Non-targeted tandem mass spectrometry enables the visualization of organic matter chemotype shifts in coastal seawater

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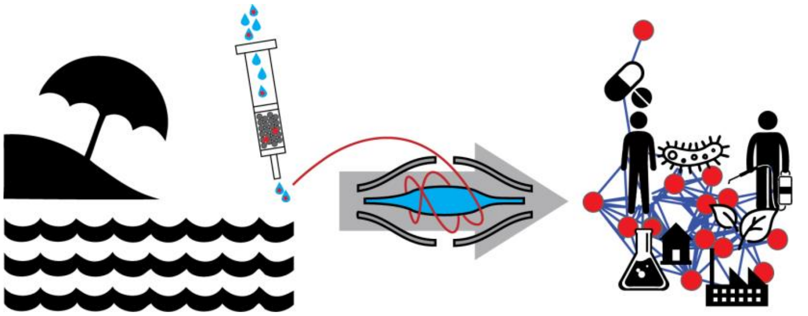
ABSTRACT

Urbanization along coastlines alters marine ecosystems including contributing molecules of anthropogenic origin to the coastal dissolved organic matter (DOM) pool. A broad assessment of the nature and extent of anthropogenic impacts on coastal ecosystems is urgently needed to inform regulatory guidelines and ecosystem management. Recently, non-targeted tandem mass spectrometry approaches are gaining momentum for the analysis of global organic matter chemotypes including a wide array of natural and anthropogenic compounds. In line with these efforts, we developed a non-targeted liquid chromatography tandem mass spectrometry workflow that utilizes advanced data analysis approaches such as feature-based molecular networking and repository-scale spectrum searches. This workflow allows the scalable comparison and mapping of seawater chemotypes from large-scale spatial surveys as well as molecular family level annotation of unknown compounds. As a case study, we visualized organic matter chemotype shifts in coastal environments in northern San Diego, USA, after significant rain fall in winter 2017/2018 and highlight potential anthropogenic impacts. The observed seawater chemotype shifted significantly after a major rain event. Molecular drivers of this shift could be attributed to multiple anthropogenic compounds, including pesticides (Imazapyr and Isoxaben), cleaning products (Benzyl-tetradecyl-dimethylammonium) and chemical additives (Hexa(methoxymethyl)melamine) and potential degradation products. By expanding the search of identified xenobiotics to other public tandem mass spectrometry datasets, we further contextualized their possible origin and show their importance in other ecosystems. The mass spectrometry and data analysis pipelines applied here offer a scalable framework for future molecular mapping and monitoring of marine ecosystems, which will contribute to a deliberate assessment of how chemical pollution impacts our oceans.

HIGHLIGHTS

- Feature-based Molecular Networking enables large-scale analysis of marine DOM
- Organic matter chemotype in coastal San Diego shifted significantly after rain
- Molecular drivers could be attributed to multiple anthropogenic compounds
- Spatial mapping highlighted different point sources as potential origin
- Repository-scale meta-analysis can further contextualize origin and importance

GRAPHICAL ABSTRACT



KEYWORDS

Dissolved Organic Matter, Tandem Mass Spectrometry, Non-targeted Screening, Molecular Networking, Coastal Environments, Anthropogenic Pollution

1. Introduction

Population growth and urbanization place increasing stress on coastal marine ecosystems, resulting in numerous negative impacts. In a meta-analysis of 216 studies, chemical contaminants were associated with a 40% reduction in biodiversity richness in marine communities(Johnston and Roberts, 2009). Chemical pollutants come in numerous organic and inorganic forms, and new compounds are constantly detected in the environment. Currently, there are over 157 million unique organic and inorganic chemical substances registered in the Chemical Abstract Service (CAS), with 30,000 to 70,000 estimated to be in daily use through human activities (Schwarzenbach et al., 2006). This incredible chemical diversity, including a myriad of potential degradation products, makes it challenging for eco-toxicologists to monitor and evaluate the impacts of all possible pollutants. In spite of this diversity, the identity and abundance of chemical pollutants are mainly established using targeted mass spectrometry (MS) methods for routine monitoring (Magi and Di Carro, 2018; Petrie et al., 2016). For example, the Clean Water Act of the Environmental Protection Agency (EPA) in the United States recommends monitoring 126 compounds(US EPA, 2013). However, as mentioned above, these compounds occupy only a small percentage of the chemical space of potential pollutants and their degradation products (Kolpin et al., 2002; Schwarzenbach et al., 2006).

In order to account for a broader range of possible chemical compounds, the use of high resolution mass spectrometry (HR-MS), including bioinformatic infrastructures, has gained momentum in the field of non-targeted analysis (Albergamo et al., 2019; Alygizakis et al., 2019; Gago-Ferrero et al., 2015; Hernández et al., 2019; Hollender et al., 2017; Huntscha et al., 2014; Schlüsener et al., 2015; Schymanski et al., 2014b; Ulrich et al., 2019; Verkh et al., 2018). HR-MS, such as direct infusion Electro Spray Ionization (ESI) Fourier-transform ion cyclotron resonance (FT-ICR) MS and orbital ion traps have also been widely applied to analyze organic compounds in various biogeochemical contexts (Dittmar and Paeng, 2009; Hawkes et al., 2016; Osterholz et al., 2016). Recent advances in non-targeted liquid chromatography high resolution tandem mass spectrometry (LC-HR-MS/MS) for the analysis of complex organic mixtures such as dissolved organic matter (DOM) may also be uniquely suited for detecting organic pollutants in marine systems that are accessible by solid phase extraction and ESI (Hawkes et al., 2018; Longnecker and Kujawinski, 2017; Lu et al., 2018; Petras et al., 2017). However, the data analysis and especially the annotation of compounds that are not covered by spectral libraries remain as major challenges.

