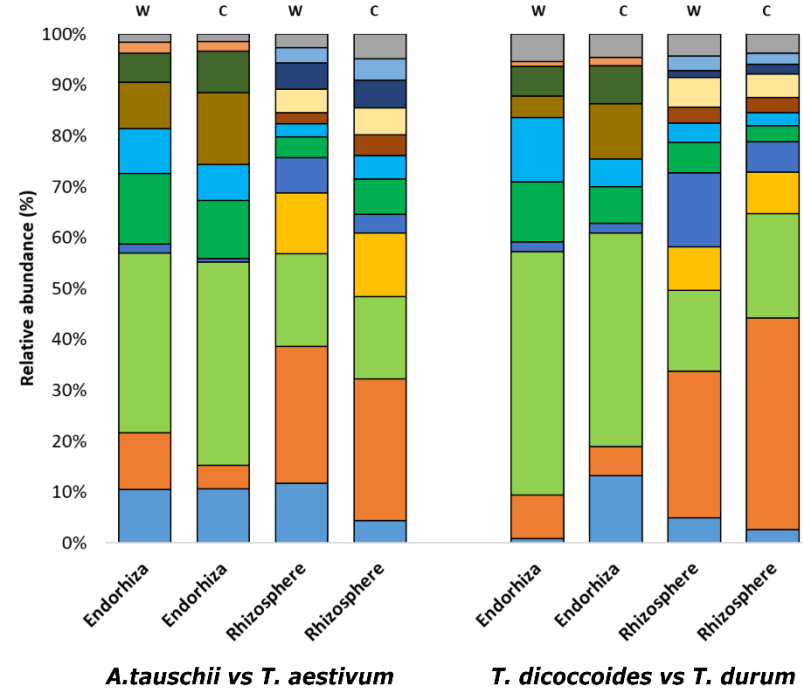
Most of the bacterial and fungal families that were found differently enriched with ALDEx2 between the endosphere of genetically related wheat groups *(A. tauschii* vs *T. aestivum* and *T. dicoccoides* vs *T. durum*) were significantly enriched in domesticated wheat species (*T. aestivum, T. durum*) (Fig. 1, Tab. S3,4). Among the enriched bacterial families, *Nocardiodaceae, Beijerinckiaceae, Acetobacteriaceae,* and *Xanthomonadaceae* enriched in the endosphere of both domesticated wheat species (*T. aestivum, T. durum*) (Fig. 1 A) and fungal families *Pleosporaceae, Didymellaceae, Chaetomiaceae* significantly enriched in the endosphere of *T. aestivum* whereas *Microdochiaceae, Mortierellaceae,* and *Phaeosphaeriaceae* enriched in *T. durum* endosphere (Fig. 1 B).

**In the rhizosphere**

Results also demonstrate that rhizosphere bacterial microbiome of wild wheat cultivars were distinct from those associated with modern cultivars (Fig. 1), with a higher differential abundance of *Nocardiodaceae, Gemmatimonadaceae, Xanthomonadaceae,* and fungal family *Coniochaetaceae* in modern cultivars compared with a higher differential abundance of *Diplorickettsiaceae, Nitrosomonadaceae, Gemmataceae,* and fungal families *Mortierellaceae, Chaetomiaceae, Piskurozymaceae*, and a family of the order *Hypocreales* in wild wheat species. However, the number of differentially abundant bacterial and fungal families was noticeably more in the genetically related group *A. tauschii* and *T. aestivum* than the other genetically related group (Fig. 1).




| | A. tauschii vs T. aestivum | T. dicoccoides vs T. durum | A. tauschii vs T. aestivum | T. dicoccoides vs T. durum |
|---|---|---|---|---|
| | Endorhiza | | Rhizosphere | |
| *Nocardioidaceae* | **0.93** | **2.26** | **2.07** | **0.91** |
| *Gemmatimonadaceae* | **1.66** | 0.87 | **2.01** | **0.52** |
| *Blastocatellaceae* | -0.01 | 0.43 | **1.58** | 0.70 |
| *Opitutaceae* | 0.31 | **0.84** | **1.97** | 0.44 |
| *Caulobacteraceae* | 0.44 | **1.89** | **1.85** | 1.00 |
| *Chitinophagaceae* | -1.05 | **-8.51** | **1.16** | 1.09 |
| *Pedosphaeraceae* | | **-4.61** | **1.36** | 0.16 |
| *Chthoniobacteraceae* | | | **1.76** | 0.37 |
| *Propionibacteriaceae* | | **-4.63** | **1.97** | 0.50 |
| *Pseudomonadaceae* | 0.18 | **1.52** | **2.03** | -0.46 |
| *Beijerinckiaceae* | **1.73** | **1.28** | | 0.29 |
| *Oxalobacteraceae* | 1.18 | **2.29** | **3.69** | 0.74 |
| *Fibrobacteraceae* | 1.10 | **1.66** | **2.06** | -0.09 |
| *Diplorickettsiaceae* | 0.39 | **0.92** | **-6.58** | 0.31 |
| *Nitrospiraceae* | | **-4.82** | **1.72** | 0.30 |
| *Comamonadaceae* | 0.87 | **1.89** | **3.09** | 0.16 |
| *Acetobacteraceae* | **8.58** | **-6.21** | **2.33** | 1.22 |
| *Nitrosomonadaceae* | | 1.13 | **-5.96** | 0.30 |
| *Latescibacterota* | | | | 0.16 |
| *Gemmataceae* | | | **-4.52** | **-4.68** |
| *Other* | | | | |




| | A. tauschii vs T. aestivum | T. dicoccoides vs T. durum | A. tauschii vs T. aestivum | T. dicoccoides vs T. durum |
|---|---|---|---|---|
| | Endorhiza | | Rhizosphere | |
| *Pleosporaceae* | **6.77** | | **3.97** | **-1.36** |
| *Nectriaceae* | **-3.40** | 0.36 | **2.99** | -0.78 |
| *Microdochiaceae* | **-2.69** | **1.08** | **3.52** | -0.92 |
| *Didymellaceae* | **4.82** | | **2.82** | **-0.97** |
| *Mortierellaceae* | **-3.82** | **8.57** | **-6.17** | **-1.74** |
| *Herpotrichiellaceae* | **-2.46** | | **3.16** | **-1.93** |
| *Lasiosphaeriaceae* | **-2.66** | | **-4.63** | **7.10** |
| *Chaetomiaceae* | **4.53** | | **-4.31** | **-1.02** |
| *Piskurozymaceae* | | | **-5.33** | **-1.34** |
| *Phaeosphaeriaceae* | **-2.45** | **2.06** | **-3.53** | |
| *Hypocreales* | | | **-4.60** | **-8.31** |
| *Leptosphaeriaceae* | -2.06 | **-7.03** | | |
| *Coniochaetaceae* | | | **12.5** | **6.64** |
| *Periconiaceae* | **-2.53** | | | |
| *Other* | | | | |

*ALDEx2*
**bold text**
*indicates significance ($p < 0.05$)*

**Figure 1.** Bacterial (A) and fungal (B) microbiome composition at the family (99 % predominant) level (root n=9, rhizosphere n=9). The median difference in centered log-ratio (*clr*) values between the microbiota of domesticated wheat species (*T. aestivum*, *T. durum*, labled with C) and their corresponding wild ancestors (*A. tauschii*, *T. dicoccoides*, labled with W) in different plant compartments is shown in the tables on the right side, which is determined from the ALDEx2 differential abundance test. Bold text indicates the significance between groups in different plant habitats. Positive numbers represent extend of particular taxon enrichment in domesticated wheat species (*T. aestivum*, *T. durum*) whereas negative numbers show the degree of enrichment in wild plants (*A. tauschii*, *T. dicoccoides*). The empty cells show no change in abundance between genetically related wheat species.

## Co-occurrence patterns of microbial communities between wild and domesticated wheat

Due to the changes in bacterial and fungal microbiome responses to domestication, general genera co-occurrence tendencies in wild and domesticated wheat are likely to differ. We used the network inference tool SpiecEasi (v0.1.4) to investigate how domestication affects inter-kingdom microbial genera interactions. We constructed inter-kingdom co-occurrence networks of bacteria and fungi in the rhizosphere of two wild and domesticated wheat groups (*A. tauschii* vs *T. aestivum*; *T. dicoccoides* vs *T. durum*) (Fig. 2, 3) from the aforementioned three sites. We selected core microbial genera that were found in >85% (bacteria) and >75% (fungi) of the rhizosphere samples across all three locations to decrease the influence of site-specific genera on network structure. The co-occurrence network of the rhizosphere of *A. tauschii* consisted of 119 nodes and 238 edges (Fig. 2 A), whereas *T. aestivum* consisted of 97 nodes and 117 edges (Fig. 2 B). *T. dicoccoides* consisted of 116 nodes and 210 edges (Fig. 3 A), while the network of *T. durum* contained 106 nodes and 168 edges (Fig. 3 B). This reflects the differences in the rhizosphere bacterial and fungal composition between wild and domesticated wheat species, resulting in poor networking between bacterial and fungal genera in modern wheat species. We also found that correlations between microbes from different kingdoms in the rhizosphere are usually positive (*A. tauschii* - 76.6%, *T. aestivum*-70.29%, *T. dicoccoides* - 59.04%, *T. durum* - 62.5%) and were dominated by interactions between bacteria.

Correlation analysis of connected nodes between bacteria and fungi showed more positive interaction between the two kingdoms in both groups of plants (wild vs cultivated) and it seems that the relationship between fungi and bacteria did not change or slightly increased as plants domesticated.

Based on betweenness centrality and connectivity scores, bacterial and fungal genera from each network were identified as "hub taxa" (Fig. 2, 3, labeled nodes). These were *Stenotrophobacter*, *Lysobacter*, and fungal genus *Plectosphaerella* in the rhizosphere of *A. tauschii* and *Stenotrophobacter*, *Blastocatellia DS-100*, and the fungal genus *Microdochium* were the most connected genera in the rhizosphere of domesticated wheat *T. aestivum*. *Ferruginibacter*, *Blastocatellia DS-100*, and the fungal genus *Dichotomopilus* were hub taxa/genera in the rhizosphere of *T. dicoccoides* and *Ferruginibacter*, *Microlunatus* and the fungal genus *Penicillium* were in the rhizosphere of *T. durum*.

*Eppicoccum*, *Cladorhium*, *Articulospora*, *Acremonium*, *Microdochium*, *Cylindrocarpon*, *Mortierella*, *Plectosphaerella* had positive interactions with bacterial genera, *Gibellulopsis Fusarium*, *Zopfiella*, *Penicillium*, *Coniochaeta*, *Dichotomopilus* had also negative connections with bacterial genera. Bacterial genera *Ferruginibacter*, *Microvirga*, *Microlunatus*, *Bryobacter*, a genus of the phylum *Candidatus* Levybacteria, *Luteolibacter*, *Lysobacter*, *Flavisolibacter*, *Brevundimonas*, a genus of the family *Vicinamibacteraceae*, a genus of the family SC-I-84 in the order *Burkholderiales* had a positive correlation with fungal genera.

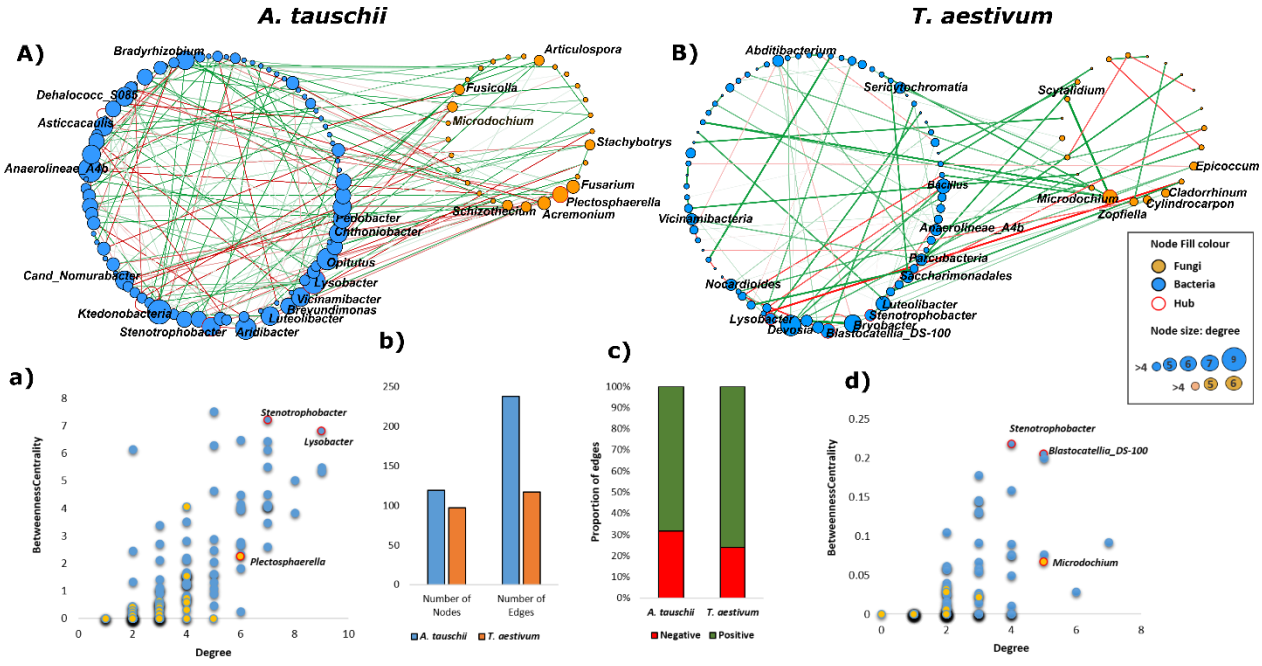



**Figure 2**. Bacteria-fungi interkingdom association of the rhizosphere microbiotas. Co-occurrence-based network of rhizosphere microbial genera detected in genetically related groups: wild *A. tauschii* (A), domesticated wheat *T. aestivum* (B). Each node corresponds to a genus, and edges between nodes correspond to either positive (green) or

negative (red) correlations inferred from genera abundance profiles using the SpiecEasi method (pseudo $p < 0.05$, correlation values $< -0.3$ or $> 0.3$). Scatter plots (a,d) show connectivity scores of the nodes and hub genera based on degree, betweenness centrality and closeness centrality. Genera belonging to different microbial kingdoms have different color codes (bacteria, blue; fungi, orange), and node size reflects their edge counts between genera. Bar graph (d) show the number of nodes and proportion among the genetic groups, and bar graphs (c) shows the proportion of intra-kingdom edges of positive (green) or negative (red) correlations in the rhizosphere network.

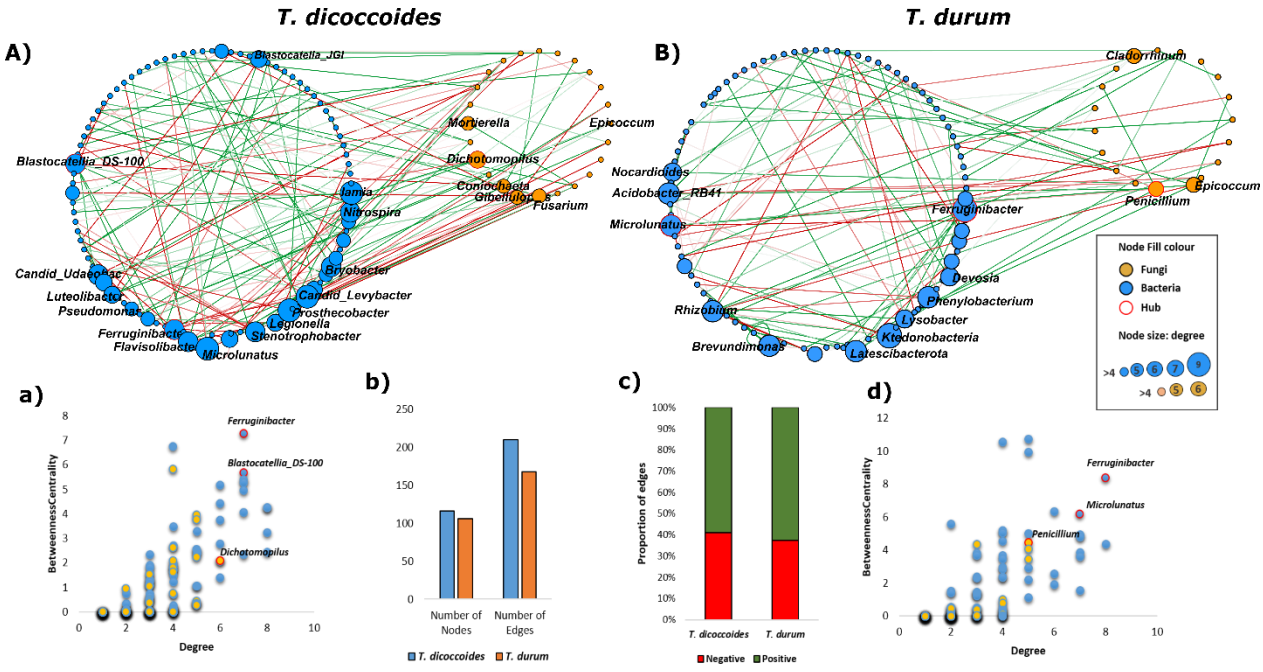



**Figure 3**. Bacteria-fungi interkingdom association of the rhizosphere microbiotas. Co-occurrence-based network of rhizosphere microbial genera detected in genetically related groups: wild *T. dicoccoides* (A), domesticated wheat T. durum (B). Each node corresponds to a genera, and edges between nodes correspond to either positive (green) or negative (red) correlations inferred from genera abundance profiles using the SpiecEasi method (pseudo $p < 0.05$, correlation values $< -0.3$ or $> 0.3$). Scatter plots (a,d) show connectivity scores of the nodes and hub genera based on degree, betweenness centrality, and closeness centrality. Genera belonging to different microbial kingdoms have different color codes (bacteria, blue; fungi, orange), and node size reflects their edge counts between genera. Bar graph (d) shows the number of nodes and proportion among the genetic groups, and bar graphs (c) shows the proportion of intra-kingdom edges of positive (green) or negative (red) correlations in the rhizosphere network.

## The effect of domestication on rhizosphere microbiome of wild and domesticated wheat involving P and N-cycling.

To evaluate whether domestication has shifted the rhizosphere microbiome involved in P and N-cycling, we quantified the abundance of microbial genes related to nitrification, denitrification, and phosphorus mineralization in the wild and modern wheat rhizosphere samples in three locations. We found variations in N-cycle genes only (Fig. 4). The significant changes in gene copies were different in locations. Such as, *nosZ* and *amoA* were significantly higher in the rhizosphere of wild *T. dicoccoides* than other wheat species, and this trend was observed only in WG. The *nirS* gene copy numbers were higher in the rhizosphere of *A. tauschii* than *T. aestivum* in all three locations (Fig. 4). The results of potential enzyme activities showed significant changes in potential alkaline PA and urease activities (Fig. 5). Although the relative abundance of *phoX* gene showed no changes, alkaline PA activity was higher in the rhizosphere of *A. tauschii* in GG, slightly higher in the rhizosphere of *T. dicoccoides* in WG compared to their modern wheat species (Fig. 5 a). Potential urease activity significantly changed between the rhizosphere of wild *A. tauschii* and modern *T. aestivum* in GG as well as in WG but the reverse was observed in RH. (Fig. 5 b). The potential urease activity was higher in the rhizosphere of wild *T. dicoccoides* than cultivated *T. durum* in GG and RH.

