Accepted Manuscript
Version of record at: https://doi.org/10.1039/D1EM00447F

Abiotic Reduction of 3-Nitro-1,2,4-triazol-5-one (NTO) and Other Munitions Constituents by Wood-Derived Biochar through Its Rechargeable Electron Storage Capacity

Danhui Xin[†], *Julián Girón*[†], *Mark E. Fuller*[‡], *and Pei C. Chiu*[†*]

[†]Department of Civil and Environmental Engineering, University of Delaware, Newark, DE 19716

[‡]Aptim Federal Services, 17 Princess Road, Lawrenceville, NJ 08648

[*]Corresponding Author

Pei C. Chiu, Department of Civil and Environmental Engineering, University of Delaware, Newark, DE 19716; Email: pei@udel.edu; https://orcid.org/0000-0003-2319-4496

Authors

Danhui Xin, Department of Civil and Environmental Engineering, University of Delaware, Newark, DE 19716; Email: danhui@udel.edu; https://orcid.org/0000-0002-5267-9727

Julián Girón, Department of Civil and Environmental Engineering, University of Delaware, Newark, DE 19716; Email: juliangp@udel.edu

Mark E. Fuller, Aptim Federal Services, 17 Princess Road, Lawrenceville, NJ 08648; Email: mark.fuller@aptim.com

Pei C. Chiu, Department of Civil and Environmental Engineering, University of Delaware, Newark, DE 19716; Email: pei@udel.edu; https://orcid.org/0000-0003-2319-4496

Accepted Manuscript
Version of record at: https://doi.org/10.1039/D1EM00447F

Abstract

The environmental fate of 3-nitro-1,2,4-triazol-5-one (NTO) and other insensitive munitions constituents (MCs) is of significant concern due to their high water solubility and mobility relative to legacy MCs. Plant-based biochars have been shown to possess a considerable electron storage capacity (ESC), which enables them to undergo reversible electron transfer reactions. We hypothesized biochar can act as a rechargeable electron donor to effect abiotic reduction of MCs repeatedly through its ESC. To test this hypothesis, MC reduction experiments were performed using wood-derived biochars that were oxidized with dissolved oxygen or reduced with dithionite. Removal of aqueous NTO, an anion at circumneutral pH, by oxidized biochar was minimal and occurred through reversible adsorption. In contrast, NTO removal by reduced biochar was much more pronounced and occurred predominantly through reduction, with concomitant formation of 3-amino-1,2,4-triazol-5-one (ATO). Mass balance and electron recovery with ferricyanide further showed that (1) the amount of NTO reduced to ATO was relatively constant (85−100 μmol per gram of biochar) at pH 6–10; (2) the fraction of biochar ESC reactive toward NTO was *ca*. 30% of that toward ferricyanide; (3) the NTO-reactive fraction of the ESC was regenerable over multiple redox cycles. We also evaluated biochar transformation of other MCs, including nitroguanidine (NQ), 2,4-dinitroanisole (DNAN), and hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX). While mass and electron balances could not be established due to sorption, DNAN and RDX reduction by reduced biochar was confirmed via detection of multiple reduction products. In contrast, NQ was not reduced under any of the conditions tested. This study is the first demonstration of organic contaminant degradation through biochar's rechargeable ESC. Our results indicate biochar

Accepted Manuscript
Version of record at: https://doi.org/10.1039/D1EM00447F

is a regenerable electron storage medium and sorbent that can remove MCs from water through concurrent reduction and sorption, and is thus potentially useful for pollution control and remediation at military facilities.

Accepted Manuscript
Version of record at: https://doi.org/10.1039/D1EM00447F

Introduction

Munitions constituents (MCs) used in explosive formulations have caused contamination of soil and groundwater at/near military sites across the U.S.[1] 3-Nitro-1,2,4-triazol-5-one (NTO), nitroguanidine (NQ), and 2,4-dinitroanisole (DNAN) are the major constituents in the insensitive formulation IMX-101, and NTO, DNAN, and hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) are the main constituents in the formulation IMX-104.[2, 3] While insensitive MCs are less prone to accidental detonation than legacy MCs such as 2,4,6-trinitrotoluene (TNT), they are more water-soluble.[4-6] For example, the solubility of NTO is 16,642 mg/L at 25 °C[7], two orders of magnitude higher than that of TNT (100–128 mg/L at 25 °C).[8, 9] This suggests that insensitive MCs are more leachable and mobile in the environment and thus may pose a significant contamination problem and health concern.[4, 10-14] Hence, it is necessary to develop cost-effective pollution control and remediation approaches for insensitive MCs at military sites.

Biochar is a subset of black carbon produced through pyrolysis of biomass such as wood and grass.[15, 16] Because of its high specific surface area, biochar has been evaluated as a sorbent for removing organic chemicals[17-19] including pesticides, dyes, and MCs[20, 21] such as TNT and RDX. However, the effectiveness of biochar as a sorbent has not been evaluated against insensitive MCs like NTO, which is an anion at circumneutral pH (pK_a = 3.76).[22-24]

In addition to being a sorbent, biochar is capable of mediating redox reactions through two different mechanisms: electron conduction[25-29] and electron storage.[30, 31] The first mechanism requires external electron donor and acceptor to be in simultaneous contact with a conductive moiety in biochar, such as graphene domains[32, 33] that conduct electrons from a donor to an acceptor such as 2,4-dinitrotoluene, RDX, and nitroglycerin.[26-29]

Accepted Manuscript
Version of record at: https://doi.org/10.1039/D1EM00447F

In contrast to the conduction mechanism, the electron storage mechanism involves redox-facile functional groups in biochar. Electrons can be stored in biochar through reduction of its electron accepting functional groups, such as quinones, or removed from biochar through oxidation of its electron donating functional groups, such as hydroquinones.[30] The capacity of a biochar to store and reversibly exchange electrons with its surroundings is termed electron storage capacity (ESC), and is operationally defined to be the sum of electron donating capacity (EDC) and electron accepting capacity (EAC).[30, 31, 34] While EDC and EAC vary with the redox state of a biochar, the ESC is constant for a given pair of reductant and oxidant used to measure ESC.[34, 35] Studies have shown that ESC is a common property of biochar prepared via pyrolysis of lignocellulosic biomass.[35] Biochar ESC can range from 0.2 to 7 mmol e^-/g, is distributed over a broad range of reduction potential, and is highly reversible over multiple redox cycles.[30, 34, 36-39]

Biochar can be an electron donor when its ESC is filled (i.e., its redox-facile functional groups reduced), and an electron accepter when its ESC is empty (i.e., its functional groups oxidized). Electrons stored in biochar have been shown to be partially available for abiotic reduction of metal(loid)s[40, 41, 42] and for microbial reduction of nitrate.[31] However, it is unknown whether electrons stored in biochar are available for organic contaminant degradation and, if so, to what extent, and whether this degradative capacity is regenerable.

We hypothesized that biochar ESC can be accessed by MCs for reductive transformation, and that this process can be repeated through recharge of biochar ESC. This study was designed to (1) test this hypothesis, (2) determine the portion of ESC that is accessible to NTO and other MCs (DNAN, NQ, and RDX), and (3) produce data to support evaluation and development of biochar-based MC treatment/remediation systems. We performed batch experiments to assess the ability of wood-derived biochars to adsorb and degrade NTO in buffered solutions at pH 6−10

and in artificial stormwater runoff (ASR) at pH 6. Parallel experiments were conducted using dithionite-reduced and air-oxidized biochars to study MC reduction and adsorption, respectively.

Materials and Methods

Chemicals. NTO and RDX were obtained from U.S. Army Armament Research Development and Engineering Center (Picatinny, NJ). The RDX contained 3.8% octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX) as an impurity. NQ (25% moisture content) and DNAN (98%) were purchased from Sigma-Aldrich (St. Louis, MO). 3-Amino-1,2,4-triazol-5-one (ATO, >99%) was purchased from Princeton BioMolecular Research (Princeton, NJ). 2-Amino-4-nitroanisole (2ANAN) and 4-amino-2-nitroanisole (4ANAN) were purchased from Apollo Scientific (Cheshire, UK), and 2,4-diaminoanisole (DAAN, 99.6%) from Honeywell Fluka (Charlotte, NC). The three nitroso reduction intermediates of RDX, hexahydro-1-nitroso-3,5-dinitro-1,3,5-triazine (MNX), hexahydro-1,3-dinitroso-5-nitro-1,3,5-triazine (DNX), and hexahydro-1,3,5-trinitroso-1,3,5-triazine (TNX), were purchased from SRI International (Menlo Park, CA). Pure standards of NTO, NQ, DNAN, RDX, and HMX were acquired from AccuStandard (New Haven, CT). Additional chemicals for buffer solutions, ASR, pH control, high-performance liquid chromatograph (HPLC), and biochar redox titration, are provided in Table S1 of the Supporting Information. All chemicals were used as received.

Biochars. Two wood-derived commercial biochars, Soil Reef biochar (SRB) and Rogue biochar (Rogue), were used. Physical-chemical properties of SRB and Rogue were measured and described in Section S1 (Supporting Information) and summarized in Table S2. SRB and Rogue are both derived from pine wood but were pyrolyzed at different temperatures (550 °C and 900 °C, respectively). SRB was chosen for this study because it has been field-tested for

Accepted Manuscript
Version of record at: https://doi.org/10.1039/D1EM00447F

stormwater treatment[43] and its ESC and reversibility were well-characterized[31, 34, 40, 44]. Rogue was included because its BET surface area (407±9 m^2/g) and ESC (7.07±0.15 mmol/g) measured with titanium(III) citrate and dissolved O_2 (DO) were the highest of all commercial biochars we have tested.[35] These values are roughly two times those of SRB (158±3 m^2/g and 3.54±0.13 mmol/g, respectively).

To facilitate MC reduction, SRB and Rogue particles in the 250–500 μm size range were ground at 4000 rpm for 3 min using a Beadbug 3 bead homogenizer (Benchmark Scientific Inc., Sayreville, NJ) to obtain a particle size of <53 μm. Ground biochars were then oxidized with DO in continuously aerated deionized water to deplete stored electrons and bring the EDC of biochar to zero with respect to DO (E_H = +0.80 V vs. standard hydrogen electrode or SHE, at pH 7 and P_{O_2} = 0.21 atm). Based on our previous work, SRB and Rogue needed to be oxidized in aerated deionized water for at least 3 d to ensure thorough oxidation of reduced functional groups[34, 35]. Oxidized biochar samples were collected on a glass microfiber filter, dried at 65 °C for 24 h, and stored in a desiccator until use.

