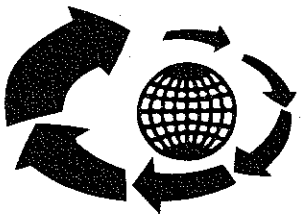
Assessment of the Consequences to Below-ground Rhizosphere and Non-rhizosphere Associated Microbiota in Terms of Community Composition, Biomass and Activity in Relation to Changes in Atmospheric CO_2 Under Varying Nutrient Concentrations and Moisture Contents

David C. White



Prepared for

Southeast Regional Center
National Institute for
Global Environmental Change

Environmental Institute

Report Number 68

FINAL TECHNICAL REPORT

Assessment of the Consequences to Below-ground Rhizosphere and Non-rhizosphere Associated Microbiota in terms of Community Composition, Biomass and Activity in Relation to Changes in Atmospheric CO_2 Under Various Nutrient Concentrations and Moisture Contents.

David C. White

University of Tennessee, Knoxville,
Center for Environmental Biotechnology/Oak Ridge National Laboratory

Submitted in Partial Fulfillment of U.A. Project No. 94UOT001SCR2 from the Southeast Regional Center of the National Institute for Global Environmental Change

July 16, 1998

TABLE OF CONTENTS

ABSTRACT

Applying signature lipid biomarker (SLB) techniques toward the assessment of below ground microbial processes in the rhizosphere and bulk soil has provided added insight into the effects of elevated CO_2 levels and soil disturbance on microbial populations. This research focused on the analysis of ester-linked polar lipid fatty acids (PLFA) to ascertain both microbial and eukaryotic biomass, community structure and nutritional/physiological status from rhizosphere and soil samples obtained from a number of collaborators. The signature biomarker technique allows the researcher to examine the microbial community *in-situ* without the necessity of culturing recoverable isolates, which have been shown to represent less than 1% of the population in soils. In terms of rhizosphere microbial populations, our research has shown there was a linear increase in microbial biomass associated with the increase in fine root mass under elevated CO_2. There was also a shift in the community structure of these rhizosphere populations favoring actinomycete type bacteria. In bulk soils it was demonstrated that after a disturbance event such as tillage or irrigation the microbial community structure that existed prior to the disturbance will be re-established.

INTRODUCTION

In order to reduce the uncertainties regarding effects of elevated atmospheric CO_2 on terrestrial ecosystems, insight into below-ground processes are needed. Traditional microbiology methodologies lack the ability todescribe quantitatively and comprehensively these diverse communities, and as a result the extant microbiota is typically over generalized in research addressing these processes. The application of molecular techniques to the study of microorganisms *in situ* overcomes a number of these deficiencies providing a better understanding of how bacterially mediated processes are effected by increasing levels of atmospheric CO_2. The knowledge gained can be related back to whole plant processes in terms of positive, negative or neutral feedbacks providing first, a detailed description of the microbial component of the terrestrial carbon cycle and second, insight into how anthropogenic influences on the atmosphere will effect ecosystem potentials as carbon sinks or sources.

In 1993 Zak *et al.*, proposed a conceptual model of the global carbon cycle, which included a below-ground microbial component. In this model, they hypothesized that exposures to elevated atmospheric CO_2 levels would result in an increase in below-ground microbial biomass as a result of increased carbon allocation by the plant to the roots. Ecosystems are bound, to varying extents, by below-ground processes, such as rates of nutrient cycling. Significant effects on the microbial component may eventually result in shifts in carbon source/sink relationships, terrestrial ecosystem structure and net primary productivity (O'Neill, 1994). Therefore a seemingly minor change (*i.e.* microbial biomass and/or community composition) from an ecosystem perspective may eventually evoke a considerable change in ecosytem function. In this study, it was hypothesized that exposures to elevated atmospheric CO_2 would result in significant changes in rhizosphere and non-rhizosphere microbial community composition, biomass and physiological status. It was also hypothesized that the manifestation of such changes may result only from the combination of elevated atmospheric CO_2 with certain environmental attributes, such as nutrient limitations (nitrogen), substrate limitations (carbon), rising temperatures and water stress. This research focused on questions relating to responses of the rhizosphere microbiota to elevated atmospheric CO_2 under various conditions of: (1) nitrogen: Does an increase in the availability of photosynthate result in an increase in the nitrification potential? (2) carbon: If competition for carbon limits soil microbial populations, how does an increase in carbon allocation to fine roots effect microbial biomass and community composition? and (3) temperature and water: Do concurrent environmental influences, such as temperature and water stress, magnify, lessen or have no effect on microbial community composition, biomass or activity in relation to carbon utilization?

The below-ground rhizosphere and non-rhizosphere microbiota of indigenous Southeastern United States plants were quantitatively described in terms of viable biomass, community composition and physiological status under conditions of ambient and elevated atmospheric CO_2. CO_2 effects (on the below-ground microbiota) were quantified in association with nutrient, temperature and water stress treatments. Various of species were analyzed, including white oak, aspen, long leaf pine, bahia grass and peanut, providing a more comprehensive overview of plant-microbe interactions than that obtainable with a single species study. Microbial biomass and community composition was determined by application of the signature lipid biomarker technology. This approach provided a quantitative measure of *in situ* community taxonomy, which was amenable to the testing of null hypotheses with multivariate

statistical tests. Previous research and the data resulting from this study have demonstrated that elevated atmospheric CO_2 levels do effect changes in the rhizosphere and non-rhizosphere associated microbiota (Ringelberg *et al*, 1998). These results have put a crack in the "black box" recital often used in the description of microbial community dynamics in models describing the terrestrial carbon cycle. Continued research into CO_2 effects on below ground microbiology will further the accuracy of global change models in predicting the consequences of elevated atmospheric carbon dioxide levels on terrestrial ecosystems.

