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Supervisor: Prof. Dr. Iris Lewandowski

The potential of miscanthus as biogas feedstock

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Andreas Kiesel

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Dean Faculty of Agricultural Science

Prof. Dr. Ralf Vögele

Thesis Committee

Prof. Dr. Iris Lewandowski
University of Hohenheim, Germany

Prof. Dr. John Clifton-Brown
Aberystwyth University, United Kingdom

Prof. Dr. Uwe Ludewig
University of Hohenheim, Germany

Content

Abstract

Of all renewable energy forms, biomass accounts for the by far largest proportion of gross inland energy consumption in Europe. As the biogas sector in particular can provide demand-driven electricity generation, energy storage and flexible utilization options including biofuels, it is likely to play an important role in future energy systems in future. In Germany, the largest biogas market in Europe, energy crops provide the highest proportion of biogas input substrates, with maize being the most dominant. The environmental impact of biogas production is mainly attributed to energy crop production, with the risks of maize cultivation being particularly criticized. Perennial biomass crops have the potential to reduce the environmental impact of the biogas sector and miscanthus is an especially promising candidate crop due to its high yields. However, preliminary observations have indicated that the green harvest of miscanthus necessary for biogas production leads to a strong yield depression in the subsequent year.

The aim of this thesis was to determine and understand the mechanisms influencing the green-cut tolerance of miscanthus and to assess the potential of different green-harvest regimes for biogas production. Here, ‘green-cut tolerance’ is defined as the crop’s ability to regrow in the year after the green harvest is performed without yield depression. A further aim of this thesis was to investigate the environmental performance of miscanthus-based biogas production and to determine its energy efficiency compared to other utilization options.

Field trials were conducted to assess the potential of miscanthus hybrids for biogas production, the green-cut tolerance of *Miscanthus x giganteus* (Mxg), and how both are influenced by management practices (harvest regime x nitrogen fertilization). A Life-Cycle Assessment was performed to evaluate the environmental impact of biogas production from perennial C_4 grasses, including miscanthus, and to assess the optimization potential compared to the standard biogas crop maize. The suitability of miscanthus biomass was investigated for the utilization options bioethanol, biogas and combustion, and the energy efficiency of these was compared based on their net energy yield.

The results revealed that Mxg harvested in October showed the highest average biomass yield, the highest methane yield (approx. 6000 m^3 methane ha^{-1}) of all harvest regimes, and a higher substrate-specific methane (SMY) yield than for biomass harvested after winter. An earlier green harvest (July, August) improved the SMY, but led to a sharp biomass and thus methane yield decline in the second year and was identified as unsuitable for Mxg. As increased nitrogen fertilization showed no effect on the yield in any of the harvest regimes, it can be disregarded as a management practice for improving green-cut tolerance. Instead, harvest date was found to have a strong influence on green-cut tolerance and sufficient time for relocation of carbohydrates needs to be allowed before a green cut is performed. This finding is crucial for the utilization of miscanthus biomass harvested green and also for the breeding of new varieties with improved green-cut tolerance. Breeding targets for optimized biogas varieties should include to increase the SMY and biomass yield and to widen the possible harvest window. Selecting genotypes that relocate carbohydrates to the rhizomes earlier

would allow an earlier green harvest without yield decline the following year, but this may involve a trade-off with the SMY.

The suitability of miscanthus for the utilization options assessed was found to be influenced by biomass composition, which in turn was affected by genotype and harvest date. Lignin content had a negative effect on biomass quality for biogas and bioethanol production and increased with later harvest dates. Hemicellulose had a positive effect on biomass quality for bioethanol production through the improvement of the saccharification potential. Low ash, potassium and chloride content enhanced biomass quality for combustion by increasing the ash melting temperatures and decreased with a delay in harvest to after winter. For the biogas and bioethanol utilization pathways, novel miscanthus varieties with low lignin content need to be developed, whereas for combustion varieties with a high lignin content are more favourable.

The Life Cycle Assessment revealed that the use of miscanthus has a high potential to reduce the environmental impacts of biogas crop production and thus the biogas sector. Miscanthus had a more favourable performance than the annual biogas crop maize in each impact category considered and the highest reduction potential compared to the fossil reference in the impact categories climate change, fossil fuel depletion and marine eutrophication.

The choice of biomass utilization pathway had a considerable effect on the energy yield per unit area, with combustion showing the overall highest energy yield potential for electricity production. However, for the combustion pathway, miscanthus is generally harvested after winter and this is accompanied by biomass yield losses of 35% compared to peak yield. In the biogas pathway, miscanthus can be harvested close to peak yield, leading to an only 10% lower energy yield than that of combustion. When considering the use of miscanthus for biofuel production, the highest area efficiency was found for the direct use of biomethane, followed by battery electric vehicles fuelled by electricity from biomass combustion, and the lowest for the direct use of bioethanol. However, the low conversion efficiency of bioethanol production did not consider energy generation from by-products.

In this thesis it was determined that the green-cut tolerance of miscanthus is influenced by the carbohydrate relocation to the rhizomes and thus by harvest date. Miscanthus harvested in October shows a high potential as feedstock for biogas production due to its high yield and sufficient digestibility, can help improve the biogas sector's environmental performance and contribute to an increase in greenhouse gas mitigation. The digestibility of miscanthus biomass for biogas production could be improved by breeding and selecting genotypes with low lignin contents and by applying suitable pretreatment methods. Increased digestibility could also help to overcome potential trade-offs between early carbohydrate relocation and SMY. The efficiency of biomass utilization greatly depends on the utilization option, with a high efficiency being identified for biomethane as a transportation fuel and for peak-load power generation. It was shown that miscanthus is a suitable crop for the provision of sustainably produced biomass as a feedstock for the growing European bioeconomy that provides additional ecosystem services, e.g. groundwater and surface water protection.

Zusammenfassung

Von allen Erneuerbaren Energieträgern stellt Biomasse den weitaus größten Anteil am Bruttoinlandsenergieverbrauch in Europa. Der Biogassektor wird voraussichtlich im zukünftigen Energiesystem eine wesentliche Rolle spielen, da dieser die Möglichkeit einer bedarfsorientierten Stromerzeugung, Energiespeicherung und flexiblen Nutzung, einschließlich Biokraftstoffen, bietet. In Deutschland, dem größten Biogasmarkt in Europa, stellen Energiepflanzen den höchsten Anteil an den Biogas Einsatzsubstraten, wobei Mais hier eine dominierende Rolle einnimmt. Ein großer Teil der Umweltauswirkungen der Biogaserzeugung sind auf den Energiepflanzenanbau zurückzuführen, wobei die Risiken des Maisanbaus besonders kritisiert werden. Mehrjährige Biomassepflanzen haben das Potenzial die Umweltauswirkungen des Biogassektors zu verringern und Miscanthus ist aufgrund seines hohen Ertragspotenzials besonders vielversprechend. Erste Beobachtungen haben jedoch gezeigt, dass die für die Biogaserzeugung notwendige Grünernte von Miscanthus im Folgejahr zu starken Ertragseinbußen führen kann.

Ziel dieser Dissertation ist es, die zugrundeliegenden Mechanismen der Grünschnitt-Toleranz bei Miscanthus zu erforschen und das Potenzial verschiedener Grünernte-Regime für die Biogaserzeugung zu bewerten. Die „Grünschnitt-Toleranz“ wird hier definiert als die Fähigkeit der Kulturpflanze im Jahr nach Grünernte ohne Ertragseinbruch wieder aufzuwachsen. Ein weiteres Ziel dieser Arbeit war es, die Umweltauswirkungen der Miscanthus-basierten Biogaserzeugung und deren Energieeffizienz im Vergleich zu anderen Nutzungsmöglichkeiten zu untersuchen.

Es wurden Feldversuche durchgeführt, in denen das Potenzial von verschiedenen Miscanthus Hybriden für die Biogaserzeugung, die Grünschnitt-Toleranz von *Miscanthus x giganteus* (Mxg) und der Einfluss des pflanzenbaulichen Managements (Ernteregime x Stickstoffdüngung) untersucht wurde. Die Umweltauswirkungen der Biogaserzeugung aus mehrjährigen C_4 Gräsern, einschließlich Miscanthus, wurden im Rahmen einer Ökobilanz untersucht und das Optimierungspotenzial im Vergleich zur Standard Biogaspflanze Mais bewertet. Die Eignung von Miscanthus Biomasse für verschiedene Verwertungsoptionen, einschließlich Bioethanol, Biogas und Verbrennung, wurde untersucht und die Energieeffizienz dieser Verwertungsoptionen anhand ihres Energieertrages miteinander verglichen.

Die Ergebnisse zeigten, dass die Ernte im Oktober bei Mxg den höchsten durchschnittlichen Biomasseertrag und den höchsten Methanertrag (ca. 6000 m^3 Methan ha^{-1}) aller Ernteregime lieferte, sowie die Biomasse einen höheren substratspezifischen Methanertrag (SMY) als bei der Frühjahrsernte im März aufwies. Eine frühere Grünernte (Juli, August) verbesserte den SMY, führte jedoch im zweiten Jahr zu einem starken Einbruch des Biomasseertrages und damit auch des Methanertrages. Eine Grünernte zu einem früheren Erntezeitpunkt wurde daher als ungeeignet für Mxg identifiziert. Eine erhöhte Stickstoffdüngung beeinflusste in keinem Ernteregime den Ertrag und stellt daher keine geeignete Managementpraxis zur Verbesserung der Grünschnitt-Toleranz dar. Stattdessen wurde festgestellt, dass das Erntedatum einen starken Einfluss auf die Grünschnitt-

Toleranz hat. Die Ernte muss hierbei im Spätherbst erfolgen, um der Pflanze ausreichend Zeit für die Einlagerung von Kohlenhydraten in die Rhizome zu ermöglichen. Dies ist eine entscheidende Erkenntnis für die Verwertung von grün geernteter Miscanthus Biomasse und für die Züchtung neuer Sorten mit verbesserter Grünschnitt-Toleranz. Züchtungsziele für optimierte Biogas-Sorten sollten darauf ausgerichtet werden den SMY und den Biomasseertrag zu erhöhen und das mögliche Erntefenster zu erweitern. Die Auswahl von Genotypen, die Kohlenhydrate früher im Rhizom einlagern, würde eine frühere Grünernte ohne Ertragseinbruch im Folgejahr ermöglichen, könnte jedoch den SMY negativ beeinflussen.

Die Eignung von Miscanthus für die betrachteten Nutzungsmöglichkeiten wurde durch die Biomasse-Zusammensetzung beeinflusst, welche wiederum abhängig vom Genotyp und Erntedatum war. Ein hoher Ligningehalt in der Biomasse wirkte sich negativ auf die Produktion von Biogas und Bioethanol aus und spätere Erntetermine führten zu höheren Ligningehalten. Hemicellulose bewirkte eine Verbesserung des Verzuckerungspotentials und erhöhte so die Qualität der Biomasse für die Bioethanolproduktion. Ein niedriger Gehalt an Asche, Kalium und Chlorid verbesserte die Qualität der Biomasse für die Verbrennung durch Erhöhung der Ascheschmelztemperaturen und eine spätere Ernte nach dem Winter führte zu einer Verringerung dieser Bestandteile. Für die Verwertungswege Biogas und Bioethanol empfiehlt es sich neue Miscanthus Sorten mit niedrigerem Ligningehalt zu entwickeln, während für die Verbrennung Sorten mit hohem Ligningehalt günstiger sind.

Die Ökobilanz ergab, dass der Einsatz von Miscanthus ein hohes Potenzial zur Verringerung der Umweltauswirkungen der Biogasproduktion und damit des Biogassektors aufweist. Miscanthus schnitt in jeder betrachteten Wirkungskategorie besser ab als der Anbau von Mais und zeigte das höchste Reduktionspotential im Vergleich zur fossilen Referenz in den Wirkungskategorien Klimawandel, fossiler Ressourcenverbrauch und marine Eutrophierung.

Die Wahl der Biomassenutzung hatte einen erheblichen Einfluss auf den Energieertrag pro Flächeneinheit, wobei die Verbrennung das insgesamt höchste Energieertragspotenzial für die Stromerzeugung aufweist. Für die Verbrennung wird Miscanthus jedoch in der Regel nach dem Winter geerntet, was mit Biomasseertragsverlusten von 35% im Vergleich zum Maximalertrag einhergeht. Für die Biogasnutzung kann Miscanthus annähernd zum Zeitpunkt des Maximalertrages geerntet werden, was in der Summe zu einem nur 10% niedrigeren Energieertrag als bei der Verbrennung führt. Bei der Verwendung von Miscanthus zur Herstellung von Biokraftstoffen wurde die höchste Flächeneffizienz für die direkte Verwendung von Biomethan ermittelt, gefolgt von batteriebetriebenen Elektrofahrzeugen, die mit Strom aus Biomasseverbrennung betrieben werden. Die direkte Verwendung von Bioethanol wies die niedrigste Flächeneffizienz auf. Allerdings wurde hierbei nicht die Energieerzeugung aus Nebenprodukten berücksichtigt, was bei der Bioethanolproduktion die Umwandlungseffizienz verbessern könnte.

In dieser Arbeit wurde festgestellt, dass die Grünschnitt-Toleranz von Miscanthus durch das Erntedatum und durch die Rückverlagerung von Kohlenhydraten in die Rhizome beeinflusst wird. Im

Oktober geernteter Miscanthus weist aufgrund des hohen Biomasse Ertrages und der ausreichenden Verdaulichkeit ein hohes Potenzial für den Einsatz in der Biogaserzeugung auf und kann zur Verringerung der Umweltauswirkungen und zur Minderung der Treibhausgas Emissionen des Biogassektors beitragen. Die Abbaubarkeit der Miscanthus Biomasse im Biogasprozess kann weiter verbessert werden, indem Genotypen mit niedrigerem Ligningehalt gezüchtet und geeignete Vorbehandlungsmethoden angewendet werden. Eine verbesserte Verdaulichkeit könnte auch dazu beitragen, mögliche negative Rückkopplungseffekte zwischen einer frühen Kohlenhydrat Rückverlagerung und dem SMY zu vermindern. Die Effizienz der Biomassenutzung hängt stark von der Nutzungsoption ab, wobei die direkte Nutzung von Biomethan als Kraftstoff und die Spitzenlast-Stromerzeugung hierbei hervorzuheben sind. Es wurde gezeigt, dass Miscanthus eine geeignete Pflanze für die Bereitstellung von nachhaltigerer erzeugter Biomasse für die wachsende europäische Bioökonomie ist, die zusätzliche Ökosystemdienstleistungen erbringt, z.B. Schutz des Grundwasser- und Oberflächengewässerschutz.

Chapter 1 - General introduction

1.1. Energy market in Europe and Germany

On 21 December 2015 at the COP21 in Paris, the United Nations agreed to keep the global temperature increase well below 2°C above pre-industrial levels until 2100 and also strengthen their efforts to keep it below 1.5°C. After achieving the required ratification threshold, the Paris Agreement entered into force on 4. November 2016. Both the European Union (EU) and Germany have ratified the Paris Agreement and are now obliged to reduce their greenhouse gas (GHG) emissions in the coming decades. To achieve the Agreement's goal, the EU announced that it would reduce its GHG emissions by 40% by 2030 and 80%-95% by 2050 compared to 1990 levels (European Commission 2018a). However, the policies currently in place are not sufficient to fulfil the EU's contribution to the Paris Agreement's temperature targets, since even if fully implemented, they are estimated to lead to emission reductions of only 60% by 2050 compared to 1990 levels (European Commission 2018a). Germany's contribution to achieving these goals comprises reducing its GHG emissions by 55% by 2030 and becoming net carbon neutral by 2050 (BMUB 2016). To meet these European and national targets, all economic sectors and the private sector need to review the GHG emissions caused by their activities and find solutions for their short-term reduction and long-term avoidance. In 2016, 78% of the EU-28's GHG emissions originated from energy use, including generation of electricity and heat and combustion of transport fuels (EEA 2018). This shows that the supply of energy still largely relies on fossil fuels, such as oil, coal and natural gas. In 2017, the total primary energy consumption in Europe was 1,969.5 million tonnes of oil equivalents (Mtoe), with carbon-intensive fuels accounting for 75.4% (fossil oil 37.1%, natural gas 23.2%, coal 15.1%), followed by nuclear energy (9.8%), renewables (8.2%) and hydro power (6.6%) (BP 2018). In the short and medium term, the reduction of GHG emissions from energy use will play a crucial role in meeting the EU's and Germany's reduction targets. However, to become a net carbon-neutral society by 2050, emissions from industrial processes, agriculture and waste management will also need to be reduced or avoided.

