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## Effects of community-accessible biochar and compost on diesel-contaminated soil

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### ABSTRACT

Petroleum pollution is a global problem that requires effective and accessible remediation strategies that takes ecosystem functioning into serious consideration. Bioremediation can be an effective tool to address the challenge. In this study, we used a mesocosm experiment to evaluate the effects of locally sourced and community produced biochar and compost amendments on diesel-contaminated soil. At the end of the 90-day experiment, we quantified the effects of the amendments on total petroleum hydrocarbons (C9–C40) (TPH) and soil pH, organic matter, aggregate stability, soil respiration, extractable phosphorus, extractable potassium, and micronutrients (Mg, Fe, Mn, and Zn). We observed significantly higher TPH degradation in compost-amended soils than in controls and soils amended with biochar. We propose that the addition of compost improved TPH biodegradation by augmenting soil nutrient content and microbial activity. Our results suggest that community-accessible compost can improve TPH biodegradation, and that implementation is possible at the community level.

### KEYWORDS



Amendments; biodegradation; bioremediation of hydrocarbons; remediation of contaminated sediments; nutrients; total petroleum hydrocarbon (TPH)

### 1. Introduction

Pollution is a pervasive problem that affects ecosystem functioning and human health around the world. Petroleum pollution releases greenhouse gases and other pollutants into the environment, endangering wildlife and altering water and soil chemistry (dos Santos and Maranhão 2018). It comes from many sources including extraction, transportation, leakage from tanks and equipment, pipeline breaks, vandalism and sabotage, consumption, and refining (Aisien et al. 2015; Lim et al. 2016). In 2014 alone, the American Petroleum Industry spent \$1.4 billion on remediating spills, and \$302 billion during 1990–2014 (American Petroleum Institute (API) 2016). In fiscal year 2015, US EPA Region 1 that includes New England, reported 311 newly found, leaked underground storage tanks (UST), increasing the total USTs awaiting cleanup to 2932 in the region (US EPA 2017). In 2017, the average cost for remediation of leaked USTs in USA was \$130 thousand when soil impact was low, increasing to

\$1 million with groundwater contamination (UST 2017).

Hydrocarbons range in their level of toxicity. Some hydrocarbons, including polycyclic aromatic hydrocarbons (PAH) and benzene, are carcinogenic to both animals and humans (Abdel-Shafy and Mansour 2016; Agency for Toxic Substances and Disease Registry (ATSDR) 2016; dos Santos and Maranhão 2018). Aromatics, organic compounds in which the carbon atoms form a ring, are generally more toxic than aliphatics, organic compounds in which the carbon atoms form open chains (Interstate Technology Regulatory Council (ITRC) 2014; von Oettingen 1942). Due to petroleum pollution's global dimension and environmental impact, remediation practitioners must consider long-term ecosystem functioning and community-accessibility of proposed techniques (Frederick and Egan 1994; Singh et al. 2017). Some approaches to petroleum remediation are expensive and energy intensive, including incineration, soil washing,

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and soil vapor extraction (Kujat 1999; Lim et al. 2016). In 2009, the US EPA Office of Land and Emergency Management established a policy on Principles for Greener Cleanups. This policy supports remediation techniques that reduce their environmental footprint and set a platform for land reuse (Lim et al. 2016; US EPA 2016). One strategy primed to reach these goals is bioremediation, the use of plants, microorganisms, and other soil inhabitants to degrade, remove, or otherwise control a contaminant (Chawla et al. 2013; Cook and Hesterberg 2013; dos Santos and Maranhão 2018). Studies have identified bacteria, archaea, fungi, protozoa, viruses, and algae among the ranks of microbial TPH degraders (Juwarkar et al. 2010; Varjani and Upasani 2017). Bioremediation provides potential to remediate sites while enhancing soil properties that support soil organismal and plant communities, as well as provides a positive esthetic for the surrounding human population (Chawla et al. 2013; Slegers 2010). Bioremediation can also remediate a site at a lower cost, often 80–90% less than that of engineered techniques, increasing its potential widespread implementation (Chen et al. 2015; Megharaj et al. 2011; Singh et al. 2017; Stephenson and Black 2014).

