

Soil aggregation and soil organic matter in conventionally and organically farmed Austrian Chernozems

Bodenaggregation und organische Substanz in konventionell und biologisch bewirtschafteten österreichischen Tschernosemböden

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Summary

In order to study the soil aggregate distributions and soil organic matter (SOM), we sampled top- and subsoils in four intensively farmed croplands (two organic (Org-OB and Org-LA), and two conventional (Con-OB and Con-LA)) on Haplic Chernozems located in Marchfeld in the east of Vienna (Austria). Soil structure and SOM quantity, quality and distribution between free and occluded particulate organic matter and aggregate size fractions (<20 µm, 20–250 µm, 250–5000 µm) were studied by following a density fractionation procedure with low-energy ultrasound treatment. The relation of the soil physicochemical (e.g., particle size distribution, pH, organic carbon, total nitrogen) and biological properties (e.g., fungal biomass, active fungi) with stable soil aggregate size fractions and SOM was studied. The mean weight diameter (MWD) showed no significant difference between all studied sites and was between 3.8 mm and 10.0 mm in topsoils and between 6.7 mm and 11.9 mm in subsoils. In topsoils, the contents of calcium-acetate-lactate (CAL)-extractable P, active fungal biomass, dithionite-extractable Fe and sand were significantly positively correlated with the amount of the macroaggregates and with the MWD. We observed that most soil organic carbon, depending on soil texture, was stored in the microaggregate size classes <20 µm and 20–250 µm.

Keywords: organic matter dynamics, aggregate stability, solid-state ¹³C NMR spectroscopy, particulate organic matter (POM), aggregate hierarchy

Zusammenfassung

Um die Verteilung der Bodenaggregate und der organischen Substanz des Bodens näher zu erforschen, wurden Ober- und Unterböden von vier intensiv landwirtschaftlich genutzten Ackerflächen (zwei biologisch-organischen (Org-OB und Org-LA) und zwei konventionell bewirtschafteten (Con-OB und Con-LA)) beprobt. Bei den Böden handelte es sich um Tschernoseme, die sich im Marchfeld, östlich von Wien (Österreich), befinden. Die Bodenstruktur sowie die Menge, die Qualität und die Aufteilung der organischen Substanz zwischen freier und okkludierter organischer Substanz (fPOM und oPOM) und die Größe der Aggregate (<20 µm, 20–250 µm, 250–5000 µm) wurden mittels Dichtefraktionierung mit niedriger Ultraschallenergie untersucht. Das Verhältnis der physikochemischen (z.B. Korngrößenverteilung, pH, organischer Kohlenstoff, Gesamt-Stickstoffgehalt) und der biologischen Eigenschaften (Pilz-Biomasse, aktive Pilze) in den Aggregaten wurde mit stabiler Bodenaggregatgrößen Fraktionierung untersucht. Der mittlere gewichtete Durchmesser (MDW) lag zwischen 3,8 mm und 10,0 mm in den Oberböden und zwischen 6,7 mm und 11,9 mm in den Unterböden und zeigte keine signifikanten Unterschiede zwischen den Standorten. Im Oberboden waren der CAL-lösliche Phosphorgehalt, die aktive Pilzbiomasse, dithionitlösliches Eisen und Sand signifikant positiv korreliert mit der Anzahl der Makroaggregate und mit dem MDW. Der meiste organische Kohlenstoff war, abhängig von der Bodenart, in den Mikroaggregaten der Größenklassen <20 µm und 20–250 µm gespeichert.

Schlagworte: Dynamik der organischen Substanz, Aggregatstabilität, Festkörper-¹³C-NMR-Spektroskopie, partikuläre organische Substanz, Aggregathierarchie

Abbreviations

ANOVA: analysis of variance
 C: carbon
 C_t : total carbon
 CAL: calcium-acetate-lactate
 CEC: cation exchange capacity
 Con-OB: conventional field in Obersiebenbrunn
 Con-LA: conventional field in Lassee
 CPMAS: cross-polarization magic angle spinning
 DFS: differential fluorescent stain
 DTAF: dichlorotriazinylaminofluorescein
 fPOM: free particulate organic matter
 HWC: hot water extractable carbon
 K: potassium
 MWD: mean weight diameter
 N: nitrogen
 N_t : total nitrogen
 NMR: nuclear magnetic resonance
 O: oxygen
 OC: organic carbon
 OM: organic matter
 oPOM: occluded particulate organic matter
 Org-OB: organic field in Obersiebenbrunn
 Org-LA: organic field in Lassee
 P: phosphorous
 POM: particulate organic matter
 SOM: soil organic matter
 NMR: nuclear magnetic resonance

1. Introduction

Cultivation of soil impacts soil organic matter (SOM) quantity and quality as well as soil structure due to tillage activities (Six et al., 2002; Lal, 2013). In addition to tillage activities, harvesting of crops also causes strong soil disturbances and subsoil compaction due to heavy machinery (Pulleman et al., 2003). The formation and stabilization of soil aggregates are influenced by drying and wetting cycles as well as physical, chemical, and biological soil properties (Six et al., 2000; Six et al., 2004; Regelink et al., 2015). According to the hierarchical aggregate model, which was first described by Tisdall and Oades (1982), macroaggregates ($>250\ \mu\text{m}$) are made of microaggregates ($<250\ \mu\text{m}$), sand particles and particulate organic matter (POM) that are bound together by transient or temporary binding agents. These aggregate binding agents include microbial-derived mucilage, as well as roots and fungal hyphae (Tisdall and

