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Ammonium and nitrate are both suitable inorganic nitrogen forms for the highly productive wetland grass *Arundo donax*, a candidate species for wetland paludiculture

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ABSTRACT

The effects of inorganic nitrogen (N) forms (NH_4^+, NO_3^- or both) at equimolar (0.5 mM) concentrations on growth, biomass allocation, photosynthesis, nitrate reductase activity (NRA) and N uptake rates of *Arundo donax* were investigated in hydroponic culture. Plants supplied with NH_4^+, or NH_4NO_3 had significantly higher above-ground biomass, leaf length, shoot number and shoot production rates than NO_3^- fed plants, whereas the relative growth rates, below-ground biomass, and other plant morphological parameters were indifferent to N nutrition. Leaf photosynthetic pigment concentrations and leaf specific area of NO_3^- −fed plants were lower than those of plants grown on NH_4^+, or NH_4NO_3. In addition, NH_4^+ and NH_4NO_3 fed plants had higher light-saturated rate of photosynthesis and stomatal conductance than NO_3^- fed plants and there were no differences in leaf dark respiration among N-form treatments. Both leaves and roots of *A. donax* had NRA, but NRA was much greater in leaves, particularly when plants were fed with NO_3^-. The N uptake rate of *A. donax* was greatest when supplied as NH_4NO_3 (1.10 ± 0.34 mg N g^{-1} root DM h^{-1}) although not different from the N uptake rate with NH_4^+ alone (0.61 ± 0.08 mg N g^{-1} root DM h^{-1}), whilst NO_3^- uptake velocities were similar among N-form treatments. Our results indicate that although some traits perform better in the presence of NH_4^+, presumably due to lower energetic costs of NH_4^+ uptake, *A. donax* grows well with either NH_4^+ or NO_3^-. This reflects its ability to grow well in both wetland and terrestrial soil types, which is an important consideration for its use as a candidate species for paludiculture.

1. Introduction

Nitrogen is one of the most important mineral elements in plant tissues, and is typically absorbed as ammonium (NH_4^+) or nitrate (NO_3^-) (Miller and Cramer, 2005). Relative amounts of NH_4^+ and NO_3^- assimilated are essential in controlling growth of plants and their yield (Greenway and Woolley, 2001; Duan et al., 2007). Generally, plants can take up either form of soluble N available for growth; however, differences in N form uptake between species (Kronzucker et al., 2000; Grassein et al., 2015) are likely to reflect differences in their efficiency of N uptake and use (Tanner, 1996; Güsewell and Bollens, 2003; Duan et al., 2007). NH_4^+ preference is common in plants occupying environments with restricted nitrification, where NH_4^+ dominates (Garnett et al., 2001; Kronzucker et al., 1997) whilst plants mostly take up NO_3^- in well-drained soils where nitrification is predominant. Within-species plasticity in response to NH_4^+ and NO_3^- are also common, with factors such as N form supplied (Aerts and Chapin, 1999; Green and Galatowisch, 2002), and environmental conditions (Dyhr-Jensen and Brix, 1996; Brix et al., 2002) affecting uptake preferences, assimilation processes and metabolism of N.

For plants that grow in wetland environments, both NH_4^+ and NO_3^- can be available, with their concentrations determined by soil moisture availability and redox potential. The presence of high NH_4^+ concentrations may be beneficial for energetic reasons, but can also be toxic if excess NH_4^+ uptake over NO_3^- uptake leads to cytoplasmic acidosis (Claussen and Lenz, 1999; Konnerup and Brix, 2010; Horchani et al., 2011). Some previous studies of preferences for the two N forms in wetland plants indicate better growth with NH_4^+ rather than NO_3^- as the N form (Jampeetong et al., 2012; Piwpuan et al., 2013), although N preferences have been studied in relatively few wetland species. These responses of wetland plants to N availability and relative preferences for NH_4^+ and NO_3^- are important in structuring natural wetland communities, but have

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also become of recent interest in managed wetlands, e.g. in paludiculture – the cultivation of rewetted organic soils (Wichtmann and Joosten, 2007) – which is an innovative concept that

allows rewetted peatlands to remain productively used. Paludiculture requires high above-ground yields of productive emergent wetland macrophytes, and hence growth responses to N are of great interest for potential paludiculture crops. Recently, various plants have been tested for their suitability as paludicrops. The cultivation of common reed, *Phragmites australis,* as a bioenergy crop or as industrial raw material illustrates the practical and economic feasibility of paludiculture (Wichtmann and Joosten, 2007). Apart from *Phragmites australis*, cultivation, harvest and use of *Typha* spp., *Alnus glutinosa* and *Sphagnum* have also shown promising results (Tanneberger and Wichtmann, 2011). Notably, altered hydrology, especially re-flooding, a precondition for paludiculture on degraded peatland areas. Depending on the condition of the peatland and its supply of ground and surface water, different degrees of water management are needed.

Arundo donax, commonly known as giant reed, is one of the most promising paludicrops, due to its capacity to produce more biomass using less fertilizer and without pesticides than many alternatives (Williams et al., 2009). Additionally, *A. donax* has a large amount of energy per unit of dry weight; hence, fuel crop producers consider *A. donax* as one of the top potential biofuel crops (Mantineo et al., 2009; Williams et al., 2009). *Arundo donax* is also a viable species for nutrient removal in constructed wetlands (Calheiros et al., 2012; Idris et al., 2012). It is considered the most suitable European grass crop species for bioenergy production, comparable in yields and agronomic framework to *Miscanthus gigantea* in North America (Lewandowski et al., 2003; Angelini et al., 2009). It has been cultivated in the Mediterranean region for the last 10 years and the research has mostly focused on traits, such as drought and salt tolerance (Cosentino et al., 2016), which can guarantee survival during the dry Mediterranean season. Although *A. donax* can grow in both terrestrial and aquatic environments, little is known about its preference and response to different forms of inorganic N. Although some growth parameters respond positively to N addition, *A. donax* remains highly productive with limited N supply (Cosentino et al., 2014; Cosentino et al., 2016) and may therefore be more suitable for paludiculture across a broader N gradient than alternative taxa such as *Typha* spp.

