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Zinc, Cadmium and Lead Accumulation and Characteristics of Rhizosphere Microbial Population Associated with Hyperaccumulator *Sedum Alfredii* Hance Under Natural Conditions

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Abstract A field survey was conducted to study the characteristics of zinc, cadmium, and lead accumulation and rhizosphere microbial population associated with hyperaccumulator *Sedum alfredii* Hance growing natively on an old lead/zinc mining site. We found significant hyperaccumulation of zinc and cadmium in field samples of *S. alfredii*, with maximal shoot concentrations of 9.10–19.61 g kg^{-1} zinc and 0.12–1.23 g kg^{-1} cadmium, shoot/root ratios ranging from 1.75 to 3.19 (average 2.54) for zinc, 3.36 to 4.43 (average 3.85) for cadmium, shoot bioaccumulation factors of zinc and cadmium being 1.46–4.84 and 7.35–17.41, respectively. While most of lead was retained in roots, thus indicating exclusion as a tolerance strategy for lead. Compared to the non-rhizosphere soil, organic matter and total nitrogen and phosphorus content, CEC and water extractable zinc, cadmium, and lead concentration were significantly higher, but pH was smaller in rhizosphere soil. The rhizosphere soil of *S. alfredii* harbored a wide variety of microorganism. In general, significantly higher numbers of culturable bacteria, actinomycetes, and fungi were found in the rhizosphere compared to bulk soil, confirming the stimulatory effect of the *S. alfredii* rhizosphere on microbial growth and proliferation. Analyses of BIOLOG data also showed that the growth of *S. alfredii* resulted in observable changes in BIOLOG metabolic profiles, utilization ability of different carbon substrates of microbial communities in the rhizosphere soil were also higher than the non-rhizosphere, confirming a functional effect of the rhizosphere of *S. alfredii* on bacterial population.

Keywords *Sedum alfredii* Hance · Rhizosphere · Soil microbial population · Hyperaccumulation

Metal hyperaccumulating plants are characterized by accumulating exceptionally high concentrations of metals in their above-ground biomass (Baker and Brooks 1989). These plants have attracted great interest of scientists because of their role in the development of phytoremediation technologies for remediation of metal polluted soils. Significant progress has been made in understanding of plant-internal processes associated with metal hyperaccumulation (Lasat 2002). However, there remains considerable uncertainty about the mechanisms by which hyperaccumulating plants obtain metals from soil, and especially the role of rhizosphere processes (McGrath et al. 2001). The rhizopshere of hyperaccumulator plants might play an important role in the enhancement of the transfer of metals from the soil to roots (Whiting et al. 2001).

Higher microbial numbers and activities are typically observed in the plant rhizosphere, although long-term exposure to heavy metals can alter the qualitative and quantitative structure of microbial communities, resulting in decreased metabolic activity and diversity. Hyperaccumulators may alter metal equilibrium in their rhizosphere, resulting in rhizosphere microbial populations that may be different from the rhizosphere of non-hyperaccumulator plants. For example, a high proportion of metal resistant bacteria persist in the rhizosphere of the hyperaccumulators

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Thalaspi caerulescens (Delorme et al. 2001), *Alyssum bertolonii* (Mengono et al. 2001), and *Alyssum murale* (Abou-Shanab et al. 2003) grown in contaminated soil. Furthermore, soil microorganisms are known to play a key role in the mobilization and immobilization of heavy metals (Wu et al. 2006), thereby changing their availability to plants and affecting plant ability to uptake heavy metal from soils. For example, the presence of rhizosphere bacteria increased concentration and accumulation of Zn in *T. caerulescens* (Whiting et al. 2001), Ni in *A. murale* (Abou-Shanab et al. 2006), and *T. geosingense* (Idris et al. 2004), Cd in *Brassica napus* (Sheng and Xia 2006), Cu, Zn, and Pb in *Elsholtzia splendens* (Wang et al. 2007), respectively.

