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Biodegradability of unheated and laboratory heated dissolved organic matter†

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Following wildfires, partially combusted biomass remains on the forest floor and erosion from the landscape can release dissolved pyrogenic organic matter (dPyOM) to surface waters. Therefore, post-fire alterations to dissolved organic matter (DOM) in aquatic systems may play a vital role in DOM stability and biogeochemical cycles. Dissolved PyOM biodegradation remains poorly understood and is expected to vary with combustion temperature and fuel source. In this study laboratory heating and leaching of forest floor materials (soil and litter) were used to compare the biodegradability of unheated, low (250 °C), and moderate (450 °C) temperature leachates. Inoculation experiments were performed with river microbes. Dissolved organic carbon (DOC) and nitrogen (DON), inorganic nitrogen, and DOM optical properties were monitored for 38 days. Inoculation experiments showed significantly greater DOC biodegradation of low and moderate temperature samples (64% and 71%, respectively) compared to unheated samples (32%). The greater DOC biodegradation may be explained by lower molecular weight DOM composition of heated leachates which was supported by higher initial E_2/E_3 ratios (absorbance at 250 nm/365 nm). Further, the observed decrease in the E_2/E_3 ratio after incubation suggests biodegradation of smaller compounds. This trend was greater for heated samples than unheated DOM. Specific ultraviolet absorbance increased after incubation, suggesting biodegradation of aliphatic compounds. Inoculated moderate temperature samples showed the greatest DON degradation (74%), followed by low temperature (58%) and unheated (51%) samples. Overall, results suggest that low and moderate temperature dPyOM was more biodegradable than unheated DOM, which may have implications for aquatic biogeochemical cycling, ecosystem function, and water quality in fire-impacted watersheds.

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Environmental significance

Erosion from burned landscapes mobilizes dissolved pyrogenic organic matter (dPyOM) to surface waters. An understanding of the biodegradability of dPyOM compared to unheated dissolved organic matter (DOM) is essential to better evaluate post-fire surface water quality. Low and moderate temperature DOM samples were more biodegradable than unheated samples. This finding suggests that following fire, the biodegradability of DOM may be altered, with implications for biogeochemical cycles, in-stream processing, and transformation of DOM composition in surface waters.

1 Introduction

Wildfires are a vital part of environmental ecosystems and biogeochemical cycles.[1] In the last several decades the occurrence of large high severity fires has increased due to climate change and the accumulation of excessive fuel loads.[2–5] Wildfires produce soil pyrogenic organic matter (PyOM) from the partial combustion of forest fuels, vegetation, organic soils, and biomass.[6–9] Post-fire rainfall and snowmelt mobilizes thermally altered organic matter to downstream water bodies as dissolved pyrogenic organic matter (dPyOM).[9,10]

dPyOM composition varies with combustion temperature and therefore is expected to influence the biodegradability of dPyOM.[9,11–13] In general, high temperatures (*i.e.*, >450 °C)[14] produce solid PyOM with low oxygen to carbon and hydrogen to carbon ratios, low alkyl chain length, high nitrogen content, high aromaticity and high aryl groups. In contrast, low-temperatures (*i.e.*, 100–400 °C)[15,16] produce carbon-rich PyOM with higher alkyl chain length, functional groups containing oxygen, and fewer aromatic and aryl groups.[9,11,17] These properties of solid PyOM will potentially influence dPyOM composition, especially for lower temperature PyOM when more

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soluble compounds are present. Further, dPyOM composition has been shown to shift from non-aromatic sugar-like structures to polycondensed aromatic structures with increasing temperature.[9] This transition toward polycondensed aromatic structures may lead to the persistence of higher temperature dPyOM in the aquatic environment because the natural degradation rate of polycondensed aromatic compounds is typically slower than aliphatic compounds.[18,19]

dPyOM was traditionally considered recalcitrant as most studies focused on the more condensed aromatic portion from high temperature dPyOM.[9,20–22] Similarly, dPyOM has largely been considered to have a low biodegradability due to its aromaticity.[9] However, recent studies demonstrated that the reactivity and degradation of dPyOM substantially vary with combustion temperature and degree of condensation and aromaticity.[9,12,23,24] Therefore, the biodegradability of low-temperature dPyOM may be greater than previously thought, because it contains more oxygenated aliphatic compounds and less condensed aromatic compounds than high-temperature dPyOM.[9] In addition, high-temperature dPyOM should not be completely bio-refractory as there is evidence of microbial availability of aromatic organic matter.[12] Many microbes (i.e., bacteria – *Pseudomonas*, *Rhodococcus*, and fungi – *Chrysosporium pannorum*, *Pleurotus ostreatus*) are capable of degrading polycyclic aromatic hydrocarbons into much simpler compounds with the help of monooxygenase, dioxygenase, and manganese peroxidase enzymes.[12,25–28] Therefore, low to high temperature dPyOM is expected to be biodegradable to some extent, depending on dPyOM composition.

Several laboratory studies have evaluated the biodegradability of dPyOM, with the majority focusing on low or high-temperature dPyOM without a comparison to unheated DOM.[12,13,23,29] Moreover, the type of microbes used for incubation is important for evaluating DOM biodegradation. Studies by Bostick *et al.*,[12] Goranov *et al.*[23] and Chen *et al.*[13] evaluated dPyOM biodegradation with soil microbes. Meanwhile the role of aqueous microbes in dPyOM biodegradation is yet to be explored. Another critical factor for dPyOM biodegradation is the nitrogen components. Black nitrogen was present in char as bioavailable pyrrole-type moieties[30] suggesting that dissolved black nitrogen that is mobilized to aquatic systems as part of dPyOM[31] may also be biodegradable.[9,31] The biodegradability of dissolved organic nitrogen (DON) present in dPyOM has yet to be studied.

