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ARTICLE

Freshwater Ecology

Competition in a changing world: invasive aquatic plant is limited by saltwater encroachment

Ashley M. McDonald[1,2] | **Charles W. Martin**[1] | **Carrie R. Adams**[3] | **Laura K. Reynolds**[2]

[1]UF|IFAS Nature Coast Biological Station, University of Florida, Cedar Key, Florida, USA

[2]Soil, Water, and Ecosystem Sciences Department, University of Florida, Gainesville, Florida, USA

[3]Environmental Horticulture, University of Florida, Gainesville, Florida, USA

Correspondence
Ashley M. McDonald
Email: ashley.mcdonald@ufl.edu

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Abstract

Competitive interactions between native and nonindigenous species occur under conditions of ongoing environmental alterations related to urbanization and climate change, presenting a challenge to understanding invasiveness. Indigenous submerged aquatic vegetation species have been negatively impacted by nutrient enrichment and saltwater intrusion, while simultaneously experiencing a rapid spread in nonindigenous competitors. To better predict future shifts in freshwater macrophyte community dominance, investigations need to consider how these multiscale disturbances influence competitiveness of nonindigenous macrophytes. Using an outdoor greenhouse mesocosm setup for approximately 19 weeks over the course of subtropical winter, we experimentally examined how nutrient enrichment, salinity, and the interaction of both factors influence the competitive interactions between a native macrophyte with moderate salinity tolerance, wild celery (*Vallisneria americana*), and a highly invasive macrophyte with lower salinity tolerance, hydrilla (*Hydrilla verticillata*). We tested the factors of macrophyte composition (hydrilla monoculture, wild celery monoculture, or biculture), salinity (0, 4, or 8), and fertilization (ambient or fertilized) on biomass production, biomass allocation, and morphological metrics as responses to environmental condition and interspecific competition. Fertilizer enhanced wild celery leaf area (more than twofold) and shifted allocation toward aboveground biomass, but did not enhance hydrilla competitiveness. Salinity negatively affected wild celery leaf area (by 7.5%–67.8%) and biomass (by 4%–15%) and also reduced hydrilla belowground biomass production (by 6%–25%) but did not influence competition. An interaction effect of fertilizer and salinity negatively influenced hydrilla growth, however the combined factors did not influence competitive outcomes. The lack of evidence for a competitive advantage by hydrilla in this study demonstrates that salinity exposure sufficiently limits a highly invasive plant's competitive abilities and subsequent suppression of a native species.

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KEYWORDS
competitive interactions, greenhouse experiment, saltwater intrusion, spring-fed rivers, submerged aquatic vegetation

INTRODUCTION

Environmental alterations enhance the invasiveness of a nonindigenous species (NIS) through increased fluctuation of resource availability (Barlow & Kean, 2004; Davis et al., 2000; Sánchez & Fernández, 2006), driven by the creation of an opportunity niche when resource levels exceed the relative background baseline (Hui et al., 2016). Opportunistic NIS succeed by taking advantage of a disturbance-related resource flux to become established and then reduce fitness of native competitors through amensalism (Catford et al., 2011; Kupferberg, 1997; Petren & Case, 1996). Urbanization-linked environmental alterations have greatly contributed to establishment of NIS (Cadotte et al., 2017), and subsequent alterations will continue to occur in tandem with elevated temperatures, rising sea levels, and altered precipitation patterns that will introduce novel stressors for both native species and nonindigenous competitors. Complex interactions of multiscale disturbances linked to urbanization, land use change, and climate change present a challenge for understanding and predicting invasiveness and competitiveness of a NIS and resilience of a native species (Fournier et al., 2017; Hellmann et al., 2008). Therefore, realistic assessments of the competitive impact of an NIS on its native competitor require consideration of both anthropogenic disturbance and plausible climate change stress factors.

Land use change adversely affects freshwater environments to such an extent that the decline of native freshwater biodiversity exceeds that of terrestrial or marine ecosystems (Jenkins, 2003; Lake et al., 2000). Urbanization directly impacts water levels and flow rates through freshwater extraction for municipal and agricultural use, and water quality concomitantly decreases with increased nutrient and sediment loads due to altered land usage of the watershed (Salerno et al., 2018). These allochthonous nutrient inputs redistribute resource availability, enhancing non-native establishment (Lambert et al., 2014; Santana Marques et al., 2020), driving declines in aquatic ecosystem function (Meyer et al., 2005), and exacerbating climate change impacts on aquatic organisms (Bond et al., 2008; Freeman et al., 2019). Salinity increases also constitute a substantial threat to freshwater ecosystems, as over half of streams in the United States are predicted to have a 50% increase in electrical conductivity by the end of this century due mainly to salt loading from human land use activities (Olson, 2019). Coastal freshwater ecosystems are at even greater risk of salt stress due to an encroaching salinity wedge that is driven by the combined effects of sea level rise, altered precipitation patterns, and aquifer extraction (Werner et al., 2013). The interacting factors of resource abundance and multiple stressors influence competitive interactions between native species with NIS in degraded aquatic habitats (Alpert et al., 2000; Lake et al., 2000), and therefore contribute to the possibility of competitive displacement of native species by NIS.

In submerged aquatic vegetation (SAV) communities, a shift in dominance from native to NIS threatens biodiversity and species composition/identity components of aquatic ecosystems (Emery-Butcher et al., 2020; Havel et al., 2015; Hill et al., 2020; Muthukrishnan & Larkin, 2020; Sala et al., 2000), which are essential to ecosystem functioning (O'Connor & Crowe, 2005), service provision (Harrison et al., 2014), and buffering against long-term perturbation impacts (Yachi & Loreau, 1999). This is primarily because SAV communities are habitat modifiers (e.g., ecosystem engineers, sensu Jones et al., 1994) that contribute multiple ecosystem functions such as influencing water column turbidity, nutrient cycling, and oxygenation (Gurbisz et al., 2017; Madsen et al., 2001), and providing forage and habitat for both invertebrate and vertebrate animals (Declerck et al., 2005; Hargeby et al., 1994). Therefore, replacement of a native macrophyte by an NIS with dissimilar biology, phenology, or growth form threatens to disrupt one or more of these functions, although this effect can be both functionally and species specific (see Alford & Rozas, 2019; Martin & Valentine, 2011; Salgado et al., 2019). Concerns of functional disruption are currently caused by an ongoing invasion of nonindigenous hydrilla (*Hydrilla verticillata*, [L.f.] Royle) into coastal spring-fed river systems where SAV communities are historically dominated by native wild celery (*Vallisneria americana* Michx.) (Langeland, 1996). Estimating potential shifts in this SAV community and determining how altered conditions will influence invasibility and competitive dominance of hydrilla in these delicately balanced ecosystems present critical research priorities for managers and conservationists.

Hydrilla and wild celery are closely related, resolving into a subclade within Hydrocharitaceae (Chen et al., 2012), yet both species exhibit marked distinctions in morphology, resource acquisition abilities, physiological tolerances, and life history. Hydrilla benefits from a resource acquisition advantage in enriched water column

nutrient conditions by allocating energy to canopy biomass production with vertical growth up to an inch per day. Hydrilla will then branch profusely until half of its standing crop occurs in the upper 0.5 m of water column, enhancing the shoot's abilities to outcompete other macrophytes for the interception of surface light and absorption of surface-diffused dissolved inorganic carbon (Haller & Sutton, 1975; Langeland, 1996; Pieterse et al., 1984; Van et al., 1999). The two macrophyte species differ in their salinity tolerances, with the invasive strain of hydrilla exhibiting production limitations above a salinity of 4 (Haller et al., 1974; Hillmann et al., 2019; Shields et al., 2012; Twilley & Barko, 1990) and the upper wild celery salinity limit ranging between 13 and 15 (French & Moore, 2003; Haller et al., 1974; Martin & Valentine, 2012). Model predictions indicate that both anthropogenic nutrient enrichment and salinity encroachment will increasingly become part of the altered physical environment of near-coastal aquatic habitats at risk of invasion (IPCC, 2019). Therefore, attempts at predicting the success of prolific nonindigenous macrophytes like hydrilla necessitate the consideration of an interaction of these two abiotic factors. Specifically, consideration of interacting factors during a period of low productivity (i.e., winter) can reveal any condition-specific trade-offs between interspecific competition for resources and abiotic stress resistance during a seasonal period of vulnerability (Dunson & Travis, 1991).

