



## Research article

# Mixed organic and inorganic amendments enhance soil microbial interactions and environmental stress resistance of Tibetan barley on plateau farmland

Xiangyu Guan<sup>a</sup>, Zhen Cheng<sup>a</sup>, Yiqiang Li<sup>a</sup>, Jinfeng Wang<sup>b,\*</sup>, Ruoyu Zhao<sup>a</sup>, Zining Guo<sup>a</sup>, Tingting Zhao<sup>a</sup>, Liying Huang<sup>c</sup>, Cheng Qiu<sup>c</sup>, Wenyu Shi<sup>b</sup>, Song Jin<sup>d,\*\*</sup>

<sup>a</sup> School of Ocean Sciences, China University of Geosciences (Beijing), Beijing, 100083, China

<sup>b</sup> College of Food Science & Nutritional Engineering, China Agricultural University, No. 17 Qinghuadong Road, Haidian District, Beijing, 100083, China

<sup>c</sup> Institute of Agricultural Quality Standards and Testing, Tibet Academy of Agriculture and Animal Husbandry Sciences, Lhasa, 850031, China

<sup>d</sup> Department of Civil and Architectural Engineering, University of Wyoming, Laramie, WY, 82071, USA



## ARTICLE INFO

## Keywords:

Environmental adaption  
Lignite-based soil amendment  
Organic fertilizer  
Soil microbiome  
Plateau farmland

## ABSTRACT

Sufficient crop yield while maintaining soil health and sustainable agricultural development is a global objective, serving a special challenge to certain climate-sensitive plateau areas. Despite conducting trials on a variety of soil amendments in plateau areas, systematic research is lacking regarding the influences of organic and inorganic amendments on soil quality, particularly soil microbiome. To our knowledge, this was the first study that compared the effects of inorganic, organic, and mixed amendments on typical plateau crop hulless barley (*Hordeum vulgare* L. var. *Nudum*, also known as “Qingke” in Chinese) over the course of tillering, jointing, and ripening. Microbial communities and their responses to amendments, soil properties and Tibetan hulless barley growth, yield were investigated. Results indicated that mixed organic and inorganic amendments promoted the abundance of rhizosphere microorganisms, enhancing the rhizosphere root-microbes interactions and resistance to pathogenic bacteria and environmental stresses. The rhizosphere abundant and significantly different genera *Arthrobacter*, *Rhodanobacter*, *Sphingomonas*, *Nocardioides* and so on demonstrated their unique adaptation to the plateau environment based on the results of metagenomic binning. The abundance of 23 genes about plant growth and environmental adaptations in the mixed amendment soil were significantly higher than other treatments. Findings from this study suggest that the mixed organic/inorganic amendments can help establish a healthy microbiome and increase soil quality while achieving sufficient hulless barley yields in Tibet and presumably other similar geographic areas of high altitude.

## 1. Introduction

Tibetan hulless barley (*Hordeum vulgare* L. var. *nudum*), also known as “Qingke” in Chinese and “Ne” in Tibetan, is one of the earliest crops known to be cultivated by humans (3600 years ago) on the Tibetan Plateau (Badr et al., 2000; Zeng et al., 2018). It has a robust tolerance to extreme environmental conditions such as drought, high salinity, cold, and high altitudes of 4000 m above sea level, including the Tibet Plateau (Chen et al., 2015; Mayer et al., 2012; Wang et al., 2020). Tibetan hulless barley is the main source of food and economic resources for local farmers, accounting for about 70% of grain crop in Tibet, as well as

playing a vital role in the agricultural ecosystem (Newman and Newman, 2006; Zeng et al., 2018).

Inorganic fertilizer use continues to increase in the Tibetan Plateau to improve crop yields. Although it can supplement and replenish vital nutrients such as nitrogen, phosphorus, and other elements in soil, excessive application of inorganic fertilizers can cause loss of soil organic matter, increase greenhouse gas emissions from soil and soil acidification, and contribute to nitrogen contamination in groundwater (Chen et al., 2018a; Guo et al., 2010; Liang et al., 2020). Organic fertilizers/amendments start to gain attention as an alternative to traditional farming practices (Mader et al., 2002). Farmland with

\* Corresponding author. No. 17 Qinghuadong Road, Haidian District, Beijing, 100083, China.

\*\* Corresponding author.

E-mail addresses: [wangjf@cau.edu.cn](mailto:wangjf@cau.edu.cn) (J. Wang), [sjin@uwyo.edu](mailto:sjin@uwyo.edu) (S. Jin).

organically-amended soils are known to have higher soil stability, better structure, and higher soil biological activities (Mader et al., 2000; Reganold et al., 2001). However, the disadvantage of organic soil amendments are low nutrient input per weight and slow-release characteristics. Studies have been conducted on combining inorganic fertilizer and organic soil amendments to maintain soil health and achieve higher crop yields (Jiang et al., 2019; Lv et al., 2020).

Soil microorganisms are essential for soil and plant health and viability (Finkel et al., 2020; Menno et al., 2016; Wei et al., 2015). Studies indicate organic/inorganic amendments (fertilizers) significantly increase the abundance, diversity, and composition of soil microbial communities (Lazcano et al., 2013; Qiu et al., 2012), where bacteria are significantly affected by amendment/fertilizer type, and fungi only respond to specific amounts of amendments/fertilizer. Organic amendments/fertilizers have been demonstrated to increase the relative abundance of functional taxa involved in heterotrophic and nitrogen fixation functions when compared to inorganic fertilizer treatments (Gu et al., 2019). Different types of soil amendments also have an impact on interactions between microorganisms. For example, addition of organic fertilizer can reshape the microbial community and fortify resistance of wheat to soil-borne disease (Chen et al., 2019; Dong et al., 2020). And Xia et al. reported that the diversity of microbial communities in the rhizosphere decreased inversely with the level of inorganic nitrogen fertilizer (Xia et al., 2021). Past studies were mostly conducted under greenhouse conditions or in the field in geographic areas with mild climates.

Studies on the impact of organic and inorganic amendments on soil in Tibet, including its relationship to the rhizosphere and microorganisms at different growth stages, are lacking (Table S1). This study was conducted on the hulless barley farm in Tibet. Key parameters included: 1) impacts of organic and/or inorganic amendments on abundance, composition, function, and interactions of bacterial and fungal communities in the rhizosphere during three key growth stages (tillering, jointing, and ripening); and 2) how these interactions and impacts affect hulless barley growth and yield in Tibet.

## 2. Materials and methods

### 2.1. Tillage experiment and sample collection

The field tillage experiment was conducted at the Institute of Agricultural Quality Standards and Testing, Tibet Academy of Agriculture and Animal Husbandry Sciences, Southern Agricultural Experiment Station of Nedong District, Shannan City, Tibet, China (28°57'N, 91°54'E, elevation 3967.3 m) (Fig. S1a). The test plot has a temperate monsoon climate, average annual temperature of 8.8 °C, atmospheric pressure of 660.4 hPa, solar radiation of 6018.9 MJ, and a precipitation of 383.2 mm.

Tibetan hulless barley was planted from mid-April to early September 2020 with a growth period of about 135 days. The soil treatments consisted of three different amendment schemes: (1) DAP, inorganic fertilizer amendment; (2) ORG, organic soil amendment; and (3) MIX, a mixture of the inorganic fertilizer and organic soil amendments. The inorganic fertilizer was diammonium phosphate (Guizhou Kailin Group Public Co., Ltd., China), which was applied at a rate of 0.10 metric ton per hectare (mt/ha) for treatment (1). The organic soil amendment was a commercially available biochemically processed lignite called Ginate (Apaxfon Baotou BioScience and Technologies Co., Ltd., China), which was applied at a rate of 15 mt/ha for treatment (2). Ginate organic soil amendment is primarily composed of slow-releasing intermediate organic compounds, mineral-associated organic matter (MaOM), and to lesser extent readily bioavailable short-chain organic compounds. This organic soil amendment was selected for its field performance in improving plant/crop growth as well as containing no pathogens, residual antibiotics, and weed seeds (which are known to be present in manures and manure composts). The MIX treatment (i.e.,

treatment 3) consist of the diammonium phosphate and ginate organic soil amendment, applied at rates of 0.05 and 7.5 mt/ha, respectively.

Each treatment was applied to separate fields (2 m × 5 m) with 1-m intervals between each field; Tibetan hulless barley was planted in each field. At each growth stage: tillering (mid-May), jointing (early July), and ripening (mid-August), six hulless barley plants were randomly sampled in each treatment (Fig. S1b). Sampling also included soil and rhizosphere (root zone) samples (the sampling time was based on that it didn't rain seven days before sampling). All samples were placed in an ice box immediately after collection and taken to the laboratory within 12 h. The soil within 2 mm from the root surface was collected to serve as rhizosphere samples after gently shaking the roots to remove loosely attached soil clumps and brushing (DeAngelis et al., 2009). The bulk soil was used as soil samples after removing large particles and plant residues. A total of 216 samples including 108 rhizosphere soil and 108 bulk soil samples were obtained. Plant height was measured for each sampling stage and the crop was harvested in early September to measure thousand-grain weights and seed numbers per plant.