The objective of this study was to evaluate the biodegradability of low (250 °C) and moderate (450 °C) temperature dPyOM and unheated DOM. Forest floor surface soils and plant litter were heated at 250 °C and 450 °C in a muffle furnace and leached. Unheated DOM samples were prepared by leaching unheated soils and litter. Heated and unheated samples were inoculated with native river microbes and monitored for DOC, ammonia (NH_3–N), nitrite + nitrate (NO_2^- + NO_3^-), ultraviolet absorbance, and functional group changes using Fourier-transform infrared spectroscopy (FTIR) for 38 days of incubation. Control experiments were performed with sterile river water inoculation.

2 Materials and methods

2.1 Soil and litter sample collection

Forest floor surface soil and litter samples were collected from the University of Idaho Experimental Forest (UIEF) within the Big Meadow Creek watershed in Troy, Idaho (46°47′49.9″N & 116°48′57.1″W). Major vegetation types include Grand fir, Ponderosa pine, Western hemlock, Subalpine fir, and Douglas fir.[32] Seven 1 m × 1 m square plots were randomly selected. Litter samples (*i.e.*, leaves, branches, needles) and surface soils were collected from the forest floor plots from the top 5 cm. Samples from all plots were homogenized. Soil and litter were stored in separate zip lock bags and transported to Washington State University (Pullman, WA) for processing. Briefly, samples were air dried at room temperature until a constant weight was reached. Samples were sieved through a 2 mm sieve separately and classified as soil (*i.e.*, passing through the sieve) or litter (*i.e.*, material retained on the sieve) and stored at 4 °C.

2.2 Preparation of unheated, low, and moderate temperature leachates

To evaluate dissolved PyOM, laboratory heating experiments were performed by adapting previously established methods.[33–35] Briefly, soil and litter samples were heated at 250 °C or 450 °C in aluminum containers (0.5 cm soil depth) using a Yamato Muffle Furnace (F0810CR) under oxic conditions. After heating, samples were immediately removed from the furnace, cooled to room temperature, and stored at 4 °C.

To prepare unheated and heated leachates, equal masses of soil and litter were added to ultrapure Milli-Q water to target approximately 10.0 ± 1.0 mg DOC per L. One 450 °C leachate was outside this range due to the low carbon content of solids. The solids and water were manually mixed in amber bottles for one minute and placed on a mechanical shaker at 110 rpm for 24 hours at room temperature (20 °C). Unheated and heated leachates were vacuum filtered through 0.7 μm glass fiber filters (GF/F) (Cytiva 1825-047) and refrigerated at 4 °C in sterile glass amber bottles until analysis and experiments. Throughout the manuscript, unheated, 250 °C, and 450 °C temperature samples are denoted as "unheated", "low temperature" and "moderate temperature", respectively.

2.3 Experimental methods

Microbial incubation experiments were conducted with filtered unheated and heated leachate samples, and water quality parameters were monitored for 38 days. After 38 days, a significant decrease in DOC and DON was generally observed and the experiment was ended. Inoculums contained river water that was collected in sterile bottles from the South Fork Palouse River (Pullman, WA) (46°43′51.9″N & 117°10′25.7″W), transported to Washington State University, and stored at 4 °C. The concentration of native microbes in the river water was determined using the spread plate enumeration method. R2A without dextrose was used as the initial culture medium and plate medium to facilitate the growth of microbes capable of using organic matter present in the river water. Serial dilutions

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were plated in triplicate to determine the initial colony forming unit per mL (CFU mL^{-1}). Based on the initial microbial concentration, 10 mL of river water were used to inoculate 1000 mL of each leachate sample (unheated, low temperature, and moderate temperature). A sterile inoculum (control) was prepared with an aliquot of river water, that was autoclaved at 121 °C and 55 psi and filtered through a 0.2 μm polyethersulfone (PES) membrane filter. Ten mL of sterile inoculum were added to each sample to serve as controls, resulting in two experimental conditions (control and inoculated) for three samples (unheated, low, and moderate). Incubation experiments were performed in duplicate for each sample. In total, there were six reactors for control experiments and six reactors for inoculation experiments.

Batch reactor incubation experiments were conducted at room temperature (20 °C) in unsealed 1 L glass amber bottles. Steel wool was placed in reactor openings to allow air exchange and prevent contamination from exogenous microbes. Microbial incubation experimental conditions were adapted from previous studies that evaluate biodegradation in aquatic systems.[36–38] The reactors were manually shaken on sampling days to homogenize. Aliquots were subsampled from the reactors for analysis throughout the incubation period similar to previous studies.[39–41] This approach was taken to avoid sample to sample variability challenges of small, sacrificial reactors. The solid materials and leachates can be highly variable, including the amount of DOC leached from the same solid sample. Consequently, the decrease in reactor volume during the incubation period may have impacted the experimental results and is a limitation of the study. The rate of change in DOC and DON concentrations, DOM optical properties, and microbial concentrations may have been influenced by the decrease in reactor volume, due to possible changes in reactant concentration and dynamics between microbes and DOM that resulted in a faster or slower reaction.[42] Therefore, the parameter concentrations near the end of the incubation period might be over- or underestimated. However, the goal of the study was to compare the biodegradability of unheated, low, and moderate temperature DOM samples and all samples and replicates were subjected to the same experimental conditions. Therefore, the biodegradability comparison of the samples should not be majorly impacted by the decrease in reactor volume with incubation time.

