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

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A B S T R A C T

In nature, seagrasses are confronted with a compound pool of low concentrations 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 ^{15}N-labelled substrates and one ^{14}N-labelled 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, nitrate) and organic substrates (urea, 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 (^{15}N and ^{13}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.

Keywords: Nitrogen uptake; seagrass; *Zostera noltii*; isotope label; inorganic nitrogen; organic nitrogen

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. Hasegawa et al. 2005; Alexandre et al., 2010), 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 and co-workers (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., 2006; 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 (Vonk et al., 2008; Van Engeland 2011, 2013). 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.

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., Touchette and Burkholder, 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. Dolch et al., 2013; Brun et al., 2003), 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 labelling, we investigated uptake by the temperate seagrass, *Zostera noltii* Horneman, of ^{15}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}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}C and ^{15}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°29'19.79"N; 6°15'53.05"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 2µm filtered water from Oosterschelde (south-west Netherlands) under controlled temperature (19°C) and light (278 µmol photons $m^{-2}s^{-1}$) conditions. Inorganic nitrogen concentration in the tank were as in Oosterschelde (NH_4^+ = 4.7µmol N, NO_x^- = 1.17µmol N, DON = 20.1 µmol 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°C and lights 254 mol photons m^{-2} 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, remineralisation). 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 labelling 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 labelled nitrogen substrate (^{15}N 99% pure ^{15}N, Cambridge Isotope Laboratories) at a concentration of 1 µM, and one background substrate in the light isotope form (^{14}N) at 1 µM (both added with a pipet). The substrates were ammonium, nitrate, urea, and glycine (Cambridge Isotope Laboratories). Urea and glycine (amino acid) also contained isotope labelled carbon (^{13}C, universally labelled 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

129 water column of seagrass ecosystems (0 – 8 µM and 0 – 3.2 µM, respectively; Touchette and
130 Burkholder, 2000). Control treatments were performed where only the substrate was added
131 without background. Each nutrient treatment was replicated 5 times.Since the cups were
132 relatively small, there may have been substrate depletion during the experiment. However,
133 because the objective of this study is to determine the uptake capacity of nitrogen from a pool
134 of nitrogen, rather than quantifying their uptake kinetics, this was not a problem.

135 After the incubation, plants were rinsed and cleaned with artificial seawater containing
136 only the nutrient background, and dabbed with paper tissues. Aboveground and belowground
137 parts were separated and immediately stored in glass vials at -20 °C. Later they were freeze-
138 dried for 48 hours. Dried samples were weighed and ground to a homogenous powder for
139 further analysis.

140

141 2.2. Sample and data treatment

142 Dried samples were analysed for their nitrogen and carbon content, and nitrogen and
143 carbon isotope composition using Thermo EA 1112 elemental analyzer coupled to a Thermo
144 Delta V Advantage isotope ratio mass spectrometer with a ConFlo II interface (EA-IRMS).
145 Specific ^{15}N uptake rates (V_{15N}; expressed µmol N g DW^{-1} h^{-1}; DW = dry weight) were
146 calculated as :

147 $$V_{15N} = \left(\left(AF_{sa} - AF_{bg}\right) * F_N\right)/(M_N * time),$$

148 where AF_{sa} and AF_{bg} are the ^{15}N fraction in the sample and the natural isotope fraction in the
149 plant tissue, respectively. F_N is the nitrogen fraction in the sample's dryweight (gN gDW^{-1}),
150 M_N is the molar mass of nitrogen (14 $gmol^{-1}$) and time is the length of the incubation period

(hours). These specific ^{15}N uptake rates were converted to specific N uptake rates using the ^{15}N fraction in the substrate (F^{15N}_{substr}) :

$$V_N = V_{15N} / F^{15}{}_{substr}$$

This fraction was 1 for all substrate-background combinations, except those where the labeled substrate (^{15}N) and the non-labeled background (^{14}N) were the same (F^{15N}_{substr} = ½). Total N uptake (ρ_N; µmol N) after incubation was calculated for individual treatment as:

$$\rho_N = V_N * time * DW_{sa}$$

where DW_{sa} is the sample's dryweight (g).

Depletion was calculated as the percentage of available substrate (N_{added}) that was taken up:

$$Depletion = (\rho_N / N_{added}) \times 100$$

Note that in the treatment where the substrate (^{15}N) was also added as background (^{14}N), this background was also taken into account. Similar formulas were used for the carbon uptake rates from the organic molecules.

2.3. Statistical analysis

Treatment and background effects were tested through variance analysis (ANOVA). When needed, asymmetry in distribution per group was compensated by log-transformations. Regression analysis (ordinary least square) was performed to compare carbon and nitrogen uptake from the organic substrates.

