***New Phytologist* Supporting Information**

Article title:
Root cap is an important determinant of rhizosphere microbiome assembly

Authors:
Lioba Rüger, Minh Ganther, Jule Freudenthal, Jan Jansa, Anna Heintz-Buschart, Mika Tapio Tarkka, Michael Bonkowski

Article acceptance date: 24 April 2023

The following Supporting Information is available for this article:

Fig. S1 Assembled rhizobox filled with loam and planted with *Zea mays*.

Fig. S2 Effects of the root cap treatment on root growth.

Fig. S3 Rarefaction curves showing the sequencing depth for protists and prokaryota.

Fig. S4 OTU richness and Pielou evenness of prokaryote and protist communities at the three root zones, with HAIR or NoHAIR, and CAP or DeCAP treatment.

Fig. S5 Group dispersion of beta diversity in prokaryote and protist communities at the three root zones, with HAIR or NoHAIR, and CAP or DeCAP treatment.

Fig. S6 Principal Component Analysis and Variance Partitioning Analysis of RNAseq root gene expression data.

Fig. S7 Root zone-specific genes and enriched GO terms.

Fig. S8 Venn diagrams of root zone differences in either HAIR or NoHAIR samples.

Table S1 Primer tag sequences used for barcoding protist sequences.

Table S2 Differentially expressed genes by root zonation, root cap, and root hair treatment.

Table S3 One-factor Analysis of Variance in root growth, between roots with HAIR and NoHAIR, and CAP and DeCAP treatments.

Table S4 Three-factor Analysis of Variance in protist and prokaryote OTU-richness, Shannon diversity, Pielou evenness and beta dispersal.

Table S5 One-factor Analysis of Variance in prokaryote abundance, protist and prokaryote OTU-richness, Shannon diversity, Pielou evenness and beta dispersal.

Table S6 Three-factor Analysis of Variance in prokaryote abundance.

Table S7 Prokaryote abundances measured by qPCR.

Table S8 Permutational Multivariate Analysis of Variance and Variance Partitioning Analysis of beta diversity of prokaryota and protists.

Table S9 Enriched Gene Ontology terms for root zone comparisons and root zone-specific gene expression.

Table S10 Number of differentially expressed genes between root zones, root hair treatments and cap treatments.

Separate files have been provided for Tables S1, S2, S7, S9, and S10.

Methods S1 Rhizobox construction and preparation of maize seedlings.

Methods S2 Quantification of prokaryota by qPCR.

Methods S3 Processing of prokaryotic, protist and root gene expression sequencing data.

Methods S4 Statistical analysis.

Fig. S1 Assembled rhizobox with six columns, filled with loam and planted with individual maize plants (*Zea mays* L.). To enable root zone-specific sampling, rhizoboxes were constructed with a removable perspex front window. The bottom openings of the columns were closed, leaving only a gap for a protruding strip of a glass fiber wicker for passive watering by immersing it in a container with water.

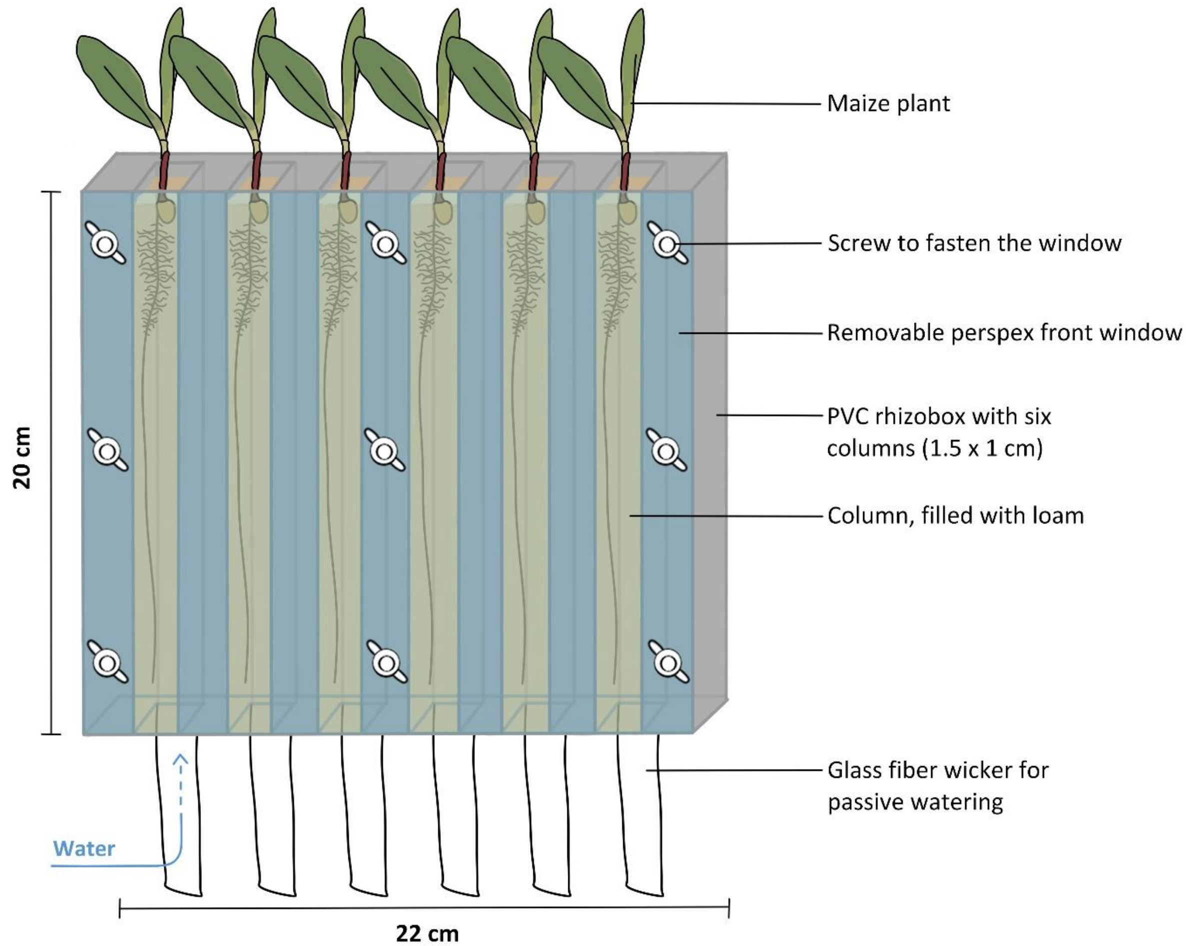

Fig. S2 Boxplots of (a) the total root length, (b) length of the primary root and (c) the average root diameter of plants (*Zea mays* L.) with (HAIR) and without root hairs (NoHAIR) and with (DeCAP) and without applied decapping treatment (CAP). The horizontal line within each box represents the median. Whiskers extend to the lowest and highest scores within 1.5 times the interquartile range from the box. Points represent individual datapoints. Lower case letters above boxplots indicate differences between means (Tukey's HSD), n=6, except in the CAP-HAIR treatment n=5.

