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The Contribution of Coniferous Canopy to the Molecular Diversity of Dissolved Organic Matter in Rainfall

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Abstract: Rainwater interacts with tree canopies in forest ecosystems, which greatly influence its quality. However, little information is available regarding how tree canopies influence dissolved organic matter (DOM) in rainwater. To examine this, we collected bulk deposition (rainfall) and throughfall in a conifer (*Chamaecyparis obtusa*) plantation, western Japan, during a rain event, and analyzed their DOM molecular compositions using ultrahigh-resolution Fourier transform ion cyclotron resonance mass spectrometry. The dissolved organic carbon flux and the number of DOM molecular species detected were approximately seven times and three times higher in throughfall than in rainfall, respectively. We found that the average proportion of molecular species shared between five sample replicates was larger in throughfall (69%) than in rainfall (50%). Nonmetric multidimensional scaling revealed that the molecular species were significantly differentiated between throughfall and rainfall, and the dissimilarity among the replicates was much smaller in throughfall. This indicates that the quality of DOM in rainwater became spatially homogeneous due to contact with tree canopies. The number of lignin-like molecules was larger than those of any other biomolecular compounds in throughfall and seven times larger than in rainfall, suggesting that many of plant-derived DOM molecules were dissolved into rainwater.

Keywords: biomolecules; biogeochemical cycle; molecular diversity; temperate forest; water conservation function

1. Introduction

Forests cover approximately 67% of land in Japan, 40% of which are conifer plantations [1]. The role of wood production of these forests has declined due to the long-term depression in forestry, in the context of the aging population and of depopulation in regional areas [1,2]. Public demand for the forests has gradually changed and diversified, such as, growing expectations for water conservation and carbon sequestration. Forest policy also has changed from an emphasis on wood production to fulfillment of the multi-functional roles of forests [1]. Particularly, forests play central roles in water availability and in regulating surface and groundwater flows while maintaining water chemistry, via interactions between rainwater and forest elements, such as, trees, litter, and soils [3]. It is thus essential to develop measures for evaluating the water conservation function of forests.

Dissolved organic matter (DOM) plays a critical role in changes in water chemistry within forest ecosystems, because it strongly affects water color and acidity [4,5]. It also forms complexes with nutrients and metals, and thereby serves as a mobilizing agent for them to move from soils to watercourses [6–9]. Additionally, it is utilized by microorganisms as an energy source [10,11]. DOM therefore has a great influence on biogeochemical cycles in forest ecosystems, indicating that understanding the quality and functions of DOM is critical to an evaluation of the water conservation function of forests.

Rainfall is an input of DOM to forest ecosystems. It is, however, intercepted and stored by tree canopies, and subsequently evaporates from there or reaches soil surfaces as throughfall. When rainfall comes into contact with tree canopies in forest ecosystems, the quality of rainwater greatly changes, as several compounds are washed and leached from the canopies [12]. Therefore, evaluating the quality of DOM in rainwater is a first step towards understanding biogeochemical cycles and subsequently the utility of forests for water quality conservation. Tree canopies provide a large amount of DOM to rainwater [13,14]. Schrumpf et al. [14] showed that annual fluxes of dissolved organic carbon (DOC) in rainfall (59–144 kg ha^{-1}) increased to 142–219 kg ha^{-1} during the passage through forest covers in a montane rainforest. Additionally, several studies have shown that the DOC concentrations and fluxes are higher in coniferous forests than in broad-leaved forests [15–19]. It is thus predicted that the chemical composition of DOM in rainwater becomes more diverse after passing through tree canopies, particularly in coniferous stands. It is also predicted that the spatial homogeneity or heterogeneity of the chemical composition differs between rainfall and throughfall. This is because rainwater contains DOM originating from several sources, such as airborne particulate matters [20], whereas throughfall water can contain much of plant-derived DOM [21–23]. However, little information is available about how the diversity in the chemical composition of DOM changes in rainwater after it passes through tree canopies.

A new approach for the evaluation of DOM in natural water has gained increasing interest in recent years. Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR MS) is an ultrahigh-resolution mass spectrometry technique that allows for the detection of hundreds or thousands of DOM molecular species in river and lake water [24,25]. It also can be used to trace changes in molecular composition in rainwater moving through a forest [26]. Additionally, molecular species detected can be classified into seven biomolecular classes, such as, lipids, proteins, and lignin-like molecules, using a van Krevelen diagram [25,27]. In this study, to examine changes in the molecular composition of DOM in rainwater passing through tree canopies, we applied FT-ICR MS to rainfall and throughfall in a plantation forest of Japanese cypress (*Chamaecyparis obtusa*), which is one of the major plantation species in Japan.

