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Development of soil organic carbon pools after vineyard abandonment

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Keywords (in English)

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Abandoned vineyard soils show quick recharge of soil organic carbon (SOC) stocks after cancellation of cultivation. In the study abandoned vineyards with six different age classes concerning the duration of postagricultural development, organized along two lines in different exposures on slope (one S and one SW exposed chronosequence) were selected. Involving an additional recently cultivated vineyard location, totally 13 sites were sampled for topsoil characteristics. In each bulk soil sample density fractions, hot water extraction, and microbial samples were separated. Accordingly the C and N content and C/N ratio of free particulate organic matter (FPOM), occluded particulate organic matter (OPOM), clay-, silt- and sand sized microaggregates, hot water soluble organic matter, and microbial biomass of were measured and discussed in the study.
We found that labile, active carbon pool (FPOM) have relatively low share of the TOC (in average 11.6% in S and 4.6% in SW sequence) and showed no increase with the time since the cancellation of cultivation. Also this pool has generally higher C/N ratio (20.6±3.7), as more stable pools (OPOM: 19.2±9.6; clay fraction: 9.2±1.2,). Highest part of TOC is stored in clay-sized microaggregates fraction (in average 37.2% in S and 41.5% SW sequence) and its amount correlates significantly with the time since the cancellation of cultivation. By comparison, in recently cultivated soil lower share of C in clay sized microaggregates and (24.0% of TOC) and higher share of labile, FPOM (26.6% of TOC) was found. C-pools in mMicrobial and hot water extractable C forms showed significant changes with the time. Based on, and exposure, and cultivation also proved differentce compared the cultivated site, anyway, their contribution to TOC are low.

Authors

Assoc. Prof. Tibor József Novák — Department of Landscape Protection and Environmental Geography, University of Debrecen, Hungary

Dr. József Incze — Department of Landscape Protection and Environmental Geography, University of Debrecen, Hungary

Almuth McLeod — Landesbetrieb, Geological Survey Nordrhein-Westfalen, Germany

Prof. Luise Giani — Department of Soil Science, Carl von Ossietzky University Oldenburg, Institute of Biology and Environmental Sciences, Germany

Tibor József Novák[1*], József Incze[1], Almuth McLeod[2], Luise Giani[3]

[1] University of Debrecen, Faculty of Sciences and Technology, Institute of Earth Sciences, Department of Landscape Protection and Environmental Geography, Debrecen, Hungary, Egyetem tér 1. H-4010, Debrecen, Hungary, Tel. +36-52-512-900/23221

[2]Geological Survey Nordrhein-Westfalen, Landesbetrieb, De-Greiff-Straße 195, D-47803, Krefeld, Germany

[3] Carl von Ossietzky University Oldenburg , Institute of Biology and Environmental Sciences, Department of Soil Science, D-26111, Oldenburg, Germany

* Associate professor, Tibor József, Novák, novak.tibor@science.unideb.hu

ORCID iD: 0000-0002-5514-9035

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Abstract

Abandoned vineyard soils show quick recharge of soil organic carbon (SOC) stocks after cancellation of cultivation. In the study abandoned vineyards with six different age classes concerning the duration of postagricultural development, organized along two lines in different exposures on slope (one S and one SW exposed chronosequence) were selected. Involving an additional recently cultivated vineyard location, totally 13 sites were sampled for topsoil characteristics. In each bulk soil sample density fractions, hot water extraction, and microbial samples were separated. Accordingly the C and N content and C/N ratio of free particulate organic matter (FPOM), occluded particulate organic matter (OPOM), clay-, silt- and sand sized microaggregates, hot water soluble organic matter, and microbial biomass were measured and discussed in the study. We found that labile, active carbon pool (FPOM) have relatively low share of the TOC (in average 11.6% in S and 4.6% in SW sequence) and showed no increase with the time since the cancellation of cultivation. Also this pool has generally higher C/N ratio (20.6±3.7), as more stable pools (OPOM: 19.2±9.6; clay fraction: 9.2±1.2,). Highest part of TOC is stored in clay-sized microaggregates (in average 37.2% in S and 41.5% SW sequence) and its amount correlates significantly with the time since the cancellation of cultivation. By comparison, in recently cultivated soil lower share of C in clay sized microaggregates and (24.0% of TOC) and higher share of labile, FPOM (26.6% of TOC) was found. C-pools in microbial and hot water extractable C forms showed significant changes with the time. Based on exposure and cultivation also proved different, anyway, their contribution to TOC are low.

Keywords: postagricultural soils, chronosequence, organic carbon fractions, free particulate organic matter, occluded particulate organic matter

1. Introduction

Soil organic matter (SOM) could be divided on different fractions according to that, how far it is physically protected against further oxidation and mineralization processes, and how sensitive it responds to changes of land use. Density fractionation of SOM allows differentiation of light, and heavy fractions. In light fractions free particulate organic matter (FPOM), and occluded particulate organic matter (OPOM) could be distinguished according to that, if the SOM is occluded and therefore slightly protected within aggregates, or not. In case of heavy fractions SOM is so inseparably connected to the mineral particles, and therefore it is usually regarded to be a passive pool within SOM. Heavy fraction can be further separated according the particle size distribution, belonging into sand (2-0.063 mm), silt (0.063-0.002 mm) and clay (<0.002 mm) size. Occlusion of SOM into stabile soil aggregates and following stronger connection to mineral particles is regarded as the most important stabilization process of the SOM (Dilly and Blume, 1998).

In consequence of vineyard abandonment and subsequent self-restoration significant C-sequestration in soils considering the total organic carbon (TOC) content (Novák et al., 2014; Spohn et al., 2015) can be observed. Nevertheless, it is unknown which kind of C pools were affected. Many studies show that the conversion of arable land to abandoned soils, or conversion of conventional tillage to no tillage management caused predominantly an increase of the FPOM (Coneição et al., 2013), which belongs to the active C pool, and considered as most sensitive indicator for land use changes.

For the dynamic of different SOM pools during self-restoration of vineyard soils no data are available so far. Hence, the aim of this study was to fill this knowledge gap, and investigate, which fractions contributes most effectively to carbon sequestration during the spontaneous restoration of soils after abandonment of cultivation.

3. Materials and methods

3.1. Study area

The study was carried out on the southest part of the Tokaj-Hegyalja Wine Region (Hungary), on Tokaj Nagy-Hill (Fig. 1). It is a traditional wine production area with almost thousand years old tradition. The physico-geographical conditions and soil forming factors of the area show big diversity within the area. The hill rises from the alluvial plain of the Bodrog, Tisza and Takta rivers starting at 100 m and reaches the elevation of 514 m a.s.l. The main mass of the hill is built up from late Miocene volcanic materials (Pécskay et al., 1995; Rózsa et al., 2006), which are mostly covered by aeolian loess sediments from lower to upper Weichselian ice age and their derivates, as colluvial materials (gravel, blocks, eroded and redeposited loess and soil). On the steepest sections of slopes volcanic rocks outcrop since the rapid erosion removed completely the overlaying loess cover. Remnants of interlayered fossil soil layers and preglacial weathering products of volcanic materials increase the spatial heterogeneity of parent materials of soils. The mean annual temperature varies between 8.5 °C on the top and almost 10 °C at the base due to the microclimatic variability influenced by the topography. The average annual number of sunny hours is about 2000-2050. The mean annual precipitation is between 580 and 617 mm (Justyák, 1981). The duration of snow cover is short, does not reach 40 days and the mean thickness is less than 20 cm. The soil temperature regime could be given in mesic, the soil moisture regime in ustic (Füleky et al., 2007) and presumably xeric in some of the years.

#Fig. 1. Location of study area#

Vegetation, soils, and geomorphology on major part of the hill has been affected by cultivation during the last centuries (Balassa, 1975, 1991; Rózsa, 2007). The changes of the socio-economic environment during the last centuries were reflected also in land use changes (Boros, 2008) resulting the fluctuation of the extent of cultivated vineyards (Nyizsalovszki and Fórián, 2007). Remarkable part of them were abandoned or restored according the changes of actual demand for wines (Boros, 2008). After abandonment of vineyards spontaneous reforestation and shrub development could start (Sendtko, 1999), which might be interrupted by later restoration of plantations during conjuncture periods.

Soils of the Hill therefore shows also frequently evidence of former or recent disturbances (Novák et al., 2014). Still the high diversity of parent materials, topography, and vegetation and land use history resulted high diversity in soils as well. Earlier studies (Kerényi, 1994; Stefanovits et al., 1999) reported about eroded Luvisols, Chernozems, Phaeozems from the Hill, later on weathering products of volcanic rocks Phaeozems, Luvisols, Cambisols, Leptosols and Umbrisols were described (Füleky et al., 2004, 2007; Madarász et al., 2013). Skeletal soils (Dövényi, 2010) and slope deposits (Kerényi, 1994) were also frequently found, which could be assigned to Leptosols and Regosols according to the WRB.

3.2. Sampling design and settings

For study site selection land use change data compiled from historical maps and remotely sensed data were used starting with the I. Military Survey (date: 1783), II Military Survey (1858), III Military Survey (1884), topographic maps from the period of World War II (1940), New Survey Topography Map (1960), Topography Map (1989) and orthophoto from 2010. The extent and location of vineyards were identified, vectorized and transformed into unified projection system (Unified National Projection, EOV). Elevation deformation of air photographs was offset. For the processing of maps and remotely sensed land use data Quantum GIS 1.7 and Erdas Imagine 8.5 software were applied.

Changes between the mapped periods described the vineyard abandonments between two consecutive maps. The abandoned vineyards were assigned into the following age classes based on the number of years since the abandonment: 193; 142; 101; 63; 39; 14 which are the midpoints of the time intervals between the releases of two consecutive maps. These age classes were delineated with polygons on the maps of the Hill. We supposed, that similarly to other regions slope grade and exposition affect development of soils and vegetation (Koulouri and Giourga, 2007; Leeschen et al., 2008) the study design was extended accordingly. Based on the topographic map the slope gradient and exposition were assigned to the abandonment data. Slope grade categories were applied as in Hungarian agricultural practices (0–5%, 5–12%, 12–17%, 17–25% and 25–35%), exposition was ranked according the four cardinal and four intercardinal directions.

Based on cross sectioning of the slope-grade data, slope-exposition, and abandonment-age map two chronosequences were constituted (Table 1). One sequence was set on 25-35% slopes exposed to south (S-sequence, sites S1-S6), and one other one on 17-25% slopes exposed to southwest (SW-sequence, sites SW7-SW12), both including sites of same ages of abandonment (193; 142; 101; 63; 39; 14 years). Depending on the succession grade and time left since abandonment the sampling sites are overgrown by grasslands, shrubs, or secondary forest vegetation. In closest vicinity of S-sequence sites a cultivated vineyard (25% slope and southwest exposition) was sampled as reference (S/SW0) site.

