Nitrogen availability modulates the host control of the barley rhizosphere microbiota

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Abstract

Background:

Since the dawn of agriculture, human selection on plants has progressively differentiated input-demanding productive crops from their wild progenitors thriving in marginal areas. Barley (*Hordeum vulgare*), the fourth most cultivated cereal globally, is a prime example of this process. We previously demonstrated that wild and domesticated barley genotypes host distinct microbial communities in their rhizosphere. Here, we tested the hypothesis that microbiota diversification is modulated by, and responds to, nitrogen (N) application in soil and assessed the impact of microbiota taxonomic and functional compositions on plant growth.

Methods:

We grew two wild (*H. vulgare* ssp. *spontaneum*) and an 'Elite' domesticated (*H. vulgare* ssp. *vulgare*) barley genotypes in an agricultural soil treated with and without N inputs. By using a two-pronged 16S rRNA gene amplicon sequencing and comparative metagenomics approach, we determined the impact of N application on taxonomic composition and metabolic potential of the microbial communities exposed to limiting and replete N supplies. We then implemented a plant-soil feedback experiment to assess microbiotas' recruitment cues and contribution to plant growth.

Results:

N availability emerged as a modulator of the recruitment cues of the barley bacterial microbiota as evidenced by the increased number of bacterial genera differentially recruited between unplanted soil and rhizosphere communities under N-limiting conditions. This recruitment pattern mirrored the impact of the host genotype on rhizosphere bacteria. The characterisation of the assembled metagenomes of plants exposed to N-limiting conditions revealed a metabolic specialisation of the rhizosphere microbiota compared to unplanted soil controls. This specialisation is underpinned predominantly by bacteria and is manifested by the enrichment of a core set of biological processes sustaining the adaptation of polymicrobial communities such as N utilisation, quorum sensing and motility across genotypes. The quantitative variation in a group of these biological processes defined host signatures in the barley rhizosphere metagenome. Finally, a plant-soil feedback experiment revealed that the host-mediated taxonomic diversification of the bacterial microbiota is associated with barley growth under sub-optimal N supplies.

Conclusions:

Our results suggest that under N limiting conditions, a substrate-driven selection process underpins the assembly of barley rhizosphere microbiota. Host-microbe and microbe-microbe interactions fine-tune this process at the taxonomic and functional level across kingdoms. The disruption of these recruitment cues negatively impacts plant growth.

Keywords:

Barley, rhizosphere, microbiota, Nitrogen, domestication, 16S rRNA gene, assembled metagenomes, functional enrichments, plant-soil feedback.

Background

To sustainably enhance global food security, innovative strategies to increase crop production while preserving natural resources are required [1-3]. This is particularly true for nitrogen (N) applications in staple crops, such as cereals, as approximately 50% of the applied fertilisers is lost either in the atmosphere or in groundwater [4]. These losses are dependent on soil characteristics and driven by soil microbes, predominantly bacteria and archaea, capable of modulating N availability for plant nutrition [5, 6]

Therefore, a better understanding of the functions provided by microbes thriving at the root-soil interface, i.e., the rhizosphere microbiota, under limiting and replete N supplies will be a key step towards economically and environmentally sustainable crop production [7, 8]. This is becoming an urgent research priority for crop wild relatives which, unlike domesticated varieties, have not been selected to respond to synthetic fertilisers and their microbiota may represent an untapped resource of plant-probiotic functions [9, 10].

Barley (*Hordeum vulgare*), the fourth most cultivated cereal worldwide[11], represents an attractive model to investigate host-microbiota interactions in crop plants: we previously demonstrated that domesticated (*H. vulgare* ssp. *vulgare*) and wild (*H.vulgare* ssp. *spontaneum*) barley genotypes host contrasting microbiotas [12, 13] and that perturbation of a root trait impacts on the recruitment of individual bacteria in the rhizosphere [14]. These data unequivocally point at the barley genotype as a determinant of the microbial communities thriving at the barley root-soil interface.

Do wild and domesticated genotypes assemble a distinct microbiota when exposed to contrasting N supplies? And, if so, what microbes and metabolic functions respond to the plants' "N-starvation"? Furthermore, what is the contribution of the rhizosphere microbiota to plant growth under sub-optimal N supplies? Addressing these questions will pave the way to the development of plant varieties capable of recruiting a beneficial microbiota for optimum plant growth under reduced N applications.

Here we addressed these questions by comparing the plant physiological responses of an 'Elite' (domesticated) two wild barley genotypes grown under controlled conditions in soils amended with and without mineral nitrogen. These data were integrated with a comprehensive characterisation of the rhizosphere microbiota using a combined Illumina 16S rRNA gene amplicon and metagenomic sequencing approaches. Finally, a

plant-soil feedback experiment was performed to determine how distinct structural and functional configurations of the barley microbiota impact on plant growth.

Results

N-limiting conditions amplify the host effect on the rhizosphere bacterial microbiota

To gain insights into the role played by N-availability on the composition of the barley bacterial microbiota, we selected one reference barley cultivar, Morex (hereafter 'Elite'), and two wild genotypes from the B1K collection [15], B1K-12 and B1K-31 (hereafter 'Desert' and 'North' respectively) previously characterised for their capacity for recruiting distinct microbiotas [12, 13]. Plants were grown under glasshouse conditions (Methods) in an agricultural soil previously used for microbiota investigations and designated 'Quarryfield' (Additional file 1: Table S1; [13, 16]). Plants, and unplanted soil controls, were supplemented with three modified Hoagland's solution preparations [17] containing all essential macro and micronutrients and three levels of mineral nitrogen (Additional file 1: Table S2): the optimum required for barley growth (N100%), a quarter of dosage (N25%) or no nitrogen (N0%). At early stem elongation (Additional file 1: Figure S1), plants were harvested, and total DNA preparations were obtained from rhizosphere and unplanted soil specimens. In parallel, we determined aboveground plant biomass, plant nitrogen content as well as concentrations of ammonium (NH_4^+) and nitrate (NO_3^-) in rhizosphere and unplanted soil.

We observed that plant performance was affected by the N application: aboveground biomass and plant nitrogen content were significantly lower at N0% compared to N100%, with N25% yielding intermediate values (Kruskal-Wallis test followed by Dunn post-hoc test, P value <0.05, FDR corrected, Additional file 1: Figure S2). Likewise, the residual nitrogen in the rhizosphere at the completion of the experiments, measured as a concentration of ammonium and nitrate respectively, displayed a significant decrease in the values recorded for N100% to N25% and from this latter to N0%. (Kruskal-Wallis test followed by Dunn post-hoc test, P value <0.05, FDR corrected, Additional file 1: Figure S2).

In parallel, we generated a 16S rRNA amplicon sequencing library from the obtained rhizosphere and unplanted soil controls and we identified 26,411 individual Amplicon Sequencing Variants (ASVs) accruing from 6,097,808 sequencing reads. After pruning *in silico* ASVs representing either host (i.e., plastid- or mitochondrial-derived sequences) or environmental contaminations, as well as low count ASVs, 5,035,790 reads were retained, representing over the 82% of the initial dataset. This sequencing

information was rarefied at equal sequencing depth across samples, agglomerated at genus level (Methods) and used to investigated between-sample diversity. As expected, Bray-Curtis distance was capable of differentiating bulk soil from rhizosphere profiles as evidenced by a segregation of either class of samples along the CAP axis accounting for the largest source of variation (Figure 1A). Furthermore, we observed a “gradient” along the axis accounting for the second source of variation aligned with the treatment effect, in particular for rhizosphere samples (Figure 1A). The microhabitat effect, i.e., either the affiliation of a sample to either bulk soil or rhizosphere, exerted the primary impact on the bacterial communities thriving at the root-soil interface (Permanova, R^2= 0.47, *P* value <0.001, 5,000 permutations, Figure 1B) followed by the treatment effect (Permanova, R^2= 0.10, *P* value <0.001, 5,000 permutations, Figure 1B). Congruently, the largest number of genera significantly enriched in rhizosphere compared to unplanted soil was observed in a nitrogen-independent manner, followed by the N0% exerting the second largest effect (Wald test, *P* value < 0.05, FDR corrected, Additional file 1: Figure S3). When we inspected the drivers of community assembly within the rhizosphere samples, we noticed that the treatment effect outweighed the genotype effect (Permanova, $R^2_{Treatment}$= 0.25, $R^2_{Genotype}$= 0.14, *P* value <0.001, 5,000 permutations, Figure 1B), motivating us in further examining the impact of the treatment on the abundance of individual bacteria underpinning genotype-mediated diversification. Strikingly, when we performed a set of pair-wise comparisons between individual genotypes at the different N levels, we observed that N0% was associated with the largest number of differentially recruited genera, while higher N levels progressively obliterated interspecific differences among genotypes (Wald test, *P* value < 0.05, FDR corrected, Figure 1C). Closer examination of the phylogenetic affiliations of the bacteria responding to plant recruitment cues at N0% revealed a relatively broad taxonomic affiliation of these treatment- and host-genotype dependent taxa (Figure 1D).

