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Effects of NH_4–N concentrations and gradient redox level on growth and allied biochemical parameters of *Elodea nuttallii* (Planch.)

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ABSTRACT

Aquatic plants frequently encounter multiple stresses under natural conditions. Nuttall's water weed, *Elodea nuttallii* (Planch.) is a submerged aquatic macrophyte which has flexible ability to use different nutrient sources from various environments. However, recently the growth of *E. nuttallii* has been declining in waters of Japan and in the Chesapeake Bay, a large estuary in the United States. In the present experiment, we studied growth and survival capabilities of the plant under a gradient of redox conditions; from highly oxic (+400 to +440 mV) to extremely reduced (−180 to −120 mV) conditions. Reduced environment was prepared by adding glucose to growth medium and nitrogen gas bubbling, while the oxic environment was brought about by atmospheric air bubbling. In comparison to the oxic environment, growth rate and carbon–nitrogen content of the plants were significantly affected negatively at hypoxic and anoxic conditions. In hypoxic and anoxic environments, indole acetic acid (IAA), tissue nitrogen and chlorophyll levels were down-regulated, whereas hydrogen peroxide (H_2O_2), indole acetic acid oxidase (IAAO) and peroxidase (POD) levels were up-regulated. It was also found that high NH_4–N concentrations (10–40 ppm) affect the growth rate and biochemical parameters of the plant; however, in hypoxic and anoxic treatments the effects were more severe. We conclude that *E. nuttallii* is poorly tolerant to hypoxia/anoxia. Moreover, oxygen stress combined with high ammonium concentration act as important factors influencing distribution and abundance of this species.

Introduction

The principal stress factor for plants in flooded soil is biochemical reduction, the intensity of which is measured as redox potential (Lissner et al., 2003). A reduced redox potential dramatically alters the chemistry and microbial metabolism at the sediment–water interface; this is a common phenomenon in water-logged soil. The reducing chemicals and biogeochemical processes induced by oxygen (O_2) depletion accelerate the sediment's oxygen demand and lower its redox potential to values at −300 mV (DeLaune et al., 1990). Wetland soils are characterized by gradients of redox conditions from totally oxidized to extremely reduced state. A lack of O_2 may negatively affect plant metabolism, including nitrogen uptake and assimilation (Gibbs and Greenway, 2003).

Water-logging of soil and consequent hypoxia induce complex morphological responses in plants. Under reducing conditions, NH_4^+ is the available form of N in environment. Several previous studies have shown that NH_4^+ enrichment can directly cause decline in aquatic plant populations in natural water (Cao et al., 2007; Goldyn, 2010). The mechanisms include the formation of reactive oxygen species (ROS), induced by NH_4^+ metabolism by the plants (Nimptsch and Pflugmacher, 2007) and the imbalance of C–N reserves in plants stemming from the incorporation of NH_4^+ into free amino acids accompanied by consumption of soluble carbohydrates (Cao et al., 2007). Mittler (2002) proposed that oxidative damage in plants is associated with many types of stresses (biotic and abiotic), and plant hormones play a major role in signaling responses and regulations of developmental processes. The common reactive oxygen species (ROS) such as the superoxide anion ($O_2^{-}\cdot$), H_2O_2, and the hydroxyl radical (HO·) can damage biological molecules (DNA, RNA, and proteins) and membranes by inducing lipid peroxidation (Weckx and Clijsters, 1996). However, ROS scavenging mechanisms exist, and it is crucial to identify key components involved in plant tolerance to strong oxidative conditions (Sinha and Saxena, 2006). On the other hand, the phytohormone indolyl acetic acid (IAA) and also H_2O_2 are considered to regulate plant growth especially in stressful environments (Pasternak et al., 2005).

Submerged macrophytes are unique among rooted aquatic plants in linking the water column and the sediment through their physical structure and are capable of taking up nutrients from both

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water column and sediment (Ottosen et al., 1999). *Elodea nuttallii* (Planchon), an exotic submerged aquatic macrophyte, has undergone explosive growth in Japan since the early 1960s, although this trend seems to be diminishing (Nagasaka, 2004). The population of *Elodea* spp. in the Chesapeake Bay, USA, has displayed similar trends (Stevenson et al., 1979). Researchers have suggested different causes for such decline, including progression of eutrophication (Riss et al., 2000), climatic factors (Hamabata, 1991) and lack of genetic variability (Kadono et al., 1997).

Elodea nuttallii is often subjected to gradient redox conditions, such as they occur in flooded soil, eutrophic lakes, and waste water. In these aquatic environments, due to the cessation of ammonium nitrification, NH_4–N level increases, as it was described, for example, in Plesne Lake in Central Europe (Kopácek et al., 2004). Chlorosis of leaves (brown–black discoloration of the leaves), suppression of growth rate, decreased photosynthetic rates, etc., were reported as NH_4^+ toxicity symptoms for *E. nuttallii* (Dendène et al., 1993), suggesting that increased NH_4–N concentrations affect negatively growth and distribution of the plant. However, it is still unknown whether this species is affected directly by gradients in oxygen levels/redox potentials or gradients of redox potentials in combination with high NH_4–N concentrations simultaneously affect the growth and distribution of the species. In the present study, *E. nuttallii* was subjected to different oxygen levels and NH_4–N concentrations, and growth and related biochemical parameters of the plants responding to these conditions were investigated.