Using a greenhouse mesocosm setup, we investigated interactions of invasive hydrilla and native wild celery under conditions of nutrient enrichment and saltwater encroachment over the period of a subtropical winter season. We examined SAV growth, biomass allocation, and nutrient incorporation as proxies for competitive outcomes under manipulations of nutrient enrichment, salinity increase, and an interaction of both factors. We tested the following hypotheses regarding competitive interactions between hydrilla and wild celery under the following influential conditions: (1) influence of nutrient enrichment and competitive interaction: nutrient enrichment enhances hydrilla competitiveness, resulting in negative wild celery responses; (2) influence of salinity and competitive interaction: salinity limits hydrilla competitiveness, due to the species' lower salinity tolerance, resulting in a neutral to positive effect on wild celery condition; and (3) influence of nutrient, salinity, and competitive interaction: wild celery competitiveness increases with nutrient addition, but only when competing at salinities over 4 (i.e., the known hydrilla tolerance threshold). Results from this competition experiment will provide novel insight into how interacting global change conditions will factor into hydrilla's competitiveness, and also clarify whether these conditions may enhance or restrict the competitive exclusion of nonindigenous hydrilla over wild celery.

MATERIALS AND METHODS

Experimental setup

We manipulated three factors in a fully crossed randomized block design: *macrophyte composition* (three levels: hydrilla monoculture, wild celery monoculture, and hydrilla–wild celery biculture), *salinity* (three levels: 0, 4, and 8), and *fertilization* (two levels: ambient and fertilized) (Figure 1). We replicated each of the 18 unique treatment combinations six times ($n = 6$) for a total of 108 replicate aquaria, organized into a randomized grid of seven columns (blocks) oriented on a northerly to southerly plane (Figure 1), contained within a temporary outdoor greenhouse at the UF|IFAS Nature Coast Biological Station in Cedar Key, FL (USA; 29.135215°, −83.032545°). We collected all macrophytes used in the competition experiment from wild cultures in Florida, with dioecious hydrilla collected from Lake Apopka, FL (USA; 28.67431°, −81.678743°) and wild celery from Lake Hartridge, FL (USA; 28.048296°, −81.749511°) in November 2018 and kept in outdoor holding tanks (less than 1 week) until experiment initiation.

The experiment commenced on November 20 and was maintained for 132 days (~19 weeks) in replicate cylindrical 38-L bucket aquaria (approximately 30.5-cm diameter and 37 cm tall) and followed the design of Martin and Valentine (2012). Upon experimental initiation, we standardized plant lengths by trimming wild celery leaf blades to 15 cm and separated hydrilla secondary apical branches from primary stems prior to removing tertiary branches and standardized by trimming to 15 cm. We conducted distinct trimmings due to varying growth physiologies between the two species (i.e., wild celery grows from the apical meristem at the base of the plant, while hydrilla grows from the tip). We removed reproductive structures prior to placement in aquaria. Each replicate contained constant densities of macrophytes, with four physiologically independent shoots planted in a square pattern. Biculture treatments had two wild celery and two hydrilla shoots planted in an alternating orientation. These densities scale up to approximately 57 shoots m^{-2}, similar to densities used in previous experiments that reported competitive responses under comparable control conditions (Sutton et al., 1980; Van et al., 1999). Shoots planted in bucket aquaria contained 17 L of conditioned tap water over an approximately 8 cm deep sediment layer that was a mixture of seven parts cleaned, azoic sand to 1.25 parts organic potting

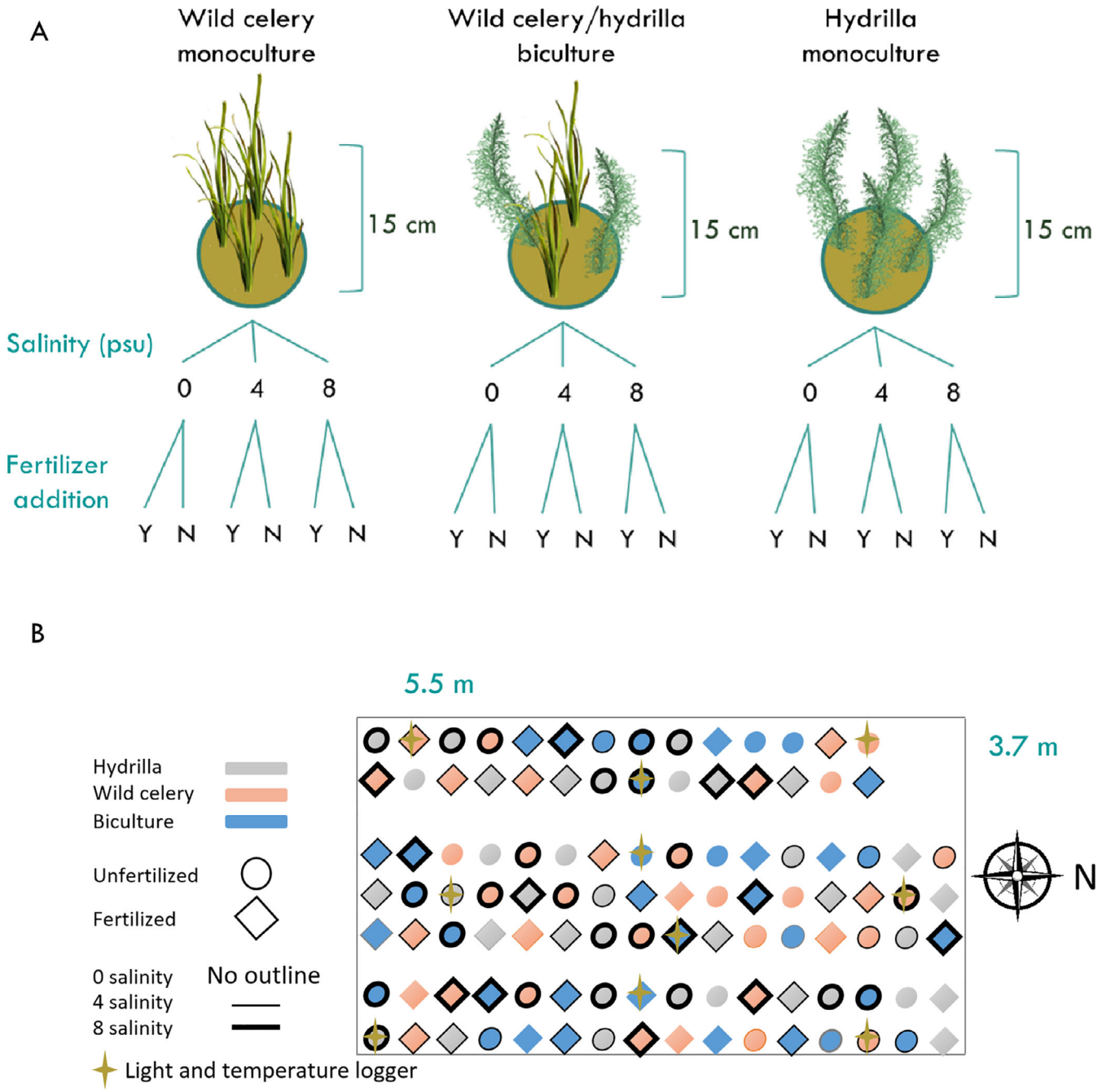


FIGURE 1 Overview of experimental factors tested during November–April 2018 (A). Three levels of macrophyte mixtures with all shoots trimmed to 15 cm at beginning of experiment; three levels of salinity tested; two levels of fertilizer addition tested. Each treatment had six replicates at the beginning of the experiment for a total of 108 experimental units. Orientation and dimensions of the outdoor greenhouse where experimental units were maintained and monitored, with columns numbered 1–7 oriented from east to west (B).

