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Effect of Arsenic Pollution Extent on Microbial Community in Shimen Long-Term Arsenic-Contaminated Soil

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Abstract In order to investigate arsenic migration and transformation behavior under the action of microorganisms in Shimen long-term arsenic-contaminated soil under the condition of avoiding any influence of complicated soil environmental factors except increasing soil arsenic pollution degree, exogenous arsenic(III) or arsenic(V) stress experiments were carried out under the same experimental condition using the same soil sample. The changes of microbial community with exogenous arsenic concentrations and stress time were regularly monitored and comparatively analyzed. The soil microbial community shows extremely high diversities, and arsenic pollution degree affects microbial community composition rather than microbial diversity due to the long-term adaptation of microorganism to the arsenic-contaminated soil. *Acidiobacteria* and *Nitrospirae* play a key role in soil arsenic migration and transformation. Nitrospirae through producing NO_3^- takes part in the oxidation of As(III), and *Acidiobacteria* oxidizing sulfide minerals, as well as the adsorption and deposition of As(V), can enhance the soil acidity to promote soil arsenic migration and transformation, which can bring about the significant change of soil microbial community composition. Finally, its microbial community should tend to maintain a new pseudo-dynamic balance after a long time and a long-term arsenic-contaminated soil must be an arsenic oxidation-state soil. This work helps us understand why total arsenic, total organic carbon(TOC), NO_3^-, and pH are the key environmental factors that indirectly control the mobilization and release of arsenic via influencing the structures of the microbial communities in Shimen arsenic-contaminated soil.

Keywords Arsenic-contaminated soil · Microbial community · Arsenic migration and transformation · Exogenous arsenic stress

1 Introduction

Shimen realgar mine located in Hunan Province, China, is the largest realgar mine in Asia with a history of more than 1500 years (Liu 2014). This mine was shut down in 2011 due to serious pollution. A survey of the soil environmental quality by the Institute of Geographic Resources of the Chinese Academy of Sciences in 2012 showed that the over-standard rate of arsenic in the realgar ore area and its surrounding soil reached 66.1%, of which 17.9% of the soil samples was heavily polluted, 8.7% and 13.2% were moderately and mildly polluted, and the over-standard rate of arsenic in vegetables in and around Shimen realgar mine was as high as 40.43% (Tang et al. 2016; Yang et al. 2016, 2018). In October 2012, a comprehensive treatment of arsenic

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pollution soils began. Serious arsenic-contaminated soils were stripped and concentrated landfill, and then uncontaminated soil of a layer of 60 cm was covered, finally, shallow root green plants were planted on the remediation soil surface (Su et al. 2015; Liu et al. 2018; Wu et al. 2017a, b; Shukla and Srivastava 2017; Singh et al. 2015). However, in 2015, the Chinese Academy of Agricultural Sciences found the arsenic content of crops tended to rise and there was arsenic migration in the soil remediation area (Su et al. 2015; Liu et al. 2018; Wu et al. 2017a, b; Yang et al. 2016).

Migration and transformation of arsenic in realgar mining areas were reviewed by Wu et al. (2017a, b). It depends on its existent forms and valences, which in turn are related to the soil biological and chemical environment (Yan et al. 2017; Wang et al. 2018; Zeng et al. 2016). Major factors influencing soil arsenic migration and transformation include pH, Eh (Huang et al. 2011), redox actions (Yamamura et al. 2014; Qiu et al. 2017; Zeng et al. 2016, 2018; Li et al. 2017; Kawa et al. 2019; Hu et al. 2019), arsenic-bound forms (Hu et al. 2015; Wan et al. 2017; Meng et al. 2017; Fan et al. 2018) as well as arsenic adsorption-desorption ability (Pan and Zhu 2013; Yan et al. 2017) and arsenic bioavailability (Kumarathilaka et al. 2018; Sarun et al. 2018).

The impact of soil microorganisms on the migration and transformation of arsenic in soil is very complex. Many environment factors affect microbial community structure and its function in heavy metal or arsenic-contaminated soils (Phan et al. 2019; Sandip et al. 2018; Song et al. 2009; Seulki et al. 2019). Most of study works have focused on the relationship between microbial community and the soil environment factors, including various heavy-metal contaminated and physicochemical property soils (Avidano et al. 2005; Angel et al. 2011; Li et al. 2017; Sherlyn et al. 2018), but these results were extremely variable. It has been proved that the soil microbial community structure and its diversity are determined to a great extent by the soil type as well as the ecosystem type (Sherlyn et al. 2018; Seulki et al. 2019). However, it is still difficult for us to understand clearly the effect of microbial interactions on arsenic migration behavior in the arsenic-contaminated soil (Wu et al. 2016) because the energy or nutrient cycles between soil microbes are still unknown (Seulki et al. 2019) and there are many environment factors of different soil types as well as the ecosystems. Arsenic migration and transformation in arsenic-contaminated soil also should be a trace and slow process under some chemical and biological actions.

