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# Implications of productivity and nutrient requirements on greenhouse gas balance of annual and perennial bioenergy crops

STEPHANE CADOUX\*, †, FABIEN FERCHAUD‡, CHARLOTTE DEMAY§, HUBERT BOIZARD§, JEAN-MARIE MACHET‡, EMILIE FOURDINIER§, MATTHIEU PREUDHOMME§, BRIGITTE CHABBERT¶, ||, GHISLAIN GOSSE\*\* and BRUNO MARY‡

\*INRA, UMR0211 Agronomie, F-78850, Thiverval-Grignon, France, †AgroParisTech, UMR Agronomie, F-78850, Thiverval-Grignon, France, ‡INRA, UPR1158 AgroImpact, site de Laon, F-02000, Barenton-Bugny, France, §INRA, UPR1158 AgroImpact, site d' Estrées-Mons, F-80203, Péronne, France, ¶INRA, UMR614 Fractionnement des AgroRessources et Environnement, F-51000, Reims, France, ||UMR Fractionnement des AgroRessources et Environnement, Université de Reims-Champagne Ardenne, F-51000, Reims, France, \*\*Agro-Transfert Ressources et Territoires, F-80200, Estrées-Mons, France

## Abstract

Biomass from dedicated crops is expected to contribute significantly to the replacement of fossil resources. However, sustainable bioenergy cropping systems must provide high biomass production and low environmental impacts. This study aimed at quantifying biomass production, nutrient removal, expected ethanol production, and greenhouse gas (GHG) balance of six bioenergy crops: *Miscanthus × giganteus*, switchgrass, fescue, alfalfa, triticale, and fiber sorghum. Biomass production and N, P, K balances (input-output) were measured during 4 years in a long-term experiment, which included two nitrogen fertilization treatments. These results were used to calculate *a posteriori* 'optimized' fertilization practices, which would ensure a sustainable production with a nil balance of nutrients. A modified version of the cost/benefit approach proposed by Crutzen *et al.* (2008), comparing the GHG emissions resulting from N-P-K fertilization of bioenergy crops and the GHG emissions saved by replacing fossil fuel, was applied to these 'optimized' situations. Biomass production varied among crops between 10.0 (fescue) and 26.9 t DM ha<sup>-1</sup> yr<sup>-1</sup> (miscanthus harvested early) and the expected ethanol production between 1.3 (alfalfa) and 6.1 t ha<sup>-1</sup> yr<sup>-1</sup> (miscanthus harvested early). The cost/benefit ratio ranged from 0.10 (miscanthus harvested late) to 0.71 (fescue); it was closely correlated with the N/C ratio of the harvested biomass, except for alfalfa. The amount of saved CO<sub>2</sub> emissions varied from 1.0 (fescue) to 8.6 t CO<sub>2</sub>eq ha<sup>-1</sup> yr<sup>-1</sup> (miscanthus harvested early or late). Due to its high biomass production, miscanthus was able to combine a high production of ethanol and a large saving of CO<sub>2</sub> emissions. Miscanthus and switchgrass harvested late gave the best compromise between low N-P-K requirements, high GHG saving per unit of biomass, and high productivity per hectare.

**Keywords:** biofuels, biomass, C3 crops, C4 crops, ethanol, greenhouse gas, legume crops, lignocelluloses, nitrogen, nutrient use efficiency

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## Introduction

To reduce greenhouse gas (GHG) emissions and limit climate change, many authors have shown the need to develop a renewable energy portfolio that could replace fossil resources, together with an improvement in energy efficiency (e.g. IEA, 2010; Teske *et al.*, 2011). Biomass is expected to contribute significantly to this portfolio by providing an important source of renewable carbon for the production of bioenergy (Berndes *et al.*, 2003; Sims *et al.*, 2006), biomaterials, and chemicals

(Ragauskas *et al.*, 2006; Bozell & Petersen, 2010; Cherubini, 2010). Biomass resources consist of bioenergy crops, agricultural and forest residues, animal manure, and organic wastes. Several authors have studied their respective potential contribution (e.g. Hoogwijk *et al.*, 2003, 2005; Smeets & Faaij, 2007; Haberl *et al.*, 2010). In a recent review, Chum *et al.* (2011) conclude that dedicated bioenergy crops have the largest technical potential.

Increasing the cultivation of dedicated bioenergy crops introduces the difficulty of reconciling food production, energy production, and conservation of the environment (Tilman *et al.*, 2009). Hence, to ensure a sustainable development of bioenergy crops, the area

Correspondence: F. Ferchaud, tel. + 33 323240775, fax + 33 323240776, e-mail: fabien.ferchaud@laon.inra.fr

dedicated to their production must be limited to minimize the competition with food production, the carbon or energy production per unit area must be high enough to replace significant amounts of fossil resources, and their global and local environmental impacts must be as low as possible (Field *et al.*, 2008; Haughton *et al.*, 2009; Rowe *et al.*, 2009; Karp & Richter, 2011). This suggests seeking new plant types and suitable cropping practices that could simultaneously fulfill all these requirements.

The possibility of using biomass for a biorefinery industry allows a wide range of candidate crops: perennial C4 crops, short rotation coppices, semiperennial forage crops and annual C4 or C3 crops (Karp & Shield, 2008; Sanderson & Adler, 2008; Zegada-Lizarazu *et al.*, 2010). Many papers report data on one crop, but few have compared a diversity of crop types in the same location to identify the most suitable crop for bioenergy production. Annual and perennial crops were experimentally compared in the studies of Cherney *et al.* (1991), Scholz & Ellerbrock (2002), Boehmel *et al.* (2008), Propheter *et al.* (2010), and Propheter & Staggenborg (2010). No consensus emerged on the superiority of any crop in terms of biomass production. These studies, including those focusing on perennial crops (Mantineo *et al.*, 2009; Kering *et al.*, 2012), did not confirm that miscanthus was the highest yielding crop as shown in review papers (e.g. Lewandowski *et al.*, 2003; Somerville *et al.*, 2010). This is due to the interaction between crop type, crop management, and soil and climatic conditions (Cadoux *et al.*, 2010), which emphasizes the need to compare crops at the same site to avoid confusion with soil and climate effects. Concerning nutrient removal at harvest, annual crops seem to remove more nutrients than perennial crops (Karp & Shield, 2008). However, few studies have addressed the removal of P and K (Propheter & Staggenborg, 2010; Kering *et al.*, 2012), despite the concerns about the depletion of these nonrenewable, nonmanufacturable resources (Neset & Cordell, 2012).

Comparisons of bioenergy crops with regard to GHG balance are mainly based on life cycle analysis (LCA) (Adler *et al.*, 2007; Hillier *et al.*, 2009; Fazio & Monti, 2011). These studies indicate lower GHG emissions of all bioenergy cropping systems compared to fossil-based energies – assuming cultivation on arable land – and lower GHG emissions of the biofuels obtained from perennial crops compared with annual crops. GHG reductions varied between 40% and 60% with annual crops and between 90% and 115% with perennial crops, according to Adler *et al.* (2007) and Fazio & Monti (2011), respectively. In these studies, the assumptions made about crop management practices are generally (i) based on data coming from various papers and thus different experimental sites; and (ii) never based on

optimized fertilization practices, i.e. defined to ensure both limiting environmental impacts and maintaining soil fertility over the long-term (OECD, 2008). This is likely to affect LCA results as nutrient fertilization is often considered as a major contributor to the GHG emissions of energy crops (Adler *et al.*, 2007; Styles & Jones, 2007) and LCA approach is highly dependent on the assumptions made about management practices and yields (Bessou *et al.*, 2011). Crutzen *et al.* (2008) have proposed a cost/benefit approach to assess the GHG balance of bioenergy crops. Their methodology focusing on the N<sub>2</sub>O emissions during the crop production phase is based on the calculation of a cost/benefit ratio (the global warming effect of N<sub>2</sub>O emissions resulting from N fertilization vs. the cooling effect due to replacement of fossil fuel). Using a global N<sub>2</sub>O emission factor of 3–5%, these authors concluded that the high level of N fertilization required by conventional annual bioenergy crops was likely to lead to higher GHG emissions than fossil fuel (up to +70% in the case of rapeseed). They also pointed out the value of perennial crops to reduce N<sub>2</sub>O emissions.

