A footptrint of plant eco-geographic adaptation on the composition of the barley rhizosphere bacterial microbiota.

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Abstract

Background:

The microbiota thriving in the rhizosphere, the thin layer of soil surrounding plant roots, plays a critical role in plant's adaptation to the environment. Domestication and breeding selection have progressively differentiated the microbiota of modern crops from the ones of their wild ancestors. However, the impact of eco-geographical constraints faced by domesticated plants and crop wild relatives on recruitment and maintenance of the rhizosphere microbiota remains to be fully elucidated.

Methods:

We grew twenty wild barley (*Hordeum vulgare* ssp. *spontaneum*) genotypes representing five distinct ecogeographic areas in the Israeli region, one of the sites of barley domestication, alongside four 'Elite' varieties (*H. vulgare* ssp. *vulgare*) in a previously characterised agricultural soil under greenhouse conditions. At early stem elongation, rhizosphere samples were collected, and stem and root dry weight measured. In parallel, we generated high-resolution 16S rRNA gene profiles of the rhizosphere and unplanted soil samples. Ecological indices and multivariate statistical analyses allowed us to identify 'host signatures' for the composition of the rhizosphere microbiota. Finally, we capitalised on single nucleotide polymorphisms (SNPs) of the barley genome to investigate the relationships between microbiota diversity and host genetic diversity.

Results:

Elite material outperformed the wild genotypes in aboveground biomass while, almost invariably, wild genotypes allocated more resources to belowground growth. These differential growth responses were associated with a differential microbial recruitment in the rhizosphere. The selective enrichment of individual bacterial members of microbiota mirrored the distinct ecogeographical constraints faced by the wild and domesticated plants. Unexpectedly, Elite varieties exerted a stronger genotype effect on the rhizosphere microbiota when compared with wild barley genotypes adapted to desert environments and this effect had a bias for *Actinobacteria*. Finally, in wild barley genotypes, we discovered a limited, but significant, correlation between microbiota diversity and host genomic diversity.

Conclusions:

Our results revealed a footprint of the host's adaptation to the environment on the assembly of the bacteria thriving at the root-soil interface. This recruitment cue layered atop of the distinct evolutionary trajectories of wild and domesticated plants and, at least in part, is encoded by the barley genome. This knowledge will be critical to further dissect microbiota

contribution to plant's adaptation to the environment and to devise strategies for climate-smart agriculture.

Keywords:
Barley, Rhizosphere, Microbiota, Domestication, 16S rRNA gene, Eco-geography, Climate-smart agriculture

Background

By 2050 the world's population is expected to reach 9.5 billion and, to ensure global food security, crop production has to increase by 60% in the same timeframe [1]. This target represents an unprecedented challenge for agriculture as it has to be achieved while progressively decoupling yields from non-renewable inputs in the environment [2] and amidst climatic modifications which are expected to intensify yield-limiting events, such as water scarcity and drought [3].

A promising strategy proposes to achieve this task by capitalising on the microbiota inhabiting the rhizosphere, the thin layer of soil surrounding plant roots [4]. The rhizosphere microbiota plays a crucial role in plant's adaptation to the environment by facilitating, for example, plant mineral uptake [5] and enhancing plant's tolerance to both abiotic and biotic stresses [6].

Plant domestication and breeding selection, which have progressively differentiated modern cultivated crops from their wild relatives [7], have impacted on the composition and functions of the rhizosphere microbiota [8]. These processes were accompanied by an erosion of the host genetic diversity [9] and there are growing concerns that, in turn, these limited the metabolic diversity of the microbiota of cultivated plants [10]. Thus, to fully unlock the potential of rhizosphere microbes for sustainable crop production, it is necessary to study the microbiota thriving at the root-soil interface in the light of the evolutionary trajectories of its host plants [11].

Barley (*Hordeum vulgare* L.), a global crop [12] and a genetically tractable organism [13], represents an ideal model to study host-microbiota interactions within a plant domestication framework, due to the fact that wild relatives (*H. vulgare* ssp. *spontaneum*) of domesticated varieties (*H. vulgare* ssp. *vulgare*) are accessible for experimentation [14]. We previously demonstrated that domesticated and wild barley genotypes host contrasting bacterial communities [15] whose metabolic potential modulates the turn-over of the organic matter in the rhizosphere [16]. However, the impact of eco-geographical constraints faced by domesticated plants and crop wild relatives on recruitment and maintenance of the rhizosphere microbiota remains to be fully elucidated. Tackling this knowledge gap is a key pre-requisite to capitalise on plant-microbiota interactions to achieve the objectives of climate-smart agriculture, in particular sustainably enhancing crop production [17].

Here we investigated whether exposure to different environmental conditions left a footprint on the barley's capacity of shaping the rhizosphere bacterial microbiota. We

characterised twenty wild barley genotypes from the 'B1K' collection sampled in the Israeli geographic region, one of the centre of domestication of barley [18, 19]. This material represents the three-major barley 'Ecotypes' adapted to different habitats in the region: the Golan Heights and northern Galilee, ('North Ecotype'); the coastal Mediterranean strip, ('Coast Ecotype'); and the arid regions along the river Jordan and southern Negev ('Desert Ecotype'). We further subdivided these 'Ecotypes' into 5 groups of sampling locations according to the average rainfall of the areas, as a proxy for plant's adaptation to limiting growth conditions. These wild barley genotypes were grown in a previously characterised soil representative of barley agricultural areas of Scotland, alongside four reference cultivated 'Elite' varieties. We used an Illumina MiSeq 16S rRNA gene amplicon survey to characterise the microbiota inhabiting the rhizosphere and unplanted soil samples. By using ecological indices, multivariate statistical analyses and barley genome information we elucidated the impact of eco-geographical constraints and host genetics on the composition of the microbial communities thriving at the barley root-soil interface.

Results

Evolutionary trajectories and eco-geographic adaptation impact on plant growth

Aboveground dry weight from the barley genotypes was measured at early stem elongation as a proxy for plant growth: this allowed us to identify a 'biomass gradient' across the tested material. The 'Elite' varieties, outperforming wild barley plants, and wild barley genotypes adapted to the more extreme desert environments (i.e., 'Desert 2') defined the uppermost and lowermost ranks of this gradient which contained the measurements of the other wild barley genotypes ($p < 0.05$, Kruskal–Wallis non-parametric analysis of variance followed by Dunn's post hoc test; Figure 1). Conversely, when we inspected the ratio between above- and belowground biomass we noticed an opposite trend: almost invariably wild barley genotypes allocated more resources than 'Elite' varieties to root growth compared to stem growth ($p < 0.05$, Kruskal–Wallis non-parametric analysis of variance followed by Dunn's post hoc test; Figure 1). As we sampled plants at the same developmental stage (Methods), these observations suggest that the exposure to the same soil type triggered differential growth responses in wild and domesticated genotypes.

Taxonomic diversification of the barley microbiota across barley genotypes

To study the impact of these differential responses on the composition of the barley microbiota we generated 6,646,864 sequencing reads from 76 rhizosphere and unplanted soil specimens. These high-quality sequencing reads yielded 11,212 Operational Taxonomic Units (OTUs) at 97% identity (Additional file 2: Supplementary worksheet 2). A closer inspection of the data indicated that rhizosphere specimens emerged as 'gated

communities' as evidenced by the fact that members of five bacterial phyla, namely *Acidobacteria*, *Actinobacteria*, *Bacteroidetes*, *Proteobacteria* and *Verrucomicrobia* accounted for more than 97.8% of the observed reads (Figure 2, Additional file 2: Supplementary worksheet 3).

Next, we investigated the lower ranks of the taxonomic assignments (i.e., OTU level) to identify host signatures on the microbiota thriving at the root-soil interface and computed the Observed, Chao1 and Shannon indexes for each sample type. This analysis further supported the notion of the rhizosphere as a 'gated community' as the Observed OTUs and Shannon index, but not the projected Chao1, identified significantly richer and more even communities in the bulk soil samples compared to plant-associated specimens ($p < 0.05$, Mann–Whitney U test; Additional file 1: Figure S2). Interestingly, when we compared the Chao1 index within rhizosphere samples, we observed members of the 'Desert 1' group assembled a richer and more even community compared with the other genotypes ($p < 0.05$, Kruskal–Wallis non-parametric analysis of variance followed by Dunn's post hoc test; Additional file 1: Figure S2).

