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ORIGINAL PAPER

Relations between labile and stable pool of soil organic carbon in drained and rewetted peatlands

Barbara Kalisz, Andrzej Łachacz

Department of Soil Science and Microbiology, Faculty of Agriculture and Forestry, University of Warmia and Mazury in Olsztyn, Poland

Abstract

Soil organic carbon (SOC) is a basic element which influences soil processes. In peatlands, carbon is stored in various organic compounds, and any alteration of peatland, especially drainage, leads to the changes in SOC pools. The aim of the study was to assess the relations between labile carbon and humus fractions, and state of topsoil transformation as well as the relative composition of labile carbon pool in organic soils affected by drainage and rewetting. We extracted labile carbon with hot water (HWC) and stable carbon in the form of humus substances (chemical extraction), and measured the amounts on CN analyser. In the labile pool, we estimated the relative amount of hydrophilic and hydrophobic fractions using chromatographic techniques. The state of soil transformation was assessed on the base of water-holding capacity index (W1). Mean HWC concentrations were higher in drained peatlands than in the rewetted one. The chromatographic analysis of labile carbon showed that HWC fraction contained more hydrophilic than hydrophobic organic compounds. High concentration of humus fractions in drained peatlands resulted in the higher humification degree, which amounted to 44.8%. The drainage and soil use influenced the quantities of studied labile and stable carbon pools. The HWC concentration proved to be a good indicator of biological changes in organic soils, and may be a good index to monitor the initiation of peat formation at rewetted peatlands which had been previously drained. The state of transformation expressed by W1 index influenced labile carbon pool and relative amounts of hydrophilic part of HWC, as well as free fulvic acids and stable carbon pool (humification degree). The release of labile organic carbon from peatlands implies a loss of sequestered carbon. The stable carbon pool, when subjected to drainage, becomes vulnerable to microbial changes, and successively becomes secondary humified, therefore the value of humification degree increases.

Keywords: Histosols, HWC, organic carbon, organic soil, humus, peatland degradation

Barbara Kalisz, PhD, Department of Soil Science and Microbiology, Faculty of Agriculture and Forestry, University of Warmia and Mazury in Olsztyn, Plac Łódzki 3, 10-727 Olsztyn, barbara.kalisz@uwm.edu.pl
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INTRODUCTION

Soil organic carbon (SOC) as a part of soil organic matter (SOM), is a basic element which influences soil processes. Human activities affect these processes and lead to carbon loss or storage/sequestration. It is well-known that climatic changes are related mainly to carbon (Duval et al. 2018), therefore, SOC is an indicator in monitoring the soil changes (Conant et al. 2008, Fenner and Freeman 2011, Kalisz et al. 2015, Duval et al. 2018, Li et al. 2018, Lorenz et al. 2019, Becher et al. 2020, Mendyk et al. 2020). Generally, soils contain (globally) approximately 2,344 Gt of organic carbon (Stockmann et al. 2013), from which even up to 40% is stored in peatlands (Dixon et al. 1994, Hugelius et al. 2020, Harris et al. 2022). Any changes in the quantity or quality of SOC, affect the carbon cycle and lead to carbon pools variations. Therefore, it is crucial to know the SOC stocks and pools in soils. The carbon in peatlands is stored in various organic compounds, from undecomposed plant remnants to humified substances of complex composition. These organic substances are transformed by microbial activity and are a part of various carbon pools. Any alteration of peatland ecosystem, especially drainage, leads to the changes in SOC pools (Heller and Zeitz 2012, Kalisz et al. 2015, Glina et al. 2016, Kalisz et al. 2021, Smolczynski et al. 2021, Lipka et al. 2022).