Recently, Chen et al. (2020) collected 24 soil samples from the representative points around the abandoned Shimen realgar mine to investigate their microbial communities and diversities, and thought that total arsenic, total organic carbon (TOC), NO_3^-, and pH were the key environmental factors, which indirectly controlled the mobilization and release of arsenic via influencing the structures of the microbial communities in the soils.

The aim of this study was to investigate arsenic migration and transformation behavior under the action of microorganisms in Shimen long-term arsenic-contaminated soil under the condition of avoiding any influence of complicated soil environmental factors except increasing the degree of soil arsenic pollution. Exogenous arsenic(III) or arsenic(V) stress experiments were carried out under the same experimental condition using the same soil sample. The changes in soil microbial communities with exogenous arsenic concentration and stress time have been monitored continuously and regularly for a long time through high-throughput Illumina sequencing technology.

2 Materials and Methods

2.1 Arsenic-Contaminated Soil

An arsenic-contaminated soil was taken from the surface soil of a farmland around realgar mining area in Shimen County, Hunan Province, China. The parent material of the soil is slate shale. The collected soil was screened by 2-mm sieving to remove the larger stones and other impurities. This soil was used for the following experiments. The physicochemical properties of this soil were analyzed, as shown in Table 1.

2.2 Exogenous Arsenic Stress Experiment

Each experimental sample of this arsenic-contaminated soil was 300 g, added 0 ppb (that is, without exogenous arsenic stress), 50 ppb, 100 ppb, and 200 ppb of arsenic(III or V valence) in the form of $NaAsO_2$ or Na_3AsO_4, respectively. The soil water content in these soil samples was adjusted to 70% of the maximum field water capacity. The culture experiments of exogenous arsenic stress were carried out in a thermostat with a constant temperature of 25 °C. During the cultivation period, the pure water was supplemented regularly to keep the soil water content constant. The change

Table 1 Physicochemical properties of the experimental soil

pH	Organic matter g/kg	Total phosphorus g/kg	Total nitrogen g/kg	Total potassium g/kg
7.26	18.27	1.38	2.72	9.69
Available phosphorus mg/kg	Available nitrogen mg/kg	Available potassium mg/kg	Total arsenic mg/kg	Available arsenic mg/kg
14.93	72.75	107.2	268.7	80.6

of microbial communities in these experimental soil samples was monitored on the 7th, 15th, and 45th days, respectively. Each experiment sample was in triplicate.

2.3 Microbial Community Analysis

2.3.1 DNA Extraction

Community DNA was extracted from experimental soil sample (0.5 g) in the Institute of Environment and Sustainable Development in Agriculture, Chinese Academy of Agricultural Sciences, Beijing, using a FastDNA SPIN Kit for soil (MP Biomedicals, USA) in accordance with the manufacturer's protocol.

2.3.2 PCR Amplification, Sequencing, and Data Process

PCR amplification and sequencing of the 16s rRNA gene fragments were carried out as described by Li et al. (2017) in our laboratory, School of Minerals Processing and Engineering, Central South University, Changsha city, Hunan province. PCR was performed on Applied Biosystems 2720 Thermal Cycler using primer pair 515F (50-GTG CCA GCM GCCGCG GTA A-30) and 806R (50-GGA CTA CHV GGG TWT CTA AT-30) together with Illumina adapter sequences. The raw data of samples for Miseq paired-end sequencing was FASTQ data format. Data process and statistical analyses also were performed as described by Li et al. (2017) in our laboratory.

3 Result and Discussion

3.1 Alpha Diversity of Soil Microbial Community

Alpha diversity indexes of all experimental samples of the soil are shown in Table 2. Alpha diversity indexes including Shannon diversity index and Simpson diversity index did not show any significant difference. Observed OTU number and Chao1 index also were basically similar among all experimental samples. However, OTU number with culture time seemingly showed a slight downward trend. Exogenous arsenic also slightly reduced the richness index (Chao1) of soil bacteria. Relative abundance of microbial phyla with exogenous arsenic(III) or arsenic(V) concentration and stress time are shown in Figs. 1 and 2, respectively. The results indicated that exogenous arsenic significantly affects microbial community composition rather than microbial diversity.

