Journal of Environmental Radioactivity 162-163 (2016) 319–327

Contents lists available at ScienceDirect

Journal of Environmental Radioactivity

journal homepage: www.elsevier.com/locate/jenvrad

Disparate radiocesium leaching from two woody species by acceleration of litter decomposition using microbial inoculation

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ARTICLE INFO

Article history:
Received 8 January 2016
Received in revised form
10 June 2016
Accepted 12 June 2016

Keywords:
Cryptomeria japonica
Prunus x yedoensis
Radiocesium
Lignin
White-rot fungus

ABSTRACT

Studies focusing on the migration of radionuclides in the forest floor have demonstrated that the ecological half-life of radiocesium on organic layer containing the debris of plant litter with various fungi and microorganisms is shorter than that in the deeper soil zone, suggesting that the litter decomposition affects radiocesium mobilization. Here, we showed the involvement of lignin, one of the major cell wall components of plant litter, in the fate of contaminated radiocesium during the process of fungal litter decomposition. In this study, litter decomposition of two different woody species, broadleaf deciduous Japanese cherry consisted of hardwood lignin and coniferous evergreen Japanese cedar with softwood lignin, were accelerated by *in vitro* fungal inoculation. *In vitro* inoculation exhibited 1.93- to 2.59-times faster decomposition than field experiment. Then, the cherry litter lost approximately 25% of initially contaminated radiocesium within 1 month of *in vitro* decomposition, whereas the cedar litter kept initial level at least for 6 month. The retention of radiocesium correlated with thioglycolate lignin content in cedar litter but not in cherry litter. Consequently, the behavior of radiocesium contaminated in litter fall may vary depending on the contamination pathway or the manner of nutrient mobilization at the stage of abscission between evergreen and deciduous trees.

1. Introduction

Radioactive material was accidentally released in a forest area following the Fukushima Dai-ichi Nuclear Power Plant (FDNPP) accident on March 11, 2011 (Hashimoto et al., 2012). The pruned woody parts and litter of trees contain considerable amounts of radionuclides (Endo et al., 2012). The fate of radionuclides deposited in forest floor is mainly governed by three physicochemical processes: 1) retention by recalcitrant components of the organic layer or mineral soil; 2) migration into the deeper soil zone; and 3) leaching into forest streams. In addition to such the dynamics, biological process also acts important role in the migration and concentration of radionuclide. For example, terrestrial detritivores are known to consume forest litter as an energy source and to bioaccumulate contaminated radiocesium (Murakami et al., 2014). Moreover, soil microorganisms profoundly influence the fate of radiocesium by leaching from organic matter, enabling microbial radiocesium immobilization and re-absorption into fungi and plant bodies followed by uptake via fungal hyphae and plant roots (de Boulois et al., 2008a, b; Kuwahara et al., 2011). Thus, the fungal and microbiological activity is very important to the environmental biocycling and the long-term retention of radiocesium in the organic layer of forest soil is consequently attributed to microbial population and habitat (Kruyts and Delvaux, 2002; Schell et al., 1996; Steiner et al., 2002). Therefore, taking into consideration of the presence of fungi and microbial activity could offer valuable information for the fate of radionuclides deposited in forests around the FDNPP. The net transport of radiocesium in forest floor is frequently explained by rate coefficients or ecological half-lives in individual soil compartments, e.g., organic layer and mineral soil components (Hashimoto et al., 2013). However, this analysis does not provide insight into the specific mechanisms determining the fate of radiocesium associated with individual litter decomposition by fungal activity.

The breakdown of litter components is the first step in the remobilization of radiocesium under bioactive natural

Abbreviations: FDNPP, Fukushima Dai-ichi Nuclear Power Plant; TGAL, thioglycolate lignin.

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http://dx.doi.org/10.1016/j.jenvrad.2016.06.010
0265-931X/

environment. White-rot fungi appear to play a key role in this biocycling process because they are the primary source of the enzymes necessary to decompose littered organic matter in forests (Harvey et al., 1987; Tuor et al., 1995). The principal component of litter is the plant cell wall, which contains cellulose, hemicellulose, and lignin (Doblin et al., 2010). Although cellulose is the most abundant organic compound in litter, the strength of the cell wall is limited by rigid hemicellulose complexes that protect the surrounding cellulose microfibrils. Subsequently, lignin fills the spaces between cellulose and hemicellulose. Unlike cellulose and hemicelluloses, the lignin polymer is highly recalcitrant to biochemical degradation due to its molecular complex architecture (Hofrichter, 2002). Importantly, increased radiocesium activity concentration resulting from litter decomposition has been reported in various environmental conditions (Brückmann and Wolters, 1994; Fukuyama and Takenaka, 2004; Rafferty et al., 1997; Witkamp and Barzansky, 1968). So, contaminated radiocesium could be associated to lignin or the other recalcitrant organic matters in decomposed litter rather than easily decomposable organic components, for example, cellulose and hemicellulose. Once the contaminated radiocesium is transiently immobilized to lignin at early phase of decomposition, it downwardly moves into mineral soil or forest floor leachate accompanied by the recalcitrant organic matter (Currie et al., 1996; McDowell and Likens, 1988; Qualls et al., 1991). Then, they can be incorporated into high-molecular-mass complex organic acids, primary categorized as humic substances (Cole et al., 1984; Cronan, 1985; Guggenberger and Zech, 1994). Generally, the ecological half-life of radiocesium is shorter in the organic layer, which is composed of recalcitrant structures of plant litter, such as lignin, than that in the deeper soil zone, suggesting that lignin breakdown contributes substantially to radiocesium mobilization. Although fungal translocation of radiocesium in decomposing plant litter has been recently reported (Huang et al., 2016), the extent to which litter components contribute to the fate of radiocesium has not been fully determined, limiting our ability to predict the effects of microorganism, which determines the lignin biodegradation activity, on the vertical migration of radiocesium.