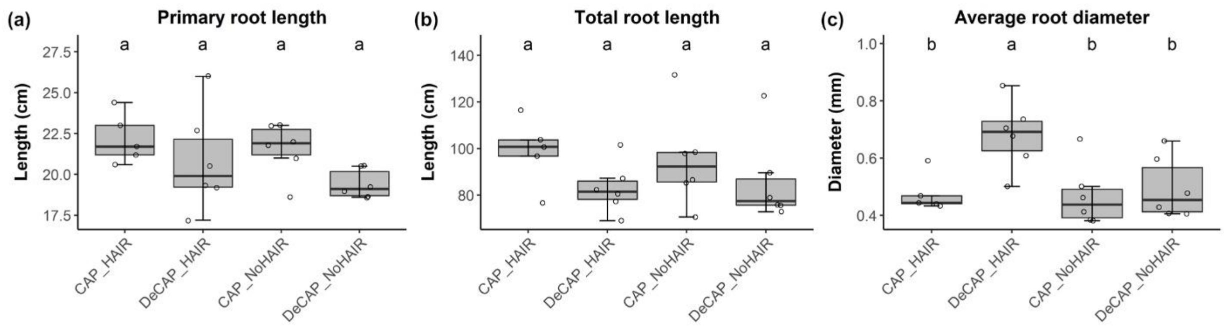

Fig. S3 Rarefaction curves for (a) protists (Cercozoa and Endomyxa) and (b) prokaryotes (bacteria and archaea) with interpolated number of OTUs for subsampled sequences (solid line) and extrapolated number of OTUs with increasing number of sequences (dashed line).

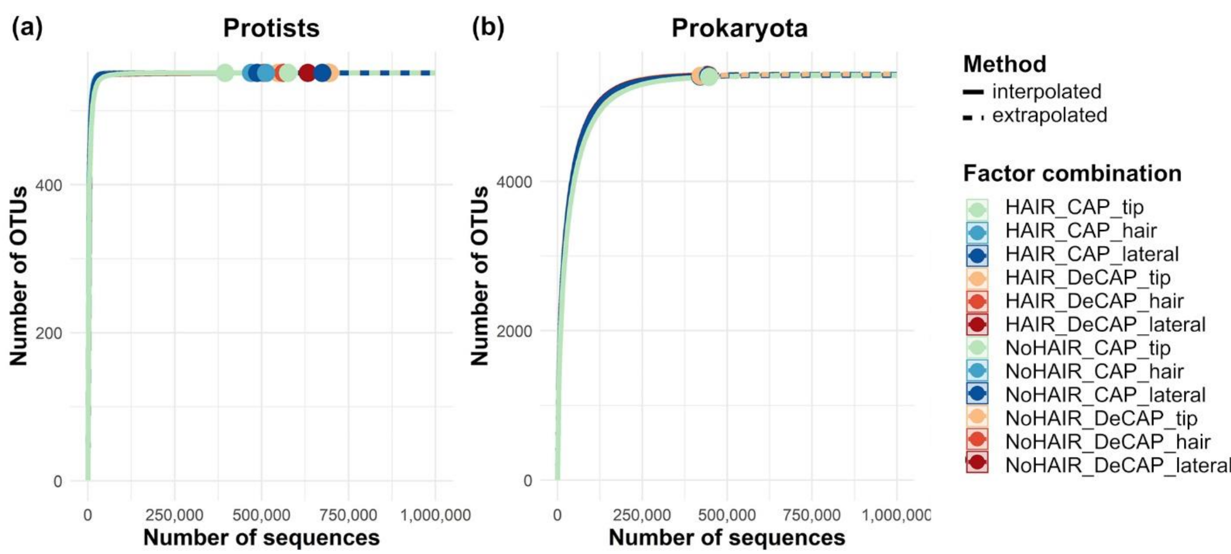

Fig. S4 OTU richness and Pielou evenness of (a) prokaryote (bacteria and archaea) and (b) protist (Cercozoa and Endomyxa) communities at the three root zones: root tip, root hair zone and lateral root emergence zone of *Zea mays* L., with HAIR or NoHAIR, and CAP or DeCAP treatment. The horizontal line within each box represents the median. Whiskers extend to the lowest and highest scores within 1.5 times the interquartile range from the box. Points represent individual datapoints. Letters indicate significant differences between root zones (Tukey's HSD).

