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Katarzyna WASAK-SĘK[1*], Wojciech SZYMAŃSKI[2] and Marek DREWNIK[2]

EFFECT OF CARBONATE ROCKS ON THE DISSOLVED ORGANIC MATTER LEACHING FROM SOIL IN MOUNTAINOUS FORESTS AFFECTED BY WINDTHROW

Abstract: An increase in the number of windthrow events has been observed in mountainous areas of central Europe. In such areas, rock fragments are transported from the subsoil to the soil surface, which in the case of carbonate rocks leads to the incorporation of Ca^{2+} into acid soils. These processes can alter the quality and quantity of dissolved organic matter (DOM). The study aimed to determine the role of carb onate rocks as a factor determining the quality and quantity of DOM released by gravity leaching under laboratory conditions in a long-term experiment using soil in its natural state. The soil was collected from the Tatra Mts. Three organic O-horizons and two A-horizons were selected. Relatively pure limestone collected in the study area was used. During the 20-week experiment, the soils with and without limestone were leached with deionised water. The chemical composition of the collected filtrate was measured at regular intervals, including the amount of dissolved organic carbon (DOC). The qualitative composition of the filtrate was determined by FTIR-ATR spectroscopy. The addition of limestone caused an increase in DOC leaching from the acidic O-horizons. In the A-horizons, the DOC leaching rate was reduced in the first stage of the experiment, demonstrating adsorption processes occurring in these horizons. The presence of limestone changed the quality of the leached DOM. Immobilisation of carbohydrates and aliphatic compounds occurred in the A-horizons. DOM was leached from both the O- and A-horizons mainly in the form of Ca-DOC complexes.

Keywords: dissolved organic carbon, SOC stabilisation, limestone, windthrow, FTIR-ATR spectroscopy

Introduction

Soils are the most important organic carbon (OC) pool in terrestrial ecosystems [1]. An important research problem is to identify the factors that influence the rate of accumulation (sequestration) of soil organic carbon (SOC) and the rate of depletion of the SOC pool, as these two processes determine the SOC stock in different soil types [2, 3]. SOC sequestration is the result of a balance between SOC influx and depletion due to mineralisation of soil organic matter (SOM) and leaching in the form of dissolved organic matter (DOM). In the case of these two processes, the factors influencing the quantity and quality of leached DOM have not been extensively studied [4, 5].

Many studies have shown that soil mineral composition and chemical properties are important factors controlling SOC stabilisation and consequently sequestration [4, 6, 7]. In acid soils, Fe^{3+} and Al^{3+} ions and the amorphous form of Fe and Al mineral phases have

[1] Institute of Geography and Spatial Organization, Polish Academy of Sciences, ul. św. Jana 22, 31-018 Kraków, Poland, phone +48 12 422 40 85, ORCID: 0000-0002-7857-4878
[2] Institute of Geography and Spatial Management, Faculty of Geography and Geology, Jagiellonian University, ul. Gronostajowa 7, 30-387 Kraków, Poland, ORCID: WS 0000-0002-5894-5176, MD 0000-0001-7400-2103
[*] Corresponding author: katarzyna.wasak@zg.pan.krakow.pl

been the main focus of SOM stabilisation, as they are strong chelating factors of SOM [8, 9]. However, little attention has been paid to non-acidic soils [3, 10], although they have a high potential for SOM sequestration [6, 11] and such soils (mostly as soils developed on carbonate rocks) represent approximately 13 % - 40 % of all soil resources in the world [12].

The stabilisation of SOM in calcareous soils may be due to the naturally occurring presence of Ca^{2+} ions. Using chemical models, it has been shown that Ca^{2+} ions can bind to SOM through key processes involving weak outer sphere interactions (exchangeable bridges) and stronger inner sphere interactions (through complexes with O-bearing ligands such as carboxylate groups) [3, 13]. This has been confirmed in laboratory studies [9, 14-16] and in catchment scale field studies [15]. Calcium can facilitate the binding of organic compounds to mineral phases, especially permanently negatively charged siloxane surfaces and hydroxyls of aluminosilicates and oxides *via* cation bridging [9]. Ca^{2+} ions also play an important role in the bonding of SOM to ferrihydrite at high pH (≥ 7) and the formation of strong Fe-Ca-OC complexes [17]. As the mineral part of soil seems to be the most important in the SOM adsorption process, the formation of supermolecular organo-metallic structures is possible in the presence of Ca^{2+}, and SOM may also be precipitated by Ca^{2+} ions [3, 13, 18]. On the other hand, Ca^{2+} affects soil functioning by increasing pH, which have an direct effect on soil biological activity [19]. In soils with pH below 4, Al^{3+} is toxic for microorganisms, thus in the case of the very acidic soils limestone addition could increase microbial activity and in turn - DOC leaching [20]. Maximum SOM adsorption occurs when soil pH is 4-5 (adsorption by Al-Fe oxides) [16]. SOM is most soluble at pH *ca*. 6.2-7.0, while at pH higher than 7 the interaction with Ca^{2+} cause the stabilisation by inner and outer-sphere interaction. At pH higher than 8 adsorption of SOM by $CaCO_3$ and cementation of aggregates occurs [3]. However, these thresholds are gradual and appear to differ in experimental research for different soils [19, 21]. Therefore, further studies are needed to determine the effect of Ca^{2+} addition to soil on SOC stabilisation in different conditions.