Sedum alfredii Hance is a new Zn/Cd-hyperaccumulator native to China (Long et al. 2002; Yang et al. 2004). Up to now, most researches on *S. alfredii* have been focused on its physiological mechanisms of uptake, transport and storage of Zn and Cd. Few information are available on their effects on soil microbial populations and processes. Therefore, the objective of this study was to investigate Zn, Cd, and Pb accumulation and rhizosphere microbial characteristics of indigenously field growing *S. alfredii* on the vicinity of an old lead and zinc mine.

Materials and Methods

The study area is located at 29°13′40″N latitude and 118°47′20″E longitude, of Quzhou City, Zhejiang Province, southeast China. It was a subtropical area with warm and humid climate. Annual average temperature and precipitation are 16.3–17.3°C and 1,632.5 mm, respectively. This mining area has been extensively mined from the early 1960s. The main plant species of the herbs in the investigated mining area are *S. alfredii* H, *Viola yedoensis* M, *Viola diffusa* G, *Prunella vulgaris* L, *Juncus effusus* L, *Ixeris sonchifolia*. While *S. alfredii* is the main plant species growing in abandoned mine slag and residuals (Fig. 1). *S. alfredii* is a perennial herb, and their seedlings are ready to obtain via asexual reproduction.

Five sampling sites (S1, S2, S3, S4, S5) were selected according to the topography of mining area and the distribution of *S. alfredii*. Individual *S. alfredii* plant specimen was collected along with the soil adhering to their roots. Roots and shoots were separated and transported in polyethylene bags, respectively. Operationally defined rhizosphere soil was separated by gentle shaking of the roots, and operationally non-rhizosphere soil was collected from non-vegetated spots at about 10–30 cm distance from *S. alfredii*. Each soil sample was filled in polyethylene bags, respectively. Each soil sample was sieved through a 2 mm screen and homogenized. A portion were air-dried and sieved (<0.45 mm) for physical and chemical analysis. Soil samples collected from site 1, 4, and 5 were chosen for microbial parameters analysis, and were stored in polythene bags at 4°C.

Soil organic matter, cation exchange capacity (CEC), clay content, total nitrogen, Kjeldahl nitrogen, total phosphorus, available phosphorus, and pH_{water} were determined following the methods described by Lu (1999). Total concentration of heavy metal (Cd, Pb, Zn) in the soils was analyzed with flame atomic absorption spectrophotometry (AAS, Z-5300) by digesting 100 mg of soil in a mixture of HF–$HClO_4$–HNO_3 (Lu 1999). Soil water or ammonium acetate-extractable heavy metal (Cd, Pb, Zn) were measured by shaking 5 g sub-samples of each soil sample with 25 mL de-ionized water or 1 mol L^{-1} NH_4OAc (pH = 7.0) at 25°C for 2 h, respectively. All extracts were filtered, heavy metal concentrations in extracted solutions were determined by AAS (Z-5300).

Shoots or roots were washed with tap water and rinsed three times with de-ionized water, then dried at 80°C to constant weight. Dry materials were ground with stainless steel grinder for Zn analysis. Approximately 0.1 g samples of plant material were weighed accurately into 50-mL borosilicate cup, and mineralized by oven incineration methods at 550°C. The ash was dissolved with 10 mL 6.0 mol L^{-1} HCl, mixed, filtered, and brought to 100 mL with 0.6 mol L^{-1} HCl. Zinc concentrations were determined by atomic absorption spectroscopy (AAS, Z-5300).

Fig. 1 Growth of *S. alfredii* at the abandoned mine slag and residuals

The total numbers of culturable heterotrophic bacteria, fungi, and actinomycetes were determined by serial dilution and plating on selective media. Serial dilutions of soil samples were made with sterile deionized water. The media used were beef extract and peptone medium (peptone 10.0 g, beef extract 3.0 g, NaCl 5.0 g, agar 20.0 g, distilled water 1.0 L, pH 7.2–7.4) for bacteria, bengal medium (KH_2PO_4 1.0 g, dextrose 10.0 g, $MgSO_4 \cdot 7H_2O$ 0.5 g, peptone 5.0 g, rose bengal 0.03 g, chloramphenicol 0.1 g, agar 15.0 g, distilled water 1.0 L, pH 7.0–7.4) for fungi, and gauze No. 1 (GA) medium (starch 20.0 g, KNO_3 1.0 g, NaCl 0.5 g, $FeSO_4 \cdot 7H_2O$ 0.01 g, K_2HPO_4 0.5 g, $MgSO_4 \cdot 7H_2O$ 0.5 g, agar 20.0 g, distilled water 1.0 L, 3.3 mL of 1% potassium dichromate as fungal and bacteria inhibitior) for actinomycetes.

