Closeout Technical Report For DOE Award Number DE-FG02-97ER62332

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Table of Content

| Summary | 2-3 |
| Justification of the work, list of objectives and hypotheses | 3-4 |
| Results from field and greenhouse experiments | 4-7 |
| OTC results | |
| Techniques for N uptake in the field | |
| Lab and Greenhouse results | |
| Determining N uptake of intact roots using HPLC | |
| Integrating other factors | 7-9 |
| Root morphology | |
| Mycorrhizal fungi | |
| Synthesis using conceptual and quantitative models | 9-11 |
| Postdocs, graduate and undergraduate students | 11-11 |
| Publications credited to this grant | 11-13 |
| Appendix | 13-|
| 1996 progress report | |
| 1997 progress report | |
| Reprints of publications credited to this grant | |

DOE Patent Clearance Granted

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Summary

This project was conducted between 1995 and 1999 during which two postdocs and numerous undergraduate students received training in research. Furthermore, the funds from this grant contributed either totally or partially to the publication of fourteen refereed journal articles (see appendix). The focus of this research was to investigate plant nitrogen budget under elevated CO₂ concentration. We were particularly interested in addressing the following: 1- Does elevated CO₂ increase root carbohydrate availability?, 2- Does such an enhancement increase kinetics of root nitrogen acquisition?, 3- Does the effect on kinetics differ between NH₄⁺ and NO₃⁻? and 4- If there are interspecific differences in 1-3, could those variation lead to changes in community composition. The following report will show that although root carbohydrate availability often increases in response to elevated CO₂, such an increase is neither necessary nor is it directly related to changes in root N uptake kinetics. The data also shows that depending upon species, the effects of elevated CO₂ on root nitrogen uptake kinetics ranges from down regulation to no changes to up regulation. Furthermore, the effects on NH₄⁺ is not always similar to the effects on NO₃⁻. Perhaps the most critical finding is the fact that in many instances a change in root N uptake kinetics alone does not provide a reliable prediction of plant N acquisition in response to elevated CO₂. Here we show that a better examination of whether plant N uptake responds to CO₂ level and whether such a responses can be scaled up to community level processes would require integration of knowledge of other root system characteristics. For example, it is well established that mycorrhizal fungi are important regulators of plant N uptake. Our data suggests that while elevated CO₂ affects root N uptake capacity this effect is highly dependent on the type and level of the mycorrhizal infection. Another root characteristic that significantly affects N uptake and could mask any potential impact of kinetics is root morphology. We will illustrate that when all else is equal, increased biomass allocation to roots is the least effective mechanism in adjusting plant N uptake under elevated CO₂. Finally, plants may be able to reduce their demand for N via increased N use efficiency (NUE). The research conducted here indicates that elevated CO₂ may evoke different responses in NUE depending upon species and that an increased NUE may be one of the most effective mechanisms in optimizing N uptake and growth responses to elevated CO₂. We conclude
that elevated CO$_2$ can have a dramatic effect on root N uptake kinetics, but viewed in isolation this observation does not provide a robust assessment of plant N economy under an enriched CO$_2$ atmosphere. Therefore, future work designed to predict whole-plant N responses to elevated CO$_2$ must consider other root system adjustments listed above, collectively.

**Justification of the work, list of objectives and hypotheses**

An important root-shoot interaction that might determine the overall response of plants is the ability of the root system to adjust nutrient acquisition capacity to meet variations in shoot demand caused by environmental changes. Plant roots can alter their nutrient acquisition capacity by adjusting their physiological, longevity, morphological and/or architectural characteristics to meet changes in shoot nutrient demand. Even though physiological capacity of root nutrient uptake is only one of the number of adjustments that influences nutrient acquisition, its response to changes in plant environment might provide a key mechanistic explanation of why some species are more sensitive to global change than others. However, it should be highlighted that the degree to which kinetics of nutrient uptake or other potential adjustments are expressed would ultimately depend on soil nutrient availability and soil factors that determine nutrient transport to the root surface.

Studies of shoot responses to global change commonly examine physiological parameters such as photosynthetic rate and stomatal conductance. By contrast, studies of root responses to global change often focus on root growth and morphological characteristics, seldom addressing changes in physiological characteristics such as hydraulic conductivity and kinetics of ion uptake. Active root nutrient absorption is a highly adaptive plant characteristic that influences acquisition of N and other nutrients in response to environmental factors. Therefore, knowledge of changes in the kinetics of nutrient uptake and the relative species differences is critical in predicting ecosystem responses to global change. Although in recent years a number of investigators have underscored the need for a better understanding of how, for example, elevated CO$_2$ changes root uptake kinetics, there has been relatively little response to this plea. This is surprising particularly when one considers that, at least in the short-term, physiological adjustments are less costly to
the plant and more likely to act as a primary signal that leads to longer term responses in root morphology and architecture.

We were testing three specific hypotheses. These were:

1. The capacity for root $\text{NO}_3^-$ absorption will increase with $\text{CO}_2$ enrichment, particularly in species that primarily assimilate $\text{NO}_3^-$ in their roots. Species that primarily assimilate $\text{NO}_3^-$ in their shoots will increase their reliance upon $\text{NH}_4^+$ as a nitrogen source.

