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Special Section:
Biogeochemistry of Natural Organic Matter

Key Points:

- Amazonian Basin headwater streams draining croplands had lower DOC concentrations than pristine forested streams
- Distinct molecular assemblages were apparent in cropland and forest headwater streams highlighting them as sentinels for detecting change
- Unique molecular formula were present in cropland headwater streams providing markers to track agricultural impacts in the region

Supporting Information:

- Supporting Information S1

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(continued)

Identifying the Molecular Signatures of Agricultural Expansion in Amazonian Headwater Streams

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Abstract Agricultural impacts on aquatic ecosystems are well studied; however, most research has focused on temperate regions, whereas the forefront of agricultural expansion is currently in the tropics. At the vanguard of this growth is the boundary between the Amazon and Cerrado biomes in Brazil, driven primarily by expansion of soybean and corn croplands. Here we examine the impacts of cropland expansion on receiving lowland Amazon Basin headwater streams in terms of dissolved organic carbon (DOC) concentration and dissolved organic matter (DOM) composition via ultrahigh-resolution mass spectrometry. Streams draining croplands had lower DOC concentrations and DOM molecular signatures enriched in N- and S-containing formula in comparison to forested streams. Cropland streams were also enriched in aliphatic, peptide-like, and highly unsaturated and phenolic (low O/C) compound categories in comparison to forest streams (enriched in polyphenolics, condensed aromatics, and highly unsaturated and phenolic [high O/C] compound categories) indicative of the shifting of sources from organic-rich surface soils and litter layers to autochthonous and more microbial biomass. Distinct molecular assemblages were strongly correlated with cropland and forest catchments, highlighting headwater streams as sentinels for detecting change. On investigation of unique molecular formulae present in only cropland sites, four cropland markers provided the ability to track agricultural impacts in the region. Overall, these patterns indicate reduced organic matter inputs in croplands and greater microbial degradation at these sites leading to declining DOC concentrations, and DOM of more microbial character in receiving streams that is more biolabile, with clear ramifications for downstream ecology and biogeochemical cycles.

1. Introduction

A major uncertainty in our ability to predict how climate change and land cover change will affect the global carbon cycle centers on how organic matter (OM) moves across the terrestrial-aquatic interface and how the quantity and quality of OM mobilized from soils to aquatic environments will change under these impacts. In recent years inland waters have also garnered increasing attention as globally important hotspots of carbon processing and export (Battin et al., 2009; Cole et al., 2007; Drake et al., 2017). Therefore, studies are urgently needed to better understand how anthropogenic activities impact the amount and composition of dissolved OM (DOM) mobilized from terrestrial ecosystems into aquatic networks. Previous studies have shown that mobilization of aged dissolved organic carbon (DOC) is positively correlated with human disturbance worldwide (Butman et al., 2015) and that anthropogenic pressures such as land cover change impact DOM composition (Graeber et al., 2015; Heinz et al., 2015; Wilson & Xenopoulos, 2008). This is fundamentally important as DOM composition controls its photoreactivity, bioavailability, and ultimately its persistence in the environment (D'Andrilli et al., 2015; Kellerman et al., 2018; Lu et al., 2014; Maizel et al., 2017; Marschner & Kalbitz, 2003; Spencer et al., 2015). Furthermore, understanding impacts on DOM composition is also important for downstream water quality and the transport and reactivity of toxic substances (Aiken et al., 2011; Chow et al., 2007).

Agriculture is now arguably the major human impact on Earth. Croplands and pastures currently occupy approximately 40% of the Earth's land surface, and their area is continuing to expand and intensify particularly in the tropics (Foley et al., 2005; Laurance et al., 2014; Tilman, 1999). By the mid-2010s within the

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Brazilian Amazon and Cerrado biomes more than 750,000 and 830,000 km^2, respectively (an area equivalent to France, Spain, Germany and Portugal combined), has undergone land cover change primarily due to expansion of agriculture, and within this region the State of Mato Grosso lies at the current forefront of expansion (Neill et al., 2017). Initial cropland expansion in Mato Grosso was primarily for single-cropped soybeans, but more recently farming practices have shifted to double-cropping within the same year of soybeans with a second crop, primarily corn (Neill et al., 2017; Spera et al., 2014). Taken together, the impact of cropland expansion and intensification in the region has led to a range of issues including increased stream temperatures, alteration of the microclimate and tree species composition in riparian forests, increased water flux, and significant modification of climate due to reduced forest cover (Coe et al., 2017; Macedo et al., 2013; Nagy et al., 2015; Silverio et al., 2015).

Interestingly, despite these large changes across the Amazon cropland region, the inorganic solute concentrations in small Amazon lowland headwater streams remain nearly identical in watersheds draining either forest or croplands (Riskin et al., 2017). This limited effect of forest conversion to croplands on stream inorganic solute concentrations in this region of the Amazon contrasts with large increases of solute concentrations in streams draining cropland watersheds in other regions (Howden et al., 2011; Johnes & Heathwaite, 1997; Mattsson et al., 2005; Mayer et al., 2002). Several factors contribute to this pattern. First, deep and highly weathered soils, particularly Oxisols rich in iron and aluminum oxides, dominate in the Amazon region of cropland expansion (Jankowski et al., 2018; Neill et al., 2017). These soils allow for rapid water infiltration and produce predominantly vertical soil hydrologic flowpaths that limit erosion, allow high water-soil contact, and produce streams dominated by groundwater inputs with very stable flows (Elsenbeer, 2001; Riskin et al., 2017; Scheffler et al., 2011). Second, phosphorus fertilizer added to croplands is strongly retained in surface soil layers (Riskin et al., 2013) and nitrogen can be strongly retained as nitrate on soil exchange sites at depths of 6 m or more (Jankowski et al., 2018). Third, crop management in the region has historically employed minimum tillage that limits soil disturbance, and relatively low levels of nitrogen fertilizer have been added to the soybean crop, although that is changing with the expansion of nitrogen fertilized corn as a second crop (Neill et al., 2017).

In this study, we examined DOC concentration and the molecular composition of DOM in headwater streams using the extreme mass accuracy and precision of Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR MS) in Amazon cropland sites and pristine forest sites to directly assess the impacts of land cover on stream DOM. Past studies that examined the effects of agriculture on the composition of DOM in receiving streams found a shift toward less aromatic, lower molecular weight, and more microbially derived material with increased bioavailability (Heinz et al., 2015; Lu et al., 2015; Riedel et al., 2016; Roebuck et al., 2018; Stanley et al., 2012; Williams et al., 2010; Wilson & Xenopoulos, 2008). However, these studies predominantly focused on temperate and subtropical ecosystems, whereas here we aim to examine how tropical croplands at the forefront of global agricultural impacts may alter stream DOM composition and thus ultimately its biogeochemical role in the environment. Furthermore, we examine DOM in both cropland and forest streams at three time periods of the year to identify source materials and address how important seasonality is in the biogeochemical cycling of DOM. Finally, unlike in previous studies examining agricultural impacts on streams, at these sites traditional inorganic solute tracers showed no change with land cover. This study aimed to see if cropland stream DOM may exhibit unique molecular signatures that would allow it to be traced through aquatic ecosystems and thus reveal the impact of agriculture at these sites where other approaches had shown no impact.

