1. Title

2	GREENHOUSE GAS BALANCE OF CROPLAND CONVERSION TO BIOENERGY
3	POPLAR SHORT ROTATION COPPICE
4	Running title: From cropland to bioenergy SRC: a GHG balance
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The production of bioenergy in Europe is one of the strategies conceived to reduce 27 greenhouse gas (GHG) emissions. The suitability of the land use change from a cropland 28 (REF site) to a short rotation coppice plantation of hybrid poplar (SRC site) was investigated 29 by comparing the GHG budgets of these two systems over 24 months in Viterbo, Italy. This 30 period corresponded to a single rotation of the SRC site. The REF site was a crop rotation 31 between grassland and winter wheat, i.e. the same management of the SRC site before the 32 conversion to short rotation coppice. Eddy covariance measurements were carried out to 33 quantify the net ecosystem exchange of CO_2 (F_{CO2}), whereas chambers were used to measure 34 N₂O and CH₄ emissions from soil. The measurements began two years after the conversion of 35 arable land to SRC: for that an older poplar plantation was used to estimate the soil organic 36 carbon (SOC) loss due to SRC establishment, and to estimate SOC recovery over time. 37 Emissions from tractors and from production and transport of agricultural inputs (F_{MAN}) were 38 modelled. The GHG emission offset due to the substitution of natural gas with SRC biomass 39 for production of heat was credited to the SRC site. Emissions generated by the use of 40 biomass (F_{EXP}) were also considered. Suitability was finally assessed by comparing the GHG 41 budgets of the two sites. CO_2 uptake was 3512 ± 224 gCO₂ m⁻² in the SRC site in two years, 42 and $1838 \pm 107 \text{ gCO}_2 \text{ m}^{-2}$ in the REF site. F_{EXP} was equal to $1858 \pm 240 \text{ gCO}_2 \text{ m}^{-2}$ in the REF 43 site, thus basically compensating F_{CO2} , while it was 1118 ± 521 gCO₂ m⁻² in the SRC site. 44 This latter could offset $379.7 \pm 175.1 \text{ gCO}_2\text{eq m}^{-2}$ from fossil fuel displacement. Soil CH₄ and 45 N_2O fluxes were negligible. F_{MAN} weighed 2% and 4% in the GHG budgets of SRC and REF 46 sites respectively, while the SOC loss weighed 455 ± 524 gCO₂ m⁻² in two years. Overall, the 47 REF site was close to neutrality in a GHG perspective $(156 \pm 264 \text{ gCO}_2\text{eg m}^{-2})$, while the 48 SRC site was a net sink of 2202 ± 792 gCO₂eq m⁻². In conclusion the experiment led to a 49 positive evaluation from a GHG viewpoint of the conversion of cropland to bioenergy SRC. 50

51 **3. Introduction**

In the articulated regulation concerning energy and climate change policies, the European Union (EU) established two targets for 2020: (i) reduction of 20% of greenhouse gas (GHG) emissions relative to the levels of 1990, and (ii) share of 20% renewable energy use in gross final energy consumption (European Commission, 2007; European Commission, 2008). For Italy the latter is modulated to 17% (European Commission, 2009).

In the context of climate mitigation, bioenergy crops are expected to play a key role in the 57 renewable energy supply in the EU in the next coming decades (Djomo et al., 2013). Short 58 rotation coppice (SRC) of fast growing trees, and especially of poplar (Populus spp.), is a 59 promising culture in this context. SRC has the potential to reduce GHG emissions to the 60 61 atmosphere during both its production (by capturing CO_2 from the atmosphere and storing it in aboveground biomass and soil) and use (by avoiding CO₂ emissions from fossil fuel 62 burning). However, the management of SRC requires energy inputs, and converting land for 63 64 SRC production (i.e. land use change, LUC) may alter the equilibrium of the existing ecosystems, causing an impact that in some cases can counterbalance the positive effects on 65 climate mitigation of the SRC (Zona et al., 2013; see also Crutzen et al., 2008; Fargione et al., 66 2008 for bioenergy crops in general). The LUC to SRC may imply losses of soil organic 67 carbon (SOC) at the installation (Don et al., 2012), especially in C-rich soils, and the 68 management of SRC requires the use of fossil fuels which in some cases can outweigh part of 69 70 the benefits of the supposed carbon neutral SRC systems (Abbasi and Abbasi, 2009). A recent study (Djomo et al., 2011), however, showed that poplar and willow SRC biomass use can 71 72 save up to 80%-90% of GHG emissions compared to using coal for energy production. Studies on the climate mitigation potential of poplar cultivations constitute an important tool 73 74 in supporting energy and environmental policies at different scales. In recent years researchers 75 approached poplar SRCs from different perspectives: ecological (Jaoudé et al., 2010; Zhou et

al., 2013), economic (Strauss and Grado, 1997; Mitchell et al., 1999; El Kasmioui and 76 Ceulemans, 2012, 2013), energetic and environmental (Jungmeier and Spitzer, 2001; 77 Cherubini et al., 2009; Devis et al., 2009; Nassi o Di Nasso et al., 2010; Arevalo et al., 2011; 78 Don et al., 2012; Dillen et al., 2013; Djomo et al., 2013). However, these studies often used 79 different approaches making it difficult to compare their results (Migliavacca et al., 2009; 80 Djomo et al., 2011). Furthermore, emphasis was mainly given to emissions from fossil fuels 81 82 compared with the biogenic emissions due to the LUC (Djomo et al., 2013). Including the different contributions of the LUC in the assessments of emission savings related to energy 83 crops is crucial (Davis et al., 2009). A full GHG budget based on long-term measurements of 84 CO₂ and non-CO₂ GHGs via eddy covariance (EC) methodology (Aubinet et al., 2012) and 85 soil chambers measurements (Allard et al., 2007), can be used to assess the GHG mitigation 86 potential of land conversion to SRC (Byrne et al., 2007; Ceschia et al., 2010). Several authors 87 88 (e.g. Ceschia et al., 2010; Osborne et al., 2010) highlighted the need for a more consistent number of studies on GHG budgets, including different types of management practices, 89 climate conditions, and soil characteristics, in order to reduce the uncertainty in GHG budgets 90 at large scale (Smith et al., 2010). A GHG budget approach was used by Gelfand et al., 2011 91 in a conversion of unmanaged lands to herbaceous biofuel crops in the US. In Europe, Zona et 92 al., 2013 estimated the GHG balance in the first year after the conversion from agricultural 93 lands to a poplar SRC in Belgium, focusing on biogenic contributions. The present study 94 considered a conversion of a cropland (hereafter indicated as "REF site") to a poplar SRC 95 (hereafter indicated as "SRC site") for bioenergy production in the Mediterranean area 96 (Viterbo, Central Italy). The aim was to extend the GHG balance to emissions generated by 97 field management and to the offset of GHG from fossil fuels substitution. The number of 98 studies on SRC systems cultivated in Mediterranean areas is limited, where water availability 99 can constitute a limiting factor for biomass yield and thus climate mitigation (Cherubini et al., 100

- 101 2009). Given that the Climate Change mitigation potential of energy crops is the main reason
- 102 for subsidies to arable land conversion, our study aimed to assess the suitability of the LUC to
- 103 SRC in terms of mitigation of GHG emissions.

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105 **4. Materials and methods**

106 *4.1 <u>GHG budgets assessment</u>*

The GHG budgets were calculated for the SRC and for the REF sites on a temporal basis of two years (24 months), corresponding to the second rotation cycle of the SRC site. They included several positive and negative GHG contributions, with the following sign convention: a positive flux indicates a release into the atmosphere, while a negative flux represents an uptake from the atmosphere. In both cases the boundary of the system was set to the farm level. For the SRC site, the net GHG budget (B_{SRC}) was calculated as the algebraic sum of all GHG contributions as indicated in Eq. (1):

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$$B_{SRC} = F_{CO2} + F_{CH4} + F_{N2O} + F_{MAN} + F_{SOC} + F_{SAV} + F_{EXP}$$
(1)

In equation (1), F_{CO2} represents the flux of CO₂, i.e. the net ecosystem exchange (NEE) of CO₂, while F_{CH4} and F_{N2O} represent the biogenic methane and nitrous oxide soil-atmosphere exchanges. F_{MAN} includes the GHG emissions related to the management of the SRC site, and F_{SOC} is the loss of soil organic carbon content due to the installation of the cuttings. F_{SAV} represents the GHG offsets, i.e. avoided GHG emissions due to the substitution of natural gas by biomass in the heat production, and F_{EXP} the biomass exported from the site at the end of the cycle and reemitted as CO₂ at burning.

Similarly, the net GHG budget of the REF site (B_{REF}) was estimated with the algebraic sum indicated in Eq. (2), where in respect to Eq. (1) there is not F_{SOC} and F_{SAV} , and F_{EXP} is the portion of the exported biomass that returns to the atmosphere as CO₂ or CH₄:

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$$B_{REF} = F_{CO2} + F_{CH4} + F_{N2O} + F_{MAN} + F_{EXP}$$
(2)

All the contributions of B_{SRC} and B_{REF} were expressed as CO₂-equivalent (CO₂eq) fluxes per unit of surface, as the functional unit of the study was one square meter of land. Finally, the net GHG cost or benefit of converting the cropland to a SRC plantation was calculated by 129 comparing B_{SRC} and B_{REF} . Displacement of food and feed production related to SRC 130 cultivation on cropland was beyond the scope of this study.

