Environ Sci Pollut Res (2017) 24:7668–7678
DOI 10.1007/s11356-017-8432-8

RESEARCH ARTICLE

Phytoremediation of strontium contaminated soil by *Sorghum bicolor* (L.) Moench and soil microbial community-level physiological profiles (CLPPs)

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Received: 10 October 2016 / Accepted: 9 January 2017 / Published online: 25 January 2017

Abstract Phytoremediation of strontium contaminated soil by *Sorghum bicolor* (L.) Moench was investigated, and the soil microbial community-level physiological profiles (CLPPs) were examined. The growth and the stable strontium (^{88}Sr) accumulations of the energy crop *S. bicolor* grown on the Sr-spiked soil at the level of 0, 50, 100, 200, and 400 mg/kg soil were characterized through pot soil system after the entire growth period (140 days). Correspondingly, the available content of strontium in soil extracted by Mehlich III extraction solution reached 42.0, 71.9, 151.8, and 242.2 mg/kg, respectively. The Sr-polluted soil microbial community was assessed by a Biolog Eco-plate method. The results showed that the spiked Sr significantly increased the height and the stem biomass weight of the plant. Sr contents in roots, stems, and leaves of the sorghum increased linearly ($R^2 > 0.95$) with the elevation of the Sr-spiked level in soil. The average Sr concentration in roots, stems, and leaves reached 68.9, 61.3, and 132.6 mg/kg dry weight (DW) under Sr-spiked 400 mg/kg soil, respectively. Sr content in tissues decreased in the order of leaves > roots > stems. The bioconcentration factor (BCF; Sr contents in shoots to soil) values of *S. bicolor* in soil system was lower than 1 (0.21~0.39) whether based on the spiked Sr level or on the available Sr level in soil. The transfer factor (TF; Sr contents in shoots to roots) values of *S. bicolor* in soil system usually is higher than 1 or near to 1 (0.92~1.29). TF values increased while BCF values decreased as the soil Sr increased. The Biolog Eco-plate assay showed that Sr at the spiked level of 400 mg/kg soil enhanced the soil microbial diversity and activity.

Keywords Strontium · *Sorghum bicolor* · Phytoremediation · Soil microbial community-level physiological profiles (CLPPs) · Biolog Eco-plate

Responsible editor: Elena Maestri

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Introduction

Radioactive strontium (^{90}Sr) is an important fission product of ^{235}U and ^{239}Pu at a relative high fission yield which can be released into the environment from nuclear facilities such as nuclear power plants, nuclear reactors, and the explosion of nuclear weapons (Entry et al. 1996; Dushenkov 2003). It owns chemical similarity with calcium. After nuclear power plant accident (Sahoo et al. 2016) such as Fukushima accident in 2011 and Chernobyl accident in 1986, ^{90}Sr, similar to ^{137}Cs, is usually regarded as a key long lived radionuclide ($T_{1/2}$ = 28.7 year) in radioactive pollution control. ^{90}Sr in soils could pose a long-term radiation effects on human health via the food chain and other pathways and increased the risk of cancer with the elevation of Sr concentration in the surroundings (Zhu and Shaw 2000). It is an urgent need for remediation of ^{90}Sr contamination (Achal et al. 2012). Phytoextraction of radionuclides from soil is considered to

be a promising and cost-effective bioremediation method using plants with high shoot accumulation abilities for the soil contaminated by low concentrations of radionuclides (Zhu and Shaw 2000). Contaminants were mainly located in the aerial part of the metal accumulating plants after the process of phytoextraction. The aboveground portions of the plant are harvested and removed after the sufficient plant growth and metal accumulation. Another phytoremediation mechanism is phytostabilization, which aims to reduce the mobility or immobilization of metals by stabilized metal in the roots (Al Chami et al. 2015). The strategy of phytostabilization appears more feasible in real polluted soil remediation because the radionuclides could be restrained in the vicinity of the plant roots.

Some plants such as trees, grass, and other herbaceous plants, as well as phytoplankton and aquatic plants grown in forest, meadow, desert area, or aquatic area were found to accumulate ^{137}Cs and ^{90}Sr (Entry et al. 1996). In heavy metal phytoremediation researches, the bioconcentration factor (BCF) values and transfer factor (TF) values are two important parameters which can describe the metal bioconcentration ability from soil to plant and metal transfer ability from roots to shoots of a plant, respectively. It is believed that a plant can efficiently phytoextract heavy metals from soil by the plant when BCF and TF values are higher than 1. However, it is necessary to notice that the terms and the calculation method of BCF values or TF values did not unify in many reported references. Metal content or radioactivity concentration in the plant or in part of the plant (shoots, or leaves, or stems, or grains), expressed in milligrams per kilogram for stable isotope or in becquerels per kilogram dry weight for radioactive elements, can be selected to calculate BCF or TF values. Total metal content in soil or in plant were mostly used. However, the available metals in soil extracted by some specialized extractant solution were also utilized or suggested. According to IAEA definition, the use of activity concentration ratio values between the organism and its associated environmental media such as soil, sediment, water, or air as a parameter is a common approach to assess the radionuclide transfer ability from environment to wild plants (IAEA 2010). For example, the term of concentration ratio (CR) or soil-to-plant transfer factor was calculated as the ratios of the activity concentration in plant material per unit dry weight (Bq/kg) to the concentration in the soil per unit dry weight (Bq/kg) and used to describe the radioactive ^{137}Cs and ^{90}Sr transfer from soil to plant (Krouglov et al. 1997; Al Attar et al. 2015).

Field or laboratory experiments or field investigation showed that the ability to accumulate radionuclides varies among a wide array of plant species (Fuhrmann et al. 2002). The ^{90}Sr BCF ratio (radionuclide in plant tissue Bq/kg/Bq/kg radionuclide in soil) of three grasses, bahia grass (*Paspalum notatum* var. saura Parodi.), johnson grass (*Sorghum halepense* L. Persoon.), and switchgrass (*Panicum virginatum* L.), reached 5.4, 12.6, and 5.9, respectively (Entry et al. 1999). However, Sr was bioexcluded by *Paxillus involutus* mushroom with the BCF < 1 in the caps and stipes (Brzostowski et al. 2011). Soil-to-plant concentration factors of ^{90}Sr varied slightly or greatly among different soils (Krouglov et al. 1997) or different plant species (Twining et al. 2004). Obviously, BCF values or TF values are influenced by soil conditions, climate and plant species.

