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The foliar spray of *Rhodopseudomonas palustris* grown under *Stevia* residue extract promotes plant growth via changing soil microbial community

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Abstract
Purpose The extract of *Stevia* residue is an ideal substitute for cultivation of the purple nonsulfur bacterium, like *Rhodopseudomonas palustris* (*R. palustris*). But the influence of *R. palustris* grown under residue extract on its downstream application is still not well-characterized. The objective of this study was to assess the effect of foliar spray of *R. palustris* grown under *Stevia* residue extract on the plant growth and soil microbial properties.
Materials and methods A pot experiment was carried out under the greenhouse condition, consisting of four treatments varying in the sprayed substances: sterilized water (control), *R. palustris* grown under the chemical medium supplemented with L-tryptophan (SyT), *R. palustris* grown under *Stevia* residue extract supplemented with L-tryptophan (ExT), and *R. palustris* grown under *Stevia* residue extract supplemented with NH_4Cl (ExT). The net photosynthesis rate of the uppermost leaves was measured with a portable photosynthesis system. Soil microbial activity was analyzed by microcalorimetry. Soil bacterial community components were determined by real-time quantitative PCR (qPCR) and high-throughput sequencing techniques.
Results and discussion Compared with SyT, the *R. palustris* grown under *Stevia* residue extract not only improved the plant biomass and the net photosynthetic rate to a large extent, but also increased soil microbial metabolic activity and altered community compositions as well. The treatments receiving *R. palustris*, especially ExT and ExN, increased the relative abundances of some functional guilds involved in C turnover and nutrient cycling in soil, including Acidobacteria, Actinobacteria, Proteobacteria, Gemmatimonadaetes, Nitrospirae, and Planctomycetes.
Conclusions *R. palustris* grown under the *Stevia* residue extract showed advantages over that under the chemical medium on both plant growth and soil microbial properties. One of the possible reasons could result from the increases in microbial activity and several bacterial keystone guilds involved into C and nutrient cycling, both of which potentially contribute to the improved plant growth. The results would be conducive to the downstream application of *R. palustris* in an economical way.

Keywords Bacterial community · High-throughput sequencing · Microcalorimetry · *Rhodopseudomonas palustris* · *Stevia* residue

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1 Introduction

Stevia rebaudiana (Bertoni) is an economic plant in huge demand by pharmaceutical and food and beverage industries (Puri et al. 2011). After sweetener extraction, the residue of *Stevia* produces large amounts of wastewater rich in organic components suitable for the growth of beneficial microbes, such as a representative purple nonsulfur bacterium, *Rhodopseudomonas palustris*. However, given the high C/N ratio of *Stevia* residue extract (Xu et al. 2013a), it is necessary to supplement nitrogen (N) sources with the aim of satisfying microbial growth, e.g., NH_4Cl or L-tryptophan. The NH_4Cl is a common N source, while L-tryptophan is the precursor of indole-3-acetic acid (IAA), a typical phytohormone regulating

various developmental and physiological processes in plants (Kim et al. 2004). Our preceding report has shown that *R. palustris* exhibits distinct growth pattern and genotypic characteristics under such N sources (Xu et al. 2013a). Yet, whether those changes would influence the downstream application of *R. palustris* is still unknown so far.

The intrinsic characteristics of *R. palustris* assured its promising use as the biofertilizer for sustainable agricultural development. It could convert sunlight to energy and absorb atmospheric carbon dioxide and convert it to biomass (Hu et al. 2011). Moreover, it harbors various functions including excretion of plant hormones, N-fixation, and solubilization of mineral nutrients (Kantachote et al. 2005; Oda et al. 2008). Those features make it feasible to be used as plant growth-promoting bacteria in agriculture (Yin et al. 2012). Often, it is the case to employ the *R. palustris* as the root inoculant (Lee et al. 2008), while the foliar application and its subsequent effect is less concerned. One of the possible advantages of foliar application is that it avoids the adverse influences of many biotic and abiotic factors on soil environment (Pandey et al. 2013). Another is the wide surface area of global terrestrial plants (an estimated 6.4×10^8 km^2) (Atamna-Ismaeel et al. 2012; Penuelas and Terradas 2014), with an immensely diverse microbes (of up to 10^6–10^7 cells per cm^2 leaf surface) for plant growth promotion (Lindow and Brandl 2003). In view of these aspects, the inoculation of *R. palustris* onto plant leaf surface would potentially improve plant growth in a more straightforward way, and such effects would inevitably reflect on soil microbial properties due to the important roles of microbes on the plant-soil ecosystem.