### 2.2. Soil chemical analysis

The soil samples were dried at 85 °C for 48 h and weighed to calculate the soil moisture (SM) content before being crushed and passed through a 2-mm sieve. Physical and chemical parameters of the soils, including pH, total phosphorus (TP), available phosphorus (AP), total potassium (TK), available potassium (AK), total nitrogen (TN), ammonia (NH<sub>4</sub><sup>+</sup>), nitrate (NO<sub>3</sub><sup>-</sup>), and nitrite (NO<sub>2</sub><sup>-</sup>) were measured using standard soil testing procedures. Soil base mineral ions (Si, Al, Fe, Ca, Na, Mg, As, Cr, Cu, Mn and Zn) were confirmed by using inductively coupled plasma optical emission spectroscopy (ICP-OES, Spectro Blue, Germany). The soil total organic carbon (TOC) content was confirmed by using a high-frequency infrared carbon-sulfur analyzer (LECO CS744, Leco Corporation, Saint Joseph, MI, USA).

### 2.3. DNA extraction, qPCR, and shotgun metagenomics sequencing

DNA was extracted using E. Z.N.A.® soil DNA Kit (Omega Bio-tek, Norcross, GA, USA) from 0.5 g soil. The concentration and purity of DNA was found using 1% agarose gel and UV-Vis spectrophotometer (NanoDrop 2000; Thermo Scientific, Wilmington, DE, USA). Genomic DNA was stored in a -80 °C freezer before subjected to high-throughput sequencing. Primer pairs 338 F (5'-ACTCCTACGGGAGGCAGCAG-3') and 806 R (5'-GGACTACHVGGGTWTC TAAT-3') and the primer pair ITS1F (5'-CTTGGTCAATTTAGAGGAAGTAAA-3') and ITS2R (5'-GCTGCGTTCTTCATCGATGC-3') were used for qPCR, gene amplification and sequencing of region V3-V4 of the bacterial 16 S rRNA gene and the ITS sequence, respectively. qPCR for 16 S rRNA and ITS genes was performed on an ABI Prism 7500 (Applied Biosystems, Foster City, CA, USA) with a reaction mixture containing 10 µL of ChamQ SYBR Color qPCR Master Mix (2X) (Vazyme Biotech Co, Ltd., China), 1 µM of each primer, ~10 ng of total DNA, and ample RNase-free water to obtain a 20 µL volume. Standard curves were obtained with serial dilutions of a known amount of pMD18-T plasmid DNA containing a fragment of the target genes. Standard curves for 16 S rRNA and ITS genes were based on serial dilutions of target genes (between 10<sup>2</sup> and 10<sup>8</sup> copies/µL). These numbers were calculated from the concentration of extracted plasmids measured with a Qubit 3.0 Fluorometer (Thermo Fisher Scientific, Carlsbad, CA, USA).

The 16 S rRNA and ITS genes amplification instrument are ABI GeneAmp® 9700 PCR thermocycler (ABI, Foster City, CA, USA) under the following conditions: 94 °C for 3 min, followed by 35 cycles of 94 °C for 30 s, 56 °C for 30 s and 72 °C for 40 s, and 72 °C for 10 min. The PCR mixtures contained 5 × TransStart FastPfu buffer 4 µL, 2.5 mM dNTPs 2 µL, forward primer (5 µM) 0.8 µL, reverse primer (5 µM) 0.8 µL, TransStart FastPfu DNA Polymerase 0.4 µL, template DNA 10 ng, and ddH<sub>2</sub>O to achieve a final volume of 20 µL. PCR reactions were performed

in triplicates. The PCR product was extracted from 2% agarose gel and purified using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA) according to the standard instructions and quantified using a Quantus Fluorometer (Promega, Madison, WI, USA). The final amplicon obtained in the same molar amount of amalgamate and paired-end sequencing ( $2 \times 250$ ) was subsequently performed, using the Illumina MiSeq PE300 platform, in Shanghai Majorbio Bio-pharm Technology Co., Ltd., China.

DNA of each three out of six rhizosphere soil samples were pooled, and two pooled samples from each of the three treatments in ripening stage were used for metagenomic sequencing. For construction paired-end library, DNA was fragmented to an average size of about 300 bp using Covaris M220 Focused ultrasonicator (Covaris, Woburn, MA, USA) according to standard instructions for metagenomic sequencing. Paired-end library was prepared by using the TruSeq DNA Sample Prep Kit (Illumina, San Diego, CA, USA). Adapters containing the full complement of sequencing primer hybridization sites were ligated to the blunt-end fragments. Paired-end sequencing was performed on the Illumina HiSeq3000 platform (Illumina Inc., San Diego, CA, USA), using HiSeq 3000 PE Cluster Kit and HiSeq 3000 SBS Kits according to the manufacturer's instructions.

#### 2.4. Sequencing data analysis

The amplicon sequencing data preprocessing was performed using Quantitative Insights Into Microbial Ecology (QIIME, version 1.9.1) software. The raw 16 S rRNA gene and raw ITS rRNA gene sequencing reads were demultiplexed, quality-filtered by Fastp (version 0.20.0) (Chen et al., 2018b), and merged by FLASH (version 1.2.11) (Magoc and Salzberg, 2011) with the following criteria: (i) the 300 bp reads were truncated at each site, receiving an average quality score of  $<20$  over a 50 bp sliding window while truncated reads shorter than 50 bp or containing ambiguous characters were discarded (ii) only overlapping sequences longer than 10 bp were assembled according to their overlapped sequence. The maximum mismatch ratio of the overlap region is 0.2. Reads that could not be assembled were discarded; (iii) Samples were distinguished according to the barcode and primers and the sequence direction was adjusted for exact barcode matching and 2 nucleotide mismatches for primer matching.

The high-quality data was clustered into operational taxonomic units (OTUs) at a 97% similarity (Edgar, 2013) with UPARSE (version 7.1, <http://drive5.com/uparse/>) and chimeric sequences were identified and removed. The taxonomy of each OTU representative sequence was analyzed by QIIME using a confidence threshold of 0.7 and the UNITE fungal ITS database with min-confidence of 0.75.

The metagenomic sequencing reads were first trimmed using Fastp (version 0.20.0) before being assembled into contigs using metaSPAdes (Nurk et al., 2017) with default parameters. For each assemblies set, metagenome-assembled genomes (MAGs) were recovered by using MetaBAT2 (Kang et al., 2019), MaxBin2 (Wu et al., 2016) and CONCOCT (Alneberg et al., 2014). All MAGs were pooled and de-duplicated using VAMB (Nissen et al., 2021). As the final step of metagenomic binning, bin\_refinement module of MetaWRAP pipeline was used to improve the quality of all the MAGs (Uritskiy et al., 2018). CheckM was used to estimate the completeness and contamination of MAGs. Total 404 MAGs were retained under the criterion  $>70\%$  completeness and  $<10\%$  contamination. Taxonomic assignment of MAGs was performed using GTDB-tk (Chaumeil et al., 2019).

Gene prediction using PROKKA for these scaffolds and the predicted proteins were assigned to the KO using the KEGG Automatic Annotation Server (Seemann, 2014). Trimmed high-quality reads locating on the given scaffolds were counted to calculate the abundance of KOs in each sample using Burrows-Wheeler Aligner (BWA) (Li and Durbin, 2009). The matrix was normalized by dividing the absolute amount of each functional gene by the total number of reads assigned to functional genes in each sample.

#### 2.5. Statistical analyses and visualization

Statistical analysis of all data was performed using different software packages in R 3.6.2 for analysis and visualization (Team, 2015). Bray-Curtis distances were calculated using the "vegdist" of the R "vegan" package, A PCoA (principal coordinate analysis) ordination plot was then implemented using the "cmdscale" package.  $\beta$  diversity as well as the similarity of sample subgroups and its impacts on the microbial community was tested using the "Adonis" package. The "randomForest" package was used to evaluate soil properties and microbial metabolic processes sensitive to soil amendments; predictor variables were tested for significance with the "rfPermute" package. Wilcoxon rank-sum tests were performed using the "compare means" of the "ggpubr" package for soil properties, microbial biomass, and samples of Tibetan hulless barley yields for different soil amendment treatments. Mental correlation test of soil properties with bacterial and fungal communities was implemented using the "ggcor" package. In order to identify which microbes responded significantly to fertilizers, we performed indicator species analysis using the "multipatt" of the "indicspecies" package (De Caceres et al., 2016).