The aim of this study was therefore to assess how *Arundo donax* responds to NH_4^+ vs NO_3^- nutrition, and to elucidate N uptake assimilation processes in this species. For this purpose, growth, biomass allocation, photosynthesis rates, nitrate reductase activity (NRA), and N uptake rate of plants were compared between plants supplied with different forms of inorganic N, i.e. NH_4^+ and NO_3^- alone or in combination.

2. Materials and methods

2.1. Plant materials and growth conditions

Shoots of *Arundo donax* were collected from the Påskehøjgård research farm located 12 km north of Aarhus, Denmark (56°13′N; 10°07′E), and placed horizontally on a table in approximate 5 cm of water in a greenhouse for 10 days to induce shoot growth at the stem nodes. New-grown, same-sized shoots, which were about 30 cm tall and had developed roots at each node, were excised and used as replicates for the experiment. The plants were transferred to individual 1.7 L black glass vessels containing 1.5 L of nutrient solution (see below). The plants were held erectly in the vessels by slices of foam in the lids of the vessels with only the roots submerged in the nutrient solution. The experimental design consisted of ten replicates of each N treatment (NH_4^+, NO_3^-, NH_4NO_3) and the plants were rotated every two days when growth solutions were also renewed to avoid edge-effects.

The plants were kept in a growth chamber (2 m × 2.4 m × 2.15m, length × width × height) and growth conditions were a 14/10-h day/night light regime, a 50/80% day/night relative humidity and a 30/22 °C day/night thermoperiod. Light was supplied by metal halide bulbs at a photon flux density of approximately 400 μmol m^{-2} s^{-1} (PAR) at the base of the plants. The N-nutrition treatments were NH_4^+ and NO_3^- supplied alone or in combination at equimolar concentrations. The N was added as $(NH_4)_2SO_4$, KNO_3, and NH_4NO_3 at equimolar N concentration (0.5 mM) in the three treatments. The basic growth medium was a full-strength standard N-free nutrient solution prepared according to Smart and Barko (1985). Since additional potassium (KNO_3) was added in the NO_3- treatment, K_2SO_4 was added to the NH_4NO_3 and NH_4^+ treatments to reach equal concentrations of K^+. Phosphorus was added as KH_2PO_4 (0.1 mM). A commercial micronutrient solution for aquarium plants (Pioneer micro plus with iron, Brøste, Kgs. Lyngby, Denmark combined with Pioneer Jernchelat EDDHA, Azelis, Kgs. Lyngby, Denmark) was added in the following concentrations (M): B^{3+} 23, Cu^{2+} 2, Fe^{2+} 35, Mn^{2+} 11, Mo 0.6 and Zn^{2+} 5. The solution pH was adjusted to 7.0 with 4 M H_2SO_4. The growth solutions were renewed every two days throughout the duration of the experiment to minimize nutrient depletion and changes in pH.

2.2. Plant growth, morphology and biomass allocation

Fresh weights of all plants were determined at the initial and final time of experiment after thoroughly blotting roots with water absorbing tissue paper. The relative growth rate (RGR; d^{-1}) was estimated as the difference in the natural logarithm of final and initial fresh weights divided by the growth period in days. Additionally, the increase in number of leaves and shoots throughout the experiment divided by the time were calculated for the average leaf and shoot production rates (LPR and SPR; no. d^{-1}). Likewise, the leaf and shoot elongation rates (LER and SER; mm d^{-1}) were calculated as the increase in the longest leaf or shoot length divided by time. After five weeks of growth, the plants were harvested and divided into leaf, stem, root and rhizome fractions for biomass allocation. Fresh mass of each part was measured before drying at 70°C to obtain a constant weight and to estimate shoot to root ratio (S/R ratio).

2.3. Leaf photosynthesis and dark respiration

After four weeks of growth, the leaf gas-exchange rates were measured on the youngest healthy, fully developed leaves (the third or the fourth from the apex) of each plant using a portable photosynthesis system (Li-6400XT; LI-COR Inc., Lincoln, NE) equipped with CO_2 and temperature-control modules and the CO_2 mixer installed. The airflow through the leaf chamber was set at 400 μmol s^{-1}, the chamber temperature at 30 °C and the CO_2 concentration at 400 μmol mol^{-1}. The light-saturated photosynthetic capacity (A_{max}) was measured at a photosynthetic photon flux density (PPFD) of 2000 μmol(photons) m^{-2} s^{-1} provided by a blue-red LED light source mounted above the leaf cuvette. To measure the dark respiration rate (R_d), the lamp was switched off and the chamber was darkened. Each reading was logged after a 3–5 min period of stabilization. Simultaneously, stomatal conductance (g_s) was also recorded by the Li-6400XT system during photosynthesis measurements.

2.4. Pigment concentrations and specific leaf area

The concentrations of chlorophyll *a* (chl *a*), chlorophyll *b* (chl *b*), total chlorophyll, and carotenoids (carotenes and xantophylls, car)

in the leaves measured for photosynthesis were determined following Lichtenthaler (1987). The youngest fully developed leaves from each plant were freeze dried and cut into small pieces and 5–10 mg samples weighed. Pigments were extracted with 8 mL of 96% ethanol and incubated in the dark at room temperature. The concentrations of pigments were spectrophotometrically determined after 24 h extraction. The leaf area was determined by a leaf area scanner (Model 3100 Li-Cor, USA).The leaf area was divided by leaf DW, which was measured from entire leaves before preparing the samples for chlorophylls extraction, to calculate specific leaf area (SLA)

2.5. Nitrogen uptake rates

NH_4^+ and NO_3^- uptake rates were estimated after thirty days of growth. Plants were transferred to individual vessels containing 1.5 L of Mili-Q water for 24 h in order to deplete internal and adsorbed pools of inorganic N prior to the uptake experiment and ensure N uptake independent of residual unassimilated internal inorganic N. Fifteen plants (five plants from each treatment) were then placed individually in vessels containing 1.5 L of 0.5 mM NH_4^+ solution and fifteen plants in vessels containing 1.5 L of 0.5 mM NO_3^- solution. Eight 5-ml samples were withdrawn every 5 min to analyse NH_4^+ uptake and every 20 min to analyse NO_3^- uptake. The concentrations of NH_4^+ and NO_3^- in all samples were analysed colorimetrically using a flow injection analyser (Quikchem method no. 10-107-06-3B and 10-107-04-1C; Lachat instruments, Mikwaukee, WI, USA). The N uptake rate was calculated from the depletion curves with linear regression analyses and related to root DM.

