Nitrogen dynamics in soils with different hydrocarbon contents planted to barley and field pea

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Xu, J. G. and Johnson, R. L. 1997. **Nitrogen dynamics in soils with different hydrocarbon contents planted to barley and field pea**. Can. J. Soil Sci. **77**: 453–458. Nitrogen dynamics and cycling are important in plant-soil ecosystems, and they may differ between hydrocarbon-contaminated and uncontaminated soils. The objective of this experiment was to study the effects of petroleum hydrocarbons and remediation methods on nitrogen dynamics and cycling in plant-soil ecosystems. The experiment involved two plant species (barley and field pea) grown in soils at four different hydrocarbon levels (0, 5, 25 and 55 g kg^{-1}). Hydrocarbon contamination significantly reduced N uptake by plants, but increased N accumulation in soil microbial biomass. It widened the C:N ratio in soil and led to more available N being immobilized by soil microorganisms, which reduced available N for plant uptake. Urease activity increased with hydrocarbon content in soil due to the increase of microbial biomass and activity.

Key words: Nitrogen dynamics, hydrocarbon contamination, microbial activity, remediation, Black Chernozem

Xu, J. G. et Johnson, R. L. 1997. **Cinétique de l'azote dans des sols de différentes teneurs en hydrocarbures sous culture d'orge et de pois sec**. Can. J. Soil Sci. **77**: 453–458. La dynamique et le renouvellement de l'azote, deux aspects importants des écosystèmes plante-sol peuvent varier sous l'effet de la contamination des sols par les hydrocarbures. Notre expérience portait sur les effets de la présence des hydrocarbures de pétrole et des méthodes d'assainissement sur ces deux mécanismes. L'expérience comportait deux espèces végétales l'orge et le pois sec, cultivées en sols contenant quatre niveaux d'hydrocarbures : 0, 5, 25 et 55 g kg^{-1}. La contamination réduisait de façon significative l'absorption de N par les plantes, mais elle accroissait l'accumulation de N dans la biomasse microbienne du sol. Le rapport C/N dans le sol était plus large et une plus grande partie du N assimilable se retrouvait dans les micro-organismes telluriques aux dépens des cultures. La présence des hydrocarbures accroissait l'activité de l'uréase, en raison de l'augmentation de la biomasse et de l'activité microbiennes.

Mots clés: Dynamique de l'azote, contamination par les hydrocarbures, activité microbienne, assainissement, chernozem noir

After the mid-1980s, hydrocarbon-contamination became a critical environmental issue in the world due to its adverse environmental and health effects. Increased attention has been given to the study and development of techniques for clean up of this contamination. Meanwhile, there has been an increased interest in use of biological assays as diagnostic and monitoring approaches to evaluating remediation and reclamation, since the final goal of a reclamation program is the establishment of a self-sustaining vegetative cover that requires minimal or no maintenance (Klein et al. 1985). Although soil chemical and physical properties are important in determining initial plant establishment and growth, biological factors are useful monitors because they are essential for successful maintenance of soil fertility, productivity and sustainability of a plant-soil ecosystem.

Nitrogen dynamics and cycling play important roles in bioremediation and reclamation of hydrocarbon-contaminated soils, because N is an essential element for microbial activity and plant growth. Nitrogen dynamics and cycling in hydrocarbon-contaminated soils may differ from those in uncontaminated agricultural soils (Xu et al. 1995) because hydrocarbons can change soil physical, chemical and biological properties. Although there has been some research done, the information and findings to date are limited and controversial (Haines et al. 1981; Griffiths et al. 1982; Bonin et al. 1990; Xu et al. 1995).

The oil and gas industry in Alberta indulge in a wide range of activities that generate many kinds of organic wastes. The most abundant is crude oil in soil, which normally results from oil spills. Therefore, the study and prediction of the effects of hydrocarbons, and also the effects of the different remediation methods on plant growth and biological activities in hydrocarbon-contaminated soils are important for successful remediation and reclamation. The objective of this experiment was to study the effects of hydrocarbons and remediation methods on N dynamics and cycling in plant-soil systems. In the experiment, the different N forms in plant-soil systems containing uncontaminated soil, crude oil-contaminated soil and two remediated soils planted to barley (*Hordeum vulgare* L.) and field pea (*Pisum arvense* L.) were examined.

