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Carbon Isotope Study of Soil Amendment with Maize Fermentation Digestate

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Authors' contributions

This work was carried out in collaboration between all authors. Author FB realized first version of manuscript including literature and statistics. Authors MG and EG were responsible for experiment realization and sampling procedures. Authors BC, IJ and ZL were responsible for all type of analysis, including composite samples, method reproducibility evaluation and all statistics. All authors participate in the final version and agreed with this final version.

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ABSTRACT

Maize digestate was applied to follow its sequestration in arable soil profiles with accent on changes in soil organic carbon (SOC) content and the production of dissolved organic matter (DOM). Two control sites with cultivated Cambisols (rye-grass, kohlrabi) were amended by the addition of digestate from maize silage fermentation. Liquid digestate was applied once in the spring in the quantity of 18 g C/ m^2. The process of amendment was checked after 4 and 12 months by the use of natural labelling of the amendment by the difference in the carbon isotope composition of the SOC (C_3 vegetation) and applied digestate (C_4 maize).

The amendment was evaluated from the SOC content, dissolved organic carbon (DOC) and their $\delta^{13}C$ isotope composition by sampling across the soil profiles (0–90 cm). An increase of the carbon content by the amendment was measurable in the upper soil layers (0–20 cm) only. The deep soil

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layers are poor in carbon content (less than 0.2% of SOC) but rich in clay micro particles, which efficiently adsorbed incoming DOC with infiltrated precipitation. Change of the $\delta^{13}C$ of soil carbon was measurable down to -80 cm.

Applied digestate increased the carbon content of top soil layer only. We did not observe significant migration of C_4 amendment from top soil to the deeper soil layers. Digestate addition increased DOC production at both sites for the upper soil layers only. DOC released from the deep soil layers originates from the upper parts of the soil profile and not from the native carbon in the layer.

Keywords: 13C; digestate; dissolved organic carbon; soil amendment.

1. INTRODUCTION

Digestate is a by-product of the anaerobic digestion process, which produces methane and heat in biogas plants. With the increasing number of installed biogas plants (after implementation of the Renewable Energy Source Act), the production of digestate has also increased. The main components of digestate are plant cell wall polymers (cellulose, hemicellulose and lignin) which are difficult to digest [1]. The main use of the digestate is a soil additive (fertilizer), solid fuel or material for production of particleboard. Digestate contains high amounts of plant nutrients (organic carbon, phosphorus, potassium and a high portion of nitrogen mineralised to ammonium), which affect C and N dynamics in soils [2]. Digestate, compared to untreated organic waste, contains stabilized microbes what stimulates microbial soil activity and enhances the soil organic matter pool (SOM) [3]. Numerous results of field or pot tests point out the positive effects of digestate in terms of both the crop yield and soil quality - it will enhance the fertility of soils due to their vast amount of plant nutrients [2,4] like plant available ammonium, potassium and phosphorus [5,6]. Besides these positive properties, digestates are suspected to have negative impacts on soils: i) the addition of easily degradable carbon together with microbes can increase organic matter degradation with increasing CO_2 production [7]; ii) digestate addition transfers recalcitrant amphiphilic substances (humic, fulvic and fatty acids) into the soil matrix [4]; a high content of hydrophobic organic material in digestate increases soil water repellency and reduces the wettability of soil particles and decrease soil water content [8,9]. The addition of soluble monovalent cations from digestate changes the ionic equilibrium in soil and microbial activity. Soils with a high surface area (i.e. those that are clay rich) are more heavily influenced by the addition of digestate than sandy soils [8].

Labelling with natural abundances of ^{13}C stable isotope is a proven technique for the examination of soil C dynamics [10]. The difference in the natural abundances of ^{13}C between C_3 vegetation ($\delta^{13}C$ = -32 to -22‰) and C_4 ($\delta^{13}C$ = -10 to -17‰) – is sufficient to provide the basis for the estimation of the contribution of ^{13}C-enriched maize digestate to SOM in ecosystems with C_3 vegetation. The reproducibility of $\delta^{13}C$ measurements 0.1‰ corresponds to the resolution 0.7% of addition of maize material to the soil sample [11].

The overall objectives of the present study were: i) to follow the sequestration of added amendment in arable soil profiles with accent on the stability of amendment, ii) to trace the changes in the production of dissolved organic matter (DOM) after amendment. To this end, we measured soil organic carbon (SOC) content and ^{13}C isotopic composition in soil profiles, digestate and dissolved organic carbon (DOC) before and after amendment.

