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Fractions of nitrogen (including ^{15}N) and also carbon in the soil as affected by different crop residues**

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Abstract. Returning crop residue can increase soil organic matter content, and residue quality has an influence over the rate of their turnover. However, there is a lack of information concerning the biochemical transformations of organic compounds of N and C present in the crop residues during subsequent crop growth. In this study, the contents of organic N and C fractions in soils obtained using acid and alkaline hydrolysis under two crop rotations (faba bean *vs*. wheat rotation) were investigated. Black fallow served as a control. The mean total N increased in the order: black fallow, wheat rotation, faba bean rotation, total C and SOM were higher in the cropped soils than in black fallow. Hydrolysable-N (1-step acid hydrolysis) reached 83.7% total N, amino acid-N and threonine+serine-N were the highest in faba bean rotation and the lowest in black fallow, ammonia-N and aminosugar-N were lower in black fallow than in cropped soils. Hydrolysable-N (2-step sequential fractionation) reached 85.3% total N and significant differences were noted between the cropped soils and black fallow, with respect to both the N and C contents. ^{15}N was mainly accumulated in the N soluble and easily hydrolysable N compounds, and these fractions were greater in cropped soils than in black fallow. N in the humic compounds increased from black fallow to faba bean rotation. A PCA analysis showed that crop rotation and soil sampling terms had a substantial influence over cluster formation. An ANOSIM test revealed significant differences between the crop rotation and term treatments. The results indicated that soil with faba bean rotation is richer in N compounds than soil with wheat as a forecrop and this may result in a reduction in N fertilizers for the succeeding crop.

Keywords: organic nitrogen fraction, soil organic matter, crop residues, faba bean, wheat, ^{15}N

INTRODUCTION

Soil organic matter (SOM) is one of the most important parameters of soil biology, it has a significant influence on the chemical and physical properties of the soil. However, its concentration in mineral soil is low, it does not exceed 5% (King *et al.*, 2020; FAO, 2017). SOM is formed by all organic compounds in the soil regardless of their origin. These compounds turnover in the soil, mainly through mineralization and synthesis as well as uptake by microorganisms, and physical and chemical processes (Hayes *et al.*, 2017; Stevenson, 1994). From an agricultural point of view, it is important to maintain the amount of SOM at a high level, because it plays a very important and positive role in soil productivity (King *et al.*, 2020; Schmidt *et al.*, 2011). SOM contains all of the macro and micronutrients which can be absorbed by plants (King *et al.*, 2020). However, the amount of nutrients delivered from SOM are not enough to cover all of the physiological and nutritional

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demands of plants (FAO, 2017) and it is necessary to deliver these elements (mainly nitrogen) to the soil-plant environment.

Nitrogen is applied to the soil in mineral and organic fertilizers, a substantial amount of it (10-20%) is usually immobilized in the form of organic N compounds such as proteins, amino acids and aminosugars during the synthesis of the microbial biomass. During decomposition (mineralization) of the soil microbial biomass, some of the N-compounds are released as mineral forms of N and some portion undergoes conversion to more stable organic N compounds, ultimately becoming a part of the SOM. Some studies have shown that the recently immobilized N organic compounds, mainly amino acids, aminosugars and amides are mineralized far more rapidly than the native organic N compounds like simple and complex proteins (Roberts and Jones, 2008). There is some evidence, that the long term application of N-fertilizers promotes the buildup of rapidly mineralizable N compounds, which reduces the response of crops to fertilizers (Bird *et al.*, 2011; Huo *et al.*, 2017; Jackson *et al.*, 2019), this is caused by the priming effect. The priming effect occurs when soil crop residues are added, this affects the rate of decomposition of the SOM, either positively or negatively. SOM consists of C, H, N and O but C and N have the most substantial effect on soil microbes, therefore adding a substrate containing different ratios of these nutrients to the soil can modify their activity. Fertilizer, plant residues and carbohydrates from living roots can all potentially have an effect on SOM (Bird *et al.*, 2011; Hou *et al.*, 2017; Jackson *et al.*, 2019). In investigations concerning the determination of the fraction of N in the soil, the Bremner method was usually used. Subsequent studies have revealed that using methods involving the recovery of amino acid-N, NH_4^+ and aminosugars-N from soil hydrolysates produces values which are underestimates (Greenfield *et al.*, 2013; Leavitt *et al.*, 1996).

Soil organic carbon (SOC) is the largest terrestrial pool of C and plays a very important role in the global C dynamic and in soil fertility (Drosos *et al.*, 2020; Yang *et al.*, 2022). The amount of C in organic compounds in soil depends on a few factors, mainly the persistence of mineralization and input into the soil (Yang *et al.*, 2022), crop rotation, tillage, climate, soil type and other parameters connected with sustainable agriculture (Man *et al.*, 2021). In general terms, SOM may be divided into three main pools: labile, partially labile and inert (Haynes, 2005). These compounds are differentiated in terms of the mineralization process in the soil. The content of C in the labile fraction of SOM is very important for the evaluation of soil fertility, but from an agricultural point of view it is far more often very important to establish the amount of C in the partially stable fraction, which contains C in fulvic and humic acids.

Non-hydrolysable fractions of SOM contain materials in various stages of decomposition and stabilization and therefore the scope of modelling it is usually considered to comprise multiple soil C pools. Schmidt *et al.* (2011) reported the content of C in this fraction to be in the range of 20-60% in most soils and therefore these results may be a source of errors in the modelling of C turnover in soils (Falloon and Smith, 2000). The amount of C in resistant soil C compounds may be estimated by using a few chemical fractionation methods (Leavitt *et al.*, 1996; Paul *et al.*, 2006; 2011). Greenfield *et al.* (2013) have shown that significant changes occur during hydrolysis resulting in C losses (up to 75% with a preferential loss of ^{13}C) and *de novo* synthesis of non-hydrolysable ^{13}C depleted materials dominated by aromatic>alkyl>carbonyl molecules.

The turnover of C in the soil is influenced by many factors, including the chemical composition of plant materials introduced to the soil, among others. Shirato and Yokozawa (2006) divided organic C compounds in plant litter into three pools according to acid hydrolysis: labile pools I (LP I), labile pools II (LP II) and recalcitrant pools (RP). For mineral soils the best and also the most rapid method is the Bremner method (Stevenson, 1982). The abovementioned factors provide the basis for the search for other methods which could be used to demonstrate the responsiveness of the soil to N-fertilization. However, the application of acid hydrolysis with hot HCl at different concentrations is one of the most widely used methods for the separation of the inactive fraction of SOM. All chemical methods have some disadvantages connected with the formation of "artifacts", and therefore another method is proposed (Bruun *et al.*, 2010).

In this study, faba bean (legume plant with N fixation capacities) and cereal (wheat) were used in crop rotation to test the effects of these crop on the nitrogen and carbon fractions in the soil during two subsequent crop cultivations. It was hypothesized that the use of different crop rotation regimes and crop residues of plants with various C/N ratios could cause changes in the N and C content in the organic fractions extracted from the soil and that faba bean is more beneficial in terms of soil N accumulation than wheat rotation. In order to determine the circulation of N which was introduced into the soil with the crop residues, an isotopic analysis of N ($\delta^{15}N$) was used, wherein a marker consisting of ^{15}N-labelled fertilizer was applied. For better characterization of the distribution of N in the mineral soil during its turnover upon the addition of N in the form of plant residue two acid hydrolysis methods were used. It is possible to achieve a better understanding of the cycling of N in the case of the 2-step method as opposed to the 1-step method.

MATERIALS AND METHODS

The field study was carried out in the Institute of Agrophysics in Lublin (Poland) (51º15'N, 22º35'E), on soil developed from loess (Haplic Luvisol according to World Reference Base, 2014). Annual precipitation is 552 mm on this site and the mean air temperature is 7.4°C. The granulometric composition of the soil was as follows: sand 640, silt 290 and clay 70 g kg^{-1}. The content of total carbon in the organic compounds and total nitrogen as determined by the 2400 Series II CHNS/O Elemental Analyser (PerkinElmer Inc., Norwalk, CT, USA) were 8.97 and 0.76 g kg^{-1} of soil, respectively. The content of the available forms of soil P, K and Mg were 142, 181 and 42 mg kg^{-1}, respectively. The available forms of P, K and Mg were extracted from the soil using the Mehlich procedure, and the amounts of the various nutrients were analysed using ICP (Inductively Coupled Plasma). The soil pH value (H_2O) was found to be 6.1. The soil did not contain calcium bicarbonate (test for the presence of $CaCO_3$ with diluted HCl). The soil parameters described above were evaluated for samples taken from the 0-20 cm layer. The experimental field was conventionally tilled for 38 years. Before the establishment of the present experiment, wheat (*Triticum aestivum* L.) (2016) or oats (*Avena sativa* L.) (2017) were cultivated.