MATERIALS AND METHODS

Soil Materials

The soil at the oil-spill site was a Black Chernozem (Typic Cryoboroll) near Erskine (52°20′N, 112°53′W), Alberta, Canada. The oil spill occurred as a result of a pipeline break in June of 1990. At the spill site, the contaminated surface soil (0–10 cm) was excavated, homogenized and stockpiled. Material 1 (oil-contaminated soil) used in the experiment was sampled at six locations from the stockpile, mixed and homogenized. It contained 55 g hydrocarbon kg^{-1} soil.

Table 1. Selected properties of the soils used in the experiment

Soils	TPH[z] (g kg^{-1})	SOM[y] (g kg^{-1})	Total N (g kg^{-1})	NH_4^+ (mg kg^{-1})	NO_3^- (mg kg^{-1})	Clay (g kg^{-1})	pH
Oil-contaminated soil	55	42	3.5	2.4	0.2	302	6.7
Bioremediated soil	25	43	3.4	1.5	0.3	309	6.8
Solvent-extracted soil	5	40	3.3	1.7	0	301	6.6
Uncontaminated soil	0	41	3.6	5.6	13.2	305	6.8

[z]TPH represents total petroleum hydrocarbons.
[y]SOM represents soil organic matter.

Material 2 (bioremediated soil) was the oil-contaminated soil treated in a bio-reactor (Alberta Environmental Centre 1993) for 15 mo, which led to a reduction in its oil content to 25 g hydrocarbon kg^{-1} soil. Material 3 (solvent-extracted soil) was the oil-contaminated soil treated by the solvent extraction soil remediation procedure (Meadus et al. 1993). This soil material contained oil and solvent (naphtha) residues at 5 g kg^{-1} soil. Material 4 (uncontaminated soil) was Ap horizon (0–10 cm) collected adjacent to the spill site. Selected properties of the soils in the experiment are listed in Table 1.

Plant Growth and Sampling

The four soil materials described above were sieved through a 2-mm screen and packed in plastic cylinders (25 cm in height and 20 cm in diameter) to a bulk density of 1.0 Mg m^{-3}. Three seeds of similar size for barley or field pea were seeded in each cylinder. Urea (300 mg N kg^{-1} soil), phosphate (65 mg P kg^{-1} soil) and potassium sulfate (125 mg K kg^{-1} soil) were placed 3 cm below the seeds at the time of seeding. The plants were grown at 22°C in a growth chamber under artificial lighting (300 E s^{-1} m^{-2}) with a 16-h light and 8-h dark cycle. During the growing period, the relative humidity of the air in the growth chamber was maintained at about 60% and the soil moisture was maintained at about 60% of soil water holding capacity (approximately –30 kPa) by watering each day.

Four sampling times based on growth stages were set up during the growing period. They were days 25, 40, 60 and 80 after seeding, which corresponded to the tillering, stem extension, heading and ripening stages of barley. Field pea was sampled at the same time. On each sampling day, the shoots were excised at the surface of the soils, and the roots were separated from the soil by hand and washed. The plant shoots and roots were dried at 70°C for 24 h and then weighed and ground for determination of N. The soils were saved for chemical and biological analysis as described below. The experiment contained two plant species, four kinds of soils, four sampling dates with three replicates in total of 96 experimental units (cylinders).

Chemical and Biological Assays

The N concentration in plant shoots and roots was determined by digesting the ground plant samples using a micro-Kjeldahl procedure (Bremner 1965), and analyzed on a Technicon Autoanalyzer (Technicon Instruments Corporation, New York). The shoot N (total N accumulated in shoots) and root N (total N accumulated in roots) were calculated by multiplying the N concentration with the corresponding biomass of the shoots or roots, respectively.

The hydrocarbon content in soils was analyzed by gas chromatography (HP 5890) after extracting the soil samples with dichloromethane (US Environmental Protection Agency 1981). Organic carbon was determined by the Walkley-Black wet oxidation method (Allison 1965) after the removal of hydrocarbons using the dichloromethane extraction. Total N was determined by the micro-Kjeldahl procedure (Bremner 1965). Soil pH was determined in water extract (soil : solution = 1:1) by the method of Peech (1965), and particle size was measured by hydrometer method (Day 1965).

