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RESEARCH ARTICLE

Insight into the dynamics of protected and non-protected carbon pools in four soils with different land uses

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Abstract
Background and aims To provide insight into the patterns of soil organic matter decomposition, changes in the quantity of biopolymers and the correlation between them were followed using 2D correlation spectroscopy (2DCOS) FTIR.
Methods Soil organic matter fractions with different vegetation/land use (grass, spruce, oak and arable) were examined in a 1-year laboratory incubation. The non-protected organic matter fraction was calculated in terms of particulate organic matter (POM), the carbon stabilized in aggregates as S + A (sand + aggregates), and the mineral-associated organic matter (MAOM) as the s + c (silt and clay) fraction.
Results Forest soils (spruce, oak) exhibited high C and N accumulation in the POM fraction (48, 43% and 29, 22% for spruce and oak, respectively) due to the limited decomposition, caused by low pH and high soil C/N ratio. The 2DCOS analysis revealed that carbohydrate-protein and carbohydrate-lignin correlations could be observed most frequently during incubation. The carbohydrate-protein correlation was negative in all cases, for all fractions and for all vegetation types, which suggests biogeochemical linkage between these biopolymers. The temporal order of the spectral changes was widely varied for the vegetation types and especially for the SOM fractions. Lipid/Lignin → Carbohydrate or Lipid → Lignin/Carboxyl/Protein sequences were found for the protected carbon pools (S + A and s + c), possibly because of the readily available abundant N compounds present in MAOM.
Conclusion Although lipids and lignin are considered as chemically stable materials that commonly remain constant during decomposition, these compounds were found to be very susceptible in all the fractions.

Keywords Laboratory incubation · Soil organic matter fractions · Decomposition · Vegetation · Land use

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Introduction

Preserving soil organic matter is a priority, both to maintain soil fertility and to reduce the impacts of global climate change. However, soil organic matter is not a homogeneous material, but can be broken down into pools with different mean residence times (MRT) ranging from days to thousands of years (Kuzyakov 2006; Schmidt et al. 2011; Ma et al. 2019). Differences in the degradability of carbon pools can be explained by the different stabilization mechanisms of the carbon. The main stabilization mechanisms in soils are biological, physical and chemical stabilization (von Lützow et al. 2007). Despite decades of research, there is still no consensus on the existence of materials that are recalcitrant due to their chemical structure, since many studies contradict this (Kleber 2010; Dungait et al. 2012). The most likely picture of carbon stabilization is that carbon binding in aggregates and on mineral surfaces is the main mechanism of stabilization (Dungait et al. 2012). In the case of carbon bound to mineral surfaces, also known as mineral-associated organic matter (MAOM), organic molecules are bound to the surfaces by strong covalent (ligand exchange) or weak intermolecular (van der Waals) chemical bonds (von Lützow et al. 2007; Lehmann and Kleber 2015). While the aggregates are a tight mixture of minerals and organic compounds that prevent microorganisms from accessing the soil organic matter (SOM) (Six et al. 2004).

In models and studies on carbon stabilization, aggregates and MAOM are usually separated from bulk soil by a combination of successive sieving and density separation steps, leading to physical fractions that have different OM characteristics (e.g. residence times, turnover times, and chemical composition) (Hassink et al. 1997; Zimmermann et al. 2007; Mueller et al. 2009). However, very few observations have been published on the dynamics of mineralisation for the different biomolecules in SOM fractions. In general, SOM mineralization is assessed by measuring the time course of CO_2 exhaled from incubated soils (e.g. Spaccini et al. 2000; Jeewani et al. 2021; Shaaban et al. 2023), but the consideration of CO_2 respiration rates alone does not provide an insight into the dynamics of organic carbon. The lack of molecular-level studies hinders the ability to model and predict changes in soil organic C stocks in response to environmental changes (Campbell and Paustian 2015).

Fourier transform infrared (FTIR) spectroscopy is a commonly used technique that can distinguish the major biopolymers in organic matter, such as proteins, carbohydrates, cellulose, lignin and lipids (Smidt and Meissl 2007; Plaza et al. 2007). Previous studies have attempted to track changes in soil organic matter structure using conventional one-dimensional FTIR (e.g. Plaza et al. 2007; Tang et al. 2011; Hao et al. 2022), however, 1D FTIR has been shown to have spectral overlaps that reduce the selectivity of the method due to the extreme heterogeneity of the molecules in organic matter. Therefore, one-dimensional FTIR spectra provide only limited structural information on the degradation of soil organic matter. Two-dimensional correlation spectroscopy (2DCOS), developed by Noda (1993), can be applied to solve the above problem. 2DCOS analysis extends the spectral data over a second dimension by means of external perturbation, i.e. the use of an additional factor in the analysed system (e.g. increasing temperature, time, etc.), which selectively excites or changes various components in the system. The resulting changes in the spectra reveal relationships between different peaks and provide information on the sequential order of changes in these peaks (Noda and Ozaki 2004; Abdulla et al. 2010). Although many investigations have been made on the degradation of organic matter in incubation experiments, the 2DCOS approach, which has been used in many fields of organic matter research, e.g. to monitor chemical changes in DOM (Chen et al. 2019) or metal binding to SOM (Sun et al. 2019), has not previously been applied for this purpose. To provide an insight into the changes in organic matter pools and the patterns of SOM decomposition, changes in chemical properties of soil organic matter fractions under different types of vegetation/land use (grass, spruce, oak and arable) were examined in a 1-year laboratory incubation. The SOM fractions were prepared according to Zimmermann et al. (2007). The non-protected organic matter fraction was calculated in terms of particulate organic matter (POM), the carbon stabilized in aggregates as S + A (sand + aggregates) and the mineral-associated organic matter (MAOM) as the s + c (silt and clay) fraction. It was hoped to reveal not only different patterns in C pools stabilised by different mechanisms, but also the effects of different plant cover on the mineralisation patterns in the soil.

One specific aim was to follow changes in the carbon and nitrogen contents of fractions with different mechanisms of carbon stabilization under different vegetation types. Another goal was to investigate the dynamics of biopolymers during incubation using the 2DCOS FTIR technique, which is able to eliminate spectral overlaps and help elucidate the sequence of biopolymer degradation. To the best of our knowledge, this is a novel approach with great potential to reveal chemical linkages between biomolecular compounds such as carbohydrates or lignin derivatives. One specific aim was to follow changes in the carbon and nitrogen contents of fractions with different mechanisms of carbon stabilization under different vegetation types. Our hypothesis is that carbon and nitrogen content and the ratio of carbon to nitrogen is affected by not only the stabilization mechanism of SOM, but also vegetation/land use. Another goal was to investigate the dynamics of biopolymers during incubation using the 2DCOS FTIR technique, which is able to eliminate spectral overlaps and help elucidate the sequence of biopolymer degradation. We also hypothesized that there will be typical patterns on the correlation of the main biomolecules categories and the 2DCOS method is useful for insight into the sequence of biopolymer degradation.

