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I am submitting herewith a thesis written by Mary Helen Ward entitled "Synthetic Fuel Oil Effects on Microbial Activity and Nitrogen Transformations in a Soil." I have examined the final copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Ecology.

Gary S. Sayler, Major Professor

We have read this thesis and recommend its acceptance:

Accepted for the Council:

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SYNTHETIC FUEL OIL EFFECTS ON MICROBIAL ACTIVITY AND NITROGEN TRANSFORMATIONS IN A SOIL

A Thesis

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ABSTRACT

The effects of a solvent refined coal oil (SRC-II) on microbial processes in a Captina silt loam soil was examined. The soil samples were maintained under environmental conditions favorable for most aerobic microbial activities. Soil was treated with four oil concentrations ranging from 0.2% to 8.6% (wt/wt). Oxygen uptake rates, total viable cell counts, numbers of nitrifying bacteria and inorganic nitrogen concentrations were monitored before oil addition and at regular intervals for three months afterwards. Organic carbon, total nitrogen and soil pH were also measured before and after application of the oil.

The SRC-II coal oil affected soil processes at all treatment levels. The lowest oil concentration (0.2%) decreased numbers of nitrifying bacteria while increasing total viable cell numbers and net nitrogen mineralization. The higher oil concentrations reduced oxygen uptake rates and total viable cells as well as nitrifier numbers. Soil treated with a 1.7% oil concentration showed significant increases in respiration rates and cell densities after two months, while no significant increases were observed at oil levels of 3.4% and 8.6%.

The application of the coal oil to soil samples raised the carbon to nitrogen ratio of the soil. The sum of nitrate and ammonium nitrogen in the oil-treated soils was never significantly lower than the control soil levels indicating that nitrogen was not limiting to decomposition. However, the toxicity of the oil towards the nitrifying bacteria resulted in an accumulation of ammonium in treated soils. This may

affect plant establishment on soils contaminated with a synthetic fuel oil.

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I. INTRODUCTION

The ability of microorganisms to utilize hydrocarbons has been known since the late 1800s. The reviews of the literature on the interaction of microbes and hydrocarbons include a great number of microbial species, habitats and an extensive range of hydrocarbons (see reviews: Zobell, 1946; Atlas and Bartha, 1973; Crow et al., 1974; Vanloocke et al., 1975; Colwell, 1978; Atlas, 1981). However, the susceptibility of hydrocarbons to microbial attack depends on their molecular structure and some hydrocarbons are actually toxic, thus interfering with important microbial processes (Colwell, 1978). The potential for hydrocarbon degradation by soil, water and sediment microorganisms has been of much interest due to concern over the tons of petroleum pollutants released each year as land and water spills (Vanloocke et al., 1975; Atlas, 1981).

As petroleum reserves are depleted, coal conversion processes will be of increasing importance for meeting U.S. energy needs Braunstein et al., 1977). These new products represent oils vastly different from conventional petroleum products (Sturm et al., 1975; Holmes et al., 1976; Giddings and Herbes, 1981). The spillage of synthetic oil is expected to be a significant environmental problem based upon off-site refining and transportation routes favored by this emerging industry (Leggett et al., 1980). Two major modes of transportation, barge and rail, will be employed with land transportation having an expected greater spillage volume on a per mile basis (Leggett et al., 1980). Current studies of freshwater systems are investigating the

environmental importance of synthetic fuel spills and have indicated that the oils have a significantly greater toxicity and content of water soluble toxic compounds than conventional petroleum products (Giddings and Herbes, 1981).

The terrestrial section of the Advanced Fossil Energy Program (AFEP) at the Environmental Sciences Division of Oak Ridge National Laboratory has been conducting studies on the effects of coal conversion products on agricultural plants and the importance of soil microorganisms in the degradation of the oil. As an integral part of that program, the purpose of this study was to assess the effects of a solvent refined coal oil on microbial activity and numbers, and nitrogen transformations in a soil. The first two areas are important because changes in both would have implications for the degradability of the oil. Disruption of nitrogen transformations could affect nitrogen biogeochemical cycling and plant growth in general.

II. LITERATURE REVIEW

The Interaction of Microorganisms and Petroleum Oils

Most of the work on the interaction of petroleum oils and microorganisms has focused on the effects of the oils in aquatic habitats, where spills have been the most frequent. Colwell and Walker (1977) in a review of the literature concluded that two major microbial responses to crude oil spills in the marine environment are increases in hydrocarbon-utilizing bacteria and the inhibition of certain other bacteria which may ultimately affect some microbial processes. Atlas et al. (1976) found that numbers of hydrocarbon-degrading psychrophilic Pseudomonas bacteria increased after exposure to a crude oil in arctic marine waters. Total numbers of pelagic bacteria had also increased above controls after a 28 day exposure to the oil. A crude and fuel oil were found to have little effect on total numbers of heterotrophs in oil-free marine sediments (Walker, Seesman and Colwell, 1975). However, certain physiological groups of microorganisms including lipolytic, proteolytic and chitinolytic bacteria were inhibited, with fuel oil (containing a high percentage of aromatics) being the more toxic. Both oils supported the growth of heterotrophic and cellulolytic bacteria.

The short-term effects of petroleum oils on microbial uptake of dissolved organic compounds has also been studied. Pelagic and benthic subarctic populations previously not exposed to hydrocarbon pollution had reduced uptake rates of glucose and glutamic acid in the presence of a crude oil (Hodson et al., 1977; Griffiths et al., 1981a). Similar

concentrations of two crude oils did not cause a reduction in glucose utilization by temperate populations previously exposed to petroleum products (Alexander and Schwartz, 1980). Although the environmental conditions may partially account for the different results of these studies, other data suggests that selection for oil-resistant species does occur in marine environments (Walker and Colwell, 1975a).

Griffiths et al. (1981b) studied the long term effects of a crude oil on heterotrophic uptake of glucose and glutamic acid in arctic and subarctic sediment communities. For periods of up to 18 months uptake rates were significantly reduced by .5 and 1 ppt of a fresh and weathered crude, although numbers had recovered by 8 months. It was found that concentrations above 1 ppt caused no further reduction in activity, but there was a lag period of 1.5 days before maximum reduction took place at a high oil concentration (50 ppt). From this long term study, they predicted reduced uptake rates for as long as six years following the initial impact of a high oil concentration. Reductions would also be expected in secondary productivity due to the important role of bacteria in mineralizing 50% of the primary productivity (Fenchel and Jorgensen, 1977).

Land spills of hydrocarbon products, although usually not as extensive as water spills, occur via pipeline leakage and accidents on site and in transportation (Vanloocke et al., 1975). Petroleum and shale oils have had a range of effects on soil microorganisms dependent on conditions such as concentration, habitat type and oil composition. Bacteria and actinomycete numbers were not affected by a 10%

concentration of shale oil, but fungal populations were reduced significantly (Hersman and Klein, 1979). A crude oil applied at high concentrations (5 and 12 $1/m^2$) on arctic tundra soils, resulted in increased heterotroph populations and oil-degrading populations with the magnitude of the response dependent on soil type and depth of oil penetration (Sexstone and Atlas, 1977). An oil high in straight chain alkanes added to soil caused an increase in bacterial numbers with counts returning to their original levels after one year (Jensen, 1975). Other instances of increases in microbial numbers in the presence of crude oil have been reported (Llanos and Kjoller, 1976; Loynachan, 1978). Atlas et al. (1976) found comparable numbers of microorganisms in soil at a natural oil seep and an uncontaminated tundra soil, but population composition was different. Most notably there were greater fungal numbers associated with the oil. Decreases in bacteria, actinomycetes and fungi on oil-polluted agricultural land have been attributed to toxic effects and to a deterioration in the soil conditions of aeration and nutrient status (Niewolak, 1978).

Increases in microbial repiration measured as oxygen uptake or carbon dioxide evolution have also been associated with oil polluted soils (Dobson and Wilson, 1964; Jones et al., 1970; Odu, 1972; Gudin and Syratt, 1975; Jensen, 1975; Vanloocke et al., 1975). A shale oil had no effect on oxygen uptake rates at a 10% concentration but significantly reduced other indices of microbial activity and ATP concentrations (Hersman and Klein, 1979). The nitrifiers are inhibited by a great number of organic compounds including some aromatics found in petroleum oils (Stafford, 1974; Powlson, 1976; Hockenbury and Grady, 1977).

The availability of soil mineral nutrients affects rates of oil degradation. The incorporation of carbon into microbial biomass involves a complementary uptake of nitrogen. The quantity of nitrogen required is expressed by the carbon to nitrogen (C:N) ratio of the cells, which is approximately 10:1 for soil microbial populations (Waksman, 1924; Alexander, 1977). The addition to soil of petroleum oils and other substances with high C:N ratios results in the immobilization of inorganic nitrogen by the microorganisms, thus making it unavailable for plant growth (Lyon et al., 1923; Stotsky and Mortensen, 1957; Udo and Fayemi, 1975; DeJong, 1980).

Optimal C:N ratios for oil degradation in soil are greater than the value for microbial cells because complete assimilation of petroleum carbon into biomass is not achieved under natural conditions. Some petroleum compounds are recalcitrant or are only metabolized slowly and the efficiency of conversion of a carbon source to cellular material is less than 100%. Also, the turnover of the microbial populations results in some recycling of nutrients. In soils a 60:1 carbon to nitrogen ratio was found to be optimal for crude oil degradation under laboratory conditions (Dibble and Bartha, 1979). This value was similar to the ratios found to be optimal for biodegradation of crude oil in seawater (Atlas and Bartha, 1972).

To achieve these optimal ratios fertilization is usually required. The addition of nitrogen and mineral nutrients has had favorable effects on oil utilization by microorganisms (Jobson et al., 1974; Gudin and Syratt, 1975; Westlake et al., 1978). In field plots treated with oil

and urea phosphate, rapid increases in bacterial numbers were found, while fertilization had no effect on fungi (Westlake et al., 1978). Revegetation was also more rapid in plots to which nitrogen had been added (Gudin and Syratt, 1975). Odu (1978) found that the addition of nitrogen and phosphates had a greater positive effect on oil degradation than nitrogen alone, but other macro- and micronutrients were ineffective. Fertilization decreased the doubling time of aerobic bacteria from 2.0 to 1.2 days in an arctic soil (Atlas et al., 1976). Addition of nitrogen and phosphorus stimulated degradation of saturated hydrocarbons more than aromatic hydrocarbons (Atlas, 1981).

Other environmental factors, although more difficult to modify than the soil nutrient status, can have a considerable influence on the interactions of an oil and microorganisms. Petroleum oils increase the soil pH (Vanloocke et al., 1975). This may have a favorable effect on degradation in acid soils as the optimal pHs for microbial activity and oil transformations are near neutrality (Alexander, 1977; Dibble and Bartha, 1979).

