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Effect of nitrogen and phosphorus addition on phenanthrene biodegradation in four soils

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Abstract

Phenanthrene mineralization rates were found to vary widely among four soils; differences in soil nutrient levels was one hypothesis to explain this variation. To test this hypothesis, phenanthrene mineralization rates were measured in these soils with, and without, added nitrogen and phosphorus. Mineralization rates either remained unchanged or were depressed by the addition of nitrogen and phosphorus. Phenanthrene degradation rates remained unchanged in the soil which had the highest indigenous levels of nitrogen and phosphorus and which showed the largest increase in phosphorus levels after nutrients were added. The soils in which degradation rates were depressed had lower initial phosphorus concentrations and showed much smaller or no measurable increase in phosphorus levels after nutrients were added to the soils. To understand the response of phenanthrene degradation rates to added nitrogen and phosphorus, it may be necessary to consider the bioavailability of added nutrients and nutrient induced changes in microbial metabolism and ecology.

Introduction

Microbial activity in soil is generally considered to be limited by the amount of available carbon and not by the levels of inorganic nutrients (Alexander 1994). A large influx of carbon compounds, as occurs during an oil spill, can reverse this situation creating an environment where biodegradation of the added carbon compounds is limited by nutrient availability. Numerous studies have been published in which nitrogen and/or phosphorus addition stimulated pollutant degradation; however, the effects of nutrients on pollutant degradation rates in soil are not consistent (Bossert & Bartha 1984; Alexander 1994; Baker & Herson 1994). Pollutant degradation rates in soil may be unaffected by added nutrients or may actually decline relative to controls (Manilal & Alexander 1991; Morgan & Watkinson 1992). Stimulation of pollutant degradation rates may not appear until days or weeks after nutrients are added (Jobson et al. 1974; Bossert & Bartha 1984). Different aspects of pollutant degradation rates such as lag time, initial rate, and degradation extent may be affected singly or as a group by nutrient additions (Thorton-Manning et al. 1987; Swindoll et al. 1988).

Differences in the bioavailability of nutrients added to soil may explain a portion of the observed differences in the response of degradation rates to nitrogen and phosphorus supplements. Just as sorbed carbon substrates are generally considered to be unavailable to soil microbes, sorbed inorganic nutrients also may be less bioavailable than nutrients dissolved in the soil solution. While nitrate is very soluble in soil, ammonium is retained by soil cation exchange sites on clays (Bohn et al. 1985). When added to soil, phosphate is adsorbed quickly to iron and aluminum oxide surfaces and may form precipitates with iron, aluminum, manganese, and calcium (Brady 1990).

The objective of this study was to determine the effect of added nitrogen and phosphorus on the biodegradation rates of low concentrations of phenanthrene in four soils. These soils were selected from a set of seven soils for which phenanthrene degradation rates had been measured over a period of six

months (Johnson 1997). One hypothesis proposed to explain the variation in phenanthrene degradation rates was that low nutrient levels limited biodegradation in the soils with low biodegradation rates. To investigate this hypothesis, four soils, Yolo, Scott, Aiken and Forbes, were selected which represented a range of degradation rates. Of the seven soils tested, phenanthrene degradation rates were the fastest in Yolo soil and the slowest in Forbes soil. Scott and Aiken soils exhibited intermediate phenanthrene degradation rates (Johnson 1997). Phenanthrene degradation rates were measured in these soils with, and without, supplemental nitrogen and phosphorus. If addition of nutrients increased phenanthrene degradation rates in the soils with slow phenanthrene biodegradation rates, it would support the hypothesis that nutrient levels limited microbial activity in these soils. In addition to measuring phenanthrene biodegradation rates, nitrogen and phosphorus levels were measured both in soils which did, and did not, receive added nutrients to try to quantity the soils' ability to sequester nitrogen and phosphorus.