Material and methods

Collection of sediment and plants

For the investigations sediment was collected from a pond in Oaso Park, 60 km northwest from Tokyo, in December 2011. The sediment was taken from the top surface (<15 cm depth) of the pond sediment. Soil material was homogenized, air-dried and sieved to <2 mm. The sediment contained 5.4 ± 0.2% (n = 4) organic matter content. *E. nuttallii* plants were collected from Moto-Arakawa River, Saitama, Japan. About 10 cm apical tips of the plants were incubated for two weeks in a growth chamber at a temperature of 25 °C, with a relative air humidity of 90% and a photon flux density of approximately 100 μmol m^{-2} s^{-1}, which was provided by fluorescent lamps in a 12/12 h light/dark cycle.

Experimental set-up

Elodea nuttallii was subjected to different redox potentials under different NH_4–N concentrations. Since it was difficult to keep a constant redox potential throughout the experiment period, certain ranges of redox potential were maintained. The three levels of redox potential (E_H values) applied were (i) +400 mV to +440 mV, (Oxic; O_1), (ii) −5 mV to +5 mV (hypoxic/moderately reduced; O_2) and (iii) −180 mV to −120 mV (anoxic/highly reduced; O_3): Fig. 1. Regarding the nitrogen source, the suitable NH_4–N concentration for *E. nuttallii* is 2.5 ppm (Ozimek et al., 1993). In eutrophic environment, the maximum reported NH_4–N concentration was 50 ppm (Kopácek et al., 2004). In our study, four different concentrations of NH_4–N {2.5 (N_1), 5 (N_2), 10 (N_3) and 40 (N_4) ppm} were used (Fig. 1). The experiment was conducted in microcosms (MCs), each consisting of a 6 L (15.7 cm × 15.7 cm × 24.5 cm) glass vessel which was hermetically sealed with an air-tight lid. Each MC was filled with 600 g of air-dried soil and deionized water in a 1:5 ratio. Then, a growth medium contained 5% Hoagland nutrient solution (Hoagland and Arnon, 1950) was mixed, and ammonium sulfate was added to adjust the required NH_4–N concentration.

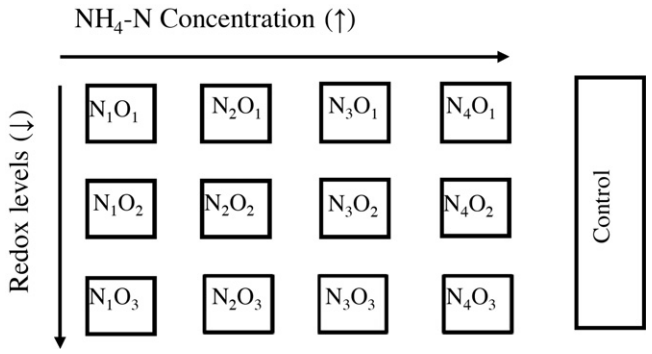

Fig. 1. Layout of the experimental set-up (13 microcosms/treatment). NH_4–N concentration and redox levels are presented as N and O, respectively. Microcosms were randomly distributed with equal spacing in the growth chamber.

Microcosms with highly reducing and moderately reducing conditions were prepared following the method developed by Yu et al. (2007). Glucose, a simple carbon source, was used in this experiment during the 22 day incubation period. At the beginning of incubation, 8.16 g glucose was added to each reduced (MC 3) and highly reduced microcosms (MC 4) at the 1st and 3rd day, and twice of that amount was repeated at 5th day. At 14th day, again, 8.16 g glucose was added to MC 4. Continuous flushing of N_2 gas was carried out for the last 3 days of the experiment for the hypoxic/moderately reduced (MC3) condition and for the last 7 days for anoxic/highly reduced (MC4) conditions, in order to reduce the E_H values to approximately −5 mV and −180 mV, respectively. For the oxic treatments, continuous bubbling with atmospheric air was used. Redox potential and pH were measured four times a day using four portable pH/ORP meters (POT-101M, SIBATA, Japan). For control, 5% Hoagland nutrient medium was used without any further treatment. The temperature was maintained at 23 ± 2 °C in a room with fluorescent lights. No attempt was made to control the pH of the sediment suspensions. After adjustment the expected redox conditions the experiment was continued for 14 days. In total three treatments, each with 13 microcosms were used (Fig. 1). In each microcosm eight plants (12–14 cm) were planted.

Plant growth parameters

At 14 DAT (day after begin of treatment) two plants from each tank were harvested, cleaned and the fresh weight and length were measured after blotting with laboratory tissue. The average relative growth (RGR) was calculated using the following equation(1)RGR = $\frac{\ln W_2 - \ln W_1}{T_2 - T_1}$ where, RGR is the average value of the specific growth rate (g DW/g/day) and W_1 and W_2 are plant weights at time (days) T_1 and T_2 respectively. Shoot elongation was calculated by Eq. (2),

$$\mathrm{SGR} = \frac{L_2 - L_1}{T_2 - T_1} \quad (2)$$

where, SGR is the shoot growth rate (cm/day). L_1 and L_2 are the initial and final shoot lengths (cm) at the time (days) T_1 and T_2 respectively.

Chlorophyll content, carotenoid content and chlorophyll fluorescence

Contents of chlorophylls and carotenoids in fresh leaves were estimated by the method of Lichtenthaler (1987). Leaf samples (50 mg) were mashed with a mortar and pestle and extracted with 80% acetone (v/v) in dark for 24 h. Afterwards the sample was centrifuged for 10 min at 8000 × g. The supernatant was collected and the light absorption read at wavelengths 665 and 649 nm for chlorophyll a (chl a) and chlorophyll b (chl b), respectively, and