Utilizing Pine Needles to Temporally and Spatially Profile Per- and Polyfluoroalkyl Substances (PFAS)

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ABSTRACT: As concerns over exposure to per- and polyfluoroalkyl substances (PFAS) are continually increasing, novel methods to monitor their presence and modifications are greatly needed, as some have known toxic and bioaccumulative characteristics while most have unknown effects. This task however is not simple, as the Environmental Protection Agency (EPA) CompTox PFAS list contains more than 9000 substances as of September 2020 with additional substances added continually. Nontargeted analyses are therefore crucial to investigating the presence of this immense list of possible PFAS. Here, we utilized archived and field-sampled pine needles as widely available passive samplers and a novel nontargeted, multidimensional analytical method coupling liquid chromatography, ion mobility spectrometry, and mass spectrometry (LC-IMS-MS) to evaluate the temporal and spatial presence of numerous PFAS. Over 70 PFAS were detected in the pine needles from this study, including both traditionally monitored legacy perfluorooalkyl acids (PFAAs) and their emerging replacements such as chlorinated derivatives, ultrashort chain PFAAs, perfluoroalkyl ether acids including hexafluoropropylene oxide dimer acid (HFPO−DA, "GenX") and Nafion byproduct 2, and a cyclic perfluorooctanesulfonic acid (PFOS) analog. Results from this study provide critical insight related to PFAS transport, contamination, and reduction efforts over the past six decades.

KEYWORDS: Biomonitoring, contamination, per- and polyfluoroalkyl substances, fluoroethers, ion mobility, mass spectrometry, PFAS

INTRODUCTION

Per- and polyfluoroalkyl substances (PFAS) are a class of manmade chemicals comprised of highly fluorinated aliphatic substances with unique surfactant properties. PFAS are commonly used in nonstick cookware, stain-resistant materials, food packaging, paint, and aqueous film-forming foams (AFFF) as well as other household and industrial materials. Therefore, important PFAS point sources include industrial sites, firefighting training areas, and wastewater treatment plants. The production of long-chain or legacy PFAS such as perfluorooctanoic acid (PFOA) and perfluorooctanesulfonic acid (PFOS) began in the United States in the 1940s. By the early 2000s, these legacy compounds had become a global concern due to their environmental persistence, mobility, and links to adverse health outcomes including cancer, thyroid disease, immune system dysfunction, and birth defects, among many others. In response, most western manufacturers have phased out the production of legacy PFAS and shifted to short-chain and structurally modified emerging derivatives such as those with ether linkages and chlorine substitutions. While these replacements were thought to be a safe alternative to legacy PFAS, similar concerns about their environmental persistence and toxicity have been raised. Thus, long-term monitoring of the presence and global distribution of this growing class of chemicals is essential.

PFAS are routinely monitored in surface and groundwater, soil, and wildlife samples; however, widespread atmospheric monitoring is less feasible due to the expensive and complex equipment required for sampling. Investigations of airborne PFAS are important in understanding the exposure, fate, and transport of these pollutants. Furthermore, atmospheric PFAS pose an important route of human exposure and can reflect environmental changes more quickly than lagged aquatic ecosystems. Air emissions are also a major source of PFAS in the environment, thus there has been increasing interest in monitoring the air emissions and fate of a rapidly expanding list of PFAS. For example, air emissions were the primary source of PFOA contamination in drinking water in Parkersburg, West Virginia near a fluorochemical manufacturer.

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following deposition and groundwater transport. Atmospheric deposition of emerging PFAS such as GenX has also been shown to be an important contributor to soil and water contamination. Recently, precipitation monitoring and Community Multiscale Air Quality modeling approaches have been applied to assess the transport and deposition of GenX via air emissions from a North Carolina fluorochemical manufacturer. Indoor and outdoor air monitoring generally targets volatile PFAS such as fluorotelomer alcohols, perfluoroalkyl sulphonamides, and perfluoroalkyl sulfonamidoethanols, which are either directly assessed using field instruments or sampled for subsequent analysis via gas chromatography–mass spectrometry (GC-MS) or other suitable methods depending on the analytes of interest. Both active and passive air sampling techniques have been applied to atmospheric PFAS assessments, collecting primarily volatile PFAS; however some samplers collect both gas and particle phase PFAS. The two most common passive air samplers applied to atmospheric PFAS are polyurethane foam (PUF) and sorbent-impregnated (SIP) PUF samplers, although many others have been developed and utilized in the field. Most ambient air studies focus on the area immediately surrounding PFAS point sources, as atmospheric long-range transport results in diffusion and low surface concentrations.

