

**Supplemental Material for: Applied and Environmental Microbiology**

**Soybean Root Nodule and Rhizosphere Microbiome: Distribution of Rhizobial and Non-rhizobial Endophytes**

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29 **Supplementary Figures:** Figures 1-9

30 **Supplementary Tables:** Table 1-4

31 **Fig. S1.** Roots from one of the soybean plants (L1P1N1-23). The 23 intact root nodules  
32 that were selected for the microbiome analysis are indicated (arrows).

33 **Fig. S2:** A comparison of bacterial genera identified by 16S rRNA gene  
34 sequences from the root nodules and rhizosphere soil of plant 1 (L1P1N1-23).  
35 The total number of DNA sequences obtained from root nodules (826,945) and  
36 rhizosphere soil (19,345) of plant 1 were used for this comparison.

37 **Fig. S3:** Species accumulation curve for the root nodules and a rhizosphere  
38 sample of plant 1 (L1P1N1-23).

39 **Fig. S4 A&B:** Nonmetric multidimensional scaling representation of 16S rRNA  
40 gene sequences from root nodules of plant 1 (L1P1N1-23). A). Describing the  
41 influence of root nodule location on a root system and B) size of the root nodules  
42 on bacterial endophytes. The two-dimensional stress was 0.11 and 0.10 for  
43 panels A and B, respectively.

44 **Fig. S5:** Maximum likelihood phylogenetic tree based on partial sequences of the  
45 16S rRNA gene of the *Nitrobacter* related sequences detected within root  
46 nodules and rhizosphere soil of soybean plants. The distribution of  
47 related *Nitrobacter* sequences from GenBank is shown next to the cluster. The  
48 numbers at the nodes reflect bootstrap support values that were above 50%.

49 **Fig. S6:** Maximum likelihood phylogenetic tree based on partial sequences of  
50 the 16S rRNA gene of the *Tardiphaga* related sequences detected within root  
51 nodules and rhizosphere soil of soybean plants. The numbers at the nodes  
52 reflect bootstrap support values that were above 50%.

53 **Fig. S7:** Soil and plant samples were collected from three locations within the  
54 Kindrick farm.

55 **Fig. S8:** Soybean root nodules intact with the root system. **A).** before cleaning  
56 and **B).** after cleaning the nodule surfaces.

57 **Fig. S9:** Description of two step PCR approach for 16S rRNA gene amplification  
58 and library preparation for Illumina MiSeq paired-end DNA sequencing.

59

#### 60 **Supplemental Tables:**

61 **Table S1:** Culture and culture-independent based studies focused on the non-  
62 rhizobial endophytes within soybean root nodules.

63 **Table S2:** Distribution of various non-rhizobial endophytes within root nodules of a  
64 plant (L1P1N1-23).

65 **Table S3:** Bacterial diversity within root nodules of a plant and rhizosphere soil. In  
66 sample ID, L1-3 represents three locations (1-3), P1-9 represents nine plant (1-9),  
67 N represents the nodules per plant (N1-23), and R is for rhizosphere samples.

68 **Table S4:** Soil physiochemical characteristics of soil attached with soybean plants  
69 from three locations within Kindrick farm.

70 **Table S5:** List of unique indices used for each of the root nodules and soil  
71 samples.

