

## Uptake of nitrogen from compound pools by the seagrass *Zostera noltii*



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### ABSTRACT

In nature, seagrasses are confronted with a compound pool of low concentrations of inorganic and organic nitrogen-containing substances of varying bioavailability. Nevertheless, the majority of research on nitrogen acquisition by seagrasses has been largely limited to studies addressing a single nitrogen substrate at a time. Using a combination of one of <sup>15</sup>N-labeled substrates and one <sup>14</sup>N-labeled background substrate, we investigated how the rate of nitrogen uptake by the seagrass *Zostera noltii* varies with nitrogen background. Leaf and root mediated uptake were studied separately for different combinations of inorganic (ammonium and nitrate) and organic substrates (urea and glycine). Ammonium uptake rates were higher than for the other substrates. However, substrate uptake was not dependent on the background nutrient. Similar patterns and uptake rates were found for above- and belowground plant parts. The dependence of uptake rate on substrate type, combined with an independence of nutrient background is explained as difference in uptake capacity, rather than substrate preference. For the dual labeled (<sup>15</sup>N and <sup>13</sup>C) urea and glycine, strong relationships existed between nitrogen and carbon uptake, but with deviations from expectations under complete uptake of the molecules. Overall, this study indicates that at realistically low ambient concentrations, seagrasses can simultaneously use inorganic and organic sources for their nitrogen needs, and do not distinguish between substrates. In other words, they take up whatever is available.

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### 1. Introduction

Like all plants, seagrasses need nitrogen (N) to maintain their high productivity. However, unlike many terrestrial plants, the resorption of N from the senescent leaves is very limited, and a lot of N is lost due to the high leaf detachment (Romero et al., 2006; Stapel and Hemminga, 1997). This makes seagrasses strongly dependent on external nutrient sources (Short and McRoy, 1984) from the sediment and water column (Short and McRoy, 1984; Stapel et al., 1996; Touchette and Burkholder, 2000).

Nitrogen is available to seagrasses as a mixture of compounds, of which some are expected to be more immediately useful to them than others. Usually, affinities for ammonium are higher than for nitrate in kinetic uptake experiments (e.g. Alexandre et al., 2010, 2011; Hasegawa et al., 2005), which is generally attributed to additional costs associated with nitrate reduction (Turpin, 1991). If this increased affinity for ammonium is inherent to the organism, and exists without external stimulus (and literature shows at least that this property is very common in seagrasses in general (Touchette and Burkholder, 2000), and in *Zostera noltii* in particular (Alexandre et al., 2010)), it

could be called a 'constitutive preference'. In addition, nutrient–nutrient interactions have been reported, where nitrate uptake is down-regulated under increasing ammonium availability (Alexandre et al., 2010). In their uptake experiments, Alexandre et al. (2010) could also demonstrate an up-regulation of ammonium uptake by *Zostera noltii* under increased nitrate concentrations, which they attributed to a signaling function of nitrate in the ammonium metabolism. The latter mechanism could be addressed as an 'induced preference', where ammonium uptake is stimulated by an external factor.

Whereas for a long time nitrogen research has solely focused on dissolved inorganic nitrogen (DIN) uptake by seagrasses (e.g. Cornelisen and Thomas, 2004; Stapel et al., 2001), recent studies suggest that seagrasses are also able to use dissolved organic matter as a nitrogen source. This enables them to shortcut N cycling (Barron et al., 2006; Evrard et al., 2005; Vonk et al., 2008). Similar to terrestrial plants (Harrison et al., 2007), seagrasses exhibit distinct uptake rates for different organic nitrogen substrate, that seem to be related to the substrate's bioavailability, molecular complexity and/or chemical stability of the molecules (Van Engeland et al., 2011, 2013; Vonk et al., 2008). For instance, urea is a very simple molecule that provides two amine groups per molecule. Amino acids with chemically very stable phenyl-groups may be less prone to breakdown and uptake. It is currently not clear if any nutrient–nutrient interactions exist in the uptake dynamics of organic nitrogen.

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In oligotrophic coastal systems (usually in tropical regions), effective use of nitrogen sources are vital to maintain a high productivity, whereas in eutrophic areas (usually in temperate regions) nitrogen overloading may occur (e.g., Burkholder et al., 2007). Recently, it was discovered that dissolved organic nitrogen pools in coastal waters are relatively high and not refractory, even in oligotrophic systems (Bronk et al., 2007). In oligotrophic systems, the availability of additional nitrogen sources may help to explain the high productivity of seagrass systems. In eutrophic systems, the availability of additional nitrogen sources may form an additional threat. Therefore, we aim to (1) quantify uptake rates of each of the dominant nitrogen sources, i.e., inorganic and organic nitrogen, and (2) detect whether the availability on one of these sources affects uptake rates of the other sources. We studied this in a temperate seagrass species, *Zostera noltii*, as this species usually occurs in meso- or eutrophic situations (e.g. Wadden Sea, Cadiz Bay e.g. Brun et al., 2003; Dolch et al., 2013), but can also be found in oligotrophic lagoons (Honkoop et al., 2008). We tested this in the lower range of nutrient concentrations as observed in Cadiz Bay in summer (Van Engeland et al., 2013).

Using stable isotope labeling, we investigated uptake by the temperate seagrass, *Zostera noltii* Horneman, of  $^{15}\text{N}$  nitrogen from different inorganic (ammonium, nitrate) and organic substrates (urea and glycine) as a function of the presence of one of the other substrates as background ( $^{14}\text{N}$ ). By adding fairly low concentrations, we focused on nutrient interactions in uptake at nutritional conditions that are realistic for the source population of the studied plants (Cadiz Bay, Spain). Dual labeling ( $^{13}\text{C}$  and  $^{15}\text{N}$ ) was used to track potential dissolved organic carbon uptake.

