Environ Sci Pollut Res (2015) 22:17151–17160
DOI 10.1007/s11356-015-4875-y

RESEARCH ARTICLE

Restoration of rare earth mine areas: organic amendments and phytoremediation

Lingyan Zhou[1] · Zhaolong Li[1] · Wen Liu[1] · Shenghong Liu[1] · Limin Zhang[1] · Liyan Zhong[1] · Ximei Luo[1] · Hong Liang[1]

Received: 31 March 2015 / Accepted: 9 June 2015 / Published online: 3 July 2015

Abstract Overexploitation of rare earth mine has caused serious desertification and various environmental issues, and ecological restoration of a mining area is an important concern in China. In this study, experiments involving dry grass landfilling, chicken manure broadcasting, and plant cultivation were carried out to reclaim a rare earth mine area located in Heping County, Guangdong Province, China. The prime focus was to improve soil quality in terms of nutrients, microbial community, enzyme activity, and physicochemical properties so as to reclaim the land. After 2 years of restoration, an increase of organic matter (OM), available potassium (K), available phosphorus (P) levels, and acid phosphatase (ACP) activity and a reduction of the available nitrogen (N) level and urease (URE) activity in soil were achieved compared to the original mined land. The nutrients and enzyme activities in soil with 5 years of restoration were close to or surpass those in the unexploited land as control. The bulk density, total porosity, water holding capacity, pH, and electrical conductivity (EC) of soil were improved, and the number of cultivable microorganisms and the bacterial diversity in soil were greatly increased with time during ecological restoration, especially for surface soil. Furthermore, the artificial vegetation stably grew at the restored mining sites. The results indicated that organic amendments and phytoremediation could ecologically restore the rare earth mining sites and the mined land could finally be planted as farmland.

Responsible editor: Zhihong Xu

✉ Hong Liang
lhofice@163.com

[1] College of Life Sciences, Zhongkai University of Agriculture and Engineering, Guangzhou 510225, China

Keywords Rare earth mine · Phytoremediation · Physicochemical properties · Microbial community · Ecological restoration

Introduction

Mining sites have eroded about 40,000 km^2 of land in China, and the abandoned mining land increases by 330 km^2 annually (Zou et al. 2012). The discarded lands usually have unfavorable soil properties, such as lack of nutrients (N, P, K); contain almost no organic matter; and have high metal toxicity, poor physical structure, and extreme pH (Ye et al. 2002; Li 2006; Rotkittikhun et al. 2006; Li et al. 2007; Mendez et al. 2007; Rosario et al. 2007; Mendez and Maier 2008). As a result, most of mine tailings are devoid of vegetative cover and just have a stressed heterotrophic microbial community (Moynahan et al. 2002; Mendez et al. 2007). Furthermore, the spread of fine particles often causes heavy metal pollution of soil and water, which poses severe health risks to humans (Wang et al. 2008).

Economically, there is an urgency to revegetate mine wasteland to improve the environment and increase available land resources. Phytoremediation is a novel, environmental-friendly, and cost-effective alternative to current remediation technologies that uses various plants to degrade, extract, contain, or immobilize contaminants from contaminated soil or water for improvement of the damaged soil ecology (Raskin et al. 1997; Berti and Cunningham 2000; Itanna and Coulman 2003; Mendez and Maier 2008; Ali et al. 2013; Mani and Kumar 2014). Phytoremediation was widely used in the restoration of mine tailings (Seenivasan et al. 2014; Canha et al. 2010; Stojanović et al. 2012; Liu et al. 2014; Mani and Kumar 2014; Marrugo-Negrete et al. 2015). The selection of appropriate plant species is a key step to successful revegetation (Stiles et al. 2011). Plants, which are endemic to mining areas, were generally thought to be

the best choice (Madejón et al. 2003). However, some introduced plant species failed in land reclamation (Martínez-Ruiz et al. 2007). The native plants are often better in terms of survival, growth, and reproduction in mining sites than introduced plants due to ecological adaption to the local climate (Yoon et al. 2006). Moreover, application of native plants to phytoremediation could avoid introduction of potentially invasive plant species (Mendez and Maier 2008; Boukhris et al. 2015). The physicochemical properties of mine wasteland are another limiting factor for successful revegetation. The establishment of plants in mine tailings always requires compost or nutrient amendments such as woodchips, composted green waste, or manure (Munshower 1994; Piha et al. 1995; Ernst 2005; Mendez et al. 2007). These amendments generally improve soil structure, water holding capacity, and nutrient contents which favor plant growth (Munshower 1994; Tordoff et al. 2000).

There are rich resources of rare earth elements in the mountain area in the North Guangdong Province, China, where many districts rich in weathering shell ion rare earth elements were found. The rare earth mine has been exploited for decades because of rising price and market demand uptrend. Overexploitation of the rare earth mine has resulted in a series of environmental issues including land aridity, water and soil erosion, headwater pollution, and downstream farmland damage. In the mined land, the soil organic poverty, soil desertification, soil water content drop, soil acidity, soil salinization, and heavy metal pollution made plants hardly grow (Wang and Liu 2008; Liang 2009). Therefore, development of economical and efficient technologies for remediation of soil is an urgent need. However, no remediation experiment and suitable plant species for phytoremediation of rare earth mine site have been reported so far. In the present work, organic amendments and different plant configurations including native plants were applied to the phytoremediation of the abandoned mining area, and the pedological characteristics and microbial communities were investigated as the groundwork for ecological restoration of this area.