#Table 1. Sampling site characteristics#

3.3 Field soil sampling

On each sampling site mixed surface samples were collected from the top 0-10 cm layer of soils, in three replicates. The same time soil profiles were prepared, sampled, and described to classify the soils according the WRB. A detailed description of soil profiles, and vegetation and the evaluation of carbon stocks was published by Novák et al. (2014). From the evaluation of carbon fractions subsequently the data of sampling site SW9 were excluded due to visible evidence of later disturbances affecting the shallow topsoil.

3.4. Standard soil analytics

Bulk soil samples were grounded to fine powder with a ball mill for 5 minutes and dried at 105 °C for 24 hours. For the exact determination of soil texture class grain size distribution was analyzed by combined wet sieving (2–0.2 mm fractions) and pipette method (<0.2 mm fractions) (Pansu and Gatheyrou, 2006). Total organic carbon of bulk samples were determined by both wet oxidation method (Ponomareva and Plotnikova, 1980) and using CHNS analyzer (Flash EA CHNS 112 series, Thermo Electron Cooperation). pH_{H2O} and pH_{KCl} were measured in 1:2.5 suspensions with standard glass electrode. Inorganic carbon in both, in bulk soil and fractionated samples were measured by volumetric calcimeter (Scheibler) method (Chaney et al., 1982).

3.5. Soil organic matter fractionation

3.5.1. Density fractions

The SOM fractionation was conducted to obtain light (<1.8 g cm^{-3}) fractions (FPOM and OPOM) and OM of the heavy (>1.8 g cm^{-3}) fractions (associated with grain size fractions as sand, silt and clay) according to Kalinina et al. (2009, 2010, 2011, 2015) (Fig. 2).

#Fig. 2. Processing of separation of OM fractions#

The fractionation was carried out on dried (105°C) and sieved (2 mm) soil samples in portions of 7 g per measurement. Fractionation and measurements were done in triplicates. To separate light and heavy fractions a sodium polytungstate (SPT) solution with a special density of 1.8 g cm^{-3} were used to soak soil aggregates and particles for 12 hours. The FPOM fractions were separated as the overfloating soil particles (lighter than 1.8 g cm^{-3}) due filtration by cellulose-nitrate membrane filters (1.2 µm). Before determining the weight of the light fractions remained on the filter, SPT solution had to be removed from the filtrate (Ahmed and Oades, 1984) therefore after each filtration, the soil was washed with deionized water and the wash-water's electrical conductivity was measured until having values lower than 100 S cm^{-1}. To determine the separate's weight, the filtered and washed soil fraction was dried at 105°C.

To gain the OPOM fractions in the next step the heavy fractions (heavier than 1:8 g cm^{-3}) were dispersed with help of ultrasonic sound treatments in order to crack aggregates and free the OPOM. Optimization of the energy intensity was necessary, to avoid destroying organo-mineral complex, and mutate grain size distribution applying to high energy, or preserving uncracked

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aggregates and underestimate of the OPOM by applying to low energy (Leifeld and Kögel-Knabner, 2005; Schmidt et al., 1999; Steffens et al., 2009). For determination of the optimal treatment time a sonotrode was carried out by measuring the heating of 100 ml H_2O during a time of 120 seconds. The resulting time for treating the samples was calculated and set to 10 minutes, whereby approximately 450 Jml^{-1} energy output had been reached. The aggregates after this treatment were cracked and the OPOM lighter than 1.8 g cm^{-3} freed. Ultrasound dispersion was followed by centrifugation (15 minutes at a speed of 10 000 spins per minute) and again filtering, washing, drying.

The left-over heavy soil fractions (heavier than 1:8 g cm^{-3}) of all three parallels were mixed together for grain size analysis without H_2O_2 pre-treatment to avoid destruction of organo-mineral complexes. Therefore the separated fractions are not in fact mineral grain particles but are denominated as i.e. sand sized (2-0.63 mm), silt sized (0.63-0.002 mm), and clay sized (<0.002 mm) microaggregates according definitions (Blume et al., 2011).

From each separated fraction 2 mg was weighed for C/N analysis in triplicates.

The amounts of OC in particular fractions ($g{\cdot}kg^{-1}$ soil) were calculated as follows:

$$OC_i = \frac{F_i}{\sum_{i=1}^{n} F_i} \cdot C_i \cdot 100$$

where OC_i is the amount of OC in i fraction in g in 1 kg of soil ($g \cdot kg^{-1}$), F_i is the mass of the i fraction in kg, C_i is the concentration of organic C in i fraction in $g \cdot kg^{-1}$. In a similar way the amounts of N for each fraction were calculated.

3.5.2. Hot water extractable C (C_{hwe})

Hot water extractable C (C_{hwe}) was gained by reflushing 5 g of soil with 25 ml of H_2O_{dest} for 60 min with an attached reflux condenser, which was prewetted previously with 25 ml moderately boiling distilled water, following the VDLUFA–Methodenbuch, (2004)). Subsequently, the samples were cooled down in a water bath to room temperature, 2 droplets of $MgSO_4$ solution (490 $g \cdot l^{-1}$) were added and was centrifuged (3500U/min with Labofuge 400/Heraeus company/swing-bucket rotor) for 10 minutes at 2600 g. The supernatant was filtered through a 0.45 µm nitrocellulose filter (Millipore), acidified to pH 2 with 6M HCl (10 ml filtrate+40 µl 6M HCl) to prevent possible CO_2 absorption from the air, and stored at 4 °C. The C_{hwe} concentration was measured using a TOC analyzer (TOC-V CSH, Shimadzu) and calculated for g kg^{-1}.

3.5.3. Microbial C (C_m)

Microbial C was determined according to Vance et al. (1987). Five g sieved, field moist soil was fumigated with chloroform for 24 h and was subsequently extracted in 0.5M K_2SO_4 for 1 hour on a shaker. Another 5 g were directly extracted in 0.5 M K_2SO_4 for 1 h on a shaker. The organic C in both extracts was determined with a CN Analyzer (Multi 2100 Analytik Jena) after acidification to pH 3 in order to remove inorganic C. The difference between the organic C in the extract of the fumigated and the non-fumigated soil was multiplied by the conversion factor 2.64 for C (Vance et al., 1987), and the result of this multiplication is considered as the microbial C.

3.6. Chemical analysis

Total carbon (TC) and total nitrogen (TN) content in bulk soil samples and in particular fractions were measured with a CHNS analyzer (Flash EA CHNS 112 series, Thermo Electron Cooperation) after the fractionations were separated by the above-mentioned way. Three repetitions were conducted per measurement. The amount of TC and TN are given in m/m % and calculated to g kg^{-1}. The standard values used for this measurement are 10.36% for N and 69.38% for C.

Inorganic carbon content in the bulk soil samples and in density fractions were measured by volumetric method (Scheibler calcimeter), and total carbon data were corrected with the measured values to calculate the total organic carbon (TOC). Since inorganic carbon was found almost exclusively in sand and silt fractions in higher amount, which could influence strongly the results of carbon measurements, in the fractionated carbon content only the clay and the light organic carbon fractions were taken into consideration.

3.7. Data analysis and evaluation

Before statistical analysis of relationships between variables, all datasets were tested for normality with Kolmogorov-Smirnov test, and the equal variance of homogeneity. Since we found the data not to follow normal distribution, non-parametric statistical tests (Spearman's rank correlation) were applied. Statistical analyses were performed using SPSS 17.0. Since carbon content of density fractions (FPOM, OPOM, and heavy fractions) are overlapping with C_m and C_{hwe}, the evaluation was carried out separately for density fractions and C_{hwe} and C_m.

4. Results

4.1. Dynamic of TOC and TN

The TOC concentrations in S-sequence were varying between 11.03 g kg^{-1} (S6) and 45.59 g kg^{-1} (S1) values were increasing with the age of abandonment (Table 2). In comparison the cultivated reference site (S/SW0) had 14.85 g kg^{-1}. In the SW-sequence lowest TOC-concentration was also measured in most recent abandonment 5.30 g kg^{-1} (SW12), and the oldest abandoned site had 26.96 g kg^{-1} (SW7). The increase of TOC concentration showed significant correlation with the duration of abandonment in both sequences. Spearman's rho was in case of S-sequence 0.786 ($p<0.05$) in SW-sequence 0.886 ($p<0.01$).

#Table 2. Amount and share of organic carbon in fractions of soil organic matter in abandoned vineyard soils#

TN-concentration in S-sequence was also increasing with years since abandonment from 0.835 g kg^{-1} in the youngest (S6), and 5.076 g kg^{-1} in the oldest abandonment (S1). The TN concentration correlates significantly with the years since abandonment (Spearman's rho=0.965; $p<0.01$). We measured the lowest TN concentration in the cultivated reference site (S/SW0) 0.276 g kg^{-1}. In case of the SW-sequence, similarly to the TOC, TN concentrations were lower consequently. In most recent abandoned site (SW12) TN concentration was 0.523 g kg^{-1}, in the oldest abandonment (SW7) 2.383 g kg^{-1}. TN increase with the time since abandonment proved to be significant in case of SW-sequence as well (Spearman's rho=0.872; $p<0.05$).

4.2. Dynamics of fractionated SOC pools

In average 82.1% of OC was found in clay and light (OPOM and FPOM) fractions, and only 17.9% in silt and sand sized particles, additionally the inorganic carbonates were found mostly in silt and sand fractions which variated on wide range depending from carbonate contents of parent material, therefore only OC in clay and light (OPOM, FPOM) fractions were evaluated separately.

OC content of OPOM and the clay fractions proved to be in significant correlation ($p<0.01$) with the TOC of the samples, therefore similarly to TOC they increased with the time since abandonment. Spearman's rho in case of heavy (clay) fraction was 0.961 ($p=0.0001$), and in case of OPOM fraction was 0.807 ($p=0.001$). The OC content of FPOM fractions do not show significant relation to the TOC content, therefore also not related with the time since abandonment (Fig. 3).

#Fig. 3. OC in fractions (clay, OPOM, FPOM) plotted against the TOC content of samples#

The clay fraction contained the highest proportion of TOC in every case, in exception of the recently cultivated S/SW0, where OC content of both OPOM and FPOM fractions exceeded the OC content of clay fraction. After the clay-sized particles, the second largest OC pool proved to be in the OPOM fraction, with exception of the 39 years old abandonment of S-sequence (S5) and the cultivated reference (S/SW0) samples, where OC content of FPOM was higher.

Highest proportion of light POM (OPOM and FPOM) within the TOC was found in the cultivated site (S/SW0) 52.5%. In abandoned soils the contribution of light POM to the TOC was substantially lower, in average 26.3% in S-sequence, and 11.8% in SW-sequence. Consequently, low proportion of TOC was found in clay sized POM in case of the cultivated S/SW0 reference site (47.5%), and much higher part of it in cases of abandoned soils: 73.3% in S-sequence, and 88.2% in SW-sequence in average.