The issue of DOC leaching is important in forest soils affected by windthrows. In recent decades, large areas of forest in Europe, including the Alps and the Carpathians, have been affected by windthrows [22, 23]. There are an increasing trend of forest disturbances in recent decades, and climatic scenarios suggest that they will continue to increase in the future [22]. Such events may disturb the relationship between SOC stabilisation and the DOC release. In the first years after a windthrow, basic cations are leached from the soil exchange complex as a result of soil acidification caused by nitrification [24]. The biological activity of the soil changes [25], as does the uptake of basic cations by plant roots [26]. Unweathered rock fragments are transported from the subsoil to the soil surface, which in the soils formed from calcareous rock in a humid climates leads to the mixing of rock fragments releasing Ca^{2+} ions with acidic soil forming the forest organic O-horizons and the A-horizons. These soils are common in the Alps, in the Pyrenees, and in the Carpathians [27-29]. The large scale of the material transport after windthrow is confirmed by studies conducted in the calcareous part of the Tatra Mountains after the windthrow in 2013 [30].

The aim of this study was to investigate the role of carbonate rocks as a factor in determining the quality and quantity of DOM released by gravity leaching under controlled laboratory conditions in a long-term natural soil experiment. It was hypothesised that the

presence of carbonate rocks would significantly reduce the amount of DOC released from the soil and also change the composition of the DOM.

Materials and methods

Materials

Samples from the O-horizons were collected from different forest stands in the Tatra Mts. (N49°15'47-50'', E19°52'19-25''; elevation: 1,050 m to 1,100 m): O-horizon formed under beech (*Fagus sylvatica*) (BO), under Norway spruce (*Picea abies*) (SO), and under dwarf pine (*Pinus mugo*) (DpO). In the case of soil under beech and Norway spruce, soil samples representing the A-horizons (0 cm - 10 cm) were also collected: A-horizons formed under beech (BA), and under Norway spruce (SA). The samples were collected from Calcaric Cambisols developed on Triassic and Jurassic limestones. The sample of carbonate rock used in the study was also collected in the Tatra Mts. from Jurassic limestone.

Experiment

The final soil sample for the experiment was prepared by mixing 10 representative subsamples of 100 g for the O-horizon and 500 g for the A-horizon. The samples were dried at 40 °C, homogenised, and then carefully sieved through a 5 mm sieve in order to preserve naturally occurring fine and preserve naturally occurring fine and medium soil aggregates. Limestone cubes of 15 mm side length were cut and washed in deionised water. Limestone cubes prepared in this way were placed in the Eppendorf tube halfway up the soil.

The laboratory experiment lasted 20 weeks and was carried out synchronously in 11 variants: (i) 5 types of soil horizons (BO, SO, DpO, BA, SA) with the addition of relatively pure limestone (15 mm x 15 mm x 15 mm cube), (ii) 5 types of soil horizons without limestone, and (iii) blanks (a laboratory filter used to prevent the release of solid particles into the filtrate studied). Each variation of the experiment consisted of 5 replicates, giving a total of 55 samples.

Five grams of soil from the O-horizon or twenty grams of soil from the A-horizon were placed in conical polyethylene Eppendorf tubes with a volume of 50 mL (test tubes) with a drilled pouring hole and a laboratory filter. Deionised water was added to the prepared test tubes (12 cm^3) every 7 days, with a total volume (on a 20-week scale) corresponding to the average precipitation in the warm half of the year in the sampling area (mean average precipitation: 1200 mm). For the duration of the experiment, the test tubes were placed in partial shade and covered with a perforated lid. The air temperature in the laboratory was ~20 °C and the humidity ~30 %. After 5, 10, 15 and 20 weeks of the experiment, the filtrate (soil solution collected from each test tube and separated by gravity) was poured into a polyethylene bottle and stored at ~4 °C prior to quantitative analysis. The experiment was continued under the above conditions until a filtrate samples suitable for FTIR-ATR analysis were obtained. Homogenised soil samples collected from the test tube were dried at 40 °C after the experiment and then prepared for further analysis.

Analysis of filtrate and soil properties

For the filtrate obtained after 5, 10, 15, and 20 weeks of the experiment, the following data were determined: DOC concentration (using infrared spectrophotometry; with the

removal of carbonates), water chemistry including Ca^{2+} and SO_4^{2-} concentration (ion chromatography with eluent conductivity suppression; Dionex ICS2000 machine equipped with a CS-16 cation column and a CG-16 5 mm guard column). Filtrate pH was measured potentiometrically. A 0.45 µm syringe filter was used to remove the suspension prior to determining the water chemistry. The concentrations of Ca^{2+}, and SO_4^{2-} ions were multiplied by the volume of the filtrate to calculate the load. All results for Ca^{2+}, and SO_4^{2-} ions are reported as the mean of five measurements minus the blank. DOC concentrations were calculated as loads per dry matter.

For the filtrate obtained after 20 weeks of the experiment, FTIR-ATR spectra were collected using a Nicolet iS50 Thermo Scientific FTIR spectrometer equipped with a Pike GladiATR (Pike Technologies, Madison, WI) accessory. Ten drops of filtrate were applied to an ATR crystal, dried at 105 °C and then 32 scans were collected for each sample in the range 400 1/cm to 4000 1/cm with a resolution of 2 1/cm. The FTIR-ATR spectra were processed using Spekwin32 software. A 0.45 µm syringe filter was used to remove the suspension before analysis.

