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Research article

Growth of floating hook-moss (*Warnstorfia fluitans*) differs with nutrient and water flow adjustments in greenhouse and cold room conditions

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ABSTRACT

Floating hook-moss (*Warnstorfia fluitans*) is a bryophyte growing in northern aquatic and peatland ecosystems. *W. fluitans* uptakes metals and excessive amounts of nitrogen from wastewater, which suggests that it may have commercial potential for use in phytoremediation. Optimization of growth conditions would allow artificial cultivation of floating hook moss in large quantities for phytoremediation applications. We tested how application of combined nutrient (NPK 7-2-2 ranging from 0.1 to 1 ml per liter of water) and water flow (ranging from 0.15 to 1.9 ml/min) treatments affect growth of *W. fluitans* in greenhouse conditions. At the end of the experiment, all treatment combinations were subjected to an additional cold room condition at low temperature (0–2 °C) without constant water flow. The moss generally produced biomass in the various treatment combinations. However, contrary to our expectations, we found that increase of nutrients and water flow had a negative effect on the growth of *W. fluitans*. The highest growth rates in the experiment were detected in the control unit that had no nutrient addition or applied water flow. Our results suggest that cold temperatures are beneficial for *W. fluitans* growth. Our results show that the commercial production of *W. fluitans* may not require nutrient or water flow manipulation, at least in the tested scale. Instead, the growth conditions should mimic the natural cold climate conditions of *W. fluitans* habitats in northern peatlands and/or spring ecosystems.

1. Introduction

Phytoremediation is an effective method that uses green plants to reduce environmental pollutants [1]. Aquatic bryophytes, including mosses, liverworts, and hornworts, can be useful in remediation processes due to their ability to accumulate elements beyond their physiological needs, owing to the lack of cuticles in their tissues and the presence of sites that have exchangeable cations in their cell walls [2,3]. Consequently, most mosses used in phytoremediation hyperaccumulate heavy metals, such as Pb, Cr, Zn and Ar [4]. Several aquatic mosses, including *Taxiphyllum barbieri*, *Funaria hygrometrica*, and *Leptodictyum riparium*, have shown the capacity for metal accumulation, and they have also been used as bioindicators of environmental conditions [4-6]. Similarly, floating hook-moss (*Warnstorfia fluitans*) has been shown to remove up to 82% of Arsenic (As) (74 μg/L in water) within an hour in controlled laboratory

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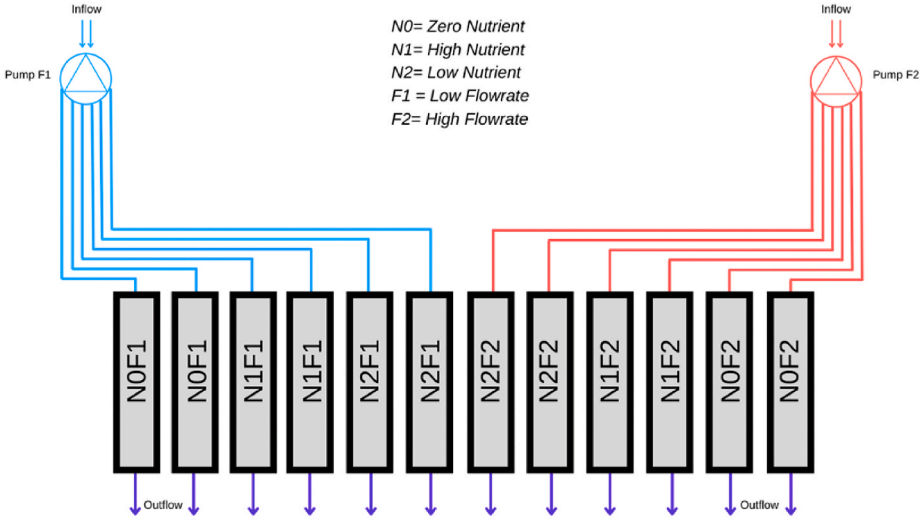

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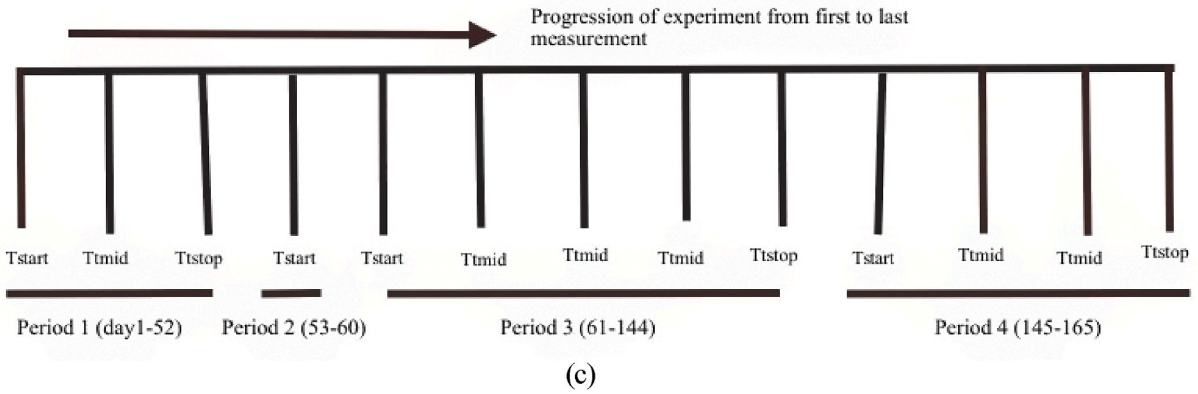

(c)

Fig. 1. Experimental setup showing (a) cross-section of experiment (b) experimental setup in the greenhouse and (c) operating conditions with the timeline of the experiment divided into four periods (each period included a different sampling scheme). Period 4 is the separate cold room treatment without nutrient addition and with stagnant water.

conditions [7]. The moss can also remove nitrogen and accumulate metals in various types of wastewaters [8,9].

