Microbial responses to elevated atmospheric CO_2 and O_3 in northern temperate forests

by

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Abstract

Rising atmospheric concentrations of CO_2 and O_3 have the potential to alter terrestrial ecosystem functioning by modifying rates of photosynthesis, plant growth and the production of plant detritus. Because soil microbial communities are structured by the amount and biochemical composition of plant detritus, rising concentrations of atmospheric CO_2 and O_3 have the potential to alter the composition and activity of soil microbial communities. The objectives of this study were to gain a basic ecological understanding of microbial metabolic responses to elevated CO_2 and O_3 and to examine how these responses are modified by different plant communities over the growing season. To test these ideas, microbial activity was studied under *Populus tremuloides* and a mixture of *Betula papyrifera* and *Populus tremuloides* exposed to experimental CO_2 (ambient and 560μL L^{-1}) and O_3 treatments (ambient and 54.5nL L^{-1}). I employed extracellular enzyme analysis of 1,4 β,D-glucosidase and cellobiohydrolase to assess microbial degradation of cellulose. Averaged among vegetation types, atmospheric CO_2 and O_3 interacted to significantly influence β-glucosidase activity of subsurface mineral soil, such that O_3 counteracted the stimulatory effects of elevated CO_2. In the aspen community, exposure to elevated CO_2 (-20%) and elevated O_3 (-20%) significantly reduced the activity of β-glucosidase, relative to the ambient condition. Additionally, exposure to elevated O_3 significantly reduced the activity of cellobiohydrolase (-40%), relative to the ambient condition, beneath aspen. Enzyme activity in the aspen-birch community did not differ by treatment. Regarding seasonality, β-glucosidase activity and cellobiohydrolase activity were greatest in May and steadily declined in July and October for both vegetation types. Different responses between aspen and aspen-birch communities possibly result from changes in plant litter chemistry or a change in microbial community

composition of the aspen community. As a result, forest community composition and ontogeny may be important factors in understanding ecosystem responses to elevated CO_2 and O_3.

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Introduction

Fossil fuel burning and land-use change has increased the concentration of trace gases in the Earth's atmosphere. For example, O_3 and CO_2 have increased in concentration since pre-industrial times (Barnola et al., 1995; Finlayson-Pitts and Pitts 1997; Brady and Weil 2002). Globally, CO_2 has increased by 30% and O_3 has increased by 36% within the last two-hundred years (Barnola et al., 1995; IPCC 2001). These changes in atmospheric chemistry have large implications for the functioning of ecosystems, because CO_2 and O_3 influence photosynthesis, plant growth and the resulting coupled relationship between plant and microbial communities (Norby et al., 1992). Specifically, these trace gases have the potential to alter ecosystem C storage (Carney et al., 2007) at local, regional and global scales.

Anthropogenic CO_2 and O_3 possess the potential to alter C cycling primarily through their impact on photosynthesis (Rogers et al., 1994; Findlay and Jones 1990). While elevated levels of CO_2 increase the rate of photosynthesis (Rogers et al., 1994), increases in O_3 concentrations decrease photosynthetic rate (Kohut et al., 1986). Plant growth is directly influenced by changes in photosynthetic rates which, in turn, impact the quantity and chemical composition of plant detritus (Rogers et al., 1994). These changes in plant litter impact microbial metabolism and community composition (Karnosky et al., 2003), as a result of heterotrophic soil microorganisms' dependence on detritus as a source of energy. Because soil microorganisms are responsible for the decomposition of plant detritus, changes in microbial decomposition impact soil C storage and nutrient supply to plants (Carney et al.,

2007). This causal chain demonstrates that plant growth and microbial activity are dependent upon one another in a feedback loop (Zak et al., 1993). As a result of this coupling, elevated levels of CO_2 and O_3 in the Earth's atmosphere have the potential to impact C cycling within forest ecosystems.