Selecting an appropriate remediation strategy, such as bioremediation, is dictated by a site's biogeochemistry, level of pollution, and other environmental factors (Lim et al. 2016). Therefore, when evaluating a site it is important to consider the following factors: contaminant concentration and bioavailability, soil pH, temperature, soil structure and texture, nutrients, the microbial growth cycle, and electron acceptors (Aisien et al. 2015; Hall et al. 2011; Juwarkar et al. 2010; Oliveira et al. 2015; Semple et al. 2003).

Biochar, produced during the pyrolysis of biomass, has been shown to increase soil pH, cation exchange capacity, and productivity; improve soil physical properties (aggregate stability, porosity, aeration, water holding capacity); and adsorb hydrophobic organics (Hale et al. 2012; Kookana et al. 2011; Sizmur et al. 2016). Studies on the use of biochar in bioremediation have shown inconsistent results. For example, Bushnaf et al. (2011) and García-Delgado et al. (2015) reported no significant increase in degradation rates as a

result of biochar addition. In contrast, Qin et al. (2013) found that biochar significantly increased degradation rates by approximately 20%. Considering the potential remediation and carbon sequestration benefits of biochar, its use in bioremediation warrants further study (Ennis et al. 2012; Fang et al. 2014; Smith 2016).

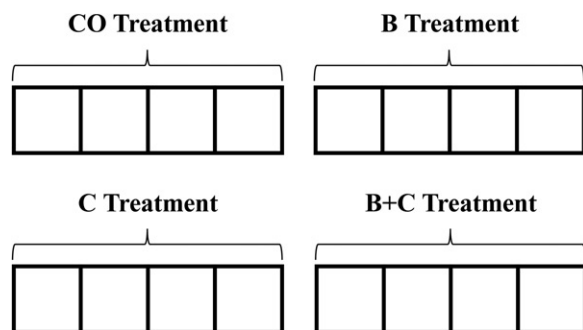
The addition of mature compost to enhance biodegradation in a polluted soil has been studied since the early 1980s. Depending on the compost's composition, it has been shown to improve soil organic carbon content, nutrient availability, pH, and water retention (Kästner and Miltner 2016; Wu et al. 2017). Compost can result in sorption of contaminants to the newly introduced organic matter and lowering sorption to parent soil. Organic matter slowly releases nutrients as degraded into its constituent parts (Agegnehu et al. 2017). In addition, compost increases soil microbial diversity (Kästner and Miltner 2016; Sayara et al. 2010) and available nutrients (Chen et al. 2015). High degradation rates have been reported for the removal of PAHs by compost addition (Kästner and Miltner 2016). Results of these studies have been consistent; however, technological optimization needs further study.

In this study, the impact of biochar and compost on diesel-contaminated soil was assessed in a 90-day greenhouse mesocosm study. The objective was to evaluate the amendments' independent and interactive effects on: (i) soil physical, chemical, and biological properties (aggregate stability, organic matter, pH, respiration, and nutrient levels) and (ii) the biodegradation of total petroleum hydrocarbons (TPH).

## 2. Material and methods

### 2.1. Contaminated soil

Bulk soil was collected on May 10, 2017 from Westmoreland, NH, USA (Figure 1). This area has a history of small-scale, organic farming. Bulk soil (~20 kg dry weight) was removed from the top ~30 cm of the soil profile, A and B horizons (Qin et al. 2013), oven dried at 80°C for 3 days, sieved through a 2-mm mesh to remove stones, and stored in a dark container at 4°C (Malachowska-Jutysz and Kalka 2010). Diesel fuel



**Figure 1.** Locator map indicating the site of soil collection in Westmoreland, NH, USA.

(Citgo No. 2 Diesel Fuel, Ultra Low Sulfur, All Grades) was added to the dried, bulk soil (150 mL per 700 g) on May 25, 2017. This fuel formulation is mostly made of C8 to C26 aromatic hydrocarbons, cycloalkanes, and *n*-alkanes that have a moderate volatility—vapor pressure of 0.27 kPa (Khudur et al. 2015; PRO-ACT 1999). The bulk diesel-contaminated soil, or initial soil (Table 1), was stored in a dark container at 4 °C until used to establish mesocosms (6 days) (Qin et al. 2013).