For each biochar, two types of samples were prepared: DO-oxidized biochar (SRB_{OX} and $Rogue_{OX}$) and dithionite-reduced biochar (SRB_{RED} and $Rogue_{RED}$). SRB_{OX} or $Rogue_{OX}$ were depleted of electrons and served as sorption controls, whereas SRB_{RED} or $Rogue_{RED}$ were used to study MC reduction. To prepare dithionite-reduced biochars, SRB_{OX} and $Rogue_{OX}$ were placed in an anaerobic glove box under 98±0.5% N_2 and 2.0±0.5% H_2 (P_{O_2} < 5 ppm) (Coy Laboratory, Grass Lake, MI) to deoxygenate. SRB_{OX} or $Rogue_{OX}$ was then reduced with freshly prepared 25 mM sodium dithionite in 100 mM citrate buffer at pH 6.4 for 3 d (measured E_H = –0.43 V vs. SHE). Dithionite was added in excess and was replenished as needed. After reduction, SRB_{RED} and $Rogue_{RED}$ were collected on a glass microfiber filter, rinsed thoroughly with copious DO-

free deionized water, vacuum-dried, and stored in a desiccator in a glove box until use. All reported data are based on dry mass.

Batch experiments. Batch reactors were set up and experiments were conducted inside an anaerobic glove box. SRB_{RED} or $Rogue_{RED}$ was used for MC reduction experiments, and SRB_{OX} or $Rogue_{OX}$ was used as nonreactive control and for MC sorption experiments. A summary of the conditions for batch experiments, including the initial concentration of MC, biochar dose, and solution matrices, is provided in Table S3.

NTO reduction in buffer solutions. Batch experiments were performed with SRB for NTO reduction in pH 6, 8 and 10 buffer solutions. To initiate an experiment, a predetermined amount of SRB_{OX} or SRB_{RED} (0.40 or 0.80 g/L) was added to amber borosilicate reactors containing 125 mL of 110 µM NTO in 50 mM pH buffer. MES, Tris, and CAPSO were used to maintain the pH at 6.0±0.1, 8.0±0.1, and 10.0±0.1, respectively. Blanks (no biochar) were prepared identically. All reactors were placed on an orbital shaker at 100 rpm. Samples of 0.625 mL were taken at different elapsed times and immediately passed through 0.22-µm PTFE syringe filters for HPLC analysis. Experiments were performed for up to 600 h until the concentration of ATO plateaued. All collected samples combined accounted for <10% of the total solution volume in each reactor. Removal of NTO and ATO due to sampling was accounted for to establish mass balance. After reaction, SRB was retrieved on a glass microfiber filter, vacuum-filtered, and rinsed thoroughly with deionized water. Since NTO and ATO (pK_a = 8.71, Figure S1) did not sorb to SRB at pH 10 (Figures 1(c) and S2), SRB samples retrieved from pH 6 and 8 reactors were extracted with 50 mL of 50 mM CAPSO solution at pH 10 to recover sorbed NTO and ATO. Extraction was performed for up to 600 h and extracts (0.625 mL) were sampled at different times for HPLC analysis.

SRB electron content measurement. The electron content (i.e., EDC) of fresh SRB_{RED} and spent SRB_{RED} recovered from NTO experiment was measured using ferricyanide as an oxidant (E_H = +0.43 V vs. SHE)[45]. Reactors containing fresh SRB_{OX} and SRB_{OX} exposed to the same NTO solution were included as controls. SRB samples were placed in 0.23 L of 1 mM ferricyanide solution in 50 mM MES, Tris, or CAPSO buffer. Electrons transferred from SRB to ferricyanide were determined based on the amount of ferricyanide consumed. Concentration of ferricyanide was measured by absorbance at 420 nm, using a Vernier LabQuest 2 UV-vis spectrophotometer (Beaverton, OR). The extinction coefficients of ferricyanide at pH 6, 8, and 10 were 1135 ± 40, 1152 ± 40, and 1058 ± 40 $M^{-1}\cdot cm^{-1}$, respectively. Each SRB sample was oxidized with ferricyanide for up to 72 h. After EDC measurement, SRB was collected by filtration and vacuum-dried at 65 °C for weight measurement.

ESC reversibility for NTO reduction. Additional experiments were performed to assess the reversibility of ESC with respect to NTO reduction over three redox cycles. To prevent sorption of NTO and ATO to SRB, experiments were run at pH 10 in 50 mM CAPSO buffer. Four pairs of duplicate reactors containing 125 mL of 100 µM NTO were prepared: 0.80 g/L of SRB_{RED} was added to the first two pairs of reactors, 0.80 g/L of SRB_{OX} was added to the third pair of reactors, and no biochar was added to the last pair of reactors. For each cycle, NTO reduction was run for 24 h following the same procedures as described above. After reaction, SRB was retrieved on a glass microfiber filter, vacuum-filtered, thoroughly rinsed with 50 mL of 50 mM CAPSO buffer three times. For the first pair of reactors containing SRB_{RED}, the retrieved SRB_{RED} was reduced with 50 mL of 1 mM dithionite (total e^- content = 100 µmol) for 24 h, washed with 50 mL of deoxygenated deionized water three times, and used in the next cycle of NTO reduction. This pair of reactors was labeled as "ESC-recharged". Note that, based on SRB

porosity, any residual dithionite remaining in the pores of SRB_{RED} would contribute to <1% of the electrons transferred to NTO. For the second pair of reactors containing SRB_{RED}, the retrieved SRB_{RED} was labeled "ESC-not-recharged". The procedures for the second cycle of NTO reduction were the same, where both "ESC-recharged" and "ESC-not-recharged" SRB_{RED} were added to 125 mL of 100 µM NTO in 50 mM CAPSO buffer and allowed to react for 24 h. After the second cycle, "ESC-recharged" SRB_{RED} was reduced again with dithionite for the third cycle. Biochar-free and SRB_{OX} controls were included in each cycle.

MC reduction in ASR. To assess the performance of biochar in synthetic stormwater, batch experiments were also carried out in ASR, which was established based on the composition of stormwater samples collected from an east coast U.S. Navy facility and consisted of 0.38 mM Na^+, 0.24 mM K^+, 0.09 mM NH_4^+, 0.08 mM Ca^{2+}, 0.05 mM Mg^{2+}, 0.65 mM Cl^-, 0.15 mM SO_4^{2-} and 0.02 mM NO_3^-.[46] Batch experiments were performed with $Rogue_{OX}$ or $Rogue_{RED}$ for NTO, NQ, DNAN, and RDX in ASR at pH 6. To compare SRB and Rogue, an additional experiment with SRB (SRB_{OX} or SRB_{RED}) was run for NTO in ASR at pH 6 under identical conditions. The pH was controlled at 6.0±0.2 during experiment using 0.05 N HCl. The solution was sampled (0.625 mL for NTO and DNAN, 6 mL for RDX) at different times. A portion of the RDX sample (5 mL) was used for NO_2^- analysis and the rest for RDX and transformation product measurement. After each experiment, biochar samples were subjected to solvent extraction. NTO, NQ, and daughter products were extracted with a 3:7 (v/v) mixture of acetonitrile and 0.1% trifluoroacetic acid, whereas DNAN, RDX, and daughter products were extracted with an 8:2 mixture of acetonitrile and 0.1% trifluoroacetic acid.[47] Each biochar sample was extracted three times. Samples of MCs and daughter products from multiple extractions were combined to obtain the total recovery.

MC sorption isotherm. Experiments were performed to obtain MC sorption isotherms for $Rogue_{OX}$ in ASR at pH 6. To obtain an isotherm, a series of duplicate amber borosilicate reactors were set up that contained ASR and an MC at different initial concentrations. The biochar doses and MC concentration ranges (Table S4) were selected based on preliminary experiments and the solubility of each MC used. Samples (0.8 mL) were collected at different incubation times and passed through 0.2-μm PTFE syringe filters for HPLC analysis. Experiments were run for up to 400 h until an apparent sorption equilibrium was reached (variations in aqueous concentrations <1% per hour). pH was maintained at 6.0±0.2 using 0.05 N HCl. For comparison, we ran an additional set of batch experiments to obtain the sorption isotherm for NQ and SRB_{OX} in 50 mM Tris buffer at pH 8.0±0.1.

For each MC, the mass sorbed per gram of biochar (C_s) was plotted against the equilibrium aqueous concentration (C_{eq}), and the data were fitted to a Langmuir isotherm (Eq. 1) using the least-square method.

$$C_s = \frac{K_L C_{eq} C_{s,max}}{1+K_L C_{eq}} \quad \text{[Eq. 1]}$$

where C_s (μmol/g) is sorbed MC mass per gram of biochar, C_{eq} is equilibrium MC concentration in water (μM), $C_{s,max}$ (μmol/g) and K_L (μM^{-1}) are fitted maximum sorption capacity and sorption affinity, respectively. After each sorption experiment, biochar samples that sorbed the maximum mass of MC were subjected to solvent extractions, as described above for MC reduction experiments.

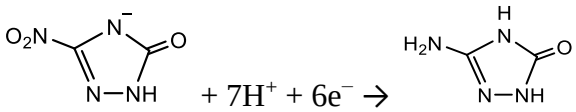
Analytical methods. MCs were analyzed using an Agilent 1200 Series HPLC (Santa Clara, CA) equipped with an Agilent 1260 diode array detector. The hydrophilic analytes NTO, ATO, and NQ were separated using a Thermo Scientific (Waltham, MA) Hypercarb porous graphitic carbon column (4.6 mm×100 mm, 5 μm particle size). A mixture of acetonitrile and 0.1%

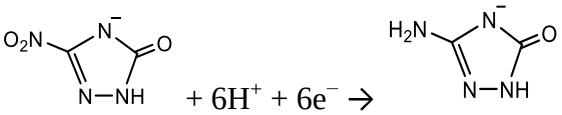
trifluoroacetic acid was used as eluent at a flow rate of 2.0 mL/min. The run time was 10 min and the temperature was 34.0 °C. NTO, ATO, and NQ were detected at 7.9, 4.3, and 5.8 min and quantified based on absorbance at 318, 210, and 260 nm, respectively. The hydrophobic analytes, including DNAN, RDX, HMX, and their daughter products, were separated using an Agilent Zorbax SB-C18 column (4.6 mm×50 mm, 3.5 μm particle size). A mixture of phosphate buffer and methanol was used as eluent at a flow rate of 1.7 mL/min. The run time was 7 min and the temperature was ambient. DNAN and RDX were detected at 4.8 and 3.4 min, respectively, and quantified based on absorbance at 214 nm. The same method was used to quantify daughter products of DNAN[48] and RDX[49, 50]: 2-ANAN, 4-ANAN, and DAAN were measured at 4.2 min (254 nm), 3.2 min (234 nm) and 2.3 min (210 nm), respectively, and MNX, DNX, and TNX were detected at 2.9, 2.4, and 1.9 min, respectively, based on absorbance at 234 nm. Nitrite, a potential RDX reduction product,[51] was measured using Hach NitriVer® 3 Nitrite Reagent (Loveland, CO) and a Vernier LabQuest 2 UV-vis spectrophotometer.