W. fluitans is an aquatic moss species endemic to the arctic and subarctic regions (Riis et al., 2010), [7,10]. *Warnstorfia* is one of the bryophyte genera that dominates in the nutrient-poor high arctic lakes and acidic streams that are free of snow and ice cover only during the summer months [11-13]. *W. fluitans* grows in nutrient-poor aquatic habitats and is well adapted to low temperatures, and growth is increased with higher nutrient availability and light density [14]. Nitrogen (N) and phosphorus (P) are generally limiting nutrients for the primary production of aquatic plants in the arctic freshwater ecosystems (Riis et al., 2010). However, the growth rate of *W. fluitans* increases more in P-rich conditions than in N-rich conditions, making phosphorus an important limiting nutrient for the growth of the moss (Riis et al., 2010). *W. fluitans* dominates in high arctic lakes with typically low nutrient levels, which potentially limits the primary production (Riis et al., 2010). This raises the question of whether the growth of *W. fluitans* can be optimized in artificial conditions with the manipulation of suitable nutrients.

Little is known of the optimal culture and growth conditions of *W. fluitans*, which is urgently needed for applying the moss for commercial purposes and to maximize its use in phytoremediation in cold conditions. In general, when considering plant growth, several parts of the plant may be targeted for maximum growth. For example, in optimization of rice growth, high nitrogen (N) application at the early vegetative stage increased the number of panicles, which contributed to an increase in the yield [15]. N and potassium (K) fertilization were also important for high yield in cotton, in terms of boll weight and number [16]. While a combination of various nutrients may complement each other in some plants, they may have different and even contradicting effects in others. In Arabidopsis, N increased carbon allocation in leaves and stem, but decreased allocation in roots and fruits, whereas phosphorus increased allocation in stem and fruits, and decreased root and leaf allocation [17]. Besides fertilization, stirring of water in the artificial growth conditions to simulate flowing water has produced a morphological response and increased growth of many aquatic plants adapted to lotic waters [18-20].

This study was focused on *W. fluitans*, which is found, besides natural aquatic ecosystems, in several metal-rich sites, such as mine areas of Nordic countries [9]. Our primary aim was to examine the effects of combined NPK fertilizer and various water flow rates on the growth of *W. fluitans* in ambient greenhouse conditions. We aimed to find out optimal conditions to maintain maximum growth of the moss biomass in controlled conditions all year round. At the end of the experiment, due to results obtained by that time, we subjected all *W. fluitans* material for cold room conditions because the moss can also be cold-adapted (Riis et al., 2010). To the best of our knowledge, this study is the first to consider and test the growth of *W. fluitans* in artificial growth conditions.

2. Materials and Methods

2.1. Sampling of Warnstorfia fluitans material

Floating hook moss material was collected from Finnish Lapland (N68°0′E25°0′) with the landowner's permission. Moss material was sampled from a natural spring ecosystem located in the middle of peatland. The moss was collected in spring water and transported to the University of Oulu where it was stored in a climate room with a temperature of 5 °C to mimic the conditions of cold water springs.

2.2. Experimental setup

The experiment was carried out at the Botanical Garden of University of Oulu in a greenhouse between March 2021 and August

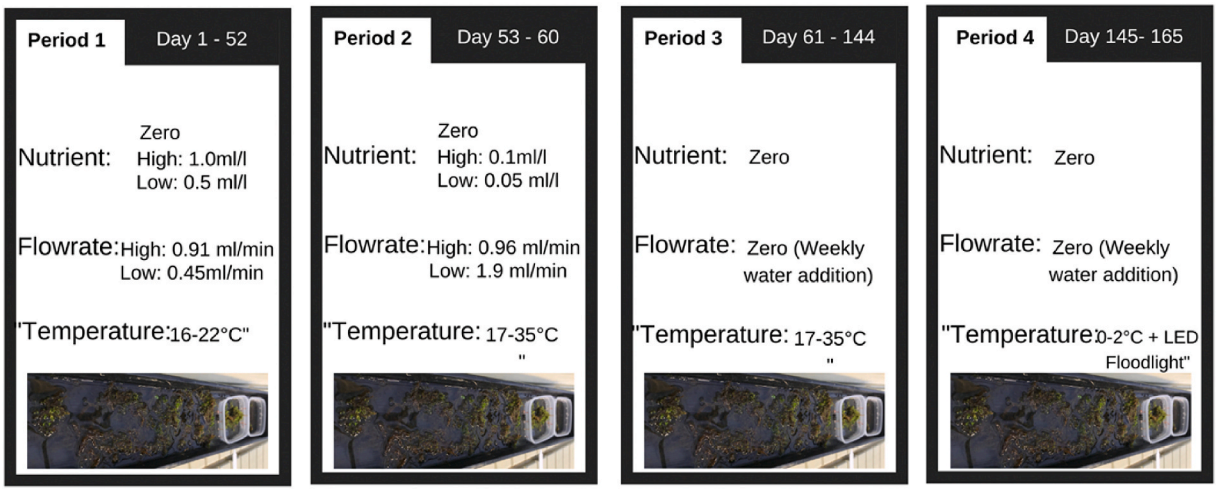

Fig. 2. Graphical illustration and details of the tested treatment combinations (nutrient addition and water flow rate) in periods 1–4. Temperature was monitored as a background factor.