The OC content of OPOM and clay fractions increased significantly with the time since abandonment of the cultivation parallel with the TOC content of the samples in both, S- and SW-sequences (Fig. 4). In S-sequence the OC content of clay fraction showed stronger relation with time and faster growth (Spearman's rho=0.929; $p<0.01$) than OC content of the OPOM fraction (Spearman's rho=0.821; $p<0.05$). In the SW-sequence the increase OC content was faster in case of OPOM (Spearman's rho=0.943; $p<0.01$), than in clay fraction (Spearman's rho=0.8771; $p<0.05$). In case of FPOM no significant relation with the duration of self-restoration was found in none of the sequences (Fig. 4).

#Fig. 4. Changes of OC content in fractions (clay, OPOM, FPOM) after abandonment in S- and SW-sequence#

Contribution of C_m to the TOC of the bulk soil proved to be evenly very low, in average 0.75% in the S, and 1.13% in the SW sequence. It was in both cases higher than in the reference cultivated S/SW0 site (0.71%). Lower C_m contribution and activity in the S sequence are related to the limited access to soil moisture on these exposed slopes. Lowest contribution in case of the cultivated S/SW0 site refers to reduced microbial life under cultivated soil conditions.

4.3. Dynamics of N in fractions

Parallel with the TOC content N content showed an increasing dynamic with the time in both S and SW sequences. Highest part of TN was stored in almost every samples in clay fraction, followed by the OPOM fraction, and lowest in FPOM (Fig. 5).

#Fig. 5. Changes of total N content in fractions (clay, OPOM, FPOM) after abandonment in S- and SW-sequence#

4.4. Dynamics of C_{hwe} fraction

C_{hwe} represents the relatively stable but small part of the TOC pool. Respectively, highest values were measured in the samples with higher postagricultural development, and it shows significant increase with the time (Fig. 6). Anyway, it contributes to the TOC only in very low amount, varying between 0.38 and 1.83 g kg^{-1} in abandoned soils and having the lowest value in the cultivated soil (0.34 g kg^{-1}).

#Fig. 6. OC_{hwe} fraction and their relation to the time in the two chronosequences#

4.5. C/N ratio in total soil and in fractions

C/N ratio of the not fractioned samples proved to be slightly different in the two sequences: 11.1 (±3.2) in S- and 10.6 (±1.9) SW-sequence (Table 3). In fractioned samples of the S sequence the highest C/N values were found in FPOM 20.1 (±1.01). In the OPOM it was lower, 16.2 (±3.1), and in clay sized fraction was the lowest 9.7 (±0.7). In the SW sequence the C/N values of FPOM and OPOM fractions were similar 21.1 (±2.5) and 22.4 (±5.9), consecutively. In clay sized fraction it was lowest, 8.7 (0.4).

#Table 3. C/N ratio in bulk soil and in investigated fractions of soil organic matter#

5. Discussion

In contrast to other studies (John et al., 2005; Poeplau and Don, 2013) with development to forest vegetation we found less C sequestered in FPOM fractions, and it is not increasing with the time. The reason for this might be, that it must be differentiated among forest types developing during the secondary succession and the quality of the produced biomass. Much

FPOM is always related to forests with 'moder' and 'rohhumus' organic layers, producing much more FPOM than forest sites with 'mull' layers, which was the case in our study.

Particulate organic matter (POM) responds often more rapidly to land use conversions or changes in management practice (Leifeld and Kögel-Knabner, 2005; Six et al., 1998), than heavy fractions. Conversion of cultivated land to forest or grasslands results in rapid growing of POM under wet temperate climatic conditions (Poeplau and Don, 2013). Peoplau and Don (2013) also found POM to be a very sensitive indicator of changed SOC sequestration pattern after land use change. Other studies show additionally an increasing change of POM in aggregates (Kalinina et al., 2014, 2015). Passive OC pools (OC in silt and clay fractions (Christensen, 2001) were found to participate in the process of OC sequestration during self-restoration (Jastrow, 1996; McLauchlan, 2006; Floote and Grogan, 2010) however others shows no substantial participation of this fractions.

Similarly to statements in other studies, smallest contribution to TOC was found in FPOM, and highest in the mineral (clay) fraction (Coneição et al., 2013), anyway differences between the S and SW sequences and the cultivated reference place were considerable. Only 4.0% of the TOC took place in FPOM in average of the samples from SW-sequence, 13.4% in average of S-sequence and 26.6% in case of the cultivated reference (S/SW) site. This relation proved to be not varying with the time since the abandonment, but the difference between the S and SW sequences was significant. In contrast of other studies, we did not find increasing amount of FPOM, which could be because of the shortest turnover time of this fraction. It seems to be, that in these conditions (dry microclimatic conditions on exposed slopes) SOM of FPOM will be either quickly decomposed or moved into the OPOM and the heavy OM fractions.

The C/N ratio was more variable within the different fractions and did not show any clear development with the time of self-restoring. Generally the lowest C/N ratio was found in clay fraction 9.2±1.2 and in both of the light fractions were more than double higher, being 19.3±9.7 in OPOM and 20.6±3.7 in FPOM.

Conclusions

The chronosequential study of the SOC sequestration after vineyard abandonment on S and SW exposed slopes showed that the separate fractions have different contributions to increase of th TOC content. Considering the duration of postcultivation development of the soils a relatively quick C sequestration rate could be pointed on, which is also influenced by the slope exposition. The well-known considerable C decrease and exhaustion of C pools caused by vineyard land use can be the reason for the fast recharge of them after leaving off it. Almost independently from the duration of postagricultural soil development, the largest part of TOC is stored in form of stable organic compounds bound in clay-sized microaggregates. The contribution of the labile fractions (FPOM, OPOM) to the TOC proved to be relatively low in abandoned vineyards soils, since in the cultivated vineyard soil it is significantly higher – besides lower TOC content. More labile pools (FPOM) represent very limited capacity, even if presumably the C in this fraction is the first steps in C sequestration, providing sources to sequester the C in further, more stable pools, but this process proved to be rapid under the conditions of our study.

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Fig. 6. OC_{hwe} fraction and their relation to the time in the two chronosequences

#Table 1. Sampling site characteristics#

Sampling site	Elevation a.s.l. (meter)	Exposition	Slope	Time since abandonment (years)	Vegetation type	WRB RSG
S1	362	S	25-35%	193	Forest	Calcisol
S2	305			142	Grassland/shrub	Leptosol
S3	203			101	Shrub	Calcisol
S4	233			63	Grassland/shrub	Calcisol
S5	155			39	Grassland/shrub	Calcisol
S6	114			14	Fallow	Regosol
SW7	426	SW	17-25%	193	Forest	Chernozem
SW8	377			142	Afforestation	Cambisol
SW9	335			101	Forest	Cambisol
SW10	275			63	Grassland	Phaeozem
SW11	236			39	Shrub	Cambisol
SW12	257			14	Fallow/shrub	Regosol
S/SW0	145	SSW	25%	0	Cultivated vineyard	Regosol

#Table 2. Amount and share of organic carbon in fractions of soil organic matter in abandoned vineyard soils#

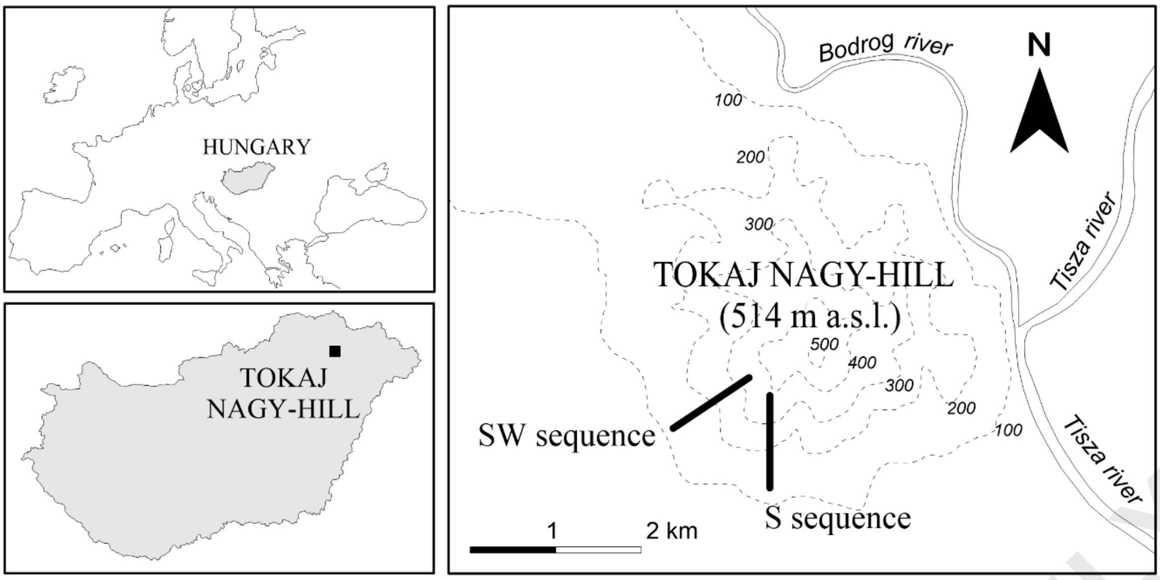
Sampling site	Density fractions						OC_{hwe}		C_m		TOC g/kg
	FPOM*		OPOM**		clay sized						
	g/kg	% of TOC	g/kg	% of TOC	g/kg	% of TOC	g/kg	% of TOC	g/kg	% of TOC	(bulk soil)
S1	9.35	20.5%	14.04	30.8%	17.18	37.8%	1.83	4.0%	0.321	0.7%	45.51
S2	3.02	9.3%	11.15	34.2%	15.86	48.6%	1.46	4.5%	0.128	0.3%	32.60
S3	3.42	7.5%	13.50	29.6%	16.47	36.1%	1.81	4.0%	0.294	0.6%	45.59
S4	4.10	11.3%	8.01	22.1%	13.99	38.7%	1.51	4.2%	0.360	0.9%	36.18
S5	4.46	19.3%	1.06	4.6%	8.17	35.3%	1.42	6.1%	0.284	1.2%	23.15
S6	1.37	12.4%	4.02	36.5%	2.97	26.9%	0.38	3.4%	0.060	0.5%	11.03
SW7	0.43	1.6%	9.71	36.0%	14.40	53.4%	1.70	6.3%	0.105	0.4%	26.96
SW8	1.37	5.5%	9.53	38.7%	6.96	28.2%	1.13	4.6%	0.146	0.6%	24.64
SW10	0.41	2.3%	8.32	46.8%	6.42	36.1%	0.87	4.9%	0.101	0.6%	17.79
SW11	1.18	6.6%	7.19	40.2%	7.27	40.7%	1.05	5.8%	0.134	0.7%	17.87
SW12	0.21	4.0%	2.09	39.5%	2.61	49.3%	0.45	8.5%	0.177	3.3%	5.30
S/SW/0	3.95	26.6%	4.76	32.0%	3.57	24.0%	0.34	2.3%	0.105	0.7%	14.85

