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Lake Shore Restoration with *Vallisneria spiralis* in Lake Como (Northern Italy) to Improve Sustainability

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Abstract: In the Anthropocene era, lake ecosystems are increasingly subjected to significant human-induced pressures, leading to declines in both biodiversity and habitat quality. However, restoration initiatives offer promising avenues for enhancing the resilience of freshwater environments. This research investigated a range of established and novel methods aimed at promoting the growth of the macrophyte *Vallisneria spiralis* in the littoral zone of Lake Como, a southern alpine lake in Italy. To conduct this study, samples of *Vallisneria spiralis* were collected and placed in tanks containing four different types of 3D-printed biodegradable substrates. The optimal conditions for the growth of this species were identified as follows: a temperature range of 25 to 27 °C, the continuous operation of a circulation pump equipped with a filter, the presence of a fertile substrate, and light cycles comprising 6 h of peak illumination followed by 6 h of darkness. Remarkably, the plants exhibited a growth rate of 4 mm per day, increasing from an initial count of 12 specimens to 400 within four months, with a total of over 700 plants by the end of the study. Among the substrates tested, the patch substrate was found to be the most effective. After their introduction into the natural environment, the survival rate of plants established on stable substrates in contact with the lakebed reached an impressive 85.7%. This research represents a pioneering step in demonstrating that *Vallisneria spiralis* may serve as a viable option for restoration projects in coastal lake habitats, particularly when employing biodegradable substrates.

Keywords: biodegradable artificial 3D printed substrates; freshwater ecosystems; lake restoration; sustainability of freshwater; macrophytes; *Vallisneria spiralis*

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1. Introduction

Earth is often referred to as the “blue planet” because about 71% of its surface is covered by salt water, primarily in oceans and seas. However, less than 5% of the Earth’s water is freshwater, most of which is in ice caps, glaciers, groundwater aquifers, and lakes [1]. Lakes are a crucial resource for life on Earth, providing essential ecosystem services to human populations [2]. Aside from a few remote lakes, they have historically been vital to human settlements, supplying food, and water for domestic and industrial use [3], and offering economic benefits in transportation, recreational activities, irrigation, and agriculture [4]. Even in remote, pristine areas like high mountains or arctic regions, lakes are exposed to increasing contamination through the passive or active long-range transport of various inorganic and organic contaminants [5–7]. Additionally, lakes continuously receive significant amounts of materials from their watersheds [8], leading to plastic pollution that impacts sediment, the water column, and biota [9–11]. In addition to natural processes of eutrophication, nutrient discharges enhanced by human activity present another major challenge for environmental sustainability and lake management [12]. Human activities have had such a significant impact that it is now crucial to take action to protect freshwater ecosystems by enhancing their resilience to various pressures, preventing any further loss of biodiversity, and improving sustainability [13–15].

In this context, habitat restoration has been identified as a sustainable and effective solution at the worldwide level [16,17]. Restoration programs mainly focus on improving communities of foundational organisms that can better structure habitats and support biodiversity. Numerous examples from marine ecosystems highlight projects aimed at restoring coral reefs in tropical areas [18–20] and *Posidonia oceanica* in the Mediterranean Sea [21–23], both fundamental habitats for marine ecosystems and human uses. For lacustrine ecosystems, restoration efforts have mainly addressed the growing issue of eutrophication, particularly through biomanipulation strategies that involve removing certain species of fish to control zooplankton density [24,25] and, to a lesser extent, introducing submerged macrophytes [26–28].

Lake Como, the deepest lake in Italy, provides substantial ecosystem services, including drinking water, recreational uses, industrial uses, and irrigation. However, its location in a heavily industrialized area means it is subject to pollution from watershed activities and even from melting glaciers [29,30]; the lake also receives water inflows from rivers and various wastewater treatment plants that struggle to effectively reduce contaminants from urban and industrial sources [31]. Despite the implementation of current water treatment practices, their inefficiency allows many pollutants to continue to enter the ecosystem and accumulate in edible fish species, including Alosa agone [32–34]. In conclusion, various human impacts, such as plastic pollution, climate change, and urbanization, have led to a significant reduction in biodiversity and a decline in key freshwater ecosystems that provide essential services. Urgent action is required to address these challenges and protect these crucial environments.

