Kuroda *et al. Journal of Wood Science* (2022) 68:16
https://doi.org/10.1186/s10086-022-02024-7

Journal of Wood Science

ORIGINAL ARTICLE Open Access

Cellular-level *in planta* analysis of radial movement of minerals in a konara oak (*Quercus serrata* Murray) trunk

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Abstract

After the Fukushima Daiichi Nuclear Power Plant accident, radiocesium, one of the main radioactive materials, has been accumulated inside konara oak trunks. Radiocesium has been thought to move radially through the trunk, but it has not been scientifically vindicated because the mechanism of the radial movement of minerals has not yet been experimentally determined. In this study, mineral radial movement was investigated in konara oak trunks of standing trees. A stable isotope cesium (Cs) solution was injected as a tracer into the outer sapwood of standing konara oak tree trunks. A trunk part was subsequently freeze-fixed with liquid nitrogen and subjected to Cs distribution analysis using cryo-scanning electron microscopy/energy-dispersive X-ray spectroscopy. By comparing normal samples that included living cells and freeze–thaw treated samples that contained no living cells, it was concluded that the injected Cs has been moving through the sapwood by the combination of rapid symplasmic movement by virtue of the living xylem parenchyma cells and slow apoplastic diffusion. Conversely, the Cs solution did not diffuse from the sapwood to the heartwood, implying that Cs is exuded from the living parenchyma cells to the apoplast, i.e., cell walls or adjoined dead parenchyma cells in the layer at the sapwood–heartwood boundary, and then diffused into the heartwood. By integrating the results of this study and our previous results obtained on Japanese cedar, we conclude that the mechanism of the radial movement of minerals through the sapwood seems to be a universal characteristic of tree species. In contrast, since mineral concentrations varied among tree species, the movement mechanism across the sapwood–heartwood boundary can differ among tree species.

Keywords: Apoplast, Cesium, Cryo-scanning electron microscopy, Energy-dispersive X-ray spectroscopy, Freeze–thaw, Mineral transport, Nuclear power plant accident, Parenchyma cell, Symplasmic

Introduction

Konara oak (*Quercus serrata*) is a tree species traditionally used for shiitake mushroom log cultivation and for obtaining charcoal. After the Fukushima Daiichi Nuclear Power Plant (FDNPP) accident in 2011, a concern regarding the effect of radioactive contamination on konara oak trees emerged [1–3]. Intensive investigations revealed that radiocesium (^{137}Cs and ^{134}Cs), one of the main radioactive materials released from FDNPP, was detected in tree trunks [e.g., 4–6]. Radiocesium was detected in both sapwood and heartwood, although the activity concentration was strikingly higher in bark. Since the leaves of konara oak were not opened at the time of the accident, it was thought that radioactive material has attached directly to the trunk surface (i.e., bark) of konara oak trees and then moved radially via the cambium to the xylem at the early phase of the FDNPP accident [4–6]. Moreover, radiocesium was detected in the leaves of noncontaminated seedlings of konara oak newly planted in contaminated soil [7], indicating that this species absorbs radiocesium from the soil like other tree species [8, 9]. Since a konara oak tree has the ring-porous type of

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vessels, sap flows through the outer part of the sapwood [10, 11]. Thus, minerals absorbed by roots move only into the outer sapwood of a konara oak trunk. To favor accumulation in the inner sapwood and heartwood, radiocesium must move in a radial direction from the bark or outer sapwood in a trunk. However, there is no scientific evidence of the mechanism of the radial movement of minerals, including cesium, through the trunk of konara oak trees. Therefore, it is necessary to unravel the route of radial mineral movement to understand not only how konara oak tree trunks were contaminated but also what future contamination trends could be expected regarding this species.

In general, minerals are thought to be moved radially in tree trunks by the activity of ray parenchyma cells [12–14]. This hypothesis is widely accepted because they are the only living cells in the sapwood, although a limited number of tree species contain living wood fiber within the sapwood. There are two routes to transport water and soluble compounds—symplasmically and apoplasmically [14]—both of which are able to achieve the transport in the radial direction [15, 16]. Symplasmic transport between parenchyma cells is known to use plasmodesmata [12, 14, 17]. For a short distance, the radial movement of soluble compounds through a tree trunk was revealed using fluorescence tracers in the stem of *Eucalyptus saligna* [18] and current-year annual rings of *Acer pseudoplatanus* and *Populus tremula* × *P. tremuloides* branches [16]. By contrast, long-distance radial movement that exists between the outer sapwood and heartwood is very limited. Hasegawa and Shiroya [19] tracked the movement of ^{14}C from the cambium, where ^{14}C labeled sucrose was injected in a trunk of *Prunus yedoensis*. Although it was detected in both the sapwood and sapwood–heartwood transition zone, the target sites of moving in the xylem were not identified because autoradiography of ^{14}C distribution was conducted only in the outer sapwood, close to the injection point. Okada et al. [20, 21] revealed that minerals were selectively transported in the radial direction from the sapwood to the heartwood of a Japanese cedar (*Cryptomeria japonica*) trunk, although its mechanism was not experimentally cognized. Recently, our group succeeded in revealing the site of the radial movement of minerals in a Japanese cedar tree trunk using direct analysis at the cellular level [22–24]—artificially injected stable isotope cesium (Cs) moved from the sapwood to the heartwood via a combination of active transport by xylem parenchyma cells and diffusion through cell walls. In this regard, can this knowledge be applied directly to other tree species such as konara oak? The pattern of radiocesium distribution between the sapwood and heartwood in a konara oak trunk is different from that of Japanese cedar [e.g., 25]. Moreover, when Cs was artificially applied into the bark, it was not detected in the heartwood of konara oak trees but it was detected in the heartwood of Japanese cedar trees [26, 27]. Evidently, the pattern of the radial distribution of minerals across a trunk is different among tree species [28, 29]. In addition to physiological differences, gymnosperms (e.g., Japanese cedar) and angiosperms (e.g., konara oak) differ in the anatomical and structural properties of xylem components such as tracheids, vessels, wood fiber, and ray and axial parenchyma cell distribution [13, 30]. Owing to these differences, the mechanism of mineral movement in the radial direction in a trunk of konara oak might be different from that of Japanese cedar.

In this study, we analyzed the radial movement of minerals in konara oak trunks. We applied our previously developed methodology, which included tracing the Cs that was artificially injected into a living konara oak trunk. A trunk part was subsequently freeze-fixed on a standing tree and subjected to distribution analysis using cryo-scanning electron microscopy/energy-dispersive X-ray spectroscopy (cryo-SEM/EDX) [22–24]. This method allowed us to analyze the distribution of ionized Cs in water-hydrated samples at the cellular level. In other words, we were able to determine the mineral movement in a living standing tree (*in planta*). Finally, we discussed and compared the mechanisms of the radial movement of minerals in trunks among different tree species.

