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Emerging investigator series: impacts of land use on dissolved organic matter quality in agricultural watersheds: a molecular perspective†

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In aquatic systems, dissolved organic matter (DOM) has important ecological and biogeochemical functions, where the molecular composition of DOM has larger-scale implications for climate change and global carbon cycles. However, there is limited information about the relationships between landscape characteristics and human disturbance that influence the molecular composition of DOM changes in watersheds. In this study, we collected water samples from 22 sites across a gradient of topographically characterized agricultural land coverage and community infrastructure development in the Kawartha region in Ontario, Canada. We employed a combination of Fourier Transform Ion-Cyclotron Resonance Mass Spectrometry (FT-ICR-MS) and absorbance spectroscopy to investigate changes in the molecular composition of DOM with increasing agricultural and community development disturbance on the optical and molecular characteristics of DOM. We found that dissolved organic carbon (DOC) concentrations in disturbed (>75%) watersheds ranged from 3.67–32.8 mg L^{-1} and were significantly higher than in watersheds with more abundant forest coverage (3.78–9.13 mg L^{-1}). In addition, watersheds with higher phosphorus concentrations had more negative nominal oxygenation state of carbon (NOSC) values, suggesting biologically processed DOM correlating with increased phosphorus levels in aquatic systems. To relate the molecular properties of DOM to landscape metrics, we used Spearman's correlation analysis to reveal that agriculturally impacted and community developments enhanced the molecular signature of unsaturated hydrocarbon. In addition, we identified 65 dissolved organic phosphorus (DOP) molecules that significantly increased in abundance with disturbance, likely due to microbial mineralization of existing DOM with the addition of phosphorus to form larger, biologically inaccessible molecules. The overall recalcitrance of the identified molecules can serve as molecular signatures when evaluating the level of disturbance of a watershed.

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Environmental significance

The molecular composition of dissolved organic matter has important ecosystem functions like impacting contaminant transport, carbon sequestration potential, and microbial metabolism. As human disturbances continue to change natural biogeochemical cycles, it is important to understand changes in DOM at the molecular level to understand how ecosystem functions are changing. In this work, we show how disturbances at the landscape level impact the molecular composition of DOM in agriculturally dominated watersheds. Together, we demonstrate how agriculture disturbance shifts the DOM pool towards favouring highly unsaturated and decomposed dissolved organic phosphorus molecules that can be used as molecular signatures in future work.

1 Introduction

Dissolved organic matter (DOM) is a mixture of heterogenous carbon-based molecules that is an essential component of the global carbon cycle. The movement of DOM, particularly in freshwater sources *via* streams, lakes, and tributaries, has critical roles in regulating water quality and carbon sequestration across inland watersheds.[1–3] While DOM concentration is important for determining carbon flux from terrestrial to aquatic systems, the chemical composition of DOM is also important to consider since the chemical characteristics of DOM are related to reactivity and functions within aquatic systems.[4,5] The overall source, reactivity, and composition of DOM have essential roles in meeting the heterotrophic nutrient needs of microorganisms and serve as a critical link in the global carbon cycle by connecting abiotic to biotic carbon.[6] DOM of relatively lower molecular weight generally originates

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from autochthonous sources and comprises proteinaceous and aliphatic fractions.[7] In contrast, allochthonous DOM external to the aquatic system originates from vegetation and humic fractions of soils that are less biologically accessible for microbial consumption and often persist longer than autochthonous DOM.[8] In addition, the molecular composition of DOM can influence the binding and transport of inorganic contaminants throughout aquatic systems and the biogeochemical fate of these contaminants.[9,10]

Disturbance to freshwater ecosystems *via* urbanization and agricultural land use has implications for the quality of DOM in streams and runoff channels.[11] In addition, the chemical transformations caused by microbial decomposition and photochemical degradation further alter the chemical composition of DOM and the overall reactivity.[12,13] Agricultural land use can significantly change the overall quality of DOM by altering hydrological flow pathways, reducing vegetation inputs, and the proportion of microbial-transformed autochthonous DOM.[11] Hydrological changes like surface runoff, discharge rate, and soil porosity also impact the type of DOM in agricultural watersheds where low discharge periods and baseflow conditions promote microbially-derived DOM formation.[14]

Excessive amounts of nutrients like phosphorus (P) and nitrogen (N) mobilized from terrestrial to aquatic systems in agriculturally impacted watersheds can reduce water quality and enhance algae proliferation, leading to rapid eutrophication.[15] For example, fertilizer application and organic amendments can also affect the quality of DOM by leading to nitrogen-containing and proteinaceous DOM signatures associated with more eutrophic conditions in agriculture-draining streams.[16] The increase in urbanization and agricultural land usage has led to enhanced carbon export from soils through soil compaction, vegetation loss, and changes in hydrology.[11] Practices such as tilling or employing heavy machinery can also affect the structural integrity of soil, resulting in higher soil organic carbon inputs that ultimately reduce soil quality and organic carbon sequestration in soils.[17]

The physical characteristics of the landscape within the watershed can also influence the transport and transformations of DOM. For example, small streams draining forested regions across flatter watersheds result in high residence times and microbially processed terrestrially derived DOM, whereas steeper watersheds can mobilize DOM faster with fewer transformations.[18,19] Forests, bogs, and wetlands can act as a source of aromatic DOM with high molecular weight, and the vegetation composition further influences DOM profiles across the watershed.[20,21] The level of vegetation near a watershed directly affects the carbon in the soil, with a diverse selection of plants correlating with soil fungi and structural integrity.[21] For example, foliage in deciduous forests acts as large pools of carbon, however, it does not exhibit the same level of carbon storage within the soil as forests that contain mixed spruce or coniferous trees.[22–25] These differences in tree coverage across watersheds can influence the polyphenolic profile of DOM in streams and, therefore carbon storage and downstream carbon mineralization by microorganisms.[26]

Traditional methods of DOM characterization involve using absorbance and fluorescence techniques to differentiate DOM sources[27] and relate agriculture land use change. While beneficial for optically active DOM molecules, optical-based techniques lose sensitivity in systems where non-optically active DOM is dominant, like in agriculture-dominated systems where microbially-derived DOM is abundant.[14] The development of Fourier Transform Ion Cyclotron Resonance Mass Spectrometry (FT-ICR-MS) allows for a high degree of resolution and mass accuracy to confidently discern isotopes of unique carbon molecules with DOM and allow for untargeted approaches to the total organic carbon pool.[28] Although FT-ICR-MS is becoming increasingly routine in characterizing DOM, there remain limited studies incorporating approaches that elucidate landscape-level predictors on the influence of DOM quality in urbanized or agriculture-dominated regions.

Therefore, this study aims to identify landscape-level predictors for DOM quality along a gradient of disturbance in watersheds draining regions undergoing human development with variable levels of agricultural disturbance, urbanization, and regions with natural swamp, bog, and forest coverage. We hypothesize that an increase in agricultural and urbanized land use will enhance hydrogenated microbial signatures in the DOM profile of streams, whereas DOM in streams dominated by wetland and forested regions will contain greater abundances of refractory and aromatic DOM. Using watershed characteristics and land cover information from existing watershed tools, we hope to provide relationships between the characteristics of watersheds and species DOM molecules in streams.

2 Methods

2.1 Study area and field methods

The area administered by the Kawartha Region Conservation Authority (KRCA), Kawartha region, is located in southcentral Ontario. The region extends from Lake Scugog in the southwest and Pigeon Lake in the east to Balsam Lake in the northwest and Crystal Lake in the northeast – a total of 2563 square kilometres (Fig. 1). The region is underlaid by sand on the Oak Ridges Morain to the South, diamicton plains in the center, and granite bedrock to the north. The diamicton plains over limestone geology have resulted in an agricultural-dominated landscape in the central region. Sites were selected based on the existing water quality monitoring program administered by KRCA and were characterized based on land cover characteristics, *i.e.*, ≥75% agricultural land use (Table S1†).

