Chemical Composition of Labile Carbon Fractions in Forest Soils as Affected by Soil Parameters

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Research Article

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Abstract

Understanding how the chemical composition of dissolved and particulate organic matter (DOM and POM) is affected by environment factors is critical because these labile pools of carbon are involved in an array of biological, chemical and physical processes. In this study, the chemical composition of DOM and POM was measured in 13 forest soils using UV-Vis spectroscopy, fluorescence spectroscopy with PARAFAC modelling and FT-IR spectroscopy.

There were significant differences between the soils for the SUVA indexes, PARAFAC components and relative intensities of different IR bands. Redundancy analysis (RDA) revealed that soil parameters had a great influence on the chemical composition of DOM and POM with high constrained variability (77.9 and 77.1 %, respectively). The pH of the soils proved to be an important controlling factor for both DOM and POM, regulating the concentration of the C3 PARAFAC component (low-molecular-weight compounds associated with biological activity) and the aromatic compounds of POM (aromaticity, $rA_{1630}$ and $rA_{1515}$). The silt content was the other main regulating factor controlling the chemical characteristics of the labile pool, having a strong negative correlation with the SUVA values of DOM due to the preferential adsorption of hydrophobic moieties. RDA analysis also revealed that, despite their different origins, there is a strong correlation between the chemical composition of POM and DOM.

Introduction

Soil organic matter (SOM) has a number of functions in the environment, in connection with soil quality functions such as fertility (Tiessen et al. 1994), buffering capacity (Ritchie and Dolling 1985) and structural stability (Six et al. 2000). Furthermore, SOM plays a potential role in the release and sequestration of CO$_2$, through the decomposition of organic matter or the accumulation of carbon by capturing plant residuals (Lal et al. 2015). However, soil organic matter is not a homogeneous material but consists of molecules ranging from the simple to the more complex in very different stages of decomposition from recognizable plant residues to low-molecular-weight carbohydrates and proteins (Lehmann and Kleber 2015). The labile pools of soil carbon can be considered as the active carbon in soils and have central role in short- to medium-term nutrient availability and soil structural stability. Also, these pools are sensitive indicators of minor changes in both the climate or local environment (Haynes 2005; Fang et al. 2005) and soil quality (Filep et al. 2015), so there is increasing interest in how it responds to such changes.

Dissolved organic matter (DOM) is a complex mixture of low- and high-molecular-weight organic molecules originating from litter, soil leachates, plant root exudates and microbial by-products (Thurman 1985; Guggenberger et al. 1994). The dissolved organic matter fraction is often defined operationally as the organic carbon fraction that can pass through a filter of about 0.45 µm pore size (Kalbitz et al. 2000). DOM cycling in terrestrial ecosystems is of particular interest in the light of a changing climate, because DOM is one of the largest sources of available organic carbon for microbes (De Troyer et al. 2011; Xenopoulos et al. 2021). Particulate organic matter (POM) is also considered as a rapidly changing pool
of organic matter, which could respond rapidly to environmental changes (Six et al. 2002). This fraction contains organic matter with a size of > 63 µm and a density of > 1.6 g/cm³ (Christensen 1992). POM makes a substantial contribution to soil organic matter, typically comprising > 50% of SOM in mineral soils and > 70% in sediments (Hayes et al. 2017). Particulate organic matter can be considered to be in a kind of metastable phase (Huang et al. 2019), meaning that although it is insoluble, it is chemically and biologically active, e.g. it can adsorb metals via several mechanisms (Guo et al. 2006; Zhao et al. 2021).

Radiocarbon dating suggests that the DOM and POM pools are not connected, as the average age of DOM is 5–40 years, while POM may be hundreds or thousands of years old (Lu et al. 2014). Furthermore, research has provided additional evidence for the lack of connection between DOM and POM, showing a clear compositional difference between them, which could be explained by their different sources and different degradation pathways (Feng et al. 2016). Matiasek and Hernes (2019) reported that fractionation may occur during the solubilisation of particulate-bound organic matter to dissolved organic forms, as indicated by the distinct amounts of amino acids and lignin in POM and DOM. However, Li et al. (2018) pointed out that despite the compositional differences in DOM and POM, they are nevertheless coupled to some extent, exhibiting a similar shift in diagenetic status. They may be linked via processes such as dissolution, sorption/desorption, aggregation and disaggregation. More direct evidence is thus needed to elucidate whether DOM and POM are connected or not.

In this study, the spectroscopic characteristics of the dissolved organic matter and particulate organic matter fractions of thirteen forest soils from Hungary were evaluated as affected by soil parameters. The chemical composition of the pools was analysed by combining UV-Vis, fluorescence and FT-IR spectroscopy, using PARAFAC modelling. Redundancy analysis was used to reveal which soil factors control the chemical compositions of these labile pools and what connection there is between them. The specific objectives were: (i) to gain an insight into the relationship between soil parameters and the chemical composition of the labile fraction of the soils, (ii) to evaluate whether the chemistry of POM and DOM is connected.

**Materials And Methods**

**Site description and sampling**

Thirteen forest topsoil samples were collected from Hungary. More details about the sites are available in Zacháry et al. (2018). Samples were taken from the upper 0 – 20 cm horizon and the soils were air-dried, homogenized, passed through a 2-mm sieve and stored at room temperature.

**Laboratory analysis**

**Soil fraction preparation**

The dissolved organic matter was extracted with ultrapure water at a 1:10 soil:solution ratio for 2 h and filtrated through a 0.45 µm membrane nylon filter. Particulate organic matter was fractionated according
to Zimmermann et al. (2007). Briefly, soil samples were added to distilled water and dispersed. After dispersion the suspension was wet-sieved over 63-µm sieve. The > 63 µm fraction was dried at 40°C and separated using NaI at a density of 1.6 g cm$^{-3}$. The POM was then rinsed with distilled water to remove NaI and subjected to further analysis.

