Table S1. Primer sequences.

| **Primer name** | **Primer sequence** |
|---|---|
| 16S-V4-515F | TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGTGCCAGCMGCCGCGGTAA |
| 16S-V4-802R | GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGTACNVGGGTATCTAATCC |
| 16S-V4-806R | GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGACTACHVGGGTWTCTAAT |
| MitoBlk-515F | TCCCCATGCTTTCGCACCCCA/3SpC3/ |
| CloroBlk-806R | GTCTCTAATCCCATTTGCTCC/3SpC3/ |

Table S2. Pielou’s evenness, Species richness and Shannon index of endophytic seed microbial communities obtained from seven different barley genotypes.

| | **Golden Promise** | **Morex** | **HOR7985** | **BCC1415** | **BCC436** | **BCC768** | **BCC1589** |
|---|---|---|---|---|---|---|---|
| **Pielou's evenness** | 0.82±0.07a | 0.71±0.10a | 0.86±0.04a | 0.84±0.06a | 0.73±0.05a | 0.76±0.09a | 0.69±0.08a |
| **Species richness** | 75.69±21.47a | 41.24±13.24b | 81.19±18.18a | 31.19±8.15b | 41.12±4.49b | 52.03±13.56ab | 25.73±7.24b |
| **Shannon index** | 3.53±0.56ab | 2.62±0.58bc | 3.75±0.35a | 2.87±0.34abc | 2.71±0.21bc | 2.99±0.47abc | 2.23±0.38c |

Letters indicate significant differences (Tukey's test $p \leq 0.05$).

Table S3. Dominant phyla in the endophytic seed microbiome of seven different barley genotypes.

| | **Golden Promise** | **Morex** | **HOR7985** | **BCC1415** | **BCC436** | **BCC768** | **BCC1589** |
|---|---|---|---|---|---|---|---|
| **Proteobacteria** | 45.36a | 64.87a | 48.00a | 38.64a | 61.60a | 50.84a | 73.30a |
| **Actinobacteria** | 26.46abc | 25.66abc | 37.78ab | 49.48a | 21.30bc | 16.91bc | 11.16c |
| **Firmicutes** | 23.67a | 9.05a | 9.48a | 10.91a | 16.05a | 30.81a | 15.50a |
| **Bacteroidetes** | 4.12a | 0.43b | 3.99a | 0.55ab | 1.03ab | 1.03ab | 0.02b |
| **Rare phyla (<1%)** | 0.39 | 0.00 | 0.76 | 0.41 | 0.03 | 0.41 | 0.02 |

Letters indicate significant differences (Tukey's test $p \leq 0.05$).

Table S4. Pielou’s evenness, Species richness and Shannon index of bulk soil and barley rhizosphere samples from seven different genotypes grown in mouldboard plough (MP) soil.

| | **MP Rhizosphere** | | | | | | | **Bulk soil** |
|---|---|---|---|---|---|---|---|---|
| | **Golden Promise** | **Morex** | **HOR7985** | **BCC1415** | **BCC436** | **BCC768** | **BCC1589** | |
| **Pielou’s evenness** | 0.96±0.00a | 0.95±0.00a | 0.95±0.00a | 0.96±0.01a | 0.95±0.00a | 0.95±0.00a | 0.95±0.00a | 0.95±0.00a |
| **Species richness** | 1625.07±130.97a | 868.48±291.52bc | 1188.08±188.91ab | 659.07±338.83c | 1062.38±148.87bc | 750.38±74.77bc | 979.73±147.30bc | 925.32±54.60bc |
| **Shannon index** | 7.07±0.08a | 6.41±0.37bc | 6.75±0.15ab | 6.10±0.51c | 6.65±0.13abc | 6.31±0.12bc | 6.56±0.15abc | 6.52±0.07abc |

Letters indicate significant differences (Tukey's test $p \leq 0.05$).

Table S5. Pielou’s evenness, Species richness and Shannon index of bulk soil and barley rhizosphere samples from seven different genotypes grown in cultivator tillage (CT) soil.

| | **CT Rhizosphere** | | | | | | | **Bulk soil** |
|---|---|---|---|---|---|---|---|---|
| | **Golden Promise** | **Morex** | **HOR7985** | **BCC1415** | **BCC436** | **BCC768** | **BCC1589** | |
| **Pielou’s Evenness** | 0.96±0.00a | 0.96±0.00a | 0.96±0.00a | 0.96±0.00a | 0.96±0.00a | 0.96±0.00a | 0.96±0.00a | 0.96±0.00a |
| **Species Richness** | 1068.03±133.05ab | 904.06±291.92b | 1101.27±128.73ab | 909.66±88.71b | 1170.68±126.99ab | 1242.16±244.61ab | 1339.95±48.74ab | 1496.15±302.94a |
| **Shannon index** | 6.68±0.13ab | 6.48±0.37b | 6.70±0.11ab | 6.51±0.08b | 6.75±0.11ab | 6.80±0.20ab | 6.88±0.04ab | 6.99±0.22a |

Letters indicate significant differences (Tukey's test $p \leq 0.05$).