Results and Discussion

NTO removal by SRB. Figure 1 shows NTO removal by SRB_{OX} and SRB_{RED} at pH 6, 8, and 10 and the mass balance at the end of each experiment. SRB_{OX} adsorbed NTO in small amounts which were largely recovered by extraction. NTO sorption decreased with increasing pH, with 80%, 93% and 99% of the initial mass remaining in solution at equilibrium at pH 6, 8, and 10, respectively (solid blue bars in Figure 1(d)). As NTO is anionic (pK_a 3.76)[22] at all three pH values, the decreasing sorption (24, 10, and 2 μmol/g, respectively) was most likely due to increasingly negative surface charge of SRB with pH.[52] Extraction of SRB_{OX} from pH 6 and 8 reactors with CAPSO buffer yielded mass balances of 94% and 101%, respectively.

In contrast, significantly more NTO was removed from solution by SRB_{RED}, and ATO was produced concomitantly, indicating NTO was reduced to ATO by SRB_{RED}, as shown in Eq. 2. Reduction of NTO to ATO was rapid in the first 24 h and continued at decreasing rates for up to 600 h. Note that Figure 1(a)–(c) are semi-log plots and therefore the changing slopes represent decreasing pseudo-first-order rate constants. The decreasing NTO reduction rate constants could be due to one of two reasons (or both). First, it has been shown that a large portion of the ESC resides in the interior of SRB particles,[34] and the rate of access is limited by pore diffusion, which is approximately two orders of magnitude slower than diffusion in the bulk solution. Second, the redox-active functional groups (or ESC) of biochar are distributed over a range of reduction potentials[34, 53] and hence would react with NTO at a spectrum of rate constants. The decreasing NTO reduction rate over time likely reflects a combination of slow diffusion through tortuous channels to access reactive ESC residing in deep pores in biochar interior, and slow reaction with functional groups of increasing reduction potentials (i.e., decreasing reactivities).





[Eq. 2]

The combined aqueous NTO and ATO masses were about 80% at pH 6 and 8 and virtually 100% at pH 10. This suggests that, in contrast to NTO, ATO was sorbed to a similar extent at pH 6 and 8 but negligibly at pH 10. This was independently confirmed, as shown in Figure S2. As the pK_a of ATO had not been reported, we performed a titration (Section S2) and determined the pK_a of ATO to be 8.71 (Figure S1). This is in agreement with the pH effect on ATO sorption, as ATO would be predominantly neutral at pH 6 and 8 and negatively charged at pH 10, where

sorption would be hindered by electrostatic repulsion between negatively charged ATO and biochar surface (pH_{zpc} = 2–3[52]).

Because complete mass balance was achieved at pH 10 based on aqueous NTO and ATO (dotted line in Figure 1(c)), all NTO removal by SRB_{RED} was attributed to reduction. This also confirms that no alkaline hydrolysis of NTO or ATO occurred at pH 10 over 600 h. Following extraction, the mass balances for all SRB_{RED} reactors were between 91% and 100% (Figure 1(d)). The remaining sorbed mass was likely sorbed through interactions that could not be reversed by pH increase, such as the π–π donor-acceptor interactions[21] between graphitic regions of SRB and the aromatic NTO^- anion[23].

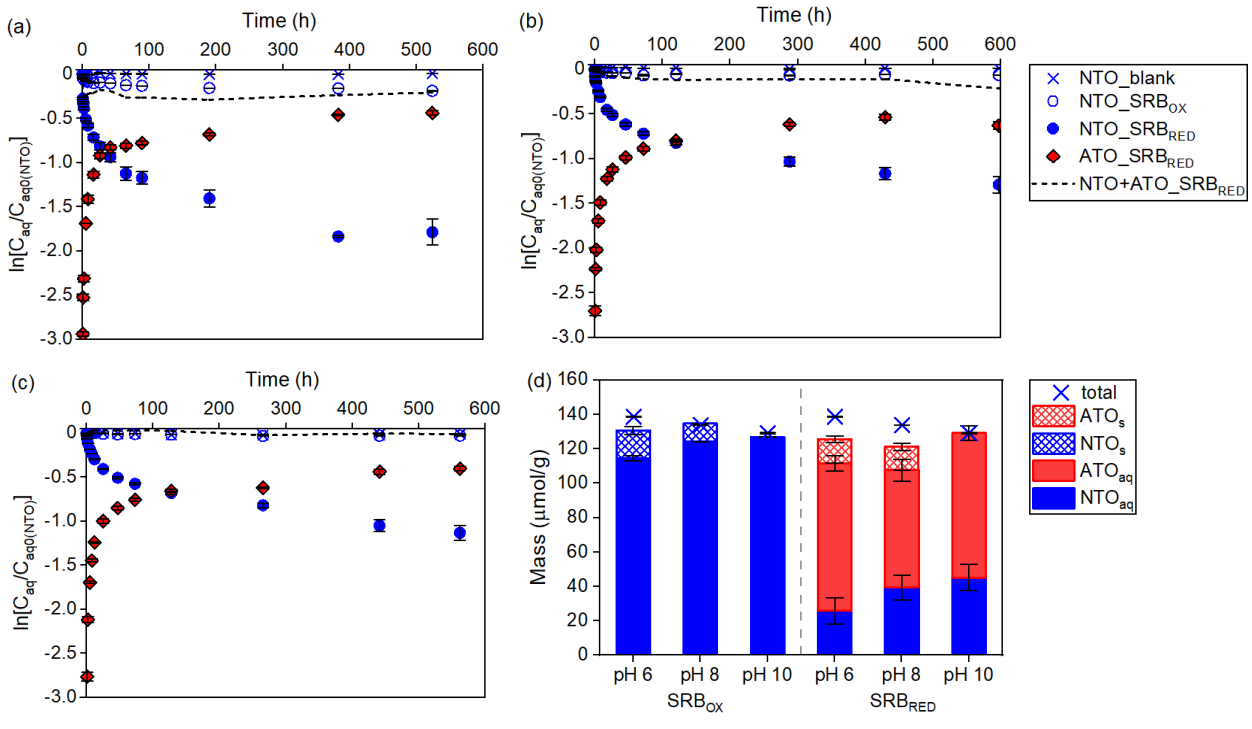
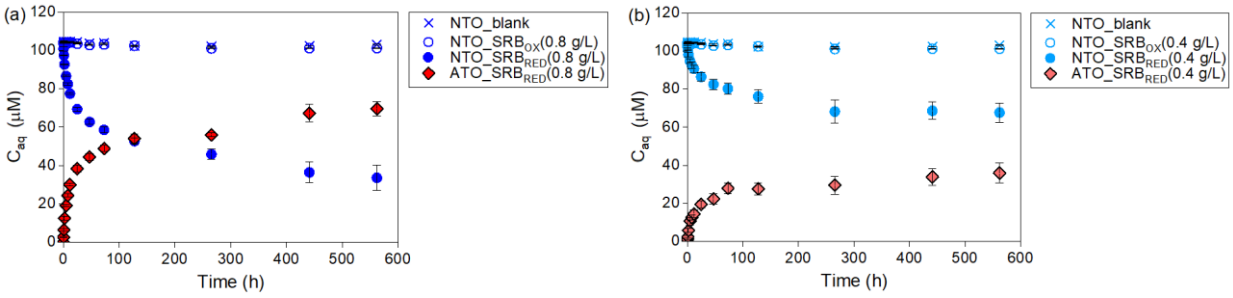


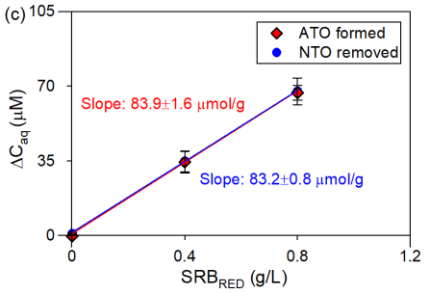
Figure 1. Aqueous concentration (C_{aq}) of NTO and ATO over time with 0.80 g/L of SRB_{OX} or SRB_{RED} at (a) pH 6 (50 mM MES) (b) pH 8 (50 mM Tris), and (c) pH 10 (50 mM CAPSO), and (d) mass balance at the end of experiment. The Y axis in panels (a)–(c) shows the natural

291 logarithm of C_{aq} of NTO or ATO relative to the initial NTO concentration ($C_{aq0(NTO)}$=110 µM).
292 The total mass is based on blank (without SRB). NTO_{aq} and ATO_{aq} are the final masses in the
293 aqueous phase, and NTO_s and ATO_s the sorbed masses extracted from solid.
294
295 *ESC available for NTO reduction.* Figure 2(a and b) shows aqueous NTO removal and ATO
296 formation with 0, 0.40, and 0.80 g/L of SRB_{OX} or SRB_{RED} at pH 10. NTO removal and ATO
297 formation were observed with SRB_{RED}, but not SRB_{OX}. When the SRB_{RED} mass increased from
298 0.40 to 0.80 g/L, the amounts of NTO removed and ATO produced both doubled (Figure 2(c)),
299 indicating the quantity of electrons per gram of SRB_{RED} available for NTO reduction within 600
300 h, that is, the fraction of SRB's ESC that was accessible to and of sufficiently low reduction
301 potential to reduce NTO, was constant. Given the fact that 6 electrons per molecule are required
302 to convert NTO to ATO (Eq. 2), the portion of ESC that was available for NTO reduction at pH
303 10 was 499 and 503 µmol e^-/g SRB, respectively, based on the amounts of NTO reduced
304 (83.2±0.8 µmol/g) and ATO formed (83.9±1.6 µmol/g). At pH 6 and 8, the total amounts of
305 ATO formed with 0.80 g/L of SRB_{RED} were 100±7 and 82±8 µmol/g (Table S5), corresponding
306 to 600 and 492 µmol e^-/g, respectively, as shown in Figure 3 (red bars).
307

308



309



310 Figure 2. (a) Aqueous concentration (C_{aq}) of NTO and ATO over time with 0.80 g/L of SRB_{OX}
311 or SRB_{RED} at pH 10. (b) C_{aq} of NTO and ATO over time with 0.40 g/L of SRB_{OX} or SRB_{RED} at
312 pH 10. (c) Concentrations of ATO produced and NTO removed by SRB_{RED}.