In order to study the N and C organic fractions transformation in the soil under the influence of crop rotation, a 3-year cycle (2018-2020) of the field experiment was established. It included crops in rotation as follows: faba bean (*Vicia faba* L.) or wheat (*Triticum aestivum* L.) in the first year, spring wheat (*Triticum aestivum* L.) in the second year and winter triticale (*Triticosecale Wittm. ex A. Camus*) in the third year. Additionally, black fallow was tested as a reference soil (these plots were maintained without any plants during the whole experimental period). The plots (2 × 3 m) were arranged using a completely randomized block design (in three replicates). The sequence of crops grown in the experiment and the terms of soil sampling are provided in Table 1.

The crop residues (3-4 cm long sections) of the first crops (faba bean and wheat) were incorporated into the soil to a depth of approx. 20 cm. The cultivation of faba bean, wheat and triticale were conducted according to the conventional production system. Mineral fertilization (phosphorus, potassium and magnesium) was applied based on an analysis of the content of these nutrients in the soil. Nitrogen fertilization was carried out as follows: after the emergence of the first crops (faba bean and wheat) 30 kg ha^{-1} was applied on both crops and at the point of stem elongation 70 kg ha^{-1} was applied on the plots with wheat. For subsequent crops (wheat and triticale) the N fertilizers were applied at the tillering and stem elongation stages at a dose of 70 and 30 kg ha^{-1}, respectively.

In order to study the content of ^{15}N in the organic fraction, in the first year of the cycles, ammonium sulphate ($(NH_4)_2SO_4$) containing 98% ^{15}N was applied after forecrop emergence at an amount of 3 g m^{-2} to the faba bean, wheat and black fallow plots. This ^{15}N-labelled fertilizer was applied in solution, dissolved in water.

The rainfall and air temperatures during the experimental period were recorded hourly at the meteorological station of the Institute of Agrophysics, Polish Academy of Sciences in Lublin, located 150 m from the experimental site.

The plant residues of the forecrops (faba bean and wheat, cultivated in 2018) that were collected at the maturity stage were analysed for total N and C using a CHNS Elemental Analyser (PerkinElmer, Inc., Waltham, MA, USA). In order to determine the ^{15}N/^{14}N isotopic ratios in the plant residues a dynamic flash combustion method was applied. Analyses of the δ^{15}N content were performed in the Stable Isotope Laboratory, in the Institute of Geological Sciences Polish Academy of Sciences in Warsaw, Poland.

Table 1. Scheme of the field experiment in terms of soil sampling

Year	Study treatment and sampling term		
	Faba bean rotation (FR)	Wheat rotation (WR)	Black fallow (BF)
2018	Faba bean (*Vicia faba*)	Wheat (*Triticum aestivum* L.)	Black fallow
		Late autumn (November)	
2019	Spring wheat (*Triticum aestivum* L.)		Black fallow
		Tillering (May)	
		Stem elongation (June)	
		Senescence (July)	
	Winter triticale (*Triticosecale Wittm. ex A. Camus*)		Black fallow
		Tillering (November)	
2020	Winter triticale (*Triticosecale Wittm. ex A. Camus*)		Black fallow
		Stem elongation (May)	
		Senescence (July)	

The analyses were performed using a Flash 1112 HT elemental analyser coupled to a Delta V Advantage IRMS (both: Thermo Scientific) with a continuous flow of He. The samples, which were wrapped in tin capsules were combusted at 1 020°C in the presence of O_2. The obtained N_2, which was purified using a water trap and separated from the other gases on a GC column was subsequently transferred to an IRMS, where its isotopic composition was measured. The measurement results were calculated by using three international standards (USGS 40, USGS 41 and IAEA 600). Finally, the results were expressed in terms of atom % ^{15}N excess (at % ^{15}N exc.) relative to air N_2.

The soil samples were collected in the following conditions: late autumn (in November 2018), two months after residue incorporation, and at the three phenological growth stages of wheat and triticale: tillering (BBCH 29 (Biologische Bundesanstalt, Bundessortenamt and Chemical Industry)), stem elongation (BBCH 33), and senescence (BBCH 99). The developmental stages were determined according to the BBCH scale (Meier, 2001). Five soil cores from each plot were collected from a 0 to 20 cm depth (from the three plots for each treatment) and then the soil samples were sieved (2-mm) and air-dried before the analyses were performed to determine the total organic C, total N, and N and C in the fractions.

The amount of N and C were determined using the 2400 Series II CHNS/O Elemental Analyser (PerkinElmer Inc., Norwalk, CT, USA). Soil organic matter (SOM) was calculated from the formula: SOM = C total×1.724.

Acid hydrolysis of the soil organic nitrogen compounds was carried out using the Bremner method (Bremner, 1965; Stevenson, 1982). In brief, the total soil hydrolysable N was obtained by using 6 M HCl. Additionally, four hydrolysates of each soil were prepared and analysed: acid-hydrolysable amino acid-N by mixing the acid hydrolysis solution and 0.5 M NaOH, acid-hydrolysable ammonium-N by using MgO, acid-hydrolysable aminosugar-N with phosphate borate buffer (pH 11.2), and also threonine and serine-N obtained using HIO_4 and Devard's alloy. Acid-unhydrolysable N was calculated by subtracting the total soil hydrolysable N from the total N. Then, the nitrogen content in all of these hydrolysates was determined using the Kjeldahl method.

The nitrogen and carbon organic compounds were isolated from the soil material using a 2-step sequential extraction as described by Kalembasa (1995) and modified by Becher (2013), Becher *et al.* (2023) and Kalembasa and Kalembasa (2016). According to the procedure mentioned above, the following fractions were obtained: a fraction of the soluble mineral and organic compounds, a fraction of the easily hydrolysable, difficult-to-hydrolyse and non-hydrolysable compounds.

In all four of the separated fractions, the content of ^{15}N was determined using a Flash 1112 HT elemental analyser coupled to a Delta V Advantage IRMS (Thermo Fisher Scientific, Waltham, MA USA) in a continuous flow mode and expressed in terms of atom % ^{15}N excess (at % ^{15}N exc.).

The chemical fractionation of the soil organic carbon was carried out following the method proposed by the International Humic Substances Society (IHSS) (Hayes *et al.*, 1989). The nitrogen and carbon compounds in the alkaline samples were obtained by soil extraction with 0.1 M NaOH with the following modifications:

1) in the first step, H_2SO_4 was used instead of HCl;

2) for the precipitation of humic acids, H_2SO_4 was used instead of HCl.

These modifications allowed for the determination of C in the extracted fraction and solution after the precipitation of humic acids using the oxidation method with the application of potassium dichromate (Kalembasa, 1991). This allowed for the following fractions to be obtained:

1) extracts of the easily soluble fraction of C and N extracted from the soil with mineral acid (0.05 M H_2SO_4) to remove the calcium and magnesium from the humic acids;

2) humic compounds including humic (HA) and fulvic acids (FA) (extracted with 0.1 M NaOH). Both acid groups contain many chemical compounds. The compounds in the humic acid group are more complicated in certain aspects of their chemistry than the fulvic acids which are very simple and are the first products of the mineralization of plant biomass. From these acids, humic acids are synthesized using a polymerization process;

3) humin (the insoluble fraction of humic compounds). They are compounds of C and N closely connected with the mineral portions of the soil. They are very stable in the soil and the synthesis of these fractions takes a very long time.

The N contents in all of the obtained fractions were determined using the Kjeldahl method and the C content through the oxidation-titrimetric method (Kalembasa, 1991).

