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**Measured and modeled C flows after land use
change to perennial bioenergy crops.**

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Summary

In the first part of this Thesis the general concepts and state of art of the research subjects are introduced, then focusing on the impact of the land use change to perennial bioenergy crops on agricultural systems' carbon stocks. Then, the specific objectives of the research that has been carried out are stated.

In the second part, the detailed methods and results of the four experiments performed are described one by one. All four papers constitute original research. The first and second papers originate from direct field measurements investigating the carbon flows subsequent to the establishment of perennial grasses for bioenergy on, respectively, a fertile agricultural land and a marginal land resembling, since it had been cultivated with poplar for the previous thirty years, the conditions of a biomass supply district. The third and fourth papers originate from model simulations investigating the potential of perennial biomass crops to produce energy and second-generation biofuels at the regional scale and their impact on soil carbon stocks and, more generally, on greenhouse gases emissions. Finally, by converging the findings of all the four experiments, general conclusions are drawn, also underlying future perspectives, current gaps and further research needed in order to fill these gaps.

General introduction

Together with water vapor (H₂O), methane (CH₄), nitrous oxide (N₂O) and ozone (O₃), carbon dioxide (CO₂) is one of the primary greenhouse gases in earth's atmosphere. Greenhouse gases (GHG) are so called because they absorb and emit radiation causing the warming of the earth's surface (i.e. greenhouse effect). The greenhouse effect is critical in supporting life on earth. But, since the industrial revolution (second half on the 18th century), human activities have produced an increase in CO₂ atmosphere concentration, which abruptly accelerated since the second half on the 20th century, therefore inducing an additional, anthropogenic-driven, global warming. Soon, global warming from the increase in CO₂ and other GHG was recognized as a scientific and political issue (Schneider, 1989) and, in 1992, the United Nations Framework Convention on Climate Change was negotiated in Rio de Janeiro with the objective to “stabilize greenhouse gas concentrations in the atmosphere at a level that would prevent dangerous anthropogenic interference with the climate system” (Sands, 1992). The international commitment to mitigate global warming has also been recently re-affirmed in Paris (UNFCCC, 2016).

Anthropogenic-driven changes in the carbon cycle triggering emissions of CO₂ are principally due to the use of fossil fuels and to changes in land use (Schneider, 1989; Sands, 1992; Houghton, 2003). Burning fossil fuels shifts the carbon cycle as vast amounts of carbon are released rapidly when, otherwise, they would slowly leak through volcanic activity over millions of years. Changing land uses disturbs the carbon cycle as big carbon pools can be cleared (e.g. deforestation; Fargione et al., 2008), can be built (e.g. re-forestation; Post and Kwon, 2000) or, in the case of soil organic carbon for example, can simply be reduced (e.g. soil tillage; Reicosky et al., 1997) or increased (e.g. retention of agricultural residues; Chivenge et al., 2007). However, the general trend of land use changes has clearly been towards clearing and reducing carbon pools for production activities and urbanization (Kalnay and Cai, 2003; Houghton, 2003; Fargione et al., 2008; Burney et al., 2010). Among the different human activities, agriculture has a primary role in causing and, at the same time, mitigating GHG, as “globally about one-third of the total human-induced warming effect due to GHG comes from agriculture and land-use change” (Paustian et al., 2006). More options are available for GHG mitigation in the agricultural field: i) adoption of low-input management practices, ii) increase of soil organic matter and biomass carbon stocks, iii) displacement of fossil fuels by renewable energy.

One strategy to achieve these mitigation potentials is to cultivate dedicated perennial biomass crops for the bioenergy industry (Lewandowski et al., 2003; McLaughlin and Kszos, 2005; Anderson-Teixeira et al., 2009; Alexopoulou et al., 2015; Agostini et al., 2015; Qin et al., 2016). In fact,

perennial biomass crops can be managed with very low inputs which, when compared to common commodity crops, result in CO₂ savings, by reducing production of fertilizers and pesticides, through lower consumption of machineries and materials, by lowering associated transportation emissions (Adler et al., 2007; Fernando et al., 2010; Fazio and Monti, 2011; Gelfand et al., 2013; Schmidt et al., 2015). Perennial biomass crops can increase carbon stocks with high productions of above- (Fuentes and Taliaferro, 2002; Lewandowski et al., 2003; Fike et al., 2006; Kering et al., 2012; Cosentino et al., 2014; Alexopoulou et al., 2015) and below-ground biomass (Monti and Zatta, 2009) and by accumulating soil organic carbon (SOC; Anderson-Teixeira et al., 2009; Monti et al., 2012; Cattaneo et al., 2014; Agostini et al., 2015). Furthermore, the harvested aboveground biomass can be used to produce renewable energy (Adler et al., 2007; Lynd et al., 2008), which in turn achieves CO₂ emissions savings by displacing fossil non-renewable energy sources (Clifton-brown et al., 2004; Gelfand et al., 2013; Hudiburg et al., 2016), since burning the latter causes higher emissions of CO₂ (Turhollow and Perlack, 1991).

Nonetheless, while CO₂ savings from lower inputs and from fossil fuels displacement can be considered more or less certain, CO₂ savings from net ecosystem carbon accumulation mainly depend on the former land use, thus on the land use change effect (Fargione et al., 2008; Anderson-Teixeira et al., 2009; Qin et al., 2016). Until now, although results vary from study to study, it has been observed, in general, that the land use change from croplands to perennial biomass crops acts as an ecosystem carbon sink, while the land use change from forests or grasslands to perennial biomass crops mostly results in ecosystem carbon losses or neutral (Post and Kwon, 2000; Guo and Gifford, 2002; Anderson-Teixeira et al., 2009; Qin et al., 2016). At the same time, it has been argued that, when croplands currently dedicated to food production are converted to biomass crops, an indirect land use change (ILUC) effect would be likely generated, as the global market may trigger the conversion of more land somewhere else (this land could be rich in C and its conversion impactful) as an answer to increased prices (Searchinger et al., 2008).

In the present work, we mainly focused on the direct effects of the land use change to perennial biomass crops (i.e. net ecosystem carbon variation). Nonetheless, although marginally treated, in order to deliver more comprehensive views on biomass crops' impact on global carbon flows, also life-cycle CO₂eq emissions due to the use of agronomic inputs were assessed by employing a dedicated software, and CO₂ uptake due to fossil fuels displacement or CO₂ emissions due to ILUC were estimated by using literature coefficients. Although data regarding the direct land use change effect of perennial biomass crops are already present in the literature (Qin et al., 2016), given the crop- and environmental-specificity of carbon variations upon land use change more studies are needed. In the present work, two experiments directly measured carbon flows after land use change

to dedicated perennial biomass crops, of which, particularly, one investigated for the first time the land use change from short rotation forestry to dedicated perennial grasses, while other two experiments assessed the regional-scale potential of dedicated perennial grasses to store carbon and displace fossil fuels by employing a process-based model.

Research objectives

The research carried out focused on the carbon stocks variations after land use change to perennial bioenergy crops, with the following specific objectives:

- to assess the net ecosystem carbon uptake performed by a perennial biofuel grass (switchgrass; *Panicum virgatum* L.) on a fertile cropland and to compare such uptake to the carbon balance of a classic succession of commodity crops on the same land
- to investigate the long-term soil carbon dynamics in a potential biomass supply district, by comparing 10-year SOC changes beneath switchgrass and giant reed (*Arundo donax* L.) on a land previously cultivated with poplar for 30 years
- to estimate, by using a biogeochemical model and spatial databases, the total carbon sequestration attainable in the Mediterranean basin by producing switchgrass on the whole surface potentially available for biofuels
- to map, through a regional modeling study, the potential position of biofuel plants supplied by switchgrass and giant reed biomass in the US Southeast, and to estimate their biogenic greenhouse gas impact

Thus, the first two experiments aimed to add more field data to the literature on the carbon variations caused by land use change to perennial bioenergy crops. The third and fourth experiments are mainly intended as a resource for decision makers and stakeholders, since biomass potential productions are quantified and mapped at the regional scale, while soil carbon and greenhouse gases uptake or release is estimated as well.

Soil respiration and carbon balance in a maize-wheat rotation and in switchgrass

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Abstract The deployment of dedicated energy crops and the related land use change (LUC) are topical issues, particularly in relation to carbon storage and climate change mitigation effects. We compared the carbon flows of the most common crop rotation in Europe (maize-wheat) and the perennial grass switchgrass for two consecutive years. Yearly mean soil respiration did not statistically differ between switchgrass and crop rotation (2.63 and $2.26 \text{ g C m}^{-2} \text{ d}^{-1}$, respectively), but interestingly, while in switchgrass the peak flux was reached during crop growth ($5.5 \text{ g C m}^{-2} \text{ d}^{-1}$), in the crop rotation system it occurred with bare soil (after harvest and soil tillage) ($4.1 \text{ g C m}^{-2} \text{ d}^{-1}$), likely due to diverse soil respiration pathways (heterotrophic/autotrophic) in the two systems. Soil organic carbon decreased by 2.2 and 1.0 Mg ha^{-1} , respectively in switchgrass and maize-wheat. Nonetheless, thanks to the carbon stored in the litter and root pools (1.9 and 2.5 Mg C ha^{-1} , respectively), switchgrass system eventually resulted in a net carbon sink. The estimation of life-cycle cultivation emissions and fossil fuels offset savings further increased the difference in carbon storage between the food (maize-wheat) and fuel (switchgrass) systems, since switchgrass used less agronomic inputs (-63% emissions) and saved 3.3 Mg C ha^{-1} , considering advanced bioethanol as end product displacing fossil fuels.

Keywords: Carbon; Biomass; Soil Respiration; Food; Fuel; Land Use.



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Introduction

Changes in land use and land management are estimated to have released 156 Pg of C to the atmosphere in the last 150 years (Houghton, 2003). In the past decades, the increase in world population has triggered cropland expansion to produce more food, thus causing most part of these land use-related emissions in the agricultural sector (Burney et al., 2010; West et al., 2010). But, presently, there is an international commitment to reverse or, at least, mitigate global warming processes (Paris Agreement; UNFCCC, 2016), both by reducing emissions to the atmosphere and by fixing atmospheric C. Agriculture has a primary role in greenhouse gas (GHG) mitigation (Paustian et al., 2006), because it can reverse detrimental land use changes (e.g. re-afforestation; Post and Kwon, 2000) and attenuate the pressure from land management (i.e. low inputs). For example, one way to achieve this mitigation is to cultivate perennial crops for the biofuel industry, which can increase the organic C stocks of agricultural systems (Lemus and Lal, 2005; Tilman, 2006; Anderson-Teixeira et al., 2009). Although such crops are best suited to marginal lands (Cai et al., 2011), in some cases (e.g. biomass supply districts) their cultivation may conflict with the cultivation of annual food crops in conventional agricultural areas. In this case, the substitution of conventionally tilled food crops with deep-rooted perennial bioenergy crops could potentially stock, for example, 4.5 or 6.3 Mt C, respectively on European set-aside lands (Freibauer et al., 2004) or low-productive Mediterranean croplands (Nocentini et al., 2015), while mitigating land management-related emissions thanks to the lower agronomic inputs required (Fazio and Monti, 2011). Nonetheless, being mandatory for biofuel crops to be at least GHG neutral, measuring their C flows against the C flows of the annual crops that would be replaced, will allow to quantify their real mitigation potential. Until now, biofuel perennial crops cultivated on former croplands have been reported to generally increase SOC stocks, by 6-14% (Qin et al., 2016), while fixing additional C through litter and root biomass (Tilman et al., 2006; Anderson-Teixeira et al., 2013).

In North Italy, switchgrass (*Panicum virgatum* L.) may be used for the production of bioenergy, since this perennial crop revealed to reach significant yields in the Mediterranean basin (Alexopoulou et al., 2015) and since, in general, it has been selected and extensively studied as a bioenergy crop since more than ten years (McLaughlin and Kszos, 2005), and found able to positively impact GHG emissions (Monti et al., 2012). At the same time, the Po Valley hosts the cultivation of several food crops, among which maize (*Zea Mays* L.) and wheat (*Triticum aestivum*), that meet favorable climatic conditions allowing substantial grain yields (ISTAT, 2010) without irrigation aids. Maize and wheat are also used in succession, especially when maize stover is reintegrated into the soil, limiting the decline of soil fertility.

We hypothesize that, within future biomass supply districts, to counterbalance the uncertain effects on C stocks of unmanaged marginal and grazed lands conversion to switchgrass (Garten and Wullschleger, 2000; Qin et al., 2016), the conversion of the least remunerative croplands may be subsidized, since this type of land use change can be expected to store elevated amounts of carbon (Freibauer et al., 2004; Nocentini et al., 2015; Qin et al., 2016). In the present study we measured, during two consecutive growing seasons, soil CO₂ respiration and changes in the organic C pools, such as aboveground biomass, litter, roots and soil organic carbon (SOC), in two large fields (farm-scale) in the Po Valley (North Italy), one cultivated with maize and wheat and another one cultivated with switchgrass. These measurements eventually allowed an estimation of the carbon balance of the two agricultural systems (food versus fuel) and delivered an insight on C flows responses to land management, since the two fields were, respectively, under conventional tillage (maize-wheat) and not tilled (switchgrass). Besides direct land use effects (C flows), other indirect C flows, such as life cycle emissions (Fazio and Monti, 2011), savings from fossil fuels displacement (Gelfand et al., 2013) and indirect land use change (ILUC) effects (Searchinger et al., 2008; Laborde et al., 2014), usually distinguish food crops from perennial biofuel crops, therefore, using a dedicated software and literature coefficients, indirect flows were also estimated.

Materials and Methods

Experiment set-up

The trial was carried out in Cadriano (Bologna, North Italy; 44° 33' N, 11° 24' E; 33 m a.s.l.), on a clay loam soil (Table 1) in the experimental farm of the University of Bologna, in 2015 and 2016. A two-ha field of switchgrass was compared with a two-ha field on a maize-wheat rotation.

Table 1 General soil characteristics of the two experimental fields (0-45 cm)

	switchgrass field	maize-wheat field
Gravel (>2 mm) (%)	ns	ns
Sand (0.05 mm<2 mm) (Particle size an.) (%)	29	21
Silt (0.002 mm<0.05 mm) (Particle size an.) (%)	39	51
Clay (<0.002 mm) (Particle size an.) (%)	32	28
pH	7.2	7.6
Total nitrogen (Dumas) (g kg ⁻¹)	1.2	1.4
Total limestone (Dietrich-Fruehling) (%)	1.0	7.5
Available phosphorus (Olsen) (mg kg ⁻¹)	57	30
Exchangeable potassium (mg kg ⁻¹)	161	161

ns= not significant

Switchgrass (cv. Alamo) was sown on 23 April 2012, 45 cm row spaced. Sugar beet and wheat were the former crops, respectively in 2010 and 2011. Seedbed preparation included plowing, harrowing and mechanical hoeing. Phosphate (P_2O_5 , 230 kg ha⁻¹) was distributed during seedbed preparation, while N fertilizer (urea, 100 kg ha⁻¹) was applied from the second year on. Switchgrass was harvested and baled in autumn (early October). Maize (cv. Pioneer 1028, FAO 500) was sown on 1 April 2015 (70 cm inter-row) on a field that had been cropped with maize and wheat since the year 2008. It was fertilized with phosphate (P_2O_5 , 70 kg ha⁻¹) at sowing, and with N (urea, 300 + 300 kg ha⁻¹) after emergence. Mechanical and chemical weed control were performed, as well as treatments against stalk borer in July. Combine harvest was carried out on 20 August without residues removal. Then the soil was plowed and harrowed in September and, on 16 October, wheat (cv. Rebelde) was sown (20 cm inter-row). N fertilizer was applied as urea in January and March (100 + 270 kg ha⁻¹), and as ammonium nitrate in April (167 kg ha⁻¹). Chemical weed control was performed and pesticides against aphids, insects and fungal diseases were also applied. Grain harvest was carried out on 24 June and, afterwards, straw was baled. Information about the management of the two fields is summarized in Table 2.

Table 2 List of field operations and agronomic inputs for switchgrass, maize and wheat cultivated at the experimental farm (North Italy) in the years 2015 and 2016. The following information was used to estimate life-cycle emissions. Emissions occurred during switchgrass establishment were annualized considering ten years of switchgrass economic life span

Inputs	Units	Switchgrass	Maize	Wheat
Plow depth	m	0.4*	0.4	0.4
Harrowing (disk-grubber)	n	1*	1	1
Harrowing (power)	n	1*	1	1
Seeds	kg ha ⁻¹	9*	23	200
N fertilizer	kg ha ⁻¹ y ⁻¹	50 [§]	275	210
P fertilizer	kg ha ⁻¹	230*	70	-
Hoeing	n	1*	1	-
Herbicides	n	2*	1	1
Pesticides	n	-	2	2
Harvesting	type	baling	combine	combine + baling

* establishment

§ from 2nd year

In both fields, sampling areas of 6 m² (3 m x 2 m) were settled in March 2015, randomly distributed on the large surfaces. Inside these sampling areas, various measurements were performed in order to study the size of the C pools and the C flows of the two systems.

Minimum, maximum, average temperature and daily precipitations were recorded by a weather station placed inside the experimental farm (Fig. 1).

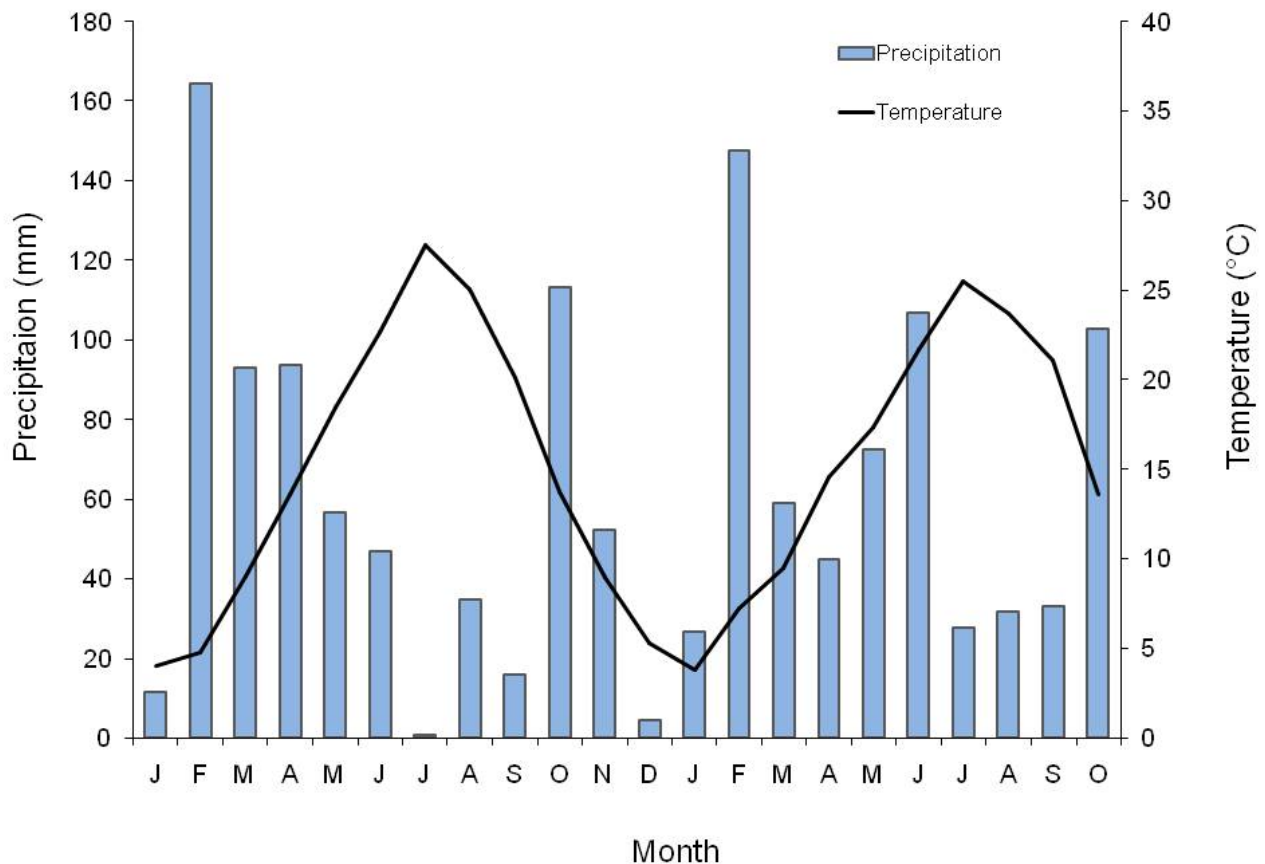


Fig. 1 Cumulative monthly precipitations and monthly average temperature at the experimental site in North Italy for the years 2015 and 2016.

Aboveground C

The aboveground biomass was manually harvested in all sampling areas in switchgrass (fall 2015 and 2016), maize (August 2015) and wheat (June 2016). For the two cereals, grains were separated from the rest of the plant using a small combine harvester (Wintersteiger AG). Harvest losses were measured after wheat mechanical harvest on 6 m² surfaces in June 2016, whereas in maize, being only the grain harvested, all the remaining biomass was considered residue. Also in switchgrass, after each mechanical harvest, losses were measured on 6 m² surfaces, while litter biomass was

collected in March, November 2015 and November 2016 on 0.25 m² areas. Biomass, grains and litter moisture content was determined on oven dried (105 °C) sub-samples after 72 h.

Belowground C

In each sampling area, at each sampling date, three soil cores (70 mm ϕ) were collected by a mechanical auger coupled with a tractor down to 0.45 m soil depth, split in three increments: upper layer (0-0.15 m), intermediate layer (0.15–0.30 m) and deep layer (0.30-0.45 m). In each sampling area, the three soil cores were collected within the row, next to the row and in the inter-row, respectively, to take into account spatial differences in root biomass and SOC deposition. In total, 252 soils cores were collected throughout the experiment (2 crops x 7 samplings x 3 replicates x 3 depths x 2 years). Samples were air-dried: then root biomass was manually separated from the soil, washed, sieved and oven dried (105 °C) for 72 h, while the remaining soil was grinded to 0.5 mm, before encapsulating sub-samples of about 15 mg. The sub-samples were pretreated with HCl to eliminate inorganic C, then finally organic C was determined by an elemental analyzer (Flash 2000 CHNS/O Analyzer, Thermo Scientific, US). Since switchgrass is a C4 species, which succeeded to C3 crops (sugar beet and wheat), its contribution to SOC could be estimated as described by Balesdent et al. (1987):

$$C_{sw} = C_t * [(\delta^{13}C_t - \delta^{13}C_0)/(\delta^{13}C_s - \delta^{13}C_0)] \quad (1)$$

where, C_{sw} , C_t and $\delta^{13}C_t$ are switchgrass-derived C, total soil organic C and soil ¹³C abundance relative to ¹²C at time t , respectively. $\delta^{13}C_0$ and $\delta^{13}C_s$ are the ¹³C/¹²C abundance in the soil prior to switchgrass and of switchgrass plant tissues. Carbon isotope composition was determined using CF-IRMS (continuous flow-isotope ratio mass spectrometry, Delta V advantage, Thermo Scientific, US), both on soil samples and switchgrass root and litter tissues. A small part of the collected switchgrass roots and dead litter (7 replicates) was sub-sampled and oven dried at 60 °C for 72 h, then grinded (0.5 mm) before isotope determinations. The measured $\delta^{13}C$ value of switchgrass tissues (-14.00‰ \pm 0.29) was used in equation 1 as $\delta^{13}C$ of plant inputs to the soil.

In order to not underestimate C accumulation (Anderson-Teixeira et al., 2013), roots were collected always at harvest of the crops and, in switchgrass, also at the beginning of the experiment (March 2015). SOC was measured, contemporarily in the two fields, in March 2015 and in November 2016; additional soil samples had been previously collected in the switchgrass field in March 2013 and March 2014, which were analyzed as well in order to study soil C dynamics since conversion. To calculate C stocks variations starting from SOC concentrations in the soil, soil bulk density was

measured in both fields at two depths (15 and 30 cm), using metal cylinders (volume=98 cm³) and collecting a total of 12 samples (3 replicates x 2 depths x 2 crops).

Soil respiration

Inside each sampling area, two plastic rings (10 cm diameter) were hammered into the soil, one in the row and one in the inter-row; the rings, 10 cm long, were hammered 5 cm into the soil. Soil respiration measurements were performed in each ring (total of 28), weekly during the growing season (April-October) and twice a month in the dormant period, between 7 and 10 am, starting from May 2015. Soil respiration was measured using an infrared gas analyzer (EGM-4 instrument, PPSystems, USA) coupled with a soil respiration chamber (volume= 1.3 l). The measurements were always performed in parallel in switchgrass and in the maize-wheat field, between 7 and 10 am, since continues measurements previously performed (data not shown) showed that in this moment of the day (together with the frame 8-10 pm) the efflux from the soil corresponded to the daily average.



Fig. 2 Soil respiration measurements in switchgrass (a) and wheat after harvest (b). Measurements were performed using an infrared gas analyzer coupled with a soil respiration chamber.

Statistical analysis

All measured data were subject to repeated measures analysis of variance (ANOVA). Fisher's LSD ($P \leq 0.05$) test was used to separate means when ANOVA revealed significant differences ($P \leq 0.05$).

Life-cycle emissions, offset credits and C balance

Equivalent CO₂ emissions from the agronomic inputs of the two fields were also estimated. Taking into account all field operations carried out and materials (i.e. seeds, fertilizers, herbicides, pesticides) used, and after interviewing the workers of the experimental farm about the machines used and the time and diesel spent per hectare for each operation, we were able to perform an analysis with the life cycle software SimaPro 8.0 (PRé Consultants, Amersfoort, NL). Processes

already present in the Ecoinvent 3.0 database were modified to better reflect the field operations observed in terms of diesel use and machines consumption. Finally, equivalent CO₂ emissions caused by each process were calculated employing the IPCC 2013 GWP methodology (IPCC, 2014). Emissions occurred during switchgrass establishment were annualized, assuming that the economic life span of the stand was ten years.

Also the CO₂ indirect uptake deriving from displacing fossil fuels was estimated, since the lignocellulosic harvested biomass could be used to produce advanced ethanol. Lynd et al. (2008) reported that 282 l of EtOH can be produced from 1 ton of dry biomass. Thus, considering an energy density for advanced (lignocellulosic) ethanol of 21.1 MJ l⁻¹ and C savings equal to 89.7 g of CO₂ MJ⁻¹ of energy produced (Gelfand et al., 2013), offset credits could be calculated.