For phytoremediation, high biomass yield could compensate the insufficient metal accumulation content compared with hyperaccumulators. The total quantity of contaminant could be comparable or even higher than some hyperaccumulators (Al Chami et al. 2015). Redroot pigweed uptake the largest accumulation quantity of ^{90}Sr because of its larger biomass even though its bioconcentration ratio was the lowest when compared with Indian mustard and tepary bean (Fuhrmann et al. 2002).

Sorghum bicolor (L.) Moench is a fast growing energy crop with high biomass in C4 photosynthesis. It is also an ideal botanical model for many tropical grasses with complex genomes such as maize and sugarcane due to its smaller genome and duplication (Calvino and Messing 2012). *S. bicolor* has been investigated at filed scale and in lab scale to accumulate heavy metals such as cadmium and zinc (Pinto et al. 2006; Marchiol et al. 2007; Zhuang et al. 2009). Sweet sorghum thus can be potentially established as an ideal model plant both in scientific research and in field application for soil phytoremediation (Wang et al. 2016a, b, 2017). However, Sr uptake by *S. bicolor* was seldom reported (Sasmaz and Sasmaz 2009).

Phytoremediation not only aims to remove pollutants but also tends to restore the soil function. Soil microbial community is a good indicator of the soil health (Epelde et al. 2009). Soil microbe will reflect the soil quality and are influenced by biotic and abiotic environmental condition. The importance of biodiversity of an effective phytoremediation system such as soil microorganisms is increasingly considered for the cleanup of the metal contaminated ecosystems (Prasad and Freitas 2003). The soil microbial community analysis may also contribute to the understandings of "metal–sorghum–soil–microbe interactions," which is a key scientific problem in phytoremediation using sweet sorghum but with a little scientific effort on it. Many available techniques for describing microbial communities such as phospholipid ester-linked fatty acid (PLFA) profiles (Frostegard et al. 2011) and PCR-based methods (such as denaturing/temperature gradient gel electrophoresis (DGGE/TGGE), amplified ribosomal DNA restriction analysis (ARDRA), ribosomal intergenic spacer analysis (RISA), automated ribosomal intergenic spacer analysis (ARISA), the random amplified polymorphic DNA (RAPD) finger prints) were reported (Khan et al. 2010; Wang et al. 2007). Among them, the methods for community-level physiological profile (CLPP) determination based on the Biolog

Eco-plates, utilizing the carbon substrate and reducing the dye to produce a red-violet coloration and then read on a standard laboratory microplate reader, are widely used to rapidly assess the soil microbial diversity at community level (Garland 1996, 1997; Garland et al. 2001; Classen et al. 2003), although the method is criticized mainly for that it depends on the growth of an extracted microbial population, which may not represent the whole soil (Chapman et al. 2007). The Biolog Eco-plate can be performed simply and conveniently without requirement of a large investment of time and monetary resources and highly specialized expertise (Garland 1996, 1997; Garland et al. 2001; Classen et al. 2003). In this study, the Biolog Eco-plates were used to analyze the Sr-contaminated soil microbial community under the sweet sorghum phytoremediation, which would roughly and rapidly to help screen the plant for the Sr-contaminated soil restoration.

The pure beta emitter radiostrontium isotopes were much less studied and reported than the gamma emitter radionuclides such as iodine, tellurium, and cesium isotopes largely because it is a more difficult measurement technique for pure beta emitter Sr (Sahoo et al. 2016). The stable ^{88}Sr cannot completely mimic radioactive ^{90}Sr in soil–plant due to their great differences in the soil concentration and in the radioactivity. However, stable element instead of radioactive element as pre or primary study is a common step in lab study. Some researchers used stable ^{88}Sr in phytoexperiment to model ^{90}Sr behavior and Sr accumulation patterns in plants, such as ^{88}Sr in wheat (*Triticum aestivum* L.), husk oat (*Avena sativa* L.), naked oat (*Avena nuda*), and barley (*Hordeum vulgare* L.), for their potential use in phytoremediation for Sr-polluted areas (Qi et al. 2015). The behavior of stable isotope ^{88}Sr was even used as an indicator of long-term behavior and processes of radioactive isotope ^{90}Sr in plants and in the environment (distribution, pathways, mobility, transfer, etc.) (Tsukada et al. 2005; Soudek et al. 2006). The research on the Sr-polluted soil phytoremediation is still very limited (Eapen et al. 2006). The effect of on the soil microbial community after cultivation of *S. bicolor* was much less reported. In this study, stable strontium ^{88}Sr instead of radioactive strontium ^{90}Sr were used in this manuscript.

In this study, the growth and stable isotope ^{88}Sr accumulation of sweet sorghum cultivated in pot after 140 days' sowing when the sorghum mature in Beijing, China was investigated. Sr is whether a nutrient or a pollutant depending on the available Sr in soil. The total metal (Sr) content in shoots (leaves + stems) or in the roots and the metal spiked level or the available Sr in soil extracted by Mehlich III solution (Mehlich 1984) were used to calculate the BCF and TF values and compared in this study (seen in the "Materials and methods" section in this study). The soil microbial community characteristics with or without Sr pressure when the plant was harvested were compared and assessed by Biolog Eco-plate methods. This paper is expected to offer new information on Sr phytoremediation from soil by *S. bicolor* and accumulate basic data for potential application of *S. bicolor* for Sr phytoremediation in future.

Materials and methods

Soil preparation, plant cultivation, and Sr analysis in plants

Seeds of *S. bicolor*, cv. "Cowly," were provided by Hebei Academy of Forestry Sciences, China. The pot experiment was performed at a farmland in Beijing, China. The soil contained high levels of organic matter (9.6%) and a pH of 7.24. The total nitrogen, phosphorus, and potassium concentration were 1.95, 8.77, and 10.10 g/kg, as well as the available nitrogen, phosphorus, and potassium were 140.05, 178.45, and 1010.57 mg/kg in the soil, respectively. The cation exchange capacity of the soil was 2.93 cmol/kg. The soil texture was sandy clay loam, which contained 57.2% sand, 16.0% silt, and 26.8% clay, respectively.