2.6. Nitrate reductase activity

After four weeks of growth, the nitrate reductase activity (NRA) in leaves and roots of *Arundo donax* were estimated following the method of Scheible et al. (1997) and Piwpuan et al. (2013). After 0.2–0.4 g of fresh samples had been ground to powder in a mortar precooled with liquid N_2 and rested on ice, 2.5 mL ice-cold extraction buffer were added. The extraction buffer contained 100 mM HEPES-KOH (pH 7.5), 5 mM $Mg(Ac)_2$, 1 mM EDTA, 1% BSA, 5 mM DTT, 10% Glycerol, 0.1% Triton X-100, 0.5 mM PMSF (in 99% ethanol), 20 μM FAD, 25 μM Leupeptin, 5 μM Na_2MoO_4 and 1% PVPP. 100 μL of the plant extract was transferred to an Eppendorf tube resting on ice before adding 0.5 mL of pre-warmed assay mix at 25◦C. The assay mix contained 100 mM of HEPES-KOH (pH 7.5), 5 mM of KNO_3, 5 mM of EDTA and 0.25 mM of NADH. Regarding the time zero control, a stop reagent containing 25 μL of 0.6 M ZnAc and 75 μL of 0.15 mM phenazine methosulphate was added before the assay mix. The reaction was stopped after incubation by adding the stop reagent to the mixture. After keeping samples for 15 min at the room temperature, the NO_2 concentrations were colorimetrically analysed using colour reagent which contained 300 μL of 1% sulfanilamide in 3 M HCl and 300 μL of 0.02% NED. Colour was enabled to develop for 30 min at the room temperature. The samples then were centrifuged at 14,000g (Eppendorf Centrifuge 5417C, Hamburg, Germany) for two minutes and NO_2^- concentration was measured in a spectrophotometer at a wave length from 480 nm to 600 nm and the corresponding. The optimal incubation time was estimated by comparing enzyme activity among 0.5, 2, 5, 10, 30, 60, 90 and 120 min of incubation. The optimal time was estimated to be 45 min for leaves and 120 min for roots of *Arundo donax*.

2.7. Plant tissue analysis

The dried plant fractions (leaves, stems, roots, and rhizomes) were finely ground in a ball mill (mixer Mill MM 400, Retsch, Haan, Germany) before the tissue nutrient concentrations were analysed. The total N and carbon (C) concentrations were analysed on a 2–5 mg sample using a CHN analyser (Na 2000, Carlo Erba, Italy). Inorganic N concentrations in leaf and root material were determined by extracting 5–10 mg of ground material in 20 mL Mili-Q water at 80 °C for 20 min. NH_4^+ and NO_3^- in the extracts were analysed colorimetrically using a flow injection analyser (Quikchem method no. 10-107-06-3B and 10-107-04-1C; Lachat instruments, Mikwaukee, WI, USA) as above described.

Table 1
Effects of nitrogen source on relative growth rate (RGR), total biomass and plant morphology (specific leaf area (SLA), number of leaves or shoots, leaf or shoot production rate (LPR, SPR), leaf or shoot length, leaf or shoot elongation rate (LER, SER), and shoot to root biomass ratio (S/R ratio)) of *Arundo donax* grown at equimolar concentrations of different inorganic nitrogen forms (NH_4^+, NO_3^- or NH_4NO_3) and results of ANOVA (*F*-ratios). Values are means ± S.D.

Parameters	N forms			*F*-ratio
	NH_4^+	NH_4NO_3	NO_3^-	
Leaf number	25.7 ± 1.5	26.2 ± 1.6	22.3 ± 0.6	2.6^{ns}
LPR (no. d^{-1})	0.64 ± 0.04	0.63 ± 0.04	0.55 ± 0.02	1.9^{ns}
Leaf length (cm)	35.5 ± 0.5b	35.1 ± 0.6b	32.4 ± 0.6a	8.5^{*}
LER (cm d^{-1})	0.53 ± 0.02	0.49 ± 0.06	0.44 ± 0.03	2.1^{ns}
Shoot number	4.4 ± 0.4b	3.9 ± 0.3b	2.8 ± 0.1a	8.6^{*}
SPR (no d^{-1})	0.097 ± 0.010b	0.083 ± 0.008b	0.051 ± 0.004a	8.7^{*}
Shoot length (cm)	82.5 ± 2.4	83.4 ± 1.9	77.6 ± 1.7	2.4^{ns}
SER (cm d^{-1})	1.55 ± 0.06	1.58 ± 0.06	1.40 ± 0.05	2.9^{ns}
S/R ratio	1.88 ± 0.16	1.83 ± 0.16	1.51 ± 0.12	1.8^{ns}
RGR (d^{-1})	0.050 ± 0.003	0.046 ± 0.002	0.044 ± 0.002	1.4^{ns}
SLA (cm g^{-1})	20.8 ± 0.2a	20.5 ± 0.2a	19.8 ± 0.2b	6.3^{**}
Total biomass (gDM)	18.0 ± 0.6	16.0 ± 0.6	15.4 ± 1.1	2.6^{ns}

Different letters superscripts between columns indicate significantly differences between nitrogen sources.
ns: non-significant.
* $P < 0.05$.
** $P < 0.01$.