On May 31, 2017, three samples of the initial soil (~100 g each) were sent to Eastern Analytical, Inc. (EAI, Concord, NH) for analysis of TPH (C9-C40), nitrate/nitrite, total kjeldahl nitrogen (TKN), and total phosphorus. On the same day, three samples of the initial soil (~200 g each) were sent to Cornell Soil Health Laboratory (CSHL, Ithaca, NY) for analysis of texture, pH, organic matter (OM), aggregate stability, soil respiration, extractable phosphorus, extractable potassium, and minor nutrients (Mg, Fe, Mn, and Zn). Aggregate stability was a measure of the percentage of dried aggregates that did not disintegrate under a simulated rainfall. Soil respiration was a measure of the total CO<sub>2</sub> emitted by a gram of soil over a 4-day incubation period. Extractable phosphorus and potassium

were measured on a modified Morgan's extractant using a rapid flow analyzer and ICP spectrometer, respectively.

## 2.2. Mesocosm experiment

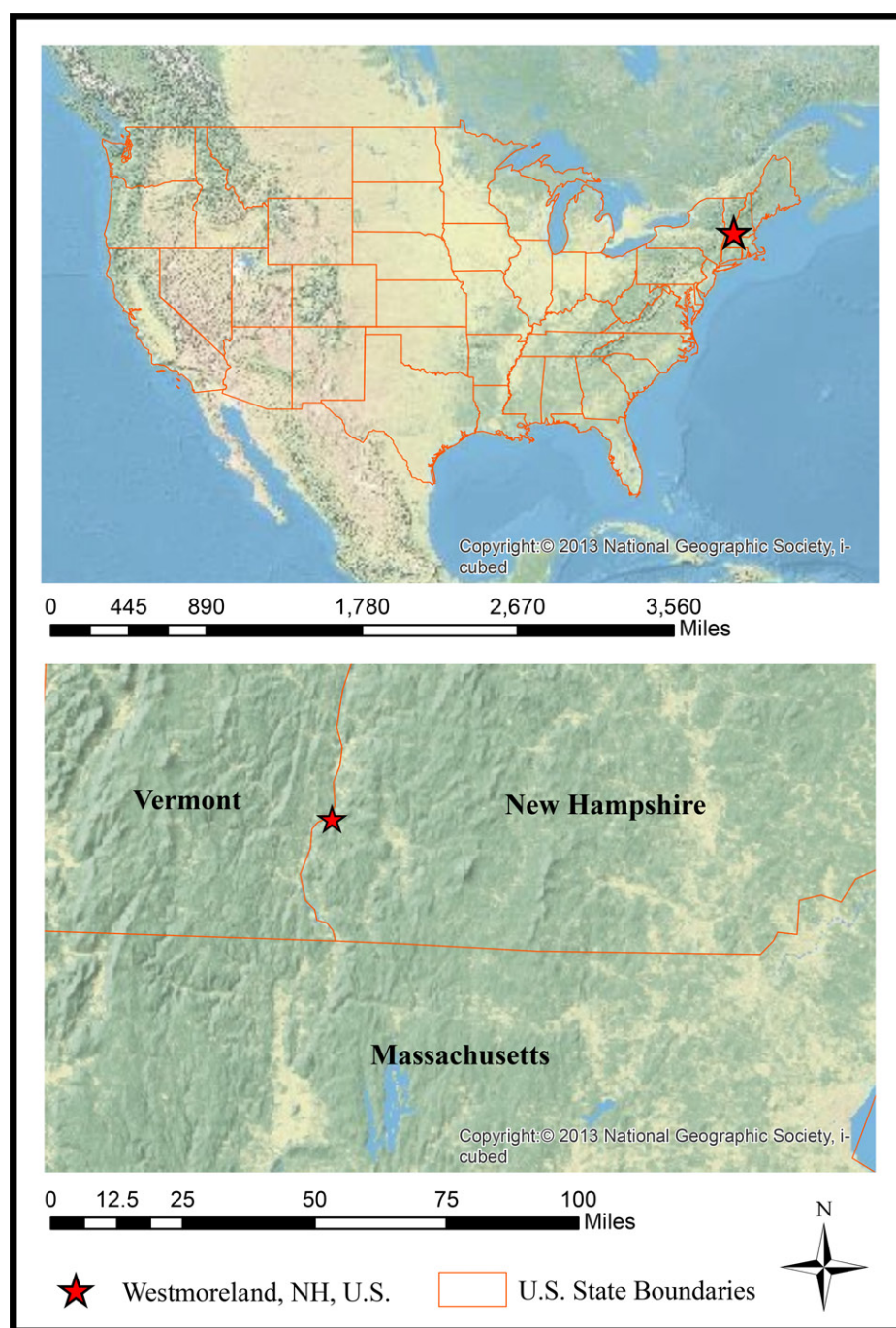
Biochar was purchased (Sullivan Center for Sustainable Agriculture, Sullivan, NH) and stored in dark, airtight containers at room temperature (20 °C) from May 11–31, 2017 (Hale et al. 2012). The biochar was made in March 2017 by cooking hardwood cordwood (mix of birch, beech, oak, maple, and ash) at 400–430 °C for 10–12 h in an adam retort, a high efficiency and low-cost kiln. Mature compost, the second amendment, was acquired from the compost pile at Antioch University New England (AUNE) in Keene, NH on May 20, 2017 and dried for 3 days at 20 °C. It was derived from kitchen scraps, yard waste, and garden waste. A full factorial experiment was established using biochar and compost amendments (Figure 2).