New developments in MS software tools, including molecular networking, comprehensive spectral and structure databases, statistical significance estimation, as well *in silico* annotation and community based annotation criteria have significantly improved the annotation of MS/MS spectra (Aron et al., 2019; Dührkop et al., 2019; Horai et al., 2010; Nothias et al., 2020; Ruttkies et al., 2016; Scheubert et al., 2017; Silva et al., 2018; Steen et al., 2020; Wang et al., 2016; Watrous et al., 2012). These technological improvements enable the annotation of a multitude of known and unknown chemical compounds which is central for a mechanistic understanding and prediction of how human actions influence ecosystems.

This case study serves as a first large scale application of Feature-based Molecular Networking for the analysis of marine organic matter composition, in which we assessed the chemical impacts of a major rain event in northern San Diego, California (USA). Our data provide insights into global chemical impacts on coastal environments in Northern San Diego after significant rain and offer complementary insights to microbiology (He and He, 2008; Steele et al., 2018) and chemical analysis of anthropogenic pollutants in the area (Patterson et al., 1976; Tran et al., 1997).

Our results show a clear shift in the organic matter composition after the rain event that could be attributed in part to the increased presence of multiple anthropogenic pollutants, some from identifiable point sources. The results serve thereby as strong case for the use of non-targeted LC-MS/MS and advanced data-analysis methods such as Feature-based Molecular Networking, and the sharing and reuse of MS/MS datasets (Jarmusch et al., 2020; Nothias et al., 2020; Wang et al., 2020).

2. Experimental Section

A detailed experimental procedure, including mass spectrometry setting can be found in the Supporting Information (SI). Between 10:00 and 13:00 (PST) on December 8th, 2017 and January 12th, 2018, we collected surface seawater samples (-10 cm) at 30 sites spaced approximately 300 meters apart and 50-100 m offshore along the San Diego coastline from Torrey Pines State Beach to Mission Bay. Geographical locations of sampling stations are shown in Figure 1. Aliquots of seawater were taken for nutrient (50 mL), total organic carbon (TOC) analysis (40 ml) and non-targeted LC-MS/MS (2 x 1L). For concentration and desalting of organic compounds, we performed solid phase extraction (200 mg PPL cartridges) of seawater adjusted to pH ~2 (1.2 mL 37% HCl) and eluted in methanol (Dittmar et al., 2008). Eluted samples were concentrated *in vacuo* and re-dissolved in 100 µL MeOH/H_2O/Formic acid (80/19/1) and 10 µL were injected for reverse phase ultra-high-performance liquid chromatography high-resolution tandem mass-spectrometry (RP UHPLC-MS/MS) analysis using positive mode electrospray ionization (ESI) quadrupole-Orbitrap mass spectrometer as described before (Petras et al., 2017). We chose to run the samples in positive mode as the larger part of MS/MS libraries are acquired in positive ESI. Retention time and m/z drifts were controlled with a quality control mix of 6 standards (Sulfamethazine, Sulfamethizole, Sulfachloropyridazine, Sulfadimethoxine, Amitryptilin, Coumarin-314) between runs and within samples with commonly observed contaminants and typical components of DOM (Dibutyl phthalate, pheophorbide A and tryptophan) which was below 5 ppm and below 0.1 min (Figure S1). For level 1 annotations, authentic standards (Irgarol, Isoxaben, Imazapyr, Hexa(methoxymethyl)melamine) were dissolved in methanol/water/ formic acid (80/19/1) with final concentrations of 0.01, 0.10, 1.00 and 10.00 µg/mL. To estimate extraction efficiencies during SPE, we spiked different amounts (0, 1, 10, 100 ng) of authentic standards that were detected in this study (Irgarol, Isoxaben, Imazapyr, Hexa(methoxymethyl)melamine) as well as other commonly observed xenobiotics in coastal ecosystems (Carbamazepine, Cocaine)(Pereira et al., 2016) to 1L seawater samples collected at Scripps Institution of Oceanography (SIO) Pier, California, USA (Feb 15 2020).

For LC-MS/MS data analysis, ion features were generated from extracted ion chromatograms (XIC) using MZmine2 and linked to their tandem mass spectra, which provides a combination of relative abundance (XIC) and qualitative (MS/MS) information (Nothias et al., 2019). Subsequently, mass spectra were analyzed by molecular networking to establish molecular (MS/MS similarity) relationships between all detected compounds and annotated against the *GNPS* library, which currently contains 74,044 MS/MS spectra (Dec 18, 2019, including Mass Bank (Horai et al., 2010), ReSpect (Sawada et al., 2012) and HMDB (Wishart et al., 2007)), as well as against the commercial NIST17 MS/MS spectral library (Wang et al., 2016). In addition to spectrum library matching, molecular formulas of selected features that could not be annotated by spectrum library matching were calculated based on exact mass and MS/MS fragmentation trees using *ZODIAC* within *SIRIUS4* (Dührkop et al., 2019; Ludwig et al., 2019) which was run as a GNPS workflow. Features from potential contaminants that were observed in the PPL process blanks with a relative peak area > 30% in comparison to the sample average were filtered out. The resulting molecular feature table was then compared to different metadata categories by means of multivariate statistical analysis and spatial visualization in a geographic heat map. A flow chart of the sample processing and LC-MS/MS analysis is shown in Figure 1. The feature table, including all levels of MS/MS annotations are provided a .csv file in the supplemental information.

