EXPLORATION OF COMPLEX INTERACTIONS AMONG ATMOSPHERIC CO_2, FOLIAR QUALITY, INSECT HERBIVORES AND DECOMPOSITION PROCESSES

by

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(Under the Direction of MARK D. HUNTER)

ABSTRACT

Atmospheric CO_2 concentrations have increased exponentially over the last century and continuing increases are expected to have significant effects on ecosystems. We investigated the interactions among atmospheric CO_2, foliar quality, litter quality, herbivory and decomposition within a scrub oak community. Sixteen plots of open-top chambers were followed, eight at ambient levels of CO_2 and eight at elevated levels of CO_2 .

To assess the effects of CO_2 on foliar quality and herbivory we focused on three oak species, one nitrogen fixing legume and six different insect herbivore feeding guilds. Plant species differed in their relative foliar chemistries over time, however, the only consistent differences were higher nitrogen concentrations and lower C:N ratios in the nitrogen fixer when compared to the oak species. Under elevated CO_2, damage by herbivores decreased for four of the six insect groups investigated. Overall declines in both foliar quality and herbivory under elevated CO_2 treatments suggest that damage to plants may decline as atmospheric CO_2 levels continue to rise.

To define links between foliar quality, herbivory and litter quality we focused on three dominant oak species and three herbivore damage categories. We found variation in litter

chemistry associated with CO_2 and herbivory. However, changes in litter chemistry from year to year were far larger than effects of CO_2 or insect damage, suggesting that these may have only minor effects on litter decomposition.

To assess the influence of litter quality on decomposition and the influence of herbivory on litter quality along with subsequent decomposition we performed two three-year decomposition experiments. We found that despite variation in litter quality associated with CO_2, herbivory and their interaction there was no subsequent effect on rates of decomposition under ambient atmospheric conditions. However, the chamber in which the decomposition took place resulted in significant differences in decomposition rates. Litter decomposing under elevated CO_2 decomposed more rapidly than litter under ambient CO_2, and exhibited higher rates of mineral nitrogen accumulation suggesting that the atmospheric conditions during the decomposition process have a greater impact on rates of decomposition and nitrogen cycling than do the atmospheric conditions under which the foliage was produced.

INDEX WORDS: Elevated CO_2, Foliar quality, Litter quality, Herbivory, Decomposition, *Quercus myrtifolia*, *Quercus chapmanii*, *Quercus geminata*, *Galactia elliottii*, Scrub oak forest, Kennedy Space Center

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

Human activities including increased fossil fuel use and deforestation have resulted in the increase of atmospheric carbon dioxide (CO_2) concentrations over the past 200 years from about 280 parts per million (ppm) in the early days of industrialization to around 370 ppm at the beginning of the 21st century. The Intergovernmental Panel on Climate Change (IPCC) projects that CO_2 concentrations will range between 540 and 970 ppm by the end of the 21st century, if current trends continue (2001). Climate models predict that increases in CO_2 with other radiative 'greenhouse' gases such as methane, nitrous oxide, chlorofluorocarbons, and ozone will result in an increase in global temperatures and changes in precipitation patterns. Global warming has earned a place in the general public's attention, however the fact that ecosystems, particularly plants may be directly affected by increases in CO_2 concentrations has received little publicity (Korner, 2000). Changes in CO_2 along with climate change could affect ecosystems. Changes in CO_2 alone could be enough to change plant communities and rising CO_2 concentrations may have mediating effects on herbivory and nutrient cycling. Recalling the overall process of photosynthesis, CO_2 and water are combined in plant leaves utilizing sunlight to produce carbohydrates and oxygen.

$$CO_2 + H_2O \xrightarrow{light} CH_2O(carbohydrate) + O_2$$

Carbohydrates are utilized for the production of proteins, lipids and structural forms of plants. This description of photosynthesis leaves out a number of variable steps and compounds, some of which are important to plant responses to increasing CO_2 (Kimball et al. 1993). As a result plant responses to elevated CO_2 concentrations vary within and among species (Williams and Whitham 1986; Lindroth et al. 1993; Lindroth et al. 1995; Curtis et al. 1996; Johnson et al. 1996; Koch and Mooney 1996; Mousseau et al. 1996; Norby et al. 1996; Cook et al. 1997; Robinson et al. 1997; Van Gardingen et al. 1997; Woodward and Beerling, 1997; Scowcroft et al. 2000; King et al. 2001; Saleem et al. 2001). Carbon dioxide mediated changes in plants may affect foliar quality, herbivory or the decomposition process and nutrient dynamics. This dissertation explores the effects of carbon dioxide on foliar and litter quality, herbivory, and the decomposition process along with associated interactions. This introductory chapter reviews the effects of CO_2 on plants, herbivores, litter quality, and decomposition and then introduces the study system and the hypotheses that form the foundation of this research.

Plant Responses to Elevated CO_2

Plants vary in their responses to CO_2. These differential responses may alter species composition in ecosystems and as a result foster changes in ecosystem function. While plant responses to elevated CO_2 concentrations vary within and among species (Lindroth et al. 1993; Lindroth et al. 1995; Robinson et al. 1997; Curtis and Wang, 1998; Saxe et al. 1998; Medlyn et al. 1999; Pritchard et al. 1999; Ward and Strain1999; Korner, 2000; Scowcroft et al. 2000; Tingley et al. 2000; King et al. 2001; Saleem et al. 2001; Poorter and Navas 2003) some generalities have emerged. Plant responses are primarily linked to the ability of species to store excess carbohydrates. Plants with the C_3 photosynthetic pathway have stronger responses to

CO_2 than plants with the C_4 photosynthetic pathway. C_3 plants are CO_2-limited and oxygen inhibits photosynthesis. Although rubisco has a high affinity for binding CO_2 if enough O_2 is present, rubisco will bind O_2 as well. The ratio of CO_2 to O_2 ultimately determines the product of the rubisco reaction. C_4 plants, on the other hand, are able to enhance the partial pressure of CO_2 at the site of rubisco to the extent that the oxygenation reaction of rubisco is nearly completely inhibited. As a result, C_4 plants have negligible rates of photorespiration. This ability to concentrate CO_2 means that C_4 plants are less sensitive to external changes in CO_2 and therefore do not react as strongly as C_3 plants to increases in CO_2. The response N-fixing plants is not well established although Reich et al. (2001) reported that aboveground (foliar) N levels declined in four legumes (*Amorpha canescens, Lespedeza capitata, Lupinus perennis, Petalostemum villosuni*), while below ground (root) N levels remained unchanged under elevated CO_2. Elevated CO_2 increases photosynthetic rates across various ecosystems (Williams et al. 1986; Bazzaz, 1990), including arctic tundra (Oberbauer et al. 1986; Tissue and Oechel, 1987) grasslands (Smith et al. 1987) and deciduous forests (Williams et al. 1986). Typically an increased photosynthetic rate translates into increased plant growth. The doubling of CO_2 without the added effects of climate change is predicted to increase plant growth (and yield) by about 30% (Lambers 1993). However temperature and CO_2 interact such that in some cases plant growth may possibly be greater or less than predicted. Elevated CO_2 also affects transpiration. It typically reduces stomatal conductance due to declining aperture and stomata frequencies (Morison, 1987). Consequently there is an increase in water use efficiency (WUE) and increased plant growth in response to elevated CO_2 is observed even when water is limited (Kimball et al. 1993). Over longer periods of CO_2 exposure the initial responses of some plants to elevated CO_2

attenuate because of photosynthetic adaptations to higher concentrations (Oberbauer et al. 1986; Williams et al. 1986; Smith et al. 1987; Tissue and Oechel, 1987; Bazzaz, 1990).

In addition to aboveground growth responses, plants often allocate additional C to roots, thus increasing the root-shoot ratio (Bazzaz 1990; Rogers et al. 1994). There may also be an increase in the size and / or number of leaves, (Lindroth et al. 1995). In cases where no growth response is observed, CO_2 enrichment may still alter the chemical composition of live plant-tissue. Bryant et al. (1983) proposed that increased secondary chemical production would result when the nutrient to C supply rates favors C. This higher carbon:nutrient balance can result from decreased nutrients (nitrogen) or increased C resources. This hypothesis is based on the fact that phenylalanine is used both for protein synthesis and as a common precursor for some polyphenols. If N availability regulates phenylalanine then, when N concentrations are high, protein synthesis should increase and phenolic production decrease. When N availability is low, then protein synthesis should decline and the production of phenols should increase. Thus plants that have a high level of C and a low level of N available are predicted to produce more defensive chemical compounds (Jonasson et al. 1986), whereas plants that have lower carbon:nutrient ratios are predicted to produce fewer defensive chemicals (Coley 1987). Chapin (1980) has shown that nonstructural carbohydrates increase in plants grown under low nutrient conditions and Gershenzon (1984) has shown that plant defenses, both spatially and temporally, are related to resource availability. Plants grown in low nutrient soils produce more allelochemicals, particularly phenolics (Janzen, 1974; Mattson, 1980). Several additional studies have found evidence supporting the carbon/nutrient hypothesis under limited nutrient supply (Phillips and Henshaw1977; Mihaliak and Lincoln 1985; Bryant et al. 1987; Takeda 1988; Margna et al. 1989; Mihaliak and Lincoln 1989). The current rapid increase of atmospheric CO_2

concentrations allows the carbon/nutrient supply hypothesis to be approached from a different angle (Lincoln 1993). The effects of nutrient availability have been intensely studied and the effects are well known, however the realization that CO_2 is increasing has made the effect of carbon supply on plant defenses of increasing interest (Lincoln 1993).

Past tests of the carbon/nutrient hypotheses have focused on nutrient supply manipulation or on indirect carbon supply manipulation. Price et al. (1989) showed that increasing or decreasing shoot growth via manipulating water supply (and in so doing manipulating carbon availability) in *Salix* resulted in changes in phenolic glycosides. When shoot growth increased, phenolic glycosides decreased and when shoot growth decreased, phenolic glycosides increased. A higher concentration of total phenolics was found in the leaves of the ant-plant *Barteria fistulosa* when exposed to sunlight than in leaves that were shaded (Waterman et al. 1984). Bryant et al. (1987) found that paper birch and green alder had lower phenolic concentrations when the trees were fertilized and that paper birch had lower phenolic concentrations when light was reduced.

Recently, experiments have been designed to test the effects of increased carbon supplied to plants directly, though this is not an entirely new idea. The first observation that increased CO_2 enhanced plant growth is attributed to De Sassure in 1804 (Kimball et al. 1993). As early as the 1930s, nurseries were reported to be using CO_2 in greenhouses in order to aid plant growth (Kimball et al. 1993). However field experiments using Free-air CO_2 enrichment (FACE) and open-top chambers (OTC) did not come into use until the 1980s. The development of FACE and OTC allowed the effects of elevated CO_2 on plants to be studied in the field.

As described above, fertilizing plants directly with carbon dioxide leads to a stimulation of photosynthesis and an increase in carbohydrates, particularly in plants with the C_3

photosynthetic pathway (Lambers and Poorter 1992). Carbon gain in plants may be directed toward defensive compounds (lignin, tannins, and phenolic glycosides) (Lindroth et al. 1993), or nonstructural carbohydrates such as starch and sugar. Photosynthetic water use efficiency and integrated nutrient use efficiency increases in those plants producing tissue with higher carbon / nutrient ratios (Williams et al. 1986; Denno et al. 1990). When nutrient supply is limited, increased photosynthesis under elevated CO_2 results in lower nutrient concentration in plant tissues. In particular, lower N concentrations and higher C:N ratios are often observed (Korner 2000). However, when nutrient supply is high the increased photosynthetic response is not as strong. Thus in addition to carbon allocation by plants, the availability of resources appears to influence the degree to which species adjust (Bazzaz 1990; Lindroth et al. 1993). The effect of elevated CO_2 on photosynthetic rates and associated increases in biomass and WUE is enhanced when other resources such as water, light, and nutrients are abundant (Bloom et al. 1985; Bazzaz 1990), and the enhancing effects of CO_2 disappear when N or P is limited (Wong 1979; Zangerl and Bazzaz 1984). Light saturation is higher under elevated CO_2 indicating that elevated CO_2 may compensate for low light (Valle et al. 1985; Bazzaz 1990) and the optimal temperature for photosynthesis is higher at elevated levels of CO_2 and the range of optimal temperatures for photosynthesis is narrower (Bazzaz 1990; Potvin 1985).

Since plants species' responses to elevated CO_2 differ considerably, species composition due to competitive shifts within plant communities is likely to occur under changing CO_2 conditions. It is thought that this indirect effect of CO_2 will have greater effects on ecosystem function than the direct effects of elevated CO_2 (Korner 2000). However a potential route by which elevated CO_2 might influence the ecosystem function is through its impact on insect herbivores or plant litter quality.

Herbivores, Plants, and CO_2

Increases in global temperatures and changes in precipitation are expected to drive changes in insect herbivore populations. Expected increases in herbivore range and densities may decrease the ability of ecosystems to response to environmental stress (Dale et al. 2001). For example, drought and insect herbivore interactions have resulted in well-documented forest die-off in the American southwest (Allen and Breshears 1998; Hanson and Weltzi, 2000; Ogle et al. 2000). Herbivory can also function as a regulator of ecosystem nutrient fluxes (Hunter, 2001; Lovett et al. 2002). Depending on the system, anywhere from 10% to 40% of plant material can be removed by herbivores, which can result in a substantial redistribution of nutrients within an ecosystem, and can in turn change the soil microclimate (Fahnestock and Knapp 1994), alter the nutrients entering the decomposition process (Chapman et al. 2003), and shift above- and belowground carbon allocation (Bardgett et al. 1998). Herbivory has been found to increase (Holland and Detling 1990; Kielland et al. 1997; Tracy and Frank 1998; Reynolds et al. 2000), decrease (Pastor et al. 1998; van Wijnen et al. 1999; Lovett et al. 2002), or have no change (Seastedt et al. 1983; Fahnestock and Detling 2002) on nutrient cycling in ecosystems. In addition to alterations of nutrient imputs to the ecosystem, herbivory can lower plant density (Fahnestock and Knapp 1994; Kielland and Bryant 1998; Kosola 2001), alter plant community structure (Brown 1994; Maron and Jefferies, 1999), and change foliar chemistry (Grace 1986; Grime et al. 1996; Chapman et al. 2003).

Given the effects of increased CO_2 on plants, plant / herbivore interactions can be anticipated to change in response to elevated CO_2. The growth of plants under elevated CO_2 alters foliar chemistry, primarily decreasing N concentrations and increasing carbohydrate availability in leaves. These changes are expected to have effects on insect herbivores and

subsequent levels of defoliation (Lincoln 1993). Leaf N is a limiting nutrient in many insect diets (Mattson 1980). When given a choice, many herbivores will preferentially feed on leaves high in nutrients and water, low in toughness and low in polyphenols (Coley 1983; Kimmer and Potter 1987; Waterman and McKey 1989; Lambers 1993). Changes in plant chemistry, such as those seen in plants exposed to high CO_2 concentrations, affect the quality of herbivore diets and may result in behavioral and / or physiological changes in insect herbivores (Koch and Mooney 1996; Lindroth 1996; Stiling et al. 1999). When the quality of a food source is low, insect herbivore responses include increased feeding rates and consumption, choosing more nutritious leaves, changing host species, lowering development rates, lowering survivorship and fecundity, increasing food use efficiency or reducing population density (Scriber and Slansky 1981). Several of these herbivore responses have been observed when herbivores feed on plants grown in CO_2 rich environments. Three species of noctuids increased their consumption of legumes by 20-40% when the plants were grown at elevated CO_2 concentrations (Lincoln et al. 1984; Lincoln and Couvet 1986; Osbrink et al. 1987). *Pseudoplusia includens* larvae consume leaves of *Glycine max* at a 50% higher rate when plants are grown at elevated instead of ambient CO_2 (Lincoln and Couvet 1986). and Fajer et al. (1989, 1991) found that the larvae of *Junonia coenia* increased their feeding on *Plantago lanceolata* when the plants were exposed to elevated CO_2. Leaf-chewing insects such as grasshoppers (Johnson and Lincoln 1990) and lepidopteran larvae (Lindroth et al. 1993,1995) generally consume more leaf area when fed plants that have been grown under elevated CO_2. A number of additional studies have found that insect herbivores compensate for lower nitrogen concentrations by increasing their consumption rates by 20-80% (Lincoln et al. 1984; Akey et al. 1988; Akey and Kimball 1989; Fajer, 1989; Fajer et al.

1989,1991; Lincoln and Couvet 1989; Lincoln et al. 1993; Johnson and Ball 1996). Likewise, the area damaged by leaf-mining insects may also increase (Salt et al. 1995).

However, increased consumption rates may not compensate fully for reductions in foliar quality. Lepidopteran larvae exhibit increased mortality and slower growth rates when feeding on elevated CO_2 plants (Akey and Kimball 1989; Fajer 1989; Fajer et al. 1989, 1991) along with reduced larval and pupal weights (Akey et al. 1988; Akey and Kimball 1989; Fajer et al. 1989,1991; Osbrink et al. 1987). Egg production by a *Melanoplus* grasshopper was significantly reduced when reared in elevated CO_2 on a C_4 grass (Montjoy 1992). Herbivores may become more susceptible to pathogens, parasitoids, and predators as a consequence of these changes in behavior and physiology (Price et al. 1980; Lindroth 1996; Stiling et al. 1999). For example, increases in mortality of leaf miners feeding on elevated CO_2 plants have been linked to increases in parasitism (Stiling et al. 1999). Observations suggest that at least some insect populations may increase under elevated CO_2 (Oechel and Strain 1985), but no response was observed for phloem-feeding whitefly on cotton in open top chambers under field conditions (Butler et al. 1986). In the longest field study to date, some insect herbivore populations have been shown to decline markedly under elevated CO_2 (Stiling et al. 1999, 2002, 2003). Thus, many of the responses exhibited by insect herbivores feeding on plants grown under elevated CO_2 are consistent with those of insect herbivores feeding on low quality plants.

Why are plants of lower quality under elevated CO_2? The increased availability of fixed carbon may not only enhance plant growth, but also increase the proportions of leaf soluble sugars and starch (Cave et al. 1981), and is thought to dilute the concentration of foliar proteins, making them a relatively poor food source (Johnson and Lincoln 1990). Accumulation of starch to greater than 10% of leaf dry weight has been found to account for a majority of the N dilution

in sagebrush plants grown under elevated CO_2 (Johnson and Lincoln 1990). Leaf carbohydrates are typically considered highly digestible and an essential energy source to many insect herbivores. However, CO_2 enrichment may increase leaf fiber content, which in turn may reduce leaf digestibility for herbivores.

Much attention has been given to the role of secondary plant compounds and their effects on insect herbivores. Secondary compounds including lignin, tannins, and terpenoids may comprise as much as 30% of leaf dry weight (Lambers and Poorter 1992). These compounds make plant tissue both unpalatable and indigestible. Tannins can impede digestion by blocking digestive enzymes and binding proteins while tannins and lignin also increase leaf toughness (Haslam 1988). Flavonoids and flavonoid-based compounds have been found to reduce growth and survivorship of herbivores (Lincoln et al. 1982). However, there is little evidence supporting the hypothesis that secondary compounds will increase in CO_2 rich environments. Elevated CO_2 had no effect on two monoterpenoid allelochemicals (aucubin and catapol) found in *Plantago lanceolata* and known to repel generalist herbivores (Fajer et al. 1989, 1991). Lincoln and Couvet (1989) investigated the effect of three levels of atmospheric CO_2 on volatile terpenes in *Mentha piperita* leaves and their consumption by the caterpillar *Spodoptera eridania* and found similar results. And Johnson and Lincoln (1990) found that atmospheric CO_2 concentration had no effect on allelochemicals in *Artemisia tridentata*. In light of the evidence, or lack thereof, it appears that an external increase in carbon availability alone may not result in increases in the production of polyphenols (Lincoln 1993).

So will levels of herbivory increase or decrease under elevated CO_2? Higher consumption could compensate for increased plant productivity observed under elevated CO_2. Herbivory in temperate forests commonly results in about 10% defoliation annually. However,

leaf area removed can be much higher particularly during outbreaks (Reichle et al. 1973; Fox and Morrow 1983). It is not yet clear whether changes in defoliation at the ecosystem scale will result from increases in atmospheric CO_2.

Although CO_2 itself could possibly have direct effects on insects, they are unlikely to be particularly strong. Some insects are adapted to high levels of CO_2 such as within the nests of social insects or soil inhabitants (Nicholas and Sillans 1989). Other insects respond directly to CO_2 gradient cues such as soil dwelling insects or the better-known hematophagous insects. Gas exchange in insects occurs via spiracles, which respond to internal levels of CO_2 (Hoyle 1960; Burkett and Schneiderman 1974). Currently there are few data on the gas exchange of insects in response to increased external levels of CO_2. Hypothetically higher external levels of CO_2 could result in increases in spiracular openings, however this is more likely to lead to a water deficiency than to a change in the CO_2 gradient within the insect. Thus at this time, given the limited information, the direct effects of CO_2 on insect herbivores are predicted to be much smaller than the potential indirect effects of CO_2 mediated by plants (Lincoln 1993).

Given that herbivory can change plant density (Fahnestock and Knapp 1994), influence community structure (Brown 1994), alter nutrient input (Belovsky and Slade 2000), and influence foliar (Karban and Baldwin 1997; Agrawall et al. 1999) and subsequently litter quality (Findlay et al. 1996), changes in herbivore density, behavior, and physiological responses to elevated CO_2 have the potential to influence decomposition and nutrient dynamics. This is considered next.

Litter Quality and Decomposition

Decomposition of litter results in a continuous supply of nutrients and is the primary process of terrestrial nutrient cycling, linking above and below ground components via resource supply (Field et al. 1992; Adams and Wall 2000; Saleem et al. 2001). The dynamics of decomposition, however, vary among ecosystems and can ultimately influence ecosystem structure and function. Decomposed litter returns nutrients to the soil, provides resources for detritus food webs, and contributes to soil formation (Field et al. 1992; Elliott et al. 1993; Gholz et al. 2000). Annual rates of decomposition vary from 30% (in some temperate grasslands) to 100% (in some deciduous forests) of senesced organic material and depend on temperature, moisture, nutrients, microbial organisms and fauna, and the quality of plant material (Meentemeyer 1978; Swift et al. 1979; Aber and Melillo et al. 1982; Coûteaux et al. 1991; Field et al. 1992; Elliott et al. 1993; O'Neill and Norby 1996; Etiope 1997; Gholz et al. 2000). Climate, particularly temperature and moisture, is considered important on a regional and global scale (Olson 1963; Meentemeyer 1978; Donnelly et al. 1990; Vitousek et al. 1994). While litter quality may be influential at large spatial scales, it is most frequently considered to have a substantial impact on the local scale. Litter quality depends on the chemical and physical structure of the litter material (Swift et al. 1979) and is determined by plant genetics, nutrient availability and carbon allocation (Chapin 1980; Vitousek 1982). In general, litter chemistry components that influence decomposition are the C:N ratio, lignin concentration, and polyphenols (Kuiters 1990; Lambers 1993). Plants growing in poor soils (i.e. limited nutrient supply) are characterized by low N concentrations and high secondary metabolite concentrations. In turn they produce litter low in N and high in secondary compounds which enter the decomposition cycle where reduced decomposition rates result in delays of nutrient release

which in turn decreases nutrient availability (Vitousek 1982). When N is available in quantities that meet the nutritional needs of detritivores, decomposition rates increase (Melillo et al. 1982; Bargali et al. 1993; Enriquez et al. 1993). Carbon-based compounds, such as lignin, cellulose, and phenolics, also regulate decomposition rates (Day 1982; Berendse et al. 1987). Because of its complex structure, lignin is one of the slowest decaying molecules in nature (Meentemeyer 1978) and numerous enzymes are required for lignin decomposition (Aber and Melillo 1991). Secondary metabolites, which often serve as herbivore deterrents, also reduce microbial activity (Horner et al. 1988). Lignin content has been positively correlated with immobilization (Berg et al. 1984; Berendse et al. 1987) while the (lignin + polyphenols):N ratio has been negatively correlated with net N mineralization rates of legume residues (Fox et al. 1990). Chemical composition of litter not only affects the rates of decomposition but also influences the fate of nutrients in the decomposition process (Berg and McClaugherty 1989). Litter that is low in N decays slowly, thus more N is immobilized (Berendse et al. 1987; Cuevas and Medina 1988).

Carbon dioxide-mediated changes in plant species composition, biomass partitioning, or chemical composition of plant foliage could directly influence the resulting litter quality which in turn could lead to changes in nutrient cycling and C storage during decomposition (Berg 1984; Ceulemans et al. 1999). While systems characterized by greater growth are often associated with litter that breaks down and mineralizes more rapidly than unproductive systems, potential changes in chemical composition of litter in CO_2 rich environments may outweigh productivity gains. Reduced N concentrations in the leaves of plants grown under elevated CO_2 imply that there will be a correspondence of low N in the leaf litter of plants grown under elevated CO_2. If true, low litter N could ultimately lead to lower rates of N mineralization and higher rates of immobilization in the soil, resulting in limited nutrient supply. Elevated CO_2 also results in

higher foliar C:N ratios and may increase secondary metabolites in some species of plants. Such changes may result in slower decomposition rates if these changes are retained in leaf litter entering the decomposition process (Lambers 1993; Lindroth et al. 1993). The rate of litter decomposition is expected to be slower under elevated CO_2 due to an increase of carbon-to nitrogen ratios and increase in lignin in plant tissue grown under elevated CO_2, both of which slow decay (Melillo et al. 1982; Williams et al. 1986). There have been reports of both increased and decreased rates of decomposition of plant litter from elevated CO_2 concentrations (Coûteaux et al. 1991; Cotrufo et al. 1994; Boerner and Rebbeck, 1995; Cotrufo and Ineson 1995; Gorissen et al. 1995), as well as reports of no changes in decomposition rates (Cotrufo et al. 1994; Cotrufo and Ineson, 1995; O'Neill and Norby 1996). Leaf litter of *Castanea sativa* grown in elevated CO_2 decomposed slower, within the first three months of decomposition, than litter of ambient grown *C. sativa* (Coûteaux et al. 1991). Cotrufo et al. (1999) found that *Quercus pubescens* leaf litter grown in a CO_2 spring (a naturally occurring out-gassing of CO_2) exhibited changes in the chemical composition of the litter. Polyphenolic concentrations decreased and lignin concentrations increased in litter from those oaks growing within the affected area of the CO_2 spring compared to those oaks grown in ambient CO_2. The oak litter decomposed slower in the CO_2 rich environment compared to *Q. pubescens* litter decomposing in ambient CO_2 environments. A microcosm experiment found that litter of *Danthonia richardsonii* grown under elevated CO_2 concentrations and a nitrogen fertilization regime (to mimic defoliation) had higher C:N ratios compared to control plant litter (Lutze et al. 2000). Decomposition rate, measured as cumulative respiration, was reduced for litter of plants grown in CO_2 rich environments. A mesocosm study by Ball and Drake (1997) found that decomposition rates of plant litter from high CO_2 environments were correlated to the litter C:N ratio. *Spartina patens*, a C_4 grass,

exhibited no differences in decomposition rates and the C:N ratio did not differ between plants grown at elevated and ambient CO_2 levels. For the C_3 sedge, *Scirpus olneyi*, however, plants grown under increased concentrations of CO_2 had higher C:N ratios than control plants and litter from enriched CO_2 environments had depressed rates of decomposition.

There can be complex interactions among elevated CO_2, site characteristics, and decomposition. Robinson et al. (1997) found that above ground litter of *Festuca vivipara* grown in CO_2 rich environments had higher C:N ratios and lower nitrogen concentrations than control plants. Soluble carbohydrates and alpha-cellulose were also higher in plants grown under elevated CO_2 than in plants grown under ambient levels of CO_2. When the litter was placed in two different arctic locations, however, decomposition differed significantly. The litter allowed to decompose at a high arctic site lost more mass from elevated CO_2 plants than ambient CO_2 plants. When litter was allowed to decompose at a low arctic site, however, the mass loss results were reversed. Thus decomposition of identical resources was not the same between the two arctic ecosystems. The authors speculated that differences in decomposer activity, temperature, or moisture differences between the two ecosystems may explain the contradictory results. As we might expect, the effects of elevated CO_2 on decomposition, and therefore the nutrient cycling process, depend on complex interactions among community composition, litter quality, and climate (Field et al. 1992; Melillo et al. 1995; Adams and Wall 2000).

To explore links among elevated CO_2, foliar quality, herbivory, and decomposition, this study took advantage of a long-term CO_2 project in a Florida scrub oak community. The field site is described below, followed by the hypotheses that were tested.

Study Site

The study site was located in the Kennedy Space Center on the east coast of central Florida (28°38′N, 80°42′W) USA. The soil consists of sand and sandy coquina and has an organic layer 20 cm deep. The scrub oak forest is xenomorphic largely consisting of evergreen or semi-evergreen trees with a mature canopy height of 3 to 5 m. This plant community is fire controlled and was last burned January 1996. Prior to site burning the plant composition consisted primarily of oak species (76% *Quercus myrtifolia*, 15% *Quercus geminata*, 7% *Quercus chapmanii*). The remaining 2% of the community included *Serenoa repens* (palmetto), *Myrica serifera* (wax myrtle), *Lyonia ferruginea* (rusty lyonia), *Ceratiola ericoides* (Florida rosemary), and *Galactia elliottii* (milk pea). Sixteen open-top chambers were conctructed on site. Eight chambers have recieved elevated CO_2 treatments (ambient + 350 ppmV) continuously since May 14, 1996 while the other eight chambers have served as ambient controls.

Overview

There are a number of potential routes that can lead to changes in litter quality and ecosystem function when CO_2 increases (Figure 1.1). Changes in foliar chemistry as a direct result of elevated CO_2 could lead to changes in litter chemistry, which subsequently affect the decomposition process and nutrient cycling. Depending on whether a negative or positive feedback system dominates will determine if decomposition rates increase or decrease. Changes in foliar chemistry could also affect insect herbivores. A low quality food source could result in increased herbivore mortality whether directly due to the low nutritional quality of the food or resulting from increased susceptibility to pathogens, parasitoids, and predators. The decrease in herbivores could lead to lower defoliation rates, less greenfall and an overall decline in litter

quality, which could have impacts on nutrient cycling within the system. Herbivore activity could also interact with increases in CO_2 resulting in an exaggerated plant defense response. If phytochemical induction is high when herbivory and CO_2 work in tandem then litter quality may decline again affecting the fate of nutrients within the ecosystem. This study attempts to explore the effects of elevated CO_2 on foliar quality, litter quality, and herbivory along with associated changes to the decomposition process.

Chapter 2 focuses on the effects of CO_2 on foliar quality and insect herbivory. In Chapter 2, three oak species comprising 98% of the scrub oak community and the nitrogen-fixing legume (*Galactia elliottii*) were used to examine the effects of elevated CO_2 on foliar chemistry and herbivore damage in the scrub oak community. There were several hypotheses for this portion of the study. Hypothesis I. The chemical composition of oak foliage, regardless of species, will exhibit decreases in nitrogen and increases in C:N ratios and secondary metabolites (condensed tannins, hydrolysable tannins, total phenolics) under elevated CO_2 and the foliage of the nitrogen-fixing legume will exhibit little change in nitrogen concentrations since the ability to fix nitrogen may result in less nitrogen dilution. Hypothesis II. Herbivore damage will decline on the oaks under elevated CO_2 because herbivores are unable to compensate for decreases in foliar nitrogen under the rigors of field conditions. Hypothesis III. There will be little change in herbivore damage on the nitrogen-fixing legume.

Chapter 3 examines the changes in litter quality due to the effects of CO_2 and herbivore damage. Chapter 3 addresses the issue of defining links between foliar quality and litter quality and the influence of insect herbivores on litter quality. Leaf litter chemistry was followed for three years and focused on the three oak species. Two hypotheses associated with this study were: Hypothesis IV. The chemical composition of plant foliage is reflected in the quality of leaf

litter grown under ambient and elevated CO_2. Hypothesis V. Herbivore damage will have a stronger effect on litter quality than the direct effects of elevated CO_2.

Chapter 4 focuses on the decomposition of litter grown in ambient and elevated CO_2 treatments along with the effects of herbivory. Chapter 4 is the culmination of a study in which the litter of a single species, *Quercus myrtifolia*, was followed throughout three years of decomposition. The litter was divided by CO_2 treatment and four categories of herbivore damage (undamaged, chewed, mined, miscellaneous damage). Hypothesis VI. Due to changes in litter quality, litter from CO_2 rich environments will decompose slower than litter from ambient CO_2 environments.

Finally, Chapter 5 ties the results of the preceeding chapters together to give a summary of the effects of CO_2 on foliar quality and any links between foliar quality and litter quality.

