

Article

Exploratory Characterization of Dissolved Organic Matter Released from Composite Leaf Litter Samples Representing Five Deciduous Tree Species Under Controlled Laboratory Conditions

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Abstract

Leaf litter decomposition is a key pathway for carbon transfer from forest ecosystems to soils and surface waters. Dissolved organic matter (DOM) released during early-stage leaching represents a potentially reactive fraction of this carbon pool; however, its molecular composition and short-term reactivity remain insufficiently characterised. This study provides a comparative characterisation of DOM released from composite leaf litter samples representing five common deciduous tree species (*Betula pendula*, *Carpinus betulus*, *Alnus glutinosa*, *Populus tremula*, and *Quercus robur*) under controlled laboratory conditions. Leaf material collected from multiple trees per species was pooled to obtain a single composite sample; therefore, replicate leaching experiments represent procedural rather than biological replication. DOM was isolated using solid-phase extraction (SPE) and analysed by gas chromatography–mass spectrometry (GC–MS) following trimethylsilyl (TMS) derivatisation, while chemical oxygen demand (COD) and biochemical oxygen demand (BOD₅) were used as indicators of oxidative reactivity and short-term biodegradability. The applied analytical approach captures a selective and operationally defined fraction of DOM, primarily low-molecular-weight and derivatisable compounds; therefore, the results are interpreted as semi-quantitative compositional fingerprints. Carbohydrates, phenolic compounds, and low-molecular-weight organic acids dominated the detected fraction of DOM, with differences observed among composite samples. The composite samples representing *A. glutinosa* and *P. tremula* contained higher relative proportions of carbohydrate-related compounds, whereas the composite samples representing *B. pendula* and *C. betulus* showed higher relative contributions of aromatic compounds. Apparent differences in BOD₅ were observed among composite samples; however, these observations likely reflect procedural variability rather than independent biological effects. The results indicate variability in DOM composition and apparent reactivity among composite litter samples under controlled laboratory conditions. Due to the lack of biological replication and the selective nature of the analytical approach, the findings should be interpreted as exploratory and not as evidence of generalised tree-species effects.

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1. Introduction

Forests play a central role in the global carbon cycle as major producers of organic matter. A portion of this carbon is transferred from forest ecosystems to aquatic systems primarily via litterfall, followed by processing on the forest floor, leaching, and lateral hydrological transport. Direct transfer from the canopy to surface waters (e.g., via throughfall or stemflow) represents a minor and spatially limited pathway. During decomposition, this organic matter is mineralised, releasing CO₂ and CH₄, which may offset a substantial fraction of the annual terrestrial carbon sink at the ecosystem scale [1,2].

It is estimated that approximately 2.9 Pg of carbon is transferred annually from terrestrial to freshwater systems. Lakes, of which there are more than 300 million worldwide, act as major biogeochemical “reactors” that transform and recycle terrestrial organic matter [2–5]. However, global estimates of lake abundance vary considerably depending on factors such as minimum size thresholds, inclusion of ponds and small or ephemeral water bodies, and the mapping approach used.

Within the land–water continuum, forest ecosystems are important contributors to carbon transfer to inland waters. Tree species composition and litter inputs are commonly associated with variation in both the quantity and quality of organic matter exported from the forest floor. Leaf litter represents a major source of labile and structurally diverse organic compounds released during early decomposition, and its chemical composition is associated with species identity and litter chemistry [6–12]. During this phase, water-soluble compounds are rapidly released through leaching, forming dissolved organic matter (DOM).

In addition to abiotic leaching processes, microbial communities associated with leaf surfaces may contribute to the initial transformation of organic matter. Leaf litter carries epiphytic and endophytic microorganisms prior to abscission, which can remain active during early stages of decomposition. These microorganisms may influence DOM composition through enzymatic transformation of organic compounds and the release of microbial metabolites [6,7]. Consequently, DOM produced during early litter decomposition reflects not only plant-derived compounds but also microbially modified and microbially derived components.

Vascular plants constitute the dominant source of organic carbon entering freshwater systems, as leaf litter is rich in organic compounds and highly susceptible to leaching during senescence and early decomposition. Numerous studies have demonstrated that DOM released from senescing leaves and decomposing litter varies with tree species, litter chemistry, environmental conditions, and decomposition stage [9–12]. Many investigations have characterised litter-derived DOM using optical techniques, including absorbance- and fluorescence-based approaches, which provide valuable information on DOM source, aromaticity, and transformation processes [13–20]. These methods have substantially improved understanding of DOM dynamics, biodegradability, and environmental fate; however, they provide only indirect information on molecular composition and generally do not identify individual compounds.

Studies employing molecular-level analytical techniques have also examined litter-derived DOM and leaf leachates, revealing the presence of carbohydrates, phenolic compounds, organic acids, and other low-molecular-weight constituents [21,22]. Nevertheless, only a limited number of studies have combined detailed molecular characterisation of litter-derived DOM with functional indicators of short-term reactivity and

biodegradability, particularly across multiple temperate deciduous tree species under controlled early-stage leaching conditions. Addressing this aspect may improve understanding of compositional variability within litter-derived DOM and its potential relationship with bulk indicators of apparent reactivity during the initial stages of decomposition. This represents an important knowledge gap, as early-stage leaching may contribute to carbon cycling, microbial activity, and oxygen consumption in soils and adjacent aquatic systems.

Despite growing interest in DOM dynamics, relatively few studies have compared molecular-level information obtained from selective analytical fractions of litter-derived DOM with bulk indicators of DOM reactivity and biodegradability under controlled early-stage leaching conditions. Because GC–MS analysis following derivatisation characterises only a limited and operationally defined fraction of DOM, such comparisons should be regarded as exploratory and descriptive rather than as direct assessments of composition–function relationships.

