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The increasing importance of distinguishing among plant nitrogen sources

Arnold J Bloom

Many studies of plant nitrogen relations assess only the total amount of the element available from the soil and the total amount of the element within the plant. Nitrogen, however, is a constituent of diverse compounds that participate in some of the most energy-intensive reactions in the biosphere. The following characterizes some of these reactions, especially those that involve ammonium and nitrate, and highlights the importance of distinguishing both among the nitrogen sources available to plants and among the nitrogen forms within plants when considering plant responses to rising atmospheric CO_2 concentrations.

Address
Mail Stop 3, Department of Plant Sciences, University of California at Davis, Davis, CA 95616-8780, United States

Corresponding author: Bloom, Arnold J (ajbloom@ucdavis.edu)

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Introduction

Nitrogen (N) is the element that organisms require in amounts greater than all others except for carbon, oxygen, and hydrogen. Indeed, N availability from the environment often limits the productivity of natural and managed ecosystems [1,2•]. N is a constituent of many stable compounds, including inorganic ones such as dinitrogen gas (N_2), ammonium (NH_4^+) salts, and nitrate (NO_3^-) salts and organic ones such as amino acids and nucleotides, the building blocks of proteins and nucleic acids, respectively. These compounds differ profoundly in their chemical properties. For example, NH_4^+ is a cation in which the oxidation state of nitrogen is –3, whereas NO_3^- is an anion in which the oxidation state of nitrogen is +5.

Most studies of plant N relations, however, do not compare the performance of plants receiving different N sources. One indication of this is that less than one-quarter of the articles on the topic of plant N cited in Thomson Reuters' *Web of Science* mention a N form such as 'ammonium,' 'nitrate,' 'amino acid,' or 'purine' in their title, abstract, or keywords. Here I argue that treating N as a single entity — so-called total soil N or total plant N — is inadequate because the specific N compounds that are available from the environment and that subsequently become engaged in plant metabolism strongly determine plant responses to the atmospheric CO_2 concentrations anticipated during the next few decades.

Nitrogen as an element

Nitrogen is one of the most common elements on Earth. About 78% of the atmosphere is composed of N_2, dinitrogen gas. For the most part, this large reservoir of N is not directly useable by living organisms because N_2 has an exceptionally stable triple bond (N≡N) that requires enormous amounts of energy to break. In particular, the process of biological N fixation involves the reaction:

$$N_2 + 8H^+ + 8e^- \rightarrow 2NH_3 + H_2 \quad 147\,\text{kcal mol}^{-1}$$

The ammonia (NH_3) produced through this reaction dissolves into water at physiological pH to form ammonium (NH_4^+).

$$NH_3 + H_2O \rightarrow NH_4^+ + OH^- \quad pK_a = 9.25$$

An alternative process is atmospheric N fixation, in which lightning converts water vapor and oxygen into highly reactive hydroxyl free radicals, free hydrogen atoms, and free oxygen atoms that attack N_2 to form nitric acid (HNO_3). This nitric acid subsequently falls to Earth with rain and disassociates into nitrate (NO_3^-). Once fixed into NH_4^+ or NO_3^-, N enters a biogeochemical cycle and passes through several organic or inorganic forms before it eventually returns to N_2 (Figure 1).

The chemical reactions, which interconvert these various N forms within plants and other organisms, are among the most energy intensive in life. For example [3],

