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PRIMARY RESEARCH PAPER

Effects of substrate and shading on the growth of two submerged macrophytes

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Abstract Excessive nutrient loading may cause a shift from submerged macrophyte dominance to free-floating macrophyte dominance. Tolerance and persistence of submerged plants in response to shade may be key characteristics in determining when/if such a shift occurs in shallow eutrophic lakes. This study examines how the cover of floating macrophyte (*Lemna minor*) and shade of dark mesh affect the growth and photosynthetic efficiency of two submerged plants (*Vallisneria natans* and *Myriophyllum spicatum*) on different nutrient substrates. We found that low- and mid-cover intensities generally enhanced the leaf/shoot growth of both submerged plants under all cover and substrate types. The relative growth rates (RGR) were slightly enhanced under the treatment of *Lemna* with low- and mid-intensity cover on both nutrient-rich substrates. The leaf/shoot growth and RGR of both submerged macrophytes generally increased more under *Lemna* cover than mesh cover. The photosynthetic efficiency (F_v/F_m value) typically increased with the duration of treatment and the cover densities. In addition, these two macrophytes with contrasting growth forms have markedly different growth and survival strategies in response to covers. These results strengthen the hypothesis that submerged plants can successfully develop under a low-intensity cover of floating vegetation on nutrient-rich substrate.

Keywords Filamentous algae · Fluorescence · Shallow lake · Free-floating macrophytes · Submerged macrophytes

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Introduction

Shallow lakes often possess two alternative stable states, a turbid and devoid of submerged macrophytes or clear and vegetated (Scheffer et al., 1993). Excessive nutrient loading has caused most lakes to shift to a turbid state dominated by phytoplankton as the main primary producers (Scheffer et al., 1993). However, nutrient-rich shallow lakes may also be dominated alternatively by free-floating macrophytes in stable states (Morris et al., 2003; Scheffer et al., 2003; Scheffer and Van Nes, 2007), especially in subtropical and tropical shallow lakes, where free-floating plant dominant states may occur frequently. Several recent papers have supported that free-floating macrophytes will profit most from enhanced eutrophication and future climate change (Feuchtmayr et al., 2009; Netten et al., 2010, 2011).

Free-floating macrophyte dominance creates dark and anoxic underwater conditions that leave little opportunity for animal or plant life in shallow lakes (Janes et al., 1996; Scheffer et al., 2003; Morris et al., 2004; Abdel-Tawwab, 2006; Meerhoff et al., 2006, 2007). However, the environmental conditions created by free-floating macrophyte mats are not at all deleterious to submerged plant growth. For instances, a high-intensity cover of free-floating macrophytes may lead to strong oxygen depletion (Caraco et al., 2006), and may benefit photosynthesis and reduce photorespiration (Simpson et al., 1980); free-floating macrophyte mats can successfully reduce the biomass of phytoplankton, periphyton and filamentous algae (Parr et al., 2002; Bicudo et al., 2007; O'Farrell et al., 2009) and subsequently improving water transparency. These suggest that the effect of free-floating macrophyte mats on the growth of submerged plants may depend on the cover intensity of free-floating macrophytes.

Besides light and oxygen, nutrient competition between submerged and floating macrophytes may largely determine the shift between floating and submerged plants. Floating macrophytes can acquire inorganic nutrients from water column only, whereas submerged rooted macrophytes can take up nutrients effectively from both substrate (Chambers et al., 1989) and water column (Carignan & Kallf, 1980; Madsen & Cedergreen, 2002). Despite a previous report pointed out that nutrient absorbed from water column was less effective (Barko & Smart, 1986), manipulative experiments on four submerged species have demonstrated that all species were able to satisfy their demand for mineral nutrients by leaf nutrient uptake alone (Madsen & Cedergreen, 2002). Moreover, periphytons attached to submerged plant may also take up nutrients and further reduce water nutrient concentrations (Eriksson & Weisner, 1997). Low nutrient availability of water column might be expected to prevent the growth of floating plants (Szabo et al., 2010). Thus, the competitive advantage of submerged macrophytes relative to floating plants will depend on the nutrient conditions of substrate. Nutrient-rich substrate may favour the persistence of submerged macrophyte dominance. In contrast, in nutrient-poor substrate submerged macrophytes will compete more strongly with floating plants for nutrient from water column. Furthermore, considering into nutrient consumption, the cover effect of floating macrophytes appear to be more complex compared to simple physical shading, such as cover with dark mesh. However, the effects substrate- and cover types on the interactions between submerged macrophytes and free-floating plants have been scarcely studied.

