

# Plant and Soil Mediation of Elevated CO<sub>2</sub> Impacts on Trace Metals

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## ABSTRACT

The cycling of trace metals through terrestrial ecosystems is modulated by plant and soil processes. Changes in plant growth and function and soil properties associated with increased atmospheric carbon dioxide (CO<sub>2</sub>) may therefore also affect the biological storage and stoichiometry of trace metals. We examined CO<sub>2</sub> effects on a suite of metal micronutrients and contaminants in forest trees and soils at two free-air CO<sub>2</sub> enrichment sites—a loblolly pine forest in North Carolina (Duke) and a sweetgum plantation in Tennessee [Oak Ridge National Laboratory (ORNL)]—and an open-top chamber experiment in a scrub-oak community in Florida [Smithsonian Environmental Research Center (SERC)]. We found that CO<sub>2</sub> effects on soil metals were variable across sites; there were significantly higher surface soil metal concentrations with CO<sub>2</sub> enrichment at Duke and ORNL ( $P < 0.05$ ), but a trend of decreased soil metal concentrations at SERC (non-significant). These impacts on metals may be understood in the context of CO<sub>2</sub> effects on soil organic matter (SOM);

changes in percent SOM with CO<sub>2</sub> enrichment were greatest at Duke (18% increase), followed by ORNL (7% increase), with limited effect at SERC (3% increase). There were significant effects of elevated CO<sub>2</sub> on foliar metal concentrations at all sites, but the response of foliar metals to CO<sub>2</sub> enrichment varied by metal, among sites, and within sites based on plant species, canopy height, and leaf age. Contrary to expectations, we did not find an overall decline in foliar metal concentrations with CO<sub>2</sub> enrichment, and some essential plant metals were greater under elevated CO<sub>2</sub> (for example, 28% increase in Mn across species and sites). Our results suggest that elevated CO<sub>2</sub> impacts on trace metal biogeochemistry can be understood by accounting for both metal function (or lack thereof) in plants and the soil characteristics of the ecosystem.

**Key words:** biogeochemical cycles; elevated CO<sub>2</sub>; free-air CO<sub>2</sub> enrichment; global change; micronutrients; soil organic matter; trace metals.

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## INTRODUCTION

Human industrial activities over the past 200 years have led to a 35% increase in atmospheric carbon dioxide (CO<sub>2</sub>) concentrations, and concentrations are expected to continue to rise through the end of the century (IPCC 2007). In addition to its role as a greenhouse gas, CO<sub>2</sub> has an essential biological role as the building block of plant biomass. Under current atmospheric CO<sub>2</sub> concentrations, photosynthesis is CO<sub>2</sub> limited (in C<sub>3</sub> plants); therefore, as

atmospheric CO<sub>2</sub> concentrations increase, plant photosynthetic rates, carbon assimilation, and dry matter production may also increase (Ainsworth and Long 2005; Curtis and Wang 1998; Ellsworth and others 2004).

Elevated atmospheric CO<sub>2</sub> not only affects the total amount of plant biomass produced, but also the chemical make-up of plant tissue (for example, Cotrufo and others 1998; Roth and Lindroth 1995; Taub and others 2008). Loladze (2002) suggests that there will be a decrease in all soil-derived nutrients in plants, because the increase in atmospheric CO<sub>2</sub> is not matched by an increase in soil-derived elements. Although plant stoichiometry is less constrained than that of organisms at higher trophic levels (Sterner and Elser 2002), plant nutrient uptake is not a completely passive process. For example, increased production of fine roots with CO<sub>2</sub> enrichment (Norby and others 2004; Pritchard and others 2008) may allow plants to match increased C assimilation with increased uptake of soil-derived elements. The extent that a specific element concentration decreases with CO<sub>2</sub> enrichment may be a function of both plant nutrient requirement and uptake capacity.

In this study we focus on CO<sub>2</sub> effects on trace metal concentrations, because trace metals are important both as micronutrients and as environmental contaminants. Six of the eight known plant micronutrients are metals [copper (Cu), iron (Fe), manganese (Mn), molybdenum (Mo), nickel (Ni), and zinc (Zn)], and they are involved in a wide scope of physiological processes. As is the case with foliar nitrogen (N), plant response to CO<sub>2</sub> enrichment may change the requirement for, and stoichiometry of, trace metals that are functionally important for plant metabolism. For example, as photosynthetic activity shifts from being CO<sub>2</sub>-limited under ambient CO<sub>2</sub> to electron transport limited under elevated CO<sub>2</sub> (Farquhar and von Caemmerer 1982), plant nutrient requirements may also change; plant requirements for trace metals involved in electron transport, such as Fe and Mn, may increase under elevated CO<sub>2</sub>, whereas N demand may decline.

We also examine non-essential trace metals [aluminum (Al), cobalt (Co), lead (Pb), and vanadium (V)]—that is, metals taken up by plants at low levels, but not required for plant metabolism. Many of these elements are important contaminants whose movement through the biosphere is modulated by plant activities. By analyzing leaves for both essential and non-essential metals, we seek to determine if and when changes in metal concentrations with CO<sub>2</sub>

enrichment are due purely to non-regulatory processes (for example, growth dilution) and when there is biological regulation of trace metal concentrations. The first hypothesis we tested in this experiment focused on plant metal stoichiometry; we expected that elevated CO<sub>2</sub> would decrease foliar metal concentrations, *but* plant essential metals would decrease less than non-essential metals because of biological regulation.

Plant foliar metal concentrations may also be affected by CO<sub>2</sub>-mediated changes in soil properties. Metal solubility in soils (and availability to organisms) is strongly influenced by soil pH and soil organic matter (SOM). The sorption capacity of most metals increases with increasing pH (Marschner 1995), and organic matter provides a large surface area and negative charge—both of which support the binding of metals (McBride and others 2004). The second hypothesis we tested focused on CO<sub>2</sub>-mediated changes in soil metal dynamics; we expected that observed and predicted changes in pH and SOM with CO<sub>2</sub> enrichment (Andrews and Schlesinger 2001; Jastrow and others 2005; Oh and Richter 2004) would affect soil metal availability and plant uptake and assimilation.

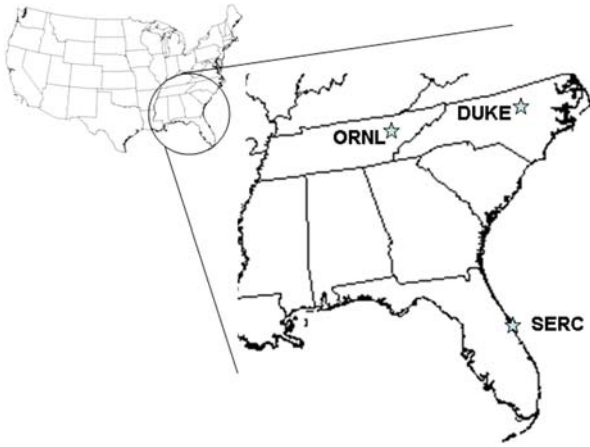
We measured concentrations of metals in plants and soils at two free-air CO<sub>2</sub> enrichment (FACE) sites—a loblolly pine forest in North Carolina (Duke) and a sweetgum plantation in Tennessee [Oak Ridge National Laboratory (ORNL)]—and an open-top chamber (OTC) CO<sub>2</sub>-enrichment experiment in a scrub-oak community in Florida [Smithsonian Environmental Research Center (SERC)]. By looking across sites that vary in climate, species composition, and soil chemistry we hope to identify CO<sub>2</sub> effects that are common across systems and those that are unique to a particular species or site.

