NO$_3^-$ Assimilation and Elevated CO$_2$

To monitor short-term responses of plants to elevated CO$_2$, we developed a system that simultaneously monitors shoot exchange of CO$_2$, O$_2$, and H$_2$O (Fig. 2) and root exchange of CO$_2$, O$_2$, pH, NH$_4^+$, and NO$_3^-$ (Fig. 1). This is the only system in the world with these capabilities. Net CO$_2$ exchange from leaves provides a measure of carbon fixation and net O$_2$ exchange provides a measure of photosynthetic electron transport. Their ratio (CO$_2$ consumed per O$_2$ evolved) is called the assimilatory quotient (AQ) and is proportional to NO$_3^-$ photoassimilation (Bloom et al. 1989). During NO$_3^-$ photoassimilation, reductant produced during photosynthetic electron transport supports NO$_3^-$, NO$_2^-$, and NH$_4^+$ assimilation rather than CO$_2$ assimilation; therefore, O$_2$ evolution occurs without CO$_2$ consumption, and the AQ declines.

For wheat grown under 360 or 700 $\mu$mol mol$^{-1}$ CO$_2$, the A-C$_t$ curves (net CO$_2$ assimilation as a function of internal CO$_2$ concentration) were typical (Fig. 3). Net CO$_2$ assimilation increased steadily with $C_t$ until there was a break in the curve near the $C_t$ maintained under the growth conditions. Net O$_2$ evolution increased initially with $C_t$ probably because photosynthetic oxygen fixation declines with increasing $C_t$. At higher $C_t$’s, net O$_2$ evolution remained relatively constant because photosynthetic electron transport under these conditions was probably limited. Net CO$_2$ assimilation exceeded net O$_2$ evolution at higher $C_t$’s. This is consistent with observations that CO$_2$ inhibits CO$_2$ evolution via the TCA cycle resulting in increased net CO$_2$ assimilation and that CO$_2$ has little effect upon O$_2$ consumption via respiratory electron transport resulting in steady net O$_2$ evolution (Gemel & Randall 1992, Oaks 1994).

A shift in nitrogen source from 100 $\mu$M NH$_4^+$ to 100 $\mu$M NO$_3^-$ produced subtle, but significant changes in the AQ (Fig. 4). Standard gas exchange systems, which monitor CO$_2$ or O$_2$ alone, could not distinguish such changes from experimental variation. Our system, unique in its ability to measure CO$_2$ and O$_2$ fluxes simultaneously under normal atmospheric CO$_2$ and O$_2$ concentrations (Fig. 2), detected that the AQ of the ambient CO$_2$ plants decreased at low $C_t$’s after a shift from NH$_4^+$ to NO$_3^-$ nutrition (Fig. 4). This reflects that the photosynthetic electron transport system at low $C_t$’s produces reductant in surplus of what is required for carbon fixation, and this reductant may be diverted to NO$_3^-$ photoassimilation. At higher $C_t$’s, AQ did not significantly differ under NH$_4^+$ or NO$_3^-$ (Fig. 4). This is consistent with the hypothesis that elevated CO$_2$ inhibits NO$_3^-$ photoassimilation. By contrast, plants grown under elevated CO$_2$ did not show any significant change in AQ when shifted from NH$_4^+$ to NO$_3^-$ (Fig. 4). Thus, NO$_3^-$ photoassimilation was negligible. This is consistent with the observation that these plants had lower NO$_3^-$-reductase activities in the shoot (Fig. 6).

We also conducted longer-term experiments to test our hypothesis that elevated CO$_2$ inhibits NO$_3^-$ assimilation (Smart et al. 1998). We grew wheat in a 28 m$^3$ controlled environment chamber for 23 days at high densities (1500 plants m$^{-2}$), light levels of 1142 ± 36 $\mu$mol photon m$^{-2}$ s$^{-1}$ for 18 h per day, and temperatures of 24°C during the light period and 18°C during the dark period. The chamber contained two nutrient flow systems that controlled NO$_3^-$ levels at 0.10 ± 0.01 mM or 1.00 ± 0.05 mM. These systems also maintained the pH at 5.8 ± 0.1. Four separate experiments were conducted at each of two CO$_2$ concentrations, 360 and 1000 $\mu$mol mol$^{-1}$. Total nitrogen content in the shoots and roots was similar among all CO$_2$ or NO$_3^-$ treatments (Table 1). In both shoots and roots, the organic nitrogen fraction declined under elevated CO$_2$, while the fraction remaining as unassimilated NO$_3^-$ increased. Although
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Fig. 2. A schematic of the gas exchange system that monitors CO2, O2, H2O, and N2O fluxes from shoots of intact plants under standard atmospheric conditions. The circles represent solenoid valves with the normally open ports labeled 'NO' and the normally closed ports labeled 'NC.'

