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Sequestration potential of energy crop *Miscanthus x giganteus* cultivated in continental part of Croatia

Sekvestracijski potencijal energetske kulture *Miscanthus x giganteus* uzgajane u kontinentalnoj Hrvatskoj

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ABSTRACT

Miscanthus x giganteus is an energy crop relatively recently introduced into Croatia, that is suitable for cultivation on marginal or abandoned agriculturl land. Energy crops can contribute to climate change mitigation both by carbon sequestration into soil/plant pool and by substitution of fossil fuels. Therefore, the aim of this paper is to evaluate the impact of *Miscanthus x giganteus* cultivation on the environment by determination of soil and soil-plant system's carbon budget, as well as to estimate the sequestration potential of Miscanthus stand on abandoned agricultural land at national level. After the 3 years of *Miscanthus* cultivation, soil pH and K_2O decreased, P_2O_5 increased and N_{tot} remained the same. Plant biomass increased from April, peaked in September, and decreased towards the spring harvest with harvested yield of 14.5 t/ha. Average soil respiration amounted 9.1 t/ha/y, and didn't show seasonal trend, or significant correlation to soil temperature and moisture. Estimated sequestration potentials of Miscanthus are 1.3 (soil system) and 2.0 (soil-plant system) t/ha/y. The highest contribution to carbon gains and losses had respectively preharvest losses and soil respiration. Between 704 520-3 251 600 t C could be sequestrated in soil/plant pool in Miscanthus lifespan if 5-15% of abandoned agricultural land would be converted to Miscanthus cultivation. Therefore, Miscanthus represents biological carbon sink due to great sequestration potential and additional fossil fuel replacement benefits.

Keywords: carbon budget, climate change mitigation, energy crops, *Miscanthus x giganteus*, sequestration potential, soil respiration

SAŽETAK

Miskantus (*Miscanthus x giganteus*) je energetska kultura relativno nedavno uvedena u Hrvatsku, a koja je pogodna za uzgoj na marginalnom ili napuštenom poljoprivrednom zemljištu. Energetske kulture mogu doprinijeti ublažavanju klimatskih promjena na dva načina: sekvestracijom ugljika u tlo i biomasu te zamjenom fosilnih goriva. Stoga je cilj rada utvrditi utjecaj uzgoja miskantusa na okoliš utvrđivanjem balance ugljika u sustavu tlo i tlo/biljka, kao i procijeniti sekvestracijski potencijal nasada miskantusa uzgajanog na napuštenom poljoprivrednom zemljištu na nacionalnoj razini. Nakon tri godine uzgoja miskantusa, reakcija tla i sadržaj K_2O su se smanjili, sadržaj P_2O_5 se povećao, a sadržaj N_{tot} je ostao isti. Biljna biomasa se povećavala od travnja, dosegnula vrhunac u rujnu te se smanjivala prema proljetnoj žetvi, s prinosom od 14,5 t/ha. Prosječno disanje tla iznosilo je 9,1 t/ha/god i nije pokazalo sezonski trend, niti značajnu korelaciju sa temperaturom i vlagom tla. Procijenjeni sekvestracijski potencijal miskantusa je 1,3 (sustav tlo) i 2,0 (sustav tlo-biljka) t/ha/god. Dobicima ugljika su najviše doprinijeli predžetveni gubici a gubicima ugljika disanje tla. Ukoliko bi se

5-15% napuštenog poljoprivrednog zemljišta prenamijenilo za uzgoj miskantusa, između 704 520-3 251 600 t C bi se moglo uskladištiti u tlu/biljci tijekom životnog vijeka miskantusa. Stoga, miskantus predstavlja biološki ponor ugljika zbog svog velikog sekvestracijskog potencijala uz dodatne prednosti zamjene fosilnih goriva.

Ključne riječi: bilanca ugljika, ublažavanje klimatskih promjena, energetske kulture, *Miscanthus x giganteus*, sekvestracijski potencijal, disanje tla

INTRODUCTION

Global temperatures have increased by approximately 1.0 °C above pre-industrial level and are likely to reach 1.5 °C between 2030 and 2052 if the level of greenhouse gasses (GHG) continues to increase at the current rate (IPCC, 2018). The largest contribution to total GHG emissions in Croatia has the energy sector with 70.2% of which fossil fuel combustion comprise majority (>90%), followed by agricultural sector with 12.1% (NIR, 2018). Therefore, in order to stabilize global warming, actions are needed to reduce anthropogenic GHG emissions both by implementation of clean energy solutions and natural climate solutions (IPCC, 2018, Anderson et al., 2019, Griscom et al., 2019). Replacement of fossil fuels by cultivation and utilization of renewable bioenergy fuel sources (energy crops) is one of the solutions for clean energy implementation. Furthermore, cultivation of energy crops represents also natural climate solution due to the energy crops potential to remove atmospheric carbon by carbon sequestration into the plant and soil pool.