A reduction in GHG emissions from energy use can be achieved by switching to low-carbon energy carriers or increasing the energy use efficiency. The latter is an important tool to meet short term GHG reduction goals and avoid increased demand for fossil fuels. However, in the longer term its potential is limited because improvements in energy use efficiency alone cannot reduce emissions to zero. In the past, gains in energy efficiency were often overcompensated for by higher increase in overall energy demand. To meet the long-term goal of a carbon-neutral society, energy provision needs to be switched to low-carbon energy carriers, such as renewable sources and nuclear power. In 2015, nuclear power and renewable energy sources provided 13.6% and 13.0% of the EU-28's gross inland consumption, respectively (Figure 1b) (European Union 2017). However, the problem of radioactive waste disposal from nuclear power plants has still not been sufficiently resolved. In addition, the construction of new nuclear power plants is challenging and the costs are likely to be underestimated, as seen in the building of new nuclear power plants with the European Pressurised Reactor (EPR) design in Olkiluoto, Flamanville and Hinkley Point. Further, in Germany at least, the public's acceptance of nuclear power is low. For these reasons, only very few new nuclear power plants are currently planned or under construction in Europe, and as shown in Figure 1a, nuclear power production has not increased in the past two decades (European Union 2017). By contrast, the gross inland consumption of renewable energy sources increased continuously in the past two decades and more than doubled from 1998 to 2015 (European Union 2017). This indicates that renewable energy sources – hydro, wind, photovoltaic and bioenergy from biomass – are currently the most relevant low-carbon energy sources in Europe.

As can be seen in Figure 1b, biomass – including the biogenic fraction from waste – is the most important renewable energy source in Europe and accounted for 8.4% of the EU-28's total gross inland consumption in 2015, followed by hydro (1.8%) and wind (1.6%) (European Union 2017). The great advantage of biomass over hydro, wind and solar is, that it can be used for mobility, heating and electricity purposes and can be easily stored for several months to be used for the production of power and/or heat on demand. Biomass has a key role to play in the transition of the energy system in the coming decades as it can complement the production of electricity from fluctuating energy sources, such as wind and solar power, and provide a sustainable renewable biofuel for the mobility sector and household heating. The role of bioenergy as an energy source may then decline in the distant future, as developments in energy storage (including batteries) and electric vehicles advance and the use of renewable electricity for heating and cooling purposes increases. However, for the transition phase and to ensure service security, bioenergy will be indispensable in the next few decades. To achieve a net carbon neutral society by 2050, materials such as plastic also need to be derived from renewable sources and biomass is a key feedstock here. Biomass as a sustainable feedstock for the biobased industry and biobased products is facilitated by the European Bioeconomy Strategy (European Commission 2018b). The chemical industry requires sustainable carbon and biomass is expected to be one major feedstock for virgin plastic production by 2050 (Carus 2018). This suggests that the demand for biomass is likely to increase in the coming decades, first primarily for energy application and then towards the second half of the century primarily for material applications. However, consideration of sustainable land use is required to avoid negative impacts on global warming, biodiversity, nitrification and acidification of terrestrial and marine ecosystems by an increased use of biomass, whether energy crops or residues. In this context, this study intends to contribute to improve availability and sustainability of biomass production and provision as feedstock for the bioenergy sector and the bioeconomy.

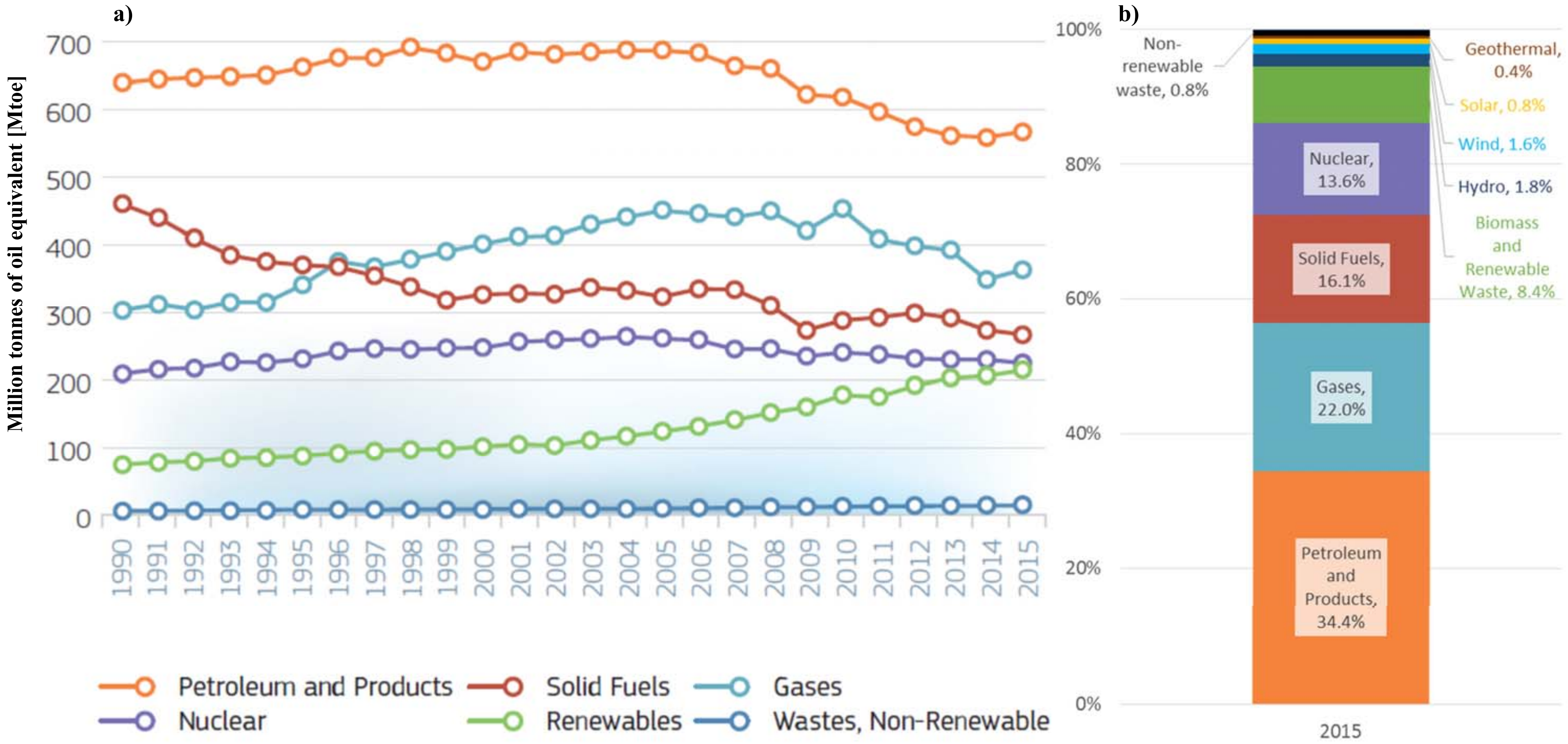


Figure 1 a) Energy supply in Europe - gross inland consumption in the EU-28 from 1990 until 2015 in million tonnes of oil equivalent (Mtoe) **b)** Proportions of individual fuels making up EU-28's gross inland consumption in 2015 (European Union 2017).

1.2. Biomass supply and energetic use

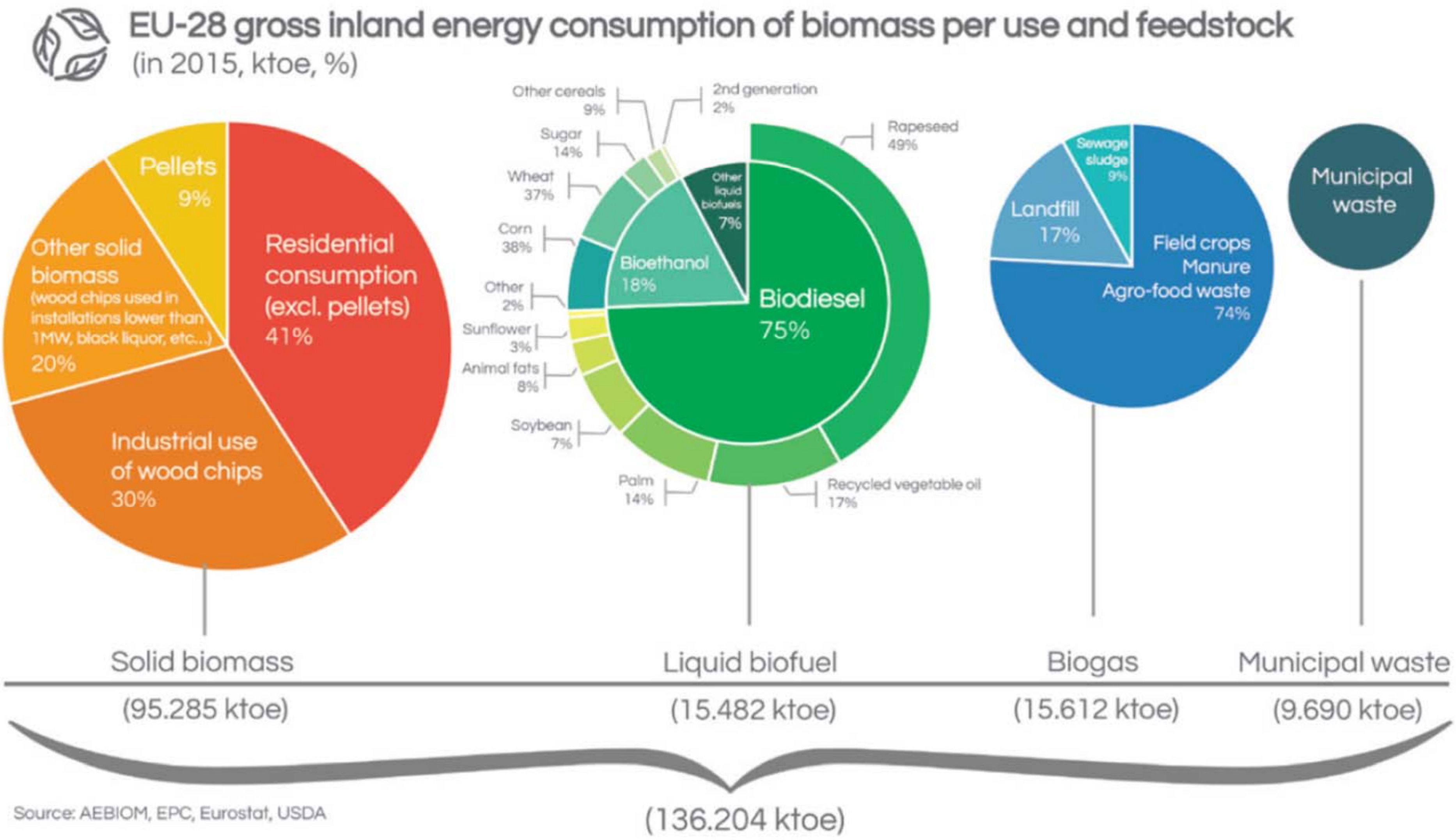
1.2.1. Current situation

Biomass encompasses all forms of agricultural, forestry and aquatic products and by-products. However, today's bioenergy systems utilize mainly terrestrial plant biomass from agriculture and forestry or residues from animal farming, crop production and forestry. The type of biomass utilized depends to a large extent on regional availability. In regions with a high proportion of forests, such as Scandinavia and parts of Central Europe, wood and forest products play a dominant role on the biomass and bioenergy market. In regions where arable farming predominates, biomass from energy crops and residues from crop production and farming are mainly used. As seen in Figure 2, solid biomass – for the most part wood and wood-based fuels – accounted for the largest share (95 Mtoe) of the gross inland energy consumption from biomass in the EU-28 in 2015 (Aebiom 2017). This is also reflected in the final energy consumption, where solid biomass provided 91% and 51% of the total biomass-based heat and electricity in the EU-28, respectively, followed by biogas with 4% and 34% (Aebiom 2017). As Figure 2 indicates, the raw material basis for liquid biofuels is quite versatile, with mainly oil-rich crops and residues being used for biodiesel production, and crops with a high starch and sugar content being used for bioethanol production (Aebiom 2017). In 2015, second-generation fuels from lignocellulosic feedstocks played a very minor role, but their importance is expected to increase considerably in the near future.

Within the EU-28, there are a number of different approaches to increasing the production of biomass-based electricity. While countries such as Germany and Italy are focusing on small- and medium-scale biogas plants, others, predominantly the UK, have put an emphasis on large-scale, wood-fired plants (Aebiom 2017). Germany is the most important biogas market with a proportion of more than 50% of the total biogas production in the EU-28 in 2015 (Aebiom 2017; Scarlat *et al.* 2018). In Germany, energy crops provided 48.9% by mass and manure 44.5% by mass of the raw material for biogas plants in 2016, with maize alone accounting for 69% of the total input mass from energy crops (FNR 2018). This illustrates both the high flexibility of biogas production in making use of different feedstocks, but at the same time its strong focus on maize as the most economic biogas crop.

1.2.2. What can biogas contribute?

In Europe, biomass is primarily utilized as solid fuel for heat and electricity production (Figure 2). However, biogas should not be seen as competition for other utilization options of solid biomass such as combustion, but can play an important complementary role in existing and future energy systems. Major advantages of biogas production are as follows:


EU-28 gross inland energy consumption of biomass per use and feedstock (in 2015, ktoe, %)

Source: AEBIOM, EPC, Eurostat, USDA

Figure 2 Composition of gross inland energy consumption from biomass in the EU-28 in 2015 in ktoe (kilo tonnes of oil equivalent) (Aebiom 2017)

- **Demand-driven electricity production**

Biogas production provides a storable and flexible resource for electricity provision with lower GHG emissions than fossil energy carriers and the average electricity mix in Germany (FNR 2018). Demand-driven electricity provision is becoming increasingly important with ongoing exploitation of intermittent renewable energy sources, such as wind and solar, and biogas is seen as a promising, cost-effective and renewable energy carrier for this purpose (Lauer and Thrän 2018). While in the past most biogas plants produced baseload power, they are increasingly being transformed to provide more demand-driven electricity production (FNR 2018). This can be achieved by increasing installed electric capacity, increasing gas storage capacity and adapted feeding management of the digester (Szarka *et al.* 2013; Thrän *et al.* 2015). Demand-driven operation of biogas plants can be a cost-effective technology to provide peak-load power with reduced GHG emissions compared to the fossil reference natural gas (Lauer and Thrän 2018; Lauer *et al.* 2017).

Another option for demand-driven electricity supply is the upgrading of biogas to biomethane and injection into the natural gas grid. The natural gas grid connects biogas plants with the conventional gas infrastructure, including peak-load gas power stations and large gas storage facilities. Gas storage facilities in Germany provide a gas storage capacity equivalent to 80 days' full supply (BMWI 2019). Injection of biomethane into the gas grid allows both long-term energy storage and flexible electricity provision using renewable biogas. Biogas plants can be also used to convert surplus electricity, e.g. if too much wind or solar power is available, into biomethane. For this purpose, the surplus electricity is used to produce hydrogen, which can react in a biotechnological process with the carbon dioxide (CO_2) from the biogas either in the biogas digester itself or in a separate reactor (Theuerl *et al.* 2019). Such a technology could be ideally combined with biomethane plants, because the connection to the gas grid would be advantageous and an increased methane content in the biogas would reduce the demand for upgrading by removing CO_2.