Many other studies have shown that total protein nitrogen declines with elevated CO2 (Curtis 1996, Cotrufo et al. 1998), this is the first to pinpoint a cause for this decline: CO2 inhibition of NO3 photoassimilation.

To contrast plant responses under NH4+ and NO3- nutrition, 16-day old wheat seedlings were transferred to CO2-controlled environmental chambers equipped with nutrient flow systems and then exposed for two weeks to 360 or 700 μmol mol−1 CO2 and to 200 μM NH4+ or NO3 as a sole nitrogen source. Plants grown at elevated CO2 had significantly more biomass than those grown at ambient CO2 (Fig. 5). The CO2 growth enhancement was nearly double under NH4+ nutrition than under NO3− nutrition (Fig. 5). Shoot and root protein were higher under NH4+ nutrition than under NO3− nutrition (Fig. 6 & Fig. 7). In the elevated vs. ambient CO2 treatment, shoot protein was 5% lower under NH4+ nutrition and 11% lower under NO3− nutrition (Fig. 6). Thus, the decline in shoot protein at elevated CO2 was more than double under NO3− nutrition than under NH4+ nutrition. This is consistent with our hypothesis that elevated...
CO₂ inhibits NO₃⁻ photoassimilation.

Without photoassimilation, assimilation of NO₃⁻ into amino acids in the shoot becomes energetically unfavorable. During NO₃⁻ assimilation, an anion is converted to a neutral compound, thus, generating an anion surplus (Raven & Smith 1976). When the root is the site of assimilation, direct efflux of carbonate or hydroxyl ions balances such a surplus. Assimilation in the shoot requires a more elaborate scheme: (a) PEP is carboxylated to form organic acids, (b) organic acids, principally malate, are translocated to the roots, (c) organic acids are decarboxylated, and (d) carbonate is exuded from the root. This scheme exacts several additional ATP (Raven & Smith 1976). As CO₂ levels rise, plants that rely heavily on shoot NO₃⁻
Table 1. Total nitrogen, organic nitrogen, and nitrate nitrogen for wheat canopies grown in controlled environments under 360 or 1000 µmol mol⁻¹ CO₂ and with 0.1 or 1.0 mM NO₃⁻ in the nutrient medium. Shown are the means ± range for four 23 day experiments. Means that are significantly different (P < 0.05) within a column are marked by different letters.

<table>
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<tr>
<th>CO₂ Treatment</th>
<th>NO₃⁻ Treatment</th>
<th>Total Nitrogen</th>
<th>Organic Nitrogen</th>
<th>NO₃⁻ Nitrogen</th>
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<td>mM</td>
<td>mg g⁻¹</td>
<td>mg g⁻¹</td>
<td>mg g⁻¹</td>
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<tr>
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</tr>
<tr>
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<td>0.1</td>
<td>49.1 ± 0.3</td>
<td>32.5 ± 0.9</td>
<td>16.0 ± 0.5</td>
</tr>
<tr>
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<td>1.0</td>
<td>49.4 ± 0.6</td>
<td>32.3 ± 1.0</td>
<td>16.7 ± 0.5</td>
</tr>
<tr>
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<td>48.8 ± 0.8</td>
<td>28.7 ± 1.2</td>
<td>19.8 ± 1.2</td>
</tr>
<tr>
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<td>50.9 ± 0.3</td>
<td>28.3 ± 1.3</td>
<td>22.6 ± 1.1</td>
</tr>
<tr>
<td>Roots</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
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<tr>
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<td>32.1 ± 1.4</td>
<td>17.1 ± 1.3</td>
<td>13.4 ± 1.4</td>
</tr>
</tbody>
</table>

assimilation such as species of tropical origin (Andrews 1986) may find themselves at a competitive disadvantage and may need to allocate more resources to root NO₃⁻ assimilation. This hypothesis is supported by a decline of NO₃⁻ reductase in shoots (Hocking & Meyer 1991) and evidence that root NO₃⁻ reductase activity increases (Fig. 6).