2. SITE EXPERIMENTALS

2.1 Soils

Sampling sites were localised in the SE part of the Czech Republic in the agricultural region of South Moravia, close to the Austrian border (49°31’N, 165°58’E). Area belongs to middle warm and humid continental climate with prevailing Cambisols. The characteristics of the sites are given in Table 1.

Rectangle fields with areas 80 and 200 m^2 were divided to the net of 1 m^2 squares and numbered. Three sampling plots were randomly selected inside of each site using a random number generator. In the center of each sampling plot we collected two discrete subsamples in the distance of 20 centimeters approximately to form one composite sample from the plot [12]. Cores were taken by the soil probe from 10 depth intervals: 0–5 cm and 5–10 cm (about 50 g each – because of a high carbon content), and further every 10 cm (about 100 g)

Table 1. Basic characteristics of the experimental sites

Site	Vatin	Habruvka
Area (m^2)	80	200
GPS coordinates	49°31’30’’N, 15°58’2’’ E	49°18’13’’N, 16°43’25’’E
Soil type	Eutric Cambisol	Mesotrophic Cambisol
Altitude (m a.s.l.)	535	505
Average temperature (℃)	6.9	7.5
Annual precipitation (mm)	617	644 + occasional irrigation
Crop	Rey grass*	Kohlrabi or celery (vegetable), grass*
Soil profile (cm)	0–90	0–90
Organic C content (%)	From 1.7 to 0.05	From 2.0 to 0.05
Sand/silt/clay content in Ap (%)**	42/36/22	41/34/25
pH (in water 25℃)	6.33	6.12
pH (in 1M KCl 25℃)	4.70	4.82
C/N ratio (w/w)	15 to 9	18 to 11
$\delta^{13}C$ (‰)	-28.2 to -25.8	-28.5 to -25.5
Amendment (gC/m^2) 8th April 2014	18	18

** no C_4 weed observed*

***according to hydrometer method [21]*

until 90 cm depth. Soil controls were taken only once at the beginning of the experiment (April 2014). On three or four squares around the control sampling plot we applied digestate (Table 2) in the quantity of 1.5 L of solution i.e 18 g C per each 1 m^2 square. After the application of digestate, the amended area was watered by additional 10 L /m^2 water. Soils with amendment were sampled randomly around the control plots, in the center of amended square, again with two discrete subsamples for each plot. Cultivated soils are regularly ploughed and natural Ap (from surface to -20 cm) and B horizons (from -20 to -50 cm) are frequently mixed together, so measured depth intervals are more useful for the observation of sequestration. Soils with amendment were sampled twice: first after 120 days (September 2014) after haymaking and kohlrabi picking, and secondly after one year (April 2015). Places after first sampling were assigned to be not sampled during the second sampling.

The soil samples were sieved through a 2 mm sieve, dried at 50℃ to a constant weight, homogenized to 60 µm and mixed together to composite samples. Composite samples used for carbon content and $\delta^{13}C$ measurements were acidified (10% hydrochloric acid) to remove carbonates, then washed and dried. We collected three composite samples from each sampling depth – all together 30 samples for each sampling (control i.e. 1.day, after 120 and 365 days).

Table 2. Chemical and isotopic composition of applied digestate

Dry matter (d.m.) (%)	3.54
pH	8.4
Organic C (% d.m)	33.9
$\delta^{13}C$ (‰)	-16.8±0.2
NH_4^+ N (% d.m.)	5.72
NO_3^- N (% d.m.)	<0.003
Ntot (% d.m.)	10.2
Ca (% d.m.)	1.7
Mg (% d.m.)	0.6
K (% d.m.)	6.65
Ptot (% d.m.)	1.11
B (% d.m.)	0.005
Cu (% d.m.)	0.012
Mn (% d.m.)	0.029
Mo (% d.m.)	<0.001
Zn (% d.m.)	0.055

2.2 DOM

Homogenised composite samples, not acidified, were mixed with distilled water (about 10 g of soil with 10 ml of water), sonified for about 10 min at room temperature, left for an additional 20 min and filtered (through 5 and 0.45 µm cellulose filters). The resulting solution was used for DOC concentration and $\delta^{13}C$ measurements.

