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Growth Responses of *Holcus lanatus* L. (Velvet Grass) in Soils Contaminated with Cesium or Strontium

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Abstract: Radiocesium (r-Cs) and radiostrontium (r-Sr) released from nuclear accidents (e.g., Chornobyl, Fukushima) and routine operations (reactors, reprocessing) pose environmental and health concerns. Their primary pathway to humans is through plant uptake and subsequent bioaccumulation within the food chain. While soil amendments with potassium (K) and calcium (Ca) are known to mitigate r-Cs and r-Sr uptake, respectively, the impact on plant growth remains unclear. This study investigates the effects of Cs and Sr on the growth of *Holcus lanatus* L. seedlings under hydroponic and soil conditions with varying Cs and Sr concentrations. Stable isotopes of Cs and Sr served as non-radioactive analogs. Seedling growth was assessed across a range of Cs and Sr concentrations ($\leq$1 and $\geq$4 mg L^{-1}). The impact of the addition of K and Ca on Cs/Sr uptake in amended soils was also evaluated. Additionally, this study examined how Cs and Sr amendments affected the influx rates of other nutrients in *H. lanatus*. Higher Cs and Sr concentrations ($\geq$4 mg L^{-1}) significantly inhibited seedling growth, while lower concentrations had no effect. Notably, *H. lanatus* exhibited moderate Cs tolerance and strong Sr tolerance. Furthermore, K and Ca supplementation in Cs/Sr-amended soils demonstrably reduced plant uptake of these elements. This study also observed alterations in the uptake rates of other nutrients within *H. lanatus* due to Cs/Sr addition. This study suggests that *H. lanatus* exhibits moderate tolerance to Cs and Sr contamination, potentially making it suitable for revegetation efforts in contaminated grasslands. Additionally, K and Ca amendments show promise as a strategy to mitigate plant uptake of these radioisotopes further. These findings contribute to the development of safer revitalization strategies for areas impacted by nuclear accidents.

Keywords: wild grass; cesium; strontium; tolerance; growth traits; contaminated land reclamation

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1. Introduction

Long-lived radioisotopes cesium-137 (^{137}Cs) and strontium-90 (^{90}Sr) pose environmental threats due to their persistence in the biosphere (half-lives > 30 yrs) and potential food chain entry via plant uptake from contaminated soils and water [1–3]. While natural levels are innocuous, elevated ^{137}Cs and ^{90}Sr in the soil can significantly alter plant growth and development [3–5]. Cs and Sr compete with their chemical analogs potassium (K) and calcium (Ca) for plant uptake through shared transport mechanisms [1,6–9]. While increased soil K and Ca generally decrease Cs and Sr uptake, respectively [5,10–14], the relationship is complex, with some studies reporting variable effects of K concentration on Cs uptake [15,16]. Therefore, Cs and Sr accumulation likely depends on plant species and environmental conditions [5,17].

Green plants with tolerance to potentially toxic elements (PTEs) are employed for phytoremediation due to their ability to exclude PTEs and maintain low uptake even in highly contaminated soils [18–21]. Wild grasses possess inherent stress tolerance compared to domesticated species, allowing them to thrive under challenging environmental conditions, such as nutrient deficiencies, water scarcity, and so forth [19,22,23]. Their diverse genomes offer potential advantages for remediation efforts [24,25]. *Holcus lanatus* L., for example, exhibits tolerance to various abiotic stresses and several PTEs (As, Cd, Pb, Zn) in contaminated soils [21,26–29]. However, its tolerance to radiocesium (r-Cs) and radiostrontium (r-Sr) remains unknown. While K and Ca application is expected to reduce r-Cs and r-Sr uptake, respectively, due to their analogous nature and shared transporters, the precise role of K and Ca in mitigating r-Cs and r-Sr uptake, along with their impact on plant growth, requires further investigation. Furthermore, limited research exists on how r-Cs and r-Sr affect plants' uptake of other nutrients and non-nutrient elements.

This study investigates the potential of *H. lanatus*, a wild grass species, as a phytoexcluder for r-Cs and r-Sr. The efficacy of K and Ca amendments in reducing r-Cs and r-Sr uptake by *H. lanatus* grown in contaminated soils has been examined, while their impact on plant growth characteristics has also been evaluated. Additionally, this study explores the influence of r-Cs and r-Sr amendments on the mobility and plant availability of nutrient and non-nutrient elements within the soil. The overall aim is to assess the suitability of *H. lanatus* for revegetation of r-Cs- and r-Sr-contaminated areas. Furthermore, we aim to identify potential r-Cs and r-Sr excluder genes within *H. lanatus*, which could represent a novel approach to phytoremediation.

2. Materials and Methods

2.1. Seed

Mature, healthy *H. lanatus* L. seeds were obtained from the Fukushima University Campus, Japan, following stringent selection criteria to exclude diseased or damaged individuals. Before experimentation, the seeds were cleaned, followed by drying and storage in airtight polyethylene bags. Finally, the seeds were de-husked.

