

# Concentration of sugars, phenolic acids, and amino acids in forest soils exposed to elevated atmospheric CO<sub>2</sub> and O<sub>3</sub>

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## Abstract

Concentrations of soluble soil sugars, soluble phenolic acids, and free amino acids were measured in three forest communities at the FACTS-II Aspen FACE Site near Rhinelander, WI, in order to better understand how elevated atmospheric CO<sub>2</sub> and O<sub>3</sub> are influencing soil nutrient availability and cycling. Sugars, phenolic acids, and amino acids are mostly derived from plant and microbial processes, and have the potential to be influenced by changes in carbon inputs. We hypothesized that concentrations in the soil would parallel increases seen in biological activity, due to greater net primary productivity under elevated CO<sub>2</sub> and seasonal patterns of root growth. Chemical analysis of soils revealed marginally significant increases of total soluble sugars and total soluble phenolic acids in the elevated CO<sub>2</sub> treatment (+27 mg kg<sup>-1</sup>, +0.02 μmol g<sup>-1</sup>), but there were no significant differences in concentrations due to elevated O<sub>3</sub> or CO<sub>2</sub> + O<sub>3</sub>. Total free amino acid concentrations were not affected by any of the treatments, but significant shifts in individual amino acids were observed. Elevated CO<sub>2</sub> and the interaction treatment (elevated CO<sub>2</sub> + O<sub>3</sub>) increased aspartic acid concentrations, while elevated O<sub>3</sub> treatment decreased the concentration of valine. Concentrations of sugars increased throughout the growing season, while phenolic acids were constant and amino acids decreased. The birch–aspen community had the highest concentration of phenolic acids and sugars overall, while maple–aspen had the lowest. These findings suggest that concentrations of soluble sugars, soluble phenolic acids, and free amino acids in the soil are strongly influenced by soil properties, plant and microbial activity, plant community composition, and to a lesser degree, changes in atmospheric CO<sub>2</sub> and O<sub>3</sub>.

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## 1. Introduction

Concentrations of CO<sub>2</sub> and O<sub>3</sub> in the Earth's troposphere have been steadily increasing since the advent of the industrial revolution and have the ability to significantly alter forest net primary productivity (Norby et al., 2005). Elevated atmospheric CO<sub>2</sub> (around 560 μl l<sup>-1</sup>) has been shown to increase carbon (C) inputs into the soil (Jastrow et al., 2005), while current levels of O<sub>3</sub> in many parts of the world are capable of directly altering belowground processes by reducing C inputs (Anderson, 2003; Loya et al., 2003). A significant portion of net primary production is allocated to root systems, resulting in large fluxes of organic compounds into the soil (King et al.,

2004). It is estimated that up to 40% of the C fixed by plants can be lost through root exudation (Lynch and Whipps, 1991). Organic compounds in the soil are derived from root exudates, root residues, microbial metabolism, and above ground litter (Kuzakov, 2001), and these compounds play an important role in soil processes (Walker et al., 2003).

Plant tissue biochemistry is an important factor that influences the rate of litter decomposition and thus the cycling of compounds in the soil. Elevated CO<sub>2</sub> has been shown to increase concentrations of non-structural carbohydrates and defense compounds in leaves, while decreasing leaf nitrogen concentrations (Liu et al., 2005; Lindroth et al., 2001; Peñuelas et al., 1996, 1997). Consequently, leaf litter with higher concentrations of carbohydrates and amino acids, and lower concentrations of phenolic acids, provides more labile C to microbes (Martens, 2000).

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Although litter tissue biochemistry and decomposition have been studied extensively, there are relatively few reports in the literature of the effects of elevated atmospheric CO<sub>2</sub> and O<sub>3</sub> on the concentration of organic compounds in soil. Terrestrial ecosystems store a significant amount of C, particularly in the soil, and it is important to understand how changes in Earth's atmosphere will alter pools and cycling of organic compounds in the soil. This study focused on three soluble pools of organic compounds in the soil: soluble sugars, soluble phenolic acids, and free amino acids.