*FPOM=free particulate organic matter;

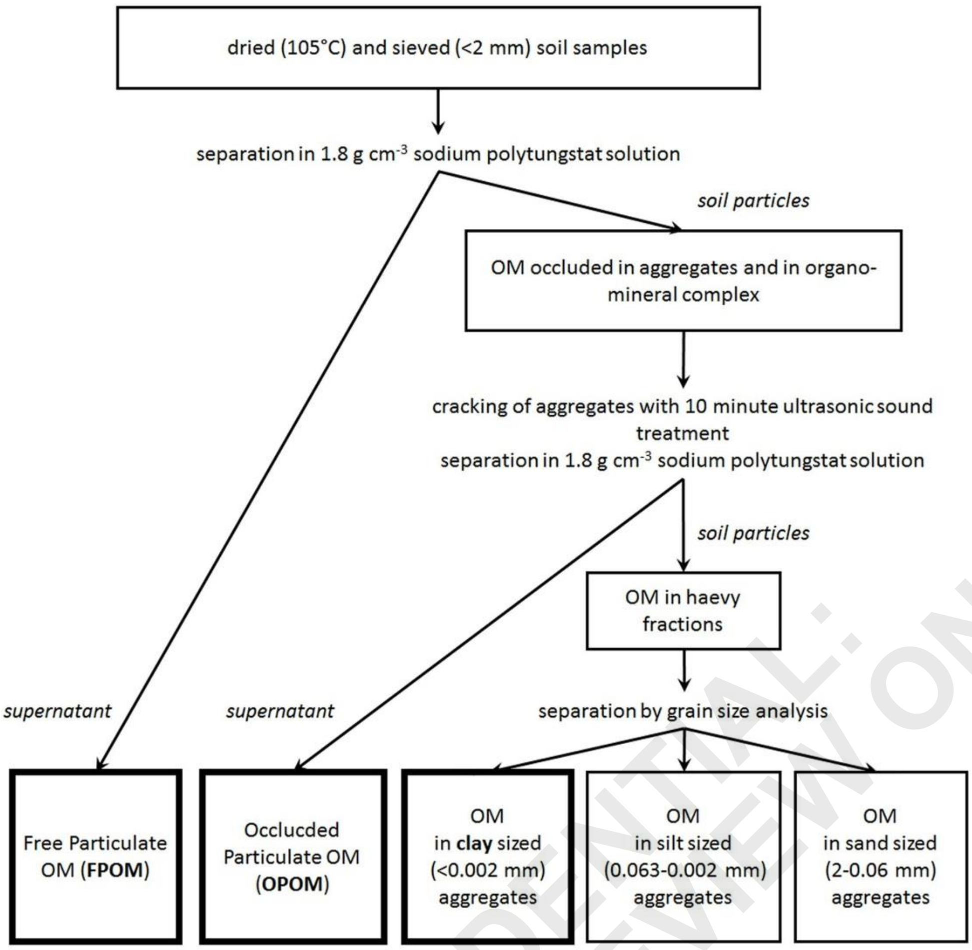
**OPOM=occluded particulate organic matter

#Table 3. C/N ratio in bulk soil and in investigated fractions of soil organic matter#

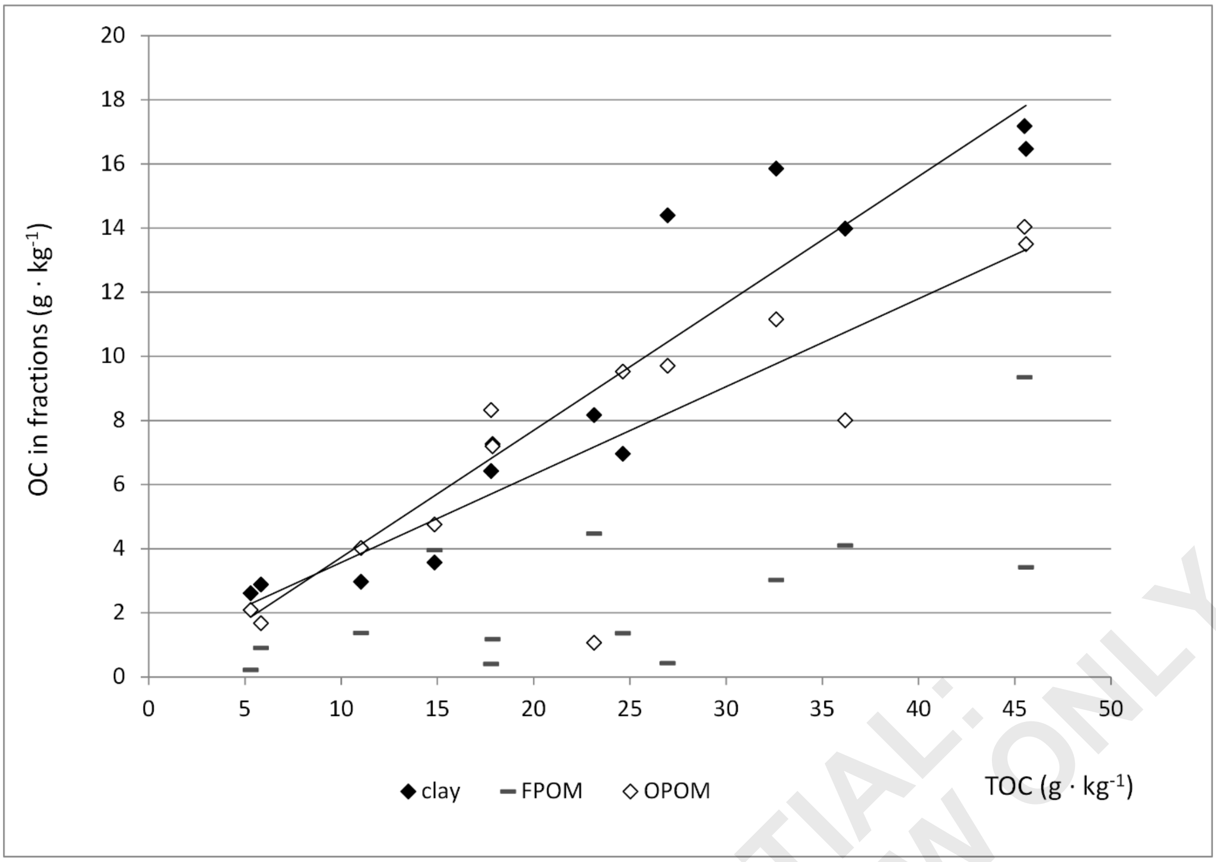
Sampling site	C/N ratio				
	Bulk soil	Density fractions			OC_{hwe}
		Light fractions		Heavy fraction	
		FPOM	OPOM	clay sized	
S1	8.97	21.50	17.08	9.61	11.92
S2	6.50	19.47	19.11	9.71	13.95
S3	9.38	19.84	4.81	9.72	13.91
S4	16.22	20.99	17.17	12.39	14.27
S5	12.32	21.02	25.26	8.69	11.81
S6	13.20	22.11	17.67	7.65	11.43
SW7	11.31	22.41	16.62	9.72	18.74
SW8	13.46	12.96	22.17	7.76	11.49
SW9	7.39	22.96	26.66	7.71	14.69
SW10	10.71	25.78	15.26	8.46	9.47
SW11	10.89	20.52	17.06	9.02	12.77
SW12	10.13	26.83	46.98	8.51	13.11
S/SW0	8.06	16.01	12.10	9.86	



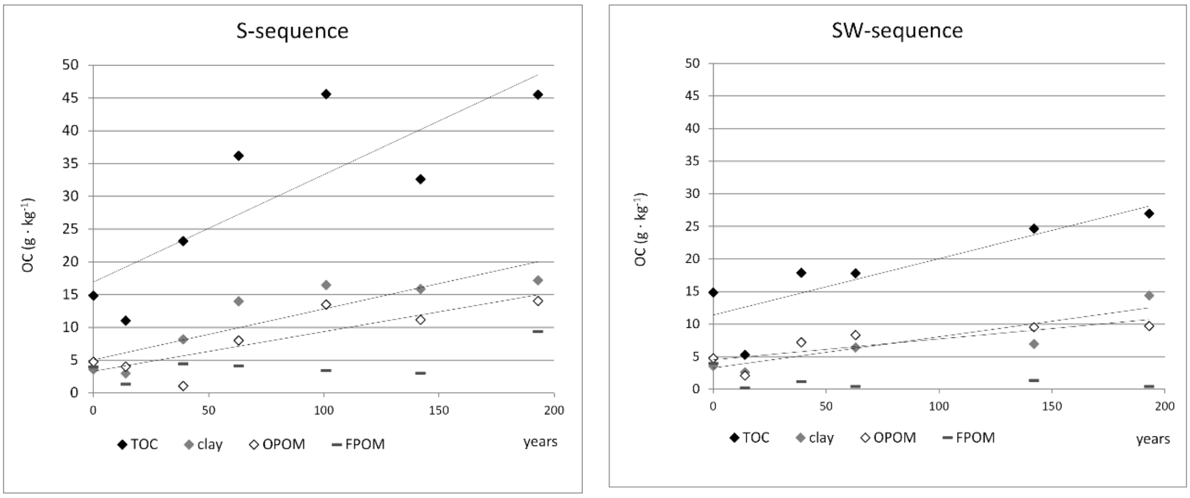
#Fig. 1. Location of study area#



#Fig. 2. Processing of separation of OM fractions#



#Fig. 3. OC in fractions (clay, OPOM, FPOM) plotted against the TOC content of samples#



#Fig. 4. Changes of OC content in fractions (clay, OPOM, FPOM) after abandonment in S- and SW-sequence#

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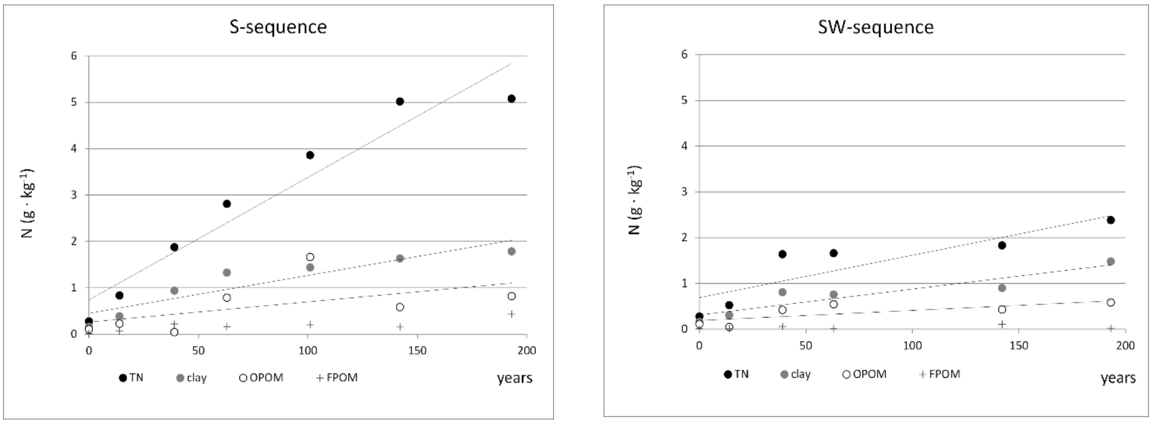

