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Sr Isotope Tracing of Differences in the Effects of Microorganisms on Weathering of Calcite and Apatite

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To study differences in the effects of microorganisms on weathering of calcite and apatite, one strain of *Aspergillus niger* (*A. niger*) and one strain of *Penicillium glaucum* (*P. glaucum*), which respectively contain the mixture of calcite and apatite were cultivated for 24 days in the sucrose-potato culture medium, supernatant was taken every three days from the culture medium, followed by the determination of Ca^{2+} and Sr^{2+} contents and Sr isotopic ratios. The results of measurement showed that the Sr isotope ratios in the supernatant from the culture medium are intermediate between those of the end-member constituents calcite and apatite (0.70721-0.70861). Results of isotope mixing equations to calculation showed that in the first 15 days *A. niger* played a dominant role in weathering of calcite in the apatite/calcite mixture. The contribution rate of apatite for Ca^{2+} in the solution increased from 39.0% on the 18th day to 61.6% on the 24th day; *P. glaucum* played a key role in weathering of apatite in the first 3 days. Ions dissolved from apatite account for 73.9% of the total. It is known from the results of Sr isotope tracing that in the prior period of fungus cultivation *A. niger* plays a key role in weathering of calcite while *P. glaucum* plays a key role in weathering of apatite. The ability of *P. glaucum* to weather calcite tends to intensify progressively over time. Therefore, Sr isotope tracing can be used to accurately recognize differences in the effects of microorganisms on weathering of minerals.

Keywords microorganism, calcite, apatite, differential weathering, Sr isotope

INTRODUCTION

Interaction between microorganism and minerals, i.e., microbial weathering, is of frequent occurrence in nature. Microorganisms are directly involved in material cycling in nature (Ehrlich 1998; Lian et al. 2006). In microbial weathering, the affected minerals are eroded through attack by some metabolic products released into solution by the microbes, or by microbial oxidation or reduction of some dissolved rock components. Thus, microbes influence the rate of rock weathering (Ehrlich 1998; Lian et al. 2006; Uroz et al. 2009). Activity of microorganisms leads to destruction of silicates, phosphates, carbonates, oxides and sulfides, thus making some important elements (Ca, Mg, K, Na, Si, A1, Fe, Mn, etc.) in the minerals to go into solution (Newman 2010).

Soil microorganisms play an important role in the process of cycling of nutrient substances in the ecological system. Ectotrophic mycorrhiza fungi can assist host plants to more effectively absorbing and utilizing Ca, K, Mg, P and other nutrient elements from soil minerals (Arocena and Glowa 2000; Blum et al. 2002; Finlay et al. 2009; Martino and Perotto 2010; Uroz et al. 2009; Wallander et al. 2006). Microbial weathering of minerals may provide a source of abundant mineral nutrients for the soil ecological system (Blum et al. 2002; Gadd and Raven 2010).

Due to differences in crystal structure, different minerals display significant differences in resistance to weathering. At the same time, the effects of microorganisms on weathering of minerals also show certain differences (Blum et al. 2002; Ehrlich 1998). Differences in the effects of microorganisms on weathering of minerals form on the basis for a better understanding of the mechanism of involvement of microorganisms in weathering, and the assessment of the capability of microorganisms to weather minerals. X-ray powder diffraction analysis, electron microscopic observation, chemical analysis and mass equilibrium calculation are the main approaches to study the effects

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of microorganisms on the weathering of minerals (Blum et al. 2002; Ehrlich 1998; Smits et al. 2009).

However, when rock contains several different mineral components, more than one of which may be a nutritional source of a specific elements (e.g., Ca) for microbe, these analytical methods are less useful in determining how much of the specific element a microbe may derive by weathering from each of the different mineral components. Instead, isotopic Sr can be used as a tracer for qualitative and quantitative determination of the sources of Ca and other cations from a mineral mixture Sr release as a result of weathering (Åberg et al. 1989; Bain and Bacon 1994; Blum et al. 2000, 2002; Blum and Erel 1995; Bullen and Bailey 2005; Capo et al. 1998; Chadwick et al. 2009; Drouet et al. 2007; Miller et al. 1993; Shand et al. 2009; Wallander 2000; Wallander et al. 2006). The Sr isotope method can be used to assess the effect of exotrophic fungi on weathering of soil minerals in the rhizosphere, and to measure Sr release as a result of weathering (Bullen and Bailey 2005; Wallander 2000).

In the present report, the weathering activity on calcite and apatite by *A. niger* and *P. glaucum* is studied by applying the Sr isotopic tracing method. An attempt is made to model the extent of weathering by the fungi of the different minerals in a mineral mixture in a soil under natural conditions. Such a model will aid an assessment of ecological conditions in soil and in enhancing environmental protection.

