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Consequences of field N2O emissions for the environmental sustainability of plant-based biofuels produced within an organic farming system


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Abstract

One way of reducing the emissions of fossil fuel-derived carbon dioxide (CO2) is to replace fossil fuels with biofuels produced from agricultural biomasses or residuals. However, cultivation of soils results in emission of other greenhouse gases (GHGs), especially nitrous oxide (N2O). Previous studies on biofuel production systems showed that emissions of N2O may counterbalance a substantial part of the global warming reduction, which is achieved by fossil fuel displacement. In this study, we related measured field emissions of N2O to the reduction in fossil fuel-derived CO2, which was obtained when agricultural biomasses were used for biofuel production. The analysis included five organically managed feedstocks (viz. dried straw of sole cropped rye, sole cropped vetch and intercropped rye–vetch, as well as fresh grass–clover and whole crop maize) and three scenarios for conversion of biomass into biofuel. The scenarios were (i) bioethanol, (ii) biogas and (iii) coproduction of bioethanol and biogas. In the last scenario, the biomass was first used for bioethanol fermentation and subsequently the effluent from this process was utilized for biogas production. The net GHG reduction was calculated as the avoided fossil fuel-derived CO2, where the N2O emission was subtracted. This value did not account for fossil fuel-derived CO2 emissions from farm machinery and during conversion processes that turn biomass into biofuel. The greatest net GHG reduction, corresponding to 700–800 g CO2 m−2, was obtained by biogas production or coproduction of bioethanol and biogas on either fresh grass–clover or whole crop maize. In contrast, biofuel production based on lignocellulosic crop residues (i.e. rye and vetch straw) provided considerably lower net GHG reductions (≤215 g CO2 m−2), and even negative numbers sometimes. No GHG benefit was achieved by fertilizing the maize crop because the extra crop yield, and thereby increased biofuel production, was offset by enhanced N2O emissions.

Keywords: bioethanol and/or biogas, carbon sequestration, digestate recycled as fertilizer, emission factor, fossil fuel displacement, grass–clover, methane, nitrous oxide, rye and vetch straw, whole crop maize

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Introduction

A future goal within organic farming is to reduce the reliance on fossil fuels and reduce the emission of greenhouse gases (GHGs) to the atmosphere via local production of renewable energy (Jørgensen & Dalgaard, 2004). This could include biofuels, such as biogas and bioethanol, produced from either dedicated energy crops, crop residues or other agricultural residuals like manure (Oleskowicz-Popiel, 2010). Agro-biofuels are often referred to as carbon dioxide (CO2) neutral because CO2 emitted during the combustion of the bio-fuels has been assimilated from the atmosphere through plant photosynthesis, and consequently there is no net CO2 emission. Thus, when replacing fossil fuels with biofuels, emission of fossil fuel-derived CO2 is avoided.

However, crop production results in emission of other greenhouse gases, especially nitrous oxide (N2O), which is formed in the soil by nitrifying and denitrifying bacteria when the nitrogen (N) availability is high (Firestone & Davidson, 1989). Besides fertilizer-derived N2O emissions, decomposition of crop residues may also enhance the direct emission of N2O (Chirinda et al., 2010). In addition to direct N2O emissions, crop production may lead to indirect N2O emissions outside the cropping system due to nitrification and denitrification of ammonium (NH4+), and nitrate (NO3−), which was...
lost from the field via volatilization or leaching. The global warming potential of N$_2$O is 298 times higher than that of CO$_2$ based on a 100-year time horizon (IPCC, 2007).

During the entire life-cycle of a biofuel, greenhouse gases are emitted from other sources, for example, loss of CO$_2$ from soil carbon due to land-use change, fossil fuel-derived CO$_2$ emissions from farm machinery, transportation and during conversion processes that turn biomass into biofuel. The number of GHG sources considered in life-cycle assessments depends on the systems boundaries chosen in each analysis (Larson, 2006).

Studies on GHG balances for biofuels produced within organic farming systems are sparse. For biogas produced on organic grass–clover silage, direct and indirect N$_2$O emissions were estimated to account for 32% of the total GHG emissions, which included: (i) cultivation of a 3-year ley crop under Finish conditions, (ii) biogas conversion process, and (iii) upgrading of biogas to transport fuel (Tuomisto & Helenius, 2008). In this life-cycle analysis, the N$_2$O loss was estimated by assuming that 2.55% of any N input was emitted as N$_2$O; a metric often referred to as N$_2$O emission factor.

For conventional farming, Smeets et al. (2009) made a life-cycle analysis of the most common combinations of energy crops and first generation conversion technologies divided into key producing world regions. The N$_2$O emissions were calculated using a statistical model that took into account not only the amount of N fertilizer applied, but also the climatic zone, the type of soil and energy crop. The analysis showed that field emissions of N$_2$O typically accounted for between 10% and 80% of the total GHG emissions due to biofuel production. In line with this, St Clair et al. (2008) estimated the preharvest GHG costs related to the production of three bioenergy crops. The assessment included GHG costs associated with all field preparations, farming practices and substances applied until the time of harvest as well as N$_2$O emissions and loss of soil carbon due to land-use change. For the crops with the largest GHG costs, ca. 50% of the GHG emissions were derived from N$_2$O emissions following N fertilization, which was estimated using the former IPCC default emission factor of 1.25% (IPCC, 1997). These studies all emphasize that direct field emissions of N$_2$O contribute significantly to total GHG emissions during biofuel production.

To what extent do N$_2$O emissions counterbalance the avoided fossil CO$_2$ emissions achieved by fossil fuel displacement in terms of global warming? Based on global data, Crutzen et al. (2008) estimated that 3–5% of the anthropogenic N input to Earth is emitted as N$_2$O when considering the full cycling of N including plant production, livestock feeding and manure management. Using this emission factor range, the authors calculated to which extent the avoided emission of fossil fuel-derived CO$_2$ was counteracted by release of N$_2$O. The investigated biofuel production systems were rape-seed biodiesel and bioethanol produced from maize grain or sugar cane. The authors concluded that N fertilization of the crops in these biofuel production systems can readily lead to N$_2$O emissions large enough to cause climate warming instead of cooling by fossil fuel displacement. In contrast, an analysis based on 9 years of field N$_2$O measurements in rye and poplar energy crops estimated that direct and indirect N$_2$O emissions, as well as GHG emissions related to fertilizer production, counteracted up to 16% of the CO$_2$ fixed in the harvested biomass (Kern et al., 2010).

Currently, 230 PJ yr$^{-1}$ of biogas is produced in the EU countries, while the bioethanol production is ca. 3000 m L yr$^{-1}$ (IEA, 2008); the latter having an energy content of 64 PJ yr$^{-1}$ (WTW, 2008). The production of biofuels is expected to rise as the EU target for 2020 is that 20% of the total energy consumption should be based on renewable resources (EU, 2009). Ethanol produced from raw materials containing starch (first generation) is a well-established technology, whereas production of lignocellulosic bioethanol (second generation) is a relatively immature technology with mainly demonstration or pilot plant facilities established (Thomsen & Hauggaard-Nielsen, 2008; Sims et al., 2010). Pretreatment of the lignocellulosic feedstock is needed before ethanol fermentation to open up the structure and make the cellulose and hemicellulose available for enzymatic hydrolyses (Thomsen et al., 2006).

For most feedstocks, the ethanol energy yield is considerably lower than the biogas energy yield, primarily because ethanol is produced from sugar only, whereas biogas may be produced from a variety of chemical compounds, i.e. sugar, proteins and fats (Oleskowicz-Popiel, 2010). The effluent from ethanol fermentation can easily serve as substrate for anaerobic digestion due to its high content of protein and remaining sugars, including C5 sugars, which enables coproduction of ethanol and biogas (Uellendahl & Ahring, 2010).

Within agriculture, potential lignocellulosic feedstocks for second generation biofuel production are either crop residues (e.g. cereal straw) or dedicated feedstocks, including purpose-grown vegetative grasses and grass–legume mixtures, short rotation forests and other energy crops (Sims et al., 2010). Whole crop maize is a dedicated feedstock that is very commonly used for biogas production, especially in Germany, because of its high crop yield and efficient conversion of biomass into biogas (Weiland, 2006; Schittenhelm, 2008). For arable organic farming, grass–clover has been suggested as a suitable feedstock available for biogas production.
Grass-clover provides high dry matter yields in unfertilized swards, where 95% of the nitrogen is supplied by the N₂-fixing clover (Høgh-Jensen & Schjoerring, 1994).

