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The Interactive Effects of Crude Oil and N forms on C Mineralization and Microbial Biomass of a Clay Soil

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Introduction

The human ever-increasing industrial activities in the last few decades have resulted in severe soil pollution. Soil contamination with petroleum in oil-producing countries is a serious problem in natural and agricultural environments. In oil fields of Iran, large areas are subjected to intense pollution. This can reduce the quality of physical, chemical and biological properties of the soil, and decrease the soil capacity to support plant growth. Soil contamination with crude oil may severely change diverse soil properties, which might then disturb microbial communities and activities. However, both inhibition and stimulation of microbial activities have been reported in petroleum hydrocarbon-contaminated soils. It seems that soil properties and the type of petroleum hydrocarbons play an important role in affecting microbial activities and biomass in oil-polluted soils. On the other hand, the availability or addition of nutrients to petroleum hydrocarbon-contaminated soils is very important. In spite of contradictory results, the application of nutrients may cause an increase in microbial activity and biomass. Due to considerable differences in chemical properties of pollutant hydrocarbons and soil conditions, the impact of various hydrocarbons on microbial activity and biomass is not identical. It is also true that contaminants may well serve as organic carbon sources for soil microbes, and an enrichment of oil-degrading microbial populations has been observed in most contaminated ecosystems.

Assessment of microbial parameters such as C mineralization and microbial biomass provide information about resistant microorganism's activity and existence as well as the influence of intensity and duration of hydrocarbon pollution on soil metabolic activities. These assessments may help in indicating oil pollution effects on soil health.

The main objective of the present study was to explore the impact of various forms of N on C mineralization rate and microbial biomass in a clay soil spiked with three levels of crude oil.

Materials and methods

The experiment was conducted as factorial (3×4) arranged in a completely randomized design with three replicates under laboratory conditions during 126 days. Three levels of crude oil (0, 5% and 10%) and four N forms (NH₄, NO₃, NH₄+NO₃ and control) were added to a clay soil. The CO₂ released from microbial respiration (C mineralization rate) was determined by titration of NaOH trap at weekly intervals and microbial biomass carbon (MBC) was determined using the fumigation-incubation procedure at monthly intervals.

Results and discussion

Total C mineralization or microbial respiration (CO₂-C)

There was no significant difference in C mineralization rate among untreated and treated soils with N fertilizer ($P>0.05$). The effect of N on C mineralization may depend on its level and availability in soil. Similar results have been reported in the literature.

Nevertheless, C limitation in the studied soil led to the stimulation of microbial activities with increasing the level of crude oil during 126 days incubation.

Soils treated with 5 and 10 % petroleum had greater C mineralization than the control soils (Table 1).

Table 1: The interactive effects of crude oil levels and N forms on CO₂-C (mgkg⁻¹) in a clay soil during 126 days incubation. Each value represents mean (n=3). Standard errors of means are included in parenthesis

LSD _{0.05}	mean	N form				Oil level (%)
		NH ₄ +NO ₃	NO ₃	NH ₄	control	
CO ₂ -C (LSD _{0.05} =247)						
	1201(10.3)C	1147(5.9)C ^a	1243(10.2)B ^a	1207(18.8)B ^a	1208(6.4)B ^a	0
	4718(101)B	4651(24.8)B ^b	4626(9.8)A ^b	4970(179)A ^a	4626(190)A ^b	5
	4862(59)A	4945(28.3)A ^a	4789(66.7)A ^a	4864(48.5)A ^a	4849(92.5)A ^a	10
142	-	3581(19.7)a	3553(29)a	3680(82)a	3561(96.5)a	mean
	123					LSD _{0.05}

The comparison of treatments is shown with the capital letters in the column and lower case letters in the row. Values sharing the same letters are not significantly different (LSD test, $P \leq 0.05$)

However, the interaction of crude oil and N form on microbial respiration was not significant ($P > 0.05$). McGill and Nyborg (1975) reported that N application, especially NO₃-N, enhanced C mineralization rate.

