Environmental and Experimental Botany 122 (2016) 94–99

Contents lists available at ScienceDirect

Environmental and Experimental Botany

journal homepage: www.elsevier.com/locate/envexpbot

Growth and C/N metabolism of three submersed macrophytes in response to water depths

Guixiang Yuan[a,b], Hui Fu[b], Jiayou Zhong[b], Qian Lou[b], Leyi Ni[a], Te Cao[a,*]

[a] Donghu Experimental Station of Lake Ecosystems, State Key Laboratory of Freshwater Ecology and Biotechnology, Institute of Hydrobiology, The Chinese Academy of Sciences, Wuhan 430072, China
[b] Jiangxi Provincial Key Laboratory of Water Resources and Environment of Poyang Lake, Jiangxi Institute of Water Sciences, Nanchang 330029, China

ARTICLE INFO

Article history:
Received 16 June 2015
Received in revised form 15 September 2015
Accepted 24 September 2015
Available online 28 September 2015

Keywords:
Water depth
Carbon
Nitrogen
Submersed macrophytes
Free amino acid
Soluble carbohydrate

ABSTRACT

For submersed macrophytes, carbon (C) and nitrogen (N) metabolism is a central factor affecting growth and survival under low light availability. Three common submersed macrophytes, *Vallisneria natans*, *Potamogeton maackianus*, and *Potamogeton malaianus*, were grown at five water depths (1.0, 2.5, 4.0, 5.5, and 7.0 m) to explore mechanism of low light adaptation in terms of C/N metabolism by examining relative growth rate (RGR) and contents of soluble carbohydrate (SC), starch, total carbon (TC), free amino acid (FAA) and total nitrogen (TN) in leaf, stem and root of the plants. With increasing water depth, *P. malaianus*, *P. maackianus* and *V. natans* initiated dying at 4.0 m, 5.5 m and 7.0 m water depths, respectively. *V. natans* showed higher RGR than the other two species. For all the plants, the FAA contents increased with increasing water depth, except for the roots of *P. maackianus*. The TN contents showed a unimodal curve along water depths with the highest in moderate water depth, except for *P. malaianus* and the roots of *V. natans*. For *V. natans* and *P. maackianus,* the C metabolic indices (SC, starch, and TC) showed a unimodal curve along water depths with the lowest in moderate water depth in the leaves and stems, except for TC contents in the leaves of *P. maackianus*. Compared with *P. maackianus* and *P. malaianus*, lower SC and FAA contents and higher starch storage in *V. natans* contributed to its higher tolerance to deeper depths. The nonlinear changes in metabolite contents along water depths for *V. natans* and *P. maackianus* demonstrated complex mechanism for low light adaptation, and thus partly explained the wider ranges of colonizing water depths than *P. malaianus*.

1. Introduction

Submersed macrophytes often experience low light stress in deep and eutrophic water and own various morphological and physiological adaptations (Chambers, 1987; Middelboe and Markager, 1997; Zhang et al., 2010). To enhance photosynthetic efficiency, rosette macrophytes generally have low light compensation points for photosynthesis (Titus and Adams, 1979; Su et al., 2004), while canopy-forming macrophytes tend to elongate their shoots toward water surface as to capture more light (Titus and Adams, 1979; Ni, 2001). Adjustments of carbon (C) and nitrogen (N) metabolism, *e.g.*, increased carbohydrate storage, low respiration rate and metabolism homeostasis, also facilitate to tolerate low light stress of submersed macrophytes (Jana and Choudhuri, 1979; Myers and Kitajima, 2007; Yuan et al., 2013; Dietze et al., 2014). In general, a trade-off exists between the relative growth rate (RGR) of plants and survival, depending on light regime (Kobe, 1997; Walters and Reich, 1999). Shade-intolerant species, typically canopy formers, are characterized by high metabolism, such as high respiration rate (Reich et al., 1998; Walters and Reich, 2000), whereas shade-tolerant species, such as rosette formers, exhibit a low respiration rate and high carbohydrate storage (Kobe, 1997; Walters and Reich, 1999; Myers and Kitajima, 2007). Compared to shade-intolerant species, shade-tolerant species usually have lower RGR in high light availability and higher survival rate in low light stress (Kobe, 1997; Niinemets, 2006).