The primary aim of the present study was therefore to provide a comparative characterisation of the SPE-extractable and derivatisable fraction of DOM released from freshly fallen leaves representing five common deciduous tree species occurring in Northern and Central Europe under controlled laboratory conditions. In parallel, bulk indicators of oxidative reactivity and apparent biodegradability were assessed using chemical oxygen demand (COD) and biochemical oxygen demand (BOD₅). Particular emphasis was placed on comparing compositional patterns of the GC–MS-detectable DOM fraction with these bulk reactivity metrics while recognising that the two approaches characterise different operational fractions of dissolved organic matter.

Based on current knowledge, we formulated the following exploratory hypotheses:

- (i) The molecular composition of DOM differs among composite litter samples representing different deciduous tree species;
- (ii) Compositional patterns observed in the GC–MS-detectable fraction of DOM may not necessarily correspond to patterns of apparent short-term biodegradability assessed using BOD₅;
- (iii) Differences in DOM composition and apparent reactivity among composite samples are more pronounced than differences in overall litter mass loss during early-stage leaching.

2. Materials and Methods

2.1. Study Site

Leaf samples were collected from natural forest stands located in the vicinity of Białystok, northeastern Poland (53.13° N, 23.16° E). The study area is situated within the temperate climatic zone, with a mean annual temperature of approximately 7–8 °C and mean annual precipitation of 550–600 mm. The forests are dominated by mixed deciduous stands typical of Central and Eastern Europe, growing on sandy and loamy soils developed on glacial deposits.

2.2. Sample Collection

The selected species are among the most widespread and ecologically important deciduous tree species in Poland and Central Europe. They represent contrasting ecological strategies and occupy different habitat types, including mesic deciduous forests (*Carpinus betulus*, *Quercus robur*), riparian and wetland environments (*Alnus glutinosa*), and pioneer or early-successional habitats (*Betula pendula*, *Populus tremula*). Previous studies have reported differences among tree species in litter chemistry, decomposition dynamics, and dissolved organic matter composition [8,10,23]. Their inclusion was intended to provide

a comparative assessment of DOM composition and oxygen-demand characteristics across a range of ecologically relevant litter types commonly occurring in temperate forest ecosystems.

The study focused on leaves of five deciduous tree species: *B. pendula*, *C. betulus*, *A. glutinosa*, *P. tremula*, and *Q. robur*. Yellowed, intact leaves were collected by gently shaking branches to obtain freshly senesced material representative of recent leaf litter input.

We acknowledge that this approach may preferentially detach leaves that are already loosely attached, potentially introducing bias related to senescence stage. However, this method was applied consistently across all species and allowed collection of freshly senesced leaves while minimising contamination from soil and surface litter. Nevertheless, species-specific differences in leaf abscission dynamics and physiological status at the time of detachment cannot be excluded and may have contributed to variability in DOM composition independently of species identity. This potential source of variability should be considered when interpreting compositional differences among the analysed samples.

Leaf material was collected from four individual trees per species ($n = 4$) located in natural forest stands within Las Zwierzyński and Puszcza Knyszyńska, near the Arboretum in Kopna Góra. Trees were selected as healthy individuals without visible signs of damage or disease.

Leaves from all trees within each species were pooled to obtain a single composite sample representing leaf litter from each species. This approach provided a composite representation of litter chemistry for each species but did not allow assessment of variability among individual trees within species. Consequently, experimental replicates represent technical rather than independent biological replicates, and the effective biological replication per species was $n = 1$.

Prior to leaching experiments, leaves were dried at 60 °C for 12 h to standardise moisture content. Leaves were not rinsed in order to preserve naturally occurring water-soluble compounds.

2.3. Experimental Design

For each species, three independent leaching experiments were conducted ($n = 3$). Because all experiments were performed on subsamples derived from a single composite biological sample, these replicates represent procedural (technical) rather than independent biological replicates.

2.4. Leaching Procedure

Leaf litter was leached under controlled laboratory conditions following a modified protocol based on [24]. The selected conditions were based on a standardised litter-leaching protocol commonly used for comparative assessment of organic matter release from plant litter. The experimental design was intended to provide controlled and comparable conditions among samples rather than to reproduce the full complexity of forest-floor hydrological processes. Approximately 6 g of dried leaf litter was placed in glass vessels containing 200 mL of distilled water. The vessels were covered with aluminium foil and incubated at 4 °C for 7 days. Samples were gently mixed every 2–3 days to maintain consistent contact between the leaf material and water. Distilled water was used to minimise variation associated with water chemistry and to facilitate comparison of DOM release among the analysed composite litter samples.

These conditions were selected to reduce microbial activity rather than to eliminate it. Microbial communities present on leaf surfaces may remain active at 4 °C and contribute to initial transformation of organic matter. Therefore, the obtained DOM likely reflects a combination of abiotic leaching and initial microbial processing.

After incubation, the leachates were pre-filtered through qualitative paper filters (Alchem, fast filtration grade; nominal pore size not specified, Alchem, Białystok, Poland) to remove coarse particulate material. Subsequently, a portion of each leachate was filtered through 0.45 µm membrane filters to obtain the operationally defined dissolved fraction of organic matter. This filtration step was applied to operationally separate dissolved and particulate-associated fractions. It should be noted that part of the high-molecular-weight or colloidal fraction of dissolved organic matter may be retained during filtration; therefore, the operationally defined dissolved fraction may not fully represent the entire DOM pool.

For GC–MS analysis, aliquots of the leachates were subjected to solid-phase extraction (SPE) on C18 cartridges prior to derivatisation and chromatographic analysis. The SPE procedure was applied as a sample preparation and concentration step prior to GC–MS analysis. Extraction efficiency was not determined because the objective of the study was qualitative characterisation of the SPE-extractable fraction of DOM rather than quantitative assessment of total dissolved organic matter recovery.