MATERIALS AND METHODS

Samples consisted of fine roots (<1.0 mm in diameter) and bulk soils ranging in mass from 100-500 mg fine root and 1-20 g soil. All sample material was collected into sterilized bags (whirl-pak,) and placed on ice for transportation to the laboratory where it was frozen at –20°C or below. Sample material was obtained from the following sources: **(1)** Dr. H. Rogers at the University of Auburn-USARC site in Auburn, Alabama, where long leaf pine was subjected to elevated atmospheric CO_2 in association with different nitrogen loads and water stresses, and bulk soil from different tillage regimes (conventional and no-till) were analyzed shortly after disturbances such as a simulated planting event or irrigation. **(2)** Dr. K. Boote at the University of Florida-IFAS Center in Gainesville, Florida, where bahia grass and peanut were subjected to elevated CO_2 levels and various incubation temperatures, **(3)** Dr. D. Zak at the University of Michigan-Kellog Biological Research Station in Pellston, Michigan, where aspen was subjected to elevated atmospheric CO_2 in association with differing soil fertilities and **(4)** Dr. R. Thomas at Duke University where *Lysiloma* and *Leucaena* were subjected to elevated atmospheric CO_2 in association with various nutrient loads as well as differing moisture contents.

The signature lipid biomarker analysis entails the quantitative recovery and identification of ester-linked phospholipid fatty acids (PLFA), lipopolysaccharide lipid-A hydroxy fatty acids (LPS-OHFA) and sterols. Bacterial and mycorrhizal PLFA were recovered from fine roots and bulk soils by extraction in a modified single phase organic solvent system (methanol: dichloromethane: phosphate buffer, 2:1:0.8, v:v:v) (Bligh and Dyer, 1959). Samples were sonicated for 2 minutes in the extractant to help remove bacteria from the soil particles and root material. Samples were then allowed to extract overnight after which they were centrifuged at 2000 rpm for 20 minutes. The supernatant was decanted into separatory funnels and the remaining sediment washed with an additional volume of chloroform. Dichloromethane extracted Nanopure water (Barnstead, Dubuque, Iowa) was then added to the funnels and the phases were allowed to separate overnight. The organic layer was drained then dried under nitrogen with rotary evaporation. The total lipid was fractionated into neutral-, glyco- and polar lipids on silicic acid columns as described in Guckert *et al.*, 1985. Polar lipids were subjected to a mild alkaline methanolysis resulting in the recovery of fatty acid methyl esters for GC/MS analysis (Guckert, *et al.*, 1985). Fatty acids are designated as described by Ringelberg *et al.*, 1989.

PLFA were used to describe the viable microbial communities in terms of total biomass (Balkwill *et al*, 1988) and community composition (Vestal and White, 1989). Phospholipids are essential components of life making up the bulk of the matter of prokaryotic cell membranes. Since phospholipids are susceptible to both endogenous and exogenous phospholipases, they are reliable measures of viable cell biomass (White *et al*, 1979). PLFA have been shown to be

sufficiently distinct to allow for the identification of individual species of bacteria (Guckert *et al*, 1991) and interpretations of microbial community composition can be made utilizing the same knowledge base (Frostegard *et al*, 1993). Community composition can be inferred from a PLFA profile by relating the type of PLFA identified to different biosynthetic pathways utilized in fatty acid synthesis (Fredrickson *et al*, 1995) or to specific species or classes of bacteria which contain a unique signature PLFA biomarker (Vestal and White, 1989). Ratios of specific PLFA have also been shown to reflect changes in bacterial physiology. An example is the ratio of *trans/cis* mono-unsaturated PLFA which has been observed to increase in direct proportion to an increase in toxicity exposure (Heipieper *et al*, 1992) and/or starvation (Guckert *et al*, 1986). Another example is the ratio of saturated/unsaturated PLFA which has been shown to increase as cell membrane fluidity is decreased in response to conditions such as desiccation (Keift *et al*, 1994).

In addition to PLFA, LPS-OHFA can provide insight into Gram-negative bacterial taxonomy (Parker *et al*, 1982). LPS-OHFA were recovered from the aqueous portion of the extraction described above. After hydrolysis in strong acid, the fatty acids were methylated using strongly acidic methanol then analyzed using the GC/MS conditions referenced above.

Similarly, sterols have been used to relate species of algae (Canuel *et al*, 1995) and fungi (Wassef, 1977) taxonomically. By determining the sterol content in each of the samples (fine root or soil), insight into the makeup of the mycorrhizal population can be gained. Trimethyl-silyl (TMSi) derivatives of 3ß-ol sterols were prepared from the neutral lipid fraction by alkaline saponification as described by Nichols *et al.*,1983. Within 24 hours of analysis, 100-500 µL of a mixture consisting of [BSTFA : (CH_2Cl_2:acetonitrile, 1:1, v:v), 1:1, v:v] was added to form the trimethyl silyl esters. Sterols were identified and quantified under the conditions referenced above.

Results of the signature lipid biomarker analysis were interpreted as described in Ringelberg *et al*, 1998. A three tiered statistical approach was used where hypothesis testing was first made using analysis of variance (ANOVA) followed by the application of multivariate analyses such as hierarchical clustering and principal components for the determination of sample relatedness based on treatment effects. The final analysis was with an artificial neural network coupled with a sensitivity test to infer which communities of microorganisms reflected the presence of elevated atmospheric CO_2 and which variables (individual PLFA or sterols) were the most sensitive to the elevated CO_2 exposure.