313

314 Although the reduction potential distribution of biochar's ESC has not been fully delineated, it
315 appears to cover a broad range of potential.[34] Because one gram of SRB_{OX} can store up to 4.0
316 mmol of e^- with dithionite as a reductant (E_H = –0.43 V vs. SHE at pH 6.4),[34, 54] the result above
317 suggests that only 500–600 μmol/g of the stored electrons in SRB_{RED}, or 12–15% of SRB's ESC,
318 had sufficiently low reduction potential to reduce NTO. An effort to establish an electron balance
319 for NTO reduction by SRB_{RED} using DO as an oxidizing agent (E_H = +0.80 V vs. SHE at pH 7)
320 was hindered by the volatile nature of DO. Therefore, ferricyanide (E_H = +0.43 V vs. SHE at pH
321 7) was used instead to retrieve electrons from SRB_{RED} before and after reaction with NTO.[55] If
322 all the electrons accessible to and reactive toward NTO could also reduce ferricyanide, the
323 amount of electrons remaining in used SRB_{RED} at the end of an NTO reduction experiment

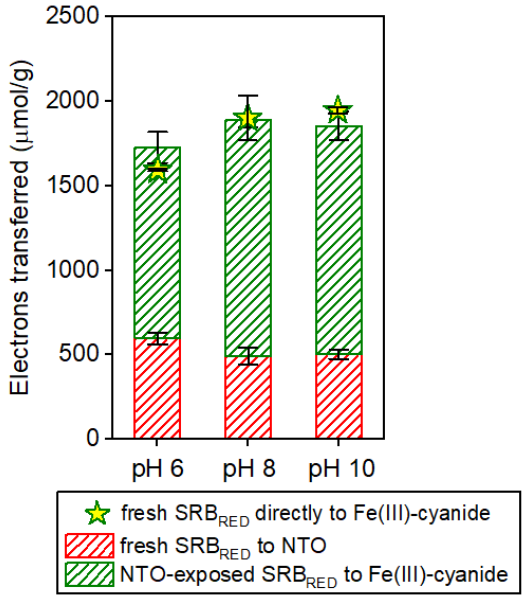
would be the difference between all retrievable electrons from unused fresh SRB_{RED} and the electrons consumed by NTO:

e^- retrieved from NTO-exposed SRB_{RED}

= (e^- retrieved from fresh SRB_{RED}) – (e^- consumed for NTO reduction) [Eq.3]

The data in Figure 3 confirm this relationship and show the ESC of SRB that is reactive towards NTO was 26–38% of that reactive towards ferricyanide, suggesting NTO is significantly more difficult to reduce than ferricyanide.

The ESC retrieved by ferricyanide increased notably from 1591 μmol/g to 1899 μmol/g when pH increased from 6 to 8. Since the reduction potential of ferricyanide is pH-independent, this result suggests that the functional groups that constitute ESC have acid-base properties and deprotonate between pH 6 and 8. To illustrate this pH effect, we use 9,10-anthrahydroquinone-2,6-disulfonate (AH_2QDS, pK_a = 7.6) as a hypothetical hydroquinone in SRB to show (Section S4 and Figure S3) that the thermodynamic driving force for the oxidation of AH_2QDS by ferricyanide is lower at pH 6 (when the hydroquinone is fully protonated) than at pH 8 and 10 (when the hydroquinone is singly deprotonated). Hence, biochar's ESC is a function of pH, even when measured using the same reductant and oxidant (e.g., dithionite and ferricyanide), and pH should be specified when ESC is reported.



342

343 Figure 3. Electron balance for NTO reduction by SRB_{RED}. Electrons of fresh SRB_{RED} consumed
344 by NTO was calculated based on ATO formation.

345

346 *Rechargeability of ESC for NTO reduction.* The ESC of biochar has been shown to be reversible
347 over multiple oxidation-reduction cycles.[34, 35] This suggests that, as a rechargeable electron
348 storage medium, biochar should be able to reduce contaminants repeatedly through recharging.
349 This is demonstrated in Figure 4. Consistent with our earlier results, removal of aqueous NTO by
350 SRB_{OX} was negligible at pH 10 (which was chosen to minimize sorption of NTO and ATO). In
351 contrast, NTO was transformed to ATO stoichiometrically by SRB_{RED}. For the first 24-h cycle,
352 the NTO removed from solution by SRB_{RED} was *ca.* 32 µM (corresponding to *ca.* 38 µmol/g
353 SRB_{RED}, Figure S4), in good agreement with the data in Figure 1(c). The used SRB_{RED} ("ESC-
354 not-recharged") recovered from the first cycle was unable to reduce NTO to any measurable
355 extent upon transfer to a fresh NTO solution, suggesting the most reactive electrons had been
356 exhausted. When the used SRB_{RED} was recharged with dithionite ("ESC-recharged"), NTO was
357 reduced to ATO in comparable quantities to those in the first cycle (Figure S4). Stoichiometric

358 conversion of NTO to ATO was also observed in the third cycle, albeit in a slightly lower
359 quantity (32 μmol/g). This indicates the fraction of the ESC that had sufficiently low redox
360 potentials (and thus sufficiently high reactivity) to reduce NTO could be recharged repeatedly.
361 The electrons in the ESC that could reduce NTO within 24 h in the second and third cycles were
362 101% and 85% that in the first cycle, respectively, demonstrating high reversibility of the ESC of
363 SRB for NTO transformation. Therefore, if placed in a sufficiently reducing environment, such
364 as a stormwater treatment or groundwater (bio)remediation system, biochar may be repeatedly
365 recharged, either chemically[34] or microbially[31, 43], and continuously degrade contaminants such
366 as NTO and other munitions compounds (shown below).

367 Much research to date on the fate and treatment of NTO has focused on sorption[56-58] and
368 biodegradation.[59-61] Because ATO can be readily mineralized to innocuous products such as CO_2,
369 NH_4^+, and N_2,[62] reduction to ATO may be a first step to achieve complete NTO mineralization.
370 Recent studies further showed that NTO can be abiotically reduced to ATO by geo-constituents
371 including the hematite-Fe^{2+} redox couple[24], FeS minerals[63], hydroquinones and humic acids[64].
372 Because ESC is a universal property of plant-based biochars,[35] our results suggest that pyrogenic
373 carbon, another redox-active geo-constituent that is ubiquitous in soils and sediments,[65, 66] may
374 also mediate the abiotic reduction of NTO through the same mechanism. Our result therefore has
375 broader implications for the roles of black carbon in controlling the fate of MCs in subsurface
376 environments as well as in engineered systems.
377

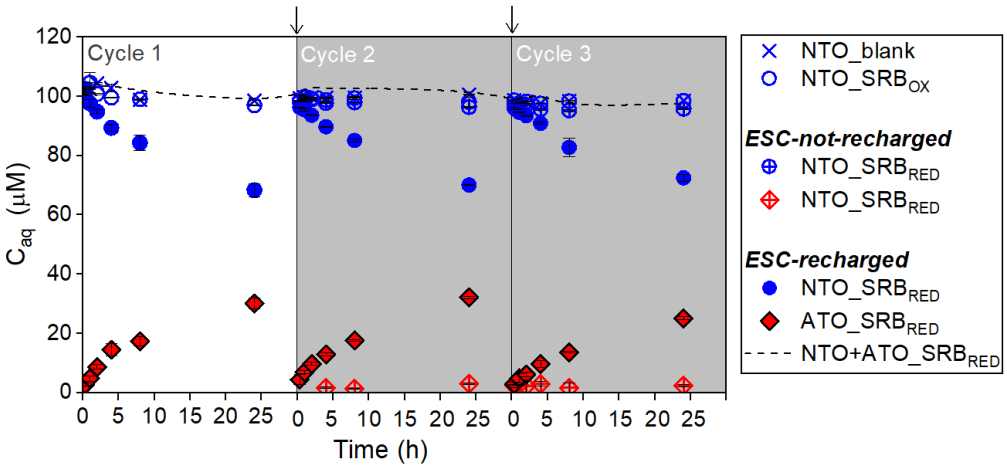


Figure 4. Rechargeability of biochar ESC for repeated NTO reduction. Aqueous concentrations (C_{aq}) of NTO and ATO are shown as a function of time with 0.80 g/L of SRB_{OX} or SRB_{RED} at pH 10. After each cycle, NTO solution was replaced and, for "ESC-recharged", the recovered SRB_{RED} was recharged with dithionite for the next reaction cycle (↓). The error bars for the SRB_{RED} data in the first cycle represent one standard deviation from four replicates.

MC sorption by biochar. A goal of this study was to evaluate the potential utility of biochar for removing MCs in treatment systems. To that end, we evaluated removal of NTO, NQ, DNAN, and RDX by biochar through two mechanisms, i.e., sorption and abiotic reduction. Unlike NTO, which is negatively charged under circumneutral conditions, NQ, DNAN, and RDX are neutral and less water-soluble, which suggests that sorption may play a greater role in their removal by biochar. Hence, we first characterized the sorption of these MCs to biochar before investigating their potential reductive transformation. Rogue, a commercial wood-derived biochar with high BET surface area and ESC, was used to assess its sorption and reduction capacities for MCs in ASR at pH 6. As shown in Figure S5, all four MCs were removed rapidly from solution as soon

as oxidized Rogue ($Rogue_{OX}$) was added. Removal subsequently slowed but continued for up to 340 h until an apparent equilibrium was reached.

The equilibrium concentration of sorbed and aqueous MCs from Figure S5 were used to construct sorption isotherms. The data for each MC were fitted to a Langmuir isotherm, as shown in Figure 5 and Table 1. The maximum sorption capacities ($C_{s,\,max}$) of Rogue for NTO, NQ, DNAN, and RDX were 154, 388, 476, and 213 µmol/g in ASR at pH 6, corresponding to 2.0, 4.0, 9.4, and 4.7% of the biochar mass, respectively. We recovered 83-88% of the removed MC mass by solvent extraction (Table S6) but did not detect any of the reduction intermediates or products (see below) throughout incubations, suggesting all MCs were removed by $Rogue_{OX}$ predominantly or exclusively through sorption.

The $C_{s,\,max}$ and K_L values, representing sorption capacity and affinity of MCs for $Rogue_{OX}$, respectively, were highest for DNAN. This was expected based on its high K_{OW} and K_{OC} values (Table 1) and the strong π–π interactions resulting from its electron-withdrawing nitro groups. Despite the low solubility of RDX, the non-aromatic structure may have contributed to the considerably lower $C_{s,max}$ compared to DNAN.[67, 68] Although NQ has high solubility and the lowest molecular weight, the $C_{s,max}$ for NQ was twice that for NTO on a mass basis, suggesting the charge of NTO hindered its sorption. Overall, Rogue showed significant sorption capacities for MCs, especially for neutral and aromatic MCs such as DNAN. When NQ sorption to SRB and Rogue was compared (Section S5, Figure S7(b)), $C_{s,max}$ for SRB was 98 µmol/g (1.0% of SRB mass), or one-fourth of the $C_{s,max}$ for Rogue. This shows that Rogue exhibits a greater sorption capacity for MCs than SRB, presumably due to its higher BET surface area (Table S2).