Tolerance and growth strategy of species are also important factors in determining the survival of submerged macrophytes below floating mats. High-growing species, for instance, *Myriophyllum spicatum*, may respond to shading by accelerating elongation, so that they rapidly colonise the subsurface layer where the greatest amount of remaining light is available. In contrast, rosette-forming species can seldom grow to subsurface layer and may be highly tolerant to low-light intensities. For example, species of *Vallisneria* is a submerged aquatic plant with basal rosettes of flexible ribbonlike leaves that can form an underwater meadow. *Vallisneria* has been reported to be efficient carbon fixers at low-light intensities (Titus & Adams, 1979) and have a very low-light-compensation point (Blanch et al., 1998, Morris et al., 2004). Here, we studied the effects of cover types, cover intensities and substrate types on the growth of two submerged plants with contrasting growth forms. We hypothesized that the (i) submerged plants can successfully develop under a low-intensity cover of free-floating macrophytes and in a nutrient-rich substrate; (ii) cover effects between free-floating plant and mesh on the growth of submerged macrophytes are different; and (iii) submerged macrophytes with contrasting growth forms have clearly different growth and survival strategies in response to covers.

Materials and methods

Species

Vallisneria natans and *M. spicatum* were selected for the experiment as the two species are known to persist in very nutrient-rich lakes still supporting submerged vegetation (Sand-Jensen et al. 2008). They have different light-compensation points with 9.4 μmol m^{-2} s^{-1} in *V. natans* and 27.5 μmol m^{-2} s^{-1} in *M. spicatum* at 20°C in studied region (Su et al., 2004), which might imply that they have different responses to cover densities of free-floating plants. Seedlings of *V. natans*, apical shoots of *M. spicatum* and fronds of *Lemna minor* were collected from

ditches near Lake Dianchi (24°29′N to 25°28′N, 102°29′E to 103°01′E), a shallow, eutrophic plateau lake located at Kunming, China. The plants were pre-incubated in experimental ponds (2 m × 2 m) filled with tap water (total nitrogen 0.61 ± 0.19, total phosphorus 0.59 ± 0.06 mg l^{-1}; $n = 5$) for 7 days.

Experimental set-up

A mesocosm experiment was performed in 274 l polyethylene tanks (height: 0.8 m; diameter: 0.66 m) from 10th July to 10th August 2010 in an unheated glasshouse at a site near Lake Dianchi (24°44′32″N, 102°35′24″E). Seedlings of *V. natans* (~8 cm length for the longest leaf) and apical shoots of *M. spicatum* (8 cm length, without lateral shoots) were weighed and planted in individual plastic pots (8 cm diameter and 13 cm high) that were filled with 10 cm of substrate. Three types of substrate (sand, clay and a 50:50 v/v mixture of the two) were used. Clay was collected from deposits in Lake Dianchi. The six pots (three substrates × two species) were then placed into a tank (Fig. 1). The sides of the tanks were covered by black foil to prevent light penetration from the sides. The initial dry weight:fresh weight ratio was determined on plants identical to the experimental material. A total of 35 tanks were designed and filled with the same tap water as above to 70 cm high.

The experiments were then designed further with a completely randomised block design of 3 × 2 factorial and a control treatment (CK) and replicated five times (Fig. 1). Treatments and levels were as follows: cover type (cover 1—free-floating plant *L. minor* and cover 2—dark mesh) and cover intensity (three densities of low, medium and high were created by adding 30, 60 and 180 g fresh biomass for *L. minor*; similar shading effects were simulated by placing a fine dark mesh on the water surface and adjusting number of layers). To maintain the similar cover intensity during whole experiment, the fresh biomasses of floating plants were weekly weighted and adjusted to its initial design value. The CK was designed without any coverage. Given that our main objective in the present study was to elucidate the growth responses of submerged macroptytes on shading under a eutrophic condition, we prioritized low-light levels by shading the experimental glasshouse with two layers of fine dark mesh.

Data collection

To evaluate shading validity, we measured the light intensities at a superficial 5 cm depth for each tank at 4:00 PM–5:00 PM after 2 days of treatment, using a submersible digital illuminometer (ZDS-10W; Shanghai, China). During the experiment, light intensity in glasshouse was measured every 4 days from 8:30 AM to 11:30 AM, every 10 min. Chlorophyll fluorescence, a non-destructive assay used to estimate the