The BIOLOG method adjusted for use in metal-contaminated soils was used (Kelly and Tate 1998). Soil samples were extracted by shaking 10 g of soil with 100 mL 0.1 M Tris buffer (pH 7.5) for 10 min. After shaking, samples were centrifuged for 10 min at 2,600 g. The supernatant liquid was diluted by tenfold, then 150 μL dilution liquid was used to inoculate the BIOLOG CE plates. Plates were incubated at 25°C for 7 days and color development was measured as absorbance using an automated plate reader (ELX808) at 590 nm and the data were collected using Microlog 4.01 software (BIOLOG). The BIOLOG data were analyzed in two ways. Firstly, the rate of color development on the BIOLOG plates over time was determined by calculating the average intensity of color on each plate (AWCD) at each reading time (Garland 1996). Secondly, AWCD at 72 h for different treatments were then compared by principal component analysis (PCA) with SAS statistical software. Each of the 31 substrates was treated as a variable, and each set of triplicate plates was treated as a sample.

All experimental data, means of three repeats, were processed by Microsoft Excel 2000. The regression analysis was conducted by using the statistical software packages of SAS 9.0. The difference (LSD) at the 5% level was used to test the significance between rhizosphere and non-rhizosphere means by *t*-test. For multivariate analysis of the Biolog data the absorbance values were first transformed by dividing by the AWCD to avoid bias between samples with different inoculum densities (Garland 1996) and were then analyzed by principal component analysis.

Results and Discussion

The basic characteristics of the tested soils are presented in Table 1. Organic matter and total N and P contcentration, and CEC were found to be generally significantly higher in rhizosphere soil than in non-rhizosphere soil, while pH in rhizosphere soil was smaller than in non-rhizosphere soil.

Table 1 Soil physical and chemical properties

Soil	pH	O-M (g kg^{-1})	TN (g kg^{-1})	TP (g kg^{-1})	Clay (%)	CEC (cmol kg^{-1})
Site 1						
Rhizosphere	6.12	91.62	2.05	1.30	12.68	19.08
Non-rhizosphere	6.54	49.31	1.41	0.47	14.56	9.76
F value	8.38***	27.38***	43.00***	1.75***	3.76***	7.53***
Site 2						
Rhizosphere	5.60	42.22	1.64	1.75	16.21	16.98
Non-rhizosphere	5.86	41.16	1.63	0.88	14.94	15.66
F value	1.08***	1.23**	2.25^{ns}	1.49***	1.11***	6.19**
Site 3						
Rhizosphere	5.44	44.27	1.73	1.65	24.75	15.25
Non-rhizosphere	5.65	35.95	1.66	0.87	16.36	11.10
F value	100.8^{ns}	7.82***	5.88^{ns}	4.00***	6.71***	2.30***
Site 4						
Rhizosphere	4.52	109.7	3.18	0.86	16.47	10.24
Non-rhizosphere	5.11	27.65	1.06	0.66	8.72	6. 61
F value	1.00***	1.19***	4.43***	195.47*	17.00***	1.94***
Site 5						
Rhizosphere	6.92	32.38	1.14	0.65	6.47	6.00
Non-rhizosphere	6.98	31.17	1.27	0.74	8.91	5.44
F value	64.33^{ns}	6.18**	4.54^{ns}	97.78*	10.22***	16.30**

^{ns} $p > 0.05$,* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

The soil from the polluted site was highly enriched in Zn, Cd, and Pb with maximal concentrations of up to 13.5 g kg^{-1} Zn, 0.13 g kg^{-1} Cd, and 38.5 g kg^{-1} Pb. A considerable sample variation of soil metal concentrations between the sites was also observed, pointing to the heterogeneous nature of the polluted area (Table 2). The highest and lowest total Zn and Cd concentration was all found on site 1 and site 4, respectively, while the highest and lowest total Pb concentration was found on site 3 and site 4, respectively. No significant difference of total Zn, Cd, and Pb concentration were found between the rhizosphere and non-rhizosphere soil at site 1, 2, 3, and 5, while a significant increase of total Zn, Pb, and Cd concentration were observed in rhizosphere soil as compared to the non-rhizosphere soil in site 4.