2.4 Analytical methods

Reactors were shaken manually to homogenize the samples before aliquoting 70 mL from each reactor on days 1, 4, 8, 14, 22, 29, and 38. The aliquots were analyzed for microbial count, DOC, NH_3–N, NO_2^- + NO_3^-, ultraviolet absorbance, and functional groups using FTIR. For microbial count analysis, 100 μL of each sample was plated on R2A media (without dextrose) in triplicate following methods described above. Plates were incubated at 30 °C for up to 24 hours until countable colonies appeared, and the resulting CFU mL^{-1} was calculated for each sample. After microbial count analysis, aliquots were filtered through 0.7 μm GF/F to remove any particles from the river inoculant. DOC and total dissolved nitrogen (TDN) were determined using a Shimadzu TOC-L_{CSH} with a TNM-L module following the non-purgeable organic carbon method. Potassium hydrogen phthalate and potassium nitrate standard solutions were used for instrument calibration. NH_3–N was measured following the Hach spectrophotometric method (TNT 830) using a Hach DR6000. A Seal Analytical AQ 400 was used for NO_2^- + NO_3^- analysis following EPA Method-126-C.[43] DON was calculated from the difference in TDN and inorganic nitrogen.[44,45] A PerkinElmer Lambda 365 UV/Vis spectrophotometer was used to measure the ultraviolet absorbance from 200 nm to 700 nm. Specific ultraviolet absorbance at 254 nm ($SUVA_{254}$) was calculated by dividing the ultraviolet absorbance at 254 nm by the DOC concentration and normalizing for path length.[46] E_2/E_3, an indicator of DOM molecular weight, was calculated by dividing the sample absorbance at 250 nm by absorbance at 365 nm.[47] The ratio of UV-absorbance at 220 nm to 254 nm (A_{220}/A_{254}) was evaluated as an indicator of sample polarity.[48–51] FTIR analysis was conducted on liquid filtered leachates using a Shimadzu FTIR for evaluating changes in sample functional groups. The spectral range was from 4000 to 600 cm^{-1} in the transmittance mode at a resolution of 4 cm^{-1} and 64 scans were taken for each sample.

2.5 Statistical analysis

Statistical analysis was conducted using Welch's two sample *t*-test and one-way analysis of variance (ANOVA) where appropriate to determine whether the observed biodegradation differences of unheated, low, and moderate temperature samples for control and inoculation experiments were statistically significant ($p < 0.05$). All statistical analyses were conducted using R-studio.

3 Results and discussion

3.1 Effect of heating temperature on DOC and DON

Heating temperature significantly influenced the mass of DOC leached from the solid material. Solid material heated at low temperature leached significantly higher DOC than moderate temperature ($p = 0.0003$) and unheated materials ($p = 0.002$) (Fig. 1A). The average DOC leached from low temperature samples was 7.3 ± 0.44 mgC per g of solids, whereas the average DOC leached from unheated materials was 1.9 ± 0.26 mgC per g of solids. Materials heated at moderate temperature leached only 0.4 ± 0.02 mgC per g of solids (Fig. 1A). These observations are consistent with previous studies.[10,52–54] Bahureksa *et al.*[52] observed that the solubility of organic carbon leached from organic soils peaked at 250 °C and reached a minimum at 450 °C. Work by Hohner *et al.*[10] demonstrated the solubility of organic carbon peaked between 250 °C and 350 °C for mineral soils. Santos *et al.*[54] also observed increasing carbon solubility up to 300 °C. Collectively, these findings suggest that low-temperature PyOM is more soluble than unheated and high-temperature PyOM, which may increase the biodegradability of dissolved PyOM.

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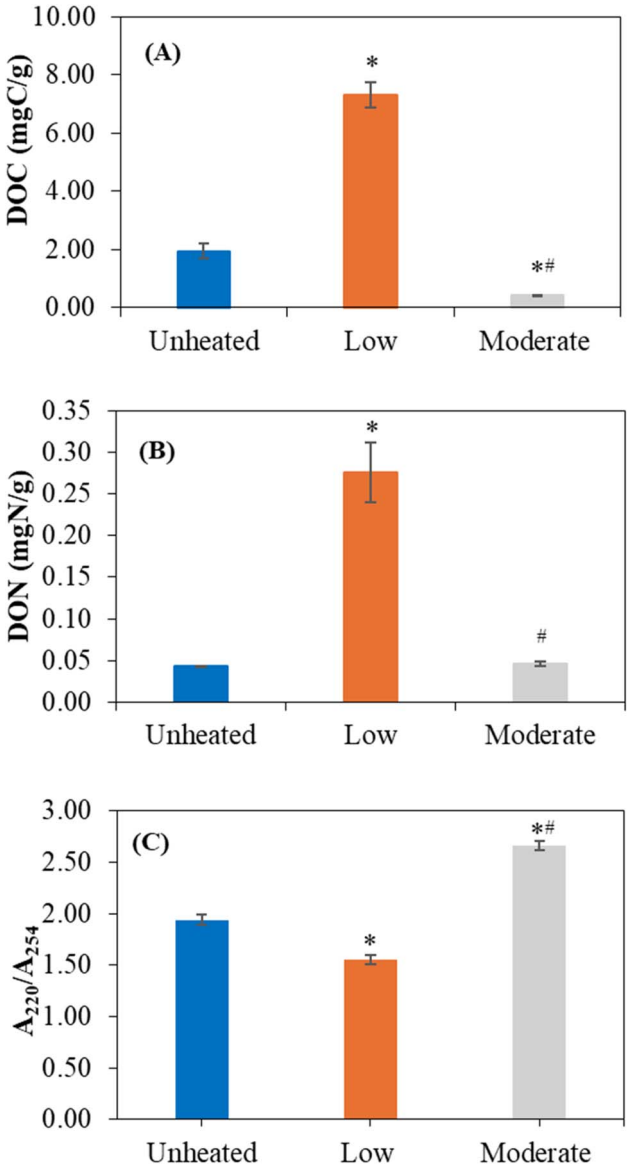