2.3 Analytical Methods

The organic C content was determined with a Fisons 1108 elemental analyzer on the homogenised and acidified samples with standard error about 3% from the measured value - according to replicates of internal reference soil with 0.8% of carbon. The sample size (maximal weight about 35 mg) is limiting for the quality of measurement of the samples with very low carbon content. Samples with carbon content below 0.1% have standard error up to 10% (according to replicates of low carbon content samples). The $\delta^{13}C$ measurements were performed by flash combustion in a Fisons 1108 elemental analyzer coupled with a Delta V isotope ratio mass spectrometer in a continuous flow regime. The isotope measurement is more sensitive than elemental analysis - external reproducibility of $\delta^{13}C$ measurement is about 0.1‰ [11] and the sample size is not limiting. The acid treatment does not change the isotopic composition of the studied soils [13].

The concentration of DOC was measured on an Apollo 9000HS analyzer; the remainder of the solution was acidified with diluted phosphoric acid to remove bicarbonates, and further concentrated by evaporation (at 50℃) for $\delta^{13}C$ of DOC measurements (Fisons 1108 and DeltaV with NBS 22 as internal reference).

Carbon isotope composition is measured in $\delta^{13}C$ delta units (Equation 1):

$$\delta^{13}C\ (‰) = \{(^{13}C/^{12}C)_{sample} - (^{13}C/^{12}C)_{standard}\}/(^{13}C/^{12}C)_{standard} * 1000 \quad (1)$$

where, $(^{13}C/^{12}C)_{standard} = (^{13}C/^{12}C)_{PDB} = 0.0112372$.

As isotopic composition is the molecular property of the monitored element, a simple mass balance is valid for carbon isotopes in soil (^{13}C isotopes in soil organic are sum of ^{13}C of all components):

$$\delta^{13}C_{tot} * n_{tot} = \Sigma\ \delta^{13}C_i * n_i \quad (2)$$

where the indices *tot* corresponds to the total soil organic C (SOC plus amended C) and *i* corresponds to its components - original SOC and amended C in our case.

2.4 Data Analysis

We use composite samples to reduce number of samples for analysis. All analyses were made from three composite samples for each sampling point. Data are presented as mean values of three samples (in the figures). The standard deviation of measurement was calculated by the standard method from three measurements. An estimate of the confidence interval for normal distribution and three replicates was calculated from Equation (3) [14]:

$$X = X_{avg} \pm t_\alpha \times s/\sqrt{3} \quad (3)$$

where X_{avg} is the mean value of measurement, s is the standard deviation and t_α equals 4.3 for the 95% confidence interval and normal distribution - the standard deviation should be multiplied by about 2.5 [14].

The quality of amendment was evaluated by two-sample t-test of control and amended soils. With three composite samples from each site and depth layer we have to simplify assumption. We assume the normal distribution of measured samples and equal means as the null hypothesis [14].

3. RESULTS AND DISCUSSION

3.1 Carbon Content of Soils

The carbon content of the control and amended soils in both localities is presented in Fig. 1. Carbon amendment is measurable in the upper layers only (down to -40 cm). The deeper layers have very low carbon content (less than 0.2%) and practically no measurable amendment. For the top soil layer, the Habruvka site has more C than Vatin; below 20 cm the Vatin site is enriched. For deeper layers, with low carbon content, a difference between the control and amended soils is overlapped by the ± 5% uncertainty range of a potential of the elemental analyzer (method has sample weight limit about 35 mg). In particular, the Habruvka site indicates for deeper layers a loss of carbon after amendment instead of addition, because of measurement error and probable inhomogeneity of deep soil layers.

In amendment, we added 18 g C per m^2, which corresponds to about 30% of total organic carbon in 5 cm of the top soil. This means that the differences in carbon content are reasonable in the upper layers, but hardly measurable in deeper layers with very low carbon content. Possible effect of the acid treatment on amendment carbon is unknown; we assume that amendment is insoluble in diluted acid as humic acids are and any possible loss would be visible on $\delta^{13}C$ value of SOC. The extent of amendment was tested by two-sample t-test (Table 3). The increase of carbon content by amendment is not significant at depth 5cm on the Vatin site (two tails probability P(T<=t) (2) is .065 against presumed .05). Increase of carbon content is significant on Hrabuvka site down to the depth 40 cm.

