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Effects of Elevated Atmospheric CO_2 on Scrub-Oak Root Carbon Pools and Soil Microbial Processes

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EFFECTS OF ELEVATED ATMOSPHERIC CO_2 ON SCRUB-OAK ROOT CARBON POOLS AND SOIL MICROBIAL PROCESSES

by

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ABSTRACT

EFFECTS OF ELEVATED ATMOSPHERIC CO_2 ON SCRUB-OAK ROOT CARBON POOLS AND SOIL MICROBIAL PROCESSES

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The levels of atmospheric CO_2 are rising and this affects the growth of plants and the ecosystems in which they reside. Plants take up additional C from the atmosphere and have potential to sequester C in the soil. I investigated the sequestration of C belowground and the microbial processes that control C retention in the soil. This study was conducted in a Florida scrub-oak ecosystem, where CO_2 levels have been elevated to twice ambient since 1996 in open top chambers. There were eight replicates of ambient CO_2 chambers and eight replicates of twice-ambient CO_2 levels. The chambers were blocked according to the vegetation present at the beginning of the study and the site was burned prior to construction of the chambers. Soil cores were taken to investigate the effects of elevated CO_2 on soil biomass pools, microbial response and nutrient limitations. Elevated CO_2 did not affect total biomass of roots as of May 2002. There was less biomass of the smallest roots (< 0.25 mm) in elevated CO_2 in the top 10 cm. The C and N contents of root and organic matter pools reflected the trends in biomass. N concentration was lower for < 0.25 mm and 1-2 mm roots in the upper portion of the soil. Dissolved organic C and soil pH were unaffected in elevated CO_2. An oxygen biosensor system was used to examine microbial function in the scrub-oak soils. Microbial response was affected by CO_2 treatments. The soil microbial communities had greater N limitation

in elevated CO_2 than ambient CO_2, while the litter community was unaffected. The rhizosphere community had greater P limitation in elevated CO_2 than ambient CO_2. Substrates for the microbes derived from roots and litter grown in elevated CO_2 seemed to have more energy available to microbes, but this was dependent upon N conditions. Overall, there was greater nutrient limitation of microbial activity in elevated CO_2 than ambient CO_2, but the scrub-oak ecosystem was nutrient limited regardless of CO_2 conditions preventing full use of the potential C available for energy.

This dissertation is dedicated to all the women who overcame obstacles to walk this path before me and to my husband and father for their support.

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CHAPTER I

INTRODUCTION

Carbon dioxide, one of the greenhouse gases that may lead to an increase in global temperature, has been on the rise for the better part of two centuries (IPCC, 2001). This rise in the concentration of atmospheric CO_2 is linked to anthropogenic activities, including agriculture, burning of fossil fuels, cement manufacturing and deforestation. A large amount of carbon was removed from the rapid biogeochemical C cycle during the Carboniferous Epoch when organic matter was buried in sediment to be transformed slowly into fossil fuels(Kump et al., 2004). Industrial society has tapped into this C reservoir as an energy source and is rapidly translocating C from the long-term geological carbon cycle to the rapid, short-term biogeochemical cycle.

Rising atmospheric CO_2 concentrations

Atmospheric CO_2 concentrations before 1750 were estimated to be 280 ± 10 ppm (IPCC, 2001), but in following years concentrations increased by 100 ppm. NOAA Climate Monitoring and Diagnostics laboratory reported in their 2002-2003 summary that atmospheric CO_2 concentrations ranged from 373 ppm in the South Pole, Antarctica Observatory to 377 ppm at Barrow Alaska Observatory in 2003 (Conway et al., 2003), with the American Samoa Observatory (374 ppm) and Mauna Loa, Hawaii, Observatory (376 ppm) falling in that range. A more recent report gave a concentration of 380 ppm (Sabine et al., 2004). The majority of this increase can be pinpointed between 1980 and

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This dissertation follows the format of Plant and Soil.

the present. From 1980 – 1989, the rate of increase was 3.3 ± 0.1 Pg C / yr (Prentice et al., 2001). The rate decreased only slightly to 3.2 ± 0.1 Pg C/yr from 1990-1999 (Prentice et al., 2001).

Rates of CO_2 input into the atmosphere were estimated at 5.4 ± 0.3 Pg C / yr from 1980-1989 and 6.3 ± 0.4 Pg C / yr from 1990-1999 (Prentice et al., 2001) from burning of fossil fuels and cement manufacturing. Deforestation in the 1980's was estimated to have contributed 0.6 to 2.5 Pg C / yr (Prentice et al., 2001) to the atmosphere in the form of CO_2, but due to the difficulty of estimating deforestation contributions, there are no rates yet available for the 1990's. Less than half of CO_2 released into the atmosphere remains there, and consequently, atmospheric CO_2 concentration is less than what it could be due to human activities.

The Earth's oceans and terrestrial systems serve as sinks for anthropogenic CO_2. Carbon dioxide is dissolved into ocean water and utilized by phytoplankton and can be precipitated into the sediment as organic detritus, calcium carbonate or remain in solution. Oceans cover 71% of the Earth's surface and have potential to take up all of the carbon dioxide that humans have released into the atmosphere(Kump et al., 2004). However, because a majority of the ocean water cycles slowly, this would not be possible for several thousand years (Sabine et al., 2004). Currently, most of the CO_2 taken up by the ocean is confined to the thermocline, while only about 7% of anthropogenic CO_2 has made it to depths greater than 1500 m (Sabine et al., 2004). Between 1800-1994, oceans are estimated to have taken up 118 ± 19 Pg of anthropogenic C (Sabine et al., 2004). Some predict that as oceans dissolve more CO_2, the rate will decrease. Estimates support that theory, where between 1980 –1989 the ocean took up CO_2 at a rate of –1.9 ± 0.6 Pg

C / yr, but between 1990 – 1999 the rate was reduced to -1.7 ± 0.5 Pg C / yr (IPCC, 2001).

Terrestrial systems have also taken up a significant portion of CO_2 released into the atmosphere due to human activities. In the 1980's -0.2 ± 0.7 Pg C / yr were estimated to be taken up by terrestrial ecosystems, while in the 1990's -1.4 ± 0.7 Pg C / yr were estimated to be taken up (IPCC, 2001). The amount taken up by terrestrial systems did not exceed the amount released due to land use change. Overall terrestrial systems were a net source, but have potential to change from a source to a sink.

Climate change

Carbon dioxide is a greenhouse gas, which means its molecular structure allows it to trap long wave radiation that would normally pass through the atmosphere to space. This essentially heats the atmosphere to a temperature higher than would otherwise be expected. Greenhouse gases have allowed life-supporting conditions to come about earlier than otherwise possible because without their heat absorbing capacity, Earth would have been frozen until the solar radiation of the sun increased (Kasting, 1993). However, that was 4 bya, and, currently, levels of CO_2 are higher than they have been in the past 420,000 yrs and probably as far back as 20 million yrs (IPCC, 2001). The existing living organisms have all evolved in lower atmospheric CO_2 concentrations than current levels. As a greenhouse gas, CO_2 has potential to disrupt global climate by raising temperature, melting sea ice, which raises sea levels, increasing droughts, and slowing or halting the oceanic conveyor belt of warm water that helps distribute heat evenly over the planet. These dire predictions have led to an increased interest in the fate of CO_2 released

into the atmosphere. Interest has been focused on terrestrial systems because heterogeneity of terrestrial landmasses makes predictions and estimations of response to a rise in atmospheric CO_2 difficult. Many studies have been initiated in the last decade in a variety of ecosystems with the goal of predicting long-term reactions of terrestrial biomes to future levels of atmospheric CO_2.

Terrestrial system uptake of CO_2

In terrestrial systems there are two mechanisms for removal of CO_2 from the atmosphere. Carbon dioxide diffuses into precipitation forming carbonic acid, followed by the weathering of mineral rocks, which removes C from precipitation and forms mineral compounds that enter the long-term geological cycle (Siever, 1974). The other mechanism is uptake of CO_2 by plants and incorporation into biomass. This process and subsequent mineralization and microbial assimilation via decomposition are of great interest. These processes remove CO_2 from the atmosphere, thereby reducing the effect on climate. It is also the least predictable mechanism for CO_2 removal from the atmosphere, as plants are dependent upon climate, water availability, nutrient abundance and a myriad of other factors for growth. To further complicate ability to predict plant response, CO_2 can directly or indirectly affect these factors. For example, as a greenhouse gas, CO_2 can cause increased temperatures, perhaps allowing some plants to grow faster and take up greater amounts of CO_2. Or an increase in temperature may also lead to drought, killing plants and thereby reducing potential uptake of CO_2. In addition to predicting a plant's potential to take up CO_2, it is also necessary to predict how quickly plant-produced biomass will be decomposed and respired back into the atmosphere.

Elevated atmospheric CO_2 concentration has potential to alter plant production, but many plants are water limited and must balance water loss through stomates with intake of CO_2 (Bazzaz, 1990). In elevated atmospheric CO_2, stomates were opened for a shorter period of time in order to take up the same amount of CO_2 (Bazzaz, 1990; Eamus and Jarvis, 1989). Elevated atmospheric CO_2 increased plant water use efficiency (WUE) (Ceulemans and Mousseau, 1994). Plants increased biomass production because water limitation was eased and CO_2 was more readily available to be incorporated into biomass (Ceulemans and Mousseau, 1994).

However, one problem plants encounter in elevated atmospheric CO_2 is nutrient limitation. Most plants are limited by one or more nutrients, but this limitation may be more pronounced when water limitations are removed. This can cause changes in quality of biomass produced in elevated atmospheric CO_2, affecting decomposition and herbivory.

Once C has been removed from the atmosphere, for it to remain out of the atmosphere it must enter long-term soil C storage. There are three forms in which plant carbon can reach the soil; aboveground litter, root litter, and root exudates (Matamala and Schlesinger, 2000). Plants respond differently to various environmental conditions, allocating new carbon differently and this impacts microbial communities (Bernston and Bazzaz, 1996b). Some plants in high nutrient soils, may invest C in aboveground biomass, increasing future litter fall. Others, in low nutrient conditions, may invest C in root biomass to increase exploration of soil volume for exploitation of nutrient resources (Chapin et al., 1987; Matamala and Schlesinger, 2000). Still others may increase rhizosphere exudates to stimulate phosphate releasing bacteria (O'Neill, 1994). The

particular stresses of a plant will dictate investment of excess carbohydrates produced in elevated atmospheric CO_2. The form of the carbohydrate leaving the plant and moving into the soil and soil surface may dictate how microbial communities utilize it. Lower quality tissue (higher C:N) may decompose slower (Rastetter et al., 1992). Rhizosphere exudates may be lost from the system through percolation or incorporated into microbial biomass for short term cycling before being respired back to the atmosphere.

Microbial communities may be indirectly affected by elevated atmospheric CO_2 through plant responses. As plants exhibit greater NPP in elevated CO_2 than ambient CO_2, there may be an increase in quantity of growth substrate for microbes, which will have an effect on microbial community structure and cycling of C (Bernston and Bazzaz, 1996b). There can also be an altered quality of growth substrate influencing subsequent growth and community structure of dependent microbes. In addition to changes in growth substrates, there may also be changes in the soil environment that can affect microbial communities. Increases in plant WUE can increase soil moisture, causing increases in decomposition rates (Field et al., 1995). Plant exploitation of soil nutrients may leave microbial communities more nutrient limited, causing them to turn to recalcitrant humus with low C:N, and thereby reducing long-term C storage in the soil. On the other hand, microbes may become nutrient starved and decomposition may slow. Responses of the plant can change soil biogeochemical cycling, thereby changing microbial community function or composition. There are two control points between removal of CO_2 from the atmosphere and long-term storage in the soil. Plant uptake of CO_2 and incorporation of C into biomass is the first. The second is decomposition of biomass by soil microbial

communities. Studies of elevated atmospheric CO_2 on ecosystems investigate factors that influence those two control points to some degree.

Overview of elevated atmospheric CO_2 studies

Effects of elevated atmospheric CO_2 on plant growth have been studied for many years, but many early studies were done in greenhouse containers under optimal conditions. Response trends under those conditions were applicable to few ecosystems, so it was necessary to conduct studies under more realistic conditions. Now there are sufficient numbers of CO_2 studies on ecosystems that some short-term predictions can be made, but the long-term responses are varied and dependent on environmental conditions of individual ecosystems.

The earliest studies revealed immediate responses of plants at the leaf and cellular level. In a review of plant responses to elevated CO_2, Bazzaz (1990) stated that elevated CO_2 reduced or eliminated photorespiration, and reduced stomatal conductance, which increased plant water use efficiency. Greenhouse managers have long known increased CO_2 stimulated plant growth, but in the natural system, this stimulation may be limited by nutrients or water (Bazzaz, 1990).

Many studies have shown stimulation of photosynthesis at the molecular level has increased production of aboveground biomass (Delucia et al., 1999; Niklaus et al., 2001; Saxe et al., 1998). This increase in foliage has led to increased litter fall in several studies (Finzi et al., 2002; Niklaus et al., 2001; Schlesinger and Lichter, 2001). In systems where N limitation may have been present, increased C uptake changed foliage tissue quality by reduced N concentration (Cotrufo et al., 1998b; Curtis and Wang, 1998; Körner and

Arnone, 1992; Owensby et al., 1993) or increased C:N ratio (Cotrufo et al., 1994; Niklaus et al., 1998; Rouhier et al., 1994). Some studies have shown the C:N ratio was altered before leaf senescence so it was no longer different from leaf litter grown in ambient CO_2 (Hall et al., 2005a; Hall et al., 2005b; Hirschel et al., 1997), while others have shown that high C:N remains and slows decomposition (Cotrufo et al., 1994).

Belowground production of plants is stimulated in elevated CO_2. Root biomass was enhanced by elevated CO_2 in many studies (Bernston and Bazzaz, 1996a; Jongen et al., 1995; King et al., 2001; Lipson et al., 2005; Matamala and Schlesinger, 2000; O'Neill, 1994; Wiemken et al., 2001). Also, many researchers predicted increased root exudation that consequently affected microbial communities in elevated atmospheric CO_2 (Allen et al., 2000; Bazzaz, 1990; Paterson et al., 1997; Pritchard and Rogers, 2000). Others have found stimulation of rhizodeposition in elevated atmospheric CO_2 (Norby, 1994; Paterson et al., 1996; Zak et al., 1993). Canadell et al. (1996) and Cheng and Johnson (1998) found a 60% increase in root-exuded carbon in elevated CO_2. Similar results were obtained in a mixed grass experiment, where elevated CO_2 caused a 56% increase in root respiration, turnover and exudation (Hungate et al., 1997). These increases in carbon allocation were also predicted to increase carbon loss from the soil, leaving roots unchanged by elevated CO_2 (Higgins et al., 2002; Körner and Arnone, 1992). The increases in surface litter, root biomass and rhizodeposition did not always lead to increased soil C. Soil C pools have been affected in various ways by treatment of plant ecosystems with elevated CO_2. Soil C or organic matter increased in some systems (Billes et al., 1993; Gorissen, 1996; Ineson et al., 1996; Schlesinger and Lichter, 2001; Williams et al., 2000), decreased in others (Cardon et al., 2001; Heath et al., 2005), or

remained unchanged (Billings and Ziegler, 2005; King et al., 2001; Niklaus et al., 1998; Niklaus et al., 2001; Rice et al., 1994).

The amount of C input into soil was not the only important CO_2 induced change in soil systems; the quality of leaf litter and roots can alter soil C and N content as well as microbial C and N content. There has been less exploration of the C:N ratio of roots, but responses have varied with reduced N concentration (Cotrufo et al., 1998a; Janssens et al., 1998), as well as increased C:N ratio (Jongen et al., 1995), while others have found C:N ratio unaffected (Matamala and Schlesinger, 2000; Rouhier et al., 1994). A greater soil C:N ratio was found in ponderosa pine in elevated CO_2 than ambient CO_2, as well as lower N concentration (Johnson et al., 2000). Hu et al. (2001) also found lower soil N in California grasslands, whereas Williams et al. (2000) found greater soil N in tall grass prairie in elevated CO_2 than ambient CO_2.

Soil microbial communities are unlikely to be directly affected by increased atmospheric CO_2, but they will be indirectly affected through changes in quantity and quality of organic energy sources and through changes in competition for nutrients. Microbial biomass increased in some ecosystems in elevated CO_2 treatment (Diaz et al., 1993; Hu et al., 2001; Williams et al., 2000; Zak et al., 1993), while others exhibited no change in microbial biomass (Billings and Ziegler, 2005; Niklaus et al., 2001; Schortemeyer et al., 1996; Wiemken et al., 2001). Microbial C:N ratio and N were also affected by elevated atmospheric CO_2. Lower microbial C:N ratio (Niklaus et al., 1998) and greater microbial N (Williams et al., 2000) were found. Also higher C:N (Hu et al., 2001) and lower microbial N (Hungate et al., 1996a), and in some cases no changes (Allen et al., 2000), have been observed.

Elevated CO_2 causes a variety of changes in microbial processes, leading to variations in the C cycle. In a review, Zak et al. (2000) found responses of soil microbes were greatly variable in every aspect studied. Responses of the soil system are dependent on soil moisture, climate, pH, soil types and so on.

Florida scrub-oak

A Florida scrub-oak ecosystem was chosen for the current study because it has a mature nutrient cycle and experiences nutrient and water limitations. Which limitation has the greatest effect and can elevated atmospheric CO_2 stimulate plants under these limiting conditions? In this system, the response of plants to elevated atmospheric CO_2 under the combined effect of these limitations can be observed. This system is also fire maintained, which presents the opportunity to predict the effects of elevated atmospheric CO_2 on a system depending on belowground C storage for regeneration. Could these plants potentially store excess C in roots and then regenerate faster in elevated atmospheric CO_2 following fire?

Study site description

The experimental site was located on Merritt Island at Kennedy Space Center on the eastern coast of Florida, USA (28°38'N-80°42'W at an elevation of 0-3 m above sea level). There were two soil types on the site; Paolo sand and Pomello sand. The Paolo sand (Spodic Quartzipsamments) is a matrix with rapid permeability, low water capacity, low natural fertility, low amounts of organic matter, and strong to medium acidity (Huckle et al., 1974). The parent materials were thick beds of eolian sand (Schmalzer and

Hinkle, 1990). The Pomello sand (Arenic haplo humods) is a matrix with layered permeability, low available water, low natural fertility, low amounts of organic matter, and strong to very strong acidity (Huckle et al., 1974). The parent materials were thick beds of marine sand (Schmalzer and Hinkle, 1990). The acidic pH of the soils, which was 3.75 to 4 in the top 15 cm and 4.25 from 16 to 30 cm deep (Schmalzer and Hinkle, 1991), has potential to limit some nutrients in the system. Possible limitations at pH below 4 are N, P, S, B, C, Mg, Fe, Ca, and Mo (Taiz and Zeiger, 1998). The climate is subtropical, with the wet season occurring from May to October. The coldest month is January with an average daily temperature of 15.72 °C and a low of 11.8°C (Huckle et al., 1974; Mailander, 1990). The warmest month is August with an average daily temperature of 27.61 °C and a high of 32.6°C (Huckle et al., 1974; Mailander, 1990). The mean annual rainfall for Merritt Island is 131 cm, but can range from 77.5 to 217.7 cm annually (Mailander, 1990).The plant community is made up of 76% *Quercus myrtifolia*, 15% *Q. geminata*, 7% *Q. chapmannii, Serenoa repense*, and *Lyonia ferreginea.* The dominant oaks are C_3 and clonal. At the initiation of the study, the community was ten years post burn.

The scrub-oak was burned in February of 1996 and again prior to the construction of experimental chambers. According to previous studies, the scrub-oak system takes longer than three years to recover to pre-burn vegetation. In other previously burned sites on Merritt Island, pH increased between 6 and 12 months post-burn (Schmalzer and Hinkle, 1991). For the first 18 months post-burn, P, K, Mg, Na, and Fe decreased, but returned to pre-burn levels (Schmalzer and Hinkle, 1991). In contrast, Al and Cu increased for 18 months post-burn, but returned to pre-burn levels (Schmalzer and

Hinkle, 1991). There was also a delayed increase in nitrate and ammonium, which extended 24 months post-burn (Schmalzer and Hinkle, 1991). While this fire was deliberately set and maintained, central Florida has one of the highest frequencies of thunderstorms in the world, which may contribute to the natural unsuppressed fire regime (Schmalzer and Hinkle, 1991).

Experimental design for study

The treatments were manipulated in open topped chambers (OTC's) (Drake et al., 1989). The OTC's were octagonal, with sides of 139.9 cm, a diameter of 356.6 cm, and a height of 365 cm. There were eight chambers with elevated (2x ambient) CO_2 air blown in, eight chambers with ambient air blown in, and eight chamberless reference plots. Treatment was initiated on May 14, 1996. The type of pre-burn vegetation determined the chamber sites. Blocks were designated according to similarity of pre-burn vegetation composition (Figure 1). Minirhizotron tubes were inserted into the soil at this time. Minirhizotrons are clear tubes constructed of cellulose acetate butyrate. They allow the periodic measurement of root length in a non-destructive way with the use of a specially designed camera. Major areas of investigation by a team of researchers included monitoring photosynthetic rates, community evapotranspiration, community structure for plants and insects, allocation of biomass, and accumulation of carbon.

Effects of elevated CO_2 on Florida scrub-oak

The open topped chambers erected over the Florida scrub-oak were continuously exposed to ambient and twice ambient CO_2 for over ten years. The most immediate effects of

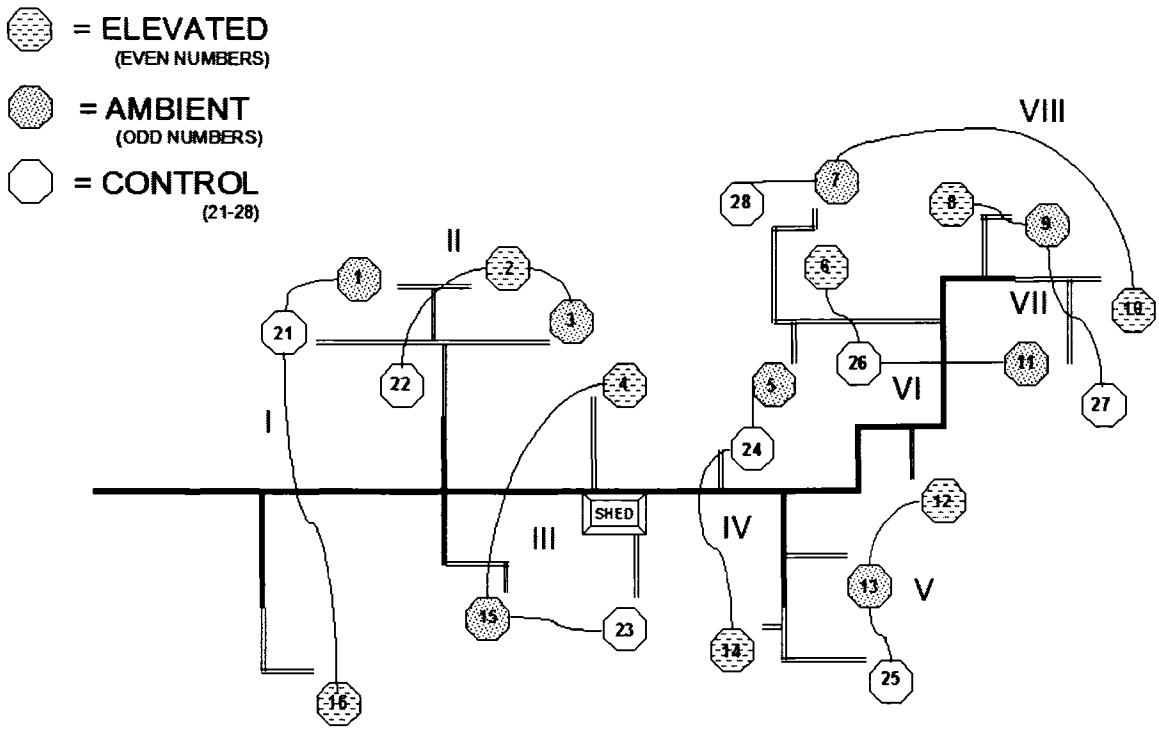

Figure 1. Site map of open top chambers in the Florida scrub-oak. Roman numerals represent the blocking of the chambers according to the vegetation present before construction of the chambers.

elevated CO_2 on the scrub-oak were observed in the photosynthetic rates and aboveground biomass. Elevated CO_2 stimulated photosynthetic rates of *Quercus geminata* by 73% and *Quercus mytifolia* by 51% for the period of July 23 to August 22, 1996 (Li et al., 1999). In this same period, there was significantly greater accumulation of starches (264%), and sugars (54%) in elevated CO_2 than ambient CO_2 for *Q. myrtifolia*, but not *Q. geminata* and reduction of Rubisco activity by 40% in *Q. myrtifolia*, but not *Q. geminata* (Li et al., 1999). The increase in photosynthesis led to a relative increase in aboveground biomass in elevated CO_2 chambers of 44% (May 1996 to January 1997) 55% (January 1997 to January 1998), 66% (January 1998 to January 1999) and 75%

(January 1999 to January 2000) (Dijkstra et al., 2002). By 2000, however, the responses were species specific, where *Quercus myrtifolia* had significantly greater biomass in elevated CO_2 than ambient CO_2, *Quercus chapmannii* tended to have greater biomass in elevated CO_2 and *Quercus geminata* was unaffected in elevated CO_2 (Dijkstra et al., 2002). From February to June of 2000, measurements showed leaf area index in elevated CO_2 was consistently higher, ranging from 20% (winter) to 55% (summer) (Hymus et al., 2002). By April of 2001, elevated CO_2 caused a 138% increase in aboveground biomass, a 31% increase in the O horizon accumulation, 82% increase in aboveground C and a significantly greater net increase in ecosystem C (Johnson et al., 2003).

As is often found for leaf litter chemistry, a decline in overall N and increase in C:N ratio of live leaf tissue was shown in elevated CO_2 (Hall et al., 2005b). Elevated CO_2 also caused a decline in herbivory of live leaf tissue by some insect groups (Hall et al., 2005b). Leaf litter contained higher condensed tannins (Hall et al., 2005a), reduced cellulose and hemicellulose concentrations in elevated CO_2 (Hall et al., 2006). Carbon dioxide did not affect the concentration of N, C:N ratio, lignin or phenolics in leaf litter (Hall et al., 2006). Despite greater accumulation in the O horizon in elevated CO_2 after seven years of exposure (Johnson et al., 2003), leaf litter fall declined in 2000 – 2002 (Hungate et al., 2006). Carbon dioxide history of leaf litter did not affect decomposition outside the chambers; however, in elevated CO_2, decomposition rates increased between 18-36 months of exposure (from November 2003 to May 2005) (Hall et al., 2006).

Elevated CO_2 altered N cycling and several other nutrients in the scrub-oak. A nitrogen fixing plant, *Galactia elliotti,* was stimulated in elevated CO_2 and the rate of N mineralization was reduced, leading to a lower rate of nitrate recovery in the first 14

months of the study (Hungate et al., 1999). However, in the 2001-2002 year, elevated CO_2 depressed N fixation by *Galactia elliotti* (Hungate et al., 2004), where it had previously stimulated fixation. This was perhaps due to increased shading or limitation of Mo or Fe (Hungate et al., 2004). There was a significant decrease in P and N concentrations in aboveground tissues, but the total biomass content for these two elements was greater in elevated CO_2 (Johnson et al., 2003). The plant accumulation of N had initially been stimulated by elevated CO_2, but in years 4 – 7, the response declined (Hungate et al., 2006). Investigations into scrub-oak roots showed significantly decreased C:N ratio (Schortemeyer et al., 2000). Belowground there was no change in N in microbial biomass in the first 14 months (Hungate et al., 1999). There was, however, an increase in the specific rate of ammonia immobilization (Hungate et al., 1999) and a significant decrease in the nitrate leaching rate for 1996-1997, but this decrease was not evident later in the study (Johnson et al., 2001a). The leaching rates of NH_4^+ and NO_3+NH_4^+ were not affected, but the available soil P was significantly reduced in elevated CO_2 (Johnson et al., 2001a). As the study progressed, a lower level of available N, Zn and extractable P was shown in the soils in elevated CO_2 (Johnson et al., 2003). It was suggested that declines later in the study were due to progressive N limitation (Hungate et al., 2006), where N becomes unavailable as it is incorporated into live plant tissue. The soil microbes and plants begin to suffer from greater N limitation in elevated CO_2.