Materials and methods

Yolo soil was collected from an organic farm at the University of California, Davis in July, 1993. Aiken and Forbes soils were collected from conifer and oak forests in the Tahoe National Forest, Placer County, CA in July 1994. Scott soil was collected from beneath an oak tree on a grassland in rural Yuba County, CA in July 1994. It is not known whether any of these soils were previously exposed to polyaromatic hydrocarbons. Soil names reflect the dominant soil series at the collection sites according to soil survey maps except for Scott soil which was collected from an area for a which a soil survey was not available (USDA 1972; National Forest Service 1986). Soil fractions passing through a 2 mm sieve were stored in sealed bags at 4 °C and maintained at field moisture levels.

Soil analyses

Organic matter, total carbon, soil texture, exchangeable ammonium and nitrate, and available phosphorus analyses were performed by the Department of Agricultural and Natural Resources Laboratory, University of California, Davis (DANR). Organic matter was measured for Yolo soil by the Walkley-Black wet combustion method (Nelson & Sommers 1982). Total carbon was measured for the other soils by combustion using a Carlo-Erba Carbon/Nitrogen Analyzer (C.S. Elantech Inc., Lakewood, NJ). Since the soils analyzed were not calcareous, these two methods of measuring soil carbon should provide comparable results (Nelson & Sommers 1982). Available phosphorus was determined by 0.5 N HCO_3^- extraction followed by reduction and spectrophotometeric measurement of the complex formed by orthophosphate and molybdate in acidic solution (Olsen et al. 1954). Exchangeable ammonium and nitrate levels were determined by potassium chloride extraction with subsequent measurement using a diffusion-conductivity analyzer (Keeny & Nelson 1982; Carlson 1978). Soil moisture content at -0.033 MPa moisture tension was determined using a pressure plate (Klute 1986). The soil percent moisture at -0.033 MPa moisture tension will be referred to as the -0.033 MPa moisture content. Soil pH was measured in a 1 : 1 (w : w) slurry. Soil properties are listed in Table 1.

Table 1. Soil properties

Soil	Total carbon (g Kg^{-1})	Texture class	pH	Percent moisture at -0.033 MPa
Yolo	11[1]	loam	6.8	20.6
Aiken	54	sandy loam	5.2	45.7
Scott	36	loam	5.1	29.9
Forbes	40	loam	4.9	40.3

[1]Organic carbon content calculated from organic matter measured by Walkley–Black method. OC = OM/0.58.

Biodegradation studies

The biodegradation rates of 50 μg [9–^{14}C]-phenanthrene per kg dry soil (Sigma Chemical Co., St. Louis, MO) were compared for Aiken, Forbes, Scott, and Yolo soils with, and without, additional nitrogen and phosphorus. Enough nitrogen and phosphorus were added so that, on a mass basis, the ratio between phenanthrene carbon and added nutrients was approximately C:N 1 : 100 and C:P 1 : 1000. Phosphorus was added at a rate of 47 mg P per kg dry soil as KH_2PO_4 and K_2HPO_4 (ACS Certified, Fisher Scientific, Fair Lawn, NJ). The ratio of KH_2PO_4 to K_2HPO_4 was 0.8. Nitrogen was added as NH_4NO_3 (Sigma Chemical Co.) at a rate of 4.7 mg N per kg dry soil. All solutions were prepared using 0.2 μm filtered ultrapure (17 M-ohm) water. Each treatment was prepared in triplicate.

Forbes, Scott, and Aiken soils were brought to approximately -0.033 MPa moisture content four days

before phenanthrene was added. Three days before phenanthrene addition, nutrients were added in aqueous solution to the soils. Yolo soils were brought to −0.033 MPa moisture content and nutrients added three days before adding phenanthrene. The soils were spiked with phenanthrene three days after nutrient additions to allow fast nutrient conversion or sequestration processes to occur before phenanthrene degradation began. Water and/or nutrients were thoroughly mixed with the soils which were then stored at 25 °C in plastic bags in the dark. The evening before phenanthrene addition, all soils were passed through a 2 mm sieve and samples were taken for moisture analysis. Soils were returned to the 25 °C incubator until the following day.

For measurement of phenanthrene biodegradation rates, 20 g dry weight of each soil was added to a modified 0.473 L (1 pint) Mason jar. Phenanthrene mineralization rates were measured by trapping evolved $^{14}CO_2$ in base as described previously (Hoyle et al. 1995). Phenanthrene biodegradation experiments prepared in this manner on separate days have been found to produce results which were not significantly different as long as care was taken to duplicate experimental conditions and no shift occurred in soil nutrient or microbial content between experiments (Johnson 1997).