Each treatment had four replicates. The experimental mesocosm was comprised of initial soil (700 g), biochar (B) at 2.5% [w/w], and/or compost (C) at 15% [w/w] as associated with each treatment, mixed thoroughly in a plastic bin by shaking and stirring, and placed in a yogurt container (height 17.5 cm, base diameter 8.5 cm, and top diameter 11.5 cm), recently washed with dish soap (Bastida et al. 2016; Marchand et al. 2016; Nwankwegu et al. 2016; Qin et al. 2013). Double-distilled water was added to the soil in each mesocosm until reaching field moisture capacity (FMC) (32%), as indicated by weight (g). FMC of the initial soil was established using standard protocol (Romano and Santini 2002). All mesocosms were placed in a greenhouse (Keene State College, Keene, NH) on May 31, 2017 and kept at temperatures ranging 23–40 °C,

**Table 1.** Soil parameters.

Soil parameter	Value	Parameter	Value
TPH, C9-C40 (mg/kg)	95,333 ± 1856	Nitrate/nitrite (mg/kg)	25
Texture	Loam	TKN (mg/kg)	1600
Sand (%)	44.2	Total phosphorus (mg/kg)	1500
Silt (%)	45	Extractable phosphorus (ppm)	51.57 ± 0.87
Clay (%)	10.8	Extractable potassium (ppm)	233.20 ± 0.95
pH	5.87 ± 0.09	Magnesium (ppm)	106.07 ± 0.28
Organic matter (%)	5.87 ± 0.07	Iron (ppm)	3.87 ± 0.07
Aggregate stability (%)	49.9 ± 4.7	Manganese (ppm)	30.63 ± 0.32
Respiration (mg CO <sub>2</sub> /g)	2.00 ± 0.00	Zinc (ppm)	4.03 ± 0.03





**Figure 2.** Each treatment contained four replicates. Each experimental unit (square) contained bulk diesel-contaminated soil (700 g) and the amendments indicated: biochar (B) at 2.5% [w/w] and/or compost (C) at 15% [w/w]. Controls (CO) contained no amendments.

at humidity ranging 24–68%, and with fan circulated air. The mesocosms were assessed for FMC every other day by weighing (g) and double distilled water was added to maintain FMC until August 29, 2017 (90 days). Mesocosm positions were rotated every other day to account for sunlight and temperature differences within

the greenhouse. At the end of 90 days, each mesocosm was destructively sampled, and soil samples (100–200 g each) were immediately sent to EAI and CSHL for analysis of TPH (C9–C40), pH, OM, aggregate stability, soil respiration, extractable phosphorus, extractable potassium, and minor nutrients (Mg, Fe, Mn, and Zn).