## MATERIALS AND METHODS

### Site Descriptions

#### *Duke FACE*

The FACE experiment at Duke Forest is located on a mixed evergreen-deciduous temperate forest dominated by loblolly pine (*Pinus taeda*) and is located in the Blackwood Division of Duke Forest in Orange County, North Carolina (35°58'N, 79°05'W; Figure 1). The stand of loblolly pine, which was planted in 1983 at a spacing of 2.0 m × 2.4 m, is located on low-fertility, acidic Hapludalf soils. The sub-canopy and understory are diverse, containing more than 50 species, but dominated by sweetgum (*Liquidambar styraciflua*). The FACE experiment



**Figure 1.** Locations of ecosystem CO<sub>2</sub> experiments included in this study. The FACE at Duke Forest is located on a mixed evergreen-deciduous temperate forest, and the FACE experiment at ORNL is located on a sweetgum plantation. The CO<sub>2</sub> experiment at SERC, a scrub-oak ecosystem, is comprised of OTCs.

began in August 1996 and consists of three 30-m-diameter ambient CO<sub>2</sub> rings ( $\sim 382 \mu\text{mol mol}^{-1}$ ) and three 30-m-diameter elevated CO<sub>2</sub> rings ( $\sim 582 \mu\text{mol mol}^{-1}$ ) that are arranged in a complete block design to account for topographic variation and potential fertility gradients. The CO<sub>2</sub> treatment is applied through a series of vertical pipes located around the perimeter of each ring. The pipes, which extend from the forest floor to the canopy, are equipped with regulated blowers that deliver a controlled amount of CO<sub>2</sub>-fumigated air to maintain ambient or elevated levels of CO<sub>2</sub> into the rings (Hendrey and others 1999). The site, experimental design, and FACE technology have been well described (Finzi and others 2001; Hendrey and others 1999).

#### ORNL FACE

The deciduous forest site (ORNL) is a sweetgum (*L. styraciflua*) plantation located in the Oak Ridge National Environmental Research Park in Roane County, Tennessee ( $35^{\circ}54'N$ ,  $84^{\circ}20'W$ ; Figure 1). Soils at the site, classified as Aquic Hapludult, have a silty clay loam texture, are moderately well drained and slightly acidic. The stand was planted with 1-year-old sweetgum seedlings in 1988 at a spacing of 1.2 m  $\times$  2.3 m. There are three ambient CO<sub>2</sub> ( $\sim 393 \mu\text{mol mol}^{-1}$ ) rings and two enriched CO<sub>2</sub> ( $\sim 549 \mu\text{mol mol}^{-1}$ ) rings, which are each 25 m in diameter. The FACE apparatus (Hendrey and others 1999) is assembled in both elevated CO<sub>2</sub>

rings and in two of the three ambient CO<sub>2</sub> rings. CO<sub>2</sub> enrichment began in 1998 and continues during the growing season through the present time. The site and experimental design are described by Norby and others (2001).

#### SERC OTC

The SERC site is an OTC CO<sub>2</sub>-enrichment experiment located in a scrub-oak ecosystem on Merritt Island Wildlife Refuge, Cape Canaveral, Florida ( $28^{\circ}38'N$ ,  $80^{\circ}42'W$ ; Figure 1). Soils are sandy, well-drained Pomello and Poala soils with low nutrient content and low pH (Schmalzer and Hinkle 1992). The experiment is comprised of 16 octagonal OTCs (3.6-m-diameter, 3.7-m-height), half of which are exposed to current ambient CO<sub>2</sub> and half to elevated CO<sub>2</sub> (ambient + 350 ppmv). CO<sub>2</sub> treatment has been in progress since May 1996. The plots are arranged in a randomized block design; blocks were established based on vegetation composition. Aboveground biomass is dominated by *Quercus myrtifolia* (76%), *Q. geminata*, and *Q. chapmanii*. Other species present include *Galactia elliotii*, *Serenoa repens*, and *Vaccinium myrsinites* (Dijkstra and others 2002). Complete description of the OTC system can be found in the study of Stiling and others (1999), and further site description can be found in the study of Hymus and others (2003).

## Field Sampling

### Soils

Soil samples were collected at ORNL from 25 to 28 July 2005, at Duke from 8 to 11 August 2005, and at SERC from 29 to 31 August 2006. A core sampler was used to collect two 2.5-cm-diameter  $\times$  20-cm-deep soil cores per ring at each FACE site; three cores were collected per chamber at SERC. Acid-washed butyrate plastic core liners were used in the soil corer to maintain an intact core during extraction. Cores were divided into 5-cm-depth increments and pooled within ring/chamber. In all of our analyses, 'ring' or 'chamber' is the unit of replication for CO<sub>2</sub> treatment, so pooling of cores within a ring has no consequence in testing for CO<sub>2</sub> or depth effects.

### Leaves

Canopy leaves were collected at ORNL from 25 to 28 July 2005, at Duke from 8 to 11 August 2005, and at SERC from 25 to 29 August 2006. Green leaves were sampled from three canopy heights—low (10–12 m), mid (12–14 m), and upper

(14–16 m)—from *L. styraciflua* at ORNL and Duke (lower and mid canopies only, pooled for analysis), and from *P. taeda* at Duke (all canopy heights). The canopy at ORNL was accessed using a stationary hydraulic lift located near the center of each ring. At Duke, the canopy was accessed by a central walk-up tower and by a mobile hydraulic lift. Both 0-year (needles that originated in 2005) and 1-year (needles that originated in 2004) needles were samples from *P. taeda*. In each canopy height, three replicate samples were collected. At SERC, three replicate samples were collected from the ground at approximately 1.5-m-height. For all leaves collected, a sample consisted of approximately 5 leaves/20 needles from an individual tree.

### Sample Analysis

After removal of roots, soils were passed through a 2-mm screen and air-dried. Leaves were dried at 60°C in a Fisher Isotemp oven and homogenized using a ball mill (using acid-washed polypropylene tubes and glass grinding balls). Soils for metal analysis were digested using repeated additions of concentrated nitric acid (HNO<sub>3</sub>) and hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) with heating (U.S. EPA 1996); leaves were digested using repeated additions of HNO<sub>3</sub>, followed by H<sub>2</sub>O<sub>2</sub> and HCl (U.S. EPA 1991).

Samples were analyzed for metal concentrations (Al, Co, Pb, V, Cu, Fe, Mn, Mo, Ni, Zn) using a Thermo-Finnegan Element2 Inductively Coupled Plasma Mass Spectrometer (ICP-MS). Percent SOM was determined by the method of percent loss on ignition (8 h combustion at 400°C). Soil pH was determined in a 1:1 ratio of soil (g) to water (ml).

