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Impacts of water depth and substrate type on *Vallisneria natans* at wave-exposed and sheltered sites in a eutrophic large lake

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ABSTRACT

Water depth, substrate type and wave exposure are major environmental factors influencing the growth and distribution of submerged macrophytes. However, we lack knowledge on the ecological reactions of submerged macrophytes to those factors during an entire growth period in a eutrophic lake with a Secchi depth of less than 50 cm. We investigated the interactive effects of water depth (60, 120 and 200 cm), waves (exposed vs. sheltered) and substrate nutrient level (fertile sludge vs. brown clay) on the survival, growth and morphology of *Vallisneria natans* using a new type of rhizotron in a 252-day field experiment. Plant length, leaf number, ramet number, root length, root number and biomass generally decreased with increased water depth at both wave conditions and in both substrates. When exposed to wave, the biomass of *V. natans* in water at 200 cm depth rapidly declined in both substrates. When sheltered to wave, aboveground biomass at 60 cm water depth first declined to zero with many buried rhizomes remained. The tuber germination rate decreased with increasing water depth during the second year of germination. No plants sprouted at 200 cm water depth except at the case of exposed wave and sludge substrate. Ramet number was influenced most by water depth, followed by aboveground biomass, leaf number, plant length, and survival rate. Wave shelter and brown clay enhanced the impacting strength of the water depth. Wave exposure exhibited no negative influence on survival percentage, whereas sludge had a positive influence on plant survival in deep water after overwintering. Waves negatively affected the rate of increase of plant growth in sludge but positively in clay. The positive joint effects of wave exposure and low-nutrient substrate were mainly on plant length and biomass. Water depth had a negative influence that predominated over substrate and waves regarding plant survival and growth. Moreover, this negative influence may be aggravated by an increasing risk of submersed macrophyte loss caused by decreased belowground growth under high water levels, high wave exposure and high-nutrient conditions. Decreasing the water depth could be an useful measure for submerged macrophyte restoration in freshwater habitats, even in turbid eutrophic water.

1. Introduction

Water depth is a major environmental factor influencing the distribution and growth of submerged macrophytes (Strand and Weisner, 2001; Xu et al., 2016). Human activities are expected to increase the probability of flooding (Blom and Voesenek, 1996), and to alter previous regular water level fluctuation in many lakes (Hu et al., 2010). An increase in water level reduces the light penetration into the lake bottom, especially in eutrophic water. The growth of submerged macrophytes tends to be light limited in most aquatic environments. However, submerged macrophytes exhibit high phenotypic plasticity in response to increasing water depth. Morphological characteristics, such as plant length, ramet number, internodal length and branch number, may react to water depth (Reckendorfer et al., 2013; Zhu et al., 2012). Such adaptations are favoured by differential photosynthetic efficiency at low light intensities (Eusebio Malheiro et al., 2013; Yang et al., 2004). Without plastic changes, a species might not be able to respond adequately to rapidly changing water levels (Clevering and Hundscheid, 1998). It is predicted that global warming will cause extreme events, such as earlier and higher flooding (means the water level increasing earlier and more), to occur more fre-

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quently (Monirul Qader Mirza, 2002), which may have important consequences on plant growth in floodplains because it will lead to a sudden rise of water level.

However, water depth does not affect plant growth in isolation and often influences plant growth in conjunction with other factors, such as mechanical stress from wave action (Madsen et al., 2001; Van Zuidam and Peeters, 2015). The hydrodynamic conditions of waves and currents also-directly and indirectly affect seagrass ecosystems (La Nafie et al., 2012). Macrophytes growing in lakes are frequently subject to hydrodynamic forces resulting from the drag of water passing along the plant. Aquatic plants in moving water experience a force more than 25 times greater than that experienced by terrestrial plant in wind of the same velocity (Denny and Gaylord, 2002). If sufficiently strong, such forces will tend to break the stems or uproot entire plants.

Some studies have shown that organic-rich sediment may be a key factor limiting the growth and distribution of submerged macrophytes in eutrophic shallow lakes (Soana et al., 2012; Yu et al., 2010). In addition, compared to water column nutrients, sediment type has more significant impacts on the growth, nitrogen (N) and phosphorus (P) content of certain plants (Xie et al., 2005). Excessive sediment nutrients and organic matter (OM) are important indicators of shallow lake eutrophication, and may have direct and indirect negative effects on aquatic plant growth (Best et al., 1996; Thiebaut, 2005). Roots are the most important parts of aquatic plants for nutrient uptake and fixing, and primarily occur in the sediment. No long-term culture of submerged plants can be performed successfully without sediments. Therefore, it is important to recognise the influence of sediment type on aquatic plants. Many large lakes have different sediment types with different nutrient levels. However, information about how aquatic plants adjust their root traits to adjust to different sediment environments remains limited (Xie et al., 2007).

Although the individual effects of water depth, substrate type and hydrodynamics on seagrasses have been studied in detail, their interactive effects may be difficult to predict for submerged macrophytes because plant responses to individual stressors are diverse (Xie et al., 2007). Many studies have been conducted on water column with water transparency of more than 1.0 m. However, in the clear-water state in shallow lakes, light penetration through the water column is not influential in regulating submerged vegetation. Currently, most experiments and studies on the growth of submerged macrophytes have covered only a short period (10 days–100 days) of submerged macrophyte life (Jiang et al., 2008). Studies on the growth strategy over the whole one or more life period of submerged macrophytes in a eutrophic lake have rarely been reported, particularly for lakes with <50 cm Secchi depth.

Eel grass, *Vallisneria natans* (Lour.) Hara., is a perennial submerged plant with a wide geographical range that occurs in freshwater lakes, ponds, and rivers. This species produces plagiotropic stolons, which spread horizontally above the ground and form large clonal populations in the field (Xiao et al., 2007). This plant provides food resources and habitats for fish and invertebrates, and has a strong positive influence on water quality. It was chosen for the current study because of its high frequency and adaptive capability in freshwater habitats.

This paper examines the effects of water depth and substrate type on the growth and ramet morphology of *V. natans* under wave-exposed and sheltered case in eutrophic water with a series of in situ experiments. The purpose of the experiments was to (1) investigate changes in the growth and phenotypic characteristics of shoots and roots in response to water depth and substrate type under wave exposure, (2) determine which growth strategy *V. natans* adopts based on its morphological traits, (3) examine plant regeneration and regrowth after winter, and (4) identify the mechanisms that cause a decline in submerged vegetation biomass in shallow eutrophic lakes in a turbid state. The results are expected to contribute helpful information towards enhancing macrophyte restoration projects in freshwater habitats.