3.2 δ^{13}C of Vatin Site

The results of amendment are presented in Fig. 2. The changes of carbon isotopic composition of SOC with amendment are more significant than the changes of soil carbon content in Fig. 1. A shift of δ^{13}C from the control soil values to less negative (i.e. more enriched by ^{13}C) amended soil values is visible down to depth 80 cm of depth profiles of the Vatin site, even four months after application. The difference decreases with increasing depth from 1 to about 0.3‰ at the depth 80 cm.

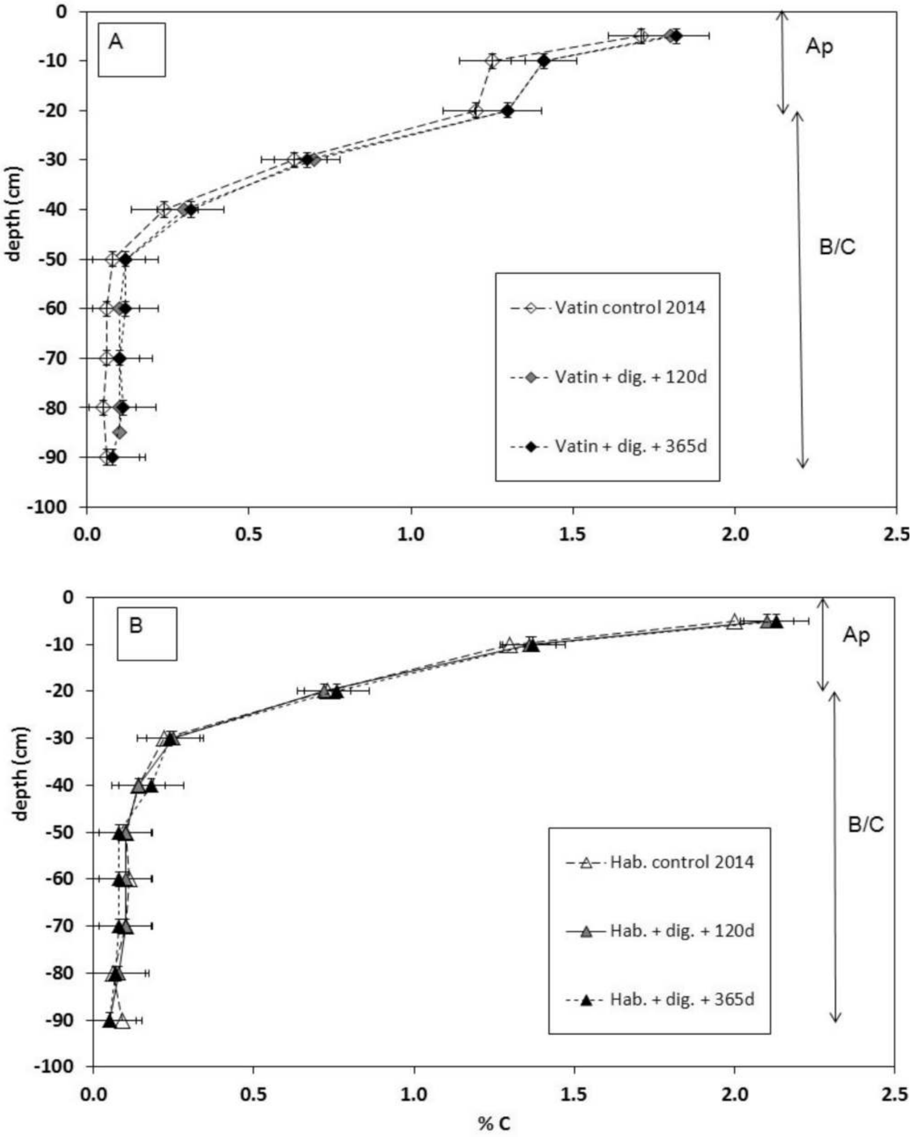

Fig. 1. Carbon content of studied sites – Vatin (A) and Habruvka (B). Empty signs correspond to control plots mean values, full signs are mean values of soils with applied digestate: grey after 120 days and black after 365 days. Horizontal error bars refer to ±5% error in carbon content; vertical error bars refer to uncertainty in sampling depth estimates. Points are localized at the bottom of sampling interval