The artificial Sr-contaminated soil was prepared by adding the aqueous solution containing strontium chloride ($SrCl_2 \cdot 6H_2O$). The spiked ^{88}Sr concentration was set as 50, 100, 200, and 400 mg/kg soil. Correspondingly, the available concentration of strontium in soil was 42.0, 71.9, 151.8, and 242.2 mg/kg, respectively, which was extracted by Mehlich III extraction solution (Mehlich 1984). The soil without Sr addition was prepared as the control group. Each treatment has four replicates. The pots (26 cm diameter and 20 cm height) were placed in open air under plastic sheeting. The seeds after sterilization were planted in each pot. The mature plants were harvested after grown for 140 days. The aerial part tissues and roots were separated, rinsed repeatedly, and dried at 70 °C. All fractions of dried plants were then grinded into fine powder and preserved in sealed polyethylene bags. The plant sample was digested using concentrated nitric acid and perchloric acid (Wang et al. 2016a, b). The Sr level in digestion solutions was determined by an atomic absorption spectrophotometer (Hitachi, ZA3000).

The phytoremediation ability of the plant was evaluated using both the bioaccumulation factor (BCF) and the translocation factor (TF) as follows (Fellet et al. 2007)

$$\mathrm{BCF} = [\mathrm{Sr}]_{\mathrm{shoot}} / [\mathrm{Sr}]_{\mathrm{soil}} \quad (1)$$

$$\mathrm{BCFs} = [\mathrm{Sr}]_{\mathrm{shoot}} / [\mathrm{Sr}]_{\mathrm{spiked\ in\ soil}} \quad (2)$$

$$\mathrm{BCFa} = [\mathrm{Sr}]_{\mathrm{shoot}} / [\mathrm{Sr}]_{\mathrm{available\ in\ soil}} \quad (3)$$

$$\mathrm{TF} = [\mathrm{Sr}]_{\mathrm{shoot}} / [\,\mathrm{Sr}]_{\mathrm{root}} \quad (4)$$

$$[Sr]_{shoot} = \left([Sr]_{leaf} \times W_{leaf} + [Sr]_{stem} \times W_{stem}\right)/(W_{leaf} + W_{stem}) \quad (5)$$

where $[Sr]_{root}$, $[Sr]_{shoot}$, $[Sr]_{stem}$, and [Sr]leaf are the Sr concentration in the *S. bicolor*'s roots, shoots, stems, and leaves, respectively (mg/kg); $[Sr]_{soil}$ is the Sr concentration in the soil (mg/kg); $[Sr]_{spiked\ in\ soil}$ is the spiked Sr in soil; $[Sr]_{available\ in\ soil}$ is the available Sr in soil extracted by Mehlich III extraction solution; W_{leaf} is the weight of leaves (g); and W_{stem} is the weight of stems (g).

Soil microbial communities assessed by Biolog Eco-plates

According to the Biolog assay procedure (Wang et al. 2016a, b), the Sr-spiked soil (400 mg/kg) and the control soil without spiked Sr were analyzed using the Biolog Eco-plate system (Biolog Inc., Hayward, CA, USA). One hundred fifty microliters of the 100-fold dilution of soil extraction by sterile sodium chloride solution (0.85 mol/L) was added to the each well of the Biolog Eco-plates using an eight-channel pipette injector. All plates were incubated at 25 °C. Automatic plate reader (Bio-Tek Instruments Inc., Winooski, VT, USA) was used to read the absorbance values (OD values) at 590 nm daily through 5 days. Each 96-well plate consisted of three replicates, each one comprising 31 sole carbon sources (C) and water blank (R). The absorbance value of the Biolog Eco-plates control well (containing no substrate) was subtracted from the absorbance of all the other wells to eliminate background color from the soil suspension. AWCD, the average well color development, was calculated from all of the net absorbance. Data are the means of three individual OD values. Shannon diversity index (H'), Simpson index (D), and substrate richness index (R) were calculated to value soil microbial functional diversity according to the following formula (Wang et al. 2016a, b):

(1) Shannon diversity index

$$H' = -\sum p_i \ln p_i \quad (6)$$

Pi is ratio of the absorbency of the *i*th well to the total absorbency of all wells.

(2) Simpson index

$$D = 1 - \sum p_i^2 \quad (7)$$

Pi is ratio of the absorbency of the *i*th well to the total absorbency of all wells.

(3) Substrate richness index (S). S is the well numbers with (C − R) value above 0.25, where C and R absorbency were in each C-source well and the control, respectively.

Statistical analysis

All statistical analysis was performed using the Statistical Product and Service Solutions (SPSS) 22.0 package. Differences in heavy metal concentrations among different varieties of sorghum or between different tissues of sorghum were analyzed with one-way analysis of variance (ANOVA) approach. A significance level of $P < 0.05$ was used and the Duncan test was performed for multi-comparisons when significant differences occurred.

Results and discussion

Growth of *S. bicolor* (L.) Moench under Sr pressure

The *S. bicolor* (L.) Moench grew higher significantly than the control when the soil was spiked with Sr at the addition level ranged from 50 to 400 mg/kg, shown in Fig. 1. Compared with the control sample (158.1 cm), the height of the sorghum increased by 39.2~63.2%. However, the difference of height caused by varied spiked Sr concentration under the experimental vales from 50 to 400 mg Sr/kg was not significant.

According to Fig. 2, the stem yield increased significantly under each Sr treatment (50~400 mg/kg) compared with the control. The maximum stem production occurred when the spiked Sr was 50 mg/kg soil, which was 3.1 times of the control with the average weight of only 18.1 g. The stem yield under 100, 200, or 400 mg Sr/kg treatment, reaching 1.9~2.1-fold of the control, did not show significant differences. The dry weight of leaves increased but not significantly when compared with the control. Generally, the existence of strontium (50~400 mg Sr/kg) did not show any harmful effects on the growth of *S. bicolor*. In fact, in the current experimental condition, Sr promoted the growth of the plants in dry biomass of stems and in the height compared with that of the control and the corresponding available Sr reached 42.0~242.2 mg/kg soil. The typical concentration of plant available Sr in soil ranges from 0.2 to 20 ppm (Bentley 2006). Strontium content of soils is highly associated with the parent rocks and climate (Nadimi-Goki et al. 2014). The background value in soil in Xiamen, China was 37 mg/kg. In fact, the background values of Sr in China varied 6~5979 mg/kg and the average value for Sr was 167 mg/kg. In this study, the added Sr at 400 mg/kg soil and the available Sr at 242.2 mg/kg soil did not only exert any toxic effect on the growth of *S. bicolor* but also promote the plant growth in height and the stem biomass weight. It was once reported that the highest allowable harmless limit of Sr in soil was reported to be 600 mg/kg (Krutilina et al. 1999). Now there is still no clear conclusion on the role of strontium on plants. However, as a calcium analog and Ca as an important macronutrient element, Sr may play a similar role in many cellular metabolic processes (Qi et al. 2015). Sr was also reported to promote the plant growth at low level but inhibited the growth of plants at high level compared with the control treatment (Roca et al. 1997; Qi et al. 2015). Most of the 26 cultivars of wheat (*T. aestivum* L.), husk oat (*A. sativa* L) and