2. Materials and methods

2.1. Experimental site and conditions

The experiments were conducted on a floating platform approximately 100 m offshore in Zhushan Bay, located at the northwest part of Lake Taihu, China (Fig. 1). Zhushan Bay has been phytoplankton-dominated zone since 2000. Because of the long-term discharge of OM from the Yincun River and Baidugang River, fertile sludge is present in the sediments, along with brown clay, due to the wind-wave induced erosion of the lake bottom and sediment resuspension. The prevailing wind direction in Lake Taihu is southeast, and thus Zhushan Bay is frequently exposed to strong wind wave. At present, water quality in the bay is worse than class IV standards (National environmental quality standard for surface water, GB3838-2002 of the People's Republic of China). Submerged vegetation covered the majority of Zhushan Bay before 1997. However, by 2012, nearly no submerged plants remained in the bay because of its poor water quality, lake eutrophication and, potentially, the occurrence of increasing water levels in some periods.

In June 2011, a wave-sheltered site was created by constructing a 70-m wave barrier composed of one row of 600 stakes positioned parallel to the shore using a professional pile driving boat (Fig. 1). Wave barriers are effective in reducing wave energy, water velocity and sediment accretion if properly designed and constructed. It reduced wave energy by 70% (Alkhalidi et al., 2015). The wave-sheltered experimental site (the sheltered site) was between the wave barrier and shoreline, 20 m distance from the wave barrier, whereas the wave-exposed experimental site (the exposed site) was in the open water equidistant from the shoreline as the sheltered site. There was no significant difference in water quality between the sheltered site and the exposed site, because the sheltered site was only approximately 80 m from the exposed site. The total N (TN) and total P (TP) concentrations ranged from 2.32 to 5.52 mg L^{-1} and from 0.133 to 0.184 mg L^{-1}, with means of 3.52 and 0.152 mg L^{-1}, respectively (Table 1). The Secchi disc depth was approximately 37.0–76.2 cm, with a mean of 48.8 cm.

2.2. Rhizotrons

Rhizotrons are important instruments to noninvasively study the dynamics of root growth and development of plants through an entire growth cycle (Busch et al., 2004). Rhizotrons have frequently been applied to both terrestrial and wetland plants (Busch et al., 2006), but rarely to submerged macrophytes. A new type of rhizotron was developed to make the traditional rhizotron suitable for the submerged macrophytes (Chinese patent number: 201310731902.8). The rhizotron was in the shape of a cross to increase its stability in water with a strong hydrodynamic disturbance and was made from clear acrylic (Fig. 2), which is one of the most favoured materials for this apparatus because of its low weight and good machinability. It was constructed of 0.4-cm clear acrylic at the front and back and 1.4-cm clear acrylic at the sides and the bottom. Each rhizotron had four arms with same inner cavity dimensions of 25 cm × 50 cm × 3.4 cm. Removable black plastic boards were inserted on the front and back and sides of each rhizotron to create a dark environment for the roots. When the removable black plastic boards were removed from a rhizotron, plant roots in the inner cavity of the rhizotron can be easy to measure and count. This newly developed rhizotron

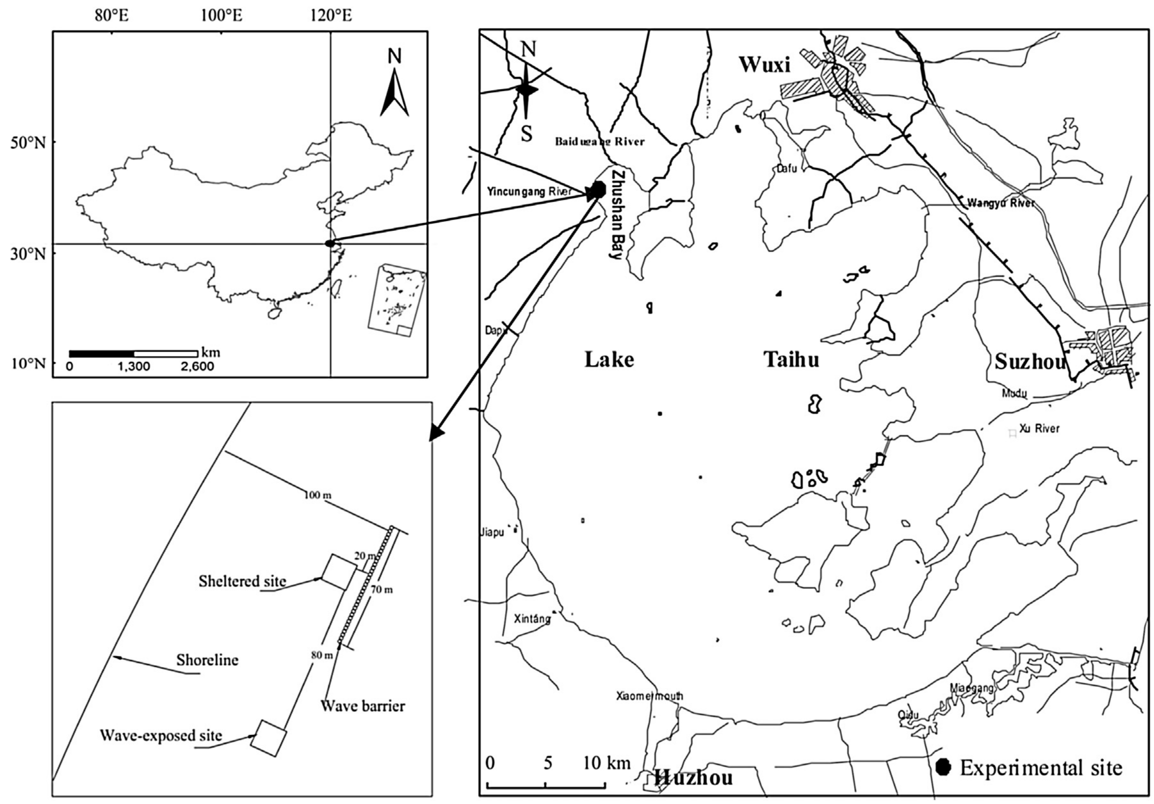

Fig. 1. Site of the field experimental system.

Table 1
TN, TP, Secchi disc depth and water temperature changes on different investigation days.