The primary objective was to identify and quantify these compounds in three different forest communities over the course of one growing season. It was hypothesized that concentrations of sugars, phenolic acids, and amino acids would be relatively low in the spring and increase during the growing season, as root growth became more active. Due to the different growth patterns of the three tree species at this site (*Populus tremuloides*, *Betula papyrifera*, *Acer saccharum*), differences in soil chemistry between the forest communities were expected. Increased fine root biomass in the elevated CO<sub>2</sub> treatment was hypothesized to positively influence the concentrations of sugars, phenolic acids, and amino acids in the soil, whereas elevated O<sub>3</sub> was expected to reduce concentrations, because it typically causes a decrease in root biomass. The interaction treatment with both elevated CO<sub>2</sub> and O<sub>3</sub> was predicted to have concentrations of sugars, phenolic acids, and amino acids similar to those of the control.

## 2. Methods

### 2.1. Site Description

The Aspen FACE site is located on a 32 ha abandoned agricultural field near Rhinelander, Wisconsin. The experiment is a completely randomized block design with four different treatments: elevated CO<sub>2</sub>, elevated O<sub>3</sub>, elevated CO<sub>2</sub> + O<sub>3</sub>, and control. Each treatment is replicated three times, for a total of 12 plots ( $N = 12$ ). Each 30 m diameter ring is divided so that half of the ring is dominated by aspen (*P. tremuloides* Michx.), a quarter is birch (*B. papyrifera* Marsh.) and aspen, and the other quarter is maple (*A. saccharum* Marsh.) and aspen. Seedlings of each species were planted in early June 1997 and have been exposed to treatment gases from budbreak to the end of each growing season since 1998. Forest succession has allowed other woody and herbaceous species to establish in the forest understory, but the overstory of each community type is still exclusively dominated by the trees originally planted. Soils consist of a Pandus sandy loam (mixed, frigid, coarse loamy Alfic Haplorthod) with a pH of 5.5. At the time of planting, soils consisted of 1.53% C and 0.12% N across treatments. A detailed soil physical and chemical analysis is reported in Dickson et al. (2000).

Treatment gases are delivered by a computer-controlled system during the daylight hours. Elevated CO<sub>2</sub> concentra-

tions within the rings are kept at a target of 200  $\mu\text{l l}^{-1}$  above current levels in the atmosphere. In the ozone treatments, diurnal concentrations are kept approximately 1.5 times greater than ambient concentrations. Ozone was not delivered on days when the maximum temperature was less than 15 °C or times when plants were wet from fog, dew, or rain. Details of the exposure system and ozone exposure profiles can be found in Karnosky et al. (2005).

### 2.2. Sampling

In order to reduce spatial variability within treatments, six cores (1 cm diameter) were collected across all three replicates of the four treatments from the top 10 cm of soil in each of the three plant communities on June 9, July 12, and August 22 of 2005. Tree arrangement is identical in each replicate subplot. Random locations were generated and soil cores were extracted from the same random location in each replicate subplot. Samples were immediately placed on ice, transported back to the lab, and stored at -20 °C until analysis. Careful measures were taken to reduce freeze and thaw events in order to minimize the amount of C and N transformations as much as possible and to assure the results reflect treatment differences and not sample handling. Samples were frozen until they were sieved. Afterwards, they were analyzed immediately. Each soil sample was sieved (2 mm) within a month of collection and analyzed for total soluble sugars, total soluble phenolic acids, and amino acids.

### 2.3. Total sugars

Sugars were extracted using a method described by Martens and Frankenberger (1990). One gram of soil was combined with 10 ml of 0.25 M H<sub>2</sub>SO<sub>4</sub>, agitated for 16 h, and titrated to pH 3.5–4.5 with 5.0 M KOH to prevent co-precipitation of saccharides. Samples were then centrifuged at 4500 rev min<sup>-1</sup> for 20 min and filtered through a 0.22  $\mu\text{m}$  nylon filter. The solution was passed again through a Supelco<sup>TM</sup> (Bellefonte, PA) solid phase extraction system consisting of a strong cation (3-propylsulfonic acid H<sup>+</sup>) column and a strong anion (3-quaternary propylammonium Cl<sup>-</sup>) column to remove ionic interferences and analyzed using the phenol–sulfuric acid colorimetric procedure (Dubois et al., 1956) on a Beckman Coulter DU640 Series spectrophotometer (Beckman Coulter, Inc., Fullerton, CA). A reference standard of D-glucose was used to generate a calibration curve.

