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Assessing the Potential of Jellyfish as an Organic Soil Amendment to Enhance Seed Germination and Seedling Establishment in Sand Dune Restoration

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Abstract: Worldwide, sandy coastlines are affected by extensive wind and water erosion. Both soil quality and periodic drought present major problems for sand dune restoration projects. Hence, soil amendments are needed to improve soil quality and enhance soil restoration efficiency. The jellyfish population has increased in some aquatic ecosystems and is often considered as a nuisance because of their negative impacts on marine ecosystem productivity as well as coastal attractiveness. Thus, development of new products derived from jellyfish biomass has received attention from researchers although utilization is still at a preliminary stage. Herein, our main objective was to test seed germination, seedling establishment, and seedling vitality of annual ryegrass (*Lolium multiflorum* L.) when supplied with organic soil amendment from two different jellyfish species (*Aurelia aurita* and *Cyanea capillata*) in comparison with an unfertilized control and mineral fertilizer treatment. We hypothesized that jellyfish dry matter as an organic soil amendment would improve seed germination and seedling establishment in sand dune environments. Germination and seedling growth experiments were conducted in the laboratory and greenhouse. The results indicate that jellyfish enhanced seedling growth and establishment in sand dune soil significantly ($p < 0.05$ and $p < 0.01$) under water scarcity conditions. Therefore, jellyfish may have potential for an auxiliary role in sand dune restoration projects in coastal areas in the future.

Keywords: seed germination; jellyfish; blue fertilizer; soil restoration; soil amendments; water use efficiency

1. Introduction

Human activities have damaged sandy beaches and coastal dunes. With further population growth, it is expected that human impacts on coastal ecosystems will only increase [1]. Furthermore, periodic environmental stress, including drought, is predicted to increase water scarcity problems worldwide. This creates additional and serious functional limitations for plant growth and also has significant adverse influences on crop yields [2,3]. Sand dune plants play an important role in dune stabilization and protect the adjacent coastal beaches from wind and water erosion but they are subjected to severe environmental stresses such as drought [4,5]. Therefore, organic soil amendments are important for ecological maintenance of the plants in sand dune restoration plans, especially in arid and semi-arid land where soils are under fragile conditions because of water scarcity and poor organic matter.

According to Donohue et al. [6], seed germination is a highly important stage in plant life cycles and factors affecting germination significantly impact subsequent seedling establishment, as well as plant environmental adaptation [7]. Seed germination is the initial process through which seeds develop into plants and it commences by the seed absorbing water under adequate temperature, which leads to the creation of a new plant [8]. According to Kildisheva et al. [9], taking seed germination into account in restoration planning is important to certify seed use efficiency and management. Seedling emergence is the most important phenological event that influences the success of plants [10] and it starts with seed germination. Seedling survival is also one of the most critical stages in plant growth and it is often adversely affected by drought and soil dryness [11]. According to Manz et al. [12] and Bewley et al. [13], water uptake by a seed includes three phases: first, the rapid initial uptake of water; second, a plateau phase (metabolic preparation for germination); and third, a further increase of water uptake. Therefore, soil moisture (available and adequate water) plays an important role in seed germination and seedling establishment.

Jellyfish, when supplied as an organic fertilizer, have a potential to promote seed germination, as reported by Emadodin et al. [14]. The term jellyfish as a group of marine invertebrates commonly refers to the medusae form of planktonic marine members of the class Scyphozoa or Cubozoa. Jellyfish populations have been a recurrent topic of debate over the past few decades, as mass aggregation (called blooms) of jellyfish are reported more frequently and have often been linked to human-induced environmental changes [15,16]. For example, blooms of some jellyfish species have been reported in the East Asian marginal seas [17], Red Sea [18], Mediterranean Sea, and Black Sea [19].