The "Hmisc" package was used to calculate the correlations between different grouped OTUs and Gephi (version 0.9.1, <https://gephi.org/>) was used to visualize the network patterns of different groups with significantly strong correlations ( $\rho > 0.6$ ,  $p < 0.05$ ). Topology data of networks was counted. The nodes with the top 1% connectivity were defined as keystone taxa.

In order to systematically understand the direct and indirect effects of fertilizers on microbial communities and plant production, partial least squares path modeling (PLS-PM) for different treatments were constructed using the "plsppm" package. All factor loadings in the model are  $>0.7$ , including soil properties, diversity of rhizosphere and bulk microbial communities, and traits of growth. PLS-PM path coefficients of three different fertilization groups are combined into a structural equation modeling structure (SEM). The "ggpmisc" package was used for regression analysis and visualization.

### 3. Results

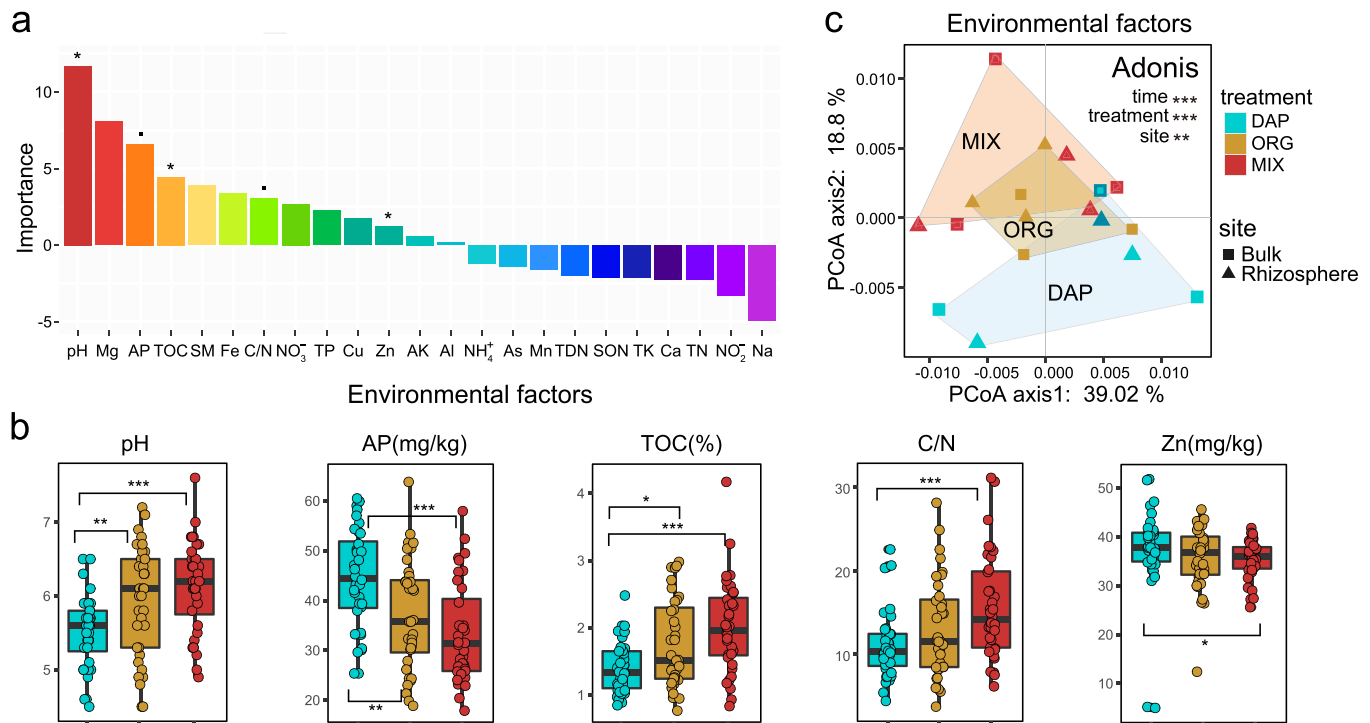
#### 3.1. Influences of soil amendments on soil properties

The Tibetan hulless barley growth cycle was completed at approximately 135 days, during which physicochemical properties of soils from different amendment treatments varied significantly (Fig. 1 and Table S2). Random forest analysis identified that 5 soil physicochemical factors: pH, AP, TOC, C/N ratio, and Zn corresponded strongly to soil amendment treatment types (Fig. 1a). The TOC and pH were significantly higher in MIX and ORG groups than the DAP group. The C/N ratio was significantly higher in MIX group than the DAP group. AP was significantly higher in the DAP group compared to the other two groups and Zn was significantly higher in the DAP group compared to the MIX group (Fig. 1b).

PCoA analysis indicated that there were significant differences ( $P < 0.001$ ) between the DAP, MIX, and ORG treated soil groups, three growth stages, and bulk and rhizosphere soils ( $P < 0.01$ ) (Fig. 1c). During the three growth stages,  $\text{NO}_2^-$  was significantly different in DAP, MIX, and ORG treated soils. TOC and metal elements such as Fe, Al, and Zn changed significantly between DAP and MIX soil groups (Table S3). Additionally,  $\text{NO}_3^-$ ,  $\text{NH}_4^+$  and TDN were significantly higher in rhizosphere than that of bulk soil (Table S4).

#### 3.2. Different soil amendments on farmland soil bacterial and fungal communities

During the hulless barley growth stage, the rhizosphere microbial biomass in the DAP treated soil decreased, and the microbial biomass in the ORG treated soil increased in contrast. Soil rhizosphere bacteria and



**Fig. 1.** Soil physicochemical parameters in soils from the Tibetan hulless barley farmland where the inorganic (DAP), organic (ORG), and mixed inorganic-organic (MIX) soil amendment treatments were applied. **a.** Soil physicochemical predictors responses to soil amendment treatments determined by random forest analysis (\*,  $p < 0.1$ ; \*,  $p < 0.05$ ). **b.** Soil physicochemical factors (parameters) and responses to different soil amendments (Wilcoxon rank-sum test \*,  $p < 0.05$ , \*\*,  $p < 0.01$ , \*\*\*,  $p < 0.001$ ). **c.** Principal coordinate analysis of soil physicochemical factors (parameters) for all soil samples. The data of six parallel samples was averaged and visualized for a clear display.

fungi increased the most in MIX treatment group (50.7% and 65.4%, respectively) (Fig. 2a). The rhizosphere bacterial and fungal biomasses were significantly higher compared to bulk soil. During the ripening stage, the difference between the bulk and rhizosphere bacteria biomass in MIX treated soil was approximately  $9 \times 10^9$  copies·g<sup>-1</sup>, and other changes in microbial biomass did not exceed  $3 \times 10^9$  copies·g<sup>-1</sup>. Changes in fungal biomass were minimal (Fig. 2a).

Through 16 S rRNA and ITS gene sequencing of 108 soil samples, 6877 bacterial OTUs and 2597 fungal OTUs were obtained. The diversity of microbial communities in the rhizosphere of MIX amendment increased (Fig. S2). The diversity of microbial communities, particularly for bacteria in bulk soil has a higher level of response to soil amendments compared to rhizosphere microbial communities.