3. Results

3.1.Seagrass DIN and DON uptake independency on nutrient background

171 Our results showed different uptake rates for different substrates (Fig. 2) with similar
172 patterns for above- and belowground tissues. Variance analyses per substrate, indicated
173 systematically higher uptake rates in aboveground than in belowground tissues (always $p <$
174 0.01), except for the labelled glycine addition. In the leaf-mediated substrate uptake, only
175 substrate type exhibited a significant effect (ANOVA; $F_{3, 80} = 75$, $p < 0.001$), but the
176 background type did not (ANOVA; $F_{4, 80} = 75$, $p > 0.05$).Tukey HSD tests indicated
177 differences between all labelled substrates ($p < 0.01$), except between nitrate and urea. Root-
178 mediated uptake rates were significantly affected by both the substrate type and the
179 background type, but the latter effect was very weak (ANOVA; $F_{3, 80} = 28$, $p < 0.001$;
180 ANOVA; $F_{4, 80} = 2$, $p < 0.05$; respectively). Ammonium uptake rates were higher than for the
181 other substrates (Tukey, always $p < 0.5$), and a significant difference existed between nitrate
182 and urea uptake rates (Tukey, $p < 0.05$). From these analyses it is clear that substrate uptake
183 showed no clear dependence on the presence and type of a background substrate (Fig. 2).

184 Considering the small volumes and low (but realistic) concentrations used, it is imperative
185 that we investigated the potential for depletion. Substrate depletion was significantly affected
186 by the tissue type and labelled substrate, but not by the background substrate (Tab. 2). The
187 amounts of ammonium taken up represent a considerable fraction of the added amounts (Fig.
188 3), indicating a strong potential for depletion-related underestimation of the corresponding
189 uptake rates. This is supported by the similar degrees of depletion in ammonium, with and
190 without ammonium background (i.e. doubling of the ammonium concentration “visible” to
191 the plant). The fraction taken up for the other substrates were far less (Fig. 3). Hence,
192 depletion-related under-estimation of the uptake rates are not likely for these substrates.

193

3.2. Carbon versus nitrogen uptake from organic N-sources

For the dual-labelled glycine and urea, a strong linear relationship existed between carbon and nitrogen uptake (Linear regression per substrate; only the slope coefficients were significant $p < 0.05$; Fig. 4). If the organic molecules would be taken up intact, one could expect that the total uptake of carbon and nitrogen by the plants occurred in proportions dictated by the C:N ratio or the substrates. This hypothetical uptake is in figure 4 indicated by the lines. The observed C:N ratios of this uptake were clearly lower than expected from the C:N ratios in the substrates (lines in Fig. 4), indicating preferential nitrogen uptake over carbon uptake. However, C:N ratio of uptake was stronger for the more carbon-rich glycine than for urea (R^2 values of 99% and 88%, from the respective regression analyses).

4. Discussion

In nature, nitrogen is available to marine macrophytes as a mixture of inorganic and organic molecules. In coastal and estuarine areas the dissolved organic nitrogen constitutes 13-18% of the nitrogen pool (except dissolved N_2; Berman and Bronk, 2003) of which substantial parts can be non-refractory (Bronk et al., 2007). It is currently established that dissolved organic matter also serves as an effective source of nitrogen to marine macrophytes (Van Engeland et al., 2011; Vonk et al., 2008). Our study supports these findings and demonstrates organic nitrogen uptake by *Zostera noltii* under conditions of a strongly reduced microbial community (epiphyte removal and artificial seawater). Our ammonium uptake rates are slightly higher than those reported by Morris et al. (2008) for *Zostera noltii* shoots from the same source population under low current conditions (data not shown). Our uptake rates

for the aboveground tissue are also in the same range as those reported by Van Engeland et al. (2011) for the same substrates, but somewhat higher for the belowground tissue. Variability between substrates also resembles those reported by Van Engeland et al. (2013) for the same seagrass species and by Vonk et al. (2008) for tropical species. These similarities with literature show that our data are of good quality. In addition, our study takes research in organic nitrogen uptake by marine macrophytes one step further by considering the role of organic substrates in nutrient-nutrient interactions in nitrogen uptake by a temperate seagrass species.

4.1. Seagrass DIN and DON uptake independency on nutrient background

With regard to the inorganic nitrogen substrates, our results agree with earlier studies that show higher uptake rates for ammonium than for nitrate (Alexandre et al., 2010; Touchette and Burkholder, 2000; Van Engeland et al., 2011) and organic N-sources (Vonk et al., 2008; Van Engeland et al., 2011; 2013). This effectively results in a 'constitutive preference' for ammonium over the other substrates. If all substrates are supplied in the same concentrations (like in this study), ammonium is taken up in higher amounts than the others. As the presence of a background substrate did not affect the uptake rates of the labelled substrate in any of the treatments (Fig. 2; comparison within panels), no down- or up-regulation was observed that favored one nitrogen source over the others (i.e. an induced preference). This contrasts with the findings of (Alexandre et al., 2010), who showed an inhibition effect of ammonium on nitrate uptake, and stimulated ammonium uptake under higher nitrate concentrations.

Considering the low (but close to ambient) nitrogen concentrations applied in our study, we may have not reached certain threshold concentrations to induce inhibition or stimulation of substrate uptake. It is likely that, at these low concentrations *Zostera noltii* is "programmed"

241 to take up whatever nutrients it can find. Clearly, our experiment was conducted in nutritional
242 conditions characteristic of the quasi-linear part of the Michaelis-Menten curve for uptake of
243 nitrogen sources.