soil. To acclimate the plants to salinity treatments, salinity was gradually raised 2 salinity units per day with the addition of an Instant Ocean aquarium salt slurry until the assigned treatment salinity was met. The nutrient enrichment treatment was attained with an addition of 1 g Osmocote slow release 14–14–14 fertilizer into each bucket's sediment mixture to the concentration of 1.2 mg L^{-1} for both nitrate and phosphate. This concentration mimicked field conditions of Florida springs groundwater enriched by inorganic nitrate, to a concentration of approximately 3.4× the nitrogen threshold set by the Florida Department of Environmental Protection for Florida's clear water streams (Harrington et al., 2010). The ambient nutrient treatment did not receive fertilizer. Each bucket was aerated continuously throughout the experiment to prevent water column stratification and conditioned tap water

was added weekly to counteract evaporative water loss and maintain salinities.

Abiotic parameter monitoring

Using a LICOR model 1400 datalogger with 4π spherical sensors, photosynthetically active radiation (PAR) was measured twice inside experimental units, once at the beginning (November 26) and once in the middle (February 11) of the experiment, to ensure light availability within buckets exceeded the minimum values estimated for *V. americana* production (based on reported values of Blanch et al., 1998). Measurements indicated adequate PAR was available to plants inside the experimental units (averages of 414 [±86 SD] μmol m^{-2} s^{-1} and 363 [±126] μmol m^{-2} s^{-1} for November and February, respectively). To characterize light environment over the entire experiment and to determine potential differences in light environment among blocks, we used Onset Hobo loggers to monitor hourly in situ light levels (in lumens per square meter) in a subset of buckets (Figure 1). While light level measured in lumens per square meter is not equivalent to values for the restricted wavelengths of PAR that are used in photosynthesis, light level measurements have a positive linear relationship with PAR (Long et al., 2012; Wall et al., 2008) and thus differences in light level measurements among blocks would indicate a similar difference in PAR. The Onset Hobo loggers also measured hourly water temperature (in degrees Celsius). Dissolved oxygen (DO; in milligrams per liter) and salinity of experimental units were measured twice a week with a YSI ProDSS handheld and associated calibrated probes.

Abiotic environment

Environmental variables were compared across blocks to test for experimental artifacts related to the influence of column (numbered 1–7 from east to west) on solar input (daily sum of lumen measurements recorded once every hour), temperature (daily average of hourly temperature measurements), and DO, with all data analyzed in RStudio v. 1.2.1335 (R Core Team, 2019). Hourly light level monitoring showed the daily sum of measurements across blocks ranged from an average of 3.7 (±1.2) lumens m^{-2} on December 14, 2018, up to 324.0 (±89.3) lumens m^{-2} on March 29, 2019. A Welch's one-way test indicated differences in solar input among blocks ($F_{6,492} = 69.7$, $p = 2.2 \times 10^{-16}$; Appendix S1: Figure S1). Furthermore, visible evidence of algal blooms in fertilized aquaria corresponded with slightly diminished light availability (17,211 [±1076] lumens m^{-2}) compared with unfertilized aquaria (19,257 [±1249] lumens m^{-2}). Temperature showed a seasonal pattern (Appendix S1: Figure S1) and ranged from an average of 9.7 (±0.6)°C on January 27, 2019, up to 25.0 (±0.4)°C on February 22, 2019. A one-way ANOVA indicated no evidence of temperature differences among blocks ($F_{6,1118} = 1.1$, $p = 0.36$). DO measurements ranged from an average of 8.7 (±0.1) mg L^{-1} on November 26, 2018, up to 16.6 (±0.8) mg L^{-1} on December 6, 2018, with a one-way ANOVA indicating no evidence of DO concentrations differing among blocks ($F_{1,144} = 0.4$, $p = 0.53$).

Response metrics sampled

Macrophyte growth and biomass allocation metrics measured on both wild celery and hydrilla included relative biomass fraction (RBF), aboveground (AG) biomass, and belowground (BG) biomass. Metrics only measured for wild celery included leaf area per shoot and new shoot production, and metrics only measured for hydrilla included stem length and new branch production. At the start of the experiment and prior to shoot planting, we measured the initial biomass for wild celery and hydrilla as the total wet biomass (in grams) of each species per replicate after spinning individual shoots in a salad spinner for 10 s to remove excess water. At the conclusion of the experiment, we measured total wet biomass (in grams) for each species per replicate following the same procedure. End of experiment wet biomass was divided by the sum of beginning and end of experiment wet biomasses to calculate the RBF, which was used to determine experimental biomass changes for both wild celery and hydrilla. RBF values >0.5 indicate a biomass gain over time and values <0.5 indicate biomass loss. End-experiment biomasses of replicates for both species were then separated into AG and BG biomass (in grams) portions and placed in a drying oven at 60°C for at least 48 h to obtain dry mass (gDM). For wild celery morphology and shoot architecture metrics, we counted the number of new shoots and calculated leaf area (in square centimeters) as the product of leaf length (in centimeters) and width (in centimeters) for each leaf on a shoot. We then divided the total leaf area (sum of leaf area for all wild celery leaves per replicate bucket) by the number of original shoots planted (two or four) for a comparable measurement of leaf area per shoot (in square centimeters per shoot) between monoculture and biculture treatments. We also divided the number of new shoots produced per replicate by the number of original shoots planted for a comparable measurement of new shoot production per original shoot. For hydrilla morphology and

architecture metrics, we measured the number of new branches produced (per shoot) and primary apical stem length (in centimeters). Flower presence was also noted, but low occurrence in wild celery (~15% of replicates) and absence in hydrilla prompted the decision to remove this response from comparative analysis. Plant response metrics were either pooled or averaged within bucket replicate, depending on the metric used (e.g., total biomass or average stem length).

To determine nutrient incorporation in plants, we dried AG biomass from a subset of four randomized end-experiment samples per treatment to a constant mass in a drying oven at 60°C for analysis of total nitrogen (in milligrams of TN per gram) and total phosphorus (in milligrams of TP per gram) content. Then, we ground tissues into a fine powder using a Benchmark BeadBlaster microtube homogenizer. Total phosphorus (TP) analysis was conducted by acid extraction followed by colorimetric analysis, using a modified method from Solórzano and Sharp (1980), then samples were run on a Shimadzu spectrophotometer, model UV-2450. Total nitrogen (TN) content was measured upon tissue ignition using a Thermo Flash EA 1112. TN and TP content of AG biomass per species per replicate was then multiplied by the end of experiment AG biomass (gDM) for that replicate, then corrected for original number of shoots planted (i.e., AG biomass divided by two or four) to obtain incorporated AG nitrogen and phosphorus (in milligrams of TN and TP).

Data analyses

Extreme high winds on December 8, 2018 disturbed 12 of the bucket replicates in the temporary outdoor greenhouse, eliminating them from the experiment. This unbalanced design required macrophyte response data to be analyzed using a linear mixed effects model comparison to determine the most parsimonious models for each of the 12 response metrics (wild celery metrics: RBF, biomass allocation, divided leaf area, new shoot production, AG-incorporated TN, AG-incorporated TP; hydrilla metrics: RBF, biomass allocation, average stem length, average number of branches produced, AG-incorporated TN, AG-incorporated TP). Additionally, differences in light regimes among blocks (described in *Abiotic environment*) warranted the inclusion of a random block effect into the correlation structure of all response variable models, to partition the variance related to this factor.