Soil and rock properties were determined before and after the experiment for homogenised and crushed (< 100 µm) subsamples. The chemical composition of each sample was determined by inductively coupled plasma optical emission spectrometry (ICP-OES) and loss of ignition (LOI) by combustion at 1000 °C. Total carbon (TC) and total nitrogen (TN) concentrations in soil samples were determined by dry combustion gas chromatography (CHN elemental analyser; Elementar vario MICRO cube). CO_2 content, derived from carbonates, was determined using a calcimeter. Soil organic carbon (SOC) was calculated by subtracting inorganic carbon from total carbon [31]. The C/N ratio was calculated as TOC/TN. Soil pH was measured in uncrushed samples in deionised water and 1M KCl (1:2.5 soil/water ratio) [32]. FTIR-ATR analyses of soil samples taken to the experiment were performed for bulk soil samples with the parameters noted above.

Statistical analysis

An analysis of Pearson correlation coefficients between the DOC load, pH, Ca^{2+}, and SO_4^{2-} load in the soil solution (filtrate) was performed ($p < 0.05$). The analysis was performed for selected weeks of the experiment (week 5, 10, 15, and 20) separately for the O-horizons and the A-horizons. The data met the normality criterion according to the Kolmogorov-Smirnov test. Data were standardised before analysis.

Results

Properties of soils and limestone used in the experiment

The O-horizons were mainly composed of SOM, as evidenced by their high LOI value and OC concentration of over 40 % (Table 1). Their C/N ratio was 32-48. The highest C/N ratio was calculated for DpO. The highest CaO content was found in BO, while the highest content of SiO_2 and Al_2O_3 was found in SO.

Soil samples collected from the A-horizons (BA and SA) consisted mainly of SiO_2, Al_2O_3, and Fe_2O_3, while the content of SOM was lower than that in the O-horizons (Table 1). The C/N ratio was similar for BA and NA (~14).

The Jurassic limestone was mainly composed of calcite (high CaO content and high LOI) (Table 1). Dolomite was a minor constituent (MgO content was 3.35 %) and all other constituents represented less than 0.5 % of the rock mass used in the experiment.

Table 1

Chemical composition of the soil samples and rocks used in the experiment

Soil sample/rock	SiO_2	Al_2O_3	Fe_2O_3	MgO	CaO	Na_2O	K_2O	TiO_2	P_2O_5	MnO	LOI	OC	C/N
	[% of dry mass]												[-]
BO	3.08	0.48	0.26	0.19	2.83	0.03	0.20	0.03	0.15	0.08	92.6	46.67	38.7
SO	13.3	3.07	1.42	0.52	1.57	0.15	0.49	0.16	0.19	0.06	79.0	41.21	32.4
DpO	2.16	0.67	0.33	0.41	1.04	0.03	0.14	0.04	0.09	< 0.01	95.1	42.23	48.4
BA	54.19	12.69	5.66	1.86	1.04	0.64	1.63	0.73	0.15	0.16	21.1	6.42	13.9
NA	49.05	12.14	5.54	1.99	1.23	0.56	1.59	0.59	0.20	0.18	26.7	9.17	14.3
Limestone	0.42	0.17	0.14	3.35	51.78	0.01	0.06	0.01	0.03	< 0.01	43.9	n.d.	n.d.

n.d. - not determined

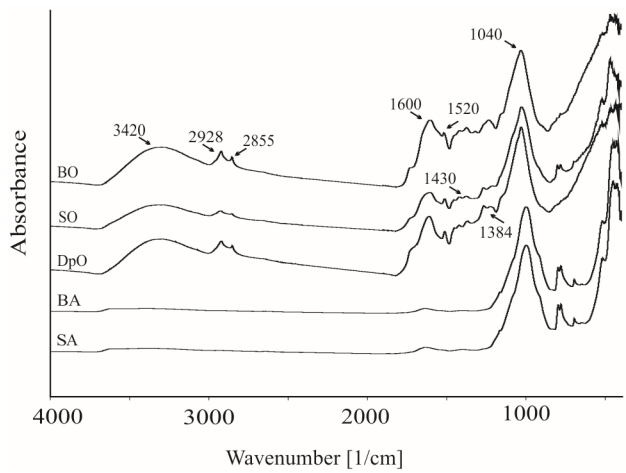

Fig. 1. FTIR-ATR spectra of the soil samples used in the experiment. Absorption bands originating from the functional groups of SOM are marked

Fourier transform infrared attenuated total reflectance (FTIR-ATR) spectra generated for the samples studied show differences between the O-horizons and the A-horizons (Fig. 1). All O-horizons has similar FTIR-ATR spectra with well-pronounced bands originating from the functional groups of SOM: a prominent and broad absorption band at ~3420 1/cm (O-H groups in alcohols, phenols and carboxylic acids), prominent bands found at ~2928 1/cm and ~2855 1/cm (aliphatic C-H), ~1600 1/cm (aromatic C=C and C=O from carboxylic acid anions), ~1520 1/cm (aromatic C=C), ~1430 1/cm (aliphatic C-H and/or symmetric vibration of COO^-), and ~1384 1/cm (aliphatic C-H deformation and/or stretching of COO^- and/or O-H deformations of phenolic and aliphatic groups). However, some absorption bands indicated the presence of minerals. Bands found at ~1165 1/cm, 1040 1/cm, 800 1/cm, 780 1/cm, 760 1/cm, 695 1/cm, 530 1/cm and 470 1/cm from Si-O stretching and O-H bending vibrations originate from silicates and aluminosilicates; but the band ~1040 1/cm may also be partly related to C-O bonds in polysaccharides, carbohydrates, nucleic acids, and proteins.