Mineral N (NH_4-N, NO_2-N and NO_3-N) was determined by extracting soil samples with 2 M KCl solution (soil : solution = 1:5) and analyzed on the Technicon Autoanalyzer. Soil microbial N was measured on 25-g soil samples using the fumigation-extraction technique (Vance et al. 1987; Jenkinson 1988) and calculated by multiplying the flush of mineral N between the fumigated and unfumigated soil samples with a k_n factor of 2.22 (Brookes et al. 1985). Soil microbial C was measured on 25-g oven-dry equivalent soil samples by the fumigation-incubation technique (Jenkinson and Powlson 1976) and calculated by dividing the flush of CO_2-C by a *kc* factor of 0.411 (Anderson and Domsch 1978).

Urease activity was measured following the procedure of Douglas and Bremner (1970). A 20-g soil sample obtained from each cylinder was dispensed into a polyethylene bottle and treated with 4 mL of urea solution (0.5 mmol urea g^{-1} soil). Soil samples were incubated at 37°C for 6 h with soil moisture contents at 60% of soil water-holding capacity. Thereafter, hydrolysis of urea was inactivated by adding 100 mL 1 M KCl-phenyl mercuric acetate (a urease inhibitor) solution. The suspension was shaken for 1 h and filtered through Whatman no. 43 filter paper. A suitable aliquot (5 mL) from the filtrate was analyzed for urea. The amount of the hydrolyzed urea was calculated by the difference between the urea added initially and that left in the solution after incubation.

Statistics

The variance of the data was analyzed using the GLM procedure of the SAS package (SAS Institute, Inc. 1987). Multiple comparisons were conducted using the Student-Newman-Keuls (SNK) procedure. The linear correlation and regression were conducted using the REG procedure.

RESULTS

Total N in Plants

For barley, the shoot N in the uncontaminated and bioremediated soils was greater than that in the solvent-extracted

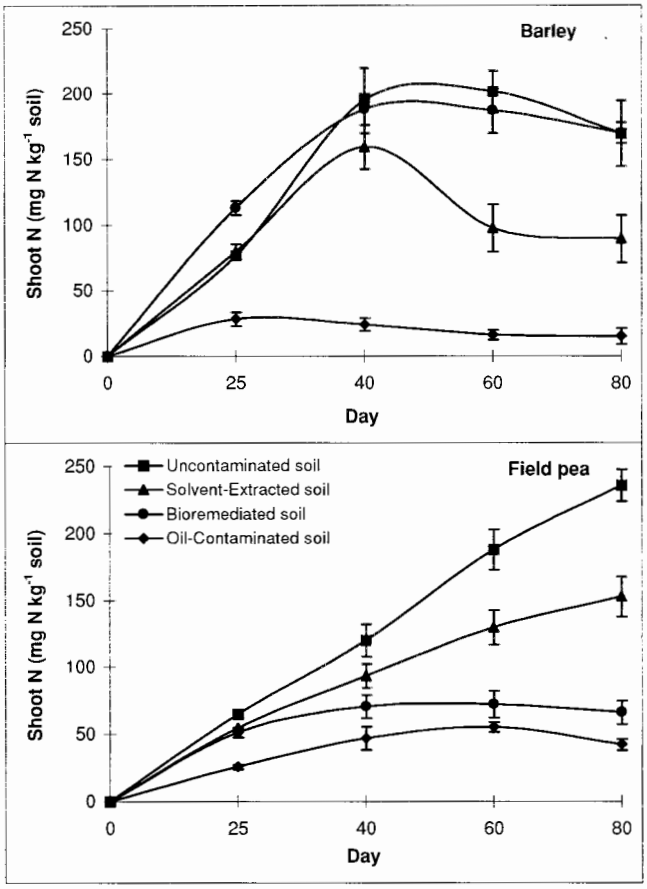

Fig. 1. Shoot N during the growing period for barley and field pea grown in four soils with different hydrocarbon contents. Error bar represents the standard deviation.