In this study, we aimed to quantify the yield and nutrient removal at harvest of six bioenergy crops and to predict their GHG balance, assuming a sustainable nutrient management of these crops. To address this question, we compiled the data obtained over 4 years in a long-term experiment, which includes two nitrogen fertilization rates and hypothesized that (i) the optimized fertilization management and corresponding crop performance could be defined by interpolating the experimental results; and (ii) the GHG balance related to nutrient fertilization could be assessed using the cost/benefit approach proposed by Crutzen *et al.* (2008).

## Materials and methods

### *Site characteristics and experimental design*

The experiment was initiated in 2006 at the INRA experimental station at Estrées-Mons in northern France (49.872°N latitude, 3.013°E longitude) in a 4.5-hectare field. The soil is a deep silt loam (Ortic Luvisol, FAO classification). The texture of the ploughed layer (0–30 cm) is 17% clay, 78% silt, and 5% sand. In 2006, the mean organic carbon content was 10.4 g kg<sup>-1</sup> and the pH in water was 7.8. The available soil water content, calculated over 1.50 m depth, is 285 mm. The climate is oceanic temperate; the average annual precipitation and temperature were 684 mm and 10.4 °C respectively for the period 2006–2010. Over the period of the experiment, 2008 was the wettest year and 2009 was the driest. In 2009, the cumulative water deficit (PET-precipitation) reached 237 mm (Table 1). Before the start of the experiment, the site was mainly cropped with winter wheat, winter barley, sugar beet, and spring pea. The previous crop was wheat, harvested in July 2005.

**Table 1** Meteorological data recorded at Estrées-Mons over the period 2006–2010

	2006	2007	2008	2009	2010
Average temperature (°C)	10.8	10.9	10.5	10.6	9.6
Precipitation (mm)	696	717	756	569	680
PET: potential evapotranspiration (mm)	796	728	701	806	756
Precipitation-PET (mm)	-100	-11	55	-237	-76
Global radiation (MJ m <sup>-2</sup> )	4372	4367	4138	4321	4219

Six crops were chosen to represent a wide range of bioenergy crop types (perennial, semiperennial, and annual; C4, C3, and legume crops). They were selected for their potential biomass yield and/or their expected low input requirements (fertilizer, pesticides, and water): miscanthus (*Miscanthus × giganteus* Greef & Deuter ex Hodkinson & Renvoize), switchgrass (*Panicum virgatum* 'Kanlow'), fescue (*Festuca arundinacea* 'dulcia' from 2006 to 2009 and 'Noria' after 2009), alfalfa (*Medicago sativa* 'Alpha' from 2006 to 2009 and 'Orca' after 2009), triticale (*×Triticosecale* Wittmack 'Triskell' from 2006 to 2008 and 'Amarillo' after 2008), and fiber sorghum [*Sorghum bicolor* (L.) Moench 'H133']. The changes in cultivars were motivated by the lower susceptibility to pests and diseases to reduce the use of pesticides. The annual crops were grown in rotation (triticale grown after fiber sorghum and vice versa) as well as the semiperennial crops (alfalfa grown after fescue and vice versa). All crops were present each year. Two harvest dates were compared for miscanthus and switchgrass: an early harvest in October (E) and a late harvest in February (L). Miscanthus E and L as well as switchgrass E and L were considered in the analysis as different crops. The experiment also included two nitrogen treatments (N<sup>-</sup> and N<sup>+</sup>) for each crop except alfalfa (Table 2). The N rates depended on the crops and were fixed *a priori*. The N<sup>-</sup> treatment was chosen to have low GHG emissions, a negative N balance (difference between fertilizer-N and N exported in

**Table 2** Mean N fertilization rates and harvest dates during the period 2007–2010. N fertilization of fescue varied between years (with the number of cuts) from 40 to 120 kg ha<sup>-1</sup> yr<sup>-1</sup> in the N<sup>-</sup> treatment and from 80 to 240 kg ha<sup>-1</sup> yr<sup>-1</sup> in the N<sup>+</sup> treatment

	N fertilization (kg ha <sup>-1</sup> yr <sup>-1</sup> )		Harvest date	
	N <sup>-</sup>	N <sup>+</sup>	E	L
Miscanthus	0	120	October	February
Switchgrass	0	120	October	February
Fescue	90	180		
Alfalfa	0	0		
Triticale	60	120		
Fiber sorghum	0	120		

the harvested biomass) and a moderate reduction in potential growth. The N<sup>+</sup> treatment was designed to produce a positive N balance and a biomass growth close to the potential allowed by the soil and climatic conditions. The N fertilization rates were calculated on the basis of the results obtained by Lewandowski & Schmidt (2006), McLaughlin & Adams Kszos (2005), Cherney *et al.* (1991), Lewandowski & Schmidt (2006) and Wiedenfeld (1984) for miscanthus, switchgrass, fescue, triticale, and fiber sorghum, respectively. The nitrogen fertilizer was applied as urea ammonium nitrate solution (UAN containing 390 g N l<sup>-1</sup>). Fescue received a fixed amount of fertilizer-N at the beginning of each cycle of regrowth, so that the total N rate per year varied according to the number of cuts. Alfalfa was not fertilized due to its N-fixing capacity, but two nitrogen treatments were distinguished as the crop was grown either after fescue N<sup>+</sup> or N<sup>-</sup> in the second rotation.

To facilitate cultural operations and limit competition between plants due to differences in canopy height, the experimental field was divided into two parts: (i) a criss-cross design for the perennial crops with four cropping systems as main plots (miscanthus E, miscanthus L, switchgrass E, switchgrass L) and nitrogen applications as subplots; and (ii) a split-plot design for the semiperennial and annual crops with species as main plots and nitrogen applications as subplots. Both parts included three replicate blocks and subplot sizes of 360 m<sup>2</sup>. This layout was preferred to a complete randomized block design, to minimize the 'edge effects' coming from differences in height between crops. Such a design has been used for comparing very different crops (e.g. Cherney *et al.*, 1991; Scholz & Ellerbrock, 2002). The pedological investigation made in 2006 showed a high homogeneity of the soil: soil depth is greater than 3 m in the whole field and textural variations are small. This indicates that soil conditions are very homogeneous between plots, making possible the comparison between all crops.

Perennial crops were established in 2006; semiperennial crops were sown in 2006 (first rotation) and 2009 (second rotation). The planting density of miscanthus was 1.5 plants m<sup>-2</sup>. The sowing density of switchgrass, fescue, alfalfa, and triticale was 15, 22, 19, and 100 bulk kg ha<sup>-1</sup> respectively, whereas fiber sorghum was sown at 22 bulk grains m<sup>-2</sup>. In 2006, miscanthus and switchgrass were not harvested, but the aerial biomass was chopped and left on the soil surface. The experiment did not receive irrigation, phosphorus, or potassium fertilizer. Pesticides were applied only when the infestation pressure was likely to significantly affect yields. Fungicides were applied once a year on triticale, generally at half dose. All crops received herbicides during the experiment: 2 years out of five for fescue, 3 years out of five for miscanthus harvested late, 4 years out of five for miscanthus harvested early, switchgrass and alfalfa and every year for triticale and fiber sorghum.

### Measurements

At harvest, the aerial biomass of each crop was collected manually and weighted. However, the harvest method and date varied according to crops. The size of the harvested areas was determined according to the amount of fresh matter per unit area and the stand homogeneity. Miscanthus and switchgrass were harvested in October for early harvested treatments and

at the end of February or early March for late harvested treatments. In the miscanthus stand, the aerial biomass was weighted in an area of 3.84 m<sup>2</sup> containing six plants and the number of stems was measured in a larger zone of 25.6 m<sup>2</sup>. The biomass production per hectare was calculated by multiplying the biomass per stem by the number of stems per ha. The switchgrass stand was very homogeneous and the biomass was collected in a 2.50 m<sup>2</sup> zone. Fescue and alfalfa were harvested at flowering for the first two cuts and at the end of growth for the third cut. Triticale was harvested at physiological maturity. The biomass of these three crops was collected in a 15 m<sup>2</sup> area. Sorghum was harvested at the end of September and the biomass was sampled in a 21.6 m<sup>2</sup> area (six rows of 8 m). For all crops, the cutting height was 7 cm and the dry matter content was obtained after drying representative subsamples at 65 °C for 96 h. The biomass production was expressed in tons of dry matter per hectare for all crops.

