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Effects of cutting on vegetative development and biomass quality of perennial halophytes grown in saline aquaponics

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ABSTRACT

The cultivation of vascular plants in aquaculture is becoming increasingly important and saline aquaponics have been centered on plants with a short life cycle (i.e. annual species). However, the cultivation of perennial halophytes can allow to produce biomass year-round by repeated cuttings and regrowth. The present study evaluated the effects of different cutting regimes on the growth and tissue nitrogen uptake of the perennial halophyte *Paspalum vaginatum* and *Salicornia neei* cultivated in saline aquaponics. Clarified water from *Litopenaeus vannamei* cultivation was used as the main source of water and nutrients to grow plants established in Nutrient Film Technique (NFT). In each trial (28 days), three groups of 22 plants were established in different NFT benches and subjected to no cutting, one harvest and two harvests. Cutting regimes applied to both halophytic species did not affect the quality of recirculating water. Tillering and stem elongation of *P. vaginatum* had increasing trends with augment of cutting frequency. *Salicornia neei* cut at 28-day intervals produced a larger number of marketable size branches with less lignified shoots than non-cut plants and branchy plants cut every 14 days, which showed the smallest branch size. Individual plants of *P. vaginatum* and *S. neei* took up in their tissues between 2.27-2.45 mg/day of N and 4.59-6.43 mg/day of N, respectively. Cutting regimes did not statistically affect the accumulation of nitrogen in the biomass of either halophytic species. One harvest treatment, every 28 days, was considered the most suitable practice for aquaponics production of both halophytes.

Keywords: *Paspalum vaginatum*, *Salicornia neei*, sea asparagus, seashore paspalum, perennial vegetables, BFT system.

RESUMO

Efeitos da poda no desenvolvimento vegetativo e qualidade da biomassa de halófitas perenes cultivadas em aquaponia salina

O cultivo de plantas vasculares na aquicultura está se tornando cada vez mais importante e a aquaponia salina tem centrado no cultivo de plantas com o ciclo de vida curto (i.e. plantas anuais). Entretanto, o cultivo de plantas perenes pode permitir a produção de biomassa durante todo o ano por cortes repetidos e rebrote. O presente estudo avaliou os efeitos de diferentes regimes de poda no crescimento e absorção tecidual de nitrogênio das halófitas perenes *Paspalum vaginatum* e *Salicornia neei* cultivadas em aquaponia salina. A água clarificada do cultivo de *Litopenaeus vannamei* foi utilizada como principal fonte de água e nutrientes para o cultivo de plantas estabelecidas em Nutrient Film Technique (NFT). Em cada ensaio (28 dias), três grupos de 22 plantas foram estabelecidos em diferentes bancadas NFT e submetidos a nenhum corte, um corte e dois cortes. Os regimes de poda aplicados a ambas as espécies halófitas não afetaram a qualidade da água de recirculação. O perfilhamento e o alongamento do caule de *P. vaginatum* apresentaram tendências crescentes com o aumento da frequência de corte. *Salicornia neei* cortada em intervalos de 28 dias produziu um maior número de ramos de tamanho comercializável com menos ramos lignificados do que plantas não cortadas e plantas ramificadas cortadas a cada 14 dias, que apresentaram o menor tamanho de ramo. Plantas individuais de *P. vaginatum* e *S. neei* absorveram em seus tecidos entre 2,27-2,45 mg/dia de N e 4,59-6,43 mg/dia de N, respectivamente. Os regimes de corte não afetaram estatisticamente o acúmulo de nitrogênio na biomassa de nenhuma das espécies halófitas. O tratamento de uma poda, a cada 28 dias, foi considerado a prática mais adequada para a produção de aquaponia de ambas as halófitas.

Palavras-chave: *Paspalum vaginatum*, *Salicornia neei*, aspargo marinho, seashore paspalum, vegetais perenes, sistema BFT.

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The cultivation of vascular plants in aquaculture is becoming increasingly important for food production. Integration of hydroponics and animal aquaculture production units is known as aquaponics. This technique allows the utilization of waters and effluents of aquatic animal production as a total or partial source of nutrients for plants, as well as generating additional revenue by producing a high value product such as an edible vegetable or fodder (Doncato & Costa, 2021). Marine shrimp produce nitrogenous waste which is toxic to these animals at high concentrations (Pinheiro *et al.*, 2017; Doncato & Costa, 2023). Recirculating the saline solution in aquaponics is a much more efficient use of water and produces less environmental waste than changing the water continuously to dilute the nitrogen. The management of plants that can tolerate salinity (i.e. halophytes) is essential for the development of saline aquaponics. In the last decade, saline aquaponics have been centered on plants with a short life cycle (i.e. annual species), such as commercial *Salicornia bigelovii*, which have high growth ratios (Ventura & Sagi, 2013), but there is the necessity to produce new propagules for each cultivation period. Perennial species have also been cultivated in aquaponics (Ventura *et al*., 2011; Ventura & Sagi, 2013; Pinheiro *et al.*, 2017; Doncato & Costa, 2018, 2021), because of their ability to produce biomass year-round by repeated cuttings and regrowth. This characteristic leads to simple growing cycles, with reduction of costs with propagule production/maintenance and consecutive biomass harvests of the same lot of plants.