### Quality Assurance/Quality Control

All sample handling and analyses were conducted using trace metal clean techniques in a class 100-laminar flow laboratory. All reagents were quartz distilled (Optima grade). One method blank was analyzed for every ten samples and two instrument blanks were run after every six samples. Instrument blank concentrations were less than 5% of any sample concentration. Indium (In) was used as an internal standard to account for any variations in the performance of the ICP-MS. The accuracy and precision of the analytical procedures were established by analyzing several standard reference materials: river water (NIST 1643d), apple leaf (NIST 1515), and San Joaquin soils (NIST 2709) (Appendix Table 1 given in supplementary materials). Relative standard deviations of all reference materials ( $n = 5$  repeated measurements) were less than 3% for all elements.

## Statistical Analyses

### Soils

We used principal components analysis (PCA; JMP, SAS Institute, Cary, North Carolina, USA) as a first step in discerning CO<sub>2</sub> effects on soil properties; PCA was used to reduce the number of soil variables (soil metals, pH, and SOM) for further statistical analysis. Variables were log-transformed to reduce the influence of outliers and to improve linear association between variables. For all PCAs, we used a correlation matrix and applied a varimax (orthogonal) rotation, retaining all components with eigenvalues above 1 (Quinn and Keough 2002).

The blocked design of the CO<sub>2</sub> experiments led us to then use the retained principal component scores for each site in an analysis of variance (ANOVA, SAS 9.0, SAS Institute, Cary, North Carolina, USA). At each site, soils were analyzed in a nested design with CO<sub>2</sub> as the main plot factor, depth as a within plot factor, and ring or chamber (random, nested within blocks at Duke and SERC) as the experimental unit for CO<sub>2</sub>. We used Hochberg's method (Hochberg 1988) for *P*-value adjustment to control family-wise error rate.

We also ran separate ANOVAs on soil pH and SOM to look at CO<sub>2</sub>-mediated changes in these factors across sites. We included site in our model as a main plot factor, with ring/chamber as the experimental unit for testing for CO<sub>2</sub> and site effects. We could not include block effects in this model because CO<sub>2</sub> chambers/rings were not blocked at all sites.

### Leaves

Foliar data were analyzed using multivariate analysis of variance (MANOVA, SAS 9.0). To determine which metals were driving significant MANOVA treatment effects, we conducted protected ANOVAs on individual metals only when MANOVA effects were significant (Scheiner 2001).

Foliar data at each site were analyzed as a partly-nested design with CO<sub>2</sub> as the main plot factor, canopy height (ORNL *L. styraciflua* and Duke *P. taeda*), needle age (Duke *P. taeda*), and species (SERC) as within plot factors, and ring/chamber (random, nested within blocks at Duke and SERC) as the experimental unit for CO<sub>2</sub>. We analyzed Duke *P. taeda* and *L. styraciflua* in separate analyses so that we could include age and canopy height into our *P. taeda* model. For the ANOVAs with unequal treatment sample sizes, degrees of freedom were estimated using Satterthwaite's approximation (Satterthwaite 1946). When ANOVA CO<sub>2</sub>

interactions were significant, we conducted planned comparisons to look at CO<sub>2</sub> effects within a species, canopy height, or needle age.

Because of the constraints on sample size of the FACE experiments and resulting low statistical power (Filion and others 2000), effects were considered marginally significant for  $P < 0.10$  and significant for  $P < 0.05$  as in other FACE and OTC studies (for example, Carney and others 2007; Ellsworth and others 2004; Jastrow and others 2005). Errors presented in the text and tables are one standard error of the mean.

## RESULTS

### Soil

#### Duke

Duke soil variables were highly correlated—the first four principal components explained 93% of the variation in the data. Only the first two components had eigenvalues above 1, so we retained these two components for factor rotation and subsequent statistical analysis. All metals had strong loadings ( $\geq 0.7$ ) onto component 1, and pH and SOM had strong loadings (pH:  $-0.75$ , SOM:  $0.87$ ) onto component 2 (Table 1). The effect of elevated CO<sub>2</sub> on soil metal concentrations at Duke was strongest in surface (0–5 cm) soils (Figure 2,

**Table 1.** Eigenvalues and Loadings (Correlations) of Duke Soil Variables onto Rotated Components

Component	Eigenvalue	Variance (%)
1	7.78	64.87
2	1.95	16.21
3	0.93	7.71
4	0.49	4.06
Variable	Rotated component 1	Rotated component 2
SOM	0.039	<b>0.872</b>
pH	0.128	<b>-0.751</b>
Al	<b>0.937</b>	-0.194
Co	<b>0.811</b>	-0.311
Pb	<b>0.870</b>	0.258
V	<b>0.870</b>	-0.415
Cu	<b>0.720</b>	-0.379
Fe	<b>0.882</b>	-0.414
Mn	<b>0.887</b>	-0.037
Mo	<b>0.907</b>	-0.282
Ni	<b>0.913</b>	0.123
Zn	<b>0.850</b>	0.301

Variables that load strongly ( $\geq 0.7$ ) are in bold.

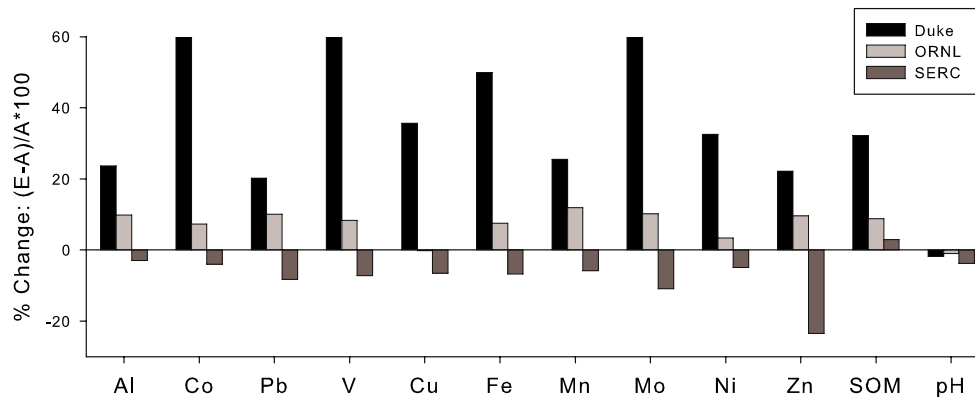
Table 2). There was a significant CO<sub>2</sub> × depth interaction effect for component 1 (Appendix Table 2 given in supplementary materials), with a significant CO<sub>2</sub> effect ( $P < 0.05$ ) in the surface soil layer (0–5 cm)—all soil metal concentrations at Duke FACE were greater in CO<sub>2</sub>-enriched surface soils than ambient soils (Figure 2). There was also a significant depth effect on component 2 (pH and SOM; Appendix Table 2 given in supplementary materials), with significant differences between surface soils (0–5 cm) and the 5–15 cm depth increments ( $p < 0.05$ ). Soil pH was lower in surface soils (0–5 cm,  $5.04 \pm 0.11$ ; 5–20 cm,  $5.39 \pm 0.04$ ) and SOM was higher (0–5 cm,  $6.93 \pm 0.47$ ; 5–20 cm,  $3.22 \pm 0.14$ ).

