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Initial soil C and land-use history determine soil C sequestration under perennial bioenergy crops

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Abstract

In the UK and other temperate regions, short rotation coppice (SRC) and *Miscanthus x giganteus* (*Miscanthus*) are two of the leading 'second-generation' bioenergy crops. Grown specifically as a low-carbon (C) fossil fuel replacement, calculations of the climate mitigation provided by these bioenergy crops rely on accurate data. There are concerns that uncertainty about impacts on soil C stocks of transitions from current agricultural land use to these bioenergy crops could lead to either an under- or overestimate of their climate mitigation potential. Here, for locations across mainland Great Britain (GB), a paired-site approach and a combination of 30-cm- and 1-m-deep soil sampling were used to quantify impacts of bioenergy land-use transitions on soil C stocks in 41 commercial land-use transitions; 12 arable to SRC, 9 grasslands to SRC, 11 arable to *Miscanthus* and 9 grasslands to *Miscanthus*. Mean soil C stocks were lower under both bioenergy crops than under the grassland controls but only significant at 0–30 cm. Mean soil C stocks at 0–30 cm were 33.55 ± 7.52 Mg C ha^{-1} and 26.83 ± 8.08 Mg C ha^{-1} lower under SRC ($P = 0.004$) and *Miscanthus* plantations ($P = 0.001$), respectively. Differences between bioenergy crops and arable controls were not significant in either the 30-cm or 1-m soil cores and smaller than for transitions from grassland. No correlation was detected between change in soil C stock and bioenergy crop age (time since establishment) or soil texture. Change in soil C stock was, however, negatively correlated with the soil C stock in the original land use. We suggest, therefore, that selection of sites for bioenergy crop establishment with lower soil C stocks, most often under arable land use, is the most likely to result in increased soil C stocks.

Keywords: bioenergy, Carbon Stocks, land-use change, *Miscanthus*, soil carbon, SRC willow

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Introduction

Tackling climate change is one of the greatest challenges facing the world (IPCC, 2014). Along with other renewable energy sources and demand reduction, the use of biomass as a low-carbon (C) replacement for fossil fuels is seen as an essential part of the move towards a more sustainable energy system (Renewable Energy Road Map 2007; DECC *et al.*, 2012). Sources of biomass are diverse and include waste streams from food, forestry and conventional agricultural crops (Rowe *et al.*, 2009; DECC *et al.*, 2012). There is, however, increasing interest and utilisation of so-called second-generation (2G) bioenergy crops, especially in temperate developed nations such as Europe and the USA (Davis *et al.*, 2012; Don *et al.*, 2012). These 2G bioenergy crops, predominantly perennial grass and woody species, are grown specifically to use as a renewable fuel source and are characterised by low input requirement and high growth rates. These traits result in a low energy requirement per unit of energy produced, limited management requirements, potentially higher C savings and reduced environmental impacts when compared to conventional food crops used for the production of first-generation biofuels (Fazio & Monti, 2011; Don *et al.*, 2012; Mohr & Raman, 2013; Walter *et al.*, 2014).

Assessing the C balance of 2G bioenergy crops presents a unique challenge as, in contrast to the use of conventional agricultural crops or waste streams, bioenergy crop production requires a major change in land use and management (Rowe *et al.*, 2009; Aylott & McDermott, 2012; Mohr & Raman, 2013). Land-use change (LUC) is known to be a primary factor affecting soil C stock (Guo and Gifford, 2002), and whilst impacts of harvesting and utilisation of these crops on the C balance are relatively well understood, impacts on soil C stocks are less well defined (Fazio & Monti,

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2011; Rowe *et al.*, 2011; Don *et al.*, 2012; Walter *et al.*, 2014).

In their meta-analysis, Don *et al.* (2012) highlighted the limited number of studies on the impacts of bioenergy crops on soil C stocks in temperate regions, and the highly variable and sometimes contradictory results reported across these. Even within single multi-site studies, impacts on soil C stock have been found to be variable between sites, with Walter *et al.* (2014), for example, reporting rates of change in soil C stocks across 21 SRC plantations in central Europe from -1.3 to 1.4 Mg C ha^{-1} yr^{-1} for transitions from arable land and -0.6 to 0.1 Mg C ha^{-1} yr^{-1} for transitions from grassland. Meanwhile, for *Miscanthus* transitions from arable land, Poeplau & Don (2014) found rates of change in soil C stocks within their study ranging from -0.17 to 1.54 Mg C ha^{-1} yr^{-1} and ranges in the literature of between -6.85 and 4.51 Mg C ha^{-1} yr^{-1}.

Some of the variations in the observed impact on soil C stocks, both between and within studies, have been related to differences in climatic conditions, original land use, soil types, management or crop genotype (Don *et al.*, 2012; Poeplau & Don, 2014; Richter *et al.*, 2015). These sources of variability can help to improve understanding of the mechanisms underlying changes in soil C stock, but comparison of studies can also be confounded by differences in quantification methods (Don *et al.*, 2012; Bárcena *et al.*, 2014). For example, LUC to SRC and *Miscanthus* can result in changes in soil C distributions within the soil profile and therefore sampling depth, which often differs between studies, can have a profound effect on the quantified impacts on soil C stocks (Poeplau & Don, 2014; Walter *et al.*, 2014). In their meta-analysis of impacts on soil C stocks of LUC to forestry, Bárcena *et al.* (2014) also highlighted the failure of many studies to adjust for change in soil bulk density (BD) that often co-occur with LUC. This results in an incorrect assessment of change in soil C stock and inflated between-study variability (Bárcena *et al.*, 2014). Apart from some notable exceptions (Walter *et al.*, 2014; Ferchaud *et al.*, 2015), few temperate bioenergy LUC studies have directly addressed the issue of changing BD (Don *et al.*, 2012).

In the context of mainland GB, and for the two dominant bioenergy crops in the UK, SRC willow and *Miscanthus* (Aylott & McDermott, 2012), we address these issues by providing a methodologically consistent data set of the impacts on soil C of land-use transitions to these crops, whilst incorporating variability in potential regulatory factors such as climate. This study aims both to assess within mainland GB the current impacts on soil C stocks of LUC to commercial plantations of either SRC or *Miscanthus*, and to provide insights and data on regulatory factors that can be incorporated into future modelling activities (see Dondini *et al.*, 2015). To meet these aims, we undertook the assessment of soil C stocks under 20 *Miscanthus* and 21 SRC commercial plantations and their paired controls. Transitions were located across mainland GB and were purposefully selected to cover a wide range of climatic and soil conditions, including soil texture, pH, initial soil C stocks, a range of bioenergy crop ages and land-use transitions from both grassland and arable land uses, thus allowing the influence of these factors on changes in soil C stocks to be explored. Soil sampling utilised a combination of 0–30-cm and 0–1-m soil cores and soil C stocks were adjusted for changes in bulk density.

Materials and methods

Site selection

A database of potentially suitable commercial SRC and *Miscanthus* plantations was populated through liaising with bioenergy companies and individual growers. Data on soil C stocks prior to the land-use change were not available for these commercial sites, thus a paired-site approach was utilised, where impacts on soil C stock are assessed through a comparison between a target land use and an adjacent paired control representing the original land use (Davis & Condron, 2002; Laganière *et al.*, 2010). The paired-site method assumes no pre-existing differences between the control and bioenergy land uses that would confound changes in soil C stock (Wellock *et al.*, 2011; Hewitt *et al.*, 2012). Bioenergy plantations were therefore selected on the basis of the availability of a suitable paired control field in addition to the bioenergy crop age (time since establishment), geographical location and the type of LUC (i.e. from arable or from grassland). Selection aimed to provide the widest range of bioenergy crop age and geographical location, and a balance of transitions from arable and grassland to SRC and *Miscanthus* (Table 1). Each control and bioenergy plantation pair is referred to as a transition. In total, 41 transitions were assessed at 28 locations across mainland GB (Fig. 1).

The 41 transitions comprised 12 arable to SRC (all willow), 9 grasslands to SRC (8 willows, 1 poplar), 11 arable to *Miscanthus* and 9 grasslands to *Miscanthus* transitions (Table 1). Grassland was defined here using Defra definitions and includes both permanent pasture (>5 years old) and temporary grassland (5 years old and under), with the majority of sites being permanent pasture (Table 1). The lower number of grassland transitions reflects the greater difficulty experienced in locating bioenergy plantations established on former grassland.