Water samples were collected in August–September 2022. All surface water sample containers were triple-rinsed with the targeted water before sampling. Surface water samples were collected from 0.15–0.3 m below the surface, and water quality field parameters, including water Temperature (Temp.), pH, Conductivity (Cond), dissolved oxygen (DO), and Turbidity (Turb) were measured *in situ* with a water quality meter (Hanna multiparameter hi9829). A combination of bridge and pole sampling was utilized to collect water in water streams unreachable by grab sampling. Samples for carbon analysis were filtered into 60 mL vials using a 0.45 μm syringe filter. All

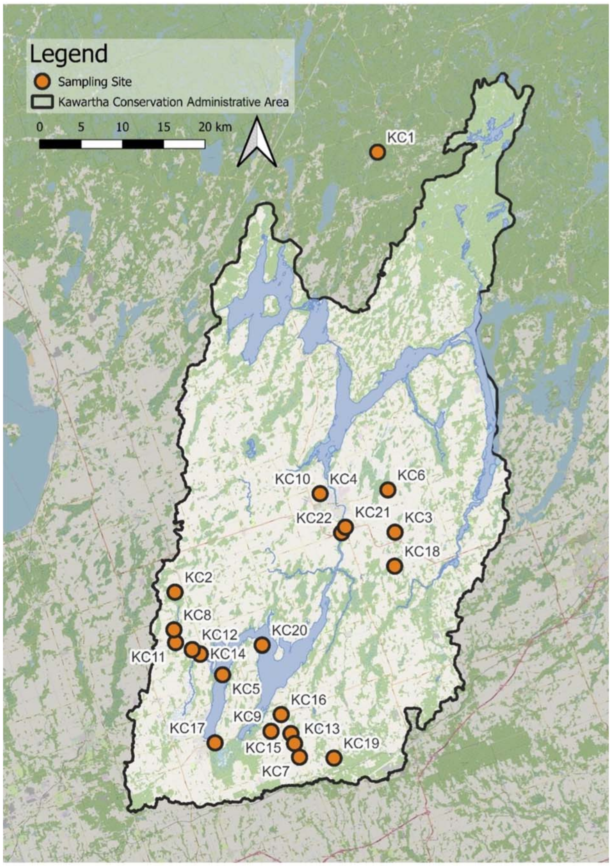

Fig. 1 Sampling map of the 22 sampled streams and tributaries (orange dots) in the Kawartha region.

samples were kept cool (<4 °C) for transport and storage. Samples were sent to Caduceon Environmental Laboratories for chemical analysis, *i.e.*, chloride (Cl), nitrite–nitrogen (NO_2–N), nitrate–nitrogen (NO_3–N), ammonia–nitrogen (NH_3–N), Total Kjeldahl Nitrogen (TKN), Total Phosphorus (TP) and Total Suspended Solids (TSS). Samples for carbon analysis were returned to Brock University absorbance spectroscopy and high-resolution mass spectrometry analyses.

2.2 Solid phase extraction and high-resolution mass spectrometry

Solid-phase extractions were conducted based on methods from Dittmar *et al.*, 2008. Briefly, cartridges were primed with 1 cartridge filling of HPLC grade methanol immediately before use.[29] Two cartridge volumes of DOM samples were loaded onto the SPE cartridges. One cartridge volume of 0.01 M HCl was added to remove salts and ionization inhibitors. Sorbents were air dried for approximately 5 minutes, and DOM was immediately eluted with 1 cartridge volume (1 mL) of methanol at a flow rate of <2 mL min^{-1} directly into muffled glass ampoules. Upon sample collection, one cartridge volume of methanol was added to collect SPE-extracted DOM samples in clean 10 mL scintillation vials. Prior to FT-ICR-MS injection, SPE extracts were normalized to 20 ppm to ensure compositional changes were based on molecular composition and not due to concentration effects across samples.

A 7 T Bruker SolariX XR FT-ICR-MS (Billerica, Massachusetts) equipped with an ESI source housed in the Trent University Water Quality Center was used for the molecular characterization of DOM. Before sample analysis, the instrument is calibrated at four different resolutions in negative ionization mode starting from 1M, 2M, 4M and 8M by co-adding 25 scans and measuring exact mass accuracy against 10 known compounds within 100 ppb accuracy in a sodium trifluoroacetate (NaTFA) tuning solution.[30,31] After calibration, MilliQ water and methanol were used for flushing peak tubing and SPE-extracted DOM samples were directly injected at a flow rate of 120 μL h^{-1}. The capillary voltage was set to 4500 V, and an ion accumulation time ranged between 0.5–1.0 s to achieve an ion count of at least 1.0×10^9 ions per scan. A total of 200 scans were co-added for each sample DOM sample and acquired from a m/z range of 150–700 at 8 M resolution and transformed using absorption mode.[32] Duplicate and blank scans were taken after every 10 and 5 samples, respectively.

Using Bruker DataAnalysis (v 4.4), baseline, background noise, and methanol/MilliQ subtractions and peaks below S/N ratios of 4 were excluded. DOM molecular formulae were determined within the elemental constraints: $^{12}C_{(1-100)}$, $^{1}H_{(1-200)}$, $^{16}O_{(1-50)}$, $^{14}N_{(0-2)}$, $^{32}S_{(0-2)}$ and $^{31}P_{(0-2)}$ with a mass tolerance of ±0.5 ppm. Isotopes of ^{13}C and ^{18}O were identified for C and O containing DOM molecules to ensure accurate formula assignment to molecules. Using a method similar to Fu *et al.*, 2020,[33] we incorporated a TRFu nested loop-based MATLAB code for formula assignments. Briefly, the optimum formula was selected based on (1) a minimum number of formulae for the black carbon, (2) the minimum number of N + S + P, (3) a minimum number of S + P, and (4) the lowest mass error. We have also calculated and included P/C ratios and ensured that annotated P-containing DOM fit within the 0.01–0.1 range of other studies.[33] Not only has this pipeline been shown to provide the accurate exact mass formula, but it has also been applied to other studies that confidently identify P-containing DOM using exact mass.[34] After formula assignment, atomic ratios, aromaticity indices (AI_{mod}),[35] and the nominal oxygenation start of carbon (NOSC)[8,36] were all calculated and exported to R[37] for data visualization using the "ggplot2" package[38] and "corrplot" package for correlation plots and heatmap analyses.[39]

2.3 Absorbance spectroscopy

Each of the 22 samples collected from different watersheds was run through the Agilent Cary 60 UV-Vis spectrometer. Absorbance was measured from 220 to 600 nm at 300 nm min^{-1} in 0.5 nm intervals using a quartz cuvette with a 1 cm path length. Samples were run at room temperature, and absorbance was baseline corrected with MilliQ water blanks. $SUVA_{254nm}$ measurements that normalize absorbance at 254 nm to DOC concentrations were calculated by dividing absorbance at 254 nm by DOC concentrations.[40,41] Spectral slopes from 275–295 nm were calculated using the linear regression of the log-transformed absorbance spectra as a proxy for DOM molecular weight.[41] Slope ratios (S_R) for DOM molecular weight and source information were calculated by dividing spectral slope values from 275–295 nm to 350–400 nm.[40,41]

2.4 Total organic carbon

A Shimadzu Total Organic Carbon (TOC) analyzer with an ASI-V automated sampler housed in the University of Toronto Scarborough TRACES center measured TOC concentrations in filtered stream samples. Briefly, potassium hydrogen phthalate was used as a standard to generate a calibration curve between 1–50 mg L^{-1} (R^2 > 0.99). After calibration, 50 mL of each sample was added to an autosampler with a carrier flow level of 150 mL min^{-1}, two injections per sample (overall standard error of 0.1 mg L^{-1}), and two washes of low TOC water between each sample. Blanks were run after every five samples.

2.5 Statistical analyses

An analysis of variance (ANOVA) was calculated in R (v 1.2.5033) to determine significant differences between DOC concentrations and absorbance properties of DOM. A Pearson correlation heatmap equipped with Bray–Curtis hierarchical clustering was conducted to relate FT-ICR-MS properties, water quality parameters, and absorbance metrics of DOM across different watersheds. Finally, a Spearman's rho (ρ) relating the relative abundances of different DOM molecules to watershed characteristics and land cover was conducted using a custom script R.[42] A total of 999 permutations were conducted to remove false positive findings, and significant correlations were observed when $p < 0.05$. Using a van-Krevelen space, we plotted significantly correlating molecules to relate DOM molecules changing in abundance in relation to different watershed characteristics and land cover properties.

