Elevated atmospheric CO₂ decreases the ammonia compensation point of barley plants

Liang Wang, Pai Pedas, Dennis Eriksson and Jan K. Schjoerring*

Plant and Soil Science Section, Department of Plant and Environmental Sciences, Faculty of Science, University of Copenhagen, Thorvaldsensvej 40, 1871 Frederiksberg C, Denmark

* To whom correspondence should be addressed. Email: jks@life.ku.dk

Received 16 January 2013; Revised 20 March 2013; Accepted 8 April 2013

Abstract

The ammonia compensation point ($\chi_{NH_3}$) controls the direction and magnitude of NH₃ exchange between plant leaves and the atmosphere. Very limited information is currently available on how $\chi_{NH_3}$ responds to anticipated climate changes. Young barley plants were grown for 2 weeks at ambient (400 μmol mol⁻¹) or elevated (800 μmol mol⁻¹) CO₂ concentration with NO₃⁻ or NH₄NO₃ as the nitrogen source. The concentrations of NH₃ and H⁺ in the leaf apoplastic solution were measured along with different foliar N pools and enzymes involved in N metabolism. Elevated CO₂ caused a threefold decrease in the $\chi_{NH_3}$ concentration in the apoplastic solution and slightly acidified it. This resulted in a decline of the $\chi_{NH_3}$ from 2.25 and 2.95 nmol mol⁻¹ under ambient CO₂ to 0.37 and 0.89 nmol mol⁻¹ at elevated CO₂ in the NO₃⁻ and NH₄NO₃ treatments, respectively. The decrease in $\chi_{NH_3}$ at elevated CO₂ reflected a lower N concentration (~25%) in the shoot dry matter. The activity of nitrate reductase also declined (~45 to ~60%), while that of glutamine synthetase was unaffected by elevated CO₂. It is concluded that elevated CO₂ increases the likelihood of plants being a sink for atmospheric NH₃ and reduces episodes of NH₃ emission from plants.

Key words: ammonia compensation point ($\chi_{NH_3}$), barley, carbon dioxide (CO₂), climate change, nitrate reductase, nitrogen metabolism.

Introduction

Ammonia (NH₃) plays a major role in atmospheric chemistry and radiative forcing (Hertel et al., 2011). When deposited in sensitive terrestrial ecosystems, NH₃/ammonium (NH₄⁺) contributes to acidification and loss of biodiversity (Dise et al., 2011). Exchange of NH₃ between the atmosphere and vegetation constitutes an important process in the global NH₃ budget, but very little information is available on how anticipated climate changes will affect this exchange. The magnitude and direction of NH₃ fluxes between vegetation and the atmosphere are controlled by plant physiological processes in interaction with environmental conditions (Schjoerring et al., 2000; Massad et al., 2008).

The fundamental parameters characterizing plant–atmosphere NH₃ exchange are the stomatal NH₃ compensation point ($\chi_{NH_3}$) together with the conductance to diffusion of NH₃ through the stomata and boundary layers surrounding the leaves (Kruit et al., 2010). Both of these parameters can be expected to be modified by future climate changes, including elevated atmospheric carbon dioxide (CO₂), rising air temperature, and more frequent drought periods (Fowler et al., 2009).

The NH₃ compensation point in plants is defined as the NH₃ concentration in the air within the substomatal cavity at which no net NH₃ exchange with the ambient atmosphere...
takes place (Farquhar et al., 1980). NH$_3$ may be emitted when the atmospheric NH$_3$ concentration falls below $\chi$NH$_3$, and absorbed by plants when the atmospheric NH$_3$ concentration is higher than $\chi$NH$_3$. The $\chi$NH$_3$ value reflects the concentration of NH$_4^+$ and H$^+$ in the leaf apoplastic solution as these two parameters control the concentration of dissolved NH$_3$ in equilibrium with gaseous NH$_3$ within the substomatal cavities (Husted et al., 1996; 2000).

There is currently very limited data on how atmospheric CO$_2$ affects $\chi$NH$_3$. Theoretically, elevated CO$_2$ would be expected to decrease $\chi$NH$_3$ due to repression of photorespiration, which is a major NH$_4^+$-generating process implicated in foliar NH$_3$ exchange (Mattsson et al., 1998; Husted et al., 2002; Kumagai et al., 2011). The more efficient photosynthetic carbon acquisition under elevated CO$_2$ will in many cases stimulate plant growth without increasing root nitrogen (N) uptake, leading to an overall decline in plant N status (Taub and Wang, 2008), which would be expected to decrease $\chi$NH$_3$. In addition, more carbon skeletons, energy and reducing power would be available for NH$_4^+$ assimilation via the glutamine synthetase/glutamate synthase cycle (Forde and Lea, 2007). An uncertain factor in the NH$_3$ budget will be how nitrate (NO$_3^-$) reduction is affected. Elevated CO$_2$ has in some cases been reported to inhibit shoot NO$_3^-$ reduction in C$_3$ species, including barley (Bloom et al., 2012), while in other cases the foliar nitrate reductase activity of barley was only slightly suppressed (Sicher, 2001) or was even significantly enhanced (Robredo et al., 2011).