The effect of a petroleum pollutant on soil organisms and the degradation rate of its components is dependent on its concentration in the soil. Low concentrations have been found to favor alkane degradation while higher applications of oil favor aromatic and asphaltic degradation (Dibble and Bartha, 1979). An optimal 5% (wt/wt) concentration of crude oil sludge was found to promote good degradation of both alkanes and aromatics. Frequent small applications had higher degradation rates than a single large application (Dibble and Bartha,

1979). Microbial numbers and activities are also concentration dependent. Jensen (1975) observed increases in bacterial numbers and oxygen uptake rates up to a 5% concentration of oil; above that treatment level differences were slight and followed no regular pattern.

The interaction of hydrophobic components of an oil with organic matter localizes these compounds and brings them into contact with the microorganisms. The presence of biological compounds such as lipids, proteins, nucleic acids and amino acids, may concentrate or solubilize some chlorinated biphenyls and many polycyclic aromatic hydrocarbons (Colwell, 1978) making them readily available for microbial attack. Water soluble hydrocarbons at high concentrations are often toxic to microorganisms (Atlas, 1978) and although their solubility allows for dilution and downward movement in the soil column, it also increases the risk of groundwater contamination (Vanloocke et al., 1975).

The composition of a petroleum oil also determines the rate and extent to which it will be degraded. Straight chain alkanes are degraded more quickly than branched alkanes and aromatic compounds are the most recalcitrant hydrocarbons (Fredricks, 1966). Metabolic pathways have been established for some of the simple alkanes and aromatics, and microbial degradation is a result of enzymatic attack by constitutive or inducible oxygenases (Vanloocke et al., 1975). Alkanes are subject to beta-oxidation as are some alkyl side chains of aromatic oil components. The ring structure of aromatics is hydroxylated and cleaved to form a dicarboxylic acid (Gibson, 1968; Atlas, 1978, 1981).

The indigenous microbial populations determine the extent to which biological degradation will occur. Although hydrocarbon-utilizing microorganisms are widely distributed and represent many microbial genera, their abundance is usually higher in environments previously exposed to hydrocarbon pollution where selection for oil tolerant species seems to occur (Colwell, 1978; Atlas, 1981). Species which are able to utilize the oil are often very specific toward oil components. Generally, there is an initial enrichment for populations capable of using the readily metabolized alkane fraction followed by populations using more recalcitrant components of the oil (Atlas, 1978). Thus a succession of populations takes place. At any one time however, mixed populations accomplish the petroleum degradation. Co-oxidation, the process by which the presence of a hydrocarbon serving as a substrate for microbial growth allows the attack on another nonsubstrate hydrocarbon, can be important (Raymond et al., 1967; Perry, 1979). Co-oxidation has been hypothesized as the reason for similar degradation rates of compounds in a mixture consisting of components considered to be both easily degradable and resistant (Horowitz and Atlas, 1977; Herbes and Schwall, 1978). Diauxie is the phenomena by which the attack on one hydrocarbon in a mixture is limited by the presence of another compound (Klug and Markovetz, 1971; Pirnik et al., 1974).

Petroleum hydrocarbon degradation is performed by a diverse group of bacteria and fungi. Zobell (1946) noted that more than 100 species, representing 30 genera, had been shown capable of utilizing hydrocarbons. Pseudomonas plays a particularly important role in oil spill

degradation (Jensen, 1975; Vanloocke, 1975; Cooper and Hedrick, 1976; Palchauduri, 1977; Kiyohara, 1979). Some other bacteria able to utilize petroleum as a growth substrate are Arthrobacter (Jensen, 1975), Alcaligenes and Achromobacter (Cooper and Hedrick, 1976), Proteus and Corynebacterium (Odu, 1978). A numerical taxonomic examination of petroleum degrading bacteria led Austin et al. (1977) to conclude that a diverse range of taxa were involved in biodegradation. Fungi are also able to use components of petroleum as a growth substrate. Some of the species which have been identified are Scoleobasidium (Pinholt et al., 1979), Graphium, Paecilomyces (Llanos and Kjoller, 1976), Aspergillus, Cochliobolus, Monilia and Trichoderma (Odu, 1978). Komagata et al. (1964) examined almost 500 yeasts for their ability to degrade hydrocarbons and found 56 species most of which were in the genus Candida. Baruach and Freitas (1978) also isolated Pichia spp. which were capable of using petroleum oils.

The Environmental Effects of Coal Oils

The composition of petroleum and synthetic fuel oils differ greatly resulting in different physical and chemical properties and toxicities. The great majority of research on the impact of synthetic fuels has been conducted on their transport, fate and effects in freshwater ecosystems (Strand and Vaughan, 1981; Giddings, 1982). The research has focused on water-soluble fractions (WSFs) of the oils for several reasons which include the geographically large effect of water-soluble components on the freshwater system and the major role of WSFs of petroleum products

in toxicity to phytoplankton, zooplankton and fish (Moore and Dwyer, 1974; Rice et al., 1977).

Comparative studies of the composition of petroleum and synthetic oils and the influence of composition on toxicity was a major objective of the aquatic studies. Major constituents of synthetic oil WSFs include phenol, cresol isomers, C_2-phenols, aniline, toluidines and C_2-anilines, while almost no phenolic or basic compounds were detected in a WSF of a petroleum crude (Giddings and Herbes, 1981). Of the synthetic oils tested, the solvent refined coal (SRC) II products had the highest concentrations of phenolics and anilines, however mono- and di-aromatics were an order of magnitude lower in the SRC WSFs than in the other coal liquids.

These differences in concentrations of WSF constituents are related to toxicity differences. Tests of WSFs on inhibition of algal photosynthesis and growth (Giddings et al., 1980; Giddings and Washington, 1981; Gray et al., 1982) showed refined petroleum products to be one to two orders of magnitude less inhibitory than coal liquids. Chronic and acute tests on the freshwater crustacean Daphnia magna, also revealed a lower or negligible toxicity of petroleum products versus synthetic products (Giddings et al., 1980; Giddings and Herbes, 1981; Strand and Vaughan, 1981). Differences in toxicity between coal and petroleum oils were even greater in chronic bioassays. The phenolic and aniline compounds cause the observed toxicity of coal liquid WSFs (J. M. Giddings, personal communication).

Ricard (1980) compared the effects of repeated inputs of a coal liquid and a petroleum marine diesel fuel on microbial activity in freshwater sediment microcosms. Dehydrogenase activity, indicative of respiration, and ATP concentrations, related to biomass, were significantly reduced by the liquefaction product, while only dehydrogenase activity was reduced by the petroleum fuel. However, at the end of 3 months of exposure other microbial functions which included mineralization of glucose, protein, starch, lipid and napthalene showed increased rates in microcosms treated with coal oil compared to controls. The petroleum diesel fuel stimulated starch, protein and lipid mineralization.

The dissolution rates of the oils in water are inversely proportional to their viscosity and the oil-water partition coefficient of each component. Two SRC-II products, three other coal-derived liquids and a petroleum crude were tested for viscosities and densities. A petroleum crude oil had a viscosity greater than twenty times the most viscous synthetic oil. The two SRC-II product oils were the densest of the six coal oils (Giddings and Herbes, 1981). Giddings and Herbes (1981) noted several trends in dissolution of 5 coal oils and a petroleum crude. The phenolic compounds dissolved most rapidly with rates inversely related to the viscosity of the oil. Substituted phenols of increasing size dissolved more slowly. Anilines measured for one SRC-II fuel oil followed the same pattern, but dissolved at lower rates than phenols.

The much lower viscosities of coal oils compared to petroleum may add significantly to speculation about the impact of a terrestrial oil

spill. Vanloocke et al. (1975) reviewed the impact of petroleum land spills and presented a formula in which maximum depth of oil penetration was directly related to soil retention capacities and viscosity of the oil. Thus, for any one soil type a coal oil would be expected to penetrate the soil to a greater depth than a similar amount of a petroleum oil. The decreased aeration and lower microbial activity associated with increased soil depths could retard degradation of the oils (Vanloocke et al., 1975; Alexander, 1977).

Information about the influence of environmental factors on petroleum degradation may be helpful in predicting the impact of a coal oil in the environment. It is likely however, that the many chemical and toxicological differences between the two oil products will result in very different interactions with the microorganisms. Therefore, knowledge of the specific interactions of synthetic oil fractions is essential to the prediction of the fate of synthetic fuels in the environment.

Measurement of Microbial Processes

The most important function of the microorganisms is often considered to be organic matter degradation or decomposition, the process by which the supply of carbon dioxide is replenished (Alexander, 1977). Decomposition is a function of all heterotrophs and because of this it is frequently measured in order to indicate the level of microbial activity in a soil (Stotsky, 1965). Several techniques have been used to measure decomposition rates including the measurement of

oxygen uptake, carbon dioxide evolution, the determination of an organic matter constituent such as lignin or cellulose. Manometric measurements give sensitive determinations of oxygen uptake and carbon dioxide evolution rates, which have been found to be well correlated with other indices of activity such as soil organic matter content (Bunt and Rovira, 1955), nitrogen and phosphorus transformations (Stotsky and Norman, 1961; Stotsky, 1965) and microbial numbers (Alexander, 1977).

Microbial counts are an indicator of population dynamics. Total viable cell (TVC) counts, usually 10-100 times less than direct counts, underestimate the soil population (Skinner et al., 1952; Clark, 1965) but provide an indication of the response of a subset of the soil population to a treatment. The agar-plate method for TVC counts estimates numbers of organisms able to grow on the specific medium used and under the conditions of incubation employed. When using soil as the inoculum, it is important that the medium be low in readily available energy materials such as sugars, in order that the few fast-growing organisms do not dominate growth on the plate and inhibit the development of a larger number of slower growing species. Soil-extract agar, widely used for total viable cell counts in soil (Lockhead, 1940; Clark, 1965), is used for this reason.

Of the major plant nutrients derived from soil, nitrogen is the most susceptible to microbial transformations. The mineralization of organic nitrogen is carried out in two processes: ammonification (the formation of ammonia) and nitrification (the oxidation of ammonia to nitrite and nitrate). Nitrification is carried out by two groups of

physiologically similar chemoautotrophic bacteria, while ammonification is brought about by a diverse, physiologically dissimilar flora (Walker, 1976; Alexander, 1977). Nitrogen mineralization is measured by the quantification of all the inorganic products of the transformation (ammonium, nitrite, nitrate).

Environmental Influences on Microbial Processes

The study of soil microbial processes and the effects of a pollutant on these processes must take into account the variety of conditions to which a soil is exposed, under natural conditions in the field, under agricultural practices or through sampling and subsequent handling in the laboratory. Such conditions include the physical phenomena of drying and rewetting, freezing and thawing, grinding and cultivation as well as other factors such as pH, organic matter and soil type. All affect the physiological status of the soil microorganisms to varying degrees.

Many studies have examined the effects of air-drying on microbial numbers and activity. In the field and in the laboratory, soils can be exposed to alternating periods of wet and dry conditions, in the first case dependent on local rainfall patterns and in the second on such common practices as air-drying soils for sieving and storage. Air-drying of soils is known to decrease respiration in comparison to field-moist controls (Cawse and Sheldon, 1972). This is accompanied by a decrease in numbers in most cases (Stevenson, 1956; Soulides and Allison, 1961; Jenkinson, 1966). Soulides and Allison (1961) also found

an increase in ammonia nitrogen during drying with no change in nitrate. In one case, partial drying was shown to have no effect on the nitrifying organisms (Quastel and Schofield, 1951), but in general, drying reduces their numbers (Alexander, 1977).