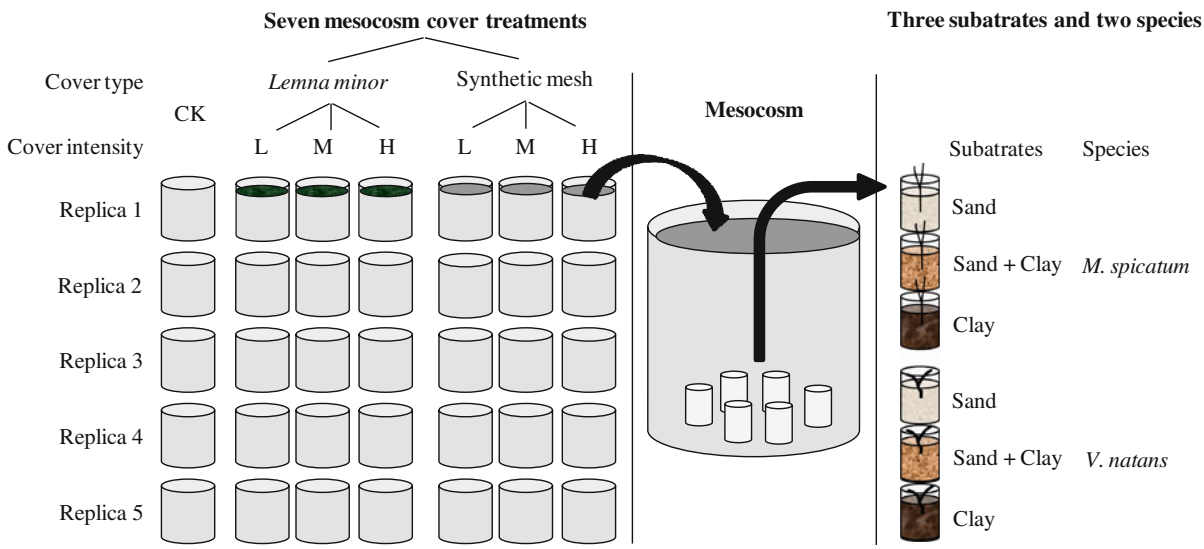

Fig. 1 Schematic diagram of experimental design. Cover intensity: *L* low, *M* medium, *H* high

intrinsic capacity of photosynthesis in green plants, was measured randomly on fully expanded leaves for each individual plant using a portable plant efficiency analyser (Handy PEA 2919; Hansatech Instruments Ltd., UK) after 10, 20 and 30 days of treatment. Variable fluorescence ($F_v = F_m - F_0$) and maximum quantum efficiency of PSII photochemistry (F_v/F_m) were calculated from F_0 and F_m, where F_v is variable fluorescence rise, F_0 is the initial minimal fluorescence level and F_m is the maximum rise fluorescence.

At the end of the experiment and before the covers were disturbed, dissolved oxygen (DO) concentration at the superficial 5 cm depth was measured using a portable DO analyser (JPBJ-609; Shanghai, China). Water was sampled to measure concentrations of total nitrogen (TN) and total phosphorus (TP) using standard methods (Huang et al., 1999). Then all the individuals were harvested. Unexpectedly, filamentous green algae had emerged around the experimental macrophytes and they were also carefully collected with tweezers and a fine mesh sieve. The longest shoot/leaf length and the number of ramets/lateral shoots were measured for each individual of *V. natans* and *M. spicatum*. All experimental individuals of both macrophytes and the total filamentous green algae in each tank were dried at 80°C for 48 h and weighed. Dry mass was used to calculate the relative growth rate of submerged plants during 31 days of incubation (relative growth rates, RGR): $RGR = (\ln DW_t - \ln DW_0)/t$ in which DW_t and DW_0 are the dry masses at time t and time 0 respectively.

Statistical analyses

The significance of cover type and cover intensity on the light intensity, DO, TN and TP was tested by two-way analysis of variance (ANOVA) using SPSS 16.0 software. The significance of cover type, cover intensity and substrate type on the relative growth (final length minus initial length) of longest leaf/shoot, the number of ramets/lateral shoots, RGR and F_v/F_m value of both submerged macrophytes were evaluated by three-way ANOVA. To further test for significant differences between *Lemna* coverage and mesh coverage at a specific treatment level, comparisons were made using a t test. The normal distribution of the variables was checked by the Kolmogorov–Smirnov test. When the data did not meet assumptions of homogeneity of variance, $\log_{10}$, cube- or square-root arcsine transformations were performed depending on the type of variables. The total biomass of filamentous green algae per cover intensity was compared between *Lemna* cover and mesh cover using a non-parametric Kruskal–Wallis test.

Results

Environmental variables

Daily irradiance from 8:30 AM to 11:30 AM in the glasshouse varied from 80 to 944 μmol m^{-2} s^{-1}, which was above the light-compensation point (Fig. 2). Significant differences of the mean irradiance (E_{mean}) were observed between the three cover densities ($F_{3,32} = 470.77$, $P < 0.01$, ANOVA) but not between the two cover types ($F_{1,32} = 3.52$, $P > 0.05$, ANOVA), suggesting that the mesh cover closely mimics the *Lemna* cover (Fig. 3a).

Subsurface DO differed significantly between the cover densities ($F_{3,32} = 115.11$, $P < 0.01$, ANOVA) and between the cover types ($F_{3,32} = 7.80$, $P < 0.01$, ANOVA) (Fig. 3b). Further analysis showed that the significant difference between the cover types only occurred in the high-cover treatment ($P < 0.01$, t test). The biomass of filamentous green algae declined significantly with increasing cover intensities in both the *Lemna* cover ($\chi^2 = 10.55$, $P < 0.05$, Kruskal–Wallis test) and the mesh cover ($\chi^2 = 11.76$, $P < 0.01$, Kruskal–Wallis test) (Fig. 3c). Paired comparison indicated that the biomass of filamentous algae was significantly higher in mesh cover than in *Lemna* cover under low- and mid-cover treatments ($P < 0.05$, t test). The filamentous algae was almost absent in the high-cover treatments of the two cover types.