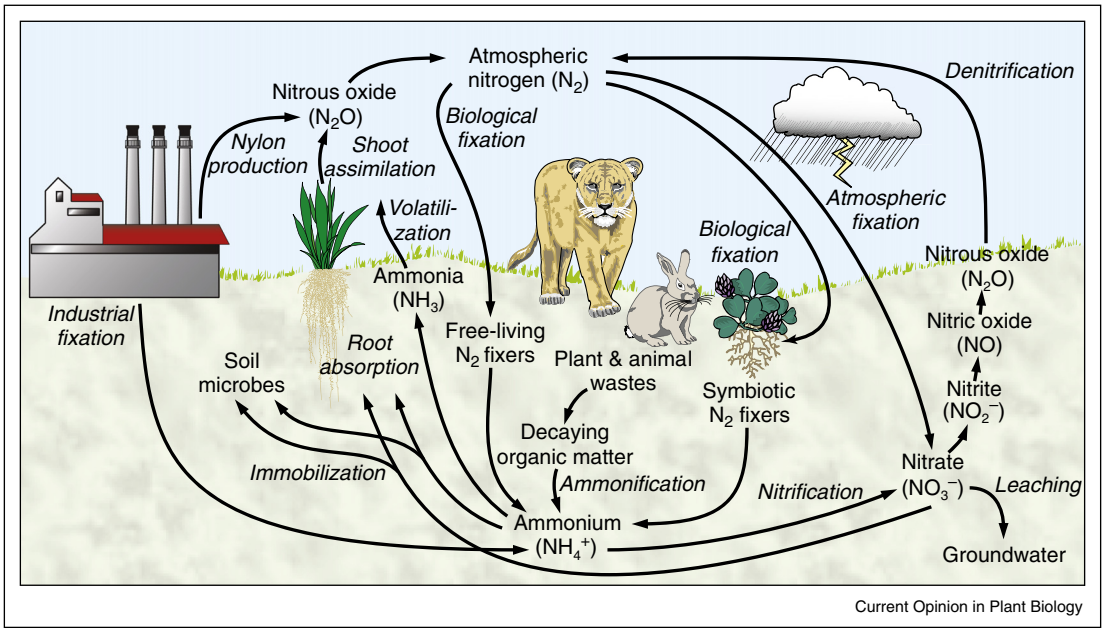
$$NO_3^- + NAD(P)H + H^+ \rightarrow NO_2^- + NAD(P)^+ + H_2O \quad 34\,\text{kcal mol}^{-1}$$

where NAD(P)H indicates either NADH or NADPH.

$$NO_2^- + 6Fd_{red} + 8H^+ \rightarrow NH_4^+ + 6Fd_{ox} + 2H_2O \quad 103.5\,\text{kcal mol}^{-1}$$

where Fd is ferredoxin and the subscripts *red* and *ox* stand for *reduced* and *oxidized*, respectively.

Figure 1



The terrestrial N-cycle.

$$NH_4^+ + \text{glutamate} + \text{ATP} \rightarrow \text{glutamine} + \text{ADP} + P_i \quad 4.3\,\text{kcal mol}^{-1}$$

$$\text{glutamine} + \text{2-oxoglutarate} + (NADH + H^+ \text{ or } \text{Fd}_{red}) \rightarrow 2\text{glutamine} + (NAD^+ \text{ or } \text{Fd}_{ox}) \quad 13.4\,\text{kcal mol}^{-1}$$

Once assimilated into glutamine and glutamate, N is incorporated into other amino acids via transamination reactions. N from amino acids is incorporated into other organic N compounds such as purines and pyrimidines.

N sources

Most organisms have the capacity to use organic N, NH_4^+, and NO_3^- as N sources. Microorganisms, however, prefer organic N forms first and then prefer the higher energy inorganic N compound NH_4^+ over NO_3^-. Phytoplankton [4], fungi [5], cyanobacteria [6], and bacteria [7] usually absorb and assimilate NO_3^- only in the absence of organic N or NH_4^+.

Higher plants use organic N, NH_4^+, and NO_3^- as N sources in proportion to their relative availability in the soil solution [1,8], but plants often cannot successfully compete with soil microorganisms for organic N [9,10,11•]. Competition for soil NH_4^+ also can be fierce because NH_4^+ adsorbs onto the cation exchange complex of many soils and because soil microorganisms use NH_4^+ not only as an N source, but also as an energy source via nitrification (microbial conversion of NH_4^+ into NO_3^-). Therefore, NO_3^- is a major N source for most higher plants [1,2•].

NO_3^- is an important N source even for plants growing in locations where soil NO_3^- concentrations tend to be low. For instance, plants that have the capability of conducting symbiotic N-fixation cease N-fixation when NO_3^- is present in the rhizosphere [12]. Forest soils in which NH_4^+ is the major N source have high rates of gross nitrification that indicate a small but ecologically important NO_3^- pool [13]. Many flooding-tolerant plants growing in wetland soils, which are subject to NO_3^- leaching and denitrification (microbial conversion of NO_3^- to N_2), develop aerenchyma that supply the rhizosphere with oxygen and thus promote nitrification on root surfaces; the roots immediately absorb this NO_3^- [14–17].

This dependence on NO_3^- as an N source persists despite the disproportionately large amount of energy required for assimilating NO_3^- into organic N compounds. Organic N compounds constitute less than 2% of plant dry mass, but plants expend about 25% of their total energy in shoots [18] and roots [19] for NO_3^- assimilation, both day [20] and night [21]. To mitigate this energy expenditure, plants employ a mechanism that other organisms lack. This mechanism is photorespiration [20,22–25,26••].