Fig. 1 Mean mass leached per gram of solid material for (A) DOC and (B) DON; (C) A_{220}/A_{254} ratios for unheated, low (250 °C) and moderate (450 °C) temperature samples. Error bars indicate the standard deviation. Significant differences ($p < 0.05$) of low and moderate temperature samples compared to unheated samples are indicated with '*' and significant differences ($p < 0.05$) between low and moderate temperature samples are indicated by '#'.

The DON concentration leached from solid materials was also influenced by heating temperature. The average DON leached from low temperature samples was 0.28 ± 0.04 mgN per g of solids, while both unheated and moderate temperature samples leached significantly less DON (Fig. 1B; $p = 0.002$). For organic soils, Bahreksha *et al.*[52] found the maximum solubility of DON at 250 °C and for mineral soils, Hohner *et al.*[10] also observed peak DON at 250 °C. The greater DON concentration from low temperature heating may point toward the formation of new, smaller compounds containing nitrogen from the destruction of larger compounds containing multiple nitrogen moieties, and may also indicate that compounds containing nitrogen are more stable at lower temperatures.[35] The lowest DON concentration was leached from the moderate temperature materials, which may be due to the increased volatilization of nitrogen containing compounds with increasing temperature. Serrasolsas & Khanna,[55] reported that DON leached from heated soil is primarily derived from degraded microbial biomass. Thus, it is also likely that most of the degraded biomass was volatilized at moderate temperatures.

Greater polarity increases organic matter solubility.[56] The A_{220}/A_{254} ratio inversely correlates with DOM polarity[48–51] and was used as an indicator of polarity. The A_{220}/A_{254} ratios of low temperature samples were 20 ± 4% lower than unheated samples ($p = 0.02$) (Fig. 1C), suggesting that low temperature samples were more polar. Therefore, greater DOC and DON leached from low temperature materials might be attributed to the greater polarity and possible presence of a higher proportion of oxygenated-aliphatic compounds. A_{220}/A_{254} indicated that moderate temperature samples were the least polar, consistent with the lowest DOC and DON leached. Research has shown that above 400 °C, DOM is generally composed of fewer oxygen-containing formulae and more aromatic compounds[35,54] suggesting lower polarity. The greater polarity and solubility of low temperature samples may influence the quality, quantity and biodegradability of dissolved PyOM.[9,24]

3.2 Biodegradation of DOC

3.2.1 Effect of heating temperature. The DOC biodegradation of unheated, low, and moderate temperature samples was significantly different. All heated samples had greater DOC biodegradation than unheated samples, with the exception of the moderate temperature control experiment (Fig. 2). The initial DOC concentration (7.0 ± 0.3 mg L^{-1}) of one moderate temperature control sample was lower than the other samples and might have impacted the biodegradation process. In control experiments, the average 38 day DOC degradation was 30.1 ± 0.7%, 59.5 ± 9.1% and 28.7 ± 2.3% for unheated, low, and moderate temperature samples, respectively (Fig. 2A). Although the control experiments contained sterile inoculum, soil/litter microbes were still present as the leachates were not autoclaved to avoid altering DOM chemical properties. Therefore, the DOC biodegradation observed in control experiments may be due to the presence of soil/litter microbes. Moreover, in control experiments DOC concentrations increased in the early stage of incubation through day 10 for the moderate temperature samples, which may be explained by organic carbon being shed from dead or lysed microbial cells. It may take time for the microorganisms to acclimate to a new substrate and as such, microbes may die at the beginning of an incubation period and contribute organic carbon.[54,57] For inoculation experiments, the average 38 day DOC degradation for unheated, low and moderate temperature samples were 32.1 ± 9%, 63.7 ± 9% and 70.9 ± 1.7%, respectively (Fig. 2B). For heated samples, DOC biodegradation was greater than the control experiments. The DOM leached from heated samples might be more readily utilized by the mixed microbial community in inoculation

