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Isotopic carbon discrimination and leaf nitrogen content of *Erica arborea* L. along a CO_2 concentration gradient in a CO_2 spring in Italy

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Summary We studied a Mediterranean species (*Erica arborea* L.) growing in a CO_2 spring in Italy that was naturally exposed for generations to a gradient of atmospheric CO_2 concentrations. The CO_2 concentration gradient to which different individual plants were exposed was determined by an indirect method based on radioisotope analysis. The stable carbon isotope ratio of sampled leaves was determined by mass spectrometry, and isotopic discrimination was then calculated. Leaf nitrogen, specific leaf area, total soil nitrogen, soil organic matter content and soil pH were also measured. In one group of plants, grown on a homogeneous soil and exposed to moderate CO_2 enrichment, isotopic discrimination was significantly reduced in response to increasing CO_2 concentrations, whereas the intercellular CO_2 concentration and leaf nitrogen content were almost unaffected. In a second group of plants, grown along a gradient of CO_2 concentration and soil nitrogen content, leaf nitrogen content was reduced when nitrogen availability was limiting. However, when soil nitrogen was available in excess, even very high CO_2 concentrations did not result in increased discrimination or reduced leaf nitrogen content in the long term. The results are discussed with respect to current theories about the long-term CO_2 response of plants based on several years of experimentation with elevated atmospheric CO_2 concentrations under controlled conditions.

Keywords: carbon isotope ratio, elevated carbon dioxide, radioisotope analysis, specific leaf area.

Introduction

Predictions about the effect of increasing CO_2 concentrations on natural ecosystems are used to develop models to assess the impacts of global change on biosystems. It is uncertain if natural vegetation will be a source or a sink for carbon under a changed climate and if the expected enhancement of atmospheric CO_2 concentrations will stimulate plant growth and net primary production. Recent studies suggest that, in the past, plants may have adjusted to increasing atmospheric CO_2 by decreasing stomatal conductance. Studies of herbaria specimens and plant material from archeological sites show that stomatal numbers (Woodward 1987, Penuelas and Matamala 1990, Paoletti and Gellini 1993) and stable carbon isotopic discrimination (Woodward 1993, Araus and Buxo 1993) have both decreased since pre-industrial times. However, little is known about the long-term response of natural vegetation to further increases in atmospheric CO_2 concentration because there have been few CO_2-enrichment experiments with natural vegetation that have lasted for more than a season (Tissue and Oechel 1987, Arp and Drake 1991). Also, it is not known if small-scale community experiments that simulate possible future climates are capable of predicting ecosystem functioning and nutrient and water cycling responses to global climate change, because these experiments often lack the necessary range of natural disturbances that occur in the long term (Woodward 1992).

The occurrence of long-existing natural CO_2 vents, termed CO_2 springs, provides a unique opportunity to study long-term response and microevolutionary adaptation of natural vegetation to elevated CO_2. In these CO_2 springs, CO_2 of deep origin is naturally released to the atmosphere by one or more vents, and the local flora is often exposed to CO_2 concentrations that can be more than twice current atmospheric concentrations (Miglietta and Raschi 1993, Miglietta et al. 1993*a*). This natural vegetation may also be assumed to have been exposed for generations to elevated CO_2 and to have been subjected, during this time, to a large range of natural disturbances of both physical and biological origin. These key factors are likely to enhance the predictive value of observations made at these sites about the long-term response of plants to elevated CO_2 despite the multiple confounding effects that may occur.

In this paper, we report long-term effects of elevated CO_2 on isotopic discrimination and leaf nitrogen content of the Mediterranean species *Erica arborea* L. growing along a complex CO_2 concentration and soil nitrogen gradient in a CO_2 spring. Based on recent theories about the relationship between leaf nitrogen content, isotopic discrimination and photosynthetic capacity of leaves (Field and Mooney 1986, Farquhar et al. 1989), we have used our results to assess the occurrence of downward regulation of potential photosynthesis in *E. arborea* after long-term exposure to enriched CO_2.