Fig. S1



Fig. S2

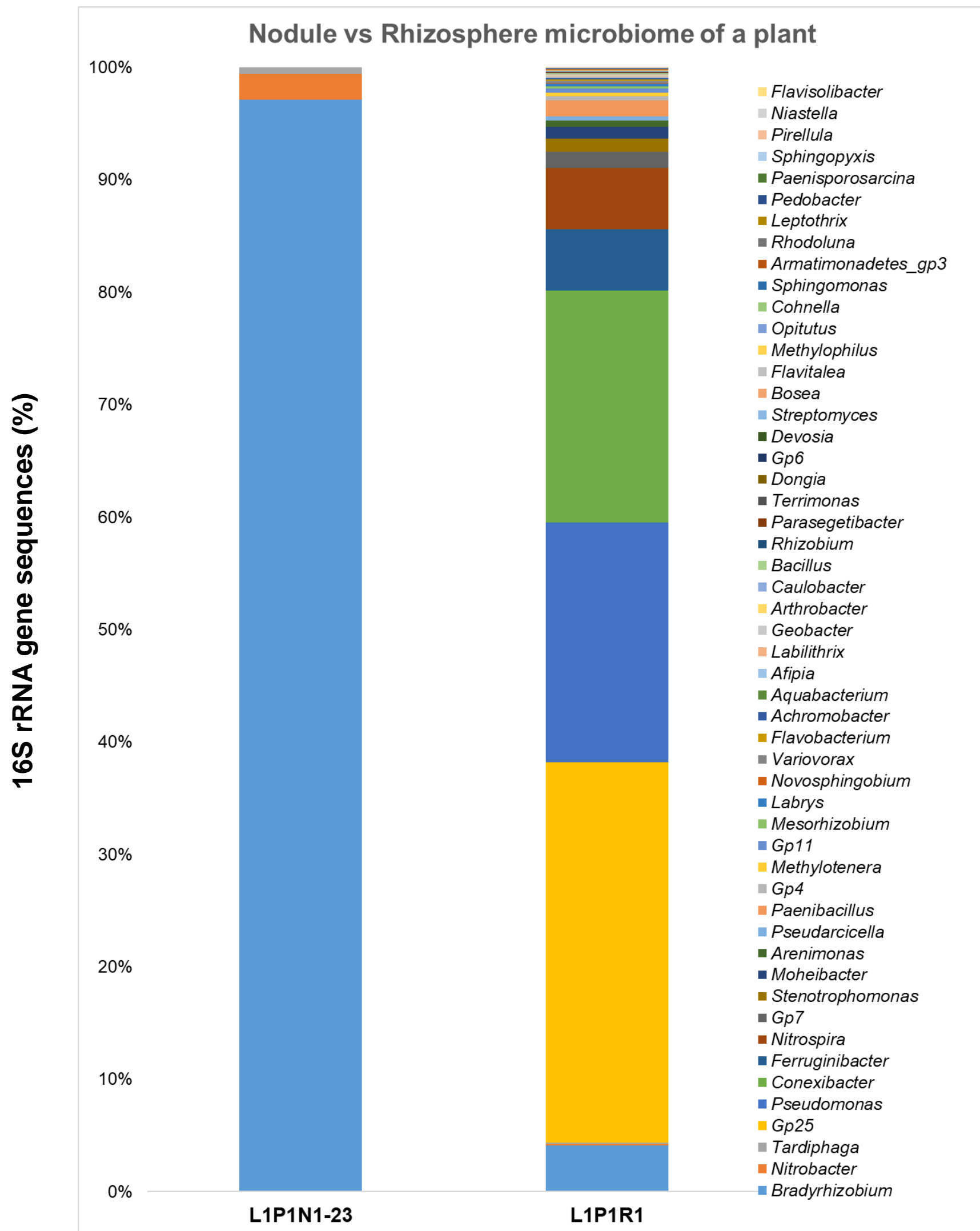


Fig. S3

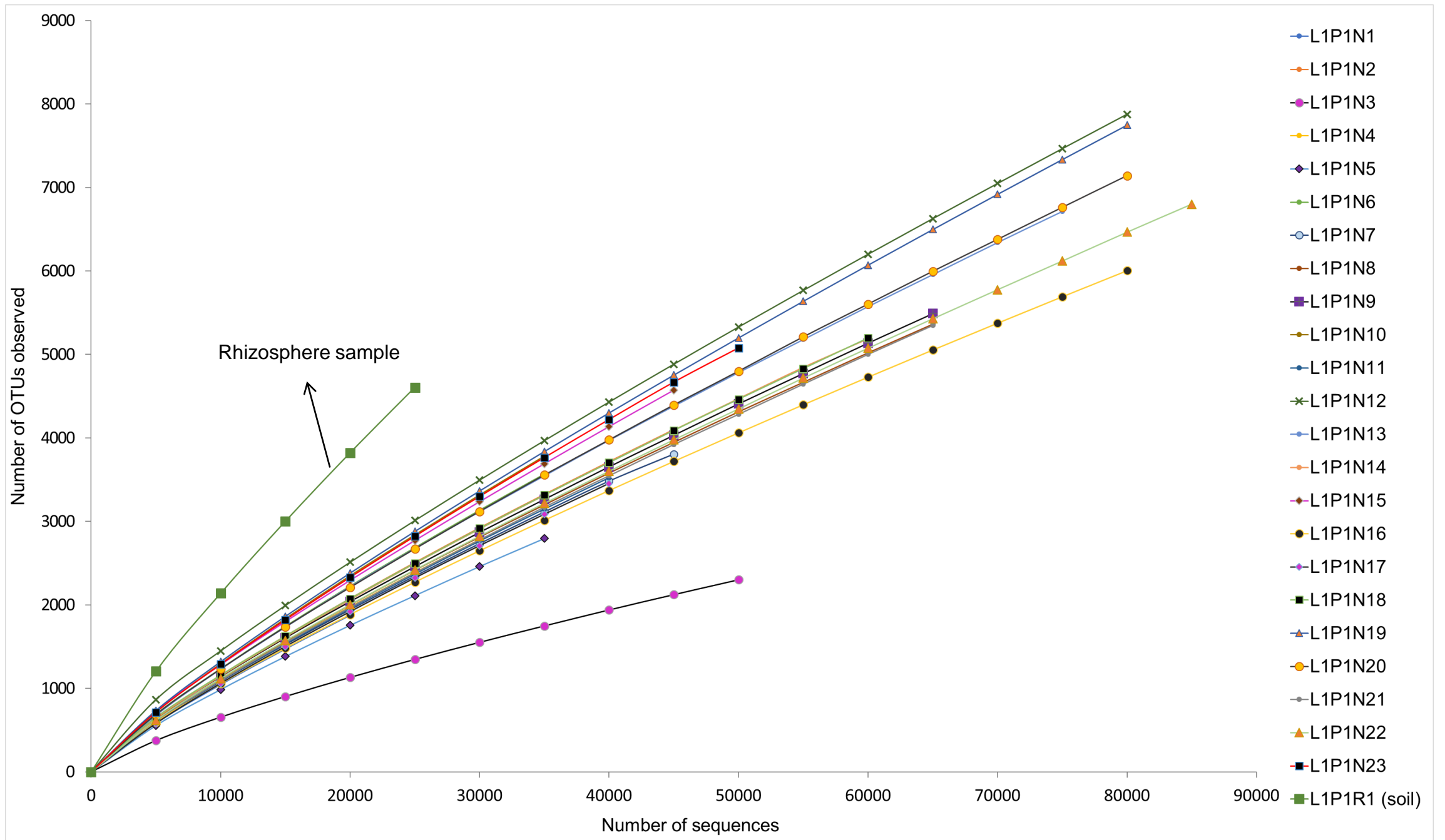