The cultivation of energy crops on the arable land suitable for agricultural food production can undermine food security, livelihoods, ecosystem functions and services and other aspects of sustainable development (IPCC, 2018). In order to avoid these negative aspects, energy crops can be cultivated on marginal or abandoned agricultural land that is unsuitable for cultivation of other traditional agricultural crops (Toma et al., 2011; Xue et al., 2016; Lewandowski et al., 2016; Prčik and Kotrla, 2016). *Miscanthus* is a sterile allopolyploid hybrid (Scallye et al., 2001). It is perennial rhizomatous energy grass with notable biomass production potential between 10–49 t dry matter per ha (Himken et al., 1997, Lewandowski et al., 2000, Kahle et al., 2001, Richter et al., 2008; Christian et al., 2008; Borzêcka-Walker et al., 2008; Hastings et al., 2009a, Hastings et al., 2009b; Zub and Brancourt-Hulmel, 2009; Majtkowski et al., 2009). In Croatia, very high yields (between 15 and 28 t) of dry matter are already obtained in the third year of Miscanthus cultivation (Bilandžija et al., 2018). *Miscanthus* has high longevity, between 15-20 years (Lewandowski et al., 2003; Anderson et al., 2011.), low nutrient (Beale and Long, 1997; Heaton et al. 2004, Lewandowski et al., 2003) and management requirements (Miguez et al., 2008; Gopalakrishnan et al., 2011; McCalmont et al., 2015) compared to other crops. Therefore, *Miscanthus* has relatively low (estimated to be between -5.40 and 0.95 Mg CO_{2eq} /ha) global warming potential (Toma et al., 2011).

Miscanthus has the potential to improve soil carbon stocks. It has been determined that a considerable amount of *Miscanthus* originated carbon was accumulating in the soil (Kahle et al. 2001; Hansen et al. 2004). From late autumn until early spring harvest, 25–50% of dry matter biomass (mostly leaves and shoot tops) falls down from the plant and enters the soil pool (Lewandowski et al., 2000). The increase in soil carbon stocks is also a result of senescent rhizomes and recycling of roots. Still, previous studies have determined that depending on specific agroecological conditions *Miscanthus* can have positive (Dondini et al., 2009; Hansen et al., 2004; Clifton-Brown et al., 2007), neutral (Robertson et al., 2017; Poeplau and Don, 2014) or even negative (Zimmermann et al., 2012; Hansen et al., 2004) sequestration potential. According to Hastings et al. (2008), in order to avoid soil C emissions, *Miscanthus* should not be cultivated on soils with high (more than 90 t C/ha) soil C content, however, if cultivated on soils with lower C content it will result in accumulation of SOC. Therefore, site-specific studies are required to evaluate the sequestration (climate change mitigation) potential of *Miscanthus* stand on national level.

Miscanthus was relatively recently introduced into Croatia, and therefore a corresponding assessment of its effects on agricultural ecosystems is needed. Previous studies on *Miscanthus* in Croatia, but also worldwide, were mainly focused on the plant establishment, nutrient requirements, productivity, harvest time, biomass properties, combustion feasibility, etc. (Christian and Riche, 1998; Lewandowski et al., 2000; Kahle et al., 2001; Lewandowski et al., 2003; Clifton-Brown et al., 2007; Christian et al., 2008; Miguez et al., 2008; Heaton et al., 2008; Bilandžija et al., 2014, Bilandžija, 2015; Leto et al., 2017; Bilandžija et al., 2018) while research on impact of *Miscanthus* production on soil respiration has rarely been investigated worldwide (Toma et al., 2011; Behnke et al., 2012; Drewer et al., 2012) and never in Croatia until today. Therefore, the aims of study were to determine:

1) changes in soil properties after three years of *Miscanthus* cultivation;
2) soil respiration and agroecological factors (soil temperature and soil moisture), their seasonal dynamic over one vegetation year and correlation;
3) average annual carbon gains/losses by aboveground biomass (harvested biomass + preharvest losses + harvested residues), belowground biomass (rhizomes, roots), soil respiration, plant CO_2 assimilation and respiration, and CO_2 emissions from field operations;
4) the sequestration potential of *Miscanthus* stand cultivated on abandoned agricultural land in Croatia according to developed scenarios (5, 10, 15% of abandoned land).