Date (day/month/year)	TN (mg L^{-1})	TP (mg L^{-1})	Secchi disc depth (cm)	Water temperature (°C)
28/9/2013	2.32	0.134	40.0	23.4
9/10/2013	2.76	0.176	45.0	21.2
22/10/2013	2.25	0.175	47.0	19.8
1/11/2013	2.50	0.151	47.2	19.0
12/11/2013	2.97	0.142	47.4	15.4
26/11/2013	3.31	0.145	54.3	13.2
12/12/2013	4.65	0.184	45.4	10.6
4/1/2014	4.55	0.145	76.2	8.2
21/1/2014	3.43	0.133	43.0	7.1
20/2/2014	5.52	0.155	75.0	7.8
16/4/2014	4.90	0.158	37.0	19.5
21/5/2014	3.43	0.133	37.0	24.1
7/6/2014	3.11	0.146	40.0	25.2

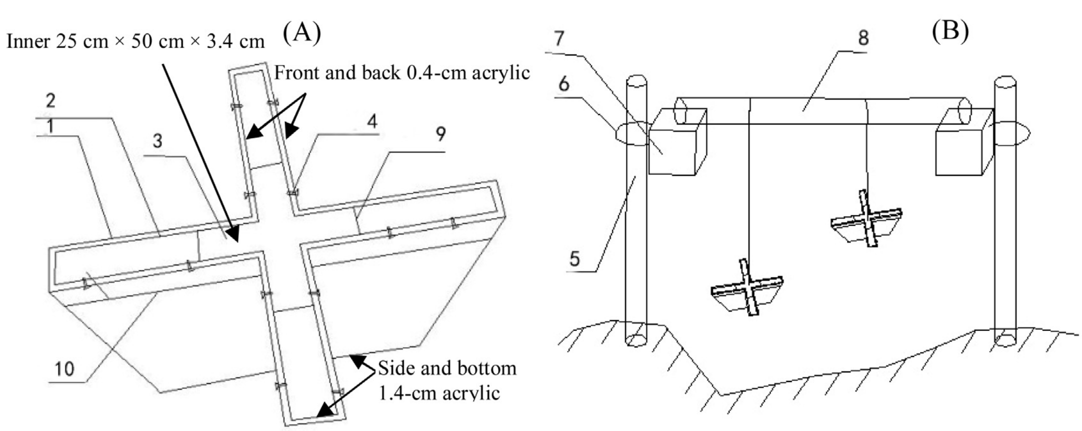

Fig. 2. Schematic of the rhizotron (the black plastic board is absent) (A) and the rhizotron in the experimental site in water(B). Numbers in A and B indicate: (1) black plastic board; (2) clear acrylic; (3) planting groove; (4) rivet; (5) steel-pipe post; (6) loop; (7) buoy; (8) beam; (9) pull rod; (10) substrate.

Table 2
Organic matter (OM), total nitrogen (TN), and total phosphorus (TP) content of the substrate (mean ± standard deviation).

Sediment type	OM (%)	TN (%)	TP (%)
Fertile sludge	1.946 ± 0.053	0.246 ± 0.004	0.168 ± 0.023
Brown clay	0.210 ± 0.051	0.042 ± 0.005	0.039 ± 0.003

facilitates the following: (1) non-destructive root morphometric measurements during experiments, (2) rhizotron-specific independent experimental units, (3) easy adjustment of water depth for the plants, (4) easy access for planting, and (5) absolute stability with adequate weight according to the law of inertia for plants in waves when the substrate is filled.

2.3. Planting and culture of submerged macrophytes

The young ramets of the submerged *V. natans* macrophytes that germinating from tubers, were collected from a pond adjacent to Dongtaihu Bay in the southeast part of Lake Taihu on 27th September, 2013. The plants were carefully washed to remove sand and soil from the roots without damaging them. One hundred and eight young ramets were selected based on size uniformity (18 cm in height, 5–6 leaves per ramet).

Mixed soils of fertile sludge and those of brown clay collected from Zhushan Bay were placed in different rhizotrons at an equal height of 50 cm. The fertile sludge was enriched with OM, TN and TP. The brown clay had low levels of OM, TN, and TP, equal to approximately 10.8%, 17.1% and 23.5% of those in fertile sludge, respectively (Table 2). The fertile sludge had lower strength, stability and smaller particle size. The soils in each rhizotron were slightly compacted to minimise further compaction. In each arm of rhizotron, 3 pockets were dug at even spacing in the soil, and the roots of 3 *V. natan* individuals were planted in three replicates. After each plant was buried 7 cm in the sediment, more soil was added to cover the roots and the shoot base. The 4th arm in the rhizotron was not planted with any submerged macrophytes, to provide a reference for comparing changes of roots and substrate. Next, each rhizotron was fixed at a water depth through a rope on a beam with each of its two ends fastened to a floating body by a screw. This type of rhizotron fixation could maintain a constant water depth because the floating bodies can move up and down with water level changes. Plants were grown at three water depths (60, 120 and 200 cm) in the experiments. Thus, 12 rhizotrons (48 arms) were used in the experiments because of the two types of substrates (fertile sludge and brown clay) and two different wave conditions (wave-exposed and sheltered). A 12 m × 12 m experimental zone at each site was protected from fish grazing by a net enclosure (mesh size = 10 mm).

2.4. Date collection and definitions of variables

The experiments lasted 252 days, extending from 28th September 2013 to 7th June, 2014. Submerged macrophyte measurements were conducted on 28th September, 9th and 22nd October, 1st, 12nd, and 26th November, and 12nd December of 2013 as well as 4th and 21st January, 20th February, 16th April, 21st May, and 7th June of 2014. The submerged macrophytes were subject to various measurements, including plant length, leaf width, root length, number of initial plants that survived, number of ramets, number of leaves per ramet, and number of roots per initial plant. The root densities in the soil at depths of 5, 10, 15, and 20 cm under water-sediment interface were measured by counting the roots that were intersected by a horizontal line. The aboveground biomass (fresh weight, B) per ramet was calculated by

$$B = 0.0202X_1 + 0.1862X_2 - 0.2235 \quad (1)$$

where X_1 and X_2 are the plant length and leaf width, respectively (Wang and Wang, 2013). On each measurement day, the water temperature was recorded using YSI 6600V2 (YSI, YSI Inc., Ohio, Yellow Springs, USA), and water samples (500 ml) were collected in plastic bottles at wave-exposed and sheltered sites respectively. The TP and TN in the original water samples were determined following standard water sample analytical programmes issued by the Chinese National Environmental Monitoring Centre.

The survival rate of aboveground parts of plants and tuber germination rate were calculated as the number of plant clumps in water divided by the initial plant number. The relative rates of increase in plant length and biomass of *V. natans* were calculated by

$$G_n = \frac{L_{n+1} - L_n}{L_{n+1}(T_{n+1} - T_n)}, \quad n = 1,\ 2,\ 3\ldots13 \quad (2)$$

where G_n is the rate of increase in plant length or biomass from the nth investigation day to the (n + 1)th investigation day (Xu et al., 2014); L_n is the plant length or biomass on the nth investigation day; and T_n is the number of days from the beginning of the experiment to the nth investigation.