#### Oak Ridge National Laboratory

ORNL soil variables were also highly correlated, and the first four components explained 90% of the variation in the data (Table 3). There was a general trend of increased soil metals with CO<sub>2</sub> enrichment at ORNL, but the increase—which was mainly in surface soils (0–5 cm)—was less than that observed at Duke FACE (Table 2, Figure 2). The first three components had eigenvalues above 1 and were retained for statistical analysis. There was a significant depth effect on component 2 (which had strong loadings from SOM, pH, Mn; Table 3) with significant differences in the surface soils (0–5 cm) compared to the 5–20 cm depth increments ( $P < 0.05$ ). Soil pH (0–5 cm,  $5.09 \pm 0.08$ ; 5–20 cm,  $4.70 \pm 0.04$ ), SOM (0–5 cm,  $5.32 \pm 0.52$ ; 5–20 cm,  $3.52 \pm 1.0$ ), and Mn concentrations were all higher in surface soils (0–5 cm,  $1198 \pm 108 \mu\text{g g}^{-1}$ ; 5–20 cm,  $1111 \pm 61 \mu\text{g g}^{-1}$ ). There was a significant CO<sub>2</sub> effect on component 3, which had strong loadings from Co and Pb, and moderate loadings (0.4–0.7) from Fe, Mo, and Zn (Table 3, Appendix Table 2 given in supplementary materials), all of which increased with CO<sub>2</sub> enrichment (Table 2, Figure 2).

#### Smithsonian Environmental Research Center

Soil variables were also highly correlated at SERC. The first four components explained 88% of the variation in the data (Table 4). Concentrations of soil metals at SERC were one to three orders of magnitude lower than at ORNL and Duke (Table 2). In contrast to ORNL and Duke, there was a general decline in metal concentrations with CO<sub>2</sub> enrichment at SERC (Table 2, Figure 2); however, there were no significant CO<sub>2</sub> effects on any of the soil components (Appendix Table 2 given in supplementary materials).



**Figure 2.** Percent change in metal concentrations, SOM, and pH in surface soils (0–5 cm) from CO<sub>2</sub> enriched plots (E) relative to ambient (A) at Duke and ORNL FACE and SERC OTC experiments. Bars with negative values represent decreased soil metal concentrations with CO<sub>2</sub> enrichment, and those with positive values represent increased concentrations. There were significant effects of elevated CO<sub>2</sub> on soil metals at Duke and ORNL (ANOVA on principal components,  $P < 0.1$ ), but not at SERC.

**Table 2.** Duke, ORNL, and SERC Mean Soil Metal Concentrations, SOM and pH ( $\pm$ Standard Errors)

	CO <sub>2</sub>	Al (mg g <sup>-1</sup> )	Co (μg g <sup>-1</sup> )	Pb (μg g <sup>-1</sup> )	V (μg g <sup>-1</sup> )	SOM (%)	pH
Duke	A	14.50 ± 1.97	27.53 ± 6.44	17.88 ± 1.71	203.91 ± 21.83	3.81 ± 0.18	5.41 ± 0.09
	E	15.48 ± 2.78	42.31 ± 12.74	18.38 ± 3.01	210.71 ± 47.37	4.48 ± 0.15	5.20 ± 0.09
ORNL	A	17.68 ± 1.08	11.38 ± 0.50	17.11 ± 0.95	25.40 ± 1.37	3.85 ± 0.28	4.85 ± 0.10
	E	17.47 ± 0.07	11.72 ± 0.11	19.13 ± 0.29	25.05 ± 0.00	4.12 ± 0.24	4.72 ± 0.06
SERC	A	0.11 ± 0.04	0.02 ± 0.001	0.97 ± 0.10	0.39 ± 0.03	1.62 ± 0.11	4.97 ± 0.06
	E	0.11 ± 0.02	0.01 ± 0.001	0.89 ± 0.05	0.37 ± 0.02	1.67 ± 0.20	4.78 ± 0.10

	CO <sub>2</sub>	Cu (μg g <sup>-1</sup> )	Fe (mg g <sup>-1</sup> )	Mn (μg g <sup>-1</sup> )	Mo (μg g <sup>-1</sup> )	Ni (μg g <sup>-1</sup> )	Zn (μg g <sup>-1</sup> )
Duke	A	16.15 ± 1.94	47.84 ± 6.32	1667.88 ± 468.17	145.85 ± 25.71	4.43 ± 1.04	26.28 ± 5.35
	E	16.77 ± 2.25	48.12 ± 11.13	1716.41 ± 323.91	148.34 ± 42.05	4.97 ± 0.85	23.69 ± 5.25
ORNL	A	10.26 ± 0.24	15.10 ± 0.64	1124.66 ± 146.79	244.93 ± 8.90	15.50 ± 1.05	58.53 ± 2.79
	E	9.55 ± 0.67	15.49 ± 0.37	1145.73 ± 146.69	263.13 ± 3.38	15.43 ± 1.36	62.23 ± 4.92
SERC	A	0.18 ± 0.03	0.11 ± 0.02	2.85 ± 0.18	25.50 ± 2.41	0.12 ± 0.01	2.52 ± 0.60
	E	0.17 ± 0.02	0.10 ± 0.01	2.68 ± 0.27	22.73 ± 1.21	0.11 ± 0.01	1.93 ± 0.37

Collected from 0 to 20-cm-depth in ambient (A) and elevated (E) CO<sub>2</sub> plots.

Results of statistical analyses on CO<sub>2</sub> effects on soil variables (reduced principal components) can be found in the text and summarized in Appendix Table 2 given in supplementary materials.

### Patterns Across Sites

CO<sub>2</sub> effects on SOM and pH were significant across all sites (SOM:  $F = 3.69$ ,  $P = 0.07$ ; pH:  $F = 4.12$ ,  $P = 0.06$ ; Appendix Table 3 given in supplementary materials), and there were no significant CO<sub>2</sub> × site interactions. Mean SOM increased with CO<sub>2</sub> enrichment at all sites, and mean pH decreased with CO<sub>2</sub> enrichment (Table 2, Figure 2).

We ran a PCA that included soil data from all sites to look at patterns among soil variables across sites. The first four components explained 99% of the variation in the data. Most of the variation

across sites was explained by the first component (82%), which had strong loadings ( $> 0.7$ ) from SOM and all soil metals (Table 5).

### Leaves

#### Duke

There was a significant effect of elevated CO<sub>2</sub> on trace metal concentrations in *P. taeda* leaves at Duke (MANOVA; Wilk's lambda = 0.47,  $P < 0.01$ ; Table 6, Figure 3). There was a significant difference in overall metal concentrations between

**Table 3.** Eigenvalues and Loadings (Correlations) of ORNL Soil Variables onto Rotated Components

Component	Eigenvalue	Variance (%)	
1	6.14	51.18	
2	2.82	23.51	
3	1.23	10.28	
4	0.63	5.30	
Variable	Rotated component 1	Rotated component 2	Rotated component 3
SOM	-0.406	<b>-0.789</b>	-0.193
pH	0.019	<b>-0.887</b>	0.144
Al	<b>0.905</b>	0.070	-0.283
Co	0.521	-0.248	<b>-0.722</b>
Pb	0.027	-0.333	<b>-0.898</b>
V	<b>0.891</b>	0.094	-0.301
Cu	<b>0.934</b>	-0.074	0.108
Fe	<b>0.858</b>	0.058	-0.451
Mn	0.253	<b>-0.806</b>	-0.345
Mo	0.454	0.277	-0.667
Ni	<b>0.783</b>	-0.453	-0.316
Zn	0.497	-0.509	-0.421

Variables that load strongly ( $\geq 0.7$ ) are in bold.

