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Uptake of sediment-derived carbon into aquatic plants:
Implications for transfer of ^{14}C from below-ground sources.

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ABSTRACT

With the increasing reliance on nuclear power generation for global energy demand, there is a growing concern over radioactive waste disposal and its environmental impacts. Geological disposal is mostly favored for its safety; however, uncertainties persist regarding routine emission of radionuclides from nuclear facilities, hazardous spent fuel, or any other unforeseen accidents. Among all the radionuclides released ^{14}C is considered significant due to its longer half-life (5730 years), and its ability to mix with stable isotopes of C makes it easily incorporated into the C compounds essential for life. The release of ^{14}C from nuclear power plants or discharge from underground repositories may enter nearby aquatic ecosystems posing a risk of incorporation by aquatic organisms and subsequent transfer into aquatic food web. Nonetheless, understanding the uptake pathway of this radionuclide in aquatic plants remains limited. In this study, we investigated the transfer of sediment-derived C into four aquatic plant species including free-floating *Lemna. minor*, submerged *Littorella uniflora*, emergent *Stachys palustris*, and *Lysimachia nummularia*, cultivated on approximately 8000-year-old residual peat, to distinguish between sediment-derived and the atmosphere C. A two-pool isotope mixing model was used to calculate the fraction contribution of sediment-derived C in the plants. Our results indicated significant variability in the transfer of sediment-derived C among the studied aquatic plant species. Free-floating, *L. minor* exhibited the highest uptake of sediment-derived C of (60%), followed by submerged *L. uniflora*, (root: 19.0%, leaves:19.7%). In contrast, emergent species *S. palustris* and *L. nummularia* showed

lower uptake percentages (roots: 7.79-9.22%, leaves 8.11-1.16%). The variability was associated with differences in plant morphology, physiology, and mechanism of C assimilation pathways. The transfer of sediment-derived C using ^{13}C has a similar trend to that observed in the ^{14}C datasets except in *L. minor* where the $\delta^{13}\text{C}$ underestimates the uptake percentage. This could be associated with the plant's photosynthetic pathways and associated fractionation. Moreover, a similar uptake of sediment-derived C was observed between roots and leaves potentially because of aerenchyma or other well-developed gas transport systems in aquatic plants. In addition to investigating ^{14}C uptake, we also determined the uptake of ^{13}C to assess whether ^{14}C could be sufficiently predicted by ^{13}C uptake in the selected aquatic plant species. Despite a slight difference in uptake of ^{13}C compared to ^{14}C in *L. minor*, a strong correlation was found between the two isotopes in most of the plants indicating that ^{13}C as a stable isotope can be used, with caution, as a substitute for relatively long-lived ^{14}C . However, further research is needed to validate these findings and explore their broader implications.

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Abbreviations and definitions

C	Carbon
¹⁴ C	Carbon -14
¹³ C	Carbon -13
DIC:	Dissolved Inorganic Carbon
DOC	Dissolved Organic Carbon
POC	Particulate Organic Carbon
IRMS	Isotopes Ratio Mass Spectroscopy
AMS	Accelerator Mass Spectrometer
AFW	Artificial Fresh Water
MOB	Methane Oxidizing Bacteria
IAEA	International Atomic Energy Agency

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1 Introduction

In recent years there has been an accelerated trend in nuclear power generation to meet global energy demands, consequently producing a substantial amount of radioactive waste (IAEA, 2020). Finland, for example, heavily relies on nuclear energy, meeting approximately 33% of its total energy demand through nuclear power plants (World Nuclear Association, 2023). Nuclear facilities discharge various radioactive wastes including both short-lived and long-lived radionuclides. The radioactive waste has raised significant concerns regarding its potential radiotoxicity effects on humans and the environment (Uchirin et al., 1997; Limer et al., 2013). Therefore, proper handling, management, and disposal of nuclear waste are crucial for ensuring the long-term safety of human health and the environment.

Currently, deep geological disposal is considered the safest and most effective way to ensure the long-term protection of both humans and the environment from the radioactivity of nuclear wastes (NEA, 1999; IAEA, 2009). Countries like Finland and Sweden follow the same deep geological disposal concept as a preferred method for the safe disposal of long-lived nuclear wastes. However, uncertainties persist regarding potential radionuclide discharges from nuclear power plants, mining operations of fuels for reactors, and the vulnerability of nuclear power plants to sabotages or other unintentional accidents. Since, even though the probability of such events is very low theoretically, it cannot be ruled out in the long run as the impact of these radionuclides on the biosphere is still unclear. Therefore, understanding the behavior, pathways, and processes of the potential release of radionuclides into the biosphere is needed for the proper assessment of future risks.

^{14}C is recognized as one of the important radionuclides released from nuclear wastes due to its significant contribution to doses in the biosphere (Posiva, 2010; IRSN, 2012). Carbon is one of the essential elements and a fundamental source of energy for all living organisms. When ^{14}C is released into the environment it mixes with stable carbon isotopes (^{12}C and ^{13}C) and enters the biological carbon pool. Considering its potential radioecological consequences and long half-life of 5730 years once it is incorporated into biota or transferred through the food web it can significantly impact the entire dynamic of the biosphere. Moreover, ^{14}C has significant resident time

in the atmosphere and hydrosphere allowing it to distribute uniformly worldwide through various processes (Pérez-Sánchez et al., 2009). As a result, its radioactivity exposure is not confined to the point of release but can have a global impact (Pérez-Sánchez et al., 2009). The ^{14}C released from nuclear reactors or geological disposal facilities are most likely in gaseous form ($^{14}\text{CO}_2$ and $^{14}\text{CH}_4$) along with dissolved carbonate species to some extent (Mobbs et al., 2014). Both gaseous and dissolved ^{14}C species follow complex pathways in the biosphere.

Overall, data on the uptake rate and mechanism of this radionuclide in many ecosystems and their species is still lacking. Such data is particularly important concerning plants as primary producers at the base of the food web, serving as food sources for animals and humans. They largely assimilate atmospheric carbon via photosynthesis but a small portion of uptake from soil carbon has also been reported (Smith et al., 2012; Mobbs et al., 2014). However, in the case of discharging ^{14}C from nuclear reactors or underground radioactive waste disposal facilities, the uptake by roots may significantly increase from soil into plants. The released ^{14}C from underground facilities might be transported upward by diffusion or dissolved in groundwater and further leach down to the neighboring aquatic ecosystems in the forms of particulate (POC) and dissolved organic matter (DOC) (Weidel et al., 2008). This allochthonous organic carbon can be incorporated into deposited sediment as particulate C or suspended in water columns in the form of dissolved inorganic carbon (DIC). ^{14}C -bearing species that end up in aquatic ecosystems can be readily available for uptake by roots of aquatic vegetation. Aquatic plants are the source of food and shelter as hiding places for many aquatic species from predators. They are also important for the general integrity of water ecosystems. Therefore, possible contamination of aquatic vegetation with ^{14}C may result in further risk through the food chain. Hence, it is significant to properly assess the proportion of sediment-derived C in these organisms. Such studies also fill the knowledge gaps on the transfer of this radionuclide in aquatic plants and develop radioecological modelling of ^{14}C in aquatic environments.

To investigate the possible transfer of sediment-derived C into aquatic plants and to distinguish its relative contribution from atmospheric C, isotopic analysis was used. In this approach, a cutaway peatland based on its distant ^{14}C signature from the air was used to determine the transfer of C from organic matter. The use of natural abundance was preferred over

artificial labelling because of its radiological hazards to the environment. Such a natural system with differences in ^{14}C content provides a valuable opportunity to distinguish sediment-derived C from atmospheric C in the plants grown on cutaway peatlands.

The primary objective of the study was to investigate the transfer of sediment-derived C into aquatic plants by estimating the proportion of sediment-derived C vs atmospheric C in the plants grown on the old peat. In this thesis, the transfer of ^{13}C was also determined as a substitute for ^{14}C to compare whether the transfer of radiocarbon is adequately predicted by its stable isotopes in the selected plant species. In general, radioecological modelling assumes that the transfer of stable and radioactive isotopes of the same element is similar, and thus using stable isotopes is expected to be sufficient to predict the transfer of radionuclides (IAEA, 2010).

2 Literature review

2.1 Geological disposal of radioactive wastes

Industries across various sectors are involved in generating radioactive wastes including, nuclear power plants, metal mining operations, medical facilities, research institutions, manufacturing, and military weapon reprocessing facilities (IAEA, 2011). Nuclear power plants are currently the major sources of nuclear waste generation (Yim and Caron, 2006). The nuclear wastes generated from the normal operation of these facilities primarily consist of spent fuel, fission products, and activated radionuclides (Bruno and Ewing, 2006). Despite the technological development to prevent long-lived radionuclides from nuclear power plants, high levels of radionuclides have been continuously observed in the vicinity of nuclear facilities (Fontugne et al., 2004; Povinec et al., 2009). The released ^{14}C from nuclear facilities may undergo various processes including atmospheric dispersion, transport in surface water, and uptake into aquatic and terrestrial organisms.

Given the potential risk associated with radioactivity, it is critical to establish a well-designed provision and effective management strategies for handling and disposal of radioactive wastes to prevent any possible release of radionuclides into the environment (IAEA, 2009). Various methods have been proposed for disposing of radioactive waste including ocean disposal, outer space disposal, and underground burial (Brunnengräber and Di Nucci, 2019). However, among these methods, geological disposal is considered the safest and most effective method, nearly by all nuclear countries for the final disposal of radioactive wastes (IAEA, 2009; Apted and Ahn, 2017; Harms et al., 2019). Finland and Sweden have made significant progress in developing disposal facilities for radioactive wastes. For instance, Finland adopted the deep geological policy for the disposal of spent nuclear waste and long-lived radionuclides as early as 1980. Following extensive environmental and technical assessment, the first site for the long-term storage of high-level radioactive waste was selected in Olkiluoto in the year 2000 (Auffermann et al., 2015). The construction work for the disposal site has been recently completed in 2022, and it is expected to be fully operational by 2023 (Posiva Oy, 2022).

Geological disposal of radioactive waste in Finland has been employed based on the multi-barrier KBS 3 system developed by the Swedish Nuclear Fuel and Waste Management Company (SKB) (Posiva, 2012). This approach involved the sealing of spent fuel or other long-lived radioactive wastes in a copper canister. Each canister is then placed vertically in the repository hole bored at a minimum depth of 400 m underground. The canister is surrounded by bentonite clay serving as an additional protective layer to prevent leakage and reduce mechanical stress on the canister resulting from surrounding bedrock shocks. Finally, after the placement of the canister, the tunnel is backfilled with swelling clay blocks to prevent any human or water access. It is important to note that, the disposal tunnel is constructed in hard bedrock ensuring that it has no contact with groundwater (Jonsson et al., 2018). The multi-barrier design of hard bedrock, canister, and chemical barrier is to minimize the potential risk of radionuclide release and the radiotoxicity hazards of nuclear waste over an extended period (Posiva, 2012).

