

Crop rotation with marigold promotes soil bacterial structure to assist in mitigating clubroot incidence in Chinese cabbage

Jinhao Zhang^{1,2,†}, Waqar Ahmed^{1,2,†}, Xinghai Zhou^{1,2,†}, Bo Yao^{1,2}, Zulei He^{1,2}, Yue Qiu^{1,2}, Fangjun Wei^{1,2}, Yilu He^{1,2}, Lanfang Wei^{1,3*} and Guanghai Ji^{1,2*}

¹State Key Laboratory for Conservation and Utilization of Bio-Resources in Yunnan, Yunnan Agricultural University, Kunming 650201, Yunnan, China

²Key Laboratory of Agro-Biodiversity and Pest Management of Ministry of Education, Yunnan Agricultural University, Kunming 650201, Yunnan, China

³Agricultural Foundation Experiment Teaching Center, Yunnan Agricultural University, Kunming 650201, Yunnan, China

†These authors contributed equally to this work.

***Correspondence:**

Guanghai Ji

jghai001@163.com

Lanfang Wei

wlfang2000@aliyun.com



Figure S1; Effect of marigold crop rotation on the incidence of clubroot in Chinese cabbage under field conditions. **Here;** Monocropping of Chinese cabbage (**CK**), Chinese cabbage seedlings were transplanted immediately after harvesting of the marigold crop (**T1**), and Chinese cabbage seedlings were transplanted with an empty period of 15 days after harvesting of the marigold crop (**T2**).



Figure S2; Chinese cabbage seedlings were grown hydroponically in the dark to investigate the effect of marigold root exudates, crude extract, and powder on the germination and death of *P. brassicae* resting spores.

Table S1; Effects of inoculation of marigold on the disease incidence, disease index, and control effect of cabbage clubroot in the greenhouse.

Treatment	Disease incidence (%)	Disease index	Control effect (%)
CK	88.89±3.85a	68.25±3.96a	-----
T1	77.78±10.18b	56.19±5.30b	17.51±8.82b
T2	48.89±3.85c	31.11±3.35c	54.13±7.56a

Note: Monocropping of Chinese cabbage (**CK**), Chinese cabbage seedlings were transplanted immediately after harvesting of marigold crop (**T1**), and Chinese cabbage seedlings were transplanted with an empty period of 15 days after harvesting of marigold crop (**T2**). Significance differences among treatments are shown by different small letters within a column according to Duncan's multiple range test at $p < 0.05$.

Table S2; Effects of inoculation of marigold on the disease incidence, disease index, and control effect of cabbage clubroot in the field.

Treatment	Disease incidence (%)	Disease index	Control effect (%)
CK	91.67±2.89a	68.57±2.85a	-----
T1	81.67±7.64b	50.47±1.80b	26.33±3.22b
T2	58.33±5.77c	25.14±3.06c	63.35±5.80a

Note: Monocropping of Chinese cabbage (**CK**), Chinese cabbage seedlings were transplanted immediately after harvesting of marigold crop (**T1**), and Chinese cabbage seedlings were transplanted with an empty period of 15 days after harvesting of marigold crop (**T2**). According to Duncan's multiple range test different small letters within a column shows significance differences among treatments at $p < 0.05$.

Table S3; Relative abundance of the top 10 dominant bacterial phyla in rhizosphere soil under different experimental conditions (\pm SEM, n=3).

Phylum	CK	T1	T2
Proteobacteria	38.80 \pm 2.07bc	46.04 \pm 4.74a	41.11 \pm 2.20b
Acidobacteria	17.60 \pm 3.31ab	14.06 \pm 3.18b	20.09 \pm 1.49a
Bacteroidetes	15.54 \pm 3.03a	14.73 \pm 2.71a	13.97 \pm 1.06a
Actinobacteria	8.45 \pm 1.36a	8.96 \pm 1.94a	6.20 \pm 0.57b
Verrucomicrobia	6.04 \pm 0.44a	5.27 \pm 0.90a	6.41 \pm 0.60a
Gemmatimonadetes	3.75 \pm 0.72a	2.83 \pm 0.69a	3.46 \pm 0.12a
Chloroflexi	3.31 \pm 0.74a	2.41 \pm 0.94a	3.07 \pm 0.37a
Firmicutes	1.80 \pm 0.68a	1.62 \pm 0.36a	1.49 \pm 0.17a
Planctomycetes	1.60 \pm 0.43a	1.30 \pm 0.37ab	1.59 \pm 0.02a
Nitrospirae	1.15 \pm 0.06a	1.00 \pm 0.17a	0.94 \pm 0.09a
Others	1.94 \pm 0.61a	1.78 \pm 0.33a	1.67 \pm 0.17a

Here; Monocropping of Chinese cabbage (**CK**), Chinese cabbage seedlings were transplanted immediately after harvesting of marigold crop (**T1**), and Chinese cabbage seedlings were transplanted with an empty period of 15 days after harvesting of marigold crop (**T2**). Different lowercase letters within a row represents the significance differences among treatments according to a least significant difference test (LSD; $p < 0.05$).