This study aims to maximize and ensure the autonomous repopulation of the littoral zone in a specific area of Lake Como using both traditional and innovative techniques in order to improve the sustainability of the littoral area and improve biodiversity. These techniques include the use of artificial biodegradable substrates for the implantation of submerged macrophytes, which play an important role in increasing littoral biodiversity.

2. Materials and Methods

2.1. Study Area

Lake Como (Figure 1), recognized for its stunning landscapes and rich history, is indeed one of the most remarkable alpine oligomictic lakes in Europe. Its unique geological formations, shaped by significant Messinian River erosion, contribute to its impressive depth and steep underwater topography. Oligomictic lakes are characterized by minimal mixing, which is evident in Lake Como's thermal stratification during the warmer months.

In summer, the lake's water layers form distinct thermal layers, with warmer water on top and colder water below, leading to different ecological conditions at various depths. The phenomenon of isothermy, in which an entire body of water reaches a uniform temperature, typically occurs at the end of winter when surface temperatures drop, and mixing can occur. This process can affect the distribution of nutrients and oxygen levels throughout the lake. In the littoral area in summer, there is no temperature stratification, and the average temperature of the water column (from the surface to a depth of 8 m) is around 24 °C.

The limited submerged areas within depths of 1.5 to 10 m suggest that the lake's surface area primarily comprises deeper water, which can heavily influence the biodiversity and habitats present in the lake. These conditions make Lake Como not only a vital ecological region but also a popular destination for tourism and outdoor activities, such as boating, hiking, and enjoying the natural scenery. The interplay of its geological history, hydrodynamics, and climatic conditions contributes to making Lake Como a unique ecological and recreational gem in Italy.

To maximize the effectiveness of the intervention, a coastal environment located between depths of 1.5 and 8 m was chosen. In this specific zone, habitats offer greater complexity and are favorable due to the presence of light, higher temperatures, and high biodiversity. In particular, the following factors were considered:

- The upper limit of the colonizable zone is 1.5 m as it is closer to the surface, and fluctuations in the water level and wave action make it difficult for macrophytes to maintain a stable presence.
- The lower limit is 8 m, as, below this depth, the vegetation gradient decreases rapidly due to lower light levels; however, macrophytes are sometimes visible at greater depths (in Lake Como, based on personal observations, never deeper than 10 m). Therefore, this is the maximum depth to target to maximize the positive effects of restoration efforts.

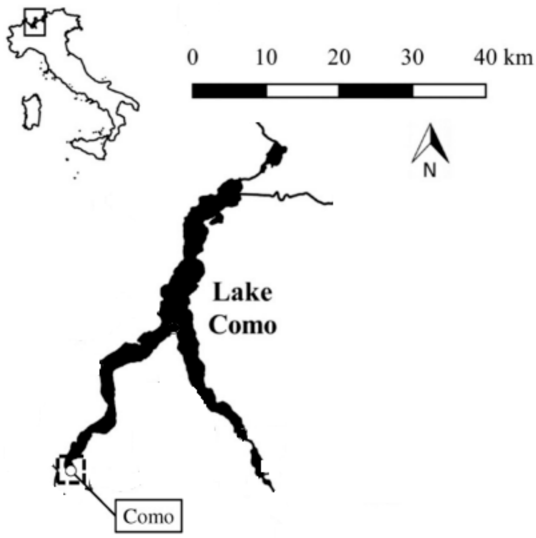

Figure 1. A map of Lake Como and the restoration area. The average temperature of the littoral area in summer is 24 °C.

Additionally, at this depth, the restoration process is relatively simple as divers do not require decompression stops. These stops would increase the duration and complexity of the dive, as well as the cost of respiratory mixtures containing helium or other diluents to lower the oxygen concentration. Conversely, it is possible to use oxygen-enriched mixtures and nitrogen-depleted mixtures, which allow for longer dive times at shallow depths with limited costs. The underwater campaign was conducted using either air or nitrox up to 32%.

2.2. Target Species

The target species in this study was *Vallisneria spiralis*, a submerged plant species which originates from Asia but is native to the Lake Como area [35]. The choice to use this species is based on two main reasons: first, it is commonly found in meso- to eutrophic water habitats [36], where it forms dense stands due to its stoloniferous growth habits [37]; second, it is widely considered as an ecological engineering species for aquatic ecosystem restoration [38]. Like the other submerged macrophytes, this species depends on sediments for nutrient uptake since the available nutrients are in higher concentrations in pore water than in the water column [39]. The presence of this plant implies a strong impact on the surrounding environment, promoting the shift of a large amount of oxygen to roots to allow their respiration; this phenomenon creates oxic conditions around roots and influences several redox-sensitive biogeochemical processes at the bottom sediment level [39,40].