The influence strengths of water depth, substrate type and wave exposure on the 5 key parameters of *V. natans* (i.e., survival rate, plant length, leaf number, ramet number and aboveground biomass) are defined as:

$$\mathrm{IS} = \frac{1}{N}\sum_i \frac{|S_{c1,i} - S_{c2,i}|}{S_{c1,i} + S_{c2,i}}$$

where IS is the influence strength, N is the total number of concomitant condition cases of conditions $c1$ and $c2$, i is the number of an element belonging to concomitant condition sets, and $c1$ and $c2$ are the two different states of the experimental condition. $S_{c1,i}$ or $S_{c2,i}$ is the value of survival rate, plant length, leaf number, ramet number or aboveground biomass under $c1$,i or $c2$,i condition.

2.5. Statistical analyses

Three-way analysis of variance (ANOVA) was used to determine whether water depth, substrate and wave exposure had joint effects on *V. natans* growth using the SPSS version 13.0 software (SPSS, Inc., Chicago, IL, USA). Differences were considered significant at $P < 0.05$. Post hoc comparisons for all analyses were made with Tukey's HSD test.

3. Results

3.1. Survival and tuber germination rates

Vallisneria natans survival rate (aboveground parts of plants) declined with decreasing water temperature from 23.4 °C at the beginning of the experiments to 7.8 °C on February 20th, 2014. Survival rate was zero in all experiments by the end of the period on February 20th, 2014 (Fig. 3). As the water temperature increased from 7.8 °C on 20th February, 2014 to 25.2 °C on 7th June, 2014, some of the tuber germination rates recovered from zero to the survival rates during the early period of the experiments. After 20th February, 2014, *V. natans* exhibited zero tuber germination rate at 200 cm water depth when planted in brown clay at both the exposed and sheltered sites, and when planted in fertile sludge at the exposed site. At the water depth of 60 cm, *V. natans* tuber germination rates all recovered to 100% when the water temperature increased from 20th February to 7th June, 2014, regardless of site and substrate. Nevertheless, *V. natans* tuber germination rates at all the water depth of 120 cm increased from 20th February to 16th

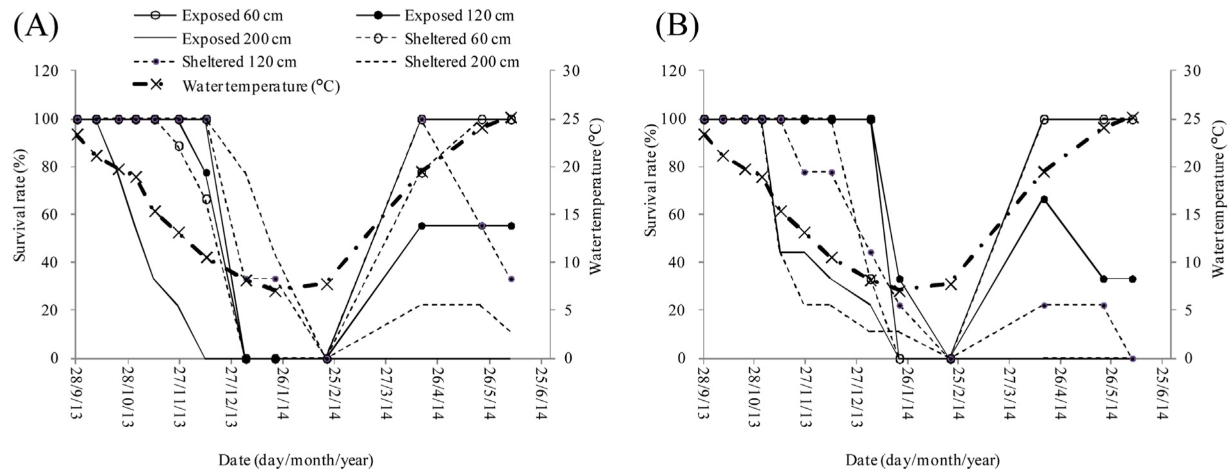

Fig. 3. Survival rate of *V. natans* (%) in fertile sludge (A) and brown clay (B).

April, 2014 at both sites and in both substrates, but none of them reached 100% from 16th April to 7th June, 2014.

3.2. Aboveground plant phenotypic plasticity and biomass variation

The plant length, leaf number, ramet number and aboveground biomass per ramet, similar to the survival rate, all showed an overall decrease when the water temperature decreased and an increase when the water temperature increased (Fig. 4). Fig. 4 shows that plant length, leaf number, ramet number and aboveground biomass at a water depth of 60 cm, regardless of the substrate and site, were greater than those at the other water depths ($P < 0.05$), particularly at 120 cm, throughout most of the experiment. The plant length was significantly higher at the exposed site than at the sheltered site ($P < 0.05$), whereas the leaf number at the exposed site was less than at the sheltered site in the same substrate. Wave exposure had a positive effect on the biomass increase in clay according to the average rate of increase in biomass. The decline of biomass in clay was delayed according to the occurred time of the negative rate value.

There were large growth rate differences among the different experimental conditions. The relative rate of increase in plant length in sludge ranged from $-0.056\,d^{-1}$ to $0.059\,d^{-1}$ at the exposed site and from $-0.044\,d^{-1}$ to $0.274\,d^{-1}$ at the sheltered site, indicating the later has a large range. In clay, it ranged from $-0.059\,d^{-1}$ to $0.158\,d^{-1}$ at the exposed site and from $-0.059\,d^{-1}$ to $0.090\,d^{-1}$ at the sheltered site (Table 3). In adverse to in sludge, the later has a relatively small range. Those results show that wave-exposed and wave-sheltered influence varies with the substrate. The biomass increase rate in sludge ranged from $-0.063\,d^{-1}$ to $0.069\,d^{-1}$ at the exposed site and from $-0.043\,d^{-1}$ to $0.140\,d^{-1}$ at the sheltered site. In clay, it ranged from $-0.059\,d^{-1}$ to $0.161\,d^{-1}$ at the exposed site and from $-0.059\,d^{-1}$ to $0.104\,d^{-1}$ at the sheltered site (Table 4).

3.3. Plant root variation

During the experiment, the temporal variation of the root length and number did not exhibit an overall reduction as water temperature decreased or increased, as did plant length, leaf number and other variables (Fig. 5A–D). The temperature appeared to have a smaller influence on root length and number than on the other variables. In fertile sludge, root length and number at 60 cm and 120 cm water depth at both sites increased during the initial stage of the experiments. The relative rate of increase in the length and number of *V. natans* roots at 60 cm water depth at both sites was greater than that at 120 cm water depth ($P < 0.05$). Root length and number at the wave-sheltered site were greater than that at the exposed site at the water depth of 60 cm. This trend was not true at the water depth of 120 cm. In brown clay, since oxidation could not change the root soil colour to a different colour from the brown clay colour and some of the roots were difficult to observe and count. Root length and number results in brown clay still exhibited similar temporal

Table 3
Rate of increase in *V. natans* (d^{-1}) plant length.