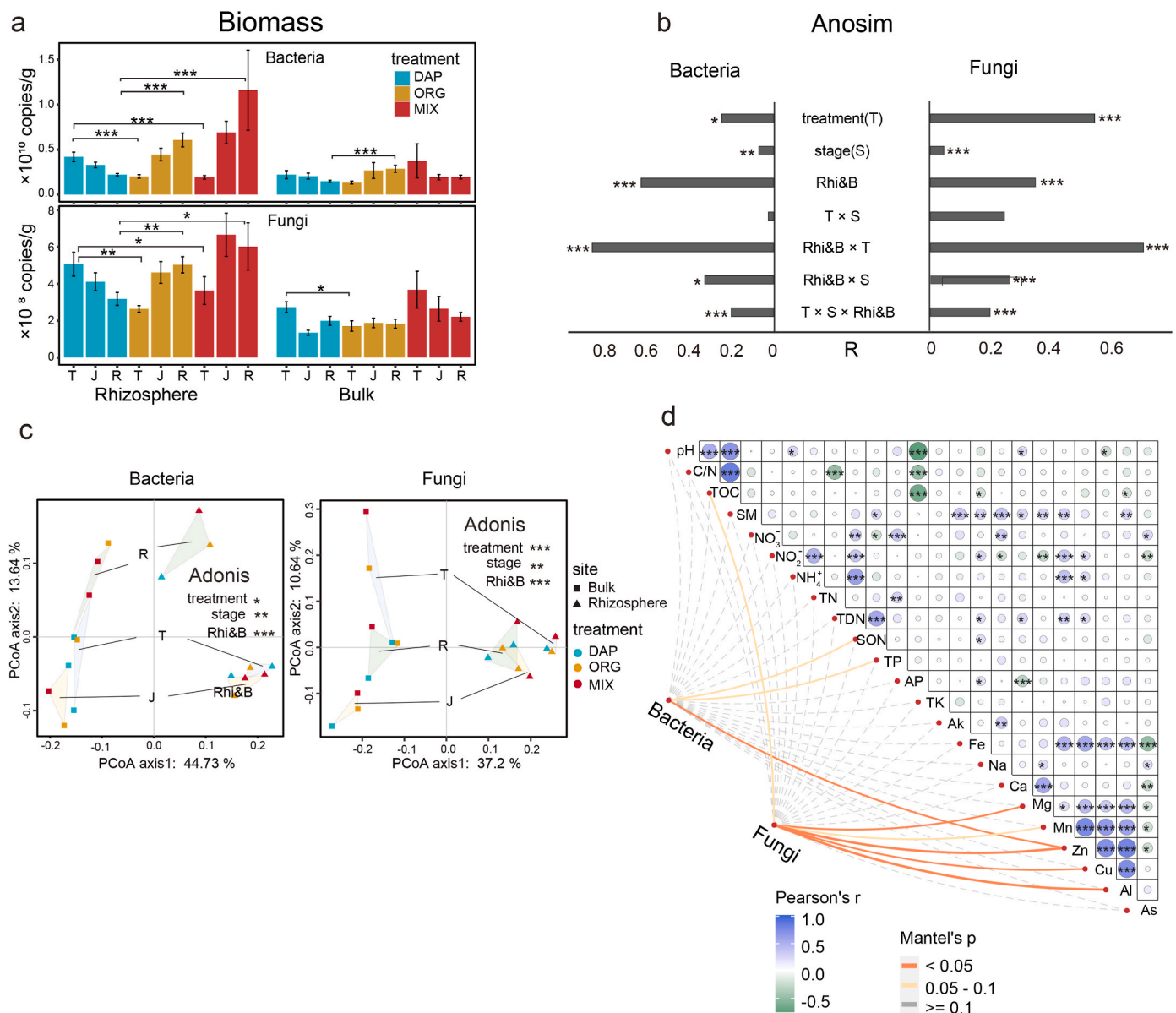
The highest differences are shown between different soil amendment treatments, sampling locations in bulk and rhizosphere soils and stages of Tibetan hulless barley growth (Fig. 2b). PCoA further identified the effect of different soil amendments on bacterial and fungal communities (Fig. 2c). Both bacterial and fungal communities were separated into different groups based on treatment, time, and site characteristics. Relative to DAP treated soils, rhizosphere bacteria communities are similar between ORG and MIX treated soils. The composition of the bacteria community was correlated with SON and TP and the fungi community with TOC and trace metal elements (Mg, Cu, Al, and Mn) (Fig. 2d). Both bacteria and fungi communities were possibly influenced by TOC, SON, TP, Zn, and Mg in soil (Fig. 2d). These environmental factors are consistent with the analysis of soil physicochemical factors (Fig. 1a).

The dominant bacteria phyla are *Actinobacteriota*, *Bacteroidota*, *Firmicutes*, and *Proteobacteria*, and dominant fungi phyla are *Ascomycota*, *Basidiomycota*, and *Mortierellomycota*. At the genus level, the relative abundance of top 20 dominant bacteria are *Arthrobacter*, *Sphingomonas*, *Rhodanobacter* and so on, and dominant fungi genera are *Gibberella*,

*Didymella*, *Plectosphaerella* and so on (Fig. S3). The number of significantly changed microbes in rhizospheric soil was lower than that of bulk soil due to plant growth and soil amendment impacts on the microbiome and rhizosphere. A total of 210 bacterial OTUs (1.76% of total soil community sequences) and 121 fungal OTUs (3.95% of total soil community sequences) were significantly altered ( $p < 0.05$ ) by soil amendment treatments across growth stages. For bulk soil, a total of 247 bacterial OTUs (3.54% of total soil community sequences) and 131 fungal OTUs (3.51% of total soil community sequences) were identified ( $p < 0.05$ ) response to fertilizer treatments (Fig. S4). *Arthrobacter*, *Microbacterium*, *Planomicrobium*, *Nocardioidea* and so on were the major differential genera. Fungal genera identified as indicator species are represented by *Saprotraph* and other guilds.

### 3.3. Soil amendment effects on microbial co-occurrence patterns

The microbial community of the MIX treated soil has the most complex microbial co-occurrence pattern with the highest node and edge counts as well as clustering coefficient, and it has the lowest ratio of negative to positive connections (Fig. 3a-b and S5). The percentage of bacterial connections was dominant (>80%). The connections between bacteria and fungi were >10%, and connections between fungi were 1%–2%. The MIX treated soil had most bacterial-fungal connections, accounting for 16.4% of the total number of connections (Fig. 4b–d). Most nodes species are present in MIX treated soil bacteria phylum *Actinobacteriota* and fungus phylum *Ascomycota* are dominant (Fig. 3c–e). The MIX treated soil has the highest average number of important species connections, followed in descending order by the ORG treated soil with the DAP treated soil possessing the least connections (Fig. 3c and d).



**Fig. 2.** Microbial communities of inorganic (DAP), organic (ORG), and inorganic and organic mixed (MIX) soil amendments in Tibetan hulless barley farmland soil. **a.** The bacteria and fungi biomass. **b.** Anosim analysis for the microbial community in rhizosphere (Rhi) and bulk (B) soil samples for the three Tibetan hulless barley growing stages for three r treatments. **c.** Principal coordinate analysis (PCoA) with Adonis (Permutational multivariate analysis of variance, PERMANOVA) tests for rhizosphere (Rhi) and bulk (B) soil samples in tillering (T), jointing (J) and ripening (R) growth stages of three treatments. The data of six parallel samples was averaged and visualized for a clear display. **d.** Correlation analysis of soil physicochemical factors with mantel tests conducted between bacterial and fungal communities (\*,  $p < 0.05$ , \*\*,  $p < 0.01$ , \*\*\*,  $p < 0.001$ ).

**3.4. Correlation between microbial communities and soil amendment treatments**

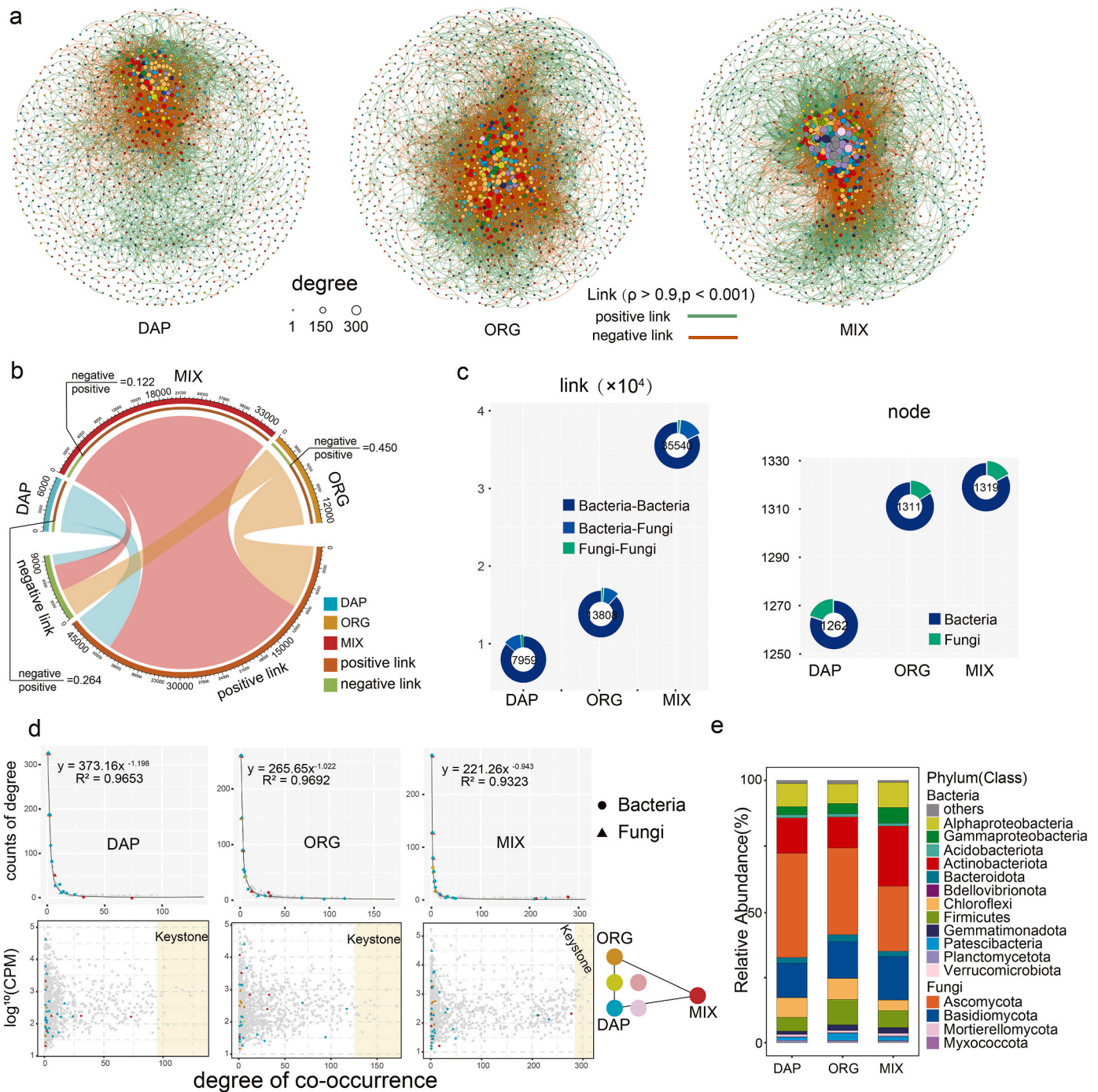
Plant traits showed that there was no significant difference in plant heights across soil amendment treatments during the tillering stage, but plant heights from DAP soil amendments were significantly lower compared to the other soil amendment treatments during the jointing and ripening stages (Fig. 4a). In addition, thousand Grain Weight (TGW) among the three soil amendment treatments were ranked MIX > ORG > DAP, and a similar trend was observed for number of plants (Fig. 4a). The estimated yield was highest in the ORG treatment with no significant difference between DAP and MIX treatments. The MIX treatment achieved the most optimal growth and crop yield (546, 973 kg/km<sup>2</sup>) (Fig. 4a).