Table S6. PERMANOVA of each barley genotype of rhizosphere samples obtained from plants grown in mouldboard plough (MP) soil.

| **PERMANOVA MP rhizosphere** | $R^2$ | $p \leq$ |
|---|---|---|
| Golden Promise *vs* Morex | 0.31 | **0.05** |
| Golden Promise *vs* HOR7985 | 0.23 | **0.05** |
| Golden Promise *vs* BCC1415 | 0.37 | **0.05** |
| Golden Promise *vs* BCC436 | 0.28 | **0.05** |
| Golden Promise *vs* BCC768 | 0.38 | **0.05** |
| Golden Promise *vs* BCC1589 | 0.31 | **0.05** |
| Morex *vs* HOR7985 | 0.18 | 0.06 |
| Morex *vs* BCC1415 | 0.15 | 0.20 |
| Morex *vs* BCC436 | 0.16 | 0.11 |
| Morex *vs* BCC768 | 0.16 | 0.17 |
| Morex *vs* BCC1589 | 0.16 | 0.12 |
| HOR7985 *vs* BCC1415 | 0.23 | **0.05** |
| HOR7985 *vs* BCC436 | 0.15 | 0.23 |
| HOR7985 *vs* BCC768 | 0.24 | **0.05** |
| HOR7985 *vs* BCC1589 | 0.19 | 0.06 |
| BCC1415 *vs* BCC436 | 0.21 | 0.06 |
| BCC1415 *vs* BCC768 | 0.17 | 0.15 |
| BCC1415 *vs* BCC1589 | 0.20 | **0.05** |
| BCC436 *vs* BCC768 | 0.20 | **0.05** |
| BCC436 *vs* BCC1589 | 0.18 | 0.06 |
| BCC768 *vs* BCC1589 | 0.17 | **0.05** |

Table S7. PERMANOVA of each barley genotype of rhizosphere samples obtained from plants grown in cultivator tillage (CT) soil.

| **PERMANOVA CT rhizosphere** | $R^2$ | $p \leq$ |
|---|---|---|
| Golden Promise *vs* Morex | 0.14 | 0.46 |
| Golden Promise *vs* HOR7985 | 0.14 | 0.42 |
| Golden Promise *vs* BCC1415 | 0.16 | 0.06 |
| Golden Promise *vs* BCC436 | 0.16 | 0.14 |
| Golden Promise *vs* BCC768 | 0.18 | **0.05** |
| Golden Promise *vs* BCC1589 | 0.21 | **0.05** |
| Morex *vs* HOR7985 | 0.14 | 0.47 |
| Morex *vs* BCC1415 | 0.14 | 0.57 |
| Morex *vs* BCC436 | 0.16 | 0.18 |
| Morex *vs* BCC768 | 0.17 | 0.06 |
| Morex *vs* BCC1589 | 0.19 | **0.05** |
| HOR7985 *vs* BCC1415 | 0.15 | 0.26 |
| HOR7985 *vs* BCC436 | 0.15 | 0.17 |
| HOR7985 *vs* BCC768 | 0.18 | **0.05** |
| HOR7985 *vs* BCC1589 | 0.18 | **0.05** |
| BCC1415 *vs* BCC436 | 0.18 | 0.06 |
| BCC1415 *vs* BCC768 | 0.18 | 0.06 |
| BCC1415 *vs* BCC1589 | 0.22 | **0.05** |
| BCC436 *vs* BCC768 | 0.17 | **0.05** |
| BCC436 *vs* BCC1589 | 0.18 | **0.05** |
| BCC768 *vs* BCC1589 | 0.15 | 0.13 |

Table S8. Dominant phyla in bulk soil and barley rhizosphere of seven different genotypes grown in two diverse soils (MP; CT).

| | **MP** | | | | | | | | **CT** | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **Golden Promise** | **Morex** | **HOR7985** | **BCC1415** | **BCC436** | **BCC768** | **BCC1589** | **Bulk soil** | **Golden Promise** | **Morex** | **HOR7985** | **BCC1415** | **BCC436** | **BCC768** | **BCC1589** | **Bulk soil** |
| **Acidobacteria** | 22.11ab | 22.47ab | 22.38ab | 24.66a | 22.62ab | 23.13ab | 22.58ab | 24.34ab | 22.52ab | 21.97ab | 21.21b | 22.29ab | 22.27ab | 23.12ab | 22.77ab | 22.75ab |
| **Actinobacteria** | 17.75f | 20.25cdef | 20.09cdef | 22.10abcd | 21.36bcde | 18.14ef | 17.27f | 23.00abc | 19.93cdef | 20.03cdef | 19.33def | 20.58bcdef | 18.79def | 23.68ab | 23.17abc | 24.96a |
| **Proteobacteria** | 19.83bcde | 21.05abc | 18.80cde | 18.20def | 20.44abcd | 22.38ab | 22.81a | **15.93f** | 20.15abcde | 19.36cde | 20.36abcd | 20.36abcd | 20.90abcd | 21.43abc | 19.91bcde | **17.46ef** |
| **Planctomycetes** | 10.54a | 8.64abcd | 9.39abc | 6.73d | 7.96bcd | 7.63cd | 9.26abc | 7.71cd | 10.21a | 10.23a | 9.99ab | 8.79abcd | 10.00ab | 8.86abcd | 8.46abcd | 8.78abcd |
| **Chloroflexi** | 5.82bc | 5.59c | 6.21bc | 6.10bc | 5.70c | 6.23bc | 6.07bc | **8.49a** | 7.23abc | 7.17abc | 7.40ab | 6.91abc | 7.15abc | 5.86bc | 6.54bc | **8.56a** |
| **Thaumarchaeota** | 5.54cde | 6.33abcd | 7.50ab | 7.56ab | 5.97abcde | 5.86bcde | 4.85de | 7.72a | 5.48cde | 6.74abc | 6.19abcde | 6.70abc | 5.78bcde | 4.45e | 6.09abcde | 6.46abcd |
| **Verrucomicrobia** | 7.36a | 6.62a | 6.20abc | 6.68a | 5.94abc | 6.30ab | 7.09a | **3.26d** | 6.15abc | 6.13abc | 6.37a | 5.95abc | 6.08abc | 4.86c | 4.93bc | **3.12d** |
| **Bacteroidetes** | 4.05a | 3.24abc | 3.18abcd | 2.06e | 3.32ab | 2.82bcde | 3.55ab | **2.31de** | 3.64ab | 3.15abcd | 3.88a | 3.52ab | 3.78a | 2.34de | 2.76bcde | **2.34cde** |
| **Rare phyla (<2.5%)** | 7.01 | 5.81 | 6.25 | 5.91 | 6.69 | 7.51 | 6.51 | 7.24 | 4.69 | 5.22 | 5.26 | 4.91 | 5.26 | 5.40 | 5.38 | 5.57 |