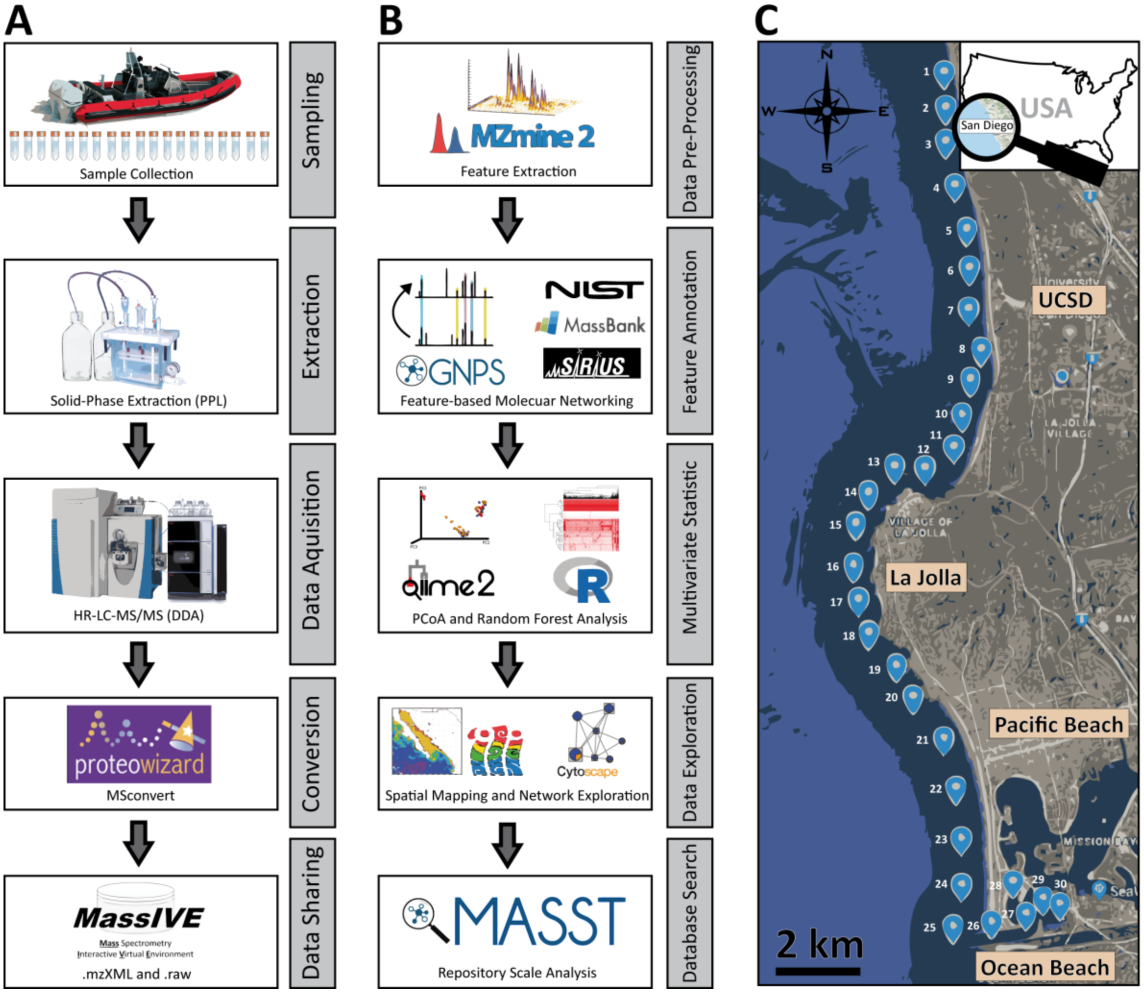


Figure 1. Experimental design and study area. Panel A shows the LC-MS/MS data acquisition and data sharing steps. Panel B shows the MS/MS data analysis workflow. Panel C indicates the geographic positions of the sample sites along the San Diego coastline.

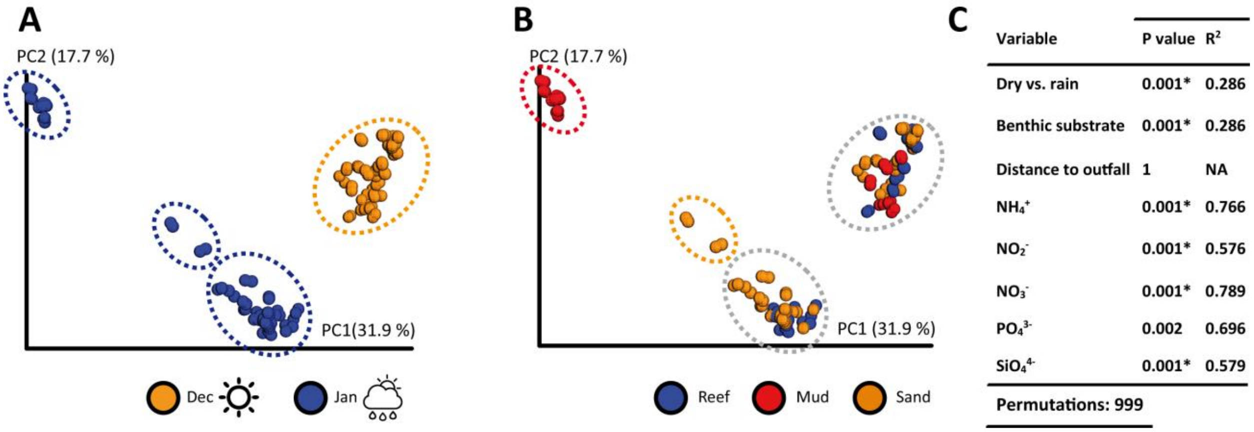
Besides total organic matter composition, we analyzed macronutrient concentrations including NH_4^+, NO_3^-, NO_2^-, PO_4^{3-}, and SiO_4^{4-} along with TOC (Figure S1), correlated their values against organic matter compositional shifts (Bray-Curtis Dissimilarity, ADONIS), and compared nutrient concentrations across samples taken during the two time points (Mann-Whitney) using *Qiime* (Bolyen et al., 2019; Caporaso et al., 2010). Selected MS/MS spectra from features that were annotated as potential pollutants and that showed distinct spatial patterns were then searched against all public *GNPS* datasets in the *MassIVE* spectral repository (massive.ucsd.edu) using the *Mass Spectrometry Search Tool* (*MASST*)(Wang et al., 2019). The first pass search matched the query MS/MS against the clustered MS/MS across all public GNPS datasets and then de-clustered the match results to reveal the exact underlying mass spectrometry files the matches were found in. To aid in sensitivity, we additionally queried the MS/MS spectra against the non-clustered data. The total number of MS/MS spectra considered was over 400 million.