Materials and methods

Study site and soil samples analysis

Composite soil samples (cca. 1500 g) were taken using a soil auger from the top 20 centimetres of Hungarian soils with different vegetation cover (grassland, spruce, oak and arable) using 5 subsamples from 1 m^2. The sampling sites were in areas very close to each other in order to prevent climatic factors from affecting the transformation of organic matter (mean annual temperature: 9.2 °C, mean annual precipitation: 725 mm $year^{-1}$). The soil samples were transported to the laboratory, dried and passed through a 2 mm sieve. The pH of the soils was determined in distilled water and KCl (Thomas 1996), and the particle size distribution by laser diffraction (Ryzak and Bieganowski 2011). The basic data of the soils are presented in Table 1.

Soil fractionation

The Zimmermann fractionation method was used, as modified by Poeplau et al. (2013). Briefly, each soil sample was subjected to ultrasonic dispersion (22 J ml^{-1}) in 100 ml distilled water, after which the samples were wet-sieved over a 63 µm screen in order to separate the silt and clay fraction (s + c) from the sand and stable aggregates (S + A) and particulate organic matter (POM). The sieved s + c fraction was centrifuged for 30 min at 2000 g and dried at 40 °C. The sieved residue (S + A, POM) was isolated by density, using sodium iodide (NaI, ρ = 1.6 g cm^{-3}). The sodium iodide solution was added to the S + A + POM fraction (> 63 µm) and centrifuged for 15 min at a speed of 1000 g. The floating POM fraction was decanted and the step was repeated to ensure the complete extraction of POM. Both fractions were carefully washed with deionised water to remove the sodium iodide and then dried at 40 °C.

Incubation experiment

In this experiment 30 g each of the s + c and S + A fractions and 1.5 g of the POM fraction were incubated for 1 year at 25 °C in three replicates (total of 36 samples). The samples were preincubated at 60% WHC at 20 °C for two weeks, because rewetting and sieving affect the availability of SOM for microorganisms and may cause an increase in the rate of

Table 1 Basic properties of the soils

Site	Vegetation	pH(H_2O)	pH (KCl)	Sand %	Silt	Clay
Faber meadow, Sopron, Hungary (N 47°39'56,508"; E 16°32'57,480")	Grass (*Festuca rubra*)	5.48	4.21	21.7	70.9	7.4
Sopron Mountains, Hungary (N 47°39'20,082"; E 16°32'28,595")	Spruce (*Picea abies*)	3.87	2.92	26.4	69.4	4.2
Sopron Mountains, Hungary (N 47°39'21,101"; E 16°32'26,645")	Sessile Oak (*Quercus petraea*)	4.28	3.40	16.8	78.7	4.5
Harka, Hungary (N 47°37'08,208"; E 16°34'58,404")	Arable *(Zea mays L.)*	5.58	5.06	25.8	69.0	5.2

respiration (Franzluebbers 1999). The moisture content of the fractions was determined using column method (Govindasamy et al. 2023) and the samples were adjusted to 60% WHC. The evaporated water was replenished with deionized water at two-week intervals.

The fractions were sampled nine times during the incubation period with micro spoon (~0.1 g), each time correcting the fraction mass by the amount of sample taken to ensure that the WHC was set accurately. Samples taken on days 0, 35, 77, 105, 133, 166, 189, 217, 273 and 357 were dried at 40 °C and stored at 4 °C prior to FT-IR analysis. The soil fractions were subjected to direct combustion (Elementar Vario MACRO Cube, Elementar GmbH) to measure total organic carbon (TOC) and total nitrogen (TN) at the initial and final stages of incubation (day 0 and day 357).

FT-IR analysis and 2DCOS

All the samples taken on days 0, 35, 77, 105, 133, 166, 189, 217, 273 and 357 were dried at 60 °C before FT-IR measurements. The FT-IR DRIFT (diffuse reflectance) measurements were carried out on a Bruker Vertex 70 spectrometer with an RT-DLaTGS detector. For each sample 128 scans were made at a spectral range of 4000–400 cm^{-1} and a resolution of 4 cm^{-1}, and three replicates were recorded. Noise in the FTIR data could cause artefacts in the 2D treatment, especially for asynchronous maps (Noda and Ozaki 2004), so the PCA denoising method was applied as proposed by Jung (2004). The spectra were subjected to 7-point 2nd order Savitzky-Golay smoothing and extended multiplicative scattering correction (EMSC). All pre-processing procedures were carried out with Quasar 1.7.0 software.

Temporal changes in the FT-IR spectra of the samples (total of 972 spectra) were evaluated using 2D correlation spectroscopy (2DCOS). Briefly, in the theory of 2DCOS (Noda 1993) the sequentially sampled spectra of the system y (x_i, p_k) described by an electro-magnetic probe (x, representing wavelength, wavenumber, frequency, and so on) are modified by an external perturbation variable (p, representing time, temperature, etc.). This external perturbation stimulates the system to cause changes in the state, order, surroundings, etc. The overall response of the stimulated system to the applied external perturbation leads to distinctive changes in the measured spectra. In 2D correlation analysis the spectral variation induced by applied perturbation is referred to as a dynamic spectrum. A dynamic spectrum ($\tilde{y}(\nu_j, t_k)$) can be defined as:

$$\tilde{y}\left(\nu_j, t_k\right) = y\left(\nu_j, t_k\right) - \bar{y}\left(\nu_j\right),$$

where $\bar{y}(\nu_j, t_k)$ is the reference spectrum, which is usually the average of all spectra, and t is the external perturbation variable (in this study, time).The synchronous correlation spectrum ($\Phi(\nu_1,\nu_2)$), which represents the simultaneous correlation between the wavenumbers ν_1 and ν_2, can be written as follows, when the spectral data are evenly spaced during the interval between t_1 and t_m:

$$\Phi(\nu_1, \nu_2) = \frac{1}{m-1}\sum_{k=1}^{m} \tilde{y}(\nu_1, t_k) \cdot \tilde{y}\left(\nu_2, t_k\right)$$

The asynchronous correlation spectrum ($\psi(\nu_1,\nu_2)$) shows the sequential (temporal) correlation of spectral intensities at wavenumbers ν_1 and ν_2:

$$\Psi(\nu_1, \nu_2) = \frac{1}{m-1}\sum_{k=1}^{m} \tilde{y}(\nu_1, t_k) \cdot \sum_{k=1}^{m} N_{ik}\tilde{y}(\nu_2, t_k)$$

where the term N_{ik} is the Hilbert − Noda transformation matrix element

$$N_{ik} = \begin{Bmatrix} if\ i = k & 0 \\ otherwise & \frac{1}{\pi(k-i)} \end{Bmatrix}$$

The 2DCOS analysis was performed using OriginPro 2020b software

PLFA analysis

The soil microbial biomass and community composition of the soil fractions were evaluated using phospholipid fatty acid (PLFA) analysis, following the procedure outlined by Ellis and Ritz (2018). In summary, PLFAs were extracted from 2 g of freeze-dried soil samples using the Bligh-Dyer extractant, consisting of a mixture of methanol, chloroform, and 50 mM K_2HPO_4 in a ratio of 1:0.5:0.4 (v/v/v). Subsequently, the fatty acid methyl ester profiles (FAME) were examined using a gas chromatograph equipped with a flame ionization detector (Agilent 8890).