Using a step-down procedure (Zuur et al., 2009), we constructed our starting models to include all fixed experimental factors (macrophyte composition, salinity, and nutrient enrichment, three two-way interactions and one three-way interaction) and grid column placement (block) as a random effect. Models were fit using R package "nlme" (Pinheiro et al., 2019) in R statistical software (v. 3.6.1) with factor selection determined by LME model fit comparisons using maximum likelihood ratio testing (LRT) (R Core Team, 2019) with an accepted α of 0.05, supported with corrected Akaike information criterion (AIC_c). LRT is also used to explain the likelihood of selected model fit for the specific response data, as a relative estimate of effect. Data residuals were checked for diversion from assumptions with Q–Q plots. To improve model testing accuracy with reduced replicate sample size (4–6 replicates per treatment), models where factor selection was based on LRT p values between 0.05 and 0.02 were resampled with a bootstrapping procedure of 10,000 iterations. Model factors where the bootstrapped confidence interval (CI) did not include zero were not considered a significant influence on the model and therefore removed from final model selection.

Nutrient incorporation data analysis of end-experiment samples of wild celery and hydrilla was conducted with a Welch's ANOVA with Games-Howell post hoc contrasts, due to limitations on tissue material necessary for the amount required for carbon, nitrogen, and phosphorus analysis. The accepted α for the Welch's ANOVA and Games-Howell post hoc contrasts was set at 0.05.

RESULTS

Response variable model construction

For response variables with heteroscedasticity among treatments, the model was constructed to include the appropriate variance structure and retained in the model if model fit was improved (Appendix S1: Table S1). Fixed effect structures of final models, AIC, Bayesian information criterion, and loglikelihood values are summarized in Appendix S1: Table S2. The model variance related to the random effect of block is reported as the intraclass correlation coefficient (ICC), summarized with random effect intercept and residual values in Appendix S1: Table S1. All experimental results are reported as average values (±SD).

Biomass production and allocation responses

At the end of the experiment, RBF indicated that wild celery was negatively affected by salinity treatments, averaging 0.49 (±0.1) in zero salinity treatments, 0.46 (±0.1) in 4 salinity, and 0.35 (±0.1) in 8 salinity (Figure 2, Table 1, and Appendix S1: Table S3). Salinity was the only fixed factor retained in the final model of wild celery RBF response, and this model was 13 times

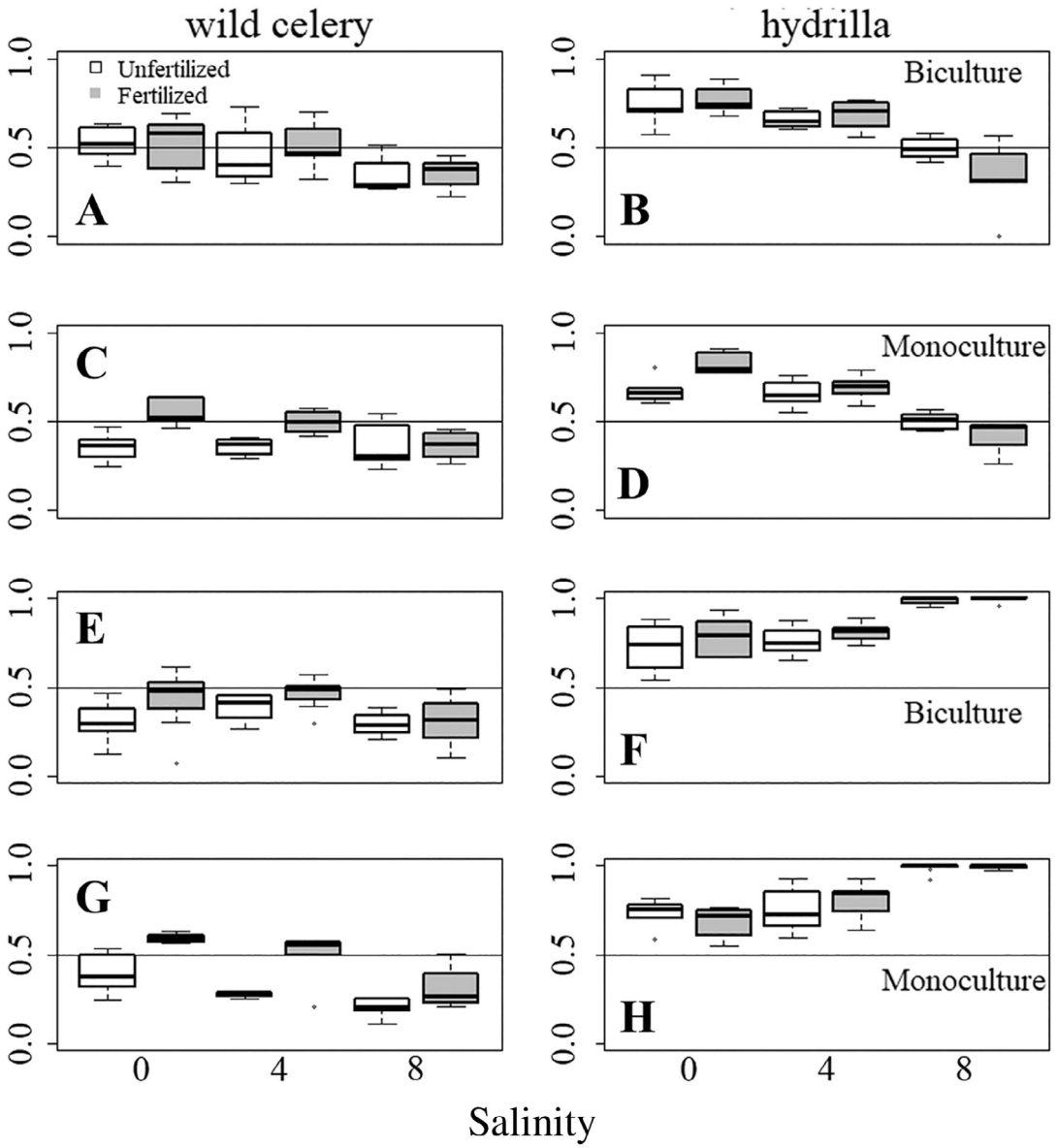


FIGURE 2 Biomass production and allocation responses for wild celery (A, C, E, and G) and hydrilla (B, D, F, and H) following 19 weeks of treatment exposure, beginning in November 2018. Relative biomass fraction for wild celery in biculture treatments (A) and monoculture treatments (C), and hydrilla in biculture (B) and monoculture treatments (D). An indicator line is set at 0.5, such that a gain in biomass at end of experiment is indicated by values above the indicator line, and biomass loss below the line. AG biomass proportion for wild celery biculture treatments (E) and monoculture treatments (G), and hydrilla in biculture (F) and monoculture treatments (H). An indicator line is set at 0.5, such that greater allocation toward AG biomass is indicated by values above the line, and greater allocation toward BG biomass below the line.

more likely to explain the data compared with a model with only the random effect of block. Wild celery RBF response model selection did not indicate any influence from competitive interaction with hydrilla. For hydrilla RBF, the interaction of salinity and fertilizer affected RBF such that it was enhanced from 0.71 (±0.1) at unfertilized, 0 salinity to 0.8 (±0.07) with fertilizer addition. However, fertilizer had little influence on RBF in 4 salinity (0.7 ± 0.07, compared with 0.66 ± 0.07 in unfertilized 4 salinity) and negative influence on RBF in 8 salinity (0.36 ± 0.16, compared with 0.5 ± 0.05 in unfertilized 8 salinity) (Figure 1, Table 1, and Appendix S1: Table S3). The final model with an interaction of fertilizer and salinity as a fixed factor is 80.5 times more likely to explain hydrilla RBF than a model with only the random block effect. Hydrilla RBF model selection did not indicate any influence from competitive interactions with wild celery.