244 Under nutritionally poor conditions, other seagrass species also seem to take up nutrients
245 from whatever source is available. *Posidonia oceanic* in Revellata Bay (Corsica) seems to
246 take up inorganic nitrogen according to the available water column concentrations (Lepoint et
247 al., 2002). However, although the same applies to *Phyllospadix iwatensis*, this species still
248 exhibits a preference for ammonium as revealed by its uptake affinities (Hasegawa et al.,
249 2005). Inorganic nitrogen concentrations in the latter study varied so much that they simply
250 drowned out the difference in affinities.This shows the value of kinetic studies in unravelling
251 nutrient preference mechanisms. To summarize, *Zostera noltii* exhibits a constitutive and
252 induced preference for ammonium under higher nutritional conditions (Alexandre et al.,
253 2010), but only a constitutive preference at lower nutritional conditions (this study). Apart
254 from that, the eventual contribution of different sources in the overall nitrogen acquisition
255 may further depend on the relative concentrations of the different sources.

256 Due to the strong depletion in the labelled ammonium additions, the true ammonium
257 uptake rates may have been underestimated, although they were roughly similar to those
258 reported by Morris et al. (2008) for *Zostera noltii* from the same source population under low
259 current conditions. Underestimating uptake rates due to depletion would imply that a potential
260 down- or up-regulation of ammonium uptake could remain undetected. However, since the
261 up-regulation, demonstrated by Alexandre et al., (2010) was more pronounced at substrate
262 concentrations of 25 µM than at 5µM, we consider such an effect at concentrations of 1 µM
263 would not likely to occur.

The organic nitrogen substances in our study did not have any effect on the uptake of any nitrogen source, nor were their uptake rates influenced by the presence of another substrate. Considering that the pattern in uptake rates for the aboveground tissue was similar to that found by Van Engeland et al. (2011), it probably reflects a 'constitutive preferential' order from ammonium as most preferred, to urea, nitrate and glycine as least preferred (note however that the differences with glycine were not statistically significant in our study). Whether an inducible preference mechanism exists in *Zostera noltii* involving organic nitrogen substances remains an open question. Note however, that amino acids concentrations of 1 µM are really at (or beyond) the upper limit of the observed range for seagrass ecosystems (e.g. Hansen et al., 2000). This implies that the chance of not detecting an existing role for amino acids in the down-regulation of the uptake of some nitrogen source is much smaller than the chance that such a role actually exists.

4.2. Carbon versus nitrogen uptake from organic N-sources

A strong relationship existed between nitrogen and carbon uptake from organic molecules in *Zostera noltii*. However, the uptake C:N ratios were lower than expected from the respective molecule C:N ratios, indicating occurrence of carbon loss. Several reasons can be put forward for this partial decoupling: 1) remineralisation outside the plant with subsequent uptake of the products (NH_4^+ and dissolved inorganic carbon), 2) uptake of the entire molecule with subsequent loss of a part of the carbon, or 3) remineralisation outside plant by epiphytic bacteria (in the boundary layer) with transport of the products influenced by boundary layer effects (i.e. coupling through limited physical transport after external remineralisation).

The fact that the uptake C:N ratio is stronger for the more carbon-rich glycine (C:N=2) than for urea (C:N=0.5) seems to support remineralisation (see Harrison et al., 2007; von Felten et al., 2008), considering that coupled uptake implies a specific uptake mechanism which is most likely not directly dependent on the molecule's C:N ratio. However, explanation 1 is problematic in the sense that the produced DIC would still enter a large background pool (micromolar versus millimolar concentrations). In contrast, explanation 3 does not suffer from this problem as remineralisation within the boundary layer would cause less dilution losses of labelled DIC in the unlabelled DIC background pool. Present study does however not provide an affirmative answer to the mechanisms causing constant uptake C:N ratios that deviate from theoretical expectations.

4.3. Summarising conclusion

Overall, this study suggests that at low ambient concentrations, *Zostera noltii* exhibits a 'constitutive preference' for ammonium over other (in)organic nitrogen sources, in-line with findings from kinetic studies. However, contrary to the demonstrated ammonium-nitrate interaction in nitrogen uptake by *Zostera noltii* at higher nitrogen concentrations, no similar regulation seems to exist in lower ambient concentrations, indicating that in low-nutrient environments *Zostera noltii* takes whatever (in)organic nutrients are available.

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LIST OF REFFERENCES

Alexandre, A., Silva, J., Santos, R., 2010. Inorganic nitrogen uptake and related enzymatic activity in the seagrass *Zostera noltii*. Marine Ecology-an Evolutionary Perspective 31(4), 539-545.

Alexandre, A., Silva, J., Bouma, T.J., Santos, R., 2011. Inorganic nitrogen uptake kinetics and whole-plant nitrogen budget in the seagrass *Zostera noltii*. J. Exp.Mar. Biol. Ecol. 401(1-2), 7-12.

Barron, C., Middelburg, J.J., Duarte, C.M., 2006. Phytoplankton trapped within seagrass (*Posidonia oceanica*) sediments are a nitrogen source : An in situ isotope labelling experiment. Limnol. Oceanogr. 51, 1648-1653.

