

Comparative assessment of ecosystem C exchange in *Miscanthus* and reed canary grass during early establishment

ÓRLAITH NÍCHONCUBHAIR^{1,2}, BRUCE OSBORNE^{2,3}, JOHN FINNAN⁴ and GARY LANIGAN¹

¹Teagasc Environmental Research Centre, Johnstown Castle, Co. Wexford, Ireland, ²UCD School of Biology & Environmental Science, University College Dublin, Dublin 4, Ireland, ³UCD Earth Institute, University College Dublin, Dublin 4, Ireland,

⁴Teagasc Crops Research Centre, Oak Park, Carlow, Ireland

Abstract

Land-use change to bioenergy crop production can contribute towards addressing the dual challenges of greenhouse gas mitigation and energy security. Realisation of the mitigation potential of bioenergy crops is, however, dependent on suitable crop selection and full assessment of the carbon (C) emissions associated with land conversion. Using eddy covariance-based estimates, ecosystem C exchange was studied during the early-establishment phase of two perennial crops, C₃ reed canary grass (RCG) and C₄ *Miscanthus*, planted on former grassland in Ireland. Crop development was the main determinant of net carbon exchange in the *Miscanthus* crop, restricting significant net C uptake during the first 2 years of establishment. The *Miscanthus* ecosystem switched from being a net C source in the conversion year to a strong net C sink ($-411 \pm 63 \text{ g C m}^{-2}$) in the third year, driven by significant above-ground growth and leaf expansion. For RCG, early establishment and rapid canopy development facilitated a net C sink in the first 2 years of growth (-319 ± 57 (post-planting) and $-397 \pm 114 \text{ g C m}^{-2}$, respectively). Peak seasonal C uptake occurred three months earlier in RCG (May) than *Miscanthus* (August), however *Miscanthus* sustained net C uptake longer into the autumn and was close to C-neutral in winter. Leaf longevity is therefore a key advantage of C₄ *Miscanthus* in temperate climates. Further increases in productivity are projected as *Miscanthus* reaches maturity and are likely to further enhance the C sink potential of *Miscanthus* relative to RCG.

Keywords: bioenergy crops, C₄ photosynthesis, carbon balance, eddy covariance, grassland, land-use change, leaf longevity, perennial rhizomatous grasses, *Phalaris arundinacea*, reed canary grass

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Introduction

Global concerns surrounding the impact of anthropogenic greenhouse gas (GHG) emissions on our climate allied with challenges to global energy security are driving interest in renewable energy, including bioenergy. In the European Union, member states have committed to increasing the contribution of renewable energy to 20% of total energy consumption by 2020 (EU, 2009). Furthermore, limitations identified in first-generation liquid biofuels have engendered an increased focus on second-generation alternatives produced from ligno-cellulosic plant materials (Sims *et al.*, 2010). Realisation of the potential of bioenergy in the EU will, however, require significant agricultural land area, estimated at between 18 and 21 million hectares (Özdemir *et al.*, 2009).

Perennial rhizomatous grasses (PRGs), such as *Miscanthus × giganteus* and reed canary grass (RCG), confer many advantages as potential nonfood bioenergy crops and have received increasing attention in Europe and the USA in recent decades (Landström *et al.*, 1996; Lewandowski *et al.*, 2003). *Miscanthus*, a C₄ plant originating from East Asia, can be highly productive, yielding 10–25 t dry matter ha⁻¹ y⁻¹ in central and northern Europe (Lewandowski *et al.*, 2000; Finnan & Burke, 2014), while yields of RCG, a C₃ perennial grass indigenous to temperate regions of Europe, Asia and North America, ranged from 5 to 12 t dry matter ha⁻¹ y⁻¹ in trials in northern Europe (Landström *et al.*, 1996; Saijonkari-Pahkala, 2001; Lewandowski *et al.*, 2003; Kandel *et al.*, 2013). As perennial species, both *Miscanthus* and RCG invest significant resources below ground, thus building reserves for more rapid canopy development in the spring compared with annual crops (Beale & Long, 1995; McLaughlin & Walsh, 1998).

Correspondence: Órlaith Ní Choncubhair, tel. +353 53 9171200, fax +353 53 9142213, e-mail: o.nichoncubhair@teagasc.ie

High productivity in *Miscanthus* has historically been attributed to the superior light-, water- and nitrogen-use efficiency afforded by the C₄ photosynthetic pathway (Long, 1983). For example, the maximum efficiency of solar energy conversion in C₄ crops has been estimated to be 40% higher than that of C₃ species (Monteith, 1978). Furthermore, studies have highlighted the crop's exceptional ability to maintain high photosynthetic productivity even in cool temperate climates (Beale & Long, 1995). This is most likely achieved through a combination of reduced susceptibility to photoinhibition and decreased sensitivity to chilling temperatures (Beale *et al.*, 1996; Naidu & Long, 2004; Wang *et al.*, 2008).

More recent studies have, however, identified extended leaf longevity and high leaf area as the driving factors contributing to greater productivity in *Miscanthus*. In a side-by-side comparison of field-scale stands of *Miscanthus* and C₄ maize (*Zea mays*) in the USA, Dohleman & Long (2009) showed that the efficiency of captured sunlight-to-biomass conversion was almost identical in both crops averaged over two growing seasons. However, light interception efficiency was 61% higher in *Miscanthus*, which developed a closed canopy a month earlier than maize and maintained it a month longer in the autumn. This resulted in substantial net carbon gains for the cold-tolerant *Miscanthus* crop due to enhanced leaf area duration (Dohleman & Long, 2009).

The question remains, however, as to whether *Miscanthus* can out-perform native C₃ bioenergy crop candidates in temperate regions in terms of productivity, leaf longevity and net C sequestration. RCG, in particular, grows vigorously after seed establishment and easily out-competes weeds after the first year of establishment (Lewandowski *et al.*, 2003). Indeed, its competitive advantages have allowed RCG to become an invasive species in certain wetland areas of the mid-western and north-western USA (Wrobel *et al.*, 2009). To date, no direct comparisons of *Miscanthus* and RCG under the same environmental conditions have been made to understand the relative dynamics of crop development and C assimilation in these ecosystems. This information would: (1) provide focus for exploiting favourable plant traits and developing superior genotypes for bioenergy production, (2) highlight the relative merits of *Miscanthus* and RCG in different climatic zones with varying growing seasons and (3) reveal the C balance implications of land-use change (LUC) to these crops.

Additional factors, such as the previous land use and the magnitude of LUC-related emissions, will significantly impact the long-term C balance of established bioenergy crops. The initial transition phase of LUC can be associated with substantially increased GHG emissions (Guo & Gifford, 2002; Fargione *et al.*, 2008;

Donnelly *et al.*, 2011; Poepflau *et al.*, 2011; Houghton *et al.*, 2012), particularly if bioenergy crop yields are low (Don *et al.*, 2012). The time taken for the crop to achieve maximum productivity is also likely to be a significant determinant of the early establishment C balance.

In mature *Miscanthus* crops, soil C sequestration rates of 0.4–0.66 Mg ha⁻¹ y⁻¹ have been reported for plantations established on former croplands (Don *et al.*, 2012; Zimmermann *et al.*, 2012; Poepflau & Don, 2014). Less information is available on grasslands converted to *Miscanthus*, but limited meta-analysis data has shown an annual carbon sequestration rate close to zero (no positive or negative effect) (Don *et al.*, 2012; McCalmont *et al.*, 2016; Qin *et al.*, 2016). Measurements of carbon exchange in RCG plantations have provided evidence of net C uptake in these ecosystems (Shurpali *et al.*, 2009; Mander *et al.*, 2012; Lind *et al.*, 2015); however, these studies were largely confined to drained peat extraction areas and there is little information on the carbon balance of RCG crop plantations established on mineral soils.

This study presents the first side-by-side field-scale comparison of *Miscanthus* and RCG to investigate comparative differences in crop development, leaf longevity and ecosystem-scale C fluxes from initial establishment to near maturity. The bioenergy crops were established on land previously under permanent grass. Conversion of grassland to bioenergy crops is of particular relevance in Ireland and in the wider European continent as over 90% and 35% of utilised agricultural area in Ireland and the EU-27, respectively, is currently used for grass production (Central Statistics Office, 2014; Huyghe *et al.*, 2014). The specific aim of the study was to address the following questions: (1) what are the C emissions associated with the initial LUC from grassland, which we define as the 'transitional phase'? (2) What are the longer term C balance implications of establishing *Miscanthus* and RCG crops on permanent grasslands (the 'post-establishment phase')? (3) How does leaf longevity in *Miscanthus* compare to that of an indigenous C₃ bioenergy crop and does it result in higher net C uptake?

Materials & Methods

Site description & management

The study was carried out from late April 2009 to the end of December 2011 at the Teagasc Environmental Research Centre, Johnstown Castle, Co. Wexford in the south-east of Ireland (52.3°N, 6.5°W, 67 m above sea level). This region has a maritime temperate climate with a mean annual rainfall of 1038 mm distributed evenly across the year and a mean annual air temperature of 10.4 °C. The seasonal range in temperature is narrow (Fig. 1), with an average summer air temperature of

14.9 °C and an average winter air temperature of 6.3 °C. The prevailing wind direction is south-westerly.

The *Miscanthus* and RCG crops were established on two former grassland sites (2 ha and 1 ha in area, respectively) (Fig. 2). Most of the experimental area had been maintained as grassland for at least 37 years and was managed organically for beef production since 2006. The *Miscanthus* site had been conventionally tilled and reseeded with perennial ryegrass in 2000 and surface seeded with white clover in 2005. The RCG site had been conventionally tilled and reseeded with perennial ryegrass and red clover in 2005. Otherwise, historical land management activities were comparable at both sites. The swards received organic fertiliser in the form of cattle slurry and farmyard manure. Grazing took place every 3–4 weeks until October 2008. Soils in this area are variable and are classified as imperfectly drained Gleys (FAO classification: Gleyic Cambisol) or moderately to well-drained Brown Earth soils (Cambisol). Selected soil physical and chemical properties of the *Miscanthus*, RCG and adjacent reference grassland sites are summarised in Table 1. The wilting point, field capacity and water content at saturation were calculated using a hydraulic properties model (<http://hydrolab.arsusda.gov/soilwater/Index.htm>) as 0.17, 0.32 and 0.59, respectively, for all sites.