2.2. Soil

This study utilizes Kuroboku, a Japanese Andosol soil characterized by its dark grayish color (Munsell soil color code: 5YR/4/1) and high organic matter content. Prevalent on terraces, hillsides, and gentle slopes throughout Japan, Kuroboku is a popular choice for gardening applications. To ensure consistency across the experiment, commercially available Kuroboku soil was obtained from the Koujiya Co. (Hitachinaka, Japan), with the specific source unspecified.

Soil pH was measured in a 1:2.5 (w/v) ultrapure water solution using a calibrated LAQUA pH meter F-71 (HORIBA Scientific, Kyoto, Japan). Total organic carbon (TOC) content was determined using a TOC-L analyzer coupled with an SSM-5000A solid sample combustion unit (Shimadzu, Kyoto, Japan). Particle size distribution was analyzed using laser light diffraction on a Malvern Mastersizer 3000E system (Malvern Instruments, Worcestershire, UK). Metal ion extraction from soil followed the EPA method 3052 [30,31] utilizing microwave-assisted wet digestion with a Multiwave 3000 microwave reaction system (Anton Paar GmbH, Graz, Austria). The total concentration of monovalent (M^+) and divalent/trivalent (M^{2+}/M^{3+}) cations was determined by either NexION 300S inductively coupled plasma mass spectrometry (ICP-MS) equipped with an S10 autosampler (PerkinElmer, Waltham, MA, USA) or MP-AES 4100 microwave plasma atomic emission spectroscopy (MP-AES) with an ASX-500 autosampler (Agilent Technologies, Santa Clara, CA, USA). Working standard solutions (mg L^{-1} to μg L^{-1}) were prepared by diluting 1000 mg L^{-1} single-element standard solutions of stable elements (Kanto Chemical, Tokyo, Japan). Table 1 shows the physical and chemical properties of the soil.

Table 1. Soil properties.

Properties		**Mean**	**SD**
Texture	Sand (%)	64.4	0.34
	Silt (%)	33.4	0.31
	Clay (%)	2.2	0.12
pH		7.15	0.02
Organic matter (%)		30.69	0.33
Element content (mg kg^{-1})	Al	53945	3502
	As	3.618	0.074
	Ca	20670	1189
	Cd	0.228	0.013
	Co	1.58	0.017
	Cr	7.37	1.304
	Cs	0.52	0.033
	Cu	79.5	4.716
	Fe	11089	491.8
	K	9232	136.8
	Mg	5318	363.5
	Mn	538.2	32.03
	Mo	1.04	0.521
	Na	3620	88.53
	Ni	6.55	0.457
	P	4035	46.56
	Pb	9.514	0.642
	Sr	119.5	6.062
	Zn	244.8	8.758

2.3. Experimental Design and Treatment Combinations for Hydroponic Experiment

H. lanatus seeds were germinated in Petri dishes lined with two moistened filter papers. Ultrapure water was used to maintain constant filter paper moisture throughout germination. Following germination, seedlings were carefully transferred to polystyrene cups containing a floating plastic net base for support. A randomized complete block design with six treatments and three replicates per treatment was employed. Treatments included control (0 mg L^{-1}) and increasing concentrations of Cs or Sr (0.5, 1, 2, 4, and 8 mg L^{-1}). Stable Cs and Sr isotopes served as analogs for r-Cs and r-Sr, respectively. Cs and Sr solutions were prepared from CsCl (Nacalai Tesque, Kyoto, Japan) and $Sr(NO_3)_2$ (Merck KGaA, Darmstadt, Germany) salts, equilibrated for 24 h before use. Working solutions were prepared by dilution with ultrapure water (resistivity > 18.2 MΩ·cm) obtained from a Milli-Q water purification system (Merck KGaA, Darmstadt, Germany). Each cup contained 25 germinated seeds. All cups were maintained at room temperature and humidity under natural light, supplemented with LED lighting as needed.

2.4. Data Recording of Seedling Growth

Seedlings were co-cultivated for two months. Following this period, three representative seedlings were randomly selected from each treatment for growth parameter measurements. Physical growth parameters were assessed at harvest. Additionally, the shoot-to-root ratio was calculated.

2.5. Experimental Design, Treatment Combinations, and Data Recording for Soil Experiment

H. lanatus seedlings were cultivated in pots containing soil and allowed to grow for one year. Established seedlings were then subjected to the following treatments, applied sequentially: T0 (control), T1 (Cs, 15 mg kg^{-1}), T2 (Sr, 15 mg kg^{-1}), T3 (Cs, 15 mg kg^{-1} + K, 200 mg kg^{-1}), and T4 (Sr, 15 mg kg^{-1} + Ca, 200 mg kg^{-1}). Following treatment application, the seedlings were allowed to grow for an additional month. Regular weeding and watering were performed throughout the cultivation period. At harvest, shoot biomass was measured.