In contrast, the A-horizons used in the experiment had FTIR-ATR spectra dominated by bands characteristic of minerals: a weak band at ~3626 1/cm originating from the stretching of O-H groups in clay minerals, and strong bands at ~1165 1/cm, 1040 1/cm,

1010 1/cm, 800 1/cm, 780 1/cm, 760 1/cm, 695 1/cm, 530 1/cm and 470 1/cm originating from Si-O stretching as well as O-H bending vibrations originating from silicates and aluminosilicates. However, absorption bands at 1040 1/cm and ~1010 1/cm may also be partly related to C-O bonds in polysaccharides, carbohydrates, nucleic acids, and proteins. Among bands characteristic of the functional groups of SOM, only weak bands at ~1630 1/cm (aromatic C=C and C=O from carboxylic acid anions) and ~1420 1/cm (aliphatic C-H) were detected.

Changes in selected soil and filtrate properties over the course of the experiment

At the end of the experiment, all the soils tested had a higher pH than at the beginning (Table 2). In the case of the A-horizons without limestone addition, the pH increased by ~0.3 pH units in both BA and SA, while in the case of the A-horizons with limestone, the pH increased more: by ~0.9 and ~0.6 pH units in BO and SO, respectively. The pH increase for the O-horizons was higher than that for the A-horizons. O horizons in contact with limestone showed the highest pH increase.

Table 2

pH of soil samples before and after the experiment

Experimental variant		Soil sample	pH_w [-]	pH_{KCl} [-]	Soil sample	pH_w [-]	pH_{KCl} [-]
Before the experiment	-	BO	5.11	4.64	BA	5.43	4.58
After the experiment	-		6.23	6.21		5.71	4.75
	L		6.37	6.12		6.37	5.42
Before the experiment	-	SO	4.77	4.24	SA	5.49	4.80
After the experiment	-		5.12	4.52		5.80	4.94
	L		6.01	5.60		6.10	5.36
Before the experiment	-	DpO	3.67	2.92			
After the experiment	-		4.10	3.24			
	L		5.38	4.73			

- soil sample without limestone, L - soil sample with limestone

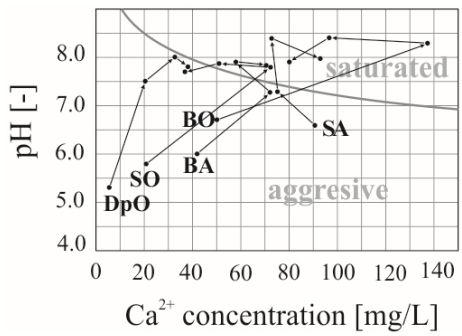

Fig. 2. Changes in the relationship between filtrate pH and Ca^{2+} concentration in filtrate on Tillmans-Trombe diagram during the experiment

During the first ~10-15 weeks of the experiment, the pH of the filtrates increased significantly and then decreased slightly (Table 3, Fig. 2). In all cases, contact with limestone increased the pH of the filtrate. In the case of the O-horizons, the difference was

greater the more acidic the soil horizon (DpO >> SO > BO). The A-horizons (BA and SA), which initially had a similar pH in both situations, also showed a similar increase in pH induced by contact with limestone.

Table 3

Changes of filtrate pH during the experiment

Experimental variant		pH [-]			
		After 5 weeks	After 10 weeks	After 15 weeks	After 20 weeks
BO	-	6.63	7.03	6.89	7.83
	L	6.66	8.31	8.41	7.92
SO	-	5.14	5.96	5.79	6.26
	L	5.82	7.80	7.86	7.74
DpO	-	4.38	4.46	4.86	4.88
	L	5.32	7.46	7.98	7.93
BA	-	4.42	5.67	6.99	7.04
	L	5.98	7.28	7.86	7.78
SA	-	5.31	7.37	7.91	7.03
	L	6.58	7.31	8.37	8.05

- soil sample without limestone, L - soil sample with limestone

The concentration of Ca^{2+} in the filtrate varied significantly over the course of the experiment depending on the experimental variant: from 2.2 mg/L to 137 mg/L (Table 4). The addition of limestone increased the Ca^{2+} concentration in the filtrate in all cases.

Most of the filtrates analysed reached a state of Ca^{2+} saturation in the 5^{th} or 10^{th} week of the experiment (Fig. 2). After reaching its maximum, the Ca^{2+} saturation in the filtrate began to decrease with a decrease in pH. Only the filtrate obtained from DpO approached, but did not reach, a state of saturation.

Table 4

Changes in Ca^{2+} concentration in the filtrates during the experiment

Experimental variants		After 5 weeks		After 10 weeks		After 15 weeks		After 20 weeks	
		Ca^{2+}							
		[mg/L]	[mmol/L]	[mg/L]	[mmol/L]	[mg/L]	[mmol/L]	[mg/L]	[mmol/L]
BO	-	48.4	1.21	68.4	1.71	48.0	1.20	57.4	1.43
	L	50.1	1.25	137.0	3.42	96.5	2.41	79.8	1.99
SO	-	9.3	0.23	27.6	0.69	20.4	0,51	24.3	0.61
	L	21.1	0.52	72.2	1.80	50.9	1.27	36.8	0.92
DpO	-	2.2	0.05	4.1	0.10	3.4	0.08	3.4	0.08
	L	5.5	0.14	20.2	0.50	32.6	0.81	38.0	0.95
BA	-	21.2	0.53	64.1	1.60	17.3	0.43	21.5	0.54
	L	41.7	1.04	72.1	1.80	57.9	1.44	71.0	1.77
SA	-	51.2	1.28	73.6	1.84	67.0	1.67	25.5	0.64
	L	90.6	2.26	75.3	1.88	72.7	1.81	92.9	2.32

- soil sample without limestone added, L - soil sample with limestone added

Quantitative changes in leached DOC over the course of the experiment

The dynamics of DOC leaching over the course of the experiment mainly depended on the type of soil used in the experiment. The concentration of leached DOC in the filtrate was higher in the O-horizons (21.03 mg/L - 192.70 mg/L) compared to the A-horizons

(12.85 mg/L - 40.63 mg/L) (Table 5). In the O-horizons it reached its maximum after 10 weeks of the experiment, while in the A-horizons no clear trend was observed.