RESULTS AND DISCUSSION

White Oak

Our research showed that the exposure of white oak to elevated atmospheric CO_2 resulted in an identifiable shift in the rhizosphere associated microbial community composition (Figure 1, Ringelberg *et al*, 1998). The shift was attributed to an increase in the relative percentage of actinomycete type microorganisms and was based on the analysis of phospholipid fatty acids (PLFA). The identification of a specific type or classification of bacteria as increasing in relative percentage poses the question of whether respiratory activity of the community as a whole might not also have been effected and how this increase might have effected rates of nitrogen fixation. Although no significant increase or decrease in the total abundance of

bacterial phospholipids was determined as a result of the elevated CO_2 exposure, bacterial biomass appeared to scale linearly with a significant increase in fine root density. This study highlighted the applicability of the signature lipid biomarker technique in defining the indirect effects of elevated CO_2 exposures on below-ground microbial community composition. This study was also the first to demonstrate a significant effect on microbial community composition due to elevated atmospheric CO_2 exposures.

Trembling Aspen (*Populus tremuloides*)

Another study involving the analysis of rhizosphere soils indicated that soil fertility, defined as soil organic carbon content, acted as a co-variable with elevated CO_2 to effect a change in the below-ground microbial community composition (Mikan, *et al.* 1998). In this experiment, trembling aspen (*Populus tremuloides*) were grown under ambient and twice ambient levels of CO_2 in soils categorized as being of either low (C horizon) or high (A horizon) fertility. The premise of the experiment was that the increased net carbon assimilation that occurs under elevated CO_2 would result in increased fine root mycorrhizal growth, microbial biomass and rates of nitrogen mineralization. The experiment attempted to answer the question of whether or not inputs of plant derived carbon to the soil would be assimilated by the soil microbes and if this increased substrate availability would result in increased microbial biomass, activity or change in community composition.

Results of a PLFA analysis illustrated that those microbial communities associated with a tree exposed to elevated CO_2 and grown in high fertility soils were of a unique and definable composition (Figure 2). The greatest observed treatment effect was, understandably, attributable to soil organic carbon content. The A horizon soils yielded a considerably greater microbial biomass. This increased biomass was also associated with a unique microbial community composition (compared to the C horizon microbial communities). Elevated atmospheric CO_2 exposures did not result in a significant increase in the below-ground microbial biomass, but did induce a shift in the microbial community of the A horizon soils. The fact that this community shift (due to elevated CO_2 levels) was identifiable in the A horizon soils only suggests that the extant microbiota was only capable of co-metabolizing any change in the quantity or quality of root exudates that occurred due to the exposure to elevated CO_2 levels. The community shift was identified, by lipid biomarker analysis, to be primarily an increase in the relative percentages of micro-eukaryotic organisms, in particular the fungi. The premise that increased carbon allocation (by the plant) to the soil would result in increased mycorrhizal growth was partially substantiated. Although the elevated CO_2 exposures did not result in a substantial increase in microbial biomass, the identified community shift has specific relevance to plant nutrient uptake. Below-ground processes invariably affect above ground process. The fact that elevated CO_2 exposures induced changes in the make up of the below-ground microbiota in high fertility soils (and not low fertility soils) highlights the potential for localized alterations to ecosystem processes, whether natural or man made.

CO_2 exposure. Although the C4 peanut may have been more suited to the environment of high temperature and CO_2, the abundance of microorganisms on the plant's fine roots did not differ significantly (in magnitude change) from that of the C3 plant. The decrease in total rhizosphere associated biomass at the higher incubation temperature may have significant consequences for terrestrial carbon sequestration under a global warming scenario. Any consequences may be magnified in the Southeastern United States if perennial peanut is increasingly used as forage or in erosion control.

In a corollary study, increases in soil organic carbon due to accelerated decomposition of organic matter as a result of global warming were examined (Zogg *et al.*, 1998). Results suggested that the perceived first order rate constant often applied to below ground microbial activity does not always hold true. For example, Zak *et al.*, 1993 presented experimental data which indicated that an increased pool of available carbon, as a result of increased temperature, had little to no effect on the rate constants describing microbial respiration in the same soils. Changes in microbial community composition were hypothesized to explain the response, which were partially confirmed by the results of this corollary study. Shifts in microbial community composition due to rapid or gradual changes in ambient temperatures can alter patterns of soil organic matter decomposition.

Changes in community composition in the rhizosphere microbiota of both bahia and perennial peanut were apparent due to incubations under elevated CO_2 levels (Figure 4). Lipid biomarkers for the fortuitous actinomycetes increased in relative percentage under elevated CO_2 levels with either ambient or elevated temperatures. Again, this same classification of bacteria was found to increase in relative percentages under elevated CO_2 atmospheres. In this study, total rhizosphere associated microbial biomass was found to increase as a result of the elevated CO_2 exposures (under ambient temperatures). In the two previously described studies, where actinomycete prevalence was also identified (white oak and long leaf pine), an increase in the rhizosphere microbiota was not as apparent. There was however, an increase in fine root mass density in both studies resulting in a net increase in below-ground microbial biomass per unit area (microbial biomass increased linearly with fine root density). The increase in actinomycetes was postulated to result from a change in the pattern of root exudation effected by the increased levels of atmospheric CO_2.