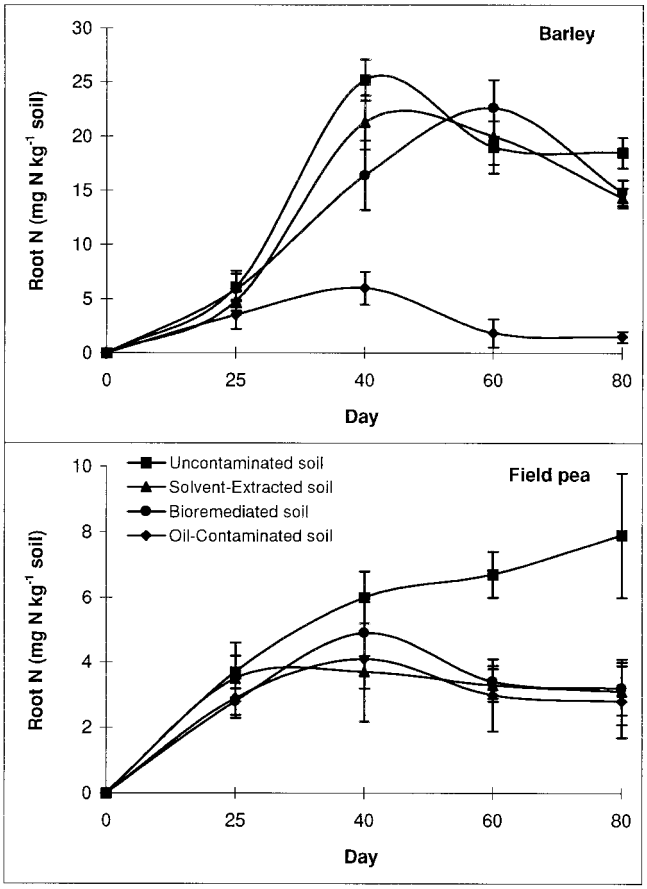

Fig. 2. Root N during the growing period for barley and field pea grown in four soils with different hydrocarbon contents. Error bar represents the standard deviation.

soil, which was in turn greater than that in the oil-contaminated soil (Fig. 1). There was no significant difference between the uncontaminated and bioremediated soils. After day 40, the shoot N of barley in the uncontaminated and bioremediated soils was stable or decreased slowly, but it decreased sharply in the solvent-extracted soil. The shoot N of barley in the oil-contaminated soil was the lowest over the growing period and it decreased slowly after day 25.

The shoot N of field pea, ranked from high to low, was in the order of uncontaminated, solvent-extracted, bioremediated and oil contaminated soils, and this pattern was different from that of barley. The shoot N of field pea continually increased over time in the uncontaminated and solvent-extracted soils, but was stable after day 25 in the bioremediated and oil-contaminated soils.

The root N of barley was greater than that of field pea (Fig. 2). For barley, there was no significant difference in root N among the uncontaminated, solvent-extracted and bioremediated soils, but root N in these three soils was greater than that in the oil-contaminated soil. For field pea, the root N in the uncontaminated soil was greater than those in the solvent-extracted, bioremediated and oil-contaminated soils. The difference among these three hydrocarbon contaminated soils was not significant.

Microbial N in Soils

Overall, the microbial N in soils under field pea was greater than that under barley (Table 2). Under barley, the microbial N was the highest in the oil-contaminated soil but lowest in the uncontaminated and solvent-extracted soils over the growing period. There was no significant difference between the uncontaminated and solvent-extracted soils.

Similar to that under barley, the microbial N in the oil-contaminated soil under field pea was the highest over the growing period, but there was no significant difference in microbial N among the uncontaminated, solvent-extracted and bioremediated soils.

The ratio of microbial C:microbial N was the highest in the bioremediated soil but the lowest in the uncontaminated soil. This ratio in the oil-contaminated soil was higher than that in the solvent-extracted soil. The variation of the microbial N was similar to that of the microbial C, and the correlation between the microbial N and the total C (organic matter-C + hydrocarbon-C) in soil was highly significant ($r = 0.94$, $n = 8$, $P \leq 0.01$).