Table 1

Changes (%) in harvested protein under elevated CO_2 (546–584 ppm) in FACE experiments (mean ± SE) [38•]

	%
C_3 grasses	
Wheat	–7.2 ± 1.4
Rice	–7.9 ± 1.0
Barley	–15.0 ± 4.2
C_3 legumes	
Peas	–2.1 ± 2.0
Soybeans	0.1 ± 0.9
C_3 tubers	
Potato	–9.0 ± 5.4
C_4 grasses	
Maize	–4.6 ± 8.7
Sorghum	–5.6 ± 7.5

Photorespiration

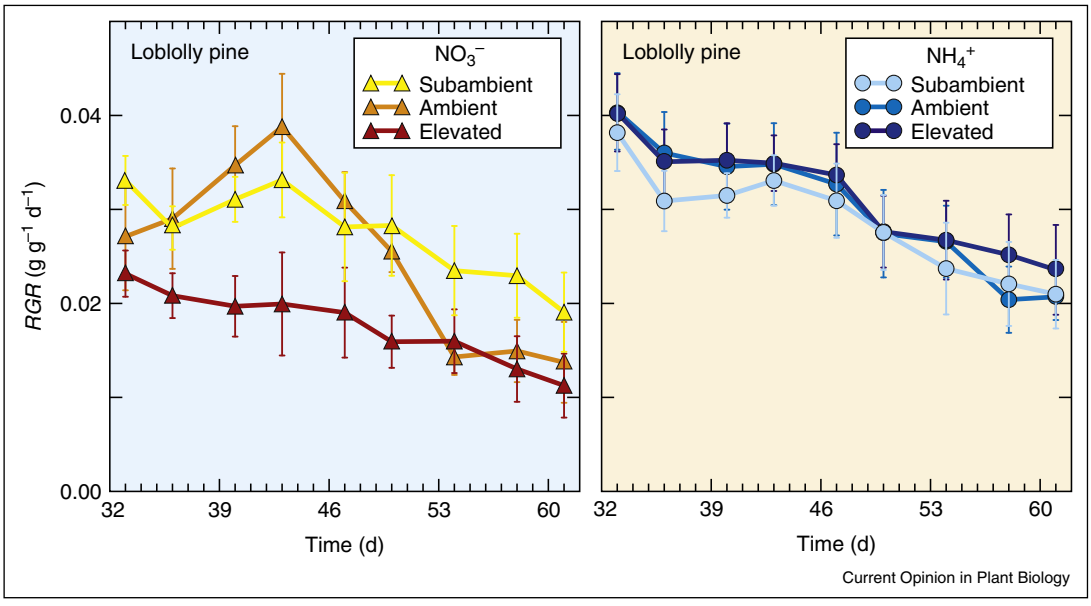
Photorespiration has been generally viewed as a wasteful process, a vestige of the high CO_2 and low O_2 atmospheres that existed when plants first evolved. Photorespiration, however, stimulates the export of malate from chloroplasts [27–29]; this malate in the cytoplasm generates NADH [28,30] that powers the reduction of NO_3^- to NO_2^-, the first step of NO_3^- assimilation [31–33]. Consequently, photorespiration supplies a significant part of the energy for NO_3^- assimilation in C_3 plants [26••].

Although C_4 plants have a CO_2 pumping mechanism that minimizes photorespiration, the first carboxylation reaction in the C_4 pathway generates ample amounts of malate and thereby NADH in the cytoplasm of mesophyll cells. This explains why NO_3^- assimilation is relatively independent of CO_2 concentration in C_4 plants [34,35] and limited to the mesophyll [36,37].

Because NO_3^- assimilation in C_3 plants depends on photorespiration, conditions that inhibit photorespiration — namely, high CO_2 or low O_2 atmospheres — impede NO_3^- assimilation [20,22–25,39]. Indeed, rising atmospheric CO_2 concentration poses a threat to food quality, whereby protein concentration in major crops will decline (Table 1) depending on their relative reliance on NH_4^+ and NO_3^- as N sources [34,35,40,41].