Plant biomass may be used for co-digestion to increase the biogas production based on animal manure (Wu et al., 2010). In the biogas reactor, microorganisms convert part of the organic carbon into biogas and CO₂. This process also implies mineralization of organically bound nitrogen. Therefore, the content of mineral nitrogen is generally higher in the anaerobic digested material than in the original, untreated manure (Clemens et al., 2006; Möller et al., 2008; Stinner et al., 2008). The digestate can be applied as fertilizer when needed by the crop, securing a high level of synchrony in time and space between soil mineral N availability and crop demand, as opposed to the traditional incorporation of untreated materials. The effect of co-digesting animal manure and plant biomass on N₂O emissions following field application of the digestate has only been investigated in a limited number of studies (e.g. Möller & Stinner, 2009).

The objective of biofuel production within organic agriculture is to create alternatives to fossil fuels in a sustainable way, which minimizes harmful emissions to the atmosphere and to the aquatic environment. The aim of this study is first of all to quantify N₂O emissions from five organically managed crops. Secondly, the aim is to evaluate the environmental sustainability when these biomasses are used for production of either (i) biogas, (ii) bioethanol or (iii) coproduction of bioethanol and biogas. To be sustainable in terms of N₂O emissions, the biofuel production systems should give significant net GHG reductions via fossil fuel displacement when field emissions of N₂O are taken into account. The five investigated feedstocks are straw of sole cropped rye, sole cropped vetch and intercropped rye–vetch, as well as fresh grass–clover and whole crop maize. The grain of rye and vetch is used for direct human consumption or animal fodder, thus it is not available for energy purposes. Based on the literature presented above, our hypotheses are: (i) that field emissions of N₂O counteract a substantial fraction (>20%) of the avoided fossil fuel-derived CO₂, and (ii) that a higher GHG benefit is obtained when the feedstocks are used for biogas rather than bioethanol production due to the more efficient carbon conversion during anaerobic digestion.

Materials and methods

Field site

The field trials were carried out from August 2007 until December 2009 at the experimental farm belonging to Faculty of Life Sciences, University of Copenhagen, which is situated in Taastrup, Denmark (55° 40' N, 12° 18' E). The 25-year mean annual precipitation is 550 mm and mean annual air temperature is 8°C with maximum and minimum daily air temperature of 16°C (July) and −1°C (February). The soil is a sandy loam (12% clay, 25% silt, 34% fine sand and 27% coarse sand) with pH(CaCl₂) of 6.8 and a content of total N and C in the 0–30 cm soil layer of 0.12% and 1.3%, respectively. Cropping history of the site was an organically managed 4-year rotation established in 1995, which consisted of barley (Hordeum vulgare L.) undersown with grass–clover, grass–clover, oat (Avena sativa L.) and fodder beet (Beta vulgaris L.). In 2003, this was changed into a rotation that included barley undersown with grass-clover, 2 years of grass-clover and finally winter wheat (Triticum aestivum L.). The grass-clover sward consisted of 8% red clover (Trifolium pretense L. cv. Rajah), 9% white clover (Trifolium repens L. cv. Klondike), 40% early ryegrass (Lolium perenne L. cv. Stefani), 20% late ryegrass (Lolium perenne L. cv. Foxtrot) and 23% hybrid ryegrass (Lolium perenne L. cv. Storm). The N₂-fixing grass–clover mixture was used as green manure by soil incorporation before sowing of the subsequent crop. The cropping system did not receive any further fertilization.

Experimental set-up for biomass production

Each growth cycle of this study was initiated late August in the first year grass–clover field by incorporating the sward into the soil in defined strips of 6 m × 14 m using a commercial rotavator (Fig 1). In between the strips, 6 m × 14 m of grass–clover sward was left as a soil-fertility building perennial strip. After rotavation, the soil was kept as a false seedbed and weeded mechanically twice before an annual crop was sown. Two identical field trials were established in two succeeding years (2007–2008 and 2008–2009) with either winter vetch (Vicia villosa Roth cv. Latiga) and winter rye (Secale cereale L. cv. Carotop) or maize (Zea Mays L. cv. Amado) as the annual crop grown in two separate fields situated ca. 50 m apart (Fig 1). The crops were managed according to organic farming practices.

In the rye and vetch field, strips were laid out in a randomized block design with four replicates and with rye and vetch sole crops (SC) and rye–vetch intercrop (IC) as the treatments (Fig 1a). In late September, rye SC and vetch SC were sown at 12.5 cm row distance (300 and 100 plants m⁻², respectively). Rye–vetch IC was established using a 75% rye plus 25% vetch replacement design, where rye and vetch seeds were mixed before sowing. The crops were harvested in late July. Following harvest and incorporation of stubbles by rotavation, the soil was kept as a false seedbed with three mechanical weeding operations prior to sowing of winter triticale (Triticosecale Wittm. cv. Dinaro) in late August.

In the maize field, an identical winter rye crop was sown at 12.5 cm row distance the same day and with the same density as in the rye and vetch field (n=4 blocks) (Fig 1b). The green rye was then harvested early May using traditional forage harvester equipment. After rotavation and seedbed preparation, maize was sown in mid May at 50 cm row distance (12 plants m⁻²). The maize crop was harvested primo October,
Fertilization experiments

Three fertilization experiments were conducted, viz. two experiments in maize crops (2008 and 2009) and one experiment in a winter rye crop (2009). The experiments were carried out using a randomized split-plot design, where an extra maize or rye strip in each block was divided into subplots of 9 m², which received a specific fertilizer (Fig. 1). All work related to fertilizer application was done by the authors and their technical staff. In the maize experiments, the fertilizers were (i) raw cattle slurry, (ii) a mixture of anaerobic digested cattle slurry (85% of the N content) and digested maize silage (15% of the N content) and (iii) green manure consisting of fresh grass-clover material was placed in and on top of similar cuts. The target fertilization rate was 150 kg inorganic N per ha (medium/fast filter no. 643; Frisenette, Knebel, Denmark) under tension using an Erlenmeyer Flask connected to a pump. The filtrate was analyzed for NH₄⁺+ and total N (AutoAnalyzer 3; Bran +Luebbe, Norderstedt, Germany) and total organic C (TOC- Vario; Shimadzu, Kyoto, Japan). The solid fraction was oven dried (105°C; 24 h), ground in a ball mill and analyzed for total N and C on an elemental analyzer (EA 1110; Carlo Erba, Milano, Italy). Samples of grass-clover were oven dried (80°C; 24 h) and analyzed for total N and C in the same way.

Soil greenhouse gas flux measurements

Emissions of N₂O, methane (CH₄) and CO₂ were measured using the static chamber method described by Chirinda et al. (2010). Briefly, a stainless steel collar (60 cm × 60 cm × 15 cm deep) was permanently installed in each plot. When measuring gas fluxes, a white PVC chamber was used that fitted into a water-filled channel on the upper edge of the soil collar, ensuring a gas-tight seal during measurements. Generally, 15 cm high chambers were used for 1.5 h enclosure periods, whereas the chamber height was extended to 60 cm with enclosure periods of ca. 2 h for measuring campaigns in spring that included rye crops. Four times during the enclosure period, a 30 mL sample of headspace air was collected through a septum using a syringe, and used to flush and pressurize a 5.9 mL pre-evacuated gas-tight syringes.
ated Exetainer vial (Labco Limited, High Wycombe, UK). The soil collar was removed and reinstalled during harvest and soil cultivation. In maize plots, the soil collar was placed across one maize row, and plants that emerged within the collar were removed in mid June by gently pulling up the plants.

Basic measurements of N₂O fluxes were conducted in unfertilized strips of vetch, rye–vetch, grass–clover and rye followed by maize from sowing in September until primo May and again from harvest of the vetch and rye–vetch crops in late July until late September. The summer break in the measurement programme was caused by the 1.5 m tall rye plants, which made flux chamber measurements impossible. However, N₂O fluxes during this season are expected to be very low because of dry soil conditions and high plant-microbial competition for nitrogen (e.g. Chirinda et al., 2010). The general frequency of the basic N₂O measurements was every fortnight and monthly during the first and second year, respectively, with increased frequency following soil cultivation. Measuring campaigns were targeted at rain events when possible. Emission of N₂O related to incorporation of the grass–clover sward was estimated from measurements conducted from late August until mid November 2008 in strips sown with rye.

In the fertilization experiments, GHG fluxes were measured daily or twice a week for the first 2–3 weeks after the fertilizer application, which was decreased to a weekly measurement frequency for the following 4 or 7 weeks in the rye and maize fertilization experiments, respectively. After harvest, measurements at 1 to 2 week intervals continued for 2 months. Fluxes of N₂O were established during all measuring campaigns, whereas CH₄ emissions were assessed during the first 1–2 weeks after fertilization. In the maize experiments, emissions of CO₂ derived from soil respiration were determined for the entire monitoring period.