On the 1st April 2009, both sites were sprayed with glyphosate to eradicate the extant vegetation. The soil was conventionally tilled using a mouldboard plough to a depth of 20 cm on the 27th April 2009 (approximately one tenth of the *Miscanthus* site) and completed on the 29th April 2009, followed by power-harrowing on the 1st and 5th June 2009. *Miscanthus* rhizomes were planted on one site on the 9th and 10th June 2009 and the soil was consolidated using a heavy roller 1 week later. Additional herbicides were applied to the *Miscanthus* site in the early establishment phase to reduce competition from grass and broad-leaf weeds. The selective herbicide MCPA was sprayed to control broad-leaf species (13th August 2009 and 27th July 2010) while glyphosate was applied soon after harvesting (5th March 2010 and 8th March 2011) to control grass weeds. The crop was cut with a conditioner mower on the 4th March 2010 and 4th March 2011 but the limited biomass material that was cut was left on the ground.

The second site lay fallow until April 2010 when RCG was established. For this, the site was power-harrowed and seeded with RCG (*Phalaris arundinacea* L.) at a rate of 30 kg ha⁻¹ on the 15th April 2010, and then consolidated with a heavy roller 1 day later. MCPA was applied on the 29th June 2010 to reduce competition from broad-leaf species. The RCG crop was harvested once during the study period, on the 12th October 2010, while harvesting of the 2011 crop was delayed until spring 2012, which is the preferred time for harvesting. No fertilisers were applied over the duration of the study.

Micrometeorological measurements

Ecosystem-scale CO₂ fluxes were measured using an open-path eddy covariance (EC) system commencing on the 28th April 2009 and 15th April 2010 at the *Miscanthus* and RCG sites, respectively. The instrumentation was identical at both sites and consisted of an open-path infrared gas analyser (IRGA)

(LI-7500, LI-COR Biosciences, Lincoln, NE, USA) coupled with a 3D sonic anemometer (CSAT3, Campbell Scientific, Logan, UT, USA). EC data were collected at a frequency of 10 Hz and averaged over 30-minute intervals. The flux tower was located in the north-eastern corner of the fields to maximise the fetch in the direction of the prevailing south-westerly wind (Fig. 2). Tower height was increased during periods of active growth to maintain its position above the canopy while restricting the flux footprint to the experimental area for as much time as possible. The maximum tower height was 4 m and 3 m at the *Miscanthus* and RCG sites, respectively, while the minimum sensor to canopy height ratios were 1.47 (*Miscanthus*) and 1.52 (RCG).

Ancillary biometric sensors included an air temperature and relative humidity probe (HMP45C, Campbell Scientific, Logan, UT, USA), a net radiation sensor (NR-Lite, Kipp & Zonen, Delft, The Netherlands) and a down-welling quantum sensor (SKP 215, Skye Instruments Ltd., Llandrindod Wells, UK). Two self-calibrating soil heat flux plates were installed at 8 cm soil depth (HFP015C, Hukseflux, Delft, The Netherlands) and averaging soil temperature probes were installed at 2 cm and 6 cm depth above the soil heat flux plates. Time domain reflectometers (CS616, Campbell Scientific, Logan, UT, USA) measured soil volumetric water content (VWC) in the upper 15 cm of soil. Daily meteorological data (mean air temperature, total rainfall and global solar radiation) were obtained from a Met Éireann synoptic weather station located 1.7 km from the field site.

Quality assurance and flux analysis

Data quality control procedures included spike removal (Vickers & Mahrt, 1997), time lag compensation using a covariance maximisation procedure and compensation for air density fluctuations using the WPL term (Webb *et al.*, 1980). The double rotation method (Kaimal & Finnigan, 1994) was used to correct for sonic anemometer tilt as the alternative planar fit method requires several weeks of measurement with constant instrumental set-up and is often not recommended for measurements over canopies with dynamic height variation (Moureaux *et al.*, 2012). Spectral attenuation effects were corrected following the analytical methods of Moncrieff *et al.* (1997). Tests on developed turbulence and stationarity were applied to the calculated fluxes (Table 13, Mauder & Foken, 2004) and data of questionable quality (QC-flag = 2) were removed, while data of moderate quality (QC-flag = 1) were retained but not included in the regression analysis performed in the gap-filling procedure. Data screening based on the results of these tests has been shown to result in a less systematic distribution of data gaps compared to the removal of fluxes below a derived friction velocity (u_*) threshold (Ruppert *et al.*, 2006). Plausible limits were also applied to net ecosystem exchange (NEE) ($-50 < \text{NEE} < 30 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$), latent heat ($-20 < \text{LE} < 600 \text{ W m}^{-2}$) and sensible heat ($-100 < \text{H} < 300 \text{ W m}^{-2}$) fluxes.

The peak location of the flux footprint, x_{max} , and the distance from the flux tower which includes 90% of the source area contributing to the measured flux, x_{R90} , were estimated for the prevailing south-westerly wind direction using the Kljun *et al.* (2004) model as 35 m and 95 m, respectively, for the *Miscanthus* site and 32 m and 87 m, respectively, for the

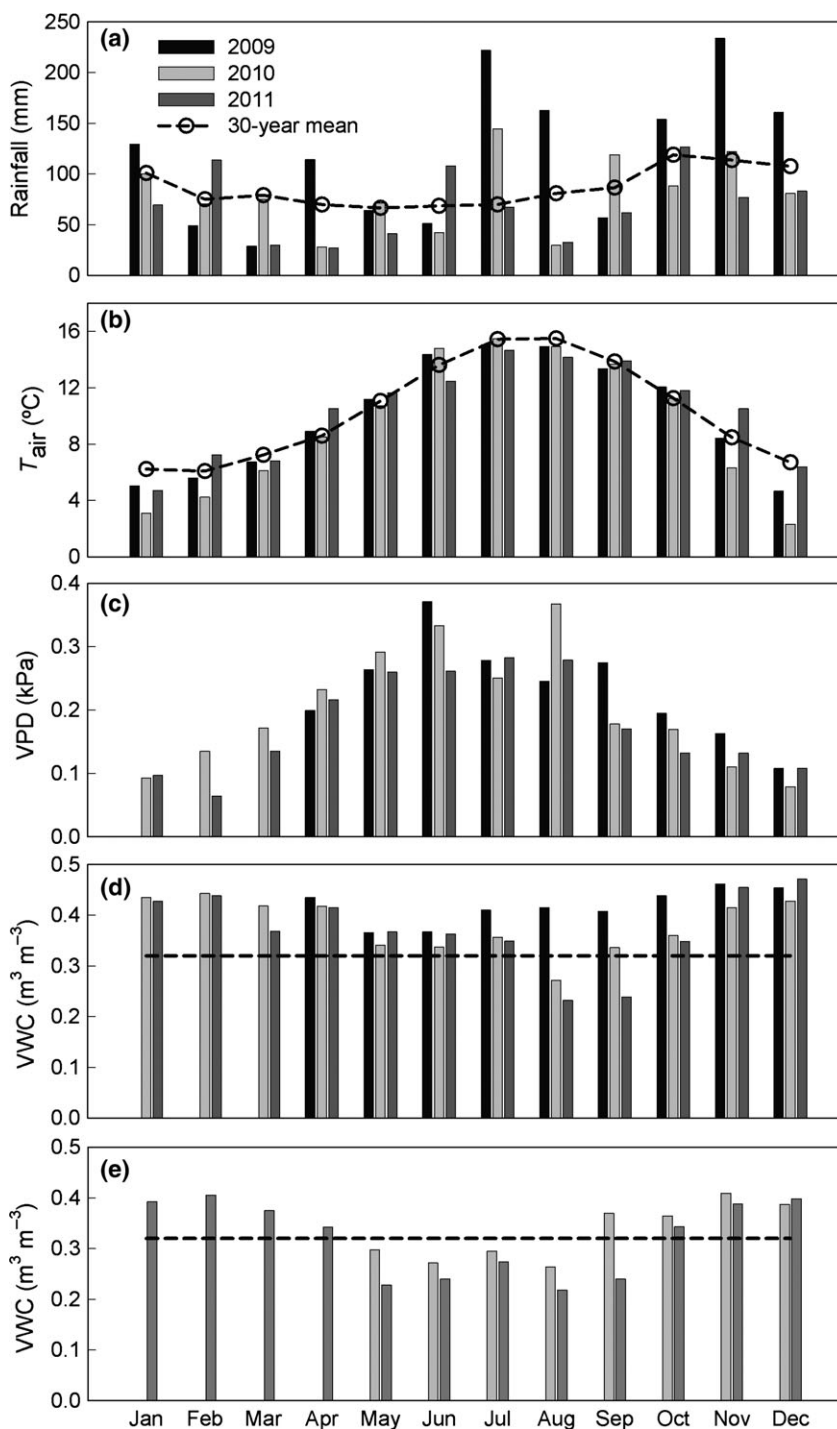


Fig. 1 Mean monthly values for total rainfall (a), air temperature (b), vapour pressure deficit (VPD) (c) and soil volumetric water content (VWC) at the *Miscanthus* (d) and RCG (e) sites for 2009, 2010 and 2011. The 30-year (1981–2010) mean monthly rainfall and air temperature values for Johnstown Castle are also shown. The soil VWC at field capacity is included in (d) and (e) as dashed black lines.

RCG site. Owing to the relatively constrained field sizes, detailed footprint analysis was conducted on half-hourly fluxes based on the analytical Kormann & Meixner (2001) footprint model. Since analytical models tend to overestimate flux footprints in comparison to more complex Lagrangian

stochastic models due to the neglect of along-wind velocity fluctuation (Kljun *et al.*, 2003), this approach is likely to be conservative. Sample flux footprints for the two towers at their maximum height under unstable conditions are shown in Fig. 2. At the maximum height, the footprint of the *Mis-*

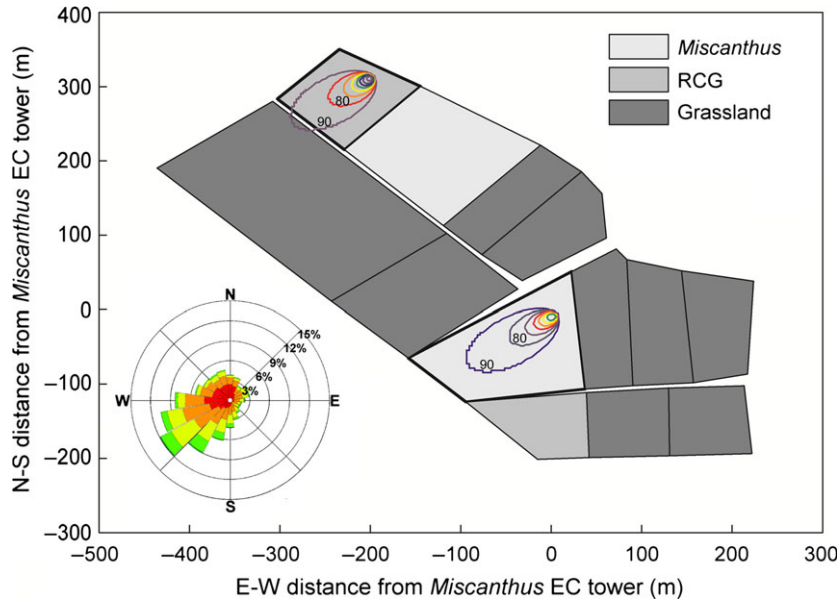


Fig. 2 Experimental site map showing the location and dimensions of the *Miscanthus* and RCG field sites and surrounding grassland fields, as well as a wind rose displaying the distribution of mean wind direction for the site. The wind rose was generated using the freeware WRPLOT View (<http://weblakes.com>). Contributions from wind speed classes 0.5–2.1 (red), 2.1–3.6 (orange), 3.6–5.7 (yellow), 5.7–8.8 (green) and $\geq 8.8 \text{ m s}^{-1}$ (dark green) are depicted. Sample footprints are shown for the *Miscanthus* and RCG eddy covariance towers at their maximum height under unstable conditions ($z/L = -0.06$, where z is the height and L is the Monin-Obukhov length). Contour lines represent the crosswind integrated cumulative contribution (%) to the estimated footprint.