Table 3. Two-sample t-test of carbon content, P = .05, equal variances

	Vatin control	Vatin amendment 1 year	Hrabuvka control	Hrabuvka amendment 1 year
-5 cm				
Mean value (%)	1.71	1.82	1.99	2.14
Variance	0.004133	0.000933	0.0049	0.0036
Hyp. Difference	0	0	0	0
t Stat	-2.51443		-2.81801	
P(T<=t) (1)	0.032873		0.023962	
t krit (1)	2.131847		2.131847	
P(T<=t) (2)	.066		.048	
t krit (2)	2.776445		2.776445	
-40 cm				
Mean value (%)	0.21	0.31	0.13	0.09
Variance	0.000633	0.002633	0.000633	0.0001
Hyp. Difference	0	0	0	0
t Stat	-3.23249		2.98481	
P(T<=t) (1)	0.01595		0.020273	
t krit (1)	2.131847		2.131847	
P(T<=t) (2)	.032		.041	
t krit (2)	2.776445		2.776445	

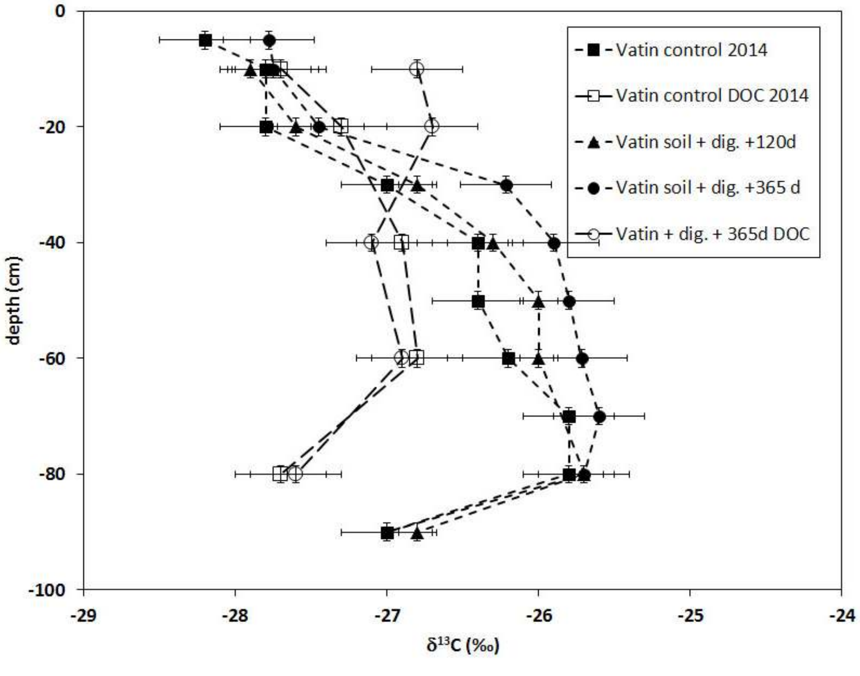

Fig. 2. Depth profiles of δ^{13}C values of the Vatin site. Full points represent control site and site with amendment (after 120 and 365 days), empty points correspond to their DOC (control and 365 days). Error bars refer to ± 2σ value

At the deepest part of the Vatin profile (-90 cm) the carbon content of soil is very low (about 0.1%) and δ^{13}C values of control and amended soils diverge to the same more negative value. Usually, the δ^{13}C of SOC changes to less negative values with increasing depth [10]. The top soil contains more “fresh” carbon from recent or young plant residues with more negative δ^{13}C values. In deeper layers the carbon content decreases and $\delta^{13}C$ value of residual "older" carbon is less negative because of carbon degradation and exhaustion [15,16].

The $\delta^{13}C$ values of DOC from top soil layers and $\delta^{13}C$ value of SOC at depth 90 cm are very close. We assume that divergence of $\delta^{13}C$ of deep SOC does not originate from subsoil or parent soil but it is adsorbed DOM originating from the top soil (Fig. 2). DOM released from the shallow soil layers moves down with the soil infiltration to be adsorbed in the layers rich of clay microparticles. The most stable forms of DOM are stabilized by sorption on mineral soils [17,18]. The extent of vertical transport of DOM from amended top soil can be estimated from the difference between $\delta^{13}C$ values of SOC measured 120 and 365 days after amendment (Fig. 2). Prolonged interval enables deeper and more intensive infiltration of soil solution with DOM from the top. It results in more significant shifts of $\delta^{13}C$ of SOC to less negative values without measurable change in carbon content of layers between depths 30 – 70 cm (Fig. 1).

