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# Mechanisms of carbon sequestration and stabilization by restoration of arable soils after abandonment: A chronosequence study on Phaeozems and Chernozems



**GEODERM** 

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#### ABSTRACT

Abandonment of croplands ongoing on 220 million ha worldwide contributes strongly to soil restoration by improvement of degraded properties and medium- and long-term carbon (C) sequestration in post-agricultural ecosystems. Two interrelated processes - decomposition and stabilization of soil organic carbon (SOC) - govern SOC dynamics and affect the C source or sink functions of former croplands. We investigated how the abandonment of arable soils affects (i) accumulation of SOC, its composition, stability, and turnover during the postagricultural restoration of soils, and (ii) microbial activity parameters. A chronosequence study was carried in two bioclimatic zones of European Russia: deciduous forest (Luvic Phaeozems, PH-chronosequence) and dry steppe (Calcic Chernozems, CH-chronosequence). Each chronosequence included an arable soil, 3-4 soils abandoned at increasing time periods (up to 35 years), and natural soil: never cropped Phaeozem and completely restored Chernozem. We combined the results of nuclear magnetic resonance (NMR), thermal analysis including Differential Scanning Calorimetry and Derivative Thermogravimetry, long-term incubation for SOC mineralization, and microbiological activity (basal respiration and microbial C content). Degraded Phaeozems with low SOC amount had much higher relative increase in SOC content (134%) during the post-agricultural restoration compared to SOC-rich Chernozems (38%). SOC gains were recorded in all organic compound classes identified by NMR and thermal analysis, but the increase of recalcitrant SOC was more pronounced in the postagricultural Chernozems than in the Phaeozems. The post-agricultural Chernozems were characterized by higher SOC aliphaticity and aromaticity than Phaeozems. Microbial activity and biodegradable SOC increased gradually during post-agricultural restoration. Being mostly a function of climate and vegetation, the soil type was the primary factor explaining the greatest portion (54-88%) of the total variance for most soil and microbial parameters. Concluding, despite SOC content increased in both Chernozems and Phaeozems during the postagricultural restoration, the mechanisms of C sequestration and stabilization were dependent on climate, vegetation, and on the degradation intensity during the agricultural use. The accumulation of organic compounds was specific for virgin soils dominating in deciduous forest and steppes, and had direct consequences for microbial activities, C turnover and sequestration.

#### 1. Introduction

Agricultural lands abandonment is a common Phenomenon occuring in many regions of the world. (Beilin et al., 2014; Ramankutty et al., 2006; Rey Benayas et al., 2007). The area of abandoned lands currently comprises about 220 million ha worldwide and ca. 1/4 of this area is located in Russia (FAO, 2010; Lyuri et al., 2010; ROSSTAT, 2017). Long-term agricultural use strongly decreases soil SOC content in the topsoil in comparison with permanent grassland or forest (Del Galdo et al., 2003; Poeplau et al., 2011; Soussana et al., 2004). The

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cessation of cropland cultivation leads to the natural vegetation recovery, restoration of agricultural soils, and substantial SOC sequestration (Kalinina et al., 2015b; Kämpf et al., 2016; Kurganova and Lopes de Gerenyu, 2008; Kurganova et al., 2010a, 2014). The abandonment of croplands and succession of natural vegetation specific for the local climate lead to higher C input and, together with absence of plowing and reduced (or absent) erosion, replenish soil organic carbon (SOC) stocks, especially in the uppermost layers (Kurganova et al., 2008, 2010b; Laganiere et al., 2010; Poeplau et al., 2011). Hence, croplands abandonment contributes strongly to restoration of former croplands by improvement of whole range of physicochemical and biological properties (Kalinina et al., 2015b) and medium- and long-term carbon (C) sequestration in post-agricultural ecosystems (Baer et al., 2010; Kurganova et al., 2015; Post and Kwon, 2000). Therefore, in contrast to the degradation of soils common during agricultural use, abandonment leads to restoration of agricultural soils.

The pattern of C sequestration and mean C accumulation rate in soils after abandonment are governed by SOC stocks in arable soils prior to abandonment and by environmental conditions: vegetation productivity, litter quality, soil type, and climate conditions (Kalinina et al., 2015b; Laganiere et al., 2010; Vesterdal et al., 2002). Metaanalyses showed that the mean increase of SOC stocks after afforestation of croplands was 10-59% (Bárcena et al., 2014; Guo and Gifford, 2002; Kämpf et al., 2016; Nave et al., 2013; Shi et al., 2013), and 18-19% after conversion of croplands to pasture or grasslands (Guo and Gifford, 2002; Kämpf et al., 2016). In temperate climate, grassland and forest establishment caused a long-lasting C sink with a relative SOC stock increase of 128  $\pm$  23% and 116  $\pm$  54%, respectively, after 120 years (Poeplau et al., 2011). Although the mineral topsoil is more rapidly affected, new C can be incorporated in deeper horizons through bioturbation and deep root growth (Del Galdo et al., 2003; Don et al., 2009; Guo and Gifford, 2002). This new soil C (SOC derived from new vegetation after land use change. LUC) has higher availability to decomposers and leading to faster biochemical reaction and intensive SOC dynamics. Therefore, abandoned lands are an excellent model to investigate restoration processes, including C sequestration and new C incorporation during the post-agricultural development of former croplands.

Two interrelated processes - SOC decomposition and stabilization contribute the most to the SOC dynamics and affect the C source or sink functions after cropland abandonment (Lal, 2004; Lutzow et al., 2006; Novara et al., 2013a). The stability of new soil organic matter is a key element in C sequestration in soils after LUC (Lal, 2008; Morris et al., 2010; Schlesinger, 1999). The LUC affects the mechanisms of C stabilization and sequestration in soils by shifts in microbial community composition and functioning (Gunina et al., 2017), by macroaggregate turnover and microaggregate formation (Six et al., 2002, 2004), interactions of SOC and aggregate dynamics (Bárcena et al., 2014; Gunina and Kuzyakov, 2014; Semenov et al., 2010). In addition, changes in vegetation caused by LUC can also modify the amount and chemical composition of the litter inputs on and into the soil (Montané et al., 2010; Pérez-Cruzado et al., 2012, 2014). The higher proportion of alkyl C components in the litter derived from woody plants leads to changes in SOC composition and properties. Chemically recalcitrant compounds can accumulate in mineral soils after an input of woody plant litter (DeMarco et al., 2016; Dümig et al., 2009). Nonetheless, it remains unclear how these changes in SOC composition affect SOC dynamics and stabilization.

These variables affecting C input, stabilization and dynamics in soils depend strongly on the climate and dominating vegetation. The C inputs in grasslands differ from those in forests by: 1) localization of the C input: aboveground litter dominates in forests, whereas grasses have mainly belowground input by roots and rhizodeposition; 2) quality of the C input: the forest litter above- and belowground have higher lignin and lipid contents and lower N content compared to grasses; 3) the roots of most tree species are associated with ectomycorrhizal fungi

(ECM), while grasses are associated with arbuscular mycorrhizal fungi (AMF). Both mycorrhizal types have distinct traits in decomposing organic matter and nutrient acquisition (Brzostek et al., 2015; Phillips et al., 2013; Smith and Read, 2009). ECM fungi have slower turnover because of the higher C/N ratio and larger fungi/bacteria ratio in the mycorrhizosphere.

Moreover, SOC dynamics, i.e. transformation, depends not only on substrate quality and amount, but also on the activity of decomposers and interactions among many microbial groups (Wutzler and Reichstein, 2013). The biochemical quality of SOC affects the activity and composition of microbial communities because various microbes utilize different organic constituents (Six et al., 2004, 2006). The SOC decomposition rate depends on several factors such as substrate quality, N availability, and physical protection by clays, which have been invoked as additional controls over microbial processing of SOC (Ryan and Law, 2005). The microbial decomposition products can be stabilized by means of organo-mineral interactions (Cotrufo et al., 2013; Schmidt et al., 2011).