### 2.4. Total soluble phenolic acids

Total soluble phenolic acids were analyzed using the Folin-Ciocalteu Method as described in DeForest et al. (2005). Compounds were extracted by agitating 5 g of soil with 25 ml of DI water for 18 h, centrifuging the samples at 4500 rev min<sup>-1</sup> for 15 min, and passing the supernatant through a 0.45  $\mu\text{m}$  nylon filter. After combining the

supernatant with 0.75 ml of  $\text{Na}_2\text{CO}_3$  and 0.25 ml of Folin-Ciocalteu reagent, samples were incubated for 1 h at 25 °C in the dark, and absorbance measured at 750 nm on the spectrophotometer. Sample concentrations were determined by comparing the solution to a standard mixture. This mixture contained  $50 \mu\text{mol l}^{-1}$  each of ferulic, *p*-coumaric, *p*-hydroxybenzoic, vanillic, and syringic acid. The standard was adjusted to a pH of 6 with NaOH and diluted to range between 3 and  $150 \mu\text{mol l}^{-1}$ .

### 2.5. Amino acids

For amino acid analysis, the six soil samples from each subplot were composited into one sample. Five grams of each composite sample was then combined with 25 ml of DI water and agitated for 20 h. Samples were centrifuged at  $4500 \text{ rev min}^{-1}$  for 15 min and passed through a  $0.45 \mu\text{m}$  nylon filter. Due to low concentrations of amino acids in our samples, the extracted solution was freeze dried to concentrate the compounds. The freeze dried samples were redissolved in 0.75 ml of DI water and filtered again through a  $0.20 \mu\text{m}$  nylon syringe filter. Amino acids were analyzed using a method described by Gratzfeld-Huesgen (1998) on a Beckman Coulter Gold Series high-pressure liquid chromatograph (Beckman Coulter, Inc., Fullerton, CA). Primary amino acids were quantified as ortho-phthalaldehyde derivatives on a  $200 \times 2.1 \text{ mm}$  AA column with fluorescence detection.

### 2.6. Statistical analyses

Data were analyzed using MINITAB® Release 14.1 software. A four-factor (day, community, treatment, sample) generalized linear model with randomized blocks, main effects, and up to four-way interactions was fit to the total sugar and phenolic acid data. Amino acids were tested similarly, but with a three-factor design (day, community, treatment) and up to two-way interactions. The interactions of blocks with other effects were placed in the error term to test the other factors and corresponding interactions since no amino acid replicates were available at the subplot level. The factors were considered as fixed, and the blocks were considered as random in the analysis of variance.

Plots of the standardized residuals versus the fitted values were used to understand the scatter of residuals and subsequent absence of significant interactions other than those deliberately used in the models. Standardized residuals greater than four in absolute value were noted as outliers, as they were four standard deviations away from the residual mean of zero and outside the 99.99% confidence interval. No residual values were considered outliers in the sugar or phenolic acid data. However, in the amino acid data, individual compounds were removed from the sample when residuals were greater than four in absolute value. Residuals were exponentially distributed,

justifying a natural log transformation of the data. *P*-values on transformed data were very similar to those of untransformed data and bolstered the decision to remove outliers of non-transformed data.

Ninety-five percent confidence intervals about the means are reported throughout the text. Post hoc statistical analysis of factor levels utilized the Fisher's LSD test. Factors and interactions with  $*P < 0.1$  were considered marginally significant;  $**P < 0.05$  were considered statistically significant, and  $***P < 0.01$  were considered highly significant according to the resulting random effects analysis.

## 3. Results

### 3.1. Sugars

Averaged across all treatments and community types, the mean total soluble sugars were  $217.2 (\pm 3.8) \text{ mg kg}^{-1}$  in June,  $232.04 (\pm 6.7) \text{ mg kg}^{-1}$  in July, and  $281.8 (\pm 6.5) \text{ mg kg}^{-1}$  in August. Sugars increased throughout the growing season, but differences were not significant. However, there was a significant interaction between date and community type ( $P = 0.04$ ). The concentration of sugars within all plant communities was significantly higher in August when contrasted to June (Fig. 1). Aspen and maple-aspen communities exhibited a gradual increase in total soluble sugars throughout the growing season, while birch-aspen had a rapid increase in concentration between July and August. The aspen community had significantly higher concentrations of sugars in July than did the birch-aspen community, while concentrations in the maple-aspen community were intermediate between the two. By the end of the growing season, the birch-aspen community had concentrations significantly higher than the maple-aspen community. Averaged across all sample dates, elevated  $\text{CO}_2$  caused sugar concentrations to increase, but these differences were only marginally significant ( $P = 0.08$ ). Elevated  $\text{O}_3$  and the interaction of  $\text{CO}_2$  and  $\text{O}_3$  had no effect on total sugars in the soil (Fig. 2).