MATERIALS AND METHODS

Site Description

The experimental site is located in Donja Bistra (N 45°55'06,2'', E 15°50'32,5'') at 144 m a.s.l., in continental part of Croatia. Experimental field was established in 2011 by planting *Miscanthus x giganteus* (Greef et Deu) rhizomes on 1m distance between and within rows what makes plant density of 10 000 plants per ha. The site has continental humid climate with mean annual temperature of 10.2 °C, mean annual precipitation of 1053.9 mm, and mean annual evapotranspiration of 659.7 mm in 30-year period (1961-1990). Soil texture is silty loam with 66.3% of silt, 21.3% of sand and 12.4% of clay in the top 30 cm of soil. The land management prior to conversion to *Miscanthus* was permanent pasture. More details on the experimental site can be found in (Bilandžija, 2015).

Soil Sampling and Analyses

Soil sampling for determination of carbon content and changes in soil properties after three years of *Miscanthus* cultivation, was conducted with Eijkelkamp soil probe in the 0 – 30 cm surface layer. Each soil sample was composed of 10 individual soil samples. Soil sampling was conducted before (April 2011) and three years after (April 2014) stand establishment in three repetitions. Preparation of soil samples was conducted according to ISO 11464:2006. Total carbon and nitrogen contents were determined by dry combustion method on the Vario, Macro CHNS analyser in accordance with ISO 10694: 1995 for carbon and ISO 13878:1998 for nitrogen. Carbon stocks were estimated in relation to site bulk density (1.4 g/cm^3 for 0-30 cm depth). Soil pH was measured using the electrometric method with the Beckman pH-meter 72, in water suspension according to ISO 10390:2005. Plant available P_2O_5 (P_{AL}) and K_2O (K_{AL}) were extracted by AL solution (ammonium lactate-acetate) according to Egner et al. (1960.).

Aboveground and Belowground Biomass Yields and Carbon Content

The study concerned 2013/2014 growing seasons, i.e. the stand's third year. Standing biomass was harvested in autumn 2013 and spring 2014 in six repetitions in order to estimate aboveground biomass (harvested biomass, harvested residues and preharvest losses) and its carbon content. The carbon content in plant material was determined by dry combustion method on the Vario, Macro CHNS analyser in accordance with ISO 10694: 1995 standard.

Harvested biomass was determined by manual cutting of biomass at 10 cm height from soil surface at randomly selected 10 m^2 large plots. The dry matter yield (t/ha) of harvested biomass was determined by gravimetric weighting of subsampled harvested biomass (~1000 g of chopped biomass) before and after oven drying at 60 °C to constant mass. For the calculation of harvested biomass yield in whole *Miscanthus* lifespan, it was assumed that the lifespan of stand is 20 years and that biomass is harvested from the third year onward as it is considered that high stable yields are achieved from the third year.

Harvested residues represent the first 10 cm of standing biomass that remains at the soil surface after the harvest. The amount of harvested residues was calculated from total plant height (that was measured from the soil surface up to sheath of the last leaf and shoot-tips) and harvested biomass yield. The amount of harvested residues in whole *Miscanthus* lifespan was calculated from the third year onward.

Preharvest losses represent dead aboveground biomass that falls to the ground over winter (basal leaves that gradually senescence from the beginning of July and shoot tips that die with the appearance of first frost). Differences between peak (autumn yield 2013) and harvested biomass yield (spring yield 2014) represents overwinter litter drop i.e. preharvest losses. The amount of preharvest losses over 20 years was calculated from the third year onward. The carbon yields obtained in the first (0.7 t C/ha) and second (5.2 t/ha) year (Bilandžija, 2015) are also taken into account as preharvest losses as they were left on the field.

Belowground biomass was estimated based on the aboveground biomass yield according to Clifton-Brown et al. (2007). According to authors, belowground biomass is 1.5 times of aboveground biomass with 66.7% of live rhizome, 14.5% of root and 18.8% of dead rhizome. Therefore, it was accepted that 18.8% of belowground biomass is entering the soil pool each year.