Materials and methods

Plant material

Konara oak trees planted in the nurseries of the Forestry and Forests Products Research Institute (Tsukuba and Kasumigaura, Ibaraki, Japan) were used (Table 1), all of which had developed colored heartwood. In this study, the heartwood was defined as the darker inner part of the trunk under the frozen condition, and sapwood and heartwood were distinguished by color.

Six trees were selected for investigation of Cs behavior applied to normal konara oak trunks. Another three trees were selected for a freeze–thaw treatment of a trunk to kill parenchyma cells in one part of a living standing tree to compare the behavior of Cs movement between standing tree trunks that contained living parenchyma cells (normal trees) and those without living cells (freeze–thaw treated trees). The freeze–thaw treatment was implemented by attaching a watertight receptacle over a tree trunk at the site where a Cs solution would be injected. The receptacle was filled with liquid nitrogen (LN_2), and the trunk was allowed to freeze for approximately 30 min, as described by Kuroda et al. [23]. The

Table 1 Description of samples

Tree number	Sample name	Injection			Annual rings at stump	**Tree length (m)	Girth at 1 m (cm)
		Period of injection	Starting date (D/M/Y)	Harvesting date (D/M/Y)			
Normal							
K01	K01_3h	3 h	29/01/2016	29/01/2016	15	14.2	39
	K01_1d	1 day	28/01/2016	29/01/2016			
	K01_4d	4 days	25/01/2016	29/01/2016			
K04	K04_3h	3 h	08/07/2016	08/07/2016	14	13.2	47
	K04_4d	4 days	04/07/2016	08/07/2016			
K06	K06_3h	3 h	18/08/2016	18/08/2016	16	13.4	43
	K06_1d	1 day	17/08/2016	18/08/2016			
	K06_4d	4 days	14/08/2016	18/08/2016			
K07a*	K07a_1d	1 day	16/07/2020	17/07/2020	17	13.4	60*
	K07a_4d	4 days	13/07/2020	17/07/2020			
K02	K02_50d	50 days	25/01/2016	15/03/2016	16	14.6	-
K05	K05_45d	45 days	04/07/2016	18/08/2016	17	12.6	44
Freeze–thaw							
K07b*	K07b_1d	1 day	16/07/2020	17/07/2020	17	13.4	55*
	K07b_4d	4 days	13/07/2020	17/07/2020			
K09	K09_4d	4 days	12/07/2021	16/07/2021	23	13.4	38
K11	K11_1d	1 day	19/08/2021	20/08/2021	7	6.4	41
	K11_4d	4 days	16/08/2021	20/08/2021			

* Biforked trunks of the same tree. Girth was measured at approximately 2 m. **Length of the main stem measured after felling. -, no data

treatment was performed twice for each tree to ensure killing the living cells.

Cs injection and sample collection

Cs injection and sample collection were performed according to Kuroda et al. [22, 23] (Fig. 1). A cesium chloride solution (1.0 M final concentration, FUJIFILM Wako Pure Chemical, Osaka, Japan) was prepared in aqueous acid fuchsin (0.1% w/v final concentration, Nacalai Tesque Inc., Kyoto, Japan). The outer bark was removed in an approximately 2 cm-wide circle from a trunk of each tree, and small holes (approximately 2 mm deep) were drilled into the sapwood. Each hole was shifted from another by either 90° or 120°. Patches on the side without holes were used as control. A 500- or 1000-mL polyethylene bottle filled with the Cs solution was attached to each trunk approximately 20 cm above each drilled hole, and the solution was injected into the holes through a plastic tube (Tygon LMT-55, Saint-Gobain, Tokyo, Japan) with a stainless-steel tube (2.84 mm ø) attached to its end. The solution was injected continuously during the period stated in Table 1, and the injection was stopped < 20 min before the trunk was frozen. For longer injection periods, a fresh Cs solution was added several times to the plastic bottle during the injection period to prevent the bottle from emptying. Following injection, the described trunk part was frozen with LN_2 for approximately 30 min and the tree was felled. Serial disks (1-cm-thick cross-sections) were cut from the frozen trunk part, immediately immersed in LN_2, and stored at − 80 °C in a deep freezer.

Cryo-SEM/EDX

Frozen disks sampled 1–3 cm above the Cs injection holes were analyzed using the cryo-SEM/EDX technique, as described by Kuroda et al. [22–24]. Approximately, 3-mm-wide strips that passed through the red area of the sapwood and pith were taken from the frozen disks and cut into small blocks. Fresh transverse surfaces of these blocks were then smoothly cut using a cryostat (Cryostar NX70, Thermo Fisher Scientific, Tokyo, Japan) at approximately − 30 °C [22, 31]. The blocks were attached to a specimen holder and transferred to a cryo-SEM/EDX system (JSM6510A, JED-2300, JEOL, Tokyo, Japan). Secondary electron (SE) images were obtained at acceleration voltages of 3 and 15 kV with gold coating in the cryo-SEM/EDX system, and the characteristic X-rays were detected at an acceleration voltage of 15 kV. Several points were selected for point analysis on each SE image, and X-rays were collected for 300 s at each point (Fig. 1 h, i). A Cs-Lα peak (4.286 keV) at each point was carefully checked and defined

