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Full Length Research Paper

Leaf vegetables for use in integrated hydroponics and aquaculture systems: Effects of root flooding on growth, mineral composition and nutrient uptake

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In recirculating aquaculture and hydroponics systems, the waste products from fish production are used to produce vegetables or other crops of economic value, and the water is recirculated to the fish tanks. We studied growth, productivity and nutrient uptake of four leaf vegetable species (*Lactuca sativa*, *Ipomoea aquatica*, *Brassica rapa* var. *chinensis* and *Brassica rapa* var. *parachinensis*) in a controlled growth experiment with three root flooding treatments (drained, half-flooded and flooded) to assess their preferred hydroponic growth requirements, biomass production and nutrient removal capacities. Growth of the two Brassica varieties was clearly best at drained root conditions, while *L. sativa* and *I. aquatica* grew best with half-flooded and flooded roots. *I. aquatica* took up 3 times more N, P and K per plant than *L. sativa*, and 4 to 6 times more than the two Brassica varieties. At a plant density of 30 plants/m^2, *I. aquatica* produced 146 g DW/m^2 aboveground biomass during a 30-day cultivation period containing 2.8, 0.9 and 6.8 g/m^2 of N, P and K, respectively. *L. sativa* produced 115 g DW/m^2 of aboveground DW during a 60-day cultivation period, containing 2.2, 0.6 and 4.6 g/m^2 of N, P and K, respectively. The two Brassica varieties produced much less aerial biomass (50-54 g DW/m^2 during a 60-day period). Both *I. aquatica* and *L. sativa* are promising species to be included in integrated hydroponic and aquaculture facilities, with *I. aquatica* showing the most promise because of its higher growth and nutrient uptake capacity.

Key words: *Lactuca sativa*, *Ipomoea aquatic*, *Brassica rapa*, phytoremediation, lettuce, choy sum, water spinach, pok choi.

INTRODUCTION

The aquaculture in the Mekong River Delta of Vietnam consists largely on extensive culture systems such as earthen ponds, where water quality is largely maintained within acceptable limits through frequent water exchange (Nhan et al., 2008). This farming practice imposes access to plenty of good-quality river water, which with the current expansion of the aquaculture industry, is becoming a limiting resource. Furthermore, the discharge of untreated aquaculture water contaminates rivers and spreads infections to downstream fish culture ponds, where it often cause disease outbreaks (Thien et al., 2007). There is therefore an urgent need to minimize the water use and the environmental impact of the aquaculture industry in Vietnam.

Phytoremediation is defined as the use of plants and their associated microbes for environmental cleanup (Pilon-Smits, 2005). One of the phytoremediation processes, in which plants are used to remove contami-

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Abbreviations: **F**, Flooded; **HF**, half-flooded; **D**, drained; **E_h**, substrate redox potential; **FW**, fresh weight; **DW**, dry weight; **IRGA**, infrared gas analyzer; **P_{max}**, light-saturated rate of photosynthesis; **PPFD**, photosynthetic photon flux density; **R_d**, dark respiration rates.

nants from soils or water into harvestable plant biomass, is called phytoextraction. Phytoextraction is used mainly for extracting heavy metals from polluted soils and water (Kumar et al., 1995), but the use of plant uptake and plant-mediated conversions also has great potential for the removal of nutrients from nutrient enriched waters. Recently, there is an increased interest in integrating treatment systems and production systems based on the common-sense approach of conversion of wastes into products.

By integrating these techniques, it is possible to reduce wastes and associated environmental impacts, and at the same time generate an additional crop (Naegel, 1977; Quillere et al., 1995; Rakocy et al., 2006). Recirculating hydroponic systems integrate fish production and plant production, and have been proposed for the control of waste nutrients accumulation from fish culture in a way that consumes less water and produces additional, saleable crops (Ghaly et al., 2005). Water quality is maintained through water treatment and water recirculation through the hydroponic units where some dissolved nutrients are taken up by plants.

Vegetables are candidate plants for use in recirculating hydroponic systems as they grow rapidly in response to the high levels of nutrients in aquaculture water. Different species of vegetables such as ice-lettuce, tomatoes, leaf lettuce, and basil have been successfully grown in aquaculture wastewater (Naegel, 1977; Quillere et al., 1995; McMurtry et al., 1997; Rakocy et al., 2006). Most research and development work have, however, been conducted in northern and western Europe, North America and other industrialized countries, in spite of the fact that the potential applicability of plants is much higher in tropical and subtropical regions because of the more favourable climatic conditions in these regions (Kutty, 1980; van Rijn, 1996).

A main task is therefore to identify useful species for the combined production of plants and fish in recirculating hydroponic systems, and to establish the required growth conditions for the plants. Focus in the present study is marketable vegetables in Vietnam, as these can be associated with an extra income for local aquaculture farmers. Lettuce (*Lactuca sativa* L.), water spinach (*Ipomoea aquatica* Forssk.), pok choi (*Brassica rapa* L. var. *chinensis* L.), and choy sum (*Brassica rapa* L. var. *parachinensis* L.) are popular edible leaf vegetables in tropical countries with a relatively high market value (Palada and Crossman, 1999). Some of the species have previously been grown in hydroponic culture (Naegel, 1977; Li and Li, 2009), but there is only limited information on their hydroponic growth requirements and their nutrient uptake capacity. In the present study, we assessed the growth, productivity and nutrient uptake of the four species in a controlled growth experiment with three root flooding treatments. We provide initial information on the potential applicability of the species in integrating hydroponics and aquaculture systems, and information on their preferred hydroponic growth requirements, biomass production and nutrient removal capacity.

MATERIALS AND METHODS

Experimental setup

A randomized block design consisting of three blocks each with four plant species and three root flooding levels was set up. The plant species were: lettuce (*L. sativa* L.), water spinach (*I. aquatica* Forssk.), pok choi (*B. rapa* L. var. *chinensis* L.), and choy sum (*B. rapa* L. var. *parachinensis* L.). The scientific names are according to Austin (2007), Larkcom (2007) and Dixon (2007). The three root flooding levels were: drained (D), half-flooded (HF), and flooded (F). The experiment was set up and run for 35 days in a growth chamber (Weiss Umwelttechnic, Lindensruth, Germany) with a light:dark cycle of 12:12 h, a temperature of 30:27°C and a relative air humidity of 90:85%. The photon flux density provided by metal halide bulbs (Osram W) in the growth cabinet was approximately 640 µmol/m^2/s PAR at the top of the plants.