**Soil analysis**

The soil pH was measured in 1:2.5 soil:water and soil:1M KCl supernatants 12 h after mixing. The total organic carbon (TOC) content was analysed using an elemental analyser (Apollo 9000, Tekmar Dohrmann). The particle size distribution was determined by the pipette method (Gee and Or 2018). The total N content was measured by the Kjeldahl method (Bremner 2019). The dissolved organic carbon (DOC) and total soluble nitrogen (TSN) were analysed in a TOC/TN analyser (TOC-L, Shimadzu). Cation exchange capacity (CEC) was determined according to the method of Gillman (1979). The basic parameters of the soils are presented in Table 1.

| Soil code | pH$_{dw}$ | pH$_{KCl}$ | TOC % m$^{-1}$ | TN % m$^{-1}$ | C/N ratio | Clay % m$^{-1}$ | Silt % m$^{-1}$ | Sand % m$^{-1}$ | CEC mol$_c$ kg$^{-1}$ |
|-----------|-----------|------------|----------------|--------------|-----------|----------------|----------------|----------------|----------------------|
| CEG       | 6.2       | 5.6        | 1.8            | 0.15         | 12.4      | 4.6            | 11.3           | 84.1           | 7.8                  |
| NYIR1     | 4.9       | 3.7        | 0.56           | 0.05         | 10.5      | 15.8           | 20.2           | 64.0           | 4.3                  |
| NYIR2     | 6.2       | 5.7        | 2.2            | 0.17         | 13.2      | 5.8            | 13.0           | 81.2           | 11.8                 |
| BAT       | 4.6       | 3.7        | 7.2            | 0.39         | 18.6      | 23.7           | 71.2           | 5.1            | 9.9                  |
| JOS1      | 5.7       | 4.8        | 9.7            | 0.68         | 14.4      | 28.9           | 55.2           | 15.9           | 29.4                 |
| JOS2      | 5.2       | 4.3        | 11.7           | 0.41         | 28.8      | 48.3           | 39.6           | 12.1           | 19.1                 |
| JOS3      | 5.9       | 5.5        | 3.9            | 0.29         | 13.4      | 20.3           | 70.0           | 9.7            | 16.9                 |
| SOP1      | 4.5       | 3.3        | 2.6            | 0.15         | 18.3      | 18.7           | 52.2           | 29.1           | 6.2                  |
| SOP2      | 3.7       | 2.8        | 11.0           | 0.24         | 46.2      | 15.3           | 35.5           | 49.2           | 6.8                  |
| SOP3      | 4.5       | 3.4        | 2.5            | 0.15         | 17.1      | 11.9           | 36.1           | 52.0           | 6.0                  |
| SOP4      | 4.3       | 5.3        | 3.0            | 0.15         | 20.5      | 17.3           | 53.5           | 29.2           | 9.7                  |
| KIS       | 5.1       | 6.0        | 3.6            | 0.25         | 14.3      | 25.1           | 58.9           | 16.0           | 18.9                 |
| KAR       | 4.5       | 5.2        | 1.4            | 0.07         | 19.8      | 17.8           | 45.5           | 36.7           | 10.1                 |

**Spectroscopic analysis and PARAFAC modelling**
UV-Vis spectrophotometric analysis of the DOM samples was performed with a UV-3600 dual-beam scanning spectrophotometer (Shimadzu Corp.) using a 1 cm quartz cuvette with deionized water as the reference. The specific UV absorption (SUVA$_{254}$ and SUVA$_{280}$, L mg$^{-1}$ m$^{-1}$) was calculated by dividing the absorption at 254 and 280 nm by the DOC concentration.

The fluorescence excitation–emission matrices (EEM) of DOM were measured with a RF-6000 instrument (Shimadzu Corp.). The EEMs were obtained by measuring fluorescence intensity excitation wavelengths ranging from 230–450 nm and emission wavelengths ranging from 260–600 nm with 2 nm increments. After blank subtraction and the correction of scattering the EEMs were Raman normalized using the area under the water Raman peak at an excitation wavelength of 275 nm. The PARAFAC modelling was conducted with MATLAB (Mathworks, Natick, MA) using the drEEM toolbox (Murphy et al. 2013). A non-negativity constraint was applied to allow only chemically relevant results. The correct number of components was determined using the core consistency diagnostic score, which should be close to 100% for appropriate models (Bro and Kiers 2003). The validity of a PARAFAC model is often evaluated using split-half analysis. However, split-half analysis is problematic for validating a model if the dataset is from geographically diverse locations, which leads to highly variable fluorescence (Dubnick et al. 2010). This is probably why none of the models explored in this study gave satisfactory results in the split-half analysis, thus preventing it from being used as a validation tool. Table 2 shows the fluorescence characteristics of the six components obtained by PARAFAC analysis.

| Comp. | Ex/Em maxima (nm) | Description |
|-------|-----------------|-------------|
| C1    | 255 (340)/444   | High-molecular-weight humic-like compounds, frequently high in forested environments |
| C2    | 260/504         | High-molecular-weight, aromatic materials |
| C3    | 310 (255)/418   | Low-molecular-weight, associated with biological activity |
| C4    | 255 (325)/384   | Very labile, associated with freshly produced DOM |
| C5    | 280/330         | Tryptophan-like, indicating proteins or less degraded peptides |
| C6    | 300 (255)/300   | Tyrosine-like, indicating more degraded peptide material |

All the POM samples were dried at 60°C before FT-IR measurement. The FT-IR ATR measurements were carried out on a Bruker Vertex 70 spectrometer with an RT-DLaTGS detector. For each sample a spectral range of 4000–400 cm$^{-1}$, a resolution of 4 cm$^{-1}$, 128 scans, and three replicates were recorded. The spectra were corrected (atmospheric water and CO$_2$ correction) and subjected to 17-point Savitzky-Golay smoothing, SNV normalization and baseline correction.
The relative absorbance (rA) and aromaticity index were calculated to evaluate relative changes in the spectra. Relative absorbance was calculated by dividing the given peak height (2920, 1710, 1630, 1515, 1270 and 1160 cm\(^{-1}\)) by the sum of the absorbance of all peaks measured and multiplying it by 100:

$$rA_x = \frac{rA_x}{\sum rA_{2920-1160}} \times 100$$

The aromaticity index (Inbar et al. 1989) was calculated by dividing the intensity of absorption at 1630 cm\(^{-1}\) (aromatic C=C) by the intensity of absorption at 2920 cm\(^{-1}\) (aliphatic C–H).

