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RESEARCH ARTICLE

Root-foraging behavior ensures the integrated growth of *Vallisneria natans* in heterogeneous sediments

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Abstract The present study was carried out to determine the efficacy of root foraging and the physiological response of *Vallisnaria natans* grown in heterogeneous sediments. *V. natans* was cultivated in two homogeneous and two heterogeneous sediments. The results suggested that *V. natans* grown in heterogeneous sediments presented a significantly higher root proportion in its total biomass, exhibited root foraging, and grew well, as indicated by a total biomass, ramet number, and plant height very close to those of plants grown in nutrient-rich clay sediment. Moreover, the more sensitive physiological response of the roots than the stems or the leaves to sediment nutrients suggested that root foraging occurred, and the approached values between the two heterogeneous sediments and the homogeneous clay sediment indicated that *V. natans* could satisfy its nutrient requirements via root foraging. The results may be useful in the recovery of macrophytes that remodel part (rather than all) of the substrate and can potentially improve habitats that are unsuitable for plant growth.

Keywords Free amino acids · Heterogeneous sediments · Phosphorus · Root foraging · Soluble carbohydrates · Submersed macrophytes · *Vallisneria natans*

Introduction

Submersed macrophytes are large aquatic plants that grow under water and can absorb nutrients via both their roots and leaves (Cao et al. 2011; Xie et al. 2005a, b). Submersed macrophytes are the primary producers in water ecosystems and provide food and habitats for many aquatic animals, in addition to being crucial for stable water clarity in shallow, mesotrophic, and eutrophic lakes (Jeppesen et al. 1998; Perrow et al. 1997; Scheffer 1998; van Donk and van de Bund 2002). The growth of submersed macrophytes is affected by many biological and abiotic environmental factors. The sediment is one of the most important factors that determine the growth, distribution, and community structure of submersed macrophytes (Barko et al. 1986, 1991; Chen et al. 2016). The sediment provides a substrate that contains the necessary nutrients for submersed macrophytes (Chambers et al. 1989), i.e., micronutrients and the macroelements nitrogen (N) and phosphorus (P), which have the greatest potential to limit macrophyte productivity in an aquatic system (Barko et al. 1988, 1991). Because of the complex conditions in the aquatic environment, sediments exist in many forms in lakes, such as homogeneous and ubiquitous heterogeneous sediments

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(Hutchings and de Kroon 1994; Hutchings et al. 2000). Hence, nutrient availability for submersed macrophytes in their natural habitat is always heterogeneous in both space and time, even on a small scale (Hutchings et al. 2000; Xiao et al. 2006; Xie et al. 2007). Many studies have examined the physiological and/or morphological responses of submersed macrophytes to heterogeneous sediments. For example, Xie et al. (2007) reported that *Vallisneria natans* tended to allocate more biomass to the roots when the clay in the top layer was a sandy loam with a poor nutrient content, and the root number and total root length in the bottom layer, with a rich nutrient content, were greater. Furthermore, the sediment type significantly impacts growth and N and P concentrations in *V. natans*, which exhibits increased N and P concentrations when growing in heterogeneous and nutrient-rich patches (Xie et al. 2005a). However, it is necessary to obtain more data concerning the root foraging of submersed macrophytes in heterogeneous sediments. Some previous studies on submersed macrophytes grown in different sediments and under different water column nutrient availability conditions have obtained inconsistent results. For example, Madsen and Cedergreen (2002) and Barko et al. (1988) found that *Elodea canadensis*, *Callitriche cophocarpa*, and *Hydrilla verticillata* presented a decreased root:shoot biomass ratio in response to nutrient availability in the sediment. However, Xiao et al. (2006) and Xie et al. (2005a, 2007) reported that *V. natans* displayed a higher root:shoot biomass ratio in response to sediment nutrient availability. The present study provides additional data on the response of submersed macrophytes to sediment nutrient availability.

Clonal plants grown in a heterogeneous nutrient environment usually exhibit foraging, such as morphological plasticity, i.e., selective placement of resource-acquiring structures in an advantageous location to enhance resource acquisition (Hutchings and de Kroon 1994; Xiao et al. 2006; Xie et al. 2006; Xie et al. 2007; Xie et al. 2013). Xiao et al. (2006) reported that *Vallisneria spiralis* allocated a higher proportion of its biomass to rich patches when established in rich patches, whereas a higher proportion of the ramet number was allocated to rich patches when this species was established in poor patches. Many submersed macrophytes can adjust their biomass allocation in sediments with heterogeneous nutrient conditions to acquire more nutrients. For example, a submersed macrophyte would allocate more biomass to the roots when grown in a nutrient-poor sediment than in a nutrient-rich sediment and would allocate greater root mass to nutrient-rich patches when grown in a sediment with a heterogeneous nutrient distribution. This phenomenon has been termed root foraging (Xiao et al. 2006; Xie et al. 2007). Root foraging enables a submersed macrophyte to optimize its nutrient utilization in a habitat with heterogeneous nutrient availability and is important for growth (Wang and Yu 2007; Xiao et al. 2006; Xie et al. 2006, 2007; Xie et al. 2013). However, the degree to which a submersed macrophyte may alter its growth in a heterogeneous sediment by root foraging is still unknown.

Changes in the carbon (C) and N metabolism of submersed macrophytes have been associated with external nutrient availability (Cao et al. 2011). The total non-structural carbohydrates (TNCs; including soluble sugars and starch) of plants are important for plant survival in an adverse environment and for normal growth (i.e., plant tissue elongation) (Cao et al. 2011; Huber et al. 2012; Xie et al. 2013). Submersed macrophytes grown in an adverse environment (such as a low-nutrient environment) usually present a higher concentration of TNC in the shoots and roots (Puijalon et al. 2008; Xie and Yu 2011), but the TNC concentration varies in different submersed macrophytes under different nutrient conditions (Cao et al. 2011). Xie et al. (2005a) noted that the plant phosphorus (P) concentration is usually correlated with external P availability, and a lack of P or other minerals can lead to alterations in the accumulation of free amino acids (FAAs) in plant tissues (Smolders et al. 1996). Hence, measurements of the nutrient content of plant tissues reflect the conditions of external resource availability as well as the response of the plant organs of a submersed macrophyte to external nutrient availability.

Vallisneria natans (Lour.) *H. hara* is an important species in the submersed macrophyte community in many freshwater habitats in China, especially in the middle and lower reaches of the Yangtze River. It is a predominant submersed vegetation species in clear water habitats and is often used for the restoration of aquatic vegetation. In this study, *V. natans* was cultured in four sediment types, which included two homogeneous sediments (i.e., sand and clay) and two heterogeneous sediments (alternating patches of sand and clay). The purpose of this study was to test the following hypotheses: (1) *V. natans* would exhibit root-foraging behavior in heterogeneous sediments due to an uneven nutrient distribution; (2) the root response to sediment nutrients would be more sensitive than that of the leaves and stems because root foraging would occur to ensure nutrient acquisition, and the roots would seek out particular patches in the sediment; and (3) *V. natans* could ensure integrated growth in heterogeneous sediments compared with its growth in homogeneous clay sediments because *V. natans* could satisfy its nutrient requirements via root foraging.