Lysiloma* and *Leucaena

The effect of CO_2 elevation (350 and 700 ppm) and nutrient treatments (high and low phosphorus both with low nitrogen) on two nitrogen fixing plants *Lysiloma* and *Leucaena* were recently examined in a study undertaken by Dr. Richard Thomas at Duke University. It was shown that CO_2 level affected the microbial biomass and the physiological status of the *Lysiloma* associated rhizosphere prokaryotic community. Specifically, viable biomass as measured by PLFA was higher with elevated CO_2, while the relative proportion of cyclopropyl PLFA was higher in the low CO_2 treatment implying a slower Gram negative bacterial turnover rate. Taken together, these results indicated that the *Lysiloma* root associated rhizosphere bacteria were better able to survive under elevated CO_2 than under the lower levels. The relative proportion of the PLFA 10me16:0, a fatty acid that is found predominately in Gram-negative sulfate reducing bacteria such as *Desulfobacter sp*, was greater with elevated CO_2. The rise in 10me16:0 with elevated CO_2 suggests an enhanced sulfate reducing population, however; this PLFA has also been detected in Gram positive bacteria, albeit in lower concentrations. The *Leucaena* species

showed no visible response statistically to elevated CO_2, instead nutrient level was responsible for the biomass effect (Figure 5).

Tillage effects

In another study conducted at the USDA Soil Dynamics Laboratory at Auburn University, Drs. Hugo Rogers and Bret Runion explored the microbial community response to different agricultural disturbances in rhizosphere and bsurface soil under different tillage and traffic regimes. The experiment was a randomized block design with traffic as the whole plot treatment and tillage as the sub-plot treatment. The soils were exposed to different disturbances (disking, irrigation and simulated planting) over a 7 day period. Soils that received the disking treatment contained lower measurable biomass at the 0-2 cm depth than did the respective controls. The most likely cause of this decrease in biomass was the turning and homogenization of the soils by the action of disking which diluted and redistributed the biomass. At the time of irrigation the biomass of the 0-2 cm disked soils was significantly lower than the non-disked soils, After irrigation, the biomass of the disked soils was not significantly different from the non-disked soils. Principal components analysis carried out on these data showed that after the disturbance (mechanical or irrigation) the soil microbial community which re-established was the same as that which existed before the disturbance (Figure 6).

CONCLUSIONS

Using phospholipid fatty acid analysis and other biomarkers (signature lipid biomarker analysis) we were free from the encumbrances associated with classical microbiological techniques. This allowed us to analyze the microbial populations *in situ* and as a result demonstrated that there is a linear increase in biomass associated with the increase in fine root mass under elevated CO_2. In terms of carbon sequestration, elevated CO_2 levels will result in more carbon sequestered in the form of microbial biomass. In terms of microbial ecology, the selection for a particular functional group of microorganisms may have far reaching effects on above ground primary productivity. The proliferation of these actinomycete type bacteria with elevated CO_2 could also have major implications for nutrient cycling in soil systems. Additional research is needed to determine the keystone species selected for by the postulated change in root exudation, to determine what their physical requirements are and how this affects the plants ability to take up nutrients.

REFERENCES

Balkwill, D.L., F.R. Leach, J.T. Wilson, J.F. McNabb and D.C. White. 1988. Equivalence of microbial biomass measures based on membrane lipid and cell wall components, adenosine triphosphate, and direct counts in subsurface aquifer sediments. *Microb. Ecol.*, **16**: 73-84.

Bligh, E.G. and W.J. Dyer. 1959. A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol. **37**(8):911-917.

Canuel, E.A., J.E. Cloern, D.B. Ringelberg and J.B. Guckert. 1995. Molecular and isotopic tracers used to examine sources of organic matter and its incorporation into the food webs of San Francisco bay. *Limol. Oceanogr.*, **40** (1): 67-81.

Fredrickson, J.K., J.P. McKinley, S.A. Nierzwicki-Bauer, D.C. White, D.B. Ringelberg, S.A. Rawson, Shu-Mei Li, F.J. Brockman and B.N. Bjornstad. 1995. Microbial community structure and biogeochemistry of Miocene subsurface sediments: implications for long-term microbial survival. *Molecular Ecology*, **4**: 619-626.

Frostegard, A., E. Baath and A. Tunlid. 1993. Shifts in the structure of soil microbial communities in limed forests as revealed by phospholipid fatty acid analysis. *Soil Biol. Biochem.*, **25**(6): 723-730

Guckert, J.B., C.P. Antworth, P.D. Nichols and D.C. White. 1985. Phospholipid ester-linked fatty acid profiles as reproducible assays for changes in prokaryotic community structure of estuarine sediments. *FEMS Microbiol. Ecol.*, **31**: 147-158.

Guckert, J.B., M.A. Hood and D.C. White. 1986. Phospholipid ester-linked fatty acid profile changes during nutrient deprivation of *Vibrio cholera*: Increases in the trans/cis ratio and proportions of cyclopropyl fatty acids. *Appl. Environ. Microbiol.*, **52** (4): 794-801.

Guckert, J.B., D.B. Ringelberg, D.C. White, R.S. Hanson and B.J. Bratina. 1991. Membrane fatty acids as phenotypic markers in the polyphasic taxonomy of methylotrophs within the proteobacteria. *J. Gen. Microbiol.*, **137**: 2631-2641.

Heipieper, H.J., R. Diefenbach and H. Keweloh. 1992. Conversion of cis unsaturated fatty acids to trans, a possible mechanism for the protection of phenol-degrading *Pseudomonas putida* P8 from substrate toxicity. *Appl. Environ. Microbiol.*, **58** (6): 1847-1852.

Keift, T.L., D.B. Ringelberg and D.C. White. 1994. Changes in ester-linked phospholipid fatty acid profiles of subsurface bacteria during starvation and desiccation in a porous medium. *Appl. Environ. Microbiol.*, **60** (9): 3292-3299.

Mikan, C.J., D.R. Zak, K.S. Pregitzer, M.E. Kubiske, D.B. Ringelberg and D.C. White. 1998. Nitrogen dynamics and microbial community composition beneath Populus tremuloides: effects of N availability and elevated atmospheric CO_2. *Biogeochemistry*, in review.

Nichols, P.D., J.K. Volkman, and R.B. Johns. 1983. Sterols and fatty acids of the marine unicellular alga, FCRG 51. Phytochemistry. **22**:1447-1452.

