EFFECTS OF PAHS ON MICROBIAL ACTIVITY AND DIVERSITY IN FRESHLY CONTAMINATED AND WEATHERED SOILS

K. Rathbone[1], J. Fuchs[1], K. Anderson[1], R. Karthikeyan[2], and N. Nurhidayat[3]

[1]Department of Agronomy, [2]Biological and Agricultural Engineering, [3]Division of Biology, Kansas State University, Manhattan, KS 66506

ABSTRACT

Polycyclic aromatic hydrocarbon (PAH)-contaminated soils are of concern to the Environmental Protection Agency (EPA) due to their mutagenic and carcinogenic nature. Bioremediation of PAHs is aimed at increasing the overall rate of natural degradation. Microbial bioremediation promotes processes by which microorganisms can take up and/or degrade organic molecules and their metabolites. Microorganisms can use organic molecules for energy or convert them into cell biomass, ultimately releasing carbon dioxide and water to the environment. Changes in microbial biomass and CO2 evolved are used as indices of degradation rates. *In situ* bioremediation utilizes indigenous microorganisms and/or involves addition of specific microorganisms with enzymatic capabilities (such as enhanced oxidative functions) to establish conditions favorable to microbial degradative activities. The overall goal of this study was to assess the biodegradation of the target PAHs, pyrene and phenanthrene, in rhizosphere soils. Comparisons of microbial diversity and activities due to PAH contamination between a weathered loam and a clean sandy loam were made. Carbon substrate utilization was the estimate of microbial functional diversity used in this study. Functional diversity was evaluated using the Biolog® assay system. Results from the study show significant differences in substrate utilization and microbial diversity between the two soil types and various treatments.

Key words: *bioremediation, functional diversity, phenanthrene, pyrene*

INTRODUCTION

Petroleum hydrocarbons are typically a complex mixture of aliphatic and aromatic organic compounds. They can be fractionated by distillation into saturates, aromatics, asphaltenes, and resins (Solomon, 1996). The saturates include n-alkanes, branched alkanes, and cycloalkanes. Polycyclic Aromatic Hydrocarbons (PAHs) are organic molecules with two or more benzene rings in which the number and arrangement of the rings result in diverse physical and chemical properties (Leahy and Colwell, 1990).

PAHs are formed during combustion reactions. Contribution of petroleum hydrocarbons to the environment results from burning of fossil fuels and subsequent atmospheric deposition, primarily from industrialized countries (Sims and Overcash, 1983). Internal combustion engines produce large amounts of petroleum hydrocarbon byproducts. In addition, many industrial activities associated with processing, production, and disposal of petroleum hydrocarbons contribute to the overall environmental load. Some of these activities include liquefaction, heat and power generation using fossil fuels, catalytic cracking procedures, carbon-black, coke, asphalt, and coal-tar production, refining and distillation of crude oil, wood-treatment processes, accidents resulting from handling and storage, open burning of tires, coal, etc., and other incineration processes (Bumpus et al., 1989; Wilson and Jones, 1993).

Soils contaminated with petroleum are classified "hazardous" and are of great importance because certain petroleum derivatives are potentially carcinogenic and mutagenic (Song and Bartha,

1990; Wilson and Jones, 1993). Remediation of soils contaminated with petroleum hydrocarbons is a global problem that consumes economic resources from both industrial and government coffers. Some petroleum hydrocarbons are carcinogenic in lab animals and mutagenic in bacteria (Wilson and Jones, 1993). Cancer induced in rabbits by injecting coal tar into dermis prompted toxicological studies resulting in benzo[a]pyrene, a target PAH, being isolated as a carcinogen (Cook et al., 1933). Other studies have confirmed that pyrene and phenanthrene, both PAHs, are mutagenic in bacterial and animal cells as well as carcinogenic in rodents (Wilson and Jones, 1993).

Soils contaminated with PAHs are remediated using a diverse set of physical and chemical methods that strip contaminants from the soil. In addition to physical and chemical treatment methods, biodegradation has become more accepted over the last two decades. Biodegradation, in general, is the decomposition of compounds by living organisms. Degradation of PAHs may be accomplished by complete mineralization, cometabolic degradation, and/or radical oxidation (Mahro et al., 1994). Complete mineralization is the total breakdown of organic compounds to water and carbon dioxide. Complete mineralization is not achieved in practice with PAHs since only two-thirds of the organic compound is mineralized and the remaining third is incorporated into new cell biomass (Leisinger et al., 1981). Cometabolic degradation is a process by which a recalcitrant compound is degraded in the presence of an analogous compound. The necessary enzymes required to degrade the recalcitrant compound are induced by the analogous compound (Leisinger et al., 1981). In mixed culture systems such as soils, cometabolic transformations may augment degradation capabilities of other organisms. In pure cultures, cometabolism ceases without any benefit to the organism (Atlas and Bartha, 1980). Radical oxidation can also occur and is a chemical transformation of an organic compound which is tied to the activity of extracellular enzymes produced by soil biota.

Factors that affect biodegradation include pollutant concentration and pre-exposure time. Microbial communities present in previously contaminated soil can metabolize PAHs at greater rates than soil microbial communities found in uncontaminated soils. An acclimation period is required for microbes after a contamination event before degradation processes commence. During this acclimation period, appropriate soil microbial communities are developed through growth and enzyme induction. Many PAH-contaminated soils have been polluted for years. Increased residence time may make the hydrocarbons unavailable to microorganisms for degradation because PAHs can be strongly sorbed to the soil matrix. Bioavailability and other interactive effects of these PAHs on microorganisms are important to know because these factors ultimately will determine the activity and diversity of microorganisms. PAH bioavailability is reduced due to its hydrophobicity and sorption onto soil minerals and soil organic matter (SOM). PAHs are relatively water insoluble and may not be available for microbial degradation. In addition, they may diffuse into soil particle sites that are inaccessible to microbes from the outside. Once inside, they are entrapped and/or continue

to be incorporated into SOM. Often, the sites in which they are entrapped have effective diameters of less than 1.0 μm. Consequently, bacteria may not be able to access these sites for PAH degradation to occur. Greater population density and diversity of microorganisms often result in increased degradation rates of PAHs in soil. The soil micro biota is capable of producing many degradative enzymes needed for mineralization of PAHs; however, a single microbial strain may not be able to produce the necessary enzymes (Lappin et al., 1985). Biodegradation of recalcitrant compounds such as PAHs is limited by soil properties, bioavailability, cometabolites, and microbial populations with degrading capabilities (Gunther et al., 1996).