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Figure 1.1. Potential routes by which changes in ecosystem function could occur under cases of increased CO_2

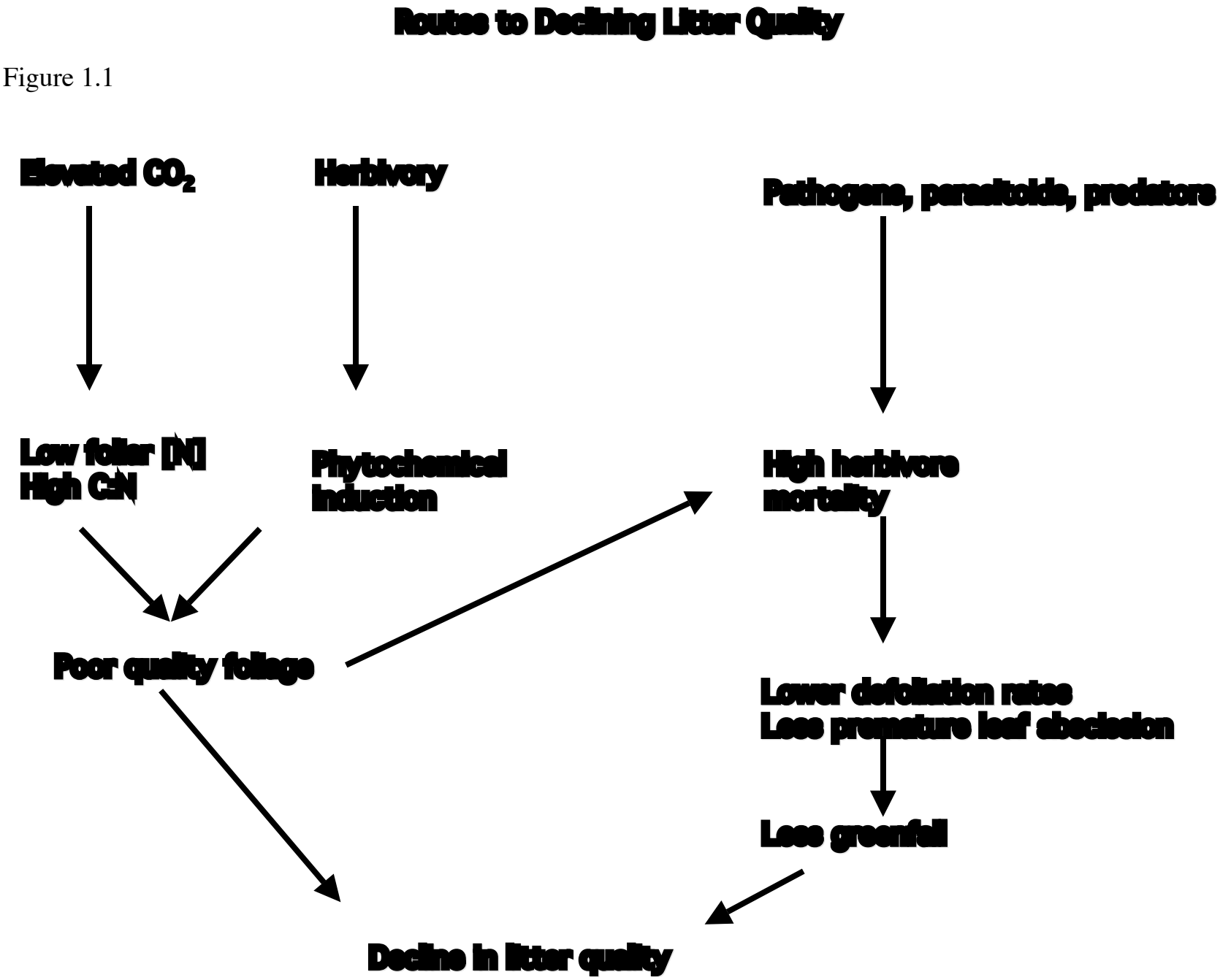
Routes to Declining Litter Quality
Elevated CO_2
Herbivory
Pathogens, parasitoids, predators
Low foliar [N]
High C:N
Phytochemical induction
High herbivore mortality
Poor quality foliage
Lower defoliation rates
Less premature leaf abscission
Less greenfall
Decline in litter quality

Figure 1.1

CHAPTER 2

EFFECTS OF ELEVATED CO_2 ON FOLIAR QUALITY AND HERBIVORE DAMAGE IN A SCRUB OAK ECOSYSTEM[1]

[1] Hall, M.C., *P*. Stiling, D. C. Moon, B. G. Drake, And M. D. Hunter. 2005. Journal of Chemical Ecology 31(2):267-286.

Abstract – Atmospheric CO_2 concentrations have increased exponentially over the last century and continuing increases are expected to have significant effects on ecosystems. We investigated the interactions among atmospheric CO_2, foliar quality, and herbivory within a scrub oak community at the Kennedy Space Center, Florida. Sixteen plots of open-top chambers were followed; eight of which were exposed to ambient levels of CO_2 (350 ppm), and eight of which were exposed to elevated levels of CO_2 (700 ppm). We focused on three oak species, *Quercus geminata*, *Quercus myrtifolia*, *Quercus chapmanii*, and one nitrogen fixing legume, *Galactia elliottii*. There were declines in overall nitrogen and increases in C:N ratios under elevated CO_2. Total carbon, phenolics (condensed tannins, hydrolysable tannins, total phenolics) and fiber (cellulose, hemicellulose, lignin) did not change under elevated CO_2 across plant species. The plant species differed in their relative foliar chemistries over time, however, the only consistent differences were higher nitrogen concentrations and lower C:N ratios in the nitrogen fixer when compared to the oak species. Under elevated CO_2, damage by herbivores decreased for four of the six insect groups investigated. The overall declines in both foliar quality and herbivory under elevated CO_2 treatments suggest that damage to plants may decline as atmospheric CO_2 levels continue to rise.

Key Words – Elevated CO_2, *Quercus myrtifolia*, *Quercus chapmanii*, *Quercus geminata*, *Galactia elliottii*, herbivory, nitrogen fixer, Kennedy Space Center.

INTRODUCTION

Atmospheric carbon dioxide (CO_2) concentrations began increasing with the advent of the industrial revolution and are continuing to increase at a rate of approximately 4 ppm annually. At the current rate of increase CO_2 levels are expected to double by the end of this century to 750 ppm. The increase in atmospheric CO_2 is expected to have significant effects on ecosystems, including short-term physiological changes in plants and long-term changes in ecosystem structure and function. Numerous studies have been conducted to determine the effects of elevated CO_2 on plants and their associated communities. It has been found that plant responses to elevated CO_2 concentrations are idiosyncratic within and among species (Williams et al. 1986; Lindroth et al. 1993; Curtis et al. 1996; Johnson et al. 1996; Mousseau et al. 1996; Cook et al. 1997; Van Gardingen et al. 1997; Woodward and Beerling, 1997). Some general trends, however, have emerged.

In the short term, elevated CO_2 increases photosynthesis across various ecosystems (Drake et al. 1997; Norby et al. 1999), including arctic tundra (Oberbauer et al. 1986; Tissue and Oechel 1997), grasslands (Smith et al. 1987), and deciduous forests (Williams et al. 1986). After a period of time, some species adjust their photosynthetic rates whereas other species show little or no adjustment (Williams et al. 1986; Smith et al. 1987; Tissue and Oechel 1987). Root to shoot ratio (Ceulemans and Mousseau 1994; Mousseau et al. 1996) and biomass tend to increase (Leadley et al. 1999; Owensby et al. 1999). Consistent plant chemical changes include increases in foliar C:N ratios and decreases in foliar nitrogen concentrations (Bezemer and Jones, 1998). According to some plant defense hypotheses, increases in carbon availability should result in

increases in carbon based secondary metabolites (Bryant et al. 1983; Tuomi et al. 1984) though there have been no consistent effects of CO_2 on secondary metabolite concentrations (Bazzaz 1990).

Resource availability appears to influence the degree to which plant species adjust to elevated CO_2 (Bazzaz 1990; Lindroth et al. 1993). The effect of elevated CO_2 on plants is enhanced when other resources such as water, light and nutrients are abundant (Bloom et al. 1985; Chapin et al. 1987), and the effects of CO_2 on plants are mitigated when nitrogen or phosphorus is limiting (Zangerl and Bazzaz 1984; Brown and Higginbotham 1986). Plant responses appear to be linked to the ability of species to store carbohydrates by increasing the size and /or number of leaves, length of roots, and fine root turnover (Ceulemans and Mousseau 1994; Johnson et al. 1996; Mousseau et al. 1996).

Changes in the nutritional and defensive characteristics of host plants may result in behavioral changes and / or physiological changes in herbivores (Lindroth 1996; Bezemer and Jones 1998; Stiling et al. 1999). Lower levels of nitrogen and higher C:N ratios in plants under elevated CO_2 have generally been associated with compensatory feeding and subsequent increases in levels of damage or defoliation (Lincoln et al. 1984,1993; Fajer et al.1989; Lindroth et al. 1993, 1995; Salt et al. 1995; Docherty et al. 1997; Kinney et al. 1997; Williams et al. 1997; but see Hamilton et al. 2004).

Leaf-chewing insects such as grasshoppers (Johnson and Lincoln 1990) and lepidopteran larvae (Lindroth et al. 1993, 1995) generally consume more leaf area when fed plants that have been grown under elevated CO_2. Likewise, the area damaged by leaf-mining insects may also increase (Salt et al. 1995). Lepidopteran larvae exhibit increased mortality and slower growth rates when feeding on elevated CO_2 plants (Akey and Kimball 1989; Fajer 1989; Fajer et al.

1989; Fajer et al. 1991). Consequently, herbivores may become more susceptible to pathogens, parasitoids, and predators (Price et al. 1980; Lindroth 1996; Stiling et al. 1999). For example, increases in mortality of leaf miners feeding on elevated CO_2 plants have been linked to increases in parasitism (Stiling et al. 1999). In the longest field study to date, some insect herbivore populations have been shown to decline markedly under elevated CO_2 (Stiling et al. 1999, 2002, 2003).

Our study used open top chambers in a field-based experiment to examine the effects of elevated CO_2 on foliar chemistry and herbivore damage in a scrub oak community. We focused on four dominant plant species within the community; three oaks and a nitrogen-fixing legume, and hypothesized that the three oak species would respond similarly with decreases in nitrogen and increases in C:N ratios and secondary metabolites under elevated CO_2. We further hypothesized that the nitrogen-fixing legume would exhibit little change in nitrogen concentrations since the ability to fix nitrogen may result in less nitrogen dilution. Additionally, we measured foliar damage by six different herbivore-feeding guilds on the four plant species. We hypothesized that herbivore damage would decline on the oak species under elevated CO_2 because we expected that herbivores would be unable to compensate for decreases in foliar nitrogen under the rigors of field conditions. We predicted that there would be little change in herbivore damage on the nitrogen-fixing legume.

METHODS AND MATERIALS

Study Site. Our study site lies within a two-hectare native scrub-oak community located at Kennedy Space Center, Florida. This woody ecosystem is controlled by a natural fire return cycle of 8 – 12 years and the mature canopy is 3 – 5 meters high. The last burn cycle was in 1996

prior to site set up. Sixteen 3.6 – m diameter plots, each enclosed with a clear polyester film open-top chamber 3.4 m in height, were utilized to control CO_2 levels. Chambers were overlaid on an octagonal framework of PVC pipe with a removable access door and frustrum to reduce dilution of air within the chamber by outside wind. All re-growth was cut to ground level in May 1996 and, since that time, the vegetation in eight of the chambers has been exposed to almost twice ambient CO_2 (700 ppm) while the other eight chambers have been exposed to ambient levels of CO_2 (350 ppm). The CO_2 is supplied 24 hours a day. Monitoring and control of CO_2 injection into each chamber is done by infrared gas analyzer in conjunction with manually adjusted needle valves. In ambient CO_2 chambers, the airflow is identical to that of the elevated CO_2 chambers but is not supplemented with CO_2. Four study species of plants dominate this community and are present in every chamber: three oak species, *Quercus myrtifolia* Willd; *Q. chapmanii* Sargent; *Q. geminata* Small; and the nitrogen fixing legume, *Galactia elliottii* Nuthall. Chambers were originally established to investigate effects of elevated CO_2 on plant productivity and nutrient cycling. Studies of herbivores and leaf chemistry were a later addition to the project.

Foliar Chemistry. Samples of fresh leaves from each of the four study species were collected for chemical analysis. We haphazardly removed four undamaged leaves from each of three individuals per chamber from each of the three oak (*Q. myrtifolia*, *Q. chapmanii*, and *Q. geminata*) species every three months (May 2001 – May 2003). While in the field, a hole punch was used to remove two disks of leaf tissue from each leaf. One disk was used to obtain the dry weight of the disk while the other disk was placed into 70 / 30 acetone / water with 1mM ascorbic acid and used for subsequent phenolic analysis. The remaining portion of the leaf was returned to the lab on ice, dried, and used to measure C, N and fiber (cellulose, hemicellulose,

lignin). Because of its small leaf size, the collection method for *G. elliottii* differed slightly. Two opposite leaflets, each from three individuals of *G. elliottii*, were collected from each chamber. One leaflet was placed in acetone to be used for phenolic analysis; the opposite leaflet was placed in a bag and used to obtain leaf weights and, subsequently, C, N and fiber content. Samples from different individual plants of a given species were pooled within chambers so that chambers (8 per treatment) acted as replicates. Dried leaves were ground to a fine powder and stored at -80^0 C prior to analysis.

Percent dry weight nitrogen and carbon were estimated from leaf powder on a Carlo-Erba NA1500 model C/N analyzer (Milan, Italy). These data also provided estimates of foliar C : N ratios. Sub-samples of leaf powder (above) were also used to assess the effects of elevated CO_2 on foliar concentrations of cellulose, hemicellulose, and lignin by sequential neutral detergent / acid detergent digestion on an Ankom fiber analyzer (Abrahamson et al. 2003).
Phenolic analysis was conducted using the leaf disks collected into 70% acetone in the field. Proanthocyanidins, an estimate of condensed tannin, were assayed using N-butanol:HCL methods described in Rossiter et al. (1988). Total phenolics were estimated using the Folin-Denis assay (Swain, 1979), and gallotannins (hydrolysable tannins) were estimated using a potassium iodate technique developed by Bate-Smith (1977) and modified by Schultz and Baldwin (1982). Standards for tannin analysis were generated by multiple sequential washes of bulk samples (one for each species) by acetone extraction. All tannin assays produced colorimetric readings, in proportion to tannin concentration, which were quantified using a BioRad microplate reader.

Damage Estimates on Green Leaves. We counted two hundred randomly selected leaves from each of the plant species from each chamber every three months starting in May 2001 and

ending in May 2002. Each leaf was scored for the presence of six types of herbivore damage; leaf gall, eye spot gall, leaf tier, chewed leaf, mined leaf, and (*G. elliottii* only) leaf mite.

Statistical Analysis. All data met the assumptions of normality (Kolmogorov-Smirnov test, $\alpha = 0.05$) and were analyzed using parametric statistics. Foliar chemistry was analyzed over a two-year period (May 2001 – May 2003). This represents 9 sampling dates for the oak species and 8 for *G. elliottii*, which was absent from all chambers during February 2003. Variation in foliar quality (carbon, nitrogen, fiber, and phenolics) under elevated and ambient CO_2 treatments across time was analyzed using repeated measures GLM with Tukey's HSD test for significant differences ($\alpha = 0.01$). Damage estimates were analyzed for a single year (May 2001 – May 2002). Variation in herbivore damage under elevated and ambient CO_2 treatments across time was analyzed using repeated measures GLM with Tukey's HSD test for significant differences ($\alpha = 0.05$)

RESULTS

Foliar Chemistry. As predicted, foliar nitrogen concentrations declined and C:N ratios increased under elevated CO_2 treatments. The mean percent dry weight of nitrogen was 1.37 (SE ± 0.02) under ambient CO_2 conditions and 1.25 (SE ± 0.02) under elevated CO_2 conditions ($F_{1,38} = 9.20$, $P = 0.004$). The mean C:N ratio was 38.57 (SE = 0.56) in the ambient treatments and 41.74 (SE = 0.58) in the elevated treatments ($F_{1,38} = 13.39$, $P < 0.001$). Contrary to our predictions, foliar phenolics, total carbon, and fiber content were unaffected by elevated CO_2 (Table 2.1). There were no significant treatment (CO_2) by date or species interactions suggesting that elevated CO_2 also caused reductions in foliar nitrogen of the nitrogen-fixer, *G. elliottii* comparable to those observed in the three *Quercus* species. Hydrolysable tannin concentrations

were greatest in *Q. chapmanii* and lowest in *Q. geminata* while total phenolic concentrations were greatest in *G. elliottii* and lowest in *Q. geminata* (hydrolysable tannins, $F_{3,33}$= 4.94, P = 0.006; total phenolics, $F_{3,21}$= 9.63, $P < 0.001$). Nitrogen concentrations were highest and carbon and C:N ratios lowest for the nitrogen fixer *G. elliottii*, while *Q. myrtifolia* had the lowest levels of nitrogen and highest levels of carbon and C:N ratio (statistical values for species effects on nitrogen, carbon and C:N ratio respectively; $F_{3,38}$= 9.74, $P < 0.001$; $F_{3,38}$= 7.09, $P < 0.001$, $P <$ 0.001; $F_{3,38}$= 12.16, $P < 0.001$). *Q. geminata* had the highest percentage of fiber (cellulose, hemicellulose). *G. elliottii* had the lowest levels of cellulose and hemicellulose (cellulose, $F_{3,37}$= 9.18, P = 0.001; hemicellulose, $F_{3,37}$=7.49, $P < 0.001$) . Species differed in their relative foliar chemistries over time (Figure 2.1). The only consistent differences among species were higher nitrogen concentrations and lower C:N ratios in *G. elliottii* compared with the three oak species (Figure 2.1a, c).

Damage Estimates on Green Leaves. Herbivore damage by chewers, miners, eye spot galls, and leaf tiers declined significantly under elevated CO_2 (chewed, $F_{1,52}$ = 29.01, $P < 0.001$; mined $F_{1,52}$=17.06, $P < 0.001$; eye spot galls, $F_{1,38}$= 14.91, $P < 0.001$; leaf tier $F_{1,38}$= 6.92, P = 0.012) (Figure 2.2). Additionally, there was a weak trend for leaf mite and leaf gall damage to decline under elevated CO_2 (Figure 2.2). Chewers and miners occurred on all species while leaf galls, eye spot galls and leaf tiers occurred exclusively on the *Quercus* species and leaf mites occurred exclusively on *G. elliottii* (Table 2.2). Chewing damage was higher under ambient than elevated CO_2 during all months except November (date * CO_2 interaction $F_{4,208}$ = 7.18, $P < 0.001$, Figure 2.3a). Leaf tier damage was higher under ambient than elevated CO2 during all months except May 2001 and February 2002 (Date * CO_2 interaction $F_{4,152}$= 2.98, P = 0.021, Figure 2.3b). Effects of elevated CO_2 on chewer damage were particularly pronounced on *Q. chapmanii*

($F_{3,52}$= 4.30, P = 0.009, Figure 2.4a), while CO_2 effects on eye spot galls were pronounced on *Q. myrtifolia* and *Q. geminata* ($F_{2,38}$= 7.31, P = 0.002, Figure 2.4b).

DISCUSSION

Though not all plants respond identically to increased concentrations of CO_2 (Bezemer and Jones, 1998; Hunter, 2001), all four of our study species responded in a similar fashion. While there were differences in overall chemical composition among the four species (Table 1), all four plant species exhibited declines in foliar nitrogen concentrations and increases in C:N ratios under elevated CO_2, while polyphenolics and fiber were unaffected. The changes in nitrogen levels and C:N ratios in the oak species are not surprising given the consistency with which this has been found in previous studies (Cipollini et al. 1993; Ceulemans and Mousseau, 1994; Luo et al. 1994; Curtis et al. 1996; Wilsey, 1996; Stiling et al. 1999; Reich et al. 2001). Finding the same response in the nitrogen fixer, however, was not expected. Reich et al. (2001) did report that aboveground (foliar) nitrogen levels declined in four legumes (*Amorpha canescens*, *Lespedeza capitata*, *Lupinus perennis*, *Petalostemum villosum*) while below ground (root) nitrogen levels remained unchanged under elevated CO_2. This suggests that even nitrogen fixers exhibit modified leaf nitrogen under elevated CO_2. More surprising, however, was the magnitude of change in nitrogen levels and C:N ratios of the nitrogen fixer when compared to the three oak species. The percent decrease in nitrogen and increase in C:N ratio of the nitrogen fixer under elevated CO_2 was more than twice the percent change seen in the three oak species (Figure 2.5). Given that lower nitrogen levels appear to be the primary driver affecting herbivores under elevated CO_2, the decrease in foliar nitrogen and increases in C:N ratios may be expected to have strong effects on herbivores that depend on nitrogen fixing plant species.

Though nitrogen levels were lower and C:N ratios higher under elevated CO_2 conditions there were no treatment effects on the secondary compounds measured (condensed tannins, hydrolysable tannins, total phenolics, cellulose, hemicellulose, and lignin). It is expected that increased availability of carbon via increased levels of CO_2 would result in changes in carbon-based compounds in plants. However, studies thus far have been inconclusive. Some species increase in levels of phenolics while others decrease or remain the same (Bezemer and Jones, 1998, Hamilton et al. 2004). Drury et al. (1998) found short term increases in phenolics in *Quercus robur*. Lindroth et. al (1993) examined seedlings of three tree species(*Populus tremuloides*, *Quercus rubra*, *Acer saccarum*) and found varying responses in phenolic concentrations with increases, decreases, and no changes being recorded. The phenolic concentrations also varied with leaf flush (i.e. time of CO_2 exposure). Williams et al. (1998) found that young leaves of *Quercus alba* had significantly lower leaf nitrogen content and significantly higher total nonstructural C:N ratio as plant CO_2 concentrations rose, while nonstructural carbohydrates and total carbon-based phenolics were unaffected by plant CO_2 treatment. In some instances total non-structural carbohydrates (TNC), especially starch, show large increases in elevated CO_2 conditions (Saxe et al. 1998). Also in woody species, available carbon might be allocated to woody tissue rather than foliar tissue. Therefore it is possible that changes in carbon concentrations occurred in our species, but were not found because we did not measure TNC or woody tissue.

In our study, herbivore damage by a number of feeding guilds was lower under elevated CO_2. Additionally, there were no treatment by plant species interactions indicating that the response to elevated CO_2 was the same across all plant species. The nutritional quality of plant foliage can have profound effects on herbivores (Feeny 1968; Coley and Aide, 1991; Feeny,

1992; Dury et al. 1998). Low nitrogen and high concentrations of polyphenolic compounds and lignin indicate low nutritional quality. Nitrogen is often a limiting resource for herbivores and limited nitrogen levels can lead to decreased growth rates, development, and fecundity (Scriber and Slansky 1981; Schultz and Baldwin, 1982; White 1984; Bazzaz, 1990). High concentrations of polyphenolic compounds, such as tannins, may act to inhibit digestion. Increased fiber can lead to tougher and / or indigestible leaves (Feeny 1968; Schultz and Baldwin, 1982; Schowalter et al. 1986; Coley and Aide, 1991; Feeny, 1992; Dury et al. 1998; Williams et al. 1998).

Studies showing compensatory feeding by herbivores on high C:N foliage have occurred exclusively under laboratory conditions (Lincoln et al. 1986; Fajer, 1989; Johnson and Lincoln, 1990). Low nitrogen foliage may increase development time and thus under field conditions increase the risks posed to herbivores by natural enemies (Stiling et al. 1999, 2002, 2003) or abiotic factors.

The decline in foliar nitrogen concentrations and increase in C : N ratios in our study are the only indication of low foliar quality under elevated CO_2 conditions. Previous studies have shown that low quality foliage has negative impacts on leafmining insects (Stiling et al. 1999, 2002, 2003). These impacts appear to result from the combined effects of nutrient limitation and increases in parasitism and predation. Stiling et al. (1999) found that decreases in plant quality due to elevated CO_2 doubled leafminer mortality while increases in parasitism due to elevated CO_2 quadrupled leafminer mortality. Insect herbivores often compensate for low foliar quality by increasing their food intake (Karban and Baldwin, 1997; Agrawal et al. 1999), yet such compensation did not act to increase the proportion of leaves damaged under elevated CO_2 in our study.

Herbivore damage can influence foliar and litter quality (Agrawal et. al. 1999; Findlay et. al. 1996) by a number of mechanisms including chemical induction and premature leaf abscission. These changes can affect vital ecosystem processes, such as decomposition and nutrient cycling. Therefore elevated CO_2 can potentially influence these ecosystem processes via direct effects through changes in foliar quality and indirect effects through herbivores that change foliar and litter quality. Future research will investigate some of these ecosystem level effects.

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TABLE 2.1. FOLIAR CHEMISTRIES OF FOUR PLANT SPECIES UNDER ELEVATED AND AMBIENT LEVELS OF CO_2

	Condensed Tannins		Hydrolysable Tannins		Total Phenolics	
Species	Ambient	Elevated	Ambient	Elevated	Ambient	Elevated
Q. myrtifolia	33.56	35.11	35.31	36.23	42.94	43.50
	(1.50)	(1.76)	(1.68	(2.12)	(2.60)	(2.70)
Q. chapmanii	41.50	44.66	38.97	39.49	42.21	44.04
	(1.89)	(1.59)	(2.50)	(2.43)	(2.35)	(2.31)
Q. geminata	25.76	28.65	28.15	30.34	33.10	35.60
	(1.43)	(1.47)	(1.20)	(1.39)	(2.13)	(2.23)
G. elliottii	37.37	38.84	32.32	36.40	48.88	52.38
	(2.38)	(2.62)	(2.87)	(3.02)	(2.95)	(3.03)

	Nitrogen		Carbon		C:N Ratio	
Species	Ambient	Elevated	Ambient	Elevated	Ambient	Elevated
Q. myrtifolia	**1.12**	**1.07**	50.73	50.78	**45.83**	**48.44**
	(0.02)	(0.02)	(0.17)	(0.27)	(0.62)	(0.77)
Q. chapmanii	**1.29**	**1.21**	49.00	49.25	**38.77**	**41.79**
	(0.02)	(0.02)	(0.17)	(0.37)	(0.71)	(0.92)
Q. geminata	**1.19**	**1.12**	49.23	49.66	**42.11**	**45.29**
	(0.02)	(0.02)	(0.22)	(0.37)	(0.62)	(0.72)
G. elliottii	**2.03**	**1.71**	48.47	48.78	**24.66**	**28.95**
	(0.04)	(0.04)	(0.29)	(0.44)	(0.65)	(0.66)

	Cellulose		Hemicellulose		Lignin	
Species	Ambient	Elevated	Ambient	Elevated	Ambient	Elevated
Q. myrtifolia	20.55	20.69	13.26	13.48	9.18	9.48
	(0.46)	(0.45)	(0.31)	(0.26)	(0.50)	(0.47)
Q. chapmanii	18.37	17.52	13.02	12.84	8.09	8.74
	(0.43)	(0.42)	(0.31)	(0.30)	(0.44)	(0.56)
Q. geminata	26.11	25.91	15.37	15.19	10.32	10.49
	(0.51)	(0.46)	(0.29)	(0.27)	(0.47)	(0.48)
G. elliottii	15.35	14.05	12.29	11.22	9.14	9.52
	(0.39)	(0.53)	(0.40)	(0.51)	(1.47)	(1.51)

Data represent % dry weights (except C:N ratio) and are the means of 72 samples except G. elliottii (64 samples). Standard errors are in parentheses. Significant treatment differences are in bold.

TABLE 2.2. HERBIVORE DAMAGE ON FOUR PLANT SPECIES AT THE KENNEDY SPACE CENTER, FLORIDA

	Chewing Damage	Mined Damage	Eye Spot Gall Damage	Leaf Tier Damage	Leaf Gall Damage
Species					
Q. myrtifolia	11.61	13.35	12.56	5.56	2.17
	(1.07)	(1.01)	(1.40)	(0.76)	(0.55)
Q. chapmanii	18.75	12.49	0.43	5.60	0.33
	(2.14)	(1.34)	(0.15)	(1.13)	(0.13)
Q. geminata	9.84	7.60	7.12	4.59	1.27
	(0.96)	(0.88)	(0.70)	(0.73)	(0.37)
G. elliottii	7.45	5.89	.	.	.
	(0.86)	(1.00)	(.)	(.)	(.)
P value	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P = 0.807$	$P = 0.018$

Not all herbivore damage occurred on all plant species, therefore data are the means of 80 samples for chewers and miners and 60 samples for eye spot galls, leaf tiers, and leaf galls. Standard errors are in parentheses and p values represent the significance of species effects on herbivore damage.

Figure 2.1. Foliar chemistry of plant species over time. Nitrogen = a, carbon = b, C:N ratios = c, condensed tannins = d, hydrolysable tannins = e, total phenolics = f, cellulose = g, hemicellulose = h, lignin = i. Data are the means of 16 samples (chambers). Bars represent standard errors. Note that *G. elliottii* was absent from all chambers in February 2003.

Figure 2.1

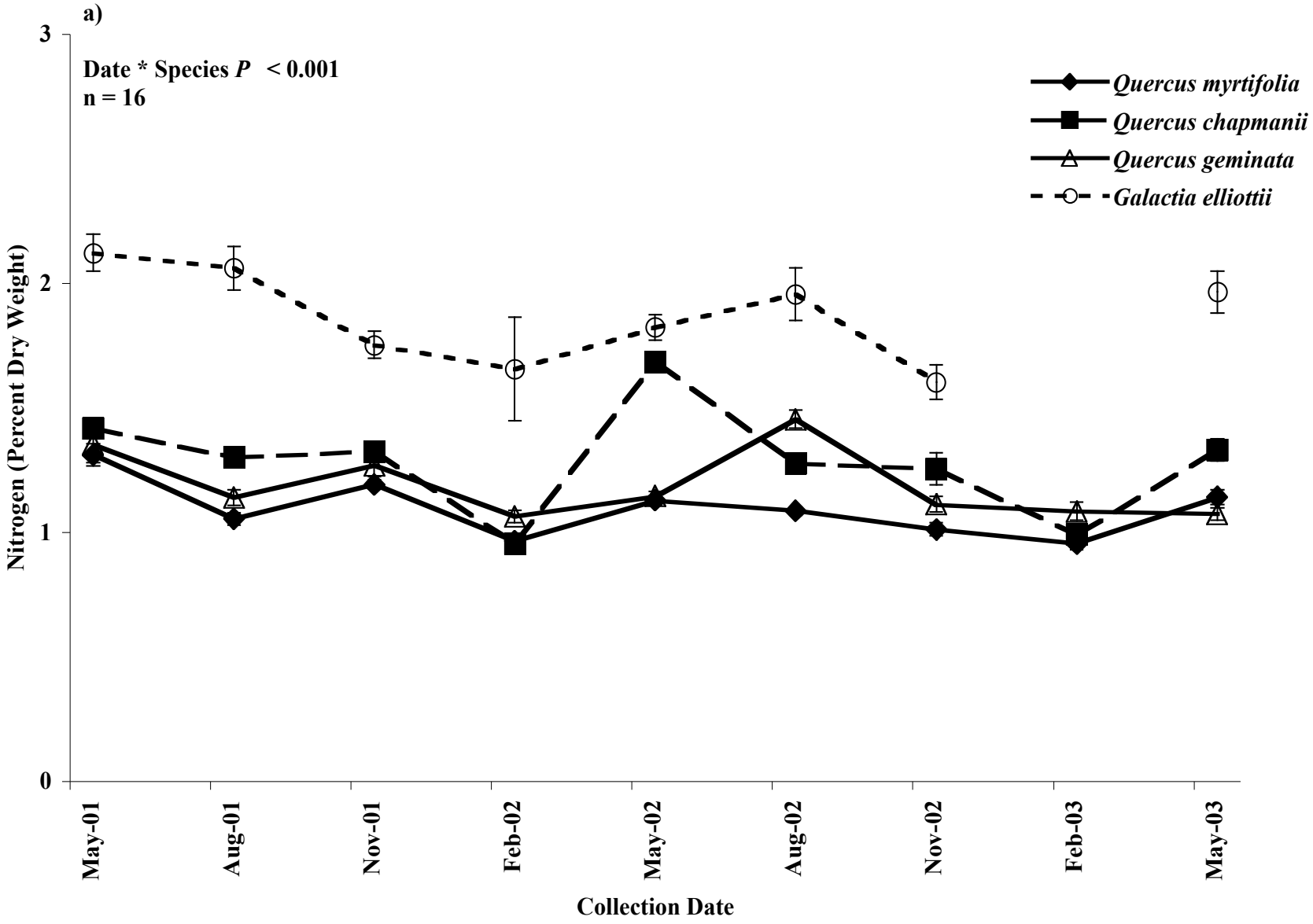

a)
Date * Species P < 0.001
n = 16
Quercus myrtifolia
Quercus chapmanii
Quercus geminata
Galactia elliottii
Nitrogen (Percent Dry Weight)
0
1
2
3
May-01
Aug-01
Nov-01
Feb-02
May-02
Aug-02
Nov-02
Feb-03
May-03
Collection Date

Figure 2.1 cont.

