

Response of Basil
(*Ocimum basilicum*)
to
Increased CO₂ Levels

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Abstract

Atmospheric CO₂ is expected to rise dramatically over the next one hundred years, with possible implications for changes in floral phenology and metabolic processes. The relationships between elevations in Carbon Dioxide and plant responses are varied and depend largely on species and habitat optimization. We grew *Ocimum basilicum* at CO₂ environments of 600ppm and 1500ppm in both nutrient enriched soil and nutrient poor soil. Above ground wet and dry biomass was quantified and shown to be significantly higher in plants treated with nutrients and in plants grown at 1500ppm CO₂ when compared to plants grown without nutrients and at 600ppm CO₂, respectively. The C/N ratio was analyzed and found to be higher in plants grown in nutrient poor soil and in plants grown in 1500ppm CO₂. We conclude that nutrients may be a more important determining factor to both biomass accumulation and C/N ratio in plant tissues than ambient CO₂ levels.

Introduction

Global climate models have projected that atmospheric CO₂ will double over the course of the next century. The increase in Carbon Dioxide and the potential climatic changes that could follow have serious implications for the future of Earth's ecosystems. It is well documented, however, that the response of plants to increases in atmospheric CO₂ can vary according to differences in plant species, genotype, and habitat (irradiance, temperature, moisture, and nutrient availability), (Pushnik et al. 1995).

The effect of elevated Carbon Dioxide levels on plant structure and properties is well documented. A study conducted by Pozo et al. in 2005 on the response of stomatal conductance and photosynthesis to wheat grown in 757 μmol⁻¹ showed that plants grown in elevated Carbon Dioxide had a higher rate of photosynthesis and stomatal conductance than plants grown in ambient levels of CO₂ (Pozo et al. 2005). Murray et al. showed that elevating CO₂ by 350 μmol⁻¹ can lead to changes in the growing season and bud set date of *Sitka* spruce trees (Murray et al. 1994). It is also acknowledged by Gifford et al. in *Plant and Soil* that elevated CO₂ leads to changes in the C/N ratio of plant tissues, and changes in C/N exchange between the plants and the soil (Gifford et al. 2000).

The species grown in this experiment was sweet basil, *Ocimum basilicum* (Lamiaceae). This low-growing dicotyledon photosynthesizes with the C₃ system. Although some basil species are perennial, *O. basilicum* is an annual herb that does not store a large percentage of its photosynthate in below-ground biomass (Duke 1991).

Sweet basil has been of cultural importance to humans for millennia. Known to the ancient Egyptians as a medicinal herb, it now brings up to \$500,000/hectare, making

this a minor but significant crop. Dietary analysis of the leaf reveals that 100g of leaf on average contains 43 calories, 86.5g H₂O, 7.0g carbohydrate, 3.3g protein, 2.0g fiber, 2.0g ash, 1.2g fat, 320mg Ca, 38mg P, 4.8mg Fe, 12mg Na, 429mg K, and a number of additional vitamins. *O. basilicum* also produces a wide array of defensive compounds in its essential oil (which is in concentrations of about 0.15%). Some of these are flavor compounds used for culinary, medicinal, and aromatic applications. Basil has also been used as traditional medicine for such diverse complaints as alcoholism, deafness, boredom, flu, gonorrhea, tumors, worms, and toothache (Duke 1991). The compounds produced include juvocimene 1 and juvocimene 2 (known hormonal toxins for juvenile insects), eucalyptol, estragol, 1,8-cineol, eugenol, borneol, ocimene, geraniol, anethole, 10-cardinols, beta-carophyllene, alpha-terpinole, camphor, 3-octanone, methyl eugenol, safrol, sesquithujene, 1-epibicyclosequiphellandrine, linalool, and methyl chavicol. Safrol and estragole (methyl chavicol) are carcinogens, found in oils of some sweet basil. The leaf juice is reported to be a slight narcotic. The compounds produced by basil give it antibiotic (active against gram-positive and mycobacteria), insecticidal, and other properties (Duke 1991). Creating such compounds may require special photosynthate requirements that make the biomass allocation of basil unique among other food plants.