2. Root carbohydrate status improves under elevated $\text{CO}_2$ and supports the energy demands of root $\text{NO}_3^-$ assimilation.

3. Interspecific differences in N acquisition and allocation are exaggerated with $\text{CO}_2$ enrichment and affect species productivity in a manner consistent with the observed shifts in community composition.

Results from greenhouse and field experiments

Previous progress reports (see appendix) had highlighted some of the preliminary results using agronomic species grown under controlled environment in walk-in growth chambers. Here we highlight results from greenhouse and field experiments conducted in Open-top Chambers using native species.

Open-top Chambers

One of our earlier challenges was to determine root physiological uptake capacity under field conditions. Traditionally, rates on ion uptake in field-grown plants are determined by using excised root segments incubated for a short period in an assay solution containing N either as a radioactive or stable isotope tracer (e.g. $^{36}\text{ClO}_3$ as a $\text{NO}_3^-$ analogue, $^{14}\text{CH}_3\text{NH}_3$ as an $\text{NH}_4^+$ analogue or $^{15}\text{NH}_4^+$ and $^{15}\text{NO}_3^-$). Although reliable, this method has several drawbacks. For example, in addition to radioactive safety issues, purchase and analysis of radioactive and stable isotopes is relatively expensive and can be a major limitation. More importantly, because excision effectively interrupts exchange of compounds between root and shoot (e.g., carbohydrate supply to root and N transport to shoot), the assay must be conducted quickly to avoid such complications. Here we present a novel field method for simultaneous measurements of $\text{NH}_4^+$ and $\text{NO}_3^-$ uptake kinetics in intact root systems. The application of this method is demonstrated using two
tree species: red maple (*Acer rubrum*) and sugar maple (*Acer saccharum*) and two crop species; soybean (*Glycine max*) and sorghum (*Sorghum bicolor*). Plants were grown in open-top chambers at either ambient or elevated levels of atmospheric CO$_2$ at two separate US national sites involved in CO$_2$ research. Absolute values of net uptake rates and the kinetic parameters determined by our method were found to be in agreement with the literature reports. Roots of the crop species exhibited a greater uptake capacity for both N forms relative to tree species. Elevated CO$_2$ did not significantly affect kinetics of N uptake in species tested except in red maple where it increased root uptake capacity, $V_{\text{max}}$, for NH$_4^+$.

**Determining N uptake kinetics using HPLC**

Another challenge in our experimental approaches involved the development of a reliable technique that could allow us to routinely measure kinetic parameters of N uptake in intact whole root systems. This was accomplished using a novel approach involving High Performance Liquid Chromatography (HPLC).

Briefly, autosamplers of almost all HPLC makes share a characteristic design feature – enclosure in relatively small units, and sampling operations are performed from samples placed inside the autosampler housing. Although this suffices for most HPLC applications, this design feature limits versatility of the autosampling operation when sample containers can not fit inside the unit. In this approach we describe a simple modification of the standard autosampler flow path that enables automated sampling and analysis form samples placed externally to the autosampler unit. An added benefit of this approach is that the modified configuration does not compromise operation in the
modified mode, and switching between the two configurations is easily achieved (See Zerihun et al. 1999 for details).

The development of this technique allowed us to address an important and fundamental question that had eluded us to that point. By this point, the few available data indicate a highly variable pattern of kinetic responses to elevated CO₂, but it was unclear if the observed inconsistencies are caused by differences in experimental protocols or by true species differences. Furthermore, if there were interspecific variations in N uptake responses to elevated CO₂, it was not clear whether these were associated with different functional groups. Accordingly, we examined intact root-system NH₄⁺ and NO₃⁻ uptake kinetic responses to elevated CO₂ in seedlings of six temperate forest tree species, representing (i) fast- vs. slow-growers and (ii) broad-leaves vs. conifers, that were cultured and assayed in otherwise similar conditions. In general, the species tested had a higher uptake capacity (Vmax) for NH₄⁺ than for NO₃⁻. Species substantially differed in their NO₃⁻ and NH₄⁺ uptake capacities, but the interspecific differences were markedly greater for NO₃⁻ than NH₄⁺ uptake. Elevated CO₂ had a species-dependent effect on root uptake capacity for NH₄⁺ ranging from an increase of 215% in Acer negundo L. to a decrease of about 40% in Quercus macrocarpa Michx (figure above). In contrast, NO₃⁻ uptake capacity responded little to CO₂ in all the species except A. negundo in which it was significantly down-regulated at elevated CO₂. Across species, the capacity for NH₄⁺ uptake was positively correlated with the relative growth rate (RGR) of species; however, the CO₂ effect on NH₄⁺ uptake capacity could not be explained by changes in RGR. The observed variation in NH₄⁺
uptake response to elevated CO₂ was also inconsistent with life-form differences. Other possible mechanisms that may explain why elevated CO₂ elicits a species-specific response in root N uptake kinetics are discussed (see Zerihun and BassiriRad 2001 for details). Despite the fact that the exact mechanism(s) for such interspecific variation remains unresolved, these differences may have a significant implication for competitive interactions and community responses to elevated CO₂ environments.