Non-structural carbohydrate (NSC) and free amino acids (FAA) act as intermediates and buffering pool of C and N metabolism, and thus closely coupled with plant growth and light availability (Niinemets and Kull, 1998; Würth et al., 2005; Myers and Kitajima, 2007; Valladares and Niinemets, 2008). For submersed macrophytes, NSC reservoir typically measured as sum of starch and soluble carbohydrate (SC) in stems and/or roots were essential for survival of the plants in adverse conditions, *e.g.*, low light and/or high nutrients stress in eutrophic lakes (White, 1973; Chapin et al., 1990; Cao et al., 2009), which sometimes also led to accumulation

* Corresponding author. Fax: +86 27 68780622.
E-mail address: caote@ihb.ac.cn (T. Cao).

http://dx.doi.org/10.1016/j.envexpbot.2015.09.009
0098-8472/

of FAA, particularly for N-rich amino acids (arginine, proline, asparagine and glutamine) in the plant tissues (Näsholm and Ericsson, 1990; Näsholm et al., 1994; Cao et al., 2009). NSC could be used for maintaining basic metabolism of plants during periods of heavy shading (Piper et al., 2009). Low light availability usually leads to decline of SC and starch contents in plants, and subsequently increased FAA content and N/C ratio of plants due to decreased carbohydrate production of photosynthesis (Cronin and Lodge, 2003; Zhang et al., 2010). Plants with high homeostatic C/N metabolism are more conservative nutrient users, usually showing low N content and high and stable biomass (Yu et al., 2010, 2011; Yuan et al., 2013). Therefore, it is interesting to reveal mechanisms underlying the distribution and succession of submersed macrophytes with regard to C/N metabolism, growth and low light tolerance in eutrophic lakes.

In this study, submersed macrophytes *Vallisneria natans*, *Potamogeton maackianus*, and *Potamogeton malaianus* were used for experiments. These three species were selected because they are different in growth forms, RGR and C/N metabolic strategies (Diao, 1990; Fu et al., 2012a; Yuan et al., 2013), and have large geographic distribution in China and inhabit in water ranging from oligotrophic- to eutrophic status (Cao et al., 2007, 2008). *V. natans* is a rosette former with low light compensation point (Titus and Adams, 1979; Su et al., 2004). *P. maackianus* and *P. malaianus* are canopy formers and capable of shoot elongation under low light stress (Ni, 2001; Liu et al., 2007; Fu et al., 2012b). Two specific hypotheses were tested: (1) in deep water depth when the plants experienced low light stress, they had increased total nitrogen (TN) contents due to insufficient C skeleton for N assimilation, and decreased total carbon (TC) contents due to limited carbohydrate production by photosynthesis. (2) *V. natans* was more tolerant to deep water depth than *P. maackianus* and *P. malaianus* owing to its advantages in C/N metabolism.

2. Materials and methods

2.1. Experimental site

The experiment was conducted in Erhai Lake (25°52′N, 100°06′E) in Yunnan Province, China. The lake is characterized with a water surface area of 250 km^2 and an average water depth of 11 m. Submersed vegetation once covered more than 40% of the lake surface in the 1980s, but less than 8% of the lake was covered in 2009 because of eutrophication (Dai, 1984; Li et al., 2011). The experiment was conducted on a floating platform (25 m in length, 20 m in width) in a bay with a water depth of 8 m during the experimental period. The floating platform was constructed with steel, anchored in this bay, and protected by a surrounding net (mesh size, 2.5 cm) to avoid herbivorous fishes.