2.3. Mesocosm Experiment

Vallisneria spiralis was taken directly from the coastal environment of Lake Como in the surrounding areas selected for replanting. The choice was made considering that resident populations may have the best adaptive characteristics for rooting. The number of individuals was kept as low as possible in order to minimize the impact of the collection on relict populations. The specimens were removed, and a preference for stolons during the detachment resulted in little chance of survival.

Mesocosm conditions were selected for the growth and reproduction of *Vallisneria spiralis* considering the autoecology of the target species and the possibility of detecting non-ideal situations, as the experimental conditions comprised a mesocosm situation rather than a strictly natural one. Two tanks were equipped with thermostatic heaters set at 26 °C, which is the maximum temperature recorded in summer at depth of up to 10 m. Therefore, this is the highest temperature to which individuals of *Vallisneria spiralis* have adapted to live in the coastal areas of Lake Como. The tanks were also equipped with LED lights with a 6000 K full spectrum growing light. The light was first kept constant, always at maximum power, but this condition favored the uncontrolled development of algae. The illumination period was then reduced to 12 h, and the conditions improved, but algae was still present. Therefore, six hours of light and six hours of alternating darkness were set. The short period of light inhibits the development of filamentous microalgae while allowing for good growth of the phanerogams. The tanks were fitted with ceramic filter rings and sponges to prevent the buildup of organic matter and to counteract Chlorophyceae blooms. Despite these precautions, one tank still experienced blooms, possibly due to the delayed asexual reproduction of plants caused by lower nutrient levels or its closeness to natural light. To stimulate *Vallisneria spiralis* growth, CO_2 was injected into the tank at a concentration of six bubbles per minute for every 180 L of water. The pearling phenomenon, in which oxygen released by plants forms visible bubbles on their leaves, occurred; however, the growth rates remained relatively similar.

Biodegradable substrates (Figure 2) were developed to establish suitable planting environments in each tank to promote the modular rooting of macrophytes. The substrates were designed, shaped, and 3D-printed using computer graphics and a combination of polylactide (PLA) and natural composite materials found on site. The goal was to minimize the alteration of natural compositions while ensuring a functional structure for agamic rooting and reproduction in the environment. The 3D models were continuously modified to improve effectiveness, with alterations made to roughness, the number of cavities, dimensions, and planting techniques. Four different substrates were produced to facilitate the rooting of aquatic plants in varying coastal environments:

(1) PATCH type: A hydrodynamic geometric module designed to be placed over sandy sediment or inside muddy ground. It features a reduced thickness and shape that provides resistance to waves and is able to withstand water turbulence up to a depth of 10 m (Figure 2A).
(2) LINEAR PATCH type: A substrate designed to facilitate the growth of plants in a linear formation, promoting rapid expansion in flat and uniform environments. This substrate is specifically intended to combat the spread of invasive species like Egeria densa (Figure 2B).
(3) BRANCH or BOULDER type: An ovoid object used to occupy the space between rocky blocks or submerged branches, equipped with a point of attachment to the bottom screw (or dowel) passing through. The geometric shape allows for the storage of large amounts of nutrient reserves and, therefore, ensures greater autonomy in environments with little organic soil available for engraftment (Figure 2C).
(4) BLOCK type: A modular object that can be used to create any structure by combining small blocks. It was specifically designed for use in mixed environments where other objects may not be suitable (Figure 2D).

All four types of substrates were designed with a primarily clustered arrangement to promote recolonization with diffuse patches. This approach was selected to increase intervention efficiency by reducing the need for multiple underwater interventions and facilitating natural recolonization between clusters. Most substrates were modular, allowing for the creation of a continuous surface. This flexibility enables the on-site adjustment of patch size to maximize functionality for root processes and recolonization. The starting substrate was enhanced with Osmocote fertilizer (Osmocote Exact Tablet 8-9M; https://icl-growingsolutions.com, accessed on 5 November 2024) at a concentration proportional to the recommended dosage for aquatic plants in order to improve the rooting phase. However, it was found that the dosage was slightly undersized and was corrected in the second moment. Additionally, a support fertilizer was developed using compounds derived from the target ecosystem of Lake Como. This fertilizer was placed within the substrate and calibrated to provide nutrients for approximately one year. The substrate's morphology also allowed for the addition of extra nutrients if needed, even after implantation in the environment through underwater intervention.