The addition of limestone affected the DOC concentration in different ways. The greatest differences between samples with and without limestone addition occurred in the 5th week of the experiment (Table 5). In this period, for filtrates from the O-horizon under beech (BO) and from the A-horizons (BA, SA), the DOC concentration in the samples with limestone was much lower than that in the samples without limestone addition (BO >> BA = SA). In the case of coniferous O-horizons (SO, DpO), the addition of limestone in the first part of the experiment (after 5 weeks) caused a significant increase in DOC concentration (SO > DpO).

Table 5

Changes in DOC concentration in the filtrates during the experiment

Experimental variant		After 5 weeks	After 10 weeks	After 15 weeks	After 20 weeks
		DOC [mg/L]			
BO	-	186.20	192.70	73.79	61.61
	L	137.10	178.60	98.64	65.73
SO	-	44.50	146.50	54.55	50.99
	L	84.32	141.20	82.60	51.41
DpO	-	21.03	62.71	48.38	45.86
	L	30.19	65.94	45.19	34.42
BA	-	30.30	14.78	12.87	12.85
	L	15.65	19.20	15.67	16.16
SA	-	40.63	24.41	28.90	13.90
	L	24.16	25.94	34.31	19.34

- soil sample without limestone added, L - soil sample with limestone added

There was a significant positive correlation between Ca^{2+} and DOC load in the filtrates studied (Table 6). Considering selected periods of the experiment duration, for the O- horizons a significant positive correlation between Ca^{2+} and DOC load in the filtrates appeared at the beginning of the experiment (in the 5th week) and was observed until the 15th week, while for the A-horizon the positive correlation between Ca^{2+} and DOC load in the filtrates appeared in the 20th week. A positive correlation between DOC load in the filtrates and filtrates pH of the O-horizons appeared in the 5th week of the experiment, while for the A-horizon it was observed in the 20th week. In contrast, there was no clear relationship between filtrate DOC and SO_4^{2-} load (data not shown).

Table 6

Correlation coefficients between loads of selected compounds in the filtrates

Correlation	After 5 weeks	After 10 weeks	After 15 weeks	After 20 weeks
Organic O-horizons (N = 6)				
DOC *vs* pH	0.887*	0.611	0.597	0.261
DOC *vs* Ca^{2+}	0.891*	0.903*	0.888*	0.666
Humus A-horizons (N = 4)				
DOC *vs* pH	–0.656	0.815	0.749	0.992*
DOC *vs* Ca^{2+}	–0.383	0.842	0.827	0.977*

* $p < 0.05$

Qualitative changes in leached DOM over the course of the experiment

FTIR-ATR analysis after 20 weeks of the experiment showed that the filtrates obtained were rich in different organic compounds. Some differences were found depending on the soil horizon, the plant community and the addition of limestone (Fig. 3).

In the filtrates obtained from all O horizons without the addition of limestone, readily distinguishable absorption bands were detected indicating O-H in alcohols, phenols and carboxylic acids (~3420 1/cm) and aliphatic C-H groups (~2928 1/cm and ~2855 1/cm for SO and DpO) (Fig. 3). Bands indicating aliphatic C-H and/or vibrations of COO^- were also found (~1400 1/cm, DpO; ~1360 1/cm and ~1420 1/cm, BO, SO) as well as a weak band indicating O-H from COOH (~1250 1/cm) and relatively broad bands indicating the presence of carbohydrates (~1050 1/cm, BO, SO and ~1075 1/cm - 1080 1/cm, DpO).

In filtrates obtained from coniferous O-horizons a band at ~1628 1/cm was detected reflecting asymmetric vibration of COO^- or aromatic C=C (Fig. 3). In BO, no band at ~1628 1/cm was detected. Instead, the appearance of a band at ~1605 1/cm reflected an asymmetric vibration of COO^-. In filtrates obtained from BO and SO, a band at ~1360 1/cm was found indicating aliphatic C-H deformation and/or stretching of COO^-.