Fig. S4 A&B

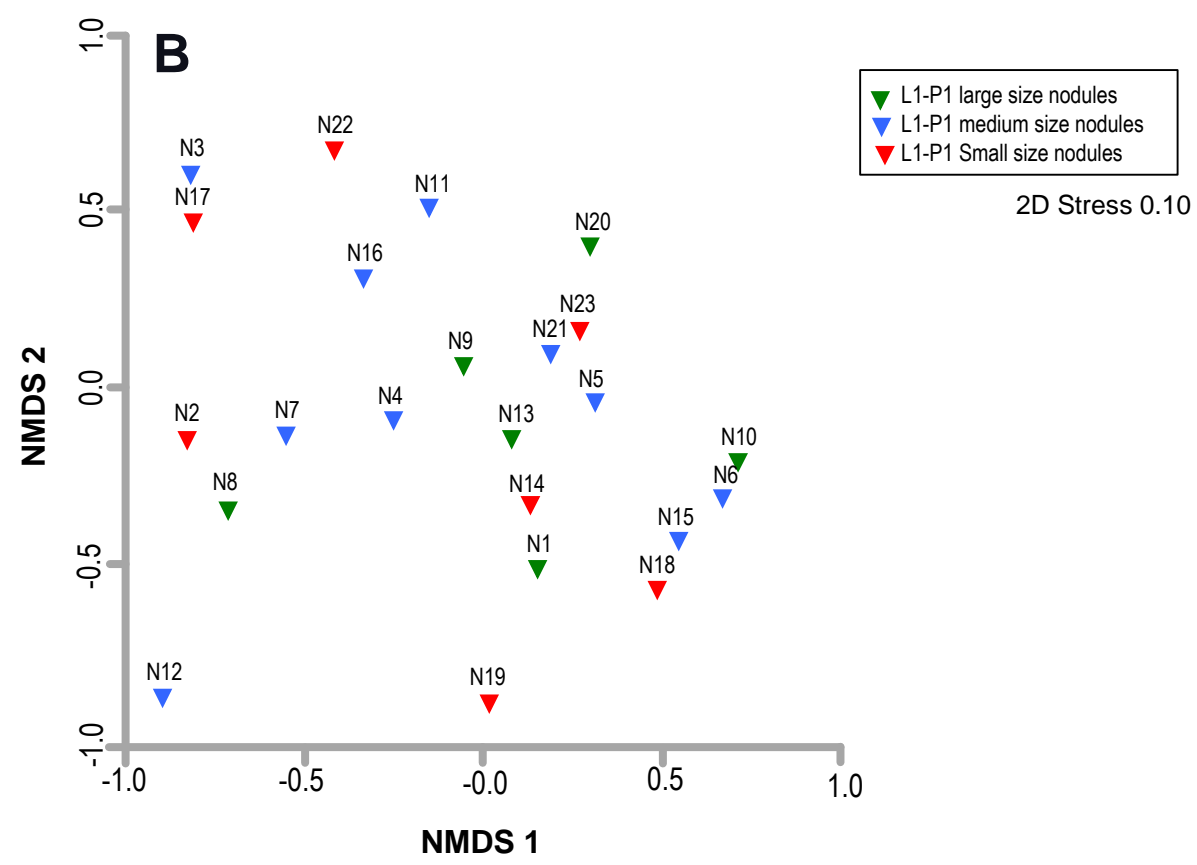
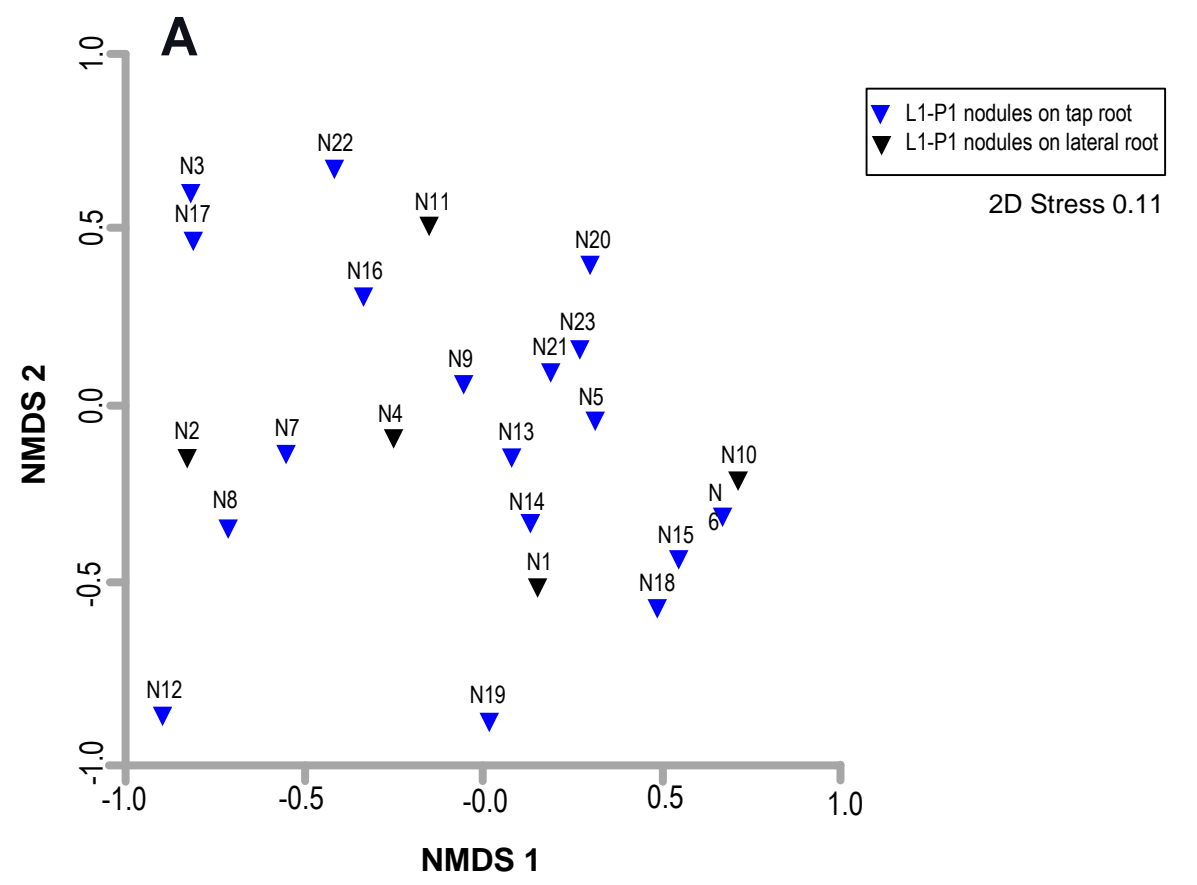