2021. There were two variable physical factors: nutrient (NPK) addition (with three levels, zero, low and high) and water flow rate (with two levels, low and high). Two replicates per each treatment combination was made, yielding 12 combined treatment units in total (Fig. 1). In addition, there was one treatment unit with stagnant water and no water flow, no nutrient addition, and no replicates set as the control unit. Altogether, there were thus 13 separate treatment units in the experiment.

A total of 13 plastic long and oblong florist trays with dimensions of 66 cm (length) × 12.4 cm (width) × 5 cm (depth) were used as the treatment units (Fig. 1b). The moss biomass mats were spread across each florist tray. Incoming water was applied from the center of one side using a peristaltic pump and collected from the other side using outlet pipes (Fig. 1). Horizontal water flow was maintained by placing the inflow pipe 1.3 cm lower from the base of the unit while the outflow pipe was at 2.0 cm from the base of the tray. A net was added to in front of the outflow pipe to avoid loss of moss biomass. Two plastic and transparent fruit containers (138 mm × 98 mm, 435 ml) with lids were placed in each florist tray. These fruit containers held smaller mats of moss biomass, and an initial sampling was taken from the containers. The moss biomass in the fruit containers served as the focal measurement units, as it was difficult to control the entire moss biomass. To control the water flow, two peristaltic pumps were used, each serving six experimental units and placed at the end of each row (Fig. 1a and b). Replicates from each experimental unit were placed side by side as opposed to a random arrangement due to the limitation of available peristaltic pumps (Fig. 1a).

The experiment ran for a total of 165 days (Fig. 2). Period 1 took place from day one to 52, period 2 lasted from day 53–60, period 3 from day 61–144, and period 4 took place between days 145 and 165. Photoperiod in the greenhouse was regulated at 16 h day, 8 h night regime, and temperature data during the period of the experiment were collected from the greenhouse repository which showed a range of 16–22 °C in period 1 and 17–35 °C in periods 2 and 3 (during summer season). At the end of the experiment, all treatment units were subjected to a separate cold room treatment without nutrient addition and stagnant water (period 4, 0–2 °C, Figs. 1c and 2, see below Cold Room Treatment). Water quality of the used tap water was pH 7, dissolved oxygen (DO) 9.1 mg/L, electric conductivity (EC) 16.8 μS/cm, and temperature (T) 7 °C. Gram-scale and volume measurements of the moss biomass were taken at each sampling point.

2.3. Nutrient addition and water flow

Nutrient addition consisted of commercially obtained NEKO NPK 7-2-2 + trace elements (composition by weight: 6.9% N, 1.6% NO3–N, 2.3% P, 2.1% K, 2.3% Urea-N, 3.0% NH4–N). The water quality for the three nutrient levels were analyzed according to the Finnish national standards (National Boards of Waters, 1981; Table 1). Furthermore, pH, Dissolved Oxygen (DO), electric conductivity (EC), and electrode redox potential (ORP) were recorded manually with Multi 3630 IDS meter in periods 3 and 4.

In total, there were seven different units labelled based on their incoming flow rate (F) and nutrient concentration (N) (Fig. 1a). The experiment was run in four separate periods (Fig. 2, see Statistical analysis). In period 1, the groups were treated with different nutrient levels and water flow combinations (denoted by N and F). In this period, the nutrient levels were 1 m/L (N1) or 0.5 ml/L (N2), whereas the tested water flows were 0.45 ml/min (F1) or 0.91 ml/min (F2). Flow rate Q was determined using the plug flow reactor method [21,22] in Eq. (1)

$$Q = \frac{V}{t} \tag{1}$$

where Q is the flow rate, v is the volume of water (ml) and t is the time (t).

Observations from period 1 were used to advance the experiment for the second period, which was then used to advance consecutive periods. In period 2, the tested water flow (F1) was 0.96 ml/min, followed by F2, which was 1.9 ml/min. The nutrient addition was reduced to 0.05 ml/L (N2) and 0.1 ml/L (N1). There was no nutrient addition at the end of the experiment, in periods 3 and 4 (Fig. 2). In period 3, all the units were subjected to the same conditions as in the control treatment, which had no water flow or nutrient addition in greenhouse conditions. However, to avoid the negative impact of evaporation, the trough was filled with tap water occasionally.

2.4. Algal growth control

Algal growth was noticed in all treatment units, except in the control unit, in periods 1 and 2, and therefore the water flow was stopped for cleaning. Algae in treatment units were broken up with a small piece of metal wire, and the algae in the peristaltic tubes were flushed, or in some cases, the tubes were changed. After running the experiment for 56 days, the experiment was halted to clean

Table 1
Water quality of the three tested nutrient levels.