To gain further insights into the impact of the sample type on the microbiota at the root-soil interface we generated canonical analysis of principal coordinates (CAP) using the weighted Unifrac distance, which is sensitive to OTU relative abundance and phylogenetic relatedness. This analysis revealed a marked effect of the microhabitat, i.e., either bulk soil or rhizosphere, on the composition of the microbiota as evidenced by the spatial separation on the axis accounting for the major variation (Figure 3). Interestingly, we observed a clustering of bacterial community composition within rhizosphere samples, which was more marked between 'Desert' and 'Elite' samples (Figure 3). Of note, these observations were corroborated by a permutational analysis of variance which attributed ~30% of the observed variation to the microhabitat and, within rhizosphere samples, ~17% of the variation to the individual ecogeographic groups (Permanova $p<0.01$, 5,000 permutations, Table 2). Strikingly similar results were obtained when we computed a Bray-Curtis dissimilarity matrix, which is sensitive to OTUs relative abundance, distance matrices (Table 2; Additional file 1: Figure S3).

Taken together these data indicate that the composition of the barley microbiota is fine-tuned by plant recruitment cues which progressively differentiate between unplanted soil and rhizosphere samples and, within these latter, wild ecotypes from elite varieties.

158 ## A footprint of host eco-geographic adaptation shapes the wild barley rhizosphere
159 microbiota

160 To gain insights into the bacteria underpinning the observed microbiota diversification
161 we performed a series of pair-wise comparisons between 'Elite' genotypes and each group
162 of the wild barley ecotypes. This approach revealed a marked specialisation of the members
163 of the 'Desert' ecotype compared to 'Elite' varieties as evidenced by the number of OTUs
164 differentially recruited between members of these groups and domesticated plants (Wald
165 test, $p < 0.05$, FDR corrected; Figure 4; Additional file 2: Supplementary worksheets 5-9).
166 Thus, the wild barley 'Ecotype' emerged as an element shaping the recruitment cues of the
167 barley rhizosphere microbiota.

168 A closer inspection of the OTUs differentially recruited between 'Desert' wild barley
169 and 'Elite' varieties revealed that the domesticated material exerted the greatest selective
170 impact on the soil biota, as the majority of the differentially enriched OTUs were enriched in
171 'Elite' varieties (Wald test, $p < 0.05$, FDR corrected; Additional file 2: Supplementary
172 worksheets 5 and 6). Next, the taxonomic assignments of these 'Elite-enriched' OTUs
173 versus the 'Desert' microbiota followed distinct patterns: while the comparison 'Elite'-
174 'Desert1' produced a subset of enriched OTUs assigned predominantly to *Actinobacteria*,
175 *Bacteroidetes* and *Proteobacteria*, the comparison 'Elite'-'Desert2' displayed a marked bias
176 for members of the *Actinobacteria* (i.e., 44 out of 104 enriched OTUs, Figure 5). Within this
177 phylum we identified a broader taxonomic distribution as those OTUs were assigned to the
178 families *Intrasporangiaceae*, *Micrococcaceae*, *Micromonosporaceae*, *Nocardioidaceae*,
179 *Pseudonocardiaceae*, *Streptomycetaceae*, as well as members of the order *Frankiales*.
180 Taken together, our data indicate that wild barley 'Ecotype' (i.e., the differential effect of
181 'North', 'Coast, and 'Desert') acts as a determinant for the rhizosphere barley microbiota
182 whose composition is ultimately fine-tuned by a sub-specialisation within the 'Ecotype' itself
183 (i.e., the differential effect of 'Desert1' and 'Desert2').

184 These observations prompted us to investigate whether the differential microbiota
185 recruitment between the tested plants was encoded, at least in part, by the barley genome.
186 We therefore generated a dissimilarity matrix using Single Nucleotide Polymorphisms
187 (SNPs) available for the tested genotypes and we inferred their genetic relatedness using a
188 simple matching coefficient (Additional file 2: Supplementary worksheet 10). With few
189 notable exceptions, this analysis revealed three distinct clusters of genetically related plants,
190 represented by and reflecting the 'Elite' material, the 'Desert' and the 'Coast' wild barley
191 genotypes (Additional file 1: Figure S4). The genetic diversity between domesticated
192 material exceeded their microbial diversity (compare relatedness of "Elite" samples in Figure

193 3 with the ones of Additional file 1: Figure S4) as further evidenced by the fact that we failed
194 to identify a significant correlation between these parameters (p value > 0.05). However,
195 when we focused the analysis solely on the pool of wild barley genotypes, we obtained a
196 significant correlation between genetic and microbial distance (Mantel test r = 0.230; p value
197 < 0.05; Figure 6).

198 Taken together, this revealed a footprint of barley host's adaptation to the
199 environment on the assembly of the bacteria thriving at the root-soil interface. This
200 recruitment cue interjected the distinct evolutionary trajectories of wild and domesticated
201 plants and, at least in part, is encoded by the wild barley genome.

202 Discussion

203 In this study we investigated how the evolutionary and eco-geographic constraints
204 faced by wild and domesticated barley genotypes impact on the recruitment and
205 maintenance of the rhizosphere microbiota.

206 As we performed a 'common garden experiment' in a Scottish agricultural soil, we
207 first determined how the chosen experimental conditions related the ones witnessed by the
208 wild barley in their natural habitats. Strikingly, the aboveground biomass gradient observed
209 in our study, with 'Elite' material almost invariably outperforming wild genotypes and material
210 sampled at the locations designated 'Desert2' at the bottom of the ranking, "matched" the
211 phenotypic characterisation of members of the 'B1K' collection grown in a common garden
212 experiment in local Israeli soil [18]. Conversely, belowground resource allocation followed
213 an opposite pattern as evidenced by an increased root:shoot dry weight ratio in wild
214 genotypes compared to 'Elite' varieties. Furthermore, as responses to edaphic stress, such
215 as drought tolerance, may modulate the magnitude of above-belowground resource
216 partitioning in plants [20] and root traits [21], our data might reflect a 'memory effect' of the
217 wild barley exposure to dry areas. Consistently, transgenic barley plants with larger root
218 systems better sustain drought stress than their cognate wild-type plants [22]. Taken
219 together, these results suggest adaptive responses to eco-geographic constraints in barley
220 have a genetic inheritance component which can be detected and studied in controlled
221 conditions.

222 These observations motivated us us to examine whether these below ground
223 differences were reflected by changes in microbiota recruitment exerted by the tested
224 genotypes. The distribution of reads assigned to given phyla appears distinct in plant-
225 associated communities which are dominated in terms of abundance by members of the

226 phyla *Proteobacteria*, *Bacteroidetes*, *Actinobacteria* and *Acidobacteria*, albeit the latter
227 appear progressively excluded from the plant-associated communities. This taxonomic
228 affiliation is consistent with previous investigations in barley in either the same [23] or in a
229 different soil type [15] as well as in other crop plants [24]. In summary, these data indicate
230 that the higher taxonomic ranks of the barley rhizosphere microbiota are conserved across
231 soil types as well as wild and domesticated genotypes.

232 The characterisation of the microbiota at lower taxonomic ranks, i.e., OTU-level,
233 revealed a significant effect of the microhabitat (i.e., either bulk soil or rhizosphere) and,
234 within plant-associated communities, a footprint of eco-geographic adaptation. For instance,
235 alpha diversity indices clearly pointed at selective processes sculpting bacterial composition
236 at the root soil interface as the number of Observed OTUs and the Shannon index indicate
237 simplified and reduced-complexity communities inhabiting the rhizosphere compared to
238 unplanted soil. This can be considered a hallmark of the rhizosphere microbiota as it has
239 been observed in multiple plant species and across soils [6]. Conversely, within rhizosphere
240 samples, alpha-diversity analysis failed to identify a clear pattern, except for the Chao1 index
241 revealing a potential for a richer community associated with plants sampled at the 'Desert1'
242 locations. This motivated us to further explore the between-sample diversity, which is beta-
243 diversity. This analysis revealed a clear host-dependent diversification of the bacteria
244 associated to barley plants manifested by ~17% of the variance of the rhizosphere
245 microbiota explained by the eco-geographical location of the sampled material. This value
246 exceeded the host genotype effect on the rhizosphere microbiota we previously observed in
247 wild and domesticated barley plants [15], but is aligned with the magnitude of host effect
248 observed in the rhizosphere microbiota of modern and ancestral genotypes of rice [25] and
249 common bean [26]. As these studies were conducted in different soil types, our data suggest
250 that the magnitude of host control on the rhizosphere microbiota is ultimately fine-tuned by
251 and in response to soil characteristics.