Fig. S5

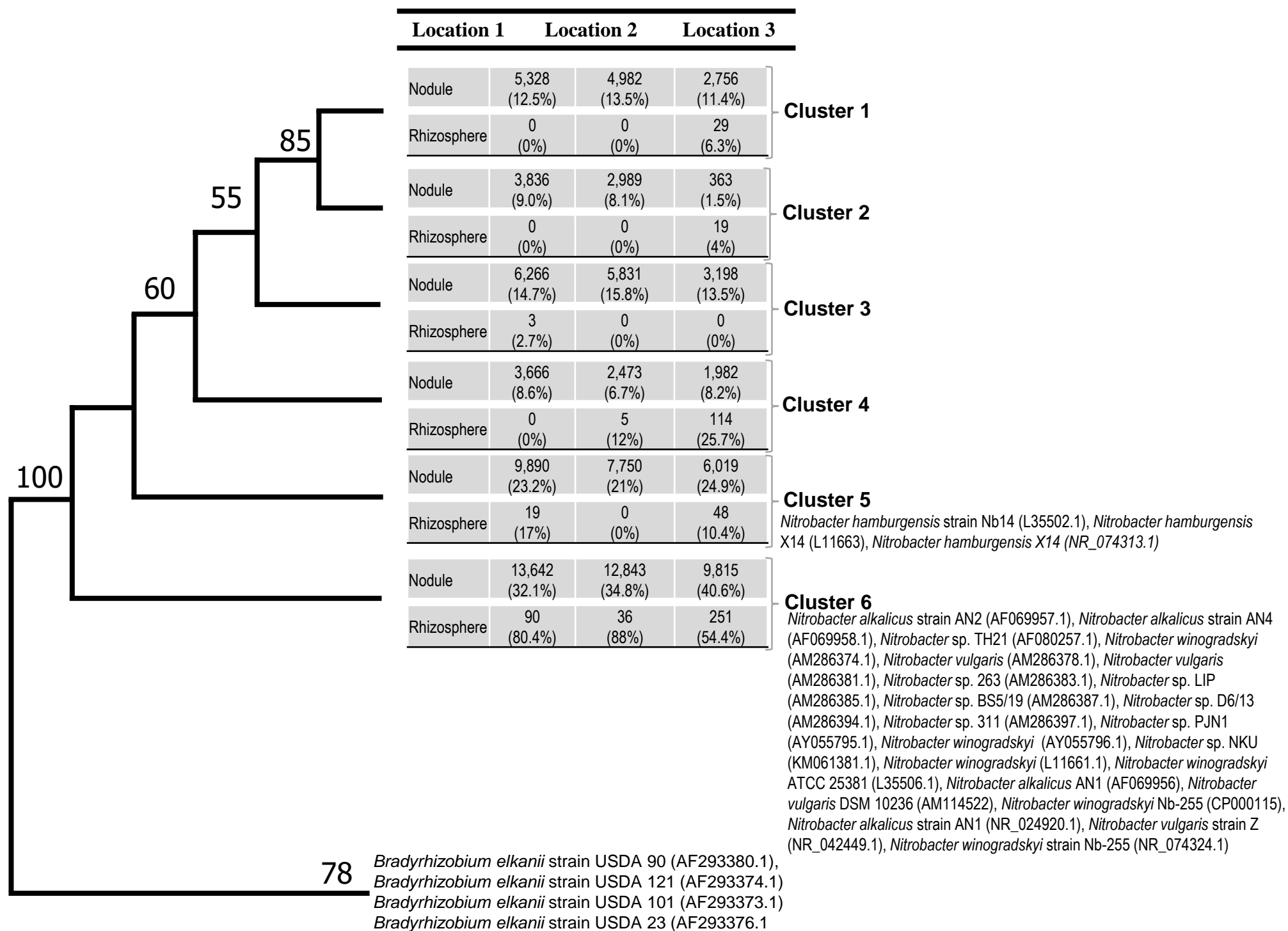




Fig. S6