Plant materials

Seeds were obtained from a commercial supplier of seeds (Trang Nong Seed Co., Ltd., Vietnam) and were germinated in plastic pots (L x W x H: 55 x 60 x 55 mm) in a mixture of sand and peat. Five seeds were placed in each pot in the growth cabinet and watered daily with tap water. Germination occurred during 5 days and the germination rates (%) of the different species were: *I. aquatica* 87%, *L. sativa* 37%, *B. rapa* var. *chinensis* 97% and *B. rapa* var. *parachinensis* 92%.

The plants were watered daily with a pH 7 nutrient solution prepared in tap water from a commercial fertilizer (Dangro NPK Makro 19-5-19+ MgO, Denmark; final concentration (mM): NO_3-N 8.5, NH_4-N 5.3, P 1.3, K 4.9, Mg 1.2, S 1.2) and a micronutrient solution (Tropica Master Grow, Tropica Aquacare, Aarhus, Denmark; final concentration (µM): K 20, Mg 16, S 28, B 0.4, Cu 0.1, Fe 1.3, Mn 0.7, Mo 0.02, Zn 0.03).

Experimental treatments

After 23 days the seedlings were thinned to a single plant per pot, and 5 similar sized plants of each species were arranged randomly in larger plastic containers (L x W x H: 370 x 270 x 110 mm) comprising the blocks for the flooding treatments. The containers were filled with a 20 mm layer of 8-11 mm diameter gravel to promote drainage from the pots. A 5 mm diameter PVC tube was fitted through a hole in the side of the containers at 20 mm height above the bottom to facilitate drainage and water exchange. In the flooded treatments, the tubes were clamped to keep the water at desired water level. The tube was opened only when the nutrient solution had to be exchanged.

In the flooded (F) treatment, the water level was controlled at the substrate surface of the pots; in the half-flooded (HF) treatment, the water level was kept 30 mm below the substrate surface, and in the drained (D) treatment, the nutrient solution was drained out of the pots 30 min after watering every second or third day. The nutrient solutions were completely exchanged twice a week to ensure adequate nutrients for plant growth. Tap water was added daily to maintain the water level in the F and HF treatments at the desired level. After 3 days of flooding, some of the plants showed symptoms of nutrient deficiency with yellowish leaves. A fertilizer stick (0.85 g: TN 10%, P 2%, K 7% and Fe 0.07%) was therefore

inserted into the substrate of each pot. The deficiency symptoms disappeared after 2 days.

Dissolved oxygen concentrations (OxyGuard Handy Beta, International A/S, Denmark) and pH (PHM 92, Radiometer A/S, Denmark) in the nutrient solutions that were drained from the containers during solution exchange were analysed. A brightened platinum electrode was inserted into the substrate at 30 mm depth in a random specimen of each species at each flooding treatment to measure substrate redox potential (E_h) using a PHM 92 Lab pH meter (Radiometer A/S, Denmark). E_h measurements were done the same day of DO and pH determination but before solution exchange. E_h readings were corrected for the potential of the calomel reference electrode (+244 mV).

Growth

At the initiation of the flooding treatments, 5 representative specimens of each plant species were sampled to determine their initial fresh weight (FW), dry weight (DW), shoot height, roots length and number of leaves per plant. Shoot height was measured and number of leaves was counted on all plants at the initiation of the flooding treatments (day 0) and thereafter at day 4, 17 and 35 when plants were harvested. At day 17, two of the 5 plants in each treatment were harvested to increase space for growth of the remaining plants.

At harvest, green leaves were counted and roots were washed carefully in tap water to remove sand and peat. Plants were then separated into roots, leaves and stems and the fractions weighed and dried at 80°C until constant weight. A sub-sample of 10 randomly selected leaves from each plant was weighed and photocopied to estimate the leaf area. The total leaf area of the plant was estimated from their weight and the area-weight relationship of the sub-sample. Specific leaf area (SLA, m^2/kg DW) was calculated from the total leaf area and the leaf DW.

Photosynthesis and respiration

Photosynthetic gas exchange was measured on an intact young and fully expanded leaf of each individual plant before initiating the flooding treatments and after 35 days of treatment. Measurements were carried out with an ADC LCA-4 infrared gas analyzer (IRGA) equipped with a Leaf Microclimate Control System (ADC BioScientific Ltd., UK). The air-conditioned PLC-4 leaf chamber was placed on a tripod at a fixed position in the growth chamber to ensure stability during readings. The leaf chamber was supplied with ambient air from the outside of the building, and light was supplied from a white halogen source (Portable Light Unit, PLU-002, ADC BioScientific Ltd., UK). Light-saturated rate of photosynthesis (P_{max}) was estimated as the average of 5 readings at a photosynthetic photon flux density (PPFD) of 2000 µmol/m^2/s. Dark respiration rates (R_d) were measured by darkening the leaf chamber by aluminium foil and a black cloth. After the IRGA-readings, the leaf was excised, weighed and its area measured with a Leaf Area Meter (Li-Cor, Model 3100, Inc. Lincoln, Nebraska, USA). Then the leaf was frozen in liquid N_2, lyophilized and its DW measured.

Chlorophyll and minerals

Concentrations of Chl *a* and Chl *b* in the freeze-dried leaf tissues were analyzed after extraction with 96% ethanol and spectrometry according to Wellburn and Lichtenthaler (1984). Total N in plant tissues was analyzed by gas chromatography after combustion of ground plant material in a NA 2000 N-protein analyzer (Fisons Instruments, Italy). Concentrations of K, P, Ca, Mg, Fe and Mn were analyzed by plasma emission spectrometry (ICP-AES, Plasma 2000, Perkin Elmer Instruments, USA) after digestion of 250 mg ground plant material in concentrated HNO_3 (4 mL) and H_2O_2 30% (2 mL) using a microwave digestion system (3000 Anton Paar, Perkin Elmer, USA). Plant nutrient uptake rates (mg/plant/d) were calculated from the nutrient concentrations in the plant fractions at start and harvest, and the biomass production during the 35-day experiment.

Statistical analysis

Data were tested for normal distribution and variance homogeneity (Levene's test) and logarithmically transformed if necessary. Differences between species and flooding treatment effects were identified by analysis of variance (ANOVA) using Type III sum of squares by the software Statgraphics Centurion XV (StatPoint, Inc., USA). As there was no significant block effect ($p>0.05$), data were subsequently analyzed by two-way ANOVA (plant species x flooding treatment). Treatment effects within species were analyzed by one-way ANOVA. Tukey Honestly Significant Differences (HSD) was used to identify significant differences between treatments at the 5% probability level. Redox potentials were analyzed by repeated measures ANOVA using the SPSS package Version 16.0.