**Table 2.** Final soil parameter results for treatments.

Soil parameter	CO	B	C	B + C
TPH (C9-C40) (mg/kg) <sup>a,b</sup>	45,250 ± 1436	45,000 ± 913	38,000 ± 408	36,250 ± 629
pH <sup>a,b</sup>	6.35 ± 0.05	6.18 ± 0.03	6.50 ± 0.04	6.45 ± 0.03
Organic matter (%) <sup>a,b</sup>	5.48 ± 0.11	5.68 ± 0.13	6.60 ± 0.17	6.65 ± 0.13
Aggregate stability (%)	82.7 ± 0.9	69.3 ± 3.2	80.4 ± 1.6	79.3 ± 2.3
Respiration (mg CO <sub>2</sub> /g) <sup>a,b</sup>	1.88 ± 0.10	1.15 ± 0.13	1.90 ± 0.17	1.68 ± 0.14
Extractable phosphorous (ppm) <sup>a,b</sup>	22.88 ± 0.23	25.55 ± 0.82	27.58 ± 0.34	28.55 ± 0.38
Extractable potassium (ppm) <sup>a,b</sup>	242.95 ± 2.64	232.45 ± 6.62	302.68 ± 4.51	285.68 ± 4.00
Magnesium (ppm) <sup>a,b</sup>	122.68 ± 1.45	117.03 ± 3.67	154.33 ± 1.76	145.53 ± 1.24
Iron (ppm)	22.43 ± 1.12	8.50 ± 1.41	9.83 ± 1.24	7.90 ± 0.54
Manganese (ppm) <sup>a,b</sup>	208.10 ± 4.67	135.53 ± 14.30	111.23 ± 16.82	93.05 ± 7.62
Zinc (ppm)	3.68 ± 0.03	3.93 ± 0.13	4.53 ± 0.09	4.53 ± 0.02

**Table 3.** Statistical results comparing initial and final soil parameters for each treatment.

Pair	SP	t	χ <sup>2</sup>	df	p	SP	t	χ <sup>2</sup>	df	p	SP	t	χ <sup>2</sup>	df	p
I v. CO	TPH*	–	4.6	1	.032	Resp.	1.0	–	5	.352	Fe*	–	4.6	1	.032
I v. B		–	4.5	1	.034		5.4	–	5	.003		–	4.6	1	.032
I v. C		–	4.6	1	.032		0.5	–	5	.637		–	4.6	1	.032
I v. B + C		–	4.6	1	.032		1.9	–	5	.114		–	4.6	1	.032
I v. CO	pH*	–	4.8	1	.028	Ext. P*	–	4.5	1	.034	Mn	–32.1	–	5	.000
I v. B		–	4.8	1	.028		–	4.5	1	.034		–6.2	–	5	.002
I v. C		–	4.6	1	.032		–	4.5	1	.034		–4.0	–	5	.010
I v. B + C		–	4.7	1	.031		–	4.5	1	.034		–6.9	–	5	.001
I v. CO	OM	2.7	–	5	.040	Ext. K*	–	4.5	1	.034	Zn	8.8	–	5	.000
I v. B		1.2	–	5	.299		–	0.0	1	1.000		0.7	–	5	.504
I v. C		–3.5	–	5	.016		–	4.5	1	.034		–4.7	–	5	.005
I v. B + C		–4.9	–	5	.004		–	4.5	1	.034		–12.1	–	5	.000
I v. CO	Agg. St.*	–	4.5	1	.034	Mg*	–	4.5	1	.034					
I v. B		–	4.5	1	.034		–	4.5	1	.034					
I v. C		–	4.5	1	.034		–	4.5	1	.034					
I v. B + C		–	4.6	1	.032		–	4.5	1	.034					

\*Non-normally distributed data.

A significant change in soil parameters (SP) was observed over the 90-day experiment. Soil treatments are: initial (I), control (CO), biochar (B), compost (C), and B + C. For normally distributed data, two-tailed *t*-tests were used, and Kruskal–Wallis tests were used for non-normally distributed data. A dash indicates that the chosen test did not give this parameter. Statistical significance was determined at  $\alpha \leq 0.05$ .

**Table 4.** Statistical results among treatments.

Pair	SP	p	Z	SP	p	Z	SP	p	Z	SP	p	Z
CO v. B	TPH	.997	–	Agg. stability*	.006	2.8	Ext. K	.419	–	Mn	.005	–
CO v. C		.001	–		.334	1.0		.000	–		.000	–
CO v. B + C		.000	–		.298	1.0		.000	–		.000	–
B v. C		.001	–		.074	–1.8		.000	–		.499	–
B v. B + C		.000	–		.087	–1.7		.000	–		.107	–
C v. B + C		.562	–		.941	0.1		.097	–		.708	–
CO v. B	pH	.028	–	Respiration	.014	–	Mg	.330	–	Zn*	.499	–0.7
CO v. C		.064	–		.999	–		.000	–		.005	–2.8
CO v. B + C		.284	–		.742	–		.000	–		.008	–2.7
B v. C		.000	–		.011	–		.000	–		.032	–2.1
B v. B + C		.001	–		.083	–		.000	–		.046	–2.0
C v. B + C		.783	–		.670	–		.070	–		.881	0.2
CO v. B	OM	.729	–	Ext. P	.011	–	Fe*	.007	2.7			
CO v. C		.000	–		.000	–		.069	1.8			
CO v. B + C		.000	–		.000	–		.008	2.6			
B v. C		.002	–		.057	–		.393	–0.9			
B v. B + C		.001	–		.005	–		.970	–0.0			
C v. B + C		.993	–		.053	–		.414	0.8			

\*Non-normally distributed data.