252 The identification of the bacteria underpinning the observed microbiota diversification
253 led to three striking observations. First, the comparison between 'Elite' varieties and the
254 material representing the 'Desert' ecotype was associated with the largest number of
255 differentially recruited OTUs, while the other wild barley genotypes appeared to share a
256 significant proportion of their microbiota with domesticated plants. A prediction of this
257 observation is that the distinct evolutionary trajectories of wild and domesticated plants *per*
258 *se* cannot explain the host-mediated diversification of the barley microbiota. As aridity and
259 temperature played a prominent role in shaping the phenotypic characteristics of barley [19,

260 27] it is tempting to speculate that the adaptation to these environmental parameters played
261 a predominant role also in shaping microbiota recruitment.

262 Second, it is the domesticated material which exerted a stronger effect on microbiota
263 recruitment, manifested by the increased number of host-enriched OTUs compared to wild
264 barley genotypes. This suggests that the capacity of shaping the rhizosphere microbiota has
265 not been "lost" during barley domestication and breeding selection. Our findings are
266 consistent with data gathered for domesticated and ancestral common bean genotypes,
267 which revealed that shifts from native soils to agricultural lands led to a stronger host-
268 dependent effect on rhizosphere microbes [28]. Due to the intrinsic limitation of 16S rRNA
269 gene profiles of predicting the functional potential of individual bacteria, it will be necessary
270 to complement this investigation with whole-genome surveys [29, 30] and metabolic
271 analyses [16, 31] to fully discern the impact of the host genotype on the functions provided
272 by the rhizosphere microbiota to their hosts.

273 The third observation is the marked enrichment of OTUs assigned to the phylum
274 *Actinobacteria* in 'Elite' varieties when compared to members of the 'Desert' ecotype, in
275 particular plants sampled at the 'Desert2' locations. At first glance, the 'direction' of this
276 bacterial enrichment is therefore difficult to reconcile with the eco-geographic adaptation of
277 wild barleys and, in particular, the fact that *Actinobacteria* are more tolerant to arid conditions
278 [32] and, consequently, more abundant in desert vs. non-desert soils [33]. However, the
279 enrichment of *Actinobacteria* in modern crops compared to ancestral relatives has recently
280 emerged as a distinctive feature of the microbiota of multiple plant species [34]. Although
281 the ecological significance of this trait of the domesticated microbiota remains to be fully
282 elucidated, studies conducted in rice [35] and other grasses, including barley [36], indicate
283 a relationships between drought stress and *Actinobacteria* enrichments. These observations
284 suggest that the wild barley genome has evolved the capacity to recognise microbes
285 specifically adapted to the local conditions and, in turn, to repress the growth of others.

286 Interestingly, we were able to trace the host genotype effect on rhizosphere microbes
287 to the genome of wild barley. This suggests that, similar to other wild species [11], microbiota
288 recruitment co-evolved with other adaptive traits. Conversely, genetic diversity in 'Elite'
289 material largely exceeded microbiota diversity. This is reminiscent of studies conducted in
290 maize which failed to identify a significant correlation between polymorphisms in the host
291 genome and alpha- and beta-diversity characteristics of the rhizosphere microbiota [37, 38].
292 Yet, and again similar to maize [39], our data indicates that the recruitment of individual

293 bacterial OTUs in the 'Elite' varieties, rather than community composition as a whole, is the
294 feature of the rhizosphere microbiota under host genetic control.

295 A prediction from these observations is that the host control of the rhizosphere
296 microbiota is exerted by a limited number of loci in the genome with a relatively large effect.
297 This is congruent with our previous observation that mono-mendelian mutations in a single
298 root trait, root hairs, impact on ~18% of the barley rhizosphere microbiota [23]. Since modern
299 varieties have been selected with limited or no knowledge of belowground interactions, how
300 was the capacity of shaping the rhizosphere microbiota retained within the cultivated
301 germplasm? The recent observation that genes controlling reproductive traits display
302 pleiotropic effects on root system architecture [40] could provide a direct link between crop
303 selection and microbiota recruitment in modern varieties. These traits, and in particular
304 genes encoding flower developments, show a marked footprint of eco-geographic
305 adaptation and have been selected during plant domestication and breeding [27]. By
306 manipulating those genes, breeders have manipulated also belowground traits, and in turn,
307 the microbiota thriving at the root-soil interface. With an increased availability of genetic [41]
308 and genomic [42] resources for wild and domesticated barleys, this hypothesis can now be
309 experimentally tested and the adaptive significance of the barley rhizosphere microbiota
310 ultimately deciphered.

311 ## Conclusions

312 Our results revealed a footprint of host's adaptation to the environment on the
313 assembly of the bacteria thriving at the root-soil interface in barley. This recruitment cue
314 layered atop of the distinct evolutionary trajectories of wild and domesticated plants and, at
315 least in part, is encoded by the barley genome. Our sequencing survey will provide a
316 reference dataset for the development of indexed bacterial collections of the barley
317 microbiota to infer causal relationships between microbiota composition and plant traits, as
318 demonstrated for *Arabidopsis thaliana* [43] and rice [44]. Furthermore, this knowledge is
319 critical for the establishment of reciprocal transplantation experiments aimed at elucidating
320 the adaptive value of crop-microbiota interactions, similar to what has recently been
321 proposed for the model plant *A. thaliana* [45]. However, our data indicated that, for crop
322 plants like barley, this will necessarily be conditioned by two elements: identifying the host
323 genetic determinants of the rhizosphere microbiota and inferring its metabolic potential *in*
324 *situ*. This will set the stage for devising strategies aimed at sustainably enhancing crop
325 production for climate-smart agriculture.

326 ## Methods

327 ### Soil

328 The soil was sampled from the agricultural research fields of the James Hutton
329 Institute, Invergowrie, Scotland, UK in the Quarryfield site (56°27'5"N 3°4'29"W). This field
330 was left unplanted and unfertilised in the three years preceding the investigations and
331 previously used for barley-microbiota interactions investigations [23]. The chemical and
332 physical characteristics of the soil are presented in Additional file 1: Table S1.

333

334 ### Plant genotypes

335 Twenty wild barley genotypes and four 'Elite' crop cultivars were used and described
336 in Table 1. Wild barley genotypes were selected representing eco-geographical variation of
337 the wild ecotypes 'North', 'Coast' and 'Desert' [18, 19] which were further subdivided in 5
338 groups according to the mean annual rainfall of the sampling locations. The 'Elite' genotypes
339 were selected as a representation of different types of spring barley in plant genetic studies.
340 The cultivar 'Morex' is a six-row malting variety whose genome was the first sequenced [46].
341 The cultivars 'Bowman' and 'Barke' are two-row malting varieties whereas Steptoe is a six-
342 row type used for animal feeding [41, 47, 48].

343 ### Plant growth conditions

344 Barley seeds were surface sterilized as previously reported [49] and germinated on
345 0.5% agar plates at room temperature. Seedlings displaying comparable rootlet
346 development after 5 days post-plating were sown individually in 12-cm diameter pots
347 containing approximately 500g of the 'Quarryfield' soil, plus unplanted pots filled with bulk
348 soil as controls. Plants were arranged in a randomised design with at least 3 individual
349 replicates per genotype, with the exception of the genotypes B1K.12 and 'Bowman' for which
350 only two replicates were available. We ensured the individual number of replicates remained
351 comparable across eco-geographic groups: 'Coast1' number of replicates n=12; 'Coast2'
352 n=12; 'Desert1' n=11; 'Desert2' n=12; 'North' n=12; 'Elite' n=13. Plants were grown for 5
353 weeks in a randomized design in a glasshouse at 18/14 °C (day/night) temperature regime
354 with 16 h day length and watered every two days with 50 ml of deionized water.

