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Effects of CO₂ and nutrient enrichment on tissue quality of two California annuals

Abstract The effects of CO₂ enrichment and soil nutrient status on tissue quality were investigated and related to the potential effect on growth and decomposition. Two California annuals, Avena fatua and Plantago erecta, were grown at ambient and ambient plus 35 Pa atmospheric CO₂ in nutrient unamended and amended serpentine soil. Elevated CO₂ led to significantly increased Avena shoot nitrogen concentrations in the nutrient amended treatment. It also led to decreased lignin concentrations in Avena roots in both nutrient treatments, and in Plantago shoots and roots with nutrient addition. Concentrations of total nonstructural carbohydrate (TNC) and carbon did not change with elevated CO₂ in either species. As a consequence of increased biomass accumulation, increased CO₂ led to larger total pools of TNC, lignin, total carbon, and total nitrogen in Avena with nutrient additions. Doubling CO₂ had no significant effect on Plantago. Given the limited changes in the compounds related to decomposibility and plant growth, effects of increased atmospheric CO₂ mediated through tissue composition on Avena and Plantago are likely to be minor and depend on site fertility. This study suggests that other factors such as litter moisture, whether or not litter is on the ground, and biomass allocation among roots and shoots, are likely to be more important in this California grassland ecosystem. CO₂ could influence those directly as well as indirectly.

Key words Elevated CO₂ · Resource partitioning · Carbon and nitrogen · Carbohydrates · Lignin

Introduction

Changes in resource availability or in the efficiency of resource gain by plants can potentially lead to changes in tissue composition (Chapin et al. 1990). Elevated CO₂ tends to increase carbohydrate levels, lower nitrogen concentration in leaves, widen the carbon:nitrogen ratio (Rogers and Runion 1994; Chu et al. 1992; Mooney et al. 1991; Bazzaz 1990) and alter carbon-based secondary metabolites (Owensby et al. 1996; Ayres 1993; Norby et al. 1986). Declines in leaf nitrogen concentration can result from a dilution by increased carbohydrate and/or decreased investment in Rubisco and other enzymes (Stitt 1991).

Tissue composition influences a range of plant and ecosystem processes, including growth potential (Gulmon and Mooney 1986), biomass allocation (Luo et al. 1994), nutrient dynamics (Swift et al. 1979) and herbivore performance (Lincoln et al. 1993; Bryant et al. 1991). Storage represents a short-term liability in terms of growth potential, but this liability may be overcome or converted to an asset in terms of survival or defense if the stored compounds are later mobilized and invested where they are in short supply (Chapin et al. 1990). Some evidence supports the idea that carbohydrate storage can be increased so much that photosynthesis and other physiological functions are impaired (Stitt 1991), though accumulation to this extreme appears to be the exception rather than the rule (Stitt 1993). Accumulated carbohydrates tend to decrease photosynthesis per unit of leaf mass, but photosynthesis per unit of leaf area tends to track nitrogen per unit area, which can either decrease or increase in plants grown at increased CO₂ (Luo et al. 1994).

Tissue composition clearly interacts with root:shoot allocation (Chu et al. 1992; Ingestad and Agren 1991), though the driving mechanisms are not well known. Nitrogen availability and shoot nitrogen are often effective predictors of shoot fraction, consistent with
the requirements for maximizing growth (Levin et al. 1989). Interactions between carbohydrate accumulation and root: shoot ratio are less clear, though the data generally support the hypothesis that allocation adjusts in plants grown under elevated CO₂ to reflect the relative stimulation of growth and photosynthesis (Luo et al. 1994). Changes in leaf composition that result in altered levels of nitrogen, starch, fiber and allelochemicals can affect consumption by herbivores, with consequent effects on growth and fitness (Lincoln et al. 1993; Marquis 1984).

The rate of litter decomposition has been correlated with initial litter quality, especially concentrations of nitrogen, carbon, and lignin (Taylor et al. 1989; Melillo et al. 1982; Swift et al. 1979), and with environmental factors such as temperature and moisture (Jansson and Berg 1985; Meentemeyer 1978). Elevated CO₂ can potentially affect decomposition through several mechanisms. These include effects on plant tissue composition especially starch, nitrogen and lignin (Mooney et al. 1991; Field et al. 1992), plant species composition (Bazzaz 1990), soil moisture (Prior et al. 1991; Schonfeld et al. 1989), and herbivory (Lincoln et al. 1993). In natural ecosystems, the primary source of mineral nitrogen for plant uptake is nitrogen released from organic material during decomposition. Decreased decomposition can slow nutrient cycling leading to indirect effects on plant growth, resource allocation, species competition, and ecosystem productivity.