Positive effects from using jellyfish as inputs for agricultural production have been recorded by several researchers. Fukushi et al. [20] indicated the potential usefulness of two jellyfish species (*Aurelia aurita* and *Chrysaora melanaster*) as a source of fertilizer for vegetable production. Hossain et al. [21] introduced desalinated-dried jellyfishes (*Nemopilema nomurai* and *Aurelia aurita* from the Sea of Japan) as an alternative material to replace herbicides and chemical fertilizers in rice production. The possible use of jellyfish as pesticides was also investigated by Hussein et al. [22]. However, some jellyfish species may have different effects on plant growth processes due to their chemical components. Therefore, in this study, we evaluated the influences of two different jellyfish species (*Aurelia aurita* and *Cyanea capillata)* from the Baltic Sea Coast of Germany, on the early growth stages of annual ryegrass (*Lolium multiflorum* L.). It was hypothesized that the addition of jellyfish dry matter to the soil will enhance seed germination as well as seedling growth and establishment in sand dunes. This was considered to be particularly important in terms of soil restoration projects in dry lands where there is a need to establish new plant communities on degraded soil with very low productivity, as well as drought conditions. However, it should be emphasized that due to various ecological conditions and different species, several experiments (under greenhouse and field conditions) are required to investigate this hypothesis. Here, we report results from a petri plate germination experiment and a greenhouse pot experiment. The experiments are carried out, which is running under a European Union (EU) project entitled: Development of products from jellyfish biomass (Gojelly) with funding by the EU Horizon 2020 Research and Innovation Program.

2. Material and Methods

Two jellyfish species (*Aurelia aurita* and *Cyanea capillata*) were collected from two sites on the Baltic Sea Coast of Germany (54°25′ N, 10°10′ E and 54°47′ N, 9°84′ E) during summer of 2018 and 2019. The samples were put into plastic bags separately and stored at −20 °C before further processing. In this investigation, two drying methods including oven-dried and alcohol-dried methods have been conducted. Jellyfish were oven-dried at 50 °C until constant weight was reached. Alternatively, the alcohol-dried method of Pedersen et al. [23] was applied in which the fresh or frozen jellyfish were exposed to ethanol (70%) then after around one-hour the jellyfish were removed and put into distilled water. After about 30 min, jellyfish were taken out of the water and dried under room temperature (around 21 ± 1 °C).

The dry matter was homogenized in a ball mill (Retsch MM2000, Haan, Germany). The carbon and nitrogen content of the material was measured via dry combustion (Vario Max CN, Elementar Analysensysteme GmbH, Hanau, Germany).

For chemical analysis, 200 mg of dried and finely ground jellyfish material was digested with 10 mL 15.6 M HNO_3 (ROTIPURAN® Supra) at 190 °C for 45 min in 1800 W microwave oven (MARS 6, Xpress, CEM, Matthews, MC, USA). After digestion, the concentrations of Ca^{2+}, Cu^{2+}, Fe^{2+}, K^+, Mg^{2+}, Na^+, and Zn^{2+} were quantified with an atomic absorption spectrometer (AAS 5EA Thermo Electron S, Carl Zeiss, Jena, Germany). The citric acid extractable P was determined by using citric acid (2% *v*/*v*) and a jellyfish/solution ratio of 1:10. After shaking with an end-over-end shaker (Type RA 20, C. Gerhardt GmbH and Co. KG, Bonn, Germany) for 30 min, the citric acid extracts were filtered (MN 619 G$\frac{1}{4}$, Machery-Nagel GmbH and Co. KG, Düren, Germany). P concentrations in all extracts were determined photometrically with a continuous flow analyzer (Skalar Analytical B.V., Breda, The Netherlands) by using the modified molybdenum–ascorbic acid blue method. Results of the chemical analysis are given in Table 1.

Table 1. Some macro- and microelement compounds of jellyfishes (*Aurelia aurita* and *Cyanea capillata*).