Elevated CO_2 initially stimulated belowground standing biomass and turnover, but these effects disappeared later in the study. By December of 1997, root length density (mm/cm^2) monitored by minirhizotron tubes was significantly greater in elevated

CO_2 (21.36 mm/cm^2) than ambient CO_2 (7.53 mm/cm^2) (Dilustro et al., 2002). Root production, mortality, and turnover significantly increased in elevated CO_2 during the initial part of the study (Dilustro et al., 2002). Root decomposition was not altered by elevated CO_2 (Dilustro et al., 2001). The root system reached closure in the third year of the study and there was no longer a significant CO_2 effect on root length density from year 3 of the study to year 7 (Day et al., 2006).

Soil C and soil moisture were both affected by elevated CO_2. Evapotranspiration was reduced in elevated CO_2 from January 1998 to August 1998 despite the increased biomass and leaf area index, and consequently, there was an increase in surface soil water (Hungate et al., 2002). Soil C seemed to vary both temporally and spatially. After a one-year period, less soil C accumulated in buried soil bags in the elevated CO_2 soil than ambient (Schortemeyer et al., 2000). There was a significantly higher amount of soluble C in the rhizosphere soil in elevated CO_2 for the soil sampled on July 9, 1998, but after heavy rains, this higher concentration had moved into the bulk soil when sampled on July 23, 1998 (Schortemeyer et al., 2000). Johnson et al. (2003) found no significant effects of CO_2 on soil C and in the most recent measurement from soil cores taken in 2002, no effect on soil C was found (Pat Megonigal P. C.).

Soil microbes, especially fungal components, were affected by elevated CO_2. In soil samples taken on July 9, and July 23, 1998, rhizosphere activity was depressed during the first sampling period, but unaffected during the second (Schortemeyer et al., 2000). Rhizosphere microbial C and N and bacterial numbers were unaffected by CO_2 treatment during these sampling dates (Schortemeyer et al., 2000). For soil samples taken from June 1998 to May 2000, there was greater ectomycorrhizal colonization frequency

in elevated CO_2 than ambient, but this did not increase ectomycorrhizal mass (Langley et al., 2003). In 2000, an increased level of ergosterol, an indicator of fungal biomass, was detected in bulk soil and root fractions (Klamer et al., 2002). Using DNA as an indicator, a shift in composition of Basidiomycetes was detected in the rhizosphere soil, but not the bulk soil, while species richness was unaffected (Klamer et al., 2002). From soil cores taken in 2002, microbial respiration in the 0-10 cm fraction was 7-19% lower in elevated CO_2, but this was not significant (Pat Megonigal P. C.).

Objectives of current study

The overall goal of this work was to increase the current understanding of conditions imposed on microbial communities by plant reactions to atmospheric CO_2, and conditions affecting microbial community utilization of dead plant organic matter for energy.

The first objective was to increase knowledge about deposition of organic matter into the soil. This included estimates of root biomass, course soil organic matter and dissolved soil carbon. This, with information gathered by other researchers, should give a clearer picture of the organic matter available as an energy source for microbes and the potential for long-term carbon storage. In chapter 2, methods for investigating root biomass and soil organic matter are described and results are shown.

The second objective was to investigate plant derived changes in the belowground biogeochemical pools of C, N, and P. This included the C:N ratio of root tissue and organic matter. These potential changes are discussed in chapter 2. Elevated atmospheric CO_2 may have altered the P dynamics of the system. Increased deposition into the rhizosphere may alter the pH of soils. In elevated CO_2, microbes may follow plant trends

and increase NUE (nitrogen use efficiency) and exhibit higher C:N. Progressive nitrogen limitation may affect microbial communities in elevated atmospheric CO_2. Knowledge about the change in biogeochemical pools is imperative for understanding microbial utilization of organic energy sources. Changes in the microbial environment are presented in chapter 5.

The third objective was to test microbial community nutrient limitations. Nutrient limitations can have an impact on microbial growth, utilization of organic matter for energy and recycling nutrients for the ecosystem. If the microbial communities are unable to function because of progressive nutrient limitation, the ecosystem's nutrient cycling and production could slow as nutrients are locked away in biomass that is sluggishly being decomposed. Or, nutrient limitation of microbes may allow a build up of organic matter in the soil, leading to long term C storage. In another scenario, the microbes may begin to break down C already in the recalcitrant soil pool because of its low C:N ratio, reducing the current level of C storage and increasing atmospheric CO_2 levels. Investigating changes in microbial community function as a result of the changes to environment, nutrients and energy sources is the final step in predicting how microbial communities might respond in the long term as decomposers in elevated atmospheric CO_2. Effects of elevated CO_2 on microbial nutrient limitation and function are discussed in chapter 5.

Hypotheses

It was hypothesized that stimulated biomass production in elevated CO_2 would lead to greater coarse soil organic matter mass, C and N content. The percentage of C and N, and

C:N ratio in the coarse soil organic matter would not be affected by elevated CO_2. It was believed there would be greater production of root biomass in elevated CO_2 and this would lead to greater amounts of dead root mass. The C and N content should reflect the biomass and dead root mass. The C percent would not be altered in the live roots, but the percent N would be less, causing lower C:N ratio. The dead roots would not have a different percent C or N or a different C:N ratio. Soil cores were taken to collect the root material and organic matter necessary to test these hypotheses and the results are presented in chapter 2.

Over the course of the study, it was hypothesized that there had been a shift towards larger roots in elevated CO_2 for greater C storage. This hypothesis was addressed in chapter 3 using the minirhizotron data collected over the course of the full study.

It was hypothesized plants would increase rhizodeposition to increase nutrient release from the soil. This would increase the dissolved organic C and change the pH of the rhizosphere soil, but not the bulk soil. The increased plant litter and increased rhizodeposition would lead to greater microbial biomass, C, N, and activity in elevated CO_2. The reduced quality of roots as an energy and nutrient source for microbial activity would lead to energy and nutrient limitations of the microbes. Progressive N limitation of the system would cause greater N limitation of the microbial communities. Stimulation of rhizodeposition would increase P availability in the rhizosphere in elevated CO_2. Microbial communities would adapt to these limitations and acclimate to the substrates from their CO_2 history. Chapter 4 demonstrates the techniques used to investigate the nutrient limitations and functions of microbial communities, without the influence of

CO_2, Following the background investigation, the above hypotheses concerning the effect of elevated CO_2 on microbes were tested and the results are presented in chapter 5.

CHAPTER II

ROOT BIOMASS AND NUTRIENT POOLS

Introduction

Root biomass is often stimulated by elevated CO_2 (Bernston and Bazzaz, 1996a; Jongen et al., 1995; Lipson et al., 2005; Matamala and Schlesinger, 2000; O'Neill, 1994; Wiemken et al., 2001). This can be driven by a greater need for nutrients to support more biomass (Chapin et al., 1987), causing plants in elevated CO_2 to invest more carbon into root systems for further exploitation of the soil (Matamala and Schlesinger, 2000). Carbon invested into the root system has a more direct route for entering long-term C storage in the soil than C deposited at the soil surface as leaf and stem litter. Stimulation of plant activity by elevated CO_2 may lead to increased C storage in the soil, increasing sink potential (Canadell et al., 1996) and having a negative feedback on potential influence of elevated CO_2 on climate. Many studies on elevated CO_2 have included the root system to some extent in observing effects of elevated CO_2 on plant ecosystems, but detailed information on the root system is often scarce. When roots were sampled for effects of elevated CO_2, they were often treated as a single unit, or at best differentiated by coarse versus fine roots and rarely by live or dead. Similar to differing functions of leaves and stems, fine and coarse roots have differing functions of nutrient/water collection and support/storage respectively. Lumping roots together may be a mistake when interpreting plant response. Also, sampling often has not differentiated effects by depth. In this study the energy values and nutrient content (C, N, P) were explored over a range of size classes of live roots and differences among live roots, dead roots, and soil organic matter. The biomass and C and N composition of roots were quantified over a

depth range of one meter. The hypotheses that elevated CO_2 would increase coarse root biomass and decrease tissue quality (i.e. raises C:N and C:P) but increase energy value (i. e. calories / g) of live root biomass, while dead root tissue would be unaffected by CO_2 treatment were addressed. Fine roots in this study observed with minirhizotrons were initially stimulated by CO_2 (Dilustro et al., 2002), but this stimulation decreased in later years as the root system reached closure (Day et al., 2006). The minirhizotron technique only sampled fine roots and was a measure of root length, not biomass. It was suspected there might be additional C storage in larger roots not observed by the minirhizotrons. A plant in elevated CO_2 may have more C to store in larger roots, providing an advantage after a burn. In addition to exploring differences in biomass, nutrient dynamics and energy values of the root system, evidence of progressive N limitation was examined.

Methods

Root extraction and processing

In the spring of 2002, five points were randomly located along five radiating transects in each chamber from which 7 cm diameter cores were removed in 10 cm increments to a meter depth. Larger roots were removed from the soil with a 1 mm mesh sieve, and the sieved soil was divided among several researchers for different analyses. A known percentage was kept in order to pick the remaining fine roots and extrapolate to the full core. The sieved roots were dried for two hours at 70° C to remove excess moisture and refrigerated at 4° C to prevent decay until further processing. The roots were sorted by hand into live root size classes of < 0.25 mm, 0.25 – 1 mm, 1 – 2 mm, 2 mm – 1 cm, > 1 cm, dead roots, and unidentifiable organic matter.

All seven classes were dried at 70° C for 48 hr and weighed. Because the root samples were not washed, a correction value for clinging soil was obtained using the methods described by Janzen et al. (2002). Briefly, a subsample was washed and analyzed separately for C content and compared to unwashed samples. T These numbers were then used to obtain conversion values that were applied to the 0.25 – 1 mm, 1 – 2 mm, 2 mm – 1 cm, and > 1 cm root size classes, correcting for adhering minerals. This correction value was 97%, which was multiplied by the weight of the unclean roots to obtain a corrected weight. Organic matter, dead roots and < 0.25 mm roots were corrected for sand contamination using the percentage of sand remaining after the samples were combusted in a calorimeter (see below). Organic matter was corrected for 23.18% sand content and dead roots were corrected for 5.82% sand content. The finest root class (< 0.25 mm) corrections were more complex. The fine roots from the top 10 cm were corrected using a 32.82% sand content from bomb calorimetry, while the < 0.25 mm roots from the 11-100 cm depths were corrected using the 3% correction from the Janzen et al. (2002) method. This seemingly high percent is due to sand clinging to the fine roots and mycorrhizal filaments in the top 10 cm. These especially fine roots and mycorrhizae were not present in lower depths. The < 0.25 mm roots were also removed from the soil portion saved from the sieved material. The miniscule roots were removed from a subset of chambers using a dissecting microscope. These fine roots were dried and weighed to approximate the root biomass underestimated by sieving. These estimates were extrapolated back to the full core and added to the < 0.25 mm root size class to get a total estimate of biomass.

Calorimetric analysis

The root samples from the five cores from each chamber were combined into depth categories (0-10 cm, 11-30 cm, 31-60 cm, and 61-100 cm) and ground. Approximately 0.75 g of roots from the 0-10 cm depth category from each chamber was mixed with DI water to make a paste, which was pressed into a pellet, dried at 70° C for 48 hrs, and weighed. The pellet was combusted under pressurized pure O_2 in a 1341 Plain Jacket Bomb Calorimeter (Parr Instrument Co.). The residual sand and ash were subtracted from the initial mass of the pellet and the caloric value of the roots was determined on an ash free basis.

C and N analysis of roots and organic matter

Once root biomass was obtained from the cores, the roots were combined into depth classes of 0-10, 11-30, 31-60, 61-100 cm and then ground and analyzed for C and N by Dumas combustion (NC 2100; CE Elantech, Lakewood, New Jersey, USA), followed by continuous flow isotope ratio mass spectrometry ($DELTA^{plus}$ –XL; Themoelectron Corporation, Bremen, Germany) at the Colorado Plateau Stable Isotope Laboratory. The percentages were applied to the biomass measurements to calculate C and N standing pools in the root system.

P analysis of roots and organic matter

Half a gram of dried roots from the top ten cm of soil taken in 2002 was measured into a crucible and ashed in a Thermoclyne muffle furnace at 500° C for 6 hrs. The cooled ash was suspended in 1 ml of concentrated H_2SO_4. Using phenolphthalein indicator, 50 µL of

H_2SO_4 suspension was neutralized with 2 N NaOH solution. The neutralized solution was brought to a 10 ml volume with deionized distilled H_2O and divided into two 5 ml portions. The control portion was acidified with 1 ml 5 N H_2SO_4. The phosphorus in the sample portion was assayed by adding 1 ml of absorbic acid reagent (50 ml of 5 N H_2SO_4, 5 ml pottasium antimonyl tartrate solution, 15 ml of ammonium molybdate solution, and 30 ml of 0.01 M absorbic acid). The color was allowed to develop in the same period of time as a standard curve (more than 10 minutes and less than 30 minutes) before measurement on a spectrophotometer at 880 nm. The absorbance of the control sample was subtracted from the sample absorbance before conversion to P concentration. This adjusted absorbance was compared to a standard curve and converted back to ug P g^{-1} material.

Statistical analyses

A split-plot MANOVA was used to analyze root biomass. Vegetation composition at the initiation of the study in 1996 was used to determine the block, and one of the two chambers within each vegetation block was assigned as elevated CO_2 or ambient CO_2. The different size classes represented the multiple response variables. ANOVAs were used as a follow up for significant MANOVA results and the Least Square (LS) means procedure was used to interpret significant ANOVA interactions using SAS (SAS Institute 1990). Depth was not a factor in the calorimetric or phosphorus data sets, so the data were analyzed using a MANOVA.

Results

Root biomass and caloric values

There was no significant CO_2 effect on root biomass ($P = 0.2665$) overall (Figure 2), but there was a significant depth effect and CO_2 by depth interaction ($P < 0.0001$). ANOVAs of all the root size classes and organic matter showed a significant CO_2 by depth interaction ($P < 0.0001$) in the smallest size class (< 0.25 mm) and a non-significant CO_2 by depth interaction ($P = 0.067$) for the dead roots. All other classes were not significant, with a range of P values between 0.24 and 0.99. The LS means analysis showed in the 0-10 cm depth increment, elevated CO_2 plants have significantly less biomass (498 g m^{-2}) invested in < 0.25 mm roots than ambient CO_2 plants (737 g m^{-2}) (Figure 3a). There was no significant difference at any other depth. Fine roots picked from the sieved soil were less than 1 g m^{-2} to a meter depth. Since it was a subsample, there were not enough replicates to run a separate statistical analysis. However, the means reflected the patterns seen in the total biomass for this category with the ambient CO_2 treatment having 0.91 g m^{-2} and the elevated having 0.77 g m^{-2}. Due to the difficulty of recovering very fine roots in the sieved material, this portion is neglected in belowground biomass studies. This sample constituted 0.014 % of the root biomass in the ambient treatment and 0.012 % in the elevated treatment. This amount was negligible and did not affect the validity of future studies at this site if not recovered.

Dead roots followed the same pattern of biomass distribution as the < 0.25 mm roots, with decreased amount of biomass in the 0-10 cm depth increment for the elevated CO_2 treated plants (Figure 3b). There was no significant effect of elevated CO_2 on the

caloric value (calories g^{-1}) of live roots, dead roots and organic matter in the top 10 cm of soil (Table 1). Calories g^{-1} decreased as root size increased.

Table 1. Calories per g of roots and organic matter from the top 10 cm of soil.

CO_2 Levels	Ambient	Elevated
<0.25 mm roots	5209 ± 71	5105 ± 94
0.25-1 mm roots	5001 ± 68	4947 ± 35
1-2 mm roots	4817 ± 47	4879 ± 50
2-10 mm roots	4613 ± 32	4589 ± 36
Dead roots	4797 ± 97	4920 ± 77
Organic matter	5341 ± 90	5316 ± 73

C and N concentration

Similar to biomass, there were no elevated CO_2 effects on C concentration (g C / g material) ($P = 0.067$) or N concentration (g N / g material) ($P = 0.238$) at the MANOVA level, but there was a significant CO_2 by depth interaction for N concentration ($P = 0.0027$). An ANOVA showed there was a significantly lower percent N in the < 0.25 mm roots in elevated CO_2 in the 0-10 cm and 11-30 depths (Figure 4a). There was also a similar trend in the 1-2 mm roots, but only in the 0-10 cm depth (Figure 4b)

C and N content

The MANOVA showed no significant elevated CO_2 effect on C content (g m^{-2}) ($P = 0.5445$) or N content (g m^{-2}) ($P = 0.0878$), but there was a significant CO_2 by depth.

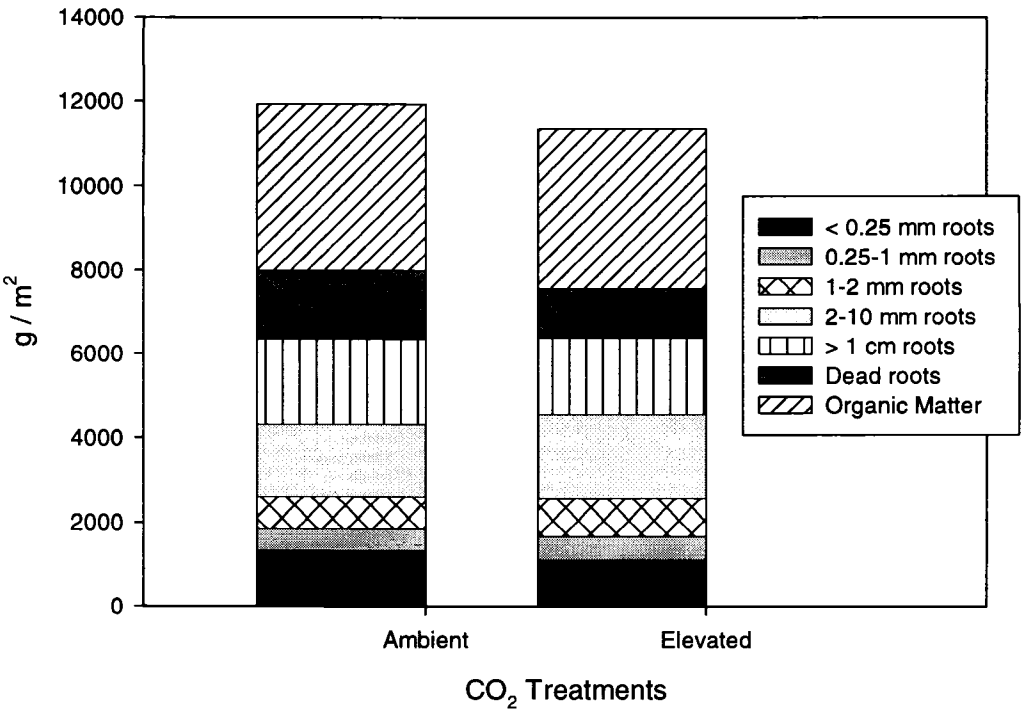

Figure 2. Below ground biomass (g m^{-2}) to a meter depth from soil cores taken in May 2002. Live root size classes by diameter are < 0.25mm, 0.25 – 1 mm, 1 – 2 mm, 2-10 mm, > 1cm. Unidentifiable matter and leaf litter make up the organic matter category.

interaction for both C and N content ($P < 0.0001$). For C content (g m^{-2}), ANOVAs showed a significant CO_2 by depth interaction ($P < 0.0001$) in the smallest size class (< 0.25 mm) roots and a non-significant CO_2 by depth interaction ($P = 0.064$) for the dead roots. For N content (g m^{-2}), there was a significant CO_2 by depth interaction ($P < 0.0001$) in the smallest size class (< 0.25 mm) and a significant CO_2 by depth interaction ($P = 0.044$) for the dead roots. All other classes were not significant with P values ranging between 0.25 and 0.99. The C and N content in the < 0.25 mm roots and dead roots reflected trend shown in the biomass, where there was less C and N in elevated CO_2 plots than ambient CO_2 treated plots in the 0-10 cm depth (Tables 2, 3, and 4).

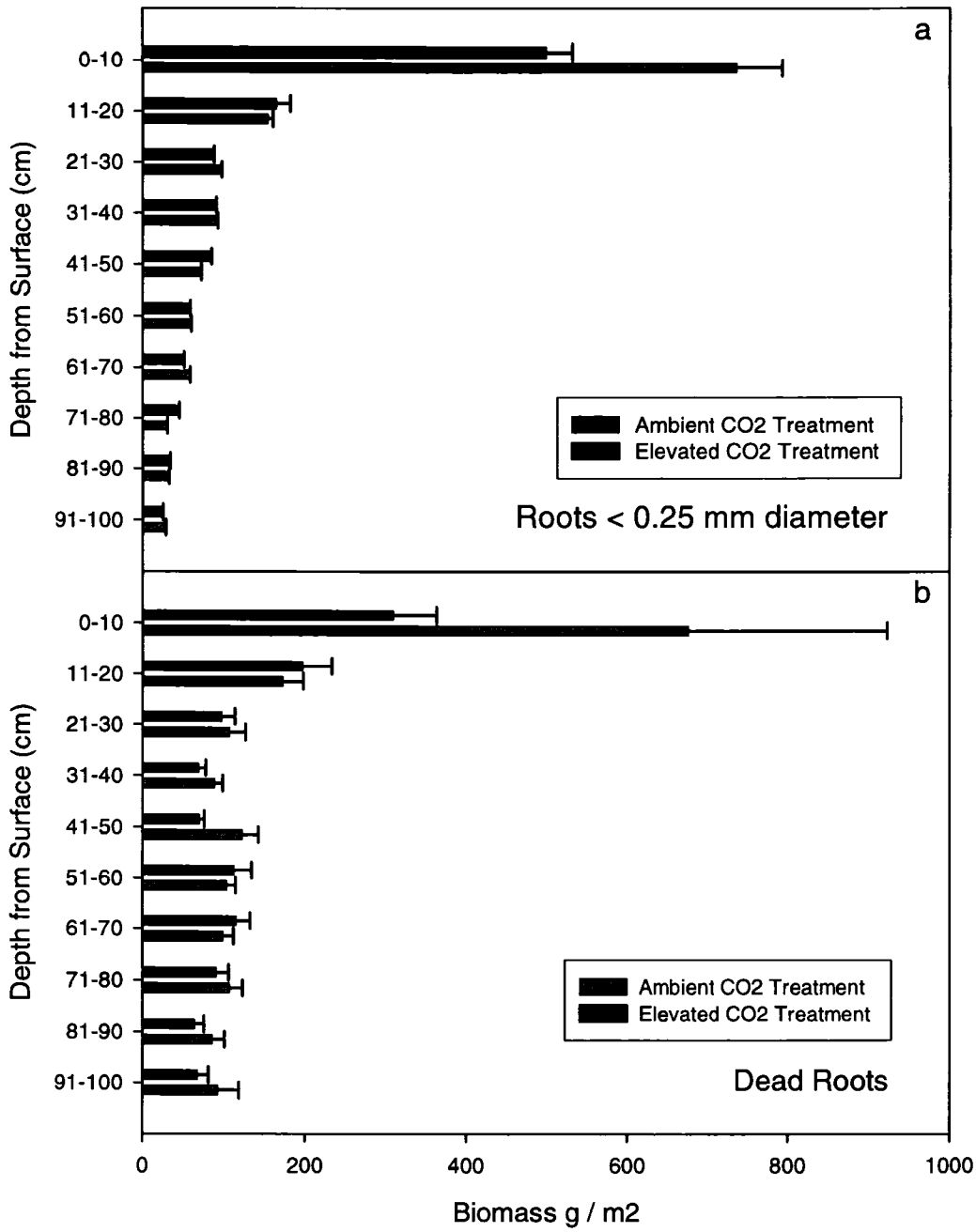

Figure 3. Biomass of roots < 0.25 mm in diameter (a) and dead roots (b) to a meter depth from soil cores taken in May 2002. Error bars represent one standard error.

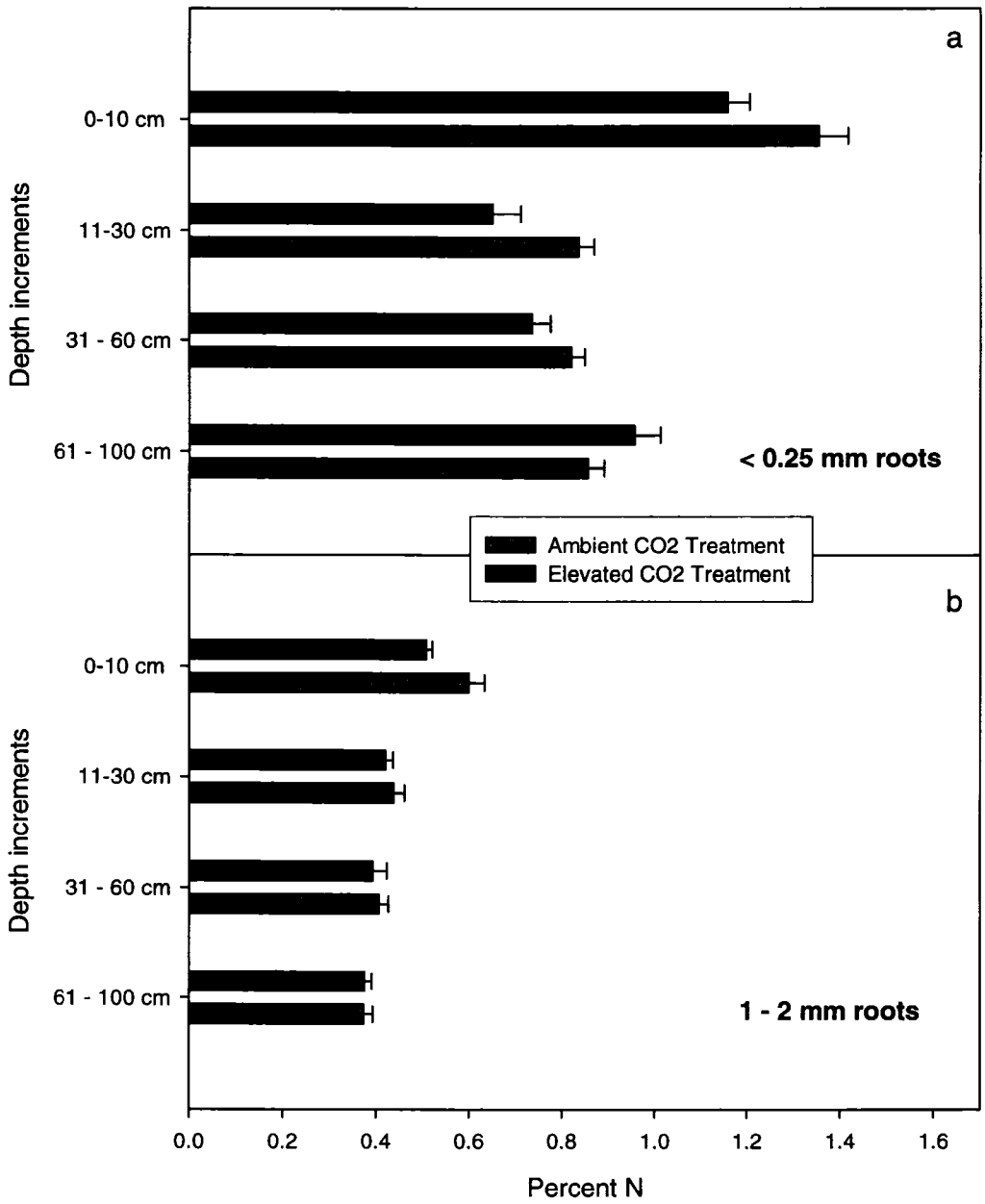

Figure 4. Percent N content of roots < 0.25 mm (a) and 1-2 mm (b) in diameter. Error bars represent one standard error.

