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EFFECTS OF ELEVATED CO$_2$ ON *BROMUS RUBENS*

AND NUTRITIONAL CONSEQUENCES

FOR THE DESERT TORTOISE

by

Kristen Marie Murphy

Bachelor of Science
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1993

A thesis submitted in partial fulfillment
of the requirements for a

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ABSTRACT

Effects Of Elevated CO₂ On *Bromus rubens* And Nutritional Consequences For The Desert Tortoise

by

Kristen Marie Murphy

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The purpose of this research was to determine the effect of elevated atmospheric carbon dioxide conditions on forage quality for the desert tortoise (*Gopherus agassizii*), using a common diet component, *Bromus rubens*. Nitrogen balance was monitored for tortoises fed pelleted diets reflecting the nitrogen levels of *Bromus* plants grown under elevated and ambient CO₂ conditions, with varying potassium levels (3.5% and 1.4% DWB). Nitrogen content in the diet approached a positive correlation with total diet intake (p = 0.11), and was positively correlated with nitrogen loss at α = 0.10 (p = 0.08) and yet remained positively correlated with nitrogen retention (p = 0.07) in high potassium diets. When consuming lower potassium diets, no significant responses were seen with respect to nitrogen content of diets, although nitrogen retention approached significance (p = 0.11). These results, suggest that under elevated CO₂-conditions, if dietary nitrogen levels decrease, nitrogen retention abilities are likely to be compromised, especially when plants simultaneously contain large amounts of potassium.
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CHAPTER 1

INTRODUCTION

Geological records of climate, physical structure, atmospheric composition, and biotic components, all reveal that change is an inherent trait of life on Earth. The extinction of one species or changes in the distribution of others are common and natural phenomena. We have yet to find, however, any historic records or evidence of change as drastic as that of human influence on the Earth’s atmosphere. Vitousek et al. (1997) state that as the human population expanded and technology developed, the scope of our influence changed drastically: where once we perceived our impacts as limited to influencing our environments locally, we now have well documented evidence of our global impacts.

Perhaps the best-documented global response to human influence is the elevation of atmospheric carbon dioxide levels. Since the late 1950’s we have direct measurements of increasing atmospheric carbon dioxide levels from the atmospheric station on the top of Mauna Loa volcano in Hawaii, U.S.A. (Keeling et al., 1989). Evidence from air bubbles trapped in glacial ice cores taken from Greenland extend our historical records over two glacial cycles (over 100,000 years) and allow us to compare natural fluctuations with current conditions (Taylor, 1999). These records show that atmospheric CO₂ levels have increased 30% since the start of the industrial revolution (Griffin and Seeman, 1996), which is roughly equivalent to 5-10 times faster than changes documented in the ice cores over the last two glacial cycles (Vitousek, 1994). The primary contribution to this
increase can be linked directly to the combustion of fossil fuels by measuring the relative proportion of $^{14}$C to the stable isotope $^{13}$C (Stuiver, 1978, Keeling et al. 1989; cited in Vitousek, 1994). This is due to the Suess effect, which states that 5,730-year half-life of $^{14}$C means that the combustion of fossil fuels adds $^{14}$C-depleted $^{\text{CO}_2}$ to the atmospheres. By measuring relative proportions of the isotopes, you can predict the contribution of fossil fuel combustion to the increased atmospheric concentrations of $^{\text{CO}_2}$. The impacts of this change on plant communities vary across species and functional groups. Generally, by increasing the abundance of a potentially limiting resource for many plant species, the elevation of atmospheric $^{\text{CO}_2}$ does invoke a plant response. The extent of this response depends on how strongly the plant was limited by atmospheric $^{\text{CO}_2}$ content. The most common responses include increased photosynthesis, water-use efficiency and biomass of plants.

The greatest effect of $^{\text{CO}_2}$ on plants is seen among plants utilizing the $C_3$ photosynthetic pathway. The reasoning behind this is due to physiological and biochemical differences in $C_3$ vs. $C_4$ plants. Although both plant groups use the same carboxylating enzyme, ribulose biphosphate carboxylase/oxygenase (Rubisco), for carbon fixation, the location of this process differs significantly. To understand the mechanism driving the different responses, it is first necessary to understand the two major processes catalyzed by the enzyme Rubisco. Rubisco binds with both oxygen and $^{\text{CO}_2}$, but has a stronger affinity for $^{\text{CO}_2}$. If Rubisco binds with $^{\text{O}_2}$, the plant will respire; if it binds with $^{\text{CO}_2}$, photosynthesis will occur. Under natural conditions, our atmosphere contains a much higher concentration of $^{\text{O}_2}$ than $^{\text{CO}_2}$ (21% vs. 0.03%) and although Rubisco has a greater affinity for $^{\text{CO}_2}$, the frequency of $^{\text{O}_2}$ binding with Rubisco is
greater because it is more readily available. Under an elevated atmospheric CO₂ state, the availability of CO₂ increases. For C₃ plants, this means that the frequency that Rubisco binds with CO₂ increases, causing an increase in the photosynthetic rate. C₄ plants, on the other hand, have specialized bundle sheath cells that compartmentalize the Rubisco/CO₂ binding process. C₄ plants initially fix CO₂ using an enzyme called PEP carboxylase that creates a four-carbon organic acid that is then transported to the bundle sheath, allowing the CO₂ levels to be maintained at a higher concentration than is currently found in the atmosphere. Therefore, within bundle sheath cells, Rubisco operates near its CO₂ saturation point, regardless of the atmospheric concentration of CO₂. C₃ plants, on the other hand, will have an increased opportunity for photosynthesis at elevated levels of atmospheric CO₂ and therefore under these conditions they exhibit photosynthetic stimulation. This photosynthetic stimulation enables plants to allocate more energy to growth and reproduction.

Additionally, stomatal conductance (the relative opening of stomata for CO₂ and water vapor exchange) can be reduced up to 40% with a doubling of CO₂ concentrations, which in turn would decrease plant water loss (Woodward et al., 1991). A simultaneous increase in CO₂ assimilation and decrease in transpiration results in a potentially large increase in plant water-use efficiency (WUE; ratio of CO₂ assimilation to water loss). Because of this dramatically enhanced plant WUE, it has been hypothesized that plant growth may be more enhanced at elevated CO₂ in water-limited environments, such as deserts, than in any other biome-type (Melillo et al., 1993; Smith et al., 1997).
Elevated Atmospheric Carbon Dioxide

and Herbivorous Insects

The ecological principle that everything is interconnected (Temple, 1996) suggests that the previously discussed changes for plants will not be limited to the Earth's flora. Studies of plants grown under elevated CO$_2$ conditions also reveal changes in foliar water content (Lincoln et al., 1986; Bezemer and Jones, 1998), allelochemical concentrations (Lindroth et al., 1993), and increased leaf toughness and reductions in nitrogen content (Fajer, 1989), all of which are qualities pertinent to herbivores. Additionally, growth stimulation caused by an increase in CO$_2$ availability in turn increases the carbon-to-nitrogen (C:N) ratio on a whole plant basis, reducing leaf nitrogen concentration on a mass basis (Hughes and Bazzaz, 1997). The increase in carbon is due to the allocation of the additional fixed carbon to pools of storage carbohydrates (Cave et al., 1981; Johnson and Lincoln, 1990). This effect, sometimes referred to as a "nitrogen dilution effect", may in turn affect organisms that feed on those plants by reducing the nutritional quality of their food source (Wong, 1979; Lincoln et al., 1984).