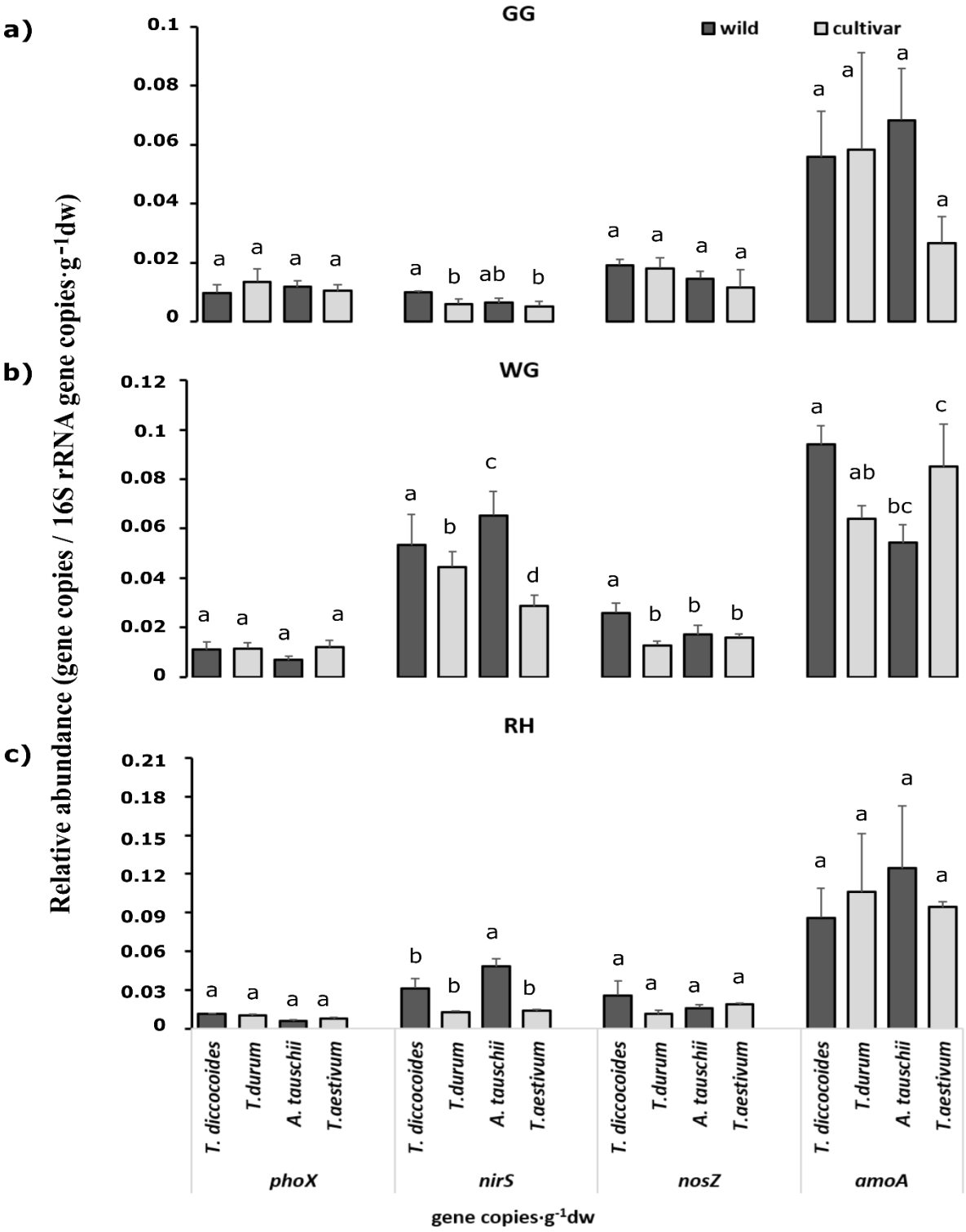



**Figure 4.** The ratio between 16S rRNA gene copy numbers per gram of dry soil weight and the *phoX*, *nirS*, *nosZ*, and *amoA* gene copies per gram of dry soil. Each bar represents 3 biological replicates and 4 technical replicates. Small letters indicate significant differences between wheat species.

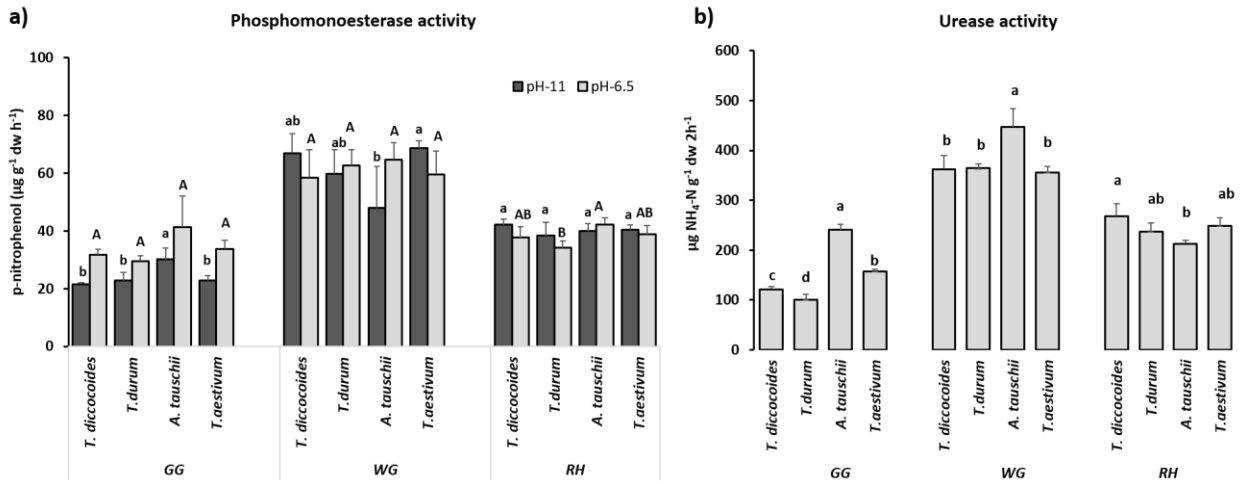



**Figure 5.** The potential activities of enzymes involved in P-, N-cycle. Figures show the potential acid and alkaline phosphomonoesterase activity **a)** and the potential urease activity **b)** in the rhizosphere of wheat species. Capital letters on figure **a)** indicate significant differences between acid phosphomonoestherases activities while small letters indicate significant differences between alkaline phosphomonoesterase activities. Small letters on figure **b)** indicate significant differences in Urease activities between different wheat species.

## Correlation

We found a correlation (Pearson) between core bacterial genera and potential enzyme activities (urease, alkaline PA) as well as gene (*nirS*, *phoX*) abundance (Tab. S5). In GG, bacterial genera *Marmoricola*, *Brevundimonas*, *Nocardioides*, *Devosia*, "*Chthoniobacter"*, *Microvirga*, and *Microlunatus* strongly positively correlated with potential urease activity, and only "*Chthoniobacter"* positively correlated with alkaline PA. On the other hand, more bacterial genera correlated with urease, alkaline PA, and *nirS* in WG. The following genera were positively correlated; *Pedobacter*, *Ferruginibacter*, *Mucilaginibacter*, *Dyadobacter*, *Terrimonas*, *Vicinamibacteraceae*, *Flavobacterium*, *Adhaeribacter*, *Microvirga*, *Stenotrophobacter*, *Opitutus*, *Flavisolibacter*, *Chloroflexi TK10*, a uncultured genus (Ellin517) of the family *"Pedosphaeraceae"*, *Dinghuibacter* with potential urease activity, *Mucilaginibacter*, *Adhaeribacter*, a genus of the family *Vicinamibacteraceae*, *Microvirga*, *Ferruginibacter*, *Dongia*, a genus of the Chloroflexi TK10 cluster, a uncultured genus (Ellin517) of the family *"Pedosphaeraceae"*, *Opitutus*, *Dyadobacter*, *Pedobacter*, *Flavobacterium*, *Flavisolibacter*, *Terrimonas*, a genus of the family "Pedosphaeraceae", a genus of the class "*Kapabacteriales*", RB41 an uncultured genus of the family *Pyrinomonadaceae* with *nirS* gene copies, a genus in the order SBR1031 of the class *Anaerolineae1*, *Reyranella*, *Solirubrobacter*, *Duganella*, *Lysobacter*, a genus of the family SC-1-84 of the order *Burkholderiales*, *Bryobacter*, *Herpetosiphon*,

*"Pajaroellobacter"* with potential alkaline PA. In RH, only the genus of the *Chloroflexi* TK10 cluster was positively correlated with bacterial genera and *nirS* gene copies.

## Discussion

We analyzed root endosphere and rhizosphere bacterial and fungal microbiome from four wheat genotypes planted at three locations and sampled at the flowering stage of the crops.

The analysis of core species from three locations showed general abundance enrichment of the most prevalent bacterial phylum/family in the endorhiza and rhizosphere of cultivated wheat species than their ancestor species (Fig. 1, Fig. S3). The enrichment of the phyla *Verrucomicrobia* and "*Bacteroidetes"* and mainly *Chitinophagaceae* in the bacterial microbiome of the rhizosphere wild wheat species, and the enrichment of the phyla *Proteobacteria*, *Acidobacteria*, and *Actinobacteria*, and *Nocardioidaceae* in domesticated crop species were also previously found in various crop plants (Cardinale et al., 2015; Pérez-Jaramillo et al., 2017; Kim et al., 2020). The reason for particular bacterial enrichment in the rhizosphere of modern wheat species can be associated with their root exudates which's content is different than their wild ancestor as shown by Iannucci et al. (2017). Furthermore, Prez-jaramillo et al. (2017) found that *Bacteroidetes* were more dominant on the fine root hairs of wild relatives of current bean (*Phaseolus vulgaris* ) whereas *Actinobacteria* and *Proteobacteria* were prevalent on thick roots of modern varieties. There was no clear trend on total bacterial gene copies per gram of rhizosphere soil in wild wheat species and modern wheat species (Fig S6). Only in Weilburgergrenze, the total 16S RNA gene copies were higher in wild wheat species. This result might indicate that fewer bacteria are supported in the rhizosphere of modern wheat species possibly due to limited exude availability. However, DNA fragments can persist for many years and can be quantified along with active DNA genes (Andersen et al., 2001) which limits our DNA-dependent approach.

In contrast to bacterial abundance, more fungal families were differently prevalent in wild species than modern wheat species (Fig. 2 B). Leff et al (2017) found fewer fungal pathogens in modern sunflower strains than the wild sunflower. Another recent study also showed a reduced abundance of potential fungal pathogens in currently cultivated tetraploid wheat compared to wild emmer (Spor et al., 2020). Our results suggest that the development of modern agriculture practices, fertilizer application, conventional agriculture practices, and changes in plant physiology and morphology during plant domestication, influenced the root-associated fungal microbiome. Our

results were in line with the results from previous wheat microbiome studies (Gdanetz & Trail, 2017; Kavamura et al., 2019; Terrazas et al., 2019; Kinnunen-Grubb et al., 2020; Chen et al., 2020). Furthermore, we also observed the reduced fungal microbiome diversity in the spermosphere of the studied modern wheat species compared to their wild relatives (Fig. S4).

In the rhizosphere (Fig. 3, 4) and root endosphere (Fig. S5), we found that inter-kingdom co-occurrence networks have distinct structural features. This structural distinction can be explained by niche differentiation among various plant compartments with significantly differing microhabitats. In the rhizosphere, readily available nutrients, organic compounds exuded by plant roots attract diverse microorganisms to the rhizosphere resulting in microbe-microbe interaction. As a result, it should come as no surprise that rhizosphere soil was the most intricate and connected habitat as reported previously (Lee et al., 2019). In contrast, the root environment contains the cortical layer and vascular tissues, where nutrients are restricted and are favored by microorganisms showing endophytic lifestyles (Sessitsch et al., 2012). This special root environment might reduce the diversity and interaction of bacterial as well as the fungal microbiome, resulting in less complex networks of the root endosphere habitat.

We also compared the bacterial and fungal genera networks and the number of connected nodes in the rhizosphere of modern wheat species with their corresponding wild ancestors and found more microbial interaction in the rhizosphere of wild wheat varieties than domesticated currently cultivated wheat species. The results indicate that wild wheat varieties have stronger inter-kingdom relationships than domesticated wheat types. Similar results have been reported previously by Kavamura et al. (2020). They studied the advanced agriculture effects on root characteristics as well as rhizosphere microbiome composition of eight wheat cultivars (*T. aestivum;* Tall and Semi-dwarf result of selective breeding) under field conditions. They found less microbial network in the rhizosphere of genetically advanced semi-dwarf wheat varieties than the Tall wheat cultivars.

There were more plant pathogenic fungi (*Fusarium* and *Microdochium*) among the most connected fungal genera in the cross-domain network of wild crops than the modern cultivars (Fig. 3, 4 A) instead more genera used as biocontrol agents (*Cladorrhinum, Epicoccum*) became more connected genera in the inter-kingdom network of domesticated species (Fig. 3, 4 B). *Microdochium* is known also as core fungal taxa in the rhizosphere together with genera of *Nectriaceae*, *Ulocladium*, *Alternaria*, and *Mortierella*. *Microdochium* is also reported as hub taxa in cross-kingdom co-occurrence networks of the wheat rhizosphere microbiome (Schlatter et al.,

2020). The members of the *Fusarium* and *Microdochium* are seed-borne wheat-pathogens and cause snow mould, seedling blight, and other serious cereal diseases that make big damage for agriculture (Ren et al., 2015). Beside seed treatments with various fungicides another potential way to repress the growth of plant pathogens is the use of biocontrol agents. Seed treatment with *Pseudomonas*, *Bacillus*, *Microbacterium*, and *Pantoea* can suppress the growth of *Microdochium nivale* and *Fusarium culmorum* (Johansson et al., 2003; Mnasri et al. 2017). Even some of the fungal species can be used as a biocontrol against plant pathogens. For example, *Cladorrhinum* species used as a biocontrol of fungal phytopathogens (Barrera et al., 2019) such as *Cladorrhinum flexuosum* SW315 reduced up to 86% of diseases of winter wheat caused by *Fusarium graminearum*, *Waitea circinata*, or *Microdochium majus* (Abaya et al., 2021). Similarly *Epicoccum* species acts as effective biocontrol agents against several plant pathogens by producing bioactive compounds like epicolactone, fluorophore, flavipin, and others (Taguiam et al., 2021). Application of *Epicoccum nigrum* and AMF promoted the growth of potatoes and decreased the severity of blackleg disease caused by *Pectobacterium carotovora* (Bagy et al., 2019). The application of different methods to reduce diseases in cereals resulted in reduced fungal diversity which in turn resulted in less network between domains. Additionally, the rhizosphere microbiome structure can be shifted as a result of seed treatments (introducing new members or removing core fungi inoculants).

The quantitative PCR data analysis of the rhizosphere microbiome showed significant reductions in the abundance of *nirS* genes, which encodes enzymes involved in nitrogen denitrification, in the rhizosphere of domesticated wheat species compared to their wild relatives in two locations. Similar results were also recorded by Spor and colleagues (2020) and the authors concluded that, the nitrifiers and AMF decreased in modern wheat species as a result of reduced microbe-host interactions. However, the study by Spor et al. (2020) was a pot study where they used the soil collected from the same location and carried out under controlled conditions. In contrast, we observed these changes in different locations under uncontrolled conditions. Therefore we found different results in each location depending on soil conditions. The most interesting finding is the variable effect of domestication between genetically related groups suggesting genetic mutations in plant genome during domestication might influence the structure, function, microbial recruitments, and microbial interactions of the rhizosphere microbiome. A small fraction of homologous *loci* harboring current hexaploid bread wheat or segmented footprints and trait *loci* selection during domestication washes or pushing phenotypic qualities show simultaneous signals

(Pont et al., 2019). The phenotypic qualities of durum wheat and bread wheat are considerably varied as compared to their wild relatives *A. tauschii* and wild emmer (Gioia et al., 2015). These genetic changes in the modern plant genome were found to be the potential sources of hologenomic diversity (Hacquard, 2016).

Most of the bacterial genera that positively correlated with fungal genera in the microbial network of wheat species were positively correlated with *nirS*, potential alkaline PA, and urease activity (Table S3). *Ferruginibacter*, *Microvirga*, *Microlunatus*, *Luteolibacter*, *Flavisolibacter*, *Brevundimonas*, genera of the family *Vicinamibacteraceae* were positively correlated with *nirS* and potential urease, *Lysobacter*, *Bryobacter*, and an unknown genus named *SC-I-84* from *Burkholderiales* on the other hand positively correlated with potential alkaline PA. Indeed, *Brevundimonas* is one of the most studied diazotrophic endophytes that can effectively fix nitrogen (Chiba et al., 2021; Johnston-Monje & Raizada, 2011; Montañez et al., 2009), *Microvirga* is also one of the genera among nitrogen-fixing bacteria groups. The genera *Ferruginibacter*, *Flavisolibacter* belong to *Micromonosporaceae* or *Chitinophagaceae* family known as plant-growth-promoting bacteria and are enriched in the rhizosphere of wild raspberry (Oszust & Frąc, 2021). The members of this family produce sphingolipids, xylanase, and trehalase encoding genes, as well as they, are considered anaerobic denitrifiers and iron-reducing bacteria (Oszust & Frąc, 2021). The high relative abundance of *Chitinophagaceae* is also found in the wild common bean rhizosphere as compared to its domesticated sort (Pérez-Jaramillo et al., 2017). The potential activity of acid and alkaline PA is differentiated by the source of production. It is believed that the origin of acid PA is both plant and bacteria, on the other hand, the alkaline PA originates from soil bacteria (Ragot et al., 2015). In this study, the potential alkaline PA activity positively correlated with *phoX* gene abundance which encodes for this enzyme (Fig. S7). Work of Luo et al. (2017) and Ragot et al. (2017) showed that *Burkholderia* and *Lysobacter* are among the dominant *phoD*-harbouring (*phoD* is another gene that encodes bacterial alkaline PA which is originally developed from forest soil) bacterial genera and were significantly correlated with alkaline PA activity (Luo et al. 2017; Ragot et al., 2017). The results suggest that the aforementioned bacterial genera (*Ferruginibacter*, *Microvirga*, *Microlunatus*, *Luteolibacter*, *Flavisolibacter*, *Brevundimonas*, *Lysobacter*, *Bryobacter*) can be the potential collaborators of fungal genera as we found a higher number of cross-kingdom connections, and this relationship can play important role in plant nutrient availability. As well as most of the bacterial genera that positively correlated belonged to

wild wheat cross-kingdom networks that imply wild plants might preserve more genetic information in their genome about the making positive microbe-microbe interactions

**Conclusions**

Genetic alterations in plant genomes have changed the physiology, morphology, phenology of crop plants during domestication but a the same time the rhizosphere microbiome structure and their function was significantly changed and the microbial network was weakened.

More bacterial families were differently abundant in the rhizosphere of modern wheat species. On the contrary, more fungal families were differentially abundant in the rhizosphere of wild relatives.

Plant domestication strongly affected the abundance of bacterial *nirS* genes in the rhizosphere that encode protein involved in denitrification due to reduced trait *loci* in the genome of modern wheat species which is responsible for establishing interaction between host plants with their associated microbes.

Relative bacterial gene abundance of other functional genes involved in the N – and P-cycle were not different in the rhizosphere of wild and domesticated wheat although the microbiome was affected. However, gene abundance of alkaline phosphatase gene *phoX* correlated with potential phosphatase activity in the rhizosphere.

The microbial cross-domain co-occurrence network analysis demonstrated that domestication reduced the interactions between bacterial and fungal communities in the endorhiza and rhizosphere as a result of reduced fungal microbiome diversity caused by modern agriculture practices.

