

1 **Higher stochasticity of microbiota composition in seedlings of domesticated wheat**
2 **compared to wild wheat**

3

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8 **Supplementary Methods**

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10 **Origin of the wheat material**

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12 Seed collections of *Triticum dicoccoides*, *Triticum boeoticum* and *Triticum urartu* were
13 collected from different locations in South- East Turkey in previous years (2004, 2005, 2006)
14 (Supp. Table 1). Seeds of *T. aestivum* from Turkey was collected by the farmer from a field in
15 Kışlak, Antakya in 2017 (Supp. Table 1) and stored in a dry and cold room. We obtained these
16 seeds in the same year from the farmer and we stored them at 4 °C until further usage.
17 Seeds of *T. aestivum* from Germany (Benchmark cv.) were collected from the experimental
18 farm of the Kiel University in Hohenschulen, Schleswig-Holstein in 2017. All the seeds in the
19 collections were stored at 4 °C until further usage and processed all the seeds in the
20 collections in 2018.

21

22

23 **Surface sterilization of seeds**

24 Seeds of *Triticum aestivum*, *Triticum dicoccoides*, *Triticum boeoticum* and *Triticum urartu*
25 were washed with sterile miliQ water. Later, each seed was treated shortly with 0.1%
26 TritonX and washed with water, then 80% EtOH and washed with water and lastly 1.2 %
27 Sodium Hypochlorite (NaClO) and finally washed with water three times. Wash-off water
28 from some seeds were kept and sequenced. Some seeds were immediately processed for
29 DNA extraction while others were grown in sterile jars.

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33 **Axenic propagation of leaves and roots**

34 After sterilization, seeds were directly transferred into the plant minimal medium (PNM). 1L
35 of PNM medium is composed 1 mL of 500 mM Potassium Nitrate, 1mL of KH₂PO₄ stock
36 solution (5g/ 100mL), 1 mL of K₂HPO₄ stock solution (2.5 g/100mL), 1mL of 2M Magnesium
37 Sulfate (MgSO₄ X 7 H₂O), 1 mL of 200 mM Calcium Nitrate, 2.5 mL of Fe-EDTA and 1mL of
38 Sodium Chloride stock solution (2.5 g/100 mL). The pH of the solution was adjusted to 6.2
39 and the medium was solidified by adding Gelrite. Finally, the medium was autoclaved at 121
40 °C for 15 minutes. After autoclaving, 10 mL 1M filtered MES was added to the medium. The
41 medium was transferred into sterile jars and after it was solidified, sterilized seeds were
42 transferred into the medium. Seeds were germinated in sterile jars in a climate chamber
43 (Percival plant growth chambers, CLF PlantClimatics GmbH, Wertingen, Germany). Light
44 period of 16/8 hours, temperature 15 °C) for two weeks corresponding to the second leaf
45 growth stage.

46

47

48 **DNA Extraction from seeds and seedling material**

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50 We followed a similar method to the Phenol-Chloroform extraction method customized by
51 Agler et al, 2016 (1). For DNA extractions, seed, leaf and root materials was processed as
52 follows: Single seeds were transferred into 7mL tubes containing sterilized zirconium beads.
53 Approximately six cm of leaves and six cm of multiple roots were used for DNA extraction.
54 We crushed samples with Bertin Precellys Instrument (Bertin Instruments, Montigny-le-
55 Bretonneux, France) pre-cooled with liquid nitrogen at 7500 rpm for 2 x 30 seconds with an
56 in-between pause of 15 seconds. To the crushed samples, we added 0.6 mL of DNA
57 extraction buffer (0.5% SDS, 50 mM TRIS buffer at pH 8, 200 mM NaCl, 2mM EDTA). Next, we
58 added 50 µg/mL of Lysozyme and 10 µg/mL of Proteinase K and incubated the samples for
59 45 min at 37°C. After incubation, samples were transferred into new tubes containing 0.1
60 mm zirconium and 0.5mm glass beads and beat-treated using Bertin Precellys (Bertin
61 Instruments, Montigny-le-Bretonneux, France) at 6500 rpm for 2 x 30 seconds with a 15

62 second pause. 10 µg/mL RNase was added to each sample before incubation at 37°C for 45
63 minutes. We next centrifuged the samples at 13000 rpm for 2 minutes to remove beads and
64 non-homogenized tissue. The nucleic acids were cleaned up with
65 phenol/chloroform/isoamyl alcohol (25:24:1) for three times, and chloroform/isoamyl
66 alcohol (24:1) one time, then precipitated by adding 1/10th of the sample volume of 3 M
67 sodium acetate and 2.5x volume 100% ethanol. Samples were then centrifuged at 4°C at
68 13000 rpm for 30 minutes. Afterwards, the pellet was washed with 70% ethanol. Finally,
69 pellets were washed in 100 µL of sterile water and stored at -20 °C until further usage. DNA
70 extracted from seeds was treated with 1:1 with 20% Chelex-100 for 30 minutes to remove
71 potential PCR inhibitors. Harvested leaves and roots were processed with the PowerSoil DNA
72 kit (Mo Bio Laboratories, Heidelberg, Germany) according to the manufacturer's instructions
73 (See Material and Methods).

74

75

76 **DNA library preparation**

77

78 We prepared libraries for replicates of individual seed as well as leaf and root samples,
79 respectively. In addition, we included three negative controls of DNA extraction and three
80 wash-off controls from the surface sterilization to the libraries.

81 PCR amplification was performed in two steps (Suppl. Table 1 and 2). For 16S, we used the
82 interfering primer approach developed by Agler and co-workers for *Arabidopsis thaliana*
83 (Suppl. Table 3). However, we modified the primers according to the wheat genome (2). In
84 the first step, universal primers primers Bacteria V5/V6/V7: B799F/ B1192R and for fungi
85 ITS1: ITS1F/ITS2 were used to amplify the targeted regions (Suppl Table 3). Interfering
86 primers for the 16S region were added in this step. Triplicates of each reaction were
87 performed for both first and second steps. First PCR was run for 15 and 20 cycles for 16S and
88 ITS, respectively. After the first reactions, three replicates were combined. 10 µL of each PCR
89 product was cleaned with 0.5 µL Antarctic phosphatase and 0.5 µL Exonuclease I in 1.22 µL
90 Antarctic phosphatase buffer (New England Biolabs GMBH, Frankfurt, Germany) at 37 °C for
91 30 minutes followed by 80 °C for 15 min. The product of the cleaned samples was used as a
92 template for the second PCR in which unique barcodes for sequencing of 12bp length were

93 added as well. Second PCRs were run for 20 and 15 cycles for 16S and ITS, respectively
 94 including in total 35 cycles for both 16S and ITS reactions.

95

96 **Table 1: PCR conditions for the V5-7 amplification**

97

V5-V7				
<u>1st PCR:</u>		<u>1st PCR</u>		
		<u>conditions:</u>		
10 µL	Phusion Master Mix	98°C	30s	
1 µL	Forward Primer	98°C	10s	
1 µL	Reverse Primer	55°C	45s	X15 cycles
2 µL	Interfering Forward Primer	72°C	30s	
2 µL	Interfering Reverse Primer	72°C	5m	
0.5 µL	DMSO			
3 µL	DNA			
0.5 µL	H2O			
Clean-up with ExoI and Antarctic Phosphatase	10 µL of the 1st PCR product is cleaned-up	37°C 80°C	30m 15m	
<u>2nd PCR:</u>		<u>2nd PCR</u>		
		<u>conditions:</u>		
10 µL	Phusion Master	98°C	30s	

	Mix			
1 µL	Forward Primer	98°C	10s	
1 µL	Barcoded Reverse Primer	55°C	45s	X20 cycles
0.5 µL	DMSO	72°C	30s	
3 µL	Clean-up product	72°C	5m	
4.5 µL	H2O			

98

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100

101 **Table2: PCR conditions for the ITS1-ITS2 amplification**

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ITS1F-ITS2				
<u>1st PCR:</u>		<u>1st PCR</u> <u>conditions:</u>		
10 µL	Phusion Master Mix	98°C	30s	
1 µL	Forward Primer	98°C	10s	
1 µL	Reverse Primer	55°C	30s	X20 cycles
0.5 µL	DMSO	72°C	30s	
1 µL	DNA	72°C	5m	
6.5 µL	H2O			
Clean-up with ExoI and Antartic	10 µL of the 1st PCR product is	37°C 80°C	30m 15m	

Phosphatase	cleaned-up			
2nd PCR:		2nd PCR conditions:		
10 µL	Phusion Master Mix	98°C	30s	
1 µL	Forward Primer	98°C	10s	
1 µL	Barcoded Reverse Primer	55°C	30s	X15 cycles
0.5 µL	DMSO	72°C	30s	
1 µL	Clean-up product	72°C	5m	
6.5 µL	H2O			

103

104 Finally, PCR products of ITS and 16S amplifications were sub-pooled in equal concentrations
105 where the concentration of each sample was estimated using the software of gel visualizer
106 (BIO RAD, Image Lab™ software 5.2.1). Subpools of 16S amplicons were run on the gel and
107 bacterial DNA was cut out of the gel. DNA concentration of the subpools were quantified
108 with Qubit. Then, we combined subpools in equal concentrations into final pools. Finally, we
109 quantified the final concentration of the final pool with Qubit 3.0 Fluorometer (Thermo
110 Fisher Scientific, Darmstadt, Germany).

111

112

113 **Amplicon Sequencing**

114 The final DNA pool spiked with 10% PhiX genomic DNA (Illumina, San Diego, CA)
115 was used for MiSeq sequencing (Illumina, San Diego, CA) using the MiSeq Reagent Kit v3
116 (Illumina, San Diego, CA). 0.5 µM of forward, reverse and index sequencing primers
117 complementary to the linker/primer region of the concatenated primers were added

118 together (sequence of primers are available in Suppl Table 3). Paired-end sequencing was
119 performed for 600 cycles for 301 bp amplicon size according to the Illumina instructions
120 (<http://os.bioprotocol.org/attached/file/20171217/miseq%20denature%20dilute%20libraries%20guide%2015039740%2003.pdf>).
121

122

123

124 **References:**

125 1. Agler MT, Mari A, Dombrowski N, Hacquard S. New insights in host-associated microbial
126 diversity with broad and accurate taxonomic resolution. bioRxiv [Internet]. 2016;
127 Available from: <https://www.biorxiv.org/content/early/2016/04/23/050005.abstract>

128 2. Zimin AV, Puiu D, Hall R, Kingan S, Clavijo BJ, Salzberg SL. The first near-complete
129 assembly of the hexaploid bread wheat genome, *Triticum aestivum*. Gigascience. 2017
130 Nov 1;6(11):1–7.

131

Table S1

Year	Location	Species	Latitude	Longitude
2017	Turkey, Kışlak	Ta (non-treated)	35° 58' 21.8"	36° 07' 41.8"
2017	Germany	Ta (benchmark)	54°18'50.9"	9°59'42.2"
2005	West population	Tb	37° 19' 31"	37° 09' 28"
2006	West population	Tb	36°54'29"	37°11'49"
2006	West population	Td	37°20'19"	37°16'50"
2006	West population	Td	37°19'50"	37°18'51"
2004	East population	Td	37°50'40"	39°47'58"
2004	East population	Tu	37°50'40"	39°47'58"
2004	East population	Td	37°47'31"	39°57'18"

Table S2

	# of samples	Total # of reads	Average # of reads	# of OTUs (b.r.)	# of OTUs (a.r.)
Seeds- bacteria	58	2356761	40633.81	1441	1157
Seeds-fungi	30	2621026	87367.53	272	124
Axenic leaves- bacteria	16	633975	84526.0	760	589
Axenic leaves- fungi	15	719618	47974.53	119	98
Axenic roots- bacteria	16	663813	41488.31	828	632
Axenic roots- fungi	16	2309064	144316.5	113	74
Leaves- bacteria	42	2460327	58579.21	3864	3676
Roots- bacteria	39	1690934	43357.28	3184	3100
Roots-fungi	42	1545621	36800.5	3757	3737
Natural soil- bacteria	3	100190	33396.67	1258	1248
Agricultural soil-bacteria	3	82333	27444.33	1270	1266

Table S3

16S, ITS universal and interfering primers

Universal_Primer- PCR1
<u>Bacteria - V5/V6/V7</u>
799F: AACMGGATTAGATACCKG
1192R: ACGTCATCCCCACCTTC
<u>Fungi ITS 1- PCR1</u>
ITS1F: CTTGGTCATTTAGAGGAAGTAA
ITS2: GCTGCGTTCTTCATCGATGC
<u>Interfering Primers: Mitochondria - V5/V6/V7</u>
Forward Primer: GGATCAGGGGCCAGCTAACGCGTGAAACA
Reverse Primer: CGGAGCGGGCGCGTACTATTACCACTACG

Bacteria 16S, ITS PCR2 primers

B5-F: AATGATACGGCGACCACCGAGATCTACACGACTGCGACTGGCGAACMGGATTAGATACCCCKG

B5-R:

