The induction of systemic resistance to barley powdery mildew by rhizosphere bacterial communities does not disrupt the structure or function of native microbial communities

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1. Abstract

Synthetic microbial communities (SynComs) could help plants withstand biotic stress and reduce the need for pesticides. With this in mind, we created two SynComs, comprising bacterial strains isolated from the rhizospheres of barley and wheat. We then studied their potential to trigger induced systemic resistance against the barley pathogen *Blumeria graminis* f. sp. *hordei (Bgh)*. To investigate the plant-microbial interactions from the perspective of both plants and microbes, we performed DAF staining to quantify *Bgh* propagation in plant leaves, analysed leaf transcriptomes and conducted rhizosp here 16S rRNA gene metabarcoding and rhizosphere metatranscriptome analysis. Our results demonstrate that the SynComs elicit defence responses in barley against *Bgh* in a manner similar to that of the positive control strain *Pseudomonas simiae* WCS417r. The SynComs act without triggering a strong gene response prior to

34 inoculation with the plant pathogen or affecting plant-associated prokaryote communities; they only
35 mildly influence bacterial gene expression in the rhizosphere. Instead, they act as priming agents,
36 preparing the plant for further pathogen attack. These findings suggest that protective SynComs can be
37 applied in the field without causing signficant disruption to native microbial communities.

38 **2. Introduction**

39 Plant-associated microbial communities have a positive effect on a plant's fitness, development, and
40 tolerance of stress (Mendes et al. 2013; Panke-Buisse et al. 2015; Lau and Lennon 2012). Plant beneficial
41 bacteria (PBB) have been isolated and characterized from the root, leaf and stem surfaces, and inside
42 plant tissues (Glick 2014). They mobilize nutrients (Israr Asghar 2023), produce phytohormones such as
43 indole-3-acetic acid, ethylene and jasmonic acid (Kejela 2023) and improve plant immunity and resistance
44 against pathogens (Fujiwara et al. 2016). Generating host-associated microbial culture collections has
45 enabled plant growth promoting (PGP) bacteria to be used as bioinoculants in the form of single strains
46 or as consortia via the synthetic microbial community (SynCom) approach (Niu et al. 2017). SynComs are
47 constructed microbial consortia, usually composed of selected commensal strains with PGP abilities. They
48 can be used to rebuild a host microbiome under controlled conditions and make it possible to study the
49 interactions between microbes and their hosts, as well as interactions inside the microbial community
50 (Northen et al. 2024).

51 Plants possess a complex innate immune system to defend against pathogenic bacteria, fungi, protists,
52 nematodes and viruses (Agrios 2009). When attacked by a pathogen, plant systemic signaling transfers
53 the information from the affected parts through the plant to tissues that are still unaffected and thus
54 triger a coordinated response (Vlot et al. 2021). More precisely, plant cells use pattern recognition
55 receptors to recognize the microbe or pathogen–associated molecular patterns. This process provokes
56 pattern-triggered immunity, which together with effector-trigerred immunity can induce salicylic acid
57 signalling and activate systemic acquired resistance (SAR) (Vlot et al. 2021; Pieterse et al. 2012).

58 Beneficial bacteria can mediate induced systemic resistance (ISR), a systemic immunity pathway distinct
59 from SAR, by priming the aforementioned defence mechanisms, a phenomenon first observed with
60 *Pseudomonas* and *Bacillus* strains (van Wees et al. 2008). This priming puts the plants under an “alerted”
61 state, enabling them to respond more quickly and/or strongly to subsequent pathogen infections (Heil
62 2001; Conrath et al. 2015; Martinez-Medina et al. 2016). Priming is suggested to be effective against a
63 wide range of pathogens, including (hemi-)biotrophic and necrotrophic types, and even herbivores (Vlot
64 et al. 2021). ISR by commensal *Pseudomonas* strains has been shown to depend on jasmonic acid and

ethylene signaling pathways (Vlot et al. 2021; Pieterse et al. 2012; Yu et al. 2022), whereas the *Bacillus*- and *Streptomyces*-elicited pathways may also employ salicylic acid signalling (Kloepper et al. 2004; Ebrahimi-Zarandi et al. 2022; van Wees et al. 2008; Kurth et al. 2014). ISR results in either subtle (Heil and Bostock 2002) or extensive (Kurth et al. 2014) changes in gene expression in the unaffected tissues that primes the plant for an improved defense response upon pathogen attack. Despite ISR mainly elicited in the roots through interaction with beneficial rhizobacteria, it can protect above-ground tissues (like leaves) by priming JA- and ethylene-dependent defence pathways (Pieterse et al. 1998; van Wees et al. 2008). Currently, it is unclear which bacterial taxa or communities are capable of inducing ISR in barley, and whether such responses are specific to particular biotic or abiotic stress conditions.

Blumeria graminis f. sp. *hordei (Bgh)* is a widespread fungal pathogen that causes the fungal disease powdery mildew. This affects a wide range of plants including cereal crops like barley (Sacharow et al. 2023) and is reported to occur in several barley growing regions (Wang et al. 2023; Cieplak et al. 2022; Cowger et al. 2018; Kloppe et al. 2022). Powdery mildew affects plant leaves and can adversely impact crop yield (Czembor and Czembor 2023) and photosynthesis (Coghlan and Walters 1992), particularly under moist and warm weather conditions which favor the pathogen (Elagamey et al. 2023; Bhardwaj et al. 2021). Efforts to study ISR have often employed using the bacterial strain *Pseudomonas simiae* WCS417r, which was recently shown to protect barley from Bgh (Pieterse et al. 2021, Sommer et al. 2026). This robust rhizobacterium has been shown to confer ISR in various host plants (Pieterse et al. 2021; Zhu et al. 2022) and is used as a positive control in this study .

The negative impacts of climate change, such as rising temperatures and prolonged droughts, strongly affect plants (Sato et al. 2024). Prolonged drought, for instance, make plants more susceptible to disease by reducing photosynthesis, transpiration and nutrient uptake (Hatfield and Prueger 2015; Nievola et al. 2017). Furthermore, climate change may support plant disease and pest dispersal (Delgado-Baquerizo et al. 2020), thus making plants more susceptible and cause damage and reduce yields (Rezaei et al. 2023; Singh et al. 2023). Such challenges suggest the need for sustainable approaches that enhance plant resilience to abiotic and biotic stress, thus ISR can work as an important tool to maintain plant health, agricultural output, and environmental sustainability under future climatic conditions.

ISR in plants has been widely described in model species like *A. thaliana* (Weller et al. 2012; Ilham et al. 2019; van Wees et al. 2000) and tomato (Suresh et al. 2022; Hu et al. 2018; Mehari et al. 2015). Even though interactions between barley and *Bgh* have been extensively studied, including host–pathogen signaling and defense responses, comparatively few studies have addressed ISR in this pathosystem,

96 particularly in the context of defined beneficial microbial communities. While recent work has started to
97 investigate ISR triggered by barley-native microbial communities, comparative studies using well-defined
98 synthetic communities from different host species (e.g., barley vs. wheat) remain limited. Bziuk et al.
99 (2022) demonstrated that a suspension of barley rhizosphere microbiome can enhance the immune
100 response and reduce the incidence of powdery mildew disease. Building on this, we tested whether
101 SynComs from the rhizospheres of barley and wheat, composed of bacterial strains enriched under
102 drought stress (each comprising Firmicutes, Proteobacteria, and Actinobacteria), could trigger ISR in
103 barley plants against the same pathogen. The ISR-eliciting bacterium *P. simiae* WCS417r (WCS417r) was
104 used as a positive control. We expected that due to the presence of Firmicutes and Actinobacteria
105 (Kloepper et al. 2004; Ebrahimi-Zarandi et al. 2022) i) both SynComs would elicit an ISR to the same extent
106 as WCS417r, but in a jasmonic acid-, ethylene- and salicylic acid-dependent manner. We also (ii)
107 hypothesized that, given that barley was used as the host plant in our experiment, the barley SynCom
108 would elicit a stronger response than the wheat SynCom due to the plant host preference of rhizosphere
109 microbiomes. Our final hypothesis was that, iii) at harvest time, only a few SynCom members would
110 remain active and abundant in the rhizosphere.

111 **3. Materials and Methods**

112 **3.1. Collection of barley and wheat rhizobacteria**

113 Barley and wheat rhizobacteria were sampled from growing cereals at the Global Change Experimental
114 Facility (GCEF) in Bad Lauchstädt (51° 23’ 123 30N, 11° 52’ 49E). GCEF is a field research station site of the
115 Helmholtz Centre for Environmental Research (https://www.ufz.de/index.php?en=42385) that has a
116 Haplic Chernozem soil type (Altermann et al. 2005). The site has a temperate continental climate with a
117 mean annual temperature of 9.7°C and mean annual precipitation of 438 mm (1993-2013) (Schädler et al.
118 2019). The bacterial strains used in this study were sampled from organic and conventional farming plots
119 that were exposed to an ambient and future climate treatment. The latter comprises besides warming a
120 10% increase in precipitation in spring (March-May) and autumn (September-November), as well as a
121 rainfall reduction by 20% in summer (June-August). This simulates the climate expected for Bad
122 Lauchstädt in 2080 (Schädler et al. 2019). The strains were isolated from the rhizospheres of the barley
123 cultivar “Antonella” and the wheat cultivar “RGT Reform” in two different stages of plant development,
124 stem elongation stage BBCH 37-39 and grain filling stage, BBCH 75-77. The isolation process is described
125 in Breitkreuz et al. (2020) and Rigerte et al. (2025). Briefly, the rhizosphere was collected by gently
126 detaching the soil from the plant roots, after which the organic material was removed manually and by

127 sieving (2 mm). The soil was then mixed and suspended to double-distilled water. The bacteria were
128 separated from the soil by sonication, and a suspension was inoculated on Pikovskaya's agar medium
129 (Pikovskaya 1948). Plates were then incubated for three weeks at 25°C and colonies showing phosphate
130 solubilization (observed as clear halos around them) were purified.

131 **3.2. Selection of bacterial isolates**

132 Polyethylene glycol 8000 (PEG) was used to lower the water potential of agar medium and thereby
133 assess the drought tolerance of the isolated strains. Drought stress was simulated by applying a 500 g/L
134 solution of PEG (PEG0.5), which corresponds to an osmotic potential of approximately −1.1 MPa (Verslues
135 et al. 2006). Drought tolerance was assessed by calculating the percentage reduction in colony diameter
136 under PEG0.5 compared to PEG0. Genomic DNA was extracted by adding PEG to the bacterial suspensions
137 and then vortexed with glass beads according to the method of Breitkreuz et al. (2019). DNA amplification
138 was performed using PCR with the primers 27F and 1492R (Lane 1991) and the Promega Green system
139 (Promega, Madison, WI, USA). Subsequently, partial 16s rRNA gene sequences were obtained by Sanger
140 sequencing using the primer BAC 341F at LGC Genomics (Berlin, Germany). The main objective of Sanger
141 sequencing was to obtain sequence regions overlapping with ASVs obtained by 16s rRNA gene amplicon
142 sequencing in previous studies. These amplicon sequencing darasets were generated from barley and
143 wheat rhizosphere samples and were used as a referene for selecting bacterial strains for SynCom
144 construction. Amplicon sequence variants (ASVs) that were more frequent under future climate
145 conditions compared to ambient conditions in barley and wheat rhizosphere samples (Breitkreuz et al.
146 2021a), as well as ASVs enriched under drought compared to well-watered conditions in a greenhouse
147 experiment using the same chernozem soil (Breitkreuz et al. 2021b), were selected from these datasets.
148 These ASVs were then compared to the 16S rDNA sequences from our cultured bacterial collection to find
149 isolates with identical or highly similar sequences. From this group, sixteen PEG-tolerant strains that
150 showed similar growth on PEG0 and PEG0.5 were selected to assemble the Drought-Tolerant Synthetic
151 Community (DT-SynCom). A full overview of the study workflow is presented in Figure 1.