Water and NH_4OAc-extractable metal was measured to estimate the bio-available metal in field site. Water extractable fractions ranged from 0.01% to 0.07% for Zn, 0.01% to 0.06% for Cd, and 0.01% to 0.05% for Pb; NH_4OAc-extractable fractions ranged from 0.85% to 6.82% for Zn, 4.55% to 26.94% for Cd, and 0.11% to 1.92% for Pb. A significant linear correlation between total and NH_4OAc-extractable concentrations was found for Zn (rhizosphere: $r = 0.919$, $p = 0.001$; non-rhizosphere: $r = 0.919$, $p = 0.001$), Cd (rhizosphere: $r = 0.942$, $p = 0.001$; non-rhizosphere: $r = 0.965$, $p = 0.001$), and Pb (rhizosphere: $r = 0.461$, $p = 0.05$; non-rhizosphere: $r = 0.739$, $p = 0.05$). At the same time, a significant linear correlation between total and water-extractable concentrations was also found for Zn (rhizosphere: $r = 0.887$, $p = 0.001$; non rhizosphere: $r = 0.603$, $p = 0.05$), and Cd (rhizosphere: $r = 0.856$, $p = 0.001$; non rhizosphere: $r = 0.914$, $p = 0.05$), but correlation between total and water extractable Pb concentration in both rhizosphere and nonrhizosphere was not significant (rhizosphere: $r = 0.246$, $p > 0.05$; non rhizosphere: $r = 0.488$, $p > 0.05$). It is also important to note that water extractable Zn, Cd, and Pb concentration in the rhizosphere were significant significantly higher than those in non-rhizosphere soil (except in site 3), that may be due to more acidity in rhizosphere.

Table 3 shows high concentration Zn, Cd, and Pb in both shoots and roots of *S. alfredii* from the polluted soils. Shoot Zn, Cd, and Pb concentrations in *S. alfredii* were 9.10–19.61 g kg^{-1} (average 15.80 g kg^{-1}), 0.12–1.23 g kg^{-1} (average 0.56 g kg^{-1}), and 0.30–0.97 g kg^{-1} (average 0.57 g kg^{-1}), respectively; while root Zn, Cd, and Pb concentrations in *S. alfredii* were 3.07–8.74 g kg^{-1} (average 6.77 g kg^{-1}), 0.03–0.37 g kg^{-1} (average 0.15 g kg^{-1}) and 0.78–15.30 g kg^{-1} (average 6.62 g kg^{-1}),