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132 *4.2 <u>Site description</u>*

Two sites close to each other located in a private farm (Gisella ed Elena Ascenzi S.A.A.S.) in 133 Castel d'Asso, Viterbo, Italy (coordinates: 42°22' N, 12°01' E), were selected during the 134 summer 2011. Two EC towers were installed in the two sites to measure the exchanges of 135 CO₂ and H₂O between the ecosystem and the atmosphere following the methodology reported 136 137 in Aubinet et al., 2000. The climate of the area is Mediterranean, with a yearly average rainfall of 766 mm, mean temperature of 13.76 °C and weak summer aridity in July-August 138 (Blasi, 1993). The SRC site was a 2-year rotation cycle managed poplar plantation of 11 ha 139 140 planted in 2010 to produce biomass for energy (heat). Poplar cultivar was *Populus x* canadensis - clone AF2 selected in Alasia Franco Vivai's nurseries. According to the 141 regional law (Rural Development Programme of Latium 2007-2013, Latium Region, 2015) 12 142 years is the maximum period to get subsidies for SRC, and corresponded to the time the 143 farmer decided to cultivate the SRC site (personal communication). For that reason the 144 145 calculations of the present study will be referred to a 12-years lifespan. The site was previously managed with a 2-year rotation between a clover grassland (Trifolium incarnatum 146 L.) in mixture with ryegrass (Lolium multiflorum Lam) and winter wheat (Triticum aestivum 147 L. emend. Fiori et Paol). The REF site was a 9 ha grassland-winter wheat rotation located at a 148 149 short distance (300 m). Having the identical land use and management of the SRC site before the installation of the poplars, it was selected to assess the GHG effects of the LUC. GHG 150 151 balances were calculated over 24 months in both sites. However, these 24-month periods did not completely overlap, as the two cultivations had different beginning times: for the SRC site 152 the GHG budget estimation went from 12 January 2012 (immediately after the first harvest of 153

the SRC site) to 11 January 2014, corresponding to the second cycle of cultivation. The 154 period of calculation of the GHG budget for the REF site went instead from 1 September 155 2011 until 31 August 2013. However, manual chamber measurements of CH₄ and N₂O in the 156 REF site started at the beginning of April 2012. The 24 months considered for the SRC site 157 corresponded to the second cycle of the short rotation coppice, and thus did not include the 158 period right after the conversion of agricultural land. This rotation was supposed to terminate 159 160 with the harvest. However, due to unfavourable climate conditions (a strong drought during summer), the harvest of the SRC site planned for 2014 was postponed to 2015. 161

The SRC site had a planting density of around 5300 cuttings per hectare, that were planted in 162 163 rows spaced 2.5 m at a distance of 0.75 m between each other. The first harvest occurred in January 2012. The SRC site was irrigated during the driest periods in summer using a system 164 of tubes installed 35 cm belowground on alternate inter-rows, summing up to about 210 mm 165 166 in 2012 and 80 mm in 2013 of equivalent precipitation added to the soil. No fertiliser was provided to the SRC site in 2012, while 40 kg per hectare of urea were dissolved in the 167 irrigation water in a single event in 2013. Insecticide (DECIS) was used in May 2012 against 168 Chrysomela populi L. In the REF site a shallow tillage (15 cm) was performed in September 169 2011 with a rotary harrow, and clover and ryegrass were sown. At the end of April 2012 half 170 of the crop was converted to sorghum (Sorghum vulgare Pers.) after a period of aridity in 171 spring time. Both the clover and the sorghum were grazed during the growing season, with 172 grazing removing all the above-ground biomass from the sorghum, while the clover was 173 harvested at the end of the cycle. At the end of October 2012 the land was tilled at 40 cm 174 depth, and winter wheat was sown in November. In April 2013 herbicide was distributed over 175 wheat (Buctril at a rate of 1 l ha⁻¹), which was harvested at the beginning of July 2013 and no 176 177 other operation was performed until the end of August. Sorghum was irrigated in several days in summer using a sprinkler with a total amount of 275 mm of equivalent precipitation, while 178

no irrigation was applied to the winter wheat. Sorghum was also fertilised twice with
150 kg ha⁻¹ of ammonium nitrate, while 200 kg ha⁻¹ of the same fertiliser were provided once
to the wheat. Apart from irrigation and fertigation, all the operations described above were
performed using two different types of tractors, generating different diesel consumptions
associated to each operation (Table 3).

An older SRC site (indicated hereafter as O_SRC site) located alongside the other one and subjected to the same type of management, but planted in 2007, was used in the estimation of SOC content loss caused by the LUC. This was necessary as the expected SOC loss following the conversion (i.e. during the first rotation) was not measured.

In the 24 months considered for the GHG budget of the SRC site, precipitations summed up 188 to 1078 mm, with an average temperature of 14.72 °C, while in the 24 months used for the 189 REF site precipitations were 1157 mm and average temperatures 15.31 °C. In both cases 190 191 yearly values of precipitation were lower than the long-term average of 766 mm (Blasi, 1993). An intense drought occurred in summer 2012, with no rain from the beginning of June until 192 193 the end of August, in contrast to the long-period average of cumulate rainfall in these months (110 mm, Blasi, 1993). Soils were classified as Chromic Luvisol according to the World 194 Reference Base classification (USS 2014), with a clay-loam texture. Values of pH ranged 195 between 5.88 in the REF site, 6.66 in the O SRC site and 6.69 in the SRC site. The stock of 196 197 nitrogen (N) up to 70 cm was not significantly different between sites, ranging from 3.16 ± 1.60 MgN ha⁻¹ to 3.19 ± 1.47 MgN ha⁻¹ and 3.25 ± 1.47 MgN ha⁻¹ respectively for 198 SRC, O SRC and REF sites. See Fig. 1 for a schematic representation of land cover and 199 management events of the two sites. 200

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4.3 <u>F_{CO2}: eddy covariance measurements</u>

The EC technique was used to determine the turbulent vertical fluxes of momentum, CO₂, 203 latent and sensible heat. A 3-D sonic anemometer was installed in each site for high-204 frequency measurements of wind speed, wind direction and sonic temperature. CO2 and water 205 vapour densities were collected using a fast-response open-path infrared gas analyser (see 206 Table 1 for models and manufacturers). These instruments were mounted on towers located in 207 the centre of the fetches. On the REF site the mast was 3 m high, while an extendible 208 209 telescopic pole was used in the SRC site in order to always measure turbulences above the roughness layer (Foken, 2008). Measurement heights ranged between 5 and 8 m, and the 210 distances of the measuring system over the $d + z_0$ plane between 2 and 5 m (d = displacement 211 height; z_0 = roughness length). Several meteorological variables above and belowground were 212 continuously measured on a 30 min basis to properly calculate fluxes and characterise the two 213 sites. In Table 1 the complete instruments setup is described, including both meteorological 214 215 and high-frequency variables.

Half-hourly fluxes were calculated with EddyPro® software (LI-COR, Lincoln, NE, USA). Several corrections to the time series (Aubinet et al., 2012) were applied as reported in Table 2. Post-processing included spike removal and friction velocity (u_*) filtering (Papale et al., 2006), gap-filling using the marginal distribution sampling (MDS) approach and partitioning of F_{CO2} into gross primary production (GPP) and ecosystem respiration (R_{eco}) components (Reichstein et al., 2005). The gap-filled F_{CO2} and its components were then cumulated along the 24-month period considered.

Uncertainty in F_{CO2} was calculated on the basis of the uncertainty in the u_* filtering, assuming that the main potential systematic error is due to advection and thus linked to the u_* filtering. One hundred thresholds were calculated using a bootstrapping technique and then applied to filter the data. For each half-hour, the median of the distribution of F_{CO2} obtained using the 227 100 thresholds was used for the GHG budget (Gielen et al., 2013), and the range of 228 uncertainty was derived as half the range $16^{\text{th}}-84^{\text{th}}$ percentile.

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4.4 Soil characteristics and SOC stock and changes

To better characterize the soil properties and to quantify the changes in SOC stocks due to the 231 installation of the poplar plantation, a number of soil analyses were performed in the three 232 sites in two different periods. In a first phase on February 2012 three soil trenches 150 cm 233 wide were opened randomly in each site and the soil sampled by depth (0-5, 5-15, 13-30, 30-234 50, 50-70, 70-100 cm) at both the opposite sides of the profiles to have six replicate samples 235 per depth. The bottom layer (70 cm - 100 cm) was absent in the REF site due to the presence 236 of the bedrock at 80 cm, rather than 100 cm as in both the SRC sites. Samples were collected 237 238 using a cylinder to determine also the bulk density. Main goals of this first sampling campaign were to describe the soil characteristics and to determine the number of replicates 239 necessary to detect with statistical significance a change in SOC content of 0.5 gC kg⁻¹ soil 240 (Conen et al., 2003). In the SRC and O SRC sites ten samples of the organic layer were also 241 taken removing all the material present over the mineral surface within a squared frame with 242 an area of 361 cm². In the REF site this sampling was not performed because a permanent 243 organic layer was missing. All samples were air-dried at room temperature and then sieved at 244 2 mm to separate the coarse fraction, and the analyses performed on the fine earth. The pH 245 was measured in deionised water with a sure-flow electrode, using a ratio soil-solution of 246 1:2.5 (w/w), and texture was determined after destruction of the cement using sodium 247 hypochlorite adjusted at pH 9 (Mikutta et al., 2005). The sand fraction was separated by wet 248 249 sieving at 53 µm while the silt and the clay fractions were separated by time sedimentation according to the Stokes law. Total carbon (C) and nitrogen concentrations were measured on 250 finely ground samples by dry combustion (ThermoFinnigan Flash EA112 CHN), while SOC 251

and N stocks were determined taking into account soil C and N concentrations and a weighed 252 253 mean of bulk density, depth of sampling and stoniness (Boone et al., 1999). During the second phase in March 2014 a new sampling was performed in the REF, SRC and O SRC 254 sites. The number of samples necessary to detect statistically a SOC change was 50, as 255 derived from the first phase. Samples were taken from the first 15 cm of soil, as most of the 256 changes in a short period occur in the shallower layers. C concentration was measured and 257 258 SOC stocks re-calculated. The normality of the distributions was checked using a Chi-squared test (Pearson, 1900). An ANOVA test (Fisher, 1919), combined with a Tukey multiple 259 comparison test were used to check if SOC stocks were different between the sites. As data of 260 F_{CO2} from the beginning of the cultivation are missing, SOC changes due to the installation of 261 the poplar cuttings were calculated building a linear regression between SOC content of the 262 SRC site (4 years old) and the O SRC site (7 years old), then estimating the SOC at the time 263 264 of plantation (year "0"). Following the "free-intercept model" described by Anderson-Teixeira et al., 2009, the SOC content change due to the plantation of the SRC was then 265 extrapolated considering the difference between the SOC content at year 0 and the one 266 measured in the REF site, assuming the SOC content in the REF site in equilibrium, as this 267 type of land use was constant in the last 30 years. Uncertainties in SOC concentration and 268 stock were calculated as standard deviations from the mean values of each repeated measure, 269 while errors were estimated using the law of error propagation as reported by Goodman, 270 1960. 271