- **Flexible use options**

Biogas plants traditionally operate a combined heat and power unit (CHP unit) on-site to produce electricity and heat. The electricity is supplied to the grid and part of the heat is used to heat the digester. The remaining heat can be used for other purposes, if there is demand nearby. Although about 90% of biogas plants use or supply excess heat for other purposes, only 56% of the total heat available is utilized (Daniel-Gromke *et al.* 2018). Upgrading biogas to biomethane enables a diversification of use options. Biomethane can be injected into the natural gas grid and used according to demand for heat production or for combined power and heat production with a higher overall efficiency. Biomethane can be also compressed and used as a transport fuel. Utilization of biomethane could help to reduce the transport sector's GHG emissions in the short term (Börjesson and Mattiasson 2008).

- **Flexible feedstock resources and the closing of nutrient cycles**

A broad spectrum of feedstocks can be utilized in biogas plants including residues such as manure and straw, grassland, perennial and flowering crops or crop mixtures and even the organic fraction from municipal solid waste. However, the process technology needs to be adapted to the feedstocks, e.g. organic waste often contains impurities such as plastic (Theuerl *et al.* 2019). Unlocking the potential of residues is also often difficult, as sources tend to be decentralized and the quantities small (Theuerl *et al.* 2019). Therefore, future biogas plants will need to be able to process diverse feedstocks to allow combined utilization of residues and purposely grown energy crops, the latter mainly to secure sufficient feedstock supply. To increase both public acceptance and the overall environmental performance of the biogas sector, future energy crop production will also need to provide additional ecosystem services compared to the current state-of-the-Art. These include protection of water quality, provision of additional habitat for wildlife or nectar and pollen for flower-visiting insects. However, a high yield potential and thus area efficiency is important to minimize potential competition with other land use options, including food production. The utilization of marginal, low-quality or contaminated land can also help to avoid such competition. Perennial crops, such as cup plant and miscanthus, are promising candidate crops for the biogas sector (Mast *et al.* 2014; Mayer *et al.* 2014; Gansberger *et al.* 2015; Wahid *et al.* 2015).

For the future, biogas plants can also be regarded as a crucial component of biorefineries, including small on-farm refineries (Theuerl *et al.* 2019; Dahmen *et al.* 2018) where the biogas process can help make use of residues and provide process electricity and heat. The biogas process is also a key process in the circular economy, since the nutrients are recycled in the form of digestate back to the field where they are again available for the production of biomass to be fed into the biorefinery (Arthurson 2009). The digestate that remains after anaerobic digestion is a valuable fertilizer with a high content of plant-available nutrients (Möller and Stinner 2010; Möller and Müller 2012). Nutrient recovery from digestate may become feasible in future, potentially allowing valuable mineral fertilizers to be produced (Ehmann *et al.* 2019). In contrast, the ash resulting from combustion generally has very low nitrogen contents and a low plant availability of other nutrients, such as phosphorus (Bhattacharya and Chattopadhyay 2002).

- **Diversification of farmers' income and increased on-farm value creation**

The average electric capacity of the 8.700 biogas plants in Germany was approx. 500 kW_{el} in 2017 (FNR 2018). As many medium- and small-sized biogas plants are owned and operated by farmers, this offers them the potential to diversify their income and enhance value creation on the farm. In a growing bioeconomy sector in particular, it is important that farmers are not only suppliers of low-value biomass, but are also able to participate in economic success and achieve a higher value creation. This is crucial to ensure job creation in rural areas and along the entire value chain. Biogas production is already a good example of such value creation and on-farm biorefineries could build upon this experience or be linked to existing biogas plants.

1.2.3. What are the current problems in the biogas sector?

Development in the German biogas sector – which represents 50% of European biogas production – has largely been driven by subsidies via feed-in tariffs (Scarlat *et al.* 2018; Theuerl *et al.* 2019). This led to a sharp rise in the number of biogas plants up until 2012, when feed-in tariffs were still high and the use of energy crops, including maize, as input substrate also subsidized. This in turn resulted in a 76.6% increase in the maize cultivation area in Germany from 2000 to 2012, which can be largely attributed to the increased feedstock use in biogas plants (Theuerl *et al.* 2019). During this phase of rapid growth, environmental aspects – in particular those related to intensive maize cultivation – were not sufficiently considered, but increasingly became the subject of controversial debate among the general public. This led to more restrictive legislation and the Renewable Energy Act was amended several times from 2012 onwards, including a reduction of feed-in tariffs (Theuerl *et al.* 2019). This reduction made the building of new biogas plants that use energy crops uneconomic, because the feedstock costs for crop production where higher than the feed-in tariffs, thus slowing the further expansion of the sector (FNR 2018).

The biogas plants built up until 2012 mainly produced low-priced base load power and often did not make sufficient use of the excess heat, because the high feed-in tariffs still made them economical. Even though heat utilization has improved in recent years, still only 56% of the available excess heat was used in 2015 (Daniel-Gromke *et al.* 2018). In a future energy system, biogas needs to provide demand-driven power production and peak-load power has been identified as a cost-effective option to integrate a higher share of fluctuating renewable energy sources, such as wind and solar power, into the electricity grid (Lauer and Thrän 2018). However, for the future installation of biogas plants, environmental aspects of energy crop cultivation will need to be increasingly considered in order to reduce the overall environmental impact and achieve public consensus on biogas utilization. In the context of biogas crop production, intensive maize cultivation has received particular criticism due to a high nitrate leaching and erosion risk and potential negative impacts on biodiversity (Altieri 1999; Svoboda *et al.* 2013; Vogel *et al.* 2016). In 2014, the so-called ‘maize cap’ was introduced and since 2016 a maximum of 50% of maize and cereal grains is allowed as input substrate in new biogas plants (Theuerl *et al.* 2019; EEG 2017). This maize cap is set to gradually decrease to 44% for new plants built in 2021 (EEG 2017).

In contrast, perennial biomass crops, such as miscanthus, cup plant and highly diverse wild flower mixtures have a more environmentally benign profile and provide added value such as carbon sequestration, a very low nitrate leaching and erosion risk, and suitable habitat conditions for mammals, insects, spiders and birds (Gansberger *et al.* 2015; Emmerling 2014; McCalmont *et al.* 2015; Schorpp and Schrader 2016; Platen *et al.* 2017; Mol *et al.* 2018). For this reason, perennial biomass crops are seen as promising energy crops for future biogas plants (Theuerl *et al.* 2019). However, area efficiency is crucial to avoid increasing pressure on other land use options and indirect land use change. Amongst the aforementioned crops, miscanthus is considered to have the highest yield potential (Mast *et al.* 2014; Gansberger *et al.* 2015; Zub and Brancourt-Hulmel 2010; Cossel and Lewandowski 2016). Miscanthus has been assessed in Europe for more than 30 years, upscaling of miscanthus crop production has been improved in recent years and the establishment and cultivation of miscanthus is meanwhile well developed (Lewandowski *et al.* 2000; Clifton-Brown *et al.* 2016). Breeding activities are being conducted to develop seed-based hybrids with improved genetic performance (Clifton-Brown *et al.* 2019). Cup plant, which is well suitable for biogas production, and wild flower mixtures have only been assessed in the last few years and up until now fewer breeding activities have been undertaken than for miscanthus. For these reasons, this work focuses on miscanthus.

1.3. Miscanthus as a biomass crop

Miscanthus is a genus of perennial, rhizomatous C4 grasses mainly originating in South-East Asia. The genus miscanthus consists of 11-12 species, of which *Miscanthus sinensis* (Msin) and *Miscanthus sacchariflorus* (Msac) are receiving considerable interest as potential bioenergy crops in Europe (Clifton-Brown *et al.* 2008). *Miscanthus x giganteus* (Mxg), a natural, sterile hybrid of Msin and Msac, is currently still the single commercially available cultivar in Europe. Mxg was introduced into Europe in 1935 in Denmark for horticultural purposes (Clifton-Brown *et al.* 2015). In Europe, three breeding programmes to optimize miscanthus germplasm are currently underway at Aberystwyth University (UK), Wageningen University (NL) and INRA d'Estrées-Mons (FR) are currently ongoing to optimize miscanthus germplasm (Clifton-Brown *et al.* 2019). The introduction of novel miscanthus varieties is expected in the coming years (Clifton-Brown *et al.* 2019). Novel varieties show high potential for utilization on marginal lands due to improved stress tolerance compared to Mxg (Lewandowski *et al.* 2016). This study analyses the potential of Mxg and novel miscanthus hybrids for biogas production.

1.3.1. Crop production and yield

Commercially, *Miscanthus x giganteus* (Mxg) is often propagated clonally via rhizome propagation. Other clonal propagation methods, such as in vitro propagation, stem cuttings and collar propagation, are often too expensive or the technologies have not yet been established (Xue *et al.* 2015; Mangold *et al.* 2018). Novel miscanthus hybrids with fertile seeds also offer the potential of seed propagation, either to produce seed-based plantlets or in future maybe even direct sowing (Clifton-Brown *et al.* 2016; Xue *et al.* 2015). In addition to cost reduction, seed-based propagation allows faster upscaling due to very high multiplication rates of >1500 propagules per m^2 of mother field compared to 10-50 for rhizome propagation (Clifton-Brown *et al.* 2016). First attempts to develop direct sowing of miscanthus have been made, but this technology requires further improvements and has not yet been established in practice (Ashman *et al.* 2018). The mixed establishment of miscanthus rhizomes with maize has been tested with the aim to avoid a yield gap in the first year and to increase the attractiveness of miscanthus cultivation for farmers, but further improvements are required before implementation in practice (Cossel *et al.* 2019). Up until now, monoculture planting of rhizomes or plantlets (seed-based or in vitro) is state of the art for the establishment of new miscanthus fields.

Rhizomes and plantlets are planted in late spring (April or early May), typically when maize is sown. The planting time needs to take the risk of late frosts into account, which is higher for plantlets than for rhizomes, due to the protection of surrounding soil. For this reason, it is recommended to cover the plantlets with a transparent plastic mulch film directly after planting, which also minimizes the risk of plant losses through drying out (Clifton-Brown *et al.* 2016). Without film application plantlets need to be irrigated directly after planting and in the following two to four weeks to avoid drying out of the plantlets. Application of film increases early growth and plant development of miscanthus plantlets, but also stimulates volunteer weed growth, making sufficient chemical weed management necessary before film application (Clifton-Brown *et al.* 2016; O'Loughlin *et al.* 2017). Plastic film can also be used to accelerate early growth of rhizomes after planting, however the costs and benefits need to be weighed up (O'Loughlin *et al.* 2017). Rhizomes are generally planted by rhizome planters or modified potato planters, while plantlets are planted using conventional vegetable planting machines. In both cases, a high seedbed quality including a fine tilth is beneficial for establishment success. Planting density ranges from 10.000-40.000 plants and is influenced by site conditions and hybrid type. Intraspecific Msin x Msin hybrids often require a higher planting density than interspecific Msac x Msin hybrids, due to the smaller basal diameters of mature Msin plants. Low planting densities would leave too much space between the single Msin miscanthus plants and lead to an insufficient utilization of the total field area.

In the establishment phase after planting miscanthus is very sensitive to weed interference and suitable weed management is crucial. A lack of weed management can lead to a 97% reduction in biomass production in the first year and sequential application of pre- and post-emergence herbicides has proved to be most effective (Song *et al.* 2016). A number of pre- and post-emergence herbicides, mainly developed for other crops such as maize or cereals, are suitable for miscanthus and allow the competition of most weed species to be managed (Song *et al.* 2016; LfL 2014). If chemical weed management is not possible or not wanted, mechanical weed protection can be also performed, e.g. using a harrow or curry comb. Weed management in the first year is very important, since a poorly established crop often has a higher risk of overwinter losses, lower competitiveness against weeds in the subsequent years and may achieve maximum yield later than a well-established crop. The biomass grown in the first year is often not harvested but mulched in late winter or early spring, helping to suppress weeds in the second year. Should perennial weeds still persist in the second year, post-emergence herbicides can be applied again in spring. In the subsequent years, weed management is often no longer required due to the high competitiveness of miscanthus. Although some pests and diseases have been reported for miscanthus, including infection of rhizomes with *Fusarium spp.*, these have so far not occurred in established miscanthus crops and typically no insecticides or fungicides are applied (Thinggaard 1997; Anderson *et al.* 2014).

Miscanthus is a very nutrient-efficient crop, due to efficient nutrient relocation from the aerial biomass into the rhizomes and low nutrient offtake by the winter-harvested biomass (Lewandowski and Schmidt 2006; Cadoux *et al.* 2012). At a yield level of approx. 15 t DM ha^{-1}, the median nutrient offtake of post-winter-harvested miscanthus has been identified as 76, 6.8 and 95 kg ha^{-1} a^{-1} of nitrogen (N), phosphorous (P) and potassium (K), respectively (Cadoux *et al.* 2012). This is considerably lower than the nutrient offtake of biogas maize at a typical yield level of 15-17.5 t DM ha^{-1}, which is approx. 205 kg N ha^{-1} a^{-1}, 95 kg P ha^{-1} a^{-1} and 265 kg K ha^{-1} a^{-1} (Kramberger *et al.* 2009; Butz *et al.* 2013). However, an earlier miscanthus harvest affects nutrient relocation and could lead to higher nutrient offtake than for winter harvests.

Although nutrients are removed from the field with winter-harvested biomass, miscanthus is in practice often cultivated without any fertilization and there are contradicting studies regarding the effect of fertilization on miscanthus yield. Several studies have shown no significant effects of nitrogen fertilization on the biomass yield (Schwarz *et al.* 1994; Himken *et al.* 1997; Christian *et al.* 2008). Other studies have shown a significant yield response to nitrogen fertilization not only in young crops and in single years, but even over a period of 10 subsequent years (Ercoli *et al.* 1999; Xu *et al.* 2017). Clifton Brown *et al.* reported positive yield response to nitrogen fertilization in 5 of 6 years (Clifton-Brown *et al.* 2007), while Strullu *et al.* only observed a response for miscanthus harvested in October, but not for harvest after winter (Strullu *et al.* 2011). Based on these findings, it can be concluded that a slight nitrogen fertilization is required to maintain maximum yield levels in the longer term, depending on soil fertility, nutrient availability and nitrogen deposition from the air. However, plant endophytes could also play a crucial role for the nitrogen supply of miscanthus. Symbiotic endophytes have been detected in miscanthus and could supply nutrients to unfertilized plants by biological nitrogen fixation from the air (Kirchhof *et al.* 2001; Rothballer *et al.* 2008; Davis *et al.* 2010). Nitrogen deposition from the air and symbiotic endophytes could be an explanation for the contradicting literature reports of fertilization effects described above.

Miscanthus is harvested annually in late winter or early spring using conventional agricultural machinery, starting with the biomass grown during the second vegetation period. The harvest time, location and hybrid type can influence the moisture content of the biomass, since late harvest, frost periods and early senescing hybrids promote drying of the crop. If the moisture content is suitable for safe storage (generally below 20%), the standing crop can be harvested directly, e.g. using a field chopper or a mower-baler combination. With higher moisture contents, swath mowing followed by wilting and baling of the dry biomass is recommended.