CAAGCAGAAGACGGCATAACGAGATXXXXXXXXXXXXCAGCCATTTAGTGTCACGTCATCCCCACCTTCC

Fungi, XXXXXXXXXXXX: 12bp barcode sequence

ITS-F: AATGATACGGCGACCACCGAGATCTACACTCACGCGCAGGCTTGGTCATTTAGAGGAAGTAA

ITS-R:

CAAGCAGAAGACGGCATAACGAGATXXXXXXXXXXXXCGTACTGTGGAGAGCTGCGTTCTTCATCGATGC

16S, ITS sequencing primers

B5-R1

ACGACTGCGACTGGCGAACMGGATTAGATACCC

B5-R2

CAGCCATTTAGTGTCACGTCATCCCCACCTTCC

B5-R3

GGAAGGTGGGGATGACGTGACACTAAATGGCTG

ITS-R1

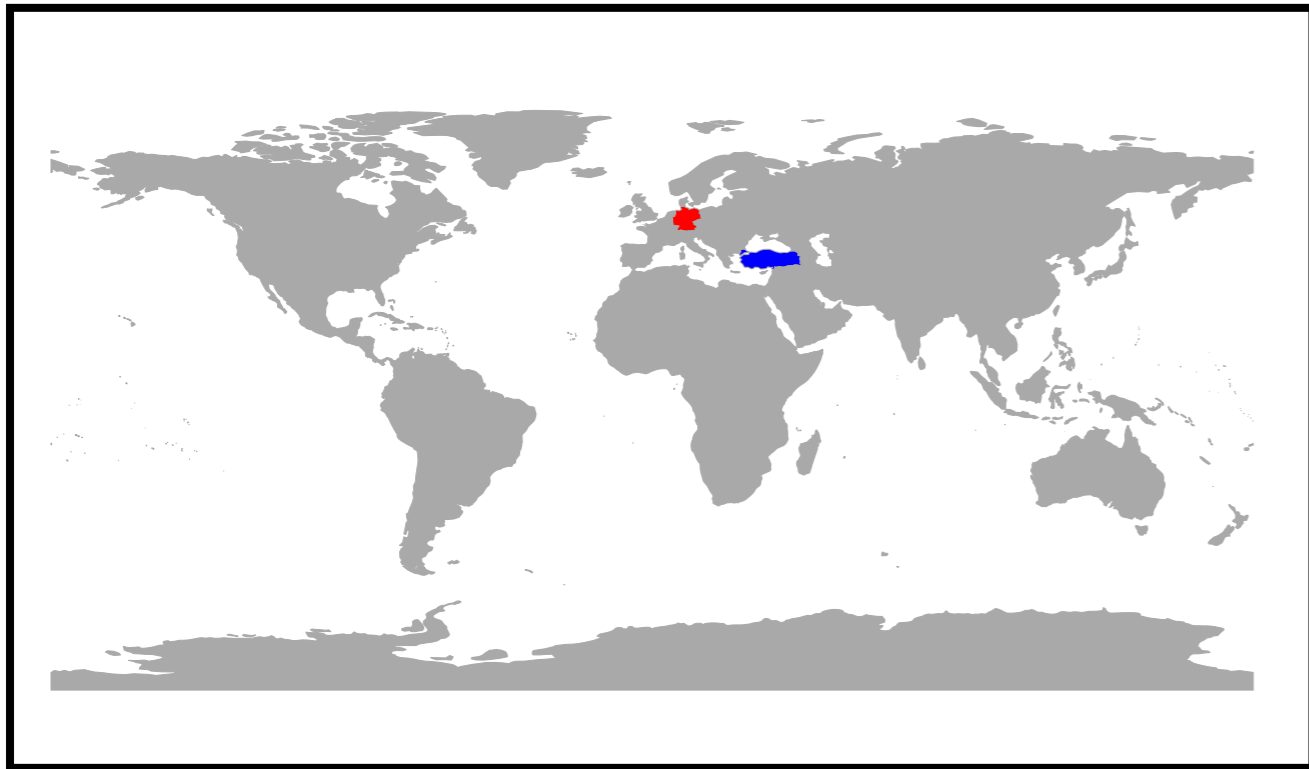