**Statistical evaluation**

One-way analysis of variance (ANOVA) with a post hoc Duncan test was used to evaluate differences between the spectroscopic parameters of DOM and POM (SUVA, PARAFAC components and relative intensities of IR bands).

The relationship between soil parameters and the chemical composition of DOM and POM was analysed using redundancy analysis (RDA). The spectroscopic parameters of DOM and POM (SUVA, PARAFAC components and relative intensities of IR bands) were selected as response variables, while the parameters of the forest soils (pH, SOC, clay, silt and sand content, carbon to nitrogen ratio) were applied as explanatory variables. All the response and exploratory variables were standardised as suggested by Braak and Smilauer (2012). The collinearity of the variables was checked using the variance inflation factor (VIF), and variables having VIF < 20 were used for further analysis.

**Results**

**Chemical composition of DOM and POM fractions**

The molar absorptivity of the DOM fraction of the samples was measured at 254 and 280 nm (Table 3). Besides the traditional 254 nm, where a group of much weaker bands appear for benzene, 280 nm was chosen because π-π* electron transitions may occur for phenolic substances, aromatic structures and polycyclic aromatic hydrocarbons (Weishaar et al. 2003). Specific UV absorbance has been shown to be a useful proxy for DOM aromatic content (Traina et al., 1990; Chin et al., 1994). The SUVA values ranged from 1.1 to 3.5 for SUVA\(_{254}\), and from 0.9 to 2.7 for SUVA\(_{280}\) in the individual soils. The highest values were obtained for the CEG, NYIR1 and JOS2 samples and the lowest values for BAT, SOP3 and KIS.
Table 3
Specific UV absorbance of DOM

| Soil  | SUVA_{254} | Soil  | SUVA_{280} |
|-------|------------|-------|------------|
|       | L mg\(^{-1}\)   m\(^{-1}\) |       | L mg\(^{-1}\)   m\(^{-1}\) |
| CEG   | 3.5 \(f\)     | CEG   | 2.7 \(f\)     |
| NYIR1 | 3.3 \(ef\)    | NYIR1 | 2.5 \(ef\)    |
| JOS2  | 3.0 \(e\)     | JOS2  | 2.3 \(e\)     |
| NYIR2 | 2.3 \(d\)     | NYIR2 | 1.8 \(d\)     |
| JOS3  | 2.2 \(d\)     | JOS3  | 1.7 \(d\)     |
| JOS1  | 2.0 \(cd\)    | SOP2  | 1.5 \(cd\)    |
| SOP2  | 2.0 \(cd\)    | JOS1  | 1.5 \(cd\)    |
| SOP1  | 1.7 \(bc\)    | KAR   | 1.3 \(bc\)    |
| SOP4  | 1.7 \(bc\)    | SOP1  | 1.3 \(bc\)    |
| KAR   | 1.7 \(bc\)    | SOP4  | 1.3 \(bc\)    |
| KIS   | 1.6 \(b\)     | KIS   | 1.1 \(b\)     |
| SOP3  | 1.5 \(b\)     | SOP3  | 1.1 \(b\)     |
| BAT   | 1.1 \(a\)     | BAT   | 0.9 \(a\)     |

Statistically homogeneous groups of sites are marked by the same letter in individual columns (Duncan post hoc test, \(p < 0.05\)).

In most of the soils the high-molecular-weight components, C1 and C2, were the dominant PARAFAC components, contributing about 65–70 % of the total fluorescence (Fig. 1). The values varied from 30.6 to 43.0 % for the C1 component and from 19.8 to 29.0 % for the C2 component. PARAFAC components that can be connected directly with microbial activity, such as C3, C4, C5 and C6, showed great variability in the individual soils. In the case of the C3 component, for example, an extremely wide range of values was observed (0.0 to 28.9 %).

The relative intensities of the bands selected for evaluating the chemical composition of POM in forest soils are given in the Table 4. In the case of the band at 2920 cm\(^{-1}\), which represents the asymmetric stretching of CH\(_2\) groups in aliphatic compounds (Niemeyer et al. 1992), the lowest relative values were measured for NYIR1 and CEG (14.5 and 15.0 %), while SOP3 had the greatest aliphatic content (25.4 %).
Table 4
Relative absorbance as a % of the sum of all selected peak heights for 13 Hungarian forest soils

| Soil  | $rA_{2920}$ | $rA_{1710}$ | $rA_{1630}$ | $rA_{1515}$ | $rA_{1270}$ | $rA_{1160}$ | Arom. index |
|-------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|
| NYIR1 | 14.5 a      | 7.5 cd      | 18.5 cd     | 7.0 abc     | 3.6 bc      | 31.0 g      | 1.3 de      |
| CEG   | 15.0 a      | 5.6 abc     | 22.9 e      | 14.0 e      | 5.9 cd      | 31.8 g      | 1.5 ef      |
| NYIR2 | 16.4 ab     | 5.9 abc     | 25.5 f      | 17.4 f      | 7.1 d       | 26.5 f      | 1.6 f       |
| JOS3  | 16.5 ab     | 4.0 a       | 19.1 cd     | 8.1 bcd     | 1.0 ab      | 24.7 ef     | 1.1 cd      |
| SOP2  | 17.7 bc     | 12.9 e      | 19.4 cd     | 9.2 cd      | 10.2 e      | 18.2 c      | 1.1 cd      |
| KIS   | 17.8 bc     | 5.4 ab      | 16.9 bc     | 10.2 d      | 5.0 cd      | 25.2 ef     | 0.9 bc      |
| SOP4  | 18.2 bc     | 6.3 bc      | 13.0 a      | 5.0 a       | 0.6 a       | 22.3 de     | 0.7 ab      |
| KAR   | 19.6 cd     | 7.3 bcd     | 12.8 a      | 4.2 a       | 0.0 a       | 24.2 ef     | 0.7 a       |
| SOP1  | 19.8 cd     | 7.4 bcd     | 15.6 b      | 5.5 ab      | 1.6 ab      | 21.1 cd     | 0.8 ab      |
| JOS1  | 20.7 de     | 7.3 bcd     | 25.8 f      | 10.2 d      | 0.4 a       | 13.8 b      | 1.2 d       |
| BAT   | 21.8 de     | 7.3 bcd     | 17.9 bcd    | 6.6 abc     | 0.5 a       | 19.8 cd     | 0.8 ab      |
| JOS2  | 22.6 e      | 8.6 d       | 26.2 f      | 10.3 d      | 1.7 ab      | 9.9 a       | 1.2 cd      |
| SOP3  | 25.4 f      | 8.8 d       | 20.4 de     | 8.0 bcd     | 1.3 ab      | 12.8 ab     | 0.8 ab      |