2.2 Radiocarbon

^{14}C is the only naturally occurring radioisotope of carbon. It is naturally produced continuously in the upper atmosphere when high-energy cosmic rays interact with the nuclei present in the atmosphere resulting in the production of high-energy neutrons. These neutrons are absorbed by ^{14}N to form ^{14}C . Additionally, it is also produced by the neutron reaction with ^{13}C , and ^{17}O in the atmosphere (IRSN, 2012; Lee and Park, 2022). ^{14}C is unstable, undergoes decay over time, and is a beta emitter (IRSN, 2012). Upon decay, it emits low-energy radiation and transforms to stable ^{14}N . ^{14}C is present in trace amounts in the atmosphere with about 1 or 1.5 atoms per 10^{12} atoms of total atmospheric carbon. Unlike ^{14}C , stable isotopes of carbon (^{12}C and ^{13}C) have no breakdown over millions of years, and thus no radiation is emitted. It is estimated that ^{12}C is the most abundant isotope making up 99% of all carbon present in nature while ^{13}C accounts for approximately 1% of total carbon.

Artificial sources of ^{14}C include human activities such as nuclear weapon testing, generation of nuclear energy, and minute sources of medical, and research increased the level of ^{14}C in the

atmosphere (Magnusson, 2007; IRSN, 2012). Currently, nuclear reactors have become the primary sources of anthropogenic ^{14}C , as weapon testing has been banned (Yim and Caron, 2006). The sharp increase in the concentration of ^{14}C was observed in the early 1960s as the result of intensive nuclear weapon testing, which peaked in 1963 (IAEA, 2004; Kutschera, 2022).

The operation of nuclear reactors for electricity generation produces various radioisotopes including ^{14}C , through the fission process in the fuel or other auxiliary processes within the operating system (Yim and Caron, 2006). ^{14}C is generated during the fuel cycle through neutron interaction with various isotopes like ^{13}C , ^{14}N , ^{15}N , ^{16}O , and ^{17}O . These isotopes can be found in nuclear fuel as well as in the moderator and coolant systems of nuclear reactors (IAEA, 2004). ^{14}C release from nuclear power plants is mainly in the form of gaseous $^{14}\text{CO}_2$ and $^{14}\text{CH}_4$ along with a small amount of low molecular weight organic compounds (Bracke and Müller, 2008; Pérez-Sánchez et al., 2009). In addition to the regular operation of nuclear power plants, the release of ^{14}C also occurs during decommissioning, management, and disposal of nuclear waste. Within geological disposal facilities, buried nuclear waste can release ^{14}C through the corrosion of carbon-containing steel or neutron-activated steel over time or degradation of organic materials present in the waste resulting in the dissolution or leaking of $^{14}\text{CO}_2$ and $^{14}\text{CH}_4$ into the surrounding environment (Berglund et al., 2013; Heikola, 2014; Mibus et al., 2018). The presence of various species of ^{14}C in the environment depends on the source and the prevailing environmental condition (Hou, 2018). As ^{14}C is a long-lived radionuclide released from nuclear facilities, it remains in the environment for a longer time and becomes integrated into the carbon cycle. Therefore, it is important to understand the behavior and transfer pathways of ^{14}C from underground waste disposal facilities into plants for proper assessment of its radioecological impact.

2.3 Transfer of ^{14}C into the biosphere

^{14}C released into the biosphere, either from nuclear reactors or from geological repositories, corrosion of metals, or failure in storage. It is released in gaseous form ($^{14}\text{CO}_2$ or $^{14}\text{CH}_4$), liquid discharges (coolant and moderator water), or as a solids waste (spent fuel rods), (IAEA, 2004). Potential receptors for released ^{14}C in the environment are terrestrial ecosystems like forests, agricultural soil, or crops as well as aquatic ecosystems such as rivers, lakes, wells, and

oceans. The behavior and transfer of ^{14}C into the biosphere follows complex processes that involve various sources and pathways. Gaseous ^{14}C in the forms of $^{14}\text{CO}_2$ mixes with stable carbon isotopes (^{12}C and ^{13}C) increasing its radioecological concern due to its high mobility and global dispersion (Pérez-Sánchez et al., 2009).

Organisms like plants fix atmospheric $^{14}\text{CO}_2$ through the process of photosynthesis, (Mobbs et al., 2014; Limer et al., 2017). Animals feeding on plants can transfer assimilated ^{14}C further in the food chain or even to the soil system after their life span. Moreover, some proportion of C assimilated by plants may be transported to roots either from the plant's growth and development or as leaf litter and subsequently incorporated with organic matter as root exudates (Lange et al., 2015).

The ^{14}C in the soil can then be taken up by plants directly through their roots. However, root carbon uptake is generally considered to be low, ranging from 1 to 2 % (Amiro et al., 1991; Yim and Caron, 2006; Van Dorp and Brennwald, 2009) some also reported up to 5% (Livingston and Beall, 1934; Majlesi et al., 2019), but a large uncertainty exists with this pathway. The efficiency of root uptake may vary depending on plant species, height, and source area (Evenden et al., 1998). ^{14}C released into the soil may also dissolve in soil water and potentially reach plant roots. This may be absorbed by plants through their roots in the form of carbonate ions (Zamanian et al., 2017). Of the C pool in the soil system, some proportion can be released back to the atmosphere and thus re-assimilated by plants as $^{14}\text{CO}_2$ via photosynthesis. The re-assimilation of released $^{14}\text{CO}_2$ in plant canopy has been hardly studied and even considered negligible in some studies (Mobbs et al., 2014; Majlesi, 2021). Therefore, more data on the re-assimilation of soil C by plant would be useful.

Another possible pathway of ^{14}C is methanotrophy, which takes place after the possible leaking of $^{14}\text{CH}_4$ from geological disposals as below-ground sources. Soil microorganisms such as methanotrophs (methane-oxidizing bacteria, MOB) utilize $^{14}\text{CH}_4$ as the sole energy source. Carbon in methane is not readily available to organisms. Methanotrophs oxidize $^{14}\text{CH}_4$ to $^{14}\text{CO}_2$ in the presence of oxygen and make inorganic carbon available to enter the food web through plants (Chanton et al., 2009; Limer et al., 2013). Therefore, they play a key role in the transfer of carbon into the food chain. The rate of methane oxidation depends upon microbial population, oxygen availability, and prevailing environmental conditions. Oxygen availability, particularly in

northern peatlands, is the major limiting factor in methane oxidation (Le Mer and Roger, 2001). In waterlogged peatlands, methane oxidation is relatively low and the majority of methane produced is emitted to the atmosphere (Sundh et al., 1995). However, some models assume that all methane is oxidized to CO₂ but in peatlands or wetlands, this assumption may not be true, as methane emission is usually reported (Thorne and MacKenzie, 2005; Wen et al., 2018). Various other factors may also be involved in complete oxidation therefore ecosystem specific data is needed.

Transfer of ¹⁴C into the aquatic ecosystem and its possible risk to the biosphere.

¹⁴C can be transferred into aquatic ecosystems either directly from nuclear facilities in the form of liquid waste or indirectly from buried radioactive waste as a result of unexpected rupture or accidents. In case of escape from geological waste facilities ¹⁴C containing gases (¹⁴CH₄, ¹⁴CO₂) may move upward in soil layers through diffusion (Ota and Tanaka, 2019). It either escapes into the atmosphere or is dissolved in soil solution and thus further transports into groundwater in the form of dissolved inorganic carbon (DIC) (mainly carbonate and bicarbonate) and water-soluble organic compounds (IAEA, 2004; Limer et al., 2017).

The atmospheric ¹⁴C may enter water bodies either directly through the air-water interface or via photosynthesis by aquatic plants. The later results in the integration of ¹⁴C into bed sediment through root exudation or as leftovers of plants. Furthermore, the ¹⁴C in the soil system, either as soil particles or soil solution can further transport and leach into the groundwater and neighboring aquatic ecosystems as particulate (POC) and dissolved organic matter (DOC), respectively (Weidel et al., 2008). Such allochthonous organic matter can be incorporated into deposited sediment as particulate C or (re)-suspended in water columns in the form of dissolved C. Once it enters into the aquatic ecosystem it can be taken up by aquatic vegetation through roots, which are subsequently consumed by other organisms (Thorne et al., 2018).

The incorporated ¹⁴C within the organisms may remain in the organism's biomass or respire back into the atmosphere as ¹⁴CO₂. Retained ¹⁴C in organisms' biomass can be incorporated into organic matter through excretion or after their life spans (Jones et al., 1998). Moreover, a sub-

stantial amount of DIC in anoxic sediments can be converted to $^{14}\text{CH}_4$ by methanogens and incorporated with organic matter (Sanseverino et al., 2012). The decomposition of organic matter supplements the aquatic carbon pool, which can then be taken up by microbial biomass (Guillemette et al., 2013). For example, MOB's are present at the oxic-anoxic interface within bed sediments which incorporate $^{14}\text{CH}_4$ into cellular biomass or oxidize it to $^{14}\text{CO}_2$, making C available for plants and other species (Jones and Grey, 2011). Moreover, MOB's serve as a source of food for other benthic invertebrates and zooplankton (Thorne et al., 2018). The movement and interactions of ^{14}C within the aquatic ecosystem are significantly influenced by the hydraulic and geochemical characteristics of water (Monte et al., 2005). The hydraulic processes including water current and turbulence motion primarily impact the transport and diffusion of radionuclides in the water. Simultaneously, the geochemical properties regulate the movement of radionuclides between the water column and sediments and re-suspension in the water column.

Among aquatic organisms, macrophytes may incorporate ^{14}C through the process of photosynthesis and convert it into a biogenic C. Therefore, there is a possibility that ^{14}C from radioactive wastes can enter water bodies and adversely affect aquatic biota. Moreover, longer half-life and bioaccumulation of ^{14}C in the environment pose a significant risk not only to non-human biota but also to humans.

2.4 Aquatic plants

Aquatic plants are characterized by their ability to grow in an abundant supply of water. They can be partly or fully submerged in water and serve as food sources for primary consumers in aquatic ecosystems, including invertebrates and fishes. As primary producers, these plants play a key role in regulating carbon dynamics and other biochemical processes within aquatic ecosystems (Thorne et al., 2018). The distribution of aquatic plants is influenced by various abiotic factors, such as temperature, light, chemical hydrology, and CO_2 availability in aquatic habitats (Cronk and Fennessy, 2016). Among these factors, light and CO_2 availability are particularly critical for the survival and growth of aquatic plants. The mechanism by which aquatic plants take up CO_2 is markedly different from that of terrestrial plants because they grow in a water medium rather than in the air (Pedersen et al., 2013a). In aquatic ecosystems, the availability of CO_2 is controlled by many factors including water movement and chemistry

(Bornette and Puijalon, 2011). Hence, the plants are distributed in the aquatic ecosystem based on the availability of CO_2 and the plant's ability to assimilate CO_2 , HCO_3^- or both. However, some species can alter their carbon assimilation pathways according to the concentration of available carbon species in the surroundings (Bornette and Puijalon, 2011). Therefore, the accumulation of ^{14}C in plants' biomass from the surrounding provides us valuable insight into the abundance and potential transfer of contaminants within the aquatic food web. Various other factors also influence the C uptake such as type of plant species, DIC composition and relative abundance in the environment, water chemistry, water depth, seasonal changes, and other ecological factors (Maberly and Madsen, 2002).

In general, plants record any contamination phenomena of the surrounding environment. This includes a variety of plants such as algae, non-vascular, and vascular plants, and some of them is commonly used in ecotoxicological research (Dixit et al., 1992; Mkandawire and Dudel, 2005; Ceschin et al., 2021). However, using model plant species for assessing the ecotoxicity of a particular contaminant is scattered in the literature (Smith, 1991). Likewise, when it comes to assessing the risk associated with ^{14}C in the biosphere, terrestrial plants mostly are focused and there is hardly any research focusing on its assimilation by aquatic vegetation. To fill this knowledge gap, research is needed on ^{14}C uptake in aquatic ecosystems.