As already discussed by Anderson-Teixeira et al. (2013), being the aboveground biomass harvested and removed every year, the net belowground C balance corresponded to the net ecosystem C balance (NECB), when considering the litter layer part of the soil system. We therefore equaled the belowground C balance to the NECB as well, always considering the C content of the biomass its 40%.

$$NECB = -\Delta root C - \Delta litter C - \Delta SOC \quad (2)$$

Finally, a more comprehensive C balance was performed by adding to the estimated NECB the indirect emissions deriving from agronomic inputs and fossil fuel offset indirect C credits deriving from the production of advanced ethanol:

$$System C balance = NEBC + Life Cycle Emissions - Fossil Fuel Offset \quad (3)$$

Results and Discussion

Biomass C

Harvested biomass was highest in wheat and corresponded to 13.7 Mg ha⁻¹, taking into account both grain and straw (7.2 and 6.4 Mg ha⁻¹, respectively). Instead, in maize, only the grain biomass was harvested (7.5 Mg ha⁻¹), while stover biomass (12.4 Mg ha⁻¹) was completely left on field as residue. Switchgrass showed comparable yields in the two consecutive years of the experiment (14.4 and 13.8 Mg ha⁻¹, respectively in the first and second year), although the composition between harvested biomass and harvest residues differed (15% and 23% of residues in the first and second year, respectively). Therefore, C inputs to the system through residues were greatly higher in maize than in switchgrass (-79%) or wheat (-87%). Moreover, switchgrass residues were not embedded into the soil with tillage, thus they were likely decomposed more rapidly and emitted to the atmosphere. On the other hand, switchgrass residues contributed to enlarge the litter layer covering

the soil which, at the end of the experiment, reached 12.3 Mg ha⁻¹ of dry biomass, an amount that equaled the litter biomass measured in a riparian buffer in central Iowa under the same vegetation (Tufekcioglu et al., 2003). Looking at root biomass, switchgrass showed a mean dry root biomass of 9.2 Mg ha⁻¹, greatly surpassing both maize and wheat (1.9 and 0.6 Mg ha⁻¹, respectively); Tufekcioglu et al. (2003) also reported switchgrass to have about five times more root biomass than maize (~10 and ~2 Mg ha⁻¹, respectively), while Anderson-Teixeira et al. (2013) measured seven times more root biomass in switchgrass than maize (9.0 and 1.2 Mg ha⁻¹, respectively). Further, while in maize and wheat most of the root biomass was found in the upper layer (77 and 86%, respectively), in switchgrass only the 48% was in the upper soil, with the 19% of root biomass (1.8 Mg ha⁻¹) measured in the deep layer. Previously, Monti and Zatta (2009) had found even more root biomass in the deep layer (43%) than in the upper (31%) and intermediate (26%) layers under switchgrass.

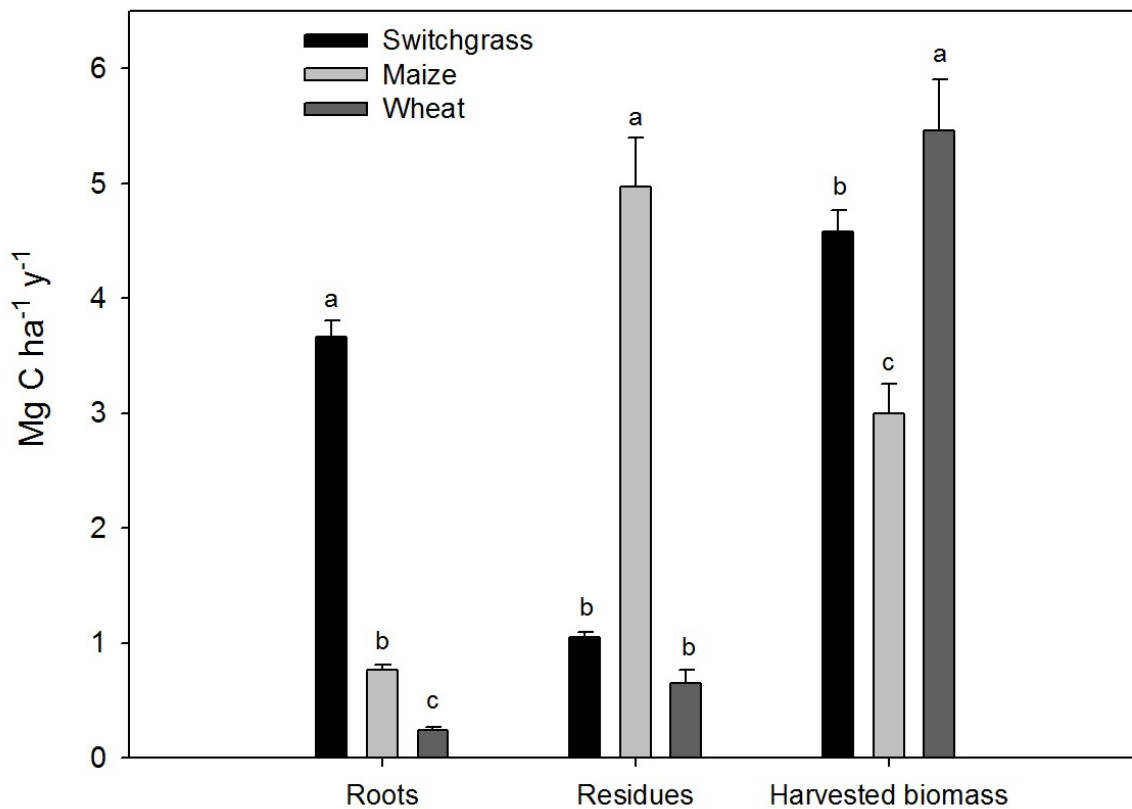


Fig. 3 Biomass C in roots, harvest residues and harvested biomass of switchgrass, maize and wheat cultivated in the years 2015 and 2016 in North Italy; for switchgrass, values of the C pools were averaged between the two growing seasons. *Error bars 1 SE; Letters Significance groupings at $p \leq 0.05$.*

Soil organic C

In the experimental frame (two years), SOC decreased by 1.0 Mg ha⁻¹ in the maize-wheat field and by 2.2 Mg ha⁻¹ in the switchgrass field. In the maize-wheat field, the loss totally happened in the upper and intermediate layers (0-0.30 m) while, on the opposite, in the switchgrass field we measured a SOC gain in the upper layer (+0.6 Mg ha⁻¹), but a considerable loss in the deeper layers (-2.7 Mg ha⁻¹). We hypothesize that tillage and no-tillage managements promoted distinct carbon dynamics within the soil profile (Baker et al., 2007). In the annual crops field, tillage events moved part of the fresh organic matter down to the deeper layer, where losses are reduced due to lower summer temperatures and moisture, whereas it enhanced organic matter decomposition and emission in the upper layers because of an increased gas diffusivity (Ryan and Law, 2005) after soil disruption. In switchgrass instead, the soil was never broken, so no major transfers of organic matter occurred between soil layers. At the same time, a higher inputs rate likely occurred close to the surface than in the intermediate and deep soil layers, as the litter pool contributed in part to soil organic matter, and fine roots, which turn over more rapidly, were more concentrated in the upper soil (direct observation). However, the isotopic analysis revealed that switchgrass-derived SOC increased by 9.0 Mg ha⁻¹ throughout the experiment (years 2015-2016; Fig. 4), while SOC derived from the previous C3 vegetation (sugar beet and wheat) decreased by 11.1 Mg ha⁻¹. Switchgrass-derived SOC was the 20% of total SOC (0-0.45 m soil depth) after 5 years of cultivation, and the 31% in the upper soil layer. Similarly, in the upper soil (0-0.15 m), Collins et al. (2010) found a 24% switchgrass contribution to SOC after 5 years on a land previously cropped with maize and potato (*Solanum tuberosum* L.), while Follett et al. (2012), on a US marginal cropland, measured a lower switchgrass contribution (12%), but the difference is in part likely due to the deeper sampling depth they adopted (0-1.5 m).

In the switchgrass field, SOC and the ¹³C abundance was monitored since after conversion. Despite the SOC loss measured during the experiment, in the five years of switchgrass cultivation, SOC significantly increased by 8.6 Mg ha⁻¹ (+17%) ($P \leq 0.05$), with an overall contribution from the C4 vegetation (switchgrass) equal to 12.2 Mg ha⁻¹ (Fig. 4). We therefore hypothesize that SOC accumulated during the first three years of switchgrass cultivation, reaching a plateau at the fourth year, then the soil C stock likely started fluctuating. Often, SOC accumulation rates measured in the short-term are likely to decrease in the long-term (Qin et al., 2016). Soil C data from the maize-wheat field of previous years were not available, however, we think that such information is less needed as the cereal field had been on the same maize-wheat succession for already ten years and, probably, was at steady-state conditions. The steady-state hypothesis was further proved by the non-significance of SOC variations within the experimental frame.

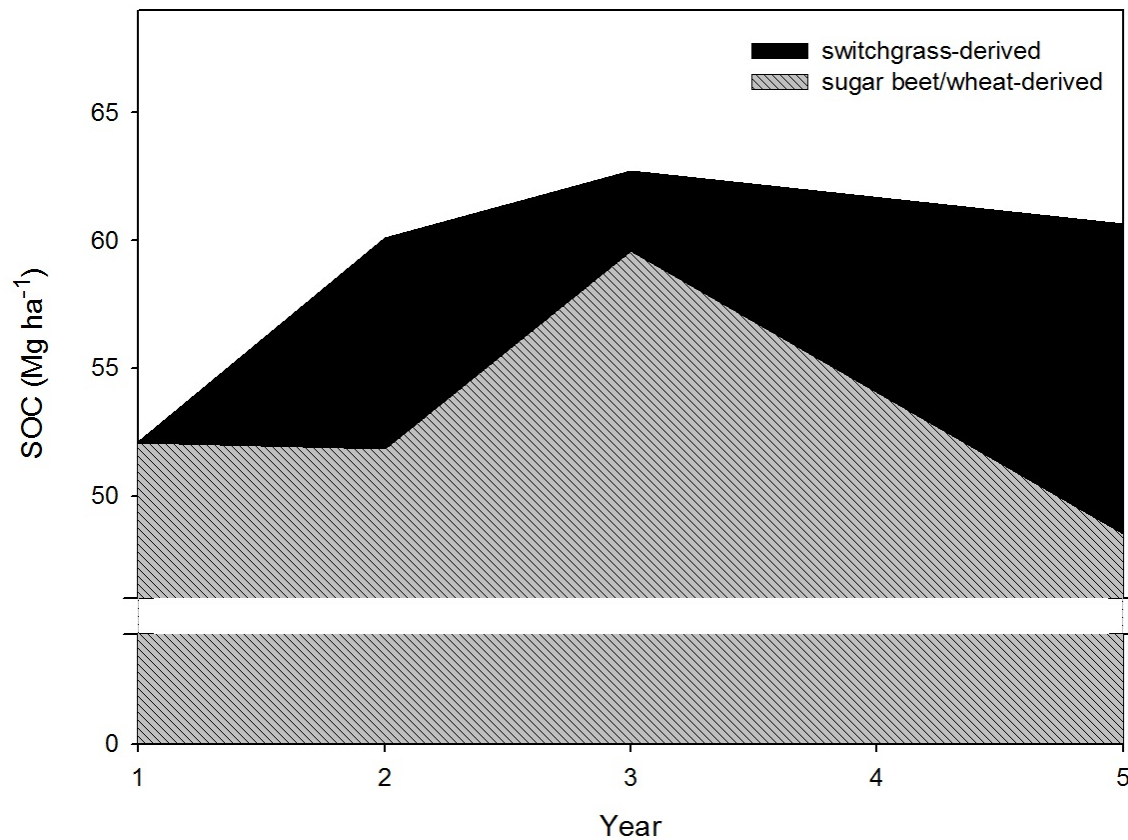


Fig. 4 Cumulative switchgrass (C4)-derived SOC in the upper 0.45 m of soil after four growing seasons (from year 2013 to year 2016) on a former cropland previously cultivated with C3 species (sugar beet and wheat) in North Italy.

C respired from the soil

Soil respiration was monitored in both fields during 18 months (May 2015 to October 2016). Although the annual soil respiration rate did not statistically differ between crops (2.63 and 2.26 g C m⁻² d⁻¹, respectively for switchgrass and maize-wheat), soil respiration in switchgrass showed a more regular trend than in the annual cereals (Fig. 5). Apparently, this was due to the reduced soil tillage of the perennial crop (Table 2). The no-till management allowed also the formation of a thick litter layer under switchgrass, which likely mitigated the effects of precipitations and temperature on soil respiration.

During the growing season, mean soil respiration was higher in switchgrass (3.4 g C m⁻² d⁻¹) than in maize or wheat (2.7 and 2.8 g C m⁻² d⁻¹, respectively). Tufekcioglu et al. (1998) also reported similar soil respiration rates under switchgrass or maize (3.7 and 2.4 g C m⁻² d⁻¹, respectively) during a growing season in Central Iowa, while Rochette et al. (1991) measured much higher mean rates in maize or wheat (~9.9 and ~6.7 g C m⁻² d⁻¹, respectively) between May and August in two

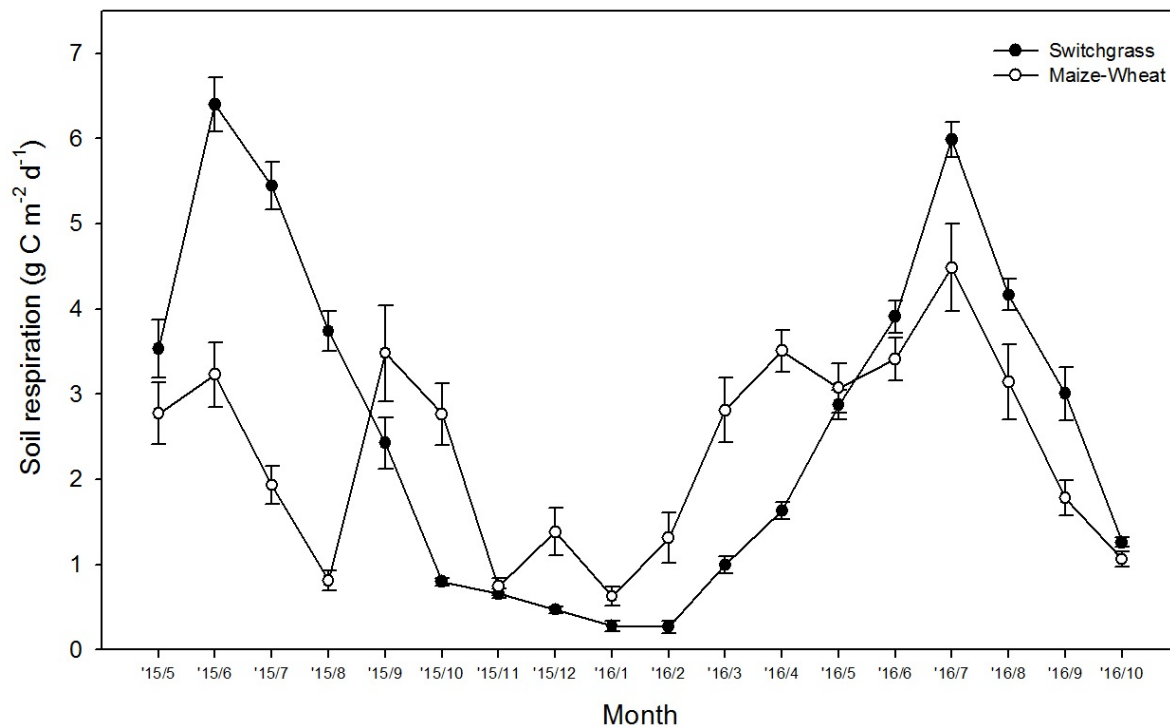


Fig. 5 Soil respiration measured at Cadriano experimental farm (North Italy) during the years 2015 and 2016. A two-ha field cultivated with switchgrass (established in the year 2012) and a two-ha field on a maize-wheat succession are compared. *Error bars 1 SE.*

large fields in Canada. On the opposite, during the dormant period (November to March), mean soil respiration was higher in the maize-wheat field (+100%) than in the switchgrass field ($0.46 \text{ g C m}^{-2} \text{ d}^{-1}$), but was however low compared to the growing periods. Noteworthy, soil respiration in the maize-wheat field reached peak mean values in the five weeks after the first year plowing ($3.7 \text{ g C m}^{-2} \text{ d}^{-1}$), and in the seven weeks after wheat harvest ($4.4 \text{ g C m}^{-2} \text{ d}^{-1}$); these increases in the soil CO_2 efflux appeared closely linked to the soil disturbance caused by the intense management. Soil respiration was significantly ($P \leq 0.001$) higher in the vegetative period (April-October) than in the dormant period (November-March) in switchgrass (Fig. 6). On the opposite, soil respiration was not statistically different in the maize-wheat field between periods of crop growth and periods with bare soil (Fig. 6).

In the entire experimental period, 14.4 and $12.4 \text{ Mg C ha}^{-1}$ were emitted from the switchgrass soil and the maize-wheat soil, respectively. It was not in the scope of this experiment to partition soil respiration (Subke et al., 2006), therefore it is not possible to estimate the amount of respired C that derived from organic matter decomposition (heterotrophic respiration) and how much of the respired C derived from root growth and maintenance (autotrophic respiration), thus, the net C loss. Some indications could be however derived. For example, if the respiration rate during growth was

divided by the amount of roots, maize and wheat had, respectively, three or eleven times higher soil respiration than switchgrass. This showed how, in switchgrass, root-associated respiration was likely high, although it could derive both from autotrophic root respiration or from enhanced microbial heterotrophic respiration within the rhizosphere (i.e. priming effect; Kuzyakov, 2010); however, switchgrass high root biomass certainly increased soil respiration, in part by boosting its growth and maintenance (autotrophic) components (Tufekcioglu et al., 2001; Ryan and Law, 2005; Subke et al., 2006). The relation between roots and soil respiration was experimented also by comparing the efflux on the row and on the inter-row in switchgrass and maize. In switchgrass, respiration on the row was +13% than on the inter-row, while this difference was much more pronounced in maize and was also significant (+118%) ($P \leq 0.01$); a significant difference between respiration on the row and on the inter-row in maize was already reported by Rochette et al. (1991). In switchgrass instead, the narrower inter-row and the larger extension of the root apparatus likely faded this difference between rows and inter-rows. Finally, as depicted in Figure 6, also seasonal patterns can help in a partial understanding of the relative contributions of roots and decomposition to soil respiration (Ryan and Law, 2005).

Subke et al. (2006), after performing a meta-analysis, found a strong relation between yearly respired C and its heterotrophic component across different vegetation types: when total soil respiration is not too low ($> 500 \text{ g C m}^{-2} \text{ y}^{-1}$), its heterotrophic component should be its 45-60%; similar results were previously discussed by Hanson et al. (2000) who found the heterotrophic contribution to average 54% annually. Following this relation, in our study, being yearly soil respiration 811 and 727 $\text{g C m}^{-2} \text{ y}^{-1}$ (switchgrass and maize-wheat, respectively), heterotrophic respiration may have been ~60% of the total (Subke et al., 2006). If we assume so, total losses from soil respiration in the experimental period were 8.7 and 7.4 Mg C ha^{-1} , respectively in switchgrass and the maize-wheat field. Thus, since net SOC variations corresponded to -2.2 and -1.0 Mg C ha^{-1} , respectively in switchgrass and the maize-wheat system, the remaining 6.5 and 6.4 Mg C ha^{-1} emitted through heterotrophic respiration must have been largely caused by fast decomposition of residues and fast root turnover (Hanson et al., 2000); interestingly, in the maize-wheat field, the sum of roots and residues returned to the soil with tillage events corresponded to 6.6 Mg C ha^{-1} (Fig. 3), which means that an amount of C equal to all organic C inputs was re-emitted to the atmosphere.

Emissions from agronomic inputs and offset credits

In this experiment, emissions from agronomic inputs were 709 and 1937 $\text{kg of CO}_2\text{eq ha}^{-1} \text{ y}^{-1}$, respectively for switchgrass and maize-wheat (Fig. 7). Estimated emissions from maize or wheat

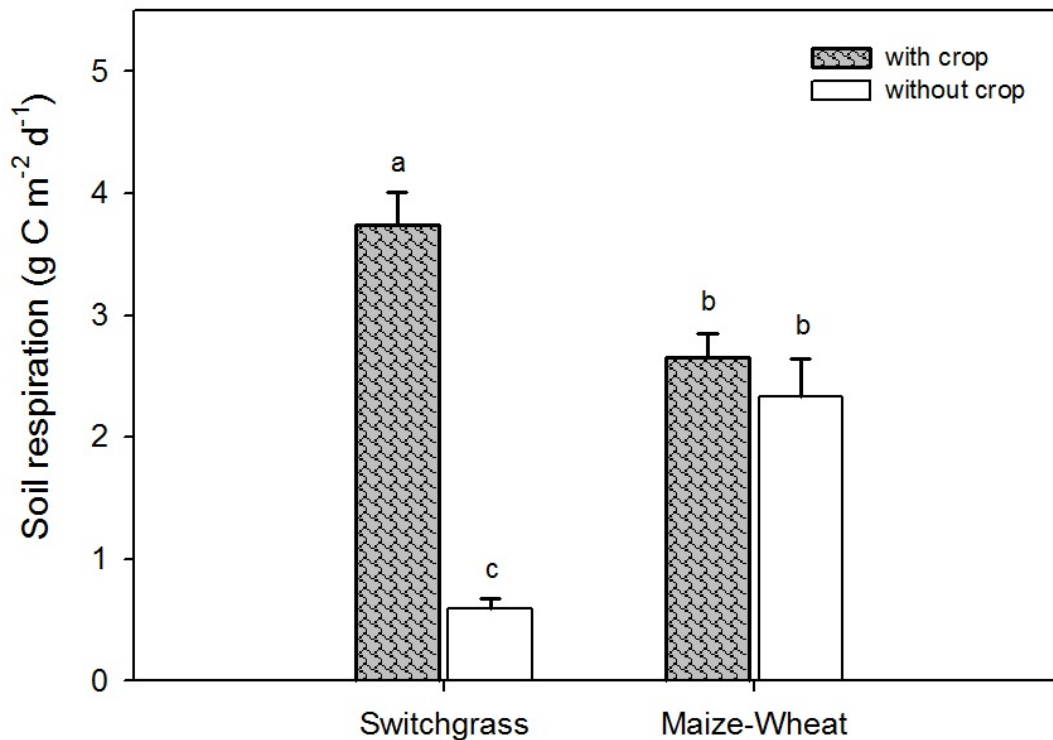


Fig. 6 Soil respiration in switchgrass and maize-wheat averaged for the periods where the soil was covered by the crop or was without the crop (or with the crop dormant). The difference between a perennial no-till system and a conventional system with annual crops is underlined: in switchgrass, the autotrophic component of yearly soil respiration might have been higher than in the maize-wheat system. *Error bars* 1 SE; *Letters* Significance groupings at $p \leq 0.05$.

cultivation were almost identical (1969 and 1905 kg of CO₂eq ha⁻¹, respectively), while, because of the no-till management after establishment and the reduced inputs demand, switchgrass emitted only the 37% of the CO₂ emitted by the annual cereals. Similar cultivation emissions for switchgrass in North Italy were also calculated by Fazio and Monti (2011), whereas they reported lower emissions for maize or wheat, mainly because they assumed much lower N fertilization rates than those actually applied in our experiment. In switchgrass, N fertilization and baling were the most impactful operations (23 and 29% of total emissions, respectively), while in both, maize and wheat, the higher emissions were those deriving from plowing and N fertilization (14 and 49% of total emissions, respectively).

Estimated C savings corresponded to 1.7 Mg ha⁻¹ y⁻¹, if switchgrass harvested biomass was converted in advanced ethanol. Although in the study region wheat straw is commonly harvested and baled for other purposes, if we assume that it was also used for advanced ethanol, it saved 0.9

Mg C ha⁻¹. However, differently from the switchgrass system, these savings would occur only every other year in the maize-wheat succession (i.e. years where wheat is cultivated).

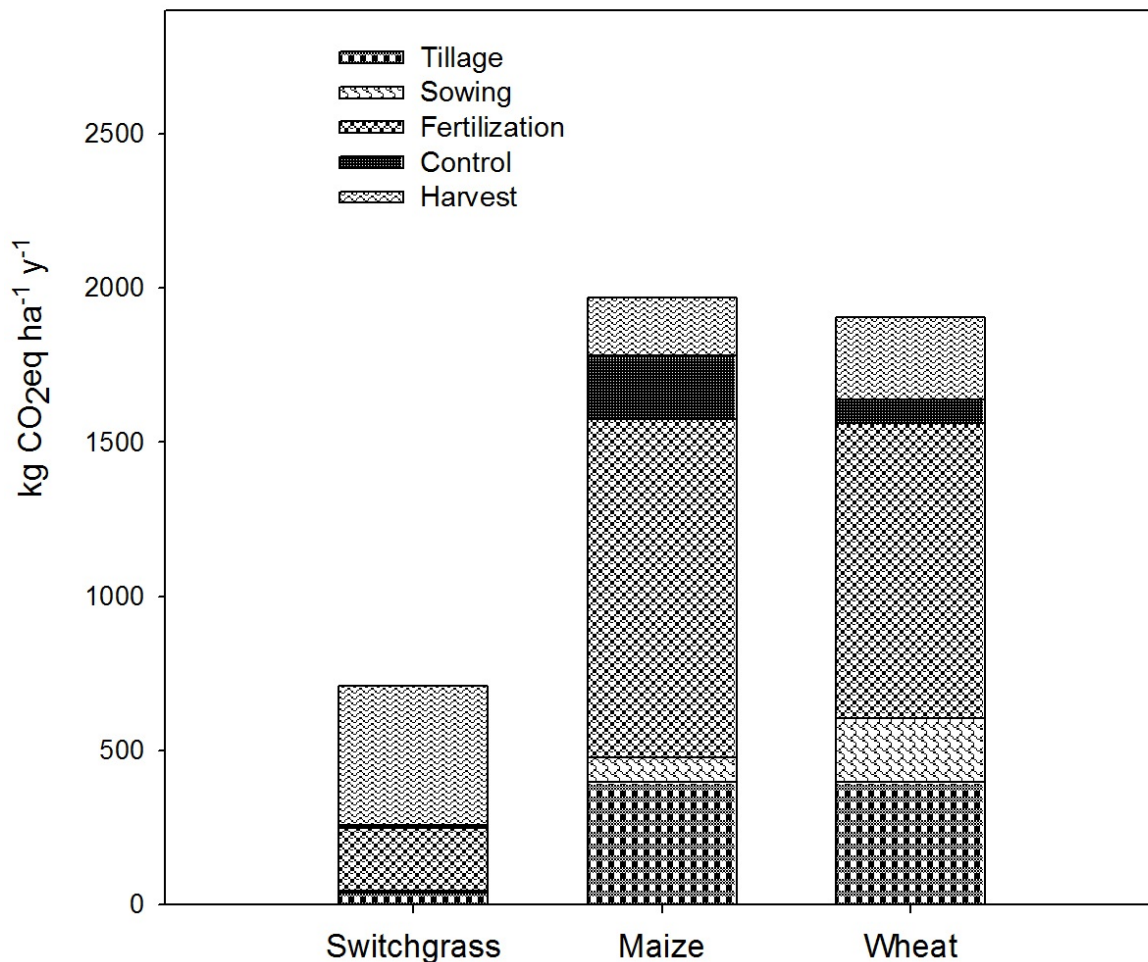


Fig. 7 Equivalent emissions of CO₂ from the use of agronomic inputs in switchgrass, maize and wheat cultivated at the experimental farm (North Italy) in the years 2015 and 2016.

