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in response to the three separate BEX pollutants, based on their chemical properties.

Materials and Methods

Microcosm experiments

e soil samples used for the microcosm experiments in this study were collected within the premises of the Botanical Garden located in the Science Complex at Albion College in Albion, Michigan, USA (42.24°N 84.76°W). e collected soil was mostly silt and sand that was slightly greyish brown in coloration and made up of mostly ne particles. e soil pool collected was then divided into three equal subsamples (10 grams each) in sterile clay pots that were then used for the microcosm experiments. Brie y, the indigenous bacterial populations in the soils were exposed to the various BEX hydrocarbon substrates by adding and mixing thoroughly up to 10% V/W of each BEX substrate to soils in the sterile clay pots before incubating at 30°C in the dark for a period of about 2 weeks as previously described in Chikere et al. and Olapade et al. [15,16].

Isolation of hydrocarbon-utilizing bacteria

A er 2 weeks of incubation0.1 g of soil subsamples were removed from each treatment into sterile 10 mL Bushnell Haas (BH) broth (Difco & BBL, USA) that was supplemented with 0.025% of each of the hydrocarbon substrate. e tubes were then incubated with shaking at 180 rpm at 30°C in the dark for at least a minimum of 48 hours. A er incubation, 0.2 mL was transferred from each broth onto sterile Bushnell Haas agar (BHA) plates (comprised per liter of: magnesium sulfate 0.2 g; calcium chloride 0.02 g; monopotassium phosphate 1.0 g; diammonium hydrogen phosphate 1.0 g; potassium nitrate 1.0 g and ferric chloride 0.05 g. 15 g of agar added to make the BH agar plates). Organic source was introduced through the vapor phase transfer by aspetically placing sterile Whatman lter papers previously impregnated {wet} with the respective hydrocarbon (BEX) substrate inside the lids of the Petri Plates, before incubating at 30°C in the dark for 48 hours. Bacterial isolates that grew on the BHA plates were then selected as putative hydrocarbon-degraders for subsequent screening and characterization according to standard methods [17]. All the isolates selected were screened for the 238 base pair long catechol 2, 3-dioxygenase [C230] gene fragment according to Mesarch et al. [18] using primers DEG-F (5'CGACCTGATC(AT)CGCA TGACCGA-3' and DEG R (5'T(CT)AGGTCA(GT)(AC)ACGGTCA3'. All the hydrocarbon substrates utilized in this study were of analytical grade and obtained from Fisher Scienti c.

Growth of bacterial isolates on hydrocarbon sources

Eleven bacterial isolates were selected based on their phylogenetic identities to represent the 11 main phyla obtained from the 45 total bacterial species in the three BEX microcosms. ese selected bacterial isolates were then individually examined for their potential growth in the presence of various organic compounds as sole sources of carbon 2shre5ewthu5(s33o)0.

bacterial phylotypes responded when exposed to toluene treatments in two separately contaminated soil microcosms that were then examined under controlled laboratory conditions [16].

e Shannon-Weiner diversity indices obtained in the three microcosms i.e. 13.07 in the benzene spiked microcosm, 8.35 (ethylbenzene) and 9.45 (xylene) appeared to be relatively higher than those previously reported in similar studies that were based on examining microbial diversity in contaminated soils [14,23,24]. Comparatively, some of these studies documented lower Shannon-Weiner diversity indices that ranged from about 1.45 to upwards of 3.93 in their studied environments. However, it should be pointed out that, the relatively high diversity indices recorded in this present study could partly be attributable to the use of top soils with mostly biologically active compositions as compared to such soils with aged petroleum contamination [25] or those mostly made of sandy and stony soils from Antarctica [23].

Furthermore, the results of both alpha and beta diversity measures probably suggest slight di erences in the preferences of the indigenous hydrocarbon degraders for the three separate hydrocarbon substrates used in the BEX-spiked microcosms. Since previous studies have also indicated individual preferences for BTEX by microbial consortia under controlled conditions typically in the order of toluene-benzene-ethylbenze-and xylene [26]. erefore it can be suggested that the di erences observed in bacterial diversity among the microcosms are partly attributable to the chemical properties of the hydrocarbon substrates. Comparatively, utilization of benzene as a substrate has been found to be most widely preferred by soil microbial communities followed closely by preference for toluene, the xylenes, styrene and naphthalene in that order [13].

e numerical dominance of bacterial members belonging to the - Proteobacteria, mainly species of Pseudomonas and Acinetobacter, in this study appeared to further corroborate previous documentations that have also revealed high occurrences or community shi towards these particular bacterial phylotype, among indigenous hydrocarbon-degrading microbial communities in response to hydrocarbon contamination [16,27,28]. Speci cally, members of the pseudomonads observed to be dominant in the three microcosms examined in this study have also been shown to be proli c hydrocarbon degraders in several studies of contaminated environments [9,15,28-30]. Probably because the pseudomonads are copiotrophic with propensity for high concentrations of nutrients [31], thereby giving them the edge in typically outnumbering other bacterial competitors within any contaminated environment. e Acinetobacter species, which were also well represented within the BEX-polluted soil microcosms examined, have been previously shown to have the potentials of producing various biosurfactants that can be used to solubilize and biodegrade hydrocarbon compounds [32]. Overall, the di erences observed in the bacterial phylotypes isolated from the three polluted soil microcosms in this study, suggests that the shi s in community compositions are probably attributable to variations in the chemical properties of the BEX pollutants on the indigenous hydrocarbon-degrading bacterial assemblages within the soil assemblages.

Conclusion

is study has documented changes in hydrocarbon-degrading bacterial assemblages within the same soil community in response to varying hydrocarbon substrates. e observation further corroborates previous reports of such shi s in indigenous microbial community structures and diversity, mostly towards the dominance of taxa with hydrocarbon-degrading capabilities. Potential shi s in microbial community compositions in polluted environments are partly attributable to the metabolic pro ciency of the assemblages as well as the type and complexity of the hydrocarbon pollutants.

Acknowledgements

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