The concentration of each phospholipid fatty acid (PLFA) was determined using the 19:0 internal standard, and all the identified PLFA concentrations were expressed as nmol PLFA g^{-1} dry weight of soil. The sum of the following PLFAs was used to measure the bacterial biomass: i15:0, a15:0, i16:0, 16:1ω7, i17:0, cy17:0, 18:1ω7, and cy19:0 (Frostegård and Bååth 1996; Kandeler et al. 2008), while e 18:2ω6,9 and 18:1 ω9 are considered as fungi (Frostegard et al. 1993). Gram-positive bacteria were characterized by iso and anteiso saturated and by branched PLFAs, while Gram-negative bacteria were represented by monounsaturated and cyclopropyl PLFAs, specifically the 17:0 and 19:0 PLFAs (Zelles 1999).

Statistical analysis

To estimate significant differences between the measured parameters (C and N content, C/N ratio and PLFA data) one-way analysis of variance (ANOVA) was used with the post hoc Tukey test. Linear regression was used for describing the relationship between POC, pH and carbon to nitrogen ratio. The ANOVA models were performed using SPSS 22 software (SPSS Inc, Chicago, IL, US), while linear regression was done using Origin 2020b software (OriginLab Corporation, Northampton, MA, US).

Results

Mass distribution and C and N storage among the soil organic fractions

The soil in the spruce forestland contained a very high mass fraction of POM, more than 13% of the total soil mass, while in oak (4.8%), grassland (2.2%) and arable soils (< 1%) with mineral organic matter fractions (S + A and s + c) accounting for 95–99% of the mass (Fig. 1a).

The accumulation of organic carbon varied considerably between the different land uses: forest soils showed a high accumulation of C and N in the POM fraction (48, 43% and 29, 22% for spruce and oak, respectively), while the soils of the grassland and arable sites had much lower accumulation (Fig. 1b). Most of the C and N accumulated in the S + A fraction, except for spruce, where most them accumulated in the POM fraction. These values were similar to those found by Zimmermann et al. (2007) for arable land, and for temperate and alpine permanent grassland sites. Compared to grassland containing similar amounts of POM, arable soil accumulated a much lower proportion of C in the S + A fraction, resulting from the weaker aggregation under more intensive soil management (Low, 1972).

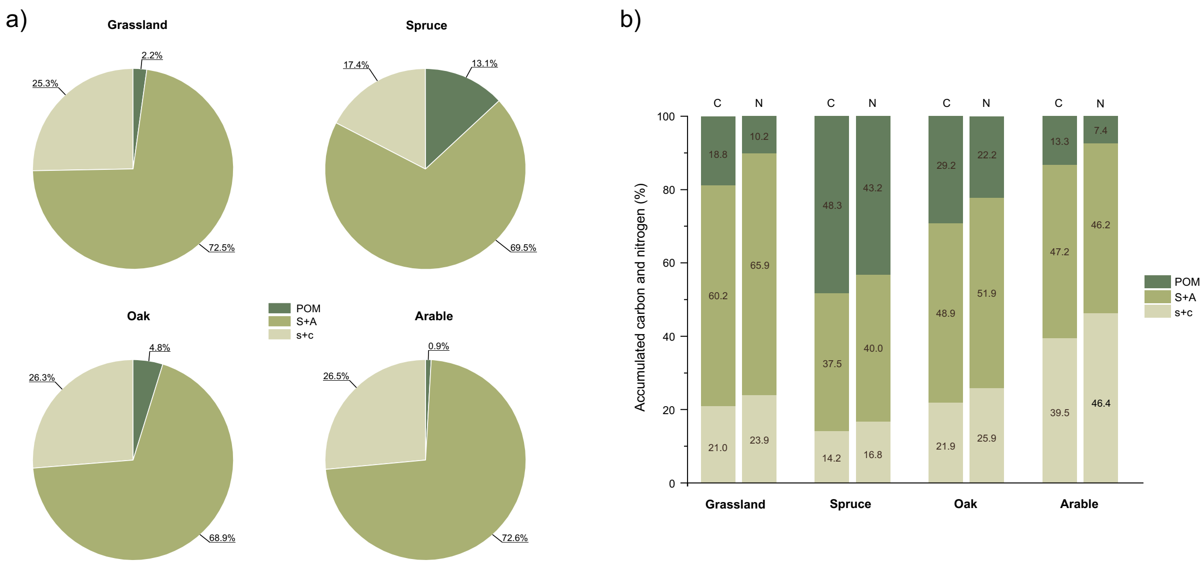


Fig. 1 Mass distribution of soil organic matter fractions (**a**) and carbon and nitrogen accumulation in the organic matter fractions (**b**) for sites with different vegetation

Significant linear relationships were also revealed between POM-C and the pH and carbon to nitrogen ratio of the soils (Fig. 2).

Carbon and nitrogen contents of the organic matter fractions during the incubation

Both the carbon and nitrogen contents of the organic matter fractions of soils with different vegetation types exhibited wide variation (Table 2). The C and N content of the POM fractions ranged from 32.9 to 39.4 and 1.26–1.90%, respectively, those of the S + A fraction from 1.7 to 5.7 and 0.12–0.26%, respectively, and those of the s + c fractions from 3.2 to 8.6 and 0.26–0.37%, respectively.

The C/N ratio for the soil fractions in the spruce area was extremely high: 31.2 for POM, 26.5 for S + A and 23.5 for s + c. In general, the C/N ratio of the fractions decreased with decreasing particle size.

During incubation, the C content of all the organic fractions decreased significantly, as did the C/N ratio, while the N content remained practically unchanged between the fractions.

2D correlation spectroscopy was performed to extract compositional changes in soil organic fractions with time as perturbation (Figs. 3, 4 and 5). The auto peaks found in the diagonals of the synchronous spectra, show which region of the spectrum was most susceptible during incubation. The correlation peaks in the synchronous spectra show the movement of two different spectral regions in the same or opposite directions; if the correlation was positive, there was movement together, and if negative, there was opposite movement. Asynchronous 2D spectra can be interpreted using Noda's rules (Noda 1993), which can be used to determine which spectral domain change precedes the other. Briefly, assuming that wavenumbers ν_1 and ν_2 are positively correlated in the synchronous spectrum, a positive cross-peak at ν_1 and ν_2 indicates that ν_1 occurs predominantly before that at ν_2 in the asynchronous spectrum. Under the same circumstances, a negative cross-peak at wavenumbers ν_1 and ν_2 indicates that a change at ν_1 occurs predominantly after that at ν_2. However, this sign rule is reversed when the synchronous correlation intensity at the same coordinates is negative.