Cycles of drying and wetting stimulate microbial respiration as measured by both oxygen uptake and carbon dioxide evolution (Cawse and Sheldon, 1972). The magnitude of the response depends on the length of the drying period; the longer a soil has been dried the greater the activity of the soil population upon rewetting (Birch, 1958; Soulides and Allison, 1961). The period of rapid decomposition lasts for several days; oxygen uptake and carbon dioxide evolution then fall to the levels of the continually moist controls (Stevenson, 1956; Birch, 1958; Soulides and Allison, 1961). This increase in activity is repeated with successive dry and wet cycles (Birch, 1958; Soulides and Allison, 1961).

During incubation of a remoistened soil, bacteria numbers measured by plate counts can increase above controls (Soulides and Allison, 1961). This rapid increase in numbers follows the burst of respiration (Stevenson, 1956; Funke and Harris, 1968). The lag time is dependent on the number of bacteria killed and whether the soil is reinoculated before incubation (Powlson, 1976). Repeated drying and wetting with incubation has a more drastic effect on microbial numbers than a single treatment, with numbers decreasing to a greater extent during the successive drying periods and not recovering to the levels of the continuously incubated controls (Soulides and Allison, 1961).

Soulides and Allison (1961) found that immediately after remoistening a dry soil there was an increase in ammonia with no significant release of nitrate nitrogen. This changes upon incubation, however, and Birch (1958) found an increase in nitrate with no accumulation of ammonia indicating that nitrification had resumed. Increases in nitrification and nitrogen mineralization have been found in the laboratory, greenhouse and field (Powlson, 1976) and occur concurrently with the respiration increase. The length of the drying period and percent carbon in the soil affect the magnitude of the flush (Birch, 1958, 1959) which is characterized by a carbon to nitrogen ratio of 10 to 1 (Birch, 1959; Jenkinson, 1966). This low C:N ratio indicates that a highly nitrogenous fraction of the organic matter is rendered decomposable. The fact that the organisms are also killed by air-drying led Jenkinson (1966) to conduct labelling experiments in which he concluded that the dead or injured microorganisms were the main substrate for the flush.

Freezing and thawing of soils causes some of the same chemical and biological changes as drying and rewetting but to a lesser degree (Soulides and Allison, 1961). Freezing with subsequent thawing results in an increase in respiration but multiple cycles do not have a cumulative effect (Soulides and Allison, 1961; Ivarson and Sowden, 1970). Freezing for over 10 weeks greatly reduces or eliminates this respiration increase upon thawing (Ivarson and Sowden, 1970). Freezing is known to result in a small or negligible release of ammonia and nitrate (Soulides and Allison, 1961).

Severe soil treatments, such as drying at high temperatures and fumigating, have a drastic effect on the microbial populations. Actinomycetes, bacteria and fungi are greatly reduced in number with fungi being the most sensitive to heat treatments (Evans, 1955; Chandra and Bollen, 1961; Gasser and Peachy, 1964; Jenkinson, 1966; Funke and Harris, 1968; Draycott and Last, 1971; Powlson, 1976). After remoistening a heat-dried soil or following fumigation, an increase in respiration occurs which is greater in magnitude than that of a remoistened air-dried soil (Funke and Harris, 1966; Jenkinson, 1966; Powlson, 1976). This respiratory increase is usually preceded by a lag period proportional to the severity of the treatment. Factors such as pH, temperature and soil type affect the extent to which a fumigant sterilizes the soil, thus influencing the lag time for recovery. Recovery time is also dependent on conditions of the treatment (drying time, fumigant concentration) and whether or not the soil was reinoculated (Powlson, 1976). Fumigants which leave residues in the soil continue to influence microbial activity long after the treatment by either continuing to kill organisms or by serving as a substrate for survivors or recolonizers.

Following heat treatments and remoistening or fumigation, there is an increase in ammonia and in some cases nitrate (Birch, 1958; Jenkinson, 1966; Draycott and Last, 1971). Nitrification is inhibited by heating and other highly biocidal treatments (Davies and Owen, 1951; Chandra and Bollen, 1961; Gasser and Peachy, 1964; Jenkinson, 1966) and even in soils which were reinoculated, nitrification was stopped for

6-10 weeks after heating with steam (Malowany and Newton, 1947). The nitrifiers are also inhibited by some treatments which have little or no effect on the rest of the soil population (Goring, 1962).

Population recovery following severe treatments is positively correlated with increased respiration. Depending on whether plate counts or direct counts are used, numbers increase above controls or return to control levels respectively. Studies on the effects of burning on soil populations (Meikljohn, 1955) and studies on soil fumigants effects (Jenkinson, 1966; Sheilds et al., 1974) using direct counts, showed that numbers at first decreased and then increased after treatment but never achieved their original levels. Comparative studies of plate counts and direct counts show the plate count method gives higher numbers after treatments, with direct counts showing no increase above control levels. With both methods numbers returned to their respective control levels with time (Meikljohn, 1955; Sheilds et al., 1974).

The interrelated environmental factors of aeration, moisture, temperature, pH, organic matter content and soil type together determine the microbial species present and their levels of activity in the soil. Aeration is affected by moisture content; water-logging of soils hinders the movement of oxygen and aerobic activities are repressed. Optimal water contents for aerobic decomposition and nitrogen transformations lie between 50 and 80% of the water-holding capacity (Miller and Johnson, 1964; Alexander, 1977). Temperatures affect the species composition and the biochemical capacity of the community as each

species has an optimal temperature range. Although decomposition takes place at temperatures down to freezing, maximum rates generally occur with temperatures in the mesophilic range between 30 and 40°C. Nitrogen mineralization however, generally occurs maximally above 40 and up to 60°C (Alexander, 1977). Seasonal differences in microbial activities are associated with temperature and the availability of easily decomposable substrates. In temperate climates activities generally reach a maximum in early spring and late summer (Alexander, 1977).

Bacteria, fungi and actinomycetes have optimal pHs for growth and a range outside of which no proliferation takes place. Decomposition and nitrogen mineralization are favored by neutral soils and liming of acid soils often has a positive effect on these processes (Alexander, 1977). Lower pHs are usually associated with an increasing proportion of fungi (Waksman, 1961; Brady, 1974; Powlson, 1976).

Organic matter influences various soil properties including water retention capacity, the exchangeable base reserves, the capacity to supply nitrogen and other elements and soil structural stability, each of which in turn affects the soil microbial activities (Brady, 1974). Organic matter content has been related to rates of carbon mineralization in soils. Bunt and Rovira (1955) found a highly significant correlation between the organic matter content and rates of oxygen uptake and carbon dioxide evolution. The proportion of readily decomposable substrates and resistant humic compounds is important for both carbon and nitrogen mineralization. Soil type influences activity particularly through the content of clay which absorbs many organic

substrates and enzymes, thus retarding decomposition. Sand and silt can serve as barriers to microbial movement by preventing substrates and cells from encountering one another (Ou and Alexander, 1974).

The nitrifying bacteria are ubiquitous wherever ammonia and nitrite are available and when conditions are favorable for other aerobic microbial processes (Walker, 1976). However, nitrification is more sensitive to environmental influences than most other processes. This fact can be attributed in part to the few physiologically similar species responsible for the transformation of ammonium to nitrate (Alexander, 1977).

Soil acidity has been cited as a chief environmental influence on nitrification rates (Morrell and Dawson, 1967; Dancer et al., 1973). Although rates generally decrease below pH 6 (Dancer et al., 1973), nitrification has been reported at pHs as low as 3.7 (Olsen, 1929) and marked accumulation of nitrate has been reported at pH levels of 4.0 (Weber and Gainey, 1962; Alexander, 1977). Morrell and Dawson (1967) analyzed 116 northern temperate soils and found patterns of ammonium oxidation related to pH. At pHs of 5.01 to 6.38, oxidation of ammonium and nitrite was rapid while in alkaline soils nitrite accumulated, being only slowly oxidized to nitrate. This accumulation of nitrite may be attributed to the sensitivity of the nitrobacter group to ammonium salts (Alexander, 1977). In more acid soils (pH <4.98), oxidation of ammonium to nitrate was slower with no accumulation of nitrite.

Other factors affecting nitrification rates are aeration and soil moisture (Justice and Smith, 1962; Parker and Larson, 1962; Sabey, 1969;

Sabey et al., 1969). Oxygen is an obligate requirement for all nitrifier species, thus the process will be inhibited under conditions of poor soil structure or water logging. Sabey et al. (1969) have listed moisture and temperature as the most important factors controlling nitrification in surface soils. Optimal temperatures usually lie between 30 and 35°C dependent on physiological differences among species while rates below 5°C and above 40°C are very slow (Alexander, 1977). The total organic nitrogen content of a soil has been related to the rate and total accumulation of nitrate (Weber and Gainey, 1962) and the initial population of nitrifiers also had an effect on the nitrifying capacity of the soil (Fredrick, 1957; Sabey et al., 1969).

III. MATERIALS AND METHODS

The interactions of a coal oil and soil microorganisms were studied under controlled environmental conditions in order that the effects of the oil on microbial processes could be separated from environmental influences. Soil temperature, aeration and water content were maintained at levels favorable for most aerobic microbial processes. Samples were incubated at mesic temperatures corresponding to the mean temperature for September (late summer) in the Oak Ridge area. The soil was packed to a bulk density of 1.1 g/cm^3, which corresponds to the density of a well aerated cultivated soil. The moisture content of the soil was kept constant at field capacity and an equilibrium period of at least twenty-four hours followed water addition in order to allow the flush of microbial activity to subside.

Site Description

Soil was sampled from the Ecology Field Area on the Oak Ridge National Laboratory reservation, Roane County, Tennessee (Fig. 1). The soil of the sampling area is a Captina silt loam which is a low to intermediate terrace soil developed as an alluvium of the Clinch River (Van Voris et al., 1977). The climate of Roane County is humid-mesothermic with 28 year means for precipitation and temperature being 140.1 cm and 14.4°C respectively (Van Voris et al., 1977). Detailed descriptions of the vegetation, geology and land use history of the site are available from previous studies of the area (Kelly et al., 1969; Van Voris et al., 1977).