The residual leaf material was dried at 60 °C for 24 h and weighed. Based on three procedural replicates, the average mass loss of the leaf litter and its standard deviation were calculated.

Drying of leaf material at 60 °C prior to leaching may have affected the composition of dissolved organic matter by altering thermolabile compounds or partially removing highly soluble fractions. However, this procedure was applied consistently across all samples to ensure comparability of results and to standardise initial moisture content. Therefore, potential biases associated with drying are expected to be systematic rather than sample-specific and should not substantially affect comparative interpretation among the analysed samples. Nevertheless, the obtained results reflect the applied laboratory protocol and may not fully represent natural leaching processes immediately following litter-fall.

2.5. DOM Extraction

For chemical characterisation, DOM was extracted from leachates pre-filtered through qualitative paper filters (Alchem, fast filtration grade) to remove coarse particulate material. This step was applied to obtain a particle-reduced fraction suitable for SPE and GC–MS analysis.

It should be noted that the applied approach does not strictly correspond to the operational definition of dissolved organic matter (<0.45 µm), as colloidal or fine particulate-associated organic compounds may still be present in the extracts. Solid-phase extraction using C18 sorbent exhibits compound-class-dependent recovery, preferentially retaining non-polar and moderately polar compounds, while highly polar constituents are likely underrepresented due to limited retention on the C18 sorbent.

The extraction efficiency was not quantified; therefore, the obtained results represent the SPE-extractable fraction of DOM rather than the entire dissolved organic matter pool. However, all samples were processed using the same extraction protocol, allowing for internally consistent comparison of DOM composition among composite samples.

Dissolved organic matter was isolated using solid-phase extraction (SPE) for subsequent chemical analysis. SPE cartridges containing 500 mg octadecyl (C18) silica-based sorbent (J.T. Baker, Radnor, PA, USA) were used.

Cartridges were conditioned sequentially with acetone, methanol, and distilled water (all solvents: acetone and methanol, analytical grade, Chempure, Piekary Śląskie, Poland). Leachate aliquots (20 mL) were passed through the cartridges at a flow rate of 0.5–1.0 mL min^{−1}. After loading, air was drawn through the cartridges for 5 min to remove residual water.

Retained organic compounds were eluted with methanol (Sigma-Aldrich, Poznań, Poland) (30 mL at 1 mL min⁻¹). Anhydrous MgSO₄ (Chempure, Poland) was added to the eluates to remove traces of water.

Dried extracts were transferred to pre-weighed glass beakers and evaporated to dryness overnight in a fume hood. The residues were rinsed from the vessel walls with 1–2 mL of pyridine (Sigma-Aldrich, Poland) to ensure complete dissolution prior to derivatisation.

An aliquot (220 µL) of the extract was transferred into a 2 mL reaction vial, and 80 µL of BSTFA containing 1% TMCS (Sigma-Aldrich, Poland) was added. The mixture was heated at 60 °C for 1 h to form trimethylsilyl (TMS) derivatives prior to GC–MS analysis.

2.6. DOM Composition Determination

Gas chromatographic separation and identification of DOM components, analysed as TMS derivatives, were performed using an HP 8860 gas chromatograph coupled with a 5977B mass selective detector (Agilent Technologies, Santa Clara, CA, USA). The instrument was equipped with an HP-5 ms fused silica capillary column (30 m × 0.25 mm i.d., 0.25 µm film thickness). Helium (purity 99.999%) was used as the carrier gas at a constant flow rate of 1 mL min⁻¹.

Samples (1 µL) were injected using an HP 7673 autosampler. The injector temperature was set to 280 °C and operated in split mode (10:1). The oven temperature programme started at 50 °C and increased to 325 °C at a rate of 3 °C min⁻¹. Electron impact mass spectra were acquired at 70 eV, and detection was performed in full-scan mode over a mass range of 41–600 m/z.

The relative contribution of each compound to the total ion current (TIC) was calculated and expressed as a percentage of the total signal. These values should be considered semi-quantitative, as no internal standards were used and response factors may differ among compound classes. GC–MS analysis following TMS derivatisation is widely used for comparative characterisation of low-molecular-weight and derivatisable DOM fractions of dissolved organic matter in environmental samples [21,22]. However, this approach does not provide comprehensive coverage of the entire DOM pool, as highly polar, high-molecular-weight, and structurally complex constituents may remain underrepresented or unresolved [22,25]. Accordingly, the obtained chromatographic profiles should be interpreted as comparative molecular fingerprints of the SPE-extractable DOM fraction rather than absolute concentrations of individual compounds.

A mixture of C₁₀–C₄₀ n-alkanes (Sigma-Aldrich, Poland) in hexane was analysed under identical conditions, and retention times of alkane homologues were used to calculate linear temperature-programmed retention indices (RI_{calc}). Organic compounds were identified based on agreement between mass spectra and RI_{calc} values, using the NIST 20 library and a dedicated database of TMS derivatives [26]. Compound assignments lacking corresponding retention index confirmation or showing substantial deviations from published RI values were treated as tentative identifications.

The GC–MS analysis was intended for qualitative molecular characterisation of the SPE-extractable fraction of DOM. SPE recoveries were not independently determined. Therefore, the reported relative abundances (% TIC) should be interpreted as comparative molecular fingerprints rather than quantitative estimates of compound concentrations or total DOM composition.