Gas analyses and flux calculations

Concentrations of CH₄ in gas samples were established by manual injection of 0.5 mL on a gas chromatograph (HP 6890; Agilent, Santa Clara, CA, USA). To measure N₂O concentrations, the Exetainer vials were pressurized to 1.4 bar with carrier gas to flush a 1 mL sample loop immediately followed by gas chromatography (GC-14B; Shimadzu). The CO₂ concentrations were established by infrared gas analysis (3300 IRGA; Milton Roy, Ivyland, PA, USA) or gas chromatography (HP 6890) using 1 mL of sample that was withdrawn from the vial by a pressure-lock syringe.

Emissions of N₂O and CH₄ were calculated using linear regression of headspace concentrations vs. time (n = 4), whereas the soil CO₂ efflux at the time of chamber closure was estimated by quadratic regression (Kutzbach et al., 2007). Cumulative emissions of N₂O, CH₄ and CO₂ for each monitoring period were obtained by linear interpolation between measurement days. For the fertilizer experiments, N₂O and CH₄ emission factors were calculated as the fertilizer-induced N₂O-N and CH₄-C emissions relative to the amount of total N and C applied, respectively. The near-term carbon sequestration caused by organic C input to the soil via fertilization was calculated as the C content in the fertilizer applied subtracted the fertilizer-induced CO₂ emissions. The latter calculation methodology was based on assumptions described by Jacinthe et al. (2002).

Soil variables and precipitation

During flux measuring campaigns, soil temperature was measured at 5 cm depth. In addition, the volumetric soil water content at 0–6 cm depth was determined using a time domain reflectometry probe (HH2 Moisture Meter; Delta-T Devices, Cambridge, UK). Inorganic N content of the soil was assessed in connection with selected measuring campaigns. Briefly, a soil sample consisting of four 2 cm diameter cores was collected from the 0–20 cm soil layer of each field plot using an auger. Soil inorganic N was extracted in 0.01 M CaCl₂ and filtered extracts were analyzed for NH₄⁺ and NO₃⁻ on an autoanalyzer (Brain+Luebbe). Soil moisture content was determined gravimetrically by oven drying (105°C; 24 h). Precipitation was recorded at 1 m height by a weather stations situated at Risø DTU, 12 km west of the experimental field (55°41’N, 12°05’E).

Biomass for biofuel potentials

Ethanol and biogas potentials were determined in batch trials on dried straw of rye, vetch and rye–vetch, as well as fresh grass–clover and whole crop maize. The crops for these trials were predominantly grown at the experimental fields in Taastrup described above. Grass–clover was harvested on 14 May 2008, whereas rye and vetch were harvested on 4 August 2009. Whole crop maize (cv. Tassilo) originated from a nearby (<1 km) conventionally managed field and was harvested on 14 November 2007.

All biomasses were milled to 1 mm particle size prior to biofuel conversion. Straw of rye and vetch were stored dry, whereas fresh grass–clover and maize were kept at −18°C. Total and volatile solids (TS, VS) were determined using standard methods (Greenberg et al., 1998). The ethanol fermentation trials were carried out on both untreated and hydrotherm pretreated (HTT) biomass. Pretreatment opens the stringent structure of lignocellulosic materials and thereby facilitates enzymatic hydrolysis, resulting in fermentable sugars (Thomsen et al., 2006). Hydrothermal pretreatment of the biomass was performed in a 2 L loop autoclave operated in a batch setup (Bjerre et al., 1996) that was fed with 1 L tap water and 60 g dry matter of biomass, which was treated at 195°C for 10 min. The biogas potential trials were conducted on untreated biomass and on the residues from the ethanol fermentation; the latter was used for the scenario on coproduction of ethanol and biogas.

Ethanol potentials

The ethanol fermentations were performed as simultaneous saccharification and fermentation (SSF). They were carried out in duplicate batch trials with 8 g total solids (TS) of substrate and 80 mL of water. The pH was adjusted to 4.8 with 0.01 M H₂SO₄. Prior to the SSF, all samples were subjected to a lique-
faction step (prehydrolysis), i.e. 24 h at 50°C with the addition of an enzyme mixture of cellulases providing 15 filter paper units (FPU) per g dry matter filter cake (celluclast and novozym 188; Novozyymes, Bagsværd, Denmark). The SSF was started by adding a pure baker’s yeast (Saccharomyces cerevisiae) and additional 20 FPU of the above mentioned enzyme mixture. The SSF were monitored continuously by assessing weight loss caused by the production of CO₂. The final ethanol contents were evaluated on a HPLC (Shimadzu) fitted with an Aminex HPX-87H column (Shimadzu).

**Biogas potentials**

For the determination of biogas potentials, duplicate SSF effluents were pooled and tested in triplicates. Triplicate-samples of all biomasses and SSF effluents were distributed in 116 mL serum flasks in amounts of 0.5 g volatile solids (VS) for biomass samples, and both 0.10 g VS and 0.35 g VS for SSF effluent samples. The samples were inoculated with 20 g of effluent from a lab-scale biogas reactor treating cattle manure, and incubated at 53°C for a period of 65 or 25 days for the biomass and SSF effluent samples, respectively. Additionally, flasks containing ethanol were incubated under the same conditions to quantify and subtract biogas production deriving from ethanol in the nondistilled SSF effluents. For subtraction of biogas produced by the inoculum, flasks containing only 20 g of inoculum were also prepared. The CH₄ production in the flasks was measured by collecting 0.5 mL of headspace air using a syringe and analyzing the CH₄ concentration in the sample by gas chromatography (HP 6890; Agilent). Measurements were carried out in increasing intervals ranging from 3 days in the beginning to 15 days in the end of the digestion trials.

**Calculation of net greenhouse gas reduction**

A global warming potential for N₂O of 298 was used when converting cumulative N₂O emissions into CO₂-equivalents (IPCC, 2007). For the rye fertilization experiment, cumulative N₂O emission from sowing in autumn to fertilization in spring was assumed to be equal to the emission measured in the rye crop that was grown in the coming maize field (Fig. 1b). The grass–clover crop was intended to be kept in the crop rotation for 4 years, and therefore the annual N₂O emissions from the established sward was added one-fourth of the emission derived from incorporation of a grass–clover sward. In these two calculations and when calculating means across the two experimental years, the standard deviation was obtained using the rule of additive variances (Sokal & Rohlf, 1995).

For rye, vetch and rye–vetch, N₂O emissions were allocated between straw and grain in proportion to the energy content of the two fractions, in line with EU recommendations (EU, 2009). The energy content of the biomasses were measured using a calorimetre (6300; Parr, Moline, IL, USA).

To compare the three biofuel scenarios with respect to fossil fuel displacement, the produced bioethanol and biogas were normalized according to fossil petrol. Petrol was chosen as a fossil fuel reference because it is used in an internal-combustion engine just like ethanol and biogas. More specifically, the avoided fossil fuel-derived CO₂ emission was obtained by calculating how much petrol the produced bioethanol and/or biogas corresponded to in terms of energy content. The calculation was based on the measured crop yields (g DM m⁻²) and biofuel potentials (g ethanol or CH₄ g⁻¹ DM) as well as lower heating values for ethanol, biogas and petrol (MJ kg⁻¹) and CO₂ emissions from combustion of petrol (kg CO₂ kg⁻¹) derived from the literature (WTW, 2008).

The net reduction in greenhouse gas emissions due to biofuel production was calculated by subtracting the N₂O emission in CO₂-equivalents from the avoided fossil fuel-derived CO₂ emission. We did not attempt to make a complete life-cycle assessment, thus for instance CO₂ emissions from farm machinery and during biomass–biofuel conversion processes were not accounted for. Likewise, we did not consider GHG emissions associated with the extraction and processing of the fossil petrol that the biofuels were assumed to replace.

**Statistics**

One-, two- and three-factor analyses of variance including interactions were conducted by fitting mixed effects models using the PROC MIXED procedure of SAS (Release 9.1; Statistical Analysis System Institute, Cary, NC, USA). Data were log-transformed as required to obtain normality and homogeneity of variance. To account for the experimental design, Block was included as random effect when analysing data within an experimental field. When cumulative N₂O emissions for two periods were compared, Plot was also present in the random statement to account for duplicate measurements in each plot. Likewise, Plot was included when the effect of the three biofuel scenarios on the net GHG reduction was tested. Main effects and interactions with P < 0.05 were considered to be significant and differences of least squares means was used to interpret significant effects.

In general, the net GHG reduction was calculated for each plot individually using plot-specific biomass yields and N₂O emissions. However, for grass–clover and rye this was not possible because the N₂O emission during some periods was estimated based on emissions measured in other plots. To compare the net GHG reduction across feedstocks, the 95% confidence intervals were therefore used, which were derived as mean ± (t × SE).