Bacterial α-diversity based at the 97% similarity level. The indexes were calculated from OTU relative abundance of each replicate. The difference among experimental samples is not significant at $p < 0.05$ level.

3.2 Change of Soil Microbial Community with Culture Time

When there was not any exogenous arsenic stress, *Firmicutes*, *Actinobacteria*, and *Proteobacteria* were three dominant phyla in the soil sample, as shown in Fig. 1 and Table 2. Major rare phyla included *Thaumarchaeota*, *Bacteroidetes*, *Verrucomicrobia*, *Planctomycetes*, *Acidiobacteria*, *Chloroflex*, *Gemmatimonadetes*, *Nitrospirae*, *Norank*, and *Armatimonadetes*. *Thaumarchaeota* is an abundant and ubiquitous phylum, including a large number of uncultured *Archaea* in the middle temperature environment, which plays critical roles in the global nitrogen and carbon cycles (Reji and Francis 2020; Pinto et al. 2020). *Planctomycetes* (e.g., members in family *planctomycetaceae*) was found in slightly, moderately, and severely heavy-metal contaminated soils (Li et al. 2017). *Nitrospirae* is a key phylum in the process of nitrogen cycle and can oxidize NO_2^--N into NO_3^--N (Wu et al. 2017a, b; Oremland et al. 2002; Lin et al. 2018; Zhu et al. 2019). *Acidiobacteria* can oxidize

Table 2 Alpha-diversity indexes of all experimental samples of the soil

			Shannon index	Simpson index	Chao1 index	OTU
Control sample	0 ppb	7 days	6.02 ± 0.29a	0.99 ± 0.00a	2560 ± 498ab	1476 ± 264a
		15 days	6.07 ± 0.24a	0.99 ± 0.00a	2531 ± 526b	1445 ± 286a
		45 days	5.91 ± 0.31ab	0.99 ± 000a	2496 ± 478b	1428 ± 258b
Samples stressed by arsenic(III)	50 ppb	7 days	5.98 ± 0.25a	0.99 ± 0.00a	2489 ± 494ab	1486 ± 274a
		15 days	6..01 ± 0.30a	0.99 ± 0.00a	2471 ± 346b	1465 ± 256b
		45 days	5.85 ± 0.28ab	0.99 ± 0.00a	2439 ± 487b	1419 ± 314b
	100 ppb	7 days	5.96 ± 0.25a	0.99 ± 0.00a	2478 ± 484ab	1469 ± 285a
		15 days	5.87 ± 0.31a	0.99 ± 0.00a	2456 ± 476b	1435 ± 256ab
		45 days	5.95 ± 0.32ab	0.99 ± 0.00a	2416 ± 487b	1399 ± 338b
	200 ppb	7 days	5.94 ± 0.27a	0.99 ± 0.00a	2486 ± 394ab	1476 ± 274a
		15 days	5.92 ± 0.29ab	0.99 ± 0.00a	2421 ± 446b	1420 ± 326b
		45 days	6.02 ± 0.26ab	0.99 ± 0.00a	2389 ± 457b	1378 ± 353ab
Samples stressed by arsenic(V)	50 ppb	7 days	6.02 ± 0.29a	0.99 ± 0.00a	2518 ± 384ab	1481 ± 285a
		15 days	6.06 ± 0.24b	0.99 ± 0.00a	2506 ± 478b	1455 ± 226a
		45 days	5.98 ± 0.15b	0.99 ± 0.00b	2486 ± 417b	1431 ± 276b
	100 ppb	7 days	5.97 ± 0.29a	0.99 ± 0.00a	2509 ± 434ab	1486 ± 274a
		15 days	6.03 ± 0.29a	0.99 ± 0.00a	2487 ± 456b	1465 ± 235ab
		45 days	5.96 ± 0.29ab	0.99 ± 0.00a	2449 ± 425b	1429 ± 289b
	200 ppb	7 days	6.04 ± 0.14ab	0.99 ± 0.00a	2496 ± 384ab	1472 ± 285a
		15 days	5.92 ± 0.25ab	0.99 ± 0.00a	2483 ± 446b	1455 ± 256ab
		45 days	6.01 ± 0.21a	0.99 ± 0.00b	2456 ± 387b	1423 ± 298ab

sulfide minerals (Yan et al. 2017; Qiu et al. 2017; Kawa et al. 2019). In terms of relative abundance change of three dominant phyla, *Firmicutes* decreased, and *Proteobacteria* and *Actinobacteria* all increased with culture time. Major rare phyla also had some variation in abundance with culture time. The change of soil microbial community with culture time should be attributed to the difference between natural condition and

