Journal of Experimental Marine Biology and Ecology 477 (2016) 23–30

Contents lists available at ScienceDirect

Journal of Experimental Marine Biology and Ecology

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

The role of dissolved organic and inorganic nitrogen for growth of macrophytes in coastal waters of the Baltic Sea

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ARTICLE INFO

Article history:
Received 17 September 2015
Received in revised form 27 November 2015
Accepted 5 January 2016
Available online xxxx

Keywords:
Dissolved organic nitrogen
Macrophytes
Translocation
Uptake
Stable isotopes
Bacterial biofilm

ABSTRACT

Macrophytes and phytoplankton compete for inorganic nitrogen during growth, even in eutrophied coastal waters containing relatively high nitrogen concentrations. In this study we investigated, whether dissolved organic nitrogen (DON) serves as an additional nitrogen source for rooted submerged macrophytes in several key species in the nutrient-rich inner coastal waters of the Darss-Zingster-Bodden chain, located in the southern Baltic Sea. The uptake and translocation of ^{15}N-labeled dissolved inorganic nitrogen (DIN, ammonium and nitrate) and DON (amino-acid mixture) were measured for three common species: *Chara aspera*, *Chara tomentosa*, and *Stuckenia pectinata*. A two-compartment-device was used to discriminate between the roles of roots and shoots in N uptake. The results showed that DON and DIN were taken up by all species, but ammonium (mean 0.116%^{15}N mg DW^{-1} h^{-1}) was preferred over amino acids (mean 0.024%^{15}N mg DW^{-1} h^{-1}) which were preferred over nitrate (mean 0.007%^{15}N mg DW^{-1} h^{-1}). To our knowledge, this is the first study to demonstrate the uptake of DON in charophytes and the submerged angiosperm *S. pectinata*. Both nitrate and ammonium, as DIN, were translocated in the basipetal and acropetal directions in Characeae, which was unexpected given the lack of vascular bundles in these species. By contrast nutrient transport was below the detection limit in the vascular macrophyte *S. pectinata*. The translocation of DON was not observed in any species or in any direction. Our findings suggest that rooted plants have an advantage over phytoplankton based on their ability to assimilate and transport nutrients not only from the water column but also from the sediments, whereas phytoplankton can only use nutrients of the water column.

1. Introduction

The nutritional pollution through human activity and agriculture in catchment areas of coastal zones and estuaries have led to the increasing eutrophication of coastal waters and thus to a shift in plant communities, from macrophytes to phytoplankton dominated systems (Gocke et al., 2003; Kovtun et al., 2009; Munkes, 2005; Schumann et al., 2006). Macrophytes can therefore be used as an indicator species in assessments of the good environmental status of a water body. However, to do so requires a detailed understanding of the mechanisms underlying nutrient uptake and the growth of these key species. Abiotic factors such as light limitation and sedimentation were shown to indirectly influence growth (Angelstein et al., 2009; Kovtun-Kante et al., 2014; Schaible and Schubert, 2008). Another important aspect is the competition for nutrients between macrophytes and phytoplankton. A number of studies have examined the role of phosphorus as a limiting factor (Angelstein and Schubert, 2008; Reid et al., 2000; Rip et al., 2007). Although nitrogen limitation is less well explored, it has been documented in freshwater and marine environments (Bianchi and Engelhaupt, 2000; Elser et al., 2007; Guildford and Hecky, 2000).

In examining the mechanisms of nitrogen limitation, both the sources (sediment vs. water column) of the different nitrogen species and the ability of primary producers to assimilate them must be considered. The two forms of nitrogen, dissolved organic nitrogen (DON) and inorganic nitrogen (DIN), differ in their availability (Stepanauskas et al., 2000). DON accounts for anywhere between 20 and 90% of the total nitrogen pool (Petrone et al., 2009; Seitzinger and Sanders, 1997). However, its concentration was previously thought to be small and was usually not included in studies of the nitrogen uptake by phototroph organism. In addition, 20–30 years ago DON was considered to be largely refractory and was thus ignored as a nutrient source. This erroneous conclusion was based on the complex composition of DON, which includes the poorly decomposable humic and fulvic fractions. However, once DON was identified as a nutrient source the conversion of DON into biomass by phytoplankton and microorganisms was demonstrated on short time by several groups (Andersson et al., 2006; Berg et al.,