#Fig. 5. Changes of total N content in fractions (clay, OPOM, FPOM) after abandonment in S- and SW-sequence#

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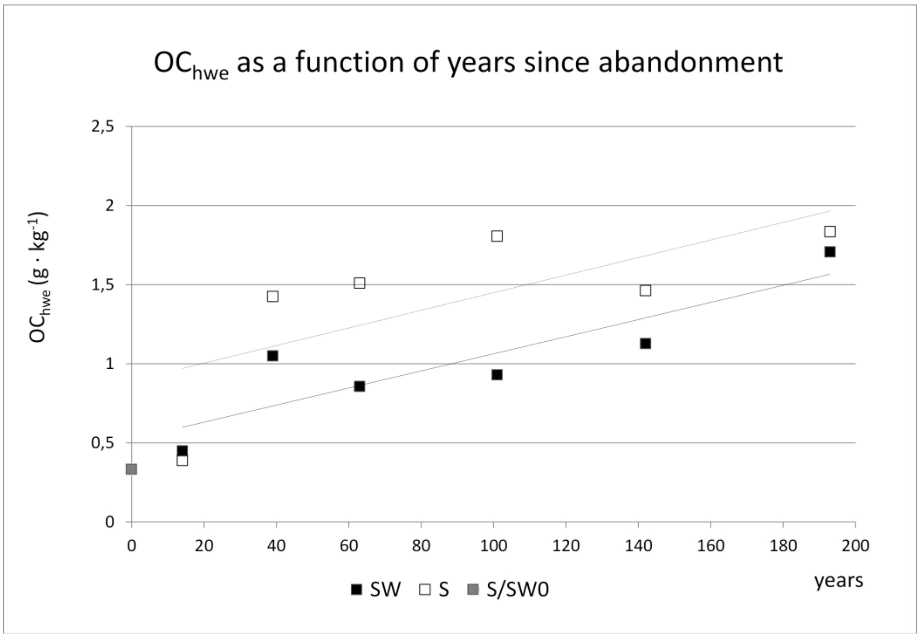

#Fig. 6. OC_{hwe} fraction and their relation to the time in the two chronosequences#

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#Table 1. Sampling site characteristics#

Sampling site	Elevation a.s.l. (meter)	Exposition	Slope	Time since abandonment (years)	Vegetation type	WRB RSG
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S4	233			63	Grassland/shrub	Calcisol
S5	155			39	Grassland/shrub	Calcisol
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SW8	377			142	Afforestation	Cambisol
SW9	335			101	Forest	Cambisol
SW10	275			63	Grassland	Phaeozem
SW11	236			39	Shrub	Cambisol
SW12	257			14	Fallow/shrub	Regosol
S/SW0	145	SSW	25%	0	Cultivated vineyard	Regosol

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#Table 2. Amount and share of organic carbon in fractions of soil organic matter in abandoned vineyard soils#

Sampling site	Density fractions						OC_{hwe}		C_m		TOC g/kg
	FPOM*		OPOM**		clay sized						
	g/kg	% of TOC	g/kg	% of TOC	g/kg	% of TOC	g/kg	% of TOC	g/kg	% of TOC	(bulk soil)
S1	9.35	20.5%	14.04	30.8%	17.18	37.8%	1.83	4.0%	0.321	0.7%	45.51
S2	3.02	9.3%	11.15	34.2%	15.86	48.6%	1.46	4.5%	0.128	0.3%	32.60
S3	3.42	7.5%	13.50	29.6%	16.47	36.1%	1.81	4.0%	0.294	0.6%	45.59
S4	4.10	11.3%	8.01	22.1%	13.99	38.7%	1.51	4.2%	0.360	0.9%	36.18
S5	4.46	19.3%	1.06	4.6%	8.17	35.3%	1.42	6.1%	0.284	1.2%	23.15
S6	1.37	12.4%	4.02	36.5%	2.97	26.9%	0.38	3.4%	0.060	0.5%	11.03
SW7	0.43	1.6%	9.71	36.0%	14.40	53.4%	1.70	6.3%	0.105	0.4%	26.96
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SW11	1.18	6.6%	7.19	40.2%	7.27	40.7%	1.05	5.8%	0.134	0.7%	17.87
SW12	0.21	4.0%	2.09	39.5%	2.61	49.3%	0.45	8.5%	0.177	3.3%	5.30
S/SW/0	3.95	26.6%	4.76	32.0%	3.57	24.0%	0.34	2.3%	0.105	0.7%	14.85

*FPOM=free particulate organic matter;

**OPOM=occluded particulate organic matter

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Table 3. C/N ratio of organic matter in bulk soil and in investigated organic matter pools

Sampling site	C/N ratio				
	Bulk soil	Fractions			
		Light fractions		Heavy fraction	OC_{hwe}
		FPOM	OPOM	clay sized	
S1	8.97	21.50	17.08	9.61	11.92
S2	6.50	19.47	19.11	9.71	13.95
S3	9.38	19.84	4.81	9.72	13.91
S4	16.22	20.99	17.17	12.39	14.27
S5	12.32	21.02	25.26	8.69	11.81
S6	13.20	22.11	17.67	7.65	11.43
SW7	11.31	22.41	16.62	9.72	18.74
SW8	13.46	12.96	22.17	7.76	11.49
SW9	7.39	22.96	26.66	7.71	14.69
SW10	10.71	25.78	15.26	8.46	9.47
SW11	10.89	20.52	17.06	9.02	12.77
SW12	10.13	26.83	46.98	8.51	13.11
S/SW0	8.06	16.01	12.10	9.86	

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#Table 3. C/N ratio in bulk soil and in investigated fractions of soil organic matter#

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	Bulk soil	Density fractions			OC_{hwe}
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SW12	10.13	26.83	46.98	8.51	13.11
S/SW0	8.06	16.01	12.10	9.86	

Dear Editors and reviewers,

thank you very much for dealing with our manuscript and improving its quality with your remarks and suggestions!
Our detailed responses to the comments are below, typesetted with italic and blue.

REVIEWER'S DETAILED COMMENTS:

Reviewer 1

Comments and suggestions
Authors: Tibor József NOVÁK, József INCZE, Almuth MCLEOD, Luise GIANI
Title: Developement of soil organic carbon pools after vineyard abandonment
Manuscript number: SSA

Summary of the manuscript:
The manuscript analyzes soil properties of abandoned vineyards of different ages. The manuscript is the original research paper of the authors. The subject of the manuscript is interesting and current.
The theme of the manuscript's scope fits the aims of the journal. This paper is new and valuable contribution to merit publication in an international journal. The manuscript is definitely worth publishing in the *Soil Science Annual* journal.
Today, there are many abandoned agricultural lands or vineyards, the earlier cultivation of which may have caused interesting and important changes in the soils. These soils are particularly characterized by changes in the content of organic matter or microbial activity, which are significantly determined by the cultivation methods or their changes.
The basic idea of the experiment was carried out precisely, the study provided large amount of high quality data. For this study the authors used well-chosen and appropriate methods. The data are generally well presented. Thus, in addition to the above, the significance of the manuscript is also emphasized by the fact that we can learn much more about the changes due to the change of cultivation methods.

Thank you very much for the reviewer the accurate and positive review and high appreciation of our work!

The **Abstract** is appropriate, well built. It is summarizes the essence of the manuscript. It summarizes the contents of the entire manuscript in a clear and concise way.
Line 42 I would suggest changing the **keyword** "vineyard abandonment" because it can already be found in the title.

We changed this keyword to 'postagricultural soils', which is more general than vineyard abandonment'

Line 42 and 43 In addition, where these abbreviations are not frequently used internationally, I suggest that the keywords " FPOM " and " OPOM " be listed in full, because the abbreviation is unlikely to search the manuscript, which could reduce the number of potential readers.

We changed these keywords according tot he reviwers suggestion

Introduction

This chapter supports and complements the research topic of the manuscript. Appropriate and timely references are built in the introduction chapter. The References more or less are up to date. The chapter detailed partly well processed (see below*). In my opinion, it is a well-structured chapter, rather short compared to the large number of valuable data. This chapter covers the most important areas of the entire topic of the manuscript.
*At the same time I miss in this chapter the comparison with previous research on carbon and nitrogen turnover, its significance. Since the vast majority of the manuscript deals with this issue, it would be useful to make a brief addition to this chapter. As this topic is very popular in general, but it is very rare and significant in this context (abandoned and undisturbed areas, climate change etc.), I would suggest that this issue be addressed in a short paragraph.
Line 65 The objective part should be included in a separate paragraph.

We changed these keywords according tot he reviwers suggestion.As far previeous studies concern to similar conditions (vineyards, abandonments), the previeous results are involved into the introduction.
The study aims are now separated into new paragraph.

Methods
The methods chapter are appropriate and sufficiently and overly detailed.
Line 70 In my opinion, the study area chapter should not be written separately, but rather moved to the Methods chapter. In this case, the Methods chapter would be augmented by a subsection. Furthermore, I suggest that the GPS coordinates of the studied areas should also be indicated.

We merged the description of the study area into the Materials and methods chapter, and reorganized the numbnering of chapters accordingly

Since the locations were choosen to represent typical habitats, and the sampling points are close to each other (within one sequence not larger distance than 1.5-2 km) we decided not to put GPS coordinates into the table, because it would make the table too spacious.

Results
The interpretation of results is generally proper. Results chapter is very detailed and not too long, even though a lot of data and research has been done. These chapters are well-structured and properly constructed. This chapters provide detailed and perfectly summarizes the new and novel results. The authors show the results in tabular and figure form and its show a lot of results. Evaluation of these results is appropriate and draws realistic conclusions in the next chapter (Discussion). On the other hand, it make useful and interesting findings that may be interested.
In my opinion, the many figures used are very clear and detailed. It is absolutely necessary to interpret the results. They are illustrative, practical and definitely needed to illustrate the results and the statements.

Discussion
Discussion is not too detailed, but it sums up the results properly. It does not draw far-reaching conclusions, only realistic conclusions. It also well-structured and properly constructed. It provides an adequate evaluation of the results obtained compared to other similar research experiences.

Conclusions

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The chapter summarizes and well sums up the essence of the manuscript. This chapter explain and justified by the data. I think that the allegations are thorough and supported by the data and results.
The **tables** are necessary and useful for the presentation of the results.
The **figures** are practical and definitely needed for illustrate the results and the statements.