All of the C parameters were calculated on the basis of the C extracted and separated from the soil in the fulvic acids fraction rather than determining the C content using 333 mM $KMnO_4$ (Blair *et al.*, 1995). The reason for this, according to our unpublished results, is that the correlation coefficient between the amount of C in the fulvic acids and the C determined using 333 mM $KMnO_4$ was R = 0.96 (n = 60).

The C management index (CMI) was calculated in order to obtain the dynamics of the system and provide an integrated indication of the quantity and quality of the soil organic carbon (Blair *et al.,* 1995). Soils with higher values of CMI are considered to be better managed.

Based on the analysis of the results obtained by applying C extraction to the soil, the following parameters were calculated (Blair *et al.*, 1995):

$$\mathrm{HI} = ((\mathrm{CHA}+\mathrm{CFA})/C_{\mathrm{total}})\ 100, \tag{1}$$

$$\text{CPI} = \text{total C (mg g}^{-1}\text{) in the treatment soil/ total C (mg g}^{-1}\text{) in the reference soil,} \quad (2)$$

$$\text{L} = \text{content of C in the labile fraction/ content of C in the non-labile fraction,} \quad (3)$$

$$\text{LI} = \text{Lability of C in the treatment/ Lability of C in the reference soil,} \quad (4)$$

$$\text{CMI} = \text{CPI} \times \text{LI} \times 100, \quad (5)$$

where: HI – humification index, CPI – C pool index, L – lability of C, LI – lability index, CMI – carbon management index.

Statistica (version 13. software.dell.com) software (Dell Inc. Tulsa, OK, USA, 2016) was used to perform statistical analyses. Data normality and the homogeneity of variance were verified using the Shapiro-Wilk and Levene tests, respectively. In the case of both assumptions being met, a comparison of means was performed based on analysis of variance (ANOVA) and the calculation of significant differences was performed using a post-hoc Tukey's HSD test at a significant level of $p<0.05$. When the assumptions of a normal distribution were met, but the homogeneity of variance was violated, an F-Welch test was used. In the case of both assumptions for an ANOVA not being met, then the Kruskal-Wallis test was used instead.

The soil chemical profile (including soil properties determined on the basis of 7 discrete sampling occasions, *e.g.* from Nov 2018 to Jul 2020) was analysed through the application of multivariate methods using PRIMER 7 software (Plymounth, UK). The transformed data ($\log(X+1)$) were normalized and an analysis of similarities (ANOSIM test) using Euclidean distance was performed. Two-way crossed ANOSIM (including crop rotation and term effects) with replicates were performed with 9999 permutations to assess whether the chemical profile differed between both the crop rotations (BF, FR, WR) and term (7 sampling terms) factors. The *R* statistic offers a very useful comparative measure of the degree of separation of the samples, therefore it was used. The value of R usually ranges between 0 (which indicates completely random separation of the groups being compared) and 1 (for complete separation). A similarity percentage (SIMPER) analysis was used to identify the contribution (%) of each chemical variable to that difference between the pairs of crop rotation and the term treatments. A principal component analysis (PCA) was also performed using PRIMER 7 software.

RESULTS AND DISCUSSION

The annual precipitation in the consecutive years of 2018-2020 amounted to 388, 509 and 702 mm, respectively, while the long-term average was found to be 552 mm (Fig. 1). The mean annual air temperatures in 2018-2020, were higher than the long-term values (7.4°C) and were 9.7, 10.3 and 10.0°C, respectively. With regard to the amount of rainfall occurring during the faba bean and wheat growing season (April-July) it was 228 mm in 2018 while the long-term average amounted to 247 mm.

The yields of the plant residues and C (g m^{-2}) were significantly higher for wheat as compared to faba bean (563 and 232 g m^{-2} for wheat and 390 and 160 g m^{-2} for faba bean, respectively). Although the N yield was higher for faba bean, it did not differ to a statistically significant extent between the crops (3.48 for faba bean and 3.05 g m^{-2} for

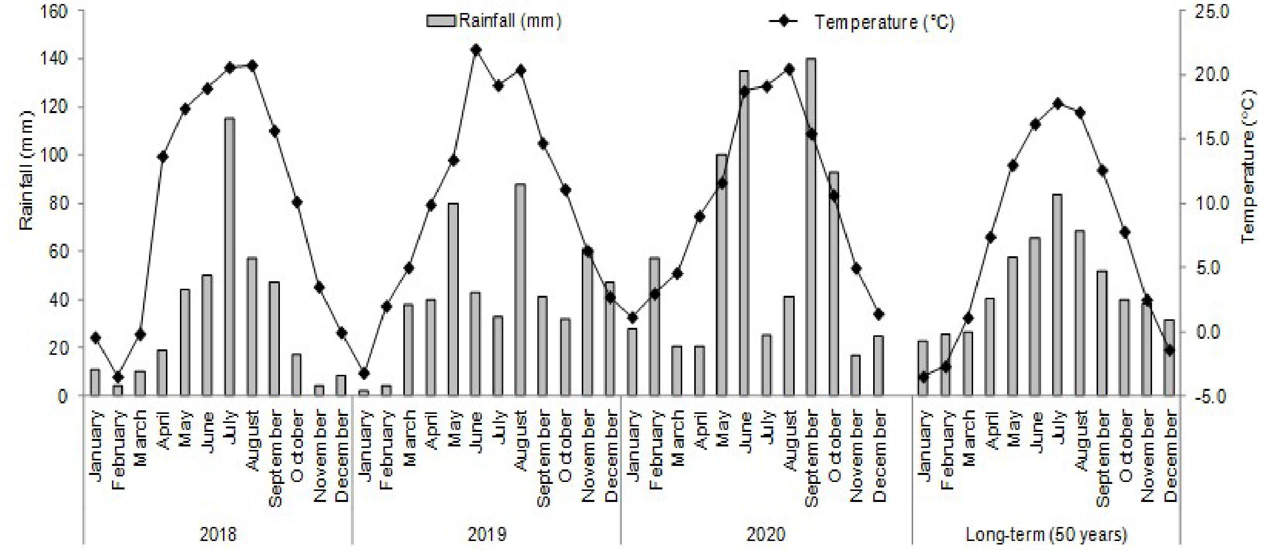

Fig. 1. Weather conditions during the experimental period (2018-2020) and the long term (50 year) average for the study site. Data were recorded hourly by the meteorological station of the Institute of Agrophysics PAS, located near the experimental field.

Table 2. Average content of total N, C and organic matter (g kg^{-1}) in the soil

Treatment	Total N (TN)	Total C (TC)	Soil organic matter (SOM)
		(g kg^{-1})	
Black fallow (BF)	0.697 ± 0.086 a	7.12 ± 0.29 a	12.8 ± 0.89 a
Faba bean rotation (FR)	0.814 ± 0.085 c	8.33 ± 0.23 b	14.0 ± 1.04 b
Wheat rotation (WR)	0.780 ± 0.098 b	8.21 ± 0.32 b	14.0 ± 0.72 b

± Standard deviation. Values followed by the same letter for a given variable are not significantly different ($p<0.05$) as calculated by F-Welch test.

wheat). The C/N value of the plant residues was greater ($p<0.05$) in the case of wheat (76.4) as compared to faba bean (46.3).

The amount of total N (TN) in the soil (as a mean from the whole period of the experiment) increased significantly from black fallow (BF) to faba bean rotation (FR) (Table 2).

The 1-step acid hydrolysis used for the separation of the N fractions has shown that the amount of N in the total hydrolysable fraction in the soil increased significantly as follows: BF, WR, FR (Table 3). Moreover, the N content in the separated fractions differed significantly between the different treatments. The largest amounts of N compounds were in the form of amino acids and their content was found to be greater ($p<0.05$) in FR and WR than in BF. Ammonium-N and aminosugar-N were both found to be lower in soil without plants than in cropped soils. The amino acids serine and threonine, which were determined separately, also differed significantly between all of the soils examined.

