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Wheat Growth in Soils Treated by Ex Situ Thermal Desorption

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Abstract

Successful remediation of oil-contaminated agricultural land may include the goal of returning the land to prespill levels of agricultural productivity. This productivity may be measured by crop yield, quality, and safety, all of which are influenced by soil characteristics. This research was conducted to determine if these metrics are affected in hard red spring wheat (*Triticum aestivum* L. cultivar Barlow) when grown in soils treated by ex situ thermal desorption (TD) compared with wheat grown in native topsoil (TS). Additionally, TD soils were mixed with TS at various ratios to assess the effectiveness of soil mixing as a procedure for enhancing productivity. In two greenhouse studies, TD soils alone produced similar amounts of grain and biomass as TS, although grain protein in TD soils was 22% (±7%) lower. After mixing TS into TD soils, the mean biomass and grain yield were reduced by up to 60%, but grain protein increased. These trends are likely the result of nutrient availability determined by soil organic matter and nutrient cycling performed by soil microorganisms. Thermal desorption soil had 84% (±2%) lower soil organic carbon than TS, and cumulative respiration was greatly reduced (66 ± 2%). From a food safety perspective, grain from TD soils did not show increased uptake of polycyclic aromatic hydrocarbons. Overall, this research suggests that TD soils are capable of producing safe, high-quality grain yields.

Core Ideas

- Wheat was grown in crude oil-contaminated soil remediated using thermal desorption.
- Remediated soil matched topsoil in grain yield, but grain protein was decreased.
- Mixing topsoil with remediated soil decreased yield but increased grain protein.
- Grain from remediated soil contained no more petroleum hydrocarbons than topsoil.
- Mixing topsoil with remediated soil decreased the C:N ratio and increased respiration.

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Accidental releases during the extraction, transport, and storage of crude oil can expose soil to high levels of petroleum hydrocarbons (PHCs). These PHCs harm soil health (Roy and McGill, 1998; Eom et al., 2007), reduce seed germination (Tang et al., 2011; Yi et al., 2016), and hinder vegetative growth (de Jong, 1980; Essien and John, 2010). When these releases occur on agricultural land, soil remediation is required to return the land to precontaminated levels of productivity. Although precontaminated levels of productivity may not be immediately feasible in some cases, restoring productivity is a long-term goal at many contaminated sites. Many techniques exist to remediate PHC contamination in soil (Lim et al., 2016), and the most appropriate technology to implement is dictated by site-specific considerations.

One established technique that may be suitable for remediating agricultural soil is ex situ thermal desorption (TD), which can be widely applied due to its versatility, relatively short treatment time, and the ability to reuse the treated soil (de Percin, 1995). Briefly, TD enhances contaminant vaporization by heating contaminated materials in a desorption unit (Lighty et al., 1990; USEPA, 1994). The vaporized contaminants are combusted in a thermal oxidation chamber, and the treated soil is rehydrated and available for reuse. This technique has been used to treat a variety of contaminants (Falciglia et al., 2011; Qi et al., 2014; Sierra et al., 2016), as the heating temperature and heating time can be manipulated to target volatile and semivolatile compounds in a variety of soil matrices.

An appealing aspect of using TD to remediate contaminated agricultural soil is the possibility of soil reuse after treatment, although the capacity of TD soils to sustain vegetation has not been fully explored. This capacity is influenced by the nature of the contaminated material, as TD is often used on industrial soils and subsoil materials, which may have decreased fertility compared to agricultural topsoil. Nonetheless, numerous studies have shown that TD soils are capable of producing more biomass than contaminated, untreated soils (Roh et al., 2000; Dazy et al., 2009; Wang et al., 2010; Ouvrard et al., 2011); however, these studies offer no comparison with noncontaminated soils. Thus, they do not quantify the differences between remediated soil

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Abbreviations: DAS, days after sowing; HRSW, hard red spring wheat; PAH, polycyclic aromatic hydrocarbon; PHC, petroleum hydrocarbon; PVC, polyvinyl chloride; SOC, soil organic carbon; SOM, soil organic matter; TD, ex situ thermal desorption; TS, native topsoil; TPH, total petroleum hydrocarbon.

and noncontaminated soil, which is essential to gauge progress toward attaining prespill productivity.

In two studies that did offer direct comparison between TD soils and noncontaminated soils, seed germination, shoot growth, and biomass were between 40 and 80% lower in TD soils (Vidonish et al., 2016; Yi et al., 2016). Notably, these plants did not grow to maturity, so TD effects on later life stages of the plant is unknown. Additionally, the effects of TD on plant growth may vary among species (Dazy et al., 2009; Yi et al., 2016), so translating this information to common agricultural crops requires species-specific research. Further, none of these studies involving TD soils quantified contaminant uptake into plant structures. Since TD may only be applied to attain environmental regulatory standards, PHC concentrations may remain above background levels. Thus, food safety must also be a consideration when assessing the viability of using TD soils for agricultural production.