Statistically significant differences among treatments occurred for final soil parameters (SP) (i.e., parameters at the end of the 90-day experiment). Soil treatments are: control (CO), biochar (B), compost (C), and B + C. For normally distributed data, the Tukey's HSD test was used at  $\alpha \leq 0.05$ . For non-normally distributed data, the Dunn test with the Bonferroni method was used at  $\alpha \leq 0.0083$ . A dash indicates that the chosen test did not give this parameter.

### 2.3. Statistical methods

Prior to running statistical tests, we normalized the TPH data by adjusting the concentration according to the mass of amendments added as

follows: Adjusted TPH =  $\text{TPH concentration} \times [(\text{mass of amendments} + \text{mass of initial soil}) / \text{mass of initial soil}]$ . Two-tailed *t*-tests were performed to determine if there was a statistically

**Table 5.** Spearman's rank correlation test.

	pH	OM	Agg.	Resp.	Ext. P	Ext. K	Mg	Fe	Mn	Zn
S	1110.8	1222.7	748.6	883.9	1189.1	1146.4	1152.5	480.9	240.4	1179.0
p value	0.008	0.000	0.710	0.259	0.001	0.003	0.003	0.271	0.007	0.001
rho	−0.63	−0.80	−0.10	−0.30	−0.75	−0.69	−0.69	0.29	0.65	−0.73

significant difference in each soil parameter between the initial soil and each treatment's soil at the end of the experiment. For data that were not normally distributed, non-parametric Kruskal–Wallis tests were performed. In addition, we used a one-way ANOVA followed by a Tukey's HSD test to evaluate if there was a statistically significant difference in each soil parameter among the four treatments using pairwise comparisons. For data that were not normally distributed, the non-parametric Kruskal–Wallis test was used followed by a Dunn test with Bonferroni adjustments at  $\alpha \leq 0.0083$ . The Spearman's rank test was used to correlate the concentration of TPH in each soil with the 10 response variables in order to assess possible influence. Statistical significance was determined at  $\alpha \leq 0.05$  unless otherwise noted.

### 3. Results

At the end of the 90-day study, the concentration of TPH had significantly decreased in controls (CO), B, C, and B + C treatments, by 52.5, 52.8, 60.1, and 62.0%, respectively (Tables 2 and 3). Among the four treatments, only compost-amended soils significantly increased TPH degradation in contrast to the control (Table 4).

In contrast to the initial bulk soil (Table 1), all treatments showed a significant increase in pH, magnesium, iron, manganese, and aggregate stability, and a significant decrease in extractable phosphorus. OM and zinc significantly decreased in controls, did not change in treatment B, and significantly increased in treatments C and B + C. Respiration significantly decreased in treatment B and did not change in the other treatments. Extractable potassium did not change in treatment B and significantly increased in all other treatments (Tables 2 and 3).

Results of the Spearman's rank correlation test showed a significant correlation between TPH and pH, OM, extractable phosphorus, extractable potassium, magnesium, manganese, and zinc

(Table 5). All correlations were negative except for manganese, which was positively correlated with TPH.

## 4. Discussion

### 4.1. Compost-amendment biodegradation mechanisms

We observed that all treatments significantly reduced TPH relative to the initial bulk soil, and the compost amendment increased TPH degradation significantly more than other treatments. Kästner and Miltner (2016) also reported that a sterilized compost improved biodegradation of TPH relative to non-amended soil. We speculate that compost improved TPH biodegradation by: (i) stimulating native TPH degrader activity through nutrient addition and promotion of non-TPH degrader activity; and (ii) increasing TPH degrader biodiversity by adding TPH degrader species that were not initially present. The relative importance of these mechanisms remains uncertain, paralleling debate in the literature.