Materials and methods

Plants were collected in a CO_2 spring located near Naples, in central southern Italy. In this CO_2 spring, several gas vents occur within a large area (Figure 1) and release more than 200 Mg CO_2 day^{-1} at the surface (Carapezza et al. 1984). The gas emitted by the vents is composed of 19% CO_2 and 80% water vapor plus traces of oxygen, nitrogen, hydrogen sulfide and carbon monoxide (Tedesco et al. 1988). As a result of gas emissions, there is a significant enhancement of atmospheric CO_2 over a large area (Carapezza et al. 1984). A gradient of decreasing CO_2 concentrations with increasing distance from major vents also occurs, although short-term fluctuations of CO_2 concentrations due to atmospheric turbulence may affect the extent of the gradient. As has been observed in other CO_2 springs (Miglietta and Raschi 1993), there are technical difficulties in the direct determination of average CO_2 concentrations by infrared gas analysis at this site.

We were, however, able to estimate average growth CO_2 by comparing the ^{14}C content of plant tissues at various locations. The CO_2 released at the spring surface originates from the dissolution of carbonate sediments and is depleted in ^{14}C (so called 'dead CO_2' because of the absence of ^{14}C). This spring-released CO_2 has a stable carbon isotope ratio close to zero ($\delta^{13}C = -0.2‰$), where:

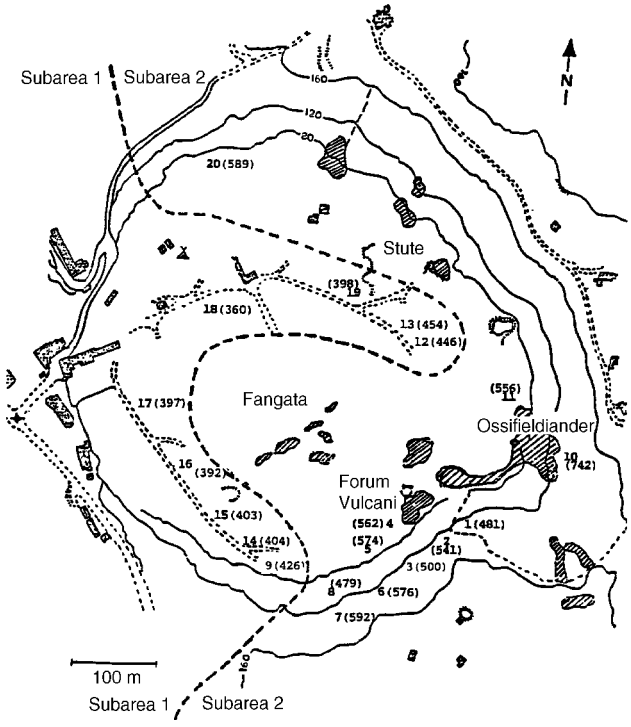

Figure 1. Map of the Solfatara CO_2 spring, Pozzuoli, Italy. The dotted line separates the two main subareas of the spring characterized by different vegetation and soil types. Numbers in the figure denote the location where plant samples were collected in 1989 and 1992. Long-term CO_2 concentration to which individual plants were exposed is given in parenthesis. The gray-colored areas indicate the principal geothermal vents.

$$\delta^{13}C = \left(\frac{R_{sample}}{R_{standard}} - 1\right)1000\ , \quad (1)$$