Table 2. Carbon content of live roots (g C m^{-2}) for different diameter classes of <0.25mm, 0.25 – 1 mm, 1 – 2 mm, 2-10 mm, >1cm for 10 cm depth increments ± standard error.

C g m-2	< 0.25 mm roots		0.25 -1 mm roots		1 - 2 mm roots		2 -10 mm roots		> 1cm roots	
Depth (cm)	Ambient	Elevated	Ambient	Elevated	Ambient	Elevated	Ambient	Elevated	Ambient	Elevated
0-10	426 ± 37.4	266 ± 12.1	81 ± 5.7	94 ± 9.4	68 ± 9.7	95 ± 34.6	181 ± 26.0	207 ± 42.5	521 ± 179.9	480 ± 173.9
11-20	75 ± 3.7	56 ± 5.4	41 ± 3.5	53 ± 8.0	51 ± 7.0	69 ± 17.5	129 ± 22.1	141 ± 19.6	277 ± 190.3	263 ± 92.9
21-30	46 ± 1.2	31 ± 3.6	27 ± 3.6	26 ± 2.2	36 ± 3.5	35 ± 9.2	81 ± 18.8	69 ± 21.9	94 ± 54.6	9 ± 8.6
31-40	41 ±1.7	34 ± 3.0	21 ± 1.8	23 ± 2.6	27 ± 3.5	39 ± 7.8	69 ± 16.1	45 ± 10.9	31 ± 21.8	14 ± 13.9
41-50	31 ± 1.5	32 ± 2.9	19 ± 2.2	19 ± 2.6	30 ± 5.5	33 ± 7.6	82 ± 30.4	54 ± 18.24	42 ± 30.9	23 ± 22.5
51-60	26 ± 1.8	22 ± 2.2	18 ± 1.2	21 ± 2.7	29 ± 4.6	41 ± 11.4	50 ± 21.3	96 ± 43.3	0	28 ± 21.5
61-70	28 ± 1.1	22 ± 0.8	18 ± 1.4	14 ± 1.7	27 ± 5.4	38 ± 11.6	61 ± 12.3	113 ± 27.8	0	0
71-80	14 ± 0.7	20 ± 0.6	13 ± 2.4	13 ± 2.2	27 ± 6.4	34 ± 9.4	64 ± 18.3	61 ± 13.2	0	18 ± 18.2
81-90	15 ± 0.8	15 ± 0.4	10 ± 1.0	12 ± 0.8	28 ± 9.7	33 ± 7.4	59 ± 17.9	75 ± 16.5	0	6 ± 6.4
91-100	13 ± 0.7	11 ± 0.3	15 ± 2.4	11 ± 2.5	46 ± 23.8	25 ± 6.0	53 ± 11.8	67 ± 9.8	0	0

Table 3. Nitrogen content of live roots (g N m^{-2}) for different diameter classes of <0.25mm, 0.25 – 1 mm, 1 – 2 mm, 2-10 mm, >1cm for 10 cm depth increments ± standard error.

N g m-2	< 0.25 mm roots		0.25 -1 mm roots		1 - 2 mm roots		2 -10 mm roots		> 1cm roots	
Depth (cm)	Ambient	Elevated	Ambient	Elevated	Ambient	Elevated	Ambient	Elevated	Ambient	Elevated
0-10	11.2 ± 1.04	6.4 ± 0.42	1.24 ± 0.10	1.34 ± 0.13	0.77 ± 0.09	0.95 ± 0.33	2.22 ± 0.32	2.55 ± 0.36	5.30 ± 1.97	4.06 ± 1.46
11-20	1.4 ± 0.10	1.2 ± 0.13	0.52 ± 0.06	0.66 ± 0.10	0.43 ± 0.05	0.56 ± 0.07	1.09 ± 0.17	1.07 ± 0.14	2.26 ± 1.59	2.01 ± 0.74
21-30	0.9 ± 0.03	0.6 ± 0.06	0.34 ± 0.05	0.33 ± 0.03	0.31 ± 0.03	0.28 ± 0.07	0.72 ± 0.17	0.56 ± 0.20	0.74 ± 0.46	0.07 ± 0.07
31-40	0.8 ± 0.03	0.7 ± 0.04	0.28 ± 0.03	0.27 ± 0.03	0.22 ± 0.03	0.28 ± 0.05	0.47 ± 0.11	0.30 ± 0.07	0.22 ± 0.15	0.12 ± 0.12
41-50	0.6 ± 0.03	0.7 ± 0.04	0.25 ± 0.03	0.23 ± 0.03	0.24 ± 0.05	0.23 ± 0.04	0.48 ± 0.14	0.37 ± 0.11	0.39 ± 0.30	0.15 ± 0.15
51-60	0.5 ± 0.02	0.5 ± 0.03	0.24 ± 0.02	0.25 ± 0.03	0.24 ± 0.05	0.29 ± 0.06	0.28 ± 0.09	0.65 ± 0.26	0	0.31 ± 0.24
61-70	0.6 ± 0.03	0.5 ± 0.02	0.22 ± 0.02	0.17 ± 0.02	0.21 ± 0.05	0.27 ± 0.08	0.32 ± 0.06	0.60 ± 0.14	0	0
71-80	0.3 ± 0.02	0.5 ± 0.02	0.18 ± 0.04	0.16 ± 0.02	0.21 ± 0.06	0.25 ± 0.06	0.31 ± 0.08	0.32 ± 0.06	0	0.16 ± 0.16
81-90	0.3 ± 0.02	0.4 ± 0.02	0.13 ± 0.01	0.16 ± 0.01	0.22 ± 0.08	0.24 ± 0.05	0.28 ± 0.08	0.41 ± 0.09	0	0.06 ± 0.06
91-100	0.3 ± 0.02	0.3 ± 0.01	0.19 ± 0.03	0.14 ± 0.03	0.36 ± 0.19	0.19 ± 0.04	0.27 ± 0.06	0.37 ± 0.05	0	0

Table 4. Nitrogen and carbon content of dead roots and organic matter for 10 cm depth increments ± standard error.

	Dead Roots				Organic Matter			
	C g m-2		N g m-2		C g m-2		N g m-2	
Depth (cm)	Ambient	Elevated	Ambient	Elevated	Ambient	Elevated	Ambient	Elevated
0-10	349 ± 131	153 ± 29	3.34 ± 1.12	1.62 ± 0.19	1237 ± 146	1079 ± 94	25.15 ± 3.23	21.20 ± 1.39
11-20	89 ± 14	101 ± 20	0.83 ± 0.17	0.81 ± 0.14	255 ± 57	271 ± 51	3.43 ± 0.68	3.43 ± 0.66
21-30	54 ± 10	49 ± 9	0.48 ± 0.08	0.39 ± 0.06	174 ± 43	128 ± 23	2.31 ± 0.55	1.64 ± 0.33
31-40	46 ± 5	32 ± 4	0.36 ± 0.04	0.26 ± 0.03	92 ± 9	79 ± 12	1.19 ± 0.16	0.97 ± 0.15
41-50	65 ± 11	33 ± 3	0.51 ± 0.10	0.26 ± 0.02	78 ± 10	56 ± 5	1.04 ± 0.18	0.69 ± 0.08
51-60	54 ± 6	54 ± 11	0.41 ± 0.03	0.43 ± 0.09	69 ± 7	83 ± 10	0.91 ± 0.12	1.04 ± 0.14
61-70	49 ± 6	54 ± 9	0.39 ± 0.04	0.48 ± 0.08	75 ± 10	79 ± 8	0.86 ± 0.15	0.85 ± 0.09
71-80	53 ± 8	44 ± 9	0.43 ± 0.06	0.37 ± 0.07	73 ± 14	61 ± 7	0.83 ± 0.18	0.68 ± 0.09
81-90	43 ± 7	31 ± 6	0.34 ± 0.05	0.27 ± 0.05	61 ± 11	69 ± 9	0.67 ± 0.12	0.75 ± 0.11
91-100	46 ± 13	33 ± 6	0.37 ± 0.10	0.29 ± 0.07	68 ± 17	64 ± 8	0.75 ± 0.20	0.69 ± 0.10

P concentration and content

There was no significant effect of elevated CO_2 at the MANOVA level on the P concentration (P = 0.57) or P content (P = 0.38) for live roots, dead roots and organic matter in the top 10 cm of soil. Although not significant, the trends for P content of the biomass in <0.25 mm size class and the dead roots reflected trends in biomass where there was significantly less in elevated CO_2.

Nutrient ratios

There was no significant elevated CO_2 effect (P = 0.334) or a CO_2 by depth interactive effect (P = 0.16) on C:N at the MANOVA level (Figure 5). There was no significant effect of elevated CO_2 on the P concentration (P = 0.57) (Figure 6), C:P (P = 0.71) or N:P (P = 0.38) for live roots, dead roots and organic matter in the top 10 cm of soil. Phosphorus content or concentration in root biomass was not affected by elevated CO_2 treatment, but an interesting duality between N and P was observed between root sizes. As roots increased in diameter, P concentrations also increased while N concentrations decreased (Figure 6). This may be related to the trend seen in caloric value, where calories g^{-1} decreased as root size increased (Table 1).

Discussion

Biomass

Based on findings of others and early minirhizotron measurements in the current project (Day et al., 2006), greater C storage in the roots of plants in elevated CO_2 was

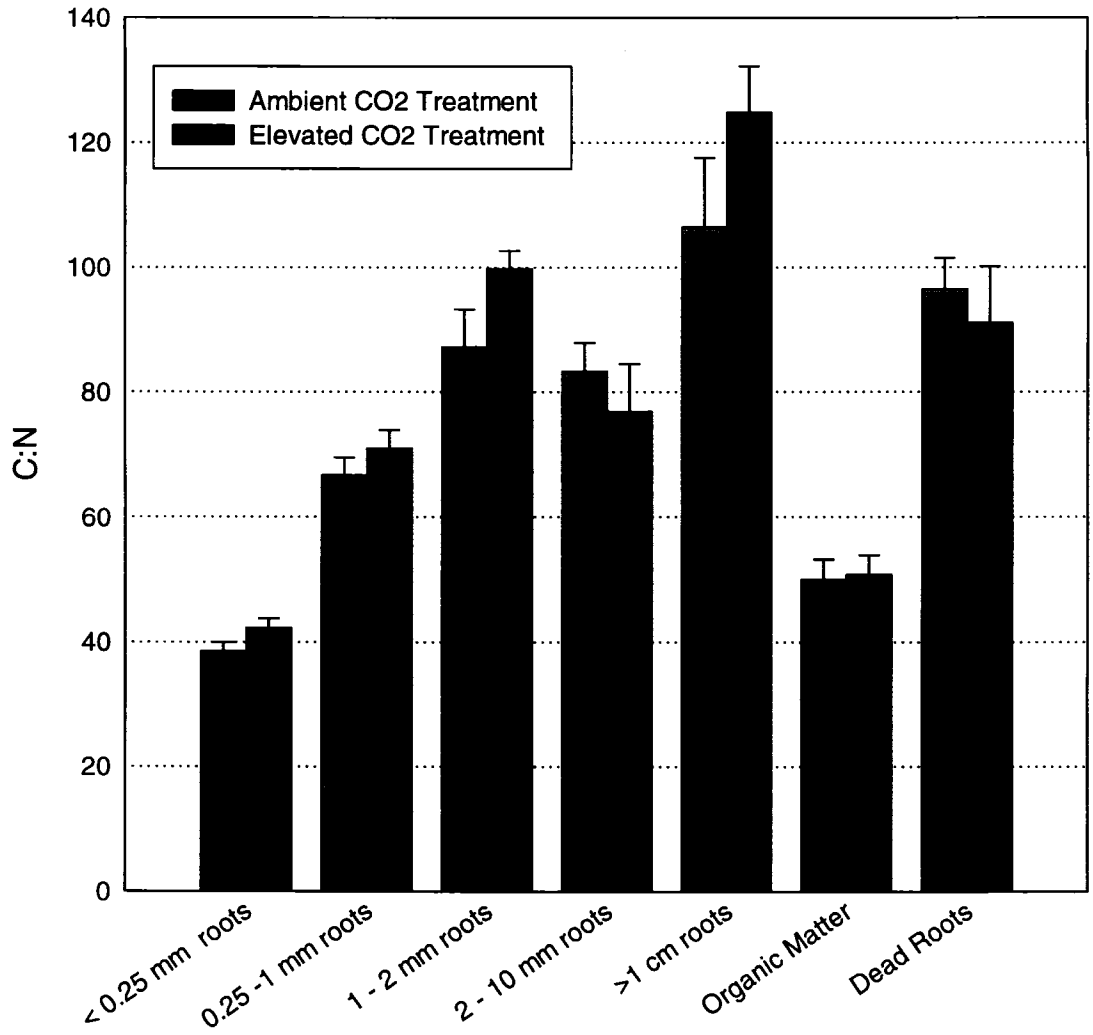

Figure 5. The C:N ratio of roots from the top 10 cm of soil. Error bars represent one standard error.

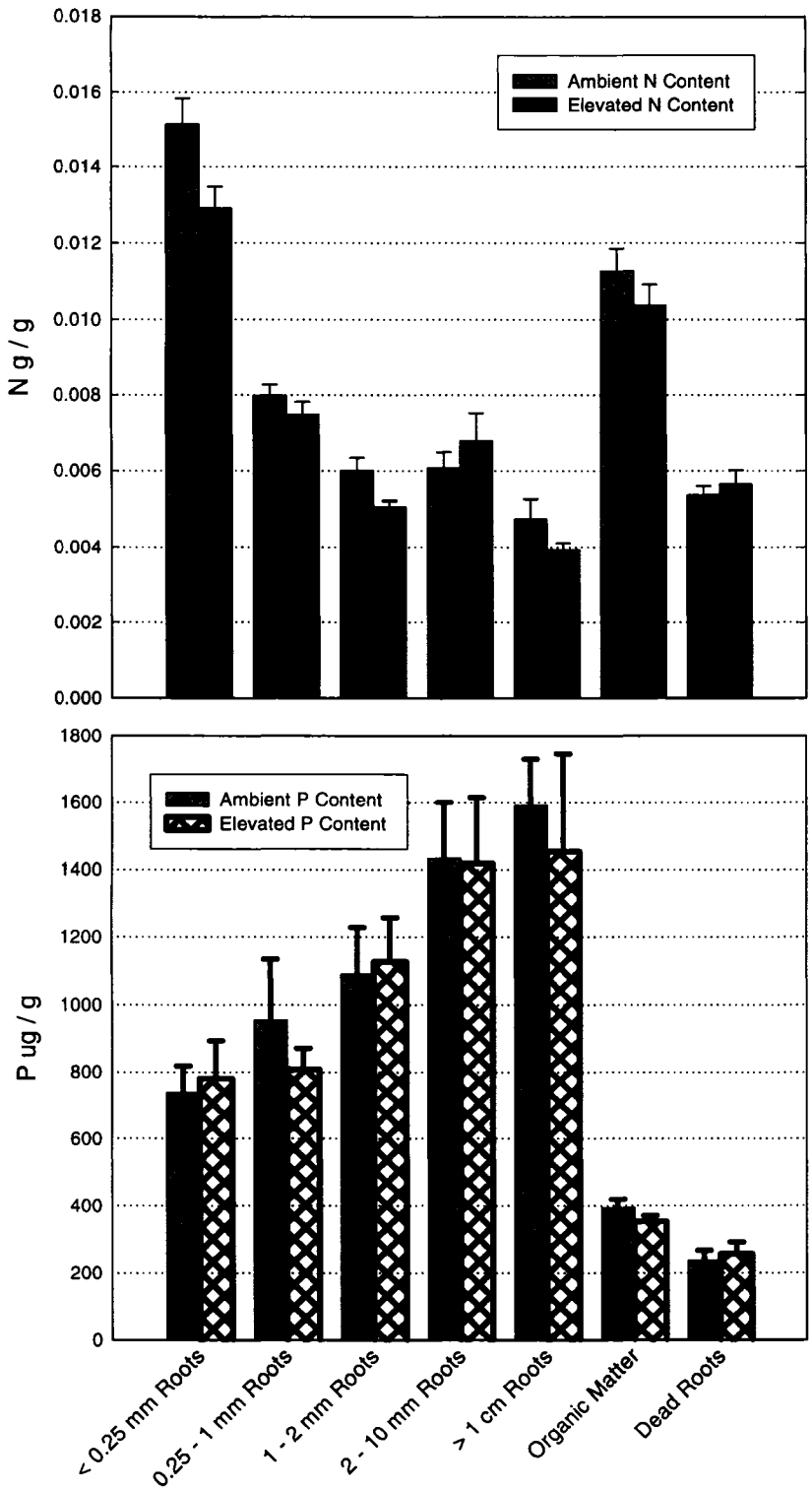

Figure 6. The N and P concentration of roots in the top 10 cm. Error bars represent one standard error.

expected. Instead, this study falls among the minority where overall root biomass is unresponsive to elevated CO_2 treatment. The literature provides support for elevated CO_2 stimulating belowground biomass. In a review by Rogers et al. (1994) of 157 studies of root biomass using several methods of CO_2 manipulation on various species, there were only 3 studies that exhibited negative responses to elevated CO_2 and 13 exhibited no response. The remaining 141 studies showed a positive response of root biomass to elevated CO_2. More recent studies seem to follow the same trends. Root biomass roughly doubled for *Adenostoma fasciculatum* in a Chaparral system (Lipson et al., 2005); increased by 96% for *Populus tremuloides* and *Betula papyrifera* (King et al., 2001); increased by 50% for *Betula pedula* (Ineson et al., 1996); increased for *Pinus echinata* seedling fine roots (Norby et al., 1987), and increased for *Populus tremuloides* under a high nitrogen treatment (Pregitzer et al., 2000). Besides biomass, fine root production increased more than 100% for ingrowth bags for *Pinus sylvestris* seedlings (Janssens et al., 1998) and increased by 85% in calcareous soils and 43% in siliceous soils for *Fagus silvatica* and *Picea abies* stands (Wiemken et al., 2001).

Other research showed no significant effect of CO_2 on root biomass including a study of *Anthyllis vulneraria* and *Plantago media* (Ferris and Taylor, 1993) and a study of *Populus tremuloides* under a low nitrogen treatment (Pregitzer et al., 2000). Overall the root biomass of this study was unaffected by CO_2 treatment, but when root sizes were examined separately by depth, it was found that biomass of < 0.25 mm diameter roots was depressed in elevated CO_2 in the top 10 cm. The fine roots, such as those < 0.25 mm, are responsible for nutrient and water uptake (Gordon and Jackson, 2000), which are important factors in controlling exploitation of the soil (Fitter, 1987; Waisel et al., 2002).

The cost of exploiting greater soil volume may not return adequate nutrients from this nutrient poor soil to be beneficial. This may be the reason < 0.25 mm roots are depressed in elevated CO_2. Several studies showed root growth substantially increased (Pregitzer et al., 2000; Zak et al., 1993), and root production and mortality increased (Pregitzer et al., 2000) under elevated CO_2 when high levels of N were available. However, in the scrub-oak ecosystem the nutrients are low. In a study of roots in this system using minirhizotrons, Day et al. (2006) presented evidence for root closure of this system. They showed the root system in elevated CO_2 reached closure sooner than ambient CO_2 treated plants, but both treatments equilibrated at the same root length density.

Some studies that measured roots may not discern a difference in biomass because more rigorous methods of soil removal may destroy fine roots with a diameter < 0.25 mm. The roots not destroyed are often lumped with larger roots, masking differences that might exist. In the study of *Populus tremuloides*, where roots were separated into classes of < 0.5 mm and 0.5 – 1 mm diameter, Pregitzer et al. (2000) showed the smaller size class was more responsive to CO_2 and N treatments. Jach et al. (2000) found a shift in percentage of fine roots (< 2 mm) and coarse roots (> 2 mm) between CO_2 treatments. They showed the percent of fine root biomass increased from 1% under ambient to 8% in elevated CO_2, whereas in the scrub-oak there was a small increase from 27% to 29%.

Contrary to the findings of Jach et al. (2000), where root litter of *Pinus sylvestris* was greater under elevated CO_2, scrub-oak dead root litter in the top 10 cm constituted a smaller fraction in elevated CO_2. The greater amount of dead root mass in the ambient CO_2 treatment was not from the < 0.25 mm diameter roots that have died. One of the basic assumptions for sorting roots was if they were < 0.25 mm and still intact, they were

alive and were sorted into the live root fraction. Any dead roots of this size would have been unidentifiable and sorted into the organic matter fraction. One possible explanation was the reduced dead root pool was evidence of a seasonal effect or of a stress on the ecosystem such as drought. Less dead root mass under elevated CO_2 may be evidence of increased decomposition. However, past studies have found no evidence of increased root decomposition in elevated CO_2 at this site (Dilustro et al., 2001).

Despite past evidence in the scrub-oak of increased litter fall (Hungate et al., 2006), there was no evidence of any CO_2 effect on coarse particulate soil organic matter (> 1 mm), which included surface litter. Previous studies of litter fall found elevated CO_2 increased litter fall in the first years of the study, but not years 5 – 7 (Hungate et al., 2006). Soil cores were taken in year 7 of the study and support the findings of Hungate et al. (2006).

There was no evidence that plants in elevated CO_2 were storing greater amounts of carbon in belowground biomass. One of the objectives was to sample roots larger than the fine roots monitored by minirhizotrons. The larger structural components of the root system were sampled, but the sampling methods may have been inadequate for the largest roots. Due to the nature of scrub-oak morphology (lignotubers and rhizomes), the belowground system is somewhat unusual. The scrub-oak has an abundance of large belowground structures, presumably for storage in preparation for regeneration post-burn. These structures have been encountered as large as 12.4-15 cm diameter using a 15 cm corer (P. C. Daniel Stover) outside of the chambers. The 7 cm corer used in this study did not capture these larger structures. Larger cores in the chambers would have been destructive to the long-term study and therefore not feasible. Interestingly, even though it

was not significant, the largest size class of roots (> 1 cm) was encountered in 20% of the cores below 50 cm in the elevated CO_2 chambers, while no roots of this size were encountered in the ambient CO_2 chambers below 50 cm. This may indicate a greater distribution of structural roots deeper in the soil in elevated CO_2.

The scrub-oak is a fire maintained system and it was hypothesized plants in elevated CO_2 may have an advantage for regeneration after a fire from the root system because they were able to store greater amounts of C in their root systems. Since there was no evidence of this in the standing rootstock, the possibility was considered that the energy was being stored in more complex carbohydrate structures that would appear as increased caloric value. There was no evidence of this.

Nutrients

The g C m^{-2} and g N m^{-2} reflected the greater biomass in the < 0.25 mm diameter roots in the ambient CO_2 treatment. Jach et al. (2000) investigated similar aspects of CO_2 effects on *Pinus sylvestris* on roots with diameters > 2mm, 1 – 2 mm and < 1 mm. Pregitzer et al. (2000) investigated *Populus tremuloides* roots according to their order, which can be comparable to size. The findings of Jach et al. (2000) and Pregitzer et al. (2000) concerning the distribution of C and N were comparable to my findings. Similar to the findings of Pregitzer et al. (2000), therewas no effect of CO_2 on C concentration in root biomass. Jach et al. (2000) found no significant effects of elevated CO_2 on N concentration in roots, but trends indicated coarse roots (> 2 mm) had lower N concentrations and fine roots (< 1 mm) had higher concentrations of N in elevated CO_2. The < 0.25 mm diameter roots had significantly lower N concentrations in elevated CO_2.

Because fine roots of < 0.25 mm diameter are the most labile components of the root system, this may be evidence of progressive N limitation in elevated CO_2 (Hungate et al., 2006). The one root size class for which Jach et al. (2000) found no changes in N concentration was the 1 – 2 mm diameter roots. There was a trend of significantly lower N concentration in the 1 – 2 mm diameter roots in elevated CO_2, again supporting the progressive nitrogen limitation theory. Between Jach et al.'s (2000) and this study, there were many effects of CO_2 that would not have been elucidated had the roots been lumped into larger categories, but this study did not match the trends found by Jach et al. (2000). The trends of N and C allocation to the different components of the root system vary according to other factors in the environment. Pregitzer et al. (2000) found a universal decrease in N concentration across all root orders of *Populus tremuloides*, which seems to be more closely related to what was found in this study.

The N and lignin contents of leaf litter affected decomposition rates (Melillo et al., 1982), and for this reason, it has been an area of interest in CO_2 studies. There are numerous examples where elevated CO_2 has altered the N content of living tissue (Körner and Arnone, 1992; McGuire et al., 1995; Norby et al., 1992; Williams et al., 1994) and it was theorized that this may slow decomposition rates in elevated CO_2 (Rastetter et al., 1992). However, using senesced leaf litter instead of living tissue, several studies showed leaf litter decomposition was not affected by environment of tissue growth (i. e. grown in elevated or ambient CO_2 conditions) (Hall et al., 2006; Van Ginkel et al., 1996). The lack of altered C:N ratios in the organic matter fraction in this study confirms other's findings that the difference in quality of living tissue does not

occur in senesced leaf tissue at this study site (Hall et al., 2005a; Hall et al., 2005b) and in other studies (Hirschel et al., 1997).

These findings on leaf litter beg the same questions about what is happening to root nutrient content and decomposition. No effects of elevated CO_2 treatment were found on the N and C concentrations of dead roots. Because there is no significant effect of elevated CO_2 on N or C concentrations of dead roots, the process of root death may be similar to leaf senescence, where nutrients and carbohydrates are withdrawn. Any differences in tissue quality that may have been present in living roots, such as those shown in the 1 – 2 mm roots, are equalized upon root death. The lack of CO_2 effect on leaf litter and dead root C:N ratios indicated that if decomposition rate are slowed in this system in elevated CO_2, it is unlikely to be due to altered tissue quality.

Conclusions

The expected greater root biomass was not found under elevated CO_2, but there is still potential for C storage in the large lignotubers and rhizomes, which are characteristic of the dominant plants in this ecosystem. Recent ground penetrating radar measurements in the open-topped-chambers indicate about 20% more biomass of coarse belowground structures in elevated CO_2 (D. Stover and F. Day, personal communication). The smallest root fraction (< 0.25 mm) was most affected by elevated CO_2 levels. The most versatile fraction (< 0.25 mm roots) may have been depressed in elevated CO_2 in the top 10 cm due to increased soil moisture and the lack of need to spread a fine root network to take up water. Living roots had a lower N concentration in the < 0.25 mm and 1-2 mm size categories in elevated CO_2, but this concentration difference was not evident in dead

roots. The literature showed that decomposition of green leaf tissue was different than decomposition of senesced leaf tissue and the same trends could apply to root decomposition. Roots may respond similarly upon death and it is necessary to use cautious interpretation of root decomposition studies because living roots are often used. Further investigation of changes in root N concentration before and after root death is needed to understand root decomposition in elevated atmospheric CO_2.

CHAPTER III

MINIRHIZOTRON ESTIMATES OF FINE ROOT BIOMASS

Introduction

There are various methods employed to study growth and biomass of roots, but many are destructive. Studies on the effects of CO_2 on plant communities are often long-term and destructive sampling must be minimized. Consequently, monitoring fine root dynamics has been notoriously difficult. Traditional methods of monitoring root dynamics include destructive soil coring and root ingrowth bags, but these approaches are not feasible over the course of a long-term study. Minirhizotron tubes, first described by Bates et al. (1937), are minimally destructive and versatile for monitoring many aspects of fine root dynamics. Minirhizotrons are clear, cylindrical tubes inserted into the ground so roots can be viewed and recorded. Minirhizotrons have been used to monitor root length density (RLD), production, and mortality in a variety of ecosystems (Hendrick and Pregitzer, 1993; Ponti et al., 2004; Tierney and Fahey, 2001; Tierney et al., 2003).

Fine root biomass and C content are critical components in models of C in ecosystems but they cannot be directly determined by minirhizotron techniques (Hendrick and Pregitzer, 1993). The basic problem is trying to estimate a 3-dimensional value (biomass) from 2-dimensional measurements (RLD). To estimate biomass from minirhizotron data, a conversion factor for length to biomass must be developed, and assumptions regarding depth of view must be made. Specific root length (SRL) is a measure of cost (mass) per return (length) (Ryser, 2006) and is used as a conversion

factor to change root length into biomass (Johnson et al., 2001b). To determine SRL's, soil cores were taken in May 2002 from the study site (see Chapter 2).