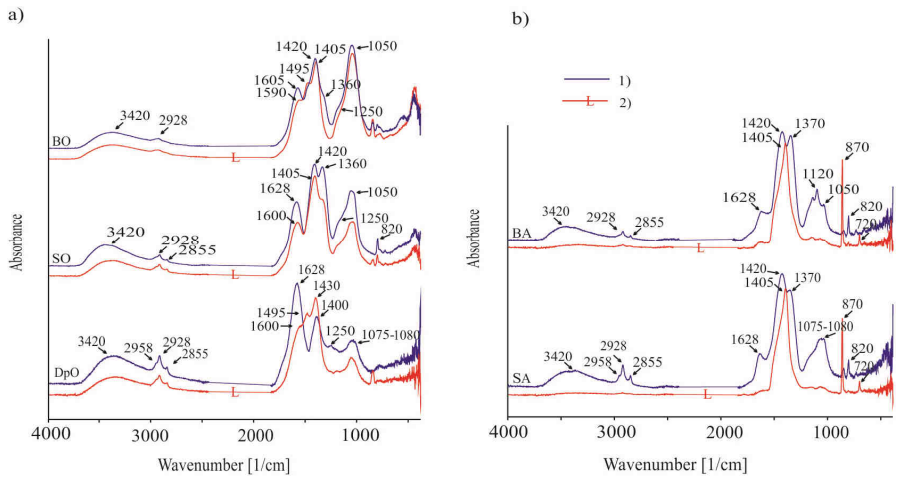

Fig. 3. FTIR-ATR spectra of filtrate leached from: a) O-horizons and b) A-horizons; 1 - without addition of limestone, 2 - with addition of limestone

The addition of limestone altered the FTIR-ATR spectra obtained from the O-horizons in different ways; however, some of the changes were common to all O-horizons (Fig. 3). The bands reflecting asymmetric vibration of COO^- (at ~1628 1/cm in SO and DpO and ~1605 1/cm of BO) shifted towards lower frequencies (~1600 1/cm, SO, DpO and ~1590 1/cm, BO), indicating the appearance of metal-DOM complexes. In all cases, bands representing aliphatic C-H deformation and/or stretching of COO^- (~1360, ~1400, ~1420) 1/cm changed their position towards ~1405 1/cm (BO, SO) or 1410 1/cm (DpO). The appearance of a band at 1405 1/cm together with a shift towards a higher wavenumber (~1380 - ~1405 1/cm) indicated SOM-metal complexes of the bridge or pseudo-bridge type. A band at ~1495 1/cm indicating C=C of aromatic rings to conjugated C=N systems and amino functionalities appeared in BO and DpO (Fig. 3).

Strong and broad absorption bands indicative of O-H in alcohols, phenols and carboxylic acids (~3420 cm-1) were found in filtrates from the A-horizon without addition of limestone (Fig. 3). Bands indicative of aliphatic C-H were detected (~2928 1/cm ~2855 1/cm for BA and SA; ~2928 1/cm for SA). Bands indicating aliphatic C-H and/or vibrations of COO^- were found at ~1370 1/cm and ~1420 1/cm. A band reflecting aromatic C=C or C=O of COO^- anions was detected at ~1628 1/cm. Bands indicating the presence of carbohydrates were detected at ~1050 1/cm (BA) and 1075 1/cm - 1080 1/cm (SA). Absorption bands at ~1120 1/cm, and ~820 1/cm were also observed, indicating the presence of mineral material (silicates and aluminosilicates).

The addition of limestone significantly changed the FTIR-ATR spectra of the filtrates obtained from the A-horizons (Fig. 3). The absorption bands representing O-H groups (~3420 1/cm), aliphatic C-H (~2928, ~2855, ~2958) 1/cm, aromatic C=O and/or C=O from COO^- (~1628 1/cm) and carbohydrates (~1050 1/cm and 1075 1/cm - 1080 1/cm) became much weaker. Furthermore, bands indicating the presence of mineral material (~1120 1/cm, ~820 1/cm), became much weaker.

In contrast to the O-horizons, the weakening of the ~1628 1/cm band was not accompanied by the appearance of prominent bands at ~1550 1/cm - 1600 1/cm typical for metal-DOM complexation (Fig. 3). However, this band may be partially overlapped by bands at lower frequencies, as they give a relatively broad absorption band. Similar to the filtrates from the O-horizons, bands at ~1420 1/cm and ~1370 1/cm disappeared, while a band at ~1405 1/cm appeared. Finally, two new distinct bands appeared at ~870 1/cm and ~720 1/cm, indicating the presence of ions derived from the dissolution of $CaCO_3$.

Discussion

Quantitative changes in the leached DOC due to the addition of limestone

The highest DOC concentration, and therefore leaching, in filtrates from the O-horizons can be explained by the properties of these horizons. Their SOM is more susceptible to decomposition than the SOM in the A-horizon, making it an important source of DOC in the soil solution [5, 33]. In the experimental variants with soils from the O-horizons, the highest DOC concentrations in the 10^{th} week of the experiment are most likely the result of peak microbial activity at that time. A decrease in DOC concentrations in the15^{th} week may be the result of a lack of influx of fresh SOM and/or nitrogen immobilisation and/or NO_2 production. NO_2 can react with certain aromatic compounds that can be toxic to soil microorganisms [33]. In our research, we did not investigate microbial activity, but the appearance of aromatic compounds on conjugated C=N systems and amino functionalities is suggested by the appearance of a band at ~1495 1/cm in filtrates from BO and SO [34] (Fig. 3).