Knowledge on the community structure of the rhizosphere microbiome and their interactions with microbial pathogens can be used in the future approaching a sustainable agriculture

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*Supplementary material to:*

# Host-dependent shifts of the inter-kingdom interactions in the wheat rhizosphere microbiota during plant domestication

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**Table S1.** The date of sampling in 2019.

| **Sampling plan of MikroNet project** | | | | | | | |
|---|---|---|---|---|---|---|---|
| **Plant species** | **Location** | **Blocks** | **Wheat accessions** | **Roots** | **Rhizosphere** | **Bulk soil** | **Date of sampling** |
| ***T.diccocoides*** | GG | a | TRI 18524 | x | x | x | 15.05.2019 |
| | GG | b | TRI 18524 | x | x | x | 15.05.2019 |
| | GG | c | TRI 18524 | x | x | x | 15.05.2019 |
| | WG | a | TRI 18524 | x | x | x | 24.05.2019 |
| | WG | b | TRI 18524 | x | x | x | 24.05.2019 |
| | WG | c | TRI 18524 | x | x | x | 24.05.2019 |
| | RH | a | TRI 18524 | x | x | x | 05.06.2019 |
| | RH | b | TRI 18524 | x | x | x | 05.06.2019 |
| | RH | c | TRI 18524 | x | x | x | 05.06.2019 |
| ***T.durum*** | GG | a | TRI 10715 | x | x | x | 15.05.2019 |
| | GG | b | TRI 10715 | x | x | x | 15.05.2019 |
| | GG | c | TRI 10715 | x | x | x | 15.05.2019 |
| | WG | a | TRI 10715 | x | x | x | 24.05.2019 |
| | WG | b | TRI 10715 | x | x | x | 24.05.2019 |
| | WG | c | TRI 10715 | x | x | x | 24.05.2019 |
| | RH | a | TRI 10715 | x | x | x | 05.06.2019 |
| | RH | b | TRI 10715 | x | x | x | 05.06.2019 |
| | RH | c | TRI 10715 | x | x | x | 05.06.2019 |
| ***T.aestivum*** | GG | a | TRI 368 | x | x | x | 14.06.2019 |
| | GG | b | TRI 368 | x | x | x | 14.06.2019 |
| | GG | c | TRI 368 | x | x | x | 14.06.2019 |
| | WG | a | TRI 368 | x | x | x | 04.06.2019 |
| | WG | b | TRI 368 | x | x | x | 04.06.2019 |
| | WG | c | TRI 368 | x | x | x | 04.06.2019 |
| | RH | a | TRI 368 | x | x | x | 12.06.2019 |
| | RH | b | TRI 368 | x | x | x | 12.06.2019 |
| | RH | c | TRI 368 | x | x | x | 12.06.2019 |
| ***A. tauschii*** | GG | a | AE 220 | x | x | x | 14.06.2019 |
| | GG | b | AE 220 | x | x | x | 14.06.2019 |
| | GG | c | AE 220 | x | x | x | 14.06.2019 |
| | WG | a | AE 220 | x | x | x | 04.06.2019 |
| | WG | b | AE 220 | x | x | x | 04.06.2019 |
| | WG | c | AE 220 | x | x | x | 04.06.2019 |
| | RH | a | AE 220 | x | x | x | 05.06.2019 |
| | RH | b | AE 220 | x | x | x | 05.06.2019 |
| | RH | c | AE 220 | x | x | x | 05.06.2019 |

**Table S2.** Origins of standard sequences, primer sequences, and real-time PCR program.

| Target | Origins of standard sequence | Primer sequence (5` → 3`) [a] | references | qPCR program |
|---|---|---|---|---|
| Bacterial 16S rRNA gene | *Verrucomicrobium spinosum* (DSM 4136) | **F:** AYT GGG YDT AAA GNG<br>**R:** CCG TCA ATT TCM TTT RAG TTT | Claesson et al. 2009 | 95°C 15 min<br>95°C 45s<br>60°C 45s<br>72°C 1min<br>84°C 20s<br>60°C 15s<br>40c |
| Fungal ITS2 fragment | *Saccharaomyces cerevisiae* (DSM 1334) | **F:** GCA TCG ATG AAG GCA GC<br>**R:** TCC TCC GCT TAT TGA TAT GC | Manter & Vivanco 2007 | 95°C 10 min<br>95°C 15s<br>55°C 30s<br>72°C 30s<br>76°C 30s<br>60°C 15s<br>35c |
| *nirS* | *Cupriavidus necator* (DSMZ 530) | **F**: CAGRTTRTGGTT<br>**R:** GAS TTC GGR TGS GTC TTG A | Throbäck *et al*. 2004 | 95°C 15 min<br>95°C 45s<br>60°C 45s<br>72°C 1min<br>84°C 20s<br>60°C 15s<br>40c |
| *nosZ* Bacteria Cl. I - typical | *Pseudomonas fluorescens* (E8) | **F:**CGC RAC GGC AAS AAG GTS MSS GT<br>**R:**CAK RTG CAK SGC RTG GCA GAA | Henry et al. 2006 | 95°C 15 min<br>95°C 45s<br>60°C 45s<br>72°C 1min<br>84°C 20s<br>60°C 15s<br>40c |
| Bacterial *amoA* | *Nitrosospira* sp. | **F:** GGG GTT TCT ACT GGT GGT<br>**R:** CCC CTC KGS AAA GCC TTC TTC | Rotthauwe et al. 1997 | 95°C 15 min<br>95°C 45s<br>60°C 45s<br>72°C 1min<br>84°C 20s<br>60°C 15s<br>40c |
| *phoX* | *Rhodococcus opacus* B4 | **F:** CAG TTC GGB TWC AAC AAC GA<br>**R:** CGG CCC AGS GCR GTG YGY TT | Ragot et al., 2016 | 95°C 15 min<br>95°C 45s<br>60°C 45s<br>72°C 1min<br>84°C 20s<br>60°C 15s<br>40c |

**a** F and R indicate forward and reverse primers, respectively.

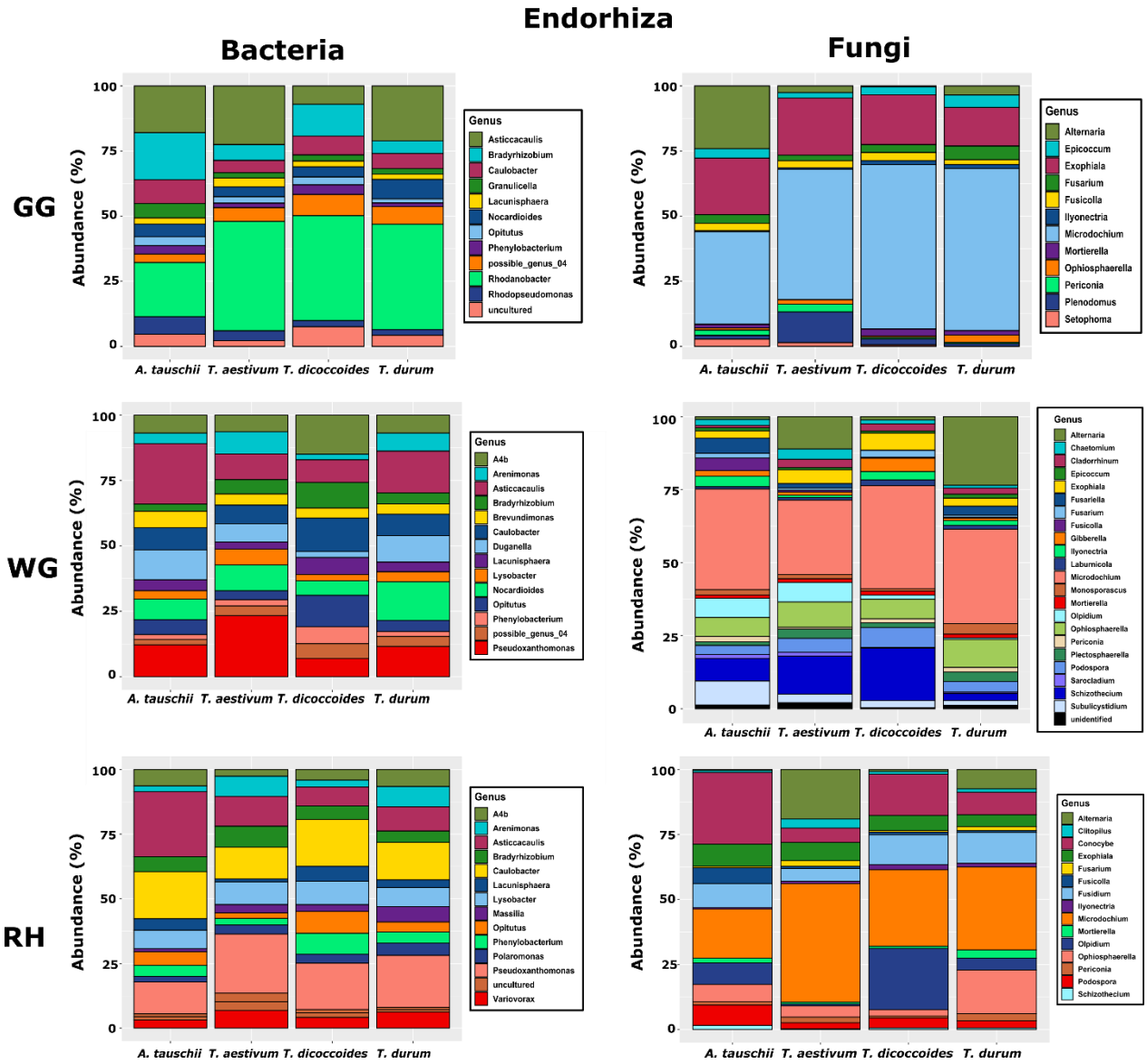



**Figure S1.** The taxonomic classification of bacterial and fungal core microbiome in endorhiza. Bacterial genera 99 %, and fungal genera 75% predominant in endorhiza samples in each location (GG- Groß-Gerau, WG-Weilburger-Grenze, and RH-Rauischholzhausen).

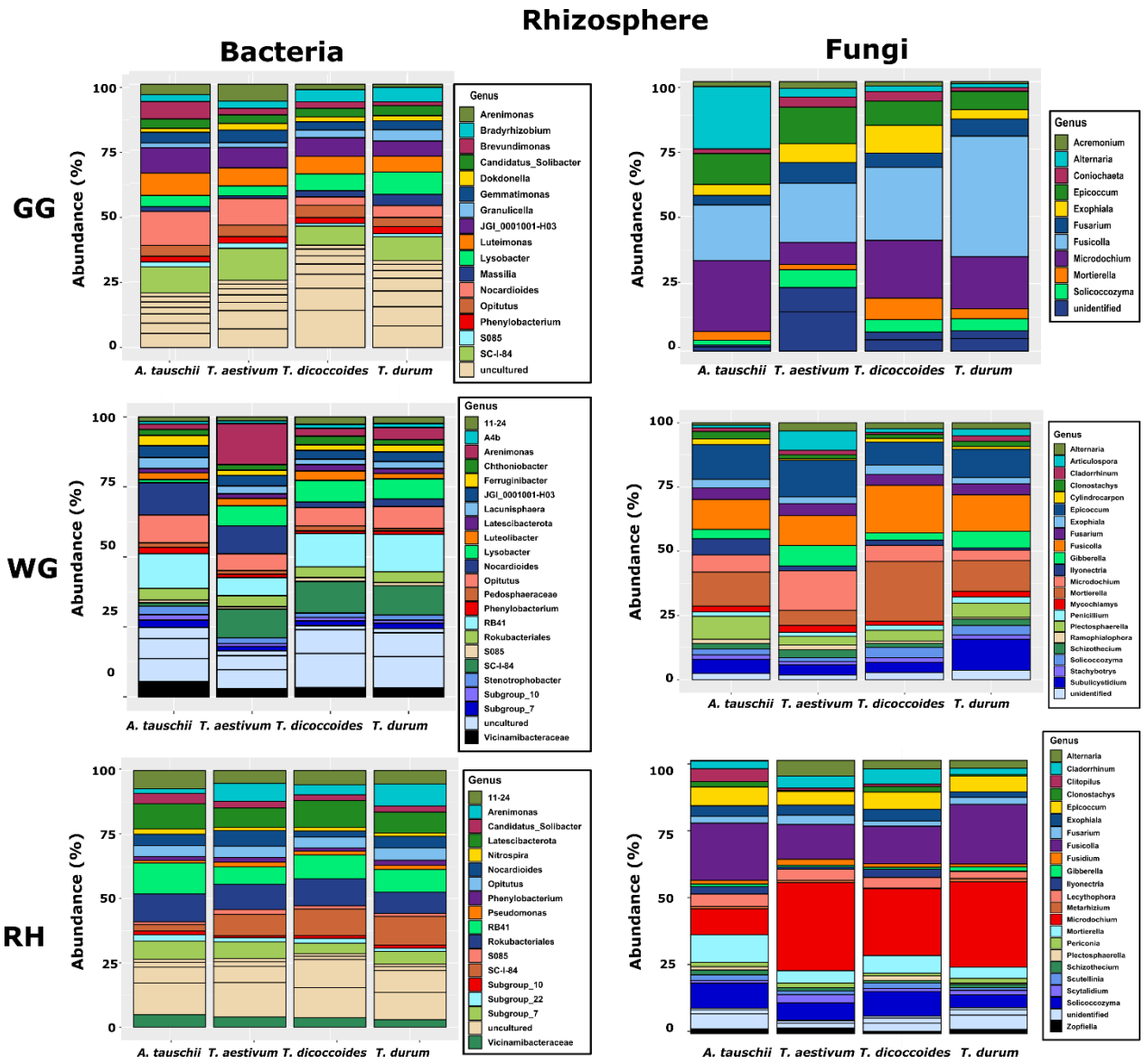
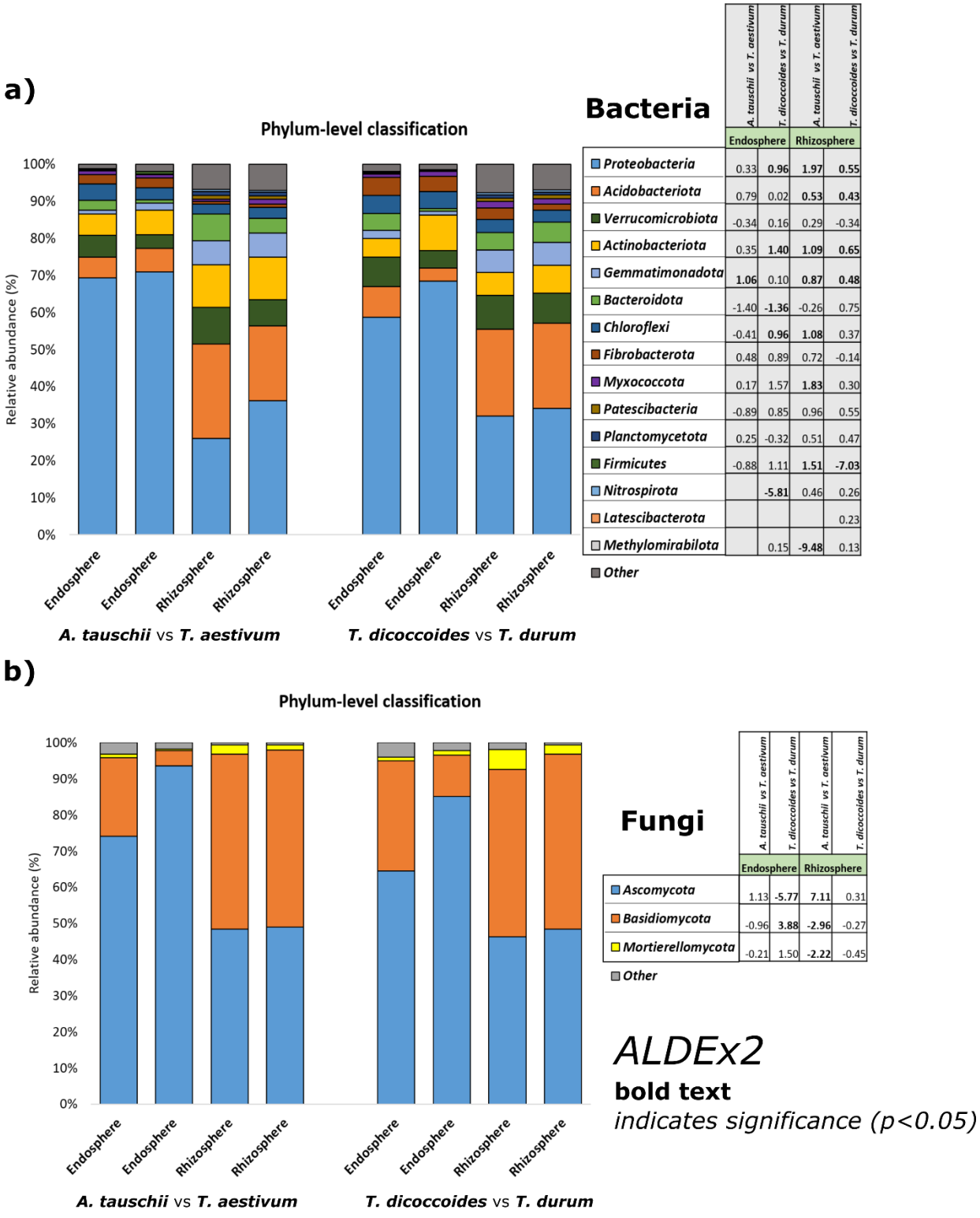



**Figure S2.** The taxonomic classification of bacterial and fungal core microbiome in endorhiza. Bacterial genera 99 %, and fungal genera 75% predominant in endorhiza samples in each location (GG- Groß-Gerau, WG-Weilburger-Grenze, and RH-Rauischholzhausen).




Bacteria

| | A. tauschii vs T. aestivum | T. dicoccoides vs T. durum | A. tauschii vs T. aestivum | T. dicoccoides vs T. durum |
|---|---|---|---|---|
| | Endosphere | | Rhizosphere | |
| Proteobacteria | 0.33 | **0.96** | **1.97** | **0.55** |
| Acidobacteriota | 0.79 | 0.02 | **0.53** | **0.43** |
| Verrucomicrobiota | -0.34 | 0.16 | 0.29 | -0.34 |
| Actinobacteriota | 0.35 | **1.40** | **1.09** | **0.65** |
| Gemmatimonadota | **1.06** | 0.10 | **0.87** | **0.48** |
| Bacteroidota | -1.40 | **-1.36** | -0.26 | 0.75 |
| Chloroflexi | -0.41 | **0.96** | **1.08** | 0.37 |
| Fibrobacterota | 0.48 | 0.89 | 0.72 | -0.14 |
| Myxococcota | 0.17 | 1.57 | **1.83** | 0.30 |
| Patescibacteria | -0.89 | 0.85 | 0.96 | 0.55 |
| Planctomycetota | 0.25 | -0.32 | 0.51 | 0.47 |
| Firmicutes | -0.88 | 1.11 | **1.51** | **-7.03** |
| Nitrospirota | | **-5.81** | 0.46 | 0.26 |
| Latescibacterota | | | | 0.23 |
| Methylomirabilota | | 0.15 | **-9.48** | 0.13 |
| Other | | | | |

**Figure S3.** Bacterial (a) and fungal (b) microbiome composition at the phylum (99 % predominant) level (root n=9, rhizosphere n=9). The median difference in centered log-ratio (*clr*) values between the microbiota of domesticated wheat species (*T. aestivum*, *T. durum*) and their corresponding wild ancestors (*A. tauschii*, *T. dicoccoides*) in different plant compartments is shown in the tables on the right side, which is determined from the ALDEx2 differential abundance test. Bold text indicates the significance between groups in different plant habitats. Positive numbers

represent extend of particular taxon enrichment in domesticated wheat species (*T. aestivum*, *T. durum*) whereas negative numbers show the degree of enrichment in wild plants (*A. tauschii*, *T. dicoccoides*). The empty cells show no change in abundance between genetically related wheat species.

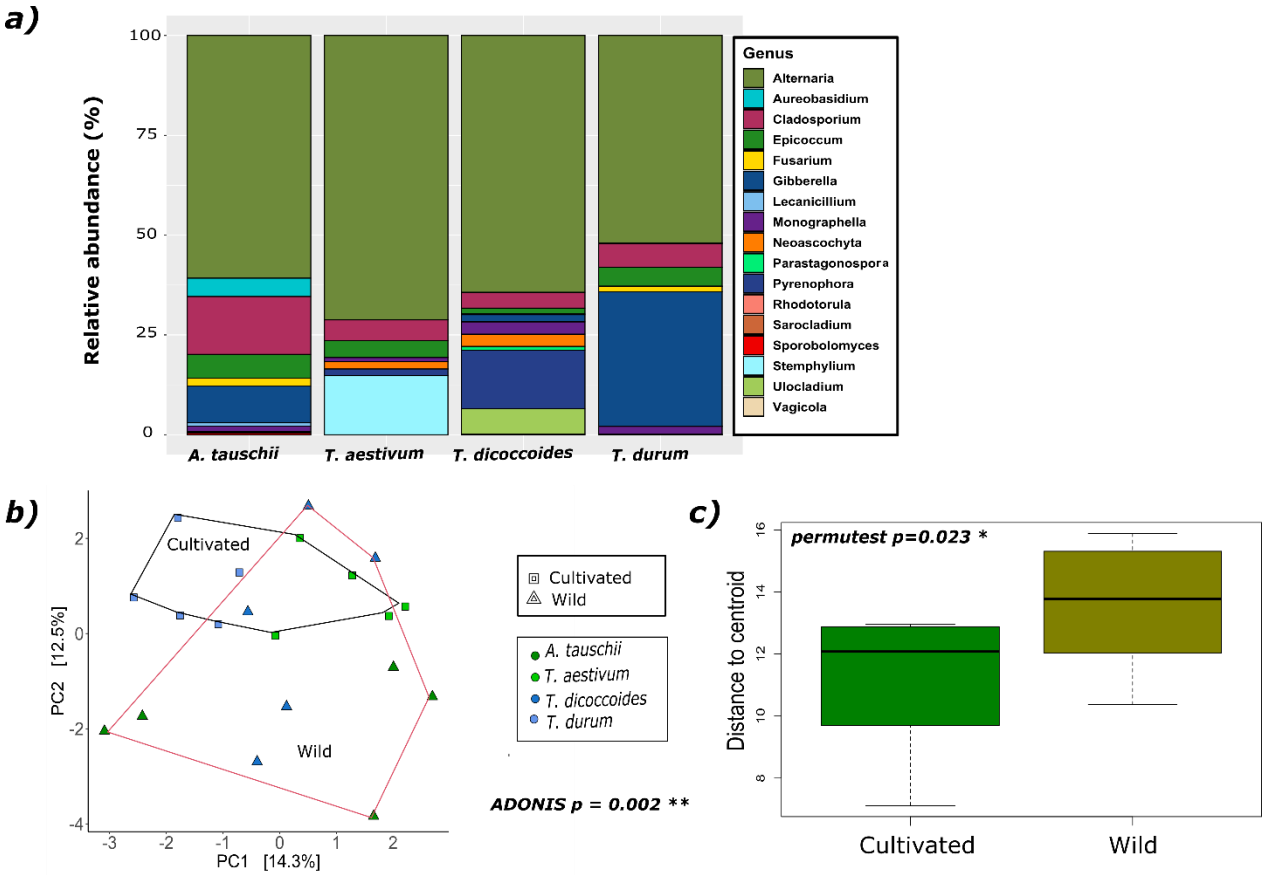



**Figure S4**. Spermosphere fungal microbiome of wild and domesticated wheat species (wild *A. tauschii*, *T. dicoccoides*, and modern *T. aestivum*, *T. durum*). Figure (**a)** shows the relative abundance of fungal genera of seed endophytes. Unconstrained ordination based on Euclidian distance matrices of spermosphere fungal microbiome (**b**). Euclidian distance calculated from the data transformed to the centered log-ratio. The box plots (**c**) represent the range of distances from the centroid based on Euclidian distance matrices of fungal compositions.