Alternative naturally occurring passive sampling material with short lifespans, such as pine needles, are ideal for global, low-cost evaluations. Passive sampling using pine needles has been applied to a wide variety of organic pollutants, including a limited number of mostly legacy PFAS. Pine needles are particularly useful for monitoring pollutants over a large space and period of time, or in remote areas, where the cost and installation logistics required for using advanced monitoring equipment is not feasible. Pine needles have waxy, lipid-rich cuticles which facilitate the accumulation of atmospheric pollutants. Previous studies have demonstrated that the uptake of PFAS occurs primarily via adsorption of atmospheric PFAS in the gas and particle phases to the needles with some uptake of short chain, ionizable PFAS from the soil (Figure 1).

Volatile neutral PFAS can undergo long-range atmospheric transport and are subject to subsequent photochemical degradation or biotransformation to produce ionizable PFAS such as perfluoroalkyl acids (PFAAs). It has also been demonstrated that PFAAs can be directly released into the atmosphere and undergo atmospheric transport in the form of aerosol particles (Figure 1). Taken together, these PFAS and pine needle properties enable highly comprehensive passive sampling compared to traditional monitoring techniques.

In this manuscript, we explore the use of pine needles and nontargeted mass spectrometry-based measurements for insight into legacy and emerging PFAS presence and distribution over time. While PFAS manufacturing began in the 1940s, routine testing of environmental contamination did not begin until the turn of the 21st century when the toxicity and persistence of these chemicals became public knowledge. Thus, PFAS presence during this gap (1940s–1990s) has been retroactively evaluated using sediments and ice cores, but these methods are limited to aquatic and artic ecosystems and may disregard atmospheric contributions or lack regional specificity. Here, archived pine needles from herbaria dating back to the 1960s were evaluated in addition to those sampled in the field for over four years. Evaluations of 75 unique PFAS (Table S1) were performed using a nontargeted platform coupling liquid chromatography, ion mobility spectrometry, and mass spectrometry (LC-IMS-MS) separations following an optimized PFAS extraction technique which requires as few as 20 needles per sample. In the drift tube IMS (DTIMS) experiments, ions are pulled through a drift tube filled with a buffer gas (in our case nitrogen) by a weak electric field. Since the smaller ions have fewer collisions due to their more compact size, they also migrate faster and have a lower drift time. The ion’s drift time is therefore directly correlated to its gas-phase size or collision cross section (CCS). Commonly used LC-MS methods distinguish analytes based on their hydrophobicity (retention time) and mass-to-charge ratio (m/
z), thus interfacing IMS with traditional LC-MS techniques allows further separation based on the gas-phase size, shape, and charge of the ions. LC-IMS-MS measurements have also been shown to greatly enhance sensitivity in highly complex samples due to the increased peak capacity possible for the measurements. This is however the first study to leverage IMS and its ability to separate fluorocarbon- and hydrocarbon-based molecules for advanced PFAS separations in complex matrices. Furthermore, by evaluating the archived and field-sampled pine needles with the nontargeted LC-IMS-MS method, we were able to assess how PFAS have changed through time while also pinpointing the primary sources of contamination.

**METHODS**

**Standards and Reagents.** Mass labeled internal standards were obtained from Wellington Laboratories (Guelph, Canada). All experimental and quality control samples as well as extraction blanks were spiked with $^{13}$C$_4$-PFBA, $^{13}$C$_3$-PFPeA, $^{13}$C$_3$-PFHxS, $^{13}$C$_4$-FPFoA, $^{13}$C$_5$-PFNA, $^{13}$C$_6$-PFDA, $^{13}$C$_6$-PFUdA, $^{13}$C$_7$-PFDoA, $^{13}$C$_7$-PFDeDA, $^{13}$C$_8$-PFBS, $^{13}$C$_8$-PFHxS, and $^{13}$C$_8$-PFOS. Additionally, archived samples and samples from location 5 were spiked with $^{13}$C$_1$-GenX. For extractions and mobile phases, Optima LC-MS grade methanol, water, ammonium acetate, ammonium hydroxide, and glacial acetic acid were obtained from Fisher Scientific (Hampton, NH).

