






Article

Coping with Stress as a Prelude of Naturalization: Different Responses of *Lagarosiphon* Species to Water Trophy

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Abstract: Horticulture is a major pathway of introduction of aquatic plants. Among traded aquatic plants, we found two species belonging to the genus *Lagarosiphon* Harv. (Hydrocharitaceae), native to South and Central Africa, *L. major* (Ridl.) Moss and *L. cordofanus* Casp. *L. major* is the main representative of the genus, having already been introduced via horticulture sale beyond its native range, and often becoming invasive. In contrast, *L. cordofanus* is a lesser-known congener that could be potentially sold as an alternative to *L. major*. It is relatively understudied, and has yet to be recorded in the wild outside its native range. Many factors can promote the invasiveness of an alien plant; among them, increased nutrient availability often facilitates opportunistic alien species such as *L. major*. In a horizon-scanning perspective, a manipulative experiment under controlled conditions was performed to test the response of *L. cordofanus* to different trophy levels using *L. major* as the tolerant alternative species. According to our results, the naturalization of *L. cordofanus* in temperate shallow waters does not seem likely, especially if considered in comparison to *L. major*.

Keywords: *Lagarosiphon*; nutrient enrichment; invasiveness; alien species for sale; prevention



Citation: Montagnani, C.; Caronni, S.; Quaglini, L.A.; Sebesta, N.; Gentili, R.; Citterio, S. Coping with Stress as a Prelude of Naturalization: Different Responses of *Lagarosiphon* Species to Water Trophy. *Diversity* **2023**, *15*, 693. <https://doi.org/10.3390/d15050693>

Academic Editor: Juan B. Gallego-Fernández

Received: 20 April 2023

Revised: 10 May 2023

Accepted: 17 May 2023

Published: 21 May 2023



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1. Introduction

Horticulture is considered a major pathway of introduction of aquatic plants [1], and the strategic importance of regulating the sector to prevent the introduction and spread of alien organisms is globally recognized [2].

Among traded aquatic plants, we found species of the genus *Lagarosiphon* Harv. (Hydrocharitaceae), native to South and Central Africa, namely, *L. major* (Ridl.) Moss and *L. cordofanus* Casp. Traditionally, the former, as easy-to-grow plant, is the main representative of the genus for sale, while among congeners, the latter is one of the few “alternatives” to *L. major* on the market, even if it is not so frequently traded.

Lagarosiphon major has been introduced via horticultural trade to multiple countries outside its native range, usually sold as an ornamental aquarium plant [3]. It has been accidentally released in the wild in different areas of the globe, and it often becomes invasive in lakes, dams and rivers. There, it can form thick mats that reduce light penetration and often oxygen availability [4]. Moreover, it can successfully outcompete native aquatic macrophyte species [4,5] and alter habitat conditions for indigenous macroinvertebrates and fish communities [5,6]. In heavy infestations, the presence of *L. major* can even negatively impact human activities, posing a serious threat to the functioning of irrigation and hydroelectric systems [7–9]. The costs of management of this aquatic weed are elevated [10] and, once established, the chances of eradication are very low (e.g., [11,12]).

On the other side, *L. cordofanus* is a quite understudied aquatic species, native to Central and Southern Africa, whose native range encompasses a much wider area than that of *L. major*, but it is not currently naturalized in other parts of the world [13]. Data about its ecology and potential impacts are still lacking.

One of the main and most-implemented measures to avoid or, at least, reduce problems relating to alien species introduction is trade embargos, complementary to banning the cultivation and release of invasive organisms in nature [14,15]. Indeed, due to its high invasive potential, embargos for *L. major* are currently active in the Member States of the European Union (Regulation EU n.1143/2014), in the United Kingdom (Invasive Alien Species—Enforcement and Permitting—Order 2019, which retains in UK law the Regulation EU n.1143/2014) and in New Zealand (National Pest Plant Accord), where it is a major water weed. In other countries, other biosecurity measures to prevent its introduction are implemented in several regions (e.g., the National Priority List of Exotic Environmental Pests, Weeds and Diseases in Australia, and the APHIS Federal noxious weed program in US).

While bans on trade may be effective for reducing invasion risks associated with *L. major*, they may also shift the attention of buyers to closely related species, such as *L. cordofanus*, as alternatives. In a horizon-scanning perspective [16], it would be prudent to forecast the likelihood of the establishment and possible invasiveness of *L. cordofanus* in case of its release in the wild. Unfortunately, this is not currently feasible based on the available data [17].

Factors promoting the invasiveness of an alien plant can be multiple, and they are also closely tied to the invasibility of an ecosystem [18]. Changes in ecosystem chemistry and resource availability (e.g., eutrophication and pollution), in particular, are expected to be the major drivers of potential future impacts of alien species [19]. Indeed, species composition can dramatically change in response to increasing levels of nutrient availability, and in many cases, opportunistic species, including many alien species, can take advantage of the situation [20]. Nitrogen and phosphorus are key elements in this process, especially in case of over-enrichment, resulting from increased agricultural intensification and urbanization [21,22]. Under such conditions, nutrient imbalance usually enhances high-biomass-forming microalgae and triggers the shift from macrophytes to phytoplankton communities [21]. Negative effects to submerged macrophytes are mainly due to alterations of light availability for photosynthesis, direct competition for resources and overgrowing phenomena (uncontrolled periphyton growth and mucilage directly on macrophytes) [23]. The latter effect can be highly detrimental for sensitive freshwater plants and their dispersal units due to mechanical damages and photosynthetic activity reductions [24]. These conditions can play a key role in alien species proliferation, as they provide an advantage to ruderal, pioneer plants compared to native species, which are less prone to rapid adaptation and resistance [25].

Therefore, the response of alien species to different trophic conditions could be an important element to consider in assessing the risk of naturalization of traded plants potentially escaping from confined environments, as in the case of *L. major* and other aquatic plants. Thanks to its broad ecological tolerance, *L. major* usually thrives in water bodies characterized by different levels of trophic [26]. No information is available in the scientific literature about the response to different trophic conditions of *L. cordofanus*.

To understand how the trophic conditions of water bodies can influence the growth of *L. cordofanus* and therefore the risk of naturalization, a manipulative experiment under controlled conditions was performed. The congener *L. major* was selected to be the alternative because of its well-known resistance to a wide spectrum of nutrient concentrations and its invasiveness in freshwater ecosystems [27].