Two groups of approaches are usually used to characterize SOC composition and stability: 1) methods of physicochemical fractionation based on SOC properties related to the potential degradation by microbial decomposition - these are indirect approaches, and 2) direct approaches based on microbial decomposition of SOC and on CO2 evolution rates (Fontaine et al., 2007; Lutzow et al., 2006). The first group focuses on the chemical composition of organics in soil and includes: an acid and alkali hydrolysis, physical fractionations (e.g. density and particle size; (Kalinina et al., 2010, 2011, 2013), the other modern instrumental methods, e.g. isotopic (Deng et al., 2014; Novara et al., 2013b, 2014) or nuclear magnetic resonance (NMR) (Campo and Merino, 2016; Merino et al., 2014), thermal analysis (Kučerík et al., 2016, 2018; Siewert, 2004; Siewert et al., 2012), and Fourier-transform spectroscopy (Ludwig et al., 2008). The second group is based on biokinetic techniques (Rovira et al., 2010; Semenov et al., 2006, 2008) and on determining the ecophysiological parameters of the microbial community (Ananyeva et al., 2009; Anderson, 1994, 2003; Susyan et al., 2011). Soil microbial properties such as basal respiration (R<sub>basal</sub>), microbial biomass carbon (C<sub>mic</sub>), metabolic quotient (qCO<sub>2</sub>), and R<sub>basal</sub>/ SOC and Cmic/SOC ratios are the early and powerful indicators of changes in the quantity and quality of SOC following the LUC or other environmental changes (Bending et al., 2002; Jinbo et al., 2007; Kurganova et al., 2012, 2018). The relationship between SOC stability and microbial activity of soil is still unknown, especially considering the broad range of pools and sources contributing variously to the CO<sub>2</sub> fluxes from soil.

This study was designed to investigate how the abandonment of arable soils alters SOC composition and stability. Specifically, we quantified the interlinking shifts in microbial activity and various functional SOC pools (biodegradable, labile, recalcitrant) during the natural post-agricultural restoration of arable Phaeozems and Chernozems located in forest and steppe regions, respectively. These two soil groups combined contribute > 50% to the total agricultural area in Russia and 18–44% to soils abandoned after 1990 (Kurganova et al., 2010a, 2014). To assess the changes in various C pools and their turnover in soils after conversion from cropland to natural vegetation, and to assess the microbial contribution to processes of C sequestration, we conducted NMR and thermal analyses along with long-term incubations to examine SOC mineralization.

We hypothesize that (1) microbial activity and the content of biodegradable SOC will increase considerably during post-agricultural restoration from arable soil to abandoned lands under natural vegetation, and (2) the recalcitrant pools of SOC will predominate in Chernozems, which are Ca-rich soils, whereas Phaeozems will accumulate more active C pools – microbial C and biodegradable C. This study combined for the first time the bio-kinetic and physicochemical fractionations to link SOC decomposition and sequestration during post-agricultural restoration of former arable soils. Understanding of the mechanisms,

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	Sand:silt:clay	1.0:2.1:1.2 (Joamy clav)	1.0:3.1:1.6 (loamy clay)
	Soil type	Luvic Phaeozem	Calcic Chernozem
	AGP/BGP <sup>f</sup> , Mg ha <sup>-1</sup> yr <sup>-1</sup>	9.64/1.43	2.44/11.56
	NPP <sup>e</sup> , Mg ha <sup><math>-1</math></sup> yr <sup><math>-1</math></sup>	11.07	14.00
	Natural vegetation	Broad-leaved forest (Populus tremula L., Tilia cordata Mill., Acer Datamoides L. Ouvrus rubur I. herbaceous cover)	promotione in generation of the production of the feature valesiaca Grass steppe (Elytrigia repeat L., Poa angustifolia L., Festuca valesiaca Schleich: ex Gaudin, Stipa capillata L.)
	HTC <sup>d</sup> (summer)	1.44	0.74
0115.	AP <sup>c</sup> , mm	652	592
egeranon and st	T <sub>Jan</sub> /T <sub>Jul</sub> <sup>b</sup> , °C	-7.5/18.8	- 3.3/23.8
climate, v	MAT <sup>a</sup> , °C	5.4	10.0
Characteristics of	Vegetation zone	Deciduous forest	Dry steppe

Climate data were extracted from (Veselov and Pribylskaya, n.d.: http://aisori.meteo.ru/ClimateR) and present the mean values for 3 decades (1986-2015).

 $T_{Jan}$  and  $T_{Jul}$  are the mean air temperature in January (the coldest month) and July (the hottest month). MAT is mean annual air temperature.

precipitation. AP is annual

HTC is a Selyaninov hydrothermal coefficient for the summer season (Kurganova et al., 2011); vegetation parameters were adapted from (Bazilevich, 1993)

NPP is Net Primary Production. AGP and BGP are the aboveground and belowground production, respectively.

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through which organic C is stabilized in soils during post-agricultural development, and of interactions between physicochemical and biological processes is necessary to increase C sequestration and thus to mitigate climate change.

# 2. Materials and methods

# 2.1. Study area characteristics

A chronosequence study was carried in two contrasting bioclimatic zones of European Russia: deciduous forest (Moscow region, 54°49'N; 37°34′E) and dry steppe (Rostov region, 47°27′N, 39°35′E). Both regions have the temperate continental climate. The difference in annual precipitation between Moscow and Rostov comprises 160 mm (Table 1). However, mean annual temperature in the Rostov region is 4.6 °C higher than in the Moscow region, which is the main reason for the strong aridity and water limitations. The hydrothermal coefficient (a ratio of the total precipitation over the summer (mm) to one tenth of the sum of the mean daily air temperatures over the same period) in the former is 2 times lower than in the latter: 0.74 vs 1.44. The study regions also have contrasting soil types (Luvic Phaeozem, PH and Calcic Chernozem, CH, respectively) and vegetation (Table 1). Although the total productivity of the natural vegetation in both regions is similar, the ratio between above- and belowground biomass is much higher in the forest vegetation than in the steppe coenosis (Table 1). Cover loam is parent material in forest cronosequence and silty loamy loess-like carbonate sediments are distributed in the steppe study area.

# 2.2. Study design and soil sampling

In each bioclimate region, a chronosequence included the current arable (0-moment) and the post-agricultural soils, which were abandoned (removed from agricultural use) at different times in the past: 6. 15, and 30 years for Luvic Phaeozems (PH-chronosequence) and 5, 11, 21, and 77 years for Calcic Chernozems (CH-chronosequence). The arable fields in both regions were sown by cereals and were cropped without fertilization for the last decades. (Table 1S, Supplementary data). The arable and abandoned sites were under cultivation for at least 150 years. The abandoned fields in Moscow region are the former experimental fields of Experimental Field Station (Institute of Physicochemical and Biological Problems in Soil Science of the Russian Academy of Sciences). Before abandonment, these fields were under cereal crops - fallow rotation. Previously, the 30-year site was regularly mowed; therefore, the wood vegetation is absent. Now, all sites are neither fertilized nor mowed. The abandoned fields in the Rostov region were the former experimental fields of the Agrobiological Station (South Federal University). These fields were under cereal crops before abandonment. Presently, they are neither fertilized nor mowed as well. Therefore, the land use history and management were the identical for abandoned fields in both regions and the studied sites differ only in their age since abandonment which affected the stage of vegetation succession. Secondary broad-leaved forest and the 77-year-old grass steppe represent the final stages of the post-agricultural succession for Luvic Phaeozems and Calcic Chernozems, respectively. The natural forest field has never been previously cultivated, but trees were completely cut by local residents during the World War II (about 75 years ago). Hence, the soil was not disturbed here. In steppe region, the abandoned land of 77 years old is the natural restored steppe ecosystem and soil. Before abandonment, this field was under cereal crops (including maize) without fertilizer application for a long period similar to other fields in this chronosequence. It's considered that 50-60 years is quite enough to reach a full recovery of vegetation and all soil properties in Chernozems after cessation of agricultural use (Lyuri et al., 2010). In both regions, all fields were located at similar geomorphological positions within a maximal distance of no > 500-700 m apart. The differences of the main soil properties (texture, mineralogy, pH,