3 Results and discussion

3.1 Water quality covariates, dissolved organic carbon concentrations, and optical properties

DOC concentrations ranged from 3.67–32.8 mg L^{-1} in watersheds with less than 75% agricultural land coverage and were significantly greater ($p < 0.05$) than in watersheds with more abundant natural land cover (3.78–9.13 mg L^{-1}) (Fig. 2). Aquatic systems with greater agricultural land use were also the systems mobilizing the highest concentrations of total organic carbon including KC 4, KC 7 and KC 9, ranging from 18.1–32.6 mg L^{-1}. KC 3 and KC 18 had the lowest levels of DOC in streams ranging from 3.78–4.78 mg L^{-1}, respectively. Across all sites, spectral slopes ranged from 0.0075–0.075, suggesting up to a 10-fold difference in proposed DOM molecular weight across sites. We found that spectral slope values were lowest at KC 13 (0.0075), one of the most agriculturally impacted watersheds, suggesting that the optical DOM profile of this catchment was relatively more aromatic DOM with higher molecular weights than other catchments.

Overall, we found that site KC 22 (Scugog River Upstream) had the highest weighted average m/z ratio of 547, followed by KC 6 (Stoney Creek at Pigeon Lake Rd). Interestingly, the lowest

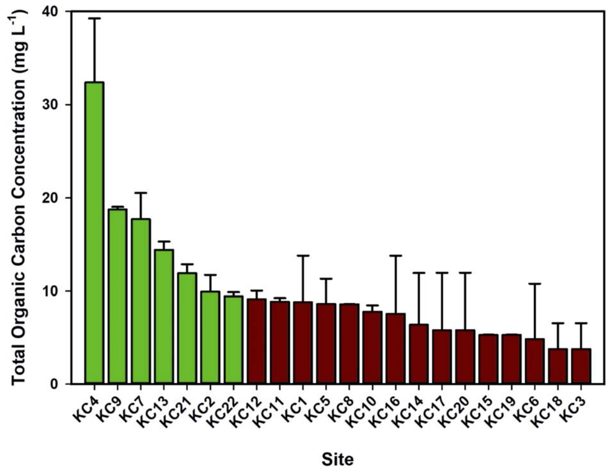

Fig. 2 Total organic carbon concentrations across the 22 sites where green bars represent regions with more than 80% of agricultural land use to indicate more agriculture disturbances across watersheds. Brown bars represent tributaries where agricultural land use was >75% of agricultural land use.

molecular weight of DOM was found in KC 3 (Stoney Creek at Settlers Rd.; m/z = 346) and KC 10 (Scugog River Downstream; m/z = 348), where DOM at these sites was relatively more positive (KC 3 NOSC = −0.553; KC 10 NOSC = −0.423) than other watersheds. The level of heteroatom content reported by FT ICR MS also varied across watersheds and was related to the overall m/z characteristics. For example, KC 10 (Scugog River Downstream) also had the lowest weighted m/z and contained the highest weighted average sulphur content of 0.44 and the highest S/C ratio of all sites at 0.036 (ESI Dataset #1†). Notably, KC 2 (Layton River) had the highest N (0.76) and P (2.09) content across all watersheds with a relatively high m/z ratio of 485. We also found that KC 13 (Blackstock Creek) and KC 14 (Nonquon River) had the highest DBE ratios of 14 and 14.9, respectively, and suggested the presence of highly unsaturated material. Interestingly, these sites both have >40% natural land cover with "fair" levels of surface water quality based on Ontario Provincial water quality guidelines.

When relating relationships between water quality and both optical and molecular characteristics of DOM, we found significant ($p < 0.05$) correlations between nutrients, m/z, and double bond equivalence of DOM in watersheds (Fig. 3). Specifically, Spearman correlation values between chloride concentrations and the overall m/z ratio of a watershed was −0.62 ($p < 0.05$), indicating that as streams in the region had greater chloride concentrations, the relative size of DOM molecules decreased. In addition, as the abundance of DOM molecules with greater NOSC values increased, so did the proportion of nitrate in aquatic systems (Spearman's value = 0.75; $p < 0.05$). However, we observed an opposite relationship between NOSC and P concentrations, where molecules had lower NOSC values as P concentrations increased (Spearman's correlation = −0.82; $p < 0.05$). Despite opposite patterns towards the degree of oxygenation of DOM, both N and P concentrations resulted in DOM with overall lower m/z ratios.

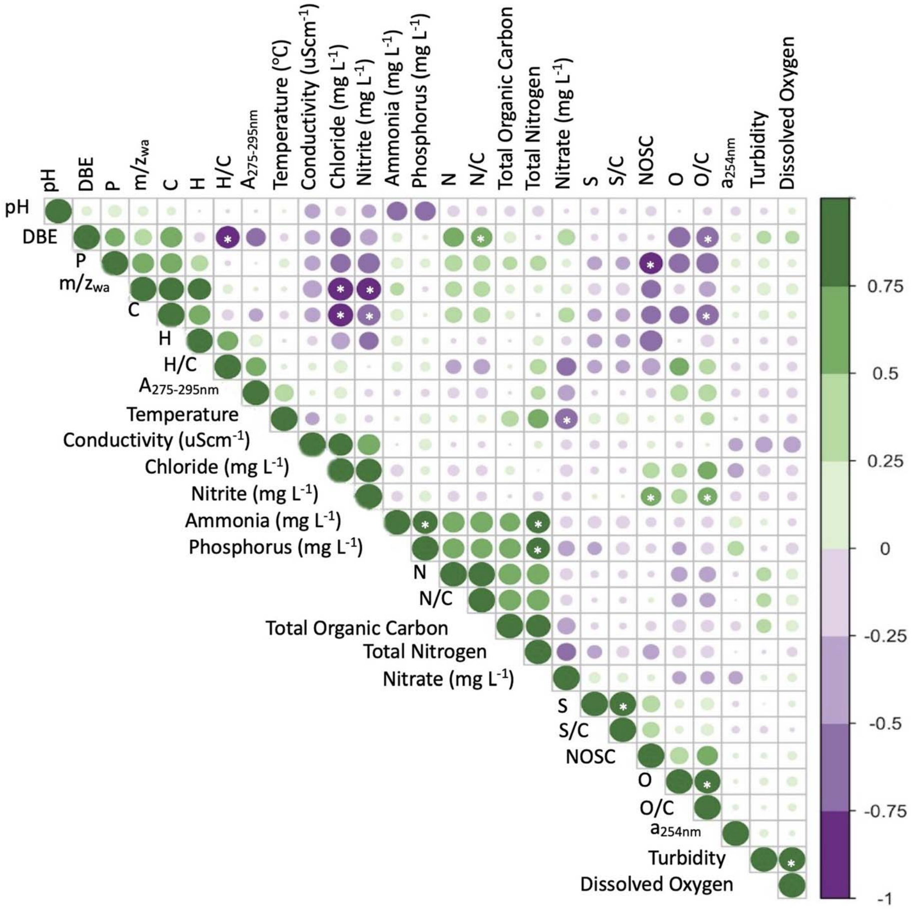

Fig. 3 Pearson correlation plot of water quality, HRMS-derived atomic metrics and absorbance properties of DOM across all watersheds where white asterisks indicate significant relationships ($p < 0.05$).

Other studies have shown a similar pattern of increased DOC export in agriculturally impacted watersheds and greater proportions of microbial humic compounds than in forested streams.[17,43,44] Headwater streams in North Germany[45] and several agricultural streams in Australia[46] also showed greater DOC concentrations in streams with greater agricultural land use. However, this may not be representative of all agricultural disturbances as other studies have found comparatively low DOC concentrations in agricultural systems,[11,20] likely due to the intensity and duration of agricultural land use. The increase in NOSC values observed in this study further aligns with other studies that found increased humification or a reduction in DOM bioavailability in agriculturally impacted watersheds.[45]

3.2 Watershed specific patterns with DOM composition

We found that agriculturally impacted watersheds generally produced a more similar DOM profile with higher molecular weight and less biologically available carbon than DOM draining systems with greater natural land cover characteristics. A Pearson correlation heatmap was created, linking the physical water chemistry properties to DOM chemical characteristics across watersheds (Fig. 4). The 22 sites were categorized into 5 groups based on Bray–Curtis hierarchal clustering, where the first group consisted of sites KC 6 and KC 9 where DOM had more negative absorbance 275–295 nm values, positive NOSC values, and DOM molecules with similar mass-to-charge ratios. Sites KC 2, KC 19, KC 14, and KC 20 were more similar, with sites KC 14 and KC 20 being closely related based on similar levels of dissolved oxygen, more comparable total organic carbon concentrations, temperature, and conductivity.