Berman, T., Bronk, D.A., 2003. Dissolved organic nitrogen: a dynamic participant in aquatic ecosystems. Aquat. Microb. Ecol. 31, 279-305.

Bronk, D.A., See, J.H., Bradley, P., Killberg. L.. 2007. DON as a sourve of bioavailable nitrogen for phytoplankton. Biogeosciences 4, 283-296.

Brun, F. G., Vergara, J. J., Navarro, G., Hernández, I., & Pérez-Lloréns, J. L., 2003. Effect of shading by *Ulva rigida* canopies on growth and carbon balance of the seagrass *Zostera noltii*. Mar. Ecol. Prog. Ser. 265, 85-96.

Cornelisen, C.D., Thomas, F.I.M., 2004. Ammonium and nitrate uptake by leaves of the seagrass *Thalassia testudinum*: impact of hydrodynamic regime and epiphyte cover on uptake rates. Journal of Mar.Syst. 49(1-4), 177-194.

De Brouwer, J.F.C., Wolfstein, K., Ruddy, G.K., Jones, T.E.R., Stal, L.J., 2005. Biogenic stabilization of intertidal sediments: the importance of extracellular polymeric substances produced by benthic diatoms. Microb. Ecol 49(4), 501-512.

335 Dolch T., Buschbaum C., Reise K., 2013. Persisting intertidal seagrass beds in the northern
336 Wadden Sea since the 1930s. J. of Sea Research 82: 134-141 2013
337 Duarte, C.M., 1990. Seagrass nutrient content. Mar. Ecol. Prog. Ser. 67(2), 201-207.
338 Evrard, V., Kiswara, W., Bouma, T.J, Middelburg, J.J. 2005. Nutrient dynamics of seagrass
339 systems : ^{15}N evidence for the importance of particulate organic matter and root
340 systems. Mar. Ecol. Prog. Ser. 295: 49-55.
341 Honkoop, P.J.C., Berghuis, E.M., Holthuijsen, S., Lavaleye, M.S.S., Piersma, T., 2008.
342 Molluscan assemblages of seagrass-covered and bare intertidal flats on the Banc
343 d'Arguin, Mauritania, in relation to characteristics of sediment and organic
344 matter. J. Sea Res. 60:255-263
345 Hansen, J.W., Udy, J.W., Perry, C.J., Dennison, W.C., Lomstein, B.A., 2000. Effect of the
346 seagrass *Zostera capricorni* on sediment microbial processes. Mar. Ecol. Prog. Ser.
347 199, 83–96
348 Harrison, K.A., Bol, R., Bardgett, R.D., 2007. Preferences for different nitrogen forms by
349 coexisting plant species and soil microbes. Ecology 88(4), 989-999.
350 Hasegawa, N., Iizumi, H., Mukai, H., 2005. Nitrogen dynamics of the surfgrass *Phyllospadix*
351 *iwatensis*. Mar. Ecol. Prog. Ser. 293, 59-68.
352 Lepoint, G., Millet, S., Dauby, P., Gobert, S., Bouquegneau, J.M., 2002. An annual nitrogen
353 budget of the seagrass *Posidonia oceanica* as determined by in situ uptake
354 experiments. Mar. Ecol. Prog. Ser. 237,87–96.
355 Morris, E.P., Peralta, G., Brun, F.G., van Duren, L., Bouma, T.J., Pérez-Lloréns., J.L., 2008.
356 Interactions between hydrodynamics and seagrass canopy structure: spatially explicit
357 effects on ammonium uptake rates. Limnol. Oceanog. 53: 1531-1539.

358 Romero, J., Lee, K.S., Pérez, M., Mateo, M.A., Alcoverro, T., 2006. Nutrient dynamics in
359 seagrass ecosystems. In: Larkum, A.W.D., Orth, R.J., Duarte, C.M. (Eds.), Seagrasses:
360 biology, ecology and conservation. Springer, the Netherlands, pp. 227-254.
361 Short, F.T., McRoy, C.P., 1984. Nitrogen uptake by leaves and roots of the seagrass *Zostera*
362 *marina* L. Bot. Mar. 27(12), 547-555.
363 Stapel, J., Aarts, T.L., van Duynhoven, B.H.M., de Groot, J.D., van den Hoogen, P.H.W.,
364 Hemminga, M.A., 1996. Nutrient uptake by leaves and roots of the seagrass Thalassia
365 hemprichii in the Spermonde Archipelago, Indonesia. Mar. Ecol. Prog. Ser. 134(1-3),
366 195-206.
367 Stapel, J., Hemminga, M.A., 1997. Nutrient resorption from seagrass leaves. Mar. Biol.
368 128(2), 197-206.
369 Stapel, J., Hemminga, M.A., Bogert, C.G., Maas, Y.E.M., 2001. Nitrogen (N-15) retention in
370 small *Thalassia hemprichii* seagrass plots in an offshore meadow in South Sulawesi,
371 Indonesia. Limnol. Oceanogr. 46(1), 24-37.
372 Touchette, B.W., Burkholder, J.M., 2000. Review of nitrogen and phosphorus metabolism in
373 seagrasses. J. Exp. Mar. Biol. Ecol. 250(1-2), 133-167.
374 Turpin, D.A., 1991. Effects of inorganic N availability on algal photosynthesis and carbon
375 metabolism. J. Phycol. 27, 14–20.
376 Van Engeland, T., Bouma, T.J., Morris, E.P., Brun, F.G., Peralta, G., Lara, M., Hendriks, I.E.,
377 Soetaert, K., Middelburg, J.J., 2011. Potential uptake of dissolved organic matter by
378 seagrasses and macroalgae. Mar. Ecol. Prog. Ser. 427, 71-81.
379 Van Engeland, T., Bouma, T.J., Morris, E.P., Brun, F.G., Peralta, G., Lara, M., Hendriks, I.E.,
380 van Rijswijk, P., Veuger, B., Soetaert, K., Middelburg, J.J., 2013. Dissolved organic
381 matter uptake in a temperate seagrass ecosystem. Mar. Ecol. Prog. Ser 478, 87-100.