Statistically homogeneous groups of sites are marked by the same letter in individual columns (Duncan post hoc test, p < 0.05). The aromaticity index was calculated by dividing the intensity of absorption at 1630 cm$^{-1}$ by the intensity of absorption at 2920 cm$^{-1}$.

For the C = O stretching of carboxyl groups (Hay and Myneni 2007), represented by the relative absorbance of the 1710 cm$^{-1}$ band, the lowest value was found for JOS3 (4.0 %) and the highest for SOP2 (12.9 %).

The broad peak around 1630 cm$^{-1}$ could be associated with several chemical structures, e.g. carbon-carbon stretching in the aromatic ring or C = O stretching in amides (Niemeyer et al. 1992; Abdulla et al. 2010). In the present study, considerably lower values were detected for samples SOP4 and KAR, while high relative absorbance was measured for NYIR2. A similar pattern was found for the main band of C-C stretching in aromatic rings at 1515 cm$^{-1}$ (Haberhauer et al. 1998), with the lowest relative absorbance values for SOP4 and KAR samples and the highest for NYIR2.

The band at 1270 cm$^{-1}$, which can be attributed to the C-OH stretching in phenolic OH (Baes and Bloom 1989; Niemeyer et al. 1992) gave the lowest values (0.0. and 0.6) for the KAR and SOP4 soils and the highest value for SOP2 (10.2).
For the band at 1160 cm$^{-1}$, which can be assigned to vibrations in the glucosidic C–O–C bond and in the whole glucose ring (Pandey 1999; Olsson and Salmén 2004), great variation was found between the soils, ranging from 9.9 to 31.8.

In most of the forest soils, a low aromaticity index was calculated by dividing the intensities of the peaks of 2920 and 1630 cm$^{-1}$. Although the band in the 1630 cm$^{-1}$ region was very mixed, representing coupled vibrations, for example the C-C stretching of aromatic rings or the C = O vibrations of amides, a strong relationship was found between the intensity ratio of 1620 and 2920 cm$^{-1}$ and the ratio of aryl and alkyl C obtained from NMR measurements (Dick et al. 2006).

**Effect of soil parameters on the composition of labile fractions**

To evaluate the effect of soil parameters on the chemical composition of the labile fractions in the soils redundancy analysis was performed for both DOM and POM (Figs. 2 and 3). Based on VIF values, the pH$_{\text{H}_2\text{O}}$, TOC, carbon to nitrogen ratio, clay and silt content, and CEC were selected as explanatory variables. The full RDA model explained more than 75% of the total variance for DOM composition (77.9%). A strong correlation was found between soil pH and the C3 component, a negative correlation between the pH and the C1 and C4 components. There was a strong negative correlation between soil texture, expressed as clay and silt content, and both the SUVA values and the C2 component of PARAFAC. The cation capacity of the soils (CEC) was found to be closely correlated with the C5 component of the DOM fraction, while the C/N ratio was correlated with the C4 and C1 components.

For POM, redundancy analysis showed that the constrained variability was 77.1%. As in the case of DOM, the pH appeared to be the main controlling factor for chemical composition in POM: several relative absorbance values, $r_{A_{1515}}^{1630}$, $r_{A_{1270}}^{1515}$, and the aromaticity index, were governed by pH, though the cation exchange capacity (CEC) was also found to be a controlling factor for these absorbance values and for aromaticity. A strong correlation was found between the carbon to nitrogen ratio of the soils and the relative absorbance of the band at 1710 cm$^{-1}$. The silt content was in close negative correlation with $r_{A_{1630}}^{1630}$, $r_{A_{1515}}^{1515}$, $r_{A_{1270}}^{1270}$ and the aromaticity index. The clay content also played an important role in regulating POM quality, as indicated by the correlation between clay content and $r_{A_{2920}}^{2920}$.

**Connection between the compositions of POM and DOM**

Redundancy analysis on the spectroscopic parameters of the DOM and POM fractions (Fig. 4) indicated that the chemical structure of the dissolved organic matter was determined by the chemical composition of POM with a constrained variance of 80.4%.

A strong, positive correlation was detected between the relative intensity of the bands at 1630 and 1515 cm$^{-1}$ (C-C stretching of aromatic rings) and both the PARAFAC component C3 (low-molecular-weight compounds) and SUVA indexes. There was also a positive relationship between the $r_{A_{1270}}^{1270}$ (phenolic OH) and $r_{A_{1160}}^{1160}$ (polysacharrides) values of POM and the C2 (high-molecular-weight, aromatic) component.
and SUVA values of DOM. Close negative correlations were found between $rA_{2920}$ (aliphatic compounds) and the SUVA indexes and between $rA_{1710}$ ($C = O$ of carboxyl) and the C5 (tryptophan-like compounds) component of DOM. There was also a negative correlation between the $rA_{1630}$ and $rA_{1515}$ values ($C-C$ stretching of aromatic rings) of POM and the C4 (very labile DOM compounds) and C1 (high-molecular-weight molecules) components of the DOM fraction.

**Discussion**

**Which primary soil parameters control the chemical composition of DOM and POM?**

Although the chemical composition of both the POM and DOM fractions in forest soils was regulated by a series of physical, chemical and biological processes, this study showed that only a small number of soil properties gave a good description of the composition, as indicated by the high constrained variances of the RDA analysis (Figs. 2 and 3).