Although biomass and quality of vegetation may be valuable indicators for meeting remediation goals, these factors alone do not encompass a holistic approach to soil remediation. Plant response is closely tied to the alteration of soil properties after TD that are, in turn, linked with long-term processes vital in maintaining soil health. Notable changes in soil properties after TD are reduced soil organic matter (SOM) (McAlexander et al., 2015; Sierra et al., 2016; Yi et al., 2016) and increased pH (Ouvrard et al., 2011; Yi et al., 2016), although the magnitudes of these alterations are dependent on heating temperature, time, and native soil properties. Soil physical properties also change after TD treatment at 350°C, including a reduction of total aggregation, a sharp increase in saturated hydraulic conductivity (O'Brien et al., 2016), and decreased water retention (Roh et al., 2000). Further, when comparing TD soils with noncontaminated soils, biological communities are altered (Cébron et al., 2011), microbial abundance is decreased (Ouvrard et al., 2011; Yi et al., 2016), and genotoxicity to earthworms is increased (Bonnard et al., 2010). Projects aimed at returning the land to precontaminated conditions need to account for these changes, as each of these properties relates to short-term plant production, as well as long-term soil health.

One possible way to mitigate these effects of TD may be to mix native, noncontaminated topsoil (TS) with TD soil. Incorporating the TS increases SOM and can rapidly reintroduce a native biological community (Marschner and Rumberger, 2004), both of which benefit long-term soil health. In some circumstances, soil mixing may be an alternative to purchasing replacement topsoil, a common practice in remediation projects. Introducing topsoil from another location may be undesirable due to unknown soil management history and possibility of weed species in the seedbank or plant pathogens. Therefore, the practice of soil mixing may both reduce overall project costs and improve soil quality.

The purpose of this research was to assess the potential of TD soils for cropland production, both as a singular product and as a mixing agent with noncontaminated topsoil. This assessment was based on two greenhouse studies conducted using TD soil to grow hard red spring wheat (HRSW, *Triticum aestivum* L.). Both studies, referred to as "Study 1" and "Study 2," included measurements of biomass production, grain yield, and grain quality. Study 1 also evaluated the accumulation of polycyclic aromatic hydrocarbons (PAHs) in grain, whereas Study 2 contextualized the trends in wheat growth with measurements of soil respiration and C:N dynamics. This research is valuable because it provides direct comparison between TD soils and native, noncontaminated soils using a commonly grown commodity crop. Thus, the findings of this study are relevant in planning future remediation projects involving agricultural soil aimed toward returning the land crop production.

Materials and Methods

Soil Source, Properties, and Preparation

The two studies were conducted in a greenhouse at North Dakota State University, Fargo, ND. The treatments for each experiment were a series of mixtures composed of two different soil materials: (i) TS: native, noncontaminated topsoil taken from 0- to 20-cm depth, and (ii) TD: PHC-contaminated soil that had been treated by ex situ thermal desorption. In Study 1, treatments were composed, by weight, of TD soils as 100 (TD), 90 (TD90), 70 (TD70), 40 (TD40), and 0% (TS). In Study 2, treatments were composed of TD soils as 100 (TD), 95 (TD95), 75 (TD75), 50 (TD50), and 0% (TS). In both Studies 1 and 2, the balance of weight in each treatment was filled by TS. Notably, the TD soil material was a mixture of contaminated subsoil from up to 15 m below ground surface, so it did not originate from the zone of soil genesis. Thus, the contaminated soil is not directly comparable with TS. Despite this distinction, for ease of reference, this material will be referred to hereafter as "TD soil." This material was thoroughly mixed in a stockpile prior to treatment, so specific depth of TD soil in the profile could not be identified.

The TS was mapped as Williams-Zahl loam (Williams: fine-loamy, mixed, superactive, frigid Typic Argiustoll; Zahl: fine-loamy, mixed, superactive, frigid Typic Calciustoll) (USDA-NRCS, 2015). The TD soils were contaminated in situ with Bakken crude oil from a pipeline leak before being excavated, mixed in a stockpile, and treated by a RS40 Thermal Desorption/Oxidation unit at 350°C for 10 min. Soil characteristics for the TS and TD soil are shown in Table 1. Finally, soils were passed through a 6-mm sieve, air dried at 25°C, and stored in plastic containers in a climate-controlled greenhouse prior to the study.

Experimental Setup

In both Studies 1 and 2, mixtures were created by adding the soil for each pot to a two shell dry blender (Patterson-Kelly Company) and mixing for 5 min. Each pot in Study 1 (40 total) held 4 kg of soil, whereas those in Study 2 (40 total) held 3 kg of soil. Plastic bags were placed within the pots to prevent leaching of water. Both studies received the same fertilizer treatments. Soil P was normalized by adding dissolved calcium monophosphate [$Ca(H_2PO_4)_2$] at varying levels per treatment to reach a target rate of 15 mg P kg^{-1} (Franzen, 2014). Each pot was then subjected to one of two N treatments: (i) no additional N, or (ii) N addition to a target rate of 62.5 mg N kg^{-1} (Franzen, 2014). To attain the target N rate, dissolved calcium nitrate [$Ca(NO_3)_2$] was added at varying rates depending on starting concentration.

In Study 1, 18 HRSW (cultivar Barlow) seeds were sown 2.5 cm deep into each pot using three rows of six seeds. Seeds were spaced 2 cm apart, rows spaced 4 cm apart, and pots were covered to retain moisture during the germination period. Fourteen days

Table 1. Selected soil properties of native, noncontaminated topsoil (TS) and contaminated subsoil material treated by thermal desorption (TD) at time of planting. Standard error included in parentheses. Both TS and TD were the same materials in Studies 1 and 2. The values for the soil mixtures in both studies can be calculated using the TS:TD ratio of each treatment.