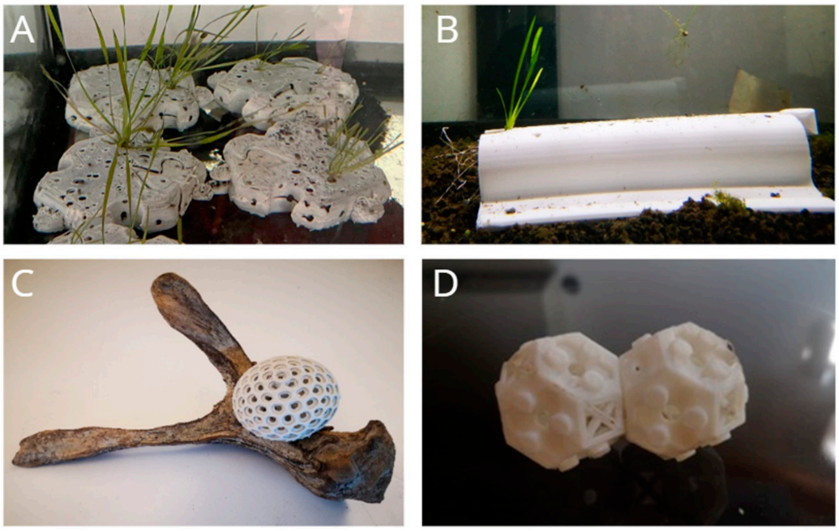

Figure 2. The four types of substrates: patch type (**A**), linear patch type (**B**), branch or boulder type (**C**), and block type (**D**).

The tanks were set up in February and March 2021, with 12 specimens of *Vallisneria spiralis* from the lake. The study proceeded with the selection of small, adventitious individuals whose roots could be easily inserted into the artificial substrate, ensuring the proper development and growth of the initial individual, as well as the colonization of the bottom by subsequent specimens which reproduced via stolons (Figure 3). All types of substrates were used for planting trials with *Vallisneria spiralis*. The different types of substrates were not differentiated with the aim of hosting different genera or species but rather to verify the functionality of anchoring based on the conditions of the restoring site or to meet the needs of scientific dissemination.

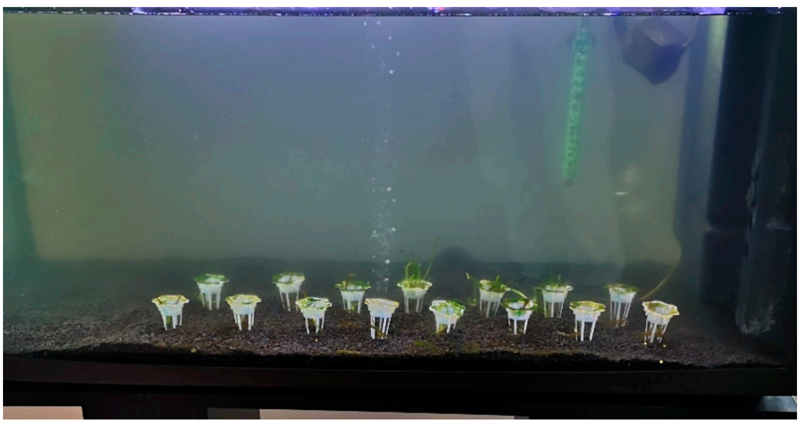

Figure 3. A tank filled with 12 starting specimens of *Vallisneria spiralis* (stolons).

After the rooting phase, which took two weeks, the growth of the stoloniferous and foliar apparatus was measured. After 11 months, it was necessary to renew the Osmocote content in order to renew reproduction.

Once fully grown, three *Vallisneria spiralis* specimens were implanted into each substrate and placed in a separate tank. In November 2023, an underwater webcam was installed to monitor the first 15 m^2 littoral area on a weekly basis, and *Vallisneria spiralis* specimens were implanted in Lake Como. A second implantation survey was carried out in February 2024. The installation was carried out by a diver (Figure 4) for seven patch-type, three branch-type, and eight block-type supports (N = 18). The patch-type supports were installed manually and attached to the substrate using a metal clamp. Two branch supports were positioned by sinking them halfway into the sandy substrate where it was loose or fixed between rock blocks to prevent displacement by currents or turbulent movement. One was removed by water turbulence, and it was immediately reinserted into the environment after the detachment. The plants remained viable despite the slippage.