Aquatic vegetation follows a specific zonation parallel to the shoreline based on the water requirement and adaptation. In deeper water zones, submerged plants are dominant, providing space for floating plants near the shoreline. It is followed by a zone of emergent plants along the water's edges and a transitional area where land water meets, and marshy plants become prevalent (Duncan, 1967). This study is therefore designed to determine the transfer of ^{14}C by various aquatic plant species including marshy emergent species like *Stachys palustris*, emergent plant species such as *Lysimachia nummularia*, submerged species such as *Littorella uniflora*, and free-floating species *Lemna minor* (Duckweed). With such a diverse category of aquatic plants, we aim to compare the transfer of sediment-derived C across various plant types. Previous data suggested possible enrichment of ^{14}C in bed sediments and marine ecosystems (Sobek et al., 2006; Tierney et al., 2018; Hyun et al., 2022). However, limited data on the potential transfer of sediment-derived C into aquatic plants is available. This work helps to increase the understanding of ^{14}C transfer into aquatic plants, by producing data, needed for radioecological

models in boreal ecosystems. A more in-depth ecological overview of these plant species is outlined separately in the next section.

2.4.1 *Lemna minor*

Lemna minor (duckweed) is a group of flowering plants belonging to the family *Lemnaceae*. It is recognized as the smallest flowering plant in the plant kingdom (Xu et al., 2015). The plant is composed of one or more leaves called fronds and a rootlet having no stem, forming a thick blanket on the surface of water (Ziegler et al., 2016). The size of a typical duckweed plant is about 2- 4 mm and grows in colonies (Saha et al., 2015). It is widely distributed in various geographical regions from the tropics to temperate. It is native to Africa, Asia, Europe, and North America but is also found in Australia and South America. In ideal growth conditions, duckweed becomes invasive and reproduces vegetatively, doubling its biomass in less than 2 days (Appenroth et al., 2015). In laboratory conditions, it can be cultured for an indefinite time providing proper growth conditions such as nutrients, water, light, and temperature. It can tolerate a wide range of pH of 5 – 9 and temperatures of 6- 33°C (Ekperusi et al., 2019).

L.minor is considered an attractive model aquatic plant due to its higher biomass production, smaller size, and quick development in the natural environment (Popa et al., 2006; Xu et al., 2015). Moreover, it is widely used for remediation to assess the effect of a wide range of organic and inorganic contaminants such as herbicides, pharmaceuticals, agriculture waste, and heavy metals in industrial and municipal wastewater, (Ziegler et al., 2016). Therefore duckweed is adopted as a model phytotoxicity test plant by many international standardization organizations including ISO, USEPA, ASTM, and OECD (OECD, 2006; Park et al., 2022). Moreover, duckweeds are used as biofuel and food source for many animals, including fish and livestock (Bog et al., 2019). Various studies in the literature reveal the successful utilization of duckweed for the removal of heavy metals and radionuclides from the laboratory solution. For instance, Ekperusi et al., (2019) it confirmed the effective removal of such contaminants by duckweeds. Another study Mkandawire and Dudel, (2005) evaluated the accumulation of Arsenic by duckweed from the tilling water of a uranium mine site and subsequent transfer in the trophic level. Research conducted by Popa et al., (2006) reveals that duckweed can accumulate radioactive metals such as

Cobalt, Zinc, and iron from water solution. The presence of these radioactive metals in the solution resulted in increased plant biomass, but the symptoms of toxicity were induced in the plant biomass, indicating duckweed's capability to uptake radioactive contaminants from the aquatic environment.

2.4.2 *Littorella Uniflora*

Littorella uniflora is a small and slow-growing aquatic plant belonging to the family *Plantaginaceae*. It is characterized by a short stem and an extensive root system of thick biomass consisting of numerous advantageous roots. The root system plays a significant role in plant structure as it comprises a large part of total plant weight and volume contributing to increased surface area. The plants have small stiff, fleshy, grass-like leaves ranging from 4 to 20 cm in length, arranged in a basal rosette. In submerged conditions, it can reproduce vegetatively through runners, while under emerged conditions it can produce flowers and seeds (Robe and Griffiths, 1998). *L. uniflora* has a wide range of distribution but is well adapted to the boreal sub-Atlantic region. It can be commonly found in western to northern Europe including, Finland, the United Kingdom, Ireland, the Czech Republic, and Scandinavia (Kolář, 2014). It is highly adaptive to a wide level of pH, ranging from 4 to nearly 9. Furthermore, the capability of growing under submerged as well as emerging water conditions classifies it as an amphibious plant (Robe and Griffiths, 2000). Under optimal growth conditions, it can form a dense vegetation bed, limiting the growth of other submerged plants (Sand-Jensen and Søndergaard, 1981). *L. uniflora* prefers sandy clay sediments with transparent, freshwater consisting of low nutrient availability, and under certain circumstances, it can also be found on peat or organic sediments (Farmer and Spence, 1986).

L. uniflora has unique adaptive characteristics that allow it to thrive in low-nutrient aquatic environments. For example, as compared to other aquatic plants it has a high radial oxygen loss (ROL) feature in the surrounding water creating an oxygenated zone around the root significantly enhancing nutrient uptake efficiency (Smolders and Roelofs, 1996). Symbiotic interaction of endomycorrhizal fungi with roots of *L. uniflora* has been highlighted in few studies (Andersen and

Andersen, 2006; Sudová et al., 2021). Such symbiosis might also enhance *L. uniflora*'s ability to effectively utilize sediments derived C and make it adaptable in an oligotrophic environment.

2.4.3 *Lysimachia nummularia*

Lysimachia nummularia commonly known as moneywort, or creeping jenny, is a vigorous semi-aquatic perennial plant, belonging to the *Primulaceae* family. It is characterized by a pattern of rounded green leaves arranged in opposite pairs along the stem and having cup-shaped yellow flowers of about 2cm in diameter bloom in summer (Bowles, 2017). Moreover, it is an ever-green species, that typically grows up to 20 cm or sometimes even longer and reproduces vegetatively through fragmentation and root nodes.

Geographically, it is native to Europe but has also been introduced to North America and Australia where it gains popularity as an ornamental plant. *L. nummularia* has an affinity for a wide range of environmental conditions and normally thrives in swamps or other damp places near ponds or low-lying wet ground (Kodala and Jobson, 2016). Under favorable conditions, it becomes invasive, spreads vigorously, and covers the whole area. However, it is a highly resilient plant and can adjust to slightly dryer conditions as well. In colder conditions, it has the capability to survive even in low temperatures such as -15 °C. In emerging conditions, *L. nummularia* typically assimilates atmospheric CO₂. However, in occasional submerged conditions, it can change its C assimilation pathways. Instead of using atmospheric CO₂, it absorbs bicarbonate from water like many other aquatic plants. The availability of bicarbonate for aquatic plant uptake is controlled by the movement and pH of the water. At neutral pH, the relative concentration of CO₂ and HCO₃⁻ is at equilibrium (Pedersen et al., 2013b).

2.4.4 *Stachys palustris*

Stachys palustris commonly known as marsh woundwort or clown's woundwort is a smelling perennial flowering plant belonging to the mint family (*Lamiaceae*) (Sell and Murrell, 2009). This plant is known for its medicinal properties and has been used for centuries by indigenous people as a remedy for healing wounds and for treating other skin injuries (Tomou et al., 2020). It is native to Europe including Finland and central Asia but has also been introduced to North

America and Canada. *S. palustris* is considered a transitional land-water plant usually found in marshy, damp, and wet meadows, along the sides of streams and rivers. It is also found in arable fields and is considered an annoying agricultural weed (Łuczaj et al., 2011).

S. palustris typically has an upright green stem, ranging from 80 to 120 cm in length. Its leaves are stalkless and hairy arranged oppositely along the stem. Furthermore, it produces clusters of 4- 10 pinkish-purple flowers that bloom from July to September, with seeds maturing from August to October (Taylor and Rowland, 2011). It primarily spreads vegetatively through horizontal tuberous roots but also produces seeds in late autumn. Marsh woundworts thrive in well-drained highly fertile soil (Darbyshire et al., 2014). However, they can also grow in flooded and poorly drained heavy soil. In northern boreal wetlands, it grows best in nutrient-rich clay and peat soil (Brändel, 2006). It has an extensive fibrous rooting system that is mainly responsible for nutrient uptake (Harley and Harley, 1987). The optimum pH is (6.0- 6.9) but it can also tolerate slightly acidic to slightly basic pH (Hill et al., 2004).

2.5 Role of mycorrhizas in aquatic plants.

Mycorrhiza is a mutualistic association between plants and fungi, where fungi reside either around the roots' surface or inside the root cortex. Based on the hyphal penetration in the root cortex cells this association is commonly classified into ectomycorrhiza and endomycorrhiza. In ectomycorrhiza, hyphae surround the root cells without penetrating them, while in endomycorrhiza, fungal hyphae penetrate the root cell membrane (Lodge, 2000). The remaining hyphae extended outside the roots into the soil, forming an extensive mycelium network in the soil. The mycelium network enhances plant access to water and essential nutrients (Leake et al., 2004). Consequently, mycorrhizal association plays an important role in plant nutrient uptake, mediating soil biology, and soil chemistry in the rhizosphere. Fungi benefit host plants by providing minerals and nutrients and in return plants supply organic molecules such as sugar or lipids for fungi (Smith and Read, 2008). However, some studies have reported the uptake and translocation of radionuclides by fungi (Baeza et al., 2005; Mahmoud, 2004; Steiner et al., 2002), while few have suggested that fungi could indeed contribute to the absorption of soil C through roots (Taylor et al., 2004; Bago et al., 2000).

Mycorrhizal associations are widespread in both terrestrial and aquatic ecosystems. It is assumed that almost all of the terrestrial plants are engaged in these associations (Van Der Heijden et al., 2015). Aquatic ecosystems are more diverse than terrestrial ecosystems, especially in plant species richness (Hawksworth, 1995). Despite the greater diversity of plant species in aquatic ecosystems, the exploration of such associations in aquatic plants is comparatively low (Shah, 2014). The role of mycorrhizae in aquatic plants is to help with high oxygen and nutrient deficiency as well as reduce eutrophication (Shah, 2014). Aquatic plants exhibit diverse life forms ranging from emergent, floating, and submerged, based on the availability and level of water. Hence the diversity suggests that the existence of mycorrhizal association in aquatic plants is possible (Wolfe et al., 2006; Shah, 2014; Fusconi and Mucciarelli, 2018).

2.6 Experimental approaches to investigate the transfer of ^{14}C from below-ground sources.

In this work, to estimate the proportion of sediment-derived C in the selected aquatic plants, isotopic techniques based on using the natural abundance of C isotopes (^{13}C and ^{14}C) in the sediment and the atmosphere were employed as possible sources of carbon. To assess the rate of C uptake, a microcosm was established at the University of Eastern Finland (Kuopio campus). The aquatic plants were added to the sediment under a fully controlled laboratory environment.