MATERIALS AND METHODS

Experimental Materials

Mineral characteristics. Calcite—The samples were taken from the Devonian strata at Longli, Guizhou Province. The rocks are greyish-white in color and exhibit granular detritus and massive structures. The content of granular detritus is about 85% and that of fillings is about 15%.

The granulous detritus is dominated by oolitic grains of calcite, and a small amount of biodetritus can be seen; the fillings are composed mainly of calcite sparite cements. The samples for this experiment were acquired by monomineral selection from the rocks. The mineral composition obtained on the basis of X-ray diffraction analysis is: calcite 96.1%;dolomite 2.7% and amorphous material 1.2%.

Apatite—The samples were taken from phosphorite in the Sinian strata in the Kaiyang phosphorite mining district, Guizhou Province. The rocks are white in color, and exhibit granulous and massive structures. Mineral thin sections showed that apatite minerals are present in the form of elliptic aggregates. The grains are within the range of about 0.05–0.1 mm in size, and the cement is collophanite. The samples for this experiment were acquired by monomineral selection from the rocks. The mineral composition based on results from X-ray diffraction analysis is: apatite 97.3%; calcite 1.3%; and, amorphous material 1.4%.

The above-mentioned two kinds of mineral samples were crushed, and washed twice with distilled water to remove small particles as fine as 0.1–0.3 mm. They were dried at 105°C to constant weight for experimental use. The content of the chief mineral component of these two different mineral samples accounted for 95%, respectively, meeting the requirement of the experiments. Results of the whole-rock analysis for the mineral samples are listed in Table 1 (the analytical precision was better than 2%).

Fungal strain. Soil samples were taken from the rhizoshpere in the Kaiyang Phosphorite Mining District, Guizhou Province.

Experimental Design

To a 250 mL conical flask, 100 mL of sucrose-potato extract medium was added. sucrose-potato extract medium was prepared by boiling 200 g of peeled potatoes in 1000 mL for 20–30 min and the separating the liquid portion by filtering (ordinary quantitative filter paper was used). To the filtrate, 20 g of sucrose per liter were added and the resultant solution sterilized for 30 min at 121°C. After cooling, 1.5 g apatite and 1.5 g calcite were added. The apatite and calcite, ground to a particle size of 150μm, had been previously sterilized in hot air at 150°C for 3 h. The sterilized medium was inoculated with 1 mL of *P. glaucum* spore-suspension 10^5 mL^{-1}. The procedure used for testing *P. glaucum* was also used to test *A. niger*, except that the culture medium was inoculated with 1 mL of *A. inger* spore-suspension (10^5 mL^{-1}) instead of *P. glaucum* spore-suspension. Controls were run with the same medium except that the medium was left uninoculated, the inoculum with an equivalent volume of sterile suspension medium.

Triplicates were set for every each treatment as mentioned above. All flasks were incubated in a stationary mode at 25°C. At 3 days intervals the supernatant was taken from the flask. The solution was filtrated by means of a millipore filter (Whatman membrane, 0.22 um pore-size).

Sample Analysis

(1) Pre-treatment of mineral samples

Apatite and calcite samples (0.2 g each) were completely digested in a Teflon beaker (volume, 25 mL) with the reagent-grade $HClO_4$ and HNO_3 (1:4). The digest was adjusted to a volume of 20 mL with 2% HNO_3.

(2) pH of culture solution and the concentrations of Ca^{2+} and Sr^{2+} after different durations of incubation

pH of the culture solution was determined from the cultures and the control at 3-day intervals using a pH meter (A420 American Thermo Orion Company) with a precision of ±0.01 pH units equipped with a glass electrode. After the pH determination, each sample was filtered (Whatman filter membrane, 0.22 um pore-size). The partial filtrate was

TABLE 1
The chemical composition (%) of the samples used in this study

	CaO	MgO	K_2O	Na_2O	Fe_2O_3	Al_2O_3	SiO_2	P_2O_5	TiO_5	MnO_2	CO_2	Others
Calcite	53.69	1.29	0.06	0.17	0.22	0.16	0.57	0.32	0.53	0.05	42.91	0.15
Apatite	54.37	0.47	0.14	0.31	0.14	0.47	0.36	39.86	0.07	0.05	—	3.76

then diluted 10-fold in a 50 mL volumetric flask. Then, 5 mL of each diluted filtrate was then transferred to a 10 mL centrifuge tube (polypropyiene) followed by addition of one drop of concentrated sulfuric acid. The Ca^{2+} and Sr^{2+} concentrations were then determined by ICP-OES (American Varian Company. Vista MPX type) (relative standard deviation <1%).