Elevated atmospheric CO_2 has the potential to alter terrestrial ecosystem functioning by supplying C as a key input to the process to stimulate photosynthesis (Rogers et al., 1994; Reich et al., 2006). An increase in plant photosynthetic rate increases the amount of above and belowground plant growth and biomass in response to elevated CO_2 levels (Norby et al., 1992; Zak et al., 1993). Specifically, studies have demonstrated that the mass of fine roots is directly influenced by an increase in concentration of CO_2, as the greenhouse gas stimulates belowground biomass by providing an input to photosynthesis (Norby et al., 1992). This plant material provides an important (and more available) energy source for microbial growth. Additionally, increased decomposition of cellulose has been observed under elevated CO_2, because cellulolytic enzymes are induced by their substrates (Chung et al., 2006; Larson et al., 2002). These observations suggest a greater input of fine root and mycorrhizal litter under elevated CO_2, connecting microbial activity to changes in atmospheric chemistry (Chung et al., 2006; Larson et al., 2002) and further supporting the idea that anthropogenic additions of CO_2 may have a dramatic impact on C dynamics in terrestrial ecosystems.

Elevated O_3, on the other hand, has the opposite effect on ecosystem C cycling by negatively affecting plant growth. Ozone decreases leaf area (Westman 1979) and causes premature leaf senescence, resulting in decreased photosynthesis and lower above- and belowground plant biomass (Kohut et al., 1986; Boirtier et al., 2000; Findlay and Jones

1990). Under conditions of elevated O_3, fine root biomass decreases (King et al., 2001; Larson et al., 2002) and limits microbial activity, as roots are an important energy source to heterotrophic soil microorganisms. Additionally, the negative impact of O_3 on plant growth can be large enough to counteract the stimulation of cellulose decomposition supplied by elevated CO_2 levels (Karnosky et al., 2003; Larson et al., 2002). The opposing influences of CO_2 and O_3 on plant growth, therefore, leave many questions regarding the impacts of elevated levels of these trace gases on ecosystem functioning, as well as C sequestration.

Within the ecosystem, soils function as an important storage sink in the global C cycle in the form of organic matter (Carney et al., 2007). For this reason, an improved ecological understanding of the impacts of elevated atmospheric CO_2 and O_3 on microbial activity may provide answers regarding the link between microbial activity and plant growth, as well as future C storage within terrestrial ecosystems. The amount of C stored within soil is a function of both plant production and microbial decomposition. In the past, research suggested that plants and soil may serve as a sink for the large amounts of CO_2 produced by anthropogenic activity through an increase in the production of plant biomass (Jastrow et al., 2005). However, one study demonstrates an overall loss of soil C in response to increased microbial metabolism under conditions of elevated CO_2 (Carney et al., 2007). This finding suggests that decomposition of soil organic matter can surpass plant biomass production in an atmosphere with a higher-than-ambient CO_2 concentration. A potential explanation for decreased C storage involves enhanced microbial decomposition in response to increased availability of C-rich detritus (from increased root growth), followed by increased consumption of the more complex substrates with lower energy yield (Reich et al., 2006). It

is apparent, therefore, that changes to photosynthetic rate of terrestrial ecosystems may alter terrestrial C dynamics, and therefore ecosystem C storage, by altering microbial activity.

To understand the C dynamics of terrestrial ecosystems in response to changing atmospheric chemistry, I measured the activity of enzymes responsible for the microbial degradation of cellulose in response to elevated CO_2 and O_3 concentrations to gain a basic ecological understanding of microbial metabolic responses. I also examined the impact that different plant communities have on microbial responses to elevated atmospheric CO_2 and O_3. Lastly, I explored the impacts of seasonality on enzyme activity, through periods of plant growth and leaf senescence, to pinpoint why enzymatic activity is changing in response to altered levels of CO_2 and O_3.

Methods

Study Site and Experimental Design

My research was conducted in the Rhinelander FACE (free-air CO_2 and O_3 enrichment) experiment, located 25 km west of Rhinelander, WI. Within the 32 ha site, twelve 30-m diameter treatment rings are placed 100 m apart. Within each ring, trembling aspen (*Populus tremuloides),* paper birch (*Betula papyrifera)* and sugar maple (*Acer saccharum)* were planted, at a 1-m by 1-m spacing. Ramets of aspen and seedlings of paper birch and sugar maple were planted in June, 1997. Aspen was planted in one half of each ring, whereas one quarter of the ring consists of aspen and birch and the remaining quarter consists of aspen and maple. Rings are grouped into three blocks, and factorial treatments of CO_2 and O_3 were randomly assigned within each block. CO_2 and O_3 treatments began in

May 1998 under ambient and elevated levels. The elevated CO_2 treatment is 560 μL L^{-1} and the elevated O_3 treatment is 54.5 nL L^{-1} (Dickson et al., 2000).