2.7. Determination of DOM Reactivity

2.7.1. COD Determination

Chemical oxygen demand (COD) of raw and 0.45 µm filtered leaf litter leachates was measured photometrically using a Hach Lange spectrophotometer (DR 3800, Hach

Company, Loveland, CO, USA) and standard cuvette tests (LCK 314). Measurements were performed at a wavelength of 620 nm, according to the manufacturer's protocol. Both bulk (pre-filtered through paper filters) and membrane-filtered samples were analysed in order to distinguish between total and dissolved organic matter fractions.

The applied cuvette tests (LCK 314) have a measurement range of 15–150 mg O₂ L⁻¹. All analyses were conducted in accordance with the manufacturer's instructions, including the use of reagent blanks and standard quality control procedures. Each sample was analysed in triplicate. Samples exceeding the analytical range of the LCK 314 cuvette test were diluted appropriately with distilled water, and final COD values were corrected using the corresponding dilution factors. Samples were heated using a Hach Lange LT 200 thermostat.

For each composite sample, measurements were performed in three procedural replicates, and specific COD values (COD_s) were normalised to the initial dry mass of leaf litter according to:

$$\text{COD}_s (\text{mg O}_2 \text{ g}^{-1} \text{ d.m.}) = \text{COD} (\text{mg O}_2 \text{ L}^{-1}) \times V/m_0$$

where V is the extract volume (L) and m₀ is the initial dry mass (d.m) of leaves (g).

2.7.2. BOD Determination

Biochemical oxygen demand (BOD₅) was used as an integrative indicator of the biodegradability of DOM released from leaf litter during early-stage decomposition. Measurements were conducted using the manometric OxiTop® method [27,28].

Prior to BOD₅ determination, the leachates were filtered through qualitative paper filters (Alchem, fast filtration grade).

Filtered leaf litter leachates were diluted with distilled water as required by the analytical range of the OxiTop® method and transferred into airtight manometric bottles equipped with pressure-sensing OxiTop® heads. No additional nutrient supplementation or external microbial inoculum was applied. A nitrification inhibitor (allylthiourea, ATU) was added to suppress oxygen consumption associated with nitrification. Sodium hydroxide pellets were placed inside the measuring head to absorb CO₂ produced during microbial oxidation.

All measurements were performed in accordance with the manufacturer's instructions, including the use of blank controls and standard quality control procedures. Each sample was analysed in triplicate (procedural replicates).

The bottles were incubated in the dark at 20 °C for 5 days. Oxygen consumption during incubation was recorded as a decrease in internal pressure, corresponding to microbial oxidation of biodegradable fractions of organic matter. BOD₅ values were calculated using manufacturer calibration factors and expressed as mg O₂ L⁻¹.

It should be noted that the absence of nutrient supplementation and external inoculum may lead to underestimation of absolute BOD₅ values. Therefore, the results are interpreted in a comparative context among composite samples rather than as absolute measures of biodegradability.

2.8. Statistical Analysis

Statistical analyses were performed to describe variability among procedural replicates. Results are presented as mean ± standard deviation (SD).

Due to the lack of independent biological replication (*n* = 1 per composite sample), results are interpreted descriptively and no species-level inference is intended.

3. Results

3.1. Loss of Foliage Mass Due to Leaching

Mass loss of leaf litter after the 7-day leaching experiment was similar across all analysed composite samples, ranging from approximately 26% to 28% (Table 1). The highest values were observed for the composite samples representing black alder and silver birch, whereas the composite sample representing aspen showed slightly lower mass loss. Composite samples representing European hornbeam and English oak exhibited intermediate values.

Table 1. Mass loss of leaf litter during the 7-day leaching experiment.

Species	Initial Mass (g)	Final Mass (g)	Mass Loss (g)	Average Mass Loss (%)	SD (%)
<i>A. glutinosa</i>	6.013	4.310	1.704	27.5	1.00
<i>B. pendula</i>	6.007	4.367	1.640	27.4	0.80
<i>C. betulus</i>	6.055	4.465	1.590	26.4	1.06
<i>Q. robur</i>	6.051	4.476	1.575	26.0	0.66
<i>P. tremula</i>	6.038	4.492	1.545	25.8	0.14

Notes: Average mass loss = mean value from three procedural replicates; SD = standard deviation.

3.2. Chemical Composition of DOM

Analysis of the group and molecular composition of the SPE-extractable fraction of dissolved organic matter released from leaf litter revealed compositional differences among the analysed composite samples (Table 2). Detailed semi-quantitative molecular-level information is provided in Supplementary Table S1.

Table 2. Relative contribution of the main groups of compounds to the total ion current of the chromatograms of DOM washed out from fallen leaves.

Group of Compounds	RI ^{Calc}	RI ^{DB}	Composition (% of TIC)				
			<i>A. glutinosa</i>	<i>B. pendula</i>	<i>C. betulus</i>	<i>Q. robur</i>	<i>P. tremula</i>
Aliphatic acids, including:			8.64	2.45	5.15	6.69	1.24
- lactic acid, di-TMS	1071	1074	3.78	1.57	-	-	-
- oxalic acid, di-TMS	1137	1141	-	-	-	0.40	-
- succinic acid, di-TMS	1472	1476	0.73	-	1.42	-	-
- β-phenyllactic acid, di-TMS	1592	1595	-	-	0.13	-	-
- dodecanoic acid, TMS	1653	1660	0.16	0.02	-	-	-
- azelaic acid, di-TMS	1805	1807	0.27	-	trace	0.43	-
- pentadecanoic acid, TMS	1949	1953	0.28	-	0.11	-	-
- 12-hydroxydodecanoic acid, di-TMS	2012	2013	0.18	-	-	-	trace
- palmitelaidic acid, TMS	2042	2038	1.04	-	-	-	-
- hexadecanoic acid, TMS	2050	2051	1.31	0.86	0.83	1.49	0.21
- traumatic acid-like, TMS	2083	-	1.10	-	-	-	-
- (E)-ferulic acid, di-TMS	2100	2103	0.25	0.16	-	-	trace
- tuberonic acid, di-TMS	2108	-	-	-	0.16	-	0.11
- octadecanoic acid, TMS	2146	2148	0.17	-	trace	-	-
Aliphatic alcohols, including:			0.61	0.56	0.75	1.13	1.39
- ethylene glycol, di-TMS	1035	1039	-	0.11	-	-	-
- propylene glycol, di-TMS	1066	1070	-	0.08	-	-	-
- glycerol, tri-TMS	1293	1293	0.22	0.37	0.44	0.29	0.89
- erythritol, tetra-TMS	1429	1432	0.39	-	-	-	-
- threitol, tetra-TMS	1476	1479	-	-	0.13	-	-