The bulk of research designed to evaluate the plant-herbivore interactions under an elevated CO$_2$ atmosphere has focused on insects, and of those studies the majority are limited to a small number of insects associated with agricultural systems (Coviella and Trimble, 1999). Of the species studied, a variety of responses were reported. Many of these studies have shown the reduction in plant quality to cause an increase in consumption levels, reduce growth (Lincoln et al., 1986) and retard development (Fajer et al., 1991) of the insect. Although there seems to be a similar trend among many herbivorous insects, all insects do not follow this pattern. Lindroth et al. (1993) found
that although growth rates for gypsy moth caterpillars increased, tent caterpillars showed no response to consuming elevated CO$_2$-grown plants. In a quantitative review of existing research at the time, Bezemer and Jones (1998) found that responses differed among feeding guilds. Leaf eaters and leaf miners were reported to increase consumption with leaf-eaters showing the greatest response, and both groups showed a decrease in pupal weights. Phloem-feeders and whole-cell feeders, however, tended to show a positive response with increased population sizes and decreased development times. As a result, on a population level, functional groups such as phloem feeders may exhibit an increase in population size, while leaf chewers and/or leaf miners will likely decrease in numbers (Whittaker, 1999). In general, where changes have been reported, insects seem to respond by either reducing their consumption or altering their food preferences (Peters et al., 2000). But caution is needed in interpretation of general trend predictions because the number of studies and range of species considered are far too limited (Diaz et al., 1998) and the results to date show variation between species as well as among individual plant-herbivore systems (Coviella and Trumble, 1999).

Given the variety of responses, it is thought that herbivores that are primarily limited by nitrogen would be expected to show the greatest response to the reduction in leaf tissue quality (Lincoln et al., 1986). Herbivores outside the insect world that are dependent upon levels of nitrogen may also be indirectly impacted by future changes in the atmospheric concentrations of CO$_2$. In order to determine the ultimate effects of elevated CO$_2$, research on plant-herbivore interactions under an elevated CO$_2$ scenario needs to be expanded to include herbivores from a variety of taxa.
The Role of Potassium in the Diet of Desert Tortoises

The desert tortoise (*Gopherus agassizii* [Xerobates; Bour and Dubois, 1984]) is a long-lived herbivore inhabiting the Mojave and Sonoran deserts of the southwestern United States and extending into Mexico. The Mojave population of the desert tortoise was federally listed as threatened in 1990. It is believed the primary causes for population decline include habitat encroachment through urban expansion, increased occurrence of a contagious upper respiratory disease that may be enhanced under conditions of stress, and alteration of food availability through habitat conversion and exotic species invasion (Fish and Wildlife Service, 1994). In accordance with the Endangered Species Act, following the listing of the tortoise a team of researchers was organized and tasked to develop a recovery plan to evaluate threats and develop conservation strategies for population recovery that could ultimately contribute to the delisting the desert tortoise. Research priorities were defined to address existing information gaps and evaluate the implementation of recovery strategies, with nutritional ecology of desert tortoises among those priorities.

Unfortunately, the tortoise’s long lifespan and extreme seasonal and annual variability of rainfall in the Mojave Desert, combined with the short-term funding cycles for research, elucidation of predictive patterns from field studies becomes extremely complex. As a result, while decades of research has been conducted on this species, comparisons between years to understand trends requires extrapolation of results. This is especially true for nutritional research.

Forage studies involving a combination of fecal analysis and bite-count monitoring have shown that the diet of the desert tortoise is composed primarily of a variety of
annual plants (Esque et al., 1990). Due to the extreme variability in quantity and
diversity of plants available yearly (as well as seasonally), the site variability within the
range of the species, and the seasonal variation in the nutritional requirements of tortoises
(Robbins, 1993), diet selection criteria is difficult, at best, to quantify. Tortoises are
consistently reported to sample a large proportion of the forage available in a given year,
but have been shown to invest considerable energy into seeking out specific individuals
(Jennings, 1993; Esque, 1994; Oftedal et al., 2002).

Until recently, evidence for specific criteria explaining diet selection among wild
populations of desert tortoises was largely unknown. Oftedal et al. (1996) proposed that
plant selection is based on a need to offset the high potassium content inherent in desert
tortoise diet with quantities of nitrogen and water. Studies on captive tortoises have
shown that when presented with a choice between diets of fixed nitrogen content, but
varying potassium levels, tortoises do in fact avoid the higher potassium diets (Oftedal,
Unlike herbivorous mammals, the kidneys of reptiles, tortoises included, are incapable of
producing concentrated urine, and therefore, they must use other means to excrete
excessive amounts of potassium. Other desert herbivores rely on salt gland (Nagy, 1972),
which are lacking in desert tortoises. As a result, they excrete excess potassium
occurring naturally in desert annuals by increasing the production of concentrated uric
acid. Uric acid is composed of almost 30% nitrogen on a dry matter basis, and although
water loss is minimized, potassium excretion requires a large nitrogen investment. Under
this theory, in order to maintain the necessary levels of nitrogen required for growth and
reproduction, a tortoise would need to balance the dietary level of potassium and nitrogen
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by selecting forage plants that contain enough excess nitrogen to allow for potassium excretion. As a result, Oftedal (1996) developed a method for calculating what is termed the potassium excretion potential (PEP) to indicate the quality of food for consumption by desert tortoises based on these physiological restrictions.

The PEP index is based on the following assumptions:

1. All water, nitrogen and potassium are absorbed from ingested foods
2. All absorbed water and nitrogen could be used to excrete potassium
3. Urine and urates produced would have the maximal concentrations of potassium (7 g K per kg H₂O and 1 g K per 1.5 g N).

and is derived using the following formula:

\[
\text{PEP (g per kg DM)} = 6.5 \times \text{Water (g/g DM)} + 0.976 \times \text{CP (% DM)} - 10 \times \text{K (% DM)}
\]

Where 6.5 g K/kg water equals the maximum urinary potassium concentration achieved by tortoises, and CP is crude protein (calculated as 6.25 * total nitrogen concentration in the plant). A high positive PEP index means that a plant contains enough nitrogen and water to allow for the excretion of potassium, while a negative value means that more nitrogen and/or water will be lost through the excretion of potassium than is contained in the plant. A positive PEP index is therefore an indication of a plant that is better nutritionally because enough nitrogen can be retained to allow allocation to processes such as growth and reproduction.

Field tests of this theory have recently been conducted on young desert tortoises during an El Niño year when a large variety of annual species were available. Results
from this study showed that not only did tortoises more frequently select plants with higher PEP index, but also selected portions of plants with the highest PEP index (Oftedal et al., 2003).