Plant–atmosphere NH$_3$ exchange is strongly affected by apoplastic pH (Husted and Schjoerring, 1995). When CO$_2$ dissolves in water, it forms carbonic acid, which is a weak acid. In addition, photosynthetic CO$_2$ fixation leads to intracellular generation of stoichiometric amounts of H$^+$. Elevated CO$_2$ would therefore theoretically be expected to decrease the apoplastic pH, assuming a low buffer capacity of the apoplastic solution (Nielsen and Schjoerring, 1998). Very high atmospheric CO$_2$ concentrations (1–16%) have been shown to rapidly decrease the apoplastic pH (Oja et al., 1999; Savchenko et al., 2000). However, transient additions of CO$_2$ in the physiologically relevant CO$_2$ range (0–800 μmol mol$^{-1}$) alkalized the apoplastic solution (Hedrich et al., 2001; Felle and Hanstein, 2002). All these studies of elevated CO$_2$ exposure were conducted over a short period (minutes to hours). Information is still lacking on whether or not the buffering system of the apoplast can compensate for the pH changes after prolonged exposure to elevated CO$_2$.

The aim of the present study was to investigate the effect of elevated CO$_2$ on $\chi$NH$_3$ and relate this effect to N metabolic responses. Our hypothesis was that elevated atmospheric CO$_2$ would enhance the capacity for NH$_4^+$ assimilation and therefore decrease $\chi$NH$_3$. Barley plants were grown in 2 mM NO$_3^-$ or 1 mM NH$_4$NO$_3$ solution at ambient (400 μmol mol$^{-1}$) or elevated (800 μmol mol$^{-1}$) CO$_2$ for 2 weeks. The apoplastic solution was extracted for determination of $\chi$NH$_3$ and related to key N pools and N-assimilatory enzymes in shoots and roots.
acid as an internal standard for amino acid measurement. After centrifugation at 20 000g at 4 °C for 10 min, the supernatant was filtered (Qmax syringe filter, 0.45 μm pore size; Frisøren Aps, Knebel, DK) and stored at –80 °C until analysis. NH₃ was determined by spectrophotometric detection (Häusler et al., 1994). Amino acids were derivatized by AccQ-Tag Ultra reagent (Waters, Milford, MA, USA) and afterwards measured on an Agilent UPLC System (BEH C18 1.7 μm column; Waters). For analysis of bulk tissue pH, plant tissues were ground in Milli-Q water and the pH recorded by use of a microelectrode (Metrohm, Herisau, Switzerland). Lyophilized samples were used for analysis of total C and N by mass spectrometry in a system consisting of an ANCA-SL Elemental Analyzer (Wang et al., 2011).

Frozen tissues were ground with a mortar and pestle for determination of nitrate reductase (NR), glutamine synthetase (GS), and glutamate dehydrogenase (GDH) activities. For NR, the shoot and root material was extracted in a buffer containing 100 mM HEPES/KOH (pH 7.6), 20 mM MgCl₂, 0.2 mM phenylmethylsulfonyl fluoride, 5 mM dithiothreitol (DTT), 10 μM leupeptin, 1 mM Pefabloc, 10 μM flavin adenine dinucleotide, 0.6% polyvinylpyrrolidone (PVPP), and 0.05% casein. The assay was performed in the presence of MgCl₂ or EDTA in order to obtain the actual and maximum activity, respectively, and the NR activation state (ratio between actual and maximum activity).

Shoot and root tissues for assays of GS and GDH activity were extracted in the same buffer solution containing 50 mM HEPES/KOH (pH 7.8), 5 mM MgCl₂, 0.5 mM EDTA-Na₂, 5 mM DTT, 20% glycerol, and 0.6% PVPP. GS activity was measured by incubation of extracts in a reaction buffer containing 100 mM HEPES/KOH (pH 7.8), 150 mM glutamate, 10 mM MgCl₂, 15 mM ATP, 10 mM hydroxylamine, and 2 mM EDTA. After 20 min at 30 °C, stop solution consisting of 8% (w/v) trichloroacetic acid, 3.3% (w/v) FeCl₃, and 2 M HCl was added. NADH-GDH activity was determined in a reaction buffer containing 100 mM Tris/HCl (pH 8), 1 mM MgCl₂, 50 mM hydroxylamine, 13 mM 2-ketoglutarate, and 0.25 mM NADH. NAD-GDH activity was determined in a reaction buffer consisting of 100 mM Tris/HCl (pH 9), 1 mM CaCl₂, 35 mM sodium glutamate, and 0.25 mM NAD. Total soluble protein in the supernatant of GS and GDH extraction was determined according to the method of Bradford (1976) using bovine serum albumin as the standard.

Calculations and statistical analysis

The X NH₃ at 25 °C was calculated on the basis of the apoplastic concentrations of NH₄⁺ and H⁺ according to the following equation (Mattisson et al., 2009):

\[ X_{NH_3} = K_H \times K_d \times \Gamma = 10^{-11.01} \times \Gamma \]

where \( K_H \) and \( K_d \) are thermodynamic constants of \( 10^{-11.76} \) atm l mol⁻¹ and \( 10^{-9.25} \) mol l⁻¹ at 25 °C, respectively. \( \Gamma \) is the NH₄⁺/H⁺ ratio, and NH₄⁺ and H⁺ are the NH₄⁺ concentration and the proton concentration (H⁺=10⁻¹⁰) in the apoplastic extracts. Bulk tissue \( \Gamma \) values were calculated as the ratio between NH₄⁺ and H⁺ in bulk tissue extracts.

Two-way analysis of variance (ANOVA) was carried out to test CO₂, N form, and their interactions. Duncan’s test was conducted to compare the mean values.