Minirhizotron images have been successfully used to predict fine root biomass (Jose et al., 2001), but there are several less successful studies where good correlations were only found below 30 cm (Bragg et al., 1983; Gregory, 1979; Samson and Sinclair, 1994). It is important to estimate root biomass over this long-term study because the overlying questions concern fate of C due to elevated CO_2. Biomass can be estimated by converting the minirhizotron root length data to a volumetric measurement (i. e. km / m^3) and applying the SRL to root length to convert to biomass. To convert the area of the minirhizotron image to a volume, the area of the minirhizotron tube is assumed to have a certain depth of view for the third dimension (2 mm by Taylor et al. (1970) and 3 mm by Sanders and Brown (1978)). Few studies have used minirhizotrons to determine fine root biomass (Hendrick and Pregitzer, 1996; Jose et al., 2001), so there is little information on the success of this method.

The intent was to obtain SRL's for the roots and compare the effects of elevated CO_2 on the SRL. The objective was to compare the biomass obtained from the cores to the biomass estimated from the minirhizotron data collected before and after the coring event. The final objective was to apply the SRL to the minirhizotron data set over the first eight years of the study to observe effects of CO_2 on fine root biomass over the course of the study. The hypothesis was that the biomass would be stimulated in the first years of the study in elevated CO_2 when a greater fine root abundance was observed (Day et al., 2006), but equalize as the fine roots reached closure.

Methods

Minirhizotron system and recordings

Two cellulose acetate butyrate tubes were installed at a 45° angle from the soil surface to a meter depth in each plot prior to construction of chambers in 1996. Each tube had been etched on one side with 159 (9 x 13 mm) stacked frames. The length of tube extending above the soil surface was taped and capped to prevent light from affecting the soil adjacent to the tubes. Images were recorded on Hi8 (mm) videotape four times a year using a Bartz Technology® BTC-2 minirhizotron camera system (Bartz Technology Co., Santa Barbara, CA, USA). Fine root length and width were measured for each root within the frame using ROOTS® version 1.05 and 2.2 (Michigan State University Remote Sensing Laboratory). Root data were recorded as root length per area (mm / frame) and converted to mm / cm^2.

Determination of specific root length

The roots were collected as described in chapter 2. Roots were sorted by hand into size classes of < 0.25 mm live roots, 0.25 – 1 mm live roots, 1 – 2 mm live roots, 2 – 10 mm live roots, > 1 cm live roots, dead roots, and unidentifiable organic matter. To obtain SRL, length of roots in the sample or subsample was measured. Dry mass was then obtained for the samples, and finally, length was divided by mass of the roots. To measure the length of live roots larger than 0.25 mm, an AgVision Monochrome Image Analysis System (Decagon Devices, Inc., Pullman, WA) was used. For roots in the < 0.25 mm and 0.25 - 1 mm size classes, it was necessary to measure a subsample to obtain a specific root length because the roots were too numerous to measure for the entire

sample. A subsample of the smallest size class (< 0.25 mm) from the lower depths was measured using a combination of the Agvision system and dissecting scope. The Agvision system was able to measure the larger roots in this range, but a dissecting scope was necessary for the finest roots because they were below the range the Agvision system could measure. Once all root length measurements were made, all seven classes were dried at 70° C for 48 hr and weighed. See chapter 2 for more details on biomass corrections. These length and weight measurements were used to obtain SRL (m/g) (Table 5).

Table 5. Specific root lengths (m/g) for different diameter roots extracted from the chambers in 2002. Standard deviations are in parentheses.

	Ambient CO_2 Chambers	**Elevated CO_2 Chambers**
< 0.25 mm Roots	56.129 (15.179)	51.537 (16.922)
0.25 - 1 mm Roots	7.643 (2.339)	7.902 (2.402)
1 – 2 mm Roots	1.651 (0.573)	1.575 (0.699)
2 mm – 1 cm Roots	0.384 (0.277)	0.353 (0.248)
> 1cm Roots	0.018 (0.010)	0.016 (0.010)

Conversion of minirhizotron RLD to biomass

The SRLs were used to determine biomass from the minirhizotron root length data. A 2 mm depth of field was assumed for the two dimensional minirhizotron images in order to convert the frame area to a soil volume for roots < 2 mm in diameter. For roots with a diameter > 2 mm, the assumed depth of field was the diameter of the root observed. This was to avoid underestimation of the volume of soil occupied by the root. The mean biomass was estimated for each chamber and compared by CO_2 treatment for each date of

minirhizotron measurement. A repeated measures ANOVA using SAS (SAS Institute 1990) was used to determine the effect of CO_2 treatment on total root biomass. Biomass estimates from minirhizotron images obtained within a few months before and after the coring were compared to the core biomass data to evaluate the differences between the two techniques.

Results

Comparison of minirhizotron to core data

Two different methods were used in the spring and early summer of 2002 to obtain estimates of fine RLD and fine root biomass: minirhizotron (MR) imaging (March and June) and soil coring (May). For the minirhizotron estimates of RLD, the smallest size class (< 0.25 mm in diameter) made up 94-96% of the total RLD observed. The RLD of this same size class estimated from the soil cores made up 90-92 % of the total RLD for all roots. The RLD for < 0.25 mm diameter roots, shown chronologically in Figure 7, was slightly less in the March minirhizotron estimate for roots from the elevated CO_2 chambers. This trend was more pronounced in the May core data (Figure 7), reflecting what was shown for biomass in Chapter 2. By June, the difference disappeared, perhaps due to a seasonal effect. The RLD of the larger root classes together made up less than 10 % of the total RLD measured, but there was close agreement between the different methods for estimates of RLD (Figure 8). The core data seemed to have slightly greater RLD estimates for the 1 – 2 mm size class than the minirhizotron estimates. There was one anomaly where the 2 – 10 mm size class disappeared by the June minirhizotron

recording in the ambient chambers (Figure 8). There seemed to be a slight trend towards greater RLD in the elevated CO_2 chambers, but this was small (Figure 8).

The SRL's developed for this study were not significantly affected by elevated CO_2, but SRL was slightly greater in elevated CO_2 for all except the 0.25 – 1 mm root size class (Table 5). When the four root size classes were converted to biomass and compared between minirhizotron and soil core methods, the total biomass was similar, except for the June minirhizotron data for ambient CO_2 chambers (Figure 9). For the June data, the elevated chamber total seemed to be greater, while the ambient was less due to the missing 2 – 10 mm size class. The individual size classes contributed to the overall biomass differently when examined by measurement technique. The < 0.25 mm size class contributed less to the overall biomass for the soil core measurements, while the 2 – 10 mm size class contributed greater biomass for the soil core measurements (Figure 9). The 0.25 – 1 mm and 1 – 2 mm estimates were consistent between the two methods. The reasonable agreement of the two methods led me to believe SRL could be applied to the root length estimated over the entire period of the study to estimate biomass of fine roots.

Root length and biomass over time

After applying the SRL of the different size classes to the minirhizotron data over eight years of the study, the conclusion was that estimates for the larger size classes were unrealistic for some measurement dates. The estimates of biomass of the < 0.25 mm size class was reasonable, so the model seems realistic for the smallest fine roots, but not larger fine roots. The root length for the < 0.25 mm size class over time (Figure 10) was similar to the RLD pattern reported by Day et al. (2006) (Figure 11), supporting the

conclusion that most of the root length is contributed by the smallest size class. There was little difference in the SRL by CO_2 treatment for this size (Table 5), and consequently the conversion to biomass does not alter the overall pattern between the CO_2 treatments (Figure 12). The average biomass from June 1996 to June 2004 was 1.19 kg / m^2 for the ambient chambers and 1.42 kg / m^2 for the elevated chambers. The < 0.25 mm roots probably had greater average biomass in elevated CO_2 due to the greater abundance of those roots early in the study. After the 3rd year of the study, the treatment effect disappeared for both RLD and biomass.

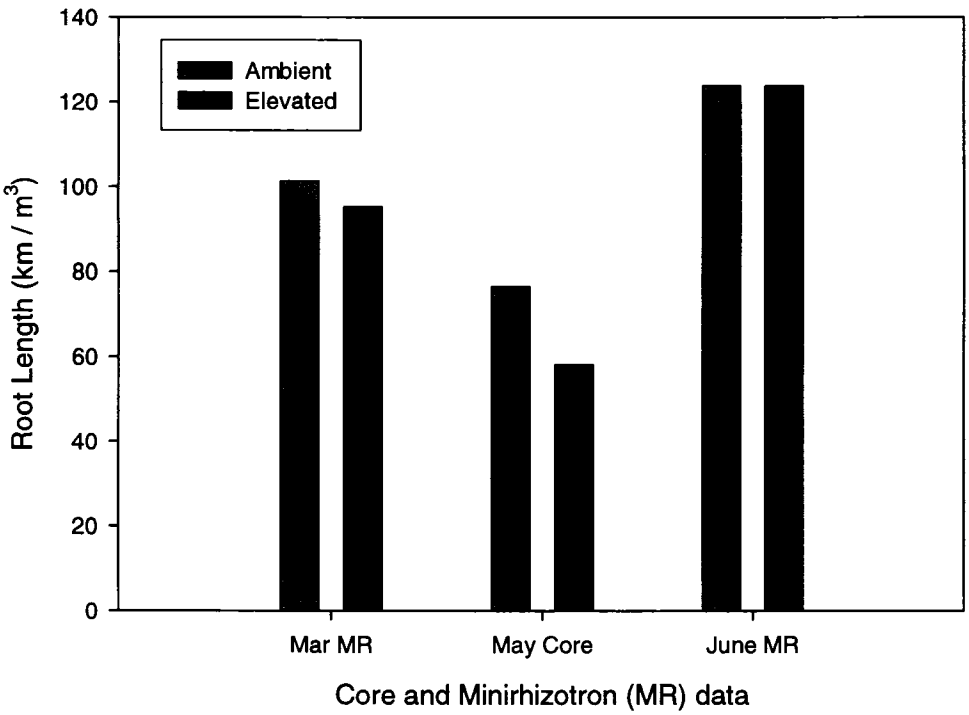

Figure 7. Comparison of root length for < 0.25 mm diameter roots estimated from soil cores to minirhizotron (MR) observations taken before and after the soil core for the two different CO_2 treatments.

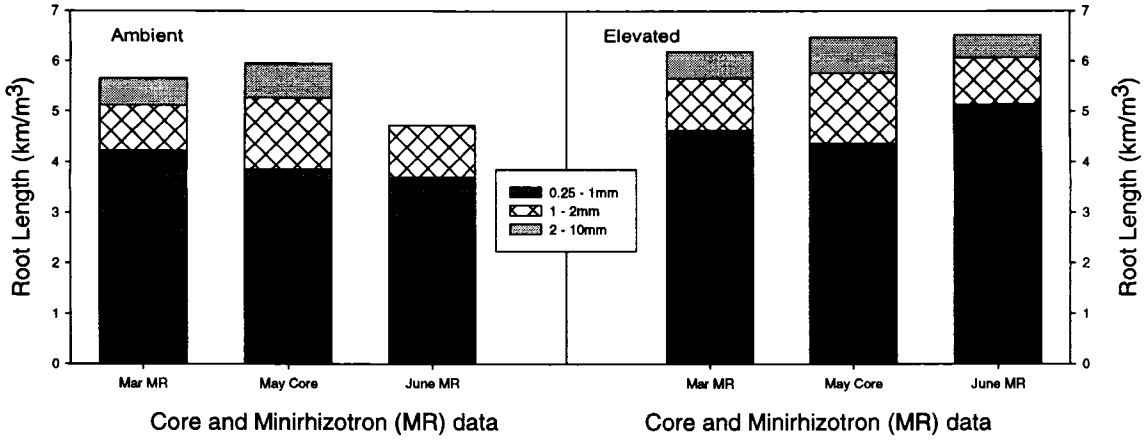

Figure 8. Comparison of root length for larger root size classes (0.25 – 1 mm, 1 – 2 mm, 2 – 10 mm) estimated from soil cores to minirhizotron (MR) observations taken before and after the soil core for the two different CO_2 treatments.

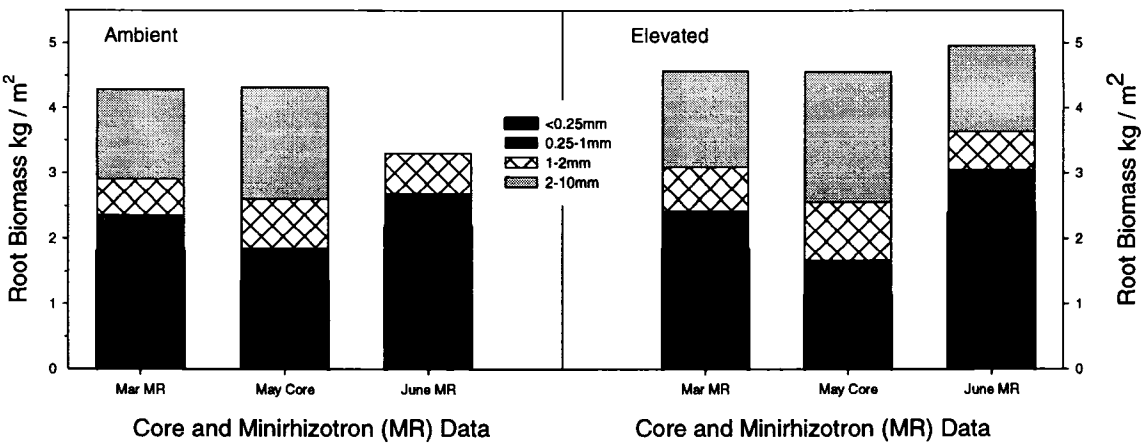

Figure 9. Comparison of root biomass estimated from soil cores and minirhizotron (MR) observations taken before and after the soil cores for the two different CO_2 treatments. Kg / m^2 is for a meter depth. Roots greater than 10 mm were not included since they are not sampled by minirhizotrons.

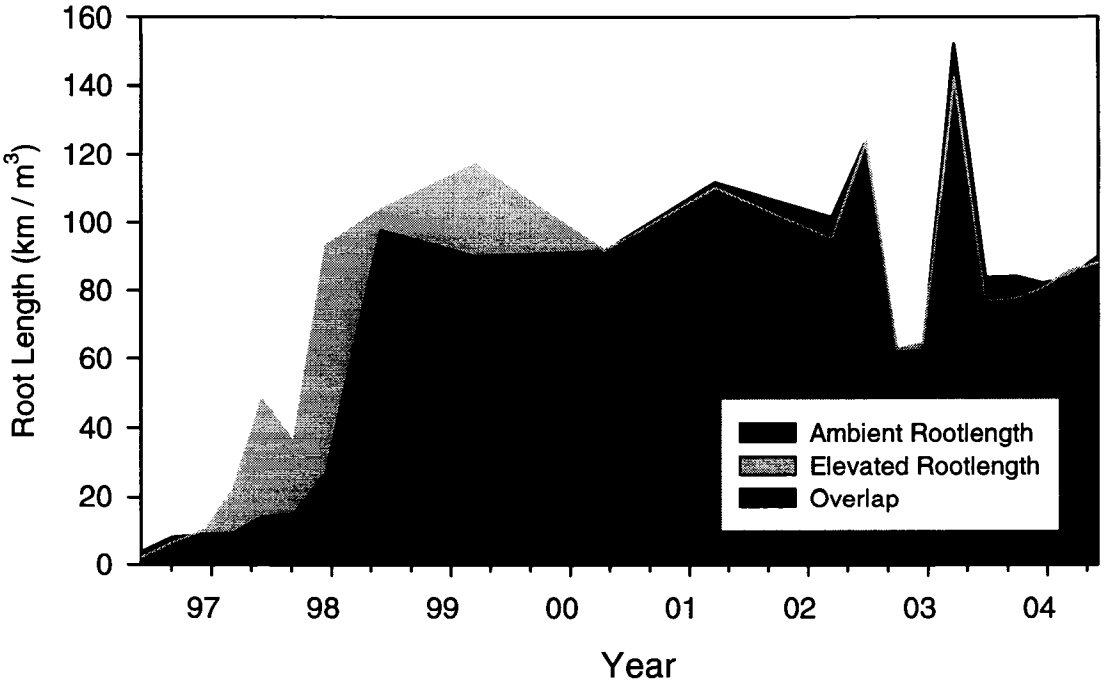

Figure 10. Fine root length for roots < 0.25 mm in diameter from the minirhizotron observation over eight years of CO_2 treatment.

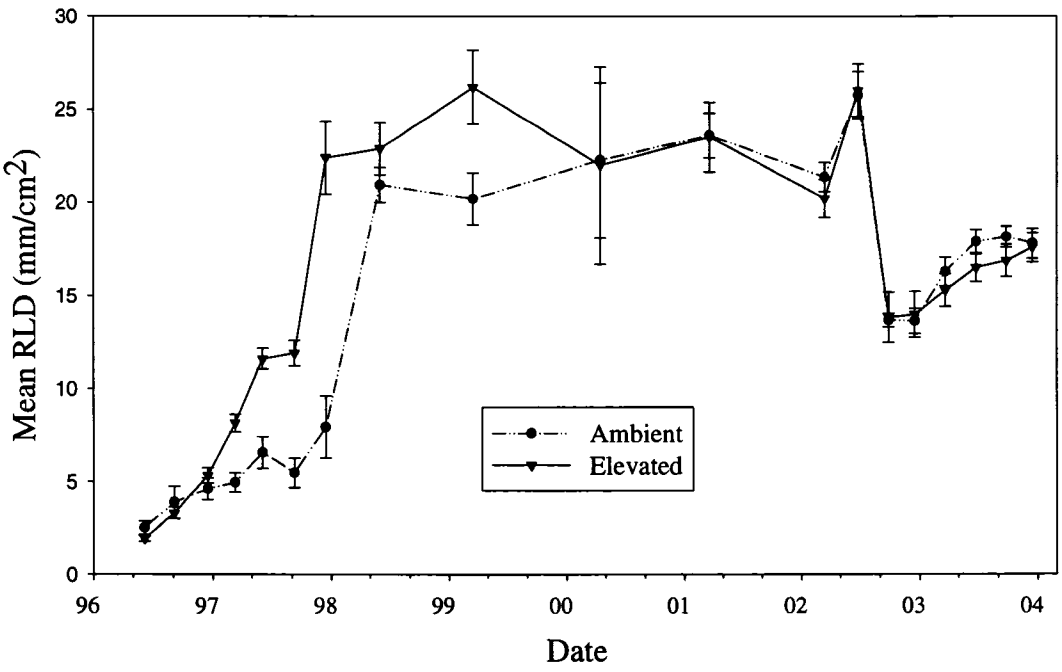

Figure 11. Root length density of Florida scrub-oak under eight years of elevated CO_2 treatment compared to ambient CO_2 treatment (from Day et al. 2006).

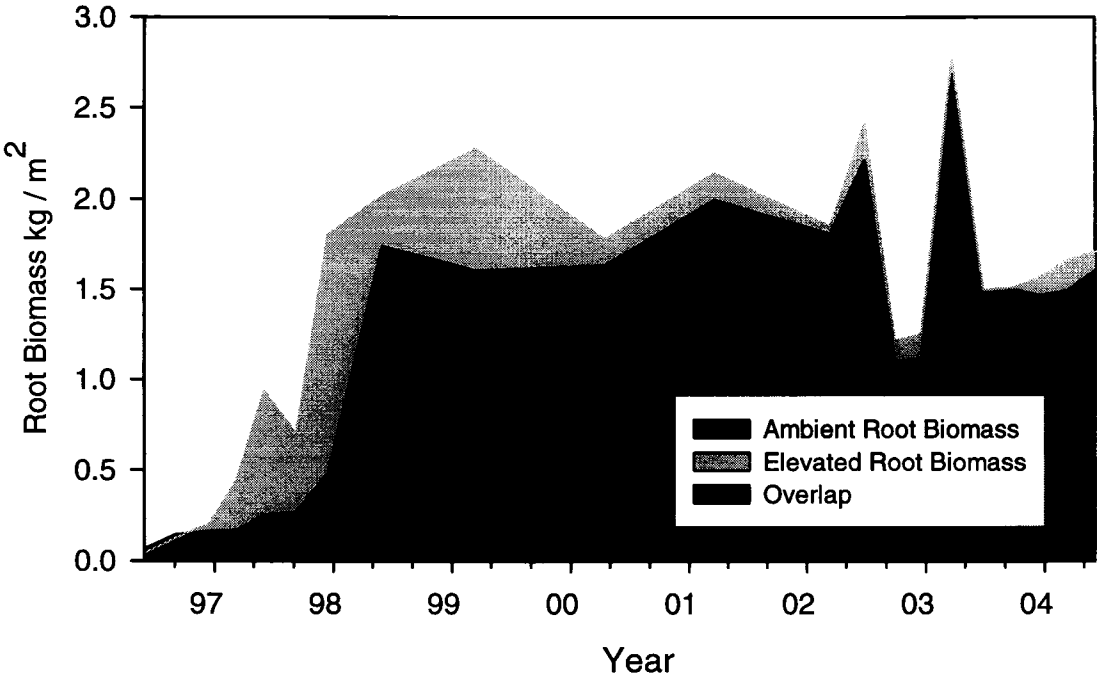

Figure 12. Fine root biomass for roots < 0.25 mm in diameter estimated from minirhizotron observations over eight years of CO_2 treatment. Kg / m^2 is for a meter depth.

Discussion

Several studies have compared root data from soil cores to minirhizotron data. For roots in two different systems, Johnson et al. (2001b) showed there was no significant difference between minirhizotron and soil core sampling of root biomass density. Jose et al. (2001) had close agreement between soil core data and biomass predicted from minirhizotron images for *Juglans nigra* and *Quercus rubra*. Similarly a close correlation between cores and minirhizotron data was observed in several other systems (Ephrath et al., 1999; Norby et al., 2004; Phillips et al., 2006; Thomas et al., 1996). Davis et al. (2004) found differences less than one standard error in homogeneous oak plots, but had

some methodological problems in mixed oak plots. For roots < 1 cm in diameter, the biomass estimates between cores and minirhizotrons are similar. The < 0.25 mm root size class was the most variable between the two methods, but it is unlikely that the minirhizotron approach overestimates RLD. As is the case for many plant systems, the fine roots were concentrated in the top 10 cm of the soil (chapter 2). One weakness of the minirhizotron techniques is the tendency to underestimate the roots at this depth due to problems such as mildew inhibited visibility (Ephrath et al., 1999; Majdi, 1996). It is more likely that the soil cores underestimate biomass, especially considering some of the very fine roots and mycorrhizal filaments may have been destroyed when the material was sieved.

Specific root length has been shown to change as nutrient availability changes. Often, SRL tends to increase as nutrient availability decreases (Ryser, 2006). Increases in SRL have been more consistently linked to P limitation than N limitation, but depending on the species, SRL can increase due to N or P limitations (Hill et al., 2006). Even though SRL's developed in this study were not significantly different, there was a slight increase in SRL for most size classes in elevated CO_2. The slight increase in SRL in this study could be due to the lower extractable P found in scrub-oak in elevated CO_2 (Johnson et al., 2003).

Our findings for total fine RLD were not different from the previous findings in the Florida scrub-oak, but there have been various responses of plant RLD in elevated CO_2. Some plants have responded with greater root length in elevated CO_2, such as *Populus grandidentata* (Zak et al., 1993). Others, such as *Larrea* and *Ambrosia* shrub interspaces in Mojave desert (Phillips et al., 2006), and California grasslands (Higgins et

al., 2002) had a significantly lower root length in elevated CO_2. Day et al. (2006) suggested the Florida scrub-oak ecosystem has reached root closure.

The results of this study suggest that the model for converting root length to biomass from minirhizotron observations is not realistic for larger fine roots. Large roots are often excluded from minirhizotron studies with ranges from < 1 mm diameter (Tierney and Fahey, 2001; Tierney et al., 2003) to ≤ 2 mm diameter (Hendrick and Pregitzer, 1993; Johnson et al., 2001b; Ponti et al., 2004). The failure of the model was probably due to the attempt to convert the minute volumes of soil to large volumes. The changes in biomass over time in the smallest most responsive size class were indicative of the changes in C investment belowground due to elevated CO_2.

CHAPTER IV

MICROBIAL METABOLISM OF WATER SOLUBLE SUBSTRATES

Introduction

As levels of atmospheric CO_2 rise, it is pertinent to investigate the effects of elevated CO_2 on plant growth and consequential effects on the C cycle. Microbial processes play a vital role in the C cycle and researchers are eager for new methods for elucidating elevated CO_2 effects on microbes and their function in C cycling. A new method of monitoring substrate-induced respiration provides increased sensitivity and a realistic estimate of substrate use and nutrient limitation, both of which can be affected by elevated atmospheric CO_2. Before examining the effects of elevated CO_2, the feasibility of using the BD Oxygen Biosensor System (BD Biosciences, Bedford, MA) (Garland et al., 2003) was evaluated on the scrub-oak ecosystem and a baseline for normal substrate use and nutrient limitations was established.

The study site is a nutrient limited system with low soil organic matter (Mulvania, 1931), making it ideal for studies on C dynamics and nutrient limitations. Also, the sandy soils of this system make it straightforward to separate the three microbial communities of interest (litter, rhizosphere and bulk soil). These Paolo and Pomello sands (Huckle et al., 1974), exhibit low pH (3.75 – 4) in the top ten cm (Schmalzer and Hinkle, 1991), facilitating an abundance of fungi in the microbial community (Madigan et al., 2000). Previous studies of the scrub-oak ecosystem showed greater soluble C, greater amounts of N contained in amino compounds and ammonia and greater microbial activity in the rhizosphere compared to the bulk soil (Schortemeyer et al., 2000). There was more

microbial DNA in the rhizosphere soil, ranging from 3.3 to 45.9 ng DNA μL^{-1}, than the bulk soil, ranging from 1.8 to 12.8 ng DNA μL^{-1} (Klamer et al., 2002), indicating greater microbial abundance in the rhizosphere.

The BD Oxygen Biosensor System (BDOBS) is a recently developed assay that utilizes a fluorescing ruthenium dye suspended in a gel in each well of a microtiter plate (BD Oxygen Biosensor system; BD Biosciences, Bedford, Mass.) (see Garland et al. in press and Garland et al. 2003 for further description). The dye fluoresces as molecular oxygen (O_2) is depleted in the microbial-substrate-nutrient solution measuring O_2 use, or less directly, substrate utilization. BDOBS was an appropriate method for examining the microbial communities in the Florida scrub-oak ecosystem because it: 1) allowed rapid assessment of the community response, 2) required low substrate concentrations, 3) allowed microbial communities to be taken directly from the environment without culturing, 4) measured oxygen consumption directly rather than CO_2 evolution, which improves sensitivity, and 5) allowed for manipulation of nutrient availability and pH (Garland et al., in press; Garland et al., 2003).

Longer incubation periods of 1 - 4 days are typical of other methods that categorize heterotrophic microbial community substrate use, but Garland et al. (2003) were able to detect a minimum response within 0.5 to 19 hrs. This made the use of BDOBS ideal for detection of community function most closely representative of field responses. The substrate concentrations can be 10 –100 fold lower than with previous methods, preventing selective enrichment of the community (Garland et al., 2003; Väisänen et al., 2005). The versatility of the system allows the use of substrates that best addressed my key questions. Natural soil carbon pools, such as leaf litter and roots were

used as substrates instead of specific carbon compounds, such as sucrose or cellobiose. This was expected to provide a realistic assessment of acclimation of microbes from a particular environment to the native substrates produced in that environment. The microbial use of C associated with different microenvironments and how nutrient limitations may regulate use of this C was of interest.