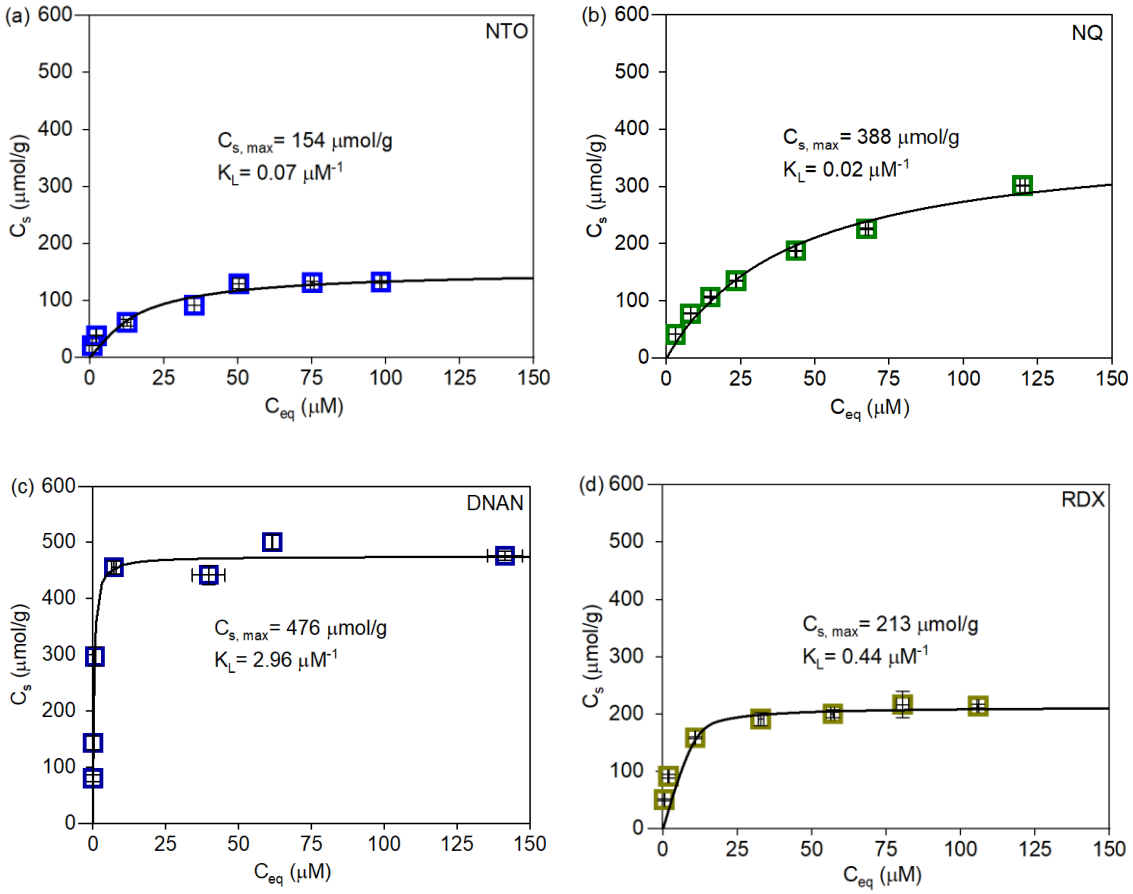


Figure 5. MC sorption to $Rogue_{OX}$ in ASR at pH 6 and the fitted Langmuir isotherms. (a) NTO (b) NQ (c) DNAN (d) RDX

MC reduction by biochar. The reactivity of biochar toward DNAN, RDX, and NQ was assessed by comparing MC removal by $Rogue_{OX}$ (sorption only) and $Rogue_{RED}$ (sorption and reduction). NQ was removed from solution at similar rates and to the same extent with SRB_{OX} and SRB_{RED} (Figure S8), suggesting NQ was removed through sorption to both oxidized and reduced SRB and was not reduced by SRB_{RED}. The similar removal also suggests that the nature and density of sorption sites for NQ were not measurably altered by dithionite reduction. NQ has been reported to undergo reduction with bimetallic particles[69], but it does not appear to react with reduced black carbon. Consistent with this result, a recent study showed that NQ was inert toward

carbonaceous reductants such as dithionite-reduced hydroquinones and humic acids.[70] Hence, abiotic reduction by carbonaceous reductants may not be an important fate mechanism for NQ even under highly reducing conditions.

In contrast to NQ, NTO, DNAN, and RDX were all reducible by reduced biochar. As shown in Figure 6(a), reduction of NTO by SRB_{RED} in ASR at pH 6 was in good agreement with that in MES buffer (Figure 1(a)), suggesting that the solution matrix did not influence the reactivity of the biochar. Under the same conditions, NTO was similarly reduced to ATO by $Rogue_{RED}$, indicating the reactivity of reduced biochar toward NTO is not specific to SRB. Interestingly, $Rogue_{RED}$ and SRB_{RED} converted comparable amounts of NTO to ATO (91 and 94 μmol/g, respectively, Table 2). This suggests that the fraction of Rogue ESC reactive toward NTO was about 564 μmol/g, approximately the same as that for SRB (546 μmol/g), despite the higher ESC of Rogue. Note that the amount of NTO reduced per gram of SRB or Rogue depends on not the total ESC, but the fraction of ESC that has sufficiently low E_H (i.e., contains sufficiently reducing electrons) to degrade NTO. Without knowing the redox potential distributions (i.e., the E_H profiles) of SRB and Rogue, it is not possible to compare the biochars' capacities to degrade NTO.

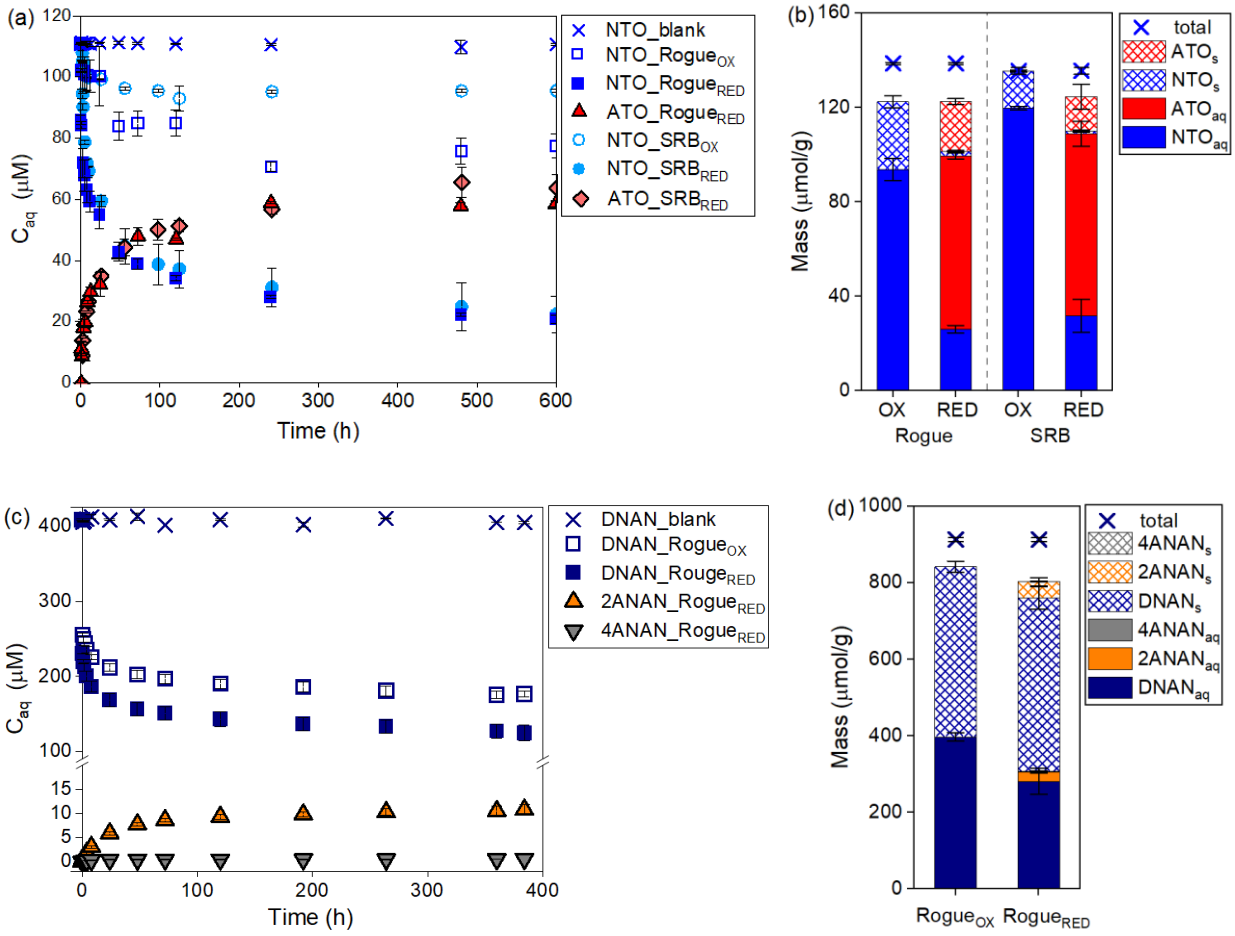
$Rogue_{OX}$ removed 518 μmol/g of DNAN and 232 μmol/g of RDX at the end of experiment (Table 2), consistent with the fitted $C_{s,max}$ values of 476 and 213 μmol/g, respectively (Table 1). In comparison, $Rogue_{RED}$ removed additional 112 μmol/g of DNAN and 100 μmol/g of RDX, suggesting these MCs were not only sorbed but chemically reduced by $Rogue_{RED}$. This was confirmed through identification of reduced products of DNAN and RDX. Unlike NTO, however, DNAN and RDX sorbed more strongly to Rogue, and the sorbed molecules might not

be readily available for reduction. Therefore, only relatively small fractions of the DNAN and RDX removed from water was recovered as reduced products.

Abiotic reduction of DNAN by $Rogue_{RED}$ was confirmed by the detection of 66 μmol/g of 2ANAN and a trace quantity (<2 μmol/g) of 4ANAN in the aqueous and solid phases combined. 2ANAN is also the major intermediate product of DNAN during chemical[63, 71] and microbial[72] reduction, because the ability of the methoxy group to be hydrated by water molecules helps to better solvate the *ortho* nitro group and enable it to bear a greater negative charge than the *para* nitro group following one electron reduction of DNAN.[73] We did not observe DAAN in either the aqueous phase or the solid phase (via extraction) throughout the experiment. Additional experiments carried out under the same conditions using 2ANAN as the starting reactant further confirmed that no DAAN was produced from 2ANAN (Figure S9(a)). Based on the yields of 2ANAN and 4ANAN and that 6 electrons were required to reduce DNAN to 2ANAN or 4ANAN, the fraction of Rogue's ESC that was reactive toward DNAN was 402 μmol/g, approximately 30% lower than that toward NTO, suggesting NTO is more reducible than DNAN at pH 6.[63]

In RDX reactors, small amounts of NO_2^- (*ca*. 5 μM) and MNX (<1 μM) were formed with $Rogue_{RED}$, but not $Rogue_{OX}$. MNX and NO_2^- are known RDX degradation products and, therefore, their detection supports the finding that RDX was degraded by $Rogue_{RED}$. Specifically, MNX and NO_2^- were formed through reduction, since an addition of two electrons is required for MNX formation and reductive denitration of RDX to form NO_2^- has been well-documented.[51, 74] RDX degradation was further confirmed by the accumulation of NO_2^- following repeated additions of RDX in reactors containing $Rogue_{RED}$, as detailed in Section S6 (Figure S10). The accumulation of NO_2^- also suggests

that Rogue could not chemically reduce nitrite, as confirmed independently by a separate experiment using NO_2^- as the starting reactant (Figure S9(b)). The mass recovery of RDX with $Rogue_{RED}$ was only 64%, considerably lower than the 96% RDX mass recovery obtained with $Rogue_{OX}$ following the same extraction procedures (Table S7). This suggests *ca*. 36% of the initial RDX was likely transformed by $Rogue_{RED}$. The incomplete mass balance with $Rogue_{RED}$ (Figure 6(f)) also suggests that RDX may have undergone ring cleavage or other reactions. Overall, our results confirm that (1) DNAN and RDX can be chemically reduced by biochar through its ESC, and that (2) DNAN and RDX can be removed from water by biochar through concurrent sorption and reduction.