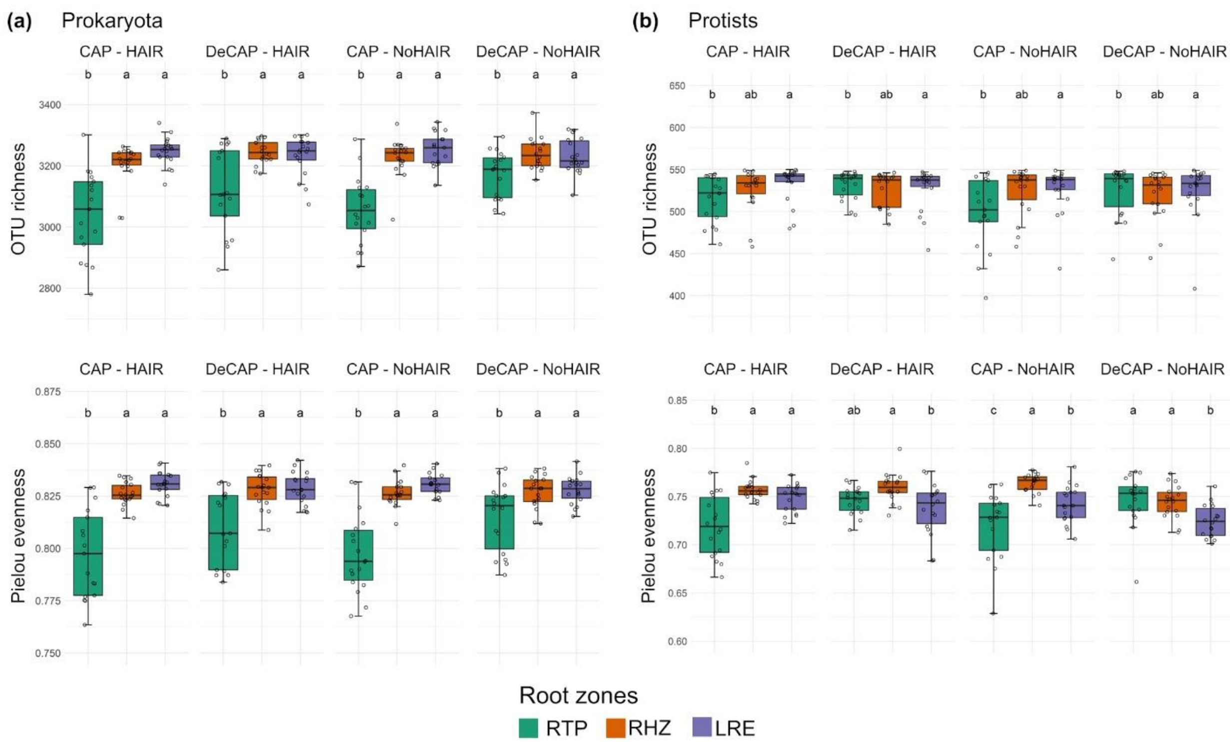

Fig. S5 Group dispersion of beta diversity in (a) prokaryote (bacteria and archaea) and (b) protist (Cercozoa and Endomyxa) communities at the three root zones: root tip, root hair zone and lateral root emergence zone of *Zea mays* L., with HAIR or NoHAIR, and CAP or DeCAP treatment. The horizontal line within each box represents the median. Whiskers extend to the lowest and highest scores within 1.5 times the interquartile range from the box. Points represent individual datapoints. Letters indicate significant differences between root zones (Tukey's HSD).

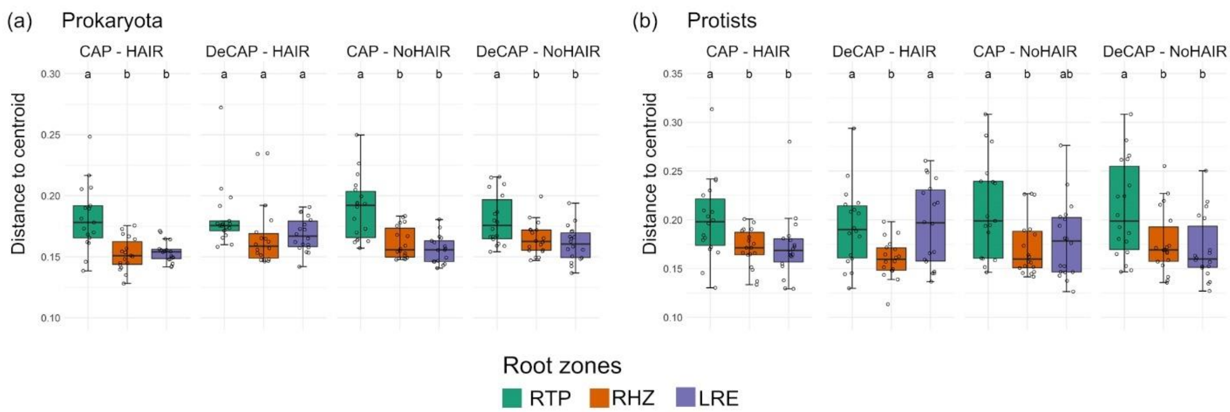

Fig. S6 (a) Principal Component Analysis and (b) Variance Partitioning Analysis of RNAseq root gene expression data. Explained variance is plotted for each gene along the y-axis boxplot, as well as the gene frequency (vertical histogram/violin shape). Factors are sorted by median explained variance, from highest to lowest.

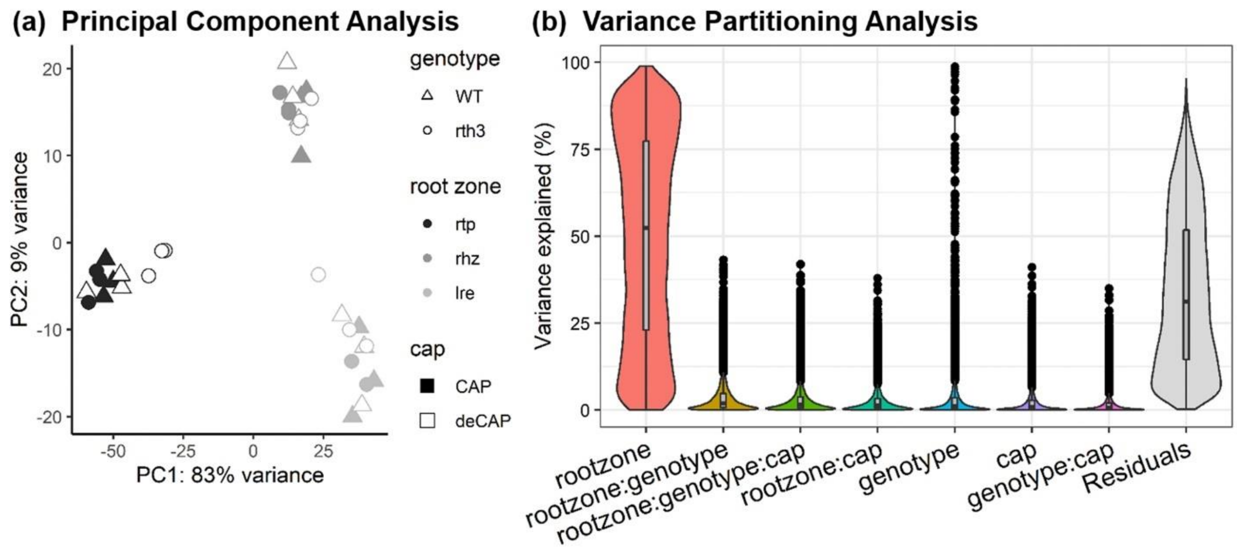

Fig. S7 Root zone-specific genes and enriched GO terms (padj<0.01, |LFC|>1). Gradient-colored boxes to the right of each GO term indicates its significance. Below are shown expression count boxplots (normalized counts in DESeq2) for selected genes related to the enriched GO term categories. The horizontal line within each box represents the median. Whiskers extend to the lowest and highest scores within 1.5 times the interquartile range from the box. Data beyond this range are represented by individual points. Colors (green, orange, blue) in the boxplots refer to the root zone (RTP, RHZ, LRE).