Elements in Dry Matter	*Cyanea capillata* % per Dry Mass		*Aurelia aurita* % per Dry Mass	
	Alcohol Dried	**Oven Dried**	**Alcohol Dried**	**Oven Dried**
N	4.4	2.9	7.8	0.7
C	15.5	10.5	27.3	3.1
P	0.22	0.8	1.0	0.2
Ca	0.75	0.77	0.75	0.84
Mg	2	1.8	1.0	1.6
Na	17.8	19.5	9.2	33.4
K	0.16	1.2	0.44	0.96
Mn	<0.01	<0.01	<0.01	<0.01
Cu	<0.01	<0.01	<0.01	<0.01
Zn	0.01	0.02	0.04	<0.01
C:N	3.5	3.6	3.5	4.4

Seed of annual ryegrass (*Lolium multiflorum* L.) was used in this investigation. The soil used in this experiment was beach sand and this was collected from the Baltic Sea Coast of Germany (54°25′ N, 10°10′ E). Nitrogen and carbon contents of the soil were around 0.006% and 0.17%, respectively, with a C:N ratio around 28. Calcium ammonium nitrate (CAN) was used as a fertilizer treatment for comparison with jellyfish with regard to impacts on seed germination, seedling plant establishment and vitality. CAN is widely used as an inorganic fertilizer on grassland and other crops.

The pH and EC values of the jellyfish liquids that were provided for each treatment in pot experiment were the same as with a petri plate experiment. In order to test seed germination rate as well as seedling growth and establishment, a petri plate experiment and pot experiment were conducted as follows.

2.1. Petri Plate Experiment

The petri plate method was used to test germination rate. This method helps to monitor the processes of germination under a controlled environment. In pre-treatment the dry matter of *A. aurita*, *C. capillata* and calcium ammonium nitrate (CAN) was dissolved in distilled-water (0.5 g DM in 40 mL distilled water). Pure distilled water was also used as a control treatment. Filter paper was put in each petri plate (8.5 cm diameter) wetted by different aqueous solutions. In total, 20 annual ryegrass (*Lolium multiflorum* L.) seeds were placed in each petri plate. All plates were covered by plastic foil to mitigate evaporation and put in darkness at 21 ± 1 °C. The plates were controlled every day and observations (seed germination number and assessment of vitality) were recorded. The petri plate experiment was carried out with eight treatments and with four replications (Table 2). The pH and electronic conductivity (EC) of the different treatments were measured with a PC60 Premium Multi-Parameter tester (Apera instruments, Europa, GmbH; Table 3). Although the petri plate test is considered as a standard work for assessment of seed germination, it may not give an accurate prediction of seedling emergence in the field [24]. Therefore, a pot cultural experiment was also conducted in the greenhouse.

Table 2. Petri plate experiment treatments.

Nr.	Treatments	Abbreviation
1	Distilled water (Control)	Control
2	*Aurelia aurita* (Oven-dried)	Aur (Ov)
3	*Aurelia aurita* (Alcohol-dried)	Aur (Alc)
4	*Cyanea capillata* (Oven-dried)	Cya (Ov)
5	*Cyanea capillata* (Alcohol-dried)	Cya (Alc)
6	*Aurelia aurita* and *Cyanea capillata* (Oven-dried)	Aur + Cya (Ov)
7	*Aurelia aurita* and *Cyanea capillata* (Alcohol-dried)	Aur + Cya (Alc)
8	Calcium ammonium nitrate	CAN

Table 3. pH and electrical conductivity (EC) values of the treatments.

Treatments	pH	EC [µS/cm]
Control	6.1	0
Aurelia (Ov)	7.5	19.00
Aurelia (Alc)	7.42	5.06
Cyanea (Ov)	6.6	13.33
Cyanea (Alc)	6.83	2.89
Aur + Cya (Ov)	6.9	14.8
Aur + Cya (Alc)	7.2	4.23
CAN	7.15	15.21

2.2. Pot Experiment

This experiment was conducted in the greenhouse in summer 2019 at the University of Kiel. Plastic pots (around 11 cm top diameter, 6.5 cm bottom diameter, 10 cm height) were filled with 700 g of sand. In total, 20 uniform seeds of annual ryegrass (*Lolium multiflorum* L.) were placed in each pot and covered by 0.5 cm of sand material and irrigated with six different solutions (oven and alcohol dried materials from *Aurelia aurita* and *Cyanea capillata* and CAN (0.5 g DM in 40 mL distilled water) and 40 mL distilled water for control (Table 4). In order to reduce evaporation, the sand was covered by seagrass. The pot experiment was conducted in six treatments with four replications (Table 4). All treatments were irrigated three times (days 10, 15, and 17) during the experimental period, with 40 mL of tap water.