Sampling method

Surface soil (0–30 cm). The surface soil of the cropped area of each bioenergy plantation or control field was sampled using a hierarchical design (Keith *et al.*, 2014), developed to capture variability across different spatial scales (Conant & Paustian, 2002; Conant *et al.*, 2003). Five sampling plots per field were

Table 1 Site details including transition location and type, current land use, duration of current land use, mean annual temperature (MAT), mean annual precipitation (MAP), soil texture (% clay) and C stocks at 30 cm and 100 cm

Site code	Transition number	Bioenergy crop	Control land use	Bioenergy planation age	Latitude, Longitude	MAP °C	MAT mm yr^{-1}	% Clay (0–30 cm; bioenergy crop)	Soil C stocks 0–30 cm (ESM reference mass of 3 Gg ha^{-1}) Bioenergy Mg C ha^{-1} ± SD	Soil C stocks 0–30 cm Control Mg C ha^{-1} ± SD	Soil C stocks 0–100 cm (ESM, reference mass of 13 Gg ha^{-1}) Bioenergy Mg C ha^{-1} ± SD	Soil C stocks 0–100 cm Control Mg C ha^{-1} ± SD
S1	1	SRC Willow	A	6	53.7, −0.8	9.63	603	8.08	54.65 ± 8.62	51.7 ± 6.92	127.72 ± 9.54	129.96 ± 10.79
S1	2	SRC Willow	A	13	53.7, −0.8	9.63	603	8.04	61.25 ± 11.08	51.7 ± 6.92	138.18 ± 8.29	129.96 ± 10.79
S2	3	SRC Willow	A	12	53.2, −0.8	9.77	580	6.73	45.62 ± 10.51	33.64 ± 3.19	85.62 ± 9.56	60.34 ± 0.98
S2	4	SRC Willow	A	8	53.2, −0.8	9.77	580	12.56	54.97 ± 7.41	33.64 ± 3.19	92.61 ± 10.05	60.34 ± 0.98
S3	6	SRC Willow	A	14	54.6, −2.7	7.64	1238	6.01	85.23 ± 10.86	73.93 ± 12.54	NA	NA
S5	9	SRC Willow	A	6	51.7, −0.9	10.04	625	5.75	57.44 ± 3.57	58.38 ± 13.00	110.75 ± 6.18	92.65 ± 8.76
S6	15	SRC Willow	A	7	51.5, −0.8	9.87	661	4.34	61.86 ± 11.30	54.38 ± 6.31	96.99 ± 6.62	99.22 ± 3.71
S7	18	SRC Willow	A	8	51.5, −1.6	9.95	663	9.86	115.79 ± 20.07	82.04 ± 12.13	143.28 ± 10.94	120.80 ± 19.95
S8	26	SRC Willow	A	5	50.7, −2.4	9.95	795	7.24	55.61 ± 9.62	47.9 ± 3.72	104.46 ± 11.12	99.95 ± 1.50
S9	33	SRC Willow	A	4	56.0, −3.6	8.36	946	4.25	87.75 ± 13.68	61.62 ± 6.41	161.83 ± 22.34	168.58 ± 13.43
S10	37	SRC Willow	A	6	54.8, −2.9	8.63	993	3.84	58.28 ± 10.15	64.36 ± 12.64	99.22 ± 16.21	71.85 ± 6.74
S11	41	SRC Willow	A	7	53.1, −0.3	9.95	582	6.76	45.70 ± 5.71	54.43 ± 8.32	132.64 ± NA	156.52 ± 26.22
S2	5	SRC Willow	PP	5	53.2, −0.7	9.77	580	9.39	124.52 ± 11.3	131.64 ± 9.78	252.05 ± 11.78	293.15 ± 15.25
S3	7	SRC Willow	PP	5	54.6, −2.6	7.64	1238	4.47	123.16 ± 19.48	127.16 ± 21.21	NA	NA
S4	8	SRC Willow	RG	5	50.9, −0.4	10.55	738	7.15	42.56 ± 6.78	61.24 ± 11.00	77.28 ± 13.31	63.84 ± 7.18
S7	17	SRC Willow	PP	23	51.5, −1.6	9.95	663	6.00	106.63 ± 8.63	184.66 ± 42.91	102.97 ± 3.51	218.08 ± 14.99
S12	20	SRC Willow	PP	10	52.2, −1.9	9.61	700	8.69	75.39 ± 12.64	95.84 ± 11.75	131.05 ± 0.80	148.76 ± 3.66
S12	21	SRC Poplar	PP	20	52.2, −1.9	9.61	700	8.82	65.59 ± 7.51	95.84 ± 11.75	105.70 ± 14.52	148.76 ± 3.66
S12	22	SRC Willow	PP	23	52.2, −1.9	9.61	700	5.98	69.00 ± 12.04	95.84 ± 11.75	99.27 ± 16.50	148.76 ± 3.66
S13	34	SRC Willow	TG	6	56.2, −3.2	8.58	810	4.69	62.57 ± 8.82	72.58 ± 16.10	106.53 ± 10.88	158.28 ± 6.03
S14	35	SRC Willow	PP	9	51.7, −4.7	10.34	882	6.65	76.49 ± 5.91	71.58 ± 12.07	103.97 ± 17.99	78.35 ± 5.58
S5	10	*Miscanthus*	A	6	51.7, −0.9	10.04	625	5.30	44.12 ± 6.58	58.38 ± 13.00	82.05 ± 5.82	92.65 ± 8.76
S15	11	*Miscanthus*	A	6	54.0, −1.2	9.17	634	4.12	39.84 ± 4.26	35.53 ± 3.87	97.56 ± 4.89	83.81 ± 6.10
S16	13	*Miscanthus*	A	3	53.4, −0.5	9.81	578	7.78	63.65 ± 7.3	59.83 ± 7.61	136.30 ± 9.22	124.95 ± 4.46
S17	16	*Miscanthus*	A	6	51.5, −1.3	10.14	633	7.05	63.98 ± 6.11	55.87 ± 5.52	102.05 ± 14.75	61.80 ± 7.21
S18	19	*Miscanthus*	A	6	51.8, −1.6	9.86	677	4.81	51.27 ± 5.82	99.38 ± 17.95	78.37 ± 6.34	146.64 ± 7.61
S19	27	*Miscanthus*	A	10	51.0, −3.1	10.22	832	8.69	45.35 ± 11.88	68.29 ± 8.61	78.57 ± 3.67	88.45 ± 4.15
S20	30	*Miscanthus*	A	8	50.4, −4.6	10.71	982	6.21	113.96 ± 23.65	87.44 ± 12.92	141.85 ± 27.88	105.30 ± 14.33
S14	36	*Miscanthus*	A	8	51.7, −4.8	10.34	882	6.57	90.89 ± 18.4	94.21 ± 16.94	139.87 ± 5.70	128.60 ± 12.53
S21	39	*Miscanthus*	A	6	52.6, 2.0	9.53	697	3.56	35.51 ± 5.68	44.37 ± 9.22	72.04 ± 9.80	90.35 ± 5.39
S22	40	*Miscanthus*	A	5	52.5, −0.5	9.78	584	9.95	82.98 ± 21.41	92.52 ± 23.4	197.89 ± 17.38	194.23 ± 7.89
S11	42	*Miscanthus*	A	7	53.1, −0.4	9.95	582	5.87	51.39 ± 9.77	54.43 ± 8.32	144.13 ± 21.54	156.52 ± 26.22
S23	14	*Miscanthus*	PP	8	53.2, 0.1	9.82	570	5.09	75.16 ± 6.6	95.89 ± 24.54	172.09 ± 13.18	241.41 ± 25.76

(continued)

Table 1 (continued)