Ventura & Sagi (2013) showed that perennial *Salicornia* species irrigated with saline water could withstand seven harvest rounds spread over one year of continuous cultivation, producing twice more biomass than annual *Salicornia* in the same period. Additionally, pruning could have effects on morphological features with marketable value and chemical composition of *Salicornia* species by generating young shoots with distinct nutritional value. For example, Costa *et al*. (2006) showed that pruned plants of *Salicornia* generate sprouting shoots that had twice the concentration of phenolic compounds compared to plants that were not pruned. Baréa *et al*. (2007) reported nitrogen enrichment of *Paspalum* spp. tiller biomass after repetitive cutting. Since the ability of plants to absorb nitrogen compounds from the recirculating water is a main issue for aquaponics integration with saline aquaculture systems (Ventura & Sagi, 2013; Lange & Paulissen, 2016; Pinheiro *et al*., 2017; Doncato & Costa, 2023), the effect of cuttings on the growth and tissue nitrogen content of plants must be better understood.

Among native perennial halophytes from Brazil, *Salicornia neei* (syn. *Salicornia gaudichaudiana* and *Sarcocornia ambigua*) and *P. vaginatum* can be highlighted for their ability to readily grow after pruning and their economic potential for multiple uses. *Salicornia neei* is a perennial shrub with leafless cylindrical main stem and branches, distributed in the intertidal marshlands along most of the Atlantic and Pacific coast of South America. The shoot biomass of *S. neei* has high nutritional quality and is rich in macronutrients and micronutrients (Doncato & Costa, 2018), as well as in phenolic acids, flavonoids and vitamin C (Costa *et al*., 2006; Souza *et al*., 2018). Vegetative biomass can be used for human and animal consumption (Costa & Herrera, 2016) and as a source of nutraceuticals (Souza *et al*., 2018). Similar to other *Salicornia* species (frequently named sea asparagus) (Ventura *et al.*, 2011; Ventura & Sagi, 2013), *S. neei* has great potential as a leafy vegetable product, particularly when the main shoots and/or branches reach a 10-cm long marketable size. Soil cultivated plants (Costa *et al*., 2014; Costa & Herrera, 2016) showed fast regrowth after cutting, and each harvest yielded over 20 t/ha. *Salicornia neei* plants growing in aquaponics with nutrient-rich recirculating water of marine shrimp cultivation (Doncato & Costa, 2018, 2021, 2023) showed staggered 82 t/ha shoot production in a 73-day growth cycle (Pinheiro *et al*., 2017).

Paspalum vaginatum is a cosmopolitan perennial grass widely spread in the Brazilian coastal salt marshes and coastal dunes (Costa & Davy, 1992). The leaf biomass of this plant is composed of 1.3% fatty acids, 10.4% crude protein and 77.1% carbohydrates (DesRochers *et al*., 2009) and has a high concentration of free phenolic compounds (Souza *et al*., 2018). Due to its vigorous growth, even when irrigated with brackish water, *P. vaginatum* is used for covering sport fields such as golf, landscaping, goat and cattle fodder and phytoremediation of saline soils (Lonard *et al*., 2015). Weekly pruning of rooted plants was satisfactorily performed under hydroponic cultivation by Pessarakli & Touchane (2006). Recirculating water of marine shrimp cultivation can supply macro- and micronutritional requirements for *P. vaginatum* (Doncato & Costa, 2021, 2023), but there are no studies evaluating the cutting effect on *P. vaginatum* growth in saline aquaponics. Cuts from this species are likely to be used for the cut-and-carry forage system, as for *Paspalum atratum* (Hare *et al*., 2001).

It is of great interest to find crops that can tolerate saline water and can rapidly take up and immobilize nitrogen from the shrimp culture medium. Perennial plants could be especially effective because of their large root systems and sustained size (they do not need to start from tiny seedlings each season) and their use could reduce the cost of plant propagation. However, few perennials have been evaluated in saline aquaponics. Therefore, we asked the following two questions: (1) Are some perennial halophytes more effective at removing nitrogen from shrimp culture water than other species? (2) Does frequent cutting of the plants stimulate growth or nitrogen uptake, making improvements in their ability to purify the culture medium? The present study evaluates the effects of harvest length interval on *P. vaginatum* and *S. neei* plants grown in saline aquaponics with water from intensive marine shrimp cultivation. Growth parameters, biomass production and tissue nitrogen content of these plants were evaluated, as well as total nitrogen uptake from the recirculating water by the plants subjected to different cutting regimes.

MATERIAL AND METHODS

Plant acquisition

All plants were obtained from the active germplasm bank of the Laboratório de Biotecnologia de Halófitas, Instituto de Oceanografia (IO), Universidade Federal do Rio Grande (FURG). *Paspalum vaginatum* plants were originally obtained from a salt flat of the estuary of Caravelas (17º45'3"S, 39º13'4"W). *Salicornia neei* plants were from lineage BTH2 selected from a wild population of Patos Lagoon estuary [32°1'S, 52°6'W; Doncato & Costa (2018)]. Both species were grown from shoot fragments, disposed individually into 50 cm^3 plugs filled with fine beach sand and placed within polyethylene trays with 10% Hoagland solution. Plants of *P. vaginatum* and *S. neei* were kept in a growth room for 6 and 12 weeks, respectively. The growth room was kept under stable conditions (average temperature ranging from 23 to 26^{0}C, 14 h light photoperiod, and average light radiation of 115.0 µmol/m^2/s of photosynthetically active radiation, by cold white fluorescent light). The nutrient solution was replaced weekly, and prior to the experiment, all plants were uprooted and roots cleaned with water to remove the substrate.

Recirculating water

The decoupled aquaponic system used saline water from a tank of *L. vannamei* breeding stock located at Estação Marinha de Aquacultura (EMA, IO, FURG). Shrimps were reared in a 40 m^3 tank with a biofloc technology (BFT) system and a density of 555 shrimps per tank (approximately 50 g each). Feeding was provided twice a day of extruded feed (Guabi®, 35% crude protein). The residence time of the tank water was one year, and there was a partial replacement of the water during the experiment.