Methods

In the study, four experimental groups of basil were grown from seed for 30 days in March and April 2008 in indoor growth chambers. The groups were: A (54 pots), B (36 pots), C (54 pots), and D (36 pots). Groups A and B were grown at CO₂ concentration 600ppm in one chamber, and groups C and D were grown at CO₂ concentration 1500ppm in a second chamber. Additionally, nutrients (Nitrogen, Phosphorous, and Potassium)

were applied to the soil of groups B and D. The chambers digitally monitored CO₂ levels, and automatically maintained consistent humidity, light, and temperature. All pots were watered as needed up to once per day, according to soil moisture as determined by feel. Because the soil in some groups dried faster than in others, the total amount of water provided differed between samples.

The seeds (Ferry-Morse Smart Start Sweet Basil) used were grown in coconut fiber (Burpee Seed Starting Greenhouse Kit--Derived from coconut fiber) uniform soil pellets in pots measuring approximately 5 by 5 cm wide and 7 cm deep. Approximately 7-10 seeds were planted in each pot. For the nutrient-added pots, a commercial NPK plant food Osmocote Smart-Release Plant Food: total Nitrogen 19% of which 10% was ammoniacal nitrogen and 9.0% was nitrate nitrogen, available phosphate (P₂O₅) 6% Soluble potash (K₂O) 12%, was used in the form of time-release pellets, applied with approximately 7-8 pellets per pot. Seeds began sprouting within 2 or 3 days for all groups. At day 21 the trays were thinned to two plants per pot, except for two pots which had only one seedling at day 21. At day 30, the groups were photographed and harvested. The seedling/s from each pot were cut at the soil line and weighed for total above-ground wet biomass per pot, then stored in paper towels and dried in an oven until the mass no longer decreased over time. The dried seedlings were then weighed again for total above-ground dry biomass per pot (i.e., for two seedlings except for two pots). In addition, 10 pots from each experimental group were selected (in an even distribution across tray locations), and biomass from these pots was reserved for elemental analysis. A small amount of leaf tissue was weighed and wrapped in tin before being placed in the analyzer. The analyzer was calibrated with standards throughout the samples. The run

resulted in a read-out with percent Nitrogen and percent Carbon by mass as well as the molar Carbon/Nitrogen ratio in each sample.

Hypotheses

Based on a review of the existing literature, several hypotheses were proposed regarding expected changes in plant growth as a result of increased CO₂ levels or increased or decreased nutrient levels in basil plants. The two variables investigated were increased CO₂ (600ppm and 1500ppm) and nutrient levels (no added nutrients compared to plants receiving 7-8 pellets of standard NPK plant fertilizer).

The first hypothesis was that plants receiving nutrients would have greater above ground biomass than plants not receiving any nutrients. Since plants require many other nutrients to grow, without additional nutrients they will not be able to effectively grow and develop. Since the seedlings will be planted in peat pellets made of coconut fibers, with no nutrients added to the soil, the plants will grow better with the addition of NPK fertilizer, regardless of CO₂ levels.

In looking at the difference in growth between the plants receiving higher levels of CO₂ (1500ppm) versus lower levels (600ppm), the plants receiving the higher level of CO₂ will have greater biomass than those plants receiving lower levels (600ppm) of CO₂. Since CO₂ is directly used by plants in the process of photosynthesis, increasing CO₂ levels should increase plant growth, but by how much? Kimball's analysis of 430 previous experiments of plants in increased CO₂ environments suggested that a doubling of CO₂ will lead to an increase in plant growth by approximately 33% (Kimball, 1983). However, more recent experiments by Stephan Long et al. suggest that this analysis, which examined primarily experiments in which plants were grown in growth chambers of

greenhouses, suggests levels much higher than occur when plants are grown in open plants and subjected to increased CO₂ levels, suggesting the increase in plant growth in real conditions may be much lower than in controlled growth chambers (Long et al. 2006). However, since this experiment utilizes growth chambers and the higher levels of CO₂ are more than double the lower CO₂ values, one would expect this experiment to yield higher growth levels at higher levels of CO₂. In terms of extrapolating this data as a possible model of what might occur if CO₂ levels rose, this limitation should be kept in mind.