Property†	Units	Soil	
		TS	TD
Sand	g kg^{-1}	454 (23)	434 (17)
Silt	g kg^{-1}	330 (90)	326 (14)
Clay	g kg^{-1}	219 (22)	239 (9)
SOC	g kg^{-1}	30 (4)	1.5 (0.7)
EC	dS m^{-1}	0.3 (0.1)	1 (0.3)
pH		7.4 (0.2)	8.2 (0.1)
TPH	mg kg^{-1}	42 (13)	101 (63)
Plant available nutrients			
NO_3–N	mg kg^{-1}	17.6 (0.8)	0.7 (0.1)
NH_4–N	mg kg^{-1}	10.2 (0.9)	7.8 (0.2)
P (Olsen)	mg kg^{-1}	8.9 (0.2)	3.3 (0.2)
K	mg kg^{-1}	248 (2)	193 (1)
Ca	mg kg^{-1}	3216 (59)	4707 (17)
Mg	mg kg^{-1}	636 (8)	690 (5)
Na	mg kg^{-1}	17.4 (0.2)	114 (2)
Fe	mg kg^{-1}	49.1 (1.7)	10 (0.1)
Mn	mg kg^{-1}	51.1 (5.2)	25.4 (0.6)
Cl	mg kg^{-1}	1.9 (0.1)	20.1 (0.3)

† SOC, soil organic carbon; EC, electrical conductivity; TPH, total petroleum hydrocarbons.

after sowing (DAS), the number of seeds per pot was reduced to six. In Study 2, 12 HRSW seeds were sown 2.5 cm deep into each pot in a circle around an open middle and covered during the germination period. At the time of seeding, a polyvinyl chloride (PVC) ring (10 cm diameter, 3 cm high) was installed to 1.5-cm depth to accommodate soil respiration sampling, described below. At 14 DAS, the number of seeds per pot was reduced to six. In both studies, pots were watered up to 80% of field capacity (volumetric water content at 33 kPa) every other day, and position in the greenhouse was rotated biweekly. Both studies were terminated after 12 wk, with all treatments having reached maturity.

Data Collection

Plant Growth and Soil Nutrients

After termination, the aboveground biomass was clipped at 1 cm above the soil surface, dried at 60°C, and weighed. Grain was dried at 60°C, weighed, and a subsample was used to quantify total N using the combustion method (Agvise Laboratories); using this total N value, a conversion factor of 5.6 (Tkachuk, 1969) was applied to determine protein content. Soil cores were taken from the center of each pot to a depth of 14 cm using a 4-cm-diameter hand probe, and subsamples from the cores were analyzed for parameters shown in Table 1. After removing the cores, the remaining soil in each pot was screened through a 2-mm sieve. Roots retained on the sieve were collected, washed, dried at 40°C, and weighed.

Soil organic C (SOC) was determined as the difference between total C and total inorganic C found using a Primacs Total Organic Carbon Analyzer (Skalar Analytical B.V.). Both pH and electrical conductivity were found using 1:1 soil-water extraction (Watson and Brown, 1998; Whitney, 1998b; Agvise Laboratories). Plant available NO_3–N and NH_4–N were determined using KCl extraction, and P was quantified by the Olsen method (Mulvaney, 1996; Frank et al., 1998; Agvise Laboratories). Plant available K, Ca, Mg, and Na were quantified by optical emission-inductively coupled plasma using the ammonium acetate extraction method (Warncke and Brown, 1998; Agvise Laboratories). Iron and Mn were found with the diethylenetriaminepentaacetic acid (DTPA) sorbitol method (Whitney, 1998a), and Cl was found with the Hg (II) thiocyanate method (Gelderman et al., 1998; Agvise Laboratories).

Study 1: Contaminant Uptake

For Study 1, subsamples from cores of TD and TS were evaluated for total petroleum hydrocarbon (TPH) concentration within the C10 to C36 range using EPA 8015 method modified with silica gel (Pace Analytical Services). Additionally, grain samples were analyzed for the presence of 16 PAHs regulated by the USEPA (Keith, 2015) using EPA 8270 by selected ion monitoring (Pace Analytical Services). Due to analysis costs, each treatment within the N-added and no-N-added blocks was pooled together for quantifying PAHs. Given the results from Study 1, as well as the high cost, the same analysis was not performed in Study 2.

Study 2: Root Analysis and Soil Respiration

For Study 2, roots were collected off a 2-mm sieve and, prior to drying and weighing, scanned and analyzed using WinRhizo 2012 software (Regent Instruments, 2012) to obtain root length, surface area, and volume (adapted from Bauhus and Messier, 1999). Soil respiration was quantified by weekly measurements of CO_2 efflux taken from each pot using an environmental gas monitor (EGM-4) equipped with a soil respiration chamber (Fouché et al., 2014; SRC-1, PP Systems) that attached to the PVC ring installed in each pot. The order of data collection from the pots was systematically rotated each week to avoid bias based on sampling time of day.

Statistical Analysis

Although the intent of using different ratios in Study 2 was to build a regression of soil mixtures by percentage, the variability inherent in greenhouse studies, as well as the differences in pots and amount of soil used, required that statistics be analyzed on each study separately. Biomass and yield parameters were reported as a relative proportion by dividing each treatment value by the value obtained for the TS to allow for better comparison between Studies 1 and 2. The remaining measurements were left in absolute terms.

All biomass and grain results were analyzed using one-way ANOVA with mean difference significance at $\alpha = 0.05$. Pairwise comparisons were conducted with a post-hoc Tukey honestly significant difference test. All statistical tests were performed with R 3.2.1 software using the stats (R Core Team, 2014) and multcomp (Hothorn et al., 2008) packages.