A radiochemical purity of 99% for the radiolabeled phenanthrene was confirmed by thin-layer chromatography. Each soil was spiked with [9–^{14}C] phenanthrene dissolved in 100 μL methylene chloride and thoroughly mixed by stirring. Adding phenanthrene to soil dissolved in either hexane or methylene chloride produced identical biodegradation kinetics in a preliminary study using Tinker soil (Johnson 1977). To each jar, 50 μg phenanthrene per kg dry soil was added except for two of the three jars containing Aiken soil plus nutrients. Due to evaporation of the spiking solvent, these jars received 60 μg phenanthrene per kg dry soil and 70 μg phenanthrene per kg dry soil. Microcosms were incubated at 25 °C in the dark.

Samples from the soils used in the biodegradation experiment were collected for nutrient analysis immediately before phenanthrene was added to the soils. These soil samples were stored at 4 °C overnight. A subsample was then taken for pH measurement. The soils were then air dried for 48 hours and submitted to DANR for nitrate, ammonium, and available phosphorus analyses.

Comparison of degradation rates

Statistical analyses were conducted to determine if phenanthrene mineralization rates in soils which received supplemental nitrogen and phosphorus differed significantly from rates in soils which did not. Phenanthrene degradation rate versus time data were used in this calculation. For experiments which measure carbon dioxide evolution as a function of time, fitting equations to cumulative plots is not statistically valid (Hess & Schmidt 1995). For each soil, mineralization rates between jars receiving nutrients and those microcosms which did not receive nutrients were compared at each sampling point. A two-tailed Student's *t*-Test was used to determine if mineralization rates for jars with, and without, nutrients differed at the 95% confidence level (Microsoft Excel for Windows 95, ver 7.0a, Microsoft Corporation).

Soil respiration

The total amount of carbon dioxide respired from each of the four soils was determined weekly for five weeks. Aiken, Forbes, and Scott soils were brought to approximately −0.0333 MPa moisture content and allowed to equilibrate at 25 °C for one week. Soils were passed through a 2 mm sieve and 25 g dry weight of each soil was placed in the same type of microcosms used to measure phenanthrene mineralization rates. Yolo soil, previously passed through a 2 mm sieve, was used at a moisture content of 16%, approximately 78% of the −0.0333 MPa moisture content. Yolo soil was removed from storage at 4 °C and 25 g dry weight soil placed in a microcosm immediately before beginning the experiment. Soils were spiked with 50 μg nonradioactive phenanthrene (Eastman Kodak, Rochester, NY) per kg dry soil using the procedure described for spiking soil with radioactive phenanthrene. Given the low concentration of phenanthrene added, the majority of the respiration was from biodegradation of organic matter rather than metabolism of phenanthrene. Four grams of a 0.45 M sodium hydroxide solution was added to a vial in each microcosm. Three microcosms without soil were prepared as described above to act as blanks.

After each week, the base was removed from the microcosms using a syringe and each base vial was rinsed with 4 mL of freshly boiled, then cooled, ultrapure water. The contents of the base trap and rinse water were combined with 5 mL 6 M $BaCl_2$ to precipitate carbonate ions trapped in the base. The entire solution

was titrated with 0.3 M HCl to a phenolphthalein endpoint. The amount of carbon dioxide respired by the soil was calculated as described by Anderson (1982). Total soil respiration measurements were performed in March 1994, while the biodegradation experiment described here were initiated in August 1995. Soils were stored sealed in plastic bags in the dark at 4 °C between experiments.