(3) Determination of Ca^{2+} and Sr^{2+} uptake by fungal mycelium

Fungal mycelium was separated from the upper culture medium by inoculation needle, and fungal mycelium in supernatant was filtrated by Whatman glass fiber filter membrane (0.22 um pore-size), in the process, to avoid the mineral particles are brought into. Then, they were dried in an oven at 105° for constant weight. The dried mycelium was weighed and then ground in agate mortar to a powder as fine as 60 mesh. Of this powder, 0.100 g were ashed in a muffle oven at 550° for 3 h. the ash was then digested completely with a mixture of reagent-grade $HClO_4$ and HNO_3 (1:4) for determination of Ca^{2+} and Sr^{2+}. The digest was then diluted to 50 mL with 2% HNO_3 for subsequent determination of Ca^{2+} and Sr^{2+} concentrations.

(4) Sr isotope analysis

First, 10 mL of culture medium filtrate was taken and digested with twice-distilled $HClO_4$ and HNO_3. The digest was transferred to a Teflon crucible and evaporated to dryness. The residue in the crucible was separated, purified and collected on 200–400 mesh Dowex 50W×8 cation exchange resin column in HCl media (Brick 1986).The Sr isotope ratios ($^{87}Sr/^{86}Sr$) were measured on a multi-collector inductively coupled plasma mass spectrometer (MC-ICP-MS). The mean value of the NBS987 Sr standard over this period was 0.71025±0.00002 ($n = 24$).

(5) Calculation

Because of their geochemical similarities, Sr is often used as a proxy for Ca in ecosystem studies (Åberg et al. 1990; Capo et al. 1998). As long as the proportion of each cation in the source materials is known, Sr isotope ratios allow calculation of the proportion of Ca derived from each source. The proportion of Sr in a mixture (in this case, in supernatant) derived from two sources (apatite and calcite) is calculated using a two-component mixing equation (Capo et al. 1998):

$$x \cdot \Delta^{87}Sr_A + (1 - x) \cdot \Delta^{87}Sr_B = \Delta^{87}Sr_M$$

One obtains:

$$x = \frac{\Delta^{87}Sr_M - \Delta^{87}Sr_B}{\Delta^{87}Sr_A - \Delta^{87}Sr_B}$$

$$\Delta^{87}Sr = \left[\left(\frac{^{87}Sr}{^{87}Sr + {}^{86}Sr}\right)_{sample} - \left(\frac{^{87}Sr}{^{87}Sr + {}^{86}Sr}\right)_{standard}\right] \times 10^4$$

where X is the fraction of Sr in the mixture derived from source A, in this case the apatite (A). B denotes the other source that, in this case, is calcite. M stands for mixture component (A and B).

RESULTS AND DISCUSSION

Identification of Fungal Strain

A number of fungi with stronge phosphorite dissolving ability were isolated by a dilution-plate method using an inorganic phosphorus-containing culture medium, with phosphorite as the sole source of phosphorous. The fungal strains with the strongest phosphorite-dissolving ability were identified by colonies that had a zone of hydrolysis with the largest diameter around, them after incubation on the phosphorite-containing agar medium. By colony morphology and biochemical identification method to get the two fungi was *A. niger* and *P. glaucum*.

Variation Characteristics of pH of the Supernatant in the Culture Medium

The changes in pH produced by *A. niger* and *P. glaucum* during growth in their respective culture medium containing the mineral powder mixture and compared to pH changes in an uninoculated control are shown in Figure 1.

Results shown in Figure 1 indicate that in the process of weathering of mineral powder by *A. niger* and *P. glaucum*, the pH of culture solution tends to change with time. Comparing the differences in pH in the process of cultivation of two fungal strains, we can see that the pH value of culture solution in which *A. niger* was inserted initially showed a tendency of decreasing

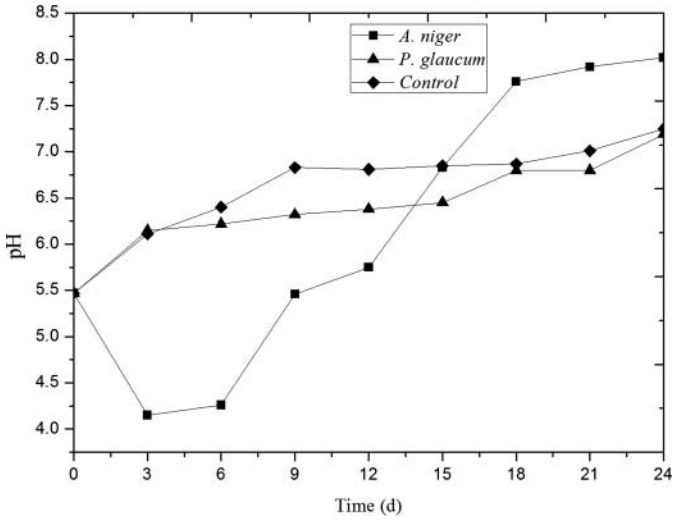

FIG. 1. Variation characteristics of pH of culture solution with time (values represent average of triplicates).