Time period (day/month/year)	Environmental conditions (substrate type, wave exposure and water depth)											
	Fertile sludge						Brown clay					
	Exposed 60 cm	Exposed 120 cm	Exposed 200 cm	Sheltered 60 cm	Sheltered 120 cm	Sheltered 200 cm	Exposed 60 cm	Exposed 120 cm	Exposed 200 cm	Sheltered 60 cm	Sheltered 120 cm	Sheltered 200 cm
28/9/2013												
–9/10/2013	0.036	−0.015	0.016	0.007	0.003	0.037	0.049	0.005	0.025	0.004	0.058	0.027
–22/10/2013	−0.019	0.018	−0.014	−0.002	−0.004	0.004	0.008	0.030	0.021	0.000	0.002	−0.024
–1/11/2013	0.022	0.004	−0.049	0.013	0.021	−0.044	0.011	−0.013	−0.006	−0.009	−0.019	0.056
–12/11/2013	−0.031	−0.024	0.004	−0.018	−0.034	−0.034	−0.032	−0.033	−0.050	0.009	−0.057	−0.055
–26/11/2013	−0.004	−0.034	−0.056	0.002	−0.015	0.005	0.003	−0.008	0.001	−0.030	0.021	0.065
–12/12/2013	−0.033	−0.016	−0.063	−0.028	0.006	0.012	−0.016	−0.034	−0.006	0.019	−0.033	0.005
–4/1/2014	−0.043	−0.043	–	−0.043	0.017	−0.021	−0.021	−0.002	−0.009	−0.022	0.011	−0.018
–21/1/2014	–	–	–	–	−0.020	−0.011	−0.059	0.004	−0.059	−0.059	0.004	−0.025
–20/2/2014	–	–	–	–	−0.033	−0.033	–	−0.033	–	–	−0.033	−0.033
–16/4/2014	–	–	–	–	–	–	–	–	–	–	–	–
–21/5/2014	0.059	0.042	–	0.274	0.030	0.031	0.158	−0.004	–	0.090	−0.005	–
–7/6/2014	0.003	0.007	–	−0.031	−0.034	0.002	0.004	0.015	–	0.006	−0.059	–

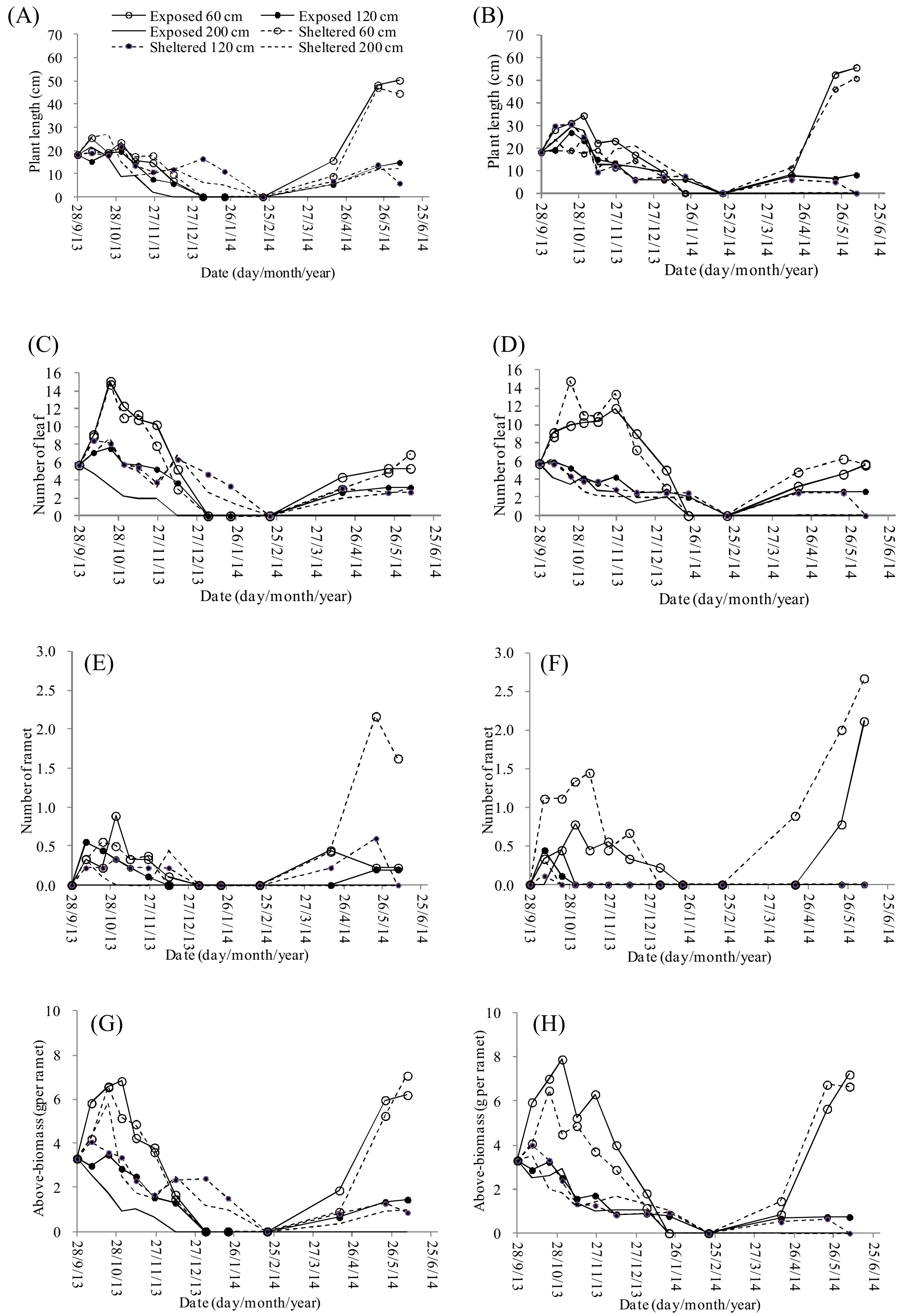

Fig. 4. Plant length (A in fertile sludge, B in brown clay), leaf number (C in fertile sludge, D in brown clay), ramet number (E in fertile sludge, F in brown clay) and aboveground biomass (G in fertile sludge, H in brown clay).

Table 4
Rate of growth in terms of *V. natans* (d^{-1}) aboveground biomass.