**Sample Collection and Treatment.** Needles were collected from North Carolina *Pinus taeda* and *Pinus palustris* trees on public land (Figure S1). At each field sampling site, needles were collected from a single tree and stored in polypropylene bags. At some sites, additional needles that had been shed the previous year were collected and stored in a separate polypropylene bag. The field samples are summarized in Table S2. The needle brachyblasts (branchlets holding needle clusters together) were separated and discarded, then the needles were dried at 37 °C for approximately 1–2 weeks to constant weight. Archived needles were obtained from either the Duke University Herbarium or the North Carolina State University (NCSC) Herbarium (Table S3). These specimens were stored at room temperature within a folded paper cover from their collection date. Approximately 20 needles were transferred from each specimen to polypropylene bags following removal of the brachyblasts and stored until extraction. The first three samples from 1938, 1947, and 1957 were discarded due to contamination from soaking the pre-1960s plant materials in various pesticides and fungicides.

Prior to extraction, the randomized and blinded needles were homogenized using a stainless-steel electric grinder. For field and archived samples, 0.5–2 g (dry weight) of sample homogenate was weighed into a 50 mL polypropylene tube. Aliquots of 15 mL of methanol and 4 ng of internal standards were mixed into the needle homogenates by vortexing for 15 s then sonicating for 30 min. Excess solid was allowed to separate for 15 min, then the supernatant was transferred to a fresh 50 mL polypropylene tube. The extraction steps were repeated with 10 mL of methanol, then the combined supernatants were vortexed for 5 s and filtered through a 0.45 μm Whatman glass microfiber syringe filter into a fresh 50 mL polypropylene tube. The extract was diluted with 25 mL of water, inverted five times, and vortexed for 15 s.

Solid-phase extraction cleanup was performed using an Oasis WAX cartridge (Waters; Bedford, CA), which was conditioned with 4 mL of 0.1% ammonium hydroxide in methanol, 4 mL of methanol, and 4 mL of water. Extracts were passed through the cartridge at one drip per second. The cartridge was dried under a vacuum for one min then washed with 4 mL of acetate buffer (25 mM, pH 4) and 4 mL of methanol. Elution was performed using 4 mL of 0.1% ammonium hydroxide in methanol. The resulting eluents were collected in polypropylene tubes and dried under a vacuum. Finally, extracts were reconstituted with 200 μL of 2 mM ammonium acetate in 40:60 methanol/water and stored at −20 °C until analysis.

**LC-IMS-MS Instrumental Analysis.** Sample analyses were carried out on an Agilent 6560 q-TOF coupled with an Agilent 1290 Infinity LC system (Agilent Technologies; Santa Clara, CA) using a previously described method. Chromatographic separation of samples (2 μL injections) was performed using a C18 Agilent ZORBAX Eclipse Plus column (2.1 × 50 mm, 1.8 μm) with the gradient summarized in Table S4 with a flow rate of 0.4 mL/min. Mobile phase A was comprised of 5 mM ammonium acetate in water, and mobile phase B was comprised of 5 mM ammonium acetate in 95:5 methanol/water. An Agilent Jet Stream ESI source (Agilent Technologies; Santa Clara, CA) was operated in negative ionization mode with the source conditions summarized in Table S5. IMS-MS settings are summarized in Table S6. Agilent ESI tune mix solution (Agilent Technologies; Santa Clara, CA) was directly injected to calibrate the instrument and calculate collision cross-section (CCS) values for the PFAS analytes using a previously described and validated single-field calibration method. Briefly, tune mix ions with known CCS values served as calibrants for relating measured analyte drift times to CCS values.