C balance

In two growing seasons, switchgrass NECB resulted significantly negative (C sink) and corresponded to -2.3 Mg C ha⁻¹, whereas the maize-wheat system lost C (1.0 Mg C ha⁻¹) (Fig. 8). Despite the SOC loss registered in the switchgrass field, the no-till management allowed a C accumulation in the litter and root pools (Anderson-Teixeira et al., 2013), finally resulting in a net C sink. On the opposite, in the annual cereals field, the harvest events killed off all the plants and, every time, tillage embedded all the residues in the soil, not allowing the formation of a litter layer; at the same time, the increased gas diffusivity and sensitivity to environmental factors given by soil disruption caused the fast re-emission of recent organic C inputs (residues and roots). In a 4-year

study, Anderson-Teixeira et al. (2013) also found switchgrass to be a C sink ($-8.0 \text{ Mg C ha}^{-1}$) and a maize-maize-soy rotation to be a C source (7.3 Mg C ha^{-1}). In their experiment, although the soil had been cultivated with annual crops since many decades, the maize-maize-soy rotation released a large amount of C, giving the impression that the soil was not at equilibrium; we think that the root exclusion technique they applied might have underestimated autotrophic respiration (Hanson et al., 2000; Subke et al., 2006) and thus overestimated C losses by microbial decomposition in the soil, eventually exaggerating the difference between the food and the biofuel system. Previously, Tilman et al. (2006) measured, during ten years of cultivation of grassland biomass, a sequestration rate ($1.2 \text{ Mg C ha}^{-1} \text{ y}^{-1}$) similar to the one measured here ($1.1 \text{ Mg C ha}^{-1} \text{ y}^{-1}$; Fig. 8) during the fourth and fifth years of switchgrass cultivation.

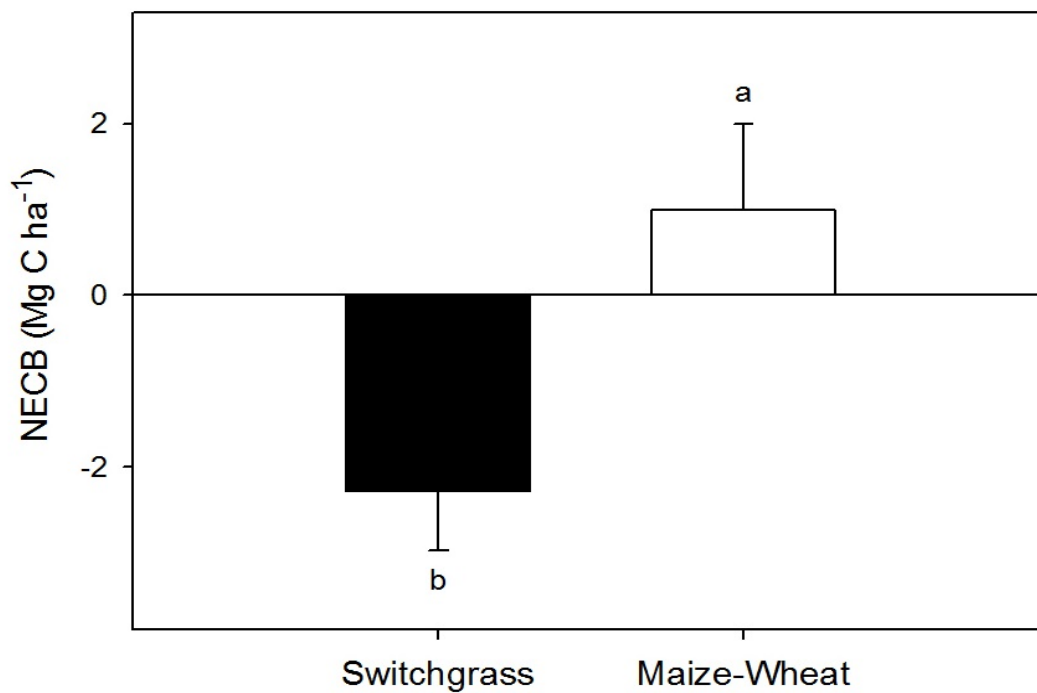


Fig. 8 Net ecosystem C balance measured at the experimental farm (North Italy) during two consecutive growing seasons (years 2015 and 2016) in an established two-ha switchgrass field and in a two-ha maize-wheat field. The balance was separately calculated for each sampling area, considering the variations occurred in the litter C pool, root C pool and SOC pool; positive values correspond to a C emission, while negative values to a C uptake. *Error bars* 1 SE; *Letters* Significance groupings at $p \leq 0.05$.

Factoring in indirect C flows, made switchgrass a greater C sink, while increased the emissions of the maize-wheat system (Table 3). In fact, agronomic inputs in switchgrass produced about one

third of the emissions that were produced by agronomic inputs in the annual cereals. Moreover, differently from the maize-wheat system where only a part of the harvested biomass was used to produce energy (30%), all switchgrass harvested biomass was destined towards advanced ethanol production, thus increasing C credits. If ILUC emissions were to be included as well, the C balance would not change substantially. ILUC effects caused by switchgrass cultivation on a primary arable land were estimated equal to 17 g CO₂eq MJ⁻¹ of wheat-ethanol (Laborde et al., 2014). The wheat-ethanol production was calculated proportionally to the potential wheat grain yield deliverable on that same land, considering 372 l ethanol Mg⁻¹ of wheat grain. So, switchgrass-derived emissions because of the displacement of food production would correspond to 0.96 Mg CO₂eq ha⁻¹ y⁻¹, eventually reducing the final C uptake by switchgrass (Table 3) to -4.6 Mg ha⁻¹. At the same time, since wheat straw is commonly harvested for other farm uses, not using it for advanced ethanol would increase the impact of the annual cereals (2.1 Mg C ha⁻¹ emitted).

Table 3 Final C balance of an established switchgrass field and a maize-wheat field, measured during two consecutive growing seasons in North Italy. The final balance includes direct (changes in litter, root and soil C pools) and indirect (emissions from agronomic inputs and estimated credits from fossil fuels offset) C flows; positive values correspond to a C emission, while negative values to a C uptake

Land use	Δ litter C (Mg C ha ⁻¹)	Δ root C (Mg C ha ⁻¹)	Δ SOC (Mg C ha ⁻¹)	Cultivation emissions (Mg C ha ⁻¹)	Offset credits (Mg C ha ⁻¹)	Net balance (Mg C ha ⁻¹)
Switchgrass	-1.9	-2.5	2.2	0.4	-3.3	-5.1
Maize-Wheat	-	-	1.0	1.1	-0.9	1.2

Considering litter, root and soil organic C pools of switchgrass at their maximum storage potential after five years (Arundale et al., 2014), we could annualize switchgrass C uptake, attempting to project its sink capacity during its entire economic life span. After five years, litter biomass was 12.3 Mg ha⁻¹, root biomass was 10.0 Mg ha⁻¹ and SOC gain corresponded to 8.6 Mg ha⁻¹. Therefore, including annualized emissions from agronomic inputs, fossil offset savings and ILUC impact, and considering ten years of economic life span, switchgrass potential as C sink on former croplands in North Italy was eventually estimated equal to -2.85 Mg C ha⁻¹ y⁻¹. On the opposite, since SOC variation in maize-wheat resulted not significant during the experiment, we considered the annual cereals soil at steady-state and estimated its annual impact equal to the cultivation emissions only (0.53 Mg C ha⁻¹ y⁻¹). So that converting one hectare of a maize-wheat succession of the Po Valley to switchgrass for advanced ethanol today would produce an overall C storage of 3.4 Mg y⁻¹ for the next ten years.

Other C flows, as the volatilization of organic compounds, methane (CH₄) emissions and C leaching to the aquifers, which normally occur in ecosystems (Smith et al., 2010), were not specifically addressed by the present study. Nevertheless, we believe that, by measuring the net C variation in each C pool (litter, roots and soil), those flows were indirectly taken into account. Instead, our measurements could not account for possible soil losses through erosion: these losses would however not be substantial (0.1 Mg C ha⁻¹ y⁻¹; Smith et al., 2010), especially in the switchgrass field where, likely, thanks to the litter layer, soil erosion did not occur.

Nitrous oxide (N₂O) emissions is another factor highly influencing the GHG balance of agricultural systems. Their estimation is outside the scope of this paper, but we can however hypothesize that, if accounted for, N₂O emissions would have probably further increased the difference in the GHG impact between the annual crops and switchgrass (Drewer et al., 2012), as yearly N fertilization was substantially lower (-79%) in the latter (Table 2).

Conclusions

Although biofuel perennial crops are thought to cause greenhouse gas emission reduction, especially when compared to annual food crops, direct estimations are necessary, also to enrich inventories employed in large-scale assessments.

In the present study, we observed that the land use change from traditional annual crops to perennial grasses, characterized by a less intensive management, lead to significant benefits in term of carbon balance. This was achieved both through organic carbon storage and by displacing fossil energy sources. However, while it is safe to rely on long-term carbon savings brought by fossil energy offset, the capacity of perennial crops to store organic C may saturate sometimes in the medium-term. Two-year continuous monitoring of the soil carbon dioxide efflux, performed also during soil tillage events and changes in crop cover, clearly underlined the direct impact on carbon flows of anthropogenic management in agricultural ecosystems.

Acknowledgements

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Land use change from poplar to switchgrass and giant reed increases soil organic carbon

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Abstract Switchgrass and giant reed can provide a dual contribution in reducing greenhouse gases emissions through displacing fossil fuels and derivatives, and increasing soil organic carbon. However, if it is generally true that displacing fossil fuels with biomass brings favorable effects, there is not as much evidence that perennial grasses increase soil organic carbon, as it mainly depends on the land use change. The present study investigated, for the first time, the effects on soil organic carbon of the land use change from poplar to switchgrass and giant reed. We addressed the soil organic carbon variation over 10 years of switchgrass and giant reed succeeding a 30-year poplar. Soil samplings were performed after three and ten years from establishment down to 0.6 m depth. The results show that, although the ability of poplar to store large quantities of soil C is widely demonstrated, the two perennial crops allowed to further increase soil organic carbon stocks; particularly, giant reed increased soil organic carbon at a double rate than switchgrass (0.19 and 0.09 g kg⁻¹ y⁻¹). The variation in soil organic carbon highly affected total greenhouse gas savings as estimated by a life cycle assessment: 11-35% and 20-42% of total savings from switchgrass and giant reed, respectively, derived from increasing soil C stocks. These results highlight the importance of understanding long-term environmental- and crop-specific land use change effects in life cycle assessments instead of applying coefficients to generic crop categories (e.g. perennial tree/crop) and crop sequences, as it normally happens.

Keywords: Lignocellulosic; Biofuels; Land Use Change; Soil Organic Carbon; Carbon isotopes; C savings.

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Introduction

Deep-rooted perennial grasses such as switchgrass (*Panicum virgatum* L.) and giant reed (*Arundo donax* L.) can provide a dual contribution to greenhouse gases emission reduction by both storing soil organic carbon (SOC) (Lemus and Lal 2007; Agostini et al. 2015) and producing biomass to displace fossil fuels (Alexopoulou et al. 2015; Schmidt et al. 2015). However, if it is true that every year a certain amount of biomass is produced, much uncertainty exists when factoring in SOC variations, which can be positive or negative depending on several intrinsic and extrinsic factors, such as climate, soil type, crop management, and former land use (Anderson-Teixeira et al. 2009; Brandão and Milà i Canals 2013). Especially the former land use impacts SOC stocks as, in most cases, the change to perennial grasses triggers the soil system towards a new equilibrium. New SOC dynamics are principally a result of a change in above- and below-ground vegetation (live C pools) and in the land management. Typically, perennial grasses increase SOC when replacing arable crops, while they decrease SOC when grown after natural ecosystems or pasturelands (Fargione et al. 2008; Anderson-Teixeira et al. 2009; Qin et al. 2016).

Ideally, dedicated perennial grasses for biofuels and biorefinery are thought to be suited to marginal lands (i.e. areas with a low economic value, considering both productive and ecological perspectives), in order to avoid competition with food crops. However, an unresolved dilemma is whether the cultivation of perennial grasses in marginal areas will maintain SOC unchanged, or if a negative SOC change will nullify the positive effects from fossil fuel displacement (Fargione et al. 2008). Understanding land use change effects on SOC will allow enhancing the life cycle assessments of agro-energy systems, since, currently, the uncertainty in SOC dynamics still makes it difficult to give any exact figure. In many cases, SOC is not even included in the analyses (Davis et al. 2009), while, more commonly, general coefficients of SOC changes are applied while implementing life cycle assessments (Brandão and Milà i Canals 2013). Some studies instead (Adler et al. 2007; Gelfand et al. 2013; Hudiburg et al. 2016) employed biogeochemical models to account for biogenic impact on CO₂ emissions, allowing a greater accuracy in assessing SOC dynamics. In fact, compared to general coefficients, such models (e.g. DAYCENT) can account for small differences in C pools, soil dynamics and land management, therefore owing the power to distinguish the effects on SOC even of crops with analogous uses and similar managements. However, in order to be properly calibrated, such models need measured, reliable data.

In general, land use changes to perennial grasses have been investigated after arable crops or after native vegetation (Qin et al. 2016). Biomass supply districts in marginal areas might not include food crops (less suitable for low-productivity marginal areas) but only perennial grasses and short rotation coppices that succeed one another. In this context, long-term SOC variations caused by

land use change from short rotation coppices to perennial grasses could constitute a valuable information for the near future.

To the best of our knowledge, although the effects on SOC of switchgrass, and to a less extent of giant reed, have been somehow investigated (Monti et al. 2012 and references therein, Ceotto and Di Candilo 2011; Sarkhot et al. 2012; Cattaneo et al. 2014; Monti and Zegada-Lizarazu 2016), this is the first study that specifically addressed SOC variation of switchgrass and giant reed following poplar in a side-by-side comparison. Sampling the soil to a depth of 0.6 m was believed effective in capturing the majority of the SOC variation as root C deposition rapidly decreases with soil depth (Garten and Wullschleger 2000; Collins et al. 2010); several other studies on SOC dynamics of switchgrass and giant reed have, in fact, considered the same soil layer or shallower (Garten and Wullschleger 2000; Kucharik 2007; Ceotto and Di Candilo 2011; Cattaneo et al. 2014; Fagnano et al. 2015; Monti and Zegada-Lizarazu 2016). The study covered a period of ten years which is considered appropriate to provide reliable results on SOC variation (Qin et al. 2016).



Fig. 1 Switchgrass and giant reed in North Italy, with poplar on the background.

Materials and methods

Experimental set up

The trial was carried out on a flat, clay-loam sub-alkaline soil (Table 1) in Campotto (North Italy, 44° 34' N, 11° 47' E; 5 m a.s.l.) over a period of ten years. The climate is characterized by cold humid winters and hot summers. During the trial (2005-2014), mean annual temperature was 13.4 ± 8.3 °C (17.8 ± 6.3 °C, March to October), and rainfall was 613 ± 150 mm (409 ± 87 mm, March to October). Switchgrass (*Panicum virgatum* L.; var. Alamo) was sown on 12th of May at seed density of 400 seeds m⁻² (48 cm row spaced), while giant reed (*Arundo donax* L.; local ecotype) was transplanted on 5th of July at a plant density of 1 x 1 m, both in replicated (n=3) plots of 2,250 m² each, arranged according to a randomized block design. Plots sides were 15m x 150m; plots were separated by 10m wide uncultivated rows. Poplar (*Populus x euramericana*, cultivar I-214) was the previous crop for 30 years, entirely covering the land where the experimental plots were set. It was completely harvested and uprooted in autumn 2003. The soil was then subject to a moldboard plowing before winter and to harrowing in spring before establishing the two perennials. In the frame of low input bioenergy systems, both grasses were never fertilized with N. Phosphate (P) was distributed at a dose of 44 kg ha⁻¹ only during field preparation, while potassium (K) was not supplied given the high soil content (Table 1). To ensure a successful establishment, 165 (switchgrass) and 210 (giant reed) mm of irrigation water were supplied during the first year. Both species were harvested annually in February.

Table 1 General soil characteristics at the experiment site (0-0.5 m)

Gravel (>2 mm) (%)	ns
Sand (0.05 mm<2 mm) (Particle size an.) (%)	21
Silt (0.002 mm<0.05 mm) (Particle size an.) (%)	51
Clay (<0.002 mm) (Particle size an.) (%)	28
pH	7.5
Total nitrogen (Dumas) (g kg ⁻¹)	1.4
Total limestone (Dietrich-Fruehling) (%)	18.3
Available phosphorus (Olsen) (mg kg ⁻¹)	12
Exchangeable potassium (mg kg ⁻¹)	315

ns= not significant

Soil organic carbon and isotope composition

Soil organic carbon content was determined in three soil layers (0-0.2, 0.2-0.4, 0.4-0.6 m) the day before sowing switchgrass and again after the third (February 2007) and tenth harvests (February 2014). Given the large plot size (2,250 m²), three samplings per plot were collected resulting in 108 soil cores (2 crops x 3 replicates x 3 samplings x 3 depths x 2 years). Soil cores (70 mm ϕ) were collected during the harvest by a mechanical auger coupled with a tractor. Soil samples were air-dried and entirely ground to 0.5 mm prior to organic C determinations. Soil sub-samples (about 15 mg) were pre-treated with HCl to eliminate inorganic C then encapsulated. SOC was determined by an elemental analyzer (Flash 2000 CHNS/O Analyzer, Thermo Scientific, US).

Since poplar has a C3 photosynthetic pathway whereas switchgrass is a C4 species, carbon isotope composition was determined (CF-IRMS continuous flow-isotope ratio mass spectrometry, Delta V advantage, Thermo Scientific, US) in order to estimate switchgrass contribution to SOC as given by Balesdent et al. (1987):

$$C_{sw} = C_t * [(\delta^{13}C_t - \delta^{13}C_0)/(\delta^{13}C_s - \delta^{13}C_0)] \quad (1)$$

Where, C_{sw} , C_t and $\delta^{13}C_t$ are switchgrass-derived C, total soil organic C and soil ¹³C abundance relative to ¹²C at time t , respectively. $\delta^{13}C_0$ and $\delta^{13}C_s$ are the ¹³C/¹²C abundance in the soil prior to switchgrass and of switchgrass plant tissues. Switchgrass roots (extracted from 70 mm ϕ soil cores) and dead litter (0.25 m² sampling areas) were collected in March 2015, separated from soil, washed, sieved, cleaned, oven dried at 60 °C for 72 h, and finally grinded (0.5 mm) before isotope determinations. The same procedure used for the soil samples was used to determine the carbon isotope ratio of the plant material, although the amount of sub-sample encapsulated for the analysis differed (~0.5 mg). Since root (-14.03‰ \pm 0.23) and litter (-13.98‰ \pm 0.44) materials showed almost identical $\delta^{13}C$ values, an average (-14.00‰ \pm 0.29) was used in the above equation as $\delta^{13}C$ of switchgrass plant inputs to the soil.

C credits from advanced bioethanol production

The greenhouse gas emissions deriving from the agricultural management of the two grasses were estimated through a life cycle analysis using SimaPro 8.0 (PRé Consultants, Amersfoort, NL), according to IPCC 2013 GWP methodology (IPCC 2014); life-cycle emissions were annualized for the ten years of cultivation of the two crops, so that the time horizon was equal and comparable with the annualized land use change SOC credits. Processes already present in the Ecoinvent 3.0 database were adjusted to better reflect the real field management of the two grasses. Cradle-to-farm gate impacts of both, switchgrass and giant reed, were then compared on a land- (hectare)

basis. Fossil fuel offset credits from the production of advanced ethanol were estimated taking into account aboveground biomass productions. Year by year yields of switchgrass and giant reed in this experiment were reported elsewhere (Alexopoulou et al. 2015). For bioethanol conversion, we assumed that 282 l of EtOH (21.1 MJ l⁻¹) are produced from 1 ton of dry biomass (Lynd et al. 2008). Estimated carbon credits due to fossil fuel offset were calculated by considering 89.7 g of CO₂ saved for MJ of energy produced from bioethanol (Gelfand et al. 2013).

Table 2 List of field operations and agronomic inputs used to estimate life-cycle emissions from switchgrass and giant reed cultivated in Campotto (North Italy) in the years 2004-2014. Besides harvest that occurred yearly, all the remaining operations and inputs occurred only during establishment, hence, the emissions deriving from them were annualized (ten years)

Inputs	Units	Switchgrass	Giant reed
Plowing	n	1	1
Harrowing (disk-grubber)	n	1	1
Rotary cultivator	n	1	1
Seeds	kg ha ⁻¹	4.2	-
Seedlings	n ha ⁻¹	-	10000
P fertilizer	kg ha ⁻¹	44	44
Irrigation	mm	165	210
Hoeing	n	1	1
Harvesting	type	baling	baling

Sensitivity analysis of SOC stocks changes

Since soil bulk density was not measured in this study, we could not accurately provide SOC stock changes due to the measured variations in SOC concentration. However, to weigh SOC storage credits on the overall C balance, we attempted to estimate, through a sensitivity analysis where soil bulk density values were varied, the range of SOC stocks variation beneath switchgrass and giant reed. First the soil water content at saturation was estimated by employing the correlation found by Saxton et al. (1986) between soil texture and soil water characteristics:

$$Saturation = [0.332 - (7.251^{-4}) \times (\%sand) + 0.1276 \times \log_{10}(\%clay)] \quad (2)$$

Where *%sand* and *%clay* are the relative content of sand and clay of the soil, respectively. Then soil bulk density (*BD*) was inferred through the following equation:

$$BD = (1 - \textit{Saturation}) \times 2.65 \quad (3)$$

We then assumed that soil bulk density could have been as low as -20% (1.06 g cm⁻³) or as high as +20% (1.58 g cm⁻³) of the calculated value (1.32 g cm⁻³) as well as that soil bulk density may have increased in time by up to 18% since establishment due to compaction (Onstad et al. 1984); we consider this latter assumption reasonable as part of the soil compaction already occurred before the initial sampling due to five months of weathering after plowing (December to May). Hence, by converting the gravimetric data to areal C stocks (Lee et al. 2009), the range within which SOC stocks changed was calculated.

Statistical analysis

All data were subject to repeated measures analysis of variance (ANOVA); the year was considered a random factor. Fisher's LSD ($P \leq 0.05$) test was used to separate means when ANOVA revealed significant differences ($P \leq 0.05$).

Results and discussion

Long-term carbon storage in the soil

There is an unquestionable consensus of scientific opinion that switchgrass and giant reed generally contribute to increase soil carbon (Anderson-Teixeira 2009; Monti et al. 2012; Cattaneo et al. 2014; Fagnano et al. 2015; Monti and Zegada-Lizarazu 2016; Ceotto and Di Candilo 2011). Nonetheless, the magnitude of SOC variation is not univocal for different environments, depending mostly on the land use change. Positive effects (SOC increase) have been generally observed when perennial grasses replaced arable crops (Anderson-Teixeira et al. 2009; Qin et al. 2016). In contrast, a reduction of SOC can be expected when perennial grasses replace natural ecosystems and pasturelands or, in general, soils with large C stocks (Garten and Wullschleger 2000; Fargione et al. 2008; Qin et al. 2016).

Poplar can be grown as a biomass crop (short rotation coppice), similarly to switchgrass and giant reed. Therefore, hypothetical specialized biofuel/biorefinery districts including only lignocellulosic crops could include poplar and perennial grasses succeeding one another in the long term. In our experiment, poplar was cultivated for 30 years before land use change to switchgrass and giant reed. As poplar is broadly recognized for its ability to accumulate high amounts of soil carbon (Rytter 2012; Agostini et al. 2015), we expected a significant reduction of SOC, especially in the first years of grasses cultivation.

Unexpectedly, we found that both perennial grasses further increased SOC after ten years (Fig. 2). Giant reed, in particular, significantly increased SOC by 2.3 ($P \leq 0.01$) and 1.5 ($P \leq 0.05$) g kg^{-1} in the upper (0-0.2 m) and intermediate (0.2-0.4 m) soil layers, respectively, showing a double mean annual SOC accumulation rate than switchgrass (0.19 and 0.09 $\text{g kg}^{-1} \text{y}^{-1}$, respectively). The higher SOC increase of giant reed compared to switchgrass can be likely explained by the higher below- and aboveground biomass development of giant reed (Monti and Zatta 2009; Alexopoulou et al. 2015). Biomass residues (unrecovered biomass) of giant reed were also more than twice those of switchgrass (+105% of leaf-litter, data not shown); although leaves debris are generally more easily decomposable than root material (Kemp et al. 2003), this contributes in explaining the higher SOC under giant reed. Root size is another major factor affecting SOC accumulation (Agostini et al. 2015), but this was found to be very similar (250-300 μm) between switchgrass and giant reed (Monti and Zatta 2009). Root tissues chemistry can also affect SOC dynamics. Liang et al. (2015) showed that giant reed roots have a slow potential decomposition (1.80 $\text{g kg}^{-1} \text{d}^{-1}$) and, among six other perennial grasses, the highest root lignin content and highest lignin/nitrogen ratio.