2. Materials and Methods

2.1. Site Description and Sample Collection

Tanguro Ranch is an ~800-km^2 farm in Mato Grosso (Brazil) that currently has ~500 km^2 of evergreen traditional Amazon forest and ~300 km^2 of cropland (Figure 1), and the Ranch lies in the headwaters of the Xingu River, which is the fifth-largest tributary to the Amazon River. Forest at Tanguro Ranch was cleared for pasture in the 1980s and then converted to soybean cropland between 2003 and 2008 (Riskin et al., 2017). Double-cropping with corn began in 2005 and to date has occurred mainly on the northern part of the farm.

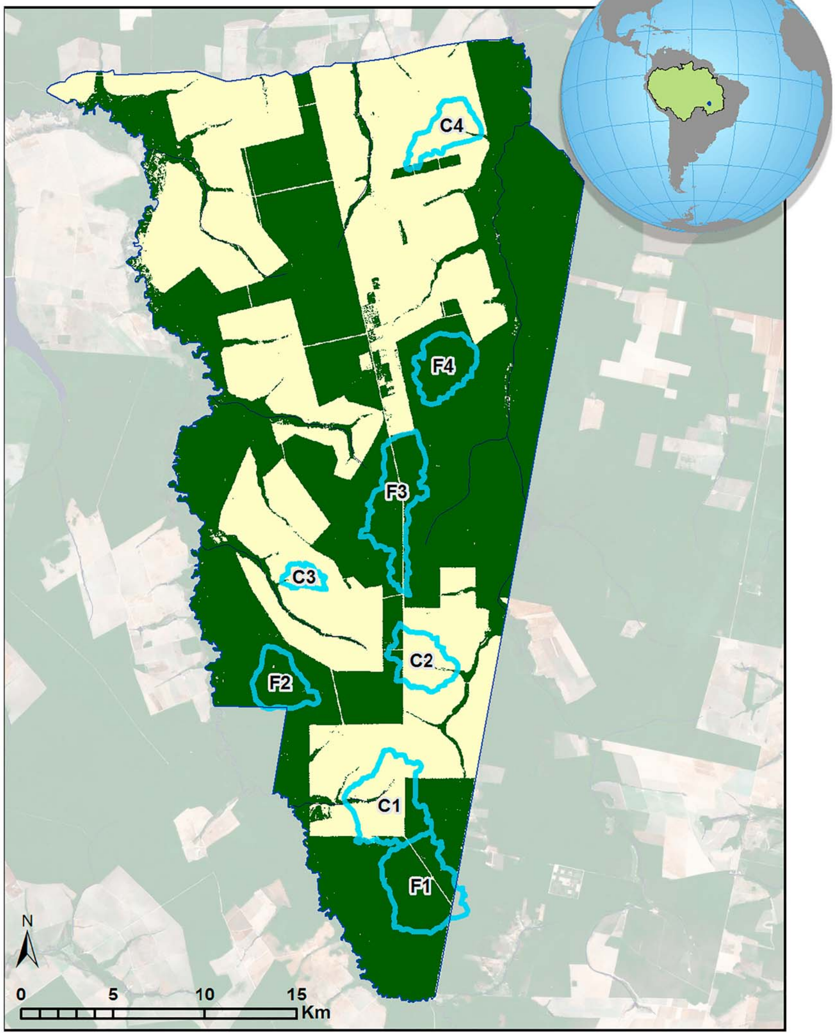

Figure 1. Map of the study site. Globe insert shows the Amazon Basin highlighted in green with the blue dot representing the location of Tanguro Ranch. The four cropland and four forested sites are labeled C1-C4 and F1-F4, respectively.

The mean annual temperature in the region is 27°C, and mean annual precipitation is 1,800 mm/year with almost all precipitation falling between September and April (Riskin et al., 2017).

We collected water samples from eight first-order headwater streams (four cropland dominated and four forest dominated; Figure 1). The catchments defined as cropland were 74-98% crop cover (soybean or soy-corn rotation), 1-23% forest, and 1-2% other land cover (Table 1). The catchments defined as forest were 90-99% forest, 0-5% cropland, and 1-5% other land-cover (Table 1). Cropland catchments ranged from 207 to 1,390 ha and forest catchments from 373 to 1387 ha. Samples were collected at three distinct hydrologic times of year in 2015-2016 (October-November: early wet season; February: late wet season; August: dry season). Samples were collected from the center of well-mixed streams and filtered in the field (0.7 μm GF/F, precombusted at 450 $^{\circ}$C) and stored frozen (in the dark at -20°C) until subsequent analyses or solid phase extraction.

2.2. Dissolved Organic Carbon Concentration

Samples for DOC analysis by high-temperature combustion on a Shimadzu TOC-L CPH using the nonpurgeable organic carbon (OC) method were acidifed to pH 2 and sparged at 75 ml/min for 8 min to remove

Table 1
Mean and Standard Deviations for Forest and Cropland Streams Land Cover %, DOC Concentrations, and FT-ICR MS Parameters

Parameter	Cropland	Forest
n	12	12
% Forest	9 ± 9	95 ± 4
% Cropland	89 ± 10	3 ± 2
% Other	2 ± 1	2 ± 2
DOC (mg/L)**	0.52 ± 0.45	1.43 ± 1.05
Formulae (#)***	8009 ± 749	6849 ± 632
Mass**	510.7 ± 9.8	525.6 ± 10.5
$\mathrm{AI}_{\mathrm{mod}}$***	0.30 ± 0.03	0.36 ± 0.02
CHO (%RA)***	81.7 ± 7.7	90.0 ± 3.5
CHON (%RA)**	12.8 ± 5.0	9.1 ± 1.6
CHONS (%RA)$^{\mathrm{n.s.}}$	0.6 ± 1.2	0.1 ± 0.3
CHOS (%RA)**	4.8 ± 5.0	0.8 ± 1.7
HUP, High O/C (%RA)***	45.7 ± 5.7	54.4 ± 3.7
HUP, Low O/C (%RA)***	36.2 ± 4.3	23.4 ± 2.6
Polyphenolics (%RA)***	8.9 ± 2.0	15.1 ± 2.3
Condensed aromatics (%RA)***	1.3 ± 0.5	3.4 ± 1.0
Aliphatics (%RA)*	5.3 ± 3.2	3.2 ± 1.5
Peptide-like (%RA)*	2.6 ± 4.2	0.4 ± 1.2
Sugar-like (%RA)***	0.1 ± 0.1	0.4 ± 0.1

Abbreviations: $\mathrm{AI}_{\mathrm{mod}}$, modified aromaticity index; DOC, dissolved organic carbon; FT-ICR MS, Fourier transform ion cyclotron resonance mass spectrometry; HUP, highly unsaturated and phenolic; n.s., not significant.
****p*-value < 0.001. ***p*-value < 0.01. **p*-value < 0.05.

dissolved inorganic carbon. DOC concentrations were calculated as the mean of three to five injections using established protocols with a coefficient of variance of <2% (Johnston et al., 2018).