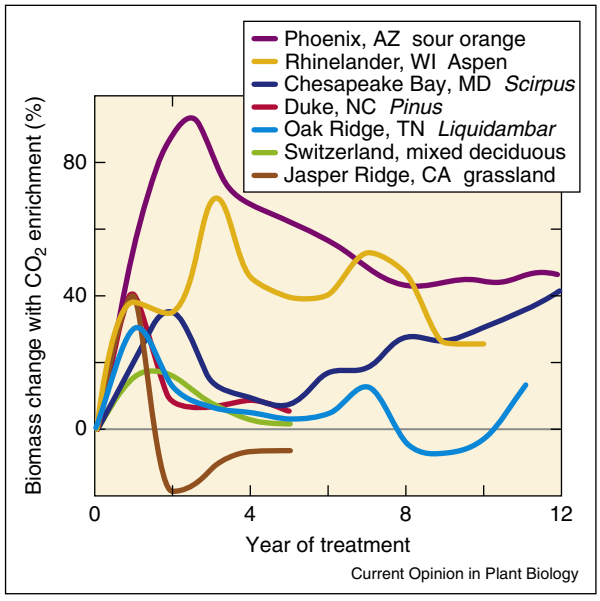
N source also determines plant growth under elevated CO_2. One to two months after exposure to differential CO_2 treatments, loblolly pine saplings that received NO_3^- nutrition grew fastest under a subambient CO_2 atmosphere approximately equal to that found 50 years ago (Figure 2). Growth of saplings that received NH_4^+ nutrition showed no differential response or a slight stimulation of growth under an elevated CO_2 atmosphere approximately equal to that expected in 50 years (Figure 2). The two inorganic nitrogen forms influenced plant growth and nutrient status so distinctly that they should be treated as separate nutrients [41].

Figure 2



Relative growth rate in grams per gram per day of loblolly pine *Pinus taeda* in controlled environment chambers under subambient (≈310 μmol mol^{-1}), ambient (≈400 μmol mol^{-1}), or elevated (720 μmol mol^{-1}) atmospheric CO_2 concentrations and receiving NO_3^- (left panel) or NH_4^+ (right panel) nutrition. These CO_2 concentrations approximate, respectively, those of fifty years ago, today, and fifty years from today. Time is in days after imposing the different CO_2 treatments. Shown are the predicted values and standard errors from mixed linear models with repeated measures on 5–10 individual plants [35].

Figure 3



Differences in biomass between elevated (≈567 ppm) and ambient (≈365 ppm) atmospheric CO_2 after years of treatment. Shown are the data from seven different studies using the designated types of plants. Data from [46,47–49,50••,51].

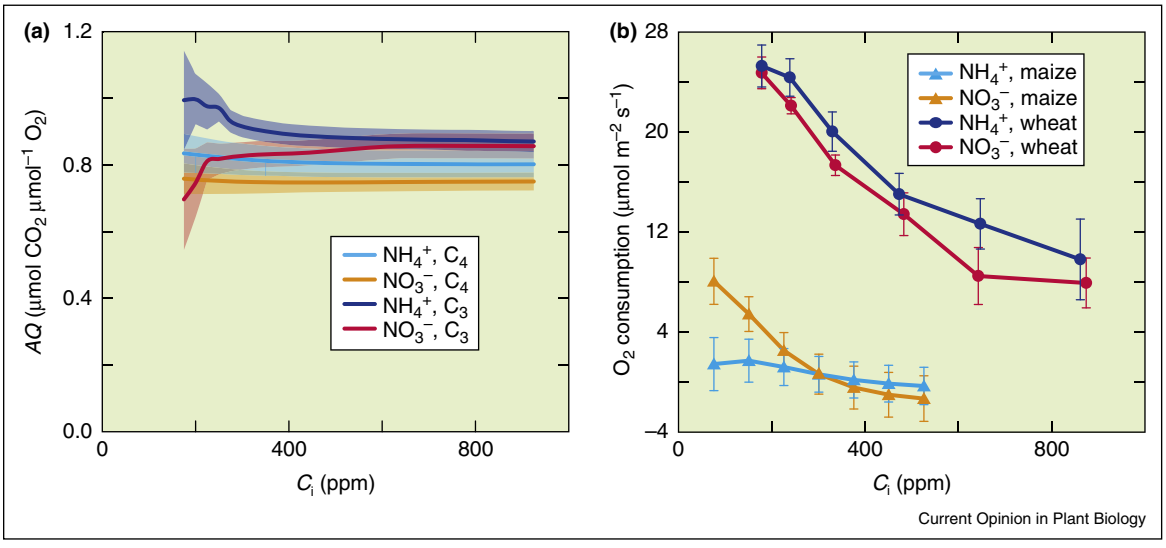
Variation in the reliance of plants on NH_4^+ and NO_3^- as N sources also explains ecosystem responses to elevated CO_2 [35,42]. For instance, *Scirpus olneyi*, the dominant C_3 plant in the Chesapeake Bay marsh, an NH_4^+-dominated ecosystem, showed a steady enhancement in photosynthesis and growth under CO_2 enrichment even after a decade of treatment (Figure 3). In an annual California grassland for which NO_3^- was the major N source, elevated CO_2 decreased net primary productivity (Figure 3) presumably because elevated CO_2 inhibited photorespiration, which in turn slowed NO_3^- assimilation until plants experienced protein deficiency. Clearly, predicting plant responses to the atmospheric CO_2 conditions anticipated in the near future will require information about the degree to which plants use each N source.

