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Effect of Nitrogen Form and Root-zone pH on Growth and Nitrogen Uptake of Tea (*Camellia sinensis*) Plants

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• *Background and Aims* Tea (*Camellia sinensis*) is considered to be acid tolerant and prefers ammonium nutrition, but the interaction between root zone acidity and N form is not properly understood. The present study was performed to characterize their interaction with respect to growth and mineral nutrition.
• *Methods* Tea plants were hydroponically cultured with NH_4^+, NO_3^- and $NH_4^+ + NO_3^-$, at pH 4·0, 5·0 and 6·0, which were maintained by pH stat systems.
• *Key Results* Plants supplied with NO_3^- showed yellowish leaves resembling nitrogen deficiency and grew much slower than those receiving NH_4^+ or $NH_4^+ + NO_3^-$ irrespective of root-zone pH. Absorption of NH_4^+ was 2- to 3·4-fold faster than NO_3^- when supplied separately, and 6- to 16-fold faster when supplied simultaneously. Nitrate-grown plants had significantly reduced glutamine synthetase activity, and lower concentrations of total N, free amino acids and glucose in the roots, but higher concentrations of cations and carboxylates (mainly oxalate) than those grown with NH_4^+ or $NH_4^+ + NO_3^-$. Biomass production was largest at pH 5·0 regardless of N form, and was drastically reduced by a combination of high root-zone pH and NO_3^-. Low root-zone pH reduced root growth only in NO_3^--fed plants. Absorption of N followed a similar pattern as root-zone pH changed, showing highest uptake rates at pH 5·0. The concentrations of total N, free amino acids, sugars and the activity of GS were generally not influenced by pH, whereas the concentrations of cations and carboxylates were generally increased with increasing root-zone pH.
• *Conclusions* Tea plants are well-adapted to NH_4^+-rich environments by exhibiting a high capacity for NH_4^+ assimilation in their roots, reflected in strongly increased key enzyme activities and improved carbohydrate status. The poor plant growth with NO_3^- was largely associated with inefficient absorption of this N source. Decreased growth caused by inappropriate external pH corresponded well with the declining absorption of nitrogen.

Key words: Ammonium, growth, nitrate, nitrogen form, nitrogen uptake, root-zone pH, tea, *Camellia sinensis*.

INTRODUCTION

Ammonium (NH_4^+) and nitrate (NO_3^-) are the most important inorganic N sources in soils readily available to plants. For many plants, NH_4^+, when supplied solely at high concentrations, is toxic and impairs plant growth (Gerendás *et al.*, 1997; Britto and Kronzucker, 2002). However, some plant species are well adapted to this N source (Britto and Kronzucker, 2002). Tea is an important beverage crop widely cultivated in subtropical and tropical areas. There is some evidence that growth of tea plants is improved with NH_4^+ as compared with NO_3^- nutrition (Ishigaki, 1974). Short-time (24 h) experiments revealed a larger absorption of NH_4^+ as compared with NO_3^- when both sources were supplied at similar concentrations (Morita *et al.*, 1998). Total N content in leaves is also increased by application of NH_4^+ together with a nitrification inhibitor as compared with the application of NO_3^- in a soil experiment (Ruan *et al.*, 2000). However, the mechanism behind the preference for NH_4^+ nutrition has not been clearly elucidated (Gerendás *et al.*, 1997; Britto and Kronzucker, 2002).

Response of plant growth and nutrient absorption to N form may further vary with change of external pH (Vessey *et al.*, 1990; Chaillou *et al.*, 1991). Tea plants prefer acid soils and are able to grow on soils below pH 5·0 and a part of China's tea fields consists of soils with pH below 4·0 (Ma *et al.*, 2000). In tea plantations, especially those destined for green tea production, large amounts of N fertilizers have been applied in the form of NH_4^+ or urea (Ruan and Wu, 2004), since it was recognized that green tea quality is closely correlated with the total N and free amino acid concentrations. Whilst nitrification in strongly acidic tea soils is expected to be low (Wickramasinghe *et al.*, 1985), several experiments indicated substantial nitrification and a large pool of NO_3^- in tea soils of very low pH (Hayatsu and Kosuge, 1993). As mentioned above, most studies on NH_4^+ and NO_3^- nutrition of tea plants used either short-time experiments (e.g. Morita *et al.*, 1998) or were done in substrate culture (e.g. Ishigaki, 1974) or under field conditions that are prone to misinterpretations due to N form transformation or changes of pH and nutrient availability. Consequently the relative uptake of NH_4^+ and NO_3^- by tea plants and the consequences for plant growth to varying external pH has not been studied in detail. It may be hypothesized that

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uptake of NH_4^+ by tea plants may decline, whereas that of NO_3^- is unaffected or even increased at slightly acid pH levels due to the involvement of protons in membrane transport as observed in other plant species (Vessey *et al.*, 1990; Marschner, 1995). Therefore the effect of N form and its interaction with external pH on growth and nutrient uptake by tea plants was investigated. Complementary measurements of metabolites related to N assimilation, intracellular pH control and carbohydrate status were also performed.