The depth profiles of the DOC $\delta^{13}C$ values from control and amended soils differ in shallow soil layers only. For the top soil part (depth 10 cm) of the control soil the $\delta^{13}C$ values of DOC and SOC are identical. The amended soil between depth 10 – 20 cm has $\delta^{13}C$ values of DOC about 1‰ less negative than SOC. With increasing depth (down to 40cm), the DOC $\delta^{13}C$ values are more negative than SOC (0.5–0.7‰ for the control soil, 1.5–1.7‰ for the amended soil). Bellow depth 40 cm the $\delta^{13}C$ values of DOC of the control and amended soils do not differ. The deepest layer (-80 cm) has $\delta^{13}C$ value of DOC the same for both the control and amended soils and identical with the DOC from the upper layer (depth 10 cm) of control soil.

Deep soil layers with low content of "old" carbon lack easily degradable components, and release only very limited amounts of DOC components. The $\delta^{13}C$ values of DOC of the control soil are very similar between 20 and 80 cm of soil depth (for amended soil between 40 and 80 cm of soil depth). According to the similarity of $\delta^{13}C$ values of DOC we assume that DOC here does not originate from the native carbon in the layers but in shallow upper layers. It means that below the depth 40 cm at the Vatin site, the amendment of maize digestate does not have effect on the DOC $\delta^{13}C$ values. Two-sample t-test (Table 4) shows significant differences between the mean values of control and amended Vatin soils. Differences are significant along the whole profile, although the carbon content below 40 cm is very low.

Table 4. Two-sample t-Test of $\delta^{13}C$ of soil carbon. P = .05, equal variances

	Vatin control	Vatin amendment 1 year	Hrabuvka control	Hrabuvka amendment 1 year
-5 cm				
Mean value (‰)	-28.2	-27.3833	-28.53	-27.76
Variance	0.123	0.063	0.13	0.063333
Hyp. Difference	0	0	0	0
t Stat	-3.20713		-2.88874	
P(T<=t) (1)	0.016339		0.02231	
t krit (1)	2.131847		2.131847	
P(T<=t) (2)	.033		.045	
t krit (2)	2.776445		2.776445	
-30 cm				
Mean value (‰)	-26.96	-26.2	-27.5	-26.83
Variance	0.063333	0.12	0.09	0.043333
Hyp. Difference	0	0	0	0
t Stat	-3.10132		-3.16228	
P(T<=t) (1)	0.018087		0.017055	
t krit (1)	2.131847		2.131847	
P(T<=t) (2)	.036		.034	
t krit (2)	2.776445		2.776445	

3.3 $\delta^{13}C$ of Habruvka Site

Data of measured $\delta^{13}C$ values are presented in Fig. 3. Depth profiles of SOM for both the control and amended soils are comparable to those of the Vatin site. The $\delta^{13}C$ values of amended soil are less negative than the control values (again about 1‰ or less), with the largest difference at 60 cm depth (about 1.5‰). The prolonged time (120 vs 365 days after amendment) increases infiltration of DOC to deeper soil layers. DOC is adsorbed on clay micro particles and shifts $\delta^{13}C$ of SOC measured here to less negative values (higher adsorption with longer time). Contribution of adsorbed DOC to the net carbon content is low and not measurable.

Similarly to Vatin site the control soil has $\delta^{13}C$ values of SOC and DOC identical at 10 cm depth. With increasing depth, the $\delta^{13}C$ value of DOC is nearly constant with increasing difference against SOC (up to 3‰) at depth 70 cm. We assume that, all DOC along the depth profile of control soil originate from the upper shallow layers only.

The amended soil has $\delta^{13}C$ values of DOC less negative than SOC in depth 10-20 cm. With increasing depth, the $\delta^{13}C$ DOC values become progressively more negative, being comparable with DOC of the control soil at about 90 cm depth. Similar to the Vatin site, the $\delta^{13}C$ values of DOC from deepest layers for both the control and amended soils are the same, i.e. there is not effect of amendment (within the range of measurement uncertainty).

The variances of $\delta^{13}C$ values of control and amended soils were evaluated by two-sample t-Test (Table 4). Measured values are quite homogenous and significantly different along the profile.