Field Sampling

Samples of the forest floor and mineral soil were collected from the Rhinelander FACE site in May, July and October 2007. Forest floor samples (n = 5) were collected from each treatment ring using 100 cm^2 template. Soil core samples, 2 cm in diameter and 5 cm in depth, were collected at depths of 0-5 cm and 5-10 cm. Three soil core samples were collected at each depth at five different locations within each ring section and were composited by ring section in the field. All samples were homogenized: forest floor samples were cut with scissors and mineral soil aggregates were broken up and samples were mixed by hand. Cores were frozen after sampling and stored at -20 °C until the initiation of enzymatic analysis.

Studies suggest that substrate availability induces the production of extracellular enzymes by the microbial community in soil (Paul and Clark 1996). As a result, the enzymes which degrade cellulose were measured in order to assess microbial metabolism of those substrates. I analyzed the activity of 1,4 β-D-glucosidase and cellobiohydrolase using methylumbelliferone (MUB) linked substrates (after Saiya-Cork et al., 2002). One gram of mineral soil sample and 0.5 g of leaf litter was placed into 125 ml of 50 mM sodium acetate buffer (pH 5.0) and mixed with a tissue homogenizer for one min (Polytron Devices Inc., Paterson, NJ). This soil solution was transferred into a 96-well microplate, with 8 replicates of each assay, including a blank, a negative control, a MUB standard and a quench standard. Quench control wells held 200 μl soil sample suspension and 50 μl reference standard. A

200 µl soil suspension and 50 µl substrate were combined in each assay. β-D-glucosidase and cellobiohydrolase were incubated for 2 h at 20 °C. Twenty-five µl of 20 mM NaOH were added to each assay at the end of the incubation period to end the enzymatic reactions. Using an f-Max fluorimeter (Molecular Devices Corp., Sunnydale, CA), fluorescence was determined at excitation energy of 355 nm and emission energy measured at 460nm. Enzyme activities were reported as nmole of substrate converted per gram per hour (nmol g^{-1} h^{-1}).

Statistical Analyses

Statistical analyses of enzyme activity were performed using a mixed-model ANOVA for a randomized complete block design. Within this model, block, CO_2 and O_3, species and time were treated as fixed effects. The appropriate expected mean squares were derived and applied to the denominator of each F-test in order to test main effects (CO_2 and O_3), split-plot effects (species), split-split plot effects (time) and their interactions. Significance for all statistical analyses was determined at $\alpha = 0.05$.

Results

Plant community, sampling date, and atmospheric CO_2 interacted to influence cellobiohydrolase activity in subsurface mineral soil (5-10 cm; $P = 0.066$), but these factors did not interact to significantly influence cellobiohydrolase activity in forest floor or surface mineral soil (0-5 cm). For example, in subsurface soil, cellobiohydrolase activity beneath aspen was 1.5 times greater under elevated CO_2 compared to ambient CO_2 on the May sampling date. In contrast, cellobiohydrolase activity beneath aspen-birch was 1.5 times

greater on the July sampling date under elevated CO_2, relative to ambient CO_2 ($P = 0.066$). β-glucosidase activity, however, was not influenced by an interaction between plant community, time and atmospheric CO_2 ($P = 0.725$).

In surface mineral soil (0-5 cm), β-glucosidase activity was influenced by an interaction between atmospheric CO_2 and O_3 (Fig. 1B), wherein under ambient O_3, β-glucosidase activity was 1.5 times greater under elevated CO_2, relative to ambient CO_2. However, under elevated O_3, β-glucosidase activity did not differ significantly between ambient and elevated CO_2 levels ($P = 0.061$, Fig. 1B). Carbon dioxide and O_3 did not interact to influence cellobiohydrolase activity in surface mineral soil ($P = 0.995$; Fig 1E). As main effects, atmospheric CO_2 and O_3 did not alter β-glucosidase or cellobiohydrolase activity; however, plant community did have a significant effect on cellobiohydrolase activity.