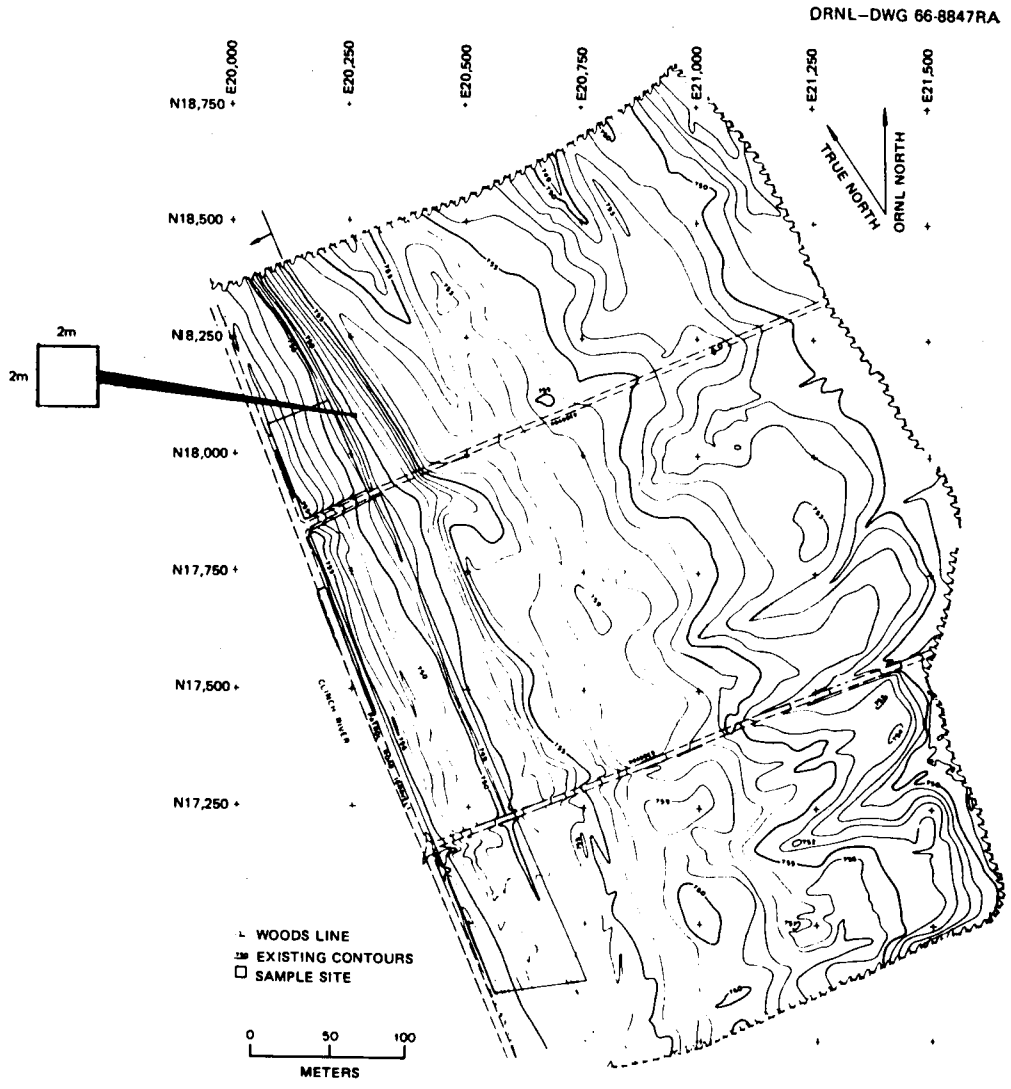

Fig. 1. Location of soil sampling site (2 × 2 m) on the Ecology Field Area at Oak Ridge National Laboratory, Oak Ridge, Tennessee.

Vegetation was removed from a 3 × 15 m area in June 1980. The ground was cleared of large debris and was disc furrowed. Disc furrowing was repeated approximately one and two months later. A topographically uniform 2 × 2 m square area was sampled in December 1980. The top 10 cm of the soil was overturned manually and mixed before random samples were taken with a spade. The soil was sieved (<0.5 cm) and brought back to the laboratory.

Soil Preparation and Field Capacity Determination

The soil was packed into 17 × 30 × 13 (high) cm plastic containers at a bulk density of 1.1 g/cm^3. The final soil volume was 3570. cm^3 (17 × 30 × 7 (high) cm). The experimental containers were randomly placed on a shelf in a walk-in environmental chamber. Mean low and high temperatures for September over a 20 year period for the Oak Ridge area were 17 and 25°C respectively. Soil samples were held at temperatures ranging between this minimum and maximum on a 24 hour cycle by the use of a programmable temperature control which changed the environmental chamber temperature a degree every three hours.

Soil moisture content was determined by oven drying at 100°C to a constant weight and was expressed as g water/g dry weight soil. It was desirable to determine field capacity or the water content after cessation of free drainage in a previously saturated soil, in order to maintain the soil samples at maximum available water and to ensure that the moisture was uniform throughout the soil depth. Field capacity can be approximated by measuring the water content of the soil at 100 cm of

water suction (10 kPa) (Marshall, 1959; Brady, 1974). Water retention curves were determined in triplicate (Appendix) according to the procedure of Vomocil (1965) and the water content of the soil at field capacity was measured as 22.2%. The soil samples were adjusted to this water content and the containers were covered with aluminum foil to reduce evaporation. This moisture content was maintained by the addition of approximately 100 ml of water at one to two week intervals.

The Oil

The synthetic fuel used in this study was a solvent refined coal oil product (SRC-II) obtained from the Fossil Fuels Research Materials Facility of the U.S. Environmental Protection Agency (EPA) and the U.S. Department of Energy (DOE), located at the Oak Ridge National Laboratory. This SRC-II product oil (EPA/DOE identification number CRM-1), the result of a pilot plant production run, was intermediate in viscosity between other raw coal liquids and distillates of a hydrotreated oil and was much less viscous than a petroleum crude. Comparative physical properties and chemical composition of the CRM-1 and a petroleum crude are shown in Table 1 (W. Griest, personal communication).

Oil Concentrations

Levels of oil application were chosen such that the highest concentration increased the "liquid" content of the soil by 0.086 cm^3/cm^3 leaving an air-filled pore space of 0.24 cm^3/cm^2. An 0.24 pore space

Table 1. Comparative physical properties and chemical composition of a coal and petroleum oil.

	CRM-1 SRC-II Product	CRM-3 Petroleum Crude A
Coefficients of Viscosity, Stokes (23°C)	8.267	300.1
Density, g/cm^3 (23°C)	.9952	.9383
% C	87.33	85.65
% N	.85	.60
% O	2.73	.29
% S	.33	1.87
% H	8.77	11.60

corresponds to a well aerated soil, which is considered to have a minimum air space of 0.10 (DeJong, 1980). Oil concentrations were 0.0, 0.1, 1.0, 2.0 and 5.0 $1/m^2$ or 0.0, 1.7, 17.2, 34.5 and 86.2 ml oil/kg dry weight soil for treatments 0 (control), 1, 2, 3 and 4, respectively (Table 2). There were four replicates at each treatment level making the total number of experimental containers twenty. Oil was added in small increments to the top 4 cm of the soil and mixed thoroughly. When all the oil and soil was mixed (the control was treated in a likewise fashion), the mixture was layered on to the remaining 3 cm of untreated soil and lightly tamped to a bulk density of 1.1 g/cm^3.

Parameters Measured

Total nitrogen (%) was determined by a modification of the semi-micro Kjeldahl method (Bremner, 1965) before oil addition (day 0) and at day 87 following oil addition. Soils were ground to pass through a 100 mesh (0.14 mm) sieve and digested with 3 ml H_2SO_4, 1.0 ml H_2O_2, 1.5 g K_2SO_4, and 7.5 mg Se. The addition of hydrogen peroxide was found to result in a more complete digestion (G. Wade, personal communication).

Soil organic carbon was oxidized with the $Cr_2O_7^{-2}$ ion using the no-heat method of Walkley-Black (Allison, 1965). Percent transmittance was read at 620 nm on a Bausch and Lomb spectronic 70, 24 hours after digestion (Graham, 1947). This determination was done at days 0 and 87. Although this method strictly determines percent organic carbon, organic matter (%) can be estimated by multiplying by 1.8 to 2.0 (the range of

Table 2. Coal oil (CRM-1) concentrations in the soil.

	l/m²	ml oil/kg dry wt. soil	% wt/wt
Control (TR0)	0.0	0.0	0.0
Treatment 1 (TR1)	0.1	1.7	0.2
Treatment 2 (TR2)	1.0	17.2	1.7
Treatment 3 (TR3)	2.0	34.5	3.4
Treatment 4 (TR4)	5.0	86.2	8.6

conversion factors for organic carbon in surface horizons of mineral soils) (Allison, 1965). Soil pH was determined by the method of Peech (1965) at days 45 and 90.

Respiration, measured as oxygen uptake rates, was determined manometrically (Umbreit et al., 1972) on a Gilson single valve differential respirometer at days 0, 4, 19, 34, 60, 73, 87 and 98. One gram quantities of soil were sampled and placed in respirometer flasks containing 10% KOH absorbed on cotton in the side arm to trap carbon dioxide. Samples were incubated at 25°C with a 45 minute equilibration time before readings were begun. For days 0, 4, 19, and 34, a two hour incubation time was used to measure accumulated oxygen uptake (ul) and uptake rates (ul/g dry wt soil/hr) were calculated after adjusting gas volumes to standard temperature and pressure (STP). After oil addition the accumulated oxygen readings for the treatments had decreased to the point that a two hour incubation period was not sufficient for estimation of the uptake rates (replicates showed a great deal of variability). Subsequently, at days 60, 73, 87 and 98 readings were taken every six hours over 48 hours. Oxygen uptake rates were estimated using a linear regression of accumulated oxygen as a function of time.

Numbers of total viable cells (TVC) were determined according to the spread plate method of Clark (1965) using a soil extract agar (Lochhead, 1940) derived from soil of the same field plot and moisture content as the samples. Counts were made 10 days after inoculation of the plates and incubation at 22-25°C (room temperature). Only plates with 30-300 colonies were counted and values reported are the average of

duplicate plates (TVC/g dry weight soil). Counts were determined on soil samples taken at days 0, 4, 45, 60, 87 and 98.

Exchangeable ammonium and nitrate were determined by the equilibrium method of Bremner (1965) at days 0, 2, 24, 38, 52, 66 and 88. Ten gram samples (moist weight) were extracted with 50 ml of a 1 N KCl solution by shaking at low speed on a reciprocating shaker for one hour. After settling, the supernatant was filtered and stored at 4°C for later analysis (Technicon Autoanalyzer II Operation Manual) on a Technicon Autoanalyzer II. Nitrite levels were one order of magnitude less than nitrate concentrations for the control and treatments and thus were not measured at every time period and are not reported. Ammonium and nitrate concentrations are expressed as ug NH_4 and ug NO_3, respectively per gram dry weight soil.

The nitrifying bacteria were enumerated by a most-probable-number (MPN) technique using an ammonium calcium carbonate medium and a nitrite calcium carbonate medium for the ammonium and nitrite oxidizers respectively (Alexander and Clark, 1965). A dilution range of 10^{-2} to 10^{-6} (g soil/g water) was used at the first two time periods. However the low numbers obtained necessitated that a lower range (10^{-1} to 10^{-5}) be used at the subsequent sampling times. The MPN estimates were obtained from an MPN table (Alexander, 1965) and are expressed on a dry soil weight basis.

All variables (except the MPN data) were analyzed separately using a two-way analysis of variance (Sokal and Rohlf, 1969). For those analyses in which significant differences were found, the Duncan's

multiple range test (Sokal and Rohlf, 1969) was used to determine the means that differed significantly for the variable of interest. The relationship between oxygen uptake and plate counts at each time period was analyzed using the Pearson product moment correlation (Sokal and Rohlf, 1969).