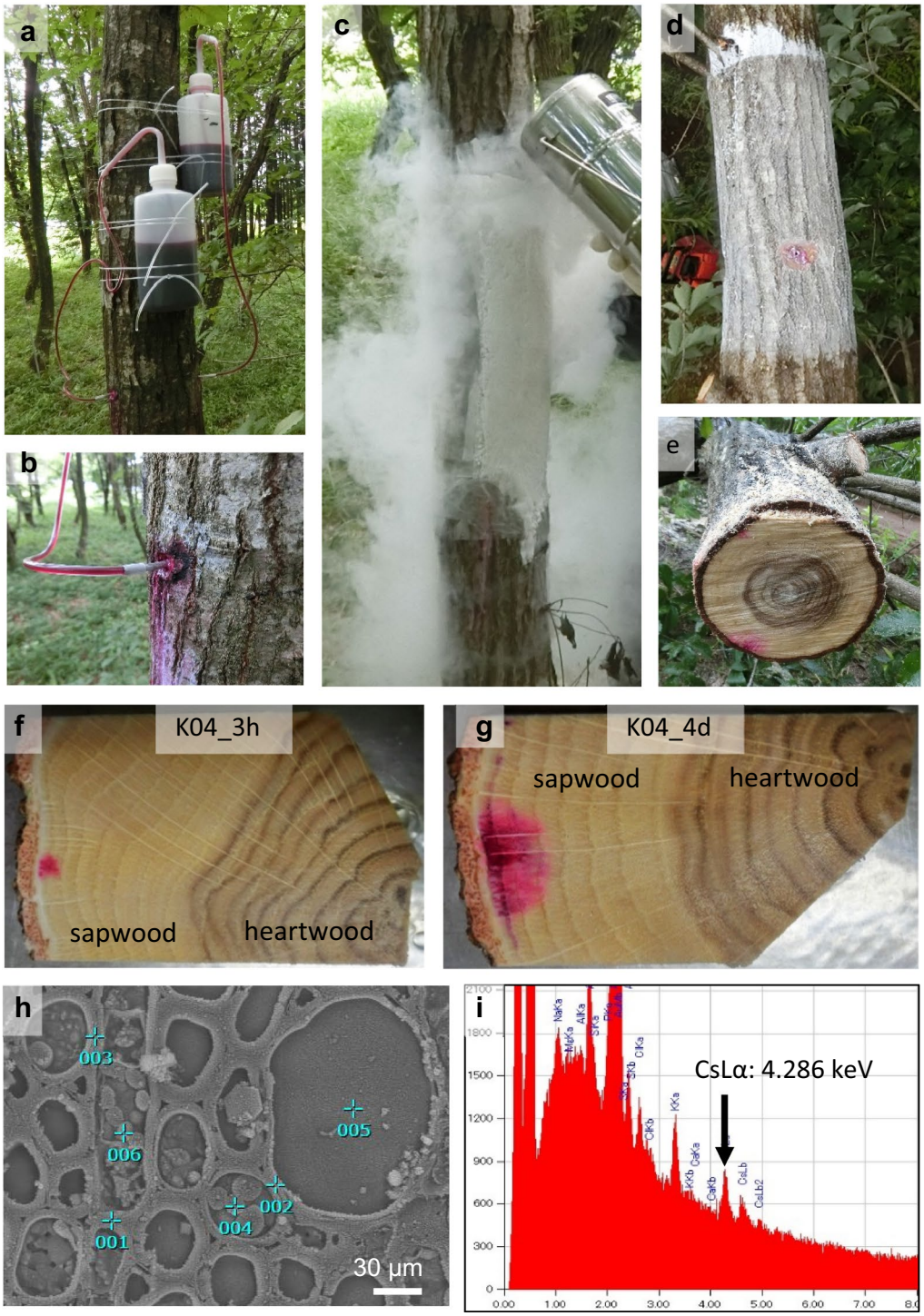

Fig. 1 Schematic representation of sample treatment and cryo-scanning electron microscopy/energy-dispersive X-ray spectroscopy (cryo-SEM/EDX) point analysis. **a**, **b** Bottles with a Cs solution were attached to a trunk approximately 20 cm above the injection hole, and the Cs solution was injected continuously during a defined period (please see Table 1). **c**, **d**, **e** After the injection, a part of the trunk was frozen by liquid nitrogen and the tree was felled. **f**, **g** The xylem region where the Cs solution was injected and its conduit were confirmed by the red color achieved by acid fuchsin staining. **h**, **i** A fresh transverse surface of the sample block was smoothly cut by cryostat and subjected to observation and analysis by cryo-SEM/EDX. Several points were selected to be analyzed on a secondary electron image, and the characteristic X-ray of each Cs-Lα peak (4.286 keV) was checked

in terms of the presence of Cs using the Analysis Station software, ver. 3.8 (JEOL, Tokyo, Japan). Vessel lumen (VL), vessel cell wall (VW), fiber lumen (FL), cell wall between fibers (FW), ray parenchyma cell lumen (RL), cell wall between ray parenchyma cells (RW), and axial parenchyma cell lumen (AL) were analyzed in several areas in each sample block.

For the observation of water distribution and cell structure, SE images were obtained after shallow freeze-etching at an acceleration voltage of 3 kV without coating, followed by deep freeze-etching that was performed by a gradual temperature increase during the cold stage of the cryo-SEM system from approximately − 120 °C to − 85 °C. This step was conducted to clearly observe the content of cell lumina by ice sublimation [11].

Soft X-ray photography

Frozen 5-mm-thick disks were placed on a film package mounted with an X-ray film (X-ray FR, FUJI Photo Film Co., Ltd., Tokyo, Japan) and irradiated by a soft X-ray apparatus (CMB-S, Softex Co., Tokyo, Japan). The irradiating conditions were the following: 20 kVp, 14 mA, 3′40″, and 1.45 m distance from the focusing plane to the film. The processed films were digitized at a resolution of 2400 dpi using a scanner (ES-G11000, Seiko Epson Co., Suwa, Japan). Ordinary light-reflected images were also recorded. After X-ray images of the water-hydrated state were recorded, frozen samples were freeze-dried and subjected to soft X-ray photography under the same conditions.

Results

Cs distribution in normal trees

The conduit path of the Cs solution in the trunk was identified as a red area stained by acid fuchsin [10, 22, 23, 32]. In all normal samples, this red area was distributed over the outer sapwood, being broader as the injection period increased and reaching the innermost sapwood after 45 and 50 days of injection (Figs. 2, 3).

Cesium distribution was determined in each cell structure that showed a Cs-Lα peak and that is represented using bars in Figs. 2–4. In the red areas, Cs peaks were detected in almost all cell structures. Along the radial profile, from the red area to the inner xylem, various Cs peaks were detected, depending on the injection duration and/or the cell type (Fig. 2). In the 3-h injected samples, Cs peaks were detected in almost the same area as the red area in all cell types, except for some VL. In the 1- and 4-day injected samples, Cs peaks of VL and FL were almost the same as those of the red area, whereas Cs peaks of VW and FW were detected beyond the detected area of VL and FL. Cs peaks of RL, RW, and AL were detected further than those of VW and FW. In some points of the inner sapwood in samples K04_4d, Cs was detected in VW and FW as dispersed.

In long injection periods such as 50 and 45 days (Fig. 3), the red area in the corresponding samples spread throughout the sapwood radially from the injection point toward the pith but stopped in the layer at the border between the sapwood and heartwood. By contrast, Cs was detected in all cell types and cell structures not only in the sapwood but also in the heartwood.

Cs distribution in freeze–thaw treated trees

The red area stained by acid fuchsin was detected in the outer sapwood of freeze-thawed samples, from where it spread wider in 4-day injected samples than in 1-day injected samples (Fig. 4). Cesium peaks of VL and FL were detected in almost the same area as the red area. Conversely, Cs peaks of VW, FW, RL, RW, and AL occupied almost the same area, being detected a little further toward the inner xylem than those of VL and FL. In some points of the inner sapwood, Cs was detected as dispersed. The same tendency was observed in 1- and 4-day injected samples.