MATERIALS AND METHODS

Plant growth conditions

Tea [*Camellia sinensis* (L.) O. Kuntze] seedlings with three or four leaves, germinated from seeds of cultivar 'Longjing 43', were transplanted to 0·2 mmol L^{-1} $CaSO_4$ solution for 5 d and then exposed to one-third-strength nutrient solution containing three different sources of nitrogen (NH_4^+, NO_3^- or 50 % NH_4^+ + 50 % NO_3^-) for 1 week. Strength of the nutrient solution was thereafter increased stepwise to two-fifths (weeks 2 and 3), half (week 4), three-quarters (weeks 5–12) and full (weeks 13–20). The composition of full-strength nutrient solution contained macronutrients (mmol L^{-1}) N (1·7), P (0·07), K (0·67) and Ca (0·53), Mg (0·67) and micronutrients (μmol L^{-1}) Zn (0·67), Cu (0·13), Mn (1·0), B (7·0), Mo (0·33) and Fe (4·2) as EDTA salt, as well as 0·07 mmol L^{-1} Al for its beneficial effect on growth of tea plants (Konishi *et al.*, 1985). The N supply used here is slightly lower than the one used by Konishi *et al.* (1985) (2·14 mmol L^{-1} NH_4^+ and 0·71 mmol L^{-1} NO_3^-), but previous experiments have shown that tea plants grown in 1·5 mmol L^{-1} produce comparable total biomass to those grown at higher concentration (e.g. 4·5 mmol L^{-1}), which may impair root growth and lead to substantial accumulation of arginine, indicative of N excess (Ruan *et al.*, 2007). To inhibit any potential nitrification in the nutrient solution (Padgett and Leonard, 1993), the nitrification inhibitor 3,4-dimethylpyrazole phosphate at 1 % of the N amount was added (Zerulla *et al.*, 2001). The pH of nutrient solutions was continuously titrated to 4·0, 5·0 and 6·0 for each of N sources with H_2SO_4 and NaOH by means of custom-built pH stat systems with an accuracy of about ±0·2. Similar systems have been described previously, but the system used here did not employ mechanical stirring for mixing the nutrient solution as described by Wollenweber (1997), but relied on the aeration system instead. Each pot contained 4 L nutrient solution that was replaced every week. Three seedlings per pot were used and thinned to two at week 15. The experiment was conducted for 20 weeks from May to September. Plants were cultivated in a glasshouse under natural light conditions supplemented with additional lighting (SON-T AGRO 400 W; Philips) until week 4 to ensure a minimum intensity of 220 μmol m^{-2} s^{-1} at canopy level. Air temperature in the glasshouse was approx. 34 °C maximum during the day and 20 °C minimum at night. Relative humidity was maintained around 70 % by a humidifier. To facilitate branching, apex buds were removed after the first-round growth (week 8). Young shoots of one bud and two leaves (as for the harvested product) were plucked thereafter. The plants were harvested at week 20, immediately frozen in liquid N_2 and freeze dried. The relative growth rate (RGR) of whole plants over the experimental duration (20 weeks) is calculated according to the equation $RGR = [\ln(W_2) - \ln(W_1)]/(t_2 - t_1)$, where W_1 and W_2 represent whole plant dry weight (g) at time weeks 1 (t_1) and week 20 (t_2).

Chlorophyll content

Chlorophyll contents of mature leaves were measured by a portable chlorophyll meter (Minolta SPAD-502, Osaka, Japan) at weeks 4 and 9. For each plant 4–12 leaves from the upper canopy were randomly selected and average readings were recorded as one replicate. The chlorophyll meter readings were calibrated ($r^2 = 0{\cdot}91$, $P < 0{\cdot}01$) with chlorophyll contents measured by Arnon's method from plants in a parallel experiment supplied with NH_4^+ or NO_3^- under similar growth conditions.

Uptake rates of NH_4^+ and NO_3^-

Specific uptake rates of NH_4^+ and NO_3^- were determined by measuring their depletion in the nutrient solutions four times, twice over 1-week intervals at weeks 10 and 11 and another two times over 5-d intervals at weeks 18 and 19. Concentrations of NH_4^+ and NO_3^- in the nutrient solutions were determined by the indophenol blue method and an ion-selective electrode (Ionplus combination; Orion Research Inc., Beverly, USA), respectively (Mulvaney, 1996). Absorption rates were expressed on a per root dry weight basis calculated from data at the final sampling (week 20) and at the beginning (week 1) assuming a constant growth rate per week. Average rates from the four measurements are reported here.

Enzyme assay

Glutamine synthetase (GS; EC 6.3.1.2) in fibrous roots and young expanding leaves (the third leaf from the bud of young shoots) was extracted with a buffer solution (pH 7·5) containing 50 mmol L^{-1} Tris, 5 mmol L^{-1} EDTA and 5 mmol L^{-1} dithioerythritol at a rate of 10 mL g^{-1} f. wt and 5 % (w/v) PVPP using a Potter S homogenizer cooled with ice (Gerendás *et al.*, 1998). Extracts were centrifuged at 12 000 *g* for 10 min and the crude enzyme was used for activity assay. GS activity was determined by the synthetase assay (Magalhaes and Huber, 1989) and a 30-min incubation time was adopted. One unit enzyme activity corresponds to the formation of 1 μmol γ-glutamyl hydroxamate per gram fresh material per minute.

Determination of free amino acids, soluble sugars, total N, nutrient concentrations and anions

Free amino acids in plant samples of finely ground powder were extracted with H_2O (1/50, w/v) in 100 °C water bath for 5 min and analysed as *o*-phthalaldehyde

derivatives on a reversed-phase C_{18} column (Hypersil ODS, 3 μm, 250 × 4·6 mm) using an automated HPLC system (Gerendás *et al.*, 1998). Standards were prepared from authentic compounds and norvaline was used as an internal standard. Soluble sugars, NO_3^- and organic anions in plant samples were extracted with chloroform : methanol (3 : 7, v/v) as previously described (Lohaus *et al.*, 2000) and separated by ion chromatography (DX 300; Dionex, Idstein, Germany) using a NaOH gradient. Carbohydrates were detected by pulsed amperometry and anions by conductivity after chemical suppression of background conductivity. Total N was determined in an elemental analyser (Carlo Erba, Milano, Italy). Plant samples were digested by mixed concentrated acids HNO_3–$HClO_4$ and measured for P, K, Mg, Ca and S by inductively coupled plasma atomic emission spectrometry (model IRIS-AP; Thermo Jarrel Ash Corp., USA). Sum of equivalents of cations (ΣCat), organic anions (ΣOrganic A) and inorganic anions (ΣInorganic A) were calculated from their concentrations and valances as $K^+ + Ca^{2+} + Mg^{2+} + Na^+$, $citrate^{2-} + malate^{2-} + oxalate^{2-}$ and $SO_4^{2-} + H_2PO_4^- + Cl^- + NO_3^-$, respectively, and expressed as mmol kg^{-1}. For anion equivalents the extracted concentrations were used, except for $H_2PO_4^-$ for which the total P concentration was considered.

Statistics

Statistical analysis was carried out using the Sigma Stat Ver 3.11 for Windows (Systat Software, Inc., Point Richmond, CA 94804-2028, USA) considering a two factorial fully randomized design. Data were subjected to analysis of variance using the *F*-test to examine the effects of N form, pH and their interaction.