A strong, positive correlation was found between soil pH and the relative amount of the C3 component, representing low-molecular-weight organic compounds. Higher soil pH created more favourable circumstances for microorganisms, leading to higher biological activity (Blagodatskaya and Anderson 1998; Pietri and Brookes 2008; Cao et al. 2016) which may in turn result in the enhanced degradation of high-molecular-weight compounds, such as carbohydrates, to low-molecular-weight degradates, represented by the C3 component. A recent study also found that the low-molecular-weight PARAFAC component (325/400) increased during incubation (Wang et al. 2019). The connection between higher microbial activity and the C3 component was confirmed by the fact that the C1 component was negatively correlated with pH, suggesting that the high-molecular-weight compounds in DOM could be the primary source for biological degradation. In addition to pH, low N availability was confirmed as a limiting factor for microbial degradation, because the C/N ratio of the soils was found to be in strong correlation with the C1 component, indicating a connection between N status and microbial activity. The biological activity, controlled by pH, also affected the concentration of the C4 component, which exhibited a negative correlation with pH.

Protein-like flourescences have been reported to be a useful proxy for measuring the biodegradibility of DOM (Fellman et al. 2008, 2009; Chen and Jaffé 2016), but a more complete picture emerged from the present analysis, the data of which not only revealed a possible connection between the PARAFAC components, but also how organic matter is degraded, with high-molecular-weight compounds (represented here as C1) being degraded into lower molecular-weight compounds (C4), after which the increased microbial activity results in a high level of microbial by-products such as peptides and amino acids (C6 component) (Figs. 1 and 2).

In this study the soil texture was found to be a significant controlling factor for the composition of the DOM fraction, as confirmed by the strong negative correlation between the silt and clay contents and the
SUVA values of DOM and the C2 component (Fig. 2) due to the preferential sorption of large, hydrophobic organic compounds (Jardine et al. 1989; Kaiser and Zech 2000). Avneri-Katz et al. (2017) found a significant reduction in SUVA\textsubscript{254} values, confirming the accelerated adsorption of hydrophobic compounds suggested by the present evaluation.

In the case of POM, the organic matter was found to be selectively decomposed, which could lead to an increase in the relative abundance of compounds with high resistance to microbial degradation, such as the aromatic and phenolic compounds represented by \( rA_{1630} \), \( rA_{1515} \) and \( rA_{1270} \), causing an enrichment in lignin-derived compounds. Although there is still debate about the preferential decomposition of organic matter in soils (Lehmann and Kleber 2015), the increasing contribution of aromatic and phenolic compounds caused by the biological degradation of plant residues is a common phenomenon. Xu et al. (2017) also found a relative increase in phenolic and aromatic carbon in residues, while Almendros et al. (2000) described the decay processes of forest biomass as the accumulation of recalcitrant, aromatic structures. In addition to pH, the cation exchange capacity of the soils proved to be a further regulator of the relative amounts of aromatic and phenolic compounds (Fig. 3). CEC is well known as a dominant factor that stimulates bacterial respiration by maintaining the pH, replacing the H\textsuperscript{+} ions produced during metabolism with basic cations (Stotzky 1966). Due to this mechanism the less degradable aromatic moieties were abundant in POM in soils with high CEC, e.g. in CEG and NYIR2.

Several studies demonstrated a strong relationship between microbial respiration and either the C/N ratio of the litter layer (Gödde et al. 1996; Michel and Matzner 2002; Spohn 2015) or the available N (Craine et al. 2007). These finding are in accordance with the present results: the strong, negative correlation between the soil C/N ratio and the relative intensity of the band at 1160 cm\textsuperscript{-1} (representing polysachharides) clearly demonstrated the enhanced degradation of easily decomposable materials, such as sugars, in soils with higher N availability. This was confirmed by the study of Gallo et al. (2005), who reported a common microbial response to higher N: the activity of cellulase and other glycosidases tended to increase. In parallel with this, the activity of oxidative enzymes tended to decline (Saiya-Cork et al. 2002). This was clearly demonstrated in the present study by the positive correlation of the C/N ratio with the relative intensity of the band at 1710 cm\textsuperscript{-1}, which represents oxidative materials; in other words, in soils with high N availability the activity of oxidative enzymes is restricted, so the amount of carboxyl groups in POM is low.

**Is the chemical composition of POM linked with that of DOM?**

Particulate organic matter is considered to be one of the major sources of dissolved organic carbon (Zsolnay 2003). It has long been known that several bacteria are able to decompose the native lignin in the soil (Brauns 1952; Sørensen 1962) and that the decomposition of POM in soils can result in several aromatic compounds such as lignin-derived materials, tannins and phenols (Kaiser et al. 2001; Kalbitz et al. 2006). It is thus not surprising that the relative amount of aromatic compounds (\( rA_{1515} \), \( rA_{1630} \) for aromatic rings and \( rA_{1270} \) for phenolic compounds) in the POM fraction of forest soils was closely
correlated with the SUVA values of the DOM fraction (Fig. 4). Based on the thermochemolysis data of forest soils Klotzbücher et al. (2013) stated that at least half the aromatic carbon comes from litter. Although Matiasek and Hernes (2019) reported different solubilisation patterns for lignin phenols, indicated by the C:V ratios (ratio of cinnamyl phenols to vanillyl phenols), which were six-fold lower in DOM, the optical characteristics of these compounds were probably identical.

The degradation of lignin-carbohydrate complexes is a prerequisite for the solubilisation of lignin, a process is linked with the degradation of cellulose, resulting in a relatively labile C source partly shielded by lignin (Jeffries 1991). Furthermore, lignin oxidation is a metabolic process that requires easily degradable carbon sources (Kirk and Farrell 1987). Klotzbücher et al. (2013) also suggested that lignin degradation and solubilisation could be related to the production of soluble carbohydrates that provide energy for microbes. In the present study, these enzyme-mediated processes were confirmed by the positive correlation between \( r_{A1515} \) and \( r_{A1630} \) in POM and the C3 component of DOM, indicative of enhanced microbial activity. In addition, the relative intensity of the band at 1160 cm\(^{-1} \), representing the carbohydrate content of POM, was in strong correlation with the C2 component and SUVA indexes of DOM, clearly revealing the connection between carbohydrates and the rate of solubilisation of lignin-derived compounds.