The bands appearing in the 2DCOS spectra (Table 3) are assigned to their corresponding chemical structures (Table 4). All major organic matter groups were found to be significantly variable during the incubation; even the amount of lipid and lignin-like substances, which are thought to be difficult to degrade, was markedly altered. No clear pattern was seen in this respect between the fractions and soils, but perhaps it is worth noting that the amount of protein-like substances in the mineral fractions was not as susceptible as in POM.

During incubation, the most common carbohydrate-protein and carbohydrate-lignin correlations were most frequently observed. The carbohydrate-protein correlations were negative in all cases, meaning that they changed in opposite directions, one

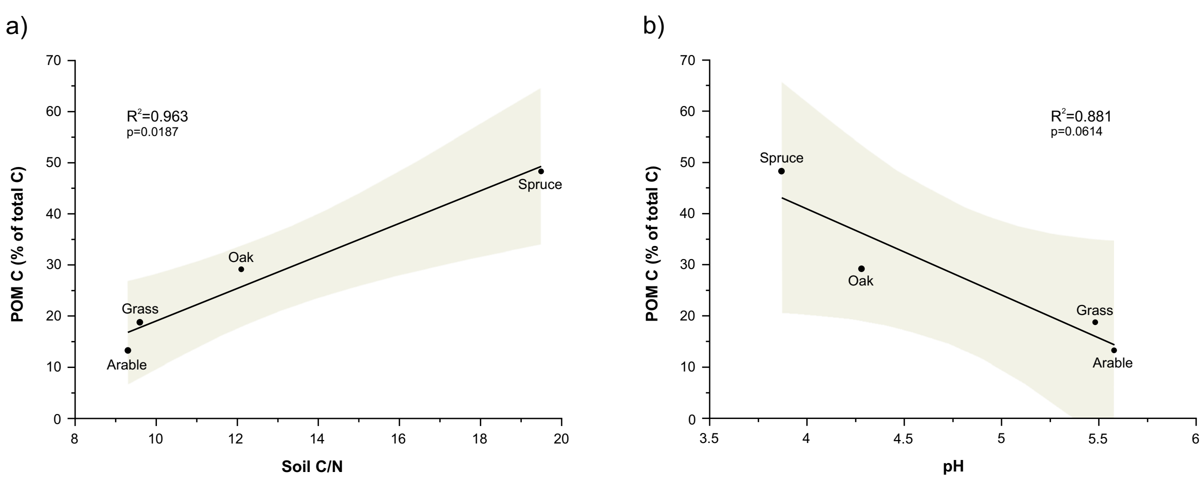


Fig. 2 Relationships between the carbon accumulated in POM fractions (POM-C) and the pH (**a**) and soil C/N ratio (**b**)

Table 2 Carbon, nitrogen and C/N ratio of whole soils and organic matter fractions FT-IR 2D COS spectra of the soil fractions

Vegetation	Fraction	C m/m%	N	C/N
Whole soil				
Grass		3.1 (0.07)[1]	0.29 (0.010)	10.9 (0.21)
Spruce		9.8 (0.24)	0.45 (0.017)	21.8 (0.32)
Oak		4.6 (0.39)	0.34 (0.025)	13.6 (0.17)
Arable		1.9 (0.04)	0.18 (0.000)	10.8 (0.21)
Before incubation				
Grassland	POM	32.9 (g)[2]	1.27 (gh)	25.9 (ij)
	S+A	3.2 (abc)	0.25 (b)	13.2 (bc)
	s+c	3.2 (abc)	0.26 (bc)	11.9 (abc)
Spruce	POM	39.2 (h)	1.26 (g)	31.2 (k)
	S+A	5.7 (cde)	0.22 (b)	26.5 (ij)
	s+c	8.6 (e)	0.37 (ef)	23.5 (fgh)
Oak	POM	39.4 (h)	1.60 (j)	24.6 (ghi)
	S+A	4.6 (bcd)	0.26 (bc)	17.5 (d)
	s+c	5.4 (cd)	0.34 (cdef)	16.0 (d)
Arable	POM	38.8 (h)	1.55 (j)	25.1 (hi)
	S+A	1.7 (ab)	0.12 (a)	13.8 (c)
	s+c	3.9 (abc)	0.33 (cdef)	11.6 (abc)
After incubation				
Grassland	POM	28.5 (f)	1.26 (g)	22.6 (efg)
	S+A	2.9 (abc)	0.24 (b)	12.5 (bc)
	s+c	2.9 (abc)	0.26 (bc)	11.3 (ab)
Spruce	POM	36.9 (h)	1.35 (hi)	27.3 (j)
	S+A	5.1 (cd)	0.24 (b)	21.5 (ef)
	s+c	7.2 (de)	0.40 (f)	18.2 (d)
Oak	POM	29.5 (f)	1.44 (i)	20.5 (e)
	S+A	3.6 (abc)	0.28 (bcd)	13.0 (bc)
	s+c	3.6 (abc)	0.36 (def)	10.3 (a)
Arable	POM	30.6 (fg)	1.39 (i)	22.1 (ef)
	S+A	1.2 (a)	0.11 (a)	11.5 (ab)
	s+c	3.0 (abc)	0.29 (bcde)	10.2 (a)

[1]standard deviation; [2]significant differences within the column according to Tukey's post hoc test based on the all value of the column

decreasing when the other increased. A similar result was obtained for the carbohydrate-lignin relationship, where there was a negative correlation for all but the arable soil S + A fraction. A carbohydrate-carboxyl correlation was also found in the mineral fractions.

The temporal order of the spectral changes was very varied both for the vegetation types and even more so for the SOM fractions. Typically, changes in compounds considered to be easily mineralizable (carbohydrate, protein) preceded those in compounds that are more difficult to break down (lipid, lignin). However, particularly in the s + c fractions, surprising sequences were found, such as Lipid/Lignin → Carbohydrate or Lipid → Lignin/Carboxyl/Protein.

PLFA analysis

High total PLFA values were observed for the POM fractions, while the mineral fractions of the soils had much lower PLFA, the exception being the s + c fraction of spruce soil, with a high 1500 nmol/g value (Fig. 6a).