Results/Discussion

Nutrient additions did not result in faster phenanthrene biodegradation rates in any of the four soils tested; thus, the experimental data did not support our hypothesis that nutrient levels limited microbial activity in soils with slow phenanthrene degradation rates. Figures 1–4 present the cumulative amount of phenanthrene mineralized versus time and the phenanthrene mineralization rate versus time for the four soils. Phenanthrene mineralization rates are expressed as the percent of total phenanthrene radioactivity added to the soil which is evolved as radiolabeled carbon dioxide per gram dry soil per hour. The cumulative amount of phenanthrene mineralized is expressed as the percent of the total amount of phenanthrene radioactivity added to the soil which has been evolved as radiolabeled carbon dioxide. The cumulative amount of phenanthrene mineralized is expressed as the percent of the total amount of phenanthrene radioactivity added to the soil which has been evolved as radiolabeled carbon dioxide. Times at which degradation rates for nutrient amended soils are significantly different than rates for soils without added nutrients are indicated by asterisks on Figures 1–4. Adding nitrogen and phosphorus had no effect on phenanthrene mineralization rates in Yolo and Scott soils. Forbes soil which received nutrients showed significantly slower degradation rates in samples collected at 56, 136, and 216 hours after phenanthrene addition and in all samples collected later than 216 hours after phenanthrene addition. Aiken soil that received nutrients exhibited significantly depressed degradation rates in samples collected 169 hours after phenanthrene addition and in all samples collected later than 216 hours after phenanthrene addition.

Table 2 presents the results of the soil chemical analyses. The difference in nitrogen and phosphorus levels between soils which did, and did not, receive extra nutrients did not equal the amount of the nutrients added for any soil. Except in Scott soil, ammonium

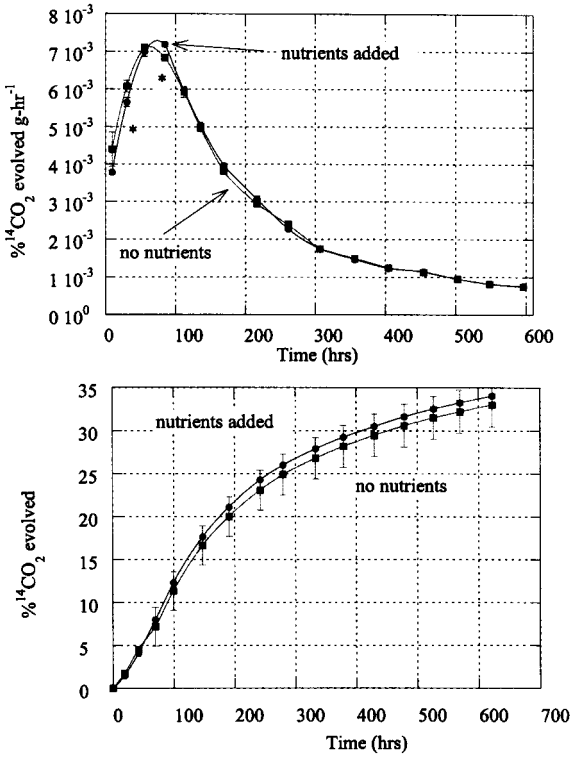

Figure 1. Phenanthrene mineralization rates in Yolo soil with, and without, additional nutrients. (a) Phenanthrene mineralization rate versus time. Asterisks indicate time points at which soils with, and without, added nutrients exhibit mineralization rates which are statistically different at the 95% significance level. (b) Cumulative amount of phenanthrene mineralized versus time.

levels either decreased or showed no change upon addition of ammonia and nitrate. Nitrate levels increased in all soils. Nitrate levels may have increased not only because nitrate was added to the soils, but also because nitrifying bacteria may have converted added or indigenous ammonia to nitrate (Paul & Clark 1989). Phosphorus levels were higher in Yolo and Scott soils than in Forbes and Aiken soils both before and after extra phosphorus was added. The increase in phosphorus levels was largest for Yolo soil, followed by Scott and Forbes soils. Aiken soil showed no increase in available phosphorus after phosphorus was added. For all soils, pH levels dropped by less that 0.1 pH units upon nutrient addition, indicating that the nutrients did not significantly affect soil acidity.