The objectives of this work were to (1) search for a simple and effective method to improve the exploited rare earth mine soil and reclaim the land, (2) evaluate change of soil physicochemical properties and microbial communities during soil ecological restoration, and (3) find plant species with high potential of phytoremediation which could be planted in the future at mined rare earth mine sites for rehabilitation. Hopefully, the data will provide reference values for phytorestoration of similar mined lands in China.

Materials and methods

Study area

The experimental site at 815# Rare Earth Mine (24° 35′ N, 114° 81′ E) is located in Heping County, Guangdong Province, China (Fig. 1). It has a subtropical monsoon climate with an average annual temperature 17.9~20.2 °C, average annual rainfall 1536~1845 mm, duration of sunshine 1704 h, and frost-free period 267~301 days. The mine land has been abandoned since 2003 when the mining operation ended. The 815# Rare Earth Mine area before exploitation was hillside land where thickset herbal vegetation with a few scattered pine, China fir, and shrubbery grew. After exploitation, the mined land has become exposed hillside and sandy soil landfill. Thus, plants can hardly grow in the mined land because of soil damage and land waste (Fig. 1a).

Soil amendment experiment

The mined land was leveled, and the soil was spiked with 600 g /m^2 dry grass (water content of 12 %) in a cave 30 cm deep and broadcasted with 500 g/m^2 chicken manure (water content of 33 %) at the soil surface. Seedlings of *Stylosanthes scabra* Vog were planted in a space of 0.5 m×1.0 m in mid-April of 2009. Seedlings of *Medicago sativa* L. were intercropped in a space of 1.5 m×3.0 m in mid-June of 2009. Seedlings of *S. scabra* Vog were planted again in a space of 0.5 m×1.0 m in mid-April 2010. Seeds of *Zea mays* Zhongnuo 2# were sown in 0.4 m× 1.2 m on March 20, 2011 and sown again on June 20, 2011, and the grown corns could flower and bear seeds normally. Seedlings of *Vetiveria zizanioides* Nash were planted, and seeds of *Digitaria sanguinalis* Scop, *Tephrosia candida* DC, and *Cajanus cajan* Millsp were sown at the slope in late March of 2009 and late March of 2010 (0.5 g /m^2 each species), respectively. There were other weeds germinated from seeds in the manure and with the wind during phytoremediation. As a control, the seeds were sown and seedlings were planted the same way as on the mined land without filling grass and broadcasting fowl manure.

Preparation of soil samples

According to the soil investigation at the mined land, soil samples were collected from the original mined land, the restored mined land in different periods, and the nearby unexploited land as control (five repeats for each sample). The experimental site at the 815# Rare Earth Mine areas was 1000 m^2, where 500 g soils in the layer between 0 and 20, 20 and 40, and 40 and 60 cm, respectively, were sampled from a sampling point each 30 m^2 on standard sampling. The soil samples were placed in plastic bags (500 g/bag) and transferred to the laboratory for analysis of soil properties and microbial community. As the soil samples were quite heterogeneous, five repetitions of each treatment and control were well mixed respectively before used. There were four treatments, namely the original mined soil, mined soil with 2 years of restoration, mined soil with 5 years of restoration, and control (unexploited soil). All the soil samples were taken in mid-October each year.