NH_4^+ versus NO_3^-

Efforts to assess the balance between plant NH_4^+ and NO_3^- use *in situ* encounter several difficulties. The rhizosphere from which plants extract these N forms is highly heterogeneous, both spatially and temporally [1]. For example, NO_3^- concentrations in a soil may range a 1000-fold over a distance of centimeters or over the course of hours [43]. Plant roots themselves modify their surroundings: they deplete nutrients, alter rhizosphere pH through ion exchange [44,45], and support soil microbes through exudates or cell death.

Once a plant root absorbs NH_4^+ or NO_3^- from the rhizosphere, these forms can undergo several fates. Some NH_4^+ or NO_3^- is stored in the root, some is assimilated into amino acids in the root, and some is translocated to the shoot where again some is stored and some is assimilated [52]. Measuring N pool sizes, employing ^{15}N-stable isotope tracers to follow fluxes from the soil and through a plant,

Figure 4



(a) Shoot *AQ* (net CO_2 consumed/net O_2 evolved) as a function of internal CO_2 concentrations (C_i) for 9 taxonomically diverse C_3 species and 3 taxonomically diverse C_4 species when they received NH_4^+ or NO_3^- as a sole N source (mean ± SE; solid ± shaded area). **(b)** Shoot O_2 consumption in the light (gross O_2 − net O_2) as a function of C_i for maize and wheat receiving NH_4^+ or NO_3^- as a sole N source.Data from [20,24,35].

and then estimating relative NH_4^+ or NO_3^- assimilation rates is not straightforward [53].

A different approach

Assimilatory Quotient (*AQ*), the ratio of net CO_2 consumption to net O_2 evolution during photosynthesis, has provided real-time, non-destructive estimates of NO_3^- assimilation for nearly a century [18,23,25,34,35,54–60]. C_3 plants under NH_4^+ nutrition down-regulate photosynthetic electron transport via the xanthophyll cycle to avoid photoinhibition at low C_i (internal CO_2 concentration). Thus, in C_3 plants reliant on NH_4^+, *AQ* increases as C_i decreases (Figure 4a, dark blue line). NO_3^- assimilation in C_3 plants accelerates the light-dependent splitting of H_2O and generates additional electrons, which are transferred first to NO_3^- and then to NO_2^-. This has little effect on net CO_2 consumption, but results in faster net O_2 evolution and thereby in lower *AQ*, especially when low C_i limits carbon fixation (Figure 4a, red line).

In C_4 plants, responses of *AQ* to C_i under both N sources have similar shapes (Figure 4a, light blue and orange lines). Shoot O_2 consumption in the light (difference between gross O_2 fluxes estimated from chlorophyll fluorescence and net O_2 fluxes monitored with an O_2 analyzer), however, increases dramatically at low C_i in C_4 plants that rely on NO_3^- (Figure 4b, orange line). This derives from the additional mitochondrial respiration required for NO_3^- assimilation when low C_i limits C_4 fixation [20].

Therefore, the responses of shoot CO_2 and O_2 fluxes to C_i can provide *in situ* estimates of the balance between plant NH_4^+ and NO_3^- use. These estimates, together with measurements of N partitioning among various compounds, offer insights about plant productivity and food security under the broad range of conditions that plants encounter today and will encounter by the end of the century. Measurements of total soil N or total plant N alone fail to account for the distinct natures of N compounds and thus cannot begin to address these crucial issues. More detailed comparisons of plant responses to different N sources will be time-consuming and expensive, but necessary.

Conflicts of interest

The author has no conflicts of interest with regards to this research.

Acknowledgement

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