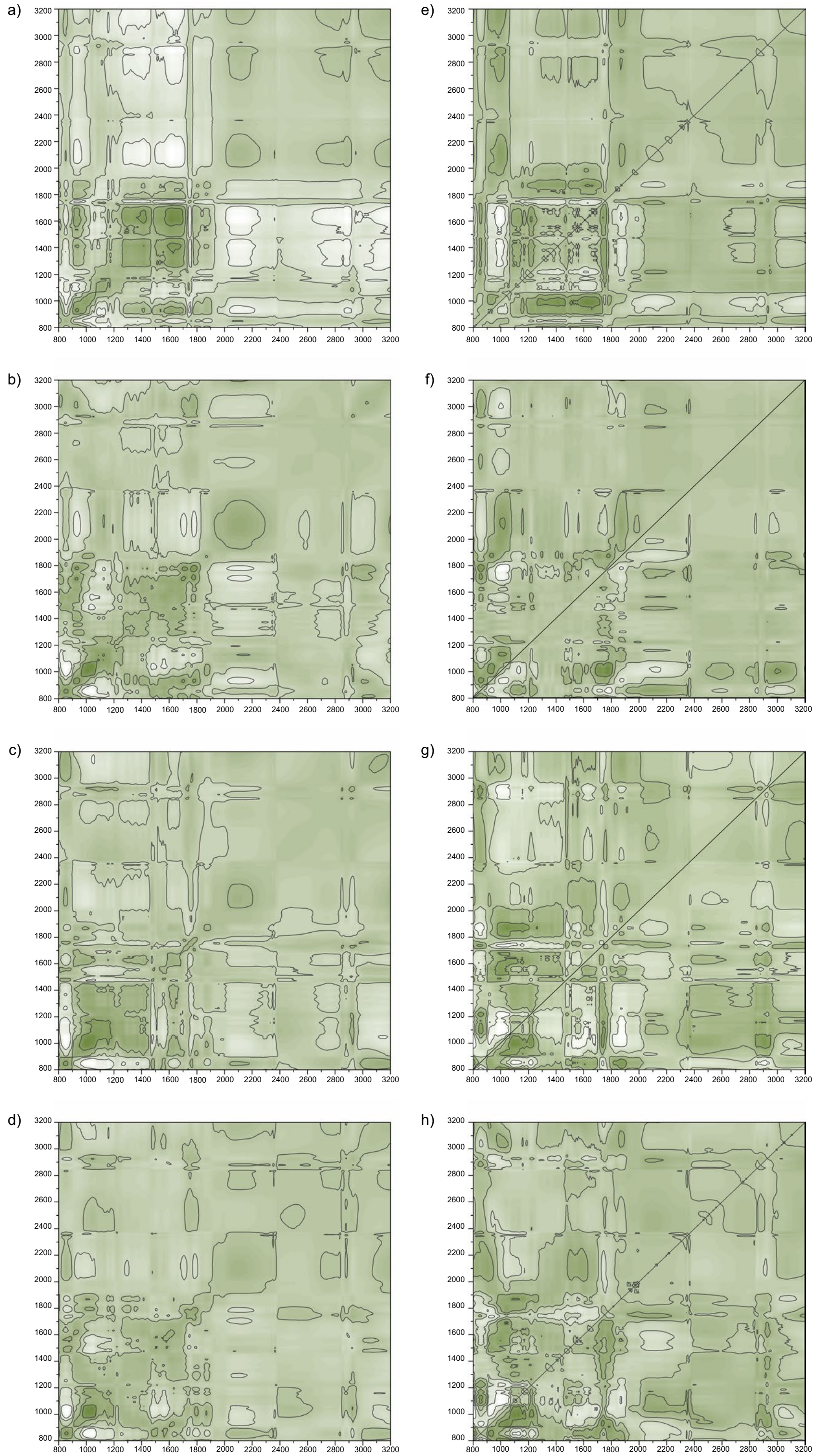
The microbial community composition in the soil organic matter fractions was assessed from PLFA biomarkers at the end of the incubation using the fungi to bacteria ratio (F/B ratio) and the Gram positive to Gram negative bacteria ratio (GP/GN ratio; Fig. 6b and c). The F/B ratio ranged from 0.15 to 0.33, with the highest value for the grass POM fraction. In most cases, the POM fractions had the highest F/B ratio, except in the case of spruce POM. Besides POM, the s + c fraction of oak and the S + A fraction of spruce had relatively high F/B ratios.There was much more significant variation in the GP/GN ratio, and in general the POM fractions had the smallest ratio (1.5–2.7). The S + A and s + c fractions had much larger ratios, typically in the range 3.0–5.0.

Discussion

Factors affecting the carbon and nitrogen accumulation in soil organic pools

Very different proportions of carbon and nitrogen pools were observed for the four soils with different vegetation types. For example, in the case of forest soils high amounts of carbon and nitrogen accumulated in the POM fractions (30–48% for C and 22–43% for N; Fig. 1b), despite the fact that the POM fraction was neither physically nor chemically protected. It is generally accepted that the degradation rate of the POM fraction is higher than that of the protected mineral C pools (Feng et al. 2016). To understand why carbon and nitrogen accumulated in the POM fraction of forest soils, the factors that influence the degradation of organic matter

Fig. 3 Synchronous and asynchronous spectra for the POM fraction for grassland (**a** and **e**), spruce (**b** and **f**), oak (**c** and **g**) and arable (**d** and **h**). The dark regions show positive, while the light ones show negative correlations