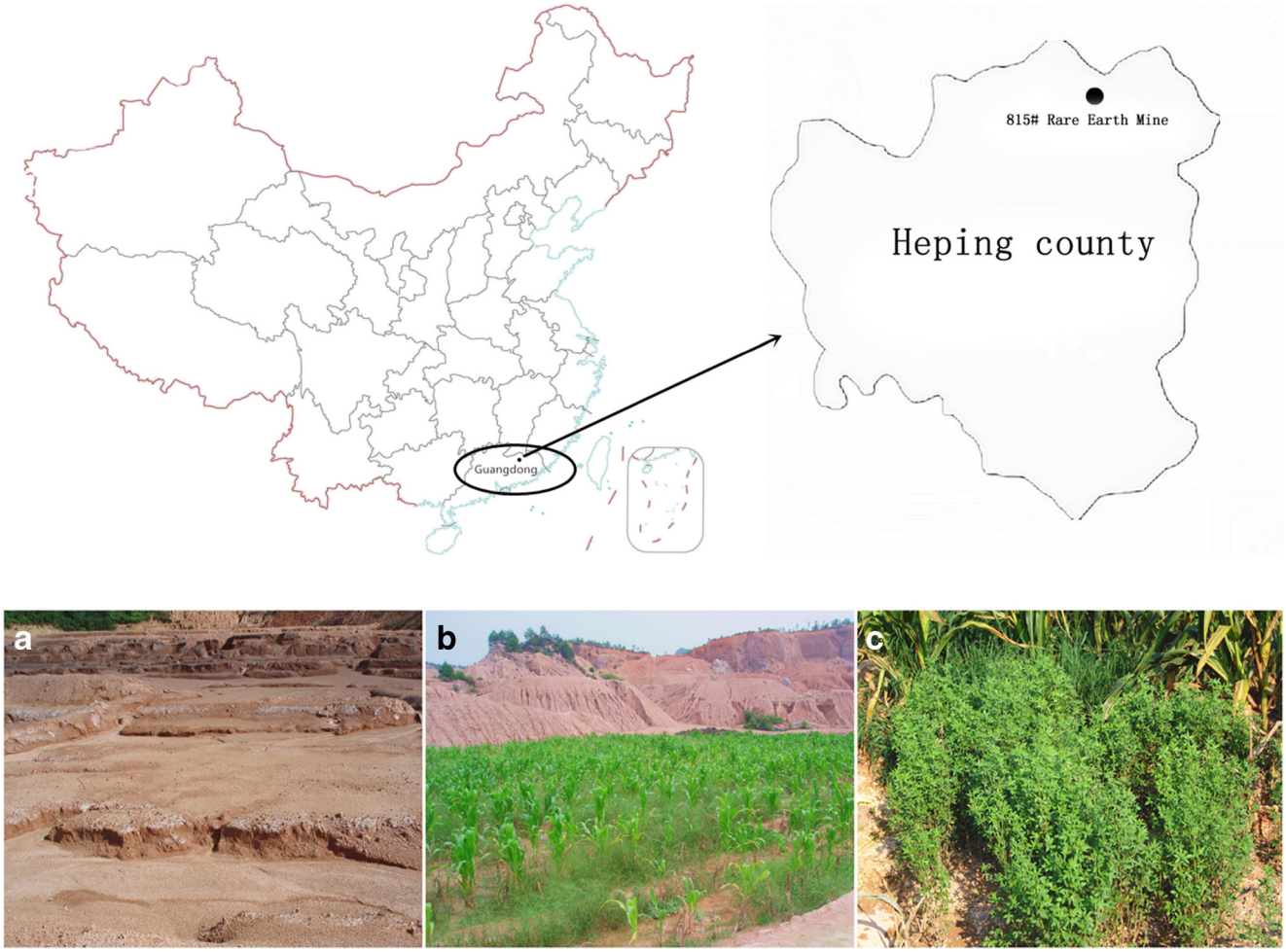

Fig. 1 The location map and ecological restoration condition of 815# Rare Earth Mine. **a** Original mined land, **b** the land with ecological restoration for 2 years, and **c** the land with ecological restoration for 5 years

Analysis of physicochemical properties of soil

The determinations of OM, available N, available P, and available K content were performed according to the protocols described by Lu (2000). Soil pH and electrical conductivity (EC) were measured in a 1:2.5 (*w*/*v*) aqueous solution using a pH meter (Sartotius PB-10, German) and EC meter (DDS-100, China). The water content, relative humidity, total porosity, and bulk density of the soil were determined and calculated according to the standard methods reported by Du and Gao (2006). Water holding capacity of soil was determined as follows: after heavy rain in late August, 100 g soil samples were placed into a 500-mL beaker and weighed daily for 7 days in succession under open-air drying condition at ambient temperature. The soil water holding capacity was estimated according to the water loss, in which lower water loss means higher water holding capacity.

Results of those 11 physicochemical properties obtained were subjected to principal component analysis (PCA) using the SPSS 17.0 software, and the PCA distribution diagram was drawn by the first and second PCA scores.

Assay of microbial activities of soil

Urease activity (URE) was measured according to the method described by Kızılkaya and Bayraklı (2005). Briefly, 5 g soil samples were mixed with 1 mL toluene, vortexed for 15 min, and then added with 10 mL of 10 % urea substrate solution and 20 mL citrate buffer (pH 6.7). The soil samples were filtered after 24 h incubation at 37 °C, and 1 mL of filtrate was diluted to 20 mL with deionized water; thereafter, 4 mL of sodium phenolate and 3 mL of sodium hypochloride solution were added. The formation of ammonium was determined using a spectrophotometer (UV 757 CRT, China) at 578 nm, and results were expressed as milligrams of N per kilogram soil. Ammonium concentrations were determined using a calibration curve of ammonium sulfate standard solutions. All the enzyme assays were performed using the air-dried soil samples in triplicate along with one control without soil. The substrate was added to blanks after the reaction stopped and before filtration of the soil suspensions.

Acid phosphatase (ACP) activities were measured according to the method previously reported by Dick et al. (1996). The intensity of the yellow color of the filtrate due to *p*-nitrophenol was determined using a spectrophotometer (UV 757 CRT, China) at a wavelength of 410 nm, and the results were expressed as milligrams of *p*-nitrophenol per kilogram of dry soil sample.