Cellobiohydrolase activity differed significantly between the two plant communities in surface mineral soil (0-5 cm; $P = 0.042$), whereas plant community had no significant influence on β-glucosidase activity ($P = 0.577$). Cellobiohydrolase activity in surface mineral soil (0-5 cm) was 1.3 times greater beneath aspen than beneath aspen-birch.

Vegetation and CO_2 interacted significantly to influence β-glucosidase activity in forest floor ($P = 0.015$), although this interaction had no significant effect on cellobiohydrolase activity ($P = 0.274$). In the aspen community, β-glucosidase activity was 1.2 times greater under ambient CO_2, compared to elevated CO_2. Whereas, in the aspen-birch community, there was no significant difference in β-glucosidase activity under ambient and elevated CO_2 concentrations (Fig. 2).

Vegetation and O_3 also interacted to influence both β-glucosidase and cellobiohydrolase activity in forest floor litter. In the aspen community, β-glucosidase

activity was 1.2 times greater under ambient O_3, relative to elevated O_3. However, in the aspen-birch community, O_3 had no influence on forest floor β-glucosidase activity (P = 0.074, Fig. 3A). Similarly, cellobiohydrolase activity beneath aspen was 1.4 times greater under ambient O_3 compared to the rate under elevated O_3, whereas O_3 had no effect on forest floor β-glucosidase activity in the aspen-birch community (P = 0.029, Fig. 3B).

Time had a significant main effect on both β-glucosidase ($P < 0.001$) and cellobiohydrolase activity ($P < 0.001$) in the forest floor. For example, β-glucosidase activity was greatest in May (6294 nmol h^{-1} g^{-1}), and steadily declined in July (5528 nmol h^{-1} g^{-1}) and October (4144 nmol h^{-1} g^{-1}). Similarly, cellobiohydrolase activity in forest floor litter was greatest in May (2075 nmol h^{-1} g^{-1}), decreasing in July (1395 nmol h^{-1} g^{-1}) and October (827.3 nmol h^{-1} g^{-1}; Fig. 4).

Discussion

The increasing atmospheric concentrations of CO_2 and O_3 resulting from human activity have the potential to alter ecosystem C dynamics by their influence on photosynthesis, plant production and microbial activity (Norby et al., 1992; King et al., 2001; Larson et al., 2002; Liu et al., 2005; Carney et al., 2007; Zak et al., 2007a). Because soil microorganisms play a key role in the cycling of C within ecosystems, it is necessary to understand how microbial activity is altered by higher concentrations of CO_2 and O_3 in order to grasp the impact of these trace gases on ecosystem function. In the Rhinelander FACE experiment, a moderately significant interaction between CO_2 and O_3 on the activity of cellulose-degrading enzymes was detected (Fig. 1B). There also was a significant interaction of each trace gas with plant community type, wherein 1,4 β,D-glucosidase and

cellobiohydrolase activity decreased in aspen community under elevated CO_2 and under elevated O_3 although no effect was observed in the aspen-birch community (Fig. 2, Fig. 3). This difference in plant community response contrasts with previous findings indicating plant communities respond in a similar fashion to elevated CO_2 and O_3 (Larson et al., 2002; Chung et al., 2005; Liu et al., 2005; Zak et al., 2007a). Moreover, the results from this study suggest enzyme activity varies on a temporal scale, as decreasing rates of cellulose degradation were noted over the growing season (Fig. 4). These findings indicate not only that elevated atmospheric O_3 and CO_2 can impact terrestrial C dynamics and future C storage, but these trace gases can generate responses dependent upon plant community composition.

The significant interaction of CO_2 and O_3 on the activity of cellulose-degrading enzyme 1,4 β-glucosidase in surface mineral soil (0-5cm; Fig. 1B) suggests that O_3 counteracts the stimulatory effects of elevated CO_2. Under ambient levels of O_3, there was greater enzymatic activity in the elevated CO_2 treatment, relative to ambient CO_2, suggesting microorganisms are stimulated by greater above- and belowground litter production under elevated CO_2 (King et al., 2001; Larson et al., 2002; Liu et al., 2005; Chung et al., 2005). Elevated CO_2 can increase above- and belowground litter production, wherein leaf litter production increased 50-100% in aspen and aspen-birch communities (King et al., 2001; Zak et al., 2007a) and fine root biomass doubled (King et al., 2001). Because cellulose is a major component of plant litter (Wilke et al., 1983), greater plant litter production under elevated CO_2 should increase cellulose availability, and hence metabolism, by microbial communities. However, under conditions of elevated O_3, rates of β-glucosidase activity were not significantly different between ambient and elevated CO_2. This observation implies that elevated O_3 concentrations could eliminate the stimulatory effects of elevated CO_2. Ample