Owing to the fact that residual nitrogen in soil at the time of sampling significantly increased from N0% to N100% (Additional file 1: Figure S2), our observations indicated that nitrogen availability for plant uptake is a factor *a*) modulating the microhabitat- and genotype-dependent recruitment cues of the barley bacterial microbiota, by *b*) promoting the selective enrichment of individual taxa in the rhizosphere and, *c*) whose magnitude is maximised when no nitrogen is applied to the system.

The metabolic specialisation of barley microbiota under N-limiting conditions

To gain insights into the functional significance of the observed treatment- and host-genotype dependent bacterial diversification of the barley microbiota we generated over

412 million paired-end metagenomic reads from 12 additional samples. These represented three biological replicates each of unplanted soil and the rhizosphere of ‘Elite’, ‘North’ and ‘Desert’ genotypes exposed to the N0% treatment in ‘Quarryfield’ soil. Upon in-silico removal of low-quality sequences and sequences matching the barley genome, likely representing a “host contamination”, we identified 72 bacterial and 8 fungal phyla, accounting for a cumulative number of 102,120,758 and 2,847,157 sequencing reads, respectively. Classes of phyla Actinobacteria, Bacteroidetes and Proteobacteria largely dominated the bacterial assemblages (Additional file 1: Figure S4A). Likewise, fungal assemblages were dominated by a limited number of classes assigned to Ascomycota and Basidomycota (Additional file 1: Figure S4B).

To further discern the metabolic capacity of barley associated communities, we assembled individual metagenomic reads in a replicated corrected manner and predicted their encoded proteins (Additional file 1: Figure S5). We observed that differences in sequencing depth among individual replicates of the same genotype led to consistent variations in assembly length and predicted proteins, likely impacting on downstream analyses (Additional file 1: Figure S6). We therefore decided to rarefy, and de-duplicate individual reads prior to assembly which led us to retain ~1 Gbp of assembled metagenome per biological replicate, corresponding to ~ 3.5 million predicted proteins per genotype on contigs exceeding 1,000 bp (Additional file 1: Figure S6; Additional file 1: Table S4).

Assembled reads were subjected to functional enrichment analysis to identify GO categories underpinning microbial establishment in the barley rhizosphere. We observed a consistent ‘rhizosphere effect’ in the functional potential of the barley microbiota manifested by the significant enrichment of multiple GO terms in each of the tested genotypes (Limma, *P* value <0.05, FDR corrected, Figure 2). The GO-slim annotation revealed a functional congruence across ‘Desert’, ‘North’ and the ‘Elite’ genotypes manifested by the simultaneous enrichment of GO-terms implicated in the regulation of microbial metabolism (e. g., nitrogen metabolism), adaptation of polymicrobial communities (e.g., quorum sensing, toxin biosynthesis), cellular motility and interactions with viruses (Limma, *P* value <0.05, FDR corrected, Table 1). Consistently, when investigating functional enrichments across genotypes we observed that a “functional core” microbiota, i.e., conserved across genotypes, represented the largest fraction of GO-terms enriched in rhizosphere samples versus unplanted soil controls with the genotype-effect, represented by GO-terms uniquely enriched in the individual genotypes, fine-tuning its

composition (Limma, *P* value <0.05, FDR corrected, Figure 3A). Closer inspection of the biological processes sustained this host-dependent diversification using Clusters of Orthologous Groups of proteins (COG) categories revealed that differences between the 'Elite' and wild genotypes (Wald test, *P* value <0.05, FDR corrected, Figure 3B). These differences revealed functional dichotomy between wild-associated communities, defined by a partial reprogramming of the metabolism of the microbiota associated to either genotypes (Figure 3C).

Taken together, our data indicate that, when N availability limits barley growth, wild and domesticated plants assemble a functional, congruent microbiota differentiating between the unplanted soil profile and plant-associated communities and, among these latter, between individual barley genotypes.

A phylogenetically diverse bacterial consortium is required for optimum barley growth

To establish a causal relationship between structural and functional configurations of the rhizosphere microbiota and plant growth, we performed a plant-soil feedback experiment by growing the 'Elite' variety in soils previously used for the growth of either a domesticated or wild genotypes amended with a N0% solution (hereafter 'conditioned soil'). For this analysis we focused on the pair 'Elite' – 'Desert' as these genotypes displayed the most contrasting microbiota (e.g., Figure 3). The obtained conditioned soils were used either in their 'native' form or subjected to a heat treatment which we hypothesized led to a disruption of the taxonomic and functional configurations of the microbiota. Consistently, at the end of the experiment, plants grown in the 'heat-treated' soil displayed a growth deficit when compared to their native counterpart, although these differences were significant only for the Desert-conditioned soil (Figure 4A, two-way ANOVA followed by TukeyHSD test, *P* value <0.05). Closer inspection of 19 chemical and physical parameters characterising the conditioned soils failed to single out a 'Desert-specific' parameter. Rather, a limited number of properties explained the majority of the variance among samples and differentiated between native soil and their heat-treated counterparts, irrespective of the initial genotype used (Additional file 1: Supplementary Figure S7; statistical values for the individual properties: *P* value < 0.01, R^2 > 0.8; 5,000 permutations). Conversely, qPCR analyses of 16S rRNA gene and ITS copy numbers performed at the end of the cultivation revealed a Desert-mediated impact on the bacterial but not on the fungal communities populating the conditioned soils (Kruskal-Wallis test followed by Dunn post-hoc test, *P* value = 0.056, FDR corrected, Additional file 1: Figure

S8). Although this observation appeared close to significance, this result motivated us to gain insights into the taxonomic composition of the bacterial communities inhabiting the conditioned soil. Strikingly, a 16S rRNA gene amplicon library constructed from the 71 samples subjected to the feed-back experiments and accruing for additional 6,770,434 sequencing reads revealed a marked compositional shift between heat-treated and native samples (Permanova, $R^2_{Treatment}$= 0.33, *P* value <0.001, 5,000 permutations, Figure 4B). When we inspected for the individual bacteria underpinning this diversification, we made two observations. First, the number of differentially enriched genera in the pair-wise comparison between native and heat-treated samples in 'Desert' conditioned soil exceeded that retrieved from the same comparison performed in the 'Elite' conditioned soil (number of differentially enriched genera N_{Desert} =24; N_{Elite} =15; Wald test, *P* value <0.05, FDR corrected, Figure 4C). Second, we identified a phylogenetically diverse group of 13 genera, assigned to 4 classes whose enrichment in native samples discriminated between these latter and both unplanted soil and heat-treated samples (Wald test, *P* value <0.05, FDR corrected, Figure 4D). These genera represented ~10% of the rhizosphere microbiota in the native samples and their sequence assignment largely overlap with genera enriched in Elite variety exposed to N0% treatments (Additional file 1: Table S5).

Taken together, our data suggest that the heat-treatment of the soil substrate led to a dysbiosis of the rhizosphere microbiota, whose magnitude is greater in the 'Desert' conditioned soil, negatively impacting on plant performance. As the median aboveground dry weight recorded in the feedback experiment is comparable to the one recorded for barley plants grown for the first time in Quarryfield soil (compare data of Figure 4A with the ones of Additional file 1: Figure S2A), this treatment appears to disrupt the capacity of Elite varieties to recruit a taxonomically diverse bacterial consortium required for optimum barley growth. Importantly, the enrichment of these putative barley growth promoting bacteria appeared sufficiently robust to withstand experiment-to-experiment variation.

Discussion

This study provided novel insights into the dynamics of the composition and metabolic capabilities of the rhizosphere microbial communities of distinct barley genotypes grown under controlled conditions in an agricultural soil and contrasting nitrogen treatments.