Results and Discussion

Wheat Growth

The trends in wheat biomass growth and grain yield were similar in both studies (Fig. 1), although total biomass and grain production were much greater in Study 1 than in Study 2 (data

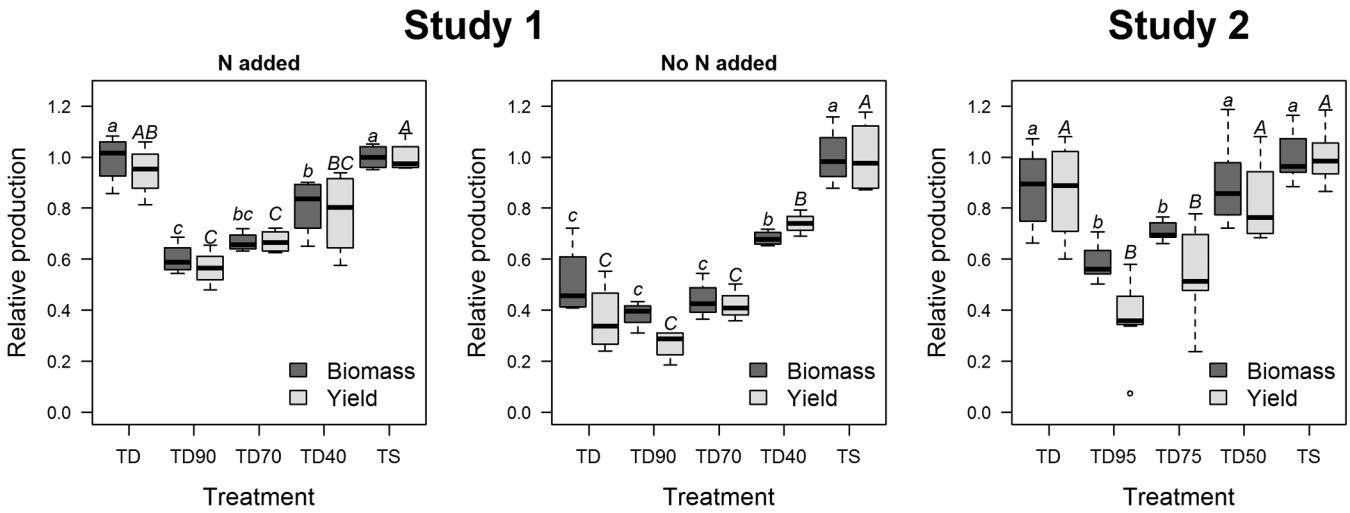

Fig. 1. Boxplots showing relative wheat production of biomass and grain yield with respect to the mean value of the native topsoil (TS) treatment for each plot, respectively. In Study 1, treatments were composed, by weight, of ex situ thermal desorption (TD) soils as 100 (TD), 90% (TD90), 70 (TD70), 40 (TD40), and 0% (TS). In Study 2, treatments were composed of TD soils as 100 (TD), 95 (TD95), 75 (TD75), 50 (TD50), and 0% (TS). In both Studies 1 and 2, the balance of weight in each treatment was filled by TS. Different letters within boxplots indicate significance at the $\alpha = 0.05$ level in Tukey's honestly significant difference test, with lowercase letters corresponding to biomass and uppercase letters corresponding to grain yield. Study 1 is divided by pots with N added (up to 62.5 mg N kg^{-1}) and no N added. Study 2 had the same fertilizer application, but no statistical response to fertilizer was evident; thus, all pots from Study 2 are shown together.

not shown). In addition to overall reduced growth, no response to N addition was evident in Study 2; thus, analyses of data from Study 2 were not partitioned by N application. Nonetheless, the relative growth trends were similar between Studies 1 and 2. The TD soils produced as much biomass as TS, except in the no-N-added pots in Study 1. Overall, wheat response was likely unaffected by TPH levels, since TPH levels of 1000 mg kg^{-1} and above have not inhibited wheat germination, root elongation (Tang et al., 2011; Shahsavari et al., 2013), biomass (Issoufi et al., 2006), or yield (Kisic et al., 2010).

In Study 1, TD soils without N addition produced ~40% as much grain as TS, which corroborates with other studies where growth in TD soils was 40 to 60% that of uncontaminated soil (Vidonish et al., 2016; Yi et al., 2016). This response is likely the result of less plant available N and SOC in the TD soils than in TS soils at time of planting (Table 2). These lower quantities of available nutrients may be the result of using subsoil material for the TD treatment and/or because plant available nutrient levels and SOM were altered by the soil heating process. Increasing the temperatures above 220°C, as occurred in this study, results in losses of organic N through the destruction of SOM (Varela et al., 2010) and increased losses of plant available N (Pape et al., 2015); consequently, plant production is also reduced (Giovannini et al., 1990).

The wheat growth in TD soils in the N-added pots from Study 1, as well as in all TD soils in Study 2, was comparable with that in TS (Fig. 1). This production is likely the result of controlled growing conditions and abundance of soil nutrients and water, so these results may not be reproduced under field conditions. Notably, this research used plastic bags to prevent leaching through the duration of the experiment. Since TD treatment sharply increases soil saturated hydraulic conductivity (O'Brien et al., 2016) and decreases water retention (Roh et al., 2000), it may likely enhance the leaching of SOC when saturated (O'Brien et al., 2016), causing losses of plant available nutrients. Under field conditions, these losses would vary and be difficult to account for with fertilizer rates in nonirrigated environments. Nonetheless, this study showed that, under appropriate nutrient management and no water stress, TD soils could match TS in wheat growth and productivity.