Here, we investigated whether *in vitro* inoculation of white-rot fungi could accelerate the leaching of radiocesium from two types of woody litter naturally contaminated with radiocesium after the FDNPP accident. We examined the fate of contaminated radiocesium and relationship between radiocesium retention and lignin content in decomposing litters from Japanese cedar and Japanese flowering cherry. This study will increase our understanding of the biorecycling of radiocesium in Japanese forest ecosystems.

2. Materials and methods

2.1. Study site, target trees, and decomposition experiment under natural conditions

Experiments were performed in Abiko (Laboratory of Environmental Science, CRIEPI), located approximately 200 km SSW from the FDNPP (35.87815°N, 140.02487°E). The gamma-radiation dose rate was 0.3–0.5 μSv h^{-1} during the experimental period. The target trees were popular ornamental species: Japanese flowering cherry trees (*Prunus x yedoensis* cv. Somei-Yoshino) and Japanese cedars (*Cryptomeria japonica*). Sampling of litterfall, consisting primarily of fallen leaves, was performed from September 5 to October 31 in 2011 and denoted as cherry litter or cedar litter, as appropriate. Samples were well dried at 80 °C for 3 days and kept in desiccators until use. Double-layered litterbags (15 × 15 cm inner bag in 20 × 20 cm outer bag) were made with conventional nylon bags with 4-mm mesh for the inner bags and 1 mm mesh for the outer bags. These double-layered bags were used for field decomposition experiments under natural conditions, i.e., on the organic layer of Japanese cedar forest in Abiko and the mineral soil layer where the organic matter was removed. The field decomposing experiments were carried out with 10 g of Japanese cedar litter and 5 g of Japanese cherry litter per litter bag (n = 4) from August 7, 2012 to October 8, 2014 (approximately 25 months). After removal from each layer, litterbags were washed and rinsed with distilled water, dried at 80 °C for 3 days, weighed the litter biomass and packed in screw-capped polystyrene U-8 containers for measurement of radiocesium activity concentration.

2.2. Evaluation of the microbial biomass in soils

Extraction of environmental DNA (eDNA) was performed using the slow-stirring method at room temperature (Aoshima et al., 2006). In brief, soil samples (1.0 g) prepared from the sites of the field decomposition experiment were gently agitated for 20 min in the presence of 8.0 mL of DNA extraction buffer (100 mM Tris-HCl, pH 8.0, 100 mM EDTA-Na, 100 mM sodium phosphate, 1.5 M NaCl and 1% hexadecylmethylammonium) and 1 mL of 20% sodium dodecyl sulfate. After centrifugation at 6000*g* for 10 min, supernatants were transferred to a new tube with an equal volume of chloroform–isoamylalchol [24:1 (v/v)]. The aqueous phase was recovered after centrifugation at 14,000*g* for 10 min and mixed with an equal volume of isopropanol. After centrifugation at 14,000*g* for 20 min, the pellet was thoroughly washed with ice-cold 70% ethanol twice at 14,000*g* for 5 min, and the air-dried pellet was dissolved in TE buffer (10 mM Tris-HCl and 1 mM EDTA-Na, pH 7.5). The microbial biomass (cells g^{-1} soil) was estimated on the basis of the linear proportion of 1.70×10^8 cells in 1 μg of eDNA (Aoshima et al., 2006).

2.3. Procedures for in vitro decomposition experiment with white-rot fungi

White-rot fungi (*Phanerochaete chrysosporium*: ATCC32629, *Pleurotus pulmonarius*: ATCC32078, *Stropharia rugosoannulata*: ATCC60010, and *Trametes versicolor*: ATCC96186) were purchased from the American Type Culture Collection (ATCC, http://www.atcc.org/). These fungi were maintained on potato dextrose agar (PDA) medium by transplanting a 5-mm^2 agar piece every 2 weeks under dark conditions at 25 °C until use for *in vitro* decomposition. At 1 week after transplantation on new PDA media, all of these fungi (four 5-mm^2 agar pieces) were inoculated together on a litter slurry consisting of 10 mL of distilled water containing 0.12 g of potato dextrose broth (PDB) and 5 g of finely milled dried litter of Japanese cedar or Japanese flowering cherry in a 60 × 60 × 100 mm polycarbonate box (plant box for tissue culture, AGC Techno Glass Co., ltd., Shizuoka, Japan). These litter-fungi slurries were incubated under dark conditions at 25 °C. After incubation for 0, 1, 2, 3, 4, and 6 months, the fungi that had proliferated on the surface of the litter slurry were carefully removed and discarded. The residual litter slurry of each box was individually transferred to a 50-mL tube and precipitated at 3000*g* for 20 min. After the supernatant was discarded, the residual litter precipitate was thoroughly washed by shaking for 24 h with 30 mL of distilled water. Then, the washed litter was precipitated at 3000*g* for 20 min and air-dried to measure the remaining biomass and radionuclides for the samples from 0, 1, 2, 3, 4, and 6 months. All of the sampling was performed in triplicate.

For measurement of radiocesium leached from contaminated plant litter, the plant litter was similarly subjected to *in vitro* decomposition after thorough washing by distilled water. After incubation for 0, 1, 4, and 6 months under dark conditions at 25 °C