Water distribution through the xylem as revealed by soft X-ray photography and cryo-SEM

Water movement through the xylem is thought to be related to mineral movement. Water distribution at the tissue and cellular levels was observed using soft X-ray photography (Fig. 5) and cryo-SEM technique (Fig. 6), respectively. On soft X-ray films, water was recognized by high X-ray absorption that was as the same level as that of cell structures such as cell walls and cellular material in a frozen-hydrated sample [33]. After drying, i.e., removal of water, cell structures and cellular material were made recognizable. Thus, a comparison between photographs captured in the water-hydrate state and after drying revealed water distribution. Cryo-SEM observation enabled revealing water distribution at the cellular level [e.g., 31]. By comparison of images taken before and after deep freeze-etching, it was visible that water was clearly distinguished from cell walls, cell contents, and tyloses because deep freeze-etching removed sublimated ice [11].

In normal trees, almost all latewood vessels and fibers as well as many earlywood vessels had their lumina filled with water, though some earlywood vessels in the annual rings of the current and the second year lacked water (Figs. 5, 6). Cryo-SEM observation revealed that many earlywood vessels in the second year and almost all earlywood vessels in older annual rings had tyloses in their lumina, where water filled in the spaces between tyloses and cell walls (Fig. 6). Almost all fibers had water in their lumina through the xylem. In

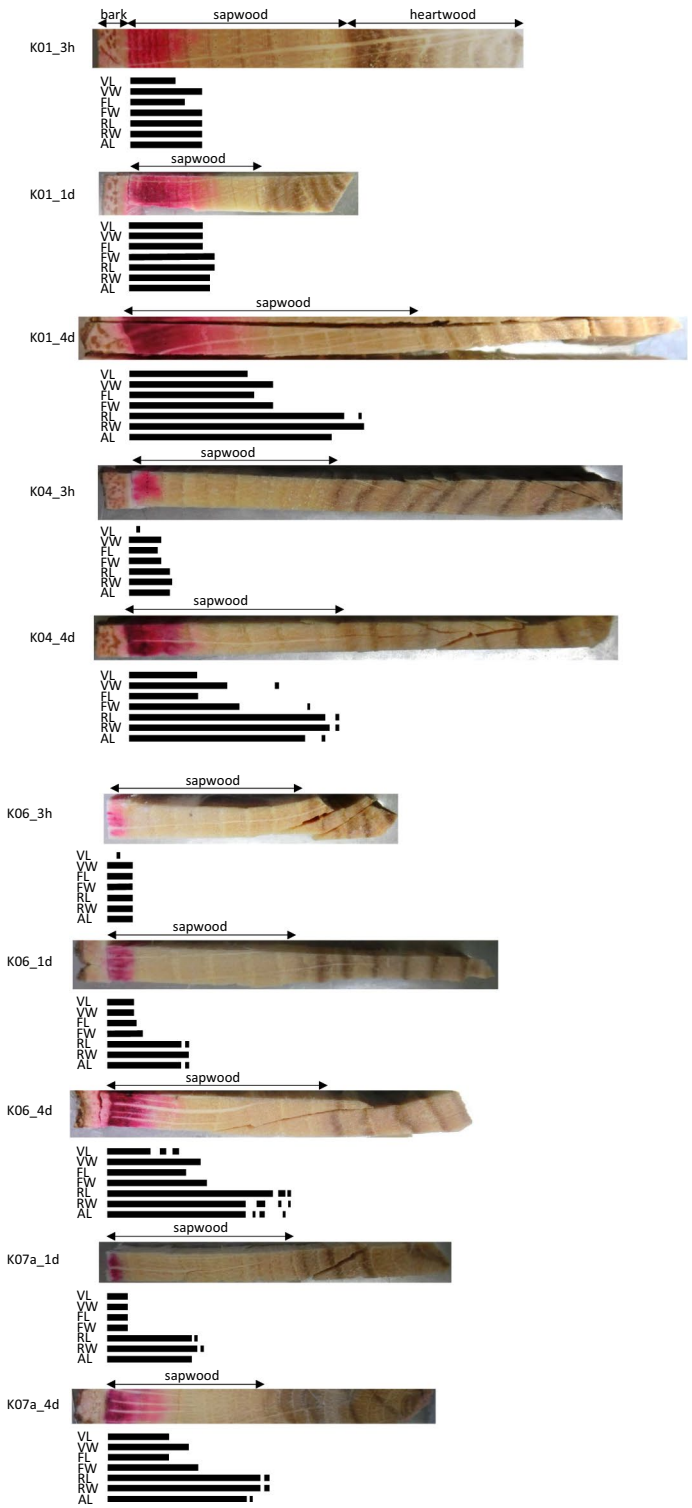

Fig. 2 Schematic representation of the radial distribution of cesium (Cs) in the xylem of normal konara oak (*Quercus serrata*) trees after 3-h, 1-day, or 4-day injection, as observed using cryo-scanning electron microscopy/energy-dispersive X-ray spectroscopy (cryo-SEM/EDX). Bars below each sample image represent the areas where Cs was detected. VL, vessel lumen; VW, vessel cell wall; FL, fiber lumen; FW, cell wall between fibers; RL, ray parenchyma cell lumen; RW, cell wall between ray parenchyma cells; AL, axial parenchyma cell lumen

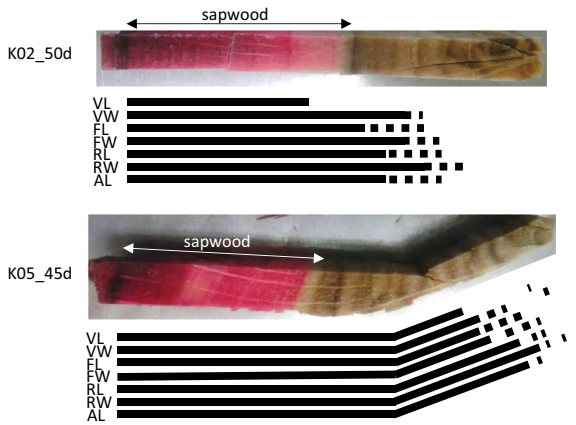

Fig. 3 Schematic representation of the radial distribution of cesium (Cs) in the xylem of normal konara oak (*Quercus serrata*) trees after long injection periods as observed using cryo-scanning electron microscopy/energy-dispersive X-ray spectroscopy (cryo-SEM/EDX). For abbreviations, please refer to Fig. 2