We hypothesized that the foliar spray of *R. palustris* grown under *Stevia* residue extract would not only promote plant growth noticeably but also change soil microbial activity and community compositions. To test this, *R. palustris* grown under different substrates, viz, the *Stevia* residue extracts and the synthetic chemical medium, were sprayed onto the leaf surfaces of Chinese pak choi cabbage (*Brassica chinensis* L.) under the greenhouse condition. Microbial metabolic activity and community composition in the soil were determined by microcalorimetry, real-time quantitative PCR (qPCR), and high-throughput sequencing based on MiSeq platform (Illumina Inc., USA). Those comprehensive assessments would overcome the knowledge gaps between foliar stimulation and soil microbial feedback and be conducive to the downstream application of *R. palustris* accompanied with the reutilization of wastewater and suchlike.

2 Materials and methods

2.1 Preparation of bacterial suspensions

R. palustris, a typical purple nonsulfur bacterium preserved in our laboratory, was used for this study. The *Stevia* residue extract was used as the culture medium for *R. palustris*. The detailed extracting procedure (at 20–25 °C) and the chemical properties of the extract were described in our previous report (Xu et al. 2013a). The concentrations of total organic carbon (C) and total N in the *Stevia* residue extract were 605 and 20.5 mg/L, respectively. In order to satisfy microbial growth, additional N sources (7 mmol/L ammonium chloride or 3 mmol/L L-tryptophan) were added to the *Stevia* residue extract to balance the C/N ratio. The pH of the medium was adjusted to 7.0 before sterilization. A chemical culture medium, the components of which were described by Li et al. (2011), was served as a contrast. *R. palustris* was cultured at 30 °C under a 60-W incandescent lamp at a distance of 25 mm. The cells of *R. palustris* were collected by centrifugation at 10,000×*g* for 15 min. The pellets were resuspended in sterile water to obtain a final concentration of 10^{10} colony forming units (CFU) per milliliter, and then, the suspensions of *R. palustris* were used for the following pot experiment.

2.2 Soil preparation and pot experimental design

Soil samples were collected from an arable field located at Zhucheng, Shandong Province, China (36° 20′ N, 119° 32′ E). The soil had a pH of 7.09 (soil/water=1:2.5) and contained 8.35 g/kg organic C, 20.2 mg/kg available P, and 145.1 mg/kg available K. The soil was air-dried and then passed through a 2-mm sieve to remove root debris and large gravels.

A pot experiment was carried out in the greenhouse at the Institute of Soil Science, Chinese Academy of Sciences in Nanjing, China. There were altogether 12 pots arranged, containing four treatments with three replicates. In each pot, 1-kg soil sample was mixed with 1-g basal fertilizers ($N/P_2O_5/K_2O$=15:15:15) thoroughly, and then, ten seeds of Chinese pak choi cabbage (*B. chinensis* L.) were directly sown into the soil. The pots were arranged in a completely randomized factorial design in the greenhouse. After the emergence of the forth cotyledons of the plants, the abovementioned suspensions of *R. palustris* were sprayed onto the leaf surfaces, separately: (i) 1~5-mL suspension grown under chemical medium supplemented with L-tryptophan (SyT); (ii) 1~5-mL suspension grown under *Stevia* residue extract supplemented with tryptophan (ExT); and (iii) 1~5-mL suspension grown under *Stevia* residue extract supplemented with NH_4Cl (ExN). The sprayed volumes of suspensions were adjusted according to the plant growth. During spraying, the soil surface in each pot was covered with a plastic film in order to prevent soil contamination. A control receiving equivalent sterile distilled water was also included (control). With the aim of magnifying the effects in the short growth period (often <40 days), the spraying procedure were conducted every 2 days. At day 30 after sowing, the net photosynthesis rate of the uppermost leaves was measured with a portable photosynthesis system (LI-6400, Li-COR, NE, USA). Photosynthesis was observed