Plate counting of cultivable colonies

Plate counting of cultivable heterotrophic bacteria, actinomycetes, and fungi was performed basically following the procedure described by Zuberer (1994) and Zhang and Guo (2007). Briefly, 1.0 g soil samples were put into in a 15-mL centrifuge tube followed by the addition of 10 mL sterile distilled water and shaken for 30 min at 250 rpm, and 10- to 300-fold dilutions from different soil extracts were prepared in centrifuge tubes. Cultivable bacteria were numerated using plate counts made on Luria-Bertani (LB) medium. Fungi were numerated using plate counts made on potato dextrose agar (PDA) medium amended with 30 mg/L streptomycin sulfate, while actinomycetes were numerated using plate counts made on Gause's No. 1 medium containing 20 g/L soluble starch, 1 g/L KNO_3, 0.5 g/L $K_2HPO_3{\cdot}3H_2O$, 0.5 g/L NaCl, 0.5 g/L $MgSO_4{\cdot}7H_2O$, 0.01 g/L $FeSO_4{\cdot}7H_2O$, and 20 g/L agar. The plates were inoculated with 100 μL of soil suspension. The plates for bacteria counts were incubated at 30 °C for 36 h, and fungi and actinomycetes were cultured in an incubator at 25 °C for 3 days. The respective media without soil suspension were also used as control plates to check any possible contamination. The colony forming units (cfu) per gram of a fresh soil sample was then calculated. The plate incubation and counting was done in triplicate of each sample.

Bacterial DNA diversity assay

Soil microbial community DNA was extracted from 0.25 g of fresh soil by a bead-beating procedure using PowerSoil® DNA Isolation Kit (MO Bio, USA) as described by the manufacturer. Four pairs of primers (Table 5 in the Appendix) were used in this study for sequence-related amplified polymorphism (SRAP) analysis according to Li and Quiros (2001). The PCR reaction mixture contained 2.5 μL of 10× buffer, 1 μL of dNTPs mix (2 mmol/L), 1 μL of DNA sample, 1 μL of forward primer (10 μmol/L), 1 μL of reverse primer (10 μmol/L), 0.4 μL of DNA polymerase (2.5 U/μL; Takara, Dalian, China), and 18.1 μL ddH_2O. The reaction procedure was as follows: 94 °C for 5 min, 5 cycles at 94 °C for 45 s, 35 °C for 1 min, 72 °C for 90 s, 30 cycles at 94 °C for 45 s, 50 °C for 1 min, 72 °C for 90 s, and then 72 °C for 10 min.

The amplification products were analyzed by electrophoresis in 20 % (*w*/*v*) polyacrylamide gel with 0.12 % (*w*/*v*) urea and detected by silver staining. The gel was data-processed according to the location of DNA bands. Data was scored as the presence (1) and absence (0) for loci with a clear and repeatable band and loci without band, respectively. The polymorphism information content (PIC) was calculated by the Little Program Procedure according to the equation: $PIC=1-\Sigma (Pi)^2$ (Botstein et al. 1980).

Classification of the cultivable bacteria

Thirty random bacterial colonies from each treatment and the control were respectively inoculated in LB liquid media and extracted DNA according to Liu et al (2008). The 16S rDNA fragments amplified with primer pair 16S-F and 16S-R (Table 5 in the Appendix) were sequenced by Invitrogen Co. (Shanghai, China). The DNA sequence data were processed and aligned by DNAMAN, the bacterial taxa to which each DNA sequence belongs were identified based on the NCBI GenBank (Li and Quiros 2001). The bacterial Venn relation diagram was drawn in http://bioinformatics.psb.ugent.be/webtools/Venn/.

Data processing and statistical analysis

The data presentation and treatment was processed with Microsoft Excel 2003, and the results were expressed as means±SD. A paired *t* test was used to check the differences between the control and mined soil with different phytoremediation time in the same soil layer with a significance defined at $p<0.05$.

Results and discussion

Vegetation restoration

In the phytoremediation process of mined rare earth mine sites, new vegetation was reconstructed, and there were 28 species of plants grown in the experimental site with 2 years of restoration and 42 species grown with 5 years of restoration, including 7 species of cultivated plants. Crops such as corn, forage grass, and some tree species could grow normally in the restored mined land (Fig. 1b, c). By contrast, sown seeds and transplanted seedlings in the leveled mined land without the addition of dry grass and chicken manure could hardly germinate and grow.

The selection of appropriate plant species was thought to be a key step to successful revegetation (Stiles et al. 2011). In this study, many plants including native plants were applied to phytoremediation, and herbs, trees, and shrubs were rationally deployed according to their own characteristics. The results showed that *M. sativa* L., *S. scabra* Vog, *V. zizanioides* Nash, *D. sanguinalis* Scop, *T. candida* DC, and *C. cajan* Millsp all exhibited a strong tolerance to stress and good growth ability, indicating that those plant species could be used to revegetate mined land and effective ecological restoration was achieved by different plant configurations. The native plants not only