Integrating other factors

At this juncture, the research had clearly lead to the conclusion that root N uptake kinetics is only one of the suite of factors that can respond to elevated CO₂. Therefore, it was critical to understand the extent to which other root system adjustments responded to elevated CO₂ and whether such responses produced feedback or feedforward interactions with the kinetics of uptake. Plants posses a suite of mechanisms that can be elicited to adjust nutrient acquisition. Among the most important ones are changes in root morphology, changes in symbiotic association with mycorrhizal fungi and changes in Nitrogen Use Efficiency (NUE). We examined these characteristics in a number of OTC and Greenhouse experiments.

Root Morphology

We examined changes in root growth and ¹⁵NH₄⁺ uptake capacity of loblolly pine (Pinus taeda L.) and ponderosa pine (Pinus ponderosa Douglas. Ex Laws.) seedlings that were grown in pots in a phytotron at CO₂ partial pressures of 35 or 70 Pa with NH₄⁺ as the sole N source (details in BassiriRad et al. 1996. Tree Physiology). Kinetics of ¹⁵N-labeled NH₄⁺ uptake were determined in excised roots, whereas total NH₄⁺ uptake and uptake rates were determined in intact root systems following a 48-h labeling of intact seedlings with ¹⁵N. In both species, the elevated CO₂ treatment caused a significant downregulation of ¹⁵NH₄⁺ uptake capacity in excised roots as a result of a severe inhibition of the maximum rate of root ¹⁵NH₄⁺ uptake (Vmax). Rates of ¹⁵NH₄⁻ uptake in intact roots were, however, unaffected by CO₂ treatment and were on average 4- to 10-fold less than the Vmax in excised roots, suggesting that ¹⁵NH₄⁻ absorption form the soil was not limited by the kinetics of root ¹⁵NH₄⁺ uptake. Despite the lack of a CO₂ effect on
intact root absorption rates, $^{15}$NH$_4^+$ uptake on a per plant basis was enhanced at high CO$_2$ concentrations in both species, with the relative increase being markedly higher in ponderosa pine than in loblolly pine. High CO$_2$ concentration increased total 15NH$_4^+$ uptake and the fraction of total biomass allocated to fine roots (< 2 mm in diameter) to a similar relative extent. We suggest that the increased uptake on a per plant basis in response to CO$_2$ enrichment is largely the result of a compensatory increase in root absorbing surfaces.

**Mycorrhizal fungi**

Studies (including many of our own) addressing plant N acquisition in response to elevated CO$_2$ often use nonmycorrhizal plants. Because most native plants are mycorrhizal and considering that elevated CO$_2$ often enhances mycorrhizal infection of the roots, it is critical to understand what role mycorrhizas can play in nutrient acquisition of plants under elevated CO$_2$. The following study was designed to address the relative importance of mycorrhizal fungi in nutrient uptake of tree seedlings. An understanding of root system capacity to acquire nitrogen (N) is critical in assessing the long-term growth impact of rising atmospheric CO$_2$ concentration on trees and forest ecosystems. We examined the effects of mycorrhizal inoculation and elevated CO$_2$ on root ammonium (NH$_4^+$) and nitrate (NO$_3^-$) uptake capacity in sweetgum (*Liquidambar styraciflua* L.) and loblolly pine (*Pinus taeda* L.). Mycorrhizal treatments included inoculation of seedlings with the arbuscular mycorrhizal (AM) fungus *Glomus intraradices* Schenck & Smith in sweetgum and the ectomycorrhizal (EM) fungus *Laccaria bicolor* (Maire) Orton in loblolly pine. These plants were then equally divided between ambient and elevated CO$_2$ treatments. After 6 months of treatment, root systems of both species exhibited a greater uptake capacity for NH$_4^+$ than for NO$_3^-$: In both species, mycorrhizal inoculation significantly increased uptake capacity for NO$_3^-$, but not for NH$_4^+$. In sweetgum, the mycorrhizal effect on NO$_3^-$ and NH$_4^+$ uptake capacity depended on growth CO$_2$. Similarly, in loblolly pine, the mycorrhizal effect on NO$_3^-$ uptake capacity depended on growth CO$_2$, but the effect on NH$_4^+$ uptake capacity did not. Mycorrhizal inoculation significantly enhanced root nitrate reductase activity (NRA) in both species, but elevated CO$_2$ increased root NRA only in sweetgum. Leaf NRA in sweetgum did not change.
significantly with mycorrhizal inoculation, but increased in response to CO₂. Leaf NRA in loblolly pine was unaffected by either treatment. The results indicate that the mycorrhizal effect on specific root N uptake in these species depends on both the form of inorganic N and the mycorrhizal type. However, our data show that in addressing N status of plants under high CO₂, reliable prediction is possible only when information about other root system adjustments (e.g., NUE, biomass allocation to fine roots and kinetics of uptake) are simultaneously considered.

Synthesis using conceptual and quantitative models

We developed a conceptual model that could illustrate the mechanisms involved in regulation of nutrient uptake and growth responses to high CO₂. The proposed conceptual model (on the right) suggests that in predicting the whole plant nutrient uptake, root system adjustments reviewed here must be considered collectively. Examination of each mechanism in isolation is likely to lead to false prediction of how nutrient acquisition may be impacted by high CO₂. For example, an increase in fine root production under high CO₂ does not automatically translate into greater nutrient uptake if the fine roots produced under high CO₂ have substantially lower physiological capacity to take up nutrients. Similarly, with respect to mycorrhizae, an increase in percent infection (which is commonly used as a measure of response to CO₂) does not necessarily mean an increase in external hyphae or N and P uptake. The fungal symbionts should be capable of affecting whole-plant nutrient uptake
much more significantly than the fractional biomass allocated to them. Their carbon metabolism is likely to be of less importance, positively or negatively.