The estimated productive life time of miscanthus is 20-25 years (Lewandowski *et al.* 2003). Long-term trials have not shown a steady yield decline even after more than 20 years, indicating a productive lifetime of 20+ years (Xu *et al.* 2017; Larsen *et al.* 2014; Gauder *et al.* 2012). However, this could be influenced by genotype, management (fertilization, harvest time) and site conditions. Fertilization could help to maintain long-term productivity and extend the productive lifetime of miscanthus (Xu *et al.* 2017). Harvestable biomass yield at spring harvest is reported in the range of 7-30 t DM ha^{-1} a^{-1} for *Miscanthus x giganteus* (Mxg) in Europe (Anderson *et al.* 2014). This large variability can be largely explained by climatic conditions (Jones *et al.* 2016). A 12-year long-term trial revealed an average yield potential of 18.3 t DM ha^{-1} a^{-1} for Mxg with a fertilization of 80 kg N ha^{-1} in south-west Germany, which was comparable to the yield of biogas maize grown in the same trial with a fertilization of 240 kg N ha^{-1} (Xu *et al.* 2017). Such a yield potential seems to be a reasonable average for Mxg on highly productive sites in Central Europe. On a nearby site with lower productivity due to more shallow soil, a 14-year trial reveal an average yield potential of 14.1 t DM ha^{-1} a^{-1} for Mxg (Gauder *et al.* 2012). Long-term trials over 15 years in Ireland and Denmark identified an average yield potential for Mxg of 9.0 and 9.7 t DM ha^{-1} a^{-1}, respectively (Clifton-Brown *et al.* 2007; Larsen *et al.* 2014). This yield potential could be classified as a reasonable average yield for Northern European climatic conditions. In Southern Europe, miscanthus shows a very high yield potential of even more than 30 t DM ha^{-1} a^{-1} under conditions without water limitation (Clifton-Brown *et al.* 2001). However, since in this climatic conditions water is often the limiting factor, reported yields are often much lower, e.g. a rainfed long-term trial in Greece reported an average yield of 13.3 t DM ha^{-1} a^{-1} for Mxg (Alexopoulou *et al.* 2015). With ongoing breeding activities, novel miscanthus germplasm is being produced which can achieve yields in a similar range as Mxg (Clifton-Brown *et al.* 2019). Germplasm with improved stress tolerances has the potential to outcompete Mxg on marginal land which often includes limited growth conditions (Lewandowski *et al.* 2016; Kalinina *et al.* 2017).

1.3.2. Ecological benefits of miscanthus cultivation

Low input character and groundwater protection

Soil cultivation is only needed before establishment of the crop and after that the soil is left untilled during the whole cultivation period of more than 20 years. Due to the perennial nature of the crop, cultivation of perennial miscanthus is less intensive compared to annual crops as maize. After successful miscanthus establishment, field operations are largely limited to harvest and biomass collection. Due to low nutrient requirements, miscanthus is commercially often cultivated without any fertilization (McCalmont *et al.* 2015). Herbicides are often only required for establishment in the first twelve months and later on the competitiveness of the crop is sufficient to suppress weeds (McCalmont *et al.* 2015). The low input character and high yield potential of miscanthus makes it a very energy efficient crop (Lewandowski and Schmidt 2006). In annual cropping systems, including maize, soil cultivation and chemical crop protection is mostly performed annually and for this reason more often than in miscanthus. A comparative 12-year trial revealed an 11% higher accumulated gross energy yield of miscanthus (80 kg N ha^{-1}) compared to annual maize (240 kg N ha^{-1}) under the same growing conditions, with the tendency to further increase in the coming years (Xu *et al.* 2017). This means an advantage for both the farmers, due to lower annual costs, and the environment, due to lower energy consumption and pesticide use during the cultivation period.

Miscanthus is a very nutrient and water use efficient crop (Lewandowski and Schmidt 2006; Clifton-Brown and Lewandowski 2000; van Loocke *et al.* 2012). The removal of macronutrients, especially nitrogen, is comparatively low compared to other crops, due to its efficient nutrient recycling system (Cadoux *et al.* 2012; Strullu *et al.* 2011). Two pathways for nutrient recycling are known: 1st Translocation from aboveground biomass to the rhizome and 2nd Relocation of nutrients to the soil by leaf fall over winter (Christian *et al.* 2006). Due to this low nutrient removal, the recommended fertilization of miscanthus is rather low compared to annual crops and in practice miscanthus is often cultivated without any fertilization over multiple years. Fully established miscanthus crop shows low nitrate leaching losses even if nitrogen fertilizer is applied (Christian and Riche 1998), this makes miscanthus a very suitable crop for water sensitive and protection areas. This ecosystem service is especially interesting for sites with high leaching risks, e.g. sandy soils, water protection areas in general or regions, where the Nitrate Directive threshold value of 50 mg nitrate l^{-1} in groundwater is exceeded (Council of the European Communities 1991; European Commission 2018c). Green-harvested miscanthus crops might partially lose the ability to recycle nutrients, leading to a higher nutrient offtake and for this reason a higher fertilizer demand (Cadoux *et al.* 2012; Strullu *et al.* 2011). However, nitrogen removal at maximum biomass yield is still below the typical nitrogen removal of a biogas maize crop and may decline further by delaying the harvest to October (Cadoux *et al.* 2012). The typical nutrient removal of green-harvested miscanthus needs to be further researched.

Stress tolerance and water use efficiency

Stress tolerance and efficient utilization of limited resources, especially water, are important traits due to expected increasing risks for stress conditions caused by climate change and for utilization of marginal land (Richards *et al.* 2014; IPCC 2018). Marginal land is characterized by biophysical and agroeconomic constraints (Richards *et al.* 2014). While the latter, including for example field accessibility and field shape, often do not impact the crop growth directly, biophysical constraints, such as high sand content, shallow sites, waterlogged soils and depleted soils with very low soil organic carbon and nutrient availability, usually directly impact the crop growth and by that the agroeconomic crop performance.

Miscanthus is a very water efficient crop and can even produce more biomass with the same amount of water than maize (van Loocke *et al.* 2012). As a perennial crop, no soil cultivation is needed once the crop is established and a mulch layer is covering the top soil, which helps to avoid evaporation of soil humidity and facilitates water infiltration during rain events (Blevins *et al.* 1971).

Compared to annual crops miscanthus establishes a deeper rooting system, which allows to access water in deeper soil layers (Neukirchen *et al.* 1999). The high water use efficiency, the deep rooting system and the reduced evaporation support drought tolerance and make miscanthus very attractive for efficient utilization of marginal land (Lewandowski *et al.* 2016). Utilization of marginal land thus helps to avoid competition with food and feed production. At the same time, soil organic carbon content often benefits from miscanthus cultivation, which can positively influence soil fertility (McCalmont *et al.* 2015). In the long term, miscanthus could thus contribute to increasing the fertility of low-productive, marginal sites and allow for economic arable crop production again.

Breeding of miscanthus aims to increase stress tolerance and yield resilience of novel miscanthus hybrids and displayed in first trials up to 30% higher yields under drought conditions than conventional *Miscanthus x giganteus* (Clifton-Brown *et al.* 2019). Important mechanisms were identified inducing increased salt tolerance of miscanthus germplasm, including Na+ exclusion mechanisms, osmotic stress tolerance and tissue tolerance (Chen *et al.* 2017). The broad genetic diversity allows for further adaptation of miscanthus to stress conditions by breeding and shows the high potential for utilization of marginal land with increased stress conditions (Clifton-Brown *et al.* 2019; Lewandowski *et al.* 2016).

Marginal land with biophysical constraints also includes contaminated areas, which are unsuitable for food or feed production, due to contamination of the soil and potential contamination of the crop. Especially on contaminated sites, the non-food crop miscanthus has two major advantages: 1) avoid introduction of contaminants into the food chain, while ensuring income for the farmer, 2) phytostablisation of the contaminant in the soil, by minimizing transportation of the contaminant through wind or water erosion (Rusinowski *et al.* 2019b). New miscanthus hybrids showed almost 40% lower contents of heavy metals in the aboveground biomass than *Miscanthus x giganteus*, which could simplify handling and utilization due to contamination potentially below threshold levels (Rusinowski *et al.* 2019a). Miscanthus was also identified as a suitable utilization option for military damaged sites (Pidlisnyuk *et al.* 2019).

While newly planted miscanthus shows a certain erosion risk, established miscanthus has a very low erosion risk, since the soil is not cultivated anymore and the leaf-fall over winter is forming a mulch layer preventing erosion (Cosentino *et al.* 2015). For these reasons, miscanthus was identified as a suitable crop for erosion control and soil stabilization on arable fields with a slope and productive use of eroded soils (Cosentino *et al.* 2015; Yost *et al.* 2017). Barrier strips alongside the slope and alongside waterbodies displayed to be an effective measure to reduce erosion from uphill annual, arable crops and to protect water quality (Ritchie *et al.* 1997; Ferrarini *et al.* 2017a). Due to increasing restrictions on pesticide and fertilizer use, the area alongside water bodies (e.g. 5m strips) can be also classified as marginal land due to agroeconomic constraints and miscanthus could be an attractive alternative. Establishment of miscanthus on such sensitive areas and in the landscape, is likely to have implications on biodiversity. These are introduced in the following paragraph.

Contribution to biodiversity aspects and potential role in the ecosystem

Biodiversity is rather complex to investigate, since it is not only influenced by the crop itself, but at least some indicators are also strongly influenced by the interaction of the crop with the surrounding landscape and can differ between regions. In this work, miscanthus is intended to replace partly maize for anaerobic digestion, for this reason mainly studies comparing biodiversity aspects of miscanthus and annual arable crops are used for comparison. In Germany, miscanthus is considered as crop for arable land and establishment on non-arable land, e.g. grasslands, needs permission and compensatory measures. Biodiversity impacts of replacing grassland by miscanthus might be completely different, depending on the species-richness of the grassland. Also the establishment of miscanthus on fallow land might have other implications on biodiversity compared to establishment on arable land, depending on the species-richness of that specific site. Sites with high species-richness should be neither converted into miscanthus nor in any other crop cultivation area to avoid negative impacts on biodiversity. For these reasons, this work is considering only biodiversity effects of miscanthus establishment on arable land.

The extensive crop production and especially the avoidance of soil cultivation offers the potential to promote biodiversity and species richness. In several studies, higher species richness was observed in miscanthus for ground invertebrates, spiders, beetles and earthworms than in annual crops (Bellamy *et al.* 2009; Dauber *et al.* 2010; Felten and Emmerling 2011; Haughton *et al.* 2016; Schrama *et al.* 2016; Emmerling and Pude 2017). Perennial nature of miscanthus and availability of biomass are potential drivers for the higher species-richness in soil life, even though C/N ratio and particle size of miscanthus litter is not ideally suited for earthworm feeding (Felten and Emmerling 2011). Positive impact on biotic soil health, including soil enzyme activity and microbial diversity compared to annual crops were reported by Cattaneo *et al.* (Cattaneo *et al.* 2014), while Schrama *et al.* found almost no differences in soil fungal and bacterial biomass comparing maize and miscanthus (Schrama *et al.* 2016). Species-richness and abundance of ground flora strongly depends on crop management and the plantation establishment success, patchy miscanthus fields provided room for a higher diversity than well-established crops (Bellamy *et al.* 2009; Dauber *et al.* 2010; Haughton *et al.* 2016; Semere and Slater 2007). Since miscanthus provides no feed sources for pollinators, abundance of pollinators depends on presence of flowering non-crop species, which is a clear trade-off between productivity and biodiversity. Abundance of non-crop species will be rather low in well-established crops, but is reported to be higher than in annual arable crops (Dauber *et al.* 2010; Semere and Slater 2007). However with ongoing improvements in crop establishment, room for non-crop plants might decline in future. Miscanthus provides shelter for small mammals during the breeding period and over winter, however abundance was higher within the field margins and boundaries than in the field (Dauber *et al.* 2010; Semere and Slater 2007). Contradictory results are published about biodiversity of birds in miscanthus and can be summarized as follows: 1. Patchy fields and young crops enhance diversity due to higher habitat variability and abundance of non-crop plants which provide feed, e.g. seeds or insects; 2. Higher abundance within field margins and boundaries than in the field; 3. Open ground birds, such as field lark (*Alauda arvensis*), are mainly found in late spring/early summer, while woodland species are mainly found in the taller crop later in the year (Bellamy *et al.* 2009; Dauber *et al.* 2010; Semere and Slater 2007; Sage *et al.* 2010; Immerzeel *et al.* 2014). Compared to arable crops, farm- and woodland species could benefit from large-scale miscanthus establishment, but habitat for open-ground birds could be lost leading to an overall neutral effect of miscanthus on bird diversity (Emmerling and Pude 2017; Sage *et al.* 2010).

The higher abundance of mammals and birds in the field margins and boundaries shows the high impact of the field size and the implementation of a miscanthus plantation on the biodiversity and ecosystem services. To maximize ecosystem services, strip establishment of miscanthus would be suitable and could replace habitat and buffer functions of hedges and trees, which were often removed in the course of rationalization of agriculture (Emmerling and Pude 2017; Baum *et al.* 2012; Ferrarini *et al.* 2017b). Economic optimization including large field sizes or a regional concentration e.g. around biorefineries might reduce ecosystem services and lead to neutral or even negative impacts on biodiversity aspects and especially open-landscape birds or pollinators could be negatively affected.

1.4. Motivation and objectives

As shown above, miscanthus has the potential to supply sustainably produced biomass, while providing additional ecosystem services. For this reason, it would make sense to replace annual biomass crops by miscanthus and increase miscanthus utilization. Miscanthus is a multi-purpose crop, which can be used for several applications, including heat and power, biofuels and material applications (Lewandowski *et al.* 2016; Lewandowski 2016). However, in Europe miscanthus is still only cultivated on approx. 20.000 ha, mainly in UK, France, Germany and Poland (Lewandowski 2016). On a larger scale, miscanthus biomass is used as feedstock for power plants in UK. Smaller scale applications include heating (single homes up to local heat grids), animal bedding and mulch material. The most important barrier for increased utilization of miscanthus is the missing market for the biomass, which can be described as a chicken-and-egg problem (McCalmont *et al.* 2015). For any large-scale application, industry needs a secure and predictable supply of substantial biomass quantities to be able to justify investments into biomass conversion plants. Farmers on the other hand are only willing to invest into the crop, if there is a mature market to sell the biomass on. To overcome this barrier, industry could closely cooperate with farmers and famer's cooperatives, which might be difficult for industry due to missing contacts, or mature markets for the biomass need to be addressed.

The biogas sector, especially in Germany, is a mature market for biomass and strongly connected to agricultural sector and farmers. In 2017, approx. 900.000 ha maize and 474.000 Mio ha other crops were cultivated only in Germany for biogas production (FNR 2018). Assuming an average fresh yield of 50 t maize per ha, the market only for biogas maize is about 45 million tons in Germany. Establishing miscanthus as a biogas crop could be a win-win situation by improving the sustainability and environmental performance of the biogas sector and achieving a “critical mass” of miscanthus cultivation in agriculture to raise interest of other biomass users.

Currently, miscanthus is not used for biogas production, since miscanthus harvest is generally performed after winter when the biomass is dry. The dry miscanthus straw is not suitable for wet fermentation biogas plants, due to high risk of forming floating layers. Further, the substrate-specific biogas yield of dry harvested miscanthus is low and a pre-treatment is required to increase methane production (Klimiuk *et al.* 2010; Menardo *et al.* 2013). Pre-treatment units are often not available at conventional biogas plants. Preponing the harvest time before winter showed the potential to improve the substrate-specific methane yield (SMY) (Mayer *et al.* 2014; Wahid *et al.* 2015; Baldini *et al.* 2017). The green-harvested miscanthus showed high yield potentials of 4000-6000 m^3 methane per ha, which is only slightly lower than that of maize (Mayer *et al.* 2014; Baldini *et al.* 2017). While these studies showed the high potential and improved suitability of green-harvested miscanthus for utilization in biogas plants, concerns were raised over negative effects of a green harvest on the crop and yield in the subsequent year (Fritz and Formowitz 2010). In this case, the green harvest in August led to a significant yield depression in the following year and miscanthus was therefore considered as unsuitable for biogas production (Fritz and Formowitz 2010). However, only one harvest time in August and no fertilization was tested in the aforementioned study, which could both influence the regrowth in the year after a green harvest has been performed.