2.2. Experimental material and design

Seedlings of *V. natans* and shoots of *Potamogeton* were collected from Erhai Lake. The shoots/seedlings were similar in size and healthy in appearance. For *P. maackianus*, the apical shoot was 35 cm in length, with 10 intact leaf blades and no flower. For *P. malaianus*, the apical shoot length was 30 cm in length, with three intact leaf blades and no flower. For *V. natans*, intact seedlings were 20–30 cm long with seven leaves. The shoots/seedlings were planted evenly in plastic pots (diameter 43 cm, height 36 cm) containing 25 cm sediments collected from the lake, with 20 shoots per pot for *P. maackianus* and five shoots per pot for *V. natans* and *P. malaianus*. The shoot/seedling density used in our study was similar to those of the natural population of the plants in this lake. All the plants were cultured *in situ* at 80 cm water depth for 7 days acclimation and then grown at water depths of 1.0, 2.5, 4.0, 5.5, and 7.0 m. The pots were hung from the floating platform to the designed water depths. Four pots were used as replicates for each species at each water depth, and there were a total of 60 pots.

The *in situ* experiment started on July 2010 and lasted for 52 days. During the experimental period, water quality and temperature were measured at each depth at noon every 5 days. Photosynthetic active irradiation (PAR) was measured by a UWQ-4341 sensor coupled with a Li-1800 data logger (Li-Cor, Lincoln, NE USA). The average PAR was 27.30%, 3.76%, 0.78%, 0.20%, and 0.07% of the surface irradiance at water depths of 1.0, 2.5, 4.0, 5.5, and 7.0 m, respectively. Secchi depth was 1.3–1.5 m, and the water temperature was 15 °C–18 °C. The concentrations of $N–NO_3$, $N–NH_4$ and $P–PO_4$ in water column were 0.42 ± 0.05 mg L^{-1}, 0.02 ± 0.006 mg L^{-1} and 0.008 ± 0.002 mg L^{-1}, respectively, and did not vary significantly with water depths.

2.3. Plant harvesting and biochemical analyses

At the end of the experiment, all the plants in each pot were collected, washed, dried with tissue paper and weighted, and then carefully separated into leaves, stems and roots and oven dried at 80 °C to constant weight. The dried samples were ground into fine powder. About 50 mg of the sample powder was extracted twice with 8 mL of 80% ethanol at 80 °C for 20 min and then centrifuged at $10{,}000 \times g$ for 15 min. The supernatant was collected, decolorized by activated charcoal, and filtered (micro-void filter film, φ 20 mm). The filtrate was used to analyze SC and FAA (Yemm and Willis, 1954; Yemm et al., 1955; Hecht and Mohr, 1990) using glucose and alanine as standards, respectively. The residues after centrifugation were used to analyze starch (Dirk et al., 1999). TN and TC of the samples were determined by an elemental analyzer (Thermo Flash 2000, Cambridge, UK).

The RGR of the macrophytes was calculated using the following formula:

$$\mathrm{RGR} = \frac{\ln X1 - \ln X2}{T}$$

where X1 is the initial biomass at the start of the experiment, X2 is the biomass at the end of the experiment, and T is the experimental time.

2.4. Statistical analysis

SPSS software was used for statistical analyses. Values were expressed as means ± standard error (SE). All data were tested for normality and homogeneity before analyses. The effects of water depths on RGR and contents of C/N metabolites were evaluated by one-way ANOVA, and means were compared by Duncan's multiple range test. Explained variances of the C/N metabolite contents were analyzed using three-way ANOVA, with contents of SC, starch, TC, FAA, and TN as dependent variables, with species, organs and depths as fixed factors, and interactions between species and depths, organs and depths considered. For *P. maackianus*, the biomass of roots in depths of 5.5 and 7.0 m was not sufficient to analyze the metabolite contents. For *P. malaianus*, the plants in depths of 5.5 and 7.0 m were dead.

3. Results

Increasing water depths inhibited growth of all the plants greatly, decreased RGR of *V. natans*, *P maackianus* and *P. malaianus* by increasing extents, and thus led to differentiation of RGR along water depths (Fig. 1). RGR ranges of the plants changed from 0.046–0.050 at 1.0 m water depth to 0.030–0.035 at 2.5 m water depth for the two *Potamogeton* species; at both water depths RGR was highest for *V. natans*, middle for *P. malaianus* and lowest for