Along a gradient of trophic, under controlled conditions, viable fragments of *L. major* and *L. cordofanus* were transplanted in water-filled tanks enriched with nitrogen (N) and phosphorus (P), simulating four different trophic conditions found in temperate water basins already invaded by *L. major* in Italy (Italian prealpine lakes). The experiment was

conducted by simulating the typical climatic conditions of summer months in temperate areas, where peaks of trophy can easily occur in shallow, slow freshwaters. The response variables considered to evaluate the tolerance of species along the water eutrophication gradient related to plant survival and growth, root emission and plant architecture.

2. Materials and Methods

2.1. Target Species

Lagarosiphon major or curly leaved waterweed is an invasive aquatic perennial plant whose native range lies in southern Africa, in an area between southern Zimbabwe, Botswana and South Africa [28]. *L. major* thrives in clear, slow, sunny water with temperatures between 10–25 °C, with an optimal growth between 18–23 °C. Photosynthetic activity can increase at higher temperatures (20–25 °C), but it is reduced above 30 °C [29]. Cold temperatures do not represent a substantial limit to *L. major* persistence in nature, as winter dormancy allows the species to overcome cold winters [7]. It preferentially grows on sandy soils in shallow waters (generally less than 6 m of depth, even with exception in relation to turbidity), as light and increased pressure constrain its growth at greater depths [5,8]. *L. major* has adaptive capacities that promote its survival in stressful environments, such as those characterized by high-nutrient levels or low inorganic carbon concentration [7,27]. Carbon, nitrogen and phosphorus, together with a significant fine sediment portion in soils, seem to be the main factors influencing *L. major* growth and competitiveness [8,27,30]. The plant is dioecious, but in its invasive range, sexual reproduction has never been recorded due to lack of male individuals. Successful propagation is vegetative, via the release of viable fragments of stem able to generate new clones [8]. These propagative units are very resistant to desiccation, and even tiny fragments root rapidly to originate new clones, especially in shallow water and unvegetated substrata [31]. They are the main unit of dispersal within and between water bodies, via overland transport by human-mediated vectors [32,33];

As *L. major* and other *Lagarosiphon* species, *L. cordofanus* is a perennial dioecious aquatic plant, but its native range is wider than that of *L. major*, and covers tropical and southern Africa (Angola, Botswana, Cameroon, Democratic Republic of the Congo, Ethiopia, Kenya, Malawi, Mozambique, Namibia, Rwanda, Somalia, South Africa, South Sudan, Sudan, United Republic of Tanzania, Uganda, Zambia, and Zimbabwe) [13,34]. In contrast to *L. major*, *L. cordofanus* is not naturalized elsewhere in the world. The information about its ecology and biology is very scarce; however, based on available descriptions, similarities with *L. major* are evident in relation to colonized habitats, at least from an ecological point of view: it occurs in lakes, dams and ponds, in permanent or temporary water on floodplains, in still or slow flowing freshwaters up to a depth of 2 m, and it prefers sheltered areas protected from wind, waves and currents with high light intensity [13,35]. It can vegetatively spread [34].

2.2. Experimental Setup

Shoots of *Lagarosiphon major* were harvested in the wild in 2017, in two different sites nearby Pavia and Varese, along the Ticino river (Lombardy region, Italy) in shallow waters (at a depth not exceeding 50 cm). Shoots of *L. cordofanus* were bought from online vendors (eBay, Aquasabi).

Shoots of both species were briefly maintained in water-filled tanks to be acclimated to the new conditions. After acclimation, shoots were cut, obtaining 108 viable fragments, each 3 cm long (9 fragments for each replicate) and with no roots. Fragments were placed in experimental tanks, maintaining *L. major* and *L. cordofanus* separated.

For the experiment, 12 tanks (size: 39 cm × 28 cm × 28 cm) for each species (3 replicates for each treatment), were filled with commercial river sand (Vaga Ticino) and tap water (15 L each tank). Sand (3 kg for each tank), extracted along the same water basin where *L. major* was harvested (Ticino river), was characterized by sand granulometry between

0.1 mm and 0.9 mm; it was a wet, siliceous sand (SiO₂ 83.3%) with other components in minor percentages (Al₂O₃ 6.6%, K₂O and Fe₂O₃ 2.1%, Na₂O 2%, MgO 1.5%, CaO 1.2%).

Water was periodically added to the tanks to maintain a constant water level. Plants were subjected to a 12 h day–night cycle in a growth chamber, with the air temperature (monitored with MadgeTech TransiTempII Temperature Data Logger) maintained at an average of 25 °C with minimal fluctuations between day and night.

In order to give an adequate supply of nutrients to the plants, for each tank, 1.5 mL of a breeding medium containing all key elements to plants was added (H₃BO₃ 2.6 g, MnCl₂·4H₂O 1.81 g, ZnSO₄·7H₂O 0.222 g, Na₂MoO₄·2H₂O 0.390 g, CuSO₄·5H₂O 0.079 g, Co(NO₃)₂·6H₂O 0.0494 g).

The experiment lasted 56 days.

2.3. Environmental Parameters

The pH, water conductivity and water temperature were monitored, once a week, with a Combo pH/Conductivity/TDS Tester. Initial (T₀) and final concentrations (T_f) of total N and P in water were estimated by means of spectrophotometric analyses.

2.4. Experimental Treatments

Controlled amounts of NH₄Cl and KH₂PO₄ salts were added to water in order to simulate four different conditions of trophic status, based on different concentrations of total N and total P. Reference concentrations for each level of trophic status were based on the classification of trophic status by the Organisation for Economic Co-operation and Development (OECD) [36] with a calibration based on the trophic status of different prealpine lakes (Lombardy region, Northern Italy) [37,38]. Reference values were established from water bodies where *L. major* is established and often invasive (e.g., Maggiore, Iseo, Monate, Garda lake) [39–41].

Accordingly, concentrations for the four treatments obtained are reported in Table 1. The pH values, monitored for the entire duration of the experiment, were initially similar to those characterizing shallow waters of the reference lakes (<https://www.arpalombardia.it/Pages/Dati/2021/Dati-analitici-corpi-idrici-lacustri-2021.aspx?tipodati=0&tema=Tema%20ambientale&sottotema=Sottotema%20ambientale&ordine=1>, accessed on 9 March 2023) and in accordance with previous experiments involving *L. major* (e.g., [9]).

Table 1. Total N, Total P and trophic classification in treatments.