The research performed for this study aims to gain knowledge beyond the status quo, to identify the suitability, potential and sustainability of miscanthus for the biogas sector, and to develop miscanthus as a crop for the bioeconomy. To achieve these overarching aims, **three major objectives** were defined and associated research questions derived for each objective:

1. Assess and improve the suitability of miscanthus for biogas production:
    a. How does the harvest time impact biomass yield, SMY and methane yield per hectare of Mxg and novel miscanthus hybrids?
    b. Can miscanthus be cut under a green-harvest regime without yield depression in the following year?
    c. Which mechanisms influence the crop’s ability to tolerate a green cut without yield depression the following year and can this be influenced by crop management?
2. Assess the environmental performance of miscanthus for biogas production:
    a. Could miscanthus improve the environmental performance of biogas production in comparison with maize?
    b. How does the environmental performance of miscanthus compare with that of other perennial crops?

3. Compare biogas production with other potential utilization options:
    a. How does the harvest time x energy conversion route affect the energy yield?
    b. Is the energy yield of biogas production comparable to that of combustion?
    c. How does the cell-wall composition influence the suitability of miscanthus for different utilization pathways?

Objective 1 is addressed in **Chapter 2** “Miscanthus as biogas substrate – cutting tolerance and potential for anaerobic digestion” and **Chapter 4** “Site-Specific Management of Miscanthus Genotypes for Combustion and Anaerobic Digestion: A Comparison of Energy Yield”. “Cutting tolerance” is defined here as the ability of the crop to tolerate a green cut without a yield depression in the following year and is used synonymously with “green-cut tolerance”.

Objective 2 is addressed in **Chapter 3** “Environmental Performance of Miscanthus, Switchgrass and Maize: Can C4 Perennials Increase the Sustainability of Biogas Production?”.

Objective 3 is addressed in **Chapter 4** “Site-Specific Management of Miscanthus Genotypes for Combustion and Anaerobic Digestion: A Comparison of Energy Yield” and **Chapter 5** “Evaluation of Miscanthus sinensis biomass quality as feedstock for conversion into different bioenergy products”.

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Chapter 2 - Miscanthus as biogas substrate – cutting tolerance and potential for anaerobic digestion

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Miscanthus as biogas substrate – cutting tolerance and potential for anaerobic digestion

ANDREAS KIESEL and IRIS LEWANDOWSKI
Department of Biobased Products and Energy Crops, Institute of Crop Science, University of Hohenheim, Stuttgart, Germany

Abstract

In the anaerobic digestion and biogas industry in Germany, the step of energy crop production accounts for a high proportion of the greenhouse gas emissions and environmental impacts. Replacing annual energy crops, for example maize, by perennial biomass crops such as miscanthus offers the potential to increase the sustainability of biogas crop production. However, the cutting tolerance of miscanthus and the mechanisms influencing it need to be investigated to assess its potential as a biogas crop. For this purpose, a field trial with different harvest regimes was conducted to identify the potential methane yield and cutting tolerance of *Miscanthus x giganteus*. Several fertilization regimes were tested under nitrogen-limited conditions in a pot trial to investigate the mechanisms behind the cutting tolerance. The refilling of carbohydrate (starch) stores in the rhizome was identified as a very important factor influencing the cutting tolerance of miscanthus, whereas the nutrient relocation appeared to be of less importance. The field trial revealed that *Miscanthus x giganteus* offers a very high methane yield potential of approx. 6000 m^3 ha^{-1} when harvested in October, which is within the range of the methane hectare yield of energy maize. The substrate-specific methane yield of *Miscanthus x giganteus* biomass decreased with later harvest dates and reached 247 ml (g oDM)$^{-1}$ in October. This harvest date delivered very high, stable yields of on average 26 t DM ha^{-1} over two years and enabled a good cutting tolerance. Green harvest in October was identified to be suitable for *Miscanthus x giganteus* and is recommended for biogas utilization. In conclusion, the perennial biomass crop *Miscanthus x giganteus* is a very promising biogas crop and offers the potential to increase the sustainability of the anaerobic digestion sector in Germany by replacing a substantial area of biogas maize cultivation.

Keywords: anaerobic digestion, biogas, carbohydrates, cutting tolerance, energy crop, green cut, *Miscanthus x giganteus* perennial, relocation of nutrients

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Introduction

In Germany, almost 8.000 biogas plants with a total installed electric capacity of 3.8 GW were under operation in 2014 (FNR 2014). At the same time, 1.27 Mha or 10.5% of the total arable land in Germany (11.9 Mha) was used for the cultivation of energy crops for biogas production (FNR 2014; Statistisches Bundesamt 2014). Maize is the most important biogas energy crop making an input proportion of 73% of crop-derived biomass (FNR 2014). This is criticized because maize cultivation can be characterized as intensive due to high fertilizer demands often combined with intensive soil cultivation. Also the environmental impact can be high, due to high erosion and nitrate leaching risk and negative impacts on biodiversity caused by pesticide use when monoculture of maize prevails (Altieri, 1999; Svoboda *et al.*, 2013; Vogel *et al.*, 2016).

When considering the entire biogas value chain, the step of energy crop cultivation accounts for a high share of the environmental impact and greenhouse gas emissions (Lijó *et al.*, 2014; Pacetti *et al.*, 2015). Perennial energy crops offer the potential to reduce the environmental impacts of crop production and thereby increase the sustainability of the biogas sector. Various perennial biogas crops are currently being researched as biogas substrates, including cup plant (*Silphium perfoliatum* L.), szarvasi (*Elymus elongatus* ssp. *ponticus* cv. Szarvasi-1), energy dock (*Rumex schavnat*) and giant knotweed (*Falopia sachalinensis* var. Igniscum). However, Mast *et al.* (2014) revealed that the overall and substrate-specific methane yield of such novel energy crops is lower than for energy maize. A lower methane yield per hectare means that a larger cultivation area is required which consequently could lead to increased competition with land for food production and biodiversity conservation. To avoid such negative effects, alternative biogas crops should ideally be higher yielding than maize, should have a better environmental profile and be able to be

Correspondence: Andreas Kiesel, tel. +49 711 459 22379, fax +49 711 459 22297, e-mail: akiesel@uni-hohenheim.de

grown under conditions that are marginal for food production.

Miscanthus is a rhizomatus, perennial C4 grass species, which originates from South-East Asia. The sterile clone *Miscanthus x giganteus* is a high-yielding genotype, which is currently the standard cultivar in commercial utilization. This high yield potential has led to miscanthus being identified as a promising energy crop in several studies (Lewandowski *et al.*, 2000; Clifton-Brown *et al.*, 2004). As its fertilizer and pesticide requirements are low, miscanthus can be also characterized as a low-input crop (Lewandowski & Schmidt, 2006). *Miscanthus x giganteus* has a good environmental profile with the potential to increase soil carbon, soil fertility and biodiversity and to reduce nutrient run-off and leaching (McCalmont *et al.*, 2015). Despite these benefits, miscanthus cultivation and the utilization of its biomass are still not widespread in Europe [approx. 38.300 ha in Europe (Elbersen *et al.*, 2012)]. Here, the biomass is mainly used for the low-value application of combustion, mostly for heat generation and therefore harvested in late winter or spring. Water content and concentration of critical elements are the major determinants of the combustion quality of biomass. For miscanthus, both are positively influenced by delaying the harvest to late winter or spring, which is however accompanied by biomass losses (Iqbal & Lewandowski, 2014). The low relevance of miscanthus production in Germany, and in Europe in general, has been described by McCalmont *et al.* (2015) as a 'chicken-and-egg' problem. There is no significant market for miscanthus biomass in Europe, and biomass production costs for the low-value application of heat production are still too high. Opening up the biogas sector as a new market for miscanthus biomass could encourage the introduction of this environmentally beneficial crop into European agriculture and thereby help reduce the ecological burden of biogas production.

Miscanthus is currently not used for biogas production on account of the low suitability of the winter-/spring-harvested biomass, which is characterized by high lignin and low water contents. A green cut increases both yield and suitability of the biomass as biogas substrate. However, harvesting miscanthus when it is still green is not recommended by Fritz & Formowitz (2010) as it negatively impacts biomass yields in the following years. Later studies had contradictory findings. Some, such as Mayer *et al.* (2014) and Wahid *et al.* (2015), consider green-cut miscanthus to be the most promising future biogas crop. Wahid *et al.* (2015) identified September to October as the ideal harvest time for miscanthus when its biomass is to be used for biogas production. However, neither of these studies looked into the cutting tolerance of miscanthus. Cutting tolerance has been defined by Kiesel & Lewandowski (2014) in this context as the ability of a crop to recover from an early green harvest without yield reductions in the following year. Miscanthus recycles a large proportion of nutrients from the aboveground biomass to the rhizomes during senescence in autumn and reuses them for the production of new shoots in spring (Lewandowski *et al.*, 2003). The prevention of this nutrient relocation could be one explanation for yield losses in miscanthus in the year following a green cut, but the mechanisms influencing the cutting tolerance are still not clear and need to be explored.

The objectives of this study were to investigate the mechanisms determining the cutting tolerance of miscanthus, to identify green-cut regimes suitable for *Miscanthus x giganteus* and to quantify the biogas yield derived from these. For this purpose, a field trial with *Miscanthus x giganteus* and a pot trial with a novel *Miscanthus sacchariflorus* genotype (OPM 19) were performed. OPM 19 indicates that the *Miscanthus sacchariflorus* genotype was genotype number 19 of the OPTIMISC (EU FP7 No. 289159) genotype set. The field trial was used to analyse the cutting tolerance of *Miscanthus x giganteus* with three different green-cut regimes and two nitrogen levels and to measure the effects on dry matter (DM) and specific biogas yield. In the pot trial, the response of miscanthus to different nitrogen levels and application dates was tested under nitrogen-limited conditions. Rhizome weight and starch production were measured to help understand the mechanisms behind cutting tolerance.

Materials and methods

Field trial

The cutting tolerance field trial was performed using a *Miscanthus x giganteus* stand established in 2008 at the research station 'Ihinger Hof' in south-west Germany (48.7° latitude, 8.9° longitude, approx. 480 m a.s.l.). The soil is classified as Haplic Luvisol with a silty clay texture and an overlay of loess loam. The site is characterized by a long-term average annual air temperature and precipitation of 8.3 °C and 689 mm, respectively. The climate data relevant for the field trial (2012–2015) are shown in Table 1 on a monthly basis. As miscanthus is a perennial crop, the 2012 data are included to show that the year preceding the cutting tolerance trial was within the range of average conditions. The original planting density was three rhizomes m^{-2}, and weeding was performed during the establishment period only. The crop was fertilized annually from 2010 onwards with 80 kg N ha^{-1} a^{-1} of stabilized ammonium nitrate fertilizer ENTEC® 26 (EuroChem Agro GmbH, Mannheim, Germany). Harvests were conducted in spring, as practised in commercial miscanthus cultivation.

The cutting tolerance field trial was set up in 2013 in the mature miscanthus crop described above as a randomized

Table 1 Climate data on monthly basis at research station Ihinger Hof for years 2012–2015. Average air temperature was measured 2 m above soil surface

	Average air temperature (°C)				Precipitation (mm)			
Month	2012	2013	2014	2015	2012	2013	2014	2015
January	2.0	0.5	3.2	1.9	56.1	21.6	36.5	76.5
February	−3.5	−1.6	4.1	−0.2	8.4	54.6	43.8	13.2
March	7.3	1.4	7.2	5.4	7.7	31.0	8.4	34.3
April	8.1	8.4	10.9	9.0	42.3	60.7	49.9	31.9
May	14.3	10.8	12.1	13.0	43.1	138.6	68.2	67.7
June	16.3	15.8	16.7	16.5	116.5	82.8	24.3	75.2
July	17.3	19.8	18.4	20.8	96.0	173.4	162.0	28.9
August	19.2	17.5	15.7	20.0	39.3	69.5	142.4	75.0
September	14.0	13.7	14.6	12.6	57.2	97	77.0	36.0
October	8.8	10.9	12.1	8.4	58.3	87.1	50.1	16.0
November	5.5	4.0	6.7	7.2	133.7	60.3	59.0	69.5
December	1.8	3.0	2.8	NA	68.1	46.3	41.7	NA
Average	9.3	8.7	10.4	10.4*	NA	NA	NA	NA
Sum	NA	NA	NA	NA	726.7	922.9	763.3	524.2†

NA, not assessed.
*Preliminary average, no data from December included.
†Preliminary sum, no data from December included.

block design with three replicates. The plot size was 9 m^2. The central 4 m^2 were harvested for yield estimation. The variants included three green-harvest regimes, two nitrogen (N) fertilization levels and a winter control (Table 2). The green-harvest regimes comprised one double-cut (July/October) and two single-cut regimes, one early (August) and one late (October). Each green-harvest regime was tested at a lower (80 kg N ha^{-1} a^{-1}) and higher (140 kg N ha^{-1} a^{-1}) nitrogen fertilization level. The winter control was only fertilized with 80 kg N ha^{-1} a^{-1}. The fertilizer used was also ENTEC® 26. The 2013 N fertilization was split into two applications: 80 kg N ha^{-1} on 22 April 2013 and 60 kg N ha^{-1} on 10 June 2013 for the plots with the higher fertilization level. In 2014, the total amount was given in one application on 10 April 2014. During the course of the field trial, the mineral content of the soil (P, K and Mg) was monitored for each plot to avoid negative effects due to nutrient limitation. The plant-available mineral supply was found to be sufficient, and therefore, no mineral fertilizer other than nitrogen was applied. The mineral nitrogen content of the soil was measured after the last green cut each year. As only very low values were detected (on average 4.4 kg N ha^{-1} in 2013 and 3.6 kg N ha^{-1} in 2014), these were neglected in the calculation of nitrogen fertilization for the following year.

The crop was harvested using a sickle bar mower at a cutting height of approx. 5 cm. The border of each plot was removed, and the central 4 m^2 were collected and weighed. In literature, a minimum sampling area of 3 m^2 is recommended (Knörzer *et al.*, 2013). A subsample of approx. 1 kg was taken and dried at 60 °C in a drying cabinet to constant weight to establish the dry matter (DM) content. The DM yield was calculated based on the fresh matter (FM) yield and the DM content. The dried subsample was milled in a cutting mill SM 200 (Retsch, Haan) using a 1-mm sieve for chemical and biogas analyses. An aliquot of five shoots was used to establish the average dry weight per shoot and the leaf-to-stem ratio. For this purpose, the five shoots were sepa-

Table 2 Experimental treatments of the cutting tolerance field trial

No.	Harvest regime	Fertilization (kg N ha^{-1})	Harvest date Year 1 (2013)	Harvest date Year 2 (2014)
1	Double cut	80	1st cut: 18.07.13	1st cut: 28.07.14
2		140	2nd cut: 24.10.13	2nd cut: 23.10.14
3	Early single cut	80	29.08.13	28.08.14
4		140		
5	Late single cut	80	24.10.13	23.10.14
6		140		
7	Winter control	80	20.02.14*	09.03.15†

*Biomass from growing season 2013.
†Biomass from growing season 2014.

rated into leaf and stem biomass, dried at 60 °C and weighed. The leaf sheath was counted as stem biomass and therefore not removed from the stems. Flowers were only present at the harvest of the late single-cut regime in 2014 and were counted as stem biomass.

Plant measurements were taken on 14 May 2014 and 10 June 2014 and on 29 April 2015, 21 May 2015 and 23 June 2015. The measurements included shoot density, stem height and diameter. The shoot density was established by counting the shoots taller than 5 cm in the central square metre. The stem height was measured as the distance from the soil surface to the point where the last fully developed leaf projects from the stem. The stem diameter was measured 5 cm above the soil surface. To take into account the fact that stems may not be perfectly round, each stem was measured horizontally from several angles at the defined height and the largest diameter was recorded. The stem height and diameter were measured on five representative shoots (minimum 60% of mean height) from each plot.