The water was conducted by gravity to a reservoir (3000 L) of the aquaponic system, solids in suspension were removed by clarification in a conical clarifier tank of 500 L and by filtration with BP-420-50 Pentair® filter bags of 50 microns. Storage water was then pumped to individual reservoirs of three hydroponic units. Water was renewed every week after cleaning with sodium hypochlorite of the clarifier tank, filter bags, pipes and reservoirs of the aquaponics systems.

Experimental design

Two experiments were carried out, one for each halophytic species. Both experiments used the same setup and were developed over 28 days, with the trial of *P. vaginatum* in summer-fall 2019 and the *S. neei* trial conducted in fall 2019. Plants of *P. vaginatum* (shoot height = 10.95 ± 0.43 cm, mean ± standard error) and *S. neei* (shoot height = 5.63 ± 0.40 cm) were transferred to net pots filled with small gravels and randomly distributed among the NFT benches (Figure 1). In each NFT unit were placed 22 plants, and 13.1 L/min of clarified shrimp water was recirculated every 15 minutes (393 L/h) between the bench tubes and water reservoir of 250 L. After two weeks of irrigation, plants of two NFT units were cut 4 cm above gravel level, and one NFT unit was left as an uncut control. Additionally, the two NFT benches with cut plants were submitted to one additional and two harvests with 28-day and 14-day intervals at the same cutting height, respectively. Thus, three periods of cutting were tested: no cutting during the cultivation period (control), one cutting after 28 days, and two cuttings over the 28 days (one every 14 days). The cutting height was long enough to preserve the branch apical meristems of *S. neei* and to ensure a viable photosynthetic area for the basal regrowth of *P. vaginatum* tillers.

Local experimental conditions (mean ± standard error) during the *P. vaginatum* trial were temperature of 22.01±0.52°C and daily solar radiation of 16.96 ± 0.82 MJ/m^2/day. During the *S. neei* trial, the average temperature was 19.87±0.42°C, and the daily solar radiation averaged 11.20±0.88 MJ/m^2/day. Meteorological data were obtained from the automatic station of Instituto Nacional de Meteorologia, INMET (32°4'43"S, 52°10'3"W).

Physical and chemical water parameters

Water physical-chemical parameters were monitored three times per week in both experiments. Water temperature was measured with a TDU-300 Unity® thermometer, pH was measured using a FEP20 Mettler Toledo® pH meter, and salinity was measured by a PAL-06S Atago® refractometer. Total suspended solids (TSS) were estimated according to Strickland & Parsons (1972). Total ammonia nitrogen (TAN) quantification followed that described at UNESCO (1983). Nitrite (NO_2) was analyzed by the methodology of Bendschneider & Robinson (1952) while nitrate (NO_3) and orthophosphate (PO_4) were measured according to Aminot & Chaussepied (1983). All sampling bottles were cleaned with 10% chloridric acid and rinsed with distilled water three times.

Vegetative parameters, biomass and tissue nitrogen content

Biometry procedures were carried out at the beginning of each experimental trial and at each cutting date. For *P. vaginatum*, we measured shoot height, number of tillers, number of leaves and shoot biomass (mg of dry matter), and for *S. neei*, we measured shoot height, length of all branches (primary, secondary and tertiary), branch number and shoot biomass. Plants were individualized by their position in the benches, and biotic data are presented as absolute growth rates between harvests. The results of the two cuttings treatment are sums of two regrowth periods. *Salicornia neei* foliar index was estimated using the sum of shoot heights and branch lengths (cm). At the beginning of the experiment, there were no roots out of the net pots, and the final root biomass of both species was measured at the final harvest by cutting all external roots to the net pots.

Shoot and root biomass components were weighed on a precision scale to determine fresh biomass and then oven dried at 60°C for 48 h to quantify dry biomass. All biomass data were presented as dry matter. The difference between fresh and dry shoot matter estimates the water content in shoot tissue. Nitrogen content in shoot and root tissues was measured by Kjeldahl digestion following the protocol of AOAC (1990). The amount of accumulated nitrogen in plant biomass under different cutting regimes was estimated by the multiplication of the average plant biomass components produced during the experiment and their tissue nitrogen content.

Statistics

For each halophyte, one-way Analyses of Variance (ANOVA) were carried out to compare the physical-chemical parameters of the recirculated water, vegetative attributes, biomass and nitrogen tissue content among cutting regime treatments (no cutting, one cutting and two cuttings). To fulfill the requirements of normality (Shapiro-Wilk test) and homoscedasticity (Levene test), some variables were transformed. Shoot height, number of tillers and number of leaves of *P. vaginatum*, as well as shoot height, foliar index and number of branches of *S. neei,* were transformed by square root (x). Log_{10} (x) was used for shoot biomass of *P. vaginatum* and shoot biomass, shoot and total nitrogen content of *S. neei*. Log_{10} (x) was applied for the TAN of the *P. vaginatum* experiment, as well as for the water temperature, TAN, NO_2, NO_3 and PO_4 of the *S. neei* experiment. When significant ($\alpha = 0.05$) results were obtained, Tukey's post hoc test was performed.

Paired t-tests were performed to contrast plant responses between the first and second cuttings in the two-cutting treatments. For these analyses, the number of tillers and leaves of *P. vaginatum* and foliar index and branch number of *S. neei* were transformed by square root (x), while shoot biomass of *P. vaginatum* and *S. neei* were transformed by log_{10} (x). Histograms of *S. neei* branch lengths at the beginning and each harvest date of all treatments illustrate the impact of the different cutting regimes on this marketable value attribute. All statistical analyses were conducted with SYSTAT 5.0 (Systat Software).