TN concentrations differed significantly between the two cover types ($F_{1,32} = 6.43$, $P < 0.01$, ANOVA) and among the four cover densities ($F_{3,32} = 4.26$, $P < 0.05$, ANOVA) (Fig. 3d), while TP concentrations differed significantly between the two cover types ($F_{3,32} = 9.93$, $P < 0.01$, ANOVA) but not among the four cover densities ($F_{1,32} = 0.27$, $P > 0.05$, ANOVA) (Fig. 3e). The two variables were significantly lower in mesh cover than in *Lemna* cover under high-cover treatments ($P < 0.05$, t test); however, TN was significantly higher for mesh cover than *Lemna* cover under mid-cover treatments ($P < 0.05$, t test).

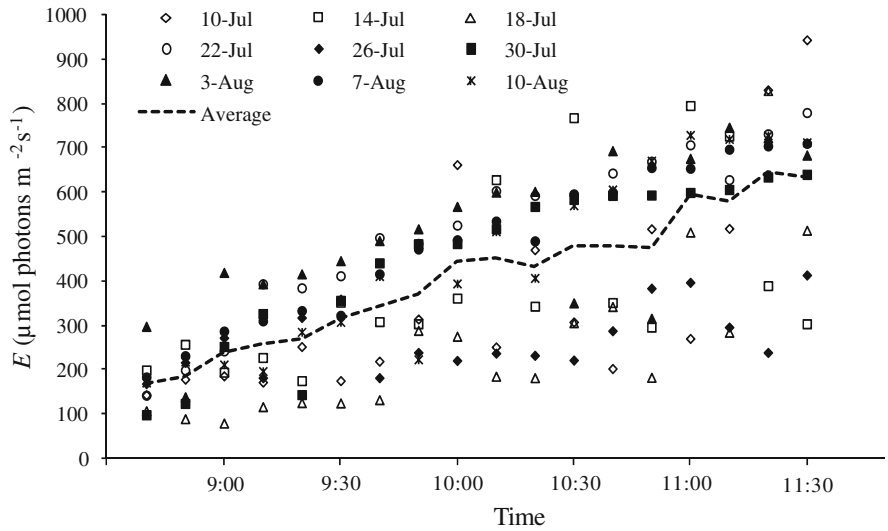

Fig. 2 Light intensity and average for every 10 min in experimental glasshouse from 8:30 AM to 11:30 AM for every 4 days over the experimental period. The light-compensation points are 9.4 µmol m^{-2} s^{-1} in *V. natans* and 27.5 µmol m^{-2} s^{-1} in *M. spicatum* at 20°C in studied region (Su et al., 2004)

Growth of submerged macrophytes

The length of the longest leaf, the number of ramets and RGR of *V. natans* varied significantly over cover intensity and substrate type (Table 1; Fig. 4). The three variables declined significantly with decreasing clay content. On clay and mixed substrates, the length of the longest leaf had a mild increase under low- and mid-cover conditions compared to CK, and then decreased sharply under a high-intensity cover. Significant differences in the number of ramets and RGR were detected between the two cover types. There were significantly more ramets in *Lemna* cover than in mesh cover ($P < 0.05$, Kruskal–Wallis test) on clay substrate with low cover and on mixed substrate with mid cover. On clay and mixed substrates, RGR declined linearly with increasing cover intensity in mesh cover, but mildly increased under low- and mid-cover conditions of *Lemna* compared to CK. RGR was significantly higher in *Lemna* cover than in mesh cover, on clay substrate with low and mid cover, mixed substrate with mid cover and sand substrate with low and mid cover ($P < 0.05$, *t* test). Significant interactions were also detected between cover type and cover intensity and between substrate type and cover intensity.

Similar to *V. natans*, the length of the longest shoot, the number of lateral shoots and RGR of *M. spicatum* showed significant differences among the cover intensities and between the substrate types (Table 1; Fig. 4). The cover types significantly influenced RGR of *M. spicatum*, which was significantly higher in *Lemna* cover than in mesh cover, on clay substrate with mid cover and mixed substrate with low and mid cover ($P < 0.05$, *t* test). Unlike *V. natans*, however, the length of the longest shoot of *M. spicatum* was significantly different between the two cover types but the number of lateral shoots did not show significant differences between the cover types, suggesting a different response model for the two species. Shoots grew significantly quicker in *Lemna* cover than in mesh cover, on clay substrate with mid cover, and mixed and sand substrates with low cover ($P < 0.05$, *t* test). On the other hand, although the shoot growth and RGR of *M. spicatum* declined significantly on sand substrate than on the two other substrates, the extent of decline was markedly smaller compared to that of *V. natans*, suggesting *M. spicatum* has a relatively stronger ability to adapt to the sand substrate.

