



Supplementary Materials

Exploring the Potential of Overexpressed *OsCIPK2* Rice as a Nitrogen Utilization Efficient Crop and Analysis of Its Associated Rhizo-Compartmental Microbial Communities

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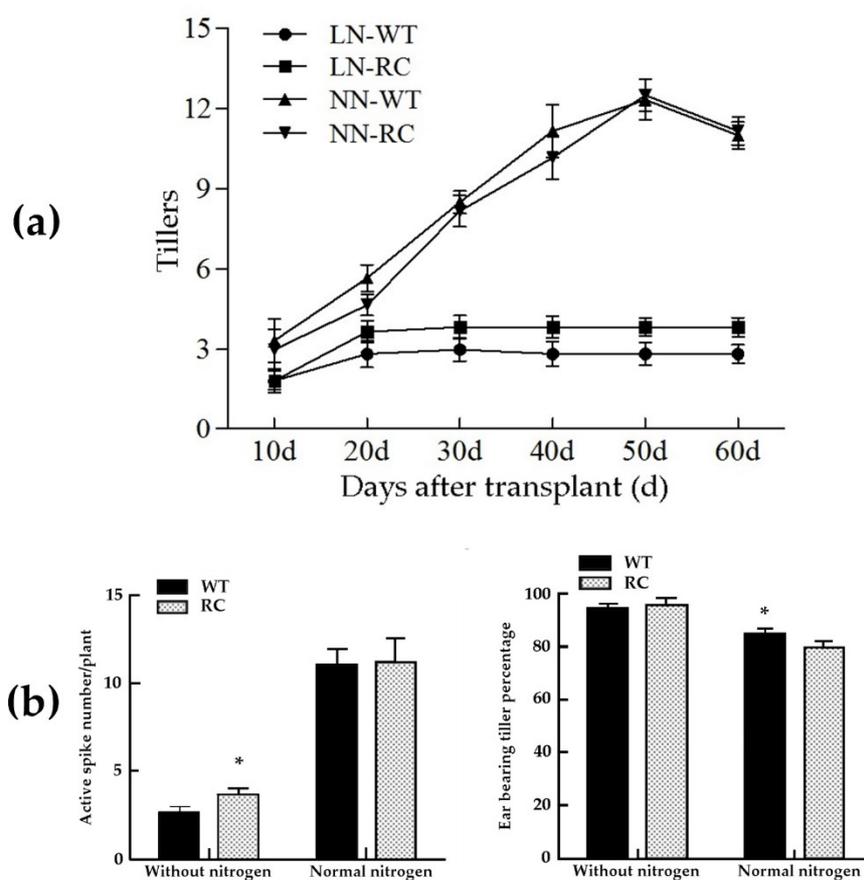


Figure S1. (a) Tillering dynamics of rice under different nitrogen treatments in pots. LN -WT: No N Wild type, LN-RC: Zero N Transgenic rice, NN-WT: Normal Nitrogen Wild type and NN-RC: Normal Nitrogen Transgenic rice. (b) Effective spike number and ear bearing tiller percentage of rice under different nitrogen treatments in pots. LN: no nitrogen treatment, NN: normal nitrogen treatment. *indicate significant difference at ($p < 0.05$) between different samples in the same treatment calculated by LSD's test.



Figure S2. Rice roots at heading stage under no nitrogen treatment in bucket.

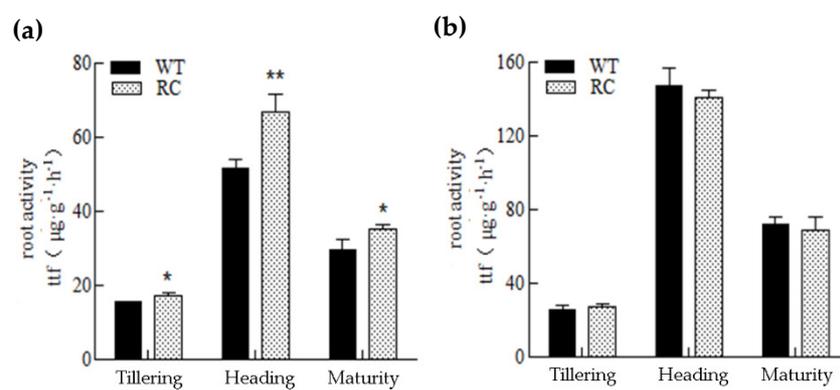


Figure S3. Root activity of rice at different growth stages under different nitrogen treatments in pots **a:** LN, no nitrogen treatment, **b:** NN, normal nitrogen treatment. *, ** indicates significant difference ($p < 0.05$) between different samples in the same growth period calculated by LSD's test.