Letters indicate significant differences (Tukey's test $p \leq 0.05$).

Table S9. Twenty most abundant genera in bulk soil and barley rhizosphere of seven different genotypes grown in two diverse soils (MP; CT).

| | | MP | | | | | | | | CT | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Phylum** | **Genus** | **Golden Promise** | **Morex** | **HOR7985** | **BCC1415** | **BCC436** | **BCC768** | **BCC1589** | **Bulk soil** | **Golden Promise** | **Morex** | **HOR7985** | **BCC1415** | **BCC436** | **BCC768** | **BCC1589** | **Bulk soil** |
| **Acidobacteria** | **Uncl. *Subgroup 6*** | 11.73 | 12.19 | 13.18 | 14.65 | 12.82 | 13.21 | 12.36 | 14.16 | 13.85 | 13.47 | 13.21 | 13.84 | 13.60 | 14.80 | 14.34 | 14.39 |
| **Thaumarchaeota** | **Uncl. *Nitrososphaeraceae*** | 4.95 | 5.85 | 6.77 | 7.13 | 5.44 | 5.48 | 4.47 | 7.04 | 4.51 | 5.73 | 5.13 | 5.51 | 4.74 | 3.83 | 5.16 | 5.29 |
| **Planctomycetes** | **Uncl. *WD2101 soil group*** | 3.45 | 2.42 | 2.68 | 1.71 | 2.27 | 2.50 | 2.87 | 1.97 | 2.84 | 2.79 | 2.45 | 2.20 | 2.54 | 1.73 | 1.80 | 1.91 |
| **Alphaproteobacteria** | ***Sphingomonas*** | 2.39 | 2.95 | 2.07 | 2.26 | 2.80 | 2.88 | 2.50 | 2.43 | 1.93 | 1.55 | 1.77 | 1.64 | 2.22 | 2.21 | 2.45 | 1.71 |
| **Actinobacteria** | **Uncl. *Micrococcaceae*** | 2.00 | 3.10 | 2.42 | 3.16 | 2.77 | 2.97 | 2.35 | 1.35 | 1.57 | 1.86 | 1.65 | 1.71 | 1.91 | 2.35 | 2.20 | 1.29 |
| **Gammaproteobacteria** | **Uncl. *Burkholderiaceae*** | 2.25 | 2.25 | 2.07 | 2.13 | 2.07 | 2.91 | 2.60 | 1.45 | 2.30 | 2.01 | 2.00 | 2.31 | 2.46 | 2.02 | 1.75 | 1.40 |
| **Acidobacteria** | **Uncl. *Blastocatellaceae*** | 2.60 | 2.73 | 1.96 | 2.86 | 2.35 | 2.24 | 2.24 | 2.06 | 1.72 | 1.68 | 1.64 | 1.75 | 1.79 | 1.71 | 1.59 | 1.47 |
| **Chloroflexi** | **Uncl. *KD4-96*** | 1.50 | 1.65 | 1.69 | 2.06 | 1.88 | 1.74 | 1.65 | 2.40 | 1.91 | 1.90 | 2.06 | 2.11 | 2.02 | 2.24 | 2.11 | 2.91 |
| **Actinobacteria** | ***Gaiella*** | 1.35 | 1.57 | 1.77 | 1.95 | 1.80 | 1.48 | 1.40 | 2.33 | 2.04 | 2.25 | 1.82 | 2.10 | 1.80 | 2.15 | 2.16 | 2.46 |
| **Acidobacteria** | ***RB41*** | 2.56 | 2.58 | 2.29 | 2.43 | 2.16 | 2.48 | 2.46 | 3.04 | 1.29 | 1.38 | 1.30 | 1.33 | 1.33 | 1.13 | 1.17 | 1.37 |
| **Gammaproteobacteria** | ***Massilia*** | 1.09 | 1.95 | 1.41 | 1.59 | 1.76 | 2.43 | 3.01 | 0.27 | 2.47 | 1.88 | 2.49 | 2.77 | 2.60 | 2.21 | 1.88 | 0.24 |
| **Actinobacteria** | ***Nocardioides*** | 1.08 | 1.63 | 1.43 | 2.00 | 1.48 | 1.35 | 1.32 | 1.78 | 1.96 | 1.98 | 1.78 | 1.95 | 1.59 | 2.10 | 2.19 | 2.22 |
| **Verrucomicrobia** | ***Chthoniobacter*** | 2.30 | 2.19 | 1.91 | 1.92 | 2.10 | 1.69 | 2.07 | 0.68 | 1.83 | 1.82 | 1.94 | 1.71 | 1.98 | 1.35 | 1.31 | 0.70 |
| **Actinobacteria** | **Uncl. *Gaiellales*** | 1.56 | 1.72 | 1.90 | 1.69 | 1.94 | 1.27 | 1.38 | 2.50 | 1.52 | 1.25 | 1.50 | 1.42 | 1.39 | 1.66 | 1.78 | 2.29 |
| **Actinobacteria** | **Uncl. *MB-A2-108*** | 1.61 | 1.85 | 2.04 | 2.00 | 2.00 | 1.79 | 1.97 | 2.44 | 1.44 | 1.50 | 1.30 | 1.44 | 1.18 | 1.36 | 1.37 | 1.49 |
| **Verrucomicrobia** | ***Luteolibacter*** | 2.09 | 1.61 | 1.43 | 2.08 | 1.31 | 1.98 | 2.07 | 0.12 | 1.76 | 1.61 | 1.73 | 1.57 | 1.33 | 1.20 | 1.23 | 0.22 |
| **Planctomycetes** | ***Pirellula*** | 1.49 | 1.31 | 1.32 | 1.01 | 1.13 | 0.96 | 1.21 | 0.81 | 1.52 | 1.29 | 1.67 | 1.41 | 1.45 | 1.69 | 1.36 | 1.17 |
| **Gemmatimonadetes** | **Uncl. *Gemmatimonadaceae*** | 1.4 | 1.08 | 1.15 | 0.83 | 1.09 | 1.49 | 1.42 | 1.97 | 1.29 | 1.29 | 1.34 | 1.22 | 1.29 | 0.85 | 1.16 | 1.46 |
| **Planctomycetes** | **Uncl. *Pirellulaceae*** | 1.31 | 1.15 | 1.30 | 1.07 | 1.09 | 0.68 | 1.17 | 1.07 | 1.19 | 1.11 | 1.07 | 1.14 | 1.17 | 1.12 | 0.98 | 1.30 |
| **Actinobacteria** | ***Streptomyces*** | 1.02 | 1.03 | 1.11 | 1.07 | 1.24 | 1.08 | 0.88 | 1.17 | 0.88 | 0.86 | 0.92 | 0.92 | 0.90 | 1.26 | 1.17 | 1.32 |