The reason for the increase in CO₂ in C3 plants is because of the plants' use of RuBisCO for photosynthetic processes, which is in contact with the external air via stomata in the leaf surface. At current levels of CO₂ the RuBisCO is not saturated with CO₂. When the levels of ambient CO₂ increase, however, the CO₂ prevents the competing oxygenation reaction from occurring and thus increases the rate of photosynthesis (Long et al. 2006). The increased rate of photosynthesis and down-regulation of the process of oxygenation will allow the plants to increase their biomass by incorporating more carbon into their structure.

In examining both parameters together (nutrients and CO₂ levels), it is hypothesized that the plants receiving high levels of CO₂ and increased nutrients will have greater above-ground biomass than those receiving increased nutrient levels but at lower levels of CO₂. Since other nutrients, including Nitrogen, Phosphorus, Sodium, Calcium, Magnesium, Iron, Zinc, and Copper are all necessary for plant growth, it is likely that since both nutrient levels and high CO₂ levels contribute to increased plant growth that both factors together would lead to the greatest growth (Loladeze, 2002).

Furthermore, if a plant does not have adequate amounts of nutrients, it will not be able to effectively utilize the increased CO₂ to increase its rate of photosynthesis. Without the nutrients necessary for growth process to occur, additional CO₂ will fail to produce significant increases in above ground biomass.

In examining the C/N ratio, it is expected that the C/N ratio will be larger for plants in an increased CO₂ atmosphere than plants in a lower CO₂ atmosphere because increased atmospheric CO₂ leads to the plant absorbing more Carbon relative to the levels of Nitrogen it is taking up (Kuehny). Another reason for a decrease in Nitrogen uptake is increased growth, since Nitrogen uptake is dependent on a plant's developmental stage. When the plant grows very quickly but does not actually develop any faster, excess growth is due to increased Carbon uptake, leading to relatively low levels of Nitrogen (Greenwood et al., 1990 and Coleman et al, 1993). The effects of the decreased relative Nitrogen levels can have implications for human nutrition as well as for the ability of the plant to propagate and reproduce. Nitrogen is needed to create proteins in plants, and if levels of Nitrogen decrease, this will impact the ability of the plant to produce proteins, with implications for both human nutrition and the plant's ability to create seed (Tauber et al. 2008).

Results

The results of the experiment consisted of several forms of data that we had to make sense of. Included in this, we had dry and wet weights for each sample, in addition to the data garnered from the C:N ratio analysis.

We first weighed the above-ground biomass of the basil as we cut it, thus giving us the “wet” weight (Fig. 1).