RESULTS AND DISCUSSION

Physical and chemical water parameters

The different cutting regimes applied to both halophytic species did not affect the quality of recirculating water in the hydroponic units. The global average conditions of the hydroponics units of the experiments with *P. vaginatum* and *S. neei* are summarized in Table 1.

The nitrate and phosphate concentrations in the water during the *S. neei* experiment were distinctly lower than those in the water used in the *P. vaginatum* experiment because

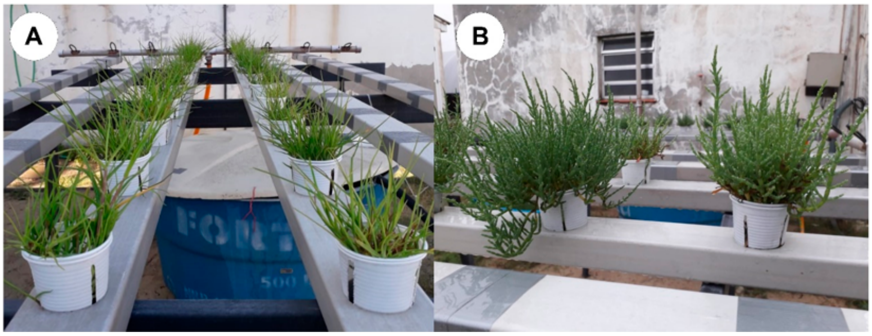

Figure 1. Plants of *Paspalum vaginatum* (A) and *Salicornia neei* (B) growing in saline aquaponics with water from intensive marine shrimp cultivation. Rio Grande, FURG, 2019.

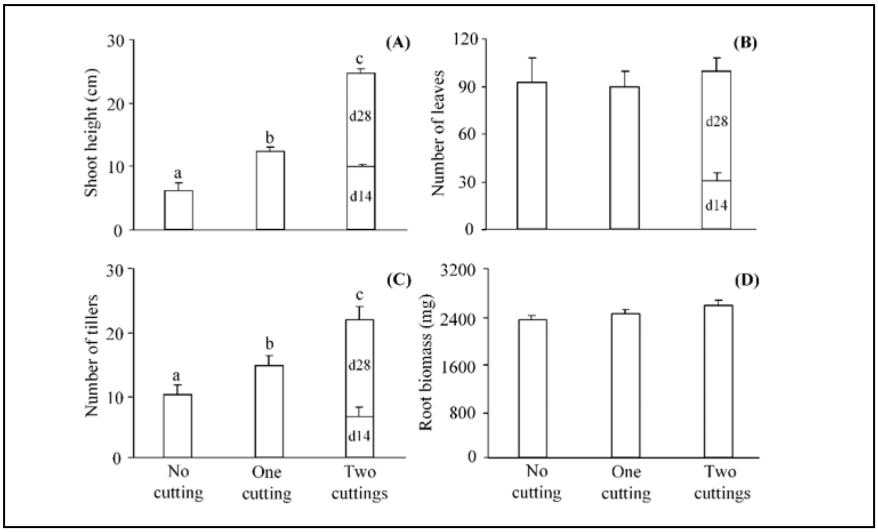

Figure 2. Mean (± standard error) cumulative shoot height (A), numbers of leaves (B) and tillers (C), and final root biomass (D) of *Paspalum vaginatum* plants subjected to three cutting regimes over 28 days growing in saline aquaponics. The codes d14 and d28 indicate data from consecutive harvests at days 14 and 28, respectively. Different lowercase letters represent significant differences between the averages (p <0.05), according to the Tukey test. Rio Grande, FURG, 2019.

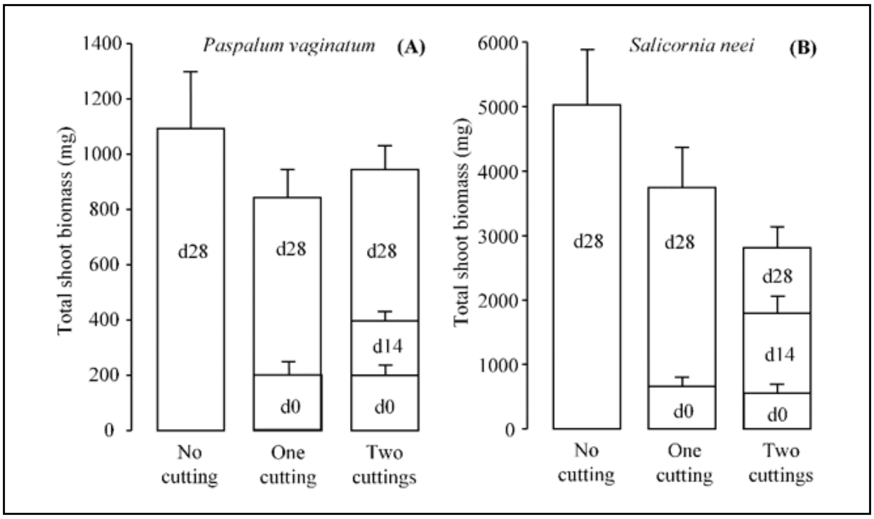

Figure 3. Mean (± standard error) cumulative shoot biomass of *Paspalum vaginatum* (A) and *Salicornia neei* (B) plants subjected to three cutting regimes over 28 days growing in saline aquaponics. The d0, d14 and d28 codes indicate data from consecutive harvests at days 0, 14 and 28, respectively. Rio Grande, FURG, 2019.

Table 1. Global average (± standard error) conditions of the water recirculating in hydroponic units during the *Paspalum vaginatum* and *Salicornia neei* experiments. Rio Grande, FURG, 2019.