2. Materials and Methods

2.1. Site Description

This study was conducted in a mountainous watershed (Ochozu Experimental Watershed: OEW; Figure S1), located approximately 15 km east of the city of Fukuoka in western Japan (33°38′ N, 130°32′ E). The watershed area is 9.5 ha. The mean annual precipitation and temperature in this area were 1797 mm and 16.2 °C, respectively. The predominant forest soil is classified as forest-brown (Cambisol) soil and the underlying bedrock consists of serpentinite and chlorite schist [28]. Approximately 46% of the OEW is covered by a Japanese cypress (*Chamaecyparis obtusa*) plantation that was planted in 1957 along a stream channel [29]. Ridges down to the middle slope are covered by a mixed forest of evergreen and deciduous species, such as *Quercus serrata*, *Myrica rubra*, *Rhus succedanea*, *Clethra barbinervis*, *Machilus thunbergii*, *Cinnamomum tenuifolium*, *Neolitsea sericea*, and *Quercus glauca*. In the plantation area, pruning and thinning were conducted in 1993 and 2012, respectively, and the stand density, mean diameter at breast height, and mean height were approximately 950 trees ha^{-1}, 21 cm, and 16 m [30], respectively.

2.2. Water Sampling

The five bulk deposition collectors were installed in an open area (7 m × 7 m plot) located in the ridges of the OEW. The five throughfall collectors were installed in a riparian area (20 m × 20 m plot) covered by a Japanese cypress plantation. In this study, bulk deposition collected in the open area was referred to as rainfall.

The collectors consisted of a brown glass bottle coupled to a plastic funnel with diameter of 300 mm. The inside of the plastic funnel was wrapped with aluminum foil, which had been pre-combusted at 450 °C for 3 h to prevent the water sample from being contaminated with DOM leaching from the funnel. The sampling was conducted in December 2015, with a focus on just one rain event that occurred over a 24 h period, though the quantity and quality of DOM can vary depending on rain event characteristics, such as rainfall amounts [31]. After the sampling, the volume of rainfall and throughfall were measured. The water samples were filtered through glass fiber filters with a nominal pore size of 0.7 µm (Whatman GF/F, GE Healthcare, Little Chalfont, UK), and stored in the dark under 4 °C till analysis.

2.3. Chemical and Data Analyses

Dissolved organic carbon (DOC) concentration was measured using the persulfate-UV oxidation method (Sievers 900 Laboratory TOC Analyzer, GE Healthcare, Chicago, IL, USA). DOC flux was calculated by multiplying the DOC concentration and the volume of water. DOM molecular species and their number were measured using electrospray ionization coupled to FT-ICR MS, after water samples were treated using the solid phase extraction method to remove inorganic salts and enrich DOM [32]. Water samples were first passed through the sorbent of styrene divinyl benzene polymer (Sep-Pak PS2 Plus Short Cartridge, Waters Inc., Milford, MA, USA), DOM was extracted from the sorbent with methanol, and then diluted with ultrapure water to yield a final sample composition of 50/50 (v/v) of water to methanol. The treated samples were injected into the FT-ICR mass spectrometer (solariX 9.4T, Bruker Daltonics Inc., Billerica, MA, USA) using a syringe pump and analyzed in the negative ion mode. All spectra (Figures S2 and S3) were externally and internally calibrated using a sodium iodine solution and fatty acids, respectively. Peak lists of the mass-to-charge ratio (m/z) were produced using a signal-to-noise ratio cut-off of 4. Isotope peaks were removed from the list. The m/z peaks derived from ultrapure water were also removed. An expected molecular formula was assigned for each m/z value with a mass accuracy of ≤1 ppm using the Molecular Formula Calculator (ver. 1.0, NHMFL, Tallahassee, FL, USA, 1998,). The target m/z values ranged from 180 to 500, based on Ide et al. [26] and Reemtsma [33], and the following conditions were adopted for formula assignment: C = 0–∞; H = 0–∞; O = 0–∞; N = 0–5; S = 0–3; P = 0–3; DBE ≥ 0 [34]. Subsequently, a van Krevelen diagram was used based on the elemental ratios of the expected molecular formulas, i.e., the oxygen-to-carbon (O/C) and hydrogen-to-carbon ratios (H/C), to identify which biomolecular class each molecular species belonged to. Each molecular species was divided into seven biomolecular classes, i.e., lipids, proteins, aminosugars/carbohydrates (As/Ch), unsaturated hydrocarbons (UH), lignin-like molecules (lignins), tannin-like molecules (tannins), and condensed aromatic structures (CAS), based on the same protocol as in Ide et al. [26].