Time period (day/month/year)	Environmental conditions (substrate type, wave exposure and water depth)											
	Fertile sludge						Brown clay					
	Exposed	Exposed	Exposed	Sheltered	Sheltered	Sheltered	Exposed	Exposed	Exposed	Sheltered	Sheltered	Sheltered
28/9/2013	60 cm	120 cm	200 cm	60 cm	120 cm	200 cm	60 cm	120 cm	200 cm	60 cm	120 cm	200 cm
–9/10/2013	0.069	−0.009	−0.019	0.025	0.021	0.026	0.073	−0.012	−0.022	0.021	0.019	0.005
–22/10/2013	0.010	0.013	−0.026	0.043	−0.009	0.028	0.014	0.010	0.004	0.045	−0.013	−0.033
–1/11/2013	0.004	−0.018	−0.048	−0.021	−0.006	−0.058	0.013	−0.022	0.013	−0.031	−0.028	−0.007
–12/11/2013	−0.034	−0.011	0.012	−0.005	−0.029	−0.027	−0.031	−0.034	−0.049	0.008	−0.040	−0.032
–26/11/2013	−0.007	−0.027	−0.026	−0.019	−0.020	−0.010	0.015	0.006	−0.018	−0.017	−0.004	0.013
–12/12/2013	−0.035	−0.010	−0.063	−0.037	0.025	0.042	−0.023	−0.031	0.001	−0.014	−0.022	0.011
–4/1/2014	−0.043	–	–	−0.043	0.001	−0.023	−0.024	0.001	0.001	−0.026	0.004	−0.009
–21/1/2014	–	–	–	–	−0.022	−0.009	−0.059	−0.007	−0.059	−0.059	0.002	−0.011
–20/2/2014	–	–	–	–	−0.033	−0.033	–	−0.033	–	–	−0.033	−0.033
–16/4/2014	–	–	–	–	–	–	–	–	–	–	–	–
–21/5/2014	0.063	0.031	–	0.140	0.016	0.050	0.161	0.003	–	0.104	0.008	–
–7/6/2014	0.002	0.005	–	0.020	−0.019	0.005	0.073	−0.012	–	0.021	0.019	–

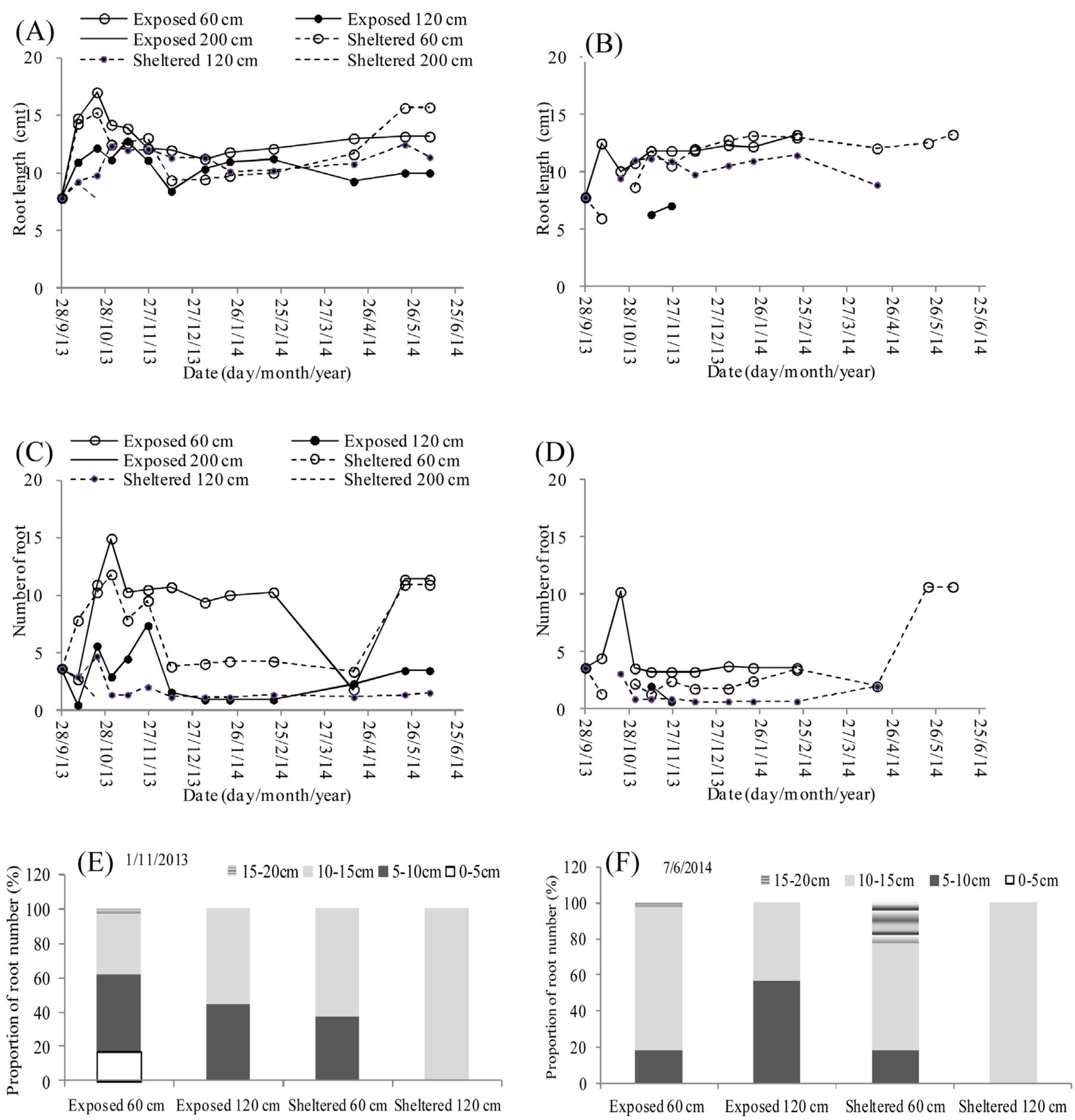

Fig. 5. Variation of root length (A in fertile sludge, B in brown clay), root number per initial plant (C in fertile sludge, D in brown clay), proportion of root number in sludge on 1st November, 2013 (E) and 7th June, 2014 (F).

variation to that of fertile sludge. The percentage distribution of the root length in the sludge showed that most of the plant roots were approximately 10–15 cm, followed by 5–10 cm, 15–20 cm, and then 0–5 cm (Fig. 5E, F). The root length of *V. natans* at a water depth of 120 cm was approximately 5–15 cm at the wave-exposed and 10–15 cm at the sheltered site, whereas the root length at a water depth of 60 cm was approximately 0–20 cm at the exposed site and 5–15 cm at the sheltered site on 1st November, 2013, 5–20 cm on 7th June, 2014 at both sites. The roots were distributed deeper at a water depth of 60 cm on 7th June, 2014 than they were on 1st November, 2013, whereas no significant influence was observed at a water depth of 120 cm.