TP concentrations ranged from 0.02–0.2 mg L^{-1} and NH_3–N concentrations ranged from 0.01–0.38 mg L^{-1} across all sites where Site KC 2 had the highest TP (mean = 0.20 mg L^{-1}) and NH_3-N (mean = 0.38 mg L^{-1}) concentrations. Groups two and three are more alike due to their shared land coverage of human disturbance. KC 5 and KC 17 possess comparable properties regarding their lack of organic nitrogen molecules, high levels of total chlorine content, high electrical conductivity, and similar pH. The most distant relation is KC 13, which is evident with the abundance of nitrate in the water, more negative spectral slope values, and lower conductivity.

Groups four and five, however, are similar due to land cover characteristics where this cluster containing KC 7, KC 10, KC 18, and KC 22 exhibited low sulfur content in DOM but possessed high levels of conductivity and DOM molecules with high m/z ratios. However, DOM draining through watersheds KC 18 and KC 22 contained more abundant organic sulfur compounds in

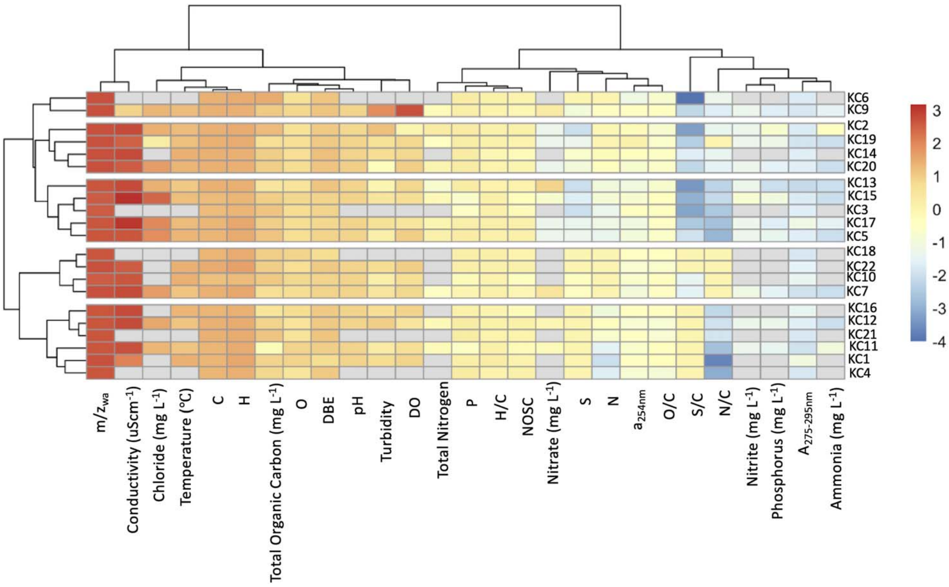

Fig. 4 Pearson correlation heatmap linking water quality, DOM absorbance properties and molecular composition of DOM across 22 different watersheds.

their water streams. The amount of total organic carbon and quantity of DOM molecules with high *m*/*z* ratios in KC 18 and KC 22 are nearly identical. Similar to the third sub-group, all the sites in the fifth sub-group display relatively low levels of nitrogen-containing DOM molecules.

The inconsistent clustering of agriculturally impacted systems (KC 4, KC 7, and KC 9) is largely dominated by differences in sulfur in DOM and S/C ratios. These findings suggest that while there are intrinsic differences across all watersheds regardless of land use, specialized practices or even sulfur containing fertilizers like ammonium sulfate, potassium sulfate, and magnesium sulfate in maize fields[47] routinely used in this region may be impacting the degree of DOM sulfurization in streams. These patterns of enhanced fertilizer use and changing DOM properties have been found in rice patty fields where enhanced sulfurization is changing the biogeochemistry of contaminants like mercury.[48]

3.3 Spearman correlation van Krevelen diagrams

To relate landscape properties to DOM in aquatic systems, Spearman rank correlation analyses were conducted to reveal unique patterns in the overall DOM profile after disturbances. In watersheds where bog coverage was relatively higher, DOM molecules within the aliphatic and highly unsaturated/phenolic compound region increased in relative abundance (Fig. 5A). Most of these compounds ranged between 0.25 > O/C > 0.5 and 1.25 > H/C > 2.0, suggesting that these molecules had relatively high microbial lability resembling low-oxidized plant material. As swamp coverage increased across the catchment area, so did the relative abundance of DOM within the vascular plant-derived polyphenol compound class and more oxidized highly unsaturated and phenolic compounds (Fig. 5B). While aliphatic molecules were present, their abundance was less than DOM molecules, increasing in abundance as bog coverage increased.

As agricultural and land management disturbances increased across catchment areas, the relative abundance of oxidized phenolic DOM decreased; however, the relative abundance of highly unsaturated DOM with O/C ratios < 0.25 increased in abundance (Fig. 5C and D). Interestingly, the high degree of similarity of DOM across both forms of disturbances suggests that disturbance can impact DOM quality in similar ways, specifically shifting the DOM pool towards less oxygenated and unsaturated molecules.

We show that disturbed ecosystems mobilize more DOC than undisturbed ecosystems but that the quality of DOM in undisturbed ecosystems resembles more polyphenol and tannin molecular signatures. These findings are consistent with other studies showing that agricultural disturbances alter stream DOM quality towards favouring humic fractions of DOM in North Germany[45] and several agricultural streams in Australia.[46] Similarly, the abundance of low-oxidized lignins, aliphatic, and phenolic compounds correlating with bog and swamp-dominated catchments has been previously reported in more coastal systems,[49] but also in boreal watersheds.[28] Although the overall chemical diversity was not evaluated in this study, others found that the overall functional diversity of DOM in agricultural landscapes was comparatively low compared to littoral inputs in forested settings.[26,50] In addition the export of DOC in agriculturally impacted watersheds produces greater proportions of microbial humic compounds than in forested streams.[43,44,51] However, our study builds upon these findings to identify that highly unsaturated DOM molecules are preferentially accumulated in the DOM pool with disturbance.

To further elucidate the specific patterns on the DOM molecules significantly correlating with agriculture and community development disturbance, we identified 65 molecules with Spearman's correlation values = 1 when $p < 0.05$ within the unsaturated hydrocarbon DOM compound class that