382 von Felten, S., Buchmann, N., Scherer-Lorenzen, M., 2008. Preferences for different nitrogen
383 forms by coexisting plant species and soil microbes: Comment. Ecology 89(3), 878-
384 879.
385 Vonk, J.A., Middelburg, J.J., Stapel, J., Bouma, T.J., 2008. Dissolved organic nitrogen uptake
386 by seagrasses. Limnol. Oceanogr. 53(2), 542-548.
387

388 **Figure captions**

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

392 Figure 2. Boxplots of the ^{15}N specific uptake rates for the different labelled substrates
393 (grouped in separate graphs) in different backgrounds of nitrogen containing substances
394 (horizontal axis) in leaves (upper panels) and roots (lower panels). Both the labelled (^{15}N) and
395 background (^{14}N) were added in final concentration of 1µM. The small lines, boxes, whiskers
396 and dots indicate median, interquartile range (IQR), 1.5×IQR and outliers (deviation from
397 median larger than 1.5 × IQR). The thick horizontal lines and grey zones indicate the mean
398 and (±) standard deviation of the uptake rate for the ^{15}N substrate in a background of the same
399 substances in ^{14}N form. NB = No Background (indicating no^{14}N-nutrient added).

400 Figure 3. Total N uptake as a percentage of the added substrate N in leaves (upper panels) and
401 roots (lower panels). The small lines, boxes, whiskers and dots indicate median, interquartile
402 range (IQR), 1.5×IQR and outliers (deviation from median larger than 1.5×IQR). Background
403 N is not taken into account unless the background was the same n species as the substrate.

404 Figure 4. Total uptake of substrate C versus substrate N for the two organic substrates. The
405 theoretically expected relationship between C and N uptake, derived from the substrate C:N
406 ratios are for urea and glycine shown by the dotted and dashed line, respectively. These
407 calculations assumed absence of fractionation. Root and leaf-mediated uptake are for both
408 substrates indicated with different symbols (*cf.* legend in figure). Equations carbon uptake (C)
409 as function of nitrogen uptake (N) are given for the theoretical lines (normal font) and the

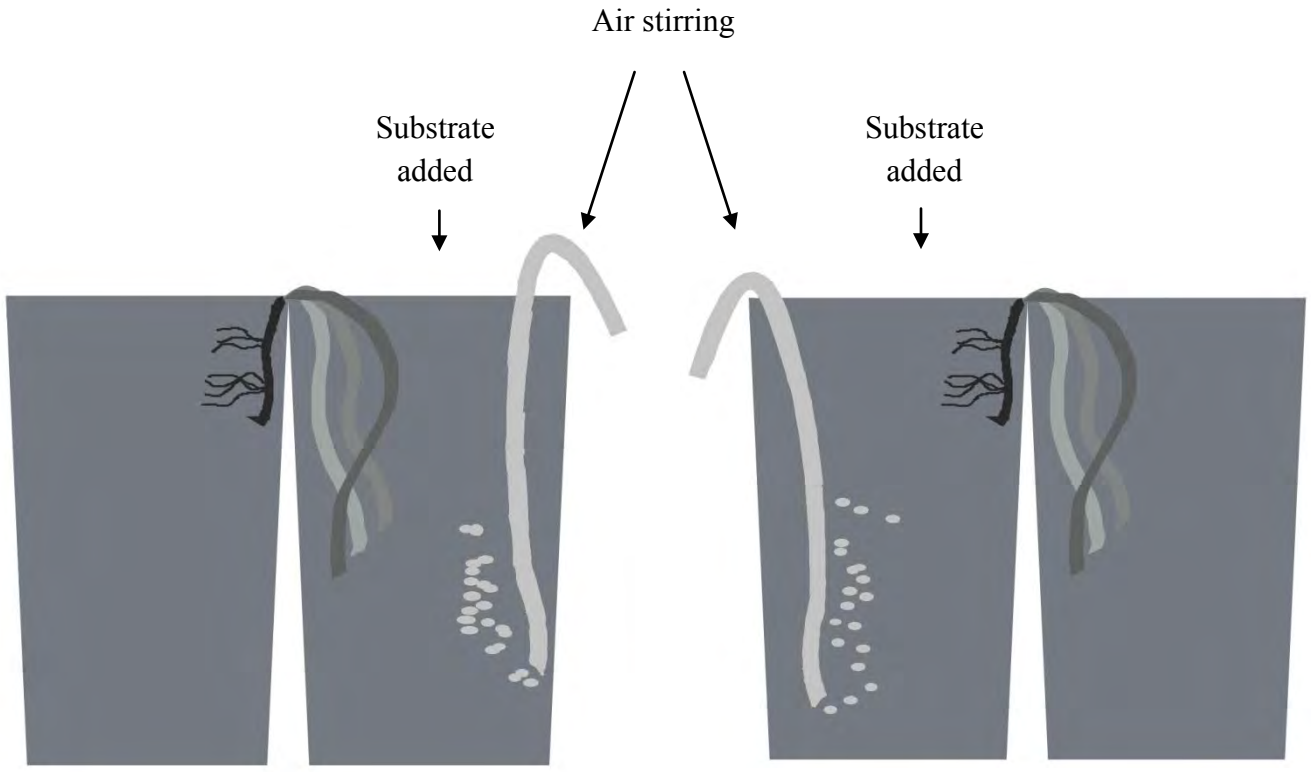
410 empirical data (bold font). In the latter case, only the slope coefficients were significant in the
411 linear regression (*cf*. text).