Treatment	Total N (µg/L)	Total P (µg/L)	Level of Trophic Status for Total N and Total P
A	870	21	Oligo-/mesotrophy
B	1610	21	Hyper-/mesotrophy
C	890	62	Oligo-/eutrophy
D	1680	64	Hyper-/eutrophy

2.5. Functional Traits

For each species under all treatments, the following traits were measured:

- Plant survival percentage;
- Main shoot length (cm);
- Final dry weight (mg);
- Number of lateral shoots (n° of shoots per plant);
- Lateral shoot length (cm);
- Branching degree (m⁻¹);
- Number of roots (n° of roots per plant);
- Root length (cm).

All traits were measured at the beginning (day 1) and at the end (day 56) of the experiment.

2.6. Periphyton and Microalgal Mucilage Cover

The mean periphyton and microalgal mucilage cover in each tank was estimated by image analysis. Specifically, the surface of each plant was photographed by a digital camera, and the mucilage and algal cover (% of the total algal surface) was estimated using the software Image J, a free public-domain software application developed by the US National Institutes of Health (NIH).

2.7. Statistical Analysis

Data regarding the environmental parameters and the functional traits were analyzed by means of univariate statistical analyses (Dependent Sample *t*-test, ANOVA and SNK tests) with the software applications GMAV5 and Past 4.11. All details of the performed analyses are available in the Supplementary Materials.

3. Results

3.1. Environmental Parameters

Total nitrogen and phosphorous concentrations varied significantly between the beginning and the end of the experiment in relation to the applied treatment (Figure 1).

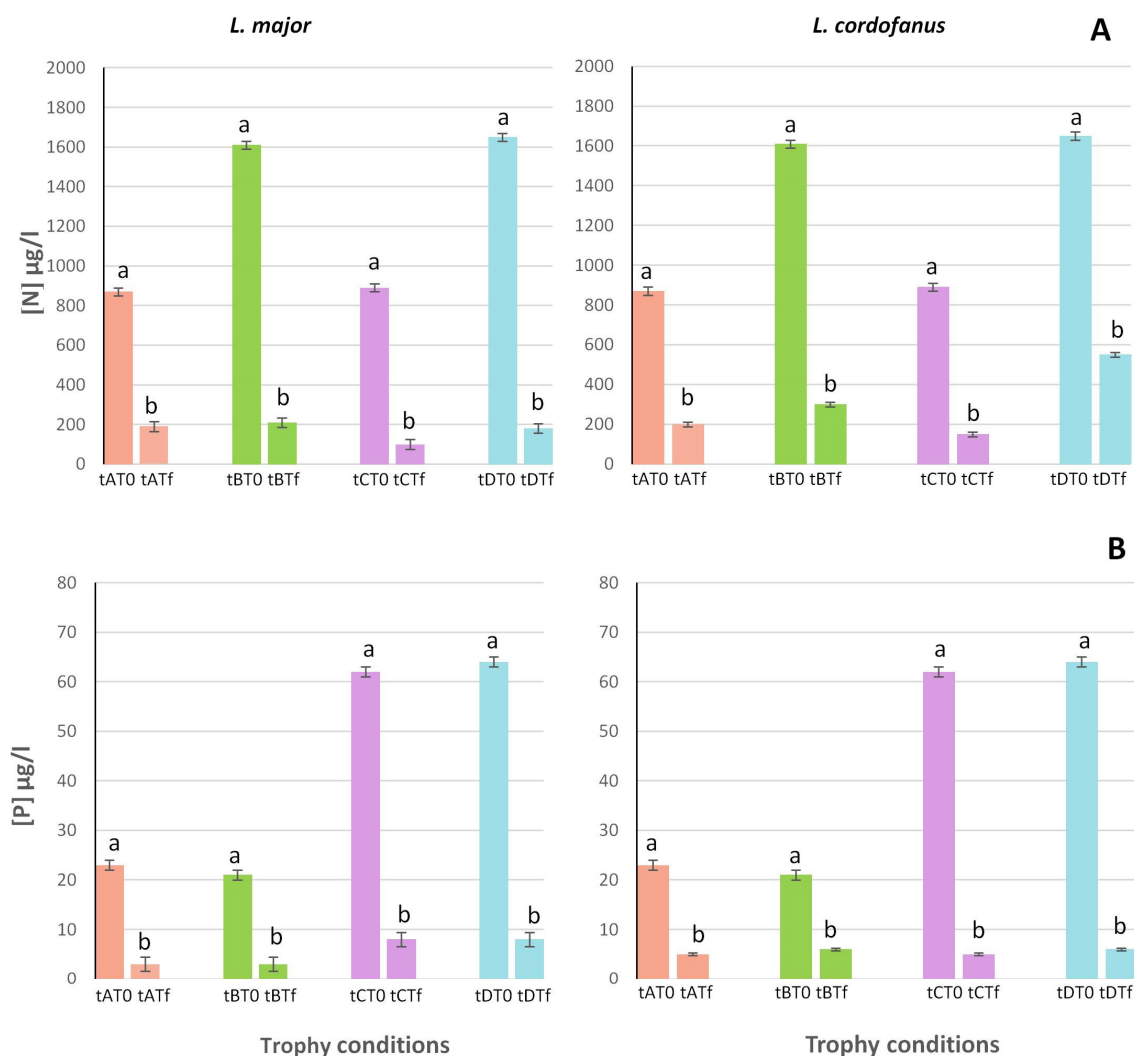


Figure 1. Mean (+SE) concentrations of total N (A) and P (B) at the beginning (T0) and at the end (Tf) of the experiment for the two species in relation to the four treatments (tA, tB, tC and tD). Lowercase letters were used to indicate statistically significant differences between the beginning (T0) and the end (Tf) of the experiment for each treatment.

Both in tanks with *L. major* and those with *L. cordofanus*, N and P depletion was proportional to their initial concentration, meaning that significantly higher values of depletion were recorded in treatments with high initial concentrations of N and/or P (B and D for N, ~1600 µg/L; C and D for P, ~60 µg/L) than in treatments with low initial concentrations (A and C for N, ~870 µg/L; A and B for P, ~20 µg/L).