Dry subsamples were analyzed each year to quantify the concentration of carbon (C), nitrogen (N), phosphorus (P), and potassium (K). Total C and N were determined by dry combustion (NF ISO 10694 and 13878) using an elemental analyzer (CNS 2000, Leco). P and K were measured after total mineralization by hydrogen peroxide and nitric acid. P was determined by UV-Visible spectroscopy (Varian Cary 50) and K by atomic absorption spectroscopy (Varian AA240FS). N, P, and K contents at harvest and ratios of each nutrient concentration to carbon concentration (N/C, P/C and K/C) were then calculated.

The soil mineral nitrogen content in the 0–150 cm layer, calculated using measurements of soil bulk density and mineral N concentrations (data not shown), was 159 ± 30 kg N ha<sup>-1</sup> (mean ± standard deviation) in April 2006. It decreased during the following 2 years in all treatments reaching 44 ± 23 kg N ha<sup>-1</sup> in March 2008 and was fairly constant thereafter.

### Assessment of GHG balance in relation to N-P-K fertilization

The experimental data were used to compare the GHG balance related to N-P-K fertilization of the different bioenergy crops using a modified version of the cost/benefit method proposed by Crutzen *et al.* (2008). This analysis was made by considering the production of ethanol from lignocellulose, which is likely to be the major biobased product from carbohydrates (Bozell & Petersen, 2010). The indicator developed by Crutzen *et al.* (2008) compares the global warming effect of N<sub>2</sub>O emissions resulting from N fertilization (*Meq*), and the cooling effect due to replacement of fossil fuel by biofuel (*M*). In our study, the calculation of *M* was similar to that of Crutzen *et al.* (2008), but we added to the calculation of *Meq* the effect of the GHG emitted during production and transport of fertilizer-N, P, and K.

Let us consider first the benefit ('saved fossil CO<sub>2</sub>') due to bioenergy crops. According to Crutzen *et al.* (2008), it is directly linked to carbon contained in biomass as 'the lower heat value per carbon and consequently the CO<sub>2</sub> emissions per energy unit are almost identical for the fossil fuels and the bio-fuels'. It can be written:

$$M = r_C \cdot \frac{\mu_{CO_2}}{\mu_C} \cdot c_v \quad (1)$$

where *M* is in g CO<sub>2</sub> kg<sup>-1</sup> DM of biomass, *r<sub>C</sub>* is the carbon concentration in biomass (g kg<sup>-1</sup>), *c<sub>v</sub>* is the yield of transformation of biomass into ethanol (g C biofuel g<sup>-1</sup> C biomass), *μ* terms refer to the molar weights of CO<sub>2</sub> and C ( $\mu_{CO_2}/\mu_C = 44/12$ ).

We calculated *c<sub>v</sub>* as:

$$c_v = EtOH \cdot \frac{C_{EtOH}}{r_C} \quad (2)$$

where *EtOH* is the conversion yield of biomass to ethanol (g kg<sup>-1</sup>) and *C<sub>EtOH</sub>* is the C content of ethanol ( $C_{EtOH} = 24/46$ ).

Lignocelluloses can be biologically converted into ethanol following either separate hydrolysis and fermentation (SHF) or simultaneous saccharification and cofermentation of C5 and C6 sugars (SSCF), the last one being favored in several technologies under development in USA (Aden, 2008) and in Europe (Gnansounou, 2010). For triticale, we assumed a separate conversion of grain using mature first generation technologies. We used the following calculation for *EtOH* derived from USDOE (2010), Vogel *et al.* (2011), and Zhao *et al.* (2012):

$$EtOH = 0.51 * (1.11 * 0.85 * starch + 1.11 * 0.65 * cellulose + 1.14 * 0.65 * hemicellulose) \quad (3)$$

where the concentrations of starch, cellulose, and hemicellulose, expressed in g kg<sup>-1</sup> of dry harvested biomass, are obtained from Da Silva Perez *et al.* (2010); 0.51 is the conversion factor of ethanol from sugars (USDOE, 2010; Vogel *et al.*, 2011; Zhao *et al.*, 2012); 1.11 and 1.14 are the conversion factors of sugars from glucan (cellulose and starch) and xylan (hemicellulose), respectively; 0.85 is the process efficiency of ethanol from starch (Zhao *et al.*, 2012) and 0.65 is a process efficiency of ethanol from lignocellulose, which seems achievable *via* SSCF for most crops (Olofsson *et al.*, 2008).

Let us consider now the cost effect of bioenergy crops. According to Crutzen *et al.* (2008), it can be written:

$$Meq = t_{N_2O} \cdot \frac{\mu_{N_2O}}{\mu_{N_2}} \cdot GWP \quad (4)$$

where *Meq* is given in kg CO<sub>2</sub> t<sup>-1</sup> dry matter of harvested biomass, *μ* represents the molar weights of N<sub>2</sub>O and N<sub>2</sub> ( $\mu_{N_2O}/\mu_{N_2} = 44/28$ ) and *GWP* is the global warming potential of N<sub>2</sub>O (296 kg CO<sub>2</sub> kg<sup>-1</sup> N<sub>2</sub>O). *t<sub>N<sub>2</sub>O</sub>* represents the amount of N<sub>2</sub>O-N emitted per unit of harvested biomass (kg N t<sup>-1</sup> DM) and is equal to:

$$t_{N_2O} = r_N \cdot \frac{y}{e} \quad (5)$$

where *r<sub>N</sub>* is the nitrogen concentration in the harvested biomass (kg plant-N t<sup>-1</sup> DM), *y* is the 'yield of N<sub>2</sub>O-N from fixed N application', i.e. the global N<sub>2</sub>O emission factor for N input (kg N<sub>2</sub>O-N kg<sup>-1</sup> fertilizer-N) and the term *e* was defined by Crutzen *et al.* (2008) as 'a surrogate for the uptake efficiency of the fertilizer by the plants'. These authors set the *e* value at 0.40, based on reviews dealing with N fertilizer recovery efficiency (RE<sub>N</sub>), i.e. the percentage of fertilizer-N recovered in the above-ground plant biomass. However, we agree with Rauh & Berenz (2007) that this value is too small and inappropriate. Indeed,

according to the definitions of  $t_{N_2O}$  and  $y$ ,  $t_{N_2O}$  can also be written:

$$t_{N_2O} = \frac{F_N \cdot y}{B} \quad (6)$$

where  $F_N$  is the amount of N fertilizer (kg ha<sup>-1</sup>) and  $B$  is the harvested biomass (t DM ha<sup>-1</sup>).

Combining Eqns (5) and (6) yields:

$$e = B \cdot \frac{r_N}{F_N} \quad (7)$$

or

$$e = \frac{Q_N}{F_N} \quad (8)$$

where  $Q_N$  is the amount of N in the harvested biomass (kg ha<sup>-1</sup>).

Thus,  $e$  cannot be equated to  $RE_N$ , but represents the ratio of the amount of nitrogen in the harvested biomass to the amount of fertilizer-N applied to the crop.

Finally, combining Eqns (4), (5), and (7) yields:

$$Meq = \frac{F_N}{B} \cdot y \cdot \frac{\mu_{N_2O}}{\mu_{N_2}} \cdot GWP \quad (9)$$