The water amount sampled, DOC flux, and the number of molecular species detected were compared between rainfall and throughfall samples using the Mann-Whitney U-test. Jaccard similarity coefficients were calculated to examine how many molecular species were the same between rainfall and throughfall samples and between sample replicates, where coefficient = 1 indicates that two water samples share all of the molecular compounds, while coefficient = 0 indicates that there are no common molecular species. Then, the two-dimensional ordination of non-metric multidimensional scaling (NMDS) for different samples were obtained for all molecular species detectable by FT-ICR MS and for each biomolecular class by calculating the Jaccard's distance between samples. Permutational multivariate analysis of variance (PerMANOVA) was used to examine whether molecular species significantly differed between rainfall and throughfall samples. Statistical analyses in this study were conducted using R (version 3.4.0) [35]. The package 'vegan' was used to conduct PerMANOVA.

3. Results

The average rainfall amount among five sample replicates (9.8 ± 0.1 mm) was approximately 3 mm higher than the average throughfall amount (6.6 ± 1 mm) (U-test, $p < 0.05$; Figure 1). On the other hand, the average DOC flux among the replicates was approximately seven times higher in throughfall (0.71 ± 0.14 kg ha^{-1}) than in rainfall (0.11 ± 0.02 kg ha^{-1}) (U-test, $p < 0.05$). The coefficient of variance (CV) of the DOC flux among the replicates was 21% in rainfall and 20% in throughfall.

The average number of DOM molecular species detected was approximately three times higher in throughfall (2679 ± 187) than in rainfall (850 ± 56) (Figure 1; U-test, $p < 0.05$). The CV of the number of molecular species among sample replicates (n = 5) was 7% in both rainfall and throughfall, which was smaller compared to those in the DOC flux. The number of molecular species shared between sample replicates was also much larger in throughfall (1859 ± 85) than in rainfall (424 ± 18) (U-test, $p < 0.001$), and accounted for on average 69% of the total number of molecular species in throughfall and 50% in rainfall. The number of lipids was larger than those of any other biomolecular classes in rainfall, whereas the number of lignins was larger in throughfall, followed by those of lipids and proteins (Figure 2). The numbers of molecular species classified into biomolecular classes (biomolecular species), excluding CAS, were significantly larger in throughfall than in rainfall (U-test, $p < 0.05$ in all cases). In particular, the number of lignins was approximately seven times larger in throughfall than in rainfall.

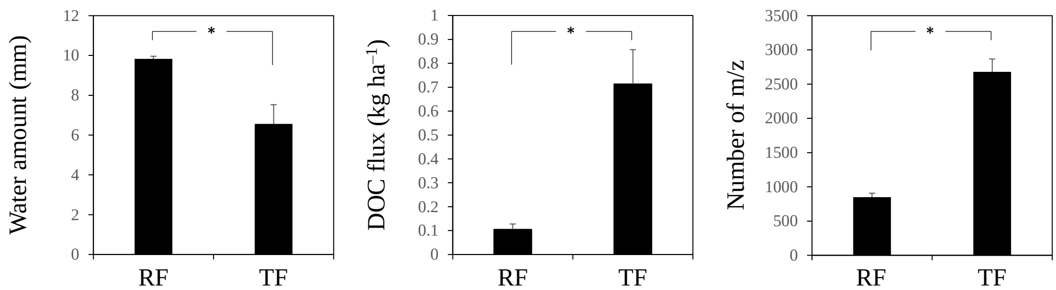

Figure 1. The amount of water sampled, dissolved organic carbon (DOC) flux, and the number of molecular species of dissolved organic matter (DOM) in bulk deposition (rainfall: RF) and throughfall (TF). Error bars represent standard deviations. An asterisk in the figure indicates that there is a significant difference between RF and TF (U-test, $p < 0.05$).