Results

Plant growth

CO₂ increased total plant biomass by 36% and 21% in the NO₃⁻ and NH₄NO₃ treatments, respectively (\( P < 0.01 \); Fig. 1). The corresponding root/shoot ratios remained unchanged in the NO₃⁻ treatment but decreased in the NH₄NO₃ treatment (\( P < 0.01 \); data not shown). The total leaf area was increased by elevated CO₂ (\( P < 0.05 \); Fig. 1), while the water consumption decreased by 8–9% (\( P < 0.01 \); Fig. 1). More water was consumed when plants were supplied with NH₄NO₃ compared with those supplied with NO₃⁻ (\( P < 0.01 \); Fig. 1).

![Fig. 1. Effect of elevated atmospheric CO₂ on plant weight (dry matter basis), leaf area (LA), and water consumption (WC). The experimental treatments were: ambient CO₂ and 1 mM NO₃⁻ (A-NO₃⁻, open bars), elevated CO₂ and 1 mM NO₃⁻ (E-NO₃⁻, grey shaded bars), 1 mM NH₄NO₃ (A-NH₄NO₃, open hatched bars), and elevated CO₂ and 1 mM NH₄NO₃ (E-NH₄NO₃, grey hatched bars). Values are means ± standard error (SE) (n=8). Different letters above columns indicate significant differences (\( P \leq 0.05 \)) between mean values inside same plant organ (shoot, root, or total). In the ANOVA table, * and ** denote significant differences at \( P \leq 0.05 \) and \( P \leq 0.01 \), respectively; n.s., no significant difference.](https://academic.oup.com/jxb/article-abstract/64/10/2713/540936/6431073640386?45?3251309202010636)
Leaf gas exchange

Elevated CO₂ promoted a clear increase in net photosynthesis, while stomatal conductance and transpiration decreased ($P < 0.01$; Fig. 2). Measurements of net photosynthesis at elevated CO₂ showed similar rates in plants previously grown at ambient or elevated CO₂, thus demonstrating that photosynthetic acclimation had not occurred (Fig. 2a). Plants receiving NH₄⁺ in addition to NO₃⁻ responded more to elevated CO₂ and obtained higher net photosynthetic rates than plants supplied exclusively with NO₃⁻ (Fig. 2a).

Nitrogen and carbon levels

The N concentration in the dry matter of both shoot and root tissues exhibited a remarkable decrease at elevated CO₂ ($P < 0.01$; Fig. 3a). This decrease was similar whether N was supplied as NO₃⁻ (~19% in shoot and ~18% in roots) or NH₄NO₃ (~15% in shoot and ~22% in roots). The decline in N concentration resulted in a marked increase in the C/N ratio at elevated CO₂ ($P < 0.01$; Fig. 3b). However, due to the
increased biomass at elevated CO$_2$, the total N content per plant was not changed (Fig. 3c).

**Apoplastic NH$_4^+$ and pH**

Elevated CO$_2$ caused about a threefold decrease in the apoplastic NH$_4^+$ concentration ($P<0.01$; Fig. 4a). At the same time, a small ($P<0.05$) acidification of the apoplastic solution occurred (Fig. 4b). These changes decreased the stomatal $\chi_{NH_3}$ from 2.25 and 2.95 nmol mol$^{-1}$ under ambient CO$_2$ to 0.37 and 0.89 nmol mol$^{-1}$ at elevated CO$_2$ in the NO$_3^-$ and NH$_4$NO$_3$ treatments, respectively ($P<0.01$; Fig. 4c). The corresponding apoplastic $\Gamma$ values, i.e. the ratio between the concentration of NH$_4^+$ and H$^+$ in the apoplastic solution, were 230 and 302 under ambient CO$_2$ and 38 and 91 at elevated CO$_2$ in the NO$_3^-$ and NH$_4$NO$_3$ treatments, respectively.

**Tissue and xylem NH$_4^+$ and pH**

Elevated CO$_2$ significantly reduced the NH$_4^+$ concentration in shoots of plants in the NO$_3^-$ treatment but not in the NH$_4$NO$_3$ treatment (Fig. 5a). In the roots, the NH$_4^+$ concentration increased in response to elevated CO$_2$ in the NH$_4$NO$_3$ treatment (Fig. 5a). Elevated CO$_2$ also led to acidification of the leaf tissues (Fig. 5b). The bulk tissue $\Gamma$ value, i.e. the ratio between bulk tissue NH$_4^+$ and H$^+$ concentration, decreased significantly ($P<0.01$) from 646 at ambient CO$_2$ to 280 at elevated CO$_2$ in plants supplied with NO$_3^-$, while in plants receiving NH$_4^+$ the corresponding decrease ($P=0.30$) was from 667 to 563 (Fig. 5c).

The NH$_4^+$ concentration in the xylem sap was around 1 mM and showed a small, statistically non-significant decrease in response to elevated CO$_2$ (data not shown). Elevated CO$_2$ reduced the xylem NO$_3^-$ concentration from around 30 to 22 mM in plants receiving only NO$_3^-$ and from around 20 to 13 mM in plants receiving NH$_4$NO$_3$ ($P<0.01$). The pH in the xylem sap was around 6.0 in all treatments and showed a small (about 0.2 pH units) increase under elevated CO$_2$ in the NO$_3^-$ treatment ($P<0.05$; data not shown).