**Results**

**Precipitation, soil moisture and temperature**

Compared with the mean annual precipitation for the field site, the two cropping years 2007–2008 and 2008–2009 were slightly wetter with an annual precipitation (September–August) of 647 and 679, respectively (Fig. 2a). Soil moisture in the 0–6 cm soil layer varied between 6 and 40 vol% during the study period (Fig. 2b) and soil temperature at 5 cm depth showed clear seasonal fluctuations (Fig. 2c).
Overall for the basic \( \text{N}_2\text{O} \) measurements (viz. vetch, rye–vetch, grass–clover and rye/maize plots), the cumulative emissions were higher in 2008–2009 than the previous year \((P = 0.0002; \text{Table 1})\). According to differences of least squares means, the established grass–clover sward had the lowest emission, whereas the largest loss of \( \text{N}_2\text{O} \) occurred from the vetch plots \((P < 0.0001)\), especially during the second period that followed harvest (Crop × Period; \( P < 0.0001 \)). Incorporation of the grass–clover sward stimulated emissions that were 2–8 times higher than the total annual emission from the established sward. Soil inorganic N measured after incorporation of grass–clover, vetch and rye–vetch appear in Table 2.

**Soil greenhouse gas emissions – fertilization experiments**

Fertilization of the winter rye crop in early spring induced a modest increase in the cumulative \( \text{N}_2\text{O} \) emission during the first months after fertilizer application (Treatment × Period; \( P < 0.0001; \text{Table 1} \)). The highest emission, though, occurred during the second period that followed harvest in August \((P < 0.0001)\).
When pooling the N$_2$O emission in the two periods, the fertilized plots were similar to the control ($P = 0.91$; Table 3). In contrast, the CH$_4$ emission from raw slurry was higher than the emission from both anaerobic digested slurry + maize and the unfertilized rye crop ($P = 0.02$). However, the CH$_4$ emission factor, which relates the emission to the amount of carbon applied, did not differ between the two fertilizers ($P = 0.36$). The same was the case for the N$_2$O emission factor ($P = 0.84$).

Fertilization of maize at sowing in May caused a substantial increase in N$_2$O emissions (Treatment × Period; $P < 0.0001$; Table 1). In contrast to the rye experiment, N$_2$O emissions declined in the second period.

### Table 1

<table>
<thead>
<tr>
<th>Cropping year and crop type</th>
<th>First period</th>
<th>Second period</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Range of N$_2$O fluxes mg N m$^{-2}$ h$^{-1}$</td>
<td>Cumulative N$_2$O emission mg N m$^{-2}$</td>
</tr>
<tr>
<td></td>
<td>28 Sep 2007 to 6 May 2008 (14 campaigns)</td>
<td>45 (8)</td>
</tr>
<tr>
<td>Vetch</td>
<td>0–47</td>
<td>45 (8)</td>
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<tr>
<td>Rye-vetch</td>
<td>0–34</td>
<td>44 (14)</td>
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<td>Grass-clover</td>
<td>–2–12</td>
<td>6 (3)</td>
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<tr>
<td>Rye/maize</td>
<td>0–62</td>
<td>36 (6)</td>
</tr>
<tr>
<td>2007-2008</td>
<td>10 Sep 2008 to 6 May 2009 (10 campaigns)</td>
<td>278 (47)</td>
</tr>
<tr>
<td>Vetch</td>
<td>0–155</td>
<td>278 (47)</td>
</tr>
<tr>
<td>Rye-vetch</td>
<td>0–118</td>
<td>149 (17)</td>
</tr>
<tr>
<td>Grass-clover</td>
<td>0–31</td>
<td>35 (13)</td>
</tr>
<tr>
<td>Rye/maize</td>
<td>0–80</td>
<td>72 (12)</td>
</tr>
<tr>
<td>2008</td>
<td>25 Aug to 17 Nov 2008 (5 campaigns)</td>
<td>8–125</td>
</tr>
<tr>
<td>Grass-clover, plough-in</td>
<td>18 Mar to 6 May 2009 (12 campaigns)</td>
<td>3 (1)</td>
</tr>
<tr>
<td>Rye, NF</td>
<td>0–20</td>
<td>3 (1)</td>
</tr>
<tr>
<td>Rye, RS</td>
<td>0–88</td>
<td>23 (3)</td>
</tr>
<tr>
<td>Rye, DSM</td>
<td>0–58</td>
<td>15 (3)</td>
</tr>
<tr>
<td>2008</td>
<td>15 May to 16 Jul 2008 (11 campaigns)</td>
<td>46 (12)</td>
</tr>
<tr>
<td>Maize, NF</td>
<td>10–129</td>
<td>46 (12)</td>
</tr>
<tr>
<td>Maize, RS</td>
<td>20–1568</td>
<td>583 (41)</td>
</tr>
<tr>
<td>Maize, DSM</td>
<td>22–2105</td>
<td>895 (138)</td>
</tr>
<tr>
<td>Maize, GM</td>
<td>15–204</td>
<td>104 (7)</td>
</tr>
<tr>
<td>2008 (n = 3)</td>
<td>13 May to 22 Jul 2009 (14 campaigns)</td>
<td>70 (17)</td>
</tr>
<tr>
<td>Maize, NF</td>
<td>7–199</td>
<td>70 (17)</td>
</tr>
<tr>
<td>Maize, RS</td>
<td>16–872</td>
<td>643 (68)</td>
</tr>
<tr>
<td>Maize, DSM</td>
<td>15–1016</td>
<td>416 (94)</td>
</tr>
<tr>
<td>Maize, GM</td>
<td>10–410</td>
<td>253 (25)</td>
</tr>
<tr>
<td>2009 (n = 1)$^2$</td>
<td>13 May to 22 Jul 2009 (14 campaigns)</td>
<td>129</td>
</tr>
<tr>
<td>Maize, NF</td>
<td>9–538</td>
<td>129</td>
</tr>
<tr>
<td>Maize, RS</td>
<td>10–1902</td>
<td>1006</td>
</tr>
<tr>
<td>Maize, DSM</td>
<td>19–4374</td>
<td>1470</td>
</tr>
</tbody>
</table>

1Abbreviations are NF for no fertilizer, RS for raw cattle slurry, DSM for mixture of digested slurry and maize, and GM for green manure.

2Block excluded due to missing biomass data.
period that followed harvest of the maize crop in October ($P < 0.0001$). In the maize fertilization experiment in 2009, an error during harvest caused missing biomass data for one block. Therefore, the $N_2O$ flux data for this specific block was also removed from the dataset. Regarding $N_2O$, the excluded block had the highest emission of all blocks (Table 1), presumably because of the position next to a windbreak strip of willow, which increased soil temperature (Foereid et al., 2002). Concerning total $N_2O$ emissions, application of green manure more than doubled the emissions, but an even further increase occurred in plots fertilized with either raw slurry or digested slurry + maize, which gave rise to similar $N_2O$ emissions ($P < 0.0001$; Table 4). For the digested slurry + maize treatment, the $N_2O$ emission deviated between the 2 years (Treatment × Year; $P = 0.027$), which was mainly caused by a higher N fertilization rate the first year in this specific treatment (Table 4). Accordingly, the $N_2O$ emission factor for the two slurry-based fertilizers was rather constant at ca. 3%, whereas that of green manure was considerably lower, namely 0.5–0.8% ($P < 0.0001$).

In line with the rye experiment, anaerobic digestion of slurry reduced the field emissions of CH$_4$ in the maize fertilization experiment in 2009, but not in 2008 (Treatment × Year; $P < 0.0001$; Table 4). In general, the CH$_4$ emissions and emission factors were substantially higher in 2008 than the following year ($P \leq 0.0004$). The soil respiratory CO$_2$ emission increased in the following order of treatments: Unfertilized < Digested slurry + maize < Raw slurry < Green manure ($P < 0.0001$). Although green manure gave rise to the highest soil CO$_2$ emission this treatment still provided the highest near-term carbon sequestration potential of the three treatments ($P < 0.0001$). The total $N_2O$ emissions associated with the cultivation of vetch, rye–vetch, grass–clover, rye and maize are presented as means across the 2 years of data in Fig. 3.

### Biomass yields

Analysing 2 years of biomass data revealed that the total biomass of vetch was generally lower than the harvested biomass of rye–vetch, grass–clover and unfertilized whole crop maize ($P = 0.0002$; Fig. 4a and c). The grain of rye and vetch were used for direct human consumption and as animal fodder, thus only the straw of these crops were available as feedstock for energy purposes. The feedstock yield increased in the order vetch, rye–vetch followed by grass–clover and unfertilized whole crop maize that were similar in terms of the harvested biomass ($P < 0.0001$). The yield of grass–clover differed considerably between the 2 years (Crop × Year; $P = 0.026$), whereas the other crops had more stable feedstock yields. For unfertilized rye, both the total biomass and the straw yield were similar to that of rye–vetch based on data from the second experimental year including all five crops (Fig. 4a–c). The winter rye crop did not response to fertilization, neither with respect to total biomass ($P = 0.27$) nor straw yield ($P = 0.27$; Fig. 4b). In contrast, the maize biomass increased when the crop was fertilized with either raw slurry or anaerobic digested slurry + maize, but not when the maize crop received green manure ($P = 0.013$; Fig. 4c).