Measurement of Soil Respiration and Agroecological Factors

Soil carbon loss from respiratory processes was determined by calculation of soil CO_2 efflux. Measurements of soil CO_2 concentrations, as well as soil temperature and soil moisture, were conducted once per month from May 2013 until April 2014 in six repetitions during 30 min of incubation period. The measurements were not conducted over winter period (November 2013-February 2014) due to unfavourable field conditions (snow cover, low temperatures, high soil moisture, frozen soil).

Soil temperature and soil moisture in the soil surface layer (10 cm depth) were measured with IMKO HD2 (2011), in the vicinity of each chamber, simultaneously with measurements of soil CO_2 concentrations in six repetitions. Measurements of soil CO_2 concentrations were conducted by *in situ* closed static chamber method with portable infrared detector of carbon dioxide (GasAlerMicro5 IR, 2011). More information's on the measurement methodology can be found in Bilandžija et al. (2016). CO_2 efflux (kg ha^{-1} day^{-1}) was afterwards calculated according to Bilandžija et al. (2014a) as:

$$FCO_2 = [M * P * V * (c_2-c_1)] / [R * T * A * (t_2-t_1)] \qquad (1)$$

where: FCO_2 – soil CO_2 efflux (kg/ha/day); M – molar mass of the CO_2 (kg/mol); P – air pressure (Pa); V – chamber volume (m^3); c_2–c_1 – CO_2 concentration increase rate in the chamber; during incubation period (µmol/mol); R – gas constant (J/mol/K); T – air temperature (K); A – chamber surface (m^2); t_2-t_1 – incubation period (day). For the calculation of carbon budget, CO_2 efflux was expressed as C-CO_2 t/ha/year. Interpreted seasons of the year imply: spring (April-May), summer (June – August) and autumn (September – October).

CO_2 Emissions from Field Operations in Miscanthus x giganteus Lifespan

As Miscanthus is perennial grass, CO_2 emissions from field operations are divided on field establishment stage and biomass production stage emissions. CO_2 emissions from field operations are calculated based on the assumption that *Miscanthus* lifespan is 20 years, that fertilization and irrigation are needed only in the first year and that harvest is conducted from the third year onward.

Data on fuel consumption per field operation in similar agroecological conditions are obtained from Filipovic et al. (2006) and Peric et al. (2018) and are presented in Table 1. It is assumed that tractor distance between the field and the storage is on average 5 km. CO_2 emissions from field operations are calculated based on machinery fuel consumption and CO_2 emission factor. According to Valsecchi et al. (2009), CO_2 emission factor is 2.67 kg CO_2 / l diesel fuel. Indirect emissions from field operations are not taken into account like emissions from fertilizer production, machinery, plant material etc.

Table 1. Field operations and fuel consumption

Operation	Diesel consumption (l/ha):
herbicide application	1.84†
plowing (20-30 cm)	35.52‡
disc harrowing x 2	15.02‡
combined implement	6.93‡
rhizomes planting	10.08†
fertilization	1.45†
irrigation	9.06†
harvesting	32.89†
baling and loading	16.12†
transport (tractor)	22.00†

Sources: Peric et al. (2018)†; Filipovic et al. (2006)‡

Sequestration Potential of Miscanthus x giganteus

Sequestration potential of *Miscanthus* is determined in two ways, by calculation of carbon balance within soil system and within soil-plant system. Sequestration potential within soil system is determined based on the changes in soil carbon stocks over three years. Sequestration potential within soil-plant system was determined based on determination of carbon gains and losses by above and belowground biomass, soil respiration, plant CO_2 assimilation and respiration rate, and CO_2 emissions from field operations.

Carbon gains represent the amount of carbon that enters the soil pool as preharvest losses, harvest residues, dead belowground biomass and plant pool by photosynthetic assimilation of carbon. The CO_2 assimilation rate of *Miscanthus* was assumed to be 30.65 μmol $CO_2/m^2/s$ i.e. 116.7 t C-CO_2/ha/year, an average of four month measurements conducted by Beale et al. (1996).

Carbon losses represent the amount of carbon that is emitted to the atmosphere by soil and plant respiration, field operations as well as the amount of carbon that is removed from the system by harvested biomass. Dark respiration rate was assumed to be 2.1 μmol $CO_2/m^2/s$ i.e. 8.0 t C-CO^2/ha/year according to Beale et al. (1996). Sequestration potential is calculated and expressed annually and for *Miscanthus* lifespan of 20 years.