Site code	Transition number	Bioenergy crop	Control land use	Bioenergy planation age	Latitude, Longitude	MAP °C	MAT mm yr^{-1}	% Clay (0–30 cm; bioenergy crop)	Soil C stocks 0–30 cm (ESM reference mass of 3 Gg ha^{-1}) Bioenergy Mg C ha^{-1} ± SD	Control Mg C ha^{-1} ± SD	Soil C stocks 0–100 cm (ESM, reference mass of 13 Gg ha^{-1}) Bioenergy Mg C ha^{-1} ± SD	Control Mg C ha^{-1} ± SD
S24	12	*Miscanthus*	TG	7	54.1, −1.1	9.17	634	7.06	51.99 ± 4.81	61.46 ± 7.36	118.03 ± 1.96	105.97 ± 4.88
S25	23	*Miscanthus*	PP	6	53.2, −3.7	8.18	1218	9.53	112.1 ± 12.64	96.89 ± 22.17	119.13 ± 7.73	68.74 ± 22.49
S26	24	*Miscanthus*	TG	1	52.4, −4.0	8.81	1502	10.51	136.3 ± 25.07	140 ± 30.68	131.86 ± NA	123.40 ± NA
S27	25	*Miscanthus*	PP	9	51.2, −2.8	10.31	765	7.55	152.7 ± 23.7	178.14 ± 39.45	232.40 ± 2.45	232.33 ± 40.35
S19	28	*Miscanthus*	PP	10	51.0, −3.1	10.22	832	6.88	49.91 ± 7.87	87.2 ± 9.71	81.30 ± 6.72	130.77 ± 11.46
S28	29	*Miscanthus*	PP	9	50.5, −4.8	10.00	1044	10.82	67.92 ± 7.78	85.95 ± 14.27	98.28 ± 9.31	108.49 ± 4.17
S20	31	*Miscanthus*	PP	7	50.4, −4.6	10.71	982	6.53	94.71 ± 14.44	146.8 ± 14.56	117.05 ± 12.65	182.47 ± 21.93
S21	38	*Miscanthus*	PP	6	52.6, 2.0	9.53	697	3.75	47.15 ± 0.51	91.65 ± 7.98	NA	NA

Control land-use classifications are based on Defra guidelines; A = arable land, PP = permanent pasture (defined as to land that is used to grow grasses or other herbaceous forage, either self-seeded or sown and has not been included in the crop rotation for 5 years or longer and has not been set aside during this 5-year period), RG = rough grazing (defined as to low-yielding permanent grassland, usually on low-quality soil, usually unimproved by fertiliser, cultivation, reseeding or drainage), TG = temporary grassland (defined as grass for grazing, hay or silage included as part of normal crop rotation, lasting at least one crop year and <5 years, sown with grass or grass mixture). With the exception of transitions 1, 2, 4, 5 and 41 and 42, none of the bioenergy crops received either inorganic or organic fertiliser; transitions 41 and 42 received wood waste and fibroflos applications, transitions 1–5 received a combination of inorganic fertiliser and treated sewage sludge. All arable fields were under conventional management receiving annual tillage and regular fertiliser applications.

Transition 32 was excluded as this was a short-rotation forestry plantation rather than a SRC planation.

Fig. 1 Map of sampling locations. Dark grey = SRC willow, light grey = *Miscanthus*; the data points of different bioenergy crops present at the same location are offset.

randomly selected from intersections of a grid overlaid on a map of the cropped area of field. The resolution of the grid was adjusted to ensure that there were a minimum of 50 grid intersections, with the condition that the resolution of the grid could not be <5 m. A 20-m perimeter buffer was also used to reduce potential edge effects. Within the five sampling plots, the three within-plot soil cores were taken using a split-tube soil sampler (Eijkelkamp Agrisearch Equipment BV, Giesbeek, The Netherlands) with an inner diameter of 4.8 cm to a depth of 30 cm. The first core was taken at the grid intersect, with two further cores taken at distances of 1 m and 1.5 m in random compass directions from the intersect. This gave a total of 15 spatially nested samples per field, accounting for both field-scale (between sampling plots) and plot-scale (cores within plots) variability. Before each core was taken, litter (L) and fermentation (L_f) horizons were collected from a 25 cm × 25 cm area centred on the coring location. Soil cores were divided in the field into 0–15 cm and 15–30 cm (measuring from the base of the core), individually bagged and returned to the laboratory. There was limited compression in some cores and this was allocated to the 0–15 cm section under the observation that most compression occurred in the upper layer of soil. The depth of the hole was always measured to ensure that the accurate core length was known.

Deep cores (0–100 cm). One of the five sampling plots was randomly selected and three 1-m cores were taken following the same spacing as the 30-cm cores, with the exact coring locations adjusted to avoid those of the 30-cm cores. Cores were taken using a window sampler system with a 4.4 cm cutting diameter (Eijkelkamp Agrisearch Equipment BV, Giesbeek, The Netherlands), allowing a full 1-m core to be extracted and subsequently transported in one section. If coring to the full depth was not possible, for example when large stones or bedrock were encountered, the precise depth of the cored hole was recorded.

Laboratory processing

Litter samples were dried at 80 °C for 24 h and dry mass of woody material (e.g. twigs, branches), leaves and undifferentiated material was recorded. Litter was assumed to have C concentration based on litter dry mass of 43% and 45% for *Miscanthus* leaves and stem, respectively (Beuch *et al.*, 2000; Robertson *et al.*, in preparation), 42% and 49% for willow leaves and stems, respectively (Chauvet, 1987; Heller *et al.*, 2003), 46% for grass litter (Ross *et al.*, 2002) and 41% for cereal litter (Aita *et al.*, 1997).

Short cores (0–30 cm). The fresh mass of the 0–15 cm and 15–30 cm core sections was recorded and sections were then cut lengthways into quarters for separate subsequent analyses. One quarter was then set aside for processing for soil C and bulk density (BD, Table S1), together with the large stones and roots (>5 mm) hand-sorted from the remaining three sections. Another quarter was used to assess soil pH (Table S1) and the remaining sections were archived as a frozen sample (−20 ^{0}C).

For the assessment of soil pH, the fresh samples were bulked within each sampling plot but not across depths giving 10 composite samples per site (five each for the 0–15 cm and 15–30 cm depths). The fresh, bulked samples were sieved to 4 mm to remove stones and roots. 10 g of bulk soil was then mixed well with 25 ml of deionised water and allowed to stand for 30 min, before the pH of the liquid layer was recorded (Hanna pH210 Meter, Hanna Instruments Ltd., Befordshire, UK).

For BD, texture and soil C assessment, the fresh soil mass was recorded and then samples were air-dried at 25 °C for a minimum of 10 days. Air-dried samples were reweighed, sieved to 2 mm and the mass and volume of stones and roots remain on the sieve recorded. A subsample of the sieved soil (15–18 g) was oven-dried (105 °C for 12 h) and moisture-loss was recorded. The oven-dried subsample of soil was grounded in a ball mill (Fritsch Planetary Mill) and a 100-mg subsample was used for the assessment of C concentration using an elemental analyser (Leco Truspec CN, Milan, Italy). Prior to analysis using the elemental analyser, soil subsamples that were either from sites located on soil types known to contain inorganic C or which had pH values > 6.5 were tested for the presence of inorganic C using acid fumigation following Harris *et al.* (2001). All samples from sites which tested positive were treated to remove inorganic C following the same procedure.

A subsample of the sieved air-dried soil was also used to assess soil texture. As for pH measurement, samples were bulked across each field but not across depth, thus giving one value per field for each depth (0–15 cm and 15–30 cm). Analysis of the bulked samples was conducted by Macaulay Scientific Consulting Ltd. (Aberdeen, Scotland) with proportions of sand, silt and clay analysed by laser diffraction (Malvern Mastersizer 2000, Malvern Instruments Ltd., Worcestershire, UK). Analysis was conducted for both the bioenergy crops and the paired controls.

Bulk density of the whole core was calculated using values of moisture-loss from the air and oven-dried subsamples following methods in the GB Countryside Survey (Emmett *et al.*, 2008; Reynolds *et al.*, 2013). These calculations accounted for the measured mass and volume in the soil cores taken up by stones, and so are corrected to represent the fine earth proportion (Schrumpf *et al.*, 2011). The Countryside Survey conducted a pilot study to compare different protocols to estimate BD in different soil types and found that the method used in this study was consistent with other protocols and within the ranges of typical values expected for each of the soil types (Emmett *et al.*, 2008).

The soil C concentration and bulk density data were used to derive mass-based values of soil C stock to account for differences in bulk density across transitions. A soil C stock was calculated based on an equivalent soil mass approach (ESM), using a reference dry soil mass of 3 Gg ha^{-1}, following the method of Gifford & Roderick (2003).