Materials and methods

Study site, sediments, and plant materials

This study was conducted from 15 June to 10 August 2012 at the Donghu Lake Ecosystem Experimental Station (30° 32′ 53.41″ N, 114° 21′ 15.63″ E) located on the edge of Donghu Lake in Wuhan City in central China. Donghu Lake was divided into six sections in the 1960s by dikes and roads, and the clay used in our experiments was obtained from the mesotrophic Tanglinhu area. The Tanglinhu area was mesotrophic; the total phosphorus (TP) content was 0.7 mg g^{-1}, and the total nitrogen (TN) concentration was 2.2 mg g^{-1} in the Tanglinhu area (Feng et al. 2006). Before the experiment, the clay was mixed well to ensure a homogeneous high nutrient concentration, and the sand was washed several times with tap water to ensure that the nutrient level was low. The *V. natans* seedlings collected from the Wuhan Botanical Garden were similar in appearance and exhibited an average height of 27.6 cm.

Experimental design

The experimental seedlings were cultured outdoors in 20 aquaria (length 50 cm, width 50 cm, height 80 cm) containing 10 cm of sediment, filled with tap water to a height of 60 cm. The bottom of each aquarium was divided into 16 patches (4 × 4) of equal area (approximately 156.3 cm^2). The 20 aquaria were allocated into 4 groups. In the first group, all of the patches were filled with clay (Ho-C). In the second group, all of the patches were filled with sand (Ho-S). In the third (He-S) and fourth (He-C) groups, the patches were alternately filled with clay or sand in a mosaic pattern, as shown in Fig. 1. Ho-C indicates the homogenous clay treatment, in which all of the sediments were clay; Ho-S indicates the homogenous sand treatment, in which all of the sediments were sand; He-S indicates the heterogeneous sediment treatment in which the seedlings were planted in sand; and He-C indicates the heterogeneous sediment treatment in which the seedlings were planted in clay. Each group contained five replicates. The four groups of aquaria were arranged side by side approximately 1 m apart. Eight seedlings were planted in eight different patches in each aquarium, with no two patches abutting each other. The seedlings were planted in the same positions in all aquaria. The seedlings were planted evenly in the Ho-C and Ho-S treatments, while they were planted in clay patches in the He-C treatment and in sand patches in the He-S treatment (Fig. 1).

In the third and fourth groups, the planting pattern was reversed. The details of the planting design are shown in Fig. 1. To provide an appropriate light intensity for *V. natans* growth, all of the aquaria were placed under a shelter covered with a black nylon net. The overlying water was replaced with tap water when algal blooms occurred during the experiment.

Measurement of environmental parameters

The TN, NH_4-N, NO_3-N, TP, PO_4-P, and chlorophyll a (Chl *a*) concentrations in the overlying water and the PO_4-P, NH_4-N, and NO_3-N concentrations in the pore water in the sand and clay sediments were measured weekly in all of the aquaria according to standard methods (Eaton et al. 1995; Golterman 1969; Lorenzen 1967). The TN and TP of water samples were measured according to Chinese standard methods (Huang et al. 1999). The water samples were filtered through a Whatman GF/C glass fiber filter (Middlesex, UK; 1.2-μm pore diameter) for the analysis of PO_4-P, NO_3-N, and NH_4-N. The PO_4-P concentration was determined using the molybdenum blue method (Golterman 1969). NO_3-N was analyzed via a UV spectrophotometric method and NH_4-N via

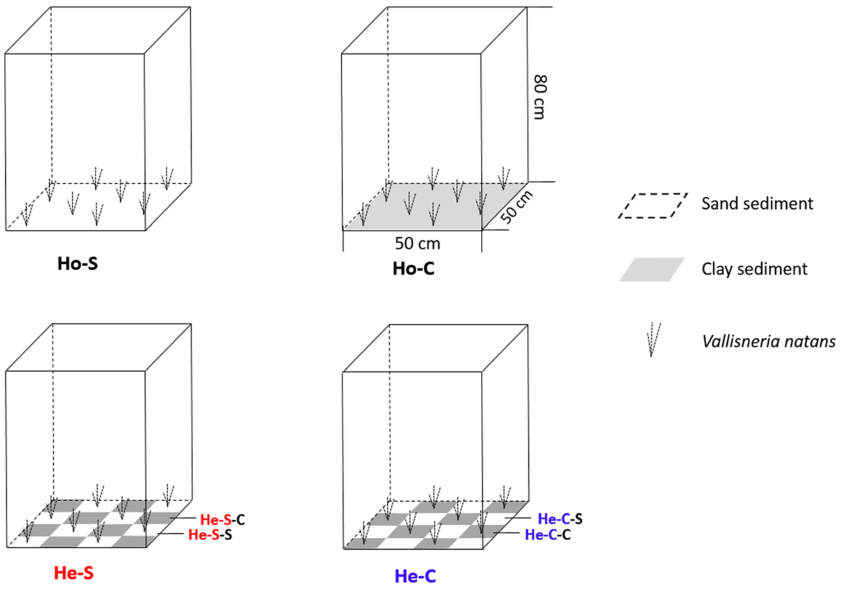

Fig. 1 The design of the experiment to measure the growth of *V. natans* in four sediment types for 57 days in aquaria filled with 10 cm of sediment and 60 cm of tap water. Ho-S and Ho-C indicate the homogeneous sand and clay sediments, respectively. He-S-S and He-S-C indicate the sand and clay sediments, respectively, in the heterogeneous treatments in which *V. natans* was planted in sand sediments, and He-C-S and He-C-C indicate the sand and clay sediments, respectively, in the heterogeneous treatments in which *V. natans* was planted in the clay sediments