Microbial activity in the environmental samples should have a detectable response using the BDOBS without substrate amendments because of its increased sensitivity. The plate is read from the bottom, allowing detection of activity despite a heavy silt load or opacity from other environmental contamination. In the first study of soil systems using this technique, Väisänen et al. (2005) used the BDOBS to successfully distinguish between fungal and bacterial components of the communities and between the microbial communities present in different aggregate sizes. They were also able to elucidate their preferred use of specific carbon sources added as amendments, and to detect N limitations of the system using N amendments. The intent was to explore N and P limitations of different microbial communities in the scrub-oak ecosystem. Because the gel containing the fluorescing dye is the only component of the BDOBS system already prepared, all other physiochemical conditions are under the control of the investigator. Factors such as pH and nutrient levels can be manipulated (Garland et al., in press). This is vital for the scrub-oak ecosystem, where low soil pH creates a unique environment for the microbial communities. The microbial response may be altered by the neutral pH necessary for the use of some other methods.

With the oxygen biosensor system, several aspects of microbial community function were explored, laying the groundwork for a study of elevated atmospheric CO_2

effects on microbial community function (Chapter 5). The three microbial communities were expected to respond differently to the natural substrates. The litter microbial community was expected be most active due to high microbial densities found in scrub-oak litter compared to the soil (Albarracin, 2005). Microbial abundance indicated the rhizosphere microbial community should be more active than the bulk soil community. It was expected that the litter microbial community would be better adapted to litter substrates as an energy source, while soil communities would be better adapted to root substrates as an energy source. Nutrient limitations of the system were explored by how the microbial communities responded to nutrient additions. It was expected that all communities would be N and P limited, but the rhizosphere microbial community would have less extensive P limitation due to rhizodeposition induced release of phosphorus from the soil (Canadell et al., 1996).

Methods

The Oxygen Biosensor System is a recently developed assay that utilizes a fluorescing ruthenium dye suspended in a gel in each well of a microtiter plate (BD Oxygen Biosensor system; BD Biosciences, Bedford, Mass.) (see Garland et al. 2003 for further description). The dye fluoresces as O_2 is depleted in a microbial-substrate-nutrient solution. This fluorescence was measured at set time increments on a Dynex MFX Microplate Fluorometer. Fluorescence, normalized to the value at one hour (Normalized Relative Fluorescent Units: NRFU) was plotted against time to show a trace of O_2 consumption for a particular microbe or community. NRFU can be thought of as a measure of O_2 utilization or, less directly, substrate use.

Preliminary pH study

There were typically three components added to each well: the microbial community, the energy substrate and a buffer/nutrient solution. The buffer/nutrient solution has typically been buffered at a neutral pH of 7 in past studies using the BDOBS system. However, the pH of the soil from the source ecosystem is low (3.75 - 4), so a buffered pH of 7 may cause a deviation from the natural response. Preliminary pH tests were done to determine the best pH at which to conduct the study. Carbonate buffer solutions were created using $KHCO_3$ (10g/L) and the pH was adjusted to 5.1 and 7.1. These two buffers (7.1 and 5.1) were compared to an unbuffered water solution. An O horizon litter community from the scrub-oak was exposed to various substrates in the two buffered and one unbuffered nutrient solution.

Preliminary energy substrate study

A comparison was done among extracts of fresh and dried roots, litter and soil. Extracts were made by warming the solution to 46°C for 2 hours. The root and litter samples were mixed with water in a 1:5 ratio. The soil was mixed in a 1:2.5 soil to water ratio. Once the extract had been heated for 2 hr, the solution was filter sterilized. Serial dilutions were made to find the ideal concentration for the substrate. Dilutions of different substrates were exposed to a litter microbial community in the BDOBS system and fluorescence was read on a fluorometer.

Microbial community collection and preparation

Soil cores and leaf litter were taken from the eight ambient CO_2 chambers (n=8) over the summer of 2004. The rhizosphere soil and roots were removed from the cores (Figure 13). The remaining soil was sieved and constituted the source of the bulk soil microbial community. The two soil microbial communities (RMC and BMC) were diluted in a 1:2.5 soil to water ratio and shaken with sterile glass beads for 2 minutes. The supernatant was immediately poured off and diluted five times for B-doxy plate inoculation.

Approximately 5 g of leaf litter was gathered from each of the ambient chambers (n=8) and mechanically broken into small pieces 3-4 mm in size. The litter was then combined with ddH_2O in a 5 ml to 1 g ratio. The water-litter mixture was shaken for 4 minutes in a 50 ml centrifuge tube with 2 mm glass beads. The solution was then diluted to a final concentration of 1 g litter to 25 ml filter sterilized deionized water for plate inoculation.

Nutrient supplements

Three microbial communities were exposed to four levels of nutrient treatments. An unbuffered, filter-sterilized mineral salt solution was mixed using: 0.01 g $CaCl_2$/L, 0.005 g $FeSO_4$/L, 0.0025 g $MnSO_4$/L, 0.0025 g $NaMoO_4$/L, 0.1 g $MgSO_4$/L. Additions of N (0.5 g $[NH_4]_2SO_4$/L) and P (0.05 g K_2PO_4/L) were added singly and in combination to give four nutrient treatments (-N-P, +N, +P, +N+P) or two high levels of P and N and two ambient levels of P and N.

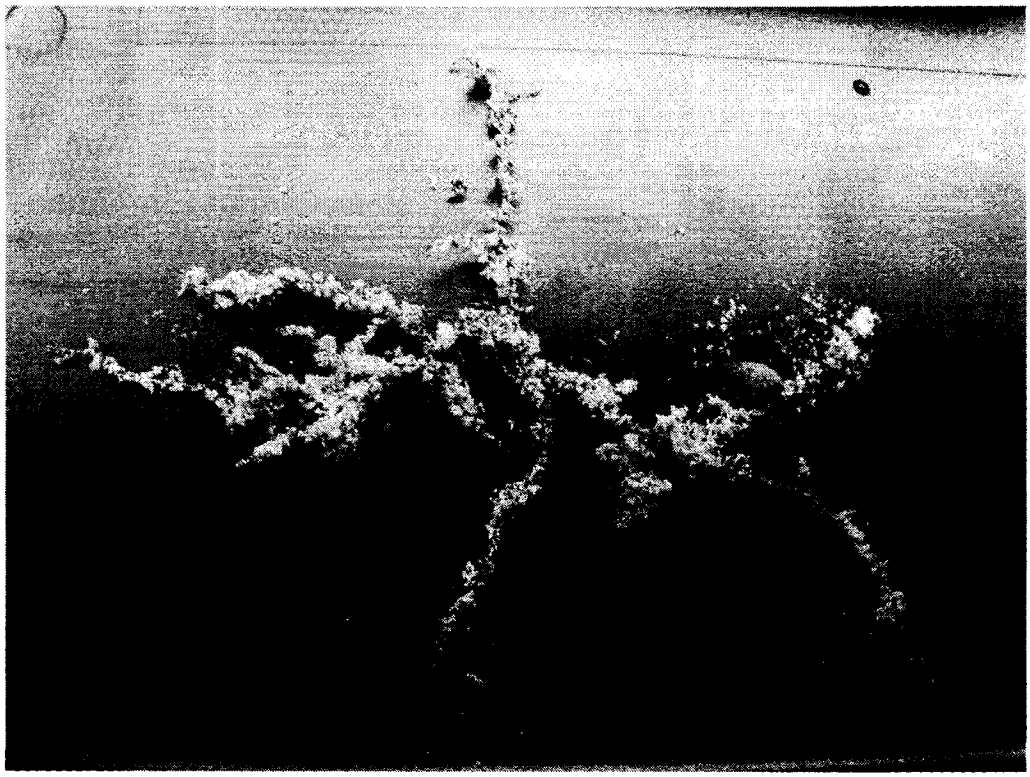

Figure 13. Roots and rhizosphere soil from scrub-oak ecosystem.

Energy substrates

Three substrates were used for energy sources for microbial growth, along with a water control to monitor the effect of background carbon associated with the microbial community. The three energy sources were made from litter extracts, root extracts, and glucose. Root and litter substrates were made from materials taken from the ambient chambers in spring 2002. These were dried at 70° C and ground to a powder. The dried tissue was mixed in a 1 g to 10 mL ratio and heated for 2 hrs at 46° C. The solution was filter sterilized through a 0.22 μm filter. After the solutions were made, preliminary tests

showed root solutions were too rich to observe the response within the parameters of the BDOBS system and consequently were diluted to 1:3 of the original concentration. Glucose substrate was mixed with water in a concentration of 600 mg/L and filter sterilized. Water was used in place of substrate as a method of monitoring the response of the microbial community to the background C associated with a community taken directly from the environment. This allowed me to distinguish how microbes responded to nutrient addition or substrate addition and helped to interpret the response of communities exposed to a natural substrate. Because the natural substrates are made from extracts of plant tissue naturally containing N and P in the tissue, the extracts presumably carry a certain level of N and P. For example, for microbes using natural substrates as an energy source, microbial response to nutrient addition is befuddled by the N and P already present in the substrate, but the microbial use of water and glucose allows the separation and interpretation of responses.

Plate loading and reading

Final dilutions for leaf litter microbial communities were 1 g litter to 25 mL water and the final dilution for soil microbial communities was 1 g soil to 12.5 mL water. B-doxy plates were loaded with 50 µl of substrate, 50 µl of nutrient solution, and 50 µl of microbial inoculums; consequently, the final solution had a concentration 1/3 of that previously described. The three microbial communities were exposed to all combinations of nutrients and substrates. The plates were put on a Dynex MFX Microplate Fluorometer plate reader and fluorescence was read every 15 minutes for 48 hours at 485-nm excitation and 604-nm wavelengths with the top reading mode. The fluorescent value

was divided by the value at one hour to obtain a normalized relative fluorescent unit (NRFU).

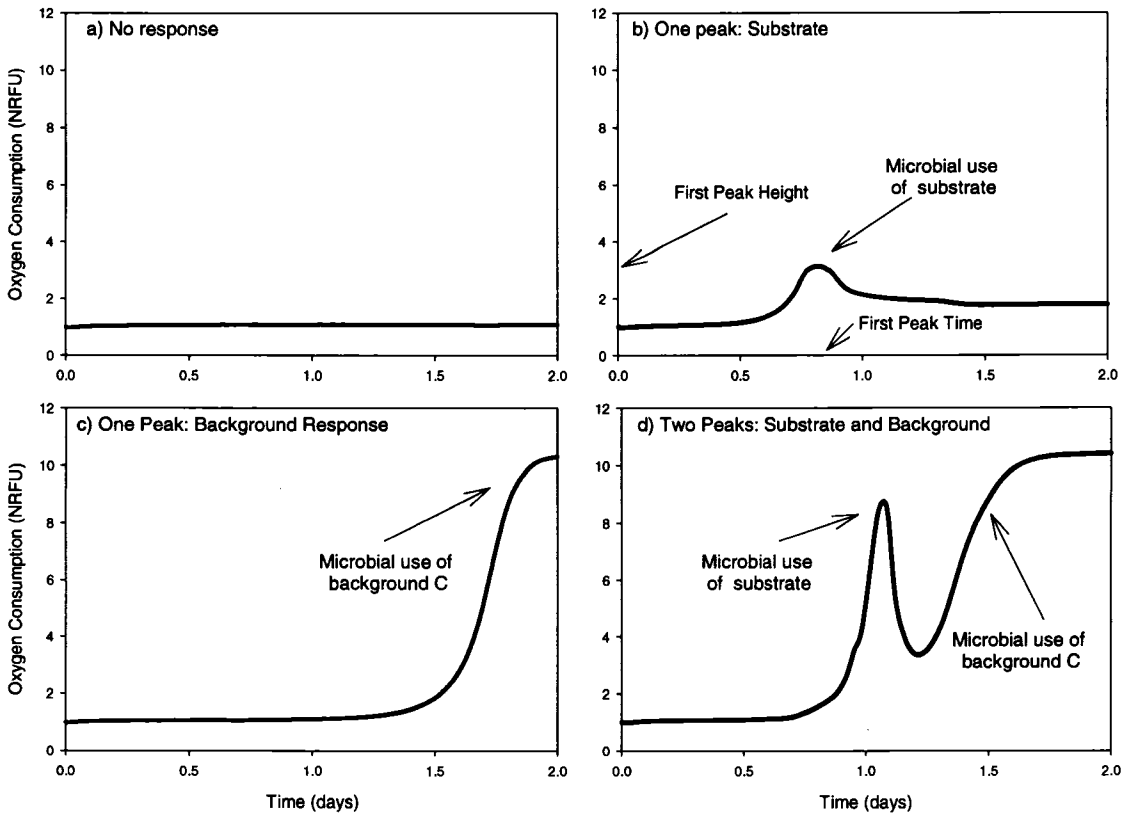

Figure 14. Four types of responses exhibited by the microbial communities. a) No response. b) One peak: when the response was a single, early peak, it indicated use of the substrate. Two of the parameters used to measure microbial response, first response and first peak height are shown. c) One peak: a late peak that continued past the 2 day mark is a response to the background C. This was only exhibited when +N+P were added, but no energy substrate was added. d) Two peaks: The first peak represents the microbial communities' use of the added substrate and the second peak represents the use of the background C.

Bdoxy data analysis

The NRFUs were plotted against time to obtain an oxygen consumption curve (Figure 14b-d). Relevant points were selected from the resulting curves of microbial community activity for analysis: 'Time to first response', 'time to first peak', and 'first peak height' (for others, see Garland et al. 2003). The 'time to first response' and 'first peak height' are illustrated in Figure 14b. Time to first response (point in time when NRFU = 1.10) is indicative of the microbial communities' readiness to utilize a substrate. The first peak height can indirectly show how much of the added substrate the microbes can use for energy. The time it takes to reach the first peak is affected by the height of the first peak, but is also indicative of the time it takes for the microbial community to maximize use of the energy source.

Statistical analyses

Three sets of data, divided by exposure to energy substrate (glucose, background carbon, and natural substrates) for each response variable (time to first response, time to first peak, and first peak height) were analyzed using an ANOVA. Significant two and three-way interactions were analyzed with the LS means post hoc test using SAS (SAS Institute 1990).

Results

Preliminary pH study

The unbuffered environment allowed the microbes to consume the most oxygen, indicating greater energy production. The litter microbial community responded faster

under natural pH environment, than under the two adjusted and buffered pH environments (pH 5 and pH 7) (Figure 15). The litter microbial community consumed the most oxygen at pH 5, but responded considerably slower and consumed less oxygen at pH 7.

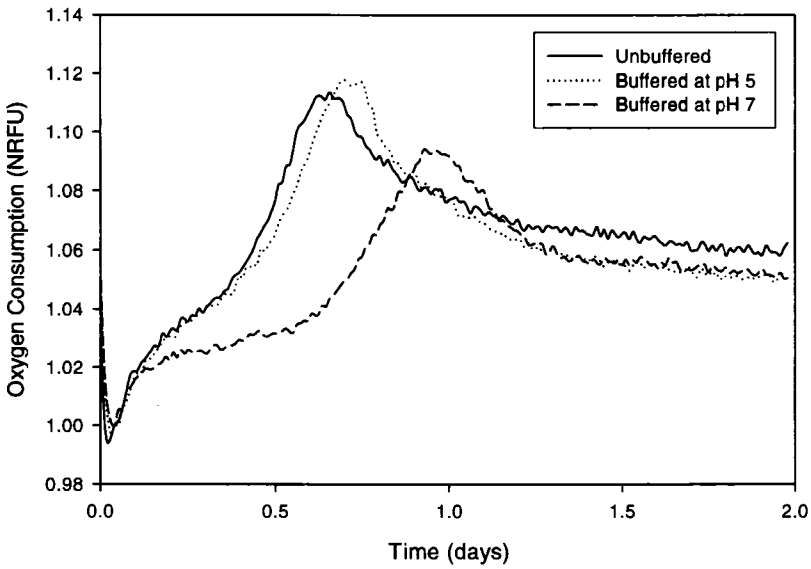

Figure 15. Example of typical litter microbial community response to soil extract under different pH environments.

Preliminary substrate studies

The microbial community respiration was highest without nutrient additions from substrates made from roots, followed by litter, with little energy being obtained from glucose and soil substrates. Root substrate was extracted from roots living at the time of harvest, while litter substrate was extracted from dead tissue, which is probably responsible for the large difference between the responses. Nutrient additions made a large difference in the microbial community's ability to use glucose. The soil solution

was a viable substrate even though the response was subtle. In soil with more organic matter, use of a soil extract may be more valuable than it appeared in this organic matter-poor soil.

Response types

There were four types of response exhibited by the microbial communities (Figure 14). First, when the microbes were unable to use a substrate, no appreciable response was exhibited (Figure 14a). Second, when the microbes were able use the given substrate, one peak was apparent early in the 48 hr period (Figure 14b). Third, when no substrate was added, the response was to the background carbon characterized by a late peak. This response was only exhibited in the high N, high P treatment (Figure 14c). The last type of response exhibited two distinct peaks. Based on the other response types, it was concluded the first peak was the response to the substrate and the second peak was the response to the background carbon associated with the microbial community inoculums (Figure 14d).

Response to glucose

Microbial community environment (i. e. litter, rhizosphere or bulk soil) ($P < 0.0001$), N level ($P < 0.0001$), P level ($P = 0.037$) and an interaction between environment and N level ($P = 0.018$) significantly affected the first response time of microbes using glucose as an energy source. High P enabled the microbial communities on average to respond faster (0.97 days) than those exposed to ambient P conditions (1.16 days). Under high N levels, the litter microbial community responded first (0.04 days), followed by the bulk

soil microbial community (0.72 days), with the rhizosphere microbial community responding last (0.96 days) (Figure 16). All microbial communities responded slower under ambient N than to high N. The litter microbial community (0.85 days) still responded before the soil communities, but the rhizosphere (1.82 days) and bulk soil (1.74 days) communities were no longer significantly different from one another (Figure 16).

Microbial community environment ($P = 0.0001$), N level ($P < 0.0001$), an interaction of N and P levels ($P = 0.0002$) and an interaction of community environment and N and P ($P = 0.046$) significantly affected the first peak time of microbes using glucose as an energy source (Figure 17). A pattern of response was exhibited by the communities where microbes from the litter responded first, followed by the soil communities. This pattern held at high N, but not at ambient N. At ambient N, the difference in response times between P levels was less distinct, while at high N, the communities under ambient P responded significantly more slowly than the communities under high P levels (Figure 17).

Nitrogen level ($P < 0.0001$), P level ($P < 0.0001$), an interaction between N and P levels ($P < 0.0001$) and an interaction microbial community environment and P ($P = 0.025$) significantly affected the first peak height of microbes using glucose as an energy source (Figure 18). These variations of response can be explained by looking at the community environment, N, and P interaction despite its lack of significance ($P = 0.08$). Only under high levels of both N and P were the rates of oxygen consumption rapid (litter microbes = 9.5 NRFU, rhizosphere microbes = 10 NRFU, bulk soil microbes = 9.3 NRFU). Under ambient N or P conditions, first peak heights ranged from 1.07 to 1.23

NRFU, with one exception. Under high N and ambient P, the litter community was able to consume significantly more oxygen (1.89 NRFU) than the soil communities. This indicated the litter community was less P limited than the soil communities, but this limitation was secondary to N limitation.

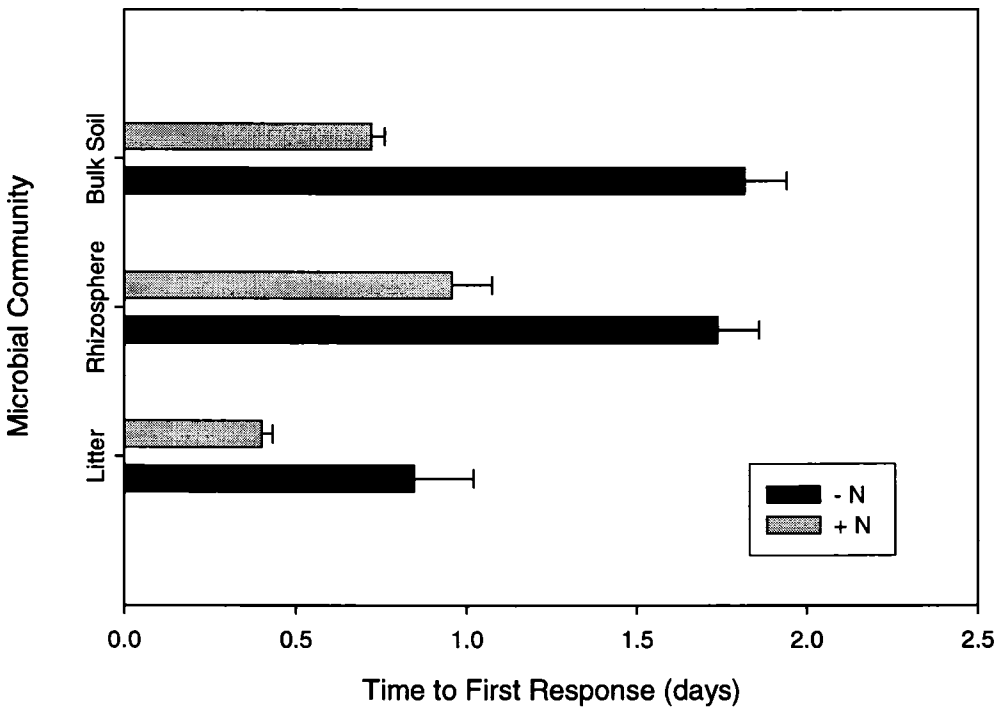

Figure 16. Effects of N and community environment on first response time of microbes using glucose as an energy source. The error bars represent one standard error.

Response to background C

Microbe environment ($P < 0.0001$) and N level ($P < 0.0001$) affected the first response time for microbes using the background C (environmental soil C associated with the microbial community) (Figure 19). The litter community responded significantly faster (0.76 days) than the soil communities while the rhizosphere microbe response (1.49 days)

was indistinguishable from the bulk soil microbe response (1.45 days). High N increased the response of all microbes (0.84 days) compared to the ambient N response (1.63 days).

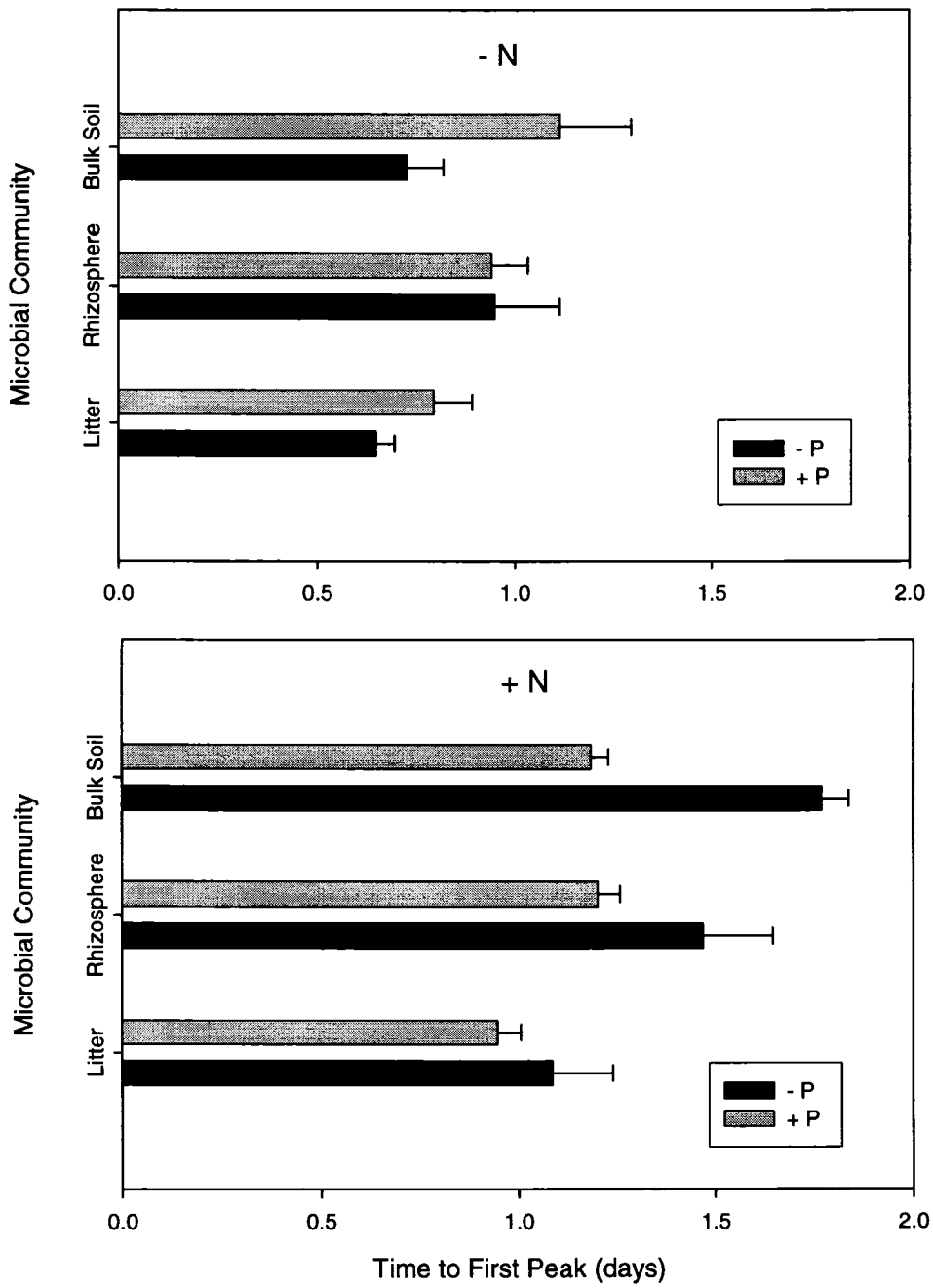

Figure 17. Effect of N, P, and community environment on first peak time of microbes using glucose as an energy source. The error bars represent one standard error.

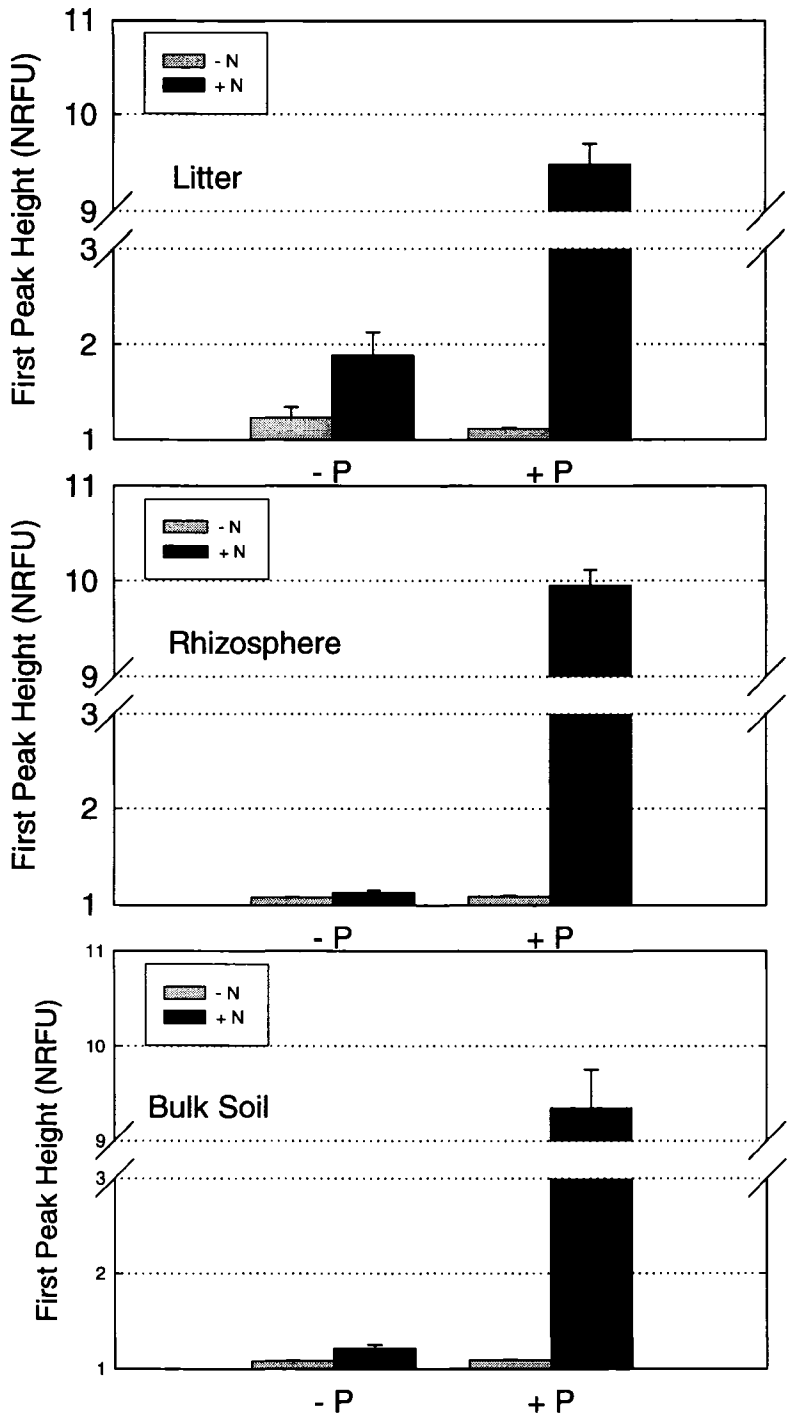

Figure 18. Effect of N, P, and community environment on the first peak height of microbes using glucose as an energy source. The effects were not significant (P = 0.08). The error bars represent one standard error.