Water parameter	*Paspalum vaginatum*	*Salicornia neei*
Temperature (°C)	23.43 ± 0.36	20.66 ± 0.29
pH	7.55 ± 0.02	7.60 ± 0.03
Salinity (NaCl g/L)	20.96 ± 0.44	24.58 ± 1.49
TSS (mg/L)	165.15 ± 3.99	193.10 ± 7.65
TAN (NH_4-N mg/L)	0.14 ± 0.03	1.40 ± 0.29
Nitrite (NO_2-N mg/L)	0.14 ± 0.01	7.06 ± 1.40
Nitrate (NO_3-N mg/L)	57.08 ± 2.51	23.71 ± 2.59
Phosphate (PO_4-P mg/L)	2.45 ± 0.07	1.27 ± 0.15

TSS = total suspended solids, TAN = total ammonia nitrogen.

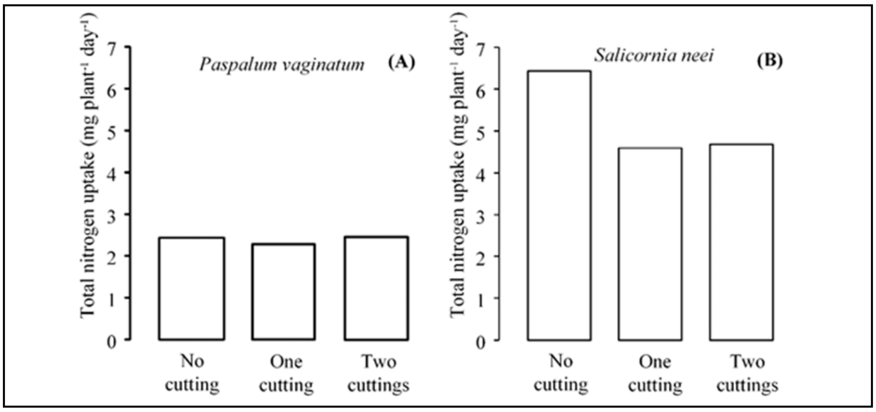

Figure 4. Estimated daily amount of nitrogen accumulated (uptake) into *Paspalum vaginatum* (A) and *Salicornia neei* (B) biomass under three cutting regimes over 28 days growing in saline aquaponics. Values were calculated by multiplication of the average biomass components produced during the experiment and their tissue nitrogen content (shoots + roots). Rio Grande, FURG, 2019.

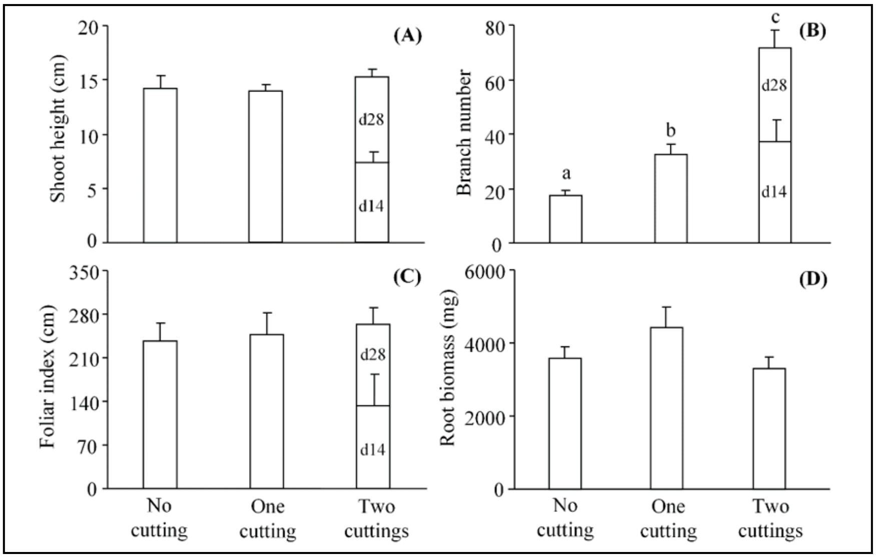

Figure 5. Mean (± standard error) cumulative shoot height (A), branch number (B), foliar index (C), and final root biomass (D) of *Salicornia neei* plants subjected to three cutting regimes over 28 days growing in saline aquaponics. The codes d14 and d28 indicate data from consecutive harvests at days 14 and 28, respectively. Different lowercase letters represent significant differences between the averages (p <0.05), according to the Tukey test. Rio Grande, FURG, 2019.

of the management practice of partial replacement of the water volume in the shrimp tank. These changes and augmentation of TAN and nitrite in the *S. neei* experiment can be explained by a new startup of biofloc formation. However, nitrogen compounds and phosphate concentrations in the recirculating water were not limiting for the growth of *P. vaginatum* and *S. neei* (Doncato & Costa, 2021) and, similarly water physical-chemical conditions, they remained within the range of saline BFT systems for *L. vannamei* production (Doncato & Costa, 2023).

Vegetative parameters, biomass and tissue nitrogen content

Few plants died during the experiments, and deaths showed no relationship with cutting treatments. The plant survival numbers of the treatments with no cutting, one cutting and two cuttings were 19, 18 and 18 for *P. vaginatum* and 22, 18 and 18 for *S. neei*, respectively.