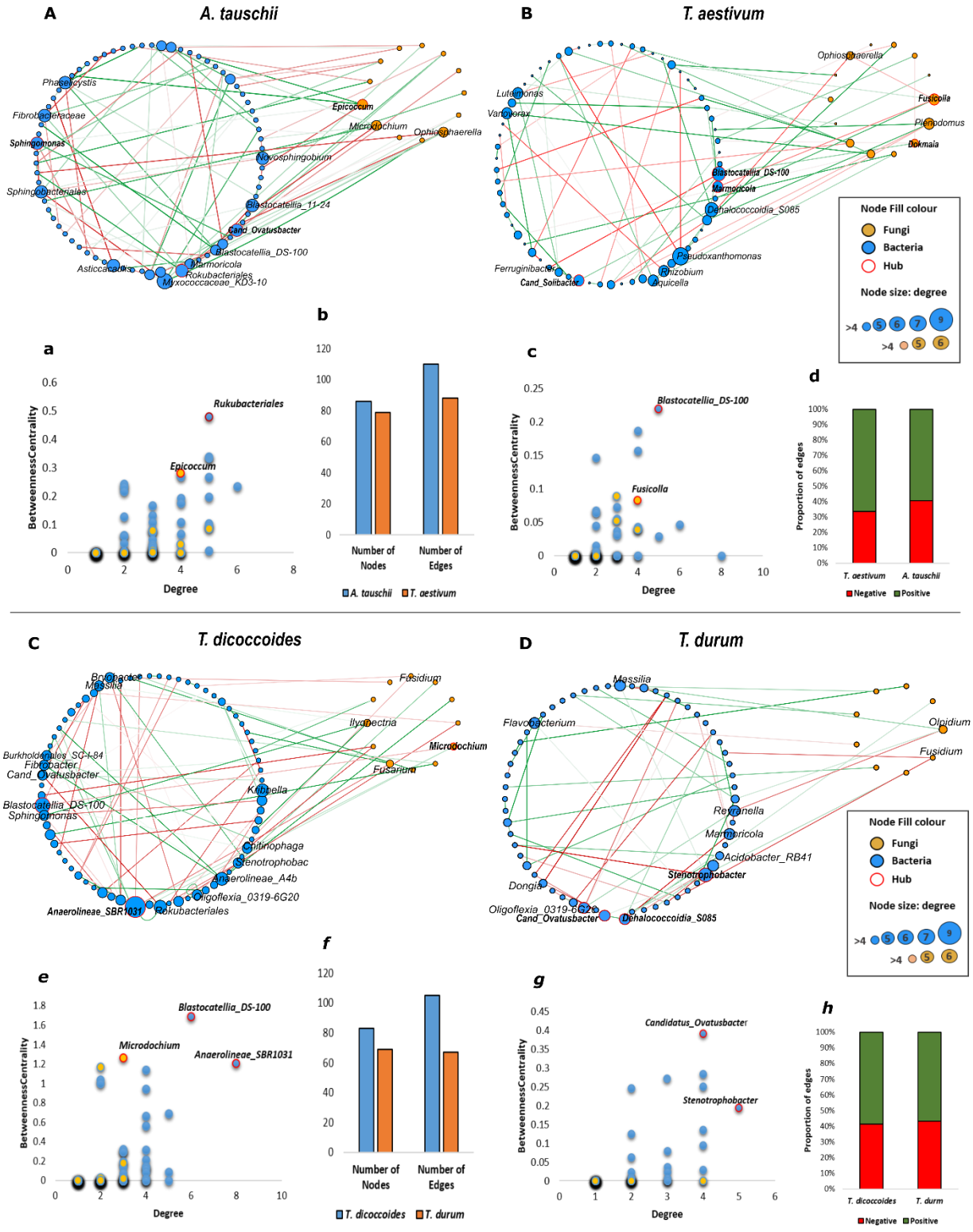



**Figure S5**. Bacteria-fungi interkingdom association of the endosphere microbiota. Co-occurrence-based network of endosphere microbial genera detected in genetically related groups: wild *A. tauschii* (A), domesticated wheat *T. aestivum* (B) and wild *T. dicoccoides* (C), domesticated wheat *T. durum* (D). Each node corresponds to genera, and edges between nodes correspond to either positive (green) or negative (red) correlations inferred from genera abundance profiles using the SpiecEasi method (pseudo $p < 0.05$, correlation values $< -0.3$ or $> 0.3$). Scatter plots

(a,c, e, g) show connectivity scores of the nodes and hub genera based on degree, betweenness centrality, and closeness centrality. Genera belonging to different microbial kingdoms have different color codes (bacteria, blue; fungi, orange), and node size reflects their edge counts between genera. Bar graph (b,f) shows the number of nodes and proportion among the genetic groups, and bar graphs (d,h) shows the proportion of intra-kingdom edges of positive (green) or negative (red) correlations in the rhizosphere network.

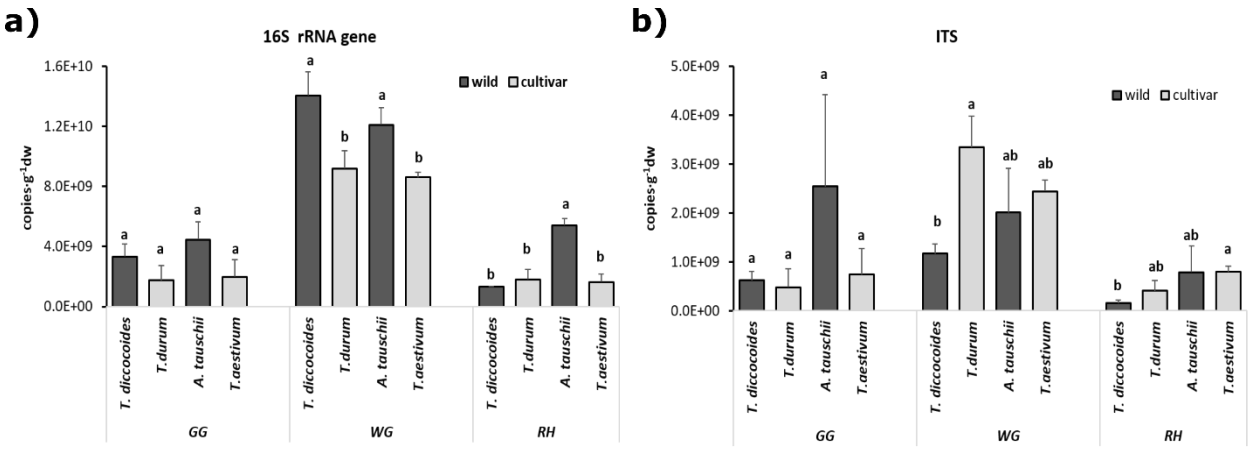



**Figure S6.** Total 16S rRNA and ITS gene copies per gram of soil dry weight (rhizosphere soil collected from four wheat species grown in GG, WG, RH research stations). Each bar represents 12 replicates (4 technical replicates from 3 biological replicates from each field). Small letters show the significant difference between wheat species. Tukey posthoc test (HSD test) and grouping if ANOVA $p <= 0.05$.

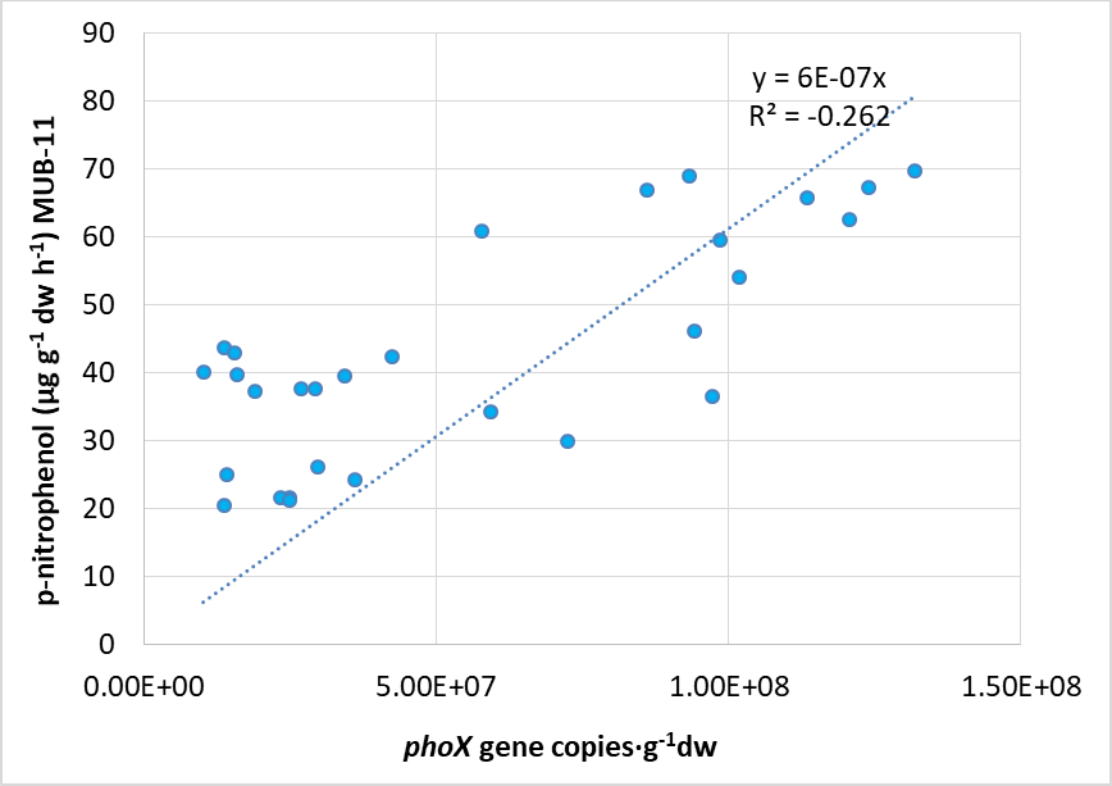



**Figure S7.** Correlation between potential alkaline phosphomonoesterase activity and gene (*phoX*) copy numbers responsible for the alkaline phosphomonoesterase enzyme production.

| **Table S3** | Differencial abundance test between wild and modern wheat species endorhiza and rhizosphere bacterial microbiome at family level. The differently abundant bacterial family are considered as significant absolute aldex affect size bigger than 1 or lower than -1. negative - *A. tauschii,* positive -*T. aestivum,* negative - *T. dicoccoides,* positive -*T. durum* | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| ***A. tauschii: T. aestivum*** | **Rhizosphere** | **Family** | **effect** | **overlap** | **we.ep** | **we.eBH** | **wi.ep** | **wi.eBH** |
| | | *Pyrinomonadaceae* | -2,9281 | 0,0001 | 0,0001 | 0,0002 | 0,0001 | 0,0002 |
| | | *Nitrosomonadaceae* | -2,4663 | 0,0001 | 0,0009 | 0,0014 | 0,0001 | 0,0002 |
| | | *BIrii41* | 3,3706 | 0,0001 | 0,0001 | 0,0001 | 0,0001 | 0,0002 |
| | | *Rhizobiaceae* | 3,6397 | 0,0001 | 0,0000 | 0,0001 | 0,0001 | 0,0002 |
| | | *A21b* | 3,6134 | 0,0001 | 0,0000 | 0,0001 | 0,0001 | 0,0002 |
| | | *Polyangiaceae* | 4,0173 | 0,0001 | 0,0000 | 0,0001 | 0,0001 | 0,0002 |
| | | *Nocardioidaceae* | 2,3973 | 0,0020 | 0,0000 | 0,0000 | 0,0001 | 0,0002 |
| | | *Diplorickettsiaceae* | -2,5501 | 0,0020 | 0,0006 | 0,0010 | 0,0001 | 0,0002 |
| | | *Rubritaleaceae* | -2,5855 | 0,0001 | 0,0004 | 0,0008 | 0,0001 | 0,0002 |
| | | *Rokubacteriales* | -2,1239 | 0,0020 | 0,0004 | 0,0006 | 0,0001 | 0,0002 |
| | | *Kapabacteriales* | -2,1527 | 0,0001 | 0,0022 | 0,0030 | 0,0001 | 0,0003 |
| | | *Xanthomonadaceae* | 2,2201 | 0,0020 | 0,0000 | 0,0000 | 0,0001 | 0,0003 |
| | | *Flavobacteriaceae* | -1,9705 | 0,0020 | 0,0011 | 0,0017 | 0,0001 | 0,0003 |
| | | *SC-I-84* | 2,0314 | 0,0020 | 0,0000 | 0,0001 | 0,0001 | 0,0003 |
| | | *Gemmatimonadaceae* | 2,4163 | 0,0059 | 0,0000 | 0,0001 | 0,0001 | 0,0003 |
| | | *TK10* | -1,9495 | 0,0020 | 0,0008 | 0,0012 | 0,0001 | 0,0003 |
| | | *Gaiellaceae* | -2,0522 | 0,0039 | 0,0013 | 0,0019 | 0,0001 | 0,0003 |
| | | *A4b* | 2,0230 | 0,0059 | 0,0000 | 0,0001 | 0,0001 | 0,0003 |
| | | *Caulobacteraceae* | 1,9061 | 0,0098 | 0,0001 | 0,0002 | 0,0002 | 0,0004 |
| | | *S085* | 1,8232 | 0,0078 | 0,0002 | 0,0005 | 0,0002 | 0,0004 |
| | | *11-24* | -1,8430 | 0,0117 | 0,0003 | 0,0005 | 0,0002 | 0,0004 |
| | | *Oxalobacteraceae* | 1,6091 | 0,0156 | 0,0004 | 0,0009 | 0,0002 | 0,0004 |
| | | *Entotheonellaceae* | -1,7206 | 0,0137 | 0,0026 | 0,0036 | 0,0002 | 0,0004 |
| | | *Dongiaceae* | 1,7551 | 0,0156 | 0,0006 | 0,0011 | 0,0002 | 0,0005 |
| | | *Propionibacteriaceae* | 1,7760 | 0,0215 | 0,0001 | 0,0003 | 0,0002 | 0,0005 |
| | | *Intrasporangiaceae* | 1,8149 | 0,0156 | 0,0002 | 0,0005 | 0,0003 | 0,0005 |
| | | *Verrucomicrobiaceae* | -1,7263 | 0,0195 | 0,0017 | 0,0025 | 0,0003 | 0,0006 |
| | | *Reyranellaceae* | 2,0222 | 0,0253 | 0,0000 | 0,0001 | 0,0004 | 0,0007 |
| | | *env.OPS_17* | -1,4953 | 0,0234 | 0,0025 | 0,0033 | 0,0005 | 0,0009 |
| | | *Unknown_Family* | -1,5571 | 0,0254 | 0,0022 | 0,0029 | 0,0006 | 0,0009 |
| | | *Solirubrobacteraceae* | -1,4690 | 0,0253 | 0,0053 | 0,0065 | 0,0006 | 0,0010 |
| | | *Sandaracinaceae* | 1,6497 | 0,0331 | 0,0018 | 0,0028 | 0,0006 | 0,0011 |
| | | *WD2101_soil_group* | 1,4314 | 0,0390 | 0,0008 | 0,0014 | 0,0006 | 0,0011 |
| | | *Candidatus_Levybacteria* | 1,6539 | 0,0370 | 0,0007 | 0,0011 | 0,0008 | 0,0012 |
| | | *Xanthobacteraceae* | 1,4876 | 0,0468 | 0,0003 | 0,0008 | 0,0011 | 0,0017 |
| | | *Anaerolineaceae* | -1,3357 | 0,0684 | 0,0053 | 0,0066 | 0,0020 | 0,0027 |
| | | *Opitutaceae* | 1,4344 | 0,0762 | 0,0008 | 0,0015 | 0,0019 | 0,0028 |
| | | *Blastocatellaceae* | 1,4222 | 0,0760 | 0,0010 | 0,0018 | 0,0021 | 0,0031 |
| | | *Comamonadaceae* | 1,5175 | 0,0955 | 0,0005 | 0,0011 | 0,0028 | 0,0039 |
| | | *SBR1031* | 1,1987 | 0,0858 | 0,0027 | 0,0039 | 0,0031 | 0,0042 |
| | | *Hymenobacteraceae* | -1,3098 | 0,0566 | 0,0067 | 0,0081 | 0,0034 | 0,0043 |
| | | *Chthoniobacteraceae* | 1,6243 | 0,0781 | 0,0026 | 0,0037 | 0,0038 | 0,0050 |
| | | *Sphingomonadaceae* | 1,4910 | 0,1016 | 0,0013 | 0,0022 | 0,0039 | 0,0051 |
| | | *Solibacteraceae* | 1,2515 | 0,0918 | 0,0021 | 0,0031 | 0,0039 | 0,0051 |
| | | *Legionellaceae* | -1,2770 | 0,0547 | 0,0101 | 0,0118 | 0,0047 | 0,0056 |
| | | *DS-100* | 1,2615 | 0,0879 | 0,0066 | 0,0081 | 0,0050 | 0,0064 |
| | | *Beijerinckiaceae* | 1,1370 | 0,1170 | 0,0033 | 0,0047 | 0,0069 | 0,0086 |
| | | *Abditibacteriaceae* | 1,1772 | 0,1152 | 0,0046 | 0,0061 | 0,0083 | 0,0102 |
| | | *Gemmataceae* | -1,1483 | 0,0938 | 0,0073 | 0,0088 | 0,0091 | 0,0105 |
| | **Endorhiza** | | **effect** | **overlap** | **we.ep** | **we.eBH** | **wi.ep** | **wi.eBH** |
| | | *Microscillaceae* | 2,62661 | 0,00012 | 0,00007 | 0,00029 | 0,00004 | 0,00016 |
| | | *S085* | 3,09603 | 0,00012 | 0,00014 | 0,00048 | 0,00004 | 0,00016 |
| | | *Thermoanaerobaculaceae* | 3,61647 | 0,00012 | 0,00002 | 0,00011 | 0,00004 | 0,00016 |
| | | *Rhizobiaceae* | -2,84878 | 0,00012 | 0,00011 | 0,00038 | 0,00004 | 0,00016 |
| | | *Sphingomonadaceae* | -2,77046 | 0,00012 | 0,00010 | 0,00036 | 0,00004 | 0,00016 |
| | | *SC-I-84* | 3,81245 | 0,00012 | 0,00002 | 0,00008 | 0,00004 | 0,00016 |
| | | *Acetobacteraceae* | 2,80994 | 0,00012 | 0,00005 | 0,00019 | 0,00004 | 0,00016 |
| | | *env.OPS_17* | -2,35211 | 0,00012 | 0,00048 | 0,00144 | 0,00004 | 0,00017 |
| | | *Streptomycetaceae* | -2,39202 | 0,00175 | 0,00035 | 0,00114 | 0,00004 | 0,00017 |
| | | *Cellvibrionaceae* | -2,15024 | 0,00012 | 0,00043 | 0,00137 | 0,00005 | 0,00019 |
| | | *Vicinamibacteraceae* | -2,10147 | 0,00175 | 0,00020 | 0,00066 | 0,00006 | 0,00021 |
| | | *DS-100* | -1,99345 | 0,00521 | 0,00055 | 0,00170 | 0,00007 | 0,00024 |
| | | *Pseudonocardiaceae* | -1,80754 | 0,01733 | 0,00018 | 0,00065 | 0,00021 | 0,00066 |
| | | *11-24* | -1,56009 | 0,02604 | 0,00062 | 0,00194 | 0,00035 | 0,00106 |
| | | *Myxococcaceae* | -1,46609 | 0,02253 | 0,00127 | 0,00362 | 0,00051 | 0,00151 |
| | | *Gemmatimonadaceae* | 1,35934 | 0,04160 | 0,00069 | 0,00224 | 0,00053 | 0,00160 |
| | | *Reyranellaceae* | 1,27212 | 0,06250 | 0,00177 | 0,00521 | 0,00138 | 0,00392 |