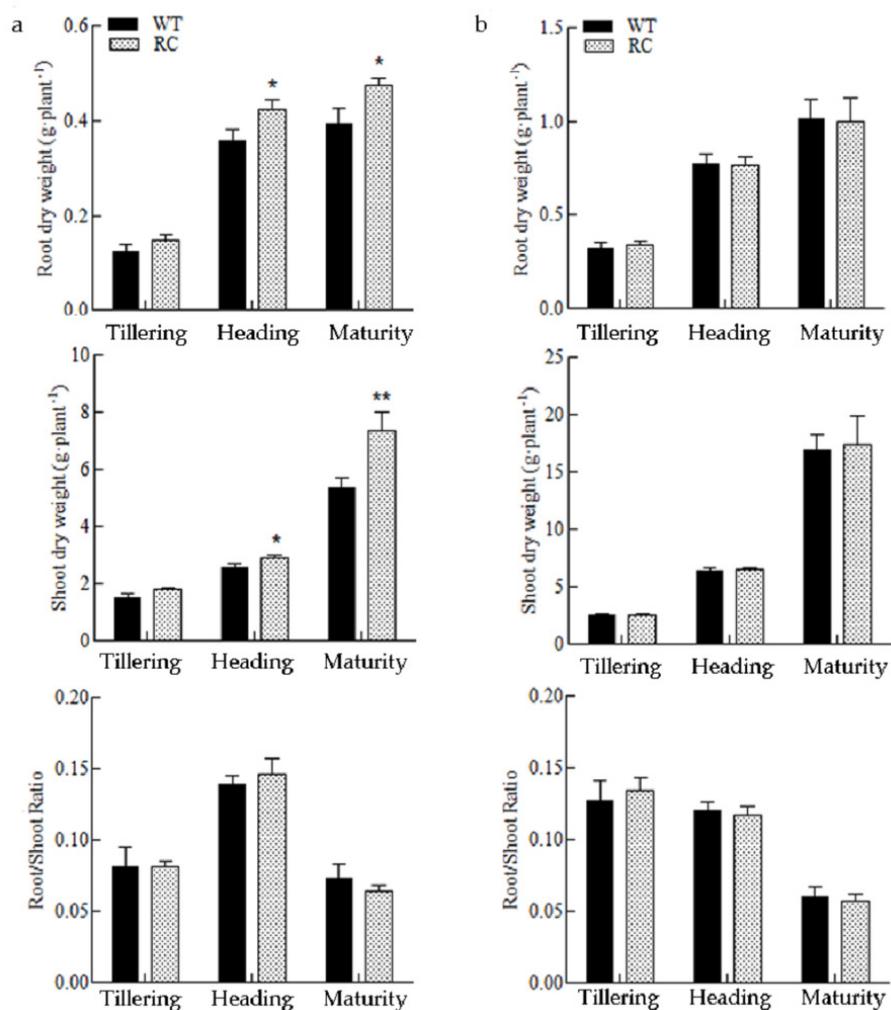


Figure S4. Dry weight and root-shoot ratio of rice at different growth stages. (a) no nitrogen treatment, (b) normal nitrogen treatment. *, ** indicates significant difference ($p < 0.05$) between different samples in the same growth period calculated by LSD's test.

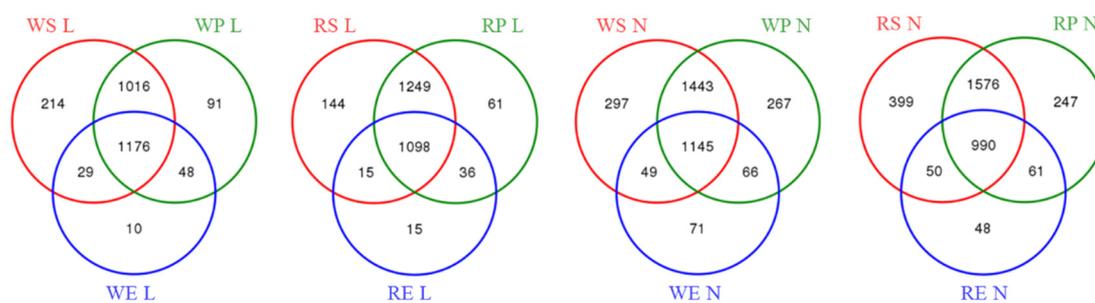


Figure S5. Venn diagram of OTUs in rice rhizo-compartments. WSL, WPL, WE L represents Wild type rhizosphere, wild type rhizoplane, wild endosphere under no nitrogen, respectively. RS L, RP L, RE L represents transgenic line rhizosphere, transgenic line rhizoplane, transgenic line endosphere under no nitrogen. WS N, WP N, WE N represents Wild type rhizosphere, wild type rhizoplane, wild endosphere under normal nitrogen, respectively. RS N, RP N, RE N represents transgenic line rhizosphere, transgenic line rhizoplane, transgenic line endosphere under normal nitrogen, respectively.