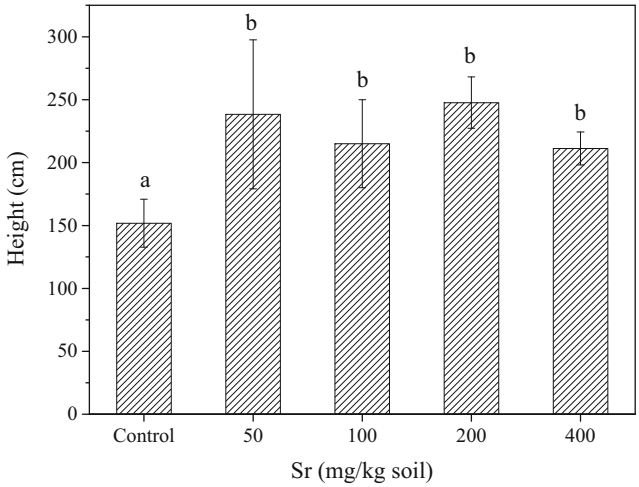

Fig. 1 Height of *Sorghum bicolor* (L.) Moench with different Sr treatments. Each value is the mean of quadruplicate determinations. The *bars* are the standard errors of the means ($n = 4$). *Same lowercase letters* mean that heights of sorghums have no significant difference in treatments ($P < 0.05$)

naked oat (*A. nuda*), and barley (*H. vulgare* L.) were reported to be not influenced on the shoot biomass by Sr treatment (26~3026 mg Sr/kg) (Qi et al. 2015). The response of organisms to Sr depends on many factors including soil type, soil properties, and the available Sr in soil (Margon et al. 2013).

The biomass of aboveground parts took up 67~81% of the total biomass. The root weight took up 19~33% of the total biomass under Sr treatment (50~400 mg/kg). The shoot biomass higher than the root biomass was universal (Wang et al. 2012). For ^{88}Sr uptake by radish, the aboveground and root parts took up about 50% each of the total biomass under 0~40 mg Sr/kg soil (Wang et al. 2012). The aboveground part biomass weight is an important indicator to assess the ability

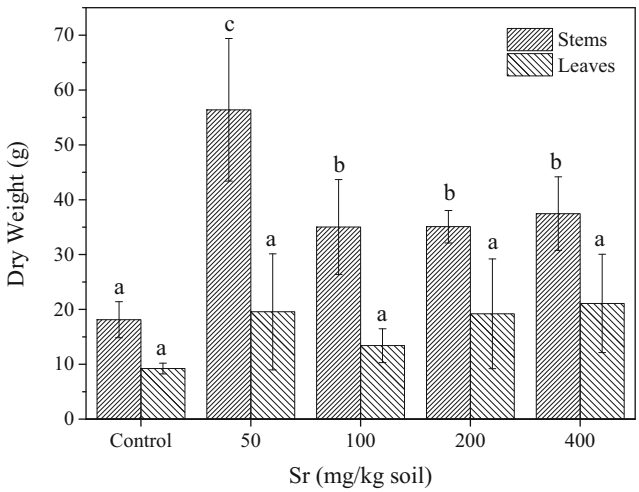

Fig. 2 The yields of shoot parts of *Sorghum bicolor* (L.) Moench in different treatment. Each value is the mean of quadruplicate determinations. *Error bar* is the standard deviation of examples ($n = 4$). *Different lowercase letters* mean that dry weights have significant difference between the same parts of sorghum ($P < 0.05$)

of a plant for phytoremediation. It is expected that *S. bicolor* grow well with a high biomass and beneficial for Sr phytoremediation which could make a reparation for the lower Sr uptake content in tissues compared with some hyperaccumulators.

Sr accumulation and distribution in *S. bicolor*

The concentration and distribution of Sr in *S. bicolor* are shown in Fig. 3. Sr in the control sample was not able to be detected. Thus, this treatment was not included when making Sr accumulation figures. When the Sr-spiked level arrived at 50, 100, 200, and 400 mg/kg soil, the average Sr concentration in roots could reach 16.5, 26.7, 40.7, and 68.9 mg/kg dry weight, respectively; Sr in stems could reach 14.9, 18.7, 30.9, 61.3 mg/kg dry weight, respectively; Sr in leaves could reach 27.6, 39.6, 91.9, and 132.6 mg/kg dry weight, respectively. The significant differences in radioactive or stable Sr uptake with the elevated Sr level in soil or in solution among different plants were observed and reported. The shoot Sr concentration (mg/kg) at maturity of four selected oat cultivars under the Sr levels of 26, 151, 776, 1526, and 3026 mg/kg in pot soil reached 16.3~19.1, 84.1~42.2, 404.4~269.5, 1141.1~588.2, and 4063.6~2413.3 mg/kg dry weight, respectively (Qi et al. 2015). The Sr contents in the green mass of vetch and oats varied in the range of 16.8~48.7 and 5.3~30.5 mg/kg, respectively, when the total strontium content in the medium loamy soddy–podzolic soil varied from 131 to 165 mg/kg (Karpova and Gomonova 2006). Mean Sr values in the shoots, roots, and soil were, respectively, 453, 243, and 398 mg/kg for *Euphorbia macroclada Boiss* (local name: Sütlegen); 149, 106, and 398 mg/kg for *Verbascum cheiranthifolium Boiss* (local name: Sigir Kuyrugu); and 278, 223, and 469 mg/kg for *Astragalus gummifer* (local name: Keven) grown in the surface soils of the arid and semi-arid Keban mining area in Turkey (Sasmaz and Sasmaz 2009). In NE Italy, the total Sr content in soil ranged from 36.4 to 137.2 mg/kg, and correspondingly, Sr in roots of rice reached 36.4~137.2 mg/kg, in stems of rice reached 3.5~10.5 mg/kg, in leaves of rice reached 15.6~38.6 mg/kg, and in grains of rice reached 0.05~13.8 mg/kg, respectively (Nadimi-Goki et al. 2014). Sr content in the shoots of *S. bicolor* was 6.2~8.2 mg/kg where the soil Sr level was 106 mg/kg in the field at a former uranium leaching heap site near Ronneburg, in Germany (Phieler et al. 2015). *S. bicolor* appeared to exert the normal Sr accumulation ability compared with the abovementioned reports.