2.6.1 Source partition technique using natural abundance of ^{14}C in a cutaway peatland complex.

In this thesis, the uptake of sediment-derived C by selected plant species was investigated using isotopic methods to distinguish sediment-derived C from atmospheric C. The concept behind this approach is to use a cutaway peatland, where a naturally large difference exists in the ^{14}C isotopic signature between the "normal" ^{14}C level in the air and the residual peat aged up to 8000 years. The difference in ^{14}C content of the residual peat stems from the removal of the uppermost layers of the peat (through peat extraction for producing energy), leaving behind

peat which is approximately 8000-year-old due to the gradual decay of this radionuclide over time (Biasi et al., 2011). The selected approach provides a great opportunity to separate the C sources and determine the proportion of each source in environmental samples by using a two-pool isotope mixing model. Using this approach may overcome the challenges of artificial labelling techniques, relevant to source partitioning. Such techniques are often associated with methodological errors and possible risks to the environment. Furthermore, using ^{14}C in labelling experiments requires a well-reasoned base and safety protocols. Therefore, isotopes natural abundance is often preferred over radiolabeling methods. For instance, the usefulness of this method is demonstrated in the previous study, using the same peat to track the contribution of soil-derived C in terrestrial plants (Majlesi et al., 2019). Here the peat is used as a sedimentary source of C implying the incorporation of ^{14}C with organic substrate in aquatic ecosystems. Henceforth, the contribution of sediment-derived C to the plants grown on residual peat sediment can be estimated. Finally, the ^{14}C contents of the plants were accurately measured by Accelerator mass spectrometer (AMS).

2.6.2 AMS

AMS is a highly sensitive technique used for measuring rare isotope atoms in a sample (Hellborg and Skog, 2008). It consists of a series of magnetic and electrostatic tandem accelerator systems designed to eliminate the undesired isotopic interfaces from the ion beam as illustrated in Figure 1. Consequently, the concentration is narrowed down to a single atom making it possible to measure several rare and interesting isotopes accurately. In the case of ^{14}C negative carbon ions are generated through caesium sputtering. Cs is used because it does not produce a negative ion of ^{14}N , effectively eliminating the isobaric interface. During the sputtering process, the charged ions are separated and filtered out by a low-energy mass spectrometer, and a negative ion beam is introduced to the accelerator. Within the accelerator, several high-energy spectrometers are employed to filter out any unwanted background ion and to ensure selectivity and sensitivity of rare isotopes beam. Finally, the ^{14}C atoms are counted with high accuracy (<0.2%) in the detector.

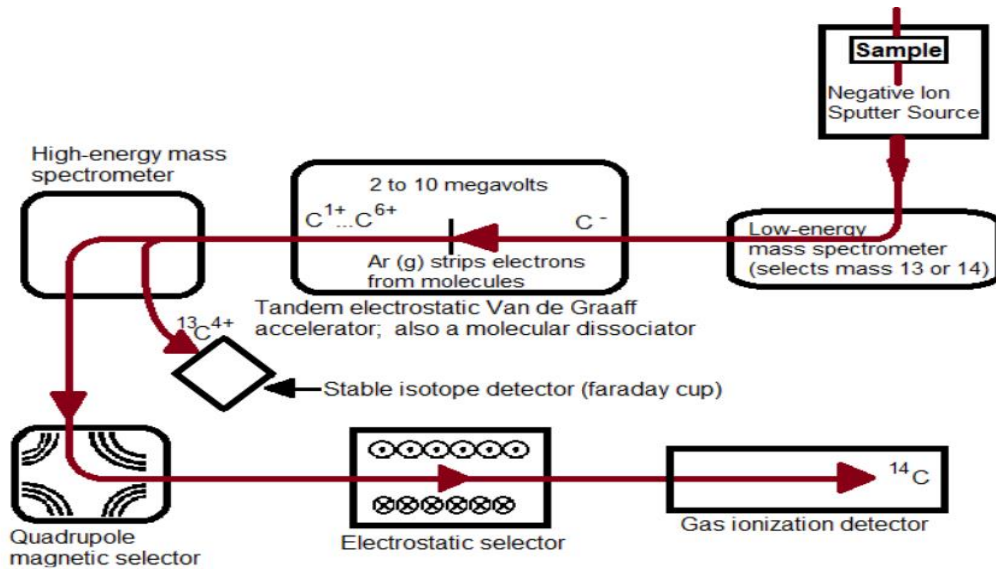


Figure 1. Systematic diagram of Accelerator Mass Spectrometry (Sources: Laura McWade, 2013).

The results of the ^{14}C analysis were expressed as percent modern carbon (pMC) providing a measure corresponding to the age of C assimilated by plants. In fact, it represents the portion of $^{14}\text{C}/^{13}\text{C}$ activity in the sample compared to the $^{14}\text{C}/^{13}\text{C}$ activity in the standard. The principle radiocarbon standard is defined as the 95 % activity of oxalic acid I from the year 1950 calibrated to the measured activity of pre-fossil fuel wood 1890 (Analytic, 2012).

$$pMC = (^{14}\text{C}/^{13}\text{C})_{s[-25]} / (^{14}\text{C}/^{13}\text{C})_{1950 s[-25]} \times 100 \quad (1)$$

The results in eq 1 are normalized to $\delta^{13}\text{C}$ value of -25‰ to correct isotope fractionation (Stuiver and Polach, 1977). Notably, the year 1950 is considered the “modern” reference point in pMC value calculation due to the atomic bomb spike. A pMC values exceeding 100 in a sample indicate that it originates from 1950 or later, while values below 100 suggest that the sample is from before 1950 (Stenström et al., 2011; Analytic, 2012). Radiocarbon dating is translated to conventional age using a radiocarbon decay equation with a mean lifetime of 8033 years for ^{14}C .

$$T = -8033 \ln (A_{sn}/A_{on}) \quad (2)$$

Where A_{on} is the activity count per minute of modern standard and A_{sn} is the count per minute activity in the sample.

The normalized pMC values of our samples are linked to age as described in eq 3.

$$pMC = 100 \times EXP\left(\frac{\delta^{14}C \text{ age}}{8033}\right) \quad (3)$$

2.6.3 Stable isotope approach using ^{13}C .

Stable isotopes represent non-radioactive forms of atoms. They do not emit radiation and are safely used in various fields such as chemistry, ecology, biogeochemistry, and climate change. Naturally occurring stable isotopes of carbon (^{13}C) are instrumental in tracing C origin, fate, and cycling in the biosphere (Fry, 2006). Ecologists mostly rely on $\delta^{13}C$ to assess the fraction contributions of different sources in the environmental components (Phillips and Gregg, 2001). Researchers used natural abundance (non-labelled) and labelling approaches for source partitioning. In labelling, studies ^{13}C isotopes are added to the system, to measure the contribution of a source in samples. However, artificially added ^{13}C may differ from that of the natural system. Natural variation of ($^{13}C/^{12}C$) in the different environmental compartments can replace artificial labelling. However, the natural abundance of ^{13}C might not always differ significantly in various environmental compartments to distinguish the sources. Various physical, chemical, and biological processes influence the stable isotope ratios differently along the pathways (Lacombe and Bazinet, 2021). The changes in ratios between stable isotopes allow us to distinguish different environmental components based on their isotopic compositions. The use of stable isotopes is also common in radioecological models, assuming their similar transfer to corresponding radionuclides (IAEA, 2010). In this work, the transfer of ^{13}C from the sediments into the plants was also determined and compared to ^{14}C data sets to examine whether it sufficiently correlated with ^{14}C uptake. The variation in stable isotope ratio ($^{13}C/^{12}C$) in biological samples can be detected and measured significantly through an Elemental Analyzer Isotopes Ratio Mass Spectrometer (EA-IRMS) and can be used to partition different C sources.

2.6.4 EA-IRMS

EA-IRMS is a conventional analytical system that is used to determine the stable isotope composition (e.g., ^{13}C and ^{15}N) of a sample. The entire system typically comprises of IRMS equipped with an elemental analyzer via a continuous flow interface as shown in Figure 2.

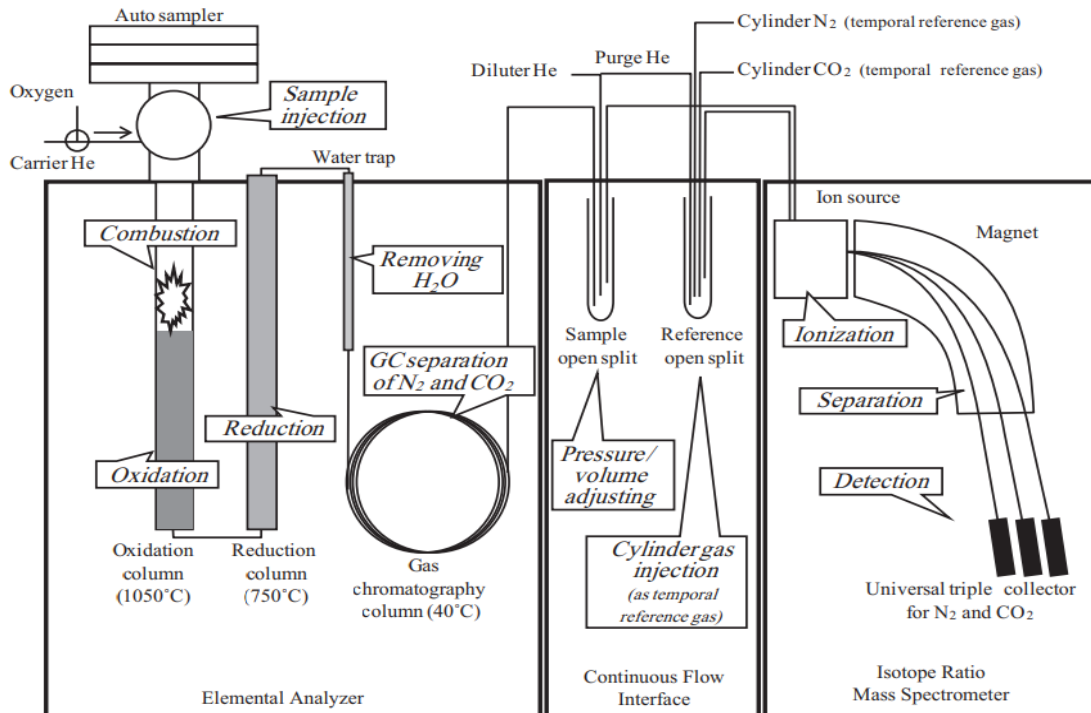


Figure 2. Schematic diagram of EA-IRMS system (source: Ogawa et al., 2010).

The isotope analysis starts with the introduction of samples into an elemental analyzer wrapped in tin cups. In the combustion column within the elemental analyzer, the sample is combusted with higher temperatures up to 1050°C. The ignited mixture is then transferred to the reduction column through helium gas for the reduction of N_2O to N_2 . Any water vapours present are removed before the introduction of CO_2 and N_2 into a continuous flow interface. The continuous flow interface split and reduced the gas sample. The gas mixture is then injected into mass spectrometry along with a standard pumped ions source. Within a mass spectrometer, the gas mixture is ionized by a beam of electrons. The ionized gas is focused, accelerated, and deflected by magnetic fields. The carbon and nitrogen are then differentiated in the sample using calibrated mass-to-charged ratio and collected in their respective Faraday cup detectors. The detectors translate the amount of carbon and nitrogen and their relative heavier isotopes in the injected samples. The isotopic composition is processed through ISODAT^{NT} software and expressed in δ notation (Ogawa et al., 2010).

Delta notation (δ) expressed the difference of measured isotope content in a sample relative to the international standard reference. The δ value of natural materials is so small that is expressed in part permil (‰). The standard reference material is Pee Dee Belemnite.