Table 1 The change of soil chemical properties during ecological restoration

Soil sample		Organic matter (g kg^{-1})	Available N (mg kg^{-1})	Available P (mg kg^{-1})	Available K (mg kg^{-1})	pH	Electrical conductivity (μS cm^{-1})
0~20 cm	Control	7.15±0.01b	253±25c	2.85±0.05c	64.95±3.5c	3.49±0.14a	19.0±4.03c
	Original mined soil	3.95±0.01d	543±20a	0.9±0.02d	38±2.65d	3.0±0.10c	195.3±2.96a
	2 Years of restoration	5.43±0.05c	227±11c	32.9±0.55a	161.1±3.37a	3.31±0.02b	37.7±3.51b
	5 Years of restoration	9.25±0.04a	367±10b	8.58±0.18b	98.9±1.58b	3.44±0.02ab	24.6±2.30c
20~40 cm	Control	11±0.1a	374±17b	2.83±0.01b	60.2±1.31b	3.77±0.05a	26.4±1.36c
	Original mined soil	2.92±0.05c	657±33a	0.78±0.03d	40±1.25d	3.06±0.02c	93.7±2.56a
	2 Years of restoration	2.62±0.02d	53±4d	1.18±0.03c	52±0.47c	3.11±0.03c	35.7±4.75b
	5 Years of restoration	5.26±0.15b	187±8c	13.1±0.18a	70.8±1.38a	3.42±0.03b	29.2±3.02bc
40~60 cm	Control	15.2±0.4a	475±30b	2.75±0.1a	59.3±0.55a	3.70±0.12a	16.0±1.71d
	Original mined soil	1.79±0.11d	925±20a	0.88±0.04b	41±0.77c	3.03±0.06c	105.2±6.01a
	2 Years of restoration	3.85±0.07b	277±9.1c	0.84±0.05b	42±1.03c	3.13±0.03c	64.7±0.86b
	5 Years of restoration	3.11±0.1c	166±10d	0.85±0.01b	45±0.59b	3.4±0.01b	32.3±2.38c

Different lowercase characters in a single column indicate significant difference ($p<0.05$) between the control and mined soils with different phytoremediation time in the same soil layer

ecologically adapted to the local climate but also avoid introduction of the potentially invasive plant species (Yoon et al. 2006; Mendez and Maier 2008; Boukhris et al. 2015). More and more mining wastelands were remediated by native plants (Yoon et al. 2006; Wang et al. 2008; Boukhris et al. 2015). Excitingly, the native plants *D. sanguinalis* Scop and its variant were found to be excellent plants for phytoremediation of mined land in terms of growth, reproduction, and tolerance to stress (Liu et al. 2015). In addition, those native plants exhibited an ability of heavy metal enrichment (Liu et al. 2015). The heavy metal levels in the mined land were effectively reduced during the phytoremediation process (Liu et al. 2015). In the subsequent phytoremediation, the native plants would gradually substitute for the introduced plants and reconstructed the plant community.

A multitude of remediation practices demonstrated that direct establishment of vegetation on barren mine lands is often difficult due to unfavorable edaphic conditions, especially high metal toxicity and extreme acidity, and the application of compost or organic amendments such as woodchips, composted green waste, or manure to improve the poor substrate conditions facilitates plant establishment and subsequent vegetation development (Munshower 1994; Ye et al. 1999; Ernst 2005; Mendez and Maier 2008; Renella et al. 2008; Yang et al. 2010). Plant community diversity can be different when soil physicochemical factors such as pH, cation exchange capacity, electrical conductivity, and metal content are different (Conesa et al. 2007). In this study, the species of grown plant increased during ecological restoration, suggesting that filling dry grass,

Table 2 The change of soil physical properties during ecological restoration

Soil sample		Bulk density	Total porosity (%)	Content of water (%)	Water holding capacity (%)	Relative humidity (%)
0~20 cm	Control	1.31±0.11a	50.44±2.01a	27.7±0.82a	29.7±0.93a	94.41±1.84a
	Original mined soil	1.33±0.18a	49.85±4.80a	18.22±0.61c	24.99±3.01b	71.06±3.80b
	2 Years of restoration	1.22±0.03a	53.83±2.23a	26.43±0.57b	28.38±1.23ab	93.18±1.23a
	5 Years of restoration	1.21±0.10a	54.23±4.50a	28.14±0.52a	30.13±1.32a	93.93±1.32a
20~40 cm	Control	1.32±0.08a	50.18±1.58a	27.63±1.35ab	32.14±0.62a	92.18±1.45a
	Original mined soil	1.31±0.05a	50.18±1.63a	25.94±0.67b	28.19±1.64ab	92.63±1.66a
	2 Years of restoration	1.29±0.09a	51.42±2.31a	25.3±1.72b	27.25±3.31b	92.84±3.31a
	5 Years of restoration	1.26±0.09a	52.57±4.85a	29.3±1.58a	31.8±3.57ab	92.49±3.83a
40~60 cm	Control	1.39±0.09a	47.66±1.38a	27.5±0.88a	29.11±0.31a	97.82±1.31a
	Original mined soil	1.32±0.04a	50.71±1.81a	27.5±0.62a	29.68±0.81a	96.02±1.81a
	2 Years of restoration	1.29±0.04a	51.46±4.72a	27.29±0.79a	28.53±0.97a	95.75±0.97a
	5 Years of restoration	1.28±0.15a	51.65±4.81a	28.31±0.89a	29.73±2.8a	95.16±2.88a

Different lowercase characters in a single column indicate significant difference ($p<0.05$) between the control and mined soils with different phytoremediation time in the same soil layer