Our study supports the hypothesis that compost enhances biodegradation of TPH by adding essential limiting nutrients. Phosphorus is highly limiting to microbial mineralization; thus, the quantity and relative abundance of P influences soil microbial biomass and activity (Kaczyńska et al. 2015; Kästner and Miltner 2016; Varjani and Upasani 2017). We observed a negative correlation between extractable phosphorus and TPH concentration, suggesting that our compost amendments increased P availability and thus microbial mineralization of organic compounds, including TPH. Similarly, Khudur et al. (2015) produced a 78–90% increase in biodegradation by adding ammonium sulfate and potassium phosphate to a diesel-contaminated soil. Further, Nwankwegu et al. (2016) observed that compost enhances TPH removal more than nutrient amendment alone. They observed that compost, fertilizer, and no amendment treatments resulted

in TPH removal of 93.31, 71.36, and 57.90%, respectively.

In our study, compost likely contributed micronutrients to soil that increased microbial degradation of TPH. For example, some of the enzymes that TPH degraders use to mineralize organic matter require iron. Das and Chandran (2011) report that multiple microbes use dioxygenases and alkane hydroxylases, both of which contain iron, to break down C10 to C30 alkanes (Ji et al. 2013; Varjani and Upasani 2017). We observed that iron was lower in biochar and compost amended treatments than in controls. As TPH degradation was higher in compost-amended treatments, we hypothesize that the TPH degraders active in these soils required iron for their metabolism, causing the declines in iron we observed. On the other hand, biochar-amended treatments degraded TPH as much as controls; thus, we hypothesize that the biochar adsorbed iron cations, contributing to the declines in iron we observed in biochar-amended soils (Xu et al. 2014). In addition to iron, the parameters extractable potassium, OM, and magnesium were negatively correlated with TPH and showed the same pattern of significance among treatments, suggesting a possible mechanistic role of these soil properties in biodegradation that warrants further study.

In addition to its influence on nutrients, compost also changes TPH degrader community activity, composition, and biomass (Chen et al. 2015; Bastida et al. 2016). We measured microbial respiration to evaluate how amendments affected microbial activity. Compost-amended treatments showed no significant increase in respiration despite the positive effect of compost on TPH removal. Further, there was no correlation between TPH and respiration. Thus, this study does not show that increased microbial activity was a significant mechanism in hastening TPH degradation. However, it is possible that compost addition facilitated more efficient TPH degrader activity via augmentation of TPH degrader biodiversity and/or interactions with other soil microbes. Compost's high organic matter content increases microbial mineralization rates and thus soil temperature, thereby increasing microbial biodiversity in compost-amended soil (Bastida

et al. 2016). This influx of diversity may increase the abundance of microbes that effectively degrade TPH, as well as non-TPH degrader microbes that enhance TPH degrader activity via interactive effects. For example, a diverse group of non-TPH degrading microbes can persist in contaminated soil and enhance TPH degrader activity through mechanisms such as metabolic cooperation and cross-feeding (Kästner and Miltner 2016; Nwankwegu et al. 2016). In fact, the TPH degrader fraction of the microbial community can be very small; Bastida et al. (2016) observed that 90% of alkane and PAH removal was attributable to only 0.55% of the soil metaproteome in a compost-amended soil. Therefore, future studies about the mechanisms by which soil amendments affect TPH biodegradation should characterize microbial community composition in addition to microbial respiration.

#### 4.2. Biochar-amended treatments

TPH declined significantly and equally in control and biochar-amended treatments, although not to the degree of compost-amended treatments. This TPH removal was likely due to the initial bulk soil providing sufficient nutrients and active TPH degraders for the control and biochar-amended treatments to result in a degree of successful TPH degradation. According to standards from the Cornell Soil Health Laboratory, initial bulk soil nitrogen, extractable phosphorus, OM, and iron results were at excellent or optimal levels for microbial functioning (Das and Chandran 2011; Khudur et al. 2015).

The significant increase of extractable phosphorus in biochar-amended soils relative to controls could be caused by a priming effect, whereby the biochar promotes the growth and/or activity of bacteria involved in phosphorus liberation (Anderson et al. 2011; Xu et al. 2014). Although this increase in P availability did not increase TPH biodegradation in our study, it may nonetheless improve the nutrient status of native soils in which phosphorus is highly limiting.