IV. RESULTS

The results of the two-way ANOVA procedure showed a significant ($p < .05$) time and treatment interaction for each parameter, prohibiting discussion of the factors separately. However, it is of interest to determine whether responses to treatments differ significantly through time and at any one time period. Results of a one-way ANOVA for each time period and treatment level showed a significant F value ($p < .05$) for all comparisons except at day 0, in which there were no significant differences among the means for oxygen uptake rates, plate counts, ammonia and nitrate measurements.

Oxygen Uptake Rates

A graph of mean oxygen uptake rates for the treatments through time (Fig. 2) shows that by day 19 the rates for the oil treated soils were lower than the control. By day 73, O_2 uptake rates for the soil treated with 1.7% oil (TR2) had increased to control levels and continued increasing so that at days 87 and 98 they were 2 and 2.5 times, respectively, control values. Results of a Duncan's multiple range test performed at each time period and treatment level are presented in Tables 3 and 4, respectively. The control was significantly higher than treatments two (1.7%) and four (8.6%) at day 19 and treatments two and three (3.4%) at day 34. At day 60 the control and treatment one (0.2%) were not significantly different but were significantly higher than the other oil treatments. The increase in rates for the soil of treatment two following day 60 was such that by day 73 they were not significantly

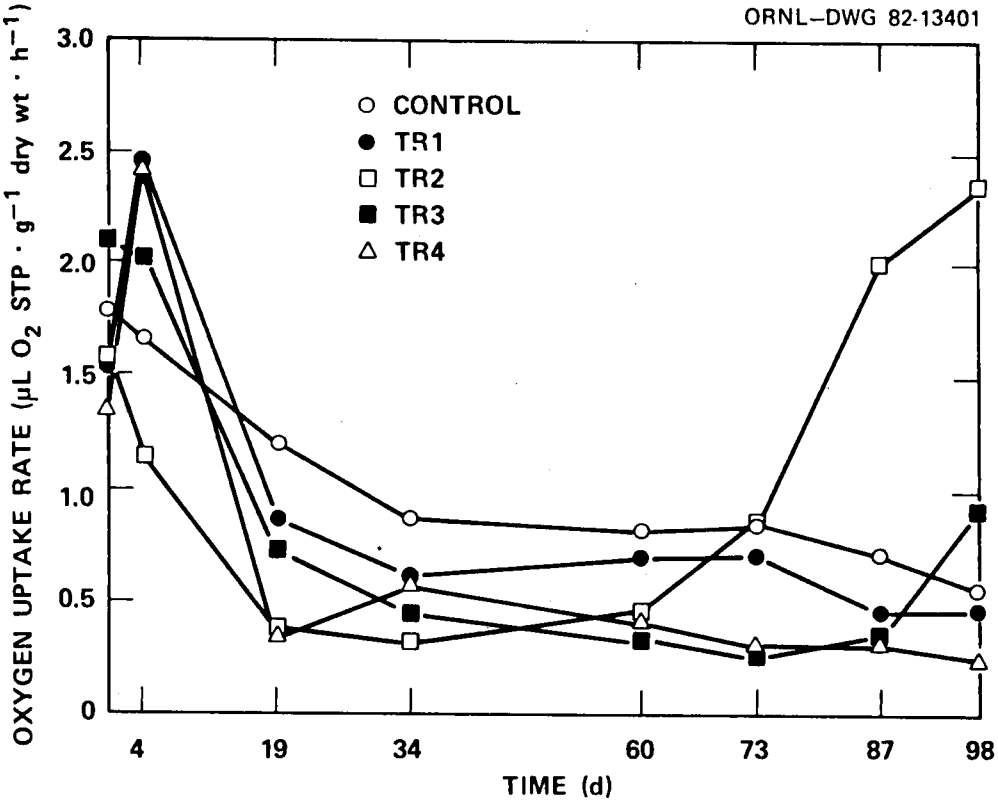

Fig. 2. Mean oxygen uptake rates (ul STP/g dry wt soil/hr) for the control and oil treatments through time. Oil levels are 0.2% (TR1), 1.7% (TR2), 3.4% (TR3) and 8.6% (TR4). Least significant difference (LSD) value at $p < .05$ is 0.675.

Table 3. The effect of coal oil concentrations of 0% (control), 0.2% (TR1), 1.7% (TR2), 3.4% (TR3) and 8.6% (TR4) on oxygen uptake rates (ul STP/g/hr) in soil. A Duncan's multiple range test was used to analyze the data at each time period. (Mean values joined by line are not significantly different at $p < .05$.)

Day 0*	Day 4	Day 19	Day 34	Day 60	Day 73	Day 87	Day 98
TR3 2.10	TR1 2.45	C 1.21	C .88	C .82	C .86	TR2 2.02	TR2 2.36
C 1.80	TR4 2.41	TR1 .87	TR1 .61	TR1 .71	TR2 .86	C .72	TR3 .92
TR2 1.57	TR3 2.03	TR3 .73	TR4 .50	TR2 .47	TR1 .71	TR1 .47	C .57
TR1 1.53	C 1.67	TR2 .39	TR2 .33	TR4 .46	TR4 .33	TR3 .36	TR1 .48
TR4 1.36	TR2 1.14	TR4 .37	TR3 .30	TR3 .34	TR3 .28	TR4 .35	TR4 .25

*Day 0 was before addition of the oil to soil.

Table 4. Coal oil effects on oxygen uptake rates (ul STP/g/hr) for each treatment level. Duncan's multiple range test results are presented. (Mean values joined by line are not significantly different at $p < .05$.)

Control		TR1		TR2		TR3		TR4	
Day	0* 1.80	Day	4 2.45	Day	98 2.36	Day	0 2.10	Day	4 2.41
Day	4 1.67	Day	0 1.53	Day	87 2.02	Day	4 2.03	Day	0 1.36
Day	19 1.21	Day	19 .87	Day	0 1.57	Day	98 .93	Day	34 .50
Day	34 .88	Day	73 .71	Day	4 1.14	Day	19 .73	Day	60 .46
Day	73 .86	Day	60 .71	Day	73 .86	Day	87 .37	Day	19 .37
Day	60 .82	Day	34 .61	Day	60 .47	Day	60 .34	Day	87 .35
Day	87 .72	Day	98 .48	Day	19 .39	Day	34 .30	Day	73 .33
Day	98 .57	Day	87 .47	Day	34 .33	Day	73 .28	Day	98 .25

*Day 0 was before oil addition.

different than the control and treatment one whereas at days 87 and 98 the rates were significantly higher than all the other samples. Uptake rates for the control and treatment one decreased significantly through time, while those of treatments three and four remained not significantly different after their decrease to day 19 levels (Table 4). This resulted in the soil samples for the control and treatment one having oxygen uptake rates not significantly different from those of treatments three and four at the last two measurements.

Total Viable Cells

A plot of the means for numbers of total viable cells (Fig. 3) shows differences between treatments as early as day 4. At days 34 and 60 cell densities for treatment one (0.2%) were almost an order of magnitude greater than for the control while levels for treatments two (1.7%), three (3.4%) and four (8.6%) were approximately an order of magnitude less. These differences are significant ($p < .05$) as indicated by the Duncan's multiple range test (Table 5). After day 60, treatment two increased population densities above the control numbers and at day 98 were significantly higher than those for treatment one also. Numbers in the control decreased significantly after day 34 (Table 6) which partially accounts for the fact that they are not significantly different from the 3.4% and 8.6% oil levels after day 98 (Table 5). Cell densities at the 3.4% oil concentration had increased by day 87 but this was not significant and the mean values never returned to initial (day 0) levels.

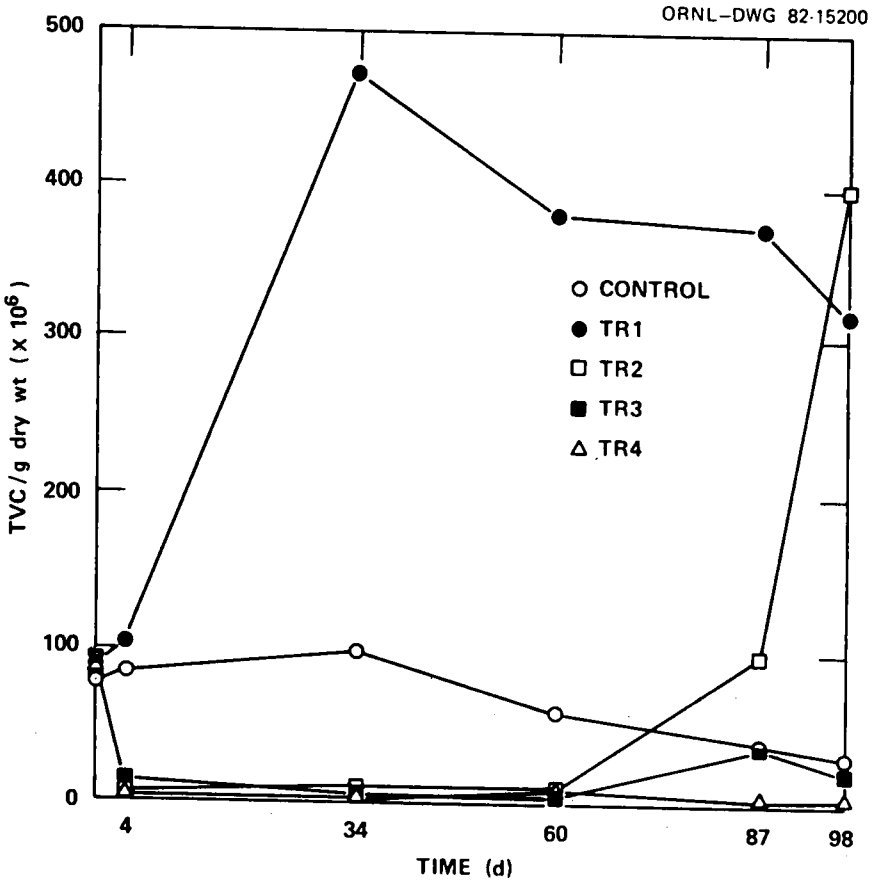

Fig. 3. Total viable cell (TVC) means through time (TVC/g dry wt soil) for the control and oil levels 0.2% (TR1), 1.7% (TR2), 3.4% (TR3) and 8.6% (TR4). Least significant difference (LSD) value at $p < .05$ is 73.2×10^6.

Table 5. Total viable cell counts (TVC × 10^6/g dry wt soil) as affected by oil concentrations of 0% (C = control), 0.2% (TR1), 1.7% (TR2), 3.4% (TR3) and 8.6% (TR4). A Duncan's multiple range test was performed at each time period. (Means joined by line are not significantly different at $p < .05$.)