Land Use Change from Abandoned Agricultural Land to Miscanthus Cultivation

The term ‘energy crops’ is used for both annual and perennial crops on agricultural land intended solely for energy purposes. A great potential for introducing energy crops without affecting the existing agricultural production is cultivation of energy crops on abandoned agricultural land. According to the digital Corine Land Cover database of Croatian environment agency (2012), total abandoned agricultural land in Croatia amounts 541930 ha. For estimation of theoretical sequestration potential, three scenarios considering conversion of abandoned agricultural land to *Miscanthus* cultivation were developed:

1) conservative scenario: 5% of abandoned agricultural land conversion (27097 ha);
2) optimistic scenario: 10% of abandoned agricultural land conversion (54193 ha);
3) progressive scenario: 15% of abandoned agricultural land conversion (81290 ha).

Statistical Analysis, Quality Management and Quality Control

Statistical analysis was conducted by statistical Software SAS. Variability was evaluated by analysis of variance (ANOVA) and tested, if necessary, with Bonferroni post-hoc t-tests. Significance level was 5%. Quality management (QM) system is in line with good laboratory practice and Internal and External quality control (QC) were included.

RESULTS

Changes in Soil Properties

Soil sampling to 30 cm revealed significant difference between sampling in 2011 and 2014 for all studied soil properties, except for total nitrogen content (Table 2). Soil pH decreased by 0.1 unit in three years' period. Plant available potassium significantly decreased and plant available phosphorous significantly increased in three years of *Miscanthus* cultivation. Due to the low pH_{H_2O}, it is assumed that amount of total carbon content represents the amount of organic carbon content (ISO 10693:1995). In terms of changes in soil carbon stocks, level of carbon content was significantly higher in 2014 than before *Miscanthus* stand establishment. Therefore, the sequestration potential of *Miscanthus* within the soil system is calculated to be 1.3 t/ha/y (Table 2).

Table 2. Changes in soil properties

Soil property	2011	2014	Difference
pH_{H_2O}	6.5A	6.4B	-0.1
C_{org} (t/ha)	51.1A	55.0B	+3.9
N_{tot} (t/ha)	6.6A	5.2A	-1.4
K_{AL} (mg/kg of soil)	99A	74B	-2.5
P_{AL} (mg/kg of soil)	19A	26B	+0.7

(-decrease; +increase); means followed by the same letter are not significantly different at the p ≤0.05 level

Soil pH, plant available potassium and plant available phosphorous did change over 3 years of *Miscanthus* cultivation significantly compared to natural grassland before the land use change in 2011. The observed changes could be attributed to the nutrients translocation to the belowground biomass (rhizomes) at the end of the growing season as nutrients may also be leached out of the aerial biomass (Cadoux et al., 2012). Himken et al. (1997) have estimated that 21-46% of N, 36-50% of P, 14-30% of K is translocated from the shoots to the rhizomes and that up to 50% of recycled potassium can return to soil after leaching from the stems. Soil N stocks did not decrease significantly over 3 years of cultivation, although experimental plot was not N fertilized at all and Lewandowski et al. (2000) have reported that 60 kg N/ha is optimal for *Miscanthus* development. The significant change of C stocks, with an average carbon sequestration rate of 1.3 t/ha/yr in the first 30 cm of topsoil is consistent with many other studies that reported increases in C stocks of more than 1 t/ha/yr (Kahle et al., 2001; Hansen et al., 2004; Clifton-Brown et al., 2007; Dondini et al., 2009).

Aboveground and Belowground Biomass

Aboveground biomass is comprised of harvested biomass, preharvest losses and harvested residues while belowground biomass is comprised of roots, dead and live rhizomes. The amounts of aboveground and belowground biomass dry matter yields and their carbon contents are presented in Table 3. Total aboveground dry matter biomass yield was comprised of harvested biomass (46.4%), preharvest losses (52.2%) and harvested residues (1.4%) (Table 3). Based on the amount of aboveground biomass, total belowground biomass was estimated. The total C content over 20 years was estimated to be 288.5 in aboveground biomass and 471.6 t/ha in belowground biomass (Table 3).