The manuscript is suitable for publication after a few above-mentioned minor revisions. ***Great job! I wish further success to the authors.***

REVIEWER'S DETAILED COMMENTS:

Reviewer 2

The paper deals with distribution of light and heavy fractions of soil organic carbon in a two chronosequences of abandoned vineyard soils. The subject is interesting, due to the fact that this kind of soil organic matter fractionation is rarely investigated. In the introduction the authors should explain what is their meaning of particulate organic matter (POM)?

> *It is quite complicated question, since many different definitions exists (see the review article about the topic below: Six et al., 2004). But we think that the description of our separation method gives the definition: every organic material which is <1.8 g cm^{-3} and not associated with mineral particles, was considered as particulate organic matter. This density-based separation of POM is in practice easily applicable.*

> *Six, J., Bossuyt, H., Degryze, S., Denef, K. 2004.* A history of research on the link between (micro)aggregates, soil biota, and soil organic matter dynamics. Soil and Tillage Research 79 (1): 7-31. https://doi.org/10.1016/j.still.2004.03.008

Usually, this fraction is considered as all soil organic matter particles less than 2 mm and greater than 0.053 mm in size (Cambardella and Elliot, 1992), that includes partially decomposed detritus and plant material, pollen, and other materials, and is commonly considered as a readily available (labile) source of soil nutrients, and a contributor to soil structure. Due to that, POM is highly sensitive to soil management.

> *This is true, that is, why we considered to investigate separate fractions in abandonment chronosequence, and not only the TOC as we did in our earlier study (Novák, et al, 2014).*

Tillage or soil disturbance increases the rate of decomposition and depleting PO, thus reduction in POM content is observed when native grasslands are converted to agricultural land. Did authors compare POM and mineral-associated C in abandoned vineyard soils?

> *We did: data are visible in Table 2, Table 3, and Fig 3, Fig 4. Anyway, we separated POM into 2 parts: occluded in ultrasonic destroyable aggregates (OPOM), and free (FPOM), and from mineral associated fractions only clay fraction was considered, since OM associated with silt and sand fractions was negligible.*

As regards conclusions. How did you find that "The well-known considerable C decrease and exhaustion of C pools caused by vineyard land use can be the reason for the fast recharge of them after abandonment"? This is not speculation not confirmed by the data obtained.

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If you consider S/SW 0 cultivated soils TOC content (14.8 g/kg) and the TOC content of soils after 200 years of abandonment (26-45 g/kg) it seems to be not only speculation. Cultivated vineyards topsoil suffers under heavy erosion, sometimes also leveling, and other landscaping works exhaust the TOC stocks of the carbon pool under vineyards.

Soils of abandoned vineyards have been probably (although this fact is not clearly indicated in the paper) overgrown by shrubs and trees, so should indicate much bigger input of a POM. How can you explain the following: "Labile fractions (FPOM, OPOM) contribution to TOC proved to be relatively low in abandoned vineyards soils, since in cultivated vineyard soil it is significantly higher. More labile pools (FPOM) represent very limited capacity, even if presumably C in this fraction is the first steps in C sequestration, providing sources to sequester C in further, more stable pools, but this process proved to be rapid under the conditions of our study."

The text is sometimes written in a too complicated manner. In addition to a few stylistic errors, sometimes there are very long sentences difficult to follow (for example lines 297 - 302). The paper should be proofread by a native English speaker.

We corrected the mentioned sentence as follows:

"The OC content of OPOM and clay fractions ~~parallel with the TOC content of the samples~~ increased significantly with the time since abandonment of the cultivation parallel with the TOC content of the samples in both, S- and SW-sequences (Fig. 4). "

and a native English lector corrected the manuscript.

Some detailed suggestions are as follows:

line 29 delete "of"

We deleted it

lines 34-35 correct "Highest part of TOC is stored in clay fraction" for "Highest part of TOC is stored in clay sized microaggregates"

We corrected it

lines 130-131 Lack of information on land use change of abandoned vineyards chronosequences. Are they left to overgrow with bushes and trees?

We completed the paragraph with this information: lines 132-134

'Depending on the succession grade and time left since abandonment the sampling sites were overgrown by grasslands, shrubs or secondary forest vegetation. '

lines 143-144 Undisturbed soil samples in metal cylinders were collected in order to calculate carbon stocks. What it was done for? No data presented in the paper.

We deleted this unnecessary information, no data if it was evaluated in the manuscript.

line 201 correct "C in in i Fraction" for "C in i fraction"

We corrected it

line 206 please provide more details of the procedure. Delete double dots after bracket

Description of the method was completed with details, and double dots were deleted.

lines 269-273 What is your meaning of particulate organic matter (POM)? How is that possible that POM (particles less than 2 mm and greater than 0.053 mm) are present in the clay fraction?

That is a mistake in the text, we corrected the sentence:

In average 82.1% of ~~particulate OM~~ OC was found in clay and light (OPOM and FPOM) fractions

lines 372-374 Which fraction indicated the fastest increase in carbon after abandonment?

Fastest increase is in the clay sized microaggregates as it is shown in Fig. 4

Table 2. Microbial carbon is signed "Cm" in the text, but "Cmicr" in the table.

We corrected it in the Table 2

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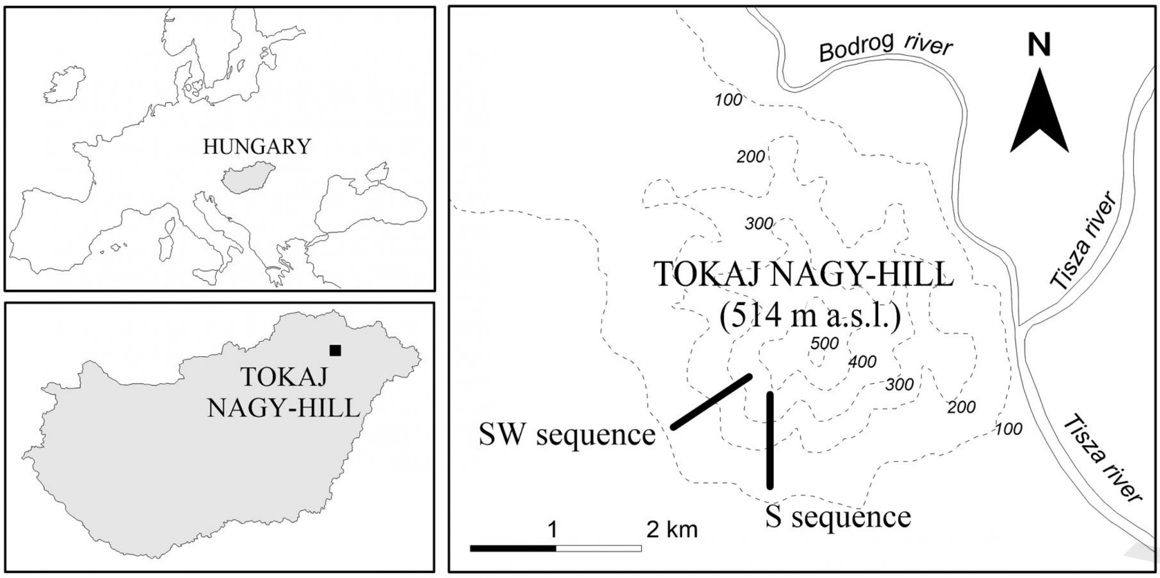

Fig. 1. Location of study area

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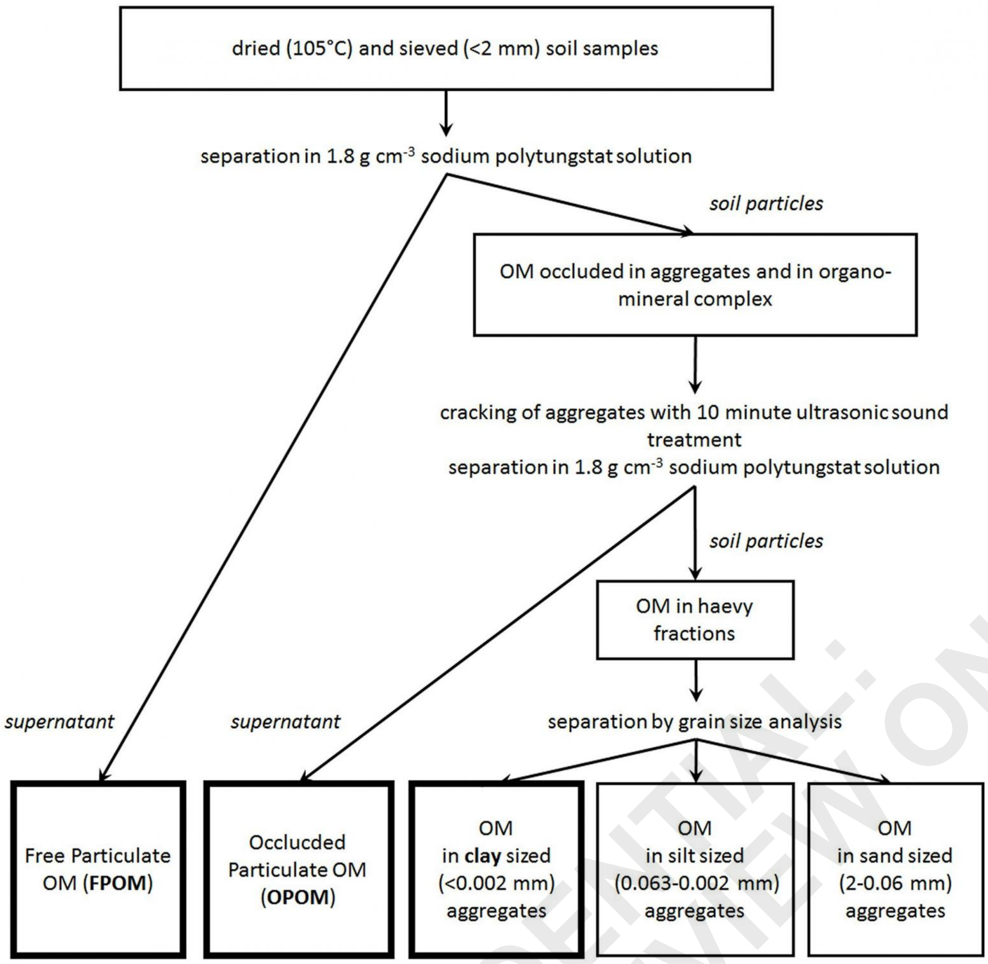