Generally, SOC can be divided into labile (active) and stable (refractory, recalcitrant) pools (Lützow et al. 2007). Soil labile organic carbon pool is composed of amino-acids, carbohydrates, microbial biomass and other simple organic compounds (Zou et al. 2018), it is also a part of SOC which is cycling fast in the environment (Liu et al. 2018), no longer than several years, while the refractory carbon cycle may last even several thousand years (Lehmann and Kleber 2015, Ouyang et al. 2018). One of labile carbon fractions is hot water-extractable carbon (HWC), which is correlated with the mass of micro-organisms simultaneously being an excellent indicator of qualitative changes in organic matter (Ghani et al. 2003, Kalisz et al. 2012, Sparling et al. 2016, Kalisz et al. 2021, Smolczynski et al. 2021, Glina et al. 2022, Mencel et al. 2022). This fraction is potentially the most susceptible to oxidation to CO_2 (Schulz et al. 2004, Hassan et al. 2016, Bojko et al. 2017, Cao et al. 2017, Duval et al. 2018), and therefore has the greatest impact on global climate change. The HWC fraction includes a broad spectrum of organic constituents with molecular weights ranging from hundreds to more than 300,000 Da (Piccolo 2002). It represents a small but highly important fraction of the SOM carbon due to its high reactivity (McKee et al. 2016), which comprises organic compounds operationally termed hydrophilic (organic substances with hydrogen bonds and polar functional groups) and hydrophobic (organic substances with aromatic cores).

The stable or recalcitrant carbon pool is supposed to be ‘microbiologically protected’ (Lehmann and Kleber 2015), hence its transformation may last much longer than in the case of labile organic compounds. The stable carbon compounds comprise humic substances, and diverse carbon compounds with low molecular mass forming dynamic associations (Sutton and Sposito 2005, Kalisz et al. 2021). Compounds that are classified as recalcitrant or stable are assumed to comprise greater percentage of SOC than the labile carbon pool (Davidson and Janssens 2006). The stable pool comprises substances which are resistant to decomposition for a long time, have high molecular weights, and which can be operationally termed humic substances (unbound or bound to cations) (Valladares et al. 2007, Kalisz et al. 2010, Smolczynski et al. 2011). The studies of SOC use methods that involve chemical fractionation for the determination of different fractions (Lützow et al. 2007). Although chemical (alkaline) extraction of humus fractions has some uncertainties, it is effective in assessing the SOC pools (Gerke 2018, Weber et al. 2018).

The aim of the study was to assess:

- the relations between labile carbon (HWC) and humus fractions, and state of topsoil transformation
- the relative composition of HWC
- in topsoils of organic soils (Histosols) affected by drainage or rewetting.

MATERIALS AND METHODS

The soil samples (n=18) were collected from surface (0–30 cm) soil layers from three peatlands in north-eastern Poland (Figure 1). The climate in NE Poland is transitional (continental-maritime), moderate, where average annual air temperature is 7.5–8.0°C, and average annual precipitation 550–650 mm. The investigated peatlands are fens, and had been formed from alder, rush and sedge peats (degree of decomposition of approx. 60–100%). In 19th and at the beginning of 20th century, they were drained for agricultural purposes and used as meadows and pastures (grasslands). Nowadays, their use is different. The P1 peatland, deeply drained (groundwater level is approx. 150 cm below the surface) and has been abandoned, not used for years (ca. 30). The P2 peatland was drained, and has been used as grassland. The groundwater level at P2 is at 60–80 cm below the surface. At the P1 and P2 peatlands the plant species typical for *Molinio-Arrhenatheretea* class prevail (at P1 high share of herbs). The P3 peatland had been drained at the end of 19th century, and since the 80s of the 20th century it has been subjected to rewetting (spontaneously) as a result of overgrowing drainage ditches. Nowadays, the groundwater level is at the surface (fluctuating at 0–15 cm above the surface), and the plant species typical for *Phragmitetea* and *Scheuchzerio-Caricetea nigrae* (with high share of *Carex elata*) classes prevail. The soils at studied sites were classified into:

P1 & P2: hemic murshic soil (drained) (Kabala et al. 2019), and Rheic Murshic Hemic Histosol (Eutric, Hyperorganic) (IUSS 2022)

P3: hemic murshic peat soil (Kabala et al. 2019), and Rheic Hemic Histosol (Eutric, Hyperorganic) (IUSS 2022).