2.3. Ultrahigh-Resolution Mass Spectrometry

Samples for FT-ICR MS analysis were solid-phase extracted using the procedure described in Dittmar et al. (2008). Filtered samples were acidified to pH 2 before solid phase extraction on 100-mg Agilent Bond Elut PPL cartridges. DOC concentrations were used to adjust sample volumes needed for extracting to a target concentration of 50 μg C/ml in the methanol elutes. Extracted samples were stored at -20° C prior to analysis on a 21 t FT-ICR MS located at the National High Magnetic Field Laboratory (NHMFL; Tallahassee, Florida; Hendrickson et al., 2015; Smith et al., 2018). Direct infusion electrospray ionization generated negative ions at a flow rate of 700 nl/min, and 100-time domain acquisitions were coadded for each mass spectrum.

Molecular formulae were assigned to signals >6σ root-mean-square baseline noise (Koch et al., 2007; Stubbins et al., 2010) with EnviroOrg ©,™ software (Corilo, Yuri. EnviroOrg. Florida State University, 2015). Elemental combinations of $C_{1-45}H_{1-92}N_{0-4}O_{1-25}S_{0-2}$ with a mass accuracy of ≤300 ppb were considered for assignment. Classification of formulae was based on their elemental stoichiometries (Santl-Temkiv et al., 2013). The modified aromaticity index ($\mathrm{AI}_{\mathrm{mod}}$) of each formula was calculated, and $\mathrm{AI}_{\mathrm{mod}}$ values of 0.5-0.67 and >0.67 were classified polyphenolic and condensed aromatic structures, respectively (Koch & Dittmar, 2006, 2016). Other compound categories were highly unsaturated and phenolic (low oxygen)=$\mathrm{AI}_{\mathrm{mod}}$<0.5, H/C<1.5, O/C<0.5; highly unsaturated and phenolic (high oxygen)=$\mathrm{AI}_{\mathrm{mod}}$<0.5, H/C<1.5, O/C>0.5; aliphatics=H/C≥1.5-2.0, O/C≤0.9, *N*=0; peptide-like=H/C≥1.5-2.0, O/C≤0.9, *N*>0; and sugar-like=H/C≥1.5-2.0, O/C>0.9. Although consistent with peptide and sugar stoichiometry, the last two compound categories are ambiguous as the formulae may also occur in alternative isomeric arrangements. While FT-ICR MS allows for the precise assignment of molecular formulae to peaks that may represent multiple isomers, they describe the underlying molecular compounds comprising DOM; thus, the term compound may be used when discussing the peaks detected by FT-ICR MS. Signal magnitude was converted to relative abundances by dividing the signal magnitude of an individual peak by the sum of all assigned signals. Then, the percent contribution of each compound category was calculated as the percent that the relative abundance in each compound category contributed to the summed abundance of all assigned formulae. A similar calculation was done to report the percent relative abundance of compounds that contained only carbon, hydrogen, and oxygen (CHO [%RA]), as well as those including nitrogen (CHON [%RA]), nitrogen and sulfur (CHONS [%RA]), and sulfur (CHOS [%RA]), where %RA stands for percent of the relative abundance.

2.4. Statistical Analysis

All statistical analyses were carried out in R (R Core Team, 2015), including Welch's *t* tests, Mann-Whitney *U* tests, principal component analysis, and Spearman rank correlation. Data used in the *t* tests were tested for heteroscedasticity using the studentized Breusch-Pagan test. If data were found to be heteroscedastic, a Welch's *t* test that does not assume equal variances was utilized. If data were not normally distributed, they were log-transformed or a nonparametric Mann-Whitney *U* test was utilized to test for significant difference. Principle component analysis was conducted using the "vegan" package (Oksanen et al., 2011) on variables that were scaled to a range of 0 to 1. Spearman rank correlation coefficients were calculated using the "Hmisc" package in R (Harrell et al., 2016). Spearman rank correlation coefficients were calculated between the relative abundance of assigned molecular formulae in each sample and DOC concentration, the percent of the catchment that is forested, and the percent of the catchment that is cropland. Relationships were considered significant if they had a false discovery rate corrected *p*-value < 0.05 (Benjamini & Hochberg, 1995).

3. Results and Discussion

3.1. Agricultural Impacts on Dissolved Organic Carbon Concentration

Concentrations of DOC in forested streams ranged from 0.66-3.63 mg/L (mean = 1.43 mg/L) exhibiting both higher concentrations and a greater range than in cropland streams (0.24-1.86 mg/L; mean = 0.52 mg/L; p-value < 0.01; Table 1 and Figure 2a). Seasonally, DOC concentrations were both highest and most variable during the late wet season period for both forest (mean = 1.97 ± 1.72 mg/L) and cropland streams (mean = 1.34 ± 0.74 mg/L; Table 2). The dry season DOC concentrations were the lowest for both stream types and exhibited the least variability (cropland: mean = 0.30 ± 0.02 mg/L; forest: mean = 1.30 ± 0.75 mg/L; Table 2). The early wet season DOC concentrations were similar to concentrations in the dry season, although for both forested (mean = 1.33 ± 1.16 mg/L) and cropland (mean = 0.38 ± 0.11 mg/L) streams were slightly elevated and more variable reflecting the transition from dry season to wet season with the onset of the first rain events. This observed seasonal variability in DOC concentration is indicative of changing hydrologic flow paths, residence times, and DOM source pools between the three sampling periods (McGlynn & McDonnell, 2003; Spencer et al., 2010).

Previous studies have shown agricultural practices to increase, not change, or decrease DOC concentrations in receiving streams (Graeber et al., 2015; Heinz et al., 2015; Krupa et al., 2012; Lu et al., 2014; Stanley et al., 2012). This wide-ranging response of DOC to agriculture is unsurprising due to the diversity of farming practices and soil types (Stanley et al., 2012). Conversion of pristine lands, such as forests, to cropland has been shown to cause substantial loss of OC from soils due to elevated erosion and decomposition (Don et al., 2011; Van Oost et al., 2007). This is particularly problematic in highly weathered soils such as the Oxisols at this study site, as these soils provide limited mineral surfaces for physical protection and stabilization of OC (Don et al., 2011; Ye et al., 2017). Multiple factors influence soil OC stocks in the Oxisols on which most new tropical cropping occurs. Cropping can lead to OC loss, particularly under frequent tilling (Durigan et al., 2017; Nagy et al., 2018). However, the minimum tillage cropping practices now in place at Tanguro Ranch and over much of the intensive cropland Amazon and Cerrado regions typically result in small soil OC losses or limited change in soil OC stocks (Nagy et al., 2018). The use of land as cattle pasture prior to cropping may even increase soil OC stocks in comparison with native forest (Desjardins et al., 2004). Importantly, the higher soil moisture and temperature observed in cropland soils due to reduced leaf area evapotranspiration compared with forests (O'Connell, 2015; Silverio et al., 2015) can influence OC decomposition rates that ultimately control soil OC stocks.