| ***T. dicoccoides : T. durum*** | **Rhizosphere** | **Family** | **effect** | **overlap** | **we.ep** | **we.eBH** | **wi.ep** | **wi.eBH** |
|---|---|---|---|---|---|---|---|---|
| | | *Rhizobiaceae* | -2,60993 | 0,00012 | 0,00012 | 0,00070 | 4,11E-05 | 2,34E-04 |
| | | *Methylophilaceae* | 2,79564 | 0,00012 | 0,00013 | 0,00072 | 4,11E-05 | 2,34E-04 |
| | | *Thermoanaerobaculaceae* | 2,72172 | 0,00175 | 0,00003 | 0,00020 | 4,11E-05 | 2,34E-04 |
| | | *Devosiaceae* | 2,76273 | 0,00012 | 0,00009 | 0,00060 | 4,11E-05 | 2,34E-04 |
| | | *Gaiellaceae* | 3,59248 | 0,00012 | 0,00004 | 0,00029 | 4,11E-05 | 2,34E-04 |
| | | *Roseiflexaceae* | 2,16880 | 0,00175 | 0,00064 | 0,00313 | 4,24E-05 | 2,39E-04 |
| | | *Candidatus_Levybacteria* | -2,28847 | 0,00175 | 0,00056 | 0,00253 | 4,43E-05 | 2,48E-04 |
| | | *SBR1031* | -2,19697 | 0,00175 | 0,00027 | 0,00142 | 4,63E-05 | 2,54E-04 |
| | | *IMCC26256* | -2,15805 | 0,00175 | 0,00070 | 0,00315 | 5,11E-05 | 2,77E-04 |
| | | *Spirosomaceae* | -2,07056 | 0,00175 | 0,00068 | 0,00309 | 5,27E-05 | 2,85E-04 |
| | | *Haliangiaceae* | 1,97745 | 0,00175 | 0,00079 | 0,00336 | 5,40E-05 | 2,89E-04 |
| | | *Cellvibrionaceae* | -1,77569 | 0,00348 | 0,00045 | 0,00222 | 5,62E-05 | 2,99E-04 |
| | | *Solimonadaceae* | -2,02432 | 0,01040 | 0,00037 | 0,00181 | 6,36E-05 | 3,31E-04 |
| | | *env.OPS_17* | -1,93381 | 0,01040 | 0,00022 | 0,00120 | 8,58E-05 | 4,28E-04 |
| | | *Ilumatobacteraceae* | -1,86753 | 0,01040 | 0,00074 | 0,00342 | 1,19E-04 | 5,78E-04 |
| | | *Parachlamydiaceae* | -1,58291 | 0,01733 | 0,00146 | 0,00622 | 1,57E-04 | 7,32E-04 |
| | | *Gemmataceae* | -1,38537 | 0,03125 | 0,00198 | 0,00804 | 5,09E-04 | 2,21E-03 |
| | | *Paracaedibacteraceae* | -1,35056 | 0,04333 | 0,00268 | 0,01066 | 9,34E-04 | 3,81E-03 |
| | | *Xanthomonadaceae* | 1,13988 | 0,10919 | 0,00334 | 0,01432 | 4,50E-03 | 1,79E-02 |
| | **Endorhiza** | | **effect** | **overlap** | **we.ep** | **we.eBH** | **wi.ep** | **wi.eBH** |
| | | *Micrococcaceae* | 3,07408 | 0,00012 | 0,00006 | 0,00026 | 4,11E-05 | 1,55E-04 |
| | | *Saccharimonadales* | 3,26357 | 0,00012 | 0,00005 | 0,00020 | 4,11E-05 | 1,55E-04 |
| | | Streptomycetaceae | 3,57697 | 0,00012 | 0,00003 | 0,00014 | 4,11E-05 | 1,55E-04 |
| | | *Intrasporangiaceae* | 2,43934 | 0,00012 | 0,00019 | 0,00054 | 4,15E-05 | 1,55E-04 |
| | | *Sandaracinaceae* | -2,49995 | 0,00012 | 0,00020 | 0,00064 | 4,18E-05 | 1,56E-04 |
| | | *Chitinophagaceae* | -2,45261 | 0,00012 | 0,00014 | 0,00039 | 4,37E-05 | 1,61E-04 |
| | | *Micromonosporaceae* | 2,71157 | 0,00175 | 0,00008 | 0,00026 | 4,40E-05 | 1,61E-04 |
| | | *11-24* | -2,00800 | 0,00012 | 0,00040 | 0,00093 | 4,43E-05 | 1,62E-04 |
| | | *Blrii41* | -2,09967 | 0,00348 | 0,00034 | 0,00085 | 4,63E-05 | 1,66E-04 |
| | | *Thermoanaerobaculaceae* | -2,19003 | 0,00175 | 0,00049 | 0,00117 | 4,79E-05 | 1,71E-04 |
| | | *Oxalobacteraceae* | 1,65839 | 0,00694 | 0,00049 | 0,00144 | 5,43E-05 | 1,85E-04 |
| | | *Microscillaceae* | -1,96989 | 0,00521 | 0,00030 | 0,00074 | 6,94E-05 | 2,20E-04 |
| | | *Nocardioidaceae* | 1,77106 | 0,00867 | 0,00018 | 0,00065 | 7,81E-05 | 2,42E-04 |
| | | *Acetobacteraceae* | -1,97583 | 0,00348 | 0,00037 | 0,00092 | 9,29E-05 | 2,72E-04 |
| | | *Vicinamibacteraceae* | -1,95959 | 0,01387 | 0,00022 | 0,00059 | 1,34E-04 | 3,61E-04 |
| | | *Devosiaceae* | 1,54320 | 0,01389 | 0,00126 | 0,00310 | 1,58E-04 | 4,22E-04 |
| | | *Caulobacteraceae* | 2,09462 | 0,02604 | 0,00002 | 0,00013 | 1,63E-04 | 4,38E-04 |
| | | *Nitrospiraceae* | -1,58041 | 0,01736 | 0,00104 | 0,00229 | 1,84E-04 | 4,67E-04 |
| | | *Microbacteriaceae* | 1,32925 | 0,02951 | 0,00138 | 0,00336 | 2,87E-04 | 7,12E-04 |
| | | *Reyranellaceae* | 1,58666 | 0,03640 | 0,00024 | 0,00083 | 3,75E-04 | 8,88E-04 |
| | | *Inquilinaceae* | -1,36411 | 0,02600 | 0,00087 | 0,00205 | 4,15E-04 | 9,20E-04 |
| | | *Propionibacteriaceae* | -1,51884 | 0,02431 | 0,00221 | 0,00420 | 6,04E-04 | 1,29E-03 |
| | | *env.OPS_17* | -1,42000 | 0,03466 | 0,00233 | 0,00435 | 6,47E-04 | 1,39E-03 |
| | | *Acidobacteriaceae_(Subgroup_1* | -1,36630 | 0,03472 | 0,00092 | 0,00226 | 6,91E-04 | 1,49E-03 |
| | | *SC-I-84* | 1,06169 | 0,08319 | 0,00548 | 0,01049 | 2,15E-03 | 4,38E-03 |
| | | *Pedosphaeraceae* | -1,13494 | 0,08492 | 0,00418 | 0,00761 | 4,35E-03 | 7,32E-03 |
| | | *Sphingomonadaceae* | 1,08673 | 0,10919 | 0,00362 | 0,00751 | 4,17E-03 | 7,78E-03 |
| | | *SBR1031* | 1,15361 | 0,11111 | 0,00339 | 0,00699 | 0,00616 | 0,01084 |
| | | Fibrobacteraceae | 1,01268 | 0,13345 | 0,00750 | 0,01359 | 0,00654 | 0,01173 |
| | | Xanthomonadaceae | 1,06491 | 0,14236 | 0,00623 | 0,01180 | 0,01104 | 0,01841 |

| **Table S3** | Differencial abundance test between wild and modern wheat species endorhiza and rhizosphere fungal microbiome at family level. The differently abundant fungal family are considered as significant absolute aldex affect size bigger than 1 or lower than -1. negative - *A. tauschii* , positive -*T. aestivum* , negative - *T. dicoccoides* , positive -*T. durum* | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| ***A. tauschii: T. aestivum*** | **Rhizosphere** | **Family** | **effect** | **overlap** | **we.ep** | **we.eBH** | **wi.ep** | **wi.eBH** |
| | | *Coniochaetaceae* | -4,7016 | 0,0002 | 0,0000 | 0,0000 | 0,0002 | 0,0004 |
| | | *Chaetothyriales* | -3,1369 | 0,0002 | 0,0000 | 0,0000 | 0,0002 | 0,0004 |
| | | *Mortierellaceae* | 2,0414 | 0,0022 | 0,0015 | 0,0022 | 0,0002 | 0,0004 |
| | | *Herpotrichiellaceae* | -2,1161 | 0,0111 | 0,0009 | 0,0016 | 0,0004 | 0,0007 |
| | | *Didymellaceae* | -2,0615 | 0,0134 | 0,0013 | 0,0020 | 0,0004 | 0,0007 |
| | | *Piskurozymaceae* | 1,6233 | 0,0156 | 0,0039 | 0,0047 | 0,0006 | 0,0009 |
| | | *Lasiosphaeriaceae* | 1,6687 | 0,0223 | 0,0043 | 0,0052 | 0,0008 | 0,0011 |
| | | *Microdochiaceae* | -1,7821 | 0,0179 | 0,0003 | 0,0008 | 0,0008 | 0,0011 |
| | | *Nectriaceae* | -2,2365 | 0,0201 | 0,0008 | 0,0014 | 0,0009 | 0,0011 |
| | | *Hypocreales* | 1,4906 | 0,0313 | 0,0079 | 0,0088 | 0,0016 | 0,0019 |
| | | *Chaetomiaceae* | 1,4196 | 0,0424 | 0,0079 | 0,0088 | 0,0023 | 0,0025 |
| | | *Helotiaceae* | 1,3033 | 0,0379 | 0,0106 | 0,0114 | 0,0033 | 0,0036 |
| | | *Phaeosphaeriaceae* | 1,1887 | 0,0757 | 0,0155 | 0,0162 | 0,0076 | 0,0079 |
| | | *Pleosporaceae* | -1,1114 | 0,12472 | 0,006189623 | 0,00728 | 0,013249836 | 0,01347 |
| | | | | | | | | |
| | **Endorhiza** | | **effect** | **overlap** | **we.ep** | **we.eBH** | **wi.ep** | **wi.eBH** |
| | | *Pleosporaceae* | -1,9435 | 0,0069 | 0,0001 | 0,0003 | 0,0001 | 0,0002 |
| | | *Melanommataceae* | -1,8682 | 0,0087 | 0,0009 | 0,0013 | 0,0001 | 0,0003 |
| | | *Periconiaceae* | 1,3901 | 0,0087 | 0,0022 | 0,0030 | 0,0001 | 0,0003 |
| | | *Nectriaceae* | 2,5268 | 0,0208 | 0,0000 | 0,0001 | 0,0002 | 0,0004 |
| | | *Mortierellaceae* | 1,5499 | 0,0243 | 0,0005 | 0,0010 | 0,0002 | 0,0004 |
| | | *Phaeosphaeriaceae* | 1,8394 | 0,0434 | 0,0001 | 0,0003 | 0,0004 | 0,0006 |
| | | *Chaetomiaceae* | -1,4302 | 0,0347 | 0,0016 | 0,0021 | 0,0005 | 0,0008 |
| | | *Microdochiaceae* | 1,6184 | 0,0556 | 0,0003 | 0,0007 | 0,0006 | 0,0009 |
| | | *Didymellaceae* | -1,2617 | 0,0520 | 0,0020 | 0,0028 | 0,0019 | 0,0025 |
| ***T. dicoccoides : T. durum*** | **Rhizosphere** | **Family** | **effect** | **overlap** | **we.ep** | **we.eBH** | **wi.ep** | **wi.eBH** |
| | | *Hypocreales* | -3,5630 | 0,0001 | 0,0000 | 0,0002 | 0,0000 | 0,0002 |
| | | *Lasiosphaeriaceae* | 2,3296 | 0,0001 | 0,0002 | 0,0005 | 0,0000 | 0,0002 |
| | | *Coniochaetaceae* | 2,4040 | 0,0018 | 0,0002 | 0,0004 | 0,0000 | 0,0002 |
| | | *Aspergillaceae* | 1,9071 | 0,0174 | 0,0004 | 0,0009 | 0,0001 | 0,0003 |
| | | *Herpotrichiellaceae* | -1,4890 | 0,0468 | 0,0005 | 0,0012 | 0,0010 | 0,0020 |
| | | *Mortierellaceae* | -1,1830 | 0,0799 | 0,0020 | 0,0040 | 0,0023 | 0,0042 |
| | | *Piskurozymaceae* | -1,2585 | 0,0816 | 0,0025 | 0,0046 | 0,0030 | 0,0053 |
| | **Endorhiza** | | **effect** | **overlap** | **we.ep** | **we.eBH** | **wi.ep** | **wi.eBH** |
| | | *Mortierellaceae* | 3,3913 | 0,0001 | 0,0000 | 0,0001 | 0,0000 | 0,0001 |
| | | *Leptosphaeriaceae* | -2,4490 | 0,0001 | 0,0001 | 0,0002 | 0,0000 | 0,0001 |
| | | *Xylariales* | -1,2778 | 0,0399 | 0,0012 | 0,0027 | 0,0009 | 0,0019 |

| **Table S4** | Differencial abundance test between wild and modern wheat species endorhiza and rhizosphere bacterial microbiome at genus level. The differently abundant bacterial genera are considered as significant absolute aldex affect size bigger than 1 or lower than -1. negative - *A. tauschii* , positive -*T. aestivum* , negative - *T. dicoccoides* , positive -*T. durum* | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| ***A. tauschii: T. aestivum*** | **Rhizosphere** | **Genus** | **effect** | **overlap** | **we.ep** | **we.eBH** | **wi.ep** | **wi.eBH** |
| | | *RB41* | -3,2119 | 0,0001 | 0,0000 | 0,0001 | 0,0001 | 0,0002 |
| | | *JGI_0001001-H03* | -3,1516 | 0,0001 | 0,0001 | 0,0003 | 0,0001 | 0,0002 |
| | | *Rokubacteriales* | -2,4555 | 0,0001 | 0,0001 | 0,0003 | 0,0001 | 0,0002 |
| | | *Stenotrophobacter* | -2,7060 | 0,0001 | 0,0002 | 0,0004 | 0,0001 | 0,0002 |
| | | *Ferruginibacter* | -2,8134 | 0,0001 | 0,0002 | 0,0005 | 0,0001 | 0,0002 |
| | | *Kribbella* | 3,2461 | 0,0001 | 0,0001 | 0,0003 | 0,0001 | 0,0002 |
| | | *BIrii41* | 3,1300 | 0,0001 | 0,0001 | 0,0002 | 0,0001 | 0,0002 |
| | | *A21b* | 3,2667 | 0,0001 | 0,0000 | 0,0001 | 0,0001 | 0,0002 |
| | | *Caulobacter* | 3,2939 | 0,0001 | 0,0000 | 0,0001 | 0,0001 | 0,0002 |
| | | *Pajaroellobacter* | 3,4969 | 0,0001 | 0,0000 | 0,0001 | 0,0001 | 0,0002 |
| | | *Massilia* | 3,9898 | 0,0001 | 0,0000 | 0,0000 | 0,0001 | 0,0002 |
| | | *Asticcacaulis* | 3,4225 | 0,0001 | 0,0000 | 0,0000 | 0,0001 | 0,0002 |
| | | *Marmoricola* | 4,1879 | 0,0001 | 0,0000 | 0,0001 | 0,0001 | 0,0002 |
| | | *Luteolibacter* | -2,8193 | 0,0001 | 0,0003 | 0,0006 | 0,0001 | 0,0002 |
| | | *Kapabacteriales* | -2,3042 | 0,0001 | 0,0009 | 0,0018 | 0,0001 | 0,0002 |
| | | *Mucilaginibacter* | 2,7924 | 0,0001 | 0,0001 | 0,0002 | 0,0001 | 0,0002 |
| | | *Gaiella* | -2,3895 | 0,0001 | 0,0005 | 0,0011 | 0,0001 | 0,0002 |
| | | *TK10* | -2,4077 | 0,0001 | 0,0004 | 0,0009 | 0,0001 | 0,0002 |
| | | *Flavobacterium* | -2,2935 | 0,0001 | 0,0004 | 0,0008 | 0,0001 | 0,0002 |
| | | *Arenimonas* | 2,4069 | 0,0001 | 0,0000 | 0,0001 | 0,0001 | 0,0002 |
| | | *Entotheonellaceae* | -2,1095 | 0,0039 | 0,0011 | 0,0022 | 0,0001 | 0,0002 |
| | | *Aquicella* | -2,0531 | 0,0039 | 0,0013 | 0,0025 | 0,0001 | 0,0003 |
| | | *Blastocatellia_11-24* | -2,1397 | 0,0020 | 0,0001 | 0,0002 | 0,0001 | 0,0003 |
| | | *env.OPS_17* | -1,9053 | 0,0059 | 0,0008 | 0,0015 | 0,0002 | 0,0004 |
| | | *Solirubrobacter* | -1,9169 | 0,0039 | 0,0018 | 0,0032 | 0,0002 | 0,0004 |
| | | *Aurantisolimonas* | -1,6665 | 0,0215 | 0,0028 | 0,0049 | 0,0004 | 0,0008 |
| | | *Lysobacter* | 1,7322 | 0,0313 | 0,0001 | 0,0003 | 0,0004 | 0,0008 |
| | | *Legionella* | -1,5647 | 0,0175 | 0,0031 | 0,0053 | 0,0006 | 0,0011 |
| | | *SC-I-84* | 1,6532 | 0,0487 | 0,0003 | 0,0007 | 0,0009 | 0,0019 |
| | | *Reyranella* | 1,4741 | 0,0448 | 0,0006 | 0,0014 | 0,0011 | 0,0022 |
| | | *A4b* | 1,3642 | 0,0703 | 0,0014 | 0,0029 | 0,0026 | 0,0049 |
| | | *Gemmata* | -1,1742 | 0,0625 | 0,0058 | 0,0095 | 0,0029 | 0,0051 |
| | | *Candidatus_Levybacter* | 1,1121 | 0,0955 | 0,0081 | 0,0133 | 0,0051 | 0,0089 |
| | | *Nocardioides* | 1,0859 | 0,1211 | 0,0053 | 0,0098 | 0,0085 | 0,0139 |
| | | *Bradyrhizobium* | 1,0613 | 0,1423 | 0,0052 | 0,0095 | 0,0120 | 0,0189 |
| | **Endorhiza** | **Genus** | **effect** | **overlap** | **we.ep** | **we.eBH** | **wi.ep** | **wi.eBH** |
| | | *Massilia* | -3,81832 | 0,00012 | 0,00005 | 0,00016 | 0,00004 | 0,00013 |
| | | *Allorhizobium-Neorhiz* | -2,70305 | 0,00012 | 0,00008 | 0,00027 | 0,00004 | 0,00013 |
| | | *Rhizobacter* | -2,77924 | 0,00012 | 0,00011 | 0,00036 | 0,00004 | 0,00013 |
| | | *Blastocatella* | -2,61687 | 0,00012 | 0,00029 | 0,00086 | 0,00004 | 0,00013 |
| | | *Stenotrophobacter* | 3,16742 | 0,00012 | 0,00007 | 0,00023 | 0,00004 | 0,00013 |
| | | *S085* | 3,21079 | 0,00012 | 0,00007 | 0,00022 | 0,00004 | 0,00013 |
| | | *Thermoanaerobaculace* | 3,50134 | 0,00012 | 0,00004 | 0,00015 | 0,00004 | 0,00013 |
| | | *Gemmatimonas* | 3,50482 | 0,00012 | 0,00003 | 0,00010 | 0,00004 | 0,00013 |
| | | *Dokdonella* | 2,51801 | 0,00012 | 0,00001 | 0,00006 | 0,00004 | 0,00013 |
| | | *SC-I-84* | 3,58198 | 0,00012 | 0,00002 | 0,00007 | 0,00004 | 0,00013 |
| | | *env.OPS_17* | -2,11624 | 0,00012 | 0,00046 | 0,00130 | 0,00004 | 0,00013 |
| | | *Pajaroellobacter* | -2,53835 | 0,00012 | 0,00013 | 0,00041 | 0,00004 | 0,00013 |
| | | *Candidatus_Paracaedib* | 2,32489 | 0,00175 | 0,00020 | 0,00060 | 0,00004 | 0,00013 |
| | | *Microvirga* | 2,71471 | 0,00012 | 0,00008 | 0,00027 | 0,00004 | 0,00013 |
| | | *Sphingomonas* | -2,22094 | 0,00012 | 0,00046 | 0,00125 | 0,00004 | 0,00013 |
| | | *Mucilaginibacter* | -2,05954 | 0,00175 | 0,00031 | 0,00092 | 0,00004 | 0,00014 |
| | | *Streptomyces* | -2,21864 | 0,00175 | 0,00038 | 0,00110 | 0,00004 | 0,00014 |
| | | *Reyranella* | -2,31945 | 0,00348 | 0,00031 | 0,00084 | 0,00005 | 0,00015 |
| | | *Cellvibrio* | -1,99392 | 0,00695 | 0,00071 | 0,00200 | 0,00007 | 0,00021 |
| | | *DS-100* | -1,79774 | 0,01387 | 0,00110 | 0,00288 | 0,00009 | 0,00026 |
| | | *Mesorhizobium* | -1,73263 | 0,01040 | 0,00104 | 0,00270 | 0,00014 | 0,00039 |
| | | *Lechevalieria* | -1,77790 | 0,02253 | 0,00028 | 0,00083 | 0,00022 | 0,00061 |
| | | *Blastocatellia_11-24* | -1,49970 | 0,02431 | 0,00086 | 0,00231 | 0,00040 | 0,00106 |
| | | *Polaromonas* | 1,12703 | 0,11979 | 0,03307 | 0,06153 | 0,00607 | 0,01436 |