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4.5 Soil CH₄ and N₂O fluxes

On-site measurements of CH_4 and N_2O soil fluxes were combined with laboratory incubation analyses, where soil samples were tested at different water contents and N addition levels. Field measurements of soil N_2O and CH_4 fluxes were carried out in the two sites using nine

manual, dark, static PVC chambers (15 cm diameter, 20 cm height, and total volume 277 0.0039 m³) per site, placed over as many PVC collars (7 cm height, 15 cm diameter) 278 permanently inserted into the soil at 5 cm depth for the period of observation. At the SRC site, 279 three collars were distributed along the tree line (between two trees), three along the irrigated 280 inter-rows and three along the non-irrigated inter-rows, while in the REF site collars were 281 placed in three different blocks of three collars each. Gas samples were collected from each 282 chamber at the closure time, and 30 and 60 minutes after closure. Samples were stored in 283 glass vials provided with butyl rubber air tight septum (20 ml) and concentration of N₂O and 284 CH₄ was measured using a trace Ultra gas chromatograph (GC) (Thermo Scientific, Rodano, 285 286 IT). The flux detection limit due to the concentration measurement was in the order of about 0.1 mg of CH₄ or N₂O m⁻² day⁻¹, and the analytical precision of the GC for standards at 287 ambient concentration was approximately 3-5 %, using one standard deviation as a measure 288 289 of mean error. Further details on GC set in Castaldi et al., 2013. Measurements started two weeks after collar insertion and samples were collected every 2-4 weeks, depending on land 290 291 management practices and weather conditions, for a total of 30 dates in the SRC site and 24 for the REF site. Similar frequencies were used in previous studies (e.g. Pihlatie et al., 2007; 292 Weslien et al., 2009), and considered pertinent on the basis of the low magnitude of the 293 measured fluxes. To test if fertilisation could trigger a peak of N₂O emission as found in 294 previous studies (e.g. Gauder et al., 2012), measurements in both sites were carried out more 295 frequently in occasion of fertilisation events (on average every two days), starting from the 296 day before the application of fertiliser and for a week. Measurements also covered different 297 soil and meteorological conditions, including periods of drought and rewetting. Measured 298 average daily soil CH₄ and N₂O fluxes were cumulated over the 24 months by linear 299 interpolation as described by Marble et al., 2013, and uncertainty calculated propagating the 300 standard deviations of the replicates. Intergovernmental Panel on Climate Changes (IPCC) 301

302 100-year global warming potential (GWP) weighed estimates of GHGs (Forster et al., 2007) 303 were used to convert F_{N2O} and F_{CH4} into CO₂ equivalents: factors 298 and 25 respectively.

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4.5.1 Laboratory incubations

Due to the fact that we don't have continuous measurements of non-CO₂ fluxes from soil, we 306 performed a laboratory analysis to verify the accuracy of field campaigns. Laboratory 307 incubations were carried out to assess the GHG emission rates under controlled laboratory 308 conditions in soil treated with both water and nitrogen addition, and to quantify the rates of 309 soil mineralization and nitrification. The rational of the incubation was to assess whether the 310 fluxes were driven by limiting conditions like water and/or nitrogen, or slow rate of organic N 311 mineralization, as found in a Mediterranean coppice site in the same region (Castaldi et al., 312 2009; Gundersen et al., 2012). Addition of N allowed to check if short-time peaks of 313 emissions occurred that could escape due to the selected frequency of sampling. Soil cores (7 314 315 cm diameter, 10 cm height) sampled in the two ecosystems were incubated at 20 °C. Water was then added to reach three different ranges of Water Filled Pore Space (WFPS%): 20% 316 (i.e. the value estimated at sampling), 50% and 90%, each of them replicated five times. The 317 sample at the highest WFPS% was also replicated with or without nitrogen supply 318 (100 kgN ha⁻¹ of NH₄NO₃). Cores were placed in gas-tight 1-litre jars and 6 ml air samples 319 were collected immediately after closure and after 3 hours of incubation for N₂O production 320 determination. Gas concentration was determined by gas chromatography the day after the 321 treatment and in the following 5 days, leaving the jars open during this period and closing 322 323 them only when N₂O production needed to be determined, in order to avoid developing of liquid oxygen tension conditions. Net mineralization and nitrification, and net potential 324 nitrification rate were determined on sieved (2 mm mesh) soil samples over 14 days of 325 326 incubation, while for the determination of potential nitrification soil was amended with

ammonium sulphate (NH₄)₂SO₄ (100 µgN g⁻¹ dry soil). A modified method (Kandeler, 1996; 327 Castaldi and Aragosa, 2002) was used to extract NH_4^+ and NO_3^- from the soil at T_0 and T_{14} 328 days for further concentration determination with calibrated specific electrodes after the 329 addition of a pH and ionic buffer 0.4 ml di ISA (Ionic Strength Adjustor; Orion cat. No. 330 951211 e Orion cat No. 930711). Mineralization rates were calculated as the total soil mineral 331 N (μ g of N-NH4⁺ + N-NO3⁻ per gram of dry soil) measured after 14 days of incubation (T₁₄) 332 minus total mineral N measured at incubation start (T_0) divided by the number of days of 333 incubation. Nitrification rates were calculated similarly, considering only the amount of N-334 NO3⁻ produced at T_{14} minus the amount of N-NO3⁻ present at T_0 . 335

In order to compare results obtained with soil cores to field conditions, in situ *WFPS%* wascalculated for the whole period of field monitoring:

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$$WFPS\% = \frac{M_{SOIL}}{1 - (\rho_{BULK}/\rho_{PART})} * 100$$
 (3)

where M_{SOIL} is the volumetric soil moisture (m³ m⁻³), ρ_{BULK} is the bulk density (Mg m⁻³) and ρ_{PART} is the particle density (Mg m⁻³). For mineral soil ρ_{PART} is approximated to that of common silicate materials (2.65 Mg m⁻³, Chesworth, 2008).

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4.6 Emissions due to management

Life cycle inventory (LCI) was used to estimate the anthropogenic GHG emissions due to farming operations (Robertson et al., 2000) in both sites (Table 3), and the GHG emissions due to grazing in the REF site (Table 4). The present study is not a full LCA, but the LCA approach was used to estimate emissions caused by field management as described in the following. In particular, indirect land use change (iLUC) was not taken into account. iLUC includes modifications in land use elsewhere in the world triggered by the local substitution of an arable land with an energy crop (Djomo et al., 2013). iLUC occurred outside the boundary

of the system we used for this analysis, i.e. the farm. Fossil fuel emissions associated with the 351 cultivation of the SRC and REF sites included on-site emissions from tractors (used to carry 352 out all the main operations: ploughing, seeding, solid fertilisation, harvesting) and irrigation, 353 354 as well off-site emissions from the production and transport of agricultural inputs (fertiliser, insecticide, herbicide). Emissions due to the production of tractors were considered negligible 355 as in Budsberg et al., 2012 and Caputo et al., 2014. On-site GHG emissions due to diesel 356 consumption were calculated as the product of the amount of fuel diesel consumed to carry 357 out a given farm activity (e.g. harvesting) and the emissions factor of diesel, 90 gCO₂eq MJ⁻¹ 358 (Table 3). This factor includes emission costs due to the combustion of diesel 359 (74 gCO₂eq MJ⁻¹), and emissions due to its production and transportation (16 gCO₂eq MJ⁻¹) 360 (Edwards et al., 2007). Considering energy density of diesel to be 38.6 MJ l⁻¹ (Alternative 361 Fuel Data Center, 2014), producing, transporting and burning 11 of diesel emitted 362 363 3474 gCO₂eq. An exception was made for harvesting in the SRC site, for which emissions for diesel consumption relative to the previous harvest (2012) were considered, as the harvest at 364 365 the end of the cycle was postponed. Emissions due to irrigation were calculated by multiplying the electricity consumed in powering the pumps by an emissions factor of 366 750 gCO₂ kWh⁻¹, calculated as the average of different emission factors for different sources 367 of electricity (Bechis and Marangon, 2011) weighted on the Italian electricity grid mix, 368 derived from the Italian energetic balance 2012 (Italian Ministry of Interior, 2013). Off-site 369 emission costs for fertilisers and insecticides were estimated as the product of applied amount 370 of fertiliser or insecticide and the emission factors for manufacturing 1 kg of 371 fertiliser/insecticide: 4018.9 gCO₂ kg⁻¹ N for urea (NPK rating 40-0-0), 4812 gCO₂ kg⁻¹ N for 372 diammonium phosphate (NPK 18-46-0)¹, 7030.8 gCO₂ kg⁻¹ N for ammonium nitrate (NPK 373 33-0-0) and 7481.9 gCO₂ kg⁻¹ N for calcium ammonium nitrate (NPK 27-0-0) (Wood and 374

¹ This includes production and transport costs of the overall fertiliser, including P.