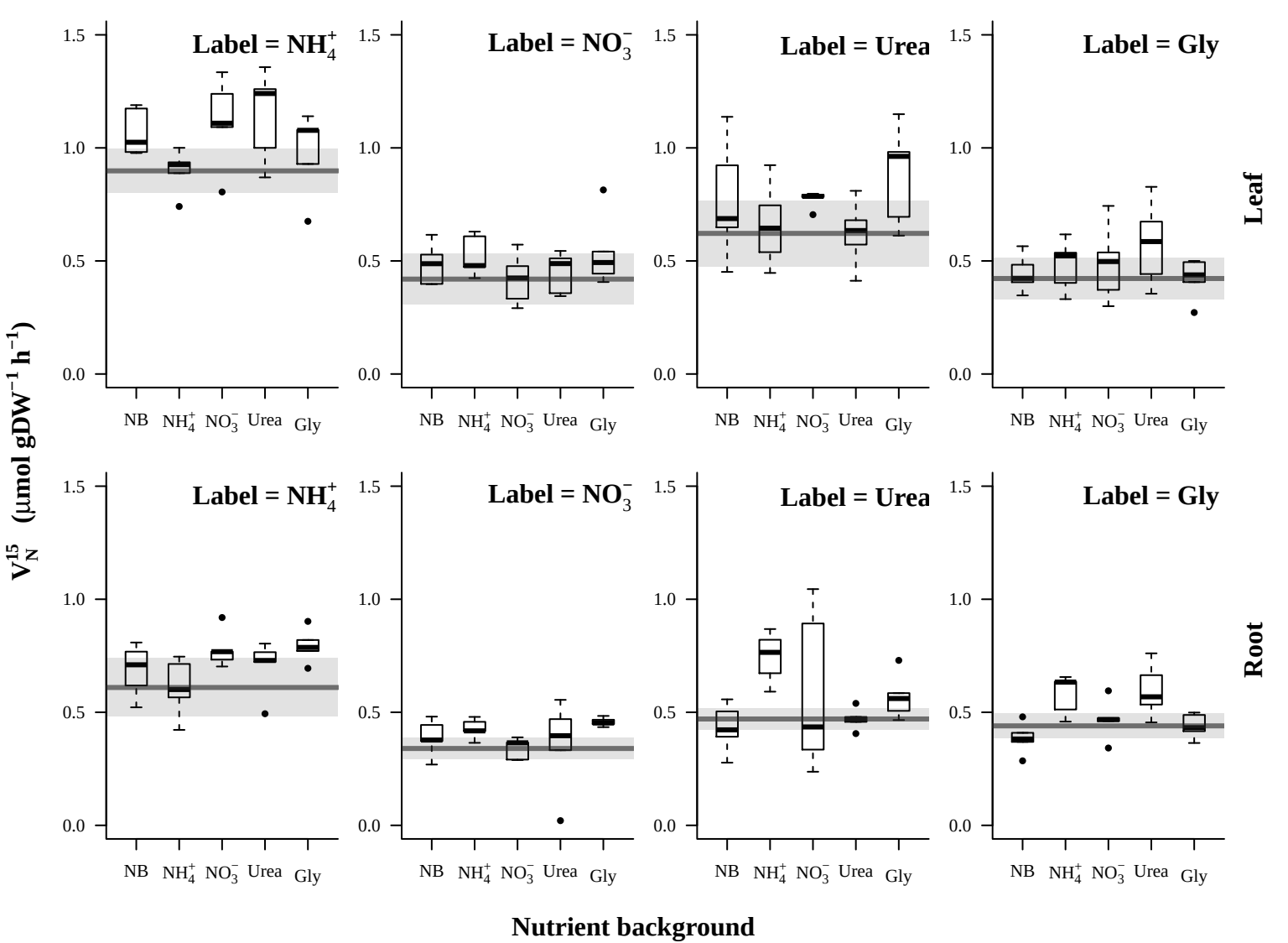
Table 1. Experimental design showing concentrations of non-labelled-background and labelled-substrate applied to both above and belowground tissues. Values should be interpreted as "background substrate concentration – labelled substrate concentration" in micromolar.