RESULTS

Plant growth and biomass production

Plants supplied with NO_3^- displayed yellowish leaves resembling symptoms of nitrogen deficiency from the very beginning of the experiment. Their leaves had lower contents of chlorophyll than plants receiving the other two N form combinations throughout the experimental period (Fig. 1A, B). Root morphology of plants was also quite different among N sources at pH 4·0 and pH 5·0 at the early stage. The plants receiving NH_4^+ and $NH_4^+ + NO_3^-$ grew well with long and white seminal roots while new root development in NO_3^--supplied plants was much slower with shorter seminal roots. The biomass production was significantly less in NO_3^-- than in NH_4^+- or $NH_4^+ + NO_3^-$-fed plants and was not statistically different between the latter two N sources (Fig. 1C, D).

Response of plant growth to root-zone pH varied with the N form supplied. Shortly after treatment onset (week 4), plants exposed to pH 6·0 exhibited stunted and brown seminal roots irrespective of the N source and showed chlorotic leaves that contained considerably less chlorophyll than those from the other two pH treatments (Fig. 1A). After a prolonged time, those plants supplied with NH_4^+ or $NH_4^+ + NO_3^-$ slowly recovered and attained comparable chlorophyll levels in the leaves (Fig. 1B), whereas those supplied with NO_3^- remained chlorotic until the final harvest (week 20). Shoot and whole plant biomass production was highest at pH 5·0 and least at pH 6·0 for all N sources tested (Fig. 1C). Shoot growth of NO_3^-- and NH_4^+-supplied plants, but not those of $NH_4^+ + NO_3^-$-supplied ones, was reduced at pH 4·0 compared with pH 5·0. Root growth was also strongly reduced at pH 4·0 in plants supplied with NO_3^-, but was unaffected in plants receiving NH_4^+ or $NH_4^+ + NO_3^-$ (Fig. 1D). Whole plant biomass production decreased significantly at pH 4·0 for NO_3^--fed plants, though only slightly (n.s. at $P > 0{\cdot}05$) for NH_4^+- or $NH_4^+ + NO_3^-$-supplied plants.

GS activity

The activity of GS in fibrous roots and young leaves was increased by the provision of NH_4^+ compared with NO_3^- (Fig. 1E, F). Plant roots receiving $NH_4^+ + NO_3^-$ had an intermediate level of GS activity, being substantially higher than those provided with NO_3^-, but slightly lower than in plants receiving NH_4^+. The effect of root-zone pH on GS activity in roots was insignificant. However, there was a significant interaction between N form and root-zone pH showing a tendency of decreasing leaf GS activity at pH 4·0 for plants supplied with NH_4^+ or NO_3^-, but at pH 5·0 for plants receiving $NH_4^+ + NO_3^-$.

Nitrogen absorption and concentrations of cations and anions

Absorption of NH_4^+ was much faster than that of NO_3^- irrespective of root-zone pH (Table 1). When NH_4^+ and NO_3^- were individually supplied, NH_4^+ absorption rates were 2·7-, 2·0- and 3·5-fold greater than rates for NO_3^- at pH 4·0, 5·0 and 6·0, respectively. The differences became larger when NH_4^+ and NO_3^- were co-provided, being 6·2-, 6·5- and 16·2-fold greater for NH_4^+ than for NO_3^-. This is because NO_3^- uptake was substantially depressed by co-provision of NH_4^+, being 3·0-, 5·1- and 6·8-fold smaller in plants co-provided than in plants solely supplied with NO_3^- at pH 4·0, 5·0 and 6·0, respectively. Absorption of NH_4^+ and NO_3^-, when supplied individually, was the largest at pH 5·0 and the least at pH 6·0 (Table 1). NH_4^+ uptake differed insignificantly between pH 5·0 and pH 4·0 whereas NO_3^- absorption was reduced by 37 % at pH 4·0 compared with pH 5·0. The absorption of NH_4^+ or NO_3^- was only marginally affected by root-zone pH when they were simultaneously supplied. With regard to N forms, the rate of total N ($NH_4^+ + NO_3^-$) uptake was in the order $NH_4^+ > NH_4^+ + NO_3^- > NO_3^-$ and pH 5·0 > pH 4·0 > pH 6·0 with respect to root-zone pH. The RGR was closely correlated to the total N absorption rate in an exponential fashion (Fig. 2).

Mature leaves and roots of plants supplied with NH_4^+ contained significantly larger total N concentrations than those supplied with NO_3^- whereas $NH_4^+ + NO_3^-$-supplied plants exhibited intermediate levels (Table 2). The specific nitrogen absorption rate closely correlated with total N concentration in mature leaves ($r = 0{\cdot}82$, $P < 0{\cdot}01$) and