The highest DOC leaching in coniferous O-horizons after limestone addition, which occurred in the first phase of the experiment (the 5^{th} week), was most likely the result of both increased microbial activity and SOM solubility caused by increasing soil pH in the previously acidic coniferous O-horizons. In soils with pH ~4-5, the adsorption of SOM by Al-Fe oxides is the strongest [3], so the pH increase caused by limestone addition in SO (pH before the experiment was 4.77, Table 2) may be sufficient for DOM deprotonation and its release into the soil solution in the first phase of the experiment, which was observed as an increasing DOC concentration in SO with limestone (Table 5). All O-horizons studied were mixed with mineral material (Table 1); therefore, adsorption of

SOM by Al/Fe oxides and clay minerals may occur. In soils with pH < 4, Al^{3+} is toxic for microorganisms, so in the case of the very acidic O-horizon DpO (pH = 3.67), the addition of limestone could be the trigger to increase microbial activity by increasing the pH and consequently the DOC concentrations, and thus leaching in the first phase of the experiment. Similarly to our case, it has been reported in a 20-month field experiment that liming ($CaCO_3$+Mg) causes an increase in DOC release into the soil solution in extremely acid soils (pH = 3.5-4.5), while in less acid soils (pH = 4.5-5.0) no increase in DOC release was detected [20]. After the 10^{th} week of our experiment, different patterns of DOC leaching were observed in the coniferous O-horizons. For DpO, the amount of DOC leached by limestone addition was lower than without limestone addition, whereas for SO, even more DOC was leached after limestone addition (Table 5). This in turn is consistent with the results of studies that have documented an increase in the amount of leached DOC due to long-term liming from both A-horizons [21] and O-horizons [19, 35] in acid soils. In these studies, the authors explain this effect by an increase in soil biological activity (measured as respiration) due to liming. It is possible that in our experiment, in the case of DpO, the biological activity did not compensate for the chemical weathering caused by the unsaturated filtrate.

In contrast to the coniferous O-horizons, no increase in leached DOC was observed in the beech (BO) samples with limestone added in the 5^{th} week of the experiment (Table 5). The pH of BO, higher than that observed in both coniferous O-horizons, was probably the result of the high natural calcium content of the O-horizon under beech (Table 2). The relatively high pH of BO and the very low pH change during the first 5 weeks after limestone addition (Table 3) suggest that there were no significant changes in the soil environment. Thus, neither the effect of increased DOM solubility nor increased microbial activity is likely to have affected DOC leaching in BO in a meaningful way. Instead, SOC stabilisation may have occurred in BO, as there was a decrease in DOC during the first 5 weeks of the experiment following limestone addition (Table 5). DOC stabilisation could occur to some extent *via* the formation of complexes and bridges between aluminosilicates and iron oxides in the presence of Ca^{2+} (mineral admixture, Table 1), or *via* the formation of organo-metal supramolecular structures with the SOM exchange sites and with DOM molecules (flocculation) [3, 16, 19, 36].

A decrease in DOC leaching reported in the 5^{th} week of the experiment after the addition of limestone to both A-horizons suggests the stabilisation of DOC in these horizons (Table 5). This can be explained mainly by the combined effect of the increase in pH and the availability of Ca^{2+} ions (Tables 3, 4). Experimental studies have shown that Ca^{2+} ions play an important role in the adsorption of DOM on ferrihydrite at high pH ($\geq$ 7) [17] and on montmorillonite [36]. Ca^{2+} ions themselves are strongly absorbed by SiO_2 and Al_2O_3 at high pH [37], suggesting the possibility of DOM complexing with these minerals *via* Ca^{2+} ions. Considering that the addition of limestone rapidly increased the pH at the 5^{th} week (Table 3), it can be assumed that adsorption of DOM by clay minerals and/or Al and Fe oxides in the presence of Ca^{2+} ions was most likely. This is in agreement with the results of other laboratory experiments [16, 33], which show that the addition of Ca^{2+} causes a significant decrease in the extent of DOC leaching. Some authors point to a competition between DOC and SO_4^{2-} ions [20], therefore we tried to take this into account in our experiment, but we did not obtain a significant correlation between DOC and SO_4^{2-} ions (data not shown).

Qualitative changes in leached DOM due to the addition of limestone

Our results show that in the A-horizons, mainly aliphatic compounds, phenolic groups and carbohydrates were retained in the soil, which is clearly confirmed by the weakening of the absorption bands indicating these groups (Fig. 3). This can be explained by the immobilisation of these compounds via the formation of Ca bridges between OM and soil minerals, given the availability of a large number of Ca^{2+} ions derived from limestone dissolution [31]. Phenolic groups are thought to be important for Ca^{2+} complexation in less acidic environments [38]. This effect is much less pronounced in the O-horizons, where there is much less mineral material (Table 1). In the O-horizons there is no clear weakening of the bands that occurred in the A-horizons (Fig. 3). We can therefore assume that after the addition of limestone, flocculation occurs and relatively stable aggregates are formed, which is indirectly evidenced by the disappearance of absorption bands in the filtrate originating from silicates and aluminosilicates (Fig. 3). The presence of Ca^{2+} promotes the flocculation of DOM with SOM and soil minerals [3, 13, 18]. Flocculation is favoured by high pH (> 7) [39] and Ca^{2+} concentration (above ~ 0.18 mM) [15] - conditions present in our experiment (Table 4). The complexation of polysaccharides with clay minerals and Fe oxides in the presence of Ca^{2+} ions has been described previously [9, 17]. These are mostly derived from microbial secretions and are usually negatively charged, making them susceptible to cation bridging [7]. The role of aliphatic C-H groups in complexation with clay minerals is less clear. The study [7] points to the role of low molecular weight organic acids in SOM stabilisation. Another suggests that aromatic compounds are those complexed with clay minerals [9]. The results of our experiment do not exclude aromatic carbon as an important factor involved in SOM complexation in the presence of Ca^{2+}. This is supported by the observed decrease in intensity in the band at ~1628 1/cm in the A and O-horizons (SO, DpO) after limestone addition (Fig. 3). However, this band may also be indicative of COO^- groups [34]. A decrease in band intensity at ~3420 1/cm occurs in soil solutions from the A-horizons after limestone addition, suggesting an important role of hydroxyl (O-H) groups in the formation of Ca-DOM complexes in these horizons. The results of quantitative changes in DOC leached with and without limestone addition, discussed above, indicate a stabilising role of the formation of insoluble bonds between SOM and soil minerals, reducing DOC concentrations in filtrates.

