The Influence of Soil Fungi on the Sorption of Cesium and Strontium in the Soil Organic Layer

Prapamon Seeprasert, Minoru Yoneda, and Yoko Shimada

Abstract—Large quantities of cesium (Cs) and strontium (Sr) released from the Fukushima accident in 2011 are still present in terrestrial ecosystems. This study addressed the contribution of microbial activity to the sorption of Cs and Sr into organic material, which is necessary for comparing non-sterile systems with sterile systems. The aim was to determine the contribution of microbial activity to the sorption of Cs and Sr in organic material. The complete potential of fungi to cycle Cs and Sr in the organic soil system was assessed in a series of experiments. Organic material was prepared under laboratory conditions from leaf litter to minimize the interference from competition by clay minerals. The results of an experimental system comparing biotic and abiotic systems conclusively demonstrate that soil fungi play an important role in the sorption and retention of Cs and Sr. In all experiments, the retention of both elements was greater in biotic systems than in abiotic systems. Soil and saprotrophic fungi make an important contribution to the sorption of Cs and Sr in organic systems and may partly account for the strong, irreversible binding observed in biotic systems. The single strains of *Fusarium* sp., *Trichoderma* sp., and *Aspergillus* sp. showed increased amounts of Cs and Sr in a fixed form compared with those found in a biotic system. This finding may partly account for the high level of retention of Cs and Sr in upland organic soil, which is not satisfactorily accounted by the physicochemical process alone. It may also partly account for the strong, irreversible binding observed in biotic systems.

Index Terms—Cesium, organic soil, soil fungi, strontium.

I. INTRODUCTION

Large quantities of cesium (Cs) and strontium (Sr) released from the Fukushima accident in 2011 are still present in terrestrial ecosystems. The fractions of Cs and Sr linked to organic matter and microorganisms in soil may be relatively small compared with the amount bound to inorganic constituents; however, the organic layers in the soil system are more sensitive to the presence of humic acid, which can induce Cs and Sr to change into their soluble forms and be persistently available for uptake by plants [1]–[3]. Previous studies have shown that the behavior (e.g., distribution and mobility in soil) of Cs and Sr is significantly influenced by organic matter [4], [5]. Thus, it can be assumed that Cs and Sr are strongly retained in the top layer of organic soil [6]. For example, 40 years after the atomic bomb explosion in Nagasaki, Japan, Cs monitoring showed that 95% of the fallout was still present in the uppermost 10 cm of local soil [7]

and that the rate of vertical migration was low [8], [9]. It can be assumed that the strong retention of Cs and Sr in the organic layer is influenced by fungal and microbial activities.

The fungal and microbial activities in the organic soil layer are likely to substantially contribute to the long-term retention of radionuclides in the organic layer [10], [11]. In the forest soil, fungi are the greatest living biomass in the decomposing organic layers. They are the primary source of enzymes necessary to degrade the litter. Griffin (1981) showed the ability of fungi to take up nutrients from the soil solution [12]. Using the fungi enzymes to break down macromolecular complex, then most substances is easily to move into the cell or being bound to specific carrier molecular. Nowadays, the ability of microorganisms which includes bacteria, actinomycetes and fungi to accumulated Cs and Sr from their external environment has been largely studied. However, the mechanisms involved in the uptake and retention of Cs and Sr which is contribution of microbial activity to the sorption of Cs and Sr in organic material by fungi has been scarce. Thus, the role of soil microbiological process in the bioavailability of Cs and Sr needs to be more completely elucidated in order to predict the fate and transfer of Cs and Sr in soil. The role of microorganisms in the biogeochemical cycling of Cs and Sr can affect the soil solution by various mechanisms, both direct and indirect. For example, microorganisms can alter the soil pH and can act as potential ligands in the form of low-molecular-weight organic compounds with their exudates. In addition, Cs and Sr may directly attach to the cell surface or be actively taken up from the soil solution [13]–[15].
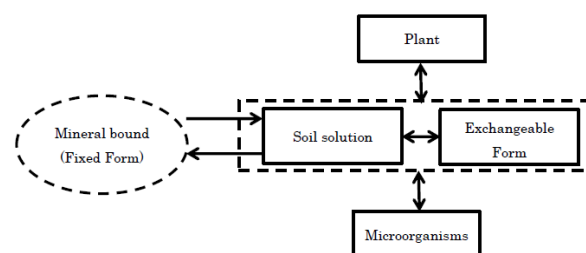

Fig. 1. A conceptual schematic summarizing Cs and Sr transfer into microorganisms and plants from soil. This figure was adapted from Avery (1996) [16].