| ***T. dicoccoides : T. durum*** | **Rhizosphere** | **Genus** | **effect** | **overlap** | **we.ep** | **we.eBH** | **wi.ep** | **wi.eBH** |
|---|---|---|---|---|---|---|---|---|
| | | *Pseudoxanthomonas* | -2,99578 | 0,00012 | 0,00004 | 0,00021 | 0,00004 | 0,00017 |
| | | *Bradyrhizobium* | -2,98351 | 0,00012 | 0,00006 | 0,00028 | 0,00004 | 0,00017 |
| | | *Devosia* | 2,99197 | 0,00012 | 0,00013 | 0,00053 | 0,00004 | 0,00017 |
| | | *Methylotenera* | 2,78517 | 0,00012 | 0,00016 | 0,00068 | 0,00004 | 0,00017 |
| | | *Flavisolibacter* | 3,25892 | 0,00012 | 0,00004 | 0,00018 | 0,00004 | 0,00017 |
| | | *Gaiella* | 3,62408 | 0,00012 | 0,00003 | 0,00014 | 0,00004 | 0,00017 |
| | | *Fibrobacter* | -2,27022 | 0,00012 | 0,00012 | 0,00049 | 0,00004 | 0,00018 |
| | | *Thermoanaerobaculace* | 2,94461 | 0,00012 | 0,00003 | 0,00014 | 0,00004 | 0,00018 |
| | | *Solirubrobacter* | -2,88536 | 0,00012 | 0,00026 | 0,00098 | 0,00004 | 0,00018 |
| | | *Stenotrophobacter* | -2,43725 | 0,00012 | 0,00012 | 0,00053 | 0,00004 | 0,00018 |
| | | *Mucilaginibacter* | 2,11941 | 0,00012 | 0,00007 | 0,00030 | 0,00004 | 0,00018 |
| | | *Haliangium* | 2,13568 | 0,00175 | 0,00054 | 0,00190 | 0,00005 | 0,00019 |
| | | *SBR1031* | -2,16818 | 0,00348 | 0,00048 | 0,00172 | 0,00005 | 0,00020 |
| | | *Candidatus_Levybacter* | -2,05658 | 0,00175 | 0,00064 | 0,00223 | 0,00005 | 0,00020 |
| | | *Caulobacter* | -2,24266 | 0,00175 | 0,00037 | 0,00120 | 0,00005 | 0,00021 |
| | | *IMCC26256* | -1,90242 | 0,00348 | 0,00075 | 0,00261 | 0,00006 | 0,00024 |
| | | *Cellvibrio* | -1,83435 | 0,00521 | 0,00063 | 0,00211 | 0,00007 | 0,00027 |
| | | *Blastocatella* | -1,82868 | 0,01040 | 0,00085 | 0,00289 | 0,00007 | 0,00028 |
| | | *Aquicella* | -1,89418 | 0,00694 | 0,00033 | 0,00124 | 0,00007 | 0,00029 |
| | | *Bauldia* | -1,80855 | 0,00348 | 0,00069 | 0,00233 | 0,00007 | 0,00029 |
| | | *env.OPS_17* | -1,79373 | 0,01213 | 0,00033 | 0,00123 | 0,00010 | 0,00037 |
| | | *Lysobacter* | 1,27931 | 0,06066 | 0,00106 | 0,00378 | 0,00161 | 0,00529 |
| | | *Nocardioides* | 1,32774 | 0,07452 | 0,00154 | 0,00528 | 0,00162 | 0,00531 |
| | | *SC-I-84* | 1,31113 | 0,07626 | 0,00102 | 0,00367 | 0,00177 | 0,00575 |
| | | *Phenylobacterium* | 1,23010 | 0,09532 | 0,00207 | 0,00685 | 0,00319 | 0,00987 |
| | **Endorhiza** | **Genus** | **effect** | **overlap** | **we.ep** | **we.eBH** | **wi.ep** | **wi.eBH** |
| | | *Marmoricola* | 3,80384 | 0,00012 | 0,00001 | 0,00006 | 0,00004 | 0,00016 |
| | | *Dokdonella* | 3,14426 | 0,00012 | 0,00002 | 0,00009 | 0,00004 | 0,00016 |
| | | *Allorhizobium-Neorhiz* | 3,49305 | 0,00012 | 0,00005 | 0,00020 | 0,00004 | 0,00016 |
| | | *Streptomyces* | 3,57960 | 0,00012 | 0,00002 | 0,00011 | 0,00004 | 0,00016 |
| | | *Saccharimonadales* | 3,08669 | 0,00012 | 0,00008 | 0,00032 | 0,00004 | 0,00016 |
| | | *Asticcacaulis* | 2,00186 | 0,00012 | 0,00004 | 0,00022 | 0,00004 | 0,00016 |
| | | *BIrii41* | -2,23893 | 0,00012 | 0,00035 | 0,00104 | 0,00004 | 0,00017 |
| | | *Mucilaginibacter* | -2,34815 | 0,00175 | 0,00024 | 0,00077 | 0,00004 | 0,00017 |
| | | *Thermoanaerobaculace* | -2,14898 | 0,00348 | 0,00027 | 0,00086 | 0,00005 | 0,00017 |
| | | *Reyranella* | -2,25938 | 0,00175 | 0,00018 | 0,00065 | 0,00005 | 0,00018 |
| | | *Blastocatellia_11-24* | -2,07211 | 0,00521 | 0,00031 | 0,00095 | 0,00005 | 0,00019 |
| | | *Candidatus_Berkiella* | -1,81602 | 0,00694 | 0,00082 | 0,00240 | 0,00006 | 0,00022 |
| | | *Tahibacter* | -2,09671 | 0,00175 | 0,00028 | 0,00089 | 0,00006 | 0,00022 |
| | | *Candidatus_Paracaedib* | -1,96432 | 0,01213 | 0,00047 | 0,00145 | 0,00007 | 0,00025 |
| | | *MND1* | -1,68703 | 0,00868 | 0,00228 | 0,00551 | 0,00012 | 0,00037 |
| | | *Nitrospira* | -1,70340 | 0,01563 | 0,00050 | 0,00152 | 0,00012 | 0,00038 |
| | | *Blastocatella* | -1,82547 | 0,01563 | 0,00066 | 0,00192 | 0,00012 | 0,00039 |
| | | *Inquilinus* | -1,49017 | 0,01389 | 0,00064 | 0,00173 | 0,00018 | 0,00057 |
| | | *IMCC26134* | -1,68135 | 0,01907 | 0,00060 | 0,00172 | 0,00027 | 0,00077 |
| | | *env.OPS_17* | -1,50760 | 0,02778 | 0,00119 | 0,00335 | 0,00039 | 0,00114 |
| | | *Duganella* | 1,38617 | 0,06076 | 0,00070 | 0,00234 | 0,00066 | 0,00191 |
| | | *Devosia* | 1,20403 | 0,06597 | 0,00366 | 0,00959 | 0,00143 | 0,00388 |
| | | *Nocardioides* | 1,15658 | 0,09705 | 0,00183 | 0,00521 | 0,00346 | 0,00868 |
| | | *Arenimonas* | 1,23366 | 0,10590 | 0,00185 | 0,00529 | 0,00409 | 0,01021 |
| | | *Massilia* | 1,03712 | 0,14038 | 0,00867 | 0,02078 | 0,00915 | 0,02081 |

| **Table S4** | Differencial abundance test between wild and modern wheat species endorhiza and rhizosphere fungal microbiome at genus level. The differently abundant fungal genera are considered as significant absolute aldex affect size bigger than 1 or lower than -1. positive - *A. tauschii* , negative -*T. aestivum* , negative - *T. dicoccoides* , positive -*T. durum* | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| ***A. tauschii: T. aestivum*** | **Rhizosphere** | **Genus** | **effect** | **overlap** | **we.ep** | **we.eBH** | **wi.ep** | **wi.eBH** |
| | | *Alternaria* | -1,4969 | 0,0513 | 0,0010 | 0,0016 | 0,0021 | 0,0026 |
| | | *Microdochium* | -2,3937 | 0,0045 | 0,0000 | 0,0001 | 0,0002 | 0,0004 |
| | | *Exophiala* | -3,5965 | 0,0002 | 0,0001 | 0,0003 | 0,0002 | 0,0003 |
| | | *Epicoccum* | -2,9185 | 0,0002 | 0,0002 | 0,0004 | 0,0002 | 0,0003 |
| | | *Fusicolla* | -2,7143 | 0,0002 | 0,0001 | 0,0002 | 0,0002 | 0,0004 |
| | | *Fusarium* | 1,5739 | 0,0267 | 0,0071 | 0,0083 | 0,0011 | 0,0013 |
| | | *Solicoccozyma* | 1,3761 | 0,0379 | 0,0057 | 0,0069 | 0,0036 | 0,0041 |
| | | *Mortierella* | 1,7172 | 0,0112 | 0,0033 | 0,0042 | 0,0006 | 0,0008 |
| | **Endorhiza** | **Genus** | **effect** | **overlap** | **we.ep** | **we.eBH** | **wi.ep** | **wi.eBH** |
| | | *Schizothecium* | 3,1247 | 0,0001 | 0,0000 | 0,0000 | 0,0000 | 0,0001 |
| | | *Alternaria* | -2,2243 | 0,0001 | 0,0000 | 0,0001 | 0,0000 | 0,0001 |
| | | *Fusicolla* | -2,1767 | 0,0001 | 0,0003 | 0,0008 | 0,0000 | 0,0001 |
| | | *Ilyonectria* | -2,1875 | 0,0018 | 0,0006 | 0,0014 | 0,0000 | 0,0001 |
| | | *Mortierella* | 1,0452 | 0,1042 | 0,0066 | 0,0118 | 0,0054 | 0,0087 |
| ***T. dicoccoides : T. durum*** | **Rhizosphere** | **Genus** | **effect** | **overlap** | **we.ep** | **we.eBH** | **wi.ep** | **wi.eBH** |
| | | *Zopfiella* | 2,7971 | 0,0001 | 0,0000 | 0,0002 | 0,0000 | 0,0002 |
| | | *Gibberella* | -1,9177 | 0,0052 | 0,0003 | 0,0011 | 0,0001 | 0,0004 |
| | | *Acremonium* | -1,8971 | 0,0087 | 0,0005 | 0,0016 | 0,0001 | 0,0004 |
| | **Endorhiza** | **Genus** | **effect** | **overlap** | **we.ep** | **we.eBH** | **wi.ep** | **wi.eBH** |
| | | *Ophiosphaerella* | -1,7223 | 0,0225 | 0,0005 | 0,0008 | 0,0002 | 0,0004 |
| | | *Fusarium* | -1,5154 | 0,0416 | 0,0012 | 0,0016 | 0,0007 | 0,0009 |
| | | *Exophiala* | 1,1482 | 0,1007 | 0,0047 | 0,0051 | 0,0054 | 0,0058 |
| | | *Microdochium* | 1,6233 | 0,0468 | 0,0006 | 0,0009 | 0,0008 | 0,0011 |
| | | *Mortierella* | 3,7182 | 0,0001 | 0,0000 | 0,0000 | 0,0000 | 0,0002 |

| Table S5 | Correlation analysis between core bacterial genera of the rhizosphere and rhizosphere soil biochemical parameters (potential enzyme activity, the abundance of genes encoding enzymes involved in N- and P-cycles).<br>Pearson's correlation coefficient was used to identify significantly correlated genera and p values were corrected using the Benjamini–Hochberg method |
|---|---|