3. Results and Discussion

3.1. Field Work and Auxiliary Measurements. In December 2017, no precipitation was reported in San Diego, CA, USA(“Weather Forecast & Reports - Long Range & Local | Weather Underground,” n.d.), whereas three days before our sampling in January 2018, a major rainfall event was measured (January 9th 2018, 39.7 mm of precipitation in San Diego). At the Scripps Pier station, salinity was measured to be 33.24 PSU on December 9th 2017 and 31.9 PSU on January 9th 2018, which was lower than any measurements made in the previous year (2017) at this site. This decrease in salinity is consistent with modest freshwater inputs following rainfall (Nezlin et al., 2008; Reifel et al., 2009). Macronutrient concentrations including NH_4^+ ($P<0.0001$), NO_3^- ($P<0.0001$, NO_2^- ($P<0.0001$), PO_4^{3-} ($P<0.0001$), and SiO_4^{4-} ($P<0.01$) along with Total Organic Carbon ($P<0.01$) were significantly higher following the rain event (Mann-Whitney, Figure S2) which we attributed to input from river water and/or runoff. At both time points, samples taken from the southern end of our study area (Mission Bay) were noticeably higher in all measured nutrient concentrations than other samples (Figure S2), which could be explained by increased influence of runoff at Mission Bay through the San Diego River. Looking at geographical differences from the samples taken before the rain, we noted that NO_3^- and NH_4^+ concentrations were generally higher in Mission Bay as compared to other sites. In the samples taken after the rain, NH_4^+, NO_3^-, NO_2^-, PO_4^{3-} as well as TOC were also higher in Mission Bay and the Los Penasquitos Lagoon outlet as compared to most other sites.

3.2. Non-Targeted MS/MS Analysis. Following non-targeted LC-MS/MS analysis of seawater PPL extracts, we obtained 5,521 MS1 ion features with assigned MS/MS spectra, which decreased to 4,384 MS1 ion features after PPL process blank subtraction (the feature matrix is provided in the supporting information). From these MS/MS spectra, we created a molecular network using the Feature-based Molecular Networking workflow in *GNPS* (Nothias et al., 2020; Wang et al., 2016). All MS/MS spectra were thereby searched against the *GNPS, Massbank* and *NIST17* spectral reference libraries, which resulted in 92 annotations after blank subtraction (142 in total). The observed annotation rate of 2.1% after blank subtraction is in a similar range to other non-targeted environmental metabolomics studies (Floros et al., 2017; Petras et al., 2017, 2016), leaving the vast majority of ions detected as unknowns. Low annotation rates are still driven by the sparse availability of reference MS/MS spectra in the public domain and highlights the tremendous knowledge gap in chemical space and the need to expand non-targeted approaches. In addition to limited spectral library coverage, the high complexity of environmental samples such as DOM can result in chimeric MS/MS spectra (i.e., spectra containing fragments from more than one compound) which can further limit spectral matching. Annotations obtained with precursor and MS/MS matching are considered level two annotations according to the 2007 Metabolomics Standards Initiative(Sumner et al., 2007) and the environmental science community (Schymanski et al., 2014a) and should be considered as putative identifications. The library matching score cut-off used in this study give rise to an 1 % false discovery rate for spectral matching (Scheubert et al., 2017; Wang et al., 2016). MS/MS mirror plots of the sample spectrum and the reference spectrum for either library or analog matches discussed here are shown in Figure S3. Precursor mass deviation and matching score (cosine) of all annotations are displayed in the Feature Table .csv file in the supplemental information and can be found online under https://gnps.ucsd.edu/ProteoSAFe/status.jsp?task=5ea78acca01b42efaa8464398c631cf6. Expanding the library search to putative analogs, we were able to annotate 1404 spectra (32%) to molecular families. The analog search algorithm matches similar MS/MS spectra with different precursor masses to library spectra, where the maximum precursor and fragment mass difference between putative analogs and library compounds is restricted by the user. Through the extended search space, analog annotations are more prone to false positive matches, but matches with high cosine scores and delta masses that correspond to known modifications (such as methylation, hydroxylation, acetylation, etc.(Hartmann et al., 2017)) can provide insight into the putative molecular family of a compound. Both spectrum library matching with strict and expanded precursor mass tolerance (analog search) that are discussed here were manually inspected for precursor mass deviation as well as number of matching fragments ions. For analog matches (spectrum library matching with expanded precursor and corresponding fragment mass tolerance(Cooper et al., 2019; Wang et al., 2016)) exact mass offsets of precursor and fragment ions where only considered if they could be explained by common chemical modifications (e.g. methylation, acetylation oxidation etc.)(Hartmann et al., 2017).

3.3. Global Comparison of Seawater Chemotypes. After feature detection and annotation, we initially displayed sample-to-sample similarity by multivariate statistical analysis of their LC-MS/MS-based chemical composition with the Bray-Curtis dissimilarity metric. Sampling date (December vs. January) significantly influenced the seawater organic matter composition (Adonis, P<0.001, Figure 2C). Besides the clear time dependency, the separation of different seawater chemotypes could also be attributed to the benthic substrate (sandy bottom vs. rocky reef bottom) of particular samples sites. Together with macronutrient concentrations, including NH_4^+, NO_3^-, NO_2^-, and SiO_4^{4-}, sampling period and location explain the differentiation of the chemical seawater composition ($p<0.001$, Figure 2C). In a principle coordinate analysis (PCoA) shown in Figure 2A, the samples (represented as dots) are separated based on their compositional similarity with the Bray-Curtis dissimilarity metric. This separation could be attributed to sampling date (blue vs. orange), with January samples being the furthest outlier. Before the rain (December), Mission Bay samples clustered closer to samples from other locations (Figure 2 B, red vs. orange and blue) when compared to the January samples. A similar trend was also observed for the four samples taken after the rain at the northern end of Torrey Pines State Beach (Figure 2 A, orange, Sand; Station 1 and 2, proximal to the Los Penasquitos Lagoon outlet). The compositional sample-to-sample distance in PCoA between those two sites and the other sites with sandy substrate were further away from each other after the rain than before the rain. Increased allochthonous inputs to particular stations (1, 2, 26-30) at each end of the sampling transect are consistent with their proximity to freshwater input through the Los Penasquitos Lagoon (north) or San Diego River and Rose Creek (south).