TABLE 1 Final model selection of restricted maximum likelihood fixed effect statistical summaries.

Dependent variable	Fixed effect	Value	SE	df	*t*	*p*
Wild celery RBF	Intercept	0.5	0.03	58	20.0	<0.0001
	Salinity	−0.02	0.004	58	−3.8	0.004
Hydrilla RBF	Intercept	0.72	0.025	65	28.8	<0.0001
	Salinity × fertilizer	−0.027	0.006	65	−3.8	0.0004
Wild celery AG biomass	Intercept	−0.04	0.04	55	17.7	<0.0001
	Fertilizer	0.13	0.03	55	−4.3	0.0001
	Competition × salinity	−0.02	0.009	55	2.3	0.0268
Hydrilla AG biomass	Intercept	0.7	0.017	66	40.9	<0.0001
	Salinity	0.03	0.003	66	9.3	<0.0001
Hydrilla stem length	Intercept	14.49	0.74	64	19.56	<0.0001
	Salinity × fertilizer	−0.89	0.22	64	−4.03	0.0002
Hydrilla new branches	Intercept	6.14	1.11	63	5.52	<0.0001
	Competition	2.1	0.88	63	−2.39	0.0197
	Salinity × fertilizer	−0.64	0.28	63	21.5	0.0246
Wild celery divided leaf area[a]	Intercept	1.5	0.08	57	−2.3	<0.0001
	Salinity	−0.07	0.01	57	−5.6	<0.0001
	Fertilizer	0.4	0.08	57	4.75	<0.0001
Wild celery new shoots	Intercept	2.7	0.22	59	12.1	<0.0001
Wild celery AG-incorporated TN	Intercept	2.39	0.46	36	5.16	<0.0001
	Salinity	−0.18	0.05	36	−3.35	0.0019
	Fertilizer	1.09	0.38	36	2.88	0.0068
Hydrilla AG-incorporated TN	Intercept	2.83	0.34	38	8.4	<0.0001
	Salinity	−0.17	0.05	38	−3.4	0.0015

Abbreviations: AG, aboveground; RBF, ratio biomass fraction; TN, total nitrogen.
[a]Indicates $\log_{10}$ transformation of dependent variable.

Fertilizer influenced wild celery biomass allocation with enhanced proportional allocation toward AG biomass by 0.13 in nutrient addition treatments (Figure 2, Table 1, and Appendix S1: Table S3). Biomass allocation of wild celery was further influenced by an interaction between competition and salinity, such that the trend of increasing BG biomass allocation in higher salinities was more prevalent in wild celery monocultures (AG proportion values of 0.49 ± 0.13, 0.37 ± 0.13, and 0.25 ± 0.11 for 0, 4, and 8 salinity, respectively) than when in competition treatments with hydrilla (AG proportion values of 0.37 ± 0.17, 0.44 ± 0.1, and 0.31 ± 0.13 for 0, 4, and 8 salinity, respectively) (Table 1 and Appendix S1: Table S3). A model with fertilizer as a fixed factor is 13.2 times more likely to explain wild celery biomass allocation than a random effect only model. Furthermore, the final model that includes both fertilizer and the interaction of competition and salinity is 22.3 times more likely than only the fixed effect of fertilizer.

Salinity influenced hydrilla biomass allocation (Figure 2 and Table 1), such that allocation toward BG biomass decreased with each increased salinity treatment (AG proportion values of 0.73 ± 0.12, 0.79 ± 0.10, and 1.00 ± 0.01 for salinity treatments of 0, 4, and 8, respectively; Table 2). The final model with salinity as a fixed effect is 58.3 times more likely to explain hydrilla biomass allocation than a model that only accounts for the random block effect.

Morphological and architectural responses

Wild celery new shoot production values were not adequately explained by any of the treatment factors (Figure 3, Table 1, and Appendix S1: Table S3). Wild celery leaf area per shoot increased with fertilizer addition from 27.17 (±25.6) to 55.7 (±38.8) cm^2 per shoot (Figure 3, Table 1, and Appendix S1: Table S3) but responded negatively at higher salinity treatments, from 55.0 (±37.8) cm^2 per shoot at 0 salinity to 50.9 (±37.7) cm^2 per shoot at 4 salinity and 17.7 (±13.8) cm^2 per shoot

TABLE 2 Comparison of experimental setup conditions and simplified results of wild celery and hydrilla competition experiments.

Experimental conditions tested (study citation)	Facility type	Source of biological material	Shoot type and planting density	Time elapsed (weeks)	Season	Competitive response evidence?
Sediment nutrient limitation (Steward, 1991)	Outdoor mesocosms	Hydrilla—wild sourced from Potomac River monoecious variety Wild celery—wild sourced from Davie, FL	Hydrilla—mature apical sections (10 cm) Wild celery—mature shoots (5 cm) Density: Two plants per pot of unknown size	~7	Late summer	Hydrilla—intraspecific competition observed (total biomass)
Sediment nutrient enrichment with limiting or saturated light levels (Smart et al., 1994)	Climate-controlled indoor greenhouse facility	Hydrilla—greenhouse culture Wild celery—commercially sourced from Wisconsin	Hydrilla—mature apical sections (15 cm) Wild celery—immature winter buds Density: 400 shoots m^{-2}	8	Mid/late summer	Hydrilla—interspecific competition observed (root biomass) with enrichment Wild celery—interspecific competition observed (density) under base conditions; (biomass) under high light
Sediment nutrient enrichment (Van et al., 1999)	Outdoor mesocosms	Hydrilla—stock culture originally sourced from Davie, FL dioecious variety Wild celery—stock culture originally sourced from Davie, FL	Hydrilla—mature apical sections (15 cm) Wild celery—mature shoots of unknown leaf length Density: 19–112 shoots m^{-2}	16	Summer	Hydrilla—interspecific competition observed (biomass) without enrichment Wild celery—interspecific competition observed (biomass) with enrichment
Sediment nutrient enrichment with increased salinity (this study)	Outdoor greenhouse canopy	Hydrilla—wild sourced from Lake Apopka, FL dioecious variety Wild celery—wild sourced from Lake Hartridge, FL	Hydrilla—mature apical sections (15 cm) Wild celery—mature shoots (15 cm) Density: ~57 shoots m^{-2}	~19	Winter/spring	Wild celery—competitor presence effect observed (biomass allocation), but only for increased salinity response

at 8 salinity (Figure 3, Table 1, and Appendix S1: Table S3). A model with fertilizer as a fixed effect is 11.7 times more likely to explain the leaf area per shoot response data than a model with only the random block effect. The final model that includes both fertilizer and salinity is 27 times more likely than a model with only fertilizer as a fixed effect. Leaf area per shoot responses did not show any effect from competition with hydrilla.

Hydrilla apical stem lengths were influenced by an interaction of salinity and fertilizer, such that fertilizer addition enhanced stem lengths from 14.1 (±3) to 21.4 (±4.8) cm at 0 salinity, with little to no evidence of fertilizer influence at 4 salinity (13.8 ± 2.1 cm, compared with 13.5 ± 1.7 cm for unfertilized 4 salinity treatment) or at 8 salinity (10.5 ± 2.9 cm, compared with 10.2 ± 2.6 cm for unfertilized 8 salinity treatment) (Figure 4, Table 1, and Appendix S1: Table S3). The final model with an interaction of salinity and fertilizer is 60.8 times more likely to explain stem length than a model with only the random effect of block. The interaction of salinity and fertilizer also influenced the average number of branches hydrilla produced, such that fertilizer addition increased the number of new branches from 4.9 (±2.9) to 11.5 (±7.9) in 0 salinity treatments, but with little to no evidence of fertilizer influence in either 4 salinity (12.9 ± 3.8, compared with 10.2 ± 6.3 for unfertilized 4 salinity treatment) or 8 salinity treatment (9.9 ± 5.2, compared with 9.4 ± 4.8 for unfertilized 8 salinity treatment) (Figure 4, Table 1, and Appendix S1: Table S3). The final model with an interaction of salinity and fertilizer is 21.3 times more likely to explain branch production than a model with only the random effect of block.