TCACGCGCAGGCTTGGTCATTTAGAGGAAGTAA

ITS-R2

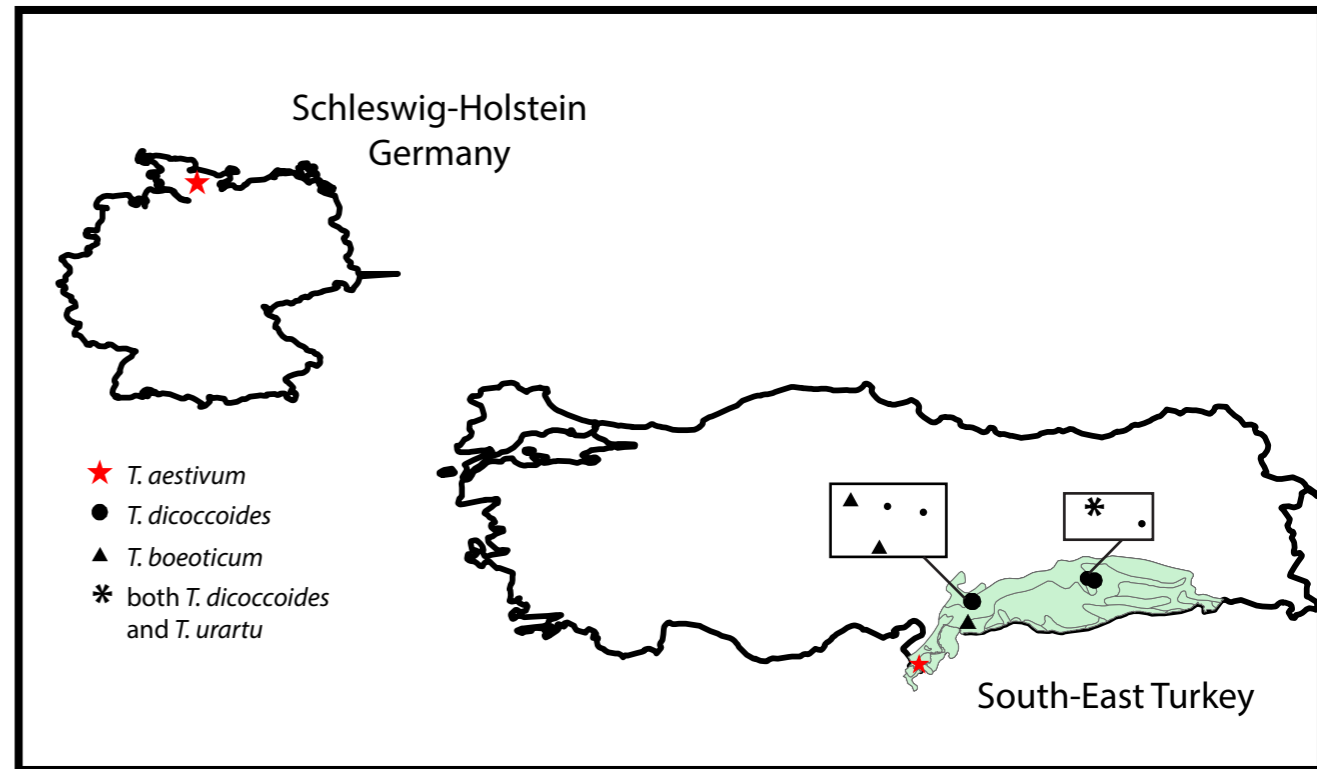
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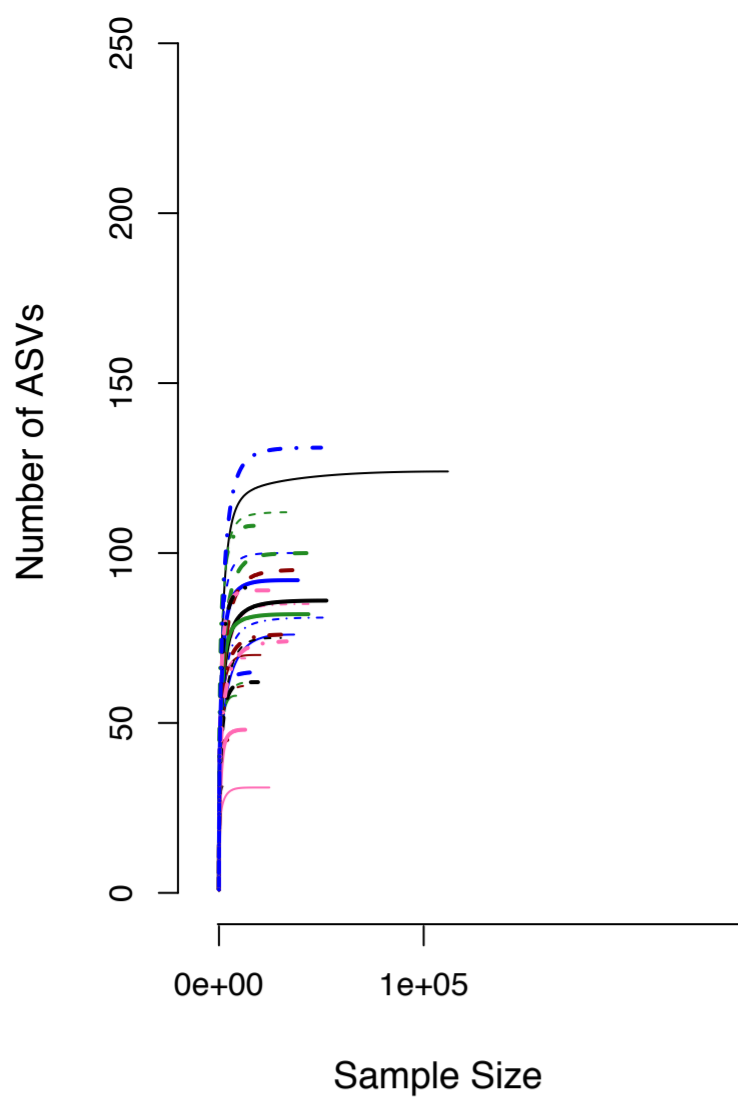
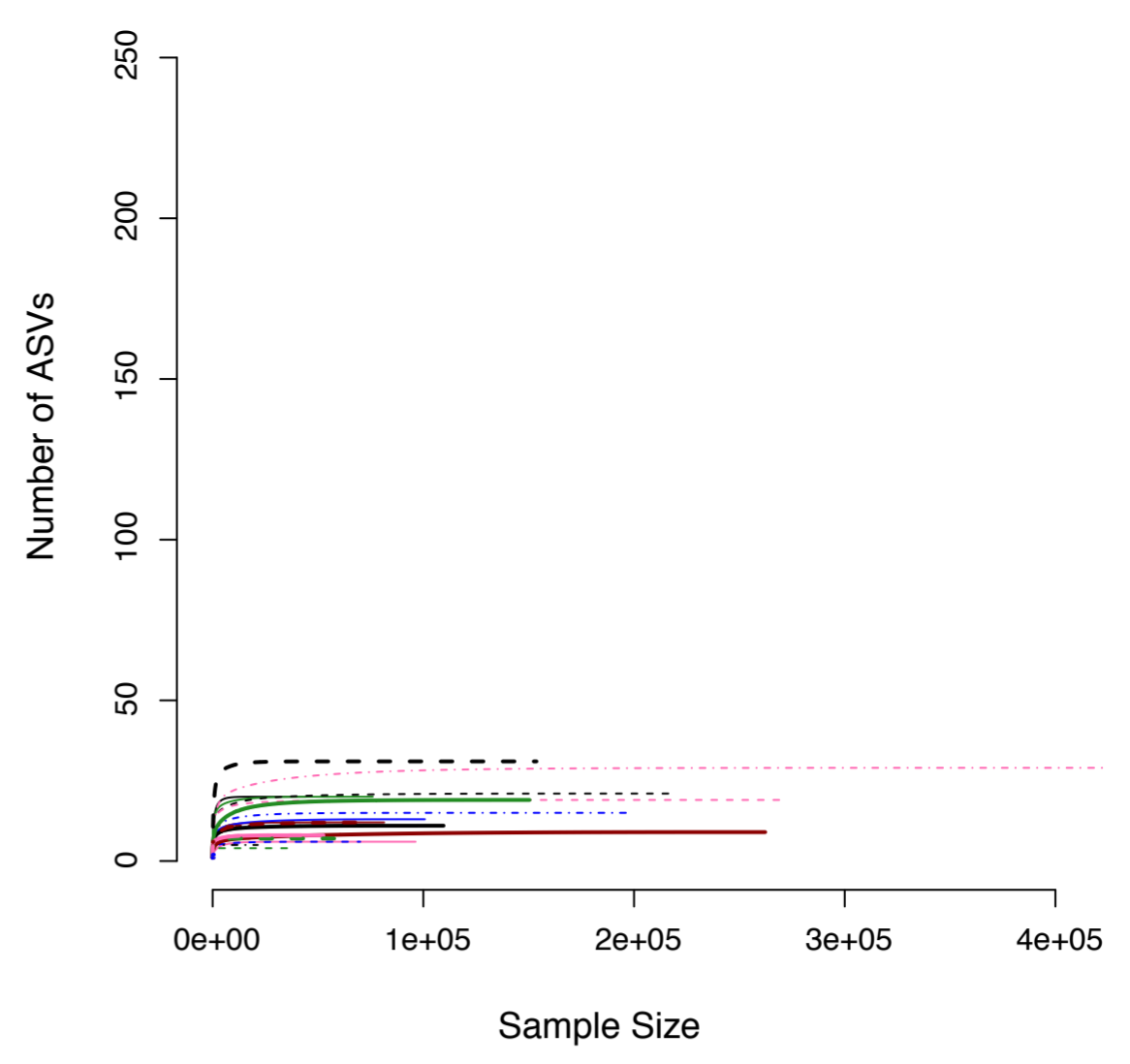
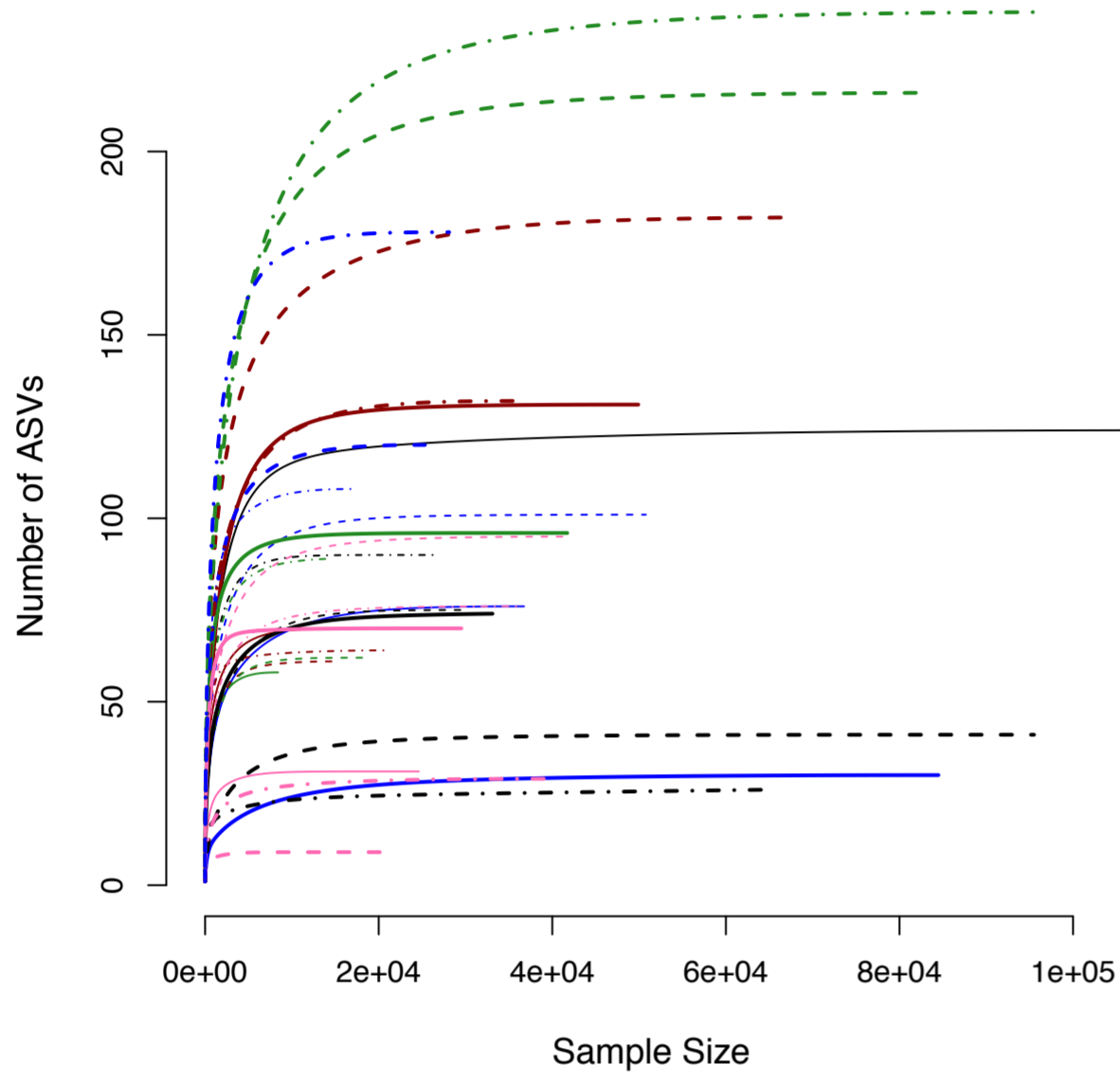
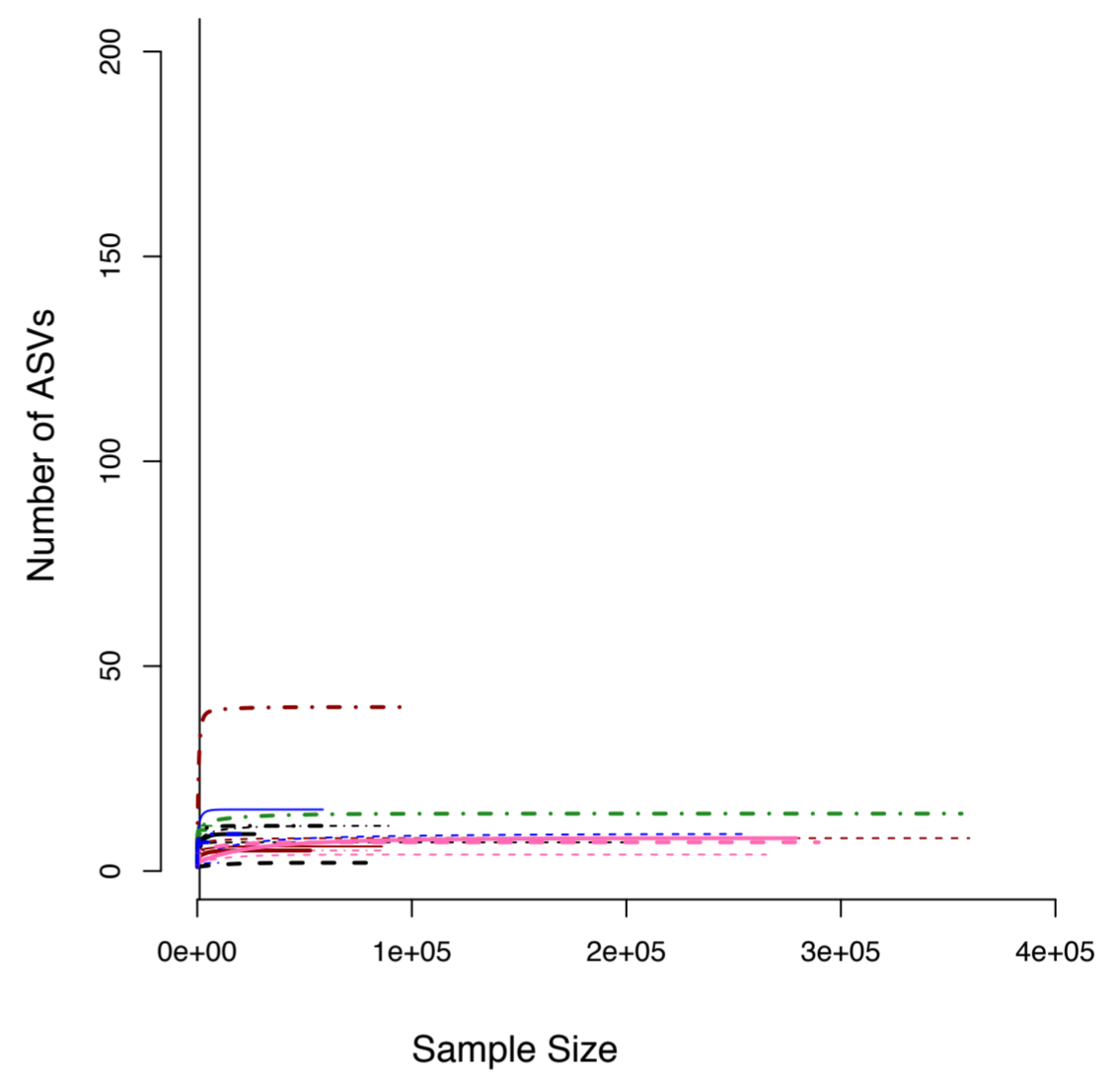
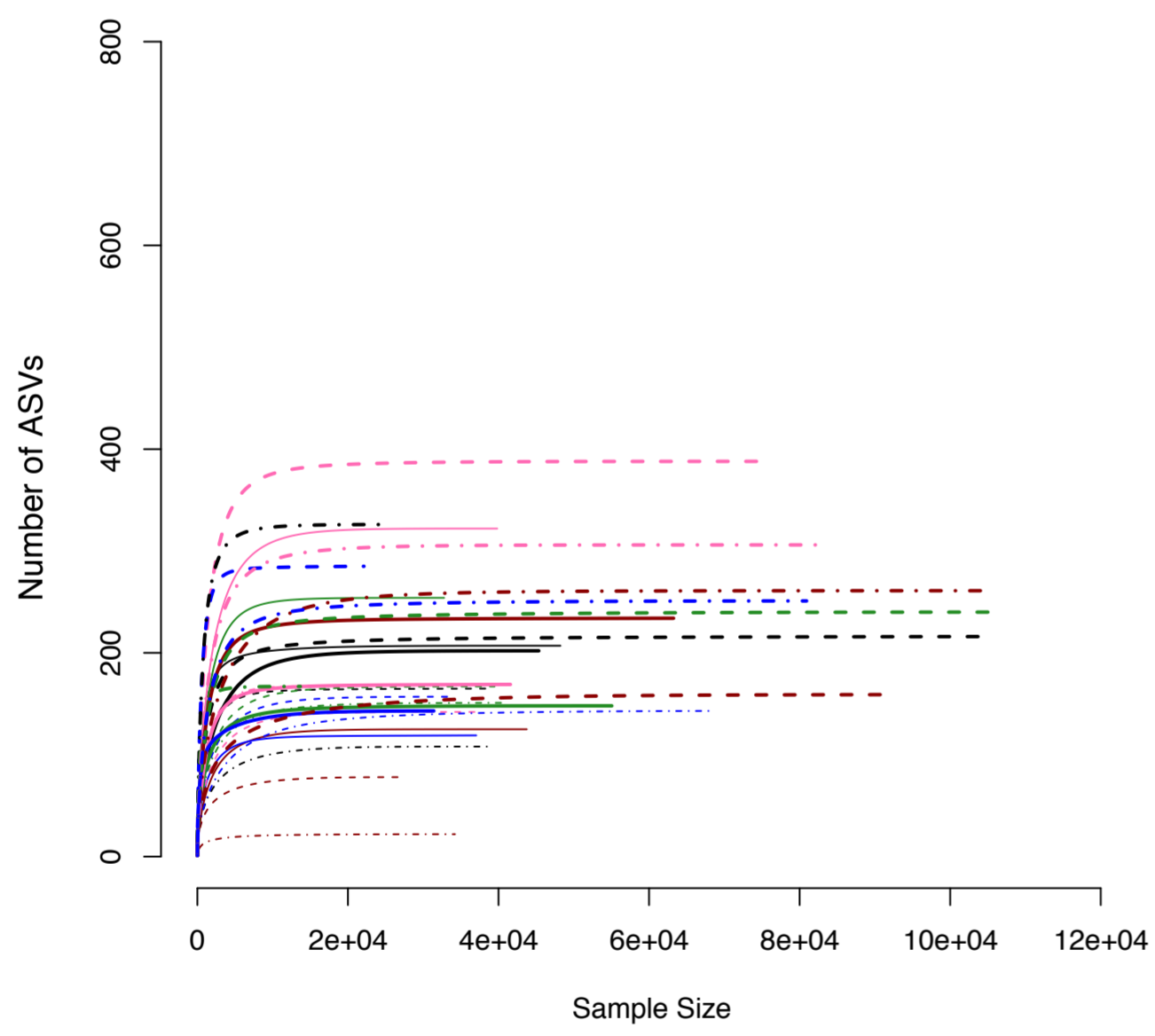
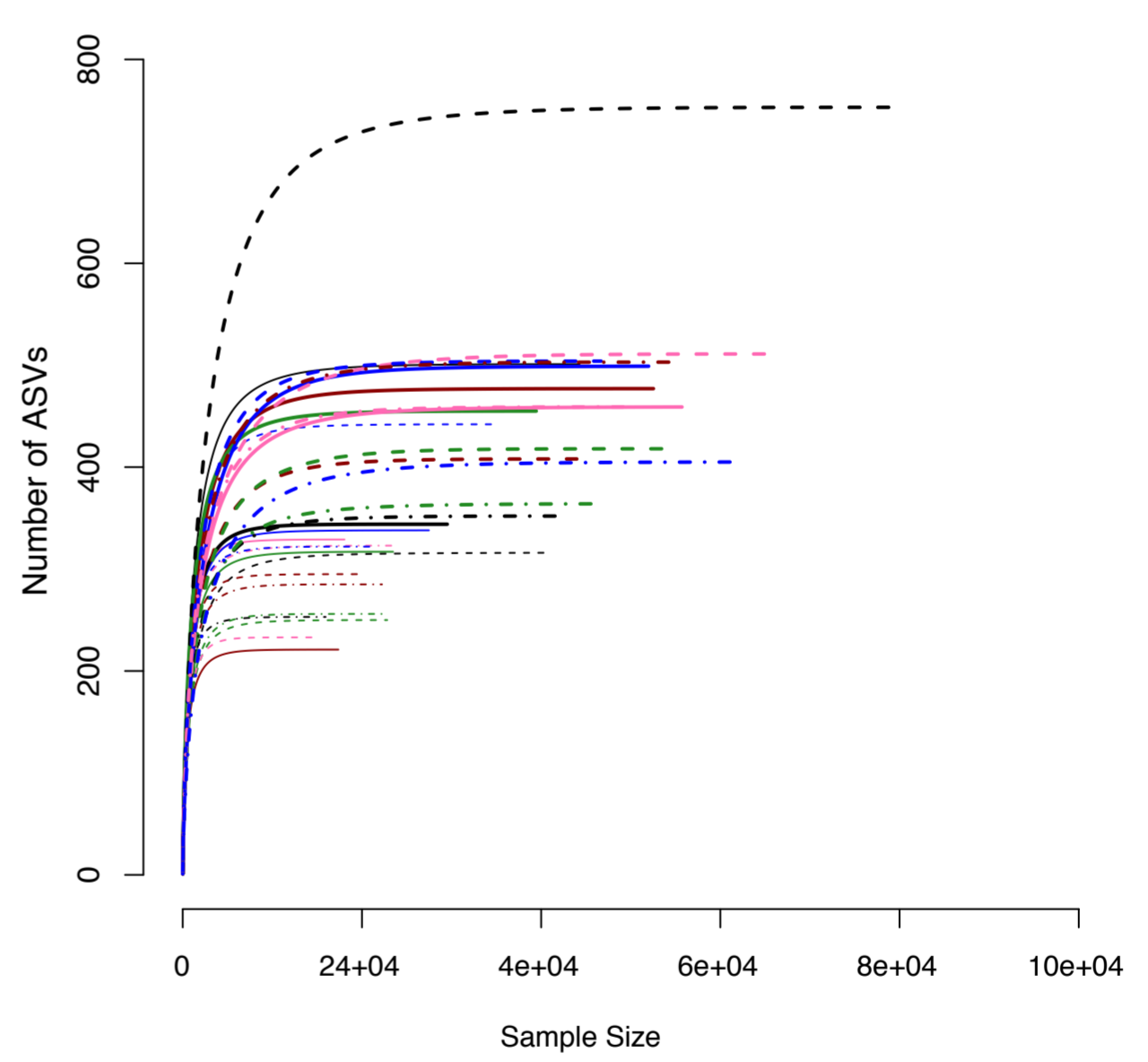
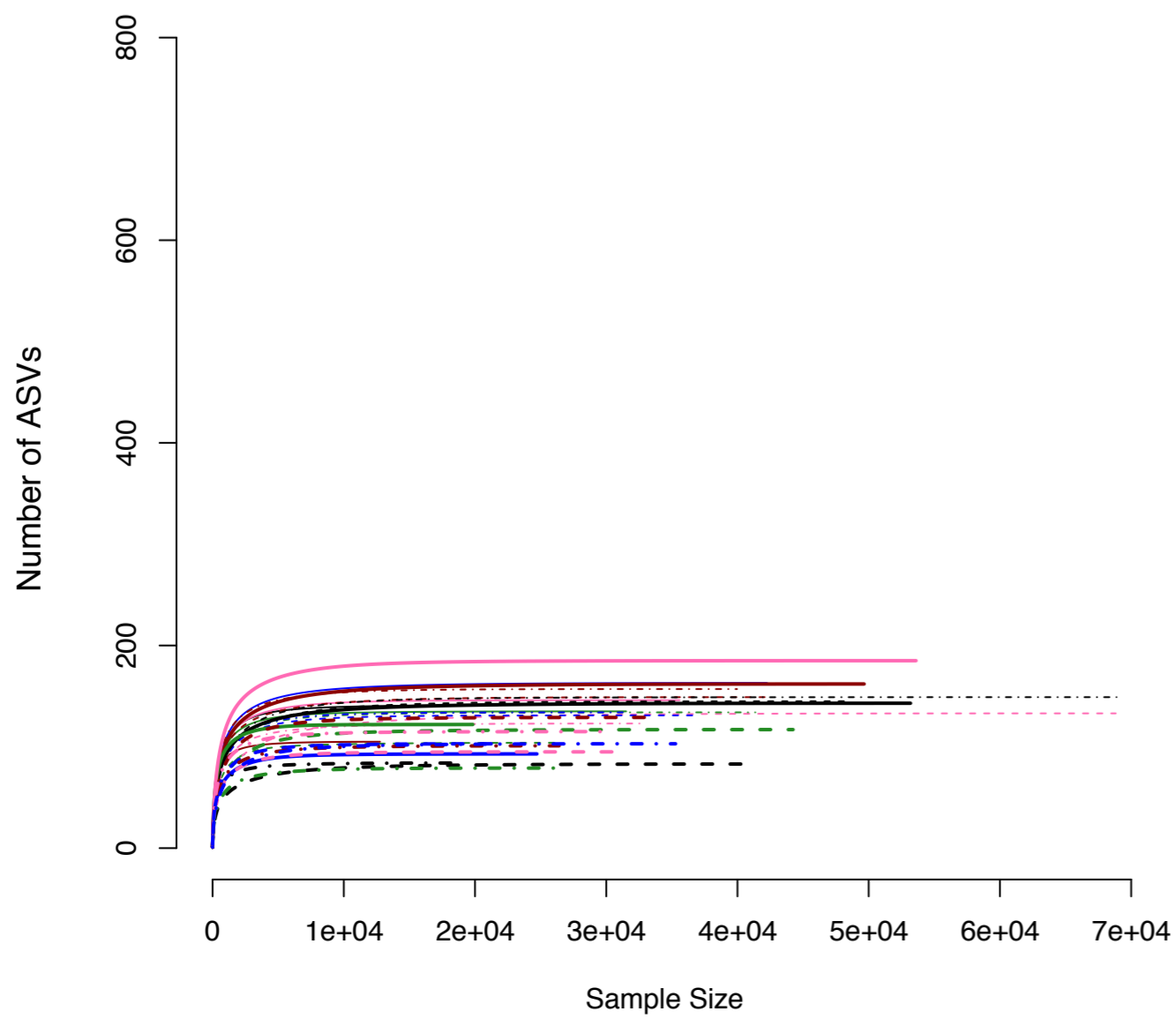
ITS-R3