N level ($P < 0.0001$) and the interaction between microbial community, N and P ($P = 0.018$) affected the time to first peak for microbes using background C as an energy source. Overall, high N delayed time to first peak of microbes (1.80 days) compared to microbes at ambient N (0.85 days). This trend generally held when the responses were categorized by P levels and microbial community environment (Figure 19). The litter community reached the first peak the fastest under ambient N conditions, significantly faster than the slowest soil response under ambient N (bulk soil under high P = 1.19 days), with the other soil responses under ambient N ranging in between (Figure 19). The times to first peak of the microbial communities under high N conditions ranged from 1.66 to 1.98 days and were all significantly slower than the time to first peak of microbes under ambient N. The one exception was the rhizosphere community under ambient P and high N (1.55 days) did not reach the first peak significantly faster than the bulk soil community under high P and ambient N (1.19 days) (Figure 19).

Nitrogen level ($P < 0.0001$), P level ($P < 0.0001$) and an interaction between the two ($P < 0.0001$) had an effect on the first peak height for microbes using the background C as an energy source. When using background C as an energy source, the microbes regardless of environment were unable to use the background carbon to any extent under ambient N with either high or ambient P. Under high N and ambient P, there was only slight oxygen consumption, but with high P and N the scrub-oak microbes had extensive oxygen consumption (Figure 20).

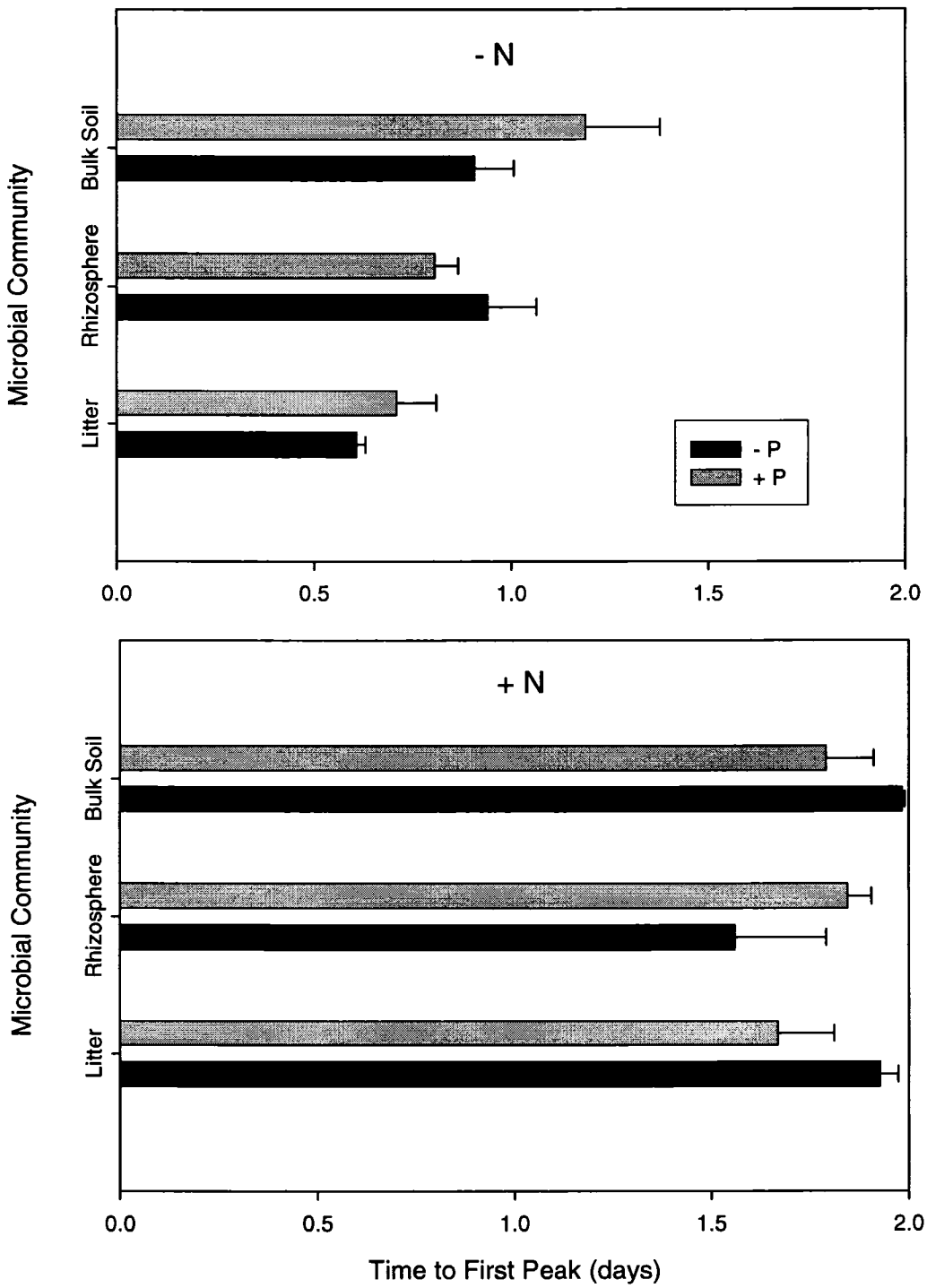

Figure 19. Effect of N, P, and community environment on first response time of microbes using background C as an energy source. The error bars represent one standard error.

Response to natural substrates

Community environment ($P < 0.0001$) and substrate source ($P < 0.004$) were the most important factors affecting time to first response of microbes utilizing natural substrates as an energy source. The litter responded the fastest (0.39 days), followed by bulk soil microbes (0.55 days) and last by rhizosphere microbes (0.61 days). The microbial communities responded significantly faster to root extract (0.50 days) than the litter extract (0.53 days).

Community environment ($P < 0.0001$) and N levels ($P < 0.0001$) were the most important factors in the time to first peak while utilizing the natural substrates as an energy source. The litter microbes reached the first peak the fastest (0.85 days), while the soil communities were indistinguishable (rhizosphere microbes: first peak time of 1 day, and bulk soil: first peak time of 0.84 days). The microbial communities under ambient N conditions responded significantly faster (0.86 days) than microbes under high N conditions (1.01 days).

Community environment ($P < 0.0001$), N levels ($P < 0.0001$), substrates source ($P < 0.0001$) and the interaction of those three were the most important factors in the first peak height while utilizing the natural substrates as an energy source. Phosphorus had no effect. The interaction of the community environment, N, and substrates source ($P = 0.01$) revealed that under ambient N, the peak heights followed the pattern of litter microbes consuming more oxygen than the soil microbes. The microbes consumed more oxygen when exposed to root substrates when compared to litter substrates (Figure 21a). However, differences in these two patterns were not significant under ambient N. Under

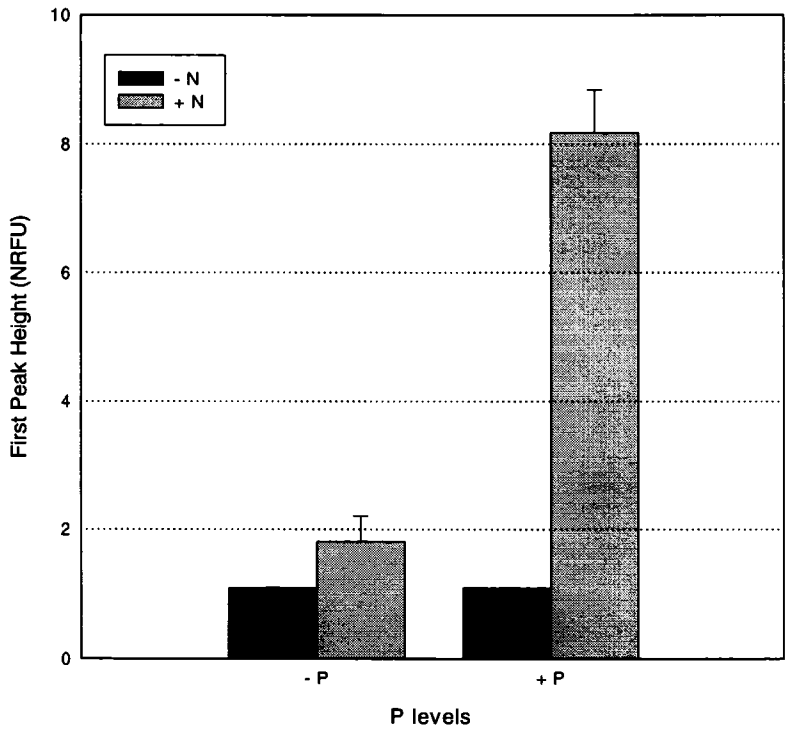

Figure 20. Effect of N and P on first peak height of microbes using natural substrates as an energy source. The error bars represent one standard error.

high N, this general pattern also held, but the differences were greater (Figure 21b). The litter microbes seem to be better adapted to using litter substrates. Under high N conditions, all microbial communities were able to obtain a large amount of energy from root substrates (Figure 21b). The litter microbes were able to utilize litter substrates to a greater extent than the soil microbes under high N (Figure 21b). The oxygen consumption of litter microbial communities utilizing litter substrate was more than twice the oxygen consumption of soil microbial communities utilizing litter substrates. This indicated an acclimation to the use of litter extracts by litter communities.

Overall, it was found that natural substrates could be used to distinguish microbial communities of different environments from one another (Figure 22). Litter microbes

responded fastest for both first response time and first peak time while the first peak height indicated litter microbes were able to obtain the most energy from natural substrates (Figure 22). The bulk soil microbes followed the litter microbes in these trends, and rhizosphere microbes were last to respond with the smallest first peak (Figure 22). However, the height of the first peak for the community response was confounded by N and substrate source, so these must be taken into consideration and first peak height alone cannot be used to distinguish microbial communities (Figure 22).

Nutrient effects

The addition of N had an effect on almost all response variables of the microbial communities using all substrates. The only response not affected by N was time to first response of microbial communities using natural substrates (Figure 23). The addition of N allowed an extensive use of the natural substrates, while the use of background carbon and glucose was conditional upon whether P was also added. If the microbial communities were utilizing the natural substrate, it was not necessary to add P to elicit a large response. To demonstrate this, responses were scored on whether or not the microbial community responded to the background C with a NRFU of ≥ 8 (Figure 14d) and the percentage of times this occurred is shown in Table 6. Microbial communities were only able to have a large response to N addition alone with the use of natural substrates. Addition of both N and P allowed the microbial communities to have a large response to background carbon when utilizing added glucose or background C alone. This large response to background C was never achieved when N was not added.

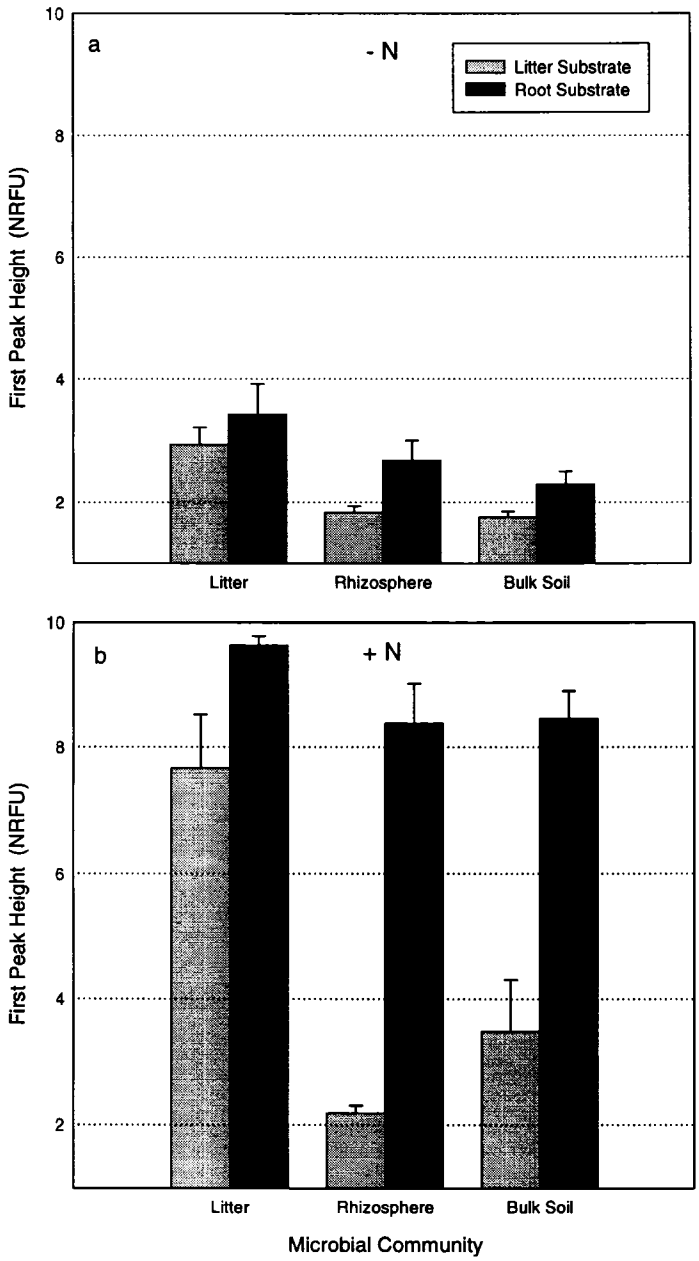

Figure 21. Effect of N, P, and community environment on first peak height of microbes using natural substrates as an energy source. The error bars represent one standard error.

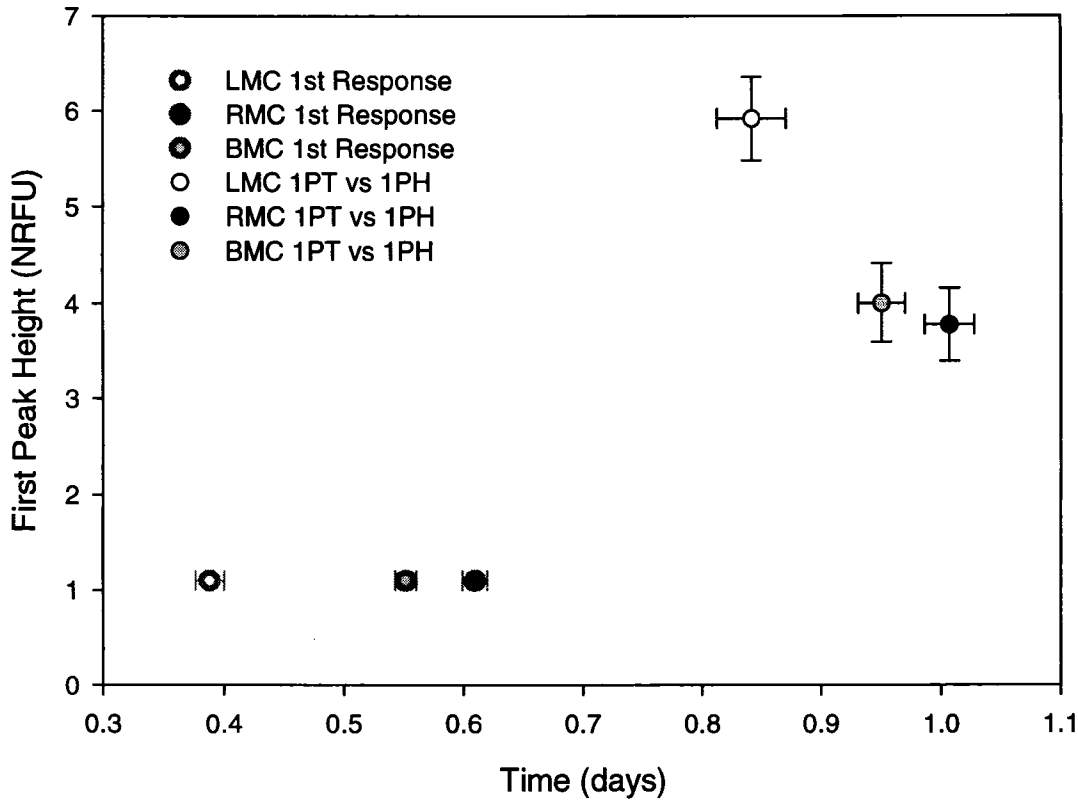

Figure 22. Microbial communities' response to natural substrates (litter and root extracts) of litter microbial community (LMC), rhizosphere microbial community (RMC), and bulk soil microbial community (BMC). 1st Response represents time to first response. The height of the first peak (1PH) is plotted against the time to reach the first peak (1PT) for each microbial community. The bars represent one standard error for each measurement.

microbial community responded to the background C with a NRFU of ≥ 8 (Figure 14d) and the percentage of times this occurred is shown in Table 6. Microbial communities were only able to have a large response to N addition alone with the use of natural substrates. Addition of both N and P allowed the microbial communities to have a large response to background carbon when utilizing added glucose or background C alone. This large response to background C was never achieved when N was not added.

The first response time and first peak time were affected by N addition, but not in a consistent pattern. This lack of consistency was probably influenced by whether a high first peak was achieved, because this would have delayed the time to the first peak. For microbial communities responding to glucose and the background carbon, N addition sped up time to first response, but did not affect first response time of microbial communities utilizing natural substrates (Figure 23). For the microbial communities using glucose, high N levels reduced the difference between the times to first response of the individual communities so litter, bulk soil and rhizosphere microbes were no longer distinguishable from one another. Nitrogen delayed overall time to first peak in many cases, regardless of whether a high first peak was achieved or not. Phosphorus addition also significantly sped up the time to first response for microbial communities utilizing glucose as an energy source, but did not affect the time to first response for the microbes using background C or natural substrates.

Table 6. Percentage of microbial community response to background C where the peak was greater than 8 NRFU.

	Nutrients	Litter Substrate	Root Substrate	Glucose	Background C
Litter Microbial Community	-N-P	0	0	0	0
	+N	100	100	0	12.5
	+P	0	0	0	0
	+N+P	100	100	100	75
Rhizosphere Microbial Community	-N-P	0	0	0	0
	+N	100	93	0	0
	+P	0	0	0	0
	+N+P	100	100	100	87.5
Bulk Soil Microbial Community	-N-P	0	0	0	0
	+N	100	93	0	0
	+P	0	0	0	0
	+N+P	93	100	100	62.5

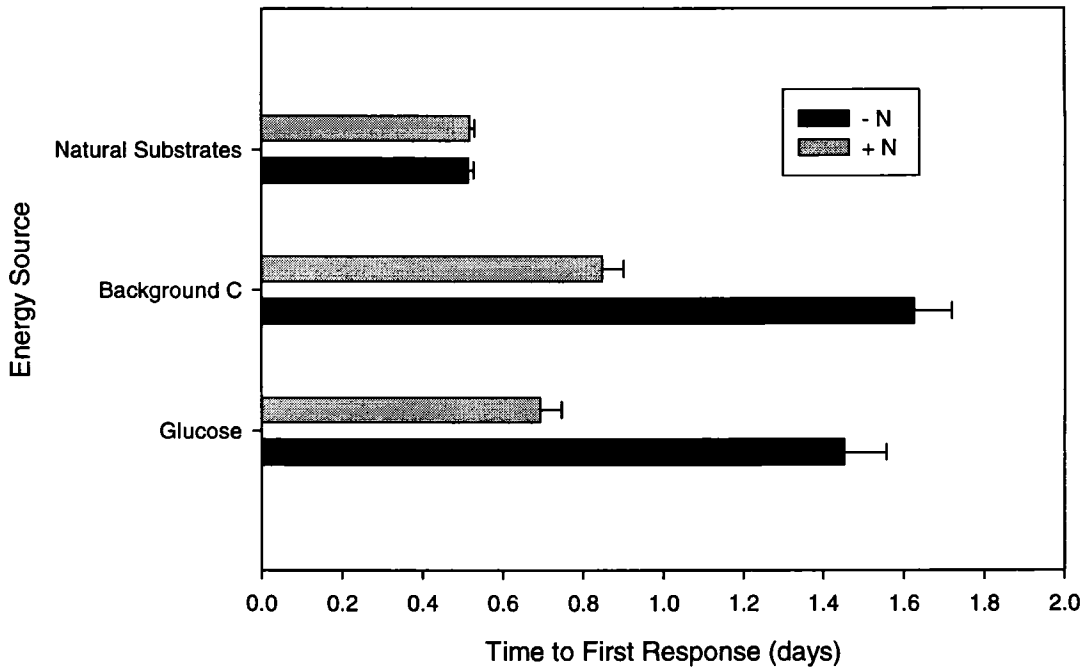

Figure 23. Effect of N addition on the first response time of microbes using glucose, background C and natural substrates as energy sources. The error bars represent one standard error.

Discussion

Preliminary pH studies

The ambient pH of the system was naturally 3.75 – 4.00, which is more acidic than the typical circumneutral pH used in microbial studies. Microbial communities responded to buffered pH of 5 or the ambient pH. The delay in response at pH 7 was probably due to microbes recovering from the change in pH. Kurzatkowski et al. (2004) found there was no relation between pH and microbial activity or biomass, but in their study the microbes were in their native pH at the time of measurement. The other explanation is that the delay may have been due to a shift in composition of microbial communities from a fungi dominated community to a bacteria dominated community as low pH favors fungi

(Madigan et al., 2000). Because the objective was to compare microbial communities at ambient pH, the unbuffered conditions were used for this study.

Effect of nutrient addition on microbial response

Addition of N affected every parameter measured regardless of which substrate was added, with the exception of the first response time of the communities exposed to natural substrates. It has long been observed that addition of N depresses or causes delays in respiration and decomposition rates or causes a reduction in microbial biomass (Agren et al., 2001; Fog, 1988). The results of this study were a mixture of responses, where N addition either delayed or accelerated the response, but a consistent delay or depression of response observed by other researchers was not demonstrated. In the cases where there was a delay of response, it was in the time to first peak and was caused by increased oxygen consumption leading to an increased first peak height. This was because the response under high N was larger overall. It has been suggested that depression of response under high N was due to a reaction with phenol to form a recalcitrant compound (Haynes, 1986), but this requires high pH (Nömmik, 1970) and was not likely to occur in the scrub-oak system. Agren et al. (2001) concluded the depression in microbial biomass may have been due to an increase in efficiency of the remaining microbes. The depression observed in this study may have been due to an increase in efficiency, a shift in composition of the community or increased competitive advantage of some members of the microbial community.

Many studies have revealed increased microbial respiration in response to N addition, often linked to C availability in the soil (Allen and Schlesinger, 2004; Jonasson

et al., 1996; Vance and Chapin, 2001). Addition of labile C stimulated microbial respiration in Allen and Shlesinger's study (2004) and Vance and Chapin's study (2001), while the addition of sugar increased microbial C in Jonasson et al.(1996). This was not observed in the current study. When glucose was added with no nutrient amendment, there was no oxygen consumption by the microbes. The addition of N alone to the soil in Allen and Schlesinger's study (2004) also stimulated microbial respiration, but again this was not observed. In the scrub-oak, addition of glucose only elicited a response in conjunction with N and P amendment. Vance and Chapin (2001) found the response to N addition was greatest when there was abundant C availability. Both Vance and Chapin (2001) and Allen and Schlesinger (2004) found a significant interaction of C and N addition, but neither required the addition of P for stimulation of microbial activity. Phosphorus addition significantly decreased the time to first response for microbial communities utilizing glucose as an energy source. This effect could be related to an increased ability to generate ATP and process glucose as an energy source for cell maintenance, but may not necessarily lead to an increase in cellular biomass or cell replication unless N limitation is also removed. Interestingly, there seemed to be C available in the soil (referred to as background C) that was not utilized due to the limitation of N and P. These results confirm the severe nutrient limitation present in this system.

The response pattern was greatly influenced by N addition when microbes were allowed to use natural substrates, but not by P addition. The natural substrate may contain enough P to release microbial communities from limitation. There was a small amount of

oxygen consumption under ambient N, but that increased greatly with N addition for the root substrates.

Effect of native environment on microbial response

As expected, the litter microbial community was the most responsive, responding faster and consuming more oxygen than the other communities. Studies have shown higher abundance of microbes in the litter layer than soil with bacterial counts (Berg et al., 1998; Pietikäinen et al., 1999) and respiration measurements (Kurzatkowski et al., 2004). Microbial attributes such as biomass (Fierer et al., 2003), capacity for substrate use (Griffiths et al., 2003), and respiration (Kurzatkowski et al., 2004) have been shown to decrease with depth. It was suggested that decreasing C availability caused changes in microbial functions as depth increased (Fierer et al., 2003; Goberna et al., 2005) and that C availability was a major factor in determining microbial community composition (Drenovsky et al., 2004). This pattern is likely to be particularly strong in the scrub-oak ecosystem, where the soil is particularly low in organic matter. Albarracin (2005) found there were significantly more culturable cells in the litter using general nutrition plates than the soil in Florida scrub-oak, and the technique used in the current study primarily observes reactions of the heterotrophic community. These specific subsets of the microbial communities overlap, leading to the conclusion that increased response of litter over soil communities observed using the oxygen biosensor system is due to increased microbial numbers observed using plating techniques.

The microbial communities of the rhizosphere and bulk soil are expected to respond differently as they have different environments, C sources and nutrient

availability. The C source for the bulk soil community is most likely leachate from the litter at the surface, while the C source for the rhizosphere may be dominated by exudates from roots (Pietikäinen et al., 1999). The rhizosphere was described by Rovira et al. (1983) as variable in extent (volume of soil surrounding the root) and composition (types of root exudates and microbes). The microbe population was always higher on the rhizoplane compared to the rhizosphere, but not always significantly so (Dhillion and Anderson, 1994). Rhizodeposition, described as organic inputs of living roots into the rhizosphere (Paterson et al., 1997), has potential to change the nutrient conditions of the surrounding soil by increasing P availability (Canadell et al., 1996). Because of these differences, a larger divergence was expected between the responses of the rhizosphere and bulk soil microbial community. Butler et al. (2003) and Steer and Harris (2000) found few differences in PLFA profiles and microbial biomass respectively between the rhizosphere and bulk soil microbes. The response of the scrub-oak communities typically revealed the bulk soil community as more responsive than the rhizosphere microbial community, but the differences were not always significant. There may be several explanations for the unexpectedly slow rhizosphere response. The rhizosphere is a gradient of compounds, nutrients and root exudates, varying in concentration from the root surface to the surrounding soil. The microbes may be specifically adapted to their position within the gradient. Disturbance to these microenvironments may have delayed recovery time. The bulk soil is a more homogeneous environment and disturbance may not have as adversely affected the microbes.