**Tissue NO$_3^-$, amino acids, and soluble protein**

The NO$_3^-$ concentration in shoot and root tissues was significantly reduced by elevated CO$_2$ ($P<0.01$; Fig. 6a). In addition, the level of soluble (free) amino acids in shoot tissue decreased under elevated CO$_2$, while no significant change was observed in the roots (Fig. 6b). There was no significant effect of elevated CO$_2$ on the soluble protein concentration (Fig. 6c).

**NR, GS, and GDH activity**

The actual activity of NR in the shoots was about 45% lower under elevated CO$_2$ ($P<0.01$; Fig. 7). Concurrently, the maximum NR activity in the shoots decreased by 55-60%, reducing the activation state from around 45% under ambient CO$_2$ to 40% under elevated CO$_2$ (Fig. 7). In roots, no significant changes in NR activity were observed (Fig. 7). GS activity was not affected by elevated CO$_2$ (Fig. 8), as was also the case for the aminating activity of GDH (NADH-GDH), while the deaminating GDH activity (NAD-GDH) increased by around 15 and 16–34% in shoots and roots, respectively (data not shown).

**Discussion**

**Plant growth and photosynthesis**

Plants exposed to elevated CO$_2$ are likely to become N limited as they achieve a faster growth rate (Conroy and Hocking,
The N limitation will cause a decrease in the N concentration or in the levels of N metabolites (Stitt and Krapp, 1999). In order to avoid N limitation, previous studies have explored a very high N supply (more than 10 mM N) to grow barley plants (Sicher and Bunce, 2008; Robredo et al., 2011), which is far above physiologically relevant N concentrations in soil solution. In the present study, plants received 2 mM $\text{NH}_4^+$ or 1 mM $\text{NH}_4\text{NO}_3$ and the N was topped up or renewed every 3 d. The N concentrations in shoot and root tissues were above 3.8% (Fig. 3) and total soluble protein remained unchanged for all treatments (Fig. 6), showing that the N status of the plants was optimum.

Elevated CO$_2$ dramatically increased leaf photosynthesis as well as shoot and root biomass (Figs 1 and 2a). Increased biomass is a general feature of CO$_2$ responses in C$_3$ crops (Kimball et al., 2002). However, the initial stimulation of photosynthesis often gradually declines with prolonged exposure to elevated CO$_2$, a phenomenon known as photosynthetic acclimation (Stitt and Krapp, 1999; Ainsworth and Long, 2005; Seneweera et al., 2011). This acclimation can mechanistically and quantitatively be attributed to a decline in apparent in vivo Rubisco activity ($V_{\text{max}}$) and reduced investment in Rubisco, accompanied by a decrease in foliar soluble protein (Rogers and Humphries, 2000). However, in the present study, the amounts of total soluble protein in shoots were similar at ambient and elevated CO$_2$ in both the NO$_3^-$ and $\text{NH}_4\text{NO}_3$ treatments (Fig. 6c). Moreover, plants grown at elevated CO$_2$ did not show any decline in photosynthesis, as evidenced by the fact that their photosynthesis was similar to that of plants grown at ambient CO$_2$ and only exposed to elevated CO$_2$ during the short measurement period (Fig. 2a).

Increased CO$_2$ assimilation at elevated CO$_2$ would be expected to stimulate N acquisition in order to match the faster growth rates (Kruse et al., 2002). However, declines in N concentration of shoot and root tissues at elevated CO$_2$, accompanied by increasing C/N ratios, are often observed in C$_3$ plants (Cotrufo et al., 1998; Kimball et al., 2002). This decrease may derive from a dilution effect accompanying the faster biomass growth (Taub and Wang, 2008). That dilution was a main factor is corroborated by the fact that the quantity of N per plant, i.e. the product of biomass and N concentration, was not different between plants grown at elevated CO$_2$. The N limitation will cause a decrease in the N concentration or in the levels of N metabolites.
glutamine in both roots and shoots (data not shown), but this was apparently not sufficient to stimulate N uptake. NO$_3^-$ uptake also depends on energy from mitochondrial respiration. The potential alterations in root respiration by elevated CO$_2$ are still poorly documented (Gonzalez-Meller et al., 2004; Leakey et al., 2009). In the present work, root biomass was increased by elevated CO$_2$ (Fig. 1), which may have increased growth respiration at the expense of energy for ion uptake. The signalling pathways involved in this regulation and their interactions with plant growth and C and N metabolism under elevated CO$_2$ is an area where further knowledge is required (Foyer et al., 2011). Bloom et al. (2010) ascribed the lack of increased N uptake under elevated CO$_2$ to decreased capacity for shoot NO$_3^-$ assimilation rather than to a direct effect on N absorption. However, provision of N in the form of NH$_4^+$ did not in the present work result in higher total N uptake (Fig. 3c), suggesting that the lack of stimulation of N acquisition under elevated CO$_2$ was not specifically associated with NO$_3^-$ assimilation.