There is still a serious debate about the  $N_2O$  emission factor  $y$  in the literature (e.g. Reay *et al.*, 2012). Crutzen *et al.* (2008) proposed a value of 0.03–0.05, estimated from a global ‘top-down’ approach. This value includes direct emissions from soil and indirect emissions from downwind and downstream ecosystems. It was further re-estimated by Smeets *et al.* (2007) who proposed a value of 0.027 and well argued by Davidson (2009) who found  $y = 0.025$  for nitrogen derived from mineral fertilizer by coupling bottom-up and top-down approaches. We used the latter value because it better accounts for the different potential sources of  $N_2O$  and is in better agreement with the direct and indirect emission factor estimates of the IPCC methodology (Davidson, 2009). We also assumed that N fertilization for biomass production will be mainly derived from mineral fertilizer. As  $y$  only includes the biological  $N_2O$  emission sources (Davidson, 2009), we propose to add to the  $Meq$  value the contribution of GHG ( $CO_2$ ,  $N_2O$ , and  $CH_4$ ) emitted during fertilizer production and transport ( $Meq_N$ ):

$$Meq_N = \alpha_N \cdot \frac{F_N}{B} \quad (10)$$

where  $\alpha_N$  is the GHG emission factor of fertilizer-N production and transport (kg CO<sub>2</sub> eq kg<sup>-1</sup> N). Similarly, GHG emissions due to production and transport of P and K fertilizers are:

$$Meq_P = \alpha_P \cdot \frac{F_P}{B} \quad (10')$$

$$Meq_K = \alpha_K \cdot \frac{F_K}{B} \quad (10'')$$

The values of  $\alpha_N$  (5.84),  $\alpha_P$  (4.63), and  $\alpha_K$  (0.60) are found in the Ecoinvent 2.2 database (Swiss Center for Life Cycle Inventories, 2010) with UAN solution, triple superphosphate, and

potassium chloride as fertilizer types for N, P, and K, respectively. These values include GHG emissions due to fertilizer production and transport ‘at regional storehouse’. Finally, the total emissions of GHG resulting from N-P-K fertilization are:

$$Meqt = Meq + Meq_N + Meq_P + Meq_K \quad (11)$$

The ethanol production (t ha<sup>-1</sup> yr<sup>-1</sup>) was calculated as:

$$EP = B \cdot EtOH \quad (12)$$

and the net benefit of the bioenergy crop (t CO<sub>2</sub> eq avoided ha<sup>-1</sup> yr<sup>-1</sup>), not considering emissions from fuel use during cultivation and from the transport and transformation of the biomass, was:

$$CO_2 \text{ avoided} = B \cdot (M - Meqt) \quad (13)$$

### Calculation of ‘optimized’ fertilization rate

The  $Meqt/M$  ratio could be calculated in the experimental treatments, but it is more relevant to calculate it in situations of sustainable bioenergy production. We assumed that the sustainability of bioenergy cropping systems involves compensating for nutrients removed in harvested biomass. Indeed, on one hand, any surplus of nutrients increases the potential losses of nutrients into the environment and, on the other hand, nutrient deficiency can impair soil fertility and future crop yield (OECD, 2008). Therefore, the calculations of  $M$ ,  $Meq$ , and  $Meqt$  were applied to ‘optimized’ situations for which nutrient fertilization was calculated to reach a balance between inputs and outputs, rather than to experimental treatments. In a first step, N, P, and K balances were calculated for each experimental treatment as the difference between fertilization inputs and harvest outputs. Then ‘optimized’ N fertilization rates were calculated empirically using a linear relationship between N balance and N fertilization rate, fitted on data from the N– and N+ treatments of each crop, to define the level of N fertilization leading to an N balance equal to 0. For the calculation of  $Meq$ , this was similar to the use of the original calculation by Crutzen *et al.* (2008) with Eqn (4), but assuming that  $e = 1$ . For alfalfa, we assumed no N fertilization, given its N-fixing capacity. The expected biomass in the ‘optimized’ situations was also calculated assuming a linear relationship between biomass and N fertilization rate. Similarly, concentrations of C, P, and K were determined assuming a linear relationship with biomass and the ‘optimized’ P and K fertilization rates were calculated to be equal to the harvest outputs. The error on the ‘optimized’ N rate with this method is likely to be small because the relationship between N fertilization and N uptake, and thus N balance, is linear when nitrogen supply is lower or close to the crop nitrogen needs (e.g. Ter Steege *et al.*, 2001). The errors on expected biomass and P and K fertilization rates are also reasonable because most estimates were interpolated between two close values of biomass and P and K concentrations (Fig. 1 and Fig. 2).

### Statistical analysis

ANOVA was used for statistical analysis of the biomass production, the N/C, the P/C, and the K/C ratios, for each year

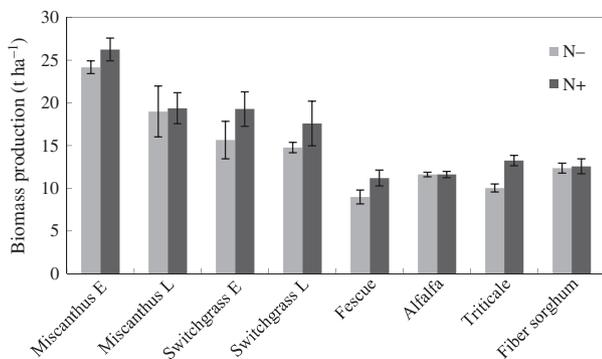
from 2007 (2006 being incomplete in terms of results), after verifying that variances were homogeneous. ANOVA was performed separately for the criss-cross of perennial crops and the split-plot of annual and semiperennial crops by using the criss-cross and the split-plot options of the statistical software Statbox 7.0. The comparison of all crops together was based on the difference in means and on the standard deviations.

## Results

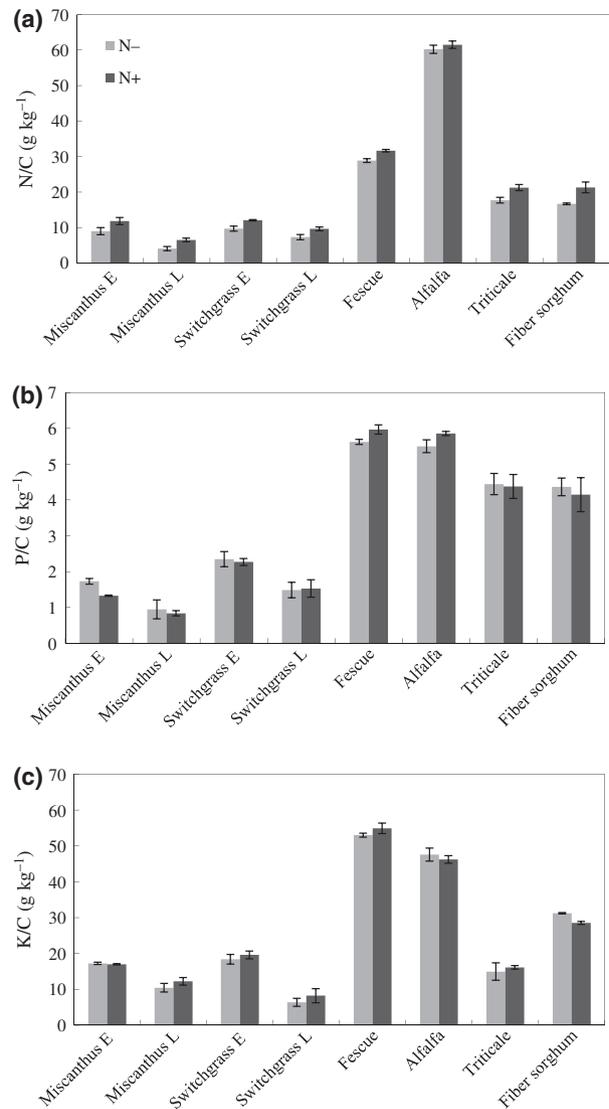
### Biomass production

Over the period 2007–2010, the biomass production varied widely between crops and experimental years (Table 3). The ANOVA showed that there was a significant crop effect among perennials every year from 2008 (Table 4). In 2008, miscanthus E treatments yielded more than miscanthus L, switchgrass E, and L. In 2009, the biomass ranked as follows: miscanthus E > miscanthus L > switchgrass E > switchgrass L. In 2010, the biomass was still the highest in miscanthus E treatments followed by miscanthus L and then switchgrass E and L. The nitrogen application rate had a significant effect in 2009, the biomass production being generally higher in N+ treatments. In 2010, an interaction appeared between crop and nitrogen, illustrating a strong and positive effect of fertilizer-N on miscanthus E, switchgrass E, and switchgrass L, but still no effect on the miscanthus harvested late.

The ANOVA indicated that a significant crop effect appeared on biomass production of annual and semiperennial crops in 2007 and remained later, but the crop ranking varied between years: fescue > alfalfa and fiber sorghum > triticale in 2007; alfalfa > triticale > fiber sorghum > fescue in 2008; fiber sorghum > triticale > fescue > alfalfa in 2009 and fiber sorghum and alfalfa > triticale > fescue in 2010. Nitrogen application rate had a significant effect on biomass in 2008. Triticale responded positively each year from 2008 and fescue responded positively in 2007, 2008,



**Fig. 1** Mean annual biomass production of the different crops and treatments over the period 2007–2010. Bars represent the standard deviations.