2.8. Data analysis

All statistical tests were performed using JMP version 12.1 (SAS Institute Inc., Cary, NC). All data were tested for normal distribution and variance homogeneity by the Shapiro-Wilk test and Levene's test respectively. When necessary, data were transformed (log or sqrt) to ensure homogeneity of variance, however, for clarity, all data are presented as means ± standard deviation of untransformed data. One-way analysis of variance (ANOVA) using type III sum of squares was performed on the data to detect differences among treatments. The effect of "N-form", "plant fraction" and their interaction on NRA, inorganic nitrogen concentrations in plant tissues were identified by a two-way ANOVA. Post hoc comparison of means was performed using Tukey HSD procedure at the 0.05 significance level.

3. Results

3.1. Plant growth, morphology and biomass allocation

The relative growth rate (RGR) ranged from 0.044 to 0.05 d^{-1} and was not significantly influenced by N supply form. However, *A. donax* had significantly higher leaf length, specific leaf area, shoot number and shoot production rate when provided with NH_4^+ or NH_4NO_3 than NO_3- alone. Other plant morphological parameters such as the number of leaves, shoot length, LPR, LER, and SER did not differ under different N forms supplied. The S/R ratio also showed no significant differences among the three N treatments (Table 1). The N forms significantly affected above-ground biomass with NH_4^+ fed plants having higher above-ground biomass

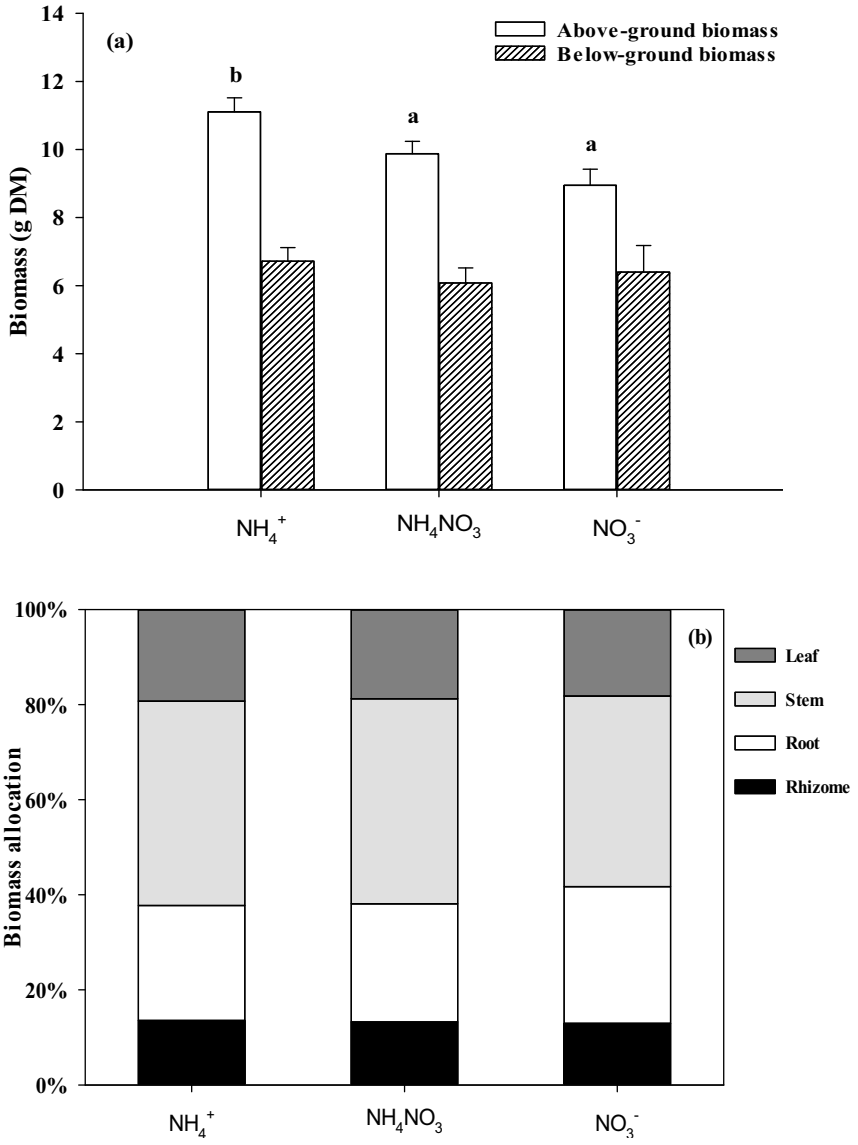

Fig. 1. Above-ground and below-ground biomass (a) and biomass allocation (b) of *Arundo donax* grown at equimolar concentrations of different inorganic nitrogen forms (NH_4^+, NO_3^- or NH_4NO_3). Mean values ± 1SD, different letter superscripts in (a) identify significant differences. Error bars omitted in (b) for clarity.

than NH_4NO_3 and NO_3^- fed plants (Fig. 1a). Meanwhile the below-ground biomass was unaffected by the N treatments (Fig. 1a). The apparent contradiction between S/R data and biomass data (Table 1 vs Fig. 1a) is a consequence of the statistically significant but small effect in Fig. 1, which disappears in S/R calculations. Fig. 1b) further confirms the absence of any strong effect of N form on biomass allocation.

3.2. Photosynthetic gas exchange and pigment concentrations

The light-saturated rate of photosynthesis (A_{max}) and the stomatal conductance (g_s) differed between N treatments, but the dark respiration rate (R_d) was not affected (Fig. 2). *Arundo donax* grown on NO_3^- solution had significantly lower A_{max} and g_s compared to plants supplied with NH_4^+ and NH_4NO_3.

Plants in the NH_4^+ and NH_4NO_3 treatments had higher concentrations of chl *a* than plants grown on NO_3^- (Table 2). Since chl *a* differed significantly among treatments, these differences also showed in the total chl. The N forms also systematically affected total carotenoids with the highest concentrations in NH_4^+ and

Table 2
Concentrations of photosynthetic pigments (Chl – chlorophyll, Car – carotenoids; mg g^{-1} DM) in leaves of *Arundo donax* grown at equimolar concentrations of different inorganic nitrogen forms (NH_4^+, NO_3^- or NH_4NO_3) and results of ANOVA (*F*-ratios). Values are means ± S.D.