Complex coupling was revealed by RDA between the rA1710 value of POM and the C5 component (tryptophan-like materials) of DOM (Fig. 4). As discussed earlier, a lower N supply could favour oxidative enzyme processes (Saiya-Cork et al. 2002). However, the low N level could also inhibit other special microbial decomposition processes, which means that the fluorescence of proteinaceous tryptophan-like components, found to be a useful indicator of a reduction in the total microbial activity in wastewater (Cohen et al. 2014), could be less useful if nitrogen is limited. This could explain the negative correlation revealed by statistical analysis between the rA1710 value of POM and the C5 component of the DOM fraction.

**Conclusions**

Significant differences were found in the chemical compositions of both DOM and POM in 13 forest soils, reflecting their different origin and biogeochemical transformation. Nevertheless, RDA analysis showed that the chemical characteristics of the two labile fractions largely determined by the basic properties of the soils.

The study also revealed that, in addition to factors directly or indirectly influencing the biodegradation of organic matter, purely physico-chemical properties also influenced the composition of the DOM pool through adsorption. A complementary pattern was found between the C1 and C2, and the C3 and C4 PARAFAC components of DOM, suggesting biological linkage between them. This was confirmed by RDA, which provided a clear picture of the transformation and coupling of various biodegradable DOM components, high-molecular-weight compounds (C1) being degraded into lower molecular-weight
compounds (C4), and increased microbial activity resulting in a high level of microbial by-products such as peptides and amino acids.

Despite their completely different origins and diagenetic status, a strong relationship was observed between the chemical characteristics of POM and DOM, suggesting that although they represent different levels of biodegradation, they are biogeochemically coupled.

**Declarations**

**Conflict of interest**

The authors declare no conflict of interest.

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**References**

1. Abdulla HAN, Minor EC, Dias RF, Hatcher PG (2010) Changes in the compound classes of dissolved organic matter along an estuarine transect: A study using FTIR and 13C NMR. Geochim Cosmochim Acta 74:3815–3838. https://doi.org/10.1016/j.gca.2010.04.006
2. Aciego Pietri JC, Brookes PC (2008) Relationships between soil pH and microbial properties in a UK arable soil. Soil Biol Biochem 40:1856–1861. https://doi.org/10.1016/j.soilbio.2008.03.020
3. Almendros G, Dorado J, González-Vila FJ et al (2000) 13C NMR assessment of decomposition patterns during composting of forest and shrub biomass. Soil Biol Biochem 32:793–804. https://doi.org/10.1016/S0038-0717(99)00202-3
4. Avneri-Katz S, Young RB, McKenna AM et al (2017) Adsorptive fractionation of dissolved organic matter (DOM) by mineral soil: Macroscale approach and molecular insight. Org Geochem 103:113–124. https://doi.org/10.1016/j.orggeochem.2016.11.004
5. Baes AU, Bloom PR (1989) Diffuse reflectance and transmission fourier transform infrared (DRIFT) spectroscopy of humic and fulvic acids. Soil Sci Soc Am J 53:695–700. https://doi.org/10.2136/sssaj1989.03615995005300030008x
6. Blagodatskaya EV, Anderson TH (1998) Interactive effects of pH and substrate quality on the fungal-to-bacterial ratio and QCO2 of microbial communities in forest soils. Soil Biol Biochem 30:1269–1274. https://doi.org/10.1016/S0038-0717(98)00050-9
7. Braak CJF ter, Smilauer P (2012) Canoco Reference Manual and User's Gguide: software for ordination, version 5.0
8. Brauns FE (1952) The Chemistry of Lignin. Academic Press
9. Bremner JM (2019) Determination of nitrogen in soil by the Kjeldahl method. J Agric Sri 55:. https://doi.org/10.1017/S0021859600021572

10. Bro R, Kiers HAL (2003) A new efficient method for determining the number of components in PARAFAC models. J Chemom 17:274–286. https://doi.org/10.1002/cem.801

11. Cao H, Chen R, Wang L et al (2016) Soil pH, total phosphorus, climate and distance are the major factors influencing microbial activity at a regional spatial scale. Sci Rep 6:1–10. https://doi.org/10.1038/srep25815

12. Chen M, Jaffé R (2016) Quantitative assessment of photo- and bio-reactivity of chromophoric and fluorescent dissolved organic matter from biomass and soil leachates and from surface waters in a subtropical wetland. Biogeochemistry 129:273–289. https://doi.org/10.1007/s10533-016-0231-7

13. Christensen BT (1992) Physical Fractionation of Soil and Organic Matter in Primary Particle Size and Density Separates. Springer, New York, pp 1–90

14. Cohen E, Levy GJ, Borisover M (2014) Fluorescent components of organic matter in wastewater: Efficacy and selectivity of the water treatment. Water Res 55:323–334. https://doi.org/10.1016/j.watres.2014.02.040

15. Craine JM, Morrow C, Fierer N (2007) Microbial nitrogen limitation increases decomposition. Ecology 88:2105–2113. https://doi.org/10.1890/06-1847.1

16. De Troyer I, Amery F, Van Moorleghem C et al (2011) Tracing the source and fate of dissolved organic matter in soil after incorporation of a 13C labelled residue: A batch incubation study. Soil Biol Biochem 43:513–519. https://doi.org/10.1016/j.soilbio.2010.11.016

17. Dick DP, Knicker H, Ávila LG et al (2006) Organic matter in constructed soils from a coal mining area in southern Brazil. Org Geochem 37:1537–1545. https://doi.org/10.1016/j.orggeochem.2006.06.017

18. Dubnick A, Barker J, Sharp M et al (2010) Characterization of dissolved organic matter (DOM) from glacial environments using total fluorescence spectroscopy and parallel factor analysis. Ann Glaciol 51:111–122. https://doi.org/10.3189/172756411795931912

19. Fang C, Smith P, Moncrieff JB, Smith JU (2005) Similar response of labile and resistant soil organic matter pools to changes in temperature. Nature 433:57–59. https://doi.org/10.1038/nature03138