Mineral N in Soils

The mineral N in soil under field pea was greater than that under barley. Under barley, the mineral N decreased expo-

Table 2. Microbial N, microbial C to microbial N ratios and mineral N in the four soils planted to barley and field pea

Sampling date	Soil materials	Microbial N		Microbial C/microbial N		Mineral N	
		Barley	Field pea	Barley	Field pea	Barley	Field pea
		(mg N kg^{-1} soil)				(mg N kg^{-1} soil)	
Day 25	Uncontaminated soil	10.6 ± 1.0[z]	17.0 ± 2.1	15.6 ± 1.2	7.5 ± 0.2	122 ± 5.6	198 ± 5.8
	Solvent-extracted soil	10.8 ± 0.2	13.4 ± 2.5	18.4 ± 0.8	9.2 ± 0.8	56 ± 3.4	77 ± 3.4
	Bioremediated soil	16.7 ± 1.0	24.7 ± 3.1	82.9 ± 2.4	36.8 ± 2.1	160 ± 7.8	139 ± 7.8
	Oil-contaminated soil	32.1 ± 1.9	32.5 ± 2.9	31.9 ± 2.1	16.9 ± 1.3	2 ± 1.1	4 ± 2.6
Day 40	Uncontaminated soil	6.3 ± 0.6	6.2 ± 0.8	14.0 ± 0.5	9.2 ± 0.4	19 ± 1.2	139 ± 6.9
	Solvent-extracted soil	7.0 ± 1.5	10.5 ± 0.5	21.3 ± 0.8	5.2 ± 0.2	12 ± 1.6	42 ± 4.2
	Bioremediated soil	10.0 ± 0.7	14.1 ± 1.9	69.6 ± 2.2	60.5 ± 2.7	57 ± 3.8	166 ± 9.4
	Oil-contaminated soil	21.6 ± 0.4	32.0 ± 0.2	63.6 ± 2.3	28.6 ± 1.4	2 ± 1.0	1 ± 1.1
Day 60	Uncontaminated soil	3.9 ± 1.9	13.3 ± 1.4	15.4 ± 1.0	3.1 ± 0.5	7 ± 0.4	74 ± 3.4
	Solvent-extracted soil	2.5 ± 0.1	9.1 ± 0.6	22.0 ± 1.3	6.2 ± 0.8	5 ± 0.8	46 ± 2.3
	Bioremediated soil	8.6 ± 1.0	11.7 ± 2.4	84.2 ± 2.5	60.0 ± 4.6	11 ± 1.2	164 ± 6.5
	Oil-contaminated soil	24.4 ± 4.2	24.7 ± 2.5	12.4 ± 0.4	23.6 ± 2.4	5 ± 2.4	2 ± 0.8
Day 80	Uncontaminated soil	13.1 ± 0.6	10.0 ± 1.3	8.2 ± 0.2	11.8 ± 2.4	1 ± 0.2	13 ± 0.8
	Solvent-extracted soil	6.4 ± 1.0	4.1 ± 0.7	22.0 ± 1.2	26.1 ± 2.5	1 ± 0.8	18 ± 0.7
	Bioremediated soil	13.9 ± 2.2	8.5 ± 1.2	42.9 ± 1.6	93.6 ± 6.8	1 ± 0.4	99 ± 3.8
	Oil-contaminated soil	25.3 ± 0.5	26.5 ± 1.3	19.9 ± 0.9	40.8 ± 3.4	1 ± 0.1	1 ± 0.4

Summary of ANOVA

Source of variance	DF	Microbial N	Microbial C/microbial N	Mineral N
Plant (P)	1	**[y]	NS	***
Soil (S)	3	***	***	***
Date (D)	3	*	*	***
P × S	3	NS	NS	*
P × D	3	NS	*	**
S × D	9	*	NS	*
P × S × D	9	*	NS	**

[z]The numbers in the table are represented by mean ± standard deviation (SD).
[y]The difference between means is significant at: * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$; NS, not significant.

nentially over time in the four soil materials used in the experiment (Table 2). The mineral N in the bioremediated soil was greater than that in the uncontaminated soil which, in turn, was greater than that in the solvent-extracted soil. The mineral N in the oil-contaminated soil was the lowest during the growing period.

Under field pea, the mineral N in the uncontaminated and solvent-extracted soils decreased with time. However, the mineral N in the bioremediated soils was the highest during the growing period and decreased only after day 60. The mineral N in the oil-contaminated soil was also the lowest over the growing period.