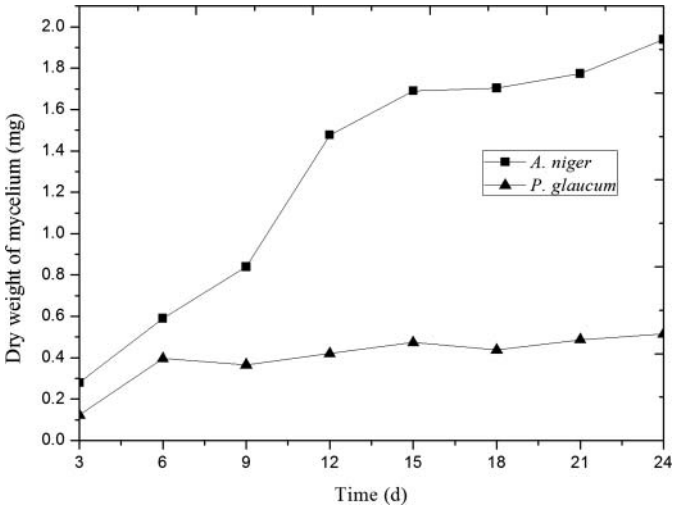

FIG. 2. Growth curves of *A. niger* and *P. glaucum* in terms of biomass change in sucrose-potato extract medium (values represent average of triplicates).

and then increasing, whereas the pH value of culture solution in which *P. glaucum* was inserted kept a steady increase; after 15 days, the pH value of culture solution in which *A. niger* was inserted was higher than that of culture solution in which *P. glaucum* was inserted. The pH value of the control group tended to increase slowly and finally came up to about 7.0.

Different types of fungi have different demands for nutrients, and their metabolites are also different. In their study of how *Penicillium rugulosum* dissolved apatite Reyes et al. (1999a, 1999b) found that different carbon and nitrogen sources would lead to different acids produced by fungi. When the nitrate was the only nitrogen source in the culture medium, citric acid would be produced, leading to dissolution of apatite in large amounts. The production of acid is more dependent on carbon source. If glucose was taken as the carbon source, only citric acid would be produced.

When sucrose was the carbon source, citric and glucose acids would be produced. Illmer observed that the fungi *A. niger*, *Penicillium simplicissimum*, *Penicillium aurantiogriseum*, etc. could effectively dissolve slightly soluble $AlPO_4$ (Illmer et al. 1995). Under experimental conditions under *A. niger* can produce acid, other fungi may be unable to produce any acid. Thus, *P. glaucum* produces little or no acid in the sucrose-potato medium we employed. Even if it produces a little amount of acid, the acid would be consumed as a result of mineral weathering. Therefore, the pH value of culture medium tends to increase steadily.

Growth of fungi requires the most suitable culture medium. As can be seen in Figure 2, the mycelium quality of *P. glaucum* tended to increase slowly with time; on the 12th day the mycelium quality of *A. niger* showed that the *A .niger's* quality was 6 times that of *P. glaucum*. The quality of *A. niger* was far more than that of *P. glaucum* over time.

Variation Characteristics of Ca^{2+} and Sr^{2+} Concentration Over Time

After the action of *A. niger* and *P. glaucum* on minerals for different time, the concentrations of Ca^{2+} and Sr^{2+} in the supernatant would change with incubation time, and the variation trend is shown in Figure 3.

The data shown in Figure 3 indicate that *A. niger* and *P. glaucum* weather mineral powder, the mass concentrations of Ca^{2+} in the supernatant tend to vary over time, but *A. niger* and *P. glaucum* show different variation characteristics. When *A. niger* acts on mineral powder, the mass concentrations of Ca^{2+} in the supernatant tend to increase first, and then decrease; the case is true for the variation characteristics of Sr^{2+} concentrations. When *P. glaucum* acts on mineral powder, the mass concentrations of Ca^{2+} in the supernatant tend to increase first and then decrease; the concentrations of Sr^{2+} tend to increase first from 0.237 mg/L on the 3rd day to 1.046 mg/L on the 18th day and then decrease to 1.010 mg/L on the 24th day. As for the control group, the mass concentrations of Ca^{2+} and Sr^{2+} show a slow increase over time.