355 ### Bulk soil and rhizosphere DNA preparation

356 At early stem elongation, corresponding to Zadoks stages 30-32 [50], plants were
357 pulled from the soil and the stems and leaves were separated from the roots. Above-ground
358 plant parts were dried at 70 °C for 72 h and the dry weight recorded, using vegetative
359 biomass as a proxy for plant growth. The roots were shaken manually to remove excess of

360 loosely attached soil. For each barley plant, the top 6 cm of the seminal root system and the
361 attached soil layer was collected and placed in sterile 50 ml falcon tube containing 15 ml
362 phosphate-buffered saline solution (PBS). Rhizosphere was operationally defined, for these
363 experiments, as the soil attached to this part of the roots and extracted through this
364 procedure. The samples were then vortexed for 30s and aseptically transferred to a second
365 50ml falcon containing 15ml PBS and vortexed again for 30s to ensure the dislodging and
366 suspension of the rhizosphere soil. Then, the two falcon tubes with the rhizosphere
367 suspension were mixed and centrifuged at 1,500 x *g* for 20min, the supernatant was
368 removed, with the rhizosphere soil collected as the pellet, flash frozen with liquid nitrogen
369 and stored at -80°C, until further use. After the rhizosphere extraction step, these parts of
370 the roots were combined with the rest of the root system for each plant, thoroughly washed
371 with water removing any attached soil particles and dried at 70°C for 72h for root biomass
372 measurement. Bulk soil samples were collected from the uppermost 6cm of unplanted pots
373 and subjected to the same procedure as above.

374 DNA was extracted from the rhizosphere samples using FastDNA™ SPIN Kit for Soil
375 (MP Biomedicals, Solon, USA) according to the manufacturer's recommendations. The
376 concentration and quality of DNA was checked using a Nanodrop 2000 (Thermo Fisher
377 Scientific, Waltham, USA) spectrophotometer and stored at -20°C until further use.

378 Preparation of 16 rRNA gene amplicon pools

379 The hypervariable V4 region of the small subunit rRNA gene was the target of
380 amplification using the PCR primer pair 515F (5'-GTGCCAGCMGCCGCGGTAA-3') and
381 806R (5'-GGACTACHVGGGTWTCTAAT-3'). The PCR primers had incorporated an
382 Illumina flow cell adapter at their 5' end and the reverse primers contained 12bp unique
383 'barcode' for simultaneous sequencing of several samples [51]. PCR, including No-
384 Template Controls (NTCs) for each barcoded primer, was performed as previously reported
385 with the exception of the BSA concentration at 10mg/ml per reaction [23]. Only samples
386 whose NTCs yielded an undetectable PCR amplification were retained for further analysis.

387 Illumina 16S rRNA gene amplicon sequencing

388 The pooled amplicon library was submitted to the Genome Technology group, The
389 James Hutton Institute (Invergowrie, UK) for quality control, processing and sequencing.
390 Amplicon libraries were amended with 15% of a 4pM phiX solution. The resulting high-quality
391 libraries were run at 10pM final concentration on an Illumina MiSeq system with paired-end
392 2x 150 bp reads [51] to generate the sequencing output, the FASTQ files.

393 ## Sequencing reads processing

394 Sequencing reads were processed and analysed using a custom bioinformatics
395 pipeline. First, QIIME (Quantitative Insights into Microbial Ecology) software, version 1.9.0,
396 was used to process the FASTQ files following default parameters for each step [52]. The
397 forward and reverse read files from individual libraries were decompressed and merged
398 using the command join_paired_ends.py, with a minimum overlap of 30bp between reads.
399 Then, the reads were demultiplexed according to the barcode sequences and joined by the
400 overlapping paired-end (PE). Quality filtering was performed using the command
401 split_libraries_fastq.py, imposing a minimum acceptable PHRED score '-q' of 20. Next,
402 these high quality reads were truncated at the 250th nucleotide using the function 'fastq_filter'
403 implemented in USEARCH [53]. Only these high-quality PE, length-truncated reads were
404 used for clustering in Operational Taxonomic Units (OTUs) a 97% sequence identity. OTUs
405 were identified using the 'closed reference' approach against Silva database (version 132)
406 [54]. OTU-picking against the Silva database was performed using the SortMeRNA
407 algorithm [55], producing in an OTU table containing the abundance of OTUs per sample
408 plus a phylogenetic tree. To control for potential contaminant OTUs amplified during library
409 preparation, we retrieved a list of potential environmental contaminant OTUs previously
410 identified in our laboratory [56] and we used this list to filter the results of the aforementioned
411 OTU-enrichment analysis. Additionally, singleton OTUs, (OTUs accounting for only one
412 sequencing read in the whole dataset) and OTUs assigned to chloroplast and mitochondria
413 (taken as plant derived sequences) were removed using the command
414 filter_otus_from_otu_tables.py. Taxonomy matrices, reporting the number of reads assigned
415 to individual phyla, were generated using the command summarize_taxa.py. The OTU table,
416 the phylogenetic tree and the taxonomy matrix, were further used in R for visualizations and
417 statistical analysis.

418 ## Statistical analyses I: univariate datasets and 16S rRNA gene alpha and beta-diversity
419 calculations

420 Analysis of the data was performed in R software using a custom script with the
421 following packages: Phyloseq [57] for processing, Alpha and Beta-diversity metrics; ggplot2
422 [58] for data visualisations; Vegan [59] for statistical analysis of beta-diversity; Ape [60] for
423 phylogenetic tree analysis; PMCMR [61] for non-parametric analysis of variance and
424 Agricolae for Tukey post hoc test [62]. For any univariate dataset used (e.g., aboveground
425 biomass) the normality of the data's distribution was checked using Shapiro–Wilk test. Non-
426 parametric analysis of variance were performed by Kruskal-Wallis Rank Sum Test, followed

427 by Dunn's post hoc test with the functions kruskal.test and the posthoc.kruskal.dunn.test,
428 respectively, from the package PMCMR.
429
430 For the analysis of microbiota data, first, a 'phyloseq object' was created allowing
431 joint analysis of the mapping file (metadata information), OTU table, phylogenetic tree and
432 the taxonomy matrix. For Alpha-diversity analysis, the OTU table was rarefied. at 11,180
433 reads per sample and this allowed us to retain 8,744 OTUs for downstream analyses
434 (Additional file 2: Supplementary worksheet 4). The Chao1, Observed OTUs and Shannon
435 indices calculated using the function estimate richness in Phyloseq package. Chao1: that
436 project sequencing saturation, considering rare OTUs; Observed OTUs: that counts unique
437 OTUs in each sample; and Shannon: that measures evenness in terms of the number of
438 OTUs presence and abundance. Beta-diversity was analysed using a normalized OTU table
439 (i.e., not rarefied) for comparison. For the construction of the normalized OTU table, low
440 abundance OTUs were further filtered removing those not present at least 5 times in 20%
441 of the samples, to improve reproducibility. Then, to control for the uneven number of reads
442 per specimen, individual OTU counts in each sample were divided over the total number of
443 generated reads for that samples and converted in counts per million. Beta-diversity was
444 analysed using two metrics: Bray-Curtis that considers OTUs relative abundance; and
445 Weighted Unifrac that additionally is sensitive to phylogenetic classification [63]. These
446 dissimilarity matrices were visualized using Canonical Analysis of Principal coordinates
447 (CAP) [64] using the ordinate function in the Phyloseq package, a constrained method of
448 ordination, was used to maximise the variation explained by a given variable at its
449 significance was inspected using a permutational ANOVA over 5,000 permutations

450 Beta-diversity dissimilarity matrices were assessed by Permutational Multivariate
451 Analysis of Variance (Permanova) using Adonis function in Vegan package over 5,000
452 permutations to calculate effect size and statistical significance.