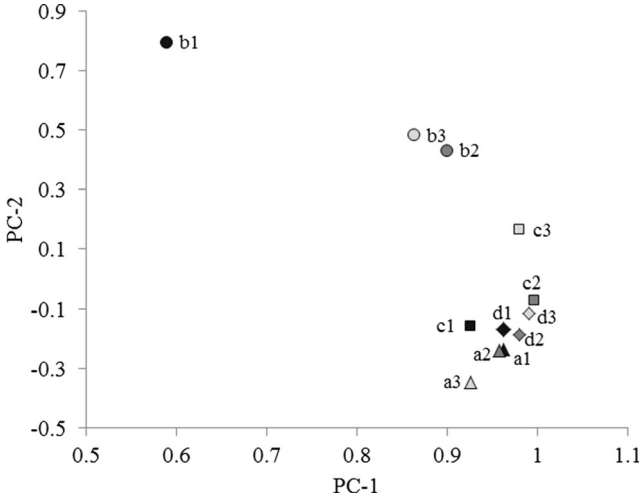

Fig. 2 Principal component analysis. *a1*, *a2*, *a3*: soils of 0~20, 20~40, and 40~60 cm depth in the control; *b1*, *b2*, *b3*: soils of 0~20, 20~40, and 40~60 cm depth in the original mined land; *c1*, *c2*, *c3*: soils of 0~20, 20~40, and 40~60 cm depth in the mined land with 2 years of restoration; *d1*, *d2*, *d3*: soils of 0~20, 20~40, and 40~60 cm depth in the mined land with 5 years of restoration

broadcasting chicken manure, cultivating plants, and introducing wild plants had ameliorated the mined soil and restored the soil ecosystem to some extent.

Amendments on soil physicochemical properties

The physicochemical analysis of the soils showed that the OM, available P, and available K levels in the original mined soil decreased significantly in contrast to the control. With the revegetation and organic amendments, the OM, available P, and available K levels in the reclaimed soil increased. However, the available N level increased in the original mined soil in contrast to the control (Table 1). It is speculated that exploitation of rare earth by ammonium oxalate extraction method resulted in a lot of residual ammonia nitrogen in the soil. With absorption by plants, the available N level decreased (Table 1). Compared to the control, the pH lowered and the EC increased in the original mined soil. Subsequently, the pH raised and the EC decreased during ecological restoration (Table 1), which was in accordance with the improvement of soil fertility. During the phytoremediation process, the bulk density decreased and the total porosity increased in all three soil layers. The natural moisture and water holding capacity increased year by year during ecological restoration, especially for surface soil (Table 2).

PCA with correlation matrix was attempted to understand the relationship between the changes in the physicochemical parameters of different phytoremediation time and soil layers (Fig. 2). The results showed that the difference of principal component between the original mined soil and control was significant, indicating that exploitation of mining impacted very obviously on the soil physicochemical properties, especially for surface soil. After 2 years of restoration, the differences of principal component from the control gradually decreased, especially for surface soil, suggesting that the consequence of restoration in top soil was better than in deep soil. After 5 years of restoration, the soil characteristics in the reclaimed soil were similar to those in the control, especially for surface and 20~40 cm depth soil, which showed that the soil was further restored by longer time phytoremediation.

Orientation and progress of ecological restoration in the damaged soil system were limited by the site climate, soil fertility, and water condition. Phytoremediation is a comprehensive technology for application of suitable combination of plants to rebuild vegetation on the basis of the site climate, soil fertility, and soil water condition. In this study, the phytoremediation which integrated soil leveling, grass filling, chicken manure broadcasting, and plant cultivation not only increased soil water holding capacity and fertility, but also improved the soil granular structure, water infiltration capacities, air permeability, and cation exchange capacity, which decided the stability and elasticity of the soil. These results were in accordance with the researches carried out in other environments (Pérez-de-Mora et al. 2007; Munshower 1994;

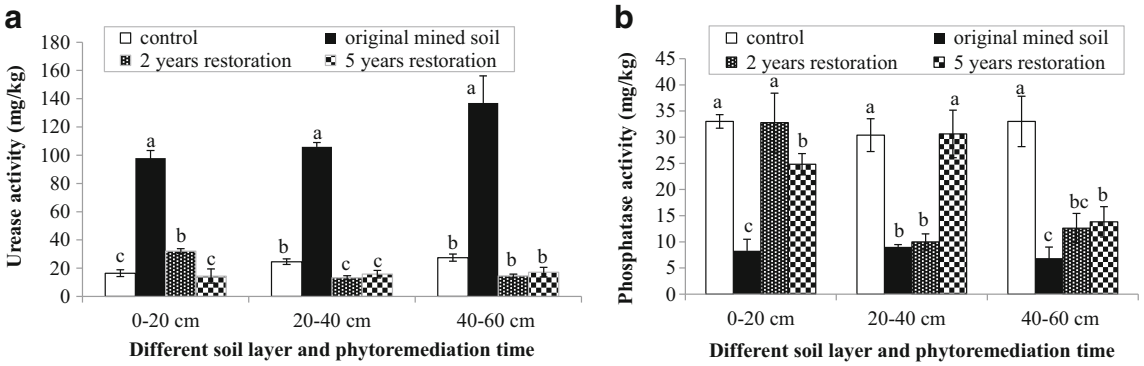

Fig. 3 The change of soil enzyme activity during ecological restoration. **a** Urease activity and **b** phosphatase activity. *Different lowercase characters* indicate significant difference ($p<0.05$) between the control and mined soils with different phytoremediation time in the same soil layer