152 **3.3 Composition of a synthetic microbial community (SynCom) from barley and wheat**

153 The final synthetic communities consisted of bacterial strains that were isolated from the rhizosphere of
154 barley and wheat. The strains were selected based on their drought tolerance and 16S rDNA sequence
155 identity as described in sections 3.1 – 3.2 The bacterial strains included in both SynComs were described
156 in a previous study (Rigerte et al. 2025) and are listed in Table 1 to document the methods used in this

157 work. Each SynCom was constructed by combining equal volumes of single-strain cultures as described in
158 the following section.

159 **3.4. Preparation of the drought-tolerant synthetic community (DT-SynCom)**

160 Bacterial strains from the rhizosphere of barley and wheat were cultivated for three days at 25 °C on yeast
161 malt extract (YME) agar. The YME medium was prepared by dissolving the constituent ingredients (7 g
162 malt extract, 2.8 g yeast extract, 10.5 g agar (2%) and 2.8 g glucose) in 700 ml water and autoclaving for
163 20 minutes. Each strain was resuspended in 10 mM magnesium chloride ($MgCl_2$) until the optical density
164 (OD) at 600 nm reached a value of 0.5. All individual suspensions were then centrifuged, the supernatant
165 decanted and the bacterial pellets resuspended in 1 ml $MgCl_2$. The barley strains were combined in equal
166 proportions into a synthetic community and the wheat strains into a separate community, with each strain
167 adjusted to a final concentration of 10^9 cfu/ml in 10 mM $MgCl_2$.

168 **3.5. Propagation of powdery mildew**

169 Barley seeds were sterilized (excluding the surface sterilization step with 75% EtOH), sown and grown for
170 three weeks as previously described. Barley plants were then inoculated with *Bgh* as describedbelow
171 (Lenk et al. 2018; Lenk et al. 2019). Plants were then grown for an additional ten days to obtain mature
172 powdery mildew spores, which were used for infections. Propagation was repeated once a week to
173 maintain a viable powdery mildew culture.

174 **3.6. Inoculation of barley plants with SynComs and powdery mildew infection assay**

175 Plant inoculation and powdery mildew infection assays were performed following established protocols
176 (Lenk et al. 2018; Lenk et al. 2019), with minor modifications as described below. Barley seeds (variety
177 "Golden Promise") were first surface sterilized by a one-minute wash with 75% ethanol (25 inversions per
178 minute), followed by a five-minute incubation in sodium hypochlorite (NaClO). The washing step was
179 followed by a ten-minute incubation in sterile water and repeated three times. The seeds were then
180 placed on sterile NB plates (Table S1) and kept in the dark at room temperature for germination. After
181 four days, the seeds were divided into four groups and inoculated by means of a two hours long incubation
182 with one of the following treatments: the wheat SynCom, the barley SynCom, or WCS417r (positive
183 control). Plants were inoculated with either freshly prepared SynComs or previously prepared SynComs
184 that were stored at -80°C in 25% glycerol and washed three times with $MgCl_2$ (10 mM) to remove glycerol
185 prior to use. The forth group was inoculated with $MgCl_2$ (10 mM) to serve as a mock control solution. The
186 barley plants were then grown for 3 weeks under a 14/10-hour day/night cycle at 20°C/16°C respectively.

187 On day 21, the rhizosphere of each plant was collected by carefully removing the root-attached soil by
188 hand, and the second and third leaves of three separate plants, were sampled and stored frozen until
189 subsequent nucleic acid extractions. The remaining barley plants and a glass slide were then placed in a
190 cardboard box and infected with *Bgh* by gently shaking the infected plant over the box. To spread the
191 spores, a styrofoam board was fanned ten times in all directions. After one hour, the spores deposited on
192 the glass slide were counted to verify the spore density of approximately 30 spores per mm^2. One week
193 later, the plant leaves were harvested to evaluate the infection intensity.

194 **3.7. Plant diaminofluorescein (DAF) staining**

195 Plant diaminofluorescein staining was performed following established protocols (Lenk et al. 2018; Lenk
196 et al. 2019). The MES-KOH (morpholinoethanesulfonic acid-potassium hydroxide) 0.5 M stock solution
197 was prepared by adding 21.32 g MES to 200 ml H_2O and adjusting the pH to 5.7 with KOH. The bottle was
198 then wrapped in an aluminum foil, as MES is light-sensitive, and then placed in the refrigerator till further
199 use (shelf life of the solution is one month). The prepared MES stock solution was then used to prepare
200 the working solution DAF-FM-DA described in Table S2. For staining, the second and third leaf of barley
201 were selected, and 16 leaf discs per treatment of inoculated plants were immersed in 4 ml of working
202 solution (without added DAF) for 45 minutes (in the dark), then the solution was removed and 4 ml of DAF
203 solution was added. The leaf discs were then incubated in the dark for 45 minutes and then vacuum
204 infiltrated 3 to 5 times to aspirate the solution into the plant tissue, followed by incubation under light for
205 105 minutes. The solution was then removed and 1 ml of working solution was added for a wash step to
206 remove any remaining DAF-FM DA. The leaf discs were then dried on a paper towel and placed upside
207 down on a 96-multiwell plate filled with 1% phytoagar (any meniscus formed by the agar medium was
208 removed by scraping to obtain a uniform surface for fluorescence measurement). The plate was then
209 sealed with qPCR-foil and the Keyence BZ-X800 fluorescence microscope was used to image the leaf discs.
210 Fluorescence was measured using a BZ-X Filter GFP (model OP-87763) with an excitation filter of 470/40
211 nm, an emission filter of 525/50 nm and a dichroic mirror with a cutoff wavelength of 495 nm.
212 Fluorescence was normalized for each replicate as the ratio of the measured intensity to the mean
213 intensity of the empty wells. A small pseudocount was added for log10 transformation to avoid undefined
214 values when intensity ratios were zero (pseudocount = 0.1 × smallest non-zero ratio observed). Values
215 were then plotted as log10(ratio + pseudocount).

216 **3.8. Nucleic acid extraction and sequencing**

217 **Rhizosphere metatranscriptome:** total RNA from barley rhizosphere samples was extracted using the
218 RNeasy PowerSoil Total RNA Kit (Qiagen, Hilden, Germany) following the extraction protocol provided by
219 the manufacturer. Sequencing was performed by Azenta Life Sciences (Leipzig, Germany) on an Illumina
220 instrument after rRNA depletion in a paired-end configuration generating approximately 20 million
221 paired-end reads of 150 bp read length per sample.

222 **Host leaf gene expression:** Second leaf of barley plant was sampled and grinded using liquid nitrogen.
223 Leaf RNA was extracted using the NucleoSpin RNA Plant Kit (Macherey-Nagel, Düren, Germany) and
224 Agilent Bioanalyzer was used to determine the RNA integrity number (RIN) which was ≥ 7 for all samples.
225 Samples were sequenced by Novogene (Düsseldorf, Germany) and Illumina sequencing was performed
226 using a NovaSeq X Plus Series sequencing platform, which generated approximately 20 million paired-end
227 reads of 150 bp length per sample (40 million reads in total).

228 **Rhizosphere 16S rRNA gene metagenomes:** barley rhizosphere DNA was extracted using the DNeasy
229 PowerSoil Pro Kit (Qiagen, Hilden, Germany) and barley leaf microbiome DNA was extracted using
230 NucleoSpin Plant II kit (Macherey-Nagel, Düren, Germany). The V4 region of the 16S rRNA gene was
231 amplified using the primers 515F (GTGYCAGCMGCCGCGGTAA) and 806R (GGACTACNVGGGTWTCTAAT)
232 (Caporaso et al. 2011), each with Illumina overhang adapters (P5:
233 TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG; P7: GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG). The
234 Illumina MiSeq platform with Reagent Kit v3 was used to generate an average of ~200,000 read pairs per
235 sample (2x 300 bp).

236 All sequencing data generated in this study, including rhizosphere metatranscriptomes, rhizosphere 16S
237 rRNA gene amplicon sequencing data, and host leaf RNA sequencing data, have been deposited in NCBI
238 under BioProject ID PRJNA1405459.

239 **3.9. Rhizosphere 16S rDNA amplicon sequencing bioinformatics and microbiome analysis**

240 The sequenced 150 bp paired-end reads were processed using the Dadasnake pipeline v0.11 (Weißbecker
241 et al. 2020; Callahan et al. 2016) with default parameters. The process included quality filtering and
242 trimming by truncating forward and reverse reads to 170 bp and 130 bp, respectively. Reads with a
243 minimum quality score of 13 were retained and a maximum expected error rate of 0.2 for both read
244 directions was allowed. Error models were learned and sequencing errors corrected using DADA2, which
245 was followed by dereplication, which collapsed identical reads into amplicon sequence variants (ASVs).
246 ASVs were subsequently clustered at 97% sequence identity to generate operational taxonomic units

247 (OTUs) (Schloss 2021; Estensmo et al. 2021). Chimeras were removed using DADA2's consensus algorithm.
248 Taxonomic classification of the produced OTUs was performed against the SILVA v138.1 database (Quast
249 et al. 2013). Additionally, OTUs that could have been potentially associated with the SynCom strains were
250 identified using MMseqs2's easy-search module by demanding a minimum sequence identity of 99% and
251 99% sequence coverage of the OTU sequence by the corresponding 16S rDNA from the SynCom genomes
252 published by our group (Rigerte et al. 2025). OTUs identified as belonging to the following taxa were
253 removed in R v4.4.1 prior to further analysis: chloroplasts, mitochondria, Rickettsiales (obligate
254 intracellular symbionts/parasites), Diploririckettsiales (not known to be plant-associated),
255 Piscirickettsiales (not known to be associated with soils/plants), fungi, algae, Escherichia spp., and
256 taxonomic assignments that were explicitly marked as "unknown" and/or "unclassified".

257 Further processing was performed using functions from the phyloseq v1.50 (McMurdie and Holmes 2013),
258 microViz v0.12.7 (Barnett et al. 2021), and vegan v2.7-1 (Oksanen et al. 2025) R packages. The OTU table
259 was pre-processed using the tax_fix() function from microViz to fill in missing taxonomic ranks at higher
260 levels from lower levels. OTUs with zero reads across all samples were removed. Alpha diversity metrics
261 (Shannon, Chao1 and observed) were calculated using the estimate_richness() function from phyloseq
262 without additional filtering of the OTU table. To calculate beta diversity, the data were first filtered to
263 remove OTUs with fewer than five reads across all samples, with no phyla being removed. Samples with
264 zero reads across all OTUs were then filtered out. The data were then rarefied to even depth using
265 phyloseq's rarefy_even_depth() function. Beta diversity was assessed using Bray-Curtis distances
266 calculated upon these data as inputs. A Principal Coordinates Analysis (PCoA) biplot was created using
267 data from phyloseq's ordinate() function to examine the distribution of the samples along the first two
268 principle coordinates. Beta dispersions of the samples were calculated using betadisper() from vegan and
269 statistically significant pairwise differences in these were assessed using TukeyHSD() (Tukey's Honestly
270 Significant Differences) from the stats package. Pairwise Permutational Multivariate Analysis of Variance
271 (PERMANOVA) between all treatments was conducted using the pairwise.adonis() function from the
272 pairwiseAdonis v0.4.1 package (Arbizu 2020), with Bray–Curtis distances as the input variable, 9999
273 permutations, and Benjamini–Hochberg (BH) multiple testing p-value correction. Alpha and beta diversity
274 of the soil microbiome samples were analyzed with 1,390 and 1,246 operational taxonomic units (OTUs)
275 across 20 samples, respectively.

276 **3.10. Leaf transcriptome analysis**

277 Sequenced reads were trimmed using Trimmomatic (Bolger et al. 2014) with the default parameters, and
278 mapped to the barley reference genome *Hordeum vulgare* cv. Morex genome assembly v3 using HISAT2
279 (Kim et al. 2015). FeatureCounts (Liao et al. 2014) was used to assess the number of reads per gene.