O'Neill, E.G. 1994. Responses of soil biota to elevated atmospheric carbon dioxide. *Plant and Soil*, **165**: 55-65.

Parker, J.H., G.A. Smith, H.L. Fredrickson, J.R. Vestal, and D.C. White 1982. Sensitive assay, based on hydroxy fatty acids from the lipopolysaccharide lipid A, for Gram-negative bacteria in sediments. *Appl. Environ. Microbiol.* **44**: 1170-1177.

Ringelberg, D.B., J.D. Davis, G.A. Smith, S.M. Pfiffner, P.D. Nichols, J.S. Nickels, M.J. Henson, J.T. Wilson, M. Yates, D.H. Kampbell, H.W. Read, T.T. Stocksdale, and D.C. White. 1989. Validation of signature polar lipid fatty acid biomarkers for alkane-utilizing bacteria in soils and subsurface aquifer materials. *FEMS Microbiol. Ecol.*, **62**:39-50.

Ringelberg, D.B. and D.C. White. 1994. Analysis of the effect of increased CO_2 on the biomass, community structure and nutritional status of the rhizosphere microbiota of white oaks, long leaf pine and cottonwood. Environmental Institute the Univ. of Alabama, NIGEC Southeastern Regional Report #33.

Ringelberg, D.B., J.O. Stair, J. Almeida, R.J. Norby, E.G. O'Neill and D.C. White. 1998. Consequences of rising atmospheric carbon dioxide levels for the belowground microbiota associated with white oak. *J. Environ. Quality*, **26** (2):495-503.

Vestal, J.R. and D.C. White. 1989. Lipid analysis in microbial ecology: Quantitative approaches to the study of microbial communities. *Bioscience*, **39** (8): 535-541.

Wassef, M.K. 1977. In: Advances in lipid research (R. Pacletti and D. Kritchevsky, eds.), Academic Press, NY.

White, D.C., W.M. Davis, J.S. Nickels, J.D. King and R.J. Bobbie. 1979. Determination of sedimentary microbial biomass by extractable lipid phosphate. *Oecologia*, **40**: 51-62.

Zak, D.R., K.S. Pregetizer, P.S. Curtis, J.A. Teeri, R. Fogel and D.L. Randlett. 1993. Elevated atmospheric CO_2 and feedback between carbon and nitrogen cycles. *Plant and Soil*, **151**: 105-117.

Zak, D.R., D.F. Grigal and L.F. Ohmann. 1993. Kinetics of microbial respiration and nitrogen mineralization in lake states forests. *Soil Sci. Soc. Am. J.*, **57**:1100-1106.

Zogg, G.P., D.R. Zak, D.B. Ringelberg, N.W. MacDonald, K.S. Pregitzer and D.C. White. 1998. Compositional and functional shifts in microbial communities related to soil warming. *Soil Sci. Soc. Am. J.*, submitted.

Table 1. Total rhizosphere associated microbial biomass, expressed as a concentration of membrane phospholipids (PLFA), in bahia and perennial peanut exposed to ambient (350 ppm) and elevated (750 ppm) CO_2 levels and ambient (+0°C) and elevated (+4.5°C) temperatures.

Temperature (°C)	CO_2 (ppm)	Bahia grass	perennial peanut
+0	350	31 (2)	168 (33)
+0	750	50 (6)	234 (35)
+4.5	350	43 (9)	162 (35)
+4.5	750	36 (9)	146 (24)

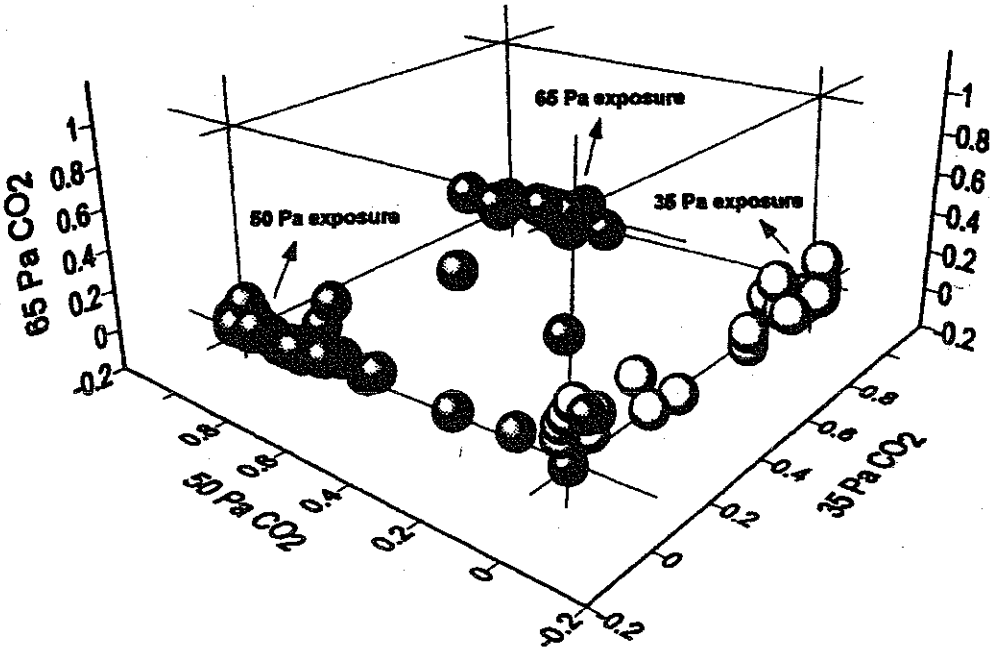


Figure 1. An illustration of the results of a feed forward artificial neural network of rhizosphere (fine root) derived ester-linked phospholipid fatty acid profiles (PLFA) from white oak showing differences which were attributable to different atmospheric CO_2 exposures. The network was trained on 10% of the data set to recognize a PLFA profile representative of 65 Pa CO_2 (x, y, z coordinates of 0, 0, 1), 50 Pa CO_2 (x, y, z coordinates of 0, 1, 0) and ambient or 35 Pa CO_2 (x, y, z coordinates of 1, 0, 0). The observed differences directly reflect a shift or change in microbial community composition as a result of the various CO_2 exposures.