**Quality Assurance and Data Analysis.** Pooled quality control (QC) samples comprised of equal amounts of each pine sample homogenate and extraction blanks were prepared and analyzed alongside experimental samples to ensure extraction quality. Additionally, Agilent ESI tune mix solution was utilized as an instrumental blank to monitor LC-IMS-MS instrument performance and confirm the absence of carryover every 10–15 injections. Each data file was demultiplexed using a PNPN PreProcessor (v2020.03.23) with a signal intensity threshold of 20 counts. The data files were single-field calibrated using Agilent ESI tune mix data collected on the same day as the sample using Agilent IM-MS Browser 10.0 software to relate measured drift times to associated CCS values. Data were processed using Skyline-daily (v21.0.9.118) with an in-house library containing PFAS class, name, molecular formula, adduct, m/z, retention time, and CCS values for >100 individual PFAS species ascertained from chemical standards. The PFAS compounds detected in at least one pine needle sample are listed in Table S1. Drift time filtering was used with a resolving power of 40 to eliminate noise due to the complex matrix. Targets that were not present in any samples were removed from the Skyline document. Annotations were based on mass accuracy, retention time alignment, CCS matching via drift time filtering, and coelution and drift time alignment with labeled internal standards (if applicable). Additionally, tentatively identified and unknown targets were added to the Skyline document based on m/z (mass defect) and class-specific CCS versus m/z trends. Extracted ion intensities were exported to Excel for further analyses. Peak areas of analytes with labeled internal standards were normalized to the respective internal standard, and those without matching internal standards were normalized to

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surrogate standards, which were assigned based on structural similarity and retention time proximity (Table S7). Normalized peak areas of analytes detected in extraction blanks were subtracted from the sample normalized peak areas to assess only the signal extracted from the needles themselves. For the archived samples, three blanks were averaged and only signals outside of three standard deviations from the blank mean were considered. Concentration estimates were made for analytes with matched labeled internal standards based on the light/heavy abundance ratio, the amount of internal standard, and

Figure 2. Historic North Carolina pine PFAS profiles. (A) Chemical structures of example legacy and emerging PFAS, perfluorooctanoic acid (PFOA), and perfluoromethoxypropionic acid (PFMPA). (B) Legend for the historic PFAS ToxPis in C. Each slice represents an individual PFAS compound colored by class, and the numbers indicate the numbers of carbon present in the PFAS. PFSAs, PFCAs, and FTSs with two hydrocarbons (n:2) are ordered clockwise by increasing chain length except for branched isomers (br-n). The height of the slice corresponds to the scaled abundance with 0 as not detected and 1 as the maximum signal detected across all samples. (C) Timeline visualizing the temporal and spatial PFAS trends across multiple North Carolina counties from 1961 to 2005. Each county is represented by a different row as shown on the right side of the graph. Historical information is also added on the timeline with stars to highlight some of the PFAS trends.
the amount of material used for extraction. Analytes without matching internal standards were related to the amount of material used for extraction (normalized peak area/g), but no estimated concentration is reported due to unknown differences in extraction and ionization efficiency without matched internal standards. These relative and semiquantitative abundance values were imported into the ToxPi GUI for visualization and further analyses. The interactive map, Spatial and Temporal ToxPi Profiles of Per- and Polyfluoroalkyl Substances (PFAS) in North Carolina Pine Needles, was created using the ToxPi*GIS Toolkit, which generates ToxPi feature layers that can be used from with ArcGIS. Note that the segments “others” and “ToxPi score” included in the interactive map do not correspond to any data and are default list items in the ToxPi*GIS legend. A walkthrough of the steps used to make this map are in the toolkit documentation (www.toxpi.org). An unpaired t test was used to determine the statistical significance of the number of PFAS per sample in the temporal study of archived needles.