Table 4. Pot experiment treatments.

Nr.	Treatments	Abbreviation
1	Distilled water (Control)	Control
2	*Aurelia aurita* (Oven-dried)	Aur (Ov)
3	*Aurelia aurita* (Alcohol-dried)	Aur (Alc)
4	*Cyanea capillata* (Oven-dried)	Cya (Ov)
5	*Cyanea capillata* (Alcohol-dried)	Cya (Alc)
6	Calcium ammonium nitrate	CAN

2.3. Statistical Analysis

The petri plate test and the pot experiment were both conducted in a completely randomized design. The data were subjected to analysis of variance (ANOVA) and Welch's *t*-test as an adaptation of student's *t*-test [25]. The level of significance was declared at $p < 0.05$, $p < 0.01$ and $p < 0.001$.

3. Results

3.1. Petri Plate Experiment

Petri plate experimentation for testing germination rate and estimating seed viability is a standard practice [24]. In the petri plate experiment, the initial evidence of radicle protrusion (germination) appeared after two days. The germination rate was significantly less ($p < 0.05$; $p < 0.001$) than the control in Aur (Alc) and Aur + Cya (Alc) (Figure 1). Other treatments showed no indication of significant differences ($p > 0.05$) in germination rates relative to the control. Germination under the control (water) treatment started earlier than other treatments. However, after four days, germination rates under Cya (Ov) showed slightly more (2.5%) than control. All shoots under jellyfish treatments showed more vitality than water and chemical fertilizer treatments (according to the visual estimation).

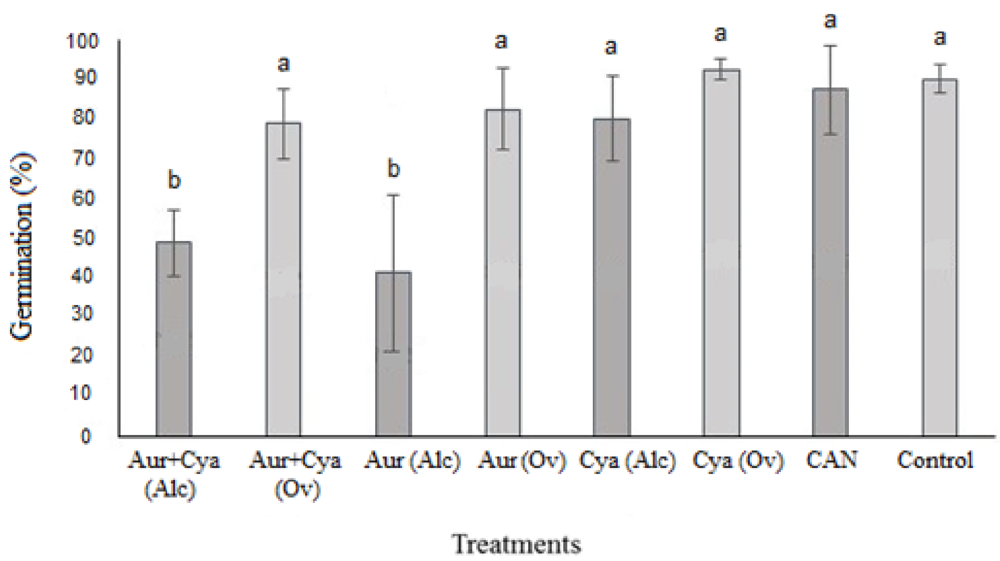

Figure 1. Total germination in percent depending on the different treatment applications in the petri plate experiment. The error bars represent the ± standard deviation. Welch test in two pairs only with control. Same letters indicate no significant differences with control.

3.2. Pot Experiment

In the pot experiment, the effect of jellyfish on seed germination and seedling growth varied with jellyfish species ($p < 0.05$). Seedling emergence occurred earliest in the control treatment, yet mortality increased sharply in the following days (Figure 2). Seedlings in the jellyfish treatment showed greater vitality with better establishment rate. The sprouts and seedlings had a stronger and greener appearance than those of the water (control) and chemical fertilizer treatments.