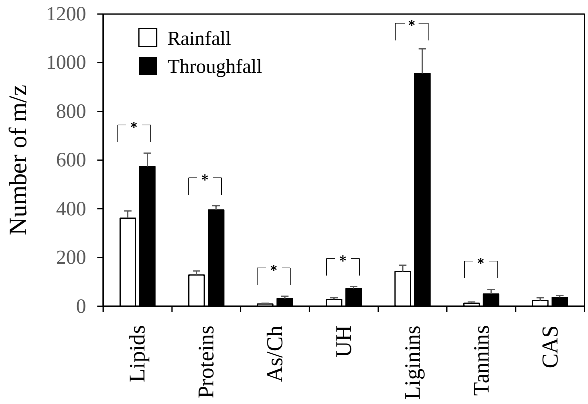

Figure 2. The number of dissolved organic matter (DOM) molecular species classified into seven biomolecular classes, i.e., lipids, proteins, aminosugars/carbohydrates (As/Ch), unsaturated hydrocarbons (UH), lignin-like molecules (lignins), tannin-like molecules (tannins), and condensed aromatic structures (CAS) in bulk deposition (rainfall) and throughfall. Error bars represent standard deviations. An asterisk in the figure indicates that there is a significant difference between rainfall and throughfall (U-test, $p < 0.05$).

The NMDS showed that DOM molecular species were significantly differentiated between rainfall and throughfall (Figure 3a; perMANOVA, $p < 0.01$). All seven biomolecular species were also significantly differentiated (Figure 3b–h; perMANOVA, $p < 0.05$ in all cases). The dissimilarity of the total molecular species among sample replicates on the two-dimensional ordination of NMDS was much smaller in throughfall than in rainfall (Figure 3a). The same trends were found for proteins and lignins (Figure 3c,f).

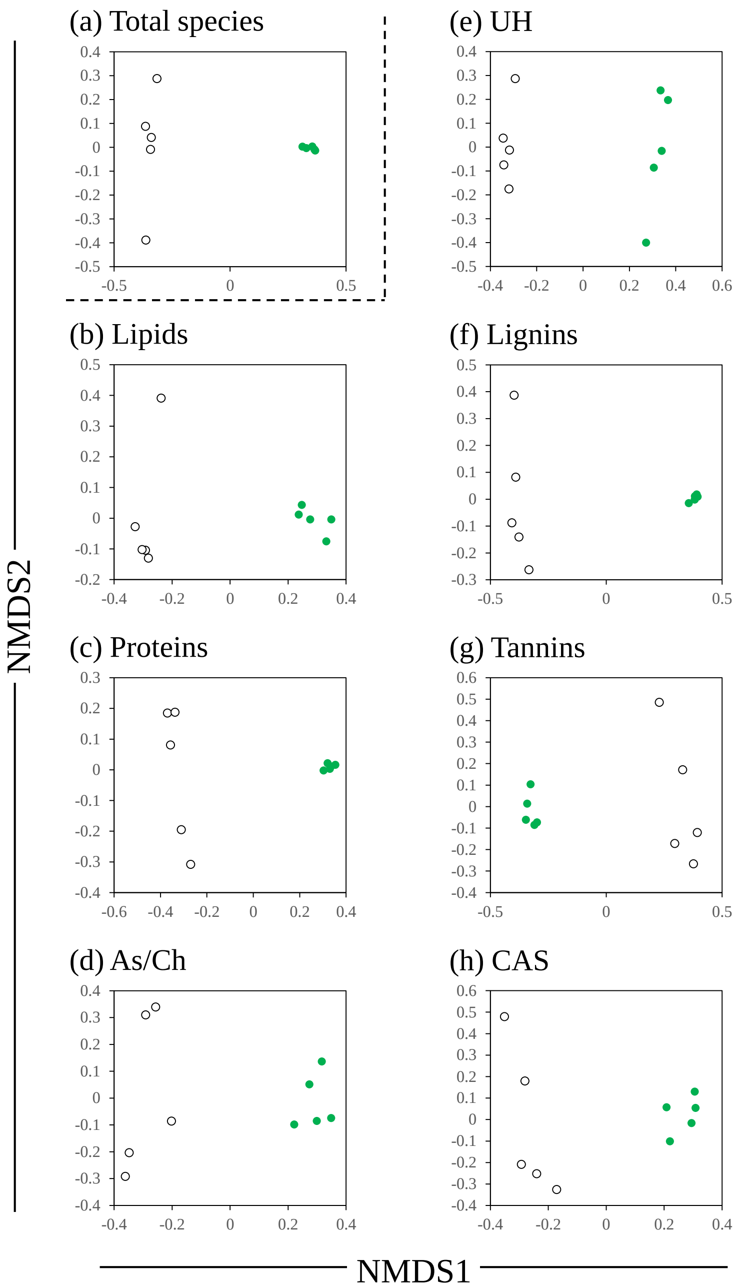

Figure 3. (**a**) Two-dimensional ordination of nonmetric multidimensional scaling (NMDS) of different sample replicates for all molecular compounds identified and (**b**–**h**) for the biomolecular classes in bulk deposition (rainfall) and throughfall. Open and closed circles represent rainfall and throughfall water samples, respectively. See Figure 2 for the abbreviations of biomolecular classes.