canthus tower was well conserved within the *Miscanthus* field while close to 90% of the flux footprint from the RCG tower was derived from the RCG field. At lower heights (April 2009 to August 2011 for the *Miscanthus* tower and April 2010 to June 2011 for the RCG tower), the flux footprints were better confined to the experimental areas. Half-hourly data were rejected if less than 70% of the derived footprint originated from the respective experimental areas, similar to previous studies (Ammann *et al.*, 2007; Baum *et al.*, 2008; Davis *et al.*, 2010; Vanderborght *et al.*, 2010). This resulted in 17.4% and 14.9% of fluxes being rejected overall for the *Miscanthus* and RCG sites, respectively.

The positioning of the EC sensors in relation to the inertial boundary layer was also monitored due to the relatively low measurement heights. The thickness of the inertial boundary layer, calculated according to equation 5 of Munro & Oke

(1975), reached maximum values of 4.9 m, 4.9 m and 7.0 m in 2009, 2010 and 2011, respectively, for *Miscanthus* and values of 2.8 m and 3.7 m in 2010 and 2011, respectively, for RCG. The lower limit of the inertial boundary layer was estimated to occur between $(z-d) = 5z_0$ and $(z-d) = 10z_0$ (Garratt, 1992), where z is the height above ground, d is the zero-plane displacement height and z_0 is the roughness length of the crop surface. This yielded maximum values in the range 1.1–1.6 m, 1.1–1.5 m and 3.0–4.2 m in 2009, 2010 and 2011, respectively, for *Miscanthus* and values of 0.8–1.1 m and 1.7–2.5 m in 2010 and 2011, respectively, for RCG. These estimates suggested that the sensors were within the appropriate inertial boundary layer for the vast majority of the experiment.

The quality of the CO₂ flux estimates was assessed by examining energy balance closure (EBC) at the site. This routine provides an independent check of the degree to which turbulent

Table 1 Physical and chemical characteristics of the soils at the *Miscanthus*, RCG and adjacent reference grassland sites in autumn 2011. Bulk density (BD), texture, pH and total nitrogen (TN) are reported for 0–15 cm soil depth. The soils at all sites are classified as loam. Total carbon (TC) and total organic carbon (TOC) are shown for three depths: 0–15 cm (A), 15–30 cm (B) and 30–45 cm (C)

	BD (g cm ⁻³)	Sand (%)	Silt (%)	Clay (%)	pH	TN (%)	TC (%)			TOC (%)		
							A	B	C	A	B	C
<i>Miscanthus</i>	0.98	50	32	18	6.4	0.3	3.2	2.9	1.5	2.4	2.2	1.1
Grassland reference	0.99	51	32	18	6.5	0.3	2.9	2.9	2.1	2.2	2.2	1.5
RCG	0.97	48	33	19	6.7	0.3	2.8	2.5	1.9	2.2	1.9	1.4
Grassland reference	0.88	52	30	19	6.2	0.3	2.7	2.5	2.1	2.0	1.8	1.5

fluxes are captured in the boundary layer and may highlight significant bias in measurements (Twine *et al.*, 2000). Energy balance closure was tested by comparing half-hourly and daily sums of net radiation with the sum of good quality (QC-flag = 0, 1) LE and H fluxes and energy storage terms using the equation

$$R_n = LE + H + G + S_s + S_p \quad (1)$$

where R_n is net radiation, LE is the latent heat flux, H is the sensible heat flux, G is the soil heat flux, S_s is soil heat storage above the heat flux plates and S_p is the energy stored in photosynthate. Heat storage in the soil surface layer (of depth Δz) was calculated as

$$S_s = \frac{\Delta T(\theta_v \rho_w c_w + \rho_s c_s) \Delta z}{\Delta t} \quad (2)$$

where ΔT is the change in average soil temperature above the heat flux plates over the time interval Δt , θ_v is the soil volumetric water content, ρ_w is the density of water, c_w is the specific heat capacity of water, ρ_s is the soil bulk density and c_s is the specific heat capacity of soil [a value of $837 \text{ J kg}^{-1} \text{ K}^{-1}$ was used for c_s (Scott, 2000)]. The energy captured during

photosynthesis and stored in biomass, S_p , was computed by equating photosynthetic fixation of $2.5 \text{ mg CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ to an energy flux of 28 W m^{-2} (Meyers & Hollinger, 2004). For this calculation, photosynthetic rates were based on gross primary productivity (GPP) estimates calculated by subtracting modelled total ecosystem respiration (TER) from measured NEE. Heat storage in plant biomass and canopy air was not measured and considered minor relative to the other terms in equation 1, particularly in the case of *Miscanthus* which had a sparse, open canopy for much of the study.

The regression analysis of half-hourly energy fluxes yielded a slope of 0.945 ± 0.004 [95% confidence interval (CI)] and intercept of 5.2 W m^{-2} for the *Miscanthus* site and a slope of 0.905 ± 0.004 and intercept of 6.3 W m^{-2} for the RCG site (Fig. 3). Calculating daily sums of energy fluxes resulted in a small increase in the slope for *Miscanthus* to 0.963 ± 0.013 and a larger increase in the slope for RCG to 0.964 ± 0.016 (Fig. 3). This suggests that there was a small contribution from additional nonestimated storage terms, such as heat storage in plant biomass or canopy air, to the energy balance of these ecosystems. These contributions tend to be negligible on a daily scale (Oncley *et al.*, 2007). Although these slopes were significantly different from one, they compare well with

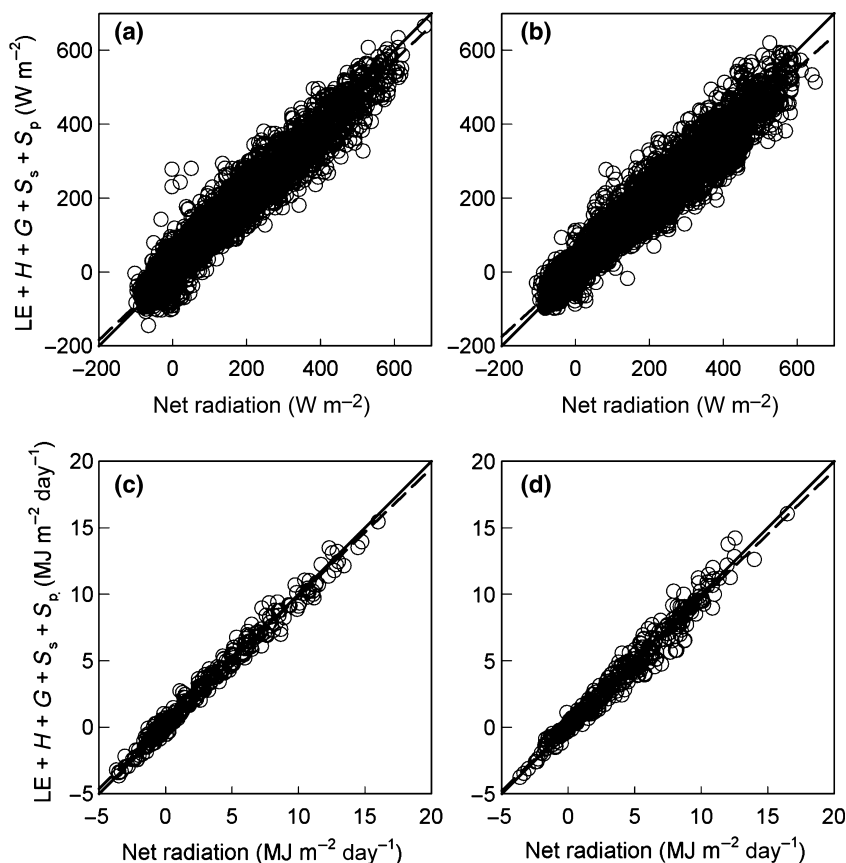


Fig. 3 Energy balance closure on a half-hourly timescale for *Miscanthus* (a) and RCG (b) and a daily timescale for *Miscanthus* (c) and RCG (d) in 2011. The dashed black lines represent the regression lines [$y = 0.95x + 5.24$, $R^2 = 0.96$ for *Miscanthus* (half-hourly); $y = 0.91x + 6.3$, $R^2 = 0.96$ for RCG (half-hourly); $y = 0.96x + 0.2$, $R^2 = 0.99$ for *Miscanthus* (daily); $y = 0.96x + 0.04$, $R^2 = 0.97$ for RCG (daily)]. The solid black lines indicate a 1 : 1 relationship. Daily sums were calculated using the available data in a 24-h period.

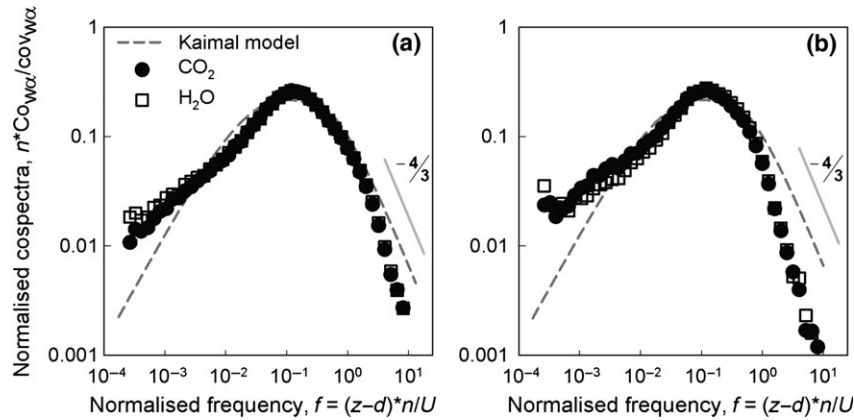


Fig. 4 Normalised ensemble-averaged cospectra of CO₂ and H₂O in unstable conditions plotted against normalised frequency, f , for the *Miscanthus* (a) and RCG (b) sites. The dashed grey curve and solid grey line represent the theoretical universal Kaimal cospectra and the $-4/3$ slope predicted by Kolmogorov's Law, respectively (Kaimal *et al.*, 1972).

reported ranges of EBC of 0.53–0.99 (Wilson *et al.*, 2002) and 0.70–0.94 (Stoy *et al.*, 2013) and suggest that the vast majority of the available energy was accounted for. Consequently, turbulent energy exchange was satisfactorily resolved by the EC measurement system.