Comprehensive knowledge of the diversity of indigenous microbial communities and their activities is considered important when assessing the strategy and outcome of bioremediation; yet, little is known about the components of functional diversity responsible for degradation of PAHs in field situations. Degradation of PAHs is facilitated by communities of numerous functional microbial populations and, therefore, microbial composition determines the degradative potential and success of any bioremediation project. A wide range of techniques is available to identify components of microbial communities to examine morphological and physiological properties of isolated pure cultures, but these methods do not describe functional diversity.

Diversity measures of the terrestrial environment are limited by methods and taxonomy. Microbial communities can be considered functional units characterized by the sum of their metabolic properties. Therefore, substrate utilization patterns of the entire community result from taxonomic diversity and abundance of each taxon. The Automated Microbial Identification System, developed by Biolog® Inc. (Hayward, CA) for rapid identification of laboratory cultures, has been shown to be useful in the characterization and classification of heterotrophic microbial soil communities.

In this study, clean and contaminated soils were examined to test the effects of pyrene and phenanthrene contamination on microbial activity and diversity. Effects of pyrene and phenanthrene on microbial activity were assessed by measuring mineralized CO2-C and NO3-N. Microbial diversity was assessed by substrate utilization patterns using Biolog® assay.

EXPERIMENTAL SECTION

Soil Microcosms

To study the effects of pyrene and phenanthrene contamination on soil microbial activity and diversity, two soil types were selected. A sandy loam (SL) with no known history of petroleum contamination was obtained near a greenhouse site affiliated with university grounds at Kansas State University, Manhattan, Kansas. A loam (L) that had been previously contaminated with jet and diesel fuels was obtained from a California naval base. This soil was weathered for two years prior to our study. Both soils were ground and air-dried prior to the experiment and the chemical properties were determined (Table 1).

Treatments

Treatment combinations consisted of contaminant(s) concentration of 230 mg kg^{-1} of dry soil with the following amendments to the SL: pyrene at 230 mg kg^{-1} (A); phenanthrene at 230 mg kg^{-1} (B); a combination of pyrene and phenanthrene at 115 mg kg^{-1} each (AB); and an uncontaminated control soil (C). Weathered-L treatments consisted of only pyrene and phenanthrene in combination at 115 mg kg^{-1} each (E) and a weathered-L control (F). The treatments were carried out in triplicate. The contaminant solutions were prepared by dissolving pyrene (Sigma P-2146) and phenanthrene (Sigma P-2528) in acetone. Each solution was applied uniformly to a thin layer of pre-weighed soil kept in a shallow foil-lined tub using a spray bottle. The soil was continuously mixed to facilitate uniform contamination. One hundred grams of each soil was weighed into 240 mL Qorpak® bottles and brought to optimum moisture content (already found in literature). The samples were then placed in 940 mL Mason jars and the jars were sealed. Soils were incubated at 25 C in a humidified environmental chamber. At 0 (Time0), 21 (Time1), 42 (Time2), and 63 (Time3) days, triplicate samples from each treatment were allocated to mineralized CO2-C and NO3-N, contaminant concentration, microbial biomass, and Biolog® assay determinations.

Determination of Mineralized CO_2-C

Each day during the first week of the study and at least weekly thereafter, CO_2-C evolved from the microcosms was sampled, by taking 0.5 mL gas samples from the head space of sealed mason jars containing the soil samples. The concentration of CO_2-C was measured on a Shimadzu Gas Chromatograph-8A (Shimadzu Inc., Kyoto, Japan). The gas chromatograph was equipped with a thermal conductivity detector (TCD) and a 2-m Porapak column. Column temperature was maintained at 70° C and Helium was used as the carrier gas at a constant flow rate of 14 mL min^{-1}. After head space gas sampling, jars were opened for approximately 15 min under a laminar flow hood to allow samples to equilibrate with atmospheric CO_2, then resealed and allowed to continue incubation.

Determination of Mineralized NO_3-N

Net nitrification rate was measured as an indicator of microbial stress and contaminant toxicity. To evaluate this response at each sampling time, 25 g of soil from each treatment was weighed into duplicate 160 mL serum bottles. Ammonium sulfate was added to one serum bottle at a concentration of 75 mg NH4-N kg^{-1} dry soil. The ammonium provided ample N to the microorganisms and thus eliminated any N limitations to nitrification. Water was added to the other serum bottle as a control. Samples were then sealed with parafilm and incubated for 7 days at 25 C. Ten g of soil from each of these samples was extracted with 50 mL of 1M KCl and analyzed for NO_3^--N and $NH4^+$-N on an Alpkem Autoanalyzer (Alpkem Corp., Clackamas, Oregon) before and after incubation. Net nitrification rates were determined by the difference between final and initial nitrate concentrations and expressed on a per-day basis.

Determination of Contaminant Concentrations

To determine contaminant concentrations, 2.5 - 3.0 g of soil from each treatment was placed (at each sampling time) in scintillation vials and spiked with 100 L of 1000 mg kg^{-1} tetracosane, a matrix recovery standard. The samples were shaken with 10 mL of high-purity acetone for 30 min, centrifuged for 10 min, and supernatants collected. These steps were repeated three times. An aliquot of this extract was transferred to an analytical vial, spiked with 5 L of 1000 mg kg^{-1} 5- - androstane (an internal standard), and the vials were sealed and stored at -20°C until analyzed. The samples were analyzed by an HPLC (Hewlett Packard 1080) set at 240 nm, 40 bw, using a C18 reverse phase column (Vydac) and diode array detector. Percent recovery for the contamination procedure was calculated by comparing contaminant concentration recovered in extract to the initial concentration.

Determination of Microbial Biomass C and N

Soil microbial biomass was determined using the chloroform fumigation-incubation technique (Jenkinson and Powlson, 1976). Five grams of sample was weighed into duplicate 160 mL serum bottles. Both samples were preincubated for 5 days at 25 C, after which one of the samples was fumigated in a vacuum desiccator with ethanol-free chloroform. Soils were saturated with chloroform by applying vacuum three times, followed by equilibration with the atmosphere. Each time, the chloroform was allowed to boil for 30 seconds. After the third evacuation, the desiccator valve was closed and the chloroform was allowed to diffuse into the soil for 24 hours. The beaker filled with chloroform was then removed from the desiccator. Desiccator with samples was evacuated and equilibrated with atmospheric pressure eight times for 3 minutes each cycle to remove residual chloroform. Samples were then removed, sealed tightly, and incubated for 10 days at 25 C. At the end of the incubation period, CO2 -C concentrations from the head space of the serum bottles were measured on a Shimadzu Gas Chromatograph-8A (Shimadzu Inc., Kyoto, Japan). After measuring CO2, 25 mL of 1M KCl was added to the soil in each bottle and shaken for 30 min on an orbital shaker. Samples were then centrifuged, filtered through nylon mesh, and analyzed for $NO3^-$-N and $NH4^+$-N on an Alpkem Autoanalyzer (Alpkem Corp., Clackamas, Oregon). Microbial biomass carbon and nitrogen were calculated according to Voroney and Paul (1984):