Fig. 2. Processing of separation of OM fractions

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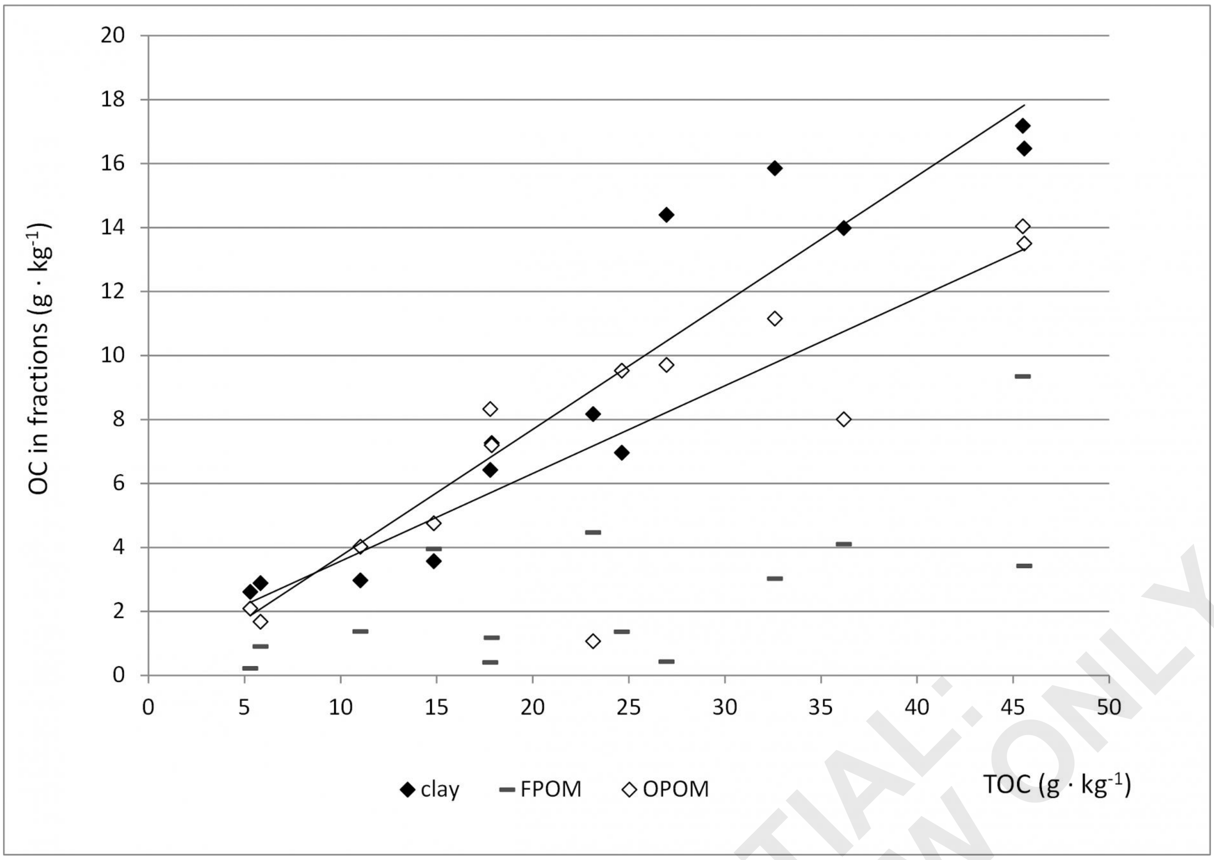

Fig. 3. OC in fractions (clay, OPOM, FPOM) plotted against the TOC content of samples

Figure 4

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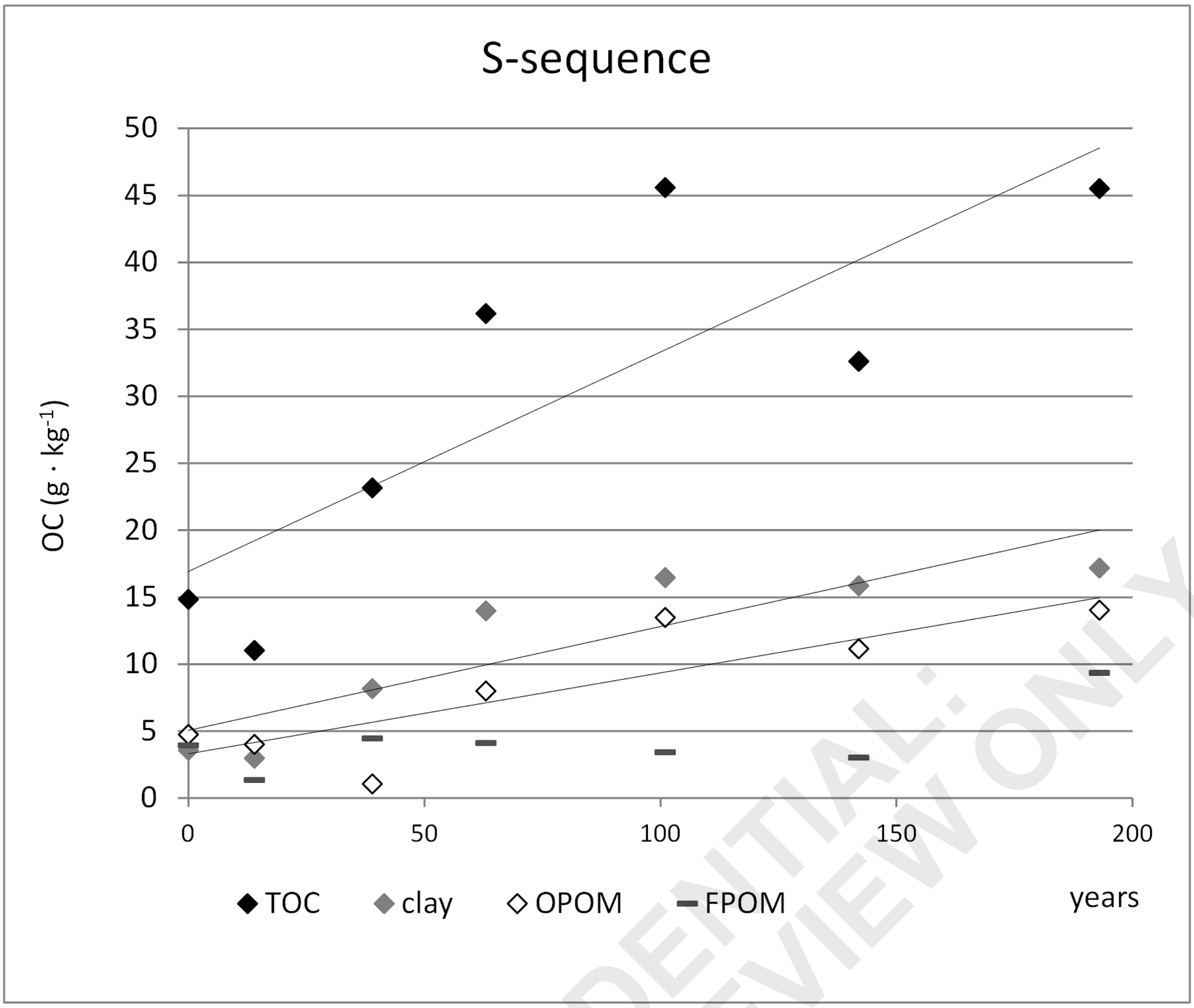

Fig. 4a. Changes of OC content in fractions (clay, OPOM, FPOM) after abandonment in S-sequence

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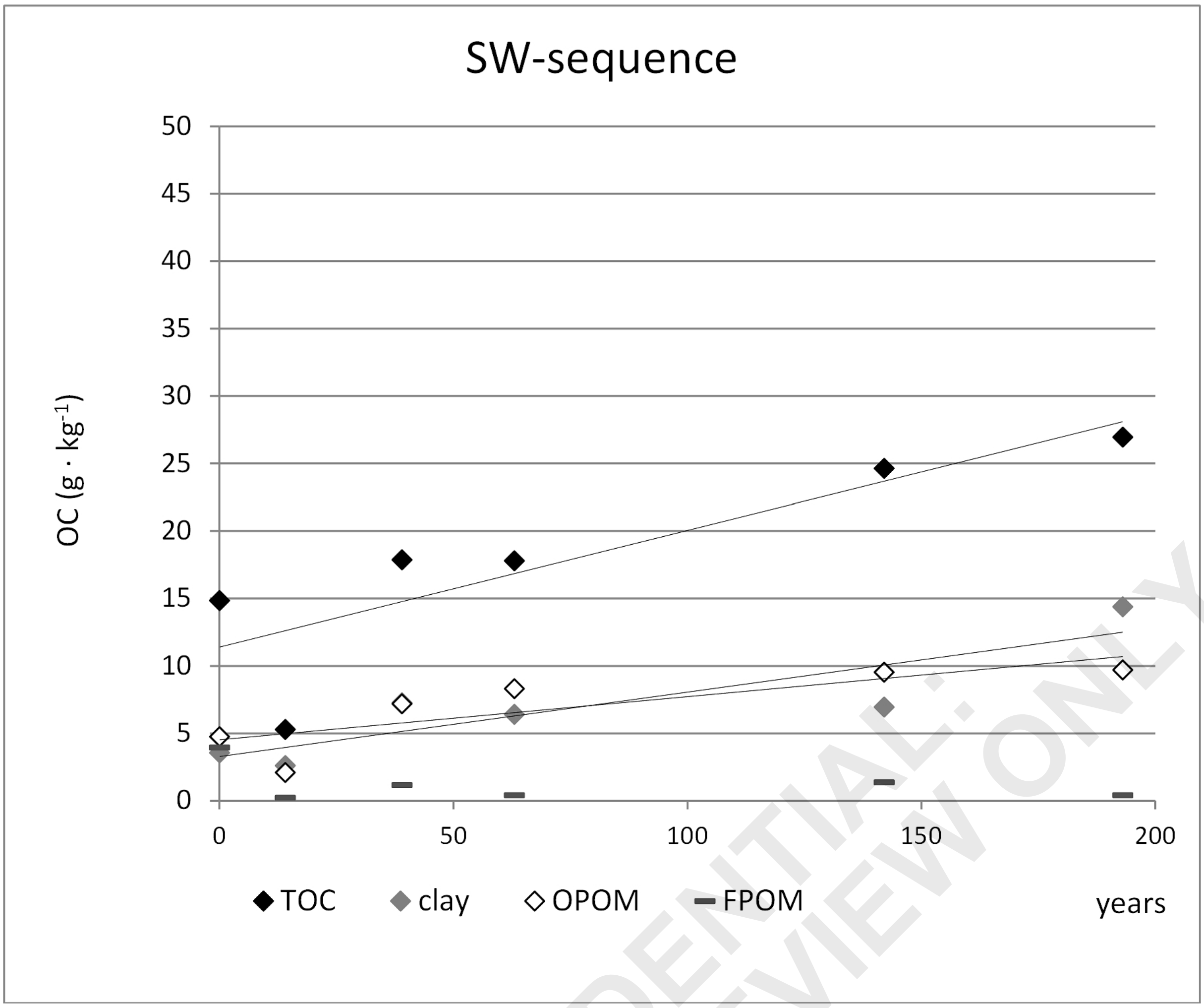