453 Statistical analyses II: analysis of OTUs differentially enriched among samples

454 The differential analysis of the OTUs relative abundances was performed a) between
455 individual eco-geographic groups and bulk soil samples to assess the rhizosphere effect
456 and b) between the rhizosphere samples to assess the eco-geographic effect. The
457 ecogeographic effect was further corrected for a microhabitat effect (i.e., for each group,
458 only OTUs enriched against both unplanted soil and at least another barley genotype were
459 retained for further analysis). The analysis was performed using the DESeq2 method [65]
460 consisting in a moderated shrinkage estimation for dispersions and fold changes as an input

461 for a pair-wise Wald test. This method identifies the number of OTUs significantly enriched
462 in pair-wise comparisons with an adjusted p value (False Discovery Rate, FDR $p < 0.05$).
463 This method was selected since it outperforms other hypothesis-testing approaches when
464 data are not normally distributed and a limited number of individual replicates per condition
465 (i.e., less than 10) are available [66]. DESeq2 was performed using the eponymous named
466 package in R with the OTU table filtered for low abundance OTUs as an input.
467 The number of OTUs differentially recruited in the pair-wise comparisons between
468 'Elite' and wild barley genotypes was visualised using the package UpSetR [67].
469 The phylogenetic tree was constructed using the representative sequences of the
470 OTUs significantly differentiating 'Elite' genotypes and either 'Desert1' or 'Desert2' samples
471 annotated with iTOL [68].
472

473 ### Statistical analyses III: Correlation plot genetic distance-microbial distance.

474 To assess the genetic variation on the barley germplasm we used the SNP platform
475 'BOPA1' [69] (Close et al., 2009) comprising 1,536 single nucleotide polymorphisms. We
476 used GenAlex 6.5 [70, 71] to construct a genetic distance matrix using the simple matching
477 coefficient. Genetic distance for the barley genotypes was visualised by hierarchical
478 clustering using the function hclust in R. Microbial distance was calculated on the average
479 distances for each ecogeographic group using the Weighted Unifrac metric. Correlation
480 between the plant's genetic and microbial distances was performed using a mantel test with
481 the mantel.rtest of the package ade4 in R. The correlation was visualised using the functions
482 ggscatter of the R packages ggpbur.

483

484

485 ## Tables and Figure legends

486 **Table 1. The domesticated and wild barley genotypes names, sampling site,**
487 **ecogeographic group and environmental parameters of the B1K collection sites:**
488 **mean annual rainfall (MAR*), mid-day temperature in January (MDT1*), Elevation, and**
489 **soil bulk density (Db*) from [19]. (**) missing data**

Eco-geo graphical group	Site	Genotype ID	MAR* (mm)	MDT1* (°C)	Elevation* (m)	Db* (g/ml)
Coast1						
	Michmoret	B1K.03.09	569	12.3	19	1.32
	Dor	B1K.20.13	543	12.3	16	1.06
	Kerem Maharal	B1K.21.11	602	11.9	92	1.04
	Oren Canyon	B1K.30.07	623	11.9	98	1.02
Coast2						
	Amatzya	B1K.17.10	366	10.5	355	1.21
	Shomerya	B1K.18.16	318	10.1	441	1.13
	Beit Govrin	B1K.35.11	386	10.8	303	0.97
	Sinsan Stream	B1K.48.06	471	10.4	358	1.05
Desert1						
	Ein Prat	B1K.04.04	388	10.4	319	m.d. (**)
	Neomi	B1K.05.13	153	13.1	-245	1.28
	Talkid Stream	B1K.08.18	215	12.7	-253	1.05
	Kidron Stream	B1K.12.10	87	14	-380	1.38
Desert2						
	Yeruham	B1K.02.18	112	9.9	535	1.41
	Shivta	B1K.11.11	88	10.7	358	1.43
	Mt. Harif	B1K.33.03	74	8.3	860	1.52
	Havarim Stream	B1K.34.20	93	10.1	485	1.32
North						
	Susita	B1K.14.04	444	10.5	51	0.93
	Hamat Gader	B1K.15.19	436	11.3	-69	0.96
	Avny hill	B1K.31.01	502	10.4	177	1.13
	Almagor	B1K.37.06	461	11.1	-37	1.11
Elite						
		Barke				
		Bowman				
		Morex				
		Steptoe				

490

491

492 **Table 2. Proportion of rhizosphere microbiota variance explained by the indicated**
493 **variables and corresponding statistical significance.**

Weighted Unifrac		
	R2	Pr(>F)
Microhabitat	0.285	<0.001
Eco-geography*	0.168	<0.001
Bray-Curtis		
	R2	Pr(>F)
Microhabitat	0.221	<0.001
Eco-geography*	0.129	<0.001

494 (*) Analysis performed in rhizosphere samples only

495

496 **Figure 1 Plant growth parameters of the wild and domesticated barley genotypes.** (**a**)
497 Distribution of the twenty wild barley genotypes used in this study in the Israeli geographic
498 region. Individual dots depict the approximate sampling location for a given genotype,
499 colour-coded according to the designated ecogeographic area. (**b**) Stem dry weight of the
500 barley genotypes at the time of sampling. (**c**) Ratio between root and shoot dry weight of the
501 indicated samples. In **b** and **c**, upper and lower edges of the box plots represent the upper
502 and lower quartiles, respectively. The bold line within the box denotes the median, individual
503 shapes depict measurements of individual biological replicates/genotypes for a given group.
504 Different letters within the individual plots denote statistically significant differences between
505 means by Kruskal-Wallis non-parametric analysis of variance followed by Dunn's post-hoc
506 test ($P < 0.05$).

507 **Figure 2 The dominant phyla of the bulk soil and rhizosphere microbiota are**
508 **conserved across barley genotypes.** Average relative abundance (% of sequencing
509 reads) of the dominant phyla retrieved from the microbial profiles of indicated samples. Only
510 phyla displaying a minimum average relative abundance of 1% included in the graphical
511 representation.

512 **Figure 3 Wild and elite barley genotypes fine-tune the composition of the rhizosphere**
513 **bacterial microbiota.** Principal Coordinates Analysis of the Weighted Unifrac dissimilarity
514 matrix of the microbial communities retrieved from the indicated sample types. Individual
515 shape depicts individual biological replicates colour-coded according to the designated
516 ecogeographic area.

517 **Figure 4 Enrichments of individual bacteria discriminates between elite varieties and**
518 **wild barley genotypes.** Number of OTUs differentially enriched (Wald test, P value < 0.05,
519 FDR corrected) in the indicated pair-wise comparisons between elite varieties and groups

520 of wild barley genotypes. In the panel, horizontal blue bars denote the total number of
521 differentially enriched OTUs for a given comparison. Vertical black bars denote the
522 magnitude of the enrichment in either the individual comparison or among two or more
523 comparison highlighted by the interconnected dots underneath the panels. Abbreviations:
524 C1 (Coast 1); C2 (Coast 2); D1 (Desert 1); D2 (Desert 2); N (North).

525 **Figure 5. Actinobacteria are preferentially enriched in and discriminate between elite**
526 **genotypes and wild barley genotypes adapted to desert environments.** Individual
527 external nodes of the tree represent one of the OTUs enriched in the rhizosphere of elite
528 genotypes compared to either (or both) rhizosphere samples desert areas (Wald test, P
529 value < 0.05, FDR corrected). The colours reflect OTUs' taxonomic affiliation at Phylum
530 level. A black bar in the outer rings depicts whether that given OTU was identified in the
531 comparisons between 'Elite' and either 'Desert 1' or 'Desert 2' genotypes, respectively.
532 Phylogenetic tree constructed using OTUs 16S rRNA gene representative sequences.

533 **Figure 6**: **Mantel test between genetic distance and microbial distance in the wild**
534 **barley rhizosphere.** Individual dots depict individual comparison of any given pair of wild
535 barley genotypes between average value of weighted unifrac distance (y-axis) and genetic
536 distance shown as simple matching coefficients (x-axis). The blue line depicts the regression
537 lines, the grey shadow the confidence interval, respectively.