The PLS-SEM model showed that the effect of soil amendment

treatments on soil pH was most observable among environmental factors (Fig. 4b). The analysis interpretation (R<sup>2</sup>) of soil organic matter sources show that MIX treatment had the most significant positive effect on the increase of soil organic matter. The growth of bacteria and fungi was positively affected by soil organic matter. Soil properties (pH, inorganic matter, organic matter) had a stronger effect on the rhizosphere bacterial community than fungi community. Bacteria are more sensitive to fertilization than fungi during plant growth. The MIX treatment contributed the most to changes in soil organic concentration for the microbiome as well as the growth and yield of Tibetan hulless barley.

**3.5. Relationships between the metabolic functions of Tibetan hulless barley rhizosphere microbiome and environmental adaptation**

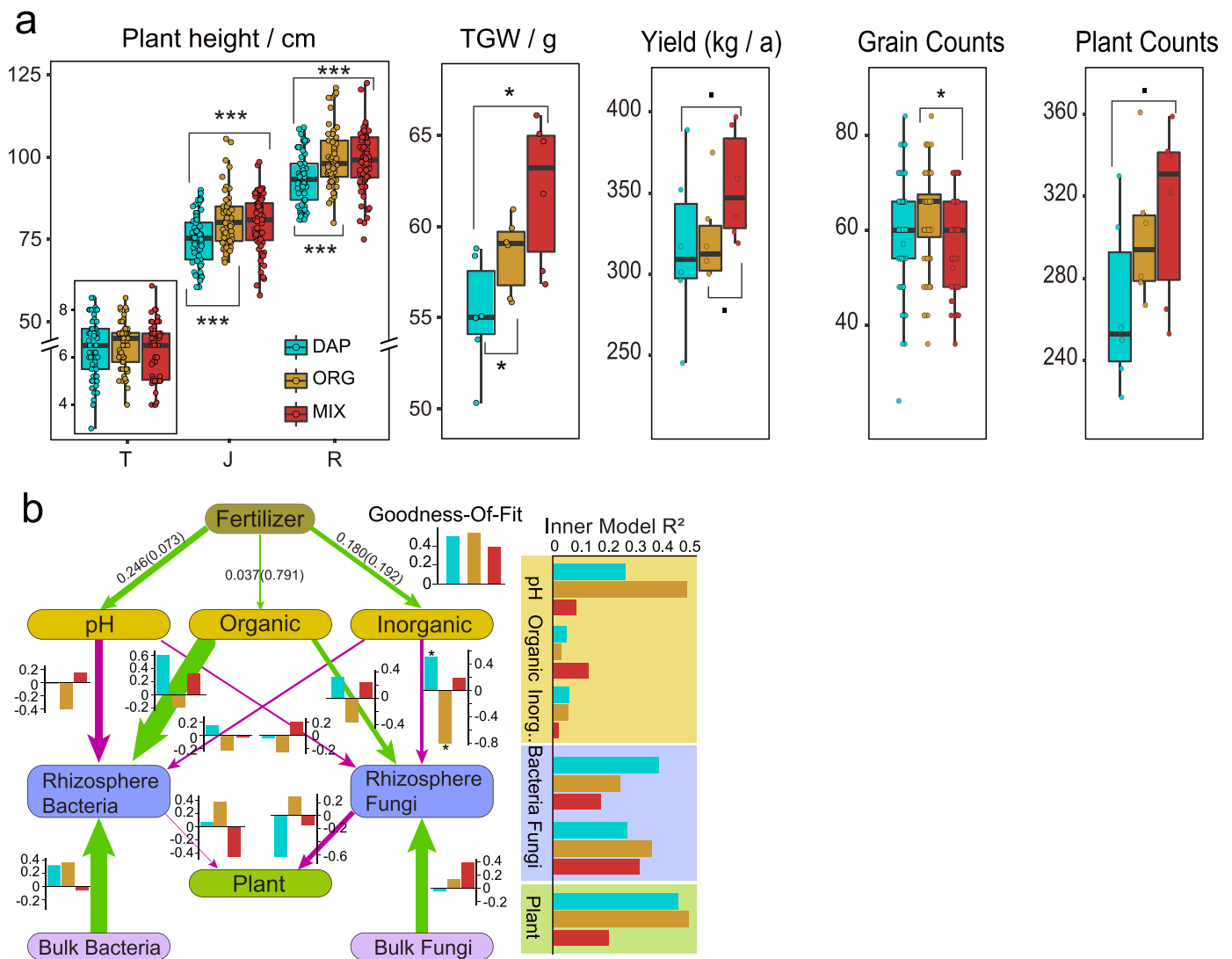
The metagenomic data was effectively assembled in 18 relative high



**Fig. 3.** Interaction patterns of rhizosphere microbial communities from Tibetan hullless barley farmland soil with different soil amendment treatments. **a.** Co-occurrence network of abundant OTUs (>0.1%) and soil amendment indicator OTUs (<0.1%) in the rhizosphere microbial community of inorganic (DAP), organic (ORG), and inorganic and organic mixed (MIX) soil amendment treatments ( $\rho > 0.9, p < 0.001$ ); orange lines mean positive correlations between bacteria, green lines mean negative correlations between bacteria), the color of node OTUs represents different phyla, and the size represents connectivity. **b.** The proportion of positive and negative connections of bacteria in three soil amendment treatments. **c.** Connections between fungi and bacteria across three soil amendment treatments based on the connectivity (top 20) in co-occurrence networks. **d.** Keystone bacteria and fungi across three soil amendment treatments based on the connectivity (top 20) in co-occurrence networks. **e.** Taxon classification of nodes at the phylum level within networks across the three soil amendment treatments. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

quality draft MAGs such as *Arthrobacter*, *Rhodanobacter*, *Sphingomona*, *Nocardioidea* and *Mycobacterium*, which were also the abundant and differential genera among three treatments (Table S5, Fig. S3). In carbon cycle, chitin-degrading genes of carbon biodegradation pathway were identified, as well as a large variety of acetic acid fermentation genes. Carbon fixation such as glycerol utilization, pyruvate metabolism, formate metabolism and so on are all related to the rTCA cycle. In

nitrogen cycle, assimilation and dissimilatory nitrate reduction and glutamate synthesis are both related to the conversion of ammonia into organic nitrogen and an ammonia transporter. In sulfur and phosphorus cycles, assimilatory sulfate reduction consumes sulfate to synthesize sulfur-containing amino acids such as cysteine, and phosphorus metabolism includes inorganic phosphorus, polyphosphoric hydrochloride, phosphate, and organic phosphorus metabolism were found.