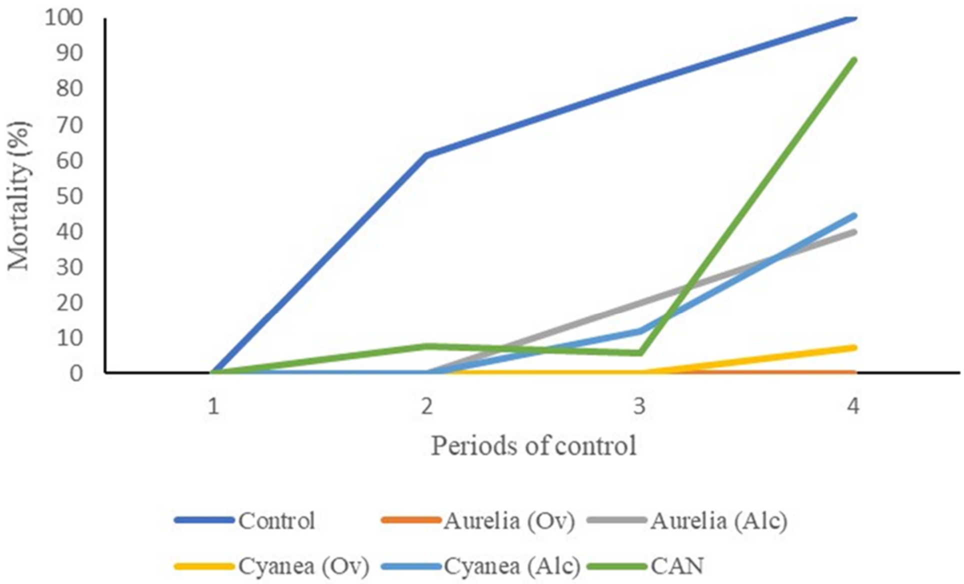

Figure 2. Mortality of seedlings (%) in the pot experiment during the first period of the control (two days).

Results of total germination and final seedling emergence, including surviving and non-surviving seedlings, for the different treatments at the end of the experiment confirmed there was less mortality and higher rates of seedling establishment in the Aur (Alc) and Aur (Ov) treatments (Figures 3 and 4). The results also indicated that the length of grass seedlings changed under different treatments significantly ($p < 0.05$ and $p < 0.01$, Figure 5). The maximum length of the grass seedlings recorded under the Aur (Alc) treatment was around 14 cm. Plant from Aur (Alc), Cya (Alc) and Cya (Ov) treatments were also significantly taller than control ($p < 0.05$; Figure 5).

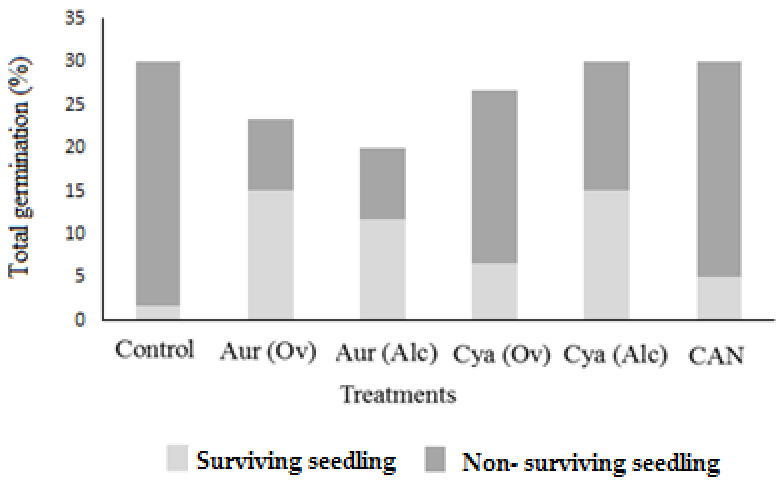

Figure 3. Total germination for final seedling emergence (including surviving and non-surviving seedlings) for each of the different treatments.