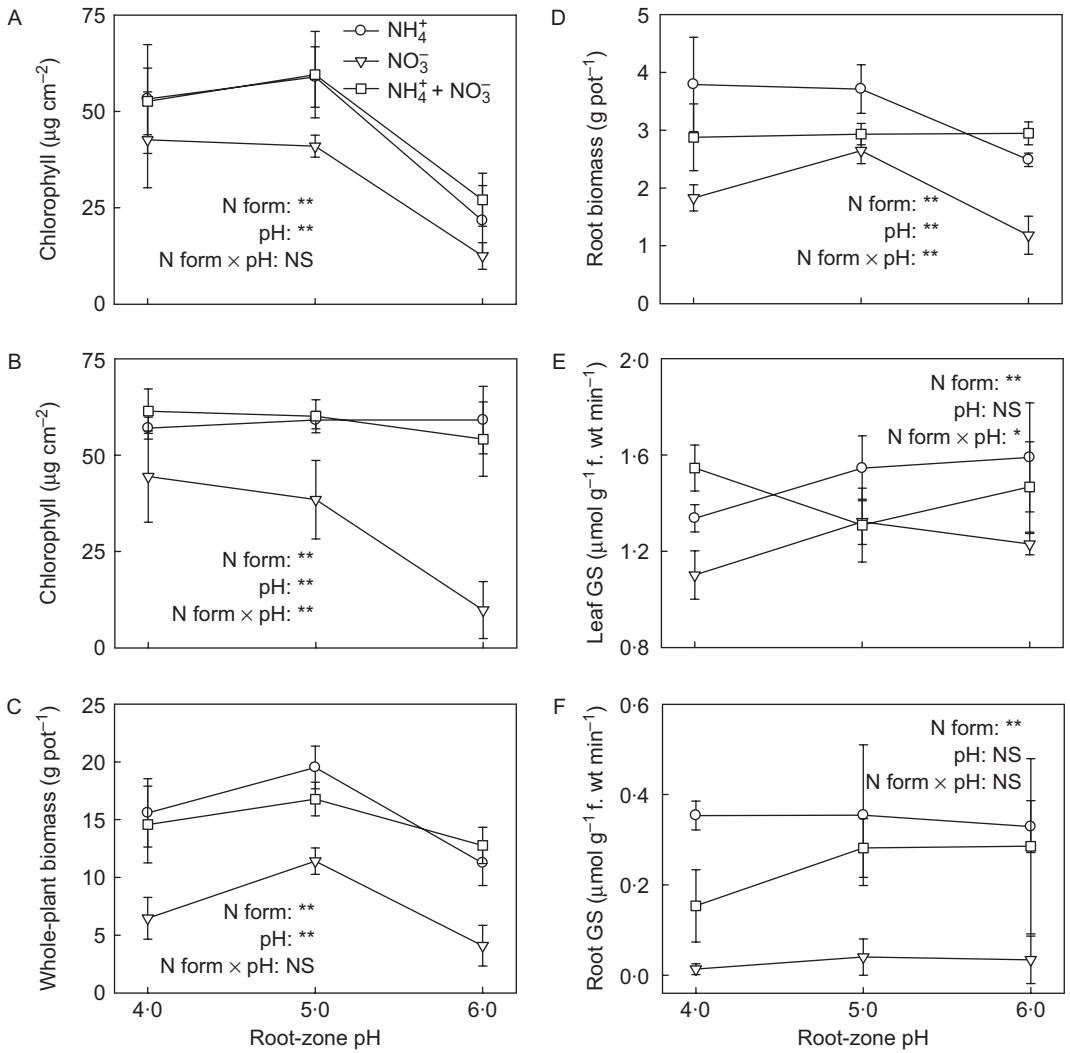

FIG. 1. Chlorophyll content in mature leaves (A, B), biomass production (C, D) and glutamine synthetase (GS) activity (E, F) of tea plants grown with different N forms and root-zone pH. (A) and (B) are measurements 4 and 9 weeks after onset of the treatments, respectively. Bars are s.d. ($n = 3$ or 4). Results of two-way ANOVA indicated as **, $P < 0{\cdot}01$; *, $P < 0{\cdot}05$; NS, not significant $P > 0{\cdot}05$.

roots ($r = 0{\cdot}72$, $P < 0{\cdot}01$). Provision of NO_3^- (NO_3^- or $NH_4^+ + NO_3^-$) significantly increased NO_3^- concentrations in roots and slightly in mature leaves, although accumulation remained insignificant in view of the total N concentration (Table 2). Both total N and NO_3^- concentrations were unaffected by root-zone pH.

Carboxylates are often discussed in relation to ion balance and pH control, and in both mature leaves and fibrous roots their contents (mainly as oxalate) were higher with NO_3^- nutrition and increased at higher external pH (Table 2). Similarly the sums of inorganic anions (SO_4^{2-}, $H_2PO_4^-$, Cl^- and NO_3^-) in fibrous roots were generally larger at high than at low pH (4·0), while in leaves no obvious relationship was apparent. A clear trend was observed between the cations (K^+, Ca^{2+}, Mg^{2+} and Na^+) and organic anion contents (Fig. 3). Highest values of both parameters were observed with NO_3^- nutrition, lowest with NH_4^+ nutrition while plants grown with $NH_4^+ + NO_3^-$ attained intermediate values.

Free amino acids and soluble reduced sugars

Mature leaves of NH_4^+-supplied plants contained substantially increased concentrations of free amino acids compared with those given NO_3^-, and those with the mixed N forms contained intermediate levels (Table 3). The profile of free amino acids was also largely changed by the application of different N forms. The predominant amino acid in the plants supplied with NO_3^- was glutamic acid (Glu), followed by theanine (Thea, N^5-ethyl-glutamine), aspartic acid (Asp) and glutamine (Gln), whereas in NH_4^+-supplied plants Thea and arginine (Arg) predominated, followed by Glu, Gln and Asp. Root-zone pH and its interaction with N form affected none of the major amino acids mentioned above.

Fibrous roots of the plants supplied with $NH_4^+ + NO_3^-$ or NH_4^+ contained similar concentrations of glucose, which were significantly larger than in NO_3^--fed plants (Table 4). Root fructose concentration was greater in

TABLE 1. *NH_4^+- and NO_3^--specific absorption rates of tea plants grown with different N forms and root-zone pH (means ± s.d.,* n = *4)*

Treatment		Specific absorption rate (μmol g^{-1} root d. wt d^{-1})		
N form	pH	NH_4^+	NO_3^-	Total N
NH_4^+	4·0	165·9 ± 21·8		165·9 ± 21·8
	5·0	194·2 ± 23·8		194·2 ± 23·8
	6·0	151·2 ± 27·0		151·2 ± 27·0
NO_3^-	4·0		60·9 ± 9·4	60·9 ± 9·4
	5·0		96·3 ± 13·5	96·3 ± 13·5
	6·0		43·6 ± 2·3	43·6 ± 2·3
$NH_4^+ + NO_3^-$	4·0	124·8 ± 39·2	20·1 ± 7·6	144·9 ± 46·0
	5·0	121·2 ± 40·7	18·7 ± 11·8	139·9 ± 51·1
	6·0	103·6 ± 13·7	6·4 ± 3·2	110·0 ± 16·6
ANOVA (*F*-value)				
N form		20·31**	200·71**	41·90**
pH		2·16	26·21**	6·72**
N form × pH		0·66	12·38**	0·61