SOC variation (g kg^{-1})

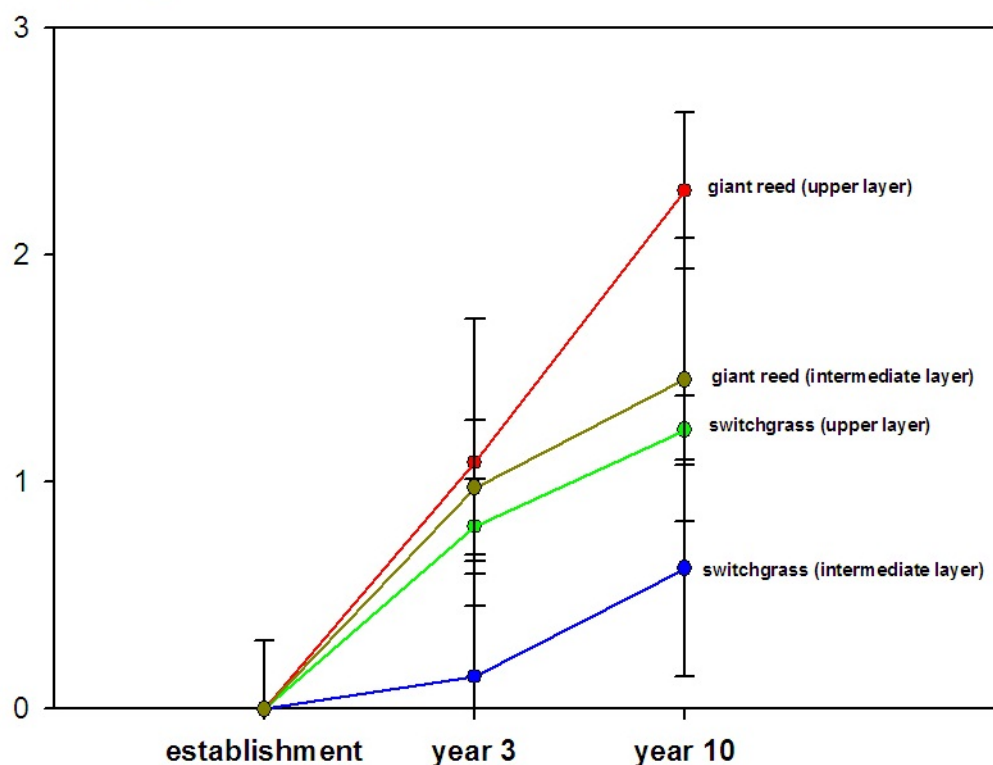


Fig. 2 Soil organic carbon (SOC) variation under switchgrass and giant reed in the upper (0-0.2 m) and intermediate (0.2-0.4) soil layers after three and ten years of cultivation. For both grasses, poplar was the previous crop for thirty years. Error bars 1 SE.

Remarkably, about the half (59% and 54% in switchgrass and giant reed, respectively) of SOC was accumulated during the first three years, then SOC accumulation rates decreased. Higher SOC accumulation rates in the early period were also observed in other studies on the same species (Kucharik 2007; Anderson-Teixeira et al. 2009). In our stands, biomass yields also showed a descending trend starting from the 4th year (Alexopoulou et al. 2015), however such a decline was irregular and difficult to associate with the decrease of SOC accumulation rates. The absence of N fertilization in our experiment may also have contributed to a progressive decrease of SOC accumulation rates. Lee et al. (2007), for example, showed that, under switchgrass, SOC gains can increase up to +67% depending on N fertilization rates and fertilizer type (organic or synthetic). Again, in a 16-year study on N fertilization of giant reed, Monti and Zegada-Lizarazu (2016) found that SOC significantly increased with increasing N fertilization rates. Garten and Wullschleger (2000) reported that the mineral-associated fraction of SOC is preponderant under switchgrass (~83%) and that it is more stable (mean residence time of about 25-38 years) than the particulate organic matter fraction (mean residence time of about 3 years), which in fact tends to stabilize C by associating it to the mineral fraction (13-15% y⁻¹ transferred). Therefore, we may hypothesize that this stabilization becomes more difficult as the soil approach steady-state conditions, thus, when a C accumulation is occurring beneath switchgrass, the overall mean residence time of SOC decreases as less C is transferred to the mineral-associated fraction.

Regardless of the cause, the decreasing SOC accumulation rate after three years also suggests that 8-10 years should be the minimal experimental frame for avoiding biased SOC estimations in perennial biomass crops (Qin et al. 2016). For example, if the present study had been limited to the first three years only (instead of ten years), the annual equivalent SOC accumulation rate would be almost double (0.16 and 0.34 g (C) kg⁻¹ y⁻¹, respectively in switchgrass and giant reed).

C4-derived SOC

SOC stocks may evolve irrespective of the contribution from the current vegetation. A high deposition of organic matter may be canceled by a fast turnover, thus apparently resulting in a null contribution from the current land use. Garten and Wullschleger (2000), for example, converted pastures to switchgrass and, although they always observed an increase in switchgrass-derived C, they did not find a positive SOC gain in all sites; thus, C losses likely counterbalanced the deposition of new C. Understanding the real contribution of the current vegetation to SOC becomes an important indication of the accumulation potential of a certain land use and gives a deeper insight on SOC dynamics.

Switchgrass-derived C (upper 60 cm soil depth) was insignificant (0.3%) after three years, but was the 9% after ten years (Fig. 3). Other authors reported a contribution ranging from 12% to 24% after nine and five years, respectively (Follett et al. 2012; Collins et al. 2010), but they considered very different soil layers of 150 and 15 cm, respectively. Although in their study the soil was analyzed down to a double depth than in our experiment, Follett et al. (2012) found a similar contribution of switchgrass to SOC revealing that the overwhelming majority of C deposition occurred in the upper soil layers. Figure 3 shows how switchgrass-derived C was higher in the upper soil layer (11%), though significantly lower than that measured by other authors (Collins et al. 2010) at the same depth.

In the present study, poplar-derived C increased ($0.15 \text{ g kg}^{-1} \text{ y}^{-1}$) over the first three years after switchgrass establishment (Fig. 3). This could be easily explained by the decomposition of pre-existing poplar carbon sources, which may be instead characterized by a mean residence time of about five years, as previously reported by Cotrufo et al. (2005) who also operated on a loam soil. This was in fact verified by observing the poplar-derived C decrease in the following seven years ($-0.08 \text{ g kg}^{-1} \text{ y}^{-1}$). So, the high contribution of poplar-derived C to SOC in the first three years seems, eventually, to clearly explain why SOC accumulation rates decreased afterwards under both crops. Overall, in the ten years, switchgrass-derived C increased at a rate that was higher than the rate of the net SOC increase (0.10 vs. $0.09 \text{ g kg}^{-1} \text{ y}^{-1}$), as expected. The deposition of switchgrass-derived C exceeded the emissions of poplar-derived C, as the system had probably not reached yet a steady-state.

Contribution to life-cycle C balance

Considerable uncertainties still persist on the use of SOC in life cycle assessments. Generic and standard SOC values are commonly used to estimate the carbon balance of agricultural systems (Brandão and Milà i Canals 2013), whereas, often, analyses are incomplete, not even including the land use change effect on SOC (Davis et al. 2009). Brandão and Milà i Canals (2013) analyzed the main drivers of the coefficients given by the Intergovernmental Panel on Climate Change commonly adopted to account for SOC variations. While initial SOC stocks are calculated using different factors (climate, soil type and native vegetation), basically, SOC changes only depend on land-use management options. For example, according to the indications of the Intergovernmental Panel on Climate Change, the land use change examined in our experiment, from poplar to switchgrass or from poplar to giant reed, should not cause any SOC change because perennial grasses and poplar are classified within the same vegetation category (Brandão and Milà i Canals 2013). On the opposite, we measured a substantial SOC accumulation, which was also substantially

SOC (g kg^{-1})

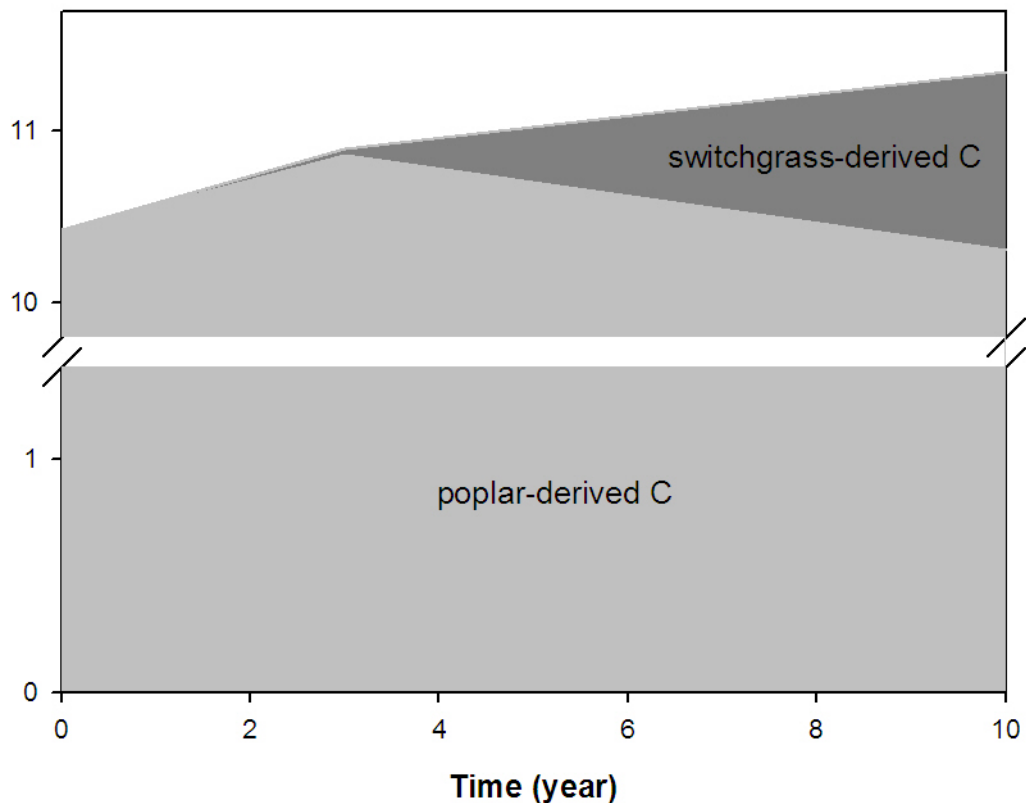


Fig. 3 Cumulative switchgrass (C4)-derived C in the upper 0.4 m of soil after ten years of cultivation following a poplar (C3) grove of thirty years.

different between switchgrass and giant reed. We can therefore conclude that more accurate and crop-specific SOC determinations are necessary to avoid biased environmental impact assessments. For example, in the present study, global warming emissions due to agronomic inputs resulted in 539 (switchgrass) and 637 (giant reed) $\text{kg (CO}_2\text{eq) ha}^{-1} \text{y}^{-1}$ (-0.15 and $-0.17 \text{ Mg of C ha}^{-1} \text{y}^{-1}$), while C savings from the conversion of lignocellulosic biomass to advanced bioethanol resulted in 2.0 and 3.1 $\text{Mg (C) ha}^{-1} \text{y}^{-1}$, since mean biomass yields of switchgrass and giant reed corresponded to 13.6 and 21.2 $\text{Mg ha}^{-1} \text{y}^{-1}$ (Alexopoulou et al. 2015), respectively. After the sensitivity analysis, we estimated that SOC stocks were increased by 0.37-1.58 and 0.75-2.18 $\text{Mg ha}^{-1} \text{y}^{-1}$, respectively beneath switchgrass and giant reed. It derives that SOC changes highly affected the overall C balance, accounting for the 11-35% (switchgrass) and 20-42% (giant reed) of total C savings.

In two recent simulation studies on biofuel production from lignocellulosic feedstocks cultivated on US marginal lands (i.e. grazing lands, idle lands), Gelfand et al. (2013) and Hudiburg et al. (2016) also underlined the relevance of SOC deposition (about 40 and 50% contribution to life-cycle C savings, respectively), assuming high accumulation rates for switchgrass ($1.4 \text{ Mg ha}^{-1} \text{y}^{-1}$) and miscanthus ($2.4 \text{ Mg ha}^{-1} \text{y}^{-1}$), whereas, previously, Adler et al. (2007) estimated a lower SOC

accumulation under switchgrass, but that corresponded to almost the totality (~80%) of the estimated greenhouse gas savings. Although SOC contribution to total savings estimated here was lower than in the aforementioned studies, in part because nitrous oxide emissions were not included in our study, our results confirm the substantial role of SOC in the sustainability of bioenergy cropping systems. Data as those presented in this study will turn out helpful in calibrating biogeochemical models (Adler et al. 2007; Gelfand et al. 2013; Hudiburg et al. 2016) during the assessment of greenhouse gases impact of established biomass supply district, as presently in the literature the initial establishment of bioenergy crops has been experimented, but no successions of managed perennial crops yet.

Conclusions

Within future biomass supply districts, a diversity of perennial species will be likely cultivated, which may succeed one another in the long term. At that stage, as a major factor affecting the sustainability of bioenergy systems, what role will be played by soil organic carbon?

To the present, it is pretty established that, when not placed onto former natural systems, perennial grasses will likely store SOC in the short term after establishment. At the same time, the Intergovernmental Panel on Climate Change estimates that managed perennial crops would not substantially differ in their C sink capacity when succeeding each other, but, actually, no measured data support this assumption.

Here, for the first time, the long-term effect on SOC of switchgrass and giant reed following poplar was investigated. Carbon stocks significantly increased over time, also differing between the two perennial grasses, and substantially affected life-cycle emissions. We finally underline the importance of punctual, accurate and crop specific determinations of SOC changes upon land use change to avoid biased estimation of C savings. Such data should be incorporated in life cycle inventories and biogeochemical models.

Acknowledgments

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Model simulation of cumulative carbon sequestration by switchgrass (*Panicum virgatum* L.) in the Mediterranean area using the DAYCENT model

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Abstract Literature lacks large-scale studies on cumulative C storage capacity of perennial grasses in Europe. At the same time, there is raising interest towards growing biomass crops in Europe, especially under marginal lands of the Mediterranean basin. In the present study we used the DAYCENT model to estimate the potential of switchgrass (*Panicum virgatum* L.) as a bioethanol crop to store soil C in the Mediterranean basin. Two scenarios were simulated: i) cultivation only on heathlands, shrublands and pastures (1.76 Mha); ii) cultivation on heathlands, shrublands and pastures, plus 5% of arable lands currently used for cereals (2.97 Mha in total). Cumulative biomass resulted in 184 and 303 Mt over 15 years, while SOC storage values were 6.1 and 12.4 Mt, respectively. Mean annual biomass yield ranged between 5.6 and 9.4 Mg ha⁻¹, while annual SOC accumulation was 0.02 to 0.62 Mg ha⁻¹. Fossil fuels displacement resulted in 54 and 89 Mt of C, i.e. 198 and 327 Mt of equivalent CO₂ in the first and second scenario, respectively. In the second scenario switchgrass SOC storage was much more pronounced. However, a loss of 54 Mt of grain commodities was also caused by switchgrass cultivation on 5% of arable lands with consequent ILUC effects. The latter were however quite low (16%) when compared to environmental benefits as stored SOC.

Keywords: marginal lands; cereal lands; biomass; SOC; direct LUC; indirect LUC; GHG.

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Introduction

There is an urgent need for strategies to reduce greenhouse gases (GHGs) emission to the atmosphere (Lehmann, 2007). The European Union (EU-28) decreased total GHGs emission by 19.2% (over 1 Gt of CO₂ eq.) in the period 1990-2012, and by 1.8% between 2012 and 2013 (European Energy Agency, 2014). In general, EU-28 is on track towards meeting GHGs reduction targets. Nevertheless the optimization of land use and management practices of the agricultural and forestry sector, currently sequestering about 9% of total EU's GHGs emission, can further enhance GHGs removal from the atmosphere.

Perennial energy crops, such as switchgrass (*Panicum virgatum* L.), are an option to reduce GHGs emissions due to low inputs requirements and high level of soil and ecosystem conservation (Fernando et al. 2010). The ecology of perennial energy crops suggests that miscanthus (*Miscanthus x giganteus*) and reed canary grass (*Phalaris arundinacea* L.), for their water and cool temperatures requirements, respectively (Lewandowski et al. 2003), are more adequate for central and northern European conditions, while giant reed and switchgrass (lowland ecotypes) for southern Europe. Compared to giant reed (*Arundo donax* L.), switchgrass is less productive, but more suitable to common farm machinery and more economical to establish (by seeds instead of rhizomes or micropropagated plants of giant reed). Thus its development can be expected to occur in a shorter term. Agronomic, economic and environmental issues of switchgrass as an energy crop have been extensively studied in the US and Europe since mid '80s (Sladden et al. 1991; Bransby et al. 1998; Muir et al. 2001; Lemus et al. 2002; McLaughlin and Kszos, 2005; Elbersen et al. 2003; Di Virgilio et al. 2007; Alexopoulou et al. 2008); however, there are no studies specifically focused on the potential of long-term switchgrass C storage at a large European scale, and particularly focused on the Mediterranean area.

Switchgrass production was found to have a great potential to reduce GHGs emission (Monti et al. 2012) due to its low inputs management (Turhollow and Perlack, 1991), considerable rhizodeposition (Tufekcioglu et al. 2003) and, as alternative renewable energy source (McLaughlin and Kszos, 2005), fossil fuel displacement. Nonetheless, this could strongly vary depending on the former land use (Fargione et al. 2008). Still it is unclear whether the higher environmental benefits deriving from the substitution of an intensive annual food crop such as wheat (*Triticum* spp.) with the cultivation of a perennial such as switchgrass can balance the negative indirect land use change effects (ILUC) (Fritsche et al. 2010) given by the food production displacement. However, it is commonly recognized that, in order to achieve considerable environmental benefits, and, at the same time, minimize the competition with food crops, switchgrass should be preferably grown on marginal lands (Gelfand et al. 2013). Switchgrass can perform well in marginal situations; Bandaru

et al. (2013), using the EPIC model, estimated that switchgrass can attain $74.61 \text{ GJ ha}^{-1} \text{ y}^{-1}$ of net energy yield (NE) when cultivated on southern Michigan's marginal lands, while sequestering $0.23 \text{ Mg ha}^{-1} \text{ y}^{-1}$ of C into the soil. Gelfand et al. (2013), studying the impact on GHGs of biofuel cropping systems when adopted in US Midwest marginal soils, analyzed that the fossil fuels offset generated was always preponderant with respect to the C debts summed up to process-based emissions. Recently, using the DAYCENT model, Wang et al. (2015) reported an average annual C storage of long-term switchgrass plots of $\sim 1 \text{ Mg ha}^{-1} \text{ y}^{-1}$ on saline marginal soils. Finally, Midbrandt et al. (2014) calculated a potential total energy production of 226 GW y^{-1} from biomasses on abandoned croplands in the US. Nevertheless, when grown on pastures or Conservation Reserve Program (CRP) lands, switchgrass environmental benefits were not that clear. For example, Bransby et al. (1998) argued that pastures can be more effective in storing C than switchgrass; Chamberlaine et al. (2011), in a simulation study using the DAYCENT model, showed that soil organic carbon (SOC) increased from 45% to 300% after 20 years when switchgrass was grown on cotton lands, whereas it did not change or raised by 27% in Conservation Reserve Program (CRP) lands.

In the present study we used the DAYCENT model (Parton et al. 1998) to estimate the effects of switchgrass cultivation on GHGs emission on both marginal and arable lands in the Mediterranean area. DAYCENT is a biogeochemical model aimed to reproduce, among others, C and N cycles of agricultural and natural systems. Here, the model was used to estimate the total above and belowground biomass of switchgrass, soil organic carbon (SOC) and net ecosystem exchange (NEE), which is the net uptake or the net emitted carbon by an ecosystem.

Methods

Model parameterization

Two switchgrass (*Panicum virgatum*, cv. Alamo) fields, 5 hectares each, established in 2012 and 2002 on a plain fertile soil ($44^{\circ} 33' \text{ N}$, $11^{\circ} 24' \text{ E}$; 33 m a.s.l.) and a hilly marginal soil ($44^{\circ} 25' \text{ N}$, $11^{\circ} 28' \text{ E}$; 80 m a.s.l.) of the Po Valley, were used for DAYCENT calibration. The plain site is a clay loam deep soil ($> 2 \text{ m}$) and was previously cultivated (period 2004-2011) with corn (*Zea mays* L.), wheat (*Triticum aestivum* or *Triticum durum*) and chard (*Beta vulgaris* L. vr. *cycla* (L.) Ulrich). The marginal site presents a clay loam (5% more sand and lower clay content than the plain site) soil, deep 1.5 m, with a slope ranging from 2 to 10%, and was previously cultivated (year 2001) with sugar beet (*Beta vulgaris* L. vr. *saccharifera* L.). Seed bed preparation, sowing time (April), annual N rate ($90 \text{ kg ha}^{-1} \text{ y}^{-1}$), harvesting time (end of September) were similar at both sites. Biomass productivity was measured annually. Since the establishment year (2012), in the plain field

an eddy covariance system was assembled for monitoring CO₂, H₂O and CH₄ fluxes, together with environmental parameters. Soil respiration was measured either by four fixed automatic chambers placed near the eddy tower, or by a portable soil respiration chamber coupled with an EGM-4 instrument (PPSystems). Root and soil samples were also collected for soil organic carbon (SOC) determinations. Actual weather data (i.e. daily minimum and maximum temperatures and daily precipitations) were collected in both sites by meteorological stations and used in the calibration process.

Normal practice with DAYCENT suggests to first calibrate the yields. According to Arundale et al. (2014) and basing on observed long-term biomass productivity (2002-2014) in the marginal site, we decided to use different sets of crop parameterization assuming 15 years of stand economic life span and four phases with different yield potentials (*prdx* parameter) that simulate a decline of yields in time. The four phases were: i) switchgrass establishment phase (year 1); ii) maximum yielding phase (years 2-6); iii) mature phase (years 7-11); iiiii) old phase (years 12-15). Observed mean yields for these four phases in the marginal site were 6.18, 10.65, 8.82, 6.3 Mg ha⁻¹, respectively. Given its relevance on C balance, the parameterization process involved also the allocation between roots and shoots, mainly adjusted through *cfrtcn (1)*, *cfrtcn (2)*, *cfrtcw (1)*, *cfrtcw (2)*. These parameters regulate the amount of assimilates that is allocated belowground, both in standard conditions and during water stress periods. Further adjustments were made using data deriving from soil respiration measures and soil C samplings. There were 29 parameters adjusted for model calibration (Table 1). This parameterization applies to switchgrass lowland ecotypes.

After the parameterization, the simulated annual yields and belowground biomass of the two sites resulted in a good approximation compared to real data (r always > 0.86). Accordingly, soil respiration, evapotranspiration, soil temperature and moisture (up to 60 cm depth), and SOC showed correlation coefficients always above 0.85.

Table 1 Parameters used to calibrate the DAYCENT model for switchgrass. The adjusted values within each range chosen after calibration are shown

Parameter	Description	Range	Adj. value
<i>prdx</i>	Potential aboveground monthly production as a function of solar radiation	-	0.8 ^a
<i>ppdf (1)</i>	Optimum temperature for production (°C)	10-40	30
<i>ppdf (2)</i>	Max. temperature for production (°C)	20-50	45
<i>ppdf (3)</i>	Left curve shape of the function of temperature effect on growth	0-1	1.0
<i>ppdf (4)</i>	Right curve shape of the function of temperature effect on growth	0-10	2.5
<i>cfrtcn (1)</i>	Max. fraction of C allocated to roots under max. nutrient stress	0-1	0.7
<i>cfrtcn (2)</i>	Min. fraction of C allocated to roots with no nutrient stress	0-1	0.37
<i>cfrtcw (1)</i>	Max. fraction of C allocated to roots under max. water stress	0-1	0.8
<i>cfrtcw (2)</i>	Min. fraction of C allocated to roots without water stress	0-1	0.37
<i>claypg</i>	No. soil layers to determine water and mineral N, P, and S available for crop growth	1-9	9
<i>crprtf (1)</i>	Fraction of N transferred to a vegetation storage pool from grass/crop leaves at death	0-1	0.65
<i>mrtfrac</i>	Fraction of fine root production that goes to mature roots	0-1	0.07
<i>cmxturn</i>	Max. turnover rate per month of juvenile fine roots to mature fine roots	0-1	0.3

<i>rdrj</i>	Max. juvenile fine root death rate	0-1	0.8
<i>rdrm</i>	Max. mature fine root death rate	0-1	0.4
<i>rdsrjc</i>	Fraction of the fine roots that is transferred into the surface litter layer	0-1	0.01
<i>cmix</i>	Rate of mixing of surface SOM and soil SOM	-	3.0
<i>npp2cs (1)</i>	GPP as a function of NPP to determine C stored in the carbohydrate pool	-	2.0
<i>ckmrspm (1)</i>	Max. fraction of aboveground live C to the maintenance of respiration	0-1	0.015
<i>ckmrspm (2)</i>	Max. fraction of juvenile live fine root C to the maintenance of respiration	0-1	0.30
<i>ckmrspm (3)</i>	Max. fraction of mature live fine root C to the maintenance of respiration	0-1	0.160
<i>cmrspnpp (1)</i>	X1 maintenance respiration based on predicted aboveground production	-	0.0
<i>cmrspnpp (2)</i>	Y1 maintenance respiration based on predicted aboveground production	-	0.0
<i>cmrspnpp (3)</i>	X2 maintenance respiration based on predicted aboveground production	-	1.25
<i>cmrspnpp (4)</i>	Y2 maintenance respiration based on predicted aboveground production	-	0.75
<i>cmrspnpp (5)</i>	X2 maintenance respiration based on predicted aboveground production	-	4.0
<i>cmrspnpp (6)</i>	Y2 maintenance respiration based on predicted aboveground production	-	1.2
<i>vlossp</i>	Fraction of aboveground plant N which is volatilized	0-1	0.04
<i>fallrt</i>	Fall rate of standing dead biomass	0-1	0.1

^a value for the “maximum yielding phase”, for the other phases the value of this coefficient was set lower

Model validation

Two separate validations were carried out to test the robustness of the model calibration. The first validation tested the modeled gross primary production (GPP), ecosystem respiration (ER) and net ecosystem exchange (NEE) against the cumulative monthly values estimated in the plain field by the eddy covariance tower. These three indexes derived from 30 second resolution measures of CO₂ fluxes entering and leaving the switchgrass field. GPP is the gross uptake, thus all the CO₂ entering the system, ER is all the CO₂ leaving the system to the atmosphere, both from plants and microbial respiration, and NEE is the balance between the previous two and gives the resulting net uptake or emission of CO₂; NEE represents a summary of the C sequestration performed by a given system. GPP, ER and NEE showed good correlation coefficients ($r=0.93^{**}$, 0.75^{**} and 0.92^{**} , respectively) (Fig. 2); the regressions were also tested for intercept=0 (GPP: $y = 0.934x$, $r=0.71$; ER: $y = 1.03x$, $r=0.91$; NEE: $y = 1.031x$, $r=0.91$).