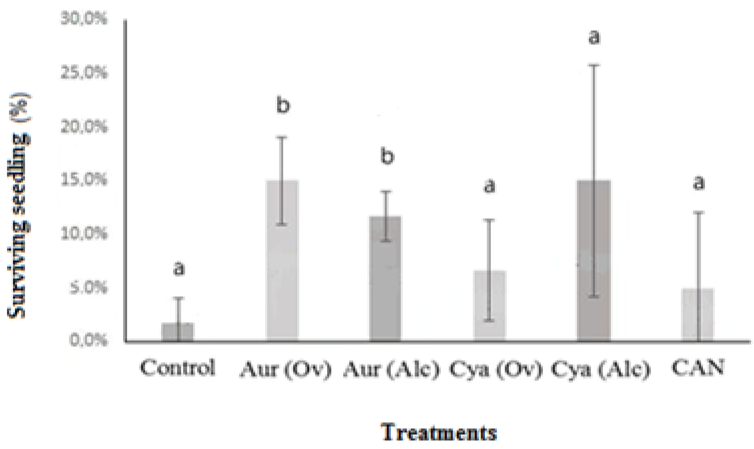

Figure 4. The proportion of surviving seedlings (as percent) among the different treatments in the pot experiment. Error bars represent the ± standard deviation. Welch test in two pairs only with control. Same letters indicate differences are non-significant with control.

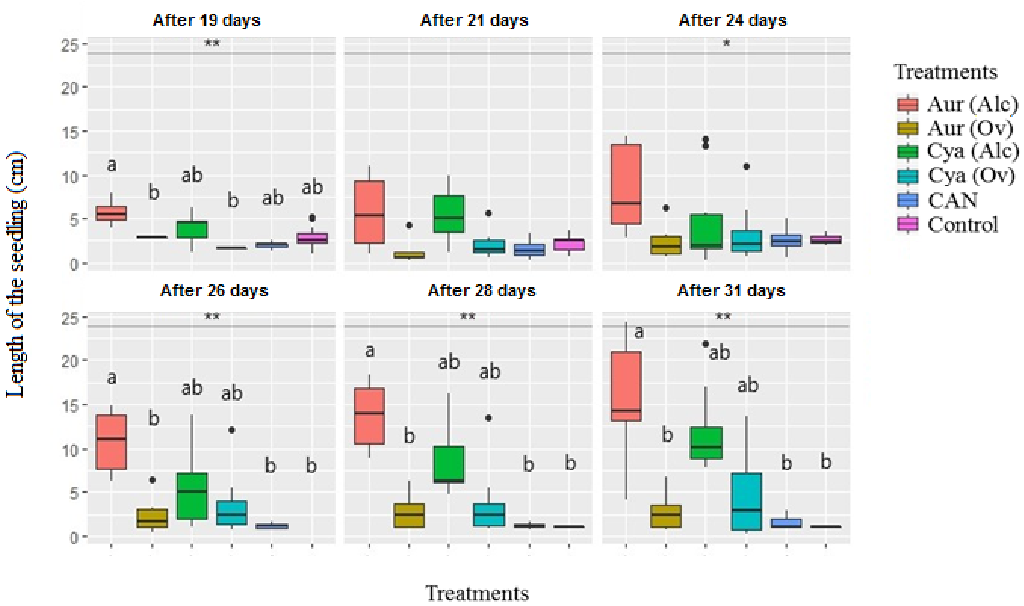

Figure 5. Average length of seedling (cm) under different treatments. Treatments followed by different letters according to Welch test significantly different at $p < 0.05$ and ANOVA test shows significantly different at * $p < 0.05$ and ** $p < 0.01$.

4. Discussion

In this investigation both of the jellyfish species *Aurelia aurita* and *Cyanea capillata* were considered as potential material for promoting seed germination. In the petri plate experiment, there were negative effects of Aur (Alc) and Aur + Cya (Alc) on germination, which may be related to the impacts of alcohol or the jelly form of Aur (Alc) liquid that may delay the time of germination. This effect did not occur in the pot experiment.