evidence suggests that elevated O_3 decreases plant above- and belowground biomass (King et al., 2001; King et al., 2005; Zak et al., 2007a) by decreasing photosynthesis through damage to stomatal function, as well as lower leaf area and premature leaf senescence (Tjoelker et al., 1995; Westman 1979; Findlay and Jones 1990). As a result, the negative growth effects of elevated O_3 have the potential to offset the positive growth effects of CO_2, thereby eliminating increases in net primary productivity of forest communities exposed to elevated CO_2 (King et al., 2005).

Recent studies have concluded that there is no interaction between CO_2 level and plant community, and no change in leaf chemistry by elevated CO_2 (Finzi et al., 2001; Zak et al., 2007a). Within my study however, the differing rates of β-glucosidase activity in the forest floor between the aspen and aspen-birch communities suggest an interaction between vegetation and CO_2 on extracellular enzyme activity. There is no significant difference between β-glucosidase activity between ambient and elevated CO_2 treatments in the aspen-birch community. However, within the aspen community, a significant decrease in β-glucosidase activity under elevated CO_2 levels, relative to ambient CO_2 conditions is observed (Fig. 2). Because similar rates of extracellular enzyme activity beneath aspen-birch, under ambient and elevated CO_2 conditions, support the idea that leaf chemistry does not change, the decrease in β-glucosidase activity in the litter of the aspen community under elevated atmospheric CO_2 is surprising and not necessarily intuitive. As assays determine enzyme activity on a per-unit basis of sample, results from the forest floor (which is nearly pure organic matter) can assess the characteristics of the litter or the decomposing community. Therefore, three possible mechanisms can explain the decrease in β-glucosidase activity in the forest floor of the aspen community. The first is a decrease in the availability

of cellulose among shed leaves. As relatively little is known about age-related shifts in the leaf chemistry of long-lived perennials (Donaldson et al., 2006), elevated CO_2 may produce a change in the leaf chemistry apparent in the more mature aspen community, where no change was previously observed. A second possibility is a change in the microbial community composition that results in decreased ability to metabolize cellulose. Lastly, a decrease in β-glucosidase activity across CO_2 treatments could be explained by both a change in litter chemistry and a change in microbial composition that have occurred after ten years of fumigation. However, analysis of litter chemistry and microbial community composition is required before further conclusions can be drawn about changes in the more mature aspen community under elevated CO_2.

Similarly, a difference in plant community response to elevated O_3 is observed between aspen and aspen-birch within this experiment. This finding contrasts previous evidence that plant community response to elevated O_3 is similar (Larson et al., 2002; Chung et al., 2005; Zak et al., 2007a) and no consistent change to leaf chemistry exists (Liu et al., 2005; Zak et al., 2007a). While no significant difference was observed in the litter of aspen-birch communities between ambient and elevated O_3 treatments in β-glucosidase or cellobiohydrolase activity, rates of both cellulose-degrading enzymes significantly decreased under elevated O_3 in the aspen community (Fig. 3). Therefore, although there appears to be no change in litter chemistry, litter mass or microbial community composition in the aspen-birch community in response to elevated O_3, either plant litter chemistry, microbial community composition, or both, are changing within the aspen community to suppress the degradation of cellulose. This difference in response between aspen-birch and aspen may be explained by differences in plant sensitivity to O_3. Evidence suggests that birch is less

sensitive to O_3 than aspen, as photosynthesis, leaf area index and aboveground growth is decreased by O_3 in aspen, but not in birch (Karnosky et al., 2003). Additionally, the negative effects of O_3 to the aspen-birch communities appear to be decreasing as they mature, whereas the aspen communities appear to be maintaining the same degree of sensitivity (King et al., 2005). Therefore, the higher sensitivity of the aspen trees to O_3 may have elicited a response to the damage in the more mature community (whereas no change in community or chemistry was noted in younger communities) via a decrease in cellulose concentration or a change in microbial community composition. Clearly, further work would be required to test these alternatives.

