Supplementary materials

Enhanced carbon sequestration in marginal land upon shift towards perennial C_4 *Miscanthus x giganteus*: a case study in North-Western Czechia

Karim Suhail Al Souki[1*], Hana Burdová[1], Jakub Trubač[2], Jiří Štojdl[1], Pavel Kuráň[1], Sylvie Kříženecká[1], Iva Machová[3], Karel Kubát[4], Jan Popelká[1], Hana Malinská[4], Diana Nebeská[1], Sergej Usťak[5], Roman Honzík[5], Josef Trögl[1]

This supplementary material was prepared to add the readers more details, for which there was not enough space in the main manuscript, about:

1. History of the study locality
2. Statistical evaluation of obtained data

1. Detailed description of the experimental site

1.1. Geological and pedological characteristics

The area of Nové Spořice lies in the Most ravine (a concave valley). Only a smaller forested part of the cadastral area northwest of Černovice belongs to the Ore Mountains themselves. The actual formation of the terrain corresponds to this division. The entire eastern and southeastern part is flatter with mild terrain waves and a ridge. In the vicinity of Černovice and Nové Spořice, the terrain gradually rises to the foot of the Ore Mountains at first, then quite sharply and reaches the highest point on a wooded terrain ridge - Jedlina 675 m above sea level. Agricultural land reaches the highest height northwest of Černovice (450 m above sea level) and the lowest in the valley to the left of the Spořice - Brány road (308 m above sea level). Due to the mining of brown coal, this road no longer exists, neither does the village of Brány. The average height of the land is 360 m above sea level.

Moreover, because of brown coal mining, most of the agricultural land from the cadastral area of Spořice was taken to landfills and used for the reclamation of dumps after mining.

The soil-forming substrate of the experimental site are terraces composed of predominantly clayed acidic material. The terraces originated as river sediments in the cold periods of the Pleistocene and their composition is dominated by hard acidic siliceous rocks, so they represent an acidic permeable substrate. In addition to fragments of quartz and other non-weatherable or difficult-to-weather minerals and rocks, they also have a weatherable admixture of surrounding rocks, especially feldspar, so that it also contains a sufficient amount of K_2O.

In the area of interest, deep brown soils have formed on these clay terraces. On clay terraces covered with slopes of acidic material or alluvial deposits, brown soils of agglomerated or floodplain soil and alluvial soils have formed. Terraces occur mainly in the vicinity of Spořice.

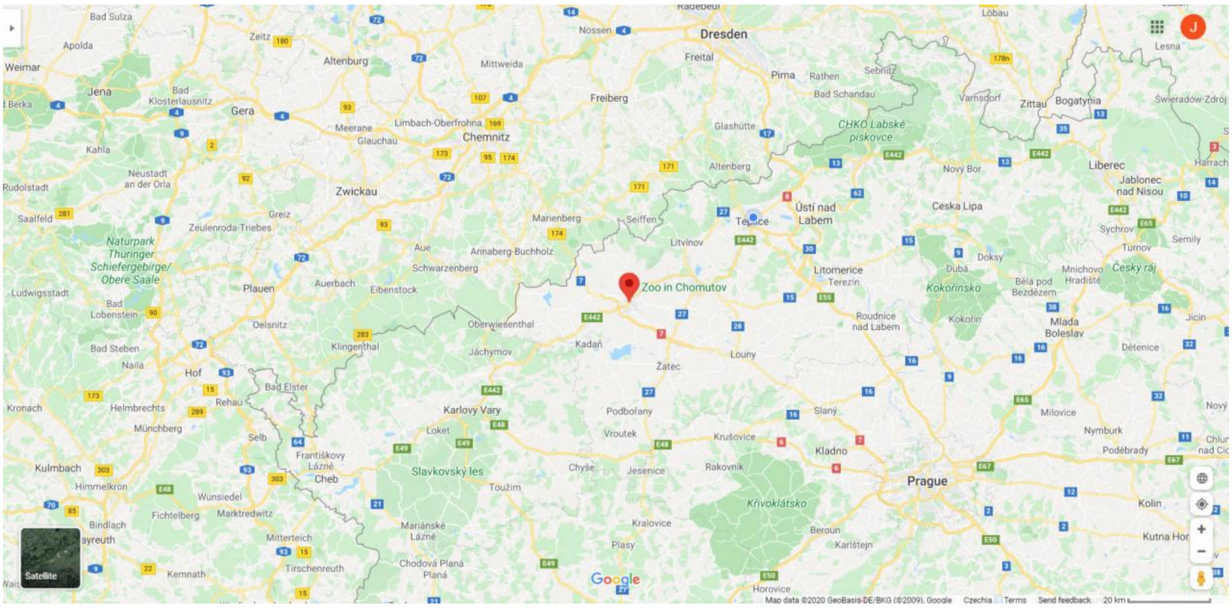
Brown soils (HP) - (Cambodia) with horizons orh - V - v / P - P / D, M represent our most widespread soil type. They are usually tied to a more sloping to strongly articulated relief on displaced weatherings with an admixture of a skeleton. Brown soils of lower positions develop at an altitude of 400-600 m with an average annual temperature of 6-9 ° C and precipitation of 600-800 mm. Brown soils of higher elevations (highlands and mountains) in areas with an average annual temperature of 4-6 ° C and precipitation of 700-1200 mm per year. The main pedogenetic process is internal soil weathering of minerals taking place in the Cambic horizon, during which the soil mass browns with released Fe and clay is formed. Properties of HP - content and quality of humus, soil reaction, saturation of sorption complex strongly depends on the nature of soil-forming substrate and climatic conditions.