Soil type, land use	Time after LUC, yrs	Symbol	pH <sub>H20</sub>	$SOC, g C kg^{-1}$	N, g N kg <sup><math>-1</math></sup>	C/N	$ m R_{basal}$ mg C kg <sup>-1</sup> day <sup>-1</sup>	SpR <sub>basal</sub> , mg C kg <sup><math>-1</math></sup> SOC day <sup><math>-1</math></sup>	$C_{mic}$ , mg C kg <sup>-1</sup>
Phaeozem chronosequ	lence								
Arable	0	PH-1	$6.96 (0.04)^{a}$	$11.6(0.1)^{d}$	$1.07 (0.02)^{d}$	$10.9 (0.05)^{\rm b}$	$6.5(0.3)^{d}$	0.56 (0.02) <sup>d</sup>	363 (9) <sup>c</sup>
Grassland	9	PH-2	$6.26(0.02)^{\rm b}$	$12.0 (0.0)^{d}$	$1.13 (0.03)^{d}$	$10.6 (0.08)^{\rm b}$	$11.8 (0.5)^{c}$	0.98 (0.04) <sup>c</sup>	555 (42) <sup>c</sup>
Grassland	15	PH-3	$5.75 (0.04)^{c}$	$14.9 (0.4)^{c}$	$1.40 (0.05)^{c}$	$10.7 (0.05)^{\rm b}$	$24.3 (0.5)^{\rm b}$	$1.62 (0.04)^{a}$	$801 (82)^{\rm b}$
Grassland	30	PH-4	$5.87 (0.02)^{c}$	$19.7 (0.2)^{\rm b}$	$1.91 (0.03)^{b}$	$10.3 (0.06)^{c}$	$23.6(0.9)^{b}$	$1.20(0.05)^{b}$	773 (18) <sup>b</sup>
Forest	60	PH-5	5.96 (0.03) <sup>c</sup>	$27.2 (0.5)^{a}$	$2.28 (0.04)^{a}$	$11.9 (0.04)^{a}$	$42.6(2.0)^{a}$	$1.56 (0.07)^{a}$	$1008 (65)^{a}$
Chernozem chronosed	uence								
Arable	0	CH-1	$7.70 (0.03)^{bc}$	$19.7 (0.2)^{bc}$	$1.63 (0.05)^{cd}$	$12.1 (0.12)^{\rm b}$	$5.5 (0.1)^{c}$	0.28 (0.01) <sup>d</sup>	pu
Grassland	ŋ	CH-2	$8.11 (0.02)^{a}$	$18.0 (0.2)^{c}$	$1.56 (0.03)^{d}$	$11.5 (0.17)^{a}$	6.5 (0.3) °	$0.36 (0.02)^{c}$	pu
Grassland	11	CH-3	$8.11 (0.01)^{a}$	$21.9 (0.6)^{b}$	$2.03 (0.02)^{bc}$	$10.8 (0.22)^{ab}$	8.9 (0.3) <sup>b</sup>	$0.40 (0.02)^{c}$	pu
Grassland	21	CH-4	$7.89~(0.01)^{\rm b}$	$22.5(0.5)^{\rm b}$	$2.14 (0.03)^{b}$	$10.5(0.07)^{\rm b}$	$13.4 (0.3)^{a}$	$0.59 (0.01)^{a}$	pu
Grassland	77	CH-5	$7.93(0.02)^{ m b}$	$27.1 (1.5)^{a}$	$2.62 (0.11)^{a}$	$10.3 (0.21)^{\rm b}$	$14.0 (0.3)^{a}$	$0.52~(0.01)^{\rm b}$	pu

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Table 2

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etc.) within each chronosequence were negligible (Kurganova et al., 2008; Lopes de Gerenyu et al., 2008).

On each plot, soil samples were collected in five points bulked within a square  $(50 \times 50 \text{ m})$  from the uppermost mineral soil layer (0–10 cm). It is considered that the upper soil layer (0–10 cm) is characterized by the most active microbial processes and SOC accumulation due to land use changes (Kalinina et al., 2009, 2011, 2018; Susyan et al., 2011; Poeplau and Don, 2013; Kurganova et al., 2018). Soil samples with natural moisture content were mixed carefully, dried at room temperature and sieved (2 mm). Fine plant root and organic residues were removed manually from the sieved soil samples. Prior to microbial analyses, the soil samples were stored in a cooler at 2–4 °C in aerated plastic bags no longer than 2-3 months. The litter layer was excluded from analysis. All laboratory analyses (except thermal and <sup>13</sup>C NMR analysis) were performed in triplicate.

## 2.3. Soil and microbiological analyses

The soil particle size distribution was determined by the pipette method (Pansu and Gautheyrou, 2006) after pretreatment with sodium pyrophosphate solution. Soil pH was measured in water extraction at a soil:solution ratio of 1:2.5. Contents of SOC and total nitrogen (N) in dry soil pellets were determined by dry combustion with an automated LECO CHNS-932 Analyzer (LECO Corporation, St. Joseph, Michigan, USA).

Basal respiration ( $R_{basal}$ ) was determined based on the CO<sub>2</sub> evolution rate from soil incubated at a moisture content of 65-70% of water holding capacity (WHC) at 22 °C. Prior to respiration analyses, the soil samples (10 g) were placed into 100-ml flasks, moistened to 70% of their WHC, and covered with films permeable for air but preventing moisture evaporation. After the preliminary incubation at 22 °C for 7 days to wait till the response of microbial community to watering (Marti et al., 2012; Susyan et al., 2011), the flasks were hermetically sealed with rubber plugs and kept in a thermostat under the same temperature for 10-12 h. Then, headspace gas samples were taken from the flasks with a syringe, and the CO2 concentrations were determined using a gas chromatograph Kristall 2000 (Russia) equipped with a thermal conductivity detector. The R<sub>basal</sub> (mg C/kg of soil/day) was estimated according to the following equation (Kurganova et al., 2012): ... ~ //

$$R_{basal} = (dC \times 12 \times V_{flask} \times 1000) / (m \times 22.4 \ t \times 100), \tag{1}$$

where dC is the CO<sub>2</sub> concentration in the flask accounting for the zero value, volumetric %;  $V_{flask}$  is the flask volume, ml; t is the incubation time, days; and *m* is the soil weight, kg.

The ratio between R<sub>basal</sub> and SOC content is the specific R<sub>basal</sub> (SpR<sub>basal</sub>) that serves as a proxy for the SOC resistance to decomposition and reflects, to some extent, the degree of the organic matter's stability (Charro et al., 2010; Kurganova et al., 2012). Lower SpRbasal values indicate that less SOC is subjected to mineralization and is thus more stable and vice versa.

Microbial biomass carbon (Cmic) was determined in the soils of the Phaeozem chronosequence by the substrate-induced respiration (SIR) method (Anderson and Domsch, 1978). The SIR was measured based on the rate of initial maximal respiration of microorganisms after amendment of soil samples with glucose (Ananyeva et al., 2009). To determine the C<sub>mic</sub>, flasks with the soils were ventilated after the R<sub>basal</sub> measurement and amended with a glucose solution at a rate of 10 mg glucose per 1 g of soil. Two hours after the glucose amendment, the flasks were ventilated again, sealed hermetically, and incubated for 1.5-2.0 h at 22 °C; then, the CO2 concentrations in the flasks were measured again. The rate of the substrate induced respiration  $(R_{SIR})$ , reflecting the response of the soil microbial community to the added glucose, was calculated according to formula (1) and then expressed in µg of C per g of soil per h. Soil microbial biomass carbon (Cmic) was derived by the  $R_{SIR}$  measurement according to the equation (Anderson and Domsch, 1978):

$$C_{\rm mic} = 40.04 \times R_{\rm SIR} + 0.37,$$
 (2)

where  $C_{mic}$  is microbial biomass carbon (µg C 100 g soil<sup>-1</sup>), and  $R_{SIR}$  is the rate of the substrate induced respiration (µg C g soil<sup>-1</sup> h<sup>-1</sup>).

The  $C_{mic}$  content in Chernozems could not be determined by SIR since those soils are characterized by slightly alkaline pH values (Table 2).