The amounts of Ca^{2+} uptake by *P. glaucum* and *A. niger* tend to vary with time and the variation characteristics are shown in Figure 4. The data shown in Figure 4 indicate that in the process of weathering of mineral powder by *A. niger* and *P. glaucum*, there are significant differences in the quantity of Ca ions uptake by the mycelium. The quantity of Ca uptake by *A. niger* tends to decrease over time. As for *P. glaucum*, it tends to decrease progressively from 91.969 mg/g on the 3rd day to 14.054 mg/g on the 24th day. Figure 5 demonstrate that amounts of Sr absorbed by *A. niger* and *P. glaucum* show a good positive correlation with those of Ca uptake by them, indicating that the mycelium display constant chemical properties when they uptake Sr and Ca.

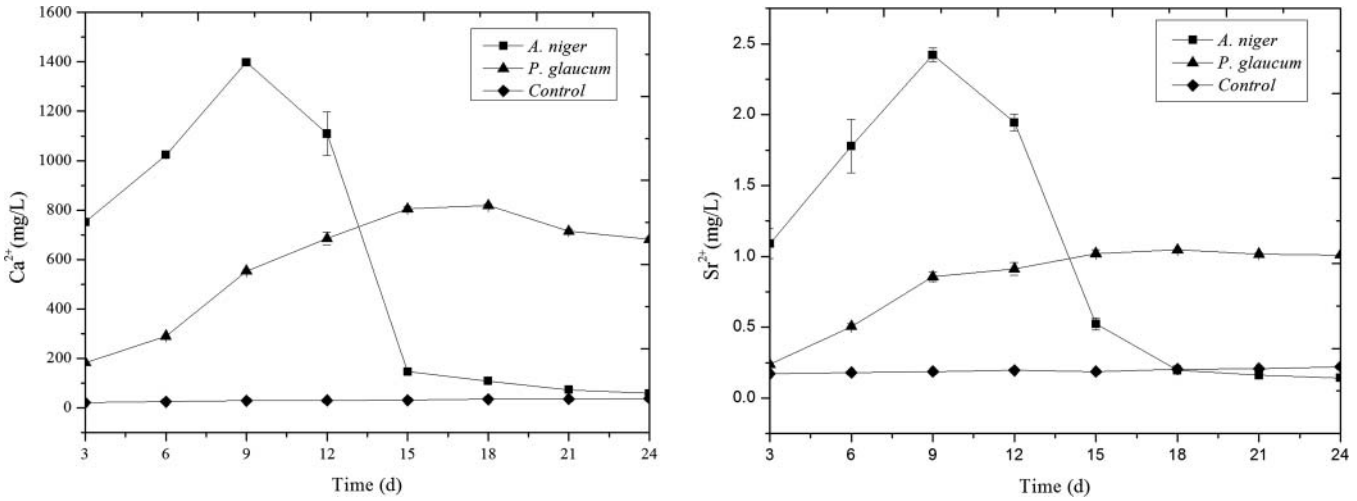

FIG. 3. Change in Ca^{2+} and Sr^{2+} concentrations during growth of *A. niger* and *P. glaucum* in the presence of calcite and apatite in sucrose-potato extract medium over time (values represent average of triplicates and error bars indicate standard deviation).

Variation Characteristics of Sr Isotope Ratios in the Culture Medium Over Time

When Sr is taken as an analog of Ca to study the geochemical behavior of Ca in the process of weathering, the geochemical behavior of Ca can indirectly reflect the geochemical behavior of P in apatite. It much be proved that Ca and Sr have similar geochemical behaviors in the process of weathering mineral powder by *P. glaucum* and *A. niger*. Correlations for the mass concentrations of Ca and Sr in the supernatant from the culture solution are shown in Figure 6.

As reflected in Figure 6, in the process of weathering of mineral powder by both *P. glaucum* and *A. niger*, Ca and Sr concentrations in the supernatant display unexceptionable correlations. The Ca/Sr concentration correlation coefficient for the mineral group under weathering by *P. glaucum* is $R^2 = 0.939$, and that for the mineral group under weathering by *A. niger* is $R^2 = 0.976$. According to the result, it is to be considered that element Sr has similarity of chemical behavior with Ca during the weathering of minerals by *A. niger* and *P. glaucum*, so Sr is treated as Calcium's Analog (Green et al. 2004).

Differences in Sr isotope ratio can be used to distinguish different types of minerals. Apatite and calcite have their own $^{87}Sr/^{86}Sr$ and Sr^{2+} concentration values. The $^{87}Sr/^{86}Sr$ ratio of apatite is 0.70859, and the quantity content of Sr^{2+} is 7.626 μg/g; the $^{87}Sr/^{86}Sr$ ratio of calcite is 0.70721 and its quantity content is 1.095×10^3 μg/g.