Variable	P value	R^2
Dry vs. rain	0.001*	0.286
Benthic substrate	0.001*	0.286
Distance to outfall	1	NA
NH_4^+	0.001*	0.766
NO_2^-	0.001*	0.576
NO_3^-	0.001*	0.789
PO_4^{3-}	0.002	0.696
SiO_4^{4-}	0.001*	0.579
Permutations: 999		

Figure 2. Principal Coordinate Analysis (PCoA, Bray-Curtis) and Statistical Analysis (ADONIS). PCoA shows compositional sample to sample distance. The color coding in A (orange: before, blue: after rain) indicates that the rain event drives chemical composition of nearshore coastal seawater. In B samples are color coded based on benthic substrate (correlating to geographical differences, red: Mud/Sludge, Orange: Sand, Blue: Rock/Reef). The dashed circles indicate clustering of similar chemotypes. C shows the results of the multivariate statistical analysis of the effects of environmental variables on chemical composition in seawater. The ADONIS permutation-based analysis indicates the significance of explanation of multivariate distance (Bray-Curtis) by different meta-data categories.

3.4. Identification of Chemical Drivers. In order to identify the main molecular drivers responsible for the differences in seawater chemotypes, we performed a Random Forest classification, a machine learning approach for compositional sample classification and regression. Two classifications between the sample groups were performed based on collection date (January vs. December) and subsequently on distance to freshwater outflows in the January samples only (close vs. far, e.g., sites 1, 2, 26-30 vs. 3-25).

Taking all features into account, the top two molecular drivers for temporal differences (December vs. January, Figure S4) did not result in any spectral library annotations. The two top drivers were a feature with *m/z* 353.2316, RT 9.0min, $C_{20}H_{32}O_5$ ($\Delta m/z$ = 1.8 ppm), followed by a feature with *m/z* 416.2473, RT 4.3 min $C_{18}H_{33}N_5O_6$ ($\Delta m/z$ = 8.6 ppm).

The top two drivers from the Random Forest classification that separated different sample sites in January (Figure S4) were two unknown compounds with *m/z* 1129.3145, RT 11.8 and 12.6 min (no confident molecular formula assignment), which were organized in a molecular network (based on MS/MS similarity) with other features were also only found in January. Besides unknowns and putative analogs, we observed a feature within the top drivers with matching MS/MS spectra to benzyl-tetradecyl-dimethylammonium, a quaternary ammonium salt, commonly used as a disinfection agent and sanitizer (all MS/MS mirror plots of spectrum library matches discussed are shown in Figure S3).

In order to prioritize the differentially abundant features with confident annotations, we filtered the Random Forest classification to features which resulted in library matches with precursor mass differences < 10 ppm. The top feature with a library match (cosine = 0.94) was Hexa(methoxymethyl)melamine (GNPS Library ID: CCMSLIB00000841617) which was confirmed by MS/MS and retention time match to an authentic standard (level1). Hexa(methoxymethyl)melamine appeared to drive the compositional distance between samples taken close to freshwater outflows. Hexa(methoxymethyl)melamine is a chemical component in resins used for coatings and plastics and has been described as a pollutant in rivers with acute fish and daphnia toxicity (Dsikowitzky and Schwarzbauer, 2015; Labunska et al., 2012). Based on an external calibration curve (Figure S6) and a standard addition experiment (Figure S7), we conclude that the extraction efficiency is only around 0.3% for Hexa(methoxymethyl)melamine. Considering the relatively poor extraction efficiency, we assume that the low recovery can be attributed to the high content of amino groups in Hexa(methoxymethyl)melamine, that most likely undergo protonation at low pH and hence show lower affinity to the hydrophobic PPL resin. However, at pH 8 the extraction efficiency was also low at ~3% (Figure S7), considerably lower than for the other compounds tested. Considering the low extraction efficiency, we estimate the highest concentrations observed in our study (Peak Area = 2.1E8) to be between 0.3 and 3 µg/L.

Besides Hexa(methoxymethyl)melamine (*m/z* = 391.2308), we detected several other compounds with analog matches to Hexa(methoxymethyl)melamine (*m/z* = 377.2128; 359.2052; 333.1842; 301.1630; 303.1730), all of which were connected in a molecular network (Figure 3 B and Figure S8).