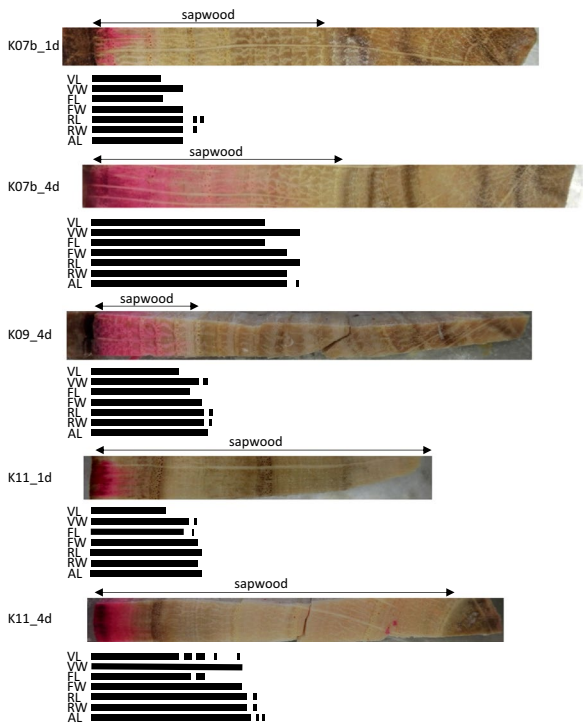

Fig. 4 Schematic representation of the radial distribution of cesium (Cs) in the xylem of freeze–thaw treated konara oak (*Quercus serrata*) trees after 1- or 4-day injection as observed using cryo-scanning electron microscopy/energy-dispersive X-ray spectroscopy (cryo-SEM/EDX). For abbreviations, please refer to Fig. 2

freeze-thawed trees, almost all vessels belonging to both earlywood and latewood lacked water (Fig. 5).

Discussion

After the FDNPP accident, many trees, including konara oaks, were contaminated by radioactive minerals, such as radiocesium, not only at their surface but also inside the tree bodies. This radionuclide was accumulated both in the sapwood and heartwood at half a year after the accident [4], and its accumulation was observed through a decade [e.g., 34, 35]. We recently reported that Cs was able to move from the outer sapwood to the heartwood by the combination of parenchyma cells' activity and apoplastic diffusion in Japanese cedar tree trunks [23]. Therefore, we hypothesized that Cs accumulated in the konara oak xylem might enter through the same way as in Japanese cedar. However, we could not anticipate its precise behavior because these two species considerably differ in both physiological and anatomic aspects, originating in differences between gymnosperms and angiosperms [13, 30]. To determine how radiocesium was accumulated in the konara oak xylem, we employed the same methodology that we developed in previous studies for standing trees, in which we used a Cs as a tracer of radial mineral movement at the cellular level [22–24]. It is known that minerals from bark are transported to the xylem via the cambium [18, 36]. Further experiments performed on konara oak revealed that Cs attached to the bark surface was transported to the xylem [27]. In addition, the sap solution flows only through the outer xylem layers, earlywood vessels of the current year, and latewood vessels of outer several years [10, 11]. Therefore, the presented experimental design is able to present how minerals move in the xylem after being absorbed either from the bark (often seen at the early phase of radioactive contamination) or from the soil via the roots (often seen at the steady-state phase of radioactive contamination) [37]. This method was proven highly suitable for investigating radial mineral movement through a konara oak trunk, with even more results than the studies on Japanese cedar.

Mineral radial movement in sapwood: parenchyma cells' activity and apoplastic diffusion

The conduit area of the injected Cs solution was confirmed by the red color of acid fuchsin [10, 11, 32] after felling the tree (Fig. 1e–g). The red area widened through the sapwood in the radial and tangential directions and across the annual ring borders as the injection duration increased. In the red area, Cs was detected in almost all cell types. To investigate whether Cs was