Plants Emit Nitrous Oxide

We recently discovered that plants may be a significant source of the greenhouse gas N₂O. A major impediment to research on N₂O emissions has been the extreme difficulty associated with N₂O analysis.

Fig. 6. Shoot total nitrogen content (mg g⁻¹ dry weight), protein content (mg g⁻¹ fresh weight), NO₃⁻ content (mg g⁻¹ fresh weight), NO₃⁻ reductase activity (µmol NO₃⁻ generated mg⁻¹ protein), and NO₂⁻ reductase activity (µmol NO₂⁻ consumed mg⁻¹ protein) of wheat grown for 14 days in controlled environment chambers at 360 or 700 µmol mol⁻¹ CO₂ and under NH₄⁺ or NO₃⁻ nutrition. Shown are mean ± SE for 4 replicates experiments, each with 8 to 10 plants per treatment. Treatments with different letters differ significantly (P < 0.05).

Fig. 7. Root total nitrogen content (mg g⁻¹ dry weight), protein content (mg g⁻¹ fresh weight), NO₄⁺ content (mg g⁻¹ fresh weight), NO₃⁻ reductase activity (µmol NO₃⁻ generated mg⁻¹ protein), and NO₂⁻ reductase activity (µmol NO₂⁻ consumed mg⁻¹ protein) of wheat grown for 14 days in controlled environment chambers at 360 or 700 µmol mol⁻¹ CO₂ and under NH₄⁺ or NO₃⁻ nutrition. Shown are mean ± SE for 4 replicates experiments, each with 8 to 10 plants per treatment. Treatments with different letters differ significantly (P < 0.05).
Fig. 8. Depiction of the system with twelve root cuvettes. The individual root cuvettes are constructed from acrylic plastic. To control root temperatures, water from a refrigerated water bath flows through the large common block on the bottom that is constructed from stainless steel. The whole unit sits atop a device with twelve motors and magnets to stir each cuvette.

Fig. 9. Nitrous oxide emissions (pmol N₂O m⁻² s⁻¹ on a log scale) from wheat leaves as a function of (A) change in assimilatory quotient (AQ) when the nitrogen source shifted from NH₄⁺ to NO₃⁻ or (B) nitrous oxide emissions (pmol N₂O m⁻² s⁻¹ on a log scale) from the roots. The change in AQ reflects the extent of shoot NO₃⁻ assimilation. Shown are the regression lines and R² statistic.

Chromatographic and isotopic methodologies suffer from interference by CO₂ that has a similar molecular weight and subliming temperature. In addition, fluxes of N₂O from soils and leaves are nearly three to six orders of magnitude smaller than the respective fluxes of CO₂, and this hampers the use of dynamic flow methods. We have overcome these technical difficulties using gas chromatography interfaced with isotope ratio mass spectrometry (GC-IRMS) to achieve a detection limit of about 0.2 pmol N₂O m⁻² s⁻¹.

In this approach, we enclose the roots of twelve intact plants in separate cuvettes (Fig. 8) and expose them to nutrient solutions enriched in ¹⁵NH₄⁺ and ¹⁵NO₃⁻. The shoots of these plants are in a common cuvette forming a canopy with a leaf area of about 0.02 m². The shoot cuvette is connected to the gas exchange system depicted above (Fig. 2). Gas from the shoot cuvette passes through an ascarite filter to remove CO₂, a KMnO₄ filter to remove moisture, and then a cryogenic trap cooled with liquid argon (-180° C). The trapped gas is then released into the GC-IRMS where it is sorbed onto a zeolite trap with a very small dead volume. The N₂O on this trap is then thermally desorbed into a slow helium carrier stream and passed into the IRMS. Using relationships developed for determination of the release of ¹⁵N₂O from soils (Mulvaney & Boast 1986) in combination with standard procedures for gas exchange analysis (Bloom et al. 1980), we estimate leaf N₂O emission rates from the mole fraction enrichment in masses 45 and 46. We developed this method at UC Berkeley in collaboration with Paul Brooks, but now conduct the analyses at the UC Davis Stable Isotope Facility.