Table 2 Concentration of heavy metal (Zn, Cd and Pb) in the tested soil

Soil	Total metal concentration (g kg^{-1})			NH_4OAc-extractable concentration (mg kg^{-1})			Water-extractable concentration (mg kg^{-1})		
	Zn	Cd	Pb	Zn	Cd	Pb	Zn	Cd	Pb
Site 1									
Rhizosphere	13.49	0.10	14.85	920.83	27.02	264.31	9.40	0.07	0.15
Non-rhizosphere	13.37	0.13	21.31	634.32	21.59	161.54	5.10	0.06	0.07
F value	$1{,}534^{ns}$	96.14**	98.60^{ns}	1.48*	1.50^{ns}	2.20***	1.76**	1.55*	1.01*
Site 2									
Rhizosphere	7.29	0.04	24.91	142.32	3.53	176.12	3.56	0.02	0.08
Non-rhizosphere	7.01	0.04	24.92	101.98	2.64	105.24	0.97	0.01	0.19
F value	1.82^{ns}	26.42^{ns}	5.18^{ns}	9.12^{ns}	1.54***	3.23**	8.53***	2.44**	18,237*
Site 3									
Rhizosphere	8.05	0.03	27.73	266.04	2.93	313.46	6.69	0.02	0.46
Non-rhizosphere	7.45	0.03	38.49	344.32	3.98	548.75	8.01	0.03	0.36
F value	2.27^{ns}	3.02^{ns}	4.16^{ns}	2.43**	1.09***	19.19***	50.01^{ns}	9.14^{ns}	1,078.8*
Site 4									
Rhizosphere	1.89	0.008	12.35	30.36	0.45	115.77	1.83	0.020	1.15
Non-rhizosphere	1.04	0.004	5.33	12.94	0.52	48.43	0.43	0.004	0.20
F value	9.68***	16.71***	5.66***	32.05^{ns}	13.10^{ns}	8.87***	1.50***	3.97^{ns}	27.22^{ns}
Site 5									
Rhizosphere	4.95	0.04	15.74	47.39	1.78	17.55	0.52	0.008	0.09
Non-rhizosphere	4.17	0.04	11.64	35.32	1.91	16.81	0.39	0.008	0.04
F value	171.5^{ns}	138.0^{ns}	7.10*	14.47^{ns}	6.82^{ns}	1.97^{ns}	1.03^{ns}	1.00^{ns}	$11{,}361^{ns}$

^{ns} $p > 0.05$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

Table 3 Heavy metal concentration in shoot and root of hyperaccumulating ecotype *Sedum alfredii*

	Shoot (g kg^{-1})			Root (g kg^{-1})			Shoot/root			Bioaccumulation factors[a]		
	Cd	Pb	Zn	Cd	Pb	Zn	Cd	Pb	Zn	Cd	Pb	Zn
Site 1	1.23	0.30	19.61	0.37	7.74	8.74	3.36	0.04	2.25	12.32	0.02	1.46
Site 2	0.59	0.63	19.40	0.17	7.75	11.11	3.45	0.08	1.75	13.88	0.03	2.67
Site 3	0.55	0.97	17.38	0.13	15.30	5.46	4.19	0.06	3.19	17.41	0.04	2.16
Site 4	0.12	0.77	9.10	0.03	0.78	3.07	3.79	1.01	2.97	14.47	0.06	4.84
Site 5	0.29	0.18	13.67	0.07	1.56	5.47	4.43	0.13	2.51	7.35	0.01	2.76
Average	0.56	0.57	15.83	0.15	6.62	6.77	3.85	0.26	2.54	13.09	0.03	2.78

[a] Bioaccumulation factor means the ratio of Zn concentration in shoot of *Sedum alfredii* to total heavy metal concentration in rhizosphere soil

respectively. Shoot/root ratios ranged from 1.75 to 3.19 (average 2.54) for Zn, 3.36 to 4.43 (average 3.85) for Cd, and 0.04 to 1.01 (average 0.26) for Pb. The mean shoot/root ratios >1 for Zn and Cd revealed very efficient transport of accumulated Zn and Cd to the shoots, whereas most of accumulated Pb was immobilized in roots. Bioaccumulation factors (BF) based on metal concentrations in shoots and total metal concentrations of the rhizosphere soils revealed the differing capacity of *S. alfredii* for the accumulation of the three metals in shoots. A significantly higher ability to accumulate Cd (BF: 7.35–17.41, average 13.09) and Zn (BF: 1.46–4.84, average 2.78) was observed in the *S. alfredii*, whereas capacity of Pb accumulation (BF: 0.01–0.06, average 0.03) was very low (Table 3). Thus, high shoot/root ratios and bioaccumulation factors for Zn and Cd in *S. alfredii* indicated that it was a Zn and Cd hyperaccumulator, which confirmed our previously reported results (long et al. 2002; Yang et al. 2004).