**, $P < 0·01$.

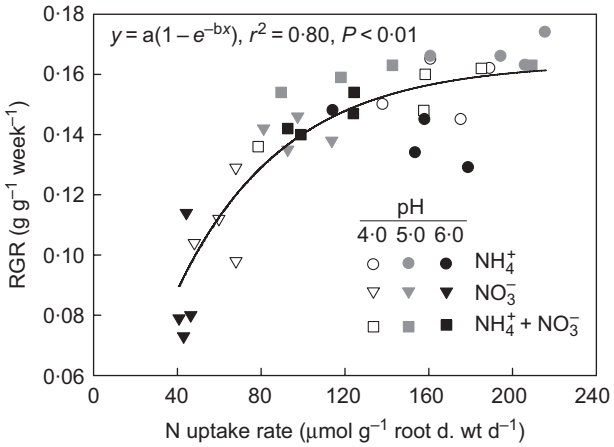

FIG. 2. Relationship of relative growth rate (RGR) of the whole plant over the entire experimental period and the specific N uptake rate of tea plants grown with different N forms and root-zone pH.

plants receiving $NH_4^+ + NO_3^-$ than in plants receiving NH_4^+ or NO_3^-. In contrast, mature leaves of plants receiving NH_4^+ had a lower glucose concentration than plants supplied with NO_3^- or $NH_4^+ + NO_3^-$. A similar observation was made with respect to the concentration of fructose in leaves, but the statistical significance was dependent on pH owing to interaction between N form and pH. Sucrose concentrations in roots and mature leaves were unaffected by N form or root-zone pH (Table 4).

TABLE 2. *Concentrations of total N, nitrate, equivalent sum of cations (ΣCat: K^+, Ca^{2+}, Mg^{2+}, Na^+), organic anions (ΣOrganic A: $citrate^{2-}$, $malate^{2-}$, $oxalate^{2-}$) and inorganic anions (ΣInorganic A: SO_4^{2-}, $H_2PO_4^-$, Cl^-, NO_3^-) in mature leaves and fibrous roots of tea plants grown with different N forms and root-zone pH (means ± s.d.,* n = *4)*

Treatment		Total N (mg g^{-1})	NO_3^- N (mg kg^{-1})	ΣCations (mmol kg^{-1})	ΣInorganic anions (mmol kg^{-1})	ΣOrganic anions (mmol kg^{-1})
N form	pH					
Mature leaves						
NH_4^+	4·0	46·6 ± 5·7	7 ± 1	620 ± 11	255 ± 35	19·1 ± 4·4
	5·0	46·1 ± 2·9	8 ± 1	657 ± 55	246 ± 36	30·9 ± 6·8
	6·0	44·6 ± 4·2	9 ± 1	668 ± 46	259 ± 35	35·9 ± 6·0
NO_3^-	4·0	30·8 ± 3·1	18 ± 14	744 ± 19	159 ± 52	35·1 ± 7·4
	5·0	30·6 ± 1·5	13 ± 3	830 ± 46	271 ± 29	48·1 ± 8·6
	6·0	26·6 ± 1·3	10 ± 5	1280 ± 378	277 ± 69	130·5 ± 28·3
$NH_4^+ + NO_3^-$	4·0	44·4 ± 4·9	10 ± 3	675 ± 22	249 ± 34	27·3 ± 2·7
	5·0	41·0 ± 2·4	28 ± 15	722 ± 15	277 ± 32	33·6 ± 6·5
	6·0	41·4 ± 3·1	18 ± 16	685 ± 67	239 ± 23	28·8 ± 3·6
ANOVA						
N form		72·81**	4·02*	18·50**	0·84	58·18**
pH		2·26	0·95	7·18**	4·12*	38·00**
N form × pH		0·47	1·80	6·12**	3·88*	26·61**
Fibrous roots						
NH_4^+	4·0	39·7 ± 7·3	9 ± 3	639 ± 63	613 ± 41	42·6 ± 14·6
	5·0	35·3 ± 2·4	11 ± 7	784 ± 97	643 ± 89	62·3 ± 16·4
	6·0	42·5 ± 2·3	10 ± 2	819 ± 42	684 ± 119	87·7 ± 12·7
NO_3^-	4·0	23·2 ± 1·5	322 ± 135	786 ± 91	569 ± 62	85·9 ± 27·9
	5·0	22·0 ± 1·5	299 ± 85	1000 ± 37	779 ± 31	145·3 ± 22·3
	6·0	19·1 ± 3·1	310 ± 145	1259 ± 145	810 ± 61	293·5 ± 71·3
$NH_4^+ + NO_3^-$	4·0	30·1 ± 1·4	293 ± 77	772 ± 94	697 ± 86	85·5 ± 23·1
	5·0	30·2 ± 1·5	401 ± 70	899 ± 100	803 ± 64	98·4 ± 20·7
	6·0	29·5 ± 1·7	303 ± 153	994 ± 96	845 ± 106	169·7 ± 29·5
ANOVA						
N form		98·64**	43·49**	26·29**	8·90**	37·47**
pH		1·13	0·38	31·24**	12·51**	41·20**
N form × pH		3·19*	0·64	3·32*	1·68	7·93*

*, $P < 0·05$; **, $P < 0·01$.

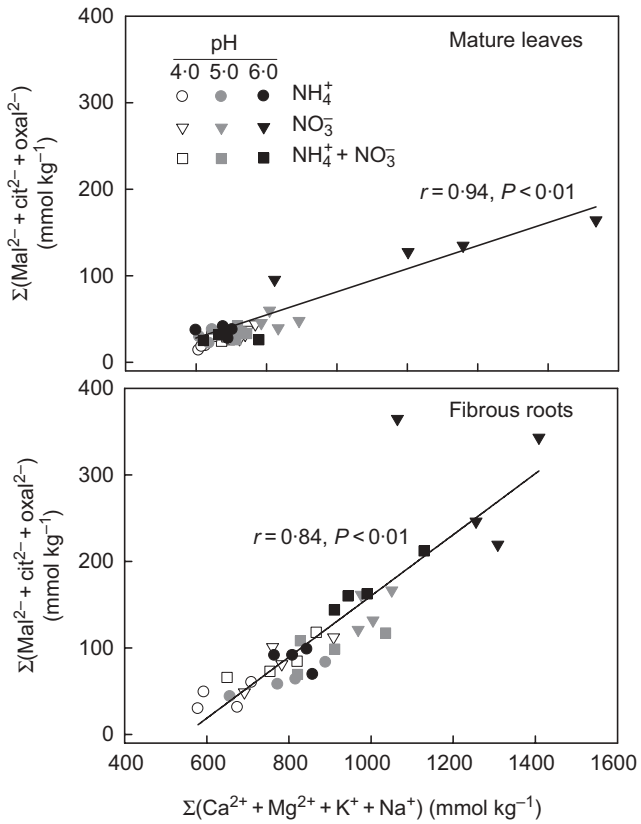

FIG. 3. Relationship of the equivalent sums of cations (K^+, Mg^{2+}, Ca^{2+}, Na^+) and the sum of organic anions (malate^{2-}, citrate^{2-}, oxalate^{2-}) in mature leaves and fibrous roots of tea plants grown with different N forms and root-zone pH.