In addition, spectral analysis of the high frequency data was performed to assess the frequency response of the EC system. Fast Fourier transforms were applied to the 10 Hz data to yield full cospectra, which were subsequently reduced into exponentially spaced frequency bins and ensemble-averaged. Normalised, ensemble-averaged and frequency-weighted cospectra for both sites are presented in Fig. 4 for unstable conditions and compared with the universal theoretical Kaimal cospectral function (Moncrieff *et al.*, 1997). The shapes of the measured cospectra are generally consistent with the Kaimal curve, except for small deviations from the theoretical $-4/3$ slope in the inertial subrange. This result is indicative of some small-scale dampening of the high frequency signal, however, the flux postprocessing routines employed included a correction for high frequency losses. The CO₂ spectral correction factor (SCF) for *Miscanthus* had a median of 1.17 and mean \pm 95% CI of 1.19 ± 0.001 . For RCG, the median was 1.15 and the mean was 1.16 ± 0.002 . These values fall in the expected range of CO₂ correction factors (1.04–1.25; Aubinet *et al.*, 2001) and compare well with an SCF of 1.12 for a similar open-path system (Haslwanter *et al.*, 2009).

Gaps in the flux data created by measurement failures and data quality analysis procedures were filled using semiempirical gap-filling techniques. TER was modelled by relating soil temperature to nocturnal measured NEE using the exponential Lloyd & Taylor (1994) equation

$$\text{TER} = R_{10} e^{E_0(1/(283.15 - T_0) - 1/(T - T_0))} \quad (3)$$

where R_{10} is ecosystem base respiration at a reference temperature of 10 °C, E_0 is an activity energy parameter, T_0 was set to 227.13 K as in the original study and T is soil temperature (K). Significant seasonality has been demonstrated in the E_0 param-

eter, particularly in summer active ecosystems (Reichstein *et al.*, 2005), and therefore bimonthly best fit estimates of E_0 were obtained in each year using equation 3. These short-term bimonthly estimates were then averaged over each year to derive more realistic long-term E_0 values. Temporal variability in the R_{10} parameter was also considered. For each available nocturnal NEE value and adopting the relevant long-term E_0 value, equation 3 was rearranged to provide an estimate of R_{10} in that half-hourly period. The weekly running mean, $R_{10\text{Mean}}$ was then computed and employed instead of R_{10} in equation 3 above to incorporate time-varying influences on R_{10} such as soil moisture status, plant phenology etc. The dependence of nocturnal TER on temperature was extended to daytime and estimates of TER were obtained for all 30-minute periods in this way.

GPP was initially computed by subtracting the estimated TER from measured daytime half-hourly NEE. Daytime GPP values were then pooled for bimonthly periods and subdivided into fixed 4 °C temperature bins within each bimonthly period. GPP model parameters were then derived for each subset using a rectangular hyperbola function (Falge *et al.*, 2001)

$$\text{GPP} = \frac{\alpha Q_{\text{PPFD}} A_{\text{max}}}{\alpha Q_{\text{PPFD}} + A_{\text{max}}} \quad (4)$$

where α is the ecosystem quantum (photon) yield ($\text{mol CO}_2 [\text{mol photon}]^{-1}$), Q_{PPFD} is the photosynthetic photon flux density ($\mu\text{mol} [\text{photon}] \text{m}^{-2} \text{s}^{-1}$) and A_{max} is the maximum assimilation rate ($\mu\text{mol CO}_2 \text{m}^{-2} \text{s}^{-1}$).

The net ecosystem carbon balance (NECB) is defined as the net rate of C accumulation or loss from an ecosystem, taking account of all physical, biological and anthropogenic sources and sinks of carbon in an ecosystem (Chapin *et al.*, 2006). NECB was calculated as $-\Sigma\text{NEE} - C_{\text{harvest}}$ where C_{harvest} is the carbon removed from the ecosystem at harvest, assuming that contributions from carbon monoxide, methane, volatile organic carbon (VOC), dissolved carbon and particulate carbon were negligible.

An assessment of the uncertainty associated with annual CO₂ flux estimates was performed in a similar way to Black *et al.* (2007). Firstly, the contribution of uniform systematic errors to the measured fluxes was evaluated following Goulden *et al.* (1996). Assuming spectral similarity between latent heat, sensible heat and CO₂ flux, the imbalance between available energy and measured latent and sensible heat, as calculated in the long-term energy balance, was used as an approximation of the underestimation of ecosystem exchange by the EC measurement system. Secondly, sampling uncertainty errors, associated with the imputation of half-hourly missing data during gap-filling procedures, were assessed in a similar way to Falge *et al.* (2001). Artificial datasets were created with 10, 25, 35 and 45% of the data replaced by gaps and gap-filling procedures were followed to fill the artificial gaps. The mean bias error was calculated as the mean difference between measured and calculated values for the complete data series. The final uncertainty estimate was computed for each measured or gap-filled half-hourly flux based on either the uniform systematic error derived from the energy balance closure, expressed as a percentage, or the mean bias error arising from sampling uncertainty (gap-filling).

Soil respiration

Soil respiration was monitored using a closed dynamic chamber coupled with a portable infrared gas analyser (Environmental Gas Monitor EGM-4, PP Systems, Hitchin, UK). Chamber measurements were made on bare soil at 10 sampling points in the *Miscanthus* and RCG sites, yielding an estimate of the flux of CO₂ from the soil surface which combines contributions from autotrophic (plant root) and heterotrophic (microbes and soil fauna) components. Measurements were carried out from early June 2010 to early September 2011 at approximately weekly intervals during the growing season and monthly intervals during the winter. Soil temperature and VWC (0–7 cm) were recorded at each sampling point using a WET sensor (Delta-T Devices, Cambridge, UK).

Crop analysis

Above-ground *Miscanthus* biomass was calculated as the product of average shoot density and total dry biomass (leaf + stem) per shoot sampled prior to harvesting and at least monthly during the growing seasons of 2010 and 2011. Shoot density estimates (shoots m⁻²) were based on the number of shoots counted in a quadrat of area 2.1 m² at 10 random locations in the *Miscanthus* plantation. Total dry biomass per shoot was measured by cutting a minimum of 35 random shoots at ground level during each sampling event and drying the leaf and stem biomass at 70 °C to constant weight. Below-ground biomass was sampled on three occasions (February 2010, January 2011 and September 2011) by excavating all below-ground plant material associated with three randomly located *Miscanthus* plants to a depth of 40 cm. Roots, live rhizomes and dead rhizomes were separated, washed free of soil over a 2 mm sieve and dried to constant weight at 70 °C. Below-ground biomass was up-scaled from the plant scale to a per unit area (m⁻²) basis

using calculated above- to below-ground ratios and average above-ground biomass on each individual sampling date. The contribution of understory vegetation was assessed by clipping all above-ground material in quadrats of area 0.25 m² placed inside the large *Miscanthus* quadrats with subsequent determination of dry matter yields after oven drying at 70 °C.

Above-ground RCG biomass was assessed by cutting all vegetation at ground level in quadrats of area 0.25 m² randomly positioned at five locations within the crop. Sampling was carried out in September 2010, prior to harvest in October 2010 and in June and September 2011. A root auger of volume 750 cm³ (Eijkelpoort Agrisearch Equipment, Giesbeek, The Netherlands) was used to sample below-ground biomass to a depth of 45 cm in January and October 2011 at 4 random locations in the crop. The intact soil cores were mixed with water in the laboratory, washed over a 0.2 mm sieve and dried at 70 °C to constant weight.

Miscanthus green leaf area index (GLAI) was determined by firstly establishing an allometric relationship between leaf area and the product of leaf length and width, following Clifton-Brown *et al.* (2000). The length and width of leaves harvested on three different occasions were measured and images of the leaves were captured with a flatbed scanner (CanoScan LiDE 35, Canon, Tokyo, Japan). Leaf area estimates were obtained using the open-source software IMAGEJ (<http://imagej.nih.gov/ij/>) to generate the linear regression equation

$$\text{leaf area} = 0.76 (\text{length} \times \text{width}) \quad (5)$$

where both measured quantities are in cm², $R^2 = 0.93$ and $n = 295$. This equation was applied to leaf length \times width measurements made during above-ground biomass sampling. *Miscanthus* GLAI was computed as the product of green leaf area per shoot and shoot density. The GLAI of understory vegetation was estimated based on an allometric relationship between dry biomass weight and calculated leaf area.

RCG leaf area index (LAI) was monitored with a Sunscan Canopy Analysis System (Delta-T Devices, Cambridge, UK) at approximately monthly intervals, providing a measure of the total leaf area per unit ground area. Individual measurements made at 40 locations within the crop were averaged on each sampling date. These measurements involved a non-destructive sampling technique based on the fraction of photosynthetically active radiation (PAR) intercepted by the canopy (Campbell & Norman, 1989). Therefore, RCG LAI values included contributions from both live and senescent leaves as both influence the transmission of light through the canopy.

Results

Environmental conditions

Mean annual air temperatures were close to the 30-year mean temperature in 2009 (10.0 °C) and 2011 (10.4 °C) but dipped in 2010 to 9.3 °C. Two prolonged periods of cold weather were experienced during these years (December 2009 to February 2010 and November 2010 to January 2011) (Fig. 1). In addition, summer mean

temperature was 7% lower than the long-term mean in 2011. Rainfall totals were highly variable, with higher than average rainfall in 2009 (1427 mm), close to the average in 2010 (972 mm) and below average in 2011 (839 mm). Exceptionally wet summer months characterised 2009, with atypically high rainfall also occurring in November. The mean monthly soil VWC at the *Miscanthus* site was high for much of the study, only reducing below field capacity ($0.32 \text{ m}^3 \text{ m}^{-3}$) in August 2010 and August and September 2011. Soil VWC in the RCG site was below field capacity from May to August of each year but remained above field capacity ($0.32 \text{ m}^3 \text{ m}^{-3}$) outside of the peak growth period. In general, soil VWC was higher in *Miscanthus* than RCG, most obviously from May to July 2011. Vapour pressure deficit (VPD) was always low, ranging from a monthly average of 0.06 kPa in February 2011 to a maximum monthly average of 0.37 kPa in June 2009 and August 2010 (Fig. 1).