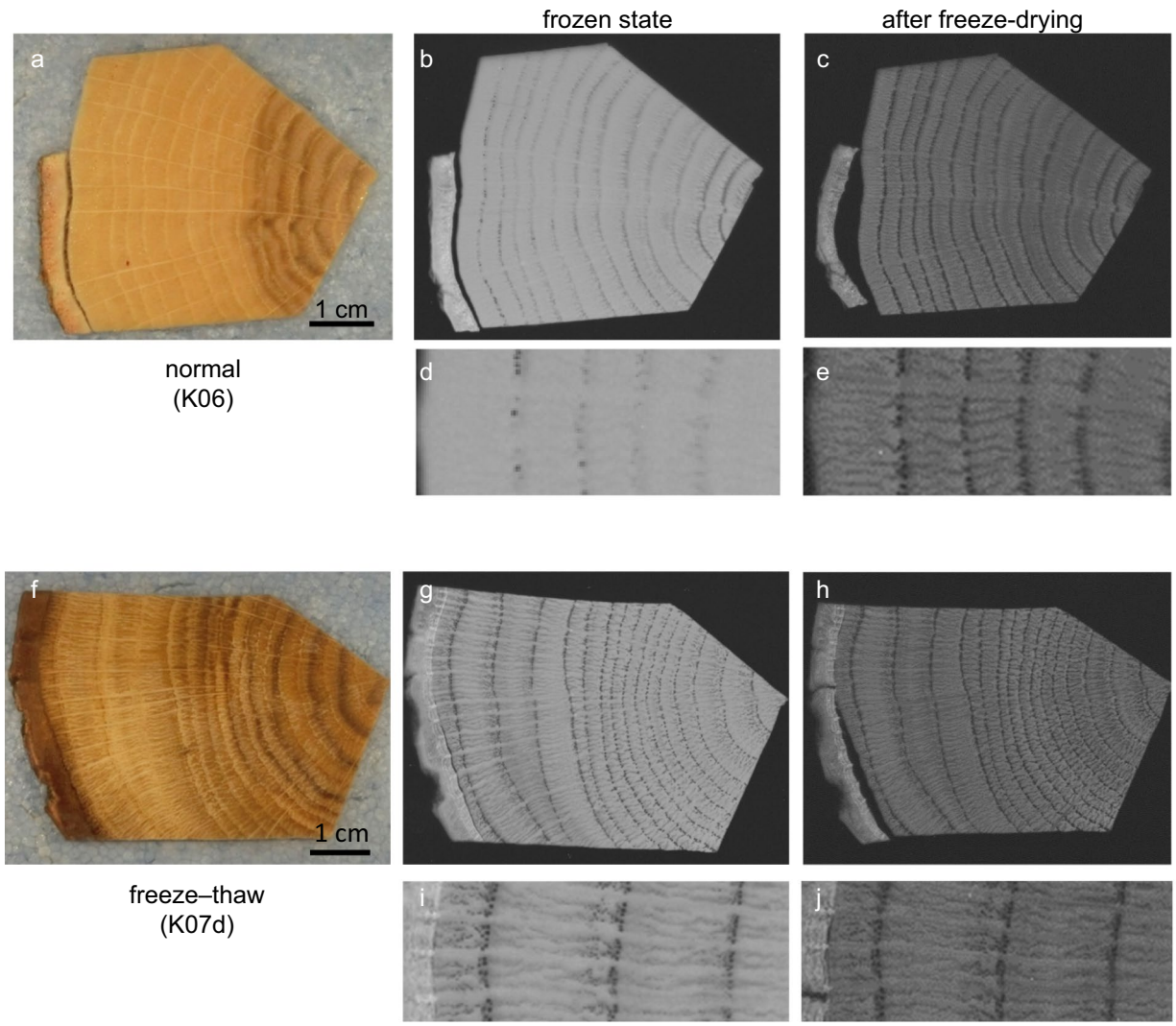

Fig. 5 Typical water distribution in a konara oak (*Quercus serrata*) trunk observed by soft X-ray photography. Optical (**a**, **f**) and X-ray photographs (**b**–**e**, **g**–**j**) of the transverse surface of controls (without Cs injection) of a normal tree (K06) and a freeze–thaw treated tree (K07b). A comparison of X-ray photographs between frozen-state wood (**b**, **d**, **g**, **i**) and that obtained after freeze-drying (**c**, **e**, **h**, **j**) reveals water distribution at the tissue level. **d**, **e**, **i**, and **j** are magnified images of **b**, **c**, **g**, and **h**, respectively

able to move by the activity of living parenchyma cells or by passive diffusion, we compared the Cs movement between trunks that contained living cells and trunks without living cells (Fig. 7). We killed living cells in tree trunks of standing konara oak trees by a freeze–thaw treatment to allow mineral movement only by diffusion [23]. A freeze–thaw treatment is known to cause freezing injuries that destroy the cell membrane of plant cells, resulting in the loss of cell functioning [38–41]. In this study, the cytoplasm of parenchyma cells was damaged after the freeze–thaw treatment (Additional file 1: Fig. S1), indicating their successful killing in a part of a standing konara oak trunk, while retaining the parenchyma cell walls. In freeze–thaw treated samples, the injected Cs moved only by diffusion. The Cs detection area was approximately the same for all cell types. It implies that there is no distinction in the area and speed of diffusion among cell types in konara oak trees. Conversely, in the normal trees, although the Cs detection area was the same for each cell type in the 3-h injection, the Cs detection areas in the 1- and 4-day injected accessions differed between the parenchyma

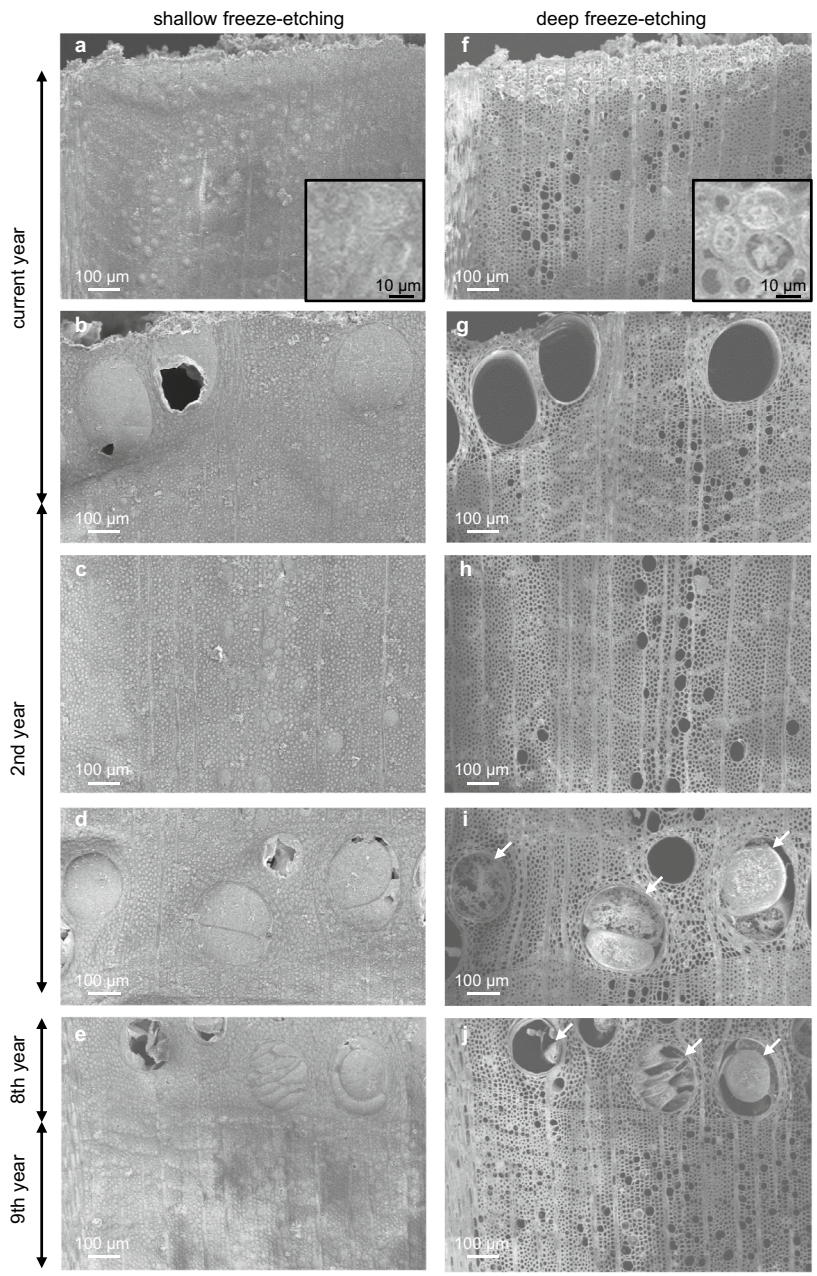

Fig. 6 Typical water distribution and cellular structures in konara oak (*Quercus serrata*) observed by cryo-scanning electron microscopy (cryo-SEM). A transverse section of annual rings from the current year, the 2nd year, and 8–9th year (innermost part of the sapwood) of a control (without Cs injection) of a normal tree (K06). Water distribution is clearly distinguished from cell walls, cell content, and tyloses (white arrows) as seen from the comparison between shallow (**a**–**e**) and deep freeze-etching (**f**–**j**) because deep freeze-etching removed sublimated ice. The boxes in **a** and **f** represent magnified images of axial parenchyma cells

cell-related structures such as RL, RW, and AL and other cell types such as VL, VW, FL, and FW, that is, Cs was detected in the inner part of the red area, and was farther in living parenchyma cell-related structures than in the other dead cells. These results indicate that parenchyma cells are the site of radial mineral movement in the konara oak xylem, and that Cs movement accomplished by the action of parenchyma cells runs faster than diffusion in konara oak trees.