280 Differential gene expression analysis was performed in R using the following pipeline. Gene counts
281 obtained by featureCounts were used for differential expression analysis using the DESeq2 package (Love
282 et al. 2014) and DESeqDataSet object was constructed using the metadata table containing treatment
283 information. Differential gene expression between different treatments was determined by fitting a
284 negative binominal generalized linear model and performing Wald tests for the pairwise comparisons. The
285 obtained *p*-values were adjusted using the Benjamini–Hochberg procedure and genes with p-value < 0.05
286 and an absolute $\log_2$ fold change ≥ 1 were considered to be significantly differentially expressed. Initially,
287 a set of the 200 genes showing the strongest variation associated with the treatment was determined by
288 using a variance-partitioning analysis. The z-scores for all treatments were then compared, and a heatmap
289 showing the 50 genes with the strongest differences was created using the ComplexHeatmap package (Gu
290 et al. 2017).

291 **3.11. Rhizosphere metatranscriptome analysis**

292 The raw metatranscriptomic reads were trimmed using Trimmomatic in paired-end mode and then
293 processed in sequential mode with SqueezeMeta v1.6.2 (Tamames and Puente-Sánchez, 2019). Contigs
294 were assembled *de novo* using MEGAHIT (k-mer sizes 41,61,81,99,121), and short contigs (<200 bp) were
295 filtered using prinseq. Taxonomic and functional annotations (minimum 50% identity) involved ORF
296 prediction using Prodigal, rRNA/tRNA detection using Barrnap and Aragorn, and DIAMOND similarity
297 searches against GenBank, eggNOG, and KEGG, supplemented by HMMER3 for Pfam. Read mapping for
298 quantification was performed using Bowtie2, with default parameters.

299 The SQM files generated by the pipeline were imported into R and processed using SQMtools (Puente-
300 Sánchez et al. 2020) and allied packages. These files contained read counts attributed to taxonomic
301 annotations as well as functional annotations (i.e., features) against the KEGG, COG, and Pfam databases.
302 Prior to analysis, raw counts (abundances) were converted to per-sample relative abundances (with non-
303 finite values being set to zero) and filtered to retain features with at least $1x10^6$ relative abundance in at
304 least 70% of the samples. Top ten functional features for the KEGG, COG, and Pfam databases grouped by
305 treatment were visualized using the plotFunctions() function from SQMtools using TPM (transcripts per
306 million) values as input. Phylum-level taxonomy summaries grouped by treatment were generated using

307 plotTaxonomy() from SQMtools with the TPM values also. Bray-Curtis distances computed from the
308 relative abundance values calculated earlier were then used to assess beta diversity and dispersion which
309 were then visualized using a PCoA (using pcoa() from ape (Paradis et al. 2004)) and tested for statistical
310 significance using TukeyHSD() as described earlier. A whole model PERMANOVA as well as pairwise
311 PERMANOVAs were also conducted as described on the Bray-Curtis distances. Finally, differential gene
312 expression analysis was performed using DESeq2 with default parameters (Love et al. 2014).

313 **4 Results**

314 **4.1 Barley and wheat SynComs reduce propagation of *Blumeria graminis f. sp. hordei (Bgh)* in barley**
315 **leaves**

316 To investigate whether barley exhibits induced systemic resistance when interacting with the SynComs,
317 its roots were inoculated with barley or wheat rhizosphere SynComs, or WCS417r or a corresponding
318 mock control solution. The barley seedlings were then grown in open-top pots with no visible changes in
319 plant phenotypes between different treatments (Figure 2A). After 21 days, the barley seedlings were
320 inoculated with *Bgh,* the propagation of which was monitored at seven days post inoculation (dpi).
321 Differences in *Bgh* growth were visualized by fluorescence microscopy following DAF-FM DA staining of
322 fungal hyphae (Figure 2B). Plants inoculated with the three inoculants exhibited comparable and
323 significantly lower fluorescence intensities than the mock-inoculated group (Figure 2C). These results
324 show that the barley and wheat SynComs induce ISR in a manner similar to the ISR eliciting bacterium
325 WCS417r.

326 **4.2 Microbial community composition in barley rhizospheres**

327 Next, we investigated whether increased tolerance to powdery mildew is mediated by changes in the
328 composition of the rhizosphere bacterial community, using amplicon sequencing of the partial 16S rDNA
329 gene. The rhizosphere microbiome comprised 1390 ASVs, which were dominated by Proteobacteria,
330 Actinobacteriota, Bacteroidota, Firmicutes, Planctomycetota, Chloroflexi, Verrucomicrobiota,
331 Myxococcota and Acidobacteriota, respectively (Supplementary Figure S1A). There was no difference in
332 either alpha diversity (see Supplementary Figure S1B) or beta diversity between treatments and the
333 control, as supported by PCoA ordination plot (Supplementary Figure S1C) and pairwise comparisons of
334 beta dispersion (Supplementary Figure S1D Table S3). Identical ASVs to the partial 16S rRNA gene
335 sequences of the inoculated strains were detected in the rhizosphere microbiomes (see Table S4, sheet
336 'taxtable', column 'insyncom'), indicating that the 16S rDNA identities were present at harvest.

337 **4.3 Barley leaf transcriptomes show no major response to inoculation (PERMANOVA)**

338 Plant transcriptome sequencing was carried out to investigate barley leaf gene expression when
339 inoculated with either $MgCl_2$ (10 mM) (mock control solution), WCS417r or SynCom consisting of strains
340 from either barley or wheat rhizosphere. Multivariate analysis of gene expression profiles after batch
341 correction using PERMANOVA did not reveal significant differences between treatments (adjusted p =
342 0.101–0.235, R^2 = 0.129–0.198), indicating that treatment explained only a small proportion of the
343 variation (Supplementary Figure S2, Supplementary Table S5).

344 **4.4 Limited contribution of treatment to variance in barley leaf gene expression**

345 To look at the sources of variability in the transcriptomic data, we quantified the variance explained by
346 the treatment, sampling date, and residuals. Due to a strong batch effect association with sampling date,
347 variance partitioning was interpreted after batch effect correction (Figure 3A). The batch effect correction
348 for the sampling date reduced its caused variance to near zero, confirming the batch effect caused by the
349 sampling date. While the treatment effect increased slightly, with a broader distribution of explained
350 variance (typically around 12% but extending up to 60% for a subset of genes) and the residual variance
351 decreased accordingly, the residual variance was still the main cause of variability between different
352 treatments, despite the explanatory power of treatment increasing after the batch effect correction.

353 The top 10 genes for which differences in gene expression were most strongly explained by the treatment
354 had variance explained values of around 78% and 90% (Figure 3B). The first four of these genes
355 (*HORVU.MOREX.r3.7HG0734910, HORVU.MOREX.r3.4HG0344820, HORVU.MOREX.r3.3HG0248440,*
356 *HORVU.MOREX.r3.7HG0659040*) exhibited different expression patterns between treatments (Figure 3C).
357 These results suggest that, after correcting for the batch effect, treatment effects on the expression of
358 the top four genes were relatively small.

359 **4.5 Differential gene expression analysis of barley leaves under different PGB treatments**

360 To investigate transcriptional changes in barley leaves caused by the inoculations, we performed
361 differential expression analysis. Inoculation with Barley SynCom, Wheat SynCom and WCS417r, resulted
362 in statistically significant sets of differentially expressed genes (7, 54, and 110, respectively), however,
363 these changes were small in magnitude related to the whole transcriptome level. Permutational analysis
364 of variance (PERMANOVA) showed that treatment did not have a significant effect on barley leaf gene
365 expression (adjusted p = 0.69–0.94; Supplementary Table S3). The pairwise comparisons showed low
366 explanatory power (R^2 = 0.015–0.023), confirming that treatment accounted for only a small fraction of

367 the total variance in gene expression. DESeq2 analysis showed that the fewest differentially expressed
368 genes (DEGs) (7 genes) were found in the comparison between barley SynCom and the control treatment.
369 This was followed by the comparison between wheat SynCom and the control (54 genes), and WCS417r
370 and the control (110 genes) (Supplementary Tables S6, S7, S8). Variance partitioning analysis was used to
371 further explore these subtle transcriptional responses. Figure 4 summarizes the 50 genes showing the
372 strongest treatment-associated expression response in this dataset, while overall effects remained small
373 at the transcriptome level.

374 The selected genes were assigned to functional categories based on their annotations and the most
375 represented categories included Metabolism & Energy (10 genes), Transcriptional & Chromatin
376 Regulation (8 genes), and Transport & Membrane Processes (7 genes). These categories include genes
377 related to primary metabolism, regulatory processes, and transport functions and are often linked to plant
378 stress adaptation and signaling. In barley SynCom inoculated plants, genes encoding a FAR1-related
379 sequence 3, SCAR family protein, RNA-binding protein, and a translation elongation/initiation factor
380 showed higher relative expression, while genes encoding a MYB and a Dof zinc finger protein showed
381 lower relative expression. In contrast, WCS417r inoculation induced higher expression of mitochondrial
382 carrier family and LanC-like protein genes, whereas a metalloprotease FtsH gene was relatively
383 downregulated. The treatment with wheat SynCom resulted in higher expression of ERAD E3 ubiquitin
384 ligase HRD3A and E3 ubiquitin-protein ligase genes, both involved in protein turnover and stress response.
385 To provide an overview of the potential biological functions of these DEGs, a summary of the
386 corresponding GO term enrichment is presented in Supplementary Figure S3.

387 WCS417r triggered transcriptional changes have also been described by a recent study (Sommer et al.
388 2026). DEG counts are sensitive to experimental and analytical approaches, therefore differences
389 between studies are expected, hence we interpret our findings within the context of this dataset.

390 **4.6 Rhizosphere metatranscriptomes show similar taxonomic and functional profiles across**
391 **treatments**

392 Metatranscriptomic analysis revealed transcripts assigned to 14 phyla across the four treatments (Control,
393 WCS417r, Barley SynCom, Wheat SynCom). No coding sequence (CDS) was annotated for the largest
394 proportion of reads (~30-60%), followed by "Unclassified" and reads mapped to the plant host
395 (Streptophyta). Bacterial phyla were detected at lower relative abundance, and most appeared
396 distributed evenly across treatments. Overall, no consistent differences in taxonomic composition at the
397 phylum level were observed between treatments (Figure 5A). The metatranscriptomic contigs were

assigned based on sequence similarity and not reference SynCom genomes, thus taxonomy was only assigned to broad bacterial groups, and therefore read counts could not be attributed to the individual SynCom strains.

Principal coordinate analysis (PCoA) of KEGG (A), COG (B), and Pfam (C) annotations showed that despite few outliers from Control plants, samples from all treatments were mostly grouped together, with ellipses overlapping in all three plots (Figure 5B). The explained variance was similar between annotations, with the highest for KEGG (PC1: 48.5%, PC2: 20.2%) and lower for COG (PC1: 43.4% and PC2: 21.%) and Pfam (PC1: 55%, PC2: 7.2%).

Alpha diversity was measured with Shannon index, richness, and evenness using KEGG, COG, and Pfam annotations (Figure 5C). The diversity values were similar between all treatments, and statistical tests showed no significant differences between all pairwise comparisons (abbreviated ns). Few outliers and variation was present, particularly for Wheat SynCom and Control samples, but no clear pattern related to treatment was detected.

4.7 Differentially expressed functional features in rhizosphere metatranscriptomes

Differential gene expression analysis of the metatranscriptomes revealed 14 functional features (KEGG, COG, and PFAM terms) to be enriched across the bioinoculant applications compared to the controls (BH adjusted *p*-value <= 0.05 & absolute $\log_2$-fold change >= 1) (Figure 6A). Application of WCS417r was associated with upregulation of genes annotated with the following features: K03520 (KEGG), COG1529 (COG), PF01778 (PFAM), and PF20256 (PFAM). These annotations include proteins associated with redox-related metabolic functions, ribosomal components, and molybdenum cofactor-binding domains, In addition, K01919 (glutamate-cysteine ligase) and K05516 (stress-induced DnaJ-like molecular chaperone) were also upregulated under WCS417r treatment.