**Fig. 2** Mean nutrient/C ratio at harvest of the different crops and treatments over the period 2007–2010 for (a) N, (b) P, and (c) K. Bars represent the standard deviations.

and 2010, whereas alfalfa and fiber sorghum were not influenced by N fertilization rate.

During the whole period 2007–2010, the average biomass production was highest for the perennial C4 crops. Miscanthus produced more than switchgrass and both crops produced more at early harvest than at late harvest (Fig. 1). Annual and semiperennial crops had the lowest biomass yield. The effect of nitrogen was visible for switchgrass E and L, fescue, and triticale.

### Nutrient content at harvest

The nutrient (N, P, and K) content at harvest was expressed, for each nutrient, per unit of C produced to give efficiencies of nutrient removal. The variability in

**Table 3** Mean biomass production, N/C, P/C, and K/C ratio measured in the different crops and treatments from 2007 to 2010

		Biomass production t ha <sup>-1</sup>				N/C g kg <sup>-1</sup>				P/C g kg <sup>-1</sup>				K/C g kg <sup>-1</sup>			
		2007	2008	2009	2010	2007	2008	2009	2010	2007	2008	2009	2010	2007	2008	2009	2010
Miscanthus E	N-	23.0	23.6	24.0	26.1	10.4	8.4	6.7	10.3	1.6	1.3	1.6	2.3	19.4	14.0	15.0	20.2
	N+	22.7	26.2	27.7	28.4	11.1	9.5	11.8	14.7	1.6	0.9	1.2	1.7	17.9	14.2	16.4	19.5
Miscanthus L	N-	14.3	18.5	20.9	22.2	3.2	3.8	3.3	5.6	0.8	1.0	0.6	1.3	5.7	10.4	8.6	15.4
	N+	15.4	19.5	20.5	22.0	4.2	4.8	6.9	9.3	0.8	0.8	0.8	1.0	10.4	11.2	12.5	14.1
Switchgrass E	N-	19.6	18.9	14.9	9.2	11.8	10.3	7.1	8.7	2.5	2.2	1.9	2.8	23.8	17.4	15.0	14.8
	N+	19.3	20.1	19.5	18.2	14.6	11.1	10.2	12.9	2.6	2.1	1.6	3.0	21.3	19.7	18.3	19.6
Switchgrass L	N-	15.9	16.7	13.8	12.6	10.6	7.5	4.4	6.1	1.9	1.7	0.7	1.6	11.2	7.2	2.7	3.5
	N+	17.5	19.4	17.5	16.0	12.0	9.0	6.8	11.1	1.8	1.6	1.0	1.7	11.3	7.9	4.8	9.1
Fescue	N-	16.6	7.5	5.8	6.0	28.4	16.9	38.7	35.9	6.0	5.1	4.6	6.1	62.0	40.1	50.8	46.8
	N+	19.1	11.4	6.4	8.0	31.1	20.0	43.6	40.3	6.6	5.1	4.9	6.7	66.5	40.1	53.2	50.4
Alfalfa	N-	14.6	15.8	3.3	12.7	64.0	58.2	57.9	59.1	5.9	5.7	4.4	5.1	58.2	43.6	34.5	44.3
	N+	14.6	16.0	3.2	12.6	64.8	61.3	56.1	59.5	6.3	5.8	4.6	5.7	58.3	43.0	29.9	40.8
Triticale	N-	12.6	11.7	7.6	8.1	19.9	19.4	14.5	14.9	4.2	4.8	4.6	4.2	12.8	15.7	15.7	16.5
	N+	13.1	14.6	10.6	12.0	20.3	25.3	18.6	19.7	4.5	4.6	4.3	4.1	13.7	18.3	15.7	16.5
Fiber sorghum	N-	14.0	11.1	12.3	12.0	23.7	18.7	12.4	11.8	5.4	5.2	3.3	3.6	44.2	24.4	27.3	27.1
	N+	14.5	12.6	12.4	13.5	25.3	27.5	14.3	16.6	4.4	4.8	3.0	4.3	37.2	23.4	24.8	27.4

**Table 4** P-values and levels of significance of the statistical analysis of the two studied factors and their interaction on biomass production, N/C, P/C, and K/C ratio obtained by a two-way ANOVA for each year and for the two experimental parts: criss-cross of perennials and split-plots of annuals and semiperennials

		Perennials				Annuals and semiperennials			
		Crop	Nitrogen	Crop*Nitrogen	Block	Crop	Nitrogen	Crop*Nitrogen	Block
Biomass production	2007	0.118	0.569	0.540	0.141	0.001**	0.133	0.414	0.475
	2008	0.005**	0.082	0.386	0.113	0.002**	0.000***	0.000***	0.424
	2009	0.001**	0.028*	0.186	0.186	0.000***	0.091	0.170	0.93
	2010	0.000***	0.104	0.027*	0.902	0.025*	0.077	0.508	0.335
N/C	2007	0.000***	0.119	0.285	0.631	0.000***	0.058	0.620	0.843
	2008	0.000***	0.213	0.968	0.702	0.000***	0.000***	0.003	0.407
	2009	0.002**	0.003**	0.033*	0.070	0.000***	0.026*	0.089	0.282
	2010	0.002**	0.001**	0.815	0.104	0.000***	0.010*	0.440	0.373
P/C	2007	0.000***	0.850	0.980	0.880	0.000***	0.727	0.145	0.022*
	2008	0.000***	0.120	0.344	0.227	0.003**	0.444	0.763	0.695
	2009	0.004**	0.647	0.028*	0.256	0.001**	0.969	0.397	0.603
	2010	0.000***	0.468	0.288	0.823	0.016*	0.107	0.555	0.975
K/C	2007	0.000***	0.446	0.038*	0.196	0.000***	0.764	0.047*	0.868
	2008	0.002**	0.447	0.780	0.391	0.000***	0.908	0.459	0.959
	2009	0.001**	0.005**	0.587	0.027*	0.000***	0.187	0.072	0.718
	2010	0.000***	0.053	0.031*	0.185	0.000***	0.931	0.124	0.271

\*Significant effect ( $0.05 > P \geq 0.01$ ).

\*\*Very significant effect ( $0.01 > P \geq 0.001$ ).

\*\*\*Highly significant effect ( $P < 0.001$ ).

the three nutrient/carbon ratios was much higher between crops than between years (Table 3) making these ratios specific for each crop. The C concentrations were very similar between N treatments and varied only slightly between crops, with higher C concentrations for perennial C4 crops than for the other crops (Table 5). Variations in the three nutrient/C ratio were

thus mainly due to variations in nutrient concentrations.

The ANOVA performed on N/C ratio showed very significant differences between perennial crops (Table 4): in 2007 and 2008, the N/C ratio of miscanthus L was lower than that of miscanthus E, switchgrass L and E (Table 3). In 2009 and 2010, miscanthus L and

switchgrass L had a significantly lower N/C ratio than miscanthus E and switchgrass E. Significant differences were also observed between the N treatments from 2009 (Table 4): low N rates always resulted in lower N/C ratios than N+ treatments, whatever the crop (Table 3). The ANOVA also showed very significant differences in P/C and K/C ratio every year: miscanthus L had the lowest P/C ratio, switchgrass E had the highest, and miscanthus E and switchgrass L had intermediate values. Nitrogen application rate had no significant effect on P/C ratio. Miscanthus L and switchgrass L had lower K/C ratios than miscanthus E and switchgrass E. No significant effect of nitrogen was observed, except in 2009.

The N/C ratio of annual and semiperennial crops varied significantly between crops every year: the N/C ratio decreased from alfalfa to fescue, triticale, and fiber sorghum in all years (Tables 3 and 4). N fertilization significantly increased the N/C ratio of crops from 2008 to 2010: the effect was noticeable for fescue, triticale, and fiber sorghum. P/C and K/C ratios varied significantly between crops every year: alfalfa and fescue had higher P/C ratio than triticale and fiber sorghum. The K/C ratio ranking was fescue > alfalfa > fiber sorghum > triticale for all years except 2008, when fescue and alfalfa had similar values. N fertilization did not affect the P/C and K/C ratios of the harvested plants.