Tortoise Nutrition Under A Changed CO₂ Environment

In order to avoid retaining potentially toxic levels of potassium or to prevent entering a negative nitrogen balance state, tortoises must depend greatly on the level of nitrogen contained in the plants they consume. Although desert annuals have relatively high concentrations of tissue nitrogen (Smith et al., 1997), altering the supply of nitrogen in the diet of desert tortoises could substantially impact their ability to tolerate the high levels of potassium found naturally in their diets. As a result, elevated CO₂ concentrations combined with the resulting nitrogen dilution effect found in many plants may have important implications on the survivorship of desert tortoises in the future. Information pertaining to how tortoises respond to these future impacts may provide managers with information that is crucial for determining the best preventative measures for avoiding further declines of this threatened reptile.

Objectives and Hypotheses

The purpose of this research was to provide an indication of the potential effects of elevated levels of CO₂ on forage quality for the desert tortoise. This study focused on a widely distributed annual grass and an annual forb for this preliminary investigation. *Bromus rubens*, an introduced annual C₃ grass, has been shown to be a dominant component in the diet of desert tortoises (Esque, 1994) at least under some circumstances. In wet years and/or on disturbed sites, this annual grass can dominate the
total annual biomass in parts of the Mojave Desert, and thus may be the most available source of food for tortoises under some circumstances. Tortoises have also been reported to actively seek out *Camissonia claviformis*, a native C$_3$ winter annual, due to its high nitrogen content (Oftedal et al., 2003). Field studies in the Mojave Desert have shown that the growth of *Bromus rubens* is particularly responsive to elevated CO$_2$ (Smith et al., 2000), and that elevated CO$_2$ causes a significant decrease in tissue N concentration in this grass (Huxman and Smith, 2001).

The focus of this study is two-fold. First we attempted to determine how CO$_2$ enrichment affects the PEP constituents of the exotic annual grass, *Bromus rubens*, and a native annual forb, *Camissonia claviformis*. Second, alfalfa-based pelleted diets were formulated to reflect the nutritional make-up of the glasshouse-grown plants and feeding trails using captive desert tortoises were conducted. Consumption and nitrogen balance were evaluated for each treatment group.

The following hypotheses were tested in regard to the effect of elevated atmospheric CO$_2$ conditions on the nutritive quality of *Bromus* and *Camissonia* for the desert tortoise:

Effect of Elevated CO$_2$ on Glasshouse Grown Plants:

1. Plants grown under elevated CO$_2$ conditions will contain less nitrogen and more carbon than plants grown under ambient CO$_2$ conditions. This will result in elevated CO$_2$-grown plants containing a higher C:N ratio when compared to those grown under ambient conditions.

2. Due to increased water-use efficiency, plants grown under elevated CO$_2$ conditions will have higher water content than ambient-grown plants.
Behavioral Responses to Diets

1. Given a constant potassium concentration, tortoises will compensate for the lower nitrogen availability by increasing consumption, or if the total K:N concentration in the diet is high enough, tortoises will avoid consuming the high K diets.

2. Tortoises will preferentially select the lower K:N diets when presented with both diets during choice trials.

Physiological Responses to Diets

1. Tortoises consuming higher nitrogen diets will have higher nitrogen retention than tortoises consuming low nitrogen diets.

2. Higher nitrogen retention will allow tortoises to allocate more nutrients to growth and will exhibit greater increases in mass and morphological measurements at the end of the trials.
CHAPTER 2

MATERIALS AND METHODS

Plant Propagation and Treatment

Seeds of *Bromus rubens* and *Camissonia claviformis* collected from a variety of field sites within the Mojave Desert, were planted in 8 one-gallon pots for each treatment (n=32 for each species), filled with a sandy loam soil, and planted at a density of roughly 25 seeds per pot to prevent root-binding from overcrowding. Pots were randomly assigned to either ambient (360 μmol mol⁻¹ ±30 ppm) or elevated (700 μmol mol⁻¹ ±10 ppm) carbon dioxide concentrations in separate glasshouse bays but with identical light, temperature and humidity conditions. Within each CO₂ treatment, half the pots were assigned a fertilization level of high or low fertility. Pots were placed randomly on benches in the University of Nevada, Las Vegas glasshouse. Light for each CO₂ treatment room was provided by natural light conditions, with maximum photosynthetic photon flux density (PPFD) of 1600 μmol m⁻² s⁻¹. All treatments received 200 ml of water every 2-3 days. Pots receiving fertilization enhancements were watered with a 1:40 Hoagland’s solution while low fertilization treatments received 1:80 solution concentration. The phenological stage of plants in each of the pots was monitored weekly and plants were harvested just prior to the production of inflorescences. Plant roots were removed, plants were weighed to determine wet weight, and aboveground portions of the
plant were stored in a freezer until mineral analysis was conducted. Because it was found that phenological stages within a pot varied, plants that had reached flowering stage were separated from non-flowering plants but were otherwise treated similarly.

Nutrient Analysis on Plant Material

Plant material was transported in sealed bags packed in dry ice to the Nutrition Lab at the Smithsonian Institution in Washington, DC. Plants were dried at 50°C for 6 days in a forced convection oven and ground to a fine powder using a mortar and pestle. Plants especially difficult to grind were reduced to a fine powder using a Wig-L-Bug mixer for 10 seconds. Using a microbalance, triplicate samples of 7-10 mg of ground plant material were placed in miniature tin foil vials. Vials were then oven dried for 2 days at 50 °C. Using a CHN gas analyzer (Model 2400 Series II; Perkin Elmer Corp., Norwalk CT, USA), carbon, hydrogen, and nitrogen content was measured (Analyzer settings: conditioner, acetanilide: C: 71.02, H: 6.71 and N: 10.36; regulator, He: 140kPA, O₂: 106kPA, N: 415kPA; fill pressure 6.492; combustion temp. 950°C; reduction temp. 640°C). Potassium and calcium content were analyzed using atomic absorption spectroscopy (AAnalyst 800, Perkin Elmer Instruments, Norwalk, CT, USA). Approximately 10 grams of dried plant matter was digested using standard perchloric acid-nitric acid solution. Then, a solution of the digestion, DI water and either a potassium modifier (CsCl 2000ppm) or calcium modifier (LaCl 4500ppm and KCl 2000ppm) was created and read in triplicate by the spectroscope. Potassium was read at a wavelength of 766.5nm and calcium was read at 422.7 nm. Phosphorus content was measured by the Gomorri method (Gomorri, 1942), using a UV spectrophotometer

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Reagents were prepared using a solution of ammonium molybdate, ammonium metavanadate and perchloric acid. Plant samples were read in duplicate at a wavelength of 400 nm with blank readings between samples to prevent cross-sample contamination.

Choice Trial

Unfortunately, the amount of fresh plant material remaining following the plant nutrient analysis was not large enough to conduct a choice trial experiment. As a result, our initial intentions of conducting a choice trial were abandoned. However, in a previous study, Christopher, et al. (1996) conducted a choice trial where juvenile tortoises were offered a choice between similarly formulated potassium diets and varying nitrogen diets and overall, tortoises did select the higher protein diets.