## 2. Materials and methods

### 2.1. Biological material and experimental setup

Shoots of *Zostera noltii* Horneman were collected from an intertidal meadow of Cadiz Bay (Southern Spain,  $36^{\circ}29'19.79''\text{N}$ ;  $6^{\circ}15'53.05''\text{E}$ ), brought to the lab in a cool box, wrapped in moist paper, and then boxed in an ice-chest to be transported to the Netherlands. The plants arrived after two days and were immediately put in a tank with  $2\ \mu\text{m}$  filtered water from Oosterschelde (south-west Netherlands) under controlled temperature ( $19\ ^{\circ}\text{C}$ ) and light ( $278\ \mu\text{mol photons m}^{-2}\ \text{s}^{-1}$ ) conditions. Inorganic nitrogen concentration in the tank were as in Oosterschelde ( $\text{NH}_4^+ = 4.7\ \mu\text{mol N}$ ,  $\text{NO}_3^- = 1.17\ \mu\text{mol N}$ ,  $\text{DON} =$

$20.1\ \mu\text{mol N}$ ). After an acclimation period of two full days, plants were cut into single complete shoots (with leaves, rhizomes and roots) and gently cleaned from epiphytes with a razor blade to minimize microbial degradation by e.g. free living bacteria, exo-enzymes etcetera (Van Engeland et al., 2011). This enabled us to focus on the ability of the seagrass itself to process or use nitrogen forms, rather than facilitation by better equipped micro-organisms. Six days after harvest in Cadiz Bay, the plants were incubated in a climate-controlled room (temperature  $19\ ^{\circ}\text{C}$  and lights  $254\ \text{mol photons m}^{-2}\ \text{s}^{-1}$ ) in 250 ml plastic cups. Plants (2–3 shoots) were left intact with their belowground and aboveground parts submersed in separate cups (Van Engeland et al., 2011) (Fig. 1). As the plants would protrude out of the water, the cups were filled almost until the edge to prevent desiccation, while exchange of water between cups was prevented. We used artificial seawater (constituents from Merck and Sigma-Aldrich) that we manually prepared to exclude unintended nutrient addition (modified F2 medium containing only the major constituents, without the nitrogen salts; see for instance De Brouwer et al., 2005), and to minimize interference of microorganisms (e.g. competition for nutrients, remineralization). In this setup, either the aboveground or the belowground tissue received a nutrient treatment, and were incubated for approximately 3 hours under continuous bubbling to prevent local depletion and the built-up of concentration gradients. For logistic reasons the labeling of the aboveground and belowground tissues were performed on consecutive days.

At the start of the experiment, the plants received a combination of one heavy isotope labeled nitrogen substrate ( $^{15}\text{N}$  99% pure  $^{15}\text{N}$ , Cambridge Isotope Laboratories) at a concentration of  $1\ \mu\text{M}$ , and one background substrate in the light isotope form ( $^{14}\text{N}$ ) at  $1\ \mu\text{M}$  (both added with a pipet). The substrates were ammonium, nitrate, urea, and glycine (Cambridge Isotope Laboratories). Urea and glycine (amino acid) also contained isotope labeled carbon ( $^{13}\text{C}$ , universally labeled 99%, Cambridge Isotope Laboratories) to track potential carbon uptake. The different substrate combinations are given in Table 1. These nutrient concentrations are similar to those found in the water column of Cadiz Bay (Van Engeland et al., 2013) and for ammonium and nitrate in the range commonly found in the water column of seagrass ecosystems ( $0\text{--}8\ \mu\text{M}$  and  $0\text{--}3.2\ \mu\text{M}$ , respectively; Touchette and Burkholder, 2000). Control treatments were performed where only the substrate was added without background. Each nutrient treatment was replicated 5 times. Since the cups were relatively small, there may have been substrate depletion during the experiment. However, because the objective of this study is to determine the uptake capacity of nitrogen from a pool

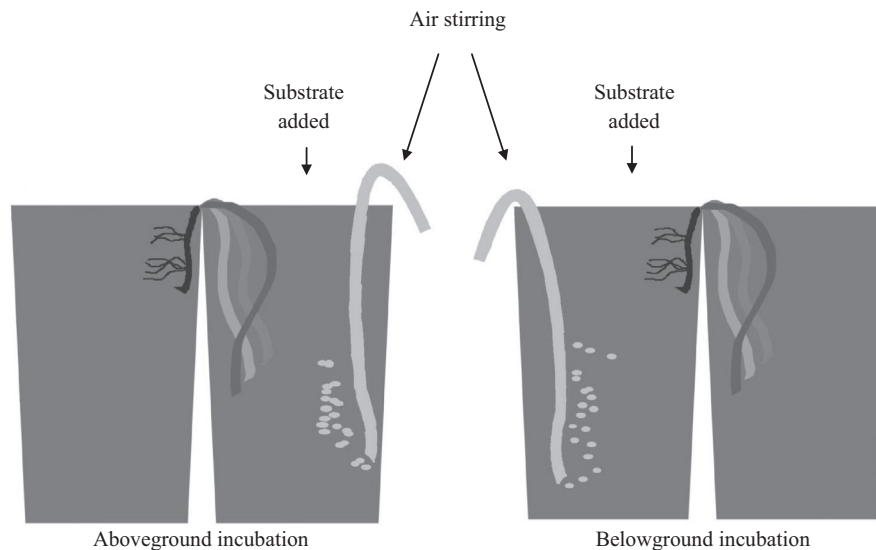


Fig. 1. Experimental setup with two cups containing the aboveground and belowground parts of intact *Zostera noltii* plants. Bubbling was used to stir the water in order to prevent concentration gradients from developing during the incubation.

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