In contrast, FTIR-ATR studies show that there may be another pattern related to the availability of large amounts of Ca^{2+} and high pH. The results of our research suggest that the addition of limestone may cause the formation of Ca-DOM complexes, which are leachable and can be removed from the soil and transferred to the filtrate [3, 40]. The results of our experiment indicate the occurrence of such complexes in filtrates obtained from both O and A-horizons. The formation of Ca-DOM complexes with carboxylic groups is confirmed by the appearance of a band at ~1405 1/cm, which is often interpreted as a side effect of the symmetric COO^- band in SOM-metal complexes of the bridge or pseudo-bridge type [8, 41-43]. This interpretation is supported by a shift of a band at ~1380 1/cm towards a higher wave number, i.e. ~1405 1/cm [43]. In our study, however, the appearance of the band at 1405 1/cm was not only associated with a weakening of the band at ~1360 1/cm - 1380 1/cm, but also at 1420 1/cm. The observed positive correlation coefficient between Ca^{2+} ions and DOC loads (Table 6) confirms the presence of Ca-DOM complexes in the soil solution [44, 45]. The formation and leaching of leachable Ca-DOM complexes could be facilitated under the conditions of complete

saturation of Ca^{2+} ions in the soil solution (Fig. 2) and in the soil exchange complex [31] occurring under the experimental conditions.

Possible environmental effects

The results of studies carried out in the Tatra Mountains indicate that the production of glycogen (carbohydrates) in the soil increases immediately after a windthrow, most likely due to a rapid decrease in the fungal population [25]. Our laboratory experiments have shown that a significant proportion of these compounds can be retained in the soil (the A-horizons) if limestone fragments are present. In this case, the soil can act as a filter, preventing selective leaching of this particular DOM fraction from the soil, which is also important because carbohydrates are considered to be the most labile fraction in the soil system [7]. In addition, SOM fractions adsorbed to the mineral fraction become much more difficult for microorganisms to degrade [46]. Both processes can be accelerated by flocculation of soil colloids in the presence of Ca^{2+} ions, which is macroscopically manifested by an increase in soil structural stability. On the other hand, limestone addition may increase DOM leaching from acidic O-horizons of coniferous forests. DOM enters the soil solution mainly in the form of soluble Ca-DOM complexes, mostly associated with carboxyl groups. This finding is important in the context of DOM transport, but also in terms of chemical weathering and Ca^{2+} transport throughout the soil profile and in denudation processes. Previous research has shown that both weathering and denudation are enhanced under conditions of contact between calcareous rocks and organic/humus soils [31].

The results obtained show that Ca-DOM complexes leached from the O-horizons could be adsorbed by soil exchange sites in the presence of Ca^{2+} ions until they are fully saturated. The A-horizons are said to have a limited available sorption capacity for DOM due to the indigenous OM already present [2]. Once the A-horizons are saturated, the B-horizons, which have a higher available sorption capacity for OM, would become the OM sink [2]. Sorption can be effective if soil solutions percolate slowly through the soil profile. However, in montane and forest soils, much of the water movement from soil to surface water occurs by preferential flow [47]. This occurs relatively rapidly through soil macropores with limited contact of the soil solution with the soil matrix. Under such conditions, Ca-DOM complexes are transported directly from the O-horizons to the surface water. As sorption in soil exchange sites facilitates DOM stability compared to DOM in soil solution [46, 48], research on the effect of DOM complexation with Ca^{2+} ions on DOM degradability in solution is scarce and gives conflicting results [48, 49]. Nevertheless, DOM originating from the O-horizons is more susceptible to microbial degradation than DOM originating from deeper soil horizons [5, 33]. Thus, the decomposition of Ca-DOM complexes may be a potential source of CO_2 to the atmosphere. The above processes may contribute to carbon loss from ecosystems following forest disturbance [50], and may be amplified in areas with calcareous soils.

Climate change may potentially increase the amount of DOM transported to surface waters through increased DOM production in soils [51] and increased water movement by preferential flow during extreme rainfall events [52]. On the other hand, prolonged draughts generally limit the mobility of DOM and reduce the amount and biological reactivity of DOM [53]. Further research on DOM dynamics in disturbed forest ecosystems is therefore needed. They should focus on the link between soil processes and water cycling in the

natural environment. The results are important in the context of the key role of forest ecosystems in DOC sequestration and mitigation of greenhouse gas emissions [54, 55].

Conclusion

Quantitative changes in DOC leached from the soil were observed following the addition of limestone to initially acid soils. The magnitude and direction of these changes were strongly dependent on the type of soil horizon. Limestone addition led to an increase in DOC leaching in the acidic O-horizons of coniferous forests, most likely due to an increase in pH and an increase in microbial activity. In the A-horizons, DOC leaching was reduced in the first phase of the experiment, indicating the occurrence of adsorption processes in these horizons.

The presence of limestone in the initially acid soil changed the composition of the DOM leached from the soil. In the case of the A-horizons, there was a strong immobilisation of carbohydrates and aliphatic compounds, which were most likely immobilised in the soil due to the presence of Ca bridges. In the case of the A and O-horizons, DOM was leached mainly in the form of Ca-DOM complexes.

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