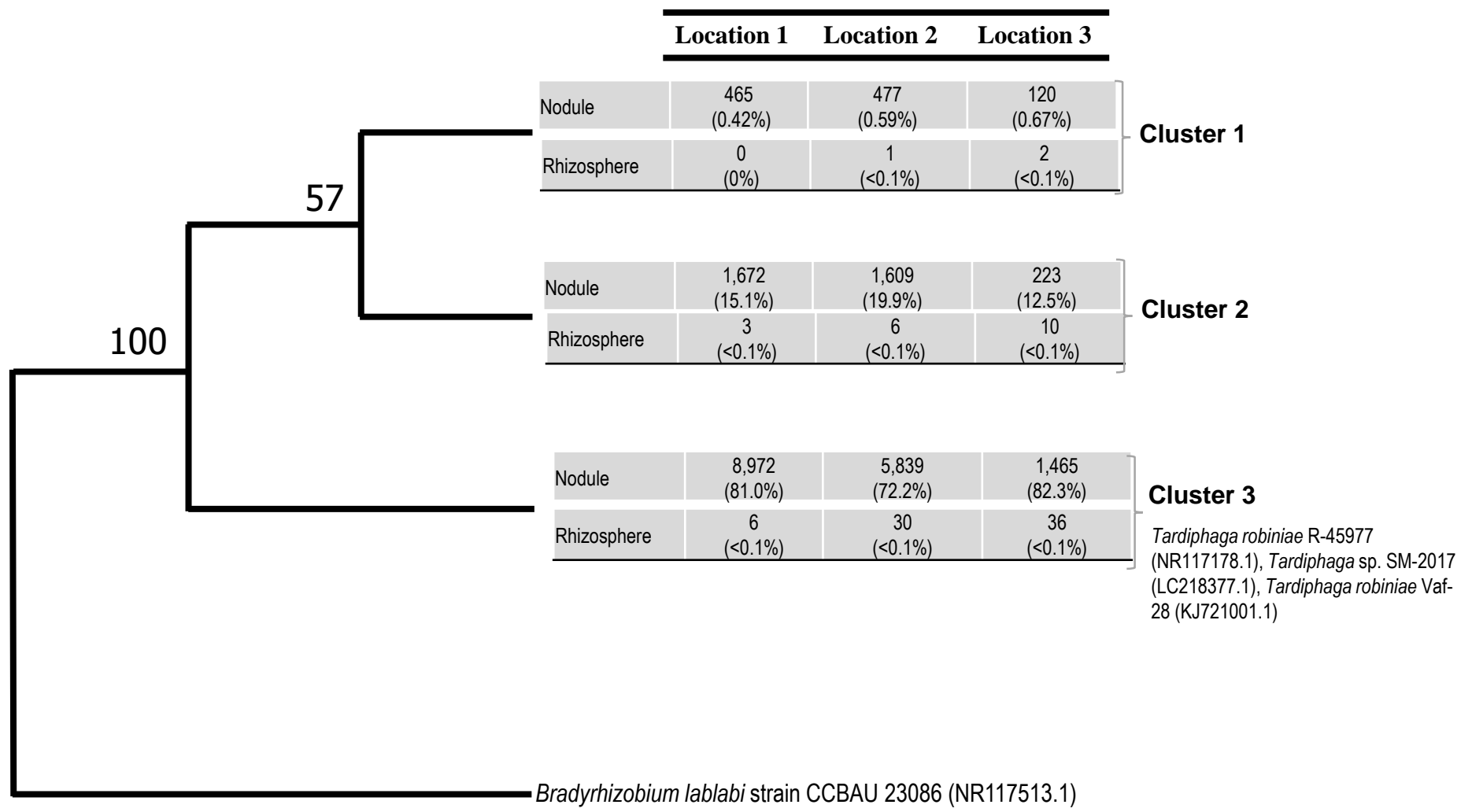


Fig. S7

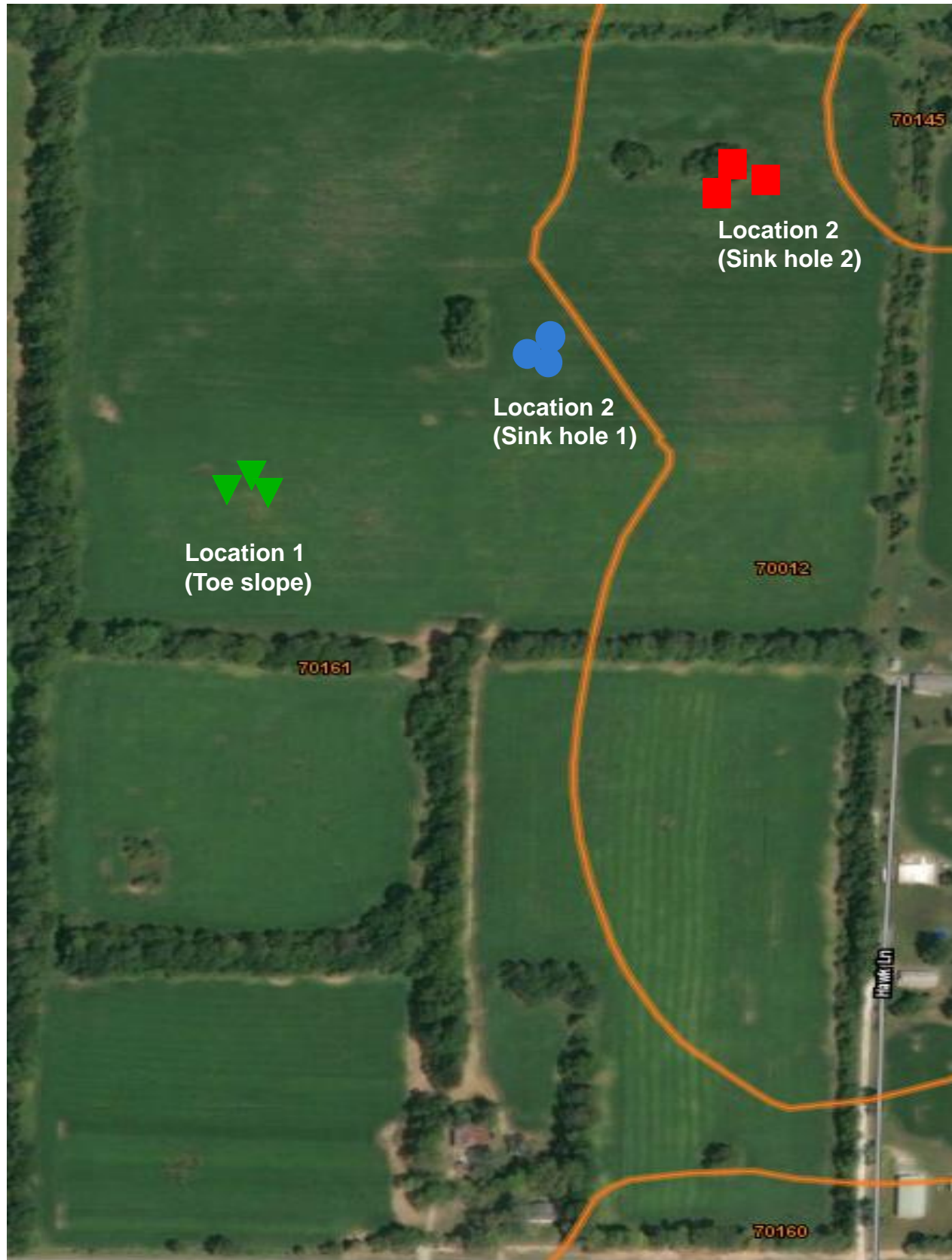