*Awena fatua* and *Plantago erecta* are the dominants in California grasslands on, respectively, sandstone- and serpentine-derived soil. These two species differ in many features, as do the ecosystems where they are abundant. Sandstone grasslands on Jasper Ridge are moderately productive (aboveground annual production 200–400 g m⁻²), with deep soil and substantial water holding capacity. In contrast, serpentine grasslands are very unproductive (aboveground annual production 100–200 g m⁻²), with shallow, rocky soils, high concentrations toxic heavy metals, and low water holding capacity (Field et al. 1996). In the experiments described here, we manipulated CO₂ and nutrient levels and determined how these changes affected the tissue chemical composition, and allocation of carbon and nitrogen to assess the potential impacts of elevated CO₂ on plant and ecosystem processes, including growth, allocation, herbivore, decomposition and nutrient dynamics. This study is one component of a larger effort to understand interactive effects of atmospheric CO₂, soil nutrient availability, and plant species characteristics on ecosystem flows of carbon and nitrogen.

**Materials and methods**

**General**

Seeds of *Avena fatua* L. and *Plantago erecta* E. Morris were collected from the annual grassland at the Jasper Ridge Biological Preserve of Stanford University, Stanford, California (37° 24' N, 122° 13' 30" W). Throughout the remainder of the paper, the two species will be referred to by genus only. In late November 1992 (approximate peak germination in the field) the seeds were planted in microcosms at natural densities for *Avena* and *Plantago* (each of microcosm contained approximately 50 or 300 plants for *Avena* and *Plantago* respectively; Chiariello and Field 1996). The microcosms are 0.2 m diameter, 0.95 m lengths of PVC pipe filled with 0.15 m of shredded serpentine topsoil over 0.8 m of crushed rock from a serpentine quarry, housed in open-top chambers at Jasper Ridge. Further details can be found in Field et al. (1996).

Jasper Ridge has a typical mediterranean-type climate with cool and wet winters, and very dry summers. Average annual precipitation over the last 10 years was 549 mm. Daily mean temperatures range from 9 °C in January to 22 °C in July.

Seven replicates of a factorial design included ambient and ambient plus 55 Pa CO₂ and soil nutrient unamended and amended with 20 g m⁻² of N, P, and K. CO₂ exposure began 2 weeks after seeding, when both of the species had germinated and were in the cotyledon stage. CO₂ exposure was continuous day and night, until plants were harvested, except for brief shut-downs for maintenance or other studies. The nutrient additions were applied as 120-day slow released Osmocote (Grace-Sierra Horticulture Product Company, Calif.) in early December.

Samples were collected at two growth phases. At the first harvest (middle of March), where we harvested shoots only, plants were growing rapidly and had not begun extensive allocation to reproduction. At the second harvest (on 22 April and 6 May 1993 for *Plantago* and *Avena*, respectively) both species were in the early stages of seed filling and the sum of live plus senesced biomass was at or near its yearly maximum. Plants were separated into above-ground and belowground parts, and soil was washed from the roots. Samples were stored in plastic bags on ice during transport to the laboratory. Plant material was oven dried at 70 °C to constant weight and weighed. Aboveground (a mixture of leaves, stems and reproductive organs) and belowground tissues from each microcosm were ground to a fine powder for chemical analysis.

**Lignin determination**

Lignin was determined only on plants collected at the second harvest. We used a semi-micro lignin assay developed for herbaceous plants by Morrison (1972a, b) and further modified by adding perchloric acid to the digestion medium to speed complete dissolution of the samples (Iiyama and Wallis 1990). This method requires prior removal of interfering phenolic compounds. To do this, approximately 15 mg finely ground plant tissue was extracted by sequentially washing with hot water, ethanol, acetone and diethyl ether, and followed by digestion of the residue in 25% acetyl bromide in acetic acid. Lignin content of each sample was analyzed in triplicate, by measuring the absorbance at 280 nm and calculated according to Morrison's equation:

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\% \text{Lignin} = 3.37 \times \text{absorbance/sample concentration (g} \text{l}^{-1}) - 1.05
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Approximately 20 mg ground plant tissue was first extracted three times with 80% (v/v) ethanol for soluble sugars followed by an enzymatic digestion at 55 °C using Amyloglucosidase from *Rhizopus* mold (Hewitt and Marrush 1966) for the starch fraction. Reducing