- xylitol, penta-TMS	1755	1750	-	-	0.18	-	-
- mannitol, hexa-TMS	2033	2036	-	-	-	0.55	-
- myo-Inositol, hexa-TMS	2065	2068	-	-	-	-	0.25
- scyllo-Inositol, hexa-TMS	2080	2084	-	-	-	-	0.14
- chiro-Inositol, hexa-TMS	2094	2098	-	-	-	-	0.11
Aromatics, including:			4.95	49.79	38.62	4.59	3.58
- 4-hydroxyphenylethanol, di-TMS	1575	1579	1.55	1.80	-	0.18	-
- phloroglucinol, tri-TMS	1659	1663	0.39	0.34	-	-	-
- betuligenol, di-TMS	1716	1721	0.27	4.31	-	-	-
- protocatechuic acid, tri-TMS	1833	1836	1.40	0.80	0.38	0.81	-
- 3,4'-dihydroxypropiophenone, di-TMS	1889	-	-	42.61	38.24	-	3.58
- syringaldehyde, di-TMS	1913	1916	1.34	-	-	3.60	-
Flavonoids, including:			0.52	0.20	0.90	0.71	3.85
- naringenin, tri-TMS	2856	2859	-	-	-	-	3.21
- epicatechin, penta-TMS	2907	2907	-	-	0.13	0.08	-
- catechin, penta-TMS	2934	2936	0.20	-	0.39	0.63	-
- kaempferol, tetra-TMS	3112	3114	-	-	0.38	-	0.64
- quercetin, penta-TMS	3128	3131	0.32	0.20	-	-	-
Carbohydrates & related compounds, including:			80.82	28.88	45.32	42.97	88.54
- arabinose, tetra-TMS	1548	1551	2.13	-	-	-	4.35
- rhamnose, tetra-TMS	1596	1599	1.88	-	2.40	-	3.92
- lyxose, tetra-TMS	1610	1614	-	-	1.36	-	-
- α -ribofuranose, tetra-TMS	1622	1626	1.95	-	-	-	2.76
- xylose, tetra-TMS	1643	1647	2.42	-	2.10	-	3.84
- levoglucosan, tri-TMS	1703	1706	9.58	-	-	-	11.24
- gluconic acid, δ -lactone, tetra-TMS	1766	1769	4.23	-	-	3.11	6.54
- α -fructofuranose, penta-TMS	1841	1845	2.40	-	3.89	2.56	4.88
- β -fructofuranose, penta-TMS	1850	1854	2.28	-	3.42	2.31	4.51
- α -glucopyranose, penta-TMS	1928	1931	0.96	0.78	6.11	2.44	4.19
- β -mannopyranose, penta-TMS	1991	1994	-	-	3.89	2.56	4.88
- β -glucopyranose, penta-TMS	2027	2032	1.45	1.24	8.65	3.00	5.23
- gluconic acid, hexa-TMS	2410	2415	5.44	-	-	4.27	7.32
- salicin, penta-TMS	2674	2679	4.67	-	1.82	4.00	8.32
- populin, tetra-TMS	2741	2746	-	-	-	-	1.66
- syringin, penta-TMS	2898	2902	-	-	-	-	1.50
- maltose, octa-TMS	3124	3129	8.11	-	5.92	6.47	9.63
- lactulose, octa-TMS	3168	3172	6.74	-	-	4.38	7.15
- sucrose, octa-TMS	3190	3195	12.36	-	6.44	7.85	14.72
Terpenes and terpenoids, including:			0.36	1.06	-	-	-
- (E,Z)-nerolidol, TMS	1683	1682	0.24	-	-	-	-
- 14-hydroxy- β -caryophyllene, TMS	1707	1705	0.11	-	-	-	-
- ursolic acid, di-TMS	3523	3523	-	1.06	-	-	-
Other, including:			1.07	-	2.68	0.46	0.50
- quinic acid, penta-TMS	1897	1900	0.82	0.17	1.62	0.46	0.14
NN			3.58	17.30	7.97	43.46	2.15

Notes: TIC—total ion current; RI^{calc}—calculated retention index; RI^{DB}—retention index from data-base; NN—unresolved or unidentified compounds; TMS—trimethylsilyl derivatives; trace—less than 0.1%; - not detected; Bold type indicates the major compound groups and their corresponding total relative abundances (% TIC).

Aromatic compounds showed substantial variation among composite samples, ranging from 3.6% TIC in the composite sample representing *P. tremula* to 49.8% in the composite sample representing *B. pendula*. Elevated aromatic contributions were also observed in the composite sample representing *C. betulus* (38.6%), whereas lower values were recorded for the composite samples representing *A. glutinosa* (5.0%) and *Q. robur* (4.6%). The dominant aromatic compound detected in the composite samples representing *B. pendula* and *C. betulus* was 3,4'-dihydroxypropiophenone.