2.6. Analysis of Plant Samples

Following cleaning and drying, plant materials from the soil experiment were subjected to acid digestion using a DigiPREP block digestion system (SCP Science, Quebec, Canada). Accurately weighed 0.1 g subsamples were transferred to digestion tubes, followed by the addition of 5 mL HNO_3 (Nacalai Tesque, Kyoto, Japan) per tube. Samples were left to stand overnight. Subsequently, 1.25 mL H_2O_2 (Kanto Chemical, Tokyo, Japan) was added to each tube, followed by 2 h of digestion at 120 °C. Digested samples were then cooled and diluted with ultrapure water to a final volume of 20 mL. Analysis of stable elements (Cs, Sr, K, Ca, P, Mg, Na, Fe, Mn, Mo, Zn, As, Co, and Ni) was performed using either ICP-MS or MP-AES.

2.7. Statistical Analysis

Univariate and multivariate general linear models (GLMs) were employed to assess potential significant differences between treatment groups. Following a one-way analysis of variance (ANOVA) with a significance level of $\alpha = 0.05$, Duncan's multiple range test (DMRT) was used for pairwise mean comparisons. Significant main effects identified by ANOVA were further evaluated using a post hoc DMRT test to determine specific treatment contrasts with statistically significant differences.

3. Results

Increasing Cs and Sr concentrations ($\geq$4 mg L^{-1}) significantly hampered *H. lanatus* seedling growth, evidenced by reductions in root length, root biomass, and shoot biomass. Conversely, lower concentrations ($\leq$1 mg L^{-1}) stimulated growth compared to the control. Root length peaked at 25 and 22 cm with 0.5 mg L^{-1} Cs and Sr, respectively, but dropped significantly to 12.7 and 13.6 cm at 8 mg L^{-1} (Cs, $p < 0.001$; Sr, $p = 0.001$). Similarly, root biomass peaked at 11.4 and 8.7 mg with 1 mg L^{-1} Cs and Sr, respectively, and decreased to 3.9 and 4.4 mg at 8 mg L^{-1} (Cs, $p < 0.001$; Sr, $p = 0.001$). Shoot biomass also displayed a concentration-dependent response, reaching maxima of 19.4 and 17.4 mg with 1 mg L^{-1} Cs ($p < 0.001$) and 0.5 mg L^{-1} Sr ($p < 0.001$), respectively. Notably, lower Cs and Sr concentrations ($\leq$ 1 mg L^{-1}) appeared to favor root development over shoot growth, as indicated by a lower shoot-to-root ratio (Figures 1 and 2; Supplementary Tables S1 and S2).

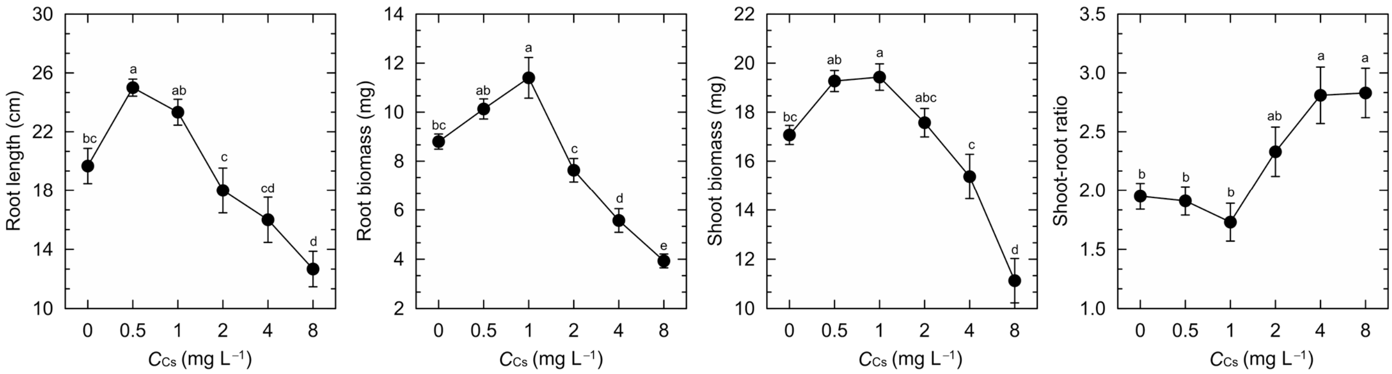

Figure 1. Effect of Cs concentrations (C_{Cs}, 0.5–8 mg L^{-1}) on the growth of *Holcus lanatus* seedlings in hydroponic conditions. Statistically significant differences ($p \leq 0.05$) are denoted by different lowercase letters.