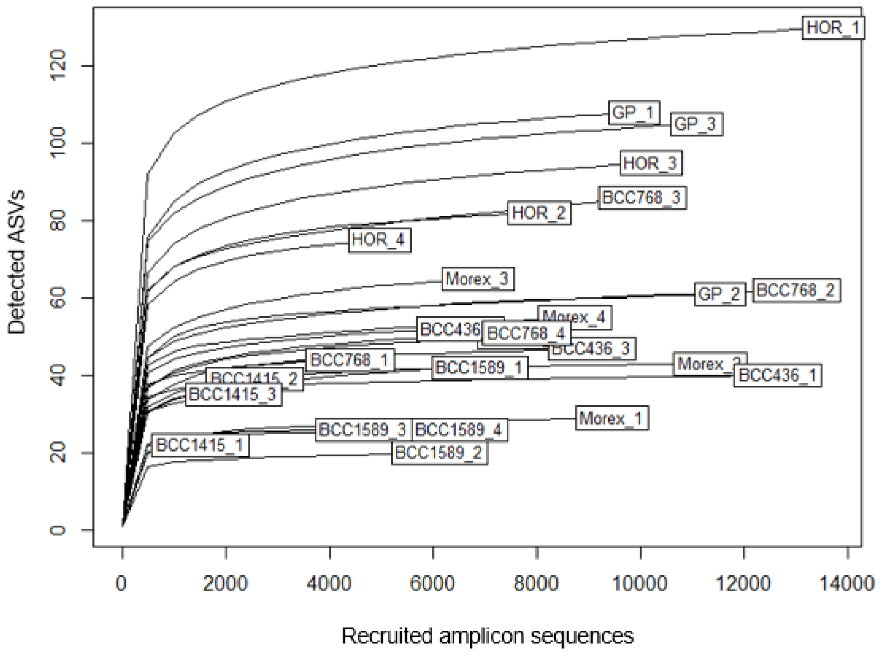

Figure S1. Rarefaction curves of 16S rRNA gene amplicon sequencing data of the barley seed microbiome.