**Table 4.** Eigenvalues and Loadings (Correlations) of SERC Soil Variables onto Rotated Components

Component	Eigenvalue	Variance (%)	
1	7.33	61.11	
2	1.74	14.48	
3	1.00	8.40	
4	0.54	4.53	
Variable	Rotated component 1	Rotated component 2	Rotated component 3
SOM	0.402	0.663	0.480
pH	-0.431	-0.696	-0.290
Al	<b>0.840</b>	0.421	0.121
Co	<b>0.810</b>	-0.058	0.332
Pb	0.577	0.467	0.584
V	<b>0.866</b>	0.178	0.411
Cu	0.185	0.210	<b>0.869</b>
Fe	<b>0.959</b>	0.029	0.170
Mn	0.228	<b>-0.863</b>	-0.018
Mo	<b>0.728</b>	0.036	0.488
Ni	0.397	0.482	0.653
Zn	0.289	0.061	<b>0.855</b>

Variables that load strongly ( $\geq 0.7$ ) are in bold.

0- and 1-year needles (Wilk's lambda = 0.14,  $P < 0.01$ ), as well as significant needle-age effects on all individual metals (ANOVA,  $P < 0.1$ ) with the exceptions of Al and Mo (Table 6, Appendix Table 4 given in supplementary materials).

**Table 5.** Eigenvalues and Loadings (Correlations) of Duke, ORNL, and SERC Soil Variables onto Rotated Components

Component	Eigenvalue	Variance (%)	
1	9.92	82.64	
2	1.40	11.63	
3	0.46	3.81	
4	0.13	1.01	
Variable	Rotated component 1	Rotated component 2	
SOM	<b>0.714</b>	-0.577	
pH	0.161	<b>0.966</b>	
Al	<b>0.995</b>	0.002	
Co	<b>0.991</b>	0.067	
Pb	<b>0.993</b>	-0.050	
V	<b>0.964</b>	0.118	
Cu	<b>0.980</b>	0.086	
Fe	<b>0.973</b>	0.129	
Mn	<b>0.988</b>	0.107	
Mo	<b>0.959</b>	-0.113	
Ni	<b>0.927</b>	-0.165	
Zn	<b>0.909</b>	-0.198	

Variables that load strongly ( $\geq 0.7$ ) are in bold.

Effects of elevated CO<sub>2</sub> on *P. taeda* foliar metal concentrations were variable across canopy heights and needle-age classes. There were significant age  $\times$  CO<sub>2</sub> (Wilk's lambda = 0.58,  $P < 0.01$ ), age  $\times$  canopy height (Wilk's lambda = 0.37,  $P < 0.01$ ), and age  $\times$  canopy height  $\times$  CO<sub>2</sub> interaction effects (Wilk's lambda = 0.37,  $P < 0.01$ ). There were significant CO<sub>2</sub> interaction effects on foliar metal concentrations for the following metals: Co, V, Cu, Fe, Mn, Ni, and Zn (ANOVA,  $P < 0.1$ , Figure 4, Appendix Table 5 given in supplementary materials).

There was a significant effect of elevated CO<sub>2</sub> on trace metal concentrations in *L. styraciflua* leaves at Duke (MANOVA; Wilk's lambda = 0.05,  $P < 0.01$ ). There was a significant CO<sub>2</sub> effect on foliar Fe concentrations, which decreased with CO<sub>2</sub> enrichment ( $P < 0.10$ ); no other metals had significantly different leaf concentrations under elevated CO<sub>2</sub> (Appendix Table 6 given in supplementary materials).

#### Oak Ridge National Laboratory

There was a significant effect of elevated CO<sub>2</sub> on trace metal concentrations in *L. styraciflua* leaves at ORNL (MANOVA; Wilk's lambda = 0.23,  $P < 0.01$ ; Figure 3, Table 6), significant difference in metal concentrations among canopy heights (Wilk's

**Table 6.** Duke, ORNL, and SERC Leaf Metal Concentrations

		CO <sub>2</sub>	Al (µg g <sup>-1</sup> )	Co (ng g <sup>-1</sup> )	Pb (ng g <sup>-1</sup> )	V (ng g <sup>-1</sup> )		
Duke	<i>P. taeda</i> , 0 <sup>1</sup>	A	144.2 ± 20.6	198.3 ± 21.8	92.4 ± 19.3	11.8 ± 2.7		
		E	106.6 ± 14.7	133.3 ± 8.6	123.4 ± 25.6	8.8 ± 0.4		
	<i>P. taeda</i> , 1	A	144.5 ± 12.4	200.6 ± 26.5	216.2 ± 23.2	30.0 ± 4.4		
		E	94.6 ± 15.8	213.7 ± 10.1	162.5 ± 23.2	30.6 ± 1.1		
	<i>L. styraciflua</i>	A	197.7 ± 22.9	65.6 ± 14.2	90.6 ± 43.6	67.8 ± 20.5		
		E	140.6 ± 21.0	47.2 ± 13.0	92.1 ± 40.0	77.7 ± 18.8		
ORNL	<i>L. styraciflua</i>	A	357.7 ± 36.4	27.0 ± 3.9	72.9 ± 5.7	26.4 ± 2.7		
		E	306.6 ± 44.6	21.7 ± 4.8	67.4 ± 5.2	27.7 ± 3.3		
SERC	<i>Q. chapmanii</i>	A	31.7 ± 4.8	4.8 ± 1.3	61.1 ± 18.1	52.1 ± 7.4		
		E	30.6 ± 2.4	4.5 ± 1.2	66.6 ± 18.6	55.0 ± 7.5		
	<i>Q. geminata</i>	A	36.3 ± 4.4	6.6 ± 1.5	64.0 ± 13.0	52.8 ± 5.0		
		E	22.9 ± 2.9	5.0 ± 1.1	65.3 ± 13.8	36.8 ± 3.7		
	<i>Q. myrtifolia</i>	A	29.7 ± 3.0	12.0 ± 2.4	35.5 ± 9.6	43.4 ± 5.7		
		E	22.5 ± 4.1	6.8 ± 1.2	74.5 ± 17.4	33.6 ± 3.0		
		CO <sub>2</sub>	Cu (µg g <sup>-1</sup> )	Fe (µg g <sup>-1</sup> )	Mn (µg g <sup>-1</sup> )	Mo (ng g <sup>-1</sup> )	Ni (µg g <sup>-1</sup> )	Zn (µg g <sup>-1</sup> )
Duke	<i>P. taeda</i> , 0	A	3.2 ± 0.3	13.9 ± 0.6	253.6 ± 19.9	35.3 ± 1.0	2.7 ± 1.6	29.7 ± 4.1
		E	3.5 ± 0.2	13.6 ± 0.6	253.0 ± 28.4	25.5 ± 2.6	2.1 ± 0.1	24.7 ± 2.7
	<i>P. taeda</i> , 1	A	2.1 ± 0.2	20.0 ± 1.6	506.7 ± 10.2	35.3 ± 0.1	0.8 ± 0.3	41.1 ± 9.0
		E	2.0 ± 0.1	21.7 ± 0.8	502.5 ± 31.6	31.9 ± 6.6	0.3 ± 0.1	37.7 ± 4.8
	<i>L. styraciflua</i>	A	5.2 ± 0.8	44.5 ± 3.3	492.8 ± 69.5	32.5 ± 20.4	5.7 ± 1.8	32.7 ± 13.8
		E	4.2 ± 0.7	34.4 ± 3.0	719.8 ± 63.7	38.6 ± 18.7	3.5 ± 1.6	31.9 ± 12.6
ORNL	<i>L. styraciflua</i>	A	4.6 ± 0.5	35.1 ± 2.1	1109.8 ± 168.4	19.1 ± 3.55	6.6 ± 0.6	22.7 ± 2.8
		E	3.8 ± 0.6	32.2 ± 2.5	1258.9 ± 194.8	24.2 ± 4.35	6.8 ± 0.8	25.8 ± 3.4
SERC	<i>Q. chapmanii</i>	A	4.9 ± 0.7	35.8 ± 3.5	39.66 ± 4.32	6.5 ± 1.7	0.8 ± 0.2	16.0 ± 4.4
		E	3.8 ± 0.2	36.5 ± 3.4	65.89 ± 5.90	8.0 ± 2.2	0.5 ± 0.2	20.9 ± 3.5
	<i>Q. geminata</i>	A	3.8 ± 0.2	35.2 ± 3.6	53.25 ± 5.87	8.76 ± 2.52	0.6 ± 0.1	26.4 ± 2.5
		E	3.9 ± 0.2	28.8 ± 2.5	64.71 ± 8.96	8.61 ± 1.75	0.7 ± 0.1	22.5 ± 1.9
	<i>Q. myrtifolia</i>	A	2.9 ± 0.1	27.8 ± 2.5	65.99 ± 6.69	3.70 ± 0.94	0.6 ± 0.1	35.9 ± 3.5
		E	2.9 ± 0.2	27.0 ± 1.4	80.96 ± 6.39	4.52 ± 1.50	0.7 ± 0.1	35.6 ± 3.5