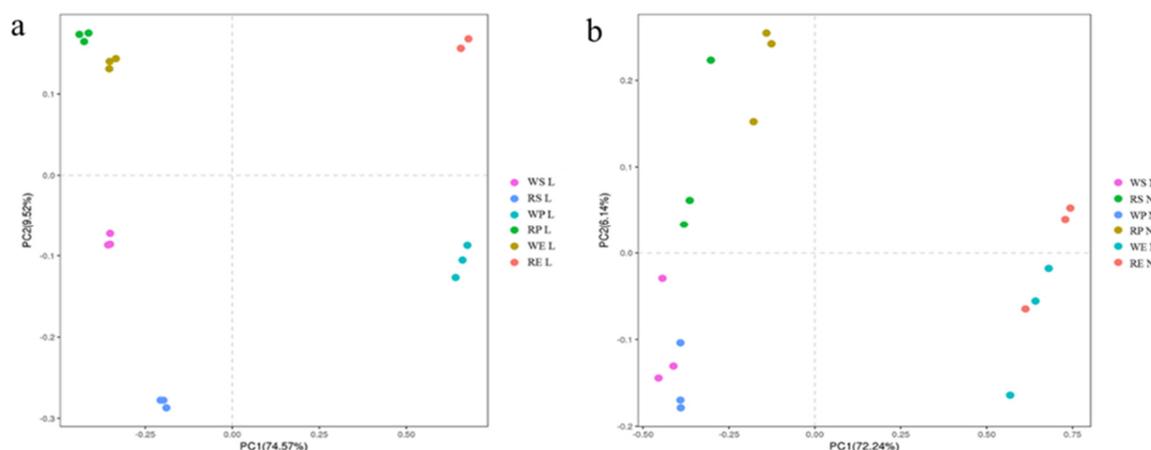


Figure S6. The results of principal component analysis of bacterial communities in rhizo-compartments of rice. WS L, WPL, WE L represents Wild type rhizosphere, wild type rhizoplane, wild endosphere under no nitrogen, respectively. RS L, RP L, RE L represents transgenic line rhizosphere, transgenic line rhizoplane, transgenic line endosphere under no nitrogen. WS N, WP N, WE N represents Wild type rhizosphere, wild type rhizoplane, wild endosphere under normal nitrogen, respectively. RS N, RP N, RE N represents transgenic line rhizosphere, transgenic line rhizoplane, transgenic line endosphere under normal nitrogen, respectively.