The δ value can be calculated with the formula given below:

$$\delta = \left[\left(\frac{R_{\text{sample}}}{R_{\text{standard}}} \right) - 1 \right] \times 1000 \quad (4)$$

δ is used for isotopes of interest (e.g., ^{14}N or ^{13}C) and the term R_{sample} refers to the ratio of heavy to light isotopes in a sample. For example, R_{sample} for carbon is the ratio of ^{13}C to ^{12}C in the sample, while R_{standard} represents the ratio of heavy to light isotopes in the standard (e.g., 0.01118 for $^{13}\text{C}/^{12}\text{C}$) (Fry, 2006). δ is the difference in carbon isotope ratio related to the standard value expressed in part permil (‰). The international standard value for δ is 0 ‰, which means a sample with a positive δ value indicates enrichment of heavy isotopes compared to the standard while a negative value δ suggests fewer heavy isotopes in the sample than the standard.

3 Aims of the thesis

The primary aim of the thesis is to investigate the transfer of sediment-derived C into aquatic vegetations under laboratory conditions, as implication for the transfer of ^{14}C from below-ground sources.

The specific objectives are:

- 1) To examine the uptake of ^{13}C and ^{14}C by the selected plant species.
- 2) To compare the uptake of ^{13}C with ^{14}C data sets, to examine whether ^{13}C as a stable isotope can be used in radioecological models to sufficiently predict the assimilation of ^{14}C in aquatic plants.
- 3) To estimate the uptake of sediments-derived C between roots and leaves.

4 Materials and Methods

4.1 Sampling site description

The peat soil used in the study was collected from the Linnansuo peatland complex N62° 53' and E30° 43', within the rural region of Joensuu City, in Eastern Finland in June 2023. Climatic data available for the region from 1971 to 2000 indicate a mean annual temperature of +2.1°C with annual precipitation of 669 mm. January records the lowest temperature with a mean of -10°C, while July is the warmest with 16°C (Shurpali et al., 2008). The peatland in this site is classified as ombrotrophic *Sphagnum fuscum* bog (Biasi et al., 2008). The process of peat extraction activities such as clearing, digging of ditches, and drainage, began in 1976. After two years, in 1978, peat extraction began and continues still in some parts of the site. However, in 2001 extraction was stopped when the thickness decreased to the range of 20- 85 cm, and perennial red canary grasses were cultivated in some areas of peatland (Shurpali et al., 2008). The organic matter content of the peat ranged between 42% and 63%. The average C content, pH, and C/N ratio were $39 \pm 2.57\%$, 4.5, and 40.3-40.2 respectively (Shurpali et al., 2008). The large difference in the natural abundance of ^{14}C in the soil-atmosphere system on this site can be used to partition the C sources in aquatic plants. Therefore, the peat sample from this site serves as below ground source of ^{14}C for aquatic plants.

4.2 Collection of soil and plant samples

The soil samples were collected at a depth of 50 cm by digging. A total of eight plastic buckets each with the capacity of holding about 7 kg, were filled with soil. The samples were collected randomly from 8 different spots. The collected samples were labelled, transported to the university, and stored at +4°C before the experiment. After that, the peat samples were mixed thoroughly and sieved at a mesh size of 4mm to remove extra materials, including visible plant debris and roots.

Plant samples were acquired from two different sources. *L. nummularia*, *L. uniflora*, and *L. minor* were purchased from the local supplier (Pieni Eläinkauppa, Kuopio, Finland).

S. palustris was the only species that was collected from the lake Kallavesi, near the University of Eastern Finland, Kuopio campus (N62° 89' and E27° 63'). After collection, the plants were transported to the laboratory, washed, and added to the aquariums.

4.3 Artificial freshwater preparation

Artificial freshwater (AFW) was prepared for the experiment according to OECD guideline 203 for the testing of chemicals (OECD, 1992). The preparation process involved the production of four stock solutions, containing the required amount of salt as outlined in Table 1. To produce 1 L of AFW, 25 ml of each of the four-stock solutions were combined in a volumetric flask, and added with Milli-Q water to reach 1 L.

Table 1. Composition of stock solutions made for artificial fresh water.

Salts	Molecular mass (g/mol)	Mass (g)	Molarity (mol/L)
CaCl ₂ × 2H ₂ O	147.01	11.76	0.8
MgSO ₄ × 7H ₂ O	246.47	4.93	0.02
NaHCO ₃	84.01	2.59	0.03
KCl	74.55	0.23	0.003

In order to make 10 L of AFW for the experiment, 250 ml of each of the four-stock solutions was mixed thoroughly in a separate plastic container and filled up to 10 L with Milli-Q waters. The pH of the AFW was recorded as neutral (pH=7).

The peat soil was acidic with average pH content of 4.5. Therefore, a pilot study was conducted by adding AFW to peat soil, and within 24 hours of introducing AFW, the pH of the water dropped significantly from 7 to as low as 4. This indicated that low pH might adversely affect the plant nutrient uptake in experimental conditions. To control the pH throughout the experiment, a buffer solution was deemed necessary. A 0.01M phosphate buffer with a pH of 7 was prepared to prevent a decrease in pH level during the experiment. The specifications and quantities of ingredients added to the buffer solution are provided in Table 2.

Table 2. Composition of 1-liter buffer solution.

Buffer	Molecular mass (g/mol)	Mass (g)	Molarity (mol/L)
NaH ₂ HPO ₄ .2H ₂ O	156	1.56	0.01
Na ₂ HPO ₄ . 2H ₂ O	178	1.78	0.01

For preparation of 10 L of buffered AFW, 250 ml of each stock solution, 15.6 g of NaH₂HPO₄.2H₂O, and 17.8 g of Na₂HPO₄. 2H₂O was thoroughly mixed with Milli-Q water in a plastic container and made the volume up to 10 L. The buffered solution ensured the optimum condition for the experiment.

4.4 Experimental setup

A microcosm study to investigate the uptake of belowground ¹⁴C by four different plant species including *L. nummularia*, *L. uniflora*, *L. minor*, and *S. palustris* was carried out in the summer of 2023, at the University of Eastern Finland, Kuopio. The experiment started in July 2023 and lasted for one month, which ended in August 2023. To establish the microcosm, 7 L of plastic aquariums (31 cm×21 cm×24 cm) were filled with 500 g of peat soil. The plants were distributed in the aquariums according to the space required for the plant development as shown in table 3. For instance, *L. uniflora* was totally submerged, had an intensive root system, and spread slowly about 15 cm in 30 days so they needed more space for development. Hence, two individuals were placed in each aquarium. For *S. palustris* and *L. nummularia*, three and four individuals were planted in each aquarium, respectively. In contrast, *L. minor* (duckweed) was the only species in which the number of individuals could not be determined since they were purchased as 100 ml solution bags. Therefore, individuals from five bags were added to one aquarium (6 aquariums in total). After adding plants to the test system, 2 L of buffered AFW was gently added to the aquariums to avoid disturbance of the peat and the plants. The aquariums were connected to an aeration system. The aeration system basically consisted of an air pump connected to the glass pipets through silicon tubes. The Pipet tips stayed 2-4 cm deep in the AFW of every aquarium releasing air constantly into the water. The experimental period was 30 days. Throughout the experimental period, the average temperature of the laboratory room was maintained at 20

$\pm 1^\circ\text{C}$, and the photoperiod was fixed under 16:8 light: dark photoperiod. Other parameters like pH, conductivity, and dissolved Oxygen were measured 5 times a week using the multi-parameter portable meter, (multi-3620 IDS, Xylem Analytic, Germany). However, it was noticed that maintaining the optimal pH in the peat soil was quite challenging, despite the addition of a buffer solution. To resolve that, the water was changed every 10 days or whenever the pH dropped below 5.

Table. 3 Experimental setups of the microcosm study with naturally depleted peat in ^{14}C . Values indicates the number of aquariums and number of individuals per aquarium for each species.

Plant species	Number of aquariums	Number of individuals per aquarium
<i>L. nummularia</i>	7	4
<i>L. uniflora</i>	18	2
<i>L. minor</i>	6	N.D*
<i>S. palustris</i>	6	3

N.D. Not determined. * The number of *L. minor* was not available since they were purchased as solution bags (100 ml per bag). The individuals from five bags were added to one aquarium.

4.5 Sample preparation for ^{13}C and ^{14}C analysis.

After 30 days of the experiment, the sediment and plant samples were collected from the aquariums. Plant samples were washed thoroughly with Milli-Q water. The roots and leaves were separated except for the *L. minor* due to its small size. The duckweeds from one aquarium were collected and pooled for oven drying at 40°C for 48 hours to remove their water content. Similarly, the roots and leaves of other species were dried separately for 48h at the same temperature of 40°C in an oven. The sediment samples were also oven-dried similar to plant samples. After drying, the sediments and the plant samples were ground in mortar and pestles to a fine powder to homogenize the samples.

For ^{13}C analysis, 2-3 mg samples of plant roots (n=10) and plant leaves (n=10), as well as sediment (n=10), were weighed into tin cups by using MX5 milligram balance (Mettler Toledo). For duckweeds, the whole plants were weighed without separation of roots from leaves (n=10) to

obtain sufficient biomass and to avoid possible damage to plants. After weighing the samples into the tin cups, they were systematically arranged within labelled well plates.

The analysis of ^{13}C and C content (%) of the samples, was performed at the University of Eastern Finland, Kuopio campus using EA-IRMS (Thermo Finnigan, FLASH, EA 1112, Milan, Italy). In addition, 40 mg of ground plant samples were separately weighed and wrapped in aluminum foil for ^{14}C analysis. The dried sediment samples (n=3), roots (n=3 for each species), and leaves (n=3 for each species) of submergent and emergent plants, as well as the whole duckweeds (n=4), were transported to the Finnish Museum of Natural History, LUOMUS, Helsinki, Finland for ^{14}C analysis. The radiocarbon dating of the plant samples was carried out by accelerator mass spectrometer (AMS) with high precision (<0.2%).

4.6 Mycorrhizal colonization of roots

At the end of the experiment, root samples from *L. nummularia*, *L. uniflora*, and *S. palustris* were collected and washed thoroughly with Milli Q water. The clean roots were placed in Petri dishes containing 10% KOH solution, at room temperature. After 24h the roots were rinsed with 1% HCl to restore acidity for staining. The samples were then stained using Lactophenol blue stain (Diagnostica Merk, E. Merk, 64271 Dramstad, Germany). After staining the roots samples were rinsed overnight (12h) in deionized water to remove the extra stain.

Stained roots were cut into approximately 1 cm long pieces, and a group of 10 × 1 cm pieces were randomly selected on a glass slide for microscopic examination. Each root sample was replicated four times per slide (n=4 for each species). The samples were examined using a Motic AE21 inverted microscope (Motic microscope, China) for the presence or absence of mycorrhizal infection.