Pelleted Diet Preparation

The diets for the feeding trials were created based on the nutrient analysis of the glasshouse grown plants. Using an alfalfa-soy based pellet, diets were formulated to reflect significant nutrient differences between the treatment groups of the glasshouse grown plants. Diets were designed to be nutritionally complete using a full compliment of vitamins and minerals, with the exception of the nutritional component being tested. Treatment groups for the plant growing conditions consisted of elevated or ambient CO$_2$ levels and low or high nitrogen enhancement. Additionally, because phenological stages varied at time of harvest, plants were also separated by phenological stage. The fresh plant analysis revealed that the only PEP constituent that showed a significant difference
was nitrogen content in *Bromus*. Growing conditions or fertilizer treatments did not have a significant effect on either potassium or water for *Bromus*. Additionally, results from fresh plant analysis for *Camissonia* did not detect any difference in any of the PEP components measured from CO₂ growing conditions. As a result, a total of four (4) treatment groups were created representing *Bromus* plants grown under elevated or ambient CO₂ growth conditions and either flowering or pre-flowering phenological stages. The nitrogen levels were based on the mean nitrogen level in *Bromus* from each treatment group. Given that potassium was similar for all groups, the potassium level for the pellet diets was based on the mean potassium level for all treatment groups (approximately 3.5% K DWB).

The formula was provided to Zeigler Bros. Inc. (Gardner, PA, USA) for the creation of pelleted diets. Diets were tested prior to the commencement of any feeding trials to ensure pelleted diets matched the nutrient quantities requested. Specifics on the diet ingredients and nutrient compositions are provided in Table 1.

### Feeding Trials

The feeding trials consisted of a 3-part procedure that included an acclimation and intake-monitoring period followed by a nitrogen balance study. The acclimation period was conducted for a total of 5-weeks, allowing tortoises to adjust to the new diets. During the first week, diet was introduced using a 50:50 mix of their previous diet and the new study diet. For the remaining four weeks, they were provided only their study diet. Pellet diet was provided on an ad libitum basis and fresh water was provided twice weekly.
Following the completion of the acclimation period, food intake was monitored for each tortoise for a total of 4 weeks. The diet provided was weighed before and after exposure to determine the quantity of intake. The results from this period were used to determine the average ad libitum quantity of food intake expressed on a body weight basis. This amount helped to determine the quantity of food to offer tortoises of similar weights to ensure that consumption was not restricted. At no pint during the study did tortoises consume all diet that was provided to them.

The nitrogen balance trial required the collection and separation of all excreta (urine, urates, and feces). At the beginning and end of the nitrogen balance study, cages were cleaned with a 1 g/L solution of lithium carbonate to be sure there was no residual excreta in the cages. The cages were then washed with a 3N HCL solution to remove any minerals. This cleaning procedure was repeated at the end of each trial. All rinses were collected, frozen and stored for assay. Similarly, samples of all excreta were collected in a sealed container, weighed, frozen and stored for assay immediately following collection. The analysis process for all excreta and rinses is detailed in the following section.

Prior to and at the conclusion of all trials, weight and morphometric measurements were recorded for all tortoises. Tortoise body mass was measured using an Ohaus electronic balance. Midline carapace length (MCL), plastron length (PL), shell width (measured between the 7th and 8th marginal scutes) and height were measured with slide calipers (Haglof Inc. Madison, MS, USA).

The initial plant analysis revealed potassium levels quite a bit higher than that typically found in field-collected plants. As a result, consumption during this trial was
extremely low. However, tortoises continued to produce excreta so we continued with
the nitrogen balance trial and extended the trial by adding a second three-week balance
period to allow data pooling if necessary. While we were conducting this trial, we re­
formulated a pelleted diet using the same nutrient composition as described previously
with the exception of potassium which was based on potassium data from previously
analyzed, field-collected Bromus plants (approximately 1.4 % K DWB). Using the new
diets, we ran an additional balance trial. The same stages were followed: an acclimation
and intake monitoring period followed by a nitrogen balance study. This trial was
conducted similar to the previously described trial with acclimation and intake lasting for
a total of four weeks, and was followed by a single three-week balance trial.

The first balance trial using potassium values obtained from our glasshouse grown
plants will be referred to as KM1 Part A (first 3 week-balance trial) and Part B (second 3-
week balance trial) or, collectively, as the High Potassium Feeding Trial. The second
balance trial using potassium values obtained from field-collected plants is referred to as
KM2 or the Natural Potassium Feeding Trial.

Preparation and Analysis of Excreta

All excreta was transported on dry ice to the Nutrition Laboratory of the Smithsonian
Institution, National Zoological Park, in Washington D.C. Fecal and urate samples were
dried at 50 °C for 7 days in a forced convection oven. Dried samples were removed from
the oven, and re-weighed to determine dry matter content. As some samples of feces
inevitably were contaminated by fractional pieces of urates or food particles, samples
were sorted as clean or contaminated. “Clean” fecal samples for each animal throughout
the entire study were then pooled and ground to a fine powder using a Wiley Mill and a #40 screen (0.38mm).

Urates were prepared similarly, but were ground using a mortar and pestle. Immediately after collection, urine sample tubes were labeled with the time of collection and whether the sample collected was clean and fresh. Clean, fresh urine samples were sorted from the others to reduce complication of results from evaporation, and samples for a single tortoise during the balance trial were pooled. Pooled samples were centrifuged to separate suspended urate particulates. The “clean” supernatant was removed and put into a separate container. The remaining urate solid was weighed and then dried at 50° C. This solid particulate was considered part of the clean urate samples and included in the total weight. The amount of liquid lost during the drying process was assumed to be urine and was added to the clean urine total weight. HCL and LiCO₃ rinses were thawed and pooled for each tortoise. 7-10 mg sub-samples of ground, pooled fecal and urate collections, 100 mL of pooled clean urine, and 400 mL of cage rinses were placed into small aluminum vials and analyzed using the Perkin-Elmer CHN analyzer as described for fresh material.

Statistical Analysis

All comparisons were analyzed by using SigmaStat v. 2.0 (SPSS Inc., Chicago IL, U.S.A) or Minitab v. 11.12 (Minitab Inc, State College, PA, U.S.A). Data were tested for normality, and homogeneity of variance. Power tests on each comparison were also run.
Due to the small sample sizes used in this preliminary study and the results of power tests conducted for all comparisons, data were considered statistically significant if p-values were significant at $\alpha = 0.10$ or lower during the interpretation of results.
CHAPTER 3

RESULTS

Fresh Plant Analysis

*Bromus* and *Camissonia* plants grown under elevated [CO$_2$] (700 µmol mol$^{-1}$) did not significantly differ from ambient-grown (360 µmol mol$^{-1}$) plants with respect to water content or potassium content (Figure 1). Additionally, *Camissonia* plants did not show a nitrogen dilution effect under elevated CO$_2$ conditions. In contrast, *Bromus* plants grown under elevated CO$_2$ did contain significantly lower amounts of nitrogen than ambient-grown plants (2.20 vs. 2.50 % DW; $p = 0.005$). It was not surprising that the PEP index for *Camissonia* didn’t differ as none of the constituents of the index changed under elevated CO$_2$ conditions. Although *Bromus* plants did show a significant reduction in nitrogen content under elevated CO$_2$, the difference in this factor alone was not enough to cause a significant difference in the calculated PEP index for this species.

