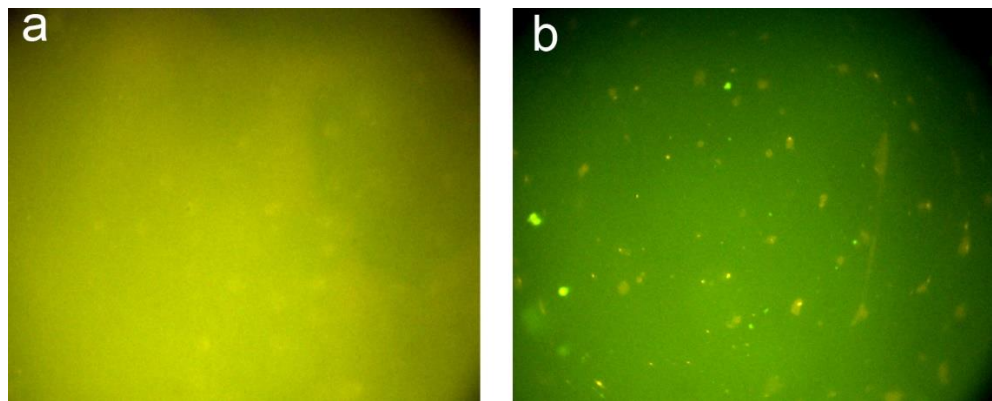


Supporting Information S. 1

The tested samples washing method before DNA extraction.

The detailed sampling procedure was as follows:

- 1) Loose soil attached to roots aseptically.
- 2) Whole plant were placed into clean and sterile beaker, with containing 1/3 volume PBS phosphate buffer (per litre: 6.33 g of $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$, 16.5g of $\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$, 200 μl Silwet L-77).
- 3) Gently shake the beaker, until the soil from plants were released most.
- 4) The whole plant were then sonicated in asepsis condition at low frequency for 1 min (five 30-s bursts followed by five 30-s rests), which further disrupted tiny soil aggregates and attached microbes.
- 5) Wiping whole body of plants with 75% alcohol, under the asepsis conditions
- 5) Again showered the plant body by sterilized PBS buffer carefully, repeat this step 6 for five times.
- 6) Finally showered the plant with DD water, and collect the final buffer.
- 7) Centrifuge the final buffer at 1000g 4°C, Supernatant were collected for an AODC test.



Supplementary Fig. S1 Photos of the wash buffer scanning by AODC. (a): The final wash buffer showed no bacterial signal were detected. (b): Buffer from the first flushing, each bright spot represented a bacteria cell.

Supplementary Table S1 General features of the 16s rDNA sequencing.

Sample ID	Raw reads	Clean reads	No. of OTU97	No. of genera	No. of family	No. of order	No. of class	No. of phylum	Good's coverage	Chao1	Shannon
FL1	30953	22402	505	226	150	85	43	19	0.98	657.0	5.1
FL2	38027	27483	531	264	179	100	47	20	0.98	719.1	5.3
FL3	35286	25562	527	248	168	100	50	22	0.98	682.0	5.1
FR1	44897	33320	484	251	163	83	41	20	0.99	544.9	5.6
FR2	40567	30233	591	259	163	83	38	18	0.99	494.4	5.8
FR3	45598	33315	560	265	170	87	41	19	0.99	557.4	5.8
FS1	32373	24656	401	220	134	75	36	17	0.99	467.1	5.7
FS2	30598	22939	402	222	142	82	44	18	0.99	513.3	5.5
FS3	33914	25790	416	225	135	72	39	18	0.99	412.3	5.5
AL1	29375	25287	288	139	90	48	22	12	0.99	249.4	2.3
AL2	38866	34204	296	149	98	53	27	14	0.99	258.5	2.2
AL3	28163	23239	263	128	90	52	23	11	0.99	278.0	2.3
AR1	46669	39565	227	153	109	57	26	12	0.99	268.1	2.3
AR2	42615	36394	243	165	109	62	30	14	0.99	292.0	2.4
AR3	39729	32355	206	155	108	58	25	13	0.99	260.6	2.6
AS1	22767	16528	456	231	155	86	44	19	0.98	732.5	2.6
AS2	25851	18032	577	266	180	99	51	22	0.97	833.3	3.0
AS3	24862	17564	507	248	167	91	42	20	0.98	755.1	2.7
S1	75658	63774	1339	398	271	144	67	26	0.97	1299.1	8.6
S2	97099	82300	1387	405	276	147	68	27	0.97	1350.1	8.7
S3	94753	80482	1401	409	277	143	66	26	0.97	1308.9	8.7

Supplementary Table S2 Observed OTUs of the whole plant soybean endophytic denitrifying bacteria.

	F1	F2	F3	A1	A2	A3	S1	S2	S3
<i>Pseudomonas</i>	120	50	294	657	474	263	8	8	9
<i>Arthrobacter</i>	17	14	21	27	23	14	180	233	217
<i>Acinetobacter</i>	3	3	9	36	8	20	0	1	4
<i>Microbacterium</i>	141	147	698	44	26	23	10	15	8
<i>Noviherbaspirillum</i>	346	416	375	15	12	15	164	182	228
<i>Leptospirillum</i>	0	0	0	0	0	0	17	7	13
<i>Stenotrophomonas</i>	8	6	5	106	10	26	12	20	18
<i>Sphingobacterium</i>	1	0	2	15	4	0	0	0	0
<i>Bradyrhizobium</i>	4606	2354	2547	146	183	139	232	252	231
<i>Propionibacterium</i>	3	1	4	86	55	42	0	0	0
<i>Eubacterium</i>	0	0	0	22	63	35	0	0	0
a total	5245	2991	3955	1134	858	547	623	718	729

Supplementary Table S3 Relative abundance of the soybean endophytic denitrifying bacteria and its distribution in different organs (%)

OTU ID	F.leaf	F.root	F.stem	A.leaf	A.root	A.stem	BS
<i>Bradyrhizobium</i>	0.067%	8.980%	0.550%	0.057%	0.140%	0.710%	0.667%
<i>Noviherbaspirillum</i>	1.013%	0.237%	0.397%	0.020%	0.003%	0.097%	0.527%
<i>Microbacterium</i>	0.407%	0.093%	3.223%	0.053%	0.050%	0.023%	0.027%
<i>Pseudomonas</i>	0.293%	0.033%	0.947%	0.533%	1.030%	0.077%	0.023%
<i>Arthrobacter</i>	0.033%	0.003%	0.033%	0.013%	0.030%	0.057%	0.477%
<i>Propionibacterium</i>	0.003%	0.003%	0.007%	0.080%	0.117%	0.013%	0.000%
<i>Acinetobacter</i>	0.013%	0.000%	0.017%	0.070%	0.030%	0.020%	0.007%
<i>Leptospirillum</i>	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.033%
<i>Stenotrophomonas</i>	0.000%	0.000%	0.000%	0.053%	0.103%	0.000%	0.050%
<i>Sphingobacterium</i>	0.000%	0.000%	0.040%	0.020%	0.003%	0.003%	0.000%
<i>Eubacterium</i>	0.000%	0.000%	0.000%	0.057%	0.043%	0.000%	0.000%