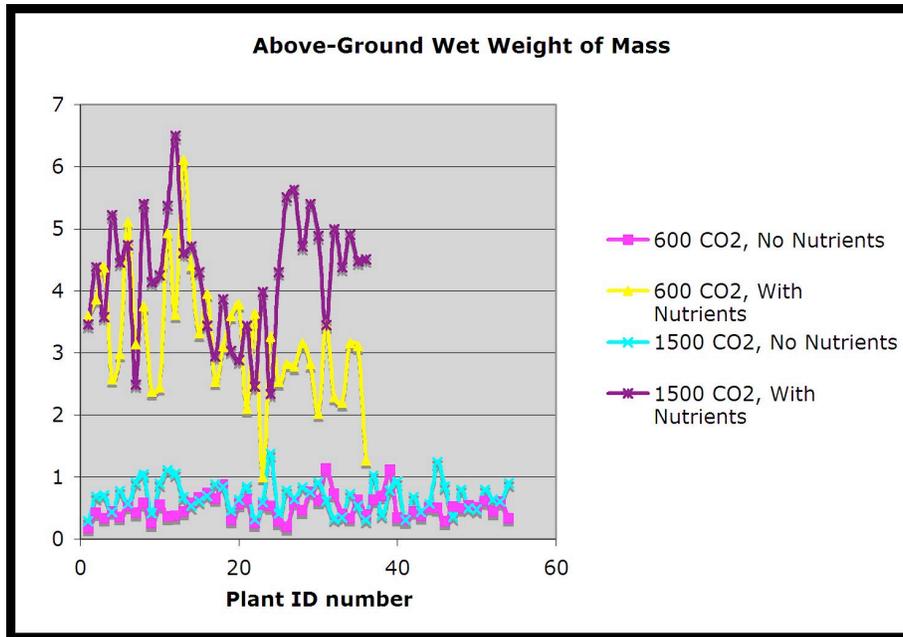


Figure 1: Wet Weights of Groups A, B, C, D

Then, we placed these samples in an oven on low heat to remove all of the water in the tissue. Again, we carefully weighed these samples and obtained the “dry” weights for the above ground biomass (Fig. 2).

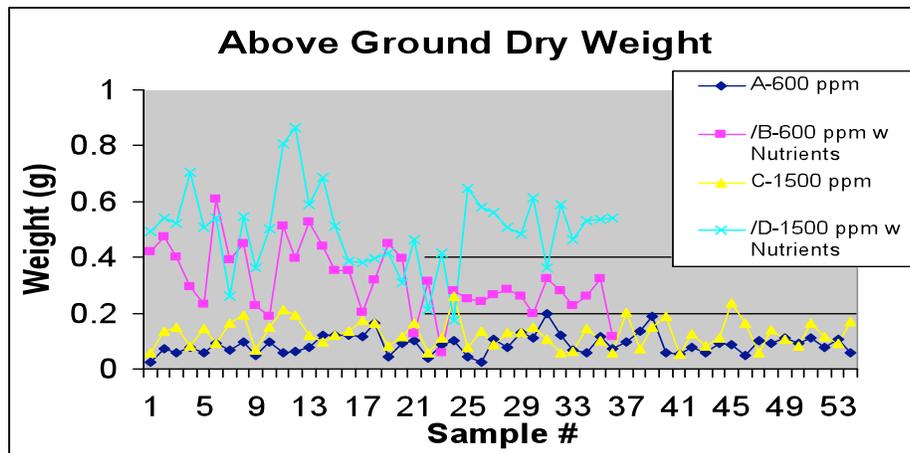


Figure 2: Dry Weight of Groups A, B, C, D

As we expected, and was visually apparent during the experiment, nutrient-enriched Groups B and D (at 600 and 1500ppm CO₂, respectively) weighed significantly more than Groups A and C, which lacked added nutrients (See Figs. 3-7).



Figure 3: Group A (600 ppm)



Figure 4: Group B (600 ppm, w/ Nutrients)



Figure 5: Group C (1500 ppm)



Figure 6: Group D (1500 ppm, w/ Nutrients)