The grain energy density for rye and vetch was 16.5 MJ kg$^{-1}$ dry matter, whereas the energy content of the straw fractions were slightly higher, more specifically 17.8 and 17.0 MJ kg$^{-1}$ dry matter for rye and vetch, respectively (Crop × Fraction; $P < 0.0001$). The C : N ratio of rye and vetch straw was 195 ± 12 and 54 ± 2, respectively (means ± SE; $n = 4$).

### Biofuel potentials

Anaerobic digestion of the five feedstocks revealed that grass–clover and whole crop maize had the highest biogas potentials, followed by straw of rye and rye–vetch with considerably lower yields and finally vetch straw that gave rise to the lowest biogas production per kilo of feedstock ($P < 0.0001$; Table 5). Expressed as volume of CH$_4$ per gram of volatile solids in the feedstock, the biogas potentials were 244, 301, 366, 374 and 397 mL CH$_4$ g$^{-1}$ VS for dried straw of vetch, rye and rye–vetch, as well as for fresh grass–clover and whole crop maize, respectively. In line with the biogas yields, the bioethanol potentials for fresh grass–clover and whole crop maize were generally higher than for the dried straw-based feedstocks ($P < 0.0001$; Table 6). Hydrothermal pretreatment of the straw biomass increased the ethanol yields, but for grass–clover and maize pretreatment had the opposite effect due to loss of organic matter (Crop × Pretreatment; $P < 0.0001$). It was therefore decided to base the bioethanol scenario on hydrothermal pretreated straw of rye, vetch and rye–vetch, whereas no pretreatment was employed for grass–clover and whole crop maize biomass. In the combined bioethanol and biogas scenario, the effluents from the ethanol fermentation were used for biogas production. Overall, pretreatment of the feedstocks prior to ethanol fermentation decreased the biogas potentials of the ethanol effluents ($P < 0.0001$; Table 6).

### Net greenhouse gas reduction

Depending on crop species and biofuel scenario, field emissions of $N_2O$ counterbalanced between 3% and
398% of the avoided fossil CO2 emission that was obtained by fossil fuel displacement (Fig. 5). The five feedstocks also varied with respect to the achieved net GHG reductions, where the N2O emission was subtracted from the avoided fossil CO2 emission. In general, the 95% confidence interval of the mean overlapped for grass–clover and unfertilized whole crop maize, which both gave rise to higher net GHG reductions (i.e. 398–868 g CO2 m–2) than the straw-based feedstocks (i.e. 176 to 215 g CO2 m–2). In all three biofuel scenarios, the N2O emission allocated to vetch straw outweighed the avoided fossil CO2 emission, resulting in a negative net GHG reduction. Consequently, the result for vetch straw was consistently lower than that for rye–vetch straw according to the 95% confidence intervals, whereas the GHG benefit of rye straw tended to lie between the two.

Fertilization of the maize crop with either raw slurry or anaerobic digested slurry + maize increased the biomass yield, but the N2O emission increased as well. Thus, the net reduction in GHG emission achieved when the maize biomass was used for biogas and/or bioethanol production did not differ between unfertilized and fertilized plots, irrespective of whether the crop received raw slurry, digested slurry + maize or green manure (P ≥ 0.45; Fig. 5b,d,f). For whole crop maize, the bioethanol scenario gave a substantially lower net GHG reduction than the two other biofuel scenarios (P < 0.0001). In accordance, for vetch and rye–vetch straw bioethanol also resulted in the lowest net GHG reduction, followed by combined ethanol and biogas and finally biogas alone that provided the highest GHG benefit (P < 0.0001). The same tendency was observed for rye straw and grass–clover, although it could not be tested statistically.

**Discussion**

**N2O emissions**

Emissions of N2O were either induced by tillage or by fertilization. Tillage leads to increased N2O emissions because the soil structure is disturbed and some organic matter, which used to be physically protected in soil aggregates, is now exposed to microbial decomposition (Jastrow *et al.*, 2007). This may in part be counteracted by newly incorporated crop residues with a high C : N ratio, which stimulate N immobilization (Rosecrance *et al.*, 2000) and thereby tend to reduce N2O losses. Rye straw had a C : N ratio of 195 in our study, whereas C : N of 78 was reported for maize stems in the literature (Johnson *et al.*, 2007). In conclusion, the main source for the N2O emissions after incorporation of rye and maize stubbles (Table 1) was probably organically bound N that had accumulated in the soil as a result of the grass–clover rich cropping history. The magnitude of N2O emissions seemed to be affected by soil tempera-

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**Table 2** Content of ammonium and nitrate in the 0–20 cm soil layer (mg N kg–1 dry soil) a few days (1–3 days) or 2 weeks (12–15 days) after incorporation of grass–clover, vetch and rye–vetch crop residues in July–August 2008 and 2009 (means; SE in brackets; n = 4)

<table>
<thead>
<tr>
<th>Time after incorporation</th>
<th>Grass–clover</th>
<th>Vetch</th>
<th>Rye–vetch</th>
</tr>
</thead>
<tbody>
<tr>
<td>NH4⁺</td>
<td>NO3⁻</td>
<td>NH4⁺</td>
<td>NO3⁻</td>
</tr>
<tr>
<td>Few days (2008)</td>
<td>0.97 (0.08)</td>
<td>0.96 (0.18)</td>
<td>1.13 (0.29)</td>
</tr>
<tr>
<td>Two weeks (2008)</td>
<td>na</td>
<td>na</td>
<td>1.19 (0.13)</td>
</tr>
<tr>
<td>Two weeks (2009)</td>
<td>na</td>
<td>na</td>
<td>5.00 (0.21)</td>
</tr>
</tbody>
</table>

na, not assessed.

**Table 3** Cumulative N2O and CH4 emissions, NH4⁺, total N and C inputs, and N2O and CH4 emission factors for two organic fertilizers applied to a rye crop in 2009 (means; SE in brackets; n = 4)

<table>
<thead>
<tr>
<th>Treatment¹</th>
<th>Cumulative emission</th>
<th>Input</th>
<th>Emission factor (EF)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N2O (mg N m–2)</td>
<td>CH4 (mg C m–2)</td>
<td>NH4⁺ (g N m–2)</td>
<td>Total N (g N m–2)</td>
</tr>
<tr>
<td>NF</td>
<td>368 (43)</td>
<td>0.4 (0.4)</td>
<td>7.2</td>
</tr>
<tr>
<td>RS</td>
<td>383 (41)</td>
<td>6.5 (2.4)</td>
<td>6.4</td>
</tr>
<tr>
<td>DSM</td>
<td>388 (65)</td>
<td>2.1 (0.9)</td>
<td>6.4</td>
</tr>
</tbody>
</table>

¹The crop received either no fertilizer (NF), raw cattle slurry (RS) or a mixture of anaerobic digested slurry and maize (DSM).
ture (Fig. 2c). For instance, emissions were much higher following incorporation of rye stubbles after harvest in August than following incorporation of maize stubbles in October. This is in line with a statistical analysis of 45 studies, which revealed that N₂O emissions derived from crop residues depended on temperature, in addition to the residue N content, application method and rainfall (Novoa & Tejeda, 2006).