In addition to the constituents of the PEP index, we also analyzed plant material for carbon, phosphorus, and calcium. *Camissonia* plants also showed no CO$_2$ response with respect to any of these variables (Figure 2). *Bromus* plants grown under elevated CO$_2$ conditions exhibited higher concentrations of carbon (40.4 vs. 39.9 % DW; $p < 0.001$), lower calcium concentrations (0.70 vs. 0.91 % DW; $p < 0.001$), and a higher carbon to nitrogen ratio (19.4 vs. 16.5; $p = 0.001$).
In contrast to the results from the effects of CO\textsubscript{2} on plants, the fertilization enhancements did not have a significant effect on any of the variables tested for \textit{Bromus} plants (Table 2). Similarly, the analysis for differences between high and low fertilization treatments showed no statistical differences in the \textit{Camissonia} species with respect to water content, phosphorus, potassium, calcium or the calculated PEP index. \textit{Camissonia} plants grown under the high fertilization enhancement did have higher percentages of carbon (39.7 vs. 39.4; \(p = 0.018\)) and nitrogen (2.26 vs. 2.05; \(p < 0.001\)) content on a dry weight basis. The proportionately large increase in nitrogen content caused significantly lower carbon to nitrogen ratios in plants receiving the high fertilization treatment (20.15 vs. 22.12; \(p < 0.001\)).

In order to quantify the differences between plants that had reached the flowering stage upon harvest and those that remained in the pre-flowering stage, the same comparisons were made between these two groups (Table 3). No statistically significant differences were found in either species with respect to the phosphorus content on a dry weight basis and \textit{Bromus} plants did not show a significant difference in the calculated PEP index. Water content was significantly lower in the \textit{Bromus} plants that had reached flowering state (3.72 vs. 4.43; \(p = 0.004\)). A similar result, although not as pronounced and not significant (\(\alpha = 0.05\)), was seen in \textit{Camissonia} (5.34 vs. 6.08; \(p = 0.090\)). In both \textit{Bromus} and \textit{Camissonia}, flowering plants had significantly lower nitrogen content (2.08 vs. 2.63 for \textit{Bromus}, and 1.44 vs. 2.86 for \textit{Camissonia}; \(p < 0.001\) for both), potassium content (3.58 vs. 4.03 for \textit{Bromus}, and 2.86 vs. 3.31 for \textit{Camissonia}; \(p = 0.027\) and 0.026, respectively), and calcium (0.69 vs. 0.92 in \textit{Bromus}, and 2.24 vs. 3.93 in \textit{Camissonia}; \(p < 0.001\) and \(p = 0.047\), respectively). In contrast to \textit{Bromus}, the PEP index for \textit{Camissonia}
was significantly higher in non-flowering plants (14.94 vs. 23.88; p = 0.002). Carbon to nitrogen ratios were higher in both species, with mean values of 20.2 and 28.6 for flowering plants and 15.7 and 13.6 for pre-flowering plants in *Bromus* and *Camissonia*, respectively (p < 0.001 for both species).

**Diet Composition**

Following receipt of the formulated diets, the diets were analyzed for nitrogen levels to ensure they met the desired target levels. The actual levels of nitrogen content in diets were 10.9, 12.8, 14.6 and 15.6 (% FWB) for diets 1-4 respectively (Table 1).

**Physical Effects of Diet**

As described in the Methods section, tortoises were randomly chosen for one of four diets in the high potassium (KM1) nitrogen balance feeding trial. Following a five-week acclimation period, mass and morphological measurements were taken for each tortoise. These measurements were repeated at the conclusion of the study. Following the conclusion of the high potassium trial, tortoises were again randomly chosen for one of four diets in the natural potassium (KM2) nitrogen balance feeding trials. Because no significant effect of elevated CO$_2$ was seen in *Camissonia* plants, the manufactured diets in both the high and natural potassium trials reflect the differences in *Bromus* plants reported in the previous section.

The effects of tortoises consuming diets of varying nitrogen levels did not cause significant changes in the body mass, midline carapace length (MCL), plastron length
(PL), shell width and shell height for any diets during the high potassium feeding trial (Table 4). Similarly, when comparing the percent change of mass and morphological measurements for the natural potassium feeding trail, no significant difference was seen among diets (Table 5). However, when we compared the pooled percent change in mass and morphological measurements between the two studies, significant differences were seen in the percent change in MCL, and in shell width and height with the natural potassium trial consistently showing higher rates of change (Figure 3). In all cases, the morphological and body mass measurements increased during the study with the exception of PL, and shell height for diet 2 in the high potassium study. It should be noted however, that as a whole, these changes are only a very small degree. Regardless, the percent change for all tortoises was much higher for the natural potassium trial than the high potassium trial in all cases that showed a significant difference between the groups. Additionally, it should be noted that despite the longer time period represented by pooling both halves of the high potassium trial, the natural potassium trial consistently showed greater increases in morphological measurements that were significantly different between the two trials (Figure 3).

Physiological Effects of Diet

The physiological parameters measured during the nitrogen balance studies include nitrogen retention and loss, as well as the partitioning of nitrogen loss through urine, urate, and fecal matter excretion. Nitrogen consumption is also included in this section. Because the sum of all excreta is pooled and used to determine total nitrogen lost, the results from the physiological responses are separated into (1) nitrogen balance, which
includes the consumption, retention and loss of nitrogen through excretion, and (2) nitrogen partitioning, which assesses differences in the partitioning of nitrogen loss between urine, urates and feces. Data presented includes the first half (KM1A) and second half (KM1B) of the high potassium trial separately and pooled (KM1 pooled), as well as the natural potassium trial (KM2).

**Nitrogen Balance**

The results from the nitrogen balance study revealed an affect of potassium level in the diet, causing the high and natural potassium results to differ. Results from both halves of the high potassium trial, KM1A and KM1B, were similar enough to justify pooling the data. As a result, data from both halves pooled of the high potassium trial, KM1 pooled, and the low potassium trial, KM2, are discussed here.

During the high potassium trial, or KM1 pooled, the total grams of diet consumed on a body mass basis approached significance (p = 0.11), with the amount consumed being positively correlated with nitrogen content of the diets. In contrast, the level of nitrogen in the diet did not affect the total grams of diet consumed in the natural potassium study, KM2 (p = 0.84) (Table 6). Similarly, the level of nitrogen in the diets showed a trend toward positive correlation with potassium intake (p = 0.11) and a significant positive correlation with nitrogen intake (p = 0.04) in the high potassium trial. This pattern was not evident in the natural potassium study, with p-values of 0.84 and 0.38 respectively (Table 6).