Temporal variation in the enzymatic degradation of cellulose was also observed in my study because both β-glucosidase and cellobiohydrolase activity in the forest floor declined over the growing season. The activity of both enzymes was highest in May, followed by a significant decrease in July and again in October. Given evidence that microbial activity is directly influenced by air temperature and soil moisture (Zak et al., 1989; Brady and Weil 2002), one would expect that highest rates of enzymatic activity to occur during the wettest and warmest months, whereas the lowest activity should occur during periods of dry and cold. However, the highest enzyme activity I observed occurred in May when soil moisture was relatively low (21.9% g/g) and average air temperature was moderate (13.7 ^{o}C), whereas soil water (55.3% g/g) and air temperature (19.1 degrees C) were higher in July, followed by October which had a moderate soil water (46.7% g/g) and low air temperature (10.5 ^{o}C; Annual Climatological Summary 2008). According to the soil water and air temperature data, it is not intuitive that microbial activity would decrease with the growing season. One possible explanation for the temporal decline in activity is lower substrate availability in July

and October as leaf litter undergoes decay. Therefore, despite the warm and wet conditions of July, enzyme activity is lower than in May due to lower levels of cellulose in leaf litter. This not only demonstrates the importance of substrate availability on the control of microbial activity, but reinforces the argument that CO_2 and O_3, through their influences on photosynthesis and plant biomass production, will influence microbial metabolism and the cycling and storage of C.

In summary, the results of my study demonstrate a moderately significant interaction between CO_2 and O_3 on the activity of cellulose-degrading enzymes, such that under elevated O_3 the stimulatory effects of elevated CO_2 was mitigated. My observations also suggest that plant communities are responding differently to elevated CO_2 and elevated O_3, because extracellular enzyme activity in the aspen community decreased under both elevated CO_2 and elevated O_3 and there was no change in enzyme activity in the aspen-birch community. Possible causes for the change in enzyme activity within the aspen community is an alteration in leaf chemistry, a change in microbial community composition, or a combination of the two. However, further analysis of litter chemistry and composition of microbial communities must be completed before any definite conclusions can be drawn. As changes in plant community response and leaf chemistry have not been previously observed, it is possible that the age of a plant community is an important factor in understanding plant responses to changes in CO_2 or O_3 concentration. Community-level responses to CO_2 and O_3 may depend on plant species and their competitive interactions (Zak et al. 2007b). Forest community composition and age, through their effects on microbial metabolism, have the potential to alter ecosystem responses to elevated CO_2 and O_3. Understanding how microbial activity is influenced by plant community composition and ontogeny in an important step

toward understanding how ecosystem C dynamics will respond to rising concentrations of CO_2 and O_3 in the Earth's atmosphere.

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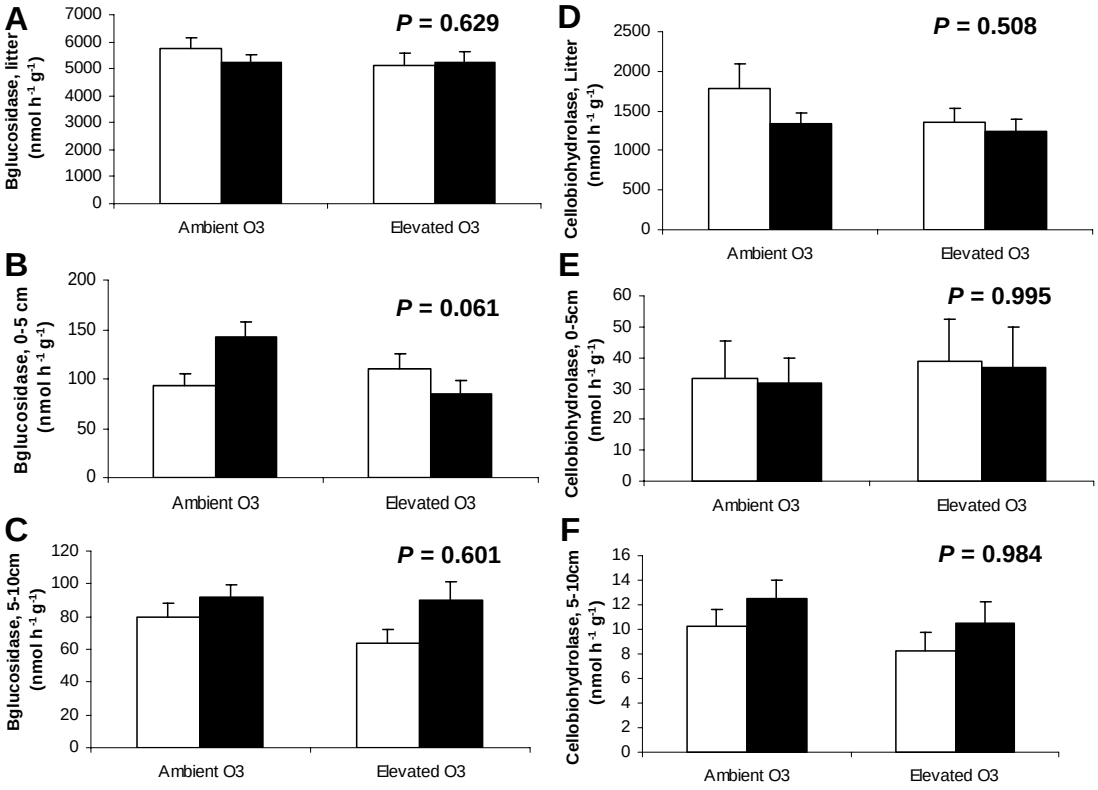