At Tanguro Ranch, two aspects of soil OC dynamics in croplands compared with forests likely influenced stream DOC concentrations. First, cropland soils received lower OC inputs and have lower OC surface stocks than forest soils (Nagy et al., 2018). Second, cropland soils were warmer compared with forest soils (Nagy et al., 2018; O'Connell, 2015). These two factors reduce OC in surface soils and thus likely lead to lower DOC concentrations measured in the cropland streams (Table 1 and Figure 2a). Headwater streams draining cropland at Tanguro Ranch have also been shown to have higher water yields than forested streams (Riskin et al., 2017), and so dilution may also likely be a factor in the observed difference between cropland and forest stream DOC concentrations.

3.2. Molecular Signatures of Headwater Stream Dissolved Organic Matter

The molecular diversity (number of assigned formula) of DOM varied between cropland and forested streams (p-value < 0.001; Table 1 and Figure 2b). Cropland streams had higher molecular diversity (6,838-9,386; mean = 8,009 assigned formulae) in comparison to forest streams (5,926-7,952; mean = 6,849 assigned formulae). Seasonally, cropland and forested streams showed reduced molecular diversity during the dry season in comparison to wet season samples (Table 2). Previous studies have also noted high molecular diversity from agriculturally impacted inland waters and attributed this in part to assignment of more heteroatomic formulae (CHON, CHONS, and CHOS; Hertkorn et al., 2016; Kellerman et al., 2014; Roebuck et al., 2018; Wagner et al., 2015). In this study we saw a similar phenomenon with significant differences between CHON and CHOS in cropland and forested streams (p-value < 0.01 for all; Table 1 and Figure 2c). The relative enrichment in S-containing, and particularly N-containing molecular formula in agricultural streams, has been linked to abiotic processes, anthropogenic nutrient inputs, and associated enhanced aquatic primary productivity (Hertkorn et al., 2016; Mattsson et al., 2005; Roebuck et al., 2018; Wagner et al., 2015; Wilson & Xenopoulos, 2008). Aquatic autochthonous sources and microbial

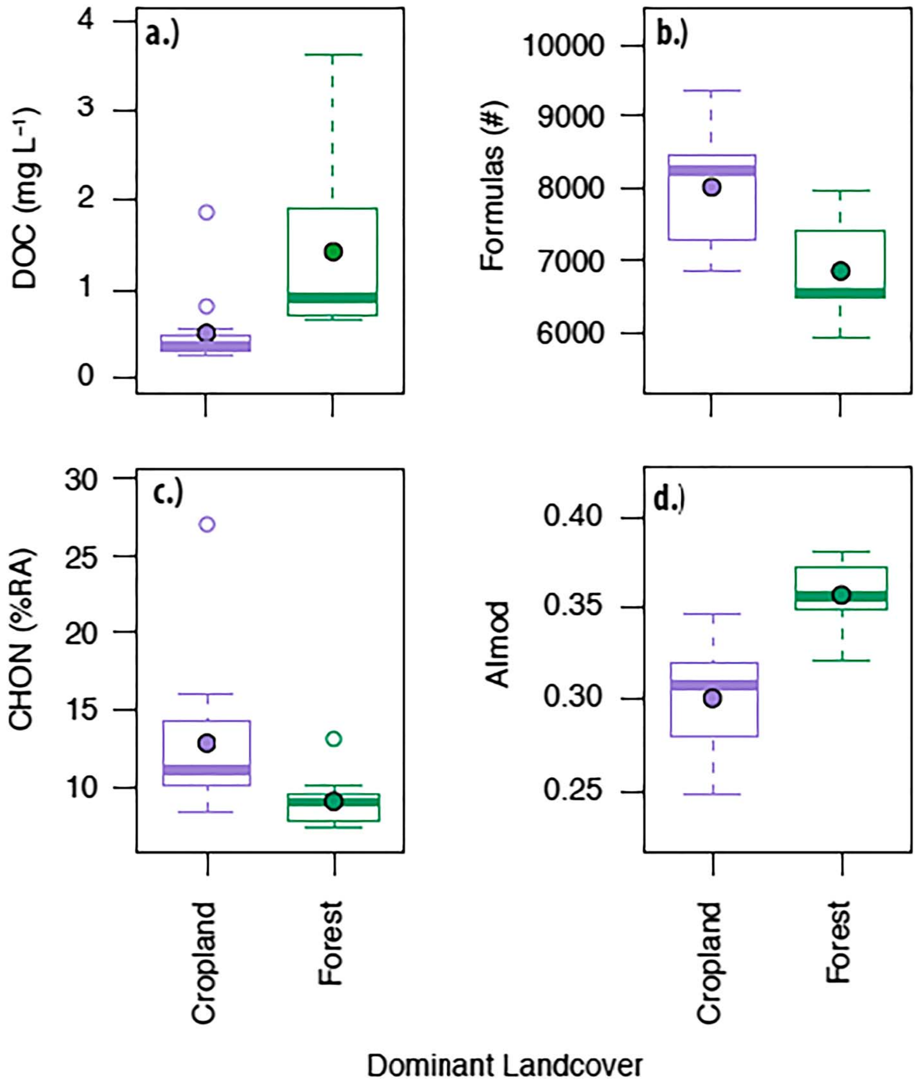

Figure 2. Box plots representing cropland (purple) and forest (green). (a) Dissolved organic carbon (DOC) concentrations (mg/L). (b) Number of assigned formulae. (c) CHON (% RA). (d) AI_{mod}. The solid circles and thick horizontal lines represent the mean and median respectively for each dominant land-cover type.

degradative processes have recently been shown to result in a diversity of N- and S-containing formula (D'Andrilli et al., 2015; Riedel et al., 2016). Small impoundments of water that exist in cropland catchments at Tanguro Ranch and throughout the region (Macedo et al., 2013; Riskin et al., 2017) may provide hotspots for DOM production, or elevated microbial decomposition due to warmer soils may be prevalent in cropland watersheds leading to this molecular signature. On a seasonal basis, N-containing formula were lowest during the late wet season samples and were highest in the early wet season samples for both cropland and forest streams (Table 2). This suggests a relative build up of N-containing formula during the dry season associated with elevated microbial degradative processes that is then subsequently flushed into streams at the onset of the wet season (Conant et al., 2011; Singh et al., 1989).