Our investigation revealed that nitrogen-availability impacts the 'rhizosphere effect' on the composition of the barley bacterial microbiota: this effect refers to a selective bacterial enrichment and is congruent with earlier studies conducted in barley [14, 18] and

other cereal crops such as maize [19, 20] and rice [21]. Our results indicate that the greater the quantity of N applied, the less plants tend to recruit a diverse microbiota. Likewise, the magnitude of the 'genotype effect', was maximal at N0%, when measurements of residual N in the rhizosphere were 0 mg Kg^{-1}, while it was nearly "obliterated" at N100%, with ~200 mg Kg^{-1} of residual NO_3 in the rhizosphere.

This is reminiscent of the observation that in *Medicago truncatula*, a model legume plant, the host control on the microbiota appear exerted in a nitrogen- and genotype-dependent manner [22]. Although the modulation of the rhizosphere microbiota in legumes has been associated to plant genes implicated in the establishment of symbiosis with N_2-fixing bacteria rather than nitrogen nutritional status *per se* [23, 24], it is conceivable that this latter impacts on, at least in part, host-microbe interactions at the root-soil interface. Although additional experiments are required to untangle the molecular mechanisms linking N-availability and uptake with microbiota diversification in barley, evidence of this interdependent relationship has been gathered in other members of the family *Poaceae*, such as perennial ryegrass (*Lolium perenne*) [25], wheat [26] and rice [27].

Interestingly, this nitrogen- and genotype-dependent diversification appears to operate beyond the dominant taxa, i.e., Actinobacteria, Bacteroidetes and Proteobacteria, we previously identified as plant-responsive in wild and domesticated barley genotypes [12, 13]. One example of this observation is the identification of members of the phyla Verrucomicrobia, Firmicutes and Myxococcota among the taxa underpinning host diversification at N0% (Figure 1D; Additional file 1: Figure S3). Although members of these phyla have not consistently been identified among rhizosphere competent bacteria [28], recent investigations conducted in maize suggest that members of the orders Pedosphaerales and Opitutales (Verrucomicrobia) can indeed respond to host genotype-dependent cues [29]. Likewise, members of the order Bacillales (Firmicutes) were identified as a 'keystone taxa' in the common bean rhizosphere microbiota [30]. Of note, members of the phylum Myxococcota have previously been characterised for their capacity of degrading cell wall material [31] and thriving under soil amended with organic nitrogen [32], two substrates likely available in the rhizosphere of plants exposed to N0%.

Our metagenomic survey revealed that the microbial communities proliferating at the barely root-soil interface are largely dominated by bacteria: more than 98% of the annotated sequences at phylum level were classified as bacteria which is strikingly similar to a previous investigation conducted in a soil with different physical and chemical characteristics [12]. This indicates that bacteria are efficient colonisers of the rhizosphere

in the tested agricultural soils. The dominance of bacterial sequences over other members of the microbiota is not unusual in soil metagenomes [33], although both the protocol used for microbial DNA preparation and the databases used for sequencing annotation [34] can 'artificially inflate' the proportion of bacteria among the analysed metagenomes. Crucially, using an independent experimental approach, we demonstrated that bacterial gene copy number exceeds the fungal one by several orders of magnitude as previously reported in soil [35] and in a recent rhizosphere metagenomic investigation [36].

The functional enrichment analysis of the assembled metagenomes revealed "signatures" underpinning the microbial adaptation to the rhizosphere environment (Figure 2; Table 1) which were previously identified in other comparative metagenomic investigations [12, 37, 38] and sustain a multi-step assembly model proposed for the diversification of the microbiota thriving at the root-soil interface [19, 28]. Importantly, the metagenomic survey allowed us to evaluate and integrate nitrogen availability into this model.

For instance, the identification of 'regulation of nitrogen utilization' among the functional categories enriched in the rhizosphere of each of the tested genotypes suggests that nitrogen availability underpins a selective process akin to the resource-ratio competition observed for other organisms [39]. Inter-organismal relationships amplify this process: for instance an increased metabolic activity of plant-associated microbes compared to unplanted soil controls [40] is likely to increase viral predation in the rhizosphere. Likewise, access to nutrients among nitrogen-competent bacteria may trigger the production of toxins, such as the one secreted by the bacterial Type-VI secretion system [41] which has previously been implicated in the regulation of plant-associated polymicrobial communities [42, 43]. Intriguingly, substrate availability appears to be a determinant also for the unplanted soil communities which, in absence of plant-derived substrates, enrich for autotrophic functions (Table 1).

This selective process is further fine-tuned by host genotype-factors (Figure 3). For instance, components of the cell machinery, likely to confer a competitive advantage to bacteria in accessing plant resources, can trigger immune responses in the roots of host plants [44] which, in turn, shape bacterial composition at the root-soil interface [45]. Likewise, host genotype-dependent factors underpinning the biosynthesis of secondary metabolites secreted during rhizodeposition which, in turn, provide an additional level of selection on the soil biota as recently demonstrated for maize [46, 47]. Consequently, microbes capable of either degrading or metabolising these, host genotype-specific,

compounds will have a competitive advantage in the barley rhizosphere. Conversely, the functional enrichment in the wild microbiota can be interpreted as an adaptive change in the transcriptional machinery required for microbial adaptation to limited nutrient availability, as observed in individual bacterial strains [48-50]. These data point at a resilient wild microbiota capable of “adjusting” to N starvation while the same condition triggers a stronger inter-organismal competition in the ‘Elite’ rhizosphere.

How does microbiota recruitment impact on plant performance? We addressed this question by establishing a plant-soil feedback experiment which suggested that, although with limited effect, a functional rhizosphere microbiota is required for optimal barley growth under soil conditions. This is conceptually similar to the well-known phenomenon of disease-suppressive soils, whereby previous crops promote the establishment of a protective microbiota for the subsequent ones in a host species-specific manner [51]. What emerged from our study is the control exerted by the recipient genotype on the resulting bacterial communities, as plants exposed to either Desert-conditioned or Elite-conditioned soil converge towards the recruitment of the same phylogenetically diverse bacterial consortium. This is in accordance with data gathered from rice, using both soil feedback experiments [52] and synthetic communities [27], indicating the host genotype as a driver of a plant-growth promoting microbiota. The current lack of representative isolates for these enriched genera makes it difficult to infer the mode of action of the bacteria, however it is striking to note that members of the genus *Devosia* have been implicated in growth promotion in plant-soil feedback experiments in rice [52]. Similarly, members of the genus *Stenotrophomonas* have been identified in the pathogen protective microbiota of sugar beet [53] while members of the phylum Actinobacteria (three of the thirteen enriched genera) have repeatedly been identified as associated to “healthy” domesticated plants [54]. Taken together, this suggests that the enriched bacteria represent a consortium of beneficial bacteria required for optimum barley growth whose recruitment is predominantly driven by the host genotype.

These results, coupled with the observation that the metabolic capacities of the microbiota are, at least in part, genetically inherited in barley [55], may set the stage for the deployment of functions of the microbiota for plant breeding purposes. This may have an immediate impact on sustainable crop production: decades of selection for barley cultivars capable of responding to fertilisers have not yet achieved the maximum nitrogen uptake efficiency for this species [56]. Our results suggest that this “gap” can be filled, at least in part, by targeting putative plant probiotic members of the soil biota.

Conclusions

Our results point at nitrogen availability for plant uptake as a modulator of the host control of the rhizosphere microbiota. This modulation is exerted at taxonomic level and underpins a functional diversification of the microbiota. Under limiting nitrogen supply, wild and domesticated genotypes retain specific functional signatures. Plant-soil feedback experiments suggest that these distinct compositional and functional configurations of the microbiota can be “overridden” by the host genotype leading to a recruitment of a phylogenetically diverse consortium of bacteria required for optimum plant growth. Our results constitute fundamentally novel insights into the adaptation of crop plants to limited nitrogen applications and will set the stage to exploit the genetic basis of plant-microbiota interactions for sustainable crop production.

Methods

Soil

Soil was sampled from the agricultural research fields of the James Hutton Institute, Invergowrie, Scotland, UK in the Quarryfield site (56°27'5"N 3°4'29"W). This field was left unplanted and unfertilised in the three years preceding the investigations and previously used for barley-microbiota interaction investigations [14]. The chemical and physical characteristics of the soil are presented in Additional file 1: Table S1.