Deep cores (0–1 m). On return to the laboratory, the 1-m cores were divided into three sections: 0–30, 30–50 and 50–100 cm. In cases where compression of the core had occurred during sampling, the length of the sections was reduced to account for the compression; a method also utilised by Walter *et al.* (2014). Depth increments of 0–30 cm, 30–50 cm and 50–100 cm were selected based on the common use of these increments in similar LUC studies (Laganière *et al.*, 2010; Don *et al.*, 2012).

Each 1-m core section was divided lengthways, one-half, and all root and stones (>5 mm) were processed for bulk density and C content as outlined for the 30-cm surface soil cores. The remaining half was retained as a frozen archive.

Soil C stocks were again calculated based on an equivalent soil mass approach (ESM), using a reference dry soil mass of 6 and 13 Gg ha^{-1} for the 0–50 cm and 0–1 m sections, respectively, following Gifford & Roderick (2003).

Treatment of under length core

In the ESM calculation, the length of the cores is not directly used to calculate soil C stocks (a reference mass is used and the deepest sections are used only to give C concentration). It is still necessary, however, to remove from the data set any cores that, due to the present of large stones or bedrock, do not reach a depth that provides a representative C concentration for the deeper soil layers. Therefore, based on inspection of the soil C profiles, cores <22.5 cm and 70 cm in length for the 30-cm and 1-m cores, respectively, were removed from the data set prior to statistical analysis (see Table S2 for details).

Statistical analysis

The difference in soil C stock and litter variables between the land uses (SRC, *Miscanthus*, arable and grassland) was tested using linear mixed-effect models with the *nlme* package in the R statistical program (Pinheiro *et al.*, 2014). Differences were observed in the control fields of the bioenergy crops with a higher overall mean soil C in the arable control sites of the *Miscanthus* transitions compared to the SRC transitions. The inclusion of site as a random factor was not sufficient to account for this underlying bias and, consequently, the SRC and *Miscanthus* transitions were analysed separately. Land use was entered as a fixed effect and field nested within site and plot nested within field entered as random effects in all models to ensure that appropriate comparisons of transition units were accounted for within site. The significance of the variable land use in the model was examined using a likelihood ratio test compared to the null model, including only random terms.

The significance of differences between the levels within 'land-use' was tested using Tukeys multiple comparison in the *glht* function in the *multcomp* package (Hothorn *et al.*, 2008). Marginal ($R_m^{2\circ}$) and conditional (R_c^2) R^2 values were calculated (Nakagawa & Schielzeth, 2013; Johnson & O'Hara, 2014) using the *r.squaredGLMM* function (Lefcheck, 2014) in the *MuMIn* package (Barton, 2015). Data on soil C for ESM at 0–30 cm and 0–1 m were log-transformed prior to testing to meet model assumptions. Litter data were x + 1 log-transformed due to high number of zero values in arable control fields. In all cases, means and standard errors given for land-use effects refer to model-estimated values, and therefore account for the random effect of site.

Difference in mean soil C stock between the controls and their paired bioenergy crops was divided by the age of the bioenergy plantation to estimate annual rates of change in soil C as Mg C ha^{-1} yr^{-1}. This procedure standardises differences in soil C stocks between the SRC and the *Miscanthus* control fields, allowing SRC and *Miscanthus* transitions to be combined into the same statistical test. Differences in annual rates of change between the 4 transitions (arable to SRC, grassland to SRC, arable to *Miscanthus* and grassland to *Miscanthus* transitions) were tested using a two-way ANOVA, with fixed factors of control land use (grassland or arable) and bioenergy crop (SRC and *Miscanthus*). Site was not included as a random factor as it was not found to improve the model fit.

Linear regression focused on the 0–30 cm depth where change was most likely and was used to explore variables influencing the impacts of transition to bioenergy crops on soil C stocks (clay content, soil pH, soil C stocks, bioenergy crop age, MAP and MAT). Data on percentage change from control were tested, again to standardise differences in soil C stocks between SRC and *Miscanthus* control fields, allowing SRC and *Miscanthus* transitions to be combined into the same statistical test.

The drivers of soil C changes were identified through model selection but the number of data points limited the complexity of candidate models. Therefore, $R_m^{2\circ}$ (Nakagawa & Schielzeth, 2013; Johnson & O'Hara, 2014) and Akaike's Information Criterion (AIC) were first used to assess the influence of each explanatory variable on the percentage change in soil C stock (Table S3). The explanatory variables were then added consecutively to the final models in the order indicated by greater $R_m^{2\circ}$ or lower AIC scores, provided the AIC of model continued to decrease. Site was included as a random variable in each model and calculation was performed in R using the *r.squaredGLMM* (Lefcheck, 2014) and *AIC* functions in the Lme4 and *MuMIn* package (Barton, 2015; Bates *et al*., 2015).

Selection based on both the $R_m^{2\circ}$ and the AIC scores resulted in the selection of the same model which included the fixed factors control soil C stock and the bioenergy crop type and the random effect of site (Table S4). The significance of the explanatory variables within this model was examined using a likelihood ratio test.

Prior to this analysis, exploration of the soil texture data showed that in contrast to the percentage sand and silt, which

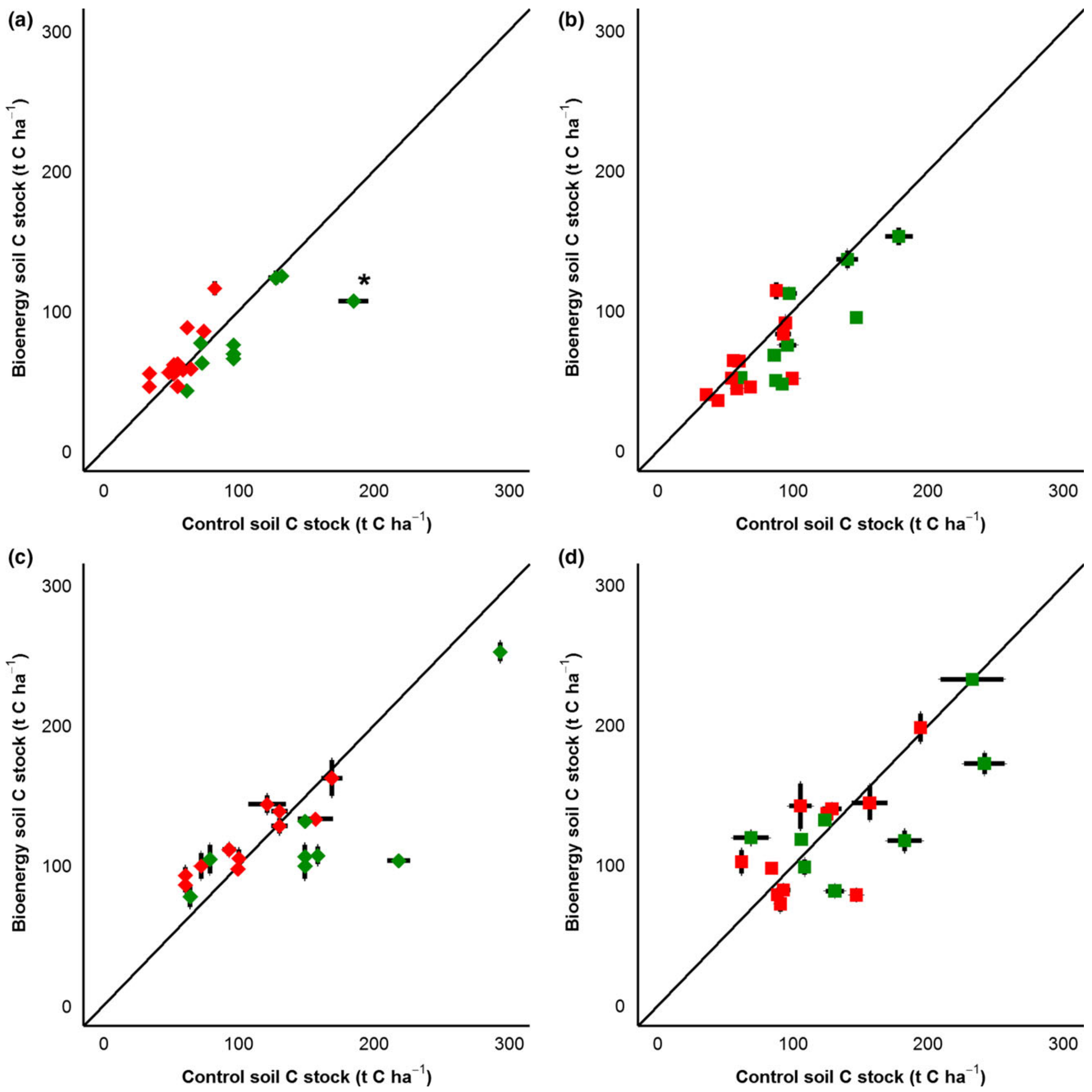

Fig. 2 Control versus bioenergy crops soil C stocks for the SRC transitions: 0–30 cm (a) 0–100 cm (c) depths, and the *Miscanthus* transitions 0–30 cm (b) 0–100 cm (d) depths; red symbols represent ex-arable transitions, and green symbols represent ex-grassland transitions. * indicates site 17 vs. 17C. Error bars give standard error.