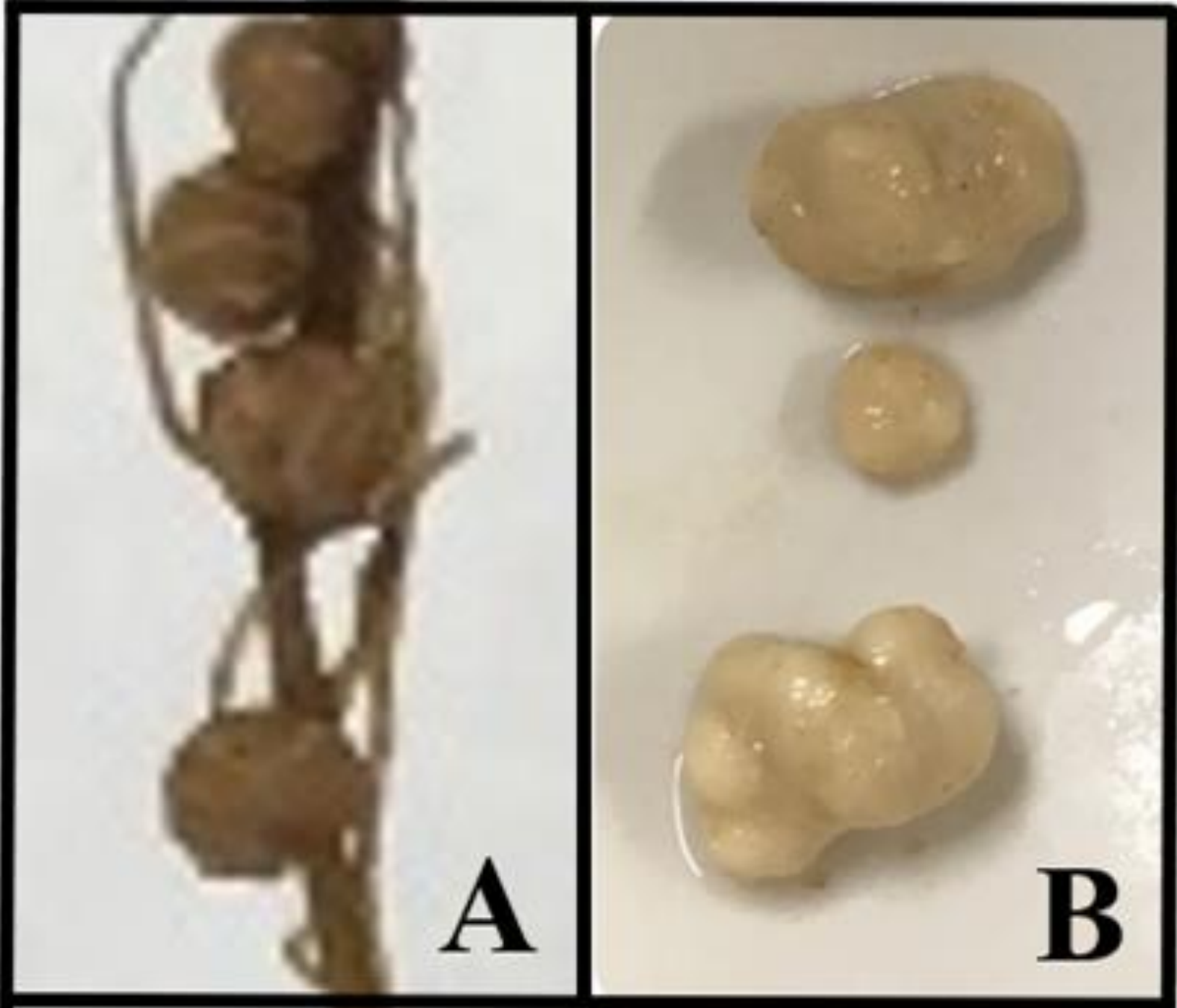
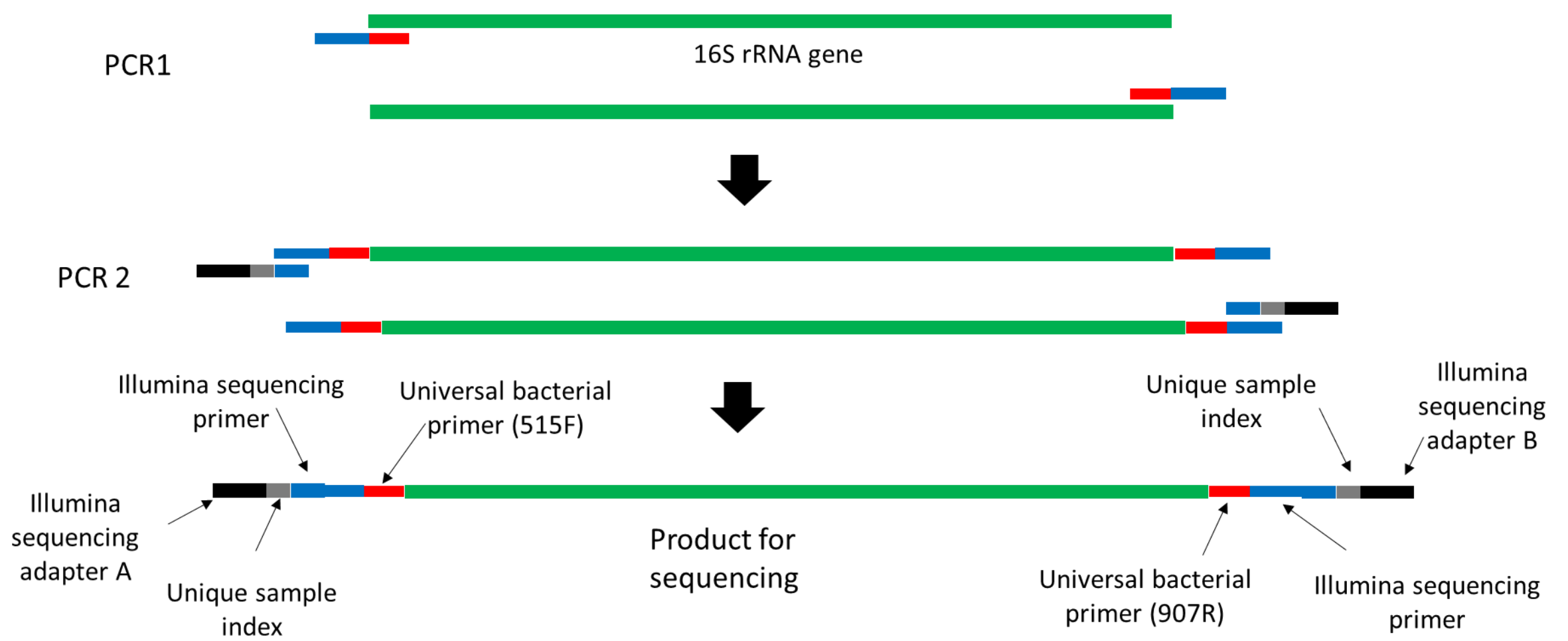