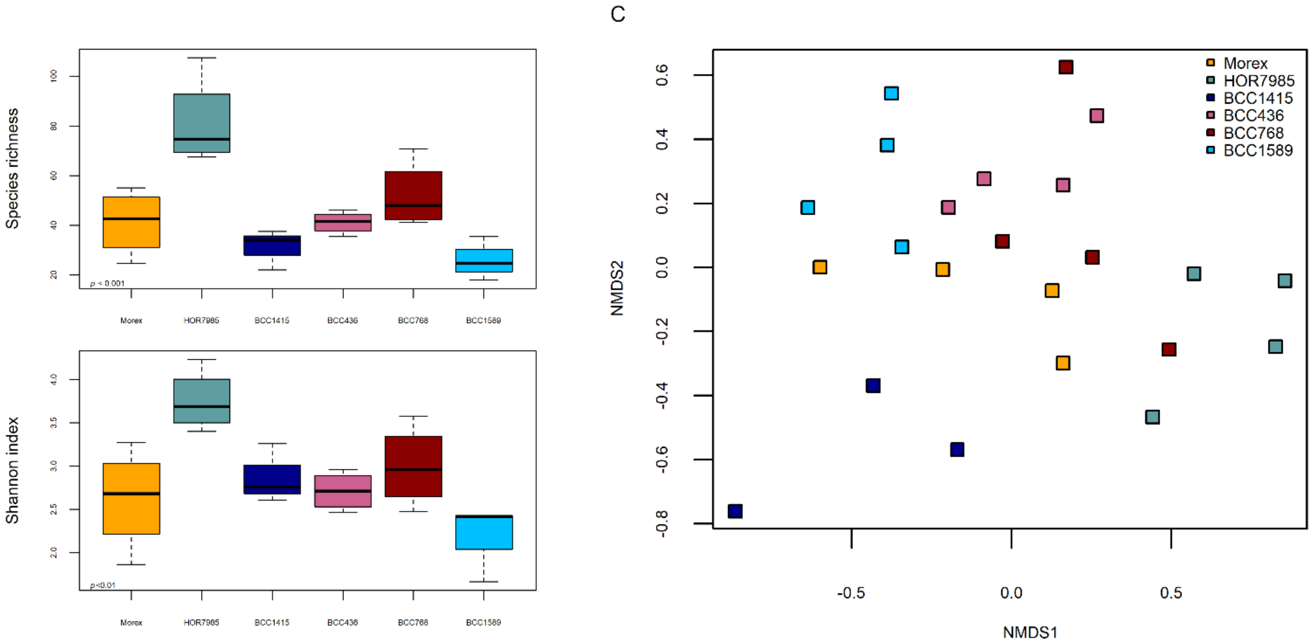

Figure S2 Microbial diversity of the endophytic seed microbiome varied between six barley genotypes originated from the same place. The microbial alpha-diversity indices Species richness (A) and Shannon diversity index (B) varied depending on the plant genotype (ANOVA for Species richness $p \leq 0.001$ and Shannon index $p \leq 0.01$). Beta-diversity of the endophytic seed microbiome was visualized by NMDS (C). The microbiome composition based on Bray-Curtis community dissimilarities (ASVs obtained from 16S rRNA gene amplicon sequencing) and was assessed from DNA of surface-sterilized barley seeds of six genotypes harvested at the same field site.