### 3.2. Phenolic acids

Total phenolic acid concentrations were relatively uniform throughout the growing season, with a slightly higher concentration in June (data not shown). Plant community was highly significant ( $P = 0.003$ ), with the birch-aspen community having a higher concentration than both aspen and maple-aspen (Fig. 3). Phenolic acids increased in the elevated  $\text{CO}_2$  treatment, but differences were only marginally significant ( $P = 0.07$ ; Fig. 4). The elevated  $\text{O}_3$  treatment had concentrations similar to those of the control, while concentrations in the interaction treatment were slightly greater than the control.

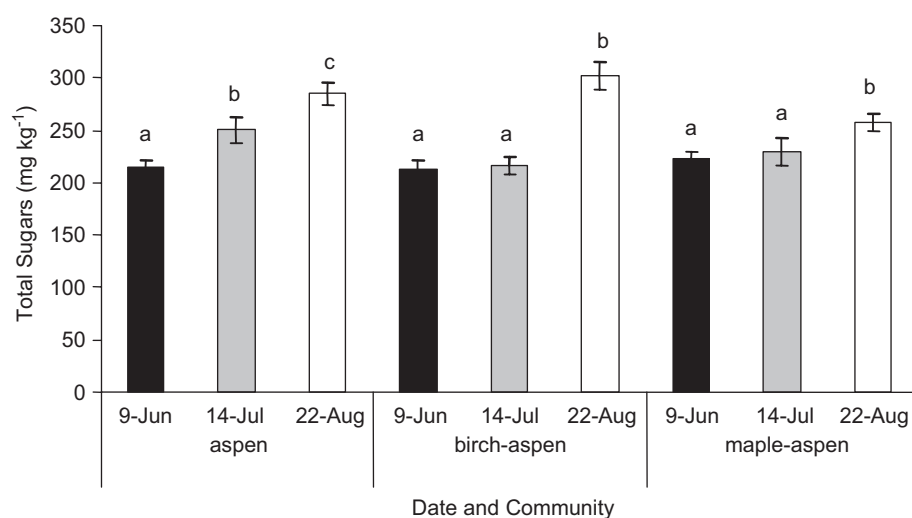


Fig. 1. Total soluble sugar concentration in the soil sampled from aspen, birch-aspen, and maple-aspen communities on three dates. Data are means  $\pm$  95% confidence interval. Within a community, different letters indicate significant differences among sample dates ( $P = 0.04$ ).

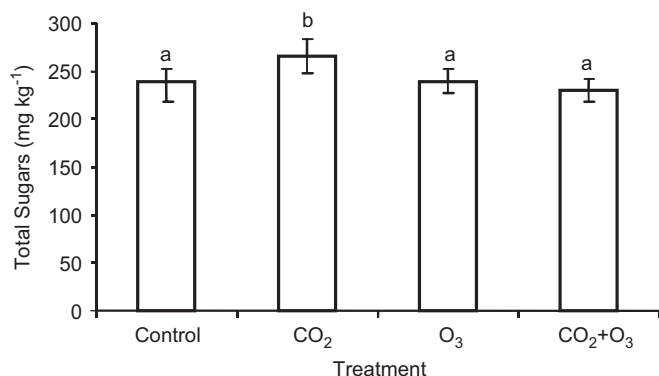


Fig. 2. Total soluble sugar concentrations in soil of forests exposed to elevated atmospheric CO<sub>2</sub> and O<sub>3</sub>. Data are means  $\pm$  95% confidence interval. Different letters indicate marginally significant differences between treatments ( $P = 0.08$ ).