Cowie, 2004). Although emission factors differ among insecticide types, in this analysis we 375 assumed that the difference is negligible as the use of insecticides was limited, and thus 376 considered the emission factor of insecticide (active ingredient: deltamethrin) as the product 377 of energy required to produce 1 kg of insecticide (310 MJ kg⁻¹) and the emission rate of 378 insecticide (60 gCO₂ MJ⁻¹) (Barber, 2004; Liu et al., 2010). The emission factor of herbicide 379 was taken from literature (Ceschia et al., 2010): 3.92 kgC per kg of product. Fuel used for the 380 application of chemical products was included in the on-site calculations described above. All 381 the contributions listed above were converted on a surface basis (Table 3). 382

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4.7 <u>Biomass use and GHG offset</u>

During the first year of cultivation the REF site was grazed by sheep, which were brought to the field in defined periods (Table 4). Hence, the aboveground biomass (AGB) from the REF site was rather grazed by sheep or provided as hay to other livestock, destined to meat and milk production, or in the case of wheat used in food (grains) and feed (foliage) production. Due to the different species cultivated throughout the two years and to the different uses of the biomass, F_{EXP} of the REF site (Eq. (2)) includes the following:

$$F_{EXP} = E_{CH4,on} + E_{CO2,on} + E_{CH4,off} + E_{CO2,off}$$
(4)

where the first subscript indicates whether the exported C is reemitted to the atmosphere as 392 CO_2 or CH_4 , and the second subscript distinguishes between emissions occurring on-site (*on*) 393 and off-site (off). In fact, the percentage of AGB ingested by herbivores on grassland varies 394 with the intensity of management (Soussana et al., 2010). In the present study, however, what 395 was left in the field by the sheep was then harvested and provided them off-site. We assumed 396 then that, apart from the grains in wheat ears, all the AGB was ingested by sheep or other 397 livestock, and that the digestible portion of the organic C ingested was respired back to the 398 atmosphere as CO₂ or emitted as CH₄ via enteric fermentation (Eq. (4)) (Soussana et al., 399

2007). Biomass in the REF site was sampled every 2-3 weeks in five plots (0.5 m x 0.5 m) 400 randomly selected within the field. In three dates samples were collected immediately after 401 grazing in a grazed area and in an undisturbed area to quantify the intensity of mowing (68%) 402 and identify the C ingested on-site and off-site. Biomass samples were oven-dried at 70 °C to 403 constant mass and weighed. Total AGB was obtained cumulating dry weights measured 404 immediately before each grazing event, subtracting each time the 32% of the dry weight of 405 406 the previous sample to consider mowing intensity. IPCC methodology (Dong et al., 2006) was then used to estimate $E_{CH4,on}$ (Eq. (4)), adjusting the methane emission factor per animal 407 considering the average weight (55 kg) of sheep (19 gCH₄ head⁻¹ day⁻¹), and multiplying it by 408 409 the daily number of sheep present on-site. The method in Soussana et al., 2007 (their Eq. (4)) was then adapted to estimate the other three components in Eq. (4): $E_{CH4,off}$ was estimated 410 applying to the C ingested off-site the ratio between the C weight in $E_{CH4,on}$ and the C ingested 411 412 on-site. The C emitted as CH₄ was subtracted from the digestible portion of the C ingested, assumed to be 65%, and the remaining converted in CO_2 as to estimate $E_{CO2,on}$ and $E_{CO2,off}$. 413 414 The remaining, non-digestible C (35%) was assumed to be returned to the SOC of the grassland (for the on-site part) or of other systems (for the off-site part) as faeces, thus not 415 contributing to the GHG balance. The portion of C that was stock in the body mass of animals 416 417 was considered negligible (Soussana et al., 2007). For the sake of simplicity, we assumed that also the C content of wheat ears will be shortly respired back to the atmosphere as CO₂, and 418 thus included in $E_{CO2,off}$ (Eq. (4)). 419

420 At the end of the cycle, poplar aboveground woody biomass (AGWB) of the SRC site was 421 supposed to be harvested and burnt, thus from one side releasing C back to the atmosphere, 422 and from the other offsetting GHG emissions for fossil fuels displacement. To estimate poplar 423 AGWB diameters were measured at the end of the cycle, after the leaves fall. Three rows of 424 trees were selected inside the plantation and the diameters of these trees were measured 425 (minimum threshold 0.5 cm) at 1 m height. A simple model considering the regression 426 between individual shoot dry weight (W_D) and 1 m diameter (D) was used:

 $W_D = b * D^c \tag{5}$

where b and c are empirical parameters, W_D is in kg DM (kg of Dry Mass), and D is in cm. 428 Parameters were set as b = 0.0847 and c = 2.112 following Mareschi, 2008 (see also Paris et 429 al., 2011) for the second rotation cycle of clone AF2 of the plantation located in Bigarello 430 (Mantua province), as the one with the more similar climatic and soil characteristics, and also 431 with the same root and shoot age. Dry combustion (1108EA, Carlo Erba, Milan, IT) was used 432 to determine the C concentration for both sites. Regarding the GHG emissions offset, it was 433 434 assumed that heat produced from SRC biomass will substitute heat produced from natural gas. The GHG offset (F_{SAV}) was estimated based on the yield of the SRC site, the energy density 435 of poplar, the conversion efficiency of typical biomass boiler in Italy, and the emission rate of 436 heat production from natural gas in Italy: 437

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$$F_{SAV} = Y * H_L * \eta_{CONV} * I_{NG} \tag{6}$$

where *Y* is the biomass yield (kg m⁻²), H_L is the low heating value of poplar (13 MJ kg⁻¹ at 30% moisture content, Boundy et al., 2011), η_{CONV} is the efficiency of conversion of poplar chips to heat, assumed in this study to be 84% (Saidur et al., 2011), and I_{NG} is the carbon emission rate (intensity) of heat produced from natural gas (i.e. 55.862 gCO₂eq MJ⁻¹) for Italy (Romano et al., 2014).

444 **5. Results**

445 5.1 <u>Biogenic fluxes of CO₂</u>

The cumulative F_{CO2} in the REF site for the two years considered was -1838 ± 107 gCO₂ m⁻², partitioned in 8032 ± 313 gCO₂ m⁻² absorbed through photosynthesis (GPP) and 6216 ± 338 gCO₂ m⁻² emitted by total R_{eco}. In the SRC site cumulative F_{CO2} summed up to -3512 ± 224 gCO₂ m⁻², with GPP equal to 8717 ± 298 gCO₂ m⁻² and R_{eco} equal to 5205 ± 425 gCO₂ m⁻² (Fig. 2). Hence, the SRC site was a larger CO₂ sink compared to the REF site over the measuring period, due to both the higher GPP and the lower ecosystem respiration of the SRC site relative to the REF site.

Seasonal differences between the sites in the net flux of CO_2 were observed (Fig. 3). The 453 main difference was the timing of the peak of CO₂ uptake, during the spring in the REF site 454 and in summer for the SRC site. In both sites, peaks of CO₂ uptake were higher in 2013 than 455 in 2012. In the latter, however, a minor peak of uptake were observed in early fall in the SRC 456 site. Periods with positive net fluxes of CO₂ appeared longer and with higher values in the 457 REF site (Fig. 3, top). Air temperatures registered in the two sites were similar, but higher in 458 summer 2012, while the SWC at 30 cm depth was higher in the REF than in the SRC site 459 (Fig. 3, bottom). 460

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5.2 Soil CH_4 and N_2O fluxes

Daily average of both F_{N2O} and F_{CH4} were very low in almost every measurement (Fig. 4), leading to low total cumulative soil F_{N2O} and F_{CH4} for both the sites: overall soil non-CO₂ fluxes were 15.5 ± 4.7 gCO₂eq m⁻² in two years for the SRC site and 0.5 ± 1.6 gCO₂eq m⁻² in two years for the REF site. Both sites were small sources of N₂O and small sinks of CH₄. CH₄ sink at the SRC site was not significantly different from the one at the REF site, although on average slightly higher, and significantly higher N_2O emissions were observed at the SRC site, although still very low. Measurements carried out in occasion of fertilisation events showed no significant increase in the emission rates of N_2O in respect to non-fertilisation periods: fluxes in the SRC site in the period of the unique fertilisation occurred in the two years of study remained low, and in the REF site none of the four measurements taken in the period of the fertilisation event of June 2012 exceeded the detection limit of the GC.

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5.2.1 Laboratory incubations

N₂O emissions determined in laboratory incubations confirmed that over most of the analysed 476 WFPS% values both soils were producing little N₂O in absence of N addition, even at 477 WFPS% normally considered to trigger N₂O emission (WFPS% 60-80%) (Fig. 5). Addition 478 of N did not seem sufficient to stimulate N₂O production. In contrast, very high WFPS%, 479 close to saturation, was able to trigger a strong increase of N₂O production in the soil of the 480 REF site. Comparing the data reported in Fig. 5 with the field data of WFPS% for the REF 481 site (Fig. 6), it can be seen that most of the time WFPS% was significantly below 70% in the 482 whole profile and that at 5 cm, where most of the interaction with added fertilizer might have 483 occurred, the WFPS% never exceeded 50%. Mineralization and nitrification rates were quite 484 low in both sites, with slightly positive mineralization rates in the SRC site 485 $(0.28 \pm 0.05 \,\mu\text{gN g}^{-1} \,\text{d}^{-1})$ and a very small net immobilization in the REF samples 486 $(-0.2 \pm 0.2 \mu gN g^{-1} d^{-1})$. Net nitrification rates calculated in the control (no N addition) were 487 also quite low and varied between $0.5 \pm 0.05 \ \mu g N g^{-1} d^{-1}$ and $-0.1 \pm 0.2 \ \mu g N g^{-1} d^{-1}$ in the REF 488 site, that might suggest either a quite slow ammonification phase as a limiting step of the 489 490 nitrification or a slow nitrification rate. However, when ammonium sulphate was added to soil samples the potential nitrification rates significantly increased reaching $1.8 \pm 0.1 \ \mu g N \ g^{-1} \ d^{-1}$ 491 and $1.4 \pm 0.3 \,\mu gN g^{-1} d^{-1}$ in the SRC and the REF sites respectively, suggesting that 492

493 mineralization might be the limiting step of subsequent nitrification and denitrification494 processes in the field.