Table 3 The change of soil microorganisms during ecological restoration

Soil sample		Culturable heterotrophic bacteria ($\times 10^3$ cfu/g)	Actinomycetes (cfu/g)	Fungi (cfu/g)
0~20 cm	Control	147.2±3.5b	250±4b	330.0±48b
	Original mined soil	4.0±0.3d	30±3c	3±1c
	2 Years of restoration	44.0±1.7c	159±3c	63±0.0c
	5 Years of restoration	1133.3±23.1a	1077±133a	2000±198a
20~40 cm	Control	85.0±2.0b	186±2b	73±11b
	Original mined soil	0.55±0.04c	10±1c	3±1c
	2 Years of restoration	32.1±3.5d	13±1c	50±10b
	5 Years of restoration	714.4±24.2a	628±9a	292±23a
40~60 cm	Control	66.4±2.5b	156±10a	25±60b
	Original mined soil	0.58±0.02d	0.0±0.0c	0.0±0.0c
	2 Years of restoration	24.3±3.8c	9±1c	22±3b
	5 Years of restoration	174.8±24.4a	73±6b	68±13a

Different lowercase characters in a single column indicate significant difference ($p<0.05$) between the control and mined soils with different phytoremediation time in the same soil layer

Tordoff et al. 2000; Ushio et al. 2010; Yang et al. 2010; Yu et al. 1996).

Soil microbial activities

ACP and URE activity analysis showed that the values of urease activity in the original mined soil were greatly increased compared to the control. With 5 years of ecological restoration, the values of urease activity were decreased and renewed near to those of the control (Fig. 3a). However, the values of ACP activity in the original mined soil were significantly decreased in contrast to the control and then increased with ecological restoration (Fig. 3b). Soil microbe is a driving force for substance transformation and nutrient cycle (Olsen et al. 1986). The microbial community physiologically links to ecosystem C and N cycling and balance (Melillo et al. 2002; Schimel et al. 2007). Soil enzyme activity, playing an important role in nutrient cycling and energy flows, reflects the strength and direction of biochemical processes in soil ecosystems (Yang and Wang 2004). Changes in microbial community composition had been well documented to modify ecosystem processes through their changes in physiological processes and, thus, could exert an important role in the functioning of terrestrial ecosystems (Malcolm et al. 2009; Compant et al. 2010). In this study, the enzyme activities are closely related to soil fertility.

Plate counting of cultivable colonies

The quantitative analysis of microbial population showed that exploitation of rare earth mine resulted in a severe decrease of cultivable colonies and ecological restoration increased greatly the cultivable colonies. For surface soil, the number of cultivable bacteria, actinomycetes, and fungi in the original mined soil decreased from 1.47×10^5 (the control) to 4.0×10^3, 250 to 30, and 333 to 30 cfu/g, respectively. However, the number in the mined soil with 2 years of restoration increased to 4.4×10^4, 159, and 63 cfu/g and in the mined soil with 5 years of restoration to 1.13×10^6, 1077, and 2000 cfu/g, respectively, which were much higher than those in the control. For all three soil layers, the deeper the soil layer was, the less the cultivable microorganism was (Table 3).

The soil microbe community structure could reflect soil quality and soil physicochemical performances (Chen et al. 2006; Amann et al. 1995). Fertilization, especially soil organic matter, plant growth, and plant community succession could constantly supply carbon source and other nutrient elements for microbial growth and affect the soil microbe community structure (Zhang and Pan 2010). In this study, as the soil ecosystem was damaged by the mining operation, the soil organic matter and soil fertility were almost lost so that the microbial population dramatically decreased (Table 3). With improvement of the mined soil in the phytoremediation

Table 4 The change of soil bacterial DNA diversity during ecological restoration

Soil sample	Amplified loci	Polymorphic loci	PIC value
Control	91	85	0.87
Original mined soil	85	81	0.87
Soil with 2 years of restoration	125	123	0.91
Soil with 5 years of restoration	156	154	0.93