$$C = (Fc - UFc)/Kc \quad (1)$$

$$N = (FN - UFN)/KN \quad (2)$$

where,

C = Microbial biomass carbon

N = Microbial biomass nitrogen

Fc = μg CO2 -C/g soil from the fumigated sample

UFc = μg CO2 -C/g soil from the unfumigated sample

Kc = 0.41, the fraction of biomass C mineralized to CO2

FN = μg NH4-N & NO3-N/g soil from the fumigated sample

UFN = μg NH4-N & NO3-N/g soil from the unfumigated sample

KN = -0.014(FC /FN) + 0.39, the proportion of microbial N mineralized during the 10-day incubation

Biolog Studies

Ten grams of each soil sample was suspended in 100 mL of 2% NaP4O7 on a rotary shaker at 300 rpm for 1 hour. After 30 minutes to allow samples to settle, a 1 mL aliquot of supernatant was diluted tenfold to obtain a final dilution of 10^{-3}. A 150 μL aliquot of this suspension was added to each well of both Biolog® G^- and Biolog G^+ microplates (Biolog Inc., Hayward, California). The microplates were kept at 22° C in a controlled environment chamber. Absorbance at 595 nm was measured for each well at 24, 48, 72, and 168 hours. Clean SL (C) from Time0 was used as control to determine the optimal incubation time.

Activity was determined by Average Well Color Development (AWCD) according to Garland and Mills (1991):

$$AWCD = (R - C)/X \quad (3)$$

Number of utilized substrates = Number of substrates which (R-C) > AWCD (4)

$$\text{Functional diversity} = -\ pi(\ln pi) \quad (5)$$

where,

R = absorbance of responsive well

C = absorbance of control well

X = number of different substrates in both Biolog Gram Negative and Biolog Gram Positive; here X = 190

pi = the ratio of activity in a particular substrate to the sum of activities on all substrates.

The AWCD, number of utilized substrates, and functional biodiversity data were used to perform principal component analysis (PCA) to profile pattern differences of each treatment.

RESULTS AND DISCUSSION

Mineralized CO_2-C

Upon wetting, both soil types (SL and L) and all treatments (A, B, C, AB, D, E, and F) demonstrated a slight increase in net mineralized CO_2-C rate during the first two days, followed by a decline over the next two to five days (Figures 1 and 2). Net mineralized CO_2-C for the control SL (C) then declined over the remaining 58 days, except for an unexpected increase which was detected on day 26 (Figure 1). Each of the treatments, pyrene (A)-, phenanthrene (B)-, and pyrene

+ phenanthrene (AB)-amended SL had similar patterns of response (Figure 1). After four or five days, there was an increase in net CO_2-C mineralization rate until day 19 and a decline throughout the remainder of the incubation (Figure 1).

Net mineralized CO_2-C from weathered L (E and F) showed a decline from the first day and throughout the entire period (from ~0.058 mg C g^{-1} day^{-1} to <0.005 mg C g^{-1} day^{-1}). Some short-lived instances of increased CO_2-C were noted on days 19, 35, and 49 (Figure 2). These short increases may be due to experimental error or variation among samples, or may reflect some changes in microbial populations. Unfortunately, these increases did not occur at times when Biolog® assays were performed. Substrate utilization pattern changes at these sampling periods may have pointed to microbial population functional shifts.

Net mineralized cumulative CO_2 -C showed significant differences between control and pyrene-amended, control and phenanthrene-amended, control and pyrene + phenanthrene-spiked, and weathered and weathered-spiked (Figure 3, statistics not shown). There were large significant differences seen between the clean and pyrene + phenanthrene-amended, and between weathered and weathered-spiked soil treatments. There was also a significant difference between clean and weathered soils (data not shown). This points to differences in microbial communities between the two soil types.

Mineralized NO_3^--N

Nitrification is the biological conversion of ammonium to nitrite and nitrate. It is a two step process carried out, primarily, by two groups of chemoautotrophic bacteria. Both groups use inorganic forms of nitrogen as their source of energy and CO_2 as their carbon source. The *Nitrosomonas* spp. are the most common ammonium oxidizers:

$$2NH_4^+ + 3O_2 \rightarrow 2NO_2^- + 2H_2O + 4H^+ + \text{energy} \quad (6)$$

Nitrobacter spp. are then able to oxidize the nitrite into nitrate:

$$2NO_3^- + O_2 \rightarrow 2\ NO_3^- + \text{energy} \quad (7)$$

In this study, nitrification inhibition was examined as a potential indicator of more sensitive microorganisms' (nitrifiers') response to environmental stress, caused by pyrene and phenanthrene contamination. Net nitrification rates (Figure 4, Table 2) indicated that all contaminated treatments significantly inhibited nitrification activity throughout the experiment. No significant increase in net nitrification rate was observed in contaminated vs. uncontaminated soils by day 42.

Decline in the net measured negative nitrification rate in uncontaminated weathered L (F) may indicate a situation where immobilization is occurring faster than mineralization due to a high microbial growth rate. The incubation time for the nitrification procedure outlined here was 7 days. This may have been too long since it appears there was a proliferation of microorganisms utilizing the

available nitrate. To minimize this effect, a shortened incubation period or an additional extraction on day 14 may have improved the results. Using the difference between the 7-day and 14-day extractions to calculate net nitrification might have helped determine when the microbial population reached equilibrium.

Nitrification inhibition results in an increase in ammonium, which did not occur in any of the treatments. Ammonium and nitrate values were negative (Tables 2 and 3). This reinforces the notion that net immobilization of nitrogen occured. In the clean SL, this may be explained by increased nitrifiers and microbial demands for ammonium when microbial populations increased. In the contaminated SL treatments, the PAHs were the primary carbon source and ammonium stimulated microbial populations.

Net nitrification rate was negligible in all contaminated soils, yet there was a positive response in microbial activity. There was no significant difference in cumulative carbon respired between clean and contaminated SL treatments. However, net nitrification rates were zero for contaminated SL and > 3 $\mu g\ N\ g^{-1}$ soil day^{-1} for clean SL. Therefore, despite indication of a stress response by nitrifiers, no difference in microbial respiration activity was measured. A similar trend holds true for the weathered L, except net nitrification rate was negligible for weathered L-spiked. Respiration in weathered L-spiked was significantly greater than the weathered L-control.