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5.3 Emissions due to management

The GHG emissions due to management practices were in total 100.9 ± 20 gCO₂eq m⁻² for the 497 SRC site and 135.7 ± 27.1 gCO₂eg m⁻² for the REF site. Analysing the single contributions, 498 differences arose between the two sites (Fig. 7): fertilisation was the main source of emission 499 of GHGs in the REF site, while its contribution to GHG emissions of the SRC site was 500 limited. Irrigation constituted a big portion of the GHG emissions from management 501 operations in the SRC site, while in the REF site, despite similar amounts of water provided, 502 irrigation played a smaller role, similar to harvesting and tillage. Emissions due to the latter 503 were more relevant in the REF site than in the SRC site. 504

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506 5.4 <u>SOC content changes</u>

In the first 15 cm of soil total C stocks were 1603 ± 376 gC m⁻² in the REF site, 1169 ± 442 gC m⁻² in the SRC site and 1403 ± 279 MgC ha⁻¹ in the O_SRC site. The statistical analysis performed on the SOC stocks showed that there were statistically significant differences between SOC data of the three sites (Table 5; p-value = $2.05*10^{-7}$). The linear regression between SOC content of SRC and O_SRC sites led to the relation:

512
$$SOC(t) = 78 * t + 857$$
 (7)

where *t* are the years from plantation and *SOC* is the soil organic carbon content expressed in gC m⁻². Estimated uncertainty was 25 gC m⁻² for the slope value, and 139 gC m⁻² for the intercept (Fig. 8), meaning that the yearly SOC accumulation after poplar plantation was 78 ± 25 gC m⁻² and the initial value (t=0) was 857 ± 139 gC m⁻², 746 ± 858 gC m⁻² lower than the REF value, corresponding to the SOC content loss due to the installation of the SRC. As this loss was a positive flux occurring only once in a LUC at the installation of the cuttings (Arevalo et al., 2011), and that the expected lifespan of the SRC site was 12 years, the value considered for the 24-month GHG budget was 1/6, corresponding to 124 ± 143 gC m⁻² (455 ± 524 gCO₂ m⁻²).

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5.5 Biomass use and GHG offset

The dry weight of AGB in the REF site summed up to 0.72 ± 0.18 kg m⁻² for the grassland, of 524 which 0.35 ± 0.07 kg m⁻² due to the clover in mixture and 0.37 ± 0.17 kg m⁻² from the 525 sorghum, while the winter wheat totalled 0.63 ± 0.09 kg m⁻², of which 0.36 ± 0.05 kg m⁻² in 526 the ears. The C content measured was 46% for all species, leading to a total of 527 $621.0 \pm 93.2 \text{ gCm}^{-2}$ in AGB, of which $265.5 \pm 79.2 \text{ gCm}^{-2}$ ingested by sheep on-site, 528 191.2 ± 49.8 gC m⁻² used by livestock off-site, and 163.9 ± 21.9 gC m⁻² converted to food. 529 The estimated emissions of CH₄ due to enteric fermentation was 4.3 ± 1.3 gCH₄ m⁻², equal to 530 3.3 ± 1.0 gC m⁻² emitted as CH₄, and thus corresponding to 109 ± 33 gCO₂eq m⁻² ($E_{CH4.on}$, Eq. 531 (4)). Hence, about 1.25% of the ingested C became CH_4 in the digestive process. Using this 532 ratio led to estimate other 2.4 \pm 0.6 gC m⁻² emitted as CH₄ off-site, i.e. 3.2 \pm 0.8 gCH₄ m⁻², or 533 80 ± 20 gCO₂eq m⁻² (*E_{CH4.off}*). Subtracting the C emitted as CH₄ on- and off-site to the 534 respective digestible C ingested by sheep and other livestock led to 621 ± 189 gCO₂eq m⁻² 535 emitted on-site ($E_{CO2 on}$) and 447 ± 118 gCO₂eq m⁻² offsite. Adding to this latter the emissions 536 expected from wheat ears use (i.e. $601 \pm 80 \text{ gCO}_2\text{eq m}^{-2}$) gave $1048 \pm 143 \text{ gCO}_2\text{eq m}^{-2}$ 537 $(E_{CO2 \text{ off}})$: in total 1858 ± 240 gCO₂eq m⁻² in two years (F_{EXP} , Eq.(4)). 538

For the SRC site, applying Eq. (5) with the diameters distribution led to estimate AGWB (dry matter) in 0.62 ± 0.29 kg m⁻², which with a C content of 49%, corresponded to a F_{EXP} of 541 1118 ± 521 gCO₂eq m⁻² per two years that are expected to be reemitted to the atmosphere at 542 the combustion. This value of AGWB then corresponded to 8.1 ± 3.7 MJ m⁻² of gross energy 543 from biomass chips, which decreased to 6.8 ± 3.1 MJ m⁻² of final heat obtainable from 544 burning biomass chips when the conversion efficiency is considered. This could offset about 545 379.7 ± 175.1 gCO₂eq m⁻² from final heat produced using natural gas.

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547 *5.6 <u>GHG budgets</u>*

All the contributions reported in the previous sections were summed up to calculate the GHG 548 budgets of the two sites. The net GHG budget of the REF site (B_{REF} , Eq. (2)) amounted to 549 156 ± 264 gCO₂eq m⁻², indicating that the REF site was close to neutrality from a GHG 550 perspective, while for the SRC site the B_{SRC} (Eq. (1)) resulted in a cumulative sequestration 551 of -2202 ± 792 gCO₂eq m⁻². The different components of the GHG budget of the two sites are 552 summarized in Fig. 9. In the REF site the F_{CO2} , weighing about 48% in the GHG budget, was 553 completely compensated by the emissions of CO₂ and CH₄ due to the biomass utilisation 554 (about 44% and 5% respectively), while the other components had a minor role (F_{MAN} around 555 4%, soil non-CO₂ <1%). F_{CO2} was the main contribution also in the SRC site, where it 556 represented the 63% of B_{SRC} , while F_{EXP} represented the 20%, SOC loss (8%) and the GHG 557 offset for the fossil fuel substitution (7%) had a similar weight, and the other contributions 558 played a minor role. As B_{REF} was almost neutral and the SRC site a sink of GHGs, the 559 difference between the two GHG budgets was favourable to the SRC site 560 $(2358 \pm 835 \text{ gCO}_2\text{eq m}^{-2} \text{ saved})$, highlighting the advantages in terms of GHGs of the LUC 561 from common agricultural to SRC of poplar in the study area. 562

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The two ecosystems behaved differently in the measuring period: they were both 565 characterised by a seasonal uptake of CO₂ (Fig. 3), driven by the timing and duration of the 566 growing season, occurring in spring at the REF site and in summer at the SRC site. The peak 567 of CO₂ uptake was similar at both sites in 2012, while it was higher at the REF site in 2013. 568 Periods with positive CO₂ fluxes were longer in the REF site, and often higher in magnitude, 569 likely as a consequence of the shorter growing season of grasses and winter wheat compared 570 to the poplars trees of the SRC site. Also the land cover of the two sites during the dormant 571 periods and the shift in time between them might have played a role on this difference: some 572 herbaceous vegetation kept growing in the SRC site in wintertime, while harvesting and 573 ploughing in the REF site in late summer/early fall might have enhanced ecosystem 574 respiration. Inter-annual differences were also observed at both sites. Both the higher air 575 temperature and the more extended period of low SWC proved the strong aridity of summer 576 2012, responsible for the autumnal increase of CO₂ uptake at the SRC site, corresponding to 577 the rewetting of the soil. At the REF site, autumn uptake was higher in 2011, while the 578 springtime uptake was much higher in 2013 than in 2012 (Fig. 3). This different behaviour 579 was mostly ascribable to the different cultivations (grassland - winter wheat), and to some 580 extent to the different climate conditions in springtime. All these differences in ecosystems 581 responses resulted in a net sink of GHGs from the SRC site and in a neutral GHG balance for 582 the REF site. 583

A GHG balance not significantly different from zero is in agreement with the average results for a set of sites in Soussana et al., 2007, where however management costs were not considered, and on-site CO_2 emissions from grazing animals were measured with EC. C sequestered by the SRC site in our study was higher than that of the Belgian site in the study of Zona et al., 2013. In the latter the net budget was positive (in a time span of one year and a

half) with a net emission of 280 ± 80 gCO₂eq m⁻², due to both the higher emission rates of 589 CH₄ and N₂O fluxes from soil $(350 \pm 50 \text{ gCO}_2\text{eq m}^{-2})$, and to the lower CO₂ sink 590 $(-80 \pm 60 \text{ gCO}_2\text{eq m}^{-2})$ as compared to the present study. Also Jassal et al., 2013 found lower 591 F_{CO2} in a 3-year-old poplar SRC in Canada (-293 gCO₂ m⁻² year⁻¹) as compared to F_{CO2} of the 592 SRC site of the present study (root age: 4 years), likely due to the lower stem density of their 593 site. All these values lied in the range found by Arevalo et al., 2011, i.e. -77 gCO₂ m⁻² year⁻¹ 594 and -4756 gCO₂ m⁻² year⁻¹ relative to a 2-year-old and 9-year-old poplar SRC respectively. 595 These results show that even in a Mediterranean area, where plants are subjected to drought 596 stress, with a proper use of irrigation there is the potential for a positive effect on climate 597 mitigation. 598