Pot trial

The objective of the pot trial was to compare different nitrogen fertilization rates and application times. It was conducted from 29 April 2014–29 September 2014 in the greenhouse using *Miscanthus sacchariflorus* (OPM19) plantlets. The greenhouse temperature was maintained at a minimum of 20 °C during the day and 15 °C during the night with no artificial lighting. The plantlets were approx. one year old (pot volume 75 cm^3) and selected according to similar plant height and shoot number. The selected plantlets were transferred into pots with a volume of approx. 5 l several months before the beginning of the pot trial and watered carefully (no excess water). The pots were placed on saucers (height 3 cm) to avoid uncontrolled leaching of nutrients. The pots were filled with a soil mixture consisting of fertilized peat, loam and sand in a volumetric ratio of 1 : 2 : 1. The soil mixture was sterilized at 80 °C for 24 h before planting. After successful establishment, the plants were watered with excess water for several weeks to remove the remaining mineral nitrogen in the soil. The excess water was able to run off the saucers, and the water remaining in the saucers was taken up by the plants within few hours to one day. No negative effects on the plants were observed during this period, and viable roots were visible at the bottom of the saucers.

At the start of the pot trial, the plants were approx. 2 years old and the pots were placed in a randomized block design with 4 replications. The treatments of the pot trial are shown in Table 3. Two fertilization levels [100 and 200 mg N (kg soil)$^{-1}$] were tested in two application regimes (single and split application). For the split applications, half of the fertilizer was applied at the beginning of the trial and the other half directly after the first cut. In addition, a control with no fertilization and a treatment with a reduced fertilizer application [50 mg N (kg soil)$^{-1}$] after the first cut were conducted. The first fertilizer application was on 23 May 2014. In the following weeks, the plants were watered according to their specific needs without excess water to avoid nitrate leaching. The plants were harvested on 15 July 2014 (after 13 weeks' growth) directly followed by the second fertilizer application. The second harvest was performed on 29 September 2014 (after 11 weeks' growth). Directly after the second harvest, the rhizomes were also harvested by washing off the soil and separating the rhizomes from the roots. The biomass samples from both harvests and the rhizomes were dried at 60 °C to constant weight and milled in a cutting mill SM 200 (Retsch, Haan) using a 1-mm sieve to be used for the chemical analysis.

Chemical analysis

Phosphor (P), potassium (K), magnesium (Mg) and calcium (Ca) contents of the field and pot trial samples were analysed according to DIN EN ISO 15510 and VDLUFA Method Book III, method 10.8.2 (Naumann & Bassler, 1976/2012). For this analysis, 0.5 g of each sample was diluted with 8 ml HNO_3 and 6 ml H_2O and digested in an ETHOS.lab microwave (MLS GmbH, Leutkirch, Germany). The extract was analysed by an ICP-OES from the State Institute of Agricultural Chemistry, Hohenheim. The nitrogen content was analysed according to the DUMAS principle (method EN ISO 16634/1 and VDLUFA Method Book III, method 4.1.2) using a Vario Macro Cube (Elementar Analysensysteme GmbH, Hanau, Germany). The starch content of the rhizomes was measured enzymatically using the starch analysis kit 10207748035 (Hoffmann-La Roche Ltd., Basel; R-Biopharm AG, Darmstadt, Gemany) at the State Institute of Agricultural Chemistry, Hohenheim. The principle of the starch analysis kit is also described in method 7.2.5 in the VDLUFA Method Book III.

Biogas analysis

The substrate-specific biogas and methane yields were measured in a biogas batch test under mesophilic conditions at

Table 3 Overview of the pot trial fertilizer treatments

Treatment	Description	Total fertilization In mg N (kg soil)$^{-1}$	1st application	2nd application
0/0	Control	0	0	0
100/0	Half amount, single application	100	100	0
200/0	Full amount, single application	200	200	0
0/50	Reduced single late application	50	0	50
50/50	Half amount, split application	100	50	50
100/100	Full amount, split application	200	100	100

39 °C according to VDI guideline 4630. The biogas batch test was certified by the KTBL and VDLUFA interlaboratory comparison test 2014. The fermentation period was 35 days. Four replicates of each sample were analysed. Standard maize was analysed alongside the miscanthus samples to monitor the activity of the inoculum. The inoculum originated from the fermenter of a commercial mesophilic biogas plant which uses the following substrates: maize silage, grass silage, cereal whole crop silage, liquid and solid cattle manure and small quantities of horse manure. The inoculum was sieved and diluted to 4% DM with deionized water. Various macro- and micronutrients were added according to Angelidaki *et al.* (2009). Afterwards, the inoculum was incubated at 39 °C under anaerobic conditions for 6 days.

For the biogas batch analysis, 200 mg organic dry matter (oDM) of milled sample was transferred into a 100 ml fermentation flask, 30 g inoculum was added, and the gas-containing headspace was flushed with nitrogen to attain anaerobic conditions. The oDM content of the milled samples was estimated by drying an aliquot of approx. 1 g at 105 °C in a cabinet dryer and incineration at 550 °C in a muffle kiln to constant weight. The weight was recorded before and after drying and incineration. The fermentation flasks were closed gastight by a butyl rubber stopper and an aluminium cap. The pressure increase in the fermentation flasks was measured by puncturing the butyl rubber stopper with a cannula attached to a HND-P pressure meter (Kobold Messring GmbH, Hofheim, Germany). The biogas production was calculated as dry gas (water vapour pressure was considered) from the pressure increase and was standardized to 0 °C and 1013 hPa using Formula (1) and (2). Formula (1) was used for the first measurement and takes into account pressure increase caused by warming from laboratory temperature to 39 °C and the water vapour partial pressure. Formula (2) was used for the subsequent 17 measurements, which were taken on a regular basis.

$$V_{\mathrm{biogas}} = V_{\mathrm{HS}} * T_{\mathrm{S}}/T_{\mathrm{F}} * ((P_{\mathrm{A1}} + P_{\mathrm{F1}}) - (P_{\mathrm{A0}} * T_{\mathrm{F}}/T_{\mathrm{Lab}}) - P_{\mathrm{WP}})/P_{\mathrm{S}} \tag{1}$$

where V_{biogas} = volume of biogas produced

V_{HS} = volume of gas-containing headspace in the fermentation flasks

T_{S} = standard temperature (=273.15 K = 0 °C)

T_{F} = fermentation temperature (=312.15 K = 39 °C)

P_{A1} = ambient pressure at first measurement

P_{F1} = overpressure in fermentation flasks at first measurement

P_{A0} = ambient pressure at sealing of the fermentation flasks (batch test start)

T_{Lab} = laboratory temperature at sealing of the fermentation flasks (batch test start)

P_{WP} = water vapour partial pressure at 39 °C

P_{S} = standard pressure (1013 hPa)

$$V_{\mathrm{biogas}} = V_{\mathrm{HS}} * T_{\mathrm{S}}/T_{\mathrm{F}} * ((P_{\mathrm{An}} + P_{\mathrm{Fn}}) - (P_{\mathrm{A}(n-1)} + P_{\mathrm{F}(n-1)}))/P_{\mathrm{S}} \tag{2}$$

where P_{An} = ambient pressure at each measurement

P_{Fn} = overpressure in fermentation flask at each measurement

$P_{\mathrm{A}(n\text{-}1)}$ = ambient pressure at previous measurement

$P_{\mathrm{F}(n\text{-}1)}$ = overpressure in the fermentation flasks at previous measurement

During the course of the biogas batch test, it was occasionally necessary to remove the produced biogas from the fermentation flasks. The overpressure in the fermentation flasks was removed using a gastight syringe once it had reached an approximate value of 500 mbar. The biogas was transferred to a gastight evacuated storage flask where it was kept until the end of the batch test. After each gas collection, the remaining overpressure in the fermentation flasks was allowed to level off to ambient pressure by injecting a blank cannula. For the subsequent measurement, $P_{\mathrm{F}(n\text{-}1)}$ was then set to zero in formula (2). At the end of the batch test, the remaining biogas in the headspace of the fermentation flasks was removed by active extraction with a gastight syringe and also transferred into the storage flask. An aliquot of the collected biogas was used to analyse the methane content by a GC-2014 gas chromatograph (Shimadzu, Kyoto). The gas chromatograph was equipped with a thermal conductivity detector (TCD), and the detection temperature was set to 120 °C. Two columns (HayeSep and Molsieve column) were used (oven temperature 50 °C) with argon as carrier gas. The gas samples were injected using a Combi-xt PAL autosampler (CTC Analytics AG, Zwingen, Switzerland).

Statistical analysis

Statistical analysis was performed using the software SAS version 9.4 (SAS Institute Inc., Cary, NC, USA). The program 'Procmixed' was used, and a mixed model was developed for the field trial according to Formula (3). A test on homogeneity of variance and normal probability of residues was performed. The effects were tested at a level of probability of $\alpha = 0.05$.

$$y = \mu + \mathrm{rep} + \mathrm{yr} + \mathrm{rep} * \mathrm{yr} + \mathrm{hr} + \mathrm{Nf} + \mathrm{hr} * \mathrm{Nf} + \mathrm{yr} * \mathrm{hr} + \mathrm{yr} * \mathrm{Nf} + \mathrm{yr} * \mathrm{hr} * \mathrm{Nf} + e \tag{3}$$

where μ = general mean effect

rep = effect of field replicate

yr = effect of year

rep * yr = effect of interaction of field replicate and year

hr = effect of harvest regime

Nf = effect of nitrogen fertilization level

hr * Nf = effect of interaction of harvest regime and nitrogen fertilization level

yr * Nf = effect of interaction of year and nitrogen fertilization level

yr * hr * Nf = effect of interaction of year, harvest regime and nitrogen fertilization

e = residual error

The effect of the nitrogen fertilization and the interactions between harvest regime and nitrogen fertilization; year and nitrogen fertilization; and year, harvest regime and nitrogen fertilization were not significant. For this reason, the model shown in Formula (3) was adapted as shown in Formula (4).

$$y = \mu + \mathrm{rep} + \mathrm{yr} + \mathrm{hr} + \mathrm{yr} * \mathrm{hr} + e \quad (4)$$

Field replicate and year were fixed effects, and the interaction between field replicate and year and the interaction between field replicate, harvest regime and nitrogen fertilization level were random effects.

The data from the pot trial were also analysed by SAS version 9.4, and a mixed model according to Formula (5) was applied. A test on homogeneity of variance and normal probability of residues was performed. The effects were tested at a level of probability of $\alpha = 0.05$.

$$y = \mu + \mathrm{treat} + \mathrm{rep} + \mathrm{treat} * \mathrm{rep} + e \quad (5)$$

where μ = general mean effect
treat = effect of treatment
rep = effect of replicate in the greenhouse
treat * rep = effect of interaction of treatment and replicate
e = residual error

The treatment consisted of nitrogen fertilization level and nitrogen application regime. Treatment and replicate were fixed effects, and no random effects were allowed.

Results

Field trial

Plant measurements. In the years 2014 and 2015 – the first and second year after the cutting regimes were first applied – nitrogen fertilization had very little effect on the regrowth in each harvest regime (Figs 1 and 2). Therefore, the following results are discussed based on the harvest regimes and not on fertilization level. The double-cut and the early single-cut regime negatively influenced the regrowth of the following vegetation period (Fig. 1). The lower shoot density and the reduced stem height and diameter revealed that the plants were growing less vigorously and sprouting started later than in the late single-cut regime and the winter control. However, no overwinter plant losses or development of gaps were observed throughout the trial. In the year after the first green cut (2014), the double-cut regime showed a high number of shoots per square metre (Fig. 1a). This could indicate that the crop reacts to this harvest regime by increased shoot numbers in the second season. However, the standard deviation in 2014 was high and this effect was no longer visible in 2015 (Fig. 1b). For this reason, the identified effect may also be caused by chance.

The average dry weight per shoot increased with later harvest in 2013, but the proportion of leaf biomass decreased sharply over winter (Fig. 2). The average dry weight per shoot of the double-cut and the early single-cut regime was lower in 2014 than in 2013. The leaf-to-stem ratio of the early single-cut regime was also lower in 2014 than in 2013. In the single late-cut regime, the average dry weight per shoot was significantly higher in 2014 than 2013, but the leaf-to-stem ratio was stable. However, the weather conditions in 2013 were not ideal for miscanthus growth (cold spring until end of May and drought during June/July), which could explain the higher average dry weight per shoot in 2014 in the late single-cut regime. In the winter control, this effect was not visible. This may be due to losses over winter.

Dry matter yield and methane yield. The double-cut regime showed a low dry matter (DM) yield in both fertilization levels and years, because the low yields of the first cut were not compensated by the second (Fig. 3). It appeared that the first cut was performed before the end of the crop's main growth phase and therefore the crop was prevented from producing a higher yield. The yield of the early single-cut regime was high in the first year (2013) in both fertilization levels. However, there was a DM yield decrease from 2013 to 2014 in the double-cut and the early single-cut regime of approx. 40% and 60%, respectively. The green cut in the first year (2013) therefore greatly influenced the yield of the second year (2014), whereas the nitrogen fertilization showed almost no effect on the DM yield in the second year. By contrast, the DM yield of the late single-cut regime was even slightly higher in 2014 than in 2013 and showed the significantly highest DM yield in both years and fertilization levels. The DM yield of the late single-cut regime was on average 39% higher than the winter control, as biomass losses occur over winter. The DM content increased with later harvest dates and was ideal for ensiling in the early single-cut regime [on average 35% of fresh matter (FM)].

The substrate-specific methane yield (SMY) decreased with later harvest dates and the significantly highest SMY was measured in the both cuts of the double-cut regime (Fig. 4). The SMY of the late single-cut regime was significantly lower than those of the early single-cut regime, but significantly higher than the winter control. The methane yield per hectare was influenced mainly by the DM yield; the SMY had only of minor influence. The DM and methane yield of the double-cut and early single-cut regime decreased sharply from 2013 to 2014. The late single-cut regime and the winter control delivered stable DM and methane yields. However, the methane yield of the late single-cut regime was about 45% higher than the winter control, due to the higher DM yield and also higher SMY.

Mineral content of and nutrient removal by the biomass. Here, 'content' refers to the concentration of the respective nutrient in the biomass (unit % DM). 'Nutrient removal' is calculated from the nutrient content and dry matter yield and expresses the amount of nutrients removed from the field (kg ha^{-1}).

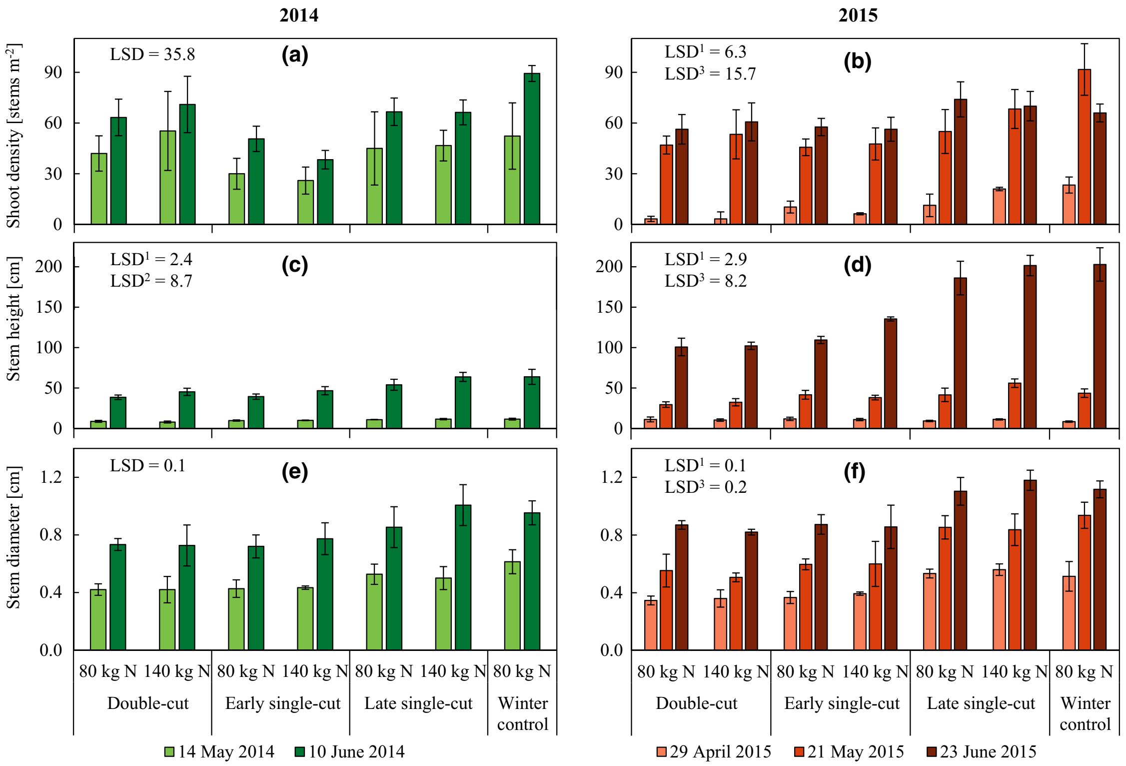

Fig. 1 Plant measurements of the regrowth of the four harvest regimes and two fertilization levels [80 and 140 kg nitrogen (N) ha^{-1} a^{-1}] in spring 2014 and 2015 in the field trial. Letters a, c and e show data from 2014 (first year after first green cut) and b, d and f from 2015 (second year after first green cut). Error bars indicate standard deviation. ^{1}LSD was calculated over the first measurement date in 2014 or 2015. ^{2}LSD was calculated over the second measurement date in 2014. ^{3}LSD was calculated over the second and third measurement date in 2015.