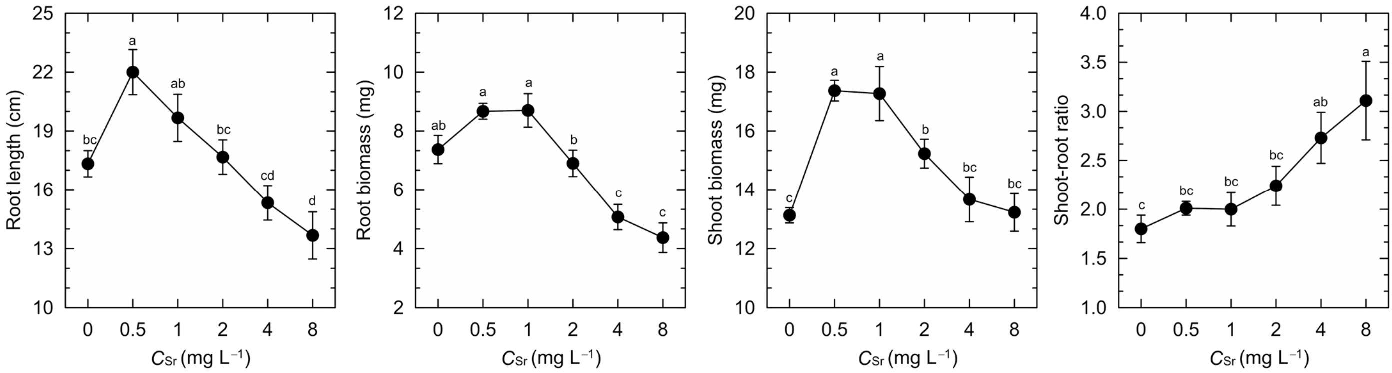

Figure 2. Effect of Sr concentrations (C_{Sr}, 0.5–8 mg L^{-1}) on the growth of *Holcus lanatus* seedlings in hydroponic conditions. Statistically significant differences ($p \leq 0.05$) are denoted by different lowercase letters.

Soil amendment with Cs led to a decrease in *H. lanatus* shoot biomass production but a significant increase ($p \leq 0.05$) in Cs influx. However, the co-application of K with Cs mitigated this effect, enhancing shoot biomass and reducing Cs influx. The transfer factor (TF) of Cs in Cs-amended soil (0.39) was less than 1, indicating Cs exclusion by this Cs-tolerant plant. Notably, K addition further reduced Cs influx by 5.1%. Interestingly, K application in Cs-amended soil also significantly decreased ($p \leq 0.05$) K influx in *H. lanatus* (Figure 3; Supplementary Table S3).

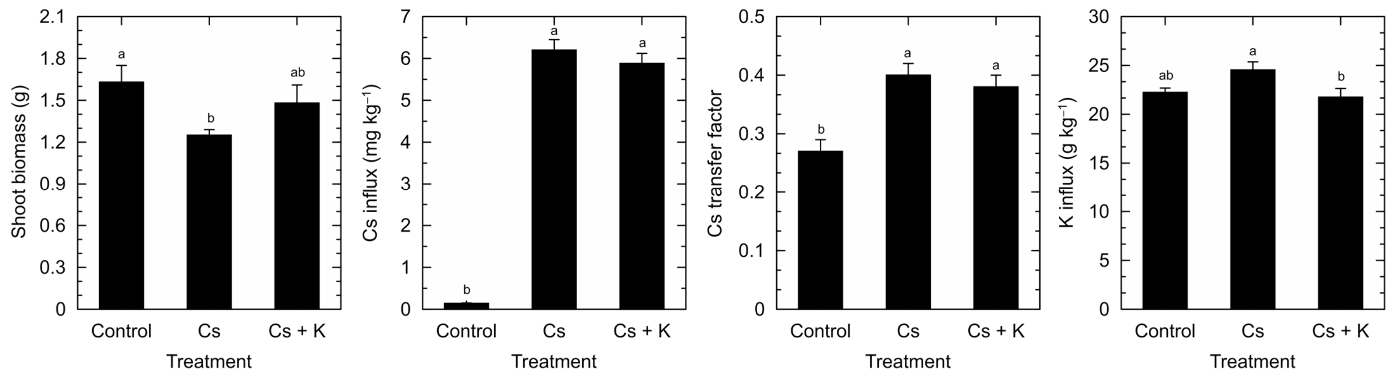

Figure 3. Effect of K-application on Cs-amended soil (C_{Cs}, 0.5–8 mg L^{-1}) on shoot biomass production, shoot Cs influx, Cs transfer factor, and K influx in *Holcus lanatus* seedlings. Statistically significant differences ($p \leq 0.05$) are denoted by different lowercase letters.