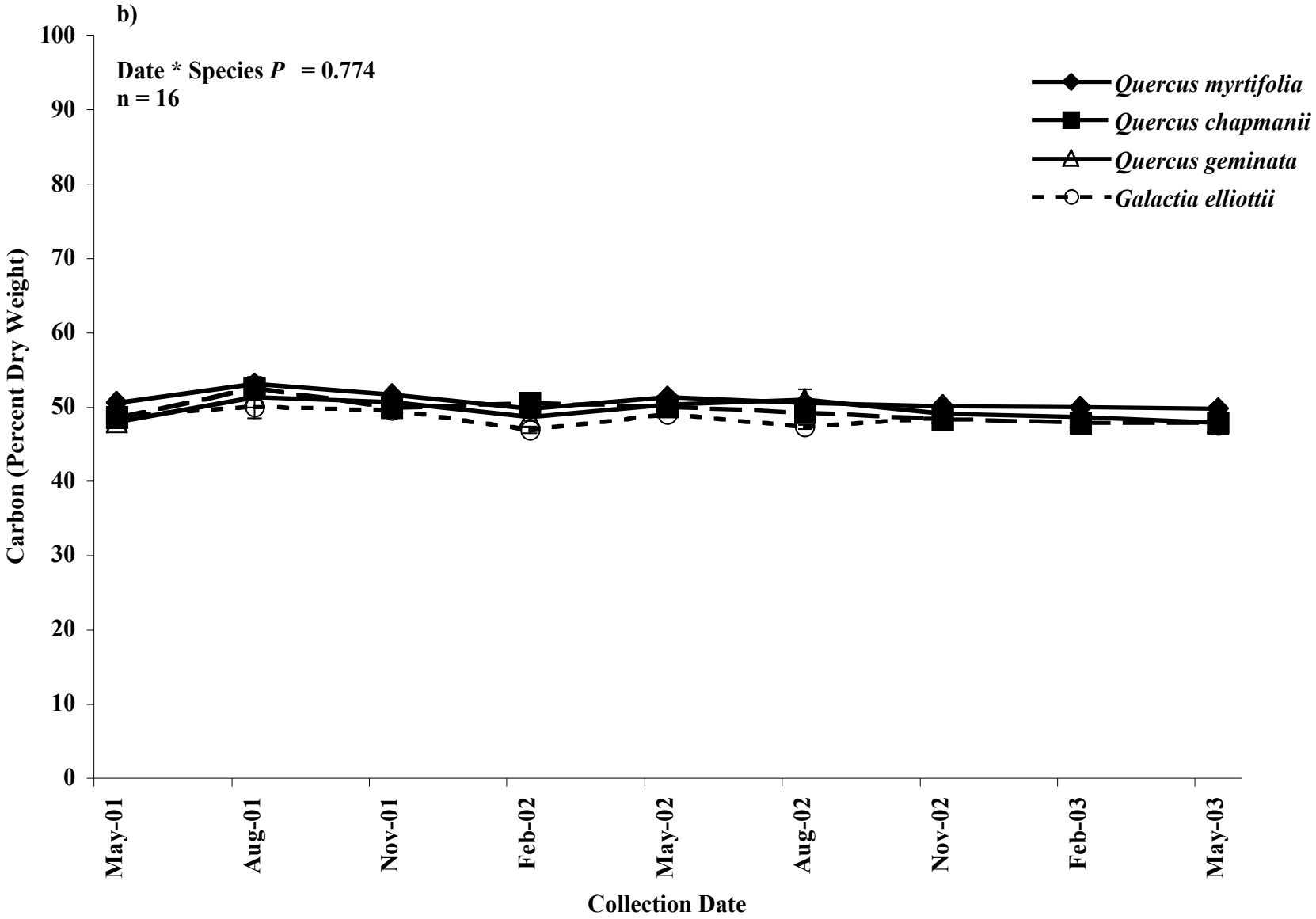


Figure 2.1 cont.

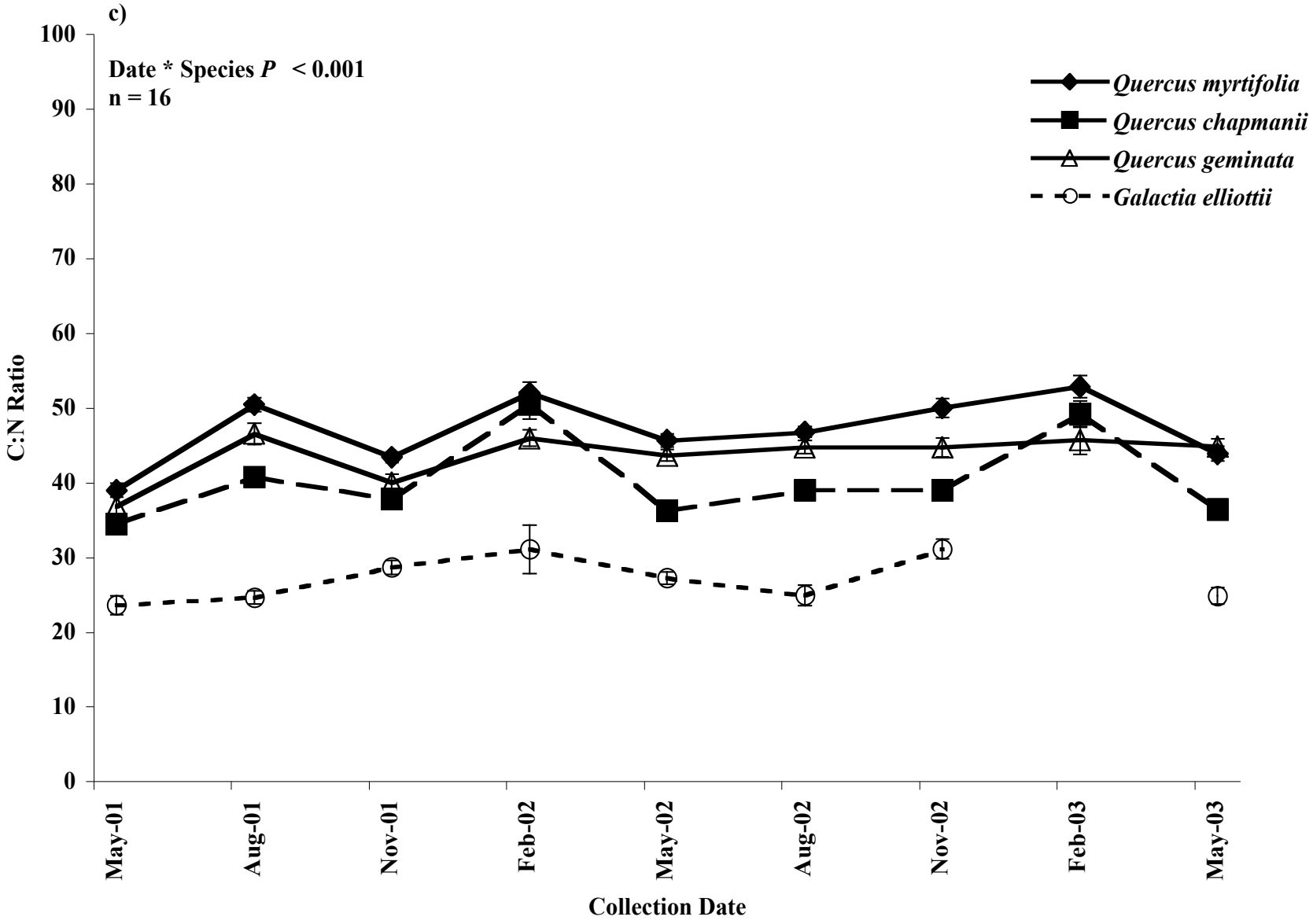


Figure 2.1 cont.

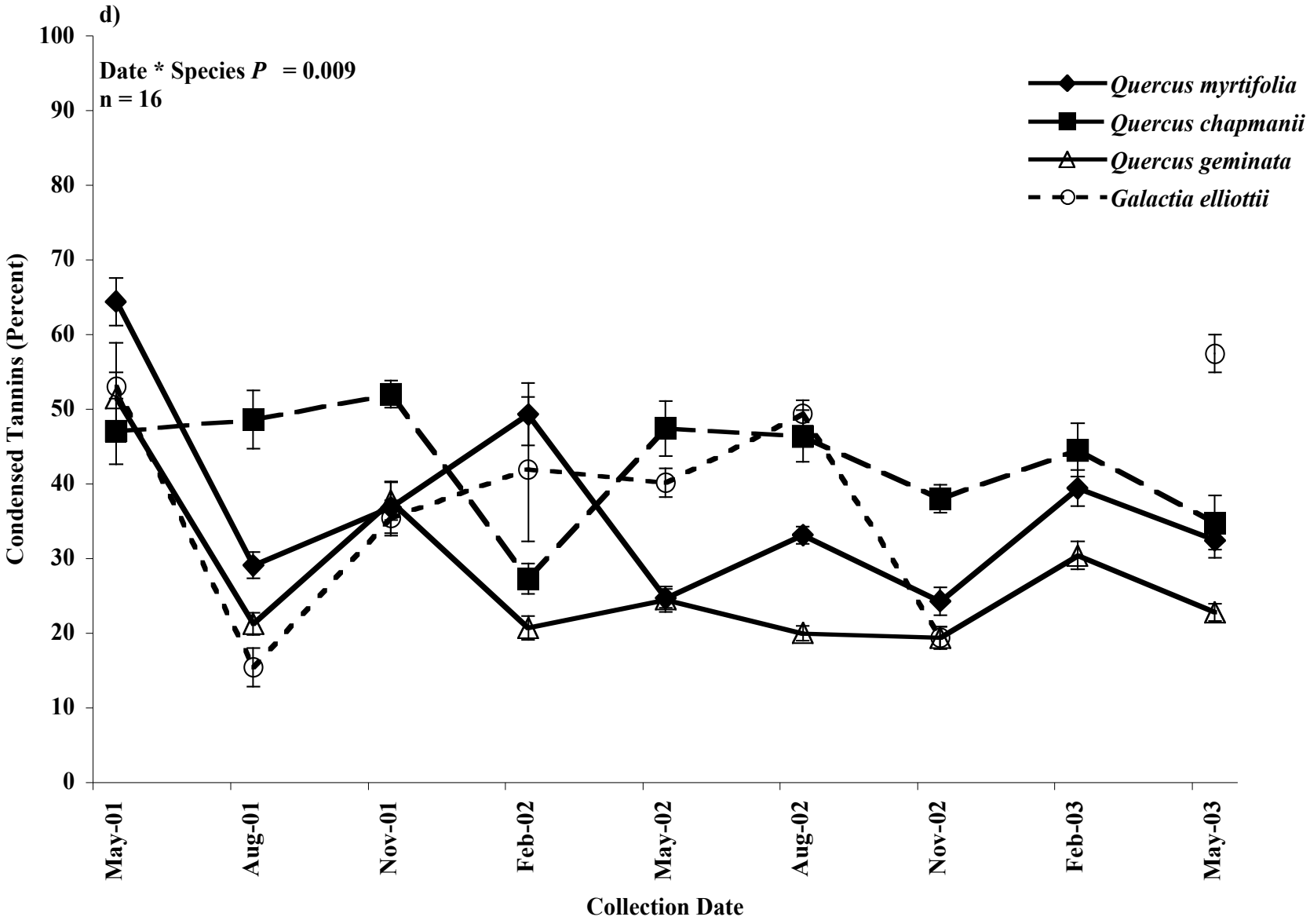


Figure 2.1 cont.

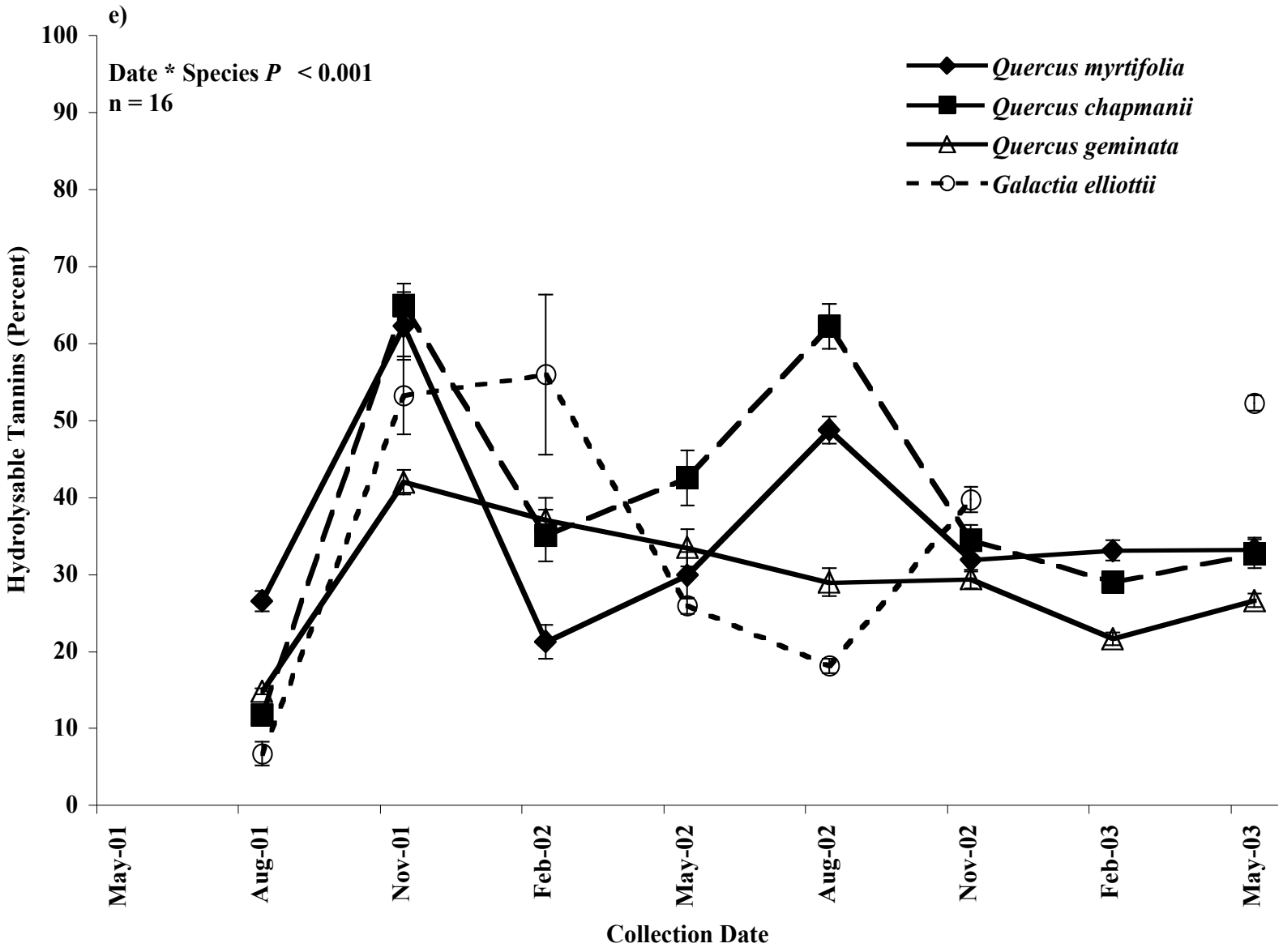


Figure 2.1 cont.

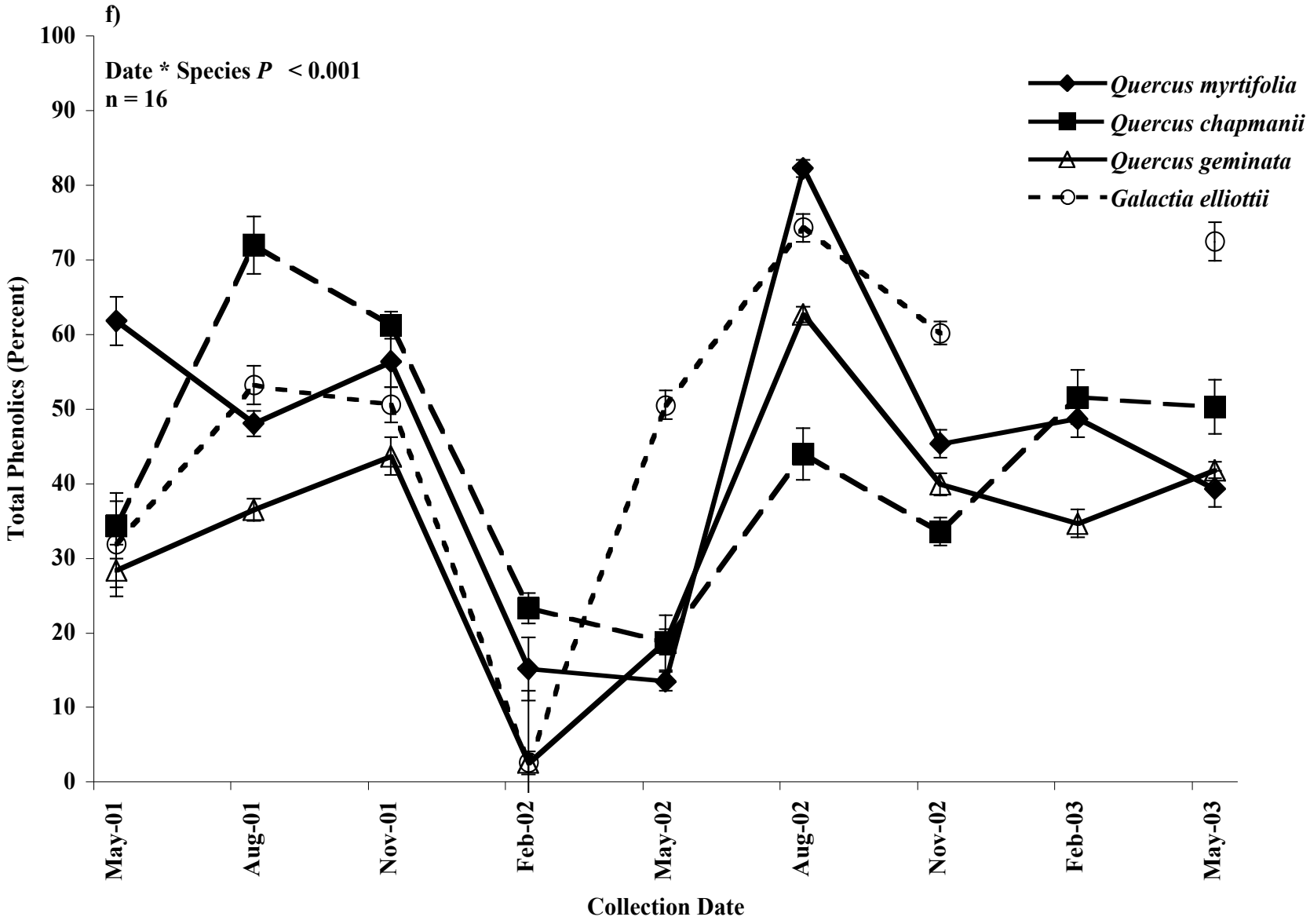


Figure 2.1 cont.

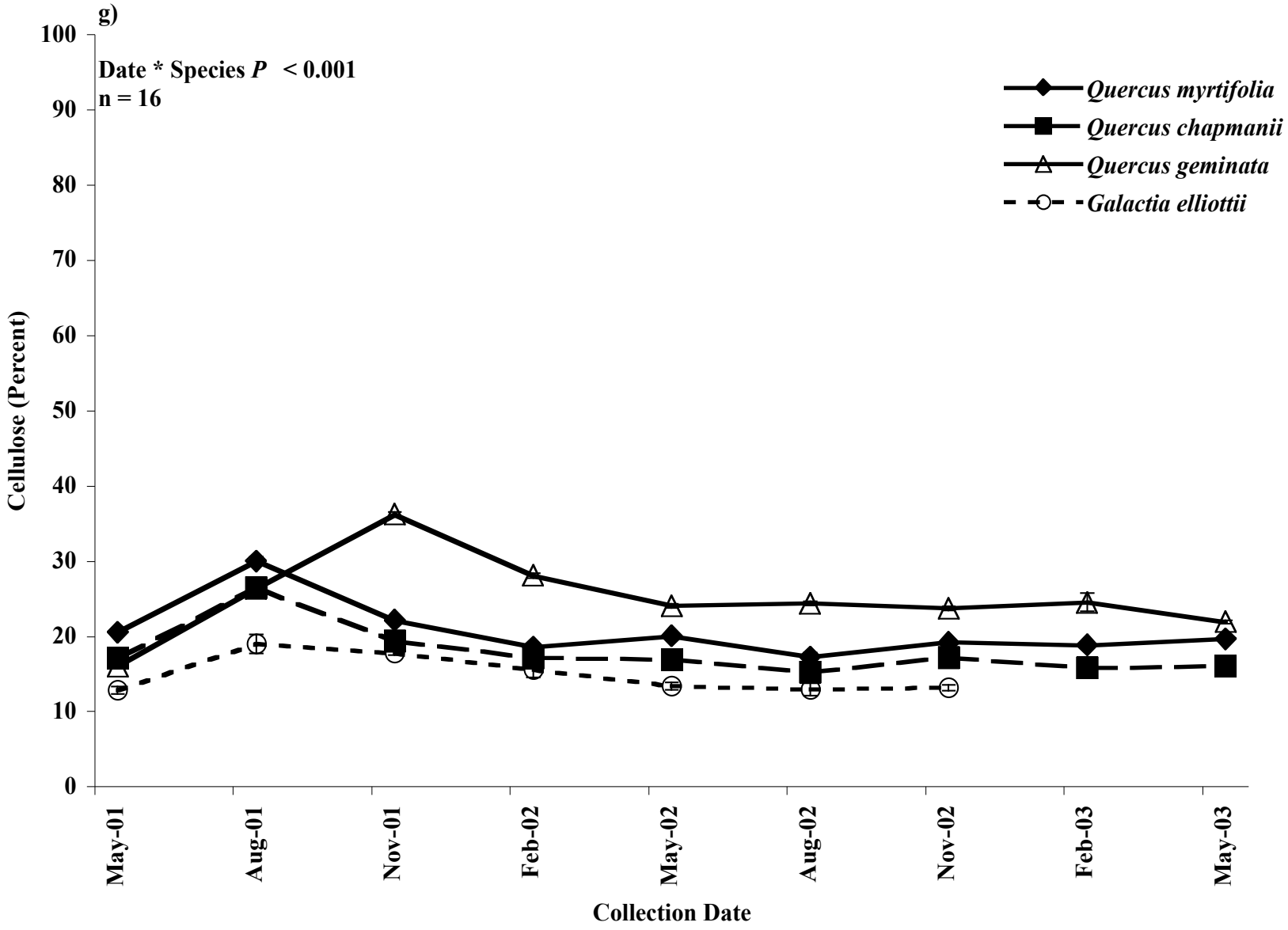


Figure 2.1 cont.

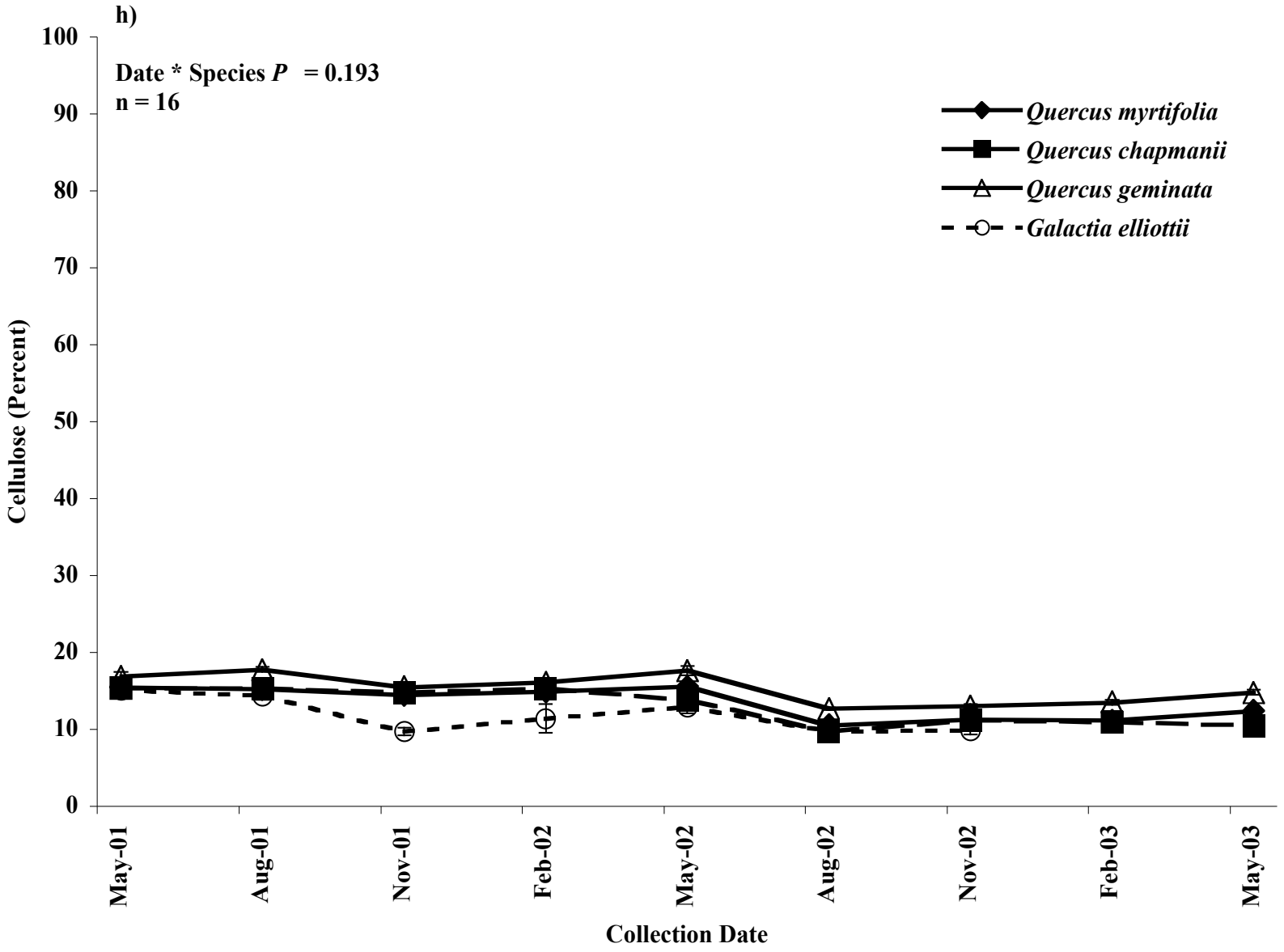


Figure 2.1 cont.

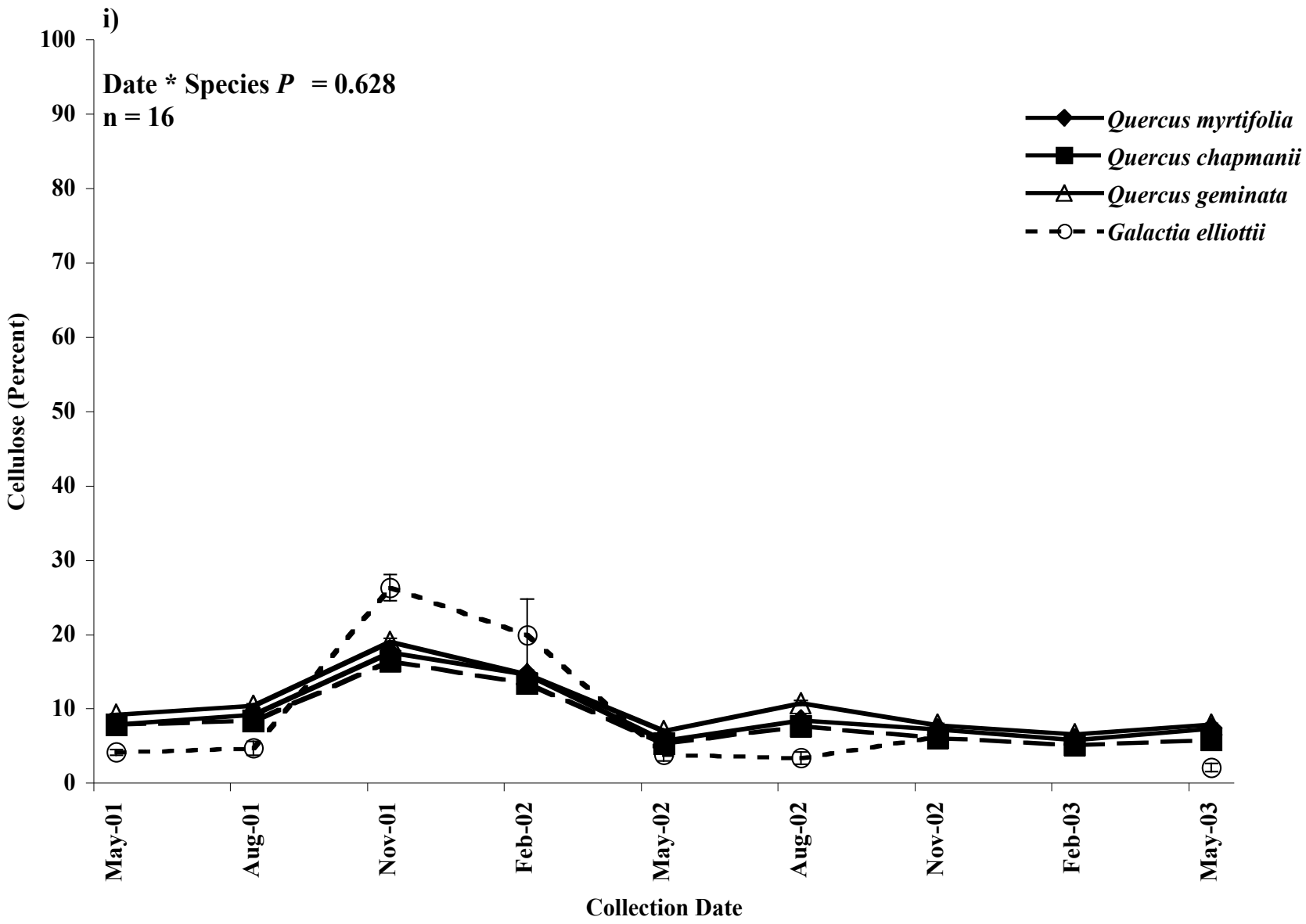


Figure 2.2. Herbivore damage across all plant species. Data are the means of 160 samples for miner and chewer damage (occurred on all plant species), 120 samples for eye spot gall, leaf gall and leaf tier damage (occurred only on oak species) and 40 samples for leaf mite damage (occurred only on the nitrogen fixer). Bars represent standard errors.

Figure 2.2

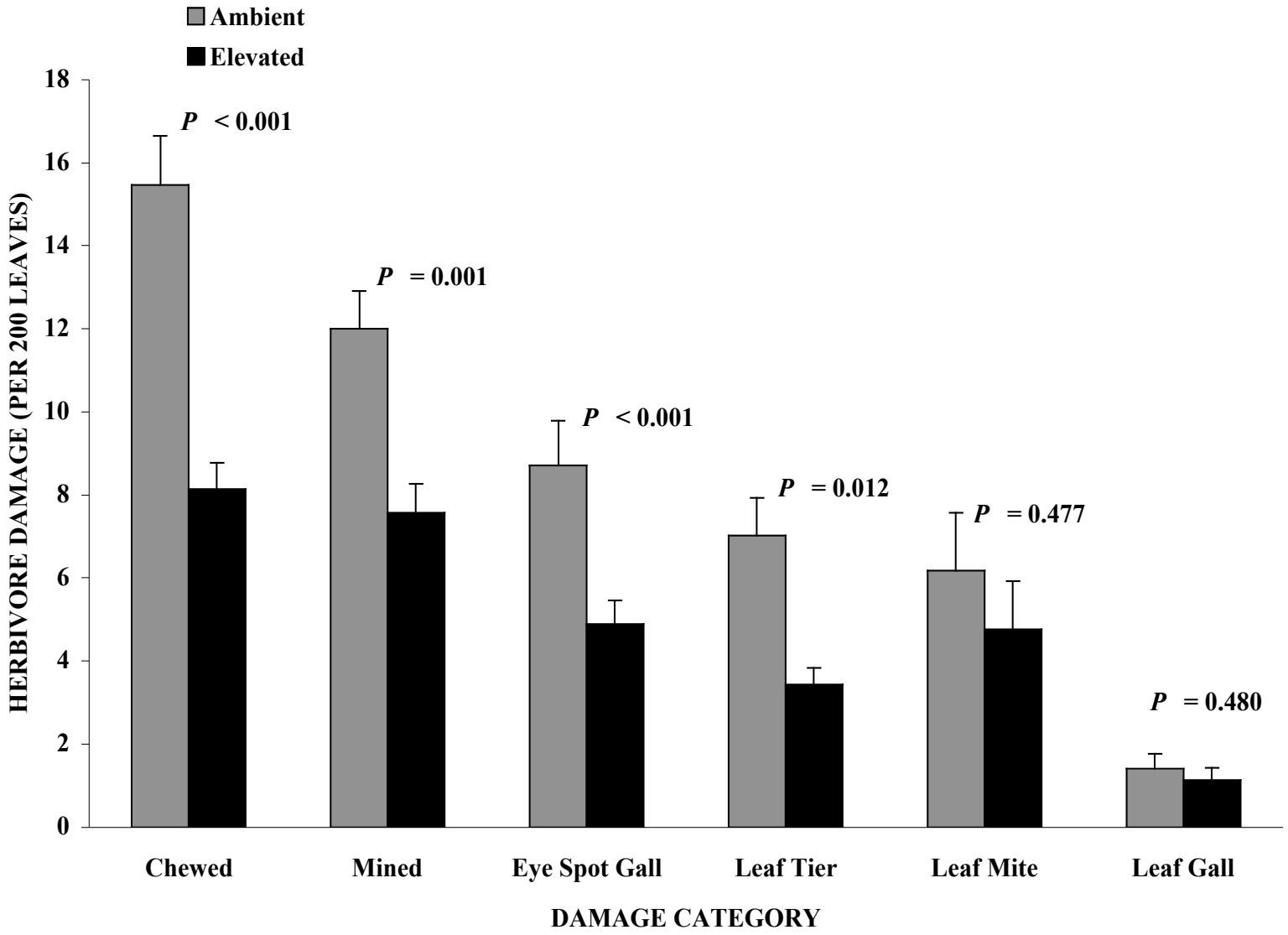

Ambient
Elevated
HERBIVORE DAMAGE (PER 200 LEAVES)
0
2
4
6
8
10
12
14
16
18
P < 0.001
P = 0.001
P < 0.001
P = 0.012
P = 0.477
P = 0.480
Chewed
Mined
Eye Spot Gall
Leaf Tier
Leaf Mite
Leaf Gall
DAMAGE CATEGORY

Figure 2.3. Damage by chewing herbivores (a) and leaf tiers (b) under elevated CO_2. Data are the means of 32 samples for chewing herbivores and 24 samples for leaf tiers and bars represent standard errors.