Total aboveground dry matter yield amounted 31.3 t/ha comprising 14.5 t/ha of harvested biomass, 16.3 t/ha of preharvest losses and 0.4 t/ha of harvested residues. Therefore, less than 50% of aboveground biomass was available for harvest as more than 50% of aboveground biomass has fallen to the ground between autumn and winter as preharvest losses. According to the literature data, there have been determined large differences in biomass yields from 2 to 44 t/ha depending on the

cv = cultivar

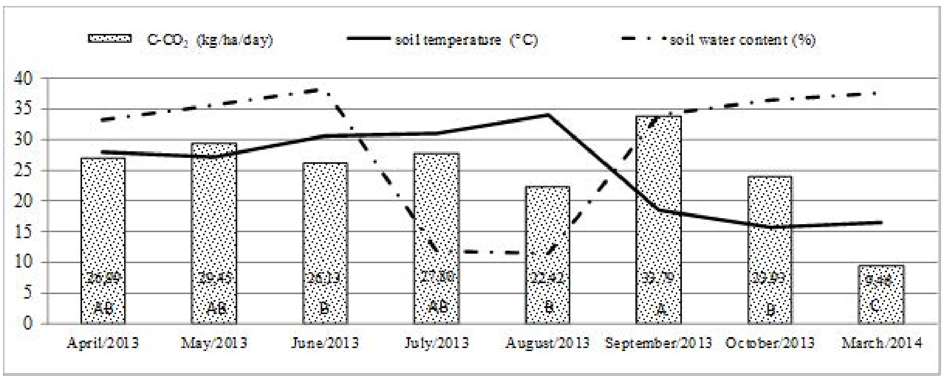
Table 3. Aboveground and belowground biomass and its carbon contents

	Dry matter yield (t/ha)	Carbon content (t/ha)	Accumulated c content (t/ha) over 20 years
harvested biomass	14.5	7.3	131.4
pre-harvest losses	16.3	8.2	153.5
harvested residues	0.4	0.2	3.6
Total aboveground biomass	31.3	15.7	288.5
live rhizome	31.3	15.7	314.6
dead rhizome	8.8	4.4	88.6
root	6.8	3.4	68.4
Total belowground biomass	46.9	23.5	471.6

cultivation location (Lewandowski et al., 2000). The amount of harvested biomass reported in this research is higher that the yields obtained by Jørgensen (1996) at spring harvest in Denmark (8 - 15 t/ha) and Kahle et al. (2001) at autumn harvest in Germany (11.7 t/ha), and it is assumed that the yield will be even higher in following years. The higher obtained amounts of aboveground biomass are attributed to different soil types and climate conditions, mostly to the higher amount of precipitation over the year (400 mm higher) and higher temperatures (1.3 °C higher). The amount of preharvest losses reported in the study is also higher compared to studies of other researches who determined biomass loss between 3 to 7 t dry matter/ha/yr (Beuch et al., 2001; Kahle et al., 2001; Miguez et al., 2008; Burner et al., 2009; Amougou et al., 2011). These differences are attributed to different harvest dates as *Miscanthus* can lose significant amount of the biomass over winter that is absorbed to the soil. The amount of harvested residues is lower compared to Kahle et al. (2001) who reported 0.7-3.1 t/ha of harvested residues. Total preharvest losses and harvested residues accounted for more than half of aboveground biomass (54%) what is in accordance with Hansen et al. (2004) who stated that total preharvest losses and harvest residues can be up to two-thirds of standing biomass.

Soil Respiration and Agroecological Factors

In 2013/2014, average seasonal soil respiration decreased respectively autumn > spring > summer (28.86>28.22>25.45 kg/ha/day). The lowest monthly soil respiration was determined in March 2014, a month before the harvest, and the highest one in September 2013 (Figure 1). Soil temperature was the lowest in October 2013 and the highest in June 2013 while soil

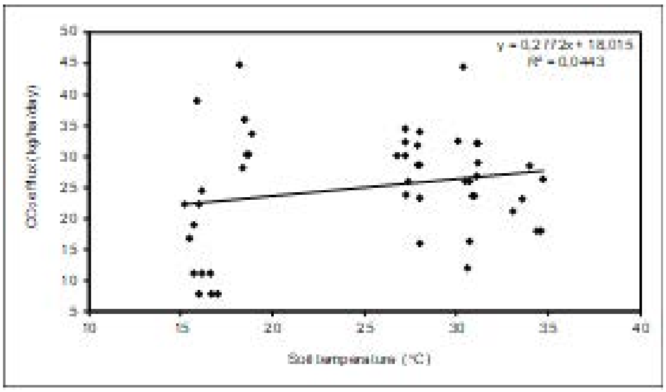


Means followed by the same letter are not significantly different at the p ≤0.05 level