Collected from ambient (A) and elevated (E) CO<sub>2</sub> plots; Duke and ORNL means are averaged across canopy heights (10–16 m).

Complete statistical results can be found in the text and in Appendix Tables 4–10 given in supplementary materials.

<sup>1</sup>*P. taeda* samples were collected from 0 and 1-year-old needles.

lambda = 0.29,  $P < 0.01$ ), and significant CO<sub>2</sub> × canopy height interactions (Wilk's lambda = 0.37,  $P = 0.05$ ). There were significant CO<sub>2</sub> × height interaction effects for several individual metals (Appendix Table 7 given in supplementary materials; Figure 5)—Al, Co, V, Fe, and Ni all had significantly lower concentrations in upper-canopy foliage with CO<sub>2</sub> enrichment ( $P < 0.1$ ; Appendix Table 8 given in supplementary materials). Although Mo had a significant CO<sub>2</sub> × height interaction effect, Mo concentrations were significantly greater in mid-canopy leaves from elevated CO<sub>2</sub> rings compared to ambient rings (Figure 5).

#### Smithsonian Environmental Research Center

There was a significant effect of elevated CO<sub>2</sub> on trace metal concentrations in leaves at SERC (MANOVA; Wilk's lambda = 0.27,  $P < 0.01$ ;

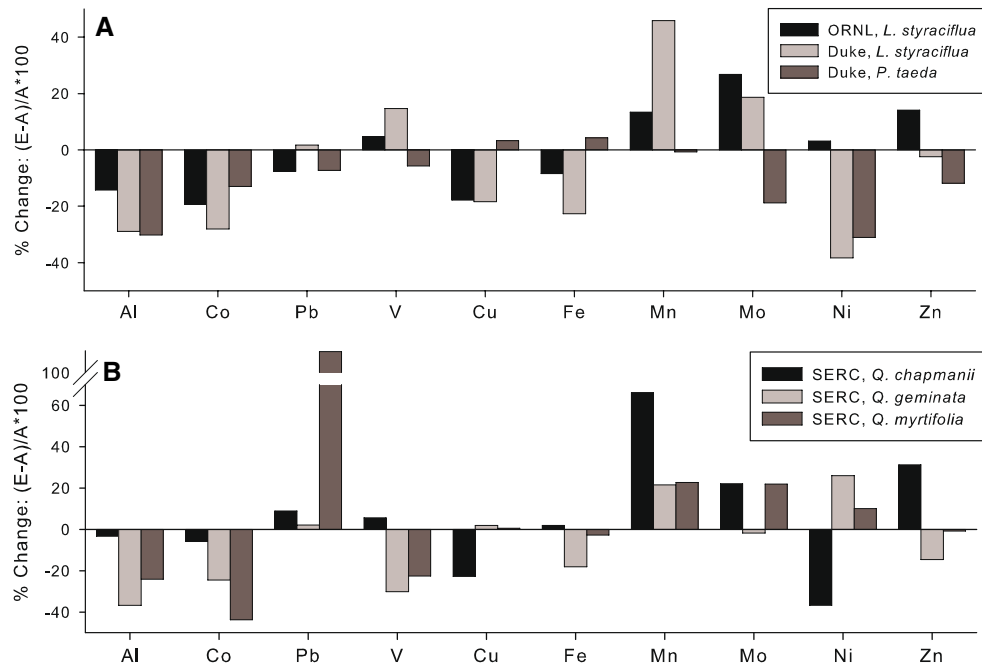
Figure 3, Table 6), significant differences across species (Wilk's lambda = 0.08,  $P < 0.01$ ), and significant CO<sub>2</sub> × species interactions (Wilk's lambda = 0.26,  $P = 0.05$ ). There were significantly lower Al concentrations in *Q. geminata*, significantly lower Ni concentrations in *Q. chapmanii*, and significantly higher Mn concentrations in leaves of all three *Quercus* species in the elevated CO<sub>2</sub> chambers compared to ambient (ANOVA,  $P < 0.10$ , Appendix Tables 9 and 10 given in supplementary materials).