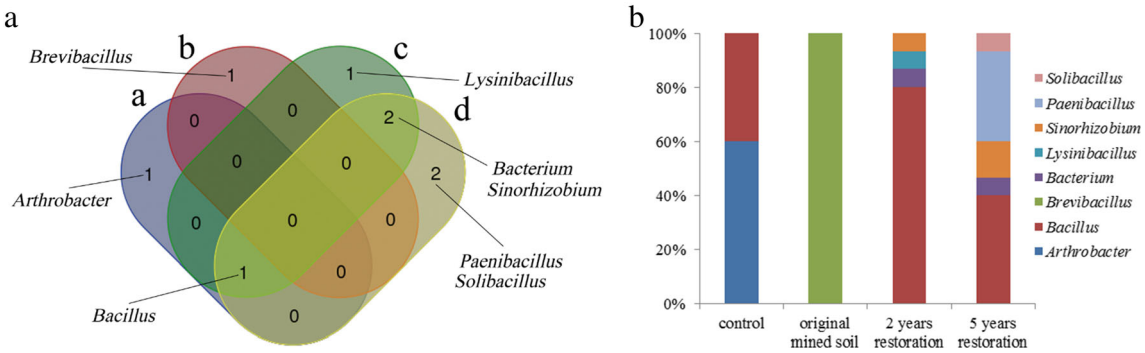

Fig. 4 Change of bacteria community during ecological restoration. **a** Venn relation graph of bacteria community during ecological restoration. **b** Dynamic change of bacteria genus during ecological restoration. *a* Control, *b* original mined soil, *c* mined soil with 2 years of restoration, and *d* mined soil with 5 years of restoration

process, the vegetation density and concentration of soil organic matter which increased the carbon source, nitrogen source, and other nutrient elements for soil microbe growth increased and made the number of microorganisms in the restored mined soil markedly increase. The results suggested that soil microbes were sensitive to the phytoremediation process, and the increase of the soil cultivable microorganism might reflect improvement of the soil ecological function, soil activity, soil organic matter, and soil fertility.

Assay of the soil bacteria diversity

Bacteria are the main components of soil microorganisms, and the plate counting of cultivable colonies showed that its change was significant in surface soil, so the bacterial diversity in surface soil was analyzed in this study. In total, 91, 85, 125, and 156 of DNA bands were amplified from bacterial DNA in the control, original mined soil, mined soil with 2 years of restoration, and mined soil with 5 years of restoration, respectively, of which, 85, 81, 123, and 154 loci existed polymorphism and the PIC was 0.87, 0.87, 0.91, and 0.93, respectively (Table 4). Sequence blast of cultivable bacteria showed that there were two genus in the control soil. These two genus disappeared and a new genus arose in the original mined soil. With the increase of vegetation species and organic amendment application during phytoremediation, one of the genus in the control reappeared and the number of genus increased greatly. The bacteria in the mined soil with 2 and 5 years of restoration belonged to four genera and five genera, respectively (Fig. 4a, b). Of these, dominant bacteria in the control belonged to *Arthrobacter* and *Bacillus*, and those in the original mined soil only were *Brevibacillus*. However, dominant bacteria in the soil with 2 years of restoration were *Bacillus* as well as in the control, and those in the soil with 5 years of phytoremediation belonged to *Bacillus* and *Paenibacillus* (Fig. 4b).

The results suggested that the changes of soil bacterial polymorphism and microbial community were closely related to the soil properties and vegetation type. The exploitation of the rare earth mine damaged the soil structure and changed the microbial community. Khan et al. (2010) found that non-tolerant microorganism species diminished, while tolerant species increased in abundance while exposed to a high concentration of heavy metals. Phytoremediation not only enhanced soil bacterial content but also increased the soil bacterial diversity (Zhang and Pan 2010; Chang and Chiu 2015; Massenssini et al. 2015). The enhancement in abundance is due to physiological adaptation, which may lead to replacement of more sensitive species (Briuns et al. 2000). Conversely, microbial activity would take part in soil organic decomposition and humus formation and make the soil release more nutrients for plant growth. The stressful tailings environment such as low pH, high metals, lack of soil structure, and normal heterotrophic microbial community resulted in low biomass production of plants grown (Ernst 2005; Audet and Charest 2007).

Conclusions

The rare earth mined land seriously lacks P, K, organic matter, and abundant microbial community, and plants can hardly grow in the mined land. By means of dry grass landfilling, organic fertilizer broadcasting, and plant cultivation in the phytoremediation process, the soil structure in the mined land was improved; the soil fertility obviously increased; the number of cultivable microorganisms and the bacterial diversity in soil greatly rose, especially for surface soil; and the artificial vegetation could stably grow at the restored mining sites. Therefore, phytoremediation could ecologically restore the exploited rare earth mine area, and the ecological restoration could be evaluated through analyzing the change of soil physicochemical properties and microbial community as well as the growth of cultivated plants.

Acknowledgments We thank Dr. Qing X. Li from the University of Hawaii at Manoa, USA, for his comments on this manuscript. This work was supported by Guangdong Provincial S & T Department under the Production-Education-Research Cooperation Project between Guangdong Province and the National Education Ministry of China (Nos. 2010B090400465 and 2012B090900010).

Appendix

Table 5 Sequences of the primers used in bacterial DNA diversity analysis

Primer name	Sequence (5′ to 3′)	Primer name	Sequence (5′ to 3′)
F1	TGAGTCCAAACCGGATA	R1	GACTGCGTACGAATTGAC
F2	TGAGTCCAAACCGGAGC	R2	GACTGCGTACGAATTTGA
F3	TGAGTCCAAACCGGAAG	R3	GACTGCGTACGAATTCAA
F4	TGAGTCCAAACCGGAAT	R4	GACTGCGTACGAATTAGC
16S-F	AGAGTTTGATCATGGCTCAG	16S-R	GGTACCTTGTTACGACTT

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