Fig. 4b. Changes of OC content in fractions (clay, OPOM, FPOM) after abandonment in SW-sequence

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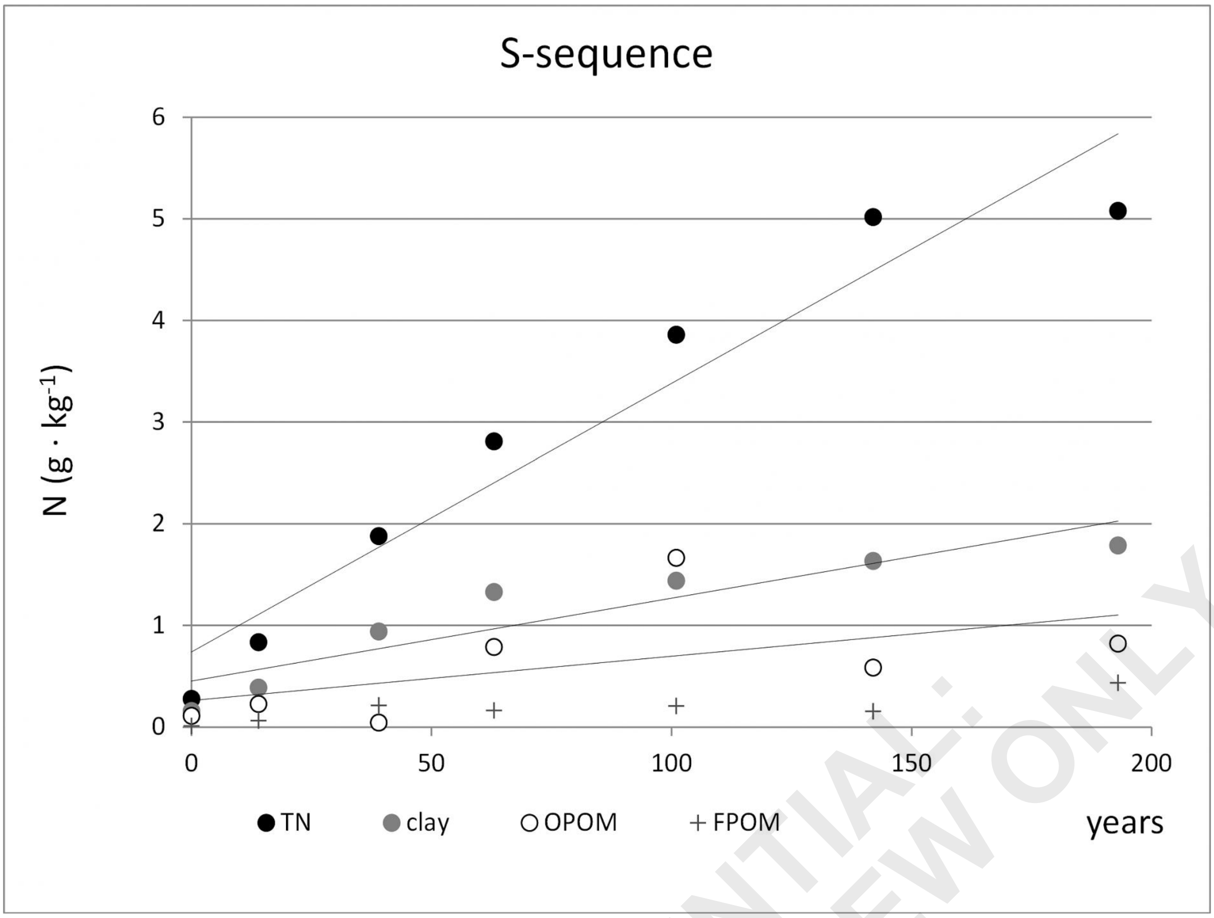

Fig. 5a. Changes of total N content in fractions (clay, OPOM, FPOM) after abandonment in S-sequence

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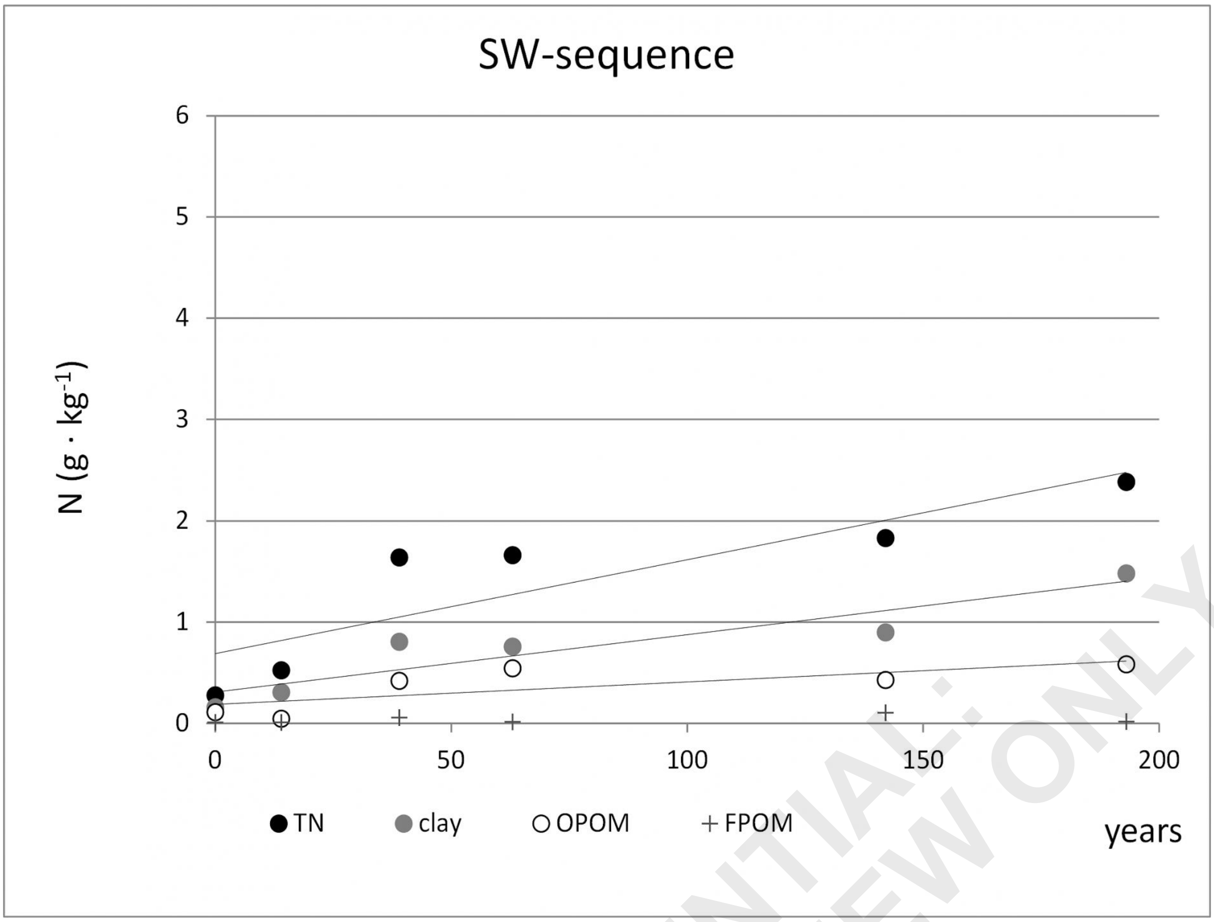

Fig. 5b. Changes of total N content in fractions (clay, OPOM, FPOM) after abandonment in SW-sequence

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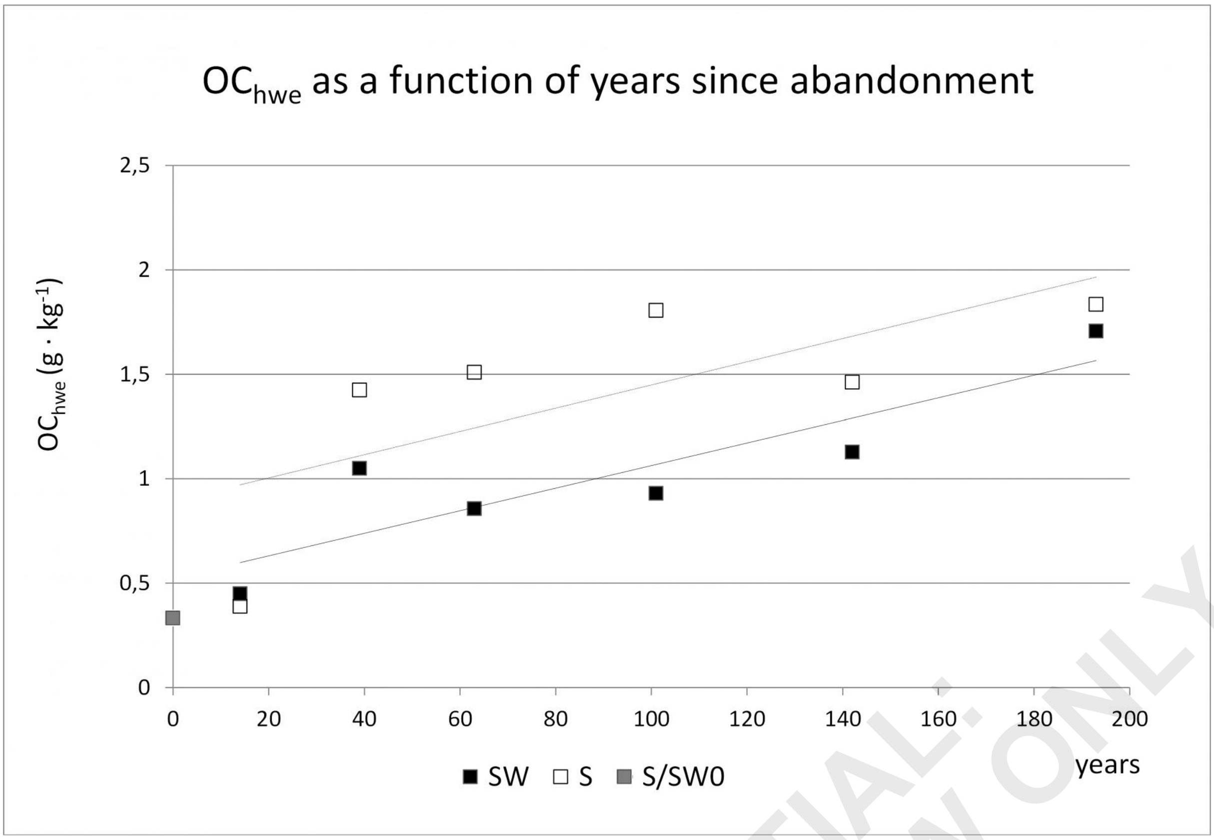

Fig. 6. OChwe fraction and their relation to the time in the two chronosequences

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