Yolo soil may have had sufficient levels of nitrogen and phosphorus to degrade the added phenanthrene; thus, addition of further nutrients did not significantly affect microbial processes. Phenanthrene degradation rates in Yolo soil, which had the highest initial nitro-

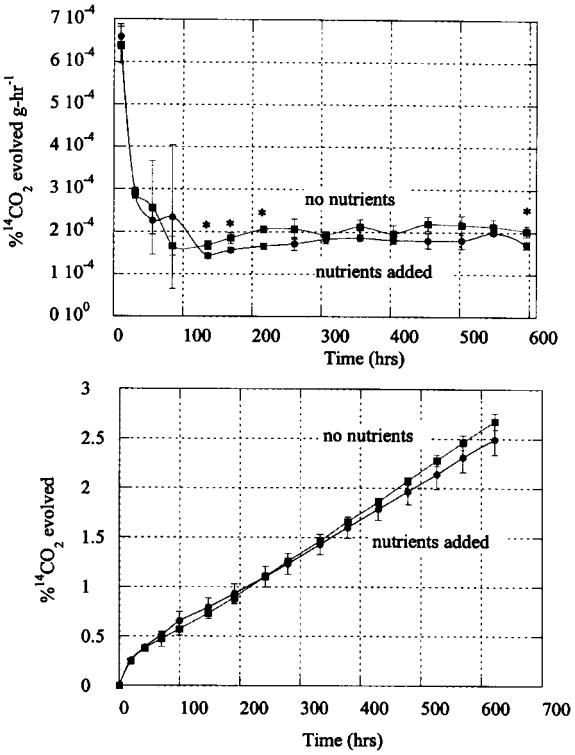

Figure 2. Phenanthrene mineralization rates in Scott soil with, and without, additional nutrients. (a) Phenanthrene mineralization rate versus time. Asterisks indicate time points at which soils with, and without, added nutrients exhibit mineralization rates which are statistically different at the 95% significance level. (b) Cumulative amount of phenanthrene mineralized versus time.

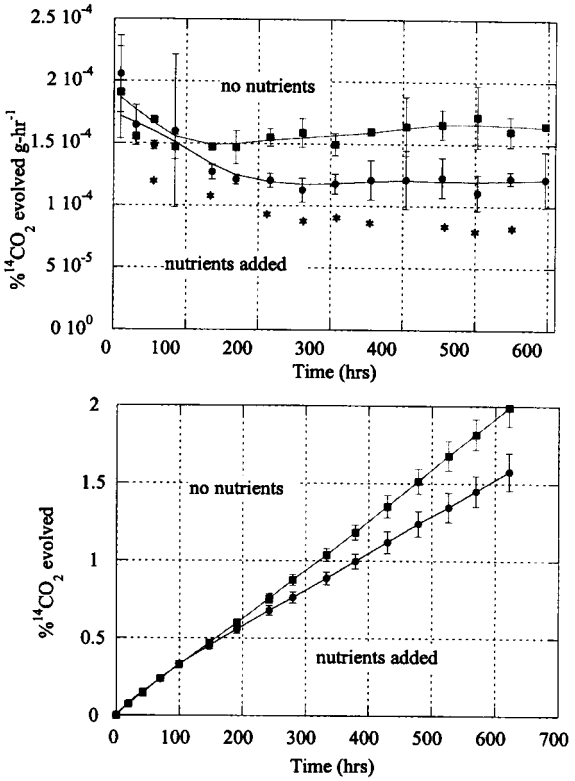

Figure 3. Phenanthrene mineralization rates in Forbes soil with, and without, additional nutrients. (a) Phenanthrene mineralization rate versus time. Asterisks indicate time points at which soils with, and without, added nutrients exhibit mineralization rates which are statistically different at the 95% significance level. (b) Cumulative amount of phenanthrene mineralized versus time.

gen and phosphorus concentrations and which showed the largest increase in phosphorus levels, did not react to added nutrients. Phenanthrene mineralization rates in Yolo soil do increase if higher phenanthrene concentrations are added to the soil, so mineralization rates are not controlled by some other factor such as pH or moisture content (Johnson 1997; Bossert & Bartha 1984). Bossert & Bartha (1984) proposed that degradation rates in contaminated soil are increased by nutrient additions only after the soil's own available nitrogen and phosphorus reserves are depleted.