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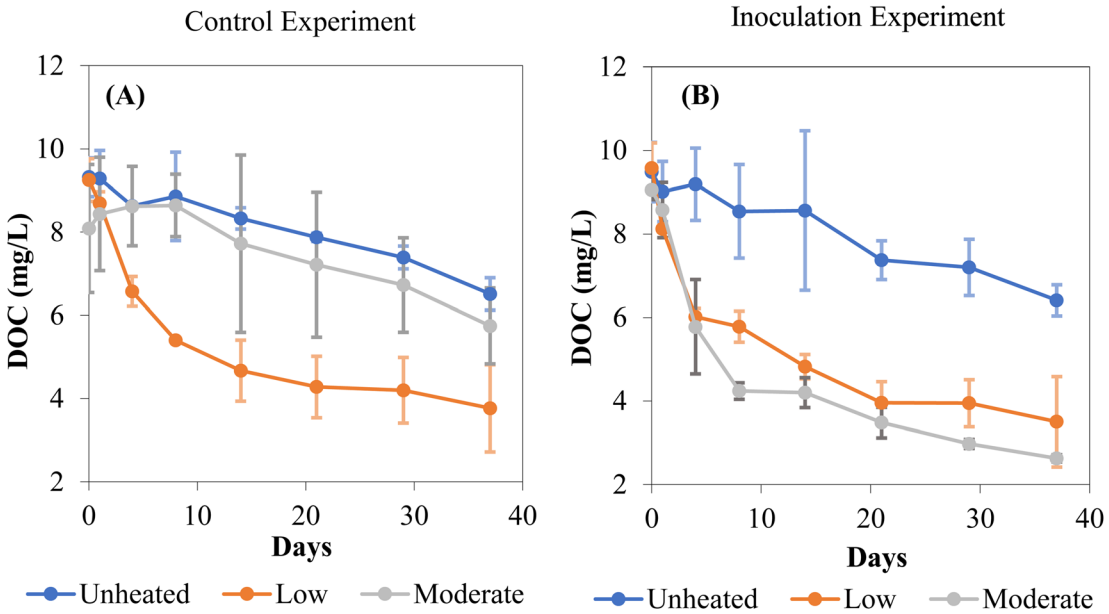


Fig. 2 DOC concentrations for unheated, low (250 °C), and moderate (450 °C) temperature samples (A) control experiments and (B) inoculation experiments. Error bars indicate the standard deviation from the respective means.

experiments than exclusively the soil microbes in control experiments.

Similar biodegradation of DOC was observed in previous studies investigating heated DOM. Norwood *et al.*[29] evaluated the biodegradation of 250 °C heated DOM and found that >50% DOC was degraded, and biomarkers of low-temperature combustion (*i.e.*, levoglucosan and free lignin phenols) were almost entirely degraded after one month of incubation. Bostick *et al.*[12] assessed the biodegradation of DOM leached from 400 °C and 650 °C oak char and found 45% and 37% DOC degradation, respectively, after 96 days of soil microbial incubation. Also, greater DOC biodegradation of 300 °C heated samples was observed compared to 450 °C and 600 °C samples.[13] Here, the observed greater DOC biodegradation of low temperature samples, and inoculated moderate temperature samples supports the hypothesis that low to moderate temperature dPyOM will be more biodegradable than unheated DOM. The greater DOC biodegradation of heated samples may be partially explained by the transition toward lower molecular weight DOM composition with increasing heating temperature.

3.2.2 DOM molecular weight and aromaticity. The E_2/E_3 ratio, inversely proportional to DOM molecular weight,[47] increased with temperature. Moderate temperature samples had significantly higher initial E_2/E_3 ratios than unheated and low temperature samples (Fig. 3A and B) suggesting the enrichment of lower molecular weight compounds at moderate temperature. After 38 days of incubation, E_2/E_3 decreased for all samples (Fig. 3A and B) signifying microbial consumption of lower molecular weight compounds, with comparatively higher molecular weight DOM remaining. In both experiments, unheated samples showed the smallest decrease in E_2/E_3, while moderate temperature samples had the greatest decrease in E_2/E_3 in inoculation experiments (Fig. 3B). These findings are consistent with the observation that lower molecular weight compounds are generally more biodegradable.[12,13,23] In previous studies, moderate to higher temperature samples were observed to contain lower molecular weight compounds that aided in DOM biodegradation.[12,13] Similarly, moderate temperature samples were enriched in lower molecular weight DOM and more readily biodegradable in this study.

The heating temperature influenced the leachate DOM aromaticity. $SUVA_{254}$ increased with temperature, indicating an increase in aromaticity.[46] Further, $SUVA_{254}$ increased after incubation for all samples (Fig. 3C and D). For unheated samples, $SUVA_{254}$ showed the smallest increase after incubation (32.5 ± 2.4%) (Fig. 3C and D) supporting the observed lower biodegradation. For low temperature samples, the increase in $SUVA_{254}$ after incubation was higher than unheated samples for both control (45.6 ± 0.04%) and inoculation (46.2 ± 1.2%) experiments. Inoculated moderate temperature samples had the greatest $SUVA_{254}$ increase (63.7 ± 2.8%) consistent with the greatest DOC biodegradation. In general, the increase in $SUVA_{254}$ after microbial incubation indicates preferential biodegradation of aliphatic organic matter, with more aromatic compounds remaining, which was also observed by Bostic *et al.*[12] and Chen *et al.*[13] for oak and corn-straw dPyOM, respectively. Therefore, the observed biodegradation of aliphatic compounds may enhance microbial growth, change aquatic biogeochemical cycles, decrease dissolved oxygen, and influence ecosystem structure and function after wildfire.