In lower positions we find mostly saturated HP, which have 2-3% humus in topsoil with HK: FK ratio less than 1, acidic to weakly acidic reaction and colloidal complex weakly saturated to saturated (V up to 50%). Mountain HPs have an acidic reaction, 4-6% humus with a significant predominance of low molecular weight components, sorption complex significantly unsaturated. A partial process of podzolization and glazing begins. HP fertility decreases from hills to mountains and from deep non-skeletal to shallow, stony. They can be sandy to clayey in grain size, which also affects their fertility and workability. Subtypes: HP typical, illimerized, glued, acidic.

The following are brief climatic and soil characteristics of the experimental plot:
Location - western edge of the town of Chomutov, locality of Nové Spořice, plain below the foot of the Ore Mountains.
Latitude 50°27´38.85´´, longitude 13°23´07.44´´
Production area: beet
Terrain configuration: flat, slightly rugged
Climate type: slightly warm, slightly dry, mostly with mild winter
Predominant wind direction: northwest
Altitude - 363 above sea level
Total precipitation: 525 mm per year, of which 300 mm per growing season
Air temperature: 7.6 ° C on average per year, 13.8 ° C per growing season
Farmland:
Soil type: brown soil, shallow, acidified
Soil type: medium-heavy, sandy-loamy, slightly stony soil
Soil condition: less structural, weakly humic
Water conditions: good water permeability
Topsoil depth: 16–18 cm, sandy loam

Bottom depth: up to 40 cm, loamy, strongly rocky
Soil-forming substrate: clayey gravel-sand terrace, strongly stony
From an agrochemical point of view, the soil of the experimental site is considered to be of lower quality (less fertile), mainly due to relatively low content of humus and basic nutrients. Nevertheless, long-term fertilization of experimental plots with mineral fertilizers, content of available nutrients grew from unsatisfactory to satisfactory. In addition, the soil is shallow, less structural, weakly humic and has a relatively high acidity (pH / KCl about 5.5) and a relatively low ratio of humic acids to fulvic acids (<1).

Reference: Veselý, V. Comprehensive soil survey: Accompanying report to the results of the survey in the economic district of JZD Spořice, Chomutov, 1967.