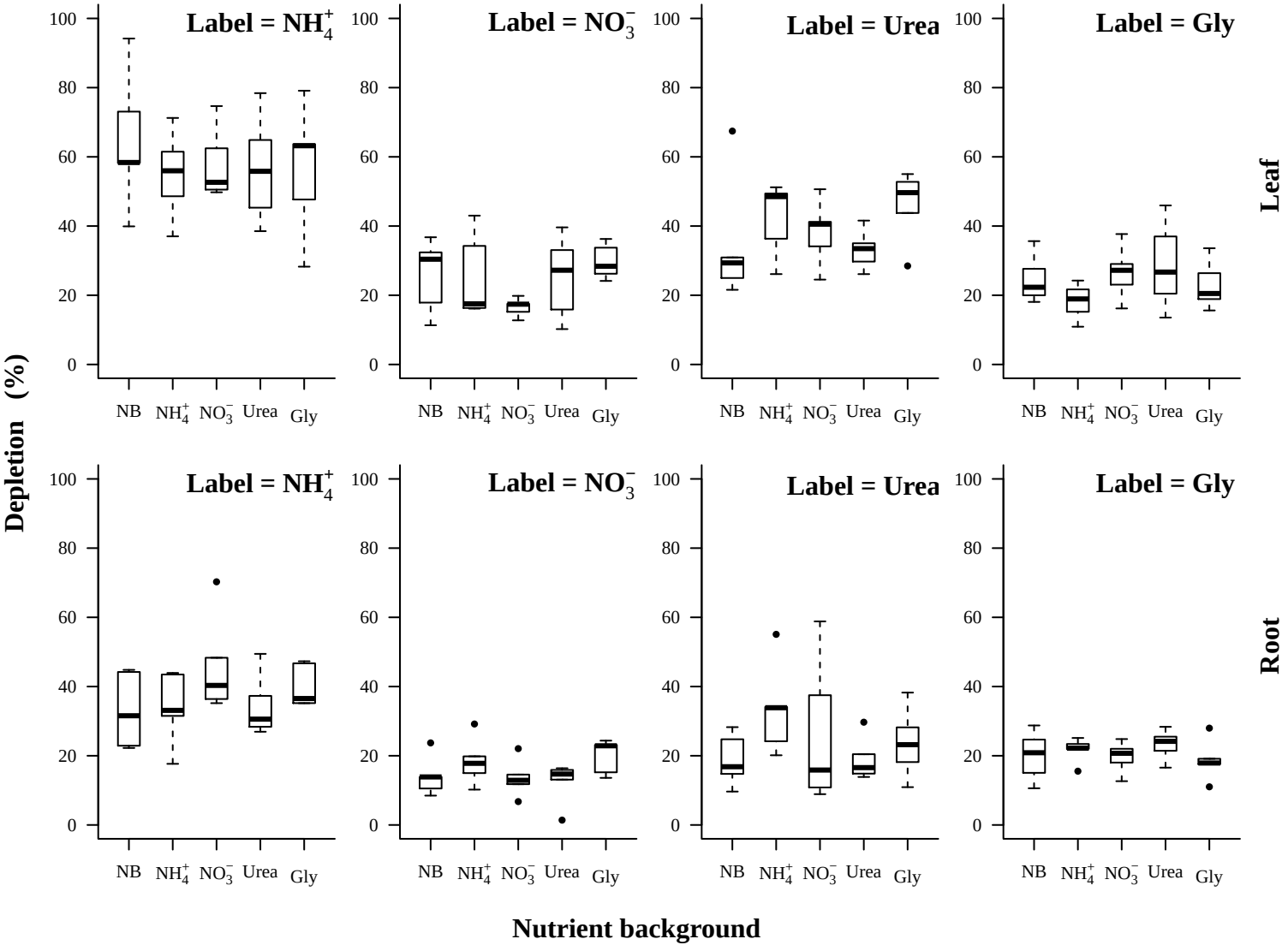
Background concentration (non-labelled) (μM)	Substrate concentration (isotope labelled) (μM)			
	NH_4^+	NO_3^-	Urea	Glycine
None (control)	0 - 1	0 - 1	0 - 1	0 – 1
NH_4^+	1 - 1	1 - 1	1 - 1	1 – 1
NO_3^-	1 - 1	1 - 1	1 - 1	1 – 1
Urea	1 - 1	1 - 1	1 - 1	1 – 1
Glycine	1 - 1	1 - 1	1 - 1	1 – 1