Oades, 1982; Amézketa, 1999; Six et al., 2004). Macroaggregates ($>250\ \mu\text{m}$) are less stable and more influenced by soil management than microaggregates ($<250\ \mu\text{m}$), due to their transient or temporary binding agents. Microaggregates consist mainly of associations of free primary particles bound together by persistent binding agents that include organic molecules, metal oxy(hydr)oxides, polyvalent cations, Ca- and Mg- carbonates, and CaSO_4 (Tisdall and Oades, 1982; Amézketa, 1999). Farmers aim to increase the nutrient level and water retention capacity of soil by applying organic inputs including manure (Siegrist et al., 1998), concurrently aiming to improve or maintain soil functions such as good physical structure with stable aggregates. Free particulate organic matter in soil (fPOM) or occluded POM within the soil aggregates (oPOM) represent plant and animal residues undergoing decomposition and are generally increasing aggregation (Six et al., 2000). POM fractions have been observed to respond more sensitively to soil management changes than to the total soil organic carbon (OC) (Golchin et al., 1994; Chan et al., 2002), especially occluded POM that may be lost from soil aggregates due to intense cultivation (Golchin et al., 1994). Cultivation has also been shown to increase SOM in clay-sized particles, in microaggregates, where SOM gets physically protected from decomposition (Six et al., 2000). Although a large body of knowledge exists about agricultural soils in the Marchfeld area (e.g., Spiegel et al., 2010), little is known about aggregate and SOM distributions in these Chernozems. This calls for a more detailed investigation of intensively cultivated soils, including detailed study of aggregate stability (Lehtinen et al., 2014), SOM distribution and its chemical quality. The objectives of our study were to assess: 1) soil aggregation in topsoils and subsoils, and 2) quality, quantity, and distribution of SOM in the different soil fractions (fPOM, oPOM, $<20\ \mu\text{m}$, $20\text{--}250\ \mu\text{m}$, and $>250\ \mu\text{m}$) in topsoils and subsoils, in four intensively managed croplands on Haplic Chernozems in the agricultural area of Marchfeld, Austria. Both topsoil (0–15 cm, in the ploughing layer) and subsoil (30–40 cm, below the ploughing layer) were investigated, in order to study soil properties as a function of ploughing.

2. Materials and methods

2.1 Site description

The sites were selected to represent one of the major cultivated soil types, Haplic Chernozem (IUSS, 2015), in

the agricultural area of the Marchfeld, located in East of Vienna in Austria. This area presents a former floodplain of the River Danube and it is one of the most important food production areas in the country. During the last 50 years, farms in the region have changed from mixed crop and animal farming to stockless farming systems (Surböck et al., 2006; Spiegel et al., 2010). We chose study sites that have the same soil type, with the same pedogenesis and the same genetic soil horizons. The mean annual temperature is approximately 9°C and mean annual precipitation about 550 mm with dry summers (Lair et al., 2009). We sampled four cropland fields in May 2011, in the villages of Obersiebenbrunn and Lassee that are located approximately 15 km apart. The selected farms represented typical farming practices in this area (description based on farmer interviews):

- a) Organic field in Obersiebenbrunn (Org-OB) (48°17'087N, 16°41'245E, Obersiebenbrunn) on an organic farm that has been managed as per the Austrian guidelines for organic farming (BIO AUSTRIA, 2010) since 1976. The studied field received biowaste compost (organic matter ca. 400 g kg⁻¹ dry matter, according to Erhart et al., 2005) produced by the city of Vienna annually as an organic fertilizer (except for the years 2001–2003). In 2009, catch crops were used and incorporated into the soil during the tillage activities in the fall;
- b) Con-OB (48°17'093N, 16°41'209E, Obersiebenbrunn) is a conventional farm located next to Org-OB and received only mineral fertilizers annually in the last decades, according to the Austrian fertilization recommendations (BMLFUW, 2006);
- c) Org-LA (48°13'556N, 16°50'051E, Lassee) received horse manure every five years as an organic fertilizer and was converted to organic farming, according to the Austrian guidelines (BIO AUSTRIA, 2010) in 1995. Catch crops were used in 2007, 2008, and 2009 and were incorporated into the soil during the tillage activities in the fall;
- d) Con-LA (48°14'153N, 16°50'090E, Lassee) is a conventional farm located close to Org-LA and receives only mineral fertilizers, according to the Austrian fertilization recommendations (BMLFUW, 2006). Catch crops were used in 2002, 2004, 2006, and 2007.

The application of pesticides and herbicides at Con-OB and Con-LA was done according to the Austrian guidelines for each crop (e.g., AGES, 2013). The crops at the time of sampling were potato (*Solanum tuberosum*) (Org-OB, Con-OB) and winter wheat (*Triticum aestivum*) (Org-LA, Con-LA). Tillage at all fields was carried out annually

in the fall to a depth of 25 cm and conventional methods for preparing the seedbed for each crop were used. If catch crops were used, additional seedbed preparation was carried out and catch crops were ploughed into the soil during the regular tillage activities. For more details of farm management, see van Leeuwen et al. (2015).

2.2 Soil sampling

The soil sampling campaign was carried out in May 2011. At each studied field, three composite soil samples were collected randomly from 0–15 cm and 30–40 cm depths, through mixing approximately 10–15 cores taken with a soil corer (diameter 8 cm, height 15 cm; root corer Eijkkamp, Agrisearch Equipment, The Netherlands) to get almost undisturbed soil samples. Hence, a total of 24 soil samples were obtained for our study. Soil samples were gently broken by hand and sieved through a 5 mm sieve in the field. Soils for microbiological analyses were only sampled from 0–15 cm soil depth. The soil samples were transported in plastic boxes, and kept at 4°C in the dark for biological analyses. Soil samples were air-dried in the laboratory prior to all other analyses.

2.3 Analyses of physicochemical bulk soil properties

Soil pH was measured electrochemically (Microprocessor pH Meter pH196 WTW, Weilheim, Germany) in distilled H₂O at a soil:water ratio of 1:2.5 (Soil Survey Staff, 2004). Particle size distribution was determined with a combined sieve and pipette method after dispersion by reciprocal shaking with sodium metaphosphate solution for 12 h (Soil Survey Staff, 2004). Ammonium-oxalate-extractable Fe, Mn, and Al (Fe_o, Mn_o, Al_o) were determined according to Schwertmann (1964). Dithionite-citrate-bicarbonate-extractable Fe, Mn, and Al (Fe_d, Mn_d, Al_d) were determined according to Mehra and Jackson (1960). Total carbon (C_t) and total nitrogen (N_t) were quantified by dry combustion (Tabatabai and Bremner, 1991), using an elemental analyzer (Carlo Erba Nitrogen Analyser 1500, Milano, Italy). Carbonate content was measured gas-volumetrically (Soil Survey Staff, 2004). Organic C (OC) was calculated as the difference of total C and carbonate C. Plant available phosphorous and potassium were determined by the calcium-acetate-lactate (CAL)-extraction (ÖNORM L1087). Cation exchange capacity (CEC) and the number of exchangeable cations were determined using an unbuffered 0.1 M BaCl₂ extraction (Soil Survey Staff, 2004). The

extracted exchangeable cations (K, Na, Ca, and Mg) were measured by flame atomic absorption spectrophotometry (Perkin-Elmer 2100, Waltham, MA, USA).