Plant growth conditions

Barley seeds were surface sterilized as previously reported [57] and germinated on 0.5% agar plates. Seedlings displaying comparable rootlet development were sown individually in 12-cm diameter pots containing approximately 500g of the ‘Quarryfield’ soil, plus unplanted pots filled with bulk soil as controls. Plantlets one week old were transferred for two weeks to a growth room at 4 °C for vernalisation. After this initial treatment, plants were grown in a randomized design in a glasshouse at 18/14 °C (day/night) temperature regime with 16 h day length (Additional file 1: Figure S1).

Nitrogen treatments

The nutrient solutions applied in this study, i.e. N100%, N25% and N0% are reported in Additional file 1: Table S2. The nutrient solution was applied with watering consisting of a weekly input of 25ml of the nutrient solution per kg of soil. The application started two days after planting, was interrupted during the vernalisation and was reinstated relapsed once the plants were transferred to the growing glasshouse and they reached early stem elongation. Fourteen treatments were applied with a total of 312.50 mg (NO_3^-);

81.4 mg (NH_4^+) for the N100% solution, 78.13 mg (NO_3^-); 20.04 mg (NH_4^+) for the N25% solution, and 0 mg of (NO_3^- and NH_4^+) for the N0% solution per pot.

Plant and Soil Nitrogen determination

To assess the N content of the plant, at the time of sampling a newly expended leaf was sectioned from every plant, freeze-dried, ball milled, and N content measured in an Elemental Analyser CE-440 (Exeter Analytical Inc, UK). The soil content of the pots was sieved through a 2mm mesh sieve. Five grams of soil was added with 25mL of 1M KCl and the resulting solution mixed in a tube roller for 1hour at ~150 rpm. Supernatant was transferred to 50mL falcon tubes and centrifuged for 15min at 5,000 rpm, then the supernatant was subject to another round of centrifugation. The supernatant was transferred to a falcon tube and analysed in the Discrete Analyser Konelab Aqua 20 (Thermo Fisher, Waltham, USA) in the analytical services of The James Hutton Institute (Aberdeen, UK). In parallel, ~10 g from the sieved soil was oven dried at 70 C for 48h and dry weight recorded to express the analytical results in NO_3^- and NH_4^+ in mg N kg^{-1} of soil.

Bulk soil and rhizosphere DNA preparation

We organised a randomised design with two factors, sample type and nitrogen treatment, having four (unplanted soil, 'Desert', 'North' and 'Elite') and three (N100%, N25% and N0%) levels, respectively and 5 replications per combinations of levels. Samples were maintained under controlled conditions (see Plant Growth Conditions) and upon cultivation of the specimens and DNA preparation we retain at least 3 biological replicates/sample type/treatment. At early stem elongation, after a 5-week growth period in the glasshouse (plus the previous 2 weeks of vernalisation) plants were excavated from the soil and the stems were separated from the roots. The uppermost 6 cm of the root system were detached from the rest of the root corpus and processed for further analysis. The sampled aboveground material was oven dried at 70°C for 48 hours and the dry weight recorded. The roots were shaken manually to remove loosely attached soil. For each barley plant, the seminal root system and the attached soil layer was collected and placed in a sterile 50ml falcon tube containing 15ml phosphate-buffered saline solution (PBS). Rhizosphere was operationally defined, for these experiments, as the soil attached to this part of the roots and extracted through this procedure. The samples were then vortexed for 30 seconds and transferred to a second 50ml falcon containing 15ml PBS and vortexed again to ensure the dislodging and suspension of the rhizosphere. Then, the two falcon tubes with the rhizosphere suspension were combined and centrifuged at 1,500g for 20 minutes to precipitate the rhizosphere soil into a pellet, then flash frozen with liquid

nitrogen and stored at -80°C, until further analysis. In addition, we incubated water agar plugs (~1 cm^3) into two unplanted soil pots and we maintained them as control samples among the experimental pots to monitor the effect of this medium on the soil microbial communities. DNA was extracted from unplanted soil and rhizosphere samples using FastDNA™ SPIN Kit for Soil (MP Biomedicals, Solon, USA) according to the manufacturer's recommendations and stored at -20°C.

Preparation of 16 rRNA gene amplicon pools

The hypervariable V4 region of the small subunit rRNA gene was the target of amplification using the PCR primer pair 515F (5'-GTGCCAGCMGCCGCGGTAA-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3'). The PCR primers had incorporated an Illumina flow cell adapter at their 5' end and the reverse primers contained 12bp unique 'barcode' for simultaneous sequencing of several samples [58]. PCR reactions, including No-Template Controls (NTCs) for each barcoded primer, were performed as previously reported [13]. Only samples whose NTCs yielded an undetectable PCR amplification were retained for further analysis.

Illumina 16S rRNA gene amplicon sequencing

The pooled amplicon library was submitted to the Genome Technology group, The James Hutton Institute (Invergowrie, UK) for quality control, processing and sequencing. Amplicon libraries were amended with 15% of a 4pM phiX solution. The resulting high-quality libraries were run at 10 pM final concentration on an Illumina MiSeq system with paired-end 2x 150 bp reads [58] to generate the sequencing output, the FASTQ files.

Amplicon sequencing reads processing

Sequence reads were subjected to quality assessment using FastQC [59]. ASVs were then generated using DADA2 version 1.10 [60] and R 3.5.1 [61] following the basic methodology outlined in the 'DADA2 Pipeline Tutorial (1.10)'.Read filtering was carried out using the DADA2 pairedFastqFilter method, trimming 10bp of sequence from the 5' of each readus using a truncQ parameter of 2 and maxEE of 2. The remainder of the reads were of high quality consequently no 3' trimming was deemed necessary. The dada method was run to determine the error model with a MAX_CONSIST parameter of 20, following which the error model converged after 9 and 12 rounds for the forward and reverse reads respectively. The DADA2 method was then run with the resulting error model to denoise the reads using sample pooling, followed by read merging, then bimera removal using the consensus method. Taxonomy assignment was carried out using the RDP Naive Bayesian Classifier through the 'assign Taxonomy' method, with the SILVA

database [62] version 138, using a minimum bootstrap confidence of 50. The DADA2 outputs were converted to a Phyloseq (version 1.26.1) [63] .

The Phyloseq objects for both the nitrogen gradient and the Plant-soil feedback experiments were initially merged. Next sequences classified as either 'Chloroplast' or 'Mitochondria' were pruned in silico from the merged object. Likewise, ASVs matching a list of potential contaminants of the lab [64] were removed as well as ASVs lacking a taxonomic classification at phylum level (i.e., 'NA'). We further applied an abundance filtering and retained ASVs occurring with at least 20 reads in 2% of the samples. Finally, ASVs were agglomerated at genus level and the objects rarefied at 25,000 reads per sample, as recommended for groups with large differences in library size [65], prior to downstream analyses.

Plant-soil feedback experiment

We grew Desert and the Elite genotypes in 'Quarryfield' soil supplemented with a N0% nutrient solution under controlled conditions (see Plant growth conditions). We selected these two genotypes since, in the tested soils, they host a taxonomic and functional distinct microbiota. At early stem elongation we removed the plants from the soil, and we harvested the residual soil and kept it separated in a genotype-wise manner. We reasoned that at the end of cultivation the soils would have been enriched for the specific taxa/functions associated to either genotypes This residual soil, either in a 'native form', i.e., not further treated after sampling, or after being exposed to a heat-treatment (126°C for 1 hour, repeated twice at an interval of ~12 hours), was used as a substrate for a subsequent cultivation of a recipient Elite barley genotype. These plants were maintained under controlled conditions (see Plant growth conditions) and supplemented with a N25% solution to compensate for the near complete depletion of this mineral in the previous cycle of cultivation (compare the NH_4^+ and NO_3^- concentrations of rhizosphere specimens at N0% and N25% in Additional file 1: Figures S2). At early stem elongation plants were harvested and their aboveground biomass determined after incubating stems and leaves at 70°C for 48 hours. For each condition we initially contemplated 5 biological replicates for each rhizosphere treatment and the entire experiment was repeated in triplicate. At the end of each replicated experiment, the residual soil was collected and arranged in a condition-wise manner and subjected to chemical and physical characterisation (Yara United Kingdom Ltd., Grimsby, United Kingdom). In total, 71 biological replicates were retained and analysed in this experiment.