The source of Cs and Sr in Fig. 1 is anthropogenic activities, which may be nuclear weapons testing, discharge of nuclear waste effluent, or accidental release that is deposited in soil via rainfall. Cs and Sr in soil are present in various solid components because of their binding capacity and in the soil solution. Thus, a wide range of soil properties such as pH, the presence of smaller ions (K^+, Ca^{2+}), clay content, and the amount of organic matter, including microbial or fungal activity, are known to affect the solid–solution exchange that controls the soil solution composition. In general, the organic

fraction of soils has a larger cation exchange capacity than the inorganic soil content. It can be assumed that Cs and Sr can be taken up from soil solution into microorganisms.

A conceptual schematic summarizing Cs and Sr transfer from soil to microorganisms and plants is presented in Fig. 1.

In a previous study, the fungal retention of Cs and Sr was assessed by measuring the amounts of Cs and Sr stored in fungal cells [17]. However, it is necessary to compare nonsterile systems with sterile systems in order to assess the complete potential of fungi to cycle Cs and Sr in soil; this is described in this paper. The aim of this study was to determine the contribution of microbial activity to the sorption of Cs and Sr in organic material. In this study, organic material was prepared from washed leaf litter to minimize interference from inorganic components and determine the true potential of litter and fungi to accumulate both Cs and Sr in situ.

II. PROCEDURE FOR PAPER SUBMISSION

A. Organic Material Preparation

The organic materials used in the experiment were prepared from leaf litter collected from four areas in Takizawa Research Forest, Iwate University, Japan, during the fall of 2014. The basic characteristics of leaf litter, such as moisture content and pH, were analyzed (Table I). The material was sorted to remove trash and washed with deionized water to reduce the interference from inorganic material. Subsequently, the leaf litter was air dried and shredded. Deionized water was then added to adjust the moisture content to 60%, and the preparation was incubated at a temperature of 25°C–30°C for 1 month.

TABLE I: BASIC CHARACTERISTICS OF THE LEAF LITTER USED IN THIS STUDY

Site	Coordinates	Moisture (%)	pH
IWT 1[1/]	39°46′55″N 141°9′22′	65.65 ± 1.9	5.11 ± 0.02
IWT 2[2/]	39°46′33″N 141°9′25′	62.16 ± 0.59	5.39 ± 0.0.8
IWT 3[2/]	39°46′33″N 141°9′10′	46.05 ± 2.37	5.73 ± 0.02
IWT 4[1/]	39°46′53″N 141°9′1′	76.48 ± 2.68	5.48 ± 0.03

[1/] coniferous forest [2/] deciduous forest

B. Soil Extraction Solution (SES) Preparation

SES was prepared using soil from the O layer from all the areas to provide inoculum for all experiments. A sample of 5 g soil dry weight equivalent was added to 50 ml of sterile distilled water (SDW) in a sterile centrifuge tube. The tube was shaken for 1 h and left to stand for 30 min. The supernatant was used as inoculum for biotic systems.

C. System Preparation

Single fungal cultures of *Fusarium* sp., *Trichoderma* sp., and *Aspergillus* sp. were obtained by isolation from soil [17]. After incubation for 7 days, each cell mat was washed to stop cell growth using SDW and subsequently resuspended in SDW. Samples of organic material measuring 5 g dry weight equivalent were set up as systems, with separate systems for abiotic and biotic processes. The biotic samples were reinoculated with 5 ml of SES. Separate systems were prepared for the three fungal genera used in this study: *Fusarium* sp., *Trichoderma* sp., and *Aspergillus* sp. For the

abiotic setup, the system was amended with an equal quantity of SDW and sterilized by autoclaving. In total, 40 µg of sterile Cs and Sr solution was added to 5 g of the organic material sample. SDW was added to adjust the moisture content to 70%–80% before sealing with a cap to avoid moisture loss and prevent contamination. Two replicates of each system were prepared. After 30 days, the subsamples of the systems were collected for measuring Cs and Sr bioavailability.