Figure 7 shows a linear correction between $^{87}Sr/^{86}Sr$ and $1/[Sr^{2+}]$ with varying action time when *P. glaucum* and *A. niger*

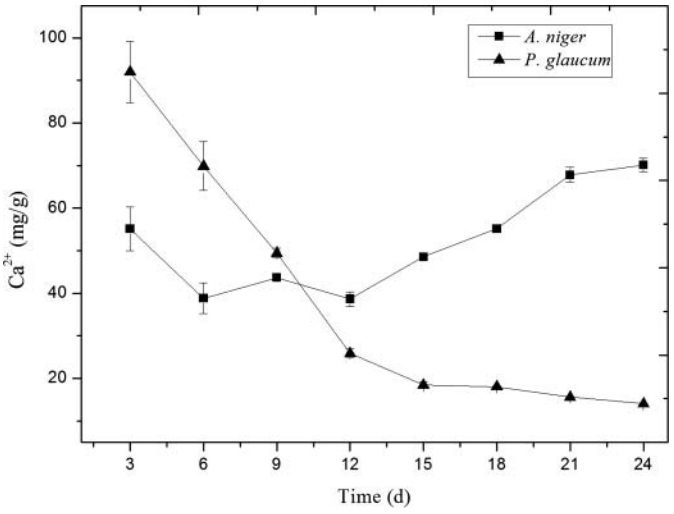

FIG. 4. Ca^{2+} uptake by mycelium of *A. niger* and *P. glaucum* over time (values represent average of triplicates and error bars indicate standard deviation).

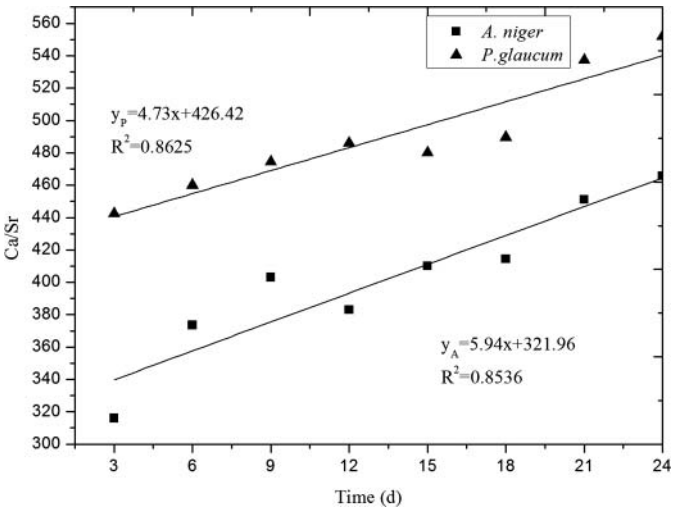

FIG. 5. The connection between the Ca/Sr uptake by mycelium over time.

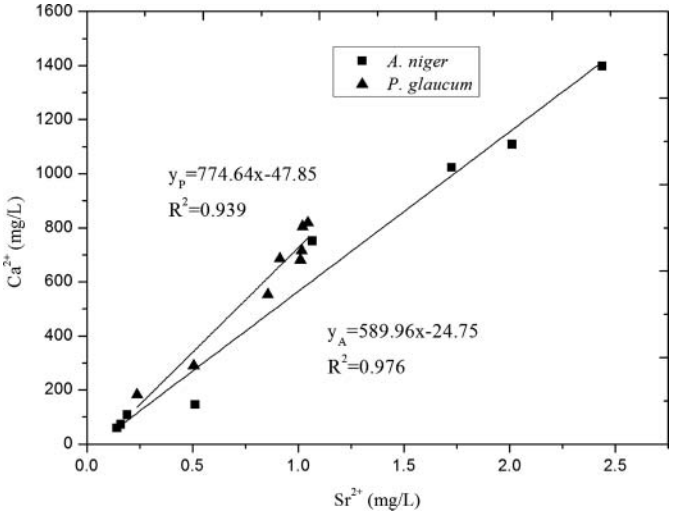

FIG. 6. Correlation between Ca and Sr concentrations in the culture medium.

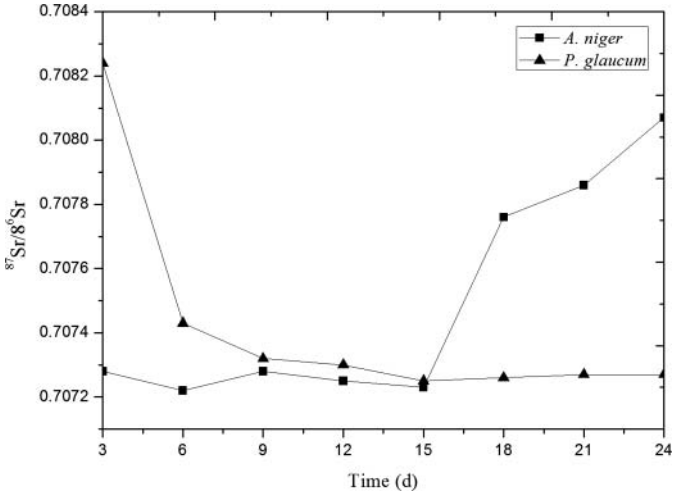

FIG. 8. Variation characteristics of $^{87}Sr/^{86}Sr$ in the supernatant.