RESULTS

Redox potential, dissolved oxygen and pH

The redox potential (E_h) in the D treatment remained positive throughout the experiment and higher than in the HF and F treatments (Figure 1). E_h initially decreased slightly in the D condition, but then increased again to similar levels as at the beginning of the experiment. In the HF and F treatments, E_h generally decreased after 4 days of flooding to negative levels, except in HF *I. aquatica* plants where E_h remained positive until day 21 (Figure 1B). The negative E_h values in the F and HF treatments indicated a reduced substrate environment.

The dissolved oxygen concentrations in the nutrient solutions drained from the experimental containers were generally <1.0 mg/L for the F treatment, <1.5 mg/L for the HF treatment and c. 5 mg/L for the D treatment throughout the experiment (data not shown). The pH of the nutrient solutions from the D treatment averaged 8.16 ± 0.05 (mean ± S.D), while it was lower, 7.31 ± 0.09 and 7.08 ± 0.12 in the HF and F treatments, respectively.

Plant growth and biomass

Flooding significantly affected growth, biomass and physiological characteristics of the plants, and the effects of flooding differed between species as shown by the significant interactions in the ANOVAs (Table 1). Growth and performance of the two Brassica varieties were clearly best at the drained condition, while *L. sativa* grew best in the half-flooded treatment and *I. aquatica* grew-best in the flooded treatment. Three specimens of *B. rapa* var. *chinensis* and four specimens of *B. rapa* var. *para-chinensis* died in the HF and F treatments. At harvest the leaves of the surviving Brassica specimens were yellow and clearly

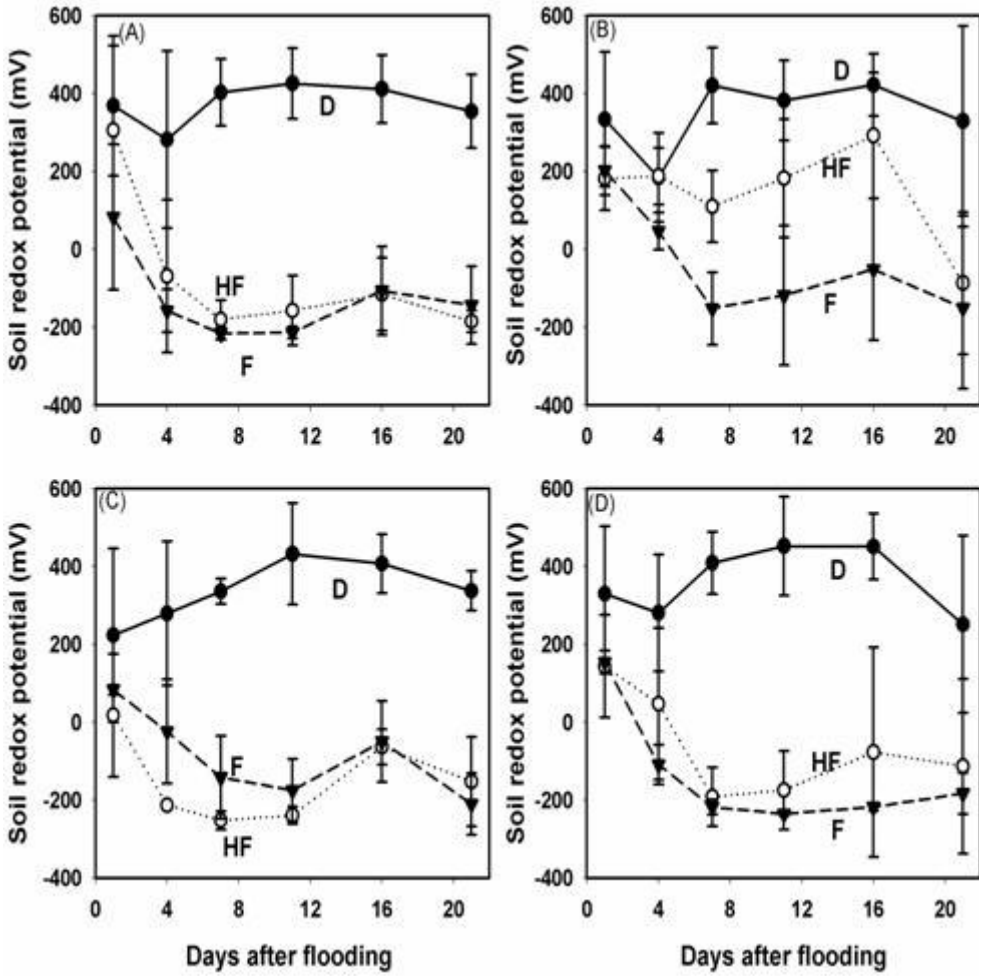

Figure 1. Substrate redox potential of *L. sativa* (A), *I. aquatica* (B), *B. rapa* var. *chinensis* (C) and *B. rapa* var. *parachinensis* (D) grown in drained (●), half-flooded (○) and flooded (▼) treatments over time. Error bars indicate standard deviation of the mean of three replicates.

Table 1. Summary of a two-way ANOVA results showing effects of plant species (*L. sativa*, *I. aquatica*, *B. rapa* var. *chinensis* and *B. rapa* var. *parachinensis*), root flooding (drained, half-flooded and flooded) and interactions between these two factors on growth and physiological parameters of the plants.

	Plant species (df = 3)		Flooding (df = 2)		Interaction (df = 6)		Error (df = 94)
	SS (%)	*p*	SS (%)	*p*	SS (%)	*p*	SS (%)
Number of leaves (per plant)	93.4	<0.001	0.5	0.018	3.0	<0.001	4.6[a]
Root length (cm)	48.9	<0.001	3.6	<0.001	37.3	<0.001	14.7[b]
Shoot height (cm)	76.4	<0.001	3.1	<0.001	12.5	<0.001	9.7[b]
Leaves biomass (g DW/plant)	63.7	<0.001	3.7	<0.001	21.5	<0.001	11.1
Stem biomass (g DW/plant)	66.0	<0.001	8.2	<0.001	17.5	<0.001	8.2
Root biomass (g DW/plant)	62.4	<0.001	6.1	<0.001	19.2	<0.001	12.5
Leaf area (cm^2)	63.3	<0.001	4.5	<0.001	17.4	<0.001	13.9
SLA (m^2/kg DW)	60.0	<0.001	0.1	0.788	14.9	<0.001	20.2
Chl *a* (mg/g DW)	31.7	<0.001	12.8	<0.001	11.9	<0.001	38.2[c]
Chl *b* (mg/g DW)	37.6	<0.001	8.6	<0.001	12.6	<0.001	35.8[c]
P_{max} (µmol/m^2/s)	17.0	<0.001	1.1	0.456	18.2	<0.001	60.7[c]
R_d (µmol/m^2/s)	32.9	<0.001	4.8	0.015	9.6	0.013	47.9[c]