Interestingly, the Cs detection areas of parenchyma cell walls and their lumina were almost the same in normal trees. It is known that the cell wall of a parenchyma cell

has blind pits that exchange substances inside and outside the cell [42, 43]. Therefore, it is possible that the injected Cs was excreted into the apoplast from parenchyma cells. In other words, parenchyma cells exude Cs into the apoplast while transporting it symplasmically. In addition, in the case of the 4-day injection of the K04 sample, Cs was sometimes detected as scattered in the cell walls of vessels or fibers in the inner sapwood (Fig. 2). These Cs detected points were too far from the injection point to be transported solely by diffusion. This result also indicates the existence of a pathway by which minerals that symplasmically move in the radial direction in parenchyma cells would be transported to the apoplast and diffuse apoplasmically. The presented results indicate that minerals in a konara oak trunk must be transported radially by the action of xylem parenchyma cells in addition to diffusion into the apoplast.

The pattern of the radial movement of minerals in konara oak, a hardwood species, is very similar to that of Japanese cedar, a conifer species [22, 23]. This resemblance suggests that the mechanism of radial mineral movement, including the activity of parenchyma cells in symplasmic movement, is universal across tree species. We did not find a difference in radial distribution of artificially injected Cs between axial and ray parenchyma cells, suggesting that both types of parenchyma cells have the same function in radial mineral movement. However, xylem anatomical features differ between konara oak and Japanese cedar. Axial parenchyma cells mostly surround vessels in both earlywood and latewood in the konara oak xylem, whereas those of Japanese cedar are distributed in latewood only. It has been reported that axial parenchyma cells surrounding vessels are involved in embolism repair in tree stems, suggesting that they may convert substances between the symplast and apoplast [14, 44, 45]. These facts may indicate the existence of a pathway by which minerals would move in the radial direction by virtue of ray parenchyma cells, being subsequently transported to axial parenchyma cells, and finally exuded into the apoplast and diffused. However, it is unlikely that axial parenchyma cells of Japanese cedar are the only place where minerals are transported from the symplast to the apoplast because they exist only in the latewood in quite a low percentage. Although we attempted to capture the moment during which Cs has moved across the plasma membrane, the spatial resolution of our cryo-SEM/EDX system was too low. The development of a method with a higher resolution is needed to visualize

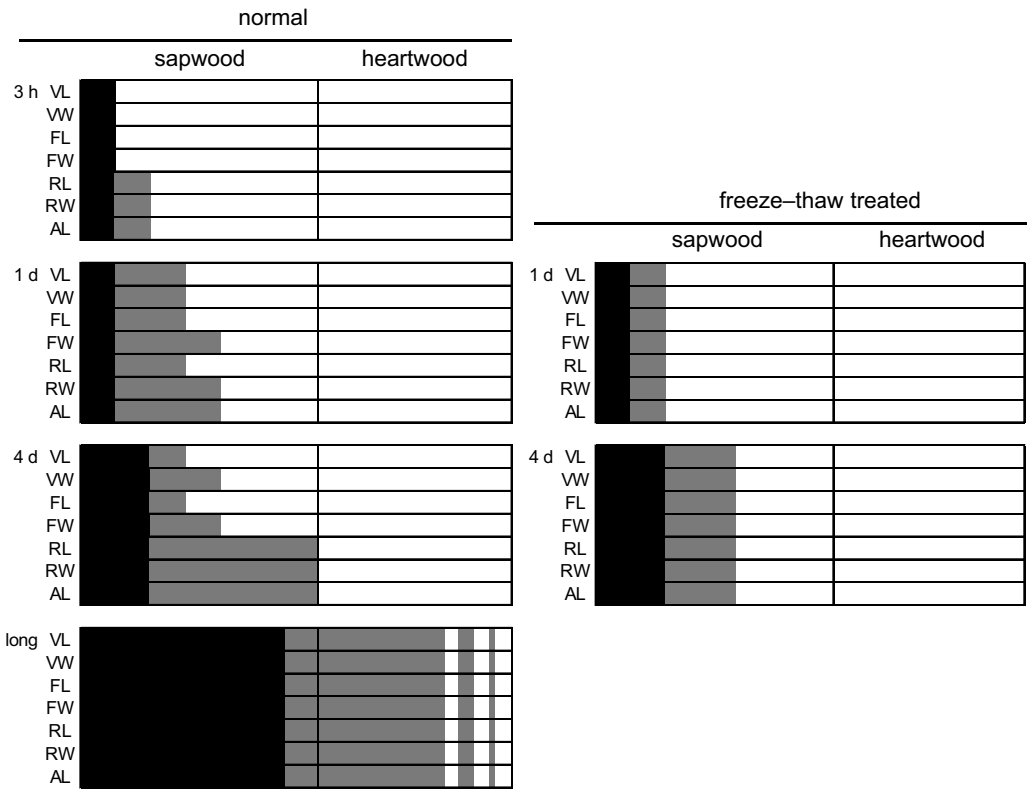

Fig. 7 Schematic representation of the radial distribution of Cs after injection into the outer sapwood of konara oak (*Quercus serrata*). Black and gray fields denote areas where Cs was detected. Black fields represent the red region stained by acid fuchsin. For abbreviations, please refer to Fig. 2

the place where minerals move between the symplast and apoplast. Elucidation of the differences in the activity of ray and axial parenchyma cells, including their function in mineral movement, remains a challenge.

Mineral radial movement from sapwood to heartwood: across a layer(s) between the two

In konara oak sapwood, Cs was found both in the symplast (in living parenchyma cells) and in the apoplast (outside the living cells). Since the heartwood consists of dead cells only (apoplast), there are two possible pathways of mineral movement from the sapwood to the heartwood: from the apoplast in the sapwood to the apoplast in the heartwood or from the symplast in the sapwood to the apoplast in the heartwood. In long-term Cs-injected samples (45 and 50 days), the red color obtained from the application of acid fuchsin reached the front of colored heartwood, and a layer immediately outside the colored heartwood was not dyed, which means that diffusion stopped in this layer. This result indicates that Cs movement from the sapwood to the heartwood is not accomplished only by simple diffusion.