According to this model, in addition to root morphology, physiology and symbiotic association with mycorrhizal fungi, plant N acquisition may be regulated by N demand. To our knowledge, a robust measure or definition of plant N demand does not exist. However, we defined photosynthetic N use efficiency (PNUE) as a measure of plant N demand. Then we expanded Gutschick's functional balanced model (which already included parameters of root morphology, kinetics and PNUE) to include the role of mycorrhizal fungi (for details see BassiriRad et al. 2001). This model allowed us to quantitatively assess the relative leverage of each of the mechanisms listed above in controlling growth and nutrient uptake responses to high CO₂. This model has been partially validated using seedlings of sunflower (for detail see Zerihun et al. 2001). The following summarizes the results of the sensitivity analysis of our functional balanced model: We suggest that the mechanisms of increased nitrogen uptake highlighted here have different weights in determining overall plant responses to high CO₂. For example, root to shoot biomass allocation should have a minor effect on RGR. This allocation is, however, expected to affect tissue nutrient content significantly, altering as well the risks of herbivory and the mineralization of litter. Increases in nutrient uptake per unit root mass, $\tilde{\nu}$, should always affect RGR and tissue $f_n$ strongly. The ability to increase $\tilde{\nu}$ by physiological adjustments in kinetic parameters is highly suppressible by soil diffusional limitation in most, but not all, ecosystems. On the other hand, photosynthetic nitrogen use efficiency should always have a strong effect on RGR, except when RGR approaches its developmental limit, $RGR_{max}$. The latter may be an important parameter to measure. Product inhibition of photosynthesis and its changes with CO₂ and nutrient treatments are expected to have only modest effects for most plants.

Even when one focuses on a single mechanism, responses to high CO₂ vary from positive to no change to negative. Some of those inconsistencies are attributable to differences in experimental protocols. A larger part of those inconsistencies may, however, be attributed to genetic differences among species. These adjustments could be elicited by CO₂ to a different extent depending on the species which, perhaps, could explain why there is a wide range of
inter species variation in growth responses to CO2. Obviously this has enormous implications for net primary productivity. However, such interspecies variation may also explain why elevated CO2 can decrease nutrient concentration in some species, but not in others. If the genetic potentials for expressing these root system compensatory adjustments are, indeed, different among species then it is abundantly plausible that high CO2 may lead to changes in species composition and biodiversity via a shift in competitive ability in nutrient acquisition. Future conceptual and quantitative models designed to predict plant and ecosystem responses to rising CO2 would require a better understanding of the factors that regulate plant nutrient status and how these factors may change in response to CO2. The FB model presented here is one such attempt.

**Postdocs, Graduate and undergraduate students trained under this grant**

Two postdocs, Ayalsew Zerihun and John Constable received partial funding from this grant. While there were no direct involvement of any of my graduate students in this grant, there were numerous undergraduate students who worked on various aspects of this research. A partial list of those students is as follows: *Meredeth Brass, Emily Cheng, Eunice Choi, *Michael Hsu, Hang Li, Yoomi Lee, *David Lindsy, Elio Oshoa, Parthiv Patal, *Menzi Tabora, *Grace Tan, Shiraz Vartanian, Partive Patel, Hemang Shah, *Jasmeen Dhaliwal. Names marked with * indicates independent study students. The remaining names are students who worked on this project as lab assistants.

**Publications credited to this grant:**


\(^{15}\)NH₄ uptake kinetics and growth in seedlings of loblolly and ponderosa pine. Tree Physiology 16: 957-962.

Appendix
July 29, 1996

Dear Dr. Elwood:

Enclosed please find a copy of my annual progress report for the DOE/PER grant; Nitrogen Budget under elevated CO₂ levels: regulation by absorption and assimilation. This grant was awarded to me through NMSU (contract number DE-FG03-95ER62126) with Vince Gutschick as CoPI. As you know, I am starting a new position at University of Illinois at Chicago (UIC) in August of 1996 and have already contacted you (my letter to you dated April 19, 96) regarding the transfer of funds to my new institution. This request is fully supported by NMSU, UIC and Dr. Gutschick.

Dr. Gutschick's operational budget for the second and third funding period will be subcontracted to NMSU from UIC. This transaction would, however, bear additional subcontract charges from UIC which would increase our original budget by roughly 7.5K for each of the remaining funding periods. You advised that this additional cost could be covered by DOE if the requested date of transfer is postponed from August of 1996 to the end of the first funding period, 10/1/96. Accordingly, I am now requesting for this transfer to become effective as of the end of the first funding period. Carol Quintana in the Office of Research at NMSU has already been notified of this change.