GCATCGATGAAGAACGCAGCTCTCCACAGTACG

A**B**

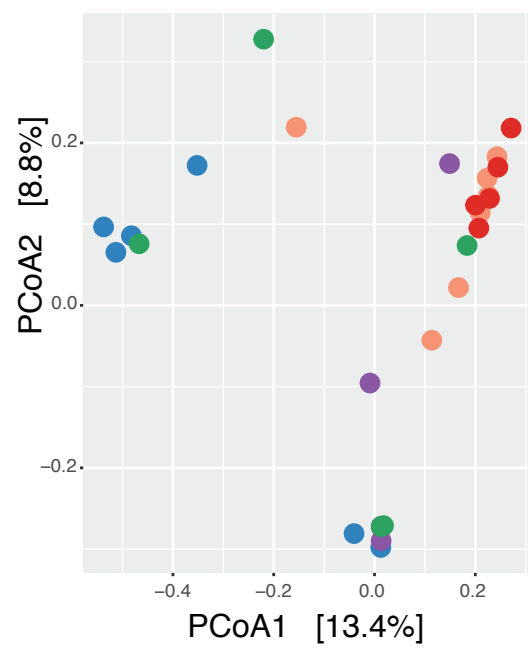
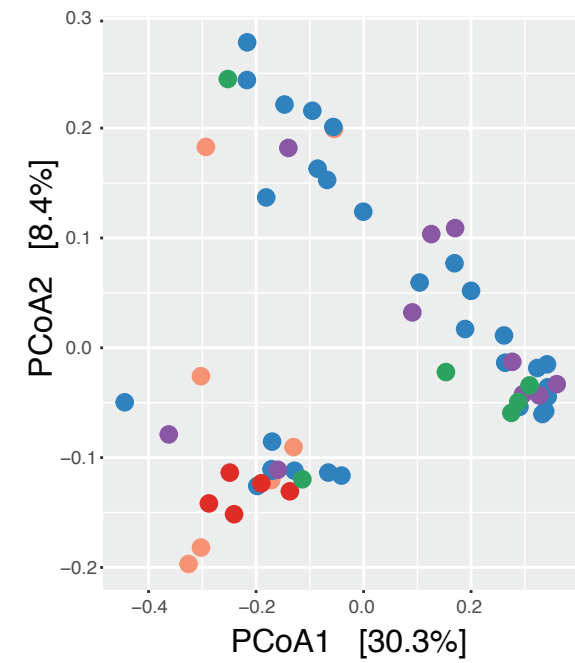
Suppl Fig 1