at 380 μmol/mol CO_2 concentration, on a saturating photosynthetic photon flux density of 1400 μmol/m^2/s. A total of three leaves were measured in each pot. After photosynthesis measurements, the fresh biomass of cabbage in each pot was weighted. Meanwhile, soil samples from each pot were collected and sieved (2-mm mesh). One proportion of soil samples was kept at 4 °C for microcalorimetric assay, and the other proportion was maintained at −40 °C for molecular study.

2.3 Microcalorimetric measurement

Microcalorimetry is a highly precise and continuous real-time measurement to monitor heat dissipation. An isothermal multichannel microcalorimeter TAM III (TA Instruments, Delaware, USA) was used for microcalorimetric measurements. The method and procedures were adopted from Zheng et al. (2009). Prior to analysis, the 4.0-mL microcalorimetric glass ampoules were sterilized in an oven at 100 °C and all soil samples were equilibrated at 28 °C for 24 h. Then, soil samples (1.2 g) were introduced in the glass ampoules and amended with 0.2 mL of aqueous solutions of glucose (5.0 mg) and ammonium sulfate (5.0 mg) in a 1:1 ratio. In order to evaluate the effect of other substrate, additional 5 mg substrate was added into the nutrient solution. The viability of soil microbial populations was continuously monitored over time, and the power–time curve was recorded electronically. Throughout the experiment, the temperature was maintained at 28 °C.

Four thermodynamic parameters, that is, growth rate constant (k), the maximum thermal power (P_{max}), the time to reach the maximum peak (T_{max}), and the total heat dissipation (Q) were obtained by integrating the power–time curves.

The microbial growth rate constant (k) was calculated by fitting an exponential growth model based on the power–time curve data that represented the microbial growth reaction by the following thermodynamic equation:

$$\ln P_t = \ln P_0 + kt$$

where t is the time, P_t is the power output at time t, and P_0 is the power at the beginning of the exponential growth phase. Q is the sum of metabolic processes that occur during substrate consumption. P_{max} and T_{max} were obtained directly from the power–time curve.

The ratio of Q/N_0 represents the dissipation of heat per cell unit (Zheng et al. 2009), where N_0 is the copy number of bacterial 16S ribosomal RNA (rRNA) gene determined by the following qPCR assay.

2.4 DNA extract and qPCR assay

Genomic DNA was extracted from 0.5-g fresh soils using a FastDNA® SPIN Kit for Soil (MP Biomedicals, Santa Ana, CA, USA) according to the manufacturer's instructions. The abundances of 16S rRNA genes in the soil samples were determined by qPCR using prime sets of 519F/907R (519F: 5′-CAGCMGCCGCGGTAATWC-3′, 907R: 5′-CCGTCAATTCMTTTRAGTTT-3′). The assay was conducted using a C1000^{Tm} Thermal Cycler equipped with a CFX96^{Tm} Real-Time system (Bio-Rad, CA, USA) using the SYBR *Premix* ExTaq^{TM} Kit (TaKaRa, Dalian, China). Plasmid standards for the quantification were generated from a cloned target gene, and the standard curves were generated according to our previous report (Xu et al. 2013b). The abundances of bacteria were expressed as gene copy numbers per gram of dry weight soil (d.w.s.).