The length of mycorrhizal infection on each root piece was assessed, averaged, and expressed in percentage as described by (Giovannetti and Mosse, 1980). Similarly, the presence or absence of infection in all the replicates was recorded and the total mycorrhizal colonization was calculated by dividing the length of infected roots by the total length of all roots and expressed in percentage.

$$\text{Total mycorrhizal colonization \%} = \frac{\text{Infected roots length}}{\text{Total roots length}} \times 100 \quad (5)$$

4.7 Data Analysis

Two-pool isotope mixing model was used both for ^{13}C and ^{14}C data to determine the contribution of two carbon sources (sediment and atmosphere) in the plant samples. This mixing model is typically used for partitioning the relative fraction of two sources in a mixture of products. Thus, the fraction contribution of source 1 (f_1) of belowground sources (sediment) in the plant sample was calculated by equation 6 (for ^{13}C analysis $\delta^{13}\text{C}$ was used rather than pMC):

$$f_1 = \frac{pMC \text{ sample} - pMC \text{ source 2}}{pMC \text{ source 1} - pMC \text{ source 2}} \quad (6)$$

Where $f_1 + f_2 = 1$ and thus the fraction contribution of source 2 is $f_2 = 1 - f_1$

In this study, the *pMC sample* represents the pMC content of the plant. Meanwhile, source 1 refers to the sediment, and source 2 corresponds to the air. The $\delta^{13}\text{C}$ value of the atmosphere was -8 ‰. The average ^{14}C content (pMC) of the atmosphere was 104.5 in 2010 according to long-term $^{14}\text{CO}_2$ observation at Jungfraujoch (Levin et al., 2011). It was assumed that the ^{14}C content of the air in Finland was equivalent to that of the measured value of (104.5 pMC) at Jungfraujoch due to the complete mixing of CO_2 in the free atmosphere. Therefore, the average ^{14}C content \pm SEM (standard error of the mean) of 104.5 ± 0.12 pMC was used for air in the mixing model as the second source of C. For ^{13}C analysis, value of -8 ‰ was used as $\delta^{13}\text{C}$ of the atmosphere.

4.8 Statistical analysis

Isoerror version 4.1 (Microsoft Excel, 2000TM spreadsheet) (Phillips and Gregg, 2001) was used to calculate the standard errors and 95 % confidence intervals for the relative contribution of source 1 and source 2. The contribution of sediment-derived C was considered significant from zero contribution (e.g., no transfer of C from the sediment) if the 95 % confidence interval of source 1 did not include zero. To compare the uptake of the sediment-derived C between roots and leaves within each species, an independent-simple t-test was used. The observed difference was considered statistically significant if $p \leq 0.05$. Finally, to investigate the correlation between ^{13}C vs ^{14}C from sediment to plants, scattered plot, using linear regression was performed. SPSS 27 for Windows (SPSS Inc., an IBM company) was used for independent-simple t-test and regression analysis.

5 Results

5.1 Physico-chemical properties of water

The physicochemical properties of the water monitored during the experiment including temperature, electrical conductivity (EC) dissolved oxygen, and pH level are shown in Table 4. Of all the parameters, pH showed significant changes, while the average temperature remained constant at $20 \pm 1^\circ\text{C}$, EC at 1.86 ± 3 mS/cm, and dissolved O₂ at 93 ± 2 %, among all aquariums.

Table 4. Average temperature ($^\circ\text{C}$), EC (mS/cm), and dissolved oxygen (%) of the experimental units during the entire experimental period.

Plant	Temperature ($^\circ\text{C}$)	EC (mS/cm)	Dissolved O ₂ (%)
<i>L. minor</i>	20 ± 1	1.86 ± 3	93 ± 2
<i>S. palustris</i>	20 ± 1	1.86 ± 3	93 ± 2
<i>L. nummularia</i>	20 ± 1	1.86 ± 3	93 ± 2
<i>L. uniflora</i>	20 ± 1	1.86 ± 3	93 ± 2

The fluctuation in the pH level after changing of buffered water can be observed in Figure 3. Higher pH was observed after 2nd day of the plantation in the aquariums or after every 10 days after changing the water. On average the pH level stayed between 5.5 to 6.3 range during the entire experimental period.

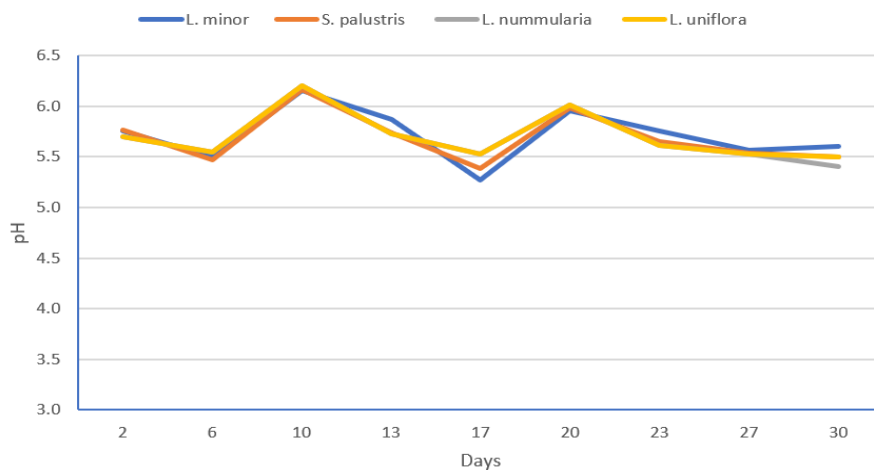


Figure 3. pH fluctuation in the water across the aquatic plant species during the experimental period.

5.2 Isotope analysis of sediment and plant species.

The results of the analysis showed that the total C content of the sediment was 29.8 ± 2.57 (Table 5). The average ^{14}C content of the sediment was 38.4 ± 0.28 pMC and the $\delta^{13}\text{C}$ value was $-26.8\text{‰} \pm 0.08$. The sediment samples were highly depleted in ^{14}C with the age of approximately 7688 years before present (BP). The ^{14}C content of the plants varied between 64.9 ± 5.00 and 99.2 ± 0.41 pMC with the highest value recorded in the *S. palustris* leaves and the lowest in *L. minor*. Similar results were found among the plants for $\delta^{13}\text{C}$ values, showing the lowest value in *L. minor* (-34.9 ± 0.04 ‰) followed by *L. uniflora* roots (-31.7 ± 0.04 ‰) and leaves ($-31.9 \pm 0.11\text{‰}$). The emergent species showed similar values, ranging from -29.5 ± 0.14 ‰ in *S. palustris* roots to -29.6 ± 0.14 ‰ in *L. nummularia* leaves. The total C content (%C) in the plant samples varied from 32.8 ± 0.97 in *L. minor* to 42.3 ± 1.00 in leaves of *S. palustris*. Overall, the emergent plants (*S. palustris* and *L. nummularia*) showed slightly higher C content in the leaves than the roots, while the opposite trend was observed in the *L. uniflora*.

Table 5. Average ^{14}C content (expressed in Percent Modern Carbon, pMC), $\delta^{13}\text{C}$ signature (‰), and carbon content (% C) of the sediment and aquatic plant used in the experiment. SEM represents the standard error mean.

Sample	pMC \pm SEM	$\delta^{13}\text{C}$ (‰) \pm SEM	C % \pm SEM
Sediment	38.4 ± 0.28	-26.8 ± 0.08	29.8 ± 2.57
<i>L. minor</i>	64.9 ± 5.00	-34.9 ± 0.04	32.8 ± 0.97
<i>S. palustris</i> roots	99.2 ± 0.41	-29.5 ± 0.12	38.9 ± 0.51
<i>S. palustris</i> leaves	99.1 ± 0.34	-29.5 ± 0.14	42.3 ± 1.00
<i>L. nummularia</i> roots	98.4 ± 0.10	-29.8 ± 0.34	33.5 ± 0.22
<i>L. nummularia</i> leaves	99.1 ± 0.23	-29.6 ± 0.14	36.3 ± 0.53
<i>L. uniflora</i> roots	91.9 ± 1.25	-31.7 ± 0.04	39.9 ± 0.54
<i>L. uniflora</i> leaves	91.5 ± 1.31	-31.9 ± 0.11	38.2 ± 1.85

5.3 Mycorrhizal colonization of the roots

The average mycorrhizal colonization level of *L. uniflora* roots was $7.75 \% \pm 0.48$ with the individual sample ranging from 6 % to 8 % (Table 6). Notably, no mycorrhizal colonization was observed in the roots of other plant species like *S. palustris*, *L. minor*, and *L. nummularia*.

Table 6. The average colonization level and standard error of the mean (SEM) in *L. uniflora* roots in (%).

Root sample	<i>L. uniflora</i>	SEM
Sample 1	7	0.02
Sample 2	8	0.02
Sample 3	6	0.02
Sample 4	8	0.03
Mean	7.25	0.48

5.4 ¹⁴C uptake by plants

The results of the mixing model revealed the highest contribution of the sediment-derived C in *L. minor* ($59.9 \% \pm 7.55$), which was significantly higher than zero contribution (Figure 4). The results also showed significant transfer of sediment-derived C into the emergent and submerged species, being the lowest for emergent species (*S. palustris* and *L. nummularia*); $7.79\% \pm 0.61$ in the roots, and $8.11\% \pm 0.51$ in the leaves of *S. palustris*, $9.22\% \pm 0.15$ in the roots, and $8.16\% \pm 0.35$ in the leaves of *L. nummularia* and $19.0\% \pm 1.89$ in the roots and $19.7\% \pm 1.98$ in the leaves of *L. uniflora*. Among the plants, studied, *L. minor* was the only species, showing a relatively higher proportion of C from the organic matter than the air. Overall, no significant difference was found between the ¹⁴C content of the roots and the leaves within the same plants. ($p= 1$ for *S. palustris*, 0.08 for *L. nummularia*, and 0.82 for *L. uniflora*).

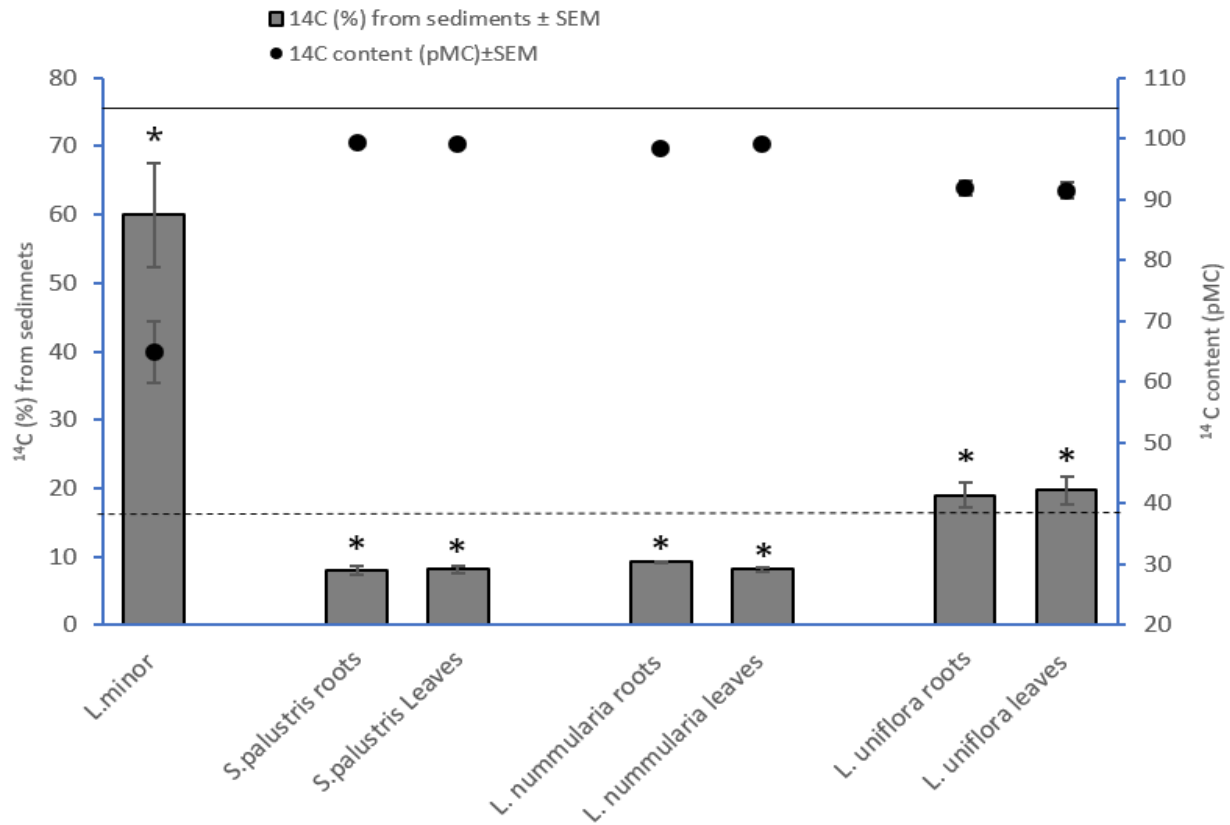


Figure 4. Contributions of sediment-derived C into the aquatic plants (%) and primary ^{14}C data in pMC. Solid black lines represent atmospheric value in pMC (104.5 ± 0.12), while the black dashed line represents sediment value in pMC (38.4 ± 0.27). * Represents a significant difference from zero contribution in the transfer of sediment-derived C into the plants. The statistical analysis showed no significant difference in the transfer of sediment-derived C between the roots and leaves of each species.