[a] df = 83; [b] df = 89; [c] df = 87

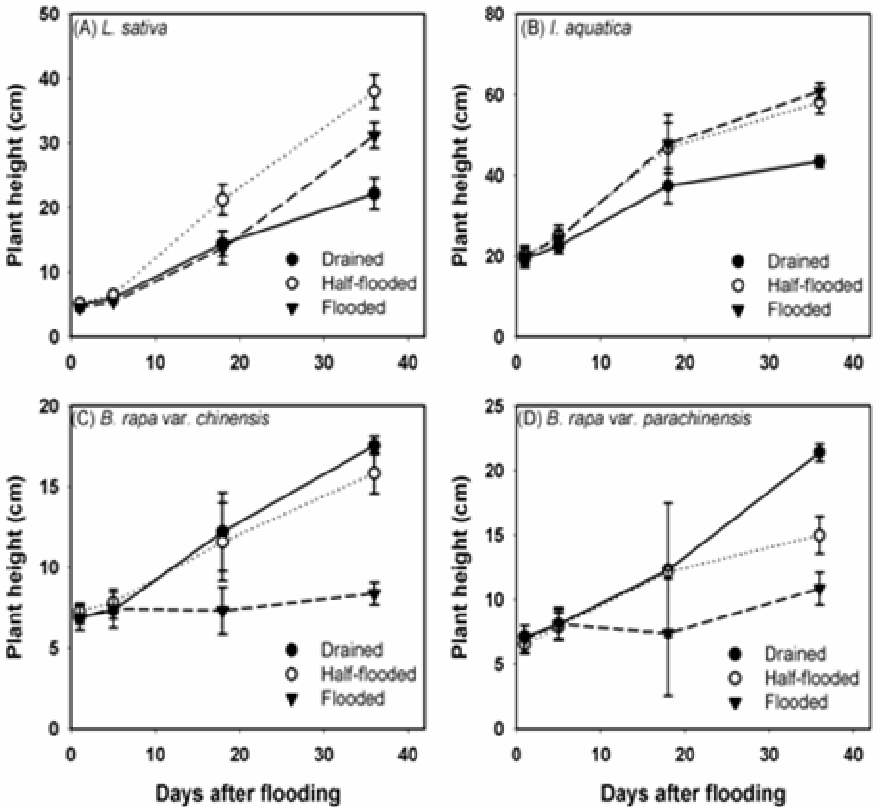

Figure 2. Plant height of *L. sativa* (A), *I. aquatica* (B), *B. rapa* var. *chinensis* (C) and *B. rapa* var. *parachinensis* (D) grown in drained (●), half-flooded (○) and flooded (▼) treatments over time. Error bars indicate standard deviation of the mean of three replicates. Note different scale on y-axes.

and clearly stressed in the F and HF treatments, and the plants had a very superficial root system. *L. sativa* also had a shallow root system at F and HF conditions, but *I. aquatica* grew well and had the largest root system at all flooding conditions.

Flooding significantly affected plant height, and the effects of flooding differed between species (Figure 2). *L. sativa* were tallest at HF condition (Figure 2A), *I. aquatica* were tallest at F and HF condition (Figure 2B), whereas both Brassica varieties were tallest at D condition (Figures 2C and D). The dissimilar effects of root flooding treatments on the species were also evident in other growth parameters (Table 2). *L. sativa* had longer roots, more leaves and a larger leaf area at HF condition than at F and D conditions. *I. aquatica* grew equally well at HF and F conditions and better than at D condition. In contrast, both Brassica varieties grew best at D condition with longer roots, more leaves and larger leaf areas than at F and HF condition (Table 2).

Plant physiological responses

Leaf Chl *a* and Chl *b* concentrations were affected by the flooding treatments, and the effects of flooding differed between the species as shown by the significant interaction in the ANOVA (Table 1). Chl *a* and *b* concentrations in *L. sativa* and both Brassica varieties did not differ significantly between flooding treatments, but for *I. aquatica* the Chl *a* and *b* concentrations were significantly lower in the HF and F plants (Table 2). *L. sativa* had significantly thicker leaves (lower SLA) at D conditions than at F and HF condition. A similar, but less pronounced trend was seen for the other species. Rates of photosynthesis (P_{max}) was significantly higher in flooded than in drained *L. sativa* and *I. aquatica,* but in the Brassica varieties no significant difference was detected. Rate of dark respiration (R_d) was also affected by flooding treatments, but not in a consistent manner (Table 2).

Mineral compositions and nutrient uptake

The concentrations of nutrient elements in the plant tissues were significantly affected by the flooding treatments (Tables 3, 4, 5 and 6). Generally, flooding significantly reduced N, P, K, Ca, Mg, Fe and Mn concentrations

Table 2. Effects of root flooding on growth and physiological characteristics of *L. sativa*, *I. aquatica*, *B. rapa* var. *chinensis* and *B. rapa* var. *parachinensis* (mean ± S.E., n=9).

Species	Treatment	Root length (cm/plant)	Number of leaves/plant	Leaf area (cm^2)	SLA (m^2/kg DW)	Chl *a* (mg/g DW)	Chl *b* (mg/g DW)	P_{max} (μmol/m^2/s)	R_d (μmol/m^2/s)
L. sativa	D	7 ± 1^{a}	21 ± 1^{a}	251 ± 47^{a}	37 ± 2^{a}	7.2 ± 0.4	2.9 ± 0.3	5.8 ± 0.9^{a}	-2.3 ± 0.5
	HF	14 ± 1^{c}	25 ± 1^{b}	830 ± 105^{b}	60 ± 4^{b}	6.8 ± 0.7	2.4 ± 0.3	10.4 ± 1.3^{b}	-2.1 ± 0.2
	F	11 ± 1^{b}	22 ± 1^{a}	522 ± 61^{a}	63 ± 5^{b}	6.8 ± 0.5	2.5 ± 0.2	11.5 ± 0.8^{b}	-1.5 ± 0.2
I. aquatica	D	17 ± 1^{a}	66 ± 4^{a}	475 ± 23^{a}	46 ± 1^{b}	11.1 ± 0.5^{b}	4.3 ± 0.2^{b}	3.2 ± 0.4^{a}	-0.8 ± 0.1^{a}
	HF	41 ± 3^{c}	102 ± 10^{b}	838 ± 56^{b}	38 ± 1^{a}	6.2 ± 0.4^{a}	2.2 ± 0.2^{a}	4.4 ± 0.9^{a}	-1.1 ± 0.1^{a}
	F	39 ± 2^{bc}	98 ± 7^{b}	1006 ± 69^{b}	42 ± 1^{ab}	6.8 ± 0.4^{a}	2.6 ± 0.1^{a}	9.2 ± 1.0^{b}	-1.6 ± 0.2^{b}
B. rapa var. *chinensis*	D	19 ± 1^{c}	22 ± 1^{b}	192 ± 18^{b}	31 ± 2^{b}	11.3 ± 0.7	4.6 ± 0.4	11.2 ± 2.1	-2.9 ± 0.4^{ab}
	HF	13 ± 1^{b}	20 ± 1^{b}	136 ± 25^{b}	23 ± 2^{a}	10.1 ± 0.5	4.1 ± 0.2	9.7 ± 1.3	-3.3 ± 0.3^{b}
	F	5 ± 0^{a}	16 ± 1^{a}	34 ± 10^{a}	24 ± 3^{ab}	9.5 ± 0.4	4.0 ± 0.2	5.9 ± 2.0	-1.7 ± 0.2^{a}
B. rapa var. *parachinensis*	D	19 ± 1^{c}	16 ± 1^{b}	204 ± 30^{c}	26 ± 3^{b}	10.8 ± 0.4	4.4 ± 0.2	11.6 ± 1.2	-2.6 ± 0.4
	HF	7 ± 1^{ab}	14 ± 1^{ab}	72 ± 10^{b}	19 ± 3^{ab}	10.5 ± 0.5	4.9 ± 0.3	10.8 ± 1.9	-3.1 ± 0.3
	F	8 ± 1^{b}	13 ± 1^{a}	18 ± 4^{a}	16 ± 1^{a}	9.1 ± 1.2	3.7 ± 0.5	9.7 ± 1.3	-2.3 ± 0.2