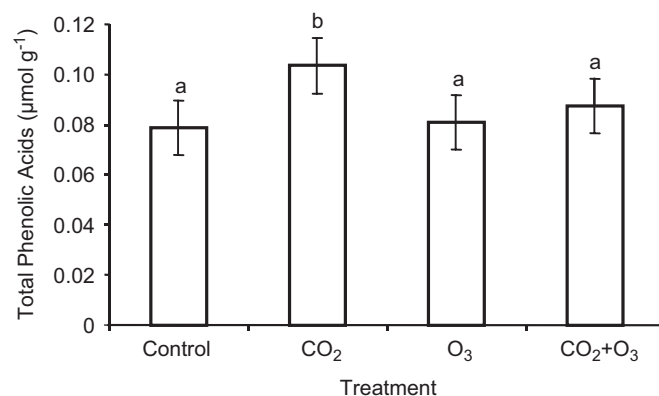


Fig. 4. Total soluble phenolic acid concentrations in soil of forests exposed to elevated CO<sub>2</sub> and O<sub>3</sub>. Data are means  $\pm$  a 95% confidence interval. Different letters indicate marginally significant differences between treatments ( $P = 0.07$ ).

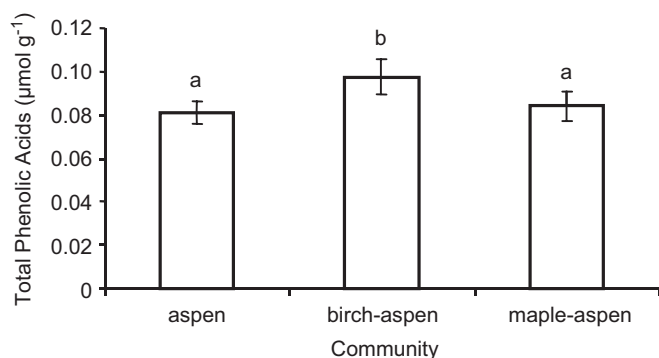


Fig. 3. Total soluble phenolic acid concentrations in soil sampled from aspen, birch-aspen, and maple-aspen communities. Data are means  $\pm$  95% confidence interval. Different letters indicate significant differences among communities ( $P = 0.003$ ).

### 3.3. Free amino acids

In total, 15 primary free amino acids were identified. However, histidine, glycine, and threonine were bulked

together into one category (HGT) because of the tendency of their peaks to clump together during analysis, making them difficult to identify as individual amino acids.

The most abundant amino acids in the soil were aspartic acid, serine, and HGT. Sampling date had a major influence on most of the compounds, with three discernible patterns (Table 1). The concentration of aspartic acid, glutamic acid, and arginine peaked in July, and by August concentrations had fallen to levels at or below those observed in June. Valine, serine, HGT, alanine, cystine, tyrosine, and isoleucine were highest in June, dropped dramatically in July, and then increased to intermediate concentrations by August. Methionine, phenylalanine, and leucine exhibited high concentrations in June, and gradually decreased throughout the rest of the growing season. Patterns were not significant for aspartic acid, glutamic acid, arginine, valine, and leucine. Total free amino acids were highest in June, dropped dramatically in July, and



Table 1  
Concentration of primary amino acids in soil sampled on three different dates

| Amino acids<br>( $\mu\text{g kg}^{-1}$ ) | June 9, 2005       | July 12, 2005     | August 22, 2005    | $P^{\ddagger}$ |
|------------------------------------------|--------------------|-------------------|--------------------|----------------|
| Aspartic acid                            | 133.45 $\pm$ 14.2  | 144.98 $\pm$ 14.9 | 130.5 $\pm$ 20.0   | ns             |
| Glutamic acid                            | 43.61 $\pm$ 4.9    | 52.27 $\pm$ 8.0   | 43.74 $\pm$ 6.7    | ns             |
| Serine                                   | 161.40 $\pm$ 32.6a | 67.47 $\pm$ 13.7b | 92.1 $\pm$ 35.2b   | ***            |
| HGT <sup>a</sup>                         | 184.80 $\pm$ 30.6a | 128.9 $\pm$ 48.0b | 202.3 $\pm$ 75.6a  | ***            |
| Alanine                                  | 40.94 $\pm$ 11.0a  | 26.17 $\pm$ 8.2b  | 45.06 $\pm$ 7.8a   | ***            |
| Arginine                                 | 15.74 $\pm$ 4.1    | 17.75 $\pm$ 8.4   | 19.78 $\pm$ 9.9    | ns             |
| Tyrosine                                 | 24.06 $\pm$ 5.4a   | 19.18 $\pm$ 7.6b  | 23.71 $\pm$ 8.3ab  | **             |
| Cystine                                  | 41.49 $\pm$ 8.8a   | 23.62 $\pm$ 7.9b  | 38.06 $\pm$ 9.5a   | ***            |
| Valine                                   | 34.51 $\pm$ 18.1   | 19.82 $\pm$ 11.0  | 21.41 $\pm$ 10.5   | ns             |
| Methionine                               | 16.63 $\pm$ 4.5a   | 8.92 $\pm$ 4.7b   | 7.96 $\pm$ 2.5b    | ***            |
| Phenylalanine                            | 20.80 $\pm$ 5.2a   | 18.16 $\pm$ 10.3b | 15.68 $\pm$ 5.2b   | ***            |
| Isoleucine                               | 25.63 $\pm$ 6.2a   | 15.49 $\pm$ 6.6b  | 23.49 $\pm$ 7.8a   | ***            |
| Leucine                                  | 4.86 $\pm$ 2.2     | 4.92 $\pm$ 2.1    | 4.12 $\pm$ 2.0     | ns             |
| Total                                    | 761.1 $\pm$ 105.2a | 456 $\pm$ 76.2b   | 577.9 $\pm$ 118.2b | ***            |