A quantitative real-time polymerase chain reaction assay was used to quantify the bacterial and fungal DNA fractions in samples from the conditioned soil experiment as follows. DNA samples were diluted to 10 ng/µl and successively diluted in a serial manner to a final concentration of 0.01 ng/µl. This final dilution was used for both the Femto Fungal DNA Quantification Kit and Femto Bacterial DNA Quantification Kit (Zymo Research) and the quantification was conducted according to the manufacturer protocol. Briefly, two microliters of the 0.01 ng/µl dilution of each sample was used together with 18 µl of the corresponding fungal or bacterial master mix. Two µl of the fungal or bacterial standards were also used to create the respective quantification curves. DNA samples from the conditioned soil experiment were randomized in the 96 well plates, using a minimum of 11 biological replicates per treatment. The quantification was performed in a StepOne thermocycler (Applied Biosystems by Life Technology) following the cycling protocols of each of the above-mentioned bacterial and fungal kits.

Statistical analyses on univariate dataset and amplicon sequencing data

Analysis of the data was performed in R software using a custom script with the following packages: Phyloseq [63] version 1.30.0 for pre-processing and beta-diversity analysis; ggplot2 version 3.3.0 [66] for data visualisations; Vegan version 2.5-6 [67] for statistical analysis of beta-diversity; PMCMR version 4.3 [68] for non-parametric analysis of variance and Agricolae for Tukey post hoc test [69]. For any univariate dataset used (e.g., aboveground biomass) the normality of the data's distribution was checked using Shapiro–Wilk test. For datasets normally distributed, the significance of the imposed comparisons was assessed by an ANOVA test followed by a Tukey post hoc test. Non-parametric analysis of variance were performed by Kruskal-Wallis Rank Sum Test, followed by Dunn's post hoc test with the functions kruskal.test and the posthoc.kruskal.dunn.test, respectively, from the package PMCMR. We used Spearman's rank correlation to determine the similarity between unplanted soil profiles and bulk soil samples amended with water agar plugs (Additional file 1: Table S3). The differential analysis of the genera relative abundances was performed a) between individual genotypes and bulk soil samples to assess the rhizosphere effect and b) between the rhizosphere samples to assess the genotype effect. The genotype effect was further corrected for a microhabitat effect (i.e., for each genotype, only genera enriched against both unplanted soil and at least another barley genotype were retained for further analysis). The analysis was performed using the DESeq2 package [70] version 1.26.0 consisting of a moderated shrinkage estimation for dispersions and fold changes as an input for a pair-wise Wald test. This method identifies the number of genera significantly

enriched in pair-wise comparisons with an adjusted *P* value (False Discovery Rate, FDR < 0.05). This method was selected since it outperforms other hypothesis-testing approaches when data are not normally distributed and a limited number of individual replicates per condition are available [65].

Metagenome sequencing, annotation and analysis.

We generated a new set of 3 bulk soil and 3 rhizosphere DNA preparations from each of the three genotypes tested (i.e., 'Desert', 'North' and 'Elite') from specimens maintained in Quarryfield soil under N0% conditions as described above. These 12 new preparations were quantified and submitted to the LGC Genomics sequencing service (Berlin, Germany) where they were used to generate DNA shotgun libraries using the Ovation Rapid DR Multiplex System 1-96 NuGEN (Leek, The Netherlands) kit following manufacturer's recommendations. These libraries were run simultaneously into an individual Illumina NextSeq500 run following manufacturer's recommendations with the 2 X150bp chemistry and generated a total of 412,385,413 read pairs. After sequencing read pairs were de-multiplexed according to the sample's barcodes using the Illumina bcl2fastq2.17.1.14 software.

Metagenome analysis was conducted according to the general approach of Hoyles *et al* [71], using updated tools where appropriate. Sequence reads were quality assessed using FastQC [72] and quality/adapter trimmed using TrimGalore [73], using a quality cut-off of 20, a minimum sequence length of 75bp and removing terminal N bases. Taxonomic classification of the sequence reads was carried out using Kraken 2.0.9 [74] with a custom database consisting of the genomic DNA sequences of all members of the Ensembl Genomes [75] release, 48 fungal and bacterial subsections. Host contamination was removed by alignment against the barley genome sequence [76] using BWA MEM [77], and non-aligning reads extracted from the resulting bam files using SAMtools [78]. Duplicate reads were removed using declumpify from the BBtools package [79] and then down sampled to 13600000 reads per sample using BBtools reformat.sh tool. Metagenome assembly was conducted using metaSPAdes [80] using default parameters in two rounds. Initial assemblies were carried out for each replicate. Reads which were not incorporated into the assembled contigs were identified by BWA alignment against the assemblies and separated from the bam files using SAMtools. Per genotype pools of unassembled reads were created by merging the reads from the individual replicates, which were then subject to a second round of assembly in order to capture lower abundance sequences. Predicted proteins were produced from all assemblies using

Prodigal version 2.6.3 [81], which were then clustered using MMseqs2 version 11.e1a1c [82] using the ‘easy_cluster’ method. Abundance of predicted proteins in each sample was determined by alignment of sequence reads against the representative cDNA sequences of the clusters using BWA MEM and determining the read counts associated with each sequence using a custom PySAM [83] script. Functional annotations, including GO and COG classifications, were obtained using EggnogMapper 2.0.1-14 [84] in conjunction with the Eggnog 5.0 database [85] using the ‘diamond’ method, a seed_ortholog_evalue parameter of 0.001 and seed_ortholog_score of 60. COG and GO terms were enumerated using a custom python script which assessed the number of occurrences of each term in each sample based upon the previously determined abundance of each annotated sequence. GO terms were mapped to the metagenomics GO slim subset dated 2020-03-23 [86] using the Map2Slim function of OWLtools [87]. Functional enrichment analysis was carried out using Limma 3.42.2 [88]. A presence-absence matrix of GO terms predicted at least once and enriched at a minimum of log fold-change 1 in the rhizosphere of each genotype was generated in R. This matrix was used to compute GO terms intersections and plotted using UpSetR [89]. The ternary plot was constructed with the function tern_E.R as previously described [90].

Table and Figure legend

GO	Function	Log2 Fold Change		
		Bulk-Desert	**Bulk-North**	**Bulk-Elite**
GO:0006282	Regulation of DNA repair	1.80	1.50	0.67
GO:0046718	Viral entry in host cell	1.80	1.80	1.80
GO:0071973	Bacterial-type flagellum-dependent cell motility	1.00	1.10	1.40
GO:0006808	Regulation of nitrogen utilisation	0.76	0.76	1.10
GO:0009372	Quorum sensing	0.72	0.57	1.00
GO:0009403	Toxin biosynthetic process	0.63	0.56	0.70
GO:0015979	Photosynthesis	-1.00	-1.10	-1.30

Table 1. Most divergent and conserved GO-slim categories at *P* value < 0.05 (Limma, FDR corrected) in the indicated pair-wise comparisons between the metagenomes of Bulk soil and the rhizosphere of the indicated barley genotypes. Positive fold changes denote rhizosphere enrichments, negative fold changes denote bulk soil enrichments.

Figure 1 Nitrogen availability drives bacterial composition in the barley rhizosphere. **A**) Canonical analysis of Principal Coordinates computed on Bray-Curtis dissimilarity matrices in the indicated samples and treatments **B**) R2 obtained from permutational analysis of variances computed in the entire dataset ('Compartment effect') or in rhizosphere samples only ('Host effect'). The factor 'Microhabitat' depicts either bulk soil or rhizosphere samples while the factor 'Genotypes' encompasses 'Desert', 'North' and 'Elite'. The factor 'Treatment' encompasses N0% N25% or N100%. Asterisks associated to the R2 value denote a significant effect (*P* value < 0.001, 5,000 permutations). **C**) Cumulative number of individual genera differentially recruited among genotypes at the three nitrogen levels (Wald test, *P* value < 0.05, FDR corrected). **D**) Taxonomic affiliation at a class level of the 31 genera exclusively recruited among genotypes at N0%. The size of the individual rectangles in the treemap is proportional to the number of genera assigned to the indicated class. Rectangle are colour-coded according to class' phylum affiliation (i.e., distinct colours denote distinct phyla).

Figure 2 Functional enrichments define the 'rhizosphere effect' on the microbiota of barley plants exposed to limiting nitrogen supply. Individual dots in the volcano plots depict individual GO-terms differentially enriched in the pair-wise comparison between the metagenomes of bulk soil and **A**) Desert, **B**) North and **C**) Elite genotypes exposed to N0% treatment, respectively. In each volcano plot the x-axis reflects the fold change (log2) of the enrichments, with positive fold changes assigned to rhizosphere enriched GO-terms and negative ones assigned to bulk-enriched GO terms, respectively. The y-axis defines the adjusted p-value (log10) assigned to each GO terms in the pair-wise comparisons. Coloured dots denote GO terms significantly enriched in rhizosphere samples (Limma, *P* value <0.05, FDR corrected) with a log2 fold change above 1.