The hypothesis that high indigenous nutrient levels result in no response to supplemental nutrients is less convincing when applied to Scott soil. Nutrient levels in Scott soil are not as high as in Yolo soil; although, phosphorus and nitrogen levels in unamended Scott soil are significantly higher than in Forbes soil. However, the increases in phosphorus and nitrate levels in Scott soil upon nutrient additions are not significantly greater than the increases observed in Forbes soil. For all soils, nitrogen and phosphorus levels measured in unamended soils would have been sufficient to degrade the added phenanthrene assuming a required carbon to nitrogen ration of 6.2 and a required carbon to phosphorus ratio of 31 (Paul & Clark 1989).

It is not clear why phenanthrene mineralization rates in Aiken and Forbes soils are depressed when nitrogen and phosphorus are added. One hypothesis is that nutrients, if added in large amounts, may inhibit microbes that are adapted to an oligotrophic soil environment. Morgan and Watkinson (1992) suggested that inhibition of oligotrophic microorganisms may have caused the 75–80% reduction in glucose mineralization rates for soils which received high levels of nutrients when compared to controls. However, the nutrient concentrations added in our study were almost one thousand times less than the concentrations reported to have no effect on biodegradation rates by Morgan and Watkinson (1992).

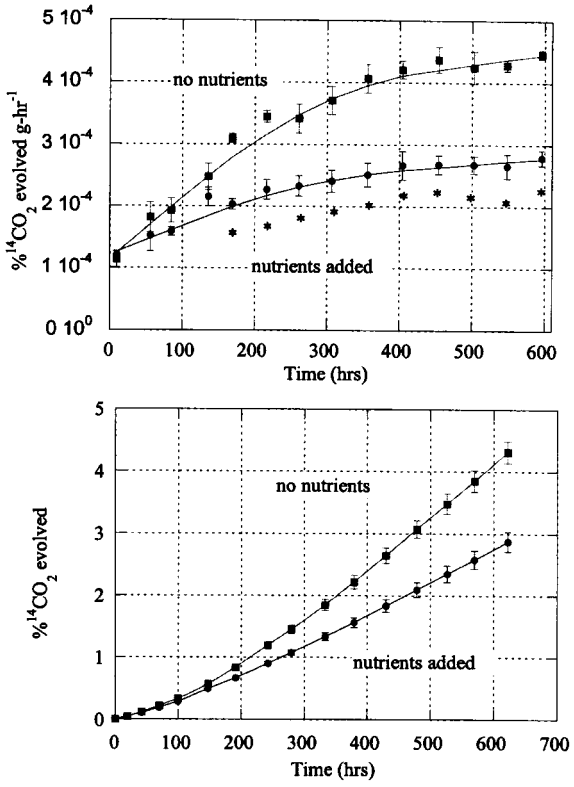

Figure 4. Phenanthrene mineralization rates in Aiken soil with, and without, additional nutrients. (a) Phenanthrene mineralization rate versus time. Asterisks indicate time points at which soils with, and without, added nutrients exhibit mineralization rates which are statistically different at the 95% significance level. (b) Cumulative amount of phenanthrene mineralized versus time.

Table 2. Nutrient levels in soils with, and without, added nitrogen and phosphorus[1]

Treatment	$N–NH_4$	$N–NO_3$	Phosphorus
Yolo + nutrients	2.3	68.4	58.8
Yolo	2.8	61.8	23.7
Difference	−0.6	6.6	35.1
Scott + nutrients	8.1	5.0	50.2
Scott	10.6	3.3	37.8
Difference	−2.5	1.8	12.5
Forbes + nutrients	5.1	7.7	14.8
Forbes	3.7	5.6	7.4
Difference	1.4	2.1	7.4
Aiken + nutrients	6.3	2.8	10.0
Aiken	6.3	0.8	10.0
Difference	0.0	2.0	0.0
Amount added to soil	2.35	2.35	47.0
Uncertainty in analysis	1.0	1.0	4.0

[1] All units are mg Kg dry soil^{-1}.