A**B****C****D****E****F****G**

A

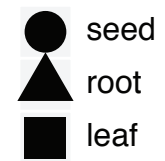
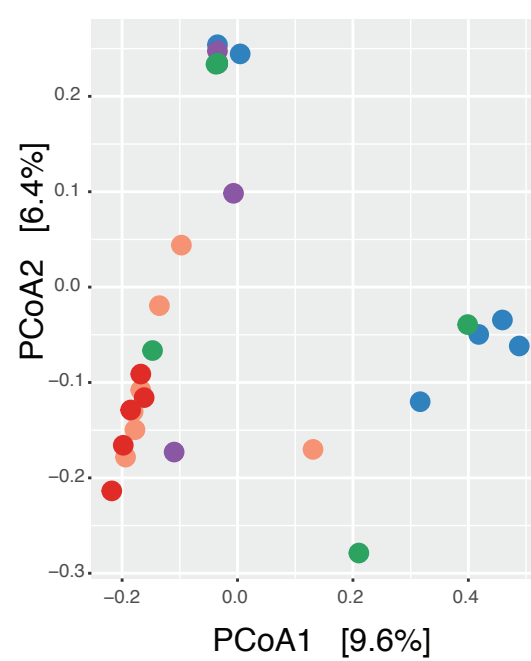
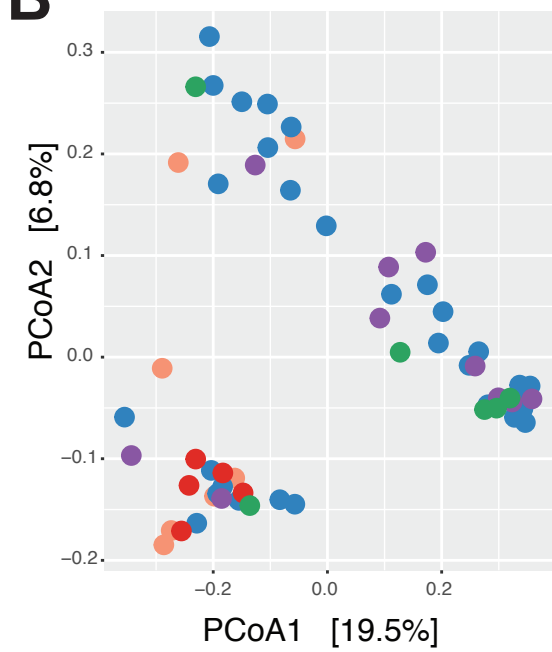
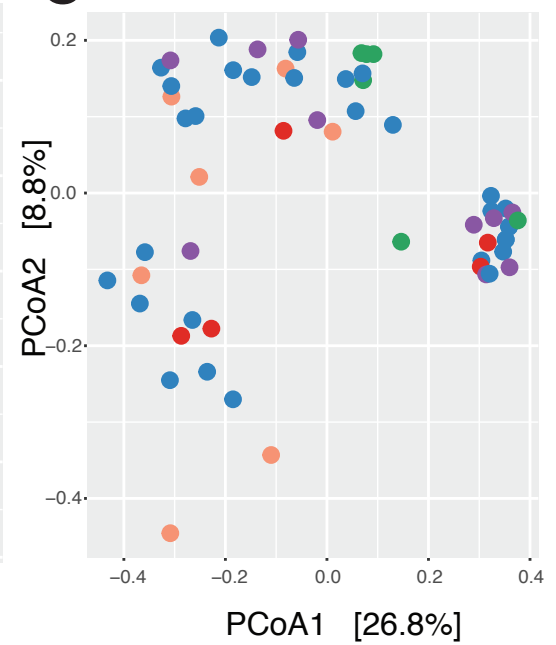
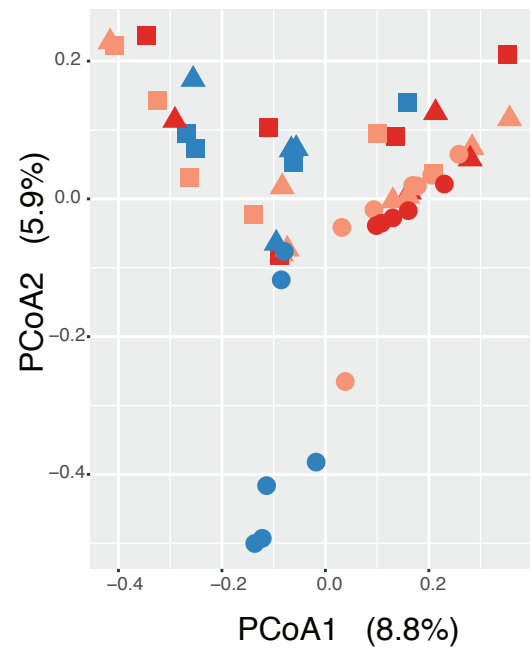
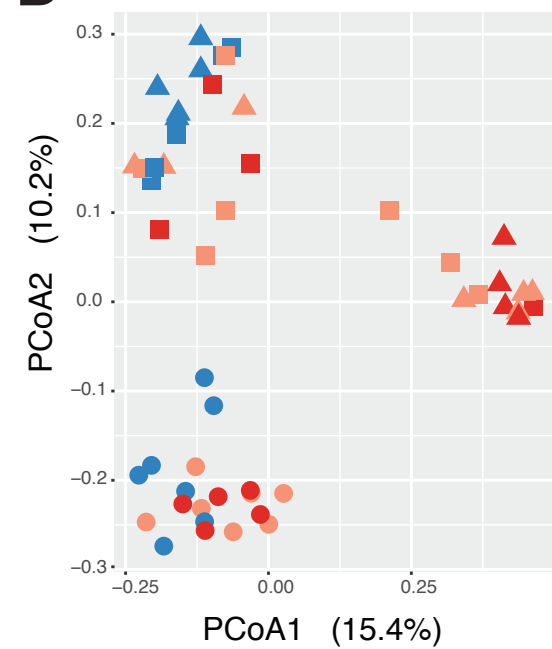
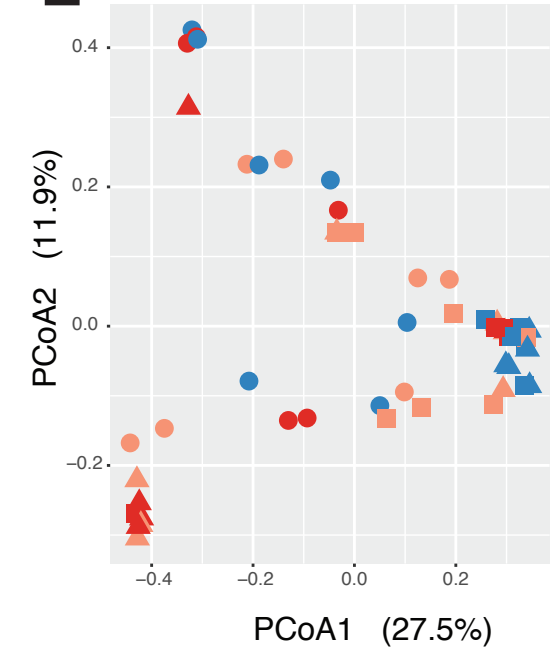
Suppl. Fig 3