| Location | Potential enzyme activity or gene abundance | Core genera | | | | | | Pearson correlation | p-value | BH-corrected p-value |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Kingdom | Phylum | Class | Order | Family | Genus | | | |
| GG | **Urease** | Bacteria | *Proteobacteria* | *Gammaproteobacteria* | *Xanthomonadales* | *Rhodanobacteraceae* | ***Rhodanobacter*** | -0,933 | 0,000 | **0,001** |
| | **Urease** | Bacteria | *Actinobacteriota* | *Actinobacteria* | *Propionibacteriales* | *Nocardioidaceae* | ***Marmoricola*** | 0,840 | 0,001 | **0,017** |
| | **Urease** | Bacteria | *Proteobacteria* | *Gammaproteobacteria* | *Xanthomonadales* | *Xanthomonadaceae* | ***Lysobacter*** | -0,804 | 0,002 | **0,027** |
| | **Urease** | Bacteria | *Proteobacteria* | *Alphaproteobacteria* | *Caulobacterales* | *Caulobacteraceae* | ***Brevundimonas*** | 0,804 | 0,002 | **0,027** |
| | **Urease** | Bacteria | *Actinobacteriota* | *Actinobacteria* | *Propionibacteriales* | *Nocardioidaceae* | ***Nocardioides*** | 0,716 | 0,009 | **0,068** |
| | **Urease** | Bacteria | *Proteobacteria* | *Alphaproteobacteria* | *Rhizobiales* | *Devosiaceae* | ***Devosia*** | 0,705 | 0,013 | **0,072** |
| | **Urease** | Bacteria | *Acidobacteriota* | *Acidobacteriae* | *Acidobacteriales* | *Acidobacteriaceae_(Subg* | ***Granulicella*** | -0,702 | 0,012 | **0,073** |
| | **Urease** | Bacteria | *Acidobacteriota* | *Holophagae* | *Subgroup_7* | *Subgroup_7* | ***Subgroup_7*** | 0,698 | 0,014 | **0,075** |
| | **Urease** | Bacteria | *Verrucomicrobiota* | *Verrucomicrobiae* | *Chthoniobacterales* | *Chthoniobacteraceae* | ***Chthoniobacter*** | 0,701 | 0,017 | **0,078** |
| | **Urease** | Bacteria | *Proteobacteria* | *Alphaproteobacteria* | *Rhizobiales* | *Beijerinckiaceae* | ***Microvirga*** | 0,690 | 0,015 | **0,079** |
| | **Urease** | Bacteria | *Actinobacteriota* | *Actinobacteria* | *Propionibacteriales* | *Propionibacteriaceae* | ***Microlunatus*** | 0,674 | 0,017 | **0,087** |
| | **Urease** | Bacteria | *Verrucomicrobiota* | *Verrucomicrobiae* | *Opitutales* | *Opitutaceae* | ***Lacunisphaera*** | -0,656 | 0,024 | **0,098** |
| | **Urease** | Bacteria | *Proteobacteria* | *Gammaproteobacteria* | *Xanthomonadales* | *Rhodanobacteraceae* | ***Dokdonella*** | -0,659 | 0,026 | **0,100** |
| | **Alkaline PA** | Bacteria | *Verrucomicrobiota* | *Verrucomicrobiae* | *Chthoniobacterales* | *Chthoniobacteraceae* | ***Chthoniobacter*** | 0,8892 | 0,000 | **0,014** |
| | **Alkaline PA** | Bacteria | *Proteobacteria* | *Gammaproteobacteria* | *Xanthomonadales* | *Rhodanobacteraceae* | ***Dokdonella*** | -0,7573 | 0,006 | **0,095** |
| WG | ***nirS*** | Bacteria | *Proteobacteria* | *Gammaproteobacteria* | *Xanthomonadales* | *Xanthomonadaceae* | ***Luteimonas*** | -0,942 | 0,000 | **0,000** |
| | ***nirS*** | Bacteria | *Acidobacteriota* | *Acidobacteriae* | *Bryobacterales* | *Bryobacteraceae* | ***Bryobacter*** | -0,931 | 0,000 | **0,000** |
| | ***nirS*** | Bacteria | *Proteobacteria* | *Alphaproteobacteria* | *Reyranellales* | *Reyranellaceae* | ***Reyranella*** | -0,922 | 0,000 | **0,001** |
| | ***nirS*** | Bacteria | *Proteobacteria* | *Gammaproteobacteria* | *Xanthomonadales* | *Xanthomonadaceae* | ***Pseudoxanthomonas*** | -0,905 | 0,000 | **0,001** |
| | ***nirS*** | Bacteria | *Proteobacteria* | *Gammaproteobacteria* | *Burkholderiales* | *SC-I-84* | ***SC-I-84*** | -0,900 | 0,000 | **0,001** |
| | ***nirS*** | Bacteria | *Proteobacteria* | *Gammaproteobacteria* | *Xanthomonadales* | *Xanthomonadaceae* | ***Lysobacter*** | -0,880 | 0,000 | **0,002** |
| | ***nirS*** | Bacteria | *Actinobacteriota* | *Thermoleophilia* | *Solirubrobacterales* | *Solirubrobacteraceae* | ***Solirubrobacter*** | -0,867 | 0,000 | **0,004** |
| | ***nirS*** | Bacteria | *Bacteroidota* | *Bacteroidia* | *Sphingobacteriales* | *Sphingobacteriaceae* | ***Mucilaginibacter*** | 0,850 | 0,001 | **0,005** |
| | ***nirS*** | Bacteria | *Myxococcota* | *Polyangia* | *Polyangiales* | *BIrii41* | ***BIrii41*** | -0,840 | 0,001 | **0,006** |
| | ***nirS*** | Bacteria | *Chloroflexi* | *Anaerolineae* | *Anaerolineales* | *Anaerolineaceae* | ***UTCFX1*** | -0,825 | 0,001 | **0,008** |
| | ***nirS*** | Bacteria | *Myxococcota* | *Polyangia* | *Polyangiales* | *Polyangiaceae* | ***Pajaroellobacter*** | -0,799 | 0,002 | **0,012** |
| | ***nirS*** | Bacteria | *Bacteroidota* | *Bacteroidia* | *Cytophagales* | *Hymenobacteraceae* | ***Adhaeribacter*** | 0,781 | 0,004 | **0,017** |
| | ***nirS*** | Bacteria | *Bacteroidota* | *Bacteroidia* | *Sphingobacteriales* | *env.OPS_17* | ***env.OPS_17*** | 0,768 | 0,004 | **0,019** |
| | ***nirS*** | Bacteria | *Proteobacteria* | *Gammaproteobacteria* | *Burkholderiales* | *Oxalobacteraceae* | ***Duganella*** | -0,765 | 0,005 | **0,019** |
| | ***nirS*** | Bacteria | *Proteobacteria* | *Gammaproteobacteria* | *Xanthomonadales* | *Xanthomonadaceae* | ***Arenimonas*** | -0,758 | 0,004 | **0,020** |
| | ***nirS*** | Bacteria | *Acidobacteriota* | *Vicinamibacteria* | *Vicinamibacterales* | *Vicinamibacteraceae* | ***Vicinamibacteraceae*** | 0,754 | 0,005 | **0,021** |
| | ***nirS*** | Bacteria | *Proteobacteria* | *Gammaproteobacteria* | *Burkholderiales* | *Nitrosomonadaceae* | ***Nitrosospira*** | -0,758 | 0,006 | **0,022** |
| | ***nirS*** | Bacteria | *Acidobacteriota* | *Thermoanaerobaculia* | *Thermoanaerobacul* | *Thermoanaerobaculacea* | ***Subgroup_10*** | 0,747 | 0,006 | **0,024** |
| | ***nirS*** | Bacteria | *Proteobacteria* | *Alphaproteobacteria* | *Sphingomonadales* | *Sphingomonadaceae* | ***Sphingomonas*** | -0,741 | 0,006 | **0,024** |
| | ***nirS*** | Bacteria | *Proteobacteria* | *Alphaproteobacteria* | *Rhizobiales* | *Beijerinckiaceae* | ***Microvirga*** | 0,752 | 0,008 | **0,025** |
| | ***nirS*** | Bacteria | *Acidobacteriota* | *Holophagae* | *Subgroup_7* | *Subgroup_7* | ***Subgroup_7*** | 0,731 | 0,008 | **0,027** |
| | ***nirS*** | Bacteria | *Bacteroidota* | *Bacteroidia* | *Chitinophagales* | *Chitinophagaceae* | ***Ferruginibacter*** | 0,714 | 0,010 | **0,032** |
| | ***nirS*** | Bacteria | *Proteobacteria* | *Alphaproteobacteria* | *Dongiales* | *Dongiaceae* | ***Dongia*** | 0,718 | 0,013 | **0,035** |
| | ***nirS*** | Bacteria | *Chloroflexi* | *TK10* | *TK10* | *TK10* | ***TK10*** | 0,707 | 0,012 | **0,035** |
| | ***nirS*** | Bacteria | *Verrucomicrobiota* | *Verrucomicrobiae* | *Pedosphaerales* | *Pedosphaeraceae* | ***Ellin517*** | 0,715 | 0,013 | **0,035** |
| | ***nirS*** | Bacteria | *Verrucomicrobiota* | *Verrucomicrobiae* | *Opitutales* | *Opitutaceae* | ***Opitutus*** | 0,693 | 0,013 | **0,038** |
| | ***nirS*** | Bacteria | *Bacteroidota* | *Bacteroidia* | *Cytophagales* | *Spirosomaceae* | ***Dyadobacter*** | 0,688 | 0,015 | **0,041** |
| | ***nirS*** | Bacteria | *Planctomycetota* | *Phycisphaerae* | *Tepidisphaerales* | *WD2101_soil_group* | ***WD2101_soil_group*** | -0,685 | 0,015 | **0,042** |
| | ***nirS*** | Bacteria | *Acidobacteriota* | *Blastocatellia* | *Pyrinomonadales* | *Pyrinomonadaceae* | ***RB41*** | 0,678 | 0,016 | **0,043** |
| | ***nirS*** | Bacteria | *Bacteroidota* | *Bacteroidia* | *Sphingobacteriales* | *Sphingobacteriaceae* | ***Pedobacter*** | 0,667 | 0,018 | **0,047** |
| | ***nirS*** | Bacteria | *Bacteroidota* | *Bacteroidia* | *Chitinophagales* | *Chitinophagaceae* | ***Flavisolibacter*** | 0,663 | 0,020 | **0,050** |
| | ***nirS*** | Bacteria | *Bacteroidota* | *Bacteroidia* | *Flavobacteriales* | *Flavobacteriaceae* | ***Flavobacterium*** | 0,661 | 0,021 | **0,051** |
| | ***nirS*** | Bacteria | *Bacteroidota* | *Bacteroidia* | *Chitinophagales* | *Chitinophagaceae* | ***Terrimonas*** | 0,656 | 0,022 | **0,054** |
| | ***nirS*** | Bacteria | *Bacteroidota* | *Kapabacteria* | *Kapabacteriales* | *Kapabacteriales* | ***Kapabacteriales*** | 0,628 | 0,031 | **0,068** |
| | ***nirS*** | Bacteria | *Actinobacteriota* | *Actinobacteria* | *Pseudonocardiales* | *Pseudonocardiaceae* | ***Lechevalieria*** | -0,650 | 0,032 | **0,069** |
| | ***nirS*** | Bacteria | *Verrucomicrobiota* | *Verrucomicrobiae* | *Pedosphaerales* | *Pedosphaeraceae* | ***Pedosphaeraceae*** | 0,605 | 0,042 | **0,086** |
| | ***nirS*** | Bacteria | *Actinobacteriota* | *Thermoleophilia* | *Gaiellales* | *Gaiellaceae* | ***Gaiella*** | -0,592 | 0,045 | **0,093** |
| | ***phoX*** | Bacteria | *Gemmatimonadota* | *Gemmatimonadetes* | *Gemmatimonadales* | *Gemmatimonadaceae* | ***Gemmatimonas*** | -0,877 | 0,000 | **0,020** |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | **Alkaline_PA** | Bacteria | *Planctomycetota* | *Phycisphaerae* | *Tepidisphaerales* | *WD2101_soil_group* | ***WD2101_soil_group*** | **0,860** | 0,000 | **0,023** |
| | **Alkaline_PA** | Bacteria | *Verrucomicrobiota* | *Verrucomicrobiae* | *Opitutales* | *Opitutaceae* | ***Opitutus*** | **-0,824** | 0,001 | **0,035** |
| | **Alkaline_PA** | Bacteria | *Chloroflexi* | *Anaerolineae* | *SBR1031* | *SBR1031* | ***SBR1031*** | **0,771** | 0,004 | **0,054** |
| | **Alkaline_PA** | Bacteria | *Proteobacteria* | *Alphaproteobacteria* | *Reyranellales* | *Reyranellaceae* | ***Reyranella*** | **0,762** | 0,005 | **0,060** |
| | **Alkaline_PA** | Bacteria | *Actinobacteriota* | *Thermoleophilia* | *Solirubrobacterales* | *Solirubrobacteraceae* | ***Solirubrobacter*** | **0,736** | 0,009 | **0,066** |
| | **Alkaline_PA** | Bacteria | *Proteobacteria* | *Gammaproteobacteria* | *Burkholderiales* | *Oxalobacteraceae* | ***Duganella*** | **0,732** | 0,009 | **0,067** |
| | **Alkaline_PA** | Bacteria | *Proteobacteria* | *Gammaproteobacteria* | *Xanthomonadales* | *Xanthomonadaceae* | ***Lysobacter*** | **0,719** | 0,009 | **0,073** |
| | **Alkaline_PA** | Bacteria | *Proteobacteria* | *Gammaproteobacteria* | *Burkholderiales* | *SC-I-84* | ***SC-I-84*** | **0,709** | 0,010 | **0,075** |
| | **Alkaline_PA** | Bacteria | *Acidobacteriota* | *Holophagae* | *Subgroup_7* | *Subgroup_7* | ***Subgroup_7*** | **-0,698** | 0,013 | **0,077** |
| | **Alkaline_PA** | Bacteria | *Acidobacteriota* | *Blastocatellia* | *Pyrinomonadales* | *Pyrinomonadaceae* | ***RB41*** | **-0,701** | 0,011 | **0,077** |
| | **Alkaline_PA** | Bacteria | *Proteobacteria* | *Gammaproteobacteria* | *Xanthomonadales* | *Xanthomonadaceae* | ***Luteimonas*** | **0,682** | 0,015 | **0,081** |
| | **Alkaline_PA** | Bacteria | *Bacteroidota* | *Bacteroidia* | *Chitinophagales* | *Chitinophagaceae* | ***Ferruginibacter*** | **-0,679** | 0,016 | **0,082** |
| | **Alkaline_PA** | Bacteria | *Acidobacteriota* | *Acidobacteriae* | *Bryobacterales* | *Bryobacteraceae* | ***Bryobacter*** | **0,674** | 0,019 | **0,085** |
| | **Alkaline_PA** | Bacteria | *Acidobacteriota* | *Blastocatellia* | *Blastocatellales* | *Blastocatellaceae* | ***Stenotrophobacter*** | **-0,668** | 0,019 | **0,086** |
| | **Alkaline_PA** | Bacteria | *Chloroflexi* | *Anaerolineae* | *Anaerolineales* | *Anaerolineaceae* | ***UTCFX1*** | **0,670** | 0,019 | **0,087** |
| | **Alkaline_PA** | Bacteria | *Bacteroidota* | *Bacteroidia* | *Sphingobacteriales* | *Sphingobacteriaceae* | ***Pedobacter*** | **-0,657** | 0,021 | **0,088** |
| | **Alkaline_PA** | Bacteria | *Chloroflexi* | *Chloroflexia* | *Chloroflexales* | *Herpetosiphonaceae* | ***Herpetosiphon*** | **0,670** | 0,024 | **0,092** |
| | **Alkaline_PA** | Bacteria | *Bacteroidota* | *Bacteroidia* | *Sphingobacteriales* | *Sphingobacteriaceae* | ***Mucilaginibacter*** | **-0,651** | 0,024 | **0,093** |
| | **Alkaline_PA** | Bacteria | *Myxococcota* | *Polyangia* | *Polyangiales* | *Polyangiaceae* | ***Pajaroellobacter*** | **0,646** | 0,026 | **0,096** |
| | **Urease** | Bacteria | *Proteobacteria* | *Gammaproteobacteria* | *Burkholderiales* | *SC-I-84* | ***SC-I-84*** | **-0,919** | 0,000 | **0,002** |
| | **Urease** | Bacteria | *Proteobacteria* | *Alphaproteobacteria* | *Reyranellales* | *Reyranellaceae* | ***Reyranella*** | **-0,890** | 0,000 | **0,003** |
| | **Urease** | Bacteria | *Proteobacteria* | *Gammaproteobacteria* | *Xanthomonadales* | *Xanthomonadaceae* | ***Luteimonas*** | **-0,888** | 0,000 | **0,003** |
| | **Urease** | Bacteria | *Myxococcota* | *Polyangia* | *Polyangiales* | *Polyangiaceae* | ***Pajaroellobacter*** | **-0,874** | 0,000 | **0,004** |
| | **Urease** | Bacteria | *Proteobacteria* | *Gammaproteobacteria* | *Xanthomonadales* | *Xanthomonadaceae* | ***Lysobacter*** | **-0,859** | 0,000 | **0,005** |
| | **Urease** | Bacteria | *Acidobacteriota* | *Acidobacteriae* | *Bryobacterales* | *Bryobacteraceae* | ***Bryobacter*** | **-0,854** | 0,001 | **0,005** |
| | **Urease** | Bacteria | *Bacteroidota* | *Bacteroidia* | *Sphingobacteriales* | *Sphingobacteriaceae* | ***Pedobacter*** | **0,826** | 0,001 | **0,008** |
| | **Urease** | Bacteria | *Bacteroidota* | *Bacteroidia* | *Chitinophagales* | *Chitinophagaceae* | ***Ferruginibacter*** | **0,825** | 0,001 | **0,008** |
| | **Urease** | Bacteria | *Myxococcota* | *Polyangia* | *Polyangiales* | *Blrii41* | ***Blrii41*** | **-0,824** | 0,001 | **0,009** |
| | **Urease** | Bacteria | *Chloroflexi* | *Anaerolineae* | *Anaerolineales* | *Anaerolineaceae* | ***UTCFX1*** | **-0,826** | 0,001 | **0,009** |
| | **Urease** | Bacteria | *Proteobacteria* | *Alphaproteobacteria* | *Sphingomonadales* | *Sphingomonadaceae* | ***Sphingomonas*** | **-0,820** | 0,001 | **0,009** |
| | **Urease** | Bacteria | *Proteobacteria* | *Gammaproteobacteria* | *Burkholderiales* | *Nitrosomonadaceae* | ***Nitrosospira*** | **-0,820** | 0,002 | **0,011** |
| | **Urease** | Bacteria | *Bacteroidota* | *Bacteroidia* | *Sphingobacteriales* | *Sphingobacteriaceae* | ***Mucilaginibacter*** | **0,800** | 0,002 | **0,012** |
| | **Urease** | Bacteria | *Proteobacteria* | *Gammaproteobacteria* | *Xanthomonadales* | *Xanthomonadaceae* | ***Pseudoxanthomonas*** | **-0,797** | 0,002 | **0,012** |
| | **Urease** | Bacteria | *Bacteroidota* | *Bacteroidia* | *Cytophagales* | *Spirosomaceae* | ***Dyadobacter*** | **0,795** | 0,003 | **0,013** |
| | **Urease** | Bacteria | *Actinobacteriota* | *Thermoleophilia* | *Solirubrobacterales* | *Solirubrobacteraceae* | ***Solirubrobacter*** | **-0,768** | 0,005 | **0,021** |
| | **Urease** | Bacteria | *Actinobacteriota* | *Thermoleophilia* | *Gaiellales* | *Gaiellaceae* | ***Gaiella*** | **-0,743** | 0,006 | **0,026** |
| | **Urease** | Bacteria | *Bacteroidota* | *Bacteroidia* | *Chitinophagales* | *Chitinophagaceae* | ***Terrimonas*** | **0,742** | 0,006 | **0,027** |
| | **Urease** | Bacteria | *Acidobacteriota* | *Vicinamibacteria* | *Vicinamibacterales* | *Vicinamibacteraceae* | ***Vicinamibacteraceae*** | **0,723** | 0,008 | **0,032** |
| | **Urease** | Bacteria | *Bacteroidota* | *Bacteroidia* | *Flavobacteriales* | *Flavobacteriaceae* | ***Flavobacterium*** | **0,720** | 0,009 | **0,034** |
| | **Urease** | Bacteria | *Acidobacteriota* | *Thermoanaerobaculia* | *Thermoanaerobacula* | *Thermoanaerobaculaceae* | ***Subgroup_10*** | **0,701** | 0,013 | **0,043** |
| | **Urease** | Bacteria | *Bacteroidota* | *Bacteroidia* | *Sphingobacteriales* | *env.OPS_17* | ***env.OPS_17*** | **0,696** | 0,013 | **0,044** |
| | **Urease** | Bacteria | *Bacteroidota* | *Bacteroidia* | *Cytophagales* | *Hymenobacteraceae* | ***Adhaeribacter*** | **0,686** | 0,018 | **0,053** |
| | **Urease** | Bacteria | *Proteobacteria* | *Alphaproteobacteria* | *Rhizobiales* | *Beijerinckiaceae* | ***Microvirga*** | **0,656** | 0,026 | **0,068** |
| | **Urease** | Bacteria | *Proteobacteria* | *Gammaproteobacteria* | *Burkholderiales* | *Oxalobacteraceae* | ***Duganella*** | **-0,648** | 0,027 | **0,071** |
| | **Urease** | Bacteria | *Acidobacteriota* | *Blastocatellia* | *Blastocatellales* | *Blastocatellaceae* | ***Stenotrophobacter*** | **0,636** | 0,028 | **0,075** |
| | **Urease** | Bacteria | *Verrucomicrobiota* | *Verrucomicrobiae* | *Opitutales* | *Opitutaceae* | ***Opitutus*** | **0,631** | 0,029 | **0,077** |
| | **Urease** | Bacteria | *Acidobacteriota* | *Holophagae* | *Subgroup_7* | *Subgroup_7* | ***Subgroup_7*** | **0,629** | 0,031 | **0,081** |
| | **Urease** | Bacteria | *Planctomycetota* | *Phycisphaerae* | *Tepidisphaerales* | *WD2101_soil_group* | ***WD2101_soil_group*** | **-0,624** | 0,034 | **0,086** |
| | **Urease** | Bacteria | *Bacteroidota* | *Bacteroidia* | *Chitinophagales* | *Chitinophagaceae* | ***Flavisolibacter*** | **0,614** | 0,036 | **0,089** |
| | **Urease** | Bacteria | *Verrucomicrobiota* | *Verrucomicrobiae* | *Pedosphaerales* | *Pedosphaeraceae* | ***Ellin517*** | **0,613** | 0,039 | **0,093** |
| | **Urease** | Bacteria | *Chloroflexi* | *TK10* | *TK10* | *TK10* | ***TK10*** | **0,614** | 0,038 | **0,093** |
| | **Urease** | Bacteria | *Bacteroidota* | *Bacteroidia* | *Chitinophagales* | *Chitinophagaceae* | ***Dinghuibacter*** | **0,618** | 0,040 | **0,093** |
| **RH** | ***nirS*** | Bacteria | *Proteobacteria* | *Gammaproteobacteria* | *Xanthomonadales* | *Xanthomonadaceae* | ***Pseudoxanthomonas*** | **-0,854** | 0,001 | **0,045** |
| | ***nirS*** | Bacteria | *Chloroflexi* | *TK10* | *TK10* | *TK10* | ***TK10*** | **0,800** | 0,004 | **0,080** |
| | **Urease** | Bacteria | *Proteobacteria* | *Gammaproteobacteria* | *Xanthomonadales* | *Xanthomonadaceae* | ***Pseudoxanthomonas*** | **0,839** | 0,001 | **0,074** |

# **Chapter 5:** General discussion

# 5. General discussion

The importance of host-microbe and microbe-microbe interactions are becoming better recognized and receiving more attention from researchers. The plant-associated microbiome is known to provide a variety of benefits, including improved nutrient uptake, disease suppression, and higher tolerance to biotic and abiotic challenges (Tiwari et al., 2016; Kuan et al., 2016; Egamberdieva et al., 2017). Among the other plant habitats, the rhizosphere is a complex ecological niche for diverse microorganisms and the place where plant and soil microbiomes interact with each other via plant roots and root excretions (Rudrappa et al., 2008). The rhizosphere assembly process begins as soon as the seed is planted in the soil. The main factors (such as plant genotype, and soil type) that determine the rhizosphere composition, structure, and abundance are well documented (Berg & Smalla, 2009; Tkacz et al., 2020). However, how and to what extend domestication and breeding impact the diversity, abundance, structure, microbial function, and network of the root-associated microorganisms (rhizosphere, endorhiza) is not yet well understood. The main goals of this thesis were: 1) evaluate the impact of cereal domestication on the diversity, structure, and co-occurrence of seed endophytes, and endorhiza in the rhizosphere microbiome and assess the co-evolution of microbes in the rhizosphere with their host plants, 2) determine the effect of domestication on the colonization of cereal rhizosphere by seed- and soil-originated microbiomes, and 3) identify the "lost plant traits" as a consequence of changes in the rhizosphere microbiome due to domestication.

In this chapter, the key findings will be discussed, as well as the directions for future investigation will be suggested.

## 5.1. The effect of plant domestication and breeding on seed-endophytes

### 5.1.1. The impact of domestication on the diversity of seed microbiome

Different microbial diversity and richness between the spermosphere of wild and domesticated cereals were found. The found results are in line with the previous studies (Perez-Jaramillo et al., 2017; Kim et al., 2020) that showed that domestication of common bean and rice plant genotype shifted the composition of the spermosphere microbiome. Furthermore, Johnston-Monje & Raizada (2011) showed differences in the abundance of particular seed endophytes between wild and domesticated *Zea* maize species. In contrast,

no difference was found in the diversity of seed microbiome between wild and domesticated maize. Özkurt et al., (2020) did not find any influence of domestication and its accompanying changes on the seed microbiome of modern wheat species. Contrastingly, a higher bacterial diversity in cultivated cereal seeds than in the seeds of the wild wheat accessions was found **(Chapter 2)**, suggesting that changes in the seed during domestication lead to microbial diversification. Exudation profiles of germinating seeds of common bean showed that the spermosphere of domesticated bean contained higher nitrogen-containing amino acids (glutamate and glutamine) than wild bean spermosphere (Perez-Jaramillo et al., 2017), which might attract more and different bacteria from the soil. Furthermore, during the domestication of the cereal, the size, form (naked) (Doebley et al., 2006), nutritional content, such as protein, micronutrients (Zn, Fe) (Cakmak et al., 2000; Chatzav et al., 2010) and macronutrients (P, N, K, Mg, and S) (Bonfil et al., 1997) of the seed dramatically changed. The alterations might slowly alter the seed habitat. Along with plant phenotypic, physiologic, morphologic changes, intensified agriculture, modern cropping strategies, and changes in root exudation patterns might be among the other contributors.

### 5.1.2. The effect of domestication on taxonomic structure of seed-endophytes

*Effects on bacteria:*

In the studied cereal seeds, the most common phyla were *Proteobacteria*, *Actinobacteria*, and *Firmicutes* (**Chapter 2**). These results are in line with previous studies of seed-associated bacteria of different crops such as bean, maize, wheat, barley (Johnston-Monje & Raizada, 2011; Pérez-Jaramillo et al., 2017; Rahman et al., 2018; Özkurt et al., 2020). The enrichment of particular phyla in the seed-endosphere suggests a change in the niche preference of microorganisms in the spermosphere. At the genus level, differences in abundances of numerous bacterial and fungal taxa between wild and cultivated wheat genotypes were found (**Chapter 2**, **Chapter 4**). The relative abundance of beneficial seed endophytes, *Pseudomonas*, *Stenotrophomonas* were reduced in currently cultivated wheat species compared to their wild relatives. On the other hand, the relative abundance of particular genera *Cutibacterium*, *Herbaspirillum, Corynebacterium, Brevundimonas,* and *Acinetobacter* were found to increase. This compositional shift can be related to modified root exudation patterns as a result of root structure change during domestication (Iannucci et al., 2017; Pérez-Jaramillo et al., 2017). For instance, a study showed that *Pseudomonas fluorescens* was

strongly attracted by some organic acids and amino acids rather than sugars (De Weert et al., 2002; Gupta Sood, 2003). In contrast, *Corynebacterium flavescens, Azotobacter chroococcum* were strongly attracted by carbohydrates including glucose, arabinose, mannose, galactose, and glucuronic acid (Bacilio-Jiménez et al., 2003; Gupta Sood, 2003).