The nitrogen, phosphorus, potassium, magnesium and calcium contents of the harvested biomass are shown in Table 4. High quantities of potassium and nitrogen in particular were removed by the biomass of the high-yielding green-cut regimes, especially in 2013. In green-cut treatments where the DM yield was low, the nutrient removal was correspondingly low, for example in the double-cut and early single-cut regime in 2014. The removal of both nutrients decreased with later harvest dates, and the lowest nutrient contents were found in the biomass of the winter control. The highest nitrogen and potassium removal by the biomass was found in the late single-cut regime and the first year of the early single-cut regime, where the DM yield was high. The removal in the early and late single-cut regime was considerably lower in 2014 than in 2013. In the early single-cut regime, this was mainly influenced by the reduced DM yield and in the late single-cut regime by a lower nitrogen and potassium content. In the first year, the potassium content of the biomass from the late single-cut regime was much lower than that from the early single-cut regime, indicating that potassium was either actively relocated to the rhizome or lost through leaf fall. In 2013, the leaf-to-stem ratio was lower at the harvest of the late single-cut regime than at the early single-cut regime (Fig. 2), but most of the dead leaves were still attached to the stem. For this reason, potassium seems to be a good indicator of how far the relocation of nutrients and carbohydrates to the rhizomes has proceeded. The nitrogen removal by the biomass in the late single-cut regime is higher than in the winter control, especially in the first year, when the plants had an oversupply of nitrogen. This can be seen from far higher nitrogen fertilization application than removal by the spring-harvested biomass in the previous year, in particular for the higher fertilization level.

Pot trial

Dry matter yield. Under conditions of limited nitrogen availability, increased nitrogen fertilization led to significantly higher biomass and rhizome production, except the treatment 0/50 (Fig. 5a–c). It is notable that the treatments with single application at the beginning of the trial (100/0 and 200/0) had a significant higher bio-

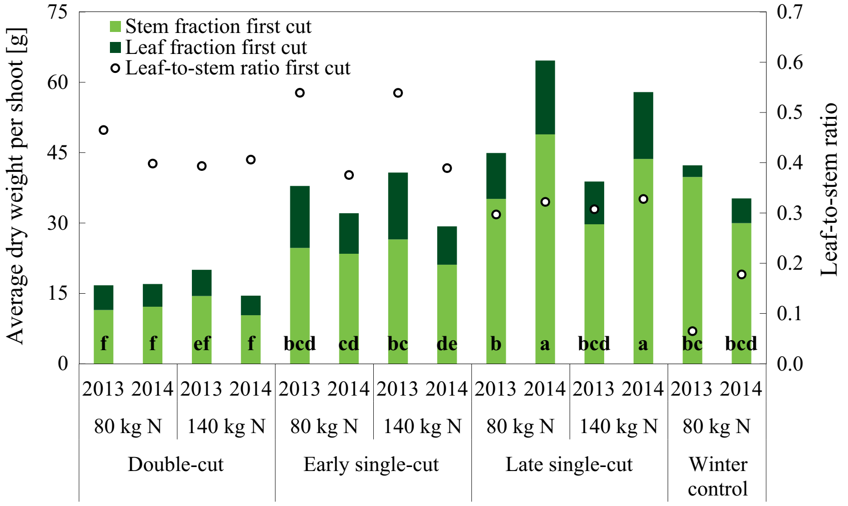

Fig. 2 Average dry weight per shoot and leaf-to-stem ratio of the first cut from the four different harvest regimes and two fertilization levels [80 and 140 kg nitrogen (N) ha^{-1} a^{-1}] in the field trial. Letter display corresponds to total average dry weight per shoot (leaf and stem biomass). The columns with different lower-case letters differ significantly from each other according to a multiple *t*-test $\alpha = 0.05$.

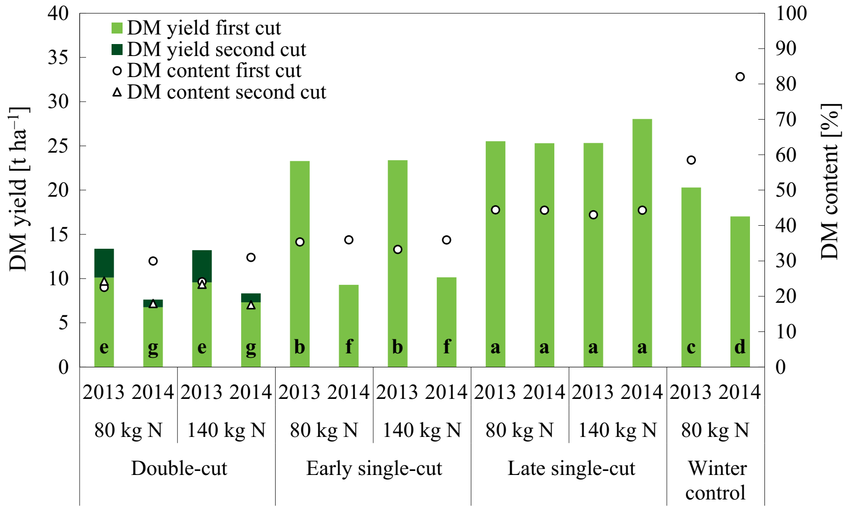

Fig. 3 Mean dry matter (DM) yield and DM content of the harvested biomass from the four different cutting regimes at two fertilization levels [80 and 140 kg nitrogen (N) ha^{-1} a^{-1}] in the field trial. Letter display corresponds to total mean DM yield (first and second cut). The columns with different lower-case letters differ significantly from each other according to a multiple *t*-test $\alpha = 0.05$.

mass production at the first cut than the split application treatments. The second nitrogen application after the first harvest stimulated the biomass production and led to nonsignificant differences between both application regimes of the same fertilization level. The rhizome production was not significantly influenced by the application regime, but by the fertilization level. Overall, single application of nitrogen fertilizer was advantageous, due to significantly higher biomass yield at the first cut and similar rhizome production and biomass yield at the second cut.

Nitrogen and starch content. Higher nitrogen fertilization resulted in higher nitrogen content in the biomass of both cuts and the rhizomes (Fig. 6). Nitrogen (N) removal was higher in the biomass of the fertilized treatments than in the unfertilized control due to both higher biomass and rhizome yield and higher N content of the biomass and the rhizomes. The N content of the aboveground biomass was higher when N fertilizer was applied in an early single application. These differences were not observed for the N content of rhizomes. Interestingly, the total N removal in the single application

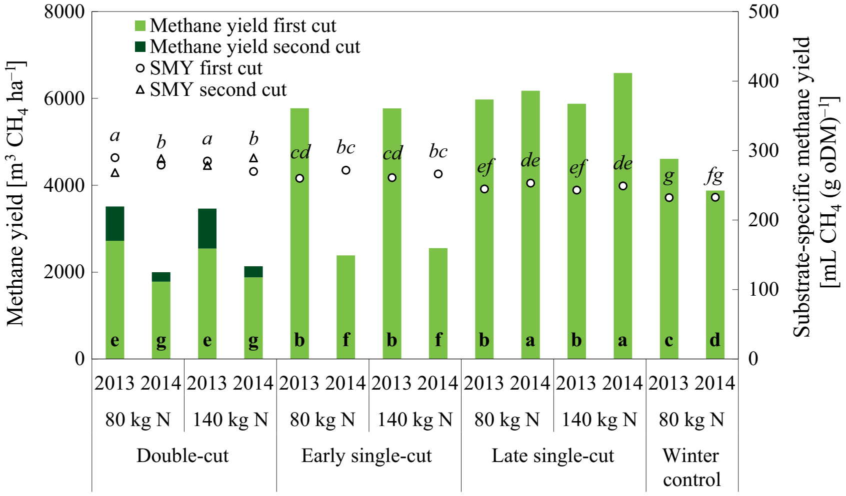

Fig. 4 Mean methane yield per hectare and substrate-specific methane yield (SMY) of the four harvest regimes at two fertilization levels [80 and 140 kg nitrogen (N) ha^{-1} a^{-1}] in the field trial. Letter display with bold lower-case letters corresponds to total mean methane yield (first and second cut). Letter display with italic lower-case letters corresponds to the SMY of the first cut. The columns with different bold or italic lower-case letters differ significantly from each other according to a multiple *t*-test $\alpha = 0.05$.

treatments was 792 and 1163 mg N in the treatments 100/0 and 200/0, respectively. This means more N was taken up by the crop than the applied N amount of 500 and 1000 mg N in the treatments 100/0 and 200/0, respectively. This could be due to residual nitrogen and mineralization in the soil or the nitrogen content of the rhizomes at the start of the trial. The split application treatments had a lower total N removal than the single application treatments. However, root biomass and soil were not analysed, so it is possible that a larger proportion of nitrogen was still attached to the roots or in the soil in the split application treatments.

Higher nitrogen fertilization resulted in lower starch content of the rhizomes (Fig. 7), due to the dilution effect of the higher biomass and protein production. The nitrogen fertilization increased growth of above- and belowground biomass production and in particular the amount of starch in the rhizome. The application of fertilizer after the first cut seemed to positively influence the amount of starch in the rhizome, whereas full application at the beginning of the trial seemed to positively influence the aboveground biomass production. However, the quantity of rhizome starch in the 200/0 treatment was not significantly lower than those of the 100/100 treatment.

Discussion

For a perennial crop, such as miscanthus, long-term productivity is the key factor for economically viable crop production. In the literature, the long-term productivity of *Miscanthus x giganteus* harvested in late winter is reported to be relatively stable with annual fluctuations due to seasonal effects (Christian *et al.*, 2008; Gauder *et al.*, 2012). It is also reported to be more stable than other perennial energy grasses such as switchgrass (Iqbal *et al.*, 2015). Long-term productivity of green-harvested miscanthus is, however, much more critical on account of the early harvest and very much depends on the cutting tolerance of the crop (Fritz & Formowitz, 2010). The results of this study indicate that *Miscanthus x giganteus* tolerates harvest in October. However, detailed knowledge of the mechanisms influencing the cutting tolerance is necessary to avoid damaging the crop and to identify optimal harvest regimes. The mechanisms driving cutting tolerance of miscanthus are discussed here, and recommendations for optimal management of miscanthus as a biogas crop are elaborated.

Mechanisms influencing cutting tolerance of miscanthus

The field and pot trial in this study were performed based on the hypothesis that nitrogen fertilization and harvest time interactively affect the cutting tolerance of miscanthus by influencing processes determining cutting tolerance. Under conditions of nitrogen limitation, as here in the pot trial, a positive effect of nitrogen fertilization on the biomass yield, biomass regrowth after the first cut and rhizome weight was indeed recorded. However, under conditions where nitrogen supply is not limited, as in the field trial, only very little effect of nitrogen fertilization on cutting tolerance was observed. The amount of nutrients removed by the harvested bio-

Table 4 Nitrogen, phosphorus, potassium, magnesium and calcium content of and removal by harvested biomass. Values in parentheses indicate standard deviation

			Nitrogen		Phosphorus		Potassium		Magnesium		Calcium	
Harvest regime	Nitrogen fertilizer level kg ha^{-1} a^{-1}	Year	Content % DM	Removal kg ha^{-1}	Content % DM	Removal kg ha^{-1}	Content % DM	Removal kg ha^{-1}	Content % DM	Removal kg ha^{-1}	Content % DM	Removal kg ha^{-1}
Double cut–first cut	80	2013	1.0 (0.1)	103 (8)	0.2 (0.0)	19 (1)	2.4 (0.1)	243 (12)	0.1 (0.0)	12 (1)	0.3 (0.0)	27 (2)
		2014	0.6 (0.1)	44 (11)	0.1 (0.0)	9 (2)	1.3 (0.2)	92 (28)	0.1 (0.0)	6 (1)	0.3 (0.1)	21 (8)
	140	2013	1.1 (0.2)	103 (20)	0.2 (0.0)	18 (3)	2.3 (0.2)	219 (45)	0.1 (0.0)	12 (2)	0.3 (0.0)	25 (3)
		2014	0.7 (0.0)	51 (5)	0.1 (0.0)	9 (0)	1.3 (0.1)	94 (3)	0.1 (0.0)	7 (1)	0.3 (0.1)	22 (7)
Double cut–second cut	80	2013	1.0 (0.1)	32 (3)	0.2 (0.0)	6 (1)	1.7 (0.2)	54 (11)	0.2 (0.0)	5 (1)	0.4 (0.0)	14 (3)
		2014	1.6 (0.0)	14 (3)	0.4 (0.0)	3 (0)	2.9 (0.4)	24 (4)	0.2 (0.0)	2 (0)	0.7 (0.1)	6 (2)
	140	2013	1.1 (0.2)	39 (11)	0.2 (0.0)	6 (2)	1.8 (0.3)	64 (14)	0.2 (0.0)	6 (2)	0.4 (0.1)	16 (6)
		2014	1.6 (0.1)	16 (0)	0.4 (0.0)	4 (0)	2.9 (0.1)	29 (2)	0.2 (0.0)	2 (0)	0.6 (0.0)	6 (1)
Early single cut	80	2013	0.6 (0.0)	132 (3)	0.1 (0.0)	28 (2)	1.4 (0.1)	319 (12)	0.1 (0.0)	18 (3)	0.2 (0.0)	44 (6)
		2014	0.5 (0.1)	47 (1)	0.1 (0.0)	12 (0)	1.0 (0.1)	96 (5)	0.1 (0.0)	7 (1)	0.3 (0.0)	27 (3)
	140	2013	0.7 (0.2)	164 (39)	0.1 (0.0)	31 (4)	1.5 (0.2)	355 (39)	0.1 (0.0)	26 (6)	0.2 (0.1)	57 (14)
		2014	0.6 (0.0)	60 (7)	0.1 (0.0)	12 (1)	1.0 (0.1)	105 (8)	0.1 (0.0)	10 (1)	0.3 (0.0)	31 (4)
Late single cut	80	2013	0.6 (0.1)	135 (22)	0.1 (0.0)	21 (4)	0.8 (0.1)	192 (44)	0.1 (0.0)	19 (3)	0.2 (0.0)	52 (6)
		2014	0.4 (0.1)	100 (24)	0.1 (0.0)	23 (4)	0.6 (0.1)	162 (32)	0.1 (0.0)	19 (2)	0.2 (0.0)	59 (14)
	140	2013	0.7 (0.2)	184 (46)	0.1 (0.0)	22 (3)	1.0 (0.2)	237 (46)	0.1 (0.0)	23 (3)	0.2 (0.0)	58 (8)
		2014	0.5 (0.1)	121 (37)	0.1 (0.0)	20 (3)	0.6 (0.1)	171 (32)	0.1 (0.0)	24 (4)	0.2 (0.0)	66 (10)
Winter control	80	2013	0.3 (0.0)	52 (12)	0.1 (0.0)	12 (3)	0.5 (0.1)	103 (34)	0.1 (0.0)	10 (3)	0.1 (0.0)	26 (8)
		2014	0.4 (0.1)	61 (17)	0.0 (0.0)	7 (1)	0.3 (0.1)	49 (9)	0.0 (0.0)	7 (1)	0.1 (0.0)	18 (5)