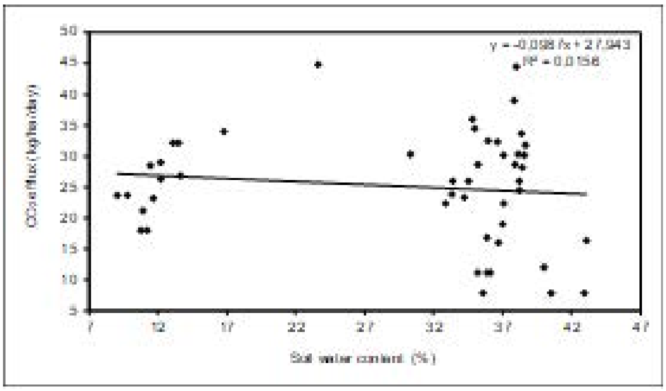
Figure 1. Seasonal dynamic of soil respiration, soil temperature and soil moisture

moisture was the lowest in summer months (July and August) and the highest in August 2013.

Soil respiration was very weakly positively correlated with soil temperatures (r=0.21) and soil moisture (r=0.12) (Figure 2). Average monthly soil C-CO_2 efflux amounts 25.0 kg/ha/day, i.e. average amount of carbon that is emitted to the atmosphere by soil respiration in one year is 9.1 t/ha. Therefore, total amount of carbon released to the atmosphere by soil respiration over 20-years of *Miscanthus* lifespan amounts 182 t/ha.



Regression (soil respiration - soil temperature)



Regression (soil respiration - soil moisture)

Figure 2. Regression for soil respiration and agroecological factors (soil temperature and soil moisture)

Soil respiration had no clear temporal trend and very weak correlation to agroecological factors i.e. soil temperature and soil moisture. According to literature data, soil respiration is usually well correlated with soil temperature and soil moisture content (Toma et al., 2011; Robertson et al., 2017) i.e. soil respiration is usually increasing when the temperatures are increasing as well. At study site, increased temperatures had no effect on CO_2 efflux probably as soil moisture has not increased as well, moreover moisture decreased in July and August. The soil moisture increased in September again and resulted in the highest soil respiration rate. The highest soil respiration rate was probably the result of *Miscanthus* peak growth phase in September 2013 what is in line with the research of Benbi et al. (2020) who also observed that peak soil respiration rates coincided with the maximum growth stage of a crop. Robertson et al. (2017) observed the highest soil respiration in September and the lowest one in January. The lowest soil respiration rate was recorded in dormant phase, a month before the harvest (May, 2014), when biomass has the highest dry matter content. Soil respiration was probably ever lower during winter months due to snow cover and very low soil temperatures and biological activity.

C-CO_2 Emissions from Field Operations

All relevant field emissions occurring from cultivation to storage of *Miscanthus* are considered and presented in Table 4. Management practices i.e. field operations that contribute the most to C-CO_2 emissions are harvesting and transport. Total estimated C-CO_2 emissions in the establishment stage and biomass production stage are respectively 0.06 and 1.2 t/ha over lifespan. Therefore, the amount of carbon emitted by field operations during whole *Miscanthus* lifespan is estimated to be 1.3 t/ha (Table 4).

The emissions from field operations have been estimated based on the fuel consumption of machinery in the studied region. The greatest contribution to emissions from field operations in the establishment stage has soil preparation and in the biomass production stage tractor transport. In the whole *Miscanthus* lifespan, emissions from soil preparation are negligible as majority of emissions are emitted by tractor transport and harvesting. The contribution of mentioned operations was expected due to the fact that these operations consume very high amounts of fuel and are conducted each year compared to other field operation that are need only in the establishment stage.

Table 4. CO_2 emissions from field operations

	Operation	Times applied in lifespan	C-CO_2 emissions (t/ha)
Miscanthus x giganteus establishment stage	herbicide application	2	0.0027
	plowing (20-30 cm)	1	0.0259
	disc harrowing x 2	1	0.01095
	combined implement	1	0.00505
	rhizomes planting	1	0.00735
	fertilization	1	0.00106
	irrigation	1	0.00660
	total		0.06
Miscanthus x giganteus biomass production stage	harvesting	18	0.4315
	baling and loading	18	0.2115
	transport (tractor)	18	0.5773
	total		1.2203
Total over 20 years of lifespan			1.3

Sequestration Potential of Miscanthus x giganteus within Soil-Plant System

In order to assess the contribution of soil / plant carbon stocks and site specific GHG emissions to carbon balance of *Miscanthus*, an annual carbon budget and afterwards the budget of *Miscanthus* lifespan was calculated (Table 5). The total carbon gains amount 362.3 t/ha over 20 years of *Miscanthus* lifespan to which preharvest losses contributed the most (42%). Total carbon losses amounted 322.7 t/ha over 20 years of *Miscanthus* lifespan with the highest contribution of soil respiration (56%). Average annual carbon balance i.e. sequestration potential within soil-plant system is 1.98 t/ha/yr i.e. 39.6 t C/ha could be sequestrated within soil-plant system during whole *Miscanthus* lifespan (Table 5).