### RESULTS AND DISCUSSION

#### Historical PFAS Evaluation Using Archived Needles.

Understanding PFAS presence during the gap between their initial production and environmental monitoring (1940s–1990s) has previously been challenging. While sediments and ice cores have been utilized, these methods are limited as they may disregard atmospheric contributions, regional specificity, and ecosystems beyond aquatic or arctic regions. Additionally, to our knowledge, these previous retroactive studies have not evaluated the shift of legacy PFAS to emerging derivatives such as perfluoroalkyl ether acids (PFEAs, Figure 2A). The nontargeted LC-IMS-MS evaluation of archived pine needles from 1961 to 2005 in this study led to the identification of 29 unique PFAS over five decades in six North Carolina (NC) counties (Tables S3, S8, S9). The resulting ToxPi profiles, which show the scaled abundance of each individual PFAS (Figure 2B), are displayed in Figure 2C for each site. While both time and location played roles in the PFAS presence, likely due to proximity to a fluorochrome manufacturer on the Cumberland−Robeson County border and other point sources such as military sites, some trends overcame regional differences. One such trend is the number of chemicals detected at each time point which is significantly different (p < 0.05) prior to and following 1985, with averages of 8 and 14 detected PFAS. Figure 2C demonstrates this progression with an increasing number of PFAS, represented as ToxPi slices, in each area with multiple time points including Durham, Cumberland, Onslow, and Brunswick counties. This
finding highlights both the increasing number of new contaminants and persistence of legacy PFAS, many of which have been phased out of use for over a decade but are still detected globally and on a regular basis. One sample which did not fit this trend was collected in Wayne County in 1967 where 11 PFAS were detected. However, this location was near an Air Force base in Wayne County and since aqueous film-forming foam (AFFF) containing concentrated PFAS mixtures was adopted by the military in the 1960s for fire training and emergencies, we expect this contributed to the high abundance of PFAS observed in this area. To further understand the regional trends, individual PFAS subclasses were also examined. The observed subclass trends appeared to be mainly due to changes in the PFAS synthesis approaches and replacement of legacy PFAS with emerging species. Starting in the 1940s, PFAS were dominantly produced via electrochemical fluorination (ECF), which yields nonspecific mixtures of even- or odd-chained and linear or branched compounds. An alternative approach that yields mostly linear species, known as fluorotelomerization, was developed in the 1970s. Fluorotelomerization has been the dominant approach since the 2000s as it is mainly used for the synthesis of short-chain emerging replacements. Overall, in Figure 2C the abundant perfluoroalkyl sulfonic acids (PFSA, pink) and perfluoroalkyl carboxylic acids (PFCA, red) shift from longer, branched chains to shorter, linear chains over time (Tables S8, S9). PFAS belonging to additional subclasses primarily emerged following 1980, including fluorotelomer sulfonates (FTSs, orange), perfluoroalkyl ether carboxylic acids (PFECAs, yellow), a perfluoroalkyl ether sulfonic acid (PFESA, green), a perfluoroalkyl sulfonamide (PFASA, aqua), and a fluorotelomer alcohol phosphate ester (blue; Figure 2C). These findings agree with the known history of each PFAS subclass. For example, fluorotelomers, or polyfluoroalkyl substances containing aliphatic chains which are not fully fluorinated, have only been produced since the 1970s, and the peak FTS levels were detected in the 2001 and 2005 needles, shortly after FTS became the principal component of AFFFs in the 2000s. Furthermore, the fluorochemical manufacturer in NC began production in 1971 but is not reported to have discharged fluorooether (PFPECA and PFESA) byproducts into the environment until 1980. The detected fluorooether levels increased beyond 1980 with their highest relative abundances in 1995 and 2001. Likewise, both samples with the highest fluoroether levels were sampled from the neighboring counties of the manufacturing plant. Temporal trends from individual compounds rather than total subclasses were more difficult to discern, likely resulting from regional differences such as population density and distance to point sources among others. Taken together, this historical study enabled snapshots of PFAS exposure through time and illuminates an increase in PFAS complexity via rising numbers of subclasses and individual species within each subclass over time. While the results of this study align well with current knowledge of PFAS production and use over time as well as previously published temporal PFAS data, this approach was limited due to the sample storage conditions. The pine needle specimens were stored in herbaria primarily for taxonomy purposes rather than molecular evaluations, therefore degradation, diffusion, and transformation of PFAS may have occurred over time.

Multidimensional Analysis Capabilities. To gain a better understanding of PFAS spatial and temporal distribution within the past 5 years, field samples were collected from 20 locations across NC from 2017 to 2020 (Figure S1, Table S2). The nontargeted multidimensional measurements greatly aided in the analysis of these samples as 75 PFAS were detected, 61 of which were annotated using a chemical standard spectral library with LC, IMS, and MS information (Tables S1, S10, S11). These annotations are considered level 1 identifications based on the Metabolomics Standards Initiative criteria of two orthogonal techniques confidently defining 2D structure by utilizing a reference standard match. Using this approach, a much more extensive and structurally diverse set of PFAS were identified compared to previous pine needle studies (Figure 3A), such as PFSAs which range from 1 to 10 carbon chains and cover linear, branched, and cyclic chemicals, or PFESA which have varying carbon chain lengths and numbers of oxygen atoms as well as two chlorinated derivatives (Table S1). Of the 61 PFAS confidently identified in the pine needles from 2017 to 2020, seven subclasses including PFSA, PFCA, FTS, PFECAs, PFESA, PFASAs, and perfluoroalkyl phosphinates/phosphate esters (PFPPs) were observed (Figure 3B). An interactive map displaying the ToxPi profiles with all 61 identified PFAS from each field sampling location at various time points is available at https://go.ncsu.edu/pfas-pine-toxpi-arcgis.