5.5 ^{13}C uptake by plants

The transfer of sediment-derived C, using the ^{13}C data set also showed a similar trend to the ^{14}C data (Figure 5). The transfer of sediment-derived C was significant in the selected species. The transfer was higher in *L. minor* ($36.6\% \pm 0.21$), followed by *L. uniflora* ($19.6\% \pm 0.23$ in the roots and $20.9\% \pm 0.61$ in the leaves) and the emergent species ($8.02\% \pm 0.61$ in the roots and 8.12 ± 0.75 in the leaves of *S. palustris*; $9.66\% \pm 1.78$ and $8.35\% \pm 0.73$ in the roots and leaves of *L. nummularia*). Moreover, the results indicated no significant differences in the transfer of sediment-derived C within the roots and leaves of the same plants ($P = 0.85$ for *S. palustris*, 0.62 for *L. nummularia*, and 0.61 for *L. uniflora*).

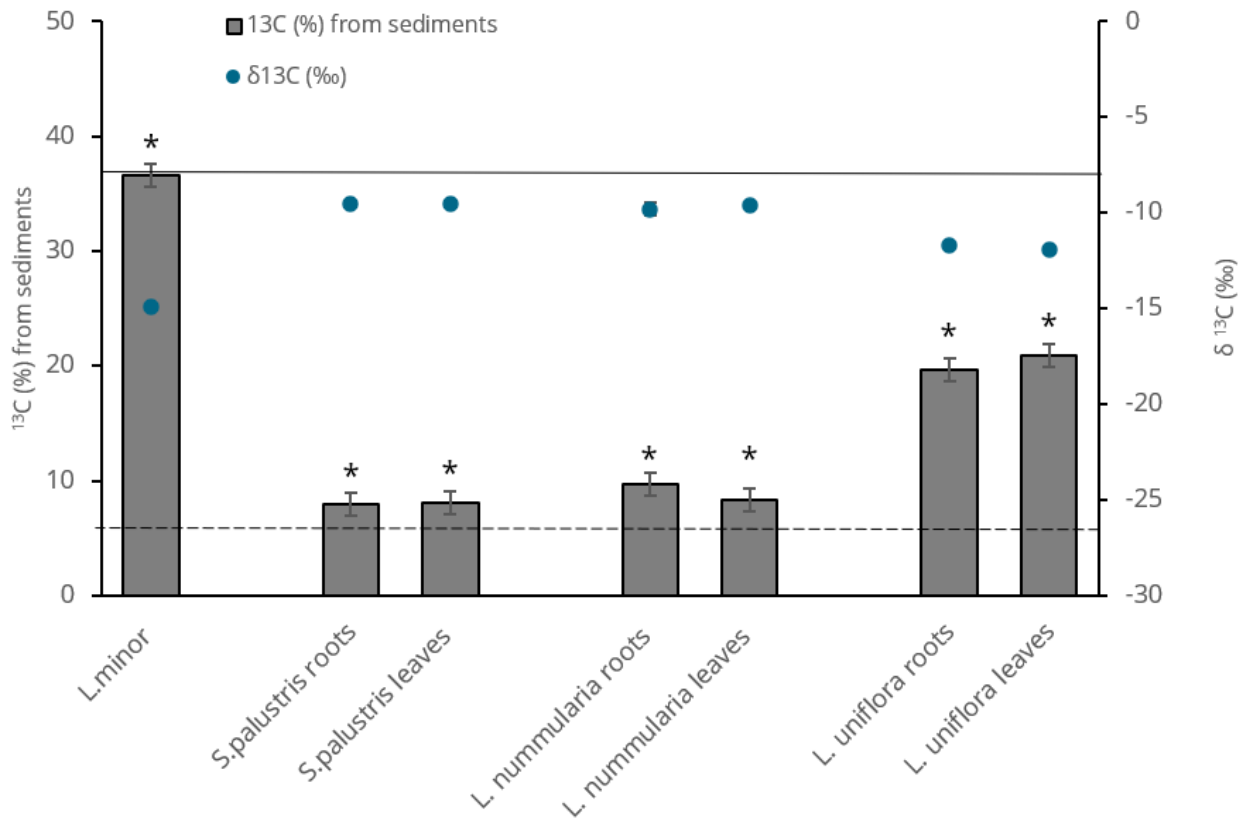


Figure 5. Contributions of sediment-derived C into the aquatic plants and primary $\delta^{13}\text{C}$ value (‰). The dashed line represents the $\delta^{13}\text{C}$ values of the sediment ($-26.8\text{‰} \pm 0.08$), while the black solid line represents the atmospheric value (-8‰). * Represent a significant difference from zero contribution in the transfer of sediment-derived C into the plants. No significant difference was observed in the transfer of sediment-derived C between roots and leaves of the same species.

5.6 Regression analysis between ^{13}C and ^{14}C data sets.

The transfer of sediment-derived C was found to be similar between the ^{13}C and ^{14}C data sets. In general, the regression analysis showed a good fit between the two data sets (Figure 6). The R^2 value was 0.93 and the slope of the regression was 0.55 (95% confidence intervals 0.38-0.72). Although the analysis demonstrated similar values for *S. palustris*, *L. nummularia*, and *L. uniflora*, the uptake of sediment-derived C in *L. minor* was slightly underestimated by the ^{13}C data set.

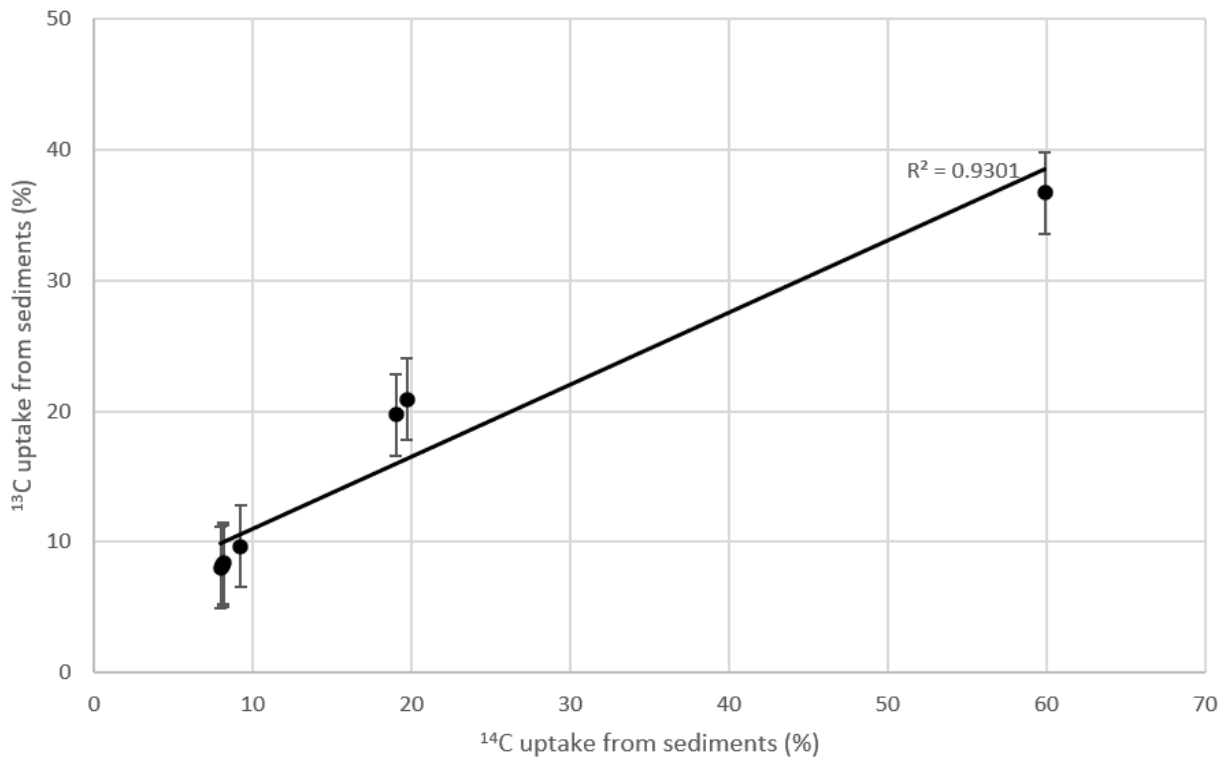


Figure 6. Relationship between ^{13}C and ^{14}C uptake by *L. minor*, *S. palustris*, *L. nummularia*, and *L. uniflora* plants. The black dots represent the uptake of ^{13}C as a function of ^{14}C in plants, and the solid line represents a linear regression (Slope = 0.55; 95% confidence interval 0.38-0.72; $R^2 = 0.93$).

6 Discussion

6.1 Natural abundance approach of using cutaway peatland.

The natural abundance approach, using cutaway peatland proved to be reliable for C source partitioning between the highly depleted sediment and the atmosphere in the studied plants. The cutaway peatland used in the study was highly depleted in ^{14}C content. The average ^{14}C value of the sediments (38.4 pMC) was comparable with the previous (39.4 pMC) value reported for the same cutaway peatlands (Biasi et al., 2011). Currently, isotope analysis is considered the preferred option for the partition of C sources in soil-plants/atmosphere systems (Kuzyakov, 2006). The current study fulfilled the important prerequisite for isotope partitioning (non-overlapping signature), as the ^{14}C signature of cutaway peatland was highly depleted as compared to that of the modern atmospheric level. This large difference in isotopic signature allowed us to trace the sources of C in the plants by applying two pool mixing model and accurately quantify their relative proportions in various parts of the plant.

The uptake of sediment-derived C was used as an implication for understanding the potential uptake of ^{14}C from below-ground sources, particularly concerning the possible release of ^{14}C from nuclear power plants or discharges from underground repositories into nearby aquatic ecosystems. Given that, plants in aquatic ecosystems are the primary sources of C for the other organisms. They can incorporate CO_2 or any other inorganic forms of C from the surroundings (possibly released ^{14}C due to identical chemistry with stable C isotopes) and convert it to organic form and thus further transport ^{14}C in the biosphere. In our study condition, artificial fresh water was used to simulate the natural aquatic conditions for the plants. It was assumed that the artificial fresh water was free of additional nutrients and all the C species originated solely from depleted peat sediments during the experimental period. consequently, the pMC value of the plant samples contained only the proportion of atmosphere and peat sediments C sources.