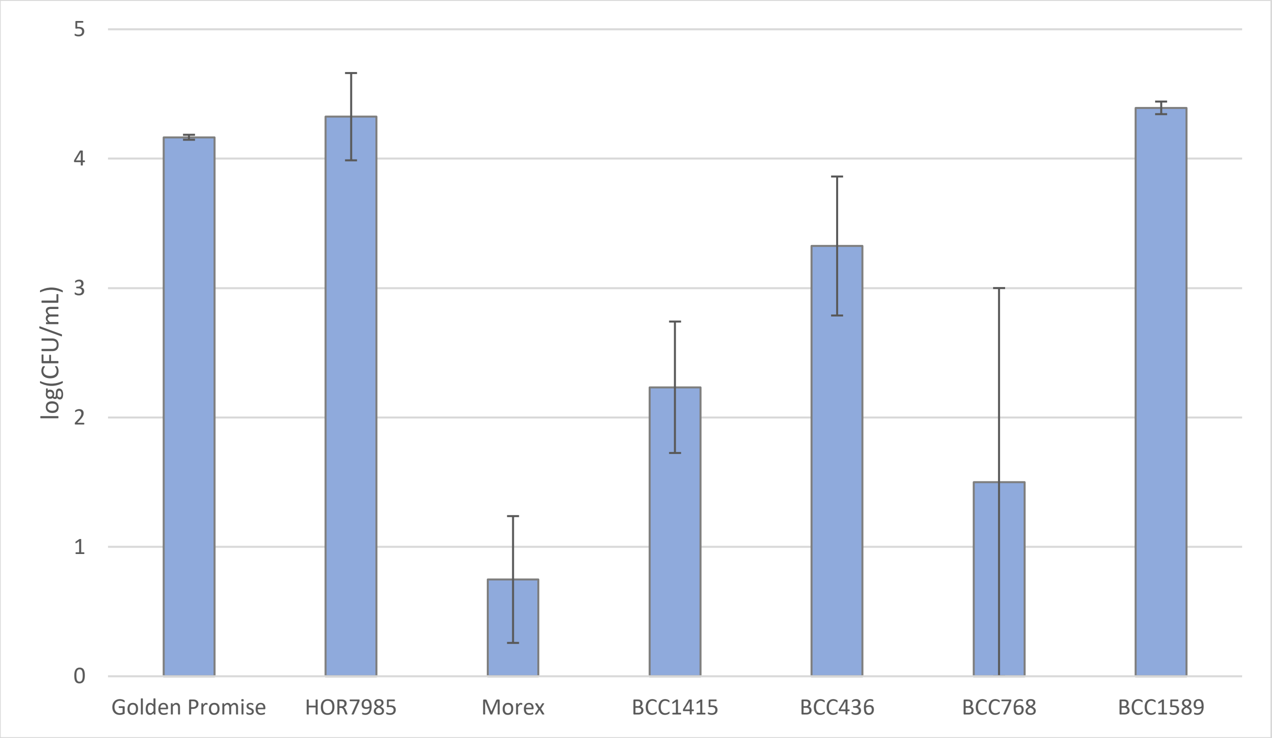

Figure S3. Logarithm of colony forming units (CFU)/g seed of seven different barley genotypes determined after 48 h incubation at 28°C. 0.5 g of surface-sterilized seeds were grounded and solved in 4.5 mL sterile double-distilled water. The seed suspension was plated on R2A supplemented with 100 µg/mL cycloheximide.

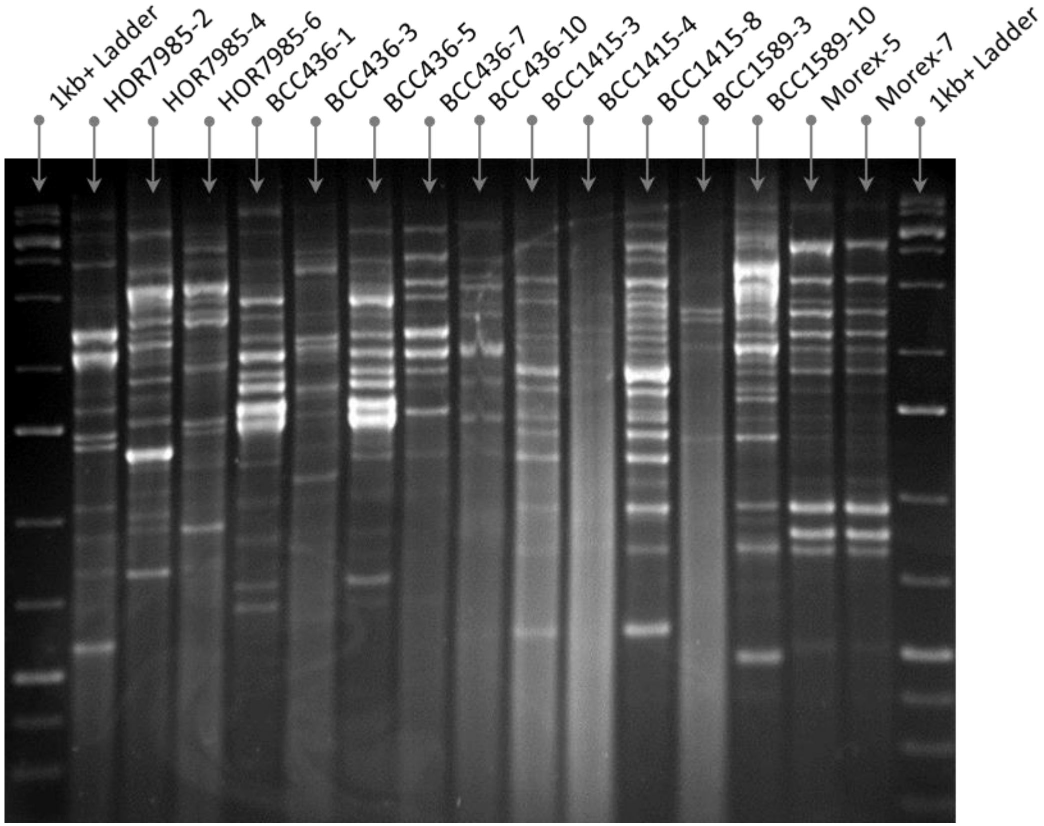

Figure S4. BOX fingerprint of isolated endophytes taxonomically affiliated to the genus *Curtobacterium*.