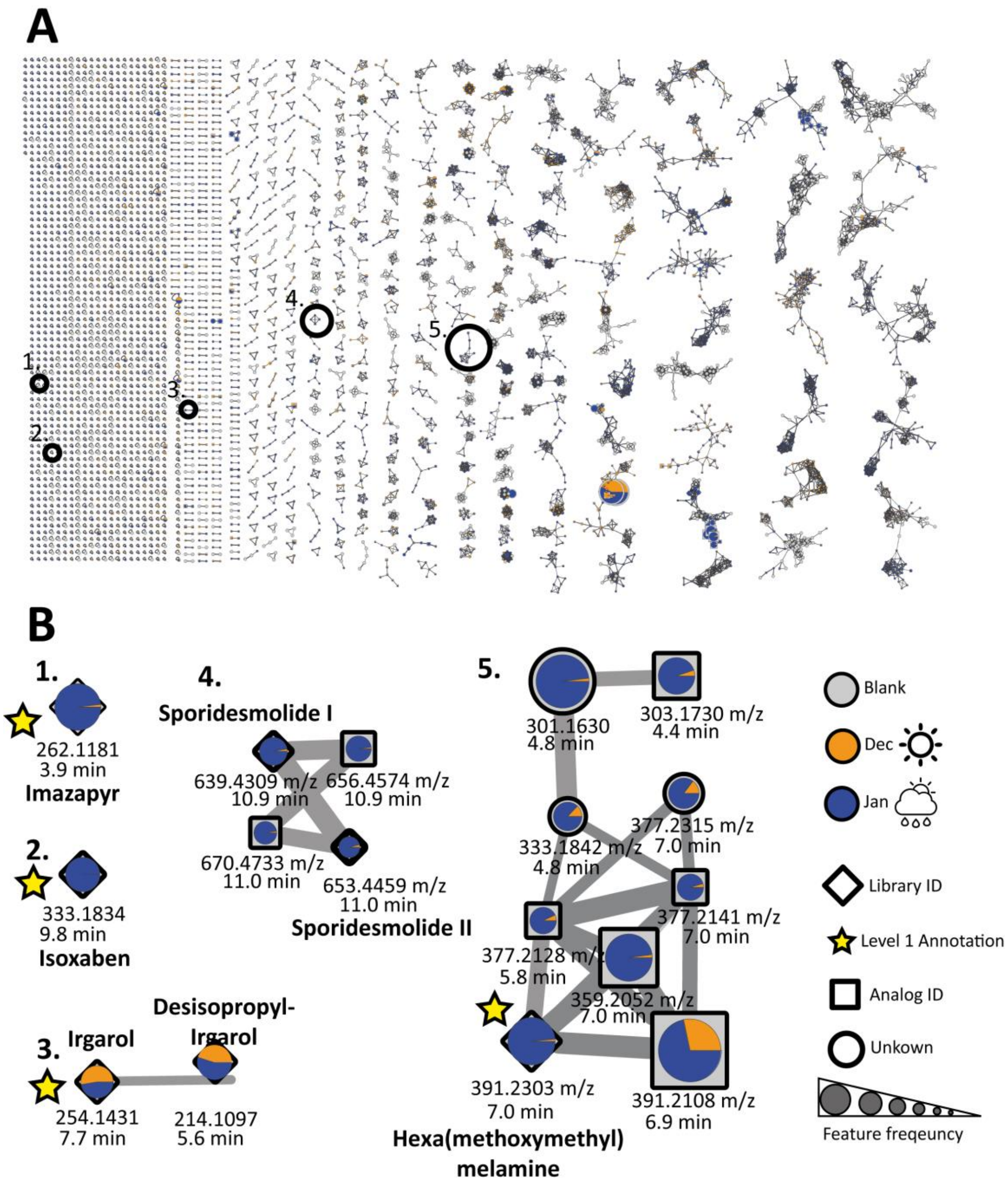


Figure 3. Molecular Network of Seawater Chemotypes. Nodes represent MS/MS spectra which are connected based on their spectral similarity (cosine > 0.7). Node size indicates the relative average abundance of a feature in the data set and the pie chart indicates the relative distribution between samples taken before and after the rain event (based on MS1 XIC). Feature shape indicates the annotation level (circle: unknown, square: analog match, diamond: level 2 library match) The star symbol indicates level 1 annotations (confirmation of retention time and MS/MS spectrum with authentic standards).

Investigating the retention times of the putative analogs, we assume that the features with m/z 359.2052, 301.1630, as well as two of the features with m/z 377.2128, are most likely in source fragments. However, derivatives with *m/z* = 377.2128; 333.1842 and 303.1730 show independent retention times (7.0, 5.8, 4.8

and 4.4 min respectively). Due to the similar spatial patterns (Figure 4 A and B and Figure S9), we assume that those compounds are most likely degradation products that underwent subsequent demethylation, dehydration and demethoxylation (Δm/z = 14.0162, CH2; 18.0089, H2O; 32.0251, COH4; 44.0299, C2OH4. Interestingly, the presence of Hexa(methoxymethyl)melamine and some of the here described derivatives (Hexamethylolmelamine pentamethyl ether, m/z 377.2128 and Tetra(methoxymethyl)melamine, m/z 303.1730) have recently also been described in storm water run-off in Seattle, USA(Peter et al., 2018) and their MS/MS spectra show high similarity to the MS/MS spectra recorded in this study. It would be interesting to further decipher if these derivatives are formed due to biological degradation, formed as side products during polymerization, or whether they are a result of ageing of Hexa(methoxymethyl)melamine-containing polymers.

After the ranking in the Random Forest classification, we focused next on compounds that showed relevant patterns in our spatial survey (higher abundance at the northern and southern extreme of the study area) and that are known as potential pollutants. For example, Imazapyr (cosine = 0.93, GNPS Library ID: CCMSLIB00003723206, level 1 annotation), a water-soluble total herbicide used for the control of a broad range of weeds, was mainly found after the rain. The spatial distribution showed several orders of magnitude higher intensities in samples taken close to the outflows of Los Penasquitos Lagoon (north) and Mission Bay (south). Based on an external calibration curve and extraction efficiency of around 65%, the highest concentration of Imazapyr in our study (Peak Area = 2.6E8) was estimated to be between 1.5 and 15 ng/L.

Besides Imazapyr, we detected several other pesticides, such as Isoxaben (cosine = 0.98, CCMSLIB00003562269, level 1) with an estimated concentration range between 0.3 and 3 ng/L (extraction efficiency ~ 35%, Figure S6 and S7), Dimethenamide-P (cosine = 0.97, CCMSLIB00003638780) and Metalaxyl (cosine = 0.95, GNPS Library ID: CCMSLIB00003135403) with similar spatial-temporal distributions (Figure S10), indicating that the Los Penasquitos Lagoon (north end) and Mission Bay, including Rose Creek and San Diego River outlet (south end), are potential point sources in our study area. Agricultural activity in the San Diego metropolitan area mainly consists of residential gardening and parks and farming activity in San Diego County (e.g. Ramona and Oceanside)(Scurlock, 2019; Sokolow et al., 2010). Imazapyr, Isoxaben, Dimethenamide-P are active ingredients in commercial herbicides used for weed control and could have been used in this context. Metalaxyl on the other hand is a fungicide that is typically used to treat root rot in vegetable crops and could originate from farmland located in north eastern San Diego (e.g., through the San Diego River).