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http://dx.doi.org/10.1016/j.jembe.2016.01.005
0022-0981/

1997; Berman and Chava, 1999; Bronk et al., 1994; Fiedler et al., 2015). The uptake of DON by macrophytes is of particular interest only since then Tyler et al. (2005) showed that the nitrogen requirement of non-rooted red and green algae can be satisfied to a significant extent by DON. Subsequently, the uptake of DON was also demonstrated in seagrasses (La Nafie et al., 2014; Van Engeland et al., 2011; Vonk et al., 2008) and seaweeds (Phillips and Hurd, 2004), but whether it also occurs in other rooted macrophytes is unknown. Unlike phytoplankton, which derives their nutrients only from the water column, rooted submerged macrophytes are also able to use nutrients from the sediments. Thus, studies of the uptake of nutrients by macrophytes must consider both the roots and shoots. Nutrient uptake by the roots of submerged aquatic plants and the mechanism of nutrient transport has been often discussed, but are still subjects of debate in the literature (Agami and Waisel, 1986a; Takayanagi et al., 2012; Wilson et al., 1988).

In comparative terms, the roots of submerged vascular macrophytes often comprise ≤10% of the total algal biomass (Brenkert and Amundsen, 1982). This low biomass of roots compared to shoots suggests that main function is to anchor plants in the sediments, with nutrient acquisition playing only a minor role (Sutcliffe, 1959). Many studies have provided support for this hypothesis, by showing that the nitrogen requirement of macrophytes can be fulfilled solely by uptake via the shoots (Madsen and Cedergreen, 2002). By contrast others have shown that both shoots and roots substantially contribute to the nutrient supply (Carignan and Kalff, 1980; Nichols and Keeney, 1976), albeit in different, species-specific proportions. Due to the lack of transpiration in submerged plants, nutrient transport must rely on alternative mechanisms (Raven, 2003) as e.g. cytoplasmatic streaming. Most submerged macrophytes have vascular bundles, which in rooted macrophytes allow the transport of phosphorus e.g. in both directions, downwards (basipetal) and upwards (acropetal) (Angelstein and Schubert, 2008; Littlefield and Forsberg, 1965). In plants such as Characeae, which lack vascular bundles, nitrogen uptake mechanism via cell wall and intracellular translocation remains to be explained.

Previous studies on uptake of nutrients have either disregarded or at least tried to remove the biofilm before the experiments. To our knowledge, there is no possibility to obtain the macrophytes axenic. In this study, under natural conditions occurring biofilm (belong to the bacteria, diatoms and attached algae) gathered at least quantitatively. Which proportions the bacterial biofilm is involved in the uptake of nitrogen components, was not an aim of this experiment. Macrophytes and its bacterial biofilm were considered together.

We hypothesized that: (1) DON provides an alternative to DIN as a nitrogen source that allows the successful growth of macrophytes, as suggested in other studies (Mozdzer et al., 2010). (2) The uptake of either nitrogen source is achieved via roots and also shoots in same ratios. Thus, the aims of this study were (1) to demonstrate the uptake of DON vs. DIN (nitrate and ammonium) by rooted submerged macrophytes. (2) to determine whether both, shoots and roots, are responsible for nutrient uptake and (3) whether transport occurs from roots to shoots and vice versa. We used ^{15}N-labeled ammonium, nitrate, and an amino-acid mixture (as DON) and examined the uptake and translocation of these nitrogen sources in three common macrophytes found in inner coastal, heavily eutrophic waters. In addition, the microbial biofilm in nutrient uptake was considered.