Figure 4. The underwater installation of different supports by a diver.

Later, eight additional block-type supports were added through remote implantation using an underwater drone. They were initially positioned using a robotic arm and then fixed in a subsequent phase with a clamp. This type proved to be the most unstable, resulting in the highest percentage of detachment due to the time elapsed between the initial placement and the second manual attachment with metal clamps. In addition to the virtual check using the underwater webcam, a comprehensive monitoring survey was carried out in the field in March 2024. The survival rate was quantified with a final check in April 2024 through underwater survey immersion, Remotely Operated Vehicle (ROV) video recording, and observation using a webcam positioned in front of the intervention area.

3. Results

For *Vallisneria spiralis*, the mesocosm conditions set in the tanks appeared to be optimal as the growth of the stoloniferous and foliar apparatus reached peaks of 4 mm/day until the tank space was filled (Figure 5). The optimal condition found for the development of the species were as follows:

- A temperature between 25 and 27 degrees Celsius;
- A constantly active circulation pump with a filter;
- The presence of a fertile substrate (tablets renewed every 6 months);

- Light cycles set to provide enough hours of darkness to counteract excessive filamentous algae growth; the cycles used were 6 h of peak light alternated with 6 h of darkness.

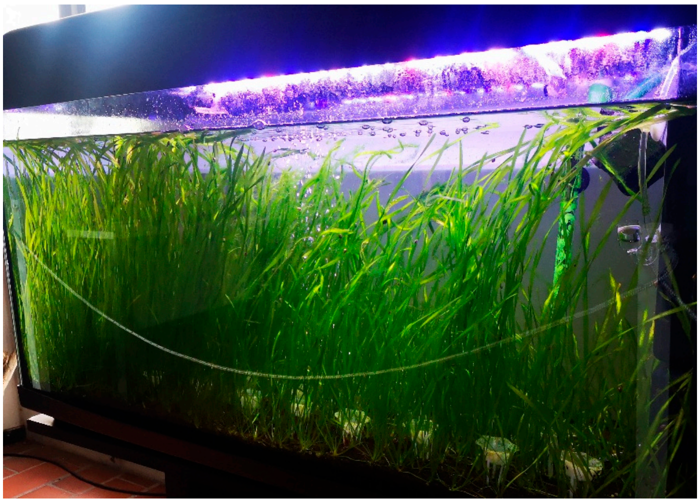

Figure 5. A tank full of completely grown specimens of *Vallisneria spiralis*.

A higher frequency of dark periods resulted in a greater control over microalgal growth, likely due to the reduced ability of the algae to tolerate short lighting periods. This significantly reduced the ongoing manual work of removing filamentous microalgae from the tanks, and the extracted biomass was also lower.

From the initial number of specimens (N = 12), the tank contained 400 *Vallisneria spiralis* individuals after 4 months and more than 700 specimens before the installation phase. Regarding the field experiment, of the 18 tutors introduced into the environment, 4 block tutors (Figure 6) were unsuccessful plantings since they were deployed during scientific outreach and citizen science activities via a Remotely Operated Vehicle (ROV) without an opportunity for immediate manual bottom fixation. Without considering those 4, 12 out of 14 remained viable (85.7%), and only 2 detached from the bottom. Thus, the main cause of *Vallisneria spiralis* death is solely linked to the detachment of the artificial substrate from the bottom and the subsequent sliding. Regarding the different substrates, it was observed that the most functional substrate seems to be the patch.

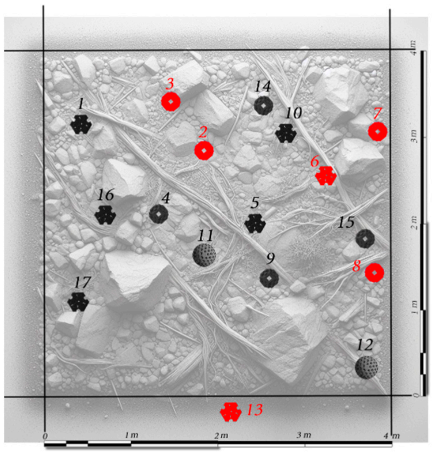

Figure 6. Patch-type, branch- or boulder-type, and block-type supports settled in the environment and monitored. Red supports indicate the ones that detached from the bottom surface and were, therefore, unsuccessful. There are three types of substrates (patch, the hexagonal one; branch, the spherical one; and block, the one with the central hole). For each, there are two colors: black when they are in place and red when they have detached from the installation site.