#### 2.4. Bio-kinetic fractionation of SOC

We use the bio-kinetic method to estimate the labile (biodegradable) and recalcitrant C pools in soils after a 12-month incubation at constant temperature and moisture (Lopes de Gerenvu et al., 2008; Semenov et al., 2010). The dry root-free soil samples (10 g) were placed into 100-ml flasks, adjusted to 70% of their WHC and pre-incubated at 22 °C for 1 week. Then soil samples were wetted with distilled water to moisture corresponding to 80-85% of WHC and incubated at the same temperature and moisture over the next 12 months. During the experiment, the permanent water content (or stable weight of vials with soils) was controlled gravimetrically every 2 weeks. The CO2 concentration in the headspace was measured daily during the first 4 days, every 2-3 days over the first month, and every week through the next 2-12 months of incubation. Before each CO<sub>2</sub> concentration measurement, the flasks were ventilated, hermetically sealed with rubber plugs, and kept in a thermostat at 22 °C for 10-12 h. Then, headspace gas samples were taken from the flasks with a syringe, and the CO<sub>2</sub> concentrations were determined using a gas chromatograph Kristall 2000 (Russia) equipped with a thermal conductivity detector.

A first order two-component model (Kätterer et al., 1998) was used to analyze cumulative  $CO_2$  losses for the 12-month period (C-loss, mg C/g of soil):

$$C\text{-loss} = \alpha \cdot C_0 \cdot (1 - e^{(-k1 \cdot T)}) + (1 - \alpha) \cdot C_0 \cdot (1 - e^{(-k2 \cdot T)}), \quad 0 \le \alpha \le 1$$
(3)

where  $C_o$  is the initial amount of total C in the soil ( $\mu g C g$  soil),  $\alpha \cdot C_o$  and  $(1-\alpha) \cdot C_o$  are the initial amounts of C in the labile and recalcitrant pools, respectively ( $\mu g C g \operatorname{soil}^{-1}$ ), k1 and k2 are the corresponding mineralization rate constants for each C-pool, and T is the time (days).

This model describes the decomposition of two separate pools with a different rate of degradation in the total SOC: the labile (biodegradable) pool ( $C_{bio}$ ) with a high degradation rate (k1) and the recalcitrant C pool ( $C_{rec}$ ) with a low decomposition rate (k2). These coefficients (k1 and k2) were used to estimate the mean residence time (MRT) for  $C_{bio}$  and  $C_{rec}$ , respectively.

#### 2.5. SOC characterization by NMR

Solid state <sup>13</sup>C-CP-MAS NMR was carried out for one replicate from 3 samples of Phaeozems (PH-1; PH-3; PH-4; see Table 2) and 3 samples of Chernozems (CH-1, CH-3; CH-5; see Table 2). Mineral soil samples were demineralized five times with 10% (w-to-w) hydrofluoric acid (HF) for 2 h. The <sup>13</sup>C-CP-MAS NMR analyses were done in an Agilent Varian VNMRS-500-WB spectrometer at a proton resonance frequency of 500 MHz and using a zirconia rotor of 160 µL. The NMR spectra were processed with MestreNova software 8.1.0 (Mestrelab Research Inc., Santiago de Compostela, Spain) to quantify the area under the shift signals. For integration, the spectra were divided into four regions representing different chemical environments of a <sup>13</sup>C nucleus: alkyl C (0-45 ppm), O-alkyl C (45-110 ppm), aromatic C (110-160 ppm) and carbonyl C (160-210 ppm). The percent contribution of each of those C groups to total C was determined. The SOC aromaticity values were calculated as a ratio aromatic C / (aromatic C + alkyl C + O - alkyl C). The SOC aliphaticity was estimated as the alkyl C to O-alkyl C ratio.

#### 2.6. SOC thermal analysis

All soil samples were analyzed for one replicate by differential scanning calorimetry (DSC) and differential thermogravimetry (DTG), with a simultaneous DSC-TG (Mettler Toledo Intl. Inc.). Thermal analyses were performed by placing soil aliquots in open aluminum pans for a temperature scan from 50 to 600 °C at 10 °C min<sup>-1</sup> under a dry air atmosphere at a flow rate of 50 mL min<sup>-1</sup> with a scanning rate of  $10 \degree C \min^{-1}$ . The heat of combustion (Q, J per g) and the weight loss (W, %) were determined by integrating the DSC over the exothermic region (150–600 °C) and from the DTG curves (first derivative of the TG traces), respectively. Samples of Indium (melting point 156.6 °C) were used to calibrate the DSC-TG device. Data recorded at < 150 °C were disregarded to exclude weight losses and energy changes associated with soil water content. Baseline correction and determination of the thermal indices were done using the STARE software (Mettler-Toledo). The areas under the DSC were divided into three groups representing the degrees of resistance to thermal oxidation (Merino et al., 2014): (i) labile organic matter, mainly carbohydrates and other aliphatic compounds (200-375 °C); (ii) recalcitrant organic matter, such as lignin or other polyphenols (375-475 °C); and (iii) highly recalcitrant (refractory) organic matter, such as polycondensed aromatic forms (475-550 °C). The resulting partial heats of combustion were designated as Q1, Q2 and Q3 (for DSC). The DTG defines SOC mass fractions with differing resistance to oxidation: W1, W2 and W3, which were determined from the temperatures at the maxima of the different DTG peaks for labile (W1), recalcitrant (W2), and highly recalcitrant organic matter (W3). The temperatures at which 50% of the energy is released  $(T50_{O})$  and 50% of SOC mass is lost  $(T50_{W})$  under the given conditions were also determined.

## 2.7. Statistical analyses

Standard and microbiological analyses were based on 3–5 replicates. The arithmetic means and standard errors for selected soil data (SOC, N, the C/N ratio,  $R_{basal}$ ,  $C_{mic}$ , C-loss,  $C_{bio}$ ,  $C_{rec}$ , k1, k2) were calculated and subjected to one-way ANOVA, testing the effect of the duration of abandonment separately for Phaeozems and Chernozems. A paired *t*-test was used to compare the mean MRT values for both C pools ( $C_{bio}$  and  $C_{rec}$ ) in Phaeozems and Chernozems.

A two-way ANOVA was used to compare the difference in response parameters using 'soil type' and 'land use' as categorial factors. A principal component analysis (PCA) was carried out and included the following variables: SOC, N, C/N ratio,  $V_{basal}$ ,  $C_{bio}$ ,  $C_{rec}$ , C-loss, k1, k2. The results were summarized using factor coordinates of the variables, based on correlations, and scatter diagrams.

The relationships between various parameters were explored with the Pearson correlation (F-test). The determination coefficient,  $R^2$  and p-levels indicated the goodness-of-fit between parameters in the regression models. Average values and standard errors (SE) are presented in the tables and figures. All statistical analyses were performed with STATISTICA 6 (StatSoft Inc., Tulsa, OK, USA) using the  $\alpha = 0.05$  level of significance.

#### 3. Results

#### 3.1. Dynamics of soil properties during post-agricultural restoration

Both soil types had a very similar texture with a predominance of silt and clay particles (Table 1), whereas the pH values differed considerably: slight-acid and neutral for Luvic Phaeozems and slight-al-kaline for Haplic Chernozems (Table 2). The pH values gradually became more acidic in the PH-chronosequence, whereas they remained nearly constant during the post-agricultural restoration in the CH-chronosequence.

The SOC and total N contents increased gradually with time after

#### Table 3

Relative intensities of signals in the solid-state <sup>13</sup>C nuclear magnetic resonance spectra (%) of the organic matter in the soils of the two chronosequences (compare Fig. 1).

Site	Alkyl C (0–45 ppm)	O-alkyl C (45–110 ppm)	Aromatic C (110–160 ppm)	Carbonyl C (160–220 ppm)	Alkyl-C/O-alkyl C	Aromaticity	O-alkyl C/aromatic C
PH-chi	onosequence						
PH-1	19	53	19	9	0.35	0.21	2.79
PH-3	20	52	20	8	0.39	0.22	2.60
PH-4	21	50	20	9	0.41	0.22	2.50
CH-chi	ronosequence						
CH-1	23	42	24	10	0.55	0.27	1.75
CH-3	21	45	25	10	0.47	0.27	1.80
CH-5	22	44	22	13	0.50	0.25	2.00

cropland abandonment in both chronosequences. However, the SOC gain was higher in the post-agricultural Phaeozems: the relative differences in SOC between arable (initial stage) and natural soils (final stage) were 134% and 38% for PH and CH, respectively. The N content showed the same tendency during post-agricultural restoration (Table 2). The relative differences in N between cropland and the final stage of post-agricultural restoration were 113 and 61% for PH and CH, respectively. In the PH-chronosequence, the C/N ratio remained rather constant (10.9–11.9) during post-agricultural restoration, whereas it tended to decrease slightly (from 12.1 to 10.3) in the CH-chronosequence.