Acclimation of litter microbial community to native substrate

Acclimation of microbial communities to their native conditions has been observed in other studies. Elliot et al. (1993) found microbes decomposed litter most rapidly in their parent forests. The acclimation of the litter microbial community to the litter substrate was evaluated by examining the substrates. Caloric content was found for each material used to make natural substrates (chapter 2). Organic matter from the O horizon of soil used to make the leaf litter substrate had a caloric value (5340.7 calories / g) greater than live roots (< 0.25 mm roots: 5208.7 cal / g, 0.25-1 mm roots: 5000.6 cal / g, 1-2 mm roots: 4816.7 cal / g, 2-10 mm roots: 4613.5 cal / g). The smallest roots (< 0.25 mm) were the only size class with a caloric value comparable to leaf litter. However, the smallest roots were not used to make the root substrate. This indicated energy in litter substrates was higher than energy in root substrates. Yet, during preliminary tests, response to the root substrates was too great to be observed within the parameters of the BDOBS system and were consequently diluted. This was not surprising considering the root substrate was made from tissue living at the time of harvest. The types of carbohydrates in roots may be more available to microbial communities than those in the litter. Readily available carbohydrates present in the litter at the time of senescence were probably used quickly, leaving material with high-energy bonds behind. Also, even though the caloric values show there is more energy in litter than roots, it is necessary to remember that substrates were made from extracts. The first few weeks of litter decomposition were characterized by leaching of elements; cations were leached in the order K>> Na > Mg > Ca > Mn ~Al > Fe and anions were leached in the order Cl >> SO_4 > ortho P ~ HCO_3 (Tietema and Wessel, 1994). Leaching of dissolved organic compounds occurs during litter

decomposition (Berg et al., 1982; Yavitt and Fahey, 1986). For this reason, the largest responses was to the root extracts regardless of microbial community environment, especially under high N conditions. The high amount of energy in the litter extract seemed to be only available to the litter microbial community, but only under high nitrogen conditions. The soil microbial communities were unable to obtain large amounts of energy from the litter substrates, even under high N conditions. They may not have the ability to produce the needed enzymes to cleave the leaf litter carbohydrates and complex humic chains. Or they may not have the necessary members of the microbial community to begin the breakdown process of this material. The conclusion was the litter microbial community had the ability to degrade the litter, but lacked the N necessary to perform the task; whereas, soil microbial communities were not primed to extensively degrade leaf litter.

Conclusions

Use of the oxygen biosensor system allowed observation of the response of nutrient additions on microbial community oxygen consumption. The system was N limited, preventing the use of C available in this system, unlike other systems where C is the limiting factor for microbial communities. The litter community was more responsive than the soil communities, probably due to higher abundance of microbes typically found in the litter layer. Differentiation between the responses of the two soil communities was less pronounced than expected, but still present. Finally, there was evidence of the acclimation to the degradation of litter extracts by litter communities under high N conditions when compared to soil communities.

CHAPTER V

NUTRIENT LIMITATION AND SUBSTRATE QUALITY EFFECTS ON SOIL MICROBES

Introduction

The assimilation of C by terrestrial systems removes CO_2 from the atmosphere, thereby potentially slowing the pace of climate change. However, this removal is temporary unless the biomass is converted to long-term storage woody tissue or soil C and there are many steps between the production of plant carbohydrates and their long-term storage in the soil. Any excess carbohydrates not directly incorporated into biomass, but allocated instead to transient pools such as rhizosphere exudates and mycorrhizal support, must be retained long enough in the system to become incorporated into a more recalcitrant C pool. Microbial communities facilitate both decomposition and C retention in the soil, and play a large role in carbon sequestration in the soil. Microbes are unlikely to be directly affected by elevated CO_2, but secondary effects such as changes in energy substrate composition and changes in nutrient cycling may influence microbial composition and community function (Paterson et al., 1996).

Surface litter, root litter and rhizosphere exudation are three energy sources for microbes that can change in quantity and/or quality in response to elevated atmospheric CO_2. Elevated atmospheric CO_2 has been shown to stimulate leaf litter production(Allen et al., 2000; Delucia et al., 1999; Niklaus et al., 2001; Schlesinger and Lichter, 2001) root biomass production (Jongen et al., 1995; Matamala and Schlesinger, 2000; Wiemken et

al., 2001) and root exudations (Norby, 1994; Paterson et al., 1996; Zak et al., 1993). Several CO_2 studies have shown effects of elevated CO_2 that include increased leaf C:N ratio (Hall et al., 2005b; Johnson et al., 2003; Rouhier et al., 1994) or decreased foliage N concentration (Cotrufo et al., 1998a; Curtis and Wang, 1998). This has potential to result in poor litter quality, thereby decreasing the rate of decomposition. However, others have found little difference in the C:N ratio once leaves are senesced (Allen et al., 2000; Hall et al., 2006; Johnson et al., 2003).

Increased root production may provide a greater energy base for microbial communities in elevated CO_2 when compared to ambient CO2 conditions. It has been shown plants produce more root length and biomass at elevated CO_2 than ambient(Curtis et al., 1990; Day et al., 1996; Dilustro et al., 2002; Ineson et al., 1996; Jach et al., 2000; King et al., 2001; Lipson et al., 2005; Norby, 1994), and this will increase the energy resources available to microbes through root litter (Jach et al., 2000). The C:N ratio of roots can be increased by elevated CO_2 (Cotrufo and Ineson, 1995; Curtis et al., 1990; Lewis et al., 1994), possibly creating a poorer quality of substrate for microbes. Rhizodeposition can also increase at elevated CO_2 (Cheng and Johnson, 1998; Norby et al., 1987; Paterson et al., 1996), stimulating rhizosphere microbial activity. I believe that in nutrient poor systems, the microbes cannot make use of all root litter and exudates and surface litter. Also high C:N ratios may further retard decomposition despite increased rhizodeposition, and surface and root litter.

As plants produce more biomass at elevated CO_2, nutrient demand increases to support the biomass and plants typically invest in roots to acquire more nutrients. Elevated CO_2 has been shown to increase root biomass (Curtis et al., 1990; Ineson et al.,

1996; Lipson et al., 2005; Norby et al., 1987), mycorrhizal associations (Norby et al., 1987), and rhizosphere exudates (Cheng and Johnson, 1998; Norby et al., 1987; Paterson et al., 1996). Root exploration of the soil increases access to nutrients in the soil. An increase in root exudations can stimulate release of P and N from the soil through changes in microbial activity (Canadell et al., 1996; Norby et al., 1986; O'Neill, 1994; Zak et al., 1993), enhancing mobilization of limiting nutrients (Farrar et al. 2003). Rhizosphere microbes can also increase the C sink strength of a system, drawing more carbohydrates from the plant into the soil, acting as important regulators of carbon flow (Canadell et al., 1996; Paterson et al., 1997). An increase in rhizosphere exudates in soil will also increase microbial activity (Paul and Clark, 1989) and breakdown of organic matter, decreasing potential C storage in the soil. Many researchers have predicted increases of root exudation and, consequently, changes in microbial communities at elevated atmospheric CO_2 (Allen et al., 2000; Bazzaz, 1990; Paterson et al., 1997; Pritchard and Rogers, 2000). Many others have found stimulation of root exudation at elevated atmospheric CO_2 (Norby, 1994; Paterson et al., 1996; Zak et al., 1996). Canadell et al.(1996) and Cheng and Johnson (1998) found a 60% increase in root-exuded carbon in elevated CO_2. Elevated CO_2 also affected nutrient availability by increasing phosphate-dissolving bacteria (O'Neill, 1994) and by increasing phosphate availability in a study of *Quercus alba* (Norby, 1994). It has been suggested that increased soil respiration observed in elevated atmospheric CO_2 was due to increased rhizosphere activity (Andrews and Schlesinger, 2001; Körner and Arnone, 1992). Elevated CO_2 stimulated microbial biomass in both the rhizosphere and bulk soil (Zak et al., 1993). Increased

nutrient uptake by plants to support greater biomass may limit ecosystem cycling of the nutrients, resulting in competition between plants and microbes.

Elevated CO_2-facilitated changes in energy or nutrient availability in an ecosystem may have different effects on soil microbial communities, depending on their environment and primary energy source. The microbial communities of interest in this study were from the leaf litter, rhizosphere soil and bulk soil. Community response to CO_2-facilitated changes may have different effects on how C is stored long-term in the soil. The purpose of this study was to examine CO_2 effects on 1) environmental factors that may influence microbes, such as soil pH, 2) microbial respiration and use of native substrates, and 3) N and P limitations of microbial metabolism.

Greatest microbial activity was expected in the elevated CO_2 litter community because this treatment has the greatest amount of litter input (Johnson et al., 2003). An effect of elevated CO_2 on nutrient limitation was not expected because the altered N concentration of live leaf tissue is no longer present once the leaves senesce (Hall et al., 2005b; Hall et al., 2006; Johnson et al., 2003). It was hypothesized that there would be a greater amount of root exudation in the rhizosphere at elevated CO_2 than ambient CO_2. Consequently, altered pH and more microbial activity in the rhizosphere microbial community were expected. It was hypothesized that greater root biomass and root litter would lead to greater soil microbial community activity and biomass at elevated CO_2. A greater response of elevated CO_2 than ambient CO_2 soil microbial communities to addition of P was expected due to the significantly lower soil extractable P (Johnson et al., 2003). Overall, evidence of progressive N limitation (reduced cycling of N because of excessive immobilization of N in biomass) of soil communities in elevated CO_2 was

expected due to increased storage of N in aboveground biomass and the O horizon (Hungate et al., 2006).

Methods

BDOBS data collection

The microbial communities were collected and prepared in the same manner as described in chapter 4, but were collected from the elevated CO_2 chambers as well. The nutrient supplements and energy substrates were the same solutions as used in chapter 4, but there were also root and litter extracts prepared from substrates grown at elevated CO_2. The elevated litter, ambient litter, elevated root, and ambient root substrates are collectively referred to as the "natural substrates". "Substrate quality" refers to the growth history of the substrate: whether the root or litter material used to make the substrate was grown in elevated or ambient CO_2 conditions. Plate loading, reading and BDOBS data analysis were identical to those described in chapter 4. Two more parameters (in addition to 'first peak height', 'first peak time' and 'time to first response') for examining the response were used in this study. To examine the effect of nutrient addition, the response without nutrient amendment was subtracted from the response with nutrient amendment to quantify the relative response of microbes using glucose or background C as a substrate. The area under the curve was also calculated as a measure of total microbial community oxygen consumption under various conditions. The units for the area under the curve are NRFU per unit time, which is referred to as the 'total O_2 consumption' to avoid confusion. Three sets of data, divided by exposure to energy substrate (glucose, background carbon, and natural substrates) for each response variable (time to first

response, time to first peak, and first peak height, area under the curve) were analyzed using an ANOVA. Significant two and three-way interactions were analyzed with the LS means post hoc test using SAS (SAS Institute 1990).

DOC and pH

Before the dilution of the microbial community for inoculation of the B-doxy plate, the soil pH was measured in a soil slurry in a 1:2 soil water ratio using a pH electrode. After plate inoculations, the soil solutions were filtered through a 0.45 μm filter, stabilized with H_2PO_4 acid and analyzed for dissolved organic carbon (DOC) using high temperature combustion on a Apollo 9000 (Tekmar Dohrmann). Both pH and DOC were analyzed using a split-plot ANOVA, where rhizosphere and bulk soil samples were subplots.

Microbial C and N

The microbial C and N from the bulk soil were determined using the chloroform-fumigation-extraction technique introduced by Brookes et al. (1985) and improved by Vance et al.(1987). Carbon and N content of microbial biomass was quantified by Dumas combustion (NC 2100; CE Elantech, Lakewood, New Jersey, USA), followed by continuous flow isotope ratio mass spectrometry ($DELTA^{plus}$ –XL; Themoelectron Corporation, Bremen, Germany) at the Colorado Plateau Stable Isotope Laboratory. Microbial C and N were analyzed using ANOVAs.

Microbial P

Microbial P was extracted using the method described by Hedley and Stewart (1982), with anionic resin strips from GE Ionics, Inc.

Results

Soil pH and soil DOC

There were no effects of CO_2 water extractable DOC or pH, both of which might alter microbial response in elevated CO_2. Dissolved organic carbon in the soil solutions was not significantly different among CO_2 treatments. The rhizosphere had a significantly lower concentration of dissolved carbon compared to bulk soil ($P = 0.01$). Soil pH was not significantly different for bulk soil verses rhizosphere soil or by CO_2 treatment ($P = 0.43$).

Microbial C, N, P

There was no significant CO_2 treatment effect on microbial C ($P = 0.84$) or N ($P = 0.81$) in bulk soil samples. This was consistent with the findings of Schortmeyer et al. (2000) in samples taken in 1998 from the scrub-oak ecosystem, where there was no CO_2 effect on microbial C or N. My attempts to sample microbial P were unsuccessful. This may have been because microbial P in this nutrient poor system was below the detection limit of the technique. It is interesting to note that of the unusable samples (6 of 16), 5 were from the elevated CO_2 treatment. This implied there was less P in the microbial biomass in the elevated CO_2 bulk soil, but further testing would be required to confirm this. It is supported by the findings of Johnson et al. (2003), who found the soil from elevated CO_2

chambers had significantly lower extractable P than ambient CO2 soils in the Florida scrub-oak ecosystem.

Microbial community total oxygen consumption

The most basic measurement of microbial heterotrophic activity was total oxygen consumption for the 48-hour period. The microbial communities were co-limited by N and P because a large response was possible only with +N+P (Figure 24a, b). The litter communities subjected to +N-P consumed considerably more oxygen than the soil communities under the same treatment (Figure 24). This indicated P limitation for the litter community was less severe that the P limitation of the soil communities, but this was secondary to N limitation. The findings here confirm another pattern established in chapter 4, where the litter community was more responsive than the soil communities. The litter community consumed an average of 8143 units of total O_2, while the rhizosphere community consumed a total of 5808 units and the bulk soil consumed 6162 units.

N, substrate quality and CO_2 effects on O_2 consumption

The two measures of the amount of microbial oxygen consumption, area under the curve and first peak height (as opposed to responsiveness; time to first response and first peak), were affected by substrate quality for the litter and rhizosphere microbial communities. Substrate quality represents the substrate's CO_2 growth history (i. e. litter or root extract made from litter or roots grown in elevated CO_2 conditions). Substrate quality effected the litter community's total oxygen consumption ($P = 0.03$) (Figure 25). The litter

microbial community respiration was greater when using elevated CO_2 substrates than ambient CO_2 substrates (Figure 25). The rhizosphere and bulk soil communities did not differentiate between substrate quality (Figure 25).

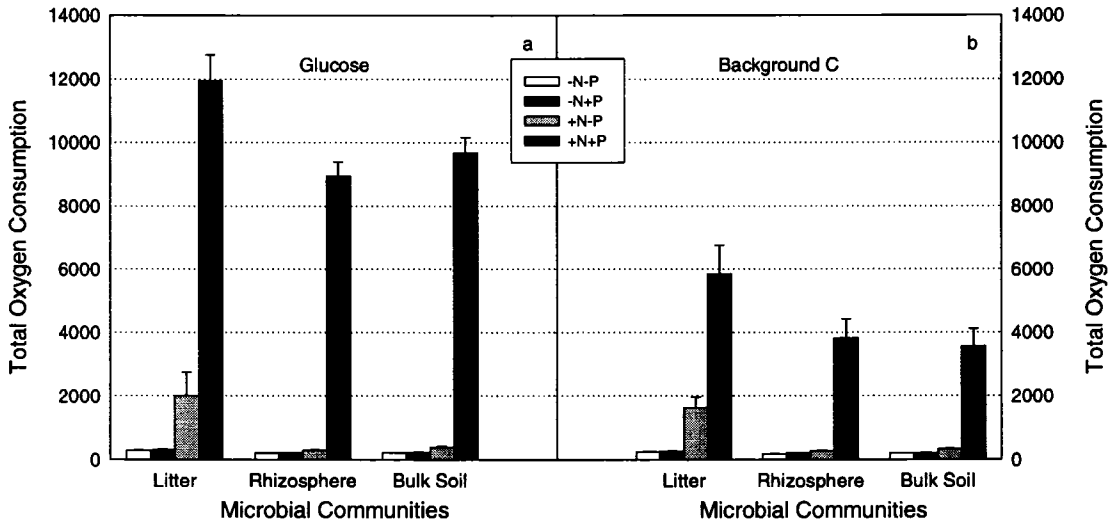

Figure 24. Area under the curve for the three Florida Scrub-oak microbial communities using glucose (a) and background C (b) as energy sources for four nutrient treatments. The bars represent one standard error for each measurement.

First peak height for both the litter ($P = 0.010$) and rhizosphere ($P = 0.002$) communities showed a significant interaction between substrate quality and N level. At high N levels, there was no significantly different response to substrate quality, but at ambient N the microbial respiration was significantly greater when using elevated CO_2 substrates than ambient CO_2 substrates (Figure 26a, b). The bulk soil community did not exhibit this same pattern (Figure 26c). Generally, the greater oxygen consumption indicated there was more energy available from substrates grown in elevated CO_2.

The bulk community did not differentiate by substrate quality, but instead had a significant interaction of CO_2 treatment*N level (P = 0.016). Under high N conditions the elevated CO_2 bulk soil community had significantly higher respiration than the ambient CO_2 bulk soil community (Figure 27a). There was no CO_2 effect at ambient N conditions (Figure 27a). These same trends held when the microbial community was utilizing glucose (Figure 27b) and background C (Figure 27c), but the differences were not significant. The litter and rhizosphere communities' total oxygen consumption did not show the same pattern. This suggested the elevated CO_2 bulk soil was more N limited than the other communities because it had a greater response under high N conditions.

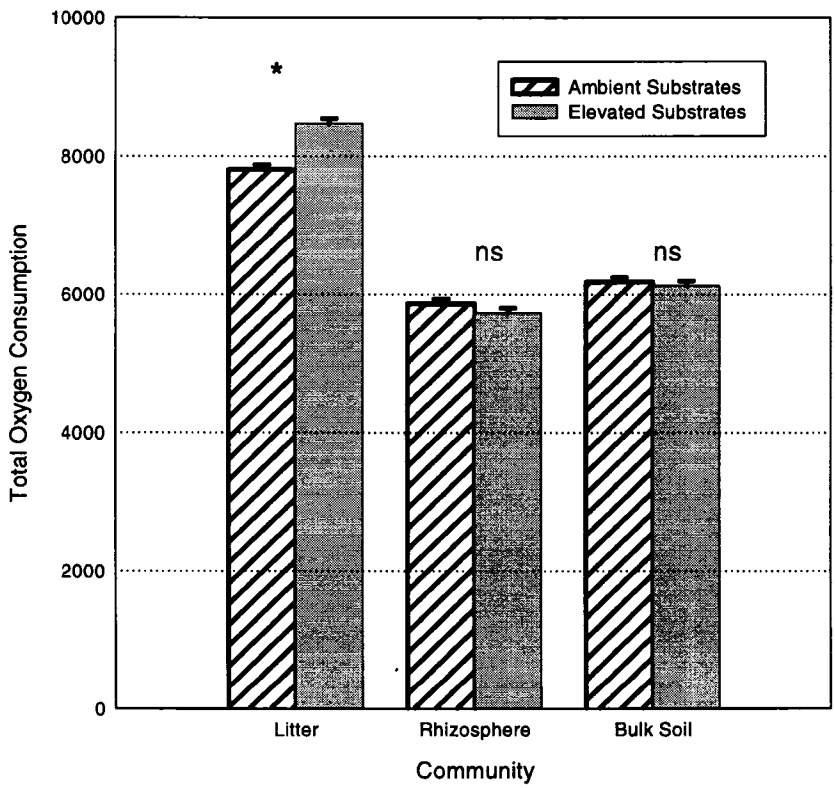

Figure 25. Effect of substrate quality on total oxygen consumption of the three microbial communities using natural substrates as energy sources. The bars represent one standard error for each measurement. (*) significant at $\alpha = 0.05$, (ns) not significant at $\alpha = 0.05$

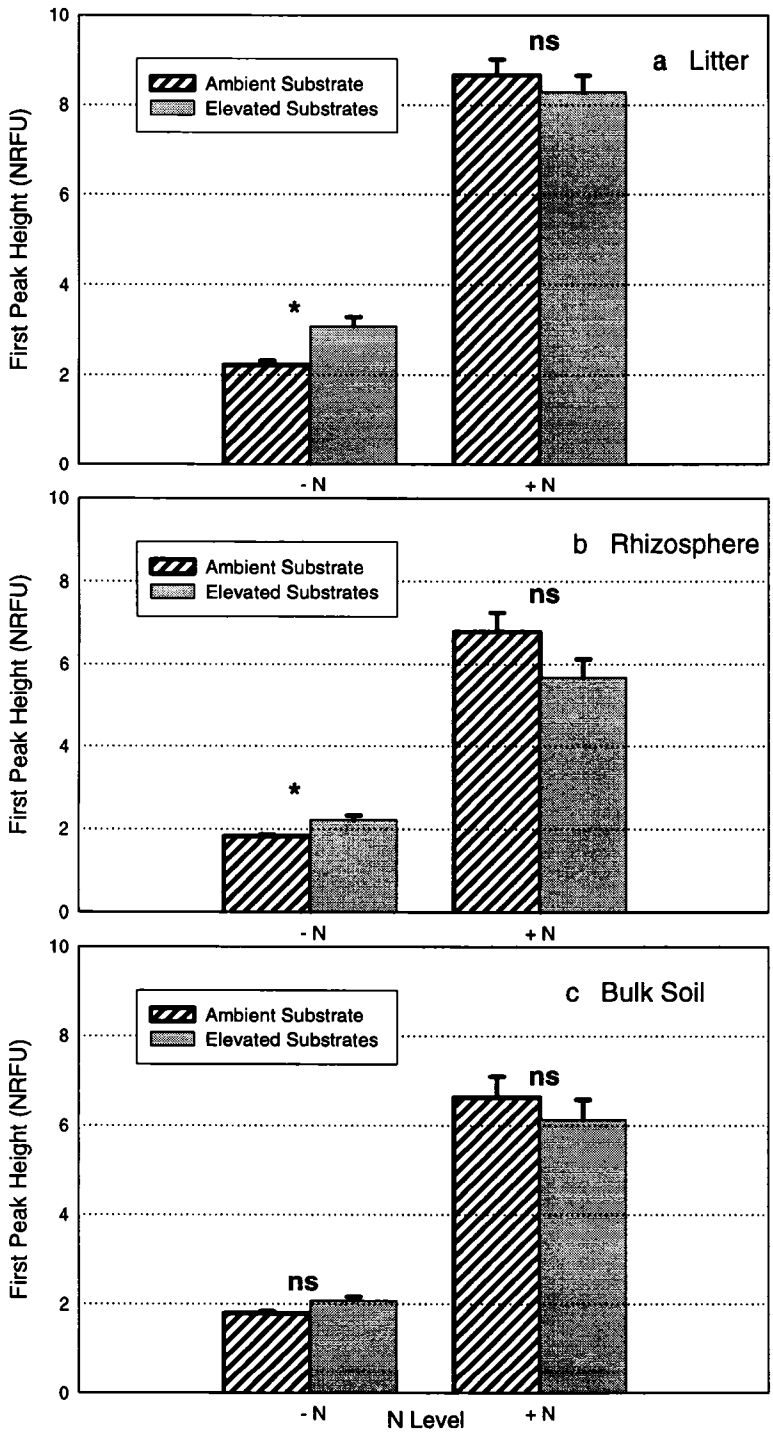

Figure 26. Effect of N and substrate quality on first peak height of the three microbial communities using natural substrates as energy sources. The bars represent one standard error for each measurement. (*) significant at $\alpha = 0.05$, (ns) not significant at $\alpha = 0.05$

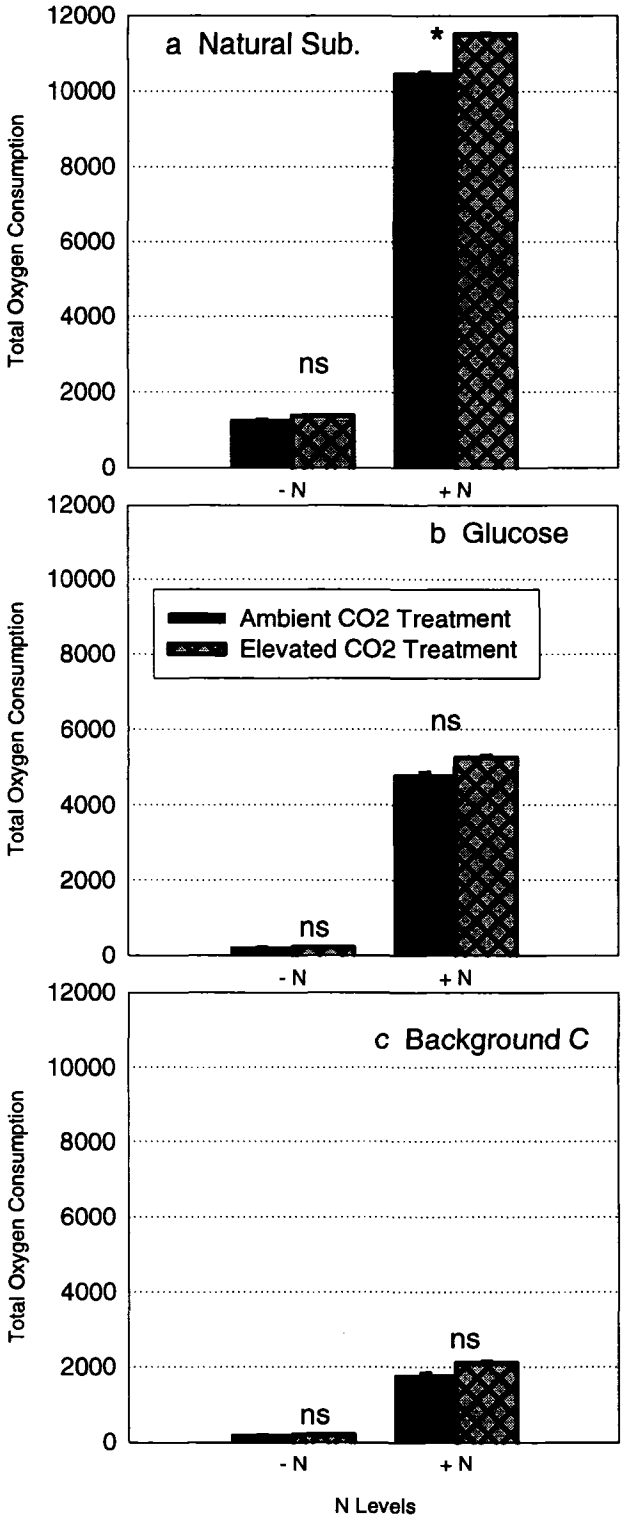

Figure 27. Effect of N level on the total oxygen consumption of the bulk soil community using (a) natural substrates, (b) glucose and (c) background C as energy sources. The bars represent one standard error for each measurement. (*) significant at $\alpha = 0.05$, (ns) not significant at $\alpha = 0.05$

CO_2 effects on response to root substrates

A CO_2 treatment and substrate source interaction significantly affected the rhizosphere microbial community's time to first response (P = 0.02). A CO_2 treatment, substrate source and substrate quality interaction significantly affected the bulk soil microbial community's time to first response (P = 0.003). The bulk soil and rhizosphere communities from the elevated CO_2 chambers responded significantly slower to root substrates grown than the ambient CO_2 soil communities (Figure 28). The elevated litter