Shoot Zn concentration significantly linearly correlated with total soil Zn concentrations ($R^2 = 0.7249$; Fig. 2a), it confirmed that *S. alfredii* had the ability to hyperaccumulation of Zn, and Zn accumulation in *S. alfredii* was greatly affected by the soil Zn concentration. However, no correlation was found between root and total soil Zn concentrations. In addition, the linear regression model could also be applied for the description of the relationship between shoot and root Cd concentrations with total soil Cd concentrations (shoot: $R^2 = 0.8918$; root: $R^2 = 0.8944$; Fig. 2b), thus confirming the hyperaccumulation of Cd as well. Shoot Pb concentration exponentially correlated with total soil Pb concentrations ($R^2 = 0.4449$), it indicates exclusion of Pb as a tolerance strategy; however, no regression model could be fitted to the relationship between root Pb concentration and total Pb concentrations (Fig. 2c).

The rhizosphere provides a complex and dynamic microenvironment where microorganism and roots form unique communities. Higher microbial numbers and activities are typically found in the plant rhizosphere (Grayston et al. 1998). The analysis of the culturable microbial population (bacteria, actinomycetes and fungi)

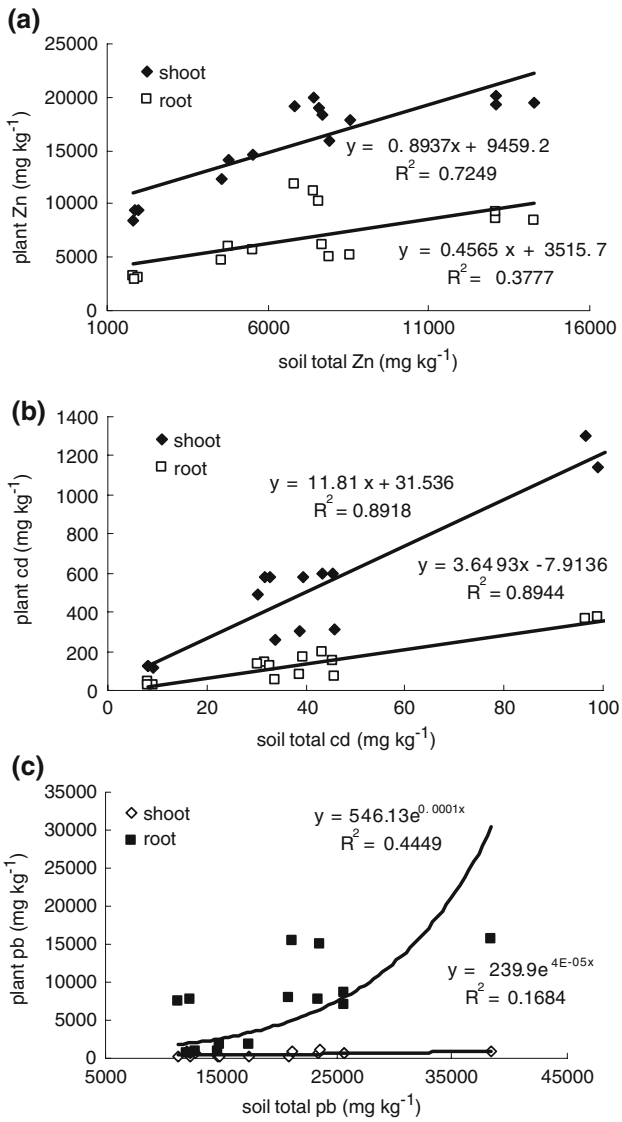

Fig. 2 Relationships between **a** Zn, **b** Cd, and **c** Pb concentrations in shoots and roots of *S. alfredii* and total metal soil concentrations

showed that the rhizosphere of indigenously field growing *S. alfredii* harbored various types of microbial populations, with the number of bacteria and actinomycetes being

higher than the fungi (Table 4). There were clear treads that higher number of bacteria, actinomycetes and fungi were found in rhizosphere compared to non-rhizosphere (Table 4). The results well agreed with other studies. For example, Delorme et al. (2001) found that Zn hyperaccumulator *T. caerulescens* increased microbial populations in rhizosphere soil compared with non-rhizosphere under controlled conditions. Aboudrar et al. (2007) proved that higher number of bacteria, actinomycetes, algae, and fungi were in the rhizosphere of the serpentine population of *T. caerulescens*.