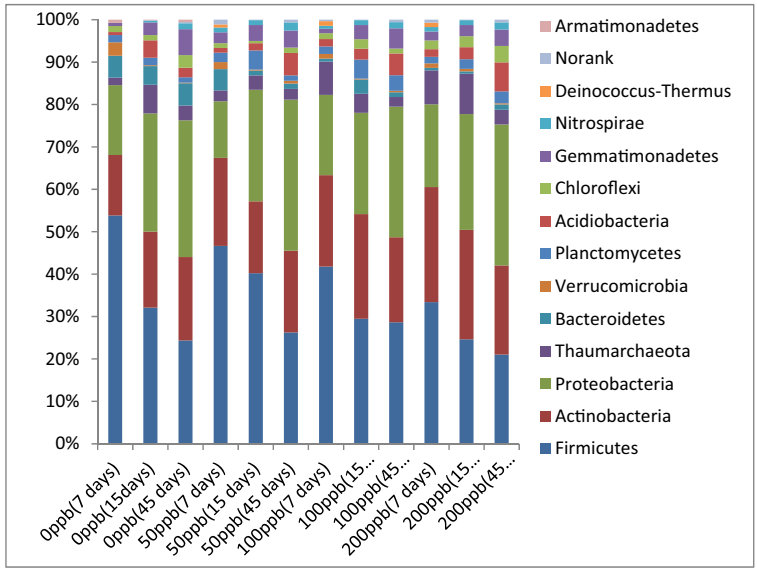

Fig. 1 The change of microbial community at Phyla level in Shimen arsenic-contaminated soil with initial arsenic(III) concentration and stress time

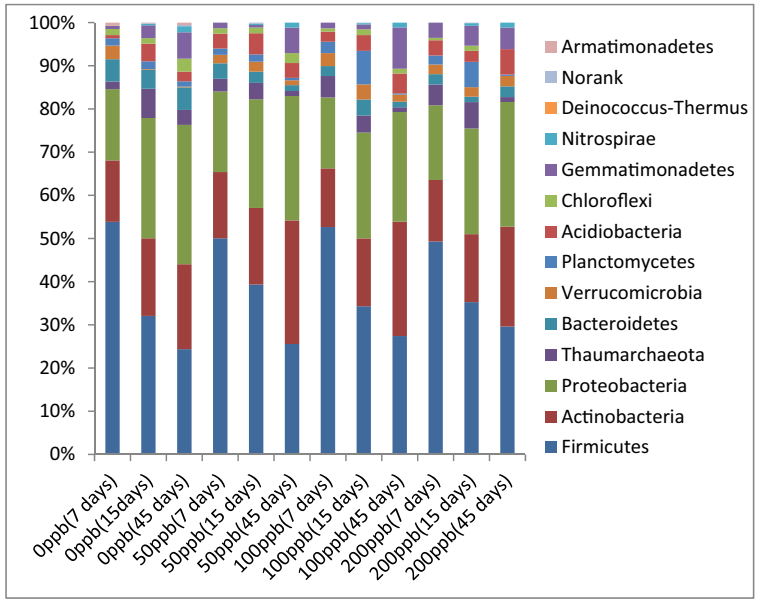

Fig. 2 The change of microbial community structure on Phyla level in Shimen arsenic-contaminated soil with initial arsenic(V) concentration and stress time

experimental condition of the soil and soil aging as well as the change of soil nutrient environment with time due to self-action of soil microorganisms. The result indicated that soil microbial community is very sensitive to the small change of soil physicochemical properties.