Chlorophyll fluorescence

The F_v/F_m values were significantly higher after 20 or 30 days of treatment than after 10 days for both species (Fig. 5; $P < 0.01$, ANOVA). The significant differences in F_v/F_m values occurred mainly among the cover intensities (Fig. 5; Table 1) and between the substrate types (Table 1). However, *V. natans* presented a significant difference in the cover types after

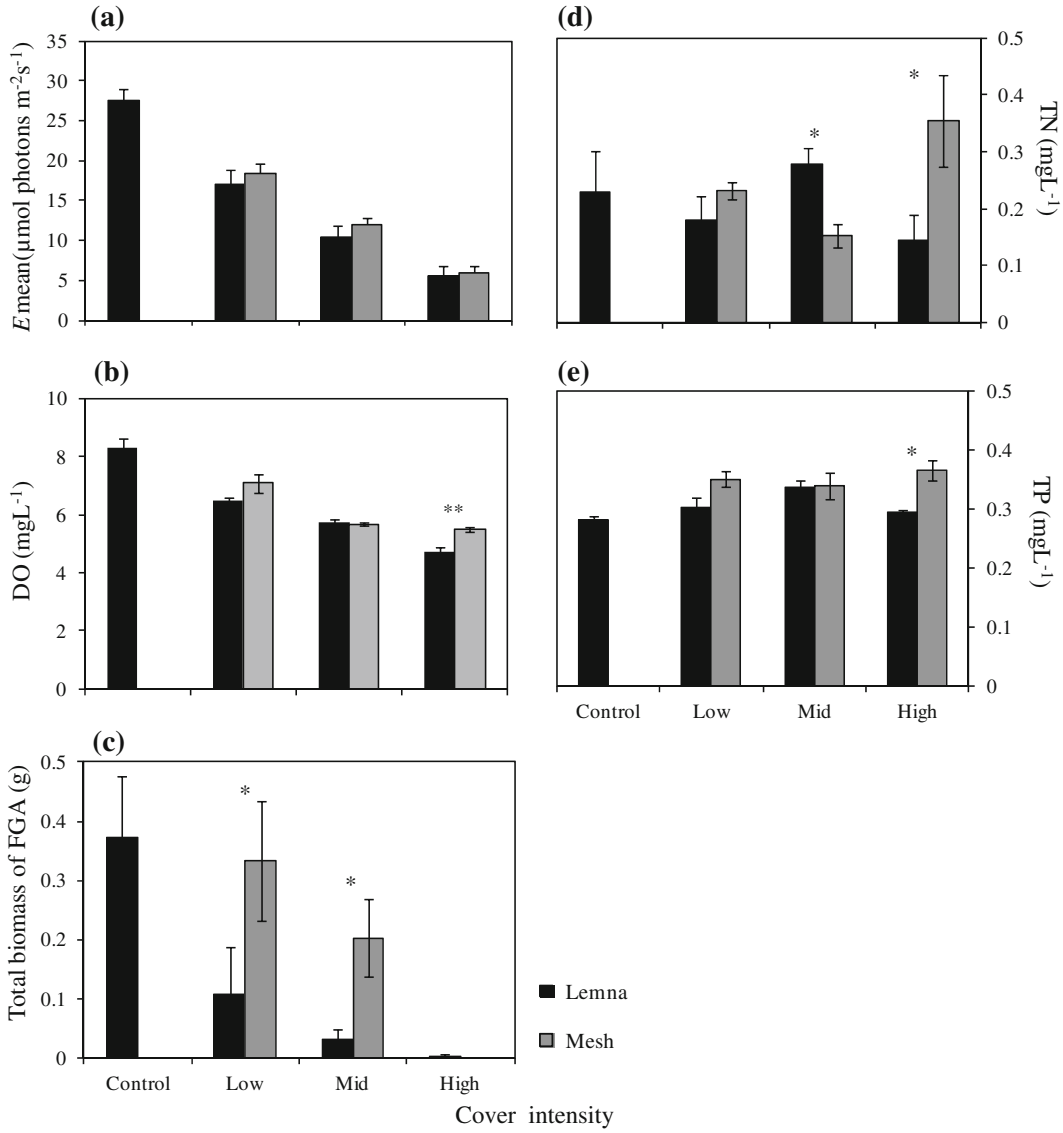

Fig. 3 Variations in **a** mean irradiances, **b** subsuperficial (5 cm depth) dissolved oxygen (*DO*), **c** total biomass of filamentous green algae (*FGA*), **d** total nitrogen (*TN*), and **e** total phosphorus (*TP*) under different treatments at the end of experiment. Values represent mean ± SE, $n = 5$. * and ** indicate significant differences between the two cover types at 0.05 and 0.01 levels by paired-sample *t* test or Mann–Whitney test, respectively

20 days of treatment, and significant interactions were also detected between cover type and cover intensity after 10 and 20 days of treatment. There were no significant correlations between F_v/F_m and cover types for *M. spicatum*, except for an interaction between cover type and cover intensity after 20 days of treatment. Moreover, it should be noted that F_v/F_m values showed a steady increasing trend with increasing cover intensities for most groups, especially after 10 days of treatment.