The monitoring involved leaving the substrates in the tank for several months in order to quantify the growth and reproduction ability of the plants. Photographic records also demonstrate the effectiveness of the mixture included in the substrates. Monitoring in the environment at the experimental site was carried out through underwater survey immersion, Remotely Operated Vehicle (ROV) video recording, and observation using a webcam positioned in front of the intervention area.

4. Discussion

At present, while *Vallisneria spiralis* is well-known as an ecological engineering species for aquatic ecosystem restoration [38], there is a lack of reviews concerning the effective restoration *Vallisneria spiralis* populations in the environment, especially when dealing with biodegradable matrices as substrates. Only a few restoration campaigns, which did not include biodegradable matrices, were carried out using *V. americana*, another species of the same genus *Vallisneria* [41,42]. Therefore, the results of this study are the first regarding a restoration project using this species in combination with biodegradable substrates. According to the literature, only three works [43–45] have examined the use of these materials in restoration programs with marine seagrass. The work of Balestri et al. [43] is the most similar to this research system; both are based on the use of biodegradable polymers, although they created biodegradable substrates by applying Poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) instead of using polylactide (PLA) polymer. However, the PLA fibers used for this experiment are considered more resistant to biodegradation in comparison with PHBV fibers [46]. With regard to the species used in restoration programs, different seagrasses have been used previously: *Cymodocea nodosa* and *Zostera noltei* [43], *Halodule wrightii* [44], and *Amphibolis* [45]. All the studies mentioned reached the conclusion that the application of sediment-filled biodegradable tubes or bio-containers resulted in a significant acceleration in seagrass recovery and restoration. Moreover, Balestri et al. [43] specified that bio-containers degraded after about three years, a period long enough to obtain well-developed plants in nurseries. As already mentioned in the Results, it was observed that the most functional substrate seems to be the patch as it adheres better to the natural bottom at the chosen site and performs better from a hydrodynamic perspective. The branch substrate is designed to be installed on branches or among rocks. However, it was also tested on sandy bottoms, which made it more susceptible to slipping. The block substrate experienced slipping. This problem was solved by manually securing it underwater, but this was often not feasible for educational activities, making it difficult to secure them once installed. As a result, these substrates are susceptible to movement, and the fact that they are not all in contact with the bottom often hinders root establishment. The path substrate was produced as a one-off; therefore, its effectiveness cannot be assessed.

5. Conclusions

The preliminary results of our efforts to restore *Vallisneria spiralis* in the littoral area of Lake Como are encouraging. Biodiversity enhancement is crucial for maintaining the environmental sustainability of lake shores, and our ongoing initiatives aim to establish a model for future restoration projects.

One particularly innovative aspect of this project is the use of substrates for cultivating *Vallisneria spiralis*. Our findings indicate several key advancements:

- Planting in specialized substrates, rather than directly in sediments, represents a significant innovation. The delicate root systems of *Vallisneria spiralis* and other aquatic plants struggle to anchor themselves in unassisted environments, making physical support essential for their establishment.
- Our comparative analysis of various forms and types of substrates has led us to identify the most effective materials for supporting plant anchoring and establishment (the patch).

- The implementation of a substrate system that functions like a small container enhances the growth medium, allowing us to easily adjust nutrient proportions tailored to the specific needs of the aquatic plants we are introducing.

Acknowledging the vital role of biodiversity in sustaining the ecological balance, our next step involves incorporating additional aquatic macrophytes, such as *Potamogeton* sp. and *Myriophyllum* spp. The forthcoming phase of our installation and monitoring will expand to cover an area of 400 m^2, facilitating a more comprehensive assessment of the restoration processes related to submerged prairies of phanerogams. By evaluating these interventions on a broader ecological scale, we aim to refine our strategies and provide valuable insights for future restoration initiatives, ultimately fostering a healthier and more resilient aquatic ecosystem.

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