In particular, for total N (Figure 1A), a double depletion occurred in tanks for which the initial concentration was two times higher (for *L. major* and *L. cordofanus*, respectively: B = 1384.6 ± 0.77 µg/L and 1304.1 ± 0.65 µg/L; D = 1495.3 ± 0.67 µg/L and 1208.8 ± 1.19 µg/L) if compared to A (Lm: 678.6 ± 0.56 µg/L; Lc: 669.9 ± 0.78 µg/L) and C (Lm: 783.2 ± 0.79 µg/L; Lc: 738.7 ± 0.91 µg/L) treatments.

For P (Figure 1B) the same depletion trend was observed, with final values even three times lower for those treatments having the highest (3×) initial P concentration (C = 53.94 ± 0.92 µg/L and 56.42 ± 0.77 µg/L; D = 55.68 ± 0.89 µg/L and 57.6 ± 0.95 µg/L for *L. major* and *L. cordofanus*, respectively) if compared to the low concentration ones (A: Lm = 19.78 ± 0.89 µg/L; Lc = 17.94 ± 0.94 µg/L; B: Lm = 17.85 ± 0.81 µg/L; Lc = 15.91 ± 1.01 µg/L).

Regarding pH (Figure 2A) and conductivity (Figure 2B), the only significant differences observed were between the beginning and the end of the experiment, while no differences either between treatments or between species were recorded. For both *L. major* and *L. cordofanus*, indeed, a constant increase was observed during the experiment in all the tanks, independently of the applied treatment.

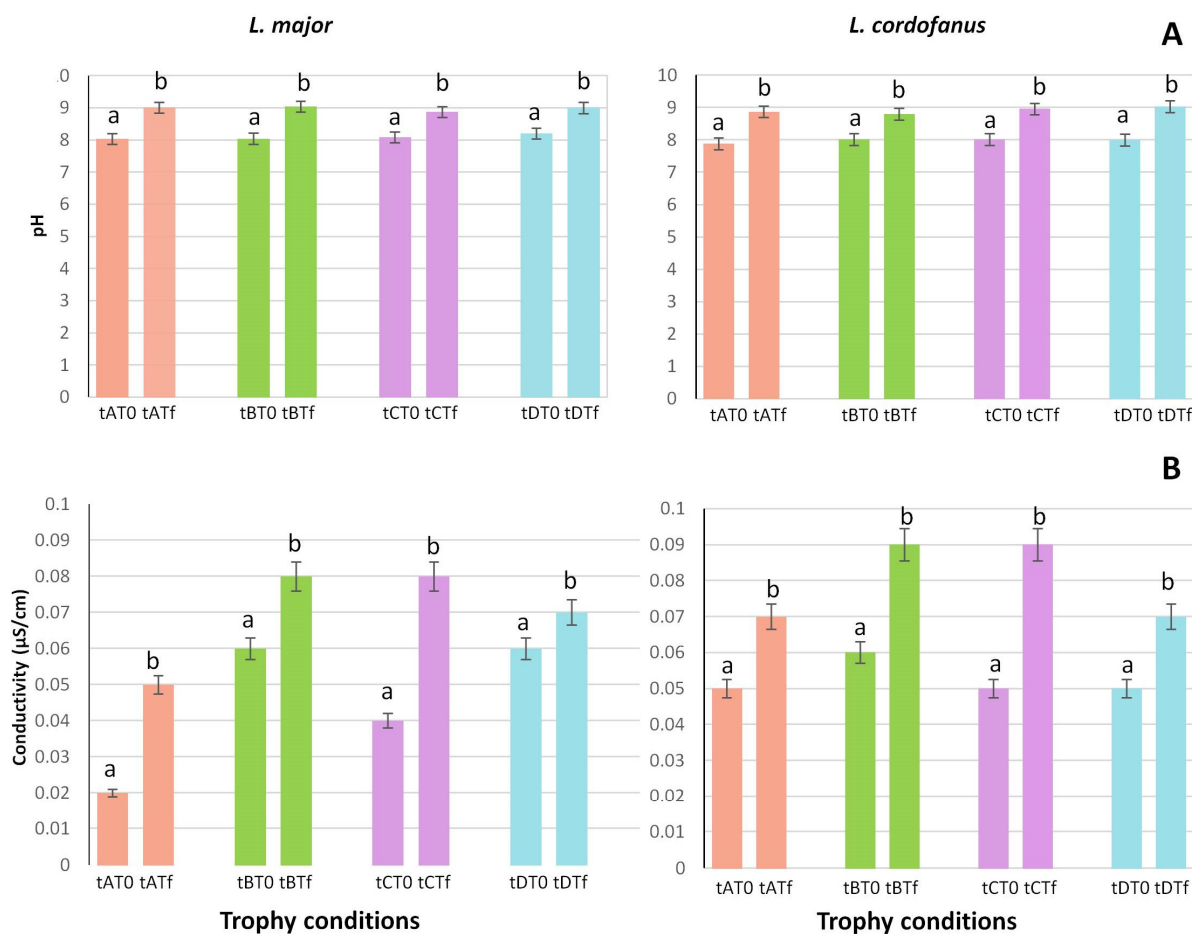


Figure 2. Mean (+SE) of pH (A) and conductivity (B) values at the beginning (T0) and at the end (Tf) of the experiment for the two species in relation to the four treatments (tA, tB, tC and tD). Lowercase letters are used to indicate statistically significant differences between the beginning (T0) and the end (Tf) of the experiment for each treatment.

3.2. Functional Traits

During the experiment, the health conditions of *L. cordofanus* plants overall worsened, with significant differences among treatments. On the contrary, *L. major* plants were healthy through the end of the experiment. These observations were confirmed by the different percentage of survival obtained for the two species at the end of the experiment (Figure 3A). For *L. major*, indeed, all the plants survived in the 4 treatments, while for *L. cordofanus*, a remarkably lower survival percentage (–35% of alive shoots) was recorded in treatments with high total P concentrations, independently of N.