2.5 DNA sequencing of 16S rRNA genes and sequencing data processing

PCR amplification was conducted for bacteria with primer set 519F/907R, respectively. To perform sequencing with the Illimina MiSeq sequencing system, the oligonucleotides of 5-bp bar-coded fused to the forward primer: barcode + forward primer and reversed primer. PCR were carried out in 50-μL reaction mixtures containing each deoxynucleoside triphosphate at a concentration of 1.25 μM, 2 μL (15 μM) forward and reverse primers, 2 U of Taq DNA polymerase (TaKaRa, Japan), and each reaction mix received 1 μL (50 ng) of genomic community DNA as a template. PCR were performed according to the following program: 35 cycles of 95 °C for 45 s, 58 °C for 45 s, 72 °C for 1 min, and a final extension at 72 °C for 7 min. Reaction products were pooled, purified using the QIA quick PCR Purification kit (QIAGEN), and quantified using NanoDrop ND-1000 (Thermo Scientific, USA). The bar-coded PCR products from all samples were normalized in equimolar amounts before sequencing by means of a MiSeq platform (Illumina Inc., USA).

After sequencing was completed, 16S rRNA data were processed using the Quantitative Insights Into Microbial Ecology (QIIME) pipeline for data set. Sequences below quality score of 25 and 200 bp in length were trimmed and then assigned to soil samples based on unique 5-bp barcodes. Sequences were binned into operational taxonomic units (OTUs) using a 97 % identity threshold, and the most abundant sequence from each OTU was selected as a representative sequence for that OTU. Taxonomy was assigned to OTUs against a subset of the Silva 104 database (http://www.arb-silva.de/download/archive/qiime/). Based on the sequences and/or OTUs, principal component analysis (PCA) was performed.

2.6 Statistical analysis

All results were the means of the triplicates and were expressed on an oven-dried soil weight basis (105 °C, 24 h). The data were subjected to ANOVA Duncan's test at significance level $P<0.05$ using SPSS 13.0 for Windows (SPSS Inc.,

Table 1 The net photosynthetic rate of plant leaf, the fresh biomass of plant, and the copy number of bacterial 16S rRNA gene in soils under different treatments

Treatment	Net photosynthetic rate (μmol $CO_2/m^2/s$)	Biomass (g/pot)	N_0 ($\times 10^{10}$ gene copy number/g d.w.s.)
Control	9.89 b	22.02 b	1.27 a
SyT	11.50 b	24.90 b	1.48 a
ExT	13.83 a	36.06 a	1.45 a
ExN	14.31 a	39.40 a	1.52 a

Different letters within a column indicate significant differences at $P<0.05$

Control foliar spray of sterilized water, *SyT* foliar spray of *R. palustris* grown under the chemical medium (L-tryptophan as N source), *ExT* foliar spray of *R. palustris* grown under *Stevia* residue extract (L-tryptophan as N source), *ExN* foliar spray of *R. palustris* grown under *Stevia* residue extract (NH_4Cl as N source)

Chicago, USA). PCA was performed on the relative abundances of the identified bacterial orders from the four treatments using Canoco for Windows 4.5 (Microcomputer Power, Ithaca, NY, USA).

3 Results

3.1 Net photosynthetic rate and plant biomass

All the *R. palustris* treatments (ExN, ExT, and SyT) consistently showed positive effects on the plant growth patterns (Table 1). The plant biomass followed the order ExN > ExT > SyT > control, with the first two treatments significantly higher than the last two ($P<0.05$). No significant difference was found between ExN and ExT as well as between control and SyT ($P>0.05$). The net photosynthetic rate showed the similar patterns as the plant biomass, with the significantly highest value observed in ExN ($P<0.05$).

3.2 Copy number of bacterial 16S rRNA genes in soil

The copy numbers of soil bacterial 16S rRNA genes ranged from 1.27×10^{10} to 1.52×10^{10}/g d.w.s. (Table 1), but there were no significant differences among all treatments ($P>0.05$).

3.3 Soil microbial heat evolution

The sets of distinguishable heat dissipation profiles obtained from power–time curves of soil samples were illustrated in Fig. 1, and the subsequent thermodynamic parameters were integrated (Table 2). Heat flows increased exponentially after the lag phase, followed by the stationary phase and then the decline phase.