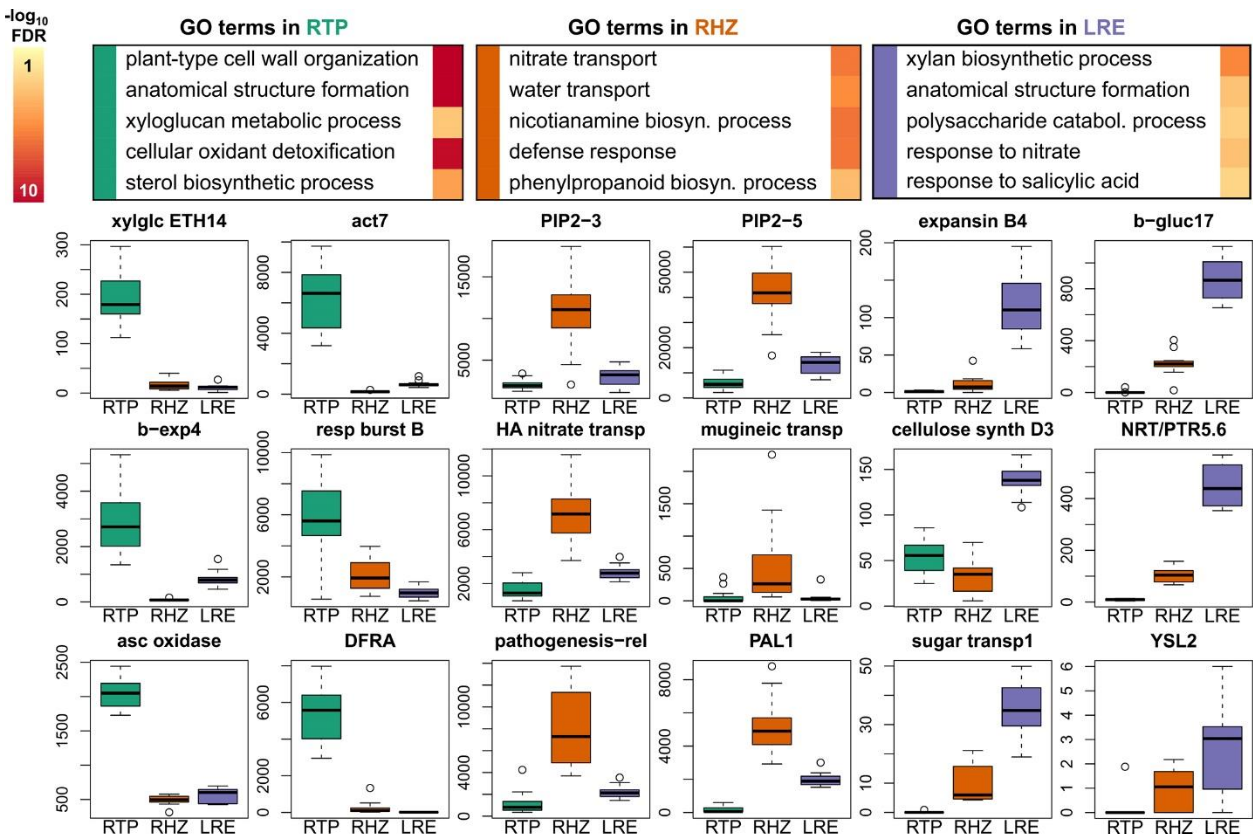

Fig. S8 Venn diagrams of root zone differences in either HAIR or NoHAIR samples. Shown are the number and the overlap of differentially expressed genes for each pairwise root zone contrast (RHZ vs RTP, LRE vs RTP, LRE vs RHZ; Condition vs baseline-Condition) in HAIR or NoHAIR samples.

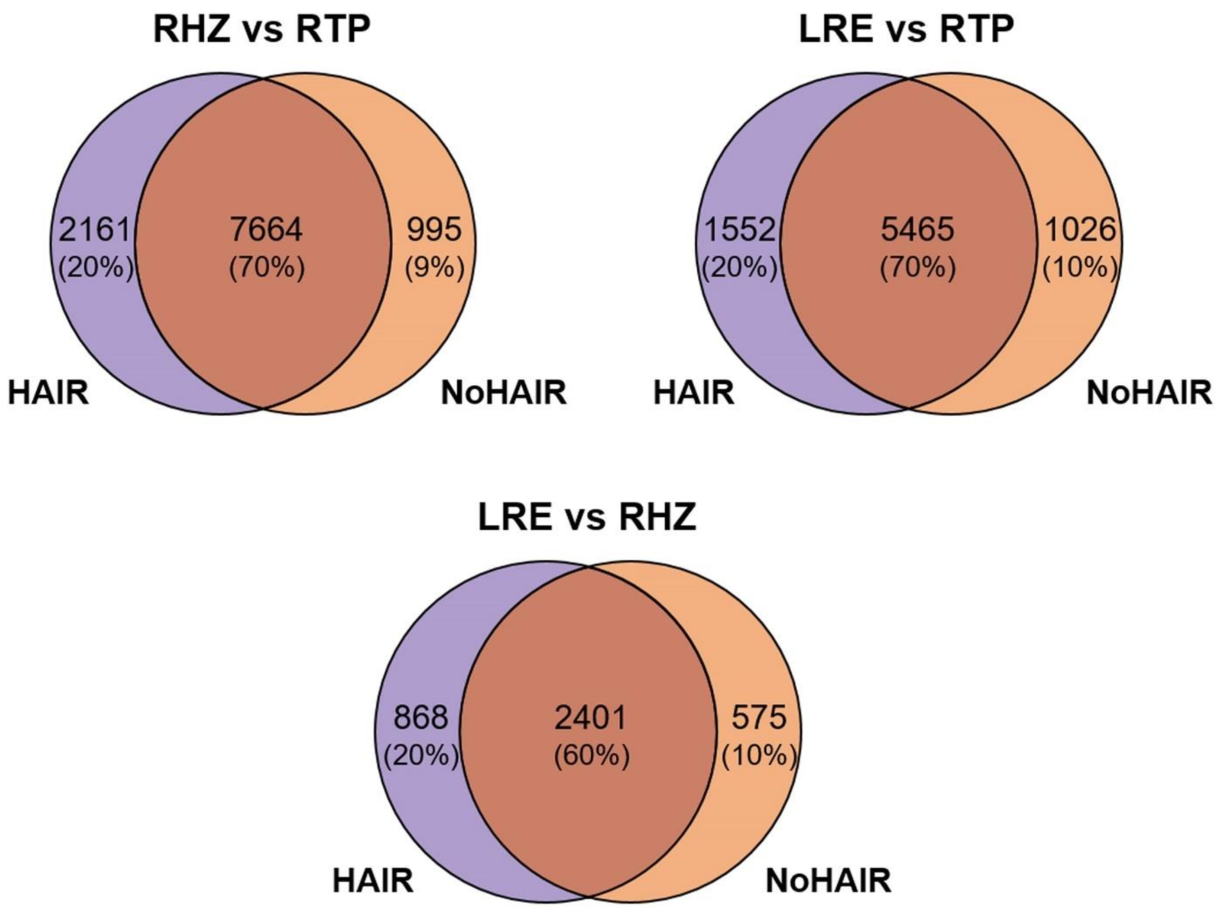

Table S1 Combinations of primer tag sequences used for barcoding of cercozoan and endomyxan sequences during the second PCR amplification.