Similar to Cs, Sr introduction to the soil negatively impacted *H. lanatus* shoot biomass production while significantly increasing Sr influx ($p \leq 0.05$). However, Ca co-application with Sr mitigated this effect, enhancing shoot biomass and significantly reducing Sr influx ($p \leq 0.05$). The TF of Sr in Sr-amended soil (0.12) was less than 1, suggesting Sr exclusion by this Sr-tolerant plant. Notably, the addition of Ca further decreased Sr influx by 28%. Interestingly, Ca application in Sr-amended soil also significantly reduced ($p \leq 0.05$) Ca influx in *H. lanatus* (Figure 4; Supplementary Table S4).

Soil amendment with Cs significantly increased ($p \leq 0.05$) the influx of several elements in *H. lanatus*, including the nutrients P, Ca, Mg, and Mn, and the non-nutrients Sr and Ni. Conversely, Cs decreased the influx of nutrients Mo and Zn, along with the non-nutrients As and Co. Similarly, Sr amendment significantly increased ($p \leq 0.05$) the influx of the nutrients Al, Ca, Mn, Mo, and Na, as well as the non-nutrient Ni. However, Sr decreased the influx of the nutrients P, Fe, Mg, and Zn in *H. lanatus*. These findings suggest that Cs and Sr accumulation influence the plant's uptake of various elements, potentially impacting plant health (Figures 5 and 6; Supplementary Tables S3 and S4).

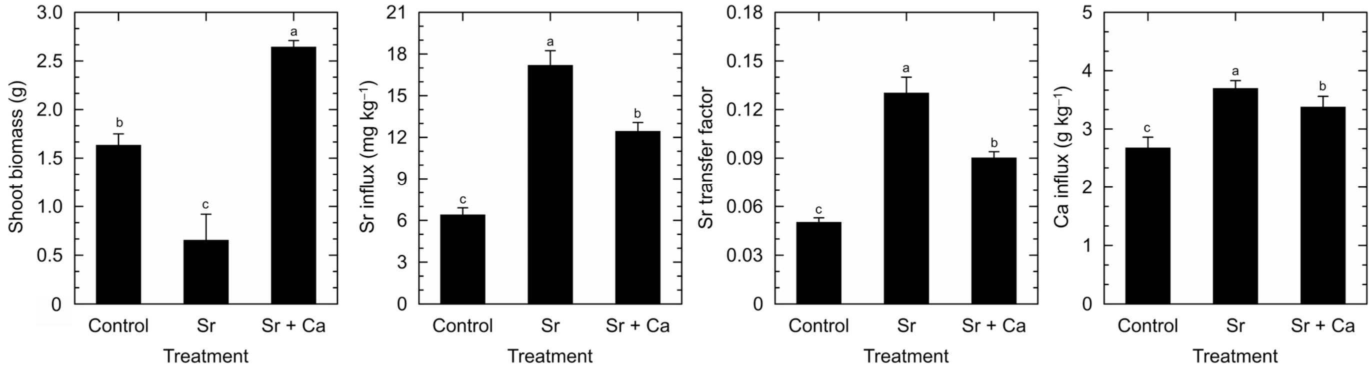

Figure 4. Effect of Ca application on Sr-amended soil (C_{Sr}, 0.5–8 mg L^{-1}) on shoot biomass production, shoot Sr influx, Sr transfer factor, and Ca influx in *Holcus lanatus* seedlings. Statistically significant differences ($p \leq 0.05$) are denoted by different lowercase letters.