Microbial Biomass

The microbial biomass procedure was not successful (data not shown). After fumigation and incubation for 10 days, fumigated samples had not responded as much as unfumigated samples for CO2 generation. This resulted in negative values for biomass carbon. Questions have arisen about the fumigating technique since this response was consistent for both contaminated and uncontaminated samples. As a check, fumigation was performed by two authors in parallel analyses and the results were similar. It is plausible that due to high soil water content, chloroform was not effectively evacuated under the protocol used. A more intensive evacuation procedure or pre-drying the samples to 25 % moisture content may have alleviated this problem. This issue was not a problem with the L soil as much as with the weathered soil, since adequate aeration and soil structure were maintained at 30% moisture content, thus enabling enough surface-to-air contact for seemingly good evacuation of residual chloroform.

A more effective way to estimate microbial biomass in this situation may have been the chloroform fumigation-extraction method (Vance et al., 1987). This would overcome the need for microbial response of the fumigated samples over unfumigated. Joergensen et al. (1995) successfully employed this technique when measuring biomass in fuel-oil-contaminated soil. They measured organic C by UV-persulfate oxidation to CO_2 by IR-detection using a Dohrman DC 80 automated system and ninhydrin-reactive N in the initial soil extracts. After fumigation, inorganic and organic N and organic C were measured by fractional distillation.

Degradation of Pyrene and Phenanthrene

Extractions from non-spiked, weathered L revealed no remaining pyrene or phenanthrene. Pyrene degradation occurred more slowly than phenanthrene degradation (Table 4). After 42 days incubation, pyrene degradation was significant only in the pyrene + phenanthrene-amended weathered L. Pyrene concentrations remained statistically similar throughout the experiment in the other two treatments. Results were inconclusive for degradation rate between soil types. The authors were unable to tease out effects of texture, organic matter, cometabolism associated with degradation of lingering, aged PAHs, and acclimation effects from previous contamination. Previous acclimation to hydrocarbon substrate enabled the weathered soil's established community, which was enzymatically ready and capable within 42 days to be effective at degrading added pyrene. To conclude that acclimation effects were a factor to degradation requires soils of similar texture and nutritional status.

Significant phenanthrene degradation was apparent only after 21 days in both SL treatments, but did not occur until day 42 in the weathered L treatment. Occurrence of cometabolism in SL cannot be concluded in this study. Given more time, differences in degradation rates between treatment combinations may become apparent. Table 4 and Figures 5 and 6 are based on the percentage of individual contaminant recovery by extraction compared to amount calculated, assuming complete and homogeneous application to the soil.

Biolog Assay

The utilized substrates absorbance pattern of clean SL (C) indicated that 3 days after inoculation was when the number of utilized substrates had reached a saturation and the Average Well Color Development (AWCD) was still increasing. By that time, further incubation did not result in a significant increase in number of utilized substrates. The absorbance of each substrate was proportional to its degree of utilization (Kreitz and Anderson, 1997).

The PCA (Figure 7) shows distinct groupings according to soil type and treatment. Pyrene and phenanthrene additions demonstrated stronger influence on substrate utilization patterns than soil type; thus, functional profiles of contaminated soil were quite similar. The clean SL and weathered L profiles developed distinctly after the 42-day incubation.

Mean comparison of AWCD, number of utilized substrates, and Shannon-Weaver diversity index between the soil samples (Figure 8) indicated there was greater activity and substrate use in the 42-day versus 21-day incubation. Microorganisms in weathered L were more active and utilized more substrate than in the clean soils. Microbial activity, substrate utilization, and diversity in contaminated soils were higher than in non-contaminated soils. However, no statistical differences between the 21- and 42-day incubations were observed for functional diversity utilization patterns. Detailed analysis of substrate use during those periods of time revealed that significant functional diversity resulted in greater utilization of amino acids. Contaminated SL (AB) had greater diversity in amino acid utilization than weathered L-spiked (Figure 9).

The results of this study indicated that microbial communities exposed to pyrene- and phenanthrene-contaminated soils produced distinctive patterns of Biolog® substrate utilization. The patterns indicated differences in community structure which resulted in a change in decomposition ability by soil microorganisms. The pyrene and phenanthrene may have induced changes in type and amount of enzymes, or composition of the microbial population. The contaminants induced enzyme response from the microorganisms under their influence. The production of aromatic ring deoxygenase, one of the PAH-degrading enzymes, was induced by the presence of PAH (Dagher et al., 1997).

The 42-day incubation allowed the microorganisms to adapt and flourish as indicated by the increased microbial activity and number of utilized substrates. Microbial communities in the weathered soils may have been selected for, or adapted to PAH exposures. The weathered soil had more organic matter, which may have increased microbial activity and thus pyrene and phenanthrene degradation rates (Kastner and Mahro, 1996).

However, organic matter did not appear to increase the population of known PAH-degrading microorganisms as much as general heterotrophic microorganisms (Carmichael and Pfaender, 1997). PAH degradation capabilities are associated with members of certain taxa such as *Pseudomonas, Sphingomonas*, and *Burkholderia*, independent of origin of the soil from which bacteria were isolated (Mueller et al., 1997). Moreover, genes responsible for PAH degradation are homologous and ordered (Dagher et al., 1997). These genetic characteristics restrict enzyme diversity in microbial communities of pyrene- and phenanthrene-contaminated soils. Biosynthesis of PAH-degrading enzymes also requires a large amount of specific enzymes for their production. The biosynthesis increases the number of substrates utilized and leads to a more homologous expression of enzymes within the community. This phenomenon might explain the insignificant differences in overall Biolog® functional biodiversity at 21- and 42-day incubations.

The role of biosynthesis enzymes is confirmed by significant differences in utilization of amino acids. The Biolog® amino acids were used as substrates for the biosynthesis of these enzymes. Microorganisms in weathered L, used a less diverse array of Biolog® amino acids than those in freshly contaminated weathered L presumably due to more amino acids already present in the weathered L.

SUMMARY

The effects of pyrene and phenanthrene on microbial activity were determined in terms of mineralized CO_2-C and NO_3^--N. Additions of pyrene, phenanthrene, and pyrene + phenanthrene enhanced microbial activity in SL. Pyrene + phenanthrene enhanced activity in weathered L as well. A wider range of amino acid substrates were utilized in weathered L-spiked compared to weathered L.

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Table 1. Chemical properties of the soils used.