Several studies (Grigal and Berguson, 1998; Price et al., 2009) confirmed that converting 599 agricultural land to SRC resulted in an initial release of SOC due to SRC establishment, and 600 601 then in a slow and continuous accumulation of SOC due to vegetation activity and wood encroachment (Arevalo et al., 2011). Despite the deep tillage at the SRC establishment, and 602 603 the fact that the REF site was ploughed every year at different depths, a gradient decreasing with depth in the C distribution of the vertical profile was evident in the three sites (not 604 shown). This suggests that the changes in SOC were attributable only to the plantation of the 605 SRC due to the effects of tillage (Anderson-Teixeira et al., 2009), and not to the mechanical 606 607 redistribution of SOC. This study indicates a SOC loss of 47% in respect to the value measured in the REF site, due to the installation of poplar cuttings. This loss was not 608 measured at the time it occurred, i.e. right after the conversion of arable land to poplar short 609 rotation coppice, but was estimated with data from the O SRC site. The reported value was 610 close to the range maximum reported in the review by Post and Kwon, 2000 (20%-50%), but 611 was higher than the results found by Arevalo et al., 2011 (7%). The absolute value, however, 612 was close to the one of this latter study (8 MgC ha⁻¹), where though the initial SOC was one 613

order of magnitude higher (114.7 MgC ha⁻¹). To correctly interpret this rapid loss of SOC for 614 a conversion of a cropland to a SRC the low degree of disturbance that characterises the REF 615 site must be taken into account. Furthermore the loss of SOC found in the present study has to 616 be considered along with its own uncertainty that was as large as the estimated value: in the 617 purposes of the GHG balance, where the uncertainty of the single components are propagated 618 to the net budget, this result is correctly interpreted as a range. We highlight that a loss of 619 SOC close to the minimum of the abovementioned range by Post and Kwon, 2000, e.g. 620 321 gC m⁻², would have changed B_{SRC} (-2202 ± 792 gCO₂eq m⁻²) by only -259 gCO₂eq m⁻². 621 Thus, even if a measured value would have probably been more accurate, the sensitivity of the 622 total GHG budget to this loss was shown to be relatively low. The estimated annual SOC 623 accumulation rate was in the range reported by Don et al., 2011 for SRCs 624 $(0.44 \pm 0.43 \text{ MgC ha}^{-1} \text{ y}^{-1})$, which explained how the frequent harvest of above ground 625 biomass was likely to facilitate the die off of the roots that contributes to SOC accumulation. 626 In our study, the low biomass yield supports the hypothesis that a big fraction of C taken up 627 628 via photosynthesis was transferred to roots and soil. In our study the break-even point, where the initial SOC content would be restored and a net SOC accumulation would start, was 10 629 years, in agreement with findings from other studies (e.g. Hansen, 1993; Arevalo et al., 2011 630 found a value of 7 years, while Grigal and Berguson, 1998, calculated a break-even point of 631 15 years). This result, not directly involved in the 24-month GHG budget, is relevant 632 considering that the SRC of the present study is expected to be used for 12 years, thus enough 633 to allow the complete recovery of the SOC loss occurred at the plantation. Different previous 634 land uses, soil types (in particular clay content), climate conditions, fertilisation rates may be 635 the main causes of differences between studies, as shown in a meta-analysis by Laganière et 636 637 al., 2010.

Our results showed that CH₄ and N₂O soil fluxes were not relevant in the GHG budgets due to 638 the combination of soil characteristics and climatic trends at both sites. Low values are 639 reported in other studies for SRCs: e.g. Gauder et al., 2012 found that the soil of different 640 energy crops acted as weak sink of CH₄ even in case of fertilisation, while emissions of N₂O 641 turned out to be higher for annual than perennial (willow) crops, which showed no significant 642 effect of fertilisation on N₂O fluxes. Agricultural sites usually have higher N₂O effluxes from 643 644 soil, though their magnitude depends on cultivations and on management practices, as shown by Ceschia et al., 2012. The SRC site as a perennial woody crop was subjected to low soil 645 disturbance during its lifespan, while the REF site was ploughed once per year, with an 646 impact on the ecosystem respiration. Zona et al., 2012 found high N₂O emissions in the first 647 growing season of a poplar SRC in Belgium: $197 \pm 49 \text{ gCO}_2\text{eq m}^{-2}$ in six months, which 648 drastically decreased to 42 ± 17 gCO₂eq m⁻² for the whole following year. This suggested an 649 influence of soil disturbance during land conversion on the stock of N in soil, which was 650 almost 1/3 lower in our study sites than in the one of Zona $(9.1 \pm 2.1 \text{ MgN ha}^{-1})$. In the 651 present experiment however, N₂O fluxes were low both in the SRC and REF sites, even 652 during periods of fertilisation, with no clear patterns. The low N₂O fluxes were confirmed by 653 laboratory analyses, as the presence of extra N did not affect the emission rates of N₂O, and 654 only very high WFPS% could trigger significant N₂O fluxes. The needed conditions of soil 655 humidity were never reached in the REF site and persisted only for a few days at 35 cm depth 656 in the SRC site (Fig. 6). At this depth fertilizer was added as fertigation in the SRC site: we 657 hypothesize that the very low porosity, the compaction and strength of the soil might have 658 favoured slow gas release and further N₂O reduction, thus leaving little N₂O to escape to the 659 atmosphere from soil surface. In the REF site, winter fertilisation was also associated with 660 low temperatures, a further constraint to microbial activity. These results provide further 661 evidence of how the simple application of the IPCC N₂O emission factor to the analysed 662

systems might have led to an overestimation of the field GHG contribution to the overall 663 664 GWP in both sites. Laboratory estimates of mineralization and nitrification rates suggested that N mineralization might be the limiting process of the chains of mineral N microbial 665 transformations, that contributed to maintain N₂O emissions low even during events of 666 intense rainfall and soil saturation. The clay content and compaction of the analysed soils 667 might be an important factor in limiting oxygen and substrate diffusion that are both 668 necessary to have optimal rates of soil organic matter mineralization. From a methodological 669 point of view, the low emissions of both CH₄ and N₂O from soil also suggest that using 4 670 samples of gas concentration per chamber instead of 3 would have not dramatically improved 671 672 the accuracy of the calculated fluxes, as a slight variation in the slope would have not induced significant changes in the results. The relevance of this result lies in the fact that fertilising a 673 poplar SRC in a Mediterranean area and in this kind of soil does not necessarily lead to 674 675 increased emissions of N₂O, with the requirement that the correct equilibrium is found between irrigation and WFPS%. Thus it is possible to maximise yield and GHG mitigation 676 677 with the right management practices (Nassi o Di Nasso et al., 2010). CH₄ and N₂O fluxes might have been enhanced by the land conversion in the first period of cultivation of the SRC 678 site, as found for CO₂. However, measurements carried out in the REF site, ploughed every 679 year, and the incubation experiment showed very low fluxes, mostly related to soil 680 characteristics and not to management activities. Thus, a low sensitivity of the total GHG 681 budget to these components can be expected. 682

683 Other components of the GHG budget related to N compounds (e.g. aerosol NH_4NO_3 , N 684 deposition and leaching) were considered negligible in this study as compared to the role of 685 N_2O emissions from soil and related to fertiliser production.

686 Regarding the use of biomass, comparisons with other studies for the REF site are 687 complicated because half of the field was converted to sorghum in spring for the low

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productivity experienced during the drought. However, the productivity of the clover in 688 mixture was found highly variable by Martiniello, 1999, and the results of the present study 689 are comparable with the lower values found by this author in non-irrigated stands in 690 Mediterranean climate (0.39 kg m⁻²). Sorghum productivity was lower than that reported by 691 Nassi o Di Nasso et al., 2011 (around 0.75 kg m⁻²) in a similar climate, likely due to the short 692 period of cultivation and to grazing. The productivity of winter wheat was similar to that of 693 Anthoni et al., 2004 $(0.32 \pm 0.03 \text{ kg m}^{-2})$. The drought in summer 2012 had an important 694 influence on the AGWB of the SRC site, which was lower compared to other studies (e.g. 695 Scholz and Ellerbrock, 2002, 0.4 to 0.7 kg m⁻² year⁻¹), and to the F_{CO2} values found with EC. 696 Our hypothesis is that the period of drought had influenced the aboveground/belowground 697 ratio, and that the herbaceous vegetation contributed to increase the F_{CO2} . In terms of C, the 698 difference $F_{CO2} - F_{EXP}$ represents to a first approximation the C stocked by each ecosystem 699 that does not return shortly to the atmosphere after utilisation, minus heterotrophic respiration 700 (Rh). While in the SRC site that difference was negative (C sink of 650 gC m⁻²), the REF site 701 acted like a small source of C (120 \pm 98 gC m⁻²). Small sources were also found by Anthoni et 702 al., 2004 (between 50 gC m⁻² and 100 gC m⁻²), while Aubinet et al., 2009 reported a 4-year 703 rotation crop being a source of 340 gC m⁻². For poplar, Deckmyn et al., 2004 found a similar 704 behaviour in a poplar SRC in Belgium. Concerning the fraction of the exports that is emitted 705 as CH₄ from enteric fermentation, our estimates were in agreement with those of Dengel et 706 al., 2011. Several studies (e.g. Gilmanov et al., 2007) used EC to measure CO2 and CH4 707 fluxes from grazed systems. Some included in the GHG budget only F_{CO2} , F_{CH4} and F_{N2O} , and 708 made a C budget for lateral fluxes like biomass export (e.g. Allard et al., 2007). However, the 709 EC method is not capable of measuring point sources of trace gases moving inside and 710 outside the footprint (data discarded by QA/QC procedures: see also Baldocchi et al., 2012). 711 Thus we adapted the method described in Soussana et al., 2007 for off-site emissions, 712

extending it also to on-site emissions, to include the effects of aboveground biomass use inthe GHG budget.