Discussion

The experimental cover and substrate treatments engendered various significant results. One conclusion is that low- and mid-cover densities generally enhanced the leaf/shoot growth of both submerged macrophytes under most cover types and substrate types. RGR did not decrease significantly, and was even mildly enhanced in several cases, in the treatment of *Lemna* cover with low- and mid-intensity covers on

Table 1 Summary of three-way ANOVAs (F-values) showing of the effects of cover type, cover intensity and substrate type on the relative length of the longest leaf/shoot, number of ramets/lateral shoots, relative growth rate and F_v/F_m after 10, 20 and 30 days of treatment

	d.f.	Relative length of the longest leaf/shoot	Number of ramets/ lateral shoots	Relative growth rate	F_v/F_m		
					10 days	20 days	30 days
Vallisneria natans							
Cover type (C)	1	3.57	15.39**	2.69	2.72	12.20**	0.96
Cover intensity (D)	2	19.33**	36.68**	56.19**	16.66**	1.29	6.10**
Substrate type (S)	2	145.50**	5.95**	132.08**	5.52**	1.36	5.99**
C × D	2	5.19**	8.94**	8.53**	12.46**	4.80*	2.59
C × S	2	3.19	2.79	1.79	0.64	1.05	0.46
S × D	4	7.18**	3.00*	5.16**	2.80*	3.05*	5.52**
C × S × D	4	0.59	2.02	1.36	0.5	0.59	0.31
Myriophyllum spicatum							
Cover type (C)	1	8.66**	0.90	7.45**	3.55	0.8	2.69
Cover intensity (D)	2	48.44**	14.20**	44.72**	15.43**	5.37**	3.65*
Substrate type (S)	2	12.61**	5.76**	4.85**	2.49	5.01**	28.55**
C × D	2	0.12	3.01	2.35	1.48	4.07*	1.59
C × S	2	1.14	0.62	2.52	0.64	2.70	0.82
S × D	4	2.13	0.85	2.62*	0.72	1.28	2.51*
C × S × D	4	1.18	1.97	1.98	0.71	0.45	1.07

* and ** indicate the significance at 0.05 and 0.01 levels, respectively

clay and mixture substrates. These results corroborate our hypothesis that on nutrient-rich substrates, submerged macrophytes can successfully develop under a low-intensity cover of floating vegetation. The biomass of both submerged macrophytes generally increased more under the *Lemna* cover than the mesh cover, which aligns with our initial hypothesis that other mechanisms, besides shade, may also be responsible for the submerged macrophyte growth. It was confirmed in this experiment that macrophytes have faster growth in length of longest leaf/shoot, RGR and ramet/shoot number on nutrient-rich clay and mixed substrates than on nutrient-deficient sand—a finding that has been well documented in the literatures (Xie et al., 2007; Jiang et al., 2008). The increased F_v/F_m values with both the duration of treatment and the cover intensity could have been caused by a higher proportion of open reaction centres (higher values of F_m), which could be attributed to an increase in Chl *a* content and PSII efficiency (Parr et al., 2002; Redondo-Gómez et al., 2007). These suggest that both studied macrophytes might be adapted fast to low solar irradiance from shading. Moreover, our results demonstrate that these two macrophytes with different growth forms have clearly different growth strategies in response to cover densities.

Our result indicated that the growth of the longest leaf/shoot of both submerged macrophytes was stimulated mildly by low- and mid-cover, but not high-cover conditions. A similar increase in shoot length at low light has been found in laboratory studies with *Potamogeton crispus* (Tobiessen & Snow, 1984) and *Vallisneria americana* (Morris et al., 2004). In a cover experiment with free-floating *Azolla filiculoides* and *Lemna minuta*, Janes et al. (1996) found that *Elodea nuttallii* significantly elongated, but *P. crispus* showed no such response under same cover conditions. Therefore, the growth stimulation in response to low light is apparently intensity-dependent and species-dependent.

Although direct light depletion seems to have an important effect on biomass production in our experiments, it is probably not the only factor explaining RGR variations of both submerged species under different cover densities. The fact that RGR of both species were much higher under *Lemna* cover than mesh cover at low- and mid-cover densities, suggests