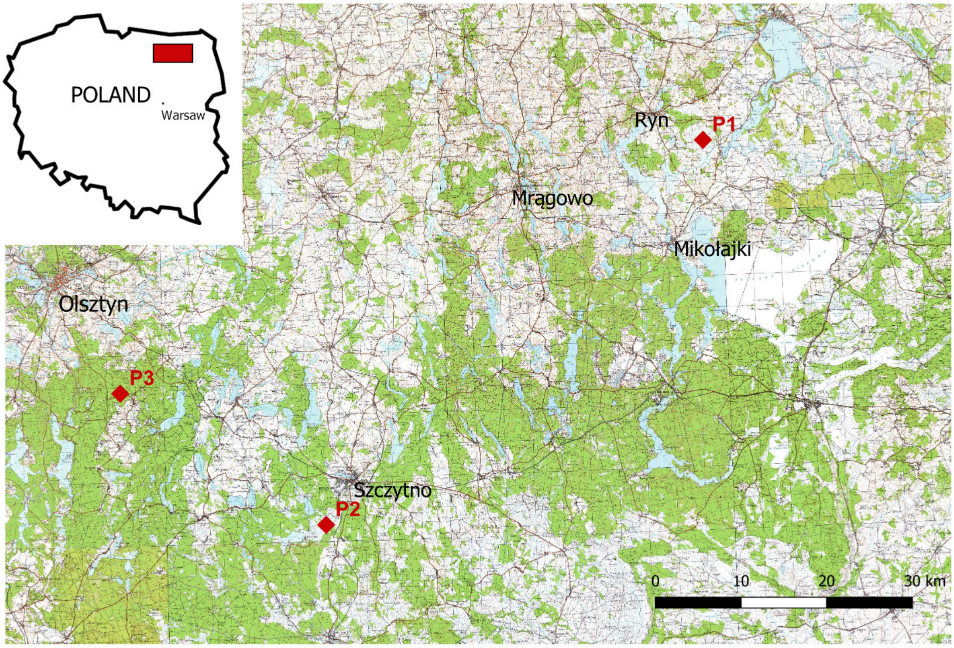

Fig. 1. Location of studied peatlands (P1 drained, abandoned; P2 drained, used as grassland; P3 rewetted, not used)

In the collected soil samples the following analyses were carried out: ash content was determined on the basis of loss-on-ignition at 550°C. Total organic carbon (TOC) and nitrogen (TN) were measured on Vario MaxCube CN Elementar analyser. Soil reaction was determined potentiometrically in potassium chloride (1 M KCl dm^{-3}; soil:KCl ratio 1:10). State of topsoil transformation was estimated with water holding capacity method – W1 index (Gawlik 1992). The W1 index was determined by dividing the water capacity of a soil sample that is absolutely dry (dried at the temp. 105°C) by its water capacity in a natural (field-moisture) state. Briefly, the fresh soil material was divided into two parts. The first one was soaked in distilled water for 7 days, put onto a sieve (of 1.0 mm in diameter) and centrifuged at 1000 g for one hour. Then the amount of water in the soil sample was determined gravimetrically (a). The second part was oven-dried at 105°C, then soaked in distilled water for 7 days, put onto a sieve and centrifuged at 1000 g for one hour. Then the amount of water in the soil sample was determined (b). The water-holding capacity index W1 was calculated according to the formula: W1 = b / a, and depending on the index value, the state of transformation was determined: 0.36–0.45 – initially transformed, 0.46–0.60 – weakly transformed, 0.61–0.75 – medium transformed, 0.76–0.90 – strongly transformed, >0.90 – degraded peatland.

For the characteristics of stable carbon pool, humus fractions (HS) were extracted using chemical extraction (Kalisz et al. 2021). Three extractants were used: 2 mol dm^{-3} phosphoric acid (H_3PO_4) was used to extract the most mobile humus compounds – free fulvic acids (FFA), 0.1 mol dm^{-3} sodium diphosphate ($Na_4P_2O_7$), used to extract humus compounds bound to cations (HS1), and 0.1 mol dm^{-3} sodium hydroxide (NaOH), used to extract strongly bound humus compounds and those with high molecular weight (HS2). Briefly, 2 mol dm^{-3} phosphoric acid (H_3PO_4) (soil/solution ratio 1:10) was mixed with soil, agitated for 30 min with the back and forth shaker and centrifuged for 5 min at 1500 g. Then the supernatant was filtered on a flat filter. The filtrate (labile pool) is termed FFA – it contained free fulvic acids and some other simple organic compounds. The remaining soil was mixed with sodium diphosphate (0.1 mol dm^{-3} $Na_4P_2O_7$), left in contact overnight (agitating several times), centrifuged for 30 min at 3000 g and filtered. Similar extraction sequence was performed with NaOH. Before every extraction, the soil was washed with inorganic water by agitating for 15 min, centrifuged for 30 min. and filtered (the filtrate of washing water was discarded). Part of organic matter which was not soluble in any of the three extractants was called residuum. The carbon in the examined fractions was measured on a Multi N/C 3100 Analityk Jena analyser.