Fig. 1 The Eddy Covariance tower measuring CO₂ fluxes during switchgrass stem elongation

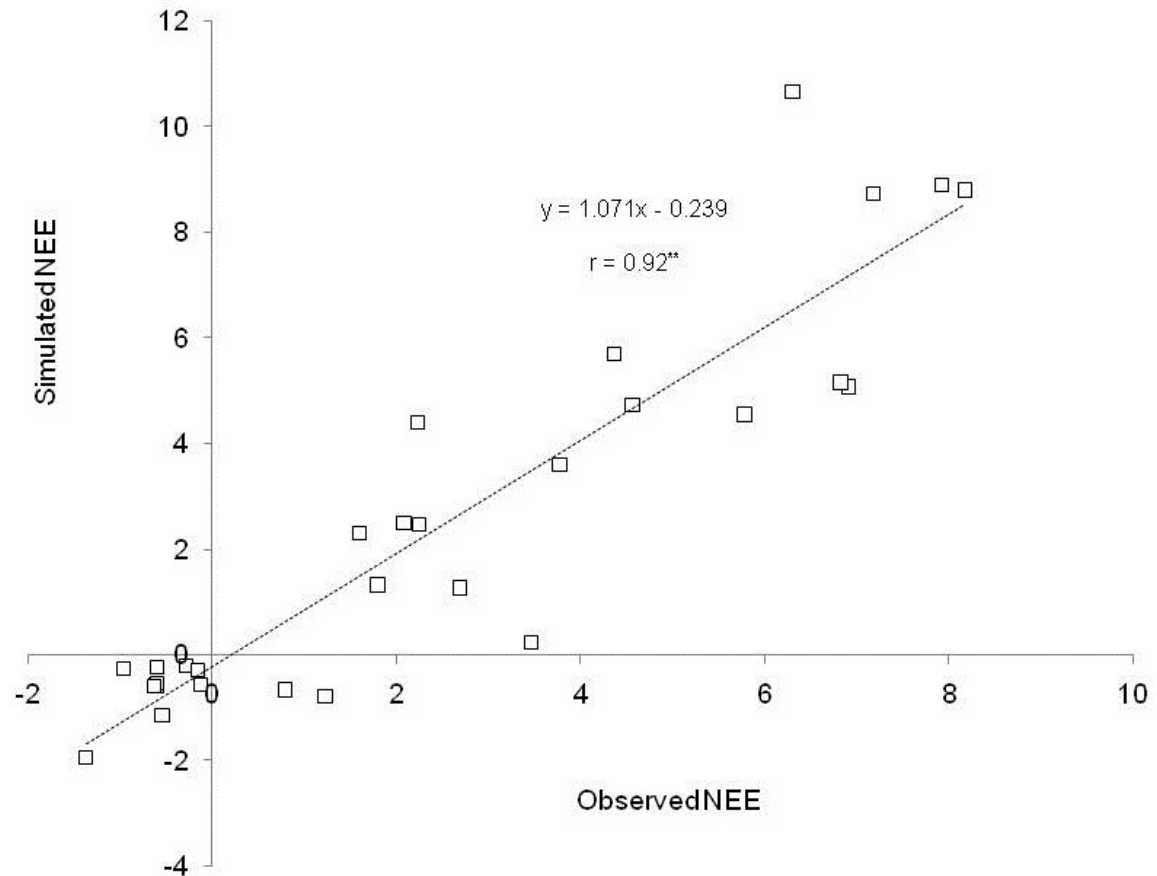


Fig. 2 Correlation between the cumulated monthly NEE values ($\text{Mg CO}_2 \text{ ha}^{-1}\text{month}^{-1}$) measured in Cadriano by the Eddy Covariance tower and simulated by the DAYCENT model. Positive values represent a net uptake from the atmosphere (growing periods), while negative values represent a net emission (dormant periods)

However, although this data were not used to calibrate the model, they derived from the same field used for calibration, thus we decided to assess the robustness of our parameterization with a supplementary validation using real yield switchgrass data (only lowland ecotypes) taken from literature referring to both European and US studies (Table 2). For northern European sites the maximum yield level was considered to be achieved in the 3rd year, thus, when simulated, switchgrass establishment phase was extended to the second year. Results from Stephenville and Clinton reported by Cassida et al. (2005) were excluded because of their exceptionally low switchgrass yields due to abnormal climate conditions (very dry environment) and low plant survival. For the EU sites switchgrass yields correlated well ($r=0.83^{**}$), while in the US locations moderately well ($r=0.72^{**}$) (Fig. 3). Taking US and EU locations together, the correlation coefficient was good ($r=0.87^{**}$); the regressions were also tested for intercept=0 (EU: $y = 0.910x$, $r=0.79$; US: $y = 0.962x$, $r=0.64$; EU+US: $y = 1.001x$, $r=0.86$).

Table 2 Data sets used to validate the DAYCENT model

Reference	Site	Years	Yield (Mg ha ⁻¹)	
			Observed	Simulated
Unpublished	Italy-Campotto ^a	2004	5.5	6.4
	Italy-Campotto	2004-2009	15.2	14.0
	Italy-Campotto ^b	2010-2014	11.1	11.2
Alexopoulou <i>et al.</i> (2008)	Italy-Trisaia ^a	1998	2.0	6.7
	Italy-Trisaia	1999-2002	11.1	14.9
	Greece-Aliartos ^a	1998	12.0	7.1
	Greece-Aliartos	1999-2002	15.9	15.8
Elbersen <i>et al.</i> (2003)	Netherlands-Polder ^a	1999-2000	2.9	3.1
	Netherlands-Polder	2001	10.7	7.4
	Germany-Braunschweig ^a	1999-2000	1.6	2.7
	Germany-Braunschweig	2001	6.3	7.0
	UK-Rothamsted ^a	1999-2000	4.8	2.8
	UK-Rothamsted	2001	12.6	7.3
Fike <i>et al.</i> (2006)	Kentucky-Princeton (US)	1993-1997	15.7	16.2
	North Carolina-Raleigh (US)	1993-1997	17.5	16.7
	Tennessee-Jackson (US)	1993-1997	13.5	17.3
	Tennessee-Knoxville (US)	1993-1997	22.0	15.6
	Virginia-Blacksburg (US)	1993-1997	13.8	11.6
	Virginia-Orange (US)	1993-1997	14.5	12.7
	West Virginia-Morgantown (US)	1993-1997	16.0	11.5
Cassida <i>et al.</i> (2005)	Arkansas-Hope (US)	1998-2001	17.2	19.1
	Texas-College Station (US)	1998-2001	17.2	20.4
	Texas-Dallas (US)	1998-2001	18.2	16.3
Muir <i>et al.</i> (2001)	Texas-Stephenville (US) (0 kg of N ha ⁻¹)	1994-1998	3.1	4.9
	Stephenville (56 kg of N ha ⁻¹)	1994-1998	9.3	9.8
	Stephenville (112 kg of N ha ⁻¹)	1994-1998	13.3	14.2
	Stephenville (168 kg of N ha ⁻¹)	1994-1998	15.4	18.5
	Stephenville (224 kg of N ha ⁻¹)	1994-1998	16.6	19.4
Sladden <i>et al.</i> (1991)	Alabama-Shorter (US)	1989-1990	22.2	16.6
Lemus <i>et al.</i> (2002)	Iowa-Chariton (US)	1998-2002	11.0	12.3
Fuentes, Taliaferro (2002)	Oklahoma-Chickasha/Haskell (US)	1994-2000	15.6	17.9
	Chickasha/Haskell ^b	1994-2000	14.3	11.4

^a establishment; ^b mature phase

Simulation approach and mapping

Setting up simulations involved four main aspects: climate, soil, former land use, and switchgrass management. For our simulations we referred to the following environmental zones as described by Metzger et al. (Metzger et al. 2005): Mediterranean North (MDN) and Mediterranean South (MDS) further divided into 11 and 8 sub-zones, respectively (Table 3). The Mediterranean Mountain (MDM) environmental zone (> 900 m a.s.l.) was excluded from the simulation because of the type of agriculture practiced at those altitudes and because of the steep slopes that would make switchgrass cultivation not feasible in that zone. Simulating it inside MDM, would have caused an overestimation of switchgrass cultivation potential. The soil database of Europe (Hiederer, 2013) was used to add the dominant soil texture within the simulation informative layers. Texture was classified in 5 groups: text1 (coarse), text2 (medium), text3 (medium-fine), text4 (fine), text5 (very fine). Corine Land Cover 2006 (European Environment Agency, 2010) was employed to draw land use data. For Greece the CLC 2000 was used as Greece did not participate to the CLC 2006.

It was assumed an economic lifespan of switchgrass stands of 15 years. Two scenarios were analyzed: i) switchgrass grown only on heathlands, shrublands and pastures (1.76 Mha), ii) cultivation on heathlands, shrublands and pastures, plus 5% of arable lands currently occupied by cereals (1.21 Mha). We considered heathlands and shrublands as those defined with the code 3.2.2 by CLC 2006 as ‘moors and heathland’. These were characterized by short vegetation (i.e. grass and shrubs). The juxtaposition of climate, texture and land use informative layers produced 104 different combinations that allowed the calculations and the creation of maps through ArcMap 10.2.2 (ESRI).

Italian Institute of Statistics (Greco and Bellini, 2010) reported a loss of arable lands in the decade between 2000 and 2010 of almost 4%. In the same period, cereals surface area (50% of the arable lands) decreased by 10%. Therefore, the second scenario was analyzed. To select arable lands we assumed that less productive arable cereal lands will be first converted to switchgrass by farmers. Therefore, precipitations during the vegetative season combined with soil texture were taken as determinant for lands conversion. Cereal lands loss was split equally between text1 and text5 areas of the MDS region. These areas present, within the Mediterranean region, more extreme conditions that could cause either water stress or saturated soil conditions, thus lowering yields or, in the case of exacerbated drought (sandy soils), requiring higher irrigation investments, making cereals cultivation less profitable. For the calculation of grain production displacement, an average grain yield of $3.0 \text{ Mg ha}^{-1} \text{ y}^{-1}$ was supposed on converted lands. Field management included autumn plowing and spring harrowing (in March), spring sowing, and annual N fertilization of 60 kg ha^{-1} .

The exception was for the establishment year in which we considered N fertilization inappropriate due to weed competition. Harvest was carried out in autumn. Eighty five percent of biomass was removed (15% biomass loss).

N fertilization has an impact, affecting: N emissions, yields and C sequestration, water quality, and profitability of switchgrass. Recently, Wang et al. (2015), analyzed the tradeoff between productivity and global warming potential (i.e. C sequestration, N₂O emissions, CH₄ emissions and chemical inputs) for different N fertilization rates in long-term plots of switchgrass. They estimated 67 kg ha⁻¹ yr⁻¹ as the best management practice (BMP). This was close to our rate (60 kg ha⁻¹ yr⁻¹), which is 25 to 33% of what is generally given to annual cereals.

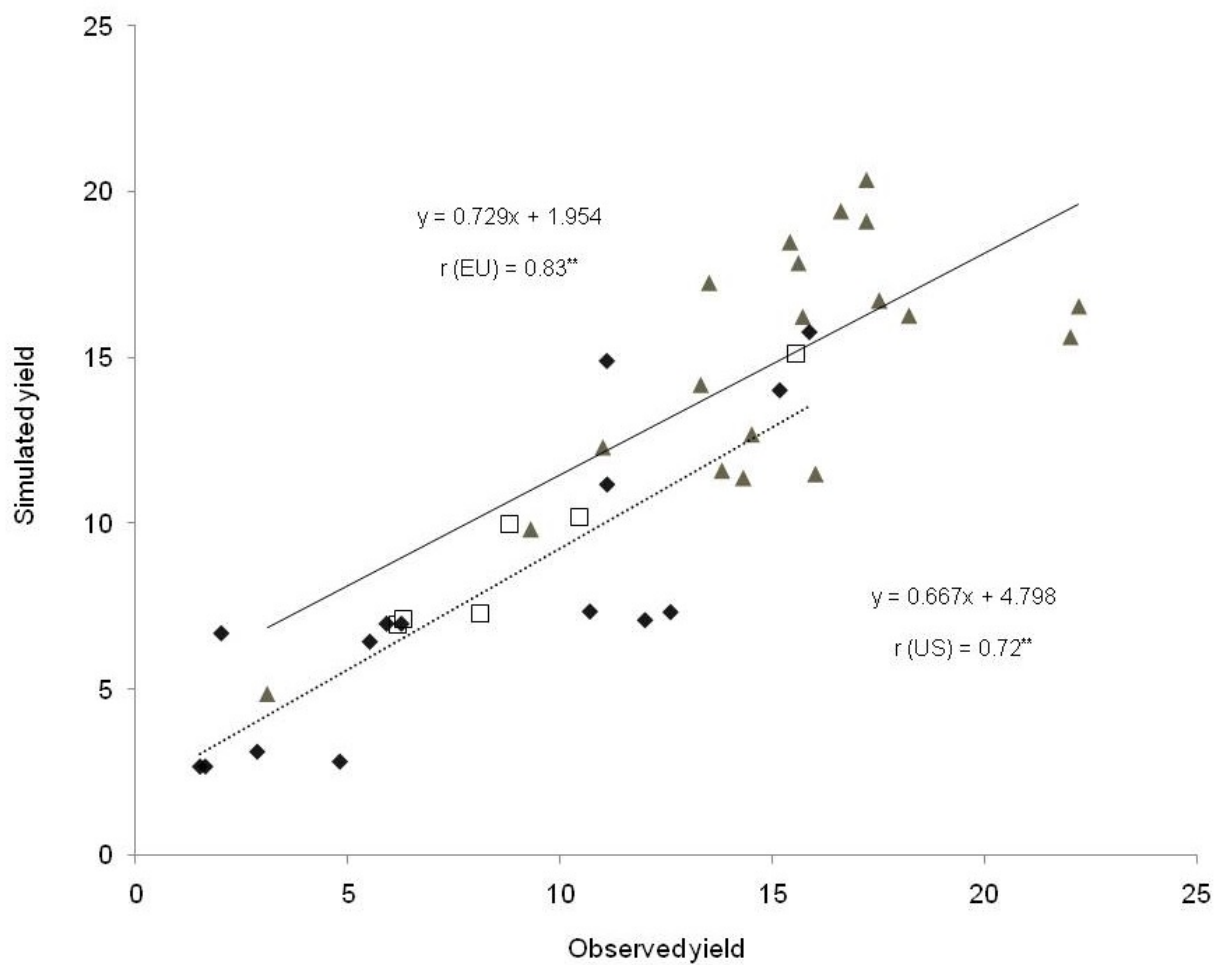


Fig. 3 Observed vs. simulated EU and US switchgrass yields (Mg ha⁻¹ y⁻¹) used for calibration and validation; all points aggregated show $r=0.87$ (calibration points = square, EU validation points = diamond, US validation points = triangle)



Fig. 4 Mechanical harvest of switchgrass in Ozzano hilly area (North Italy)

Results and Discussion

Carbon storage

First scenario (switchgrass cultivation only on heathlands, shrublands and pastures) and second scenario (1st scenario plus 5% of arable cereal lands) resulted in 1.76 and 2.97 Mha of land cultivated with switchgrass, respectively (Fig. 5). The 15-year cumulative switchgrass harvested biomass was, respectively for the two scenarios, 184 and 303 Mt, while SOC sequestration was 6.1 and 12.4 Mt. Therefore, although switchgrass surface was 69% higher in the second scenario, total SOC sequestration doubled (+103%) because of the higher C fixation rate under arable lands compared to marginal soils.

In the two scenarios, mean long-term yields (including harvest losses) ranged between 5.6 and 9.4 Mg ha⁻¹ y⁻¹. Similar values were obtained by Wang et al. (2015), who also used the DAYCENT model, for long-term irrigated (9.6 Mg ha⁻¹ y⁻¹) and rainfed (5.2 Mg ha⁻¹ y⁻¹) switchgrass. The biomass productivity values estimated in the present study were lower than generally reported in literature (Sladden et al. 1991; Lemus et al. 2002; Alexopoulou et al. 2008; Fike et al. 2006). This be explained by the assumption of a decline in yields in the long-term that begins from the 7th year

of production. Moreover, we set a medium-low level of N fertilization compared to those commonly reported (Muir et al. 2001; McLaughlin and Kszos, 2005) as we assumed that lower input cropping systems will be likely adopted by farmers on marginal lands (Wang et al. 2015).

As irrigation was not included in our simulation, in the driest regions such as MDS7 switchgrass productivity was considerably lower. In MDS8 the establishment even failed. The best yielding sub-zones were MDN2, and secondarily MDS5. Soil texture also affected switchgrass biomass productions with the highest yields being reached on clay soils, especially on text5 (avg. of 108 Mg ha⁻¹ of cumulative biomass).

Table 3 Characterization of the environmental sub-zones of the Mediterranean North (MDN) and Mediterranean South (MDS) zones as given by Metzger et al. (2005). Minimum and maximum temperatures and precipitation refer to switchgrass growing season (April-September)

Env. sub-zone	Temperature (°C)		Precipitation (mm)
	Min	Max	
MDN1	11.1	23.3	304
MDN2	14.0	24.7	506
MDN3	12.6	23.8	344
MDN4	12.6	23.2	356
MDN5	12.1	24.6	225
MDN6	11.3	24.2	251
MDN7	13.9	24.7	252
MDN8	13.7	23.1	279
MDN9	12.2	25.2	205
MDN10	14.5	25.6	204
MDN11	13.3	26.5	188
MDS1	14.5	24.3	203
MDS2	15.1	25.9	190
MDS3	13.9	27.1	166
MDS4	15.1	27.2	140
MDS5	15.6	26.7	161
MDS6	16.5	27.0	120
MDS7	16.5	29.0	113
MDS8	19.6	32.8	62

(a)

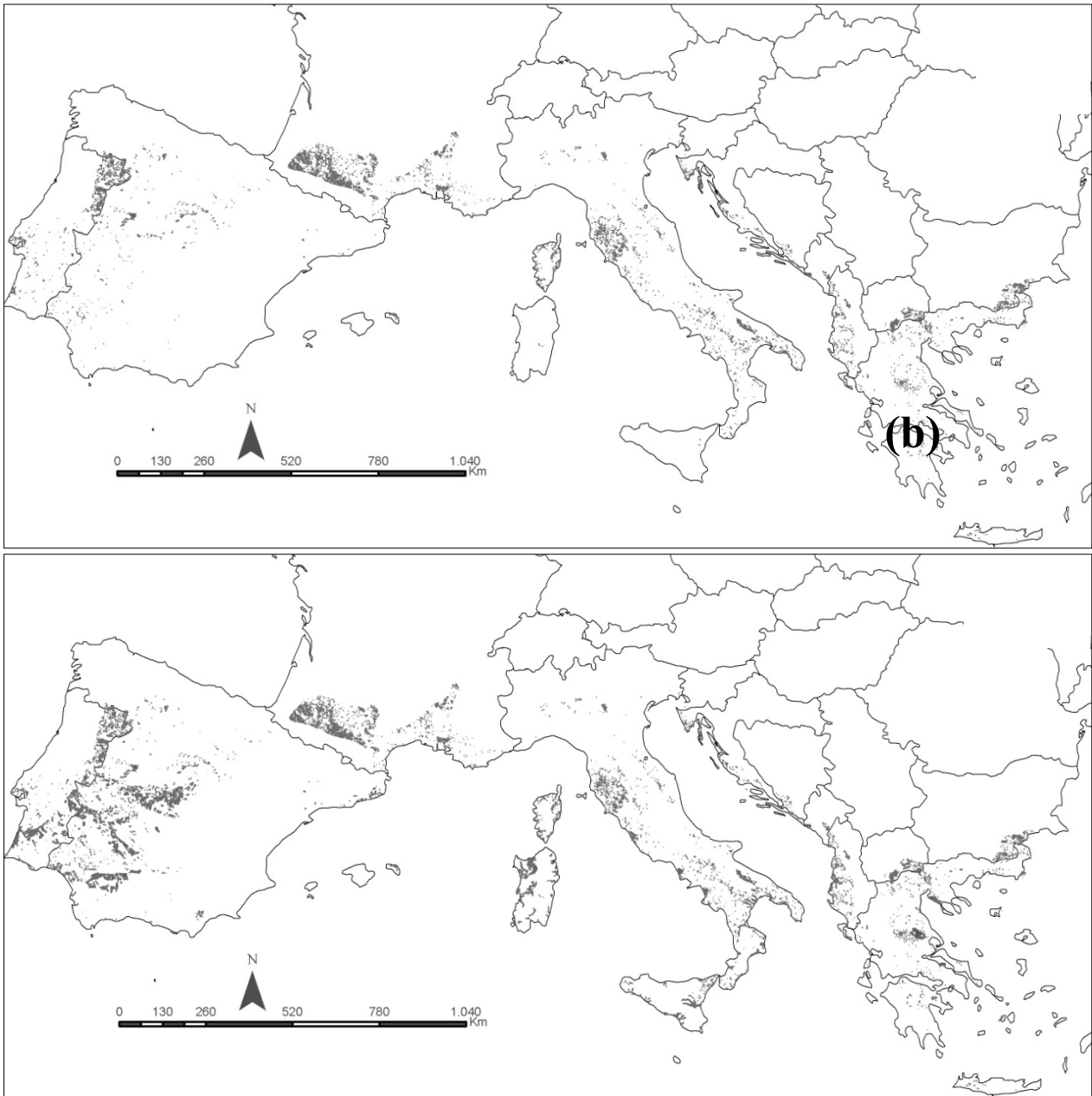


Fig. 5 Switchgrass cultivation on marginal (heathlands, shrublands and pastures) lands (1.76 Mha) in the 1st scenario (a), and on marginal lands plus 5% of the less productive cereal arable lands (2.97 Mha in total) in the 2nd scenario (b). Maps were drawn basing on the Corine Land Cover 2006 (CLC 2006)

In the model runs, before switchgrass establishment, initial soil C stocks varied among environmental sub-zones, soil textures and former land uses (Fig. 6). Initial soil C stocks values ranged between 16.8 and 42.2 Mg ha⁻¹ for heathlands, shrublands and pastures, similar to previous studies. Bransby et al. (Bransby et al. 1998) reported 17.7 Mg ha⁻¹ of belowground C in a bahiagrass pasture, Chamberlaine et al. (2011) and Wang et al. (2015) set up their DAYCENT runs with a soil C baseline of 11 and 32 Mg ha⁻¹, respectively for CRP lands and saltgrass (*Distichlis spicata* (L.) Greene). Finally, Fargione et al. (2008), in their estimation of the carbon debt from the conversion to biofuels of grassy cerrado lands, used a SOC content of 43.6 Mg ha⁻¹. In the present

study, the establishment of switchgrass caused a direct land use change (LUC) impact which released between 0.43 and 1.56 Mg ha⁻¹ of soil C in the unmanaged marginal lands, and between 0.05 and 0.42 Mg ha⁻¹ of soil C in the annually cropped cereal lands. Consequently, the soil C debt accumulated during the first year of establishment was always recovered at the end of the following year in the former cereal lands. In contrast, it took from 3 to 5 years to offset the first year emissions occurred in the newly tilled marginal lands. Fifteen-years patterns of soil C sequestration ranged between 0.3 and 9.3 Mg ha⁻¹ (i.e. 0.02 to 0.62 Mg (C) ha⁻¹ y⁻¹). Switchgrass C storage changed considerably depending on environmental conditions, soil type and field management. For example, Lee et al. (2007) reported that switchgrass sequestered up to 4.0 Mg C ha⁻¹ y⁻¹ using manure as fertilizer, while Wang et al. (2015) reported a soil C storage of 1.13 Mg ha⁻¹ y⁻¹ with irrigation of plots in a semi-arid area. Under different environmental conditions, Mehdi et al. (1999) found a negative C offset. Reviewing several site-treatment combinations, Anderson-Teixeira et al. (2009) estimated a mean sequestration of 0.4 Mg (C) ha⁻¹ y⁻¹ (0.68 Mg ha⁻¹ y⁻¹ forcing the intercept in a regression model). Bandaru et al. (2013) estimated lower switchgrass SOC storage capacity (0.23 Mg ha⁻¹ y⁻¹) for US marginal soils. Our data fall within the left side of the Gaussian curve of literature data, which is consistent with the areas considered in the present study (fallow or pasture lands have C content generally closer to saturation than arable lands; Lal, 2004; Bandaru et al. 2013). Accordingly, the minimum predicted rates for former cereal lands converted to switchgrass (second scenario) were 0.26 to 0.48 Mg ha⁻¹ y⁻¹. As for the biomass, Text5 showed the highest potential of C storage, whereas text1 the lowest. MDS3, MDS5 and MDN9 (0.62, 0.58 and 0.55 Mg ha⁻¹ y⁻¹, respectively) resulted as most favorable zones. DAYCENT algorithms bind the soil clay content to the stabilization of organic matter (Parton et al. 1987): the finer the texture, the lower the decay rate of active SOM. This explains why text5 always accumulated more SOC. Weather operated on SOC changes in multiple ways: affecting the production of above and belowground biomass, affecting the C:N ratio and lignin concentration of roots and the lignin concentration of stems, and affecting SOM decay rates through soil temperature and moisture (Parton et al. 1987). Biomass is in fact the principal source of organic C return to the soil and, at the same time, its chemistry relates to its stability (e.g. a higher lignin concentration of biomass makes the deriving organic matter more recalcitrant).

Live roots constitute another important pool of C transitorily sequestered into the soil (McLaughlin and Kszos, 2005; Trumbore and Gaudinski, 2003). In this study roots were treated separately from SOC due to their uncertain turnover rate. After 15 years, switchgrass roots were 9.2 and 15.5 Mt (3.7 and 6.2 Mt of C), respectively for the two scenarios, with a range of 3.3-6.0 Mg ha⁻¹. As expected, in time, root biomass linearly decreased with aboveground biomass. Belowground

component accounted for 40% to 50% of total biomass, also depending on stress induced allocation proper to each environmental zone, and it was always below 40% in the first two years, when root system was still building up. The simulated root to shoot biomass partition agreed with other findings (McLaughlin and Kszos, 2005; Bowden et al. 2010).