The contents of N in the fractions separated by sequential extraction were differentiated between the treatments, moreover, a significant difference between the fractions was also observed (Table 4). The lowest amount of N (3.0-3.8%, depending on the treatment) was detected in the fraction of soluble mineral and organic N compounds, about 10 times more in the fraction of easily hydrolysable compounds and the highest amount in the fraction which was difficult to hydrolyse. This means that the sum of the three fractions of total N were in a hydrolysable form (0.58, 0.67 and 0.67 g kg^{-1} in the fallow, faba bean and wheat treatments, respectively). The sum of these three fractions may be expressed as the active part of the soil which undergoes the mineralization process to various degrees. In our study, it was observed that the median value of the soluble mineral and organic N compounds fraction was significantly greater for FR and WR than for BF.

In general terms, the amount of nitrogen compounds in this fraction (mineral forms, amino acids, aminosugars, amides) is not greater than 5% of the total N (Kalembasa and Kalembasa, 2016). The fraction of SOM which are known as the low molecular weight (LMW) compounds have been gaining attention due to their potentially important contribution to soil respiration (Boddy *et al.*, 2007) and the assumed role of organic N compounds like amino acids as an N resource for plant growth (Owen and Jones, 2001).

The fraction of easily hydrolysable compounds of N and C contained compounds which were mineralized by enzymes released by microbes. As a mean from the date of sampling in all treatments, the amount was very similar and equal on average to 35% of TN. The main compounds of this fraction are simple proteins, some partially decomposed plant parts, microorganisms, complexes of amino acids and aminosugars (Kalembasa, 1995). Becher and Kalembasa (2011) determined a value of 28% nitrogen in the easily hydrolysable fraction in mineral soil. The results of this experiment revealed a lower amount of easily hydrolysable compounds in BF than in both crop rotations.

The difficult-to-hydrolyse N compounds accounted for 45% of TN on average. The concentration of protons H^+ in the acid used (H_2SO_4 (VI)) for extraction had a value of 6 M HCl, this is commonly used for the chemical hydrolysis of protein (Bremner, 1965; Stevenson, 1982). In our experiment the amount of this fraction differed between treatments and was lower in BF than in the cropped soils.

The fraction which was not hydrolysable contained values ranging from 14.7% in WR to 17.2% in FR of the total N in the investigated soils and no statistically significant differences were noted. The type and quantity of complex nitrogen in this fraction is not fully recognized.

Hydrolysable and non-hydrolysable compounds obtained through a 1-step acid hydrolysis with 6 M HCl and a 2-step sequential fractionation with 3 M H_2SO_4 showed a close relationship (Fig. 2). The correlation coefficient between these two methods used for the separation of hydrolysable soil N compounds amounted to 0.633 and for the non-hydrolysable compounds it amounted 0.870, $p<0.05$ (coefficients of determination: 0.400 and 0.757, respectively).

A similar accumulation of ^{15}N (as % ^{15}N exc.) was found in the most labile fraction, in the fraction extracted from the soil using 0.5 M K_2SO_4 (28-34% of the total ^{15}N, depending on the treatment) and in the easily hydrolysable samples (30-32%, depending on the treatment), next, in terms of difficulty, came the difficult-to-hydrolyse samples (21-24%) and the lowest value was attained by the non-hydrolysable fraction (13-16%) (Table 5). These results show that there

Table 3. Average or median values of N content (g kg^{-1}) in the soil fractions separated by 1-step acid hydrolysis

Treatment	Amino acid N	Serine+threonine N	Ammonium N	Amino sugar N	Hydrolysable unidentified N*	Total hydrolysable N	Acid insoluble N
					(g kg^{-1})		
Black fallow (BF)	0.198 ± 0.028 a	0.087 ± 0.009 a	0.146 ± 0.016 a	0.050 ± 0.010 a	0.108; min 0.046 max 0.142 a	0.581 ± 0.026 a	0.116 ± 0.095 a
Faba bean rotation (FR)	0.231 ± 0.018 b	0.096 ± 0.007 c	0.164 ± 0.013 b	0.058 ± 0.003 b	0.131; min 0.097 max 0.176 b	0.682 ± 0.027 c	0.133 ± 0.097 a
Wheat rotation (WR)	0.244 ± 0.020 b	0.091 ± 0.010 b	0.163 ± 0.011 b	0.058 ± 0.007 b	0.100; min 0.062 max 0.151 a	0.656 ± 0.023 b	0.124 ± 0.098 a

± standard deviation, * – median value, min – minimal value, max – maximal value. Values followed by the same letter for a given variable are not significantly different ($p<0.05$) as calculated by F-Welch test (amino acid N, total hydrolysable N, acid insoluble N), ANOVA test (ammonium N, amino sugar N, serine+threonine N) or Kruskal-Wallis test (hydrolysable unidentified N).

Table 4. Average or median values of N content (g kg^{-1}) in soil fractions separated by 2-step sequential fractionation

Treatment	Soluble mineral and organic N*	Easily hydrolysable N	Difficult hydrolysable N*	No hydrolysable N
		(g kg^{-1})		
Black fallow (BF)	0.020; min 0.018 max 0.025 a	0.249 ± 0.027 a	0.319; min 0.220 max 0.372 a	0.113 ± 0.064 a
Faba bean rotation (FR)	0.026; min 0.021 max 0.030 b	0.285 ± 0.030 b	0.372; min 0.254 max 0.431 b	0.140 ± 0.069 a
Wheat rotation (WR)	0.026; min 0.021 max 0.090 b	0.278 ± 0.035 b	0.358; min 0.299 max 0.403 b	0.115 ± 0.068 a

Values followed by the same letter for a given variable are not significantly different ($p<0.05$) as calculated by F-Welch test (Easily hydrolysable N, No hydrolysable N), or Kruskal-Wallis test (Soluble mineral and organic N, Difficult hydrolysable N). Explanations as in Table 3.

Table 5. Median values of the content of ^{15}N (atom % ^{15}N excess) in fractions separated by 2-step sequential fractionation

Treatment	Soluble mineral and organic N	Easily hydrolysable N	Difficult hydrolysable N	No hydrolysable N	All hydrolysable N	Mean weighted for all fractions
Black fallow (BF)	0.280; min 0.155 max 0.533 a	0.255; min 0.213 max 0.453 a	0.196; min 0.126 max 0.279 a	0.119; min 0.060 max 0.184 a	0.692; min 0.510 max 1.147 a	0.215; min 0.162 max 0.314 a
Faba bean rotation (FR)	0.537; min 0.399 max 0.663 b	0.601; min 0.433 max 0.631 b	0.432; min 0.384 max 0.542 b	0.307; min 0.237 max 0.400 b	1.555; min 1.308 max 1.753 b	0.472; min 0.380 max 0.563 b
Wheat rotation (WR)	0.716; min 0.425 max 0.819 b	0.614; min 0.446 max 0.725 b	0.426; min 0.273 max 0.595 b	0.294; min 0.230 max 0.377 b	1.601; min 1.385 max 2.034 b	0.465; min 0.343 max 0.636 b

Min – minimal value, max – maximal value. Values followed by the same letter for a given variable are not significantly different ($p<0.05$) as calculated by Kruskal-Wallis test.

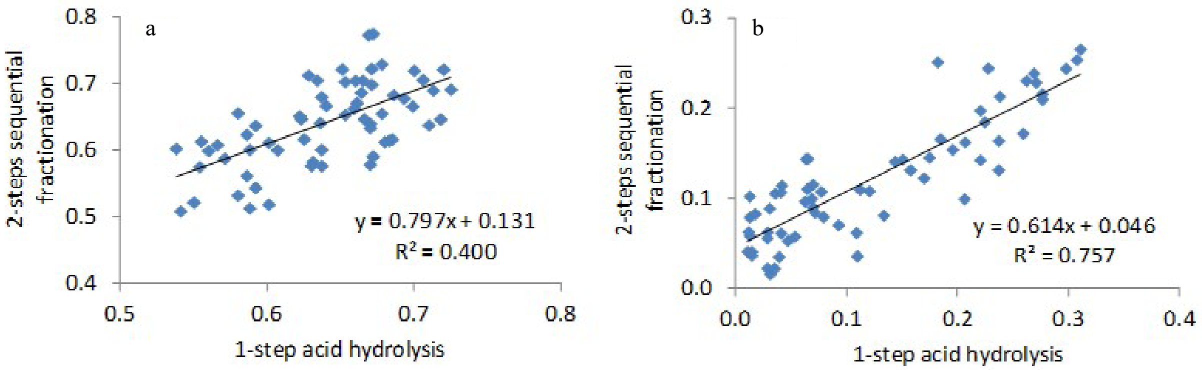

Fig. 2. Relationship between the amount of hydrolysable N (sum of the soluble mineral and organic N compounds, the easily hydrolysable and the difficult to hydrolyse) (a), the non-hydrolysable (b) the soil N compounds, were determined using a 2-step sequential fractionation with 3 M H_2SO_4 and also using a 1-step acid hydrolysis with 6 M HCl (n=63).

was not enough time to form stable compounds of N with carbon and minerals of soil. The median value of the content of ^{15}N in all of the hydrolysable and non-hydrolysable fractions was lower ($p<0.05$) in the reference soil as compared with both of the cropped soils. The accumulation of ^{15}N in soil expressed as a mean weight value was higher for the faba bean and wheat rotations than for the black fallow. Therefore, the most noteworthy difference is probably caused by the leaching of ^{15}N-ions from the top layers into the deeper layers of the profile, and the accumulation in the top layer of ^{15}N in crop residues.