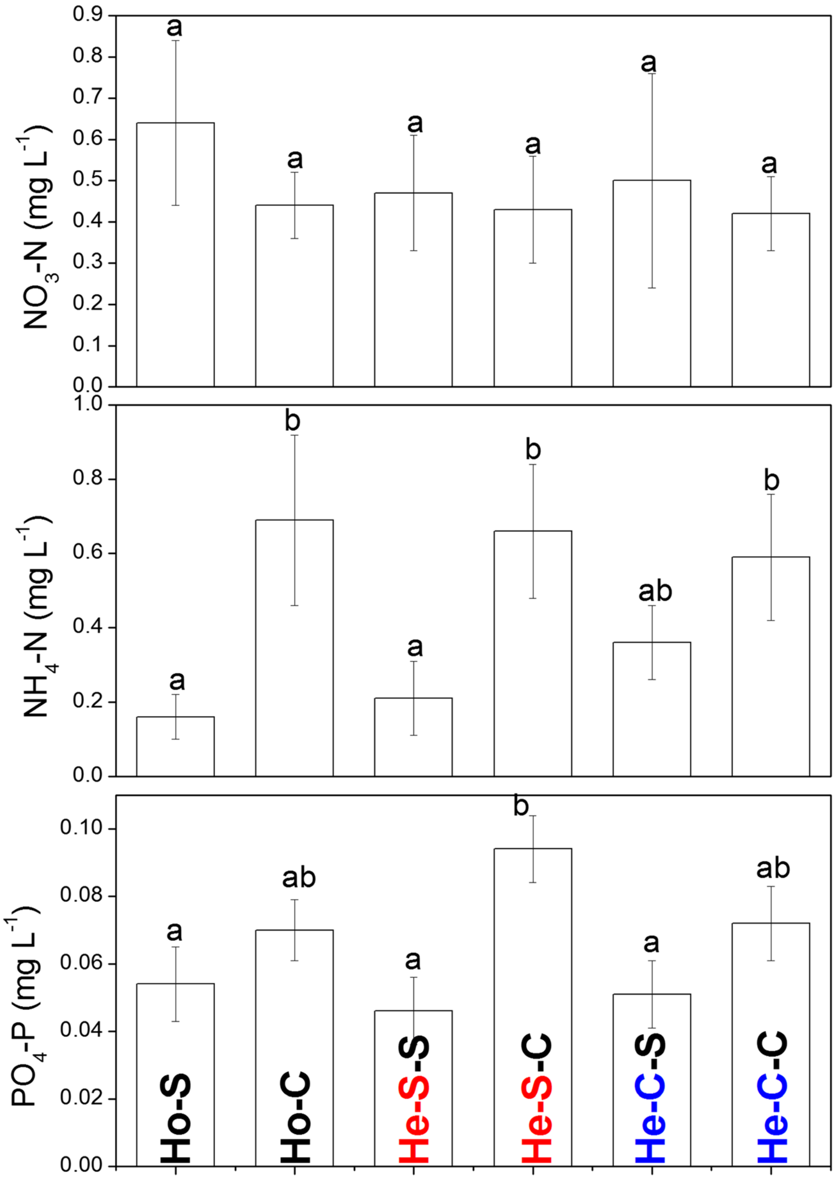

Fig. 2 Chemical contents (mean ± SE; *n* = 5) of the pore water in the sand and clay sediments in the four treatments. Ho-S and Ho-C indicate the homogeneous sand and clay sediments, respectively. He-S-S and He-S-C indicate the sand and clay sediments, respectively, in the heterogeneous treatments in which *V. natans* was planted in sand sediments, and He-C-S and He-C-C indicate the sand and clay sediments, respectively, in the heterogeneous treatments in which *V. natans* was planted in clay sediments. *Different letters* indicate the significance according to Duncan's test at the 0.05 level

the Nessler method (Eaton et al. 1995). Chl *a* was determined with a spectrophotometer (Lorenzen 1967) at 24 h after 90% acetone extraction of the residue on the glass fiber filter. pH, dissolved oxygen (DO), and water temperature (*T*) were measured with a multifunctional YSI meter (Yellow Springs Instruments, OH, USA), and the photosynthetically active radiation (PAR) at the overlying water surface was measured with a Li-COR UWQ-192S sensor coupled to an Li-1400 data logger (Li-Cor, Lincoln, NE, USA).

Plant harvest and measurements

We determined the ramet number and measured the plant height of *V. natans* in each aquarium on the 28th day. All of the plants were harvested at the end of the experiment and were then washed; dried with tissue paper; carefully divided into leaves, stems, and roots; and oven dried to constant weight at 80 °C for 72 h, followed by weighing to determine the dry weight (DW). The dried plant samples were ground into fine powder with a Planetary Micro Mill PULVERISETTE 7 premium line machine (Pulverisette 7, serial no. 07.5000/01458, FRITSCH Company, Germany) for the determination of FAA; soluble carbohydrates (SCs); starch; and C, N, and P concentrations. The C and N concentrations were determined with an elemental analyzer (Flash EA 1112, CE Instruments, Italy). P was measured via sulfuric acid/hydrogen peroxide digestion and using the ammonium molybdate ascorbic acid method (Sparks et al. 1996). Approximately 50 mg of the powder was extracted twice with 8 mL of 80% ethanol at 80 °C for 20 min. The extracts were then pooled and centrifuged at 10,000*g* for 15 min. The SC and FAA contents in the supernatant were analyzed using