538 **Additional files**

539 Additional file 1:

540 **Table S1:** Chemical and physical characteristic of the 'Quarryfield' soil used in this study.
541 **Figure S1**: Barley plants during the glasshouse growth. **Figure S2:** Alpha-diversity
542 calculations of Observed OTUs (A), Chao 1 (B), and Shannon (C) recorded for the indicated
543 eco-geographic groups and 'Elite' genotypes Data computed using OTUs clustered at 97%
544 similarity. Asterisks denote statistically significant differences between microhabitat by non-
545 parametric Wilcoxon rank sum test (* P < 0.05; ** P < 0.01; ns not significant). Different red
546 letters within rhizosphere samples denote statistically significant differences between barley
547 groups means by Kruskal-Wallis non parametric analysis of variance followed by Dunn's
548 post-hoc test (P < 0.05); ns, no significant differences observed**. Figure S3**: Wild and 'Elite'
549 barley genotypes fine-tune the composition of the rhizosphere bacterial microbiota. Principal
550 Coordinates Analysis of the Bray-Curtis dissimilarity matrix of the microbial communities
551 retrieved from the indicated sample types. Individual shape depicts individual biological
552 replicates colour-coded according to the designated eco-geographic area. **Figure S4**:
553 Genetic relatedness of the individual barley genotypes used in this study. Hierarchical

554 clustering of the genetic distance expressed as simple matching coefficient of the indicated
555 barley samples colour-coded according their ecogeographic area. Genetic distance
556 computed using the SNP information of the BOPA1 markers.

557 Additional file 2:

558 **Supplementary worksheet 1**: Experimental design and sample description for the 16S
559 rRNA gene sequencing experiment. **Supplementary worksheet 2**: OTUs and individual
560 counts identified the amplicon sequencing survey. **Supplementary worksheet 3**: Matrix
561 depicting the phylum relative abundances of the microbes identified in the amplicon
562 sequencing survey. **Supplementary worksheet 4**: OTUs and individual counts of the
563 rarefied table. **Supplementary worksheets 5-9**: Taxonomic information of the OTUs
564 differentially recruited in the pairwise comparison 'Elite' and 'Desert 1'; 'Elite' and 'Desert 2';
565 'Elite' and 'Coast 1'; 'Elite' and 'Coast 2'; 'Elite' and 'North', respectively. **Supplementary**
566 **worksheet 10**: Genetic information of the tested germplasm. **Supplementary worksheet**
567 **11**: Mapping file of the samples for the genetic distance/microbial distance analysis.

568 ## Declarations
569

570 ### Abbreviations

571 16S Ribosomal subunit (S = Svedberg sedimentation coefficient = 10-13 s); B1K: Barely 1K
572 collection; CAP: Canonical Analysis of Principal Coordinates; cm: Centimetres; cv.: cultivar;
573 FDR: False discovery rate; NTC: No-template control; OTU: Operational taxonomic unit;
574 PCR: Polymerase Chain Reaction; PERMANOVA: Permutational multivariate Analysis of
575 Variance; PHRED score: 'Phil's read editor', quality score for base pair in FASTQ files;
576 QIIME: Quantitative Insights Into Microbial Ecology: rRNA: ribosomal ribonucleic acid; SNP:
577 Single Nucleotide Polymorphisms.

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588 **Availability of data and materials**

589 The sequences generated in the 16S rRNA gene sequencing survey and the raw
590 metagenomics reads reported in this study are deposited in the European Nucleotide
591 Archive (ENA) under the accession number PRJEB35359.

592 The version of the individual packages and scripts used to analyse the data and
593 generate the figures of this study are available at https://github.com/BulgarelliD-
594 Lab/Barley_B1K

595

596 **Authors' contribution**

597 The study was conceived by RAT and DB with critical inputs from EP and EB. RAT
598 and KBC performed the experiments. JM and PH generated the 16S rRNA sequencing
599 reads. JR provided access to the molecular marker information of the barley genome. RAT
600 and DB analysed the data. All authors critically reviewed and edited the manuscript and
601 approved its publication.

602 **Ethics approval and consent to participate**

603 Not applicable.

604 **Consent for publication**

605 Not applicable.

606 **Competing Interests**

607 The authors declare that they have no competing interests.

608

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610

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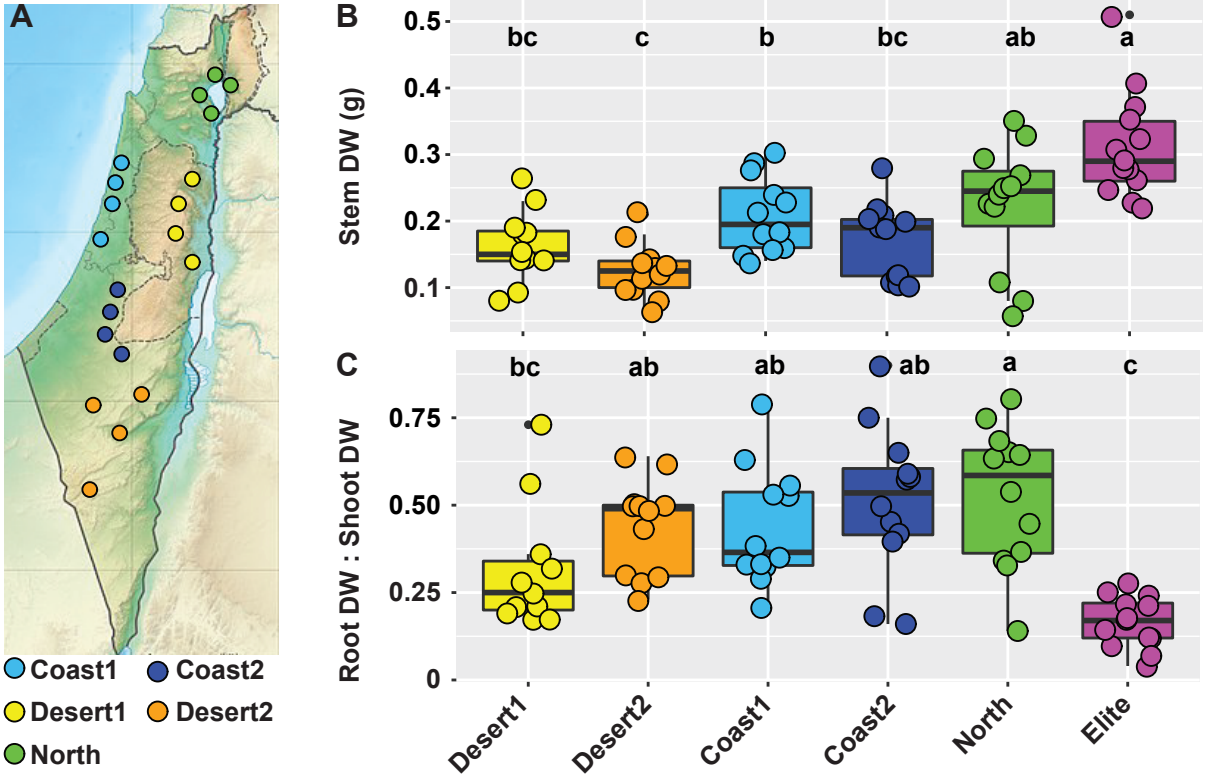
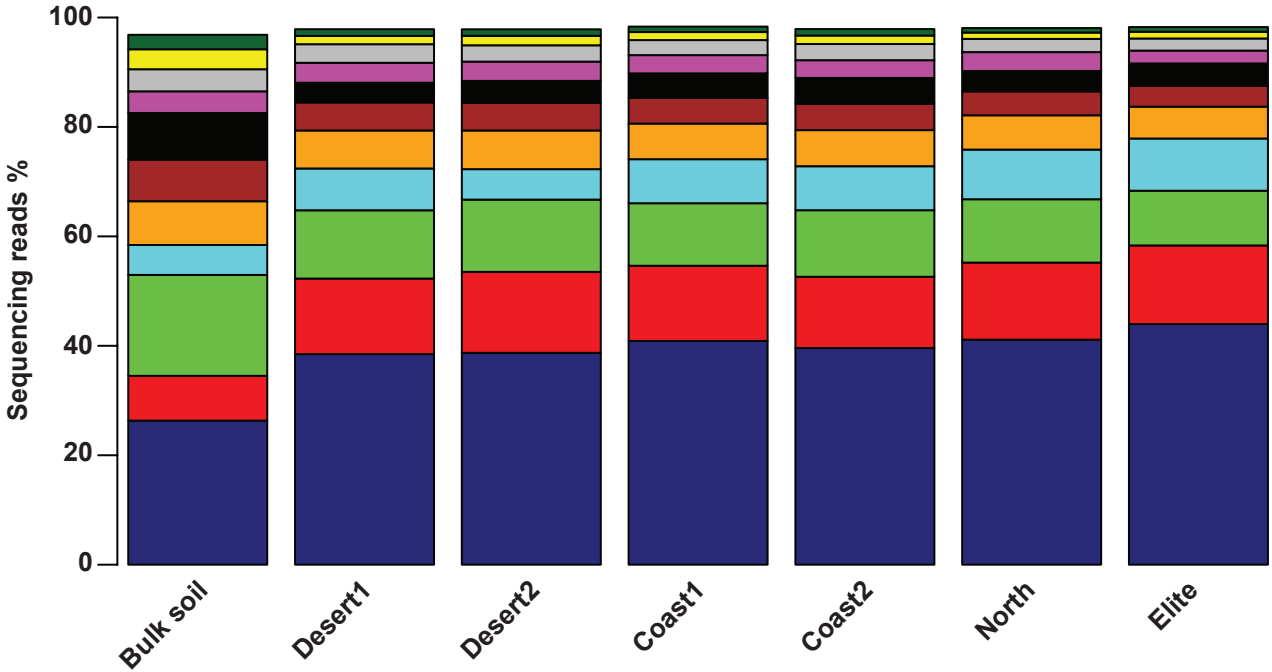
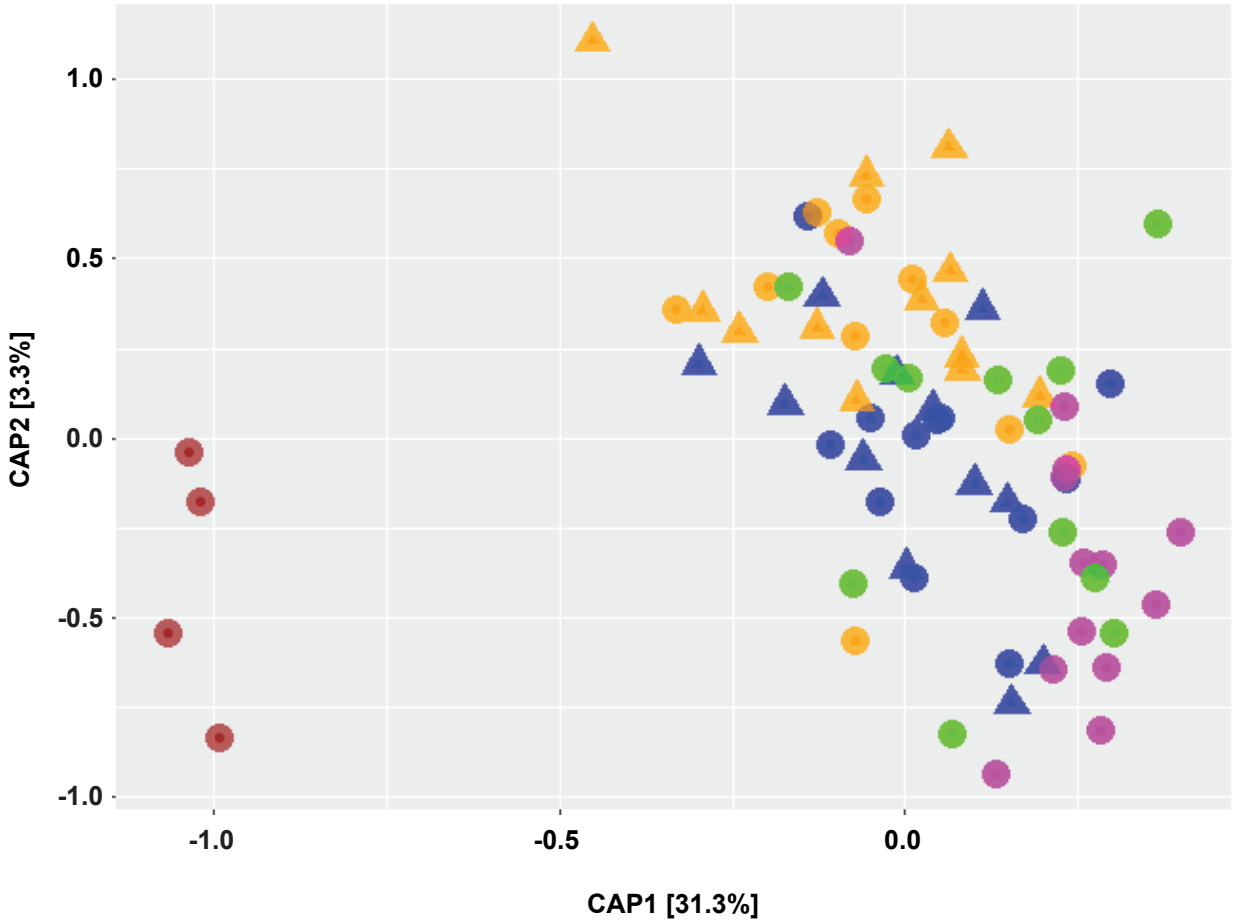
827