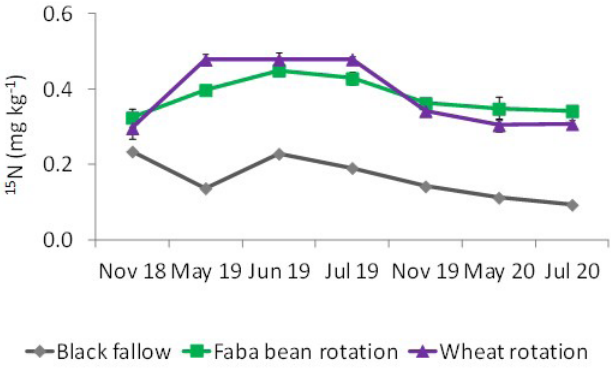

Fig. 3. Changes in the amount of ^{15}N (g kg^{-1}) in the soil during the experimental period with a standard deviation (n=3).

The amount of ^{15}N accumulated in the top layer of soil differed greatly between treatments (Fig. 3). The lowest value was determined for the black fallow sample during the whole analyses period. The differences between the forecrop (faba bean and wheat) varied in the period analysed, during the subsequent cultivation of wheat they were greater in wheat than in faba bean and later, during the growth of the second crop, the opposite result was noted. The mean content of ^{15}N in the soil was the same in cropped soils and lower in BF (Table 6). However, the percentage of ^{15}N introduced with nitrogen fertilizer decreased as follows: FR, WR, BF.

The stabilization of ^{15}N in organic carbon compounds depends upon the C3/C4 of the plants (Ellerbrock and Kaiser, 2005), changes in vegetation (Solomon *et al.*, 2002), the soil tillage system and minimizing disturbance (Wick *et al.*, 2009). Huygens *et al.* (2008), and Sollins *et al.* (2009) reported that beyond the C3/C4 pools, this phenomenon is also caused by isotope discrimination during microbial processing in which microbes consume the most labile fraction of organic matter (for respiration, the light C and N isotopes are preferentially used) and also the heavy isotopes of C and N are incorporated into their biomass, which are subsequently accumulated in soil organic matter complexes.

Table 6. Parameters calculated for ^{15}N content in soil in the study treatments. Means followed by the same letter for a given variable are not significantly different ($p<0.05$) as calculated by ANOVA test

Treatment	^{15}N (mg kg^{-1})	^{15}N (g m^{-2})	^{15}N left in soil (%)
Black fallow (BF)	0.16 ± 0.001 a	0.048 ± 0.0003 a	0.95 ± 0.05 a
Faba bean rotation (FR)	0.38 ± 0.003 b	0.114 ± 0.0008 b	3.49 ± 0.11 c
Wheat rotation (WR)	0.38 ± 0.004 b	0.115 ± 0.0013 b	3.14 ± 0.09 b

± standard deviation.

The mean values of total C (TC) and soil organic matter (SOM) for the whole experiment were lower ($p<0.05$) in BF than in both cropped soils as a result of the plant residues and plant roots in the soil (Table 2).

The amount of organic C compounds in the fraction separated using the sequential method was characterized by a wide range of variability (Table 7). The lowest content of C was determined in the fraction of soluble organic compounds (on average 1.3%) and the highest in the fraction of non-hydrolysable compounds obtained through strong acid hydrolysis (53% in each treatment). For the easily hydrolysable fraction, the amount of C was higher than in the difficult-to-hydrolyse one. This means that some of the organic C compounds are not very closely bound with the mineral portions of the soil. The sum of C extracted from the soil by both weak and strong sulfuric acid (as a mean from the whole period) was significantly differentiated between each treatment and amounted to 3.32 g kg^{-1} in BF and was higher in both cropped soils (3.90 g kg^{-1}).

The ratio of C/N was very narrow for the hydrolysable fraction and it was very similar in all treatments (mean 4.0 for soluble organic C, 7.1 for the easily hydrolysable fraction and 4.9 for the difficult-to-hydrolyse fraction), which means that chemical compounds of the soil organic matter which were calculated on the basis of the results given in Tables 4 and 7 are easily mineralized and deliver N into the soil solution. The most stable soil constituents are the organic compounds in the non-hydrolysable fraction, in which the C/N values were above 30: 33.5, 31.7 and 37.6 for the black fallow, faba bean and wheat rotations, respectively.

Among the few reagents applied, the most often used for the extraction of N and C organic compounds are NaOH and $Na_4P_2O_7$ (Kayler *et al.*, 2011; Kumar and Sharma, 2014). NaOH solution was also used as it is recommended by the International Humic Substances Society. The amount of mineral and dissolvable compounds of N in the extracts of 0.5 M K_2SO_4 was low, not more than 3.8% of TN in the investigated treatments (Table 4). However, the amount of N in the extractable part of SOM in humic compounds (fulvic and humic acids) was far more differentiated (Table 8). The largest amount was determined in soil sampled from FR and it was significantly lower in WR and BF. This means that the N in soil organic matter compounds in a stable (not extractable) form is accumulated in compounds which are synthesized in the soil and can remain in forms that are not easily mineralized. It is good practice in agriculture to maintain a high level of fertility in the soil. It is worth noting that in the extractable fraction of soil organic matter, more was accumulated in humic than in fulvic acids. From the point of view of their chemistry, fulvic acids are simple in structure and not stable in soils. They are easily decomposable by microorganisms. They are formed in the soil during the breakdown of plants and animals (Alvarez-Puebla *et al.*, 2006). Fulvic acids are

Table 7. Average or median values of the C content (g kg^{-1}) in soil in fractions separated by sequential analysis under the study treatments

Treatment	Soluble organic C	Easily hydrolysable C	Difficult hydrolysable C*	No hydrolysable C*
	(g kg^{-1})			
Black fallow (BF)	0.088 ± 0.018 a	1.744 ± 0.134 a	1.196; min 0.953 max 2.464 a	3.929; min 2.737 max 4.428 a
Faba bean rotation (FR)	0.109 ± 0.021 b	1.996 ± 0.060 b	1.442; min 1.144 max 3.651 a	4.712; min 3.061 max 5.130 b
Wheat rotation (WR)	0.109 ± 0.017 b	2.006 ± 0.134 b	1.451; min 1.144 max 3.316 a	4.780; min 2.279 max 5.410 b

Values followed by the same letter for a given variable are not significantly different ($p<0.05$) as calculated by F-Welch test (Soluble organic C), ANOVA test (Easily hydrolysable C) or Kruskal-Wallis test (Difficult hydrolysable C, No hydrolysable C). Explanations as in Table 3.