3.3 Effect of Arsenic(III) Stress on Soil Microbial Community

When there was exogenous arsenic(III) stress, three dominant phyla also were *Firmicutes*, *Actinobacteria*, and *Proteobacteria,* and the change of relative abundances of *Firmicutes* and *Proteobacteria* with stress time showed the same law as the situation of 0 ppb arsenic stress, as shown in Fig. 1 and Table 3. However, *Actinobacteria* seemed almost unchanged with stress time under the conditions of 50 ppb and 100 ppb exogenous As(III), and slowly decreased in the case of 200 pb exogenous As(III). The effect of exogenous As(III) concentration on three dominant phyla was more complex. *Firmicutes* decreased and *Actinobacteria* and *Proteobacteria* all increased with the increment of arsenic(III) concentration from 50 to 200 ppb on the 7th day. *Firmicutes* also decreased, but *Actinobacteria* just slowly increased and *Proteobacteria* was almost unchanged on the 15th day. The impact of arsenic(III) concentration on *Firmicutes*, *Actinobacteria*, and *Proteobacteria* became very small on the 45th day. As *Proteobacteria* has a stronger resistance to arsenic(III), its abundance increment should be attributed to the abundance reduction of *Firmicutes*. Therefore, exogenous arsenic(III) migrating into the long-term arsenic-contaminated soil has a great impact on native *Firmicutes*, but a little on native *Actinobacteria*, and the least on native *Proteobacteria*.

The effect of exogenous arsenic(III) concentration and stress time on major rare phyla was very complex. But some obvious change laws were found. (1) *Acidiobacteria* and *Nitrospirae* showed regular change. (2) There was phylum *Deinococcus-Thermus* obviously in the sample with exogenous arsenic(III) only on the 7th day. These experimental results indicated that a trace of exogenous arsenic(III) migrating into the arsenic-contaminated soil still could result in the change of soil microbial community composition even if the soil has been contaminated by the arsenic for a long time.

Deinococcus-Thermus was obviously detected on the 7th day and was not detected on the 15th and 45th days when there was exogenous arsenic(III) stress. It probably was caused by the easy oxidation of trivalent arsenic and its reaction exothermic. At the same time, this also implied that exogenous arsenic(III) has been completely oxidized into arsenic(V) after15 days.

Acidiobacteria was 0.740%, 1.11%, 1.72%, and 1.77% on the 7th day; 4.13%, 1.69%, 2.58%, and 2.84% on the 15th day; and 2.26%, 5.44%, 5.16%, and 6.86% on the 45th day when exogenous arsenic(III) concentration was 0 ppb, 50 ppb, 100 ppb, and 200 ppb,

Table 3 The abundance changes of three dominant phyla in Shimen arsenic-contaminated soil with initial arsenic(III) concentration and stress time

Bacterial phyla	Arsenic(III) concentration	Abundance on the 7th day, %	Abundance on the 15th day,%	Abundance on the 45 day,%
Firmicutes	0 ppb	53.9 ± 5.2	32.0 ± 5.4	24.5 ± 5.6
	50 ppb	47.8 ± 4.8	40.2 ± 4.8	26.3 ± 4.7
	100 ppb	41.8 ± 4.3	29.5 ± 4.5	28.6 ± 4.5
	200 ppb	34.1 ± 4.1	24.1 ± 4.6	20.0 ± 4.5
Actinobacteria	0 ppb	14.3 ± 3.2	17.8 ± 3.2	19.8 ± 3.5
	50 ppb	20.8 ± 3.4	16.9 ± 3.5	19.3 ± 3.4
	100 ppb	21.5 ± 3.9	24.6 ± 3.6	20.0 ± 3.8
	200 ppb	27.7 ± 4.0	25.2 ± 3.8	20.0 ± 3.9
Proteobacteria	0 ppb	16.5 ± 3.6	27.7 ± 3.5	32.4 ± 3.7
	50 ppb	13.3 ± 3.9	26.3 ± 3.8	35.6 ± 3.8
	100 ppb	19.0 ± 4.1	23.9 ± 3.9	30.8 ± 3.8
	200 ppb	19.9 ± 4.0	26.7 ± 3.8	31.5 ± 3.9

respectively. *Acidiobacteria* always increased with the increment of exogenous arsenic(III) concentration from 50 to 200 ppb. It indicated that a trace of exogenous arsenic(III) migrating into the soil should be low toxicity to *Acidiobacteria* due to its adaptability to the long-term arsenic-contaminated soil. However, *Acidiobacteria* were lower on the 15th day and very higher on the 45th day with exogenous arsenic(III) of different concentrations than without arsenic(III) (0 ppb stress). Here, exogenous arsenic was only variable because the same soil sample was used in all experiments. So the result should be only explained as follows: (1) The cumulative soluble arsenic(V) from the oxidized exogenous arsenic(III) probably inhibited *Acidiobacteria* oxidizing sulfide minerals on the 15th day; (2) complex adsorption and deposition behaviors of arsenic(V) in the soil have taken place due to excess soluble accumulation of arsenic(V) on the 45th day, which probably resulted in the enhancement of soil acidity to promote *Acidiobacteria* activity.