It is well known that root exudates serve as a source of carbon and energy to soil microorganism, therefore, the continuous input of organic matter in the rhizosphere might stimulate the microorganism growth. In this study, we really found that concentration of organic matters, total N and P in rhizosphere soil were significant higher than in non-rhizosphere soil (Table 1). A significant linear correlation also existed between number of bacteria and concentration of organic matter and total P, between number of fungi and concentration of organic matter, total N and P (Table 5).

Biolog system has previously proved to be a useful tool to compare different bacterial communities and to individuate shifts of bacterial communities under environmental stresses based on their metabolic profile (Grayston et al. 1998). In this study, the community level physiological profiles of the bacterial communities were determined by examining the microbial utilization of 31 different carbon sources. Results showed that there were qualitative differences in the soil microbial metabolic activities between rhizosphere and non-rhizosphere of *S. alfredii* grown under natural conditions (Fig. 3). AWCD profiles were significantly higher in the rhizosphere of *S. alfredii* compared with the non-rhizosphere soil at site 1 and site 4, but no significant difference of AWCD was found between rhizosphere and non-rhizosphere at site 5. The number of bacteria showed a similar change pattern as AWCD profiles. Therefore, it seems that this difference in population size may explain the difference in rate of color development on the BIOLOG plates.

The AWCD standardized data after 72 h incubation in the Ecoplate were used for the PCA. The results revealed a separation of all the soil samples, indicating that they had different patterns of potential C utilization and different microbial communities (Fig. 4). The rhizosphere and non-rhizosphere of site 1 was significantly separated along the PC1 axis (29.87% of the variance), with a negative value for the non-rhizosphere. The rhizopshere and non-rhizosphere of site 4 can be separated along both the PC1 and PC2 axis (18.76% of the variance), with a higher positive value for the rhizopshere compared to the non-rhizosphere. However, at site 5, the rhizopshere and non-rhizosphere soil could not be separated, and there were great variability in the metabolic profile of bacteria isolated from both the rhizosphere and the non-rhizosphere soil. The separation of soil samples along PC1 axis could be explained by the metabolism of the carbohydrates (D-xylose, i-erythritol, D-mannitol, D,L-α-Glycerol Phosphate, D-cellobiose, α-D-lactose), Carboxylic acid (D-GalactonicAcid-γ-Lactone, D-Galacturonic Acid, γ-Hydroxybutyric Acid, 4-Hydroxy Benzoic Acid, D-Glucosaminic Acid), amino acids (L-Serine, Glycyl-L-Glutamic Acid, L-Arginine, L-Asparagine), and others (Tween 40 and Putrescine), while the separation of soil samples along PC2 axis were attributed to metabolism of the Polymer (Tween 80, α-cyclodextrine, Glycogen), Carbohydrate (β-methyl-D-glucoside, i-erythritol, α-D-lactose, Glucose-1-phosphate, D,L-α-Glycerol Phosphate), Amino acid (L-Threonine, L-phenylalanine), and α-Ketobutyric Acid.

Table 4 Total culturable microbial populations in the rhizosphere and non-rhizosphere soil of *Sedum alfredii*

	Site 1	Site 4	Site 5
Bacteria ($\times 10^6$ CFU g^{-1}soil)	37.94	3.10	1.75
Rhizosphere			
Non-rhizosphere	0.57	1.43	1.79
F value	2,996.56***	2,207.00**	5.50^{ns}
Actinomycetes ($\times 10^6$ CFU g^{-1}soil)	9.13	6.98	13.17
Rhizosphere			
Non-rhizosphere	2.33	6.68	2.19
F value	4.53*	3.82^{ns}	14.59***
Fungi ($\times 10^5$ CFU g^{-1}soil)	1.38	7.94	3.14
Rhizosphere			
Non-rhizosphere	0.32	0.89	1.25
F value	96.80*	298.15**	2.05^{ns}

^{ns} $p > 0.05$,* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

Table 5 Linear correlation coefficients between some soil physico-chemical properties and number of microbial populations