Table S2 Differentially expressed genes (padj<0.01) by root zonation, decapping, and root hair treatment. Contrasts are denoted as Condition vs Baseline-Condition. Differential expression by root hair genotype and decap treatment was also compartmentalized for each root zone (denoted as ROOTZONE_Condition vs Baseline-Condition (e.g. RTP_DeCAP vs CAP).

Table S3 Analysis of Variance (ANOVA) conducted on primary root length, total root length and average root diameter of *Zea mays* L. plants with HAIR, and CAP treatment. Nominator, denominator degrees of freedom (df), F-values and p-values of one-way ANOVA.

ANOVA									
	Primary root length			***Total root length***			***Average root diameter***		
	df	***F***	***P***	***df***	***F***	***P***	***df***	***F***	***P***
CAP	1, 19	4.38	0.0501	1, 19	3.11	0.094	1, 19	7.27	0.014
HAIR	1, 19	1.55	0.229	1, 19	0.001	0.97	1, 19	5.34	0.032
CAP : HAIR	1, 19	0.23	0.635	1, 19	0.227	0.639	1, 19	4.15	0.056

Table S4 Analysis of Variance (ANOVA) conducted on protist (Cercozoa and Endomyxa) and prokaryote (archaea and bacteria) OTU-richness, Shannon diversity (H’) and Pielou evenness (J’) in the rhizosphere of *Zea mays* L. The factors root zone, CAP, and HAIR were included in the analysis. Nominator, denominator degrees of freedom (df), F-values and p-values.

		ANOVA								
		OTU-richness			**H’**			**J’**		
		df	***F***	***P***	***df***	***F***	***P***	***df***	***F***	***P***
Prokaryota	root zone	2, 201	65.89	< 0.001***	2, 201	92.924	< 0.001***	2, 201	91.56	< 0.001***
	CAP	1, 201	9.82	0.002**	1, 201	8.76	0.003**	1, 201	7.82	0.006**
	HAIR	1, 201	1.6	0.207	1, 201	0.32	0.571	1, 201	0.15	0.7
	root zone : CAP	2, 201	9.33	< 0.001***	2, 201	11.37	< 0.001***	2, 201	10.97	< 0.001***
	root zone : HAIR	2, 201	0.67	0.513	2, 201	0.46	0.634	2, 201	0.38	0.686
	CAP: HAIR	1, 201	0	0.99	1, 201	0.05	0.816	1, 201	0.08	0.783
	root zone : CAP: HAIR	2, 201	0.77	0.466	2, 201	0.47	0.627	2, 201	0.38	0.685
Protists	root zone	2, 203	2.42	0.092	2, 203	22.71	< 0.001***	2, 203	23.85	< 0.001***
	CAP	1, 203	1.9	0.17	1, 203	0.49	0.484	1, 203	0.12	0.733
	HAIR	1, 203	2.94	0.088	1, 203	3.73	0.055	1, 203	2.37	0.125
	root zone : CAP	2, 203	4.72	0.0099**	2, 203	21.76	< 0.001***	2, 203	18.77	< 0.001***
	root zone : HAIR	2, 203	0.45	0.641	2, 203	0.65	0.523	2, 203	1.03	0.359
	CAP: HAIR	1, 203	0.22	0.64	1, 203	2.01	0.158	1, 203	2.68	0.103
	root zone : CAP: HAIR	2, 203	0.31	0.736	2, 203	1.32	0.27	2, 203	1.13	0.324

Table S5 Analysis of Variance (ANOVA) conducted on prokaryote (archaea and bacteria) abundance, protist (Cercozoa and Endomyxa) and prokaryote (archaea and bacteria) OTU-richness, Shannon diversity (H'), Pielou evenness (J') and beta dispersal in the rhizosphere of *Zea mays* L. The root regions root tip (RTP), root hair zone (RHZ) and lateral root emergence zone (LRE) were compared in treatments with (CAP) and without (DeCAP) root caps and root hairs (HAIR and NoHAIR) individually. The table contains F-values with nominator, denominator degrees of freedom (df) and p-values.

ANOVA treatment combinations

		Abundance (qPCR)			**OUT-richness**			**H'**			**J'**			**Beta dispersal**		
	Treatment	***df***	***F***	***P***	***df***	***F***	***P***	***df***	***F***	***P***	***df***	***F***	***P***	***df***	***F***	***P***
Prokaryota	CAP HAIR	2, 50	2.03	0.143	2, 50	23.8	< 0.001 ***	2, 50	34.6	< 0.001 ***	2, 50	35.0	< 0.001 ***	2, 50	14.97	< 0.001 ***
	DeCAP HAIR	2, 51	3.18	0.050	2, 50	9.9	< 0.001 ***	2, 50	13.8	< 0.001 ***	2, 50	13.9	< 0.001 ***	2, 50	2.2	0.12
	CAP NoHAIR	2, 50	0.32	0.729	2, 50	32.5	< 0.001 ***	2, 50	45.2	< 0.001 ***	2, 50	42.6	< 0.001 ***	2, 50	17.9	< 0.001 ***
	DeCAP NoHAIR	2, 51	1.09	0.344	2, 51	6.1	0.004 **	2, 51	8.0	< 0.001 ***	2, 51	7.8	0.001 **	2, 51	7.1	0.002 **
Protists	CAP HAIR				2, 51	2.4	0.105	2, 51	20.4	< 0.001 ***	2, 51	16.1	< 0.001 ***	2, 51	4.9	0.011 *
	DeCAP HAIR				2, 51	0.3	0.72	2, 51	5.8	0.005 **	2, 51	5.8	0.005 **	2, 51	5.1	0.0095 **
	CAP NoHAIR				2, 50	4.0	0.025 *	2, 50	14.0	< 0.001 ***	2, 50	14.9	< 0.001 ***	2, 50	4.2	0.021 *
	DeCAP NoHAIR				2, 51	0.1	0.891	2, 51	5.1	0.0098 **	2, 51	6.1	0.004 **	2, 51	5.7	0.006 **

Table S6 Analysis of Variance (ANOVA) conducted on prokaryote (archaea and bacteria) abundance in the rhizosphere of *Zea mays* L. The factors root zone, CAP, and HAIR were included in the analysis. The table contains nominator, denominator degrees of freedom (df), F-values and p-values.