The humification degree (HD) was calculated according to the following formula:

$$HD = (\Sigma HS / TOC) \times 100 \, [\%]$$ (Klavins et al. 2008)

For the characteristics of labile carbon pool, hot water-extractable carbon (HWC) was used (Sparling et al. 1998). Briefly, 4 g of air-dried soil was incubated with 20 ml demineralized water in a capped test-tube at 70°C for 18 h. The tubes were shaken by hand at the end of the incubation and then filtered through Whatman ME 25/21 ST 0.45 µm membrane filters (mixed cellulose ester). The hot-water extractable carbon (HWC) was measured on Multi N/C 3100 Analytik Jena analyzer.

In HWC extracts, using aqueous solutions of various pH, selective elution of the polar fraction of organic compounds that are the hydrophilic fraction (HL) and the hydrophobic fraction (HF) was carried out. The fractions obtained in this way were tested by the RP HPLC-C18 chromatographic technique. From extracts prepared on the basis of hot water (HWC), the hydrophilic fraction (HL; considered polar), and hydrophobic fraction (HF; considered aromatic) were separated using STRATA 30 mg 30 µm columns (Solid Phase Extraction). The columns were conditioned sequentially in 1 ml of methanol, 1 ml of water, and 10 ml of water acidified to pH 1.5 with hydrochloric acid. Then, 5 ml of the sample acidified to pH 1.5 were applied to the columns, then, the column was washed with 10 ml of water at pH 1.5. Fractions were eluted with 0.1 mM and 50 mM NaOH.

All statistical analyses were performer using STATISTICA 13. Most of the studied parameters (TOC, C/N, W1 index, HWC, FFA, HS2, HD) were normally distributed (determined by Shapiro-Wilk test). The relationships between soil transformation parameters and soil carbon pools were analysed using ANOVA and host-hoc Tukey test (α=0.05). Pearson's correlations were used to determine correlation coefficients between variables. Principal component analysis (PCA) was applied to show the structure of relationships between studied variables. A set of 10 original variables was transformed into a set of orthogonal variables (principal components). Due to the differences in units of the variables, the data were subjected to standardization, and the principal components were calculated based on the correlation matrix.

RESULTS

The ash content in studied topsoils of the three peatlands was similar, approx. 18–25% (Table 1), being the highest in rewetted peatland (P3). The TOC content was the highest in the topsoil of drained peatland used as grassland (P2), and lower in topsoils of abandoned peatland (P1) and rewetted peatland (P3). The contents of TOC in the studied peatlands were similar (approx. 420–444 g kg^{-1}). More varied concentrations were noticed for TN (approx. 20–26 g kg^{-1}), and affected the C/N ratio, which was the widest in rewetted peatland and lower in the drained ones (Table 1). The soil reaction of studied topsoils was slightly acidic (pH 5.65–6.13). The state of transformation expressed as the W1 index (water-holding capacity index) was the highest in drained abandoned peatland, and the lowest in the rewetted one (Table 1).

Table 1

Basic properties of studied topsoils of peatlands (mean ± standard deviation)

Peatland	Ash	TOC	TN	C/N	pH (KCl)	W1
	g kg^{-1}					
P1	179.9±48.7	421.8±68.41	26.32±2.06	16.27±3.75	5.66	0.72±0.15
P2	206.1±83.2	444.6±46.57	24.99±1.74	17.93±2.89	6.13	0.68±0.04
P3	249.1±47.3	420.5±26.48	20.41±0.17	20.60±1.30	5.65	0.37±0.01

Mean HWC concentration was the highest (5.27 g kg^{-1}) in drained, abandoned peatland (P1), and the lowest (3.23 g kg^{-1}) in the rewetted one (P3). In both peatlands the variation of the results was also high (Figure 2A). In agriculturally used peatland (P2), the mean concentration of HWC amounted to 4.72 g kg^{-1}, and was least varied (Figure 2A). The drainage