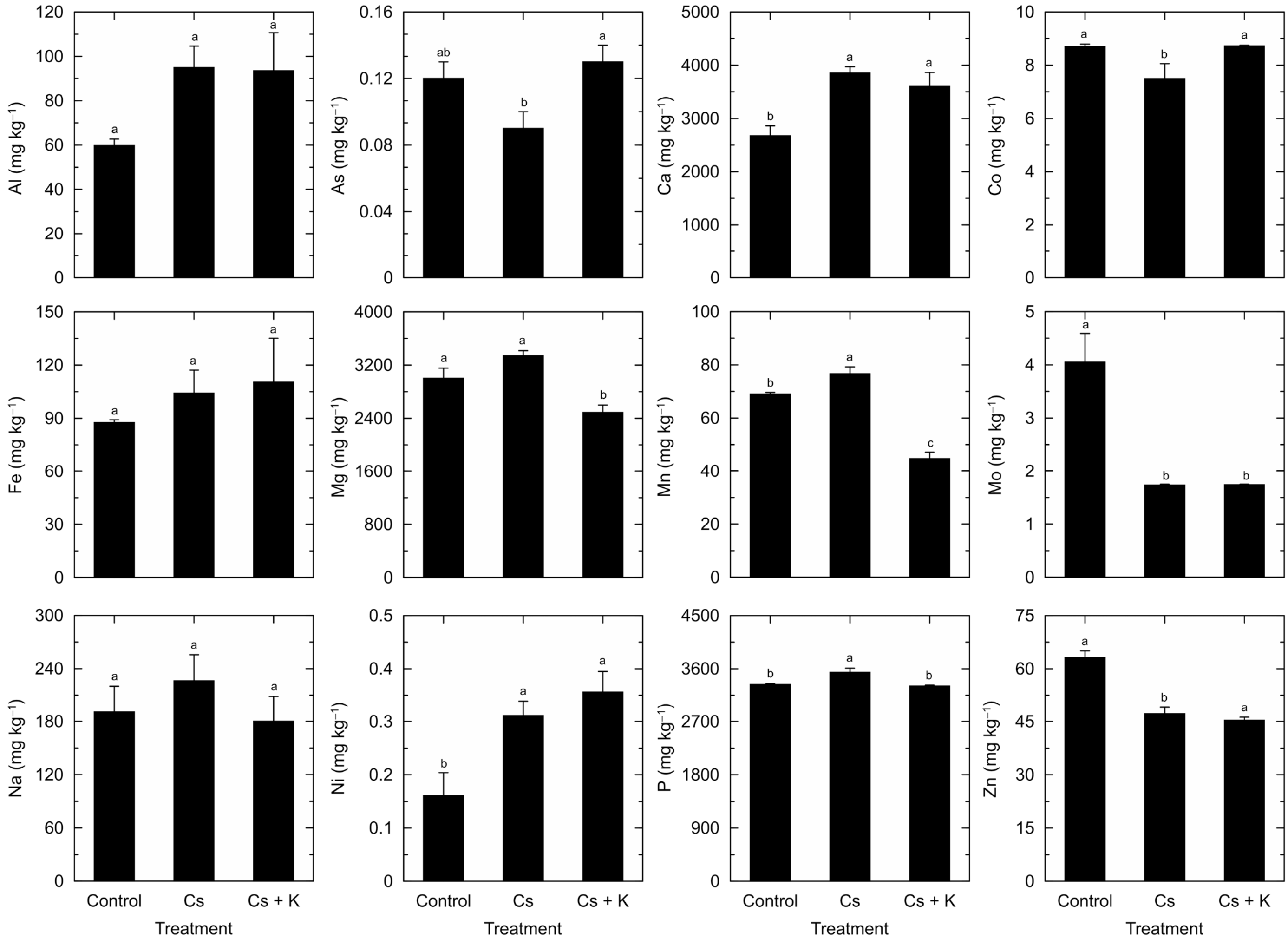

Figure 5. Effect of K application on Cs-amended soil on shoot nutrient and non-nutrient elemental influx in *Holcus lanatus* seedlings. Statistically significant differences ($p \leq 0.05$) are denoted by different lowercase letters.