The high potassium trial revealed a significant effect of nitrogen content in the diets at \( \alpha < 0.10, p = 0.08 \). In this trial, tortoises on the higher nitrogen diets lost more nitrogen (Table 6). The natural potassium trial did not show a significant pattern (p = 0.21).
The effects of nitrogen content in the diet also had a significant effect (at $\alpha < 0.10$) on the total amount of nitrogen retained in high potassium trial ($p = 0.07$), and approached significance in the natural potassium trial ($p = 0.11$). In both trails, the trend showed a positive relationship between nitrogen retention and level of nitrogen in the diets (Table 6 and Figure 4).

**Nitrogen Partitioning**

The level of nitrogen in the diets did not have a significant effect on the quantity of nitrogen lost through any form of excreta in both the pooled high potassium trial and the natural potassium trial (Table 7). When each half of the high potassium study was evaluated separately however, some significant results were evident. In the first half of the high potassium trial, KM1A, the data approached significance when comparing the affect of nitrogen content in the diet on nitrogen lost through production of feces ($p = 0.09$). In the second half of the high potassium trial, KM1B, the amount of nitrogen lost through the “Other” category did differ significantly with respect to level of nitrogen in the diets (Table 7).
CHAPTER 4

DISCUSSION

Fresh Plant Responses

The primary focus of this portion of the study was to determine the potential effect of elevated atmospheric CO$_2$ on the constituents known to be important nutritive indicators for desert tortoise. These include the components water, nitrogen, and potassium, which together are used to calculate the PEP index. In addition, the effect of elevated CO$_2$ on C:N ratio of plant tissues was evaluated. C:N ratio has been shown to be an indicator of nutritional quality of plants for many herbivorous insects (Lincoln et al., 1984) and increases of the carbon to nitrogen ratio have caused behavioral alterations based on food preference in the insects feeding on them (Lincoln et al., 1996). As discussed before, these results are not always consistent and may be absent in some feeding guilds (Bezemer and Jones, 1998), but are thought to be more likely for species strongly limited by nitrogen availability. We also measured two additional components, calcium and phosphorus, that have been reported to change under elevated CO$_2$ conditions (Lindroth et al., 1993; Kanowski, 2001).

Atmospheric [CO$_2$] did not have a significant effect on any nutrient measured in *Camissonia claviformis*, a dicot forb. *Camissonia* has been reported to have the highest photosynthetic rate of any C$_3$ plant ever recorded (Ehleringer, 1979), and is attributed to
an extremely high tissue N concentration (Mooney et al., 1976). While many other C₃ plants tend to “down-regulate” photosynthesis at elevated [CO₂] (Sage et al., 1989), *Camissonia* appears to have such a high CO₂ fixation capacity (and concomitant leaf N-content) that it can apparently adjust to higher [CO₂] without shifting leaf nutrient concentration (Sage, 1994).

In contrast, *Bromus* plants did respond under elevated CO₂ conditions with a lower nitrogen content and higher C:N ratio. These results are consistent with reports for other species (Wong, 1979; Lincoln et al., 1984; Griffin and Seemann, 1996; Conrufo et al., 1998), and confirm previous findings that *Bromus* plants grown under a glasshouse-simulated, elevated CO₂ environment will indeed produce plants with lower leaf nitrogen content (Huxman et al., 1998). Numerous reports suggest that the effect of reduced nitrogen content as a result of elevated CO₂ will be enhanced in C₃ species (Woodward et al., 1991; Ehleringer and Monson, 1993; Lindroth et al., 1993; Diaz et al., 1998), although species-specific variation is inherent in plant responses to [CO₂] even within C₃ plants. In spite of the decrease in nitrogen content for elevated CO₂-grown *Bromus*, the difference in nitrogen content alone did not change enough to cause a significant difference in the calculated PEP index for this species. An explanation for this may be due to the fact that nitrogen is only a small component of this index, and with no change in water or potassium content, the change in nitrogen was not large enough to greatly alter the PEP index.

With respect to the mineral nutrients beyond those reported for the PEP index constituents, we also found no difference between elevated and ambient CO₂ *Camissonia* plants. We did find a higher C:N ratio and lower calcium in *Bromus* plants, however.
Kanowski (2001) reported interspecific variation of phosphorus, calcium, and potassium in response to elevated CO$_2$ concentrations. His results showed a decrease in all three nutrients under elevated CO$_2$ conditions for *Flindersia brayleyana*, a mid-successional tree of Australia, but no change in *Alphitonia petriei*, a pioneer species. Interspecific responses of mineral nutrients in plants is not unusual and may be an indication that the response of mineral nutrients may be influenced by environmental and physiological factors (Saxe et al., 1998).

The reduction in nitrogen in *Bromus* is well within the range reported in other species and is similar to that reported for leaf nitrogen in previous studies on this species (Huxman et al., 1998, 2000). The increase in C:N ratio, however, is less than previously reported (Huxman et al., 2000). This is not surprising because the previous study analyzed plants that had reached full seed maturation. Although phenological state varied in this study, the plants analyzed here were either in pre-flowering or early flower stages. Water content in plants has been reported to increase (Lincoln et al., 1986), decrease (Lindroth et al., 1993) or not change (Johnson and Lincoln, 1990), making the lack of change for both species not extraordinary given such a wide range of species-specific responses to elevated CO$_2$.

With respect to the fertilization treatment, only *Camissonia* was affected, and those effects were limited to increased carbon and nitrogen contents in the high fertilization treatments. These results suggest that *Camissonia* may be more limited by the availability of nitrogen than atmospheric CO$_2$ concentration.

As mentioned above, the initial goal of this portion of the project was to determine the effect of elevated atmospheric CO$_2$ on the nutritive indicators for annual plants.
known to be components of the diet of desert tortoises. The ultimate application of this information, however, was to try to predict how future atmospheric CO$_2$ concentrations will alter the nutritive quality of desert tortoise forage. It is apparent from these results that the large seasonal, annual and spatial variation of annual plant diversity in the Mojave Desert, combined with the species-specific responses, that it is necessary to include a wider variety of annual plants in this type of investigation. Another important factor in this equation is how these nutrients change during the lifespan of annual plants. And third, we do not know how accurate the step-change increase in [CO$_2$] used in this experiment may simulate a doubling of [CO$_2$] over the next half-century with regard to plant nutrient relations. In any case, we currently have only a limited understanding of the nutritive quality of annual plants available for tortoises in a given season or how the nutrients change with phenological stage of plants through the season under ambient [CO$_2$]. Ultimately, developing an estimate of how the seasonal change in the nutritive quality of diet constituents is altered under an elevated atmospheric CO$_2$ scenario will be extremely important.