## DISCUSSION

### Soils

CO<sub>2</sub> effects on soil metals were greatest in surface soils (0–5 cm), where there was an overall increase in metal concentrations at Duke, a slight increase at



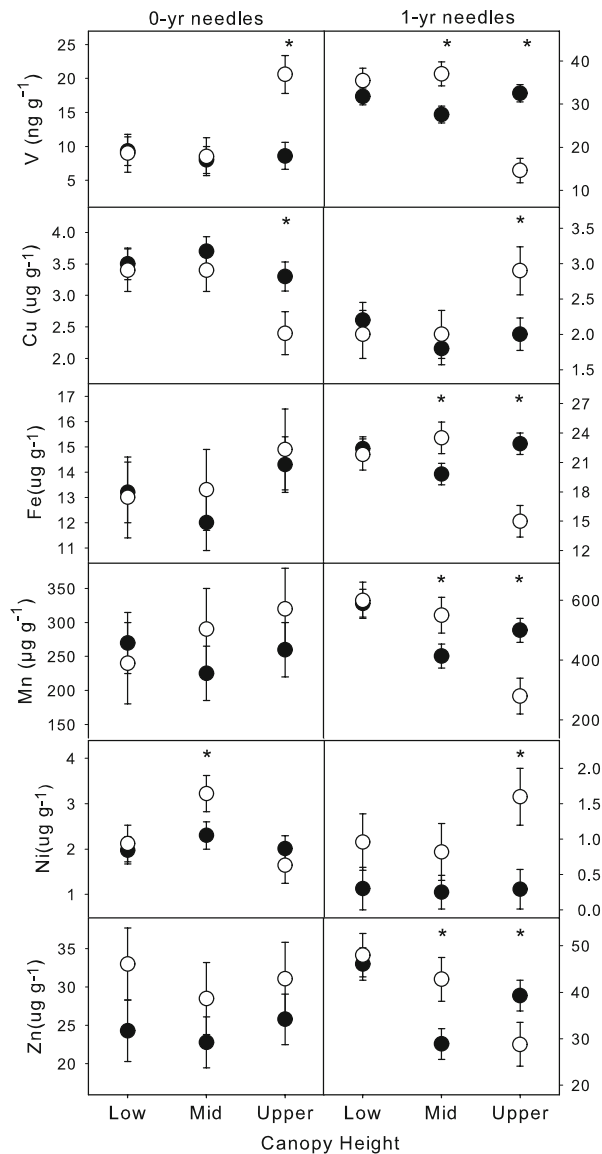


**Figure 3.** CO<sub>2</sub> effects on leaf metal concentrations at Duke and ORNL FACE, and SERC OTC experiments. **A** Percent change in foliar metal concentrations in CO<sub>2</sub> enriched plots (E) relative to ambient (A) for *P. taeda* at Duke, and *L. styraciflua* at ORNL and Duke. *P. taeda* concentrations are averages for 0- and 1-year needles. All species are averaged across canopy heights. **B** Percent change in foliar metal concentrations in *Q. chapmanii*, *Q. geminata*, and *Q. myrtifolia* at SERC. Bars with negative values represent decreased foliar metal concentrations with CO<sub>2</sub> enrichment and those with positive values represent increased concentrations. There was a significant effect of elevated CO<sub>2</sub> on foliar metal concentrations in leaves of all species at all sites (MANOVA,  $P < 0.05$ ).

ORNL, and a decrease in metal concentrations (non-significant) at SERC (Figure 2). These effects were similar to the pattern of CO<sub>2</sub> effects on SOM across sites; changes in percent SOM with CO<sub>2</sub> enrichment were greatest at Duke (18% increase), followed by ORNL (7% increase), with limited effect at SERC (3% increase). Although elevated CO<sub>2</sub> has been shown to increase soil carbon across a range of sites (Jastrow and others 2005), Carney and others (2007) found decreased carbon in SERC soils with CO<sub>2</sub> enrichment. CO<sub>2</sub>-mediated changes in SOM are important to soil metal dynamics because SOM is a key factor governing the sorption of trace metals onto soils (Linde and others 2007; Bradl 2004). For example, CO<sub>2</sub>-mediated changes in SOM were shown to be an important driver of increased soil Hg concentrations with CO<sub>2</sub> enrichment at Duke and ORNL FACE (Natali and others 2008). Although we did not find strong positive correlations between SOM and soil metals *within* each site (based on principal component loadings, Tables 1, 3, and 4), we did find broad patterns *across* sites (Table 5) that highlight the role of SOM in soil metal distributions; concentrations of soil metals

and SOM at SERC are markedly lower than concentrations at either Duke or ORNL FACE, where differences between soil metal concentrations and SOM are less pronounced (Table 2). CO<sub>2</sub> effects on soil metal concentrations may vary across sites that differ in soil carbon pools and soil carbon responses to elevated CO<sub>2</sub>.

Another important driver of metal distributions in soils is pH. We found decreased soil pH with CO<sub>2</sub> enrichment at all sites (Figure 2). Elevated CO<sub>2</sub> may increase soil acidity through increased inputs of carbonic acid from root and microbial respiration (Oh and Richter 2004) or through changes in leaf litter chemistry (but see Johnson and others 2004). Increased soil acidity tends to decrease adsorption of metals onto organic matter and metal oxides (Bradl 2004) and increase trace metal availability to and uptake by plants (Öborn and others 1995). As with SOM, we did not find strong correlations between pH and soil metal concentrations within sites. Unlike SOM, however, pH variation across sites did not strongly correlate with soil metal concentrations (Table 5). Although pH may potentially interact with SOM to drive site-level



**Figure 4.** CO<sub>2</sub> × canopy height effects on *P. taeda* foliar metal concentrations at Duke FACE. Concentrations of metals in 0- and 1-year canopy needles from low (10–12 m), mid (12–14 m), and upper (14–16 m) canopy positions in elevated (filled circles) and ambient (open circles) CO<sub>2</sub> rings. Only metals with significant CO<sub>2</sub> × height × age interaction effects (\*,  $P < 0.10$ ) are shown in figure. Symbols and error bars represent means and standard errors.

responses of soil metals to increased CO<sub>2</sub>, it appears that SOM is a more important determinant of soil metal concentrations.

Concentrations of most metals in SERC soils were one to three orders of magnitude lower than concentrations at Duke and ORNL, and the low metal binding capacity of these sandy soils may be exacerbated by elevated CO<sub>2</sub>. Hungate and others

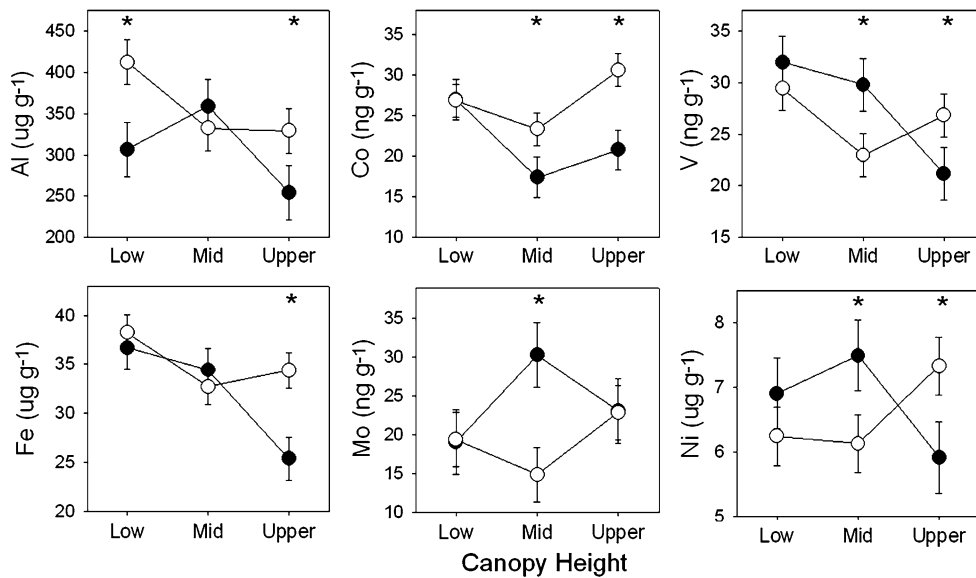
(2004) suggest that the decreased soil Mo-availability at SERC may have led to a decline in N<sub>2</sub>-fixation by the leguminous vine *G. elliotii* under elevated CO<sub>2</sub>. Although we found no significant CO<sub>2</sub> effects on soil principal components at SERC, potential decreases in essential metal concentrations (suggested by a general decline in all SERC soil metals, Figure 2) at this low-fertility site may be an important regulator of ecosystem response to elevated CO<sub>2</sub>.