Creating soil mixtures by addition of TS to TD soils resulted in a severe decrease in biomass growth and grain yield, although these metrics recovered with a greater proportion of TS added to the mixtures. In both studies, the addition of any TS to TD soils resulted in significant declines in biomass growth (up to 40%) and yield (up to 60%). This growth reduction after soil mixing agrees with findings of Roh et al. (2000), where fescue (*Festuca arundinacea* Schreb.) grown in a 1:1 mixture of TS and TD soil produced half as much biomass as the TD soil alone. This growth trend is likely the result of biological interactions associated with the reintroduction of soil microorganisms into the TD soil and alterations of total soil N and total soil C pools; this interaction is explored more fully later. Briefly, the exposure of soil with low biological activity, TD, to a soil with a fully functioning biological community, TS, resulted in the recolonization of the entire pot. During this recolonization, soil microbial populations used resources (i.e., nutrients, water) that may otherwise have been allocated to the wheat. Thus, mixing the soil reduced overall wheat growth, while wheat growth in unmixed TD soil remained high because it had less competition from soil microorganisms.

Wheat Quality

Grain protein content also showed a response to N application in Study 1, but not in Study 2. In both studies, the TD soils produced grain with less protein than other treatments (Fig. 2). Although nearly all values reported in this study are >152 g kg^{-1}, the typical protein content in field-grown Barlow HRSW (Mergoum et al., 2011), the comparatively low values in TD soils indicate an underlying issue. In Study 1, this reduced protein content in the grain is likely due to N deficiency at the grain filling stage. Protein content in grain grown in TD soils was less than (unfertilized pots) or very close to (fertilized pots), a critical

Table 2. Concentration of 16 USEPA priority polycyclic aromatic hydrocarbons (PAHs) in wheat grain grown in soil mixtures from Study 1, divided by those treatments with nitrogen added (NA) and those with no added nitrogen (NN). Bold values indicate detection of compound within wheat grain. Italic values indicate the method detection limit for each compound that was not detected. These limits change between treatments due to limits of grain sample size. Thus, the ΣPAH (detected) is the lowest possible value of total ΣPAH concentration, whereas ΣPAH (possible) is the highest possible value of total ΣPAH concentration.

PAH	TD†		TD90		TD70		TD40		TS	
	NA	NN	NA	NN	NA	NN	NA	NN	NA	NN
	μg kg^{-1}									
Acenaphthene	*0.40*	*2.6*	*0.84*	*5.9*	*0.65*	*1.9*	*0.53*	*0.76*	*0.37*	*0.52*
Acenaphthylene	*0.89*	*5.7*	*1.8*	*13.0*	*1.4*	*4.2*	*1.2*	*1.7*	*0.82*	*1.1*
Anthracene	*1.1*	*7.0*	*2.2*	*16.0*	*1.8*	*5.1*	*1.4*	*2.0*	*1.0*	*1.4*
Benzo(a)anthracene	*0.50*	*3.2*	*1.0*	*7.3*	*0.80*	*2.4*	*0.66*	*0.93*	*0.46*	*0.63*
Benzo(a)pyrene	*0.69*	*4.4*	*1.4*	*10.1*	*1.1*	*3.2*	*0.90*	*1.3*	*0.63*	*0.87*
Benzo(b)fluoranthene	**0.44**	*2.7*	*0.88*	*6.2*	*0.69*	*2.0*	**1.0**	**0.91**	*0.39*	*0.54*
Benzo(g,h,i)perylene	*0.81*	**16.7**	*1.7*	*11.9*	*1.3*	**44.4**	*1.1*	*1.5*	*0.74*	*1.0*
Benzo(k)fluoranthene	*0.90*	*5.8*	*1.9*	*13.3*	*1.5*	*4.3*	*1.2*	*1.7*	*0.83*	*1.2*
Chrysene	*0.62*	*4.0*	*1.3*	*9.1*	*1.0*	*2.9*	*0.81*	*1.2*	*0.57*	*0.79*
Dibenz(a,h)anthracene	**2.2**	*4.2*	**2.8**	*9.7*	**1.4**	*3.1*	**6.2**	**3.8**	**1.8**	**5.0**
Fluoranthene	*0.64*	**5.4**	*1.3*	**11.2**	*1.0*	**4.6**	**0.86**	**1.7**	*0.59*	*0.81*
Fluorene	*1.4*	*8.8*	*2.8*	*19.9*	*2.2*	*6.4*	*1.8*	*2.5*	*1.2*	*1.7*
Indeno(1,2,3-cd)pyrene	**37.7**	**14.0**	**51.9**	*15.5*	**26.1**	**28.6**	**78.9**	**47.7**	**26.5**	**59.8**
1-Methylnaphthalene	*1.1*	*7.2*	*2.3*	*16.3*	*1.8*	*5.2*	*1.5*	*2.1*	*1.0*	*1.4*
2-Methylnaphthalene	*1.0*	*6.7*	*2.2*	*15.3*	*1.7*	*4.9*	*1.4*	*2.0*	*0.96*	*1.3*
Naphthalene	*0.56*	*3.6*	*1.2*	*8.2*	*0.90*	*2.6*	**1.0**	*1.0*	*0.51*	*0.71*
Phenanthrene	**0.97**	**16.1**	**2.7**	**33.1**	**1.5**	**14.1**	**1.7**	**1.9**	**0.80**	**1.1**
Pyrene	**0.56**	*2.5*	*0.80*	*5.7*	*0.62*	*1.8*	**0.90**	**1.4**	*0.35*	*0.49*
ΣPAHs (**detected**)	**41.9**	**52.2**	**57.4**	**44.3**	**29**	**91.7**	**90.6**	**57.4**	**29.1**	**65.9**
ΣPAHs (*possible*)	52.5	121	81.0	228	47.2	142	103	76.1	39.5	80.4

† Treatments were composed of ex situ thermal desorption (TD) soils as 100 (TD), 90 (TD90), 70 (TD70), 40 (TD40), and 0% (native topsoil, TS). The balance of weight in each treatment was filled by TS.

value of 130 g kg^{-1} for HRSW grown in the northern Great Plains (Selles and Zentner, 2001); protein content below these levels at harvest normally indicates that the wheat is N deficient.