Figure 2.3

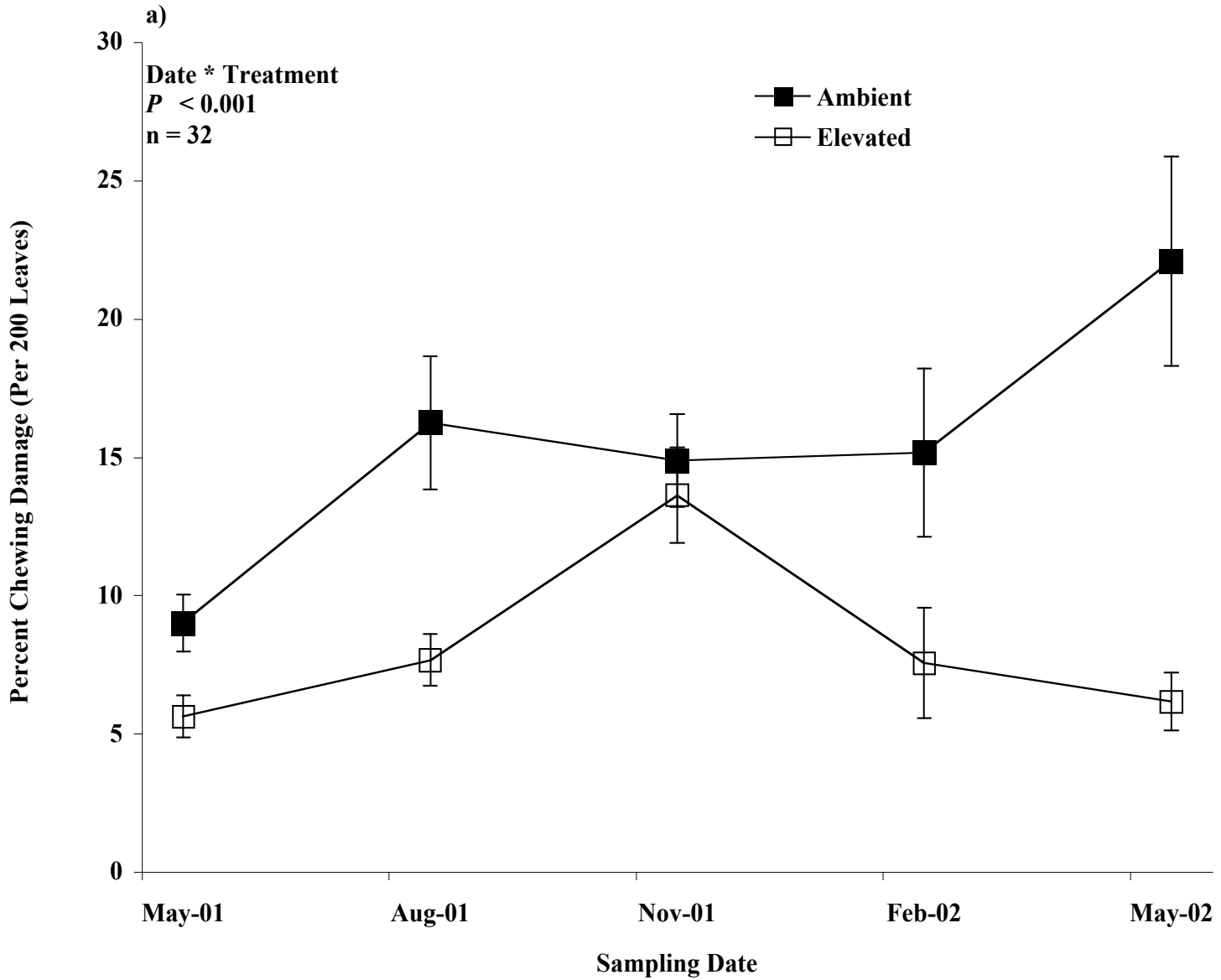

a)
Date * Treatment
P < 0.001
n = 32
Ambient
Elevated
Percent Chewing Damage (Per 200 Leaves)
30
25
20
15
10
5
0
May-01
Aug-01
Nov-01
Feb-02
May-02
Sampling Date

Figure 2.3 cont.

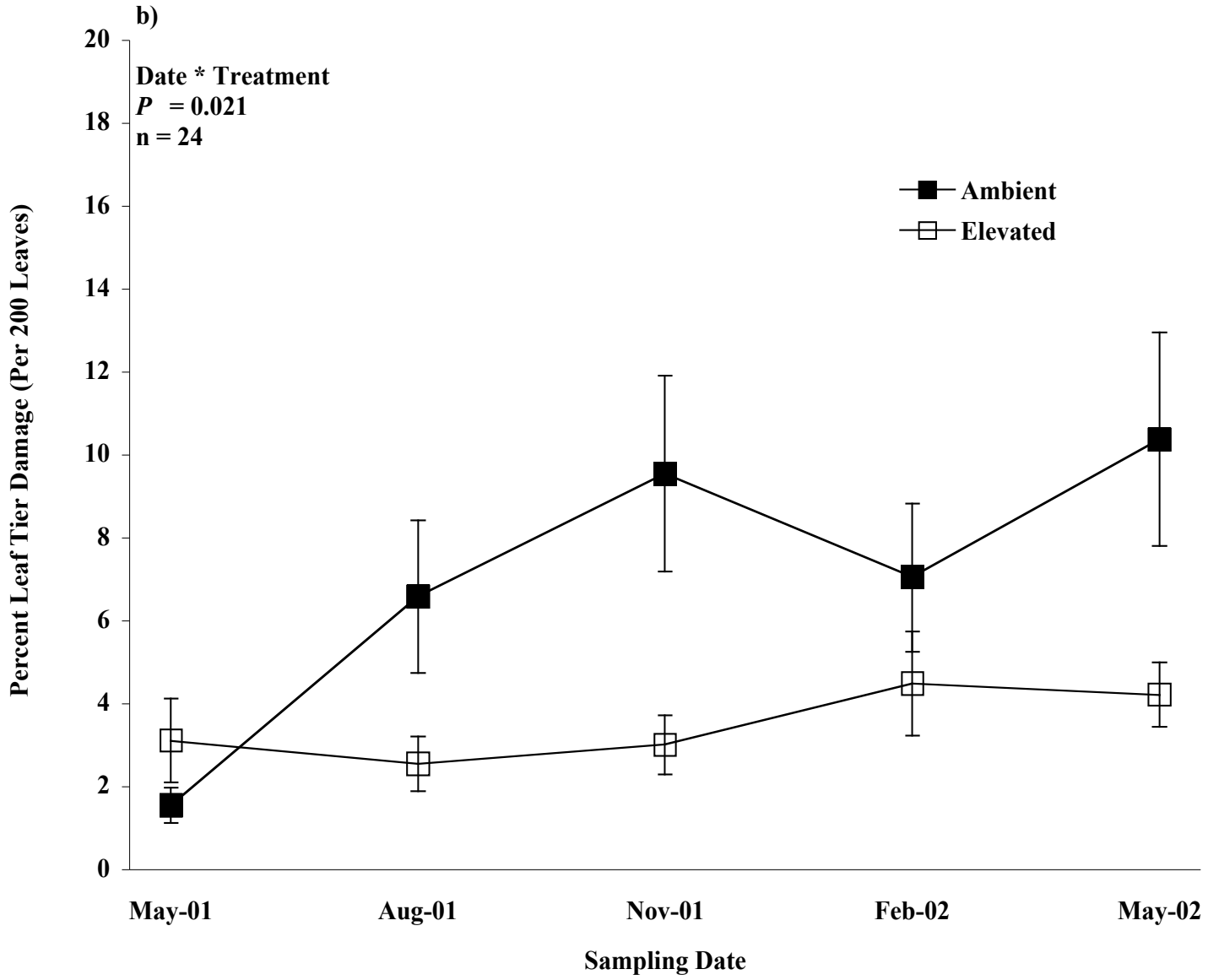


Figure 2.4. Treatment effects by plant species for a) chewing damage and b) eye spot gall damage. Data are the means of 40 samples and bars represent standard errors.

Figure 2.4

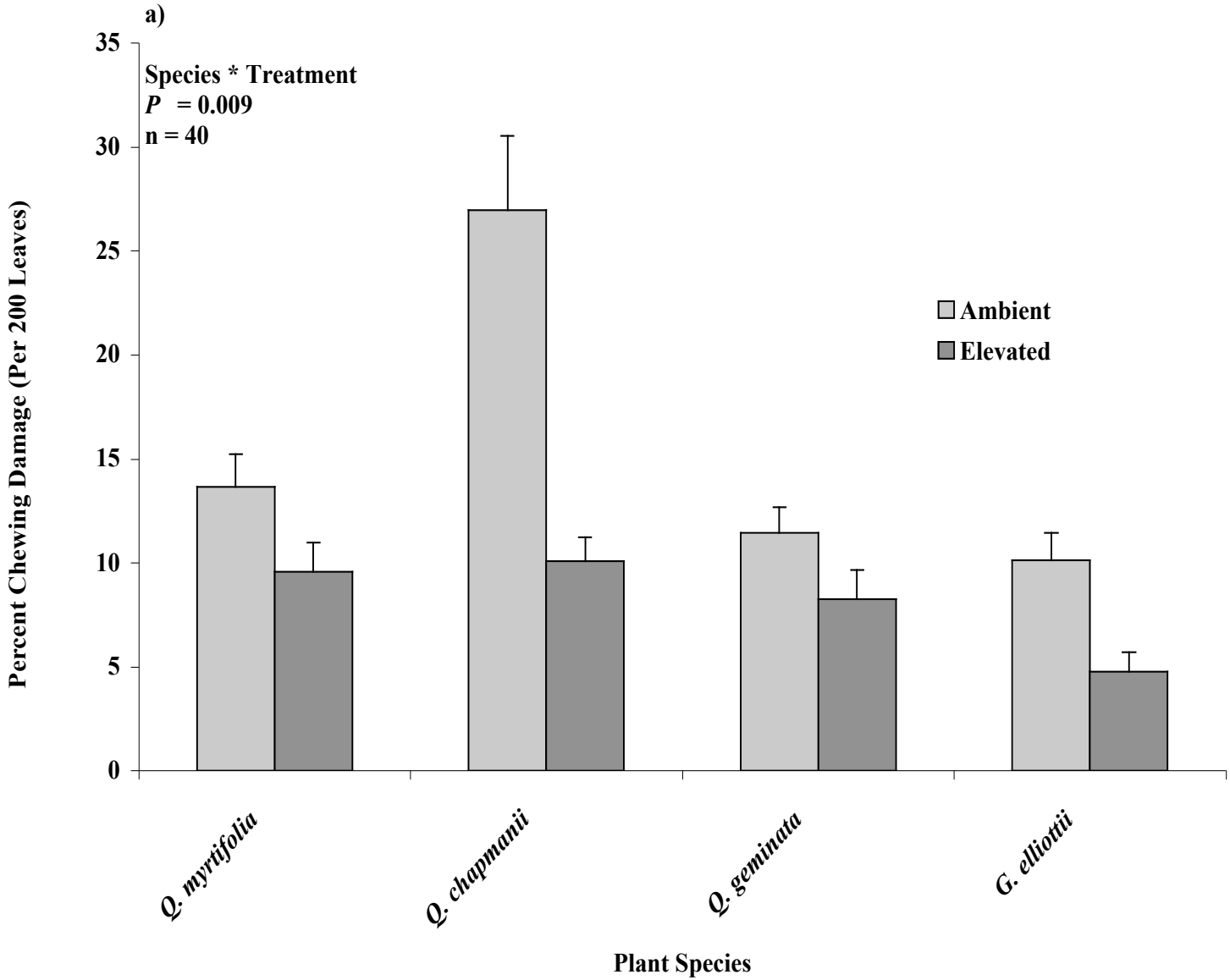

a)
Species * Treatment
P = 0.009
n = 40
Percent Chewing Damage (Per 200 Leaves)
35
30
25
20
15
10
5
0
Ambient
Elevated
Q. myrtifolia
Q. chapmanii
Q. geminata
G. elliottii
Plant Species

Figure 2.4 cont.

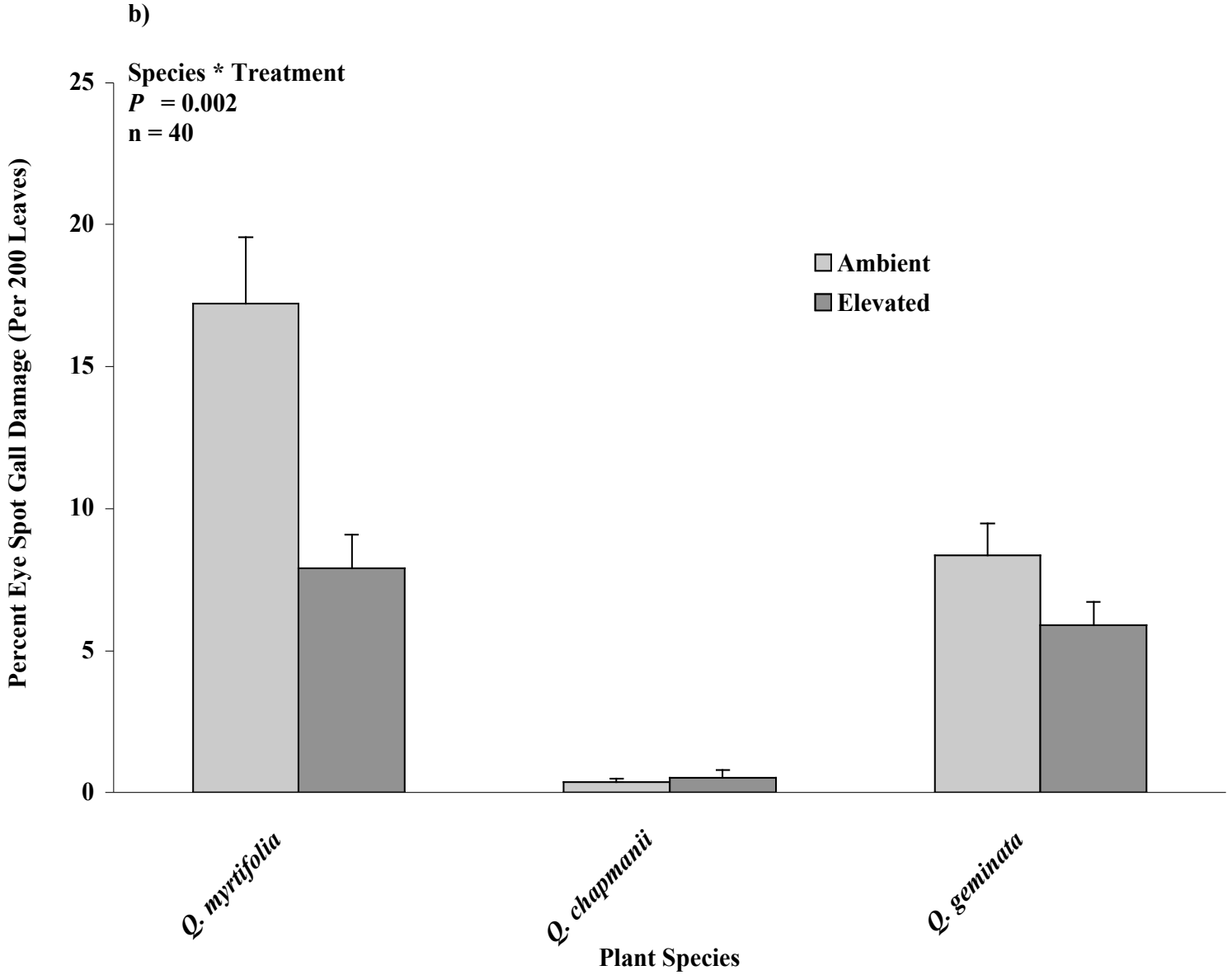
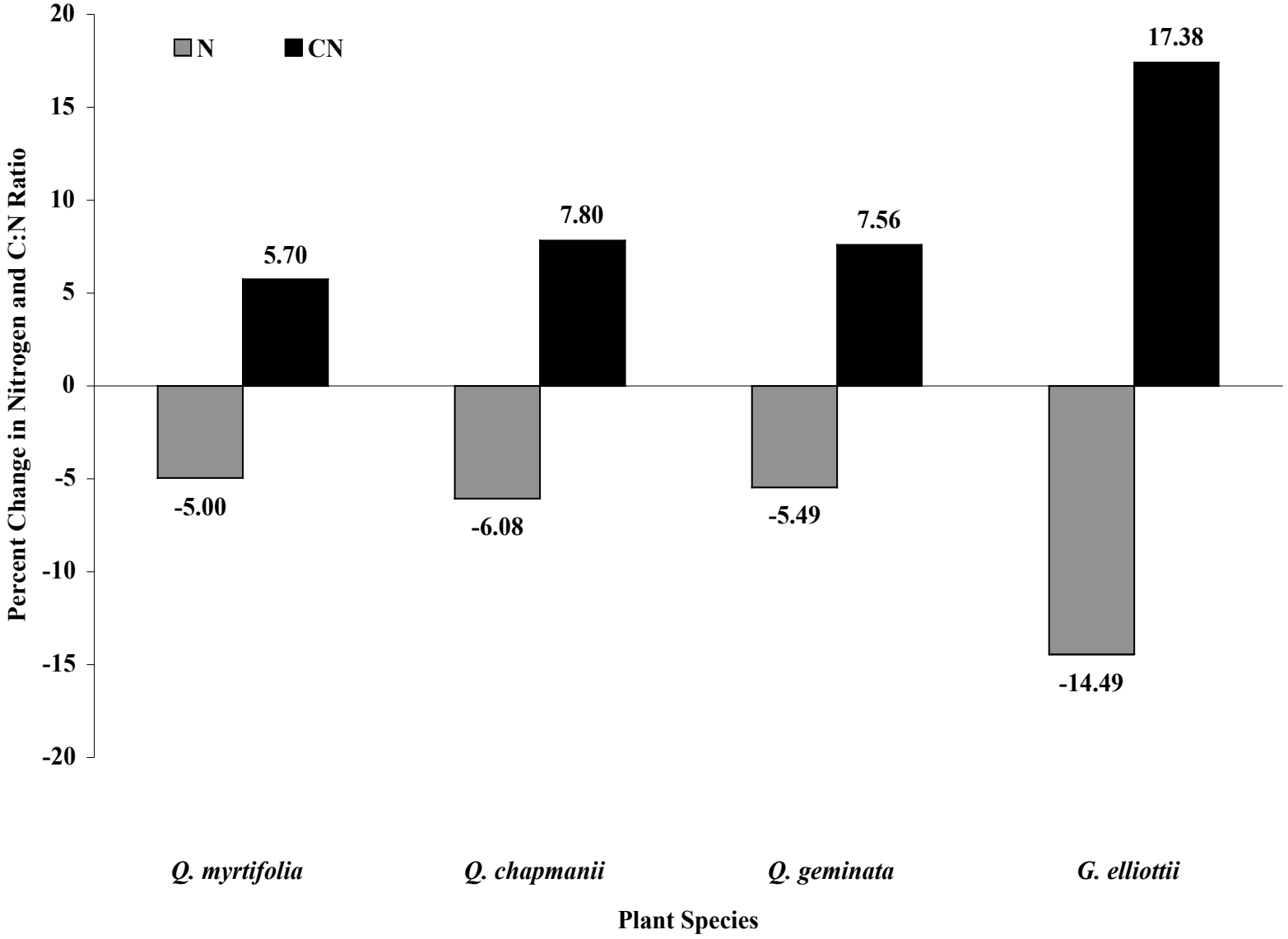


Figure 2.5. The percent change in nitrogen and C:N ratio under elevated CO_2 treatments for *Q. myrtifolia*, *Q. chapmanii*, *Q. geminata*, and *G. elliottii*. Percent change values are shown.

Figure 2.5


N
CN
Percent Change in Nitrogen and C:N Ratio
20
15
10
5
0
-5
-10
-15
-20
5.70
7.80
7.56
17.38
-5.00
-6.08
-5.49
-14.49
Q. myrtifolia
Q. chapmanii
Q. geminata
G. elliottii
Plant Species

CHAPTER 3

EFFECTS OF ELEVATED CO_2 AND HERBIVORE DAMAGE ON LITTER QUALITY IN A SCRUB OAK ECOSYSTEM[1]

[1] Hall, M.C., P. Stiling, B.A. Hungate, B.G. Drake, and M.D. Hunter. Journal of Chemical Ecology. (In Press).

Abstract – Atmospheric CO_2 concentrations have increased dramatically over the last century and continuing increases are expected to have significant, though currently unpredictable, effects on ecosystems. One important ecosystem process that may be affected by elevated CO_2 is leaf litter decomposition. We investigated the interactions among atmospheric CO_2, herbivory, and litter quality within a scrub oak community at the Kennedy Space Center, Florida. Leaf litter chemistry in sixteen plots of open-top chambers was followed for three years; eight chambers were exposed to ambient levels of CO_2, and eight chambers were exposed to elevated levels of CO_2 (ambient + 350 ppmV). We focused on three dominant oak species, *Quercus geminata*, *Quercus myrtifolia*, and *Quercus chapmanii*. Condensed tannin concentrations in oak leaf litter were higher under elevated CO_2. Litter chemistry differed among all plant species except for condensed tannins. Phenolic concentrations were lower while lignin concentrations and lignin:nitrogen ratios were higher in herbivore damaged litter independent of CO_2 concentration. However, changes in litter chemistry from year to year were far larger than effects of CO_2 or insect damage, suggesting that these may have only minor effects on litter decomposition.

Key Words – Elevated CO_2, herbivory, litter quality, *Quercus myrtifolia*, *Quercus chapmanii*, *Quercus geminata*, Kennedy Space Center.

INTRODUCTION

Increasing atmospheric carbon dioxide (CO_2) is likely to alter ecosystem processes, yet most research to date has focused on the direct effects of CO_2 on plant physiological processes, with far less attention to indirect effects on ecosystem processes, including those mediated by plant herbivores. Elevated CO_2 can alter foliar chemistry directly, for example reducing nitrogen (N) concentrations in green leaves (Lincoln et al., 1993; Agrell et al., 2000; Hall et al., 2005). Elevated CO_2 could also alter leaf chemistry indirectly, by affecting the feeding behavior of herbivores, in turn eliciting plant synthesis of chemical defenses. For example, elevated CO_2 can increase foliar concentrations of polyphenols, possibly a response to a CO_2-induced increase in herbivore feeding (Agrell et al., 2000). Changes in plant chemistry affect the quality of herbivore diets and may result in behavioral and physiological changes that influence subsequent herbivory (Lindroth, 1996; Stiling et al., 1999). Whether such changes in foliar chemistry persist in senesced leaves is not clear (Curtis et al., 1989; Coûteaux et al., 1991; Kemp et al., 1994; Finzi et al., 2001), but is important to determine given the importance of litter chemistry for decomposition and nutrient cycling.

The quality of leaf litter is an important determinant of decomposition rates and nutrient dynamics in many systems (Swift et al., 1979). Litter nitrogen, lignin concentrations, and C:N ratios are often good predictors of decay rates within and among species (Cotrufo et al., 1994). Carbon-based compounds such as polyphenols also decrease litter quality and may exert some control on litter decomposition (Hättenschwiler and Vitousek, 2000).

Elevated CO_2 may affect litter quality through several mechanisms including chemical changes in green leaf tissue that persist beyond leaf abscission or changes in patterns of herbivory that influence subsequent chemistry. The quality of green leaf tissue can change as a

result of elevated concentrations of atmospheric CO_2 (Lindroth et al., 1995; Hall et al., 2005). Generally, nitrogen concentration in green leaves declines and C:N ratios increase under elevated CO_2 (Lincoln et al., 1993; Hall et al., 2005). There may also be increases in polyphenolic concentrations in green leaves under elevated CO_2 (Agrell et al., 2000). It is unclear, however, if these changes persist in senesced leaves (Curtis et al., 1989; Coûteaux et al., 1991; Kemp et al., 1994; Finzi et al., 2001). Given the significance of litter chemistry to decomposition processes and nutrient dynamics (Heal et al., 1997), it is important to study the links among elevated CO_2, foliar chemistry, and litter chemistry.

In addition to possible direct effects of elevated CO_2 on litter quality another potential route by which elevated CO_2 might influence litter quality is through its impact on herbivores. Changes in plant chemistry affect the quality of herbivore diets and may result in behavioral and / or physiological changes that influence subsequent herbivory (Lindroth, 1996; Stiling et al., 1999). For example, to compensate for lower nitrogen concentrations in leaves under elevated CO_2, insect herbivores often increase their consumption rates by 20 – 80% (Lincoln et al.,1993). However, lepidopteran larvae can exhibit slower growth rates when feeding on elevated CO_2 plants (Fajer et al., 1991) and become more susceptible to pathogens, parasitoids, and predators (Lindroth 1996; Stiling et al., 1999). At our field site which hosts the longest continuous study of the effects of elevated CO_2 on insects, herbivore populations decline markedly under elevated CO_2 (Stiling et al., 1999, 2002, 2003; Hall et al., 2005).

Changes in the feeding behavior of herbivores alter ecosystem processes. Consumption of plant tissue by insect herbivores can have direct effects on ecosystem productivity. Herbivores can also influence ecosystem function by changing organic matter added to the soil (Chapman et al., 2003; Frost and Hunter, 2004). These changes may come from herbivore byproducts (Stadler

et al., 2001; Frost and Hunter, 2004), alteration of the plant community via selective herbivory (Ritchie et al., 1998; de Mazancourt and Loreau, 2000), or by altering the chemical properties of plant litter (Chapman et al., 2003).

Plant foliage responds to herbivore activity in multiple ways including changes in nitrogen concentrations and induction of secondary compounds (Schultz and Baldwin, 1982). If these changes carry over to litter, subsequent decomposition rates and related nutrient transformations may be altered (Melillo et al., 1982; Scott and Binkley, 1997). Chemical alteration of litter quality may also occur when herbivory instigates premature leaf abscission, which effectively limits nutrient resorption and results in litter with higher nutrient concentrations (Kahn and Cornell, 1989).

Given that herbivore damage can influence subsequent foliar and litter quality, declines in herbivore density under elevated CO_2 have the potential to influence decomposition and nutrient dynamics. Therefore, elevated CO_2 has the potential to cause changes in litter quality through direct effects on foliar chemistry and indirect effects mediated by herbivores. In this study we explore the impacts of CO_2, herbivory and their interactions on scrub oak litter chemistry.

METHODS AND MATERIALS

Study Site. Our study site lies within a two-hectare native scrub-oak community located at Kennedy Space Center, Florida. The scrub oak forest is xenomorphic largely consisting of evergreen or semi-evergreen trees with a mature canopy height of 3 to 5 m. This plant community is fire controlled and was last burned January 1996. Prior to site burning the plant composition consisted primarily of oak species (76% *Quercus myrtifolia*, 15% *Quercus geminata*, 7% *Quercus chapmanii*). The remaining 2% of the community included *Serenoa*

repens (palmetto), *Myrica serifera* (wax myrtle), *Lyonia ferruginea* (rusty lyonia), *Ceratiola ericoides* (Florida rosemary), and *Galactia elliottii* (milk pea). Continuous ground cover and longleaf pine (*Pinus palustris),* wiregrass (*Aristida beyrichiana*), and turkey oak (*Quercus laevis*) are absent. Fire in scrub communities is a stand-replacing disturbance that typically removes all aboveground vegetation. Plant re-growth is rapid and there is little change in species composition. When fire is suppressed, the scrub community transitions into a pioneer xeric hammock, which is defined by the retention of some scrub species and the lack of traditional hammock species. The last burn cycle was in 1996 prior to site set up. Sixteen 3.6 – m diameter plots, each enclosed with a clear polyester film open-top chamber 3.4 m in height, were utilized to control CO_2 levels. Chambers were overlaid on an octagonal framework of PVC pipe with a removable access door and frustum to reduce dilution of air within the chamber by outside wind. After burning, all re-growth was cut to ground level in May 1996 and, since that time, the vegetation in eight of the chambers has been kept at ambient levels of CO_2 while the other eight chambers have been exposed to elevated CO_2 (ambient + 350 ppmV). Carbon dioxide is constantly supplied to the elevated CO_2 chambers. In ambient CO_2 chambers, the airflow is identical to that of the elevated CO_2 chambers but is not supplemented with CO_2. See Dijkstra et al. (2002) for a detailed description of the site setup. Three oak species dominate this community and are present in every chamber, *Quercus myrtifolia* Willd, *Q. chapmanii* Sargent and *Q. geminata* Small.

Litter Chemistry. Leaf litter was collected quarterly for three years (2000,2001, 2002) from litter trays placed inside each chamber. Litter was then sorted by species (*Q. myrtifolia, Q. chapmanii, Q. geminata*) and by herbivore damage type (undamaged, chewed, mined). The oak species are evergreen and abscise leaves throughout the year; samples within a year were

combined. This resulted in 144 samples (16 chambers x 3 plant species x 3 damage categories) per year. Chambers (8 per treatment) acted as replicates. Litter samples collected in 2000 were assayed prior to litter samples collected in 2001 and 2002, which were assayed concurrently. Otherwise all samples were processed identically. The air-dried litter was ground to a fine powder and stored at -80^0 C prior to analysis.

Percent dry weight nitrogen and carbon were estimated from leaf powder on a Carlo-Erba NA1500 model C/N analyzer (Milan, Italy). These data also provided estimates of litter C:N ratios. Sub-samples of leaf powder were used to assess the effects of elevated CO_2 on litter concentrations of cellulose, hemicellulose, and lignin by sequential neutral detergent / acid detergent digestion on an Ankom fiber analyzer (Abrahamson et al., 2003).

Proanthocyanidins, an estimate of condensed tannin concentration, were assayed using N-butanol:HCL methods described in Rossiter et al.(1988). Total phenolics were estimated using the Folin-Denis assay (Swain, 1980), and gallotannins (hydrolyzable tannins) were estimated using a potassium iodate technique developed by Bate-Smith (1977) and modified by Schultz and Baldwin (1982). Standards for tannin analysis were generated by multiple sequential washes of a bulk sample by acetone extraction. The small amount of available litter material for some species resulted in a bulk sample that was a mix of all species of litter for each year. All tannin assays produced colorimetric reactions, in proportion to tannin concentration, which were quantified using a BioRad microplate reader.

Statistical procedures. Data were initially analyzed with the GLM procedure of SAS 8.2. However, the residuals of the ANOVA models failed the test for normality (Kéry and Hatfield, 2003). Data were transformed and reanalyzed and again failed the test of normality. Data were finally analyzed with the repeated measures GENMOD procedure of SAS 8.2 (SAS Inst. 1999)

and the log likelihood ratio was maximized. With the development of generalized estimating equations (GEE), GENMOD is a non-parametric alternative for repeated measures data (Littell et al., 2002), though posthoc analyses are not performed. The GENMOD procedure allows the explanatory variables to be selected and changes in the goodness-of-fit statistics are used to evaluate the contribution of each variable to the model. Thus the importance of each additional variable and interaction can be assessed allowing a sequence of models to be tested while taking into account main effects as well as interactions. The data presented contain only those effects that made significant contributions to the model.

RESULTS

Except for condensed tannins, all measures of litter chemistry varied among the species of oak (Table 3.1). The only consistent effect of elevated CO_2 on litter chemistry was higher condensed tannin concentrations under elevated CO_2 ($\chi^2 = 19.34$, $P < 0.001$) (Figure 3.1). All other effects of elevated CO_2 on litter chemistry were either not significant (Table 3.2) or were inconsistent among years (Figure 3.2a –c). The latter was one of our dominant findings; that interannual variation has a profound influence on the effects of elevated CO_2 on litter chemistry. A second important result to emerge was that elevated CO_2 has no influence on litter nitrogen concentration or C:N ratio (Table 3.2). We have previously shown that in living green leaves of these oak species, nitrogen concentrations decrease while C:N ratios increase under elevated CO_2 (Hall et al., 2005). Apparently, these changes are lost by the time that leaves senesce.

Phenolic concentrations (condensed tannins, hydrolyzable tannins, total phenolics) were generally lower in chewed and mined litter than in undamaged litter (condensed tannins, $\chi^2 = 22.88$, $P < 0.001$; hydrolyzable tannins, $\chi^2 = 10.07$, $P = 0.006$; total phenolics, $\chi^2 = 15.55$, $P =$

0.004) (Figure 3.3a – c). However, mining only reduced hydrolyzable tannins and total phenolics in *Q. myrtifolia* (Figure 3.3b, c), and had no effect for the other species. Effects of damage on litter C:N ratios were inconsistent among the oak species ($\chi^2 = 20.73$, $P = 0.002$, Figure 3.3d) suggesting no dominant impact of damage on C:N ratios. Lignin concentrations and lignin:nitrogen ratios were higher in damaged litter from *Q. myrtifolia*, but not from the other oak species (Damage * Species $\chi^2 = 30.35$, $P < 0.001$, and $\chi^2 = 30.06$, $P < 0.001$ for lignin and lignin:nitrogen ratio, respectively, Figure 3.3e,f). As with the effects of CO_2 (above) effects of damage on litter chemistry was relatively minor when compared to yearly variation from one growing season to the next (Figure 3.4a – e).