Along with the progress report and request for transfer of funds, you have also advised that a new budget sheet be immediately submitted from UIC. Obviously the submission of the budget sheets must officially be carried out by UIC, but since I am not physically on that campus yet the logistics are less than straightforward. UIC has already prepared the budget sheets and sent them to me for my signature. I have returned the budget sheet to them plus a copy of the annual progress report as well as this cover letter to be included in the official submission. The official paperwork should be sent to you from UIC within a few days. However, to ensure you receive my progress report for the July 31 deadline, I am sending you this package before my departure for UIC. An unofficial copy of the budget sheets from UIC are also enclosed.
As per your request, instead of a complete version of the original proposal, the following summarizes the scope of the project for the second and third funding period, reiterating that the scope of the project is essentially unchanged. Our major hypotheses regarding the effects of elevated CO$_2$ on plant N uptake and assimilation will be tested by conducting both growth chamber and field experiments culminated by the incorporation of the data into a comprehensive functional growth model. Our major goals are to evaluate 1) How does high CO$_2$ affect root uptake capacity for N, 2) How does high CO$_2$ affect the proportion total N taken up as NO$_3$, and 3) Whether interspecies differences in root N uptake responses can explain different species responses to elevated CO$_2$. Field experiments are exclusively designed and performed through my collaboration with three national sites. These sites are currently funded to do CO$_2$ research using open-top chambers and are headed by Hugo Rogers at Auburn University, Rich Norby at ORNL and Clenton Owensby at KSU.

I am encouraged by the level of cooperation from these collaborative efforts and novel approaches that we have already utilized in Oak Ridge and Auburn. We may also have the possibility to expand the field kinetic experiments by linking with two ongoing FACE projects at Duke and Phoenix. The open-top chamber experiments at the national sites are currently all out of state operations. Therefore, the location of my base institution should not negatively impact any of our commitment to this project.

We will also continue the more detailed work on kinetics and N assimilation responses to elevated CO$_2$ in growth chamber facilities both at UIC and the Duke university Phytotron. One of our most immediate priority is to complete the kinetic experiments detailed in the enclosed progress report for a total of 8 species. Data from these experiments would be analyzed in a comprehensive functional growth model using both alometric and physiological parameters. The goal of the modeling effort is to test relations among all the changes that (a) are predicted from physiological principles and that (b) promise to reduce radically the number of "free parameters" needed to predict performance at high CO$_2$.

Thank you for considering this request.

Sincerely,

Hormoz BassiriRad

Hormoz BassiriRad
Plant nitrogen budget under elevated carbon dioxide level: regulation by nitrogen absorption and assimilation

Progress Report
US Department of Energy/PER contract # DE-FG03-95ER-62126
October 1, 1995-July 31, 1996

Hormoz BassiriRad and Vincent Gutschick
New Mexico State University

Collaborators:
Rich Norby, Oak Ridge National Laboratories, Oak Ridge, TN
Hugo Rogers, National Soils Dynamic Lab, Auburn, AL
Clenton Owensby, Kansas State University, Manhattan, KS
Objective: The overall objective is to assess root physiological and morphological characteristics that may alter plant N acquisition capacity in response to rising atmospheric CO₂ concentration. There is increasing evidence that plant and ecosystem responses to elevated levels of CO₂ will ultimately depend on availability and acquisition rate of other resources such as N (Bazzaz 1990, Eamus and Jarvis 1989). Therefore knowledge of any changes in root capacity to acquire N is crucial in predicting plant and ecosystem responses to high CO₂. Here we are testing two major hypotheses: 1) elevated CO₂ will enhance root N uptake kinetics and 2) CO₂ enrichment will increase root preference for NO₃⁻ as opposed to NH₄⁺. High CO₂ enhances root energy status (BassiriRad et al. 1996a) which should in turn favor energy-intensive processes such as NO₃⁻ uptake and assimilation. The above hypotheses are being tested on a range of species from native and agricultural ecosystems using a combination of field, lab and growth chamber studies. We have demonstrated a considerable interspecies variation in root N uptake responses to CO₂ enrichment (BassiriRad et al. 1996a, b and c, and in current work) and attempts are now underway to evaluate if such variations are correlated with different functional groups (e.g., C₃ vs C₄ or grasses vs. shrubs).

A comprehensive growth model, using physiological and allocation parameters, has been largely completed and will be used to analyze the completed experimental data. The goal of the modeling effort is to test relations among all the changes that (a) are predicted from physiological principles and (b) promise to reduce radically the number of “free parameters” needed to predict performance at high CO₂. A major physiological prediction for item (b) is that the fraction of N taken up as nitrate increases at high CO₂, based on the alleviation of energetic limitations on nitrate reduction. More deeply, we are using a model of functional balance of root and shoot (Gutschick, 1993; Gutschick and Kay, 1995) that strongly relates diurnally-averaged uptake rate, v (or the detailed kinetic parameters, Vₘₐₓ and Kₘ), to root:shoot ratio, r, and which predicts tissue N content from both v and r. The model uses the photosynthetic utility of N in leaves, p*, to make the prediction.