	Organic matter	TN	TP	pH	CEC	Clay
Bacteria	0.519*	0.265	0.913***	0.010	0.919***	0.189
Actinomycetes	0.121	0.051	0.330	−0.043	0.159	−0.358
Fungi	0.654**	0.814***	0.158	−0.595**	0.003	0.425

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

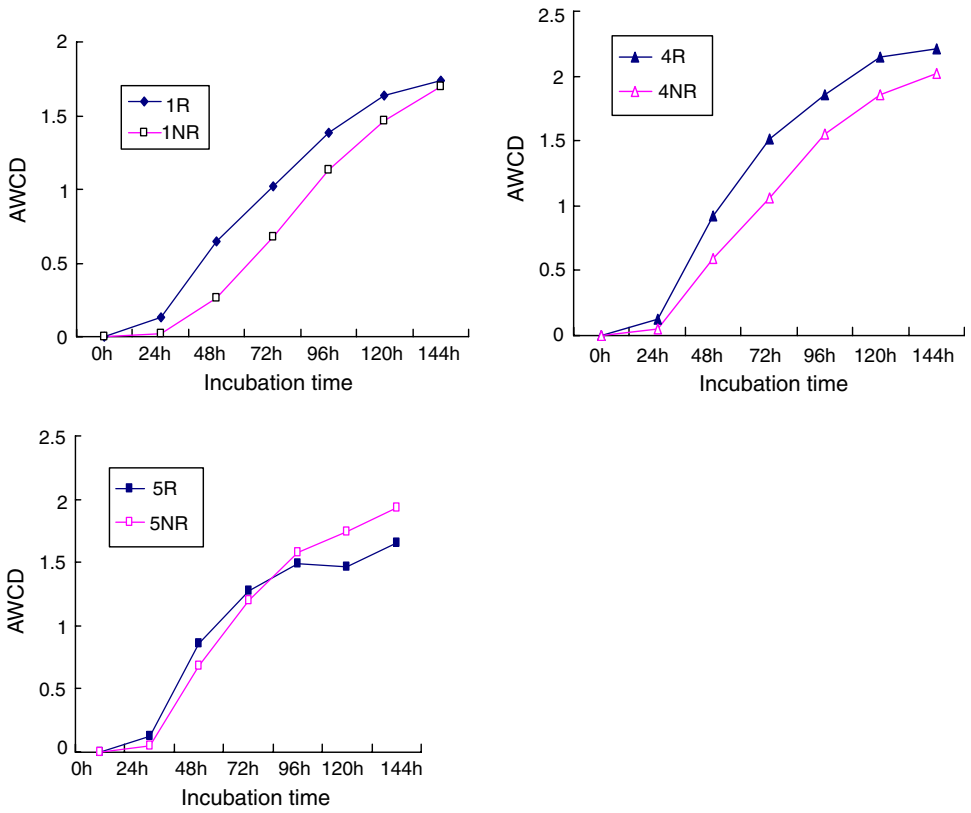

Fig. 3 Average well color development (*AWCD*) of community level physiological profiles (*CLPP*) in the rhizosphere and non-rhizosphere of *S. alfredii*

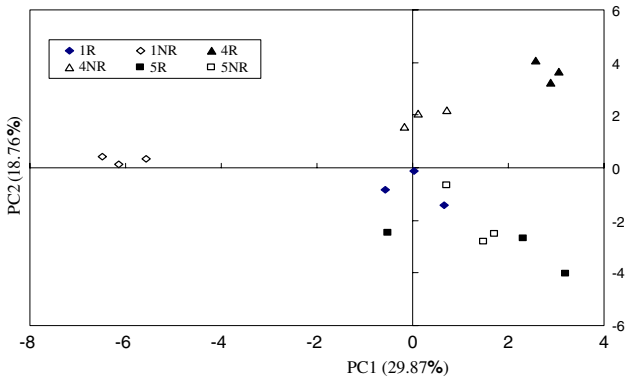

Fig. 4 Principal component analysis of BIOLOG readings 72 h after incubation

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