4. Discussion

4.1. Pattern and influence strength of water depth, wave exposure and substrate type on V. natans

The results indicate that water depth, substrate type and wave exposure influenced not only plant phenotypic plasticity but also biomass and roots of *V. natans*. Water depths exhibited more significant influences, compared to substrate type and wave exposure (Table 5), particularly at the end of the experiment (7th June, 2014). Water depth and substrate had extremely significant joint influences ($P < 0.001$) on plant length, leaf width, and root number on 1st November, 2013, in addition to plant length, leaf width, and ramet number on 7th June, 2014. Water depth or substrate type and wave exposure had extremely significant joint influences ($P < 0.001$) on plant length, leaf width, and biomass on 1st November, 2013.

Comparing the significance level of the influences induced by the 3 factors, water depth may have the strongest influence, followed by substrate. Table 6 shows that the overall influence strengths of water depth on the survival rate, plant length, leaf number, ramet number and aboveground biomass were the greatest (range: 0.643–1.204), followed by the overall influence strengths of substrate (0.382–0.849), and then the influence strengths of waves (0.345–0.555). Among the 5 parameters, ramet number was influenced most by water depth, then aboveground biomass, leaf number, plant length, and survival rate. With increasing water depth, less light penetrates the submerged macrophytes, inducing aquatic macrophytes to switch energy and biomass allocations to produce taller plants to maximally utilise light energy for photosynthesis. This strategy reduces investment in branch number and belowground biomass (i.e. the roots), which is verified by Xiao et al. (2007) and Xu et al. (2014). The counteraction between the response of plant length and the limitation of the weak light on plant growth lessens the influence strength of water depth on plant length, whereas ramet number was influenced most by water depth because of the synergism of the response of reduction of branches and the limitation of the weak light on plant growth.

Wave exposure and substrate type may alter the influence strength of water depth on *V. natans* parameters. Wave shelter and brown clay both enhanced the influence strength of water depth, whereas wave exposure and fertile sludge both decreased influence strength of water depth. Additionally, water temperature decrease reduced the influence strength of water depth, and vice versa. This means that the influence of water depth would be counteracted by wave exposure and fertile sludge.

The influence strengths on survival rate induced by substrate type and wave action were the lowest among those on the 5 parameters. Similarly to the impact of water temperature on the water depth influence on *V. natans*, decreasing water temperature reduced the influences of substrate type and wave exposure on the 5 parameters, while increased temperature amplified the effects of substrate type and wave exposure. Both substrate type

Table 5
Effect of water depth, substrate and wave condition on plant growth (plant length, leaf width, leaf number, ramet number, aboveground biomass, root depth and root number) on 1st November, 2013 and 7th June, 2014 using three-way ANOVA.

Date (day/month/year)	Parameter	Water depth (D)		Substrate (S)		Wave condition (W)		D × S		D × W		S × W	
		F	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
1/11/2013	Plant length	**124.38**	**<0.001**	**20.0**	**<0.001**	**9.03**	**0.003**	**30.97**	**<0.001**	**38.95**	**<0.001**	**34.54**	**<0.001**
	Leaf width	**312.40**	**<0.001**	**181.0**	**<0.001**	**45.34**	**<0.001**	**23.40**	**<0.001**	**51.73**	**<0.001**	**54.23**	**<0.001**
	Leaf number	**14.90**	**<0.001**	**253.49**	**<0.001**	0.21	0.649	1.77	0.176	3.92	0.051	1.42	0.248
	Ramet number	0.01	0.914	**39.23**	**<0.001**	0.11	0.745	**5.50**	**0.005**	3.41	0.068	0.11	0.900
	Biomass	0.19	0.663	**149.91**	**<0.001**	**11.84**	**<0.001**	**3.80**	**0.026**	**16.89**	**<0.001**	**20.77**	**<0.001**
	Root length	**30.45**	**<0.001**	0.44	0.512	2.75	0.104	4.81	0.033	0.16	0.693	**8.62**	**0.005**
	Root number	**166.84**	**<0.001**	**209.58**	**<0.001**	**12.02**	**<0.001**	**56.85**	**<0.001**	2.16	0.147	1.66	0.203
7/6/2014	Plant length	**342.18**	**<0.001**	0.05	0.832	**19.22**	**<0.001**	**15.33**	**<0.001**	0.91	0.347	0.02	0.875
	Leaf width	**182.82**	**<0.001**	0.05	0.832	0.05	0.832	**30.33**	**<0.001**	0.74	0.395	**31.42**	**<0.001**
	Leaf number	**71.46**	**<0.001**	**6.28**	**0.016**	0.29	0.591	1.42	0.240	**10.87**	**0.002**	**8.84**	**0.005**
	Ramet number	**23.26**	**<0.001**	**11.50**	**0.002**	**4.08**	**0.050**	**17.17**	**<0.001**	**9.92**	**0.003**	**4.66**	**0.037**
	Biomass	**172.88**	**<0.001**	0.63	0.433	0.81	0.375	**5.32**	**0.026**	3.84	0.057	**6.22**	**0.017**
	Root length	**33.47**	**<0.001**	**9.30**	**0.005**	**9.06**	**0.006**						
	Root number	**123.81**	**<0.001**	0.06	0.813	2.33	0.136						

Table 6
Influence strength of water depth, type of substrate and wave on *V. natans*.

	Characteristic	Concomitant condition						
		Wave action		Substrate type		Temperature period		Overall strength
		Exposed	Sheltered	Fertile sludge	Brown clay	Decreasing	Rising	
Water depth	Survival rate	0.568	0.718	0.581	0.705	0.407	1.353	0.643
	Plant length	0.674	0.771	0.689	0.756	0.487	1.427	0.722
	Leaf number	0.856	0.935	0.777	1.014	0.809	1.157	0.896
	Ramet number	1.191	1.216	1.028	1.379	1.077	1.582	1.204
	Aboveground biomass	0.965	0.938	0.877	1.026	0.765	1.510	0.952

	Characteristic	Concomitant condition						
		Water depth		Wave action		Temperature period		Overall strength
		60 cm	120 cm	Exposed	Sheltered	Decreasing	Rising	
Substrate type	Survival rate	0.199	0.565	0.427	0.337	0.276	0.698	0.382
	Plant length	0.376	0.666	0.621	0.422	0.464	0.692	0.521
	Leaf number	0.338	0.668	0.574	0.431	0.458	0.637	0.503
	Ramet number	0.694	1.004	0.781	0.917	0.799	1.000	0.849
	Aboveground biomass	0.348	0.579	0.496	0.431	0.373	0.737	0.464