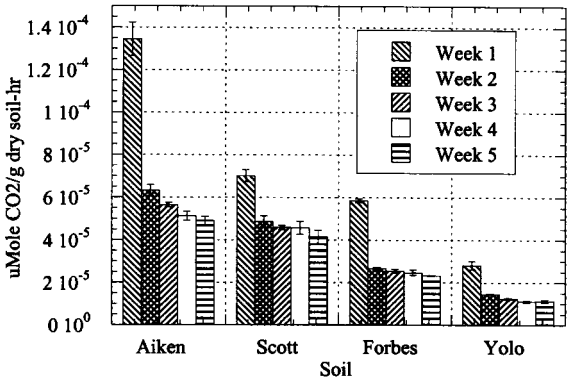

Figure 5. Total carbon dioxide evolved from Yolo, Scott, Forbes, and Aiken soils.

A second hypothesis to explain the observed repression of phenanthrene degradation rates is that addition of nutrients may stimulate degradation of carbon compounds other than phenanthrene. The utilization of carbon compounds other than phenanthrene could be due to nutrient induced shifts in the metabolism of phenanthrene degrading microorganisms (Fog 1988; Entry et al. 1993). It could also result from the growth of microorganisms which do not degrade phenanthrene, but which compete with the phenanthrene degraders for available nutrients (Swindoll 1988). In this experiment, however, it is unlikely the presence of other carbon compounds affected phenanthrene degradation rates in Aiken and Scott soils. Figure 5 presents total respiration levels for the four soils measured over a period of five weeks. For all soils, initial respiration levels are the highest during the first week. The increased respiration reflects the increase in bioavailable carbon compounds which results from disturbance of the soil by sieving and stirring (Rovira & Greacen 1957). Consistent repression of phenanthrene degradation rates in Aiken and Scott soils, however, was not observed until seven to nine days after phenanthrene was added. Repression was not consistently observed at the start of the experiment when the concentration of alternative carbon substrates would have been greatest in the soils.

If biodegradation rates are quantified by measuring evolved carbon dioxide, a repression in biodegradation rates upon nutrient addition might be explained by a

change in the ratio of assimilated to respired substrate carbon. When environmental carbon/nutrient ratios exceed those found in microbial biomass, the ratio of substrate carbon evolved as carbon dioxide versus substrate carbon incorporated into microbial biomass may increase (Bosatta & Berendse 1984; Amador & Jones 1993). When environmental carbon/nutrient ratios fall below those found in microbial biomass, carbon dioxide evolution may drop as a larger percentage of the metabolized carbon is incorporated into microbial biomass. It is not known whether the magnitude of such a shift in microbial metabolism would be able to account for the depression in phenanthrene mineralization rates observed in Aiken and Forbes soils.

Conclusions

Phenanthrene mineralization rates in four soils were either unaffected or slightly depressed by the addition of nitrogen and phosphorus. Nutrient analyses of the soils with, and without, additional nutrients showed large differences in the amount of nitrogen and phosphorus in the soils and in the increases observed in available phosphorus upon addition of nutrients. We hypothesize that phenanthrene mineralization rates in Yolo soil did not respond to added nitrogen and phosphorus because the levels of these nutrients in Yolo soil were relatively high. The reasons for depression of phenanthrene mineralization rates in Forbes and Aiken soils and the lack of response in Scott soils when nitrogen and phosphorus were added are uncertain. In order to understand the effect of nutrients on degradation rates, the bioavailability of added nutrients and of alternative carbon substrates as well as the effect of added nutrients on microbial metabolism and ecology must be considered.

Acknowledgments

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References

Alexander M (1994) Biodegradation and Bioremediation. Academic Press, New York

Amador JA & Jones RD (1993) Nutrient limitations on microbial respiration in peat soils with different total phosphorus content. Soil Biol. Biochem. 25: 793–801

Anderson JPE (1982) Soil respiration. In: Page AL (Ed) Methods of Soil Analysis: Part 2: Chemical and Microbiological Properties. 2nd edn. (pp 831–871). American Society of Agronomy-Soil Science Society of America, Madison, WI

Baker KH & Herson DS (1994) Bioremediation. McGraw-Hill, New York

Bohn HL, McNeal BL & O'Connor GA (1985) Soil Chemistry, 2nd edn. John Wiley & Sons, New York