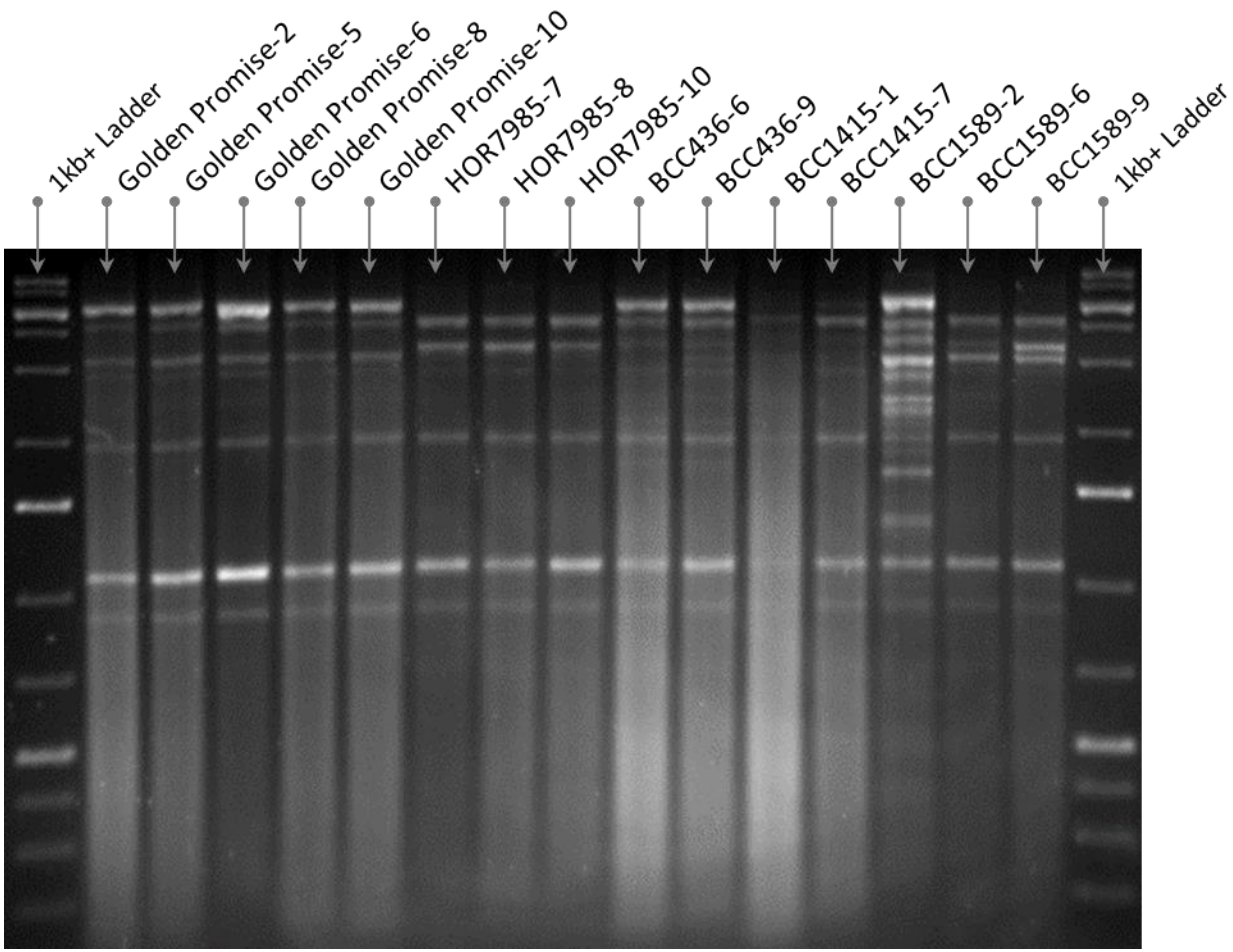

Figure S5. BOX fingerprint of isolated endophytes taxonomically affiliated to the genus *Paenibacillus.*

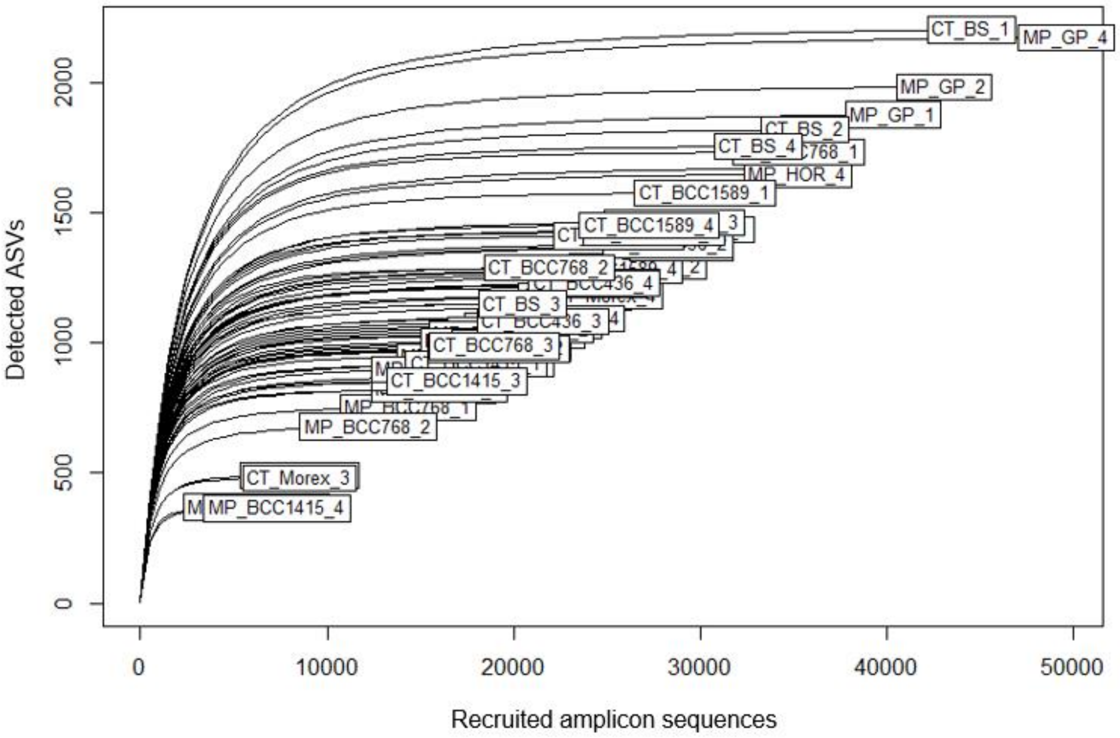

Figure S6. Rarefaction curves of 16S rRNA gene amplicon sequencing data of barley rhizosphere and bulk soil samples from two different soils (MP: mouldboard plough; CT: cultivator tillage) and seven different genotypes.