DISCUSSION

Effects of N form on tea plant growth and nutrient uptake

NH_4^+ and NO_3^- are the most important inorganic N sources for plants, but they induce different growth effects in most plants studied (Britto and Kronzucker, 2002). These responses have been discussed with respect to several hypotheses concerning mainly (*a*) uptake of N and other nutrients, (*b*) energetics of N uptake and assimilation, (*c*) ion balance and pH regulation, and (*d*) osmotic homeostasis, and will be considered in the following paragraphs (for reviews, see Gerendás *et al.*, 1997; Britto and Kronzucker, 2002).

Results of this study clearly demonstrate superior tea plant growth with NH_4^+ (Fig. 1), indicating that tea is well adapted to this N source irrespective of the root-zone pH considered here. Observations from other plant species suggest that reduced plant growth with NO_3^- compared with NH_4^+ as sole N source could be due to low rates of NO_3^- absorption (Lavoie *et al.*, 1992), inefficient assimilation owing to low nitrate reductase activity (Poonnachit and Darnell, 2004), or a combination of both. Although it is well established that N uptake is controlled by the demand imposed by growth rate, present data apparently indicate that the inefficient absorption of NO_3^- (Table 1) likely played an important role because the low RGR of these plants was closely related to their low NO_3^- absorption rate (Fig. 2). The low concentration of total N in mature leaves of NO_3^--fed plants, which was close to the critical deficiency level of around 30 mg g^{-1} (Bonheure and Willson, 1992), indicated that these plants may have suffered from N deficiency (Table 2). The visual N deficiency-like symptoms (yellowish leaves with low chlorophyll contents; Fig. 1) and lower concentrations of free amino acids (Table 3) are also supporting indicators of inadequate N status of these plants. Tissue N concentration significantly correlated with specific N (NH_4^+ and NO_3^-) absorption rate (Tables 1 and 2), suggesting that the reduced total N concentration in NO_3^--supplied plants was related to the low NO_3^- absorption rate. The reduction of NO_3^- was not determined here, but only small amounts of NO_3^- accumulated in mature leaves of NO_3^--fed plants, with similar concentrations in the roots of plants supplied with NO_3^- or $NH_4^+ + NO_3^-$, regardless of their very

TABLE 3. *Concentrations of free amino acids in mature leaves of tea plants grown with different N forms and root-zone pH (means ± s.d.,* n = *4)*

Treatment		Amino acid (μmol g^{-1})					
N form	pH	Thea	Gln	Arg	Glu	Asp	Sum
NH_4^+	4·0	35·5 ± 14·0	19·5 ± 8·2	48·9 ± 37·8	25·1 ± 5·2	16·9 ± 4·4	163 ± 53
	5·0	24·4 ± 10·4	15·8 ± 8·9	42·5 ± 21·9	28·5 ± 4·2	16·7 ± 2·8	145 ± 41
	6·0	49·1 ± 25·9	21·5 ± 11·2	34·6 ± 20·0	30·6 ± 3·1	17·6 ± 1·8	170 ± 53
NO_3^-	4·0	10·4 ± 4·5	3·8 ± 3·0	0·9 ± 0·7	21·3 ± 5·4	9·5 ± 3·4	54 ± 14
	5·0	5·7 ± 4·6	4·4 ± 1·0	1·7 ± 1·7	20·1 ± 1·5	9·4 ± 0·7	46 ± 7
	6·0	6·6 ± 8·7	4·2 ± 2·6	2·5 ± 2·0	23·0 ± 6·2	6·6 ± 1·0	49 ± 18
$NH_4^+ + NO_3^-$	4·0	25·4 ± 17·8	6·0 ± 5·3	16·6 ± 15·2	22·4 ± 2·9	12·4 ± 1·8	101 ± 44
	5·0	7·6 ± 2·4	4·1 ± 2·6	8·4 ± 4·5	20·8 ± 2·9	13·7 ± 1·4	66 ± 9
	6·0	19·2 ± 7·9	6·8 ± 6·7	11·1 ± 7·1	25·9 ± 4·9	14·5 ± 4·5	89 ± 28
ANOVA (*F*-value)							
N form		35·47**	19·32**	18·06**	7·80**	29·13**	31·44**
pH		1·83	0·56	0·41	2·66	0·06	1·17
N form × pH		1·70	0·23	0·27	0·51	1·03	0·25

**, $P < 0{\cdot}01$.

TABLE 4. *Concentrations of carbohydrates (mg g^{-1}) in mature leaves and fibrous roots of tea plants grown with different N forms and root-zone pH (means ± s.d.,* n = *4)*

Treatment		Mature leaves			Fibrous roots		
N form	pH	Glucose	Fructose	Sucrose	Glucose	Fructose	Sucrose
NH_4^+	4·0	7·9 ± 2·9	5·3 ± 1·7	68·3 ± 2·0	2·7 ± 0·6	1·8 ± 1·1	18·4 ± 10·2
	5·0	7·8 ± 3·3	5·9 ± 2·8	68·7 ± 3·3	2·6 ± 0·3	1·6 ± 0·8	24·6 ± 11·9
	6·0	8·8 ± 2·7	6·4 ± 1·6	74·5 ± 7·8	3·0 ± 0·8	2·4 ± 2·2	19·5 ± 5·4
NO_3^-	4·0	15·4 ± 3·3	11·3 ± 3·0	68·1 ± 2·4	1·6 ± 0·2	1·5 ± 0·5	21·1 ± 9·1
	5·0	11·6 ± 2·4	8·0 ± 1·3	68·7 ± 6·6	1·3 ± 0·5	1·4 ± 1·0	19·3 ± 7·4
	6·0	11·0 ± 3·9	6·7 ± 3·1	62·0 ± 8·6	1·4 ± 0·4	1·3 ± 0·7	23·8 ± 8·1
$NH_4^+ + NO_3^-$	4·0	11·8 ± 2·5	7·8 ± 2·3	72·0 ± 5·4	3·5 ± 1·2	2·9 ± 0·4	21·7 ± 7·0
	5·0	15·2 ± 4·0	10·6 ± 2·4	64·8 ± 5·2	3·1 ± 0·2	2·7 ± 0·7	27·0 ± 8·8
	6·0	9·1 ± 1·7	7·0 ± 1·8	60·9 ± 9·8	2·7 ± 0·4	2·7 ± 0·7	23·5 ± 4·2
ANOVA (*F* value)							
N form		7·66**	5·78**	1·96	25·19**	5·37*	0·52
pH		1·75	1·58	1·06	0·48	0·19	0·45
N form × pH		0·47	2·48	2·84*	2·44	0·32	0·45