**Fig. 4.** The growth traits of Tibetan hulless barley and interaction relation factors of microbial communities and physicochemical factors of farmland soil with applied inorganic (DAP), organic (ORG), and inorganic and organic mixed (MIX) soil amendment treatments on the Tibetan Plateau. **a.** Plant height and thousand-grain weight (TGW). **b.** Partial least squares structural equation modeling (PLS-SEM) examined the impacts of soil amendment treatments on soil properties (pH, C/N, TOC), soil inorganic components (TN, NH<sub>4</sub><sup>+</sup>, TP, AP, AK nutrients), rhizosphere and bulk soil microbial communities (bacterial and fungal richness and Shannon index), and direct or indirect effects of hulless barley traits (the column chart on the right shows the coefficients of determination “R<sup>2</sup>” of the inner model). The width of the arrow describes the size of the average path coefficient (three types of fertilizer treatments), the green and red arrows represent positive and negative effects, respectively, and the bar graph represents the path coefficient of soil amendment treatment groups (\*, P < 0.05). The GOF (Goodness of Fit) represents the model prediction effect. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Additionally, some functional genes in rhizospheric microorganisms such as heat shock protein genes, genes related to heavy metal resistance, and genes that resist oxidative stress help plants adapt to harsh environments and promote plant growth as well.

Amendments promote changes in microbial abundance and significantly impact various metabolic functions such as carbon, nitrogen, phosphorus, and sulfur cycles (Fig. S6). Genes in higher abundance within the MIX treatment include fructose and mannose metabolism genes *mtkK*, *pjp*, and *mtlA* in the carbon degradation pathway, TCA cycle genes such as pyruvate metabolism genes *fumA* and *fumB*, and glyoxydicarboxylic acid metabolism genes *acnB* and *PCCA* in the carbon fixation pathway and acetate fermentation gene *aldH*. Additionally, *nrtC* and *narB* as well as amino acid metabolism-related genes were more abundant in the MIX treatment group. Sulfur-related sulfate-reducing genes *cysA* and *ssuD* are more abundant in the MIX treatment group. The abundance of nucleotide metabolism-related gene *xdhB*, cofactor and vitamin metabolism-related genes *cobN* and *cobIJ*, and terpenoid

metabolism-related gene *atuF* in the MIX group is higher compared to the other treatment groups.

#### 4. Discussion

Fertilization often changes soil properties, thereby altering the structure, interaction, and function of soil microbial communities (Tao et al., 2020; Xun et al., 2015). The increase of soil properties such as TOC and C/N ratio in ORG is attributable to the rhizosphere microorganisms that benefit plant growth by participating in the nitrogen fixation and carbon source acquisition (Philippot et al., 2013; Stokstad, 2016). The microbial biomass of the DAP treated soil peaked during the tillering stage and decreased following hulless barley growth (Fig. 2). In contrast, the microbial biomass in ORG and MIX amendments increased with crop growth. Inorganic fertilizers tend to be less stable in soils over time and are readily consumed, mobilized, and leached out. Organic soil amendments exhibit slow-release characteristics that last over a longer

period of time (Mader et al., 2002; Reganold et al., 2001). This long-term supply of organic carbon and nutrients optimally benefit and sustain microbial biomass growth and activities. Among the diversity of microbial communities, bacteria sensitively responded to soil amendments compared to fungi. This response was similarly observed in other studies connecting microbial community responses to various soil amendments (Guan et al., 2020; Wu et al., 2007). It was reported that bacterial metabolism altered nitrogen-phosphorus synergy while percentage of fungi increase with higher of organic carbon content (Cheng et al., 2020; Ren et al., 2020). Nitrogen and phosphorus were possible limiting factors for bacterial growth in Tibetan hulless barley farmland soil. Fungi were possibly impacted by the presence of TOC and trace metal elements in fertilizer.

Soil amendments enhanced the interaction of microbial community. The interaction of microbial community in MIX group became closer than other two groups (Fig. 4). This indicates that microbial cooperation is dominant, improving the metabolic efficiency of microorganisms and the utilization rate of nutrients by plant uptake as a result (Tao et al., 2018). The MIX group significantly increases the abundance of key species, major soil ecological groups in relation to key taxon, and improves interrelated interactions between microbes and their functionalities. These complex and tight interactions can enhance the utilization of resources and resistance to environmental stressors and disease, thus promoting the healthy growth of Tibetan hulless barley in an inarable plateau environment (Li et al., 2019; Shi et al., 2016; Tilman, 2004; Wei et al., 2015).

The health and quality of farmland is driven by soil physicochemical factors and the microbiome in rhizosphere. Through linking soil amendment treatments with soil physicochemical parameters, growth stages, and the microbiome, we can establish a relationship between the response model of the complex soil-microbe-crop system and Tibetan hulless barley (Fig. 4). Specific microbial groups from the soil around the root zone of Tibetan hulless barley, more specifically unique bacteria including *Arthrobacter*, *Microbacterium*, *Planomicrobium*, *Nocardioides* and so on were enriched. Previous studies on wheat or barley (*Hordeum vulgare* L.) include these genera *Arthrobacter*, *Devosia*, *Bacillus* and so on as significant PGPR (Bulgarelli et al., 2013; Pontes et al., 2015; Upadhyay et al., 2012). Compared to fungi, bacteria are more diverse in their response. However, fungi can more robustly maintain ecological plateau farmland systems. Rhizosphere microorganisms form complex interactions and are subjected to a dynamic process impacted by host type, plant growth environment, and physiological status (Philippot et al., 2013).

MAG coverage of the dominant and representative rhizosphere bacteria helps us to infer presence and absence of pathways and understand comprehensive metabolic functions of rhizosphere microorganisms. In carbon biodegradation pathway, chitin-degrading genes degrade inactive carbon in the soil, replenishing the supply of carbon sources under extreme environments and conditions (Beier and Bertilsson, 2013). There is increasing evidence that acetic acid production plays an important role in the organic carbon cycle in extreme microaerobic or anaerobic habitats (Utsumi et al., 2019). This cycle is prevalent in anaerobic bacteria that thrive in the low-oxygen conditions of the Tibetan Plateau. Nitrogen cycling driven by the rhizosphere microbiome is important for crop growth (Guan et al., 2020). Assimilation and dissimilatory nitrate reduction and glutamate synthesis in nitrogen cycle help plant roots absorb ammonium nitrogen (Palatinszky et al., 2015; Yuan et al., 2007). A consistent input of ammonia will be crucial for a thriving microbial community. Sulfur-containing amino acids such as cysteine can be degraded and catalyzed by cysteine desulfhydrase to generate hydrogen sulfide, pyruvate, and ammonia-all of which possess regulatory effects on plant growth (Al Ubeed et al., 2019; Yuan et al., 2015). Energy is released, promoting plant resistance to harsh environmental stressors and inhibitors to growth (Richardson et al., 2009). These pathways related to carbon, nitrogen, sulfur and phosphorus and resistance to low temperature, low oxygen and ultraviolet light play a

very important role in the growth of highland barley in plateau environment.

Differences in gene abundance demonstrated the effect of different soil amendments on functions of microbial community. Genes in higher abundance within the MIX treatment indicates that the microbial community of the MIX treatment group has more carbon source pathways. Amino acid metabolism-related genes was likely converted into organic nitrogen, which is beneficial for crop production (Tyson et al., 2004). Sulfur-related sulfate-reducing genes could fix arsenic, reducing its effectiveness in rice plants and providing an effective way to inhibit the infiltration of arsenic into the food chain (Xu et al., 2019). Vitamins and terpenoid metabolites play an important role in promoting the production of essential compounds in plants and bacteria, inducing resistance to pathogens and promoting plant growth as a result (Palacios et al., 2014; Tholl, 2015). Therefore, mixed fertilizer can be used optimally to promote Tibetan hulless barley resistance to negative plateau environmental stressors and inhibitors to plant health.

## 5. Conclusions

In extreme ecological environments like the Tibet Plateau, quality farmland is scarce and key soil viability parameters such as organic matter and microbial populations are more vulnerable and sensitive to climatic changes. Data from this study suggests that the treatment of MIX group can achieve the greatest hulless barley yield and establish healthy soil. DAP treatment resulted in a short-term and rapid rise of soil nutrients level (N and P) as well as the population density of rhizospheric microbes. ORG treatment promoted a higher abundance of microorganisms in the rhizosphere that favors beneficial, growth-promoting bacteria that are essential for the growth of Tibetan hulless barley. The key to establishing an optimal microbiome in the rhizosphere is the balance between bacterial sensitivity and fungal stability. Mixed ORG and DAP amendments appear to have achieved those goals during the study period. Bacteria and fungi show phase differences in their rhizosphere in response to fertilizer treatments. Therefore, longer-term performance can provide further confirmation through examining the intricacies of more growth cycles and seasons of farmland systems.