Sr concentration in *S. bicolor* decreased in the order for different treatment: leaves > roots > stems, which agrees well with other studies of ^{90}Sr accumulation by many plants (Marchiol et al. 2007; Phieler et al. 2015). This order did not change under various Sr treatments in this study. The ^{90}Sr accumulation was found to be higher in leaves over other plant

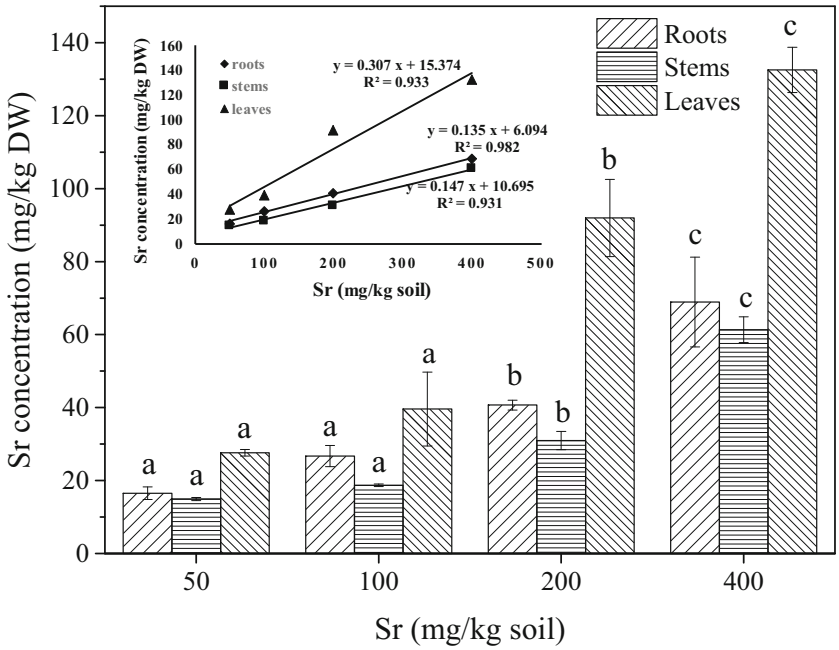

Fig. 3 Sr accumulation in roots, stems and leaves of *Sorghum bicolor* (L.) Moench in different treatments. The *bars* are the standard errors of the means ($n = 3$). The *different lowercase letters* mean that the concentration in same parts of sorghum has significant difference ($P < 0.05$)

parts for *Salix viminalis* (Von Fircks et al. 2002) and Indian mustard (*Brassica juncea*) (Su et al. 2007). Similarly, leaves of the four oak varieties had the highest Sr concentrations at each Sr level (26~3026 mg/kg) for compared with other organs of the plants, following the order of leaves > straw and roots > grain (Qi et al. 2015). The elements of Sr, Mg, and Ba were observed to coprecipitate with calcium mainly in phyllodes (i.e., petioles functioning as leaves) of the four *Acacia* species (*Acacia stipuligera* F. Muell., *Acacia ancistrocarpa* Maiden & Blakely, *Acacia stellaticeps* Kodela, Tindale & D. Keith, and *Acacia robeorum* Maslin) (He et al. 2012). Sr level in leaves was greater than in the stem for the rice grown in 12 regions of Taiwan (Wang et al. 1998). In China, Wen et al. (2009) found lower Sr concentrations in stems of sunflower than in roots and leaves.

In this study, 71~82% of Sr was distributed in the aboveground parts of *S. bicolor* (leaves plus stems) and 19~28% of Sr in the roots of *S. bicolor*. Qi et al. (2015) also reported that at least the 81% of Sr was distributed in the aboveground parts (leaves plus straw) of oat plants. This is because the biomass of the shoots was higher than the roots.

Obviously, Sr concentration in roots, stems, and leaves elevated linearly with the increase of Sr-spiked concentration from 50 to 400 mg/kg. Linear formula is shown in Fig. 3 with all R^2 above 0.93 ($P < 0.01$), where, y means Sr level in the plant (mg Sr/kg dry weight) and x means the Sr-spiked concentration to soil (mg Sr/kg soil). Many references also reported that the pollutant such as cesium, zinc, and cadmium content in plants correlated well with the pollutant content in soil, such as oak plants (Qi et al. 2015). The ratio of Sr to calcium in the soil that increased with increasing soil Sr level could contribute to the more opportunity for plants to absorb Sr at higher concentration of Sr in soil (Qi et al. 2015). However, ^{90}Sr uptake in three plant species redroot pigweed (*Amaranthus retroflexus* L.), Indian mustard (*B. juncea* (L.) Czern.), and tepary bean (*Phaseolus acutifolius* A. Gray) exhibited no apparent relationship to ^{90}Sr concentrations in the associated soil (Fuhrmann et al. 2002). Different Sr uptake mechanisms could play roles. Sr uptake mechanisms of a plant were complex even for the same plant species. The uptake of Sr^{2+} by Zea may, also depending on the external Sr^{2+} tested, result from at least two saturating (in the range 0.04–25 mM) and one nonsaturating (for Sr^{2+} content up to 100 mM) transport components (Moyen and Roblin 2010).