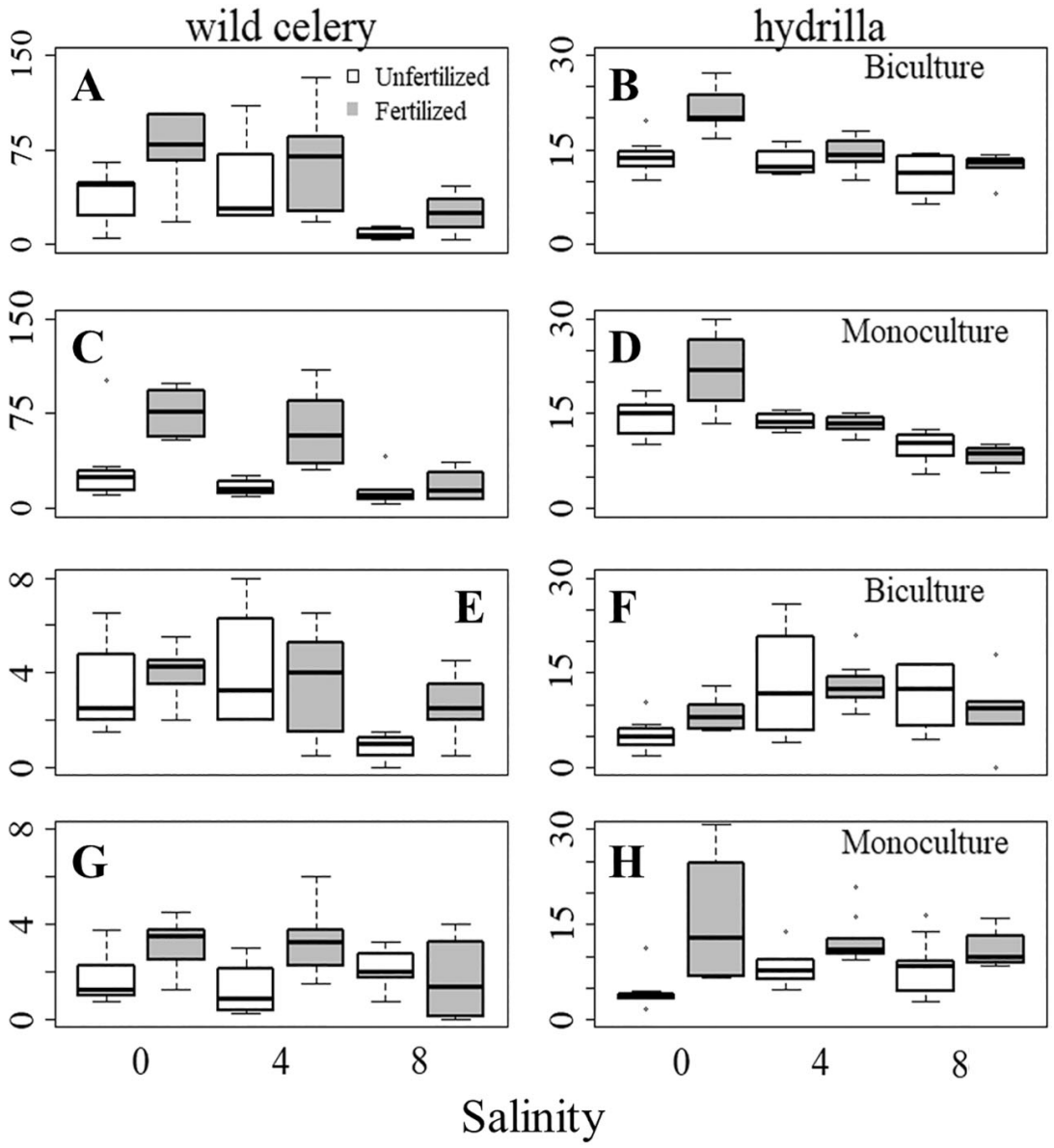


FIGURE 3 Morphological and architectural responses for wild celery (A, C, E, and G) and hydrilla (B, D, F, and H) following 19 weeks of treatment exposure, beginning in November 2018. Total leaf area per replicate divided by number of original shoots planted for wild celery in biculture (A) and monoculture treatments (C). Average stem length for hydrilla in biculture (B) and monoculture treatments (D). Number of new shoot growth for wild celery in biculture (E) and monoculture treatments (G). Average number of new branches for hydrilla in biculture (F) and monoculture treatments (H).

Leaf nutrient incorporation

Fertilizer influenced incorporation of nitrogen into wild celery leaf biomass ($Fs_{1,42.4} = 142.9$, $p = 3.7 \times 10^{-15}$), averaging 2.8 (±0.5) mg nitrogen in fertilized wild celery leaf biomass and 1.3 (±0.4) mg nitrogen in unfertilized leaf biomass. Phosphorus incorporation into wild celery leaves, however, did not have a detectable fertilization signature ($Fs_{1,10} = 0.17$, $p = 0.69$), averaging 0.6 (±0.2) mg phosphorus in fertilized wild celery leaf biomass and 0.6 (±0.2) mg nitrogen in unfertilized leaf biomass. Hydrilla nitrogen incorporation into AG biomass (stems, branches, and leaves) did not show a fertilization signature ($Fs_{1,41.9} = 0.42$, $p = 0.52$), averaging 2.5 (±0.5) mg nitrogen in fertilized hydrilla AG biomass and 2.4 (±0.7) mg nitrogen in unfertilized AG biomass. Similarly, phosphorus values did not indicate a fertilization signature ($Fs_{1,6.3} = 0.79$, $p = 0.41$), averaging 0.7 (±0.3) mg phosphorus in fertilized hydrilla AG biomass and 0.6 (±0.1) mg phosphorus in unfertilized AG biomass. Molar ratios of carbon to nitrogen averaged 16.0 (±1.4) and 19.9 (±2.1) for fertilized and unfertilized wild celery leaf biomass, respectively, and nitrogen to phosphorus ratios averaged