Urease Activity

Overall, urease activity in soil under barley was significantly higher than that under field pea (Fig. 3). Under barley, the urease activity beyond day 60 was higher than that before day 60 in the uncontaminated, solvent-extracted and bioremediated soils. There was no significant difference in urease activity in the oil-contaminated soil among the four plant growth stages. The urease activity in the oil-contaminated soil was significantly higher than that in the uncontaminated, solvent-extracted and bioremediated soils on day 25 and day 40, but there was no significant difference among the four soil materials on day 60 and day 80.

Under field pea, the urease activity in the uncontaminated soil was greater than that in the solvent-extracted soil which, in turn, was greater than that in the bioremediated soil over the growing period. The urease activity in the oil-contaminated soil was greater than that in the uncontaminated soil on day 25 and day 40. There was no significant difference between day 60 and day 80.

DISCUSSION

The shoot N and root N decreased as the hydrocarbon content in soil increased, suggesting that hydrocarbon contamination reduced N uptake by plants. This partly supported the hypothesis that hydrocarbons reduce plant growth by coating plant roots thus influencing water and nutrient uptake (Baker 1970). The lower shoot N and root N in the hydrocarbon-contaminated soils may be also due to the competition for available nutrients between the plants and microorganisms in soils (Amadi et al. 1992). In this experiment, the microbial N increased but the mineral N decreased with an increase in hydrocarbon content. This indicates that hydrocarbon contamination caused available N to be immobilized into microbial biomass, thus reducing the availability of N for plant uptake, because once immobilized, N is remineralized slowly (Reeder and Berg 1977).

The severity of the effects of hydrocarbons on N uptake and plant growth varies with the plant species involved. In this experiment, the two plant species had different responses to the hydrocarbon content in soil. For barley, there was no significant difference in shoot N between the uncontaminated and solvent-extracted soils and there was no significant difference in root N among the uncontaminated,

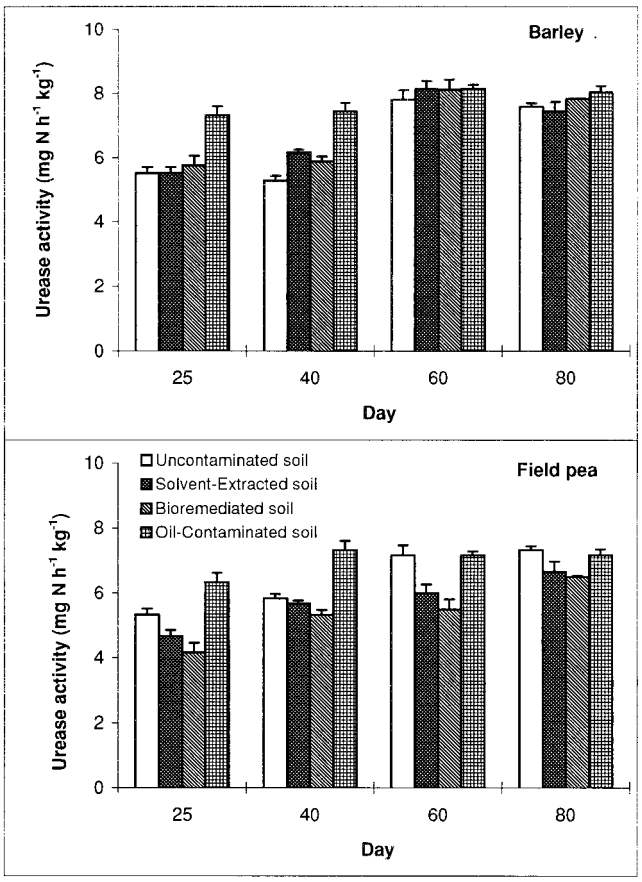

Fig. 3. Urease activity in the four soils with different hydrocarbon contents during the growing period under barley and field pea. Error bar represents standard deviation.

solvent-extracted and bioremediated soils. This indicates that N uptake by barley was not sensitive to the hydrocarbon content in soil within a certain range (0 to 25 g kg^{-1}). Beyond that range, there was a significant reduction in N uptake by barley. Field pea had a different response from barley to hydrocarbon content in soil. The shoot N decreased with an increase in hydrocarbon content in soil up to 55 g kg^{-1} in the experiment, but there was no significant difference in root N among the solvent-extracted, bioremediated and oil-contaminated soils, indicating root N of field pea was not sensitive to an increase in hydrocarbon content between 5 and 55 g kg^{-1}. The roots of field pea could survive in relatively higher concentrated hydrocarbon-contaminated soils than barley roots. This is good for reclamation of hydrocarbon-contaminated soils since leguminous species of plants can be used to fix atmospheric N and establish a vegetative cover rapidly.