Figure 1. The interaction of CO_2 and O_3 on β-glucosidase and cellobiohydrolase activity in forest floor (A,D), surface mineral soil (0-5 cm; B,E) and subsurface mineral soil (5-10 cm; C,F). Values are means across sampling dates and species. White bars indicate ambient CO_2 and black bars indicate elevated CO_2. One standard error is indicated by the length of each error bar.

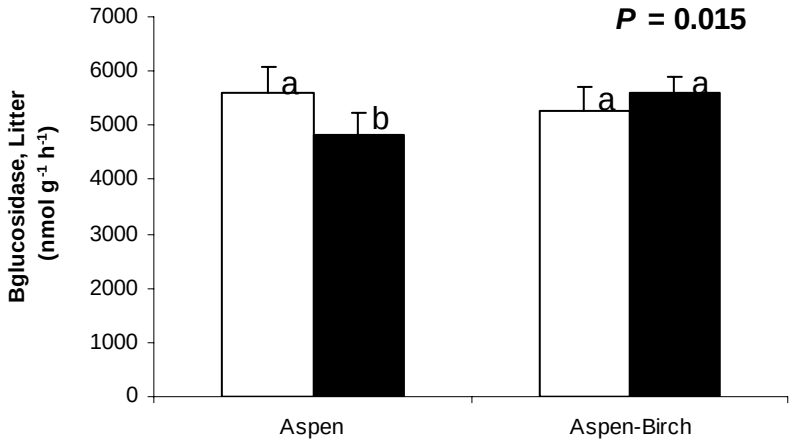

Figure 2. Mean forest floor β-glucosidase activity in aspen and aspen-birch communities growing under ambient and elevated CO_2. White bars indicate ambient CO_2 and black bars indicate elevated CO_2. One standard error is indicated by the length of each error bar. Means with the same letter are not significantly different ($\alpha = 0.05$).