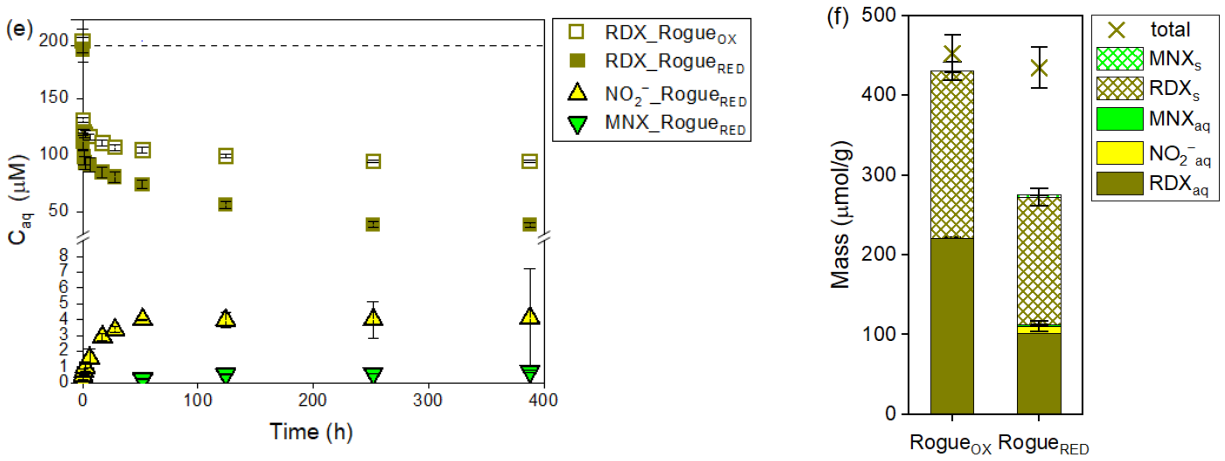
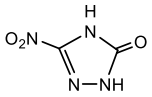
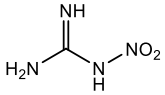
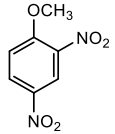
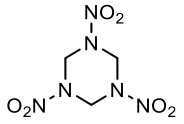


Figure 6. MC reduction by biochar in ASR at pH 6. (a) Aqueous concentration (C_{aq}) of NTO and ATO over time with 0.80 g/L of SRB or Rogue. (b) NTO mass balance. (c) C_{aq} of DNAN and 2ANAN/4ANAN over time with 0.44 g/L of Rogue. (d) DNAN mass balance. (e) C_{aq} of RDX and MNX/NO_2^- over time with 0.44 g/L of Rogue. (f) RDX mass balance. "total" is the DNAN or RDX added to blank. Compound names with the subscripts "aq" and "s" represent the masses in the aqueous phase at the end of experiment (*ca.* 400 h) and the extracted masses from the solid phase, respectively.

Table 1. Sorption of MCs by Rogue in ASR at pH 6

MC		NTO	NQ	DNAN	RDX
Structure					
Formulation		IMX-101 IMX-104	IMX-101	IMX-101 IMX-104	IMX-104
Physical Properties [a]	M.W. (g/mol)	130.08	104.07	198.13	222.26
	Solubility (mg/L)	16642[5]	2600[75]–5000[69]	276[5]	60[5]
	Log K_{OW}	0.37-1.03[5]	0.10[75]	1.64[5]	0.81-0.87[5]
	Log K_{OC}	3.03[5]	–	3.11[5]	0.88-2.40[5]
Isotherm Parameters [b]	$C_{s,\,max}$ (μmol/g)	154	388	476	213
	$C_{s,\,max}$ (%, w/w)	2.0	4.0	9.4	4.7
	K_L (μM^{-1})	0.07	0.02	2.96	0.44
	R^2	0.94	0.96	0.97	0.98

[a] M.W.: molecular weight, K_{OW}, K_{OC}: octanol-water and organic carbon-water partition coefficients, respectively. Solubility at 25 °C.

[b] Parameters of the isotherms are obtained through Langmuir isotherm fitting, as shown in Figure 5.

498 Table 2. Summary of MC reduction by biochar at pH 6

MC		NTO	NTO	NTO	DNAN	RDX
Biochar		SRB	SRB	Rogue	Rogue	Rogue
Background solution		50 mM MES	ASR	ASR	ASR	ASR
Removal by RED [a]	(µmol/g)	112±8	103±8	112±2	630±20	332±19
Removal by OX [b]		24±0	15±2	44±6	518±5	232±20
Δ Removal [c]		88±8	88±6	68±6	112±16	100±6
Product(s) formed		100±7 [d]	91±6 [d]	94±2 [d]	67±7 [e]	14±6 [f]
e^- transferred [g]		600±40	546±40	564±14	402±40	–

499 [a] RED: reduced biochar (SRB_{RED} or $Rogue_{RED}$)

500 [b] OX: oxidized biochar (SRB_{OX} or $Rogue_{OX}$)

501 [c] Δ Removal: additional MC removal by reduced biochar than oxidized biochar (Removal by RED – Removal by OX)

502 [d] ATO formed

503 [e] 2ANAN and 4ANAN formed

504 [f] MNX and NO_2^- formed

505 [g] e^- transferred (µmol e^-/g) =Product(s) formed (µmol/g) × 6 (mol e^-/mol product)

Accepted Manuscript
Version of record at: https://doi.org/10.1039/D1EM00447F

Conclusion

To the best of our knowledge, this is the first study to demonstrate the ability and capacity of biochar to reductively degrade organic pollutants through its ESC. This degradative capacity can be regenerated *in situ* through chemical or microbial reduction[31] and utilized repeatedly for pollution mitigation. Electrons stored in wood-derived biochar could reduce NTO to ATO stoichiometrically at pH 6−10. The fraction of biochar ESC reactive toward NTO was significant, *ca*. 500-600 µmol/g, or 26-38% of the ESC measured with ferricyanide, and 12-15% of that measured with DO. In addition to NTO, biochar was able to reduce DNAN and RDX as well as adsorb these MCs. Taken together, these results suggest biochar may serve as both a sorbent and regenerable reactive medium to support simultaneous removal *and* degradation of MCs and potentially other contaminants.

This study supports further investigation and development of biochar-based remediation strategies for military facilities. In particular, the ESC and surface area of biochar may be useful properties for selecting char materials for field applications. This study also offers insights into how pyrogenic black carbons, such as those produced globally from wildfires and deforestation, may represent enormous electron reservoirs and sorbents that influence chemical transformation and transport in the environment. The role of pyrogenic carbons in contaminant geochemistry hence warrants further investigation.

Supporting Information

Sections for of biochar characterization, determination of the pK_a of ATO, ATO sorption, thermodynamic calculations for the redox reactions involved, NQ sorption, and nitrite production from RDX reduction; tables summarizing chemicals used, biochar properties, and experimental

Accepted Manuscript
Version of record at: https://doi.org/10.1039/D1EM00447F

conditions; tables describing MC reduction, sorption, and extraction efficiency; figures illustrating biochar ESC reversibility for NTO reduction, batch MC sorption experiments, and removal of MC reduction daughter products by biochar.

Author Contributions

Conceptualization, P. C. Chiu; investigation, D. Xin and J. Girón; writing-original draft, D. Xin; writing-review & editing, all authors, funding acquisition, M. E. Fuller and P. C. Chiu.

Conflicts of Interest

There are no conflicts of interest to declare.

Acknowledgments

This research was supported by the Strategic Environmental Research and Development Program (SERDP) under project ER19-1106. The authors thank Jimmy Murillo-Gelvez for the method optimization of HPLC. The authors thank Delaware Environmental Institute (DENIN) for the Environmental Fellowship and University of Delaware Graduate College for the Dissertation Fellowship for Xin.

References

1. J. C. Pennington and J. M. Brannon, Environmental fate of explosives, *Thermochimica Acta*, 2002, **384**, 163-172. 10.1016/S0040-6031(01)00801-2
2. J. D. Arthur, N. W. Mark, S. Taylor, J. Šimůnek, M. L. Brusseau and K. M. Dontsova, Dissolution and transport of insensitive munitions formulations IMX-101 and IMX-104

in saturated soil columns, *Science of The Total Environment*, 2018, **624**, 758-768. 10.1016/j.scitotenv.2017.11.307

3. S. Taylor, E. Park, K. Bullion and K. Dontsova, Dissolution of three insensitive munitions formulations, *Chemosphere*, 2015, **119**, 342-348. 10.1016/j.chemosphere.2014.06.050

4. M. R. Walsh, M. E. Walsh, C. A. Ramsey, S. Thiboutot, G. Ampleman, E. Diaz and J. E. Zufelt, Energetic residues from the detonation of IMX‐104 insensitive munitions, *Propellants, Explosives, Pyrotechnics*, 2014, **39**, 243-250. 10.1002/prep.201300095

5. K. Dontsova, S. Taylor and R. Pesce-Rodriguez, *SERDP Project ER-2220: Dissolution of NTO, DNAN and insensitive munitions formulations and their fates in soils*, University of Arizona, Tuscon, United States, February 2018.