and R_{sample} and $R_{standard}$ are the $^{13}C/^{12}C$ ratios of the CO_2 and Pee Dee Belemenite (PBD) reference standard, respectively. When this CO_2 mixes with ambient air, CO_2 concentration and $\delta^{13}C$ of the mixture increase, whereas its ^{14}C content decreases. As a consequence, the effective CO_2 exposure of vegetation can be calculated from measurements of the abundance of ^{14}C in leaf tissues. It has been established that when plants are exposed to air artificially enriched with ^{14}C-depleted CO_2, the radiocarbon content of plant tissues becomes proportional to the average ^{14}C content of the source air (Enoch et al. 1984). If it is assumed that, at the CO_2 spring, ^{14}C abundance in air is a function of CO_2 concentration, ^{14}C abundance in plant tissues must equal ^{14}C in source air minus a constant discrimination term (Geyh and Scheicher 1990). Accordingly, effective average CO_2 exposure of a leaf, integrated over its lifetime is given by:

$$[CO_2] = [CO_2]_a(M_a/M_p), \quad (2)$$

where $[CO_2]_a$ is the mean CO_2 concentration in the normal atmosphere, M_a is the ^{14}C abundance in leaves grown at ambient CO_2 concentration, and M_p is the ^{14}C abundance in leaves grown at elevated CO_2 concentrations. It has been shown that growth CO_2 concentration calculated in this way correlates well with long-term CO_2 concentration measured by gas analysis (van Gardingen et al. 1995).

The CO_2 spring considered in this study is partly covered by natural vegetation and can be divided into two main subareas (Figure 1). Subarea 1 is located at some distance from the major vents where atmospheric CO_2 enrichment is moderate, and several Mediterranean plant species occur on a flat and homogeneous area (Table 1). Subarea 2 is located closer to the vents, and the vegetation is exposed to much higher CO_2 concentrations; isolated shrubs of *E. arborea*, *Myrtus communis* L. and a pure population of *Agrostis canina* L. are the only species growing in Subarea 2.

Samples of sun leaves were taken from the top of the crown of 18 plants of *E. arborea* growing in Subareas 1 and 2 in July 1989 and 1992. The location of selected plants is reported in the map of Figure 1. Nitrogen concentration was determined on leaf samples by a modified micro-Kjeldhal system. Specific leaf area (SLA, g m^{-2}) was determined in 1992 only. Dried leaf material was then used for ^{14}C absolute content and $\delta^{13}C$ determinations. Abundances of ^{14}C were determined at the Department of Geochemistry, Rome University, on the samples collected in 1989. Stable carbon isotopic ratios were determined by mass spectrometry at the Department of Geochemistry, Rome University (1989 sample), and at the Istituto di Agroselvicoltura, CNR , Porano, Italy (1992 sample), using the same standard.

Stable carbon isotope discrimination (Δ) was calculated from $\delta^{13}C$ of leaves (δ_p) and $\delta^{13}C$ of air (δ_a) according to Equation 3 (Farquhar et al. 1989):

Table 1. List of species growing in Subarea 1. Nomenclature is according to Pignatti (1982).

Agrostis canina L.
Arbutus unedo L.
Asparagus officinalis L.
Calycotome spinosa (L.) Link
Carex hirta L.
Cistus salvifolius L.
Cytisus scoparius (L.) Link
Dacus carota L. s.l.
Erica arborea L.
Hedera helix L.
Imperata cylindrica (L.) Beauv. var. *europaea* (Pers.) Anderss.
Inula viscosa (L.) Aiton
Myrtus communis L.
Quercus cerris L.
Quercus ilex L.
Rubia peregrina L.
Rubus cfr. *ulmifolius* Schott

$$\Delta = (\delta_a - \delta_p)/(1 + \delta_p). \quad (3)$$

A simplified model relating leaf Δ to the intercellular (c_i) and ambient (c_a) CO_2 concentrations (Farquhar et al. 1989) was used to calculate the c_i/c_a ratio:

$$c_i/c_a = (\Delta - a)/(b - a), \quad (4)$$

where a is the discrimination associated with CO_2 diffusion through air (−4.4‰), and b is the net discrimination associated with CO_2 fixation in C_3 plants (−27‰) (Farquhar et al. 1989). This relationship links leaf gas exchange processes with the isotopic composition of plants.