The reduction in the relative abundance of *Pseudomonas*, *Stenotrophomonas* with known plant-promoting characteristics (nutrient acquisition or mitigating stress conditions) suggests that the reduced stress for nutrient availability is due to advances in agriculture during domestication reduced the need for these beneficial microbial interactions. Özturk et al (2020) also found a reduced proportion of the *Halomonadaceae* family members known to promote plant salt tolerance and growth in the spermosphere in modern wheat seed.

Moreover, in modern cereals, new bacterial species have emerged as compared to the wild counterparts, like *Acinetobacter* which was previously reported as a novel seed-endophyte of new lineages of *Phaseolus vulgaris* (López-López et al., 2010). Genus *Acinetobacter* is often associated with wheat rhizosphere and found in agricultural fields with several plant growth-promoting traits including mineral solubilization, nitrogen fixation, iron acquisition, and siderophore production (Egamberdieva et al., 2008; Zimbler et al., 2009; Sachdev et al., 2010; Eijkelkamp et al., 2011). However, some of the *Acinetobacter* strains are opportunistic fish and human pathogens that can cause severe health problems (Howard et al., 2012; Dekić et al., 2018). Furthermore, *Corynebacterium,* another human-associated pathogen as well as root endophyte (Bernard, 2012), was found enriched in the spermosphere of modern wheat species. One of the most prevalent bacterial species found on human skin, *Cutibacterium* (*Propionibacteriaceae*) was found significantly enriched in seeds of modern cereal accessions. This result suggests the microbial inter-kingdom transfer from humans to plants as a result of the direct involvement of humans during seed and plant domestication. The presence of *Cutibacterium* and other human-associated microbes have been previously reported in different plant habitats of grapevine (Campisano et al., 2014), lemon (Faddetta et al., 2021), wheat (Kuźniar et al., 2020; **Chapter 2**), and orchid species (Alibrandi et al., 2020). Furthermore, the obtained results in this study might also indicate that the adaptation of new lineages to modern agriculture and the new communities are already incorporated in the seed spermosphere.

*Effects on Fungi:*

Among the fungal taxa, fungal diversity was reduced in modern cereal species and only a few fungal genera increased in dominance in cereal spermosphere such as *Alternaria* spp (**Chapter 4**). The found results agree with Ofek-Lalzar et al. (2016), who showed the reduced fungal diversity in modern bread wheat compared to wild wheat species *Triticum dicoccoides* and *Aeigelops sharonensis*. Furthermore, Ofek-Lalzar et al (2016) found that a few core species including *Alternaria* spp. dominated the spermosphere microbiome of all wheat species. Likewise, our results corroborate previous work by Kim et al. (2020) proving that particular fungal genera dramatically differed between wild and domesticated rice. The dramatic changes of particular fungal species might indicate a reduced competition for particular resources between microbial groups due to alterations in seed nutritional composition (Chatzav et al., 2010). Moreover, modern agriculture practices against wheat pathogens might induce the resistance of some other fungal species. For example, *Alternaria infectoria* is able to produce melanin in response to antifungals (Fernandes et al., 2016) as well as *Alternaria alternata* can develop cross-resistance to fungicides such as mancozeb, difenoconazole, propiconazole, and tebuconazoleas (Avenot et al., 2016; Yang et al., 2019). In this study, *A. alternata* and *A. infectoria* were the two of the most dominant seed-transmitted fungal species in the rhizosphere of wheat species (**Chapter 3**).

## 5.2. The effect of domestication on the seed-transmitted microbiome in different locations

More seed-transmitted microbes in the rhizosphere of wild *A. tauschii* than currently cultivated wheat species were found and *A. tauschii* presented the most distinct rhizosphere microbiome composition compared to other wheat species. These results suggest that domestication might affect the transmission of seed endophytes. However, the variable results in three field locations were observed (**Chapter 3**) indicate a strong effect of soil on the seed-borne rhizosphere microbiome. Previous studies found that soil strongly affected seed transmission (Hardoim et al., 2012; Özkurt et al., 2020; Morales Moreira et al., 2021). The microbial seed transmission can depend on the available resource as shown by Torres-Cortés et al. (2019). Although, seed-endophytes dominated in the early stage of plant development, later seed-borne microbial populations significantly reduced in the mature plant rhizosphere (Yang et al., 2017) probably due to dominant soil-originated rhizobacteria.

Furthermore, a higher proportion of microbes were found transmitted from seeds to endorhiza and rhizosphere of wild *A. tauschii* in Gross-Gerau (GG) where soil nitrogen level was relatively lower than in soil of the other study locations (**Chapter 3**). Besides, the seed-borne microbes found in those particular locations are directly or indirectly involved in N-cycling (**Chapter 3**). Moreover, the potential urease activity was significantly high in the rhizosphere of *A. tauschii* in GG (**Chapter 3**, Fig. 5). These results suggest that diploid *A. tauschii* can mediate seed-transmission of beneficial endophytes when there is a need for a particular nutrient. For conclusive proof in this aspect, more experimental validations are required.

### 5.3. The impact of cereal domestication on their endorhiza and rhizosphere microbiome assembly

Wheat was used in this thesis because it has the longest history of domestication and provides a unique opportunity to evaluate the consequence of domestication on the diversity of the root-associated microbiome. Differential abundance test showed that domestication shifted the microbial abundance from slow-growing oligotroph microorganisms: *Bacteroidetes (Chitinophagaceae)*, *Verrucomicrobia*, and *Gemmatimonadetes*, "*Planctomycetes*" (*Gemmataceae*), fungal phylum *Basidiomycota* more towards fast-growing copiotroph microbes like *Proteobacteria (Xanthomonadaceae*), *Firmicutes*, *Actinobacteria ("Nocardiodaceae")*, and fungal phylum *Ascomycota (Coniochaetaceae, Microdochiaceae, Nectriaceae)* (**Chapter 4**). The found results agreed with previous studies of Pérez-Jaramillo et al. (2017), who linked the increase of *Bacteroidetes* to change in root exudate patterns due to root structure modification of common bean during plant domestication. Similarly, bacterial families *Planctomycetes* and *Bacteroidetes* were found enriched in the wild beat rhizosphere (Zachow et al.,2014).

According to the study results, modern wheat species tend to recruit more from the surrounding bulk soil than wild species, which indicates the loss of some traits needed for host-specific recruitment. Instead, the modern wheat rhizosphere was colonized by a few common soil microbes in a significant amount. For example, the relative abundance of *Arenimonas*, a genus (SC-1-84) of the order *Burkholderiales,* was higher in the modern wheat species (**Chapter 3, 4**), which belongs to the core genera of the soils where crops were grown. Furthermore, the comparison between the differential abundance of the rhizosphere

microbiome of wheat species that were grown in the same site also showed the significant enrichment of *Arenimonas*, as well as, *Lysobacter*, *Reyranella, Luteimonas* in the rhizosphere of *T. aestivum* and *T. durum* compared to wild relative A*. tauschii* (**Chapter 3**). The host-specific selection of microbes is important for the host's innate immune system as shown by Bulgarelli et al. (2015). Bulgarelli et al. (2015) explained the differences between bacterial host-specific recruitment patterns between wild and domesticated barley with the supply and demand of functions of root metabolism and host innate immune system by investigating bacterial traits such as siderophore production, pathogenicity, sugar uptake, virulence regulation and, type III secretion system T3SS. The fast colonization of the roots of modern wheat species by given soil microbes also indicates the weakened requirements of plants for root colonization or reduced plant dependency on specific microbial interactions. The less complex microbial network in the rhizosphere of modern wheat species compared to wild relatives obtained in this study (**Chapter 4**) further supported the idea.

At the genus level, the rhizosphere microbiome of wild diploid *A. tauschii* was found enriched with more diverse bacterial and fungal genera than other wheat species including tetraploid wild emmer. We also observed that the rhizosphere of genetically related wild *T. dicoccoides* and domesticated *T. durum* were similarly enriched and the enriched microbial genera were different consistently across three locations (**Chapter 4**). The differential abundance results (**Chapter 3**) agree with the phylogenetic distance of the wheat species used in this study. In our previous studies, UPGMA dendrogram showed a higher phylogenetic similarity between *T. dicoccoides*, *T. aestivum,* and *T. durum* than *A. tauschii* (**Chapter 2**). This shows that *T. aestivum,* used in this study, is more closely phylogenetically related to *T. dicoccoides* than *A. tauschii*. However, the beta-diversity analysis showed that the root-associated microbiome structure of *T. aestivum* was more similar with *A. tauschii* than *T. dicoccoides* and *T. durum* (**Chapter 4**) showing the stronger influence of the D genome of *A. tauschii* on the rhizosphere microbial assembly of *T. aestivum*. Indeed, Tkacz et al. (2020) discovered that *T. aestivum*'s enhanced plant selection for *Glomeromycetes* and Nematoda was linked to the D genome from the wild progenitor *A. tauschii*. Moreover, whole-genome analysis showed that agronomically relevant gene family expansion in *A. tauschii* was linked with abiotic stress tolerance, disease resistance, and grain quality (Jia et al., 2013). However, hexaploid bread wheat seems to lose most of the genes after polyploidization (Chantret et al., 2005; Reif et

al., 2005; Haudry et al., 2007) in particular from D subgenome which might result in removal or modification of some important traits that are responsible for the establishment of host-microbe, microbe-microbe interactions in the rhizosphere. For instance, using reference genome sequences of 93 accessions of bread wheat and its diploid and tetraploid wild relatives from all over the world, Zhao et a., (2020) revealed that the three subgenomes of bread wheat showed similar mutation types, whereas D subgenome showed the highest mutation rate. Furthermore, the largest genomic deletion occurred in hexaploid wheat as a result of changes in *Ha* locus which is responsible for the quality of seed (Chantret et al., 2005). These changes in the hexaploid genome led to specific and non-specific down-regulation and activation of some gene expression (He et al., 2003). These previous studies indicate that modifications in the genome of wheat species are partially responsible for the rhizosphere microbiome variations between wheat species depending on ploidy level (2n, 4n, 6n). Similarly, Bouffaud et al. (2014) demonstrated that the phylogenetic distance between *Poaceae* genotypes was highly connected to the rhizosphere bacterial microbiome. Another study also showed that the crop genotype explained 43% of the variance in the fungal phyllosphere microbiome in cereals (Sapkota et al., 2015).

The beta diversity analysis further showed the host-specific effects on microbial diversity of cereal endorhiza and rhizosphere microbiome in all three locations. Our results indicate the genotype-specific selection from the available soil microbiota, which might benefit the host plant through beneficial interactions. For example, the root-associated microbiome of wheat and cucumber showed similar specific physiological capabilities such as motility and chemotaxis, different two-component systems, polysaccharide deterioration, and several secretion systems for root colonization, and the specific colonization was dependent on niche properties such as soil organic matter (Ofek-Lalzar et al., 2014). It has been proposed that changes in water and nutrient availability, as well as pH value, can modulate plant photosynthesis and growth, which in turn regulates the composition of the rhizosphere bacterial and fungal communities by inducing changes in exudation pattern (Xiong et al., 2020). However, Vieira et al. (2020) demonstrated that soil factors, notably soil texture, water content, and soil type, had a stronger influence on the content of polar root exudates of primary metabolism than plant attributes. Also, in our study, soil characteristics seem to induce plant traits that are needed for microbial interactions. In this study, the soil

characteristics of one location Groß Gerau(GG) were different from than other two locations Weilburger grenze and Rauischholzhausen (WG, RH) (**Chapter 3**), which was reflected in the microbial structure. Moreover, the plant ability to employ microbes depending on soil properties seems to be preserved more in wild crops than modern wheat species.

### 5.4. Microbial co-evolution

The effect of domestication on both, bacterial and fungal diversity in the endorhiza microbiome was found in all three locations. However, no difference in beta diversity in the rhizosphere of wild and domesticated wheat species was found in any location. This result indicates the co-evolution of endophytes (root/seed) with their host plants as it was hypothesized that wild crops have co-evolved more with their associated microbes than modern crops. The observed results in line with the results of Yeoh et al. (2017) showed that the host phylogeny is related to the variations between root community compositions. Yeoh et al. (2017) also proved that some of the root core microbiomes have co-evolved with their host plants. In the previous study on seed-endophytes, a higher phylogenic connectedness between wild plants and their seed microbiome was found (**Chapter 2**). Kim et al. (2020) also found a significant effect of the rice genome on seed endophytes as was observed in this study. Johnston-Monje & Raizada (2011) found that some seed endophytes of the wild ancestor can be conserved in domesticated maize even after many years. The previously observed results and the results of the current study suggest that the information for establishing microbe-host interactions is conserved in the host plant genome. For example, specific plant traits that are responsible for establishing symbiotic interaction between mycorrhizal fungi and plants were found in the genome of winter wheat (Lehnert et al., 2017) and legumes (Pawlowski et al., 2020). Furthermore, some microorganisms can be transferred over generations such as heritable symbiont *Epichloë coenophiala* (Nissanen et al., 2019), *Bradyrhizobium*, *Rhizobium*, and *Burkholderia* (Yeoh et al., 2017). However, the observed less phylogenic connectedness between modern cereals with their seed microbiome in this study indicates that this microbe-host interaction can be interrupted due to changes in plant genome during domestication. For example, Martın-Robles et al. (2017) studied the effect of domestication on the AMF symbiosis of wild and domesticated 27 different crops under available and restricted phosphorus Martın-Robles et al. (2017) concluded that domesticated plants benefit less from AMF than wild plants as a result of

artificial fertilizers supply. Furthermore, Spor et al. (2020) found that the relative abundance of a plant symbiont, *Glomeromycetes,* reduced in the elite wheat rhizosphere species compared to their wild relatives.

Furthermore, this study results suggest that certain microbial species in the spermosphere and endosphere of wild cereals may have been better adapted to specific plant habitats and had more time to form mutual interactions than present wheat species. For example, Emmett et al. (2017) showed that some of the specific plant characteristics, such as the age of the plant, high nitrogen use efficiency, and bigger seed size explained bacterial microbiome assembly variation (Emmett et al., 2017). It is known that similar microbes occupy specific niches such as spermosphere, rhizosphere, or endorhiza (**Chapter 3, 4**) due to their specific niche adaptations. This niche adaptation also seems to lead to co-evolution between the host plant and its associated microbes (Beckers et al., 2017). This requires microbes (Gunawardena et al., 2005) to have specific traits so they can live inside the root or seed such as tolerance or preference for limited nutrient sources, high osmotic pressure (Elbeltagy et al., 2000), and dehydration (Mano et al., 2006). Moreover, plants can specifically recruit some microbes for root colonization for their defense mechanisms (De-la-Peña et al., 2010; Doornbos et al., 2012), and the contact between microbes and plant host through the years might lead to co-evolution.

### 5.5. The effect of domestication on the microbial functional gene abundance and potential microbial enzyme activities in the rhizosphere of wheat species

Domestication can also affect the potential enzyme activities of soil microorganisms and they are encoded in functional genes. In this thesis, a significantly less abundance of microbial N-cycling genes, particularly *nirS* in the rhizosphere of modern wheat species compared to their wild progenitors was found (**Chapter 4**). Microbial urease activity was at the highest level in the rhizosphere of wild *A. tauschii* in two locations (GG, WG). Although no difference was found in P-cycling gene *phoX* in the rhizosphere of wild and domesticated wheat species, microbes related to the formation of alkaline PA (phosphomonoestherase activity) were significantly high in the rhizosphere of *A. tauschii* in GG. The abundance of genes encoding for proteins involved in the N-cycle supports our finding that the microbiome structure has changed in modern wheat species. The observed results in this study are also in good

agreement with Spor et al. (2020), who showed reduced N-cycle guilds in modern wheat species.

Due to progressively selection by breeders toward high quality and yield, domestication involves phenotypic and physiological expansions, such as improved nutrient use efficiency in modern genotypes (Roucou et al., 2017; Lei et al., 2021). Modern crops invest more energy for shoot biomass expansion instead of root biomass (Qin et al., 2013; Szoboszlay et al., 2015) due to higher selection for yields, suggesting that modern crops may receive a lower impact from soil properties and P cycling microbes (Qin et al. 2012). In contrast, wild species allocate more nutrients for root biomass (Qin et al., 2013) and might have a higher potential for scavenging more P and N, as we found in this study.

### 5.6. The effect of domestication of cereals on microbial network

The modular structured networks based on co-occurrence patterns in microbiomes provide knowledge about the potential positive or negative interactions between microbial groups. The comparison of cross-kingdom microbial networks in the rhizosphere of wild and modern crops allowed us to identify keystone microbes in bacterial and fungal interactions. A more complex cross-kingdom microbial network in all plant habitats (spermosphere, endorhiza, and rhizosphere) of wild cereals compared to modern cereals was found (**Chapter 4**). The found results are in agreement with Rossmann et al. (2020), who showed that the rhizosphere microbiomes of recent *T. aestivum* cultivars were less connected than the microbiome of older landraces. A similar study demonstrated a higher microbial network in the rhizosphere of high-growing, *T. aestivum* cultivars than genetically advanced semi-dwarf low-growing wheat varieties.

Furthermore, higher positive connections in the rhizosphere of modern cultivars than wild ancestors were found which might indicate competitive and stabile interactions. A positive association between microbial communities indicates the presence of mutualistic connections, while a negative association may indicate the presence of host competition or a predatory connection between microorganisms (Steele et al., 2011). Interestingly, similar hub species between genetically related wheat species was found (**Chapter 3**), which indicates the regulation of host genotype on hub microbes, which play a key role in shaping the microbial network structure (Agler et al., 2016). Characterization and comparison of

microbiome composition of spermosphere, endosphere, and rhizosphere of wild and modern wheat species showed that the diversity of fungi was reduced, and the habitats are dominated by few genera (**Chapter 2**, **Chapter 4**). The microbial shift during domestication seems to influence the cross-kingdom network structure.

### 5.7. Future perspectives in microbiome studies and concluding remarks

According to the findings of the thesis, significant differences in microbial diversity and composition were found between the seed microbiome of wild and domesticated cereal species. Specific bacterial and fungal species were found differently prevalent in the rhizosphere of wild and domesticated wheat species. Moreover, wild *A. tauschii* showed a different microbiome assemblage as compared to the other wheat species. Furthermore, the abundance of microbial N-cycling genes in the rhizosphere and cross-kingdom network were reduced in the modern wheat species compared to wild relatives. These observed differences can be a result of modified seed, root traits that shapes the root-associated microbiome. Furthermore, we observed different results in different locations suggesting the variable effect of domestication depending on soil and plant genotype characteristics.

Further studies might consider involving stress conditions in an experiment to explore the full potential of microorganisms of wild crops. In fact, some of the beneficial microbial interactions in the rhizosphere only take place under stress conditions (Yang et al., 2011; Liu et al., 2021). As shown by Martín-Robles et al. (2018), wild ancestors of 27 domesticated crops established AMF symbiosis under phosphorus deficit conditions compared to domesticated crops.

This study was carried out under field conditions to better investigate microbial changes under real situations. However, some interactions can only be proven under more controlled environments to reduce the effect of soil and other environmental factors on microbial responses. Therefore, further studies should also include experiments under controlled conditions to verify the effect of domestication on microbial interactions in the rhizosphere microbiome. For example, a group of “key” microbial species can be brought into controlled conditions depending on their relative abundance and co-occurrence patterns by using the sequencing data obtained from the field experiments and further examining their microbe-microbe and host-microbe interactions under controlled conditions.

Exploring the beneficial interactions in the various microbial habitats of wild relatives under natural conditions can reveal important information about the crop traits that were lost during domestication, which could then be re-established by using wild crops as a source of germplasm to improve the long-term performance of currently cultivated crops. Endophytes, in particular, can form close bonds with their hosts and benefit them more than other non-plant-associated microbes. The knowledge can be used in modern agriculture to develop environmentally friendly biocontrol agents and reduce the use of artificial fertilizers by effectively utilizing microbes in crop production.

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