Texture	pH	% OM	NH_4-N	NO_3-N	Total N	CEC
				(mg/kg)		(meq/100g)
Sandy Loam (SL)	7.77	0.5	2.0	9.2	224	6.95
Weathered Loam (WL)	5.82	2.3	39.9	56.0	1284	20.29

Table 2. Mean net nitrification rates derived from ammonium amended samples in nitrification analysis.

Treatment	$Time_0$	$Time_1$	$Time_2$
	($\mu g\ NO_3$-N g^{-1} dry soil day^{-1}		
SL Pyrene (A)	0.132 + 0.039 a	-014 + 0.007 a	-0.34 + 0.004 a
SL Phenanthrene (B)	0.122 + 0.018 a	0.003 + 0.003 a	-0.22 + 0.006 a
SL Pyrene + Phenanthrene (AB)	-1.282 + 0.01 d	0.123 + 0.028 b	0.096 + 0.021 b
Clean SL (C)	2.792 + 0.307 c	3.348 + 0.268 c	3.376 + 0.477 c
Weathered L Phyrene + Phenanthrene (E)	0.782 + 0.685 ab	0.015 + 0.014 a	-0.016 + 0.008 a
Clean Weathered L (F)	1.423 + 0.030 b	-0.201 + 0.255 ab	-10.28 + 0.767 a

Statistical significance was calculated among treatments within each time period, $p<0.05$.

Table 3. Mean net nitrification production of ammonium, derived from ammonium sulfate amended samples in nitrification analysis.

Treatment	$Time_0$	$Time_1$	$Time_2$
	($\mu g\ NO_3$-N g^{-1} dry soil day^{-1}		
SL Pyrene (A)	2.461 + 1.22	-9.240 + 0.976	-5.793 + 0.987
SL Phenanthrene (B)	1.544 + 1.55	-8.084 + 1.122	-5.856 + 1.000
SL Pyrene + Phenanthrene (AB)	-2.176 + 1.58	-6.767 + 0.662	-2.529 + 0.98
Clean SL (C)	-1.661 + 0.651	-1.869 + 1.845	-3.475 + 1.084
Weathered L Phyrene + Phenanthrene (E)	1.028 + 1.872	-15.123 + 0.317	-9.348 + 0.911
Clean Weathered L (F)	-1.568 + 0.812	-21.164 + 1.086	-6.440 + 1.181

Table 4. Mean % of original contamination concentration recovered.

Mean Pyrene Concentration (%)			
Treatment	$Time_0$	$Time_1$	$Time_2$
SL Pyrene (A)	94-539 + 2.004 a	94.525 + 0.241 a	95.106 + 0.972 a
SL Phenanthrene (B)	69.678 + 2.133 a	77.265 + 1.906 b	70.508 + 2.352 ab
Weathered L Phyrene + Phenanthrene (E)	121.701 + 7.349 a	99.625 + 4.156 b	90.957 + 4.236 b
Mean Phenanthrene Concentration (%)			
SL Phenanthrene (B)	92.654 + 1.387 a	17.362 + 6.690 b	41.887 + 4.434 c
SL Pyrene + Phenanthrene (AB)	64.989 + 2.112 a	51.944 + 3.038 b	23.115 + 2.729 c
Weathered L Phyrene + Phenanthrene (E)	104.554 + 5.305 a	79.985 + 8.522 a	53.883 + 4.329 b

Figure 1. Daily CO_2 respiration from Clean SL (1a), Pyrene (1b), Phenanthrene (1c), and Pyrene and Phenanthrene (AB) Amended SL, obtained from University Grounds, Kansas State University, Manhattan, Kansas.