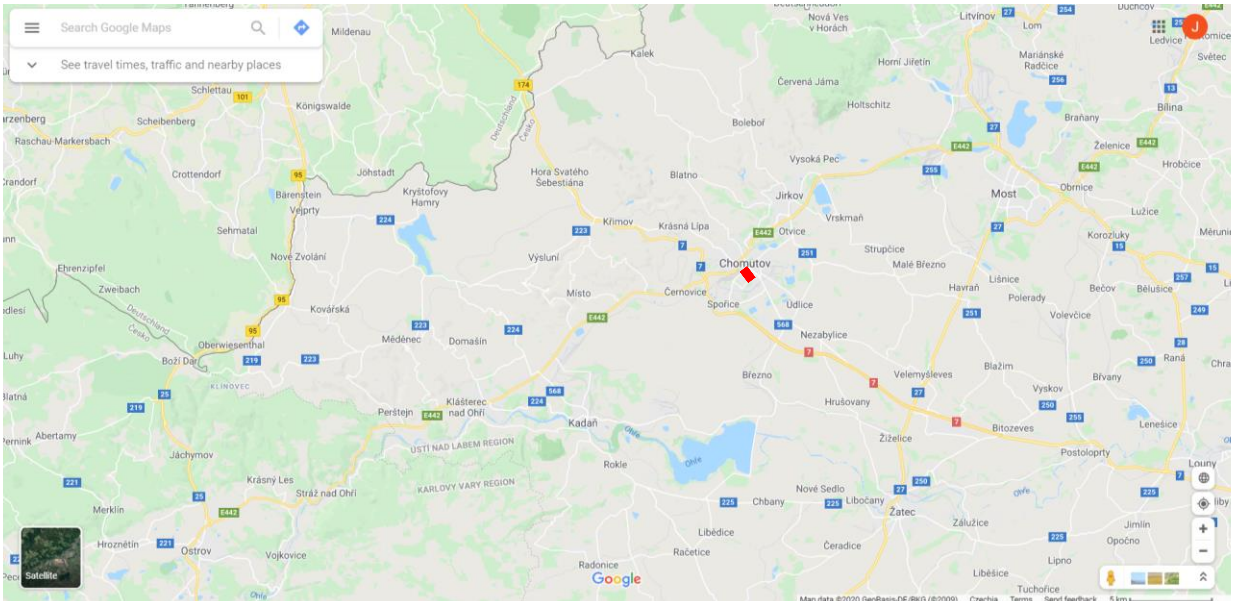


Figure S1: Map of the locality in EU context.

1.2. Agriculture history of the area

The following images show the study area with the miscanthus plantation shown as a red rectangle, the green-filled sub-rectangle represents the miscanthus plot used for sampling. The yellow-filled rectangle the selected portion of the wheat field from which the samples were collected. Finally, the brown-filled rectangle shows the unplanted pathway separating the miscanthus and wheat fields and is taken as a reference representing the initial soil state.

As part of the detailed description of our experimental plots, we also included historical images from 1953 in the analysis, where craters of bomb blasts after the Allied bombing of the Chomutov railway station in April 1945 are visible on our currently used plots.

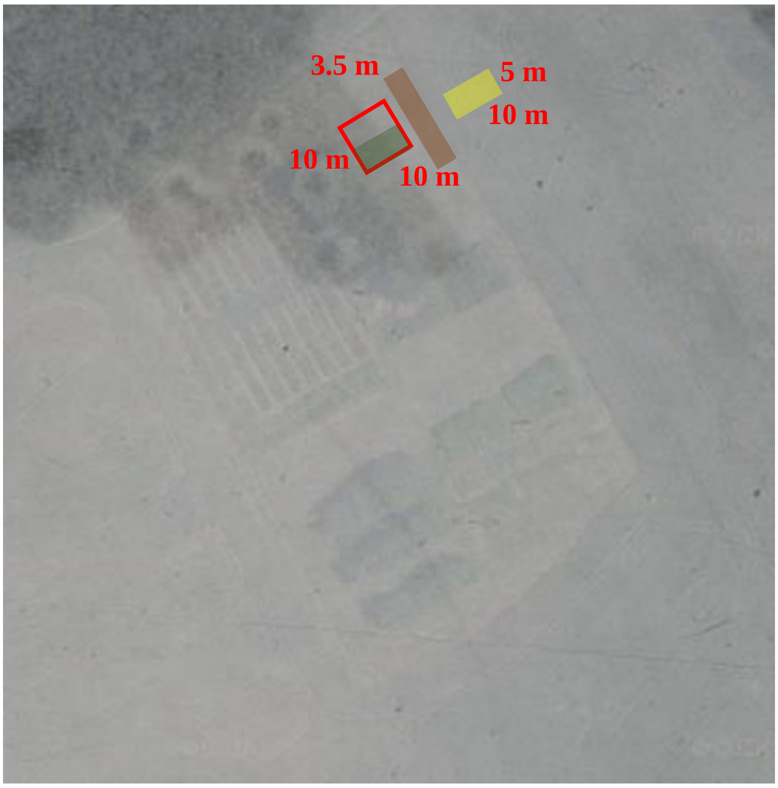


Figure S2 a: The orthophoto map of the locality in 1953

The following images with the given data of the imaging document prove that since 2010, when there were various annual crops on the experimental area intended for miscanthus, mowed permanent grassland was maintained on this area. The neighboring areas were first sown in 2010 with a rattle reed, and since 2016, when our lease was terminated, winter wheat has been sown continuously.