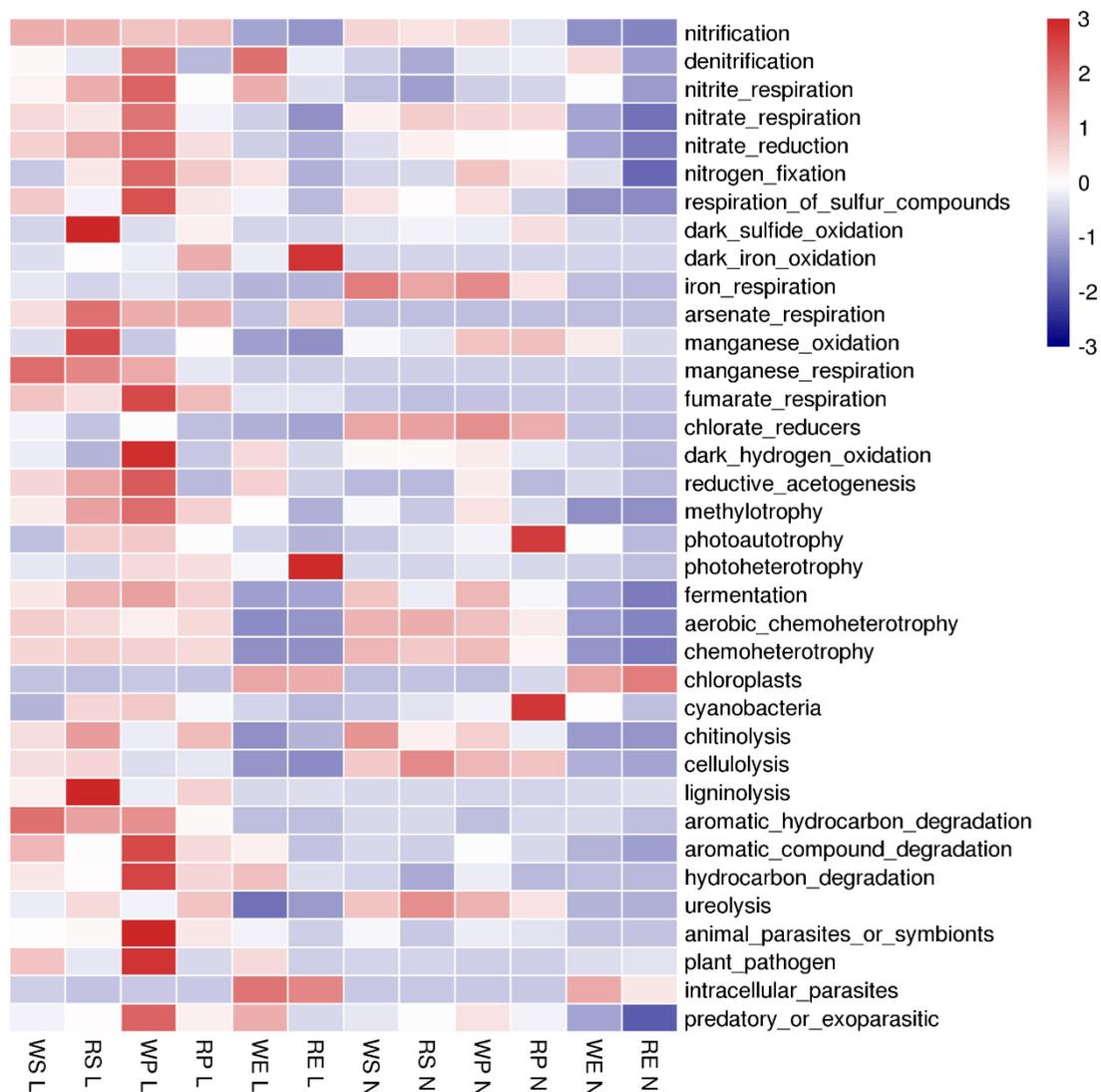


Figure S7. Heat map of OTUs abundance of different functional bacteria in rhizo-compartments of rice under different nitrogen treatments. The heat map shows the Z-score of relative abundance of each functional bacteria, the relative values for bacteria of different function are indicated by color intensity.

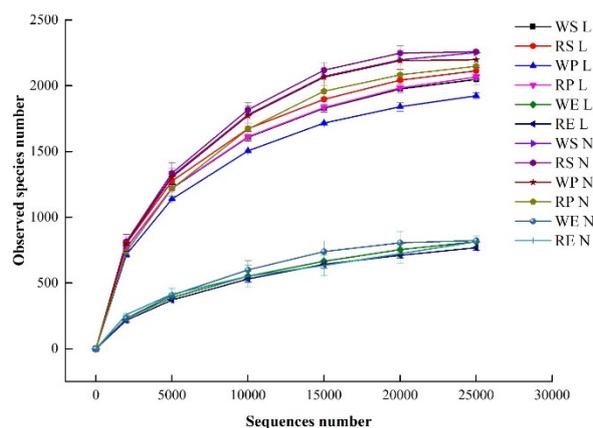


Figure S8. Rarefaction curves of rhizosphere, rhizoplane, endosphere samples of rice. WS, WE, WS respectively represent rhizosphere, rhizoplane, endosphere of WT; RS, RP, RE refers to rhizosphere, rhizoplane, endosphere of root specific expression CIPK2 rice (RC), L represents no nitrogen treatment, N represents normal nitrogen treatment, the same as below.

Table S1. Primers and Conditions *OsCIPK2* and *actin* gene.

The primer sequence is as follows	
Actin Forward primer	5'GGAAGTGGTATGGTCAAGGC 3'
Actin Reverse primer	3'AGTCTCATGGATAACCGCAG 3'
<i>OsCIPK2</i> Forward primer	5' GCCCGTAACACCGAGACTT 3'
<i>OsCIPK2</i> Reverse primer	5' GCCTCACCAACTTCATCACA 3'
The reaction system is as follows	
2× qPCR mastermix	7.5μL
Forward primer	0.5μL
Reverse primer	0.5μL
cDNA	0.5μL
Rnasefree water to make up the total system of 15μL	
<i>Actin</i> gene PCR amplification reaction profile: 95°C 5min;	
95°C 15s	} 35 cycles
55°C 20s	
72°C 30s	
<i>OsCIPK2</i> gene PCR amplification reaction profile: 95°C 5min;	
95°C 15s	} 35 cycles
55°C 20s	
72°C 30s	

Melting curve analysis refers to the standard set value of Bio-rad CFX Connect (Bio-rad) of PCR instrument. The expression of *CIPK2* gene among different rice genotypes was calculated by $2^{-\Delta\Delta ct}$ method.