Physical Responses of Desert Tortoises

The results from the physical responses of desert tortoises during the nitrogen balance trials in both the high and natural potassium studies showed no significant differences among the four diets differing in nitrogen content. This is not surprising given the narrow margins in nitrogen content variation among the four diets, the slow growth rate of tortoises, and the large individual variation. The pooled percent change for all diets, when compared between the two studies did differ significantly for midline carapace
length (MCL), plastron length (PL), and shell width and height. Additionally, although positive growth was recorded for all diets in both trials, the percent change was much higher for the natural potassium trial than the high potassium trial in all cases. The differences in growth between the two studies is further emphasized because the duration for the natural potassium trial was one week less than that of the high potassium trial, and yet tortoises still had greater overall growth. However, it should be noted that as a whole, these changes were very small. The reasoning for the differences in growth between the two studies is likely the result of differences in consumption. Tortoises in the high potassium trial consumed very little during the entire study, and with less consumption tortoises will not have as much energy to allocate to growth. But again, results should be interpreted cautiously since the changes in these morphological measurements, although potentially significant over time, were very small. The differences in consumption and retention will be discussed further in the physiological response section.

In contrast to the changes seen in MCL, PL, width and height, the percent change in body mass did not differ significantly between the two studies. It is not surprising that body mass did not differ significantly either within or between studies. The body mass of an individual tortoise can vary greatly due to differences in bladder content alone (Peterson, 1996). During this study some tortoises were more prone to drink than others, and water consumption was not restricted in any manner. Therefore, individual variation due to water consumption alone may have masked any changes in body mass resulting from different nitrogen consumption.
Physiological Responses of Tortoises

Nitrogen Balance

Nitrogen balance is the comparison between the amount of nitrogen consumed and the amount lost through excreta. The difference between these two values for the purposes of this study is considered to be the total amount of nitrogen retained. The sensitivity of tortoises to the amount of potassium in the diets was revealed in the nitrogen balance portion of the study, and is evidenced by the vastly different results obtained between the high and natural potassium trials.

In the high potassium trial, tortoises consuming diets lower in nitrogen content limited intake. It is likely that the potassium content of the diets was responsible for these results. Potassium content did not vary between diets; the higher nitrogen diets contained a higher proportion of nitrogen to potassium than the lower nitrogen diets. As a result, tortoises on the lower nitrogen diets, consumed less than those on the higher nitrogen diets (p = 0.11). This is further emphasized by the lack of response in consumption to nitrogen content in diets of the natural potassium trial. In both trials however there was a large amount of variation among tortoises in each diet. Larger sample sizes may be needed in such trials to demonstrate statistical significance at $\alpha = 0.05$ were used in the trials and perhaps would have caused results in the natural potassium trial to be significant at $\alpha = 0.05$.

Both potassium and nitrogen intake were positively correlated with nitrogen content in the diet in the high potassium trial, although only significant at $\alpha = 0.05$ with respect to nitrogen intake (p = 0.04). This is not surprising as tortoises consuming more diet are also taking in more potassium and nitrogen.
In the high potassium trial, the amount of nitrogen in the diets had an affect on the amount of nitrogen lost. Tortoises in the high potassium trials consuming the higher nitrogen diets lost more nitrogen overall than those on the lower nitrogen diets (significant at \( \alpha = 0.10 \)). Surprisingly however, even though tortoises on the higher nitrogen diets lost more nitrogen than those on the lower nitrogen diets, they were still able to retain more nitrogen overall than those on the lower nitrogen diets (\( p = 0.07 \)).

The natural potassium trial did not show a significant affect of diet nitrogen content with respect to total amount of nitrogen lost. Interestingly however, the amount of nitrogen retained did approach significance (\( p = 0.11 \))

The results from the nitrogen balance portion of the study reconfirm previous studies indicating that potassium levels may alter nitrogen retention in desert tortoises (Oftedal, 1995, 1996, 2002a, 2002b). Tortoises do indeed limit intake of high potassium diets and retain less nitrogen in diets containing high proportions of potassium to nitrogen in a lab setting, and appear to do so under natural circumstances when optimal plants are available (as measured by the PEP index). This result is also consistent with our predictions based on previous studies, that tortoises consuming high potassium diets will incur a greater nitrogen cost from the production of urates used to excrete excess potassium.

**Nitrogen Partitioning**

With regard to partitioning of nitrogen in this study, we compared the relative contribution of nitrogen loss from the different modes for excretion. This was included to see if tortoises on different diets utilized different methods for nitrogen excretion. As with total nitrogen lost, very few of the data show significant differences between the
diets. Neither the pooled data from the high potassium trial or the natural potassium trial show strong significance between nitrogen content of the different forms of excreta.

When evaluating the results from both halves of the high potassium trial, there was a significant effect of nitrogen content in the diets on loss of nitrogen through production of feces for the first half of the trial. In the second half of the trial, the amount of nitrogen lost through "Other" also showed a strongly significant effect of level of nitrogen in the diet.

As a whole, in both studies, the tortoise's primary method for nitrogen excretion was through the production of urates (ranged from 24-85% of total nitrogen lost). Nitrogen loss through fecal matter represented the second largest method for excretion (ranged from 14-54% of total nitrogen lost). This is consistent with results reported by Oftedal (1995, 1996).

The partitioning of nitrogen loss through various forms of excreta is likely to be highly dependent on the availability of water during periods of high potassium consumption. Tortoises in this study were provided opportunities to drink on a regular basis so these results do not adequately reflect the conditions desert tortoises are faced with in wild conditions, especially in years with limited rainfall.

Conclusions

Little is known about the diversity of nutritive quality of the food available to desert tortoises at any given point, and whether the foods that individuals select are indeed among the most nutritive of those available. We also know relatively little about how the nutritive quality of desert annuals may change throughout the phenological stage of the
plant or the tortoise active season (but see Oftedal et al., 2003). The response of plants to elevated atmospheric concentration is largely unknown and oftentimes species specific. This information is crucial to being able to predict the impact of elevated [CO$_2$] on the diet components and how this in turn may affect desert tortoise nutrition.

The criteria for diet selection in desert tortoises do indeed seem to be related to the relative proportions of potassium, nitrogen, and water. Although the method that tortoises use to determine the relative quantities of those components is unknown, they typically avoid foods high in potassium and low in nitrogen. One of the most common responses of C$_3$ plants to elevated [CO$_2$] is a reduction in tissue nitrogen, as was seen in Bromus but not Camissonia in this study. Further studies are needed in this area to determine whether a reduction in tissue nitrogen is common in other desert annuals, especially those of importance to desert tortoise nutrition.

The results from this study, as well as those from previous studies, provide some indication of how tortoises would respond to consuming diets if nitrogen levels were reduced, and therefore C:N and K:N ratios increased. As predicted, tortoises predictably reduce consumption of diets that have high K:N ratio, especially on diets with extremely high potassium levels. This result was evident both within studies, when comparing the different diets, as well as between studies when comparing the total consumption of all tortoises combined. In addition to reducing consumption of high K:N diets, tortoises were less able to retain nitrogen from diets with a higher K:N ratio. As a result, although all groups showed growth, tortoises on the high K:N diets showed a smaller percent increase in all morphological measurements except body mass (which as a whole varies greatly between individuals at any given time).
Desert tortoises are generalist herbivores, and with the variation among plant species in response to changes in the atmospheric CO$_2$ concentration, it is difficult to predict how this change will affect the future of this threatened species without more information on the specific response of their common forage components. Results from this study (and others) show that it is not likely that all annual C$_3$ plants will respond similarly. Nor is it likely that a nitrogen reduction will be common among all diet components, as this did not occur for *Camissonia* in this study. There is evidence however that if changes in nitrogen content resulting from elevated CO$_2$ conditions impair a tortoise's ability to excrete potassium, tortoises will likely avoid consuming that plant. The degree to which this affects the species as a whole will depend on the response of other diet components as well as the availability of a diverse array of nutritious species.