In contrast, several metatranscriptomic features were downregulated under WCS417r treatment, including ENOG4112351 (eggNOG), ENOG410ZRFR (eggNOG), and PF02868 (PFAM). ENOG4112351 and ENOG410ZRFR are annotated as proteins of unknown or uncharacterized function, while PF02868 corresponds to a thermolysin metallopeptidase–related domain.

Application of the wheat SynCom resulted in statistically significant downregulation of PF02868; no KEGG or eggNOG features were significantly downregulated in this treatment. In contrast, K05516 (KEGG; stress-induced DnaJ-like molecular chaperone) and PF01614 (PFAM; IclR-family transcriptional regulator, C-terminal domain) were significantly upregulated under wheat SynCom application.

428 Application of the barley SynCom was associated with the upregulation of genes annotated with K02109
429 (KEGG; F-type H^+-transporting ATPase subunit b), COG0711 (COG; ATP synthase–related protein), and
430 PF01061 (PFAM; ABC-2 type transporter). In addition, K05516 (KEGG; stress-induced DnaJ-like molecular
431 chaperone) and ENOG410XQKZ (eggNOG; oxidoreductase-related domain protein) were significantly
432 upregulated under barley SynCom application.

433 Several features were downregulated in the barley SynCom treatment, including ENOG4112351 (eggNOG;
434 protein of unknown function) and PF02868 (PFAM; thermolysin metallopeptidase–related domain).

435 Comparison across treatments revealed that PF02868 was significantly downregulated and K05516
436 significantly upregulated in all three treatments (WCS417r, barley SynCom, and wheat SynCom).
437 ENOG4112351 was downregulated under WCS417r and barley SynCom application but not under the
438 wheat SynCom treatment.

439 **5 Discussion**

440 In this study, we investigated the ability of SynComs, which are composed of native bacteria from barley
441 and wheat rhizospheres, to promote resistancein barley plants against barley pathogen *Blumeria graminis*
442 f. sp. *hordei (Bgh)*. SynCom-trigerred ISR reduced *Bgh* propagation in infected plant leaves, which is
443 consistent with the positive effect of the model strain WCS417r, which is known to induce ISR in barley
444 (Sommer et al. 2026) and is therefore used as a positive control in ISR experiments.

445 **5.1 The potential of SynCom as a biological control agent against plant pathogens**

446 SynComs are used in studies of plant-microbe interactions and have the potential to be used as
447 bioinoculants, as they can enhance crop disease resistance and resilience under environmental stress,
448 reduce the need for chemical pesticides and modulate host phenotype (Naik et al. 2019; Vorholt et al.
449 2017; Chesneau et al. 2025). Unlike single-strain inoculants, SynComs more accurately reflect the
450 complexity of a natural microbiome and have sometimes been shown to have a stronger effect on plant
451 health than individual strains applied alone (Liu et al. 2023). SynComs are often assembled using
452 rhizobacteria, whose composition differs from the bulk soil microbiome, partly due to root exudates
453 released by the plant (Shayanthan et al. 2022; Koprivova and Kopriva 2022). A certain proportion of these
454 strains are root-associated plant growth promoting rhizobacteria. Among other plant beneficial traits,
455 some of them activate plant immune responses through ISR, a mechanism triggered in the plant root that
456 can protect above-ground parts of the plant rom pathogen invasion (Pieterse et al. 2014).

457 The potential to act as a biocontrol agent against plant pathogens has been well studied using the model
458 strain WCS417r (Pieterse et al. 2021; Sommer et al. 2024; Vorholt et al. 2017). In addition, there are
459 studies applying SynComs for crop resistance. For instance, the application of a SynCom consisting of
460 bacterial isolates from the endosphere of the maize variety Jala landrace was found to improve plant
461 growth and inhibit the growth of phytopathogenic fungi, including *Pestalotia* sp. and *Colletotrichum* sp.
462 (La Vega-Camarillo et al. 2023).

463 Furthermore, it has been reported that microbial inoculants can help plants tolerate abiotic stress. This is
464 achieved by increasing nutrient availability, fixing nitrogen, producing plant growth promoting (PGP)
465 hormones such as indole-3-acetic acid, and decreasing ethylene production (Mastouri et al. 2010; Kejela
466 2023; Pan and Cai 2023; Aasfar et al. 2024). These additional characteristics can promote plant growth in
467 challenging environmental conditions when employed in SynComs (Wallenstein 2017; Shayanthan et al.
468 2022; Rigerte et al. 2025). The barley SynCom used here was previously characterized in the context of
469 drought tolerance (Rigerte et al. 2025). The relationship between drought tolerance and ISR is still to be
470 explored, however this broader potential further strengthens the idea of SynComs as a sustainable tool in
471 agriculture, not only against pathogens but also under future climate stress scenarios.

472 Studies using SynComs as bioinoculants are thus far limited, and their outcomes often depend on the host
473 plant, the pathogen and environmental conditions. This is demonstrated by the context-dependent
474 effects of SynComs on disease progression and microbiome composition (Pfeilmeier et al. 2024; Liu et al.
475 2023). Transferring successful results from *in vitro* or greenhouse experiments to the field is challenging
476 due to the interaction with the native microbiome and other factors that can strongly influence SynCom
477 performance. Still, SynCom approaches are a promising way forward for sustainable alternatives to
478 chemical pesticides. Our work adds to this evidence by testing their potential in the barley–*Bgh* system.
479 Our data reveal that exposure of barley roots to either barley- or wheat-derived SynComs enhanced the
480 resistance of the above-ground tissues of the plants against powdery mildew, suggesting the induction of
481 ISR by the SynComs used.

482 **5.2 Reduced fungal growth in barley leaves after SynCom and WCS417r treatments**

483 Our DAF staining results show that inoculation with both SynComs and WCS417r reduced fungal growth
484 in barley leaves following infection with *Bgh* , showing no major difference between different inoculants.
485 Lower fluorescence levels observed in our treatments suggest that plants inoculated with beneficial

486 bacteria had reduced pathogen growth (Lenk et al. 2018; Lenk et al. 2019; Brambilla et al. 2022; Brambilla
487 et al. 2023).

488 Restriction of fungal growth is a common outcome of ISR , in which root-associated beneficial bacteria
489 prime the plant for enhanced defense response upon pathogen attack which results in reduced
490 colonization instead of complete resistance (van der Ent et al. 2009; Pieterse et al. 2014). In barley,
491 reduced hyphal growth and colony establishment of *Bgh* are well-established phenotypic indicators of
492 enhanced resistance and are widely used to quantify disease suppression in both host and non-host
493 interaction studies (Hückelhoven et al. 2001; Panstruga and Schulze-Lefert 2002).

494 The reduced fungal growth after inoculation with SynCom and WCS417r suggests that these treatments
495 enhance plant's ability to restrict pathogen development in leaf tissues. Such results are consistent with
496 ISR-mediated priming in which immune responses are deployed more rapidly upon pathogen attack,
497 resulting in lower pathogen biomass (Pieterse et al. 2014). Together, these results suggest that both
498 SynComs and the model strain WCS417r limit the growth of *Bgh* in barley leaves, highlighting their
499 potential usage in microbiome-mediated disease suppression.

500 **5.3 Limited effects of synCom inoculation on barley rhizosphere microbiota**

501 The alpha or beta diversity of the barley rhizosphere prokaryotes did change significantly between
502 treatments, suggesting that the addition of SynCom did not significantly alter richness or community
503 composition. The relative abundances of bacterial ASVs in rhizosphere samples remained consistent
504 across batches of the same treatment. The available literature on changes to the microbiome after
505 inoculation with plant beneficial bacteria (PBB) is inconclusive. For example, (Hao et al. 2023)
506 demonstrated adding a SynCom to desert soil containing a mixture of tall fescue and ryegrass resulted in
507 an increase in beneficial bacteria and greater bacterial diversity. In contrast, (Qiao et al. 2024) reported
508 that SynCom addition to grafted watermelon plants led to a significant increase in the relative abundance
509 of *Pseudomonas* in the rhizosphere. Conversely, research on *Zea mays* B73 revealed that the seed
510 inoculation with PGP bacterium mitigated drought stress in maize without altering the composition of the
511 rhizosphere bacterial community (Yim et al. 2025). Similarly, (Montoya et al. 2025) demonstrated that
512 although inoculation of tomato seedlings with a bacterial SynCom induced the expression of numerous
513 genes in the roots and shoots, the composition of the rhizosphere communities was not significantly
514 altered.

515 **5.4 Subtle but specific transcriptional responses in barley leaves Following SynCom and WCS417r**
516 **inoculation**

517 Minimal transcriptomic differences in barley leaves were observed for all four inoculations. Treatment
518 explained only a minor proportion of the variance for most genes, and PERMANOVA or GO enrichment
519 analyses didn't detected clear separation between Control, WCS417r and both SynComs. This suggests
520 that most of the systemic transcriptional responses in leaves are subtle, affecting only a small number of
521 genes. This is consistent with the recent findings of A. S. and A.C.V. (Sommer et al. 2026) and the existing
522 literature. For example, none of the genes tested in *A. thaliana* leaves showed a consistent change in gene
523 expression during WCS417r-mediated ISR (Verhagen et al. 2004), suggesting that ISR activation in plant
524 leaves is not associated with strong changes in gene expression. Similarly, a study on the barley root
525 colonization of a beneficial endophyte *P. indica* showed clear physiological evidence of enhanced systemic
526 resistance against *Bgh*, without resulting in strong transcriptional response in barley leaves under non-
527 challenged conditions (Molitor et al. 2011). Furthermore, barley growth-promoting and fungal-root-
528 pathogen-inhibiting effects of *Sebacina vermifera* and rhizosphere bacteria are accompanied by a mild
529 transcriptional response in the host upon early colonization by *S. vermifera* and bacteria (Mahdi et al.
530 2022). The findings from literature and our results are consistent with the priming effect associated with
531 ISR (Conrath et al. 2001; Conrath et al. 2015; Sommer et al. 2026).

532 Our analysis of differentially expressed genes showed that mock treated plants exhibited higher activation
533 of several defense-related genes, including those encoding MYB, NB-ARC proteins, PPR proteins, as well
534 as lipid- and cell wall–related enzymes. As these samples were collected prior to pathogen inoculation,
535 this elevated defense gene expression may reflect a higher basal stress or defense state in mock
536 inoculated plants. In comparison, SynCom inoculation reduced the expression of these genes, which mat
537 reflect an ISR-like priming, where the plants show lower basal defense gene expression while remaining
538 prepared for a response under pathogen infection. WCS417r and both SynComs also influenced several
539 metabolic and signaling pathways, for example, Barley and Wheat SynCom both downregulated
540 phosphatidate cytidylyltransferase and phosphoglucose isomerase 1, indicating changes in lipid and
541 central carbon metabolism during microbe-mediated priming. These enzymes typically increase during
542 strong plant defense activation, suggesting that SynCom inoculated plants invested reduced amount of
543 lipid and carbon resources into plant basal defense. In addition, WCS417r upregulated LEA proteins and
544 MTA cycle–related enzymes, while both WCS417r and Barley SynCom increased laccase and MYB-like
545 transcription factor expression, which may support cell-wall–related defense. Together, these results

546 suggestthat different beneficial bacteria activate partially overlapping but distinct transcriptional
547 responses linked to ISR and pathogen protection.