D: Drained; HF: Half-flooded; F: Flooded

[a,b,c] Different superscript letters within columns indicate significant differences within species between root flooding treatments based on a Tukey HSD test ($p<0.05$).

in the aerial parts of the plants (leaves and stems), whereas for roots the response differed between elements and species. Fe and Mn concentrations in roots were consis-tently higher in flooded treatments than in the drained treatments, but for N, Mg, Ca, K and P the effects differed between the species, and no consistent pattern could be observed. The concentrations of N, P, K and Mg were generally higher in leaves than in roots, whereas the concentrations of Fe were up to an order of magnitude higher in roots than in leaves, particularly at flooded conditions. Concentration differences of Ca and Mn between roots and leaves differed between species.

Uptake rates of N, P and K during the treatment period are presented in Figure 4. As roots only constituted from 8 to 30% of the total plant biomass of the species (Figure 3), and as the concentrations of N, P and K mostly were higher in aboveground tissues than in roots, the majority of nutrients were located in the harvest-able aboveground biomass (Tables 3, 4, 5 and 6). Nutrient uptake rates were higher in *I. aquatica* than in the other species, particularly at F and HF conditions, largely as a consequence of the higher biomass production of this species. Nutrient uptake rates in *L. sativa* were not affected by flooding conditions, but for the two Brassica varieties, nutrient uptake rates were low at F and HF con-ditions largely because of the low biomass production (Figure 4).

DISCUSSION

In integrated hydroponic and aquaculture systems, plants are grown in a neutral medium such as vermiculite, sand or coconut fiber in small pots rather than soil. The pots are placed in a tray with the recirculating water from the fish tanks, or may be held in place by a float of Styrofoam that rests directly on the water surface. The degree of saturation of the root substrate depends on the depth in which the plants are placed. Water availability is maximal when the pots are fully flooded, but low oxygen levels around the roots may restrict plant growth. When the pots have the lower portion of the pot flooded, only a small portion of the substrate at the bottom of the pots are saturated, while the upper parts are moist and contain plenty of air.

We found that *L. sativa* and *I. aquatica* grew best at half-flooded and flooded conditions, where as the two Brassica varieties had slower growth

Table 3. Effects of root flooding on mineral concentrations in leaves, stems and roots of *L. sativa* and results of one-way ANOVA (*F-ratios*) (mean ± S.E., n=9, except for the drained *L. sativa* plants where n = 7).

Plant fractions	Flooding	N (mg/g DW)	Mg (mg/g DW)	Ca (mg/g DW)	K (mg/g DW)	P (mg/g DW)	Fe (mg/g DW)	Mn (mg/g DW)
Leaves	D	42 ± 2^{c}	6.3 ± 0.2^{b}	21 ± 1^{b}	61 ± 2^{b}	8.8 ± 0.6^{b}	0.49 ± 0.09^{b}	0.29 ± 0.05
	HF	28 ± 2^{a}	5.2 ± 0.3^{a}	17 ± 1^{a}	51 ± 3^{a}	6.1 ± 0.4^{a}	0.27 ± 0.07^{ab}	0.32 ± 0.03
	F	31 ± 1^{ab}	4.6 ± 0.1^{a}	18 ± 1^{ab}	52 ± 2^{ab}	5.9 ± 0.3^{a}	0.14 ± 0.02^{a}	0.28 ± 0.01
	F-ratios	11.7^{***}	10.3^{***}	4.2^{*}	3.8^{*}	14.9^{***}	7.0^{**}	0.3^{ns}
Stems	D	39 ± 4^{b}	2.9 ± 0.4^{b}	8 ± 1	70 ± 1^{b}	9.7 ± 0.4^{b}	0.09 ± 0.02^{b}	0.10 ± 0.03^{b}
	HF	7 ± 1^{a}	1.6 ± 0.2^{a}	7 ± 2	28 ± 4^{a}	4.9 ± 0.6^{a}	0.03 ± 0.01^{a}	0.04 ± 0.00^{a}
	F	8 ± 1^{a}	1.5 ± 0.1^{a}	6 ± 0	37 ± 4^{a}	4.8 ± 0.4^{a}	0.02 ± 0.01^{a}	0.05 ± 0.00^{a}
	F-ratios	68.5^{***}	12.6^{***}	0.7^{ns}	43.1^{***}	29.5^{***}	10.8^{***}	12.6^{***}
Roots	D	32 ± 4^{c}	1.8 ± 0.1^{a}	33 ± 6^{b}	23 ± 2^{b}	7.0 ± 0.7^{a}	1.17 ± 0.07^{a}	0.26 ± 0.06^{a}
	HF	13 ± 1^{a}	2.8 ± 0.2^{b}	31 ± 3^{b}	15 ± 1^{a}	7.1 ± 0.8^{a}	3.11 ± 0.35^{b}	0.65 ± 0.11^{b}
	F	20 ± 2^{b}	2.8 ± 0.3^{b}	17 ± 2^{a}	32 ± 4^{b}	10.3 ± 0.8^{b}	5.15 ± 0.43^{c}	0.97 ± 0.13^{b}
	F-ratios	20.6^{***}	4.7^{*}	7.9^{**}	12.2^{***}	5.5^{*}	43.6^{***}	17.2^{***}

D: Drained; HF: Half-flooded; F: Flooded.
Number of asterisks (*) indicates level of significance: $^{*}p<0.05$; $^{**}p<0.01$; $^{***}p<0.001$; $^{ns}p>0.05$.
^{a,b,c} Different superscript letters within columns indicate significant differences within species between root flooding treatments based on a Tukey HSD test ($p<0.05$).