Approaches: Effects of elevated atmospheric CO₂ concentration on root N uptake capacity and preference was tested in both growth chamber and field-grown plants. Dr. Goyal of the Agronomy Department at UC Davis provided the host lab for the N uptake kinetics. Plants were grown at CO₂ partial pressures of either 35 or 70 Pa at the Controlled Environmental facility of UC Davis. Both NH₄⁺ and NO₃⁻ were present at an equimolar concentration in a circulating hydroponic system. The first set of kinetic experiments examined the CO₂ effects on soybean, sunflower and sorghum at various stages of development. We used a novel approach involving a fully automated computer-based system to determine NO₃⁻ and NH₄⁺ uptake kinetics simultaneously by intact root systems. Other growth and physiological parameters such as biomass allocation, tissue N concentration and leaf photosynthesis were also measure in order to address the mechanisms by which CO₂ may alter N uptake kinetics. We have also completed a set of experiments at two open-top field sites in Oak Ridge National Laboratories (with Rich Norby) and National Soils Dynamics Lab in Auburn, AL (with Hugo Rogers). Both of these experiments involved a novel in situ technique to determine uptake kinetics of NH₄⁺ and NO₃⁻ in intact root systems of red and sugar maple (in ORNL) and sorghum and soybean (in SNDL).

The data on uptake kinetics, root:shoot allocation, photosynthetic performance, and growth are being incorporated into a model of CO₂-induced changes in plant performance.
The model of physiology and growth that we seek is an analytic model, that is, with explicit equations in closed form and preferably algebraic solutions. In contrast to common numerical simulations, the closed formula allows predictions of performance upon inspection.

Results to date: Root N uptake property is highly responsive to the growth CO₂ partial pressure, however, this response is highly dependent on the species, inorganic N form and growth stages (Fig. 1). The important implication is that information obtained from one species at one stage of development using only one form of inorganic N can not adequately predict plant and stand-level N uptake responses to CO₂ enrichment. Contrary to our hypothesis 1, elevated CO₂ did not enhance root absorption rate of N in either species (Fig. 2) when rates were estimated from the kinetics parameters at an external NH₄NO₃ concentration of 100 µM. Obviously the biomass data (Fig. 3) indicate a greater demand for N in response to CO₂ enrichment particularly at the end of the experimental period. However, CO₂ may in fact, reduce N demand via increased Photosynthetic N Use Efficiency (PNUE is currently being processed for these two species). In addition, the apparent downregulation of photosynthesis observed here (Fig. 4) may form another basis for a lower plant demand for N. Alternatively, a higher N demand in response to high CO₂ can be met by a greater allocation of biomass to roots. In fact, root:shoot ratio increased significantly in soybean, but remained virtually unchanged in sunflower in response to high CO₂ (Fig. 3) indicating that soybean may be better able to acquire N than sunflower as the CO₂ partial pressure increases.

The increased preference for NO₃⁻ Vs NH₄⁺ in sunflower (Fig. 2) at high CO₂ is consistent with hypothesis 2 and our previous findings (BassiriRad et al. 1996a, b) though greater preference for NO₃⁻ was only apparent at the later ontogenic stages. In fact, rates of NO₃⁻ absorption changed very little with time (Fig. 1) indicating that the apparent shift in preference is most likely caused by a decrease in NH₄⁺ uptake capacity of the older roots than an increase NO₃⁻ uptake capacity per se. In contrast to sunflower, soybean roots took considerably more NH₄⁺ than NO₃⁻ in response to CO₂ enrichment at all except one date (Fig. 2). It is unclear how CO₂ may increase root and plant preference for NH₄⁺. Uptake and assimilation of NH₄⁺ is considerably less energy requiring than NO₃⁻. However, elevated CO₂ may enhance the supply of C skeleton as substrates required for NH₄⁺ assimilation. The better ability to assimilate NH₄⁺ may in turn, act as a feedforward for NH₄⁺ uptake.

Our combined model, to date, is analytic in describing uptake kinetics (Michaelis-Menten), functional balance, and shifts in photosynthetic utility. Thus far, modeling has been used to enhance analysis of uptake data for kinetic parameters.

Literature cited:


Publications:


BassiriRad H, Griffin KL, Reynolds JF and Strain BR. 1996c. Effects of CO$_2$ enrichment on root $^{15}$NH$_4$ uptake kinetics and growth in seedlings of loblolly and ponderosa pine. Tree Physiol (in Press)


Student Participation: Joe Kidd 25%; Mostafa Elhamy 10%, Phoung Troung 10%
Figure legends:

Fig. 1  Kinetic parameters ($V_{\text{max}}$ and $K_m$) for root $\text{NH}_4^+$ and $\text{NO}_3^-$ uptake in soybean (top 4 graphs) and sunflower (bottom 4 graphs) plants at various stages of growth at different CO$_2$ concentrations.

Fig. 2  Estimated total N absorption rate (left) and percent N taken as $\text{NO}_3^-$ for soybean and sunflower at various stages of growth at different CO$_2$ concentration.

Fig. 3  Total biomass, relative growth rate and root:shoot ratio responses of soybean and sunflower to ambient and double the ambient CO$_2$ concentration at various stages of growth.

Fig. 4  Photosynthetic and leaf area responses of soybean and sunflower to ambient and double the ambient CO$_2$ concentration at various stages of growth.
Fig. 4

The graphs illustrate the net assimilation rate and leaf area over days after germination for Sunflower and Soybean under different conditions.