We observed that microbial respiration and aggregate stability significantly decreased in biochar-amended treatments relative to controls, without impacting TPH degradation. This



suggests that TPH degrader activity in biochar-amended soils was not responsible for overall declines in microbial respiration and did not require the micropores in aggregates to thrive. This hypothesis warrants further study, as results may differ over a longer experiment or if biochar is inoculated with microbes before its addition to soil. For example, Qin et al. (2013) reported a time lag in which biochar amendment did not start to significantly increase TPH degradation until day 60 of a 180-day study. They observed 77.8 and 61.2% TPH reduction in biochar-amended and non-amended treatments, respectively. Further studies about the effects of biochar amendment on aggregate stability are warranted, as soil aggregation is critical to soil restoration because it diversifies soil habitat, sequesters organic compounds, and supports a diverse soil organismal community that is more resilient to ecological degradation and perturbation (Hillel 2008).

Another possible mechanism of TPH decline in all of our experimental treatments was TPH evaporation from soils over the 90-day experiment. Villa et al. (2010) reported that 8% of original diesel mass is evaporated from soils (Khudur et al. 2015). Notwithstanding, the contribution of evaporation was likely the same for all treatments because all treatments originated from the same bulk contaminated soil (Megharaj et al. 2011).

#### **4.3. Future research needs and field-scale considerations**

Increasing field-scale bioremediation is necessary for the practice to occupy a larger place in remediation. To further optimize bioremediation practice and improve its implementation at the field scale, researchers can work to understand the most important mechanisms by which compost facilitates biodegradation and how its effectiveness varies among soil types and polluted site conditions. Using techniques such as metaproteomics, which characterize microbial community composition, can help researchers to better understand how compost affects microbial species interactions and supports TPH degrader activity (Bastida et al. 2016). Microbiologists can

then work with practitioners to determine the most appropriate compost types and amounts to apply to different contaminated sites. Nutrients can benefit nutrient-poor native soils, but excessive nutrient addition can cause leaching that contaminates ground and surface water (Chen et al. 2015). In addition, further research is needed on the use of biochar in bioremediation; longer-term experiments in nutrient poor soils that compare raw biochar with microbe-inoculated biochar would be especially informative.

As researchers and practitioners scale up to field-scale bioremediation, additional considerations are warranted. For example, the mixing of biochar and/or compost amendments into an open-air, contaminated soil would make that soil highly susceptible to erosion from wind and rain. Therefore, practitioners must develop appropriate methods to mitigate soil erosion if they mix physical amendments into soils in the field. One way to prevent soil erosion is to use plants that survive in contaminated soil and, better yet, enhance contaminant removal through phytoremediation (Banks et al. 2003; dos Santos and Maranhão 2018). In addition, the inclusion of plants in remediation efforts can improve social acceptance of bioremediation (Weir and Doty 2016) and thus garner greater public support and funding for bioremediation projects. Initially in our study, we attempted to investigate the TPH removal capacity of a native grass, Canada wild-rye (*Elymus canadensis*) because we hypothesized that its fibrous root system would support a diverse community of active TPH degraders (Edenborn et al. 2015; Phillips et al. 2009). However, the seeds did not germinate in our diesel-contaminated soil, likely because they were coated with diesel and thus unable to take up water. Thus, we recommend that phytoremediation practitioners plant seeds in a top layer of compost to eliminate seed exposure to contaminants (Phillips et al. 2009), or plant seedlings. Further, practitioners must consider carefully whether to apply biochar and phytoremediation strategies simultaneously, as biochar has been shown to both restrict (Han et al. 2016) and support (Ogbonnaya and Semple 2013) the growth of phytoremediators in diesel-contaminated soil.

#### 4.4. Conclusion

Several studies of TPH biodegradation report increases in contaminant removal with compost amendment. The results of our study support the continued use of compost to remediate diesel-contaminated soil, and suggest that locally produced compost can be an effective and accessible remediation strategy for many communities. Future bioremediation efforts should test large-scale, field-based amendment application using various biological elements including fungal and plant remediation. These studies would further shed light on the conditions under which amendments such as compost and biochar are effective, and could increase public awareness and acceptance of bioremediation strategies. This study advances the case for bioremediation as a strategy that supports community remediation efforts, particularly where funding for more expensive remediation is limited. Further, bioremediation may also maintain and improve the ecological functioning of a polluted and previously-polluted soil.

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