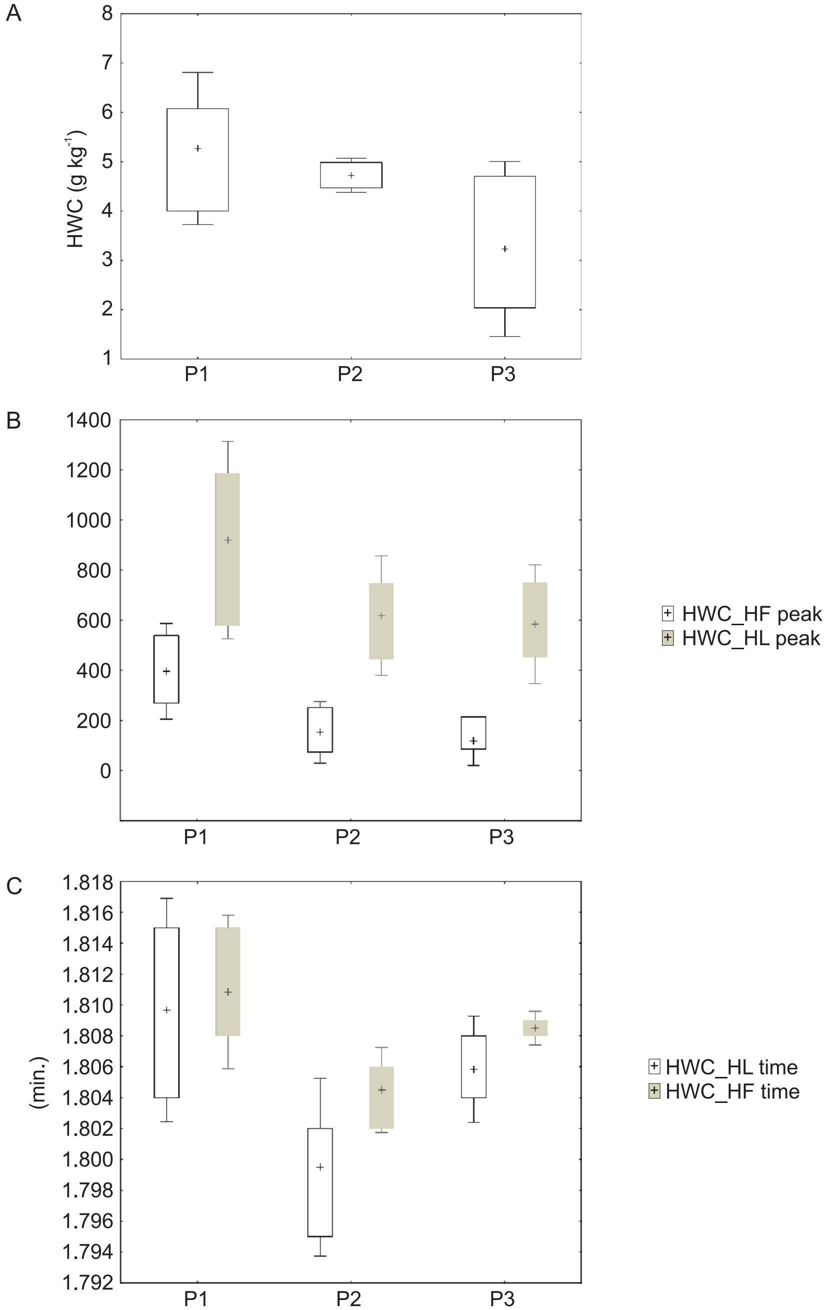

Fig. 2. Mean concentration of HWC in topsoil of peatlands (A) and relative composition of HWC expressed with hight of peak at the chromatogram (B) and retention time (C) of hydrophobic (HF) and hydrophilic (HL) fractions (+ is mean value, box is min-max, whiskers are mean ± 2 standard deviation)

of peatland increased the HWC content in P1 in relation to P2 or P3, which was related to the ongoing mursh-forming process (and also groundwater level). Re-wetting of peatland (P3) initiated series of soil processes, i.e., peat formation, but the HWC concentration was not as high as in case of mursh formation.