6. L. Le Campion, A. Vandais and J. Ouazzani, Microbial remediation of NTO in aqueous industrial wastes, *FEMS Microbiology Letters*, 1999, **176**, 197-203. 10.1111/j.1574-6968.1999.tb13662.x

7. A. Halasz, J. Hawari and N. N. Perreault, New insights into the photochemical degradation of the insensitive munition formulation IMX-101 in water, *Environmental Science & Technology*, 2018, **52**, 589-596. 10.1021/acs.est.7b04878

8. K. S. Ro, A. Venugopal, D. D. Adrian, D. Constant, K. Qaisi, K. T. Valsaraj, L. J. Thibodeaux and D. Roy, Solubility of 2, 4, 6-trinitrotoluene (TNT) in water, *Journal of Chemical Engineering Data*, 1996, **41**, 758-761. 10.1021/je950322w

9. J. Park, S. Comfort, P. Shea and T. Machacek, Remediating munitions‐contaminated soil with zerovalent iron and cationic surfactants, *Journal of Environmental Quality*, 2004, **33**, 1305-1313. 10.2134/jeq2004.1305

10. M. S. Johnson, W. S. Eck and E. M. Lent, Toxicity of insensitive munition (IMX) formulations and components, *Propellants, Explosives, Pyrotechnics*, 2017, **42**, 9-16. 10.1002/prep.201600147

11. E. M. Lent, A. M. Narizzano, K. A. Koistinen and M. S. Johnson, Chronic oral toxicity of 3-nitro-1, 2, 4-triazol-5-one (NTO) in rats, *Regulatory Toxicology and Pharmacology*, 2020, **112**, 104609. 10.1016/j.yrtph.2020.104609

12. C. L. Madeira, J. A. Field, M. T. Simonich, R. L. Tanguay, J. Chorover and R. Sierra-Alvarez, Ecotoxicity of the insensitive munitions compound 3-nitro-1, 2, 4-triazol-5-one (NTO) and its reduced metabolite 3-amino-1, 2, 4-triazol-5-one (ATO), *Journal of Hazardous Materials*, 2018, **343**, 340-346. 10.1016/j.jhazmat.2017.09.052

13. S. G. Dodard, M. Sarrazin, J. Hawari, L. Paquet, G. Ampleman, S. Thiboutot and G. I. Sunahara, Ecotoxicological assessment of a high energetic and insensitive munitions compound: 2, 4-dinitroanisole (DNAN), *Journal of Hazardous Materials*, 2013, **262**, 143-150. 10.1016/j.jhazmat.2013.08.043

14. C. I. Olivares, R. Sierra‐Alvarez, L. Abrell, J. Chorover, M. Simonich, R. L. Tanguay and J. A. Field, Zebrafish embryo toxicity of anaerobic biotransformation products from the insensitive munitions compound 2, 4‐dinitroanisole, *Environmental Toxicology & Chemistry*, 2016, **35**, 2774-2781. 10.1002/etc.3446

15. O. Mašek, W. Buss and S. Sohi, Standard biochar materials, *Environmental Science & Technology*, 2018, **52**, 9543-9544. 10.1021/acs.est.8b04053

16. J. J. Pignatello, W. A. Mitch and W. Q. Xu, Activity and reactivity of pyrogenic carbonaceous matter toward organic compounds, *Environmental Science & Technology*, 2017, **51**, 8893-8908. 10.1021/acs.est.7b01088

17. M. Inyang and E. Dickenson, The potential role of biochar in the removal of organic and microbial contaminants from potable and reuse water: A Review, *Chemosphere*, 2015, **134**, 232-240. 10.1016/j.chemosphere.2015.03.072

18. L. Beesley, E. Moreno-Jiménez, J. L. Gomez-Eyles, E. Harris, B. Robinson and T. Sizmur, A review of biochars' potential role in the remediation, revegetation and restoration of contaminated soils, *Environmental Pollution*, 2011, **159**, 3269-3282. 10.1016/j.envpol.2011.07.023

19. G. Chu, J. Zhao, Y. Zhang, K. Sun, X. Liu, Y. Si, B. Pan and C. E. Steinberg, Inherent Minerals Facilitated Bisphenol A Sorption by Biochar: A Key Force by Complexation, *ACS EST Water*, 2021, DOI: 10.1021/acsestwater.1c00344. 10.1021/acsestwater.1c00344

20. S. Y. Oh and Y. D. Seo, Sorptive removal of nitro explosives and metals using biochar, *Journal of Environmental Quality*, 2014, **43**, 1663-1671. 10.2134/jeq2014.02.0097

21. S. Y. Oh and Y. D. Seo, Factors affecting sorption of nitro explosives to biochar: Pyrolysis temperature, surface treatment, competition, and dissolved metals, *Journal of Environmental Quality*, 2015, **44**, 833-840. 10.2134/jeq2014.12.0525

22. K. Y. Lee, L. B. Chapman and M. D. Cobura, 3-Nitro-1, 2, 4-triazol-5-one, a less sensitive explosive, *Journal of Energetic Materials*, 1987, **5**, 27-33. 10.1080/07370658708012347

23. N. J. Harris and K. Lammertsma, Tautomerism, ionization, and bond dissociations of 5-nitro-2, 4-dihydro-3 H-1, 2, 4-triazolone, *Journal of the American Chemical Society*, 1996, **118**, 8048-8055. 10.1021/ja960834a

24. P. A. Cárdenas-Hernández, K. A. Anderson, J. Murillo-Gelvez, D. M. Di Toro, H. E. Allen, R. F. Carbonaro and P. C. Chiu, Reduction of 3-nitro-1, 2, 4-triazol-5-one (NTO)

by the hematite–aqueous Fe (II) redox couple, *Environmental Science & Technology*, 2020, **54**, 12191-12201. 10.1021/acs.est.0c03872

25. S. Y. Oh, D. K. Cha and P. C. Chiu, Graphite-mediated reduction of 2,4-dinitrotoluene with elemental iron, *Environmental Science & Technology*, 2002, **36**, 2178-2184. 10.1021/es011474g

26. S. Y. Oh and P. C. Chiu, Graphite- and soot-mediated reduction of 2,4-dinitrotoluene and hexahydro-1,3,5-trinitro-1,3,5-triazine, *Environmental Science & Technology*, 2009, **43**, 6983-6988. 10.1021/es901433m

27. W. Q. Xu, K. E. Dana and W. A. Mitch, Black carbon-mediated destruction of nitroglycerin and RDX by hydrogen sulfide, *Environmental Science & Technology*, 2010, **44**, 6409-6415. 10.1021/es101307n

28. S. Y. Oh, J. G. Son, S. H. Hur, J. S. Chung and P. C. Chiu, Black carbon-mediated reduction of 2,4-dinitrotoluene by dithiothreitol, *Journal of Environmental Quality*, 2013, **42**, 815-821. 10.2134/jeq2012.0411

29. S. Y. Oh, J. G. Son and P. C. Chiu, Biochar-mediated reductive transformation of nitro herbicides and explosives, *Environmental Toxicology & Chemistry*, 2013, **32**, 501-508. 10.1002/etc.2087

30. L. Klüpfel, M. Keiluweit, M. Kleber and M. Sander, Redox properties of plant biomass-derived black carbon (biochar), *Environmental Science & Technology*, 2014, **48**, 5601-5611. 10.1021/es500906d

31. J. M. Saquing, Y. H. Yu and P. C. Chiu, Wood-derived black carbon (biochar) as a microbial electron donor and acceptor, *Environmental Science & Technology Letters*, 2016, **3**, 62-66. 10.1021/acs.estlett.5b00354

644 32. X. Cao, J. J. Pignatello, Y. Li, C. Lattao, M. A. Chappell, N. Chen, L. F. Miller and J.
645 Mao, Characterization of wood chars produced at different temperatures using advanced
646 solid-state 13C NMR spectroscopic techniques, *Energy & Fuels*, 2012, **26**, 5983-5991.
647 10.1021/ef300947s
648 33. X. Xiao and B. Chen, A direct observation of the fine aromatic clusters and molecular
649 structures of biochars, *Environmental Science & Technology*, 2017, **51**, 5473-5482.
650 10.1021/acs.est.6b06300
651 34. D. Xin, M. Xian and P. C. Chiu, New methods for assessing electron storage capacity and
652 redox reversibility of biochar, *Chemosphere*, 2019, **215**, 827-834.
653 10.1016/j.chemosphere.2018.10.080
654 35. D. Xin, N. Saha, M. T. Reza, J. Hudson and P. C. Chiu, Pyrolysis creates electron storage
655 capacity of black carbon (biochar) from lignocellulosic biomass, *ACS Sustainable*
656 *Chemistry & Engineering*, 2021 **9**, 6821-6831. 10.1021/acssuschemeng.1c01251
657 36. A. Prévoteau, F. Ronsse, I. Cid, P. Boeckx and K. Rabaey, The electron donating
658 capacity of biochar is dramatically underestimated, *Scientific Reports*, 2016, **6** pp.11.
659 10.1038/Srep32870
660 37. N. Saha, D. Xin, P. C. Chiu and M. T. Reza, Effect of pyrolysis temperature on acidic
661 oxygen-containing functional groups and electron storage capacities of pyrolyzed
662 hydrochars, *ACS Sustainable Chemistry & Engineering*, 2019, **7**, 8387-8396.
663 10.1021/acssuschemeng.9b00024
664 38. Y. Zhang, X. Y. Xu, P. Y. Zhang, L. Zhao, H. Qiu and X. D. Cao, Pyrolysis-temperature
665 depended quinone and carbonyl groups as the electron accepting sites in barley grass
666 derived biochar, *Chemosphere*, 2019, **232**, 273-280. 10.1016/j.chemosphere.2019.05.225

667 39. Y. Zhang, X. Y. Xu, L. Z. Cao, Y. S. Ok and X. D. Cao, Characterization and
668 quantification of electron donating capacity and its structure dependence in biochar
669 derived from three waste biomasses, *Chemosphere*, 2018, **211**, 1073-1081.
670 10.1016/j.chemosphere.2018.08.033
671 40. D. Xin, T. Barkley and P. C. Chiu, Visualizing electron storage capacity distribution in
672 biochar through silver tagging, *Chemosphere*, 2020, **248**, 125952.
673 10.1016/j.chemosphere.2020.125952
674 41. Y. Yao, B. Gao, F. Wu, C. Z. Zhang and L. Y. Yang, Engineered biochar from biofuel
675 residue: Characterization and its silver removal potential, *ACS Applied Materials &*
676 *Interfaces*, 2015, **7**, 10634-10640. 10.1021/acsami.5b03131
677 42. P. Liu, C. J. Ptacek, D. W. Blowes, Y. Z. Finfrock and Y. Y. Liu, Characterization of
678 chromium species and distribution during Cr(VI) removal by biochar using confocal
679 micro-X-ray fluorescence redox mapping and X-ray absorption spectroscopy,
680 *Environment International*, 2020, **134**, 105216. 10.1016/j.envint.2019.105216
681 43. J. Tian, J. Jin, P. C. Chiu, D. K. Cha, M. X. Guo and P. T. Imhoff, A pilot-scale, bi-layer
682 bioretention system with biochar and zero-valent iron for enhanced nitrate removal from
683 stormwater, *Water Research*, 2019, **148**, 378-387. 10.1016/j.watres.2018.10.030
684 44. S. A. A. Nakhli and P. T. Imhoff, Models for predicting water retention in pyrogenic
685 carbon (biochar) and biochar‐amended soil at low water contents, *Water Resources*
686 *Research*, 2020, **56**, e2020WR027726. 10.1029/2020WR027726
687 45. M. Aeschbacher, M. Sander and R. P. Schwarzenbach, Novel electrochemical approach
688 to assess the redox properties of humic substances, *Environmental Science &*
689 *Technology*, 2010, **44**, 87-93. 10.1021/es902627p

46. M. E. Fuller, E. M. Farquharson, P. C. Hedman and P. C. Chiu, Removal of munition constituents in stormwater runoff: Screening of native and cationized cellulosic sorbents for removal of insenstive munition constituents NTO, DNAN, and NQ, and legacy munition constituents HMX, RDX, TNT, and perchlorate, *Journal of Hazardous Materials*, 2021, **424**, 127335. 10.1016/j.jhazmat.2021.127335

47. T. Temple, S. Cipullo, E. Galante, M. Ladyman, N. Mai, T. Parry and F. Coulon, The effect of soil type on the extraction of insensitive high explosive constituents using four conventional methods, *Science of The Total Environment*, 2019, **668**, 184-192. 10.1016/j.scitotenv.2019.02.359

48. J. Liang, C. Olivares, J. A. Field and R. Sierra-Alvarez, Microbial toxicity of the insensitive munitions compound, 2, 4-dinitroanisole (DNAN), and its aromatic amine metabolites, *Journal of Hazardous Materials*, 2013, **262**, 281-287. 10.1016/j.jhazmat.2013.08.046

49. Y. Tong, M. J. Berens, B. A. Ulrich, J. Bolotin, J. H. Strehlau, T. Hofstetter and W. Arnold, Exploring the Utility of Compound-Specific Isotope Analysis for Assessing Ferrous Iron-Mediated Reduction of RDX in the Subsurface, 2020.