Table 8. Average or median values of the N and C contents (g kg^{-1}) in soil organic matter fractions

Treatment	Easily soluble fraction	Humic compounds	Humic acids*	Fulvic acids**	Humin**
			N content (g kg^{-1})		
Black fallow (BF)	0.030 (0.007) a	0.481 (0.071) a	0.237; min 0.168 max 0.404 a	0.225; min 0.140 max 0.362 a	0.211; min 0.025 max 0.318 a
Faba bean rotation (FR)	0.032 (0.008) ab	0.548 (0.085) c	0.294; min 0.192 max 0.360 b	0.243; min 0.169 max 0.431 a	0.249; min 0.023 max 0.409 a
Wheat rotation (WR)	0.033 (0.007) b	0.520 (0.081) b	0.282; min 0.145 max 0.329 a	0.253; min 0.159 max 0.408 a	0.206; min 0.067 max 0.391 a
			C content (g kg^{-1})		
Black fallow (BF)	0.124 (0.013) a	3.149 (0.423) a	1.740; min 1.622 max 2.718 a	1.318 (0.248) a	3.843 (0.367) a
Faba bean rotation (FR)	0.146 (0.015) b	3.524 (0.265) b	2.090; min 1.871 max 2.310 b	1.466 (0.207) a	4.663 (0.364) b
Wheat rotation (WR)	0.151 (0.019) b	3.548 (0.248) b	2.092; min 1.760 max 2.392 b	1.464 (0.190) a	4.511 (0.388) b

Values followed by the same letter for a given variable are not significantly different ($p<0.05$) as calculated by F-Welch test (C in Humic compounds, Fulvic acids and humin), ANOVA test (Easily soluble fraction of N and C, N in Humic compounds) or Kruskal-Wallis test (N in Humic acids, Fulvic acids and Humin, C in Humic acids). **Median value for N content and average value for C content. Other explanations as in Table 3.

used for the synthesis of humic acids which have a far more complicated chemical structure, and are much more stable than fulvic acids (Schulten and Schnitzer, 1992; Stevenson, 1994). These compounds may be isolated and characterized using many methods (Hayes and Swift, 2020).

The amount of C in the fraction extracted from SOM was very highly differentiated (Table 8). The lowest amount of C was determined in the easily soluble fraction, in the range 1.74-1.84% of TC. The extractable fraction of C was differentiated according to the crop rotation used. A larger amount of C in the humic compounds was extracted from soil with plants rather than from black fallow. In these conditions, organic compounds in the soil are mineralized with a lack of synthesis (no crop residue). For both of the crop rotations analysed, the median value of the C in the humic acids was higher than that in the fulvic acids. This was also the case concerning the amount of nitrogen in the soil samples.

The amount of C in the form of compounds which are not extractable from soil (humin) was higher ($p<0.05$) in soils sampled from crop rotations as opposed to fallow soil. It amounted to 56% of TC in soil sampled from the crop rotation with faba bean, the cereal rotation produced a slightly lower value and the lowest value came from the black fallow sample (54%). This fraction is typical of the best cultivated soils, indicating a close connection between the organic and mineral elements of the soil and it has positive effects on the physical and chemical properties of cultivated soils. In our study, the amount of organic C which was not an extractable fraction was over 50%. This probably means that this fraction should be classified as non-humified organic carbon compounds rather than humin. It should be emphasized that this fraction contains residue crop material which is decomposed with some degree of difficulty by soil microorganisms, for the most part it consists of lignin, cellulose and hemicellulose, and in each year of the experiment it was introduced into the soil.

The study of the turnover of N and C in soil mixed with plant residues is very important as a substantial proportion of these elements are not contained within an extractable fraction. This fraction is very closely bound with minerals in the soil (Stevenson, 1994). It should be pointed out that the synthesis of this soil organic C fraction takes a long time (Kalembasa and Kalembasa, 2016) and in our case, when crop residues are ploughed into the mineral soil, a year is a relatively short time for the complex development of the humin fraction.

The values of the C/N ratio in the separated SOM compounds depended upon the fraction being analysed. The lowest values were obtained in the easily soluble fraction and ranged from 4.2 (black fallow) to 4.7 (crops rotations). This means that the C and N compounds extracted from these fractions are very simple in chemical and structural terms and are very easily decomposed in the soil (Haynes, 2005; Kalembasa and Kalembasa, 2016). The values of

C/N for humic acids and fulvic acids were similar in all treatments and amounted to 7.8 and 6.0, respectively. The non extracted residues of the soil had C/N values of 29.2, 25.3 and 24.3 for BF, FR and WR, respectively. These values are similar to those in developed and agricultural soils. This means that they are stable and difficult to mineralize but given this situation we should be awere that there are also residues of plant crops in this fraction. Also, the C/N ratios are higher in humic than in fulvic acids, showing that humic acids are more stable than fulvic ones. The values of C/N have an influence over the mineralization process of organic compounds in the soil. High values above 10 indicate that compounds are stable, but below this value the compounds are easily decomposable. From the agrochemical point of view, more stable compounds are better, because they determine the structure and the chemical properties of the soil (Hayes and Swift, 2020).

In some cases, it is worth while obtaining the values of the carbon ratio in humic and fulvic acids which are very similar in our study in all treatments and amounted to 1.4. The values of this ratio below one unit indicate that the organic materials ploughed into the soil are at the initial stage of humification whereas the values above one mean that this fraction is rather stable with the humic acids prevailing.

Soil organic matter content, which is known as the pool size of carbon is a result of the balance between the addition and decomposition rates which are also referred to as the turnover rates. In agricultural practices, both of these processes can have a serious influence over the content of SOM which also has an influence over the content of available plant nutrients. The maintenance of the content of SOM at a high level is the key to the sustained productivity of the soil agricultural system. Turnover rates depend to a high degree upon the activity of the soil microbial biomass which is mainly driven by mobilization/immobilization/decomposition processes (Mwafulirwa *et al.*, 2017; Tate, 2017).

The parameters describing the fate of carbon in the study soils are not differentiated to a substantial extent between the various crop rotations (Table 9). The fallow soil showed a higher amount of labile carbon than the cropped soils. The carbon pool, humification, liability and carbon management indexes were not significantly differentiated between the treatments. It should be pointed out that small changes in the carbon content of the fraction are difficult to determine because cultivated soil contains high background levels of carbon and the natural content of this element shows a high degree of soil variability. These are the reasons why many attempts have been made to use some modification and subpools of carbon as more sensitive indicators of changes to the carbon pool size (Coleman and Jenkinson, 1996).

A Principal Component Analysis (PCA) was used to group the samples based on their entire chemical profile. The PCA of the environmental variables clustered the samples according to the sampling terms and crop rotation. The first and second principal component respectively explained 37.8 and 20% of the total variance (Fig. 4). All of the parameters were positively correlated with PC1, and the parameters connected with the ^{15}N measurements, C content, total hydrolysable N, and easily hydrolysable C contribute most to PC1. The acid insoluble N, difficult-to-hydrolyse C, non-hydrolysable N, N content, humin N

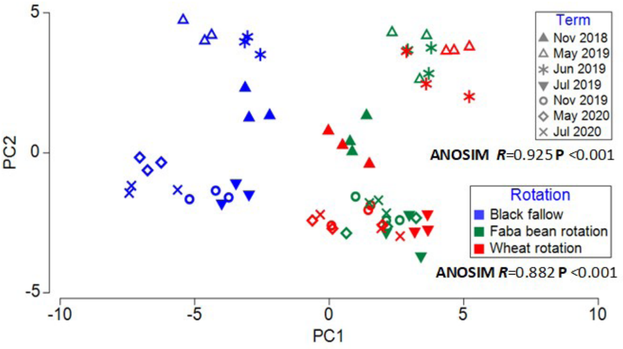

Fig. 4. Principal component analysis (PCA) grouping the samples based on the soil variables (triplicates shown for each rotation and sampling time). The first principal component explained 37.8% and the second 20% of the total variance. The results of the ANOSIM test are provided for the sampling time and rotation treatments. Different shapes of marks indicate the terms of soil sampling, while the marker colours indicate the crop rotation treatments.

Table 9. Parameters calculated for C in the soil organic matter

Parameter	Black fallow (BF)	Faba bean rotation (FR)	Wheat rotation (WR)
Carbon pool index		1.171 ± 0.007 a	1.154 ± 0.014 a
Humification index (%)	44.3 ± 0.8 a	42.3 ± 0.5 a	43.2 ± 1.0 a
Labile carbon	0.227 ± 0.004 b	0.213 ± 0.002 a	0.217 ± 0.001 a
Lability index		0.936 ± 0.019 a	0.955 ± 0.019 a
Carbon management index		110.0 ± 2.7 a	110.2 ± 3.4 a

± Standard deviation. Means followed by the same letter for a given variable are not significantly different at $p<0.05$ as calculated by ANOVA test.