Intriguingly, in Japanese cedar, the red color of acid fuchsin stopped at the intermediate wood, a layer between the sapwood and heartwood, and did not move to the heartwood [23], suggesting the existence of a barrier layer that would prevent diffusion between the sapwood and heartwood, which would be common for konara oak and Japanese cedar. In Japanese cedar, the waterless layer in the intermediate wood limited diffusion because water from the earlywood tracheid lumina in the intermediate wood was lost [31]. Therefore, it was concluded that Cs moved in parenchyma cells (symplast) from the outer sapwood to the intermediate wood and diffused into the heartwood apoplast. Although the intermediate wood may be an important factor for transition from the symplast to the apoplast, a konara oak tree does not have a clear layer of intermediate wood as seen in Japanese cedar that is represented by an easily distinguishable white zone [31, 33]. In addition, unlike in Japanese cedar, a change in water distribution was not observed at the boundary between the sapwood and colored heartwood in konara oak. During the transition from the sapwood to the heartwood, living parenchyma cells are submitted to cell death; hence, a place where living and dead parenchyma cells are adjoined must exist. Therefore, we speculate that minerals are being moved actively from living cells to dead cells at the layer of the innermost sapwood, which needs confirmation.

Thus, what are the common and different mechanisms that block diffusion at the sapwood–heartwood boundary among tree species? Abrupt changes in concentrations of various minerals at the sapwood–heartwood boundary have been reported for many tree species, including konara oak and Japanese cedar [28, 29, 46]. As observed, Mg, K, and Rb concentrations were decreased during transition from the sapwood to the heartwood in konara oak [29], whereas they increased in Japanese cedar [28, 46]. A similar tendency was obtained with mineral distribution in the sampled trees (Additional file 1: Fig. S2). If simple diffusion operates in these layers, such a drastic change in mineral concentration should not have occurred. These facts indicate that simple diffusion of minerals at the sapwood–heartwood boundary does not function, suggesting that a layer(s) blocking diffusion between the sapwood and heartwood is universal across tree species. However, the pattern of a mineral's concentration change varies among tree species, which indicates that the movement of minerals from the sapwood to the heartwood is different among tree species. For example, konara oak had a lesser accumulation of alkali metals in the heartwood than in sapwood, contrary to the pattern observed in Japanese cedar [28, 29], suggesting the suppressed movement of Cs from the sapwood to heartwood in konara oak. Wang et al. [27] failed to detect Cs in the heartwood. This inability can be due to the low quantity of Cs applied to the bark during the konara oak experiment because the same Cs quantity was used during the Japanese cedar experiments [26, 27]. To that end, there must be a mechanism that would select minerals to be moved into the heartwood, and this selectivity might be species dependent. In a Japanese cedar trunk, Cs injected into the sapwood moved to the heartwood, but Cs injected into the heartwood was difficult to move to the sapwood [24]. This result suggests that the selection of minerals occurs during the transport from the sapwood to the heartwood and vice versa. In this study, we revealed that Cs moves from the sapwood to the heartwood, but we could not obtain sufficient results to propose the mechanism by which minerals move. A possible elucidation of this unknown mechanism will clarify tree species specificity that concerns the accumulation of minerals in the heartwood.

How radiocesium has been accumulated in konara oak trunks after the FDNPP accident

Radiocesium was accumulated in the xylem of konara oak trunks from half a year to up to 10 years after the FDNPP accident [4–6, 25, 34, 35]. So far, there are no experimental data that would explain how radiocesium was accumulated there. To understand the pathway of radiocesium contamination, we need to know the mechanism of the radial movement of minerals in a konara oak trunk. Some reports suggest that radiocesium was directly attached to the bark surface and then moved inside the trunk because, owing to the lack of foliage, the surface

of the konara oak bark was directly exposed to contamination by radiocesium [5, 6, 8]. Minerals attached to the outer bark reach the inner bark by diffusion, and subsequently, those present in the inner bark move to the outer sapwood by symplasmic transportation via the cambium [14, 18, 26, 27, 36]. Moreover, this study reveals that minerals from the outer sapwood move to the inner sapwood and heartwood by symplasmic movement accomplished by the activity of parenchyma cells as well as by diffusion in the apoplast. Therefore, it can be concluded that the route of radiocesium accumulation in konara oak trunks can be explained by scientific evidences.

More than 10 years have passed since the FDNPP accident happened. Radionuclide contamination might have already reached its steady-state phase [37], in which radioactive compounds circulate between tree bodies and the soil, which means that their distribution in trees is strongly affected by plant physiology. Thus, radiocesium distribution would be similar to the natural distribution of stable Cs, K, and Rb that belong to the alkali metals [47]. Since the minerals accumulated in the heartwood are different among tree species, they should also differ in the accumulation pattern of radiocesium. To understand and explain future trends of tree trunks' contamination by radiocesium, further investigations are needed to unravel the mechanism of the radial movement of minerals, including the mechanism by which minerals are selected at the boundary between the sapwood and heartwood.

Conclusions

The motivation for this study was to scientifically decipher the mechanism by which the inside of a trunk can be contaminated after a nuclear accident. In this study, we succeeded to visualize at the cellular level the route of experimentally injected Cs moving in the radial direction through konara oak trunks, by prolonging the state of a standing tree. Our results showed that radiocesium deposited on the bark of a konara oak trunk moved by a combination of a rapid movement accomplished by the activity of ray and axial parenchyma cells and slower diffusion. Movement of minerals from the sapwood to the heartwood is considered a common mechanism across tree species because the same pattern was also exhibited by Japanese cedar. Nevertheless, this congruous pattern may have a mechanism for selecting minerals to limit their movement from the sapwood to the heartwood, which would be species specific, because the minerals that accumulate in the heartwood were shown to differ among tree species. Resolving this unknown mechanism that controls radial mineral movement would be useful to predict future trends of tree contamination by radionuclides for any tree species.

Abbreviations

FDNPP: Fukushima Daiichi Nuclear Power Plant; cryo-SEM/EDX: Cryo-scanning electron microscopy/energy-dispersive X-ray spectroscopy; LN_2: Liquid nitrogen; SE: Secondary electron; VL: Vessel lumen; VW: Vessel cell wall; FL: Fiber lumen; FW: Cell wall between fibers; RL: Ray parenchyma cell lumen; RW: Cell wall between ray parenchyma cells; AL: Axial parenchyma cell lumen.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s10086-022-02024-7.

Additional file 1. Additional figures.

Acknowledgements

We would like to thank Dr. T Miyama, Dr. T Ihara, and the Arboretum and Nursery Office of FFPRI for using sample trees. This work was supported by the Support program of FFPRI for researchers having family obligations.

Authors' contributions

KK contributed to the conceptualization and design, investigation, and writing the draft of this manuscript. KY and YI contributed to the experiments. All the authors read approved the final manuscript.

Funding

This work was supported by JSPS KAKENHI (Grant Numbers JP16H04936, JP18H02258).

Availability of data and materials

Not applicable.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Received: 9 November 2021 Accepted: 21 February 2022
Published online: 14 March 2022

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