D. Sample Analysis

After incubation, approximately 1 g dry weight equivalent of organic material was removed from systems. The samples were then flushed with 20 ml SDW (pH 6.3–7.3) for 24 h under shaker conditions. Following this, 0.1 mg/l of cation mix contain (K^+, Ca^{2+}, and Mg^{2+}) was used as an extractant to determine the more tightly bound fractions of Cs and Sr. The amounts of Cs and Sr in the solution were measured by inductively coupled plasma mass spectrometry (ICP-MS; XSeries 2, Thermo Scientific). Sample preparation followed the standard methods ISO17294-2 and EPA 6020a [18], and the percentage of spiked elements extracted was calculated. The total amount extractable represents the exchangeable and potentially bioavailable fraction of Cs and Sr spiked.

III. RESULTS AND DISCUSSION

A. Effects of Soil Fungi

To interpret the experimental results, two sets of observations were used to infer whether soil microorganisms play an important role in Cs and Sr sorption and retention. The experimental data consisted of a comparison between the amounts of each element: Cs and Sr were extracted using distilled water following the incubation period (1 month). Fig. 2 shows that 27%−50% of Cs spiked into the abiotic systems was extracted by the distilled water, whereas only 8%−24% of Cs was extracted from the biotic systems that were reinoculated with SES. The amount of Sr extracted was less than that of Cs: 10%−20% for the abiotic systems, and only 5%−10% for the biotic systems.

The results showed the same trend for all locations: ITW1 showed a larger amount of Ca and Sr extracted than IWT3. Water flushing removed more Cs than Sr. A previous study that investigated the extraction of Cs and Sr as radioactive isotopes, ^{137}Cs and ^{85}Sr also showed the same trend of result in both abiotic systems, followed by autoclaving and using a fungicide such as streptomycin [19]. In this study, we also used a different extractant, a mixed cation solution containing 1 mg/l of K^+, Ca^{2+}, and Mg^{2+}, to determine the levels of exchangeable Cs^+ and Sr^{2+} in the organic material for the abiotic and biotic systems. The results are shown in Fig. 3.

The amounts of Cs bound to organic material extracted by distilled water from the abiotic treatment were 50%, 48%, 28%, and 40% of the initial Cs concentration. These values are higher than those for the mixed cation solution, which were 31%, 30%, 23%, and 27% of the initial Cs concentration for the locations IWT1–IWT4, respectively. However, the results for biotic treatment show that the percentage of Cs extracted using the mixed cation solution decreased to 14%–18% of the initial Cs concentration. These results

indicate that the sorption of Cs was weak, whereas the sorption of Sr was much stronger, which corresponds with that observed previously; Sr is mostly bound by organic material, whereas Cs is strongly bound by clay minerals in soil [20], [21].
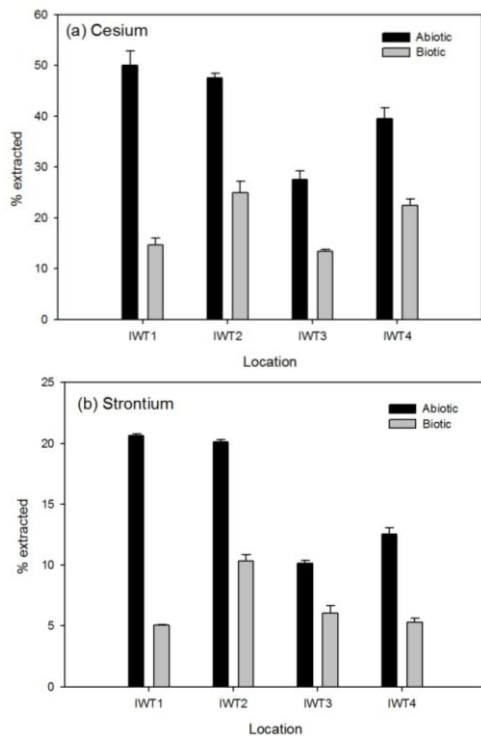

Fig. 2. Percentages of (a) Cs and (b) Sr extracted using distilled water from organic material in abiotic and biotic systems for four different soil locations.