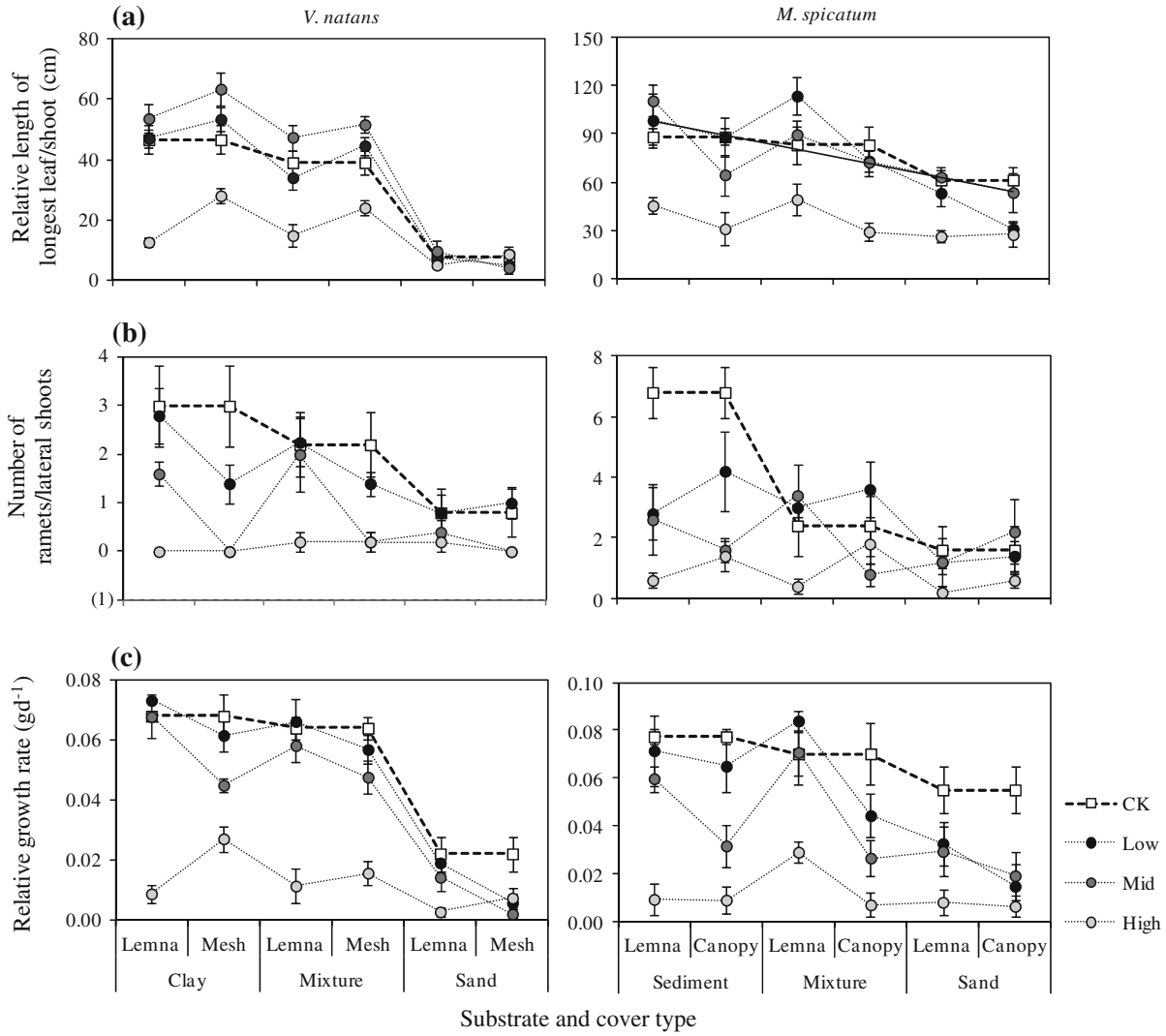

Fig. 4 Variations in **a** relative growth of the longest leaf or shoot, **b** number of ramets or lateral shoots, and **c** relative growth rate of *V. natans* and *M. spicatum*, respectively, under different treatments. Values represent mean ± SE, $n = 5$

that, additional mechanisms may play a role. In the current experimental system, despite the small differences in light intensity and DO concentration that occurred between both cover types, both variables were generally higher under mesh cover than *Lemna* cover and thus should be more conducive to RGR. Thus, we assume that the different responses of RGR between both cover types could indeed be attributed to the emergence of unexpected filamentous green algae. Experimental evidence has reported that filamentous algae have negative effects on the growth of submerged plants (Phillips et al., 1978; Simpson & Eaton, 1986; Ozimek et al., 1991; Asaeda et al., 2004; Sultana et al., 2010). In the current experiment, the biomass of filamentous algae was significantly higher in mesh cover than in *Lemna* cover at low- and mid-cover densities. The filamentous algae that appeared may further shade submerged plants. However, the mechanism that causes the difference in biomass of filamentous algae between the two cover types is unclear. We suppose that filamentous algae might suffer more from nutrient limitation under *Lemna* cover than mesh cover, since both free-floating macrophyte and filamentous algae can only acquire nutrients from the water column. This hypothesis is supported by the fact that both TN and TP