showed a correlation between the bioenergy crops and paired control (R^2 = 0.72 and R^2 = 0.71, respectively; Fig. S1), the correlation for the percentage clay content was poor (R^2 = 0.26; Fig. S1). This poor correlation appeared to be related to high soil inorganic C in five of the sites (2, 5, 7, 17 and 19), a factor known to affect laser assessment of clay content (Kerry *et al.*, 2009). Removal of these sites resulted in an improvement to an R^2 of 0.62 but did not improve the explanatory power of the percentage clay in regard to the percentage change in soil C stocks (Table S3). Thus, this subset was not used in any subsequent analysis (Table S3).

Results

Soil C stocks 0–30 cm

Land use was found to affect surface (0–30 cm) soil C stock (Mg C ha^{-1}) in both the SRC ($\chi^2(3)$ = 15.30, P = 0.001, R_c^2 = 0.86) and *Miscanthus* transitions (χ^2 (3) = 13.71, P = 0.001, R_c^2 = 0.92) (Fig. 2a,b). The greatest differences in soil C stocks were in the grassland transitions, with mean soil C stocks 33.55 ± 7.52 Mg C ha^{-1} and 26.83 ± 8.08 Mg C ha^{-1} lower under the SRC (P = 0.004) and *Miscanthus* plantations (P = 0.001), respectively (Fig. 2a,b, Table 2).

Differences between the arable controls and bioenergy crop were smaller than those seen in the grassland transitions, with greater variation between sites, and not significant (P = 0.071 and P = 0.846 for SRC and *Miscanthus* transitions, respectively) (Fig. 2). The non-significant differences in mean soil C stocks are being 16.27 ± 7.18 Mg C ha^{-1} higher under SRC, and 2.26 ± 8.18 Mg C ha^{-1} lower under *Miscanthus* plantations compared to arable controls.

Within the SRC data, the grassland control at site 17 had exceptionally high soil C compared to its paired bioenergy crop (Fig. 2a). This transition unit was located at a site with highly complex underlying geology and variable soil types. Removing this transition from the analysis of soil C stock reduced the difference between the SRC and the grassland control. The mean soil C stock under the SRC, however, was still significantly lower (−23.34 ± 8.37 Mg C ha^{-1}) than the grassland controls (P = 0.047).

Differences in soil C stocks between the bioenergy crops and the controls were reflected in the annual rates of change (Mg C ha^{-1} yr^{-1}) in the surface soil (0–30 cm) with effects of both the original land use ($F_{1,37}$ = 11.99, P = 0.001) and also bioenergy crop type ($F_{1,37}$ = 6.59, P = 0.014) but there was no interaction between these factors ($F_{1,37}$ = 0.326, P = 0.571) (Table 3). Rates of change in the transitions from grassland, as would be expected by the differences in soil C stock, were consistently negative and significantly lower than observed in the arable transitions. Unlike the differences in soil C stock, annual rates of change also allowed the comparison of the two bioenergy crops and showed that the rates of change for the SRC transitions were more positive that those for the *Miscanthus* transitions (Table 3).

Soil C stocks 0–1 m

Over 0–1 m, soil C stocks (Mg C ha^{-1}) and annual rates of change followed a similar pattern to those seen in the surface soils (Fig. 1c,d, Tables 2 and 3). Unlike the surface soil, however, differences in soil C stocks between the controls and bioenergy crops were not significant in either the SRC (χ^2 (3) = 1.93, P = 0.3813, R_c^2 = 0.92) or *Miscanthus* transitions [χ^2 (3) = 2.10, P = 0.350, R_c^2 = 0.90)] (Table 2). Annual rates of change were not significantly different between the bioenergy crops ($F_{1,34}$ = 0.015, P = 0.902), nor was there any impact of the original land use ($F_{1,34}$ = 2.432, P = 0.128) or an interaction between these factors ($F_{1,34}$ = 1.166, P = 0.287) (Table 3).

Over a shallower depth of 0–50 cm, there were differences in soil C stocks in the SRC transitions (χ^2 (3) = 7.16, P = 0.028, R_c^2 = 0.91) but not the *Miscanthus*

Table 2 Mean litter and soil C stocks (Mg C ha^{-1}) and standard error for the bioenergy crops (SRC and *Miscanthus*) and controls

	C stock (Mg C ha^{-1})			
Land use	Litter	0–30 cm	0–50 cm	0–100 cm
SRC	0.97 ± 0.18^a	70.31 ± 6.57^a	91.16 ± 8.98^a	116.91 ± 11.65^a
Arable	0.38 ± 0.19^b	54.04 ± 8.18^a	76.41 ± 12.14^a	107.22 ± 16.14^a
Grassland	0.21 ± 0.19^b	103.87 ± 9.5^b	129.03 ± 12.6^{b*}	147.19 ± 16.56^a
Miscanthus	2.09 ± .0.24^a	74.31 ± 7.84^a	108.77 ± 7.70^a	124.31 ± 11.39^a
Arable	0.78 ± 0.33^b	76.57 ± 8.53^a	94.41 ± 9.51^a	120.98 ± 12.69^a
Grassland	0.06 ± 0.36^b	101.14 ± 8.89^b	123.47 ± 10.14^a	140.49 ± 13.75^a

0–50 cm ESM and 0–100 cm ESM refer to soil C stock based on reference soil mass for these depths of 6 and 13 Gg ha^{-1}. Same litter indicates nonsignificant difference > P 0.05; * indicates that there was a near-significant difference (P = 0.063) between the grassland and the SRC. Test conducted on *Miscanthus* and SRC transitions separately and within each depth division.

Table 3 Annual rates of change in soil C stocks for 0–30 cm, 0–50 cm and 0–1 m soil cores based on ESM. Annual rates of change are estimated by dividing change in mean soil C compared to control by the years since transition. n = 15 and 3 for the 30-cm cores and 1-m cores, respectively

Land-use Change	Rate of change Mg C ha^{-1} yr^{-1} (SE)		
	0–30 cm	0–50 cm	0–1 m
SRC vs. Arable	1.54 ± 0.70	1.93 ± 1.37	1.26 ± 1.41
SRC vs. Grassland	−1.69 ± 0.81	−2.98 ± 1.61	−2.74 ± 1.65
Miscanthus vs. Arable	−0.93 ± 0.74	0.18 ± 1.37	0.05 ± 1.41
Miscanthus vs. Grassland	−3.17 ± 0.81	−2.11 ± 1.52	−0.69 ± 1.65

transitions (χ^2 (3) = 4.34, P = 0.114, R_c^2 = 0.85) (Table 2). The significant difference in the SRC transitions was, however, related to differences in soil C stocks between the grassland and the arable control (P = 0.008), although there was also nonsignificant trend for lower soil C stocks within the grassland controls compared to the SRC (P = 0.063).

Rates of change reflected the absence of a significant difference in soil C stock, which were similar in both bioenergy crops ($F_{1,35}$ = 0.188, P = 0.667). There was no interaction between the current land use and the control land use ($F_{1,35}$ = 0.761, P = 0.388) but rates of change were lower in the grassland compared to arable transitions ($F_{1,35}$ = 5.952, P = 0.019; Table 3), highlighting a difference that was less clear with soil C stock.