Moreover, IMS aided in the classification and identification of unknown PFAS features (Figure S2). Spectral features with negative mass defects are considered PFAS candidates in traditional nontargeted LC-MS studies, whereas features with a combination of negative mass defect and characteristically low CCS can be confidently classified as PFAS when LC-IMS-MS is utilized. The subclass-specific CCS versus m/z trendlines (Figure S2A), which have been extensively characterized previously, are also informative when attempting to classify or identify unknowns. For example, two unknown features detected here with similar m/z values of 488.958 and 485.964 have vastly different observed CCS values of 157.3 and 172.8 A², respectively. These features therefore fall among different regions of the CCS versus m/z conformational space, indicating that Unknown 489 is likely a PFAA whereas Unknown 486 may be a PFASA or polyfluorinated species, which would have larger CCS values for a given m/z than PFAs. Several PFAS detected in this study were initially annotated as unknown fluorinated species or given tentative identifications but were ultimately confidently identified following authentic standard confirmation. One such example is the ultrashort chain PFAA perfluoropropanesulfonic acid (PFPrS). PFPrS was not present in the initial library used for data annotation, yet this feature was able to be identified as it had an excellent fit with the existing PFSA homologous series CCS versus m/z trendline (Figure S2B, R² = 0.999). This identification and several others made in a similar fashion were verified with commercially available standards. Unfortunately, due to a lack of standards for many possible PFAS, a standard for perfluoro-2-methoxyacetic acid (PFMOAA) was not available at the time of analysis, and thus PFMOAA remains tentatively identified as a level 3 identification. While the method utilized here is readily applicable to the discovery of novel PFAS, the remaining 14 unknown features were also unable to be identified. Further studies are therefore required to identify these unknowns, which will be extremely valuable as some have unique regional trends or were detected near known point sources (Table S11).
While filtration and solid-phase extraction (SPE) cleanup steps were performed prior to analysis, the biological matrix of the pine samples remained quite complex, having many metabolites, lipids, and other small biomolecules, which lead to interferences and increased noise in traditional LC-MS analyses. These interferences may impact the extracted ion abundances and subsequent quantitation and limit the number of low-abundance molecules that can be identified using traditional methods. When IMS-MS spectra are extracted from the striped peak at approximately 10.5 min in Figure 3C, as displayed in Figure 3D, PFAS have much lower drift times than hydrocarbon-based biomolecules of similar mass-to-charge (m/z), such as lipids, due to the larger mass of fluorine in comparison to hydrogen but similar size. Using Skyline, an open-source software program able to match the observed multidimensional features to the LC, IMS, and MS library, any signal outside of a set range of IMS drift times is filtered out of the extracted ion abundance, allowing improved relative quantitation of the PFAS.\(^{32}\) The IMS dimension can also be leveraged to tease apart isomers that coelute in the LC dimension, such as linear PFOS (L-PFOS) and its branched isomers such as P1MHpS, which have previously been separated using derivatization or advanced sample extraction and isomer isolation procedures (Figure 3E).\(^{43,44}\) These constitutional isomers would both be integrated as L-PFOS in traditional LC-MS analyses, but their near-baseline separation in the IMS drift time dimension due to the smaller size of the branched isomer allows them to be treated as two separate entities here.\(^{29}\) This was an important distinction as P1MHpS was only detected directly outside of the NC National Guard facility, confirming previous findings that PFAS branched isomer profile fingerprinting can be utilized to pinpoint unique contamination sources.\(^{44-47}\) Additional PFOS isomers which are separated by LC are labeled in Figure 3C as methylated or singly branched (m-PFOS) and demethylated or doubly branched (dm-PFOS). However, these peaks were unable to be further deconvoluted; thus both the m-PFOS and dm-PFOS signal were summed for subsequent analysis. Figure 3E also demonstrates that the native ion, L-PFOS in this case, and the corresponding mass-labeled internal standard (\(^{13}\)C\(_8\)-PFOS) align in both the LC and IMS dimensions, allowing straightforward annotation and quantitation of PFAS with matching standards. In this study, a relative quantitation approach was taken to compare spatial and temporal changes in PFAS abundance. Individual PFAS abundances were normalized to matching or surrogate internal standards (Table S7) and the amount of sample material used for the extraction procedure. Those with matched internal standards were given estimated concentrations based on the ratio of the native to internal standard signals, a common approach in IMS-based omics measurements known as simultaneous discovery and targeted monitoring (DTM).\(^{48}\) The calculated values were then used to benchmark the observed abundances in comparison to previous studies, which reported similar PFAS amounts at high pg/g to low ng/g dry weight (Tables S8 and S10).\(^{22,24,25}\) Abundances for PFAS without matched internal standards are reported as normalized peak area/g dry weight rather than estimated concentrations, as extraction and ionization efficiency differences between the native molecule and surrogate internal standard were unknown (Tables S9 and S11). Importantly, while the current study focused on discovery and relative changes, this nontargeted multidimen-