2.4 Analyses of microbiological bulk soil properties

For determination of fungal hyphal length and bacterial numbers, microscopic slides were prepared as described by Bloem and Vos (2004) after a pre-incubation period for 2 weeks at 20°C. To estimate fungal biomass, we used the equation of a cylinder with spherical ends ($V = (\pi/4) W^2 (L - (W/3))$), where V = volume (μm^3), L = length (μm) and W = width (μm), a mean hyphal diameter of 2.5 μm and a specific C content of 130 $\text{fg C } \mu\text{m}^{-3}$. Total and active fungi were distinguished using differential fluorescent stain (DFS), where cell walls (polysaccharides) were stained blue with fluorescent brightener and DNA and RNA (presumably actively growing hyphae) were stained red with Europium chelate. Bacteria (proteins) were stained with dichlorotriazinylaminofluorescein (DTAF). Bacterial cell numbers and volume were determined by confocal laser scanning microscopy combined with an image analysis system and bacterial biomass was calculated using a specific C content of 320 $\text{fg C } \mu\text{m}^{-3}$ (Bloem et al., 1995). Potentially mineralizable nitrogen was measured as the accumulation of NH_4 during one week anaerobic incubation in slurry at 40°C (Canali and Benedetti, 2006). Hot water extractable C (HWC) was determined as the C present in solution after 16 h at 80°C according to Ghani et al. (2003).

2.5 Density and aggregate fractionation

A three-step density and aggregate fractionation procedure described in Lehtinen et al. (2015) was carried out in triplicate. In short, the free particulate organic matter (referred to as fPOM, 20–5000 μm) was separated from soil using the sodium polytungstate solution (density of 1.8 g cm^{-3}). To obtain POM occluded in aggregates (referred to as oPOM, 20–5000 μm), the subsequent heavy fraction ($>1.8 \text{ g cm}^{-3}$) was treated by ultrasound. Energy of 8 J ml^{-1} was used to disrupt all macroaggregates and to minimize artefacts, a procedure that was established in a former study using similar soils from the Marchfeld (Lehtinen et al., 2014). The calibration of output power of the sonicator was done calorimetrically as per North (1976). With a subsequent density fractionation step (sodium polytungstate solution, 1.8 g cm^{-3}), the oPOM floating on the suspension was obtained after centrifugation (10 minutes at

4350 rpm). All POM fractions were washed with deionized water on a 20 μm sieve until the electric conductivity dropped below 5 $\mu\text{S cm}^{-1}$ (Mueller et al., 2009; Steffens et al., 2009) and freeze-dried for further analyses (C_t , OC and N_t analyses as described in section 2.3 of this paper). The sediment with a density of $> 1.8 \text{ g cm}^{-3}$ (i.e., mineral particles and organomineral associations) was sieved at 250 μm and 20 μm to obtain macroaggregates (250–5000 μm) and two microaggregate fractions (20–250 μm and $< 20 \mu\text{m}$). All aggregate fractions were washed in a steel pressure filter apparatus (mesh size 0.45 μm) with deionized water until the electronic conductivity dropped below 5 $\mu\text{S cm}^{-1}$, then oven dried at 105°C, weighed, and ground for further analyses (C and N_t analyses as described above in section 2.3). The masses of aggregates were corrected for their respective silt and sand content (for aggregates 20–250 μm , and $>250 \mu\text{m}$), in order to exclude single silt and sand particles from being weighed as an aggregate (Lehtinen et al., 2014). Mean weight diameter (MWD, mm) of the single particle-corrected aggregates was calculated according to Kemper and Rosenau (1986) as follows:

$$\text{MWD} = \sum_{i=1}^n w_i * \bar{X}_i$$

where, $\bar{X}_i$ is the geometric mean of aggregate size on sieve i , and w_i is the mass of aggregates on sieve i .

2.6 Solid-state ^{13}C NMR spectroscopy

The chemical quality of selected POM fractions and bulk soils was analyzed by solid-state ^{13}C nuclear magnetic resonance (NMR) spectroscopy (DSX 200 NMR spectrometer, Bruker, Karlsruhe, Germany). Composite samples were prepared by mixing equal amounts of the three replicates. To improve the signal-to-noise ratio, the bulk soil samples were treated with 10% HF (Schmidt et al., 1997). The cross-polarization magic angle spinning (CPMAS) technique with a ^{13}C -resonance frequency of 50.32 MHz and a spinning speed of 6.8 kHz was applied. A ramped ^1H -pulse starting at 100% to 50% of the initial power was used during a contact time of 1 ms in order to circumvent the spin modulation during the Hartmann-Hahn contact. Pulse delays between 0.8 and 1 s were used for all spectra. Depending on the C contents of the samples, between 11.000 and 525.000 scans were accumulated and a line broadening of 50 Hz was applied. The ^{13}C chemical shifts were calibrated relative to tetramethylsilane (0 ppm).

The relative contributions of the various C groups were determined by integration of the signal intensity in their following respective chemical shift regions (Knicker et al., 2005) assignable to alkyl C (-10 to 45 ppm), N-alkyl-C (45 to 60 ppm), O-alkyl C (60 to 110 ppm), olefinic and aromatic C (110 to 160 ppm), and carbonyl (aldehyde and ketone) and carboxyl/amide C (160 to 220 ppm).