DISCUSSION

Climate and chemical composition of litter strongly determine decomposition rates and nitrogen mineralization (Swift et al.,1979). Components influencing the quality of the litter include concentrations of nitrogen, lignin, and polyphenols. Typically, high concentrations of nitrogen are positively correlated with decomposition rates while high lignin and polyphenolic concentrations are negatively correlated with decomposition rates (Swift et al., 1979). Low litter quality generally decreases decomposition, reducing nitrogen mineralization and soil nitrogen availability (Swift et al., 1979; Melillo et al., 1982). Previous studies of the effect of elevated CO_2 on litter quality have been equivocal. In some studies, increased levels of atmospheric CO_2 affected litter quality for several species (Coûteaux et al., 1991; Cotrufo et al., 1994; Parsons et al., 2004; Henry et al., 2005) while other studies have found no effect of elevated CO_2 on litter quality (Curtis et al., 1989; Finzi et al., 2001). Parsons et al. (2004) found that nitrogen concentrations declined and C:N ratios and condensed tannin concentrations increased under

elevated CO_2 in paper birch (*Betula papyrifera).* Likewise they found that mass loss was lower and decay rates were higher for birch litter from CO_2 environments compared to the control. Henry et al. (2005) found that lignin concentrations increased in grass and forb litter under elevated CO_2, however they found no differences in total phenolic concentrations or percent nitrogen in the litter from elevated CO_2 environments. They found that the increase in lignin concentrations due to elevated CO_2 did not affect decomposition rates.

In our study, condensed tannin concentrations increased under elevated CO_2 regardless of species, herbivore damage, or growing season. While the traditional measures of litter quality focus on lignin and nitrogen concentrations, there is growing consensus that polyphenols can have a large effect on decomposition processes. Palm and Sanchez (1990) found that soluble polyphenolic concentrations were a better measure for predicting decomposition rates in leguminous litter in the tropics than were lignin or lignin:nitrogen ratios. Polyphenols may affect the rates of decomposition by influencing the composition and activity of the detritivore community (Hättenschwiler and Vitousek, 2000). In addition polyphenols may alter the availability of nitrogen by binding with proteins making the litter resistant to some detritivores and reducing the rates of nitrogen mineralization (Bernays et al., 1989). In our system, increased condensed tannin concentrations under elevated CO_2 have the potential to affect ecosystem processes by slowing down litter decomposition and nutrient turnover. However, increases in condensed tannins were quite low (2-5%, Figure 3.1) and considerably smaller than interannual variation.

Prior work on green leaves in this system established that plant growth under elevated CO_2 reduced foliar nitrogen concentrations and increased C:N ratios by an average of 6% and 7%, respectively, across all three oak species (Hall et al., 2005). In contrast, secondary metabolites

were unaffected by elevated CO_2 in the green leaves of the oak species (Hall et al., 2005). In our current study of leaf litter, however, there was no evidence of lower nitrogen concentrations or higher C:N ratios. Rather the strongest CO_2 effects were seen in the secondary metabolites, particularly condensed tannins (Figure 3.1, Figure 3.2a-c). Thus, in this system differences in green leaf chemistry caused by growth under elevated CO_2 disappeared by the time leaves senesced. Few studies have directly compared foliar and litter chemistry under elevated CO_2. Curtis et al. (1989) found that nitrogen concentrations were lower and C:N ratios higher in green leaves of *Scripus olneyi* under elevated CO_2 but differences did not persist in senesced leaves. Finzi et al. (2001) found no effect of elevated CO_2 on total nonstructural carbohydrates or nitrogen in green leaves or in leaf litter of five tree species. Kemp et al. (1994), on the other hand, found significantly lower nitrogen concentrations in senesced foliage from *Poa pratensis* L. exposed to elevated levels of CO_2 compared to senesced foliage from ambient levels of CO_2. Similarly, Coûteaux et al. (1991) found that nitrogen concentrations were significantly lower in leaf litter of chestnut trees grown in elevated CO_2 compared to ambient CO_2. The studies by Kemp et al. (1994) and Coûteaux et al. (1991) did not apply elevated CO_2 continuously to field plants and this may account for the differences in their results from ours and other authors.

Phenolic concentrations were lower while lignin concentrations and lignin:nitrogen ratios were higher in litter following herbivore damage. Given that herbivores on oak generally induce increases in foliar phenolics (Schultz and Baldwin, 1982), it seems unlikely that lower phenolic concentrations in damaged litter resulted directly from herbivore feeding. Rather, insect herbivores may be avoiding high phenolic leaves (Cooper-Driver et al., 1977; Bernays et al., 1989), leading to a preponderance of damage on low phenolic litter. However, this would need to be explored experimentally by manipulating herbivore abundance. Likewise, the apparent

induction of lignification following damage (Figure 3.3e) requires experimental verification. Chapman et al. (2003) found that two insect herbivores from different feeding guilds (*Matsucoccus acalyptus* and *Dioryctria albovittella*) increased nitrogen concentrations and decreased C:N and lignin:nitrogen ratios in pinyon pine (*Pinus edulis*) litter. Moreover, these herbivore-induced changes in litter chemistry resulted in increased litter decomposition rates. However, damaged litter in our study was lower in condensed tannins yet higher in lignin and lignin:nitrogen ratios, changes that should have counteracting effects on decomposition rates. Herbivore damaged litter may decompose more rapidly due to the decline in phenolic concentrations, particularly condensed tannins, compared to undamaged litter. Conversely damaged litter may decompose more slowly due to higher concentrations of lignin and higher lignin:nitrogen ratios than undamaged litter.

However, in our study, growing season had the dominant impact on litter chemistry so that yearly variation overwhelmed most effects of CO_2 or herbivore damage. Therefore, relative to the influence of seasonal variables, elevated CO_2 and herbivore activity may be less significant factors in leaf litter decomposition and ecosystem function. While it is well known that climate and litter chemistry control the dynamics of decomposition (Swift et al., 1979), there has been little research on changes in litter quality based on yearly variation in climate. It is known, however, that the same plant species grown on different sites can vary in its litter chemistry (Vitousek et al., 1994; Scowcroft et al., 2000). Therefore, it is reasonable to expect within-site variation in litter chemistry from year to year due to climatic and other differences among the growing seasons. At our study site climatic data available for precipitation and temperature did not offer insight to the yearly difference found in the litter chemistry. Yearly precipitation, based on mean monthly data, varied only slightly with an average of 240 mm of precipitation recorded

for 2000, 242 mm for 2001, and 2002 had a slight increase in precipitation with a recorded mean precipitation of 263 mm. The average daytime temperature, based on mean monthly data did not vary among years. The daytime mean temperature within CO_2 chambers was 27 degrees Celsius for all three years. The daytime mean temperature outside of chambers was 26 degrees Celsius for 2000 and 2002 and 25 degrees Celsius for 2001. Though precipitation and temperature did not vary among years at our study site variation in monthly precipitation patterns based on timing of precipitation and litter fall may result in differential leaching of soluble compounds from year to year leading to differences in litter chemistry. In addition to temperature and precipitation, differences in macro – and micronutrient supply as well as UV-B levels may affect litter quality (Horner et al., 1988; Heal et al., 1979). Given the magnitude of changes in litter chemistry from one growing season to the next, it is possible that decay rates and nitrogen mineralization will vary over time depending upon the cohort of leaves that serve as substrate. Future work on the effects of climate change and herbivory on decomposition and nutrient cycling should occur over several years of study so that annual variation can be taken into account and key abiotic variables that drive variation in litter chemistry should be identified.

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TABLE 3.1. LITTER CHEMISTRY OF THREE OAK SPECIES AT THE KENNEDY SPACE CENTER, FLORIDA

	Q. myrtifolia	*Q. chapmanii*	*Q. geminata*	*P*- value
Condensed Tannins	19.41	18.02	18.02	N.S.
	(0.68)	(0.81)	(0.71)	
Hydrolysable Tannins	20.46	23.38	19.68	$P = 0.001$
	(0.62)	(1.01)	(0.67)	
Total Phenolics	23.93	25.53	18.36	$P < 0.001$
	(0.58)	(1.05)	(0.57)	
Nitrogen	0.76	0.81	0.75	$P = 0.018$
	(0.01)	(0.02)	(0.02)	
Carbon	48.71	46.97	46.59	$P < 0.001$
	(0.36)	(0.38)	(0.39)	
C:N Ratio	66.44	60.58	65.23	$P = 0.002$
	(1.10)	(1.24)	(1.14)	
Cellulose	22.62	19.05	25.09	$P < 0.001$
	(0.17)	(0.21)	(0.17)	
Hemicellulose	14.56	13.59	15.07	$P = 0.001$
	(0.19)	(0.22)	(0.35)	
Lignin	14.27	10.54	12.47	$P < 0.001$
	(0.29)	(0.29)	(0.32)	
Lignin:Nitrogen Ratio	19.75	13.79	17.70	$P < 0.001$
	(0.56)	(0.40)	(0.55)	

Mean differences in chemical concentrations among species were significant for all measurements except condensed tannins. Means are the results of 144 samples. Standard errors are shown in parenthesis.

TABLE 3.2. RESULTS OF ANALYSES OF LITTER CHEMISTRY FOR CO_2 AND CO_2 * YEAR INTERACTIONS AT THE KENNEDY SPACE CENTER, FLORIDA

LITTER CHEMISTRY	CO_2	CO_2 * YEAR
Condensed Tannins	$\chi^2 = 19.34$, $P < 0.001$	N.S.
Hydrolysable Tannins	N.S.	$\chi^2 = 20.39$, $P < 0.001$
Total Phenolics	N.S.	N.S.
Nitrogen	N.S.	N.S.
Carbon	N.S.	N.S.
C:N Ratio	N.S.	N.S.
Cellulose	N.S.	$\chi^2 = 9.54$, $P = 0.023$
Hemicellulose	N.S.	$\chi^2 = 11.86$, $P = 0.008$
Lignin	N.S.	N.S.
Lignin:Nitrogen Ratio	N.S.	N.S.

Figure 3.1. Condensed tannin concentrations in litter across plant species. Data are the means of 72 samples and bars represent standard errors.

Figure 3.1

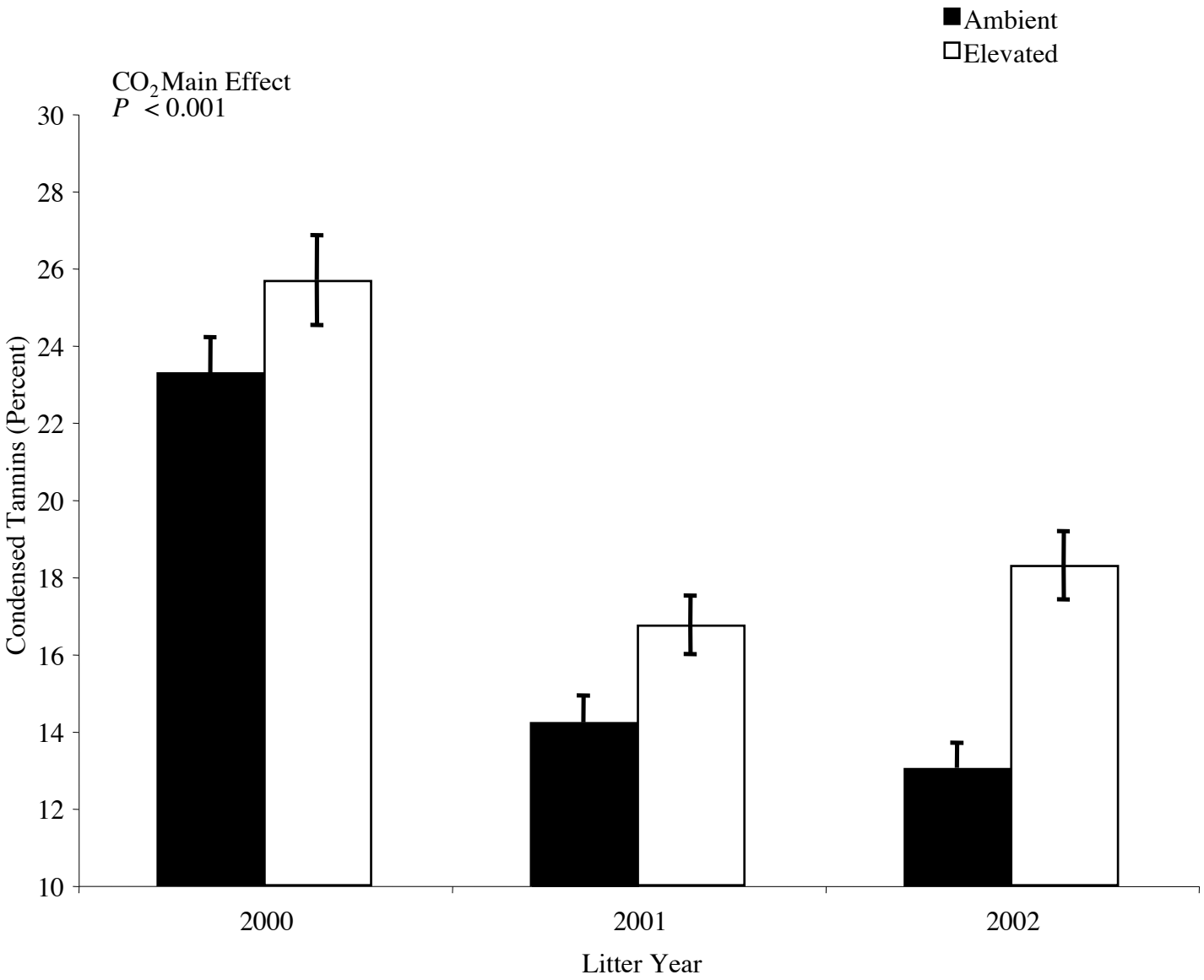

Ambient
Elevated
CO_2 Main Effect
$P < 0.001$
Condensed Tannins (Percent)
30
28
26
24
22
20
18
16
14
12
10
2000
2001
2002
Litter Year

Figure 3.2. Differences in litter chemistry between ambient and elevated CO_2 treatments across plant species by growing season (a) hydrolyzable tannins, (b) cellulose, (c) hemicellulose. Data are the means of 72 samples and bars represent standard errors.

Figure 3.2

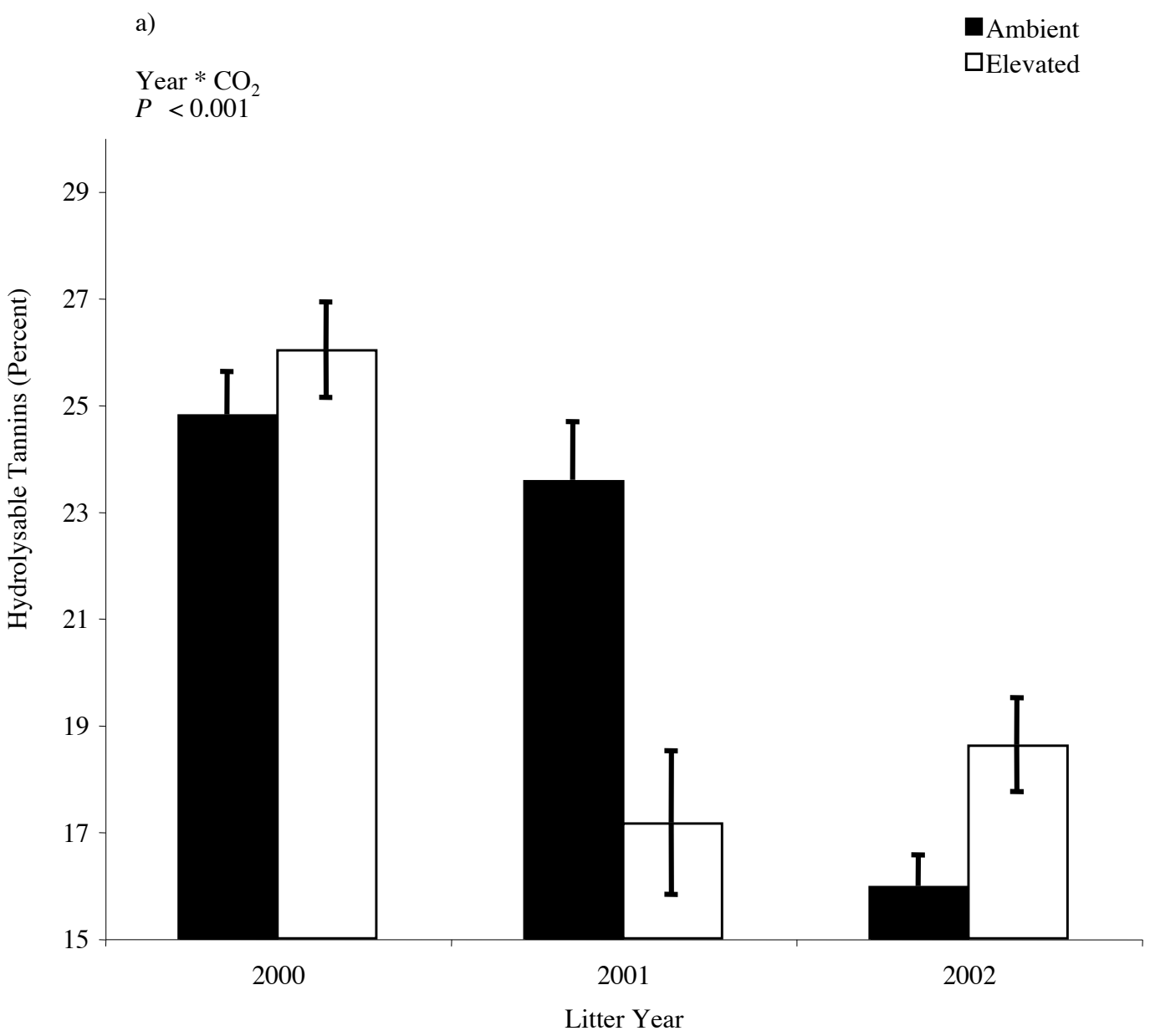

a)
Ambient
Elevated
Year * CO_2
$P < 0.001$
Hydrolysable Tannins (Percent)
29
27
25
23
21
19
17
15
2000
2001
2002
Litter Year

Figure 3.2 cont. b)

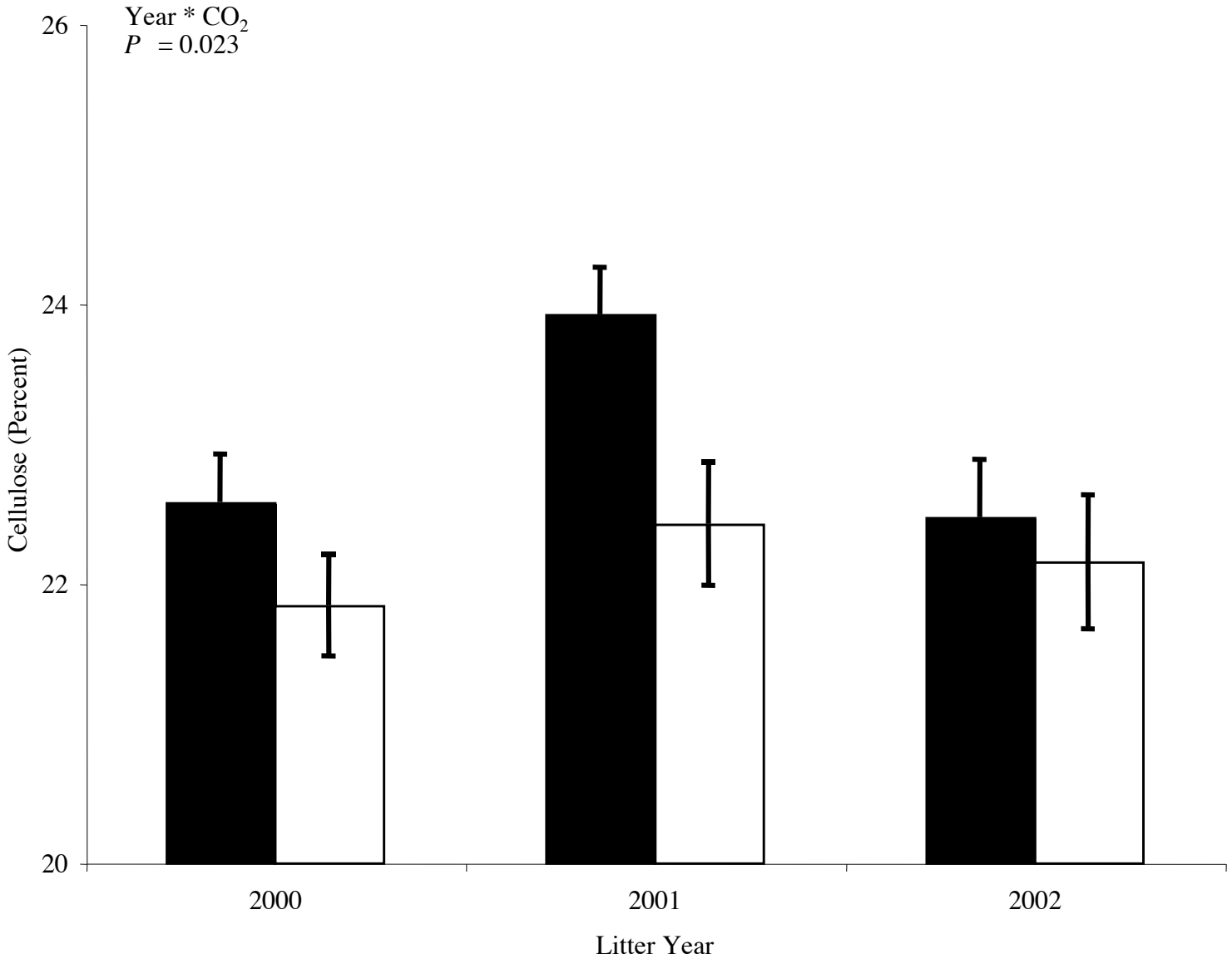


Figure 3.2 cont. c)

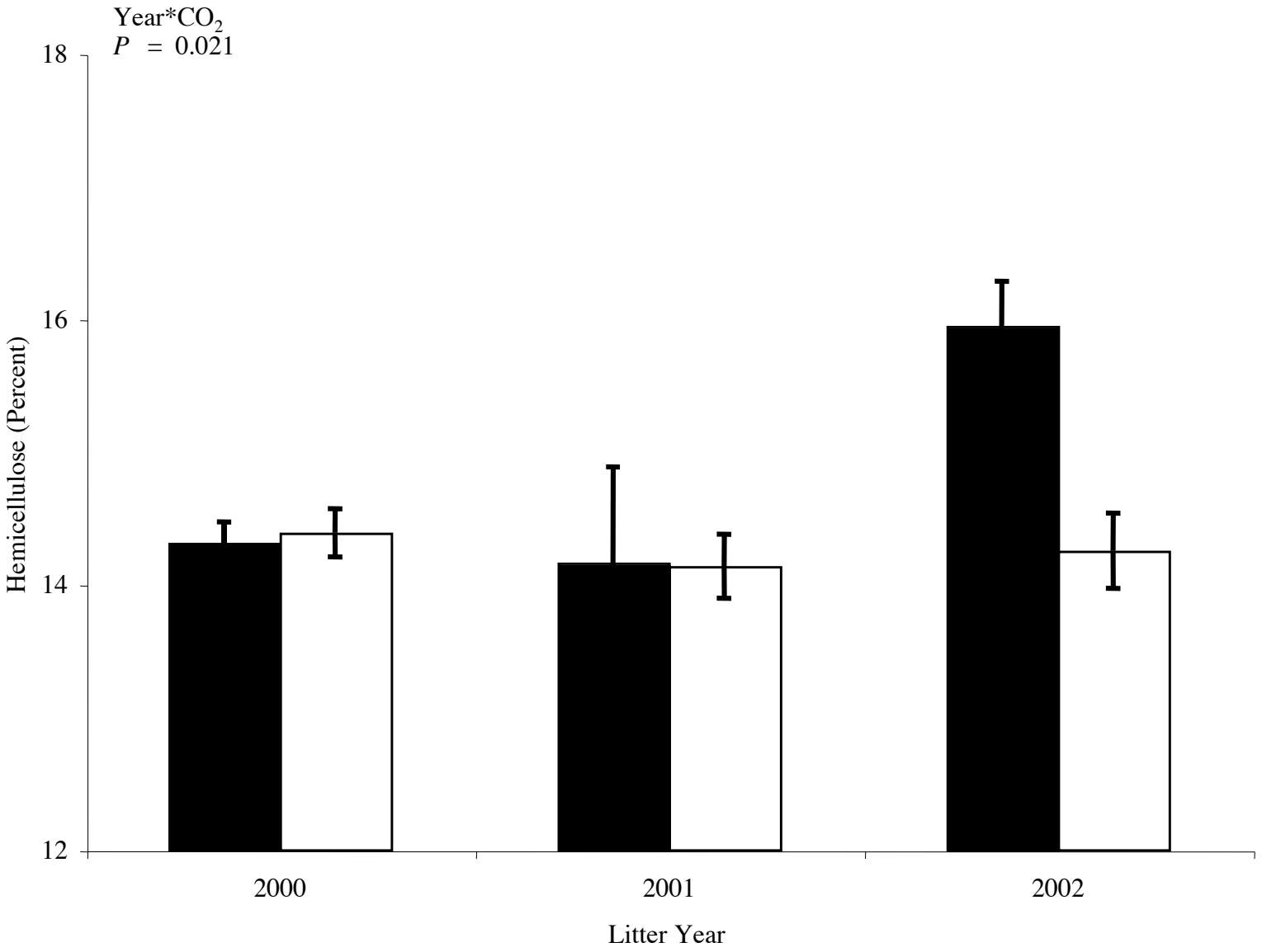


Figure 3.3. Differences in litter chemistry among undamaged, chewed, and mined litter by plant species for a) condensed tannins, (b) hydrolyzable tannins, c) total phenolics, (d) C:N ratio, (e) lignin, (f) lignin:nitrogen ratio. Data are the means of 48 samples and bars represent standard errors.

Figure 3.3

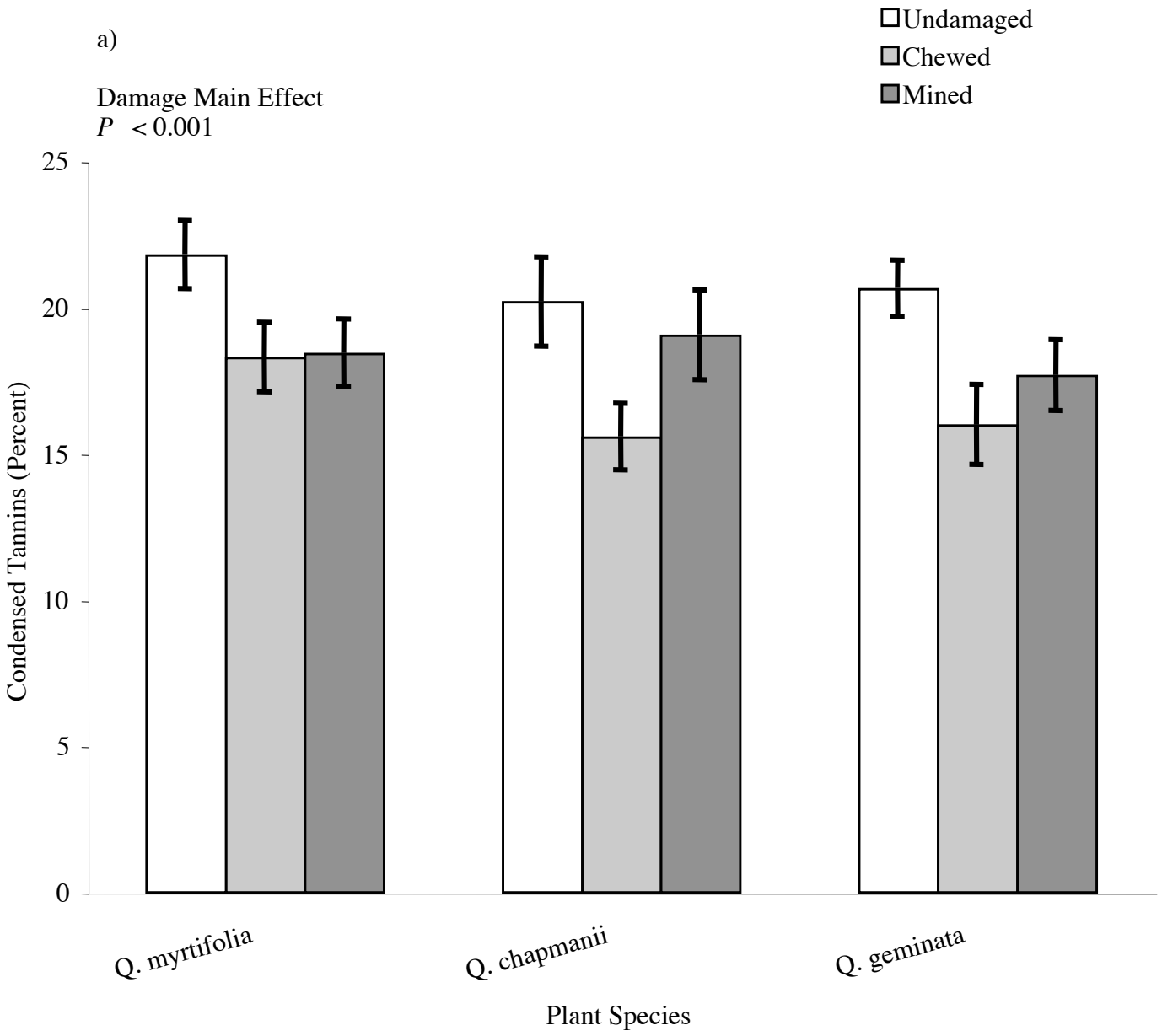

a)
Damage Main Effect
P < 0.001
Undamaged
Chewed
Mined
Condensed Tannins (Percent)
25
20
15
10
5
0
Q. myrtifolia
Q. chapmanii
Q. geminata
Plant Species

Figure 3.3 cont.

b)

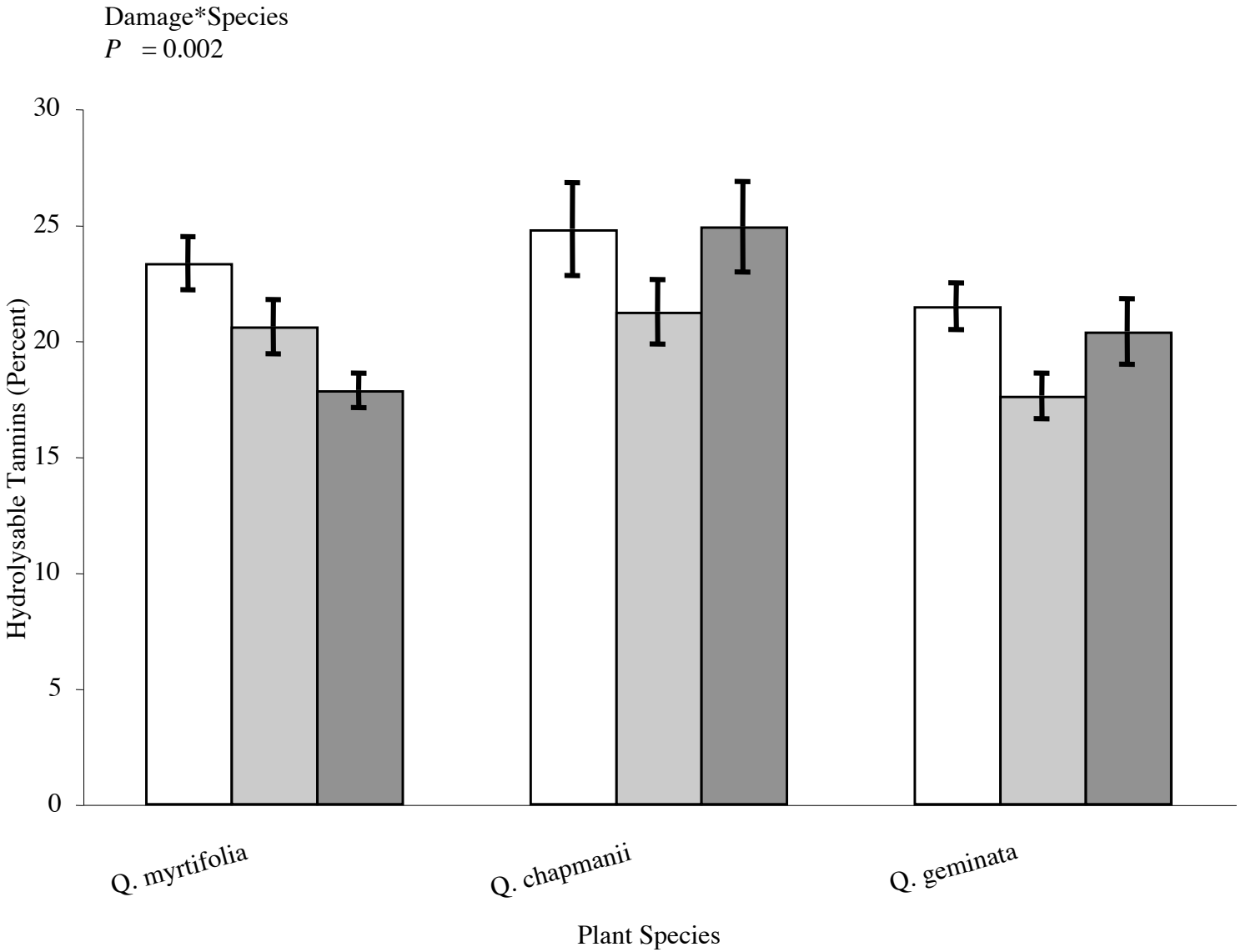


Figure 3.3 cont.