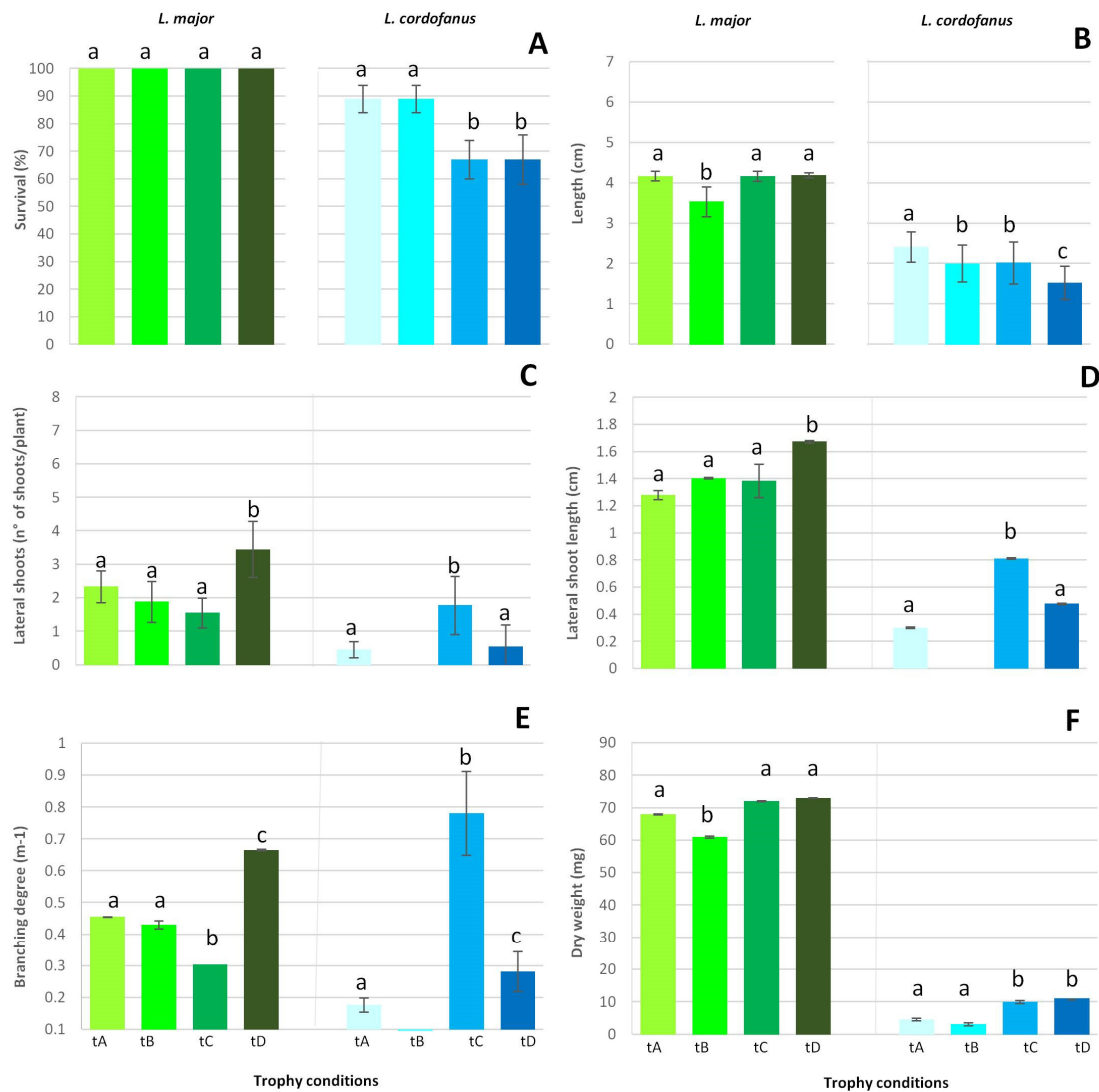


Figure 3. Mean (+SE) survival percentage (A), main shoot length increase (B), lateral shoot number (C) and length increase (D), branching degree (E) and final dry weight increase (F) for the two species in relation to the four treatments (tA, tB, tC and tD). Lowercase letters were used to indicate statistically significant differences among treatments for each species.

Regarding the traits measured in alive individuals, according to the results of the statistical analyses (see Supplementary Materials), higher values were found for *L. major* in comparison to *L. cordofanus* for all the considered traits (Figure 3). The only exceptions observed were in the treatment C (N:P = 890:62 $\mu\text{g/L}$), where the number of lateral shoots did not differ between species (treatment C), but a significantly higher branching degree was recorded for *L. cordofanus* (treatment E).

Moreover, some significant differences among treatments were recorded for each species. Specifically, for *L. major*, the number of lateral shoots, as well as their length and

the associated branching degree significantly increased at high concentrations of both N and P (treatment D, N:P = 1680:64 $\mu\text{g/L}$). A remarkably lower lateral shoot development was observed for the other treatments, especially at low N and high P (treatment C, N:P = 890:62 $\mu\text{g/L}$). For *L. cordofanus*, in contrast, the highest lateral shoot development was overall observed at low N and high P (treatment C). Moreover, for *L. cordofanus*, differently to *L. major*, almost no lateral shoots were present at high N and low P concentrations (treatment B, N:P = 1610:21 $\mu\text{g/L}$).

With regard to the main shoot length, the less remarkable increase occurred in tanks with a high N and low P concentration (treatment B, N:P = 1610:21 $\mu\text{g/L}$) for *L. major*. In all treatments, *L. major* doubled the length of the main shoot, but at high N and low P concentrations (treatment B, N:P = 1610:21 $\mu\text{g/L}$), the percentage increase was +116%, while for other treatments, it ranged from 139% (treatment C) to 141% (treatment D). Additionally, under not eutrophic conditions (treatment A), the percentage increase was greater than in treatment B (+140%). On the other hand, for *L. cordofanus*, the main shoot length increased less than for *L. major* (maximum +80% in treatment A), growing the least (+50%) when both N and P concentrations were high (treatment D, N:P = 1680:64 $\mu\text{g/L}$). For both species, no differences were observed for the other treatments. Growth determined by final dry weight increase, for *L. major*, differed significantly among treatments with high N and low P (treatment B, N:P = 1610:21 $\mu\text{g/L}$) where a lower increase was overall observed if compared to that of the other treatments, for which no differences were significant. Coherently with the percentage increase in the main shoot length, the dry weight increased slightly more than 100% (+103%) in treatment B, while in other treatments, it ranged from +130% (treatment A) to +140% (treatment D). For *L. cordofanus*, a significantly greater increase in dry weight occurred in tanks with high P concentrations, independently of N (treatments C and D, N:P = 890:62 $\mu\text{g/L}$ and N:P = 1680:64 $\mu\text{g/L}$, respectively). Considering the weight increment in percentage, in treatments C and D, the increase ranged from +36% to +41%, respectively, much more than +9% in treatment B and +14% in treatment A. In spite of moderate growth for every trait, dry weight showed a relatively large increase in treatment D; this was probably due to both the intrinsic variability of plant fragments and abundance of leaves, a trait not analyzed by the present analysis.