Figure 3 The host-genotype fine-tunes the functional potential of the barley microbiota. A) Vertical bars denote the number of GO terms differentially enriched in the indicated rhizosphere metagenomes compared to unplanted soil samples unique for or shared among two or more comparisons highlighted by the interconnected dots underneath the vertical bars (Limma, *P* value < 0.05, FDR corrected; log10 Fold Change above 1). Numbers above the bars depict GO terms retrieved from the indicated set of comparison. Coloured vertical bars depict GO terms uniquely enriched in 'Desert' (yellow), 'North' (green) and 'Elite' (magenta), respectively. **B**) A ternary plot illustrating the distributions of COG categories in the indicated metagenomes. Each shape in the plot depicts an individual COG, whose position in the bi-dimensional space reflects its distribution in each of the indicated metagenomes. Coloured shapes depict COGs significantly enriched in either 'Elite' (magenta shapes) versus both wild 'North' and 'Desert' genotypes or (dark yellow shapes) (Wald test, *P* value <0.05, FDR corrected) for which the respective annotation is indicated in **C**). Note that in **B** shape size does not reflect its sequence abundance.

Figure 4 The enrichment of a phylogenetically diverse bacterial consortium is required for optimum barley growth. A) Above-ground biomass of 'Elite' barley plants sampled at early stem elongation in conditioned soil in either native or heat-treated form as indicated by colour-coding at the bottom of the figure. Dots depict individual biological replicates and letters denote significant differences at *P* value <0.05 in a two-way analysis of variance followed by a Tukey's post hoc test. **B**) Canonical analysis of Principal Coordinates computed on Bray-Curtis dissimilarity matrices in the samples indicated by shapes and color-coding at the bottom of the figure. **C**) Venn diagram illustrating the number of bacteria significantly enriched in and discriminating between rhizosphere of

plants grown in native, conditioned soil versus both unplanted soil controls and the cognate heat-treated conditioned soils (Wald test, *P* value <0.05, FDR corrected). **D**) Cumulative abundances, expressed as number of sequencing reads, assigned to each of the bacteria significantly enriched in and discriminating between rhizosphere of plants grown in native, conditioned soil versus both unplanted soil controls and heat-treated conditioned soils (Wald test, *P* value <0.05, FDR corrected). Each vertical bar corresponds to an individual biological replicate of a sample/treatment depicted underneath the graph. Each segment in the vertical bar depicts the sequencing reads assigned to an individual bacterial genus, color-coded according to its affiliation at class level.

Additional files

Additional file 1:

Table S1: Chemical and physical characteristic of the 'Quarryfield' soil used in this study. **Table S2**: Composition of the nutrient solutions used in this study. The solution was applied with watering of the plants at a rate of 25 ml of the nutrient solution per Kg of soil. *Micronutrients concentration: 6 µM $MnCl_2$, 23 µM H_3BO_3, 1.6 µM $CuSO_4$, 0.6 µM $ZnSO_4$, 1 µM Na_2MoO_4, 1 µM $CoCl_2$. **Table S3:** Spearman's rank correlations computed between the average relative abundances (phylum level) of the communities retrieved from unplanted soil samples and unplanted soil amended with "water agar plugs". **Table S4**: Cumulative assembly statistics for the metagenomes of the four genotypes of this study. **Table S5:** Taxonomic assignment of the genera significantly enriched in rhizosphere of plants exposed to native soil in the plant-soil feedback experiments and relatedness with genera enriched in 'Elite' rhizosphere at N0% (Wald test, *P* value <0.05, FDR corrected). **Figure S1**: Barley plants at the time of sampling. Genotypes and N treatments are arranged as rows and columns, respectively, for data recording. Top row 'Elite', middle row 'North' bottom row 'Desert' genotypes. From left to right: first column 100%N treated plants, second column 25%N treated plants, and third column 0% N treated plants. **Figure S2**: Nitrogen availability impacts on above- and below-ground traits. Cumulative data gathered at early stem elongation in wild barley genotypes, 'Desert' and 'North', and the 'Elite' variety at three nitrogen fertilization regimes (N0%, N25% and 100%) as indicated in the x-axis. Individual dots depict individual replicates. A) aboveground biomass of the tested plants. B) nitrogen content in the aboveground tissues of the tested plants. Residual concentration of C) ammonium and D) nitrate retrieved from rhizospheric soil at the time of sampling. Letters denote significant differences at *P* value <0.05 in a Kruskal–Wallis non-parametric analysis of variance followed by Dunn's post hoc test. **Figure S3**: Nitrogen

availability modulates the 'rhizosphere effect' in the barley microbiota. A) Cumulative number of individual genera significantly enriched in the rhizosphere of the three genotypes (Wald test, *P* value < 0.05, FDR corrected). D) Taxonomic affiliation at a class level of the 41 genera exclusively recruited among genotypes at N0%. The size of the individual rectangles in the treemap is proportional to the number of genera assigned to the indicated class. Rectangle are colour-coded according to class' phylum affiliation (i.e., distinct colours denote distinct phyla). **Figure S4**: Taxonomic affiliation at class level of the metagenomic reads assigned to bacteria and fungi. Individual bars in each plot depicts the individual biological replicates of the indicated samples whose color-coding reflect the proportion of metagenomic reads (within kingdom % of reads) assigned to the dominant A) bacterial or B) fungal classes. **Figure S5**: Metagenome assembly, annotation and functional enrichment workflow. **Figure S6**: Impact of sequencing depth on assembly metrics across samples. Individual dots depict individual replicates color-coded according to the metagenomic sample of origin. **Figure S7**: Impact of the heat treatment on soil chemical and physical parameters. Non-metric multidimensional scaling illustrating the relationships of soil samples from the indicated sample type. Arrows depict the most significant (R2 > 0.83, p <0.001) parameters explaining the ordination, namely the concentration of ammonium, phosphorus, manganese, sulphur, sodium, iron, copper, zinc and the pH. Arrows point at the direction of change while their length is proportional to the correlation between the ordination and the indicated variables. **Figure S8:** Bacterial and fungal DNA concentration in samples from the plant-soil feedback experiment. Boxplots depicting the logarithm of the concentration (fg/ul) of the A) bacterial or B) fungal DNAs retrieved from the metagenomic preparations of indicated samples. Individual dots depict individual biological replicated. Different letters denote significantly different groups (Kruskal–Wallis and post-hoc Dunn's test, *P* value ≤0.056).

Declarations

Abbreviations

16S Ribosomal subunit (S = Svedberg sedimentation coefficient = 10-13 s); ANOVA: Analysis of Variance; ASV: Amplicon Sequencing Variant; B1K: Barely 1K collection; CAP: Canonical Analysis of Principal coordinates; cm: Centimetres; COG: Clusters of Orthologous Groups of proteins; cv.: cultivar; FDR: False discovery rate; GO: Gene Ontology; ITS: Internal Transcribed Spacer; N: Nitrogen; N0%: Nutrient solution treatment at 0% Nitrogen; N25% Nutrient solution treatment at 25% Nitrogen; N100%: Nutrient solution treatment at 100% Nitrogen; NH^{+}_{4} : Ammonium; NO^{-}_{3} Nitrate; NTC: No-template

control; PCR: Polymerase Chain Reaction; PERMANOVA: Permutational multivariate Analysis of Variance; PHRED score: 'Phil's read editor', quality score for base pair in FASTQ files; QIIME: Quantitative Insights Into Microbial Ecology; qPCR: quantitative PCR; rRNA: ribosomal ribonucleic acid; TukeyHSD: Tukey Honest Significant Differences.

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Availability of data and materials

The sequences generated in the 16S rRNA gene sequencing survey and the raw metagenomics reads reported in this study are deposited in the European Nucleotide Archive (ENA) under the accession number PRJEB30847. Individual metagenomes are retrievable on the MG-RAST server under the IDs mgm4798244.3; mgm4798274.3; mgm4798349.3; mgm4798388.3; mgm4798507.3; mgm4798563.3; mgm4798641.3; mgm4798894.3; mgm4799467.3; mgm4799972.3; mgm4801514.3; mgm4801719.3.