In order to determine the sequestration potential of *Miscanthus*, carbon budget of soil and soil-plant system was determined. Difference of 0.7 t C/ha/yr between studied sequestration potentials has been determined and is attributed to the uncertainty of estimated values

Table 5. Carbon balance over 20 years of *Miscanthus x giganteus* lifespan

	Source	C content (t/ha) over 20 years
carbon gains	preharvest losses	153.5 (42.4% of total)
	harvest residues	3.6 (1.0% of total)
	dead belowground biomass	88.6 (24.4% of total)
	Miscanthus C-CO_2 assimilation	116.7 (32.2% of total)
	Total C gains	362.3
carbon losses	soil respiration	182.0 (56.4% of total)
	field operations	1.3 (0.4% of total)
	harvested biomass	131.4 (40.7% of total)
	Miscanthus C-CO_2 respiration	8.0 (2.5% of total)
	Total C losses	322.7
	Carbon balance	39.6

Table 6. Sequestration potentials of *Miscanthus x giganteus* considering cultivation on abandoned land according to developed scenarios

Abandoned agricultural land area (ha)	Conservative	Optimistic	Progressive
	27 097	54 193	81 290
Sequestration potential of soil system (1.3 t/ha/yr)			
sequestration potential (t/y)	35 226	70 451	105 677
sequestration potential (t over 20 y)	704 520	1 409 020	2 113 540
Sequestration potential of soil-plant system (2.0 t/ha/yr)			
sequestration potential (t/y)	54 194	108 386	162 580
sequestration potential (t over 20 y)	1 083 880	2 167 720	3 251 600

of belowground biomass, *Miscanthus* assimilation/respiration rates and CO_2 emissions from fossil fuel combustion by field operations that were obtained from studies conducted in similar but still different agroecological conditions. However, both sequestration potentials are in accordance with other studies (Kahle et al., 2001; Hansen et al., 2004; Clifton-Brown et al., 2007; Dondini et al., 2009., Poeplau and Don, 2014). For example, Hansen et al. (2004), Clifton-Brown et al. (2007) and Dondini et al. (2009) reported that C accumulation in the soil is 0.8-1.1; 0.6-1.1 and 2-3 t/ha/yr, respectively.

Land Use Change from Abandoned Agricultural Land to Miscanthus Cultivation

According to conservative, optimistic and progressive scenario, respectively 27 097, 54 193 and 81 290 ha of abandoned agricultural land could be converted to *Miscanthus* cultivation. According to developed scenarios and sequestration potentials, between 35 226 and 162 580 t C could be annually sequestrated i.e. between 704 520 and 3 251 600 t C could be sequestrated during whole *Miscanthus* lifespan (Table 6) on abandoned land converted to *Miscanthus* cultivation.

Taking into account both sequestration potentials, if 5-15% of currently abandoned agricultural land would be converted to *Miscanthus x giganteus* cultivation, between 5-21% of total annual Croatian GHG emissions from agricultural sector could be removed annually from atmosphere into the plant and soil pool where it remains after the harvest of aboveground biomass.

CONCLUSIONS

Sequestration potentials within soil system was determined by changes in soil C stocks and within soil-plant system by evaluation of carbon gains and losses. The evaluation of sequestration potential within soil-plant system brought some uncertainties as part of the data was estimated according to literature data. Still, both calculations have shown that studied site acts as carbon sink. Moreover, it was estimated that between 1.2-5.5% of total annual Croatian GHG emissions from Agriculture sector could be annually removed from atmosphere if 5-15% of currently abandoned agricultural land would be converted to *Miscanthus x giganteus* cultivation. Therefore, beside diversifying fuel sources, perennial energy crop *Miscanthus x giganteus*, grown in continental humid climate of Croatia, has great climate change mitigation potential as it reduces GHG emissions by sequestration of atmospheric carbon into the soil and plant pool.

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