Increasing cutting regimes significantly affected some vegetative characteristics of *P. vaginatum* (Figure 2A-C, Table 2 and 3). Tillering ($F = 10.64$, $p<0.001$) and stem elongation ($F = 90.80$, $p<0.001$) of *P. vaginatum* had an upward trend with increasing cutting frequency. Previously, Duncan & Carrow (1999) showed that the cutting of *P. vaginatum* shoots stimulates tillering and thus lateral expansion of its turfs. *Paspalum dilatatum* ecotype André da Rocha also increased the number of tillers after two consecutive cuttings (Beck, 2012), as a result of the removal of dead material naturally accumulated in the center of turfs. Light-improving conditions stimulate basal buds to form new tillers (Baréa *et al*., 2007; Beck, 2012). In contrast, *P. atratum* (Hare *et al*., 2001) and *P. dilatatum* [biotype Virasoro; Scheffer-Basso *et al*. (2007); ecotype Bagual; Beck (2012)] showed no cutting effect on tiller production, and *Paspalum guenoarum* ecotype Azulão decreased the number of tillers after two consecutive cuts (Lopes *et al*., 2016). The plant species may also differ in their capacity to regrow and produce new leaves. Repetitive cuttings are a common management practice on *Paspalum* species, and likewise *P. vaginatum* in our aquaponics experiment. Some species can improve shoot height (vertical) regrowth with increasing cutting intervals [*P. atratum*; Hare *et al.* (2001); *P. dilatatum*; Scheffer-Basso *et al.* (2007)]. *Paspalum vaginatum* demonstrates powerful cut recovery even in a short 14-day period, a response better than *P. atratum* cv. Pojuca (Leite *et al.*, 2001).

The shoot water content and nitrogen content in the two cutting treatments were higher than those in the no cutting treatment (Table 2). For the two cuttings treatment, averages of all vegetative parameters, shoot biomass and shoot tissue water content of *P. vaginatum* plants showed statistically higher values in the second harvest than in the first one (Figure 3A, Table 3). The shoot biomass response of the second harvest (175% higher than the first harvest) was mainly determined by the production of new tillers (increased 124%) and leaves (increased 120%) (Table 3).

No effect of cutting regimes was observed on *P. vaginatum* biomass production. A similar lack of biomass response was observed in *P. dilatatum* (Bungenstab *et al.*, 2013). *Paspalum guenoarum* improved its biomass when subjected to two cuttings rather than one cutting (Lopes *et al.*, 2016). Vigorous regrowth of aerial structures does not always generate a similar gain in biomass due to the different energetic and structural prices of plant modules that need to be formed. In our experiment, the new tiller production seemed to compromise the production of leaves, plants of treatments with no cutting, one cutting and two cuttings showed averages of 9.4, 6.2 and 4.6 leaves per tiller at the final harvest (data not shown). Thus, although *P. vaginatum* shoot height and number of tillers were improved by frequent cuttings (Figure 2A-C), no expected accumulation of biomass occurred (Figure 4A) due to a negative effect on leaf formation per tiller (previously informed).

Considering the forage production and the utilization of *P. vaginatum* biomass generated in aquaponics for cut-and-carry forage systems (Hare *et al.*, 2001), one cutting treatment (every 28 days' harvest) can be considered the most suitable practice due to its intermediate tillering and new leaf production for animal feeding. *Paspalum vaginatum* tillering can become an issue in aquaponic practice since intensive tillering rapidly fills net cups, and periodic thin out may be needed. From the biomass quality point of view, the water and nitrogen content of *P. vaginatum* shoots subjected to cuttings every 14 days characterize a nutritious young biomass (Table 2), which can be used as a supplement to animal diets or for the extraction of bioactive compounds, such as phenolics (Souza *et al.*, 2018). Baréa *et al.* (2007) and Leite *et al.* (2001) reported similar nitrogen enrichment of *P. atratum* and *P. dilatatum* new tiller biomass after repetitive cutting. Furthermore, the shoot nitrogen content of *P. vaginatum*