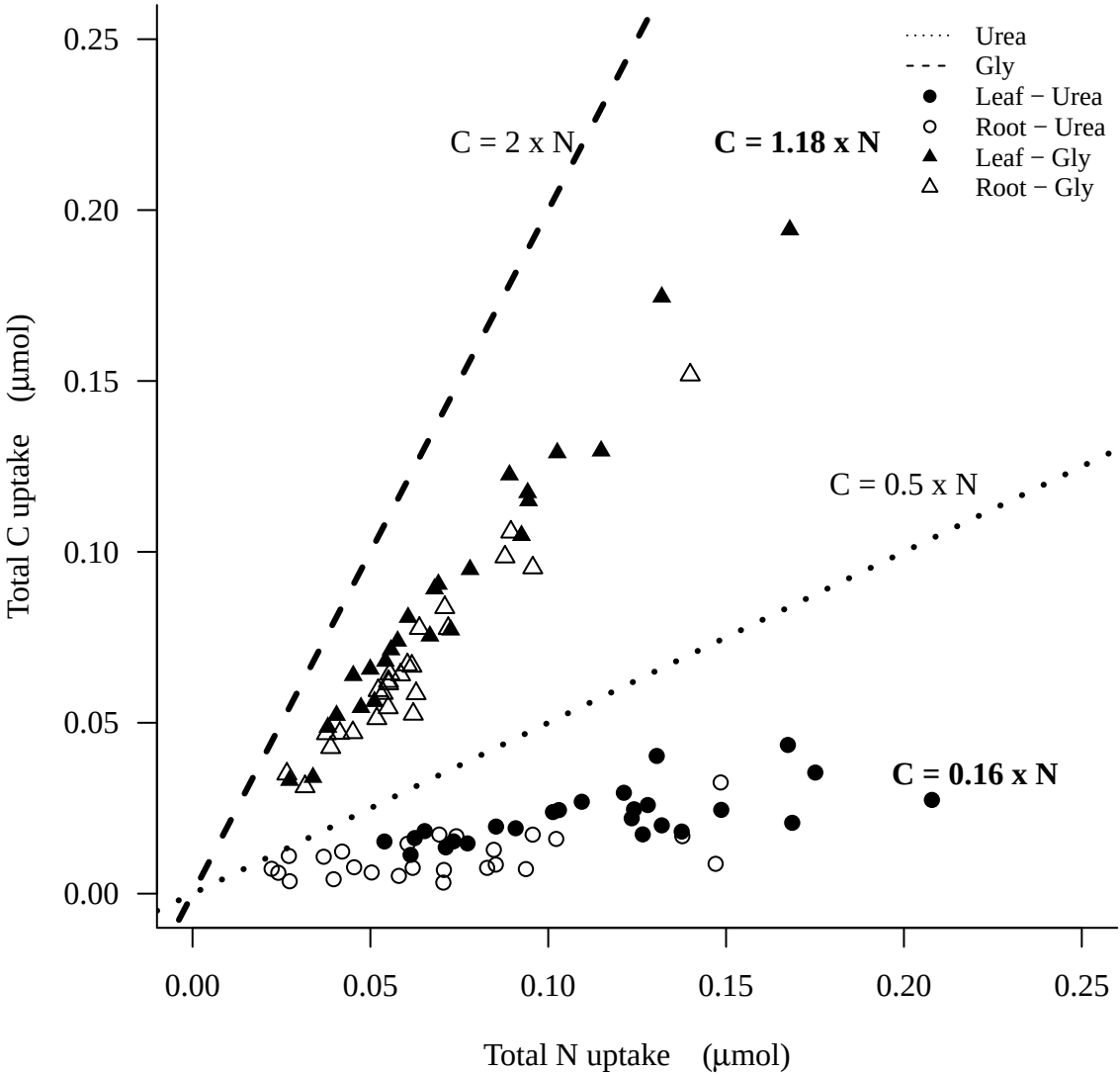
Table 2. Analysis of variance (ANOVA) table for the substrate depletion, indicating the degrees of freedom of the F statistic (df), the value of the F statistic, and the corresponding probability value (*p*).

Factor	df	F	*p*
Intercept	1, 160	1633	< 0.001
Label (L)	3, 160	70	< 0.001
Tissue (T)	1, 160	62	< 0.001
Background (B)	4, 160	0.5	0.7
L x T	3,160	5.8	< 0.001
L x B	12, 160	1.4	0.2
T x B	4, 160	1	0.4
L x T x B	12, 160	0.5	0.9


Air stirring
Substrate added
Substrate added
Aboveground incubation
Belowground incubation


Label = NH_4^+
Label = NO_3^-
Label = Urea
Label = Gly
Leaf
Root
V_N^{15} (µmol gDW^{-1} h^{-1})
1.5
1.0
0.5
0.0
NB
NH_4^+
NO_3^-
Urea
Gly
Nutrient background


Label = NH_4^+
Label = NO_3^-
Label = Urea
Label = Gly
Leaf
Root
Depletion (%)
0
20
40
60
80
100
NB
NH_4^+
NO_3^-
Urea
Gly
Nutrient background


Urea
Gly
Leaf − Urea
Root − Urea
Leaf − Gly
Root − Gly
C = 2 x N
C = 1.18 x N
C = 0.5 x N
C = 0.16 x N
0.25
0.20
0.15
0.10
0.05
0.00
Total C uptake (µmol)
0.00
0.05
0.10
0.15
0.20
0.25
Total N uptake (µmol)