Finally, the development of roots (Figure 4) was not observed in *L. cordofanus*; however, in *L. major*, a maximum of four roots *per* plant were observed, and this root emission was accompanied by some significant differences among treatments. In particular, despite a significantly higher number of roots observed on plants in tanks with low and high concentrations for both N and P (A and D treatments, N:P = 870:23 $\mu\text{g/L}$ and N:P = 1680:64 $\mu\text{g/L}$, respectively), they were longer where a high concentration of N occurred, independently of P (treatments B and D, N:P = 1610:21 $\mu\text{g/L}$ and N:P = 1680:64 $\mu\text{g/L}$, respectively).

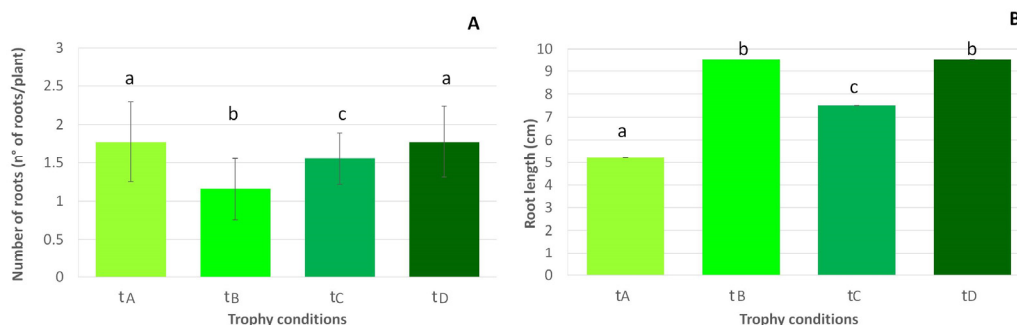


Figure 4. Mean (+SE) *L. major* main root number (A) and length increase (B), in relation to the four treatments (tA, tB, tC and tD). Lowercase letters were used to indicate statistically significant differences among treatments. Data about *L. cordofanus* roots are not shown since the values are zero in all of the treatments.

3.3. Periphyton and Microalgal Mucilage Cover

Periphyton and microalgal mucilage appeared in every tank, but with different cover. From the visual analysis, plants in treatment B, C and D were the most affected (Table 2).

Table 2. Periphyton and microalgal mucilage cover (mean%) for each species in each treatment.

Treatment	Periphyton and Microalgal Mucilage Cover	
	<i>L. cordofanus</i>	<i>L. major</i>
A	30%	22%
B	48%	45%
C	60%	50%
D	65%	60%

4. Discussion

Our work gives a preliminary overview of the potential of *L. cordofanus* to thrive under different levels of nutrient enrichment as a proxy of the likelihood that the species may become naturalized in water bodies characterized by different trophic conditions. As counter-altar species, its congener *L. major*, a highly tolerant species invasive in different parts of the globe, was subjected to the same treatments. Treatments simulated a gradient of trophic conditions with regard to enrichment of N and P up to levels found in different temperate natural water bodies of the Italian prealpine belt where *L. major* already occurs and it is often invasive.

In terms of both survival and growth (Figures 3 and 4), under all treatments, the performance of *L. cordofanus* was inferior in comparison to *L. major*. In fact, the mortality of plants was indicative of this, ranging from 10 to 35% of total shoots depending on the treatment. In contrast, *L. major* shoots experienced no mortality. (Figure 3A). Moreover, *L. cordofanus* did not invest in adventitious roots at all, while *L. major* was quite productive in this regard (Figure 4), as well as in shoot growth. During the experiment, *L. cordofanus* invested in the development and elongation of shoots (both main and lateral) differing from *L. major* and among treatments (Figure 3B–E). *L. cordofanus* showed the best vegetative performance, especially under high concentrations of total P (treatment C). When high P and N were combined (treatment D), *L. cordofanus* mainly invested in new shoots, while in low N and P conditions (treatment A), energies were channeled to the elongation of the main shoot. *L. cordofanus* seemed to suffer the imbalance of N and P in favor of N (treatment B) as it had a weak vegetative response. Basically, the increase in dry weight (Figure 3F) followed the investment in new lateral shoots, although the relevant increment at high N and P concentrations (treatment D) was likely to relate to a major leaf emission, a trait not specifically not recorded in the present analysis. In contrast, *L. major* responded vegetatively more strongly when both P and N were at high concentration (treatment D), while a weaker production of lateral shoots and roots occurred when P was at a high concentration (treatment C) and when N was at a high concentration (treatment B), respectively. However, differences among treatments were slight for *L. major*, confirming its broad tolerance to different nutrient concentration.

4.1. Species Response to Nutrient Enrichment

The response of *L. cordofanus* to treatments in our experiment was in agreement with studies that highlighted that elevated dissolved phosphorus levels promote the biomass formation in submerged macrophytes and that additional phosphorus supplies can be invested in stem elongation or in the formation of lateral branches [42–44]. Thiébaud [45] indicated that primarily phosphorus and, when phosphorus is in excess, nitrogen, plays an important role in controlling the abundance and development of aquatic plants. Nutrient-use efficiencies depend both on the plant P metabolism and on its ability to assimilate this nutrient within its vegetative structures [46]. Interestingly, the vegetative response to nitrogen at a high concentration did not have the same magnitude as that at a high P

concentration for *L. cordofanus*. In fact, in this condition, *L. cordofanus* had a good survival percentage and growth, both comparable to other treatments, but it did not invest in lateral shoots at all. Although its primary role together with phosphorus is beyond question [47,48], this may indicate that, when in excess, nitrogen had an inhibiting effect on *L. cordofanus*, an effect also observed under the condition of high nitrogen combined with high phosphorus concentrations. Even if it is often difficult to distinguish the effects of nitrogen from those of phosphorus due to the close nitrogen and phosphorus relationship [49], in the case of *L. cordofanus*, this seems to be quite evident. This situation can be partially explained considering the potential toxic effect of nitrogen to more sensitive species, when it is at critical concentrations (e.g., around 2 mg N L⁻¹), together with the effect of the increase in both phytoplankton and algae due to nitrogen loading [50–52]. Macrophytes are partially resilient to abrupt increases in nitrogen loading at moderate phosphorus concentrations; however, especially after prolonged exposure, a complete collapse often occurs, not only in relation to competition for resources, but also for the increase in turbidity and the inhibition of photosynthesis [53].