*, $P < 0·05$; **, $P < 0·01$.

different specific NO_3^- absorption rates, implying that the reduction of NO_3^- might not have been limited (Table 2). On the other hand, growth responses induced by different N forms have been frequently attributed to altered contents of nutrients and it has been postulated that poor growth of NO_3^- compared with NH_4^+-fed *Pinus pinaster* is due partly to induced deficiencies of other nutrients (Warren and Adams, 2002). In the present experiment the concentrations of macro- (P, K, Mg and Ca) and micro-nutrients (B, Fe, Zn, Cu and Mn; data not shown) in the plants fell in normal ranges and did not appear to be directly associated with poor plant growth (Table 2).

With respect to the interaction of both N forms the higher NH_4^+ absorption in plants receiving only NH_4^+ as compared with a mixed supply (Table 1) corresponds well to the altered total N concentrations in roots and mature leaves (Tables 2 and 4). These data suggest that the N supply in plants receiving $NH_4^+ + NO_3^-$ was not sufficient as NO_3^- contributed only a minimal fraction to the overall N uptake. Moreover, the concentrations of free amino acids, particularly Thea, Arg and Gln, in the mature leaves of plants supplied with $NH_4^+ + NO_3^-$ were lower than those grown on NH_4^+ alone. These observations were especially evident at pH 5·0 and provide an explanation for the smaller biomass production of these plants compared with those receiving only NH_4^+ at this root-zone pH. Thus, synergism benefits from mixed N supply, as observed in other plants (Britto and Kronzucker, 2002, and references therein), were not apparent in tea in the present study. NO_3^- absorption in $NH_4^+ + NO_3^-$-supplied plants was inhibited by the simultaneous provision of NH_4^+ when compared with plants solely supplied with NO_3^- (Table 1), an effect that has been reported previously and attributed to repressive action on NO_3^- influx, which is likely accompanied by the down-regulation of transporters for NO_3^- by NH_4^+ and/or their downstream metabolites at transcriptional and post-transcriptional levels (Kronzucker *et al.*, 1999; Glass *et al.*, 2007). In the present experiment, the depression of NO_3^- absorption rates by NH_4^+ occurred concomitantly to a substantial increase of many free amino acids, particularly Thea, Arg and Gln in leaves and roots (Ruan *et al.*, 2007), agreeing with previous findings that net NO_3^- uptake is depressed by elevated intracellular concentrations of free amino acids (Glass *et al.*, 2002).

NH_4^+ is assimilated principally in roots via the glutamine synthetase–glutamine-oxoglutarate aminotransferase (GS-GOGAT) pathway, and tea plants were able to increase root GS activity substantially under conditions of high demand due to NH_4^+ supply (Fig. 1), reaching levels typically found in herbaceous plants exhibiting high growth rates. This response to NH_4^+ nutrition provides essential capacity to assimilate the majority of NH_4^+ in the roots in order to avoid any excessive accumulation of lethal concentrations (Magalhaes and Huber, 1989; Raab and Terry, 1995). In contrast the GS activity of NO_3^--fed plants was down-regulated due to N deficiency resulting from declining absorption. It was reported from other plant species that a benefit of NH_4^+ plus NO_3^- nutrition results from additional assimilatory flux potential arising from the specific induction by NO_3^- of the plastidic GS-GOGAT pathway that is not available to plants grown on pure NH_4^+ (Redinbaugh and Campbell, 1993; Kronzucker *et al.*, 1999). However, due to the very limited NO_3^- uptake (Table 1) resulting in only a moderate input into the metabolic N pool, this synergistic effect of N sources is not generally observed in tea (Fig. 1). The less pronounced influence of N form on NH_4^+-assimilating enzymes in leaves exhibiting a much higher activity level overall (Fig. 1) agrees with the view that recycling of NH_4^+ from endogenous sources such as photorespiration represents their major task (Redinbaugh and Campbell, 1993).

On a molar basis N represents a considerable proportion of total ion uptake, and consequently the form of N absorbed exerts a strong impact on the ion balance. Views differ on the precise proton balance for assimilating the two N forms (e.g. Kosegarten *et al.*, 1997; Gerendás and Ratcliffe, 2000; Britto and Kronzucker, 2002), but it is generally agreed that growth with NH_4^+ results in a more

positive proton balance when regeneration of the co-factors is taken into consideration (Gerendás and Ratcliffe, 2002). In fact, the maintenance of appropriate carboxylate levels has been frequently considered a prerequisite for NH_4^+ tolerance (Salsac *et al.*, 1987), which most likely stems from either its anaplerotic function (reviewed by Britto and Kronzucker, 2005), its involvement in pH homeostasis (Gerendás and Ratcliffe, 2000), or its osmotic function (Salsac *et al.*, 1987). This also provides an explanation for the effect of high root-zone pH, where organic acids substantially accumulated to close the charge gap aroused from significantly larger uptake of cations (Marschner, 1995; Table 2 and Fig. 3).