(positive correlation) and non-hydrolysable C, ammonium N and fulvic acids C (negative correlation) make the largest contribution to PC2.

Both crop rotation and the timing of the soil sampling had a substantial influence over cluster formation. Based on the soil parameters, a clear separation of two groups on the biplot is visible: one group of samples from the black fallow and in the second group samples from faba bean and wheat rotations are clustered (Fig. 4). Samples from black fallow are generally grouped separately according to their sampling time, while the samples from plots with crops create three separate groups for: Nov 2018, May 2019 – June 2019, and Jul 2019 – Jul 2020. In general terms, a relatively large distance is apparent between the samples taken in May 2019 – Jun 2019 and Jul 2019 – Jul 2020 under all of the rotation treatments. Big dissimilarity in the chemical profile between Nov 2018 and May 2019 – Jun 2019 in both cropped soils were also noted.

The ANOSIM test proved that the entire soil chemical profile was significantly different between the crop rotation treatments ($p<0.001$, $R = 0.882$), but the size of the effect was slightly lower than that of the sample time ($p<0.001$, $R = 0.925$) (Fig. 4). This indicates that the biochemical transformation of the N and C compounds in the soil was more substantially affected by the sampling time than by crop rotation. The ANOSIM pairwise tests revealed a complete separation between BF and FR ($R = 1$, $p<0.001$) and a significant separation of FR and WR ($R = 0.661$, $p<0.001$), and also BF and WR ($R = 0.974$, $p<0.001$). A pairwise test for the sampling time (7 sampling terms) for most cases indicated a complete separation ($R = 1$), and for all comparisons, the test was significant ($p<0.05$).

SIMPER analyses showed that the soluble mineral and organic N and hydrolysable unidentified N explained the largest proportion of the differences between the two crop rotations (faba bean and wheat), this was followed by the SOM content and serine + threonine N (a contribution of 11.8, 11.3, 6.5 and 5.5%, respectively) (Table 1S). Some of these parameters were significantly greater in FR than in WR (hydrolysable unidentified N, serine + threonine N). The differences between WR and BF were mainly caused by the parameters connected with the ^{15}N isotope variables (33.8% in total) and the C content (5.2%), while the differences between FR and BF were mainly determined by total hydrolysable N, SOM, C contents, and ^{15}N isotope variables (6.3, 6.2, 6.2 and totally 26%, respectively).

The PCA biplot showed a large distance between the samples taken in May 2019 – Jun 2019 and Jul 2019 – Jul 2020, and a dissimilarity in the soil chemical profile between Nov 2018 and May 2019 – Jun 2019, as well. The differences between Nov 2018 and May 2019 – Jun 2019 may be explained for the most part by changes in the soil organic matter fractions, especially the decrease in C in fulvic acids and in C in the humic compounds, and also the increase in N in the humic acids during this period (Table 2S). In general terms, acid insoluble N, soluble mineral and organic N, non-hydrolysable N, difficult-to-hydrolyse C and non-hydrolysable C contributed the most to the differences between May 2019 – Jun 2019 and Jul 2019 – Jul 2020, and non-hydrolysable C increased from May 2019 – Jun 2019 to Jul 2019 – Jul 2020, while other variables increased during this period.

CONCLUSIONS

1. In this study we assessed the differences between the N and C soil fractions obtained using different methods of extraction under the influence of crop rotation and the significant differences between treatments were also noted. Over a 3-year period it was observed that there was a greater total of N content in legume rotation than that produced by cereals, however, the total C did not differ between cropped soils and it was lower in the reference soil (black fallow).

2. In the hydrolysate obtained using HCl, the amount of N in the whole hydrolysable fraction amounted to 83.3-84.1% of total nitrogen, depending on the treatments used, and this value increased significantly from the reference soil to the faba bean rotation. Among the hydrolysable compounds, over 49-52% of the N originated from amino acids (probably from proteins) and NH_4^+ which probably existed in the form of free, exchangeable, amide cations.

3. In the hydrolysate obtained using a 2-step hydrolysis (with H_2SO_4), the amounts of hydrolysable N compounds were generally lower in the black fallow than in the cropped soils. It would seem that the amount of N hydrolysable compounds determined by the two methods (with HCl and H_2SO_4) used in this experiment were very similar.

4. For the nitrogen fractions separated by a 2-step sequential method, ^{15}N (at % ^{15}N exc.) was found to be highest in the most labile fractions and in the easily hydrolysable fractions. All fractions showed a higher level in the cropped soils than in the reference soil. These results show that there was not enough time to form stable compounds of N with the carbon and minerals of the soil. Crop rotation differed significantly with the accumulation of ^{15}N in the soil.

5. On the basis of the results obtained in our experiment it may be stated that soil with faba bean rotation is richer in N compounds than soil with wheat rotation and this may result in a reduction in N fertilizer application during the subsequent crop cultivation in the case of the former. The presented results are very useful in terms of fertilization husbandry in plant production.

Conflict of interest: The authors declare no conflict of interest.

REFERENCES

Alvarez-Puebla R.A., Valenzuela-Calahorro C., and Garrido J.J., 2006. Theoretical study on fulvic acid structure, conformation and aggregation. A molecular modelling approach. Sci. Total Environ., 358(1-3), 243-254. https://doi.org/10.1016/j.scitotenv.2004.11.026.

Becher M., 2013. Organic matter transformation degree in the soils of the upper Liwiec River (in Polish). Dissertation, Siedlce University of Natural Sciences and Humanities, Poland.

Becher M. and Kalembasa D., 2011. Fraction of nitrogen and carbon in humus horizons of arable Luvisols and Cambisols located on Siedlce upland (in Polish). Acta Agrophysica, 18(1), 7-16.

Becher M., Kalembasa D., Kalembasa S., Symanowicz B., Jaremko D., and Matyszczak A., 2023. A new method for sequential fractionation of nitrogen in drained organic (peat) Soils. Int. J. Environ. Res. Public Health, 20, 2367. https://doi.org/10.3390/ijerph20032367

Bird J.A., Herman D.J., and Firestone M.K., 2011. Rhizosphere priming of soil organic matter by bacterial groups in a grassland soil. Soil Biol. Biochem., 43(4), 718-725. https://doi.org/10.1016/j.soilbio.2010.08.010.

Blair G.J., Lefroy R.D.B., and Lisle L., 1995. Soil carbon fractions based on their degree of oxidation, and the development of a carbon management index for agricultural systems. Aust. J. Agr. Res., 46(7), 1459-1466.

Boddy E., Hill P.W., Farrar J., and Jones D.L., 2007. Fast turnover of low molecular weight components of the dissolved organic carbon pool of temperate grassland field soils. Soil Biol. Biochem., 39, 827-835.

Bremner J.M., 1965. Organic forms of nitrogen. In: Methods of soil analysis (Ed. C.A. Black). American Society of Agronomy, Madison, USA.

Bruun S., Ågren G.I., Christensen B.T., and Jensen L.S., 2010. Measuring and modeling continuous quality distributions of soil organic matter. Biogeosciences, 7, 27-41. https://doi.org/10.5194/bg-7-27-2010.

Coleman K. and Jenkinson D.S., 1996. RothC-26.3 – A Model for the turnover of carbon in soil. In: Evaluation of Soil Organic Matter Models (Eds D.S. Powlson, P. Smith, J.U. Smith). NATO ASI Series (Series I: Global Environmental Change), Springer, Berlin, Heidelberg, https://doi.org/10.1007/978-3-642-61094-3_17.

Drosos M., Vinci G., Spaccini R., and Piccolo A., 2020. Molecular dynamics of organic matter in a tilled soil under short term wheat cultivation. Soil Till. Res., 196, 104448. https://doi.org/10.1016/j.still.2019.104448.

Ellerbrock R.H. and Kaiser M., 2005. Stability and composition of different soluble soil organic matter fractions – evidence from δ^{13}C and FTIR signatures. Geoderma, 128(1-2), 28-37.