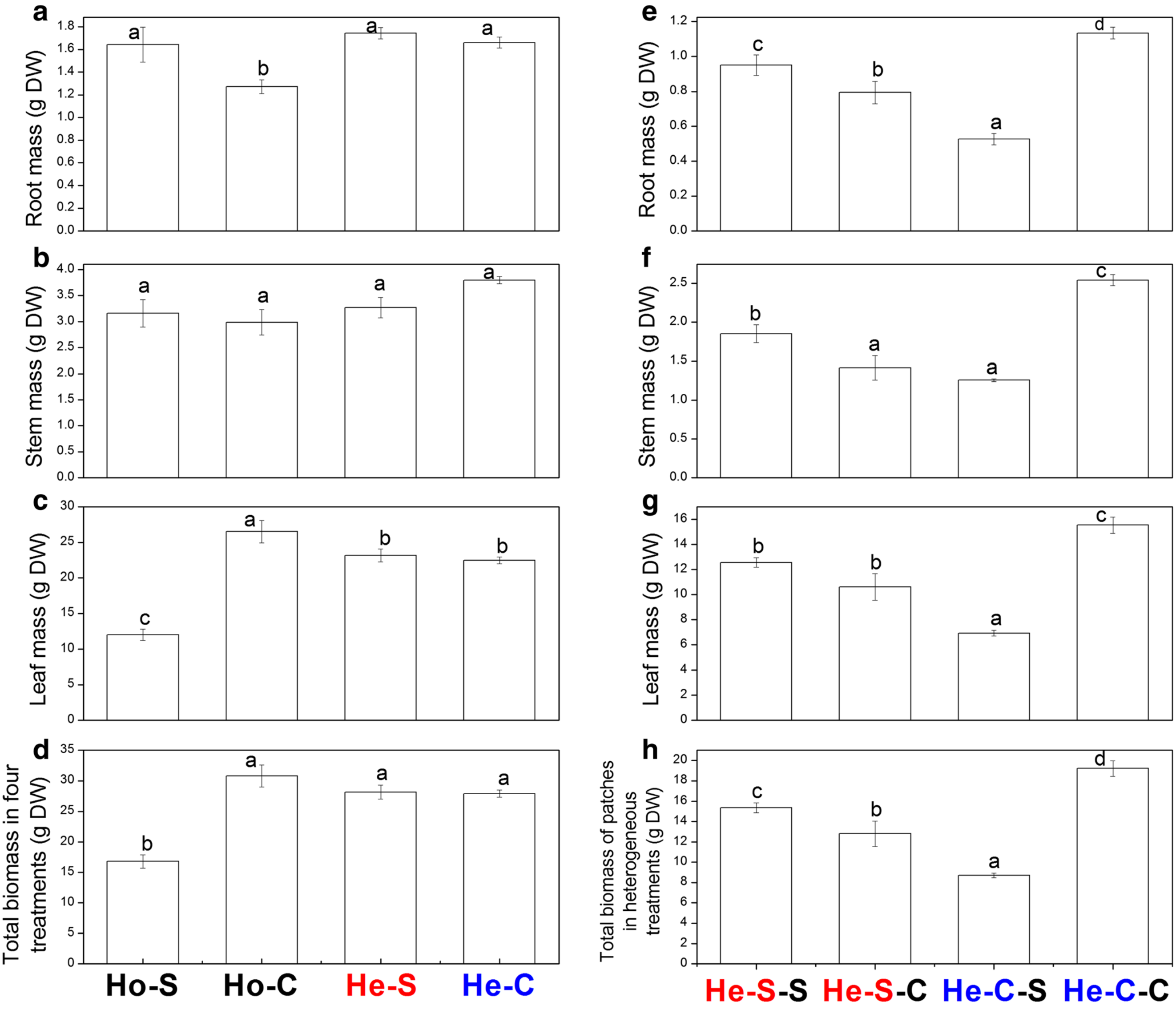

Fig. 3 Biomass accumulation (mean ± SE; $n = 5$) in *V. natans* in the four treatments. Ho-S and Ho-C indicate homogeneous sand and clay sediments, respectively; He-S and He-C indicate the two heterogeneous sediments, in which *V. natans* was planted in sand and clay sediments, respectively. He-S-S and He-S-C indicate the sand and clay sediments in the He-S treatment, respectively; He-C-S and He-C-C indicate the sand and clay sediments in the He-C treatment, respectively. *Different letters* indicate the significance according to Duncan's test at the 0.05 level

alanine and glucose as standards, respectively (Yemm and Willis 1954; Yemm et al. 1955). The starch content in the pellet was analyzed with the method of Dirk et al. (1999).

Statistical analysis

Statistical analysis was carried out with SPSS 19.0. One-way analysis of variance (ANOVA) was conducted to determine the differences in the overlying water, pore water, and plant indices among treatments. The ANOVA results were considered significant at $P < 0.05$, and multiple mean comparisons were conducted with Duncan's test (at the 0.05 significance level) to identify differences among treatments. All of data were tested for normality and homogeneity prior to one-way ANOVA. Non-normal data were Ln(x) transformed to achieve normality.

Results

Environmental parameters

The physicochemical parameters were not significantly different among the four treatments (Table S1). In the four treatments, the average values (mean ± SD) for T, DO, pH, TN, NH_4-N, NO_3-N, TP, PO_4-P, and Chl a were 29.57 ± 1.81 °C, 10 ± 2.31 mg L^{-1}, 9.23 ± 0.45, 1.51 ± 0.41 mg g^{-1}, 0.02 ± 0.01 mg g^{-1}, 1.15 ± 0.45 mg g^{-1}, 0.03 ± 0.01 mg g^{-1}, 0.005 ± 0.005 mg g^{-1}, and 25.93 ± 49.24 μg L^{-1}, respectively.

The NO_3-N concentration in the sediment interstitial water was not significantly different between the clay and sand sediments, but the PO_4-P and NH_4-N concentrations were higher in the clay than in the sand (Fig. 2).

Biomass accumulation and biomass allocation

Biomass accumulation by *V. natans* showed significant treatment differences (Fig. 3a–d). *V. natans* accumulated a greater root mass in the sand sediment than in the clay; the root mass (DW) in Ho-S was 29.13% higher than in Ho-C. The values between the two heterogeneous treatments and Ho-S were very similar (Fig. 3a). No significant differences in stem mass (DW) accumulation were apparent among the four treatments (Fig. 3b). However, the leaf mass and total biomass (DW) in Ho-C and the two heterogeneous treatments were much higher than in Ho-S, and the values between the two heterogeneous treatments and Ho-C were nearly approached (Fig. 3c, d).

The leaf mass in Ho-C was 121.28% higher than in Ho-S and was 16.22% higher than in the two heterogeneous treatments. The total biomass in Ho-C was 83.37% higher than in Ho-S but was only 9.70% higher than in the two heterogeneous treatments (Fig. 3c, d).

In the two heterogeneous treatments, biomass accumulation was significantly different between the sand and clay sediments. When *V. natans* had previously been established in clay patches, it accumulated much more biomass in clay sediment than in sand sediment. However, when it had previously been established in sand patches, *V. natans* tended to escape and accumulate more biomass in the clay sediment (Fig. 3e–h).

Biomass allocation in *V. natans* also showed significant treatment differences (Fig. 4a–c). *V. natans* allocated more biomass to the roots and stems in the nutrient-poor sediments. The root mass/total biomass proportion (DW) was highest (0.098) in Ho-S, intermediate (mean 0.061) in He-S and He-C, and lowest (0.042) in Ho-C (Fig. 4a). The stem mass/total biomass proportion (DW) was highest (0.188) in Ho-S, intermediate (mean 0.097) in He-S and He-C, and lowest (0.126) in Ho-C (Fig. 4b). In contrast, *V. natans* allocated more biomass to the leaves in nutrient-rich sediments. The leaf mass/total biomass proportion (DW) was highest (0.862) in Ho-C, intermediate (mean 0.813) in He-S and He-C, and lowest (0.714) in Ho-S (Fig. 4c). However, the biomass allocation between the sand and clay patches was very similar in the two heterogeneous patches, and no significant differences in the root mass/total biomass proportion between the patches were evident (Fig. 4d–f).

Physiological responses of *V. natans* to sediment nutrients

V. natans grown in Ho-S exhibited a significantly higher root SC concentration than in Ho-C. Furthermore, the root SC concentration in the sand patches was also significantly higher than in the clay patches in the two heterogeneous treatments. Specifically, the SC concentration in Ho-S was 201.66% higher than in Ho-C (Fig. 5a). However, the SC concentration in the stems did not vary significantly among the treatments (Fig. 5a). The leaves of individual plants displayed a much higher SC content in Ho-S than in the other treatments, but no significant differences were apparent among the other treatments (Fig. 5c). The root starch concentration was significantly affected by the treatment, with the highest values (32.0 mg g^{-1}) being in the two heterogeneous treatments, an intermediate value (24.5 mg g^{-1}) in Ho-C, and the lowest value (17.1 mg g^{-1}) in Ho-S (Fig. 5d). The leaf starch concentrations in Ho-C, He-S-S, He-S-C, He-C-S, and He-C-C were similar and significantly lower than in Ho-S (Fig. 5f). Similar to the root SC content, the root FAA concentration in the sand sediment was significantly higher than in the clay sediment, with root FAA being 50% higher in Ho-S than in Ho-C, whereas the values between the two heterogeneous treatments and Ho-C were very similar (Fig. 5g). However, no significant differences in the stem FAA and leaf FAA concentrations were apparent between the clay and sand sediments (Fig. 5h, i).