Aliphatic acids accounted for between 1.24% and 8.64% TIC across the analysed composite samples, whereas aliphatic alcohols contributed between 0.56% and 1.39% TIC. Flavonoids and terpenoids occurred in relatively low proportions (less than 4% of the TIC and less than 1.1% of the TIC, respectively), although their relative contributions differed among composite samples.

A substantial unidentified fraction (NN) was observed, particularly in the composite sample representing *Q. robur*, where unidentified compounds represented more than 43% of the total ion current (TIC). In the remaining composite samples, the NN fraction ranged from 2.1% to 17.3% TIC. This unidentified fraction may reflect the presence of structurally complex compounds; compounds not represented in available spectral libraries; or limitations associated with SPE, derivatisation, and GC–MS analysis.

3.3. Chemical and Biological Oxygen Consumption of Leaf Litter Leachates

Chemical oxygen demand (COD) varied among the analysed composite samples (Table 3). In general, unfiltered leaf litter leachates exhibited higher COD values than filtered leachates, indicating the contribution of particulate-associated organic matter to overall oxygen demand. This pattern suggests that a portion of the released organic matter was associated with suspended material retained during filtration.

Composite samples representing *A. glutinosa* and *P. tremula* tended to exhibit higher COD values in unfiltered leachates, whereas the composite sample representing *B. pendula* showed lower values. In filtered leachates, the composite sample representing *C. betulus* exhibited comparatively higher COD values than the remaining analysed samples.

Table 3. Chemical oxygen demand of leaf litter leachates.

Species	Unfiltered Leachate			Filtered Leachate		
	Total COD (mg O ₂ L ⁻¹)	Specific COD (mg O ₂ g ⁻¹ d.m.)	Average Specific COD (mg O ₂ g ⁻¹ d.m.)	Total COD (mg L ⁻¹)	Specific COD (mg O ₂ g ⁻¹ d.m.)	Average Specific COD (mg O ₂ g ⁻¹ d.m.)
<i>A. glutinosa</i>	5480	907	692 ± 155	2600	430	375 ± 52
	3750	624		2340	389	
	3270	545		1830	305	
<i>B. pendula</i>	3440	569	534 ± 40	2370	392	341 ± 38
	2870	478		1980	330	
	3360	556		1830	303	
<i>C. betulus</i>	3440	559	584 ± 27	2960	481	507 ± 59
	3760	621		3560	588	
	3550	573		2800	452	
<i>Q. robur</i>	3520	584	573 ± 17	2980	495	419 ± 69
	3550	587		2630	435	
	3310	549		1980	328	
<i>P. tremula</i>	3410	560	616 ± 43	2180	358	391 ± 35
	3760	623		2270	376	
	4000	665		2640	439	

Notes: COD—chemical oxygen demand; d.m—dry mass; SD—standard deviation.

Five-day biochemical oxygen demand (BOD₅) also varied among the analysed composite samples (Table 4). Higher BOD₅ values were recorded for the composite samples representing *Q. robur* and *B. pendula*, whereas lower values were observed for the composite sample representing *C. betulus*. Composite samples representing *A. glutinosa* and *P. tremula* exhibited intermediate values.

BOD₅/COD ratios ranged from 0.24 to 0.58 among the analysed composite samples. The highest ratios were observed in the composite samples representing *Q. robur* and *B. pendula*, whereas lower values occurred in the composite sample representing *C. betulus*. These observations indicate differences in oxygen-demand characteristics among the analysed composite samples under the applied experimental conditions.

Table 4. Summary of five-day biochemical oxygen demand of DOM leached from deciduous tree leaves.

Species	Total BOD ₅ (mg O ₂ L ⁻¹)	Specific BOD ₅ (mg O ₂ g ⁻¹ d.m.)	BOD ₅ /COD
<i>Q. robur</i>	2000 ± 471	333 ± 78	0.58
<i>B. pendula</i>	1833 ± 289	306 ± 48	0.57
<i>A. glutinosa</i>	1333 ± 577	222 ± 96	0.32
<i>P. tremula</i>	1333 ± 471	222 ± 78	0.36
<i>C. betulus</i>	833 ± 289	139 ± 48	0.24

Values are expressed as mean ± standard deviation. Specific BOD₅ values were normalised to dry leaf mass. COD values used to calculate BOD₅/COD ratios refer to unfiltered leaf litter leachates.

4. Discussion

This study provides an exploratory characterisation and comparison of DOM released from composite leaf litter samples under controlled laboratory conditions.

The applied GC–MS/SPE approach captures only a selective and operationally defined fraction of DOM, primarily low-molecular-weight and derivatisable compounds. Consequently, the presented results should be interpreted as comparative compositional fingerprints rather than a comprehensive representation of dissolved organic matter.

4.1. Early-Stage Leaching as a Rapid Carbon Release Pathway

The 7-day leaching experiment demonstrated that freshly fallen leaves lost approximately 26%–28% of their initial dry mass, confirming that, under controlled laboratory conditions, the earliest stage of litter decomposition is associated with rapid leaching of water-extractable compounds. These initial losses are commonly attributed to the release of low-molecular-weight carbohydrates, phenolics, and organic acids from senescent foliage [6,23,29].

Observed differences in mass loss among composite samples were relatively small, suggesting that early-stage leaching may represent a broadly similar pathway for carbon export from temperate deciduous leaf litter under the applied experimental conditions. The observed magnitude of initial mass loss is consistent with previous studies indicating that freshly senesced leaf litter may lose a substantial proportion of its mass during the initial leaching phase under controlled laboratory conditions [24].

The relatively small variability in mass loss among composite samples suggests that compositional differences observed in the analysed DOM fraction may have been more pronounced than differences in the overall magnitude of mass loss during the initial phase of decomposition. This observation is consistent with previous studies indicating that litter characteristics may be more strongly associated with DOM composition and quality

than with the overall magnitude of carbon release during the initial phase of decomposition [6,10,29].