Photosynthetic pigments	N-form			*F*-ratio
	NH_4^+	NH_4NO_3	NO_3^-	
Chl *a*	4.96 ± 0.30b	4.66 ± 0.29ab	3.88 ± 0.2a	4.4*
Chl *b*	1.65 ± 0.09	1.56 ± 0.11	1.36 ± 0.07	2.6ns
Total Chl	6.61 ± 0.40b	6.22 ± 0.38ab	5.24 ± 0.26a	3.9*
Total Car	0.96 ± 0.06b	0.89 ± 0.05b	0.72 ± 0.04a	6.6**
Total Chl/Total Car	6.93 ± 0.12	6.98 ± 0.12	7.31 ± 0.11	3.2ns

Different letters superscripts between columns indicate significantly differences between nitrogen sources.
ns: non-significant.
* $P < 0.05$.
** $P < 0.01$.

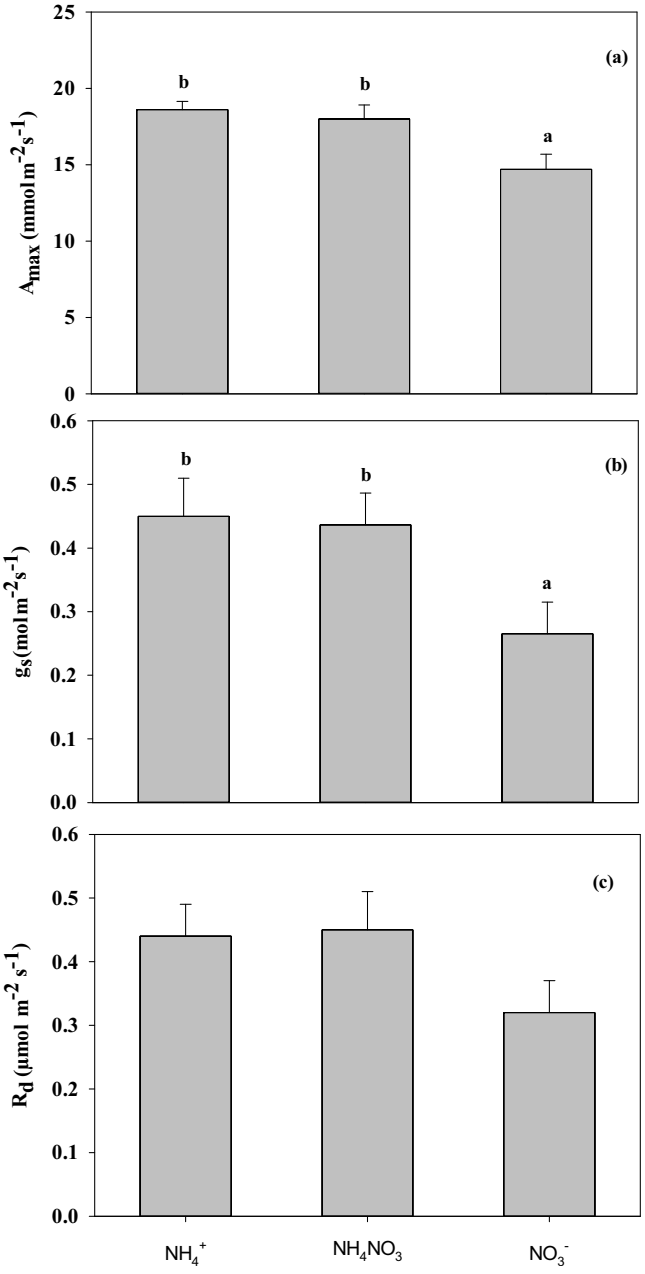

Fig. 2. (a) Light-saturated rate of photosynthesis, (b) stomatal conductance, and (c) dark respiration rate of *Arundo donax* grown at equimolar concentrations of different inorganic nitrogen forms (NH_4^+, NO_3^- or NH_4NO_3). Mean values ± 1SD. Different letters indicate significant differences between treatments ($P < 0.05$).

NH_4NO_3 fed plants, followed by plants grown on NO_3^- treatment. However, the concentrations of chl *b*, and the total chlorophyll/total carotenoid ratio were not significantly different among treatments.

3.3. Nitrogen uptake rates

The NH_4^+ uptake rate of *Arundo donax* was affected by N forms whilst NO_3^- uptake rate did not differ among treatments (Fig. 3a). The NH_4^+ uptake rate of *A. donax* was highest when supplied as NH_4NO_3 (1.10 ± 0.34 mg N g^{-1}root DM h^{-1}) although not different from the N uptake rate with NH_4^+ alone (0.61 ± 0.08 mg N g^{-1}root DM h^{-1}) and the lowest rates (0.38 ± 0.04 mg N g^{-1}root DM h^{-1}) were in plants solely supplied with NO_3^-. Meanwhile the NO_3^- uptake rate ranged unsignificantly from 0.19 to 0.35 mg N g^{-1}root DM h^{-1} among three N treatments (Fig. 3a).