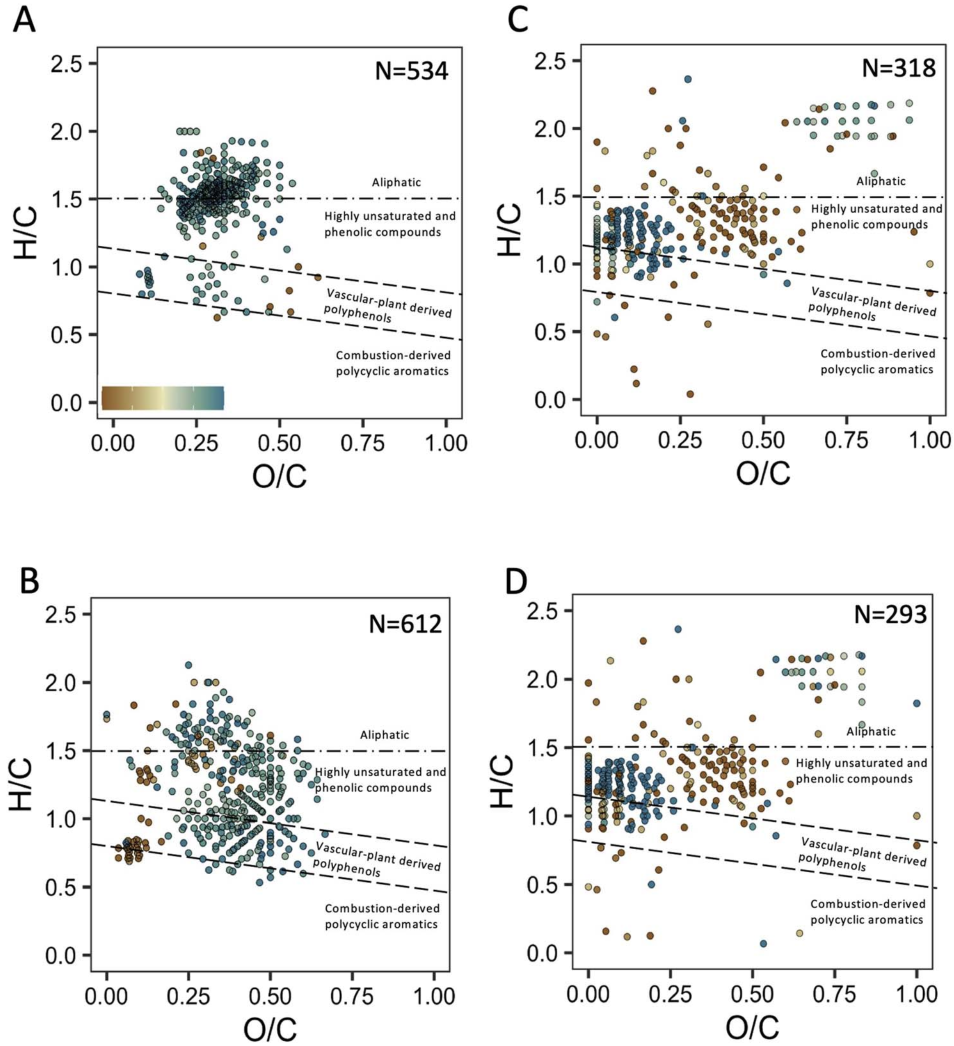

Fig. 5 Spearman correlation van Krevelen diagrams linking the relative abundance of DOM molecules significantly ($p < 0.05$) correlating with land cover and watershed characteristics including bog coverage (A), swamp coverage (B), agricultural land use (C) and community infrastructure (D) after 999 permutations. Each dot represents a unique carbon-based molecule where darker blue colours indicate a positive relationship with watershed characteristics, whereas a darker brown indicates a more negative relationship.

also contained P atoms (ESI Dataset 1†). We used ^{13}C isotopes to help reduce false positive formula assignments that may arise from exact mass alone. While ^{32}P isotopes are extremely trace, other studies have confidently assigned P-containing molecules using a similar TRFu approach for formula assignment.[34] The majority of these dissolved organic phosphorus (DOP) molecules positively correlating with increased disturbances were found across watersheds with high proportions of agricultural land use (KC 4, KC 7, KC 9) and developed spaces (KC 15), watersheds with the highest DOC concentrations in aquatic systems (Fig. 2). The highly conserved nature of these highly unsaturated P-containing molecules suggests that disturbed systems may be used as molecular signatures of areas impacted by agricultural or land use disturbance. In addition, the identified DOP molecules had more negative NOSC values (Fig. 6), suggesting a higher degree of reduction than more positive NOSC values. NOSC values tend to decrease with increased DOM decomposition, further suggesting that the identified DOP molecules have been extensively decomposed and processed.[36]

Our findings suggest that DOM in watersheds with higher agricultural land use and community development contain more phosphorus than natural land cover. DOP species are consistent with observations in other watersheds of agriculturally impacted runoff and surface waters. Specifically, the high degree of DOP compounds is readily associated with unsaturated hydrocarbon compound classes.[15] These unique phosphorus-containing DOM molecules found in agriculturally impacted sites resembling unsaturated hydrocarbon molecules are also associated with molecules that generally have high DBE configurations, suggesting lower reactivity of these molecules when compared to aliphatic fractions of DOM coinciding with bogs and swamp coverage. The identification of DOP substrates suggests long-term agricultural practices as the accumulation of soluble condensed aromatics and recalcitrant fraction of DOM tends to occur in agricultural soils and river sediment with

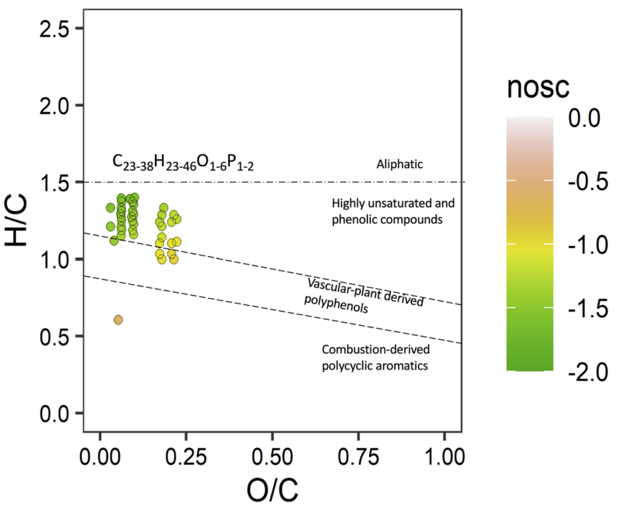

Fig. 6 van Krevelen diagram of the 65 dissolved organic phosphorus molecules significantly ($p < 0.05$) correlating with increased agriculture and landscape disturbance. Dashed lines represent boundaries for various compound classes based on O/C, H/C ratios and DBE. The NOSC value of each dissolved organic phosphorus molecule is colour coded where darker green values represent a more negative NOSC value and pink values show more positive NOSC values.

aging in the environment.[52] Since these DOP molecules have high DBE and more positive values, they may have been byproducts of existing DOM that were already microbially processed towards a more recalcitrant state.[53,54]

Yang *et al.* (2021) reported that about 10% of the molecules in the total DOM pool resembled DOP molecules that were within the unsaturated hydrocarbon compound classes, whereas most of the DOP molecules within the lignin region comprised ∼70% of the molecules observed.[54] While this observation is contrary to our findings where more than 90% of DOP molecules observed were within the unsaturated hydrocarbon region (Fig. 5), there is ongoing evidence suggesting that over 96% of DOP molecules likely span within unsaturated hydrocarbon, lignin, or tannins regions and are not readily bioavailable.[54,55] Recent research has shown that a large fraction of P-containing DOM in groundwater is found in both the aliphatic and unsaturated DOM compound class and that these sources of P-containing DOM are intermediates to end products of organic matter biodegradation.[34,56,57] In addition, up to 40% of DOP species in stormwaters are found in low molecular weight, aromatic fractions below 1 kDa.[58] One possible explanation for the difference in DOP molecules can be linked to the type of agriculture. Yang *et al.* (2021) sampled agricultural watersheds with corn-soybean crop rotation, whereas data obtained from the Annual Crop Inventory (GovCan) for 2022 (Fig. S1†) indicate that most watersheds sampled in this study included a combination of wheat, corn/soybeans, livestock farms, wheat, and barley. Manure extracts from livestock farms also have a relatively high DOP molecular signature (10–13%) and have been observed in surrounding agricultural streams,[55] suggesting that the high DOP, particularly in agriculturally impacted watersheds in this study, may have been with surrounding livestock or field where manure was recently applied. We hypothesize that because the sampled agricultural watersheds in this study were a combination of cash crops and livestock farmland, there is less of a phenolic and lignin-like signature than in cash crop-specific agricultural fields sampled in other studies.[54,55]

Agriculturally impacted and disturbed watersheds correlating with high proportions of phosphorus-containing DOM may be more impacted by precipitation due to less stable soils than forested systems. Surface runoff is a major transport mechanism of organic matter from soils that have also been treated with fertilizers. In addition, nutrient inputs from either point or nonpoint sources are continuously photochemically and microbially transformed and incorporated with organic material as they move with groundwater and surface runoff.[55] Since many of the sampled systems were shallow and close to baseflow conditions in the late summer months, the relatively high precipitation measurements for agriculturally impacted sites may have increased discharge and turbidity by remobilizing phosphorus-containing DOM substrates from sediments.[59] Enhanced surface runoff from higher precipitation levels, the highest DOC concentrations (Fig. 2), and high nutrient richness observed in the sampled agriculturally impacted watersheds can increase the degree of microbial processing in aquatic systems.[60,61] The majority of organic phosphates and organophosphonates require greater enzymatic activity and energy for microorganisms to degrade than other organic and inorganic phosphorus forms. Although organophosphonate concentration was not specifically tested in this study, since organophosphonates have been shown to slow microbial growth and accumulate in natural systems,[62,63] it is possible the enrichment of unsaturated hydrocarbons observed may be organic matter derivatives from microbial organophosphonate degradation. Together, we hypothesize that enhanced DOP signatures in more agriculturally impacted watersheds result from changes in the extent of surface runoff, nutrient loading, and *in situ* microbial remineralization of DOM.