#### 3.2. SOC composition: solid state <sup>13</sup>C CP-MAS NMR

The O-alkyl C was the major functional group in the arable soil from both chronosequences, varying between 42 and 53% of total SOC (Table 3; Fig. 1). A signal at 73 ppm, attributed to cellulose and hemicelluloses, was found in both chronosequences (Fig. 1). The intense signal at 30 ppm, assigned to methylene C from lipids and aliphatic biopolymers such as cutin and suberin, was also recorded in all spectrums. A prominent peak at 128 ppm, attributed to lignin, was also evident in all spectra. The main difference between the soils of both chronosequences was the higher aromaticity and the higher aliphaticity (alkyl C to O-alkyl C ratio) in Chernozems versus Phaeozems. The C

# gains were observed in most of the organic compounds in both chronosequences. The aliphatic compounds in soils of the PH-chronosequence increased continuously after the abandonment. According to the NMR spectra, O-alkyl C and carbonyl C components built up in the CHchronosequence, whereas the alkyl C and alkyl C/O-alkyl C ratio increased in the PH-chronosequence.

#### 3.3. SOC thermal stability: DSC-DTG

The soils from the two locations had very different DSC curve shapes (Fig. 2). A single peak at 340 °C was documented in the all soils from PH-chronosequence. Two smaller shoulders (375 and 420 °C) were distinguished only in the arable and the recently abandoned (6-year-old) soils. The content of thermolabile SOC (W1) increased considerably (from 1.8 to 4.3%) in the PH-chronosequence (Fig. 3A) and its relative content made up 50% of the total SOC pool. Abandonment also led to progressive increase SOC losses in the temperature range between 375 and 475 °C (Fig. 3A), revealing that more stable SOC compounds such as lignin or other polyphenols contribute to the SOC gain. The T50w values gradually decreased from 373 °C in arable soil to 344 °C in the forest soil, reflecting this higher relative content of thermolabile compounds (Fig. 3A).

The soils from the CH-chronosequence showed three peaks, with maxima at 344, 426, and 504 °C. The temperature of the third peak



Fig. 1. Solid-state <sup>13</sup>C Nuclear Magnetic Resonance (<sup>13</sup>C NMR) spectra of the soil samples in the chronosequences of Phaeozems (PH) and Chernozems (CH) depending on abandonment duration. PH1 and CH1 – arable, PH3 and PH4 – Phaeozems abandoned 15 and 30 years ago; CH3 and CH5 – Chernozems abandoned 11 and 77 years ago, respectively.



Fig. 2. Differential Scanning Calorimetry (DSC) curves of the heat released by increasing temperature of mineral soils from the two chronosequences (A, B) and  $\Delta$ DSC between arable (PH1 or CH1) and post-agrogenic soils: Phaeozems (PH, left) and Chernozems (CH, right). PH1 and CH1 – arable, PH2, PH3, and PH4 – Phaeozems abandoned 6, 15 and 30 years ago, PH5 – natural Phaeozems; CH2, CH3, CH4, and CH5 – Chernozems abandoned 5,11, 21, and 77 years ago.

suggests the presence of refractory C, and this peak did not appear in the PH-chronosequence. Unlike the previous chronosequence, the CH abandonment led to an increase in all thermal fractions. The thermolabile and recalcitrant organic matter (W1, W2, and W3) increased proportionally in this chronosequence (Figs. 2 and 3). The content of labile SOC content (W1) increased during post-agricultural restoration from 2.6% to 3.7%, and its relative content made up around the 50% of the SOC (Fig. 3A), although it was lower than in the PH-chronosequence. The thermo-recalcitrant SOC fractions (W2 and W3) also increased in both chronosequences, but their relative gain decreased due to the more pronounced gain of labile fractions (Fig. 3B). The T50w values decreased gradually in the post-agricultural Chernozems after abandonment, but this drop was smaller compared with the PHchronosequence (Fig. 3).

The whole sample set was characterized by relationships between the signal at the chemical-shit regions obtained by <sup>13</sup>C-CP-MAS NMR and thermal indexes measured by DSC-TG. The intensity of the signal for the O-alkyl region was associated with the thermolabile SOC (the correlation coefficients for Q1 + Q2 and W1 + W2 were 0.87 and 0.78, respectively). The signal intensity of aromatic SOC was positively related to the refractory SOC (the correlation coefficients for Q3 and W3 were 0.91 and 0.95, respectively) and T50 (for T50<sub>Q</sub> and T50w the correlation coefficients were 0.79 and 0.70, respectively).

#### 3.4. Post-agricultural shifts of microbial respiration

The rate of basal respiration ( $R_{basal}$ ) in both chronosequences increased gradually from the initial stage (arable) to the final restoration stage – virgin natural or completely restored soils. This increase was stronger in the PH- than in the CH-chronosequence: 6.6 vs 2.5 times (Table 2). The  $R_{basal}$  of post-agricultural Phaeozems was much higher than of post-agricultural Chernozems: 11.8–23.6 vs 6.5–13.4 mg Ckg<sup>-1</sup> day<sup>-1</sup>. The specific rate of basal respiration

(SpR<sub>basal</sub>) of post-agricultural Phaeozems was 2–3 times higher than of post-agricultural Chernozems (Table 2). The increase in SpR<sub>basal</sub> during post-agricultural restoration was not regular in both chronosequences. Total C losses as CO<sub>2</sub> during 1-year incubation at 22 °C without moisture limitation C-loss) due to the SOC mineralization reflected the amount of biodegradable carbon. They were much higher in post-agricultural Phaeozems (1.18–2.43 mg C kg<sup>-1</sup> yr<sup>-1</sup>) than in post-agricultural Chernozems (0.72–1.51 mg C kg<sup>-1</sup> yr<sup>-1</sup>) and gradually increased from the arable to final stage of post-agricultural restoration (Table 4).

## 3.5. Biodegradable and recalcitrant carbon and its turnover in postagricultural soils

The amount of C<sub>bio</sub> was higher in post-agricultural Phaeozems than in post-agricultural Chernozems:  $0.63-1.32 \text{ g C kg}^{-1}$ soil VS  $0.22-0.78 \text{ g C kg}^{-1}$  soil (Table 4). In both chronosequences, the higher C<sub>bio</sub> and C<sub>rec</sub> contents were observed at the late stages of post-agricultural restoration (Table 4), demonstrating C accumulation in both pools after cropland abandonment. The mineralization constants of both pools of SOC (k1 for C<sub>bio</sub> and k2 for C<sub>rec</sub>) were much higher in Phaeozems than in Chernozems (Table 4). Accordingly, the mean residence time (MRT) of Cbio changed from 15 to 30 days in post-agricultural Phaeozems and was 2-2.5 times longer (32-87 days) in postagricultural Chernozems. The MRT values for  $C_{\rm rec}$  varied from 5.6 to 12.1 years in soils of the PH-chronosequence and from 17.3 and 26.2 years in soils of the CH-chronosequences. Although the mineralization constants and MRT of both pools did not depend clearly on the restoration period, the MRT of both SOC pools in Chernozems was much higher than in Phaeozems (p < 0.0001; *t*-test). Therefore, SOC in post-agricultural Chernozems is more stable than in post-agricultural Phaeozems.



**Fig. 3.** Comparison of the T50w index (temperature at which 50% of the total SOC are lost) and distribution of the SOC thermal fractions (W1-W3) obtained by Derivative Thermogravimetry (DTG) analysis of the mineral topsoils of the two chronosequences (PH: Phaeozems, CH: Chernozems) of abandoned soils: A – absolute weight loss (% of soil weight) and T50w index (black line); B – relative weight loss (% of total losses). W1, W2, and W3 are the SOC weight loss at < 375 °C (labile), 375–475 °C (recalcitrant) and 475–600 °C (refractory C), respectively.