The root C concentration was significantly higher in the sand sediment than in the clay sediment. The value in Ho-S was 8.37% higher than in Ho-C, and this concentration was 13.48% higher in the sand patches than in the clay patches in the two heterogeneous treatments (Fig. 6a). Except for leaf C in He-S-S, no significant differences in the leaf C concentration were evident among the treatments (Fig. 6c). The root N concentration was higher in the clay sediment than in the sand sediment, but this difference was not significant, and the values did not differ among treatments (Fig. 6d). The root P concentration in Ho-C was 227.78% higher than in Ho-S and 43.90% higher than in the two heterogeneous treatments (Fig. 6g). The stem and leaf P concentrations were similar to the root P concentration (Fig. 6h, i).

The individual SC and starch concentrations were significantly higher in Ho-S than in the other treatments (Fig. 7a, b). The value in Ho-S was 64.7% higher than the average value for the two heterogeneous treatments, and the values for the SC concentration were similar between the two heterogeneous treatments and Ho-C (Fig. 7a). The value for the starch concentration in the Ho-S treatment was 70.8% higher than the average value for the two heterogeneous treatments, which was 25.6% higher than the value in Ho-C (Fig. 7b). The individual FAA concentrations were not significantly different among treatments, and the C concentration was similar among treatments (Fig. 7c, d). The individual N concentration in Ho-S was significantly lower than in the other treatments, and the value in the two heterogeneous treatments was similar to the value in Ho-C and 12.8% higher than in Ho-S (Fig. 7e). The individual P concentration in Ho-S was significantly lower than in the other treatments,

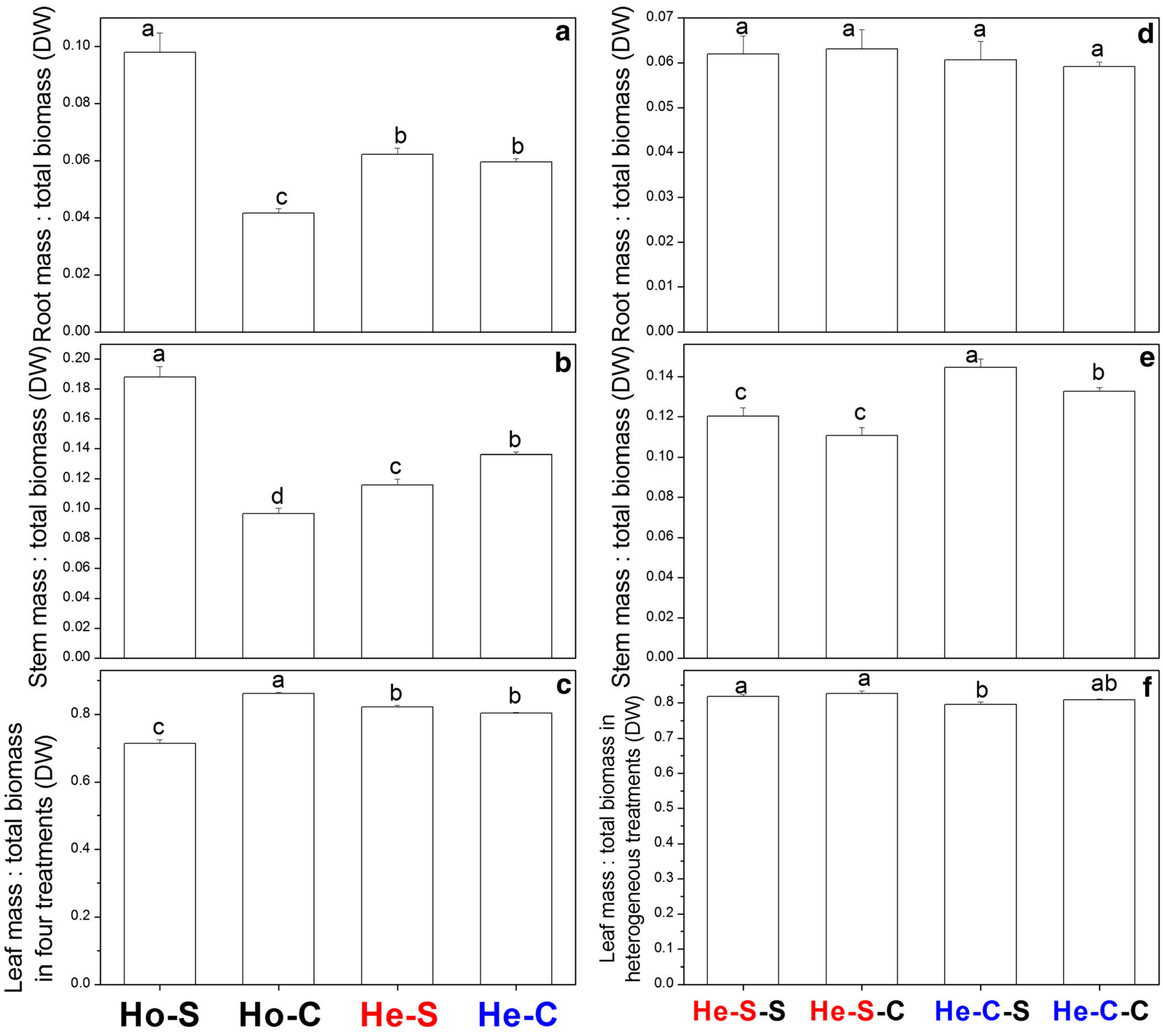

Fig. 4 Biomass allocation (mean ± SE; $n = 5$) in *V. natans* grown in the four treatments and in sand and clay sediments in the two heterogeneous treatments. Ho-S and Ho-C indicate the homogeneous sand and clay sediments, respectively; He-S and He-C indicate the two heterogeneous sediments in which *V. natans* was planted in sand and clay sediments, respectively. *Different letters* indicate the significance according to Duncan's test at the 0.05 level

and the values between two heterogeneous treatments and Ho-C were similar. However, the values in the two heterogeneous treatments were 133.3% higher than in Ho-S (Fig. 7f).

Ramet number and plant height

Ramet number and plant height showed significant treatment effects (Fig. 8). The ramet number was highest (33) in Ho-C, intermediate (mean 25.3) in He-S and He-C, and lowest (18.4) in Ho-S (Fig. 8a). Plant height was highest (37.5 cm) in Ho-C, intermediate (mean 36.2 cm) in He-S and He-C, and lowest (28.3 cm) in Ho-S (Fig. 8b).