		ANOVA		
		Abundance (qPCR)		
		df	***F***	***P***
Prokaryota	root zone	2, 202	2.44	0.099
	CAP	1, 202	0.31	0.576
	HAIR	1, 202	1.52	0.219
	root zone : CAP	2, 202	0.73	0.481
	root zone : HAIR	2, 202	1.3	0.275
	CAP : HAIR	1, 202	0.05	0.825
	root zone : CAP : HAIR	2, 202	0.84	0.434

Table S7 Prokaryote abundances measured by qPCR, given in number of copies per gram soil dry weight.

Table S8 Permutational Multivariate Analysis of Variance (PERMANOVA) using Bray-Curtis dissimilarities, permuted 999 times, and variance partitioning to test for differences in beta diversity of prokaryota (bacteria and archaea) and protists (Cercozoa and Endomyxa) in the rhizosphere of *Zea mays* L. The factors root zone, CAP and HAIR and their interactions were included in the analysis. The table contains F-values with nominator, denominator degrees of freedom (df) and R^2 (PERMANOVA) or variance explained (variance partitioning) and p-values.

		PERMANOVA			
		df	***F***	***R²***	***P***
Prokaryota	root zone	2, 201	18.5	0.15	0.001 ***
	CAP	1, 201	4.0	0.02	0.001 ***
	HAIR	1, 201	2.2	0.01	0.016 *
	root zone : CAP	2, 201	2.1	0.02	0.002 **
	root zone : HAIR	2, 201	1.0	0.01	0.361
	CAP : HAIR	1, 201	1.7	0.01	0.040 *
	root zone : CAP : HAIR	2, 201	1.0	0.01	0.306
Protists	root zone	2, 203	17.1	0.13	0.001 ***
	CAP	1, 203	6.3	0.02	0.001 ***
	HAIR	1, 203	2.2	0.01	0.007 **
	root zone : CAP	2, 203	3.8	0.03	0.001 ***
	root zone : HAIR	2, 203	1.1	0.01	0.255
	CAP : HAIR	1, 203	2.1	0.01	0.008 **
	root zone : CAP : HAIR	2, 203	1.6	0.01	0.014 *
		PERMANOVA treatment combinations			
		df	***F***	***R²***	***P***
Prokaryota	CAP - HAIR	2, 50	6.6	0.21	0.001 ***
	DeCAP - HAIR	2, 50	3.7	0.13	0.001 ***
	CAP - NoHAIR	2, 50	7.0	0.22	0.001 ***
	DeCAP -NoHAIR	2, 51	2.9	0.10	0.001 ***
Protists	CAP - HAIR	2, 51	7.7	0.23	0.001 ***
	DeCAP - HAIR	2, 51	4.6	0.15	0.001 ***
	CAP - NoHAIR	2, 50	8.1	0.25	0.001 ***
	DeCAP -NoHAIR	2, 51	3.5	0.12	0.001 ***
		Variance partitioning			
		Variance expl. (%)	***df***	***F***	***P***
Prokaryota	root zone	7.49	1, 211	18.16	<0.001
	CAP	0.76	1, 211	2.36	<0.001

	HAIR	0.22	1, 211	1.47	0.029
	total	8.55	1, 211	7.61	<0.001
Protists	root zone	8.36	1, 213	20.22	<0.001
	CAP	1.84	1, 213	4.80	<0.001
	HAIR	0.39	1, 213	1.75	0.019
	total	10.47	1, 213	9.34	<0.001

Table S9 Enriched Gene Ontology terms for root zone comparisons and root zone-specific gene expression. Genes included in the analysis were differentially expressed between root zone contrasts (padj<0.05, |LFC|>1). Contrasts are denoted as Condition vs Baseline-Condition. Root zone-specific genes were considered genes that were significantly higher expressed in one root zone compared to the remaining root zones.

Table S10 Number of DEG between root zones, root hair genotypes and decap treatments. Genes included in the analysis were differentially expressed between root zone contrasts (padj<0.05, |LFC|>1). Contrasts are denoted as Condition vs Baseline-Condition.

Methods S1 Rhizobox construction and preparation of maize seedlings.

To enable root zone-specific sampling, rhizoboxes made of PVC were constructed with a removable perspex front window. The bottom openings of the columns were closed by tape, leaving only a gap for a protruding strip of a glass fiber wicker for passive watering by immersing it in a container with water (Supporting information Fig. 1). Ethanol-sterilized rhizobox columns were filled with sieved loam (1 mm) from a Haplic Phaeozem close to Schladebach in Saxony-Anhalt with a sand/silt/clay ratio of 33/48/19 (for soil parameters see Vetterlein *et al.*, 2021), harboring its original microbial community (the same soil was used in Rüger *et al*., 2021). Maize seeds were surface-sterilized with 10% H_2O_2 under vacuum for 10 min, rinsed with sterile water and subsequently placed on sterile wet filter paper in Petri dishes. Sealed Petri dishes were placed in a 30° vertical position in the dark at 20 °C for germination. Next, root caps of half of the seedlings with straight vertical primary roots were removed as in Humphris *et al.* (2005). Each cap junction was checked and only roots whose caps came off cleanly at the first attempt were used for further experimentation. Special care was taken that the root tip was not harmed by the dissection, and successful dissection of the cap was reflected by root gene expression with low numbers of DEG and the induction of only few stress response genes in the DEG palette when comparing DeCAP and CAP treatments at RTP. Roots of seedlings were then placed into holes in the center of each soil column, which were prepared using a sterile needle. To ensure root growth along the rhizobox windows, boxes were placed on racks at an angle of 30° to the vertical.

Methods S2 Quantification of prokaryota by qPCR.

Quantitative polymerase chain reaction (qPCR) to quantify prokaryote abundance was performed as described in Bukovská *et al*. (2021). Briefly, calibration was conducted with amplicons generated from soil DNA extracts with the same primers which were then used for the qPCR analyses (Eub338F/Eub518R), using the *TP HS DNA-free 2x Master Mix* (TopBio, Vestec, Czech Republic). Amplicons were purified from the PCR mixture by *QIAquick PCR purification kit* (Qiagen, Holden, Germany), quantified by Picogreen fluorescence, and serially diluted to reach a broad range of fragment copy numbers (from dozens to hundreds of millions per microliter). The qPCR was performed in a total volume of 20 µl containing 10 µl *Luna Universal qPCR 2x Master Mix* (New England Biolabs, M3003), 2 µl template DNA, 0.5 µl of 10 µM forward primer Eub 338 (5'-ACTCCTACGGGAGGCAGCAG-3') and 0.5 µl of 10 µM reverse primer Eub518 (5'-ATTACCGCGGCTGCTGG-3'), using a LightCycler 480 II Instrument (Roche Molecular Systems, Rotkreuz, CHE). The following cycling steps were repeated for 55 cycles, after initial denaturation at 95°C for 5 min: denaturation at 95 °C for 10 s (ramp 4.4 °C s^{-1}), annealing at 55 °C for 20 s (ramp 2.0 °C s^{-1}), and amplification at 72 °C for 25 s (ramp 4.4°C s^{-1}). The qPCR results were recorded as the second derivation maximum of the amplification curves. Melting curve profiles were checked to eliminate false positives showing unusually low melting temperatures (Tm) (e.g., due to non-specific primer dimer formation).