c)

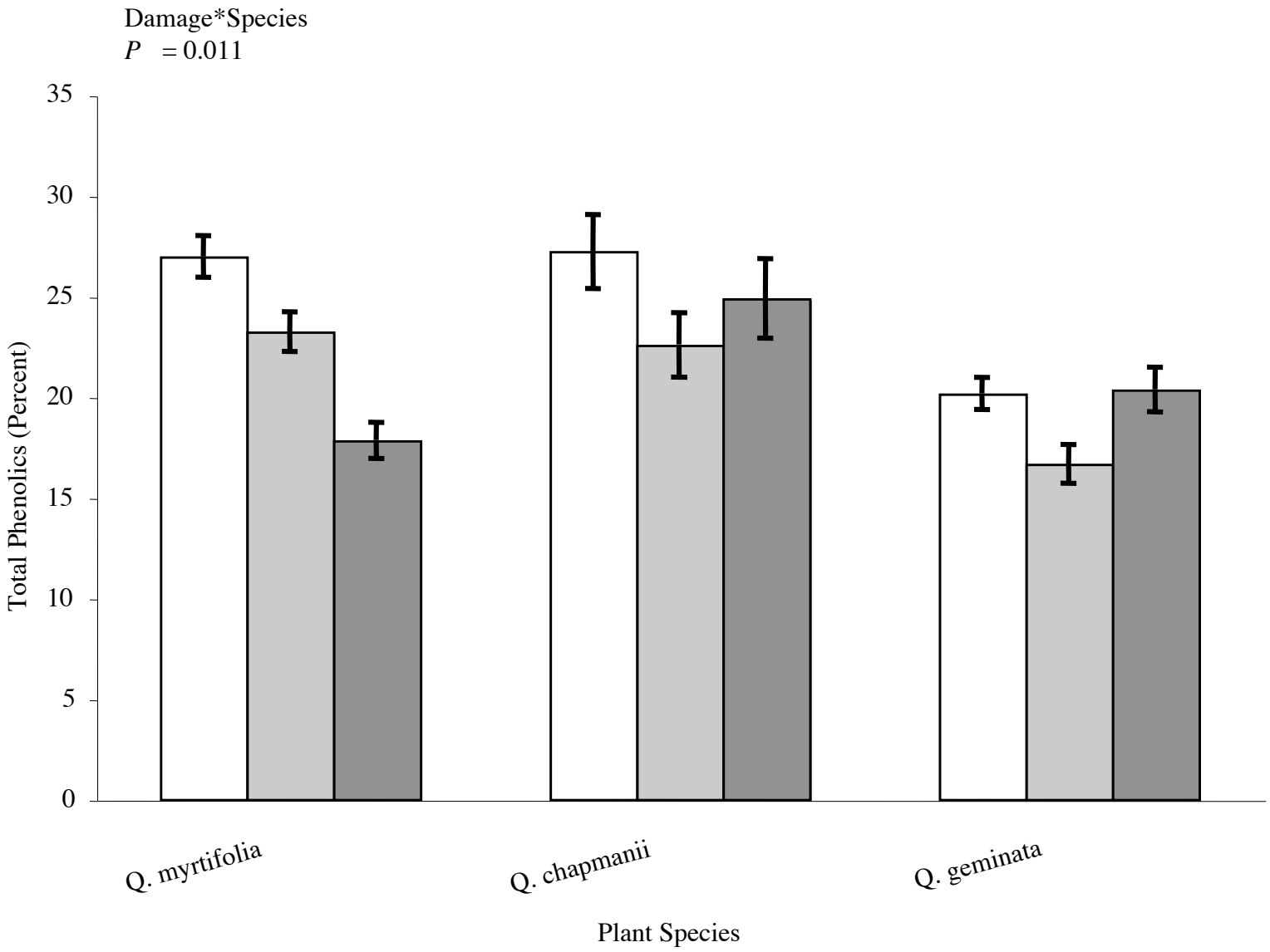


Figure 3.3 cont.

d)

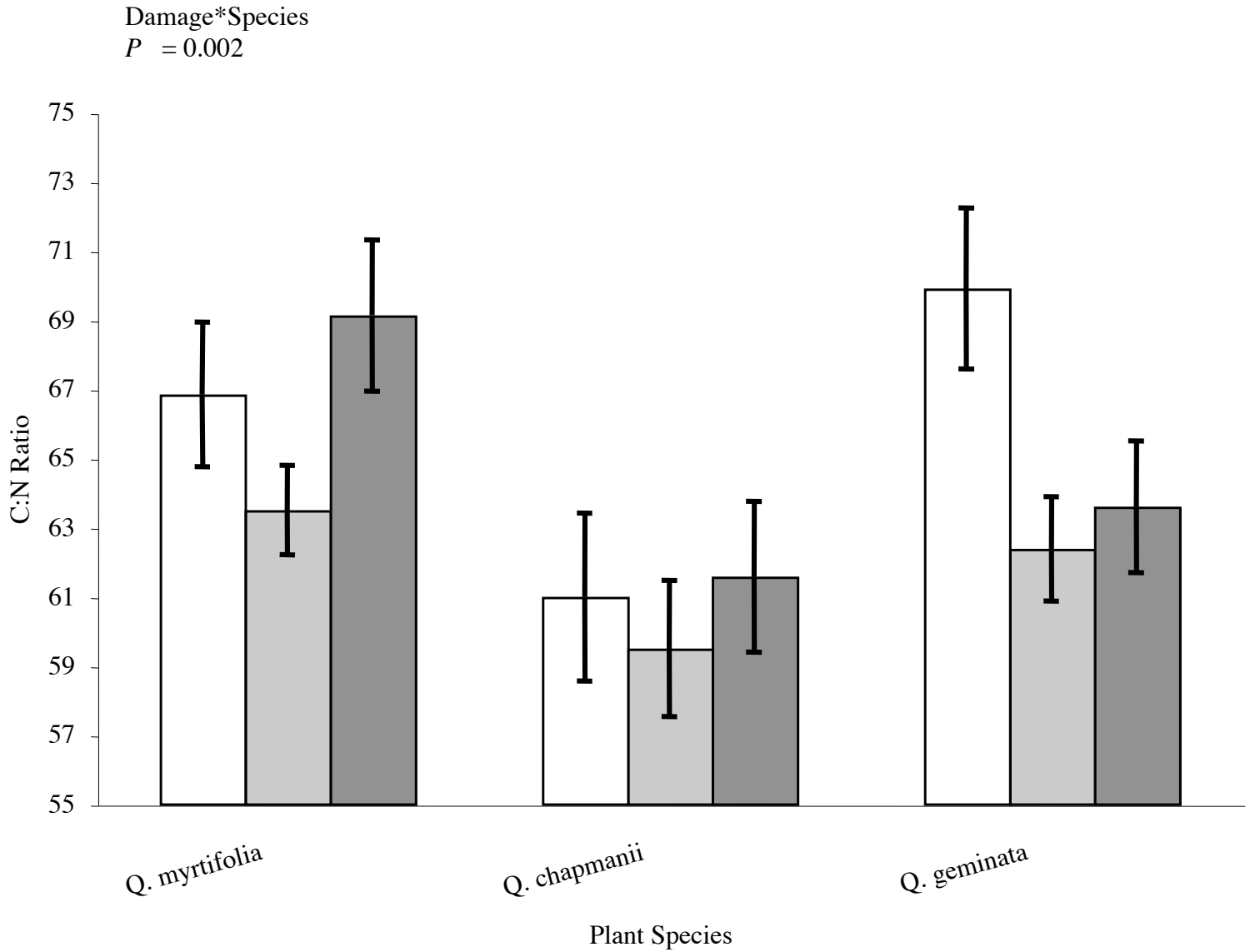


Figure 3.3 cont.

e)

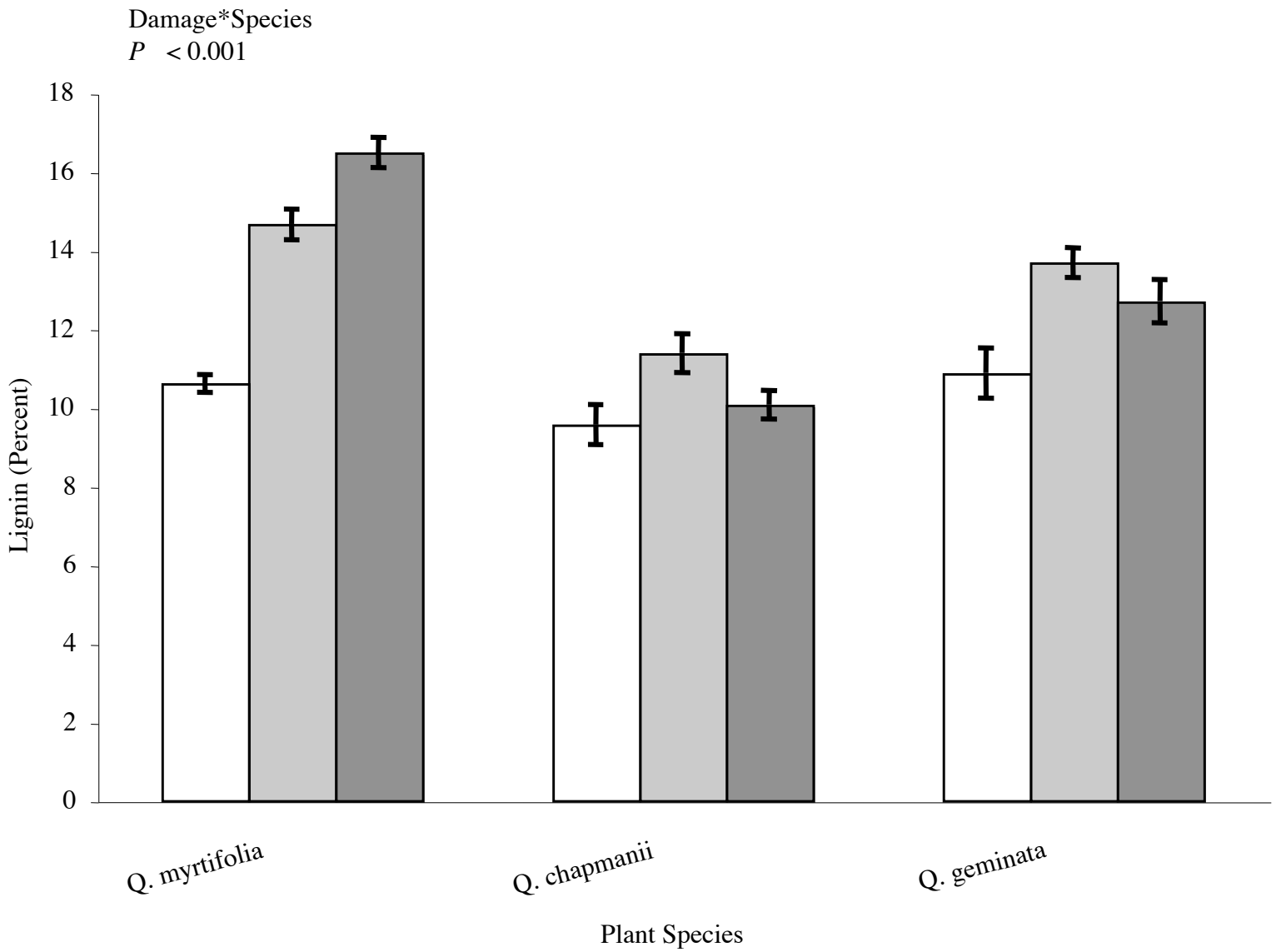


Figure 3.3 cont.

f)

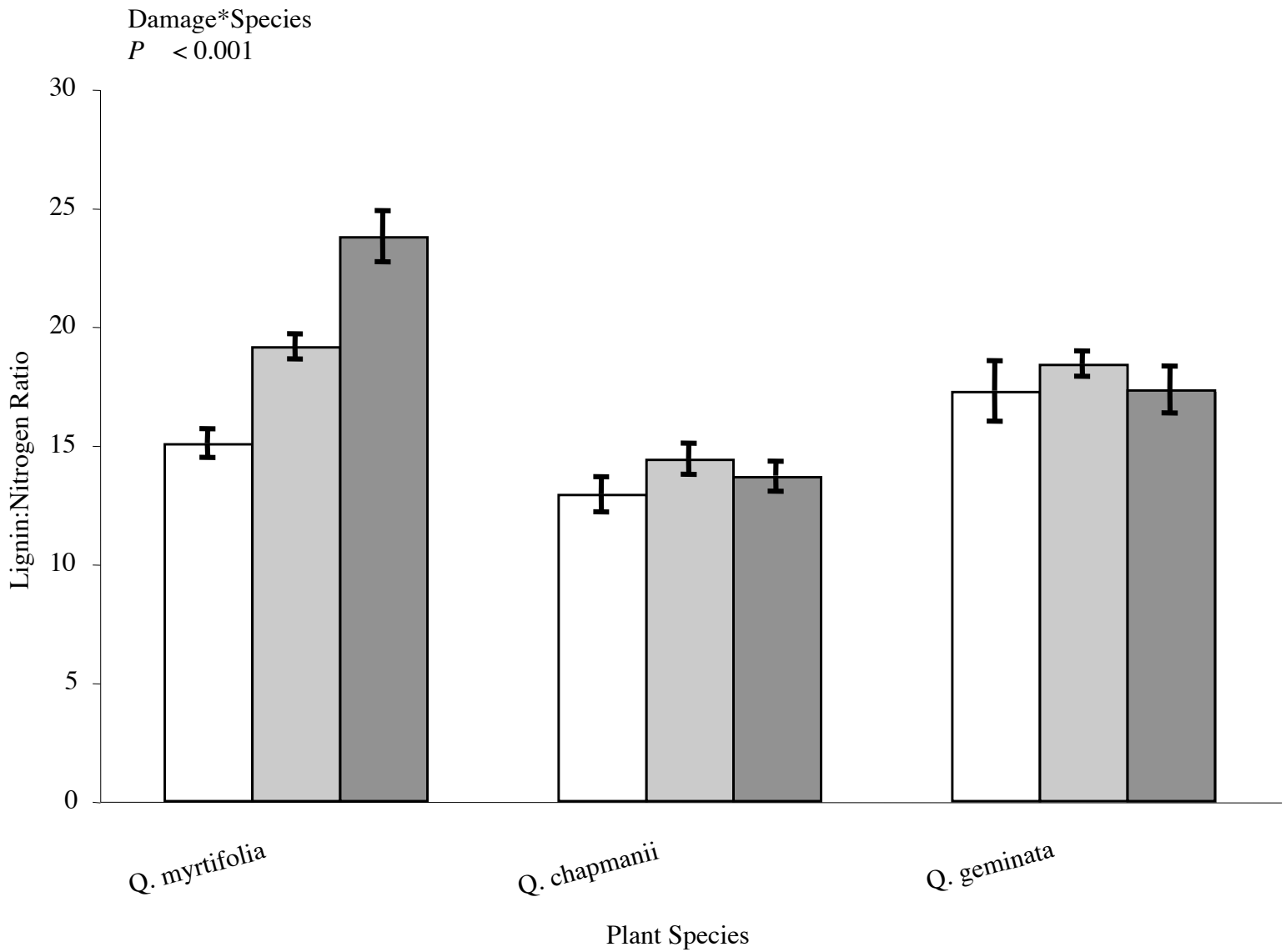


Figure 3.4. Differences in litter chemistry of undamaged, chewed, and mined oak litter over time (a) condensed tannins, (b) hydrolyzable tannins, (c) total phenolics, (d) lignin, (e) lignin:nitrogen ratio. Data are the means of 48 samples and bars represent standard errors.

Figure 3.4

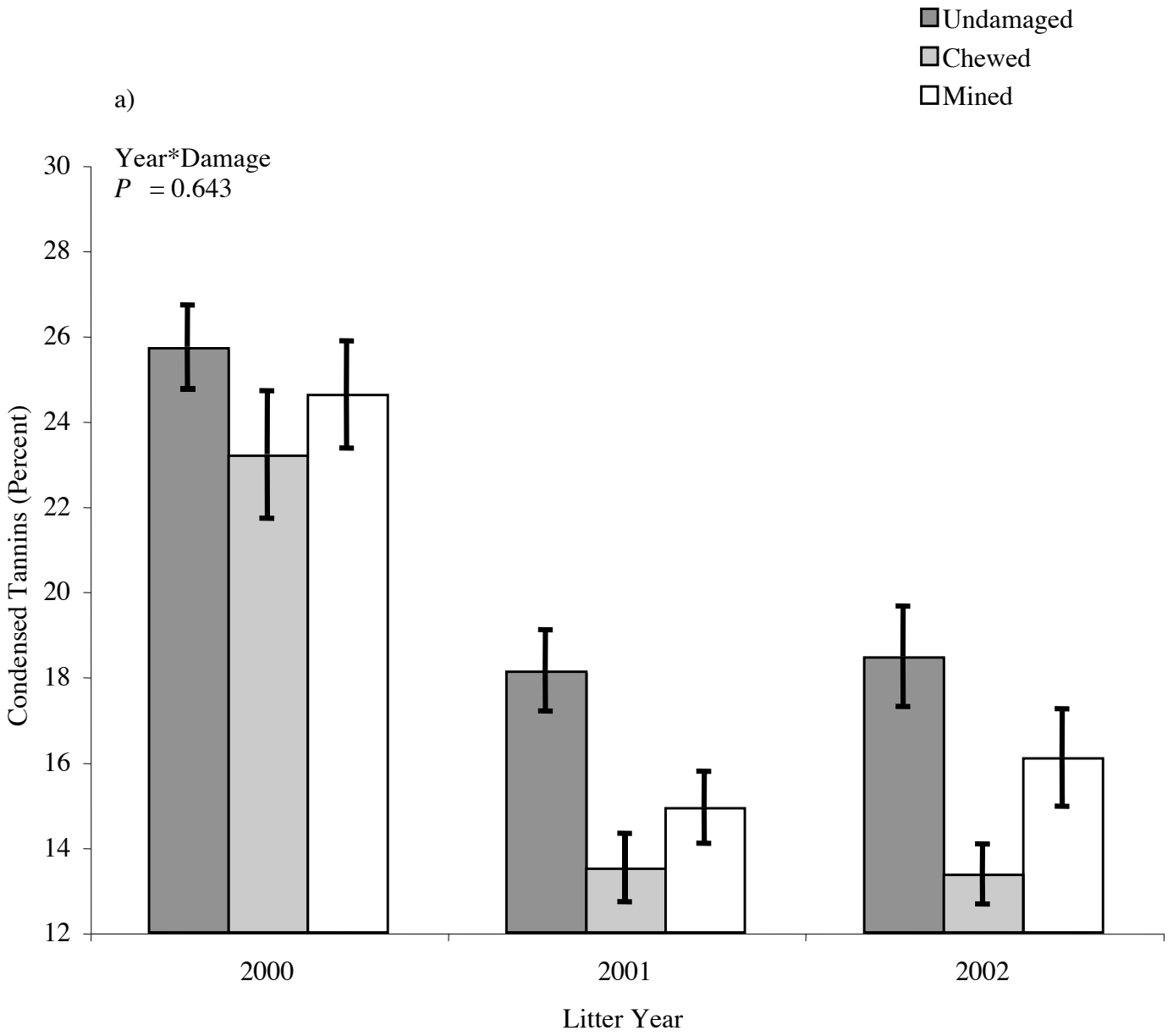

Undamaged
Chewed
Mined
a)
Year*Damage
P = 0.643
Condensed Tannins (Percent)
30
28
26
24
22
20
18
16
14
12
2000
2001
2002
Litter Year

Figure 3.4 cont.

b)

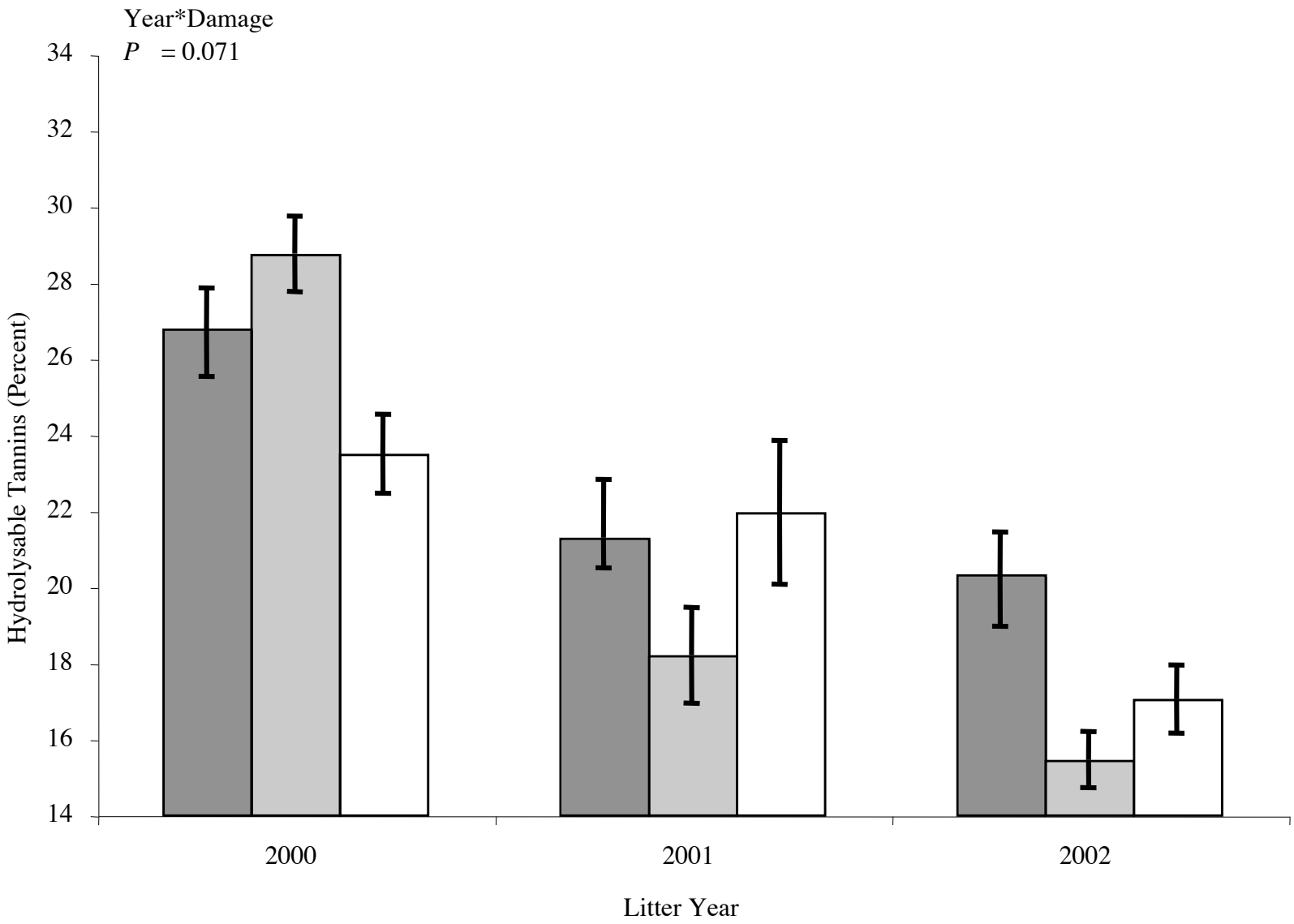


Figure 3.4 cont.

c)

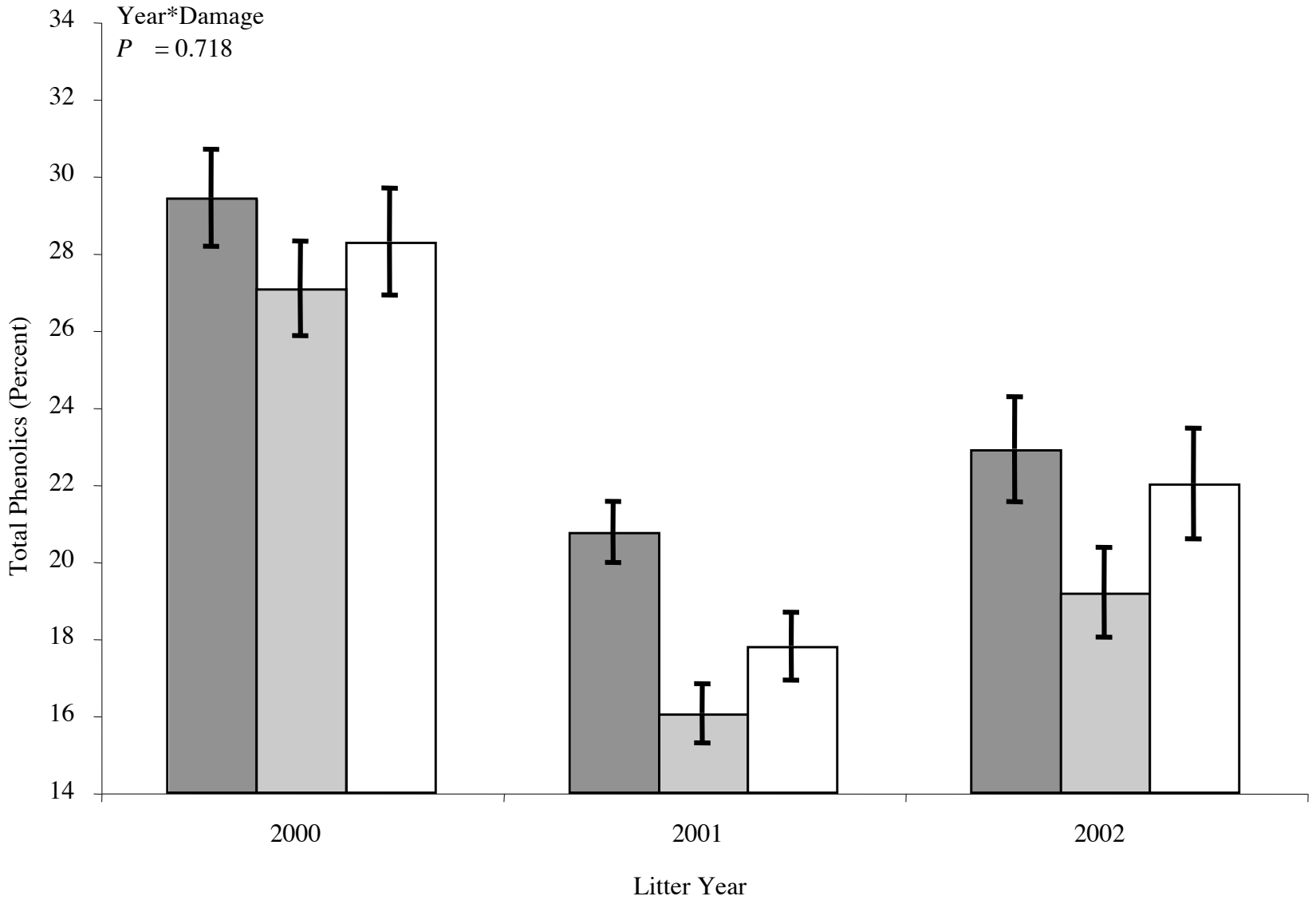


Figure 3.4 cont.

d)

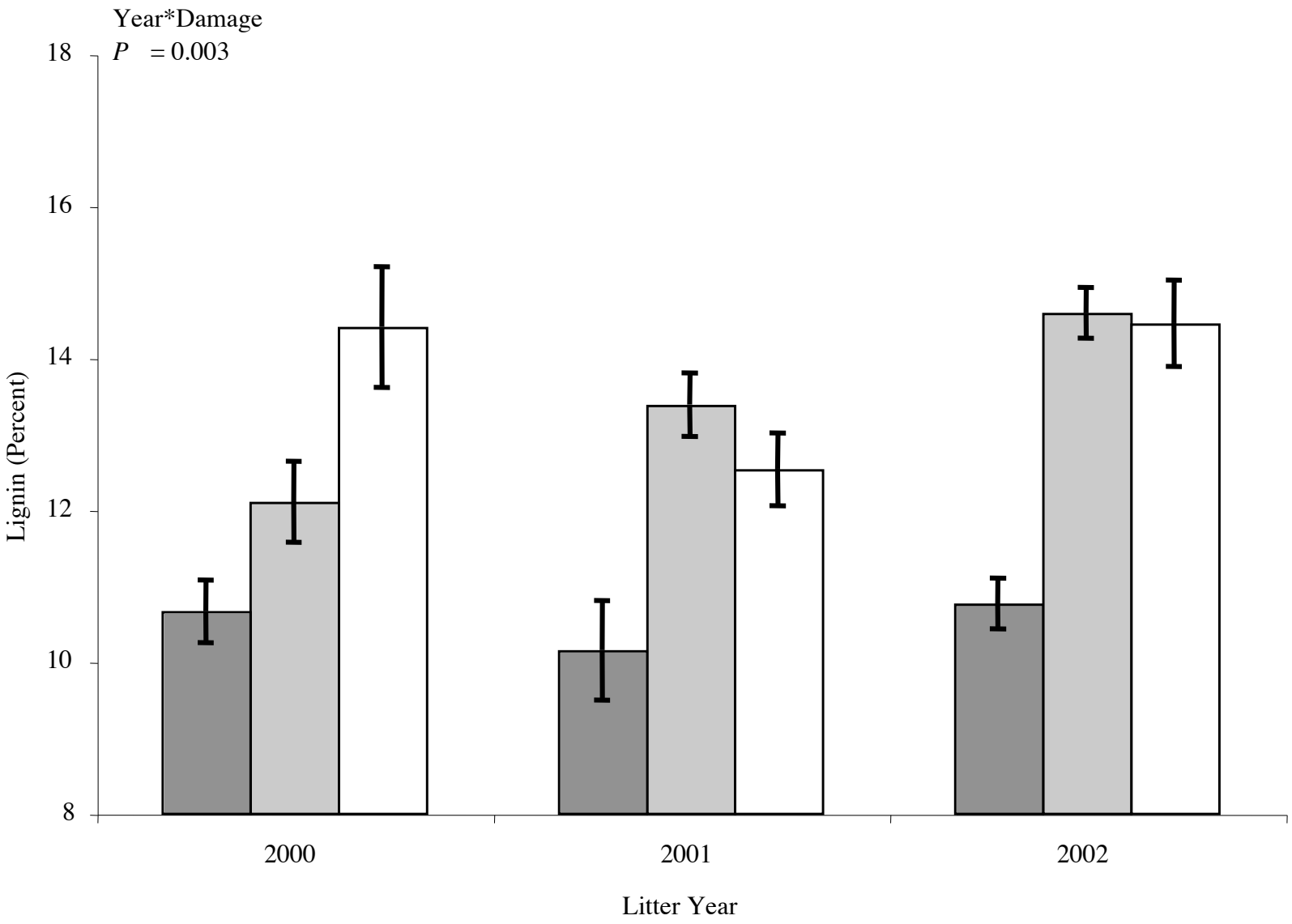
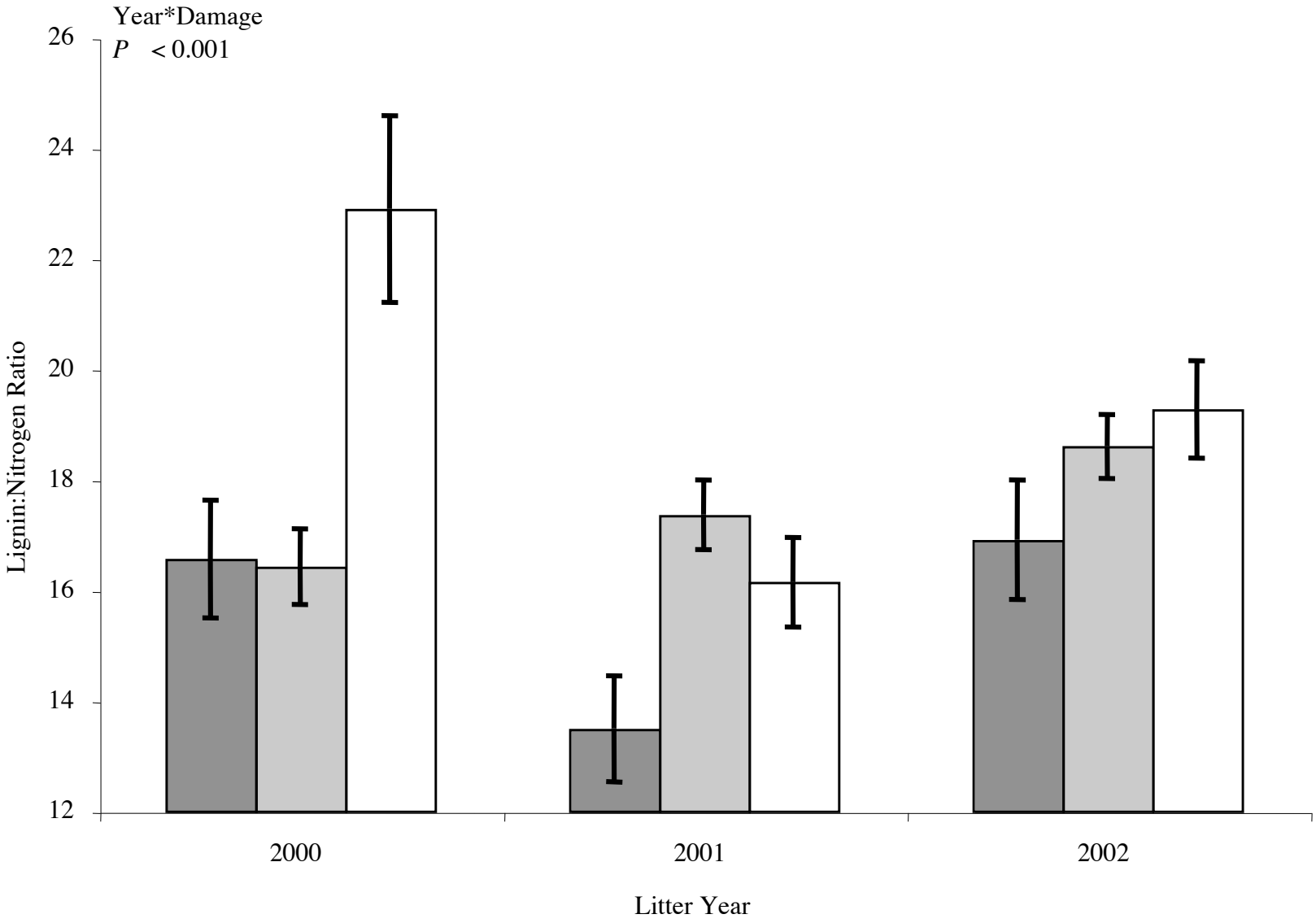


Figure 3.4 cont.

e)



CHAPTER 4

EFFECTS OF ELEVATED CO_2 AND HERBIVORE DAMAGE ON DECOMPOSITION OF *QUERCUS MYRTIFOLIA* LEAF LITTER[1]

[1] Hall, M.C., P. Stiling, D. C. Moon, B. G. Drake, and M. D. Hunter. To be submitted to *Global Change Biology*.