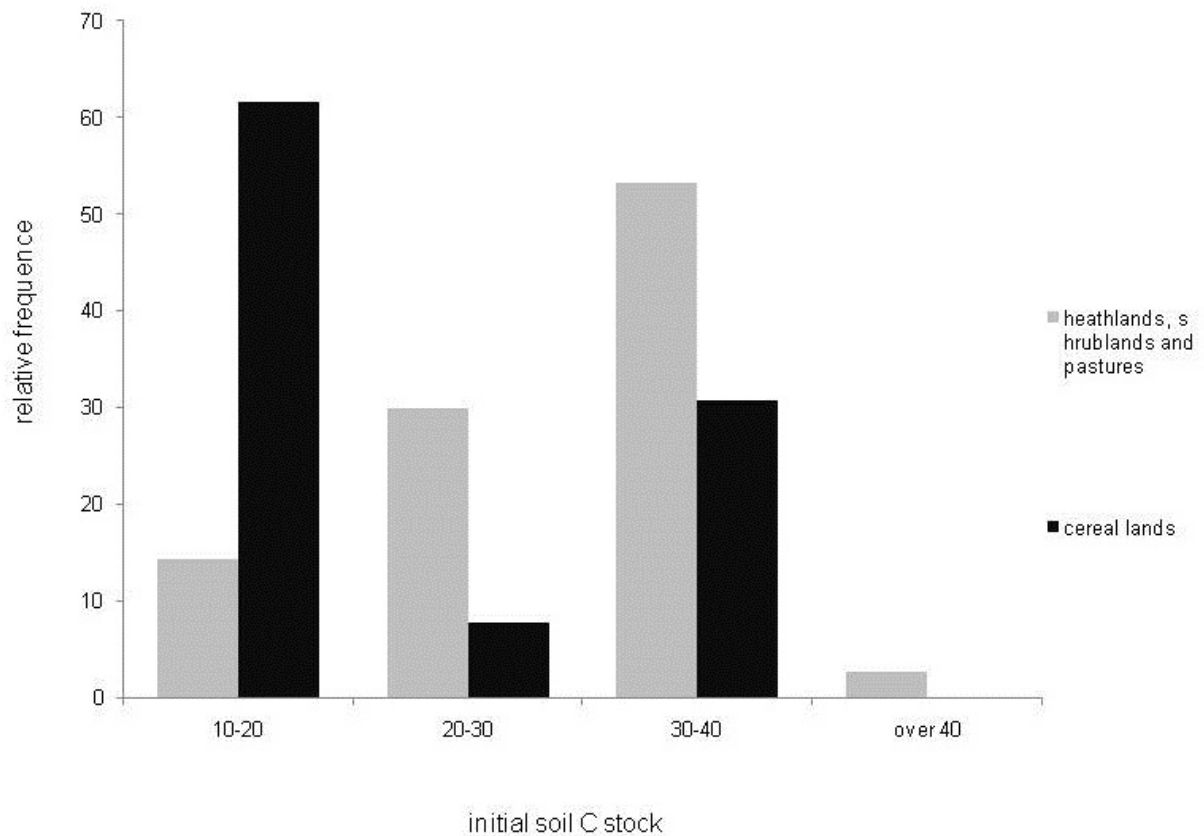


Fig. 6 Relative frequency (%) of simulated initial soil C stocks (Mg ha⁻¹) divided by classes for heathlands, shrublands and pastures (grey bars) and for arable cereal lands (black bars) before conversion to switchgrass. Each population unit is represented by an environmental sub-zone-texture combination

Indirect impacts

In this section, starting from our modeled results of switchgrass cultivation in the Mediterranean basin, an attempt to calculate the indirect impacts on C emissions is pursued. The potential to offset fossil fuels through the conversion into energy of the modeled harvested biomass and the ILUC caused by the displacement of food/feed productions are estimated.

Our results show that growing switchgrass and displacing food commodities from 5% of cereal lands in the semi-arid regions of the Mediterranean basin can lead to a considerably higher amount of C storage (Table 4), thus GHGs savings, than growing switchgrass on heathlands, shrublands or pastures only. It has been reported that pastures can be even more effective than switchgrass in storing soil C (Bransby et al. 1998), thus their conversion to switchgrass can lead to more uncertain

benefits. However, there is a heated debate about food vs. fuels land competition, displacement of food commodities and ILUC effects, even if a significant number of studies (Durham et al. 2012; Langeveld et al. 2014) report that energy crops are unlikely to affect the European commodity market, thanks to their environmental benefits that are high in terms of GHGs reduction (Don et al. 2012). In this study we calculated that 5% of arable lands converted to switchgrass caused a loss of 54 Mt of grain commodities that should be replaced somewhere else. At the same time, the direct LUC from pastures or non-arable carbon rich soils brings unsteady effects on GHGs emission (Bransby et al. 1998; Lal, 2004), due to increased soil respiration (Fritsche et al. 2010) once the spontaneous vegetation is removed and the soil tilled for switchgrass establishment. In our simulation, in the least favorable circumstances (e.g. lower yielding areas), switchgrass barely accumulated soil C ($0.02 \text{ Mg ha}^{-1} \text{ y}^{-1}$) when cultivated on pastures since it was hardly able to recover the depletion of the initial C stock occurred during the establishment. Therefore, if on the one hand, converting cereal lands to biofuels may raise ILUC concerns, on the other hand, the conversion of unmanaged systems causes greater direct LUC effects. In addition, biomass yield on marginal land, as an alternative energy source to displace fossil fuels, is generally considerably lower than that on arable lands (Bandaru et al. 2013). Therefore, whether environmental benefits from switchgrass cultivation on arable lands will prevail over negative ILUC effects is still controversial. We therefore attempted to compare negative ILUC effects and benefits from fossil fuel displacement on the 5% of arable lands converted to switchgrass. Considering the ILUC emissions equal to $18 \text{ g CO}_2 \text{ eq. MJ}^{-1}$ of wheat-ethanol, the energy content of ethanol of 21.2 MJ l^{-1} , and 372 l of ethanol per metric ton of wheat grain (Laborde et al. 2014), 54 Mt of wheat grain loss cause $7.7 \text{ Mt CO}_2 \text{ eq.}$ (2.09 Mt of C) emissions as ILUC. Total C mitigation potential, after ILUC, results in 39 Mt of C (or $144 \text{ Mt of CO}_2 \text{ eq.}$). Therefore, basing on our results, GHGs emission savings derived from switchgrass cultivation on 5% of arable lands can be significant even after deducting the ILUC effects. ILUC should also be estimated for the food offset (milk, meat) from displaced animal grazing by converting pastures to switchgrass, but unfortunately no coefficients are available in the current literature to make this estimation. We however argue that most likely this displacement would have not affected our results significantly. In the simulation area, in effect, the converted pastures respect to converted cereal lands occupied only half of the simulated surface (0.66 Mha and 1.21 Mha , respectively). We also hypothesize that establishing or exploiting one hectare of grassland somewhere else will have less impact in terms of C emissions than establishing one hectare of cereals somewhere else. Therefore, accounting for the hectares considered in this simulation and hypothesizing lower ILUC impact per hectare respect to wheat displacement, total

ILUC from pastures conversion to switchgrass would probably cause less than half of the C emissions that we estimated for cereal lands conversion to switchgrass (< 1 Mt).

Cumulative soil C storage potentials of the two scenarios are highly relevant if weighted on the EU's (EU-28) GHGs emission reduction target by 2020 (265 Mt of CO₂ eq.; European Environment Agency, 2014). In the first scenario about 83% of reduction targets would be met, while the second scenario would totally fulfill the target, with 97 Mt surplus of CO₂ eq. savings. Such achievements are probably an overestimate of what would actually happen as we analyzed the potential C storage by covering almost 2 Mha of marginal lands with switchgrass. However our estimation confirms that switchgrass may have an important role in reducing net emissions because of its potential to store soil C and displace fossil fuels. Switchgrass biomass, in fact, can be used for advanced bio-ethanol or energy production, thus displacing energy from fossil sources. Using the CO₂ and energy conversion coefficients for biomass and fossil fuels given by Turhollow and Perlack (1991), which also include emissions from switchgrass establishment, fertilization and harvest, and taking a switchgrass latent heat converter (LHC) of 17.0 MJ kg⁻¹ (Lewandowski et al. 2003), C savings due to fossil fuels displacement were, depending on the fossil fuel displaced (coal, gas, petroleum), 37 to 71 and 61 to 117 Mt, respectively for the two scenarios.

Table 4 Starting from the current land use situation in the Mediterranean basin (CLC 2006), in the 1st scenario only heathlands, shrublands and pastures were grown with switchgrass, while in the 2nd scenario a 5% of cereal lands was added. C impacts after 15 years of switchgrass cultivation are shown for both scenarios, including the estimation of the indirect land use change (ILUC) effects from the conversion of cereal lands in the second scenario. Although switchgrass surface was 69% higher in the 2nd scenario, the initial soil C stock was only 44% higher. Cereal lands were more C depleted than unmanaged lands. Therefore, total SOC sequestration doubled (+103%) in the 2nd scenario

	1 st Scenario	2 nd Scenario
	(Mt)	(Mt)
Harvested dry biomass	184	303
C savings (fossil fuels offset)	54	89
Initial soils C stock	43	62
C sequestration in soils	6.1	12.4
Food/feed displacement (grain)	-	54
C emissions from ILUC	-	2.1

Finally, other tradeoffs must be discussed when speaking of conversion of marginal lands to perennial crops. Wildlife refuges, aquifers status, soil structure and erosion will be affected by the

change in land use. Although probably not as much as unmanaged systems (heathlands, shrublands and pastures), perennial biomasses like switchgrass can still furnish a shelter for the fauna because of their low level of disturbance, reduced use of chemicals and their tall and dense vegetation, especially if the harvest time and residues removal are programmed towards an enhanced ecosystem conservation (Fargione et al. 2009). As a perennial crop, switchgrass is not tilled after the initial establishment year. Thus it covers the soil most of the time with its rhizomes, roots and litter, maintaining the soil structure and preventing soil erosion and water run-off. Its deep root system can however cause a high depletion of water reserves (Fernando et al. 2010). Hence, switchgrass can procure other ecosystem services than just GHGs mitigation, although it probably cannot fully replace those given by natural or semi-natural undisturbed systems (marginal lands). However, it would be able to partially restore these services in lands previously cropped with annual cereals.

Conclusions

Using the DAYCENT model, we estimated the C storage potential of switchgrass grown on marginal lands or marginal lands plus 5% of the less productive cereal lands in the Mediterranean region. Given the EU-28 targets on GHGs emission reduction, potential emissions savings from switchgrass cultivation in the Mediterranean area can be highly relevant.

This constitutes an example of what is meant by “agriculture impacts greenhouse gases” (Paustian et al. 2006). As land use (including agriculture) produces a high percentage of the human induced GHGs emission worldwide (about one-third) (Paustian et al. 2006), it also owns a great mitigation potential. Some of the ways for achieving it were adopted in this study: restoration of degraded lands, reduced tillage cropping, perennial soil cover, high level of field residues, low N management and fossil fuels displacement. Growing switchgrass in the Mediterranean area could result in a great potential for the future sequestration of atmospheric carbon. Carbon sequestration programs could also be an income opportunity for farmers, generating revenue once available as ‘environmental credits’.

Acknowledgements

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Biofuel production and biogenic greenhouse gas impact of switchgrass and giant reed in the US Southeast

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Abstract United States mandated the production of biofuel from lignocellulosic feedstocks. But the cultivation of these feedstocks may produce debates, as agricultural land is scarce and is primarily needed for food production and grazing. Thus, it is thought that bioenergy production should be placed on lands with low economical value (i.e. marginal lands). At the same time, depending on what land is considered marginal and therefore available for lignocellulosic crops, different greenhouse gas impacts will be generated upon land use change. Here, we attempted to estimate the impact on biofuel production and biogenic greenhouse gas emissions of the cultivation of perennial switchgrass (*Panicum virgatum* L.) and giant reed (*Arundo donax* L.) in the US Southeast. We employed the NLCD database to select grasslands, shrublands and marginal croplands. Then we allocated switchgrass and giant reed on these lands using either the land capability classification (SSURGO) or another criterion involving economical aspects and social acceptance. After calibration, the DAYCENT model was employed to simulate 15-year cultivation of both crops in the US Southeast. Florida, Georgia, Mississippi and South Carolina were the States with the highest availability of lands, thus the highest potential for biofuel production. Among scenarios, the one converting marginal grasslands, shrublands and marginal croplands yielded the greatest benefits: converting 3.6 Mha of land, 44 Mt y⁻¹ of dry biomass could be produced, storing 0.05 Mt y⁻¹ of soil organic C at the same time. In this scenario, considering 80-km supply areas, nineteen biorefineries could deliver 7124 Ml y⁻¹ of advanced ethanol across the region. When minimizing giant reed invasion risks through re-allocating giant reed outside flooded areas, 4695 Ml y⁻¹ of advanced ethanol could be still delivered from thirteen biorefineries, but the scenario turned in a biogenic greenhouse gas source (3.2 Mt CO₂eq y⁻¹).

Keywords: DAYCENT; Switchgrass; Giant reed; Biofuel; Greenhouse Gas; Land Use Change.

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Introduction

In order to achieve energy security (uninterrupted availability of energy sources at an affordable price) and a reduction in greenhouse gases (GHG) emissions (sustainability), policies have been promulgated in the US for the production of bioenergy from lignocellulosic feedstocks (The Energy Independence and Security Act; 110th Congress of the United States, 2007). Despite a longer transformation process required compared to first-generation biofuels (e.g. corn ethanol), the main advantages of lignocellulosic crops (e.g. switchgrass) used to produce advanced ethanol are the lower environmental loads caused during cultivation (Adler et al., 2007; Fazio and Monti, 2011), the possibility to reduce biogenic GHG emissions through soil organic carbon (SOC) storage (Agostini et al., 2015) and the opportunity to avoid competition for land, since they can satisfactorily grow also in marginal situations (Quinn et al., 2015) that would not be suited for the cultivation of conventional food crops. If fertile croplands currently dedicated to food production would be converted to biofuels, an indirect land use change (ILUC) effect would be likely generated in fact, as the global market may trigger the conversion of more land somewhere else (this land could be rich in C and its conversion impactful) as an answer to increased prices (Searchinger et al., 2008). The conversion of lands with high amounts of C (e.g. forests) should be as well avoided in order to not generate a barely reparable C debt (Fargione et al., 2008) given by a direct land use change impact (LUC; i.e. loss of aboveground biomass C and soil organic C upon conversion). Land allocation of lignocellulosic feedstocks is therefore essential for their own sustainability: to present, it is quite established that lignocellulosic crops should be best allocated on marginal lands (Fargione et al., 2008; Gelfand et al. 2013; Quinn et al., 2015). Defining marginal lands is still challenging but, in general, they can be identified as lands with a low economical value (“economical” embeds the productive, environmental and social values). Grasslands and shrublands, especially if characterized by pedo-climatic limitations, can be considered marginal lands. Although grasslands and shrublands are natural ecosystems, they do not store as much C as forests (Fargione et al., 2008), and their conversion could generate a C debt promptly repayable by the elevated C inputs from the lignocellulosic perennial vegetation (Agostini et al., 2015). On the other hand, marginal croplands (low productivity lands) could be converted. This conversion might generate ILUC effects, which could be however avoided through a possible intensification of food production in non-marginal croplands (Matson et al., 1997; Burney et al., 2010; Heaton et al., 2013). Agricultural intensification would certainly increase GHG emissions, but not as much as agricultural expansion (Tilman et al., 2011); at the same time, fossil fuels offset credits, SOC storage and lower agronomic inputs in the croplands converted to biofuels would probably achieve an additional reduction in GHG emissions (Adler et al., 2007). So, converting marginal grasslands

and shrublands will avoid ILUC impacts, but will give more uncertain benefits in terms of GHG emissions because of less predictable SOC trends (Qin et al., 2016) and because of an increase in management-related emissions, including N fertilizers use which causes an increase in direct and indirect nitrous oxide (N₂O) emissions (Del Grosso et al., 2006; Erisman et al., 2010), while converting marginal croplands may generate ILUC effects, but will likely yield great GHG benefits, mainly given by a substantial reduction of tillage and other agronomic inputs (which increase also biogenic emissions) compared to the former annual crops cultivation.

Cai et al. (2011) performed an analysis to estimate the global potential to produce biofuels from marginal lands and found out that the US, depending on the scenario considered, may have 43-127 Mha of available marginal land (i.e. abandoned land, wasteland, degraded land), with the eastern part of the country containing most of these territories. The US Southeast seems therefore to own a high potential for the cultivation of lignocellulosic feedstocks for advanced ethanol. Currently, bioethanol production plants are scarce in the region: only the 2.5% of US bioethanol was produced in the Southeast in the year 2016, while most of it (91%) was produced in the Corn Belt region (EIA, 2016). Nonetheless, regions should always aim to the production of renewable energy “in loco” in order to not let the environmental loads of transportation reduce sustainability of the whole process. Further, the climate in the US Southeast seems ideal as well for yielding the high biomass supplies required by the bioenergy industry: in fact, low temperature regimes are never reached (9.2-25.4 °C, as yearly mean), nor the region is characterized by low precipitation regimes (400-1600 mm y⁻¹) (Mesinger et al., 2006).

Both, switchgrass (*Panicum virgatum* L.) lowland ecotypes and giant reed (*Arundo donax* L.), find ideal conditions for growth in warm climates with sufficient water availability (Lewandowski et al., 2003; Alexopoulou et al., 2015), while being able to tolerate several pedo-climatic limitations, as high temperatures, drought or salinity (Quinn et al., 2015). Therefore we believe they could be successfully cultivated in the US Southeast, on those lands that present different limitations to the agricultural use, and can be therefore defined marginal (low productive and grazing value). Switchgrass is a US indigenous grass and it has been at the center of national projects for the production of bioenergy (McLaughlin and Kszos, 2005), as well as being selected as the leading US bioenergy crop (Wright and Turhollow, 2010). Giant reed has been instead mainly investigated in the Mediterranean Europe (Hidalgo and Fernandez, 2000; Angelini et al., 2005; Cosentino et al., 2014; Alexopoulou et al., 2015; Monti and Zegada-Lizarazu, 2015) and not as much in the US, also because considered a problematic invader (Herrera and Dudley, 2003; Ceotto and Di Candilo, 2010), especially in determinate areas (e.g. California). Nevertheless, giant reed owns a great potential for bioenergy production (Lewandowski et al., 2003), as it is able to even reach yields

over 40 Mg ha⁻¹ of dry biomass in the proper environment (Hidalgo and Fernandez, 2000). Moreover, invasion risks can be minimized by properly allocating and managing giant reed (Ceotto and Di Candilo, 2010). Miscanthus (*Miscanthus x giganteus* Greef et Deuter) is another valuable candidate for producing biofuel in the US, and it has previously been utilized in several simulation studies together with switchgrass (Davis et al., 2012; Qin et al., 2014; Hudiburg et al., 2016). However, giant reed is more heat tolerant than miscanthus (Quinn et al., 2015), thus, we believe, more suited to the US Southeast where mean yearly temperatures can reach up to 25.4 °C (Mesinger et al., 2006); no surprise that, when compared side-by-side in a long-term experiment in the Mediterranean, giant reed showed a higher yielding potential than miscanthus (Alexopoulou et al., 2015). Furthermore, there are evidences that switchgrass and giant reed can, in certain conditions, be more effective in storing SOC compared to miscanthus: in fact, they can potentially sequester C into the deeper soil layers (Qin et al., 2016), probably thanks to their evenly distributed roots down to 200 cm (Monti and Zatta, 2009).

But, which of the two crops would be more convenient to cultivate in the US Southeast? Switchgrass or giant reed? Although its high potential (Monti et al., 2012), switchgrass does not reach, on average, the yields and SOC storage rates achieved by giant reed (Hidalgo and Fernandez, 2000; Alexopoulou et al., 2015; Monti and Zegada-Lizarazu, 2015; Nocentini and Monti, 2017). Giant reed owns a higher potential to displace fossil fuels and to increase soil C stocks. However, we assume that switchgrass, despite its lower yields, would be cultivated by most farmers rather than giant reed for the following reasons: the availability of the genetic material in the US, social acceptance (giant reed is thought to be invasive and is anyway less known than switchgrass, which in the US is the selected model bioenergy crop; Wright and Turhollow, 2010) and production costs. If costs for land rent, soil tillage, fertilizer application and weeding were assumed equal for the two crops, annualized costs to produce giant reed would still be almost four times higher (Perrin et al., 2008; Soldatos et al., 2004), without taking into account the probable investments in new farm machineries needed to harvest giant reed. Therefore, although giant reed is expected to yield two or three times more biomass than switchgrass in the US Southeast, it still would be less remunerative. Nonetheless, more expensive but higher yielding biomass crops, as also miscanthus is (Soldatos et al., 2004), have been demonstrated in other influential studies to be able to positively impact the US biofuel industry, GHG balance and economy (Davis et al., 2012; Qin et al., 2014; Hudiburg et al., 2016). Thus, we believe that a mix of more biofuel crops, with different characteristics would be eventually beneficial, taken into account other factors as biodiversity sheltering and the production risks linked to monocultures. Moreover, the use of a higher yielding crop together with switchgrass, such as giant reed, will reduce the land requirements for biofuel production and will allow

production within a smaller radius around the transformation plants, mitigating at the same time the impact of transportation.

In the present study, we employed the biogeochemical model DAYCENT (Parton et al., 1998) to simulate the cultivation of switchgrass and giant reed in the US Southeast aimed to the production of advanced bioethanol. Besides converting marginal lands, we converted non-marginal grasslands and shrublands as well, in order to analyze possible tradeoffs between different land use change options. The simulation outputs allowed the estimation of dry biomass yields, SOC stocks changes and total biogenic N₂O emissions, while, using Geographic Information Systems, we attempted to predict the position of future potential bioethanol plants. While switchgrass has been extensively experimented in other US simulation studies (Davis et al., 2012; Qin et al., 2014; Hudiburg et al., 2016; Field et al., 2016), to our knowledge, this is the first regional-scale simulation involving giant reed as a biofuel crop.

Methods

Calibration-evaluation process

Calibration of the DAYCENT model for switchgrass (lowland ecotypes) has already been achieved and has been evaluated for both US and Europe environments in our previous work (Field et al., 2016; Nocentini et al., 2015). To obtain the parameterization of the model for giant reed, besides using field data from our own long-term experiments in north Italy (Monti and Zegada-Lizarazu, 2015; Alexopoulou et al., 2015), a literature research has been carried out to select those studies which reported significant information on giant reed aboveground and belowground C pools. Since the aim of this study was a simulation at regional-scales, characterized by gradients in climate and soil types, data recorded in a variety of pedo-climatic conditions were used for the calibration-evaluation process (Table 1). Long-term studies (showing the evolution in time of above- and below-ground biomass), studies from sites with different climatic conditions (to understand the growth of the crop as related to temperature and precipitations amount and distribution) and studies where fertilization and irrigation levels varied (analyzing the response of the crop to nutrient and water inputs) were included in the calibration dataset. For the evaluation dataset, studies with marked longitudinal and latitudinal differences (South Italy, North Italy, Spain, Germany, Texas, Oklahoma) were taken, as well as studies with varying agronomic inputs (nitrogen and irrigation levels). Unpublished data on aboveground yields from the long-term trial described by Cattaneo et al. (2014) were also used during calibration.

Table 1 List of literature studies used during DAYCENT calibration and evaluation for giant reed

Reference	Place	Years	Data type	Use
Alexopoulou et al. (2015)	North and South Italy	2004-2015	Yield	calibration
Angelini et al. (2005)	Central Italy	1996-2001	Yield	evaluation
Bacher et al. (2001)	Germany	1997-2001	Yield	evaluation
Cattaneo et al. (2014)	North Italy	2002-2011	SOC	calibration
Ceotto and Di Candilo (2011)	North Italy	2002-2009	SOC	evaluation
Cosentino et al. (2014)	South Italy	1998-2001	Yield	calibration
Di Candilo et al. (2010)	North Italy	2007-2009	Yield; SOC	evaluation
Fagnano et al. (2015)	South Italy	2004-2012	Yield; SOC	evaluation
Hidalgo and Fernandez (2000)	Spain	1997-1999	Yield	evaluation
Kering et al. (2012)	Oklahoma	2008-2010	Yield	evaluation
Mantineo et al. (2009)	South Italy	2002-2006	Yield	evaluation
Monti and Zatta (2009)	North Italy	2002-2007	Root biomass	calibration
Monti and Zegada-Lizarazu (2015)	North Italy	1997-2014	Yield; SOC	calibration
Nassi o Di Nasso et al. (2013)	Central Italy	2009-2011	Yield; Root biomass	calibration
Nocentini and Monti (2017)	North Italy	2004-2014	SOC	calibration
Sarkhot et al. (2012)	Texas	1970-2008	SOC	evaluation

A recently improved version of DAYCENT was employed for this study (Zhang, 2016), in which, among other parameters, K_{cet} , the crop coefficient (K_c) for evapotranspiration, has been implemented, allowing to more accurately simulate crop water use and phenology. Therefore, new adjustments to switchgrass parameterization for lowland ecotypes were also made in parallel with the calibration of the parameters for giant reed (Table 2). We decided to simulate only switchgrass lowland ecotypes because more likely to be adopted by farmers for their higher yields at the lower latitudes of the US Southeast. As previously for switchgrass (Nocentini et al., 2015), giant reed growth was divided in phases, since a decline in yields in time has been observed in our field experiments (Monti and Zegada-Lizarazu, 2015), and in the literature (Angelini et al., 2009), both showing the decline to occur after the eighth year after establishment. Several papers also show how giant reed reaches its maximum yielding capacity in the third year (Hidalgo et al., 2000; Nassi o Di

Nasso et al., 2013; Alexopoulou et al., 2015; Monti and Zegada-Lizarazu, 2015). Thus, giant reed growth phases were: i) “establishment” (years 1-2), ii) “maximum yielding phase” (years 3-8), iii) “mature phase” (years 9-15). Expert judgment was used to identify individual growth model parameters in need of adjustment to better represent giant reed growth patterns, then parameter values were adjusted by hand (Table 2) to best match empirical data on harvested biomass yields, root biomass, and SOC changes as summarized in Table 1. To simulate establishment, the *pltmrf* parameter was set lower (0.1) in order to reproduce limited growth of the new seedlings and more C was allocated to roots through the *cfrcn* (2) and *cfrcw* (2) parameters (0.50). Root:shoot ratio of giant reed was in fact shown to be ~2 at the end of the first year and ~0.6 in the following years (Nasso o Di Nasso et al., 2013). To simulate the mature phase, the *prdx* value was set lower (0.225) to reduce the yielding capacity of giant reed. The *snfxmx* (1) parameter was set slightly higher than 0 only to simulate switchgrass and giant reed capacity to achieve considerable yields without N fertilization (Alexopoulou et al., 2015; Monti and Zegada-Lizarazu, 2015).