**Figure 4.** Fluoroether distribution and spread from a fluorochemical manufacturer. Map of southeastern North Carolina with averaged PFECA and PFESA ToxPi profiles of pine needles collected from 14 locations in 2017–2018 (red outer circles) and 2019–2020 (blue outer circles). The factory icon signifies the location of the fluorochemical manufacturer, and the airplane icon points to the Fayetteville airport. 1 and 2 denote the sampling sites located approximately 1 and 2 miles from the fluorochemical manufacturing plant. The slices correspond to the labeled pie chart legend in both color and position.
ional method can be utilized for absolute quantification of regularly monitored PFAS using traditional techniques such as external calibration or isotope dilution.

Spatial and Temporal Monitoring of Point Sources Using Field Samples. To further evaluate important chemical changes occurring in the pine needle field samples collected over the past five years, fluoroether distributions were investigated. These chemicals are of particular interest due to the presence of a fluorochemical manufacturer in NC which is known to discharge fluoroether byproducts into the environment, specifically the Cape Fear River, a source of drinking water for 200,000 southeastern NC residents. Moreover, fluoroethers have been detected worldwide and linked to similar health outcomes to legacy PFAS.5,4,5-3,6 The amount and distribution of PFECA and PFESA chemicals in southeastern NC in 2017−2018 were compared to those in 2019−2020 and are displayed in Figure 4 as a ToxPi®ArcGIS map. Of the 13 detected fluoroethers, only perfluoro-3-methoxypropanoic acid (PFMPA), NVHOS, and Nafion byproduct 2 (NB2) were detected outside of Cumberland and Robeson counties, or further than 11 miles from the manufacturing plant. The highest abundance of each compound was detected at the two sampling sites within 2 miles of the manufacturer except two chlorofluoroether sulfonic acids (CI-PFESAs), F53-B major (9CI-PF3ONS) and minor (11CI-PF3OudS), which were only detected at a nearby regional airport in 2020. The fluorochemical manufacturer has not reported production of the chlorofluoroethers, therefore these are likely a component of industrial materials at the airport or another unknown point source in the area. Furthermore, in December 2019, the NC fluorochemical manufacturing plant was required to install a thermal oxidizer to control PFAS emissions from the facility. This explains the drop in fluoroether abundance from 2017−2018 to 2019−2020, particularly at the sampling site approximately 2 miles from the manufacturing plant (Figure 4). Additionally, PFMOAA was no longer detected in 2019−2020, and the remaining fluoroethers, GenX, perfluoro-2-methoxypropanoic acid (PFMPA), R-EVE, Nafion byproducts 4 and 6 (NB4, NB6), and NVHOS, decreased in abundance by 76−99%. While several fluoroethers at the sampling site approximately 1 mile outside of the manufacturing plant saw decreases in abundance over time, including GenX, PFMOAA, perfluoro-2-ethoxypropanoic acid (PFPA), NB2, and NB6, the abundance of PMPA increased, and Nafion byproduct 1 (NB1) was only detected at this location in 2019−2020. Taken together, these results show a decreasing trend in overall fluoroether abundance and spread from the manufacturing plant, except for a few species which may be recent replacements for the fluoroethers under considerable scrutiny and local regulations such as GenX.5 Importantly, these results demonstrate the capability of this approach for monitoring the introduction of new chemicals and tracking the success of PFAS reduction and remediation efforts, particularly due to the evident spatial and temporal resolution of this method.