Drying of leaf material at 60 °C prior to leaching may have influenced the composition of dissolved organic matter by altering thermolabile compounds or promoting partial degradation of certain constituents. It may also have altered the relative abundance of some thermolabile and volatile compounds. However, because the analytical approach targeted derivatisable low-volatility compounds, including carbohydrates, organic acids and phenolics, the extent to which drying affected the composition of the analysed DOM fraction remains uncertain. Although this step was applied to standardise moisture content across samples, it may also have affected the relative abundance of easily soluble compounds. Nevertheless, the procedure was applied consistently to all analysed samples, allowing comparative assessment under controlled experimental conditions.

4.2. Variation in DOM Composition Among Composite Leaf Litter Samples

Clear variation among the analysed composite samples was observed in the group-level and molecular composition of dissolved organic matter. These compositional differences are consistent with variation in litter chemistry reported among deciduous leaf litter types [9,10,21]. Leaf biochemical traits, including concentrations of soluble carbohydrates and phenolics, are commonly associated with DOM composition during early decomposition [6,12,23].

Carbohydrates and related compounds constituted the dominant fraction of DOM across composite samples, ranging from 28.9% TIC in samples representing *B. pendula* to 88.5% in samples representing *P. tremula*. Particularly high proportions in composite samples representing *A. glutinosa* and *P. tremula* were associated with elevated contributions of mono- and disaccharides and sugar acids. These compounds are commonly reported as important constituents of litter-derived DOM and are frequently detected during the early stages of litter decomposition [14,30,31].

Aromatic compounds exhibited strong variability among the analysed composite samples, reaching nearly 50% TIC in samples representing *B. pendula* and 38.6% in samples representing *C. betulus*. In contrast, samples representing *A. glutinosa* and *Q. robur* showed much lower aromatic contributions (5.0%–4.6% TIC). The dominant aromatic compound detected in samples representing *B. pendula* and *C. betulus* was 3,4'-dihydroxypropiofenone.

Although some similarities were observed between samples representing *B. pendula* and *C. betulus*, the limited number of analysed samples and the lack of phylogenetic or multivariate analysis do not allow robust conclusions regarding taxonomic or mechanistic controls on DOM composition. Notably, samples representing *A. glutinosa*, also belonging to Betulaceae, exhibited low aromatic content, suggesting that the observed patterns may not be consistent at the family level.

Increased aromaticity is commonly associated with greater structural complexity and has been linked to differences in the environmental behaviour and transformation of dissolved organic matter [32–34].

A substantial unidentified fraction (NN), particularly in the composite sample representing *Q. robur* (>43% TIC), likely includes structurally complex organic molecules that could not be resolved due to analytical limitations [22,34]. Such unresolved components are commonly reported in forest-derived DOM and may reflect incomplete derivatisation, compounds not represented in available spectral libraries, or limited resolution of complex compounds by GC–MS [22,34]. Because the chemical composition of this fraction remains unresolved, its potential contribution to oxygen-demand characteristics cannot be evaluated within the framework of the present study.

Overall, variation in the chemical composition of the SPE-extractable fraction of DOM released during early-stage litter decomposition was observed among the analysed composite samples. However, direct relationships between molecular composition and oxygen-demand characteristics cannot be established because the analytical approaches characterise different operational fractions of dissolved organic matter.

The results of this study provide insight into the potential contribution of litter-derived DOM to oxygen demand and carbon cycling under controlled experimental conditions. However, processes such as dissolved oxygen depletion in natural waters, stream-scale BOD loading, and catchment-level carbon fluxes were not directly assessed. These processes depend on complex interactions among hydrology, microbial activity, and environmental conditions, which are not fully captured under laboratory settings.

4.3. Oxidative Reactivity and Biodegradability

Variation in chemical oxygen demand (COD) among the analysed composite samples was relatively small, although consistent numerical differences were observed. Unfiltered leachates of composite samples representing *A. glutinosa* and *P. tremula* tended to exhibit higher mean COD values than the remaining samples. Because COD reflects the total pool of chemically oxidisable organic matter irrespective of its biological accessibility, these results indicate differences in oxygen-demand characteristics among the analysed leachates but do not directly reflect short-term microbial utilisation [24,31].

Five-day biochemical oxygen demand (BOD₅) also varied among the analysed composite samples. Higher specific BOD₅ values were recorded for the composite samples representing *Q. robur* and *B. pendula*, whereas lower values were observed for the composite sample representing *C. betulus*. Composite samples representing *A. glutinosa* and *P. tremula* exhibited intermediate values. These observations indicate differences in oxygen consumption during the five-day incubation period under the applied experimental conditions.

The BOD₅/COD ratio provided an additional comparative descriptor of oxygen-demand characteristics. Higher ratios were observed in the composite samples representing *Q. robur* and *B. pendula*, whereas lower values occurred in the composite sample representing *C. betulus*. Intermediate ratios were recorded for the composite samples representing *A. glutinosa* and *P. tremula*. In environmental studies, the BOD₅/COD ratio is commonly used as an operational indicator of the proportion of organic matter that may be utilised during short-term microbial incubation [30–32]. However, interpretation of this parameter should be approached with caution because BOD₅ depends not only on substrate composition but also on microbial inoculum, nutrient availability, incubation conditions, and the fraction of DOM that remains analytically unresolved [14,15,31]. Despite these limitations, BOD₅ remains one of the most widely used operational indicators of short-term microbial oxygen demand and provides complementary information to molecular-level compositional analyses [14,15,31].