Furthermore, in our maize fertilization experiments that were conducted at sowing in May, the N₂O emission factor for the slurry-based fertilizers was ca. 3%, whereas the factor was one order of magnitude lower in the winter rye fertilization experiment conducted in March (Tables 3 and 4). One reason for this difference could be the simulated application techniques, which was direct injection in maize and trail hose application in rye. In line with our results, previous studies showed higher N₂O emission factors for slurry applied by direct injection as opposed to trail hose application (Wulf et al., 2002; Thomsen et al., 2010). However, in these studies, the emission factors varied two- to fivefold between application technique, thus less than in the current study. Besides application technique, we therefore suggest that the difference in fertilizer-induced N₂O emission between our experiments was also caused by other factors, such as soil temperature, crop type, and whether the crop was established at the time of application. Moreover, the wind speed was rather high, ca. 10 m s⁻¹, when fertilizers were surface applied in the rye field, thus significant amounts of N may have

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Table 4 Cumulative N₂O, CH₄ and CO₂ emissions, input of NH₄⁺, total N and C, N₂O and CH₄ emission factors and near-term carbon sequestration for three organic fertilizers applied to a maize crop (means; SE in brackets; n = 4 in 2008 and n = 3 in 2009)

<table>
<thead>
<tr>
<th>Year</th>
<th>Fertilizer¹</th>
<th>N₂O (mg N m⁻²)</th>
<th>CH₄ (mg C m⁻²)</th>
<th>CO₂ (g C m⁻²)</th>
<th>NH₄⁺ (g N m⁻²)</th>
<th>Total N (g N m⁻²)</th>
<th>Total C (g C m⁻²)</th>
<th>N₂O EF (% of N input)</th>
<th>CH₄ EF (ppm of C input)</th>
<th>Near-term C sequestration (g C m⁻²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2008</td>
<td>NF</td>
<td>106 (23)</td>
<td>0.2 (0.2)</td>
<td>259 (14)</td>
<td>7.5</td>
<td>16.5</td>
<td>182</td>
<td>3.2 (0.4)</td>
<td>169 (27)</td>
<td>74 (10)</td>
</tr>
<tr>
<td></td>
<td>RS</td>
<td>641 (48)</td>
<td>30.6 (4.7)</td>
<td>367 (18)</td>
<td>11.7</td>
<td>26.4</td>
<td>211</td>
<td>3.2 (0.5)</td>
<td>199 (23)</td>
<td>176 (18)</td>
</tr>
<tr>
<td></td>
<td>DSM</td>
<td>943 (137)</td>
<td>41.6 (5.0)</td>
<td>294 (16)</td>
<td>11.7</td>
<td>26.4</td>
<td>211</td>
<td>3.2 (0.5)</td>
<td>199 (23)</td>
<td>176 (18)</td>
</tr>
<tr>
<td></td>
<td>GM</td>
<td>235 (16)</td>
<td>0.0 (0.3)</td>
<td>445 (16)</td>
<td>na</td>
<td>23.9</td>
<td>390</td>
<td>0.5 (0.1)</td>
<td>1 (1)</td>
<td>205 (13)</td>
</tr>
<tr>
<td>2009</td>
<td>NF</td>
<td>88 (15)</td>
<td>0 (0.2)</td>
<td>235 (17)</td>
<td>8.1</td>
<td>17.9</td>
<td>189</td>
<td>3.3 (0.4)</td>
<td>17 (3)</td>
<td>96 (36)</td>
</tr>
<tr>
<td></td>
<td>RS</td>
<td>670 (72)</td>
<td>2.2 (0.7)</td>
<td>328 (19)</td>
<td>6.1</td>
<td>12.6</td>
<td>95</td>
<td>2.8 (0.7)</td>
<td>9 (4)</td>
<td>37 (32)</td>
</tr>
<tr>
<td></td>
<td>DSM</td>
<td>444 (105)</td>
<td>0.2 (0.16)</td>
<td>292 (22)</td>
<td>6.1</td>
<td>12.6</td>
<td>95</td>
<td>2.8 (0.7)</td>
<td>9 (4)</td>
<td>37 (32)</td>
</tr>
<tr>
<td></td>
<td>GM</td>
<td>277 (26)</td>
<td>0.1 (0.6)</td>
<td>444 (3)</td>
<td>na</td>
<td>23.8</td>
<td>389</td>
<td>0.8 (0.2)</td>
<td>2 (2)</td>
<td>180 (15)</td>
</tr>
</tbody>
</table>

¹The crop received either no fertilizer (NF), raw cattle slurry (RS), a mixture of anaerobic digested slurry and maize (DSM) or green manure (GM).

²na, not assessed.
been lost via NH₃ volatilization (Sommer et al., 2004). Finally, N₂O emissions from maize were potentially overestimated because plants that emerged within the gas-flux collars were removed halfway through N₂O monitoring in the growing crop, resulting in increased soil moisture and N availability within the collars compared to outside. We estimated the missing maize N uptake during this 1-month period to be 1.4 g N m⁻² based on N analyses of shoot biomass harvested 50 and 100 days after sowing (data not shown). Accordingly, N₂O emissions from maize plots were possibly overestimated by 22 mg N m⁻² if we assume that the N₂O emission factor for half of this monitoring period was 1.6%, corresponding to 50% of the overall N₂O emission factor for slurry (Table 4). However, if emissions from all treatments were equally overestimated this would not bias the reported N₂O emission factors for maize.

Previous studies indicate that the effect of anaerobic digestion of liquid manure on N₂O emissions following field application is a complex interaction between soil type, soil moisture and application method (Petersen, 1999; Clemens et al., 2006; Möller & Stinner, 2009; Chantigny et al., 2010; Thomsen et al., 2010). In our study conducted on sandy loam soil, the N₂O emission factor was similar for raw cattle slurry and anaerobic digested slurry mixed with digested maize silage (Tables 3 and 4). For comparison, a German organic farming study reported N₂O emission factors of 0.57%, 1.38% and 1.25% for raw cattle slurry, digested slurry, and digested slurry mixed with digested plant biomass, respectively (Möller & Stinner, 2009). In that study, the liquid fertilizers were applied to a silty loam soil by direct injection into growing maize. During anaerobic digestion, the content of labile carbon in the feedstock is reduced, resulting in lower oxygen consumption following field application and thereby possibly reduced N₂O emissions derived from denitrification (Petersen, 1999). However, concurrently the ammonium content increases in the digestate. Möller & Stinner (2009) suggested that this higher supply of NH₄⁺ stimulated N₂O losses from nitrification under the aerobic soil conditions that prevailed during their trial, and thereby resulted in higher N₂O emission factors for the digestates than for the raw slurry. The deviation in the

Table 5 Biogas potentials for untreated feedstocks (means; SE in brackets; n = 3)

<table>
<thead>
<tr>
<th>Feedstock</th>
<th>Biogas potential (g CH₄ kg⁻¹ DM feedstock)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rye straw</td>
<td>138 (2)</td>
</tr>
<tr>
<td>Vetch straw</td>
<td>119 (3)</td>
</tr>
<tr>
<td>Rye-vetch straw</td>
<td>149 (6)</td>
</tr>
<tr>
<td>Grass-clover</td>
<td>249 (11)</td>
</tr>
<tr>
<td>Whole crop maize</td>
<td>231 (6)</td>
</tr>
</tbody>
</table>

Table 6 Bioethanol potentials (n = 2) and biogas potentials for ethanol effluents (n = 3) for untreated (UT) and hydrothermal pretreated (HTT) feedstocks (means; SE in brackets)

<table>
<thead>
<tr>
<th>Feedstock</th>
<th>Bioethanol potential (g ethanol kg⁻¹ DM raw feedstock)</th>
<th>Biogas potential for ethanol effluent (g CH₄ kg⁻¹ DM raw feedstock)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rye straw, UT</td>
<td>23.3 (1.5)</td>
<td>99.1 (3.5)</td>
</tr>
<tr>
<td>Rye straw, HTT</td>
<td>82.2 (2.1)</td>
<td>54.3 (0.5)</td>
</tr>
<tr>
<td>Vetch straw, UT</td>
<td>35.8 (3.7)</td>
<td>80.1 (2.2)</td>
</tr>
<tr>
<td>Vetch straw, HTT</td>
<td>76.7 (1.8)</td>
<td>41.4 (1.3)</td>
</tr>
<tr>
<td>Rye-vetch straw, UT</td>
<td>23.5 (1.5)</td>
<td>125.2 (0.9)</td>
</tr>
<tr>
<td>Rye-vetch straw, HTT</td>
<td>106.2 (3.3)</td>
<td>64.9 (1.2)</td>
</tr>
<tr>
<td>Grass-clover, UT</td>
<td>234.7 (3.8)</td>
<td>87.9 (1.6)</td>
</tr>
<tr>
<td>Grass-clover, HTT</td>
<td>90.1 (2.4)</td>
<td>32.9 (2.2)</td>
</tr>
<tr>
<td>Whole crop maize, UT</td>
<td>220.5 (18.7)</td>
<td>99.4 (3.7)</td>
</tr>
<tr>
<td>Whole crop maize, HTT</td>
<td>102.4 (5.1)</td>
<td>na</td>
</tr>
</tbody>
</table>

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response to anaerobic digestion between their study and our maize fertilization experiments could relate to differences in soil moisture, as well as other parameters such as soil texture (sandy vs. silty loam) and quality of the organic fertilizers.