Crop development

Establishment and growth of the *Miscanthus* crop was slow in the first 2 years after planting. Prior to the first harvest in March 2010, above-ground dry biomass totalled just 10 ± 4.0 (standard error of the mean, SEM) g m^{-2} while below-ground biomass stocks were estimated at $13.8 \pm 7.1 \text{ g m}^{-2}$ (Table 2). Following the growing season of 2010, above-ground biomass reached a seasonal maximum of over $100 \pm 20.2 \text{ g m}^{-2}$ in September and a maximum GLAI of $0.55 \pm 0.05 \text{ m}^2 \text{ m}^{-2}$ was recorded in October (Fig. 5). The contribution of understory vegetation to ecosystem productivity was substantial at this time. Above-ground biomass stocks of understory vegetation, dominated mostly by grass species (*Agrostis stolonifera*, *Poa trivialis* and *Alopecurus geniculatus*), totalled $110 \pm 21.7 \text{ g m}^{-2}$ in September 2010, with an associated GLAI of $3.56 \pm 0.70 \text{ m}^2 \text{ m}^{-2}$.

Table 2 Above- and below-ground biomass stocks in the *Miscanthus* and RCG crops in g (dry matter) m^{-2} . Total above- and below-ground stocks in *Miscanthus* are divided into their constituent pools. Values in parentheses represent the standard error of the mean

Date	Above-ground (g m^{-2})			Below-ground (g m^{-2})			
	Stem	Leaf	Total	Live Rhizome	Dead Rhizome	Root	Total
<i>Miscanthus</i>							
February 2010	8.2 (3.3)	1.6 (0.7)	9.9 (4.0)	10.7 (6.2)	0	3.0 (0.9)	13.8 (7.1)
September 2010	63.9 (17.3)	36.6 (5.2)	100.5 (20.2)				
January 2011	33.1 (13.9)	6.5 (2.2)	39.6 (16.1)	73.6 (3.8)	9.3 (2.1)	22.3 (2.7)	105.2 (8.5)
March 2011	53.3 (15.2)	4.9 (1.4)	58.2 (16.4)				
June 2011	52.3 (23.3)	31.5 (9.2)	83.8 (32.5)				
September 2011	1001.6 (362.3)	308.9 (102.6)	1310.5 (464.6)	331.1 (138.4)	16.0 (6.3)	63.7 (21.8)	410.8 (166.4)
Reed Canary Grass							
September 2010			653.0 (76.4)				
October 2010			589.3 (28.5)				
January 2011							489.0 (46.3)
June 2011			675.4 (93.9)				
September 2011			706.4 (61.2)				834.5 (72.5)

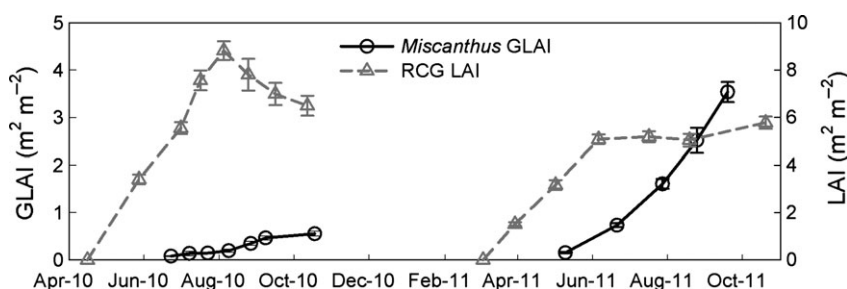


Fig. 5 Temporal pattern of green leaf area index (GLAI) in *Miscanthus* and leaf area index (LAI) in RCG during the 2010 and 2011 growing seasons. Vertical bars represent the standard error of the mean.

Following initial slow development in 2011, growth rates increased exponentially towards the end of the summer, yielding $1311 \pm 465 \text{ g m}^{-2}$ in late September 2011. GLAI increased steadily over this time (Fig. 5, Table 2). Above-ground crop expansion was facilitated by a four-fold increase in below-ground biomass between January and late September 2011 when total below-ground *Miscanthus* biomass was $410.8 \pm 166.4 \text{ g m}^{-2}$, comprising 80.6% live rhizome, 3.9% dead rhizome and 15.5% root (Table 2). Correspondingly, stocks of understory vegetation declined, associated with herbicide application in spring 2011 and increased competition from *Miscanthus* plants. Estimated above-ground biomass from weeds was $5.8 \pm 2.2 \text{ g m}^{-2}$ and $14.5 \pm 6.3 \text{ g m}^{-2}$ in late June and late September 2011, respectively, while their GLAI was calculated as $0.19 \pm 0.07 \text{ m}^2 \text{ m}^{-2}$ and $0.47 \pm 0.20 \text{ m}^2 \text{ m}^{-2}$ at the same times, representing significant reductions from 2010 values.

In contrast with the slow establishment of *Miscanthus*, rapid growth was observed in the new RCG plantation after sowing in mid-April 2010. A dense green canopy developed early in the first growing season resulting in a seasonal maximum LAI of $8.8 \pm 0.4 \text{ m}^2 \text{ m}^{-2}$ at the start of August 2010 (Fig. 5). A reduction in LAI was then observed as the crop senesced. At the time of the first harvest in October 2010, $589 \pm 29 \text{ g m}^{-2}$ had accu-

mulated in above-ground biomass and the LAI was $6.5 \pm 0.4 \text{ m}^2 \text{ m}^{-2}$. In the second year of establishment, above-ground growth began earlier than in the conversion year and, by early June 2011, a growing season maximum LAI of $5.1 \pm 0.2 \text{ m}^2 \text{ m}^{-2}$ was recorded and above-ground biomass totalled $675 \pm 94 \text{ g m}^{-2}$. Flowering and senescence occurred earlier in 2011 than in 2010 with the result that, by August 2011, the crop had completely senesced. Significant below-ground biomass stocks were recorded in the RCG crop in the second year of establishment (Table 2), with the maximum below-ground stock in September 2011 ($835 \pm 73 \text{ g m}^{-2}$) exceeding above-ground biomass estimates. Understory vegetation made a minor contribution to above-ground biomass in the RCG ecosystem, accounting for just 3.4% and 0.5% of total above-ground biomass in June and late September 2011, respectively.

Carbon fluxes

Flux measurements at the *Miscanthus* site began 1 day before the majority of the grassland field was tilled. Ecosystem exchange of carbon was dominated by respiratory fluxes in the first month post-disturbance. Half-hourly NEE fluxes \pm SEM averaged 4.0 ± 0.1 , 3.3 ± 0.1 , 3.3 ± 0.1 and $2.7 \pm 0.1 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ in the first

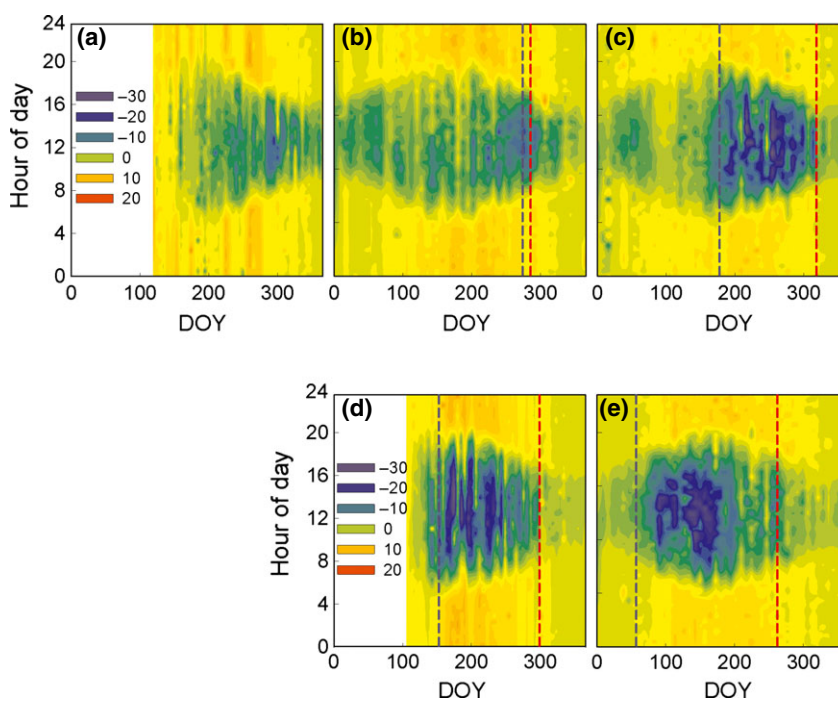


Fig. 6 Diurnal course of half-hourly fluxes of net ecosystem carbon exchange in $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ in the *Miscanthus* ecosystem during 2009 (beginning in late April) (a), 2010 (b) and 2011 (c) and in the RCG ecosystem during 2010 (beginning in mid-April) (d) and 2011 (e). Dashed vertical dark blue lines mark the 'break-even point' at which cumulative NEE becomes negative (net C uptake). Dashed vertical red lines indicate the timing of the end of net C uptake.

4 weeks following tillage, respectively, with positive fluxes indicating a net release of C to the atmosphere. Monthly cumulative NEE \pm uncertainty amounted to 104.7 ± 3.1 , 62.7 ± 2.7 and -12.1 ± 3.1 g C m⁻² in the first 3 months, respectively, post-tillage, representing significant initial net losses of carbon from the ecosystem.

A small recovery in photosynthetic activity was apparent in subsequent months, concurrent with the slow development of the newly-established *Miscanthus* crop and the emergence of understory vegetative species prompted by a flush of germination after the initial soil disturbance. However, seasonal maximum rates of instantaneous net C assimilation, recorded in mid-October 2009, were no greater than -15 $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ (Fig. 6). The magnitude of photosynthetic uptake was similarly modest in 2010, peaking at -17 $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ in late September. The seasonal pattern of NEE changed dramatically in 2011, however, concomitant with significant increases in above-ground *Miscanthus* biomass, large-scale leaf expansion and higher GLAI. Maximum instantaneous rates of net C assimilation were -30 to -35 $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ between mid-August and late September 2011.

Measurements of NEE in the RCG crop began the same day the site was harrowed and seeded in mid-April 2010. Early half-hourly fluxes of NEE were dominated by ecosystem respiration but C uptake became evident from mid-May onwards (Fig. 6). Weekly-averaged NEE was 2.6 ± 0.1 , 1.7 ± 0.1 , 0.8 ± 0.1 and

-0.2 ± 0.1 $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ in the first 4 weeks following planting, respectively. As a result, the ecosystem represented a net source of carbon over the first month (cumulative NEE was 33.3 ± 2.4 g C m⁻²) but switched to a net sink during the second (-88.0 ± 4.8 g C m⁻²) and third month (-127.3 ± 13.9 g C m⁻²).