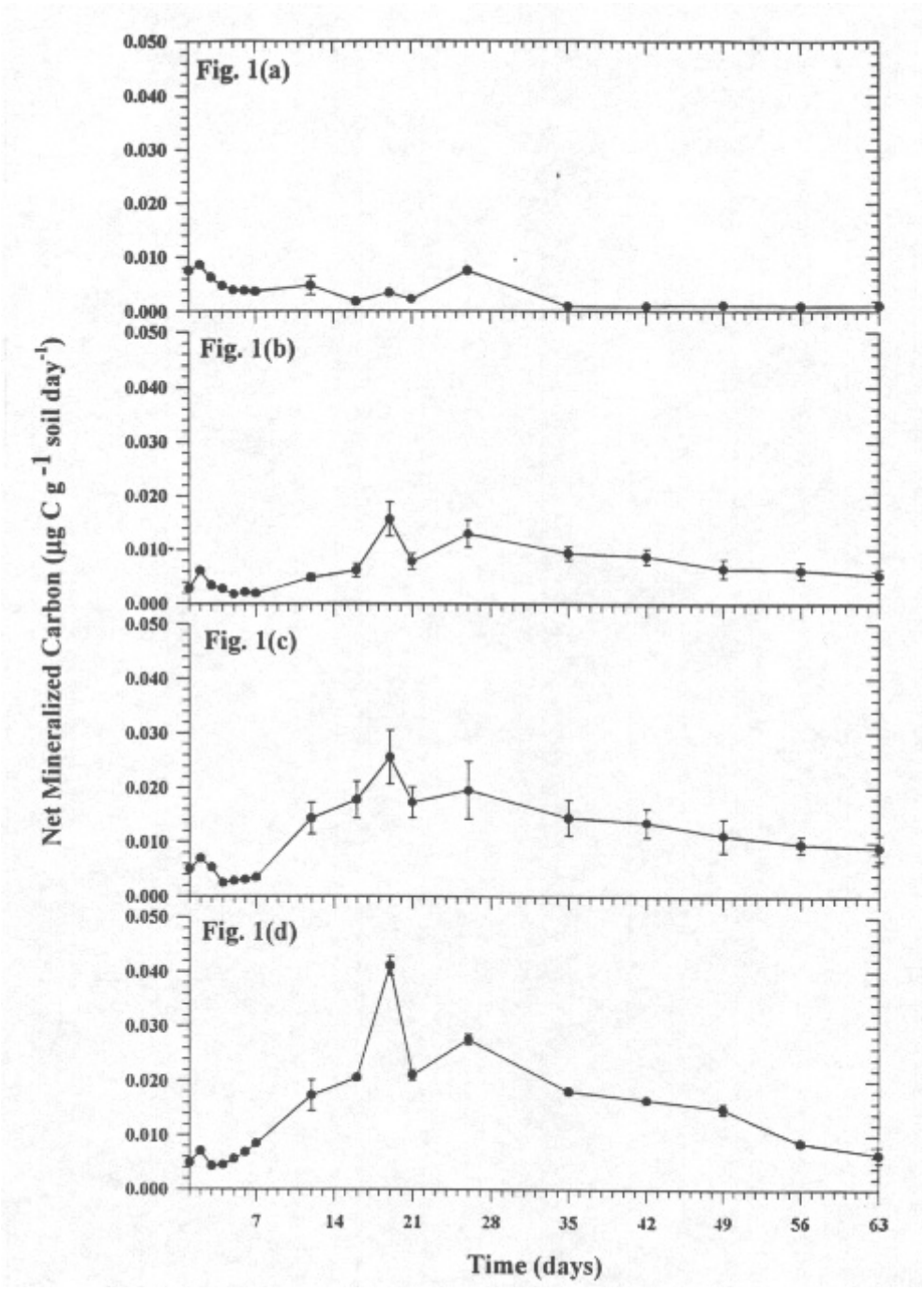

Figure 2. Daily CO_2 respiration from Clean (2a) and Pyrene and Phenanthrene (2b) Amended Weathered L, obtained from California Naval Base.

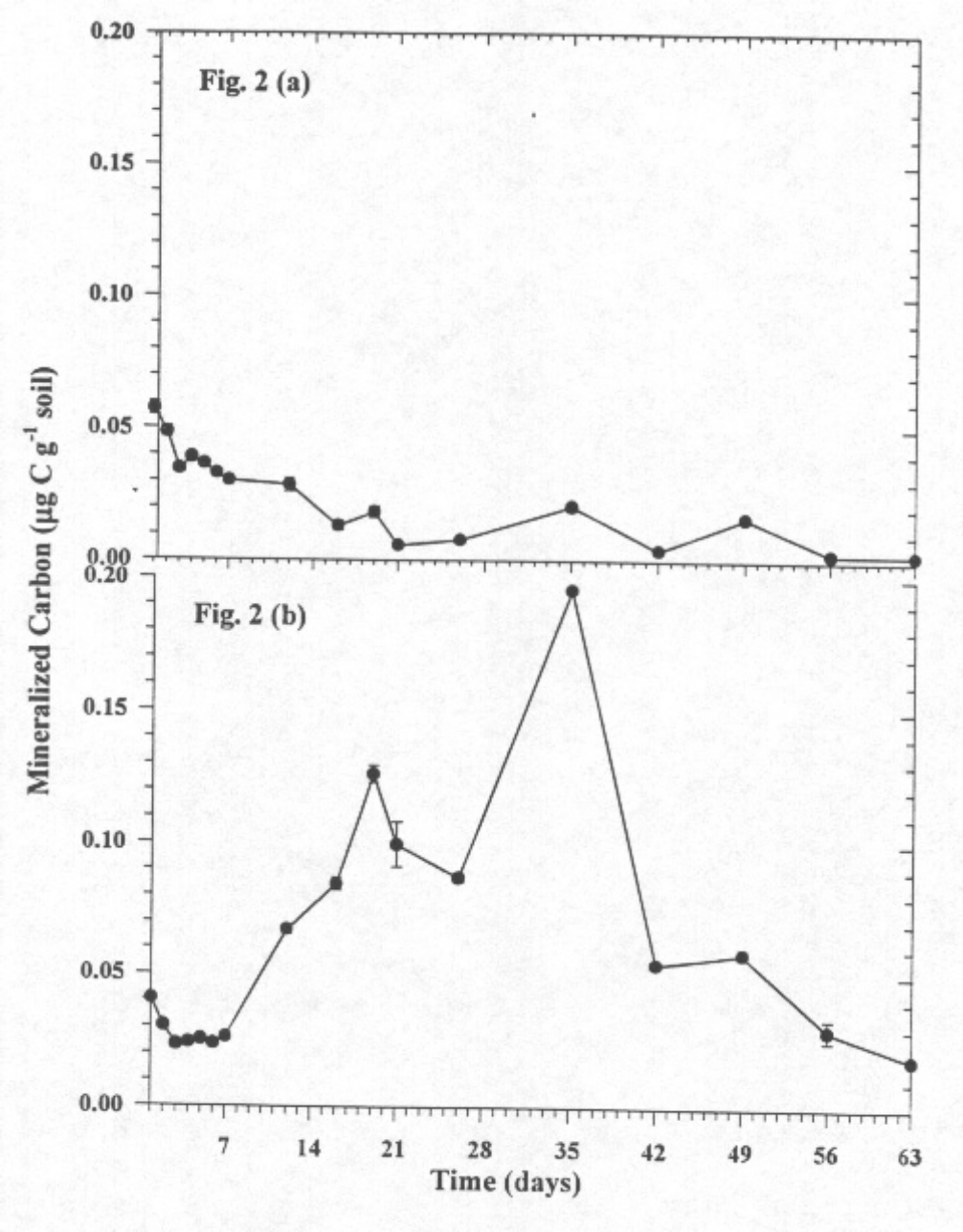

Figure 3. Cumulative CO_2 respiration from SL and Weathered L.

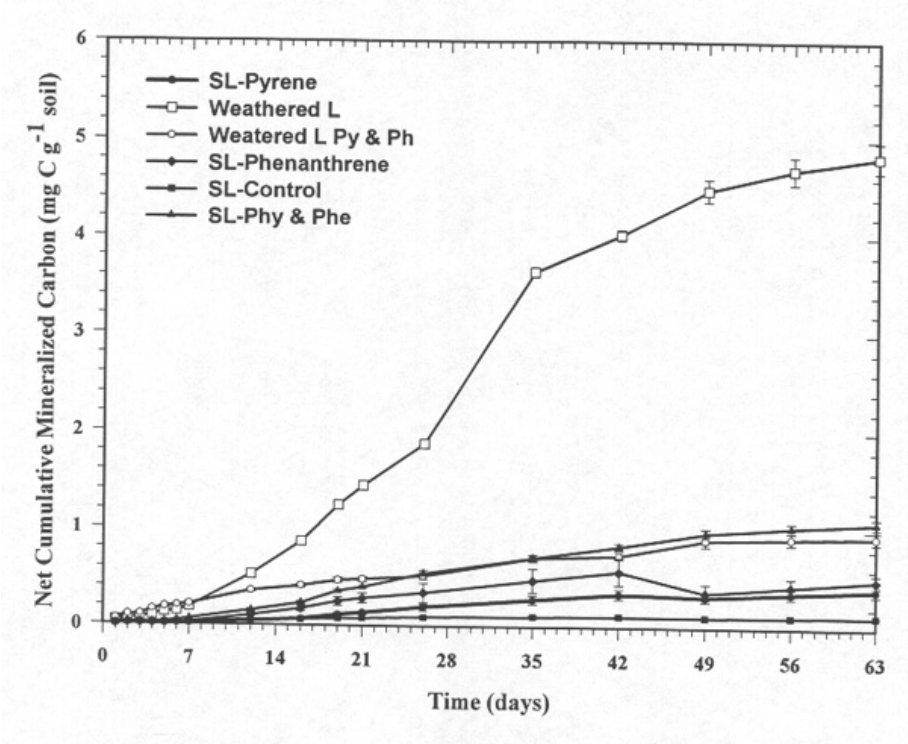

Figure 4. Nitrification Rates from SL and Weathered L.