4. Discussion

The much higher DOC flux in throughfall than in rainfall (Figure 1) indicates that tree canopies of Japanese cypress added a large amount of DOM to the rainwater. The larger number of DOM molecular species in throughfall than in rainfall (Figure 1) also indicates that the tree canopies added a diverse array of DOM molecules to the rainwater. Because lignins are phenolic polymers that originate mainly from vascular plants [36], the high proportion of lignins in the total number of molecular species and the much larger number of lignins in throughfall than in rainfall (Figure 2) suggest that many of the plant-derived DOM molecules leached from the tree canopies into the rainwater. This is supported by previous studies conducted by Ide et al. [26] and Stubbins et al. [21]. Ide et al. [26] investigated DOM molecular species in rainfall, throughfall, soil water, groundwater, and stream water in a Finnish boreal forest catchment, and suggested that lignins regulate spatial variations in molecular diversity in boreal forest ecosystems. Stubbins et al. [21] analyzed optical properties and molecular signatures of DOM in throughfall and stemflow in subtropical forests in the USA, and showed that DOM molecules in the throughfall and stemflow were mainly composed of autochthonous plant-derived organic matters.

Our results (Figure 1) show that variability of both the DOC flux and the number of molecular species among the sample replicates was small, and did not differ notably between rainfall and throughfall. These indicate that spatial variations in these analytical items could be small at a stand scale. On the other hand, the results of NMDS revealed (Figure 3) that DOM molecular species were explicitly different between rainfall and throughfall, but were more similar among throughfall sample replicates, particularly for proteins and lignins. These findings suggest that spatially heterogeneous molecular species in rainwater are converted into spatially homogeneous ones during the process where rainwater passes through the tree canopy of the conifer plantation and thereby dissolves and leaches plant-derived molecular species. Proteins are relatively labile and biodegradable compounds [37]. Given that the DOC flux and the number of proteins were much higher in throughfall than in rainfall, the spatially homogenized quality of throughfall water supports the indication of Qualls and Haines [10] that tree leaves leach readily-mineralisable DOM to the rainwater, which in turn can stimulate microbial activities in the forest floor. In contrast, the large number of lignins common to throughfall samples might be retained in forest soils due to their refractory properties and thereby contribute to the molecular diversity of DOM in soil water [26].

The spatially homogenized quality of throughfall water is partly attributable to the fact that throughfall samples were collected within a plot of the same tree species. Stubbins et al. [21] showed that molecular species of throughfall DOM were explicitly separated between oak (*Quercus virginiana* Mill.) and cedar (*Juniperus virginiana* L.) stands, suggesting that each throughfall water contains molecularly distinct types of plant-derived DOM. It is therefore possible that molecular species in throughfall greatly vary depending on tree species.

Some studies have indicated that rainfall amounts increase the throughfall DOC flux [14,38]. This is because wash-off of dry deposits from the tree canopy and leaching from the plant tissues make a large contribution to DOM in throughfall as rainfall increases [14,31]. Therefore, the number of molecular species in throughfall can increase with rainfall amounts due to the increased addition of a diverse array of DOM molecules to the rainwater. Seasons also can affect the molecular composition of DOM in throughfall, because the decomposition rate of DOM molecules should increase with ambient temperature [39]. This also would be supported by the suggestion of O'Donnell et al. [40] that the magnitude of the transformation of biodegradable DOM was partly determined by temperature. Taken together, it is likely that molecular species and their numbers in throughfall change depending on rainfall amounts and seasons.

This study presented the new biogeochemical process wherein the tree canopy can add a diverse array of DOM molecules to rainwater in a Japanese cypress plantation, based on the data obtained during a rain event. To generalize this, further studies are needed on inter-event or seasonal variations in DOM molecular species leached from the tree canopy.

Supplementary Materials: The following are available online at http://www.mdpi.com/2073-4441/11/1/167/s1. The supplementary material describes the location and map of the study area (Figure S1), and mass spectra of m/z peaks of five replicates in rainfall and throughfall (Figures S2 and S3).

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