Although low protein indicates N deficiency, high protein does not necessarily indicate sufficient N levels (Selles and Zentner, 2001). Despite overall higher protein contents in Study 2, wheat in TD soils may still have been N deficient at harvest. Since biomass production was comparatively high, these lower protein levels for the TD treatment suggest that timing plays a role in the differences in N availability in TD soils. This timing indicates that the wheat grown in TD soils depleted the available N during biomass production, and the soil did not have sufficient buffer capacity or mineralization rate to replenish plant available N at the time of grain filling. Conversely, the TS and mixtures all have higher protein levels, which could indicate higher levels of available N at the grain-filling stage (Beres et al., 2008). Therefore, these findings imply a mechanism associated with N cycling that differs between the TD-only pots and the pots containing some TS. This mechanism is likely biological and related to SOM, as soil physical and chemical characteristics

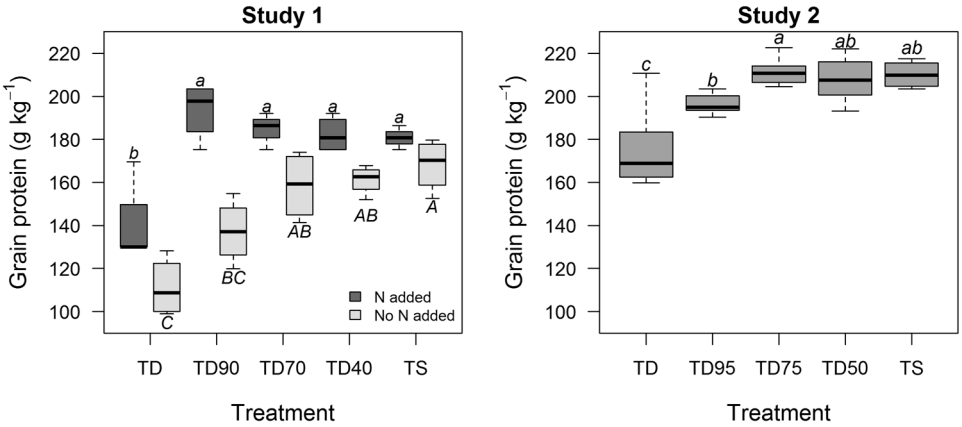

Fig. 2. Protein content of wheat grown in Studies 1 and 2. Study 1 is separated by pots with N added (up to 62.5 mg N kg^{-1}) and no N added. In Study 1, treatments were composed, by weight, of ex situ thermal desorption (TD) soils as 100 (TD), 90 (TD90), 70 (TD70), 40 (TD40), and 0% (native topsoil, TS). In Study 2, treatments were composed of TD soils as 100 (TD), 95 (TD95), 75 (TD75), 50 (TD50), and 0% (TS). In both Studies 1 and 2, the balance of weight in each treatment was filled by TS. Different letters within boxplots indicate significance at α = 0.05 level in Tukey's honestly significant difference test, with lowercase letters corresponding to pots with N added, and uppercase letters corresponding to pots with no N added. Study 2 had the same fertilizer application, but no statistical response to fertilizer was evident; thus, all pots from Study 2 are shown together.

are not drastically changed after TD treatment (Roh et al., 2000; O'Brien et al., 2016; Sierra et al., 2016). Consequently, Study 2 incorporated soil respiration and analysis of total C and total N pools to contextualize some of the biomass trends.

Soil Respiration and C and N Pools

Soil respiration may be used as an indicator for microbial abundance and activity associated with nutrient cycling (Luxhoi et al., 2006). However, these relationships may not be exact due to total respiration being the sum of several sources of CO_2 efflux (Kuzyakov, 2006), including soil fauna, root, and microbial respiration. In this study, no soil macrofauna were in the pots, and no aboveground plant structures were in the chamber during measurement, so plant respiration was limited to the root structures. Generally, as root biomass increases, respiration increases (Kocyigit and Rice, 2006; Qiao et al., 2009). However, no metrics of root growth varied significantly between treatments (data not shown), so differences in overall respiration in this study are likely not associated with root respiration but with microbial respiration.

The cumulative respiration was three times higher in TS than TD soils (Fig. 3). Total respiration values determined for TS were similar to those found under a wheat system in a field experiment (Frank et al., 2006) and slightly lower than values found under winter wheat–soybean [*Glycine max* (L.) Merr.] rotation in a field experiment (Hu et al., 2013). In comparison with these field experiments, the respiration under TD soils in this study was greatly diminished. Further, the reduction grew more severe over time, as the respiration rate decreased sharply in the final 3 wk in both TD and TD95 pots, whereas the rates remained steady in TS, TD50, and TD75. Relative to TS, mean values for respiration during the first 9 wk in TD and TD95 were 32 (±4%) and 34% (±5%), respectively. In the final 3 wk, these mean values dropped to 17% (±4%) for TD and 24% (±5%) for TD95. This stagnation in respiration may represent a point in time in which resource stores from fertilization were depleted. Thus, these values may be more indicative of respiration in TD soils without additional resource input.