The chromatographic analysis of labile carbon also showed that HWC fraction contained more compounds of lower molecular weight (conventionally called hydrophilic) than particles with aromatic (hydrophobic) rings, as evidenced by the relative content of these fractions measured by the height of the peak in the chromatogram (Figure 2B). The hydrophilic and hydrophobic fractions did not differ significantly in terms of polarity and mass, as their retention times were similar (Figure 2C). The retention time of the fractions depends on polarity and molecular weight, and the more polar the fraction and the higher its mass is, the longer the retention time will be.

The mean concentration of FFA was very similar in studied peatlands (although it varied in wide ranges in P1). Recognizable differences were found for HS1 concentrations – the lowest concentrations were in rewetted peatland, and distinctively higher in the drained (P1 and P2) ones (Figure 3). The concentrations of HS2 were similar to HS1, i.e., the lowest in the rewetted peatland and higher in drained peatlands. However, in the agriculturally used peatland (P2) the concentrations of HS2 were higher than concentrations of HS1 and much wider than in case of other two peatlands. High concentration of humus fractions in drained peatlands resulted in the higher humification degree, which amounted to 44.8% in drained abandoned peatland, 31.1% in drained, agriculturally used peatland and only 2.7% in the rewetted peatland (Figure 3).

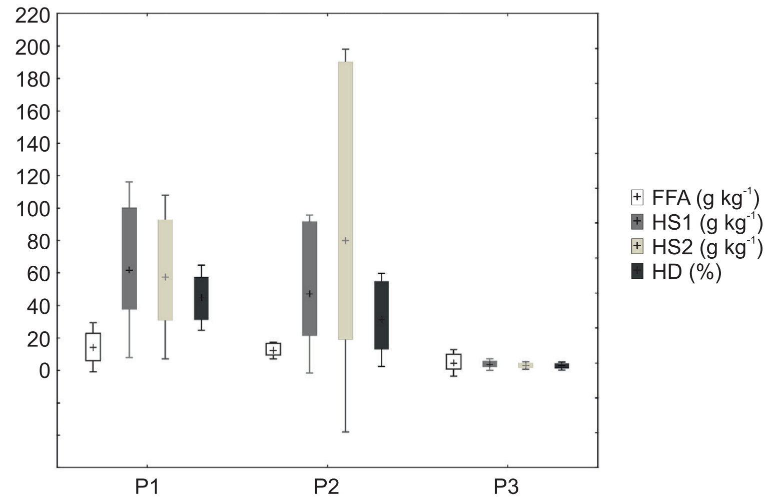

Fig. 3. The concentrations of humus fractions (FFA – free fulvic acids, HS1 and HS2; g kg^{-1}) and humification degree (HD) in the topsoils of peatlands

Table 2

Correlation between indicators of soil transformation (water holding capacity index (W1) and C/N) and total organic carbon (TOC) on labile (HWC) and stable (FFA, HS1, HS2) carbon fractions and humification degree (HD)
(*Pearson's correlation coefficient significant at p<0.05, n=18)

	HWC_HF time	HWC_HF peak	HWC_HL time	HWC_HL peak	HWC	FFA	HD	HS1	HS2
W1	0.12	0.64*	0.10	0.41	0.85*	0.82*	0.82*	0.56*	0.42
TOC	-0.63*	-0.52*	-0.59*	-0.31	-0.36	-0.65*	-0.45	0.16	0.24
C/N	-0.38	-0.59*	-0.36	-0.23	-0.57*	-0.62*	-0.54*	-0.18	-0.04

0.80 ÷ 0.99
0.60 ÷ 0.79
0.40 ÷ 0.59
0.01 ÷ 0.39
-0.39 ÷ -0.01
-0.59 ÷ -0.40
-0.79 ÷ -0.60

The TOC content and state of transformation (expressed by C/N ratio and W1 index) influenced the quantities of studied labile and stable carbon pools (Table 2). The state of transformation expressed by W1 index influenced HWC fraction, relative amount of hydrophilic part of HWC, FFA and HS1 fractions, as well as humification degree (positive significant correlation). The C/N ratio was negatively correlated with HWC and HD, which means that the wider C/N was (as in rewetted peatland P3), the lower HWC and HD. The studied parameters were also correlated with each other, as illustrated in Figure 4. The principal component analysis (PCA) showed that drainage/rewetting of peatland and soil use are dependent on labile and stable carbon pools. The first principal component PC1 explained more than 55% of the total variability of data, represented HWC, W1, FFA, HD (negative correlation with these variables) and C/N (positive correlation with this variable). The second principal component PC2, which explained 19% of the total variance, indicated TOC and HS2 (positive correlation with these variables).