Agricultural land use has been shown to increase the humification degree and molecular weight of DOM *via* microbial humification processes,[64] consistent with our findings of DOM with greater m/z ratios and lower spectral slopes in disturbed watersheds (Fig. 3 and 4). While humified species may be either autochthonous or allochthonous in origin, the high association of unsaturated hydrocarbons, DOC concentrations, and nutrients observed in this study suggests that these molecules may be the byproduct of microbial degradation or algae-derived organic matter due to the mobilization of carbon and nutrients.[15,20,64] While increasingly condensed DOM may initially enhance carbon sequestration in sediments, nutrient inputs can stimulate microbial productivity and the metabolism of DOM stored in sediments.[11,65]

4 Conclusion

In this study, we investigated the molecular characteristics of DOM across watersheds with varying landscape characteristics and land cover to statistically link DOM molecules with agricultural and developmental disturbance. We found an enrichment of DOP molecules in watersheds impacted by agricultural and developmental disturbance, whereas undisturbed ecosystems with higher forest and bog coverage resulted in increased proportions of phenolic and aliphatic DOM, likely originating from leaf litter. We also identified 65 highly recalcitrant DOP molecules that can be used as molecular signatures unique to disturbed watersheds. We show that the increased supply of DOC concentrations and nutrients and presumably, greater soil disruption in human-impacted watersheds can yield increasingly humified and unsaturated DOP molecules.

Future studies could consider identifying the source of DOP molecules from upstream sources or molecular signatures within surrounding soils of agriculturally impacted systems that contribute to the formation of DOP molecules. While these molecules were identified across watersheds in the Kawartha region, these molecular signatures may apply to additional agricultural and urbanized watersheds, but additional studies should consider how different forms of agricultural inputs (livestock, crops) and urbanization (infrastructure development *vs.* urban centers) impacts the molecular composition of DOM, DOP, and water quality. In addition, future studies should incorporate the use of enriched ^{31}P isotopes to confirm DOP molecules, similar to other studies.[66] As fertilizer use continues to increase in agricultural-dominated watersheds, tracking and identifying DOP molecules within aquatic systems will be particularly useful in understanding how aquatic systems store or mobilize P sources. Together, improving our understanding of the lability and transformation of DOP molecules would also allow for better predictions of how P is cycled in agriculturally impacted watersheds. While we show the landscape metrics have important predictive capabilities when elucidating the molecular composition of DOM, more refined spatial mapping with greater resolution should be considered using a similar statistical approach as this study. Further exploring how DOM at the molecular level is impacted by increasing disturbance and climatic perturbations is important for maintaining ecosystem services of terrestrial and aquatic systems.

Conflicts of interest

There are no conflicts of interest to declare.

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References

1 Y. Pan, R. A. Birdsey, J. Fang, R. Houghton, P. E. Kauppi, W. A. Kurz, O. L. Phillips, A. Shvidenko, S. L. Lewis, J. G. Canadell, P. Ciais, R. B. Jackson, S. W. Pacala, D. A. McGurie, S. Piao, A. Rautiainen, S. Sitch and D. Hayes, A Large and Persistent Carbon Sink in the World's Forests, *Science*, 2011, **333**(6045), 988–993.

2 B. P. Koch, G. Kattner, M. Witt and U. Passow, Molecular Insights into the Microbial Formation of Marine Dissolved Organic Matter: Recalcitrant or Labile?, *Biogeosciences*, 2014, **11**(15), 4173–4190, DOI: 10.5194/bg-11-4173-2014.

3 G. Hensgens, H. Laudon, M. S. Johnson and M. Berggren, The Undetected Loss of Aged Carbon from Boreal Mineral Soils, *Sci. Rep.*, 2021, 1–10, DOI: 10.1038/s41598-021-85506-w.

4 J. A. O'Donnell, G. R. Aiken, K. D. Butler, F. Guillemette, D. C. Podgorski and R. G. M. Spencer, DOM Composition and Transformation in Boreal Forest Soils:The Effects of Temperature and Organic-Horizon Decomposition State, *J. Geophys. Res.*, 2016, **121**, 2727–2744, DOI: 10.1002/2016JG003431.

5 J. D'Andrilli, J. R. Junker, H. J. Smith, E. A. Scholl and C. M. Foreman, DOM Composition Alters Ecosystem Function during Microbial Processing of Isolated Sources, *Biogeochemistry*, 2019, **142**(2), 281–298, DOI: 10.1007/s10533-018-00534-5.

6 T. J. Battin, L. A. Kaplan, S. Findlay, C. S. Hopkinson, E. Marti, A. I. Packman, J. D. Newbold and F. Sabater, Biophysical Controls on Organic Carbon Fluxes in Fluvial Networks, *Nat. Geosci.*, 2009, **2**(8), 595, DOI: 10.1038/ngeo602.

7 C. K. Remucal, R. M. Cory, M. Sander and K. McNeill, Low Molecular Weight Components in an Aquatic Humic Substance as Characterized by Membrane Dialysis and Orbitrap Mass Spectrometry, *Environ. Sci. Technol.*, 2012, **46**(17), 9350–9359, DOI: 10.1021/es302468q.

8 V. N. Roth, T. Dittmar, R. Gaupp and G. Gleixner, The Molecular Composition of Dissolved Organic Matter in Forest Soils as a Function of pH and Temperature, *PLoS One*, 2015, **10**(3), 1–23, DOI: 10.1371/journal.pone.0119188.

9 M. Haitzer, G. R. Aiken and J. N. Ryan, Binding of Mercury(II) to Dissolved Organic Matter: The Role of the Mercury-to-DOM Concentration Ratio, *Environ. Sci. Technol.*, 2002, **36**(16), 3564–3570, DOI: 10.1021/es025699i.

10 Y. Louis, B. Pernet-Coudrier and G. Varrault, Implications of Effluent Organic Matter and Its Hydrophilic Fraction on Zinc(II) Complexation in Rivers under Strong Urban Pressure: Aromaticity as an Inaccurate Indicator of DOM-Metal Binding, *Sci. Total Environ.*, 2014, **490**, 830–837, DOI: 10.1016/j.scitotenv.2014.04.123.

11 M. A. Xenopoulos, R. T. Barnes, K. S. Boodoo, D. Butman, N. Catalán, S. C. D'Amario, C. Fasching, D. N. Kothawala, O. Pisani, C. T. Solomon, R. G. M. Spencer, C. J. Williams and H. F. Wilson, How Humans Alter Dissolved Organic Matter Composition in Freshwater: Relevance for the

Earth's Biogeochemistry, *Biogeochemistry*, 2021, **154**(2), 323–348, DOI: 10.1007/s10533-021-00753-3.

12 R. D. Bardgett, C. Freeman and N. J. Ostle, Microbial Contributions to Climate Change through Carbon Cycle Feedbacks, *ISME J.*, 2008, **2**(8), 805–814, DOI: 10.1038/ismej.2008.58.

13 R. Benner and K. Kaiser, Biological and Photochemical Transformations of Amino Acids and Lignin Phenols in Riverine Dissolved Organic Matter, *Biogeochemistry*, 2011, **102**(1), 209–222, DOI: 10.1007/s10533-010-9435-4.

14 O. Pisani, D. D. Bosch, A. W. Coffin, D. M. Endale, D. Liebert and T. C. Strickland, Riparian Land Cover and Hydrology Influence Stream Dissolved Organic Matter Composition in an Agricultural Watershed, *Sci. Total Environ.*, 2020, **717**, 137165, DOI: 10.1016/j.scitotenv.2020.137165.

15 Y.-Y. Yang, M. M. Tfaily, J. L. Wilmoth and G. S. Toor, Molecular Characterization of Dissolved Organic Nitrogen and Phosphorus in Agricultural Runoff and Surface Waters, *Water Res.*, 2022, **219**, 118533, DOI: 10.1016/j.watres.2022.118533.

16 A. Tiefenbacher, G. Weigelhofer, A. Klik, M. Pucher, J. Santner, W. Wenzel, A. Eder and P. Strauss, Short-Term Effects of Fertilization on Dissolved Organic Matter (DOM) in Soil Leachate, *Soil Discuss.*, 2020, 1–28, DOI: 10.5194/soil-2019-97.