The decreased respiration in TD soils may also be explained by lower microbial biomass (Colman and Schimel, 2013) associated with lower SOC, especially as the soil mixtures showed increasing respiration as more TS was added. Addition of SOC to TD soils via TS mixing likely resulted in microbial recolonization (Marschner and Rumberger, 2004), since microbial biomass and activity are diminished in the TD process (Cébron et al., 2011; Yi et al., 2016). In other cases of recolonization of microbial communities after soil heating, the microbial reestablishment is normally accompanied by a burst of soil respiration (Bárcenas-Moreno and Baath, 2009; Bárcenas-Moreno et al., 2014). In this study, the burst would be expected after soil mixing due to the addition of SOC to the TD soils, as well as the fertilizer application.

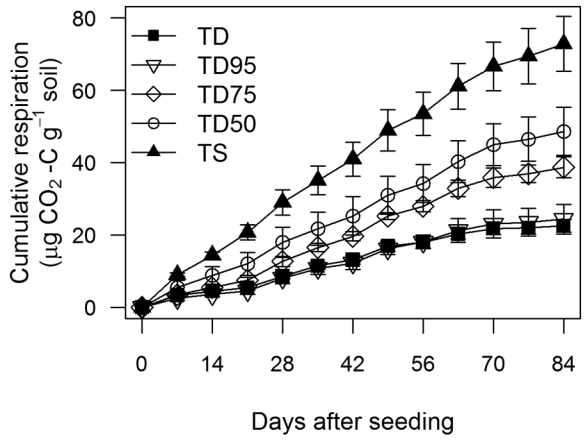

Fig. 3. Mean cumulative respiration for each treatment throughout the course of Study 2. Treatments were composed of ex situ thermal desorption (TD) soils as 100 (TD), 95 (TD95), 75 (TD75), 50 (TD50), and 0% (native topsoil, TS), and the balance of weight in each treatment was filled by TS. Each data point is the mean value of all pots within a given treatment for each week and is shown with standard error bars.

Notably, this study did not find an initial burst, as the magnitude of respiration did not change greatly between weeks until the final 3 wk (Fig. 3). This lack of response is likely because measurements were taken every 7 d, so any response between measurements was not observed. The timeframe for this recolonization can be very short, as bacteria levels may recover and stabilize within 5 d (Bárcenas-Moreno et al., 2011). Although this reestablishment and stabilization can occur rapidly (Guerrero et al., 2005; Bárcenas-Moreno and Baath, 2009), microbial populations may require more than a year to reach background levels (Hamman et al., 2007). Further, these soils were heated up to 18 mo before the mixing, so some level of stabilization after recolonization by wind deposition and dust particles may be expected prior to this study. Once stabilized, the long-term respiration trends and associated biological processes were likely regulated by soil N and C pools rather than the effects of the TD process.

Respiration was correlated with both total soil N and SOC (Fig. 4A and 4B). These figures identify clear group separation on the basis of treatment, with the exception of TD and TD95, which suggests that addition of only 5% TS may be insufficient for recovery of microbial communities in one growing season. These respiration levels may also be used to make inferences about N cycling, as several studies have identified correlations between respiration and both gross immobilization and gross mineralization (Hart et al., 1994; Bengtsson and Bergwall, 2000; Luxhoi et al., 2006). Thus, much more immobilization and mineralization are likely occurring in the TS than the TD soil, and these metrics increase in TD soil as more TS is added.

Conversely, C:N ratios show an inverse correlation with respiration, but the group separation is still evident (Fig. 4C). Although respiration values may serve as good indicators of gross mineralization and immobilization, they are not good predictors of net mineralization and immobilization (Hart et al., 1994; Song et al., 2011), so the partitioning of those values is unclear. However, net N mineralization and immobilization may be correlated with C:N (Barrett and Burke, 2000; Accoe et al., 2004). Generally, C:N ratios >20 will result in net immobilization, whereas lower ratios will result in net mineralization. This study identified values that indicate a close balance of mineralization and immobilization in the TS and TD50, whereas the remainder of the treatments tended toward net immobilization.

Despite reduced overall cycling and a tendency toward net immobilization in TD and TD95, mineralization was occurring in all treatments throughout the experiment at varying rates. The growth of the wheat was then dependent on the ability of the plant to outcompete soil microorganisms for the available N. Assuming a constant ability to compete throughout the treatments, this interaction explains the wheat growth in the pots.