Discussion

The overlying water was replaced during the experiment when an algal bloom occurred. The physiochemical parameters in the overlying water that might have affected *V. natans* growth, including PAR, *T*, DO, pH, NO_3-N, NH_4-N, PO_4-P, TN, TP, and Chl *a*, showed no significant differences among the four treatments. However, the concentrations of NH_4-N and PO_4-P in pore water were significantly different among the treatments, and we therefore concluded that the sediment type was the major factor determining the results.

V. natans exhibited root foraging, as indicated by differential biomass accumulation and biomass allocation among the

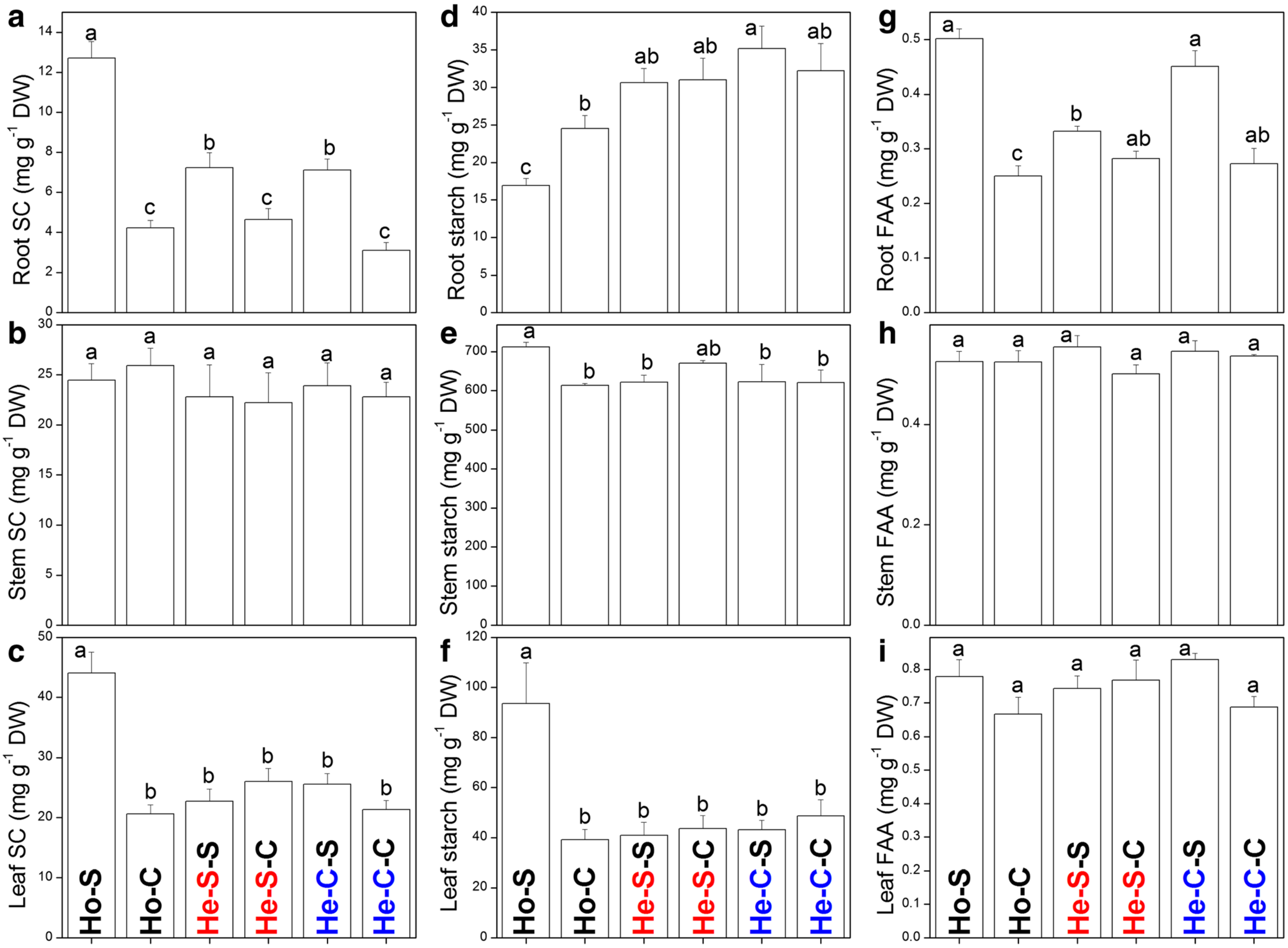

Fig. 5 Contents (mean ± SE; $n = 5$) of soluble carbohydrate (SC), starch, and free amino acids (FAAs) in the organs of *V. natans*. Ho-S and Ho-C indicate the homogeneous sand and clay sediments, respectively. He-S-S and He-S-C indicate the sand and clay sediments, respectively, in the heterogeneous treatments in which *V. natans* was planted in sand sediments, and He-C-S and He-C-C indicate the sand and clay sediments, respectively, in the heterogeneous treatments in which *V. natans* was planted in clay sediments. *Different letters* indicate the significance according to Duncan's test at the 0.05 level

treatments. In this experiment, *V. natans* showed both a global response in the four artificial soil treatments and a local response to the patches in the two heterogeneous treatments. The global response was characterized by the accumulation of more root and stem mass and the allocation of more biomass to the roots and stems in nutrient-poor sand sediments, which was consistent with previous studies (Barko et al. 1988; Wang and Yu 2007; Xie et al. 2005a, 2007; Xie et al. 2013) and presumably allowed more effective nutrient acquisition. Shifts in biomass allocation due to foraging under different environmental conditions ensure that macrophytes can minimize resource imbalances and maximize their growth rate (Barko et al. 1991; Xie et al. 2005a). An increase in the proportion of roots to the total mass could increase the exposed absorptive surface area in nutrient-poor sediment, which would be advantageous for nutrient acquisition in a non-fertile environment. An increase in the stem mass proportion could increase the chance of placing ramets in nutrient-rich patches during growth in nutrient-poor patches (Barko et al. 1991; Xie et al. 2007). The global response of *V. natans* in the four artificial soil treatments indicated the occurrence of root foraging.