Figure S2 b: The orthophoto map of the locality in 2010

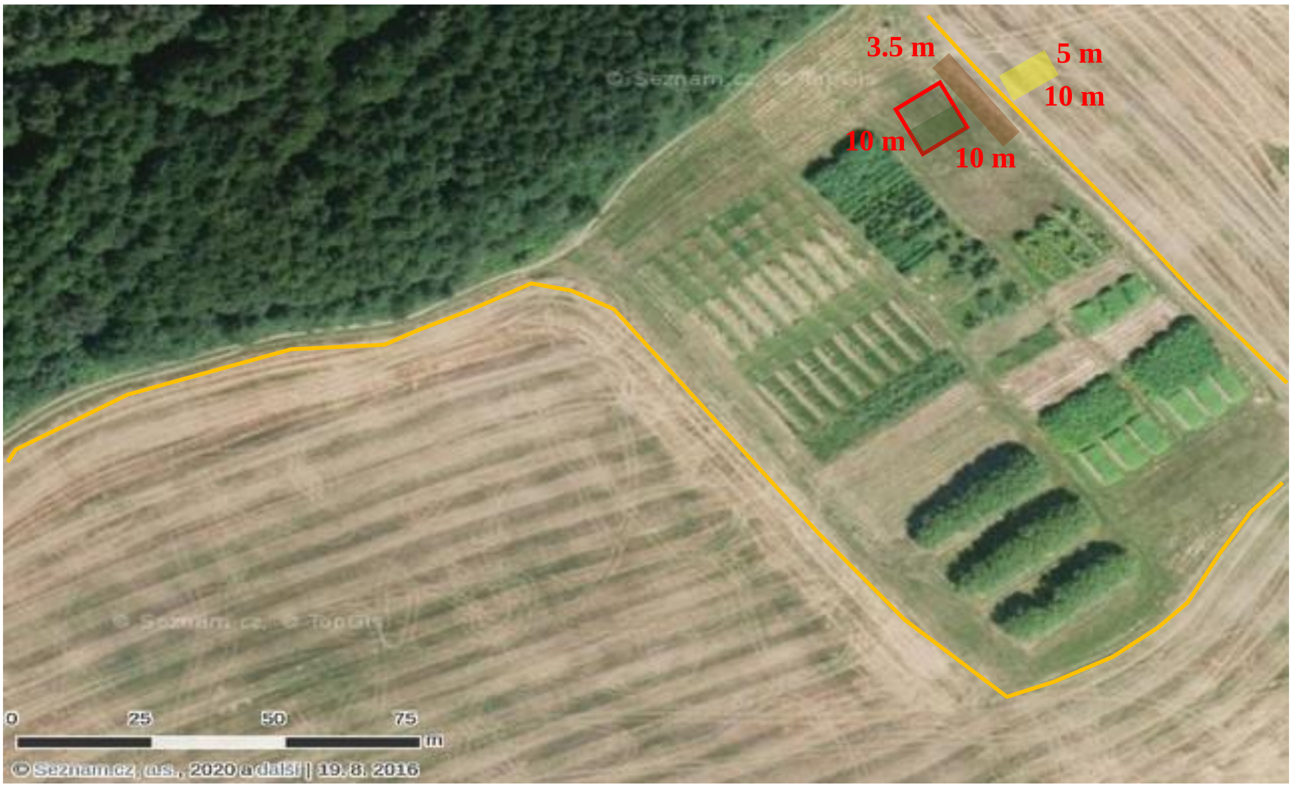


Figure S2 c: The orthophoto map of the locality in 2016

The last image number 4 is from 2019, where, after detailed observation, the growth of the ornament is also visible. Next to the ornament, a growth of wheat is visible.

Figure S2 d: The orthophoto map of the locality in 2019

2. Detail data statistics

The data (various parameters) were first subjected to normality test (Jarque-Bera test), since normal distribution of data is prerequisite for some statistical methods such as ANOVA.

Despite that, the correlation was calculated non-parametrically (Spearman correlation) especially because of missing some of the data pairs and thus making the findings stronger.