For roots, we sample the nutrient solution and analyze the headspace gas for ¹⁵N₂O and ¹⁵N₂. The rates of N₂O and N₂ production in the root zone are estimated from the relationships for N₂O and N₂ release from soils (Mulvaney & Boast 1986) in combination with known solubilities for these gases (Wilhelm et al. 1977).

Wheat, a nonaerenchyma forming vascular land plant, was grown on nutrient solutions containing NH₄⁺ plus NO₃⁻ and was deprived of NO₃⁻ for a 24 h period to deplete their internal reserves. Twelve plants were placed in root and shoot cuvettes and subjected to root temperatures of 15°C, shoot temperatures of 22.5°C, photosynthetic photon flux densities of approximately 600 μmol m⁻² s⁻¹, and CO₂ concentrations of 330 μmol mol⁻¹. Leaf N₂O emissions from the twelve plants were determined while the plants metabolized either ¹⁴NH₄⁺ or ¹⁵NO₃⁻. The plants absorbed 0.37 ± 0.04 μg NH₄⁺-N m⁻² s⁻¹ (mean ± SE, n = 5) but emitted only 1.2 ± 0.7 pmol N₂O m⁻² s⁻¹ from their leaves. This small emission may have resulted from ammonium-oxidizing bacteria on the root surface that converted the NH₄⁺ to NO₃⁻.
the plants were switched to NO₃⁻, they absorbed it at a rate of 0.43 ± 0.07 μg NO₃⁻N m⁻² s⁻¹, and the N₂O emission rate increased by an order of magnitude to an average of 13.1 ± 2.7 pmol N₂O m⁻² s⁻¹.

The assimilation quotient (AQ, the ratio of shoot CO₂ influx to O₂ efflux), shifted downward when the nitrogen source changed from NH₄⁺ to NO₃⁻ reflecting the amount of shoot NO₃⁻ assimilation. Shoot N₂O emissions were correlated with this shift in AQ (Fig. 9A), but not with root zone N₂O emissions (Fig. 7B). This indicates that shoot NO₃⁻ assimilation produces N₂O. Because more than 70% of the NO₃⁻ absorbed is assimilated in the shoot (Bloom et al. 1992), the N₂O emissions that we measured would represent about 0.2% of the NO₃⁻ assimilated. Shoot N₂O emissions, thus, represent a significant biogenic N₂O source.

**Foliar Emissions Associated with NO₃⁻ Assimilation**

Foliar emissions from both natural and agricultural ecosystems could represent a significant source of N₂O. We found that N₂O emissions from wheat canopies correlate with NO₃⁻ assimilation in shoots (Fig. 9) and N₂O has been detected as a product of in vitro nitrate reductase assays (Osretkar 1971, Harper 1981, Dean & Harper 1986). It is estimated that NO₃⁻ is the nitrogen source for as much as 50% of the inorganic N assimilated by plants and other photolithotrophs (Ajtay et al. 1979, Stewart et al. 1988, Read 1991, Raven et al. 1992). If it can be assumed that 60% of this is photoassimilated (data from our laboratory indicates that over 70% of NO₃⁻ absorbed by wheat is assimilated in leaves), then our preliminary data suggest that leaf N₂O emissions could contribute 0.44 to 0.82 Tg N₂O-N annually. This would represent from 18 to 34% of the global imbalance of N₂O production (Matson & Vitousek 1990, Schlesinger 1991) and argues that leaf N₂O emission may represent a significant biogenic N₂O source. If root NO₃⁻ assimilating activity also produces N₂O and if it is released through leaves to the atmosphere, then leaf N₂O emissions could represent up to 1.1 Tg N₂O-N annually or approximately 12% of global production. These are only rough estimates, but they highlight the potential importance of these processes.

**References:**


Osnetkar A (1971) Gaseous and other nitrogen losses from cultures of *Chlorella*, Mariland, College Park.