A farmer can produce the same cereal yield in a rye–vetch intercrop as in a rye sole crop, and in addition gain increased N input to the agroecosystem via vetch N2 fixation (Rochester & Peoples, 2005). In contrast, vetch sole cropping as a means to increase the soil N status for subsequent crops, as suggested by Rochester & Peoples (2005), is not recommended according to our results showing a risk of severe N2O emissions (Fig. 3a). More specifically, legumes such as vetch generally have higher N contents than nonlegumes (IPCC, 2006) and especially the second year of our study, incorporation of vetch roots and stubbles increased the availability of soil inorganic N (Table 2), resulting in extremely large N2O emissions (Table 1). The rye–vetch intercrop caused less N2O emission, possibly because the N-rich vetch residues were mixed with the more C-rich rye residues, which seemed to increase N immobilization in the soil (Table 2) as reported by Rosecrance et al. (2000). In a Scottish study, enhanced N2O emissions also occurred after incorporation of crop residues with a low C : N ratio compared to residues with a high C : N ratio (Baggs et al., 2000). In line with our results, Pappa et al. (2011) measured higher post harvest N2O emissions from a legume sole crop than from a barley–legume intercrop, when the legume was represented by clover.

The grass–clover sward gave rise to the lowest total N2O emission of all the investigated crops for two reasons (Fig. 3). Firstly, N2O emissions from the established sward were negligible, viz. only 0.1–0.4 kg N2O-N ha−1 during the 9-month monitoring period (Table 1), which is comparable with emission levels measured in other grass–legume swards (Kammann

Fig. 5 Avoided fossil fuel-derived CO2 emission, where field emission of N2O was subtracted (g CO2-equivalents m−2), for biofuels produced from straw of rye, vetch and rye–vetch in addition to grass–clover (a, c, e) and whole crop maize receiving either (i) no fertilizer, (ii) raw cattle slurry, (iii) mixture of anaerobic digested slurry and digested maize silage or (iv) green manure (b, d, f). The biofuel scenarios are biogas (a, b), bioethanol (c, d) and coproduction of bioethanol and biogas, where the effluent from the bioethanol fermentation is used for biogas production (e, f). The black part of the bars indicates the net greenhouse gas (GHG) reduction due to fossil fuel displacement when emissions of N2O (grey part) are taken into consideration (means ± SE; rye, n = 4; vetch, rye–vetch, grass–clover, n = 8; maize, n = 7). SE for N2O is shown as wide error bars.
et al., 1998; Simek et al., 2004). Secondly, grass–clover was the only perennial crop in the study and the intention was to keep the sward in the crop rotation for 4 years. Emissions related to ploughing in a grass–clover sward in early autumn was estimated to be 0.9 kg N₂O-N ha⁻¹ (Table 1), which was based on measurements done in the newly established rye crop that followed a grass–clover ley. For comparison, a Scottish study conducted on an imperfectly drained sandy loam soil reported that 1.5 and 0.9 kg N₂O-N ha⁻¹ was emitted during the first month after incorporation of ungrazed grass–clover swards in July and November, respectively (Ball et al., 2007). However, since the grass–clover sward in our crop rotation was meant to provide biomass for 4 years before it would be ploughed in, only 25% of the N₂O emission derived from incorporation was included in the total N₂O emission (Fig. 3) as this value was calculated on an annual basis.

Some uncertainty on the estimated net GHG reductions (Fig. 5) is connected to the N₂O emission measurements after grass–clover soil incorporation. Firstly, this N₂O source was only assessed in 2008. Secondly, comparison of cumulative emissions between crop species could be biased due to the general 14-day measurement frequency following soil incorporation, which potentially neglected differences between crops in the temporal dynamics of N₂O fluxes. For instance, the deviation between the cumulative N₂O emission after ploughing in of grass–clover (89 mg N m⁻²) and vetch sole crop (214 mg N m⁻²) in the autumn of 2008 is striking (Table 1). However, data on soil inorganic N measured 1–3 days after incorporation indicated a five times higher nitrate content in the former vetch plots compared with the grass–clover plots (Table 2), which supports a higher N₂O emission from the vetch plots. Finally, only 25% of N₂O emissions related to ploughing in of the grass–clover sward were included in the annual N₂O emission estimate, and thereby the higher uncertainty had less impact on the final net GHG reduction.

Relative magnitude of N₂O emissions

Field emissions of N₂O counterbalanced between 3% and 398% of the avoided fossil CO₂ emission depending on crop species, fertilizer type and biofuel scenario (Fig. 5), and our results highlight that N₂O emissions during biomass cultivation may affect the environmental sustainability of agro-biofuels to various extents. Thus, we were both right and wrong in our hypothesis that N₂O emissions would offset more than 20% of the CO₂ emission reduction that was obtained by fossil fuel displacement. The hypothesis was primarily based on the findings by Crutzen et al. (2008), who estimated that fertilizer-derived N₂O emissions would counteract between 50% and 170% of the avoided fossil CO₂ emissions for three biofuel production systems, including bioethanol based on maize grain. The net GHG reduction, where N₂O emissions were subtracted from avoided fossil CO₂ emissions, varied greatly between the five plant-based feedstocks. This was due to differences in three factors, viz. (i) N₂O emission (discussed above), and avoided fossil fuel-derived CO₂ emission determined by (ii) the biomass yield and (iii) the biofuel potential per kilo of harvested dry matter, which are both discussed below.

All crop yields were comparable with results obtained in other studies conducted under similar growth conditions (Danish National Trials, 2010). Interestingly, fertilization of the rye crop did not increase the crop yield, neither in 2009 (Fig. 4b) nor in 2008 (data not shown). A potential reason could be that considerable NH₃ volatilization possibly occurred following application of the slurry-based fertilizers, as mentioned above, or simply that the soil generally had a high level of plant available N originating from the grass–clover rich cropping history. In contrast to rye, maize responded to the slurry-based fertilizers (Fig. 4c), which supports several other studies documenting that increase in maize yields are closely associated with increased levels of N fertilizer application (e.g. Sistani et al., 2010; Nannen et al., 2011). However, green manure did not produce increased crop yields during the first growing season following application, presumably due to lack of appropriate soil incorporation, and thereby limited soil microbial decomposition (Ambus & Jensen, 2001). The grass–clover swards were cut four times per year, but the annual dry matter production varied considerably between the 2 years (Fig. 4a). Despite the expected complementarity between grasses and clovers, interannual variation in yields is common for grass–clover leys (Frankow-Lindberg et al., 2009).

Concerning the production of biofuel feedstock, straw yields of rye and vetch were lower than the crop yields of the two dedicated bioenergy crops, maize and grass–clover (Fig. 4). In addition, the biofuel potentials per gram of harvested dry matter were generally lower for the dried, straw-based feedstocks (Tables 5 and 6). Without pretreatment, grass–clover and whole crop maize gave the highest bioethanol potentials, where more than 20% of the feedstock mass was converted to ethanol (Table 6). This was due to the high concentration of free sugars, mainly fructose, in the grass–clover and starch in whole crop maize (Oleskowicz-Popiel et al., 2011), which are easily converted to ethanol by the yeast. Hydrothermal pretreatment of the straw biomasses clearly increased the ethanol yields (Table 6), but the yield of ca. 80 g kg⁻¹ raw material for rye and vetch was still quite low. Considering a cellulose
content of 40%, a cellulose-to-glucose factor of 0.9, and a glucose-to-ethanol factor of 0.51, then the theoretical ethanol yield would be 183.6 g kg\(^{-1}\) raw material, which indicates that the pretreatment could be improved. Overall, our biogas potentials fitted well with previous batch trial studies (Petersson et al., 2007; Oleskowicz-Popiel, 2010). It should be emphasized, however, that such studies only give an indication of the potential yields that may be obtained in a full-scale biogas plant, since the individual choice of process parameters such as retention time, substrate ratios and substrate size reduction may influence the actual biogas yields (e.g. Wu et al., 2010). Moreover, the biogas potential for grass–clover was established on cuttings from a 1½-year-old sward, which may not fully reflect the 4-year production period. For instance, another study at the field site showed a clear decline in biogas yield over time as the grass–clover sward grew older, possibly due to an increase in the lignin content of the plants (Henrik Bangsø Nielsen, personal communication).

In our study, the higher biomass yields and biofuel potentials were the reasons for a substantially higher avoided fossil CO\(_2\) emission per area for whole crop maize and grass–clover compared to straw of rye, vetch and rye–vetch. The net GHG reduction for the different biomass–biofuel combinations were calculated using the avoided CO\(_2\) emission obtained via fossil fuel displacement and subtracting N\(_2\)O emissions (in CO\(_2\) equivalents) allocated towards the feedstock. The allocation of N\(_2\)O emissions between grain and straw for the rye, vetch and rye–vetch crops was based on the energy content in the two fractions (EU, 2009), and generally 70% of the N\(_2\)O emission was allocated towards the straw feedstock. This approach enabled us to compare net GHG reductions for biofuels based on lignocellulosic crop residues with biofuels based on dedicated bioenergy crops (i.e. grass–clover and whole crop maize) (Fig. 5). In Denmark, straw is not considered as a waste product, but is a resource in great demand typically used for animal bedding, in straw-fired combined heat and power plants, or to maintain appropriate organic carbon levels in agricultural soils (Larsen & Haastrup, 2009).