Methods S3 Processing of sequencing data

Prokaryota

Based on the index combinations, sequences were demultiplexed to individual samples. Thereafter, sequencing adapters were removed using cutadapt (Martin, 2011). All subsequent steps were carried out in SEED2 software (Větrovský *et al.*, 2018). First, forward and reverse sequence reads were paired when an overlap of 20 bp was detected, with a maximum allowed mismatch set at 15%. Sequences with an average quality score of less than 30, a per-base quality score of less than 7, and a length of less than 200 bp were removed. Further, potentially chimeric sequences were removed, and remaining sequences clustered at 97 % similarity level (using usearch v. 8.1.1861 embedded in SEED2 software). All resulting OTUs were identified by comparing (using Blastn) with the SILVA SSU database. All sequences belonging to Eukaryota (mitochondria, chloroplasts) were then removed and remaining data resampled (rarefied) to 26,000 sequences per sample (except for three individual samples which were slightly below this threshold). The resulting sequences were clustered again at 97% similarity level, and the most abundant sequences from each OTU were used for identification via RDP classifier with the SILVA database as reference. All OTUs represented by less than 55 reads (accounting for < 0.001% of all reads) were discarded.

Protists

Reads were processed using the customized MOTHUR pipeline version 1.45.3 (Schloss *et al.*, 2009). First paired-end reads were merged with a minimum overlap of 200 bp and only if there were no mismatches in primer and barcode sequences and maximum two mismatches and one ambiguity in the target sequence. All sequences which did not meet the default parameters of the quality control, or which were smaller than 300 bp were removed. Sequences were clustered into operational taxonomic units (OTUs) using VSEARCH (Rognes *et al.*, 2016) according to the abundance-based greedy algorithm (agc) with a similarity threshold of 97%. OTUs represented by less than 1000 reads were removed as likely to represent amplification or sequencing noise (Fiore-Donno *et al.*, 2018). OTUs were assigned to taxa using BLAST+ (Camacho *et al.*, 2009) with an e-value of 1^{-50} and the PR2 database (Guillou *et al.*, 2013), keeping only the best hit. Cercozoan and endomyxan sequences were aligned with the template provided by Fiore-Donno *et al.* (2018) allowing gaps of maximum 5 nucleotides. Chimeras were identified using UCHIME (Edgar *et al.*, 2011) and removed. Samples were re-sampled (rarefied) to 5290 sequences per sample, with no further OUT exclusion.

Root gene expression

Data processing and analysis were conducted as described before (Ganther *et al.*, 2020). Briefly, adapter removal and quality trimming were performed using the default parameters of 'Trimmomatic' (Bolger *et al.*, 2014). Cleaned reads were aligned onto the maize B73_RefGen_v4 genome with 'HISAT2' (Kim *et al.*, 2019). Reads were assigned to genes using 'featurecounts' of the 'subread' package (Liao *et al.*, 2019). Gene descriptions from the Plants Ensembl database (plants.ensembl.org) were assigned to the gene identifiers.

Methods S4 Statistical analysis.

Microbial abundance and diversity

All analyses, data handling and visualization were performed in R version 4.0.3 (R Core Team, 2020) using the packages: 'dplyr' (Wickham *et al*., 2018), 'agricolae' (de Mendiburu, 2021), 'vegan'

(Oksanen *et al.*, 2017), ‘car’ (Fox and Weisberg, 2019), ‘gridExtra’ (Auguie, 2017) ‘RColorBrewer’ (Neuwirth, 2014) and ‘ggplot2’ (Wickham, 2016). Rarefaction curves were calculated with the package ‘iNEXT’ (Hsieh *et al.,* 2020), to confirm sufficient sequencing depth of microbial diversity. One sample each of the protist and of the prokaryote dataset were identified as outliers and removed. Calculations of alpha and beta diversity were based on relative abundances of OTUs per sample. OTU richness, Pielou evenness, Shannon diversity and prokaryote abundance were compared by analysis of variance (ANOVA) with Tukey’s Honestly Significant Difference (agricolae: HSD.test). Permutational Multivariate Analysis of Variance (PERMANOVA) using Bray-Curtis dissimilarities, permuted 999 times, tested for differences in beta diversity (vegan: adonis (Anderson, 2001)), visualized by Non-Metric Multidimensional Scaling (NMDS, vegan: metaMDS). In order to analyze if root cap or hair status or differences between root zones had stronger effects on microbial community composition, variance partitioning of beta diversities (vegan: varpart) was applied. Variation of microbial beta diversity on respective root zones was calculated as multivariate homogeneity of group dispersions and compared by PERMDISP2 (vegan: betadisper (Anderson *et al.*, 2006)) and Tukey’s HSD. To assess how much variance in beta diversity of prokaryotes could be explained by diversity of their protistan predators, the first two axes of a principal coordinates analysis (PCoA) of cercozoan Bray Curtis dissimilarities were extracted and used as explanatory variables for Bray Curtis dissimilarities of prokaryote data in a db-RDA (vegan: capscale).

Root gene expression

Differential gene expression was determined with the R package ‘DESeq2’ (Love *et al.*, 2014). After filtering genes with low-expression counts (< 10 counts in all samples combined), 29,981 genes remained for differential gene expression analysis. Genes with a corrected p-value (Benjamini-Hochberg adjustment, padj) <0.01 were considered as differentially expressed. Gene set enrichment analysis was conducted using the R package ‘GOseq’, utilizing the maize-GAMER gene ontology (GO) annotation by Wimalanathan *et al.*, (2018). For this, differentially expressed genes with an absolute $\log_2$ fold change (LFC) > 1 were included. Variance partitioning analysis was conducted with the R package ‘VariancePartitioning’ (Hoffman & Schadt, 2016).