Fig. S8



**Fig. S9**

PCR amplification and library preparation for Illumina MiSeq paired-end DNA sequencing



**First PCR primer set**

<b>515F</b>	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGTGCCAGCMGCCGCGG
<b>907R</b>	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCGTCAATTCMTTTRAGTTT

**An example of a 2<sup>nd</sup> PCR primer set**

<b>U701</b>	CAAGCAGAAGACGGCATACGAGATTCGCCTTAGTGACTGGAGTTCAGACGTGTG
<b>U501</b>	AATGATACGGCGACCACCGAGATCTACACTAGATCGCACACTCTTTCCCTACACGACG

**Table S1:** Important bacterial genera identified as non-rhizobial endophytes within soybean root nodules

Isolates (genera)	Proposed Beneficial Feature	Reference
<b><u>Culturing-based studies</u></b>		
<i>Pseudomonas, Burkholderia, Enterobacter, Agrobacterium, Ochrobactrum, Acinetobacter, Proteus</i>	Biocontrol agents	(1)
<i>Pseudomonas, Bacillus, Enterobacter, Klebsiella, Acetobacter Burkholderia, Rhizobium, Xanthomonas</i>	Phytohormone and Siderophore production, Biocontrol agents	(2)
<i>Enterobacter, Microbacterium, Agrobacterium, Serratia marcescens, Chryseobacterium, Xanthomonas.</i>	Increase plant growth	(3)
<i>Pseudomonas, Acinetobacter, Enterobacter, Bacillus, Ochrobacterum</i>	BNF, IAA synthesis, nutrient availability	(4)
<i>Agrobacterium, Bacillus, Pantoea, Serratia, Burkholderia, Acinetobacter</i>	BNF, IAA synthesis, nutrient availability	(5)
<i>Bacillus, Paenibacillus</i>	IAA synthesis and biocontrol agent	(6)
<i>Tardiphaga, Afipia</i>	-	(7)
<i>Bacillus</i> spp. ( <i>subtilis</i> and <i>thuringiensis</i> )	PGPR activity	(8,9)
<i>Bacillus endoradicis</i>	-	(10)
<i>Achromobacter, Agrobacterium, Burkholderia, Cronobacter, Enterobacter, Novosphingobium, Pantoea, Pseudomonas, Rahnella, Serratia, and Variovorax.</i>	PGPR properties in vitro	(11)
<i>Achromobacter xylosoxidans</i>	BNF	(12)
<i>Paenibacillus polymyxa</i>		(13)
35 Non-rhizobial endophytes	IAA synthesis, cellulose, pectinase	(14)
<i>Enterobacter ludwigii, Variovorax paradoxus</i> (isolates from roots)	IAA synthesis, P-solubilization, PGPR	(15)
<i>Pseudomonas, Ralstonia, Enterobacter, Pantoea, Acinetobacter.</i>	IAA synthesis, P-solubilization, BNF	(16)
<b><u>Culture-independent studies</u></b>		
<i>Pseudomonas, Enterobacter, Bacillus, Paenibacillus</i> and some others (pooled root nodules from multiple plants) 1-40%		(17)
Members of <i>Proteobacteria, Actinobacteria, Firmicutes</i> and <i>Bacteroidetes</i> . (pooled root nodules from multiple plants) 435 bacterial genera of 21 phyla		(18)
<i>Pseudomonas, Agrobacterium, Bacillus, Burkholderia, Microbacterium, Streptomyces</i> and others (pooled root nodules from multiple plants) 2434 OTUs of NREs		(19)

BNF = Biological N fixation; IAA = Indole acetic Acid; PGPR = Plant growth promoting rhizobacteria



**Table S3:** Bacterial diversity within root nodules of a plant and rhizosphere soil. In sample ID, L1-3 represents locations 1-3, P1-9 represents plant 1-9, N represents the nodules per plant, and R is for rhizosphere samples.

Sample ID	Nodules per plant or soil samples	Average number sequences per plant	Chao 1 estimator	Shannon Diversity
L1P1N1-23	23	56781 ± 4064	29263 ± 2547	2.60 ± 0.09
L1P2N1-23	23	44829 ± 7763	27932 ± 2986	2.83 ± 0.15
L1P3N1-20	20	25356 ± 2873	13846 ± 1161	2.55 ± 0.10
L2P4N1-22	22	42484 ± 6579	20072 ± 2681	2.50 ± 0.08
L2P5N1-24	24	35969 ± 3513	22991 ± 2238	2.49 ± 0.09
L2P6N1-24	24	40697 ± 4773	19554 ± 2210	2.32 ± 0.11
L3P4N1-21	21	20031 ± 1995	9051 ± 853	2.03 ± 0.12
L3P5N1-17	17	14221 ± 1698	8327 ± 451	2.04 ± 0.19
L3P6N1-19	19	43637 ± 7497	17939 ± 2412	2.27 ± 0.07
L1RP1-3	3	55296 ± 17176	45861 ± 11477	5.78 ± 0.28
*L2RP6	1	104715	55208	5.72
L3RP7-9	3	61554 ± 24622	46193 ± 16634	6.40 ± 0.29

\* Location 2, the two of three rhizosphere samples resulted in less than 5,000 sequences. These two samples were not included the diversity analysis.

**Table S4:** Soil physiochemical characteristics of soil attached with soybean plants from three locations within Kindrick farm.

Sample ID	pH	OM	NO <sub>3</sub> -N	NH <sub>4</sub> -N	P	K	Ca	Mg	CEC
<b>Location 1 (Toe slope)</b>	6.6±.2	3.5±0.1	9.7±.8	22.4±4.5	33±1.5	104±3	1223±216	31±8	9.3±1.8
<b>Location 2 (Sink hole 1)</b>	6.5±0.1	3.1±0.2	8.7±1	11.4±3.5	16±2	103±2	1113±114	24±5	8.7±1
<b>Location 3 (Sink hole 2)</b>	6.8±0.1	3.5±.02	26.7±1.8	8.2±2.5	34±2.7	134±7	1358±12	28±4	10±0.2

**Measured soil variables:** pH, organic matter (OM %), Nitrate N (ppm), Ammonium N (ppm), P (phosphorus); K (potassium); Ca (calcium); Mg (magnesium); P, K, Ca, Mg, are expressed as mg/L, CEC, potential cation exchange capacity (meq/100g).

**Sampling locations:** Location 1 & 2 were related to (Keeno-Bona complex, Karst, 2 to 5 % slopes), whereas, Location 3 was related to (Hoberg, silt loam, 2 to 5 percent slopes).