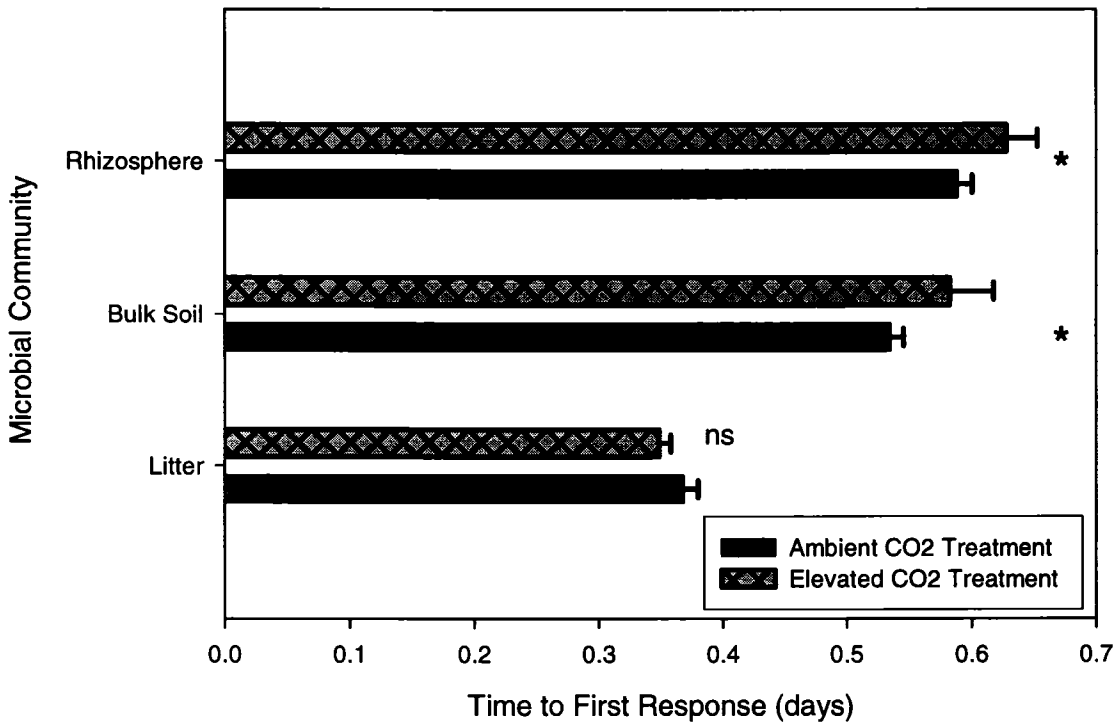

Figure 28. The effect of atmospheric CO_2 on the time to first response of the three microbial communities using root substrates as an energy source. The bars represent one standard error for each measurement. (*) significant at $\alpha = 0.05$, (ns) not significant at $\alpha = 0.05$

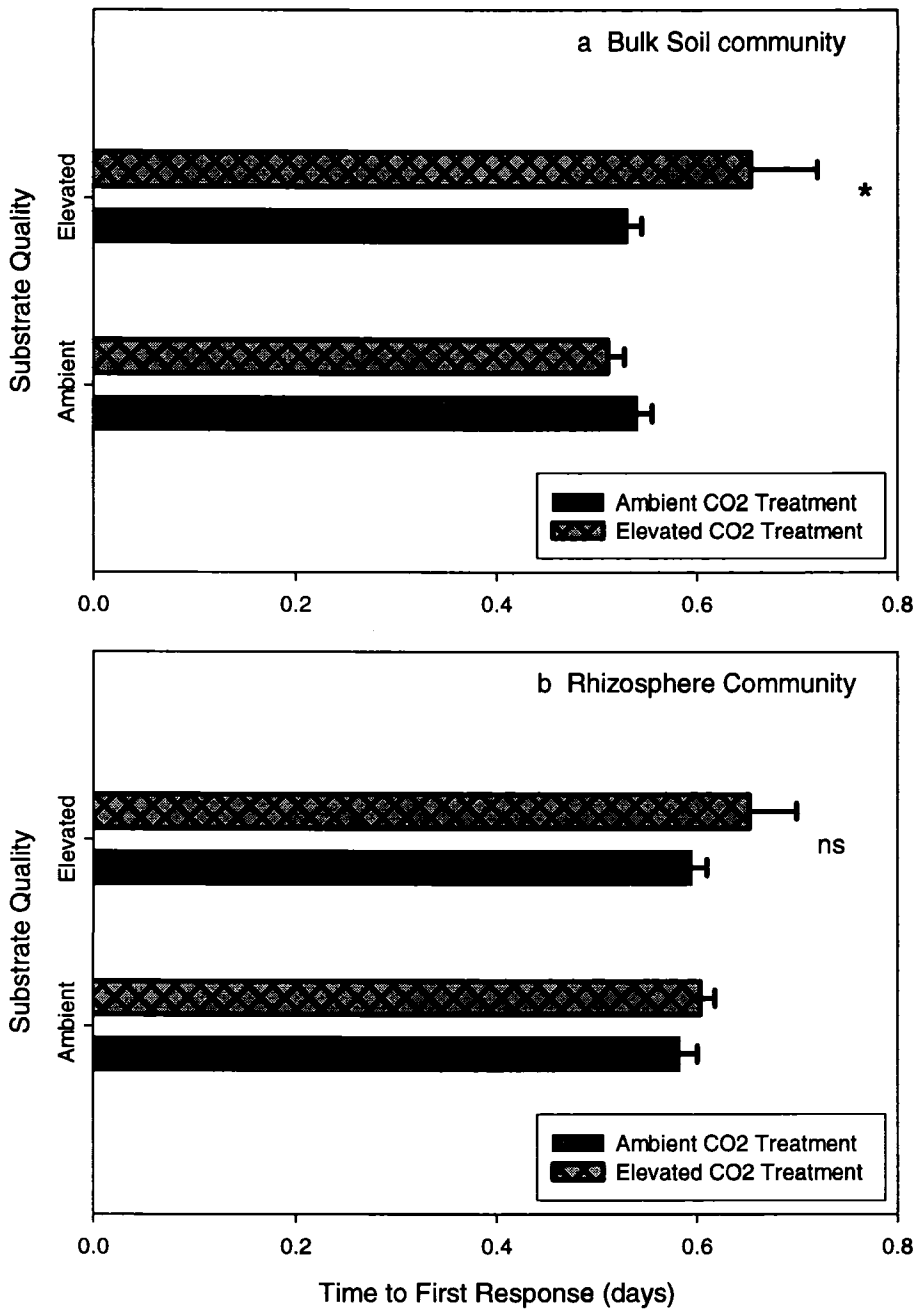

Figure 29. The effect of elevated CO_2 and substrate quality on time to first response of soil communities (a: bulk soil community, b: rhizosphere community) using root substrates as an energy source. The bars represent one standard error. (*) significant at $\alpha = 0.05$, (ns) not significant at $\alpha = 0.05$

community did not exhibit this delayed response to root substrates (Figure 28). The driving force behind this delay seemed to be the response of elevated soil communities to root substrates grown in elevated CO_2 (Figure 29). This interaction was significant for the bulk soil (Figure 29a), but not for the rhizosphere community, although the rhizosphere responses followed a similar trend (Figure 29b). The elevated CO_2 soil communities responded slower to their native substrates (roots grown in elevated CO_2) than ambient soil communities to their native ambient root substrates or the elevated root substrates.

CO_2 effects on P limitation

The addition of P interacted with CO_2 treatment, affecting the litter and rhizosphere microbial communities' response but not the bulk soil community response. A CO_2 treatment, P, and substrate source interaction significantly affected the litter community's time to first response ($P = 0.034$). The litter community from the elevated CO_2 treatment responded significantly faster to root substrate at ambient P (0.33 days) than the ambient CO_2 microbes at ambient P (Figure 30). Carbon dioxide also affected the litter community's relative time to first peak ($P = 0.039$) with P addition and utilization of glucose as a substrate. I quantified the relative response as the difference between the microbial community response with and without nutrient addition. Relative to the first peak time for litter microbes with no nutrient addition, P addition to litter microbes from elevated CO_2 sped up the response (-0.1310 days), while P addition slowed the response of microbes from ambient CO_2 (+0.1458 days).

A CO_2 treatment, N, and P interaction significantly altered the time to first peak for the rhizosphere microbial community utilizing natural substrates ($P = 0.024$). The first

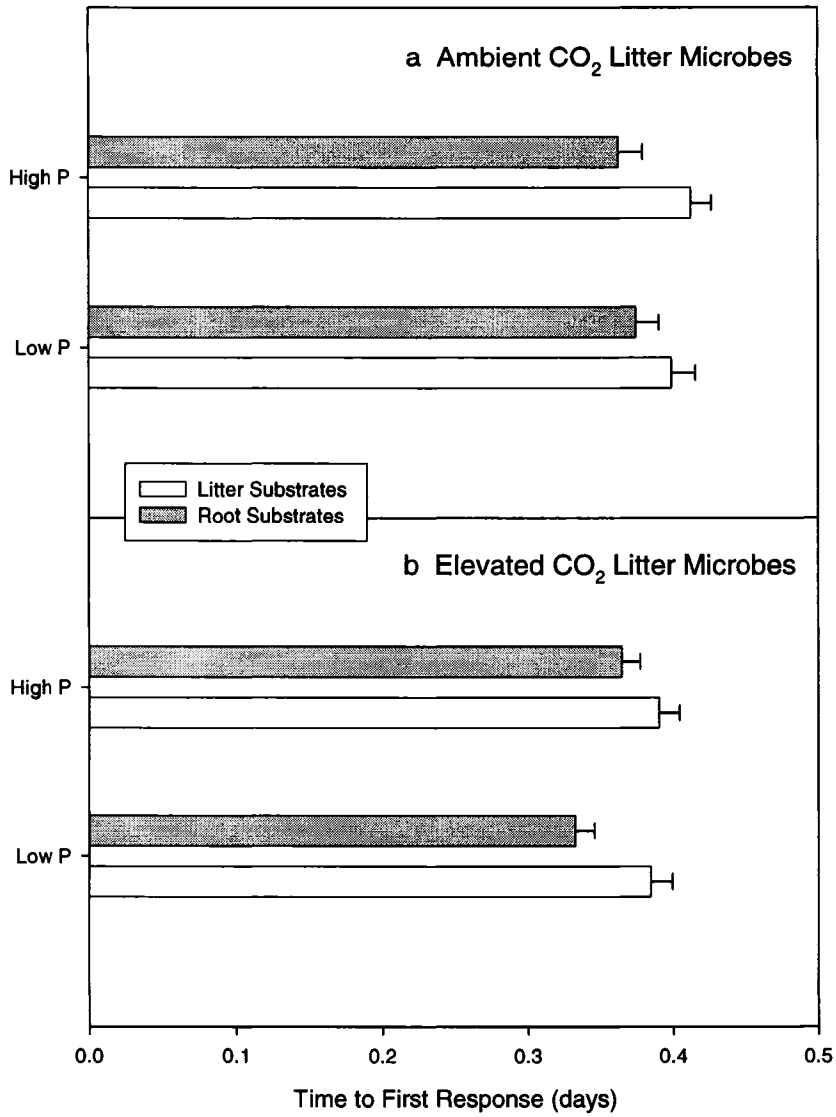

Figure 30. The effect of P and substrate source on time to first response of litter microbial community using natural substrates as energy sources. The bars represent one standard error.

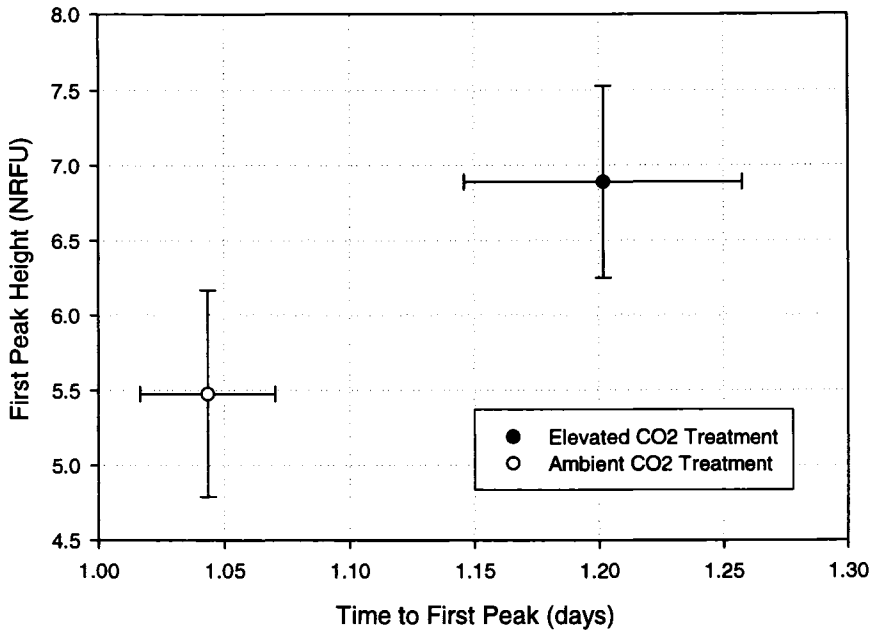

Figure 31. The effect of elevated CO_2 on the time and height of the first peak for rhizosphere community response to natural substrates and +N+P amendment. The bars represent one standard error for each measurement.

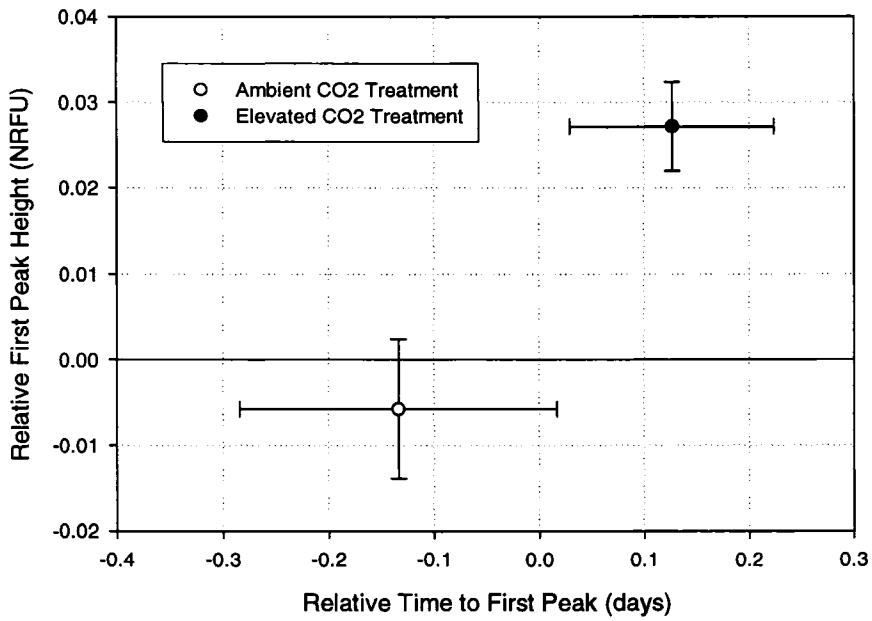

Figure 32. Relative response of rhizosphere microbial community to P addition while utilizing background C as an energy source. The relative response is the response of the microbial community with nutrient addition compared to the response without nutrient addition. The bars represent one standard error for each measurement.

peak time for ambient CO_2-treated microbes was less under +N+P conditions than the elevated CO_2-treated microbes under the same conditions (Figure 31). The time to first peak is related to the first peak height and the early response of the ambient microbes was probably due to a lower peak height for ambient CO_2 microbes at +N+P, where the elevated CO_2 microbes took longer to reach the first peak because of its increased height (Figure 31). However, the first peak height was not significantly different under these circumstances. There was a similar trend for the relative peak height in response to P addition of rhizosphere microbes utilizing background C as an energy source ($P = 0.019$). The addition of P depressed the first peak height for the ambient CO_2 rhizosphere community, but increased the height for the elevated CO_2 rhizosphere community (Figure 32). Also, although not significant, the relative time to first peak is delayed by elevated CO_2, while the P addition sped up the response of microbes from the ambient CO_2 (Figure 32). Overall the addition of P to the elevated CO_2 rhizosphere community delayed the time to first peak, but this was probably due to a larger peak under high P conditions.

Discussion

Despite the varied effects of elevated CO_2 on microbial response, there were several general responses that were observed. In elevated CO_2, soil communities were N limited and the rhizosphere community was P limited. The root substrates grown in elevated CO_2 were less rich in N, but may have had more labile carbon than roots grown under ambient CO_2.

Elevated CO_2 induced N limitation

Greater N limitation of elevated CO_2 soil communities than ambient soil communities explains the greater total oxygen consumption of the bulk soil community at high N conditions and the delayed, yet larger, first peak of the rhizosphere community at +N+P. This may partially explain the delayed response of the bulk soil and rhizosphere communities to root substrates grown in elevated CO_2 conditions.

Nitrogen limitation of microbial communities in elevated CO_2 has been observed in previous (Hu et al., 2001), whereas others have found such limitations (Finzi et al., 2002). Addition of N to the bulk soil community released the microbes from limitation and allowed the use of the substrates, which is consistent with previous studies (Allen and Schlesinger, 2004; Vance and Chapin, 2001) The greater response of the elevated community was not due to an abundance of microbes because the microbial biomass in the bulk soil was not affected by CO_2 (see results). The relatively large response of the bulk soil community from the elevated CO_2 treatment at high N indicates the community had greater N limitation. The rhizosphere community had a greater first peak under +N+P condition, indicating it was co-limited by N and P. The elevated soil microbial communities responded later to roots grown in elevated CO_2 than the ambient community to elevated root substrates and later than both elevated and ambient soil communities' responding to ambient substrates. This was due to a combination of reduced substrate quality and increased N limitation. One explanation for this may be the C:N ratio of the root substrates used to make the extracts. The N concentration was significantly lower for roots in the 1 – 2 mm diameter size class for roots grown in elevated CO_2, but not different for the other size classes (see Chapter 2). This created an increased C:N ratio (i.

e. poorer quality) for the elevated CO_2 substrate, perhaps leading to a delayed response of the microbial community. If this were solely the driving force behind the delayed responses, then the ambient bulk soil community should have responded equally late, but it did not. One possibility was progressive nitrogen limitation (PNL) of the elevated CO_2 soil communities in combination with a lower C:N ratio causing a delayed first response time.

All of these indicators support the hypothesis that the Florida scrub-oak ecosystem was developing progressive nitrogen limitation due to elevated CO_2 (Hungate et al., 2006).

Progressive nitrogen limitation is a concept developed by Luo et al. (2004). As plant communities exposed to elevated CO_2 accumulate C in biomass, N is removed from the rapid part of the N cycle, reducing N available to soil organisms and thus future plant growth. It also increases the competition between plants and microbes for N. Progressive nitrogen limitation has been observed in one other system besides Florida scrub-oak. In a Texas grassland, N was relocated from soil to biomass and C was relocated from recalcitrant pools to labile pools (Gill et al., 2006). Some studies have shown the effects of N limitations operating through the reduction of N concentration in microbial biomass in elevated CO_2 (Hu et al., 2001; Hungate et al., 1996b), while others have observed no change (Allen et al., 2000; Finzi et al., 2002). The litter microbial community has the first opportunity to assimilate N from surface litter fall, so it's reasonable that the effects of PNL were strongest in soil communities. The bulk soil community would be dependent upon N leached from the surface litter layer, as well as that from root litter and exudates. The rhizosphere community may be competing with the root for N.

Elevated CO_2 induced P limitation

Greater P limitation of the rhizosphere community in elevated CO_2 than ambient CO2 explained the delayed, yet greater, response at +N+P conditions and the delayed time, yet greater, height of the relative first peak to background C for P additions. It is reasonable that the first effects of P limitation of the microbial community would be strongest in the rhizosphere, where the microbes are competing with the root for P uptake. Pine seedlings at elevated CO_2 increased P uptake (Conroy et al., 1990). Warren and Adams (2002) found a relationship between P concentrations and Rubisco concentration in the leaf, supporting the hypothesis that increased P uptake facilitated increased photosynthesis. Duchien et al. (1993) found that CO_2-stimulated growth ceased after 4 – 12 days in low P conditions. In a study of tall grass prairie response to elevated CO_2, Owensby et al. (1993) showed CO_2 effects on P changed temporally. When there was a significant difference, either P concentration in tissue decreased and/or standing crop P increased in elevated CO_2, indicating the plants were increasing P use efficiency or total P uptake. It was hypothesized the rhizosphere microbes in the Florida scrub-oak would be less P limited than litter or bulk soil microbes, assuming that in elevated CO_2, would stimulate of P release, as shown by other workers (Norby, 1994; O'Neill, 1994). Instead the greatest P limitation was in the rhizosphere. Although the plants are drawing more P from the soil to support increasing photosynthetic machinery and biomass, there may not be other mechanisms for increasing P availability from this nutrient poor soil. Past studies of the microbial communities in the scrub-oak forest elevated CO2 studies showed there were increased levels of ergosterol, indicative of increased ectomycorrhizae activity (Klamer et al., 2002) and colonization (Langley et al., 2003) in elevated CO_2.

Mycorrhizae have long been associated with increased P uptake, and perhaps these past increases in fungal activity indicated greater P uptake. More recently Johnson et al. (2003) found soil-extractable P in the elevated CO_2 soils of the scrub-oak was reduced. He also showed there was significantly more P content in the vegetation in elevated CO_2, but no effect on P content in the O horizon. It was shown in Chapter 2 that the P concentration of live roots, dead roots and surface litter were not significantly affected by CO_2 treatment. However, Johnson et al. (2003) found at times there were significant effects of CO_2 on P concentration in leaf tissue. Together these findings suggest that the P present in live leaf tissue is probably withdrawn in a similar manner to N during senescence; the altered concentrations in living tissue in elevated CO_2 were no longer present in senesced tissue. To my knowledge, no other CO_2 studies showed evidence of altered P limitations in elevated CO_2.

P affected the litter microbial community, but the effects were not congruent with greater P limitation in elevated CO_2. This does not change the conclusion of P limitations on the ecosystem in elevated CO_2, because with regular fresh influx of senesced leaf litter, P limitation would be less likely to appear in the litter microbial communities than the bulk soil or rhizosphere microbial communities. The faster first response of the elevated litter microbes at ambient P and the faster relative time to first peak at high P conditions contradict each other, so it seems the litter community's P dynamics were beyond explanation. However, the litter community is less P limited than the soil communities regardless of CO_2 effect.

Response to substrate quality

The litter and rhizosphere microbial communities consumed more oxygen utilizing the substrates grown in elevated CO_2 than ambient, indicating there was more energy available from the elevated substrates. I suspect this is due to higher levels of extractable carbohydrates in elevated CO_2 substrates. Many studies have shown that the most consistent responses of plants to elevated CO_2 is an increase in non-structural carbohydrates, such as sugars and starches (Ceulemans and Mousseau, 1994; Eamus and Jarvis, 1989; Janssens et al., 1998; Körner and Arnone, 1992; Li et al., 1999). The interactions of substrate quality with N were curious but not beyond explanation. There may be no effect of substrate quality at high N because N allows the use of all available C regardless of CO_2 growth history, including the more recalcitrant forms in the ambient substrates. At low N, the communities were able to only utilize the more readily available forms of C that are often produced in excess in elevated CO_2. Regardless of the hypothesized explanations, it was clear the microbes respond differently to the substrates grown in different CO_2 conditions. It cannot be clearly attributed to a change in C:N ratios of the substrates since the microbial communities did not differentiate between litter and root substrates in regard to total oxygen consumption. The leaf litter used to make the litter extracts did not have a different C:N ratio between CO_2 treatments and only one of the root size categories had an altered C:N ratio due to CO_2. There was some common factor between the elevated CO_2-grown litter and roots substrates that altered it use by microbes and has yet to be elucidated for the Florida scrub-oak.

Conclusions

Changes in the environment, such as increased soil C or pH, that would have led to a change in microbial community composition or total microbial biomass were not detected. The CO_2 environment did not alter dissolved organic C and soil pH. There was no evidence of greater root exudation in this study or past studies of root biomass or litter accumulation. The microbial biomass remained unchanged in the bulk soil.

However, there were numerous examples in the literature using varied techniques where the microbial community response has been altered by elevated CO_2. Most of these were due to changes in nutrient use in the ecosystem, but there was also discrimination of substrate quality. There was evidence the system is N limited more extensively in elevated CO_2 than ambient CO_2. Also, the rhizosphere microbes may have depleted the root zone of P in the elevated CO_2 treatment. It was apparent that the elevated substrate extracts contained more available energy for microbes. Finally, the elevated CO_2 soil communities respond significantly more slowly than ambient CO_2 soil communities to native root substrate, perhaps indicating there may be a delay in the decomposition of roots under elevated CO_2.This may be due to a reduced C:N of the root material or greater N limitation of elevated CO_2 microbial communities than ambient communities. This pertains to live roots and this discrimination may not be present when microbes decompose dead roots, which is more likely in the field.

CHAPTER VI

CONCLUSIONS

Initially, productivity of the scrub-oak was stimulated in elevated CO_2. This stimulation of plant production seems to be decreasing over time. This may be due to progressive N limitation (PNL). There is also evidence that P was a limiting factor in the soil, perhaps in the future limiting plant growth in the same manner as N. If this is the case, then regular inputs of C into the soil as surface litter will decrease and production will decrease due to nutrient limitations. If this point is reached, there are a couple potential paths that the scrub-oak growth and production could take. First, plant growth and leaf cover could die back, dropping leaf and woody litter to the soil and returning some nutrients to the cycle and allowing plant growth to once again be stimulated in elevated CO_2. Second a reduction in plant cover could allow stimulation of *Galactia elliotti,* the N fixing vine, to increase N availability in the system.

There is some evidence of reduced substrate quality under elevated CO_2, but this is also unlikely to affect microbial community decomposition. The reduced quality (higher C:N ratio) was seen in living roots, but microbes will most likely decompose dead roots. However, in the event of a disturbance, the root quality may affect microbial activity. The Florida scrub-oak is a fire maintained system, with fires occurring frequently. Following a fire, there is likely significant fine root death. These dead roots in elevated CO_2 would have a decreased quality and may decompose slower, limiting microbial growth. On the other hand, there was also less fine root biomass for roots < 0.25 mm in diameter in the top 10 cm of the elevated chambers. Assuming this does not

change, then there will be a decreased amount of fine root biomass with a lower quality available for microbes to decompose in the event of a disturbance. In a fire event, elevated CO_2 plots may lose greater amounts of N, compared to ambient plots. This would be due to volatilization of N contained in the greater aboveground biomass. Plants recovering from fire in elevated CO_2 may have greater resources for recovery in large below ground structures, but may suffer greater N limitation due to a volatilization of a greater portion of the system N.

There was not a greater accumulation of C in soils of the scrub-oak, but this did not include the largest root structures. The lack of accumulation indicates that either C is not being put into the soil through fine root growth and rhizodeposition, or C is leaving the soil at a greater rate through increased decomposition and leaching. If there is more C input into the soil, it is not possible to count on the microbial biomass to retain C in the soil. My studies showed there were plenty of C energy sources available for microbial communities, but the microbes lacked N and P to utilize them. Most water-soluble C input will be leached from the soil without retention by microbes. However, the microbes are likely to retain N and P in the soil system.

Excess C leaching in elevated CO_2 would not be a problem specific to the scrub-oak, but one that may be present in any nutrient limited system. It may be more pronounced in the scrub-oak because it has a lower amount of nutrients available compared to some ecosystems. One future area of investigation may concern the fate of leached dissolved organic C. This will depend on where it originates and where it travels. It is possible terrestrial soils are sequestering C through this leachate if it moves to an anaerobic environment such as belowground water tables or wetland environments. In the

Florida scrub-oak it is likely the leachate would flow into the nearby lagoons or sit as a fresh water lens on top of the nearby salt water.

Overall, though there were few changes to the soil environment in elevated CO_2, subtle changes in energy sources and nutrient availability were able to affect microbial community response.

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Professional Experience

Research Assistant: Fall 2001 to Spring 2006 at ODU in the lab of Dr. Frank Day studying root growth and carbon accumulation in soils as it is affected by elevated atmospheric CO_2 on Merritt Island, FL at the Kennedy Space Center.

NASA Planetary Biology Internship: Summer of 2004 under Jay Garland investigating the response of Florida Scrub-oak soil microbial communities to elevated atmospheric CO_2 based on community level physiological profiles at Kennedy Space Center.

Field Intern: Summer of 2001 at Cedar Creek Long Term Ecological Research site in MN under Dr. Dave Tillman maintaining long-term studies and nitrogen/species interaction plots.

Botany Teaching Assistant: Spring Semester of 2001 at MTU under Dr. Janice Glime, lecturing, instructing botany lab and grading papers.

Biotechnology Lab Technician: Fall 1998-Spring 2001 at MTU under Dr. Chandrashekhar Joshi and Dr. Vincent Chiang extracting DNA, running PCR and other general laboratory work.

Publications

Hungate, B. A., D. W. Johnson, P. Dijkstra, G. Hymus, P. Stiling, J. P.Megonigal, **A. L. Pagel,** J. L. Moan, F. P. Day, J. Li, C. R. Hinkle, and B. G. Drake. 2006. Nitrogen cycling during seven years of atmospheric CO_2 enrichment a scrub oak woodland. Ecology 87: 26-40.

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