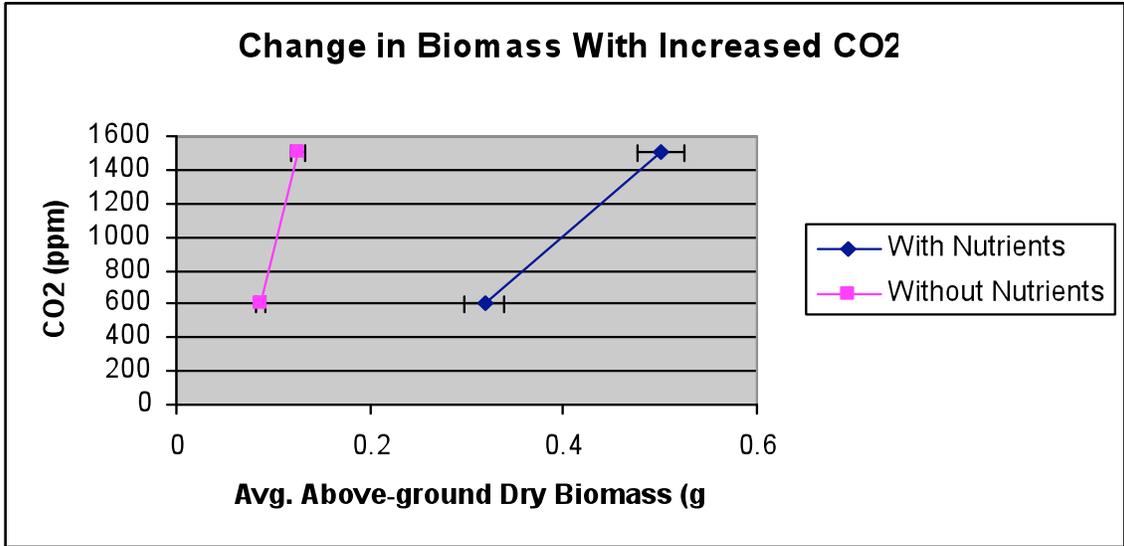


Figure 7: Average above-ground dry biomass for Groups A, B, C, D at 600ppm and 1500ppm CO₂

	A	B	C	D
Average Dry Weight	.088 g	.318 g	.126 g	.500 g

Table A: Average Dry Weight; Groups A, B, C, D

Table A shows the average dry weights for the samples in each of the four groups. With this data, we conducted a T-test that tested the null hypotheses $H_0: \text{Mean A} = \text{Mean C}$, and $H_0: \text{Mean B} = \text{Mean D}$. Both of these hypotheses were rejected through the T-test, which proved that there was a statistically significant difference between groups that had the same treatment of nutrients. Thus, this test supported our idea that CO_2 increase resulted in an increase in the above ground biomass.

An interesting comparison was made between the wet and dry weights for each group. By subtracting the dry weights from the original wet weights, we could calculate the weight of the water lost (Fig 8). Table B also shows that indeed the plants with added nutrients had more total water weight lost than those without. In addition, the elevated levels of CO_2 also increased the total water weight of the above-ground biomass. The increased water weight for groups with nutrients and higher levels of CO_2 indicates that more photosynthetic activity was occurring for the groups that had fewer limiting factors (i.e. nutrients, in particular Nitrogen, and availability of CO_2). A possible error may have occurred in potentially making water a limiting factor because we gave the plants an unregulated amount based on what was deemed necessary, and they were not watered equally, meaning some plants in the trays were not watered the same amount as others receiving the same treatments.

Ten samples were selected at random from each of the four groups for C:N ratio analysis. The results showed that the groups lacking in nutrients (A and C) had a higher

C:N ratio. This was expected because these groups were deficient in Nitrogen, meaning that their relative concentration of Carbon would be higher than Groups B and D, which had Nitrogen added.

We then performed an ANOVA test on the results of the C:N ratio analysis, controlling for nutrients. The ANOVA produced high 'F' values, which indicated there was a statistically significant variation between Groups A and C (no nutrients) in addition to between Groups B and D (with nutrients). This result, like those of the 'T'-test, indicated that one of our treatments was causing the variation. Since we had controlled for nutrients added, it was apparent that CO₂ was causing a statistically significant difference between the groups.

C:N Ratio Results (molar):

Group:	A	B	C	D
Average C:N Ratio	39.42	8.31	48.64	12.65

Discussion

As seen in our results section, basil grown with nutrients had more wet biomass in both 600ppm and 1500ppm CO₂ than the basil grown without nutrients. Elevated CO₂ is known to increase the biomass of C3 plants, and the work of Jablonski et al. (2002) has shown that agricultural species are more responsive than wild species. Their research indicates that humans may have unwittingly been selecting for plants that have increased production rates at higher CO₂ levels for years, as domestic plants are bred to increase productivity. As CO₂ levels increased over time, the plants that were most productive

under those conditions were selected for propagation. Elevated CO₂ was also shown by Jablonski et al. (2002) to increase reproductive effort, but not necessarily reproductive success—additional nutrients, particularly nitrogen, were found to be necessary for seed success.

The dry biomass of our basil grown with nutrients was also higher than the dry biomass of the basil grown without nutrients. The highest average dry biomass was found in the with-nutrient 1500ppm CO₂ basil. Lack of nutrients in soil is a commonly known limiting factor, as well as water availability. Here again, nitrogen plays a major role in the net product of biomass. Nitrogen is necessary for the formation of Rubisco, which has been linked to leaf nitrogen concentrations (Newbery et al., 1995). Our samples that did not receive additional nutrients were clearly limited, while those that had received nutrients showed a marked difference in mass.