6.2 Uptake of ^{14}C by the aquatic plants.

The transfer of sediment-derived C was significant in the selected aquatic plant species. However, the uptake varied greatly among the aquatic plant species. *L. minor* showed the highest proportion of sediment-derived C compared to other aquatic plants. It is typically assumed that (free-floating) aquatic plant leaves in direct contact with the atmosphere can uptake more CO_2 from the atmosphere (Maberly and Madsen, 2002), but surprisingly *L. minor* displayed the highest contribution of sediment-derived C (59.9%) relative to the atmospheric contribution, indicating efficiency in the uptake of sediment-derived C in dissolved forms. A similar higher uptake of 86 % dissolved inorganic C was also reported by Filbin and Hough, (1985), for *L. minor*. However, we partitioned the proportion of the sediment-derived C with isotope analysis, which is the more sensitive and accurate source partitioning method, suggesting that 59.9% uptake of sediment-derived C could be possible in *L. minor*. The significantly higher uptake of *L. minor* could also be attributed to its suspended roots for more efficient incorporation of the most readily available form of DIC such as CO_2 (Pedersen et al., 2013b). However, the concentration of CO_2 in the water column is pH-dependent. At a relatively low pH or pH below 6, the concentration of CO_2 prevails in the medium (Vuorinen et al., 1989). Thus, the relatively low pH (5.5 during the experimental period) made it more accessible for *L. minor* to uptake the readily available form of C from the dissolved phase. Moreover, (Majlesi et al., 2020) reported up to 60% transfer of organic C from sediment into DOC, using the same peat. This finding together with the results of this study (e.g., 60% uptake of sediment-derived C in *L. minor*) indicates the possible release and mineralization of DOC in the water column and availability of C in the inorganic form to *L. minor*.

In contrast, emergent species such as *S. palustris*, and *L. nummularia*, exhibited lower contribution of sediment-derived C. This could be due to variability in plant physiology and the mechanism of C uptake. Emergent plant leaves are above the water surface (in direct contact with the atmosphere), while the roots are deposited in the bed sediment, suggesting a preference for atmospheric C (Maberly and Madsen, 2002). Since the roots of emergent plants were beneath the water attached to the sediment, direct uptake of DIC from sediment pore water occurred to a weaker extent and thus they are proportionally less dependent on C from the organic matter (Vapaavuori and Pelkonen, 1985; Mommer and Visser, 2005). Our data showed no significant

difference between the ^{14}C content of roots and leaves in the emergent plants. A possible explanation could be the presence of aerenchyma. In general, partially and fully submerged hydrophytes possess a well-developed tissue structure (aerenchyma), which contains air spaces between and within roots and shoots (Takahashi et al., 2014). Such structure can facilitate rapid exchange of gases, including CO_2 , allowing hydrophytes to survive under stressful conditions to promote photosynthesis (Herzog and Pedersen, 2014; Takahashi et al., 2014).

L. uniflora being completely submerged displayed nearly double the proportion of sediment-derived C compared to emergent species with 19 % and 19.7 % being assimilated by roots and leaves respectively. The comparatively higher uptake may be associated with its extensive rooting system facilitating higher direct uptake of CO_2 from sediment pore water. Previous studies also reported 70 to 80% of the total assimilated C by isoetid directly from sediment (Smolders et al., 2002). The special adaptation of *L. uniflora* of high radial oxygen loss (ROL) oxygenates the root zone and supports microbial decomposition of organic matter, consequently enhancing CO_2 concentration in sediment pore water (Smolders and Roelofs, 1996). The presence of a higher amount of CO_2 in the rhizosphere might enhance the direct uptake of sediment C by *L. uniflora* through the process of diffusion or mycorrhizal transfer. Notably mycorrhizal association was detected exclusively in *L. uniflora* roots. Interestingly our results revealed no significant difference in the contribution of sediment-derived C between the roots and leaves of *L. uniflora*. This uniform distribution of C may be attributed to aerenchyma transports of gases between roots and photosynthetic sites in the leaves (Hostrup and Wiegleb, 1991). While the leaves of *L. uniflora* can also fix dissolved CO_2 directly from the water, but majority of C in the leaves originates from the roots (Wetzel et al., 1985).

6.3 Uptake of ^{13}C by the aquatic plants.

The transfer of sediment-derived C using ^{13}C has a similar trend to that observed in the ^{14}C data set. The highest contribution of sediment-derived C (36.6 %) was found in *L. minor*. This value was comparatively lower than the corresponding value observed in the ^{14}C data set, which potentially can be related to the plant photosynthetic pathways, and associated fractionation (O'Leary, 1981; Mendonça et al., 2013). Parallel to the ^{14}C data set, the lowest uptake percentage

was noted in the emergent plant species. Despite the direct aerial exposure of *L. minor* and emergent species leaves to the atmosphere there was a notable variability in the uptake of sediment-derived C through roots. Aquatic plants have different anatomies and C assimilation pathways which determine the sources of C used by plants during photosynthesis (Marčenko et al., 1989; Mendonça et al., 2013). In general, *L. minor* is a free-floating plant with no direct root contact with the sediment potentially acquired dissolved C from the water column. Moreover, the high biomass production and shortened doubling time might have contributed to the higher uptake of sediment-derived C in *the L. minor*.

Emergent plant roots attached to the sediment and displayed lower uptake of sediment C than free-floating. This might be attributed to the more efficient assimilating of DIC from water medium in *L. minor* than the rooted plants. Furthermore, the uptake of sediment-derived C in *L. uniflora* was double that of the emergent species which could be due to the extensive rooting system and well-developed gas transport mechanism between root and shoots. *L. uniflora* oxygenates the root zones enhancing CO₂ uptake through roots (Smolders and Roelofs, 1996). Besides physiological disparities, among plant species, DIC concentration and water pH influence sediment C uptake, because they determine the availability of dissolved C and the relative proportion of different C forms in water. CO₂ predominates at relatively low pH and diminishes at a pH level exceeding 8 (Vuorinen et al., 1989). In the current study, plants that utilized only CO₂ for photosynthesis had enough available CO₂ for uptake because the pH was recorded as less than 6.3 during the study period. However, it should be noted that different plant species exhibit varied discrimination against heavier isotopes, particularly under elevated concentrations based on photosynthetic reactions (Šturm et al., 2012; Chappuis et al., 2017). In our case, discrimination against ¹³C seems to be lower in *L. minor* in comparison to the remaining plant species. ¹³C data also reveals no significant difference between the root and leaves of the selected plant species same as the ¹⁴C data set. Our data further indicate that the uptake of sediment-derived C through roots can be species-specific as the higher uptake was observed for *L. minor* than the remaining plant species in both data sets.

6.4 Relationship between ^{13}C and ^{14}C uptake.

The transfer of sediment-derived C was found to be similar between the ^{13}C and ^{14}C data sets. Among the studied species, *S. palustris*, *L. nummularia*, and *L. uniflora* the uptake of sediment-derived C exhibited consistent values between ^{13}C and ^{14}C datasets. This suggests that these species may share similar C assimilation pathways resulting in comparable uptake of both isotopes. However, the ^{13}C dataset for *L. minor* slightly underestimated the uptake of sediment-derived C compared to the ^{14}C data sets (approximately 1.5-fold difference).

Despite the differences in *L. minor* uptake a strong correlation was found between ^{13}C and ^{14}C uptake among most of the species (Figure 6). This indicates the reliability of using stable isotopes as a proxy for predicting the transfer of ^{14}C into aquatic plants. This approach is widely used in the current paradigm in radioecological modeling that assumes that stable isotopes of the same element can sufficiently predict the transfer of radionuclides in organisms (IAEA, 2010).

Stable isotopes offer several advantages over radioactive isotopes, including not needing radiation protection measure concerns associated with radioactivity, and the practicality of measurement using mass spectrometry (Michener and Lajtha, 2007). While using ^{13}C as a proxy for ^{14}C uptake offers several advantages, it is important to acknowledge that ^{13}C may not perfectly replicate the behavior of ^{14}C in biosphere assessment studies. Fractionation processes, species-specific physiology, and environmental conditions can influence the relationship between ^{13}C and ^{14}C uptake, (O'Leary, 1981; Roussel-Debet et al., 2006). Therefore, while ^{13}C can serve as a useful alternative to ^{14}C in many cases, caution must be exercised when interpreting results, and further research is needed to clarify the underlying mechanisms driving carbon assimilation in aquatic plants.

6.5 Atmospheric values of ^{14}C

The use of the isotopes approach in the present study was based on the difference in the ^{14}C content of sediment and the atmosphere. The results were reliable for the partitioning of sediment-derived C in the selected aquatic plant species. However, it should be recognized that the estimated proportion of sediment-derived C in the plants depends not only on the ^{14}C value used for the sediment but also on the atmosphere. In our calculation, we used the average value

of 104.5 pMC for the year 2010 in the mixing model instead of the latest reported value of (100 pMC for 2022) in the long-term $^{14}\text{CO}_2$ observation at Jungfraujoch (Levin et al., 2011, 2022). The value of the ^{14}C in the atmosphere has not been constant since the bomb peak in the 1950s and early 1960s and showed a decreasing trend. The choice of using 104.5 pMC for our primary calculation was based on the assumption that the atmosphere ^{14}C values are not static but may potentially be higher in the vicinity of the operational power plants, nearby geological repositories, or in the event of unexpected accidents. Various studies such as those conducted by (Fontugne et al., 2004; Povinec et al., 2009; Bolsunovsky and Dementyev, 2011) have documented a higher concentration of ^{14}C in the vicinity of nuclear-reprocessing plants and nuclear fallout areas. However, using the latest value of atmospheric ^{14}C (100 pMC) resulted in a slightly lower uptake of sediment-derived C into aquatic plants. This suggested that the potential contamination of aquatic plants with ^{14}C may be more pronounced in the area with a higher atmosphere ^{14}C concentration.

7 Conclusions

In this thesis, the transfer of sediment-derived C into three categories of aquatic plants was investigated by applying the isotopic technique. The use of isotope analysis proved to be reliable in the partitioning of C sources between the sediment and the air in aquatic plants. The results showed a significant contribution of C from organic matter into the selected species. The free-floating species (*L. minor*) displayed the highest contribution of sediment-derived C (60%) compared to the emergent *S. palustris* and *L. nummularia* (8-10%) and submerged species *L. uniflora* (20%). The variability in the transfer of sediment-derived C among the species might be associated with the different morphology, physiology, and mechanism of C assimilation in the plants. Moreover, a similar uptake of sediment-derived C was observed between roots and leaves potentially because of aerenchyma or other well-developed gas transport systems.

The results of this study also showed a strong correlation between ^{14}C and ^{13}C data sets for transferring sediment-derived C across most of the species, meaning that ^{13}C as a stable isotope can effectively serve as a proxy for ^{14}C uptake on species-specific bases. However further research is needed to validate these findings and explore the relationship between ^{13}C and ^{14}C uptake. These findings are important for the radioecological modelling of ^{14}C as data on aquatic plants are limited. It seems that the magnitude of DIC uptake from the organic matter can be larger by roots of aquatic plants than previously assumed for terrestrial plants. That means if the aquatic reservoirs are contaminated with ^{14}C there might be a relatively higher risk to the aquatic food webs. Henceforth, further studies on the transfer of ^{14}C into aquatic ecosystems are recommended.

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