3.2.3 Effect of initial microbial community type. As expected, differences in DOM biodegradation were observed between control and inoculation experiments. For all samples and experiments, the microbial concentration (CFU mL^{-1}) initially increased and then decreased at the end of the incubation period (Fig. S1†), consistent with general microbial

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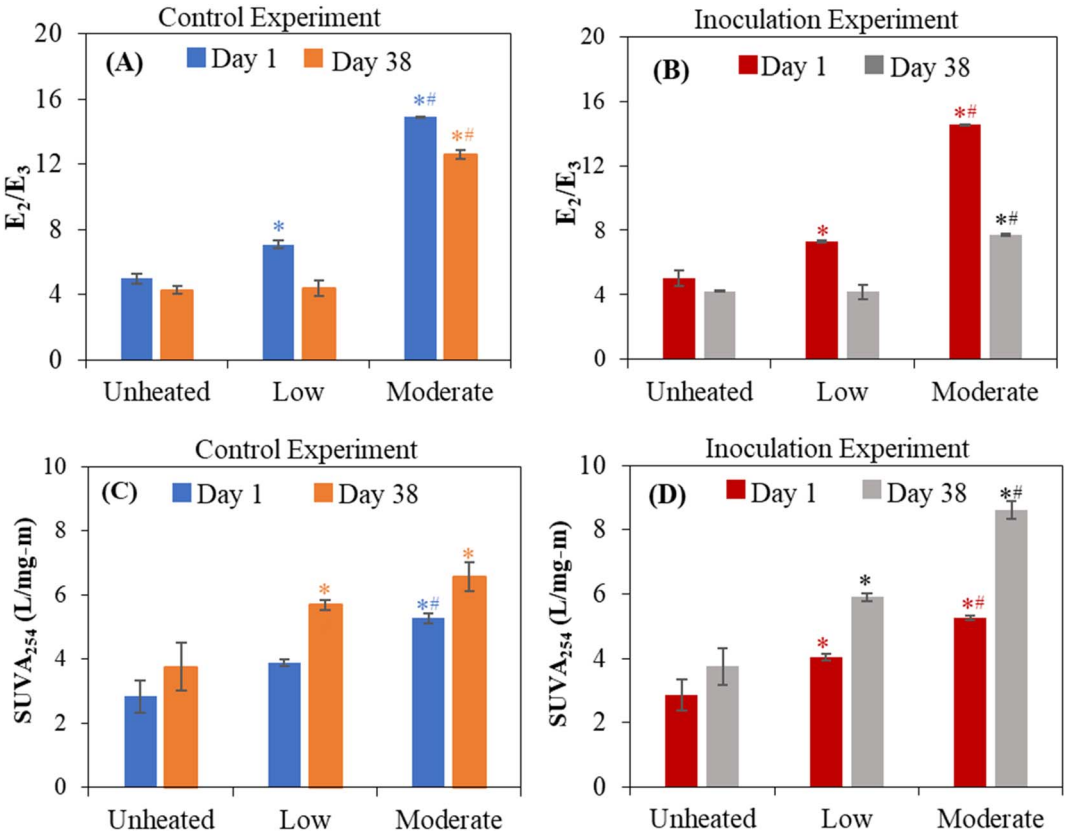


Fig. 3 Initial (day 1) and after incubation (day 38) E_2/E_3 for (A) control and (B) inoculation experiments, and $SUVA_{254}$ for (C) control and (D) inoculation experiments. Error bars show standard deviation. Significant differences ($p < 0.05$) of low and moderate temperature samples compared to unheated samples are indicated with *, and significant differences ($p < 0.05$) between low and moderate temperature samples are indicated by #.

growth patterns.[57] In control experiments unheated samples had a lower microbial concentration than the inoculation experiment (Fig. S1†). However, the DOC biodegradation of unheated samples was similar ($p = 0.81$), which indicates that the aquatic microbes did not have a considerable impact on DOC biodegradation. Perhaps, the aquatic microbes could not efficiently utilize the available substrate in unheated samples, or the amount of nutrients available may not have been sufficient for the microbes to metabolize the substrate.[57,58] DOC degradation of low temperature samples was significantly greater than the unheated control ($p = 0.01$) and inoculation ($p = 0.02$) experiments, suggesting that the heated DOM might have been more readily utilized by the microbes than unheated DOM. The microbial concentration of low temperature samples increased gradually with time for control experiments, whereas for inoculation experiments, the increase was sudden, likely due to the addition of exogenous aquatic microbes. This increase in microbial concentration supports the gradual DOC degradation with time, while the decrease in the microbial concentration at the end of the incubation may be due to less biodegradable substrate remaining.

DOC biodegradation of inoculated moderate temperature samples was significantly higher (Fig. 2B) than control experiments (Fig. 2A) ($p = 0.003$) and may be due to the significantly higher microbial growth observed (Fig. S1†). In addition to the lower molecular weight composition of moderate temperature samples, there may be fewer soil microbes present after 450 °C heating. Therefore, in the control experiment, the surviving soil microbes could not degrade DOC as well as a mixed community of soil and aquatic microbes in the inoculation experiment. Additionally, the mixed community of soil and aquatic microbes may be able to overcome the aromaticity hindrance of the heated DOM, and it appeared that the lower molecular weight of the moderate temperature samples was the main driving force for biodegradation.