Data are means  $\pm$  95% confidence interval.

$^{\ddagger}$ \*\*\* $P$  < 0.05, \*\*\* $P$  < 0.01, ns = nonsignificant. Means followed by different letters indicate significant differences.

<sup>a</sup>HGT-histidine/glycine/threonine

Table 2  
Individual amino acids ( $\mu\text{g kg}^{-1}$ ) that differed significantly among treatments

| Treatment                        | Aspartic acid <sup>***</sup> | Arginine*         | Valine***        | Methionine***    |
|----------------------------------|------------------------------|-------------------|------------------|------------------|
| Control                          | 121.8 $\pm$ 14.0a            | 19.4 $\pm$ 9.2ab  | 39.1 $\pm$ 19.4a | 12.1 $\pm$ 4.2ab |
| CO <sub>2</sub>                  | 151.1 $\pm$ 22.0b            | 20.6 $\pm$ 7.6a   | 19.6 $\pm$ 8.4ab | 18.0 $\pm$ 6.8b  |
| O <sub>3</sub>                   | 126.1 $\pm$ 19.6ac           | 11.8 $\pm$ 12.6ab | 7.6 $\pm$ 4.4b   | 6.4 $\pm$ 3.4a   |
| CO <sub>2</sub> + O <sub>3</sub> | 146.3 $\pm$ 18.0bc           | 12.8 $\pm$ 5.4b   | 35.0 $\pm$ 21.6a | 8.5 $\pm$ 3.2a   |

Data are means  $\pm$  95% confidence interval.

$^{\ddagger}$ \*\*\* $P$  < 0.1, \*\* $P$  < 0.05, \*\*\* $P$  < 0.01. Means followed by different letters indicate significant differences.

increased to intermediate concentrations by August; mimicking the pattern of the more abundant compounds.

Although aspen had the highest concentration of total amino acids, and birch–aspen the lowest, there were no significant differences in community patterns for the total amino acids or individual amino acids. Aspartic acid was positively influenced by elevated CO<sub>2</sub> and the interaction of CO<sub>2</sub> and O<sub>3</sub>, while O<sub>3</sub> negatively influenced valine (Table 2).

## 4. Discussion

### 4.1. Sugars and phenolic acids

Solid phase extraction columns used during preparation for sugar analysis greatly increases accuracy of total sugar detection by decreasing ionic interferences that often overestimate concentrations while using spectrophotometric methods (Martens and Frankenberger, 1990). While use of these columns in our experiment gave a more accurate

calculation of total sugars, concentrations were much lower than other reports from forest soils (Martens and Frankenberger, 1993; Piccolo et al., 1996). The FACE experimental site has a long history of agriculture, and the forest communities are only nine years old. This complicates comparisons with mature forest soils. Piccolo et al. (1996) tested four types of Italian surface forest soils using the same method, and concentrations of total sugars ranged from 929 mg kg<sup>-1</sup> to 1540 mg kg<sup>-1</sup> which is 3–6 times higher than concentrations measured here (Fig. 1). While the texture and pH of one of their samples was comparable to ours, organic C (%) was much higher in their soils.