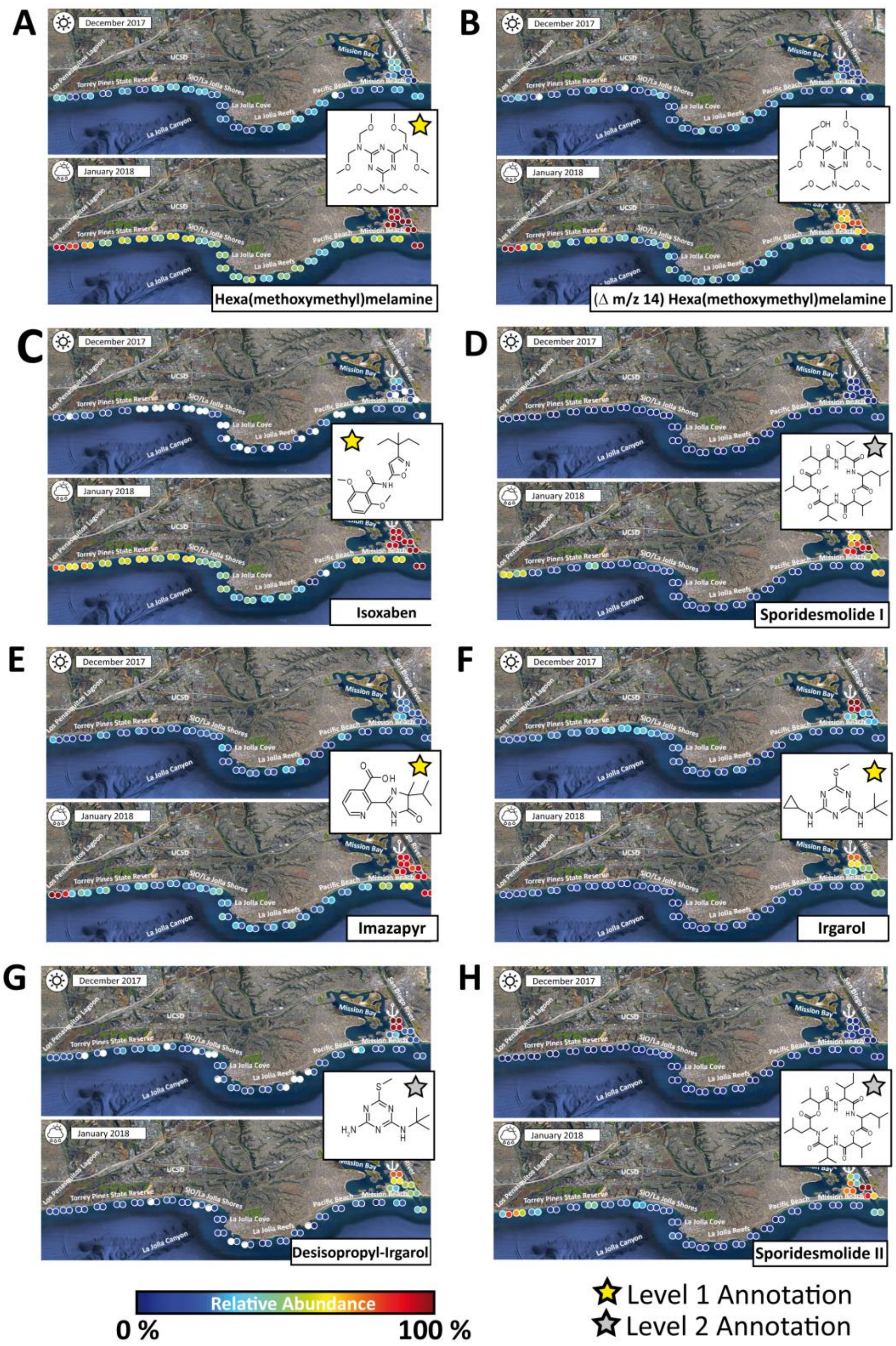


Figure 4. Extracted Ion Maps (EIM) of relative xenobiotic and natural product concentrations in seawater samples. The spatial maps indicate the relative abundance of 8 selected molecular features (Xenobiotics: Hexa(methoxymethyl)-Melamine (CCMSLIB00000841617), Desmethyl-Hexa(methoxymethyl)-Melamine, Isoxaben (CCMSLIB00003562269), Imazapyr (CCMSLIB00003723206), Irgarol (CCMSLIB00000208263), Desisopropyl-Irgarol and Sporidesmolide I and II (CCMSLIB00000507910 and CCMSLIB00000577642) from two replicate samples. Absolute peak areas can be found in the feature table in the supplemental information.

Other xenobiotics, such as the herbicide and anti-biofouling agent Irgarol (cosine = 0.99, GNPS Library ID: CCMSLIB00000208263, level 1 annotation), were detected both in December and January (Figure 3 B and 4 F), indicating that the rain event did not have a major impact on the presence of these particular compounds. The spatial distribution further indicates that the marina in Mission Bay may also be a potential point source, which is consistent with the usage of Irgarol as an antifouling agent in boat paint. Interestingly, the relative abundance of Irgarol was higher in December before the rain (max Peak Area = 1.4E8) but only in the sample sites directly in the marina. Based on an external calibration curve (Figure S6 and S7, extraction efficiency ~40%), we estimate the highest concentrations to be in the range between 0.3 and 3 ng/L. After the rain, Irgarol was detected with higher abundance at the sample sites outside of the marina and the Mission Bay channel. The wider distribution could be explained by more freshwater influx into Mission Bay and hence higher dilution and efflux into the ocean.