3.2.4 Organic matter functional groups. Peak intensities of DOM functional groups observed in the FTIR spectra changed after 38 days of incubation (Fig. S2†). The FTIR spectra were divided into a functional group region (4000–1500 cm^{-1}) and a fingerprint region (1500–600 cm^{-1}).[59] For all samples, peaks were observed in the OH-stretch region (3700–3100 cm^{-1}) and around 1660 cm^{-1} (Fig. S2†). The comparatively sharp peak in the OH-stretch region may be due to the presence of alcoholic bonds. The peak at 1660 cm^{-1} may either represent C=O or C=C bonds as it falls both in the C=O (1650–1830 cm^{-1}) region[59] and in the C=C (1640–1680 cm^{-1}) region.[60] The peak at around 650 cm^{-1} may represent vinylic carbon bond (670–700 cm^{-1}),[61] also consistent with the possible presence of the C=C (sp^2) in the functional group region.

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After 38 days of incubation, the intensity of the OH-stretch region decreased, and the decrease was greater for heated samples compared to unheated, indicating greater biodegradation of alcoholic groups (Fig. S2†). The peak intensity around 1660 cm^{-1} slightly increased for most heated samples, suggesting a possible increase in the oxygenated and alkyl carbon groups after incubation (Fig. S2†). The increase in the intensity of the oxygenated and alkyl groups may be explained by the biodegradation of lower molecular weight compounds,[12] by the presence of heat-altered microbially stable cellulose compounds,[62] or by a combination of both processes. This observation is consistent with previously reported increases in the oxygenated-C and alkyl-C after 10 days of soil microbial incubation of oak dPyOM using ^{1}H-NMR spectroscopy.[12] The observed peaks for vinylic-C on day 1 were absent on day 38 for two samples in control experiments and two samples in inoculation experiments. The absence of these peaks suggests the biodegradation of the vinylic-C groups and the presence of a wide range of enzymatic activities that are required for vinylic-C degradation.[12,62] These findings suggest that biodegradation may influence the functional groups present in unheated and heated samples differently with higher biodegradation of