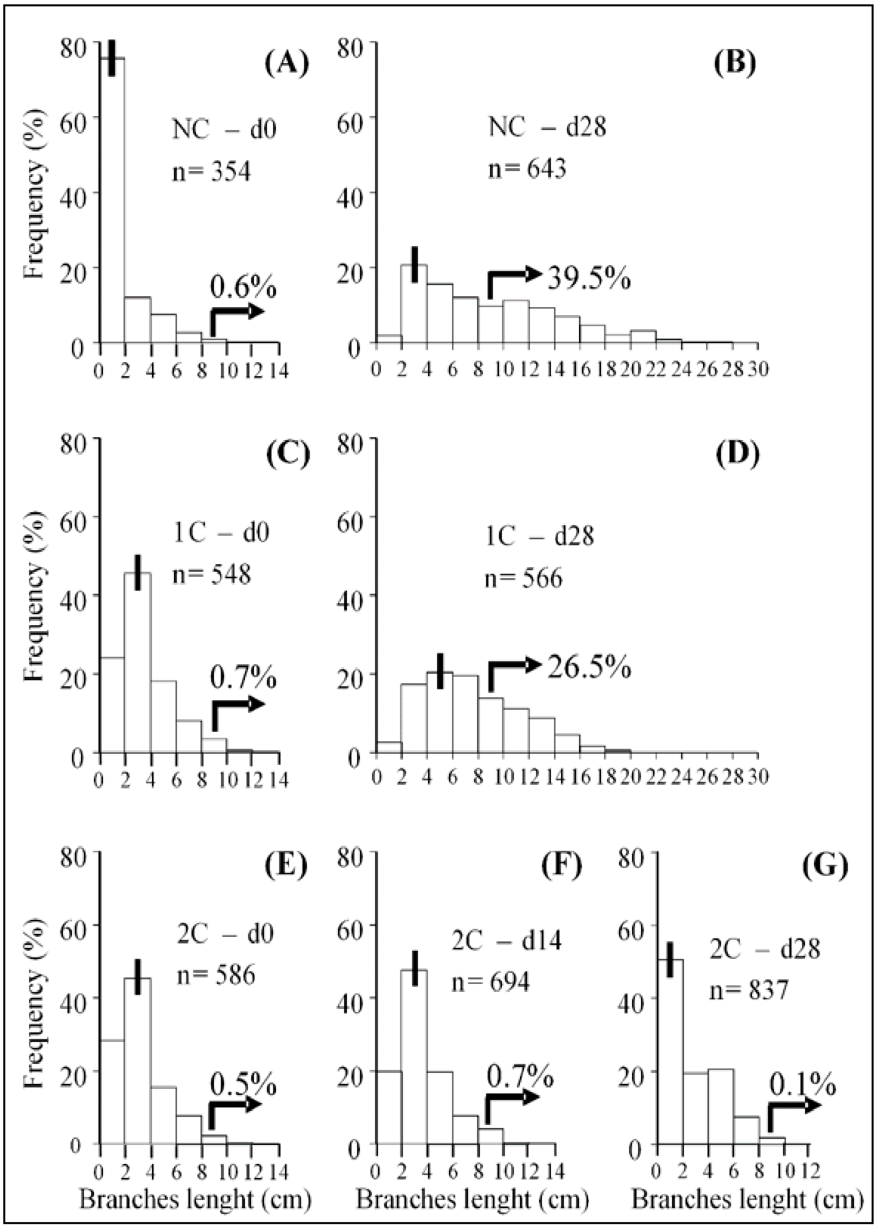

Figure 6. Histograms of *Salicornia neei* branch lengths at the beginning (A, C, E) and each harvest date of all cutting regime treatments growing in saline aquaponics. The d0, d14 and d28 codes indicate data from consecutive harvests at days 0, 14 and 28, respectively. Treatment codes: no cutting (NC), one cutting (1C) and two cuttings (2C). Rio Grande, FURG, 2019.

Table 2. Mean (± standard error) water and nitrogen contents in tissues of shoots and roots of *Paspalum vaginatum* and *Salicornia neei* subjected to three cutting regimes over 28 days growing in saline aquaponics. Rio Grande, FURG, 2019.

Parameters	***Paspalum vaginatum***			
	No cutting	**One cutting**	**Two cuttings**	**F**
Water content (%)	75.68 a (0.63)	77.73 ab (0.77)	78.99 b (0.30)	7.90 ***
Shoot N (%)	3.19 a (0.01)	3.21 a (0.08)	3.39 b (0.04)	6.15 *
Root N (%)	1.56 a (0.05)	1.73 a (0.02)	1.67 a (0.05)	4.03 ns
	Salicornia neei			
Water content (%)	90.33 ab (0.42)	90.86 b (0.16)	89.65 a (0.25)	3.40 *
Shoot N (%)	2.39 a (0.07)	2.56 a (0.06)	2.58 a (0.09)	1.32 ns
Root N (%)	1.96 a (0.09)	1.76 a (0.11)	1.64 a (0.08)	3.23 ns

The results of one-way ANOVA among cutting regimes are presented (significance: *p<0.05, **p<0.01, ***p<0.001, ns: non-significant). Different lowercase letters (within a row) represent significant differences between the averages (p<0.05), according to Tukey's test.

was higher than that observed in *P. atratum* [1.48-1.94% N, 14-28 days of cultivation; Leite *et al.* (2001)] and *P. dilatatum* [1.81% N; Baréa *et al.* (2007)].

Salicornia neei was less responsive to cuttings than *P. vaginatum* (Figure 5A-D, Table 2 and 3). The only growth parameter affected was the number of branches (F = 45.06, p<0.001, Figure 5B). Due to the apical dominance of *Salicornia* (Costa *et al.*, 2006), cutting of the main stem can stimulate the development of lateral branches, which assume a role as the primary apical meristem. When cut every 14 days (i.e. two cuttings), this species showed a massive production of new branches that had a small development in size. These plants with young tiny branches, not acceptable for the market as vegetables, could be used for the production of nutraceuticals (Souza *et al.*, 2018). Otherwise, plants subjected to no cutting or only one cutting every 28 days had approximately 26 - 40% of their branches reaching commercial size [≥10 cm; Ventura *et al.* (2011)]. Previously, Ventura & Sagi (2013) highlighted that frequent harvests led to shorter shoot sizes of *Salicornia* species cultivated in Israel. The choice of time intervals between subsequent harvests must consider the plant regrowth pattern, and in the present study, cuttings at 28-day intervals produced large amounts of branch with marketable size and less undesired lignified ("wooden") shoot parts than non-cut plants.

Salicornia neei grow vigorously, withstand periodic cutting, and was able to sustain continuous production of biomass in saline aquaponics. Previously, Costa *et al.* (2014) and Costa & Herrera (2016) showed that *S. neei* growing in field plots irrigated with saline waters/effluent from shrimp farming was able to withstand periodic cuttings. No cutting effect was observed on the shoot biomass production of *S. neei* in the present study (Figure 3B), since there was a very small elongation for the great number of branches sprouted (Figure 6). Initially, *S. neei* plants of all treatments had less than 1% of their branches with 10 cm length [international marketable size; Ventura *et al.* (2011)] and modal values of branch length lower than 5 cm. At the final harvest, 39.5% and 26.5% of the branches of the no cutting and one-cutting treatments reached marketable size, respectively. In contrast, plants of the two-cutting treatment had only 0.7% and 0.1% of their sprouted branches after the first and second harvests with marketable size, respectively. Ventura *et al.* (2011) tested cutting intervals of 14, 21 and 28 days over 5 months of cultivation and reported no effect of cutting regime on the accumulated biomass of *Salicornia fruticosa* (ecotype EL). In contrast, *Salicornia persica* (ecotype RN) accumulated more biomass than the former and showed the highest total production with cutting every 21 days. Concerning biomass quality, the nitrogen content of *S. neei* shoots produced in saline aquaponics with BFT system water was higher than values found for the same lineage cultivated in field plots [2.05% N, *S. neei* lineage BTH2; Doncato & Costa, (2018)], *Salicornia europaea* [2.40% N; Tikhomirova *et al.* (2008)] and the commercial *S. bigelovii* [0.72 to 1.36% N; Coronado (1991)], but lower than *S. neei* lineage BTH1 [2.92% N; Doncato & Costa (2018)]. Thus, saline aquaponics with BFT system water generate a high-quality *S. neei* biomass.