Table 2 List of the main DAYCENT parameters involved in switchgrass (SG) and giant reed (GR) parameterization and their respective values

Parameter	Description	SG value	GR value
<i>prdx</i> *	Potential aboveground monthly production as a function of solar radiation	0.250	0.280
<i>ppdf</i> (1)	Optimum temperature for production (°C)	30	30
<i>ppdf</i> (2)	Max. temperature for production (°C)	44	45
<i>ppdf</i> (3)	Left curve shape of the function of temperature effect on growth	0.75	0.35
<i>ppdf</i> (4)	Right curve shape of the function of temperature effect on growth	2	3.8
<i>pltmrf</i> *	Planting month reduction factor to limit seedling growth	0.4	0.4
<i>fulcan</i>	Value of <i>aglive</i> (aboveground live C) at full canopy cover	700	900
<i>kcet</i>	Crop coefficient used to calculate evapotranspiration	0.54	0.60
<i>cfrcn</i> (1)	Maximum fraction of C allocated to roots under max. nutrient stress	0.70	0.83
<i>cfrcn</i> (2) *	Minimum fraction of C allocated to roots with no nutrient stress	0.36	0.28
<i>cfrcw</i> (1)	Maximum fraction of C allocated to roots under max. water stress	0.80	0.73
<i>cfrcw</i> (2) *	Minimum fraction of C allocated to roots with no water stress	0.36	0.28
<i>claypg</i>	Number of soil layers to determine water and mineral N available for crop growth	9	9
<i>biomax</i>	biomass level above which the minimum and maximum C/E ratios of the new shoot increments equal <i>pramn</i> (* ,2) and <i>pramx</i> (* ,2) respectively (g biomass/m ²)	200	100
<i>pramn</i> (1, 1)	Minimum C/N ratio with zero biomass	37	47
<i>pramn</i> (1, 2)	Minimum C/N ratio with biomass greater than or equal to <i>biomax</i>	57	67
<i>crprtf</i> (1)	Fraction of N transferred to a vegetation storage pool from grass/crop leaves at death	0.6	0.73
<i>snfxmx</i> (1)	Symbiotic N fixation maximum for grassland/crop	0.002	0.008
<i>fligni</i> (1, 1)	Intercept for equation to predict lignin content fraction based on annual rainfall for aboveground material	0.02	0.04
<i>fligni</i> (1, 2)	Intercept for equation to predict lignin content fraction based on annual rainfall for	0.06	0.08

	juvenile live fine root material		
<i>fligni (1, 3)</i>	Intercept for equation to predict lignin content fraction based on annual rainfall for mature live fine root material	0.13	0.15
<i>mrtfrac</i>	Fraction of fine root production that goes to mature roots	0.4	0.4
<i>cmxturn</i>	Maximum turnover rate per month of juvenile fine roots to mature fine roots	0.5	0.3
<i>rdrj</i>	Maximum juvenile fine root death rate	0.95	0.90
<i>rdrm</i>	Maximum mature fine root death rate	0.80	0.45
<i>rdsrfe</i>	Fraction of the fine roots that is transferred into the surface litter layer	0.2	0.2
<i>cmix</i>	Rate of mixing of surface SOM and soil SOM	0.5	0.5
<i>npp2cs (1)</i>	GPP as a function of NPP to determine C stored in the carbohydrate pool	2.0	2.0
<i>fallrt</i>	Fall rate of standing dead biomass	0.1	0.1

*Values for the “maximum yielding phase”

The DAYCENT model was able to simulate giant reed yields (Fig. 1), root biomass and SOC (Fig. 2) with good accuracy. Unfortunately, very few studies reported the root biomass of giant reed, which however seems to reach values significantly over 10 Mg ha⁻¹, both in fine (Monti and Zatta, 2009) and sandy soils (Nassi o Di Nasso et al., 2013), once the crop is established.

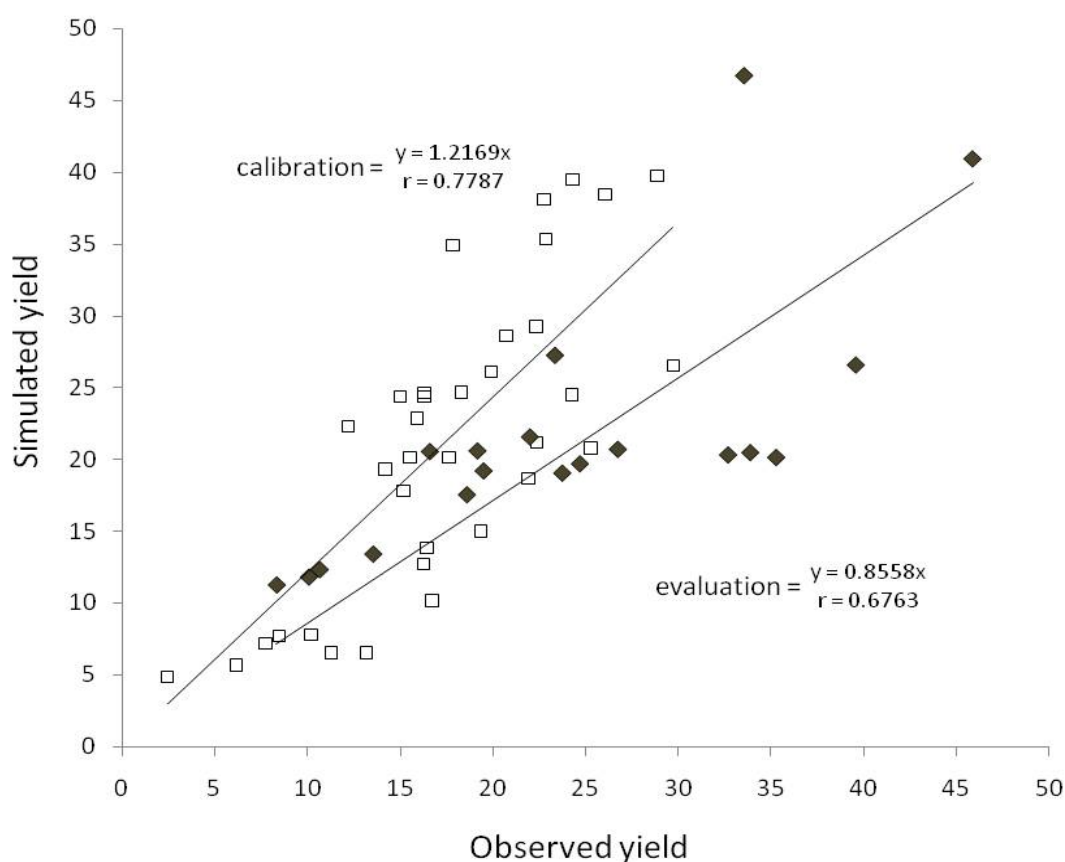


Fig. 1 Observed versus simulated giant reed yields (Mg ha⁻¹ y⁻¹) used for calibration and evaluation; all points aggregated show r=0.64 (calibration points = square, evaluation points = diamond)

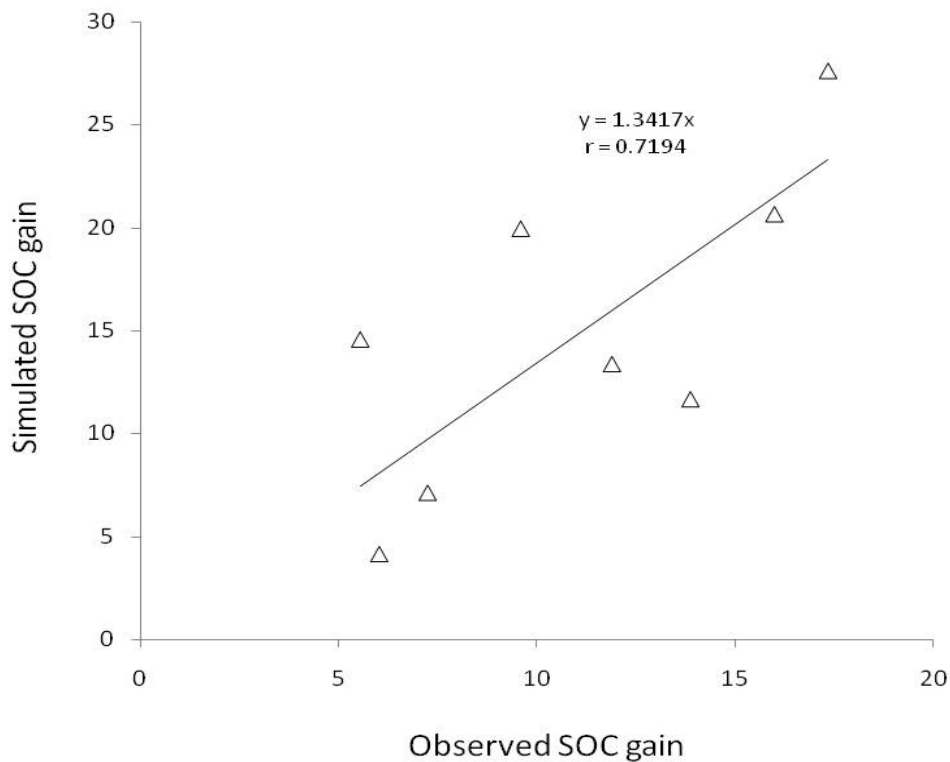


Fig. 2 Observed versus simulated giant reed total SOC gains (Mg ha⁻¹)

Land selection and crop allocation

The study was conducted in the US Southeast and the following States were included: Alabama, Arkansas, Florida, Georgia, Kentucky, Louisiana, Mississippi, North Carolina, South Carolina, Tennessee and Virginia. One of the goals of this study was to assess trade-offs among distinct land use change options for the cultivation of biofuel crops in the US Southeast. Three LUC strategies were simulated: conversion of i) grasslands and shrublands with considerable marginal traits, ii) grasslands and shrublands without major constraints for agriculture, and iii) croplands with considerable marginal traits. In order to identify the lands with the above written characteristics, two databases were principally used: the National Land Cover Database (NLCD) 2006 (Wickham et al., 2013) and the Land Capability Classification which is included in the SSURGO database (Ernstrom and Lytle, 1993). NLCD's selected classes were: 52 (areas dominated by shrubs less than 5 meters tall, with shrub canopy typically greater than 20% of the total vegetation), 71 (areas dominated by herbaceous vegetation, which is generally greater than 80% of total vegetation), 82 (areas being actively tilled and used for the production of annual crops and also perennial woody crops). The Land Capability Classification uses 8 classes, from I to VIII, to express growing limitation of a certain land for agricultural use: a land in class I has no limitations for agricultural use, while, on the opposite, a land in class VIII has severe limitations that avoid any type of

agricultural use. We estimated that lands in classes from I to VI were suitable for the cultivation of switchgrass and giant reed. Although lands in classes V and VI can already have some serious limitations, we considered that the low management required by the two perennial crops (Lewandowski et al., 2003) and their suitability for marginal lands (Quinn et al., 2015) would still render their cultivation feasible and economically sustainable; for example, in their analysis, Gelfand et al. (2013) converted to biofuel also lands in capability class VII. Five scenarios were eventually simulated: 1A) conversion of grasslands and shrublands in capability classes between IV and VI; 2A) conversion of 50% of grasslands and shrublands in capability classes between I and III; 1B) conversion of grasslands and shrublands in capability classes between IV and VI plus conversion of croplands in capability classes V and VI; 2B) conversion of 50% of grasslands and shrublands in capability classes between I and III plus conversion of croplands in capability classes V and VI; B) only conversion of croplands in capability classes V and VI. We decided to convert only 50% of grasslands and shrublands in capability classes between I and III to avoid possible ILUC effects given by the displacement of livestock grazing that occurs in part on these lands (U.S. Department of Agriculture, 1997); moreover, the total surface occupied by this land use in the US Southeast is large (4.7 Mha), thus, maintaining half of it to livestock grazing, allowed us to deliver more plausible outcomes at the regional scale. Further, although croplands in capability classes V and VI occupy a small fraction of the tilled surface in the Southeast (4.9%), their conversion could still generate ILUC effects. We however considered these effects avoidable by intensifying food production in non-marginal croplands (Matson et al., 1997; Burney et al., 2010; Heaton et al., 2013).

Federally-owned lands were identified using the USGS Federal Lands of the United States data layer (U.S. Geological Survey, 2015) and excluded from the study because not likely to be converted. Also areas with slope $> 15\%$ were excluded because considered not suitable for cropping. After filtering for federally-owned and high slope lands, the simulation area was reduced by 10.9%.

In this study, differently from previous US regional simulations that modeled biofuel crops cultivation (Davis et al., 2012; Qin et al., 2014; Hudiburg et al., 2016), switchgrass and giant reed were not cultivated on all the selected land, but were spatially allocated following two different criterias. The first was a criteria of “spatial intensification”, as described by Heaton et al. (2013): basing on some of the characteristic of the two crops, we tried to identify those marginal traits of the lands that could be best overcome by either switchgrass or giant reed. In order to do that, we used the following Land Capability Classification subclasses, which attribute the specific major limitation of a certain land ranked from II to VIII: subclass “e” is for soils where the susceptibility

to erosion is the dominant problem or hazard in their use, “w” is for soils where excess water is the dominant hazard, “s” is for soils that have limitations within the rooting zone (i.e. shallowness, stones, low moisture-holding capacity, low fertility, salinity), and “c” is for soils where there are climatic limitations (temperature or lack of moisture). Switchgrass was allocated on lands ranked “e” because of the lower soil disruption that is brought with seeding at establishment compared to the implant of rhizomes required by giant reed and for its higher tillering that covers the soil more completely (direct observation), resulting in lower erosion risks. Giant reed was allocated on lands ranked “w” because it is also a riparian species that survives and performs well in flooded conditions (Herrera et al., 2003; Quinn et al., 2015).

Both, switchgrass and giant reed, have deep and dense root systems (Monti and Zatta, 2009) that can allow them to overcome rooting zone limitations. Further, switchgrass can better grow in drier soils whereas giant reed reacts better in saline soils (Quinn et al., 2015), while both significantly yield despite the lack of soil nitrogen (Lewandowski et al., 2003). Thus, it was not possible to allocate either one of the two crops following the “spatial intensification criteria” on lands ranked “s”. On these lands we therefore decided to allocate switchgrass, applying what we called an “economical/consensus criteria”. In fact, as pointed out above, the availability of the genetic material, social acceptance and the lower production costs would likely encourage farmers to cultivate switchgrass.

Climatic limitations are really negligible in the study region and even where they occur are not strong limitations (capability classes II or III): in fact lands ranked “c” were only about 1% of the total land selected for the simulation (Fig. 3). Switchgrass was then allocated on these lands, following again the “economical/consensus criteria”, since none of the two crops seemed to have any significant ecological advantage.

Regional simulation set-up and runs

Unique combinations of weather, soil type and land use were identified within the study region. Each unique combination represented a DAYCENT modeling “strata”, which is a distinct model run. Climate data were derived from the North American Regional Reanalysis (NARR) database (Mesinger et al., 2006) (32 km grid). To identify soils with different characteristics (sand and clay contents, pH, rock fragments, depth), the SSURGO database was used (Ernstrom and Lytle, 1993). For land use, the above mentioned National Land Cover Database (NLCD) 2006 (Wickham et al., 2013) was employed. In total, 106340 unique combinations of weather, soil type and land use were identified.

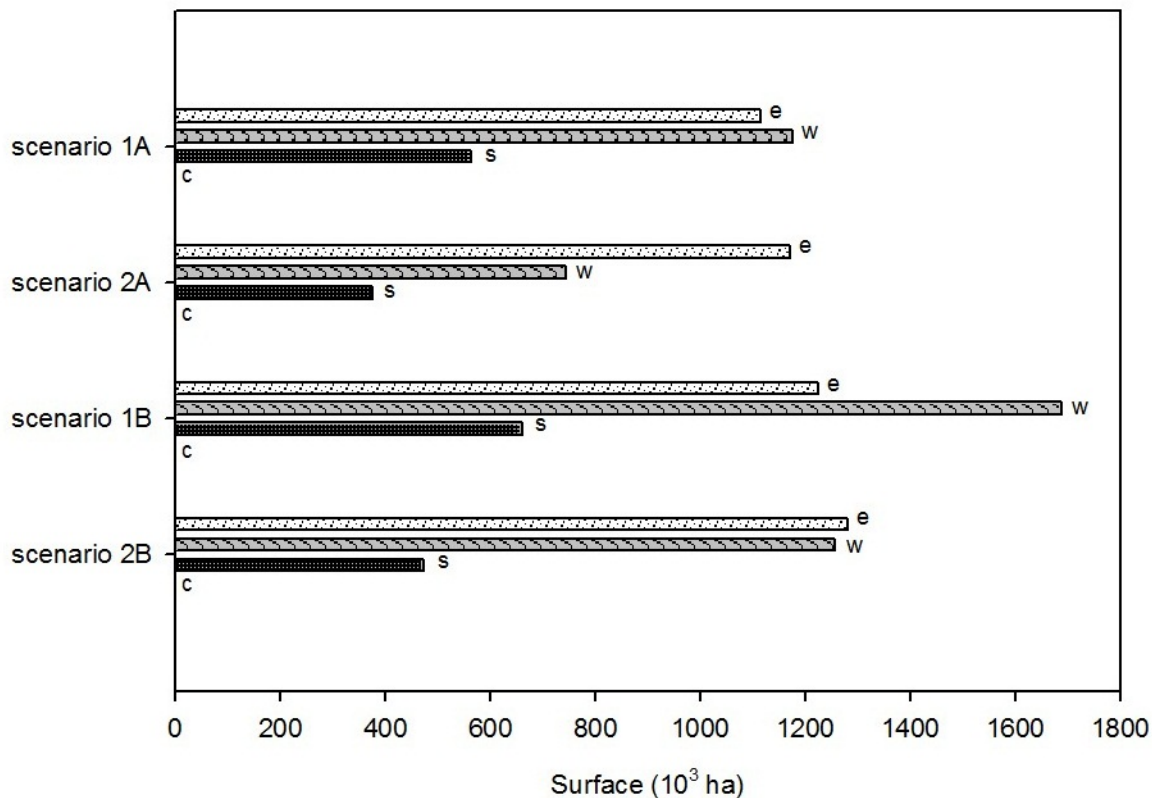


Fig. 3 Within each simulated scenario in the US Southeast, the total surface (10^3 ha) belonging to each subclass of the USDA capability classification is shown. Lands ranked “e” are susceptible to erosion, lands ranked “w” are subject to periodic flooding events; lands ranked “s” have limitations within the rooting zone (i.e. shallowness, stones, low moisture-holding capacity, low fertility, salinity), lands ranked “c” have climatic limitations (temperature or lack of moisture). The subclasses of the capability classification were used as criteria to allocate the biofuel crops switchgrass and giant reed

For each strata, the initial values of soil C and N were initialized by an equilibrium phase during which DAYCENT simulated, for several thousand years, what was assumed had been the historical land use (Ogle et al., 2010). Basically, the equilibrium phase was split in two parts: a first one, up to year 1850 (this phase extended to the present for grasslands), where the original natural vegetation was simulated and soil steady-state was reached, and a second one (only for croplands), up to the present, where first plow-out and crop rotations and managements were simulated according to various sources (Ogle et al., 2010).

Following the initialization, 15 years of cultivation of switchgrass or, depending on the modeling strata, giant reed were simulated. Sowing of switchgrass, or rhizomes implant of giant reed, occurred in May, harvest was carried out in October every year (harvest losses ~15%), the crops were not fertilized in the establishment year to avoid weeds competition, while $67 \text{ kg ha}^{-1} \text{ y}^{-1}$ were given from the second year on. This N fertilization rate was shown to be the most beneficial for switchgrass production in marginal areas, taking into account economical and environmental

aspects (Wang et al., 2015). No such data on the best N fertilization rate for giant reed was found in the present literature, thus, also to facilitate a comparison between the two perennials after the simulation, the same amount of N was given to both crops.

Sensitivity analysis of crop allocation

Two sensitivity analysis were performed, changing the allocation criteria for the two crops. In the first analysis, a part of the lands cultivated with switchgrass was allocated to giant reed. Being giant reed more productive, the scope of this analysis was to narrow the biomass supply area around the potential new biorefineries and to possibly predict the position of other biorefineries (see the next section). In this case, all lands in capability subclass “s” were cultivated this time with giant reed instead of switchgrass. In the second analysis, the aim, differently from the previous, was not to simulate more biomass production or to predict more potential biorefineries, but to simulate scenarios with a reduced invasion risk brought by giant reed. In fact, although the risk of giant reed invasion is low outside the riparian environments and it is further lowered by the annual harvest carried out when managed as an energy crop (Ceotto and Di Candilo, 2010), the diffusion risk is higher in periodically flooded areas. Therefore, in this second analysis, all lands ranked “s” were cultivated with giant reed while all lands ranked “w”, where the risk of invasion is more probable, were cultivated with switchgrass.

Biorefineries position

Total mean yearly harvested biomass was calculated at the county level (1001 counties in total). Then, using ArcMap 10.2.2 (ESRI), an analysis was carried out to discover the potential position of new biorefineries. We assumed the supply of bioethanol production plants with a capacity of 286 Ml ethanol y^{-1} . Although at present the biggest working biorefineries in the US supplied by lignocellulosic feedstocks reach a capacity of 95 Ml ethanol y^{-1} (Bacovsky et al., 2013), in the future will be economically advantageous to build big plants, which is feasible, taking into account that currently in the US there are thirteen first-generation ethanol refineries with a capacity over 500 Ml ethanol y^{-1} , and three of them with a capacity over 1000 Ml ethanol y^{-1} (EIA, 2016). Thus, we decided to use the average size of all working US ethanol plants at present (286 Ml ethanol y^{-1} ; EIA, 2016) as our target for future plants in the US Southeast, which seemed a reasonable size. Such plants would demand ~ 1.02 Mt y^{-1} of dry biomass (under current technology, 282 l ethanol Mg^{-1} of dry biomass are to be produced; Lynd et al., 2008). A 80-km radius around the potential new biorefineries was used for biomass supply, as it was estimated as the economically feasible transportation distance in Alabama, and various other southeastern States (Bailey *et al.*,

2011). Only in the first sensitivity analysis, where giant reed was allocated on more surface, the supply district was reduced to a radius of 50 km, according to IEA (2007).

To identify potential supply areas of 20096 km², a moving window (Focal Statistic) included in ArcMap's "Neighborhood Toolset" was employed. The sum of the yearly yields of each spatial unit (1 ha) was calculated within the specified neighborhood (circles with a 80-km radius) of the simulation region: when the sum was equal to 1.02 Mt y⁻¹ of dry biomass or higher, that specific neighborhood was designed as potential supply area of a biorefinery. Biomass within a supply area was then considered sufficient (between 1.02 and 1.3 Mt y⁻¹), abundant (>1.3 Mt y⁻¹) or very high (>2.1 Mt y⁻¹). This analysis was performed for each of the basic scenarios and for each scenario resulted from the two sensitivity analyses, to finally compare their potential to produce bioethanol in the US Southeast.

Greenhouse gas accounting

Starting from the model outputs, SOC changes and system N losses were converted in GHG emissions (CO₂ equivalents) as follows (IPCC, 2014):

$$CO_2eq = -(SOC\ change \times 3.67) \quad (1)$$

$$CO_2eq = [(vN \times 0.01) + (lN \times 0.0075) + (NO \times 0.01) + N_2O] \times 298 \quad (2)$$

where *vN* is the volatilized nitrogen and *lN* is the nitrogen leached; negative values correspond to a GHG uptake, while positive values correspond to a GHG emission.

Greenhouse gas intensity was calculated as the ratio between GHG emissions and dry biomass yield.

Results

Simulation of switchgrass and giant reed in the US Southeast

Mean simulated long-term (15 years) yields were, across the study region, higher for giant reed (16.3 Mg ha⁻¹ y⁻¹) than switchgrass (7.9 Mg ha⁻¹ y⁻¹), and higher on former grasslands than on former croplands, especially when switchgrass was cultivated (+14%); this was likely due to the fertilizing effect of the aboveground residues embedded in the soil upon conversion, as well as to the fact that only croplands that were marginal, thus with lower yield potential, were converted. Mean SOC change after 15 years of cultivation was significantly positive after croplands conversion (0.27 and 0.57 Mg ha⁻¹ y⁻¹, respectively for switchgrass and giant reed) while was

negative or null after grassland conversion (-0.23 and $0.01 \text{ Mg ha}^{-1} \text{ y}^{-1}$, respectively for switchgrass and giant reed). Mean N_2O emissions did not differ much between the two crops and between distinct land use transitions ($1.6\text{-}1.9 \text{ kg ha}^{-1} \text{ y}^{-1}$, on average), since N fertilization, the main trigger of N emissions in agriculture (Del Grosso et al., 2006; Erismann et al., 2010), was maintained constant in each simulation strata.

Giant reed long-term yields fluctuated more than switchgrass long-term yields across States: the lowest yields, on average, were achieved in Virginia (7.7 and $12.9 \text{ Mg ha}^{-1} \text{ y}^{-1}$, respectively for switchgrass and giant reed), while the highest yields, on average, were reached in Louisiana (8.6 and $18.1 \text{ Mg ha}^{-1} \text{ y}^{-1}$, respectively for switchgrass and giant reed). In general, lower yields were simulated in Virginia, North Carolina and Kentucky for both crops, while higher yields were simulated in Louisiana, Mississippi, Alabama and Florida for giant reed, or in Louisiana, South Carolina, Georgia, Mississippi for switchgrass. A latitudinal gradient within the US Southeast was evident in giant reed productivity: average giant reed yields, in fact, varied by 40% passing from Virginia to Louisiana, whereas varied by only 11% in switchgrass; this temperature-dependence of giant reed well agrees with the literature that describes giant reed as a warm-temperate or subtropical species (Lewandowski et al., 2003).

Table 3 Mean long-term yield, peak yield (reached in the second or third year after establishment, respectively in switchgrass and giant reed), mean SOC change and mean N_2O emissions for switchgrass (SG) and giant reed (GR) cultivated in the US Southeast after conversion of either grasslands or croplands

Crop	Former land use	Mean yield ($\text{Mg ha}^{-1} \text{ y}^{-1}$)	Peak yield ($\text{Mg ha}^{-1} \text{ y}^{-1}$)	Mean SOC change ($\text{Mg ha}^{-1} \text{ y}^{-1}$)	Mean N_2O emissions ($\text{kg ha}^{-1} \text{ y}^{-1}$)
SG	grassland	8.4	24.2	-0.23	1.9
GR	grassland	16.5	28.8	0.01	1.7
SG	cropland	7.4	21.3	0.27	1.7
GR	cropland	16.2	28.4	0.57	1.6

Performing regressions of the simulated yields against pedo-climatic factors, we observed that, on average: i) the optimum temperature was higher for giant reed than for switchgrass, as set during calibration, ii) giant reed showed a stronger response to increasing yearly total rainfalls ($r = 0.73$, $y = 4.953x + 11.63$), iii) passing from 0.3 to 2.1 m soil depth, yields increased by more than 40% in both crops ($r = 0.94$, $y = 54.61 \ln(x) + 70.49$, switchgrass; $r = 0.95$, $y = 99.89 \ln(x) + 149.8$, giant reed), iv) yields of switchgrass decreased as the USDA capability class increased from I to VI ($r = 0.93$, $y = -4.004x + 356.2$), underlying how the model was able to capture some of the limitations to the agricultural use of lands determined by the capability classification. On the opposite, giant

reed showed an unclear trend respect to the capability classes, due to the fact that, in some parts of the region, water flooding constituted rather an advantage for giant reed and increased its productivity. Further, since rooting depth was set high in both crops during parameterization (Table 2), soil depth affected yields because of the variation in nutrients and water availability.