The scripts used to analyse the data and generate the figures of this study are available at https://github.com/BulgarelliD-Lab/Barley-NT-2020

Authors' contribution

The study was conceived by RAT and DB with critical inputs from EP and LB. RAT, AMC, MEM and KBC performed the experiments. JM and PH generated the 16S rRNA sequencing reads. JA conceived and implemented the metagenomic analysis approach. RAT, SRA, AMC, CEM and DB analysed the data. All authors critically reviewed and edited the manuscript and approved its publication.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing Interests

The authors declare that they have no competing interests.

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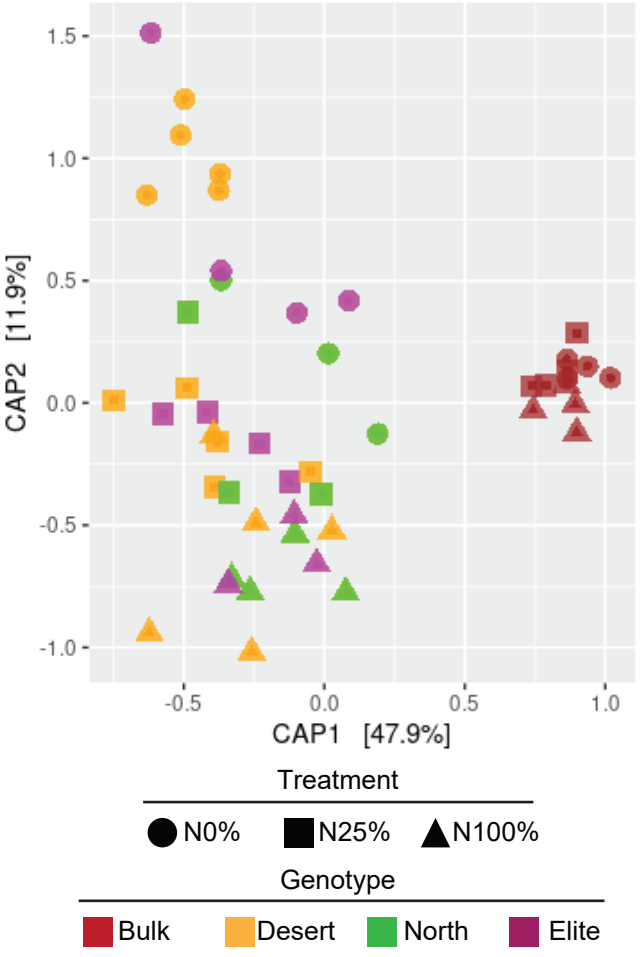
A

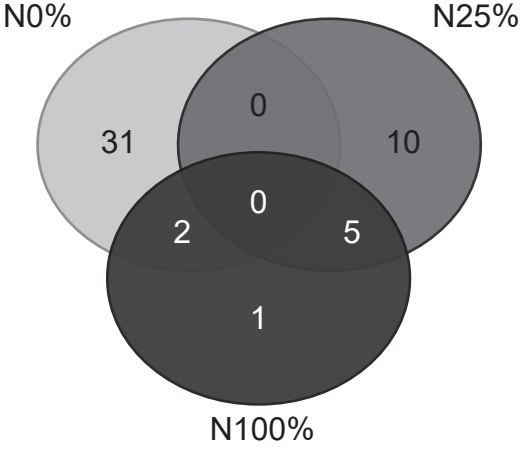


B

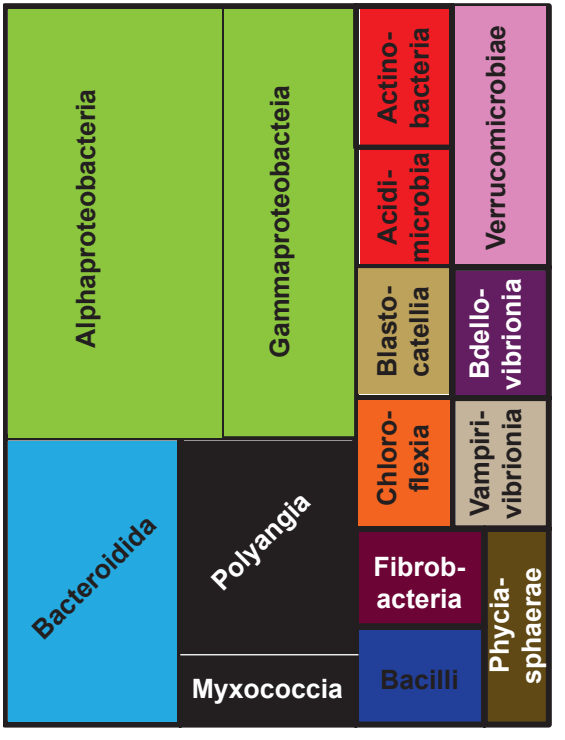
'Compartment' effect	R2
Microhabitat	0.47***
Treatment	0.10***
Treatment * Microhabitat	0.04

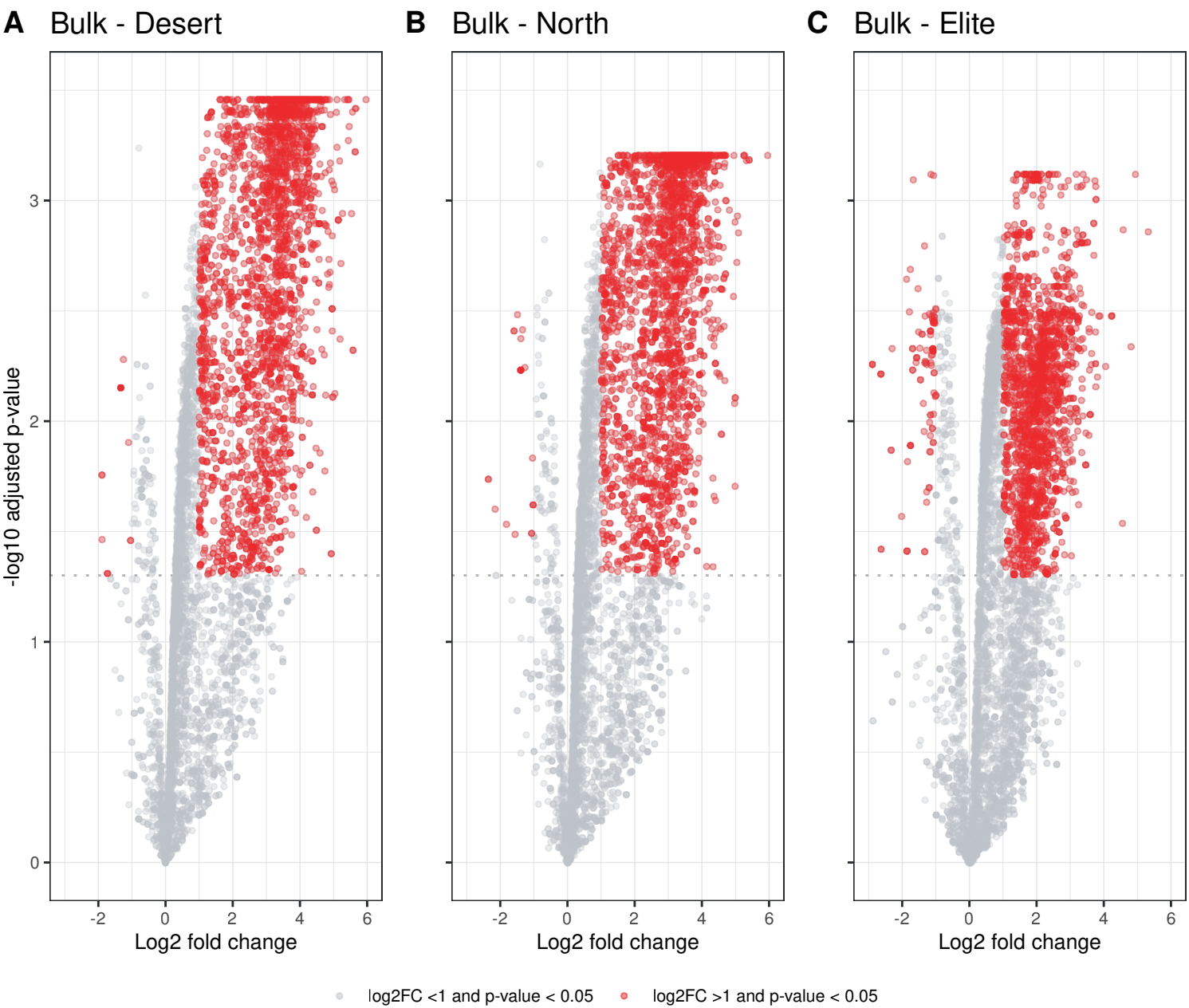
'Host' effect	R2
Genotype	0.14***
Treatment	0.25***
Treatment * Genotype	0.09