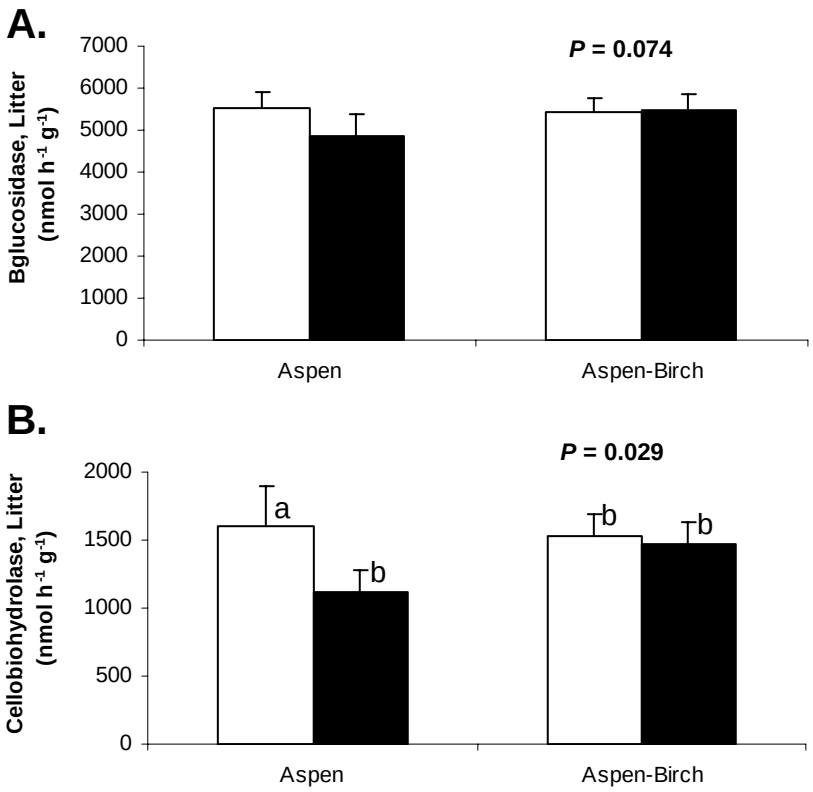

Figure 3. Mean β-glucosidase and cellobiohydrolase activity in forest floor beneath aspen and aspen-birch growing under ambient and elevated O_3. White bars indicate ambient O_3 and black bars indicate elevated O_3. One standard error is indicated by the length of each error bar. Means with the same letter are not significantly different ($\alpha = 0.05$).

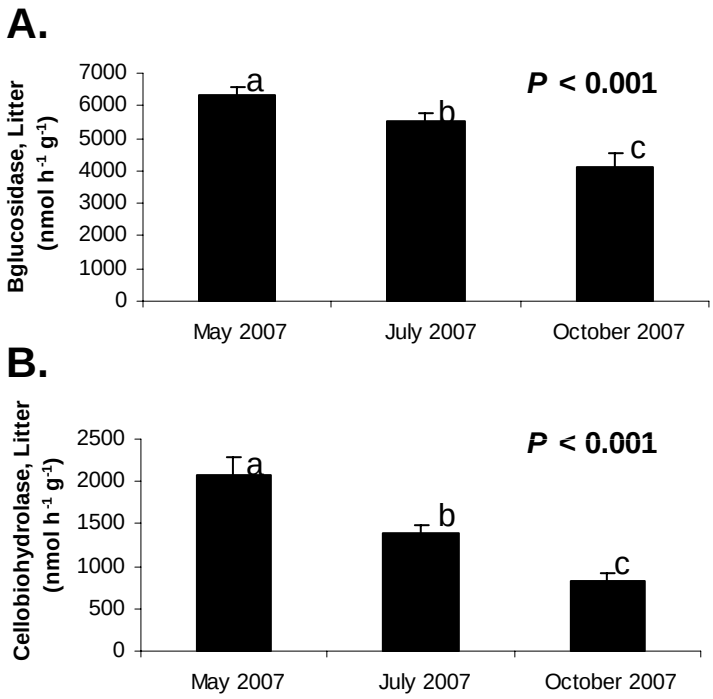

Figure 4. Mean β-glucosidase and cellobiohydrolase activity in forest floor for May, July and October sampling dates. One standard error is indicated by the length of each error bar. Means with the same letter are not significantly different ($\alpha = 0.05$).

Chapter 2: Conclusions

- The moderately significant interaction of CO_2 and O_3 on enzyme activity suggests that elevated O_3 has the potential to eliminate the stimulatory effects of elevated CO_2 on plant detritus production and hence substrate availability for microbial metabolism in soil.
- Plant communities are responding differently to elevated CO_2 and O_3. As changes in plant community response have not been previously observed, it is possible that both plant community composition and ontogeny are important to understanding plant responses to changes in CO_2 or O_3 concentration. Understanding how these factors influence microbial metabolism is an important step toward understanding how terrestrial systems are responding to rising concentrations of CO_2 and O_3.
- Microbial metabolism declines with the growing season, demonstrating the importance of substrate availability on the control of microbial activity and reinforcing the reasoning that CO_2 and O_3, through their influences on litter production, will influence microbial metabolism and the cycling of C in soil.