Compared to control, the treatments of SyT, ExT, and ExN significantly increased the microbial growth rate constant (k) and peak power (P_{max}) and decreased the time to reach the peak (T_{max}) ($P<0.05$), suggesting the high microbial activities in those treatments. The most profound effects were observed for the treatments of ExN, with significantly higher P_{max} and lower T_{max} in comparison with control, suggesting that ExN could stimulate the microbial activity to a large extent. Besides, low values of Q/N_0 were observed in ExN and ExT, while a high value was observed for control, though the differences of Q/N_0 were not significant among all the treatments ($P>0.05$).

3.4 Soil bacterial taxonomic distribution

High-throughput sequencing technique allowed us to track overall shifts in the taxonomic distribution of the soil bacterial community. A total of 94,348 high-quality sequences were obtained across all samples. The relative abundances of the

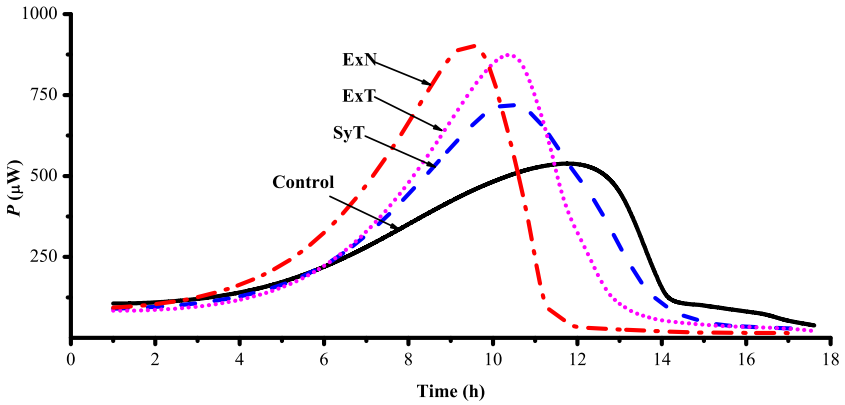

Fig. 1 Power–time curves recorded for the soil samples amended with glucose and ammonium sulfate. *Control*, foliar spray of sterilized water; *SyT*, foliar spray of *R. palustris* grown under the chemical medium (L-tryptophan as N source); *ExT*, foliar spray of *R. palustris* grown under *Stevia* residue extract (L-tryptophan as N source); *ExN*, foliar spray of *R. palustris* grown under *Stevia* residue extract (NH_4Cl as N source)

Table 2 Thermodynamic parameters from power–time curves measured by microcalorimetric method

Treatment	P_{max} (μW)	T_{max} (h)	k (/h)	Q (J/g)	Q/N_0 ($\times 10^{-10}$ J/cell)
Control	538.32 c	11.77 a	0.35 b	15.35 a	12.01 a
SyT	719.44 b	10.38 b	0.42 a	16.22 a	10.96 a
ExT	873.88 a	10.38 b	0.47 a	15.77 a	10.87 a
ExN	904.51 a	9.45 c	0.42 a	15.03 a	9.89 a

Different letters within a column indicate significant differences at $P<0.05$

P_{max} thermal power at the maximum of the peak; T_{max} peak time, is the time to reach the maximum of the peak; Q total heat evolution recorded from soil samples; k the microbial growth rate constant; Q/N_0 the heat output per cell unit

various phyla in each treatment were shown in Fig. 2. The most dominant phyla across all treatments were Proterobacteria, Actinobacteria (mainly alpha-, beta-, gamma-, and delta- classes), Acidobacteria, Chloroflexi, and Firmicutes, accounting for 35.1, 14.9, 10.3, 6.5, and 5.5 % of all bacterial sequences obtained, respectively.