Beyond the fluorochemical manufacturing plant, other identified PFAS point sources in this study included airports and a military site. One such example is the NC National Guard within the Raleigh–Durham International Airport (RDU) International Airport area. In our study, the four sampling sites around RDU had unique ToxPi profiles, showcasing the spatial resolution and specificity of this approach, as three of the locations are barely 1 km (0.63 mi) apart. Of these four locations, the site located directly outside of the NC National Guard and RDU maintenance facility had extremely elevated levels of some PFASAs, PFCAs, and PFASAs (Figure 5). Several important observations to note were that the amount of the linear C4 and C6 PFCAs; branched C5, C6, and C8 PFCAs; linear C4, C6, and C8 PFASAs; and branched C6 and C8 PFASAs at the National Guard sampling site ranged from approximately 100 to 90,000 times higher than the average values for the entire 2019−2020 sample set. Additionally, linear C5 PFCA; P1MHpS; branched C4, C5, and C7 PFASAs; perfluoro-1-hexanesulfonamide (FHxSA); and two unknown PFAS were only detected at this location. As previously stated, the highly unique isomer profiles at this site as well as other point sources (the fluorochemical manufacturer and a second airport) demonstrate the contamination fingerprinting capabilities of PFAS branched isomers (Figures S3 and S4).46−47 These unique species, isomer profiles, and elevated levels are likely due to firefighting training at the National Guard base with AFFF, which are commonly formulated with these classes of compounds and their precursors.7,58,51 Further studies of the mode of PFAS transport from the AFFF source to the pine needles are needed.

The other three RDU International Airport sampling sites exhibited unique PFAS profiles as well, with elevated levels of long-chain PFCAs that were either not detected or detected at much lower abundance at the site located nearest the National Guard facility. Moreover, the emerging cyclic PFOS analog perfluoroethylohexanesulfonate (PFECHS) was only de-
ected at the four locations near the RDU airport (Figure 4). PFCHS was excluded from the previous C8-based compound restrictions due to its lack of alternatives and critical function as an erosion inhibitor in aircraft hydraulic fluids, and it continues to be detected worldwide.52,53 Another emerging C8-based compound which was detected only at RDU and Fayetteville Regional (FAY) Airports in 2020 is the chlorinated derivative 8Cl-PFOS. Aside from this similarity, the FAY Fayetteville Regional (FAY) Airports in 2020 is the chlorinated C8-based compound which was detected only at RDU and as an erosion inhibitor in aircraft hydraulic fluid.

PFECHS was excluded from the previous C8-based compound list.

By using both archived and field sampled pine needles in conjunction with nontargeted multidimensional analyses, we were able to monitor the spatial and temporal distribution of diverse PFAS. Our results illuminate historic PFAS profiles, identify point sources, track the introduction of new chemicals, and evaluate the success of contaminant reduction efforts. Further analysis of the unknown PFAS detected here as well as recent cationic and zwitterionic PFAS of interest, requiring alternative sample extraction, ionization, and fragmentation procedures, would be insightful. This method was limited to the detection of ionizable PFAS, such as perfluoroalkyl acids, thus utilizing alternative methods to analyze neutral volatile precursors such as perfluorotelomer alcohols (FTOHs) would aid in understanding the gas-phase transport and subsequent transformation of PFAS. While the scope of this study was limited to North Carolina, this approach can be applied globally wherever pine trees exist to gain insight into both historic and modern PFAS profiles worldwide.

ASSOCIATED CONTENT

Supporting Information
The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.est.1c06483.

Pine field sample set (Table S2); pine field sampling locations (Figure S1); archived pine sample set (Table S3); LC gradient settings (Table S4); ionization source settings (Table S5); IMS-MS settings (Table S6); internal standards used for signal normalization or estimating semiquantitative levels (Table S7); IMS-MS CCS versus m/z trendlines (Figure S2); PFCA isomeric composition (Figure S3); PFSA isomeric composition (Figure S4); FAY Regional Airport trends (Figure S5) (PDF)

LC-IMS-MS parameters for detected PFAS (Table S1); archived pine concentration estimations (Table S8); archived pine relative abundances (Table S9); field sample concentration estimations (Table S10); field sample relative abundances (Table S11) (XLSX)

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Notes
The authors declare no competing financial interest.

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