**Plant NO$_3^-$ content and assimilation**

The decline in NO$_3^-$ concentration in shoot and root tissues was pronounced for all plants at elevated CO$_2$ (Fig. 6a). Approximately 30% lower NO$_3^-$ concentration in the xylem sap at elevated CO$_2$ in parallel with lower transpiration rates (Figs 1 and 2c) indicated that less NO$_3^-$ was transported to the shoot. In terms of NO$_3^-$ assimilation, elevated CO$_2$ resulted in a marked decrease (45–60%) in shoot maximum and actual NR activities compared with ambient CO$_2$. (Fig. 7). This decline may reflect the lower tissue NO$_3^-$ levels promoting degradation of NR transcripts and protein (Galangau et al., 1988) and leading to de-induction of NO$_3^-$ transporter and NR activities (Stitt and Krapp, 1999). Elevated CO$_2$ inhibited NO$_3^-$ assimilation in leaves of wheat, tomato, and Arabidopsis (Bloom et al., 2002, 2010; Searles and Bloom, 2003). In a recent study, Bloom et al. (2012) demonstrated that elevated CO$_2$ inhibited NO$_3^-$ assimilation in eight different C$_3$ species including barley, whereas it remained unchanged in three different C$_4$ species. This indicates that the mode of C fixation is a main factor determining the response in NO$_3^-$ assimilation to elevated CO$_2$. In the work of Bloom and co-workers, plants were grown at a relatively low N supply (<1mM N). However, at higher N supplies (>10mM N), the foliar NR activity of barley was only slightly suppressed (Sicher, 2001) or even significantly enhanced (Robredo et al., 2011) in response to elevated CO$_2$. In the latter case, plants were grown at very high NO$_3^-$ supply (20mM) to ensure that any possible down-regulation of photosynthesis resulting from elevated CO$_2$ was unrelated to nitrogen limitation.

In many plant species, including barley, NO$_3^-$ assimilation predominantly occurs in root tissue when the NO$_3^-$ supply is low (<1mM), and the importance of shoot assimilation increases with increasing NO$_3^-$ concentrations (Andrews et al., 1992). Root NR activity only accounted for a small proportion of the NO$_3^-$ assimilation in the present work (Fig. 7). Elevated CO$_2$ did not increase the activity of NR in roots, but, due to the relatively large decrease in shoot NR...
a larger proportion of total plant NR was present in roots (Fig. 7; see also Kruse et al., 2002, 2003). This change in NR distribution may partly alleviate inhibition of shoot NO$_3^-$ assimilation under elevated CO$_2$ due to reduced availability of NADH and ferredoxin (Bloom et al., 2010).

**NH$_3$ compensation point, tissue NH$_4^+$, and NH$_3$ exchange potential**

Elevated CO$_2$ showed a clear negative effect on $\chi_{NH_3}$, which declined from 2.25 and 2.95 nmol mol$^{-1}$ under ambient CO$_2$ to 0.37 and 0.89 nmol mol$^{-1}$ at elevated CO$_2$ in the NO$_3^-$ and NH$_4$NO$_3$ treatments, respectively (Fig. 4c). The decrease was derived mainly from lower apoplastic NH$_4^+$ concentrations, while apoplastic H$^+$ concentrations only became marginally lower (Fig. 4a, b).

The apoplastic NH$_4^+$ concentration is controlled by the balance between processes generating and assimilating NH$_4^+$ (Schjoerring et al., 2002). Root NH$_4^+$ uptake is an important process leading to increased shoot NH$_4^+$ levels (Mattsson and Schjoerring, 2002) and NH$_3$ emission from young barley plants (Mattsson and Schjoerring, 1996). NH$_4^+$ translocated from roots to shoots via the xylem sap enters the leaf apoplastic solution and ultimately the leaf cells (Finnemann and Schjoerring, 1999). Elevated CO$_2$ caused only a non-significant decrease in the xylem NH$_4^+$ concentration but markedly decreased the transpiration rate (Figs 1 and 2c), thereby suppressing the root-to-shoot NH$_4^+$ transport and thus lowering the apoplastic NH$_4^+$ concentration.

Photorespiration is quantitatively one of the most important processes generating NH$_4^+$ in young leaves of C$_3$ plants (Carvalho et al., 2011) and repression of photorespiration is widely accepted to explain the decreased foliar NH$_4^+$ concentration at elevated CO$_2$ (Geiger et al., 1999; Sicher, 2001). Suppression of photorespiration has been shown to lead to reduced tissue NH$_4^+$ and decreased NH$_3$ emission (Mattsson et al., 1998; Husted et al., 2002; Kumagai et al., 2011). However, the decrease in tissue NH$_4^+$ was only observed in shoots receiving NO$_3^-$ and not in the NH$_4$NO$_3$ treatment (Fig. 5a), showing the importance of NH$_4^+$ derived from root uptake for bulk tissue NH$_4^+$ levels (Finnemann and Schjoerring, 1999; Mattsson and Schjoerring, 2002).

The activity of the main NH$_4^+$ assimilating enzyme, GS, has been shown to be essential for tissue and apoplastic

Fig. 7. Effect of elevated CO$_2$ on (a) NR activity expressed on the basis of fresh weight or (b) per unit soluble protein. Full-length columns represent maximum NR activities (Max.), while the horizontal line within columns shows actual NR activities (Act.). Filled circles above columns in (a) show the NR activation state. Plant growth conditions and symbols are as in Fig. 1. Values are means ± SE ($n$=5–8).
Elevated atmospheric CO$_2$ decreases $\chi_{\text{NH}_3}$ in barley

Mattsson et al. (1997) observed a decrease in NH$_3$ concentrations in barley. The GS activity did not show a significant response to elevated CO$_2$ in either shoots or roots (Fig. 8). This suggests that GS may not be the key factor regulating apoplastic NH$_3$ under elevated CO$_2$. The enzyme GDH was for many years assumed to be involved in NH$_4^+$ assimilation, but seems to play a role in the liberation of NH$_4^+$ by catalysing the oxidative deamination of glutamate, thereby providing carbon skeletons for respiration and oxidative phosphorylation (Labboun et al., 2009). In agreement, elevated CO$_2$ did not significantly alter the aminating activity of GDH (NADH-GDH), but significantly increased its deaminating activity (NAD-GDH) in shoots and roots (data not shown). Increasing NAD-GDH activity could be relevant to meet energy requirements for CO$_2$ assimilation in competition with NH$_3$ production via NO$_3^-$ reduction and photorespiration (Bloom et al., 2010).