17 D. Graeber, I. G. Boëchat, F. Encina-Montoya, C. Esse, J. Gelbrecht, G. Goyenola, B. Gücker, M. Heinz, B. Kronvang, M. Meerhoff, J. Nimptsch, M. T. Pusch, R. C. S. Silva, D. Von Schiller and E. Zwirnmann, Global Effects of Agriculture on Fluvial Dissolved Organic Matter, *Sci. Rep.*, 2015, **5**, 1–8, DOI: 10.1038/srep16328.

18 P. C. Frost, J. H. Larson, C. A. Johnston, K. C. Young, P. A. Maurice, G. A. Lamberti and S. D. Bridgham, Landscape Predictors of Stream Dissolved Organic Matter Concentration and Physicochemistry in a Lake Superior River Watershed, *Aquat. Sci.*, 2006, **68**, 40–51, DOI: 10.1007/s00027-005-0802-5.

19 A. Worman, A. I. Packman, L. Marklund, J. W. Harvey and S. H. Stone, Fractal Topography and Subsurface Water Flows from Fluvial Bedforms to the Continental Shield, *Geophys. Res. Lett.*, 2007, **34**, 1–5, DOI: 10.1029/2007GL029426.

20 H. F. Wilson and M. A. Xenopoulos, Effects of Agricultural Land Use on the Composition of Fluvial Dissolved Organic Matter, *Nat. Geosci.*, 2009, **2**(1), 37–41, DOI: 10.1038/ngeo391.

21 P. Shang, Y. Lu, Y. Du, R. Jaffé, R. H. Findlay and A. Wynn, Climatic and Watershed Controls of Dissolved Organic Matter Variation in Streams across a Gradient of Agricultural Land Use, *Sci. Total Environ.*, 2018, **612**, 1442–1453, DOI: 10.1016/j.scitotenv.2017.08.322.

22 A. T. Kuiters and H. M. Sarink, Leaching of Phenolic Compounds from Leaf and Needle Litter of Several Deciduous and Coniferous Trees, *Soil Biol. Biochem.*, 1986, **18**(5), 475–480, DOI: 10.1016/0038-0717(86)90003-9.

23 K. E. Mueller, D. M. Eissenstat, S. E. Hobbie, J. Oleksyn, A. M. Jagodzinski, P. B. Reich, O. A. Chadwick and J. Chorover, Tree Species Effects on Coupled Cycles of Carbon, Nitrogen, and Acidity in Mineral Soils at a Common Garden Experiment, *Biogeochemistry*, 2012, **111**(1–3), 601–614, DOI: 10.1007/s10533-011-9695-7.

24 M. Saifuddin, J. M. Bhatnagar, D. Segrè and A. C. Finzi, Microbial Carbon Use Efficiency Predicted from Genome-Scale Metabolic Models, *Nat. Commun.*, 2019, **10**(1), 1–10, DOI: 10.1038/s41467-019-11488-z.

25 L. Thieme, D. Graeber, D. Hofmann, S. Bischoff, M. T. Schwarz, B. Steffen, U. N. Meyer, M. Kaupenjohann, W. Wilcke, B. Michalzik and J. Siemens, Dissolved Organic Matter Characteristics of Deciduous and Coniferous Forests with Variable Management: Different at the Source, Aligned in the Soil, *Biogeosciences*, 2019, **16**(7), 1411–1432, DOI: 10.5194/bg-16-1411-2019.

26 K. M. Yakimovich, E. J. S. Emilson, M. A. Carson, A. J. Tanentzap, N. Basiliko and N. C. S. Mykytczuk, Plant Litter Type Dictates Microbial Communities Responsible for Greenhouse Gas Production in Amended Lake Sediments, *Front. Microbiol.*, 2018, **9**(NOV), 1–13, DOI: 10.3389/fmicb.2018.02662.

27 D. J. Lapworth, D. C. Gooddy, D. Allen and G. H. Old, Understanding Groundwater, Surface Water, and Hyporheic Zone Biogeochemical Processes in a Chalk Catchment Using Fluorescence Properties of Dissolved and Colloidal Organic Matter, *J. Geophys. Res.*, 2009, **114**(G3), 1–10.

28 P. Herzsprung, K. Osterloh, W. von Tümpling, M. Harir, N. Hertkorn, P. Schmitt-Kopplin, R. Meissner, S. Bernsdorf and K. Friese, Differences in DOM of Rewetted and Natural Peatlands – Results from High-Field FT-ICR-MS and Bulk Optical Parameters, *Sci. Total Environ.*, 2017, **586**, 770–781, DOI: 10.1016/j.scitotenv.2017.02.054.

29 T. Dittmar, B. Koch, N. Hertkorn and G. Kattner, A Simple and Efficient Method for the Solid-Phase Extraction of Dissolved Organic Matter (SPE-DOM) from Seawater, *Limnol Oceanogr. Methods*, 2008, **6**(6), 230–235, DOI: 10.4319/lom.2008.6.230.

30 V. Mangal, Y. X. Shi and C. Guéguen, Compositional Changes and Molecular Transformations of Dissolved Organic Matter during the Arctic Spring Floods in the Lower Churchill Watershed (Northern Manitoba, Canada), *Biogeochemistry*, 2017, **136**(2), 151–165.

31 V. Mangal, B. R. Stenzler, A. J. Poulain and C. Guéguen, Aerobic and Anaerobic Bacterial Mercury Uptake Is Driven by Algal Organic Matter Composition and Molecular Weight, *Environ. Sci. Technol.*, 2019, **53**(1), 157–165.

32 Y. Qi, M. P. Barrow, H. Li, J. E. Meier, S. L. Van Orden, C. J. Thompson and P. B. O'Connor, Absorption-Mode: The next Generation of Fourier Transform Mass Spectra, *Anal. Chem.*, 2012, **84**(6), 2923–2929, DOI: 10.1021/ac3000122.

33 Q.-L. Fu, M. Fujii and T. Riedel, Development and Comparison of Formula Assignment Algorithms for Ultrahigh-Resolution Mass Spectra of Natural Organic Matter, *Anal. Chim. Acta*, 2020, **1125**, 247–257, DOI: 10.1016/j.aca.2020.05.048.

34 Y. Tao, Y. Du, Y. Deng, T. Ma and Y. Wang, Degradation of Phosphorus-Containing Natural Organic Matter Facilitates Enrichment of Geogenic Phosphorus in Quaternary Aquifer Systems: A Molecular Perspective, *J. Hydrol.*, 2023, **620**, 129513, DOI: **10.1016/j.jhydrol.2023.129513**.

35 B. P. Koch and T. Dittmar, From Mass to Structure: An Aromaticity Index for High-Resolution Mass Data of Natural Organic Matter, *Rapid Commun. Mass Spectrom.*, 2006, **20**(5), 926–932, DOI: **10.1002/rcm.2386**.

36 T. Riedel, H. Biester and T. Dittmar, Molecular Fractionation of Dissolved Organic Matter with Metal Salts, *Environ. Sci. Technol.*, 2012, **46**(8), 4419–4426, DOI: **10.1021/es203901u**.

37 R. R. Core Team, *A Language and for, for Statistical Computing*, R Foundation Statistical Computing, 2020.

38 H. Wickham, *Ggplot2: Elegant Graphics for Data Analysis*, 2016.

39 W. Taiyun, *S. R. P. ViliamCorrplot, Visualization of a Correlation Matrix (Version 0.92)*, 2021, **https://github.com/taiyun/corrplot**.

40 J. Peuravuori and K. Pihlaja, Molecular Size Distribution and Spectroscopic Properties of Aquatic Humic Substances, *Anal. Chim. Acta*, 1997, **337**(2), 133–149, DOI: **10.1016/S0003-2670(96)00412-6**.

41 J. R. Helms, A. Stubbins, J. D. Ritchie, E. C. Minor, D. J. Kieber and K. Mopper, Absorption Spectral Slopes and Slope Ratios as Indicators of Molecular Weight, Source, and Photobleaching of Chromophoric Dissolved Organic Matter, *Limnol Oceanogr.*, 2008, **53**(3), 955–969, DOI: **10.4319/lo.2009.54.3.1023**.