Soil pH and soil nitrogen concentration were determined in soil samples collected in October 1992 within 1 m of each sampled plant. Soil cores (0–20 cm) were collected and oven dried at 30 °C. Leaf samples were taken in 1992 from plants growing closest to and furthest away from vents and immediately frozen in liquid nitrogen and subsequently stored at −80 °C until analyzed for cysteine and glutathione as described by Badiani et al. (1993).

Results and discussion

An increase in cellular nonprotein sulfyhdryl compounds such as cysteine and glutathione has been proposed as a reliable indicator of H_2S accumulation and toxicity (de Kok et al. 1986). We therefore determined foliar cysteine and glutathione contents in plants growing close to the vents and in plants growing further away from the vents where the CO_2 concentration was lower. We found no difference in the contents of these two nonprotein sulfhydryl compounds between the two groups of plants (data not shown), suggesting that the presence of H_2S in the geothermal emissions did not substantially alter the cellular sulfur balance or cause sulfur accumulation or stress in the plant material studied. Similar findings were obtained in a study of the H_2S-sensitive species *Glycine max* Merill exposed to the emission of an H_2S-rich spring (Miglietta et al. 1993*b*, Badiani et al. 1993). The absence of sulfur stress in *G. max* was explained by the fact that hydrogen sulfide venting at the surface in CO_2 springs is rapidly diluted and oxidized when in contact with the atmosphere so that its concentration rapidly falls below phytotoxic levels (Miglietta et al. 1993*a*). A similar explanation may account for the absence of sulfur stress in *E. arborea*.

On the basis of calculations made using Equation 2, we determined that the plants sampled in 1989 were exposed to a CO_2 concentration gradient ranging from 360 to 742 μmol mol^{-1}. Plants growing in Subareas 1 and 2 were exposed to CO_2 concentration gradients ranging from 360 to 450 μmol mol^{-1} and from 450 to 742 μmol mol^{-1}, respectively.

Soils of Subarea 1 are more or less homogeneous, moderately acidic (mean pH = 4.3) and have a mean total nitrogen content of 0.39% (Table 2). Soils of Subarea 2 are acidic (pH < 3) and soil nitrogen content varies significantly among the sampling points (Table 2). It is not known why the soil nitrogen content is higher in Subarea 2 than in Subarea 1, but it is possible that nitrogen has leached into Subarea 2 from agricultural soils located above the site. The large differences in soil acidity between Subareas 1 and 2 were assumed to be the major factor explaining the differences in species composition between the two sites.

In plants in Subarea 1, isotopic discrimination (Δ), calculated by Equation 3, decreased with increasing CO_2 concentrations, whereas Δ was largely independent of CO_2 concentration

Table 2. Measured values of organic matter (OM, %), total nitrogen (N, mg g^{-1}) and pH of soils in the proximity of sampled plants (see Figure 1 for number legend). Asterisks indicate missing values (locations not sampled).

N	OM	N	pH
Subarea 1			
18	7.10	0.43	4.10
16	*	*	*
17	5.90	0.37	4.40
19	*	*	*
15	6.40	0.39	4.50
14	6.50	0.40	4.40
9	6.20	0.38	4.30
12	*	*	*
13	*	*	*
Subarea 2			
8	1.30	0.09	2.50
1	1.70	0.11	2.60
3	2.10	0.14	2.70
2	2.90	0.19	3.20
11	3.60	0.22	2.10
4	5.00	0.33	2.40
5	8.40	0.51	3.10
6	10.30	0.64	3.10
20	13.80	0.81	2.60
7	20.40	1.24	2.80
10	23.00	1.38	2.50

in plants in Subarea 2 (Figure 2). Consequently, increasing CO_2 concentrations had little effect on the long-term average c_i, calculated by solving Equation 4, in plants in Subarea 1, whereas average c_i increased with increasing CO_2 in plants in Subarea 2 (Figure 3).