Different studies (e.g. Cherubini et al., 2009; Djomo et al., 2013) confirmed the advantages of 715 using biomass from SRC over fossil fuels in mitigating the increase of atmospheric GHG 716 concentrations, while Abbasi and Abbasi, 2010 found that the SRC management led to GHG 717 emissions that compensate the gain due to the fossil substitution. The low yield of the SRC 718 site led to lower GHG savings compared to those found by Cherubini et al., 2009 for 719 production of heat from woody products $(379.7 \pm 175.1 \text{ gCO}_{2}\text{eg m}^{-2})$ in two years against 720 600 gCO₂eq m⁻² per year). These latter found GHG mitigation to be directly proportional to 721 crop yield for dedicated bioenergy crops. In a GHG budget perspective, however, the yield is 722 also proportional to C emissions from combustion, and correlated with F_{CO2} . The same study 723 reported GHG savings of other bioenergy systems, showing that the performance of wood-724 based systems is lower in terms of GHG offset than the one of other bioenergy crops, e.g. 725 switchgrass (1300 gCO₂eq m⁻² year⁻¹), Miscanthus (1600 gCO₂eq m⁻² year⁻¹) and fibre 726 sorghum (1800 gCO₂eq m^{-2} year⁻¹). In the present study the role of GHG offset was relevant 727 in the GHG balance; however it's important to consider that natural gas, while being the most 728 used fossil fuel for heating systems in Italy, has also a lower carbon intensity for heat 729 production (55.862 gCO₂eq MJ⁻¹) as compared to coal (76.188 gCO₂eq MJ⁻¹) and oil (73.693 730 731 gCO₂eq MJ⁻¹) (Romano et al., 2014). A different scenario, where biomass would substitute the use of other energy sources with higher emission factors (like coal) would lead to a higher 732 GHG offset. 733

Our study confirmed that farming operations have only a limited importance in the overall GHG budget when conditions of relevant CO_2 uptake by vegetation are met, and the values we found were similar to the ones found by Gelfand et al., 2011. In the SRC site irrigation was more important than other contributions and caused more emissions than irrigation in the REF site. This suggests that belowground irrigation was less efficient in terms of GHG emissions than the sprinkler. Fertilisers and other chemical products often have a higher impact on the GHG balance as compared to other field operations due to the off-site GHG emissions (Ceschia et al., 2010). At the study sites the amount and frequency of applications were relatively small, and this justifies the minor role of fertilisation in the total GHG budget. Thus the importance of farming operations can vary from year to year, depending on climate conditions and on farmer decisions.

This study reports on the GHG budget of poplar SRC in Mediterranean areas. However, when 745 considering the implications of SRC in a wider perspective, other factors should also be 746 considered to assess the overall sustainability of this type of LUC. Among them, irrigation is 747 one of the most important (Dougherty and Hall, 1995), as poplar cultivations in 748 Mediterranean climate require considerable amounts of water. In the LUC presented here, 749 both the SRC and the REF sites were irrigated with similar amounts of water, using a less 750 efficient technique at the REF site (sprinkler system) than at the SRC site (belowground drip 751 752 system; e.g. Camp, 1998). The impact of the LUC on the local water balance is thus expected to be small in this particular case, but not in general. An appropriate design of these systems 753 is also crucial to avoid water dispersion: in the present study we observed that irrigation could 754 not compensate the drought stress experienced by the SRC site in 2012, thus concerns arise on 755 756 the proper location of the belowground tubes and on the amounts of water applied. The aim of this study was to analyse the LUC from a GHG perspective at farm level: the boundary of our 757 system constituted the main difference with a full LCA analysis, where the iLUC is 758 considered in addition to the direct land use change. iLUC can cause GHG emissions 759 elsewhere, thus reducing the mitigation potential of the studied SRC on a global scale. 760

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This study analysed a land use change (LUC) for biomass production from a greenhouse gas 763 (GHG) perspective. The conversion of a traditional cropland (REF site) to a short rotation 764 coppice (SRC site) of poplar hybrids in a Mediterranean climate (Central Italy) was 765 considered. Different fluxes were included in a GHG budget calculated for both sites: to and 766 from vegetation and soil; caused by management; due to the loss of soil organic Carbon 767 (SOC) at the installation of the cuttings; caused by the use of the biomass and the 768 displacement of GHG emissions from fossil fuel. Other environmental aspects than the GHG 769 balance were not considered, such as the water balance, the minor components of the nitrogen 770 cycle and the contribution of the indirect land use change (iLUC: Djomo et al., 2013). The 771 772 direct LUC contributions, like the SOC content loss at the installation of the SRC and the corresponding disadvantages in terms of GHG, were instead included in the calculation. 773

Our study showed how poplar SRC cultivation for biomass production was overall suitable 774 from the point of view of the climate mitigation. The most important components of the GHG 775 budgets were the net ecosystem exchange (F_{CO2}) and the C export (F_{EXP}) at the end of the 776 cultivation cycle. Interestingly, fluxes of CH₄ and N₂O from soil were not relevant, likely due 777 to physical soil characteristics. No significant effects were observed even in case of 778 779 fertilisation, irrigation or rain events, in contrast with findings from other studies (e.g. Zona et al., 2012). Results demonstrated that poplar clones have the ability to stock high rates of C 780 from the atmosphere even on Mediterranean climate, where the cultivation of poplar SRC 781 may be critical for its dependence on water availability. Estimated uncertainty was however 782 783 quite large, confirming the need of large efforts in terms of data collection to correctly estimate the different components. Benefits of the LUC from common agriculture to SRC 784 derived from the interaction between the diverse components of the budget. Climate 785 786 conditions and farmer needs are the most important factors controlling the single

contributions of the GHG budget. An equilibrate combination of clones selection, irrigation
and management activities, depending also on soil properties, is thus crucial to achieve an
efficient contribution to Climate Change mitigation by LUC for bioenergy crops.

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9. Tables

1100 Table 1 – Instrumental setup of the two towers. SRC = short rotation coppice site; REF = refe	erence site; T =
1101 temperature; RH = relative humidity; PAR = photosynthetically active radiation; <i>M_{SOIL}</i> = soil	water content; P =
1102 precipitation; EC = eddy covariance; prof = profile. 4-component radiometers were used to m	easure short- and long-
1103 wave radiations, and derive net radiation. SRC site soil profiles were located in irrigated and	not-irrigated inter-rows.
1104 Precipitation was assumed to be consistent in the two ecosystems.	

	SRC	REF
Meteo		
Air T and RH	MP-100, Rotronic AG, Bassersdorf, CH	MP-100, Rotronic AG, Bassersdorf, CH
PAR	Li-190, LI-COR, Lincoln, NE, USA	-
Radiations	CNR-1, Kipp&Zonen, Delft, NL	NR01, Hukseflux, Delft, NL
M _{SOIL}	CS616, Campbell Scientific, Logan, UT, USA (2	CS616, Campbell Scientific, Logan, UT, USA (1
	prof.)	prof.)
Soil T	107, Campbell Scientific, Logan, UT, USA (2 prof.)	107, Campbell Scientific, Logan, UT, USA (1
		prof.)
Soil heat flux	HFT3, REBS Inc., Seattle, WA, USA	HFP01, Hukseflux, Delft, NL
Р	-	ARG100, EML, North Shield, UK
Logger	CR3000, Campbell Scient., Logan, UT, USA	CR1000 Campbell Scient. Logan, UT, USA
EC		
Anemometer	CSAT3, Campbell Scientific, Logan, UT, USA	USA-1, Metek GmbH, Elmshorn, DE
Gas-Analyser	LI-7500, LI-COR, Lincoln, NE, USA	LI-7500A, LI-COR, Lincoln, NE, USA

Table 2 – Correction steps applied to the time series using LICOR EddyPro software.

Correction	Reference	
Despiking	Vickers and Mahrt (1997)	
Density fluctuations	Webb et al. (1980)	
Maximisation of covariance for time lag compensation	Aubinet et al. (2000)	
Linear detrending for trend removal	Gash and Culf (1996)	
2-D coordinate rotation	Wilczak et al. (2001)	
High-pass filtering effect	Moncrieff et al. (1997)	
Low-pass filtering effect	Ibrom et al. (2007)	

- 1110 Table 3 Farming activities. Three tractors of different power were normally used to collect chips: two of the type 1,
- 1111 and one of the type 2. DAP = diammonium phosphate; AN = ammonium nitrate; CAN = calcium ammonium nitrate.