548 Our leaf transcriptome results are in line with the literature. For example, (van de Mortel et al. 2012)
549 found that *A. thaliana* root inoculation with WCS417r did not result in consistent transcriptional changes
550 in plant leaves prior to the infection, which suggests that ISR results via priming rather than changes in
551 plant gene expression. Similarly, (Lakkis et al. 2019) studied the ability of *Pseudomonas fluorescens* PTA-
552 CT2 to induce ISR in grapevine plants against *P. viticola* and *B. cinerea* and demonstrated that plant
553 inoculation did not result in consistent changes of basal defenses prior the infection. To conclude, these
554 results highlight the importance of sampling during pathogen infection to further investigate how PGP
555 treatments modulate ISR against *Bgh*.

556 **5.5 Rhizosphere microbiome gene expression analysis detects treatment-specific and shared functional**
557 **changes**

558 Metatranscriptomics offers a practical way of discovering the mechanisms underlying the ISR process,
559 rather than merely identifying genes in bacterial genomes that may contribute to it (Yang et al. 2025). In
560 all three treatments, two features were consistently affected: K05516 (stress-induced DnaJ-like molecular
561 chaperone), which was upregulated, and PF02868 (thermolysin metallopeptidase–related domain), which
562 was downregulated. This impliesthat the application of these bioinoculants elicited a shared microbial
563 response involving stress adaptation and changes in specific functional activities (Yamashino et al. 1994).
564 The wheat SynCom treatment induced fewer transcriptional changes in barley rhizosphere compared to
565 the other treatments, but included the upregulation of PF01614, a bacterial transcriptional regulator. This
566 indicates that although fewer features were enriched, the SynCom also stimulated microbial signaling and
567 stress response functions.

568 Inoculation with the barley SynCom was associated with increased expression of features annotated as
569 ATP synthase-related proteins (K02109, COG0711) and ABC-type transporters (PF01061). This implies that
570 inoculation with the barley SynCom may influence microbial energy related functions and transport
571 processes in the rhizosphere. Furthermore, the upregulation of K05516 and ENOG410XQKZ suggests
572 enhanced microbial stress adaption and redox-related functional responses under this treatment
573 (Yamashino et al. 1994). At the same time, downregulation of PF02868 and ENOG4112351 was observed,
574 similar to WCS417r, indicating shared regulation of specific, non-central metabolic features.

575 Inoculation with WCS417r was associated with the upregulation of stress related and transport associated
576 functions, suggesting that microbial activity is functionally adjusted instead of generally increased
577 (Andrade et al. 2023). The enrichment of detoxification and transport related functions indicates an
578 increased microbial capacity to manage environmental stress and to support nutrient exchange, which
579 may contribute to plant stress protection prior to pathogen infection (Andrade et al. 2023; Desrut et al.
580 2020). Additionally, increased expression of functions related to nitrogen metabolism and microbial
581 signalling suggests that WCS417r promotes a more responsive and metabolically active rhizosphere
582 microbiome (Pieterse et al. 2021).

583 Conversely, genes related to energy and ribosomes were downregulated in this treatment. This indicates
584 reduced metabolic activity, likely due to the community allocating resources towards specialised functions
585 rather than growth (Blazewicz et al. 2013).

586 The taxonomic overview of the metatranscriptomes showed that most of the transcripts could not be
587 assigned to coding sequences or were put in the group "unclassified." This is similar to earlier studies,
588 where functional metatranscriptomic datasets often had many unannotated reads. For example, (Howe
589 et al. 2023) described a large community of unclassified active microbes in metatranscriptomic profiles
590 and (Jiang et al. 2016) showed that only 19–76% of mRNA reads were annotatable across diverse
591 microbiome samples. Host-derived Streptophyta transcripts were detected and removed prior to
592 downstream taxonomic analyses.

593 The observed differential gene expression patterns in the rhizosphere microbiome suggest that microbial
594 responses to biostimulant inoculation are selective and function specific, rather than reflecting
595 widespread changes across the entire community. For instance, a study on (RNA) of the rhizosphere
596 microbiome of sugar beet seedlings grown in pathogen-suppressive soil showed restricted pathogen
597 triggerred microbial response, mainly in stress- and signaling-related transcripts, further supporting the
598 idea that inoculated treatments are more associated with specific transcripts, rather than major changes
599 in entire microbial community (Chapelle et al. 2016; Schlatter et al. 2017). Similarly, a review on soil
600 microbial ecology and metatranscriptomics concluded that metatranscriptomic responses are most
601 apparent under strong environmental changes, while weaker disturbances result in milder changes (Peng
602 et al. 2024). Together these results show that inoculation with different SynComs and WCS417r strain
603 elicits shifts in rhizosphere microbial functional activity, pointing towards modulation of energy
604 metabolism, signalling and nutrient exchange instead of mere growth-boosting.

605 **Conclusions**

606 Our study demonstrates that SynComs derived from drought stressed barley and wheat rhizospheres are
607 capable of inducing systemic resistance in barley against powdery mildew infection. Furthermore, the
608 wheat SynCom induced ISR response in barley that was comparable to the one induced by the barley
609 SynCom, suggesting that ISR can be elicited by synthetic microbial communities originating from a related,
610 but distinct host species.

611 Although the two SynComs were composed of strains from different genera, all bacterial members belong
612 to PGB, e.g. *Pseudomonas, Bacillus/Priestia, Rhizobium*-related taxa, and *Streptomyces*. The overlap of the
613 PGP traits may explain the ability of SynComs to induce ISR in barley. At the same time, treatment-specific
614 differences observed in rhizosphere metatranscriptomes indicate that distinct microbial communities
615 may achieve similar ISR outcomes through different functional and metabolic strategies.

616 A further conclusion of this study is the detection of treatment-specific shifts in rhizosphere microbial
617 gene expression, including for the well-established ISR model strain *Pseudomonas simiae* WCS417r. These
618 results provide novel insights into how ISR-eliciting bacteria and communities adjust rhizosphere microbial
619 activity beyond effects on host gene expression.

620 Finally, application of both SynComs did not affect host gene expression, as demonstrated by the absence
621 of differential gene expression in host leaf tissues under SynCom application. In the context of ISR, this
622 suggests that SynCom application alters the behavior of the soil microbial community, priming the host
623 plant to defend itself against potential pathogen attacks.

624 6 Data Availability Statement

625 Metatranscriptomic and 16S rRNA gene amplicon sequencing data of soil bacterial communities, as well
626 as host leaf RNA sequencing data of barley exposed to beneficial bioinoculants, are available under NCBI
627 BioProject ID PRJNA1405459.

628 7 Conflict of Interest

629 The authors declare that the research was conducted in the absence of any commercial or financial
630 relationships that could be construed as a potential conflict of interest.

631 8 Author Contributions

632 LR: Formal Analysis, Investigation, Methodology, Visualization, Writing – original draft, Writing – review
633 & editing. AS: Investigation, Methodology, Writing – review & editing. ACV: Investigation, Methodology,

Writing – review & editing. LDPS: Formal Analysis, Writing – review & editing. AH: Conceptualization, Funding acquisition, Writing – review & editing. TR: Conceptualization, Funding acquisition, Writing – review & editing. MTT: Conceptualization, Funding acquisition, Methodology, Project administration, Resources, Supervision, Validation, Writing – review & editing.

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1010

1011 **Figure 1. Workflow of the study**, illustrating the work described in the Materials and methods section 3.1,
1012 3.2 and 3.5 (created in BioRender).

1013 **Figure 2. Barley and wheat SynComs enhance resistance against barley powdery mildew (*Bgh*).** (A)
1014 Barley plants prior to Bgh infection, inoculated with either mock, WCS417r, wheat SynCom or barley

1015 SynCom inocula. (B) Representative fluorescence microscopy images of *Bgh* hyphae on leaf discs stained
1016 with DAF-FM-DA at 7 days post inoculation (dpi) in seedlings treated with mock, WCS417r, wheat SynCom
1017 and barley SynCom. (C) Quantification of *Bgh* on DAF-FM-DA-stained leaf discs. The *Bgh*-associated
1018 relative fluorescence units (RFU) were calculated by normalizing the measured fluorescence values
1019 against those of the uninfected controls (relative fluorescence intensity; RFU_infected / RFU_uninfected)
1020 and are shown as $\log_{10}$(RFU_infected / RFU_uninfected). Each data point represents a single measurement
1021 from a single experiment. The experiment was repeated five times with comparable results. Different
1022 letters indicate significant differences ($p < 0.05$) according to the ANOVA and Tukey HSD tests.

1023 **Figure 3. Variance partitioning of gene expression by treatment in barley leaves subjected to four**
1024 **treatments (mock/control, WCS417r, barley SynCom, wheat SynCom)**. (A) Violin plot showing variance
1025 partitioning in barley leaf transcriptomes. The violin plots show the distribution of variance explained
1026 across all genes by (i) treatment and (ii) residuals (unexplained variance). Wider regions indicate a greater
1027 density of genes with the corresponding proportion of variance explained. Treatments accounted for a
1028 small proportion of variance, while most gene expression variation remained unexplained (residuals). (B)
1029 Bar plot showing the top ten genes with the highest variance explained by treatment. The x-axis shows
1030 the percentage of variance explained, the y-axis lists the gene IDs. Each bar is divided into the variance
1031 explained by treatment (pink), date (blue) and by residuals (grey), highlighting how strongly each gene's
1032 expression is associated with treatment . (C) VST-normalised expression of the four most treatment-
1033 associated genes. Boxplots show expression values (x-axis) across the four treatments, each panel
1034 represents one gene, illustrating differences in expression patterns between treatments.

1035 **Figure 4. A clustered heatmap showing normalized gene expression levels** of 50 out of 200 genes that
1036 explain the maximum variance in barley leaf gene expression after inoculation with WCS417r, barley
1037 SynCom or wheat SynCom.

1038 **Figure 5. Metatranscriptomic profiles of the barley rhizosphere inoculated with WCS417r, Barley**
1039 **SynCom, and Wheat SynCom compared to controls.** (A) Taxonomic composition across different
1040 treatments, showing the relative abundance (%) of 13 phyla in five replicates per treatment. (B) Principal
1041 coordinate analysis (PCoA) based on KEGG (A), COG (B), and Pfam (C) annotations. Axes indicate the
1042 percentage of variance explained by PC1 and PC2. (C) Alpha diversity calculated from KEGG, COG, and
1043 Pfam functional annotations, represented by Shannon index, richness, and evenness (nine plots total).
1044 The y-axis shows the corresponding diversity metric, while the x-axis shows replicates per treatment.

1045 **Figure 6. Differential gene expression analysis of barley rhizosphere metatranscriptomes highlighting**
1046 **significantly upregulated and downregulated functional features.** (A) Volcano plots showing significantly
1047 upregulated and downregulated terms across KEGG, COG, and PFAM categories. The x-axis represents
1048 $\log_2$(fold change), and the y-axis represents $-\log_{10}$(BH-adjusted *p* value). Significantly changed terms are
1049 highlighted compared to all other terms within each category. (B) Boxplots of representative significantly
1050 upregulated and downregulated terms, displayed by treatment condition. Each boxplot shows $\log_{10}$(TPM)
1051 values for the Control versus the respective treatment (e.g., KEGG term K05516 in Control vs. Barley
1052 SynCom).