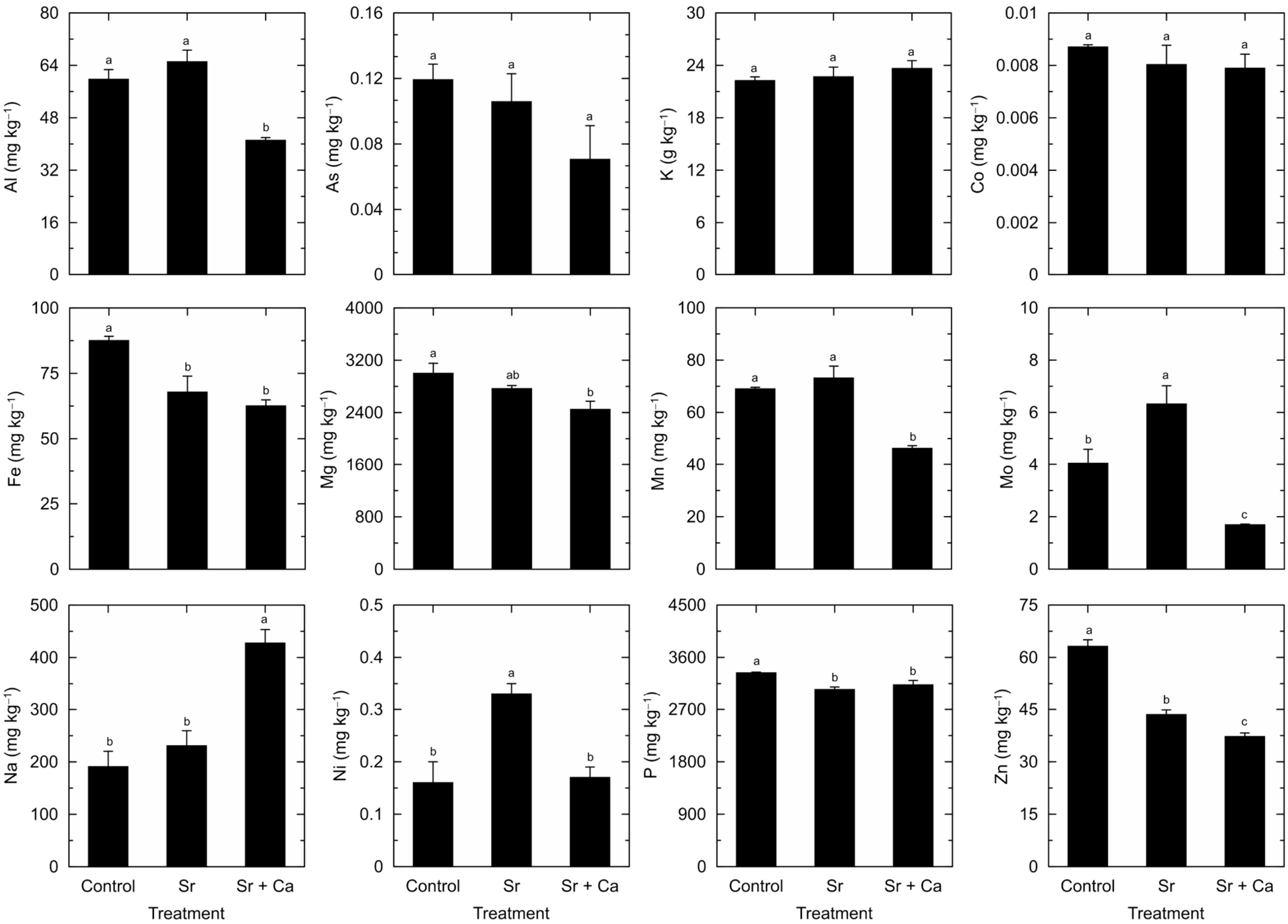

Figure 6. Effect of Ca application on Sr-amended soil on shoot nutrient and non-nutrient elemental influx in *Holcus lanatus* seedlings. Statistically significant differences ($p \leq 0.05$) are denoted by different lowercase letters.

4. Discussion

This study explored the response of *H. lanatus* to varying Cs and Sr concentrations and the influence of co-occurring K and Ca on tolerance in Cs- and Sr-contaminated soils. Hydroponic experiments revealed that higher Cs and Sr concentrations ($\geq$4 mg L^{-1}) significantly inhibited seedling growth, primarily affecting root length and biomass production. Conversely, lower concentrations ($\leq$1 mg L^{-1}) stimulated seedling growth compared to the control. Notably, at lower Cs and Sr levels, the shoot-to-root ratio suggested preferential allocation of biomass towards root development.

The soil experiments corroborated findings from the hydroponic experiments, demonstrating decreased shoot biomass and increased Cs/Sr uptake with soil contamination. However, co-application of K and Ca with Cs/Sr mitigated these negative effects, enhancing shoot biomass and reducing Cs/Sr uptake. This aligns with established knowledge on plant nutrient uptake, as reported by Rinaldi et al. [13] and Burger and Lichtscheidl [32], who documented efficient Cs/Sr absorption from the soil. While natural soil and water harbor low Cs/Sr levels, posing no visible toxicity threats, elevated concentrations become detrimental to plant growth [3–5]. The primary mechanism for this growth inhibition likely involves competitive exclusion. Chemically similar to K and Ca, respectively, Cs and Sr compete with these essential nutrients for uptake, hindering their acquisition by the plant [33]. Supporting this notion, Mohamed et al. [34] observed internal Cs inhibiting root elongation in rice.

This study observed that lower Cs and Sr concentrations resulted in a greater biomass allocation to roots than shoots in *H. lanatus*. This aligns with findings by Hill et al. [35], who reported increased root biomass production in pasture species under nutrient-scarce conditions. Such a response is often accompanied by modifications in root architecture, including enhanced root surface area through root hair development, and upregulation of nutrient transporter expression [36–41]. Moreover, Gojon et al. [42] and Lynch [43] suggest that nutrient limitations can also induce alterations in root growth patterns.

The influence of radionuclide concentration on plant root biomass is mediated by a complex interplay of factors. These factors include characteristics of the soil environment, bioaccumulation processes within the plant, and the differential distribution of radionuclides across various plant tissues [44–47]. The observed root morphological changes may be linked to K and Ca uptake optimization. Co-application of K and Ca with Cs/Sr in contaminated soil reduces the influx of both Cs/Sr and their respective counterparts. This competitive uptake likely stems from the chemical resemblance between Cs/K and Sr/Ca, potentially leading to the suppression of high-affinity transporters for these elements within the plant [1,4,5,8]. Such suppression could induce K and Ca deficiency, contributing to Cs and Sr tolerance development. While the specific transporters involved in Cs and Sr uptake can vary based on plant species and cultivation conditions [32,48], existing transport mechanisms generally demonstrate effectiveness even under low or high K and Ca concentrations. Furthermore, increased root biomass at lower Cs/Sr concentrations might be a strategy to enhance the uptake of alternative nutrients. Under Cs stress, *H. lanatus* may prioritize P, Ca, Mg, and Mn uptake, while Sr stress may trigger increased Ca, Mg, and Mo uptake [49]. This could be a compensatory mechanism to address K and Ca deficiency. It is important to note that plant tolerance to Cs and Sr varies. Low levels of some non-essential elements, like As, can even stimulate plant growth, including root development [50–52]. The existing literature, including Smith et al. [53] and references cited therein, suggest that PTEs in contaminated soil generally do not decrease root length percentages.