Rapid establishment and extensive early canopy development in the RCG crop accompanied strong photosynthetic activity early in the first growing season. Just over 2 months after planting (late June 2010), a seasonal maximum NEE of -35 $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ was recorded. Net C uptake continued until late September 2010 at a decreasing rate (Fig. 6). Winter NEE fluxes were small as a result of the exceptionally cold temperatures recorded from November 2010 to January 2011. However, photosynthetic activity resumed when daily mean temperatures began to exceed about 5 °C in February 2011. Net ecosystem C accumulation continued until a seasonal maximum NEE of -38 $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ was recorded in mid-May 2011, after which rates of C uptake declined.

Daily fluxes of TER and GPP are shown in Fig. 7. Maximal daily values of GPP in the *Miscanthus* crop increased from -7.3 and -9.2 g C m⁻² d⁻¹ in September 2009 and June 2010, respectively, to -14.1 g C m⁻² d⁻¹ in August 2011. Maximal daily TER was 10.0, 9.5 and 7.4 g C m⁻² d⁻¹ in September 2009, June 2010 and July 2011, respectively for *Miscanthus*. In the RCG crop, maximal daily GPP values of -17.6 and -16.3 g C m⁻² d⁻¹ were recorded in late June 2010 and

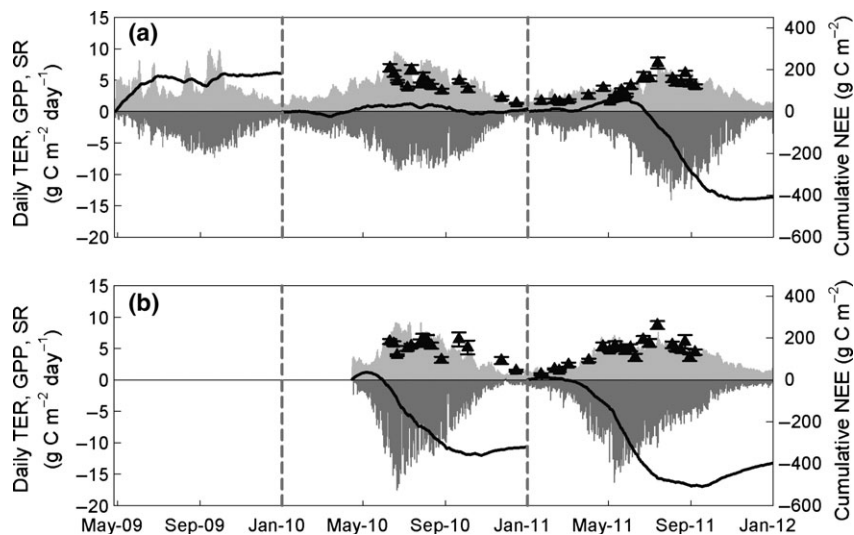


Fig. 7 Daily total ecosystem respiration (TER) (light grey area), daily gross primary productivity (GPP) (dark grey area) and cumulative net ecosystem exchange (NEE) (solid black line) over the duration of the *Miscanthus* (a) and RCG (b) studies. Cumulative NEE was reset to zero at the end of each calendar year (indicated by a dashed vertical grey line). Measured soil respiratory (SR) fluxes are also shown (black triangles), representing below-ground respiration from both autotrophic and heterotrophic sources. Vertical bars represent the standard error of the measurement means.

mid-May 2011, respectively, while maximal daily TER values of 9.2 and 7.6 g C m⁻² d⁻¹ were observed in mid-July 2010 and 2011, respectively. Higher TER values were generally associated with warmer soil temperatures and higher ecosystem productivity.

The cumulative effect of the measured NEE fluxes over the course of the study is also shown in Fig. 7. In the *Miscanthus* crop, cumulative NEE was 183 ± 28 g C m⁻² from late April to the end of December 2009, 13 ± 45 g C m⁻² in 2010 and -411 ± 63 g C m⁻² in 2011 (Table 3). GPP increased from -797 g C m⁻² in 2009 to -1684 g C m⁻² in 2011. Cumulative TER peaked in 2010 (1514 g C m⁻²) and decreased in 2011 to 1273 g C m⁻², concomitant with below average summer temperatures. In the RCG crop, cumulative NEE values were -319 ± 57 g C m⁻² from mid-April to the end of December 2010 and -397 ± 114 g C m⁻² during 2011. An increase in GPP was observed from the conversion year (-1430 g C m⁻², 8½-month period) to the second year of establishment (-1708 g C m⁻²), while TER also increased over this period from 1112 to 1311 g C m⁻².

The 'break-even' point at which cumulative NEE became negative (net C uptake) occurred in early October in 2010 and late June in 2011 for *Miscanthus* (Fig. 6). For RCG, the 'break-even' point occurred much earlier in the year than for *Miscanthus*: in early June in the conversion year (2010) and late February in 2011. With regard to winter fluxes, net C uptake did not cease in *Miscanthus* until mid-October and mid-November in 2010 and 2011, respectively, and the *Miscanthus* ecosystem subsequently remained relatively C-neutral until year end. In contrast, net C loss was observed from late October onwards in the conversion year for RCG and at a higher rate in 2011 from late September onwards.

The net rate of long-term C accumulation in an ecosystem is better described by the NECB. Since no

removal of C through harvesting occurred during the *Miscanthus* experiment, the cumulative NEE values quoted for 2009, 2010 and 2011 are representative of the NECB of this ecosystem. For the RCG crop, the NECB was calculated as 66 ± 58 g C m⁻² (a net C sink), while the NECB for the second year of RCG establishment was 397 ± 114 g C m⁻², as no biomass was harvested in this year. These values assume that contributions from leaching, methane, VOC, carbon monoxide and particulate carbon were negligible. Leaching of dissolved organic carbon (DOC) and dissolved inorganic carbon (DIC) are likely to be the most significant of these nonestimated fluxes (Osborne *et al.*, 2010; Smith *et al.*, 2010). Kindler *et al.* (2011) reported average DOC and biogenic DIC losses from European croplands of 4.1 ± 1.3 and 14.6 ± 4.8 g C m⁻² y⁻¹, respectively. This study included an Irish cropland (Carlow) with well-drained, sandy loam soil which lost 2.6 ± 0.5 and 15.2 ± 4.1 g C m⁻² y⁻¹ as DOC and biogenic DIC, respectively. Following tillage disturbance of a grassland lysimeter at Johnstown Castle with comparable soil to the *Miscanthus* and RCG sites (low to medium drainage capacity), DOC leaching amounted to 1.6 g C m⁻² over a 33-week period (Ó. Ní Chonchubhair, B. Osborne, K. Richards and G. Lanigan, unpublished results). While such C losses comprise a small fraction of the annual NEE estimates for *Miscanthus* and RCG in 2011, their significance for the long-term NECB is far greater.

Based on chamber measurements, soil respiration (below-ground autotrophic and heterotrophic respiration) accounted for the majority of TER, with small contributions from leaf and stem respiration (Fig. 7). This was particularly evident in 2011 when significant below-ground biomass had developed in both crops. Soil respiration in *Miscanthus* varied from 1.3 ± 0.1 (SEM) g C m⁻² d⁻¹ in December 2010 to 7.7 ±

Table 3 Annual cumulative fluxes of gross primary productivity (GPP), total ecosystem respiration (TER), net ecosystem exchange (NEE) and net ecosystem carbon balance (NECB) for the *Miscanthus* and RCG ecosystems

	GPP	TER	NEE* ± uncertainty	NECB† ± uncertainty
<i>Miscanthus</i>				
28th Apr 2009 – 31st Dec 2009 (Ploughing – year end)	-797	979	183 ± 28	-183 ± 28
2010	-1501	1514	13 ± 45	-13 ± 45
2011	-1684	1273	-411 ± 63	411 ± 63
RCG				
15th Apr 2010 – 31st Dec 2010 (Harrowing/planting – year end)	-1430	1112	-319 ± 57	66 ± 58
2011	-1708	1311	-397 ± 114	397 ± 114

*Positive NEE values represent a net release of C to the atmosphere, negative values a net uptake of C by the ecosystem.

†NECB = -ΣNEE - C_{harvest} where C_{harvest} is the carbon removed from the ecosystem at harvest.

$0.9 \text{ g C m}^{-2} \text{ d}^{-1}$ in July 2011. In RCG, fluxes ranged from 0.9 ± 0.1 in January 2011 to $8.7 \pm 1.0 \text{ g C m}^{-2} \text{ d}^{-1}$ in July 2011. During intervals of active RCG growth and slow *Miscanthus* development, soil respiration in RCG exceeded that of *Miscanthus*, most notably in autumn 2010 and in April and May 2011. Outside of these periods, soil respiration fluxes were comparable in both ecosystems.

Controls on ecosystem carbon fluxes

Net assimilation of carbon by the *Miscanthus* ecosystem was closely related to crop development during the growing season, as shown in Fig. 8(a). When *Miscanthus* GLAI values were $0.5 \text{ m}^2 \text{ m}^{-2}$ or lower, gross photosynthesis was cancelled out by a comparable ecosystem respiration rate and monthly sums of NEE fluctuated around zero. As leaf expansion progressed and a greater fraction of incident light was intercepted by the *Miscanthus* crop, large increases in monthly NEE were recorded, reaching a peak of $-161.8 \text{ g C m}^{-2}$ during August 2011.

A strong positive relationship was observed between monthly sums of TER and GPP for much of 2009 and 2010 and the winter months of 2011 (Fig. 8b). Notable deviations from a 1 : 1 linear relationship in May and June 2009 correspond to significant reductions in photosynthetic capacity after conventional inversion tillage. Monthly TER also exceeded monthly GPP in September 2009. This month was characterised by a 3-week period when rainfall was significantly reduced (total 1.2 mm) after a summer of exceptionally wet weather. In addition, respiration outweighed photosynthesis in March and April 2010 and in April 2011 following systemic

herbicide application. However, the largest departures from slopes close to unity were observed in the growing season of 2011, when GPP was approximately 1.5 times greater than TER.

The relationship between monthly sums of TER and GPP in the RCG ecosystem is shown in Fig. 9(a) for 2010 and 2011. A strong positive association was observed between the two variables in both years, with respiratory fluxes representing roughly 50% of GPP across the full measurement period. The only months when TER exceeded GPP were April 2010 (after planting), November – December 2010 and October – December 2011. The impact of soil VWC on monthly TER fluxes in the RCG crop can be seen in Fig. 9(b). A negative relationship was evident in 2010 as VWC increased and reached values in excess of $0.4 \text{ m}^3 \text{ m}^{-3}$, presumably due to oxygen-limitation. Monthly sums of TER in 2011 (drier year) were comparable to 2010 values at high moisture contents, but when soil VWC decreased below a mid-range level ($\sim 0.26 \text{ m}^3 \text{ m}^{-3}$), a positive relationship was observed between TER and VWC.