3.4 DOC Production

The depth profiles of $\delta^{13}C$ of DOC at both experimental sites differ because of different DOC production. We calculate the DOC production of the soil layer from measured DOC concentration and carbon content of given soil layer (Fig. 4). Evaluation of DOC production enables direct comparison of available DOM in soil layers.

The Vatin site has lower DOC production than the Habruvka site along all profiles. At the depth 10 cm, amended Vatin soil produces more DOC than the control. The remainder of the profile is comparable for both soils. Also, $\delta^{13}C$ DOC values of the control and amended soils are comparable (see Fig. 2), which means that there is no amendment effect on DOC production below the depth 10 cm. An increase of the Vatin DOC production in the deepest part (Fig. 4) results

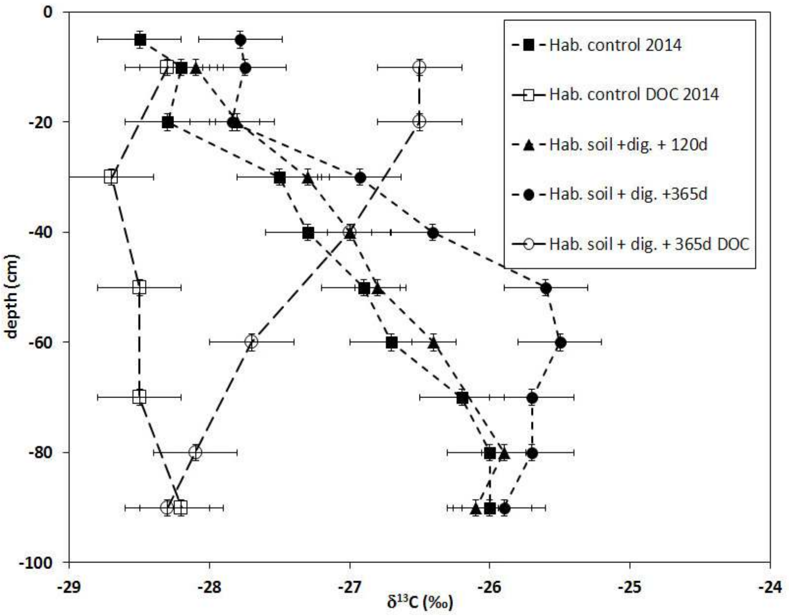

Fig. 3. Depth profiles of $\delta^{13}C$ values of the Habruvka site. Full points represent control site and site with amendment; empty points correspond to their DOC. Error bars refer to ± 2σ value

probably from the instability of adsorbed DOM here (the same $\delta^{13}C$ of DOC in the control and amended soils) and is not significant within given error range.

The Habruvka site has higher DOC production from amended soil down to depth 60 cm. The deepest layers have comparably low production for both control and amended soils. The decrease of DOC production with depth corresponds with change of $\delta^{13}C$ values of DOC (Fig. 3) from less negative values at shallow layers (a mixture of control DOC and leached DOC from top soil amendment) soil DOC production) to more negative at deeper layers (control soil DOC production only).

While evaluation of DOC production per gram of soil is related to the soil quality, that of DOC production per gram (or milligram) of available carbon is related to the carbon quality (Fig. 5). Fresh “young” carbon in the upper soil layers is easily degradable and produces more DOC than “old” complex carbon from the deep soil layers [19,20]. For our control soils, DOC production from top soil is below 1 µg.mg^{-1} for the Vatin site and 1–2 µg.mg^{-1} for the Habruvka site. Higher DOC production in the deeper layers does not originate from native carbon but instead from released adsorbate: see the Habruvka amended soil DOC production in Fig. 4.

Table 5. Estimates of carbon isotope composition of the residual amendment (calculated for amendment higher than 0.1% C)

Depth	Vatin site		Habruvka site	
	$\delta^{13}C$ amend	C amend	$\delta^{13}C$ amend	C amend
(cm)	(‰)	(%)	(‰)	(%)
-5	-15.3	0.11	-13.1	0.13
-10	-28.1	0.16		
-20	-23.2	0.10		

Using Equation (2), we could estimate $\delta^{13}C$ of residual amendment after a year. Unfortunately, the carbon content data do not allow such a calculation for most of the samples. We calculated estimates of isotopic composition of amendment for carbon content higher than 0.1% only (Table 5). We see that, the $\delta^{13}C$ values of amendment vary around -13 to -15‰ at depth 5 cm, the remainder of the estimates is close to DOC values. In spite of inaccuracy of such estimates this is the most important point of our