DISCUSSION

The content of TOC in studied peatlands was similar and related to their origin (fens), which is important for further assessments because the quantities of SOC fractions (HWC, and humus) are frequently correlated with TOC (Kalisz et al. 2010, Kalisz et al. 2015, Kalisz et al. 2021, Lachacz et al. 2023, Łachacz et al. 2023). Both, labile and stable SOC pools, affect global carbon cycle. The stability of SOC is basically regulated by the characteristics of humus and the quantities of organic carbon that can be extracted

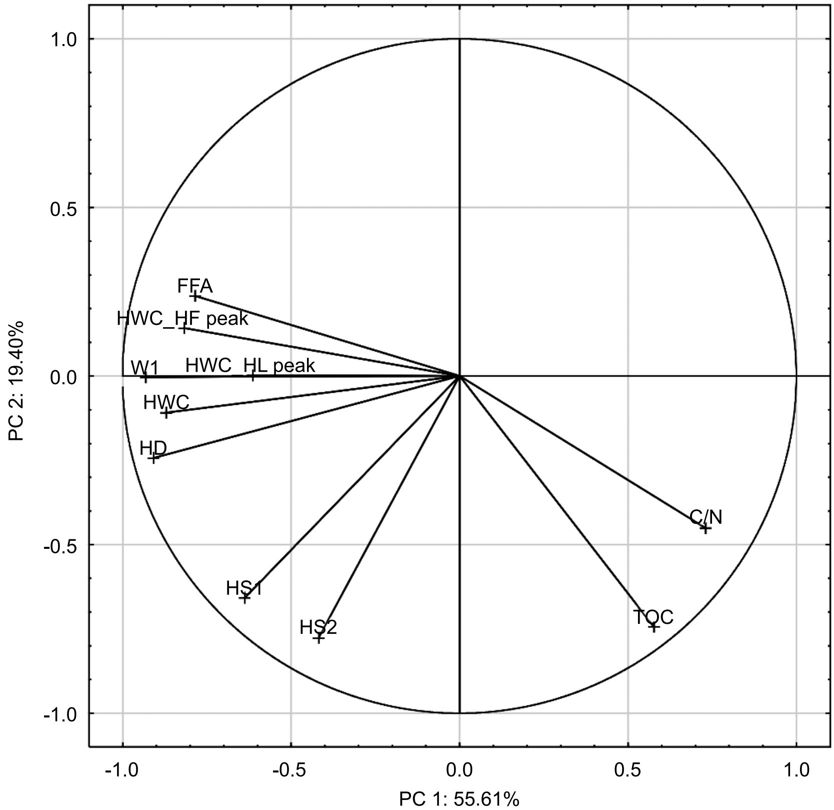

Fig. 4. The PCA ordination conducted on labile and stable carbon fractions, humification and transformation indices. Abbreviations: FFA – free fulvic acids, HD – humification degree, HS1 – humus compounds bound to cations, HS2 – strongly bound humus compounds and those with high molecular weight, HWC – hot water-extractable carbon, HL – hydrophilic fraction, HB – hydrophobic fraction, TOC – total organic carbon, W1 – water-holding capacity index)

(Wang et al. 2023). Labile SOC is a measure of the biological activity of soils (Kalisz et al. 2015, Smolczynski et al. 2021, Smreczak and Ukalska-Jaruga 2021). The relations between these two SOC pools are therefore related to the SOM transformations.

The indicators of transformations of SOM in studied peatlands were C/N ratio and water-holding capacity index – W1. The narrowing of C/N ratio suggests mineralization of SOM, and when it ranges between 10 and 15 it suggests accelerated mineralization of SOM and release of N (Watson et al. 2002). The C/N ratio in studied rewetted peatland was typical for unaltered fen soils (Ilnicki and Zeitz 2003, Kalisz et al. 2010, Heller and Zeitz 2012, Kalisz et al. 2015, Kalisz et al. 2021), whereas in drained peatlands it was lower, however still quite high, not suggesting rapid SOM mineralization. The W1 index, a quantitative indicator of transformation of peat soils reflects the degree of peat changes after drainage and the degree of degradation of organic soils (Zajac et al. 2018). Its lower value indicates lesser changes of SOM. It depends on soil water conditions, and is not basically related to the time that elapsed since drainage (Gawlik 1992, Sokolowska et al. 2005, Glina et al. 2016, Zajac et al. 2018). The above was also confirmed in the studied peatlands, i.e. the topsoil of rewetted peatland should be regarded as not transformed, the drained agriculturally used peatland as medium transformed, and drained abandoned peatland as strongly transformed. The Tuckey test confirmed that there are significant differences between P1 and P3 as well as between P2 and P3 (but not significant between P1 and P2) considering W1 index, the labile carbon pool (HWC), humification degree (HD) and humus fraction bound to cations (only HS1).