References

Anderson MJ. **2001**. A new method for non-parametric multivariate analysis of variance. *Austral Ecology*. doi: 10.1111/j.1442-9993.2001.01070.pp.x

Anderson MJ, Ellingsen KE, McArdle BH. **2006**. Multivariate dispersion as a measure of beta diversity. *Ecology Letters*. doi: 10.1111/j.1461-0248.2006.00926.x

Auguie B. 2015. *gridExtra: Miscellaneous functions for "Grid" graphics*. R package version 2.3. https://CRAN.R-project.org/package=gridExtra [accessed 1 June 2022]

Bukovská P, Rozmoš M, Kotianová M, Gančarčíková K, Dudáš M, Hršelová H, Jansa J. 2021. Arbuscular mycorrhiza mediates efficient recycling from soil to plants of nitrogen bound in chitin. *Frontiers in Microbiology*. doi: 10.3389/fmicb.2021.574060

Bolger AM, Lohse M, Usadel B. **2014**. Genome analysis Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics*. doi: 10.1093/bioinformatics/btu170

Camacho C, Coulouris G, Avagyan V, Ma N, Papadopoulos J, Bealer K, Madden TL. **2009**. BLAST+: architecture and applications. *BMC Bioinformatics*. doi: 10.1186/1471-2105-10-421

de Mendiburu F. 2021. *Agricolae: statistical procedures for agricultural research.* R package version 1.3-3. https://CRAN.R-project.org/package=agricolae [accessed 1 June 2022]

Edgar RC, Haas BJ, Clemente JC, Quince C, Knight R. **2011**. UCHIME improves sensitivity and speed of chimera detection. *Bioinformatics*. doi: 10.1093/bioinformatics/btr381

Fiore-Donno AM, Rixen C, Rippin M, Glaser K, Samolov E, Karsten U, Becker B, Bonkowski M. **2018**. New barcoded primers for efficient retrieval of cercozoan sequences in high-throughput environmental diversity surveys, with emphasis on worldwide biological soil crusts. *Molecular Ecology Resources*. doi: 10.1111/1755-0998.12729

Fox J, Weisberg S. 2019. *An R companion to applied regression, third edition. USA: Sage publications.* https://socialsciences.mcmaster.ca/jfox/Books/Companion/ [accessed 1 June 2022]

Ganther M, Yim B, Ibrahim Z, Bienert MD, Lippold E, Maccario L, Sørensen SJ, Bienert GP,

Vetterlein D, Heintz-buschart A, *et al.* 2020. Compatibility of X-ray computed tomography with plant gene expression , rhizosphere bacterial communities and enzyme activities. *Journal of Experimental Botany*. doi: 10.1093/jxb/eraa262

Guillou L, Bachar D, Audic1 S, Bass D, Berney C, Bittner L, Boutte C, Burgaud G, Vargas C de, Decelle J, *et al.* 2013. The protist ribosomal reference database (PR 2): a catalog of unicellular eukaryote Small Sub-Unit rRNA sequences with curated taxonomy. *Nucleic Acids Research*. doi: 10.1093/nar/gks1160

Hoffman GE, Schadt EE. **2016**. variancePartition: interpreting drivers of variation in complex gene expression studies. *BMC Bioinformatics*. doi: 10.1186/s12859-016-1323-z

Hsieh TC, Ma KH, Chao A. 2020. *iNEXT: interpolation and extrapolation for species diversity*. R package version 2.0.20. http://chao.stat.nthu.edu.tw/wordpress/software_download/ [accessed 1 June 2022]

Humphris SN, Bengough AG, Griffiths BS, Kilham K, Rodger S, Stubbs V, Valentine TA, Young IM. 2005. Root cap influences root colonisation by Pseudomonas fluorescens SBW25 on maize. FEMS Microbiology Ecology. doi: 10.1016/j.femsec.2005.03.005

Kim D, Paggi JM, Park C, Bennett C, Salzberg SL. **2019**. Graph-based genome alignment and genotyping with HISAT2 and HISAT-genotype. *Nature Biotechnology*. doi: 10.1038/s41587-019-0201-4

Liao Y, Smyth GK, Shi W. **2019**. The R package Rsubread is easier, faster, cheaper and better for alignment and quantification of RNA sequencing reads. *Nucleic Acids Research*. doi: 10.1093/nar/gkz114

Love MI, Huber W, Anders S. **2014**. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biology*. doi: 10.1186/s13059-014-0550-8

Martin M. 2011. Cutadapt removes adapter sequences from high-throughput sequencing reads. *EMBnet.journal*. doi: 10.14806/ej.17.1.200

Neuwirth E. 2014. *RColorBrewer: ColorBrewer palettes.* R package version 1.1-2. https://CRAN.R-project.org/package=RColorBrewer [accessed 1 June 2022]

Oksanen J, Blanchet FG, Friendly M, Kindt R, Legendre P, McGlinn D. 2017. *Vegan: community ecology package*. R package version 2.5-7. https://CRAN.R-project.org/package=vegan [accessed 1 June 2022]

Rognes T, Flouri T, Nichols B, Quince C, Mahé F. **2016**. VSEARCH: a versatile open source tool for metagenomics. *PeerJ*. doi: 10.7717/peerj.2584

Schloss PD, Westcott SL, Ryabin T, Hall JR, Hartmann M, Hollister EB, Lesniewski RA, Oakley BB, Parks DH, Robinson CJ, *et al.* 2009. Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. *Applied and Environmental Microbiology*. doi: 10.1128/AEM.01541-09

Rüger L, Feng K, Dumack K, Freudenthal J, Chen Y, Sun R, Wilson M, Yu P, Sun B, Deng Y, *et al*. 2021. Assembly patterns of the rhizosphere microbiome along the longitudinal root axis of maize (*Zea mays* L.). *Frontiers in Microbiology*. doi: 10.3389/fmicb.2021.614501

Vetterlein D, Lippold E, Schreiter S, Phalempin M, Fahrenkampf T, Hochholdinger F, Marcon C, Tarkka M, Oburger E, Ahmed M, *et al.* 2021. Experimental platforms for the investigation of spatiotemporal patterns in the rhizosphere—Laboratory and field scale. *Journal of Plant Nutrition and Soil Science*. doi: 10.1002/jpln.202000079

Větrovský T, Baldrian P, Morais D. **2018**. SEED 2: a user-friendly platform for amplicon high-throughput sequencing data analyses. *Bioinformatics*. doi: 10.1093/bioinformatics/bty071

Wickham H, François R, Henry L, Müller K. 2018. *dplyr: a grammar of data manipulation*. R Package Version 1.0.6. https://CRAN.R-project.org/package=dplyr. [accessed 1 June 2022]

Wickham H. 2016. ggplot2: elegant graphic for data analysis. New York, USA: Springer-Verlag.

Wimalanathan K, Friedberg I, Andorf CM, Lawrence-dill CJ. **2018**. Maize GO annotation—methods, evaluation ,and review (maize-GAMER). *Plant Direct*. doi: 10.1002/pld3.52