Either a little (≤ 215 g CO\(_2\) m\(^{-2}\)) or no GHG benefit was obtained when producing biofuels from the straw-based feedstocks (Fig. 5). Especially for vetch straw, the biofuel production per area was low and N\(_2\)O emissions were high, which resulted in a negative net GHG reduction in all three biofuel scenarios. Due to the recalcitrant structure of straw, it would probably be more efficient in terms of energy output to use the rye and vetch straw for direct combustion. More specifically, the energy density of the straw was ca. 17 MJ kg\(^{-1}\) dry matter, which should be compared with biofuel yields for straw corresponding to 2 and 6 MJ kg\(^{-1}\) dry matter for bioethanol and biogas, respectively (WTW, 2008). However, by burning the straw biomass in centralized heat and power plants, the farmers lose the possibility to return nitrogen in the feedstock back to the field, which would conflict with the fundamental principle of nutrient recycling in organic farming (DARCOF, 2000).

In contrast, our study indicates that significant net GHG reductions may be obtained by replacing fossil fuels with biogas or coproduced bioethanol and biogas based on either fresh grass–clover or whole crop maize (Fig. 5). These biofuel production systems gave net GHG reductions of ca. 700–800 g CO\(_2\) m\(^{-2}\), when fossil fuel displacement and N\(_2\)O emissions were taken into account. However, biofuel production is also associated with other GHG sources that were not included in the present analysis, for instance fossil fuel-derived CO\(_2\) emissions from farm machinery and during conversion processes that turn biomass into biofuel. If these sources were considered, the net GHG reduction, indicated by the black part of the bars in Fig. 5, would be smaller.

From a GHG mitigation perspective, no benefit was achieved by fertilizing the maize crop because the extra crop yield, and thereby larger avoided fossil CO\(_2\) emission, was offset by increased N\(_2\)O emissions (Fig. 5b,d, f). A similar result was obtained in a German poplar plantation, where high fertilizer-derived N\(_2\)O emissions caused that unfertilized poplar gave rise to a larger net GHG gain than fertilized poplar, when considering net CO\(_2\) uptake in above-ground biomass, and GHG emissions derived from production and use of N fertilizer (Kern et al., 2010). However, the opposite result was reported for rye. Callesen et al. (2011) also highlighted that perennial bioenergy crops should only be fertilized if the overall GHG balance, accounting for the full effect of cascading N, indicates an additional GHG sink compared with the unfertilized case. Obviously, substantial crop yields cannot be maintained without input of N. The idea of our organic strip intercropping system is that strips of grass–clover and strips hosting annual crop rotations swap place every 4 years (Fig. 1). In this way, the grass–clover strips function as a fertility building strip for the annual crops, in addition to a source of green manure and biofuel feedstock. Furthermore, it should be emphasized that the results obtained in the current study are very specific for the soil and growth conditions at our experimental site, and conclusions regarding the effect of fertilization may be different if the study was conducted on less fertile soils.

The three biofuel scenarios

In line with our hypothesis, bioethanol production gave a lower biofuel energy yield compared with biogas, and
thereby a smaller net GHG reduction (Fig. 5). The lower energy yield was typically compensated if bioethanol fermentation was combined with biogas production on the ethanol effluent. This was anticipated since a techno-economical evaluation of bioethanol and biogas production within organic farming showed that using the effluent from bioethanol fermentation will increase the energy output of the biofuel production system by ca. 50% (Klein-Marcuschamer et al., 2010). Furthermore, a pilot scale study revealed that implementation of biogas production on the effluent from bioethanol fermentation based on both C6 and C5 sugars resulted in 30% higher carbon utilization compared to a system with bioethanol production only (Uellendahl & Ahring, 2010). However, the similarity in net GHG reduction for the biogas scenarios compared with coproduction of bioethanol and biogas (Fig. 5) may disappear if other GHG sources were included in the analysis as significant amounts of energy is needed for distillation of bioethanol.

**Perennial N₂-fixing crops for biofuel production**

Future agro-biofuel production could be based on perennial N₂-fixing cropping systems such as grass–clover instead of monocultures of whole crop maize, which currently dominate on large areas of European farmland, for instance in Germany (Weiland, 2006; Schittenhelm, 2008), with negative impacts on important ecosystem functions and services (e.g. Matson et al., 1997). Nitrogen input via biological N₂ fixation has the advantage of not leading to immediate N₂O emissions, in contrast to mineral and organic fertilizers (Carter & Ambus, 2006). However, significant amounts of N₂O may be emitted following soil incorporation of N-rich leguminous crop residues, as discussed above. An advantage of perennial cropping is that this tillage-induced GHG emission only occurs once every 4–6 years, depending on how long the crop is kept in the crop rotation. At least three precautions can be taken to reduce N₂O emissions derived from incorporation of N-rich crop residues, which are to (i) only plough in leguminous crop residues during cool and dry periods as also emphasized by Ball et al. (2007), (ii) grow legumes in mixture with nonlegumes in order to increase the C : N ratio of the crop residues, and (iii) ensure the presence of a growing crop to take up mineralized N. Other recent papers also highlight advantages of perennial bioenergy crops as opposed to annual crops (Erisman et al., 2010; Kern et al., 2010). For instance, grass–clover perennials tend to increase carbon sequestration via enhanced root biomass production, formation of stable aggregates and the increased residence time of carbon due to reduced tillage (Soussana et al., 2004).

**CH₄ emissions and near-term carbon sequestration**

Our results from 2009 showed that anaerobic digestion of cattle slurry and maize silage may reduce slurry-derived field emissions of CH₄ following surface application and direct injection. Previous field studies also reported lower CH₄ efﬂux from digested slurry than from untreated slurry (Wulf et al., 2002; Clemens et al., 2006). The same positive effect, however, was not observed in our 2008 experiment. Generally, CH₄ emissions closely followed the amount of carbon applied being a source for methanogenesis (Tables 3 and 4). In addition, the CH₄ emission factor seemed to increase logarithmically with the volume of slurry applied, which probably indicated the extent of anaerobiosis in the soil. More specifically, the slurry application rate was ca. 10 L m⁻² for both treatments in 2008, whereas 6 and 4 L m⁻² of raw slurry and digested slurry + maize were applied in the 2009 experiments, respectively. The considerably higher CH₄ emission factor during the maize experiment in 2008 was probably also caused by the very low soil moisture on the day of fertilization in mid May (i.e. only 11 vol% as opposed to 15 vol% in 2009; Fig. 2b), which slowed down slurry infiltration from the simulated injection slots, prolonging anaerobiosis. Overall, emission of CH₄ only occurred during the first 1–2 days after fertilization, and as a GHG source CH₄ was negligible compared with N₂O.

Green manure provided the highest near-term carbon sequestration potential of the three organic fertilizers, which were applied to maize (Table 4). Calculated as CO₂-equivalents, this GHG sink was ca. 700 g CO₂ m⁻². This was similar to the net GHG reduction obtained via biogas production, or coproduction of bioethanol and biogas, based on the harvested maize biomass (Fig. 5b and f). However, most likely mineralization of the applied organic fertilizers continued beyond our CO₂ flux monitoring period, thus the long-term carbon sequestration potentials would be lower than the near-term potentials reported here.

**Conclusions**

Life-cycle assessments of biofuel production systems often make use of the default IPCC N₂O emission factor to estimate field emission of N₂O from various crops. This is problematic because emission factors may vary greatly depending on crop species and management of anaerobic digestate as fertilizer, as we show in the present work. If decisions regarding crop management and choice of biomass for future biofuel production are based on these models, we may end up choosing the worst solutions concerning GHG savings. Whenever available, we therefore advocate the use of crop species, management
and soil type-specific N\textsubscript{2}O emission factors in life-cycle assessments of biofuel production systems. Focus should be on maximizing the net GHG reduction rather than optimizing biomass yields via fertilizer input, as demonstrated in our maize fertilization experiments.

Our work reveals that perennial N\textsubscript{2}-fixing crops, like grass–clover swards, could be a suitable alternative to maize when producing feedstock for biofuels within organic farming. Sole cropping of annual legumes such as vetch, however, should be avoided due to the risk of high N\textsubscript{2}O emissions. Increased establishment of grass–legume swards would enhance the crop diversity in certain regions of Europe. The study shows that biofuel production based on lignocellulosic crop residues provides substantially lower net GHG reductions on an area basis compared to dedicated bioenergy crops, and even negative numbers sometimes. However, the present results are very specific for the soil and growth conditions at our experimental site, and consequently there is a great need for similar studies including a range of crop species, soil types and climatic conditions.

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