To identify the taxa that significantly responded to the foliar spray application of *R. palustris*, pairwise comparisons were made among all treatments. All taxa with significantly different relative abundances were considered ($P<0.05$, Table 3). Mainly the increased members at phylum level affiliated to Acidobacteria, Actinobacteria, and Deltaproteobacteria were found in the soils treated with *R. palustris* as compared to control ($P<0.05$), with ExN harboring the highest abundances. At a finer division, more detailed information was revealed. Evident variations were presented in the orders of *Entotheonellales* (Deltaproteobacteria), *Syntrophobacterales* (Deltaproteobacteria), *Burkholderiales* (Betaproteobacteria), *Pirellulales* (Planctomycetes), *Gemmatimonadales* (Gemmatimonadetes), and *Nitrospirales* (Nitrospirae). Of particular note, *R. palustris* grown under the *Stevia* residue extract (ExN or ExT) exhibited more apparent influences than that grown under synthetic medium.

The PCA results allowed us to separate the different treatments according to the taxonomic distributions of soil bacterial community (Fig. 3). The first two principal components (PC1 and PC2) explained 55.8 % of the total variances in the bacterial community compositions. ExT and ExN clustered together, but separated from SyT and control, indicating the shifts in bacterial community composition led by the different *R. palustris* applications.

4 Discussion

4.1 Promotion effects of *R. palustris* on plant growth

The application of *R. palustris*, irrespective of root inoculation and foliar spray, has been proven to be a feasible strategy to improve the yields of crops like rice (Harada et al. 2005) and Chinese dwarf cherry (Yin et al. 2012). In support, the plant biomass and net photosynthetic rate of Chinese pak choi cabbage (*B. chinensis* L.) in this study were elevated due to the foliar spray of *R. palustris*. Particularly, *R. palustris* grown under *Stevia* residue extract showed more remarkable influence, which might be attributed to the individual characteristics of *R. palustris*. As shown in our preceding report, *R. palustris* grown under *Stevia* residue extract has higher cytochrome content in live cells as compared to that grown under the chemical medium (Xu et al. 2013a). Besides, our unpublished data showed that the ability of excreting 5-aminolevulinic acid (5-ALA) was also high in the *R. palustris* grown under *Stevia* residue extract. 5-ALA is a potential plant growth regulator and is usually thought of as a precursor for chlorophyll synthesis (Hotta et al. 1997). These features jointly contributed to the increased net photosynthetic rates of plant leaf and the subsequent plant biomass in ExT and ExN (Table 1).

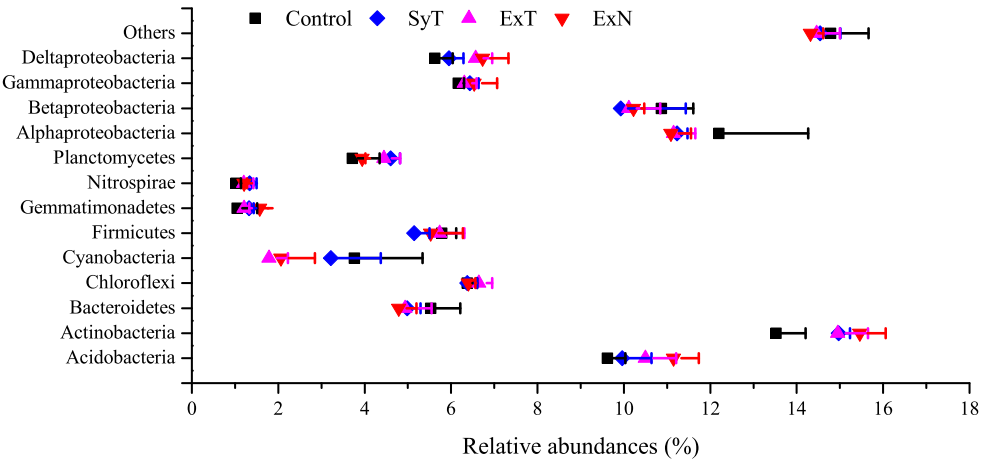

Fig. 2 The percent changes of the relative abundances of the major bacterial phyla in the soils under different foliar spray treatments. The values are the means of three replicates ($n=3$). *Horizontal error bar* indicates the standard deviation