As shown in Table 1, in pot system, the TF values of *S. bicolor* for Sr accumulation were 1.10~1.29 under the Sr-spiked level of 50~400 mg/kg soil, which indicated that *S. bicolor* (L.) Moench basically owns the good ability to transfer Sr from roots to shoots, except under the pressure of the 100 mg Sr/kg soil with TF of 0.92 near to 1. BCF values were simultaneously estimated based on the spiked Sr in soil (BCFs) and the available Sr in soil extracted by Mehlich III extraction solution (BCFa) shown in Table 2. The spiked Sr in soil linearly related well with the available Sr in soil ($R^2 > 0.96$). The BCFs and BCFa vales were close to each other. Totally, the BCF values for all the Sr treatment was only 0.21~0.39, lower than 1, which implied that it is difficult for *S. bicolor* to accumulate Sr from soil. Similarly, the field experimental data showed a depressed radiostrontium accumulation by four grain crops like winter rye, wheat, spring barley, and oat grown at the heavily contaminated sites (Krouglov et al. 1997). The increased TF values may imply that *S. bicolor* prefers to transfer Sr from roots to shoots under the elevated Sr level pressure. In soil system (50, 200, and

Table 1 TF and BCF values (within each column, values followed by the same lowercase letter are not significantly different, $P > 0.05$)

Spiked Sr in soil (mg/kg soil)	TF (mean ± SD)	BCFs (mean ± SD)	Available Sr in soil (mg/kg)	BCFa (mean ± SD)
50	1.10 ± 0.07ab	0.36 ± 0.06b	42.0	0.39 ± 0.05b
100	0.92 ± 0.19a	0.24 ± 0.05a	71.9	0.25 ± 0.03a
200	1.29 ± 0.11b	0.26 ± 0.03a	151.8	0.26 ± 0.02a
400	1.26 ± 0.05b	0.22 ± 0.04a	242.2	0.21 ± 0.02a

400 mg Sr/kg soil), BCF decreased with the Sr level meant that *S. bicolor* tended to decrease the Sr accumulation in *S. bicolor* under higher Sr concentration, which may be a protection mechanism for *S. bicolor* under Sr stress. Usually, a plant is believed to be a potential hyperaccumulator if its BCF and TF values for the metal were higher than 1. Here, with the BCF values less than 1, *S. bicolor* did not show the potential phytoextraction ability for Sr-contaminated soil. Thus, *S. bicolor* could be assumed as an excluder plant (i.e., metal in the roots < metal in soil) for Sr (Nadimi-Goki et al. 2014), similar to rice for the element of Li, Sn, and Tl. All the transfer factors from soil to plants (15 plant species) collected from a uranium mill tailings repository in South China were only 0.04~0.60 except for *Parthenocissus quinquefolia* (1.06) and *Cyperus iria* (1.10) (Li et al. 2011). The translocation factors (TFs) of Sr from root to leaf for rice grown in paddy field of NE Italy under the different rotations systems were reported to be 0.54~0.89; TFs of Sr from soil to root were also lower than 1 (0.4~0.9) in which the total Sr in soil reached 36.4~137.2 mg/kg (Nadimi-Goki et al. 2014).

Despite that the transfer from soil to root lower than 1, Sr translocation from root to shoot of *S. bicolor* was higher than 1. Thus, it appeared that the shoots of *S. bicolor* can be an efficient bioaccumulator plant for Sr, and it can be used in cleaning or rehabilitating of the Sr-contaminated soil and areas due to its high translocation factors. Sr is a moderately mobile element. It is reported that Sr is readily transported to the shoots with the xylem sap although the transport of Sr is greatly restricted in the phloem (Sasmaz and Sasmaz 2009). For the maize plants (*Zea mays* L. cv. "Liberal"), once taken up by the roots, part of the Sr^{2+} was translocated to the leaves where it affected the chlorophyll a/b ratio mainly by decreasing the chlorophyll a content (Moyen and Roblin 2010).

Under the elevation of the Sr-spiked level in soil from 50 to 400 mg/kg soil, TF values tended to increase, whereas BCF values tended to decrease except under the Sr pressure of 100 mg/kg soil. TF and BCF values roughly showed the opposite trend with Sr content in the environment. Similarly, the translocation of Sr^{2+} from solution to root of maize was inversely related to the external Sr^{2+} content in solution increased from 0.1 to 10 mM (Moyen and Roblin 2010). The transfer factor of Sr^{2+} from root to shoot of maize also increased with the increase of the external Sr^{2+} content in solution from 0.1 to 10 mM before the 24 h incubation time. However, during the incubation of 48~168 h, the transfer factor of Sr^{2+} from root to shoot of maize and the external Sr^{2+} exhibited more complex relationship (Moyen and Roblin 2010).

Sr uptake characteristics and mechanisms by plants depend on the plant species and soil properties such as organic matter content, pH, and ionic composition (Moyen and Roblin 2010). Despite that the familiarity of calcium and strontium has many effects on plants, Sr^{2+} could not simply be considered to mimic Ca^{2+}. Now there are not many details on Sr uptake by plants and more work is necessary in future (Moyen and Roblin 2010).

Community-level physiological profile analysis

The CLPP was tested by Biolog Eco-plate method to assess the potential metabolic diversity of soil microbial communities under the Sr-spiked level of 400 mg/kg. In each of the 96-μL wells in a Biolog Eco-plate, a single carbon compound (three replicate wells for each of 31 carbon sources and control well with no-carbon) and a redox dye (tetrazolium violet) reveal oxidative catabolism. After a specific incubation

Table 2 Diversity indices of soil microbial communities compared with 400 mg Cs/kg-spiked soil and control

Time (h)	48		72		96		120	
Index	Control	Sr	Control	Sr	Control	Sr	Control	Sr
AWCD	0.374 ± 0.040	0.482 ± 0.017*	0.652 ± 0.055	0.758 ± 0.053	0.813 ± 0.058	0.839 ± 0.177	0.916 ± 0.055	0.941 ± 0.188
H′	2.783 ± 0.148	2.887 ± 0.069	2.923 ± 0.089	3.086 ± 0.055	2.987 ± 0.057	3.134 ± 0.054*	3.096 ± 0.021	3.179 ± 0.040*
R	13.667 ± 2.517	18.000 ± 1.000*	18.000 ± 2.000	21.333 ± 1.155	20.667 ± 2.082	23.667 ± 0.577	23.667 ± 0.577	26.333 ± 1.528*
D	0.921 ± 0.011	0.935 ± 0.006	0.935 ± 0.006	0.948 ± 0.004*	0.941 ± 0.004	0.951 ± 0.003*	0.948 ± 0.002	0.954 ± 0.002*