Discussion

In the discussion below, we address the three questions posed, relating to (1) the transitional-phase C fluxes, (2) the postestablishment phase C fluxes associated with the *Miscanthus* and RCG crops and (3) the role of leaf longevity in regulating net C exchange. We discuss how our results compare with previous studies involving land-use transitions and examine the implications of our findings for bioenergy crop selection.

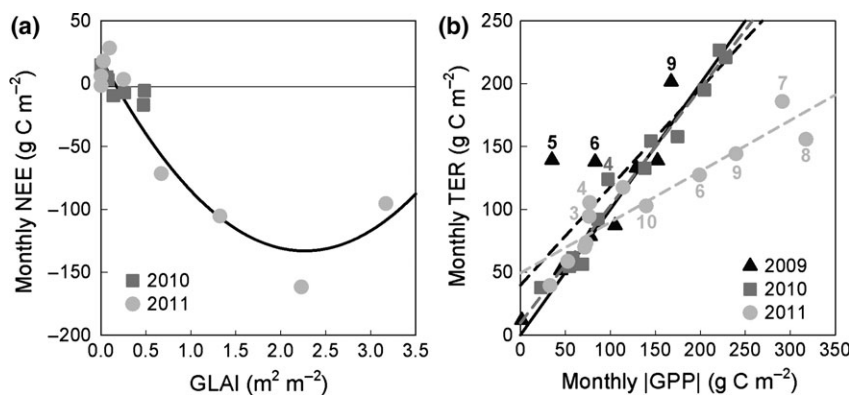


Fig. 8 Monthly sums of net ecosystem exchange (NEE) in the *Miscanthus* ecosystem are plotted against green leaf area index (GLAI) for 2010 and 2011 in (a). Panel (b) shows the correlation for the *Miscanthus* ecosystem of monthly sums of total ecosystem respiration (TER) with monthly sums of gross primary productivity (GPP), plotted in absolute values (|GPP|) to facilitate the comparison. The black solid line indicates a 1 : 1 relationship while the dashed lines represent regression lines: 2009 (black line) $y = 0.78x + 39.6$, $R^2 = 0.58$; 2010 (dark grey line) $y = 0.93x + 9.6$, $R^2 = 0.97$; 2011 (light grey line) $y = 0.40x + 49.3$, $R^2 = 0.85$. Months showing deviations from the 1 : 1 line are marked with their respective month number in the calendar year.

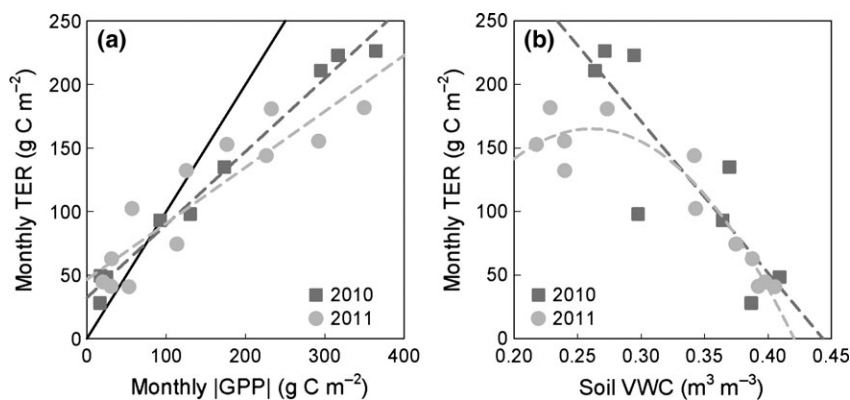


Fig. 9 Correlation of monthly sums of total ecosystem respiration (TER) with monthly sums of gross primary productivity (GPP) for 2010 and 2011 in the RCG ecosystem (a). GPP is plotted in absolute values ($|GPP|$). The black solid line indicates a 1 : 1 relationship while the dotted lines represent regression lines: 2010 (dark grey line) $y = 0.57x + 32.1$, $R^2 = 0.98$; 2011 (light grey line) $y = 0.44x + 46.5$, $R^2 = 0.81$. Panel (b) shows the relationship between TER and soil volumetric water content (VWC) during 2010 and 2011 in the RCG ecosystem. The dashed lines represent regression lines: 2010 (dark grey line) $y = -1194.2x + 529.4$, $R^2 = 0.72$; 2011 (light grey line) $y = -6451.4x^2 + 3362.2x - 273.1$, $R^2 = 0.91$.

Carbon fluxes during land-use transition to bioenergy crops

Significant carbon emissions and changes in SOC have been attributed to land-use transitions such as deforestation and conversion to cropland (Davidson & Ackerman, 1993; Houghton *et al.*, 2012; Poepflau & Don, 2013). Net C losses in the early stage of LUC can arise as a result of diminished photosynthetic capacity following herbicide application, enhanced mineralisation after tillage as well as reduced C uptake while the new crop develops to full maturity.

Table 4 summarises findings from previous research on C fluxes during land-use transitions. In a reseeded experiment in Johnstown Castle, cumulative TER from a grass sward with similar soil type to the bioenergy crop sites was $278 \pm 29 \text{ g C m}^{-2}$ in the month following herbicide application (Ó. Ní Choncuibhair, B. Osborne, K. Richards and G. Lanigan, unpublished results). Including photosynthetic uptake (albeit at a diminishing rate) would reduce this value significantly. For example, Zenone *et al.* (2011) recorded a cumulative NEE of $19\text{--}46 \text{ g C m}^{-2}$ in the month after herbicide application to long-term grassland. However, further variable net C losses occur in the posttillage period, dependent on the former land use, environmental conditions and tillage intensity. Cumulative NEE in the first 3 months posttillage in the *Miscanthus* ecosystem was $155.4 \pm 8.9 \text{ g C m}^{-2}$ and falls in the mid-range of estimated short-term tillage-induced emissions. These losses were primarily driven by minimal photosynthetic activity and slow above-ground development of the rhizome-propagated plants. This slow establishment is typical of Irish and many north-

ern European trials where 3–5 years is generally required for full establishment (Lewandowski *et al.*, 2000; Clifton-Brown *et al.*, 2007).

Following RCG planting in April, net C losses of $33.3 \pm 2.4 \text{ g C m}^{-2}$ were recorded in the first month. However, the ecosystem became a strong net C sink after this. A number of factors may have contributed to this result. Firstly, the RCG site had been ploughed 1 year previous to crop establishment. Therefore, any increased mineralisation of SOC made available by tillage is most likely to have occurred in the months following the initial soil disturbance (Vellinga *et al.*, 2004; Willems *et al.*, 2011). Secondly, a high seeding rate was employed to guarantee successful establishment. Thirdly, RCG is considered a highly competitive plant that grows rapidly and pre-empts the development of other vegetation early in the growing season (Lavergne & Molofsky, 2004). For example, Adams & Galatowitsch (2005) highlighted a shift from a low root:shoot ratio (< 1) in the first 4 months of development to a higher ratio (> 2) in the remainder of the 2-year study, which enabled RCG to initially monopolise above-ground space and later to spread vegetatively below ground.

Rapid early colonisation and crop development can therefore reduce the early transitional C losses associated with land-use change. However, our results suggest that significant net ecosystem C losses can be generated as a result of reduced photosynthetic assimilation and enhanced decomposition rates, particularly in C-rich grasslands. This loss represents a short-term land conversion carbon debt that must be overcome before net C sequestration is attained in the newly established bioenergy crop.

Table 4 Transitional-phase and post-establishment phase C fluxes associated with different land-use transitions. Annual fluxes from permanent grasslands are included for comparison. Cumulative fluxes refer to net ecosystem exchange (NEE), total ecosystem respiration (TER) or soil respiration (SR). For transitional-phase fluxes, the time period refers to the sampling duration in months (M). For long-term fluxes, the length of establishment is given in years (Y), where Y1 is the conversion year

Original land use	New land use	Location	Time period	Cumulative NEE ^A , TER ^B or SR ^C (g C m ⁻²)	Research note	Reference
Transitional-phase fluxes						
Post-herbicide						
Grass	Grass	Ireland	1 M	278 ± 29 ^B	Summer reseeded	Unpublished results*
Grass	Soybean	USA	1 M	19–46 ^A		Zenone <i>et al.</i> (2011)
Post-tillage						
Grass	<i>Miscanthus</i>	Ireland	3 M	155 ± 9 ^A		This study
Wheat	Wheat	USA	2 M	29–41 ^C		Dao, 1998
Grass	Spring barley	Denmark	3 M	260 ^C		Eriksen & Jensen (2001)
Conversion year (Y1)						
Grass	<i>Miscanthus</i>	Ireland	8 M	183 ± 28 ^A	From tillage	This study
Arable	<i>Miscanthus</i>	USA	7 M	-58 ^A	From planting	Zeri <i>et al.</i> (2011)
Grass	RCG	Ireland	8.5 M	-319 ± 57 ^A	From planting	This study
Grass/Arable	RCG	Finland	5.3 M	-57 ^A	From 1.5M post-planting	Lind <i>et al.</i> (2015)
Grass/Arable	Poplar	Belgium	7 M	75 ± 4.4 ^A	From 2M post-planting	Zenone <i>et al.</i> (2015)
Arable	Poplar	Canada	12 M	312 ^A	From 4M post-tillage	Cai <i>et al.</i> (2011)
Grass/Wheat	Switchgrass	USA	12 M	-31 ^A	From 4M post-planting	Skinner & Adler (2010)
Grass	Soybean	USA	12 M	205–262 ^A	No-till	Zenone <i>et al.</i> (2013)
Post-establishment (long-term) fluxes						
Grass	<i>Miscanthus</i>	Ireland	Y2	13 ± 45 ^A		This study
Grass	<i>Miscanthus</i>	Ireland	Y3	-411 ± 63 ^A		This study
Arable	<i>Miscanthus</i>	USA	Y3	-554 ± 20 ^A		Zeri <i>et al.</i> (2011)
Grass	RCG	Ireland	Y2	-397 ± 114 ^A		This study
Grass/Arable	RCG	Finland	Y2, 3†	-259 ^A	Fertilised	Lind <i>et al.</i> (2015)
Drained peat	RCG	Finland	Y4–7	-9 to -211 ^A		Shurpali <i>et al.</i> (2009)
Drained peat	RCG	Estonia	Y4	-91 to -163 ^A ‡	± Fertiliser	Mander <i>et al.</i> (2012)
Arable	Switchgrass	USA	Y3	-485 ± 20 ^A		Zeri <i>et al.</i> (2011)
NA	Switchgrass	USA	Y3, 4†	-448 ^A	Growing season only	Wagle <i>et al.</i> , 2015;
Grass/Arable	Poplar	Belgium	Y2	-96 ± 15 ^A		Zona <i>et al.</i> (2013)
Arable	Poplar	Canada	Y5	-17 ^A		Cai <i>et al.</i> (2011)
Permanent grassland						
Grass		Ireland	Annual	-200 to -385 ^A	Johnstown Castle; similar soil	Peichl <i>et al.</i> (2012)
Grass		Ireland	Annual	-193 to -258 ^A	South-west region	Jaksic <i>et al.</i> (2006)
Grass		Europe	Annual	-240 ± 70 ^A	9 sites	Soussana <i>et al.</i> (2007)

NA, not available.