2.7 Statistical analyses

Statistical analyses were performed using IBM SPSS Statistics 20 software package for Mac. Normality was tested with Shapiro-Wilkinson's test and confirmed that no data transformations were necessary before statistical analyses, except for OC and N_t distribution in the POM and aggregate fractions due to non-normal distribution (which were log-transformed). One-way analyses of variance (ANOVA) followed by Tukey's- significant difference ($p < 0.05$) as a post-hoc test, was used to compare the means of the different sites and soil depths for soil physicochemical properties, SOM and aggregate size distribution. Correlations between variables were calculated with the Pearson correlation coefficient.

3. Results

3.1 Bulk soil properties

The sand contents in top- and subsoil were higher in the fields located in Obersiebenbrunn (Org-OB, Con-OB), whereas silt was dominating in Lassee (Org-LA, Con-LA; Table 1). Also, $CaCO_3$ contents were higher at Obersiebenbrunn compared to Lassee. No statistical differences between all sites were observed for OC and N_t , only higher OC contents were found in topsoil compared to subsoil. CAL-extractable K content at 0-15 cm depth was significantly higher at Con-OB compared to Org-OB, while for CAL-extractable P no differences were detected. A higher content of Fe_d in Obersiebenbrunn compared to Lassee was found, while no differences were detected for Al_d and Al_o contents. Active fungi content was higher at Con-OB compared to Org-OB, whereas between Con-LA and Org-LA, no significant difference was observed. Fungal and bacterial biomass did not differ between sites, while contents of mineralisable N and HWC were both higher in Obersiebenbrunn compared to Lassee (Table 1). Significantly lower numbers in subsoil compared to topsoil were observed for pH (H_2O), OC, N_t , CAL-extractable K and

P, hydroxides (Md_o , Fe_d , Mn_o), fPOM, oPOM and 20-250 μm sized microaggregates (Tables 1 and 2).

3.2 Distributions of soil fractions and correlations with soil properties

Total soil losses during the fractionation procedure into fPOM, oPOM, $<20 \mu m$ aggregates, 20-250 μm aggregates and $>250 \mu m$ aggregates were negligible, indicated by $>99\%$ mass recoveries for all the sites. There were no significant differences between the sites in the contents of soil fractions (Table 2, Figure 1). The amount of microaggregates ($<20 \mu m$) ranged between 260 $g\ kg^{-1}$ and 337 $g\ kg^{-1}$. The amount of fPOM ranged between 3.4 $g\ kg^{-1}$ and 5.9 $g\ kg^{-1}$, and the amount of oPOM ranged between 2.5 $g\ kg^{-1}$ and 3.8 $g\ kg^{-1}$ (Table 2). The amount of macroaggregates ($>250 \mu m$) and the MWD at 0-15 cm soil depth were most strongly and positively correlated with CAL-extractable P, sand content, active fungi and Fe_d (Table 3). However, these correlations were not observed at 30-40 cm soil depth. fPOM at 0-15 cm soil depth was most strongly and positively correlated with mineralizable N content, HWC content, pH and bacterial biomass; whereas oPOM showed no significant correlations with any soil properties in the topsoil (Table 3).

3.3 Distribution and chemical quality of SOM

Total loss of OC and N_t during fractionation was negligible (recoveries $>98\%$ for all sites). The distribution of OC and N differed among sites (Figure 2). In Lassee, where we found a silty texture, the microaggregates 20-250 μm in topsoil contributed the greatest quantities of OC and N to bulk soil (46% and 50% for OC; 45% and 45% for N_t , respectively). In the sandier soil in Obersiebenbrunn, microaggregates $<20 \mu m$ contributed the largest quantities of OC and N to bulk soil (51% and 46% for OC; 51% and 47% for N_t , respectively). Similar observations were done in subsoils. The C:N ratio in the different soil fractions was highest in fPOM, followed by that in oPOM and lowest in the different aggregate size fractions at all sites (Figures 2E, 2F).

Solid state ^{13}C NMR spectra reflected this order with an increasing Alkyl-C/O-alkyl C ratio in the order fPOM $<$ oPOM $<$ bulk soil, in all analyzed fractions (Table 4). Aryl-C increased in the order: fPOM $<$ oPOM $<$ bulk soil, except in the fields in Obersiebenbrunn, the differences

Table 1. Physicochemical and biological properties of the bulk soils studied (n=3). Different letters indicate significant differences (Tukey's post-hoc test, $p < 0.05$).

Tabelle 1. Physiko-chemische und biologische Eigenschaften der Gesamtböden (n=3). Unterschiedliche Buchstaben zeigen statistisch signifikante Unterschiede (Tukey's Post-Hoc-Test, $p < 0,05$).