Day 0	Day 4	Day 34	Day 60	Day 87	Day 98
TR3 95.5	TR1 103.0	TR1 470.0	TR1 379.0	TR1 372.0	TR2 397.0
TR1 87.9	C 86.7	C 99.7	C 60.4	TR2 97.0	TR1 318.0
TR4 86.5	TR3 12.7	TR2 11.3	TR4 7.44	C 41.8	C 34.1
TR2 83.7	TR2 11.7	TR3 10.6	TR2 7.34	TR3 40.1	TR3 22.3
C 77.8	TR4 7.25	TR4 9.75	TR3 6.86	TR4 6.33	TR4 5.23

Table 6. The effects of a coal oil on total viable cell counts (TVC × 10^6/g dry wt soil). Results for each treatment level were analyzed using a Duncan's multiple range test. (Mean values joined by line are not significantly different at $p < .05$.)

Control		TR1		TR2		TR3		TR4	
Day 34	99.7	Day 34	470.0	Day 98	397.0	Day 0	95.5	Day 0	86.5
Day 4	86.7	Day 60	379.0	Day 87	97.0	Day 87	40.1	Day 34	9.75
Day 0*	79.8	Day 87	372.0	Day 0	83.7	Day 98	22.3	Day 60	7.44
Day 60	60.4	Day 98	318.0	Day 4	11.7	Day 4	12.7	Day 4	7.25
Day 87	41.8	Day 4	103.0	Day 34	11.3	Day 34	10.6	Day 87	6.33
Day 98	34.1	Day 0	87.9	Day 60	7.34	Day 60	6.86	Day 98	6.23

*Day 0 was before addition of the oil.

The cell densities for treatment one increased rapidly after oil addition, while oxygen uptake rates decreased slightly with time. A Pearson's product moment correlation analysis of oxygen uptake rates and plate counts after oil addition showed no significant ($p < .05$) correlation until day 98 (Table 7). At day 98, both cell densities and oxygen uptake rates for a 1.7% oil concentration (TR2) were higher than the control or other oil treatments. The r value (.67) is significant at the $p < .0001$ level.

Nitrifying Bacteria

At day 4 following oil addition nitrifying bacteria were not detected in any of the oil treated samples (dilution range 10^{-2} to 10^{-6}) (Table 8). Subsequently, a lower dilution range was used (10^{-1} to 10^{-5}) and ammonia oxidizers, although not nitrite oxidizers, were detected in soils of treatment one (0.2%) at day 30. By day 88, however, no nitrifying bacteria were detected in any oil-treated soil. Numbers in the control soil decreased with time yet remained above levels for treatment one throughout the experiment and the bacteria were detectable in some replicates at each time period.

Soil Ammonia

The inhibition of the nitrifying bacteria is reflected in the accumulation of ammonia in the soils containing coal oil Mean ammonia concentrations for treatment one soils increased significantly (Table 9) after day 2 to be approximately 5 times the other treatments and the

Table 7. Results of a Pearson's product moment correlation analysis of oxygen uptake rates and total viable cell counts at each time period after oil addition.

	Day 4	Day 34	Day 60	Day 87	Day 98
r value	.14	.21	.41	-.18	.67
P*	.560	.379	.072	.442	.001

*P (> |r|) if Ho: r = 0 is true.

Table 8. Most-probable-number (MPN) of the nitrifying bacteria (MPN/g dry wt soil) as affected by oil treatments of 0% (control), 0.2% (TR1), 1.7% (TR2), 3.4% (TR3) and 8.6% (TR4).

Treatment and Replicate		NH_4-Oxidizers				NO_2-Oxidizers			
		Day 0	Day 4	Day 30	Day 88	Day 0	Day 4	Day 30	Day 88
Control	1	5800	200	300	55	51	5.1	6.4	2.6
	2	840	580	620	6	11	-[a]	4.2	-
	3	1200	120	300	27	-	-	3.0	-
	4	580	330	230	13	20	51	4.4	-
TR1	1	430	-[a]	2.7	-	51	-	-	-
	2	560	-	2.8	-	4300	-	-	-
	3	5800	-	2.9	-	81	-	-	-
	4	5800	-	2.6	-	51	-	-	-
TR2	1	430	-	-	-	33	-	-	-
	2	2000	-	-	-	5.1	-	-	-
	3	5300	-	-	-	4300	-	-	-
	4	560	-	-	-	3300	-	-	-
TR3	1	280	-	-	-	5.1	-	-	-
	2	430	-	-	-	2.0	-	-	-
	3	560	-	-	-	2.0	-	-	-
	4	120	-	-	-	4.2	-	-	-
TR4	1	430	-	-	-	430	-	-	-
	2	200	-	-	-	2.0	-	-	-
	3	580	-	-	-	2.0	-	-	-
	4	280	-	-	-	110	-	-	-

[a]No nitrifiers were detected.

Table 9. Soil ammonium concentrations (ug NH_4^+/g dry wt soil) as affected by oil levels of 0% (C = control), 0.2% (TR1), 1.7% (TR2), 3.4% (TR3) and 8.6% (TR4). Results of a Duncan's multiple range test at each time period are presented. (Means joined by line are not significantly different at $p < .05$.)

Day 0*	Day 2	Day 24	Day 38	Day 52	Day 66	Day 88
TR4 2.4	TR4 2.8	TR1 20.7	TR1 16.8	TR1 31.0	TR1 23.4	TR1 26.8
TR2 2.1	TR3 2.2	TR4 4.1	TR2 5.3	TR4 9.4	TR2 13.6	TR2 20.8
C 1.2	TR2 2.0	TR2 4.0	TR4 5.2	TR2 8.2	TR4 12.0	TR3 18.6
TR3 1.2	TR1 2.0	TR3 3.9	TR3 5.0	TR3 7.0	TR3 11.6	TR4 17.4
TR1 1.2	C 1.5	C 1.2	C 1.4	C 1.2	C 0.9	C 0.7

*Day 0 was before oil addition.

control by day 24 (Fig. 4). The ammonia levels of soil treated with the other oil concentrations were not significantly different from each other throughout the experiment (Table 9). Levels for soil of treatments two, three and four (oil concentrations 1.7%-8.6%) increased significantly so that at days 38 through 98 they were significantly higher than ammonia levels in the control soil but remained significantly lower than levels in treatment one soil. Soil ammonia concentrations for the control were never significantly higher than initial levels (day 0) and at days 66 and 88 were significantly lower than previous values (Table 10). Concentrations in the oil-treated soils, however, were significantly higher than initial levels at these days.

Soil Nitrate

The inhibition of ammonium oxidation prohibited nitrate accumulation in the oil-treated soil (Fig. 5). Consequently only the control soil showed any significant increase in nitrate (Table 11). The oil treatments actually decreased soil nitrate significantly from initial values at some measurement periods. Nitrate levels in the controls were significantly higher than those of all the oil treatments at days 38, 66 and 88 (Table 12). At these time periods the treatments did not differ significantly from each other.

Nitrogen, Carbon and pH

The sum of the ammonia and nitrate levels for each treatment represents the amount of inorganic nitrogen in the soil at each time period.

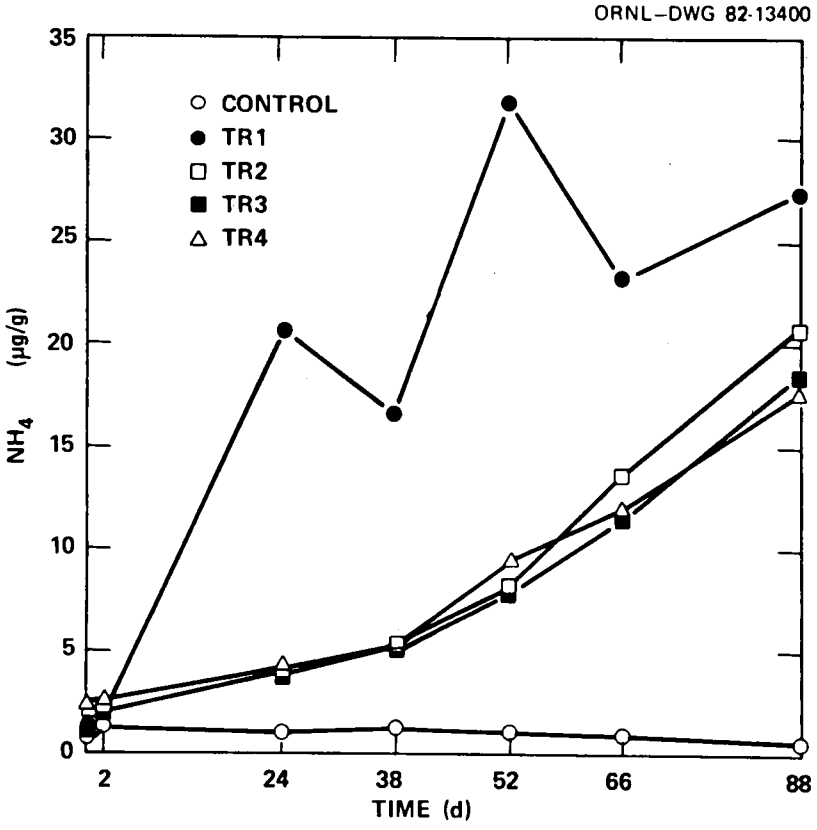

Fig. 4. Mean ammonium concentrations (ug NH_4/g dry wt soil) at each time period for oil levels of 0% (control), 0.2% (TR1), 1.7% (TR2), 3.4% (TR3) and 8.6% (TR4). Least significant difference (LSD) value at $p < .05$ is 0.816.

Table 10. The effects of a coal oil on soil ammonium concentrations (ug NH_4^+/g dry wt soil). Data for each treatment level was analyzed using a Duncan's multiple range test. (Means joined by line are not significantly different at $p < .05$.)