The shift in cropland streams to autochthonous and microbial signatures is further supported by the modified aromaticity index (AI_{mod}) that identifies DOM aromatic components (Koch & Dittmar, 2006, 2016). The comparatively lower AI_{mod} (p-value < 0.001; Table 1 and Figure 2d) in the cropland streams in comparison to the forest streams (cropland mean = 0.30 versus forest mean = 0.36) is indicative of DOM of lower aromaticity from autochthonous and microbially derived material (Kellerman et al., 2018). This finding is in agreement with past studies utilizing optical parameters to examine changing DOM sources between cropland and pristine sites (Graeber et al., 2015; Lu et al., 2014; Wilson & Xenopoulos, 2008). For context, a 0.06

Table 2

Mean and Standard Deviations for Forest and Cropland Streams DOC Concentrations and Weighted Average Relative Abundance FT-ICR MS Parameters by Season: October-November (Early Wet Season), February (Late Wet Season), and August (Dry Season)

Parameter	Cropland (early wet season)	Cropland (late wet season)	Cropland (dry season)	Forest (early wet season)	Forest (late wet season)	Forest (dry season)
DOC (mg/L)	0.38 ± 0.11	1.34 ± 0.74	0.30 ± 0.02	1.33 ± 1.16	1.97 ± 1.72	1.30 ± 0.75
Formulae (#)	7983 ± 913	8386 ± 276	7819 ± 591	7087 ± 645	7021 ± 809	6406 ± 396
Mass (Da)	506.7 ± 9.1	516.4 ± 11.0	516.1 ± 9.5	527 ± 9.1	533 ± 13.5	520 ± 10.6
AI_{mod}	0.29 ± 0.03	0.33 ± 0.02	0.31 ± 0.01	0.36 ± 0.02	0.36 ± 0.01	0.35 ± 0.02
CHO (%RA)	78.1 ± 8.4	87.0 ± 0.4	86.8 ± 0.7	89.2 ± 4.7	90.8 ± 2.7	90.8 ± 1.3
CHON (%RA)	14.4 ± 6.3	10.5 ± 1.4	10.7 ± 0.1	9.4 ± 2.0	8.3 ± 1.5	8.9 ± 1.0
CHONS (%RA)	1.0 ± 1.4	0.0 ± 0.0	0.0 ± 0.0	0.2 ± 0.4	0.0 ± 0.0	0.0 ± 0.0
CHOS (%RA)	6.5 ± 6.1	2.6 ± 1.7	2.5 ± 0.7	1.2 ± 2.3	0.9 ± 1.3	0.3 ± 0.5
HUP, High O/C (%RA)	42.3 ± 3.6	54.5 ± 5.8	47.7 ± 0.4	53.8 ± 4.0	53.2 ± 4.7	55.9 ± 3.5
HUP, Low O/C (%RA)	37.1 ± 2.3	29.8 ± 7.9	38.3 ± 1.5	22.1 ± 1.7	26.9 ± 3.8	22.6 ± 2.0
Polyphenolics (%RA)	8.0 ± 1.7	11.5 ± 2.3	9.0 ± 1.0	15.9 ± 2.2	14.4 ± 0.6	14.3 ± 2.9
Condensed aromatics (%RA)	1.2 ± 0.5	1.8 ± 0.6	1.3 ± 0.3	3.7 ± 1.1	2.8 ± 0.4	3.2 ± 0.9
Aliphatics (%RA)	7.0 ± 3.3	2.2 ± 0.9	3.5 ± 0.2	3.3 ± 1.9	2.3 ± 1.2	3.5 ± 0.9
Peptide-like (%RA)	4.4 ± 4.9	0.0 ± 0.0	0.3 ± 0.0	0.7 ± 1.7	0.0 ± 0.0	0.2 ± 0.2
Sugar-like (%RA)	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.4 ± 0.1	0.3 ± 0.1	0.4 ± 0.2

Abbreviations: AI_{mod}, modified aromaticity index; DOC, dissolved organic carbon; FT-ICR MS, Fourier transform ion cyclotron resonance mass spectrometry; HUP, highly unsaturated and phenolic.

difference in AI_{mod} as seen here between cropland and forest streams was comparable to the differences between DOM from the blackwater terrestrial dominated Congo River and the agriculturally impacted Danube River (Spencer et al., 2016; Wagner et al., 2015), or the autochthonous DOM end-member of Lake Fryxell and the Yukon River (Kellerman et al., 2018).

The FT-ICR MS data from both forest and cropland streams showed the dominance of highly unsaturated and phenolic (HUP) compounds (mean: 77.8 % and 81.9 % respectively; Table 1 and Figure 3) as is typical of DOM samples globally (Kellerman et al., 2018; Riedel et al., 2016; Spencer et al., 2014, 2015). Croplands contributed proportionally more HUP low O/C compounds (mean: 36.2 % versus 23.4 %; p-value < 0.001), whereas forest streams were enriched in HUP high O/C compounds (mean: 54.4 % versus 45.7 %; p-value < 0.001; Table 1 and Figure 3). The forest streams were also relatively enriched in polyphenolic (mean: 15.1 % versus 8.9 %; < 0.001) and condensed aromatic (mean: 3.4 % versus 1.3 %; p-value < 0.001) compounds in comparison to the cropland sites (Table 1 and Figure 3). Past studies utilizing a host of optical parameters to assess DOM composition exhibited similar trends indicating the dominance of terrestrial sources at pristine versus cropland sites (Lambert et al., 2017; Lu et al., 2014; Williams et al., 2010). Conversely, the cropland streams were relatively enriched in aliphatic (mean: 5.3 % versus 3.2 %; p-value < 0.05) and peptide-like (mean: 2.6 % versus 0.4 %; p-value < 0.05) compounds (Table 1 and Figure 3). In both stream types, assignments as sugar-like were minor and contributed to less than 0.5 % of the total relative abundance (Table 1).

The molecular-level variability of DOM in forest and cropland streams reflects a more terrestrial source of DOM, typical of organic-rich surface soils and litter layers, draining into the forest streams as evidenced by higher relative contributions of polyphenolics, condensed aromatics, and HUP high O/C compounds (O'Donnell et al., 2016; Stubbins et al., 2010; Wagner et al., 2015). This is particularly pronounced in forest streams during the early wet season when the first rains flush these organic-rich source materials resulting in the highest relative abundances of polyphenolics and condensed aromatics at this time for any site or season (Table 2). The dominance of this terrestrial source to the forest streams throughout the year was supported by the AI_{mod} data, as well as the weighted average mass that was higher in the forest streams (mean: 525.6 versus 510.7; p-value < 0.01; Table 1) and both indicated higher molecular weight, more aromatic DOM entering forest streams.