Results showed that Cs is more strongly bound in the presence of soil microorganisms in the biotic system than in the abiotic system, indicating that the microorganisms associated with Cs accounted for a significant proportion of Cs in the contaminated upland soil. This is thought to be because of microbial activity, which can accumulate Cs though the active transport of monovalent ions such as K^+ and NH_4^+ [10], [16]. This experiment indicates that bacterial and fungal microbiota that was re-inoculated from SES can enhance the retention of Cs, particularly in the organic layer.
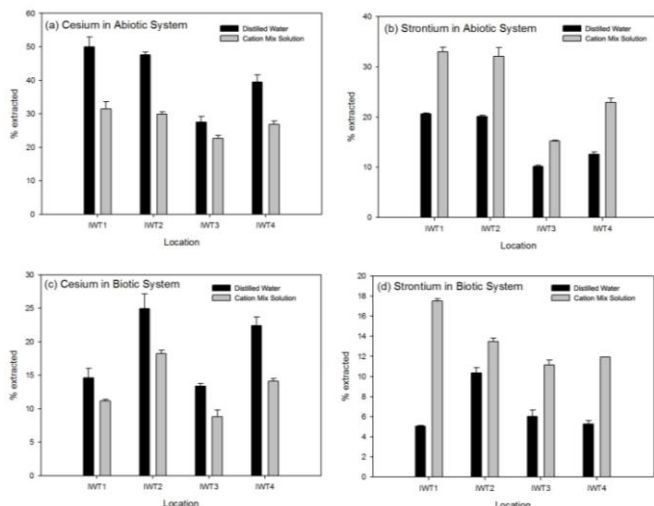

Fig. 3. Percentage of (a), (c) Cs and (b), (d) Sr extracted using different solutions from organic material in abiotic (a), (b) and (c), (d) biotic systems for four different soil locations.

B. Effects of Single Fungal Culture

These experiments were performed to determine the importance of saprotrophic fungal cultures for the sorption of Cs and Sr in the organic soil system. The results are shown in Fig. 4. As in the previous study [17], three soil fungal cultures were isolated from a soil sample, confirming that these organisms are present in the biotic organic layer. The inoculation of the organic material with each of the three soil fungal strains increased the sorption of both Cs and Sr, as demonstrated by the amount of element remaining after extraction compared with that in the abiotic system.
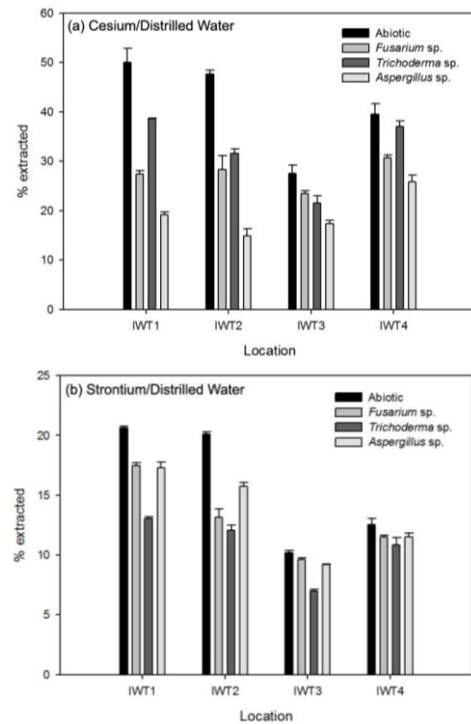

Fig. 4. Percentages of (a) Cs and (b) Sr extracted by distilled water from organic material in biotic systems inoculated by single fungal cultures for four different soil locations.