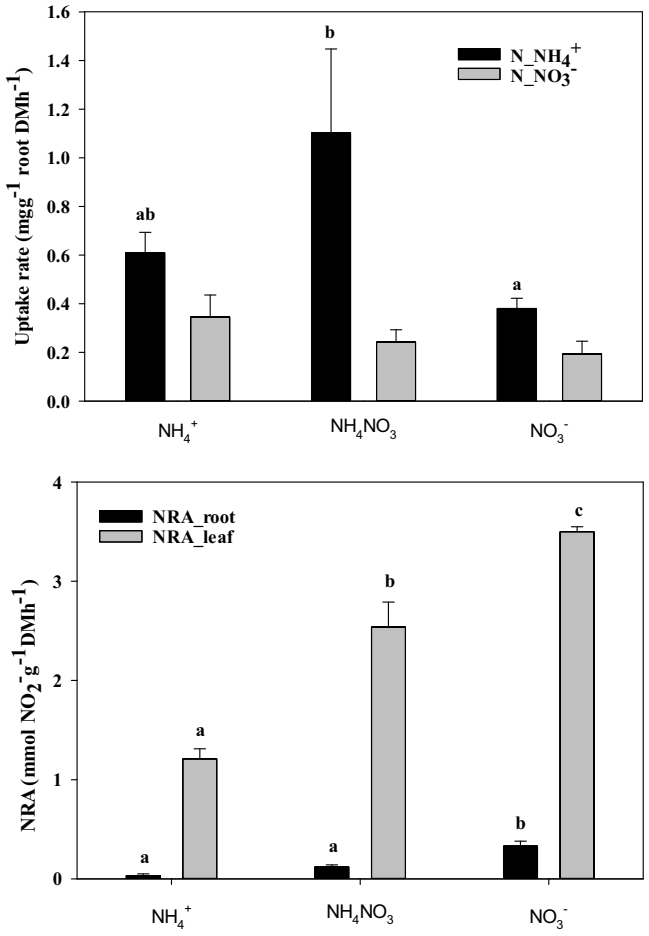

Fig. 3. (a) Nitrogen uptake rates of NH_4^+ and NO_3^- in *Arundo donax* grown at equimolar concentrations of different inorganic nitrogen forms (NH_4^+, NO_3^- or NH_4NO_3). Mean values ± 1SD. Different letters indicate significant difference between treatments ($P < 0.05$). (b) Nitrate reductase activity (NRA) in roots and leaves of *Arundo donax* grown at equimolar concentrations of different inorganic nitrogen forms (NH_4^+, NO_3^- or NH_4NO_3). Mean values ± 1SD. Different letters indicate significant difference between treatments ($P < 0.05$).

3.4. Nitrate reductase activity

Arundo donax had NRA in both leaves and roots, but activities in leaves were an order of magnitude greater than in roots. Leaf NRA was significantly lower in plants supplied with NH_4^+ alone than in plants supplied with either NO_3^- or NH_4NO_3. The highest NRA was detected in leaves of plants grown on NO_3^- with a mean rate of 3.5 μmol $NO_2^-g^{-1}$DM h^{-1} that was significantly higher than the mean rate of 2.54 and 1.21 μmol $NO_2^-g^{-1}$DM h^{-1} in leaves of plants fed with NH_4NO_3 and NH_4^+ respectively (Fig. 3b). Roots NRA was higher in NO_3^- fed plants than in NH_4NO_3 or NH_4^+ Two-way ANOVA revealed significant main effects of N form and plant fraction, and also a significant interaction between the two main factors (Table 3) that was consistent with the differences identified in Fig. 3(b).

3.5. Tissue N content

Overall, roots had higher inorganic N concentrations than leaves and their concentrations of NH_4^+ were higher than NO_3^- (Table 3, Fig. 4). Form of supplied N affected the concentration of NH_4^+ in roots significantly, but not in leaves. The NH_4^+ concentrations in roots of plants supplied with NH_4^+ were significantly higher compared to those of plants grown on NO_3^- and NH_4NO_3 solutions

Table 3
Results of two-way ANOVA for nitrate reductase activity and inorganic nitrogen concentratios in roots and leaves (plant fraction) of *Arundo donax* grown at equimolar concentrations of different inorganic nitrogen forms (NH_4^+, NO_3^- or NH_4NO_3).

Parameters	N-form (df = 2)		Plant fraction (df = 1)		N-forms × plant fraction (df = 2)	
	SS (%)	F-ratio	SS (%)	F-ratio	SS (%)	F-ratio
NRA	7.89	48.98***	36.68	455.22***	4.68	29.06***
NH_4^+	0.12	7.49**	0.01	1.24ns	0.07	4.31*
NO_3^-	0.05	2.92ns	0.06	6.48**	0.03	1.51 ns

(Fig. 4). The N form significantly affected NH_4^+ concentration of *A.donax* and there was a significant interaction between N form and plant fraction on NH_4^+ concentrations whereas the effect of plant fraction was shown only on NO_3^- concentrations (Table 3).

Tissue N concentrations and allocation of N are shown in Table 4. The tissue concentrations of C were largely unaffected by N treatment other than some minor variations in rhizomes, whereas the effects of N sources on tissue concentrations of N were significant in both roots and stems with higher concentrations in NH_4^+ and NH_4NO_3 fed plants respectively. Tissue N content was highest in leaves and lowest in rhizomes. NH_4NO_3 and NO_3^- fed plants had also higher C/N ratios than plants supplied with NH_4^+.

4. Discussion

The present study showed that *Arundo donax* performed well when fed with different inorganic nitrogen forms. Above-ground biomass was higher in the absence of NO_3^-. Plant morphology was not affected by the form of inorganic N supplied, nor was growth rate significantly lower when NO_3^- was supplied either with or without NH_4^+, despite the greater energetic costs of NO_3^- vs NH_4^+ assimilation. Plants grown on NH_4^+ did not show any toxicity symptoms. In comparison with other wetland plants, ammonium was the preferred form of N taken up by *Salvinia natans* (Jampeetong and Brix, 2009). The RGR of *Cyperus laevigatus* was higher when being supplied with NH_4^+, either alone or in combination with NO_3^-, than when being supplied with NO_3^- alone. On the contrary, the RGR of *Phormium tenax* did not change with nitrogen source (Piwpuan et al., 2013). The higher growth rates observed for plants grown on NH_4^+ could be due to increased CO_2 assimilation rates induced by NH_4^+ nutrition, as documented for *Canna indica* L., among others Konnerup and Brix (2010). In our study, the light-saturated photosynthetic rate of NH_4^+-fed plants was significantly higher than that of NO_3^- fed plants. Similarly *Rubus ideaus* (Claussen and Lenz, 1999) and *Phaseolus vulgaris* (Brück and Guo, 2006) had higher CO_2 assimilation rates when grown on NH_4^+ rather than on NO_3^- The reduced photosynthetic capacity of NO_3^- fed plants compared to NH_4^+ fed plants could be caused by NO_3^- reduction activity in leaves which requires electrons from either NADH or NADPH and competes for electrons with the photosynthetic activity (Britto and Kronzucker, 2002; Konnerup and Brix, 2010; Piwpuan et al., 2013). In agreement with previous studies our research showed that the higher rates of photosynthesis in NH_4^+ fed plants com-

Table 4
Plant fractions dry weight and concentrations of N, C and the C/N-ratio of *Arundo donax* grown at equimolar concentrations of different inorganic nitrogen forms (NH_4^+, NO_3^- or NH_4NO_3) and results of ANOVA (F-ratios). Values are means ± S.D.