Host Species

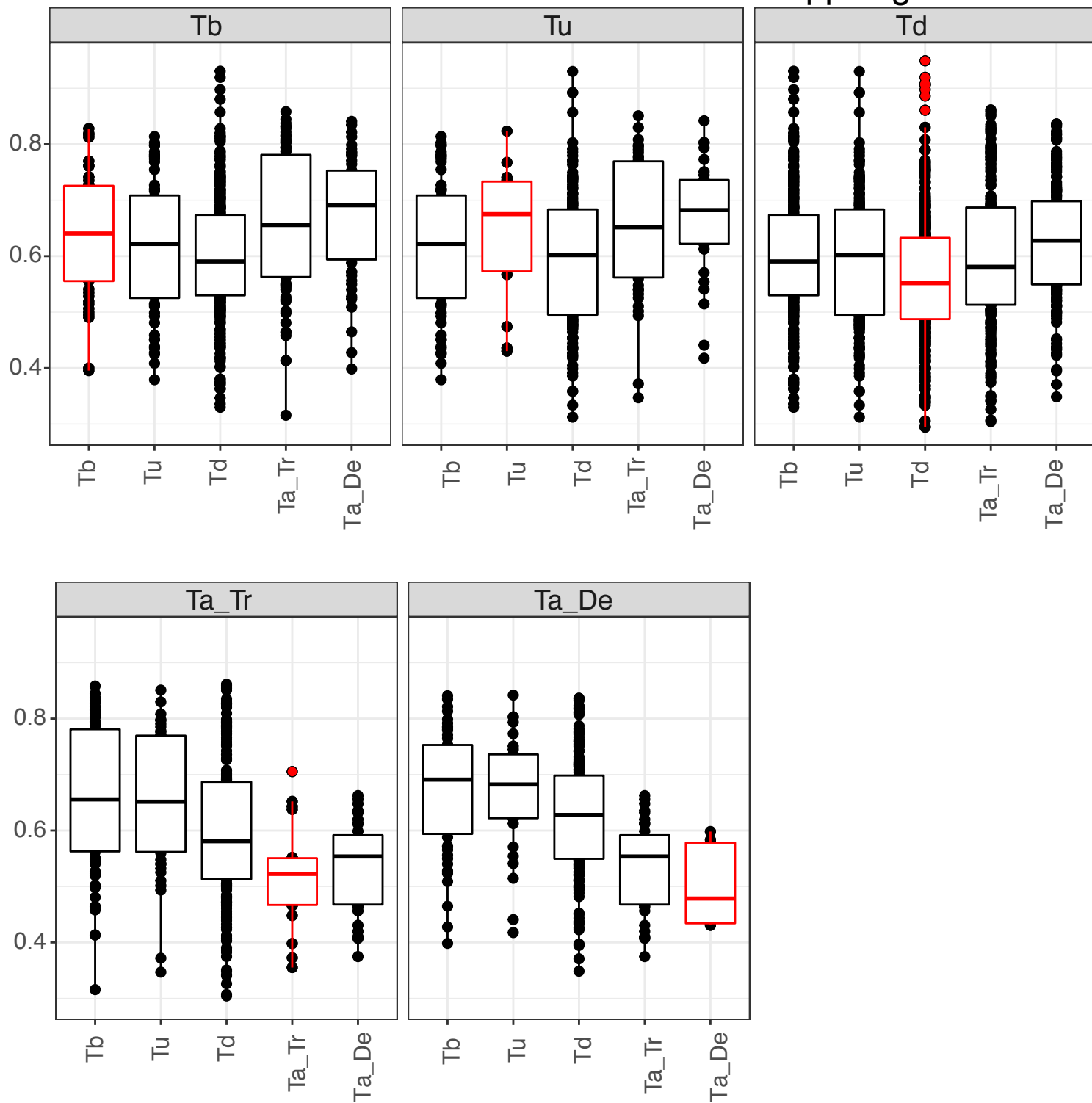


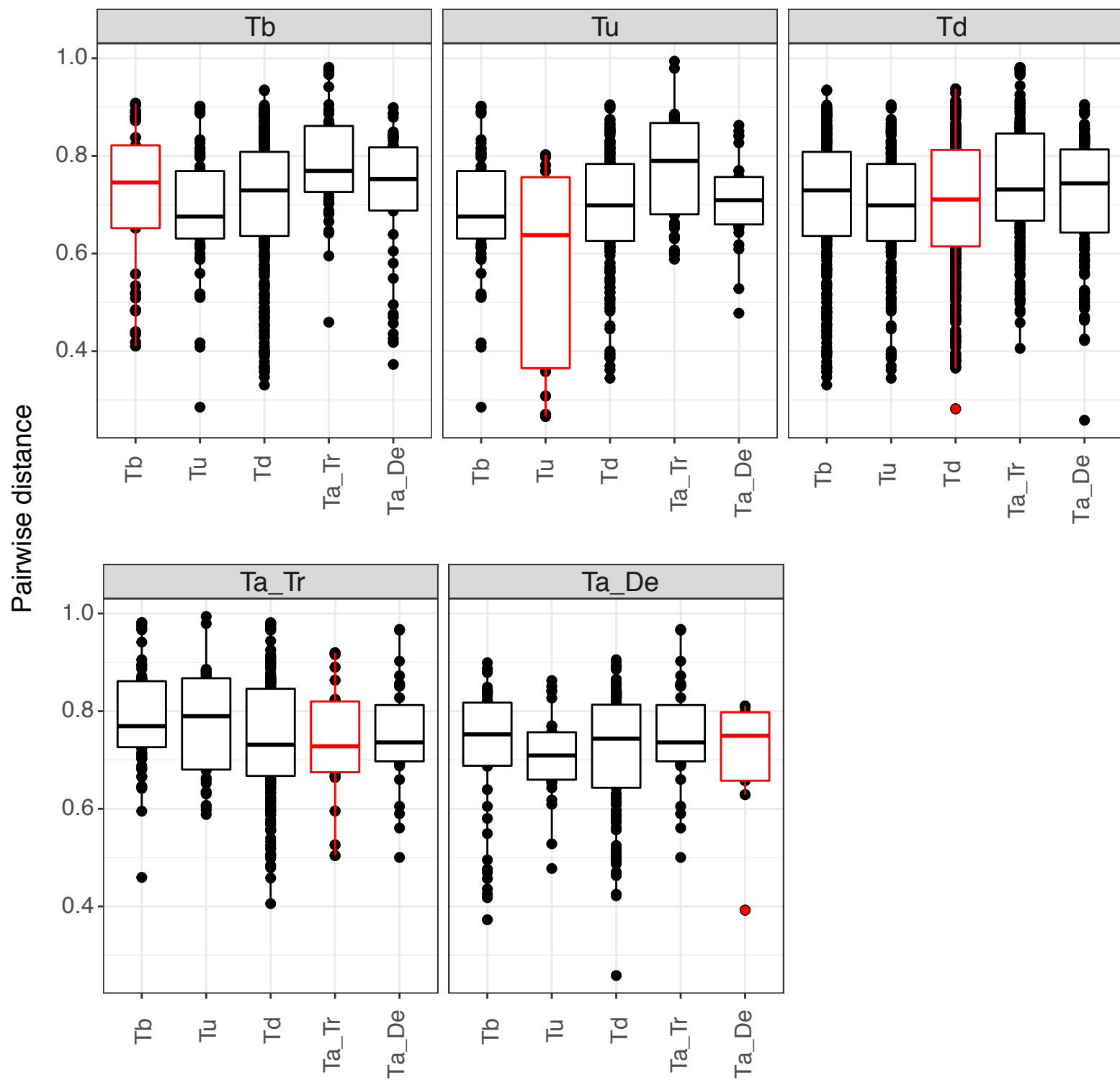
Tissues

**B****C****D****E**

Distance Metric = Bray-Curtis

Suppl Fig 4

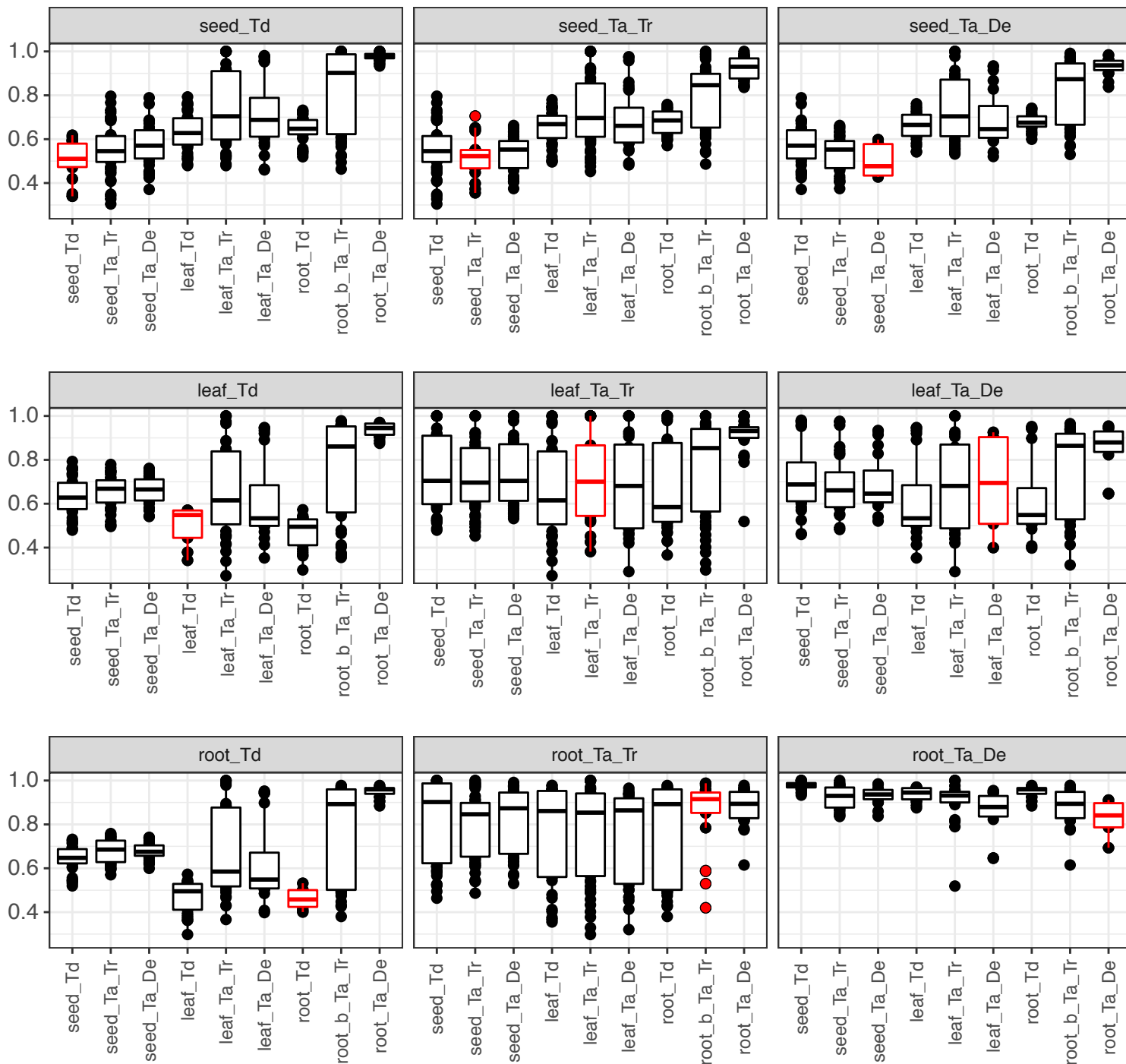




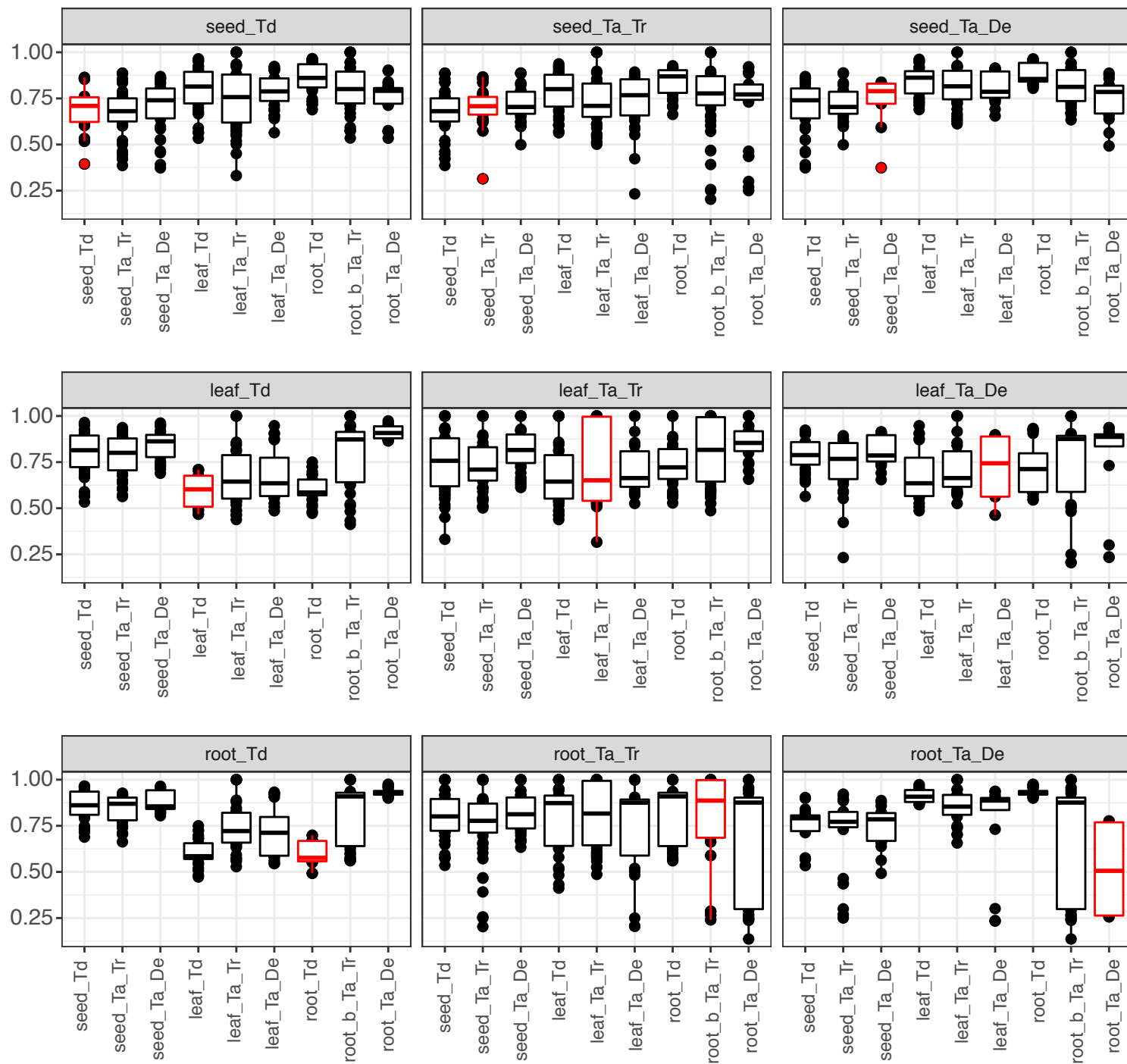
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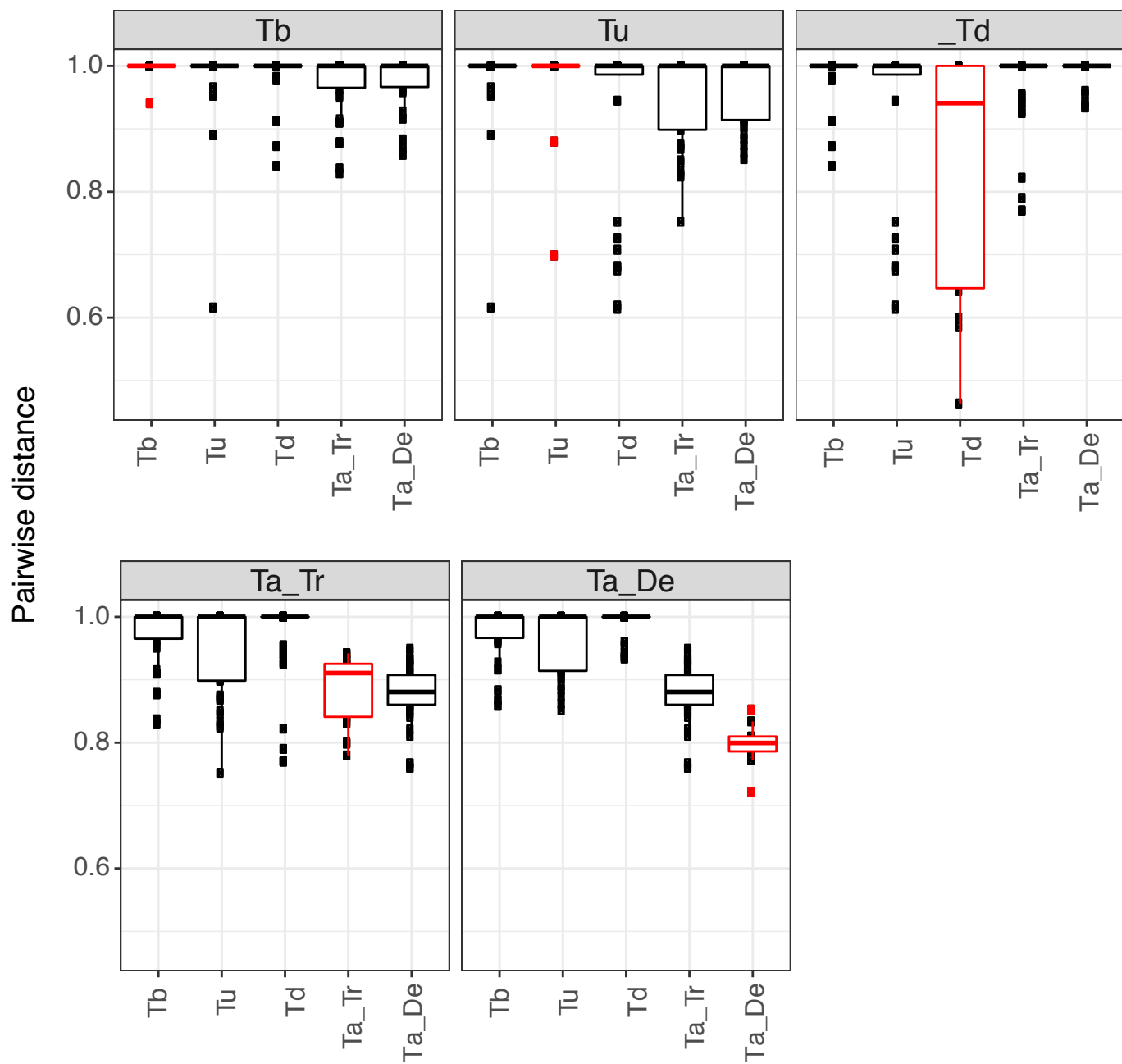
Suppl Fig 6