The local response of *V. natans* to the patches in the two heterogeneous treatments was characterized by more roots in clay patches in plants that were initially established in clay, which was consistent with a previous findings reported by Xie et al. (2007). Xie's study showed that *V. natans* allocated a higher proportion of its root biomass to nutrient-rich patches when it was established in rich patches. However, in contrast to Xie's results, we found that *V. natans* allocated less root biomass to the clay sediment when initially established in sand. Although significant differences in root mass accumulation between the sand and the clay patches were apparent in He-S, the difference between the two sediment types was much smaller than in He-C, which indicated that root foraging by *V. natans* selectively resulted in greater root mass in clay

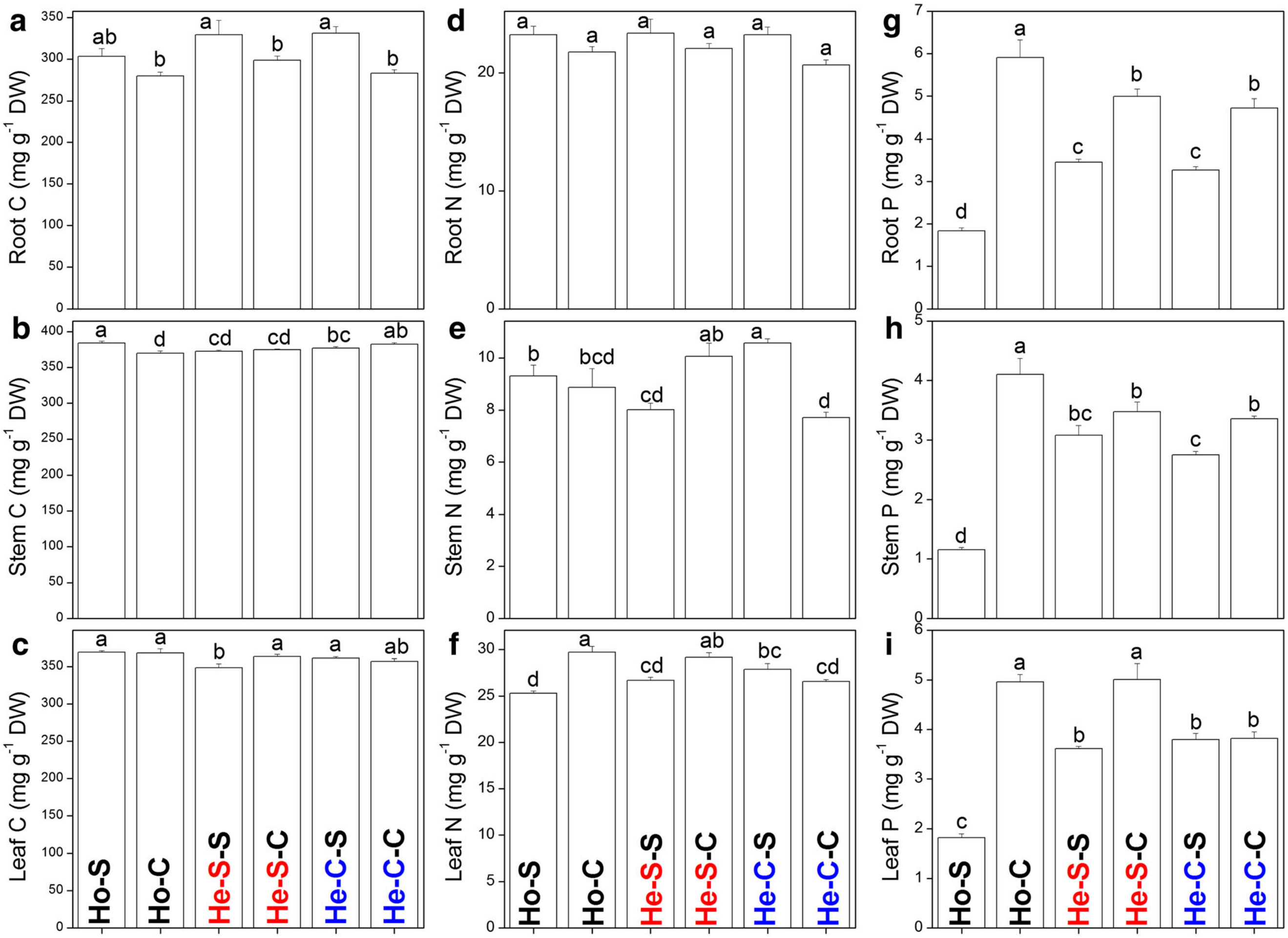

Fig. 6 Contents (mean ± SE; *n* = 5) of carbon (C), nitrogen (N), and phosphorus (P) in the organs of *V. natans*. Ho-S and Ho-C indicate the homogeneous sand and clay sediments, respectively. He-S-S and He-S-C indicate the sand and clay sediments, respectively, in the heterogeneous treatments in which *V. natans* was planted in sand sediments, and He-C-S and He-C-C indicate the sand and clay sediments, respectively, in the heterogeneous treatments in which *V. natans* was planted in clay sediments. *Different letters* indicate the significance according to Duncan's test at the 0.05 level

when initially established in sand. *V. natans* likely accumulated less root mass in clay than in sand because the patches were relatively small (the radius of each patch was only 6.25 cm). Therefore, *V. natans* could easily utilize the nutrients in the neighboring clay patches, as the root length of *V. natans* can exceed 40 cm when initially established in a nutrient-poor sediment in a heterogeneous treatment according to Xie et al. (2007). The local response to the patches further supported the occurrence of root foraging by *V. natans* in the heterogeneous treatments.

V. natans accumulated more total biomass in the nutrient-rich clay patches in the homogeneous treatments and tended to accumulated more total biomass in nutrient-rich clay patches in heterogeneous treatments, and this tendency can be viewed as foraging with a minimum metabolic cost, allowing more nutrient absorption (Xie et al. 2005b, 2007). These results were similar to a study on foraging in *V. spiralis* by Xiao et al. (2006).

SC is the primary energy substrate for a growing plant that can be directly used by the plant, and it plays an important role in sustaining root growth. A high SC concentration ensures root growth in nutrient-poor patches (Huber et al. 2012; Xie et al. 2013). The significantly higher root SC concentration observed in the sand patches indicated faster root growth than that observed in the clay patches in the heterogeneous treatments, which also supported the occurrence of root foraging. However, the starch in plant tissues must first be transformed to SC before it can be utilized (Bloom et al. 1985; Hajirezaei et al. 2003). The low starch concentration recorded in Ho-S could have been due to the consumption of the SC for root growth, during which starch is transformed to SC to support root growth. The P concentration in macrophytes is usually correlated with P availability in the sediment it they are rooted (Xie et al. 2005a). The marked difference in the P concentration in the roots between the sand and clay sediments reflected the sensitive response of *V. natans* to external P availability. A