* There is a significant difference ($P < 0.05$) between the treatment and control group

period, the plates varied in the overall extent of color development (expressed as average well color development, or AWCD). Figure 4 shows the calculated AWCD values reflecting the microbial community activity of the Sr-spiked soil with 400 mg/kg and the control sample without Sr addition. After 24 h, AWCD values obviously changed with the incubation time. AWCD for both two soils increased rapidly, basically obeying the logarithmic growth with $R^2 > 0.97$, indicating the high microbial metabolic activity during the 9 days' incubation time for two soil samples. AWCD of Sr-spiked sample increased more rapidly than the control sample, implying the higher microbial carbon source utilization ability and the higher microbial abundance (Classen et al. 2003). Under the sorghum phytoremediation, the existence of strontium favored the soil microbial activity.

Thirty-one carbon sources of a Biolog Eco-plate can be grouped as six different carbon sources: polymers, amino acids, amines, aromatic compound, carbohydrates, and carboxylic acids. The utilization of six different C-sources by the soil microbial community during 9 days is shown in Fig. 5.

The Sr-contaminated soil microbe roughly owned the higher optical density and higher utilization capacity in four kinds of carbon sources during the incubation time of about 0~120 h: polymers, amino acids, amines, and aromatic compounds, which are generally similar to the profile with the sum of AWCD values. However, Sr with the spiked level of 400 mg/kg obviously decreased the microbial community activity in carbohydrates metabolism. Carboxylic acid utilization capacity between Sr-contaminated soil and the control were near. Generally, Sr could promote the higher soil microbial diversity in metabolism of polymers, amino acids, amines, and aromatic compounds and could decrease the soil microbial activity in carbohydrates metabolism.

Here, three functional diversity indices, Shannon diversity (H′), Simpson (D), and the substrate richness (R) are calculated to reflect the functional diversity of soil microbial communities after 48, 72, 96, and 120 h of cultivation, shown in Table 2. There is no certain answer on which the incubation time point is selected to calculate the diversity indices for best assessment on the soil microbial activity. A 72- or 120-h time point is frequently selected to calculate the diversity indices, which is believed to allow the best discrimination between treatments (Wang et al. 2016a, b). The 54 h incubation time was used to perform the statistical analysis and be regarded as the shortest incubation time that allowed the best resolution among different soil samples (Gomez et al. 2004). However, longer incubation time such as 256 h was also reported to well distinguish the different treatment (Pessacq et al. 2015). According to Table 2, the values of AWCD, the substrate richness (R), Shannon diversity (H′), and Simpson (D) increased after Sr treatment, indicating that the Sr-spiked soil owned the higher microbial community diversity and activity in carbon utilization. This conclusion was consistent with the

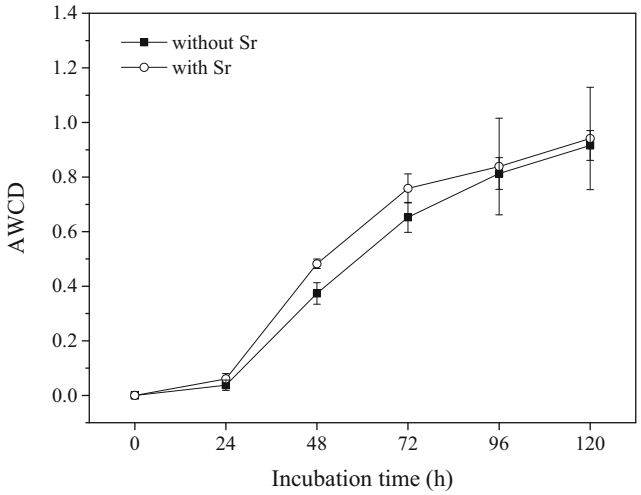

Fig. 4 The variation of average well color development (AWCD) for different treatments at different culture periods. The *bars* are the standard deviation of values ($n = 3$)

promotion effect of Sr addition to soil on the growth of *S. bicolor*. In combination of the analysis of six carbon utilizations, Sr-spiked soil microbes were more active in utilization of polymers, amino acids, amines, and aromatic compound.

The richness (R), Simpson (D), and Shannon diversity (H′) at 120 h were significantly higher for the Sr-treated soil than the control. It meant that Sr exerted obvious positive effects on soil microbial diversity and activity at community level. The result showed that the incubation time point of 120 h offered the best discrimination between Sr and the control. It is reasonably deduced that Sr at the spiked level of 400 mg/kg benefits the soil microbial community.

Soil pollutants can affect key microbial processes. The toxic metals such as cadmium and lead in soil were reported to inhibit the microbial activity and significantly change the soil microbial community structure particularly at a high level of contamination (Khan et al. 2010; Wang et al. 2007). Many studies also improved that heavy metals in low level promoted the activity of organisms. In fact, the increasing metal stress in soils may lead to an increase or decrease in microbial diversity, depending on many factors such as the initial state of the soil system, the type and concentration of metal, and the incubation time (Khan et al. 2010). The elevated DTPA-extractable Sr level in soil (50~350 mg/kg) was reported to increase the microbial biomass carbon (Margon et al. 2013). It is reported that contaminations with ^{137}Cs or ^{90}Sr up to 50-fold that of the hotspots occurring in Chernobyl caused minor changes in soil microbial functions (Niedree et al. 2013). Biolog assay results showed that AWCD of the microbial communities, community richness, and diversity indices were changed to some degree in the Sr-polluted soils and generally increased under Sr stress in this study. The element strontium here showed the beneficial but not harmful effects to organisms in soil although Sr is a nonessential element for life.