Falloon P. and Smith P., 2000. Modelling refractory soil organic matter. Biol. Fertil. Soils, 30, 388-398. https://doi.org/10.1007/s003740050019.

FAO, 2017. Soil Organic Carbon: the hidden potential. Food and Agriculture Organization of the United Nations. Rome, Italy.

Greenfield L.G., Gregorich E.G., van Kessel C., Baldock J.A., Beare M.H., Billings S.A., Clinton P.W., Condron L.M., Hill S., Hopkins D.W., and Janzen H.H., 2013. Acid hydrolysis to define a biologically-resistant pool is compromised by carbon loss and transformation. Soil Biol. Biochem., 64, 122-126.

Hayes M.H., McCarthy P., Malcolm R.L., and Swift R.S., 1989. Structures of humic substances: the emergence of forms. In: Humic Substance II: In Search of Structure: Setting the Scene (Eds M.H. Hayes, P. McCarthy, R.L. Malcolm, and R.S. Swift). John Wiley and Sons, New York, USA.

Hayes M.H.B., Mylotte R., and Swift R.S., 2017. Humin: its composition and importance in soil organic matter. Adv. Agron., 143, 47-138.

Hayes M.H.B. and Swift R.S., 2020. Vindication of humic substances as a key component of organic matter in soil and water. Adv. Agron., 163, 1-37. https://doi.org/10.1016/bs.agron.2020.05.001.

Haynes R.J., 2005. Labile organic matter fractions as central components of the quality of agricultural soils: An overview. Adv. Agron., 85, 221-268. https://doi.org/10.1016/S0065-2113(04)85005-3.

Huo C., Luo Y., and Cheng W., 2017. Rhizosphere priming effect: A meta-analysis. Soil Biol. Biochem., 111, 78-84. https://doi.org/10.1016/j.soilbio.2017.04.003.

Huygens D., Boeckx P., Templer P., Paulino L., Van Cleemput O., Oyarzún C., Müller C., and Godoy R., 2008. Mechanisms for retention of bioavailable nitrogen in volcanic rainforest soils. Nature Geosci., 1, 543-548. https://doi.org/10.1038/ngeo252.

Jackson O., Quilliam R.S., Stott A., Grant H., and Subke J.-A., 2019. Rhizosphere carbon supply accelerates soil organic matter decomposition in the presence of fresh organic substrates. Plant Soil, 440, 473-490. https://doi.org/10.1007/s11104-019-04072-3.

Kalembasa S., 1991. Quick method of determination of organic carbon in soil. Polish J. Soil Sci., 24(1), 17-22.

Kalembasa S., 1995. Application of ^{15}N and ^{13}N isotopes in soil science and agriculture chemistry research (in Polish). WNT, Warsaw, Poland.

Kalembasa S. and Kalembasa D., 2016. Conversions and pathways of organic carbon and organic nitrogen in soils. In: Bioactive Compounds in Agricultural Soils (Ed. L. Szajdak). Springer, Cham., https://doi.org/10.1007/978-3-319-43107-9_3.

Kayler Z.E., Kaiser M., Gessler A., Ellerbrock R.H., and Sommer M., 2011. Application of δ^{13}C and δ^{15}N isotopic signatures of organic matter fractions sequentially separated from adjacent arable and forest soils to identify carbon stabilization mechanisms. Biogeosciences, 8, 2895-2906.

King A.E., Ali G.A., Gillespie A.W., and Wagner-Riddle C., 2020. Soil organic matter as catalyst of crop resource capture. Front. Environ. Sci., 8:50. https://doi.org/10.3389/fenvs.2020.00050.

Kumar A. and Sharma M., 2014. Review of methodology for estimation of labile organic carbon in reservoirs and lakes for GHG Emission. J. Mater. Environ. Sci., 5(3), 653-660.

Leavitt S.W., Follett R.F., and Paul E.A., 1996. Estimation of slow- and fast-cycling soil organic carbon pools from 6N HCl hydrolysis. Radiocarbon, 38(2), 231-239.

Man M., Wagner-Riddle C., Dunfield K.E., Deen B., and Simpson M.J., 2021. Long-term crop rotation and different tillage practices alter soil organic matter composition and degradation. Soil Till. Res., 209, 104960. https://doi.org/10.1016/j.still.2021.104960.

Meier U., 2001. Growth stages of mono- and dicotyledonous plants. BBCH Monograph, Federal Biological Research Centre for Agriculture and Forestry, Bonn, Germany.

Mwafulirwa L.D., Baggs E.M., Russell J., Morley N., Sim A., and Paterson E., 2017. Combined effects of rhizodeposit C and crop residues on SOM priming, residue mineralization and N supply in soil. Soil Biol. Biochem., 113, 35-44. https://doi.org/10.1016/j.soilbio.2017.05.026.

Owen A.G. and Jones D.L., 2001. Competition for amino acids between wheat roots and rhizosphere microorganisms and the role of amino acids in plant N acquisition. Soil Biol. Biochem., 33, 651-657.

Paul E.A., Morris S.J., Conant R.T., and Plante A.F., 2006. Does the acid hydrolysis-incubation method measure meaningful soil organic carbon pools? Soil Sci. Soc. Am. J., 70, 1023-1035.

Paul E.A., Follett R.F., Haddix M., and Pruessner E., 2011. Soil N dynamics related to soil C and microbial changes during long-term incubation. Soil Sci., 176(10), 527-536. https://doi.org/10.1097/SS.0b013e31822ce6e8.

Roberts P. and Jones D.L., 2008. Critical evaluation of methods for determining total protein in soil solution. Soil Biol. Biochem., 40(6), 1485-1495. https://doi.org/10.1016/j.soilbio.2008.01.001.

Schmidt M., Torn M., Abiven S., ***et al.*****, 2011.** Persistence of soil organic matter as an ecosystem property. Nature, 478, 49-56. https://doi.org/10.1038/nature10386.

Schulten H.R. and Schnitzer M., 1992. Structural studies on soil humic acids by Curie-point pyrolysis-gas chromatography/mass spectrometry. Soil Sci., 153, 205-224.

Shirato Y. and Yokozawa M., 2006. Acid hydrolysis to partition plant material into decomposable and resistant fractions for use in the Rothamsted carbon model. Soil Biol. Biochem., 38(4), 812-816. https://doi.org/10.1016/j.soilbio.2005.07.008.

Sollins P., Kramer M.G., Swanston C., Lajtha K., Filley T., Aufdenkampe A.K., Wagai R., and Bowden R.D., 2009. Sequential density fractionation across soils of contrasting mineralogy: evidence for both microbial- and mineral-controlled soil organic matter stabilization. Biogeochemistry, 96, 209-231. doi:10.1007/s10533-009-9359-z.

Solomon D., Fritzsche F., Lehmann J., Tekalign M., and Zech W., 2002. Soil organic matter dynamics in the subhumid agroecosystems of the Ethiopian Highlands: evidence from natural ^{13}C abundance and particle-size fractionation. Soil Sci. Soc. Am. J., 66, 969-978.

Stevenson F.J., 1982. Organic forms of soil nitrogen. In: Nitrogen in agriculture soil (Ed. F.J. Stevenson). Madison: ASA-CSSA-SSSA, USA.

Stevenson F.J., 1994. Humus Chemistry: Genesis, Composition, Reactions. 2nd Edition, Wiley, New York, USA.

Tate K.R., 2017. Microbial biomass: a paradigm shift in terrestrial biogeochemistry. Singapore, World Scientific Publishing Company.

Wick A.F., Ingram L.J., and Stahl P.D., 2009. Aggregate and organic matter dynamics in reclaimed soils as indicated by stable carbon isotopes. Soil Biol. Biochem., 41, 201-209.

World Reference Base, 2014. World Reference Base for Soil Resources International Soil Classification System for Naming Soils and Creating Legends for Soil Maps. World Soil Resources Reports No. 106. update 2015, Rome: FAO.

Yang S., Jansen B., Absalah S., Kalbitz K., Chunga Castro F.O., and Cammeraat E.L.H., 2022. Soil organic carbon content and mineralization controlled by the composition, origin and molecular diversity of organic matter: A study in tropical alpine grasslands. Soil Till. Res., 215, 105203, https://doi.org/10.1016/j.still.2021.105203.