Increasing c_i had little effect on leaf nitrogen content (expressed on either a leaf mass or a leaf area basis) in plants in Subarea 1, whereas leaf nitrogen content increased with increasing c_i in plants in Subarea 2 (Figure 4).

Within a species, photosynthetic capacity of sunlit leaves is proportional to the amount of nitrogen invested in photosynthetic proteins and, therefore, to total leaf nitrogen content (Field and Mooney 1986, Sage et al. 1989, Evans 1989). It is also well known that increases in carbon assimilation rate in response to short-term exposure of C_3 species to elevated CO_2 are only realized if photosynthetic capacity is not limiting and c_i rises in response to atmospheric CO_2 enrichment (Long et al. 1993).

The absence of significant increases in average c_i (Figure 3) of plants in Subarea 1 and the constant nitrogen content of their leaves (Figure 4) is consistent with a constant photosynthetic rate along this CO_2 gradient, i.e., the reduction in the c_i/c_a ratio was a result of reduced stomatal conductance rather than increased assimilation capacity. Recent studies have shown that leaf N is a good predictor of photosynthetic capacity for plants grown in both elevated and ambient CO_2 concentrations (Petterson and McDonald 1994). Increased nitrogen-use efficiency (carbon uptake rate per unit leaf nitrogen content) in plants exposed to elevated CO_2 is caused by partial suppression of photorespiration, which only occurs if c_i rises with rising c_a (Long et al. 1993). This implies that for a given c_i, a rise in net assimilation rate necessitates an increase in photosynthetic capacity and, consequently, a change in leaf N content (Webber et al. 1994, Stitt and Schulze 1994). Farquhar and Sharkey (1982) postulate that c_i is maintained at a specific value to maintain a chloroplast-level balance between ribulose-1,5-bisphosphate consumption and ribulose-1,5-bisphosphate regeneration during photosynthesis so that both processes are colimiting. We did not measure leaf conductances; however, the decreases in leaf Δ values (Figure 2) while both c_i (Figure 3) and leaf nitrogen content remained constant (Figure 4) could only have arisen as a result of decreased stomatal con-

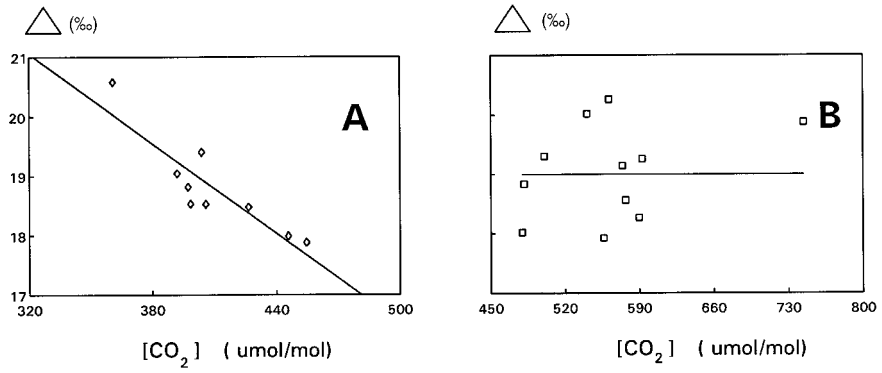

Figure 2. Isotopic discrimination (Δ) of leaves of plants growing in Subarea 1 (A) and Subarea 2 (B) plotted against mean effective CO_2 exposure calculated from ^{14}C content as described in the text.