Table S2. Morphological characteristics of roots under different nitrogen treatments in pots.

Stage	Treatment	Genotype	Adventitious root length(m)	Fine lateral root length(m)	Coarse lateral root length(m)	Total Rhizoplane area(cm ²)	Total root volume (cm ³)
Heading	LN	WT	6.29c	1.08c	0.90c	159.50c	2.627c
		RC	9.05b	2.28b	1.98b	231.18b	2.964b
	NN	WT	17.64a	4.10a	3.12a	452.02a	6.007a
		RC	17.83a	4.19a	3.03a	445.67a	5.750a
Maturity	LN	WT	4.85c	2.04c	0.93b	101.10b	1.012b
		RC	5.91b	2.75b	1.48b	124.58b	1.181b
	NN	WT	14.72a	6.65a	3.37a	356.10a	4.244a
		RC	14.61a	7.18a	3.49a	350.09a	4.068a

Table S3. Rice plant nitrogen and nitrogen utilization ratio.

Biomass nitrogen use efficiency (BNUE) = dry matter accumulation/total nitrogen uptake per plant
Nitrogen grain utilization efficiency (NGUE) = grain yield/total nitrogen uptake per plant
Nitrogen harvest index (NHI) = grain nitrogen accumulation/total plant nitrogen uptake
Nitrogen Physiological Efficiency (PE) = (Grain Yield in Nitrogen-Free Zone) / (Total Nitrogen Uptake in Nitrogen-Free Zone) / (Total Nitrogen Uptake in Nitrogen-Free Zone)
Nitrogen agronomic efficiency (AE) = (grain yield in nitrogen-free area) / nitrogen application rate
Nitrogen recovery efficiency (RE) = (total nitrogen uptake by plants in nitrogen-free zone) / nitrogen application *100

Table S4. Nitrogen cycle related gene primer sequences and amplification conditions.

Gene	Primer Sequences (5'-3')	conditions
<i>nifH</i> [1]	nifH-F: CCTACGGGAGGCAGCAG	95°C 5min;
	nifH-R: ATTACCGCGGCTGCTGGCA	95°C 45s, 59°C 45s, 72°C 45s; 39 cycles
<i>amoA</i> (AOB) [2]	AOB-F: GGGTTTCTACTGGTGGT	95°C 15min;
	AOB-R: CCCCTCKGSAAAGCCTTCTTC	94°C 45s, 58°C 45s, 72°C 45s; 39 cycles
<i>amoA</i> (AOA) [3]	AOA-F: ATGGTCTGGCTWAGACG	95°C 10min;
	AOA-R: GCCATCCABCKRTANGTCCA	94°C 45s, 55°C 45s, 72°C 45s; 39 cycles
<i>nosZ</i> [4,5]	noSZ-F: CGCRACGGCAASAAGGTSMS	95°C 15min;
	nosZ-R:	95°C 15s, 65-60°C 30s, 72°C 30s, 80°C
	SGTCAKRTGCAKSGCRTGGCAGAA	15s, 6 cycles; 95°C 15s, 64°C 30s, 72°C 30s, 80°C 15s; 40 cycles

<i>nirS</i> [5]	nirS-F: AACGYSAAGGARACSGG	95°C 15min; 95°C 15s, 65-60°C 30s, 72°C 30s, 80°C 15s, 6 cycles; 95°C 15s, 63°C 30s, 72°C 30s, 80°C 15s; 40 cycles
	nirS-F: GASTTCGGRTGSGTCTTSAYGAA	95°C,15min;
<i>narG</i> [6]	narG-F: TAYGTSGGGCAGGARAAACTG	95°C 15s,65-60°C 30s, 72°C 30s, 80°C 15s, 6 cycles; 95°C 15s, 60°C 30s, 72°C 30s, 80°C 15s; 40 cycles
	narG-R: CGTAGAAGAAGCTGGTGCTGTT	95°C 15min;
<i>nirK</i> [7]	nirK-F: ATYGGCGGVCA YGGCGA	95°C 15s, 63-58°C 30s, 72°C 30s, 80°C 15s; 6 cycles; 95°C 15s, 60°C 30s, 72°C 30s, 80°C 15s, 40 cycles
	nirK-R: GCCTCGATCAGRTRTGGTT	

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