## Credit author statement

Xiangyu Guan: Conceptualization, Methodology, Formal analysis, Writing – original draft, Writing- Reviewing and Editing. Zhen Cheng: Data curation, Visualization, Writing – original draft, Writing- Reviewing. Yiqiang Li: Visualization, Formal analysis, Data curation, Writing – original draft. Zining Guo: Data curation, Visualization. Ruoyu Zhao: Data curation, Investigation. Tingting Zhao: Data curation, Investigation. Wenyu Shi: Methodology, Data curation, Visualization. Chen Qiu: Supervision, Investigation. Liying Huang: Supervision, Investigation. Jinfeng Wang: Conceptualization, Methodology, Supervision, Visualization, Project administration, Writing- Reviewing and Editing. Song Jin: Conceptualization, Methodology, Supervision, Project administration.

## Declaration of competing interest

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

## Data availability

The data that has been used is confidential.

## Acknowledgments

This research was supported by the National Natural Science



Foundation of China (Grant No. 42172336 awarded to X.Y. Guan, Grant No. 32170659 awarded to W. Y. Shi), the Starting Grants Program for Young Talents at China Agricultural University (Grant No. 10092004 awarded to J.F. Wang), and the Fundamental Research Funds for Central Universities (Grant No. 2652019077 awarded to X.Y. Guan).

## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jenvman.2022.117137>.

## References

- Al Ubeed, H., Wills, R., Bowyer, M., Golding, J., 2019. Inhibition of postharvest senescence of green leafy vegetables by exogenous D-cysteine and L-cysteine as precursors of hydrogen sulphide. *J. Hortic. Sci. Biotechnol.* 94, 620–626.
- Alneberg, J., Bjarnason, B.S., de Bruijn, I., Schirmer, M., Quick, J., Ijaz, U.Z., Lahti, L., Loman, N.J., Andersson, A.F., Quince, C., 2014. Binning metagenomic contigs by coverage and composition. *Nat. Methods* 11, 1144–1146.
- Badr, A., Muller, K., Schafer-Pregl, R., El Rabey, H., Effgen, S., Ibrahim, H.H., Pozzi, C., Rohde, W., Salami, F., 2000. On the origin and domestication history of Barley (*Hordeum vulgare*). *Mol. Biol. Evol.* 17, 499–510.
- Beier, S., Bertilsson, S., 2013. Bacterial chitin degradation—mechanisms and ecophysiological strategies. *Front. Microbiol.* 4, 149.
- Bulgarelli, D., Schlaeppi, K., Spaepen, S., Ver Loren van Themaat, E., Schulze-Lefert, P., 2013. Structure and functions of the bacterial microbiota of plants. *Annu. Rev. Plant Biol.* 64, 807–838.
- Chaumell, P.A., Mussig, A.J., Hugenholtz, P., Parks, D.H., 2019. GTDB-Tk: a toolkit to classify genomes with the Genome Taxonomy Database. *Bioinformatics* 36 (6), 1925–1927.
- Chen, Dong, Zhang, 2015. Agriculture facilitated permanent human occupation of the Tibetan Plateau after 3600 B.P. *Science* 347, 248–250.
- Chen, S., Waghmode, T.R., Sun, R., Kuramae, E.E., Hu, C., Liu, B., 2019. Root-associated microbiomes of wheat under the combined effect of plant development and nitrogen fertilization. *Microbiome* 7, 136.
- Chen, S., Wang, F., Zhang, Y., Qin, S., Wei, S., Wang, S., Hu, C., Liu, B., 2018a. Organic carbon availability limiting microbial denitrification in the deep vadose zone. *Environ. Microbiol.* 20, 980–992.
- Chen, S., Zhou, Y., Chen, Y., Gu, J., 2018b. fastp: an ultra-fast all-in-one FASTQ preprocessor. *Bioinformatics* 34, i884–i890.
- Cheng, H., Yuan, M., Duan, Q., Sun, R., Shen, Y., Yu, Q., Li, S.J.I.C., Products, 2020. Influence of phosphorus fertilization patterns on the bacterial community in upland farmland. *Ind. Crop. Prod.* 155, 112761.
- De Caceres, M., Jansen, F., De Caceres, M.M., 2016. Package 'indicpecies'. indicators 8.
- DeAngelis, K.M., Brodie, E.L., DeSantis, T.Z., Andersen, G.L., Lindow, S.E., Firestone, M. K., 2009. Selective progressive response of soil microbial community to wild oat roots. *ISME J.* 3, 168–178.
- Dong, M., Zhao, M., Shen, Z., Deng, X., Shen, Q., 2020. Biofertilizer application triggered microbial assembly in microaggregates associated with tomato bacterial wilt suppression. *Biol. Fertil. Soils* 56, 551–563.
- Edgar, R.C., 2013. UPARSE: highly accurate OTU sequences from microbial amplicon reads. *Nat. Methods* 10, 996–998.
- Finkel, O.M., Salas-Gonzalez, I., Castrillo, G., Conway, J.M., Law, T.F., Teixeira, P., Wilson, E.D., Fitzpatrick, C.R., Jones, C.D., Dangi, J.L., 2020. A single bacterial genus maintains root growth in a complex microbiome. *Nature* 587, 103–108.
- Gu, S., Hu, Q., Cheng, Y., Bai, L., Liu, Z., Xiao, W., Gong, Z., Wu, Y., Feng, K., Deng, Y., 2019. Application of organic fertilizer improves microbial community diversity and alters microbial network structure in tea (*Camellia sinensis*) plantation soils. *Soil Tillage Res.* 195, 104356.
- Guan, X., Gao, X., Avellan, A., Spielman-Sun, E., Xu, J., Loughton, S., Yun, J., Zhang, Y., Bland, G.D., Zhang, Y., Zhang, R., Wang, X., Casman, E.A., Lowry, G.V., 2020. CuO nanoparticles alter the rhizospheric bacterial community and local nitrogen cycling for wheat grown in a calcareous soil. *Environ. Sci. Technol.* 54, 8699–8709.
- Guo, J.H., Liu, X.J., Zhang, Y., Shen, J.L., Han, W.X., Zhang, W.F., Christie, P., Goulding, K.W., Vitousek, P.M., Zhang, F.S., 2010. Significant acidification in major Chinese croplands. *Science* 327, 1008–1010.
- Jiang, S.Q., Yu, Y.N., Gao, R.W., Wang, H., Zhang, J., Li, R., Long, X.H., Shen, Q.R., Chen, W., Cai, F., 2019. High-throughput absolute quantification sequencing reveals the effect of different fertilizer applications on bacterial community in a tomato cultivated coastal saline soil. *Sci. Total Environ.* 687, 601–609.
- Kang, D.D., Li, F., Kirton, E., Thomas, A., Egan, R., An, H., Wang, Z., 2019. MetaBAT 2: an adaptive binning algorithm for robust and efficient genome reconstruction from metagenome assemblies. *PeerJ* 7, e7359.
- Lazcano, C., Gómez-Brandón, M., Revilla, P., 2013. Short-term effects of organic and inorganic fertilizers on soil microbial community structure and function. *Biol. Fertil. Soils* 49, 723–733.
- Li, H., Durbin, R., 2009. Fast and accurate short read alignment with Burrows-Wheeler transform. *Bioinformatics* 25, 1754–1760.
- Li, M., Wei, Z., Wang, J., Jousset, A., Friman, V.P., Xu, Y., Shen, Q., Pommier, T., 2019. Facilitation promotes invasions in plant-associated microbial communities. *Ecol. Lett.* 22, 149–158.
- Liang, J.L., Liu, J., Jia, P., Yang, T.T., Zeng, Q.W., Zhang, S.C., Liao, B., Shu, W.S., Li, J. T., 2020. Novel phosphate-solubilizing bacteria enhance soil phosphorus cycling following ecological restoration of land degraded by mining. *ISME J.* 14, 1600–1613.
- Lv, F., Song, J., Giltrap, D., Feng, Y., Yang, X., Zhang, S., 2020. Crop yield and N<sub>2</sub>O emission affected by long-term organic manure substitution fertilizer under winter wheat-summer maize cropping system. *Sci. Total Environ.* 732, 139321.
- Mader, P., Edenhofer, S., Boller, T., Wiemken, A., Niggli, U., 2000. Arbuscular mycorrhizae in a long-term field trial comparing low-input (organic, biological) and high-input (conventional) farming systems in a crop rotation. *Biol. Fertil. Soils* 31, 150–156.
- Mader, P., Fliessbach, A., Dubois, D., Gunst, L., Fried, P., Niggli, U., 2002. Soil fertility and biodiversity in organic farming. *Science* 296, 1694–1697.
- Magoc, T., Salzberg, S.L., 2011. FLASH: fast length adjustment of short reads to improve genome assemblies. *Bioinformatics* 27, 2957–2963.
- Mayer, K.F., Waugh, R., Brown, J.W., Schulman, A., Langridge, P., Platzer, M., Fincher, G.B., Muehlbauer, G.J., Sato, K., Close, T.J., Wise, R.P., Stein, N., 2012. A physical, genetic and functional sequence assembly of the barley genome. *Nature* 491, 711–716.
- Menno, V.D.V., Kempenaar, M., Van Driel, M., Raaijmakers, J.M., Mendes, R., Classen, A., 2016. Impact of soil heat on reassembly of bacterial communities in the rhizosphere microbiome and plant disease suppression. *Ecol. Lett.* 19, 375–382.
- Newman, C., Newman, R.K., 2006. A brief history of barley foods. *Cereal Foods World* 51, 4–7.
- Nissen, J.N., Johansen, J., Allesøe, R.L., Sønderby, C.K., Armenteros, J.J.A., Grønbech, C. H., Jensen, L.J., Nielsen, H.B., Petersen, T.N., Winther, O.J.N.B., 2021. Improved metagenome binning and assembly using deep variational autoencoders. *Nat. Biotechnol.* 39, 555–560.
- Nurk, S., Meleshko, D., Korobeynikov, A., Pevzner, P.A., 2017. metaSPAdes: a new versatile metagenomic assembler. *Genome Res.* 27, 824–834.
- Palacios, O.A., Bashan, Y., de-Bashan, L.E., 2014. Proven and potential involvement of vitamins in interactions of plants with plant growth-promoting bacteria—an overview. *Biol. Fertil. Soils* 50, 415–432.
- Palatinszky, M., Herbold, C., Jehmlich, N., Pogoda, M., Han, P., von Bergen, M., Lagkouvardos, I., Karst, S.M., Galushko, A., Koch, H., 2015. Cyanate as an energy source for nitrifiers. *Nature* 524, 105–108.
- Philippot, L., Raaijmakers, J.M., Lemanceau, P., van der Putten, W.H., 2013. Going back to the roots: the microbial ecology of the rhizosphere. *Nat. Rev. Microbiol.* 11, 789–799.
- Pontes, A.P., Souza, R.d., Granada, C.E., Passaglia, L.M., 2015. Screening of plant growth promoting bacteria associated with barley plants (*Hordeum vulgare* L.) cultivated in South Brazil. *Biota Neotropica* 15.
- Qiu, M.H., Zhang, R.F., Xue, S.S.S.Q., Shen, Q.R., 2012. Application of bio-organic fertilizer can control Fusarium wilt of cucumber plants by regulating microbial community of rhizosphere soil. *Biol. Fertil. Soils* 48 (7), 807–816.
- Reganold, John P., Glover, Jerry D., Andrews, Preston K., Hinman, 2001. Sustainability of three apple production systems. *Nature* 410, 926–930.
- Ren, F., Zhang, Y., Yu, H., Zhang, Y.A., 2020. *Ganoderma lucidum* cultivation affect microbial community structure of soil, wood segments and tree roots. *Sci. Rep.* 10, 3435.
- Richardson, A.E., Barea, J.-M., McNeill, A.M., Prigent-Combaret, C., 2009. Acquisition of phosphorus and nitrogen in the rhizosphere and plant growth promotion by microorganisms. *Plant Soil* 321, 305–339.
- Seemann, T., 2014. Prokka: rapid prokaryotic genome annotation. *Bioinformatics* 30, 2068–2069.
- Shi, S., Nuccio, E.E., Shi, Z.J., He, Z., Zhou, J., Firestone, M.K., 2016. The interconnected rhizosphere: high network complexity dominates rhizosphere assemblages. *Ecol. Lett.* 19, 926–936.
- Stokstad, E., 2016. The nitrogen fix. *Science* 353, 1225–1227.
- Tao, C., Li, R., Xiong, W., Shen, Z., Liu, S., Wang, B., Ruan, Y., Geisen, S., Shen, Q., Kowalchuk, G.A., 2020. Bio-organic fertilizers stimulate indigenous soil *Pseudomonas* populations to enhance plant disease suppression. *Microbiome* 8, 137.
- Tao, J., Meng, D., Qin, C., Liu, X., Liang, Y., Xiao, Y., Liu, Z., Gu, Y., Li, J., Yin, H., 2018. Integrated network analysis reveals the importance of microbial interactions for maize growth. *Appl. Microbiol. Biotechnol.* 102, 3805–3818.
- Team, C.R., 2015. R: A Language and Environment for Statistical Computing.
- Tholl, D., 2015. Biosynthesis and biological functions of terpenoids in plants. *Adv. Biochem. Eng. Biotechnol.* 148, 63–106.
- Tilman, D., 2004. Niche tradeoffs, neutrality, and community structure: a stochastic theory of resource competition, invasion, and community assembly. *Proc. Natl. Acad. Sci. USA* 101, 10854–10861.
- Tyson, G.W., Chapman, J., Hugenholtz, P., Allen, E.E., Ram, R.J., Richardson, P.M., Solovyev, V.V., Rubin, E.M., Rokhsar, D.S., Banfield, J.F., 2004. Community structure and metabolism through reconstruction of microbial genomes from the environment. *Nature* 428, 37–43.
- Upadhyay, S.K., Singh, J.S., Saxena, A.K., Singh, D.P., 2012. Impact of PGPR inoculation on growth and antioxidant status of wheat under saline conditions. *Plant Biol.* 14, 605–611.
- Uritskiy, G.V., DiRuggiero, J., Taylor, J., 2018. MetaWRAP—a flexible pipeline for genome-resolved metagenomic data analysis. *Microbiome* 6, 158.
- Utsumi, Y., Utsumi, C., Tanaka, M., Ha, C.V., Takahashi, S., Matsui, A., Matsunaga, T.M., Matsunaga, S., Kanno, Y., Seo, M., Okamoto, Y., Moriya, E., Seki, M., 2019. Acetic acid treatment enhances drought avoidance in cassava (*Manihot esculenta* Crantz). *Front. Plant Sci.* 10, 521.
- Wang, Y., Sang, Z., Xu, S., Xu, Q., Zeng, X., Jabu, D., Yuan, H., 2020. Comparative proteomics analysis of Tibetan hull-less barley under osmotic stress via data-independent acquisition mass spectrometry. *GigaScience* 9, g1aa019.