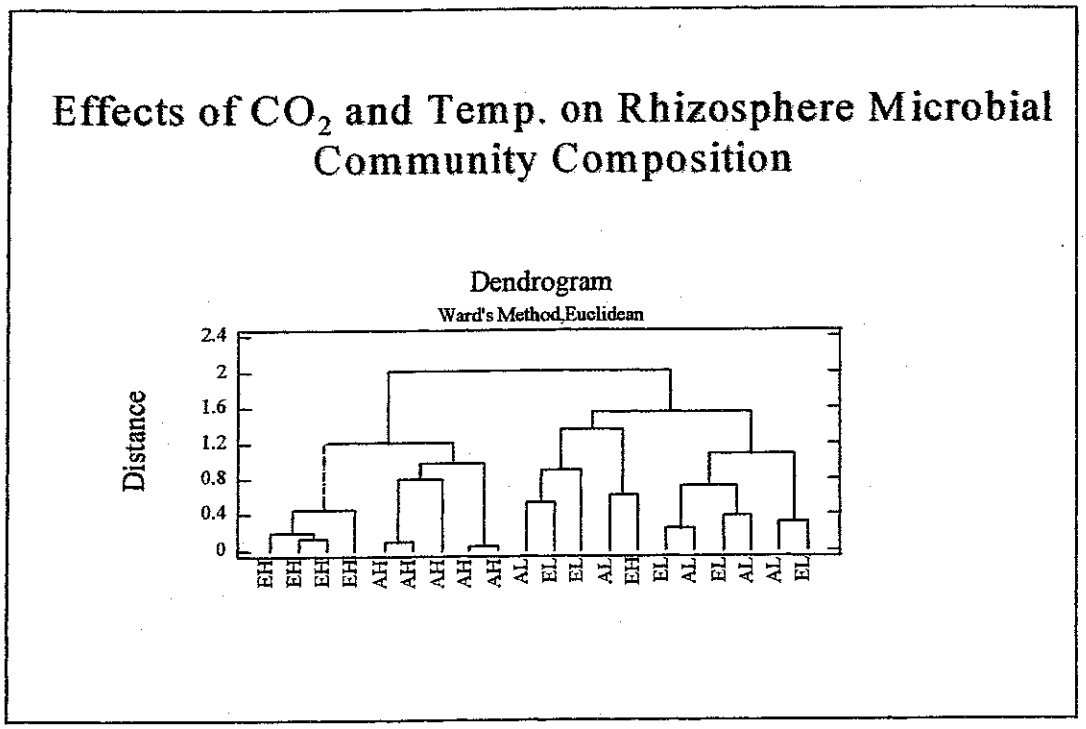


Figure 2. A hierarchical cluster analysis (Wards method) based on Euclidean distance developed from arcsin transformed ester-linked phospholipid fatty acid (PLFA) profiles recovered from the rhizosphere soil of aspen trees exposed to ambient and twice ambient levels of atmospheric CO_2 and low and high levels of soil fertility. Soil fertility is defined as organic carbon content where low fertility soils are composed of 20% type A and 80% type C soils and high fertility of 100% type A soil. The dendrogram illustrates that a relatedness exists among those samples recovered under conditions of high fertility and elevated CO_2 as does one among samples collected under ambient CO_2 levels and high fertility and, to a lesser extent, among samples collected under ambient or twice ambient CO_2 levels but all under conditions of low soil fertility. The linkages formed suggest that microbial community composition differs among the three groups of samples just described.