Table 4

Total CO<sub>2</sub> losses during one-year incubation, content, mineralization coefficients, and mean residence time (MRT) of biodegradable ( $C_{bio}$ ) and recalcitrant ( $C_{rec}$ ) carbon pools in both chronosequences. Standard errors in brackets. Letters indicate significant differences at p < 0.05.

Site	C-loss, $g C kg^{-1} yr^{-1}$	$C_{bio}$ g C kg <sup>-1</sup> soil	$C_{rec}$ g C kg <sup>-1</sup> soil	C <sub>bio</sub> k1/MRT,	C <sub>rec</sub> k2/MRT,
				day <sup>-1</sup> /days	yr <sup>-1</sup> /yrs
PH-chronosequence					
PH-1	0.96 (0.06) <sup>d</sup>	$0.26 (0.02)^{d}$	$11.36 (0.02)^{d}$	$0.033^{\rm b}/31^{\rm a}$	$0.072^{c}/14^{a}$
PH-2	1.18 (0.03) <sup>c</sup>	0.63 (0.04) <sup>c</sup>	11.37 (0.04) <sup>d</sup>	$0.031^{\rm b}/32^{\rm a}$	0.092 <sup>bc</sup> /11 <sup>ab</sup>
PH-3	2.52 (0.07) <sup>b</sup>	$1.23 (0.03)^{a}$	13.72 (0.03) <sup>c</sup>	$0.031^{\rm b}/32^{\rm a}$	0.151 <sup>a</sup> /7 <sup>c</sup>
PH-4	2.43 (0.05) <sup>b</sup>	0.98 (0.04) <sup>b</sup>	18.74 (0.04) <sup>b</sup>	$0.056^{a}/18^{b}$	$0.159^{a}/6^{c}$
PH-5	3.12 (0.08) <sup>a</sup>	1.32 (0.02) <sup>a</sup>	25.92 (0.02) <sup>a</sup>	$0.063^{a}/16^{b}$	$0.110^{b}/9^{bc}$
CH-chronosequence					
CH-1	$0.77 (0.01)^{d}$	0.22 (0.02) <sup>c</sup>	19.51 (0.02) <sup>c</sup>	$0.016^{\rm bc}/62^{\rm b}$	$0.052^{\rm a}/19^{\rm b}$
CH-2	$0.72 (0.01)^{d}$	0.25 (0.02) <sup>c</sup>	17.73 (0.02) <sup>d</sup>	$0.011^{\rm c}/88^{\rm a}$	$0.038^{\circ}/26^{a}$
CH-3	0.88 (0.02) <sup>c</sup>	0.22 (0.04) <sup>c</sup>	$21.72 (0.04)^{b}$	$0.034^{a}/29^{d}$	$0.058^{\rm a}/17^{\rm b}$
CH-4	1.51 (0.05) <sup>b</sup>	$0.78 (0.06)^{a}$	$21.68 (0.06)^{b}$	$0.012^{\rm c}/87^{\rm a}$	$0.047b/21^{ab}$
CH-5	$1.37 (0.03)^{a}$	0.42 (0.04) <sup>b</sup>	26.69 (0.04) <sup>a</sup>	$0.020^{\rm b}/49^{\rm c}$	0.055a/18 <sup>b</sup>



**Fig. 4.** Relationship between parameters of microbial activity and T50w in post-agricultural soils of both chronosequences (green squares – Phaeozems; red triangles – Chernozems). All regression lines are significant at least at p < 0.005. T50w index – the temperature at which 50% of total SOC are lost by Derivative Thermogravimetry (DTG);  $R_{basal}$  – basal respiration rate; C-loss – cumulative CO<sub>2</sub> losses for 1-year incubation,  $C_{bio}$  – biodegradable C-pool; SpR<sub>basal</sub> – specific basal respiration rate ( $R_{basal}$ /SOC); k1 and k2 – mineralization rate constants for various C-pools. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

# 3.6. Relationship between microbial activity and various SOC pools in postagricultural soils

#### Table 5

Negative relationships were observed between thermal soil stability (T50w) and most microbiological parameters:  $R_{basab}$ ,  $SpR_{basab}$ , C-loss, and  $C_{bio}$  for the whole set of soil samples ( $R^2 = 0.66-0.88$ , p < 0.005; Fig. 4). Thus, the SOC thermal stability explained 66–88% of the variability of the microbial activity in post-agricultural soils.

Various thermal fractions (W1, W2, W3, W2 + W3) correlated closely with chemical shift regions in the <sup>13</sup>C NMR spectra (Table 5). Thus, O-alkyl C correlated negatively with W1, W2, W3, W2 + W3 ( $R = -0.80 \div -0.94$ ; p = 0.001-0.05), whereas Carbonyl C and C<sub>rec</sub> content showed positive correlations with all SOC components (R = 0.81-0.86; p = 0.01-0.05).

Pearson's correlation coefficients for the relationships between thermal p	bara
meters, chemical shift regions of <sup>13</sup> C NMR spectrometry and microbial p	bara
meters in post-agrogenic soils (both chronosequences).	

Parameters	W1	W2	W3	W2 + W3
Alkyl C	0.74	0.76	0.78	0.77
O-alkyl	-0.80	-0.91	-0.94	-0.92
Aromatic C	0.64	0.84	0.86	0.85
Carbonyl C	0.83	0.81	0.81	0.81
R <sub>basal</sub>	0.64	-0.06	-0.34	-0.19
SpR <sub>basal</sub>	0.23	-0.45	-0.66	-0.55
C-loss	0.53	-0.16	-0.42	-0.28
C <sub>bio</sub>	0.47	-0.11	-0.37	-0.23
Crec	0.94	0.97	0.95	0.96

The significant coefficients are indicated in bold, p < 0.05.

Abandonment period (AP)



Soil Type (ST)

■ AP \* ST

Unexplained

# **Fig. 5.** Contributions of the two factors: Abandonment period and Soil Type as well as their interactions to the variability of soil properties, SOC-pools, and microbial characteristics. Results of two-way ANOVA with Eta squared ( $\eta^2$ , %) are shown. Both factors and their interactions are significant at *p* < 0.001. Asterisk indicates the only non-significant factor (*p* > 0.05).

3.7. Effects of soil type and abandonment period on microbial activity and various SOC pools

"Soil type" was the primary factor and contributed from 54 to 88% to the total variances of microbial parameters and soil organic matter characteristics (except C/N ratio) of all soils studied (Fig. 5). Soil type was the most important factor for parameters such as k1 and k2, explaining 81–88% of their total variances. The 'abandonment period' factor plays a secondary role and explained 7–39% of total variability for the above parameters (Fig. 5). The effect of abandonment was most pronounced for SOC and N content, contributing 35–38% to their total variances. The interactions of factors played the most significant role for the C/N ratio and explained 60% of its variances.

To elucidate interrelations between microbial activity, chemical properties, and various SOC pools, we performed a principal component analysis (PCA). The analysis clearly separated the soil types, as a function of environmental conditions (mainly climate and vegetation) in relation to the several principal components (PC, Fig. 6). We extracted two components (factors) that accounted for a total of 88.6% of the variability in the original data and distinctly separated Phaeozems and Chernozems. Factor 1 (PC-1) reflected the effects of soil type, which are closely connected with climate. It was composed of parameters connected with microbiological activity (SpR<sub>basal</sub>, C-loss, C<sub>bio</sub>), pH<sub>H2O</sub>, and indicators of SOC recalcitrance (W3, T50w, k2). Factor 2 (PC-2) was composed of total SOC and N content, and various SOC pools (W1 and C<sub>rec</sub>) which reflected distinctly the effect of time since abandonment.