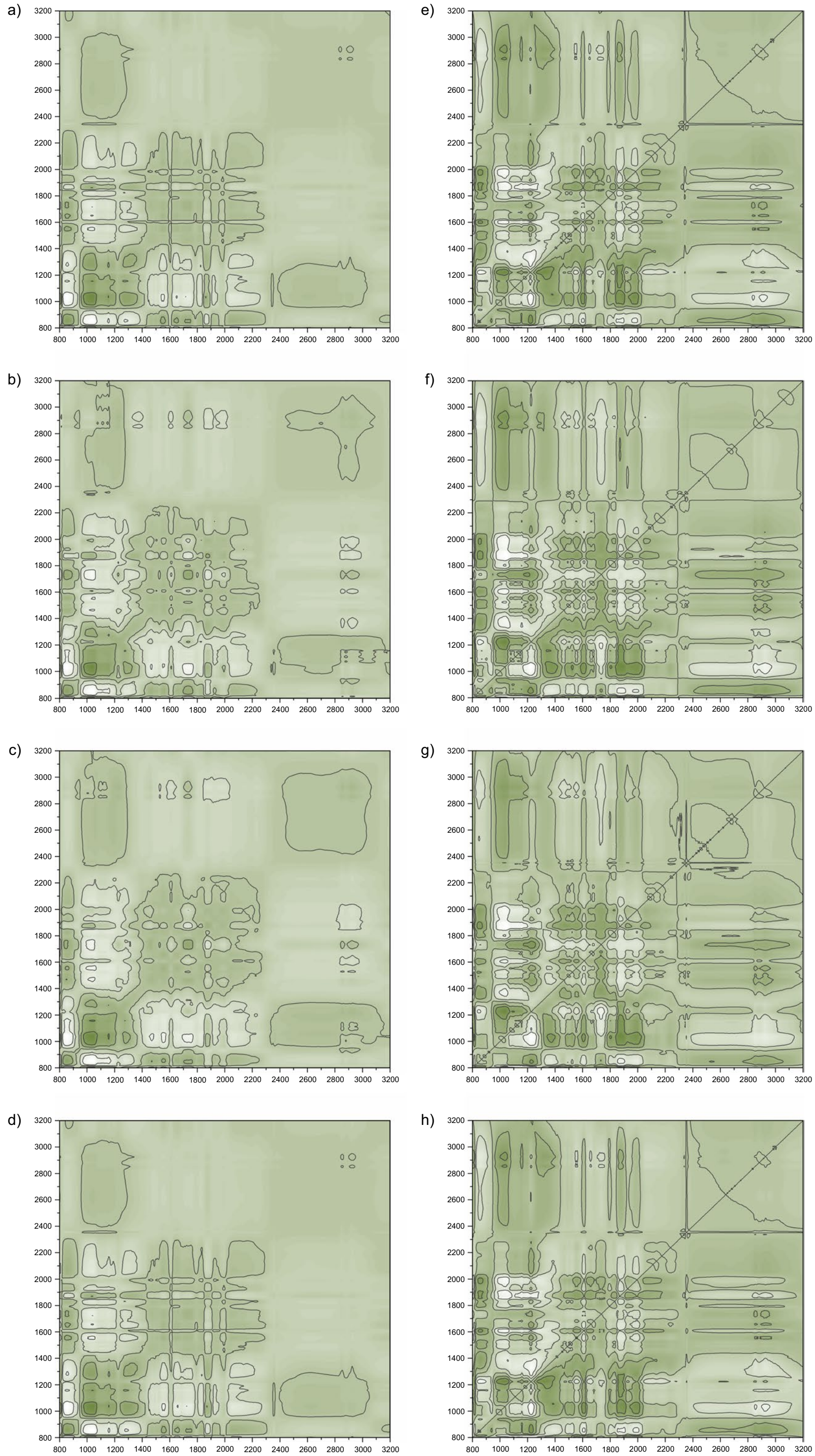


Fig. 4 Synchronous and asynchronous spectra for the S + A fraction for grassland (**a** and **e**), spruce (**b** and **f**), oak (**c** and **g**) and arable (**d** and **h**). The dark regions show positive, while the light ones show negative correlations

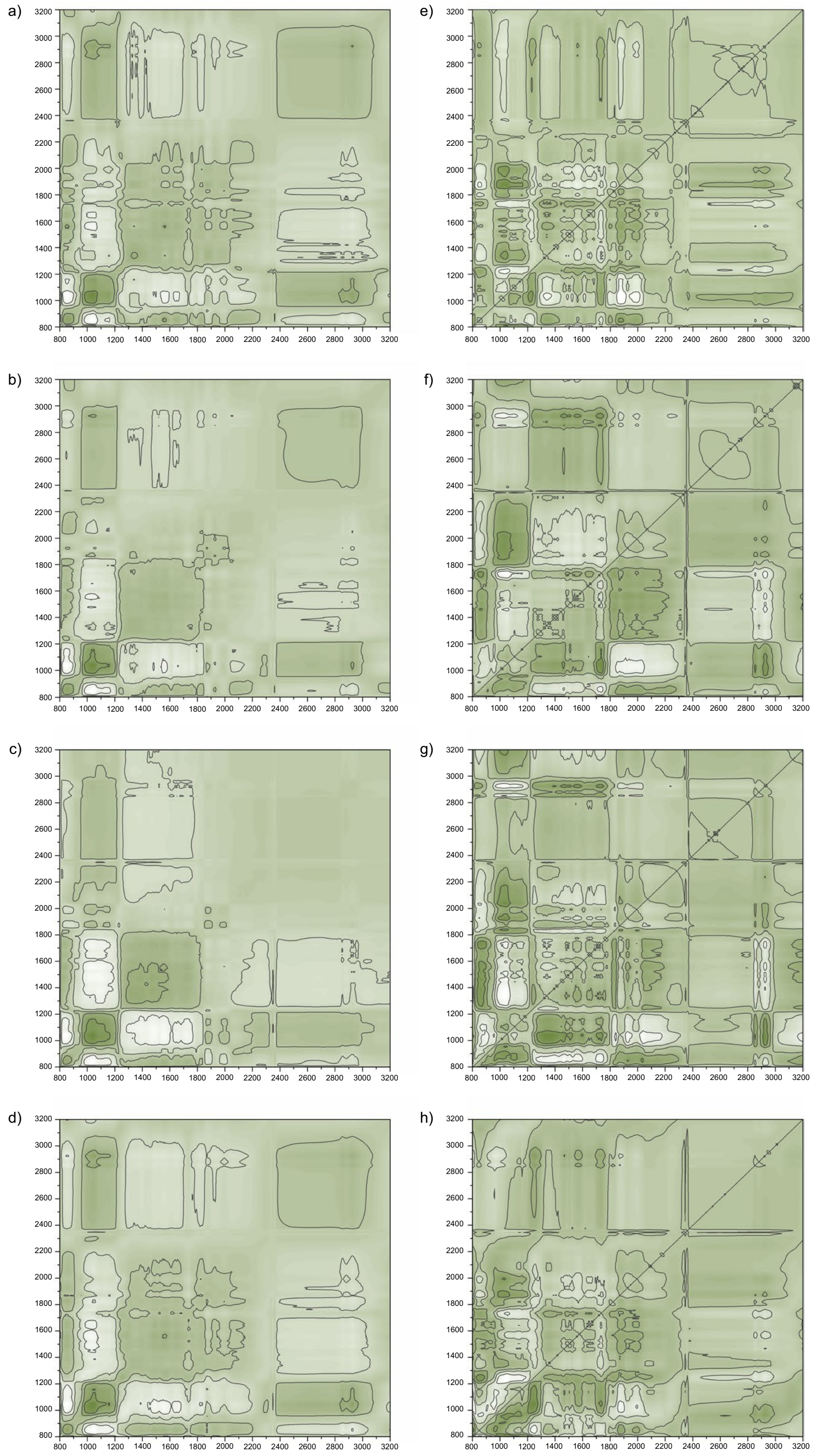


Fig. 5 Synchronous and asynchronous spectra for the s + c fraction for grassland (**a** and **e**), spruce (**b** and **f**), oak (**c** and **g**) and arable (**d** and **h**). The dark regions show positive, while the light ones show negative correlations

Table 3 Bands assigned for organic matter compound class

Compound class	Band	Description	Reference
Carbohydrate			
	1027	C–O stretching of polysaccharides	Grube *et al.*, 2006
	1160	C-O stretch of cellulose	Calderón *et al.*, 2013
	1415	bending (CH2) in polysaccharides	Calderón *et al.*, 2013
Carboxyl			
	1725	C=O stretch of COOH	Hay and Myneni, 2007
	1770	C=O stretch of COOH H-bounded	Hay and Myneni, 2007
Lignin	1270	phenolic C-OH bending	Niemeyer *et al.*, 1992
	1470	ring vibrating modes of ortho-substituted aromatics	Bellamy, 1975
	1613	aromatic C=C stretching	Baes and Bloom,1989
	3031	Aromatic C-H stretching	Niemeyer *et al.*,1992
Lipid			
	2852	aliphatic symmetric C-H stretch	Calderón *et al.*, 2011
	2924	aliphatic asymmetric C-H stretch	Calderón *et al.*,2011
Protein	1642	C=O starching of amid	Guhra *et al.*, 2020
	1577	N-H bending of amid	Guhra *et al.*, 2020

in ecosystems need to be taken into account. Both inhibiting soil environmental parameters (e.g. pH, texture) and high C input into a given vegetation type may lead to the accumulation of the POM fraction (Soucémarianadin et al. 2019; Hayashi et al. 2023). Several studies have also shown MAP and MAT to be very powerful factors in the decomposition of organic matter (e.g. Moore et al. 1999). However, the sample sites in the present study were located within a radius of a few km, so it is unlikely that the weather data differed greatly.