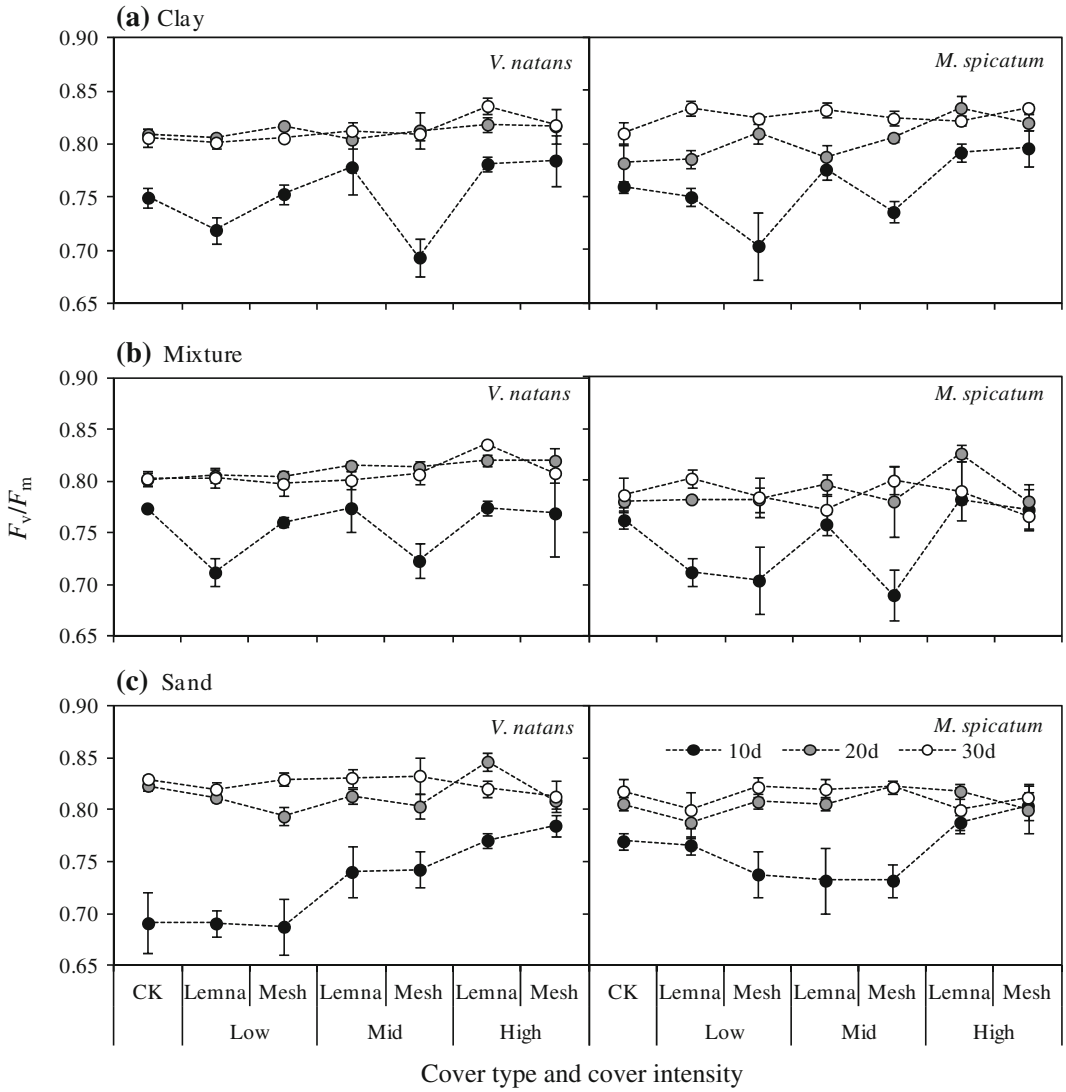

Fig. 5 Maximum quantum efficiency of PSII photochemistry (F_v/F_m) at mid-day in randomly selected, fully expanded leaves of *V. natans* and *M. spicatum* in response to different treatments for 10, 20 and 30 days. Values represent mean ± SE, $n = 5$

concentrations were significantly lower in mesh cover than in *Lemna* cover in high-cover treatments, but inconsistent with the fact that a higher TN concentration was present in the medium *Lemna* cover condition. It is unclear whether such an increase was due to the decomposition of *L. minor* and/or filamentous algae, which could result from nutrient-limited death. Moreover, *L. minor* that was present may also have an allelopathic effect on the alga (Parr et al., 2002). Our reported results appear to be the first describing the inductive effect of free-floating macrophytes on the growth of submerged macrophytes. However, the interaction mechanism among free-floating macrophyte, filamentous algae and submerged plants still needs further clarification.

Our results confirm that these two submerged species with different growth forms have clearly different tolerance levels and growth strategies in response to cover shade. The rosette-forming *V. natans* increased its biomass by producing more ramets in relatively favourable low- and mid-cover conditions and nutrient-rich substrates, while the canopy-forming *M. spicatum* adopted a strategy of rapid elongation. In line with these results, *Vallisneria* is an acknowledged

low-light-tolerant species. It has been reported that *Vallisneria* can persist under a dense canopy of *M. spicatum* (Boylen et al., 1999), and can even survive over 3 months beneath 100% shading (Blanch et al., 1998, Morris et al., 2004). It should be noted that despite the fact that these two species with different growth forms adequately tolerated severe light attenuation, the current conclusion is obtained from a small spatial scale and short-term experiment. However, the special capability of canopy-forming species with rapid elongation should allow them to rapidly colonise the subsurface layer and hence more effectively avoid damage imposed by a reduction of light intensity and dissolved oxygen. If allowed a larger temporal or spatial scale, we presume that the canopy-form species should adapt even better to free-floating cover.

Increased nutrient loading and asymmetric competition between floating and submerged species may initiate a switch to floating plant domination (Scheffer et al., 2003). However, such a switch could be misjudged if the competitive capability or tolerance of submerged plant to free-floating macrophyte is underestimated. Our results, in conjunction with current research in this field, demonstrate that substantial submerged growth is still possible under a low- and mid-intensity cover of free-floating macrophytes. Mechanisms of rapid stem elongation or high tolerance to low-light intensity might mainly contribute to the response and adaption of submerged plants to cover of free-floating macrophytes.

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