Soil amendments containing radionuclides can influence plant uptake of essential elements through complex interactions. These interactions involve root exudates, competition or synergy between essential elements, hydrological conditions within the soil, and inherent soil properties [54–58]. Soil amendment with Cs resulted in increased plant uptake of P, Ca, Mg, Mn, Sr, and Ni, while decreasing Mo, Zn, As, and Co uptake in *H. lanatus*. Similarly, Sr addition increased Al, Ca, Mn, Mo, and Na uptake, but decreased P, Fe, Mg, and Zn uptake. These changes likely reflect Cs and Sr influencing element mobilization in the soil, which can directly and indirectly impact plant health through altered nutrient availability (uptake). Previous studies have shown that element mobility in soil is influenced by the presence or absence of specific elements [59–64]. The observed increase in plant uptake of certain nutrients (P, Ca, Mg, and Mn with Cs; Ca, Mn, and Mo with Sr) might be a compensatory response to K and Ca deficiency caused by competitive uptake with Cs and Sr, respectively [49]. This competition arises from the chemical similarity between Cs/K and Sr/Ca, potentially leading to plant K and Ca deficiency.

Future research efforts should prioritize understanding the mechanisms underlying plant tolerance to various environmental stressors, such as salinity [65–67], drought [68,69], and heat [70,71]. These stress responses often involve common pathways that may also be relevant to radionuclide tolerance. Moreover, visualizing mineral element dynamics within plants [72] using real-time radioisotope imaging systems [73,74] would help in studying radionuclide uptake and distribution with high spatial and temporal resolution. Exploring the role of mycorrhizal fungi in plant tolerance, particularly their stress tolerance mechanisms [75], can contribute to enhancing plant resilience against environmental stressors, including radionuclides. Additionally, research on ontogenetic switches from plant resistance to tolerance [76] can provide a more comprehensive understanding of plant responses to stress. To effectively identify radionuclide-tolerant plant species, future research should embrace interdisciplinary approaches. This necessitates the integration of molecular biology, plant physiology, and cutting-edge imaging techniques. By leveraging existing knowledge of plant stress responses and adaptation mechanisms, researchers can elucidate the specific mechanisms underlying plant tolerance to radionuclides. This deeper understanding will pave the way for the targeted selection and development of radionuclide-tolerant plant species.

5. Conclusions

This study identifies *H. lanatus* as a promising candidate for phytoremediation and bioengineering applications in Cs- and Sr-contaminated environments. The wild grass exhibits tolerance (exclusion) towards both Cs and Sr, with a moderate Cs transfer factor (TF = 0.39) and a high Sr transfer factor (TF = 0.12). Furthermore, co-application of K and Ca with Cs or Sr in contaminated soils significantly reduces Cs and Sr influx in *H. lanatus*. These findings suggest two potential applications: (a) *H. lanatus* could be a suitable choice for revegetation efforts in r-Cs- and r-Sr-contaminated grassland soils, as it minimizes the uptake of these elements into animal fodder; and (b) genes responsible for Cs and Sr exclusion in *H. lanatus* could potentially be transferred into crop tissues, enabling the development of crops that produce safe food products even when grown in polluted soils.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/soilsystems8020057/s1, Table S1: GLM output demonstrating the effects of various Cs-concentrations on the growth parameters of *Holcus lanatus*; Table S2: GLM output demonstrating the effects of various Sr-concentrations on the growth parameters of *Holcus lanatus*; Table S3: GLM output demonstrating the effects of K-application in Cs-amended soil on the *Holcus lanatus* growth and elemental uptake; Table S4: GLM output demonstrating the effects of Ca-application in Sr-amended soil on the *Holcus lanatus* growth and elemental uptake; Figure S1: Growth characteristics of *H. lanatus* at different Cs-concentrations. From left to right: 0 (Control), 1, 2, 4, and 8 mg Cs L^{-1}; Figure S2: *Holcus lanatus* seedling growth in soil: Control (right), Sr (middle), and Sr + Ca (left).

Author Contributions: B.M.K.: Conceptualization, Methodology, Software, Validation, Investigation, Data Curation, and Writing—Original Draft; M.F.A.: Investigation, Formal Analysis, Data Curation, and Writing—Review and Editing; Z.A.B.: Investigation, Formal Analysis, Data Curation, and Writing—Review and Editing; I.M.M.R.: Conceptualization, Methodology, Software, Validation, Investigation, Resources, Writing—Review and Editing, Visualization, Supervision, Project Administration, and Funding Acquisition. All authors have read and agreed to the published version of the manuscript.

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