The stomatal NH$_3$ compensation point, $\chi_{\text{NH}_3}$, is a central driver in the exchange of NH$_3$ between plant leaves and the atmosphere (Personne et al., 2009; Massad et al., 2010; Zhang et al., 2010). Lower $\chi_{\text{NH}_3}$ in response to elevated CO$_2$ will increase the sink strength of plant leaves for atmospheric NH$_3$ and reduce the likelihood of foliar NH$_3$ emissions. Besides $\chi_{\text{NH}_3}$, the actual stomatal NH$_3$ flux at a given atmospheric NH$_3$ concentration will also depend on the stomatal conductance. This parameter decreases markedly under elevated CO$_2$ (Fig. 2b; Leakey et al., 2009), implying that the reduction in $\chi_{\text{NH}_3}$ under elevated CO$_2$ will not result in a proportional increase in NH$_3$ flux.

**Conclusions**

It is concluded that elevated atmospheric CO$_2$ decreased $\chi_{\text{NH}_3}$ in barley leaves. This reflected a pronounced decrease in the NH$_4^+$ concentration in the apoplastic solution, while the pH only changed marginally. The primary reasons for the lower apoplastic NH$_4^+$ concentration were decreased NH$_4^+$ input via the transpiration stream and decreased NH$_3$ production via NO$_3^-$ reduction and photorespiration. The activity of GS,
the first step in the assimilation of $\text{NH}_4^+$, did not increase in response to elevated $\text{CO}_2$.

**Acknowledgements**

This work was supported by the Danish Ministry of Food, Agriculture and Fisheries (3304-FVFP-08) and the Danish Research Council for Technology and Production. The valuable assistance of PhD student Lizhi Long with measurements of enzyme activities is gratefully acknowledged.

**References**

Ainsworth EA, Long SP. 2005. What have we learned from 15 years of free-air $\text{CO}_2$ enrichment (FACE)? A meta-analytic review of the responses of photosynthesis, canopy. *New Phytologist* **165**, 351–371.

Andrews M, Morton JD, Lieffering M, Bisset L. 1992. The partitioning of nitrate assimilation between root and shoot of a range of temperate cereals and pasture grasses. *Annals of Botany* **70**, 271–276.

Bloom AJ, Burger M, Rubio-Asensio JS, Cousins AB. 2010. Carbon dioxide enrichment inhibits nitrate assimilation in wheat and Arabidopsis. *Science* **326**, 899–903.

Bloom AJ, Rubio-Asensio JS, Randall L, Rachmilevitch S, Cousins AB, Carlisle EA. 2012. $\text{CO}_2$ enrichment inhibits shoot nitrate assimilation in $\text{C}_3$ but not $\text{C}_4$ plants and slows growth under nitrate in $\text{C}_3$ plants. *Ecology* **93**, 355–367.

Bloom AJ, Smart DR, Nguyen DT, Searles PS. 2002. Nitrogen assimilation and growth of wheat under elevated carbon dioxide. *Proceedings of the National Academy of Sciences USA* **99**, 1730–1735.

Bradford MM. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein using the principle of protein-dye binding. *Analytical Biochemistry* **72**, 248–254.

Carvalho JDC, Madgwick PJ, Powers SJ, Keys AJ, Lea PJ, Parry MAJ. 2011. An engineered pathway for glyoxylate metabolism in tobacco plants aimed to avoid the release of ammonia in photorespiration. *BMC Biotechnology* **11**, 111.

Conroy J, Hocking P. 1993. Nitrogen nutrition of $\text{C}_3$ plants at elevated atmospheric $\text{CO}_2$ concentrations. *Physiologia Plantarum* **89**, 570–576.

Cotrufo MF, Ineson P, Scott A. 1998. Elevated $\text{CO}_2$ reduces the nitrogen concentration of plant tissues. *Global Change Biology* **4**, 43–54.

Dise NB, Ashmore M, Belyazid S et al. 2011. Nitrogen as a threat to European terrestrial biodiversity. In: Sutton MA, Howard CM, Erisman JW, Billen G, Bleeker A, Grennfelt P, van Grinsven H, Grizzetti B, eds. *The European nitrogen assessment*, New York: Cambridge University Press, 463–494.

Farquhar GD, Firth PM, Wetselaar R, Weir B. 1980. On the gaseous exchange of ammonia between leaves and the environment: determination of the ammonia compensation point. *Plant Physiology* **66**, 710–714.

Felle HH, Hanstein S. 2002. The apoplastic pH of the substomatal cavity of *Vicia faba* leaves and its regulation responding to different stress factors. *Journal of Experimental Botany* **53**, 73–82.

Finnemann J, Schjoerring JK. 1999. Translocation of $\text{NH}_4^+$ in oilseed rape plants in relation to glutamine synthetase isoenzyme expression and activity. *Physiologia Plantarum* **105**, 469–477.

Forde BG, Lea PJ. 2007. Glutamate in plants: metabolism, regulation, and signalling. *Journal of Experimental Botany* **58**, 2339–2358.