As the assimilation of NH_4^+ requires substantial amounts of 2-oxoglutarate obtained from glucose (ultimately sucrose imported from leaves) it has been proposed that tolerance of plants to NH_4^+ nutrition is associated with adequate carbohydrate status of roots (Schortemeyer *et al.*, 1997). Indeed, most studies showed reduced sugar contents in roots of NH_4^+-grown plants (e.g. Chaillou *et al.*, 1991), and only occasionally when root growth was severely impaired, were higher sugar levels observed in NH_4^+-grown roots (e.g. Walch-Liu *et al.*, 2001). In contrast, the present experiment showed a higher concentration of glucose in roots of tea plants supplied with NH_4^+ (NH_4^+ or $NH_4^+ + NO_3^-$) than with NO_3^- (Table 4). No differences in the maximum photosynthetic rates (per leaf area) were detected between NH_4^+- and NO_3^--supplied plants at pH 5·0 in a parallel experiment. However, NH_4^+-treated plants developed more leaves and thus a larger total leaf area than NO_3^--supplied plants, indicating a stronger source capacity. Since sucrose concentrations were at similar levels in roots grown with different N forms, higher glucose concentration there suggests increased sucrose import from leaves when demand for carbon skeletons is high under NH_4^+ nutrition. Alternatively, low glucose levels could result from larger consumption under NO_3^- nutrition compared with NH_4^+ due to additional cost for NO_3^- reduction (Bloom *et al.*, 1992). However, this seems unlikely to be of significant importance in the present study since, compared with NH_4^+, both absorption and assimilation of NO_3^- were limited (Tables 1 and 4). The large capacity for NH_4^+ assimilation of tea plants was additionally reflected by the abundance of amides (Thea and Gln) in roots supplied with NH_4^+, which were 3- to 25- and 16- to 44-fold larger than in NO_3^--supplied plants (Ruan *et al.*, 2007) that were suffering from N deficiency as discussed before. The high C drain towards amino acid synthesis may also explain efforts to save carbon skeletons like the particularly strong accumulation of Arg in NH_4^+-grown tea plants, owing to its low C : N ratio (Table 3). Collectively, data indicate that tea plants have a high capacity to assimilate NH_4^+ in their roots by strongly increasing key enzyme activities and improving carbohydrate status in the roots.

Effect of pH on tea plant growth and nutrient uptake

The pH of the rooting medium is of paramount importance for plant growth as a large number of processes (e.g. nutrient availability and uptake rate, availability of toxic ion species, soil structure) are closely related to this parameter (Marschner, 1995). Even under more controlled conditions of hydroponic systems, as used here, plant biomass production was largest at pH 5·0 regardless of the N form supplied (Fig. 1), while growth was reduced more strongly at pH 6·0 than at pH 4·0, indicating that tea plants were sensitive to higher external pH. Whilst a pH of around 6·0 is considered to be more or less optimal for many plants because they are best adapted to availability and uptake of nutrient and toxic agents prevailing under these conditions, detrimental effects have been reported for some plant species. For instance, root elongation of *Lupinus angustifolius*, a plant species well adapted to acid soils, is markedly decreased by pH $\geq$6·0 (Tang *et al.*, 1996).

The mechanism for the negative effect of higher pH is not understood, but it has been suggested that the effect of inappropriate external pH on nutrient absorption may be responsible for the observed growth phenomena (Vessey *et al.*, 1990; Brix *et al.*, 2002). At pH 6·0, specific absorption rates of NH_4^+ and NO_3^- diminished by 22 % and 55 %, respectively, compared with pH 5·0 (Table 1), which corresponds to the growth reductions of 42 % and 64 % at pH 6·0 observed for plants receiving either NH_4^+ or NO_3^-, respectively. However, the growth reduction overcompensating the reduced N absorption, suggests that other factors might also be involved even though effects of root-zone pH on total tissue N concentrations of plants were rather small (Table 2). The more pronounced reduction of NO_3^- absorption with increasing pH is in line with the general view that cation uptake is usually increased at high pH while anions respond in the opposite way (Vessey *et al.*, 1990; Marschner, 1995; Brix *et al.*, 2002).

Nitrate is actively taken up by an H^+-co-transport system in the plasma membrane and is therefore dependent on the membrane H^+ gradient generated by ATPase. Consequently absorption of NO_3^- increases as the external pH is reduced because of the higher H^+ gradient (e.g. Vessey *et al.*, 1990). The present data showed, however, that plant growth and absorption of NO_3^- were not improved at low root-zone pH. The absorption rate of NO_3^- was diminished by 37 %, but only by 15 % ($P > 0{\cdot}05$) for NH_4^+ at pH 4·0 compared with pH 5·0 when the two N forms were individually supplied. With extended exposure, high H^+ activity in the root solution can disrupt the electrochemical gradient by increasing plasma membrane permeability or decreasing the efficiency of H^+-ATPase pumping activity, leading to reduced NO_3^- uptake capacity (Brix *et al.*, 2002). Indeed, NO_3^--supplied plants displayed higher sensitivity to low root-zone pH (Table 1). In addition, when plant growth is adversely affected by low external pH its effect on NO_3^- absorption may become superimposed by altered demand (demand-driven uptake) as shown for cereals (Zsoldos *et al.*, 1999). The root biomass production of plants receiving NO_3^- at pH 4·0 was reduced by 31 %, whereas those of NH_4^+- or $NH_4^+ + NO_3^-$-supplied plants was unaffected and their whole plant biomass decreased slightly ($P > 0{\cdot}05$). The latter case therefore indicates that even though tea plants prefer NH_4^+ nutrition, decreasing pH to

an extremely low level in the rooting environment could impose a detrimental effect on plant growth and nutrient uptake (Tables 1 and 2). Such phenomena have been reported in other plants, including acid tolerant ones preferring NH_4^+ nutrition such as *Typha latifolia* (Brix *et al.*, 2002), and the mechanisms involved have been well reviewed (Britto and Kronzucker, 2002).

A limited nitrification of NH_4^+ has frequently been observed under acidic soil conditions, making it a more prevalent N source under these conditions (Schmidt, 1982; Kronzucker *et al.*, 2003). Considering these ecological circumstances it seems fair to assume that most, though not all, plants that are tolerant to low soil pH are generally NH_4^+-tolerant (Britto and Kronzucker, 2002). From an evolutionary perspective, the tea plant is believed to originate in south-west China, where it co-dominates with other species to form forest climax vegetation (L. Chen, pers. comm.). The preference in tea for NH_4^+, and its poor utilization capacity for NO_3^-, may reflect its ecological position as a typical representative of climax vegetation species that is adapted to forest soils enriched with NH_4^+ as the predominant inorganic N form, according to recent opinion (Kronzucker *et al.*, 1997, 2003).

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