Driving factors determining changes in soil C

Based on the model selection, soil C stocks of the control field and the current land use (SRC, *Miscanthus*) were tested for their effect on the percentage change in soil C stocks resulting from the transition the bioenergy crops (Tables S3, S4). Soil C stocks was found to be negatively related to the percentage difference in soil C in bioenergy fields (χ^2 (1) = 8.70, P = 0.003, R_c^2 = 0.52). There was no interaction between current land-use type (SRC, *Miscanthus*) and soil C stock (χ^2 (1) = 2.138, P = 0.144, R_c^2 = 0.51), suggesting a similar relationship in both SRC and *Miscanthus*, but a near-significant effect of land-use type was observed (χ^2 (1) = 3.216, P = 0.073, R_c^2 = 0.22) likely resulting from the different intercepts of the linear relationships in the two bioenergy crops (Fig. 3 a & b). Examination of the residuals highlighted that three transitions (*Miscanthus* transitions 24 and 25, and SRC willow transition 17) had a large influence on the results. Removal of these transitions influenced the slope of the linear relationships (Fig. 3 c & d), but did not change the overall significance of any of the factors.

Time since bioenergy establishment and the clay content of the bioenergy crop were the third most important factors influencing the change in soil C stock based on the marginal R^2 and AIC scores, respectively (Table S3). However, there was no clear relationship between the percentage change in soil C stock and either time since bioenergy establishment or clay content (Fig. 4).

Litter C stocks

Litter C stocks were different between the land uses in both the *Miscanthus* (χ^2 (2) = 25.42., P = 0.001, R_c^2 = 0.84) and the SRC plantations (χ^2 (2) = 43.68, P < 0.001, R_c^2 = 0.69), with *post hoc* testing showing that litter stock was higher in the bioenergy crops than in either the arable or grassland controls (Table 2). The addition of these relatively small litter C stocks to the surface soil C stocks (0–30 cm, Table 2) has little effect on the impact of the bioenergy crops on C stocks. C stocks remain lower in the bioenergy transitions than in grassland controls and are not significantly different to the arable controls.

Discussion

Soil C stocks in arable transitions

In this study, annual rates of change in the surface soil were more positive for the arable transitions than for the grassland transitions. Although, as soil C stocks in the SRC and *Miscanthus* plantations were not significantly different to the arable controls, the difference in the rates of changes is most likely related to the negative impacts on soil C stocks of transition from grassland, rather than any positive impacts of arable. This absence of a positive impact is contrary to a number of studies which have reported increases in topsoil C stocks following transitions from arable land uses to these bioenergy crops (Jug *et al.*, 1999; Dondini *et al.*, 2009; Schmitt *et al.*, 2010; Felten & Emmerling, 2012). These studies used a fixed depth method (FD) to calculate soil C stock which, unlike the ESM used in this study, makes no adjustment for changes in bulk density (BD) (Bárcena *et al.*, 2014). Applying FD methods to our data leads to a similar result to these studies with significantly lower surface soil C stock in the arable controls (Tables S5 and S6). The use of a FD method appears to inflate the differences between the arable control and the bioenergy crops, something that has been noted in a similar land-use change study (Bárcena *et al.*, 2014). The

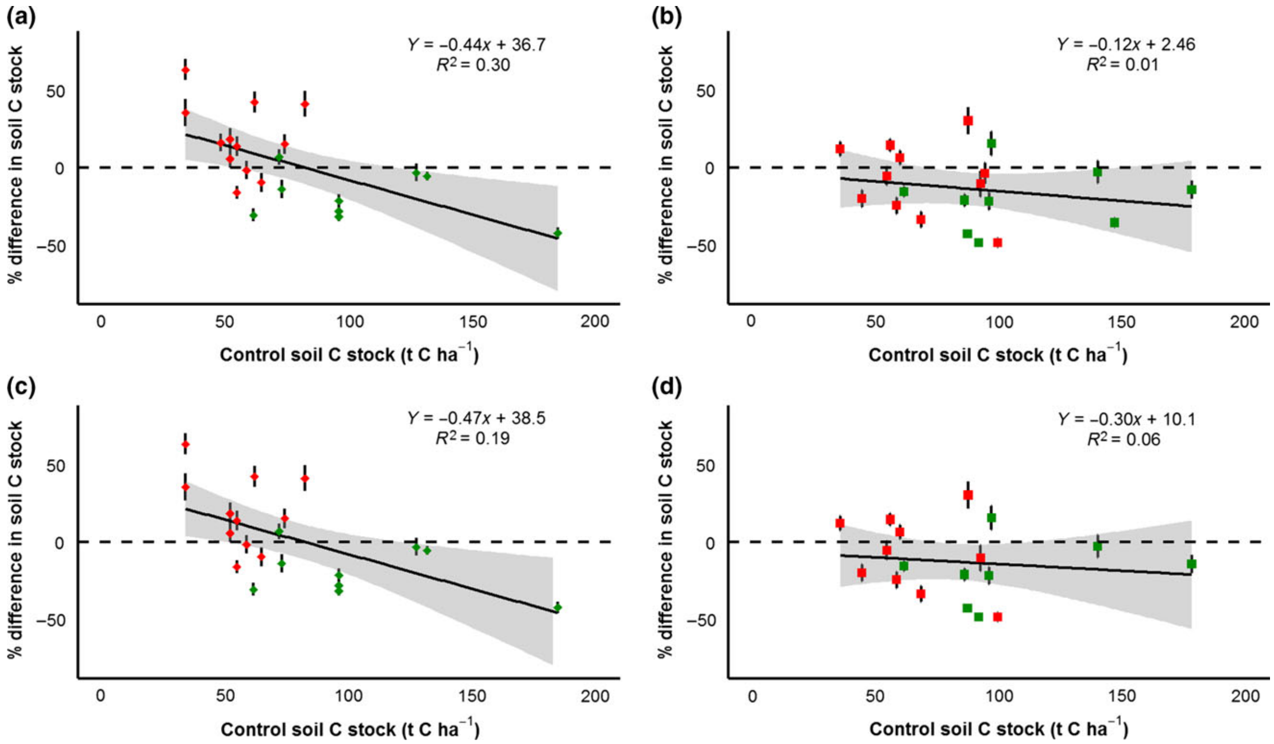

Fig. 3 Relationship between 0–30 cm soil C stocks in control crops and percentage differences for control in soil C resulting from land-use change for: SRC transitions (a), *Miscanthus* transitions (b), SRC transition without site 17 (c), *Miscanthus* transition without transitions 24 and 25 (d). Red markers indicate arable transition green grassland transition. The line shows linear regression of change in soil C stock with C stocks of the control fields; shaded area shows 95% of confidence interval, R^2 gives values for individual regression lines.

use of an ESM method is not widespread in bioenergy studies, and in the case of arable transitions, the only comparable study is that by Walter *et al.* (2014). Using an ESM method and a paired-site approach to assess impacts of arable to SRC transitions, Walter *et al.* (2014) also reported consistent changes in surface (0–30 cm) soil C stock.

Below the plough layer, BD is more consistent between land uses, and differences in C stock estimation due to method are less apparent. This is possibly reflected by the studies that have assessed soil C stock below 30 cm and reported no significant changes in transitions to either SRC (Coleman *et al.*, 2004; Lockwell *et al.*, 2012; Bonin & Lal, 2014; Walter *et al.*, 2014) or *Miscanthus* (Felten & Emmerling, 2012).

The age of the plantations studied may also have an impact on the soil C stock change. Hansen *et al.* (2004) reported higher soil C stocks under *Miscanthus* plantation compared to arable controls but only under the older of two plantations sampled (9 and 16 years old). A study of SRC by Dimitriou *et al.* (2012) also reported an increase in soil C stock compared to arable controls, but only one of the 14 sites sampled was under 15 years old. In addition, many were not in optimum condition leading the authors to suggest that some of the increase in soil C concentration could be related to C inputs from decaying stools and roots.