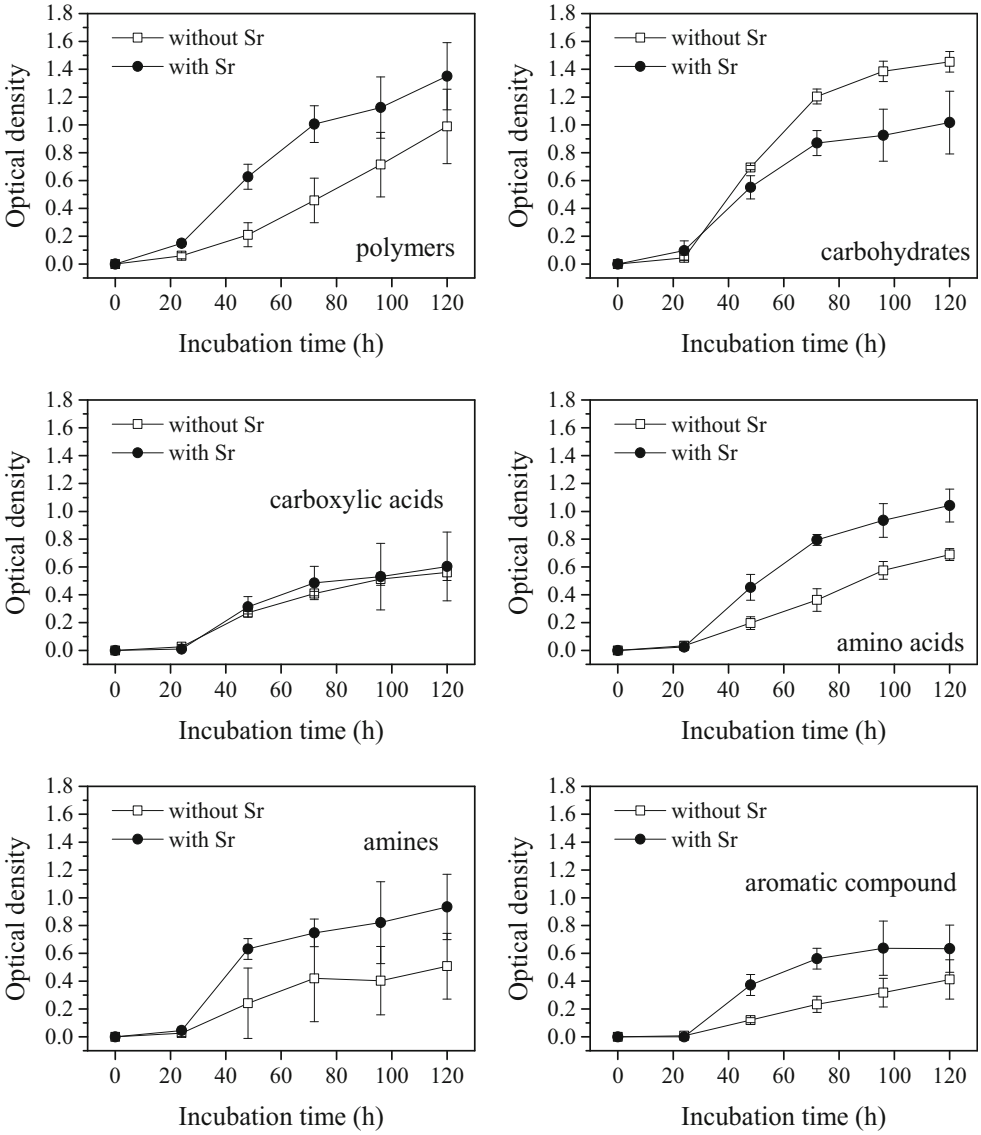

Fig. 5 The development of average optical density for each group of carbon sources. The *bar on the symbol* indicates standard error of values ($n = 3$)

Comparison of the CLPP results associated with the plant growth showed that the CLPPs were sensitive to distinguish the soil samples between Sr-treated sample and the control under *S. bicolor* phytoremediation assessed by Biolog method. However, both Sr treatment soil and CK showed that the high carbon source metabolism despite some functional diversity of soil microbial communities changed due to Sr pollution in this experiment, consistent with the good growth of *S. bicolor* under two soils.

Biolog Eco-plate method for assessment of soil microbial community also encountered some specific problems, such as the time duration required to observe a measurable response, high substrate concentrations, a restrictive indicator dye, and the presence of unknown proprietary ingredients, as well as severe selection (Ros et al. 2008; Lehman et al. 2013). Other improvements such as multi-SIR based on MicroResp™ (Chapman et al. 2007) or other traditional or molecular techniques were suggested (Khan et al. 2010; Wang et al. 2007). From our results, CLPPs based on incubations of soil suspensions assessed by BIOLOG method could still be used as a rapid and simple screening method, although other techniques were suggested to be accompanied for more precise community analysis.

Conclusions

Compared with the control plant sample after cultivated for 140 days, Sr in pot soil system could promote the growth of *S. bicolor* in the height and the shoot weight under Sr addition to soil at the level of 50~400 mg/kg. The available Sr in soil extracted by Mehlich III extraction solution linearly related well to the spiked Sr level in soil and reached 42.0~242.2 mg/kg ($R^2 = 0.96$). The total Sr level in the

roots, stems, and leaves of *S. bicolor* increased linearly with the increase of the Sr-spiked level in soil. For *S. bicolor*, the BCF values for Sr from soil to root under various Sr levels in soil were lower than 1. The TF values for Sr from root to shoot were usually higher than 1 except the Sr-spiked level of 100 mg/kg (TF was only 0.92 but also near to 1). Roughly, TF values increased and BCF values decreased when Sr concentration in soil increased, which is favorable for the plant to transfer Sr from roots to the aerial part of *S. bicolor*, showing that *S. bicolor* has the potential extraction ability for Sr-polluted soil.

The Sr-spiked level at 400 mg/kg soil increased the soil microbial diversity and activity at community level according to the CLPP analysis based on Biolog assay. As a whole, both Sr-spiked soil at 400 mg/kg soil and the control soil showed the high microbial activity and diversity. The three indices, richness (R), Simpson (D), and Shannon diversity (H′), showed the significant higher values for Sr-treated sample than the control at the incubation time of 120 h. Data at 120 h was suggested to be selected to perform the statistical analysis to allow the best resolution among different samples. CLPPs were sensitive to show the soil microbial community in Sr-treated soil.

Acknowledgements The research was supported by Program for Changjiang Scholars and Innovative Research Team in University (IRT-13026) and the National S&T Major Project (2013ZX06002001). The authors would also like to thank the financial support provided by the Open Research Fund Program of Key Laboratory of Advanced Reactor Engineering and Safety, Ministry of Education of China, and the National Natural Science Foundation of China (51338005).

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