Nitrospirae was 0%, 1.11%, 1.11%, and 1.04% in relative abundance on the 7th day; 0.338%, 1.13%, 1.13%, and 1.14% on the 15th day; and 1.41%, 1.96%, 1.96%, and 1.67% on the 45th day when exogenous arsenic(III) concentration was 0 ppb, 50 ppb, 100 ppb, and 200 ppb, respectively. It was noted that *Nitrospirae* was not detected as one of main rare flora in the case of 0 ppb arsenic(III) stress but became one of main rare flora when there was exogenous arsenic(III) stress on the 7th day, and Nitrospirae always was higher with exogenous arsenic(III) stress than without arsenic(III) stress. This result implied that Nitrospirae should play an important role in arsenic(III) oxidation, at the same time, a trace of exogenous arsenic(III) could continually stimulate *Acidiobacteria* oxidizing sulfide minerals in the soil.

It was observed that relative abundances of three dominant phyla (Table 3) and most of major rare flora (Fig. 1) seemingly tended to be consistent on the 45th day whether there was a trace of exogenous arsenic(III) of different concentrations or not. Therefore, even if exogenous arsenic(III) migrating into the long-term arsenic-contaminated soil, exogenous arsenic(III) could be oxidized into arsenic(V) by soil microbes, finally, soil microbial community and diversity should tend to maintain a new pseudo-dynamic balance after a long time.

3.4 Effect of Arsenic(V) Stress on Soil Microbial Community

When there was exogenous arsenic(V) stress, three dominant phyla still were *Firmicutes*, *Actinobacteria*, and *Proteobacteria*. *Firmicutes* in abundance decreased and *Actinobacteria* and *Proteobacteria* increased with stress time, as shown in Fig. 2 and Table 4. The change law is very consistent with that without exogenous arsenic stress (0 ppb). Exogenous arsenic(V) concentration almost had no influence on native *Firmicutes* and *Actinobacteria* on the 7th and 15th days and on native *Proteobacteria* from the 7th day to the 45th day due to low toxicity of arsenic(V). However, *Firmicutes*

showed slowly uptrend but *Actinobacteria* showed slowly downtrend with the increment of exogenous arsenic(V) concentration from 50 to 200 ppb on the 45th day. It should be attributed to the adsorption and deposition of arsenic(V) in the soil.

The effect of exogenous arsenic(V) concentration and stress time on major rare phyla also is very complex. However, it also was found that the change of relative abundances of *Acidiobacteria* and *Nitrospirae* is the most regular among major rare phyla.

Acidiobacteria was 0.740%, 3.43%, 2.32%, and 3.52% in relative abundance on the 7th day; 4.13%, 4.91%, 3.69%, and 2.55% on the 15th day; and 2.26%, 3.43%, 4.60%, and 5.89% on the 45th day when exogenous arsenic(V) concentration was 0 ppb, 50 ppb,100 ppb, and 200 ppb, respectively. *Acidiobacteria* was higher with exogenous arsenic(V) stress than without exogenous arsenic(V) stress, and seemingly was not affected by exogenous arsenic(V) concentration on the 7th day. So a trace of exogenous arsenic(V) migrating into the soil also could stimulate *Acidiobacteria* activity but the chemical behavior of arsenic(V) in the soil was not affected at an early stage. *Acidiobacteria* decreased with the increment of exogenous arsenic(V) concentration on the 15th day, however, the relative abundance was 4.13% at 0 ppb, lower than 4.91% at 50 ppb, and higher than 3.69% and 2.55% at 100 ppb and 200 ppb respectively. This should be attributed to the inhibition of cumulatively soluble arsenic(V) to *Acidiobacteria* oxidizing sulfide minerals in the medium term. *Acidiobacteria* increased with the increment of exogenous arsenic(V) concentration on the 45th day, it probably should be related to complex adsorption and deposition behaviors of excess soluble arsenic(V) in the soil, which enhanced soil acidity to promote *Acidiobacteria* activity.

Nitrospirae was 0.338%, 0.340%, 0.332%, and 0.658% in relative abundance on the 15th day, and 1.41%, 1.14%, 1.04%, and 1.14% on the 45th day when exogenous arsenic(V) concentration was 0 ppb, 50 ppb, 100 ppb, and 200 ppb, respectively. However, it was found that *Nitrospirae* was not one of main rare flora on the 7th day whether there was exogenous arsenic(V) stress or not, but it was one of main rare flora on the 7th day when there was exogenous arsenic(III) stress. This further proves the fact that *Nitrospirae* plays an important role in arsenic(III) oxidation.