Table S4; Relative abundance of the top 10 dominant bacterial families in rhizosphere soil under different experimental conditions (\pm SEM, n=3).

Family	CK	T1	T2
Pseudomonadaceae	4.49 \pm 2.65b	12.75 \pm 10.44a	3.02 \pm 1.92c
Sphingomonadaceae	6.80 \pm 1.03a	6.42 \pm 0.96a	6.77 \pm 0.07a
Burkholderiaceae	5.45 \pm 0.07a	4.37 \pm 0.82a	5.73 \pm 1.81a
Chitinophagaceae	3.56 \pm 0.56ab	3.11 \pm 0.56b	4.12 \pm 0.28a
Flavobacteriaceae	3.92 \pm 1.03a	3.96 \pm 1.54a	2.38 \pm 0.32b
Micrococcaceae	2.95 \pm 1.14b	4.72 \pm 1.27a	1.47 \pm 0.15c
Gemmatimonadaceae	3.37 \pm 0.64a	2.53 \pm 0.59a	3.00 \pm 0.12a
Rhizobiaceae	3.28 \pm 0.46a	2.69 \pm 0.75ab	1.99 \pm 0.07b
Xanthomonadaceae	2.20 \pm 0.13a	3.00 \pm 0.75a	2.61 \pm 0.51a
Uncultured bacterium	10.66 \pm 2.27ab	8.97 \pm 2.06b	13.34 \pm 0.96a
Others	53.34 \pm 0.91a	47.47 \pm 7.11a	55.57 \pm 1.29a

Here; Monocropping of Chinese cabbage (**CK**), Chinese cabbage seedlings were transplanted immediately after harvesting of marigold crop (**T1**), and Chinese cabbage seedlings were transplanted with an empty period of 15 days after harvesting of marigold crop (**T2**). Significance differences among treatments are shown by different small letters within a row according to a least significant difference test (LSD; $p < 0.05$).

Table S5; Relative abundance of the top 15 dominant bacterial genera in rhizosphere soil under different experimental conditions (\pm SEM, n=3).

Genus	CK	T1	T2
<i>Pseudomonas</i>	0.045 \pm 0.027b	0.127 \pm 0.104a	0.030 \pm 0.019c
<i>Sphingomonas</i>	0.047 \pm 0.006a	0.040 \pm 0.007a	0.043 \pm 0.002ab
<i>Flavobacterium</i>	0.039 \pm 0.010a	0.039 \pm 0.015a	0.024 \pm 0.003b
<i>Pedobacter</i>	0.035 \pm 0.006a	0.016 \pm 0.006b	0.014 \pm 0.003b
<i>Allorhizobium-Neorhizobium</i>	0.025 \pm 0.004a	0.017 \pm 0.005b	0.012 \pm 0.001c
<i>Bryobacter</i>	0.013 \pm 0.001ab	0.010 \pm 0.002b	0.015 \pm 0.002a
<i>Luteolibacter</i>	0.016 \pm 0.002a	0.012 \pm 0.003b	0.011 \pm 0.001b
<i>RB41</i>	0.014 \pm 0.003a	0.011 \pm 0.003a	0.011 \pm 0.002b
<i>Nitrospira</i>	0.012 \pm 0.001a	0.010 \pm 0.001b	0.010 \pm 0.001b
<i>Chthoniobacter</i>	0.009 \pm 0.001a	0.009 \pm 0.001a	0.010 \pm 0.001a
<i>MND1</i>	0.008 \pm 0.001b	0.007 \pm 0.004b	0.010 \pm 0.000a
<i>Ramlibacter</i>	0.009 \pm 0.001a	0.006 \pm 0.002b	0.009 \pm 0.001a
<i>Novosphingobium</i>	0.004 \pm 0.001b	0.009 \pm 0.002a	0.008 \pm 0.000a
<i>JGI_0001001-H03</i>	0.008 \pm 0.001a	0.005 \pm 0.001a	0.009 \pm 0.001a
<i>Delftia</i>	0.000 \pm 0.000b	0.0008 \pm 0.0005b	0.009 \pm 0.015a

Here; Monocropping of Chinese cabbage (**CK**), Chinese cabbage seedlings were transplanted immediately after harvesting of marigold crop (**T1**), and Chinese cabbage seedlings were transplanted with an empty period of 15 days after harvesting of marigold crop (**T2**). Different lowercase letters within a row represents the significance differences among treatments according to a least significant difference test (LSD; $p < 0.05$).

Table S6; Characteristics of the co-occurrence network.

Network properties	Value
Number of nodes	79
Number of edges	451
Modularity	0.442
Number of communities	6
Network diameter	5
Network Density	0.146
Average shortest path length	2.418
Average clustering coefficient	0.503