**Table S5:** List of unique indices used for each of the root nodules and soil samples.

Sample	F (indices)	R (indices)	Sample	F (indices)	R (indices)	Sample	F (indices)	R (indices)	Sample	F (indices)	R (indices)	Sample	F (indices)	R (indices)	Sample	F (indices)	R (indices)	Sample	F (indices)	R (indices)
L1P1N1	U701	U509	L1P2N12	U734	U509	L2P4N3	U739	511	L2P5N12	U724	512	L2P6N21	U709	513	L3P8N11	U718	515	L3P9N19	U742	515
L1P1N2	U702	U509	L1P2N13	U735	U509	L2P4N4	U740	511	L2P5N13	U725	512	L2P6N22	U710	513	L3P8N14	U719	515	L3P9N20	U743	515
L1P1N3	U703	U509	L1P2N14	U736	U509	L2P4N5	U741	511	L2P5N14	U726	512	L2P6N23	U711	513	L3P8N15	U720	515	L3P9N21	U744	515
L1P1N4	U704	U509	L1P2N15	U737	U509	L2P4N6	U742	511	L2P5N15	U727	512	L2P6N24	U712	513	L3P8N18	U721	515	L3P9N24	U745	515
L1P1N6	U705	U509	L1P2N16	U738	U509	L2P4N7	U743	511	L2P5N16	U728	512	L3P7N1	U737	514	L3P8N19	U722	515	LP1R1	U725	524
L1P1N7	U706	U509	L1P2N17	U739	U509	L2P4N8	U744	511	L2P5N17	U729	512	L3P7N2	U738	514	L3P8N20	U723	515	LP2R2	U726	524
L1P1N8	U707	U509	L1P2N18	U740	U509	L2P4N9	U745	511	L2P5N18	U730	512	L3P7N3	U739	514	L3P8N21	U724	515	LP3R3	U727	524
L1P1N9	U708	U509	L1P2N19	U741	U509	L2P4N10	U746	511	L2P5N19	U731	512	L3P7N6	U740	514	L3P8N22	U725	515	LP4R4	U728	524
L1P1N10	U709	U509	L1P2N20	U742	U509	L2P4N11	U747	511	L2P5N20	U732	512	L3P7N7	U741	514	L3P8N23	U726	515	LP5R5	U729	524
L1P1N11	U710	U509	L1P2N21	U743	U509	L2P4N12	U748	511	L2P5N21	U733	512	L3P7N8	U742	514	L3P8N11	U718	515	LP6R6	U730	524
L1P1N12	U711	U509	L1P2N22	U744	U509	L2P4N13	U701	512	L2P5N22	U734	512	L3P7N9	U743	514	L3P8N14	U719	515	LP7R7	U731	524
L1P1N13	U712	U509	L1P2N23	U745	U509	L2P4N14	U702	512	L2P5N23	U735	512	L3P7N10	U744	514	L3P8N15	U720	515	LP8R8	U732	524
L1P1N14	U713	U509	L1P2N24	U746	U509	L2P4N15	U703	512	L2P5N24	U736	512	L3P7N11	U745	514	L3P8N18	U721	515	LP9R9	U733	524
L1P1N15	U714	U509	L1P3N1	U747	U509	L2P4N16	U704	512	L2P6N1	U737	512	L3P7N12	U746	514	L3P8N19	U722	515			
L1P1N16	U715	U509	L1P3N2	U748	U509	L2P4N17	U705	512	L2P6N2	U738	512	L3P7N13	U747	514	L3P8N20	U723	515			
L1P1N17	U716	U509	L1P3N3	U701	U510	L2P4N18	U706	512	L2P6N3	U739	512	L3P7N14	U748	514	L3P8N21	U724	515			
L1P1N18	U717	U509	L1P3N4	U702	U510	L2P4N19	U707	512	L2P6N4	U740	512	L3P7N15	U701	515	L3P8N22	U725	515			
L1P1N19	U718	U509	L1P3N5	U703	U510	L2P4N20	U708	512	L2P6N5	U741	512	L3P7N16	U702	515	L3P8N23	U726	515			
L1P1N20	U719	U509	L1P3N6	U704	U510	L2P4N21	U709	512	L2P6N6	U742	512	L3P7N18	U703	515	L3P9N2	U727	515			
L1P1N21	U720	U509	L1P3N7	U705	U510	L2P4N22	U710	512	L2P6N7	U743	512	L3P7N19	U704	515	L3P9N3	U728	515			
L1P1N22	U721	U509	L1P3N8	U706	U510	L2P4N23	U711	512	L2P6N8	U744	512	L3P7N20	U705	515	L3P9N4	U729	515			
L1P1N23	U722	U509	L1P3N9	U707	U510	L2P4N24	U712	512	L2P6N9	U745	512	L3P7N21	U706	515	L3P9N6	U730	515			
L1P1N24	U723	U509	L1P3N10	U708	U510	L2P5N1	U713	512	L2P6N10	U746	512	L3P7N22	U707	515	L3P9N7	U731	515			
L1P2N1	U724	U509	L1P3N13	U709	U510	L2P5N2	U714	512	L2P6N11	U747	512	L3P7N23	U708	515	L3P9N8	U732	515			
L1P2N2	U725	U509	L1P3N14	U710	U510	L2P5N3	U715	512	L2P6N12	U748	512	L3P7N24	U709	515	L3P9N9	U733	515			
L1P2N3	U726	U509	L1P3N15	U711	U510	L2P5N4	U716	512	L2P6N13	U701	513	L3P8N1	U710	515	L3P9N10	U734	515			
L1P2N5	U727	U509	L1P3N16	U712	U510	L2P5N5	U717	512	L2P6N14	U702	513	L3P8N2	U711	515	L3P9N11	U735	515			
L1P2N6	U728	U509	L1P3N17	U713	U510	L2P5N6	U718	512	L2P6N15	U703	513	L3P8N3	U712	515	L3P9N13	U736	515			
L1P2N7	U729	U509	L1P3N19	U714	U510	L2P5N7	U719	512	L2P6N16	U704	513	L3P8N6	U713	515	L3P9N14	U737	515			
L1P2N8	U730	U509	L1P3N20	U715	U510	L2P5N8	U720	512	L2P6N17	U705	513	L3P8N7	U714	515	L3P9N15	U738	515			
L1P2N9	U731	U509	L1P3N21	U716	U510	L2P5N9	U721	512	L2P6N18	U706	513	L3P8N8	U715	515	L3P9N16	U739	515			
L1P2N10	U732	U509	L1P3N22	U717	U510	L2P5N10	U722	512	L2P6N19	U707	513	L3P8N9	U716	515	L3P9N17	U740	515			
L1P2N11	U733	U509	L1P3N23	U718	U510	L2P5N11	U723	512	L2P6N20	U708	513	L3P8N10	U717	515	L3P9N18	U741	515			

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