Sugars were predicted to increase in the soil throughout the growing season as biological activity in the soil progressed. Our sampling scheme did not allow us to specifically detect a rhizosphere effect. However, roots were collected when we sampled the soil, and our observations of soluble sugar concentrations, which increased throughout the growing season (Fig. 1), correspond with annual root activity and growth. This trend matches rates of new root production, which peaks in July at this latitude (Hendrick and Pregitzer, 1992). Increases in fine roots result in higher surface areas and the chance to lose more compounds into the soil through active and passive diffusion (Bertin et al., 2003; Jones et al., 2004; Walker et al., 2003); sugars are the dominant component of such root exudates (Jones and Darrah, 1996). Additionally, the microbial demand for energy may have been lower. Working at this same site in previous years, Larson et al. (2002) showed that microbial biomass C peaked in May (83  $\mu\text{g C g}^{-1}$  soil) and had dropped by 80% (15–18  $\mu\text{g C g}^{-1}$  soil) in July and October. With lower microbial demands, sugars would be more abundant in the soil.

Elevated CO<sub>2</sub> often increases root growth and biomass (Calfapietra et al., 2003; Norby et al., 1992; Pregitzer et al., 1995; Zak et al., 2000). Cotton plants grown at elevated CO<sub>2</sub> have been shown to have a higher concentration of carbohydrates in their roots compared to those grown at ambient CO<sub>2</sub> (Wong, 1990). An increase in carbohydrates in the root system may lead to higher rates of root respiration and increased exudation of soluble sugars into the soil (Norby, 1994). The marginally significant increase in sugars (Fig. 2) seen in this study's elevated CO<sub>2</sub> treatment may be due to increased root biomass.

Similar to sugar concentrations, total soluble phenolic acids were also much lower when compared to other forest soils. Soils studied across several mature sugar maple forests averaged 103  $\mu\text{mol g}^{-1}$ , compared to this study's mean of 0.9  $\mu\text{mol g}^{-1}$  (DeForest et al., 2005). Whitehead et al. (1981) found that soils with a pH < 7.5 contained much lower concentrations of total phenolic acids, but once the pH reached 7.5–10.5, concentrations increased dramatically. The pH of our soils is only 5.5, and soil C (1.53%) and N levels (0.12%) are both low. Our results suggest that the concentrations of total sugars and phenolic

acids depend in part on stand age and soil properties (pH, %C, %N). Unlike the trend we observed with sugars, phenolic acid concentrations were stable throughout the growing season. Most phenolic acids are hypothesized to come from plant litter and have slow degradation rates. These compounds may be added at a relatively steady rate to the soil and lack the seasonal patterns like those observed in our sugar concentrations.

Plant community significantly affected phenolic acid and sugar concentrations. The birch–aspen community had a significantly higher concentration of total phenolic acids and sugars than either aspen or maple–aspen communities. Aspen and birch are early successional tree species that are adapted to high light levels and grow quickly, whereas maple is adapted to lower light intensities and grows slower. At the Aspen FACE site, elevated CO<sub>2</sub> has been shown to increase leaf litter biomass, more so in the birch–aspen community than in the aspen community (Liu et al., 2005), possibly accounting for more phenolic compounds in the soil in the birch–aspen community. Although elevated CO<sub>2</sub> has caused a change in leaf litter quantity among the communities, the quality in terms of sugars and phenolic acids in the litter has not changed with elevated CO<sub>2</sub>. However, O<sub>3</sub> and the interaction of CO<sub>2</sub> + O<sub>3</sub> has significantly increased concentrations of sugars and phenolic acids in the leaf litter (Liu et al., 2005). This change in litter chemistry was not reflected in soil chemistry; concentrations of both sugars and phenolic acids were highest in the elevated CO<sub>2</sub> treatment, with no differences in either the O<sub>3</sub> or the interaction treatment. Since elevated CO<sub>2</sub> has been shown to increase root biomass (King et al., 2005), it may be that soil sugars and phenolic acids are more strongly influenced by root biomass than leaf litter.