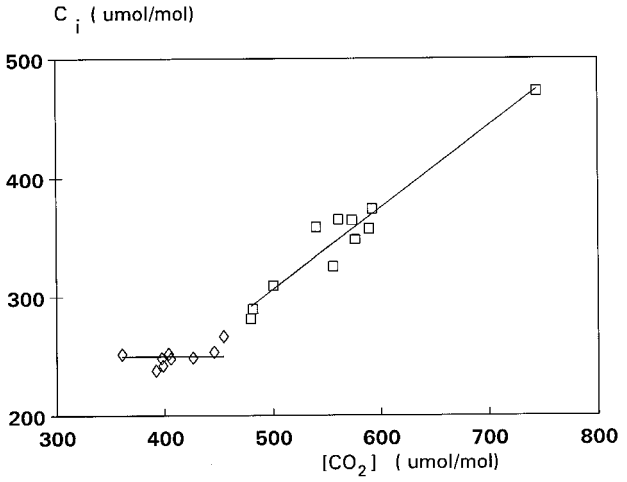

Figure 3. Mean effective intercellular CO_2 concentration (c_i), calculated by solving Equation 4, is plotted against mean effective CO_2 exposure, calculated by the indirect isotopic method described in the text. Data points for plants in Subarea 1 ◇; data points for plants in Subarea 2 □.

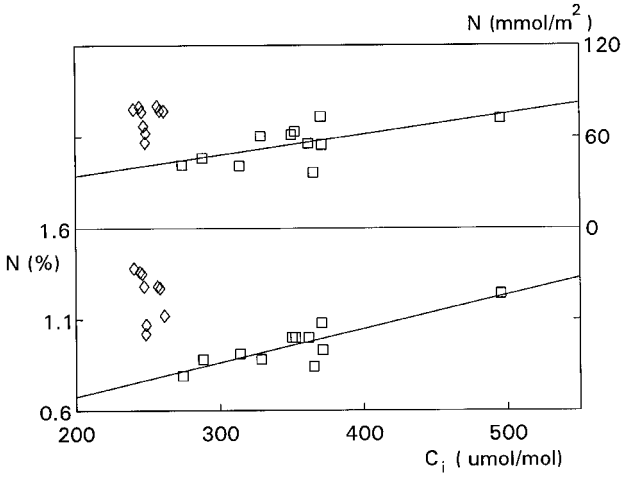

Figure 4. Leaf nitrogen (N) expressed on a leaf mass (%) and leaf area (mmol m^{-2}) basis plotted against calculated mean effective intercellular CO_2 concentration (c_i). Data points for plants in Subarea 1 ◇; data points for plants in Subarea 2 □.

ductance in response to atmospheric CO_2 enrichment. Because there is no evidence that feedback inhibition of stomatal conductance by c_i is sufficient to keep c_i constant (Morison 1987), the decrease in stomatal conductance must have occurred as a result of changes in stomatal density or morphology. This interpretation is consistent with studies of herbaria specimens that have shown a pattern of reduced stomatal densities since pre-industrial times in response to elevated atmospheric CO_2 (Woodward 1987, Peuelas and Matamala 1990, Paoletti and Gellini 1993).

Leaf nitrogen concentration of plants growing in Subarea 2 was generally low, but within this group of plants, it was positively correlated with both c_i (Figure 4) and soil nitrogen concentration (Figure 5). A multiple regression analysis undertaken to determine whether both soil N and c_i had an effect on leaf N yielded a regression coefficient that was only significantly different from zero for soil N (leaf N (mmol m^{-2}) = 56.6 + 28.5 soil N (%) – 0.045 c_i (μmol mol^{-1}), r^2=0.78), suggesting that limited nitrogen availability had a major effect on leaf N, imposed a major limitation on photosynthetic capacity, and interacted with stomatal conductance in regulating isotopic discrimination of plants along the CO_2 gradient. Thus, when soil nitrogen availability was limiting, the photosynthetic capacity of the leaves was reduced, and both c_i and isotopic discrimination increased in response to reduced carbon uptake by the mesophyll. However, when soil nitrogen availability was high, leaf nitrogen concentration increased even though the plants were exposed to higher CO_2 concentrations (Figure 5). Under these conditions, leaf nitrogen concentration and the photosynthetic capacity of sunlit leaves were similar to those observed in plants growing in Subarea 1. Accordingly, the increase in c_i observed in plants growing in Subarea 2 should have led to a significant increase in carbon uptake rates.