Table 4. Effects of root flooding on mineral concentrations in leaves, stems and roots of *I. aquatica* and results of one-way ANOVA (*F-ratios*) (mean ± S.E., n=9).

Plant fractions	Flooding	N (mg/g DW)	Mg (mg/g DW)	Ca (mg/g DW)	K (mg/g DW)	P (mg/g DW)	Fe (mg/g DW)	Mn (mg/g DW)
Leaves	D	44 ± 1^{c}	4.9 ± 0.2^{b}	15 ± 1^{a}	53 ± 2^{b}	7.1 ± 0.6	0.28 ± 0.03^{b}	0.59 ± 0.05^{b}
	HF	29 ± 1^{a}	3.8 ± 0.2^{a}	18 ± 1^{b}	38 ± 2^{a}	5.9 ± 0.3	0.11 ± 0.02^{a}	0.35 ± 0.01^{a}
	F	35 ± 1^{b}	3.8 ± 0.1^{a}	17 ± 0^{b}	43 ± 1^{a}	6.1 ± 0.2	0.09 ± 0.01^{a}	0.33 ± 0.02^{a}
	F-ratios	38.2^{***}	16.7^{***}	5.6^{*}	13.7^{***}	2.5^{ns}	21.3^{***}	27.1^{***}
Stems	D	21 ± 2^{b}	4.8 ± 0.2^{c}	21 ± 1^{a}	65 ± 3^{c}	7.0 ± 0.3^{b}	0.16 ± 0.06^{b}	0.15 ± 0.03^{b}
	HF	8 ± 1^{a}	2.3 ± 0.1^{a}	25 ± 1^{ab}	42 ± 1^{a}	5.4 ± 0.2^{a}	0.01 ± 0.00^{a}	0.05 ± 0.00^{a}
	F	9 ± 0^{a}	2.8 ± 0.1^{b}	29 ± 1^{b}	53 ± 1^{b}	6.5 ± 0.2^{b}	0.04 ± 0.02^{a}	0.06 ± 0.00^{a}
	F-ratios	41.1^{***}	67.6^{***}	8.9^{**}	31.4^{***}	12.8^{***}	14.9^{***}	19.3^{***}
Roots	D	18 ± 1^{b}	2.9 ± 0.1^{b}	11 ± 0	35 ± 4^{c}	5.4 ± 0.4^{b}	0.76 ± 0.08^{a}	0.21 ± 0.03^{a}
	HF	11 ± 0^{a}	2.5 ± 0.1^{a}	12 ± 1	14 ± 1^{a}	3.7 ± 0.2^{a}	2.02 ± 0.20^{b}	0.38 ± 0.05^{a}
	F	11 ± 1^{a}	2.6 ± 0.1^{ab}	13 ± 1	19 ± 1^{b}	4.0 ± 0.1^{a}	3.24 ± 0.61^{b}	0.61 ± 0.06^{b}
	F-ratios	34.6^{***}	5.9^{**}	2.0^{ns}	42.3^{***}	13.3^{***}	34.2^{***}	16.6^{***}

D: Drained; HF: Half-flooded; F: Flooded.
Number of asterisks (*) indicates level of significance: $^{*}p<0.05$; $^{**}p<0.01$; $^{***}p<0.001$; $^{ns}p>0.05$.
^{a,b,c} Different superscript letters within columns indicate significant differences within species between root flooding treatments based on a Tukey HSD test ($p<0.05$).

rates and grew best at drained condition. *I. aquatica* plants in flooded pots produced five-fold higher root biomass than in drained pots, and 4 to 13-fold higher root biomass than the other species. Flooded *I. aquatica* plants produced plenty adventitious roots at the stem bases that promote oxygen diffusion to the roots and facilitate nutrients uptake (Yamamoto et al, 1995). *L. sativa* grew best at half-flooded condition and produced plenty thin roots in the upper moist substrate only.

The roots of *L. sativa* were generally thin and appeared not to be able to grow in the oxygen depleted substrate. The root systems of the two Brassica varieties did not tolerate a completely water saturated substrate as roots rotted when flooded, and the plant did not develop new adventitious roots. Furthermore, aboveground parts wilted and had epinastic growth as a symptom of root anoxia.

A typical and early stress symptom in waterlogged soil is chlorosis as observed three days after the flooding treatments was initiated in all studied species. Similar symptoms has been reported for barley (*Hordeum vulgare*

Table 5. Effects of root flooding on mineral concentrations in leaves, stems and roots of *B. rapa* var. *chinensis* and results of one-way ANOVA (*F-ratios*) (mean ± S.E., n=9).

Plant fractions	Flooding	N (mg/g DW)	Mg (mg/g DW)	Ca (mg/g DW)	K (mg/g DW)	P (mg/g DW)	Fe (mg/g DW)	Mn (mg/g DW)
Leaves	D	51 ± 2	4.3 ± 0.2^{a}	28 ± 2^{a}	49 ± 3^{b}	8.9 ± 0.7	0.25 ± 0.07	0.34 ± 0.03^{a}
	HF	44 ± 5	4.7 ± 0.3^{ab}	31 ± 2^{ab}	38 ± 1^{a}	8.1 ± 0.4	0.15 ± 0.03	0.41 ± 0.03^{ab}
	F	41 ± 3	5.7 ± 0.4^{b}	39 ± 2^{b}	39 ± 4^{ab}	7.8 ± 0.6	0.23 ± 0.02	0.46 ± 0.03^{b}
	F-ratios	1.9^{ns}	5.8**	5.5*	3.9*	1.1^{ns}	1.2^{ns}	3.5*
Stems	D	28 ± 3	3.4 ± 0.2	26 ± 3	62 ± 2^{b}	8.0 ± 0.5	0.05 ± 0.01	0.12 ± 0.03
	HF	21 ± 4	3.6 ± 0.3	29 ± 3	42 ± 3^{a}	7.5 ± 0.6	0.06 ± 0.01	0.08 ± 0.01
	F	31 ± 4	3.5 ± 0.3	27 ± 3	53 ± 3^{b}	8.2 ± 0.8	0.06 ± 0.02	0.07 ± 0.01
	F-ratios	1.9^{ns}	0.2^{ns}	0.4^{ns}	16.1***	0.4^{ns}	0.1^{ns}	1.2^{ns}
Roots	D	18 ± 1	2.0 ± 0.1	17 ± 1^{ab}	16 ± 1	7.8 ± 0.7	2.17 ± 0.17^{a}	0.47 ± 0.04
	HF	18 ± 1	2.4 ± 0.2	14 ± 1^{a}	16 ± 1	6.7 ± 0.7	4.38 ± 0.42^{ab}	0.63 ± 0.06
	F	21 ± 1	2.5 ± 0.2	19 ± 1^{b}	16 ± 2	6.1 ± 0.7	4.78 ± 0.95^{b}	0.58 ± 0.09
	F-ratios	2.8^{ns}	2.3^{ns}	4.5*	0.1^{ns}	1.4^{ns}	3.9*	1.7^{ns}