Table 3 Relative abundances of selected bacteria groups in soils under different foliar spray treatments

Treatment	Acidobacteria	Actinobacteria	Betaproteobacteria	Deltaproteobacteria		Gemmatimonadaetes	Nitrospirae	Planctomycetes
			Burkholderiales	Entotheonellales	Syntrophobacterales	Gemmatimonadales	Nitrospirales	Pirellulales
Control	9.62±0.41 b	13.52±1.19 b	4.16±0.25 b	0.44±0.11 b	1.88±0.20 b	0.89±0.36 b	1.02±0.11 b	1.40±0.18 b
SyT	9.96±0.68 ab	14.97±0.26 ab	4.84±0.68 ab	0.48±0.09 ab	1.85±0.07 b	1.13±0.10 ab	1.33±0.16 a	1.83±0.05 a
ExT	10.50±0.71 ab	14.95±0.70 ab	4.71±0.55 ab	0.49±0.07 ab	2.27±0.32 a	1.06±0.10 ab	1.20±0.22 ab	1.85±0.14 a
ExN	11.15±0.59 a	15.46±1.10 a	5.78±0.49 a	0.63±0.04 a	2.15±0.06 ab	1.37±0.19 a	1.21±0.07 ab	1.66±0.28 ab

Results are expressed with means±standard errors for different foliar spray treatments (n=3), and different letters within a column indicate significant differences at P<0.05 based on Duncan's test

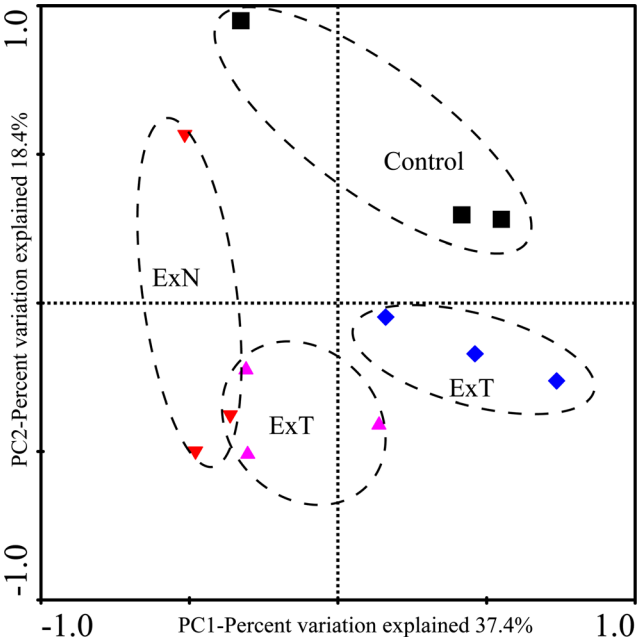

Fig. 3 Principal component analysis (PCA) of soil bacterial community composition under different foliar spray treatments

4.2 Soil microbial activity to foliar spray of *R. palustris*

Soil microorganisms play an important role in maintaining and improving soil fertility by carrying out almost all known biological reactions (Hu et al. 2010). The enhanced plant growth (Table 1) would influence the amounts and qualities of root exudates in the *R. palustris* treatments directly, because up to 40 % of photosynthetically fixed C is secreted into the rhizosphere (Bais et al. 2006). Accordingly, more incorporated root exudates into soil function as nutrient and C sources for microbial metabolisms (Huang et al. 2014), resulting in the shifts in soil microbial properties.

Microcalorimetric results showed that samples from ExN and ExT had higher microbial metabolic activities than that from SyT, suggesting the advantages of *Stevia* residue extracts over the chemical substrate. Besides, a decreasing trend of Q/N_0 was found as follows: control > SyT > ExT > ExN. Lower ratio of Q/N_0 means a more efficient metabolism and higher percentage of C that is kept as microbial biomass in the soil (Zheng et al. 2009). In this regard, the application of *R. palustris* grown under the *Stevia* residue extract would potentially benefit for soil C sequestration by enhancing metabolic efficiency of soil microorganisms.