Parameters	N form			F-ratio
	NH_4^+	NH_4NO_3	NO_3^-	
Tissue DW (g)				
Leaves	3.4 ± 0.1b	3.0 ± 0.1a	2.8 ± 0.1a	9.6**
Stems	7.7 ± 0.3b	6.9 ± 0.3ab	6.2 ± 0.4a	5.2*
Roots	4.3 ± 0.3	4.0 ± 0.3	4.4 ± 0.7	0.3ns
Rhizomes	2.4 ± 0.1	2.1 ± 0.2	2.0 ± 0.2	1.7ns
Tissue N conc. (%DW)				
Leaves	1.61 ± 0.01	1.53 ± 0.05	1.51 ± 0.02	2.6ns
Stems	0.73 ± 0.04ab	0.89 ± 0.08b	0.68 ± 0.04a	3.8*
Roots	1.03 ± 0.06b	0.97 ± 0.03ab	0.86 ± 0.03a	3.8*
Rhizomes	0.46 ± 0.02	0.42 ± 0.02	0.39 ± 0.03	2.1ns
Tissue C conc. (%DW)				
Leaves	45.9 ± 0.1	44.5 ± 1.3	44.4 ± 0.3	1.4ns
Stems	46.7 ± 1.2	50.7 ± 2.6	43.4 ± 2.2	3.0ns
Roots	45.5 ± 2.4	47.4 ± 0.7	44.9 ± 1.1	0.7ns
Rhizomes	47.5 ± 0.2b	46.9 ± 0.1a	47.7 ± 0.1b	10.7***
C/N- ratio				
Leaves	28.6 ± 0.2	29.2 ± 0.4	29.5 ± 0.3	2.1ns
Stems	66.0 ± 3.9	59.3 ± 3.4	65.3 ± 3.7	1.0ns
Roots	44.4 ± 1.0a	49.0 ± 1.1b	52.5 ± 1.6b	10.2**
Rhizomes	105.0 ± 5.0	115.4 ± 5.2	126.4 ± 8.1	2.9ns

Different letters superscripts between columns indicate significantly differences between nitrogen sources.
ns: non-significant.
* $P < 0.05$.
** $P < 0.01$.
*** $P < 0.001$.

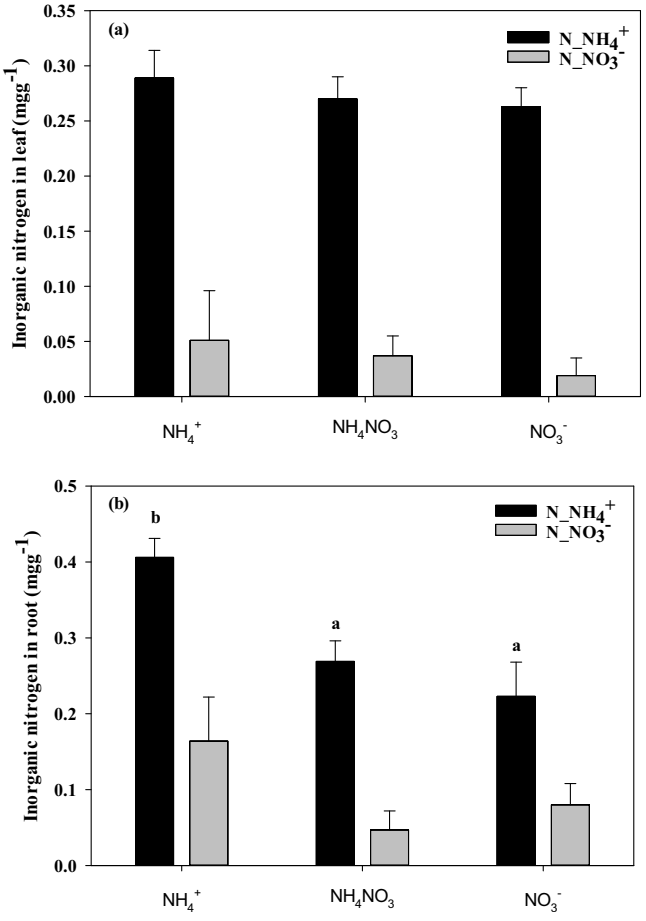

Fig. 4. Inorganic N concentrations in (a) leaves and (b) roots of *Arundo donax* grown at equimolar concentrations of different inorganic nitrogen forms (NH_4^+, NO_3^- or NH_4NO_3). Mean values ± 1SD. Different letters indicate significant difference between treatments ($P < 0.05$).

pared to NO_3^- fed plants were accompanied by higher stomatal conductance (but see also *Canna* indica which did not respond in the same way to NH_4^+ nutrition L. (Konnerup and Brix, 2010)). Our findings also revealed no effects of inorganic N on dark respiration rate and this is consistent with the findings of Rothstein and Cregg (2005), who studied the effects of nitrogen form on nutrient uptake and physiology in *Abies fraseri*.

Chlorophyll concentration is affected by a number of factors, primarily N status (Netto et al., 2005). This can be seen in our study in which the photosynthetic pigments were influenced by the inorganic N forms supplied. The photosynthetic pigments (chl *a* and car) and the light-saturated rate of photosynthesis of *Arundo donax* responded consistently with NH_4^+ nutrition. The low total chlorophyll/total carotenoids ratio may indicate limitation in the photosynthetic activity (Piwpuan et al., 2014) when plants are supplied with high NH_4^+ concentration. However, the high NH_4^+ concentration used in this study did not inhibit photosynthesis of *Arundo donax* which maintained high total chlorophyll/total carotenoids ratios. Like most previous studies, the chl *a/b* ratios were ca. 3:1, indicating healthy plant development and growth (Bojovic and Stojanovic, 2005; Netto et al., 2005).