1053

1054 **Table 1.** Composition of barley and wheat SynComs (refer Materials and Methods for details

Strain ID	SynCom	Host origin	Genus	Species name*	Acc. Number (NCBI)
B1	Barley	Barley	*Aeromicrobium*	*Aeromicrobium ginsengisoli*	NR_041384.1
B2	Barley	Barley	*Arthrobacter*	*Arthrobacter humicola*	NR_041546.1
B3	Barley	Barley	*Mesorhizobium*	*Mesorhizobium ciceri*	NR_113894.1
B4	Barley	Barley	*Mycolicibacterium*	*Mycolicibacterium montmartrense*	NR_151955.1
B5	Barley	Barley	*Phyllobacterium*	*Phyllobacterium brassicacearum*	NR_043190.1
B6	Barley	Barley	*Phyllobacterium*	*Phyllobacterium loti*	NR_133818.1
B7	Barley	Barley	*Priestia*	*Priestia aryabhattai B8W22*	NR_133818.1
B8	Barley	Barley	*Priestia*	*Priestia megaterium NBRC 15308 = ATCC 14581*	NR_112636.1
B9	Barley	Barley	*Pseudomonas*	*Pseudomonas poae*	NR_028986.1
B10	Barley	Barley	*Rhizobium*	*Rhizobium alamii*	NR_042687.1
B11	Barley	Barley	*Rhizobium*	*Rhizobium viscosum*	NR_042253.1
B12	Barley	Barley	*Rhodococcus*	*Rhodococcus fascians*	NR_037021.1
B13	Barley	Barley	*Streptomyces*	*Streptomyces nigrescens*	NR_117748.1
B14	Barley	Barley	*Streptomyces*	*Streptomyces resistomycificus*	NR_117748.1
B15	Barley	Barley	*Streptomyces*	*Streptomyces rishiriensis*	NR_112392.1
B16	Barley	Barley	*Streptomyces*	*Streptomyces tauricus*	NR_112405.1
W1	Wheat	Wheat	*Buttiauxella*	*Buttiauxella sp.—*	MK638102
W2	Wheat	Wheat	*Pedobacter*	*Pedobacter sp.*	MK638129
W3	Wheat	Wheat	*Arthrobacter*	*Arthrobacter sp.*	MK638058
W4	Wheat	Wheat	*Bacillus*	*Bacillus sp.*	MK638067
W5	Wheat	Wheat	*Mesorhizobium*	*Mesorhizobium sp.*	MK638163
W6	Wheat	Wheat	*Chitinophaga*	*Chitinophaga sp.*	MK638107
W7	Wheat	Wheat	*Arthrobacter*	*Arthrobacter sp.*	MK638059
W8	Wheat	Wheat	*Pseudomonas*	*Pseudomonas sp.*	MK637939

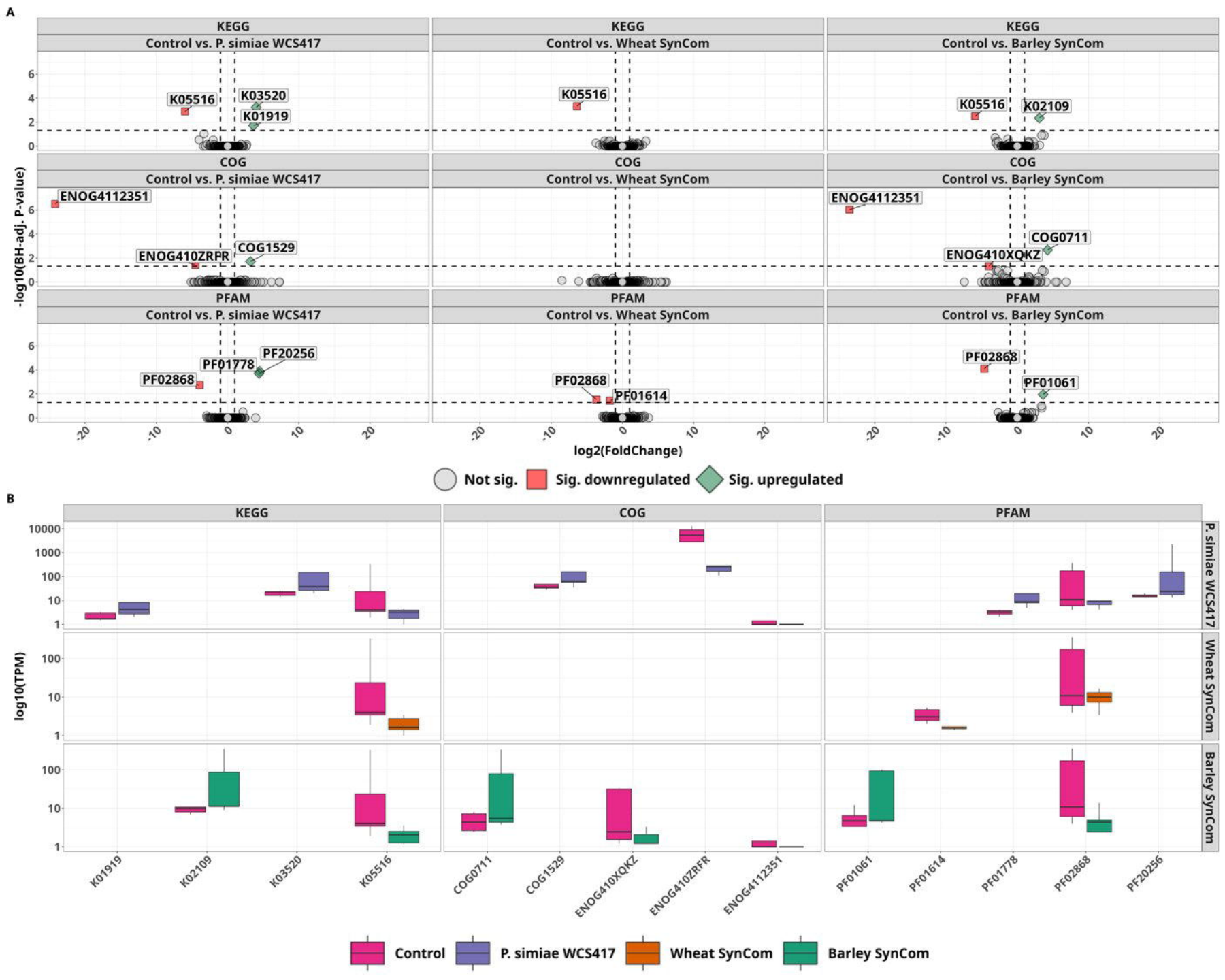
W9	Wheat	Wheat	*Plantibacter*	*Plantibacter sp.*	MK638378
W10	Wheat	Wheat	*Pseudomonas*	*Pseudomonas sp.*	MK637993
W11	Wheat	Wheat	*Microbacterium*	*Microbacterium sp.*	MK638191
W12	Wheat	Wheat	*Inquilinus*	*Inquilinus sp.*	MK638140
W13	Wheat	Wheat	*Variovorax*	*Variovorax sp.*	MK638657
W14	Wheat	Wheat	*Streptomyces*	*Streptomyces sp.*	MK638538
W15	Wheat	Wheat	*Phyllobacterium*	*Phyllobacterium sp.*	MK638310
W16	Wheat	Wheat	*Ensifer*	*Ensifer sp.*	MK638128