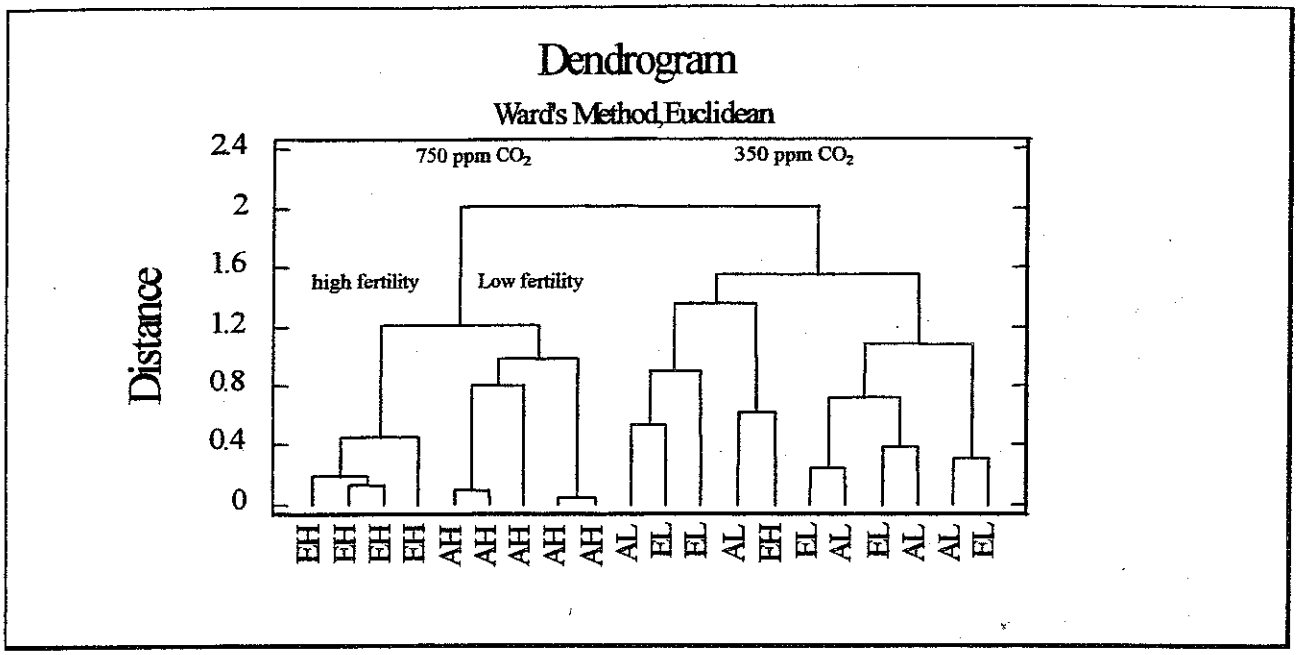


Figure 3. A hierarchical cluster analysis (complete linkage method) based on Euclidean distance developed from arcsin transformed ester-linked phospholipid fatty acid (PLFA) profiles recovered from the fine roots of longleaf pine exposed to ambient and twice ambient levels of atmospheric CO_2, low and high levels of N and with or without water stress over a period of one year (four samplings). Nitrogen loads were ~40 kg hr^{-1} yr^{-1} for the low N treatment and ~400 kg hr^{-1} yr^{-1} for the high N treatment. The dendrogram illustrates that PLFA profiles recovered from samples collected during bud set of the second year and bud break of the first year differed from those collected during bud break and mid-season growth of the first year. Water stress appeared to be the predominant factor in distinguish profiles collected during the two periods of bud set and N the predominant factor during the periods of bud break and mid-season growth.

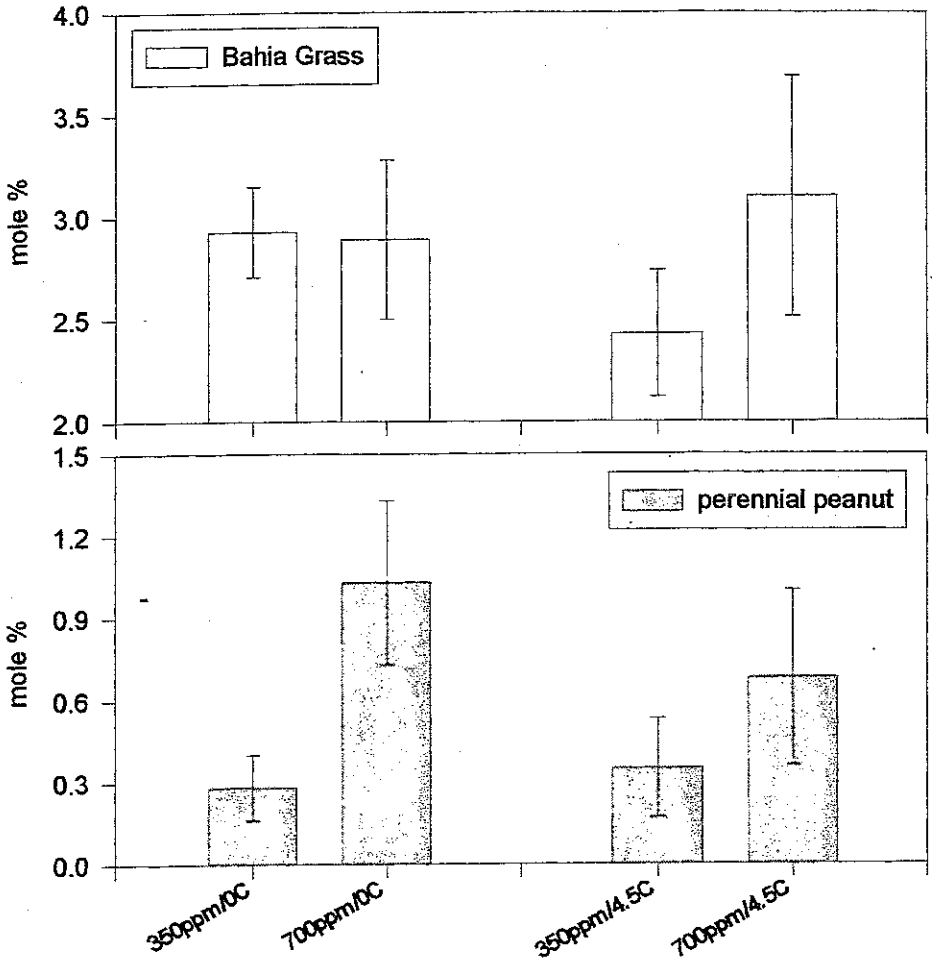


Figure 4. Bar graph illustration of the relative percentage (mole %) of the actinomycete lipid biomarker (10me18:0) in the rhizosphere of bahia grass and perennial peanut exposed to ambient (350 ppm) and elevated (750 ppm) CO_2 levels and ambient (+0°C) and elevated (+4.5°C) temperatures