Importantly, differences in the relative abundance of major compound groups identified by GC–MS did not correspond directly to the observed BOD₅ values. For example, composite samples representing *A. glutinosa* and *P. tremula* contained high proportions of carbohydrates and related compounds, yet did not exhibit the highest BOD₅ values. Similar observations have been reported in previous studies showing that broad molecular classes alone do not necessarily predict short-term DOM biodegradability because compounds within the same class may differ substantially in structure, accessibility, and susceptibility to microbial transformation [21,30,31].

The carbohydrate-related fraction identified in the present study comprised not only simple mono- and disaccharides but also sugar acids, alcohols, lactones, and glycosidic derivatives. These compounds may differ considerably in their susceptibility to microbial

utilisation and transformation [6,23]. Furthermore, BOD₅ reflects oxygen consumption during a relatively short incubation period and therefore characterises only one aspect of DOM reactivity rather than its complete biodegradation potential [14,15,31].

A substantial unidentified fraction was observed in several composite samples, particularly in the sample representing *Q. robur*. Because the chemical composition of this fraction remains unresolved, its potential contribution to oxygen-demand characteristics cannot be evaluated within the framework of the present study. Unresolved compounds are frequently reported in studies of forest-derived DOM and may reflect analytical limitations associated with extraction, derivatisation, chromatographic separation, or library-based compound identification [22,34].

Taken together, the results suggest that oxygen-demand characteristics of litter-derived DOM are influenced by multiple factors and cannot be explained solely by the relative abundance of broad molecular classes identified by GC–MS. The present results indicate that the composition of the GC–MS-detectable fraction alone does not fully explain variation in BOD₅ observed among the analysed composite samples. Because the applied analytical approaches characterise different operational fractions of dissolved organic matter, direct mechanistic relationships between molecular composition and oxygen-demand characteristics cannot be unequivocally established from the present dataset.

Although differences in COD among composite samples were relatively small, variation in BOD₅ and BOD₅/COD ratios indicates differences in oxygen-demand characteristics under the applied experimental conditions. Such variation may be relevant for understanding the behaviour of litter-derived DOM during the initial stages of decomposition and transport through terrestrial and aquatic environments [1,2,4]. However, processes such as oxygen depletion in natural waters, stream-scale BOD loading, and catchment-level carbon fluxes were not directly assessed in the present study and depend on numerous environmental factors that were not considered in the experimental design.

The release of water-soluble organic compounds during the initial stages of litter decomposition may provide a source of organic substrates for microbial communities in soils and aquatic environments [6,30,31]. However, these processes were not directly measured in the present study. Therefore, the above considerations should be regarded as potential implications rather than direct evidence, and further studies under field conditions are required to evaluate the role of litter-derived DOM in ecosystem-scale carbon cycling and oxygen dynamics.

This study has several limitations that should be considered when interpreting the results. First, the applied GC–MS analysis following TMS derivatisation provides selective information on the molecular composition of DOM, favouring compounds that are amenable to derivatisation and volatilisation. Consequently, highly polar, high-molecular-weight, or structurally complex constituents may be underrepresented or remain undetected.

Second, the use of solid-phase extraction (C18) further constrains the analysed fraction of DOM, preferentially retaining non-polar and moderately polar compounds, while more hydrophilic constituents may be excluded. Consequently, some highly polar low-molecular-weight compounds that may represent a substantial fraction of litter-derived DOM were likely underrepresented in the molecular characterisation.

An additional source of uncertainty is related to the collection of freshly senesced leaves by branch shaking. Because leaf detachment may occur at different physiological stages among species and individual trees, part of the observed variation in DOM composition may reflect differences in senescence status rather than species identity alone.

In addition, leaf material from four trees was pooled to obtain a single composite sample for each species. Consequently, within-species variability could not be assessed and the effective biological replication was $n = 1$.

Finally, the leaching experiments were conducted under controlled laboratory conditions using distilled water and fixed incubation conditions. These conditions facilitated comparison among samples but do not fully reproduce natural hydrological, chemical, and biological processes occurring in forest ecosystems. Therefore, the results are best interpreted as reflecting comparative patterns of DOM release and oxygen-demand characteristics under the applied experimental conditions rather than direct field-scale behaviour.

5. Conclusions

Under the applied laboratory conditions, leaching of freshly fallen leaves was associated with substantial mass loss and release of water-extractable organic matter during the initial stage of decomposition. Differences among the analysed composite litter samples were observed in the composition of the SPE-extractable DOM fraction, as well as in oxygen-demand characteristics measured by COD and BOD₅.

Comparison of molecular fingerprints obtained by GC–MS with oxygen-demand parameters showed that differences in the composition of the GC–MS-detectable fraction did not correspond directly to variation in BOD₅. This finding suggests that oxygen-demand characteristics of litter-derived DOM cannot be explained solely by the relative abundance of broad molecular classes identified by the applied analytical approach. At the same time, the combined use of GC–MS, COD, and BOD₅ provided complementary information on compositional and functional aspects of DOM released during early-stage leaching.

The applied GC–MS/SPE approach provided comparative molecular-level information on a selective and operationally defined fraction of dissolved organic matter and should be regarded as a molecular fingerprinting tool rather than a comprehensive characterisation of the entire DOM pool.

Because each tree species was represented by a single composite biological sample, within-species variability could not be assessed. Future research should incorporate independent biological replication, broader molecular characterisation approaches, and field-based validation in order to improve understanding of litter-derived DOM dynamics under natural environmental conditions. Future research should incorporate independent biological replication, broader molecular characterisation approaches, and field-based validation in order to improve understanding of litter-derived DOM dynamics under natural environmental conditions.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/f17070762/s1>, Table S1: Semi-quantitative molecular composition of the SPE-extractable fraction of dissolved organic matter released from composite deciduous leaf litter samples analysed by GC–MS after TMS derivatisation.

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