Control		TR1		TR2		TR3		TR4	
Day	2 1.5	Day	52 31.0	Day	88 20.8	Day	88 18.6	Day	88 17.4
Day	38 1.4	Day	88 26.8	Day	66 13.6	Day	66 11.6	Day	66 12.0
Day	52 1.2	Day	66 23.4	Day	52 8.2	Day	52 7.9	Day	52 9.4
Day	0 1.2	Day	24 20.7	Day	38 5.3	Day	38 5.0	Day	38 5.2
Day	24 1.2	Day	38 16.8	Day	24 4.0	Day	24 3.9	Day	24 4.1
Day	66 0.9	Day	2 2.0	Day	0 2.1	Day	2 2.2	Day	2 2.8
Day	88 0.7	Day	0 1.2	Day	2 2.0	Day	0 1.2	Day	0 2.4

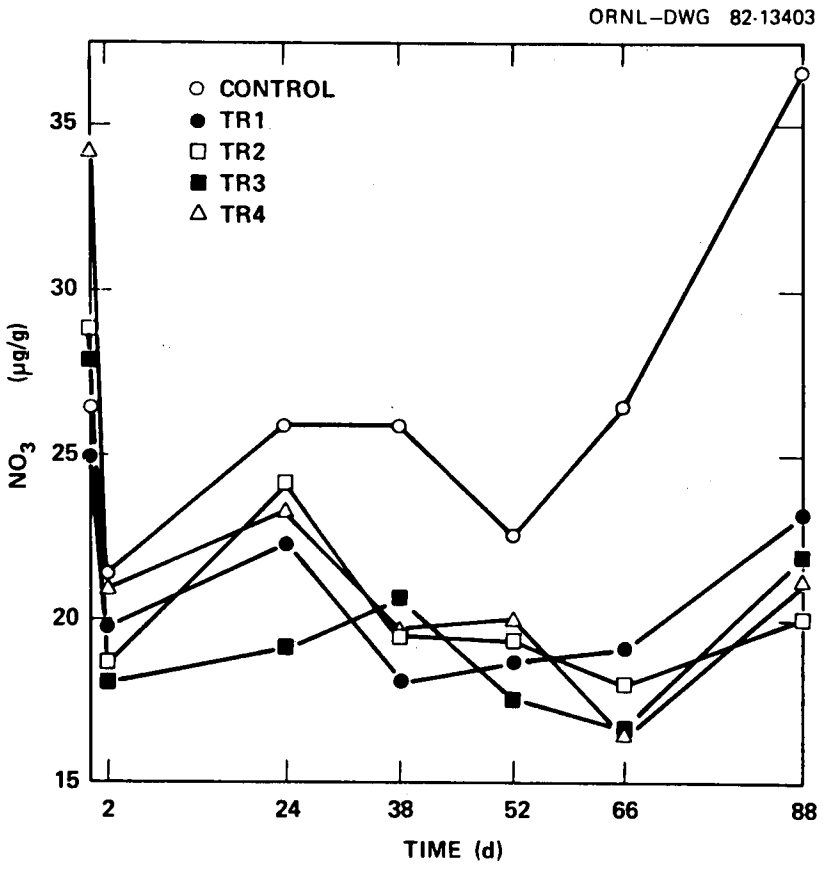

Fig. 5. Mean nitrate concentrations (ug NO_3/g dry wt soil) for each treatment through time. Oil concentrations were 0% (control), 0.2% (TR1), 1.7% (TR2), 3.4% (TR3) and 8.6% (TR4). Least significant difference (LSD) value at $p < .05$ is 1.134.

Table 11. A coal oil's effects on soil nitrate levels (ug NO_3^-/g dry wt soil). Oil concentrations are 0% (control), 0.2% (TR1), 1.7% (TR2), 3.4% (TR3) and 8.6% (TR4). Results of a Duncan's multiple range test at each treatment level are presented. (Means joined by line are not significantly different at $p < .05$.)

Control	TR1	TR2	TR3	TR4
Day 88 36.6	Day 0 25.0	Day 0 28.8	Day 0 27.9	Day 0 34.2
Day 0 26.6	Day 88 23.2	Day 24 24.5	Day 88 22.0	Day 24 23.4
Day 66 26.4	Day 24 22.4	Day 88 20.3	Day 38 20.8	Day 88 21.2
Day 24 26.0	Day 2 19.6	Day 38 19.5	Day 24 19.3	Day 2 21.1
Day 38 26.0	Day 66 19.2	Day 52 19.4	Day 2 18.2	Day 52 20.0
Day 22 22.6	Day 38 18.8	Day 2 18.6	Day 52 17.8	Day 38 19.5
Day 2 21.3	Day 52 18.6	Day 66 18.0	Day 66 16.6	Day 66 16.6

Table 12. Nitrate concentrations in the soil (ug NO_3^-/g dry wt soil) as affected by a coal oil. Data at each time period was analyzed using a Duncan's multiple range test. (Means joined by line are not significantly different at $p < .05$.)

Day 0*	Day 2	Day 24	Day 38	Day 52	Day 66	Day 88
TR4 26.6	C 21.3	C 26.0	C 26.0	C 22.6	C 26.4	C 36.6
TR2 25.0	TR4 21.1	TR2 24.5	TR3 20.8	TR4 20.0	TR1 19.2	TR1 23.2
TR3 28.8	TR1 19.6	TR4 23.4	TR2 19.5	TR2 19.4	TR2 18.0	TR3 22.0
C 27.9	TR2 18.6	TR1 22.4	TR4 19.5	TR1 18.6	TR3 16.6	TR4 21.2
TR1 24.2	TR3 18.2	TR3 19.3	TR1 18.8	TR3 17.8	TR4 16.6	TR2 20.3

*Day 0 is before oil addition.

Although the oil had significant effects on the form of inorganic nitrogen in the soil, the mean amount of mineral nitrogen was never greatly below control levels for any oil treatment (Fig. 6). The lowest oil application increased nitrogen levels with increases ranging from 1.3 to 2.1 times the control values after day 2 (Fig. 6).

There were no significant differences in organic carbon content and total nitrogen among the soil samples before oil addition. The mean of the carbon to nitrogen ratios for each treatment group was 15.42 (Table 13). Addition of the oil, which has a carbon to nitrogen ratio of 102.7, caused an approximately proportionate increase in organic carbon and total nitrogen levels in the soil. Expected carbon and nitrogen concentrations, calculated from the known composition of the oil and from control soil values, corresponded closely to actual percentages measured at day 87 (Table 14). Soil pH was also increased by addition of the oil (Table 15) which had an alkaline pH (24 hr water extracts with an oil:water ratio of 1:1 had pHs of 8-9; D. Brown, personal communication).

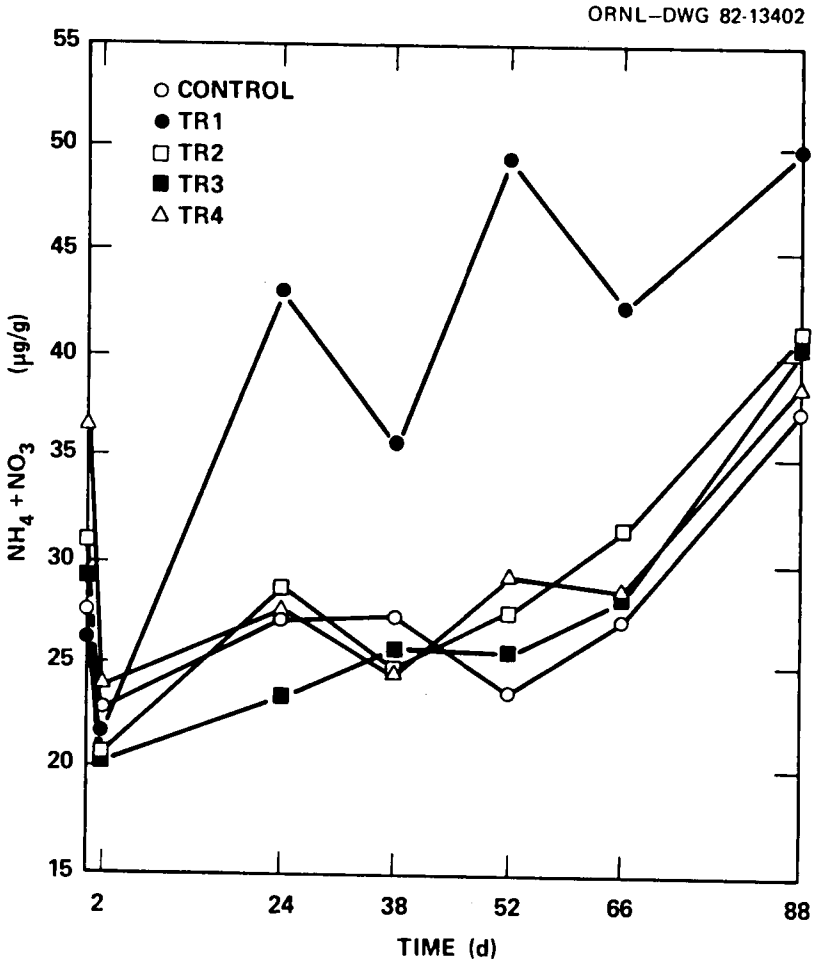

Fig. 6. The sum of the mean ammonium and nitrate concentrations before oil addition (day 0) and at days 2, 24, 32, 52, 66 and 88 for oil levels of 0% (control), 0.2% (TR1), 1.7% (TR2), 3.4% (TR3) and 8.6% (TR4).

Table 13. Percent organic carbon, total nitrogen and the resultant C:N ratios for a Captina silt loam soil before application of a coal oil (day 0).

	% Organic Carbon	Total Nitrogen %	C:N
Control	1.7 ± .2[a]	.10 ± .008[a]	17.00
TR1	1.7 ± .2	.11 ± .010	15.45
TR2	1.8 ± .2	.11 ± .010	16.36
TR3	1.6 ± .2	.12 ± .005	13.30
TR4	1.8 ± .3	.12 ± .006	15.00

[a] ± one standard deviation.

Table 14. Actual and calculated percent organic carbon (OC) and total nitrogen (N) in a Captina silt loam soil after addition of a coal oil (day 87). Oil levels were 0% (control), 0.2% (TR1), 1.7% (TR2), 3.4% (TR3) and 8.6% (TR4).

	Calculated*			Actual		
Treatment	%OC	%N	OC:N	%OC	%N	OC:N
Control	-	-	-	1.8	.11	16
TR1	2.0	.11	18	2.2	.12	18
TR2	3.3	.13	25	3.5	.12	29
TR3	4.8	.14	34	4.6	.14	33
TR4	9.3	.18	51	8.0	.16	50

*Calculated with mean values for the controls and known percentage of carbon and total nitrogen in the oil.

Table 15. Mean soil pHs at two time periods after oil addition. Oil concentrations are 0% (control), 0.2% (TR1), 1.7% (TR2), 3.4% (TR3) and 8.6% (TR4).

	Day 45	Day 90
Control	4.52 ± .06[a]	4.57 ± .08[a]
TR1	5.12 ± .05	4.98 ± .04
TR2	5.34 ± .07	5.34 ± .15
TR3	5.48 ± .09	5.38 ± .04
TR4	5.70 ± .10	5.68 ± .03

[a] ± one standard deviation.

V. DISCUSSION

The inorganic nitrogen concentrations in the soil were never reduced by any concentration of the coal oil. Petroleum oils also appear to have no detrimental effect on nitrogen mineralization because levels of ammonium and nitrate increased after the fertilization of oil spills with organic nitrogen sources (Jobson et al., 1974; Gudin and Syratt, 1975; Westlake et al., 1979).

The ten to twelve weeks required for oxygen uptake rates and viable cell counts in the oil treatments to return to control levels indicates a toxicity of the coal oil towards the microorganisms. The comparison by Ricard (1980) of the effects of a coal and petroleum oil on respiration and microbial biomass showed that the coal oil initially had a greater negative effect on sediment microorganisms. The toxicity of the SRC-II oil to the microorganisms may have been caused by the phenolic components of the oil. The toxicity of coal liquids to aquatic organisms has been attributed to the high phenolic content of the oils (Giddings et al., 1980) and phenols obtained from coal tars have been used extensively as disinfectants (Sykes, 1965).

Petroleum oils, which are much lower in phenolic compounds than coal oils, are also less toxic towards microorganisms. Increased oxygen uptake rates were the result of a two month exposure to concentrations of a petroleum oil comparable to those used in this study (Jensen, 1975; Vanloocke et al., 1975). After inputs of petroleum oils under a wide range of experimental conditions, numbers and microbial activities sometimes decreased but generally returned to control levels in a period

of days. Subsequently, increases above controls have often been observed (Dobson and Wilson, 1964; Jones et al., 1970; Odu, 1972; Llanos and Kjoller, 1976; Sexstone and Atlas, 1977).