4.3 Representative bacterial groups

The changed microbial activity in the soil is accompanied by the variations in bacterial community composition (Pascault et al. 2013). The predominance of Proterobacteria, Actinobacteria, Acidobacteria, Chloroflexi, and Firmicutes in the tested soils was expected, because these bacterial

members have been described as common inhabitants of arable soils (Rampelotto et al. 2013). Numerous reports have shown that environmental disturbances exert important influences on the soil bacterial compositions, such as land use, fertilization, and inoculation of beneficial microbes as well (Lee et al. 2008; Jangid et al. 2008; Pedraza et al. 2009). However, the employment of the low resolution technique, e.g., denaturing gradient gel electrophoresis (DGGE), fails to present the tiny variations in bacterial community components after root inoculation of *Rhodopseudomonas* (Lee et al. 2008). By contrast, with the aid of high-throughput sequencing technique, more detailed information was revealed in the microbial community compositions in the present study.

Specifically, compared with control, *R. palustris*, especially those grown under *Stevia* residue extracts, increased the abundances of the phyla Acidobacteria and Actinobacteria, as well as those of the orders *Burkholderiales*, *Entotheonellales*, *Syntrophobacterales*, *Nitrospira*, and *Pirellulales*. Such patterns suggested that the inoculation of *R. palustris* provided selective advantages for those taxa. Acidobacteria and Actinobacteria play fundamental parts in soil organic matter turnover and significantly contribute to the terrestrial C cycle (Acosta-Martinez et al. 2008; Berg et al. 2014). *Burkholderiales* involves in biological suppression of pathogen, plant-growth promotion, and N-fixation (Estrada-De los Santos et al. 2001; Coenye and Vandamme 2003; Gyaneshwar et al. 2011). The genera *Nitrospira* (Nitrospirae) hosts nitrite oxidizers and ferrous iron oxidizers, stimulating the N and Fe cycles in soil (Attard et al. 2010; Lopes et al. 2014). *Planctomycetes* includes chemolithotrophs uniquely participating in the anammox metabolism and the degradation of sulfated polysaccharides of plant origin (Fuerst and Sagulenko 2011; Cai et al. 2013). Gemmatimonadetes is recognized as a polyphosphate-accumulating microorganism (Zhang et al. 2003). Within Deltaprotepbacteria, the identified orders possess versatile functions. For example, *Entotheonellales* presents the ability of producing compounds with antifungal activity (Schmidt et al. 2000; Pidot et al. 2014). Many of the family *Syntrophobacteraceae* are sulfate-reducing (Liu et al. 2014). Based on those, it can be speculated that the foliar spray of *R. palustris* could enhance C turnover and essential nutrients cycling, which potentially contributed to soil fertility and plant growth efficiency (Chaudhry et al. 2012). However, in consideration of the complex interaction between soil microbe and plant, further research should be devoted to understand the functions of the specific microbial groups relative to plant growth promotion after foliar spray of *R. palustris*.

5 Conclusions

The foliar spray of *R. palustris* grown under *Stevia* residue extract not only significantly increased the plant biomass and net photosynthetic rate of Chinese pak choi cabbage (*B. chinensis* L.) but also enhanced soil microbial metabolic activity and altered specific bacterial groups slightly. The increased relative abundances of some functional guilds in the *R. palustris* treatments might accelerate C turnover and nutrient cycling in the soil, including Acidobacteria, Actinobacteria, Proteobacteria, Gemmatimonadaetes, Nitrospirae, and Planctomycetes. Those features potentially contributed to the improved plant growth. The most marked effects of *R. palustris* grown under the *Stevia* residue extracts suggested the potential use of wastewater and suchlike in an economical way.

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