2. Material and methods

2.1. Cultivation of macrophytes

Representative species of the three most abundant communities of macrophytes were collected at the Darss-Zingster-Bodden chain (DZBK), an inner coastal basin of the southern Baltic Sea: *Chara aspera* C.L. Willdenow, 1809 (small Characeae community), *Chara tomentosa Linnaeus*, 1753 (large Characeae community), and *Stuckenia pectinata* (syn. *Potamogeton pectinatus*) (*Potamogeton–Myriophyllum* community) (Schubert et al., 2003). The plants were collected in spring, when the presence of undesirable epiphyton and bacterial biofilms is relatively low. These algae were grown in the laboratory under controlled conditions (light:dark 12:12 h, temperature 15.5 °C) to keep those biofilm low. Habitat water was used as medium and filtered (mesh width 55 μm) at least every 2nd week in all treatments. Species were planted in a cylindrical glass vessel (height 20 cm, Ø = 6 cm). A fragment of Characeae, which is less demanding for nutrients, was embedded in artificial, autoclaved pure sea sand (Applichem). Only phosphate was added to the sand (12 g P kg^{-1} sand). A thin layer of phosphate-free sand was spread on top as a barrier to inhibit the development of phytoplankton in the water column (Wüstenberg et al., 2011). A minimum of two nodes of Characeae were planted to ensure growth. Rhizoid development required the removal of the apical part above the first developed ring of branchlets. *S. pectinata* was cultivated using roots, which were planted in natural sediment, because it is impossible to grow the plants on pure nutrient-free sediment. Here we used sediment from the natural habitat.

Roots are organs of vascular plants which anker the plant in sediment and are responsible for nutrient uptake. By contrast rhizoids are organs of non-vascular plants and the only function per definition is to anker. Some studies have shown that they have additional functions like uptake nutrients too (Agami and Waisel, 1986b). In the following, we refer to the upper and lower compartments of all species as the 'shoots' and 'roots', even though in charophytes they are correctly denoted as phylloids and rhizoids.

2.2. Experimental set-up

The uptake and translocation of nutrients were investigated using three different ^{15}N-labeled substrates. Sodium nitrate and ammonium chloride (both 99% ^{15}N) were added as inorganic compounds. An amino-acid solution (Sigma Aldrich, 17 amino acids, 99% ^{15}N) represented easily accessible DON. To compare the different substrates directly, the same concentrations were used in all treatments. In the highly eutrophied DZBK, the typical ammonium concentration in the water column during the growing season is ~10 μmol L^{-1}, which was therefore the concentration used in all treatments, with 10% of each solution enriched with ^{15}N. Subsamples of macrophytes were taken at the beginning of the experiment (natural abundance) and after 5 h of incubation (enrichment determinations). Incubation time based on preliminary experiments to uptake kinetics. The samples were briefly rinsed with deionized water and dried in an oven overnight at 60 °C. For measurements, the macroalgae were ground to a fine powder, weighed, and wrapped into tin caps. Nitrogen stable isotope measurements were done with Thermo Scientific instruments. The IRMS (Delta V Advantage) was connected to an elemental analyzer (Flash 2000) via an open split interface (Interface Conflo IV). N-contents were determined along with the δ^{15}N analysis (EA-IRMS). The standard substance acetanilide (Merck) was used for calibration of particulate nitrogen measurements. N_2 as standard gas was calibrated against the IAEA standard substances (N1, N2, N3). The precision of the analyses was ±0.2 ‰ for δ^{15}N and 1% for the elemental analysis.

To compare the uptake and translocation of roots and shoots, a two-compartment device (Frank and Hodgson, 1964) made up of an Erlenmeyer flask and glass funnel with an impermeable plug (Fig. 1), was assembled. The macroalgae were carefully inserted into the plug. We used two different treatments, mimicking above- and below-ground. For the former, ^{15}N-labeled substrates were added to the upper (shoot) compartment at a concentration of 10 μmol L^{-1}. To get a defined diffusion gradient, non-labeled substrates were added to the roots at a concentration of 1 μmol L^{-1}. The opposite was done for the below-ground (roots) treatment. Substrate uptake was determined by measurements of the labeled tissues (above-ground: shoots, below-ground: roots), and translocation by analyzing the non-labeled compartments (above-