	Depth (cm)	Obersiebenbrunn Org-OB	Obersiebenbrunn Con-OB	Lasseo Org-LA	Lasseo Con-LA
Physical soil properties					
sand (g kg ⁻¹)	0-15	444 ^a	414 ^a	216 ^b	194 ^b
	30-40	426 ^a	404 ^a	195 ^b	153 ^b
silt (g kg ⁻¹)	0-15	412 ^b	447 ^b	617 ^a	636 ^a
	30-40	420 ^b	442 ^b	602 ^a	653 ^a
clay (g kg ⁻¹)	0-15	144	139	167	170
	30-40	154	155	203	194
Chemical soil properties					
pH (H ₂ O)	0-15	7.96 ^b	7.92 ^b	8.12 ^a	8.04 ^{ab}
	30-40	8.14 ^b	8.14 ^b	8.39 ^a	8.18 ^{ab}
OC (g kg ⁻¹)	0-15	18.0	15.2	18.6	20.0
	30-40	13.1	14.0	13.7	17.3
N _t (g kg ⁻¹)	0-15	1.43	1.37	1.50	1.47
	30-40	1.13	1.17	0.97	1.37
CaCO ₃ (g kg ⁻¹)	0-15	68.3 ^b	65.3 ^b	196 ^a	197 ^a
	30-40	143 ^b	126 ^b	240 ^a	219 ^a
CAL-extractable K (mg kg ⁻¹)	0-15	70.8 ^b	219 ^a	205 ^a	115 ^a
	30-40	41.7	67.9	97.5	97.6
CAL-extractable P (mg kg ⁻¹)	0-15	107	125	89.4	88.5
	30-40	44.2	34.6	29.3	43.8
CEC (mmolc kg ⁻¹)	0-15	193	183	210	244
	30-40	175	174	180	246
BD (g cm ⁻³)	0-15	1.54	1.45	1.38	1.40
	30-40	1.44	1.53	1.46	1.44
Fe _d (g kg ⁻¹)	0-15	5090 ^a	5150 ^a	3250 ^b	3910 ^b
	30-40	4690 ^a	4870 ^a	3530 ^b	3920 ^b
Mn _d (g kg ⁻¹)	0-15	310	315	218	259
	30-40	234	251	177	238
Al _d (g kg ⁻¹)	0-15	554 ^a	575 ^a	396 ^b	492 ^{ab}
	30-40	499	575	435	480
Fe _o (g kg ⁻¹)	0-15	972	1060	844	940
	30-40	749	868	848	902
Mn _o (g kg ⁻¹)	0-15	280	295	197	239
	30-40	211	228	136	228
Al _o (g kg ⁻¹)	0-15	1290 ^a	1380 ^a	1010 ^b	1160 ^{ab}
	30-40	1230 ^a	1290 ^a	934 ^b	1140 ^{ab}
Biological soil properties					
Fungi (µg C g ⁻¹ dry soil)	0-15	12.7	12.7	10.9	15.1
Active fungi (% of hyphal length)	0-15	2.49 ^b	14.0 ^a	0.72 ^b	1.79 ^b
Bacterial biomass (µg C g ⁻¹ dry soil)	0-15	44.4	38.3	68.9	38.3
Mineralizable N (mg kg ⁻¹)	0-15	8.07 ^b	9.40 ^b	31.0 ^a	15.2 ^{ab}
HWC (µg C g ⁻¹)	0-15	317 ^b	346 ^b	510 ^a	403 ^{ab}

Table 2. Mean weight diameter (MWD) of ultrasound stable sand corrected aggregates (<5 mm), free particulate organic matter (fPOM) and occluded particulate organic matter (oPOM) in the studied sites (n=3).

Tabelle 2. Mittlerer gewichteter Durchmesser (MWD) von Ultraschallstabilen Aggregaten (<5 mm), freier partikulärer organischer Substanz (fPOM) und okkludierter partikulärer organischer Substanz (oPOM) an den untersuchten Standorten (n=3).

	Depth (cm)	Org-OB	Con-OB	Org-LA	Con-LA
fPOM (g kg ⁻¹)	0-15	3.57	3.36	5.86	3.85
	30-40	1.57	2.52	2.84	2.22
oPOM (g kg ⁻¹)	0-15	3.34	3.05	3.79	2.46
	30-40	1.51	1.86	1.27	1.35
MWD (mm)	0-15	7.64	9.98	3.82	4.50
	30-40	6.65	8.85	8.42	11.9

were in the opposite direction at 30-40 cm soil depth. Carboxyl-C increased in the order of fPOM < oPOM < bulk soil at all sites.

4. Discussion

4.1 Soil structure in the cropland sites

Evidence for the hierarchical model of aggregates was observed at 0-15 cm soil depth due to different aggregating

agents for micro- and macroaggregates. Several correlations with bulk soil properties were found for micro- and macroaggregates in topsoil (HWC in 20-250 µm aggregates vs. CAL-extractable P, sand, active fungi and Fe_d in >250 µm aggregates), but those were not observed at 30-40 cm depth. Regelink et al. (2015) also observed positive correlations between Fe(hydr)oxides and water stable aggregates in soils from the Marchfeld. The correlation with Fe_o supports the strong aggregating power of (hydr)oxides and is supported by a study by Duiker et al. (2003), which

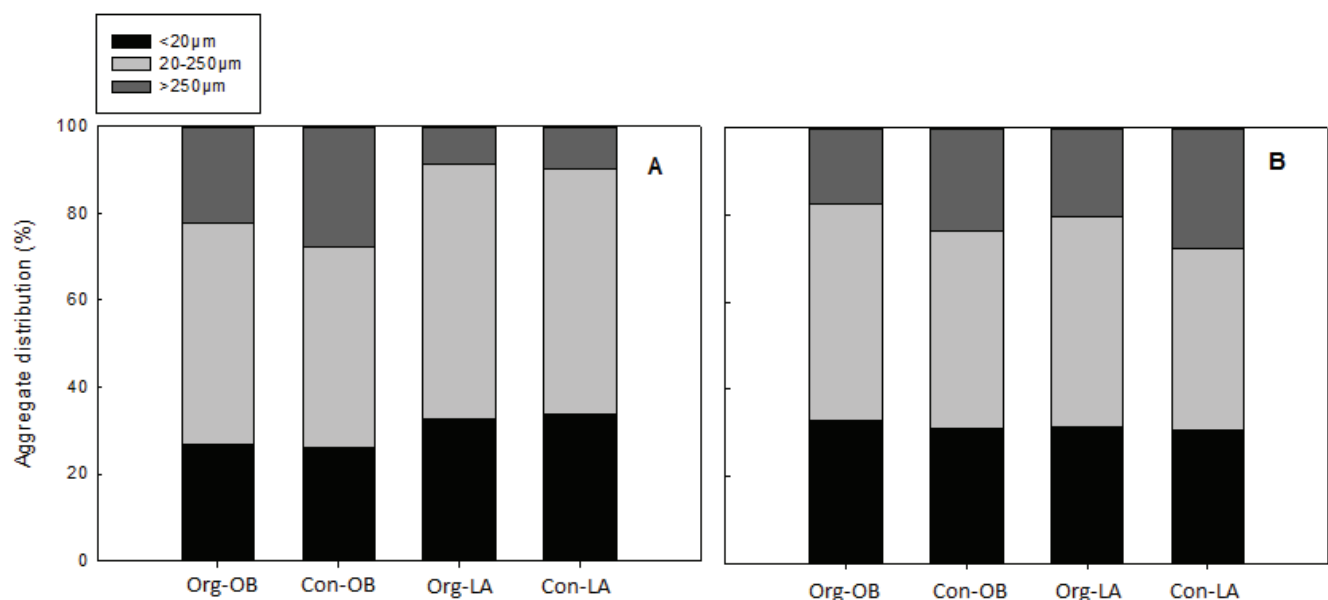


Figure 1. The distributions of micro- (<20 µm, and 20-250 µm) and macroaggregates (>250 µm) in A) 0-15 cm, and B) 30-40cm.