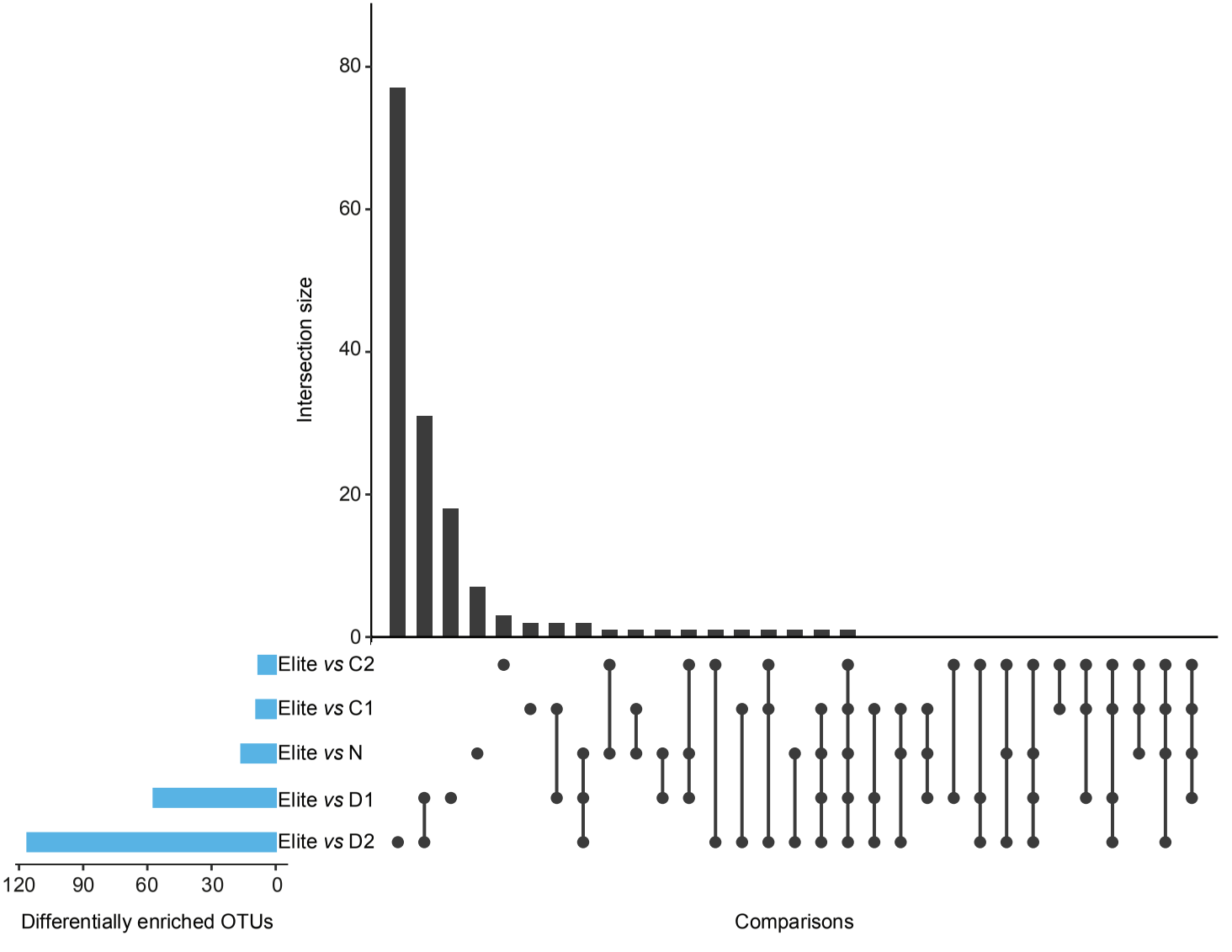

A
Coast1
Coast2
Desert1
Desert2
North
B
Stem DW (g)
0.5
0.4
0.3
0.2
0.1
bc
c
b
bc
ab
a
C
Root DW : Shoot DW
0.75
0.50
0.25
0
bc
ab
ab
ab
a
c
Desert1
Desert2
Coast1
Coast2
North
Elite