Nitrogen accumulated into biomass

Paspalum vaginatum and *S. neei* did not show marked differences in the total uptake of nitrogen into shoot and root biomass among cutting regimes (data not shown, total nitrogen uptake presented in Figure 4). Considering individual average values of biomass production and nitrogen tissue content, the total nitrogen uptake by *P. vaginatum* and *S. neei* ranged between 2.27-2.45 mg/day of N and 4.59-6.43 mg/day of N, respectively (Figure 4). Due to their differential growth patterns, *P. vaginatum* accumulated 62.8% of the total nitrogen uptake into its root biomass, whereas *S. neei* concentrated 53.2% of the total nitrogen absorption into shoot biomass.

Cutting regimes did not markedly affect the accumulation of nitrogen in the biomass of either halophytic species. This result occurs because of the very

Table 3. Mean (± standard error) of all the vegetative parameters of *Paspalum vaginatum* and *Salicornia neei* subjected to the first and second in the two cutting regimes (every 14 days) over 28 days growing in saline aquaponics. Rio Grande, FURG, 2019.

Parameters	*Paspalum vaginatum*				
	1st cutting		2nd cutting		t
Shoot height (cm)	10.14 (0.40)	a	14.49 (0.61)	b	7.62 ***
Number of leaves	31.06 (3.81)	a	68.22 (6.51)	b	5.20 ***
Number of tillers	6.72 (0.90)	a	15.06 (1.70)	b	6.57 ***
Shoot biomass (mg)	198.41 (23.96)	a	545.54 (62.90)	b	7.71 ***
Water content (%)	78.03 (0.50)	a	79.95 (0.26)	b	3.66 *
Shoot N (%)	3.37 (0.05)		3.41 (0.06)		0.82 ns
	Salicornia neei				
Shoot height (cm)	7.14 (0.75)		8.09 (0.32)		1.11 ns
Branch number	37.94 (6.82)		33.44 (2.54)		0.11 ns
Foliar index (cm)	135.39 (29.74)		127.57 (8.73)		1.22 ns
Shoot biomass (mg)	1,239.48 (254.89)		1,017.73 (75.26)		0.47 ns
Water content (%)	89.54 (0.42)		89.76 (0.24)		0.38 ns
Shoot N (%)	2.75 (0.11)		2.41 (0.05)		2.22 ns

The results of the t-student tests between cutting dates are presented (significance: *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ns: non-significant). Different lowercase letters (within a row) represent significant differences between the averages ($p < 0.05$).

small effect of cuttings on the growth and tissue nitrogen content of the studied plants. Although *P. vaginatum* had high shoot nitrogen contents in plants subjected to two cuttings (3.41%), the values of shoot and root biomass and root nitrogen content were similar with the other cutting treatments, resulting in very similar total nitrogen uptake into the plant biomass of all treatments. Both halophytes accumulate less nitrogen per day than *Aster tripolium* (shoot = 4.41 mg/day of N and roots = 8.75 mg/day of N), *Bolboschoenus maritimus* (shoot = 3.49 mg/day of N and roots = 11.56 mg/day of N) and *Spartina anglica* (shoot = 3.02 mg/day of N and roots = 6.86 mg/day of N), evaluated for remediation of eutrophicated coastal waters (Lange & Paulissen, 2016). The above cited results were obtained with plants grown for 63 days and reaching large total dry individual biomass of 78, 122.5 and 138 g, respectively. Additionally, *P. dilatatum* showed an uptake of 3.62 mg/day of N cultivated for 270 days, but plants produced a total of 243.5 g of shoot dry biomass (Baréa *et al*., 2007), which explains its much larger nitrogen accumulation in tissues than *P. vaginatum* plants growing in saline aquaponics (shoots reached 700-800 mg). On the other hand, *S. neei* lineage BTH2 cultivated with clarified BFT system water accumulated more nitrogen than previous cultivation of *S. neei* in saline aquaponics [3.93 mg/day of N; Pinheiro *et al*. (2017)], but similar *S. neei* lineage BTH2 growing in field plots [5.80 mg/day of N; Doncato & Costa (2018)] and *S. europaea* in hydroponics [shoot uptake nitrogen, 5.83 mg/day of N, nitrogen rich solution with urea and nitrate; Tikhomirova *et al*. (2008)]. Our results suggest that periodic cuttings of *S. neei* do not affect the ability of this plant to remove nitrogen compounds from the recirculating water of saline aquaculture systems.

Overall, both studied halophytes were able to regrow after cutting under saline aquaponics with clarified BFT system water of *L. vannamei* breeding stock. The one cutting treatment, every 28 days, allowed foliar structures of *P. vaginatum* and *S. neei* plants with large amounts of branches with marketable sizes. Cutting practices did not modify the capacity of halophytes to take up nitrogen into their biomass from the water of saline aquaponics. Further studies on the nutritional quality among the vegetative growth stages might provide a better understanding for the cutting management aiming to upscale this practice for halophytes' commercialization.

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