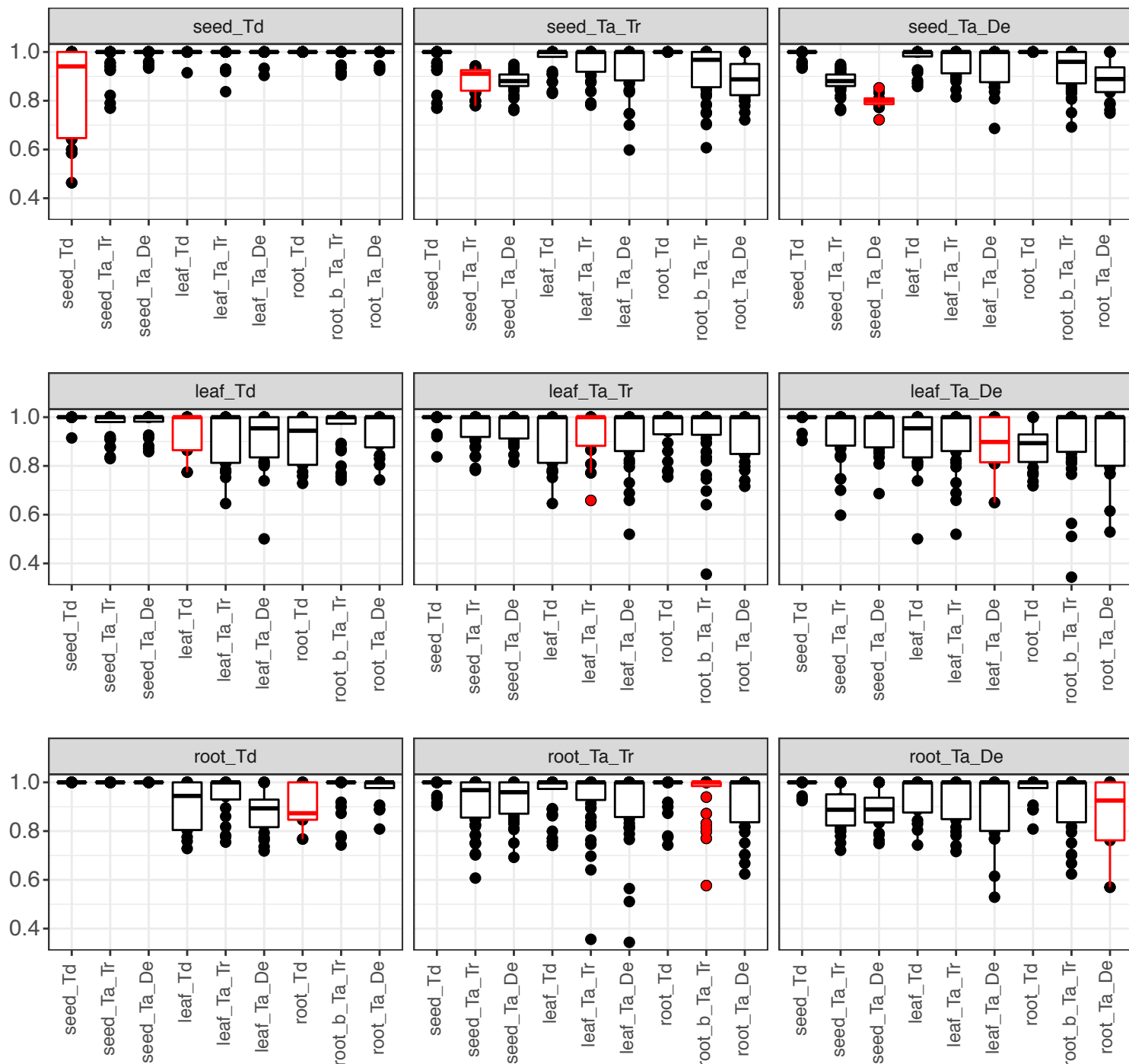
Pairwise distance

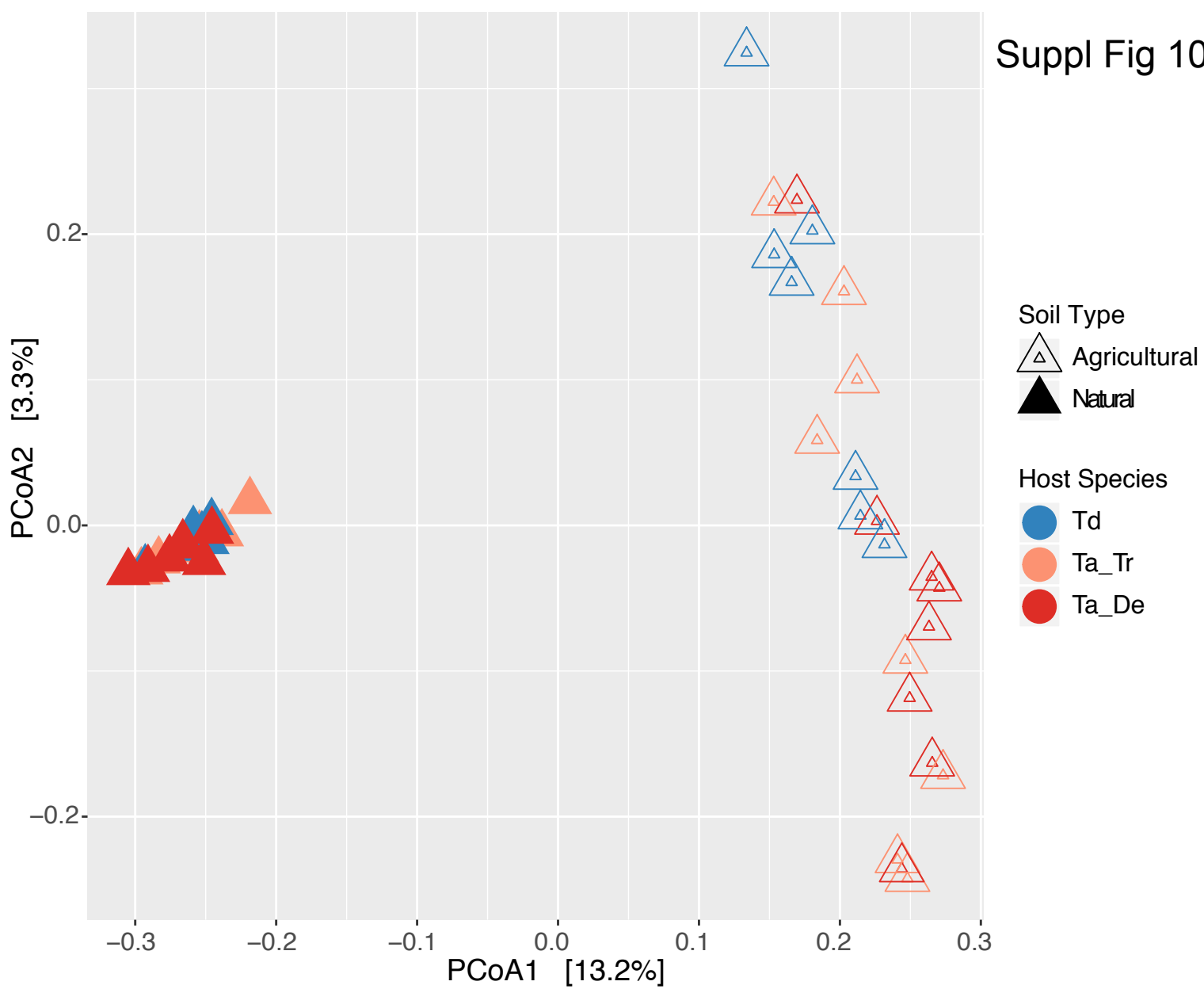


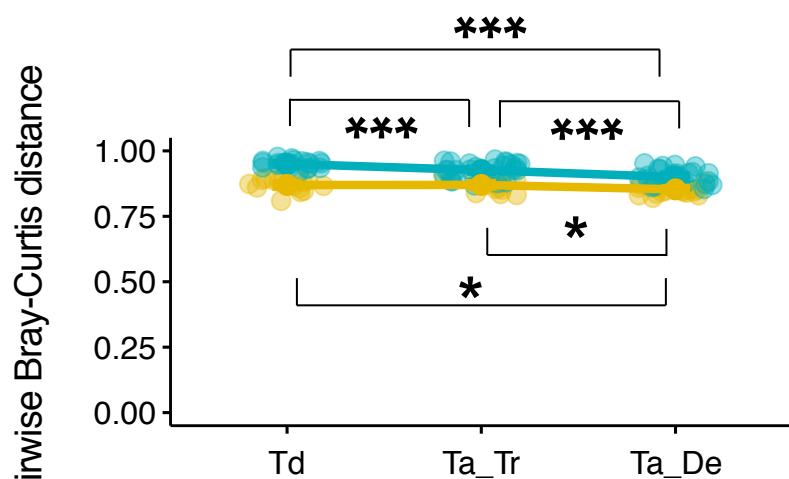
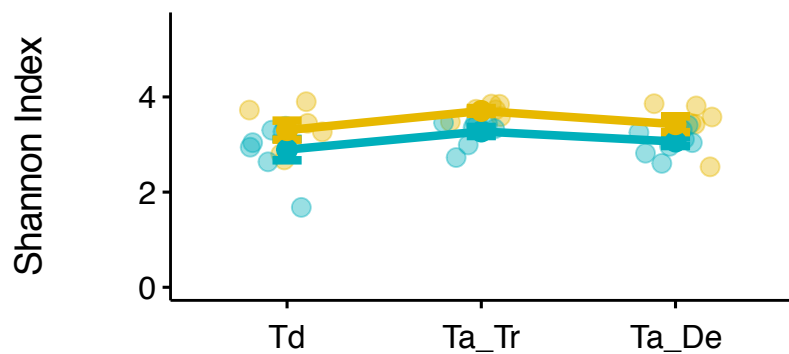
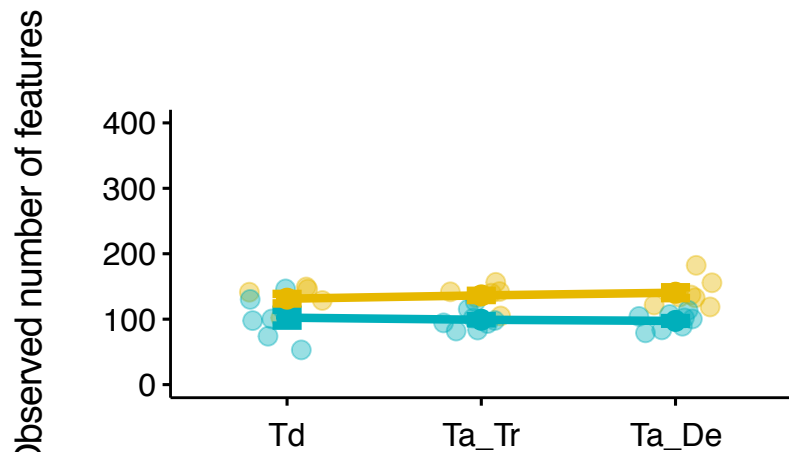
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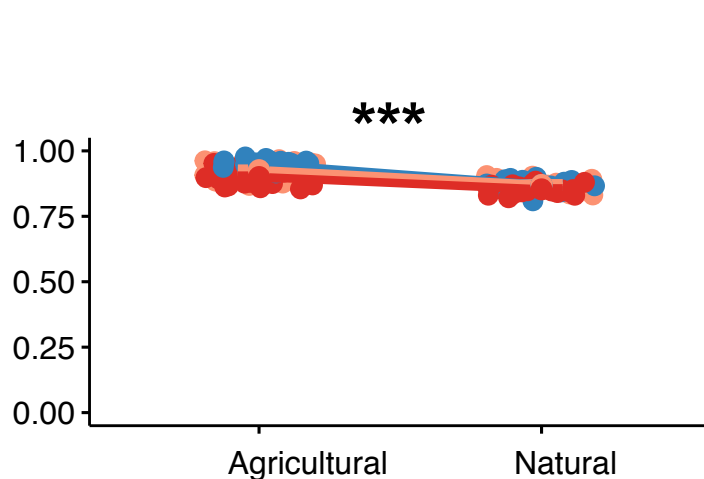
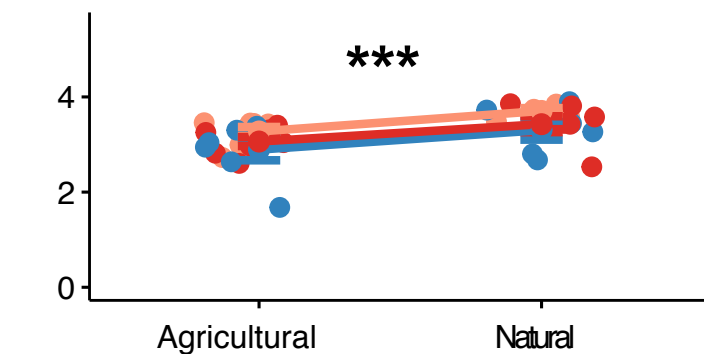
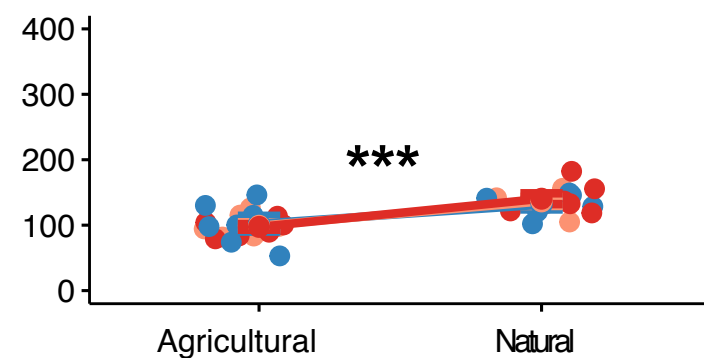