Arundo donax had higher uptake capacity for NH_4^+ than NO_3^-. In the present study, the uptake velocity of NH_4^+ was generally twice as high as that of NO_3^- regardless of the form of inorganic nitrogen the plants were previously grown on. This is a common phenomenon of plants adapted to growth in wetland environments (Piwpuan et al., 2013). The uptake rate of NO_3^- did not vary significantly among N treatments, indicating that the NO_3^- transportation in plasma membranes of the root system was not controlled by the presence of NO_3^- at the contents provided in our study. The uptake rates of NO_3^- in *A. donax,* regardless of N treatments, were similar to other macrophytes, such as *Canna indica* (Konnerup and Brix, 2010), *Cyperus laevigatus* and *Phormium tenax* (Piwpuan et al., 2013) and different from other macrophyte growth forms such as *Salvinia natans* (Jampeetong and Brix, 2009), *Ipomoea aquatica, Lolium multiflorum,* and *Sorghum sudanense* (Zhou et al., 2011). In contrast, the uptake velocity of NH_4^+ differed among N treatments with the highest and lowest records in plants grown on NH_4NO_3 and NO_3^- respectively, although the uptake rates of plants grown in the NH_4^+ and NH_4NO_3 treatments did not differ significantly. This suggests that up-regulation of NH_4^+ uptake capacity for NH_4^+ and NH_4NO_3 fed plants compared to NO_3^- fed plants was caused by a larger number of NH_4^+ transporters in the plasma membrane of the root systems. However, the higher uptake capacity could also result from a higher capacity to assimilate NH_4^+ in the roots of the NH_4^+ and NH_4NO_3 fed plants, as NH_4^+ assimilation and NH_4^+ uptake seem to be linked together, and assimilation ability is affected by transmembrane fluxes of NH_4^+ in plants (Loqué and Von Wirén, 2004; Konnerup and Brix, 2010). For comparison, *Arundo donax* had similar NH_4^+ uptake capacity to *Phormium tenax* and both species have higher NH_4^+ uptake velocities than thaose of NO_3^- (Piwpuan et al., 2013). Munzarova et al. (2006) also found higher uptake rates for NH_4^+ than NO_3^-in *Phragmites australis* and *Glyceria maxima*; nevertheless the uptake rate was not affected by N treatment in that study. Overall the results of our study indicate that *Arundo donax* prefers NH_4^+ over NO_3^- and this agrees with the results of previous studies of other macrophytes (Jampeetong and Brix, 2009; Konnerup and Brix, 2010; Zhou et al., 2011; Piwpuan et al., 2013).

Plants grown on NH_4^+ generally contained higher concentration of total N in roots and stem tissues than plants grown on NO_3^-, which is in agreement with the results of studies on other plant species such as *Typha latifolia, Sesbania sesban, Salvinia natans, Canna indica, Cyperus laevigatus* and *Phormium tenax* (Brix et al., 2002; Dan and Brix, 2009; Jampeetong and Brix, 2009; Konnerup and Brix, 2010; Piwpuan et al., 2013). This further supports that NH_4^+ is the preferred inorganic N form of *A. donax* because energy lost for uptake and assimilation of ammonium is lower than for nitrate (Raven, 1985; Lambers et al., 2008).

Even though *Arundo donax* can take up NH_4^+ faster than NO_3^-, its growth response when being supplied with different N forms showed that it developed well with either NH_4^+ or NO_3^-. Generally, plants reduce NO_3^- to NH_4^+ by the enzyme nitrate reductase, which is inducible by the presence of nitrate (Cedergreen and Madsen, 2003; Piwpuan et al., 2013) to fully assimilate uptaken NO_3^-. In our study, *A. donax* grown on NO_3^- or NH_4NO_3 solution had higher NRA than plants grown on NH_4^+ in both leaves and roots. This result was consistent with previous observations of higher NRA in plants grown with NO_3^- as opposed to NH_4^+ (Claussen and Lenz, 1999; Jampeetong and Brix, 2009; Konnerup and Brix, 2010; Piwpuan et al., 2013). Moreover, regardless of the N form, *A. donax* had very low NRA in roots compared to the leaves. In combination with relatively higher concentrations of water extractable NO_3^- relative to NH_4^+ in roots of this species compared to leaves, our study supports a significant contribution of leaf NO_3^- reduction in *A. donax*. Likewise, *Canna indica* has higher NRA in leaves compared to roots (Konnerup and Brix 2010). *Phragmites australis,* another emergent wetland plant, also has higher NRA in leaves than in roots when supplied with different N forms (Munzarova et al., 2006). The fact that detectable NRA was measured in *A. donax* supplied with NH_4^+ as the sole N source may reflect that nitrification could have been present in the solution or on the surface of the roots, in spite of the frequent solution renewal every two days.

In conclusion, our results reveal that *A. donax* grows well with either NH_4^+ or NO_3^- as the N source. *Arundo donax* had higher uptake capacity for NH_4^+ than NO_3^-, higher photosynthetic rates and greater N-tissue concentrations when provided with NH_4^+. This suggests that *A. donax* is well-adapted to growth in wetland conditions where NH_4^+ prevails as inorganic nitrogen form. However, it also possesses NO_3^- uptake features and inducible NRA in the leaves like fast-growing species in terrestrial soils high in NO_3^-. Thus, *Arundo donax* seems to be plastic with respect to utilization of different inorganic N sources, explains the distribution of this species in inundated as well as terrestrial conditions (DiTomaso and Healy, 2003). The study also highlights the suitability of *A. donax* for paludiculture, as rewetted agricultural soils are likely to have high and variable availability of both inorganic N forms as the water table fluctuates seasonally and oxidation status becomes spatially and temporally variable. The ability of *A. donax* to thrive well with both forms of N is therefore an important trait likely to confer high growth and yield potential in paludiculture practice.

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