20. Fellman JB, D’Amore DV, Hood E, Boone RD (2008) Fluorescence characteristics and biodegradability of dissolved organic matter in forest and wetland soils from coastal temperate watersheds in southeast Alaska. Biogeochemistry 88:169–184. https://doi.org/10.1007/s10533-008-9203-x

21. Fellman JB, Hood E, D’Amore DV et al (2009) Seasonal changes in the chemical quality and biodegradability of dissolved organic matter exported from soils to streams in coastal temperate rainforest watersheds. Biogeochemistry 95:277–293. https://doi.org/10.1007/s10533-009-9336-6

22. Feng X, Feakins SJ, Liu Z et al (2016) Source to sink: Evolution of lignin composition in the Madre de Dios River system with connection to the Amazon basin and offshore. J Geophys Res Biogeosciences 121:1316–1338. https://doi.org/10.1002/2016JG003323

23. Filep T, Draskovits E, Szabó J et al (2015) The dissolved organic matter as a potential soil quality indicator in arable soils of Hungary. Environ Monit Assess 187:. https://doi.org/10.1007/s10661-015-
24. Gallo ME, Lauber CL, Cabaniss SE et al (2005) Soil organic matter and litter chemistry response to experimental N deposition in northern temperate deciduous forest ecosystems. Glob Chang Biol 11:1514–1521. https://doi.org/10.1111/j.1365-2486.2005.01001.x

25. Gee GW, Or D (2018) 2.4 Particle-Size Analysis. John Wiley & Sons, Ltd, pp 255–293

26. Gillman GP (1979) A proposed method for the measurement of exchange properties of highly weathered soils. Aust J Soil Res 17:129–139. https://doi.org/10.1071/SR9790129

27. Gödde M, David MB, Christ MJ et al (1996) Carbon mobilization from the forest floor under red spruce in the Northeastern U.S.A. Soil Biol Biochem 28:1181–1189. https://doi.org/10.1016/0038-0717(96)00130-7

28. Guggenberger G, Zech W, Schulten HR (1994) Formation and mobilization pathways of dissolved organic matter: evidence from chemical structural studies of organic matter fractions in acid forest floor solutions. Org Geochem 21:51–66. https://doi.org/10.1016/0146-6380(94)90087-6

29. Guo X, Zhang S, Shan X et al (2006) Characterization of Pb, Cu, and Cd adsorption on particulate organic matter in soil. Environ Toxicol Chem 25:2366. https://doi.org/10.1897/05-636R.1

30. Haberhauer G, Rafferty B, Strebl F, Gerzabek MH (1998) Comparison of the composition of forest soil litter derived from three different sites at various decompositional stages using FTIR spectroscopy. Geoderma 83:331–342. https://doi.org/10.1016/S0016-7061(98)00008-1

31. Hay MB, Myneni SCB (2007) Structural environments of carboxyl groups in natural organic molecules from terrestrial systems. Part 1: Infrared spectroscopy. Geochim Cosmochim Acta 71:3518–3532. https://doi.org/10.1016/j.gca.2007.03.038

32. Haynes RJ (2005) Labile organic matter fractions as central components of the quality of agricultural soils: An overview. Adv Agron 85:221–268. https://doi.org/10.2136/S0065-2113(04)85005-3

33. Huang YN, Qian TT, Dang F et al (2019) Significant contribution of metastable particulate organic matter to natural formation of silver nanoparticles in soils. Nat Commun 10:1–8. https://doi.org/10.1038/s41467-019-11643-6

34. Inbar Y, Chen Y, Hadar Y (1989) Solid-state carbon-13 nuclear magnetic resonance and infrared spectroscopy of composted organic matter. Soil Sci Soc Am J 53:1695–1701. https://doi.org/10.2136/sssaj1989.03615995005300060014x

35. Jardine PM, McCarthy JF, Weber NL (1989) Mechanisms of dissolved organic carbon adsorption on soil. Soil Sci Soc Am J 53:1378–1385. https://doi.org/10.2136/sssaj1989.03615995005300050013x

36. Jeffries TW (1991) Biodegradation of lignin-carbohydrate complexes. In: Physiology of Biodegradative Microorganisms. Springer Netherlands, pp 163–176

37. Kaiser K, Guggenberger G, Haumaier L, Zech W (2001) Seasonal variations in the chemical composition of dissolved organic matter in organic forest floor layer leachates of old-growth Scots
pine (Pinus sylvestris L.) and European beech (Fagus sylvatica L.) stands in northeastern Bavaria. Germany Biogeochemistry 55:103–143. https://doi.org/10.1023/A:1010694032121

38. Kaiser K, Zech W (2000) Dissolved organic matter sorption by mineral constituents of subsoil clay fractions. J Plant Nutr Soil Sci 163:531–535. https://doi.org/10.1002/1522-2624(200010)163:5<531::AID-JPLN531>3.0.CO;2-N

39. Kalbitz K, Kaiser K, Bargholz J, Dardenne P (2006) Lignin degradation controls the production of dissolved organic matter in decomposing foliar litter. Eur J Soil Sci 57:504–516. https://doi.org/10.1111/j.1365-2389.2006.00797.x

40. Kalbitz K, Solinger S, Park J et al (2000) Controls on the dynamics of dissolved organic matter in soils: a review. In: Soil Sci

41. Kirk TK, Farrell RL (1987) Enzymatic “combustion”: The microbial degradation of lignin. Annu Rev Microbiol 41:465–501. https://doi.org/10.1146/annurev.mi.41.100187.002341

42. Klotzbücher T, Kaiser K, Filley TR, Kalbitz K (2013) Processes controlling the production of aromatic water-soluble organic matter during litter decomposition. Soil Biol Biochem 67:133–139. https://doi.org/10.1016/j.soilbio.2013.08.003

43. Lal R, Negassa W, Lorenz K (2015) Carbon sequestration in soil. Curr Opin Environ Sustain 15:79–86

44. Lehmann J, Kleber M (2015) The contentious nature of soil organic matter. Nature 528:60–68

45. Li X, Liu Z, Chen W et al (2018) Production and transformation of dissolved and particulate Organic matter as indicated by amino acids in the Pearl River Estuary, China. J Geophys Res Biogeosciences 123:3523–3537. https://doi.org/10.1029/2018JG004690