A

Host Species

B

Suppl Fig 11

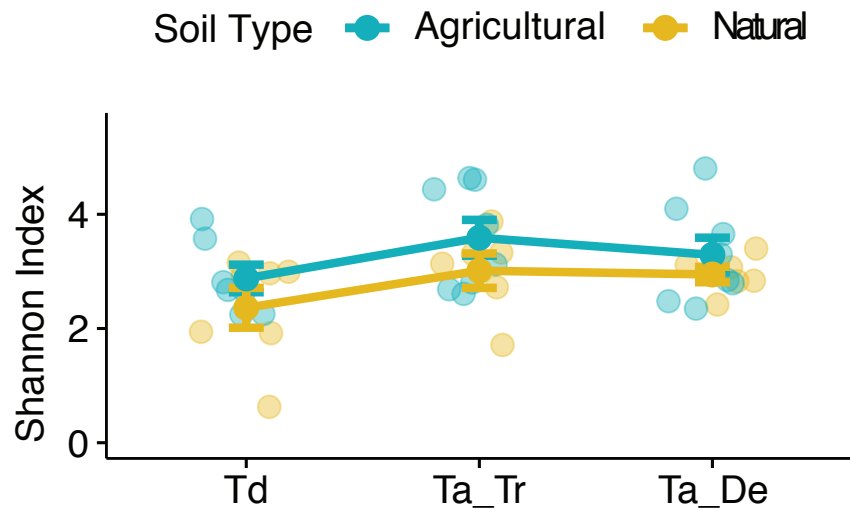


Soil Type

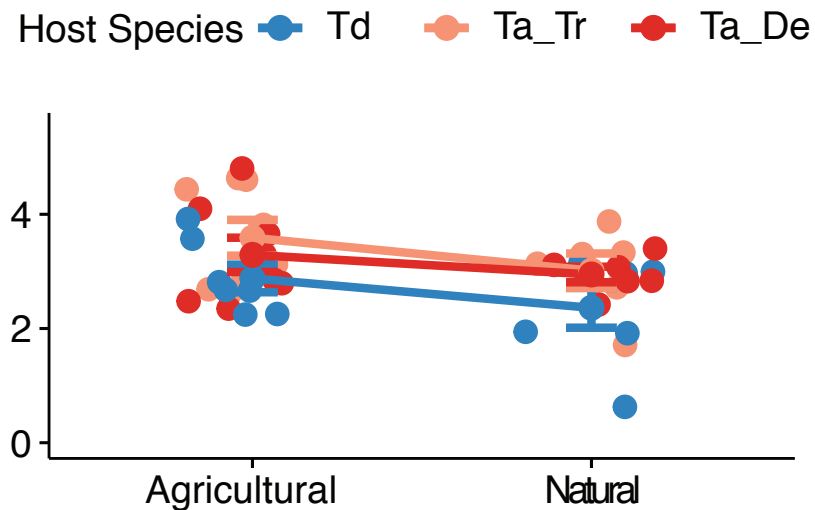
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Host Species ● Td ● Ta_Tr ● Ta_De

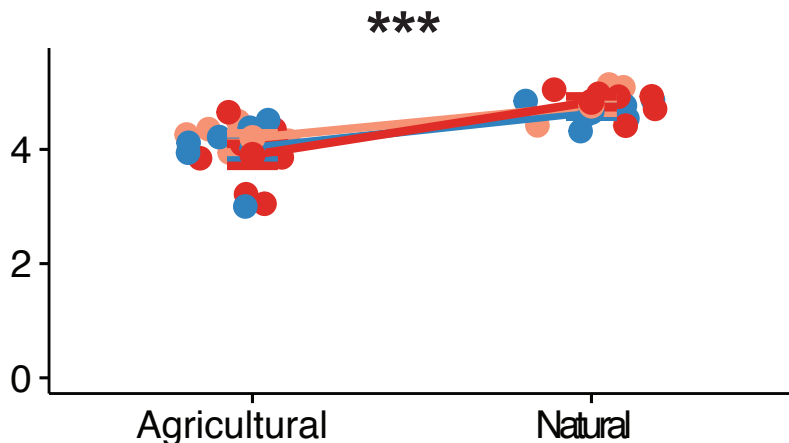
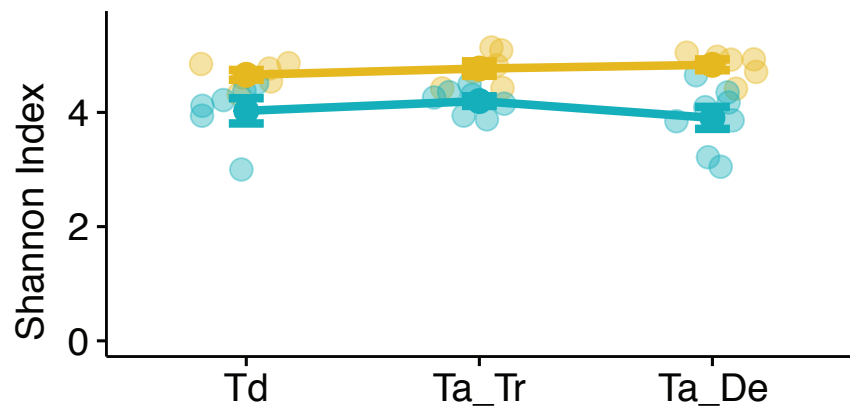
A) LEAVES



Suppl Fig 12



B) ROOTS



A

	Leaf			Root			Soil	
	Td	Ta_Tr	Ta_De	Td	Ta_Tr	Ta_De	natural soil	agr. soil
Proteobacteria; Oxalobacteraceae	22.2	12.2	21.1	18.6	26.5	24.4	1	0.9
Actinobacteria; Streptomyetaceae	0.3	2	3	32.6	27	30	0.7	10.3
Proteobacteria; Comamonadaceae	7.4	19.9	16.1	8.3	9.8	8.8	2	3.5
Proteobacteria; Rhizobiaceae	21.3	12.8	12.1	1	1.3	1.1	0.2	0
Proteobacteria; Halomonadaceae	13.6	15.9	15.7	0	0.1	0	0	0
Proteobacteria; Vibrionaceae	14.7	14	8.4	0	0.1	0	0	0
Actinobacteria; Micromonosporaceae	0.1	0.5	0.6	5.6	5.9	7.7	3.4	1.9
Actinobacteria; Nocardoidaceae	0.3	0.9	1.5	5.1	4.2	4	4	5.4
Bacteroidetes; Flavobacteriaceae	0.2	0.4	0.2	5.9	4.6	4.7	0.3	2.1
Proteobacteria; Xanthomonadaceae	1.3	1.6	1.3	3	2.7	2.7	1.5	5.9
Proteobacteria; Burkholderiaceae	3.5	2	3.2	0.4	0.5	0.3	0.1	2.1
Proteobacteria; Phyllobacteriaceae	3.5	1.6	3.1	0.3	0.3	0.4	0.2	0.5
Proteobacteria; Pseudomonadaceae	2.7	1.7	1.6	0.6	0.6	0.5	0.2	0.9
Actinobacteria; Geodermatophilaceae	0	0.2	0.2	0.5	0.5	0.4	21.8	0.2
Proteobacteria; Hyphomicrobiaceae	0.2	0.4	0.3	1.4	1.4	1.4	0.7	6.9
Actinobacteria; Actinosynnemataceae	0	0.1	0.1	2.5	1.8	1.9	1.6	0.4
Proteobacteria; Caulobacteraceae	0.1	0.2	0.2	1.7	1.6	1.4	1	1.6
Actinobacteria; Rubrobacteraceae	0	0.2	0.1	0.3	0.2	0.2	12.9	0.1
Actinobacteria; Microbacteriaceae	0.1	0.2	0.3	1	0.8	0.9	1.4	1.5
Proteobacteria; [Chromatiaceae]	1.4	1.5	0.6	0	0.1	0	0	0

B

	Agricultural			Natural		
	Td	Ta_Tr	Ta_De	Td	Ta_Tr	Ta_De
Proteobacteria; Oxalobacteraceae	44.1	20.3	36.2	0.3	1.3	1
Proteobacteria; Rhizobiaceae	0.1	0.1	0	42.5	29.6	28.2
Proteobacteria; Halomonadaceae	11.2	10.5	8.1	16.1	23.2	25.8
Proteobacteria; Comamonadaceae	10.4	21.3	17.1	4.4	18.1	14.8
Proteobacteria; Vibrionaceae	8.1	16.3	4.5	21.2	11	13.6
Proteobacteria; Burkholderiaceae	7	2.9	5.6	0	0.7	0.1
Proteobacteria; Phyllobacteriaceae	0.1	0.1	0.2	7	3.7	6.9
Proteobacteria; Pseudomonadaceae	4.4	2.6	2.4	1.1	0.4	0.4
Actinobacteria; Streptomyetaceae	0.5	3.1	5.1	0	0.6	0.1
Proteobacteria; Xanthomonadaceae	1.5	2.2	1.9	1	0.8	0.6
Proteobacteria; [Chromatiaceae]	0.6	2.1	0.2	2.3	0.8	1.3
Proteobacteria; Neisseriaceae	1.5	1.7	1.8	0.7	0.5	0.7
Actinobacteria; Nocardoidaceae	0.5	1.2	2.4	0	0.6	0.3
Actinobacteria; Propionibacteriaceae	0.8	2.1	0.8	0.1	0.3	0.3
Firmicutes; Streptococcaceae	0.8	1.1	0.7	0.6	0.3	0.5
Proteobacteria; Enterobacteriaceae	0.5	1.1	0.5	0.7	0.4	0.5
Proteobacteria; Methylobacteriaceae	0.5	0.9	0.5	0.1	0.7	0.5
Actinobacteria; Micromonosporaceae	0.1	0.5	1	0.1	0.5	0.1
Firmicutes; Staphylococcaceae	0.3	0.7	0.7	0	0.2	0.2
Proteobacteria; Methylophilaceae	0.2	0.5	0.6	0.1	0.4	0.2

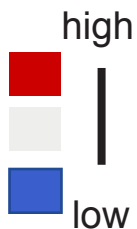
C

	Agricultural			Natural		
	Td	Ta_Tr	Ta_De	Td	Ta_Tr	Ta_De
Actinobacteria; Streptomyetaceae	49.7	40.2	46.2	15.5	11.5	8.5
Proteobacteria; Oxalobacteraceae	11.6	22	23.3	25.7	31.8	25.9
Proteobacteria; Comamonadaceae	2.7	5.2	3.3	14	15	16.1
Actinobacteria; Micromonosporaceae	3.6	3.5	3.3	7.6	8.8	13.6
Bacteroidetes; Flavobacteriaceae	0.5	0.5	0.3	11.2	9.4	10.5
Actinobacteria; Nocardoidaceae	8	6.2	5.4	2.2	1.8	2.1
Proteobacteria; Xanthomonadaceae	4.1	3.6	3.1	2	1.6	2.2
Actinobacteria; Actinosynnemataceae	1.4	0.9	0.5	3.7	2.9	3.9
Proteobacteria; Caulobacteraceae	1.7	1.5	1	1.7	1.7	1.8
Proteobacteria; Hyphomicrobiaceae	2	2	1.7	0.7	0.7	0.9
Proteobacteria; Rhizobiaceae	0.6	1.2	0.8	1.5	1.4	1.5
Actinobacteria; Thermomonosporaceae	1	1.2	1.1	1.1	1.3	0.8
Actinobacteria; Microbacteriaceae	1	0.8	0.7	0.9	0.8	1.2
Actinobacteria; Promicromonosporaceae	0.4	0.5	0.3	1.7	0.9	1.4
Chloroflexi; [Kouleothrixaceae]	0.4	0.4	0.4	0.7	1.4	1.1
Actinobacteria; Pseudonocardiaceae	0.3	0.4	0.1	1.1	1.1	0.9
Proteobacteria; Pseudomonadaceae	0.5	0.4	0.2	0.8	0.9	0.8
Firmicutes; Paenibacillaceae	0.8	1.3	0.8	0	0	0
Proteobacteria; Bradyrhizobiaceae	0.8	0.7	0.5	0.3	0.4	0.4
Actinobacteria; Micrococcaceae	0.5	0.2	0.5	1	0.5	0.4

high
|
low

ROOTS

Suppl Fig 13



	Agricultural			Natural		
Ascomycota; Pseudeurotiaceae -	33.4	28.4	33.9	0	0.1	0.1
Ascomycota; Phaeosphaeriaceae -	1.9	8.2	3.4	12.2	15.4	12.7
Mortierellomycota; Mortierellaceae -	14.8	3.9	2.2	7.2	13.8	7.3
Ascomycota; Onygenales (IS) -	12.4	15	12.5	0	0.3	0.7
Ascomycota; Helotiaceae -	11.4	13.7	10.8	0.7	0.2	1.1
Ascomycota; Nectriaceae -	0.2	1.1	0.1	10.6	18.1	15
Ascomycota; Pleosporaceae -	0	0	0	10.8	16.6	14.3
Basidiomycota; Entolomataceae -	4.2	9.7	16.3	0	0	0
Ascomycota; Lasiosphaeriaceae -	2.9	0.2	0.2	12.2	4.8	8.9
Basidiomycota; Psathyrellaceae -	0	0	0	16.3	3.8	6.7
Basidiomycota; Ceratobasidiaceae -	0	0	0	10.5	1.5	11.5
Ascomycota; Myxotrichaceae -	6.4	3.1	3.5	0	0	0
Ascomycota; Chaetomiaceae -	0.2	0.1	0	7.4	3	3.1
Ascomycota; Cephalothecaceae -	2.5	3.8	3.5	0.1	0.4	0.2
Ascomycota; Helotiales (IS) -	2.7	3.4	2.6	0	0	0
Ascomycota; Pyronemataceae -	0	0.3	0.5	1.8	4.1	3.5
Ascomycota; Diatrypaceae -	0	0	0	2	5.1	2.4
Basidiomycota; Stephanosporaceae -	0	0	0	2.3	4.8	1.6
Ascomycota; Myrmecridiaceae -	0.9	3.7	1.5	0	0	0
Ascomycota; Aspergillaceae -	1.4	1.7	1.5	0.4	0.5	0.4
	Td -	Ta_Tr -	Ta_De -	Td -	Ta_Tr -	Ta_De -

Suppl Fig 14