Over the whole period 2007–2010, the average N/C ratio was lowest in miscanthus L followed by miscanthus E, switchgrass E and L < triticale and fiber sorghum < fescue < alfalfa (Fig. 2a). Most N+ treatments

had slightly higher N/C ratios than N– treatments. The P/C ratio was lowest in miscanthus L followed by miscanthus E and switchgrass L < switchgrass E < triticale and fiber sorghum < fescue and alfalfa (Fig. 2b). The ranking of the K/C ratio was slightly different, with the lowest values for switchgrass L, followed by miscanthus L < miscanthus E, switchgrass E and triticale < fiber sorghum < fescue and alfalfa (Fig. 2c).

#### Nutrient balances

The annual inputs and outputs of N, P, and K are shown in Table 5. The nitrogen output/input ratio ( $e$ ) was calculated in the fertilized treatments. It was greater than 1 in miscanthus E N+, fescue N– and triticale N–, indicating that N fertilization did not compensate for the N removed at harvest. The  $e$  ratio was close to 1 for triticale N+ and fiber sorghum N+ receiving 120 kg fertilizer-N ha<sup>-1</sup> yr<sup>-1</sup>, meaning that the N balance was close to 0. It was below 1, i.e. N inputs exceeded N outputs, in miscanthus L N+, switchgrass L N+, and to a lesser extent in switchgrass E N+ and fescue N+. The average N balance can be calculated from Table 5. It was almost nil or positive (from –2 to +59 kg ha<sup>-1</sup> yr<sup>-1</sup>) in all N+ treatments except for miscanthus E (–24 kg ha<sup>-1</sup> yr<sup>-1</sup>), whereas it was negative in all N– treatments (from –19 to –100 kg ha<sup>-1</sup> yr<sup>-1</sup>). This result is fully consistent with our objective defined earlier, except for Miscanthus E in which both N treatments had a negative N balance and for alfalfa, which derived most of its N from the atmosphere.

**Table 5** Measured (mean ± standard deviation) inputs and outputs of N, P, and K, carbon concentration in the harvested biomass ( $r_C$ ) and nitrogen output/input ratio ( $e$ ) in the experimental treatments (mean over the period 2007–2010)

		Input N kg ha <sup>-1</sup> yr <sup>-1</sup>	Output			$r_C$ g kg <sup>-1</sup>	$e$
			N kg ha <sup>-1</sup> yr <sup>-1</sup>	P	K		
Miscanthus E	N–	0	100 ± 10	19 ± 0	192 ± 22	463 ± 2	
	N+	120	144 ± 16	16 ± 2	206 ± 12	463 ± 4	1.20 ± 0.13
Miscanthus L	N–	0	38 ± 10	9 ± 2	95 ± 20	479 ± 3	
	N+	120	61 ± 8	8 ± 1	112 ± 3	478 ± 3	0.50 ± 0.06
Switchgrass E	N–	0	70 ± 4	17 ± 1	133 ± 3	462 ± 1	
	N+	120	107 ± 10	20 ± 1	175 ± 33	462 ± 2	0.89 ± 0.08
Switchgrass L	N–	0	51 ± 6	10 ± 1	45 ± 6	474 ± 1	
	N+	120	80 ± 3	13 ± 1	68 ± 12	473 ± 2	0.67 ± 0.02
Fescue	N–	90	112 ± 4	22 ± 1	207 ± 12	434 ± 0	1.25 ± 0.05
	N+	180	153 ± 8	29 ± 1	266 ± 10	433 ± 0	0.85 ± 0.04
Alfalfa	N–	0	313 ± 17	29 ± 1	248 ± 19	448 ± 2	
	N+	0	315 ± 17	30 ± 0	236 ± 13	440 ± 10	
Triticale	N–	60	79 ± 4	20 ± 2	67 ± 5	444 ± 6	1.31 ± 0.06
	N+	120	119 ± 16	25 ± 3	90 ± 8	445 ± 5	0.99 ± 0.14
Fiber sorghum	N–	0	88 ± 8	23 ± 3	164 ± 10	424 ± 15	
	N+	120	122 ± 26	24 ± 4	162 ± 20	431 ± 6	1.02 ± 0.22

The annual P and K balances calculated over the 2007–2010 period were negative in all crops as no fertilization was applied during the 5 years of the experiment. The average P balance varied between  $-8$  and  $-30$  kg ha<sup>-1</sup> yr<sup>-1</sup>, and the lowest P removal was found in miscanthus L. The average K balance was more variable ( $-45$  to  $-266$  kg ha<sup>-1</sup> yr<sup>-1</sup>) and dependent on the crop: switchgrass and triticale had the smallest balances, while the greatest K removal occurred with miscanthus E, fescue, and alfalfa. The difference between P and K removals at harvest was likely due to the combination of two phenomena: (i) the lower crop requirements for P compared to K; and (ii) the luxury K uptake due to high levels of potassium in this soil (254 mg K<sub>2</sub>O kg<sup>-1</sup> measured in the 0–30 cm soil layer in 2005).

#### GHG balance in relation to N-P-K fertilization

The main characteristics of the 'optimized' situations used to calculate GHG balance are shown in Table 6. Nutrient fertilization was calculated so that the nutrient balance of each element was equal to 0 and the nitrogen output/input ratio ( $e$ ) was 1 in the crops receiving 'optimized' fertilization rates. The calculation indicated that the annual requirements of fertilizer-N ranged from 0 for alfalfa to 157 kg ha<sup>-1</sup> for miscanthus E. The requirement was less than 70 kg ha<sup>-1</sup> for miscanthus L and switchgrass L, and more than 120 kg ha<sup>-1</sup> for switchgrass E, fescue, triticale, and fiber sorghum. The annual requirements of fertilizer-P ranged from 8 kg ha<sup>-1</sup> for miscanthus L to 29 kg ha<sup>-1</sup> for alfalfa. The annual requirements of fertilizer-K were the highest and ranged from 57 kg ha<sup>-1</sup> for switchgrass L to 233 kg ha<sup>-1</sup> for fescue and 242 kg ha<sup>-1</sup> for alfalfa.

Table 7 summarizes the results of the calculations of the GHG balance. The conversion yield of the harvested biomass to ethanol ( $EtOH$ ) varies from 114 g kg<sup>-1</sup> for alfalfa to 276 g kg<sup>-1</sup> for triticale. Only 13–32% of the carbon content of the harvested biomass is thus converted into ethanol ( $c_v$ ). Alfalfa has the lowest conversion yield because of its low lignocellulose content and its high protein content, which was not converted into ethanol but would rather be used for human or animal food, as is often assumed (Sanderson *et al.*, 2007; Sanderson & Adler, 2008). Replacing fossil fuel by ethanol would save between 219 g CO<sub>2</sub> kg<sup>-1</sup> for alfalfa and 528 g CO<sub>2</sub> kg<sup>-1</sup> for triticale ( $M$  value). The CO<sub>2</sub> saved is higher for perennial crops than for semiperennial and annual crops, except for triticale.

The highest GHG emissions resulting from N-P-K fertilization are due to biogenic N<sub>2</sub>O emissions following N fertilization ( $Meq$ ), except for alfalfa which does not receive N fertilization. GHG emissions resulting from N-fertilizer production and transport ( $Meq_N$ ) are also

important: they are equivalent to approximately 50% of the biogenic N<sub>2</sub>O emissions following N fertilization ( $Meq$ ). Emissions resulting from P and K-fertilizer production and transport ( $Meq_P$  and  $Meq_K$ ) are much smaller. If we exclude alfalfa, the N-related GHG emissions ( $Meq$  and  $Meq_N$ ) represent more than 90% of the total GHG emissions resulting from N-P-K fertilization ( $Meqt$ ).  $Meqt$  range from 24 g CO<sub>2</sub> kg<sup>-1</sup> for alfalfa to 255 g CO<sub>2</sub> kg<sup>-1</sup> for fescue.

The  $Meqt/M$  ratio is less than 1 in all cases, meaning that GHG emissions resulting from N-P-K fertilization are smaller than the savings of CO<sub>2</sub> due to replacement of fossil fuel. Nevertheless, the different crops exhibit a wide variability: the lowest  $Meqt/M$  ratio is observed in miscanthus L (0.10) and the highest in fescue (0.72). This result indicates that GHG emissions resulting from N-P-K fertilization could offset from 10% (miscanthus L) to 72% (fescue) of the GHG emissions saved by replacing fossil fuels, depending on the crop. Miscanthus, switchgrass and alfalfa have a lower  $Meqt/M$  ratio than the other crops. The date of harvest is particularly important for the perennial crops: late harvest reduces the  $Meqt/M$  ratio by 2.7-fold for miscanthus and 1.6-fold for switchgrass compared to early harvest.