- Wei, Z., Yang, T., Friman, V.P., Xu, Y., Shen, Q., Jousset, A., 2015. Trophic network architecture of root-associated bacterial communities determines pathogen invasion and plant health. *Nat. Commun.* 6, 8413.
- Wu, T., Chellemi, D.O., Martin, K.J., Graham, J.H., Roskopf, E.N., 2007. Discriminating the effects of agricultural land management practices on soil fungal communities. *Soil Biol. Biochem.* 39, 1139–1155.
- Wu, Y.W., Simmons, B.A., Singer, S.W., 2016. MaxBin 2.0: an automated binning algorithm to recover genomes from multiple metagenomic datasets. *Bioinformatics* 32, 605–607.
- Xia, Y., Chen, X., Zheng, S., Gunina, A., Ning, Z., Hu, Y., Tang, H., Rui, Y., Zhang, Z., He, H., 2021. Manure application accumulates more nitrogen in paddy soils than rice straw but less from fungal necromass. *Agric. Ecosyst. Environ.* 319, 107575.
- Xu, X., Wang, P., Zhang, J., Chen, C., Wang, Z., Kopittke, P.M., Kretzschmar, R., Zhao, F. J., 2019. Microbial sulfate reduction decreases arsenic mobilization in flooded paddy soils with high potential for microbial Fe reduction. *Environ. Pollut.* 251, 952–960.
- Xun, W., Huang, T., Zhao, J., Ran, W., Wang, B., Shen, Q., Zhang, R., 2015. Environmental conditions rather than microbial inoculum composition determine the bacterial composition, microbial biomass and enzymatic activity of reconstructed soil microbial communities. *Soil Biol. Biochem.* 90, 10–18.
- Yuan, H., Zhang, Y., Huang, S., Yang, Y., Gu, C., 2015. Effects of exogenous glutathione and cysteine on growth, lead accumulation, and tolerance of *Iris lactea* var. *chinensis*. *Environ. Sci. Pollut. Res. Int.* 22, 2808–2816.
- Yuan, L., Loque, D., Ye, F., Frommer, W.B., von Wiren, N., 2007. Nitrogen-dependent posttranscriptional regulation of the ammonium transporter AtAMT1;1. *Plant Physiol.* 143, 732–744.
- Zeng, X., Guo, Y., Xu, Q., Mascher, M., Guo, G., Li, S., Mao, L., Liu, Q., Xia, Z., Zhou, J., Yuan, H., Tai, S., Wang, Y., Wei, Z., Song, L., Zha, S., Li, S., Tang, Y., Bai, L., Zhuang, Z., He, W., Zhao, S., Fang, X., Gao, Q., Yin, Y., Wang, J., Yang, H., Zhang, J., Henry, R.J., Stein, N., Tashi, N., 2018. Origin and evolution of qingke barley in Tibet. *Nat. Commun.* 9, 5433.