act on mineral powder. The figure also shows an excellent linear correlation between $^{87}Sr/^{86}Sr$ in the supernatant and $1/[Sr^{2+}]$ when *P. glaucum* and *A. niger* act on mineral powder. This indicates that Sr in the supernatant is derived from mixing of the two component mixture of apatite and calcite (Green et al. 2004).

Shown in Figure 8 are $^{87}Sr/^{86}Sr$ ratios in supernatant at the time when *P. glaucum* and *A. niger* act on mineral powder with the duration of incubation. It is known from Figure 8 that when *P. glaucum* and *A. niger* act on weathering of minerals, $^{87}Sr/^{86}Sr$ ratios in the supernatant into which *P. glaucum* is inserted and those in the supernatant into which *A. niger* is inserted show different tendencies of variation with time. When *A. niger* weathers minerals, $^{87}Sr/^{86}Sr$ ratio in the supernatant tends to increase progressively with the duration of cultivating time.

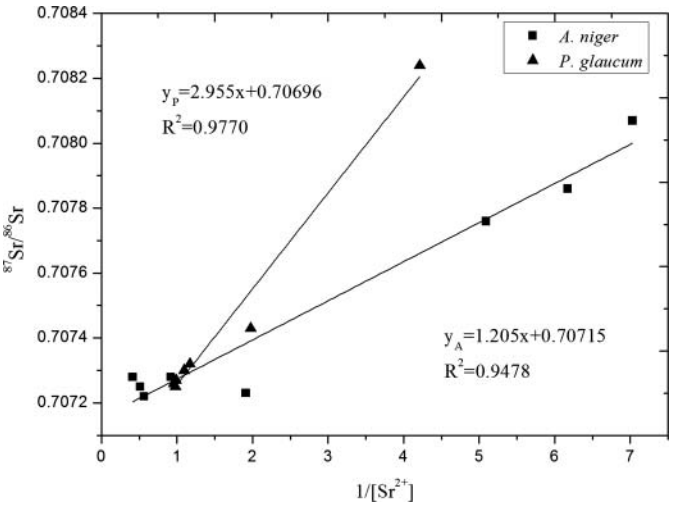

FIG. 7. Variation connection between $^{87}Sr/^{86}Sr$ and $1/[Sr^{2+}]$ in the process of weathering of minerals by *P. glaucum* and *A. niger*.

Between on the 15th day (0.70776) and the 18th day (0.70786) the ratio increased most rapidly. Till the 24th day, the ratio came up to 0.70807. In regard to *P. glaucum*, $^{87}Sr/^{86}Sr$ ratio in the supernatant tended to decrease with the duration of cultivating time. From the 3rd day to the 6th day the ratio decreased most rapidly, from 0.70824 on the 3rd day to 0.70743 on the 6th day, followed by a slow decrease to 0.70725 on the 15th day, and a small-magnitude increased to 0.70727 on the 24th day. The reasons that lead to the two clearly contrary results are: from the variation of pH of supernatant (Fig. 1) it can be seen that the pH value of culture solution of *A. niger* decreased initially from 5.47 to 4.26 on the 6th day, confirming that *A. niger* produced a large amount of organic acid at the initial stage, thus leading to a decrease in the pH of culture medium, favorable to weathering and dissolution of calcite.

With the consumption of calcite due to progressive weathering, the pH value of culture solution tended to increase progressively, the rise of pH of the culture solution would promote bacterial cells to absorb metallic ions (De Rome and Gadd 1987), thus enhance weathering process. Later, the weathering of apatite by *A. niger* intensified progressively, probably because the complexation caused by large-molecule ecto-polysaccharides and other substances secreted by *A. niger* made apatite dissolved. In regard to *P. glaucum*, as is known from Figure 2, *P. glaucum* is much less in quantity than *A. niger*, thus leading to the low intensity of weathering. On the other hand, it may be attributed to the fact that *P. glaucum* produced a little amount of acid at the initial stage, but more large-molecule ecto-polymers.

Large-molecule substances may be effective for weathering of apatite, leading to weathering-erosion of apatite. When the dissolved P can meet satisfactions for growth, with no further weathering-dissolution of apatite, the weathering of apatite was intensified at the initial stage and became weak at later stages. That is probably because the biological features of *P. glaucum*

led to relatively significant differences in the intensity of weathering of minerals.