D: Drained; HF: Half-flooded; F: Flooded.
Number of asterisks (*) indicates level of significance: $^{*}p<0.05$; $^{**}p<0.01$; $^{***}p<0.001$; $^{ns}p>0.05$.
^{a,b} Different superscript letters within columns indicate significant differences within species between root flooding treatments based on a Tukey HSD test ($p<0.05$).

Table 6. Effects of root flooding on mineral concentrations in leaves, stems and roots of *B. rapa* var. *parachinensis* and results of one-way ANOVA (*F-ratios*) (mean ± S.E., n=9).

Plant fractions	Flooding	N (mg/g DW)	Mg (mg/g DW)	Ca (mg/g DW)	K (mg/g DW)	P (mg/g DW)	Fe (mg/g DW)	Mn (mg/g DW)
Leaves	D	43 ± 2	4.7 ± 0.1^{a}	29 ± 2^{a}	48 ± 2^{b}	8.8 ± 0.4^{b}	0.22 ± 0.05	0.41 ± 0.04
	HF	43 ± 3	6.4 ± 0.3^{b}	42 ± 2^{b}	39 ± 4^{ab}	9.4 ± 1.0^{b}	0.39 ± 0.08	0.49 ± 0.04
	F	36 ± 3	6.2 ± 0.4^{b}	40 ± 2^{b}	31 ± 3^{a}	6.2 ± 0.3^{a}	0.25 ± 0.06	0.44 ± 0.03
	F-ratios	2.2^{ns}	10.5***	12.8***	7.1**	8.7**	1.6^{ns}	0.9^{ns}
Stems	D	25 ± 3	3.8 ± 0.1	24 ± 2	54 ± 3^{b}	6.9 ± 0.3^{ab}	0.09 ± 0.02	0.11 ± 0.01
	HF	31 ± 4	4.1 ± 0.3	23 ± 3	48 ± 4^{b}	8.1 ± 0.6^{b}	0.20 ± 0.09	0.10 ± 0.04
	F	23 ± 4	3.3 ± 0.2	31 ± 5	30 ± 4^{a}	5.6 ± 0.6^{a}	0.08 ± 0.02	0.12 ± 0.03
	F-ratios	1.4^{ns}	3.0^{ns}	1.6^{ns}	8.9**	6.3**	1.3^{ns}	0.1^{ns}
Roots	D	19 ± 1	2.1 ± 0.1^{a}	16 ± 1^{a}	20 ± 1	7.1 ± 0.4^{b}	1.78 ± 0.19^{a}	0.41 ± 0.06^{a}
	HF	26 ± 2	3.1 ± 0.2^{b}	18 ± 1^{a}	21 ± 1	7.3 ± 0.6^{b}	3.04 ± 0.67^{ab}	0.46 ± 0.05^{ab}
	F	23 ± 2	2.6 ± 0.3^{ab}	27 ± 2^{b}	19 ± 2	4.9 ± 0.3^{a}	4.21 ± 0.69^{b}	0.65 ± 0.06^{b}
	F-ratios	2.8^{ns}	5.8**	17.5***	0.4^{ns}	7.4**	4.0*	5.0*

D: Drained; HF: Half-flooded; F: Flooded.
Number of asterisks (*) indicates level of significance: $^{*}p<0.05$; $^{**}p<0.01$; $^{***}p<0.001$; $^{ns}p>0.05$.
^{a,b} Different superscript letters within columns indicate significant differences within species between root flooding treatments based on a Tukey HSD test ($p<0.05$).

L. cv. Proctor) grown in waterlogged soil (Drew and Sisworo, 1977). One of the first responses to the flooding of roots in sensitive species is stomatal closure (Pezeshki, 1994) which causes a reduction in the rate of photosynthesis and leads to a reduction of growth and biomass production (Huang et al., 1994; Yamamoto et al., 1995; Boru, 2003; Pociecha et al., 2008). An increased nutrient supply to waterlogged plants have been found to alleviate some of the adverse effects of waterlogging, including the reduction of photosynthetic rate, chlorophyll content and concentration of N in leaves (Huang et al., 1994). This is consistent with the observation in the present study, where the addition of fertilizer sticks into the substrate at day 3 and 16 after flooding, clearly alleviated the stress symptoms.

Plants grown in flooded substrates had higher concentrations of Fe and Mn in roots probably as a consequence of the reduced substrate conditions which led to increased concentrations of Fe^{2+} and Mn^{2+} in the soil solution (Armstrong, 1982). But in the aerial parts of the plants,

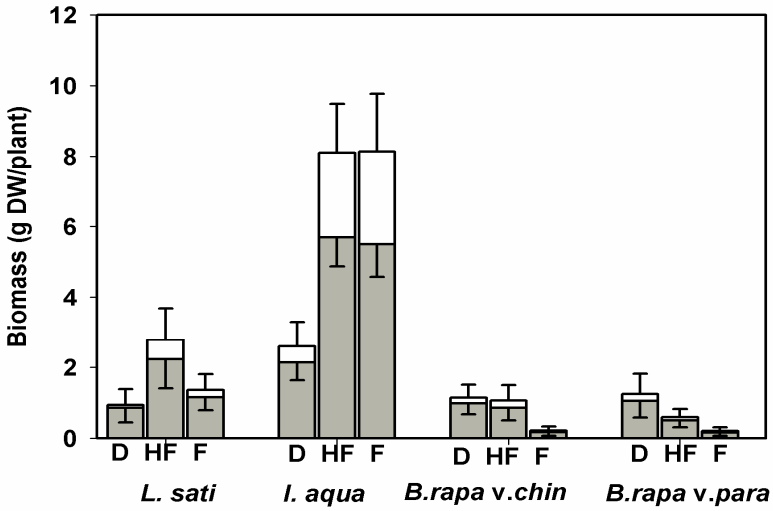

Figure 3. Total plant (white bars) and aboveground (grey bars) biomass of *L. sativa, I. aquatica, B. rapa* var. *chinensis* and *B. rapa* var. *parachinensis* grown in drained (D), half-flooded (HF) and flooded (F) treatments. Error bars indicate standard deviation of the mean of three replicates.

the concentrations of all studied nutrient and mineral elements were generally lower for plants grown in flooded compared to drained substrates. This indicates that the flooding enhanced anoxic conditions in the substrate which reduced the nutrient uptake rate. The capacity of plants to take up nutrients from the aquaculture water is important for determining the size of the hydroponic system in relation to the aquaculture system and the fish production. The plant uptake of nutrients should ideally balance the release of nutrients from the aquaculture. The nutrient uptake capacity of the tested vegetable species was clearly highest for *I. aquatica*, which took up 3 times more N, P and K per plant than *L. sativa*, and 4 to 6 times more than the two *Brassica* varieties. For I. *aquatica* uptake was highest in the HF and F treatments, for *L. sativa* highest in the HF treatment, whereas the two *Brassica* varieties had the highest uptake in the D treatment.