Besides being affected by the previous land use, SOC change was affected by soil texture, specifically by the clay content, as expected (Parton et al., 1987). SOC change and soil clay content were correlated when cultivating both switchgrass ($r = 0.41$, $y = 0.018 x^2 - 0.701 x - 20.08$) and giant reed ($r = 0.66$, $y = 0.029 x^2 - 1.154 x + 6.032$).

Land use change scenarios

Summing up total surfaces cultivated with switchgrass and giant reed, 2.9, 2.4, 3.6 and 3.1 Mha of the study region were converted, respectively in scenarios 1A, 2A, 1B and 2B (Fig. 4). The corresponding total dry biomass production, total SOC variation and total N₂O emissions for each scenario are reported on a yearly basis in Table 4.

Scenarios 1A and 1B were more efficient than scenario 2A and 2B in terms of dry biomass production and SOC change per hectare, but this was due to the allocation strategy adopted between the two crops. In scenario 1A less land was ranked “e” and more land was ranked “w” compared to scenario 2A. Thus, in scenario 1A and 1B, respectively the 41 and 47% of the surface was cultivated with giant reed, while, in scenario 2A and 2B, less surface was dedicated to giant reed (respectively, 32 and 41%). As shown in the previous section, higher long-term yields were simulated for giant reed than switchgrass on average (+99%) and, moreover, when converting grasslands, giant reed was neutral to beneficial while switchgrass lost SOC: therefore, more land dedicated to giant reed meant more benefits in terms of GHG savings.

Compared to only grasslands conversion (scenarios 1A and 2A), adding former croplands to biomass production turned soils from a source to a sink of C (scenario 1B). In fact, the conversion of 0.7 Mha of croplands produced a SOC gain of the magnitude of 0.40 Mt y⁻¹ (0.57 Mg ha⁻¹ y⁻¹, on average), whereas grasslands conversion (5.3 Mha) produced a SOC loss of -0.79 Mt y⁻¹ (-0.15 Mg ha⁻¹ y⁻¹, on average).

We also estimated the C debt deriving from the loss of permanent aboveground vegetation after conversion of grasslands/shrublands systems. This conversion debt corresponded to -0.67 Mg (C) ha⁻¹ on average. However, we considered this C debt abundantly counterbalanced by the enormous root biomass production of switchgrass and giant reed, corresponding, respectively, to 2.4 and 3.9 Mg (C) ha⁻¹ on average in the mature stands.

Table 4 Total dry biomass production, total SOC variation and total N₂O emissions for the scenarios simulated in the US Southeast. Scenarios 1A (conversion of grasslands in capability classes IV, V, VI), 2A (conversion of grasslands in capability classes I, II, III), 1B (conversion of grasslands in capability classes IV, V, VI plus conversion of croplands in classes V and VI) and 2B (conversion of grasslands in capability classes I, II, III plus conversion of croplands in classes V and VI) differed in the land use selection used to allocate the biofuel crops switchgrass and giant reed. Besides the basic scenarios, results after giant reed expansion (1st sensitivity analysis) and after re-allocation of giant reed to minimize invasion risks (2nd sensitivity analysis) are shown. Allocation of switchgrass (%) is complementary to the allocation of giant reed shown

Scenario	Surface (Mha)	Allocation (giant reed %)	Dry biomass (Mt y ⁻¹)	SOC change (Mt y ⁻¹)	N ₂ O emissions (Mt y ⁻¹)
1A (basic)	2.9	41	34	-0.35	0.005
2A (basic)	2.4	32	26	-0.44	0.004
1B (basic)	3.6	47	44	0.05	0.007
2B (basic)	3.1	41	36	-0.04	0.006
1A (1st sensitivity)	2.9	61	39	-0.22	0.005
2A (1st sensitivity)	2.4	48	29	-0.36	0.004
1B (1st sensitivity)	3.6	66	49	0.21	0.007
2B (1st sensitivity)	3.1	56	40	0.07	0.006
1A (2nd sensitivity)	2.9	20	29	-0.54	0.005
2A (2nd sensitivity)	2.4	16	23	-0.56	0.005
1B (2nd sensitivity)	3.6	19	35	-0.29	0.007
2B (2nd sensitivity)	3.1	15	29	-0.31	0.006

After performing the first sensitivity analysis (Table 4), giant reed was cultivated on more land. This time, the 61 and 66% of the surface, respectively in scenarios 1A and 1B, were converted to giant reed, while it was cultivated on the 48 and 56% of the surface, respectively in scenarios 2A and 2B. Compared to the basic scenarios, in the new scenarios an increase in total biomass production was registered (+11-15%), less SOC (-18-37%) was lost after grasslands conversion (scenarios 1A and 2A, respectively) and both, scenarios 1B and 2B, registered positive SOC gains (0.21 and 0.07 Mt y⁻¹, respectively); on the opposite, total N₂O emissions were not significantly affected by the change in crop allocation.

In the sensitivity analysis aimed to minimize giant reed's invasion risks, giant reed was cultivated, depending on the scenario, on the 15-20% of the surface converted, thus on much less land than in the basic scenarios (Table 4). This change in crop allocation caused a reduction in biomass

production (-12-20%) and made each scenario result in a greater SOC loss, even scenario 1B, which had registered a positive SOC gain in the previous two analyses, lost SOC (-0.29 Mt y⁻¹). Again, in both re-allocations of the two crops, scenarios 1A and 1B were more efficient in terms of biomass production and SOC change than scenarios 2A and 2B. This can finally be explained by the higher amount (+5%) of lands ranked “e” in non-marginal (scenario 2A) than in marginal (scenario 1A) grasslands (land ranked “e” were cultivated in all the analyses with switchgrass, which yielded less than giant reed and was detrimental on SOC when replacing grasslands).

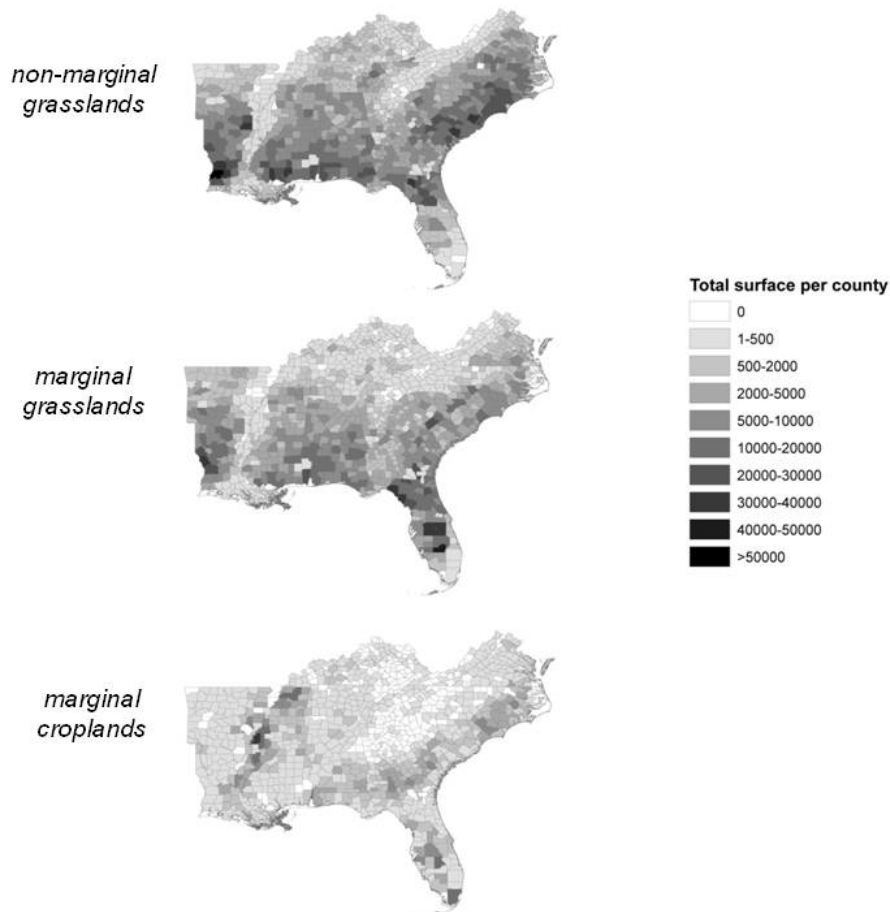


Fig. 4 For each county of the US Southeast, shows the total surface (ha) occupied by non-marginal grasslands (grasslands in capability classes I, II, III), marginal grasslands (grasslands in capability classes IV, V, VI) and marginal croplands (croplands in classes V and VI)

Biorefineries potential position

The highest biomass concentration was simulated in scenario 1B (Fig. 5a) where Ware County (GA) produced enough biomass in its surroundings (20096 km²) to supply a bioethanol plant with a capacity of 838 Ml ethanol y⁻¹. Across the different scenarios, Florida, Georgia, Mississippi and South Carolina were the States with the highest biomass supply potential, which means with high land availability too. Table 5 summarizes some of the data resulting from the GIS analysis

regarding the maximum number of bioethanol plants and the counties with the highest biomass supply potential in each scenario.

Table 5 For each scenario, the maximum number of potential bioethanol plants with a 80-km radius supply area, the highest biomass supply within the radius and the counties with the highest biomass production potential are presented

Scenario	1A	2A	1B	2B	B
Biorefineries number	12	7	19	16	3
Highest supply (Mt)	2.92	1.41	2.97	1.79	1.64
Top counties	Columbia (FL) Suwannee (FL) Gilchrist (FL)	Berkeley (SC) Orangeburg (SC) Colleton (SC)	Ware (GA) Columbia (FL) Suwannee (FL)	Washington (MS) Bladen (NC) Sunflower (MS)	Washington (MS) Sunflower (MS) Coahoma (MS)

Scenario 2A performed worse than scenario 1A. In fact, despite a -17% of land converted to biofuel production, a maximum number of 7 bioethanol plants was estimated for scenario 2A, while up to 12 bioethanol plants could be built in scenario 1A. If we were to convert only marginal croplands (scenario B), Mississippi would still have the potential to supply up to 3 bioethanol plants and Washington County (MS) could supply a bioethanol plant with a capacity of 462 Ml ethanol y^{-1} .

Expanding giant reed cultivation while reducing the radius of the biomass supply area to 50 km still allowed the production of enough biomass to supply 6 or 10 bioethanol plants, respectively in scenarios 1A and 1B (Fig. 5c), while, on the opposite, made scenario 2A completely inefficient (not enough biomass within a radius of 50 km for average size bioethanol plants). The conversion of solely marginal croplands still produced enough biomass (~ 1.05 Mt y^{-1}) for an average size bioethanol plant in Sunflower County (MS). The districts with the highest potential were identified, similarly to the previous analysis, in northern and central Florida (Columbia, Suwannee, Lafayette, Gilchrist and Highlands counties), in southern Georgia (Ware and Bacon counties) and eastern Mississippi (Sunflower County).

Reducing the surface cultivated with giant reed to minimize its invasion risks also reduced the potential for bioethanol production in each scenario. Nonetheless, scenarios 1A and 1B maintained very high or abundant biomass supplies in northern Florida, southeastern Georgia and southern

Alabama (Fig. 5e); Columbia (FL) and Alachua (FL) counties showed very high biomass availability ($\sim 2.4 \text{ Mt y}^{-1}$) in their surroundings. Scenarios 2A and 2B yielded biomass just sufficient ($< 1.3 \text{ Mt y}^{-1}$) for, respectively, 7 or 9 bioethanol plants in southern South Carolina, eastern Georgia, northern Florida and southern Mississippi, with only Screven (GA) and Allendale (GA) counties showing abundant biomass supplies ($\sim 1.4 \text{ Mt y}^{-1}$). While in the basic scenarios converting to biomass production only marginal croplands was still sufficient to supply up to three ethanol plants (two in eastern Mississippi and one in southern Georgia), after changing crop allocation to minimize giant reed invasion risk, that was not achievable anymore and only smaller biorefineries ($140\text{-}200 \text{ Mt y}^{-1}$) could be eventually built in eastern Tennessee, eastern Mississippi or central Florida.

Discussion

Some things emerged from the present analysis: i) giant reed showed a higher potential than switchgrass to produce biomass and to store SOC in the US Southeast, ii) converting arable lands yielded greater GHG benefits than converting grasslands and shrublands to perennial lignocellulosic crops, iii) the US Southeast owns the potential to host several large size plants for the production of advanced ethanol from marginal lands, particularly, Florida, Georgia, South Carolina and Mississippi, iv) among all scenarios, 1B (conversion of marginal grasslands and shrublands plus conversion of marginal croplands) resulted as the most beneficial option.

A few direct comparisons of switchgrass and giant reed are currently present in the literature (Monti and Zatta, 2009; Kering et al., 2012; Alexopoulou et al., 2015; Nocentini and Monti, 2017). Nevertheless, in these previous studies, giant reed was always reported to show higher yields (Kering et al., 2012; Alexopoulou et al., 2015), higher root biomass (Monti and Zatta, 2009) or higher SOC accumulation rates (Nocentini and Monti, 2017). Kering et al. (2012) reported that in the US giant reed yielded +58% biomass than switchgrass after that both crops were fully established, and Alexopoulou et al. (2015) observed +56% of mean biomass yield in giant reed than in switchgrass during ten years of side-by-side cultivation in Northern Italy. Monti and Zatta (2009), at the sixth year of cultivation of both perennial crops, found that giant reed had +61% root biomass, while Nocentini and Monti (2017) measured +102% SOC storage in giant reed than in switchgrass after ten years of cultivation, pointing out that organic inputs to the soil deriving from giant reed harvest residues were also greater. No surprises then if, even in our study, we simulated a higher yielding and C sink capacity for giant reed. This difference between the two crops was however less substantial as the cultivation was moved to the northern part of the study region, and in fact, giant reed, more than switchgrass, prefers warm climates (Lewandowski et al., 2003).

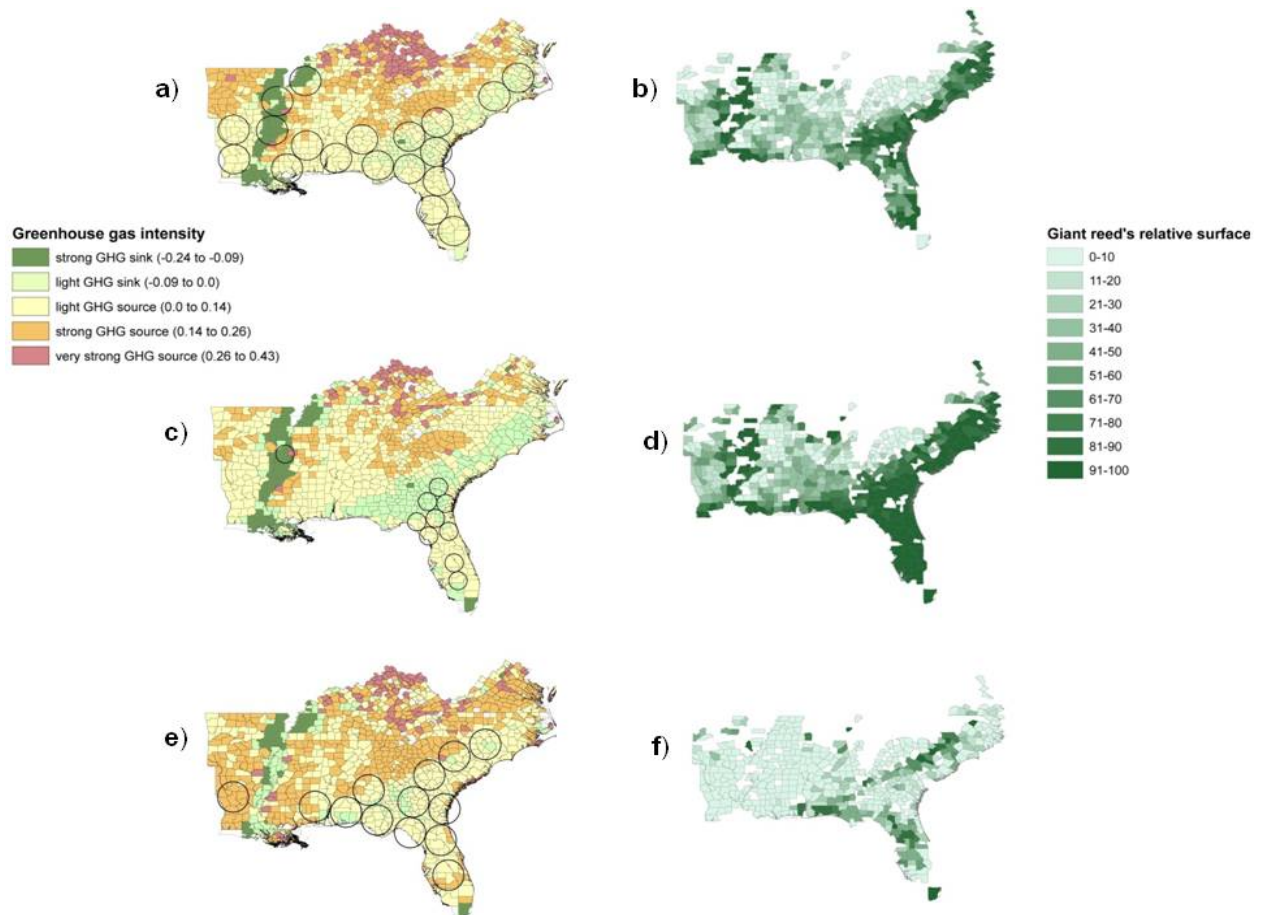


Fig. 5 *a*) Greenhouse gas intensity ($\text{Mg CO}_2\text{eq Mg}^{-1}$ of dry biomass) for each county of the US Southeast in scenario 1B; *b*) Giant reed's relative surface (%) respect to the total surface converted to bioethanol production for each county of the US Southeast in scenario 1B; *c*) Greenhouse gas intensity for each county of the US Southeast in scenario 1B after giant reed expansion (first sensitivity analysis of crop allocation); *d*) Giant reed's relative surface respect to the total surface converted to bioethanol production for each county of the US Southeast in scenario 1B after giant reed expansion (first sensitivity analysis of crop allocation); *e*) Greenhouse gas intensity for each county of the US Southeast in scenario 1B after giant reed contraction (second sensitivity analysis of crop allocation); *f*) Giant reed's relative surface respect to the total surface converted to bioethanol production for each county of the US Southeast in scenario 1B after giant reed contraction (second sensitivity analysis of crop allocation). Potential biomass supply areas for average size bioethanol plants ($286 \text{ Ml ethanol y}^{-1}$) are identified by 80-km radius circles in sub-figures *a* and *e*, while identified by 50-km radius circles in sub-figure *c*. In sub-figures *b*, *d* and *f* only counties with at least 2000 ha converted to bioethanol production are shown

Analyzing different land use change options further underlined the distinct potentials of switchgrass and giant reed. Both crops increased SOC after replacing marginal croplands and thus had a positive impact, but when grasslands and shrublands were converted, they behaved differently: on average, switchgrass lost SOC while giant reed was neutral (-0.23 and $0.01 \text{ Mg ha}^{-1} \text{ y}^{-1}$, respectively), meaning that only giant reed was able to recover the initial SOC loss occurring upon grasslands conversion and to maintain it in the long term. Interestingly, Qin et al. (2016), after a meta analysis on SOC storage by biofuel crops, reported similar results comparing switchgrass with the higher

yielding miscanthus: they found that, after grasslands conversion, on average, the former lost SOC ($-0.16 \text{ Mg ha}^{-1} \text{ y}^{-1}$), while the latter showed a positive SOC gain ($0.28 \text{ Mg ha}^{-1} \text{ y}^{-1}$).

In our study, giant reed cultivation positively affected biofuel production and greenhouse gases emissions. In fact, an evident pattern was observable (Fig. 5): the counties with a higher proportion of lands converted to giant reed were the counties with the highest biomass supplies and where greenhouse gas intensity assumed negative values, which correspond to a GHG sink capacity. Table 5 shows the States of Florida, Georgia, South Carolina and Mississippi with a high potential for advanced ethanol production, so, analyzing more deeply the land uses of these four States, we find that: in scenario 1A (conversion of marginal grasslands and shrublands), Florida and Georgia owned the 38% of the total land converted to advanced ethanol, of which 0.65 Mha (59%) were dedicated to giant reed; in scenario 2A (conversion of non-marginal grasslands and shrublands), South Carolina owned the 12% of the total land converted to advanced ethanol, of which 0.13 Mha (48%) were dedicated to giant reed; in scenario B (conversion of marginal croplands), Mississippi and Georgia owned the 44% of the total land converted to advanced ethanol, of which 0.28 Mha (89%) were dedicated to giant reed. Cai et al. (2011) also showed Florida, Georgia, South Carolina and Mississippi to have high land availability (from map), when considering marginal/abandoned croplands and grasslands discounted by the grazing land at present.

Even more importantly, our analysis, as our previous DAYCENT simulation work in the Mediterranean basin (Nocentini et al., 2015), resulted in a basic difference in biogenic emissions between land use change strategies. On average, SOC increased when converting croplands while decreased when converting grasslands and shrublands (0.57 and $-0.15 \text{ Mg ha}^{-1} \text{ y}^{-1}$, respectively). The literature already reports that SOC storage is foreseeable when converting croplands to biofuel perennial crops (Davis et al., 2012; Qin et al., 2016), while less predictable SOC dynamics occurs after converting unmanaged systems (Corre et al., 1999; Garten and Wullschleger, 2000; Qin et al., 2016). Moreover, when converting unmanaged grasslands and shrublands, no matter how low-input the succeeding biofuel crop may be, management-related GHG emissions will increase, on the opposite, when converting croplands, emissions from agronomic inputs are likely to diminish passing from annual crops to perennial crops production (Adler et al., 2007; Fazio and Monti, 2011; Gelfand et al., 2013), together with N_2O emissions that should diminish following the lower N fertilization rates given to perennial crops (Del Grosso et al., 2006; Drewer et al., 2012). Additional aspects regard plants diversity and wildlife refuges, which are likely to be reduced upon grasslands and shrublands conversion but to be enhanced with the establishment of switchgrass or giant reed on former tilled croplands (Fernando et al., 2010). For these reasons, the use of marginal croplands for biofuel production would be rather beneficial, also considering that ILUC emissions could be

either avoided through intensification of food production on non-marginal croplands (Matson et al., 1997; Burney et al., 2010; Heaton et al., 2013) or be however low (Nocentini et al., 2015) compared to the aforementioned GHG savings. Considering the ILUC impact previously calculated for the cultivation of switchgrass in low-productive former croplands in the Mediterranean basin ($0.11 \text{ Mg C ha}^{-1}$; Nocentini et al., 2015) and the total croplands surface converted in the present analysis (0.72 Mha), ILUC emissions in this study would correspond to 0.08 Mt C y^{-1} , thus substantially lower than SOC storage (0.40 Mt y^{-1}) simultaneously happening on those same croplands.

So, we eventually point out that the best scenario would be one that includes the conversion of marginal croplands: among the simulated scenarios that included conversion of croplands, we selected scenario 1B (conversion of marginal grasslands and shrublands plus conversion of marginal croplands) as the most efficient one. In fact, scenario B (only conversion of marginal croplands), although highly beneficial as GHG sink, was inefficient in terms of land availability (a high land availability that would allow a substantial production of ethanol was only found in Mississippi), while scenario 2B (conversion of non-marginal grasslands and shrublands plus conversion of marginal croplands) performed worse than 1B in terms of mean biomass yield, mean SOC storage rate and also mean N_2O emissions (Table 4), allowing, at the same time, a lower production of biofuel regionally (Table 5). One likely explanation for this lower efficiency of scenario 2B compared to scenario 1B is that the former had a higher share of lands where switchgrass was allocated (Table 4), thus with lower yields and more depleted SOC stocks.

Scenario 1B could potentially produce 7124 Ml y^{-1} of advanced ethanol from nineteen biorefineries (Fig. 5a), and, even after confining giant reed to minimize invasion risks, 4695 Ml y^{-1} could be still produced from thirteen biorefineries (Fig. 5e). Currently there are five working bioethanol plants in the study region (EIA, 2016): Ergon Biofuels LLC (Vicksburg, Mississippi; 204 Ml y^{-1}), Flint Hills Resources LP (Camilla, Georgia; 454 Ml y^{-1}), Green Plains Obion LLC (Obion, Tennessee; 416 Ml y^{-1}), Commonwealth Agri-Energy (Hopkinsville, Kentucky; 114 Ml y^{-1}) and Green Plains Hopewell LLC (Hopewell, Virginia; 235 Ml y^{-1}). All these five plants are supplied by corn ethanol feedstocks but, if converted to the production of advanced ethanol from perennial lignocellulosic feedstocks, great GHG benefits could be achieved (Davis et al., 2012). For example, our results show that Vicksburg's plant, if only being supplied by switchgrass and giant reed cultivated on marginal lands within a 80-km radius (scenario 1B), could produce even more ethanol (291 Ml y^{-1}) than it currently does, while fixing $1.1 \text{ Mt CO}_2\text{eq y}^{-1}$ through SOC storage (Fig. 5a). As for Camilla, Obion and Hopewell plants, respectively 1.1, 0.9 and 0.5 Mt y^{-1} of dry biomass would be available in their surroundings (scenario 1B), and could substantially contribute to their ethanol production, after switching to advanced ethanol technologies.

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Conclusions and future perspectives

The research carried out and presented in this thesis provides new insights on soil carbon dynamics upon land use change to perennial grasses. Field experiments fed model studies aimed at supporting decisions on future deployment of such crops in the Mediterranean area and in the US Southeast.

The carbon sink capacity of switchgrass and giant reed in North Italy were quantified. Switchgrass carbon accumulation was high in the fertile Po Valley while the carbon balance of conventional commodity crops was close to neutrality. We also discovered that giant reed could substantially and significantly store soil organic carbon, even in hypothetical biomass supply districts where dedicated perennial crops had already been cultivated for decades. Therefore, the two perennial grasses confirmed their ability to fix carbon from the atmosphere to the geosphere, principally by increasing soil stocks.

Our regional-scale investigations emphasized the considerable potential of perennial grasses to accumulate carbon when grown on marginal lands. Converting marginal unmanaged lands compared to converting marginal croplands always led to significant differences in greenhouse gases emissions.

Giant reed was identified as a very promising biofuel crop being able to produce high amounts of biomass with limited resources. But there are very few data on root biomass production of giant reed; additional studies would allow to refine the parameterization of this crop into process-based models.

One of the experiments presented long-term soil organic carbon data measured in a site where perennial biomass crops succeeded to a previous biomass plantation. To our knowledge, it was the first of its kind. We believe that an increased number of studies on long-term sequences of perennial bioenergy crops will help a deeper understanding of soil carbon dynamics and the real potential of future biomass supply districts to function as carbon sinks (as carbon accumulation tends to decrease in the longer period), as well as identifying those successions which would be highly beneficial from that standpoint.

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