The lignin/N ratio has been accepted as a proxy for organic matter decomposition (Bonanomi et al. 2013). An unfavourably high C to N ratio (lignin/N ratio) has been found for coniferous leaves (Vesterdal et al. 2008), which was supported by the present data: the POM fraction of spruce was characterized by a very high C/N ratio (Table 2), which may be one of the reasons for inhibited decomposition. However, not only the C/N ratio of POM, but the C/N ratio of the whole soil may be limiting factor for decomposition. Springob and Kirchmann (2003) found that when the C/N value in the soils was >20 it could limit SOM mineralization. Furthermore, the quantity of mineralized CO_2 derived from SOC was found to be lower in soils with a high C/N ratio (Ostrowska and Porębska 2015; Finn et al. 2015). Consistent with this, the present study revealed a significant correlation between soil C/N ratio and POM-C (Fig. 2a).

Another environmental factor that should be taken into account in the present case is soil acidity: the unfavourable pH of pine soil reduces microbial activity and thus litter degradation, which can lead to POM accumulation (Fig. 2b), as reported by John et al. (2005). Leifeld et al. (2009) suggested that higher POM content in the soil means weak aggregation, though, this hypothesis was not confirmed in the present work because the amount of aggregates did not change considerably in the soils tested (Fig. 1a).

Carbon to nitrogen ratio of the organic matter fractions

The C/N ratios of the spruce fractions were extremely high: 31.2 for POM, 26.5 for S + A and 23.5 for s + c (Table 2). These values indicate high amounts of incompletely decomposed organic matter (Buyanovsky et al. 1994; Six et al. 2001), which is a consequence of limiting environmental factors such as pH or soil C/N ratio (see previous section). The C/N ratios of the fractions were quite similar for grassland and cropland, a result in agreement with previous studies (Schneider et al. 2021).

It has been known for a long time that the C/N ratio increases with increasing particle density (Turchenek and Oades 1979). Consistent with previous research, the C/N ratio of the fractions showed a

Table 4 Susceptible compounds, correlations and sequential order of biopolymers revealed by 2D COS analysis during 1-year incubation

	POM	*S+A*	*s+c*
Grass			
Susceptible peaks	Carbohydrate Lignin Protein	Carbohydrate Lipid Lignin	Carbohydrate Lipid Lignin
Correlations	(-) Carbohydrate – Protein (-) Lipid – Protein	(-) Carbohydrate – Protein (-) Carbohydrate – Carboxyl	(-) Carbohydrate – Protein (+) Carbohydrate – Lipid
Sequential order	Protein → Carbohydrate → Lipid Protein → Lignin	Carbohydrate → Lipid Carbohydrate → Protein Carbohydrate → Lignin → Protein	Lipid/Lignin → Carbohydrate → Carboxyl Protein → Carbohydrate
Spruce	*POM*	*S+A*	*s+c*
Susceptible peaks	Carbohydrate Lipids Lignin Carboxyl	Carbohydrate Carboxyl	Carbohydrate
Correlations	(-) Carbohydrate – Protein (-) Carbohydrate – Lignin	(-) Carbohydrate – Carboxyl (-) Carbohydrate – Lignin (-) Carbohydrate – Protein	(-) Carbohydrate – Protein (+) Carbohydrate – Lipid
Sequential order	Carbohydrate → Lignin Carboxyl → Carbohydrate Carbohydrate → Protein	Carbohydrate → Lipid/Lignin/Protein Lignin → Protein → Carboxyl	Lipid → Carbohydrate Lipid → Lignin/Carboxyl/Protein Carbohydrate → Carboxyl Carbohydrate → Lignin
Oak	*POM*	*S+A*	*s+c*
Susceptible peaks	Carbohydrate Protein Carboxyl Lipid	Carbohydrate Carboxyl	Carbohydrate Lignin
Correlations	(-) Carbohydrate – Lignin	(-) Carbohydrate – Carboxyl (-) Carbohydrate – Protein (-) Carbohydrate – Lignin	(-) Carbohydrate – Carboxyl (-) Carbohydrate – Protein (-) Carbohydrate – Lignin
Sequential order	Carbohydrate → Protein Carbohydrate → Lipid Carbohydrate → Carboxyl	Carbohydrate → Lipid/Lignin/Protein Protein → Carboxyl	Lipid → Carbohydrate Lipid → Lignin/Carboxyl/Protein Carbohydrate → Carboxyl Carbohydrate → Lignin
Arable	*POM*	*S+A*	*s+c*
Susceptible peaks	Carbohydrate Protein Lignin Carboxyl	Carbohydrate Lignin Lipid	Carbohydrate Protein
Correlations	(-) Carbohydrate – Protein (+) Carbohydrate – Lipid	(-) Carbohydrate – Carboxyl (-) Carbohydrate – Protein (+) Carbohydrate – Lignin	(+) Carbohydrate – Lipid (-) Carbohydrate – Protein (-) Carbohydrate – Lignin
Sequential order	Carboxyl → Carbohydrate → Protein	Carbohydrate → Lipid/Lignin/Protein Lignin → Protein	Protein → Lipid Carbohydrate → Protein Carbohydrate → Carboxyl

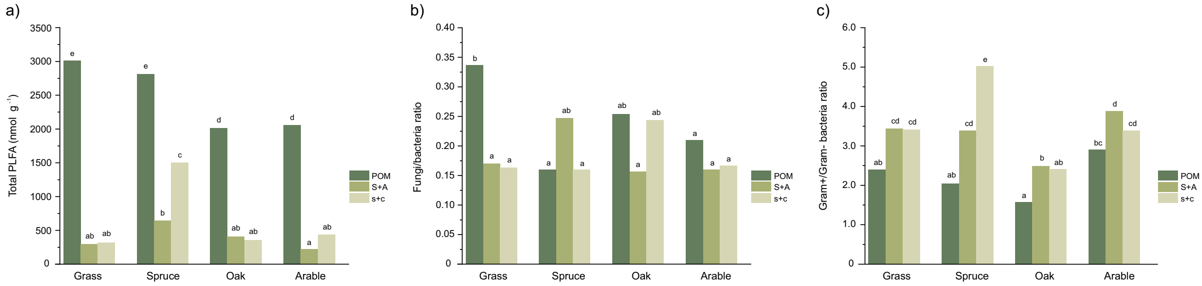


Fig. 6 Total PLFA (**a**), fungi to bacteria ratio (**b**) and Gram + to Gram- bacteria ratio (**c**) of the organic matter fractions

decreasing trend towards smaller particle size in the present study, the lowest C to N ratios are seen in the s + c fractions (Table 2). Several processes contribute to a decrease in the C/N ratio: (i) the degradation of non-protected carbon leads to the formation of compounds with high C/N ratios, so the C/N ratio of non-protected SOM decreases (Kramer et al. 2003), (ii) in parallel, N-rich compounds bind with high affinity to mineral surfaces (Oades 1988; Kleber et al. 2007; Kopittke et al. 2020) by preferential adsorption of N-rich compounds in stable amidic forms (Lehmann and Kleber 2015).