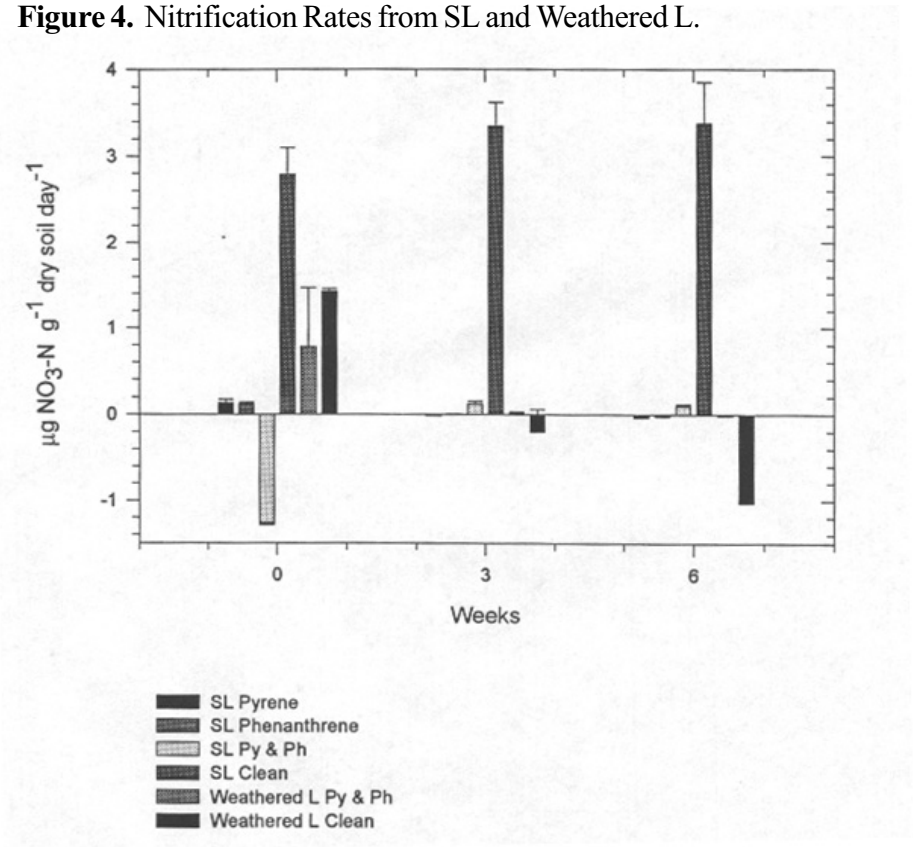

Figure 5. Pyrene degradation in SL and Weathered L.

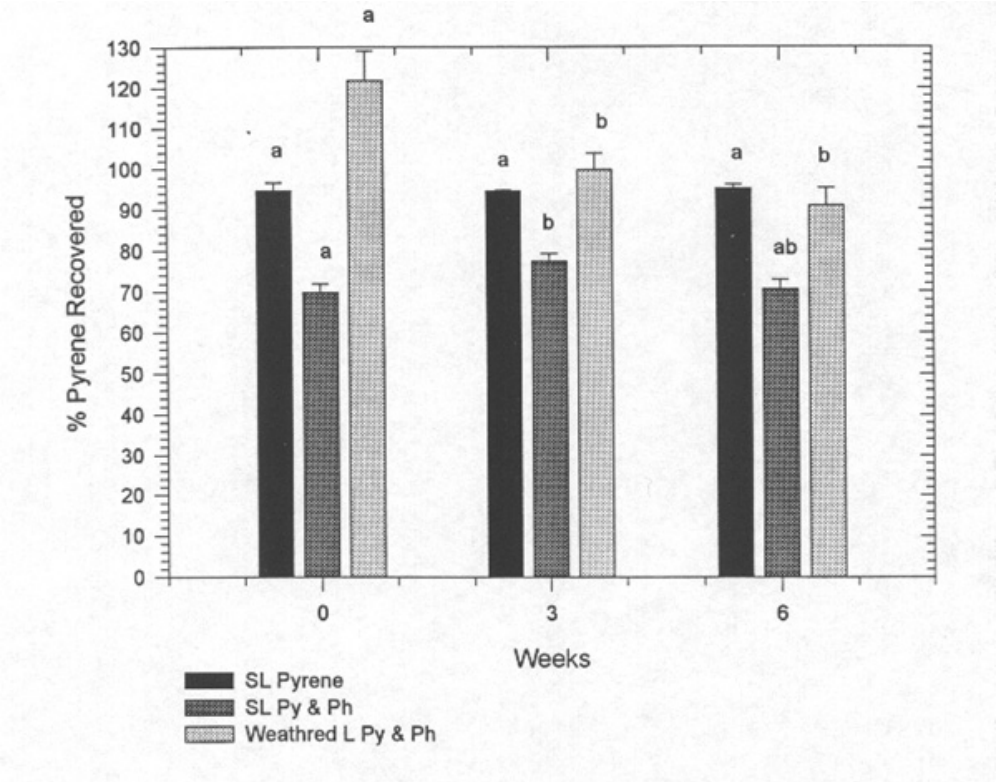

Figure 6. Phenanthranene degradation in SL and Weathered L.