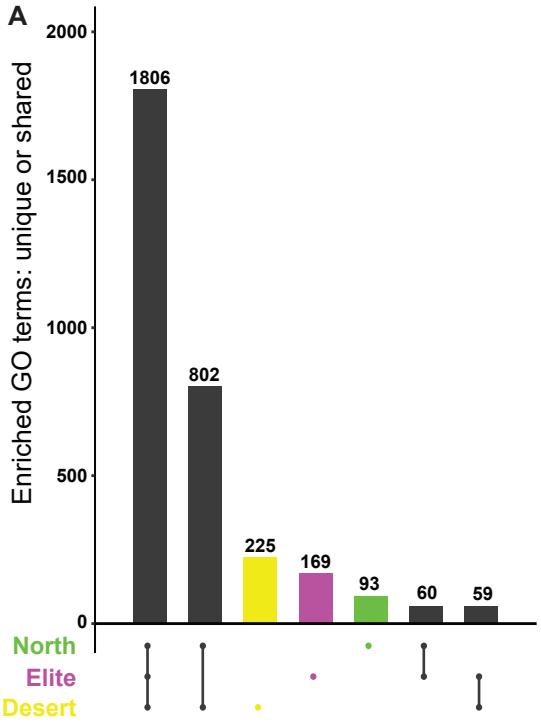
C

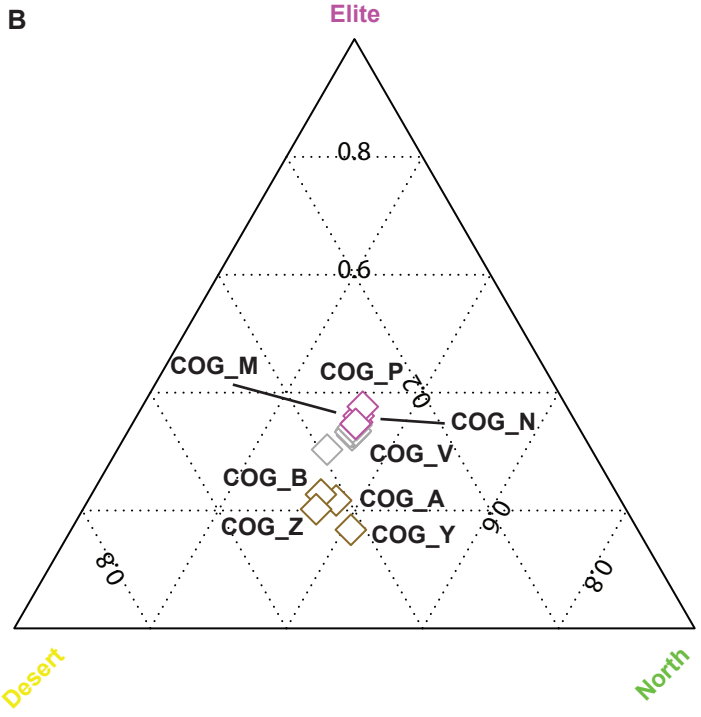


D



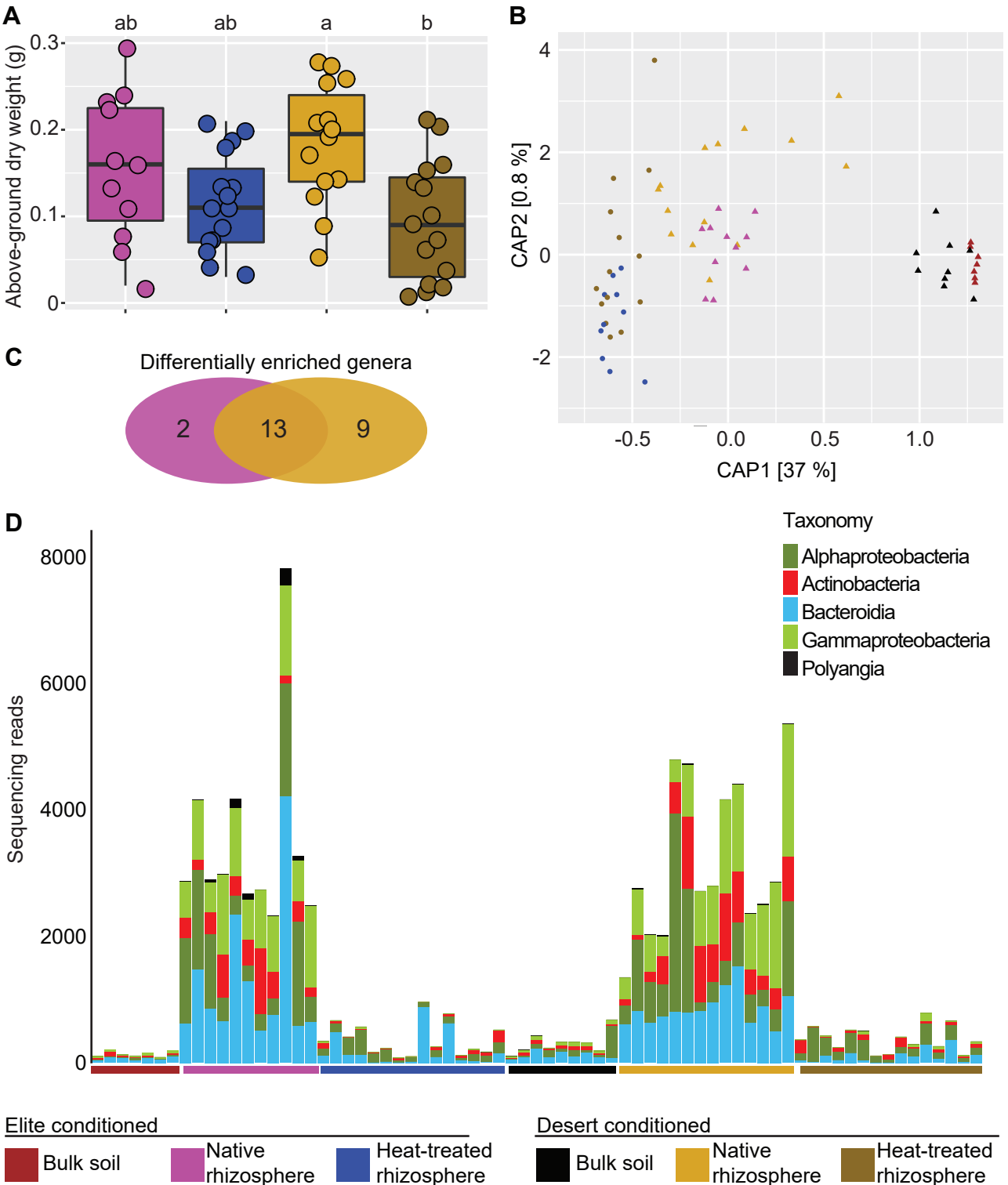

A
Bulk - Desert
B
Bulk - North
C
Bulk - Elite
-log10 adjusted p-value
0
1
2
3
-2
0
2
4
6
Log2 fold change
log2FC <1 and p-value < 0.05
log2FC >1 and p-value < 0.05





C

Wild enriched	Elite enriched
COG_A: RNA processing and modification	COG_M: Cell wall/membrane/envelope biogenesis
COG_B: Chromatin structure and dynamics	COG_N: Cell motility
COG_Y: Nuclear structure	COG_P: Inorganic ion transport and metabolism
COG_Z: Cytoskeleton	COG_V: Defense mechanisms


A
ab
ab
a
b
Above-ground dry weight (g)
0.3
0.2
0.1
0
B
CAP2 [0.8 %]
4
2
0
-2
-0.5
0.0
0.5
1.0
CAP1 [37 %]
C
Differentially enriched genera
2
13
9
D
Taxonomy
Alphaproteobacteria
Actinobacteria
Bacteroidia
Gammaproteobacteria
Polyangia
Sequencing reads
8000
6000
4000
2000
0
Elite conditioned
Bulk soil
Native rhizosphere
Heat-treated rhizosphere
Desert conditioned
Bulk soil
Native rhizosphere
Heat-treated rhizosphere