*Bromus rubens* is an exotic annual grass that has recently become a large portion of annual biomass in many areas of the Mojave Desert. *Bromus* readily invades disturbed sites and is often associated with increasing occurrences of fires (Brooks, 1999), and responds much more vigorously to elevated CO$_2$ than do native annuals of the Mojave Desert (Smith et al., 2000). This study showed that a reduction in tissue nitrogen occurred under elevated [CO$_2$] for this species, which resulted in reduced consumption and ultimately a greater loss in nitrogen for tortoises consuming a diet representing this change. By out-competing native annual plants, areas dominated by *Bromus* will contain less nutritious forage available to desert tortoises. The best way to avoid encroachment of *Bromus* is to restore areas following disturbance and limit disturbances to areas that are less sensitive ecologically.
Implications For Management of the Species

The results from this preliminary analysis suggest that diet components exhibiting a nitrogen dilution effect in response to elevated atmospheric [CO\textsubscript{2}] will negatively impair nitrogen retention in desert tortoises. This reduces the ability for tortoises to allocate energy toward growth and reproduction, which ultimately may have implications for survival of the species. However, these results are preliminary and without information on the response of other common diet components to elevated [CO\textsubscript{2}], especially those species with a high PEP index, we compromise our ability to ensure that current management practices will remain effective under an elevated atmospheric CO\textsubscript{2} scenario.

Desert tortoise nutrition was listed as a research priority in the desert tortoise recovery plan. Although research has provided us with the framework for focusing our efforts, much more information is needed in this area. Managers should work with scientists to ensure future research studies are designed to further our knowledge in this relatively unexplored area in order to provide us with the information necessary for making predictions for response of this threatened species under a changing environment. It is all too common in species and habitat management to work reactively to manage and mitigate impacts to species. It is increasingly important for research to be focused on information that will not only enhance our understanding of ecological processes under today’s environmental conditions, but will also provide us with the tools we need to avoid future conflicts under an environment influenced by human activities.
APPENDIX I

TABLES
Table 1. Pelleted diet composition for the natural potassium feeding trials (KM2). High potassium trials (KM1) contained similar ingredients with the exception of potassium bicarbonate (3.22), potassium chloride (2.20), potassium citrate (2.00) with a target potassium level of 3.5 (vs. 1.42 for natural trial) for all diets.

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<th>Diet 3</th>
<th>Diet 4</th>
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Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.
Table 2. Effects of high and low fertilization enhancement during seed propagation on water, carbon, nitrogen, phosphorus, potassium, calcium content, calculated PEP index, and the carbon to nitrogen ratio of *Bromus* and *Camissonia* plants. All comparisons analyzed by ANOVA. SEM = standard error of least square mean, DW = dry weight, Sig. = Statistical Significance, NS = Not Significant.

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Table 3. Effects of phenological state following plant harvest on water, carbon, nitrogen, phosphorus, potassium, calcium content, calculated PEP index, and the carbon to nitrogen ratio of *Bromus* and *Camissonia* plants. Statistics and abbreviations as in Table 2.

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* Not significant at α = 0.05
Table 4. Percent change in body mass and morphometric measurements in response to consuming High Potassium (KM1) diet. All comparisons analyzed by ANOVA. % Prot = percent protein (N*6.25), MCL = midline carapace length, PL = plastron length, SEM = standard error of least square mean, Sig. = Statistical Significance, NS = Not Significant, N = 6 for all treatments.

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Table 5. Percent change in body mass and morphometric measurements in response to consuming Natural Potassium (KM2) diet. Statistics and abbreviations as in Table 3.

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Table 6. Effect of nitrogen content in diet on consumption, potassium intake, and nitrogen intake, loss, and retention for high and natural potassium study diets. All comparisons analyzed by ANOVA and data represented as g per kg Body Mass. KM1 Pooled represents the pooled values from KM1A and B. Std. Dev = standard deviation, Sig. = Statistical Significance, N = 6, 4, 6, and 5 for diets 1-4 respectively.

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Table 7. Effect of nitrogen content in diet on nitrogen partitioning for high and natural potassium study diets. All comparisons analyzed by ANOVA. Data represented as nitrogen (mg) lost per body mass (kg) through variable listed per day. Urine includes only clean urine, “Other” includes nitrogen lost through cage rinses and unclean urine. Std. Dev = standard deviation, Sig. = statistical significance, N = 6, 4, 6, and 5 for diets 1-4 respectively.

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Figure 1. Effects of \([\text{CO}_2]\) on the PEP constituents and calculated PEP index of \textit{Camissonia} and \textit{Bromus} plants grown under ambient (360 \(\mu\text{mol mol}^{-1}\), white bars) and elevated (700 \(\mu\text{mol mol}^{-1}\), shaded bars) carbon dioxide conditions. All comparisons analyzed by ANOVA. Significant results are shown with corresponding p-value. N=16 for both treatment groups.

Figure 2. Effects of \([\text{CO}_2]\) on carbon, phosphorus, calcium, and the carbon to nitrogen ratio for \textit{Camissonia} and \textit{Bromus} plants grown under ambient (360 \(\mu\text{mol mol}^{-1}\), white bars) and elevated (700 \(\mu\text{mol mol}^{-1}\), shaded bars) carbon dioxide conditions. All comparisons analyzed by ANOVA. Significant results are shown with corresponding p-value. N=16 for both treatment groups.
Figure 3. Comparison of percent change in mass and morphological measurements of tortoises during the high potassium (KM1) and natural potassium (KM2) diets. All comparisons analyzed by repeated measures ANOVA. Significant results are shown with corresponding p-value. N=24 for both study groups.
Figure 4. Mean Total Nitrogen Lost □ and Retained ■ per body mass per day by diet. Values combined equal the total Nitrogen Consumed per Body Mass. Diets 1 – 4 contain increasing total Nitrogen content (details provided in text). N=6, 4, 6, 5 for diets 1-4 respectively.
BIBLIOGRAPHY


Christopher, T.E., Oftedal, O.T., Schulkin, J. 1996. Desert tortoises prefer diets that are high in nitrogen and low in potassium. Symposia of the Comparative Nutrition Society, No. 1, pp. 21-23. Leesburg, VA, USA


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    Keene State College, Keene, New Hampshire

Thesis Title: Effects of Elevated CO$_2$ on *Bromus rubens* and Nutritional
    Consequences for the Desert Tortoise

Thesis Examination Committee:
    Chairperson, Dr. Stan Smith, Ph. D.
    Committee Member, Dr. Dawn Neuman, Ph. D.
    Committee Member, Dr. Olav Oftedal, Ph. D.
    Graduate Faculty Representative, Dr. Rik Orndorff, Ph. D.