Fowler D, Pilegaard K, Sutton MA, et al. 2009. Atmospheric composition change: ecosystems-atmosphere interactions. *Atmospheric Environment* **43**, 5193–5267.

Foyer CH, Noctor G, Hodges M. 2011. Respiration and nitrogen assimilation: targeting mitochondria-associated metabolism as a means to enhance nitrogen use efficiency. *Journal of Experimental Botany* **62**, 1467–1482.

Galgau F, Danielvedele F, Moureaux T, Dorbe MF, Leydecker MT, Caboche M. 1988. Expression of leaf nitrate reductase genes from tomato and tobacco in relation to light–dark regimes and nitrate supply. *Plant Physiology* **88**, 383–388.

Geiger M, Haake V, Ludewig F, Sonnewald U, Stitt M. 1999. The nitrate and ammonium nitrate supply have a major influence on the response of photosynthesis, carbon metabolism, nitrogen metabolism and growth to elevated carbon dioxide in tobacco. *Plant, Cell & Environment* **22**, 1177–1199.

Gonzalez-Meller MA, Taneva L, Trueman RJ. 2004. Plant respiration and elevated atmospheric $\text{CO}_2$ concentration: cellular responses and global significance. *Annals of Botany* **94**, 647–656.

Häusler RE, Blackwell RD, Lea PJ, Leegood RC. 1994. Control of photosynthesis in barley leaves with reduced activities of glutamine synthetase or glutamate synthase. Part I. Plant characteristics and changes in nitrate, ammonium and amino acids. *Planta* **194**, 406–417.

Hedrich R, Neimanis S, Savchenko G, Felle HH, Kaiser WM, Heber U. 2001. Changes in apoplastic pH and membrane potential in leaves in relation to stomatal responses to $\text{CO}_2$, malate, abscisic acid or interruption of water supply. *Planta* **213**, 594–601.

Hertel O, Reis S, Skjeth CA, et al. 2011. Nitrogen processes in the atmosphere. In: Sutton MA, Howard CM, Erisman JW, Billen G, Bleeker A, Grennfelt P, van Grinsven H, Grizzetti B, eds. *The European nitrogen assessment*, New York: Cambridge University Press, 177–207.

Husted S, Mattsson M, Mollers C, Wallbraun M, Schjoerring JK. 2002. Photosynthetic $\text{NH}_4^+$ production in leaves of wild-type and glutamine synthetase 2 antisense oilseed rape. *Plant Physiology* **130**, 989–998.

Husted S, Mattsson M, Schjoerring JK. 1996. Ammonia compensation points in two cultivars of *Hordeum vulgare* L. during vegetative and generative growth. *Plant, Cell & Environment* **19**, 1299–1306.

Husted S, Schjoerring JK, Nielsen KH, Nemitz E, Sutton MA. 2000. Stomatal compensation points for ammonia in oilseed rape plants under field conditions. *Agricultural and Forest Meteorology* **105**, 371–383.

Husted S, Schjoerring JK. 1995. Apoplastic pH and ammonium concentration in leaves of *Brassica napus* L. *Plant Physiology* **109**, 1453–1460.
Kimball BA, Kobayashi K, Bindi M. 2002. Responses of agricultural crops to free-air CO2 enrichment. Advances in Agronomy 77, 293–368.

Kruit RJW, van Pul WAJ, Sauter FJ, van den Broek M, Nemitz E, Sutton MA, Krol M, Holtslag AAM. 2010. Modeling the surface-atmosphere exchange of ammonia. Atmospheric Environment 44, 945–957.

Krusse J, Hetzger I, Hansch R, Mendel RR, Walch-Liu P, Engels C, Rennenberg H. 2002. Elevated pCO2 favours nitrate reduction in the roots of wild-type tobacco (Nicotiana tabacum cv. Gat.) and significantly alters N-metabolism in transformants lacking functional nitrate reductase in the roots. Journal of Experimental Botany 53, 2351–2367.

Krusse J, Hetzger I, Mai C, Polle A, Rennenberg H. 2003. Elevated pCO2 affects N-metabolism of young poplar plants (Populus tremula × P. alba) differently at deficient and sufficient N-supply. New Phytologist 157, 65–81.

Kumagai E, Araki T, Hamaoka N, Ueno O. 2011. Ammonia emission from rice leaves in relation to photospiration and genotypic differences in glutamine synthetase activity. Annals of Botany 108, 1381–1386.

Labboun S, Terce-Laforgue T, Roscher A, et al. 2009. Resolving the role of plant glutamate dehydrogenase. I. In vivo real time nuclear magnetic resonance spectroscopy experiments. Plant and Cell Physiology 50, 1761–1773.

Languar V, Loque D, Hormann F, Yuan LX, Bohner A, Engelsberger WR, Lalonde S, Schulze WX, von Wieren N, Frommer WB. 2009. Feedback inhibition of ammonium uptake by a phospho-dependent allosteric mechanism in Arabidopsis. Plant Cell 21, 3610–3622.

Laugier E, Bouguyon E, Mauries A, Tillard P, Gojon A, Lejay L. 2012. Regulation of high-affinity nitrate uptake in roots of Arabidopsis depends predominantly on posttranscriptional control of the NRT2.1 transport system. Plant Cell 108, 1381–1386.

Leakey ADB, Ainsworth EA, Bernacchi CJ, Rogers A, Long SP, Ort DR. 2009. Elevated CO2 effects on plant carbon, nitrogen, and water relations: six important lessons from FACE. Journal of Experimental Botany 60, 2859–2876.