50. A. Bernstein, Z. Ronen and F. Gelman, Insight on RDX degradation mechanism by Rhodococcus strains using ^{13}C and ^{15}N kinetic isotope effects, *Environmental Science & Technology*, 2013, **47**, 479-484. 10.1021/es302691g

51. Y. Tong, M. J. Berens, B. A. Ulrich, J. Bolotin, J. H. Strehlau, T. B. Hofstetter and W. A. Arnold, Exploring the utility of compound-specific isotope analysis for assessing ferrous iron-mediated reduction of RDX in the subsurface, *Environmental Science & Technology*, 2021, **55**, 6752-6763. 10.1021/acs.est.0c08420

Accepted Manuscript
Version of record at: https://doi.org/10.1039/D1EM00447F

52. A. Mukherjee, A. R. Zimmerman and W. Harris, Surface chemistry variations among a series of laboratory-produced biochars, *Geoderma*, 2011, **163**, 247-255. 10.1016/j.geoderma.2011.04.021

53. M. Aeschbacher, D. Vergari, R. P. Schwarzenbach and M. Sander, Electrochemical analysis of proton and electron transfer equilibria of the reducible moieties in humic acids, *Environmental Science & Technology*, 2011, **45**, 8385-8394. 10.1021/es201981g

54. L. Selwyn and S. Tse, The chemistry of sodium dithionite and its use in conservation, *Studies in Conservation*, 2008, **53(sup2)**, 61-73. 10.1179/sic.2008.53.Supplement-2.61

55. D. Xin, M. Xian and P. C. Chiu, Chemical methods for determining the electron storage capacity of black carbon, *MethodsX*, 2018, **5**, 1515-1520. 10.1016/j.mex.2018.11.007

56. B. R. Linker, R. Khatiwada, N. Perdrial, L. Abrell, R. Sierra-Alvarez, J. A. Field and J. Chorover, Adsorption of novel insensitive munitions compounds at clay mineral and metal oxide surfaces, *Environmental Chemistry*, 2015, **12**, 74-84. 10.1071/En14065

57. R. Khatiwada, L. Abrell, G. Li, R. A. Root, R. Sierra-Alvarez, J. A. Field and J. Chorover, Adsorption and oxidation of 3-nitro-1, 2, 4-triazole-5-one (NTO) and its transformation product (3-amino-1, 2, 4-triazole-5-one, ATO) at ferrihydrite and birnessite surfaces, *Environmental Pollution*, 2018, **240**, 200-208. 10.1016/j.envpol.2018.04.034

58. N. Mark, J. Arthur, K. Dontsova, M. Brusseau and S. Taylor, Adsorption and attenuation behavior of 3-nitro-1,2,4-triazol-5-one (NTO) in eleven soils, *Chemosphere*, 2016, **144**, 1249-1255. 10.1016/j.chemosphere.2015.09.101

59. M. J. Krzmarzick, R. Khatiwada, C. I. Olivares, L. Abrell, R. Sierra-Alvarez, J. Chorover and J. A. Field, Biotransformation and degradation of the insensitive munitions

Accepted Manuscript
Version of record at: https://doi.org/10.1039/D1EM00447F

compound, 3-nitro-1,2,4-triazol-5-one, by soil bacterial communities, *Environmental Science & Technology*, 2015, **49**, 5681-5688. 10.1021/acs.est.5b00511

60. C. L. Madeira, S. A. Speet, C. A. Nieto, L. Abrell, J. Chorover, R. Sierra-Alvarez and J. A. Field, Sequential anaerobic-aerobic biodegradation of emerging insensitive munitions compound 3-nitro-1, 2, 4-triazol-5-one (NTO), *Chemosphere*, 2017, **167**, 478-484. 10.1016/j.chemosphere.2016.10.032

61. M. E. Fuller, R. T. Rezes, P. C. Hedman, J. C. Jones, N. C. Sturchio and P. B. Hatzinger, Biotransformation of the insensitive munition constituents 3-nitro-1, 2, 4-triazol-5-one (NTO) and 2, 4-dinitroanisole (DNAN) by aerobic methane-oxidizing consortia and pure cultures, *Journal of Hazardous Materials*, 2021, **407**, 124341. 10.1016/j.jhazmat.2020.124341

62. C. L. Madeira, K. V. Jog, E. T. Vanover, M. D. Brooks, D. K. Taylor, R. Sierra-Alvarez, L. A. Waidner, J. C. Spain, M. J. Krzmarzick and J. A. Field, Microbial enrichment culture responsible for the complete oxidative biodegradation of 3-amino-1, 2, 4-triazol-5-one (ATO), the reduced daughter product of the insensitive munitions compound 3-nitro-1, 2, 4-triazol-5-one (NTO), *Environmental Science & Technology*, 2019, **53**, 12648-12656. 10.1021/acs.est.9b04065

63. O. Menezes, Y. Yu, R. A. Root, S. Gavazza, J. Chorover, R. Sierra-Alvarez and J. A. Field, Iron (II) monosulfide (FeS) minerals reductively transform the insensitive munitions compounds 2, 4-dinitroanisole (DNAN) and 3-nitro-1, 2, 4-triazol-5-one (NTO), *Chemosphere*, 2021, **285**, 131409. 10.1016/j.chemosphere.2021.131409

64. J. Murillo-Gelvez, D. M. Di Toro, H. E. Allen, R. F. Carbonaro and P. C. Chiu, Reductive transformation of 3-nitro-1, 2, 4-triazol-5-one (NTO) by leonardite humic acid

and anthraquinone-2, 6-disulfonate (AQDS), *Environmental Science & Technology*, 2021, **55**, 12973–12983. 10.1021/acs.est.1c03333

65. A. I. Coppola, D. B. Wiedemeier, U. M. Hanke, M. Reisser, S. Abiven, M. W. I. Schmidt, V. Galy, B. Voss, B. Peucker-Ehrenbrink, T. I. Eglinton, N. Haghipour, G. S. Nascimento, M. Usman, T. M. Blattmann, C. V. Freymond, L. Wacker, M. Zhao and E. Schefuss, Global-scale evidence for the refractory nature of riverine black carbon, *Nature Geoscience*, 2018, **11**, 584-588. 10.1038/s41561-018-0159-8

66. M. Reisser, R. S. Purves, M. W. I. Schmidt and S. Abiven, Pyrogenic carbon in soils: A literature-based inventory and a global estimation of its content in soil organic carbon and stocks, *Frontiers in Earth Science*, 2016, **4**, pp.14. 10.3389/Feart.2016.00080

67. P. B. Hatzinger, M. E. Fuller, D. Rungmakol, R. L. Schuster and R. J. Steffan, Enhancing the attenuation of explosives in surface soils at military facilities: Sorption‐desorption isotherms, *Environmental Toxicology Chemistry: An International Journal*, 2004, **23**, 306-312. 10.1897/03-186

68. C. E. Schaefer, M. E. Fuller, J. M. Lowey and R. Steffan, Use of peat moss amended with soybean oil for mitigation of dissolved explosive compounds leaching into the subsurface: insight into mass transfer mechanisms, *Environmental Engineering Science*, 2005, **22**, 337-349. 10.1089/ees.2005.22.337

69. A. Koutsospyros, J. Pavlov, J. Fawcett, D. Strickland, B. Smolinski and W. Braida, Degradation of high energetic and insensitive munitions compounds by Fe/Cu bimetal reduction, *Journal of Hazardous Materials*, 2012, **219**, 75-81. 10.1016/j.jhazmat.2012.03.048

Accepted Manuscript
Version of record at: https://doi.org/10.1039/D1EM00447F

70. J. Murillo-Gelvez, D. M. Di Toro, H. E. Allen, R. F. Carbonaro and P. C. Chiu, Abiotic reduction of the insensitive munitions compounds DNAN, NQ, and NTO by humic acid and a model quinone, *2020 SERDP and ESTCP Symposium, Oral Presentation*.

71. M. J. Berens, T. B. Hofstetter, J. Bolotin and W. A. Arnold, Assessment of 2, 4-dinitroanisole transformation using compound-specific isotope analysis after in situ chemical reduction of iron oxides, *Environmental Science & Technology*, 2020, **54**, 5520-5531. 10.1021/acs.est.9b07616

72. C. Olivares, J. Liang, L. Abrell, R. Sierra-Alvarez and J. A. Field, Pathways of reductive 2, 4-dinitroanisole (DNAN) biotransformation in sludge, *Biotechnology Bioengineering*, 2013, **110**, 1595-1604. 10.1002/bit.24820

73. S. E. Barrows, C. J. Cramer, D. G. Truhlar, M. S. Elovitz and E. J. Weber, Factors controlling regioselectivity in the reduction of polynitroaromatics in aqueous solution, *Environmental Science & Technology*, 1996, **30**, 3028-3038. 10.1021/es960004x

74. S. Y. Oh, D. K. Cha, B. J. Kim and P. C. Chiu, Reductive transformation of hexahydro-1, 3, 5-trinitro-1, 3, 5-triazine, octahydro-1, 3, 5, 7-tetranitro-1, 3, 5, 7-tetrazocine, and methylenedinitramine with elemental iron, *Environmental Toxicology & Chemistry*, 2005, **24**, 2812-2819. 10.1897/04-662R.1

75. W. R. Haag, R. Spanggord, T. Mill, R. T. Podoll, T. W. Chou, D. S. Tse and J. C. Harper, Aquatic environmental fate of nitroguanidine, *Environmental Toxicology & Chemistry*, 1990, **9**, 1359-1367. 10.1002/etc.5620091105