Abstract – We studied the effects of elevated CO2 on the dynamics of leaf litter decomposition in a Florida scrub oak Community. Decomposition of *Quercus myrtifolia* leaf litter was followed for three years in two separate experiments. In the first experiment, we examined the effects CO_2 and herbivore damage on litter quality and subsequent decomposition. Undamaged, chewed and mined litter generated under ambient and elevated (ambient + 350 ppm V) CO_2 was allowed to decompose under ambient conditions for three years. Initial litter chemistry indicated that CO_2 levels had minor effects on litter quality with slightly higher cellulose and hemicellulose concentrations in ambient litter. Litter damaged by leaf miners had higher initial concentrations of condensed tannins and nitrogen and lower concentrations of hemicellulose and C:N ratios compared to undamaged and chewed litter. Initial condensed tannin concentrations exhibited an interaction between herbivore damage and CO_2 treatment. Litter from ambient CO_2 had higher concentrations of condensed tannins in undamaged litter and lower concentrations in chewed litter. Despite variation in litter quality associated with CO_2, herbivory, and their interaction, there was no subsequent effect on rates of decomposition under ambient atmospheric conditions.

In the second experiment, we examined the effects of source (ambient and elevated) of litter and decomposition site (ambient and elevated) on litter decomposition and nitrogen dynamics. Litter was not separated by damage type. This miscellaneous litter from both elevated and ambient CO_2 was then decomposed in both elevated and ambient CO_2 chambers. Initial litter chemistry indicated that concentrations of carbon, hemicellulose, and lignin were higher in litter from elevated than ambient CO_2 chambers. Despite differences in carbon and fiber concentrations, litter from ambient and elevated CO_2 decomposed at comparable rates. However, the atmosphere in which the decomposition took place resulted in significant differences in rates of decomposition. Litter decomposing under elevated CO_2 decomposed more

rapidly than litter under ambient CO_2, and exhibited higher rates of mineral nitrogen accumulation. The results suggest that the atmospheric conditions during the decomposition process have a greater impact on rates of decomposition and nitrogen cycling than do the atmospheric conditions under which the foliage was produced.

Key Words – Decomposition, elevated CO_2, herbivory, litter quality, *Quercus myrtifolia*.

INTRODUCTION

Carbon dioxide (CO_2) concentrations in the atmosphere are currently increasing due to increased fossil fuel use and deforestation. These increases in CO_2 along with associated climate changes are expected to continue and to have significant impacts on terrestrial ecosystems (IPCC 2001). There is abundant evidence that increased levels of CO_2 change the chemical composition of plant foliage (Curtis et al. 1989; Ceulemans and Mousseau 1994; Koch and Mooney 1996; Hall et al. 2005a). The most commonly reported changes in CO_2 grown plant foliage, increased C:N ratios and decreased nitrogen concentrations, might be predicted to change the rate of decomposition and alter nutrient cycling because these variables are associated with decomposition in natural systems (Floate 1970; Berg and Ekbohm 1983;Seastedt 1988; Taylor et al. 1989). However, it remains to been seen whether changes in plant foliage induced under elevated CO_2 are retained in senesced leaves. Curtis et al. (1989) found that increased C:N ratios in the green foliage of the sedge, *Scirpus olneyi* under enriched CO_2 were not maintained in the senesced foliage. Finzi et al. (2001) found no effect of elevated CO_2 on total nonstructural carbohydrates or nitrogen in green leaves or in leaf litter of five tree species. In our scrub oak ecosystem, previous work has shown that elevated CO_2 reduces foliar nitrogen concentrations and increases C:N ratios by an average of 6% and 7%, respectively, in green foliage across three oak species (Hall et al. 2005a). However, the senesced litter of these oaks does not express lower nitrogen concentrations or higher C:N ratios. Rather, the strongest CO_2 effects are seen in the secondary metabolites, particularly condensed tannins (Hall et al. 2005b).

In other studies, chemical differences in senesced foliage between ambient and elevated CO_2 have been observed. Changes in nitrogen (Coûteaux et al. 1991; Kemp et al. 1994; Cotrufo et al. 1994, 1998; Robinson et al. 1997), C:N ratios (Cotrufo et al. 1994, 1998; Ball and Drake,

1997; Robinson et al. 1997;Lutze et al. 2000; Parsons et al. 2004) lignin:N ratios (Cotrufo et al. 1994, 1998, 1999; Parsons et al. 2004), condensed tannins (Cotrufo et al. 1999; Parsons et al. 2004), lignin (Cotrufo et al. 1994, 1999; Henry et al. 2005), and non-structural carbohydrates (Lutze et al. 2000) have been found in litter from enriched CO_2 compared to the controls.

In addition to changes induced by elevated CO_2, other factors can influence litter chemistry, one of which is the effects of insect herbivores. Insect herbivory can change the chemical composition of plant foliage, which may lead to changes in litter quality (Grace 1986; Findlay et al. 1996; Chapman et al. 2003). Grace(1986) showed that gypsy moth defoliation resulted in increased nitrogen concentrations in Pennsylvania oak forest litter. Chapman et al. (2003) found that scale and moth herbivory increased litter nitrogen concentrations in pinyon pines and subsequently C:N and lignin:N ratios decreased. Also scale herbivory increased annual needle litterfall. This herbivore-induced increase in litter quality translated into higher decomposition rates. Though there are a number of studies that have examined the effects of elevated CO_2 on insect herbivores (Lincoln et al. 1986; Fajer et al. 1991; Roth and Lindroth 1995; Stiling et al. 1999, 2002, 2003) few studies have examined the interactions among CO_2, insect herbivory , litter chemistry and decomposition.

In this study, we conducted two experiments to explore the links among elevated CO_2, herbivore damage, litter chemistry, decomposition, and nitrogen dynamics. In the first experiment, we tested the effects of CO_2 growth conditions and herbivore damage on the decomposition of *Quercus myrtifolia* leaf litter. In the second experiment we tested the effects of CO_2 growth conditions (source) and CO_2 decomposition conditions (site) on the decomposition of *Q. myrtifolia* litter without regard to herbivore damage.

METHODS AND MATERIALS

Study Site. Our study site lies within a two-hectare native scrub-oak community located at Kennedy Space Center, Florida. This woody ecosystem is controlled by a natural fire return cycle of 8 – 12 years and the mature canopy is 3 – 5 meters high. The last burn cycle was in 1996 prior to site set up. Sixteen 3.6 – m diameter plots, each enclosed with a clear polyester film open-top chamber 3.4 m in height, were utilized to control CO_2 levels. Chambers were overlaid on an octagonal framework of PVC pipe with a removable access door and frustrum to reduce dilution of air within the chamber by outside wind. All re-growth was cut to ground level in May 1996 and, since that time, the vegetation in eight of the chambers has been exposed to almost twice ambient CO_2 (700 ppm) while the other eight chambers have been exposed to ambient levels of CO_2 (350 ppm). The CO_2 is supplied 24 hours a day. Monitoring and control of CO_2 injection into each chamber is done by infrared gas analyzer in conjunction with manually adjusted needle valves. In ambient CO_2 chambers, the airflow is identical to that of the elevated CO_2 chambers but is not supplemented with CO_2. The study species, *Q. myrtifolia*, accounts for 76% by biomass of the species composition within this community.

Experimental Design. Rates of decomposition focused on litter from the most common tree in the system, *Quercus myrtifolia*. Litter was collected and pooled (by chamber) from litter trays in all chambers during April-May of 2002, when litter was most plentiful. The litter was sorted into four categories: mined, chewed, undamaged and miscellaneous damage (more than one type of damage or damage other than chewers or miners). This resulted in eight litter types (elevated CO_2 and ambient CO_2 x 4 damage types). Litterbags consisted of 5 cm x 4 cm x 1.5-mm mesh and contained 0.1000 g to 0.5000 g of litter from each of the eight treatments depending on availability.

Experiment I. The undamaged, chewed and mined litter (UCM) was used to examine the effects of "source" (ambient or elevated CO_2) and herbivore damage on litter decomposition. Litter bags were placed in six 3.6- m diameter open (unchambered) PVC rings adjacent to the CO_2 chambers, with three replicates of each treatment per ring (n = 18 per ring). One set (replicate) was collected from each ring annually for three years (four dates including time zero, May 2002). Time zero samples were analyzed for litter chemistry, and sample sizes were: 16 undamaged – (7 ambient, 9 elevated), 20 chewed, 20 mined (10 from each CO_2 treatment) with sample sizes based on litter availability from pooled samples.

Experiment II. Each of the sixteen chambers received six bags (one for each collection date) of the miscellaneous damaged (MD) litter from each of the ambient and elevated CO_2 treatments (n =12 bags per chamber). Four bags (two each representing litter from ambient and elevated CO_2) were placed within each of three PVC rings (12 cm diameter) fixed into the soil of each chamber. Each ring was covered by a one leaf thick layer of litter, available from the forest floor in each chamber. One bag of each CO_2 treatment was removed from each chamber every six months for three years from time six months (November 2002) to time three years (May 2005) (seven dates including time zero, May 2002). The total number of bags to be sampled was 36, however, some bags were not recoverable or had 100% mass loss and nitrogen analyses (below) could not be performed on all samples. Thus sample sizes varied among collection dates. Actual sample sizes are noted on tables and figures. Time zero samples sizes were: 10 ambient and 10 elevated from pooled samples. Our design allowed us to consider the effects of both source (ambient and elevated CO_2) and "site" (ambient and elevated) on the decomposition process.

On each collection date for both experiments, litter samples were weighed to determine mass loss. In addition during Experiment II, two nitrogen pools in the litter were followed over three years of decomposition: the mineral pool (NH_4^+ and NO_3^-) and dissolved organic nitrogen (DON) pool (methods below).

Initial Litter Chemistry. Subsamples of litter used in both experiments were retained for measures of initial litter chemistry. The litter was air dried then ground to a fine powder and stored at – 80 degrees Celsius prior to analysis. Cellulose, hemicellulose, and lignin concentrations were determined by sequential neutral detergent / acid detergent digestion on an Ankom fiber analyzer (Abrahamson et al. 2003). Proanthocyanidins, an estimate of condensed tannin concentration, were assayed using N-butanol:HCl methods described in Rossiter et al. (1988). Total phenolics were estimated using the Folin-Denis assay (Swain, 1980), and gallotannins (hydrolysable tannins) were estimated using a potassium iodate technique developed by Bate-Smith (1977) and modified by Schultz and Baldwin (1982). The standard for tannin analysis was generated by multiple sequential washes of a bulk litter sample by acetone extraction. The percent dry weight nitrogen and carbon were estimated using a Carlo-Erba NA1500 model C/N analyzer (Milan, Italy).

Decomposition. Litter was air-dried before weighing and weights were used to determine mass loss. The air-dried litter was ground to a fine powder and stored at – 80^0 C prior to analysis for N-dynamics (Experiment II only).

Ground litter (0.05 g) was hydrated in 20 ml of distilled water and agitated overnight in a shaker at room temperature. The “leachate” was then filtered through a 0.4 □l filter. The filtrate was analyzed for NO_3^- -N and NH_4^+ - N using the automated cadmium reduction and phenate assays, respectively, on an Alpkem segmented flow autoanalyzer (Alpkem RFA 300, Alpkem

Corporation, Clackamas, Oregon, USA). Total dissolved nitrogen (TDN) of the filtrate was estimated following persulfate oxidation and DON was calculated as the difference between TDN and the sum of NO_3^- - N and NH_4^+ - N.

The residue (anything that did not pass through the filter) was oven dried at 60° C for 24 hours then used to estimate percent dry weight nitrogen (DWN) on a Carlo-Erba NA1500 model C/N analyzer (Milan, Italy). Total nitrogen was calculated as DWN plus percent TDN.

Data Analysis. All data were tested for normality and log transformed where appropriate. The initial litter chemistry was analyzed using ANOVA models generated by the GLM procedure of SAS 8.2 (SAS Institute 1998). The UCM litter was analyzed using the repeated measures GLM (SAS Institute 1998). The MD litter nitrogen was analyzed using the MIXED procedure of SAS 8.2 (SAS Institute 1998). The Student-Neuman-Keuls (SNK) post hoc test (α = 0.05) was used to distinguish among treatment means.

RESULTS

EXPERIMENT I - UCM LITTER

Litter Chemistry. The initial chemical composition of undamaged, chewed, and mined *Q. myrtifolia* litter derived from elevated and ambient CO_2 chambers is shown in Table 4.1. Litter from elevated CO_2 chambers had lower concentrations of cellulose ($F = 7.07_{1,48}$, $P = 0.003$), and hemicellulose ($F = 17.03_{1,50}$, $P < 0.001$) than litter from the ambient chambers. The litter from the two CO_2 treatments had similar nitrogen concentrations as well as similar C:N ratios. Similarly, lignin and phenolic concentrations, often used as indicators of litter quality, were unaffected by CO_2 levels (Table 4.1).

Herbivore damage was associated with variation in initial litter chemistry (Table 4.2). Condensed tannin concentrations were higher in mined litter than in undamaged or chewed litter ($F = 6.30_{2,50}$, $P = 0.004$) and nitrogen concentrations were also higher in mined litter than in undamaged litter ($F = 6.82_{2,50}$, $P = 0.002$). The C:N ratio was higher in undamaged litter than in mined litter ($F = 10.36_{250}$, $P < .001$) and hemicellulose was higher in undamaged litter than in either chewed or mined leaf litter ($F = 6.25_{2,50}$, $P = 0.004$) (Table 4.2).

Only condensed tannin concentrations showed an interaction between CO_2 and herbivore damage (Figure 4.1). Mined litter was consistent in the concentrations of condensed tannins between CO_2 treatments. Undamaged litter from elevated CO_2 chambers had lower concentrations of condensed tannins than litter from elevated CO_2 chambers while chewed litter was the opposite with higher concentrations of condensed tannins in chewed litter from elevated CO_2 chambers ($F = 5.46_{2,50}$, $P = 0.007$) (Figure 4.1).

Litter Decomposition. Despite initial differences in litter chemistry associated with CO_2 treatment (Table 4.1) and herbivore activity (Table 4.2, Figure 4.1), neither CO_2 treatment ($F_{2,56}$= 0.18, $P = 0.833$)(Figure 4.2a) nor herbivore damage ($F_{2,56}$=0.38, $P = 0.824$)(Figure 4.2b) influenced the rates of litter decomposition.

EXPERIMENT II - MD LITTER

Initial Chemistry. The initial chemical composition of miscellaneous damage litter is summarized in Table 4.3. Litter from elevated CO_2 chambers had higher concentrations of carbon, hemicellulose and lignin than did the litter from ambient chambers (carbon $F = 15.89_{1,17}$, $P < 0.001$; hemicellulose $F = 11.80_{1,18}$, $P = 0.003$; lignin $F = 23.85_{1,18}$, $P < 0.001$).

Litter Decomposition. The rates of decomposition were comparable for litter originating from elevated and ambient CO_2 chambers ($F_{1,123}$=1.38, P = 0.235)(Figure 4.3a), however the CO_2 level under which litter was decomposing did have an effect on mass loss. Litter decomposition was accelerated under elevated CO_2 ($F_{1,123}$=11.66, $P < 0.001$) (Figure 4.3b).

Ammonium accumulated to higher levels ($F_{1,121}$ = 12.66, $P < 0.001$) in litter decomposing under elevated CO_2 than in litter decomposing under ambient CO_2 (Figure 4.4a). Likewise, nitrate concentrations accumulated more rapidly in litter decomposing under elevated CO_2 ($F_{1,121}$= 2.67, P = 0.025)(Figure 4.4b). There were no effects of source chamber on the accumulation of mineral nitrogen in litter. Total litter nitrogen concentrations tended to increase over time ($F_{5,118}$ = 41.42, $P < 0.001$) (Table 4.4). In general DON and mineral pools also increased during decomposition (Table 4.4) but these increases in concentration largely reflect the relative loss of carbon. The percent of initial litter carbon remaining on each sample date ($F_{5,118}$, P = 0.005)(Figure 4.5a) declined faster in litter decomposing under elevated CO_2. The percent of initial litter nitrogen remaining on each sample date ($F_{,5,118}$, P = 0.579)(Figure 4.5b) did not differ between litter decomposing under ambient and elevated CO_2 and did not vary systematically over time.

DISCUSSION

Contrary to expectations, the source of leaf litter (ambient or elevated), its degree of damage, and its initial chemistry did not predict decomposition rates. Rather, litter decomposing under elevated CO_2 decomposed more rapidly than litter decomposing under ambient CO_2 (Figure 4.3b). This effect of decomposition site was most pronounced from 18 to 36 months; prior to 18 months, decomposition rates under elevated and ambient CO_2 were comparable.

Coupled with the increase in decomposition rate under elevated CO_2 was an increase in rate of mineral nitrogen accumulation in leaf litter (Figure 4.4) and an increase in the rate of carbon loss (Figure 4.5a). Litter carbon may therefore be driving the accumulation of mineral nitrogen. Given that overall levels of nitrogen varied little over the experiment (Figure 4.5b), it is tempting to conclude that rates of nitrogen mineralization were higher under elevated than ambient CO_2. However, we cannot rule out the possibility that organic nitrogen export was balanced by mineral nitrogen import.

In this study (Experiment I) initial litter quality of UCM litter exhibited only minor differences between ambient and elevated CO_2 and none of the primary regulators (nitrogen, C:N ratio, lignin) of decomposition were affected by CO_2 concentrations. Rather, litter cellulose and hemicellulose concentrations were higher in litter from ambient CO_2. In line with other studies, which have documented little or no change in leaf litter quality (Finzi et al. 2001), there was no change in decomposition rates. Conversely, MD litter (Experiment II) had higher concentrations of carbon, hemicellulose and lignin in litter originating from elevated CO_2 chambers compared to that from ambient CO_2. Although lignin concentrations are often a good predictor of decomposition rates (Meetenmeyer 1978), the origin of litter and thus litter quality did not affect decomposition rates in Experiment II. Overall, decomposition rates were unaffected by the source (ambient or elevated) of the litter regardless of whether or not litter quality was affected.

Though a number of studies have examined decomposition of litter from various CO_2 concentrations, few have examined the interactions between herbivory and CO_2 and their effects on nutrient cycling. Prior work on the three oak species in the scrub oak system found that herbivore damaged litter was lower in phenolic concentrations and higher in lignin

concentrations and lignin:N ratios (Hall et al. 2005b). Whether these effects represented choice by herbivores and / or induction was not determined in the current or previous studies in the scrub oak system because herbivore densities could not be manipulated experimentally in the multiple-project chambers. Whether the result of induction or not, variation in litter quality associated with herbivore activity did not affect decomposition rates.

The higher rates of decomposition and nitrogen cycling in elevated CO_2 chambers that we observed in this study could have been the result of differences in microclimate or biota. Elevated CO_2 chambers at this site have greater productivity (Dijkstra et al. 2002) and litter cover on the chamber floors (Stiling et al. 2003), which may increase moisture levels above those in the ambient CO_2 chambers. As a radiative gas the addition of CO_2 to chambers may have resulted in an increase in temperatures in elevated CO_2 chambers. If changes in the microclimate occurred, then activity rates of the biota may have altered leading to increased decomposition and nitrogen accumulation. Unfortunately, no systematic comparisons of microclimate between ambient and elevated chambers have been made. Most reports of temperature differences related to open top chambers focus on interior and exterior temperature measurements (Drake et al. 1989; Whitehead et al. 1995). However a review of open top chamber design and function by Leadley and Drake (1993) found no differences in long-term air temperature between elevated and ambient CO_2 chambers.

Changes in detritivore populations and activity levels other than those associated with microclimatic changes could also explain the acceleration of decomposition and nitrogen accumulation seen under elevated CO_2. There are two primary hypotheses concerning nitrogen availability under elevated CO_2. The first suggests that increased carbon availability under

elevated CO_2 could boost microbial biomass and lead to increased immobilization of nitrogen (Diaz et al. 1993). Alternatively, Zak et al. (1993) suggested that increased carbon input would result in increased microbial activity leading to increased nitrogen availability. This is consistent with our results and some previous work. For example, Zak et al. (1993) found that rhizosphere and bulk soil of *Populus grandidentata* grown in elevated CO_2 had greater microbial biomass carbon than that found in ambient CO_2. Likewise, De Graaff et al. (2004) found that soil respiration increased in *Lolium perenne* soil after exposure to elevated CO_2. However there was not an increase in microbial biomass and soil respiration increases did not depend on incorporation of plant material. Cotrufo et al. (1999) found that litter of *Quercus pubescens* grown under elevated CO_2 decomposed slower in elevated CO_2 than did litter from ambient or elevated CO_2 decomposing under ambient CO_2. They also found that nitrogen concentrations in the three litter types were similar and increased with time and concluded that net nitrogen mineralization occurred in all decomposing litter. The conflicting results between this study and the findings of Cotrufo et al. (1999) could be attributed to a number of factors. They utilized a natural CO_2 spring which varied in CO_2 concentrations, did not include litter from ambient CO_2 in the elevated CO_2 decomposition site, used a different oak species, and ran the decomposition experiment for a shorter period of time. Coûteaux et al. (1991) found changes in litter quality including lower nitrogen concentrations and higher C:N ratios in chestnut litter grown under elevated CO_2. Litter from elevated CO_2 decomposed more slowly than litter from ambient CO_2. However, when soil fauna complexity was increased litter from elevated CO_2 displayed higher decomposition rates than ambient CO_2 litter.

Our major finding is that the site of decomposition (ambient or elevated CO_2) has a greater impact on litter decomposition and nitrogen cycling than does variation in initial litter quality caused by elevated CO_2 or herbivore activity. As such, our results add to a growing body of work that suggests that increased concentrations of CO_2 may generate long-term shifts in decomposition processes and rates of nutrient cycling. Such shifts have the potential to change both ecosystem structure and function.

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TABLE 4.1. INITIAL CHEMICAL COMPOSITION OF UNDAMAGED, CHEWED, AND MINED *Q. MYRTIFOLIA* LITTER FROM AMBIENT AND ELEVATED CO_2 ACROSS DAMAGE CATEGORIES

Sample	Condensed Tannins (%)	Hydrolyzable Tannins (%)	Total Phenolics (%)	Nitrogen (%)	Carbon (%)	C:N	Cellulose (%)	Hemicellulose (%)	Lignin (%)
Elevated	26.00	27.37	28.05	0.58	50.65	88.30	19.93	13.24	8.91
	(1.25)	(0.95)	(1.77)	(0.01)	(0.98)	(2.04)	(0.32)	(0.27)	(0.34)
Ambient	23.34	25.90	27.15	0.58	50.21	87.94	21.04	14.54	9.71
	(1.67)	(0.97)	(1.07)	(0.01)	(0.11)	(1.97)	(0.16)	(0.22)	(0.19)
	NS	NS	NS	NS	NS	NS	$P = 0.003$	$P < 0.001$	*NS*

Values are means, $n = 29$ for elevated and $n = 27$ for ambient, with SE in parentheses.

TABLE 4.2. INITIAL CHEMICAL COMPOSITION OF UNDAMAGED, CHEWED, AND MINED *Q. MYRTIFOLIA* LITTER BY DAMAGE CATEGORY ACROSS CO_2 TREATMENTS

Sample	Condensed Tannins (%)	Hydrolyzable Tannins (%)	Total Phenolics (%)	Nitrogen (%)	Carbon (%)	C:N	Cellulose (%)	Hemicellulose (%)	Lignin (%)
Undamaged	22.40	26.13	27.50	0.54	51.34	95.47	20.81	14.71	8.60
	(1.29)	(1.51)	(1.77)	(0.018)	(1.76)	(2.09)	(0.32)	(0.31)	(0.40)
Chewed	22.30	27.77	28.24	0.58	50.51	88.76	20.74	13.64	9.51
	(1.74)	(1.24)	(1.37)	(0.02)	(0.14)	(2.19)	(0.23)	(0.35)	(0.31)
Mined	28.98	26.00	27.10	0.62	49.65	81.57	19.93	13.41	9.64
	(1.79)	(0.81)	(2.22)	(0.01)	(0.24)	(2.0)	(0.43)	(0.28)	(0.35)
	$P = 0.004$	NS	NS	$P = 0.002$	*NS*	$P < 0.001$	NS	$P = 0.004$	*NS*

Values are means, $n = 16$ undamaged, $n = 20$ chewed, $n = 20$ mined, with SE in parentheses.

TABLE 4.3. INITIAL CHEMICAL COMPOSITION OF MISCELLANEOUS DAMAGED *Q. MYRTIFOLIA* LITTER

Sample	Condensed Tannins (%)	Hydrolyzable Tannins (%)	Total Phenolics (%)	Nitrogen (%)	Carbon (%)	C:N	Cellulose (%)	Hemicellulose (%)	Lignin (%)
Elevated	24.07	28.91	31.88	0.62	50.79	82.63	20.42	14.51	10.55
	(2.59)	(1.80)	(2.47)	(0.02)	(0.09)	(3.27)	(0.37)	(0.13)	(0.44)
Ambient	23.60	25.77	29.89	0.63	49.48	80.67	20.70	13.42	8.07
	(2.32)	(1.21)	(2.14)	(0.04)	(0.37)	(4.92)	(0.41)	(0.30)	(0.26)
	NS	NS	NS	NS	$P < 0.001$	NS	NS	$P = 0.003$	$P < 0.001$

Values are means, $n = 10$, with SE in parentheses

TABLE 4.4. TOTAL NITROGEN AND NITROGEN POOLS IN MISCELLANEOUS DAMAGED LITTER OF *Q. MYRTIFOLIA* ON EACH COLLECTION DATE FROM SOURCE AND SITE OF DECOMPOSITION

Source	Ambient				Elevated			
Site	Ambient		Elevated		Ambient		Elevated	
Total N								
6	0.41	(0.05) 8	0.40	(0.03) 8	0.38	(0.07) 8	0.41	(0.05) 8
12	0.46	(0.03) 8	0.46	(0.03) 8	0.37	(0.03) 8	0.44	(0.06) 8
18	0.75	(0.05) 8	0.80	(0.04) 7	0.71	(0.03) 8	0.85	(0.05) 7
24	0.72	(0.03) 6	0.93	(0.16) 2	0.56	(0.04) 7	0.80	(0.11) 3
30	1.04	(0.08) 4	1.31	(.) 1	0.73	(0.04) 3	0.64	(.) 1
36	0.76	(0.12) 4	0.83	(0.10) 8	0.78	(0.08) 3	0.96	(0.07) 8
DON								
6	1.08	(0.13)	1.15	(0.19)	0.84	(0.07)	0.88	(0.07)
12	1.09	(0.12)	1.45	(0.28)	0.83	(0.04)	1.44	(0.28)
18	2.04	(0.35)	2.22	(0.47)	1.23	(0.13)	2.34	(0.42)
24	2.17	(0.44)	1.39	(0.40)	1.99	(0.41)	1.56	(0.24)
30	4.52	(0.26)	3.91	(.)	4.69	(0.44)	3.95	(.)
36	5.74	(0.26)	6.10	(0.43)	5.21	(0.15)	6.30	(0.57)
NO_3^-N								
6	0.0240	(0.0100)	0.0042	(0.0030)	0.0025	(0.0020)	0.0002	(0.0003)
12	0.0031	(0.0023)	0.0200	(0.0090)	0.0141	(0.0074)	0.0038	(0.0014)
18	0.0160	(0.0028)	0.0145	(0.0037)	0.0160	(0.0055)	0.0111	(0.0035)
24	0.0116	(0.0012)	0.0258	(0.0198)	0.0142	(0.0024)	0.0158	(0.0023)
30	0.0210	(0.0048)	0.0510	(.)	0.0150	(0.0055)	0.0281	(.)
36	0.0197	(0.0022)	0.0216	(0.0011)	0.0129	(0.0004)	0.0168	(0.0012)
NH_4^+-N								
6	0.005	(0.005)	0.016	(0.014)	0.015	(0.008)	0.009	(0.007)
12	0.070	(0.021)	0.090	(0.038)	0.061	(0.034)	0.085	(0.033)
18	0.157	(0.036)	0.142	(0.043)	0.093	(0.032)	0.177	(0.031)
24	0.177	(0.079)	0.276	(0.276)	0.160	(0.076)	0.266	(0.096)
30	0.092	(0.016)	0.272	(.)	0.067	(0.034)	0.435	(.)
36	0.594	(0.009)	0.211	(0.125)	0.062	(0.015)	0.187	(0.053)

Total Nitrogen is given as mean percent. DON, NO_3^-N, and NH_4^+-N are given in mg/kg. Standard errors are in parentheses. Sample size is given following SE for Total Nitrogen and are consistent for all subsequent measures of nitrogen. Source is the CO_2 level (ambient or elevated) in which litter originated (grew) and site is the CO_2 level (ambient or elevated) in which litter decomposed.

Figure 4.1. Effects of herbivore damage and CO_2 treatment on concentrations of condensed tannins in *Q. myrtifolia* leaf litter. Data are the means of 9 samples (undamaged ambient) 7 samples (undamaged elevated) or 10 samples (chewed or mined from ambient or elevated CO_2). Bars represent standard errors, and different letters denote significantly different means.

Figure 4.1

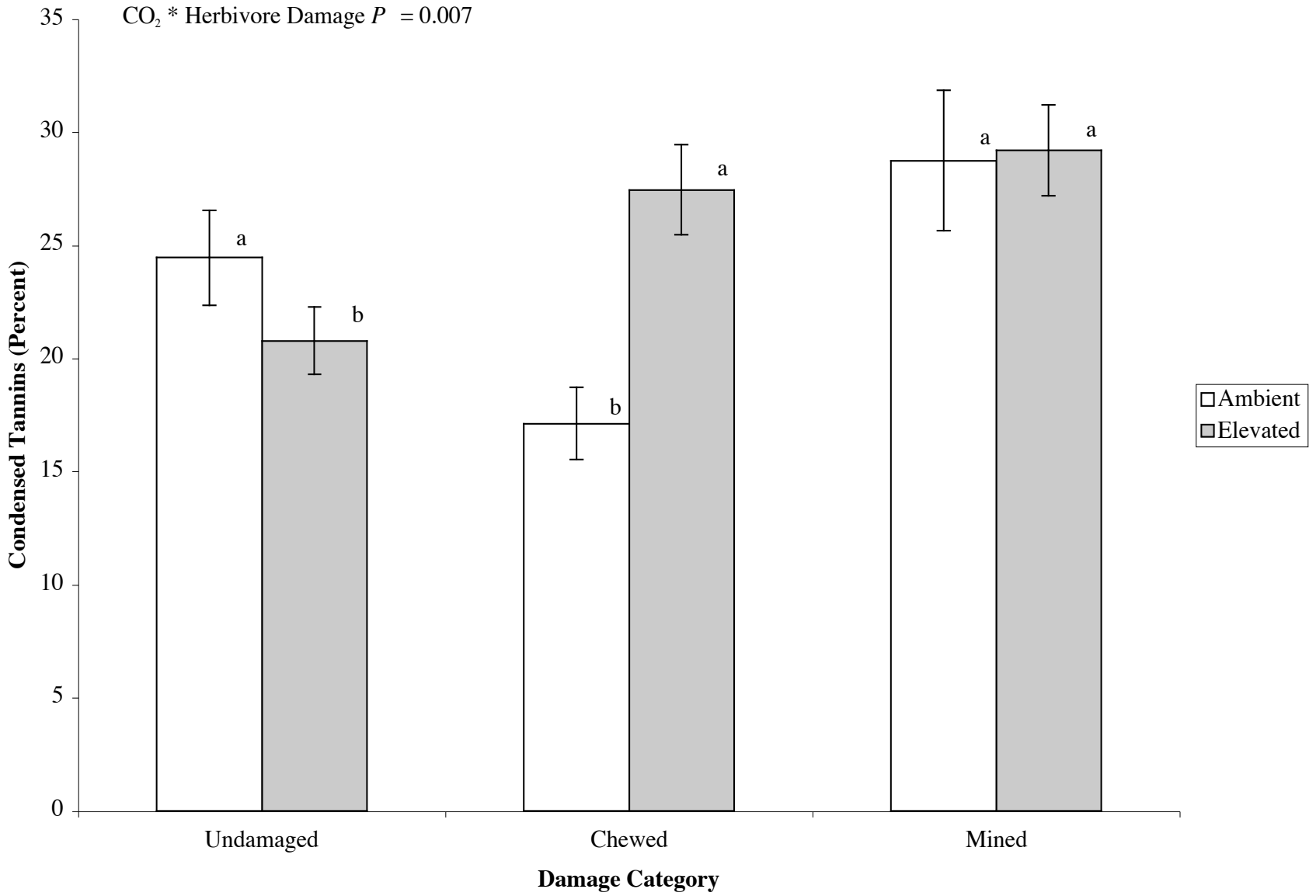

CO_2 * Herbivore Damage P = 0.007
Condensed Tannins (Percent)
35
30
25
20
15
10
5
0
a
b
b
a
a
a
Undamaged
Chewed
Mined
Damage Category
Ambient
Elevated

Figure 4.2. Decomposition rates for a) UCM *Q. myrtifolia* litter derived from ambient and elevated CO_2 and decomposing under ambient conditions for three years and b) by herbivore damage category decomposing under ambient conditions for three years. Bars represent standard errors. Data are the means of 18 samples for ambient and elevated CO_2 treatments and 12 for undamaged, chewed and mined samples.