Bosatta E & Berendse F (1984) Energy or nutrient regulations of decomposition: implications for the mineralization-immobilization response to perturbations. Soil Biol. Biochem. 16: 63–67

Bossert I & Bartha R (1984) The fate of petroleum in soil ecosystems. In: Atlas RM (Ed) Petroleum Microbiology (pp 441–473). Macmillan Publishing Co., New York

Brady NC (1990) The Nature and Properties of Soils, 10th edn. Macmillan Publishing Co., New York

Carlson RM (1978) Automated separation and conductimetric determination of ammonium and dissolved carbon dioxide. Anal. Chem. 50: 1528–1531

Entry JA, Mattson KG & Emmingham WH (1993) The influence of nitrogen on atrazine and 2,4-dichlorophenoxyacetic acid mineralization in grassland soils. Biol. Fertil. Soils. 16: 179–182

Fog K (1988) The effect of added nitrogen on the rate of decomposition of organic matter. Biol. Rev. Cambridge Philos. Soc. 63: 433–462

Hess TF & Schmidt SK (1995) Improved procedure for obtaining statistically valid parameter estimates from soil respiration data. Soil Biol. Biochem. 27: 1–7

Hoyle BL, Scow KM, Fogg GE & Darby JL (1995) Effect of carbon:nitrogen ratio on kinetics of phenol biodegradation by *Acinetobacter johnsonii* in saturated sand. Biodegradation 6: 283–293

Jobson A, McLaughlin M, Cook FD & Westlake DWS (1974) Effect of amendments on the microbial utilization of oil applied to soil. Appl. Microbiol. 27: 166–171

Johnson CR (1997) Phenanthrene biodegradation kinetics in soil. Ph.D. Thesis. University of California, Davis

Keeney DR & Nelson DW (1982) Nitrogen-inorganic forms. In: Page AL (Ed) Methods of Soil Analysis: Part 2: Chemical and Microbiological Properties. 2nd edn. (pp 643–698). American Society of Agronomy-Soil Science Society of America, Madison, WI

Klute A (1986) Water retention: laboratory methods. In: Klute A (Ed) Methods of Soil Analysis: Part 1. Physical and Mineralogical Methods. 2nd edn. (pp 635–662). American Society of Agronomy-Soil Science Society of America, Madison, WI

Manilal VB & Alexander M (1991) Factors affecting the microbial degradation of phenanthrene in soil. Appl. Microbiol. Biotechnol. 35: 401–405

Morgan P & Watkinson RJ (1992) Factors limiting the supply and efficiency of nutrient and oxygen supplements for the in situ

biotreatment of contaminated soil and groundwater. Wat. Res. 26: 73–78

National Forest Service (1986) Draft Soil Resource Inventory for the Tahoe National Forest

Nelson DW & Sommers LW (1982) Total carbon, organic carbon, and organic matter. In: Page AL (Ed) Methods of Soil Analysis: Part 2: Chemical and Microbiological Properties. 2nd edn. (pp 539–579). American Society of Agronomy-Soil Science Society of America, Madison, WI

Olsen SR, Cole CV, Watanabe FS & Dean LA (1954) Estimation of available phosphorus in soils by extraction with sodium bicarbonate. US Dept. of Agr. Cir. 939: 1–19

Paul EA & Clark PE (1989) Soil Microbiology and Biochemistry. Academic Press, New York

Rovira AD & Greacen EL (1957) The effect of aggregate disruption on the activity of microorganisms in the soil. Aus. J. Agric. Res. 8: 659–673

Swindoll, CM, Aelion CM & Pfaender, FK (1988) Influence of inorganic and organic nutrients on aerobic biodegradation and on the adaptation response of subsurface microbial communities. Appl. Environ. Microbiol. 54: 212–217

Thorton-Manning, JR, Jones, DD, & Federle, TW (1987) Effects of experimental manipulation of environmental factors on phenol mineralization in soil. Environ. Toxicol. Chem. 6: 615–621

US Dept. of Agriculture (1972) Soil Survey of Yolo County. California. US Dept. of Agriculture, Soil Conservation Service. Washington, DC