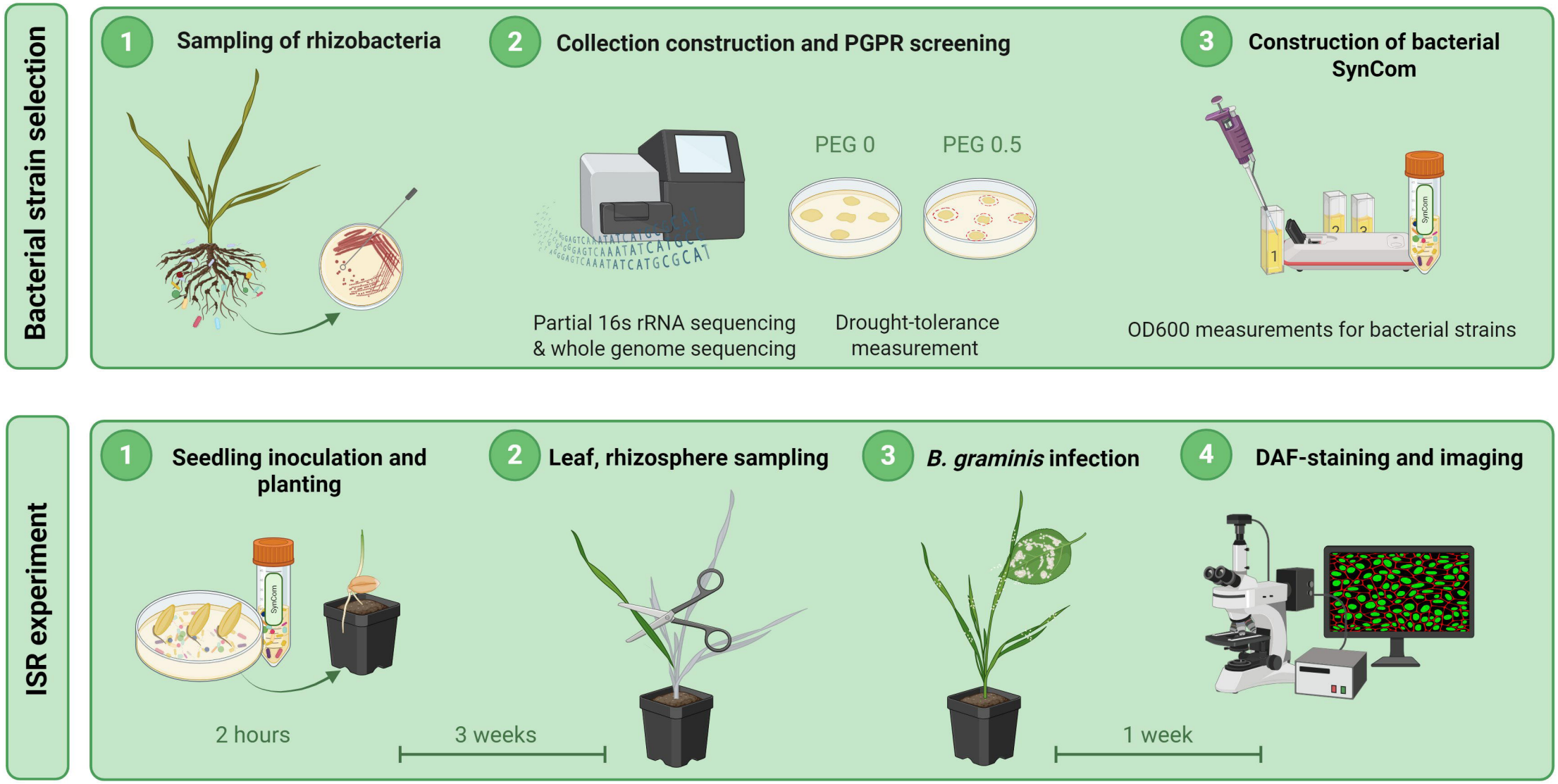
1055

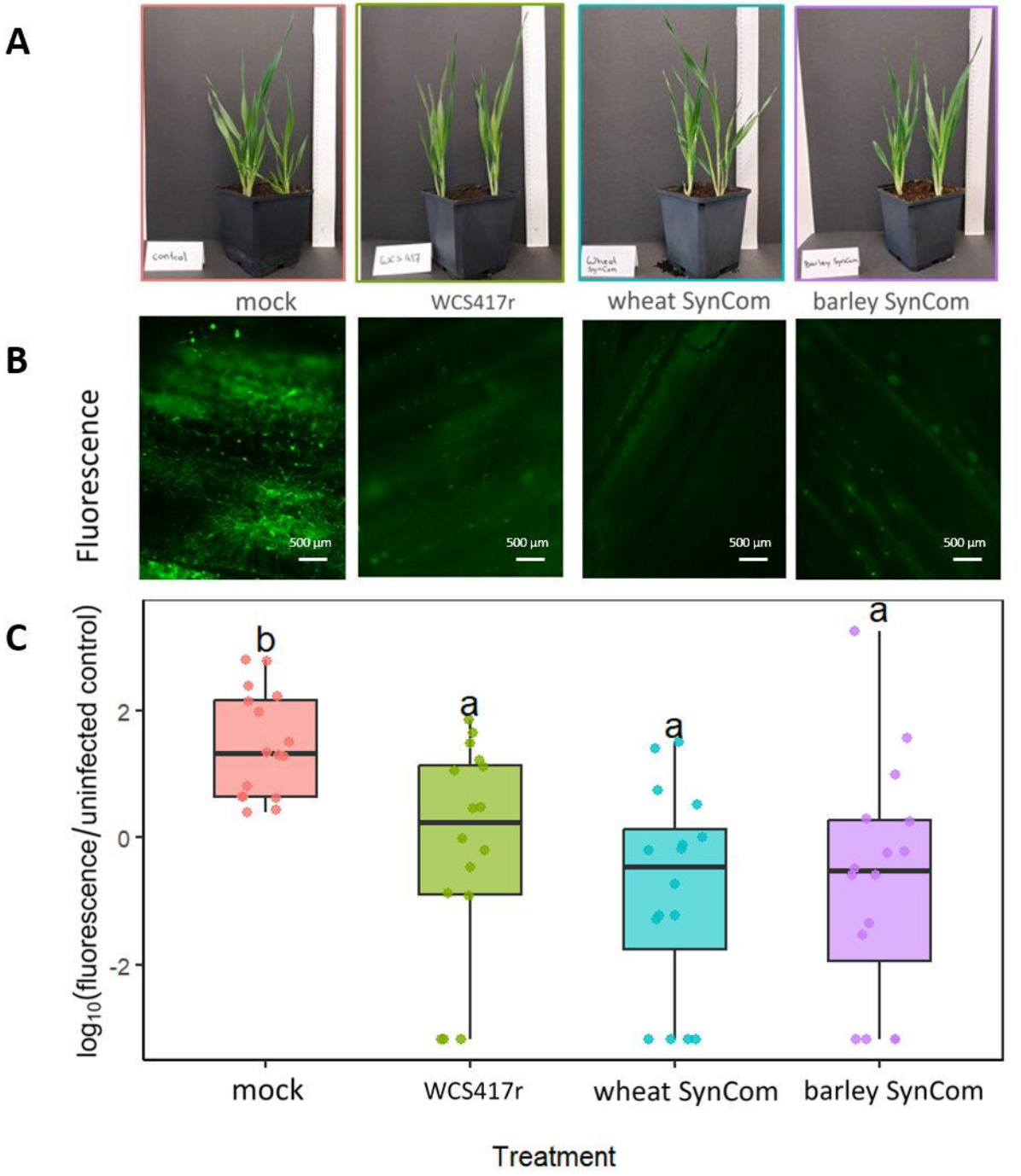
1056 * Species unresolved based on 16S rRNA gene sequence; designated as *Genus sp.*