This supports the hypothesis that down-regulation of photosynthesis due to an increase in c_i in response to CO_2 enrichment is unlikely, even in the long term, if the supply of nutrients from the soil is not limiting. Moreover, it suggests that, even in the presence of down-regulation of stomatal conductance, mean effective c_i will rise if the CO_2 concentration is more than double the current ambient concentration.

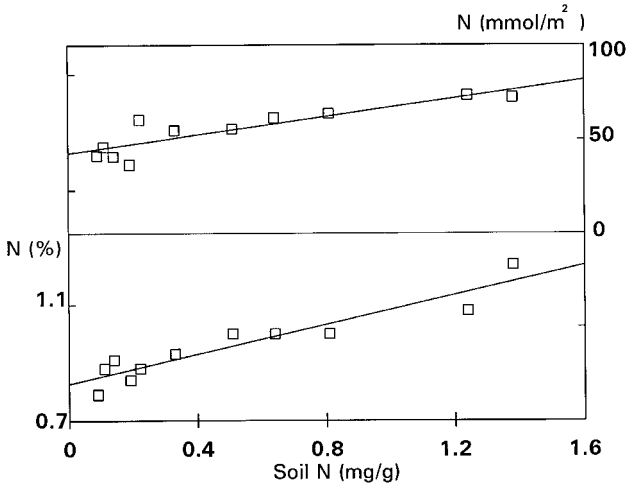

Figure 5. Leaf nitrogen (N) expressed on a leaf mass (%) and leaf area (mmol m^{-2}) basis is plotted versus corresponding soil nitrogen concentration (N_{soil}, mg g^{-1}) for plants growing in Subarea 2. Slopes of regressions N(%) = 0.83 + 0.26 N_{soil}, r^2 = 0.88 and N (mmol m^{-2}) = 42.8 + 24.2N_{soil}, r^2 = 0.78 are all statistically significant for $P < 0.01$.

Conclusions

Despite the lack of appropriate experimental replicates and the occurrence of confounding effects of elevated CO_2 and soil nitrogen, our results support the hypothesis that long-term adjustments occur in plants exposed to elevated atmospheric CO_2.

The occurrence of gas exchange adjustment driven by reductions in stomatal conductance in response to moderate CO_2 enrichment has been postulated in several studies (e.g., Woodward 1987, Penuelas and Matamala 1992, Paoletti and Gellini 1993, Miglietta and Raschi 1993, Woodward 1993, Araus and Buxo 1993). Similarly, the absence of down-regulation of photosynthetic capacity in response to CO_2 enrichment in plants well supplied with nitrogen, as well as the occurrence of a significant increase in c_i in response to a doubling of the ambient CO_2 concentration, have been observed previously in CO_2-enrichment studies made under controlled conditions and in the field (Long and Drake 1992).

Our observations illustrate a scenario between current ambient CO_2 concentrations and atmospheric CO_2 concentrations of 450 μmol mol^{-1}. We conclude that there will be little stimulation of growth in natural vegetation as a result of increasing atmospheric CO_2 because decreased stomatal conductances will tend to keep c_i values close to their current set point. An increase in atmospheric CO_2 concentration will have limited or no effect on vegetation growing under conditions of limited soil nitrogen availability, because the reduction in photorespiration as a result of increased c_i in plants with limited photosynthetic capacity will not be sufficient to increase carbon assimilation. On the other hand, in vegetation well supplied with nitrogen, an increase in atmospheric CO_2 will increase carbon uptake, because c_i will increase and the biochemical capacity for CO_2 fixation will not be limiting. However, it is uncertain if such an increase will result in increased plant growth and structural biomass accumulation or in the accumulation of nonstructural carbohydrates and alterations in the biochemical composition of plant tissues, thereby affecting decomposition rates of organic matter in the soil (Körner 1993). A continuing study of vegetation that has been exposed for generations to elevated CO_2 concentrations in the proximity of CO_2 springs has the potential to provide further information.

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