Figure 4.2

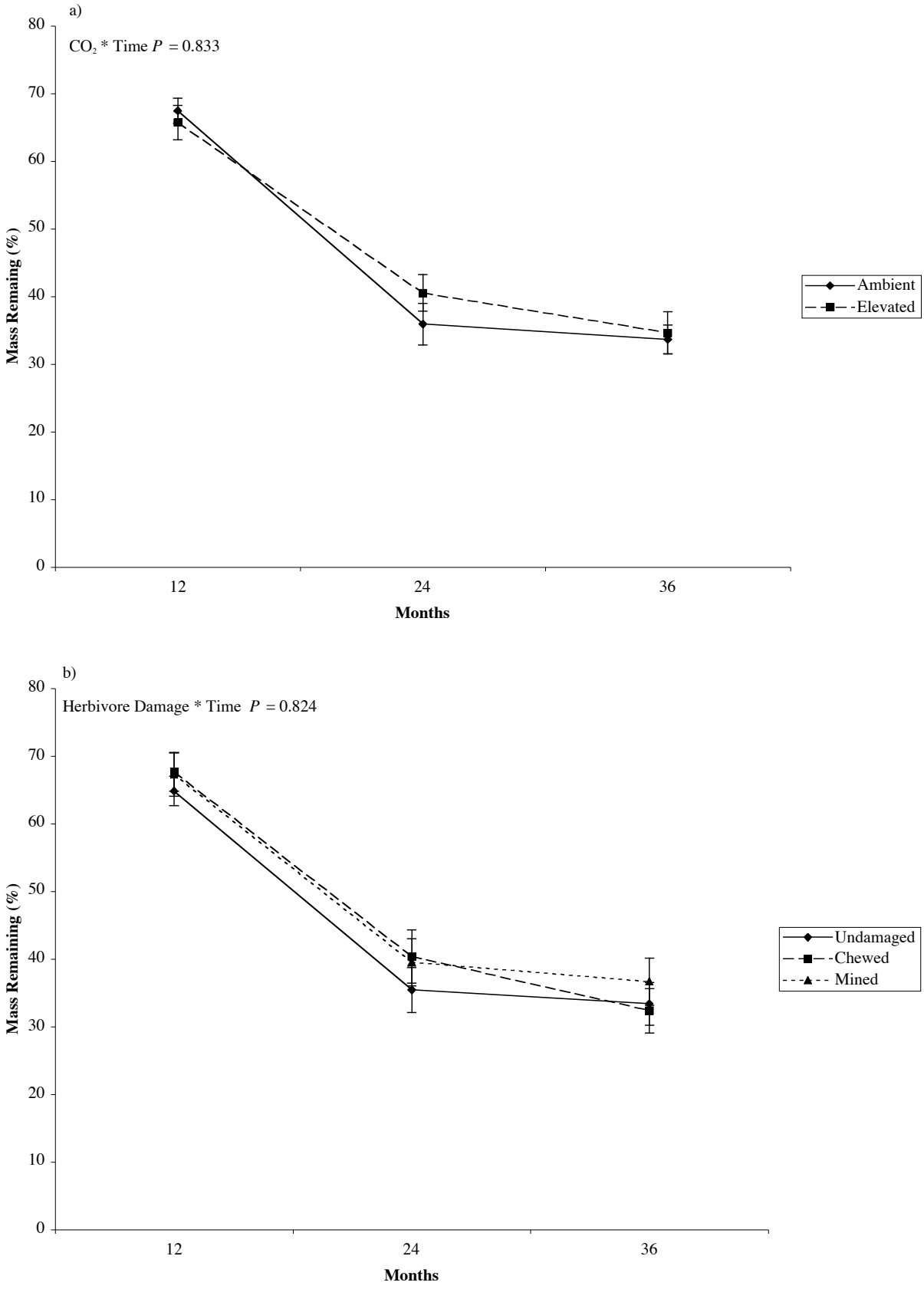
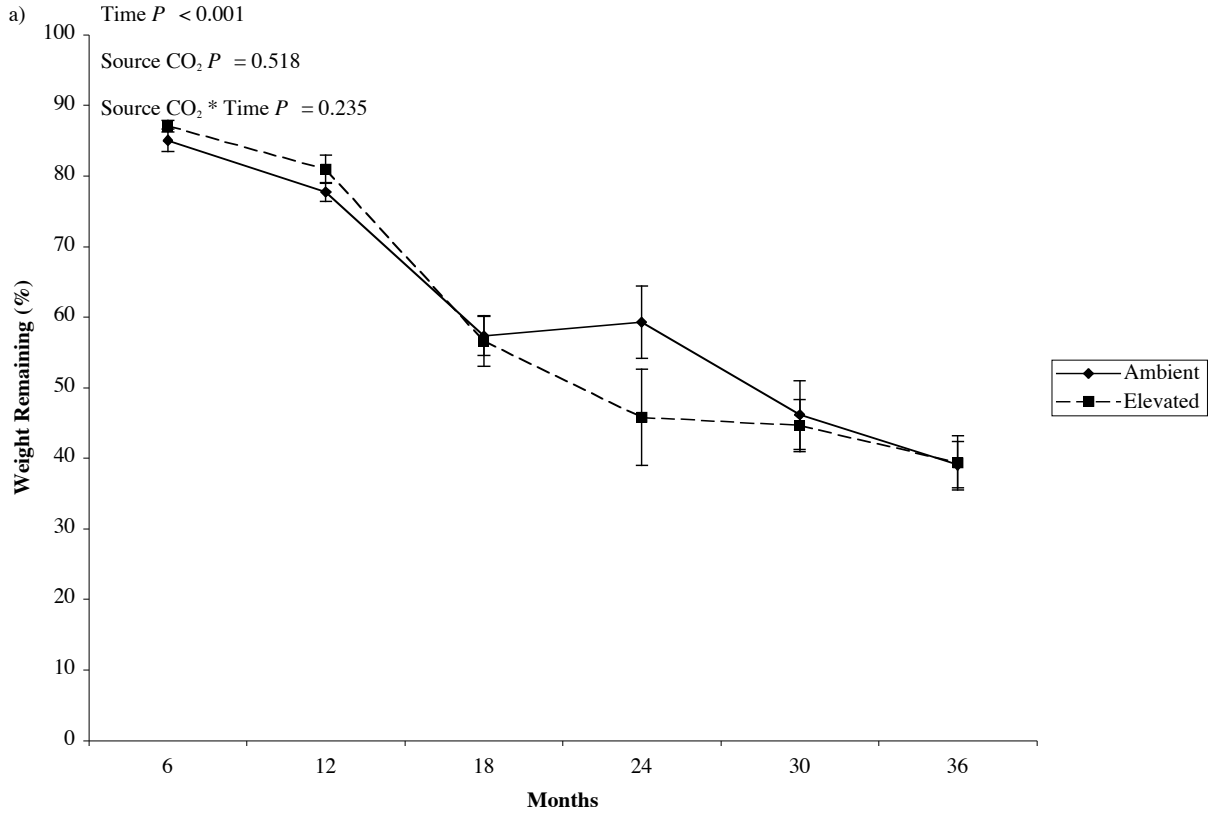

a)
CO_2 * Time P = 0.833
Mass Remaing (%)
80
70
60
50
40
30
20
10
0
12
24
36
Months
Ambient
Elevated
b)
Herbivore Damage * Time P = 0.824
Mass Remaining (%)
80
70
60
50
40
30
20
10
0
12
24
36
Months
Undamaged
Chewed
Mined

Figure 4.3. Decomposition rates for MD *Q. myrtifolia* litter for a) litter from ambient and elevated CO_2 treatments (source) and b) litter decomposing in ambient and elevated CO_2 treatments (site). Sample sizes vary by time and treatment and are as follows in order of time : Source – ambient = 16, 16, 15, 8, 5, 12; Source – elevated = 16, 16, 15, 12, 4, 12; Site – ambient = 16, 16, 16, 14, 7, 8; Site – elevated = 16, 16, 14, 6, 2, 16. Bars represent standard errors.

Figure 4.3


a)
Time P < 0.001
Source CO_2 P = 0.518
Source CO_2 * Time P = 0.235
Weight Remaining (%)
100
90
80
70
60
50
40
30
20
10
0
6
12
18
24
30
36
Months
Ambient
Elevated

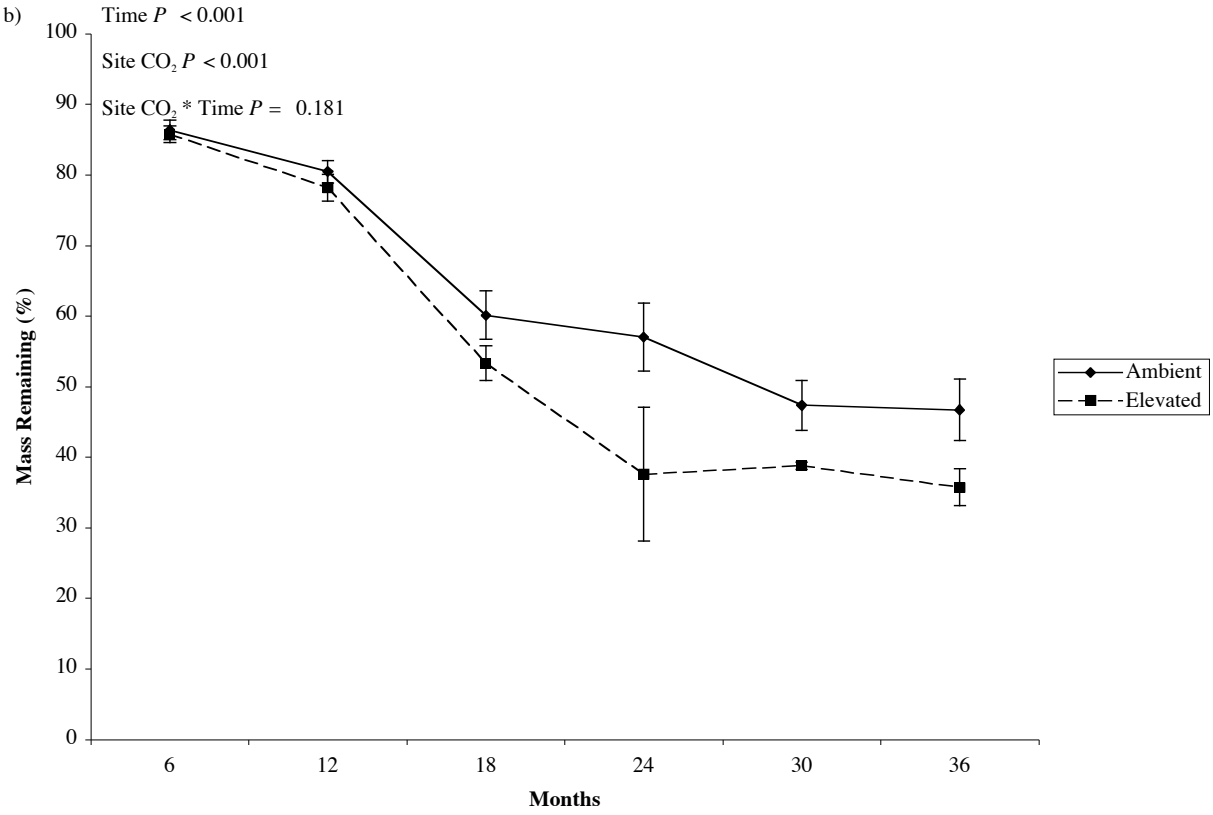

b)
Time P < 0.001
Site CO_2 P < 0.001
Site CO_2 * Time P = 0.181
Mass Remaining (%)
100
90
80
70
60
50
40
30
20
10
0
6
12
18
24
30
36
Months
Ambient
Elevated

Figure 4.4. Ammonium concentrations (a) and nitrate concentrations (b) in litter decomposing under ambient and elevated CO_2 (site) for all collection dates of MD *Q. myrtifolia* litter. Bars represent standard errors. Sample sizes varies by time and treatment and are as follows in order of time: Ambient = 16, 16, 16, 13, 7, 7; Elevated = 16, 16, 14, 5, 2, 16.

Figure 4.4

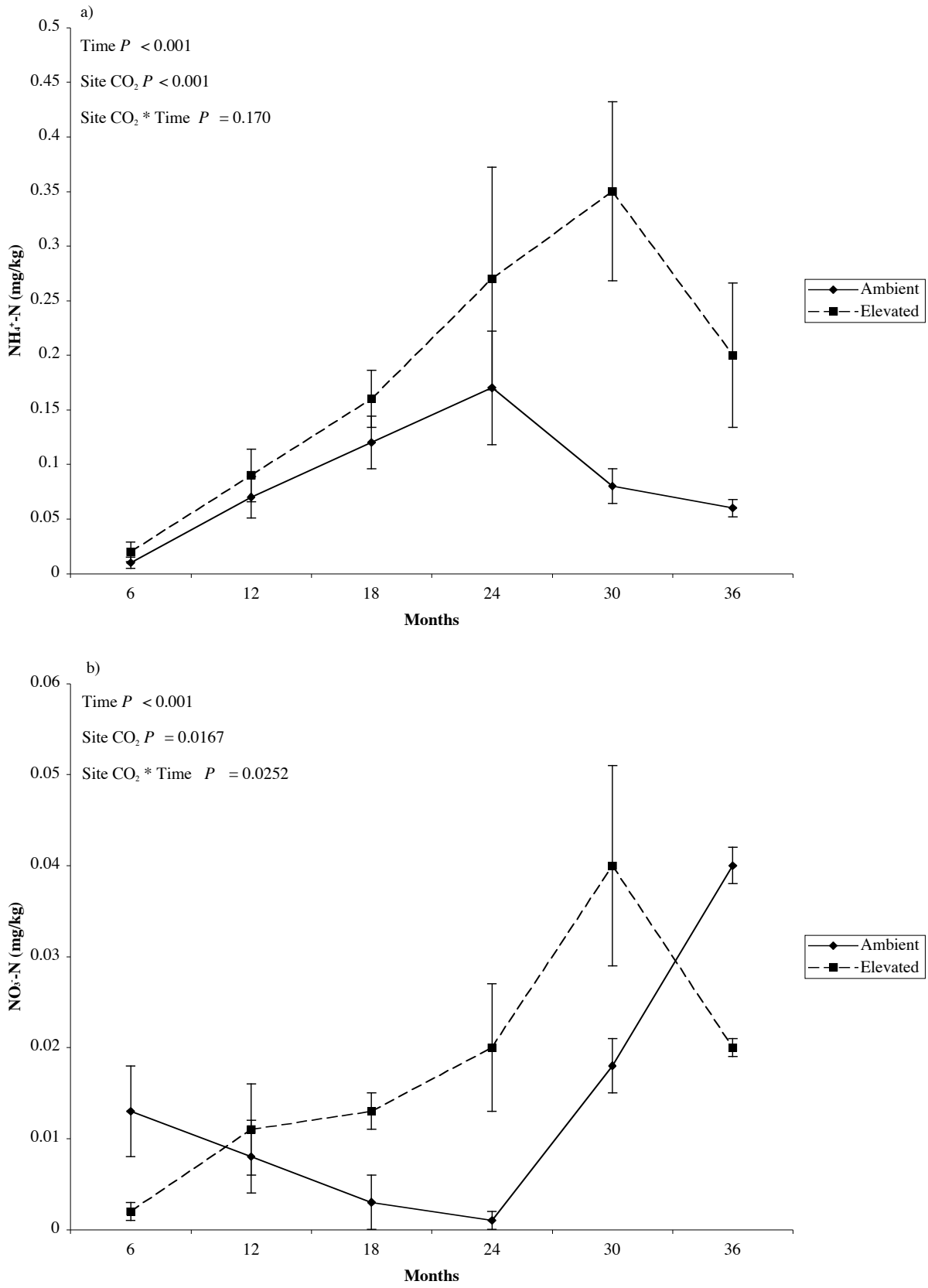
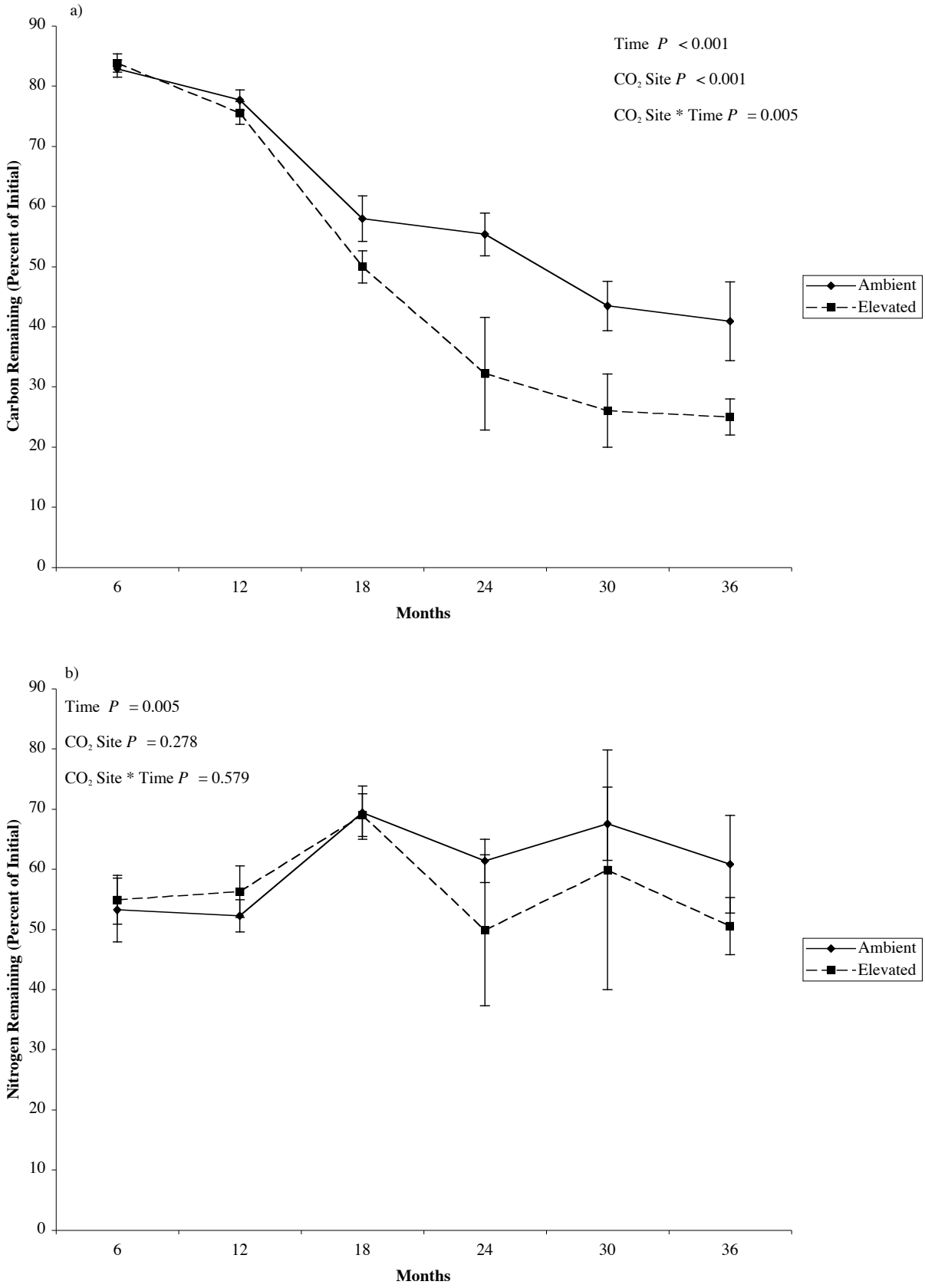

a)
Time P < 0.001
Site CO2 P < 0.001
Site CO2 * Time P = 0.170
NH4+-N (mg/kg)
0.5
0.45
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
6
12
18
24
30
36
Months
Ambient
Elevated
b)
Time P < 0.001
Site CO2 P = 0.0167
Site CO2 * Time P = 0.0252
NO3-N (mg/kg)
0.06
0.05
0.04
0.03
0.02
0.01
0
6
12
18
24
30
36
Months
Ambient
Elevated

Figure 4.5. Dynamics of C (a) and N (b) concentration during decomposition of *Q. myrtifolia* leaf litter decomposing under ambient and elevated CO_2 (site) for all collection dates of MD *Q. myrtifolia* litter. Bars represent standard errors. Saample sizes varies by time and treatment and are as follows in order of time: Ambient = 16, 16, 16, 13, 7, 7; Elevated = 16, 16, 14, 5, 2, 16.

Figure 4.5


a)
Time P < 0.001
CO2 Site P < 0.001
CO2 Site * Time P = 0.005
Carbon Remaining (Percent of Initial)
Months
Ambient
Elevated
b)
Time P = 0.005
CO2 Site P = 0.278
CO2 Site * Time P = 0.579
Nitrogen Remaining (Percent of Initial)
Months
Ambient
Elevated

CHAPTER 5

CONCLUSIONS

The goals of this research were to 1) explore the effects of CO_2 on foliar quality, 2) define links between foliar quality and litter quality, 3) determine the influence of CO_2- mediated changes in litter quality on decomposition and soil nutrient dynamics, and 4) determine the influence of herbivory on litter quality along with subsequent decomposition.

Elevated CO_2 affects foliar quality in a number of plant species and the effects vary in magnitude and direction (Williams et al., 1986; Lindroth et al.., 1993; Curtis et al., 1996; Johnson et al., 1996; Ineson and Cotrufo, 1997; Cook et al., 1997; Van Gardingen et al., 1997). There is evidence of interspecific variation compounded by effects of available resources and environmental aspects (Mousseau et al., 1996; Norby et al., 1996; Woodward and Beerling, 1997; Lindroth et al., 1993; Bazzaz, 1990). However, as seen in Chapter 2 of our study, the chemistry of all four plant species responded similarly to elevated CO_2 including the chemistry of the nitrogen fixer. Nitrogen concentrations declined and C:N ratios increased in the foliage of plants grown in elevated CO_2. The result for the oak species was not unexpected, however, it was surprising to find sharp declines in nitrogen in the nitrogen-fixing legume. Few studies to this point have examined the effects of elevated CO_2 on nitrogen fixers. Thus far the results have been mixed, however differences in methodology make comparisons difficult. Some studies measure whole plant nitrogen, others foliar nitrogen, and others belowground nitrogen. Arnone (1999) found no CO_2 effect on symbiotic N_2 fixation or nitrogen per unit land area in *Trifolium alpinium* and concluded that N_2 fixation and above ground nitrogen content were not affected by

increases in CO_2. Lee et. al., (2001) found declines in leaf nitrogen concentrations in four different functional groups, C_3 grasses, C_4 grasses, legumes and nonleguminous forbs. Reich, et al., (2001) found that above ground nitrogen concentrations declined in four species of legumes (*Amorpha canescens, Lespedeza capitata, Lupinus perennis, Petolostemum villosum*) and belowground nitrogen concentrations declined in all but one of the species. Measures of total nitrogen declined for all four of the legumes. The limited and conflicting information about the effects of CO_2 on nitrogen fixers, whether on plant traits or symbiotically mediated fixation, remains unclear and requires further examination.

A number of studies have found evidence of decreased foliar quality in plants grown in elevated CO_2 (Melillo et al., 1982; Williams et al., 1986; Bazzaz 1990). Logically the assumption follows that poor quality foliage equals poor quality litter and thus predictions are made that plant material derived from elevated CO_2 environments will decompose slowly and nitrogen turnover within an ecosystem will decline, possibly resulting in a feedback cycle of declining plant quality and nutrient availability (Bazzaz 1990; Saxe et al., 1998). However, very few studies have examined both foliar and litter quality. Curtis et al. (1989) found that nitrogen concentrations were lower and C:N ratios higher in green leaves of *Scirpus olneyi* under elevated CO_2 but differences did not persist in senesced leaves. Finzi et al. (2001) found no effect of elevated CO_2 on total nonstructural carbohydrates or nitrogen in green leaves or in leaf litter of five tree species.

As this study has shown, predictions about litter quality based on foliar quality may not be successful. While foliar quality declined in plants under elevated CO_2, litter quality behaved in a different manner.

This study encompassed three different measures of litter quality. The first, discussed in Chapter 3, measured the mean chemical composition of undamaged, chewed, and mined litter from three oak species, collected on a quarterly basis throughout an entire year and was repeated for three years. In this measure of litter quality we found that a) litter quality responses to elevated CO_2 varied among plant species and differed from living plant foliage in which all plants responded similarly and b) the only consistent effect of CO_2 on oak litter chemistry was increased condensed tannins.

The second measure of litter quality (Experiment I, Chapter 4) focused on undamaged, chewed, and mined litter of a single dominant oak species, *Q. myrtifolia* collected in the spring of a single year when litter fall was most abundant. We found that litter from elevated CO_2 was lower in cellulose and hemicellulose concentrations. These findings differ from earlier findings across oak species in which condensed tannins were the only significant chemical changes in plant litter. These differences could be attributed to a number of things. The first study utilized litter from three different oak species while Experiment I used a single oak species. The first study also combined litter that senesced throughout an entire year whereas Experiment I litter was collected in the spring. Feeny (1970) has shown that tannins in oak leaves increase during the growing season. Thus changes in condensed tannins seen in litter collected from multiple species throughout the year may be due to seasonal differences in leaf chemistry.

The third examination of leaf litter (Experiment II, Chapter 4), similar to the second (Experiment I, Chapter 4), examined the leaf litter from a single oak species, *Q. myrtifolia*. However, this litter included miscellaneous damage. Litter had higher concentrations of carbon, hemicellulose, and lignin, again differing from the other two measures of litter quality. The differences noted between the first and second measures of litter quality could equally be

affecting the results seen in this third measure of litter quality. In addition the damage in this litter may have had some effect. In addition to herbivore damage this litter could also include abiotic mediated damage from such causes as wind, rain, and hail. Damage to plant tissue can lead to changes in leaf chemistry, morphology, and physiology (Findlay et al., 1996; Mooney et al., 1991; Tallamy and Raupp, 1991). Regardless of the differing results of litter quality the fact remains that no measure of litter quality was equivalent to the foliar quality found in any of the four species studied.

Decomposition was measured in two experiments. In Experiment I, undamaged, chewed, and mined litter was generated under ambient and elevated CO_2 and decomposed in ambient conditions in order to examine the effects of herbivory on litter quality and decomposition. Litter chemistry was weakly affected by CO_2. Litter generated in elevated CO_2 had lower concentrations of cellulose and hemicellulose. Though cellulose is sometimes cited as a regulator of decomposition processes (Berendse et al., 1987; Day, 1982) it is not considered a primary regulator such as lignin, nitrogen, and C:N ratios (Kuiters 1990; Lambers 1993). Given the minor changes in litter chemistry, our finding that decomposition rates were unaffected by CO_2 was expected.

Experiment II included all damage types. This litter was generated under ambient or elevated CO_2 and samples from both growth treatments decomposed under ambient and elevated CO_2. The quality of litter from ambient and elevated CO_2 varied, with elevated litter having higher concentrations of carbon, hemicellulose, and lignin. Given that carbon compounds can decrease decomposition rates and lignin is frequently and successfully used to predict rates of decomposition (Berendse et al., 1987; Berg et. al., 1984; Fox et al, 1990) we expected to see slower rates of decomposition of litter generated under elevated CO_2. However, over a period of

three years litter generated under elevated and ambient CO_2 decomposed at comparable rates under ambient CO_2. As surprising as that is, a more unexpected result was accelerated decomposition rates of litter decomposing under elevated CO_2 regardless of from where the litter originated. Along with increased decomposition rates, litter under elevated CO_2 also exhibited increased rates of mineral nitrogen accumulation, perhaps due to increased rates of mineralization. This means that elevated CO_2 is promoting increased microbial activity in *Q. myrtifolia* litter in the Florida scrub oak forest.

There have been reports of both increased and decreased rates of decomposition of plant litter from elevated CO_2 concentrations (Boerner and Rebbeck 1995; Cotrufo and Ineson 1995; Cotrufo et al 1994; Couteaux et al 1991; Gorrissen et al 1995), as well as reports of no changes in decomposition rates (Cotrufo and Ineson 1995; Cotrufo et al. 1994). In this study the elevated CO_2 decomposition environment had a greater impact on decomposition than changes in litter quality instigated by CO_2 growth environment or herbivory.

Beyond measuring direct effects of elevated CO_2 on foliar and litter quality we also were interested in the effects of herbivory and their interactions with elevated CO_2. Given that foliar quality at our site declines under elevated CO_2, we expected to find some effects on herbivores. Prior work determined that leaf-miner populations at this study site declined in elevated CO_2 (Stiling et. al., 1999, 2002, 2003). We measured six feeding guilds (chewers, miners, leaf galls, eye spot galls, leaf tiers, and leaf mites) and found that damage by four of these different feeding groups declined in elevated CO_2 under field conditions. Given that foliar growth in elevated CO_2 results in poor quality foliage on plants it is not surprising to see effects on herbivores. Increased mortality, slower growth rates and decreased egg production have all been documented in insect herbivores under elevated CO_2 (Akey and Kimball, 1989; Fajer 1989; Montjoy, 1992; Stiling et

al., 1999, 2002, 2003). This study reiterates that herbivores may be negatively affected by increasing concentrations of CO_2.

Chapter 3 focused on only two groups of herbivores (chewers and miners) and found that phenolic concentrations were lower while lignin concentrations and lignin:nitrogen ratios were higher in litter following herbivore damage. This may be a case of avoidance / induction but we were unable to make this determination. However, of note is that herbivory and CO_2 did not interact to affect litter quality. In contrast, Chapter 4 (Experiment I) discusses the effects of herbivory on litter quality of a single oak species and found that there were interacting effects. Herbivory altered C:N ratios and hemicellulose in litter independent of CO_2 treatment. However, condensed tannins did differ with herbivore damage and CO_2. While mined litter was highest in condensed tannin concentrations independent of CO_2 treatment, undamaged and chewed litter varied with CO_2 treatment. Undamaged litter from enriched CO_2 was lower in condensed tannins than litter from the control and chewed litter from elevated CO_2 was higher in condensed tannins than litter from the ambient. Herbivore damage had no effect on decomposition rates despite elevated levels of condensed tannins. A large number of studies have examined the effects of CO_2 on plant quality and subsequently on insect herbivores (Bezemer and Jones 1998). Likewise there are a number of studies on the effects of herbivores on plant quality and leaf litter (Findlay 1996; Chapman et al., 2003; Lambers 1993). However, none have been found that examined interactions of insect herbivory and CO_2 on the decomposition process. Given the results of accelerated decomposition, mineralization, and nitrification in elevated CO_2 independent of litter quality, it would be worthwhile to explore the effects of multiple types of herbivore damaged litter and CO_2 on decomposition in both ambient and elevated CO_2 environments to determine what, if any, effects herbivores have on litter decomposing in enriched CO_2 environments.

Ecosystems are dynamic and are constantly influenced by environmental variability. As the global climate is altered, local ecosystem effects will be seen in the concentrations of atmospheric CO_2, which may in turn be expected to have cascading effects on the chemical composition of plants, behavior and abundance of insect herbivores, and the decomposition process. In light of the rapid global changes, ecosystem distribution and composition may be altered. It is important to have a better understanding of ecological and physiological effects of increasing concentrations of CO_2 and plant communities taking account of complex interactions among atmospheric CO_2, foliar quality, insect herbivores, and decomposition. Thus far this study has shown that increased CO_2 alters foliar quality, litter quality, herbivore damage, and rates of decomposition, mineralization and nitrification. Though some effects of the direct interactions of CO_2 and insect herbivores have been elucidated there are more uncertainties than defined relationships. Still uncertain are affects of herbivore-mediated changes in litter quality under elevated CO_2 as an influential component in ecosystem processes. Also mechanisms driving accelerated decomposition under elevated CO_2 need to be explored.

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