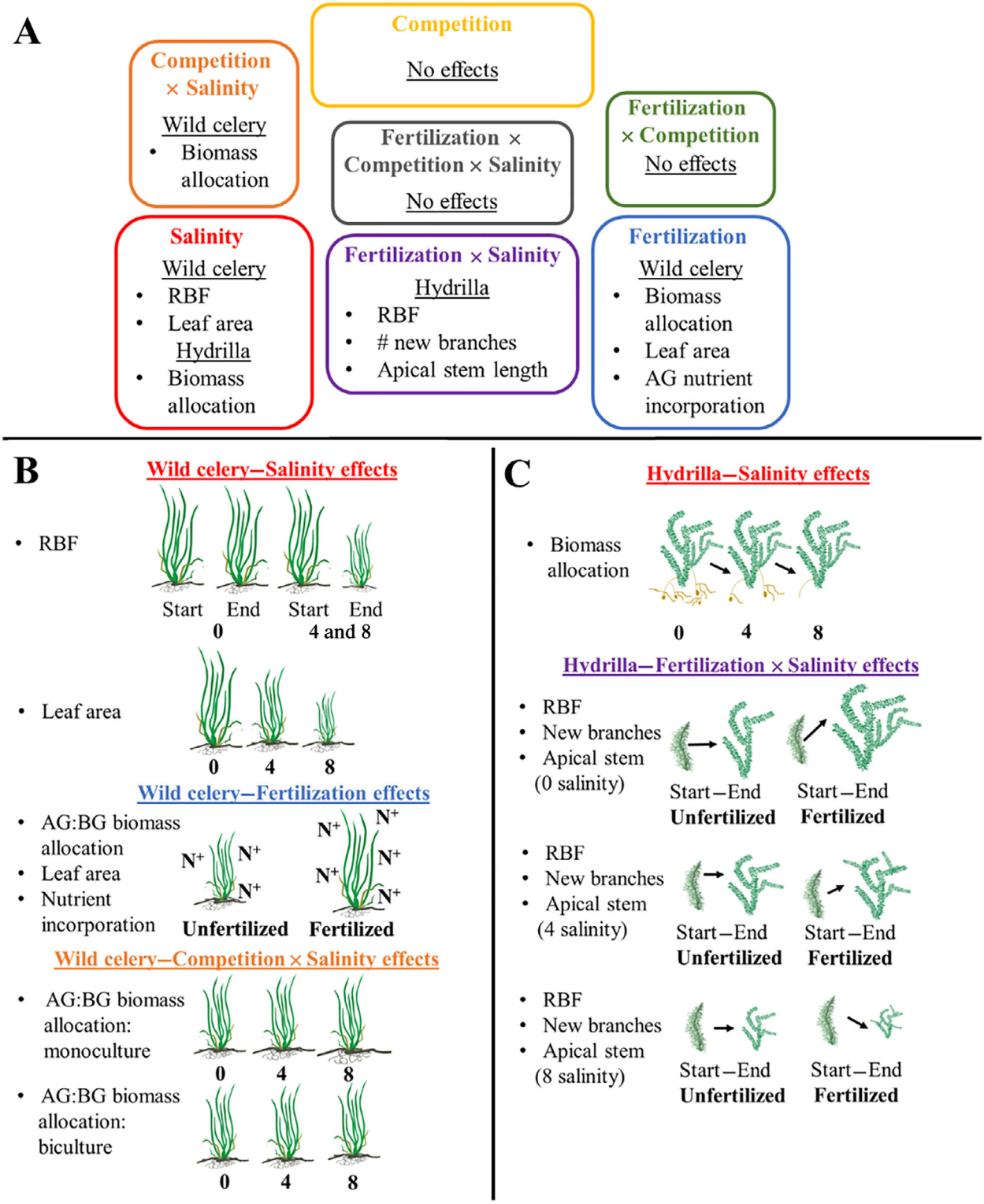


FIGURE 4 Graphic summary of treatment effects detected in macrophyte responses to the main factors and interaction factors tested. Summarized results of all treatment effect comparisons (A) and illustrations of responses of wild celery (B) and hydrilla (C) to treatment factors. AG:BG, aboveground: belowground biomass proportion; N^+, total nitrogen incorporation; RBF, relative biomass fraction.

2.4 (±0.9) and 1.2 (±0.4). Hydrilla molar ratios of carbon to nitrogen averaged 18.8 (±2.1) and 20.3 (±4.4) for fertilized and unfertilized AG biomass, respectively, and nitrogen to phosphorus ratios averaged 2.0 (±1.1) and 2.0 (±0.3) for fertilized and unfertilized AG biomass, respectively.

DISCUSSION

Contrary to expectation, our experimental results (summarized in graphic form in Figure 4) do not indicate superior competitive dominance of hydrilla over native wild celery under conditions of simulated environmental changes. This finding is unexpected specifically because researchers anticipate enhanced invasion potential of hydrilla and competitive dominance over wild celery concomitantly with climate-change-associated disturbances (Zhu et al., 2017). Factors contributing to predictions of hydrilla's competitive dominance include evidence of competitive superiority for limiting nutrient accumulation (Zhang & Liu, 2011), a post-invasion environmental niche shift of the North American invasive genotype (Zhu et al., 2017), and the high degree of somatic mutation (Grajczyk, 2009) driven by rapid clonal growth and high-yield vegetative propagules (Langeland, 1996). We conclude that hydrilla competitive efforts did not benefit from a resource-enhanced environment when inhibited by exposure to salinity. Our results suggest the stress of salinity encroachment into aquatic systems, particularly during a period of low productivity like the subtropical winter season, will have a superior influence on the likelihood of hydrilla dominance by limiting the competitive ability of resource acquisition, incorporation, growth, and storage that typically confers an advantage to hydrilla under nutrient-enriched conditions (Kennedy et al., 2009).

The dearth of strong interspecific interaction responses in this experiment are unique findings that contribute to the various results of previous experimental efforts, which have shown a wide range of responses related to the interplay of these two species. Prior experimental results range from a lack of evidence for interspecific competitive effects under base conditions (unfertilized native sediment, nonstressor; Steward, 1991), to evidence of contrasting competitive effects of wild celery and hydrilla under varying light and sediment fertilization conditions (Smart et al., 1994)—but only when the two species' BG tissues interacted, suggesting BG resource competition drove competitive responses. Furthermore, Smart et al. (1994) found that wild celery became the better competitor when sediments were fertilized under both low and high light conditions. However, this evidence for competitive superiority of wild celery under fertilized sediment conditions differs from a study by Van et al. (1999) that determined hydrilla was the superior competitor under fertilized sediment conditions, whereas wild celery was superior to hydrilla at low sediment fertility conditions. Distinct differences in supporting evidence for strong interspecific interactions across experimental trials (study comparisons summarized in Table 2) likely result from experimental differences in planting densities, origins of biological material, elapsed time of each experiment, and the seasonal period of the experiment. The lack of strong biological interaction responses in this study is particularly relevant in the context of translating experimental results to invasive management strategies as these findings suggest that, regardless of enrichment, hydrilla is likely not a substantive competitor when exposed to salinity stress.

Nutrient and salinity effects on competitive interaction responses

Contrary to expectations, our results provided no evidence for the hypothesis that nutrient addition would enhance the competitive influence of hydrilla on wild celery, thereby reducing wild celery condition (fertilization × competition bubble; Figure 4). Absence of hydrilla competitive influence was possibly due to seasonally diminished productivity of hydrilla that resulted in reduced biomass production and nutrient demands. Although both wild celery and hydrilla remain productive during winter in subtropical climates, it is a seasonally reduced rate of production (Barko et al., 1982). Winter conditions of shorter daylight hours and lower temperatures typically drive declines in macrophyte shoot biomass, canopy height, and density metrics to conserve stored resources (Barko & Michael Smart, 1981; Bartleson et al., 2014; Dawes & Lawrence, 1989; Doering et al., 1999; Hillmann et al., 2019; Sutton et al., 1992). This seasonal decline is followed by a period of rapid growth in spring months that is essential in the race to gain access to quickly diminishing limited resources upon the return of more amenable conditions (Nichols & Shaw, 1986).

The lack of a nutrient pulse signature in hydrilla AG tissues, plus the presence of algal blooms in fertilized aquaria, suggests hydrilla's competition for nutrient resources was uncharacteristically low during this period rather than these resources being unavailable for acquisition by hydrilla. Seasonality has been shown to affect nutrient content of AG tissues of hydrilla (Madsen & Owens, 1998) and to be highly relevant to hydrilla vegetative growth, with substantial declines in growth rates and biomass allocation shifts toward BG tissues during colder periods in subtropical climates (Haller et al., 1976; Mony et al., 2007). BG tissues are necessary for hydrilla to form the tubers required for regrowth following a dieback and the carbohydrate storage organs used for boosted spring growth (Owens & Madsen, 1998). The trade-off for investing resources into BG material at the expense of AG tissues may be beneficial for hydrilla in the conservation of resources until more amenable conditions arise (Owens & Madsen, 1998). However, this seasonal allocation strategy resulted in minimal nutrient resource

competitive effects with the consequence of greater nutrient acquisition by wild celery during this period, as evidenced by the latter macrophyte's fertilization signature across salinity treatments in nutrient samples, growth, morphology, and biomass allocation (fertilization bubble; Figure 4). In the absence of a salinity stressor, the shift toward rapid seasonal production by hydrilla in spring (Owens & Madsen, 1998) would likely overcome this temporary decline in resource competition, but our experimental results suggest salinity encroachment during a subtropical winter season could limit the carbohydrate storage available to hydrilla for boosted spring regrowth (detailed further in the section *Nutrient and salinity effects on noncompetitive responses*).