Massad RS, Loubet B, Tuzet A, Cellier P. 2008. Relationship between ammonia stomatal compensation point and nitrogen metabolism in arable crops: current status of knowledge and potential modelling approaches. Environmental Pollution 154, 390–403.

Massad RS, Nemitz E, Sutton MA. 2010. Review and parameterisation of bi-directional ammonia exchange between vegetation and the atmosphere. Atmospheric Chemistry and Physics 10, 10359–10386.

Mattsson M, Hauser RE, Leegood RC, Lea PJ, Schjoerring JK. 1997. Leaf-atmosphere NH3 exchange in barley mutants with reduced activities of glutamine synthetase. Plant Physiology 114, 1307–1312.

Mattsson M, Herrmann B, David M, Loubet B, Riedo M, Theobald MR, Sutton MA, Bruhn D, Neftel A, Schjoerring JK. 2009. Temporal variability in bioassays of the stomatal ammonia compensation point in relation to plant and soil nitrogen parameters in intensively managed grassland. Biogeoosciences 6, 171–179.

Mattsson M, Husted S, Schjoerring JK. 1998. Influence of nitrogen nutrition and metabolism on ammonia volatilization in plants. Nutrient Cycling in Agroecosystems 51, 35–40.

Mattsson M, Schjoerring JK. 1996. Ammonia emission from young barley plants: influence of N source, light/dark cycles and inhibition of glutamine synthetase. Journal of Experimental Botany 47, 477–484.

Mattsson M, Schjoerring JK. 2002. Dynamic and steady-state responses of inorganic nitrogen pools and NH3 exchange in leaves of Lolium perenne and Bromus erectus to changes in root nitrogen supply. Plant Physiology 128, 742–750.

Miller AJ, Fan XR, Shen QR, Smith SJ. 2008. Amino acids and nitrate as signals for the regulation of nitrogen acquisition. Journal of Experimental Botany 59, 111–119.

Nielsen KH, Schjoerring JK. 1998. Regulation of apoplastic NH4 concentration in leaves of oilseed rape. Plant Physiology 118, 1361–1368.

Oja V, Savchenko G, Jakob B, Heber U. 1999. pH and buffer capacities of apoplastic and cytoplasmic cell compartments in leaves. Planta 209, 239–249.

Personne E, Loubet B, Herrmann B, Mattsson M, Schjoerring JK, Nemitz E, Sutton MA, Cellier P. 2009. SURFATM-NH3: a model combining the surface energy balance and bi-directional exchanges of ammonia applied at the field scale. Biogeoosciences 6, 1371–1388.

Robredo A, Perez-Lopez U, Miranda-Apodaca J, Lacuesta M, Mena-Petite A, Munoz-Rueda A. 2011. Elevated CO2 reduces the drought effect on nitrogen metabolism in barley plants during drought and subsequent recovery. Environmental and Experimental Botany 71, 399–408.

Rogers A, Humphries SW. 2000. A mechanistic evaluation of photosynthetic acclimation at elevated CO2. Global Change Biology 6, 1005–1011.

Savchenko G, Wiese C, Neimanis S, Hedrich R, Heber U. 2000. pH regulation in apoplastic and cytoplasmic cell compartments of leaves. Planta 211, 246–255.

Schjoerring JK, Husted S, Mack G, Mattsson M. 2002. The regulation of ammonium translocation in plants. Journal of Experimental Botany 53, 883–890.

Schjoerring JK, Husted S, Mack G, Nielsen KH, Finnemann J, Mattsson M. 2000. Physiological regulation of plant-atmosphere ammonia exchange. Plant and Soil 221, 95–102.

Searles PS, Bloom AJ. 2003. Nitrate photo-assimilation in tomato leaves under short-term exposure to elevated carbon dioxide and low oxygen. Plant, Cell & Environment 26, 1247–1255.

Seneweera S, Makino A, Hirotsu N, Norton R, Suzuki Y. 2011. New insight into photosynthetic acclimation to elevated CO2: the role of leaf nitrogen and ribulose-1,5-bisphosphate carboxylase/oxygenase content in rice leaves. Environmental and Experimental Botany 71, 128–136.

Sicher RC. 2001. Responses of nitrogen metabolism in N-sufficient barley primary leaves to plant growth in elevated atmospheric carbon dioxide. Photosynthesis Research 68, 193–201.

Sicher RC, Bunce JA. 2008. Growth, photosynthesis, nitrogen partitioning and responses to CO2 enrichment in a barley mutant lacking NADH-dependent nitrate reductase activity. Physiologia Plantarum 134, 31–40.
Stitt M, Krapp A. 1999. The interaction between elevated carbon dioxide and nitrogen nutrition: the physiological and molecular background. *Plant, Cell & Environment* 22, 583–621.

Taub DR, Wang XZ. 2008. Why are nitrogen concentrations in plant tissues lower under elevated CO$_2$? A critical examination of the hypotheses. *Journal of Integrative Plant Biology* 50, 1365–1374.

Wang L, Xu YC, Schjoerring JK. 2011. Seasonal variation in ammonia compensation point and nitrogen pools in beech leaves (*Fagus sylvatica*). *Plant and Soil* 343, 51–66.

Zhang L, Wright LP, Asman WAH. 2010. Bi-directional air–surface exchange of atmospheric ammonia: a review of measurements and a development of a big-leaf model for applications in regional-scale air-quality models. *Journal of Geophysical Research* 115, D20310.