#### 4. Discussion

# 4.1. Changes in the amount and composition of SOC during postagricultural restoration

The two soil groups – PH and CH – followed different patterns of post-agricultural alterations in SOC content and quality, which are main mediators of C sequestration. Both arable soils contained low amounts of labile C. The O-alkyl C made up ~40% of the total SOC (Table 3) and the thermo-labile compounds (W1) comprised 48–51% (Fig. 3B). These properties are usually common for degraded soils with

low SOC content (Poeplau and Don, 2013). The SOC of arable Chernozems, however, was less degradable and more stable than in arable Phaeozems due to the higher content of aromatic and carbonyl C in SOC (Table 3, Fig. 1).

The abandonment of arable Phaeozems with lower SOC resulted in larger increase in SOC content (by 1.7 times during 30 years) than in the C-rich Chernozems (by 1.4 times during 77 years) (Table 2). This difference may reflect the higher input of aboveground litter in PH under the forest coenosis than in CH under steppe vegetation (Table 6). A second reason is the quality of aboveground litter. Forest litter contains more lignin and lipids (stable components) and less carbohydrates compared to steppe litter (Kögel-Knabner, 2000, 2002; Bonanomi et al., 2013). Moreover, the lower rate in SOC gain in the post-agricultural Chernozems might be partly connected with lower microbial activity and, hence, slower mineralization of fresh plant residues. The lower microbial activity of Chernozems is due to the more arid climate in the steppe region than in the deciduous forests zone, where Phaeozems were located (Table 6).

The abandonment of arable lands changed the SOC composition and degradability in both chronosequences (Figs. 1–3). The labile SOC fraction contributed most to the total SOC gains during the post-agricultural restoration, especially in the PH chronosequence, where the increases in O-Alkyl, most-thermolabile compounds, and  $C_{bio}$  were predominant (Table 3; Fig. 3). Hence, our second hypothesis that the recalcitrant SOC pools predominate in Chernozems, which are Ca-rich soils, whereas Phaeozems accumulate more active C pools, was also fully confirmed. A similar pattern has been described in afforested agricultural lands (Pérez-Cruzado et al., 2014). This is due to direct input of plant residues because > 50% of the litter is made up of O-alkyl compounds. Both active (labile) and passive (recalcitrant) C pools increased after conversion of cropland to grassland or forest in similar climates (Kalinina et al., 2009, 2010, 2011, 2013, 2015a, 2015b; Lopes de Gerenyu et al., 2008; McLauchlan et al., 2006).

#### 4.2. Microbiological activity, SOC decomposition and turnover

The biochemical parameters of SOC and plant residues are key determinants of microbial decomposition (Bending et al., 2002, 2004; Wang et al., 2016). Microorganisms influence SOC cycling not only via



Fig. 6. Principal component analysis (PCA) showing the grouping of soils in response to vectors reflecting soil microbial and biochemical properties. The analysis was separated into two plots: a vector plot (A, factor coordinates of the variables, based on correlations) and the grouping of data in two soil types (B). Large arrows show the effects of aridity and time after LUC (abandonment period) on soil properties.

decomposition, but also because microbial products, e.g. microbial necromass, are themselves important components of SOC (Apostel et al., 2018; Schmidt et al., 2011). The long-term agricultural use of soils drastically reduces microbial activity due to the depletion of easily available OM (Harrison and Bardgett, 2010; Ivashchenko et al., 2015). After cropland abandonment, the microbiological activities increase due to a higher input of fresh organic materials by recovering natural vegetation (Ananyeva et al., 2009; Jia et al., 2005; Susyan et al., 2011). More than 15–25 years after abandonment are needed to reach the microbial activity of natural ecosystems in soils of temperate climate (Lopes de Gerenyu et al., 2008; Mostovaya et al., 2015; Susyan et al., 2011).

Low microbial activities (R<sub>basal</sub>, SpR<sub>basal</sub>) were characteristic for arable soils in both chronosequences. The particularly low respiratory rates of arable Chernozems mainly reflected the lack of labile C fractions and the accumulation of organic compounds, conferring resistance to microbial attacks (González-Pérez et al., 2004) as well as binding on Ca<sup>2+</sup> (Rowley et al., 2018). All indexes of microbial activity (R<sub>basal</sub>, SpR<sub>basal</sub>, C<sub>mic</sub>, C<sub>bio</sub>, C-loss) increased gradually during the post-agricultural restoration in both soils (Tables 2 and 4). Some of microbial activity indexes correlated negatively with T50w, which is an indicator of SOC stability (Fig. 4). Thus, our first hypothesis that microbial activity and the content of biodegradable SOC increase considerably during post-agricultural restoration from arable to abandoned soils was fully supported. Other researchers have also shown the role of SOM thermal stability in microbial activity in different climates (Campo and Merino, 2016; Dorodnikov et al., 2007; Harvey et al., 2012; Kučerík and Siewert, 2014; Siewert et al., 2012). The low thermal stability of SOC corresponds generally to its higher microbial availability. Lower microbial activity of Chernozems (and hence a slower SOC turnover) is clearly related to their higher SOC recalcitrance compared with Phaeozems. The relationship between microbial properties and SOC quality parameters indicates that this slower turnover is related to the chemical recalcitrance of the SOC. The gain of easily biodegradable SOC (Cbio) was much slower in post-agricultural Chernozems with constant and higher alkyl-C/O alkyl-C ratios and higher T50w values than in PH-chronosequences (Table 3). We explain the lower T50w values and higher CO<sub>2</sub> losses for 1-year incubation in PH by both the increased microbial activity in former arable Phaeozems and the predominance of labile fractions in SOC after abandonment.

Accordingly, both chronosequences showed important differences in SOC composition, which affected C stability and turnover. The postagricultural Chernozems were characterized by higher C/N ratio, higher thermal recalcitrance, higher aromaticity, and alkyl C/O-alkyl C ratios in comparison with post-agricultural Phaeozems. In agreement with this, the Chernozems showed a lower microbial activity (i.e.  $R_{basal}$ ,  $SpR_{basal}$ ,  $C_{bio}$  content). Because the previous cropland management of both soils was comparable, the lower SOC turnover in the arable Chernozems explains its higher initial SOC content before abandonment. In contrast, the faster SOC decomposition in the Phaeozems resulted in higher SOC losses during cultivation. We conclude that climate and natural vegetation succession were the two crucial factors affecting the direction of soil properties development after abandonment (Fig. 6 right). The duration of the abandonment period however, affected the rate of the processes specific for individual soils properties and the closeness of these properties in restored soils to that in the natural undisturbed Phaeozem and Chernozem.

Abandoning agricultural soils resulted in considerable C sequestration and confirms the strong mitigation of CO<sub>2</sub> emissions by abandonment. Nonetheless, the two chronosequences showed specific patterns in the chemical properties of SOC, which led to different SOC turnovers. The C sink capacity of Phaeozems was rapid, but due to the higher turnover this SOC is subjected to rapid decomposition after perturbation, e.g. by new agricultural use. The new C sequestered after abandonment during post-agricultural evolution is more microbially available for mineralization compared to the C remained after agricultural use. This C availability is especially high in Phaeozems, because Chernozems have more C stabilization mechanisms like high Ca<sup>2+</sup> content (Rowley et al., 2018) and distinct drying-rewetting events, both leading to much better aggregation and so, to spatial inaccessibility of microorganisms to the organic compounds (Dungait et al., 2012; Yan et al., 2012). Consequently, Phaeozems need longer abandonment period than Chernozems to form stable SOC which will be resistant to mineralization. Therefore, new agricultural use will lead to much faster SOC in Phaeozems compared to Chernozems and so, reused Phaeozems are more vulnerable for any intensive cropland, especially for management (e.g. frequent tillage) leading to aggregate destruction. We conclude that the climate with respective vegetation and soil type are responsible for the direction of pedogenesis (Fig. 6), SOC composition and stability as well as microbial activities, while the duration of abandonment is crucial for the process rates and the closeness of restored soils properties to their natural analogs.

#### Table 6

Comparison of climatic, vegetation (litter) and soil parameters for Phaeozems and Chernozems under natural vegetation of deciduous forest and dry steppe in European Russia.