	Unit	Nutrient 0 (Tap water)	Nutrient 1	Nutrient 2
Dissolved carbon (DOC)	Mg/l	1.7	11	6.6
Nitrogen (N)	μg/l	230	81,000	38,000
Nitrogen Nitrate ($N0_3$–N)	μg/l	140	17,000	8900
Nitrogen Nitrite	μg/l	<2	5500	2400
Ammonium nitrogen (NH_4–N)	μg/l	<10	38,000	18,000
Phosphorus Phosphate (PO_4–P)	μg/l	89	27,000	13,000

the moss from algae. The algae in the containers was washed away by placing the moss material in a sieve, and by rinsing with tap water. The container was also washed while the moss material was still in the sieve. Following the washing of the moss, tap water was allowed to run through the units at a high peristaltic speed (3.43 ml/min) for 24 h.

2.5. Cold room conditions

At the end of the experiment (day 145, starting of period 4), all treatment units were transferred to a climate chamber with cold conditions. The temperature in the cold room was between 0 and 3 °C and light was provided with an illuminated LED floodlight (Luminatec led flood light, Model: Luminatec Areal 200-60-850-IP65; 200 W, 500 K). In the cold room, nutrient addition was omitted, and water was kept stagnant in all treatment units. The cold room treatment (period 4) was run for 20 days, in total (Fig. 2).

2.6. Growth measurements and water quality analysis

The growth measurements were carried out on the moss in the transparent fruit containers in each unit. The containers A and B in all 13 units were removed from the tray trough and placed on a rectangular loft net and allowed to dry for an hour. Then the containers with moss samples were dried for another hour. The containers labelled B were weighed with a compact CX621 g scale to determine biomass change in grams (g).

Containers labelled A were subjected to upthrust measurement. A transparent, water storage container of 5-L volume was filled with 1 L of tap water. A ruler was used to mark the 1-L capacity of the container in centimeters and glued to the container. A stone of 41.2 g was used as a weight and placed in the fruit container A with moss, and the container A was then covered with a lid. Then it was placed inside the storage container with 1 L of water. The upthrust was measured as the difference, in centimeters (cm), between the 1-

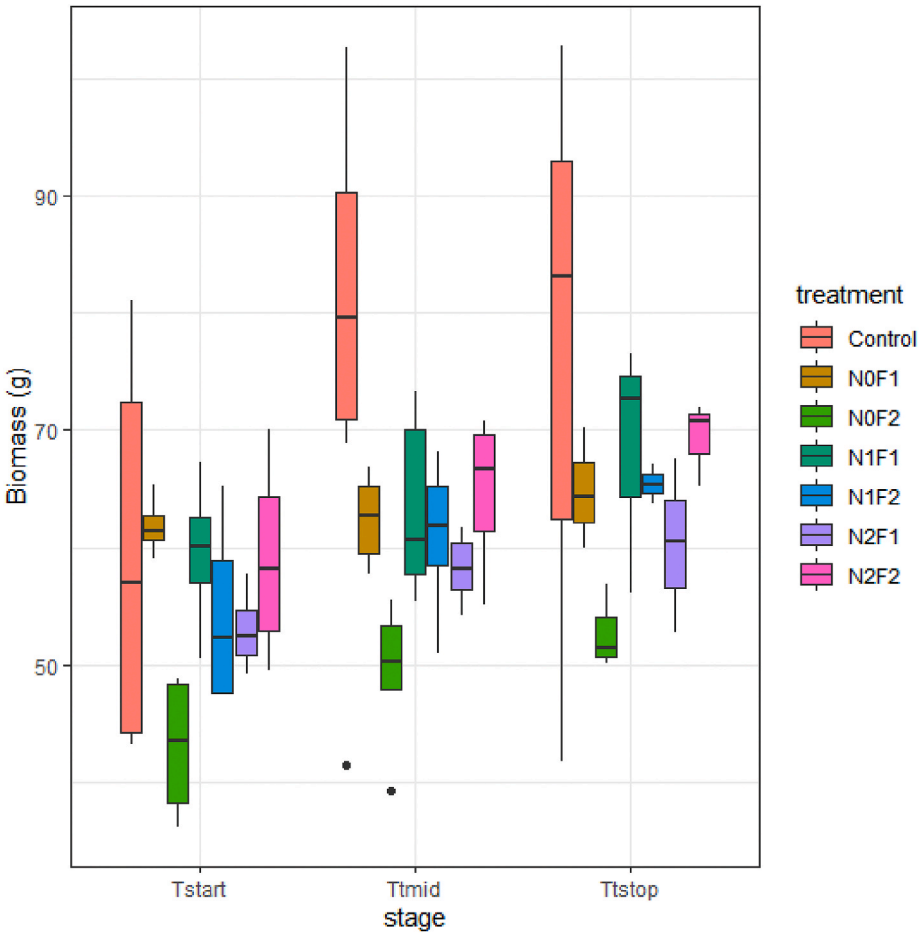

Fig. 3. Mean biomass (±SE) of *Warnstorfia fluitans* in the treatment combinations (control = zero flow, zero nutrient, N0 = zero nutrient, N1 = high nutrient, N2 = low nutrient, F1 = low flow rate, F2 = high flow rate). Stage (T) refers to measurement timing, made at the start, middle or end (stop) of the experimental period. Numbers are pooled values throughout the experiment. Treatments are classified according to period 1. N = 2 for each treatment combination (except for control N = 1).