Abbildung 1. Die Verteilung der Mikro- (<20 µm und 20-250 µm) und Makroaggregate (>250 µm) in A) 0-15 cm und B) 30-40cm.

Table 3. Pearson correlation coefficients between the free particulate organic matter (fPOM), occluded particulate organic matter (oPOM), soil aggregate fractions, mean weight diameter (MWD) and soil properties (n=12).

Tabelle 3. Korrelationskoeffizienten (nach Pearson) zwischen der freien partikulären organischen Substanz (fPOM), der okkludierten partikulären organischen Substanz (oPOM), Bodenaggregatgrößen, mittlerem gewichteten Durchmesser (MWD) und allgemeinen Bodenparametern (n=12).

	0-15 cm					30-40 cm						
	fPOM	oPOM	<20 μm	20-250 μm	>250 μm	MWD (mm)	fPOM	oPOM	<20 μm	20-250 μm	>250 μm	MWD (mm)
sand	-0.385	0.111	-0.364	-0.643*	0.697*	0.622*	0.052	0.373	0.059	0.407	-0.401	-0.592*
silt	0.407	-0.110	0.413	0.615*	-0.717**	-0.644*	-0.029	-0.282	-0.111	-0.373	0.410	0.586*
clay	0.196	-0.105	0.020	0.711**	-0.475	-0.398	-0.119	-0.597*	0.138	-0.423	0.268	0.465
pH (H ₂ O)	0.723**	0.296	0.352	0.527	-0.620*	-0.601*	0.186	-0.447	0.214	0.004	-0.164	-0.041
OC	-0.050	-0.357	-0.018	0.467	-0.284	-0.261	-0.279	-0.132	-0.190	-0.168	0.297	0.401
N _t	-0.109	-0.060	-0.299	0.640*	-0.181	-0.115	-0.301	0.021	-0.319	-0.053	0.295	0.313
CaCO ₃	0.466	-0.036	0.354	0.580*	-0.650*	-0.593*	0.022	-0.634*	0.333	-0.502	0.185	0.393
K	0.336	0.189	0.190	-0.010	-0.144	-0.125	0.249	0.008	-0.243	-0.120	0.283	0.308
P	-0.611*	-0.040	-0.427	-0.698*	0.784**	0.762*	-0.027	0.702*	-0.591*	0.278	0.203	0.073
CEC	0.020	0.004	-0.194	0.512	-0.181	-0.131	-0.147	-0.211	-0.321	-0.048	0.292	0.330
BD	-0.104	-0.194	0.111	-0.381	0.162	0.103	0.388	0.505	-0.319	0.000	0.229	0.091
Fe _d	-0.648*	-0.117	-0.404	-0.544	0.668*	0.636*	-0.300	0.242	-0.168	0.189	-0.032	-0.183
Mn _d	-0.682*	-0.216	-0.255	-0.435	0.485	0.468	-0.196	0.554	-0.582*	0.326	0.159	0.040
Al _d	-0.727**	-0.283	-0.394	-0.392	0.565	0.563	-0.294	0.003	0.083	0.082	-0.128	-0.152
Fe _o	-0.651*	-0.403	-0.239	-0.594*	0.576	0.576*	0.274	0.600*	-0.688*	0.141	0.390	0.338
Mn _o	-0.689*	-0.245	-0.183	-0.427	0.425	0.411	-0.154	0.467	-0.425	0.191	0.157	0.081
Al _o	-0.641*	-0.215	-0.285	-0.454	0.519	0.513	-0.373	0.313	-0.284	0.163	0.080	-0.041
Fungal biomass	-0.194	-0.419	-0.260	0.164	0.099	0.102						
Active fungi	-0.433	-0.053	-0.438	-0.523	0.678*	0.707*						
Bacterial biomass	0.679*	0.547	0.263	0.275	-0.390	-0.439						
Mineralizable N	0.872***	0.275	0.504	0.499	-0.719**	-0.734**						
Hot-water extractable carbon (HWC)	0.789**	0.111	0.328	0.679*	-0.699*	-0.682*						