*Órlaith Ní Choncubhair, Bruce Osborne, Karl Richards, Gary Lanigan.

†Mean value given for these years.

‡Chamber measurements.

C balance implications postestablishment

The *Miscanthus* ecosystem switched from a net C source of 183 ± 28 g C m⁻² in the conversion year (8-month period) to C-neutral in the second year and a strong C sink in the third year (-411 ± 63 g C m⁻²). In contrast, rapid and early development of the RCG crop resulted in the ecosystem being a net C sink both during the conversion year (-319 ± 57 g C m⁻², 8½-month period

post-planting) and in the second year of establishment (-397 ± 114 g C m⁻²). Previous assessments of C fluxes in the conversion year show highly variable results (Table 4), with cumulative NEE ranging from -58 to +312 g C m⁻² depending on crop type and establishment technique, location and duration of measurements. Only a limited number of studies revealed small C sinks in the conversion year, similar to RCG in this study. Even after harvested biomass losses were

incorporated, the NECB of this RCG ecosystem post-planting was still positive, indicating a net C sink of $66 \pm 58 \text{ g C m}^{-2}$ in the conversion year.

Net C fluxes in 2011 compare favourably with long-term fluxes from previous studies (Table 4), which range from close to C-neutral for poplar and RCG in certain years to in excess of $-450 \text{ g C m}^{-2} \text{ y}^{-1}$ for *Miscanthus* and switchgrass. Good agreement exists between our results and the studies on *Miscanthus* in the USA ($-554 \pm 20 \text{ g C m}^{-2} \text{ y}^{-1}$, Zeri *et al.*, 2011) and RCG on mineral soil in Finland ($-259 \text{ g C m}^{-2} \text{ y}^{-1}$, Lind *et al.*, 2015), taking account of location and comparative differences in season length and environmental controls. Studies focussed on the LUC transition from permanent grassland to *Miscanthus* or RCG are severely lacking, however, despite the fact that almost one-third of utilised agricultural land in Europe is grassland (Fischer *et al.*, 2010).

In this study, the key driver of ecosystem C fluxes in *Miscanthus* was crop development, constraining monthly-cumulated NEE in the first 2 years close to zero as primary production was counterbalanced by ecosystem respiration. In addition to the inherent limitations of slow establishment in northern Europe, various other factors may have contributed to the poor agronomic performance observed including relatively late planting, possible rhizome failure and competition from understory grass species. However, in the third growing season, exponential above-ground growth and leaf expansion was accompanied by a 4-fold increase in below-ground biomass, resulting in strong net C uptake from June to October of that year.

In the case of RCG, environmental conditions and crop phenology were the main drivers of ecosystem C exchange. Since crop establishment was rapid and early canopy development was uninhibited by competition from other species, physiological activity was dictated by soil temperature, moisture and incident radiation. Net C uptake of $-35 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ was observed just over two months after planting, culminating in a strong net C sink of $-319 \pm 57 \text{ g C m}^{-2}$ in the conversion year and an increased net sink in the second year ($-397 \pm 114 \text{ g C m}^{-2}$). Furthermore, below-ground biomass represented a significant C stock of 3.4 t C ha^{-1} , similar to the results of Xiong & Kätterer (2010).

During periods when RCG productivity exceeded that of *Miscanthus* (autumn of the conversion year and early in its second year), higher soil respiration (below-ground autotrophic and heterotrophic) was recorded in RCG than *Miscanthus*. This may indicate that there was a larger contribution from the autotrophic component to total below-ground respiration in RCG at these times. Soil respiration fluxes were similar in both crops outside of these periods. In a Finnish RCG crop which had 70%

of total plant biomass below ground during the peak growth period, autotrophic respiration was the dominant component (about 55%) of TER (Shurpali *et al.*, 2008). Soil respiration constituted the majority of TER in this study, particularly in the final year. While recognising the methodological differences between chamber and EC estimates, this result highlights the significant and increasing contribution of below-ground processes to C cycling in these developing perennial crops. Enhanced below-ground C allocation and root-associated C cycling may favour long-term soil carbon sequestration (Xiong & Kätterer, 2010; Anderson-Teixeira *et al.*, 2013), boosted also by the absence of regular soil disturbance through tillage (Freibauer *et al.*, 2004).

For the early postestablishment phase, our findings suggest that RCG provides a more favourable C balance than *Miscanthus*. However, the outperformance of RCG by *Miscanthus* in the third growth year, both in terms of yield and net C accumulation, indicates that the C sink potential of *Miscanthus* will improve further as the crop matures and above-ground productivity approaches the maximum yield.

Leaf longevity in Miscanthus and RCG

Light-use efficiency is a critical determinant of primary production and crop yield (Monteith, 1977) and is closely related to the crop's ability to maintain a closed canopy during the growing season (Beale & Long, 1995). Greater leaf longevity and higher leaf area have been demonstrated in *Miscanthus* compared with C_4 maize (Dohleman & Long, 2009). However, little information exists on the relative performance of *Miscanthus* when compared to native C_3 plants well-adapted to the temperate conditions of northern Europe.

In this study, strong coupling of GPP and crop phenology was highlighted by a striking 3-month difference in the timing of peak C assimilation in RCG (May) and *Miscanthus* (August) in 2011. RCG emerged early in the spring and achieved net C uptake (the 'break-even' or compensation point) by late February. This is consistent with the temperate climatic conditions of the current study and the associated long growing season. However, the subsequent occurrence of peak RCG productivity in mid-May resulted in the early onset of senescence in August. Crop phenology, therefore, introduced an asynchrony between the timing of maximum leaf area and maximum solar radiation for RCG, which could have implications for the ability of RCG to fully exploit peak irradiances and to maximise yields under some environmental conditions. Furthermore, RCG TER fluxes tended to exceed GPP in the winter months and reduced the cumulative C sink by 113 g C m^{-2} in the second year of establishment.

In contrast with RCG which exhibited early season leaf development, significant increases in leaf area did not occur until June for *Miscanthus*. As a result, the 'break-even' point of net C uptake did not occur until late June in 2011. However, *Miscanthus* maintained growth and substantial leaf area late into the autumn and sustained net ecosystem C accumulation until mid-November. After this, the crop remained C-neutral until the end of the year, similar to the findings of Zeri *et al.* (2011). Indeed, strong coupling of TER and GPP was observed during much of the current study, which meant that significant C losses did not occur.

Dohleman & Long (2009) demonstrated a 59% longer growing season in *Miscanthus* (199 days on average) than C₄ maize (126 days on average). Our results show that the longevity of photosynthetically active leaves in *Miscanthus* is comparable even to native C₃ crops. Indeed, the number of recorded days with net C uptake in 2011 was almost identical for both crops (215 and 212 days for *Miscanthus* and RCG, respectively). This result compares very favourably with an average cropping season length of 149 days in Irish spring barley (Davis *et al.*, 2010) and 212 days of net C uptake (six-year average) in an Irish grassland (Peichl *et al.*, 2011). This further highlights the exceptional performance of C₄ *Miscanthus* in cool temperate climates where C₄ metabolism should be temperature-limited and implies that *Miscanthus* may confer an advantage as a bioenergy crop in the long term. If leaf photosynthetic capacity and longevity is comparable to native C₃ crops and C emissions outside of the growing season are close to zero, the net C balance of *Miscanthus* is likely to be favourable.

Implications of the study

Bioenergy crop cultivation in northern Europe may focus more on grassland conversion to avoid a reduction in the area of croplands dedicated to food and feed production. However, permanent grasslands in northern Europe show strong annual net C uptake, with NEE values ranging from -193 to -385 g C m⁻² y⁻¹ (Table 4). This highlights the potential negative impacts associated with disturbing grasslands that are highly productive and supply substantial amounts of stabilised C to the soil (Jackson *et al.*, 1996; Jones & Donnelly, 2004; Poeplau *et al.*, 2011). Our study demonstrated that a significant C debt can be associated with the early-establishment phase of these bioenergy crops but highlighted the future potential of *Miscanthus* to surpass RCG and possibly long-term grasslands in terms of its C sink strength. Additional measurements of other associated

GHGs, such as N₂O and CH₄, will be necessary, however, to assess the full GHG implications of land-use change to this crop.

Furthermore, the duration of the full crop production cycle must be considered. In the case of *Miscanthus*, productive yields may be achievable for up to 15 years or more (Clifton-Brown *et al.*, 2007; Christian *et al.*, 2008; Arundale *et al.*, 2014) but this is likely to be much shorter for RCG (approximately 7–10 years; Saijonkari-Pahkala, 2001; Finnan, 2007). Therefore, more cultivation and replanting will be required in long-term RCG plantations and this has associated C balance implications due to more regular soil disturbance and concomitant reductions in productivity.

Although the theoretical light-use efficiency benefits afforded by the C₄ photosynthetic pathway are often highlighted, the empirical results of this work suggest that high biomass productivity will be controlled more by leaf- or canopy-related factors, both genetic and environmentally derived, rather than the photosynthetic characteristics of individual leaves. Further to this, the distinct difference in the timing of peak C uptake between the two crops is significant and provides information on the suitability of these crops in different climatic zones. While the late season performance of *Miscanthus* may constrain its productivity in regions with a short growing season, RCG may be a better candidate in these regions due to its early emergence in spring and subsequent rapid development. Growing *Miscanthus* under a clear, plastic film, as is common practice for maize production in parts of northern Europe, may also be a valuable tool to encourage earlier emergence in spring and enhance further the duration of net C uptake in the crop (Clifton-Brown *et al.*, 2011).

A final point worth noting is the relative allocation of biomass above and below ground in these two bioenergy crops. At the time of peak biomass yield in 2011, more than 50% of total RCG biomass was below ground compared with 24% in *Miscanthus* and above-ground biomass in *Miscanthus* was almost double that recorded in RCG. Greater investment of resources below ground could enhance long-term C sequestration; however, lower above-ground yields have significant implications for the economic viability and C-offsetting potential of the RCG crop.

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