L mark by the ruler and the new height after the moss container was placed in the storage container. The measurements were recorded and then converted into volume. The volume of water (dV) dispersed in upthrust was calculated according to Ref. [23] in Eq. (2).

$$dV = A \times B \times dH \tag{2}$$

where A is the length of the upthrust container, B is the breadth of the upthrust container, and dH is the difference in height. In addition, water quality data was collected, water temperature, pH, dissolved oxygen, electric conductivity, and redox potential in the third and fourth periods.

2.7. Statistical analysis

The statistical analyses were done in R [24] using R Commander for analysis and the ggplot2 for visualization [25]. Data were analyzed using the Linear Mixed Effect model using RCommander in R, followed by a 2-way ANOVA to test model coefficients. The response variables in the analysis were moss biomass and upthrust volume. The explanatory variables were fully combined, nutrient addition (levels: zero, low, high) and water flow rate (levels: low, high), named as "Treatment", and the stage of the experiment (levels: start = Tstart, middle = Ttmid, end = Ttstop; Fig. 1c, see below) was used as another main factor in the analysis. Period, which represents the sequential progression of the experiment (Figs. 1c and 2) was used as a random factor in the analysis. We also carried out a separate 2-way ANOVA for period 4. The explanatory variables were the same as in the Linear Mixed Effect model.

The measurements in each period were divided into 3 stages denoted as Tstart, Ttmid, and Ttstop which represent the start, middle, and end of the measurements for the periods, respectively (Fig. 1c). However, in the analysis, each stage was combined for all periods and treated as the main factor, *i.e.*, the first measurements in all periods were combined and analyzed as Tstart. The middle measurements in all periods were combined as Ttmid, and the last measurements in all periods were combined as Ttstop.

Water quality was analyzed using the multivariate Principal Component Analysis (PCA) in R. The PCA analysis of pH, water

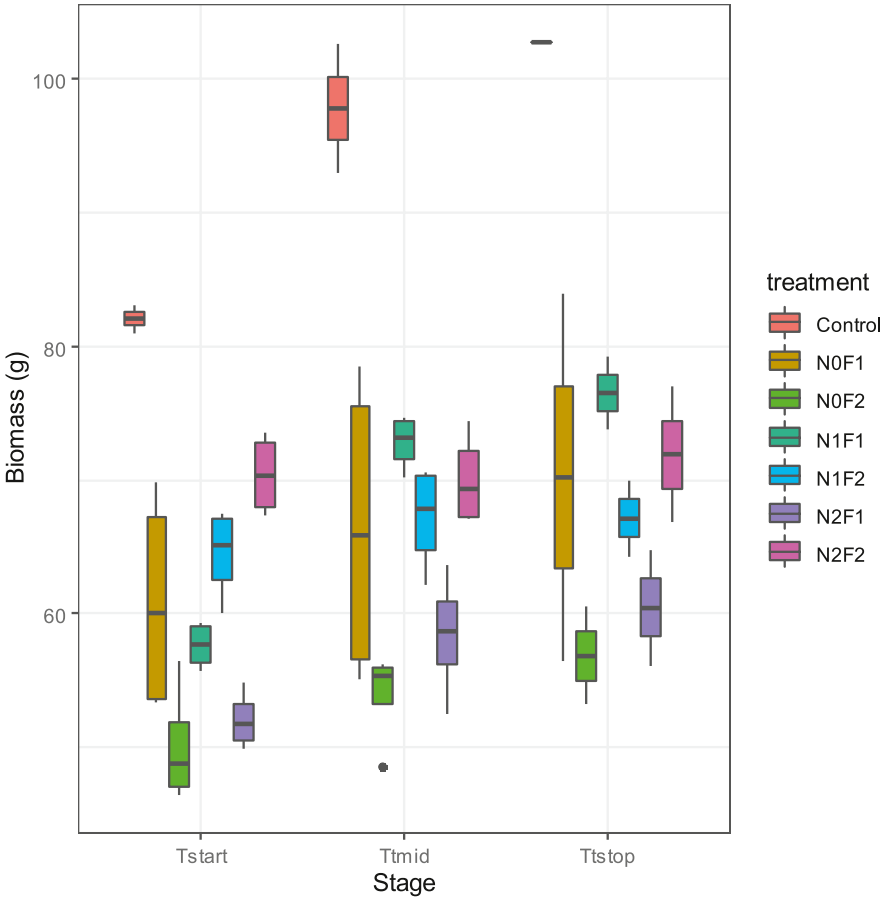

Fig. 4. Mean biomass of *Warnstorfia fluitans* (±SE) in cold-room conditions (period 4) after different treatment combinations (control = zero flow, zero nutrient, N0 = zero nutrient, N1 = high nutrient, N2 = low nutrient, F1 = low flow rate, F2 = high flow rate). Treatment refers to the treatments used in period 1. N = 2 for each treatment combination (except for control N = 1).

temperature, moss biomass, and electric conductivity, measured in the third and fourth periods of the experiment, were done in R.