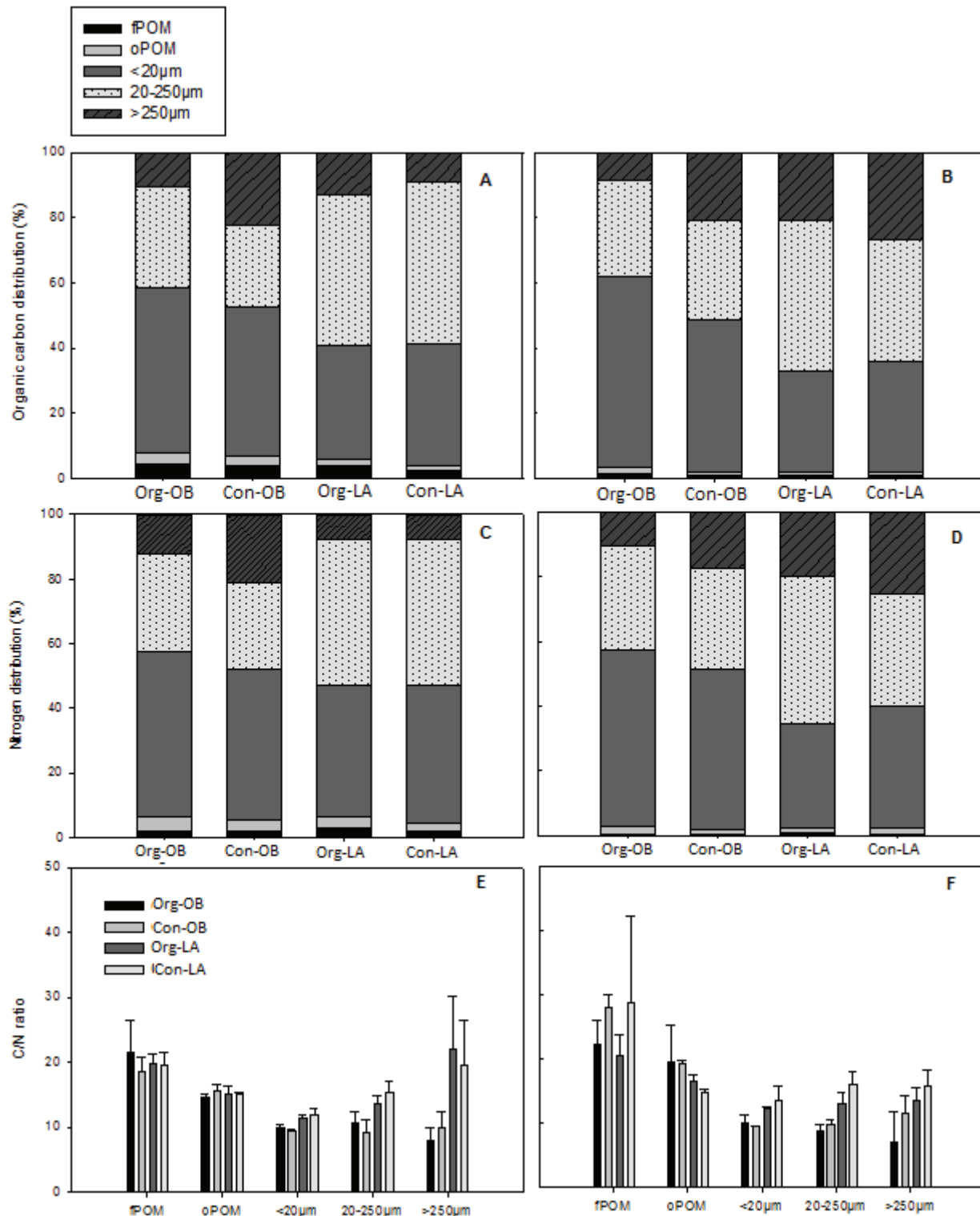


Figure 2. The C and N distribution within particle-size fractions and C:N ratios of different soil fractions in A, C, E) 0-15 cm, and B, D, F) 30-40 cm. (Note: The C content of each fraction was calculated by taking total soil C as the sum of the C associated with all separate particle-size fractions, including POM fractions).

Abbildung 2. Verteilung von C und N in den Korngrößenfraktionen und das C/N-Verhältnis der verschiedenen Bodenfraktionen in A, C, E) 0-15 cm und B, D, F) 30-40 cm. (Anmerkung: Der C-Gehalt jeder Fraktion wurde berechnet, indem der gesamte C aus der Summe des mit allen Korngrößenfraktionen, einschließlich POM-assoziierten C, berechnet wurde).

Table 4. Integrated chemical shift regions (% of total signal intensity) obtained by ^{13}C CPMAS NMR spectroscopy for the extracted free particulate organic matter (fPOM), occluded particulate organic matter (oPOM), and bulk soil for the studied sites.

Tabelle 4 Integral der chemischen Verschiebungen (% der Gesamtsignalintensität) in ^{13}C CPMAS NMR-Spektroskopie für die extrahierte freie partikuläre organische Substanz (fPOM), die okkludierte partikuläre organische Substanz (oPOM) und den Gesamtboden der untersuchten Flächen.

	Depth (cm)	Org-OB	Con-OB	Org-LA	Con-LA
fPOM					
Alkyl-C (%)	0-15	15.0	16.7	15.8	16.0
	30-40	15.0	19.0	16.0	17.0
O-Alkyl-C (%)	0-15	49.3	48.7	53.0	43.4
	30-40	37.0	38.1	45.0	48.0
Aryl-C (%)	0-15	23.9	23.1	21.4	26.5
	30-40	33.0	29.4	27.0	25.0
Carboxyl-C (%)	0-15	11.8	11.8	9.84	14.1
	30-40	15.0	13.4	12.0	10.0
Alkyl-C/O-Alkyl-C	0-15	0.34	0.34	0.30	0.37
	30-40	0.41	0.50	0.36	0.35
oPOM					
Alkyl-C (%)	0-15	20.1	19.4	18.1	19.0
	30-40	22.5	25.0	17.0	20.0
O-Alkyl-C (%)	0-15	44.6	48.5	46.0	43.5
	30-40	41.5	36.0	36.0	38.0
Aryl-C (%)	0-15	23.9	22.2	24.9	27.1
	30-40	24.4	26.0	32.0	30.0
Carboxyl-C (%)	0-15	11.5	9.92	11.0	10.3
	30-40	11.5	13.0	15.0	12.0
Alkyl-C/O-Alkyl-C	0-15	0.45	0.40	0.39	0.44
	30-40	0.54	0.69	0.47	0.53
Bulk soil					
Alkyl-C (%)	0-15	21.4	23.5	20.9	21.4
	30-40	21.9	23.3	19.7	21.4
O-Alkyl-C (%)	0-15	35.6	36.4	36.2	35.4
	30-40	32.5	33.6	29.9	31.6
Aryl-C (%)	0-15	26.4	24.6	27.7	28.3
	30-40	28.1	26.5	32.6	30.5
Carboxyl-C (%)	0-15	16.7	15.5	15.2	14.9
	30-40	17.5	16.6	187.9	16.5
Alkyl-C/O-Alkyl-C	0-15	0.60	0.65	0.58	0.61
	30-40	0.68	0.69	0.66	0.68

showed the more active role of Fe_0 over Fe_d in aggregation, in contrast to our results. Very stable aggregates can be formed when amorphous $\text{Fe}(\text{OH})_3$ (estimated as Fe_0 in our study) and SOM interact (Barral et al., 1998). Oxides have high surface areas and can adsorb organic material on their surface by electrostatic binding, and thereby enhance

aggregation (Six et al., 2004). According to Amézqueta (1999), soil structure is improved in the presence of oxides due to them acting as flocculants in solution, their ability to bind clay particles to organic matter (OM), and their ability to precipitate as gels on particle surfaces.