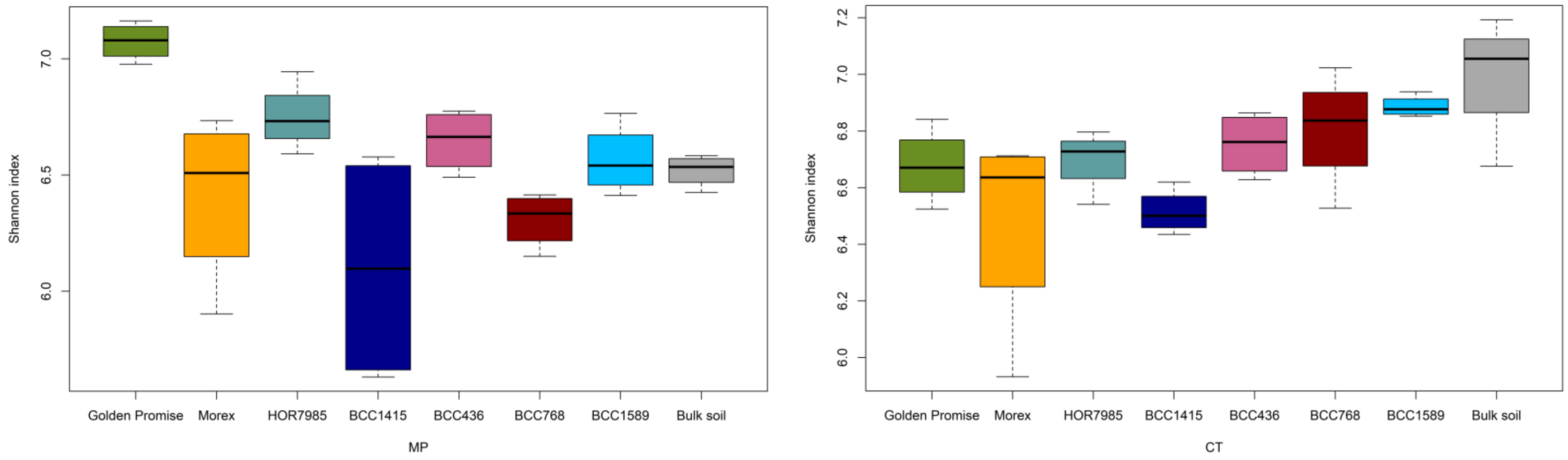

Figure S7. Shannon index of bulk soil and barley rhizosphere samples of seven different genotypes grown in MP and CT soils.

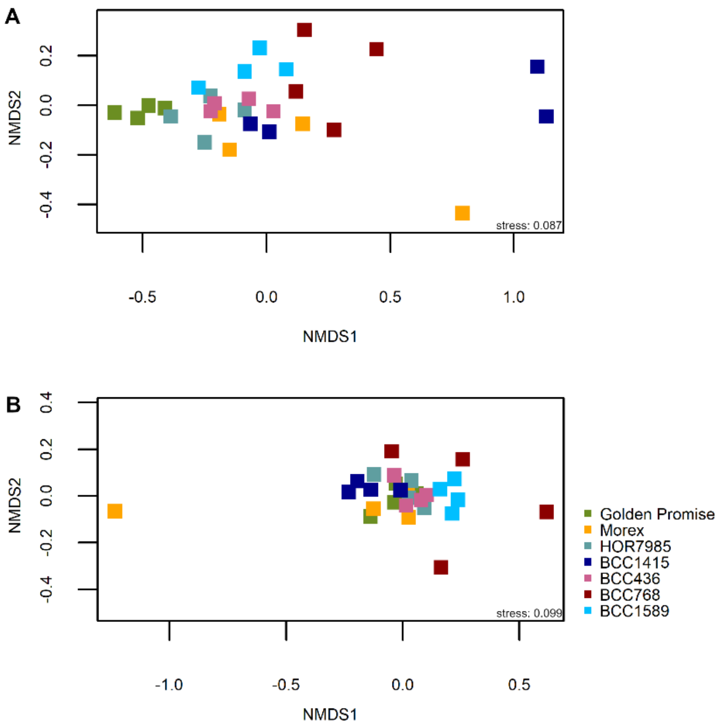

Figure S8. Non-metric multidimensional scaling (NMDS) of barley rhizosphere microbial communities from seven different genotypes grown in MP (A) and CT (B) soil. The rhizosphere community composition based on Bray-Curtis dissimilarities and was obtained from ASVs. The seven different genotypes were grown in MP (mouldboard plough) and CT (cultivator tillage) soil until BBCH13. Respective bulk soil samples are not shown. ANOSIM verified significant differences between the genotypes (*p* ≤ 0.001).