Response data for salinity effects did not support the hypothesis that salinity additions would indirectly enhance wild celery responses through competitive release from the less tolerant hydrilla (competition × salinity bubble; Figure 4). However, we did find novel evidence that the biomass allocation strategy of wild celery in response to increased salinity was altered in the presence of hydrilla. In wild celery monoculture treatments, allocation between AG and BG biomass shifted toward more BG tissues with increased salinity, but in biculture treatments biomass ratios were more conserved across salinity. An allocation shift away from AG photosynthetic material is a previously measured wild celery response to salinity stress (French & Moore, 2003), possibly as a resilience strategy to reduce the carbon burdens of these tissues during stress exposure (Koch et al., 2007; Touchette & Burkholder, 2000). By maintaining biomass allocation proportions in the presence of hydrilla across salinity treatments, wild celery may be exhibiting signs of a trade-off between interspecific competition for resources and abiotic stress resistance. In this context, maintaining the ratio of biomass allocation to both AG and BG tissues would be a competitive strategy that wild celery employs and maintains despite increasing stress addition. Possible benefits of this strategy under the experimental conditions of this study can only be speculated, but a more relevant question for future investigation is whether the same strategy would be adopted by wild celery under increased salinity conditions in higher productivity seasons—and the implications for production, growth, and storage.

Finally, we found no evidence to support the hypothesis that wild celery competitiveness is only enhanced by nutrient addition when competing at higher salinities (fertilizer × competition × salinity bubble; Figure 4). We found no evidence that biculture wild celery was limited by hydrilla competitive interactions at 0 and 4 salinities, therefore the benefit of nutrient addition to biculture wild celery was consistent across salinity treatments. Lack of evidence for competitive dominance by hydrilla in 0 and 4 salinity treatments with nutrient addition was possibly driven by temperature- and solar-related seasonal declines in growth rate and nutrient demands (Mony et al., 2007), production declines related to salinity stress (Twilley & Barko, 1990; Xia et al., 2020), or a combination of both seasonal and salinity stress factors.

Nutrient and salinity effects on noncompetitive responses

An interaction effect of fertilization with salinity on hydrilla RBF, apical stem length, and branch production provided evidence that additional nutrients in salinity 4 and 8 treatments did not alleviate salinity stress-driven production inhibitions (fertilization × salinity bubble; Figure 4). This lack of compensation for salinity stress likely ultimately hindered a valuable competitive tactic that has been shown to simultaneously allow access to surface light while restricting light to competitors (Haller & Sutton, 1975; Steward, 1991). Furthermore, salinity alone had an overwhelming influence on wild celery production and morphology, and hydrilla biomass allocation (salinity bubble; Figure 4), despite the notable differences in salinity tolerances between the two species. Previous research that examined salinity effects on wild celery did not find low-concentration salinity effects on these parameters (Doering et al., 1999; Frazer et al., 2006; Martin & Valentine, 2012; Twilley & Barko, 1990), suggesting the responses measured here are possibly related to a synergistic interactive effect of increasing salinity and low temperatures over the experimental period (Appendix S1: Figure S1). Although speculative, this point is supported by research on shoots native to South Florida that determined growth in freshwater treatments halted at 13°C but shoots in treatments at a salinity of 10 indicated a minimum growth threshold at 17°C (Bartleson et al., 2014).

Thus, despite higher salinity tolerances than hydrilla, wild celery may still be impacted by salinity during periods of vulnerability, such as the subtropical winter season during which our experiment occurred. A caveat to our speculation is that both the hydrilla and wild celery shoots used in this study originated from lakes that do not experience saltwater intrusion, and that plants from habitats that are subjected to occasional salinity encroachment may have a higher salinity tolerance threshold. Unlike the consistent reports of salinity limitations for the North American invasive strain of hydrilla (Haller et al., 1974; Hillmann et al., 2019; Shields et al., 2012; Twilley & Barko, 1990), reported tolerance thresholds for wild celery have been highly variable in the literature, ranging from as low as approximately 6.6 (Haller et al., 1974) up to salinities greater than 15 (Kraemer

et al., 1999), suggesting wide physiological variability in salinity tolerance thresholds among wild celery populations. In this study, salinity treatments for both wild celery and hydrilla showed declines in production and shoot size in addition to shifted biomass allocation strategies, suggesting impacts to production capabilities or soluble carbohydrate reserves (Kraemer et al., 1999; Madsen & Owens, 1998). Wild celery RBF values for salinity treatments indicate biomass declines from beginning to the end of experiment, although new shoot number was not affected by salinity. Therefore, ramet expansion under combined effects of low temperatures and salinity stress may have come at the expense of stored resources (Titus & Adams, 1979). For hydrilla, increased salinity drove declines in allocation of resources to BG tissues, likely resulting in limitations on spring growth capabilities that generally rely on carbohydrate resources stored in BG storage organs (Madsen & Owens, 1998). We speculate that such a limitation could result in a future competitive disadvantage for hydrilla at the start of the growing season, even at salinity levels within hydrilla tolerance, if competing with other macrophytes that are more tolerant to salinity encroachment (Twilley & Barko, 1990).

CONCLUSION

Although our results gave no indication of competitive dominance of either species during the experimental period, we found evidence that supports the notion that the competitive influence of a highly invasive species in a higher resource environment can depend strongly on mitigating factors such as stressor presence. We speculate that competitive capabilities of hydrilla were likely diminished during the low production season and, despite nutrient enrichment, further inhibited by an added salinity stressor. Physiological limitations caused by salinity offset the competitive strategies typically employed by hydrilla, such as vertical growth and branching, to outcompete other macrophytes for resources. Our findings suggest that hydrilla invasiveness may be weakened by saltwater intrusion, particularly if this exposure occurs in conjunction with a seasonal period of low production. This hypothesis is supported by observations in the lower Chesapeake Bay system, where periodic salinity intrusions combined with low productivity conditions during the critical early growing season led to subsequent declines in hydrilla dominance (Shields et al., 2012). However, if competitive interactions prior to a salinity intrusion resulted in hydrilla vastly outcompeting wild celery shoots for resources, both species may become overstressed by salinity exposure and decline. Furthermore, a severe enough reduction in macrophytes competing for resources could facilitate a state shift to opportunistic filamentous algae or cyanobacterial mat dominance (Heffernan et al., 2010; Hudon et al., 2014). Such transitions to algal and cyanobacterial mat dominance are believed to have occurred following hurricane-driven extreme saltwater encroachment events that severely diminished estuarine river macrophyte stands dominated by hydrilla (Evans et al., 2007; Frazer et al., 2006). Therefore, to reduce the likelihood of such a state shift and loss of all SAV ecosystem functions, it is essential to support wild celery production by ensuring water quality during the growing season exceeds the species' light environment requirements.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

Data (McDonald et al., 2022) are available from Figshare: https://doi.org/10.6084/m9.figshare.19067432.v1.

ORCID

Ashley M. McDonald https://orcid.org/0000-0002-0569-4334
Charles W. Martin https://orcid.org/0000-0001-9240-3824
Carrie R. Adams https://orcid.org/0000-0001-7707-7506
Laura K. Reynolds https://orcid.org/0000-0002-4498-4641

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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