3. Results

3.1. Growth of W. fluitans in greenhouse and cold room conditions

All treatment units showed biomass increase along experimental conditions and throughout the experiment (stage: $F_{1,69} = 4.33$, $P = 0.017$, Fig. 3). The nutrient level and water flow rate treatment combinations also affected the moss growth either positively or negatively during different stages within each period (treatment: $F_{1,69} = 9.54$, $P < 0.001$, Fig. 3). There was no significant interaction between the treatment and the stages ($F_{1,67} = 0.53$, $P = 0.88$). The control unit had the highest biomass at the end of the experiment (Fig. 3). As seen in Fig. 3, the flow rate may have adjusted the nutrient effect, as a higher flow rate often had a negative impact on moss growth. However, this was not consistent in all treatments, as the results suggest that with intermediate nutrient addition, the impact of flow rate may be positive (Fig. 3).

During periods 1 and 2, varying levels of algal growth was observed in the treatment units with the exception of the control unit (N0F0), and measures were taken to remove the algae from the system (see Materials and Methods). To further control the algal growth and test the impact of cold conditions on moss growth, the treatment units were taken to a cold room conditions at the end of the experiment (period 4, days 145–165). Moss biomass increased in all treatment combinations in the cold room conditions (stage: $F_{2.2} = 4.33$, $P < 0.0001$, Fig. 4). In addition, the treatment combinations differed from each other (treatment: $F_{2.6} = 29.789$, $P < 0.0001$, Fig. 4). The control treatment unit, which had not been subjected to any nutrient or water flow treatments, had the highest biomass increase in the cold room, from 81 to 102.8 g with a growth rate of 0.76 g/day (Fig. 4). As seen in Fig. 4, the previous nutrient and water flow treatments still had after effects in the cold room: the previously applied high water flow rate generally had a negative impact on growth in the cold room, but in some cases, especially when associated with low nutrient level (N2), high flow rate had a positive effect on moss growth (Fig. 4).

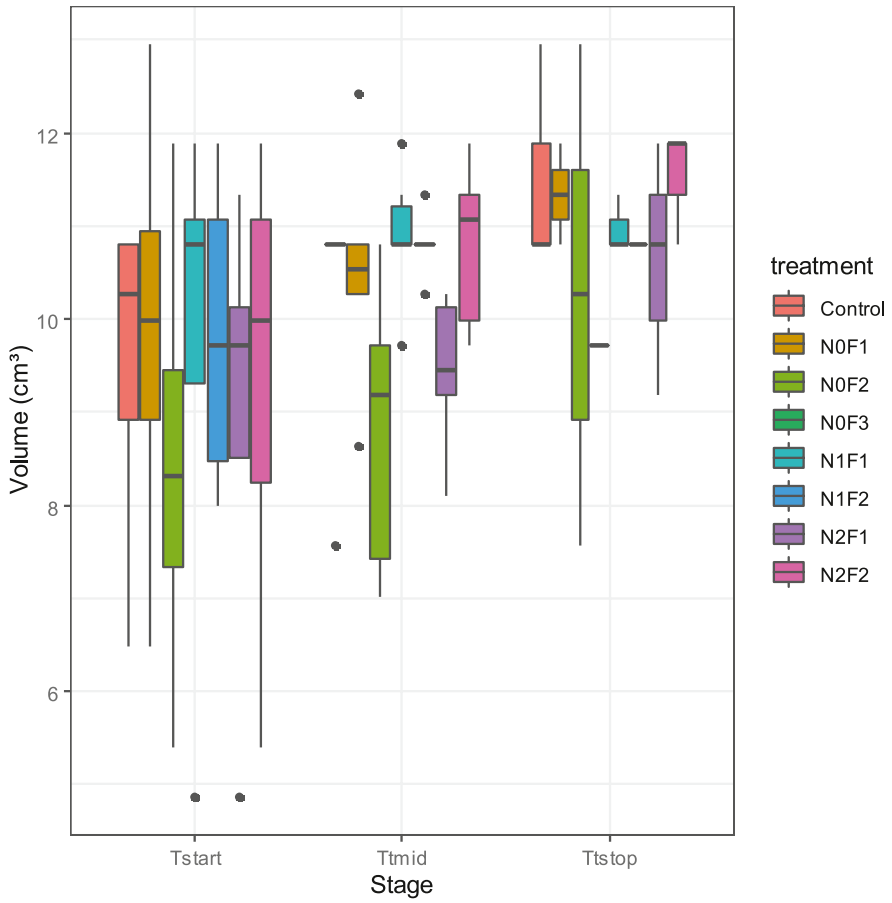

Fig. 5. Mean upthrust volume (cm^3) (±SE) of *Warnstorfia fluitans* biomass in different treatment combinations (control = zero flow, zero nutrient, N0 = zero nutrient, N1 = high nutrient, N2 = low nutrient, F1 = low flow rate, F2 = high flow rate). Stages (T) are pooled values throughout the experiment and treatments are classified according to period 1. N = 2 for each treatment combination (except for control N = 1).