The dry weights of the biomass in our experiment reflect the fluctuations in wet weight of biomass between individual plants. The percentage of water lost varied between the groups, with the highest average percentage lost in the added-nutrients, 600ppm CO₂ group. This is puzzling, as the rest of our data reflects an upward trend in water loss (no nutrients 600ppm—76.8%, no-nutrients 1500ppm—81.7%, and with nutrients 1500ppm—88.31%). After reviewing the literature on the subject, we now feel that a possible reason for this is increased water use efficiency of the tray that received the nutrients and 600ppm CO₂.

The more photosynthesis there is occurring, the more water is necessary to maintain the rate (as photosynthesis requires the H⁺ for energy). This is likely why there

were higher water percentages as we went to higher levels of CO₂, particularly where nutrients were not a limiting factor.

Plants that are grown in elevated CO₂ can increase their water use efficiency (WUE)—particularly plants that are not receiving enough water (Ferris and Taylor, 1995). It is possible that the with-nutrients 600ppm tray in our experiment was not receiving enough water (as we did not measure how much water we gave each tray, gauging water needs by soil moisture rather than plant size) and that tray may have developed increased water efficiency. The plant responses gathered by Ferris and Taylor (1995) led them to believe that the future survival of plants in higher CO₂ climates (with the water loss that is associated with higher CO₂) will have a lot to do with a plant's ability both to grow and to maintain its stores of water simultaneously.

Our C\N (Carbon\Nitrogen) averages were higher for the no-nutrient groups (600ppm CO₂—39.42; 1500ppm CO₂, 48.64) than for the with-nutrients groups (600ppm CO₂—8.31; 1500ppm CO₂—12.65), leading us to assume that Nitrogen was a limiting factor in higher CO₂ groups, and that the increased CO₂ availability led to an increased C\N disparity between the groups with nutrients versus the groups without nutrients.

The C\N ratio for the no-nutrients 600ppm CO₂ group was lower than that of the no-nutrients 1500ppm CO₂ group, which we believe stems from an increased demand for Nitrogen in the higher CO₂ groups, which was not being met in the no-nutrients 1500ppm CO₂ group. A similar difference was seen between the with-nutrients groups at 600ppm and 1500ppm CO₂—there was an increase in the C\N ratio at the 1500ppm group, implying that the more than doubled CO₂ levels led to increased Nitrogen demands.

As mentioned earlier, Nitrogen is necessary for forming Rubisco (Newberry et al., 1995), which is required for the photosynthetic process, but another important role of Nitrogen is in seed nutrition and quality (Jablonski et al., 2002). If more Nitrogen is being required simply for photosynthesis in higher CO₂ levels, that may affect the amount of Nitrogen that is used for reproduction, which could negatively affect a species' ability to germinate and compete with other species (Jablonski et al., 2002).

We noted the presence of withering (group D) and darkened spots (group C) on leaves on some plants in some of the pots. That the ailments were apparent only in the CO₂ 1500ppm groups suggests that the increased CO₂ levels or a confounding variable may be causing some increase in susceptibility to disease. One inadvertent effect of watering inconsistencies may have been the dark spots on some leaves. This could be a condition, known in basil cultivars, called "black spot", often caused by *Colletotrichum* sp. It is known to thrive in conditions of high relative humidity, high temp, high Nitrogen, long periods of wetness, and, especially, in dense growth (Garibaldi et al., 1997). Although these factors were kept consistent between groups (except Nitrogen and, to some extent, water and density), there may have been differences due to error. The plants from different groups may have been more or less susceptible to the disease. The leaf withering could be a condition caused by water or nutrient imbalance or another factor. The conditions were only casually observed in this study; further research is needed to clarify the relationship.