	Characteristic	Concomitant condition						
		Water depth		Substrate type		Temperature period		Overall strength
		60 cm	120 cm	Fertile sludge	Brown clay	Decreasing	Rising	
Wave action	Survival rate	0.135	0.555	0.437	0.253	0.207	0.760	0.345
	Plant length	0.314	0.568	0.571	0.311	0.304	0.853	0.441
	Leaf number	0.194	0.514	0.485	0.223	0.240	0.696	0.354
	Ramet number	0.636	0.475	0.581	0.530	0.481	0.777	0.555
	Aboveground biomass	0.316	0.448	0.486	0.278	0.213	0.888	0.382

and wave exposure had the greatest influence on ramet number compared to the other 4 parameters. However, water depth differed in its influence on plant parameters from substrate type and water depth. Substrate type had the second to fourth greatest influences on plant length, leaf number and aboveground biomass, while for wave exposure these parameters were plant length, aboveground biomass and leaf number. Decreasing water depth reduced the influence of substrate type on plant growth, but did not alter the actual pattern of impact of substrate type. Wave exposure altered the pattern in which substrate type influenced *V. natans*. Decreasing water depth reduced the effect of wave exposure on *V. natans*, except for ramet number, and vice versa. Furthermore, it changed the impact pattern of waves on *V. natans*. Decreasing substrate nutrient levels reduced the influence of wave exposure on *V. natans*, altering the pattern of wave influence on *V. natans*.

Wave exposure affects a wide range of morphological characteristics. Some kelps respond to wave exposure by exhibiting drag-reducing (narrow laterals and blades) and strength-increasing (relatively large holdfast, thick stipe and thick blades) morphological traits, as documented by small-scale studies (Wernberg and Thomsen, 2005). Such morphological adaptations to the hydrodynamic environment may reduce plant mortality. However, powerful waves will break the stems or uproot entire plants. In our experiment, wave exposure enhanced plant length growth at a water depth of 60 cm in both substrates. The results implied that the tensile strain of plants may be promoted by wave exposure, resulting in leaf elongation.

Roots are quite different from the aboveground parts of aquatic macrophytes. They do not directly suffer from wave action and current disturbance. The number and length of roots in sediments often affect the plant nutrient absorption and fixation, which in turn affect plant growth. The disappearance of roots inevitably causes a decline in submerged vegetation biomass. Previous studies on macrophyte growth have showed that submerged plants incorporate most of their required N and P from the bottom sediments, except under highly eutrophic conditions (Xie et al., 2005). It is not necessary for plants to obtain their nutrients exclusively from the substrate under eutrophic conditions; thus, the *V. natans* growth measured by the increasing rate of aboveground biomass was not markedly affected by low-nutrients clay compared to nutrient-rich sludge at the same water depth. The greater number of ramets in clay than in sludge at a water depth of 60 cm also indicates that roots are not a necessary site of nutrient uptake in shallower water. However, nutrient availability of the substrate appeared to regulate leaf number since it was closely related to sediment before overwintering.

4.2. Nutreint and submerged macrophyte vegetation restoration by water-level management in a eutrophic lake

The experimental results showed that, regardless of substrate and wave conditions, at 60 cm water depth, *V. natans* was able to increase its plant length, leaf number, ramet number, aboveground biomass, root number, and root length during the growing period. In addition, *V. natans* at the depth was able to retain its root number and root length during the decay of its aboveground parts due to low temperature, and germinate again when the water temperature rose in the subsequent spring. This implies that *V. natans* could be recovered in Zhushan Bay, even under severe eutrophication conditions, because high N and P concentrations did not limit plant growth at suitable water depths. However, the results indicated that *V. natans* could not grow at a water depth of 200 cm regardless of substrate type and wave exposure conditions, although, parts of the planted plants were able to grow for some or all of the experiment period. The results of these 3 water depth experiments indicate that a suitable water depth is less than 120 cm, and it is possible to restore *V. natans* vegetation in the eutrophic bay. As the water transparency is among 37.0–76.2 cm, the euphotic depth of the bay approximate 2-fold water transparency about the water depths between 60 and 120 cm, implying that a suitable water depth for *V. natans* could be the euphotic depth of the water body.

Many studies show that the euphotic depth of water body is not only related to the suspended silt in water column, but also the nutrient content and phytoplankton particulate. Over high nutrient would do harm to directly submerged plant organism (Yuan et al., 2013) and boost the growth of phytoplankton. The latter effect would then increase the suspended particulate in water column and result in attenuation of the light on the submerged plant, even inducing the submerged plant death. Zhang et al. (2003) suggested that a shift to a turbid water state may take place in a lake at phosphorus concentration between 0.16 and 0.25 mg L^{-1} resulting in a significant decrease in the submerged plants. In the lake with low concentration of nutrient nitrogen and phosphorus, *V. natans* could grow well in a water depth of 2 m. This is due to low concentration of nutrient which would limit the growth of phytoplankton and decrease the particulates in the water column, resulting in the increasing of water transparency and deeper penetration of light. Thus water column nutrient reducing is an important measure for the growth of submerged plant. The nutrients in water column is usually influenced by advection, settling, diffusion and re-suspension processes linked to the sediment. As the shallow lake bottom is easily disturbed by hydrodynamic processes, nutrients settling and accumulating in the bottom would release to the water column and increase the nutrient content in water. Zhang et al. (2003) thought that recovery of submerged macrophytes will take a very long time because the lake performs long resilience probably owing to the accumulation of phosphorus in the sediment.

Currently, three methods exist to retain the water depth at or below the euphotic depth. First, to increase euphotic depth by using physical and biological engineering techniques, which reduce the amount of suspended sediments in the water. This technique has proven successful in some instances, but it is very expensive and labour intensive, as well as being quite complicated and unsTable Second, to raise the water body bottom elevation. This could be applied to only local-scale bay and lake shore area, as it would reduce the storage capacity of flood water. In addition, it would need a large amount of soil, a large amount of manpower and material resources. Third, to lower the water level of the water body. Water-level is a decisive element of hydrology, notably in shallow lakes that are particularly sensitive to rapid changes in water level. This technique was applied to Lake Okeechobee (Florida, USA) in the spring of 2000 and was followed by a drought in 2000 and 2001. The water levels lowering stimulated greater light penetration to the sediments, leading to the increased growth of charophytes (Havens et al., 2004). At present a number of water conservancy projects have been constructed around the lake where Zhushan Bay is located, thus, it is possible to lower the water level of the lake to keep the water depth to less than 120 cm *V. natans*, particularly during spring. Therefore, water-level management may serve as a tool to restore the shallow lakes. Nevertheless, the water levels of shallow lakes naturally vary seasonally and annually and the height of submerged plant is different at its different life stage. Submerged plant restoration will be benefited from water-level management that requires deeply study to reduce flooding and water supply risks in Taihu Basin.

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