1112 SRC and REF as defined previously. Reported units are intended per hectare and activity.

O rrenting	Fuel consumption	Input rates		
Operation	(unit ha ⁻¹)	(unit ha ⁻¹)	Site	
Harvesting – wood chipper	30 l diesel	-	SRC	
Harvesting – Tractor type1	20 l diesel	-	SRC	
Harvesting – Tractor type 2	10 l diesel	-	SRC	
Shallow tillage	8 l diesel	-	SRC, REF	
Application of insecticide	1.125 l diesel	1.25 kg DECIS®	SRC	
Mechanical weeding	4 l diesel	-	SRC	
Ploughing	8 l diesel	-	SRC, REF	
Sowing	2 l diesel	-	REF	
Seed covering	4 l diesel	-	REF	
		a. 150 kg DAP	a. REF	
		b. 150 kg AN	b. REF	
Application of fertiliser	2 l diesel	c. 200 kg CAN	c. REF	
		d. 40 kg Urea	d. SRC	
Reaping	20 l diesel	-	REF	
Chemical weeding	1.125 l diesel	1 l Buctril®	REF	
Bale	7.5 l diesel	-	REF	
Turingt	a. 471 kWh electricity	a. 161H ₂ O	a. SRC	
Irrigation	b. 149 kWh electricity	b. 461H ₂ O	b. REF	

1113

1115 Table 4 – Grazing calendar and methane emissions in the REF site. Graz_days = number of days with grazing; Num =

Months	Graz_days	Num (per 9 ha)
Dec 2011	10	800
Jan 2012	7	400
Jun 2012	2	580
Aug 2012	1	580
Sep 2012	2	580
Oct 2012	5	400

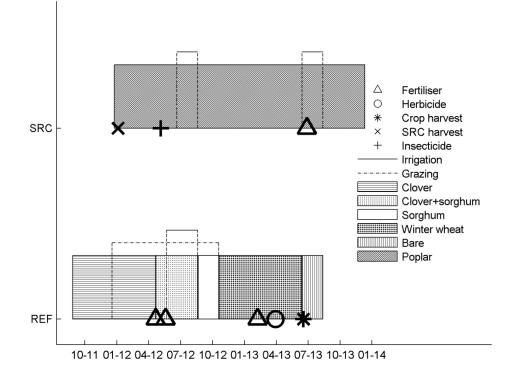
number of sheep in the cropland

1119	Table 5 – Soil characteristics of the layer 0-15 cm. SRC and REF as previously defined; SOC = soil organic carbon;				
1120	ρ_{BULK} = bulk density. Supers	scripts a-	c indicate statisticall	y significant difference	s between the means of SOC.
1121		Site	Variable	Value ± dev. std.	
			C (%)	1.46 ± 0.34	
1122		REF	ρ_{BULK} (Mg m ⁻³)	1.00 ± 0.11	
		H	SOC (MgC ha ⁻¹)	$16.03 \pm 3.76^{(a)}$	

	SOC (MgC ha ⁻)	$16.03 \pm 3.76^{(3)}$
	C (%)	1.05 ± 0.40
SRC	ρ_{BULK} (Mg m ⁻³)	1.12 ± 0.15
	SOC (MgC ha ⁻¹)	$11.69 \pm 4.42^{(b)}$
	C (%)	1.38 ± 0.27
0_SRC	$\rho_{BULK}~(Mg~m^{-3})$	1.02 ± 0.11
0	SOC (MgC ha ⁻¹)	$14.03 \pm 2.79^{(c)}$

10. Figures

1124Fig. 1 – Scheme of the chronological land cover during the cultivation cycle taken into account for GHG budget1125calculation in the two ecosystems. The expected harvest of poplar at the beginning of 2014 was postponed of one year:1126for that reason data from the previous harvest (beginning 2012) were taken into account for GHG budget calculation.1127Textures indicate different land cover type, symbols mark the most important management practices, straight lines1128indicate the periods in which sites were irrigated, dashed line period of grazing. SRC = short rotation coppice site;1129REF = reference site; in the x axis dates are reported as month-year (mm-yy)1130



1132Fig. 2 – Boxplot of the 24-month cumulative fluxes of net ecosystem exchange of CO_2 (F_{CO2}) (a), gross primary1133production (GPP) (b) and ecosystem respiration (Reco) (c) from eddy covariance (EC) data in the REF and SRC sites.1134Each box represents the range 16th-84th percentile: the central mark is the median, while the whiskers extend to the11355th and 95th percentiles

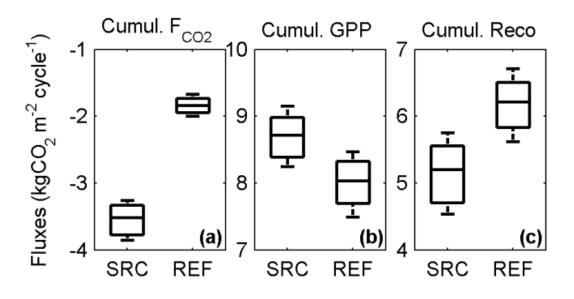
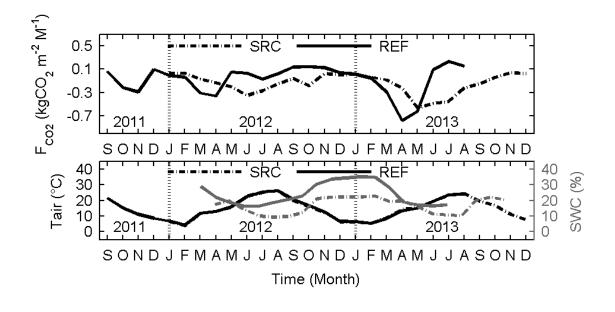


Fig. 3 – Monthly averages of F_{CO2} in the REF and SRC sites (top panel). The bottom panel shows monthly averages of
air temperature (Tair) and soil water content (SWC) at 30 cm depth. In both subplots dotted lines are used for the
SRC site and continuous lines for the REF site, while in the bottom panel SWC is in grey and the Tair in black.



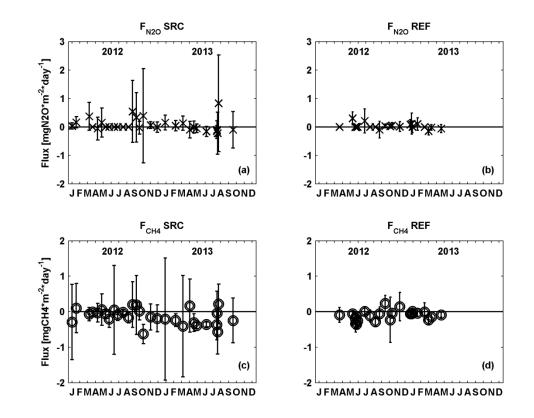


1143 Fig. 4 – Fluxes of soil N_2O (crosses) and CH_4 (circles) in the SRC (a –c) and the REF (b – d) sites. Each marker

1144 represents the average of the nine chambers, with bars indicating their standard deviation. First letter of month in the

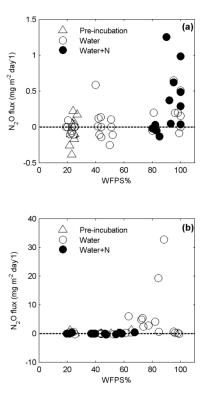
x-axis

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- 1148 Fig. 5 N₂O fluxes from incubation experiment reported in function of the water filled pore space estimated for each
- single replicate. In (a) data from samples taken in the SRC site are shown, in (b) data from REF site samples



1151

- 1152 Fig. 6 WFPS% in the REF site at three different depths for the 24-month integration periods. Dashed line points to
- 1153 the threshold (70%) unleashing N₂O from lab incubations. First letter of month in the x-axis
- 1154

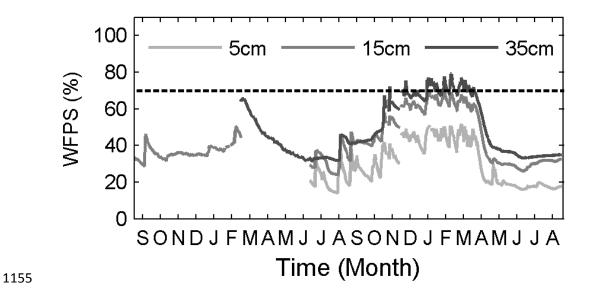
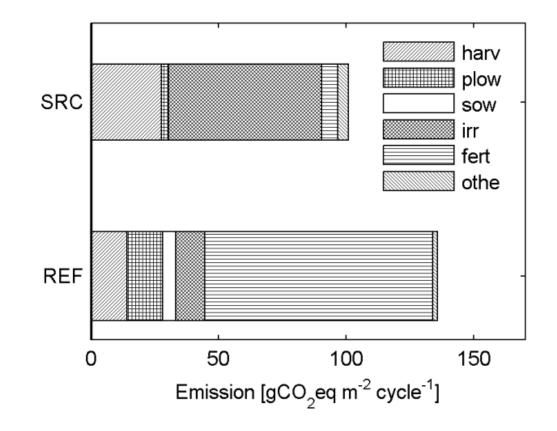


Fig. 7 –GHG emissions of the different farming operations. Harv = harvesting; plow = ploughing; sow = sowing; irr =
irrigation; fert = fertilisation; othe= minor contributions. SRC and REF as previously defined



- 1160Fig. 8 Regression line of SOC content in time t (years). The gap between SOC(0) and SOC content in the1161REF site represented the loss of SOC for the land use change. Est = estimated values; meas = measured values; SRC1162and REF as previously defined; O_SRC is the older short rotation coppice site used to build the regression

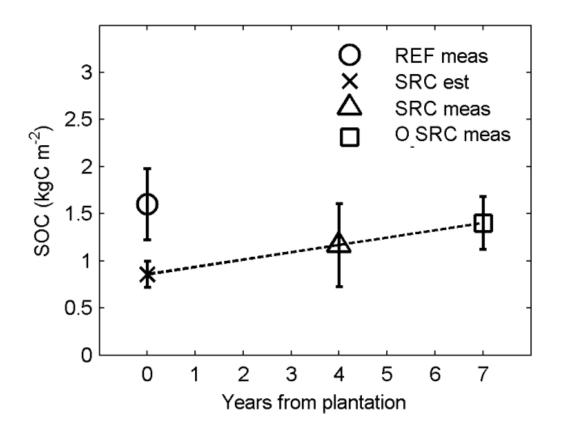


Fig. 9 – GHG balances of the SRC and the REF sites: components (left) and net (right). F_{CH4} and F_{N20} from soil are negligible and not inserted in the graph. F_{M4N} = management; E_{CH4} = exported biomass reemitted as CH₄ by enteric fermentation; E_{C02} = exported biomass reemitted as CO₂ by sheep respiration; F_{S0C} = initial SOC change at the installation of cuttings; F_{SAV} = GHG savings for replacement of fossil fuel use; F_{CO2} as previously defined

1170