3.5 Discussion

Whether there was exogenous arsenic stress or not, our soil microbial community showed extremely high diversities due to the long-term adaptation of microorganisms to the arsenic-contaminated soil. It is consistent with previous results (Avidano et al. 2005; Angel et al. 2011; Chen et al. 2020; Seulki et al. 2019). However, our microbial community compositions apparently are different from microbial community compositions from the tailings of Shimen realgar mine (Zeng et al. 2016) and the representative points around the abandoned Shimen realgar mine (Chen et al. 2020; Chen et al. 2016) due to the difference between our soil sample and their soil samples. Therefore, it further indicates that the effect of soil physicochemical properties on soil microbial community structure is very remarkable.

Generally speaking, As(III) is more toxic compared with As(V). Arsenic(III) indeed had a great influence on *Firmicutes* and a little influence on *Actinobacteria*, and As (V) really had just a little influence on *Firmicutes* and *Actinobacteria*, and both As(III) and As(V) had the least influence on *Proteobacteria* due to its stronger arsenic resistance according to our experimental result. However, there were obviously different impacts on major rare flora between exogenous As(III) and As(V). Thus, the impact mechanism between As(III) and As(V) on soil microbes is different in some degree according to our experimental results. Angel et al. (2011) noticed that the proportion of *Firmicutes* increased as soil arsenic-contaminated levels increased. Our experimental result showed that *Firmicutes* slowly increased in abundance with the increment of arsenic(V) concentration on the 45th day (Table 4). Therefore, his result is consistent with our experimental result. The abundance of *Firmicutes* always showed obvious downward trend on the 7th and 15th days and approximate downward trend on the 45th day with the increment of arsenic(III) concentration in our experimental results (Table 3). From the results mentioned above, we conclude that a long-term arsenic-contaminated soil must be an arsenic oxidation-state soil.

Microbial community composition rather than microbial diversity was significantly affected by increasing soil arsenic concentration (arsenic pollution degree) in Shimen long-term arsenic-contaminated soil according to our experimental results. The result is consistent with Li's conclusion (Li et al. 2017; Shigeki et al. 2018). However, besides three dominant bacterial phyla

Table 4 The abundance changes of three dominant bacterial phyla in Shimen arsenic-contaminated soil with initial arsenic(V) concentration and stress time

Bacterial phyla	Arsenic(V) concentration	Abundance on the 7th day, %	Abundance on the 15th day,%	Abundance on the 45 day,%
Firmicutes	0 ppb	53.9 ± 5.2	32.0 ± 5.4	24.5 ± 5.6
	50 ppb	50.0 ± 4.9	39.3 ± 5.0	25.6 ± 4.8
	100 ppb	52.7 ± 5.1	34.3 ± 5.3	27.4 ± 4.7
	200 ppb	49.3 ± 5.0	35.3 ± 4.7	29.6 ± 4.8
Actinobacteria	0 ppb	14.3 ± 3.2	17.8 ± 3.2	19.8 ± 3.5
	50 ppb	15.4 ± 3.5	17.8 ± 3.3	28.6 ± 3.8
	100 ppb	13.6 ± 3.1	15.7 ± 3.5	26.5 ± 3.8
	200 ppb	14.2 ± 3.3	15.7 ± 3.4	23.1 ± 3.7
Proteobacteria	0 ppb	16.5 ± 3.6	27.7 ± 3.5	32.4 ± 3.7
	50 ppb	18.6 ± 3.5	25.1 ± 3.8	28.8 ± 3.9
	100 ppb	16.4 ± 3.6	24.5 ± 3.9	25.4 ± 4.0
	200 ppb	17.3 ± 3.7	24.5 ± 3.8	28.8 ± 3.8

Firmicutes, *Actinobacteria*, and *Proteobacteria*, the relative abundances of *Acidiobacteria* (*Acidithiobacillus* genus) and *Nitrospirae* showed the most regular change among major rare phyla. Although exogenous arsenic(III) or (V) migrating into the long-term arsenic-contaminated soil brought about the significant change of soil microbial community composition, finally, its microbial community should tend to maintain a new pseudo dynamic balance after a long time due to the oxidation of arsenic(III) and the adsorption and deposition of arsenic(V).