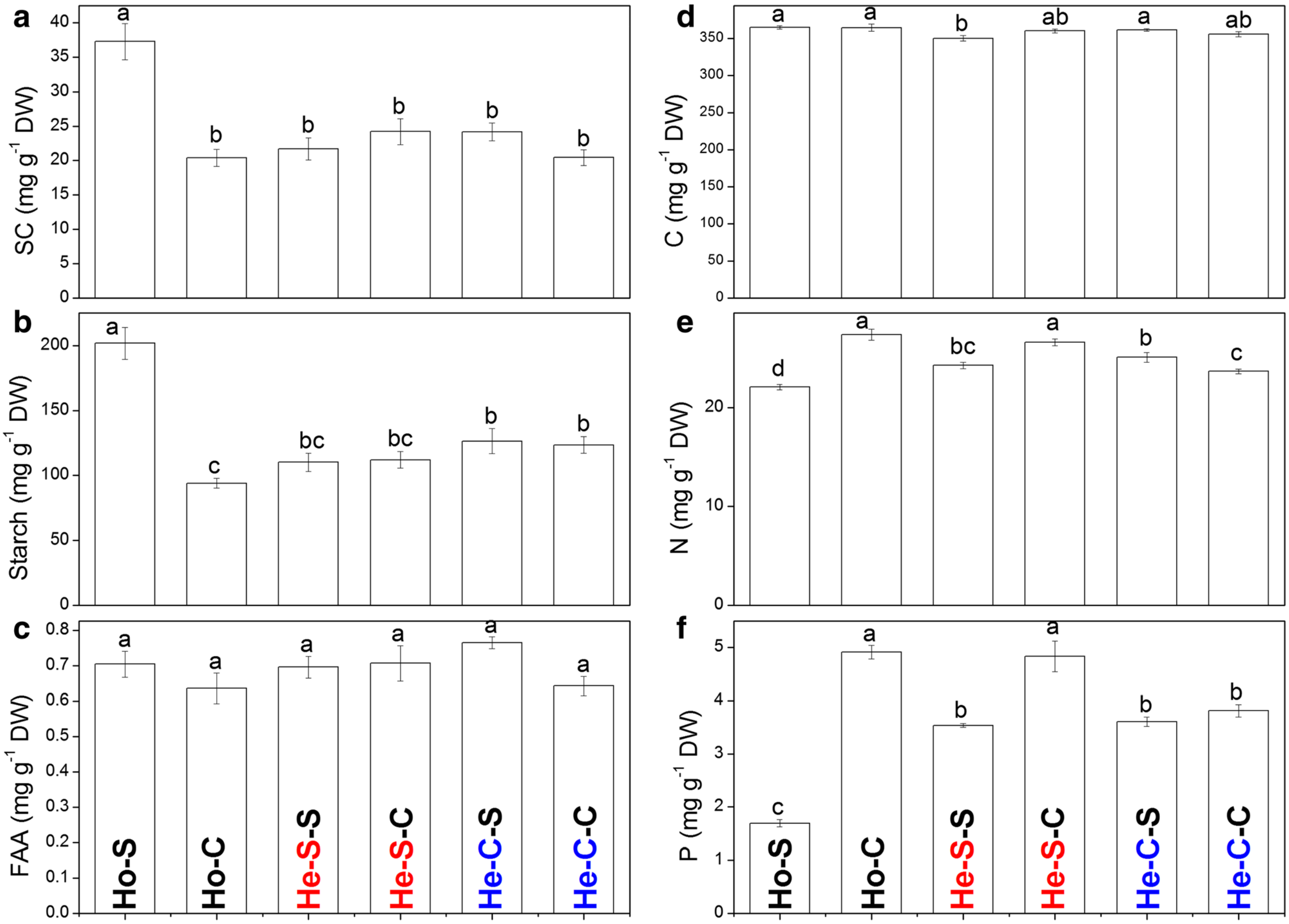

Fig. 7 Contents (mean ± SE; $n = 5$) of individual soluble carbohydrates (SCs), starch, free amino acids (FAAs), carbon (C), nitrogen (N), and phosphorus (P) in *V. natans*. Ho-S and Ho-C indicate the homogeneous sand and clay sediments, respectively. He-S-S and He-S-C indicate the sand and clay sediments, respectively, in the heterogeneous treatments in which *V. natans* was planted in sand sediments, and He-C-S and He-C-C indicate the sand and clay sediments, respectively, in the heterogeneous treatments in which *V. natans* was planted in clay sediments. *Different letters* indicate the significance according to Duncan's test at the 0.05 level

shortage of one or more minerals can result in increased accumulation of FAA (Smolders et al. 1996). In this study, the sand sediment was washed several times, and the high FAA concentration in the sand patches can probably be explained by the low P concentration or some other minerals in sand patches. Smolders et al. (1996) reported that the total nitrogen content in plants was predominantly FAA and that the roots exhibited higher C and N concentrations under phosphorus-

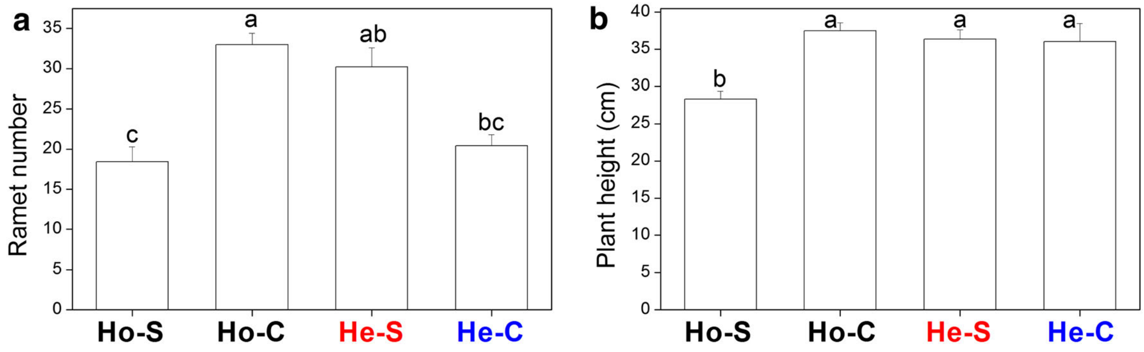

Fig. 8 Ramet number and plant height (mean ± SE; $n = 5$) in *V. natans* grown under four different treatments for 28 days. Ho-S and Ho-C indicate the homogeneous sand and clay sediments, respectively. He-S and He-C indicate the two heterogeneous sediments in which *V. natans* was planted in sand and clay sediments, respectively. *Different letters* indicate the significance according to Duncan's test at the 0.05 level

deficient conditions. The more sensitive physiological response of the roots than of the stems and leaves to sediment nutrients and the finding that the individual physiological nutrient concentrations in two heterogeneous treatments were closer to the results obtained in Ho-C than in Ho-S indicated that *V. natans* could achieve trade-offs among its organs to satisfy its nutrient requirements in the two heterogeneous treatments.

Conclusions

The present study demonstrated the occurrence of root foraging by *V. natans*, as indicated by a global response to four artificial soil treatments and a local response to the nutrient concentrations in sediment patches in the two heterogeneous treatments. The individual physiological nutrient concentrations in the two heterogeneous treatments were close to the values obtained in Ho-C, indicating that *V. natans* can satisfy its nutrient requirements via root foraging. The total biomass, ramet number, and plant height recorded in the two heterogeneous treatments were nearly approached to those observed in Ho-C, which indicated that *V. natans* could ensure integrated growth through root foraging in heterogeneous sediments.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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