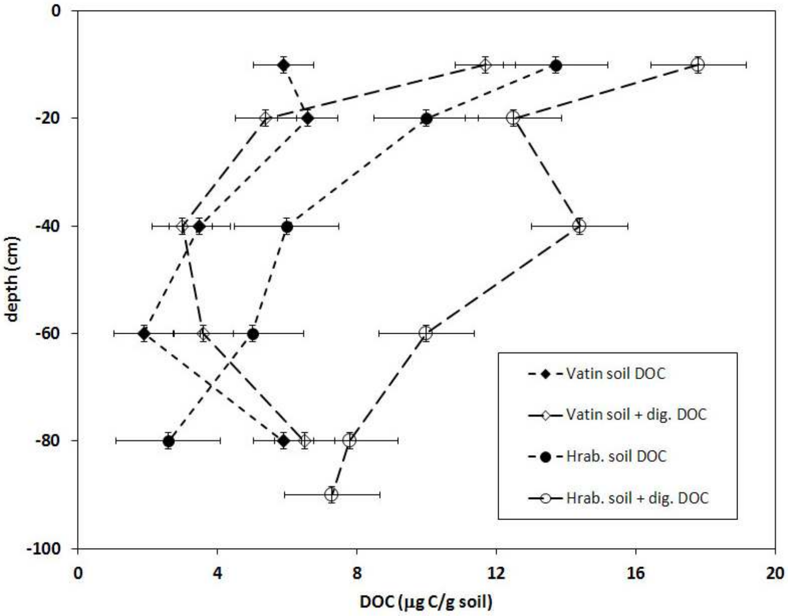

Fig. 4. Production of DOC calculated as µg of DOC per gram of dry soil. Control production in 2014 and a year after amendment are compared. Error bars correspond to standard error of calculation

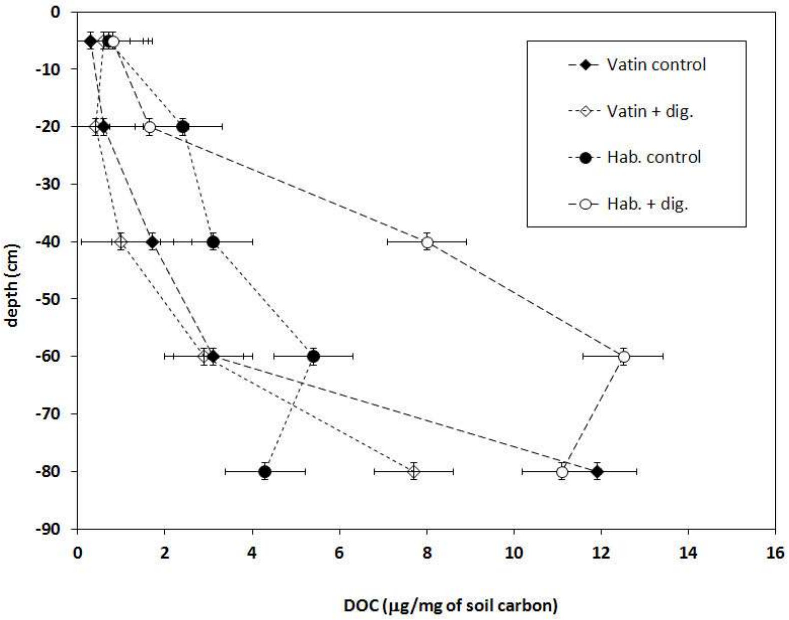

Fig. 5. Production of DOC calculated as µg of DOC per milligram of available soil carbon. Control production in 2014 and a year after amendment are compared. Error bars correspond to standard error of calculation

study – after a year we do not observe any significant migration of C_4 amendment from top soil to the deepest layer. Very limited leaching of amendment was detected as adsorbed DOM in deeper parts of the soil profile.

4. CONCLUSIONS

Applied digestate increased the carbon content of top soil layer only. We did not observe significant migration of C_4 amendment from top soil to the deeper soil layers. We have identified limited leaching of digestate to deeper soil layers which was measurable by $\delta^{13}C$ measurement only not by elemental analysis. Amended soil layers produce more DOC per gram of soil than the control soils but the quantity of the released DOC per gram of carbon is comparable for control and amended soils. Released DOC is transferred to deeper soil layers and adsorbed according to content of clay particles.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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