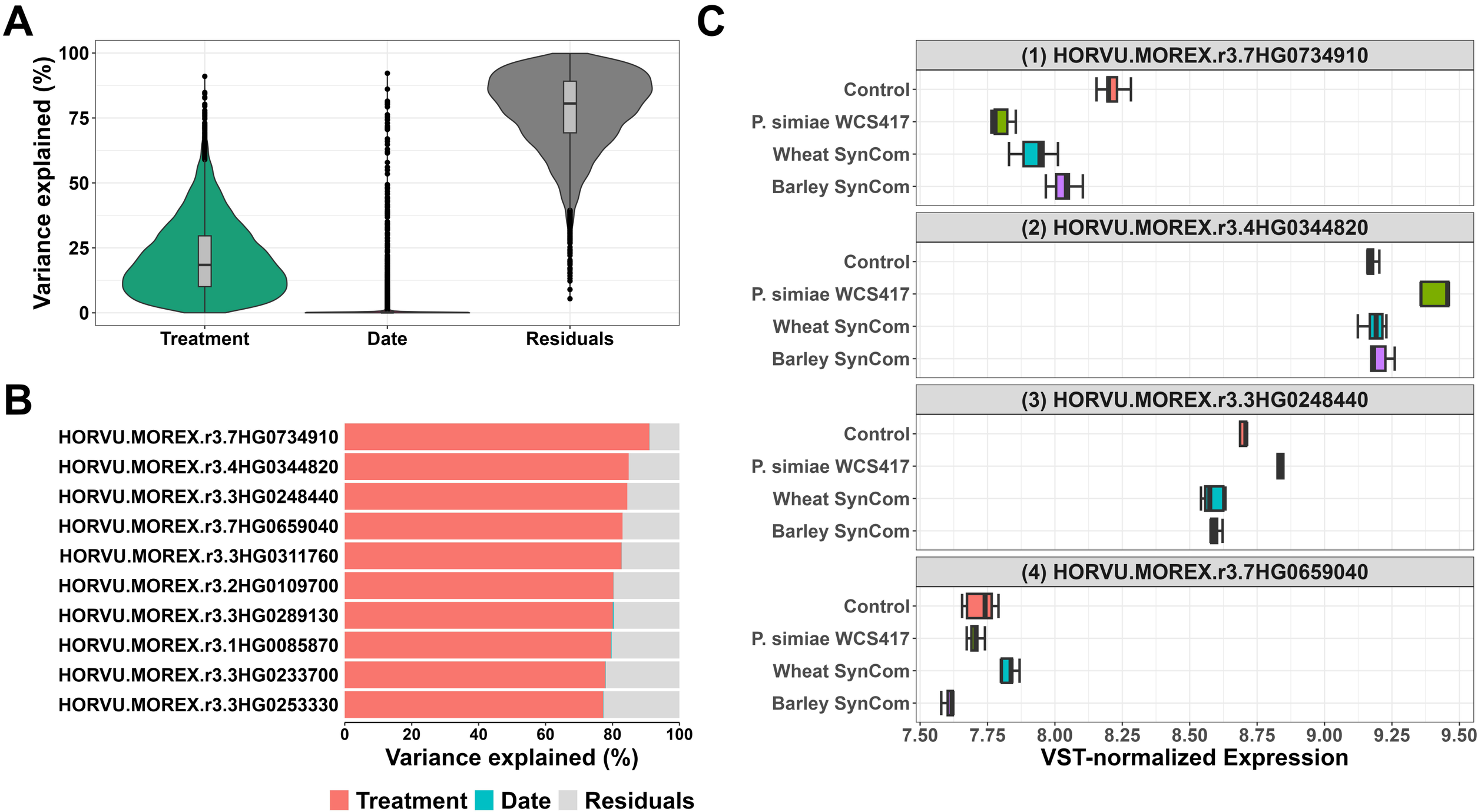
1057

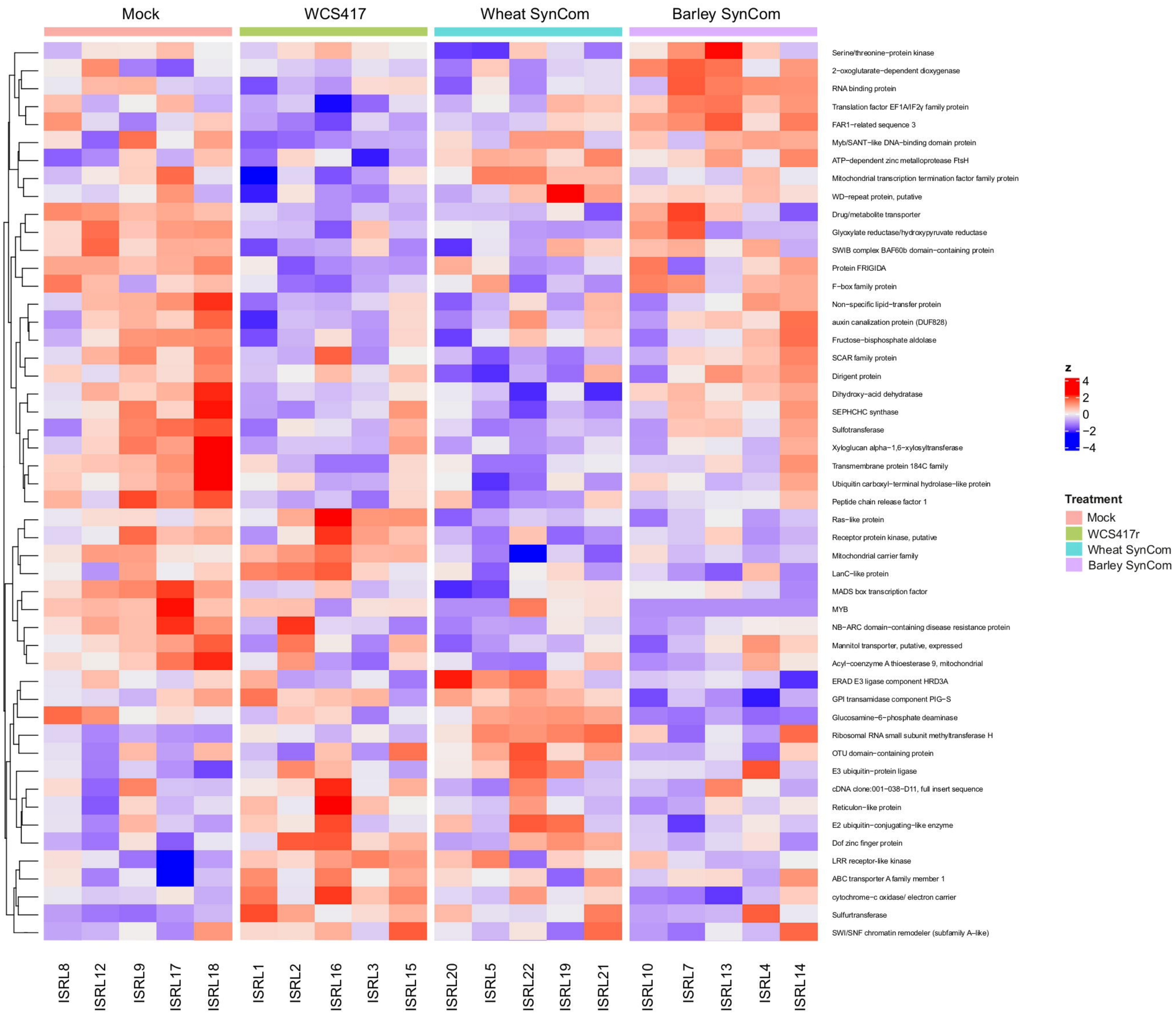
1058

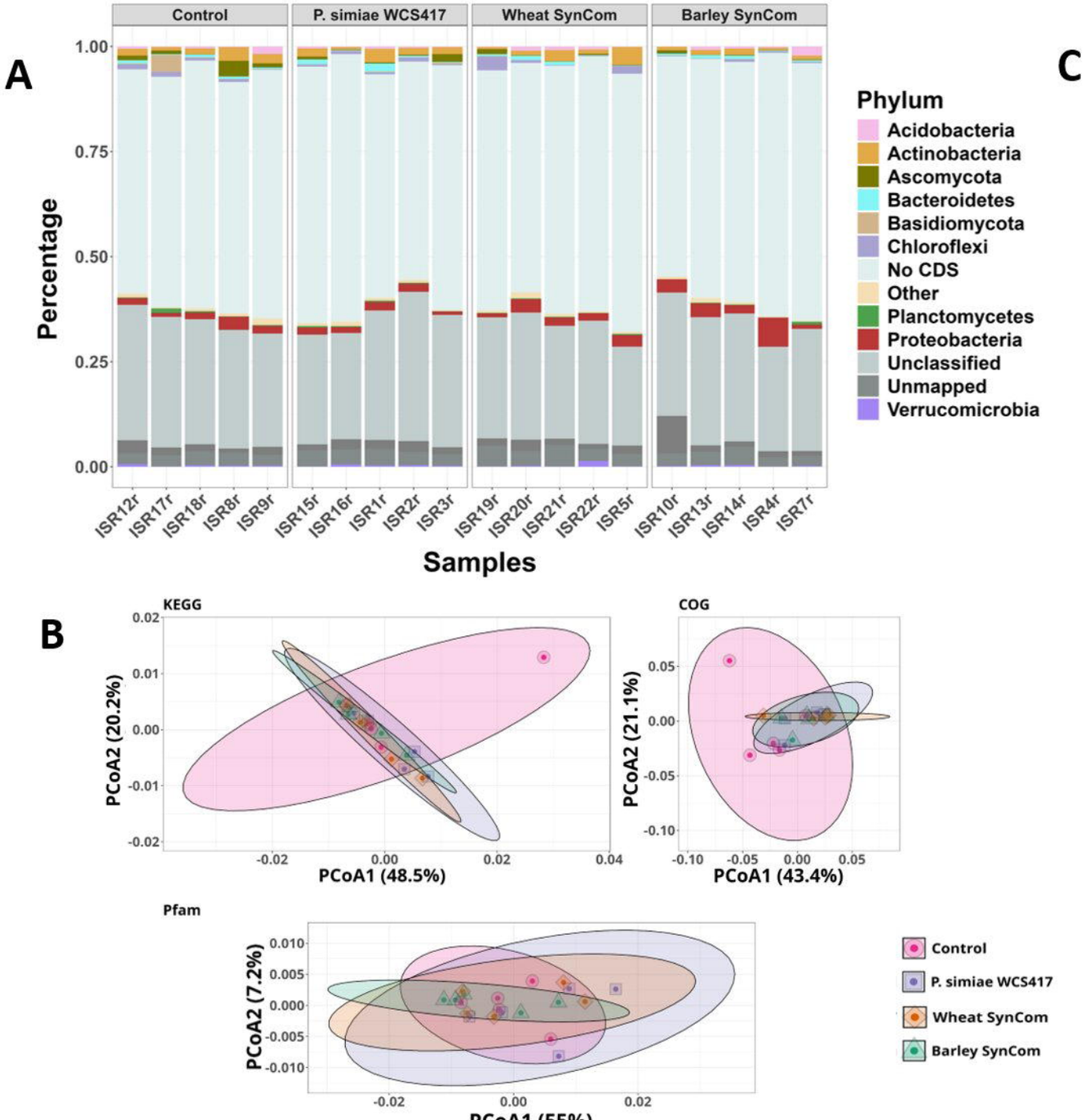

A
KEGG
Control vs. P. simiae WCS417
K05516
K03520
K01919
Control vs. Wheat SynCom
K05516
Control vs. Barley SynCom
K05516
K02109
COG
Control vs. P. simiae WCS417
ENOG4112351
ENOG410ZRPR
COG1529
Control vs. Wheat SynCom
Control vs. Barley SynCom
ENOG4112351
COG0711
ENOG410XQKZ
PFAM
Control vs. P. simiae WCS417
PF20256
PF01778
PF02868
Control vs. Wheat SynCom
PF02868
PF01614
Control vs. Barley SynCom
PF02868
PF01061
-log10(BH-adj. P-value)
log2(FoldChange)
Not sig.
Sig. downregulated
Sig. upregulated
B
KEGG
COG
PFAM
P. simiae WCS417
Wheat SynCom
Barley SynCom
log10(TPM)
K01919
K02109
K03520
K05516
COG0711
COG1529
ENOG410XQKZ
ENOG410ZRFR
ENOG4112351
PF01061
PF01614
PF01778
PF02868
PF20256
Control
P. simiae WCS417
Wheat SynCom
Barley SynCom

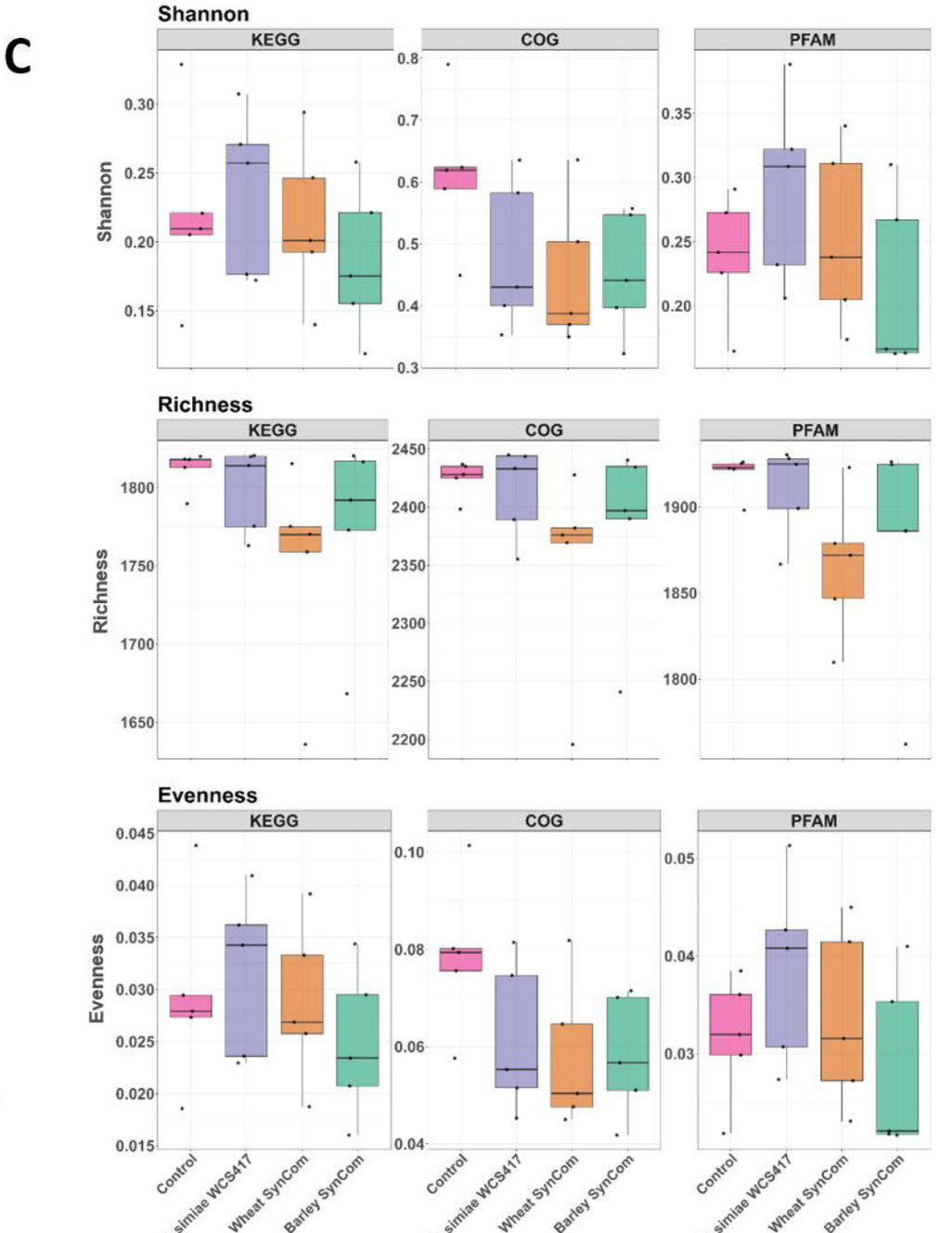

Bacterial strain selection
1
Sampling of rhizobacteria
2
Collection construction and PGPR screening
PEG 0
PEG 0.5
Partial 16s rRNA sequencing
& whole genome sequencing
Drought-tolerance
measurement
3
Construction of bacterial
SynCom
OD600 measurements for bacterial strains
ISR experiment
1
Seedling inoculation and
planting
2 hours
2
Leaf, rhizosphere sampling
3 weeks
3
B. graminis infection
1 week
4
DAF-staining and imaging


A
mock
WCS417r
wheat SynCom
barley SynCom
B
Fluorescence
500 µm
500 µm
500 µm
500 µm
C
log10(fluorescence/uninfected control)
b
a
a
a
2
0
-2
mock
WCS417r
wheat SynCom
barley SynCom
Treatment


A
Variance explained (%)
100
75
50
25
0
Treatment
Date
Residuals
B
HORVU.MOREX.r3.7HG0734910
HORVU.MOREX.r3.4HG0344820
HORVU.MOREX.r3.3HG0248440
HORVU.MOREX.r3.7HG0659040
HORVU.MOREX.r3.3HG0311760
HORVU.MOREX.r3.2HG0109700
HORVU.MOREX.r3.3HG0289130
HORVU.MOREX.r3.1HG0085870
HORVU.MOREX.r3.3HG0233700
HORVU.MOREX.r3.3HG0253330
0
20
40
60
80
100
Variance explained (%)
Treatment
Date
Residuals
C
(1) HORVU.MOREX.r3.7HG0734910
(2) HORVU.MOREX.r3.4HG0344820
(3) HORVU.MOREX.r3.3HG0248440
(4) HORVU.MOREX.r3.7HG0659040
Control
P. simiae WCS417
Wheat SynCom
Barley SynCom
7.50
7.75
8.00
8.25
8.50
8.75
9.00
9.25
9.50
VST-normalized Expression


Mock
WCS417
Wheat SynCom
Barley SynCom
Serine/threonine−protein kinase
2−oxoglutarate−dependent dioxygenase
RNA binding protein
Translation factor EF1A/IF2γ family protein
FAR1−related sequence 3
Myb/SANT−like DNA−binding domain protein
ATP−dependent zinc metalloprotease FtsH
Mitochondrial transcription termination factor family protein
WD−repeat protein, putative
Drug/metabolite transporter
Glyoxylate reductase/hydroxypyruvate reductase
SWIB complex BAF60b domain−containing protein
Protein FRIGIDA
F−box family protein
Non−specific lipid−transfer protein
auxin canalization protein (DUF828)
Fructose−bisphosphate aldolase
SCAR family protein
Dirigent protein
Dihydroxy−acid dehydratase
SEPHCHC synthase
Sulfotransferase
Xyloglucan alpha−1,6−xylosyltransferase
Transmembrane protein 184C family
Ubiquitin carboxyl−terminal hydrolase−like protein
Peptide chain release factor 1
Ras−like protein
Receptor protein kinase, putative
Mitochondrial carrier family
LanC−like protein
MADS box transcription factor
MYB
NB−ARC domain−containing disease resistance protein
Mannitol transporter, putative, expressed
Acyl−coenzyme A thioesterase 9, mitochondrial
ERAD E3 ligase component HRD3A
GPI transamidase component PIG−S
Glucosamine−6−phosphate deaminase
Ribosomal RNA small subunit methyltransferase H
OTU domain−containing protein
E3 ubiquitin−protein ligase
cDNA clone:001−038−D11, full insert sequence
Reticulon−like protein
E2 ubiquitin−conjugating−like enzyme
Dof zinc finger protein
LRR receptor-like kinase
ABC transporter A family member 1
cytochrome−c oxidase/ electron carrier
Sulfurtransferase
SWI/SNF chromatin remodeler (subfamily A−like)
ISRL8
ISRL12
ISRL9
ISRL17
ISRL18
ISRL1
ISRL2
ISRL16
ISRL3
ISRL15
ISRL20
ISRL5
ISRL22
ISRL19
ISRL21
ISRL10
ISRL7
ISRL13
ISRL4
ISRL14
z
4
2
0
−2
−4
Treatment
Mock
WCS417r
Wheat SynCom
Barley SynCom


A
Control
P. simiae WCS417
Wheat SynCom
Barley SynCom
Percentage
1.00
0.75
0.50
0.25
0.00
ISR12r ISR17r ISR18r ISR8r ISR9r ISR15r ISR16r ISR1r ISR2r ISR3r ISR19r ISR20r ISR21r ISR22r ISR5r ISR10r ISR13r ISR14r ISR4r ISR7r
Samples
Phylum
Acidobacteria
Actinobacteria
Ascomycota
Bacteroidetes
Basidiomycota
Chloroflexi
No CDS
Other
Planctomycetes
Proteobacteria
Unclassified
Unmapped
Verrucomicrobia
B
KEGG
PCoA2 (20.2%)
PCoA1 (48.5%)
COG
PCoA2 (21.1%)
PCoA1 (43.4%)
Pfam
PCoA2 (7.2%)
PCoA1 (55%)
Control
P. simiae WCS417
Wheat SynCom
Barley SynCom


C
Shannon
KEGG
COG
PFAM
Richness
Evenness
Control
P. simiae WCS417
Wheat SynCom
Barley SynCom