#### 4.2. Amino Acids

In the current study, the most abundant free amino acids in the soil were the acidic amino acid aspartate; the neutral amino acids glycine, threonine, and serine; and the basic amino acid histidine. This is consistent with other studies (Monreal and McGill, 1985; Kielland, 1995). However, our study's range of total free amino acid concentrations were relatively low; 456–761 µg kg<sup>-1</sup> versus 320–4720 µg kg<sup>-1</sup> found by Monreal and McGill (1985). Monreal and McGill's (1985) results are from cultivated and virgin soils, and results here suggest that our soils are still recovering from years of agriculture.

Free amino acids make up a considerable amount of the nitrogen leached out of leaf litter (Chapin et al., 1986). Total free amino acid concentrations were highest in the spring and may be a result of leachate from the previous fall's leaf litter. Alternatively, the high concentration of free amino acids in the spring could result from seasonal fluctuations in nitrogen mineralization (Kielland, 1995) or in amino acid uptake (Gupta and Rorison, 1974; Abuarqhub and Read, 1988). Increasing temperatures have also

been shown to increase amino acid uptake and assimilation (Jones, 1999). In mid-July, we observed a dramatic decrease in total free amino acid concentrations in the soil (Table 1). This decrease coincides with the peak growing season, when plant and microbial demand for nitrogen is high. Free amino acids in the soil are a direct source of nitrogen for plants and microorganisms.

Bacteria are thought to increase rapidly in the rhizosphere until they reach population levels limited by organic substrates (Newman, 1985). It is possible that plants and microorganisms utilized the abundant amino acid substrates in June in order to maximize growth by July, resulting in a rapid drop in free amino acid concentrations in the summer. By August, growth rates may have slowed down due to substrate limitations, allowing for a rebound of amino acid concentrations in the soil. These results suggest that microbial and plant demand for amino acids was highest in July, when we measured the lowest total amino acid concentration (Table 1). However, there were no significant responses of total amino acid concentrations to plant community, CO<sub>2</sub>, or O<sub>3</sub>. These results are similar to Larson et al. (2002), who did not detect any effect of plant community, CO<sub>2</sub>, or O<sub>3</sub> on patterns of amino acid metabolism.

A few individual amino acids exhibited significant treatment effects (Table 2). Specifically, valine was significantly lower in the elevated O<sub>3</sub> treatment, while aspartic acid was highest in the CO<sub>2</sub> and CO<sub>2</sub> + O<sub>3</sub> treatments. Studies have shown that CO<sub>2</sub> and O<sub>3</sub> enrichment decreases foliar nitrogen (McGuire et al., 1995; Curtis and Wang, 1998; Norby et al., 1999), which might ultimately decrease the amount of free amino acids leached into the soil. Liu et al. (2005) found that elevated CO<sub>2</sub> reduced the amount of nitrogen in the litter, more so in the birch–aspen community than in the aspen community. The decrease in litter nitrogen under CO<sub>2</sub> enrichment did not carry over to our study's total free amino acid nitrogen and we observed no differences related to community type. However, a decrease in one compound, valine, was visible in the elevated CO<sub>2</sub> treatment, which would not have been apparent if only looking at total nitrogen or total free amino acids. It is possible that elevated CO<sub>2</sub> could be causing a shift in the proportions of specific free amino acids, rather than a change in the overall concentration.

#### 5. Conclusions

These data suggest that concentrations of soluble sugars, soluble phenolic acids, and free amino acids in the soil of forests exposed to elevated CO<sub>2</sub> and O<sub>3</sub> are linked to changes seen in plant and microbial biomass at this study site. All the compounds studied are low molecular weight compounds. However, sugars and amino acids are much more labile than phenolic acids, and as a result were influenced by increases in biological activity in the soil throughout the growing season. The aspen and aspen–birch forest communities are both dominated by early

successional tree species. These communities exhibited the highest concentrations of sugars and phenolic acids. The data suggest that plant community type and time of sampling have a greater effect than changes in atmospheric concentrations of CO<sub>2</sub> or O<sub>3</sub>. Changes in atmospheric concentrations of CO<sub>2</sub> or O<sub>3</sub> may play a more indirect role in altering soil chemistry by shifting the composition of plant communities over time, which could ultimately influence pools of soluble compounds in the soil (Bradley and Pregitzer, 2007).

Results imply that the specific type of dominant vegetation is the primary factor controlling the concentration of phenolic acids in the soil, while the seasonal supply and consumption of microbial substrates is the main factor influencing amino acids; concentrations of sugars are affected by both.

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