The energy performance of the crop is highly dependent on the N/C ratio of the crop: a strong positive correlation ( $r^2 = 0.93$ ) was observed between the  $Meqt/M$  ratio and the N/C ratio of the harvested biomass of the nonlegume crops (Fig. 3). Triticale fell below the average relationship with a lower  $Meqt/M$  ratio than fiber sorghum despite a similar N/C ratio. This is due to a higher  $c_v$  value explained by a more efficient conversion of starch into ethanol. Alfalfa was not included in this relationship because it had the highest N/C ratio (61 g kg<sup>-1</sup>) and the second lowest  $Meqt/M$  ratio (0.11). This is because we assumed no N fertilization for this legume crop.

Finally, we calculated the net CO<sub>2</sub> emissions avoided by the bioenergy crops due to the substitution of ethanol production for fossil resources (Fig. 4). This calculation took into account (i) the difference between the GHG emissions saved by replacing fossil fuels and the GHG emissions resulting from N-P-K fertilization per ton of biomass; and (ii) the biomass production per hectare. Net CO<sub>2</sub> emissions ranged from 1.0 in fescue to 8.6 t CO<sub>2</sub> ha<sup>-1</sup> yr<sup>-1</sup> in miscanthus E and L. The crops which avoided the smallest amounts of CO<sub>2</sub> per hectare had either a low biomass conversion yield to ethanol (alfalfa) or a low GHG saving per ton of biomass (fescue). Conversely, the perennial crops which saved the highest amounts of CO<sub>2</sub> per hectare had high biomass production (miscanthus E) and/or high GHG saving per ton of biomass (miscanthus L). To save as much CO<sub>2</sub> as miscanthus L, the other crops, miscanthus E,

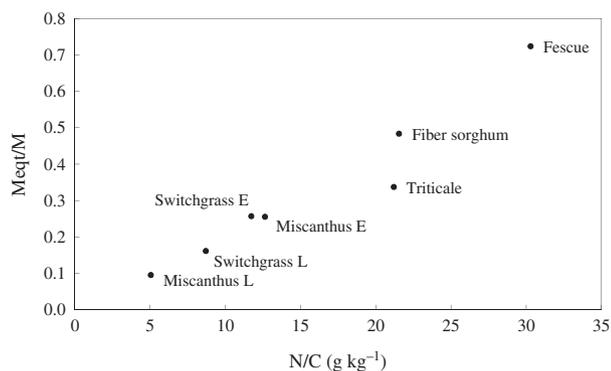
**Table 6** Calculated inputs and outputs of N, P, and K, biomass production ( $B$ ), carbon concentration in the harvested biomass ( $r_C$ ) and nitrogen output/input ratio ( $e$ ) in the 'optimized' fertilization situations (mean over the period 2007–2010)

	Input = output* kg ha <sup>-1</sup> yr <sup>-1</sup>			$B$ t ha <sup>-1</sup> yr <sup>-1</sup>	$r_C$ g kg <sup>-1</sup>	$e$
	N	P	K			
Miscanthus E	157	15	210	26.9	462	1.00
Miscanthus L	46	8	102	19.1	479	1.00
Switchgrass E	101	20	168	18.7	462	1.00
Switchgrass L	68	12	57	16.3	473	1.00
Fescue	131	25	233	10.0	434	1.00
Alfalfa	0	29	242	11.6	444	
Triticale	118	24	89	12.5	445	1.00
Fiber sorghum	123	24	163	13.3	431	1.00

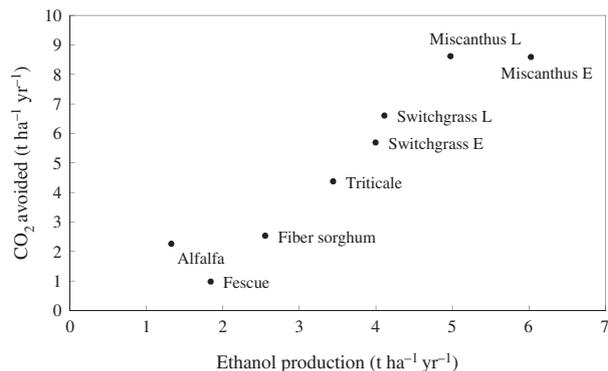
\*Except for alfalfa for which N input = 0.

**Table 7** Calculated conversion yields of biomass to ethanol on a DM basis ( $EtOH$ ) or a carbon basis ( $c_V$ ), saved fossil CO<sub>2</sub> by lignocellulosic ethanol ( $M$ ), biogenic N<sub>2</sub>O emissions resulting from N fertilization ( $Meq$ ), GHG emissions resulting from N-fertilizer production ( $Meq_N$ ), P-fertilizer production ( $Meq_P$ ), K-fertilizer production ( $Meq_K$ ), total GHG emissions resulting from N-P-K fertilization ( $Meq_t$ ) and cost/benefit ratio ( $Meq_t/M$ )

	$EtOH$ g kg <sup>-1</sup> DM	$c_V$ g g <sup>-1</sup> C	$M$ g CO <sub>2</sub> kg <sup>-1</sup> DM	$Meq$	$Meq_N$	$Meq_P$	$Meq_K$	$Meq_t$	$Meq_t/M$
Miscanthus E	224	0.25	429	68	34	3	5	109	0.25
Miscanthus L	260	0.28	498	28	14	2	3	48	0.10
Switchgrass E	214	0.24	409	63	32	5	5	105	0.26
Switchgrass L	251	0.28	481	48	24	3	2	78	0.16
Fescue	184	0.22	353	153	77	12	14	255	0.72
Alfalfa	114	0.13	219	0	0	12	12	24	0.11
Triticale	276	0.32	528	110	55	9	4	178	0.34
Fiber sorghum	192	0.23	368	108	54	8	7	178	0.48

**Fig. 3** Relationship between the cost/benefit ratio ( $Meq_t/M$ ) of the bioenergy crops and their N/C ratio in 'optimized' fertilization conditions.

switchgrass E, switchgrass L, Fescue, alfalfa, triticale, and fiber sorghum had to produce 41, 48, 12, 359, 131, 29, and 137% more biomass, respectively, than miscanthus L (not shown). Only miscanthus E did so in our experimental conditions. The ethanol production ranged from 1.3 t ha<sup>-1</sup> yr<sup>-1</sup> for alfalfa to 6.0 t ha<sup>-1</sup> yr<sup>-1</sup> for

**Fig. 4** Relationship between net CO<sub>2</sub> emissions avoided per hectare and ethanol production of bioenergy crops in 'optimized' fertilization conditions.

miscanthus E. We observed a positive and strong correlation between the net CO<sub>2</sub> emissions avoided and the ethanol production ( $r^2 = 0.91$ ) mainly due to the contribution of biomass production in both calculations. In our conditions, miscanthus was the crop which produced the most ethanol per hectare, with an advantage

for early harvests, and which avoided the most CO<sub>2</sub> emissions per hectare.

## Discussion

### *Biomass production*

The production of miscanthus in our conditions was in the range of what is reported in the literature (Heaton *et al.*, 2004; Miguez *et al.*, 2008; Cadoux *et al.*, 2012). As shown in other studies (Schwarz *et al.*, 1994; Beale & Long, 1997; Himken *et al.*, 1997; Danalatos *et al.*, 2007; Christian *et al.*, 2008), no effect of fertilization was observed during the 5 years in the late harvest treatments. From 2008, the slight effect of N fertilization on the biomass production in early harvested treatments illustrated a likely N deficiency. Indeed, Strullu *et al.* (2011; 2013) showed that cumulative early harvests led to depletion of rhizome N reserves associated with a decrease in spring N remobilization and then to a decrease in biomass production.

The yield of switchgrass was in the upper range of what is reported in the bibliography (Heaton *et al.*, 2004; McLaughlin & Adams Kszos, 2005; Fike *et al.*, 2006). The weaker response of L treatments to N fertilization is likely to be due to the same phenomenon as miscanthus i.e. the N cycling in the crop (Yang *et al.*, 2009).