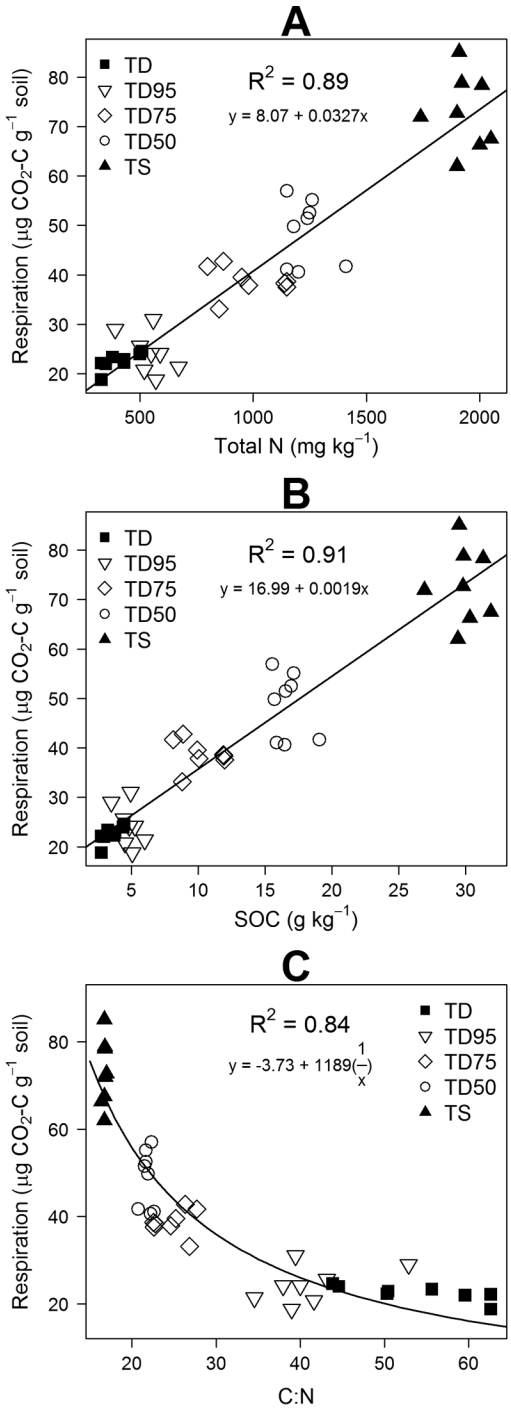

Fig. 4. Cumulative respiration plotted against (A) total soil N, (B) total soil organic carbon (SOC), and (C) soil C:N for each treatment in Study 2. Treatments were composed of ex situ thermal desorption (TD) soils as 100 (TD), 95 (TD95), 75 (TD75), 50 (TD50), and 0% (native topsoil, TS), and the balance of weight in each treatment was filled by TS.

The pool of available N was lowest in the TD95 pots, so a wheat plant that competed for a proportion of that pool received the least N. As the pool of available N increased, the total N that the plant was able to compete for increased and facilitated greater growth. Although this study identified important trends in wheat growth and soil respiration in controlled conditions, further research evaluating how TD soils respond to field conditions over successive growing seasons is required to determine how long it takes to return the land to prespill levels of crop productivity and soil health.

Contaminant Uptake

Whereas the soil contamination was measured using TPH, wheat grain contamination was measured using 16 PAHs identified by the USEPA (Zelinkova and Wenzl, 2015). These PAHs are commonly used to assess grain safety (Jones et al., 1989; Kobayashi et al., 2008; Ciecierska and Obiedzinski, 2013), as they are a significant threat to human health. Table 2 shows the concentrations of each PAH for the grain samples from N-added pots and no-N-added pots for each treatment. Notably, the concentration of many compounds was below the detection limit (shown in italics), which fluctuated according to sample size of grain. The possible ΣPAH concentration is then reported as the summation of detections (bold) and the method detection limits. This conservative approach is appropriate when describing food safety, and it avoids dangers involved with omitting nondetects (Helsel, 2006).

The ΣPAH levels in this study were much higher than wheat grain found in the United Kingdom (4.3 μg kg^{-1}; Jones et al., 1989), Poland (2.4 μg kg^{-1}; Ciecierska and Obiedzinski, 2013), and California (<5 μg kg^{-1}; Kobayashi et al., 2008), although they were comparable with wheat grain from agricultural fields in China (80 μg kg^{-1}; Li and Ma, 2016) and Syria (154 μg kg^{-1}; Khalil and Al-Bachir, 2015). Despite these relatively elevated levels, the ΣPAHs may not indicate that this grain is unsuitable for human consumption. In fact, no standards for PAHs in foodstuffs exist in the United States (ATSDR, 2013). Further, these levels are comparable with those found in other food, such as carrots (*Daucus carota* L.), which ranged from 48 to 94 μg kg^{-1} (Kipopoulou et al., 1999), and much less than is often found on leafy vegetables, which reach up to 294 μg kg^{-1} in lettuce (*Lactuca sativa* L.; Kipopoulou et al., 1999) or 850 μg kg^{-1} in spinach (*Spinacia oleracea* L.; Khan and Cao, 2012). Thus, the ΣPAHs are comparable with food directly consumed by humans on a daily basis (Menzie et al., 1992; Martí-Cid et al., 2008).

Conclusions

The results from these greenhouse studies indicate that oil-contaminated soils treated by TD may be able to safely produce grain at similar levels to native TS when supplied with sufficient nutrients and water, although grain protein may be diminished. However, the differences in soil properties, especially biological processes, suggest that soil health in TD soils was not equivalent to TS. These differences may not have been entirely the result of TD treatment, as the TD material was taken from up to 15 m below ground surface and would not be comparable with TS prior to treatment. Thus, the effects of TD are heavily dependent on the nature and origin of the treated soil. Nonetheless, until SOM and soil respiration recover on these soils, they may be susceptible to nutrient and water stress that will likely occur under field conditions. Despite substantially less wheat production, mixing the TS with TD soils increased SOC, total N, and, consequently, respiration, which shows that mixing may enhance recovery of soil health. This study suggests that these TD-treated soils are capable of producing safe, high-quality grain yield, but the recovery of long-term soil health may be accelerated by mixing TD soils with agricultural topsoil.

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