According to Bewley [8], the time for germination and post-germinative growth varies from several hours to many weeks, and it depends on the plant species and the germination conditions (Figure 6). Results from our investigation indicated that jellyfish amendments provided conditions that are likely to have caused a delay in germination in comparison with the control. However, evidence showed that in phase 3 (post-germinative growth), seedlings appeared to show greater vitality in treatments with the jellyfish amendments compared with the control. The delay in germination could be related to the salinity of jellyfish, which is indicated by high sodium concentrations in the jellyfish dry matter (Table 1). Delayed germination has been shown previously in many crops as a consequence of high salinity [26].

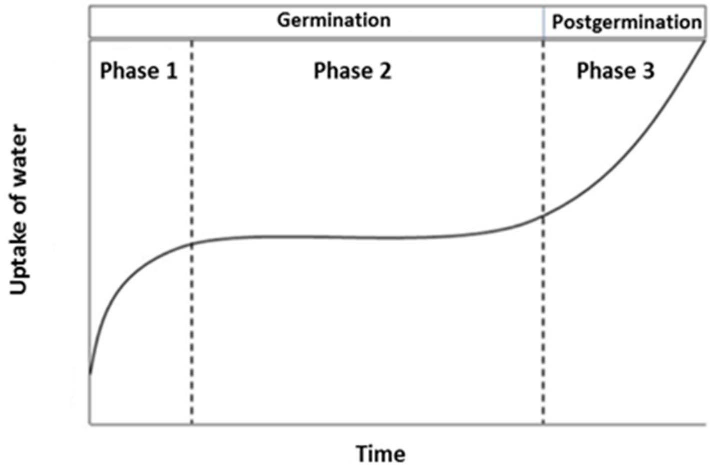

Figure 6. Germination and post-germinative growth divided into three phases regarding water uptake (adapted after Bewley [8]).

The electrical conductivity of alcohol dried jellyfish was shown in Table 3 to be less than other treatments. This could be related to the washing out of the ions by alcohol. The morphology of sprouts and seedlings under the jellyfish treatment shows greater vitality than in the control. This may be related to the presence of additional essential elements provided by jellyfish as well as enhancing soil water holding capacity. There is a good correlation between soil biological activities and soil water content [27]. Thus, there could be a key role for the use of jellyfish as an organic amendment in this context. The germination under control (water) treatment commenced earlier or proceeded faster to seedling emergence than other treatments. Therefore, it is assumed that jellyfish may affect germination by absorbing water and, if so, this may reduce the amount of water available for the seed in the initial stage of germination.

According to Smith and Doran [28], pH values from 5 to 8 represent the optimum range for most soil microorganisms. Hence the pH rates measured for different jellyfish liquids show no harmful effects in this case. The electrical conductivity was lower in treatments dried with alcohol. This may be attributed to different salinity levels and cation exchange capacities. According to Rawls et al. [29], soil organic matter content has impacts on soil structure as well as water adsorption properties. Thus, the application of jellyfish may also enhance soil water retention through enhancing soil organic carbon and collagen content, as well as provide some essential bio-, macro-, and microelements. According to Carter [30], using chemical fertilizer under conditions of low soil moisture content has harmful effects on seedling establishment. Our investigation also showed the same result. According to Killham [27], the most commonly used index to show resource quality is C:N ratio, and low C:N ratios indicate rapid rates of decomposition. The jellyfish used in this study also showed low C:N ratio = 3.5 (Table 1) in comparison with the green manures such as seagrass (C:N = 14) [16,31] that were also used as soil amendment materials.

5. Conclusions

Jellyfish generally did not reduce the germination rate and provided favorable conditions for seedling survival in sand dunes. However, the positive effects might depend on the species of jellyfish, drying process methods, natural environment (e.g., temperature), edaphic conditions, and plant types. In this study, a positive effect of *Aurelia aurita* was observed on seedling establishment of *Lolium multiflorum*, seedling length, and the vitality of seedlings under conditions of water scarcity.

In the context of this investigation, where there is a local surplus of jellyfish, it can be regarded as a local sustainable resource and its use can be considered an innovative organic soil amendment for sand dune restoration projects.

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