42 V. Mangal, W. Y. Lam, H. Huang, E. J. S. Emilson, R. W. Mackereth and C. P. J. Mitchell, Molecular Correlations of Dissolved Organic Matter with Inorganic Mercury and Methylmercury in Canadian Boreal Streams, *Biogeochemistry*, 2022, 0123456789, DOI: **10.1007/s10533-022-00944-6**.

43 A. Baker and R. G. M. Spencer, Characterization of Dissolved Organic Matter from Source to Sea Using Fluorescence and Absorbance Spectroscopy, *Sci. Total Environ.*, 2004, **333**(1), 217–232, DOI: **10.1016/j.scitotenv.2004.04.013**.

44 D. Graeber, B. Gücker, R. Wild, N. S. Wells, C. Anlanger, N. Kamjunke, H. Norf, C. Schmidt and M. Brauns, Biofilm-Specific Uptake Does Not Explain Differences in Whole-Stream DOC Tracer Uptake between a Forest and an Agricultural Stream, *Biogeochemistry*, 2019, **144**(1), 85–101, DOI: **10.1007/s10533-019-00573-6**.

45 D. Graeber, J. Gelbrecht, M. T. Pusch, C. Anlanger and D. von Schiller, Agriculture Has Changed the Amount and Composition of Dissolved Organic Matter in Central European Headwater Streams, *Sci. Total Environ.*, 2012, **438**, 435–446, DOI: **10.1016/j.scitotenv.2012.08.087**.

46 K. C. Petrone, J. B. Fellman, E. Hood, M. J. Donn and P. F. Grierson, The Origin and Function of Dissolved Organic Matter in Agro-Urban Coastal Streams, *J. Geophys. Res.*, 2011, **116**(G01028), 1–13.

47 J. L. Kovar, Maize Response to Sulfur Fertilizer in Three Iowa Soils, *Commun. Soil Sci. Plant Anal.*, 2021, **52**(8), 905–915, DOI: **10.1080/00103624.2020.1869773**.

48 W. Tang, Y. Su, Y. Gao and H. Zhong, Effects of Farming Activities on the Biogeochemistry of Mercury in Rice–Paddy Soil Systems, *Bull. Environ. Contam. Toxicol.*, 2019, **102**(5), 635–642, DOI: **10.1007/s00128-019-02627-9**.

49 J. B. Fellman, E. Hood, D. V. D'Amore, R. T. Edwards and D. White, Seasonal Changes in the Chemical Quality and Biodegradability of Dissolved Organic Matter Exported from Soils to Streams in Coastal Temperate Rainforest Watersheds, *Biogeochemistry*, 2009, **95**, 227–293, DOI: **10.1007/s10533-009-9336-6**.

50 C. Fasching, B. Behounek, G. A. Singer and T. J. Battin, Microbial Degradation of Terrigenous Dissolved Organic Matter and Potential Consequences for Carbon Cycling in Brown-Water Streams, *Sci. Rep.*, 2014, **4**, 1–7, DOI: **10.1038/srep04981**.

51 K. Wang, Y. Pang, Y. Li, C. He, Q. Shi, Y. Wang and D. He, Characterizing Dissolved Organic Matter across a Riparian Soil-Water Interface: Preliminary Insights from a Molecular Level Perspective, *ACS Earth Space Chem.*, 2021, **5**(5), 1102–1113, DOI: **10.1021/acsearthspacechem.1c00029**.

52 W. C. Hockaday, A. M. Grannas, S. Kim and P. G. Hatcher, The Transformation and Mobility of Charcoal in a Fire-Impacted Watershed, *Geochim. Cosmochim. Acta*, 2007, **71**(14), 3432–3445, DOI: **10.1016/j.gca.2007.02.023**.

53 A. Spence and B. P. Kelleher, Photodegradation of Major Soil Microbial Biomolecules Is Comparable to Biodegradation: Insights from Infrared and Diffusion Editing NMR Spectroscopies, *J. Mol. Struct.*, 2016, **1107**, 7–13, DOI: **10.1016/j.molstruc.2015.11.025**.

54 Y.-Y. Yang, S. Asal and G. S. Toor, Residential Catchments to Coastal Waters: Forms, Fluxes, and Mechanisms of Phosphorus Transport, *Sci. Total Environ.*, 2021, **765**, 142767, DOI: **10.1016/j.scitotenv.2020.142767**.

55 M. R. Brooker, K. Longnecker, E. B. Kujawinski, M. H. Evert and P. J. Mouser, Discrete Organic Phosphorus Signatures Are Evident in Pollutant Sources within a Lake Erie Tributary, *Environ. Sci. Technol.*, 2018, **52**(12), 6771–6779, DOI: **10.1021/acs.est.7b05703**.

56 W. Qiao, H. Guo, C. He, Q. Shi and B. Zhao, Unraveling Roles of Dissolved Organic Matter in High Arsenic Groundwater Based on Molecular and Optical Signatures, *J. Hazard. Mater.*, 2021, **406**, 124702, DOI: **10.1016/j.jhazmat.2020.124702**.

57 L. K. McDonough, D. M. O'Carroll, K. Meredith, M. S. Andersen, C. Brügger, H. Huang, H. Rutlidge, M. I. Behnke, R. G. M. Spencer, A. McKenna, C. E. Marjo, P. Oudone and A. Baker, Changes in Groundwater Dissolved Organic Matter Character in a Coastal Sand Aquifer Due to Rainfall Recharge, *Water Res.*, 2020, **169**, 115201, DOI: **10.1016/j.watres.2019.115201**.

58 E. C. Ged and T. H. Boyer, Molecular Weight Distribution of Phosphorus Fraction of Aquatic Dissolved Organic Matter, *Chemosphere*, 2013, **91**(7), 921–927, DOI: **10.1016/j.chemosphere.2013.01.113**.

59 T. Van Dael, L. Xia, K. Van Dijck, S. Potemans and E. Smolders, Internal Loading of Phosphate in Rivers Reduces at Higher Flow Velocity and Is Reduced by Iron

Rich Sand Application: An Experimental Study in Flumes, *Water Res.*, 2021, **198**, 117160, DOI: 10.1016/j.watres.2021.117160.

60 M. Juckers, C. J. Williams and M. A. Xenopoulos, Land-Use Effects on Resource Net Flux Rates and Oxygen Demand in Stream Sediments, *Freshw. Biol.*, 2013, **58**(7), 1405–1415, DOI: 10.1111/fwb.12136.

61 M. I. Stutter, D. Graeber, C. D. Evans, A. J. Wade and P. J. A. Withers, Balancing Macronutrient Stoichiometry to Alleviate Eutrophication, *Sci. Total Environ.*, 2018, **634**, 439–447, DOI: 10.1016/j.scitotenv.2018.03.298.

62 M. M. Adams, M. R. Gómez-García, A. R. Grossman and D. Bhaya, Phosphorus Deprivation Responses and Phosphonate Utilization in a Thermophilic Synechococcus Sp. from Microbial Mats, *J. Bacteriol.*, 2008, **190**(24), 8171–8184, DOI: 10.1128/jb.01011-08.

63 D. M. Karl, Microbially Mediated Transformations of Phosphorus in the Sea: New Views of an Old Cycle, *Ann. Rev. Mar. Sci.*, 2014, **6**(1), 279–337, DOI: 10.1146/annurev-marine-010213-135046.

64 S. Chen, Y. Du, P. Das, A. F. Lamore, N. T. Dimova, M. Elliott, E. N. Broadbent, J. A. Roebuck, R. Jaffé and Y. Lu, Agricultural Land Use Changes Stream Dissolved Organic Matter via Altering Soil Inputs to Streams, *Sci. Total Environ.*, 2021, **796**, 148968, DOI: 10.1016/j.scitotenv.2021.148968.

65 C. Gudasz, D. Bastviken, K. Premke, K. Steger and L. J. Tranvik, Constrained Microbial Processing of Allochthonous Organic Carbon in Boreal Lake Sediments, *Limnol Oceanogr.*, 2012, **57**(1), 163–175, DOI: 10.4319/lo.2012.57.1.0163.

66 M. R. Kurek, M. Harir, J. T. Shukle, A. W. Schroth, P. Schmitt-Kopplin and G. K. Druschel, Seasonal Transformations of Dissolved Organic Matter and Organic Phosphorus in a Polymictic Basin: Implications for Redox-Driven Eutrophication, *Chem. Geol.*, 2021, **573**, 120212, DOI: 10.1016/j.chemgeo.2021.120212.