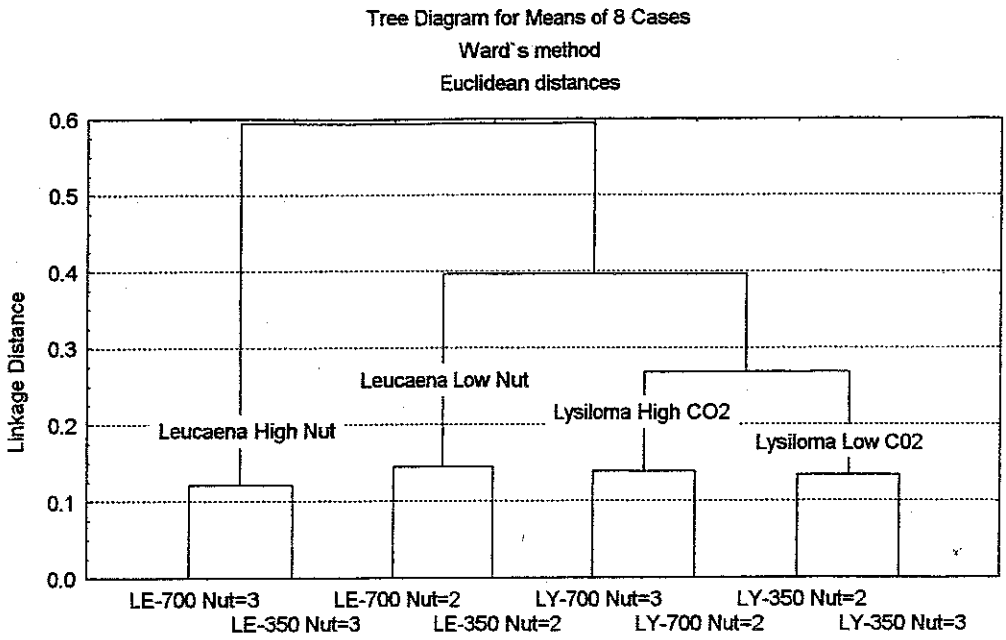


Figure 5. A hierarchical cluster analysis (Wards' method) based on Euclidean distance developed from arcsin transformed ester-linked phospholipid fatty acid (PLFA) profiles recovered from the fine roots of *Lysiloma* and *Leucaena* species exposed to 350 ppm or 700 ppm CO_2 and high or low nutrient levels. This dendogram illustrates the affect of the nutrient elevation on the *Leucaena* species microbial biomass and the low affect of the CO_2 elevation on the *Lysoloma*.

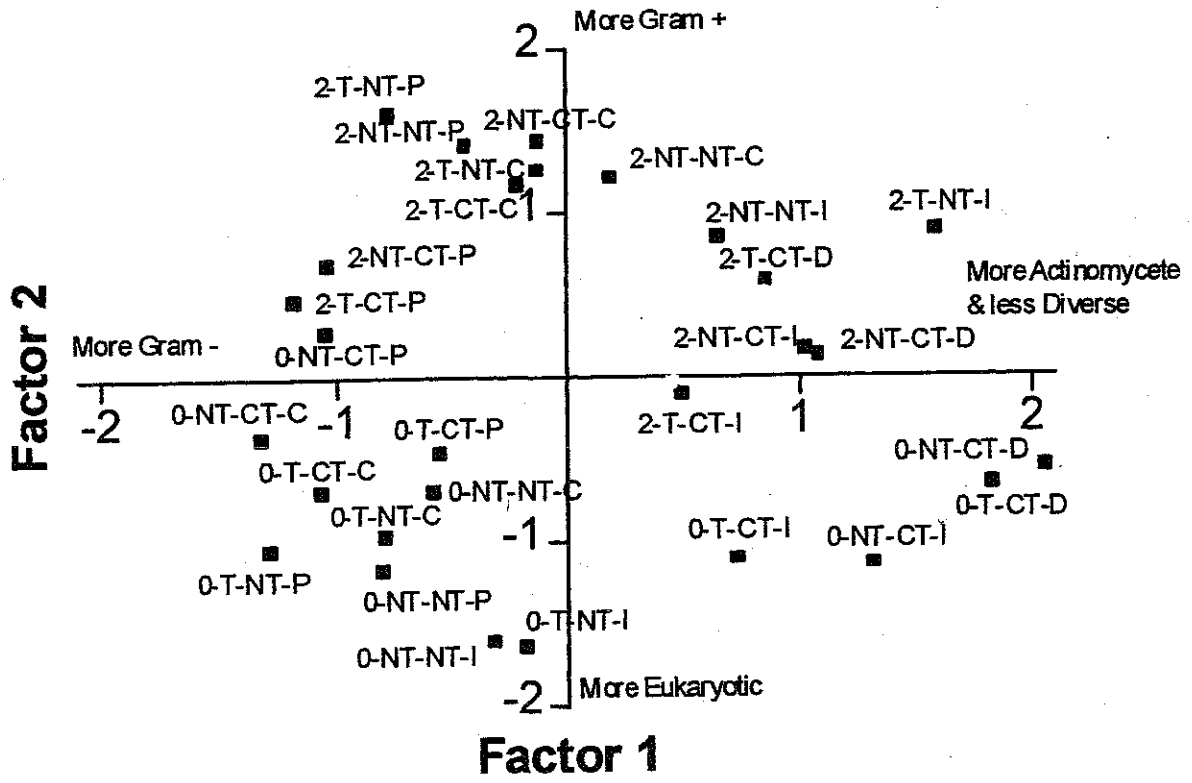


Figure 6. Factor analysis of means of PLFA profiles from soils receiving disturbances in the form of disking, irrigation and planting events. This analysis shows relative differences between depths and disturbances. The microbial communities that received disking scored most positive on factor 1 and when irrigated the same communities shifted slightly back towards the controls. After the simulated planting event (five days later) the microbial communities had re-established the community structure which existed prior to disking.

Key:
First number
0 = 0-2cm, 2 = 2-4 cm
First letter(s)
T = Traffic, NT = No Traffic
Second letters
NT = No-till, CT = Conventional tillage
Third letter
D = Disking, I = Irrigation, P = Simulated planting