The increase in oxygen uptake rates and microbial numbers after a 3 month exposure to the oil for treatment two (1.7% oil) indicated that the microorganisms were finally able to utilize the soil organic matter or some component of the oil. This change may have come about through the adaptation of the populations to the presence of the oil and/or a stimulation of decomposition by the oil, causing numbers to increase. Oxygen uptake rates for a 3.4% oil concentration were also above control rates at day 98 (although not significantly) indicating that the microflora were carrying out increased decomposition or were degrading the oil carbon. Similarly, Ricard (1980) found that mineralization rates for a number of different compounds increased at the end of a three month exposure of sediment microorganisms to a coal liquid.

Microbial activities and numbers were reduced to the same extent at oil levels above 1.7%. Griffiths et al. (1980b) also observed no further decreases in microbial activity above a particular concentration (1 ppt) of petroleum in water. Increases in both activity and numbers were concentration dependent in this study. An increase in plate counts was not observed for treatment two (1.7%) until nine weeks after the same increase associated with treatment one (0.2%). Oxygen uptake rates however, were not affected by 0.2% oil but were doubled (compared to the control) by a 1.7% oil concentration (treatment two).

The fact that no significant correlation was found for oxygen uptake rates and plate counts until day 98, might be explained by the great increase in numbers for treatment one (0.2%) while there was no corresponding increase in respiration. The uptake rates and numbers for the other oil treatments followed approximately the same pattern of decreases and increases. At day 98, cell densities for treatment one had fallen slightly and both cell densities and respiration rates were high for treatment two. Inorganic nitrogen levels in the soil were higher than controls in soil treated with 0.2% oil. The plate count values probably reflect an increase in nitrogen mineralizing bacteria.

Comparisons of cell densities obtained by both the plate and direct count methods after biocidal treatments and subsequent incubation, showed that direct count values do not increase above controls while those for plate counts increase greatly (Meikljohn, 1955; Sheilds et al., 1974). However, these same studies found decreases in numbers immediately after biocidal treatments by both methods. This indicates the usefulness of the plate count method for reflecting decreases in microbial numbers. The interpretation of the plate count results is aided by the comparison of plate count trends with those of parameters that estimate the activities of a diverse section of the microflora. Thus the increase in cell densities for treatment two which was associated with an increase in oxygen uptake rates was probably indicative of an increase in a large section of the soil community.

Unlike the other parameters measured, nitrification was depressed for all treatment levels throughout the duration of the experiment. The

drastic decrease in the number of the nitrifying bacteria after application of the SRC-11 product corroborates other information about their sensitivity to many organic compounds and soil treatments (Chandra and Bollen, 1961; Gasser and Peachy, 1964; Powlson, 1976). The presence in the coal oil of such known nitrification inhibitors as aniline compounds (Stafford, 1974; Hockenbury and Grady, 1977) may account for some of the observed inhibition. The resultant accumulation of ammonia, although not affecting the microbial populations which preferentially utilize it over nitrate (Alexander, 1977), may have consequences for plant nutrition.

It has been suggested that, because ammonium is less easily leached from the soil column than nitrate, increased nitrogen uptake and plant growth may result from the inhibition of nitrification (Draycott and Last, 1971). Ammonium is also used more efficiently within the plant, but once it is taken up it must be complexed immediately or a toxic reaction ensues. Laboratory experiments have showed that plant yields are maximized with low rates of ammonium uptake and a nitrate supply, but the conditions associated with beneficial ammonia assimilation and concentrations exhibiting toxicity are largely unknown (Reisenauer, 1978).

Ajayi et al. (1970) suggested that the inhibition of nitrification may contribute to plant injury by ammonium toxicity although field instances of this are thought to be rare. This rarity may be attributable to the short term effects of presently used nitrification inhibitors (Goring, 1962).

If an accumulation of ammonium is significant for the potential revegetation of a contaminated soil, the lowest oil treatment (0.2%) would have the greatest impact on plant establishment and growth due to its stimulation of net nitrogen mineralization. However, petroleum oils are known to be toxic to plants (Baker, 1970) and to cause reduced germination (Udo and Fayemi, 1975) even at very low concentrations (0.2%) (DeJong, 1980). It is likely that synthetic fuels may also have a direct effect on plant growth and that the accumulation of ammonium would have only a secondary effect.

The increase in the C:N ratio of the soil by the addition of the coal oil could affect the degradation rates of oil components if nitrogen became limiting. For the 3 month duration of this study, mineral nitrogen was never less than controls and at a 0.2% oil oil concentration soil inorganic nitrogen levels were much higher than controls. Thus, inorganic nitrogen was not limiting organic carbon utilization. The increases in decomposition, which were observed for a 1.7% oil concentration would probably result in a decrease in mineral nitrogen after some time period.

Addition of inorganic nitrogen may not be necessary to maximize oil degradation for the oil concentrations used in this study. Dibble and Bartha (1979) found that optimal degradation rates of a petroleum crude under laboratory conditions similar to those used in this experiment occurred with a C:N ratio of 60:1. The highest oil concentration (8.6%) increased the soil C:N ratio from 16:1 to 50:1. However, the great differences in composition between a coal oil and a petroleum product

would probably result in differences in rates of utilization of the organic carbon and nitrogen in the oils. This may result in the optimal C:N ratios for degradation being different as well.

The high water solubility of the toxic components of synthetic fuels (Giddings and Herbes, 1981) and the greater depth of penetration of coal oils in the solid column compared to a more viscous petroleum oil, would make the impact of a coal oil spill much greater than that of a petroleum product. Components of the oil would be moved away from the surface soils where the greatest amount of potential degradation might take place. The disruption of normal microbial processes in a surface soil for three months due to the presence of a 1.7% concentration of coal oil indicates that degradation of the oil would probably occur only after a considerable lag period in which numbers and activities are reduced. Thus, land spills of coal oils are expected to have a significant environmental impact on the soil processes and one which could not have been predicted from our knowledge of petroleum oils.

VI. CONCLUSIONS

The SRC-II product used in this study disrupted microbial activities for three months in a Captina silt loam soil in which the environmental conditions of aeration, moisture and temperature were optimal for most microbial processes. All oil concentrations used in this study (0.2-8.6%) affected some aspect of the soil carbon or nitrogen transformation. Nitrification was the process most sensitive to the input of the synthetic fuel oil. A 0.2% oil level was the only concentration which caused any increases in cell numbers and activities over controls before three months. The inorganic nitrogen concentrations in the soil were not affected by any oil concentration. However, soil ammonium and nitrate concentrations were significantly higher and lower than controls, respectively, in soil treated with a coal oil. This different proportion of inorganic nitrogen could have significant effects on plant growth and species composition and may have implications for plant-plant and animal-plant interactions. The recovery time of heterotrophic microbial populations after input of a coal oil for the soil and environmental conditions of this study, was determined as three months at an oil concentration of 1.7%. Three months was not long enough to detect any recovery of the nitrifying populations at any oil concentration or of the heterotrophs at oil concentrations of 3.4 and 8.6%.

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APPENDIX

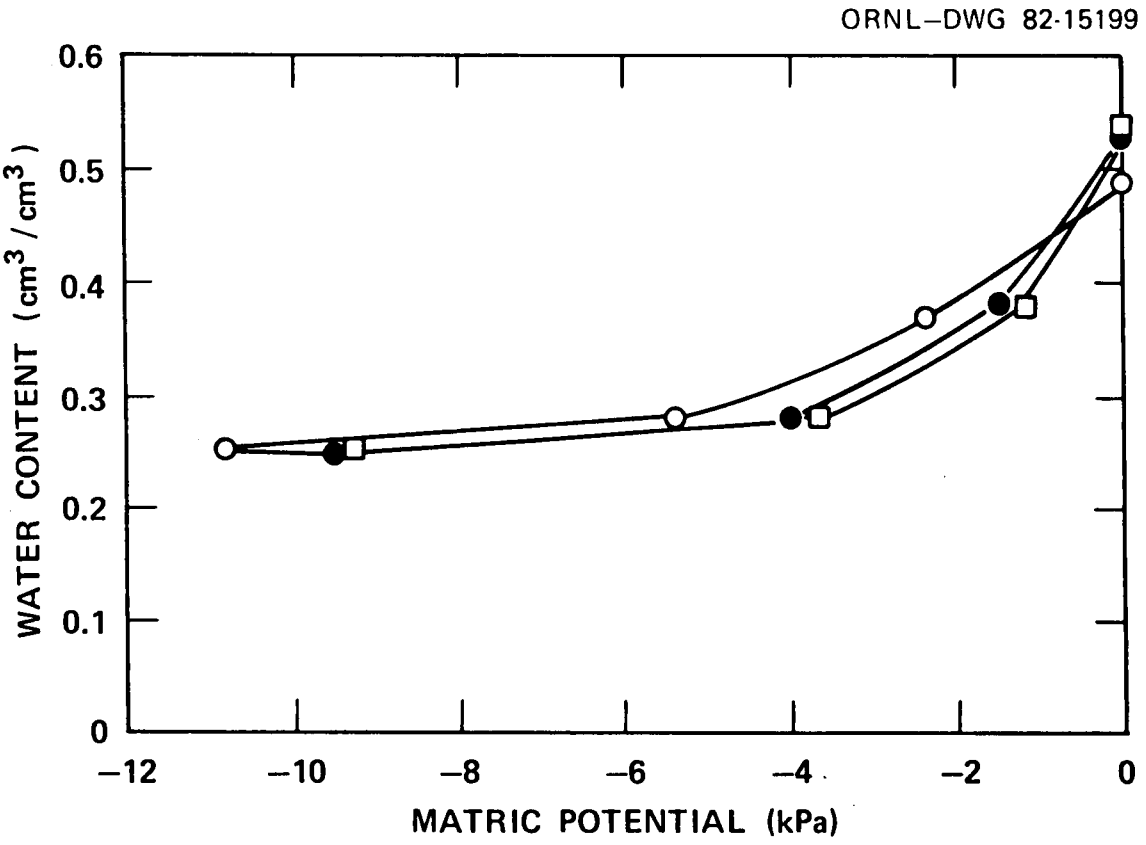

Fig. A-1. Water retention curves for three replicate samples of a Captina silt loam soil at a bulk density of 1.1 g/cm³.

VITA

Mary Helen Ward was born in Geneva, Illinois and grew up in Glen Ellyn, Illinois where she graduated from high school in 1975. She obtained her Bachelor of Science degree, cum laude in biochemistry from the University of Dallas. An interest in the effects of pollutants on ecosystems and ecology in general led to her enrollment in the Graduate Program in Ecology at The University of Tennessee, Knoxville. An opportunity to do research on the effects of synthetic fuels on microbial processes at Oak Ridge National Laboratory resulted in the attainment of a Master of Science degree in Ecology from UT in March 1983.

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