Conclusion

In conclusion, nutrients are an important limiting factor for basil growth in both 600ppm and 1500ppm Carbon Dioxide levels. Nutrient enriched basil also accumulated more above-ground biomass than basil grown in soil lacking nutrients at both 600ppm and 1500ppm CO₂. It was shown that the above-ground wet and dry biomass in basil grown in 1500ppm CO₂ environment was statistically greater than basil grown in 600ppm environment. Finally, it was shown that nutrient enriched basil had a higher C/N ratio than basil grown in soil without nutrients. Also, basil grown in the 1500ppm CO₂ environment had a higher C/N ratio than basil grown in a 600ppm environment.

Future Implications

The changes that occur in plant phenology and chemical composition could have profound implications for the future of earth's ecosystems. It is possible that changes in the C/N ratio of plant tissues as a result of changes in Carbon Dioxide could alter the interactions between plant litter and soil. Although Ceulemans et al. have shown the short term C/N ratios in the soil are not significantly changed by increasing atmospheric CO₂, accumulation of plant litter of altered Carbon and Nitrogen content over extended periods of time could have long term ramifications for soil biota, organic matter, and Carbon and Nitrogen cycling within the soil (Ceulemans et al., 1995). The augmentation of photosynthetic rate and the subsequent increase in biomass as a result of elevated CO₂ could also contribute to changes in the magnitude of the atmospheric Carbon sink represented by plant biota. As more Carbon is absorbed by plants and processed during photosynthesis, the already cryptic processes of atmosphere/biosphere relationships could

change in unpredictable ways (Poza 2005). Changes in the chemical composition of plants as a result of elevated CO₂ could also potentially lead to changes in nutritional value. Decreases in protein content similar to those observed in studies conducted by Taub et al. in 2008 might require herbivores and omnivores to ingest a greater amount of food in order to meet their nutritional needs (Taub et al., 2008). The impact of such a result could have potentially dubious consequences for organisms competing for the same resources. It is very difficult to determine whether or not the potential increase in plant biomass could offset the increase in competition for food in plant eating organisms because such changes are so closely related to certain species and habitat.

The future of population size and diversity of plant species in an elevated CO₂ atmosphere is expected to vary significantly based upon differences in plant species and habitat optimization. Studies conducted by Jorgen and Jones in 1998 have documented that certain C3 plants species have shown higher levels of biological success than C4 plants in environments with elevated CO₂ (Jorgen and Jones 1998). As different species of plants respond in different ways, certain species may be better suited for competition than others when grown in a higher CO₂ environment. A plant that shows a greater response to elevated Carbon Dioxide in terms of biomass accumulation may be better suited to compete for light, space, and nutrients than a plant with a less pronounced response. Changes in the length of growing seasons could also select for certain populations of plant species. A study conducted by Murray et al. in 1998 showed that because early budding of *Sitka Spruce* is more directly stimulated by increases in CO₂ than temperature, increased atmospheric CO₂ levels could potentially lead to widespread frost damage of buds during colder months of the year (Murray et al. 1998). The

increases in Carbon Dioxide similar to those that are expected to occur over the next century will select for certain plant species in ways that are unknown and very difficult to predict.

Future Research

Future research should be conducted to show the impact of current CO₂ levels (450ppm) on aboveground biomass accumulation and C:N content in basil to provide a comparison among current CO₂ levels and two increases in CO₂ (600ppm and 1500ppm). Also, an analysis of below ground biomass could be conducted using equipment that removes soil particles from below ground biomass while preserving root structure. F2 and F3 generations could also be examined to determine the phenotypic response of basil plants that exhibited biological success in elevated CO₂ environments and determine to what degree changes in fitness as a result of elevated CO₂ is a heritable trait. The Nutritional value of basil grown atmospheres of different Carbon Dioxide content could be compared in order to establish a proxy for protein or carbohydrate content of plant tissues and CO₂ levels. The amount of water could also be carefully monitored and controlled in order to eliminate the possible human error that may have occurred in this experiment when applying water in a more haphazard manner. In order to prevent disease that was observed in some basil plants, sterile air could be used and basil plants could be isolated from other plant species. Finally, more basil samples could be planted in open containers to establish how basil responds to elevated levels of CO₂ when grown in a medium other than a closed container.

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