Although SOM and pH are two important factors governing metal mobility in soils (Bradl 2004; Kalbitz and Wennrich 1998), other soil variables that may be affected by increased CO<sub>2</sub> such as mineral weathering rates (Andrews and Schlesinger 2001) may also be important in determining metal concentrations in soils. Further study is needed to determine other mechanisms driving changes in soil metals observed with CO<sub>2</sub> enrichment.

## Leaves

Elevated CO<sub>2</sub> significantly altered foliar trace metal stoichiometry in all species in this study (MANOVA,  $P < 0.05$ ; Figure 3). CO<sub>2</sub>-mediated changes in soil metals, however, do not appear to be driving changes in foliar metal concentrations. In contrast to the above-mentioned site-specific patterns of CO<sub>2</sub> effects on soil metals, patterns of metals in leaves were more variable within sites.

It is expected that elevated CO<sub>2</sub> may lead to a general decline in elemental concentrations in plants if the increase in carbon uptake and assimilation with CO<sub>2</sub> enrichment (and increase in non-structural carbohydrates) is not matched by increased uptake of soil-derived elements (Loladze 2002). Although many metals did have lower concentrations with CO<sub>2</sub> enrichment, consistent with the ‘dilution’ effect, in some cases there was no change and even an increase in foliar metal concentrations under high CO<sub>2</sub>. For example, Mn concentrations in leaves were significantly higher with CO<sub>2</sub> enrichment in all three *Quercus* species at SERC and tended to increase (non-significant) in *L. styraciflua* at both Duke and ORNL (Figure 3). Although overall Mn concentrations did not increase in *P. taeda*, there were significantly greater concentrations in 1-year upper-canopy elevated leaves compared to upper-canopy ambient (Figure 4). This trend of increased foliar Mn with CO<sub>2</sub> enrichment may be a function of changes in plant requirement for Mn and soil chemistry under high CO<sub>2</sub>. Mn serves a redox role in a number of plant enzymes, most notable in the oxygen evolving



**Figure 5.** CO<sub>2</sub> × canopy height effects on *L. styraciflua* foliar metal concentrations at ORNL FACE. Concentrations of foliar metals from low (10–12 m), mid (12–14 m), and upper (14–16 m) canopy positions in elevated (filled circles) and ambient (open circles) CO<sub>2</sub> rings. Only metals with significant CO<sub>2</sub> × height interaction effects (\*,  $P < 0.10$ ) are shown in figure. Symbols and error bars represent means and standard errors.

complex of photosystem II (Yachandra and others 1996), and increased photosynthetic activity with CO<sub>2</sub> enrichment may increase plant demand for Mn. Increased soil acidity (Oh and Richter 2004) and root exudation with CO<sub>2</sub> enrichment (Vanveen and others 1991) may also increase Mn availability to plants (Sims 1986); however, it does not appear that these processes are the primary drivers of changes in plant foliar Mn concentrations because other metals with similar chemical properties (that would, therefore, also have higher availabilities) did not increase with CO<sub>2</sub> enrichment across sites, as did Mn. Canopy specific differences, as found in *P. taeda*, and increased foliar Mn concentrations across sites with different soil properties and whose soil metals (including Mn) responded differently to CO<sub>2</sub> enrichment, suggest biological regulation of foliar Mn concentrations.

Based on the principles of growth dilution, we expected that any decrease in foliar elemental concentrations with CO<sub>2</sub> enrichment would be greatest in upper-canopy leaves, because CO<sub>2</sub> effects on leaf mass per unit area (LMA) and leaf density are often more pronounced in the upper canopy. For example, LMA and density in upper-canopy leaves of *L. styraciflua* at ORNL increased in CO<sub>2</sub> enriched plots (Norby and Iversen 2006), as did foliar content of non-structural carbohydrates (Sholtis and others 2004). In agreement with the growth dilution hypothesis, we found significantly lower concentrations of Al, Co, V (non-essential metals), Fe, and Ni (metal micronutrients) in upper-canopy *L. styraciflua* leaves at ORNL (Figure 5). In upper-canopy *P. taeda* needles at Duke, there were significantly lower concentrations of V (0-year

needles) and Cu and Ni (1-year needles). However, there were increased concentrations of several essential metals in upper-canopy *P. taeda* needles: Cu (0-year needles), Fe, Mn, and Zn (1-year needles; Figure 4). This increase in concentration may be driven by up-regulation of metals needed to support increased physiological activity in the upper canopy under high CO<sub>2</sub>.

## Conclusions

It has been well recognized that plant macronutrient stoichiometry is regulated by physiological and biochemical requirements (for example, foliar C:N differences among sun and shade leaves) and that such regulation constrains the potential for CO<sub>2</sub>-mediated dilution, though such dilution may occur (Taub and Wang 2008). Our results suggest that metal micronutrient stoichiometry is also governed, in part, by plant functional requirements and that dilution effects for these metals are likely to be less than for non-essential trace metals. This result should alleviate some concerns that rising CO<sub>2</sub> concentrations will result in broad-scale decreases in the concentrations of all elements essential for plant function and animal nutrition (Loladze 2002). However, decreased elemental concentrations may be of particular concern for metals that are non-essential in plants but are required nutrients for higher trophic-level organisms (for example, selenium). The effect of elevated CO<sub>2</sub> on metal stoichiometry may also be important in mediating plant and ecosystem toxicity levels. Although our experimental sites were not affected by toxic levels of trace metals, our results may be

applicable to contaminated systems; for example, elevated CO<sub>2</sub> may, through dilution effects, alleviate Al toxicity.

Our results emphasize the importance of elemental identity and function in understanding CO<sub>2</sub> effects on foliar stoichiometry, and suggest that we can develop a general model of elevated CO<sub>2</sub> impacts on trace metal biogeochemistry by accounting for both metal function (or lack thereof) in plants and the soil characteristics of the ecosystem. In principal, the logic underlying such a model could also be extended to examine the impacts of other environmental parameters expected to change in the future, for example, temperature. As temperatures increase, plant functional biology and stoichiometric demands will change, and the impacts of these changes on metal cycling may be predictable, given the functional roles of the metals. An important caveat to such an effort is that, in as much as a particular parameter, for example, temperature, affects soils directly, the impacts on metals will also be affected. Both warming and drought have been shown to have species- and element-specific effects on aboveground biomass accumulation of contaminants (Sardans and others 2008), and there is a general increase in the export of trace metals from soils exposed to drought (Sardans and Peñuelas 2007). Therefore, CO<sub>2</sub> effects on metal cycling will be driven not only by the direct effects observed in this study, but also by indirect effects of climate change on metal cycling. Such complexity poses both a challenge and an opportunity for future efforts to understand global change impacts on the ecology of metals in terrestrial ecosystems.

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