Within this study, the difference in the mean age of the bioenergy crops may also explain the differences in the rate of change between the SRC and the *Miscanthus* transitions. The mean annual rate of change in the surface soil for the SRC to arable transitions (1.43 ± 0.71 Mg C ha^{-1} yr^{-1}), with a mean age of 8.5 years, was within the upper range of reported values from 0.38 to 1.59 Mg C ha^{-1} yr^{-1} (Kahle *et al.*, 2010, 2013; Chimento *et al.*, 2014). In contrast, the annual rate of change for transitions to *Miscanthus* from arable (-0.93 ± 0.74 Mg C ha^{-1} yr^{-1}), with a mean age of 6.4 years, was more negative than the mean reported values for topsoil changes of 0.28–2.24 Mg C ha^{-1} yr^{-1} (Clifton-Brown *et al.*, 2007; Dondini *et al.*, 2009; Zimmerman *et al.*, 2012; Chimento *et al.*, 2014). This possibly reflects the mature plantations in some of these studies (16 years and 14 years in Clifton-Brown *et al.*, 2007 and Dondini *et al.*, 2009; respectively) compared to this study. It is also clear that impacts on soil C vary greatly between sites, even within individual studies. For example, although mean rates of change in the study by Zim-

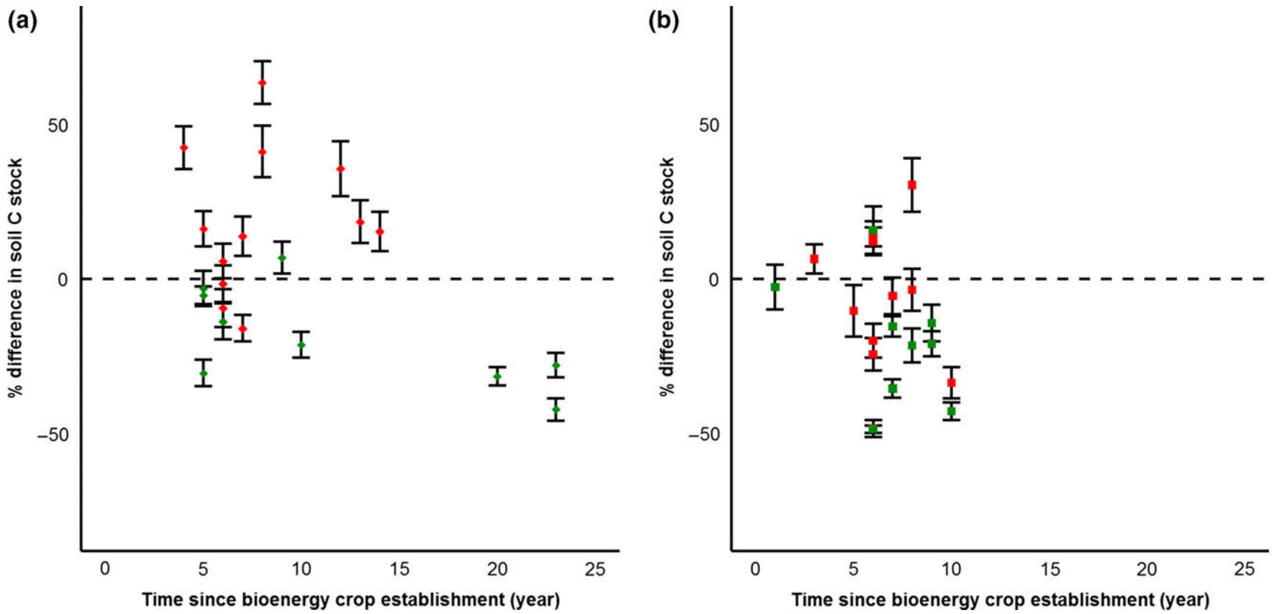

Fig. 4 Relationship between time since establishment (a) and bioenergy clay content, (b) and the percentage change in 0–30 cm soil C. Red markers indicate arable transition green grassland transition, diamond indicates SRC transitions and squares *Miscanthus* transitions. Error bars show pooled SE.

merman *et al.* (2012) were 1.79 Mg C ha^{-1} yr^{-1} from arable, the rates of change across sites within this study ranged from −6.85 to 7.7 Mg C ha^{-1} yr^{-1}. Site-specific factors clearly influence the impacts on soil C stocks, as reflected in the between-site variability observed within this study and reported in other multi-site studies (Coleman *et al.*, 2004; Dimitriou *et al.*, 2012; Don *et al.*, 2012; Walter *et al.*, 2014).

One possible additional source of variability between sites could be related to the willow clones selected. Nearly all the sites visited were planted by a single contractor whose records do not contain details of the clones planted at each site (F. Walters, Coppice Resources Ltd, Retford, pers.com.) but only that the mixed willow will contain 4–5 different clones. The lack of detailed information coupled with the practice of mixing clones throughout a single plantation (e.g. clones are not planted in uniform strips) for pest control purposes means that it is not possible within this study to examine differences between the influence of individual clones. However, any differences in soil C stock resulting from different clones are likely to be smaller than the impact resulting from the LUC from arable or grassland land uses.

Soil C stocks in grassland transitions

In contrast to the findings for arable soils, the lower soil C stocks in the topsoil (0–30 cm) and the negative rates of change of the SRC and *Miscanthus* plantations compared to the grassland controls reflect findings in other studies (Don *et al.*, 2012; Rytter, 2012; Zimmerman *et al.*, 2012). The mean annual rate of change in the transition to SRC (−1.69 ± 0.82 Mg C ha^{-1} yr^{-1}, 0–30 cm) compares well, once again, with the values reported in a study of a 9-year-old SRC willow plantation by Lockwell *et al.* (2012) of −2.22 and −1.11 Mg C ha^{-1} yr^{-1} over 0–20 cm and 0–40 cm depths, respectively. The mean rate of change for transitions to *Miscanthus* from grassland, however, was again more negative (−3.17 ± 0.81 Mg C ha^{-1} yr^{-1}) than those reported from −1.66 and 0.83 Mg C ha^{-1} yr^{-1} by Zimmerman *et al.* (2012) and Zatta *et al.* (2014).

Over the greater depth of 1 m, the magnitude of differences in soil C stocks observed was similar to those seen in the surface soil, especially for the SRC transitions (−33.55 ± 7.52 Mg C ha^{-1} and −30.28 ± 10.96 Mg C ha^{-1} for 0–30 cm and 0–1 m, respectively) but differences were no longer significant. Fewer 1-m cores were taken compared to the 30-cm cores, resulting in reduced statistical power to detect impacts at greater depth. Walter *et al.* (2014) and Lockwell *et al.* (2012), however, reported similar findings in transitions from grassland to SRC, concluding that soil C losses in the surface soil were offset by increases lower in the soil profile, resulting in no significant changes in soil C stocks overall. *Miscanthus* shares the tendency of SRC to be deep rooting and *Miscanthus*-derived C inputs have been detected at depths of up 1.5 m (Felten & Emmerling, 2012), and thus, there is a mechanism by which both crops could alter soil C stocks at depth. In this study, mean difference in soil C stocks between both the SRC and *Miscanthus* and their grassland controls was less negative over 0–1 m than over 0–30 cm. Differences in sampling intensity between 0–30 cm and 0–1 m cores mean that it is not possible to directly attribute any redistribution of soil C within the soil profile.

Alternatively to a redistribution of soil C stocks, it is possible that changes in soil C stock were limited to the surface soil and that difficulties in detecting changes in soil C stock 0–1 m are instead due to the dilution of the impacts in the surface soil when including soil C stock at greater depths. This would agree with studies which report slower turnover times in the subsoil, with reported mean C resident times in soil layers below 20 cm of 2000–10 000 years (Fontaine *et al.*, 2007). Sampling subsoil is, however, still extremely valuable as although C stocks at depth may be characterised by long residence times, they have also been found to be susceptible to priming resulting from labile C inputs such as root exudates (Fontaine *et al.*, 2007; De Graaff *et al.*, 2014). Deep soil coring therefore provides a mechanism to detect both increase in soil C and any losses due to C priming.

Regardless, if losses in the surface soil are replaced with gains at depth or just diluted, any step taken to reduce surface soil C loss would be beneficial. Grassland soil C stocks have been shown to be negatively affected by tillage (Poeplau & Don, 2014). Thus, it has been suggested that the intensive cultivation undertaken prior to the bioenergy crop establishment may account for a substantial proportion of soil C losses observed (Don *et al.*, 2012; Walter *et al.*, 2014). A move to new, less intensive establishment methods may provide one option to reduce impacts on soil C stocks. However, it is unclear what role other factors, such as changes in the quality or quantity of inputs to soil, may play in addition to the effects of cultivation. For example, Poeplau & Don (2014) reported that transitions from grassland to forestry resulted not only in changes in soil C stocks but also a shift in soil C from stable to labile pools.