In the system inoculated with single fungal cultures, approximately 18%–39% of the initial Cs was extracted, whereas in the abiotic system, 28%–50% of the initial Cs was extracted. The system inoculated with *Aspergillus* sp. showed a slightly higher ability to retain Cs than those inoculated with *Fusarium* sp. and *Trichoderma* sp. However, for Sr in biotic systems, only 7%–17% of the initial Sr was extracted, whereas for abiotic systems, approximately 10%–20% of the initial Sr was extracted. In contrast, the system inoculated with *Trichoderma* sp. showed a slightly higher ability to retain Sr than those inoculated with *Fusarium* sp. and *Aspergillus* sp. From this limited analysis, biosorption by fungal cells appears to be species specific, depending on the components of the fungal cell walls that provide binding sites for elements [22]. The results of Cs and Sr extraction using the mixed cation solution from single fungal cultures are illustrated in Fig. 5.

The results of extraction using the mixed cation solution (Fig. 5) showed that biotic systems initially retained a higher proportion of each element. Dighton *et al.* (1991) estimated the accumulation of Cs by pure cultures of soil fungi and demonstrated that all studied strains were able to immobilize Cs. Efflux studies showed that more than 40% of the Cs was bound within hyphae [10]. The *Fusarium* sp. culture used in

this study was similar to that used in a previous study [19]. In this study, fungal retention increased the amount of non-extractable Cs and Sr in comparison with the abiotic system. In batch experiments using single fungal culture solutions, retention was enhanced in systems inoculated with *Aspergillus* sp. for Cs and *Trichoderma* sp. for Sr compared with abiotic systems. Clint et al. (1991) demonstrated that pure cultures of various saprotrophic and mycorrhizal fungi can take up Cs [23]; in that study, there was no indication of permanent binding of elements. The experimental results from two fungal cultures, *Aspergillus* sp. and *Trichoderma* sp., demonstrated that the majority of biosorbed Cs and Sr within organic material remained in an exchangeable form. We infer that these Cs and Sr are available for uptake by plants.
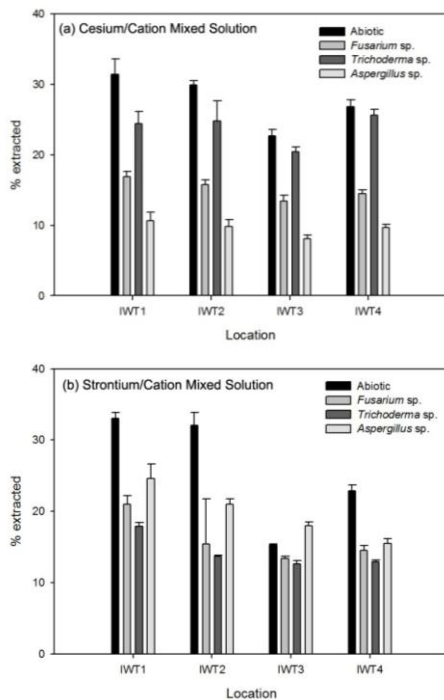

Fig. 5. Percentages of (a) Cs and (b) Sr extracted using mixed cation solution from organic material in biotic systems inoculated with single fungal cultures for four different soil locations

IV. CONCLUSION

The aim of this study was to determine the contribution of microbial activity to the sorption of Cs and Sr into organic material, which is necessary for comparing nonsterile systems with sterile systems. This study highlights the need to develop a new experimental approach to characterize the maximum potential of soil fungi to accumulate Cs and Sr in soil. The results in an experimental system comparing biotic and abiotic systems conclusively demonstrate that soil fungi play an important role in the sorption and retention of Cs and Sr. In all experiments, the retention of both elements was greater in biotic systems than in abiotic systems. Soil and saprotrophic fungi make an important contribution to the sorption of Cs and Sr in organic systems and may partly account for the strong, irreversible binding observed in biotic systems. This finding may account for the high level of radioactive Cs and Sr retention in the in situ upland soil organic layer, which cannot be satisfactorily accounted for by physicochemical processes.

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