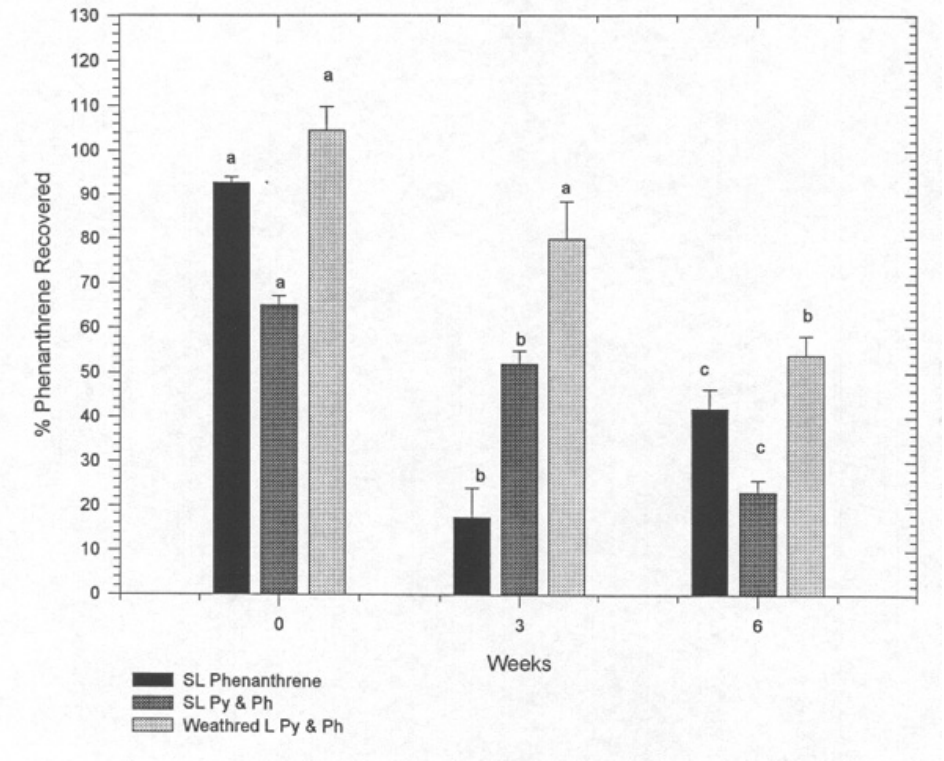

Figure 7. Substrate utilization pattern by principal component analysis.

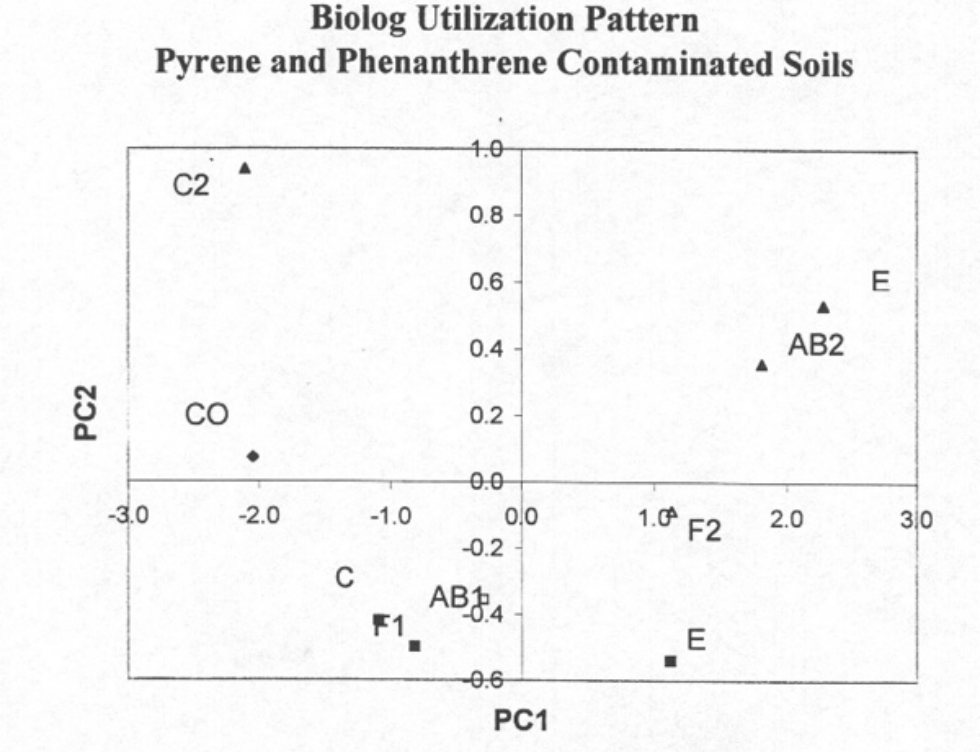

Figure 8. Correlation of substrate utilization with time for SL and Weathered L.

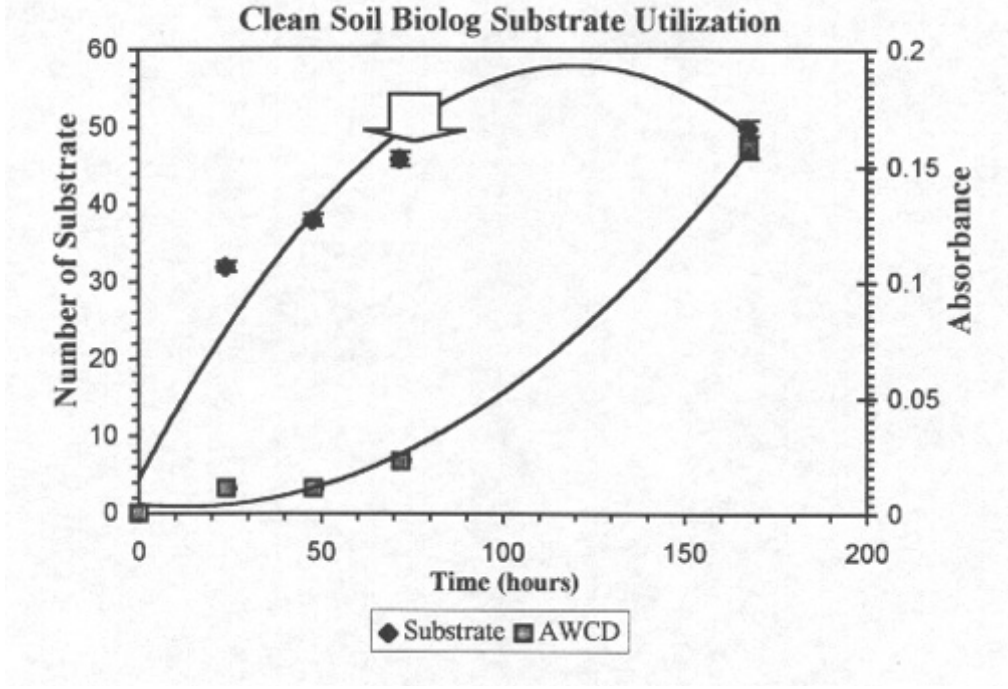

Figure 9. Microbial activity, substrate utilization, and functional biodiversity in SL and Weathered L.