The positive correlation between active fungal biomass and the amount of macroaggregates and MWD at the 0–15 cm soil depth may be explained by organic inputs entering the soil and providing substrate for soil fungi (Eash et al., 1994). Fungi exude polysaccharides that adhere to minerals in the soil (Saccone et al., 2012; Gazzé et al., 2013). This will physically aid the association of soil particles into larger aggregates when fungal growth increases and hyphae enmesh soil particles (Eash et al., 1994). The soils of our study had a high pH of approximately 8, which was more favorable for bacterial biomass compared to fungal biomass. A study by Rousk et al. (2010) confirmed that the relative abundance of bacteria was positively related to pH, which agrees with our results on high bacterial biomass in these high pH soils. In addition, soils in our study were annually ploughed, which may have further decreased the abundance of fungi. Con-OB had higher alkyl-C (lipids) contents compared to Org-OB, which can improve the aggregation due to their hydrophobic nature (Monreal et al., 1995; Dinel et al., 1997; Pare et al., 1999).

The difference in the amount of macroaggregates at 0–15 cm soil depth among sites (Obersiebenbrunn versus Lassee) may be a result of significant differences in the concentration of carbonates and particle size fractions of the soils. In general, aggregation may decrease with an increase in the concentration of carbonates because they mainly contribute to the silt and sand fractions that have a negative effect on aggregation (Dimoyiannis et al., 1998; 2012), which is in accord with results obtained in our study. However, carbonates may also act as cement compounds and bind microaggregates together (Lehtinen et al., 2014) and, thus, increase aggregation, as may be seen in Lassee between the soil depths.

4.2 SOM in the cropland sites

There were no significant differences in OC and N_t concentrations between sites, which contradicts recent studies that have found difference between different soil managements (Leifeld and Fuhrer, 2010; Gattinger et al., 2012), but are in accord with some earlier studies (Kirchmann et al., 2007; Leifeld et al., 2009; Spiegel et al., 2010). This may be explained by the fast decomposition of SOM in the Pannonian environmental zone. Organic inputs were 10 Mg ha⁻¹ year⁻¹ of biowaste compost at Org-OB and 20 Mg ha⁻¹ of horse manure every fifth year at Org-LA, respectively. Erhart and Hartl (2010) concluded that 6–7 Mg ha⁻¹ year⁻¹ of compost should be sufficient to maintain the

SOM content in soils under similar climate and cultivation (16 Mg ha⁻¹ year⁻¹ when aiming to maintain N_{org} levels (Hartl and Erhart, 2005), however, the amounts at Org-LA were slightly lower in our study. The soils at all studies fields were ploughed annually, which causes oxidation of OC (West and Post, 2002), and prevents OC accumulation. Besides, harvesting as well as seedbed preparation at all sites causes severe additional disturbance to the annual ploughing which increases SOM decomposition and mineralization (Pulleman et al., 2003). At all sites, relatively small amounts of POM were observed, which showed no differences among sites. In a previous study (Simonsson et al., 2014), the higher variation in POM than in OC was limiting the use of POM as an indicator of OC dynamics; that may contribute to the present study as well.

4.3 SOM distribution and chemical quality in the cropland sites

No significant differences in the SOM distributions among the sampled fields were observed in the present study. In the sites with significantly higher sand content (Obersiebenbrunn) as compared to the other sites (Lassee), the 20–250 µm aggregate associated OC and N_t fractions were the highest and less susceptible to mineralization. In contrast, in the sites with significantly higher silt content and slightly higher clay content, Lassee compared to Obersiebenbrunn, the <20 µm aggregate associated OC and N_t fractions had the greatest OM storage capacity. These results are in accord with those reported by Poll et al. (2003). The distribution and dynamics of N_t content paralleled those of the OC content. fPOM and oPOM associated OC and N_t were the smallest fractions in all soils, reflecting the annual tillage activities that result in fast decomposition of easily available OM. Further, the C:N ratio of soil fractions decreased from POM fractions to the aggregates, indicating that the nitrogen-rich organic matter was associated with mineral particles and reflecting the plant-like character of fPOM and oPOM (Baldock et al., 1997; Golchin et al., 1997). Because the C:N ratios for the different aggregate classes were fairly similar, no clear aggregate hierarchy existed in our soils. In case of a clear aggregate hierarchy, a higher C:N ratio in macroaggregates compared to microaggregates indicates a higher content of labile OC (Six et al., 2004).

The higher proportion of alkyl-C of the total OC in fPOM in Con-OB compared to Org-OB, may reflect the differences in fertilization. The biowaste compost used

as a fertilizer consists of humified organic matter (Erhart and Hartl, 2010). Therefore, alkyl-C that represents lipids and hemicelluloses (Golchin et al., 1994), was observed in lower proportion of the total OC in Org-OB compared to Con-OB. The analyzed fractions showed an increasing degree of decomposition in the order fPOM < oPOM < bulk soil, shown as increased Alkyl-C to O-Alkyl-C ratio (Baldock et al., 1997). The data presented herein indicate that the fPOM and oPOM consisted mainly of plant material at different stages of decomposition, and were less decomposed compared to the SOM in the bulk soil.

5. Conclusions

Our study has demonstrated that microaggregates in the range of 20–250 µm were the most prominent soil aggregates in both topsoils and subsoils in the studied Haplic Chernozem cropland soils in Austria. The content of macroaggregates and MWD were correlated with CAL-extractable P, fungal activity, sand content and dithionite-extractable Fe. The <20 µm aggregates in Obersiebenbrunn and 20–250 µm aggregates in Lassee contained the most OC and Nt. The distribution of Nt content paralleled those of the OC content. The data support the aggregate hierarchy model in topsoils due to different aggregating agents for micro- and macroaggregates but not in subsoils due to lacking aggregating agents. Additional research is needed on cultivated Chernozems to obtain quantitative basis for evaluating whether it may be beneficial to use biowaste compost and horse manure as organic inputs, in order to increase SOM content and macroaggregation as well as long-term soil fertility.

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