The aerial parts (leaves and stems) of the studied species, which constituted from 70 to 92% of the total plant biomass, are edible and can be marketed. The species can be grown all year round in the tropics and require a relatively short growth period to reach a marketable size. At optimal growth conditions, *I. aquatica* requires 25–30 days to reach a marketable size whereas *L. sativa* and the two Brassica varieties need 60–65 days from sowing. Using the growth and nutrient uptake rates found at the best flooding treatments: *I. aquatica* (F treatment), *L. sativa* (HF treatment) and the two Brassica varieties (D treatment), and assuming a plant density of 30 plants/m^2, it was calculated, that *I. aquatica* during a 30-day cultivation period would produce 146 g/m^2 of aboveground DW (equal to a marketable fresh yield of 1220 g/m^2), containing 2.8, 0.9 and 6.8 g/m^2 on a DW basis of N, P and K, respectively. *L. sativa* would during a 60-day cultivation period produce 115 g/m^2 of above-ground DW (equal to 1150 g FW/m^2), containing 2.2, 0.6 and 4.6 g/m^2 of N, P and K, respectively. The two Brassica varieties would produce much less aerial biomass (50-54 g DW/m^2 during a 60-day period), containing proportionally lower amounts of N, P and K (approximately 2.1, 0.4 and 2.6 g/m^2 of N, P and K, respectively). The plant nutrient uptake rates in integrated hydroponic and aquaculture systems might deviate somewhat from the rates found in the present study depending on the quality of the aquaculture water. However, the calculations show that particularly *I. aquatica*, because of its high growth and nutrient uptake capacity, has the highest potential for inclusion in an integrated hydroponic and aquaculture system. But also *L. sativa*, which has a longer growth cycle and a higher market value, shows some promise. The ease of propagation and handling of the vegetable crop plants, including sensitivity to heavy rain during the rainy season and susceptibility against fungus and insect infestations, remain to be studied.

In conclusion, the growth chamber study indicates that both *I. aquatica* and *L. sativa* are promising species to be included in integrated hydroponic and aquaculture facilities, with *I. aquatica* showing the most promise because of its high growth and nutrient uptake capacity. *I. aquatica* grow best in a completely water-saturated substrate, and *L. sativa* grow well in hydroponic culture with partly flooded roots. In contrary, the two Brassica varieties did not grow well at water-saturated conditions and therefore has less potential. However, further studies must be conducted using real aquaculture water for plant growth in practical full-scale aquaculture system to further assess the feasibility

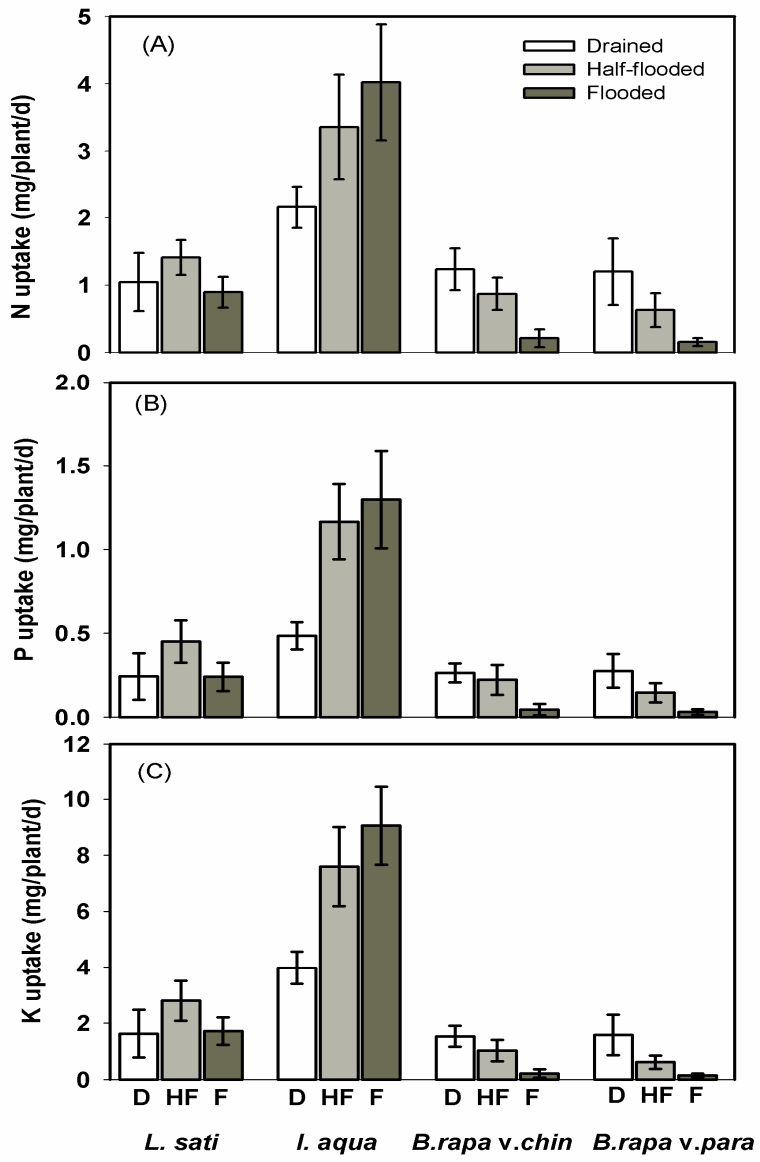

Figure 4. Nitrogen (A), phosphorus (B) and potassium (C) uptake by *L. sativa, I. aquatica, B. rapa* var. *chinensis* and *B. rapa* var. *parachinensis* grown in drained (unfilled bars), half-flooded (light-grey bars) and flooded (dark-grey bars) treatments. Error bars indicate standard deviation of the mean of three replicates.

of using these species in integrated hydroponics and aquaculture systems.

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