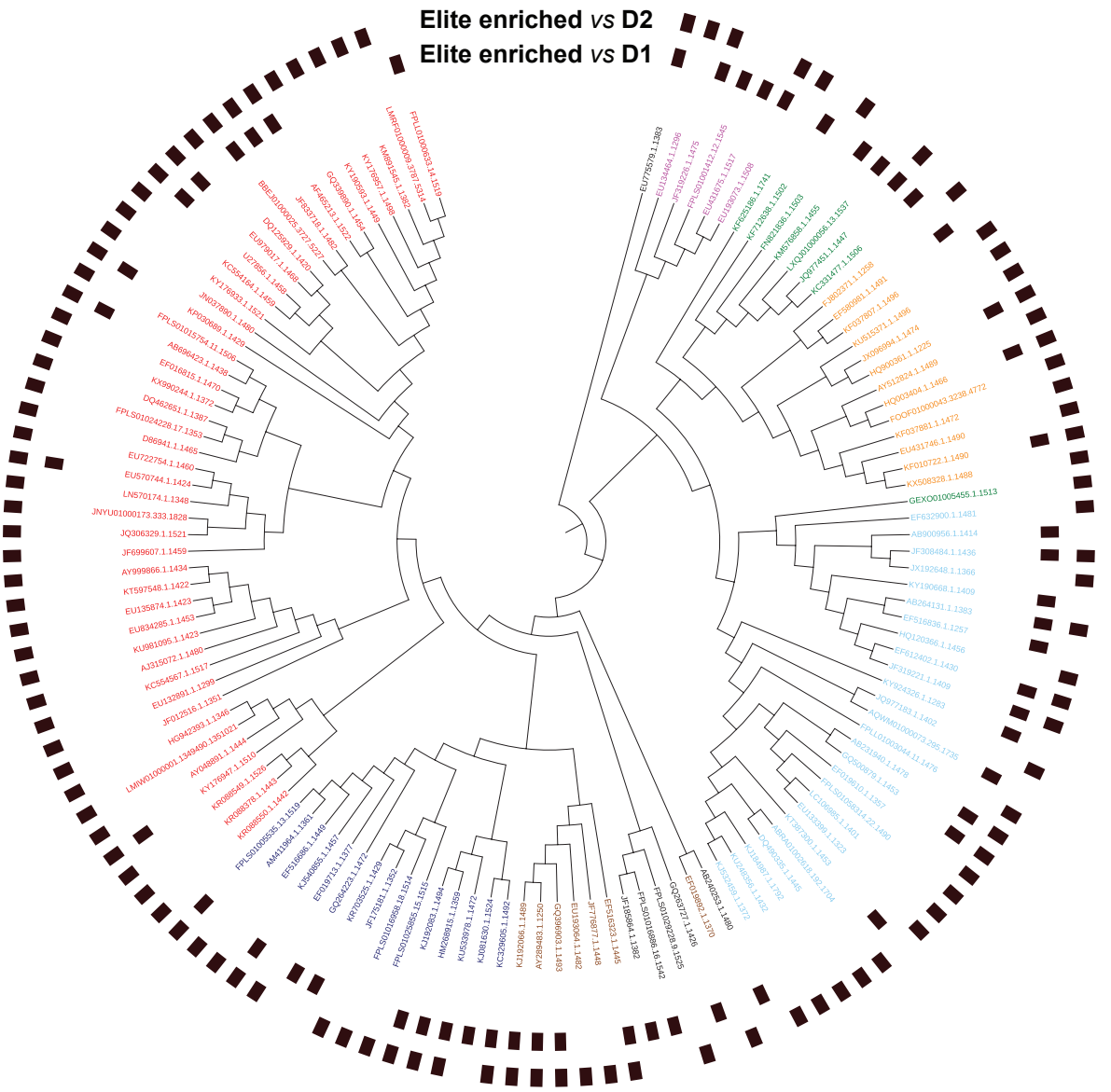


Taxonomy

- Acidobacteria
- Actinobacteria
- Bacteroidetes
- Chloroflexi
- Latescibacteria
- Gemmatimonadetes
- Planctomycetes
- Proteobacteria
- Rokubacteria
- Verrucomicrobia
- Thaumarchaeota

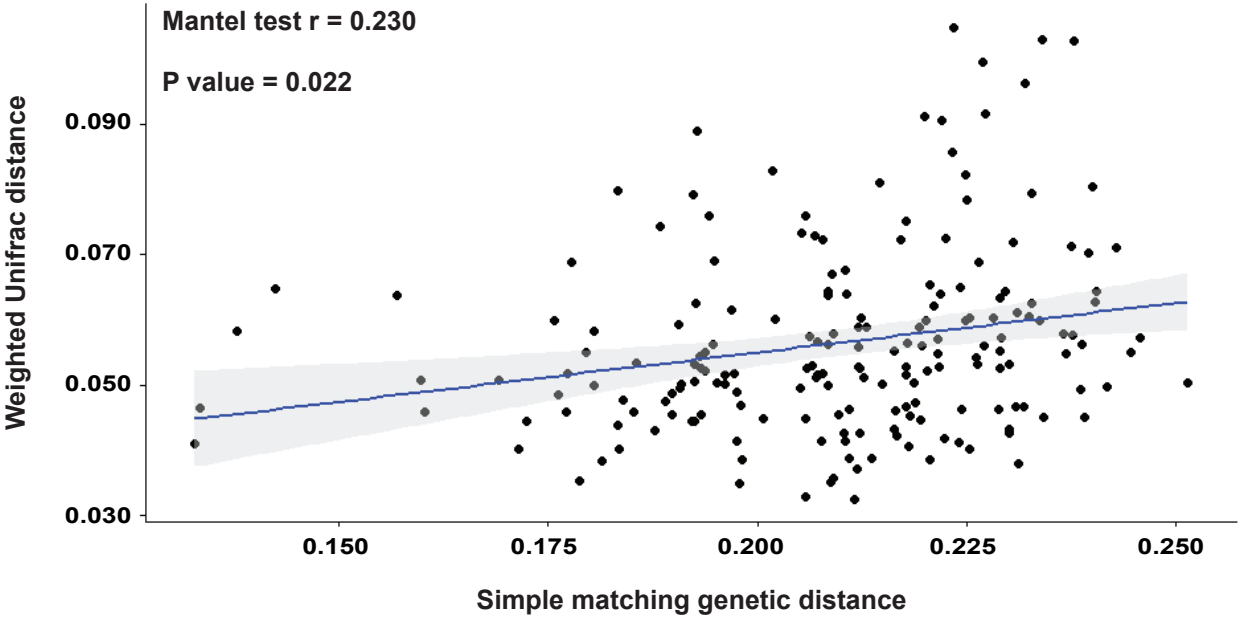

1.0
0.5
0.0
-0.5
-1.0
CAP2 [3.3%]
-1.0
-0.5
0.0
CAP1 [31.3%]
Sample type
Bulk soil
North
Coast1
Coast2
Desert1
Desert2
Elite


Intersection size
80
60
40
20
0
Elite *vs* C2
Elite *vs* C1
Elite *vs* N
Elite *vs* D1
Elite *vs* D2
120
90
60
30
0
Differentially enriched OTUs
Comparisons



OTU taxonomy

- **Actinobacteria**
- **Alphaproteobacteria**
- **Bacteroidetes**
- **Betaproteobacteria**
- **Deltaproteobacteria**
- **Gammaproteobacteria**
- **Gemmatimonadetes**
- **Other phyla**


Mantel test r = 0.230
P value = 0.022
Weighted Unifrac distance
0.090
0.070
0.050
0.030
0.150
0.175
0.200
0.225
0.250
Simple matching genetic distance