The DOM in cropland streams, however, was enriched in aliphatic, peptide-like, and HUP low O/C compounds that is representative of autochthonous and microbially derived material (Kellerman et al., 2018; Wagner et al., 2015). In cropland streams at the onset of the wet season, aliphatic and peptide-like compounds were at the highest relative abundances observed in this study, supporting the flushing of microbially derived material into these streams early in the wet season (Kellerman et al., 2018; Singh et al.,

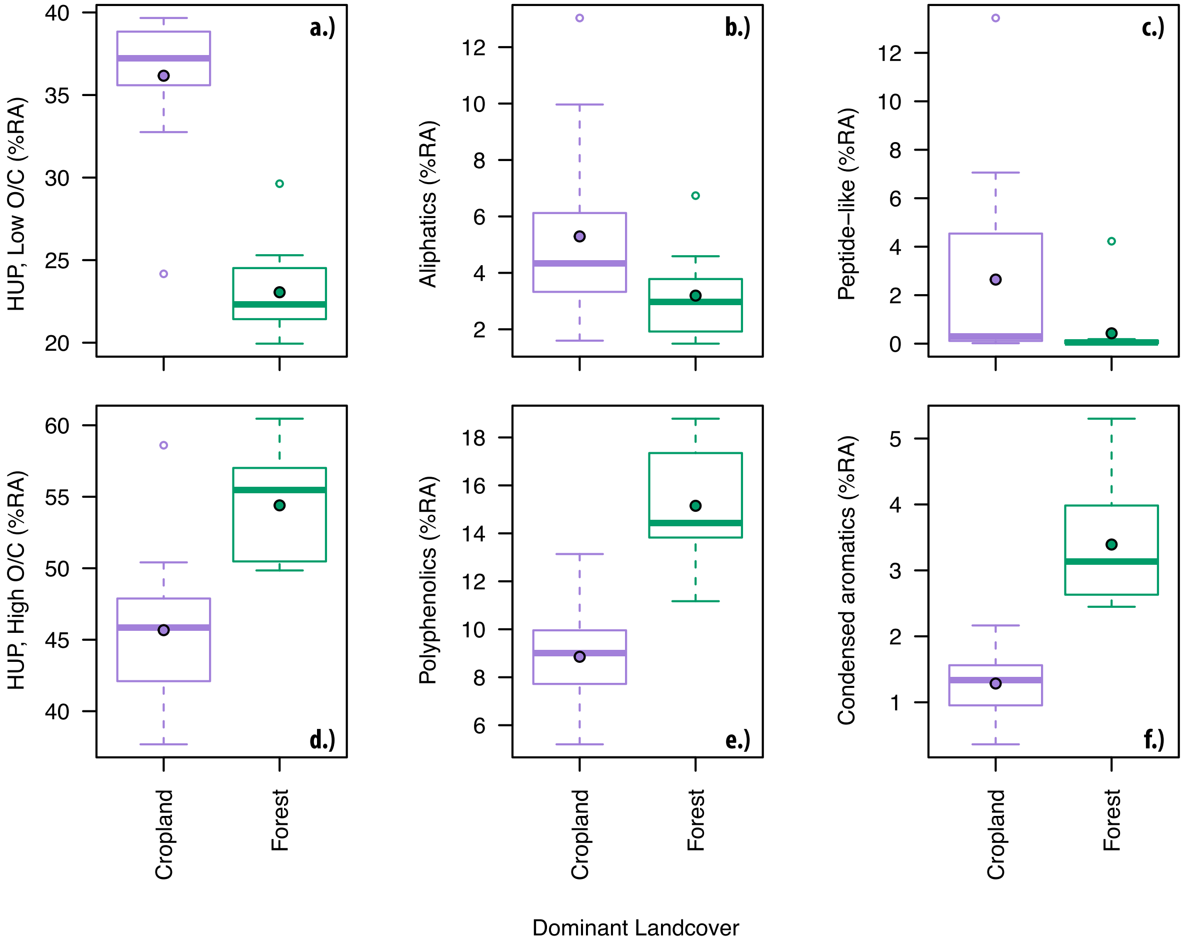

Figure 3. Box plots representing cropland (purple) and forest (green) by Fourier transform ion cyclotron resonance mass spectrometry compound class. (a) Highly unsaturated and phenolic low O/C (%RA). (b) Aliphatics (%RA). (c) Peptide-like (%RA). (d) Highly unsaturated and phenolic high O/C (%RA). (e) Polyphenolics (%RA). (f) Condensed aromatics (%RA). The solid circles and thick horizontal lines represent the mean and median respectively for each dominant land cover type.

1989). These compounds have been shown to be biologically labile and to be rapidly utilized in aquatic ecosystems (D'Andrilli et al., 2015; Riedel et al., 2016; Spencer et al., 2015; Textor et al., 2018). Therefore, shifting land cover from forest to cropland in this region will not only reduce DOC concentrations but also release DOM that will likely be more rapidly utilized and ultimately outgassed as carbon dioxide (CO_2) in receiving aquatic ecosystems. As croplands in this area of Mato Grosso have exhibited higher water yields than forested streams (Riskin et al., 2017), this biologically labile DOM is also more likely to be rapidly exported from headwater streams to higher-order rivers where it could play an active role in stream and river metabolism. Ultimately, in the cropland streams DOM export is accompanied with changing hydrology and sediment yields (Riskin et al., 2017), leading to a host of shifting biogeochemically pathways for the fate of this material, as there are clear ramifications for physico-chemical processes including sorption and photochemical degradation of DOM (Aufdenkampe et al., 2001; Hernes et al., 2013; Lu et al., 2013). Future research could elegantly address this question by linking DOM compositional studies throughout river continuums draining from different land use types.

Past studies that showed changes in DOM optical properties and C/N ratios in inland waters influenced by agricultural practices found a shift toward export of lower molecular weight, less aromatic, more microbial-like DOM composition (Graeber et al., 2015; Heinz et al., 2015; Williams et al., 2010; Wilson & Xenopoulos, 2008). Here we show the molecular-level underpinnings driving this impact due to agriculture (Table 1 and Figures 2 and 3). A microbial-like signature of DOM in agricultural streams has been attributed to a host of causes including changing inputs of organic material to the soils, elevated microbial decomposition and erosion in agricultural soils, addition of fertilizers and agrochemicals, and impacts of water management