In terms of $^{87}Sr/^{86}Sr$ ratios in supernatant and apatite and calcite the mass equilibrium equation was adopted to calculate the contribution rates of free ions in supernatant from the two kinds of minerals. At the time of weathering of apatite and calcite by *P. glaucum* and *A. niger,* Ca and Sr in the supernatant have two sources, but these two sources possess different contribution rates due to differences in weathering of minerals by different fungi (Fig. 8). By using the isotope mixing equations it is possible to quantitatively estimate Sr and Ca ratios in supernatant from different sources (Åberg et al. 1989; Bain and Bacon 1994; Blum and Erel 1995; Blum et al. 2000; Capo et al. 1998; Wallander 2000).

As calculated by the isotope mixing equations, the contribution rates of apatite and calcite in the supernatant over time are given in Table 2. Table 2 shows that in the early 15 days, calcite in the apatite and calcite mixture is preferentially weathered by *A. niger*. In combination with the analysis of variation characteristics of mass concentrations of Ca^{2+} in the supernatant (Fig. 3), it can be seen that the mass concentrations of Ca^{2+} in the supernatant are relatively high in the early 15 days, but the pH value of the solution is relatively low. The mechanism of weathering of calcite by *A. niger* is dissolution.

With the proceeding of weathering the pH value of the solution tends to increase progressively, weathering of apatite by *A. niger* tends to intensify. As the dissolved Ca from apatite increased progressively, till the 18th day, the contribution rate of apatite came up to 39.0%, and the pH value of the solution continued to rise, while the contribution rate of apatite also increased progressively. Until the 24th day, the contribution rate came up to 61.6%. Therefore, a comprehensive analysis of the data in Figure 3 and Table 2 shows that under this culture condition the weathering of apatite by *A. niger* is of time selectivity. The reason may be that low-molecule-weight organic acids may make calcite weathered first. The rise of pH value is favorable to the weathering of apatite. As for weathering of apatite and calcite mixture by *P. glaucum*, in the first 3 days *P. glaucum* was highly effective for weathering of apatite, among the dissolved ions the contribution 73.9% was contributed by apatite. As viewed from the variation tendency of Ca ions in the supernatant obtained when *P. glaucum* acts on minerals (Fig. 3) and the contribution rates of apatite and calcite (Table 2), under this cultivating condition *P. glaucum* is more or less effective for weathering of calcite, but poorly effective for weathering of apatite.

TABLE 2
The contribution rates of apatite and calcite in the supernatant over time

Time (d)	$^{87}Sr/^{86}Sr$	$X_{apatite}$ (%)	$X_{calcite}$ (%)
A. niger			
3	0.70728	5.2	94.8
6	0.70722	0.2	99.8
9	0.70728	4.9	95.1
12	0.70725	3.0	97.0
15	0.70723	1.7	98.3
18	0.70776	39.0	61.0
21	0.70786	46.4	53.6
24	0.70807	61.6	38.4
P. glaucum			
3	0.70824	73.9	26.1
6	0.70743	16.0	84.0
9	0.70732	8.0	92.0
12	0.70730	6.6	93.4
15	0.70725	2.6	97.4
18	0.70726	3.3	96.7
21	0.70727	4.5	95.5
24	0.70727	4.6	95.4

SUMMARY

The contribution rates of the two kinds of minerals to the ion concentrations in the supernatant were calculated in terms of the study of weathering of apatite and calcite mixture by *A. niger* and *P. glaucum*, quantitative analysis of the variation characteristics of pH of the supernatant in the process of weathering with incubation time, the amounts of Ca and Sr absorbed by mycelium and the variations of Ca mass concentrations and $^{87}Sr/^{86}Sr$ ratios in the supernatant, in combination with the isotope two-end-member model. Through the preceding analysis, the conclusions are drawn as follows:

1) In the mixture system composed of apatite and calcite *A. niger* is more effective for weathering of apatite than *P. glaucum*. The relatively low pH value of cultivating environment is favorable to weathering of calcite. Weathering of apatite may be mainly dependent on the complexation of large-molecute organic substances. This phenomenon is related to the textural characteristics of minerals themselves and the process of metabolism of *A. niger*.
2) In the apatite and calcite mixture system the effect of *P. glaucum* is relatively strong on weathering of calcite, but relatively weak on weathering of apatite. This is closely related to the biological characteristics of *P. glaucum* and the mineralogical characteristics of calcite.

In accordance with the above studies, it is concluded that Sr isotope tracing can be used to better distinguish the effects of different fungi in the mixture system on weathering of apatite and calcite and the differences in the effects of different fungi on weathering of minerals. This method can also be used to further study the influence of dominating microorganism in soil and the mineral composition of soil on the environmental quality of soil.

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