L. major seemed not to suffer excess of either nitrogen or phosphorus; nevertheless, concentrations in favor of one or the other did not promote its vegetative response in the same way as when both nutrients were simultaneously at a high concentration.

4.2. Strategic Investment into Shoots and Roots

Particularly for canopy-forming species, the investment in shoot biomass, often rather than roots, is considered key for their competitiveness. This has been shown to be valid for *L. major*: especially during the first phase of colonization of new sites, this strategy allows the plant to rapidly increase the leaf surface, forming dense beds and quickly establishing itself shading out the other species [26]. Increasing the leaf surface area has direct benefits for photosynthesis and nutrient uptake. In fact, increasing the shoot biomass facilitates enhanced nutrient uptake from the water column: in rooted submerged aquatic plants, leaves as roots contribute to nutrient absorption, and biomass is strategically invested into shoots or roots, depending on whether nutrients are more abundant in the water column or in the sediment, respectively [48,54]. Mainly investing in shoot biomass, *L. cordofanus* seemed to follow the same strategy as *L. major*. Even so, differences from *L. major* were evident, and they mainly related to a complete absence of roots and a definitely lower growth of shoot for *L. cordofanus*, together with a lower survival percentage.

Regarding the first point, *L. major* emitted both new shoots and adventitious roots, while for *L. cordofanus*, no roots developed at all. A low production of roots has already been recorded for *L. cordofanus*, even if conducted under different experimental conditions [55]. In fact, testing the ability of coping with the scarce availability of CO₂ and using HCO₃⁻ as a source of carbon, Hussner et al. [55] already found that *L. cordofanus* had a lower investment into roots with respect to *L. major* (and other macrophytes). In submerged aquatic plants, beyond their role in nutrient storage and anchorage, adventitious roots can facilitate the absorption of nutrients directly from the water column [8,56,57]. However, rooted submerged macrophytes can thrive even without roots, if the nutrient concentration in the water is sufficient to satisfy their requirements [58]. This could indicate that the leaf uptake is the preferential pathway for nutrient absorption of *L. cordofanus*. Nonetheless, the exclusive emission of new shoots likely did not ensure *L. cordofanus* an alternative pathway of absorption in case of inhibition of leaf uptake when the plants were covered by mucilage and algae, as happened with *L. major* [59].

The investment in shoots rather than in roots can be also seen in an ecological perspective as a different strategy of establishment of species. In this regard, it is important to underline that the experiment analyzed the response to nutrients of fragments of *L. cordofanus* and *L. major* (not fully acclimated rooted plants) simulating conditions typical of the initial phases of colonization, when viable vegetative units of dispersal “drifted to a new environment”. Initially, aquatic plants can invest differently in shoots and/or adventitious roots depending on species-specific strategies of regeneration and colonization [31]: a pref-

erence for early formation of new shoots emphasizes a higher potential for the production of new propagules, which in turn may be further dispersed, while fast root formation indicates an increased likelihood of rapid colonization [60]. According to this, along the experimental gradient of trophy, the vegetative response of *L. cordofanus* would seem to be more prone to develop and “potentially” disperse vegetative units moving away to new conditions, rather than to colonize the “new” environment. On the contrary, *L. major* excelled in both the strategies (regeneration and colonization), as already tested by [31].

4.3. Factors Influencing the Growth and Survival

Considering the dramatically lower growth of shoots and the loss of viable fragments for *L. cordofanus* with respect to *L. major*, different factors might have played a role during the experiment—both intrinsic to the species and external—mainly relating to processes of simulated eutrophication. An intrinsic factor may relate to the fact that we compared two diverse species, congeners, but they are likely to be different in morphology and metabolism. For example, even if it is a quite variable species, *L. cordofanus* has a filiform stem and thin, narrow, almost-transparent leaves, while *L. major* is characterized by a thicker stem and broad, firm, mostly opaque leaves [34]; plant and leaf morphology can influence nutrient uptake in some species [61]. Then, the size of fragments used as a starting material and their position (apical, central, etc.) might have influenced the vegetative response by macrophytes. Due to limitations in the availability of plants of *L. cordofanus*, the length of fragments was quite short (3 cm) with respect to more recent experiments in which the species was used (10 cm; [55]), even if it was retained sufficient based on data regarding *L. major*, whose fragments can remain viable and produce new shoots and roots when ≥ 10 mm [32]. However, the size of clonal fragments under which species retain the viability can be a sensitive species-specific element [62], and a full vegetative response of *L. cordofanus* could have been prevented or inhibited if fragments were too small.

An external factor influencing the growth of species was the proliferation of periphyton and mucilage, which affected both species, especially at high P and N concentrations. Due to non-sterile conditions and direct release of nutrients into the water column, during the experiment, periphyton and mucilage appeared in the tanks but particularly on the plants. Plants experienced the effect of eutrophication: the increase in plankton and algae is usually a consequence of nutrient enrichment [49,53], and in eutrophic water, it is a major cause of macrophyte decline and the shift to communities dominated by tolerant, fast-growing species or, in the more extreme cases, to planktonic communities [63,64]. Based on visual evidence and measured traits, *L. major* was less affected by mucilage and periphyton, while *L. cordofanus* suffered from a high cover of algae. To our understanding, at least at the initial stage of the microorganism colonization, a key factor that made the difference between the two species could relate to the rapidity of growth: considering the length increase at the end of the experiment, *L. major* had rapid and more abundant growth in shoots that likely improved plants' photosynthesis and increased the surface area-to-volume ratio, enabling the plants to outpace algal growth and its effects [54]. On the other hand, a lower and more restrained growth such as that of *L. cordofanus* left the plant exposed to the rapid colonization of fast-growing mucilage and periphytic organisms, dramatically reducing photosynthesis and nutrient uptake through shoots [65,66]. Moreover, *L. major* can release allelopathic compounds which likely inhibit the growth of phytoplankton and possibly also epiphytic algae and microorganisms in general [67].