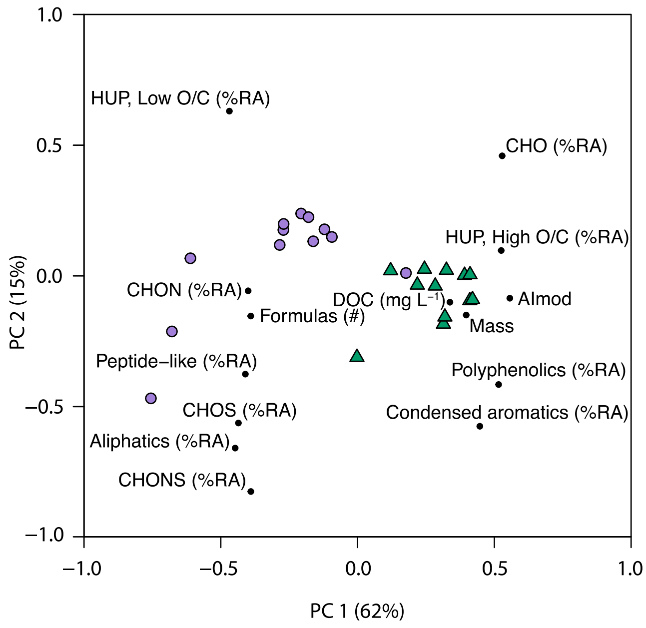

Figure 4. Principle component analysis functions (see Table 3 for structure matrix). The purple circles represent cropland stream samples, and the green triangles represent forest stream samples.

techniques such as drainage, irrigation, and impoundment (Don et al., 2011; Graeber et al., 2015; Heinz et al., 2015; Sickman et al., 2010; Van Oost et al., 2007).

Historically, soybean cropland in Mato Grosso has used minimum-tillage practices, 40-50 kg/ha of phosphorus fertilizer per year, and little if any nitrogen fertilizer; however, as double-cropping has expanded in the region nitrogen fertilizer application has also increased (≥60 kg/ha) to support the second crop (Neill et al., 2017; Roy et al., 2016). Warmer soil temperatures in croplands as well as increasing fertilizer additions under double-cropping (Nagy et al., 2018; Neill et al., 2017) likely stimulate faster decomposition of OM leading to a more microbial DOM signature. When this increased degradation is coupled with lower OC inputs from soybean cropland and past tillage that has resulted in the standing stock of surface OC been reduced at these sites (Nagy et al., 2018), this results in further microbial reworking of OM. Additionally, small water impoundments in the cropland catchments (Riskin et al., 2017) may also be sources of autochthonous DOM; however, the low DOC concentrations in the cropland streams (Table 1) argue against this as a major contributor. Moreover, the shift in molecular signatures observed in this study is similar to that seen in recent work examining DOM compositional changes between forested and agricultural sites in the Democratic Republic of Congo where no impoundments of water are present (Drake et al., 2019). Therefore, the change in litter layer and surface soils caused by cropping appears likely to be the primary driver of the observed differences in molecular signatures of cropland and forest headwater stream DOM.

3.3. Linking DOM Molecular Signatures to Land Cover

To assess the DOM parameters that distinguish the cropland and forest streams, we conducted a principle component analysis on all samples (Figure 4 and Table 3). Principle component 1 (PC1) explained 62.0% of the variance in the data and correlated positively with AI_{mod}, HUP high O/C, polyphenolic, and condensed aromatic compound classes, as well as weighted average mass and DOC, and negatively with HUP low O/C compounds, assignment of heteroatomic formulae (CHON, CHONS, and CHOS), molecular diversity (number of formula), aliphatic, and peptide-like compounds (Figure 4 and Table 3). There was a clear split of samples on PC1 between forest samples dominated by a terrestrial signature (O'Donnell et al., 2016; Roebuck et al., 2018; Stubbins et al., 2010; Wagner et al., 2015) and cropland samples with a more microbial-like signature (Gonsior et al., 2011; Kellerman et al., 2018; Roebuck et al., 2018). Principle component 2 (PC2) explained a further 15.0% of the variance in the data and correlated positively with HUP low O/C compounds and negatively with assignment of S-containing formulae (CHONS and CHOS), aliphatic and peptide-like compounds (Figure 4 and Table 3). This highlights the greater seasonality shown in these parameters for cropland sites (Table 2) with samples separating on PC2 from the core cluster of samples relating to samples taken in the early wet season (October-November). Some of the cropland stream samples from this time of year exhibit a much greater contribution and variability with respect to the relative abundance of CHONS, CHOS, aliphatic, and peptide-like compounds in comparison to any other samples in this study (Table 2), further indicative of the mobilization of microbially derived DOM from soils into these streams in the early wet season (Kellerman et al., 2018; Singh et al., 1989). Additionally, recent work examining the DOM composition of vegetation and soil leachates by FT-ICR MS has shown fresh

Table 3
Principle Component Analysis Stricture Matrix for the DOC Concentration data and FT-ICR MS Parameters

Parameter	PC1	PC2
DOC (mg/L)	0.341	-0.050
Formulae (#)	-0.381	-0.073
CHO (%RA)	0.527	0.224
CHON (%RA)	-0.312	-0.022
CHONS (%RA)	-0.371	-0.382
CHOS (%RA)	-0.417	-0.263
Mass	0.354	-0.065
AI_{mod}	0.584	-0.044
HUP, high O/C (%RA)	0.562	0.051
HUP, low O/C (%RA)	-0.672	0.441
Peptide-like (%RA)	-0.369	-0.165
Condensed aromatics (%RA)	0.443	-0.279
Polyphenolics (%RA)	0.546	-0.215
Aliphatics (%RA)	-0.390	-0.280
% Variance Explained	62	15

Abbreviations: AI_{mod}, modified aromaticity index; DOC, dissolved organic carbon; FT-ICR MS, Fourier transform ion cyclotron resonance mass spectrometry; HUP, highly unsaturated and phenolic; PC1, principle component 1; PC2, Principle component 2.