46. Lu YH, Bauer JE, Canuel EA et al (2014) Effects of land use on sources and ages of inorganic and organic carbon in temperate headwater streams. Biogeochemistry 119:275–292. https://doi.org/10.1007/s10533-014-9965-2

47. Matiasek SJ, Hernes PJ (2019) The chemical fingerprint of solubilized organic matter from eroded soils and sediments. Geochim Cosmochim Acta 267:92–112. https://doi.org/10.1016/j.gca.2019.09.016

48. Michel K, Matzner E (2002) Nitrogen content of forest floor Oa layers affects carbon pathways and nitrogen mineralization. Soil Biol Biochem 34:1807–1813. https://doi.org/10.1016/S0038-0717(02)00170-0

49. Murphy KR, Stedmon CA, Graeber D, Bro R (2013) Fluorescence spectroscopy and multi-way techniques. PARAFAC Anal Methods 5:6557–6566

50. Niemeyer J, Chen Y, Bollag J-M (1992) Characterization of humic acids, composts, and peat by diffuse reflectance Fourier-transform infrared spectroscopy. Soil Sci Soc Am J 56:135–140. https://doi.org/10.2136/sssaj1992.03615995005600010021x

51. Olsson AM, Salmén L (2004) The association of water to cellulose and hemicellulose in paper examined by FTIR spectroscopy. Carbohydr Res 339:813–818. https://doi.org/10.1016/j.carres.2004.01.005
52. Pandey KK (1999) A study of chemical structure of soft and hardwood and wood polymers by FTIR spectroscopy. J Appl Polym Sci 71:1969–1975. https://doi.org/10.1002/(SICI)1097-4628(19990321)71:12<1969::AID-APP6>3.0.CO;2-D

53. Ritchie GSP, Dolling PJ (1985) The role of organic matter in soil acidification. Aust J Soil Res 23:569–576. https://doi.org/10.1071/SR9850569

54. Saiya-Cork KR, Sinsabaugh RL, Zak DR (2002) The effects of long term nitrogen deposition on extracellular enzyme activity in an Acer saccharum forest soil. Soil Biol Biochem 34:1309–1315. https://doi.org/10.1016/S0038-0717(02)00074-3

55. Six J, Conant RT, Paul EA, Paustian K (2002) Stabilization mechanisms of soil organic matter: Implications for C-saturation of soils. Plant Soil 241:155–176

56. Six J, Paustian K, Elliott ET, Combrink C (2000) Soil structure and organic matter I. Distribution of aggregate-size classes and aggregate-associated carbon. Soil Sci Soc Am J 64:681–689. https://doi.org/10.2136/sssaj2000.642681x

57. Sørensen H (1962) Decomposition of lignin by soil bacteria and complex formation between autoxidized lignin and organic nitrogen compounds. J Gen Microbiol 27:21–34. https://doi.org/10.1099/00221287-27-1-21

58. Spohn M (2015) Microbial respiration per unit microbial biomass depends on litter layer carbon-to-nitrogen ratio. Biogeoosciences 12:817–823. https://doi.org/10.5194/bg-12-817-2015

59. Stotzky G (1966) Influence of clay minerals on microorganisms. 3. Effect of particle size, cation exchange capacity, and surface area on bacteria. Can J Microbiol 12:1235–1246. https://doi.org/10.1139/m66-165

60. Thurman EM (1985) Transport, origin and source of dissolved organic carbon. In: Organic Geochemistry of Natural Waters. Springer Netherlands, pp 67–85

61. Tiessen H, Cuevas E, Chacon P (1994) The role of soil organic matter in sustaining soil fertility. Nature 371:783–785. https://doi.org/10.1038/371783a0

62. Wang M, Tian Q, Liao C et al (2019) The fate of litter-derived dissolved organic carbon in forest soils: results from an incubation experiment. Biogeochemistry 144:133–147. https://doi.org/10.1007/s10533-019-00576-3

63. Weishaar JL, Aiken GR, Bergamaschi BA et al (2003) Evaluation of specific ultraviolet absorbance as an indicator of the chemical composition and reactivity of dissolved organic carbon. Environ Sci Technol 37:4702–4708. https://doi.org/10.1021/es030360x

64. Xenopoulos MA, Barnes RT, Boodoo KS et al (2021) How humans alter dissolved organic matter composition in freshwater: relevance for the Earth's biogeochemistry. Biogeochemistry 1–26. https://doi.org/10.1007/s10533-021-00753-3

65. Xu Y, Chen Z, Fontaine S et al (2017) Dominant effects of organic carbon chemistry on decomposition dynamics of crop residues in a Mollisol. Soil Biol Biochem 115:221–232. https://doi.org/10.1016/j.soilbio.2017.08.029
66. Zacháry D, Filep T, Jakab G et al (2018) Kinetic parameters of soil organic matter decomposition in soils under forest in Hungary. Geoderma Reg 14:. https://doi.org/10.1016/j.geodrs.2018.e00187

67. Zhao Q, Qiu Y, Lan T et al (2021) Comparison of lead adsorption characteristics onto soil-derived particulate organic matter versus humic acid. J Soils Sediments 1–15. https://doi.org/10.1007/s11368-021-02911-4

68. Zimmermann M, Leifeld J, Schmidt MWI et al (2007) Measured soil organic matter fractions can be related to pools in the RothC model. Eur J Soil Sci 58:658–667. https://doi.org/10.1111/j.1365-2389.2006.00855.x

69. Zsolnay Á (2003) Dissolved organic matter: Artefacts, definitions, and functions. In: Geoderma. Elsevier, pp 187–209

Figures

![Figure 1](image-url)
Relative contribution of PARAFAC components in the 13 forest soils

Figure 2

RDA triplot for DOM characteristics (F=3.52, p=0.005)
Figure 3

RDA triplot for POM characteristics (F=3.37, p=0.002)
Figure 4

RDA triplot for the connection between POM and DOM (F=4.09, p=0.007)