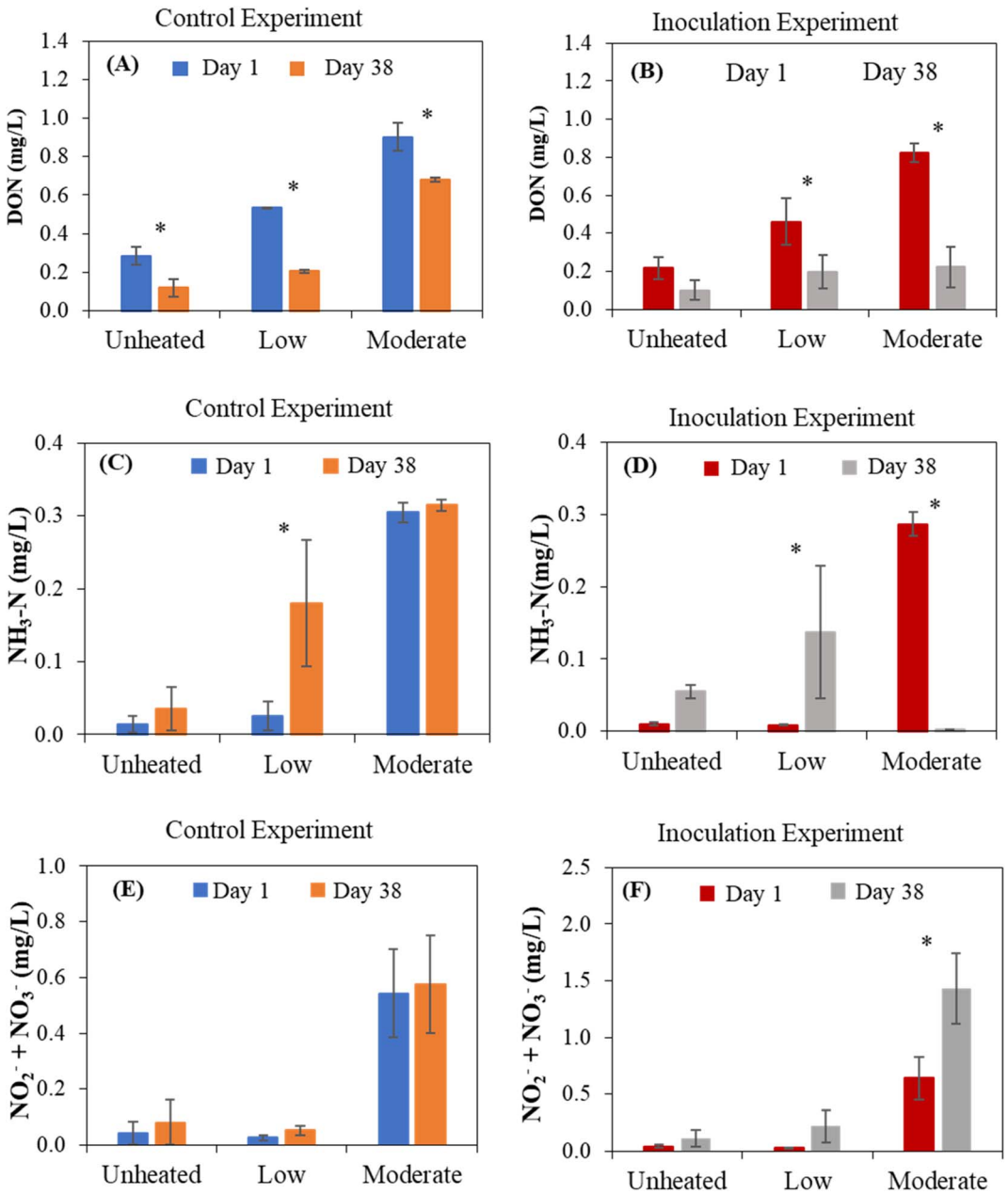


Fig. 4 Initial (day 1) and after incubation (day 38) concentration of nitrogen species (A) DON – control; (B) DON – inoculation; (C) NH_3–N – control; (D) NH_3–N – inoculation; (E) NO_2^- + NO_3^- – control and (F) NO_2^- + NO_3^- – inoculation for unheated, low (250 °C) and moderate (450 °C) temperature samples. Error bars show the standard deviation between replicate samples. Significant differences between day 1 and day 38 are indicated with * where appropriate.

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alcoholic groups and increased generation of oxygenated and alkyl groups in heated samples.

3.3 Biodegradation of DON

The initial DON concentration (mg L^{-1}) of the samples varied (unheated < low-temperature < moderate-temperature). The DON concentration of all samples decreased after 38 days for control and inoculation experiments (Fig. 4A and B). In control experiments, the DON decrease was the greatest for both unheated (54.0 ± 25.8%) and low temperature (54.6 ± 3.6%) samples, while moderate temperature samples had the lowest DON decrease (23.0 ± 5.9%) (Fig. 4A). The high variability of unheated samples may be explained by heterogeneity in soil composition and microbial community composition. The lower DON biodegradation of moderate temperature samples may be due to the lower number of soil microbes that survived 450 °C heating. Additionally, the DON composition might have become less readily mineralizable by the soil microbial community impacting the biodegradation process. Alternatively, inoculated moderate temperature samples showed the greatest DON degradation (74.4 ± 24.6%), followed by low temperature (58.0 ± 7.4%) and unheated samples (51.3 ± 9.3%) (Fig. 4B). Consistent with DOC degradation, the aquatic microbes might have degraded the moderate temperature DON more easily. DON degradation observed in this study strengthens previous work of Chen *et al.*,[13] which suggested microbial decomposition of DON from an observed increase in inorganic nitrogen after 56 days of incubation with soil microbes. Control and inoculated unheated and low temperature samples had similar DON degradation. In contrast, control moderate temperature samples had the lowest DON degradation, while the inoculation experiment had the greatest DON degradation, which may be partially attributed to the highest initial DON concentration.

Biodegradation also influenced nitrogen speciation. Generally, with the decrease in DON, ammonia-N increased after 38 days of incubation (Fig. 4C and D), as microbes converted DON to ammonia/ammonium through ammonification. However, this trend was not observed for inoculated moderate temperature samples. NO_2^- + NO_3^- also increased after incubation (Fig. 4E and F) suggesting some ammonia-N was converted to NO_2^- + NO_3^- through nitrification. Nitrifying bacteria are common in microbial communities, and under aerobic conditions first transform free ammonium into nitrite and then into nitrate.[57,58,63] For the inoculated moderate temperature samples, ammonia-N was entirely converted to NO_2^- + NO_3^-(Fig. 4D and F). Additionally, for control experiment low and moderate temperature samples, the NO_2^- + NO_3^- concentration decreased on day 21 and day 8, respectively (Fig. S3†). It is possible that these reactors may have become anaerobic, and that N_2 or N_2O gas was produced from NO_2^- + NO_3^- through denitrification.[58,64] However, the N_2/N_2O gas concentration was not measured, and the occurrence of denitrification was not confirmed. Further discussion of the temporal changes in nitrogen species during the incubation period is in the ESI (Fig. S3).† The observed increase in nitrite + nitrate may decrease the nitrogen limitation in surface waters, which could give rise to harmful algal blooms after wildfire.[65] Therefore, biodegradation of DON may influence post-fire nutrient concentrations and speciation in aquatic systems which may have important implications for ecosystem health and water quality.

4 Conclusions

Understanding the biodegradation of dissolved PyOM compared to unheated DOM is crucial for determining wildfire effects on aquatic biogeochemistry, such as dissolved oxygen and nutrient levels, aquatic microbiome structure and function, and potential influences on source water quality. To address potential post-fire alterations to DOM in aquatic systems, the biodegradation of unheated, low, and moderate temperature DOM was compared. Low and moderate temperature samples had significantly greater biodegradability than unheated samples. The greater biodegradation of heated samples may be due to the shift toward lower molecular weight DOM composition with increasing heating temperatures. DOM aromaticity was also impacted by biodegradation, as microbes metabolized lower molecular weight, aliphatic compounds during the incubation period with higher molecular weight aromatic compounds remaining. Moreover, biodegradation impacted nitrogen speciation. The observed biodegradation of heated samples may imply the potential for increased microbial concentrations in surface waters after fire, and impact the in-stream processing and transformation of DOM. Further research should focus on verifying biodegradability of unheated and heated DOM using different experimental methods and reactor sampling approaches to overcome limitations of this study. Additionally, research to identify the microbial communities responsible for biodegradation is vital to elucidate wildfire effects on ecosystems.

Conflicts of interest

There are no conflicts to declare.

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