An enlarged microbial N pool in the oil-contaminated soil sequestered a large amount of N as a result of hydrocarbon contamination. The sequestration of N in microbial biomass may prevent losses of mineral N to the atmosphere and groundwater (Wood et al. 1991). This sequestered N can also be released slowly for recycling by microorganisms during the process of hydrocarbon degradation, or may be used for plant uptake, if applicable. This hypothesis was supported by our other experimental results (unpublished data). When 800 mg N kg^{-1} was initially added to the oil-contaminated soil, there was no response (CO_2 evolution) in the degradation rate of hydrocarbons to any further addition of N fertilizer during the bioremediation process, even when the KCl extractable N (NH_4-N and NO_3-N) was close to zero. This suggests the N pool was being internally recycled by soil microorganisms during the bioremediation process. This internal N cycling is not normally found in uncontaminated agricultural soils.

The ratio of microbial C:microbial N is an indicator of the composition of microbial community. The microorganisms in the hydrocarbon-contaminated soils may be different from those in the uncontaminated soils due to changes in soil environment caused by the hydrocarbon contamination, especially the difference in C substrate for microorganisms (Pfaender and Buckley 1984) since certain microorganisms in soil are substrate specific. In general, this ratio was higher in hydrocarbon-contaminated soils than that in uncontaminated soils. It may decreased with a decrease in hydrocarbon content in soil. The data we obtained from the uncontaminated soil under both plants in the experiment were similar to the values obtained from normal agricultural soils reported by Franzluebbers et al. (1995). The wider ratios in the hydrocarbon-contaminated soils were anticipated, but the highest ratio in the bioremediated soil was not expected. Further study is needed.

Plant species also had a significant effect on the mineral N content in soil. The mineral N in soil under field pea was greater than that under barley. This could be because more mineral N was taken up by barley than by field pea, since the shoot N and root N of barley were greater than those of field pea over the growing period. The differences in mineral N in the four soils at different sampling dates under two plant species indicated that the interaction among the hydrocarbon content, plant growth stages and plant species was significant (Table 2).

Biochemical reactions in soil, including transformation and cycling of plant nutrients, depend on the activities of different enzymes (Kanazawa and Filip 1986; Xu et al. 1993). Urease activity deserves special attention since it plays a key role in transforming urea into ammonia for plant uptake (Gianfreda et al. 1994). In this experiment, the urease activity was higher under barley than under field pea. This may be because of the bigger root mass of barley, since plant roots, as well as microorganisms produce urease (Tabatabai and Bremner 1972). The urease activity in the oil-contaminated soil was higher than those in the other three soils, especially at the first two growth stages. This may be due to a larger microbial biomass (represented by microbial N) in the oil-contaminated soil and the influence of plant roots was relatively smaller at the early growth stages. Higher urease activity could hydrolyze urea into ammonium carbonate faster (Bremner and Mulvaney 1978; Mulvaney and Bremner 1981), however, the high immobilization rate in the hydrocarbon-contaminated soils sequestered the mineral N quickly. Therefore, we did not find higher mineral N in the Oil-contaminated soil, even at

the early growth stages. The urease activity in the solvent-extracted and bioremediated soils was lower than that in the Oil-contaminated soil, indicating that both remediation methods reduced urea hydrolysis rate. This effect is greater under field pea than under barley.

CONCLUSIONS

Hydrocarbon contamination widens the C : N ratios in soil, leads to more available N to be immobilized by soil microorganisms, and reduces N uptake by plants. The difference in ratios of microbial C : microbial N among the four soils implies that the species of microorganisms may be different in the four soils because of the hydrocarbon contamination and remediation processes. Both remediation methods reducing hydrocarbon content in soil had significant effects on N dynamics and cycling. Urease activity is the highest in the oil-contaminated soil because of the highest microbial biomass, however, the higher urease activity under barley is due to its bigger root mass. The N dynamics, transformation and cycling in soil is influenced by hydrocarbons. The interactions between hydrocarbon content and plant species are significant.

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