Dynamics of mineralization in soil organic matter fractions

2D correlation spectroscopy was performed to give a better understanding of changes and patterns in the mineralization of different organic matter pools during a 1-year incubation.

Surprisingly, not only the IR regions that characterize organic matter were found to be variable during incubation, but also regions that clearly indicate the susceptibility of the mineral constituents. These include the autopeaks at 860, 930 and 1000 cm^{-1} (Figs. 3, 4 and 5), which can be associated with the silicates and Fe-Al-oxide hydroxides in the soil (Xiao et al. 2018). Organic matter oxidation has previously been shown to lead to an increase in the specific surface area of soils (Oades 1984; Kaiser and Guggenberger 2003), suggesting that some of the organic matter coating mineral particles decompose during oxidation, increasing the amount of free mineral surface area.

Although lignin and lipids are considered as the most recalcitrant compounds in the soil because of their chemical structure, recent studies have confirmed that lignin and lipids are altered relatively quickly and do not appear to be stabilized in the long term in any soil fraction (Gleixner et al. 2002; Mikutta et al. 2006; Woolf and Lehmann 2019). Thus, the soil dynamics of aliphatic compounds or lignin derivatives is not caused by intrinsic recalcitrance, but by the stabilization mechanisms that determine the dynamics of these compounds, such as surface interactions with minerals (Huang et al. 2019) or hydrophobicity (Lützow et al. 2006). Dao et al. (2022) found that, even in permafrost soils, climate-induced degradation may promote carbon losses from lignin. The fact that all four soil fractions studied showed a high variability in both lipid and lignin-like substances confirms these results.

Although no clear pattern emerges for either vegetation types or carbon stabilization mechanisms (fractions), it is clear that the most dominant correlation was between the amount of carbohydrates and the amount of proteins (Table 4). As this is a negative correlation, it means that the amount of protein-like substances varies inversely with the amount of carbohydrate. The carbohydrate pool in soils is considered to be the primary source of microbes (Gunina and Kuzyakov 2015), so its variation is clearly due to an increase in microbial activity, which releases proteinaceous substances into the soil when microorganisms die (Schulten and Schnitzer 1997). This linkage is clearly shown by the negative correlation between the two types of compounds. Furthermore, a strong correlation was found between carbohydrates and amino compounds in study of Abdelrahman et al. (2016) in three soil fractions (light fraction, POM and mobile humic acid fraction) and concluded that the content of carbohydrates and amino compounds in these fractions can depict carbon cycling pattern and response to land management.

The carbohydrate-lignin correlation can also be considered typical, as it occurred in almost all fractions except grassland (Table 4). Although incubation experiments show that the amount of lignin-derived compounds does not change during organic matter decomposition (Sjöberg et al. 2004; Angst et al. 2017), clear changes and biogeochemical coupling between carbohydrates and lignin were found in the present study. The negative correlation between the two groups of compounds can be interpreted as an increase in aromatic compounds with increasing microbial activity, indicating the enhanced depolymerisation of plant-derived organic matter, which may result in the production of large amounts of aromatic lignin derivative compounds.

The 2DCOS technique allows us to determine the temporality of changes in spectra, i.e. to track which quantitative changes in one type of compound precede another. A very diverse picture was obtained in this respect, with very typical sequences of carbohydrate → protein, carbohydrate → lignin, carbohydrate → lipid/lignin/protein, which is in line with the idea that labile pools have higher variability and can be directly linked to microbial processes (Breulmann et al. 2012; Rousk et al. 2016). The soil POM fraction of grass is characterised by protein -> carbohydrate → lipid and protein → lignin sequences, possibly due to the more continuous input of plant material, lower C/N ratios and higher soil pH, resulting in more favourable conditions for the accumulation of microbial residues compared to forest or agricultural soils (Angst et al. 2021). Canarini et al. (2016) also found that POM-C was correlated with increasing microbial biomass, with a greater abundance of fungi relative to bacteria (F/B ratio), as confirmed by the present study (Fig. 6b).

An unusual order was found for the aggregate and for MAOM in particular, with changes in the amount of lipids and lignin preceding changes in the amount of carbohydrate or protein, which were observed for all the s + c fractions with the exception of the arable site (Table 4). Compared with aggregates or POM, MAOM is enriched in low-molecular-weight N compounds originating from microbial processes (Schmidt et al. 2011; Kopittke et al. 2020; Bagcilar et al. 2024). This can promote N mineralization via microbial N mining (Sollins et al. 1984; Daly et al. 2021), including processes such as (i) the liberation of adsorbed N compounds from the mineral surfaces of MAOM by desorption or exoenzymes, (ii) an increase in microbial biomass, and (iii) an increase in necromass inputs that can replenish MAOM-N pools. It can be assumed, therefore, that it is the readily available, abundant N compounds in MAOM, and partly in aggregates, that cause this particular sequence. Indeed, the C/N ratio of this fraction is quite low (Table 2). It is important to note that in the aggregates of the soils studied, such sequences were present to a lesser extent, suggesting that N mining is not as dominant a phenomenon, due to the different nature of the stabilization of organic compounds, as the adsorption of organic molecules is not the dominant mechanism in aggregates. Differences in the mineralization of non-protected and protected organic matter were also illustrated by the PLFA analysis, as the GP/GN ratio differed significantly between the POM fractions and the mineral fractions (s + c and S + A; Fig. 6c). GN bacteria preferentially mineralize easily degradable plant-derived compounds, while GP bacteria mineralize more complex molecules such as older SOM-derived C (Fanin et al. 2014). This was clearly demonstrated by the significantly higher GP/GN ratio of protected carbon pools compared to that of non-protected POM for all sites.

Conclusions

The close relationship between POM content and soil parameters, such as pH and C/N ratio, demonstrated that environmental parameters could be significant factors controlling the decomposition of organic matter. The results also demonstrated that, although lipids and lignin are considered as chemically stable materials that commonly remain constant during decomposition, these compounds were found to be very susceptible in all the fractions. Major variations were detected in the dynamics and patterns of mineralisation for non-protected and protected carbon pools:

- linkage between carbohydrate and carboxyl compounds was observed especially in the aggregate fractions of the soils, because the oxidised subunits of lignin can bind strongly to MAOM surfaces.
- an unusual sequence was found for the protected carbon pools, especially for MAOM, which was due to the lower C/N ratio of these fractions compared to that of non-protected C pools and which may promote N mineralization via microbial N mining.

PLFA analysis clearly demonstrated the differences in the mineralization of non-protected and protected organic matter by the significantly higher GP/GN ratio of protected carbon pools compared to that of non-protected POM for all sites.

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Declarations

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