Another compound in our dataset (*m/z* = 214.1097) was annotated as desisopropyl-Irgarol (Figure 3 B). Desisopropyl-Irgarol has been described as a microbial and photo degradation product from Irgarol(Liu et al., 1997; Zhang et al., 2008), which is in line with the compounds' identical spatio-temporal patterns (Figure 4 G) in our survey.

In addition to the above described xenobiotics, we detected and annotated multiple natural products in this dataset. The fungal non-ribosomal cyclo-depsipeptides sporidesmolide I and II(Bertaud et al., 1965), for example, were detected with almost identical spatial patterns (Figure 4 G and H). Highest relative abundances were observed in the January samples, which were collected close to outlets of the Los Penasquitos Lagoon and Mission Bay. The two derivatives differ by one amino acid (isoleucine vs. valine) both of which are produced by the same biosynthetic machinery and organisms (Süssmuth et al., 2011; Wang et al., 2018).

3.5. Repository-Scale Meta-Analysis of identified pesticides. In order to further investigate potential origins of the pesticides discussed above, we searched their MS/MS spectra against the entire *GNPS* spectral database, containing 1,239 public available MS/MS dataset with more than 400 million MS/MS spectra and 30 terabytes of data (1st August 2019, gnps.ucsd.edu) using the *Mass Spectrometry Search Tool* (*MASST*) (Wang et al., 2019). The matching datasets are listed in Table S1.

The MS/MS spectra from Irgarol for example was exclusively found in datasets from different coastal environments from California and Hawaii. All samples, in which Irgarol was present, were collected near marinas, ports or anchor sides. The spectra from the degradation product Desisopropyl-Irgarol was only present in datasets from San Diego (this study) and Hawaii. An interesting question resulting from this observation is whether Desisopropyl-Irgarol was not detected in other studies due to an overall lower concentration of Irgarol and hence a lower abundance of its breakdown product or whether differences in microbial and environmental conditions lead to less breakdown in other studies. The mass spectrum of Imazapyr was only found in the MassIVE dataset from this study. Dimethenamide-P was additionally found in samples from Imperial Beach (San Diego/Tijuana) close to the Tijuana River estuary, an area known to suffer from extreme anthropogenic pollution(Johnsen, 2018). The mass spectrum from Isoxabene was found in the dataset from this study, in data collected from office and human skin swab samples, and from *Euphorbiaceae* plants from New Caledonia. The mass spectra of the fungicide Metalaxyl on the other hand was found in more than 10 datasets including samples from citrus trees, corn, food as well as metabolomics studies with mice and human subjects. These results indicate that the pesticides found in our study could originate from direct agricultural deployment but also that these compounds are transferred through fruits and other plant-based foods to humans and animals. Nevertheless, it should be noted that MASST searches are inherently biased to environments and samples types that are present in datasets in the public domain and relies on the scientific community to share their MS/MS data in publicly accessible databases.

4. Conclusion

We explored the spatio-temporal shift of the organic seawater chemotype along the northern San Diego coastline before and after a major rain event in winter 2017/2018 by means of non-targeted LC-MS/MS. We detected several thousand distinct ion features contributing to the total organic matter composition. Compositional changes in the seawater chemotype could be attributed to seasonal and spatial differences. Within this diverse and complex mixture of compounds, we could annotate several xenobiotics and potential derivatives and degradation products, and pinpoint the Mission Bay Marina, freshwater influx from Rose Creek and San Diego River, and the Los Penasquitos Lagoon as potential point sources. The repository scale meta-analysis of selected MS/MS spectra from our study could further contextualize the presence and potential origin of certain compounds and highlight other potential anthropogenic stress on environments. These results thus offer an effective example of how our data analysis pipeline and repository scale meta-analysis can assist in prioritizing, contextualizing, and tracking potential sources of anthropogenic compounds and complement other community efforts such as NORMAN (Alygizakis et al., 2019). The deposition of mass spectrometry data in public databases not only facilitates the accessibility of data and reproducibility of data analysis by other researchers, but also allows for constant retro-analysis and annotation. Data from this study for example, were first analyzed via *GNPS* in January 2018, resulting in a total of 142 library IDs (2.5% annotation rate). In August 2019, the same dataset resulted in 190 library IDs (4.6 % annotation rate, Figure S11).

We anticipate that the use of non-targeted LC-MS/MS workflows to measure and monitor anthropogenic pollution in marine environments-will further increase in the future. We anticipate that the analytical and data analysis approaches presented here will contribute to obtaining new insights into the cycling of xenobiotics and their interactions in complex environmental systems.

5. Data Sharing

All MS/MS data can be found on the *Mass spectrometry Interactive Virtual Environment* (*MassIVE*) at https://massive.ucsd.edu/ with the identifier MSV000082312. Molecular Networking and Spectrum Library Matching results can be found online at GNPS under the following links:

https://gnps.ucsd.edu/ProteoSAFe/status.jsp?task=5ea78acca01b42efaa8464398c631cf6
https://gnps.ucsd.edu/ProteoSAFe/status.jsp?task=f9ae547fe4a141a58d3214462e97f9e0
https://gnps.ucsd.edu/ProteoSAFe/status.jsp?task=a17b86fa6240493fbd4b906cb00ff1fc

6. Author Information

Author Contributions

DP and JJM conceived the study and designed the experiments. DP and JJM performed the sample collection. MEW performed the TOC measurements. EK assisted in calculating distances to storm drain outfalls. DP, LBC and RRT performed the solid phase extraction and mass spectrometry experiments. MW, EEA, KAP, LIA, and PCD provided equipment, materials and software. DP and JJM analyzed and interpreted the data. DP, JJM, LIA and PCD wrote the manuscript. All authors read, discussed and approved the manuscript.

Conflict of Interest Statement

Pieter C. Dorrestein is a scientific advisor for Sirenas LLC. Mingxun Wang is the founder of Ometa labs LLC.

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