Factors influencing changes in soil C stock

Explaining variations in soil C stock changes within this study was explored through assessment of relationships between changes in soil C stock and selected factors. A negative relationship was found between changes in soil C stock and the soil C stock of the control field, suggesting that establishment of bioenergy crops on sites with low initial soil C provided the best opportunity to derive positive impacts on soil C stocks. Such a negative relationship was predicted for SRC poplar plantations in modelling work by Garten *et al.* (2011) and generally agrees with the conclusions of Don *et al.* (2012) and Walter *et al.* (2014) that conversion of arable lands, which generally have low soil C stock, is preferable to conversion of grassland for bioenergy plantations. It is difficult to separate the impacts of original soil C stocks and original land use because they are highly correlated (e.g. higher soil C stocks are generally associated with grassland sites). As land use also affects soil C stability and turnover, as well as soil C stocks (Poeplau & Don, 2014), impacts of land-use transitions could be influenced by both the stability of the soil C and the total soil C stocks.

The relationships between control soil C and changes in soil C stock following bioenergy crop establishment are relatively weak, especially for *Miscanthus*. Transitions to SRC and *Miscanthus* have R^2 values of 0.30 and 0.01, respectively, which indicate considerable unexplained variability related to the impacts on soil C stocks at individual sites. Part of this unexplained variability may reflect the challenge of finding paired sites with no pre-existing differences in soil C stocks between the two land uses before conversion. In many cases, the bioenergy crops and paired sites were adjacent but the soil texture analysis does suggest, even for the more reliable sand and silt data, that in a few of the sites there may be some underlying differences between some of the transition pairs. In addition, whilst finding sites with generally similar land-use histories was relatively straightforward, the normal crop rotation practices (rotations wheat, barley, beans, etc.) and the variable nature of farming (fertiliser inputs, harvest times, etc.) combined with the limited nature of long-term data held by land owners meant that some variability between the bioenergy crop and the paired control was inevitable. A better understanding of between-site variability is also clearly needed. For example, in this study, the rates of change in soil C stock range from -3.75 to 0.58 Mg C ha^{-1} yr^{-1} for grassland to SRC transitions, and from -7.44 to 2.53 Mg C ha^{-1} yr^{-1} for grassland to *Miscanthus* transitions.

It is worth noting that whilst underlying differences between the paired sites could have influenced the analysis of the potential factors driving soil C stock change, and the rates of change, where the percentage change was calculated at the transitions level, in the assessment of soil C stocks individual core data rather than transition level mean were used. When using this core data, the mixed model is less sensitive to variation between the bioenergy crop and the control.

No relationship was found between bioenergy planation age or clay content and changes in soil C stock. In the case of clay content difficulties with both the analysis method and a limited range of clay content across the sites (3.50–12.56% Table 1 Fig. S1) may have reduced our ability to detect a relationship. However, the absence of any relationship between soil texture and changes in soil C stocks has also been reported for SRC (Walter *et al.*, 2014) and *Miscanthus* (Poeplau & Don, 2014). Clay content tends to be positively associated with soil C stock (Stockmann *et al.*, 2013) and the absorption of C compounds to clay minerals, together with occlusion into clay aggregates, has been shown to stabilise soil organic matter (Dungait *et al.*, 2012; Stockmann *et al.*, 2013). Therefore, there could be an expectation that higher clay content would protect soil C during LUC, and aid its accumulation post LUC (Laganière *et al.*, 2010). One possible reason why this is not seen could be that the current practice of intensively tilling prior to bioenergy crop planting could reduce the protection afforded by occlusion into clay aggregates (Stockmann *et al.*, 2013).

A relationship between changes in soil C stock and time since bioenergy crop establishment was also absent, something which has been reported in a number of other multi-site studies (Don *et al.*, 2012; Walter *et al.*, 2014). This is despite general agreement across a wide range of land-use transitions that time since LUC is an important factor in determining soil C stocks (Bárcena *et al.*, 2014; Poeplau & Don, 2014; Walter *et al.*, 2014). Bárcena *et al.* (2014) suggested that the time taken for soil C stocks to recover from any initial soil C loss following land-use transitions, and to reach a new equilibrium, may vary between sites. Thus, any assessment made between sites that are yet to near a new equilibrium will lead to highly variable results (Bárcena *et al.*, 2014). In case of transitions from arable to forestry, Bárcena *et al.* (2014) found that increases in soil C were only detectable in a chronosequence of independent sites after 30 years. The time required for soil C recovery in SRC and *Miscanthus* plantations is, as yet, unknown. Walter *et al.* (2014) did select older plantations (15–35 years) in their study of 21 SRC plantations, but were still unable to detect any relationship between plantation age and impacts on soil C stock. Therefore, it may be that the time period required to detect an effect of age on soil C under bioenergy crops will exceed the expected 25–30 year life span of these plantations.

The time taken to reach a new soil C equilibrium has potential to impact on the 'payback time' required for any decreases in soil C stocks within the soil to be replaced (Mello *et al.*, 2014). In contrast to the transitions from arable, where changes in soil C stock were not significant, there is not a soil C debt to be paid. A soil C debt was detected in the surface soil, at least in grassland transitions. To replace this debt through increases in the soil C stock, the bioenergy crops must in theory reach a new soil C equilibrium that is equal to or greater that than of the grassland. The time it takes to reach this new equilibrium is also critical because, if it takes longer than the lifetime of the bioenergy crop, it may not be possible to repay the soil C debt through changes in soil C stock alone (Bárcena *et al.*, 2014; Mello *et al.*, 2014). Although it must be recognised that over greater depths this and other studies have found no significant negative impact of planting on grassland (Walter *et al.*, 2014). Although requiring a detailed life-cycle assessment to confirm, the C saving attributed to using biomass to offset fossil fuel use may be greater than any soil C loss as has been found to be the case for sugarcane planted on pasture in Brazil (Mello *et al.*, 2014).

It is possible that the difficulties in detecting a clear chronosequence may also result from different sites having different linear relationships between age and soil C and/or more complex nonlinear relationships. In addition, the C stock within the control field may not be in equilibrium, and for this reason, it is best to view controls as counterfactuals rather than a time zero. Long-term studies utilising both repeated sampling and the use of counterfactual paired sites, soil fractionation (Poeplau & Don, 2014) and process base modelling (Dondini *et al.*, 2015) are all methods which could help to provide a better understanding of the time it will take to reach a new equilibrium, and allow the comparison to other land-use options. The data collected in this study are highly suited for process models, which can be used to understand key drivers of soil C change, and such models can be used to predict impacts of future climate scenarios (Dondini *et al.*, 2015).

We conclude that where choices exist, the selection of arable land for bioenergy transitions to SRC and *Miscanthus* is likely to be more positive for soil C stocks than conversion from grassland, at least for soil C stocks within the surface soil. Whilst changes in soil C stocks at 0–1 m were not significant in any of the transitions types, the direction of changes mirrored those in the surface soil. Questions still remain as to why transitions from grassland can lead to negative changes in soil C, and work on soil C stability, especially during bioenergy crop establishment, would both address this question and potentially provide insight into management solutions that would maximise the soil C sequestration potential of these crops. Whilst these conclusions are valid for soil C, the findings also need to be considered in the wider context of other ecosystem services such as productivity, greenhouse gas regulation and water quality.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Fig. S1. Relationships between clay, silt and sand in the bioenergy crops and paired controls.
Table S1. Site data on pH and Bulk density.
Table S2. Sites and transitions under length cores.
Table S3. Marginal and conditional R^2, and the AIC score for the potential explanatory variables.
Table S4. Model selection based on $R_m^{2\circ}$ and AIC scores.
Table S5. Comparison of soil C stocks for the surface soil (0–30 cm cores) calculated using fixed depth (FD) and equivalent soil mass (ESM) methods.
Table S6. Additional site details including soil texture (% silt and sand) and C stocks based on fixed depth.