The labile SOC pool (HWC) was the lowest in rewetted peatland, which indicates that the biological activity of this peatland has been hampered. The largest labile pool was reported for drained, abandoned peatland, suggesting high microbial activity and mineralization of SOM, which is also in line with the values of C/N ratio and W1 index in this peatland. Drainage of fens has been shown to increase microbial activity in European peatlands (Straková et al. 2011, Mpamah et al. 2017). In the peatlands of tropical climate the relation may be inverse, and the drained peatlands may show lower biological activity than the unaltered ones (Kononen et al. 2018). The labile carbon pool contains more hydrophilic compounds than the hydrophobic ones, as evidenced by the chromatographic analysis, although the differences in the amounts were not high. The polarity and mass of both fractions were similar in drained, not used peatland, whereas in the drained agriculturally used and rewetted peatlands, hydrophobic fraction had higher molecular weight (as evidenced in Figure 2B). The higher molecular mass and aromaticity in hydrophobic fraction indicate more recalcitrant carbon compounds (Mustafa et al. 2022), whereas lower mass indicates more aliphatic, simpler compounds, and higher activity of organic matter (Smreczak and Ukalska-Jaruga 2021).

The stable organic compounds, which were extracted as humus substances comprised greater proportions than labile ones, which was also reported in other studies (Davidson and Janssens 2006). In the rewetted peatland with lower biological activity, the extracted humus fractions were low with prevalence of mobile humus compounds (FFA fraction), whereas in drained peatlands more humus compounds were extractable, with prevalence of humus bound to cations (HS1 and HS2). Generally organic materials with higher C/N ratios decompose slower (Kleber et al. 2015) and less humus compounds may be extracted, as reported in this study in rewetted peatland. After peatland's drainage SOM oxidation takes place and relations between SOC fractions are altered (Heller and Zeitz 2012, Kalisz et al. 2021), and the humification degree increases. In drained peatlands, the not humified part of SOM (plant remnants, non-extractable) is subjected to secondary humification and the quantities of FFA, HS1 and HS2 increase (Kalisz et al. 2021)

CONCLUSIONS

The labile carbon pool, indicated by the quantities of HWC was the highest in drained, not used peatland, lower in drained, used as grassland and the lowest in the rewetted peatland. The HWC concentration proved to be a good indicator of biological changes in organic soils, and may be a good index to monitor the initiation of peat formation at drained peatlands which have been subjected to re-wetting. The state of transformation expressed by W1 index influenced labile carbon pool and relative amounts of hydrophilic part of HWC, as well as free fulvic acids and stable carbon pool (especially HS1 and humification degree). The peatlands with higher labile pool, and higher biological activity, had higher share of humic substances and higher humification degree (secondary humification degree which occurs after drainage). Therefore the release of labile organic carbon from peatlands implies a loss of sequestered carbon from humus. In the context of climatic changes and soil carbon sequestration this issue is of great importance. The studies of SOM suggest that the longer an organic matter persists in soil, the more likely it is to become resistant to decomposition, which in unaltered peatlands or rewetted peatlands is expressed as low value of humification degree. However this resistant SOM, when subjected to drainage, becomes vulnerable to microbial changes and successively becomes secondary humified, and therefore the value of humification degree increases.

AUTHOR CONTRIBUTIONS

B.K. – conceptualization; B.K., A.Ł. – funding acquisition; B.K., A.Ł. investigation; B.K. – methodology; B.K. – visualization; B.K., A.Ł. – writing – original draft preparation; B.K., A.Ł. – writing – review & editing. All authors have read and agreed to the published version of the manuscript.

CONFLICTS OF INTEREST

The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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