Chen et al. (2020) investigated the microbial communities and diversities of 24 soil samples from the representative points around the abandoned Shimen realgar mine. His results indicated that total arsenic, TOC, NO_3^-, and pH were the key environmental factors that indirectly controlled the mobilization and release of arsenic via influencing the structures of the microbial communities in his soil samples.

Acidiobacteria can oxidize sulfide minerals in the soil (Yan et al. 2017; Qiu et al. 2017;.Kawa et al. 2019; Shi et al. 2018), and As(III) can play the role of an electron donor in the reaction as follow:

$$H_2AsO_3^- + NO_3^- \rightarrow HAsO_4^{2-} + NO_2^- + H^+ \quad (1)$$

Nitrospirae was detected as one of main rare flora in the presence of exogenous arsenic(III) but not detected in the presence of both exogenous arsenic(V) and no exogenous arsenic on the 7th day. It proves that *Nitrospirae* takes part in the oxidation of As(III) (Wu et al. 2017a, b; Oremland et al. 2002; Lin et al. 2018; Zhu et al. 2019). *Nitrospirae* abundances all were slightly higher with exogenous As (III) of different concentrations and slightly lower with exogenous As(V) of different concentrations than without exogenous As (0 ppb As stress). The experiment result reveals the following two points. (1) *Nitrospirae* takes part in the oxidation of As (III); (2) soluble As(V) slightly inhibits *Nitrospirae* activity, that is, the reaction (1) according to chemical equilibrium theory.

As(V) from the exogenous arsenic(III) oxidized by *Acidiobacteria* and *Nitrospirae* and exogenous arsenic(V) added artificially all slightly inhibited *Acidiobacteria* activity to some extent in the medium term (on the 15th day). *Acidiobacteria* abundances all were higher with both exogenous As(III) and As(V) of different concentrations than without exogenous As (0 ppb As stress) at the later stage of cultivation (on the 45th day). As our experiment was carried out only through increasing exogenous arsenic concentration under the same experiment condition using the same soil sample, the effect of complicated soil physicochemical properties on the soil microbial community has been excluded. So the behaviors of exogenous As(III) and As(V) in the soil can only be interpreted as the accumulation of soluble arsenic(V) inhibits the reaction (1) according to chemical equilibrium theory, and then, final adsorption and deposition of excess soluble As(V) in the soil can enhance the soil acidity to promote soil *Acidiobacteria* activity. Thus, our experiment result not only proves his conclusion (Chen et al. 2020) but

also is very helpful for understanding why total arsenic, TOC, NO_3^-, and pH are the key environmental factors that indirectly control the mobilization and release of arsenic via influencing the structures of the microbial communities in Shimen arsenic-contaminated soil. TOC is related to the carbon sources for the soil microbial growth. NO_3^- and pH are related to some microbial oxidations such as *Acidiobacteria* and *Nitrospirae* as well as the adsorption and deposition behaviors of As(V) in the soil. The adsorption and deposition of As (V) are related to the binding capacity and ability of soil to arsenic. However, his total arsenic only can be understood as the sum of reactive, soluble, reversible adsorption and deposition type' arsenic, that is, the soil available arsenic according to our experimental results. Generally, soil available arsenic is positively relative to soil total arsenic.

4 Conclusion

(1) The soil microbial community shows extremely high diversities. Exogenous arsenic(III) and arsenic(V) all affect microbial community composition rather than microbial diversity due to the long-term adaptation of microorganism to the arsenic-contaminated soil.

(2) Besides the three dominant bacterial phyla *Firmicutes*, *Actinobacteria*, and *Proteobacteria*, relative abundances of *Acidiobacteria* and *Nitrospirae* show the most regular change with exogenous As(III) or As(V) concentration and stress time among major rare phyla. *Acidiobacteria* and *Nitrospirae* play a key role in soil arsenic migration and transformation. *Nitrospirae* through producing NO_3^- takes part in the oxidation of As(III) produced by *Acidiobacteria* oxidizing sulfide minerals. *Acidiobacteria* oxidizing sulfide minerals as well as the adsorption and deposition of As(V) in the soil can enhance the soil acidity, which can promote arsenic migration and transformation in Shimen long-term arsenic-contaminated soil.

(3) A long-term arsenic-contaminated soil must be an arsenic oxidation-state soil. Soil arsenic migration and transformation can bring about the significant change of soil microbial community composition. Finally, the soil microbial community should tend to maintain a new pseudo-dynamic balance after a long time.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

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