**Net Assimilation Rate (µmole m⁻² s⁻¹)**
- Sunflower
- Soybean

**Leaf Area (cm²)**
- Sunflower
- Soybean

Conditions:
- Sunflower: 350, 700
Fig. 3
Total N absorption rate at 100 μM NH₄NO₃

(μmol gdw⁻¹ h⁻¹)

% N taken as NO₃ at 100 μM NH₄NO₃

Fig. 2
BassiriRad and Gutschick 1996/1997 Progress Report on Grant # DE-FG02-97ER62332
Plant N Budget Under Elevated CO₂: Regulation by N Absorption and Assimilation

Dr. Jerry Elwood
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Germantown, MD 20545

Dear Jerry:

I am submitting this progress report for 96/97 as requested for our DOE funded TECO grant # DE-FG02-97ER62332. This is continuation of the DOE grant# DE-FG03-95ER62126. Enclosed are five published papers that have been credited to this and other grants resulting from joint efforts. As you know since the last progress report, I have moved to a new position here at UIC and have proceeded rapidly to set up my lab and greenhouse facilities to accommodate the major objectives of this grant. Using funds from my start up, we have successfully fabricated and installed 20 (1 m³) open-top chambers linked to an automated system for CO₂ monitoring and control.

The Chambers, depicted on the left, are currently housed inside one of the greenhouses at UIC, but they have been designed for maximum portability so they can be used for small-scale field experiments as well. Currently our lab is also equipped with a state of the art HPLC system which allows us to simultaneously measure NO₃⁻ and NH₄⁺ concentrations with a detection limit of several nanomoles. Funds for the HPLC were also provided from my start up funds. In the following section, I shall present our progress to date which will include field, lab/green house and modeling data that has not been published yet.

Field work and collaboration with national sites:

An important objective of our proposed research was to determine if elevated CO₂ enhances root N uptake capacity and whether this effect is differentially expressed for NO₃⁻ Vs. NH₄⁺. To address these questions under the most realistic scenario, we developed a novel technique which allowed us to measure N uptake kinetics on intact field-grown roots. In the summer of 1996, with a great help from Rich Norby at ORNL and Hugo Rogers/Steve Prior at NSDL in Auburn, we used this technique successfully to measure N uptake kinetics of red maple, sugar maple, sorghum and soybean roots grown in the field at either ambient or double the elevated CO₂ levels. Briefly, In this method, we gently teased fine active roots out of soil without breaking them. Separate cohort of fine were then placed in a microfuge tube containing 2 mls of well-aerated assay solutions containing NH₄NO₃ at one of the following concentrations: 10, 25, 50, 75, 100, 150, 200 μM. The assay period was 1 to 2 hours and uptake rates were calculated from the depletion of N from the solution during that period. Rates were expressed on root dry wt basis. At the end of the uptake period, depletion of NH₄⁺ and NO₃⁻ from was measured by an automated HPLC system tooled with a UV and a fluorescence detector.
For the purpose of this report we have presented only the maximum uptake rates ($V_{\text{max}}$). $V_{\text{max}}$ values were calculated for each species by fitting the uptake rates to a Michaelis-Menten at ambient and elevated CO$_2$ were calculated (BassiriRad et al. 1996 a and b). $V_{\text{max}}$ is simply an indication of root uptake capacity and data in Figure 2 indicate that elevated CO$_2$ affects N uptake capacity differently for the two inorganic N forms. For example, in soybean, elevated CO$_2$ did not significantly change NH$_4^+$ uptake capacity, but significantly increased $V_{\text{max}}$ for NO$_3^-$. On the other hand, in sorghum roots uptake capacity for both N forms increased significantly in response to CO$_2$ enrichment. Furthermore, uptake kinetic responses to CO$_2$ are highly species dependent. In the crop species examined here CO$_2$ tended to enhance total root N uptake capacity whereas, $V_{\text{max}}$ for both NO$_3^-$ and NH$_4^+$ decreased in red and sugar maple in response to CO$_2$ enrichment.

Lab and greenhouse experiments

In order to evaluate whether and how elevated CO$_2$ alters root capacity to acquire N, we had proposed to conduct our experiments with several species that represented a wide range of growth habit and functional groups. In the last year report we had presented data showing that the effects of elevated CO$_2$ on root N uptake capacity was distinctly different between soybean and sunflower, but more importantly, this effect was highly sensitive to different stages of development. Figure 3 clearly illustrates that changes in root N uptake kinetics (reported last year) are not likely to be regulated by photosynthetic N use efficiency. In addition, in the initial proposal we hypothesized that elevated CO$_2$ will lead to increased preference for NO$_3^-$ Vs. NH$_4^+$. This increased preference for NO$_3^-$ has so far been realized only in sunflower, but even in sunflower, the change in preference appears to be unrelated to root carbohydrate status (Figure 4). Our most recent inventoried species is sorghum. $V_{\text{max}}$ for both N forms were unaffected by CO$_2$ except for nitrate at the second sampling date (Figure 6). This lack of response in N uptake capacity may simply reflect the apparent lack of increased demand at high CO$_2$ in sorghum. Once again, at this point we define demand in its crudest sense as reflected in lack of a positive response in growth, photosynthesis and photosynthetic use efficiency in response to CO$_2$ enrichment (Figure 5).