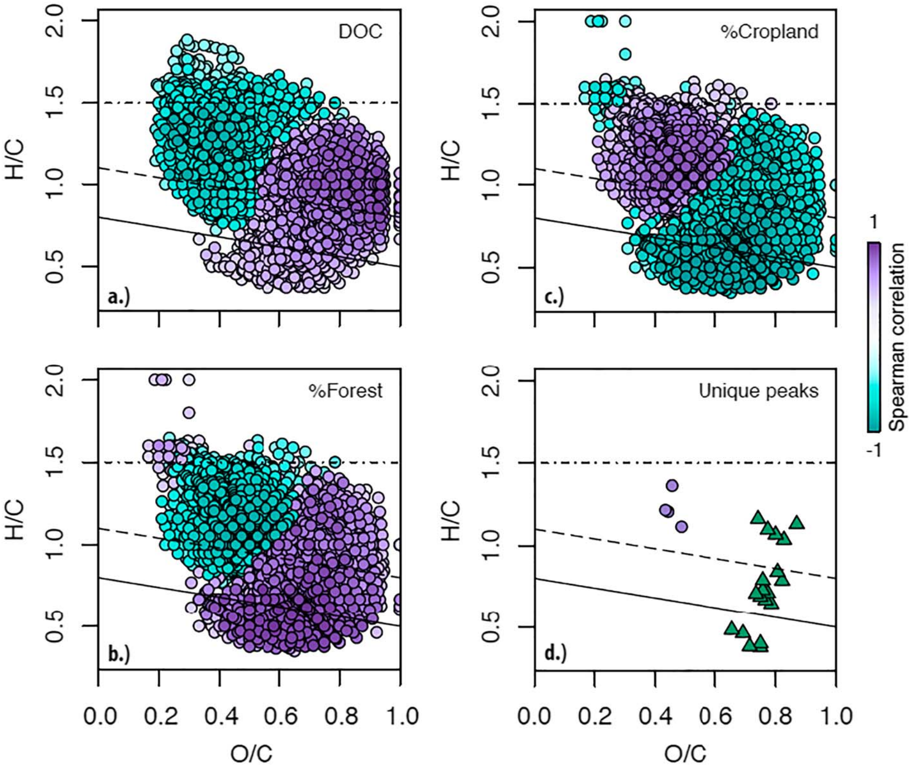

Figure 5. Spearman-rank correlations between the relative abundance of assigned molecular formulae and (a) dissolved organic carbon (DOC) concentration, (b) % forest in the catchment, and (c) % cropland in the catchment. Colors represent the correlation coefficient (ρ_s) between the relative abundance of each molecular formula and the respective variable with purple formulae exhibiting positive correlations and green formulae exhibiting negative correlations. (d) Unique molecular formulae found solely in cropland samples (purple circles) and forest samples (green triangles).

leachates to contain enriched relative abundances of aliphatic and peptide-like compounds, and lower relative abundances of HUP low O/C compounds relative to adjacent aquatic ecosystems (Textor et al., 2018), further suggesting that this signature reflects leached inputs of DOM from the terrestrial environment. Thus, future studies with higher temporal resolution, particularly capturing the transition from dry to wet season, may be able to distinguish between different seasonal inputs of DOM in cropland streams. This also underscores another impact of agriculture, particularly with respect to these biologically labile compounds: not only is the relative contribution greater from cropland streams but also the range of inputs is more seasonally variable (Tables 1 and 2 and Figures 3 and 4).

To further examine how relative abundances of individual molecular formula correlated with DOC concentration, percent cropland, or forest catchment coverage and thus different DOM sources, we conducted Spearman's rank correlations for each of the 11,279 formulae present in at least three samples (n=24). Streamwater DOC concentrations were significantly correlated (absolute-value Spearman's rank correlation coefficient, $\rho_s \geq 0.49$; $p < 0.05$) to 5,155 molecular formula (45%) with a clear separation in van Krevelen space of formula with similar ρ_s values and signs into two groups (Figure 5a). Percent forest coverage was similarly significantly correlated with 5,390 molecular formula (48%) and exhibited a similar pattern in van Krevelen space to DOC (Figure 5b). Molecular formula that were positively correlated with DOC concentration and percent forest ($\rho_s > 0$) typically had intermediary to high O/C ratios and intermediary to low H/C ratios, and negatively correlated formula ($\rho_s < 0$) exhibited the opposite trend. Percent cropland cover exhibited the same molecular formula assemblage split and was correlated with 5,390 molecular formula (48%); however, cropland extent showed the inverse coefficient signs to DOC and percent forest catchment coverage (Figure 5c). In addition to the primary delineation of molecular formula assemblages in van Krevelen space, a small cluster of low O/C and high H/C formula was observed and these molecular formulae did not contain nitrogen or sulfur and correlated positively with forest and negatively with cropland catchment coverage (Figures 5b and 5c). This region is commonly delineated as lipids (D'Andrilli et al., 2015; Lu et al., 2015), which have been shown to be particularly abundant in forest soils in this region, compared with agricultural soils (de Assis et al., 2011).

The distinct compositional assemblages in van Krevelen space (Figures 5a–5c) identified the molecular signatures of land-cover change in this area of rapid agricultural expansion and intensification in the Brazilian Amazon. The assemblages correlated well to fresh litter layers and organic-rich soil horizons as the source of forest DOM as this is typically high O/C and low H/C (Figure 5b) in nature (D'Andrilli et al., 2015; Lu et al., 2015; O'Donnell et al., 2016). Microbially derived material, likely from extensively worked soil OM, appears as the source of cropland DOM as this has low O/C and high H/C (Figure 5c), as well as is enriched in nitrogen (Figures 2c and 3c and Table 1; D'Andrilli et al., 2015; Gonsior et al., 2011; Kellerman et al., 2018). The molecular signature of Amazonian cropland stream DOM is comparable to that observed for agricultural impacts in other recent subtropical and tropical studies (Drake et al., 2019; Roebuck et al., 2018), highlighting that as soils in these watersheds all formed in areas with high annual temperatures and rainfall and are deeply weathered, their response to agricultural impacts is similar. The analogous compositional assemblages in van Krevelen space between DOC and percent forest cover in the catchment also strongly suggest that the sources of DOM composition and the higher DOC concentrations (Figure 2a and Table 1) observed in forest streams are the same. Ultimately, the clear delineation observed in DOM molecular formula shows the ability to now use ultrahigh-resolution mass spectrometry techniques such as FT-ICR MS to fingerprint land cover change.

We examined the unique molecular formula present in all cropland sites but no forest sites, and conversely those present in all forest sites but no cropland sites, to assess the truly exclusive markers for these two land-cover types (Figure 5d and Table S1). Interestingly, the four cropland markers were all N-containing HUP low O/C compounds and the 19 forest markers were all CHO molecular formula at high O/C. These unique markers offer potential for future studies to assess and trace agricultural impacts in these headwaters into downstream receiving aquatic ecosystems.

At this site in the Brazilian Amazon, traditional geochemical approaches to assess impacts on stream geochemistry (nitrogen, phosphorus, and sediment loading) appeared to suggest limited impacts of agriculture on downstream aquatic ecosystems because of the high inorganic nutrient fixation and retention capacity of the Oxisols in the region (Neill et al., 2017; Jankowski et al., 2018; Riskin et al., 2013, 2017). However, our results show that crop agriculture causes divergent DOC concentrations and a distinct DOM molecular composition compared with forested streams. Despite declining OM inputs in croplands, soil OC stocks between cropland and forest sites below the upper 10 cm were not different in this region (Nagy et al., 2018). Coupled with the fact that streams are typically dominated by groundwater inputs (Elsenbeer, 2001; Riskin et al., 2017), this suggests that the soils in this region are acting passively to the changing OM inputs from conversion of forest to croplands. Therefore, the changing signature derived from shifting OM sources in surface soils and litter layers from forest to cropland is then directly translocated to streams making them sentinels for detecting change. As this shifting DOM compositional signature is associated in other locations with an increase in the biolability of the exported DOM, this has clear implications for biogeochemical cycles, metabolism, and food webs as it is transported downstream in surface waters.

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