Factors, parameters	Phaeozem (deciduous forest)	vs	Chernozem (dry steppe)	Reference
Climatic factors				
• $\Sigma T > 10^{\circ}C$	2200 - 2700	<	2650 – 3000 350 –	Dobrovolsky (2004)
<ul> <li>Precipitation (P, mm)</li> </ul>	450 – 550	>	450	
• Evaporation (E, mm)	500 - 580	<	550 - 750	
Aridity (P/E)	0.77 – 1.10	<	0.44 - 0.77	
• Period with $T > 10$	140 – 157	<	155 – 166	
°C (days)				
Litter aboveground				
<ul> <li>Input amounts, Mg</li> </ul>	3.0 - 5.0	>	0.4 -1.2	Aleksandrova
ha <sup>-1</sup>				(1980); Bazilevich
Quality				(1993); Rodin and
- C/N	> 20	>	> 15	Bazilevich (1967)
- N (%)	1.46 – 2.12	≥	0.75 - 2.43	
- Ca (%)	1.18 – 2.46	>	0.48 - 1.60	
- Si (%)	0.67 – 1.06	<	0.59 - 2.85	
- Lignin (%)	20 - 30	>	15 – 20	
- Carbohydrates (%)	35 – 45	<	50 – 75	
- Lipids (%)	5 – 15	>	2 -10	
- Lignin/N	10 - 20		6 – 26	
Litter belowground				
Roots	Medium/		Mainly fine	Bazilevich and
	large		6.7 –	Titlyanova (2008);
<ul> <li>Input amounts*, Mg ha<sup>-1</sup></li> </ul>	1.4 – 1.5	<	8.0	Rodin and Bazilevich (1967)
Quality (fine roots)				
- C/N	> 30		> 30	
- N (%)	0.76 – 1.46	≥	1.08 - 1.50	
- Ca (%)	0.60 - 1.06	=	0.66 – 1.17	
- Si (%)	0.10 – 1.29	=	0.22 - 1.23	
<u>Soil</u> ** (0-10 cm)				
• C stock (0-20 cm), Mg ha <sup>-1</sup>	60 –110	<	130 –160	Kaurichev et al. (1989); Orlov et al.
• C/N	14 – 17	>	10 – 14	(1996)
• pH (H <sub>2</sub> O)	5.5 - 6.0	<	6.5 – 7.5	
• CEC, mg-eqv 100 g soil <sup>-1</sup>	15 - 30	<	30 - 70	
• Ca <sup>2+</sup> , mg-eqv 100 g soil <sup>-1</sup>	10 – 20	<	40 - 50	

\*fine roots only; \*\*because of very similar clay content and composition, the effects of mineral compounds on C stabilization are not mentioned here; CEC - cation exchange capacity.

#### 4.3. Pathways of SOC sequestration and stabilization during postagricultural restoration

The mechanisms of SOC sequestration and stabilization during postagricultural restoration depended on soil type and vegetation, and hence on climate. Principal component analysis revealed the dominant role of soil type in post-agricultural changes of former croplands. Being the function of climate and vegetation, soil type reflected mainly for the post-agricultural changes in microbiological activity, which is responsible for decomposition processes and content of more resistance fractions in SOC, which reflects the stabilization mechanisms. The effect of abandonment period was observed within each chronosequence. The progressive recovery of natural vegetation due to cessation of agricultural use increased in C input to soil from plant residuals and led to the C sequestration in post-agricultural soils. Factor 2 (Fig. 6), therefore, reflected the effect of time after abandonment and was mainly responsible for SOC content and its thermolabile components (W1).

Previous studies comparing soils of various rainfall regimes clearly showed higher SOC recalcitrance in soils with longer drought periods (Campo and Merino, 2016; DeMarco et al., 2016; Pisani et al., 2014). The higher recalcitrance was related to the higher content of resistant biopolymers such as cutin and suberins in the root litter of dry climates. Such compounds are especially abundant in underground plant tissues of drought-adapted plants, helping make cell walls water- and air tight (Boom et al., 2005). These recalcitrant compounds persist during litter decomposition and transformation (Zech and Kögel-Knabner, 1994) and, as a consequence of selective preservation, can accumulate in the soil (Ostertag et al., 2008). Consequently, the SOC composition in Chernozems is mainly connected with initial litter input. C allocation by plants therefore plays an important role in SOC dynamics and sequestration (Schmidt et al., 2011). Comparing the isotopic composition of root and shoot biomarkers showed the dominance of root-derived molecular structures in soil (Mendez-Millan et al., 2010) and soil microorganisms (Kramer et al., 2010). This means that root-derived C is stored in Chernozems much more efficiently than are aboveground plant materials - the main input in Phaeozems (Table 6). The mineralization of aboveground inputs occurs in the litter layer (common for Phaeozems), whereas root and mycorrhizal inputs interact with mineral soil particles - more common in Chernozems (Rasse et al., 2005).

The shifts in above-/belowground inputs, quantity and quality of plant residues, soil aggregation, and composition of destructors mediated the patterns of C sequestration for various functional SOC pools (Kalinina et al., 2009; Six et al., 2002; Susyan et al., 2011). The active C pool and the free particulate organic matter are the most susceptible to land use changes in temperate regions (Kalinina et al., 2015b; Poeplau and Don, 2013). The main mechanism of SOC stabilization here is the physical protection of SOC due to an increase in the proportion of C in microaggregates i.e. physical occlusion organic matter in aggregates (Awad et al., 2013; Erokhova et al., 2014; Gunina et al., 2015). The preferential increase of labile SOC pools resulted from the higher input of plant residues during forest or grassland establishment after the cessation of agricultural use. At the late stage of post-agricultural restoration in deciduous and forest-steppe zone, the new vegetation is composed mainly of woody shrubs, which implies a higher input of lignin, suberin, and waxes (Helfrich et al., 2006; Otto and Simpson, 2006). This suggests that the higher recalcitrance of litter after the woody vegetation invasion favor the changes in SOC composition (DeMarco et al., 2016; Pérez-Cruzado et al., 2014). Nevertheless, other processes, such as microbial biosynthesis and polymerization can also produce aliphatic compounds (Huang et al., 2011).

The higher stability of SOC in Chernozems was likely related to the formation of calcium-humus complexes, which play an important role in protecting SOC (Orlov et al., 1996; Rowley et al., 2018; Wang et al., 2016). The content of exchangeable of calcium (Ca) and the cation exchange capacity in Chernozems are 2–3 times higher than in Phaeozems (Table 6). Associations of SOC with minerals, sesquioxides, and for these soils especially with  $Ca^{2+}$  are the main mechanisms protecting SOC from microbial decomposition in soils of semi-arid climates (Rowley et al., 2018; Schmidt et al., 2011; Wiesmeier et al., 2014).

Therefore, the pattern of SOC sequestration and its stability during post-agricultural restoration are governed by initial SOC stocks in former arable soils, the biochemical composition of above-/belowground inputs, the chemical feature of soils, and the environmental conditions, mainly by aridity.

#### 5. Conclusions

Intensive cultivation of the two soils – Chernozems and Phaeozems – led to SOC losses and to dramatic changes in SOC composition, especially of easily available fractions. The SOC increased after 50–70 years abandonment for 1.5–2.5 times compared to that in arable soils. The soil type as well as intrinsic pedogenic processes, and time since abandonment determine the C sequestration dynamics and the SOC properties. This is important because the new SOC will determine the long-term source-sink C functions. The post-agricultural Chernozems were characterized by higher C/N ratios, higher thermal stability, higher aromaticity, and higher alkyl C/O-alkyl C ratios than the Phaeozems. In contrast, the Phaeozems showed a remarkable increase in microbial activity (i.e. total and specific basal respiration, microbial biomass content) and a predominant accumulation of labile components in SOC during the post-agricultural restoration. Although SOC composition explained 66–88% of the variability of the microbial activity in the post-agricultural soils, other factors such as aridity and plant residue quality should also be considered. The changes were documented in the SOC composition after croplands abandonment and affected the C stability and turnover in both soil types. The considerable differences in the SOC sequestration rates and in SOC quality during the post-agricultural restoration of Phaeozems and Chernozems are attributable to (i) climate-vegetation effects, (ii) soil properties and processes, and (iii) abandonment period.

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#### Appendix A. Supplementary data

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