Table S1: Jarque-Bera normality test

	pH	Bulk density	SOC	δ ^{13}C	Plant derived OC	BR	FDHA	PLFA-tot	Fungi [%]	G+ [%]	G- [%]	Actin. [%]	Other bacteria [%]	*cy/pre* stress
N	36	36	36	36	27	9	9	12	12	12	12	12	12	12
Jarque-Bera JB	0.4802	2.293	1.017	4.013	2.633	0.7959	0.4008	0.6849	0.08972	0.8677	1.828	1.733	0.4429	0.726
p(normal)	0.7865	0.3178	0.6015	0.1344	0.268	0.6717	0.8184	0.71	0.9561	0.648	0.4009	0.4204	0.8014	0.6956
p(Monte Carlo)	0.7539	0.1461	0.481	0.0614	0.1008	0.3853	0.7653	0.5444	0.9644	0.4032	0.0997	0.1074	0.7409	0.5139

Table S2: Spearmann correlation of determined parameters

	Soil parameters		Soil organic carbon			Microbial activities		Microbial parameters						
	1.	2.	3.	4.	5.	6.	7.	8.	9.	10.	11.	12.	13.	14.
1. pH		**0.79**	**-0.46**	**-0.63**	**-0.70**	**-0.78**	-0.63	0.17	0.33	**-0.78**	**0.58**	-0.37	**-0.84**	**0.79**
2. Bulk density	**0.79**		**-0.53**	**-0.71**	**-0.84**	**-0.95**	**-0.88**	-0.27	0.12	**-0.61**	**0.62**	-0.57	**-0.65**	**0.92**
3. SOC	**-0.46**	**-0.53**		0.21	**0.42**	**0.73**	0.67	-0.17	-0.10	0.45	-0.48	0.24	0.48	-0.52
4. δ ^{13}C	**-0.63**	**-0.71**	0.21		**0.86**	**0.88**	**0.75**	0.17	-0.20	**0.64**	**-0.60**	0.50	0.73	**-0.91**
5. Plant derived OC	**-0.70**	**-0.84**	**0.42**	**0.86**		**0.98**	**0.92**	0.06	0.01	0.57	**-0.64**	0.40	0.60	**-0.86**
6. BR	**-0.78**	**-0.95**	**0.73**	**0.88**	**0.98**		**0.92**	0.33	-0.17	0.50	-0.55	0.57	0.52	**-0.87**
7. FDHA	-0.63	**-0.88**	0.67	**0.75**	**0.92**	**0.92**		0.55	-0.05	0.33	-0.60	0.62	0.42	**-0.87**
8. PLFA tot	0.17	-0.27	-0.17	0.17	0.06	0.33	0.55		0.34	-0.15	-0.17	**0.59**	-0.19	-0.32
9. Fungi [%]	0.33	0.12	-0.10	-0.20	0.01	-0.17	-0.05	0.34		-0.24	-0.22	0.06	-0.38	0.03
10. G+ [%]	**-0.78**	**-0.61**	0.45	**0.64**	0.57	0.50	0.33	-0.15	-0.24		**-0.70**	**0.66**	**0.71**	-0.51
11. G- [%]	**0.58**	**0.62**	-0.48	**-0.60**	**-0.64**	-0.55	-0.60	-0.17	-0.22	**-0.70**		**-0.72**	**-0.70**	**0.72**
12. Actinobacteria [%]	-0.37	-0.57	0.24	0.50	0.40	0.57	0.62	**0.59**	0.06	**0.66**	**-0.72**		0.35	-0.55
13. Other bacteria [%]	**-0.84**	**-0.65**	0.48	0.73	0.60	0.52	0.42	-0.19	-0.38	**0.71**	**-0.70**	0.35		**-0.73**
14. *cy/pre* stress	**0.79**	**0.92**	-0.52	**-0.91**	**-0.86**	**-0.87**	**-0.87**	-0.32	0.03	-0.51	**0.72**	-0.55	**-0.73**	

SOC: Soil organic carbon; δ ^{13}C: Ratio of carbon isotopes; OC: Organic carbon; BR: Basal respiration; FDHA: Fluorescein diacetate hydrolytic activity; PLFA tot: total PLFA (living microbial biomass); G+: Ratio of Gram-positive bacteria; G-: Ratio of Gram-negative bacteria; *cy/pre* stress: Indicator of cyclopropylated fatty acids to their precursors, indicator of transition of bacteria to stationary growth phase.