Percentage of C mineralized to total soil organic C (CO₂-C/C_{org})

The effects of N form, oil levels and their interaction on CO₂-C/C_{org} ratio were highly significant. NH₄+NO₃ treatments reduced this ratio compared to the other two forms. CO₂-C/C_{org} decreased with an increase in crude oil level because of the high C:N ratio of crude oil. The addition of a large amount of organic C to soil resulted in decrease of CO₂-C/C_{org} despite an increase in CO₂-C production from 0 to 10%. The greatest enhancement (31%) in C mineralization/organic C occurred in NO₃ treatments without crude oil and the least (7.6%) was observed in all N treatment plus 10% crude oil.

Microbial biomass carbon (MBC)

The effects of N form, oil levels and their interactions on MBC are highly significant after 60 and 90 days incubation ($P < 0.001$). The medium level of crude oil (5%) enhanced MBC more than the other levels in soil without N fertilizer. This indicates that C is needed for MBC enhancement. If N, as an essential nutrient exists in soil, microorganisms would increase MBC at high level of crude oil (10%) and especially by using NH₄-N. Simply, lower levels of crude oil in soils treated with any form of N had no effects on MBC. So, in addition to high C contents, N should be abundant and available too. The current results are in agreement with those reported by other workers who found N application increased the microbial biomass C. This is because the microorganisms' usage of hydrocarbon pollutants and organic matters would increase their requirement for N and even P as these two major nutrients are required for the incorporation of carbon into the biomass. However, in a fertilization experiment, despite increased C contents in N fertilized soils, no changes were observed in the microbial biomass C.

Percentage of microbial biomass C to total soil organic C (MBC/C_{org})

MBC/C_{org} is one of the important soil biological indices. The increasing ratios indicate more transformations of soil organic C to microbial biomass and consequently, C sequestration in soil. Under stress conditions, a high portion of adsorbed C will be used for respiration and to supply the necessary energy for microbial maintenance and as a result no new microbial cell will be formed. The addition of N to soil caused the enhancement of this ratio by 75, 141 and 195% for NH₄+NO₃, NO₃ and NH₄, respectively. In general, N additions to soil in any form supply microorganisms' N requirements and hence more organic C is incorporated into MBC.

Results show that MBC/C_{org} decreased with increasing oil levels because of high C:N ratio of soils treated with crude oil which may cause N limitation to microorganisms' growth. The minimum (0.1%) and maximum (3.1%) effects on MBC/C_{org} are related to all N treatments and 10% and NH_4 treatment without oil, respectively.

Metabolic quotient (qCO_2)

This quotient indicates the respiration rate per unit of microbial biomass. Under stress conditions (such as pollution) qCO_2 was increased as the microorganisms required consuming more energy for maintenance than for activity. In non-fertilized treatments, the 10% oil level enhanced qCO_2 relative to 5% level, whereas N treatments induced greater qCO_2 at 5% oil level and lower qCO_2 at 10% oil level. This reduction is due likely to the N supply needed for microorganism's growth and for increasing C use efficiency.

Conclusion

In general, it can be concluded that N application does not affect total C mineralization or microbial respiration, apparently since it is not a limiting factor for microorganisms in the studied soil. However, microbial activities and consequently total C mineralization are increased by the addition of crude oil to soil. In addition, the effect of various levels of crude oil on soil microbial community and activities does not depend upon N application and its form. All N treatments and 10% oil level enhanced MBC, but this enhancement in 5% oil level was just happened in the control and NO_3 treatments. This study showed that soils containing high amounts of clay and organic matter would not be highly affected by hydrocarbon pollutants to alter microbial activity and C turnover. Nonetheless, the application of N fertilizer in petroleum-polluted environments may enhance soil microbial biomass.

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Key words

Carbon mineralization, crude oil, nitrogen forms, microbial biomass carbon, soil pollution