3.2. Upright growth: upthrust volume of W. fluitans

We measured moss growth also via upright growth as upthrust volume. Despite considerable variation between samples, moss upright growth increased in all treatment combinations statistically significantly during the experimental periods (stage: $F_{2,67} = 17.04$, $P < 0.0001$). There was also a significant difference between the treatment combinations (treatment: $F_{2,67} = 2.62$, $p = 0.0242$). We did not observe an interaction between the treatment and the stages ($F_{2,67} = 0.23$, $P = 0.99$) (Fig. 5).

3.3. Cold room conditions and moss growth

To investigate the temperature effect in more detail, we tested the effect of period on moss growth. Average moss growth was the highest in the cold conditions (period 4) but the difference between the periods was not statistically significant ($F = 0.12$, $P = 0.07$) (Table 2). However, when compared to the periods 2 and 3, there was an average increase in biomass in all treatments after moving to cold room except for low nutrient (N2) treatment.

3.4. Water quality and moss growth

The Electric conductivity (EC) ranged between 365 and 948 μS/cm during the third period of the experiment (Days 61–144), and it decreased to 266–512 μS/cm when the temperature dropped from 17-35 °C to 0–2 °C in the cold room (days 145–165). Similarly, the pH reduced from 8.4 to 9.9 in period 3 closer to neutral (7.2–8.8) in period 4. PCA analysis revealed that PC1 explained 60.19% of the variation. Temperature, pH, and electric conductivity all had a negative correlation with biomass (Fig. 6), suggesting that the weight increased when temperature, pH, and electric conductivity decreased.

4. Discussion

Floating hook-moss (*Warnstorfia fluitans*) has been proven to be efficient in the bioremediation of metal and nitrogen-rich wastewaters [7–9], and it could be commercially produced as a nature-based treatment solution for arctic wastewaters. Therefore, our aim was to test growth conditions of *W. fluitans* by using different concentrations of NPK nutrients, water flow rates, and towards the end of the experiment, also low temperatures. Although we were able to detect a positive growth rate in the treatment combinations across time, our results suggest that moss growth is highest in control conditions without nutrient addition or water flow manipulation. We also found that algal growth creates a problem in warm conditions with added nutrients. Finally, our results suggest that cold temperature, low pH and low electric conductivity enhance moss growth.

Our study showed that *W. fluitans* is sensitive to addition of high levels of nitrogen, whereas, at low levels, the impact of nitrogen was not that clear and may have related to the water flow rate. Of the added nutrients, N had the highest ratio in our experiment. In earlier studies, the growth rate increased in P-rich rather than in N-rich conditions, suggesting that phosphorus is the growth-limiting element (Riis et al., 2010). Therefore, the N:P ratio of 3:1 that was used in our experiment is likely too high in terms of Nitrogen presence and low for Phosphorus. The high nitrogen ratio could be the reason for eutrophication, accelerated by the high ambient temperatures in our experiment, as nitrogen has been proven as a factor of eutrophication in several previous studies [26,27]; (Riis et al., 2010). The nutrient addition with increasing light and temperature in periods 1 and 2 likely caused algal overgrowth and disruption of the symbiosis between moss and epiphytic cyanobacteria, which fix nitrogen on moss surfaces [28-30]. The high N:P ratio at the beginning of the experiment may have limited the growth of the moss throughout the experiment.

Added nitrogen can be harmful for mosses due to their nitrogen fixing symbiosis. For example, adding of 30 kg N ha^{-1} yr^{-1} in the boreal forests of Northern Sweden over a ten-year period reduced *Sphagnum* moss cover [31]. Our results were largely in line with the results of [28]; who noticed the sensitivity of moss-associated cyanobacterial nitrogen fixation towards repeated nitrogen input. In their study, the cyanobacterial nitrogen fixing activity was clearly dependent on the nitrogen load. When nitrogen was added at high concentration (320 kg N ha^{-1} yr^{-1}), an increased N sensitivity and reduced nitrogen fixation activity was observed [28]. They also observed that the nitrogen fixation capacity was recovered after a short period of N deprivation. Our nitrogen addition in periods 1–2 is likely the reason for the decrease in moss growth. However, the growth was not recovered after nitrogen deprivation in periods 3–4, as N2F2 treatment, which had the low nutrient and high water flow in periods from 1 to 2, experienced low growth in the cold-room conditions (Fig. 4). Furthermore, harmful algal blooms that thrive in the presence of excessive N fertilizer application [32] can increase after nutrient addition. Therefore, the repeated application of nutrients, especially nitrogen, may explain our finding that algae were thriving at the expense of the moss in the first period (Fig. 3). Also, the tap water that was used in the experiment already had high background nutrient levels compared to those reported by Riis et al. (2010).

Table 2
Average growth rate of *Warnstorfia fluitans* (g/day) in different periods of the experiment (Control = zero flow, zero nutrient, N0 = zero nutrient, N1 = high nutrient, N2 = low nutrient, F1 = low flow rate, F2 = high flow rate). N = 2 for each treatment combination (except for control N = 1).