The biomass production of fescue and alfalfa was rather low compared with perennial crops. One reason is that the biomass production of fescue varied widely between years, with low yields in 2008, 2009, and 2010. The low yield in 2009 could be due to the first year of establishment of the crop while the spreading of an inappropriate herbicide might have impeded the crop growth in 2010. No clear explanation can be offered for the low biomass yield of fescue in 2008. The effect of N fertilization each year except 2009 at the establishment of the crop confirms the high N requirements of this crop (Bélanger *et al.*, 1992). The lower production of alfalfa observed in 2009 and 2010 is attributed to the establishment of the crop in 2009 and to either delayed effects of the water deficit observed in 2009 or a variety effect.

Biotic and abiotic stresses may have occurred and may explain the relatively low yield of triticale. Indeed, in northern France, Brancourt-Hulmel *et al.* (2003) showed that the biomass production of wheat, a species similar to triticale, could reach 20 t ha<sup>-1</sup> on average. They also showed that the yield dropped to 14 t ha<sup>-1</sup> in the absence of N fertilization and fungicide. The biomass production of fiber sorghum was lower than often reported in the literature; biomass yields of about 20 t ha<sup>-1</sup> and up to 30 t ha<sup>-1</sup> are reported, but mainly

in southern Europe (Mastrorilli *et al.*, 1995, 1999; Cosentino *et al.*, 2012). The susceptibility of sorghum to low temperatures impedes sowing before mid-May in our conditions. The consequence is a considerably shorter growing period with likely reduced radiation interception.

The hierarchy of crops in terms of biomass production differs from that reported in other studies. Boehmel *et al.* (2008) observed the following hierarchy: maize (annual C4), miscanthus and triticale > willow (short rotation coppice) > switchgrass and wheat. In the study of Scholz & Ellerbrock (2002), the biomass ranked as: hemp (annual C3) > poplar (short rotation coppice) > rye and cocksfoot (annual and semiperennial C3, respectively) > triticale. These differences underline that biomass production varies widely according to soil and weather conditions as well as crop management, as shown by Cadoux *et al.* (2010). This is particularly true for the C4 crops, especially perennials, which maximize the interception of radiation (Dohleman & Long, 2009) and are potentially the most productive, but which can produce less than C3 crops if they are grown at unsuitable latitudes (Gosse *et al.*, 1986).

### *Nutrient removal at harvest*

The low and comparable nutrient/C ratios of miscanthus and switchgrass are consistent with the findings of Monti *et al.* (2008) and Prophet & Staggenborg (2010). Late harvest resulted in lower nutrient removal than early harvest; this is due to nutrient translocation from leaves and stems to the rhizomes and/or to leaf fall during winter and/or stem leaching occurring in autumn/winter (Christian *et al.*, 2002; Adler *et al.*, 2006; Yang *et al.*, 2009; Amougou *et al.*, 2012; Cadoux *et al.*, 2012). In contrast, semiperennial crops (alfalfa and fescue) are characterized by a high nutrient concentration (e.g. Scholz & Ellerbrock, 2002) and a large amount of N removed at harvest, which has been identified as a major disadvantage of using grasslands for bioenergy (Ceotto, 2009). Our study confirms this finding and shows that the high removal of K at harvest puts semiperennial forage crops (legume and nonlegume) at a disadvantage. In accordance with the results of Scholz & Ellerbrock (2002), we showed that the nutrient removal at harvest of annual crops (triticale and sorghum) was intermediate between those of perennial and semiperennial crops.

### *GHG balance in relation to N-P-K fertilization*

The GHG emissions resulting from N-P-K fertilization were assessed and compared with the CO<sub>2</sub> emissions saved by replacing fossil fuel to evaluate the contribu-

tion of the fertilization to the GHG balance of the biofuels. This cost/benefit methodology cannot replace a full life-cycle analysis to make a complete GHG balance of biofuel because it does not take into account the GHG emissions resulting from cropping operations during biomass production (except those for fertilizer production and transport) and the GHG emissions during transportation and conversion of the biomass. However, it allows a useful comparison between crops to discriminate which crops are best suited for biofuel production to obtain a largely positive GHG balance and what are the main factors involved. Indeed, GHG emitted during fertilizer production and transport are not likely to change the hierarchy between crops, and GHG emissions resulting from the use of fossil fuel and machinery during biomass production are rather small compared with fertilization-related emissions (St Clair *et al.*, 2008; Fazio & Monti, 2011). Soil carbon changes with time are also not considered in our methodology; they could lead to underestimates of GHG savings by perennial crops, which are expected to favor carbon sequestration in soils, at least when harvested late (Bessou *et al.*, 2011). Finally, our results are linked to the use of the global worldwide emission factor from mineral N fertilizer ( $y$ ) proposed by Davidson (2009) and to the emission factors of fertilizer production and transport. LCA studies comparing bioenergy crops often use the 0.01 emission factor of the IPCC methodology for direct emissions (Hillier *et al.*, 2009; Fazio & Monti, 2011) and few of them include the indirect emissions (Adler *et al.*, 2007), which leads to underestimates of N<sub>2</sub>O emissions in most cases. The 0.025 global emission factor estimated by Davidson (2009) and used in this study was close to the results of using IPCC guidelines based on both direct and indirect emissions (Davidson, 2009; Reay *et al.*, 2012). However, this methodology does not take into account the variability in N<sub>2</sub>O emissions due to differences in local conditions of biomass production (e.g. soil type, climate). The emission factors for fertilizer-N production and transport ( $\alpha_N$ ) can vary widely between fertilizer types: from 3.3 kg CO<sub>2</sub> eq kg<sup>-1</sup> N for urea to 8.65 kg CO<sub>2</sub> eq kg<sup>-1</sup> N for calcium ammonium nitrate (Swiss Center for Life Cycle Inventories, 2010). We chose to use the emission factor for UAN solution (5.84) because it corresponds to the fertilizer used in the experiment and it represents an intermediate value between urea and ammonium nitrate, which are the most widely used N fertilizers in Europe (IFA, 2012). Furthermore, choosing another fertilizer type would have changed the values of  $Meqt/M$  for all crops, but not the crop hierarchy.

In spite of the addition of GHG emissions resulting from fertilizer production and transport in our study, our  $Meqt/M$  values were generally much lower than the

$Meqt/M$  values obtained by Crutzen *et al.* (2008) for maize and sugarcane ethanol (0.9–1.5 and 0.5–0.9, respectively, with  $y = 0.03$ –0.05). This discrepancy can be partially explained by the difference in the nitrogen output/input ratio ( $e$ ) (0.4 for Crutzen *et al.* (2008) vs. 1 in our study for ‘optimized’ situations), and by the fact that we used a lower emission factor for N<sub>2</sub>O emissions ( $y$ ) than Crutzen *et al.* (2008). Our value of  $e = 1$  was obtained assuming an increase in the sustainability of crop management through optimizing agronomic inputs, which was considered by Fazio & Monti (2011) as a way to significantly improve the overall sustainability of bioenergy chains. The low  $Meqt/M$  ratios obtained for the perennial C4 crops and alfalfa can be explained by their low nutrient requirements, particularly nitrogen, per unit of C produced. The possibility of using crops with a very low N/C ratio makes second-generation biofuels potentially much more efficient than first-generation biofuels in terms of GHG balance. This is consistent with the results of the LCA studies on the GHG balance of lignocellulosic ethanol (e.g. Adler *et al.*, 2007; Schmer *et al.*, 2008; Fazio & Monti, 2011).

The N/C ratio of harvested biomass was very stable over time for a given crop, very discriminating between crops and strongly correlated with the  $Meqt/M$  ratio. It is likely to rank the crops robustly and we propose to use it as a simple indicator to compare nonlegume bioenergy crops. Biomass production per hectare is an additional indicator, but it seems to be much more site-specific. In our conditions, miscanthus was the crop which both produced the most ethanol and avoided the most CO<sub>2</sub> emissions per hectare. The advantage of early harvests compared with late harvests because of higher ethanol production per hectare and the same CO<sub>2</sub> emissions saved should be treated with caution. Indeed, other consequences of nutrient fertilization than GHG emissions are not taken into account. Finally, miscanthus L and switchgrass L allow the best compromise between low N-P-K requirements, low  $Meqt/M$  ratio and high biomass and ethanol production per hectare. A more integrated environmental impact assessment, taking into account changes of soil carbon stocks with time, nitrate leaching, water consumption, etc., is now necessary to complete the comparison of the different cropping systems. Further research studies are needed to extend this assessment to other soil and climatic conditions and to consider cropping systems including food and nonfood crops.

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