Modeling synthesis

The broad question addressed in modeling is assessing how plant N economies change under elevated CO$_2$, with ramifications that include how changes in the C economy of a plant may ameliorate metabolic limitations in N use. The first test of (degree of) optimality in physiological responses (especially N uptake velocities) and allocation to roots is the test that functional balance has been maintained between root and shoot. A qualitative estimate is that root:shoot ratio ($r$) should increase, given that shoot photosynthetic function has increased with elevated CO$_2$. This is observed in the data from soybeans in growth chambers, as a typical example. For these plants in mid-growth (day 28), $r$ increases from 0.29 in the control to 0.39 at elevated CO$_2$. A rigorous formulation is given by Gutschick and Kay (1995), relating root:shoot ratio to N uptake rate, photosynthetic nitrogen use efficiency, and plant N content. This formulation assumes that the root and shoot are in functional balance - that all N taken up is assimilated and used primarily for photosynthetic function. One may test that functional balance is attained. Rather than examining changes in $r$, to be addressed separately for optimality, one compares incremental N content ($f_N$, in gN gDM$^{-1}$) in new tissue (calculated from root uptake rates $V$, multiplied by root:shoot ratio, divided by whole-plant dry-mass gain) with $f_N$ computed...
with the assumption of functional balance, \( f_N (F_B) = \sqrt{r*v/(\beta*\alphaL*PNUE)} \).

Here, \( \beta \) is the conversion from photosynthetic rate of leaves to dry mass gain (0.67 gDM [g glucose]^{-1}), \( \alphaL \) is the ratio of leaf mass to whole-shoot mass, and \( PNUE \) is the photosynthetic N-use efficacy (g glucose photosynthesized d^{-1} [g leaf N]^{-1}). This comparison is superior to comparing new \( f_N \) with \( f_N \) in extant tissue, in that it allows for ontogenetic changes in \( f_N \). For the soybean plants in growth chambers, elevation of CO2 is predicted to multiply \( f_N \) of control plants by the ratio \( \sqrt{f_r/r_0}*(v/v_0)/ (PNUE/PNUE0) \), with zero suffices indicating control levels of CO2. Changes in \( \beta \) and \( \alphaL \) are considered as minor. The predicted change shift is that \( f_N (F_B) \) is 0.80 as large at elevated CO2 as at control CO2. The observed change is 0.67, at day 28 of growth, indicating that decreases in \( f_N \) moderately exceed those required by functional balance.

The growth model used above does not directly assess if root allocation (as \( r \)) and N uptake rates (\( v \)) are optimized jointly. It is both difficult and minimally relevant to assess the optimality of \( r \) for the plant’s N economy alone (not considering water economy; see later). As noted in Gutschick and Kay (1993), the response of RGR to \( r \) is exceptionally weak. Assessing the optimality of uptake rate \( v \) is also difficult but highly relevant to the N economy. Briefly, RGR should scale as the square root of \( v \), if one ignores metabolic costs of assimilating N. Even accounting for energetic costs of nitrate reduction (ibid.), \( v \) should attain values much higher than ever observed in plants, values that would drive plant N contents to the order of 15%. Hypotheses in the original proposal that elevated CO2 may ameliorate energetic limitations to N use become moot in this light. Alternative hypotheses must be developed, two of which may be stated here. H1: The plant is limited in the amount of N that can be deployed functionally. For example, 15% N in the leaf would make the leaf over 80% protein. The limits likely arise primarily from leaf-internal limitations on triose-P transport, and, perhaps most likely, on phloem loading (Koerner et al., 1995). The most direct way to explore these limits is with combinations of environmental and genetic alterations (see, e.g., Stitt and Schulze, 1994), which are beyond the attainable scope of the research. H2: The activity of the pH-stat (Raven, 1992) is limited. For plants taking up nitrate, the pH-stat operates by the accumulation of acid anions derived from carbohydrates and the extrusion of OH- ions. Limited ability to transport acid anions such as pyruvate to roots for decarboxylation requires greater anion accumulation, which may downregulate nitrate reduction. For plants taking up ammonium, the pH-stat operates by decreasing the uptake of metal cations and by extrusion of H+ at the roots. Limited extrusion capacity generates a deficit in cations, known to adversely affect many metabolic functions of ammonium-intolerant plants. To test this hypothesis, we will require, at a minimum, more resolution of tissue carbohydrates by chemical species and cation content analyses.

Publications credited to this grant


Bassirirad H, Norby RJ, Prior SA, Roger HH (in preparation) Assesment of ammonium and nitrate uptake kinetics in field grown intact roots in response to CO$_2$ enrichment.

References


Figure 2.
Figure 3

Photosynthetic N Use Efficiency (μmol m⁻² s⁻¹/mmol N)

Soybean

Sunflower

Days after germination

O-O 35

- - 70

- - 70

- - 70
Figure 4

Soybean

Sunflower

Total Nonstructural Carbohydrates (% dry weight)

Days after germination

O-O 35

-•- 70
Figure 5

Days after germination

Leaf area (cm²)

PS NUE

Sorghum

O O 0.35
○ ○ 7.0
Figure 6

![Graph showing V_max (µmol g⁻¹ h⁻¹) over Days after germination for NO₃ and NH₄](image)

Days after germination

Days after germination