	Control (g/day)	N0F1 (g/day)	N1F1 (g/day)	N1F2 (g/day)	N2F2 (g/day)	N2F1 (g/day)	N0F2 (g/day)
Period 1 (16–22 °C)	−0.02	0.06	0.22	0.27	0.22	0.34	0.27
Period 2 and 3 (17–35 °C)	0.34	0.007	0.05	0.03	0.18	0.14	0.11
Period 4 (0–2 °C)	0.76	0.4	0.78	0.3	0.04	0.13	0.21

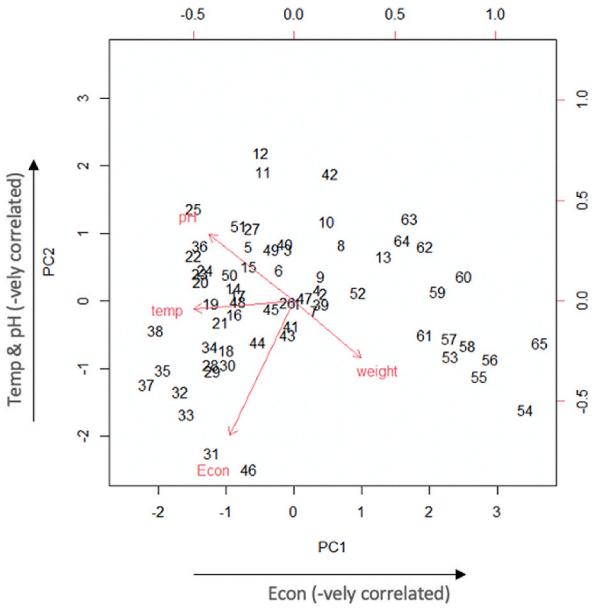

Fig. 6. Principal Component Analysis (PCA) of pH, temperature, conductivity, and biomass in Periods 3 and 4 (Econ: Electric conductivity, weight: weight of biomass, temp: temperature).

The culture in a cold room had a positive impact on moss biomass in general, although the increase was statistically significant only when compared with periods 2 and 3. The cold room temperature we used is close to, or even below the annual temperatures of *W. fluitans* in the natural habitat [9]. *W. fluitans* samples in our experiment originated from a groundwater-dependent spring ecosystem, where water temperature stays at a constant 4 °C all year-round. By comparison, the temperature regime for most algae is 15–30 °C [33], and in the Northern Hemisphere, between 10 and 19 °C [34]. Therefore, as the algae are unable to survive in cold conditions, low temperature may give a competitive advantage for the moss to survive and grow better than in higher temperatures. The control treatment unit had the best growth, perhaps due to its growth being unhindered throughout the experiment (Fig. 3). In contrast, the treatment units in other treatment groups had to recover from algae's effect, which had hampered their growth during previous periods. From the PCA analysis (Fig. 6), it can also be inferred that low temperature, neutral pH, and low electric conductivity have a positive impact on the growth of the moss. Similar conditions are observed in pristine subarctic spring ecosystems, which are natural habitats of *W. fluitans* [35].

We initially expected that the nutrient uptake is affected by the rate of water flow, because flow rate and hydraulic retention time affect uptake efficiency of nutrients in wetlands, in general [36,37]. The water flow rate in our experiment was observed to have an effect on the growth rate. Low nutrient, when combined with high flow rate, was more beneficial than low nutrient and low flow rate. The higher flow rate may have neutralized the negative effect of nutrients on moss growth.

Our results suggest that the best biomass growth was obtained in low-temperature conditions with stagnant water, based on results shown in Figs. 4 and 7. Neither the addition nor combination of nutrients and water flow helped to maximize biomass growth of the moss during the tested periods. Constant water flow was not beneficial for optimal growth of the moss, as the moss biomass increased the most in stagnant water conditions and not in continuous water flow. In future experiments, independent application of N and P (for example, NH_4NO_3 and KH_2PO_4) at suggested ratios between 17 and 640 should be tested. It is also important to apply very low amounts of N fertilizer, if at all, and it would be crucial to also determine the threshold for N application for the moss.

5. Conclusion

In our experiment, the control treatment with tap water at nitrogen concentration of 230 μg/L provided the best growth conditions for *W. fluitans*. This concentration could be used as a nitrogen threshold for the moss in future experiments. Our results also suggest that flowing water is not a requirement for *W. fluitans* growth, at least within the timescale of this experiment (5–6 months). Overall, cold-room conditions with a temperature of 2 °C and stagnant water, which is close to the natural growth temperature requirements of the moss, benefits moss growth. Our results thus suggest that in the condition of zero nutrient addition, 16-h constant light and low temperatures near 0 °C, *W. fluitans* can grow all year round in artificial conditions. Although the nutrient and water flow treatments had no expected effects on moss growth, our study serves as a starting point, and the results show that the commercial production of *W. fluitans* may not require nutrient or water flow manipulation. Instead, the growth conditions should mimic the natural cold climate conditions of *W. fluitans* habitats in northern peatlands and/or spring ecosystems. However, further studies are needed to analyze moss growth at low temperature with stagnant water for a longer timescale. For example, a study should be carried out to compare the growth rate of *W. fluitans* in its natural environments (subarctic lake and spring ecosystems) over different periods of the year (summer,

winter, spring and autumn) and when in a cold room, maintaining the same temperature of 0–2 °C over the same period throughout the year. We hypothesize that the moss will grow significantly better over constant cold temperature. It would also be useful to check the growth response when very low nutrient concentrations are added in cold room conditions, and to analyze the growth rate in a condition of either a similar or closely manipulated N–P ratio of the natural environment.

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