The aggressive proliferation of periphyton and mucilage seemed to have a direct effect on the mortality of *L. cordofanus*, which was higher in P- and P-and-N-rich treatments (C and D) than in low-nutrient (A) and N-rich (B) treatments. Paradoxically, the highest mortality of *L. cordofanus* occurred in those conditions in which the species (at least the surviving individuals) performed the best in terms of growth. In fact, in spite of high mortality rates, the surviving fragments grew and developed new shoots. This can be seen as a strategic response to stress (e.g., from periphyton). Considering the importance of investing in shoot biomass, as previously discussed, a resilient response of traits relating

to this mechanism could be key in the face of novel environmental conditions. Despite this, for an alien species to succeed in a new environment is a complicated process, [68], especially when the mortality rate is high, as is the case for *L. cordofanus*. Even so, a resilient response in shoots development may not be enough if many clones die, and only repeated introductions of the plant would eventually compensate the loss, by increasing the propagule pressure [69].

4.4. Change in Environmental Parameters

At the end of the experiment, the concentrations of nitrogen and phosphorus were significantly lower than at the beginning, while both pH and conductivity had increased.

Both nitrogen and phosphorus loading and/or their combination can promote the proliferation of microorganisms (phytoplankton, algae, etc.). The depletion of nutrients was especially evident when their availability was high; in that case, the final concentration of both nitrogen and phosphorus was two or three times lower than the initial concentration. The depletion of nutrients can be attributed both to plant activity and to microorganisms that proliferated due to eutrophic conditions. In this regard, it can be supposed that the activity of microorganisms was highly relevant, especially in tanks with *L. cordofanus*. Assuming that *L. cordofanus* absorbed nutrients only via leaf uptake, we would expect a lower absorption with respect to *L. major* due to the former's lower growth and lack of adventitious roots. However, the final concentration of nutrients was comparable between species. Therefore, unless *L. cordofanus* has an outstanding ability to assimilate nutrients via leaf uptake, microorganisms may have strongly contributed to nutrient absorption. It is likely that the scarce growth of *L. cordofanus* corresponded to a major availability of nutrients that promoted the increase in microorganisms.

Together with nutrient depletion, pH and conductivity increased during the experiment. Increased conductivity might relate to an increase in plant and microorganism debris (especially for *L. cordofanus*, due to plant death). Regarding pH, the experiment was conducted at quite a high pH (>7), though the values were compatible with values of natural water bodies where *L. major* was found (see Materials and Methods). Increasing pH values can relate to the activity of plants and microorganisms, as photosynthesis is an alkalization process [70]. Specifically, at elevated pH (7–10), bicarbonate (HCO_3^-) is the dominant carbon form instead of CO_2 [54,66,70], and the alkalization of water relates to the release in OH^- [70]. In relation to plant survival, these conditions can be detrimental for CO_2 users; however, both *L. cordofanus* and *L. major* have an affinity to bicarbonate, and their persistence at high pH and low CO_2 suggests that both species are relatively resilient to these conditions [55]. Even so, utilizing bicarbonate is an active uptake process that incurs energy costs and may require increased investments to process the bicarbonate (e.g., carboxylating enzymes) with a consequent increase in the costs of synthesis, maintenance and operation of the cells [54,71,72].

In conclusion, according to our findings, *L. cordofanus* responded well to nutrient enrichment, especially to phosphorus, but it had difficulty with stress, especially due to competition with periphyton and mucilage, both important factors in the eutrophication of waters. The low growth of the plants, as well as the lack of diversification of absorption pathways (shoots and roots), especially in comparison with *L. major* performance, were seen as limits to the potential naturalization of *L. cordofanus* under conditions that resemble those simulated during the experiment and those similar to natural areas already invaded by *L. major*.

5. Conclusions

In case of release in the wild of viable propagules, the naturalization of *L. cordofanus* in temperate shallow waters does not seem so obvious, especially if considered in comparison to *L. major*. In any case, further experiments are needed to define the naturalization potential of *L. cordofanus* under different environmental conditions. For this reason, from a

prevention perspective, the species should be treated carefully, and any release in the wild, whether voluntary or accidental, should be avoided.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/d15050693/s1>. Table S1. Results of dependent sample *t*-tests used to compare N, P, pH and conductivity between T0 and Tf for each species and treatment; Table S2. Results of the ANOVAs and SNKs to test for differences in N and P depletion among treatments (tA vs. tB vs. tC vs. tD) and species (*L. major* (Lm) vs. *L. cordofanus* (Lc)). Significant results are given in bold; Table S3. Results of the ANOVAs and SNKs to test for differences in each of the considered functional traits in relation to the species (*L. major* (Lm) vs. *L. cordofanus* (Lc)) and the applied treatment (tA vs. tB vs. tC vs. tD) at the end of the experiment. Significant results are given in bold; Table S4. Results of the ANOVAs and SNKs to test for differences in the root number and length for *L. major* (Lm) in relation to treatments (tA vs. tB vs. tC vs. tD) at the end of the experiment. Significant results are given in bold [73,74].

Author Contributions: Conceptualization, C.M. and S.C. (Sarah Caronni); methodology, C.M. and S.C. (Sarah Caronni); validation, C.M., S.C. (Sarah Caronni) and S.C. (Sandra Citterio); formal analysis C.M. and S.C. (Sarah Caronni); investigation C.M., S.C. (Sarah Caronni), L.A.Q., R.G. and S.C. (Sandra Citterio); resources, S.C. (Sandra Citterio); writing—original draft preparation, C.M.; writing—review and editing, C.M., S.C. (Sarah Caronni), L.A.Q., N.S., R.G. and S.C. (Sandra Citterio); visualization, S.C. (Sarah Caronni); supervision, S.C. (Sandra Citterio); project administration, S.C. (Sandra Citterio); funding acquisition, S.C. (Sandra Citterio). All authors have read and agreed to the published version of the manuscript.

Funding: Project funded under the National Recovery and Resilience Plan (NRRP), Mission 4 Component 2 Investment 1.4—Call for tender No. 3138 of 16 December 2021, rectified by Decree n.3175 of 18 December 2021 of Italian Ministry of University and Research funded by the European Union—NextGenerationEU. Award Number: Project code CN_00000033, Concession Decree No. 1034 of 17 June 2022 adopted by the Italian Ministry of University and Research, CUP, H43C22000530001 Project title “National Biodiversity Future Center—NBFC”.

Institutional Review Board Statement: Not applicable.

Data Availability Statement: All the research data are shareable by the authors if requested.

Acknowledgments: The authors would like to thank Giulia Salerno for her invaluable support, as well as Fabio Moia, Enrico Casati, Valentina Soler and Barbara Leoni for their technical help during the experiment.

Conflicts of Interest: The authors declare no conflict of interest.

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