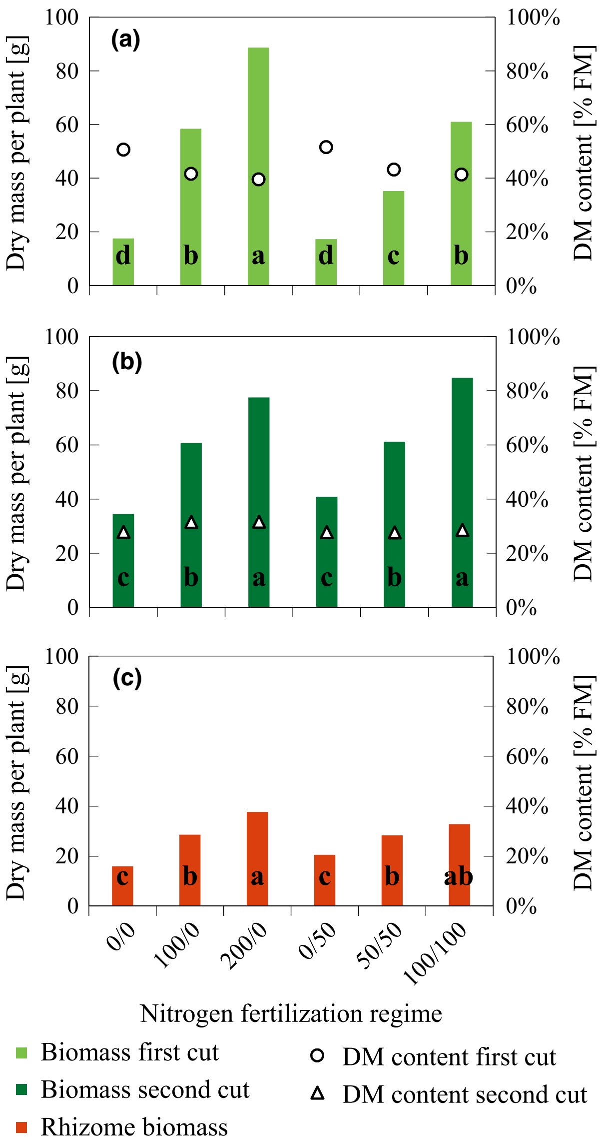

Fig. 5 Mean dry matter (DM) production and DM content of the first and second cut and the rhizomes in the pot trial treatments. The treatments comprised two nitrogen fertilization levels [100 and 200 mg N (kg soil)$^{-1}$] in two application regimes [single (full fertilizer amount at beginning) and split application (half at beginning and other half after first cut)]. Additionally, a low nitrogen application after the first cut was tested [50 mg N (kg soil)$^{-1}$]. The labelling of the treatments refers to the application rate of nitrogen [0, 50, 100 or 200 mg N (kg soil)$^{-1}$] and the fertilizer regime (fertilizer application at beginning/fertilizer application after first cut). Letter display corresponds to mean DM production. Columns with different lower-case letters differ significantly from each other according to a multiple *t*-test $\alpha = 0.05$.

mass does not seem critical for the cutting tolerance of miscanthus, as removed nutrients can easily be replaced by fertilization and taken up by the efficient, deep rooting system characteristics of miscanthus (Strullu *et al.*, 2011; Cadoux *et al.*, 2012). From these observations, it is concluded that the relevance of nitrogen fertilization increases with nitrogen limitation, for example on soils poor in nutrients or organic matter.

Nitrogen fertilization also positively affects starch production in the rhizomes, as was measured in the pot trial. This is likely to be the effect of more photosynthetically active biomass present when nitrogen fertilizer is applied. Purdy *et al.* (2015), and also the observations made in both the pot and the field trial of this study, indicate that carbohydrate – in this case starch – reserves in the rhizomes play an important role in the cutting tolerance of the crop. In the first year, the yield of the late single-cut regime was only slightly higher than that of the early single-cut regime, although the crop had two months more time for yield formation. This leads to the hypothesis that the crop invested a large proportion of the biomass accumulation into belowground biomass to refill the carbohydrate stores in the rhizomes with starch. Purdy *et al.* (2015) found that starch concentration in miscanthus rhizomes is likely to reach a peak in late autumn (November) and concluded that an earlier harvest could have a negative effect on the crop. The optimal harvest date for a green cut would therefore be at the time of maximum starch content in the rhizomes. Mutoh *et al.* (1968) revealed that, under Japanese growth conditions, the largest proportion of the net production was used to build up rhizomes, roots and reserve material in August and September. Later studies found that the starch content in the rhizomes increases until the end of the vegetation period and peaks in late autumn (Masuzawa & Hogetsu, 1977; de Souza *et al.*, 2013). Our study found the optimal harvest date for a green cut of *Miscanthus x giganteus* to be October and indicated that potassium seems to be a good indicator of how far the relocation of nutrients and carbohydrates to the rhizomes has proceeded. Himken *et al.* (1997) revealed that the content of potassium and nitrogen in the rhizome increased from June to late autumn, which supports this hypothesis. However, senescence and starch storage in the rhizomes are influenced by climatic conditions and accelerated by low daily minimum temperatures (Purdy *et al.*, 2015). In 2013, the first frost occurred at the beginning of October and several cold nights with temperatures around 0 °C were recorded before harvest. Both could have triggered carbohydrate transport to and starch formation in the rhizomes (Purdy *et al.*, 2015). This could explain why *Miscanthus x giganteus* tolerated cutting as early as October in this study and suggests that not only date, but also daily minimum temperature or first frost should be considered when determining the optimal harvest time for a green cut.

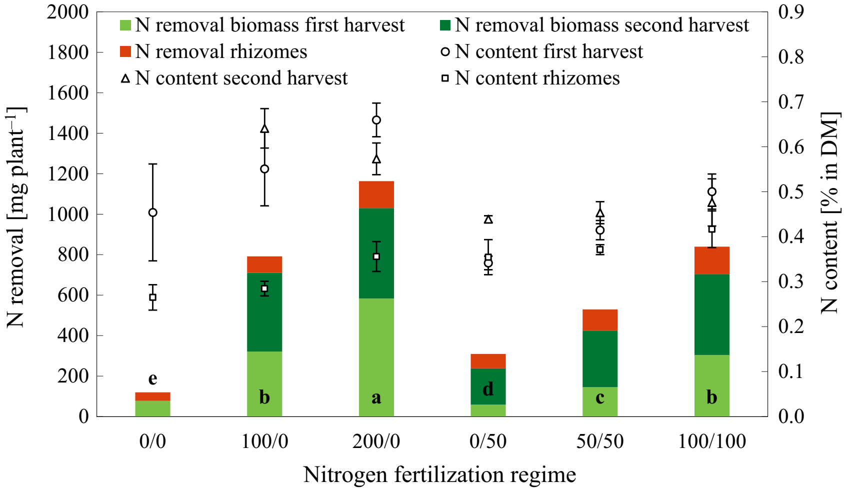

Fig. 6 Average nitrogen content and removal of the first and second cut and the rhizomes in the pot trial treatments. The treatments comprised two nitrogen fertilization levels [100 and 200 mg N (kg soil)$^{-1}$] in two application regimes [single (full fertilizer amount at beginning) and split application (half at beginning and other half after first cut)]. Additionally, a low nitrogen application after the first cut was tested [50 mg N (kg soil)$^{-1}$]. The labelling of the treatments refers to the application rate of nitrogen [0, 50, 100 or 200 mg N (kg soil)$^{-1}$] and the fertilizer regime (fertilizer application at beginning/fertilizer application after first cut). Figure display corresponds to total nitrogen removal (by biomass of first and second cut and rhizomes). Columns with different lower-case letters differ significantly from each other according to a multiple *t*-test $\alpha = 0.05$. Error bars indicate standard deviation of the respective nitrogen content.

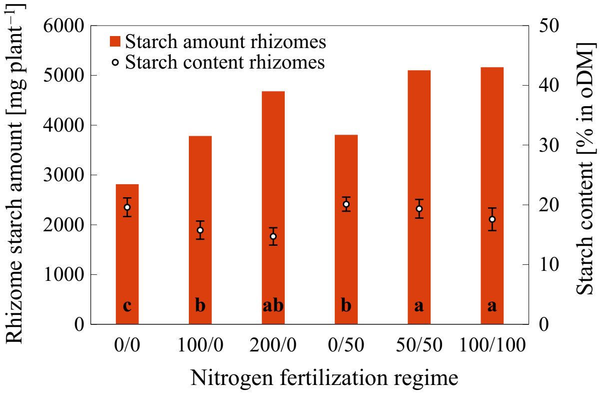

Fig. 7 Average quantity and content of starch in the rhizomes of the pot trial treatments. The treatments comprised two nitrogen fertilization levels [100 and 200 mg N (kg soil)$^{-1}$] in two application regimes [single (full fertilizer amount at beginning) and split application (half at beginning and other half after first cut)]. Additionally, a low nitrogen application after the first cut was tested [50 mg N (kg soil)$^{-1}$]. The labelling of the treatments refers to the application rate of nitrogen [0, 50, 100 or 200 mg N (kg soil)$^{-1}$] and the fertilizer regime (fertilizer application at beginning/fertilizer application after first cut). Figure display corresponds to average quantity of starch. Columns with different lower-case letters differ significantly from each other according to a multiple *t*-test $\alpha = 0.05$. Error bars indicate standard deviation of the respective starch content.

The results of Purdy *et al.* (2015) indicate that the seasonal fluctuations in rhizome starch content also differ between genotypes and the starch content of the rhizomes increases with ongoing senescence. Therefore, the potential of other genotypes that senesce earlier than *Miscanthus x giganteus* should be explored for biogas production. These genotypes may be characterized by earlier flowering. The carbohydrate sink in such genotypes may switch earlier from stems to rhizomes, and consequently, the starch stores in the rhizomes are refilled earlier. Such genotypes may tolerate earlier cutting than *Miscanthus x giganteus*. The advantage of genetic variation in ripening would be the option of broadening the harvest window of miscanthus to enable harvest under ideal climatic and soil conditions.

Cutting tolerance and yield of Miscanthus x giganteus

In the field trial, a significant yield decline was observed in the year after the early single-cut and the double-cut regime with harvests in July and August, indicating that these regimes are not sustainable. Both cutting regimes affected the regrowth of the crop in the second year negatively. This can be seen in a reduced leaf-to-stem ratio and average dry weight per shoot, especially in the early single-cut regime. Negative effects of an early green cut in August on the yield the following year have also been reported by Fritz & Formowitz (2010). The double-cut regime showed noteworthy regrowth after the first cut in 2013, but considerably less than in the late single-cut regime. In the field trial, nitrogen fertilizer was applied in spring and no fertilizer was applied after the first cut of the double-cut regime. In the pot trial, the split nitrogen application mainly increased the biomass yield of the second cut, but also promoted rhizome starch production. Therefore, we suggest investigating the effect of nitrogen fertilization on the yield of the second cut and the cutting tolerance of the double-cut regime when applied directly after the first cut under field conditions. However, the genotype used in the pot trial was not *Miscanthus x giganteus*, as in the field trial. Therefore, it cannot be ruled out that the *Miscanthus sacchariflorus* genotype used starts to relocate carbohydrates to the rhizomes earlier than *Miscanthus x giganteus*. In addition, the conditions in the greenhouse (e.g. higher temperatures) may have affected the generative development of the plants and thereby promoted the carbohydrate relocation to the rhizome.

In contrast to the early single- and double-cut regime, the late single-cut regime with harvest in October showed a stable or even slightly increased biomass yield from 2013 to 2014. Here, the slightly higher biomass yield and the higher average dry weight per shoot in 2014 may have been influenced by better weather conditions in 2014 than in 2013. Yield stability, with no negative effects on the yield in the following year, has also been reported for October harvest regimes by Mayer *et al.* (2014) and Yates *et al.* (2015). Based on these findings, it appears that a green-harvest regime with harvest in October is possible for *Miscanthus x giganteus*. However, as discussed above, the interactions between the processes of senescence and rhizome starch production with temperature should be further investigated. Such investigations should also include locations characterized by climates different from those for which an October harvest was examined in this study.

It is concluded that, with proper nutrient management and harvest timing, *Miscanthus x giganteus* tolerates green cutting in October. However, long-term studies are required to assess the cutting tolerance over a longer period and identify the fertilization requirements. As the harvest time is determined here by cutting tolerance, the biomass quality for biogas production may not be ideal. Therefore, the overall suitability of *Miscanthus x giganteus* for biogas production will be discussed in the following section.

Potential of Miscanthus x giganteus for biogas utilization

For combustion, *Miscanthus x giganteus* is conventionally harvested in late winter with dry biomass. A major advantage of a green harvest is the higher biomass yield. The average dry matter yield of the winter control (18.7 t DM ha^{-1}) was about 28% lower than the yield of the late green harvest in October (26.0 t DM ha^{-1}). Similar biomass losses over winter have been reported in the literature (Lewandowski *et al.*, 2003; Cadoux *et al.*, 2012). Therefore, the utilization of green biomass has the potential to substantially increase the biomass yield per unit area and to exceed that of maize.

The results presented confirm that *Miscanthus x giganteus* has a high potential for biogas utilization. The methane yield of the October harvest was very high (on average 6153 m^3 ha^{-1}) and within the range of the methane hectare yield of energy maize (6008 m^3 ha^{-1}) (Mast *et al.*, 2014). The ability of miscanthus to compete with maize has also been reported by Mayer *et al.* (2014). *Miscanthus x giganteus* could thus replace a significant share of maize cultivation for biogas production without increasing the cultivation area required. Schorling *et al.* (2015) revealed a total potential for *Miscanthus x giganteus* cultivation in Germany of 4 million ha. This large potential cultivation area indicates that miscanthus could make a significant contribution to substrate production for anaerobic digestion and help increase the sustainability of the biogas sector.

Before using *Miscanthus x giganteus* biomass in commercial biogas plants, its performance and substrate-specific methane yield (SMY) needs to be further investigated on a larger scale. As its SMY was analysed here in a batch test using milled biomass, the particle size may have positively influenced the SMY and in particular the rate of biogas production. For this reason, further research with commercially chopped biomass is required, also to assess the risk of floating layers forming in wet fermentation plants. As *Miscanthus x giganteus* biomass has a lower rate of biogas production than maize in anaerobic digestion (data not shown), it may require larger digester volumes or additional pretreatment. For commercial application, the biomass needs to be preserved by ensiling and the high dry matter content in October (on average 44% of fresh matter) may be problematic. However, the successful ensiling of miscanthus biomass has been reported in several studies (Huisman & Kortleve, 1994; Klimiuk *et al.*, 2010; Mayer *et al.*, 2014). On a commercial scale, additional silage additives can be used for efficient ensiling of *Miscanthus x giganteus* biomass or mixed ensiling with biomass of other crops, such as ryegrass or maize, may be performed.

The SMY decreased with later harvest dates and reached the lowest values at winter harvest [on average 233 ml (g oDM)$^{-1}$]. This is due to the effect of progressing lignification, relocation of easily degradable carbohydrates to the rhizomes and losses of faster degradable leaves over winter. As early green cuts are not tolerated by the crop, the SMY of the October harvest [on average 247 ml (g oDM)$^{-1}$] is suggested here as a reference SMY of *Miscanthus x giganteus* biomass. There are diverging findings for SMY of *Miscanthus x giganteus* biomass in previous studies. Wahid *et al.* (2015) and Mayer *et al.* (2014) found similar values for biomass harvested in autumn as in this study. Menardo *et al.* (2013) measured the SMY of pretreated miscanthus biomass and reported a very low SMY of 84 ml (g oDM)$^{-1}$ for the untreated, winter-harvested control. Klimiuk *et al.* (2010) analysed ensiled *Miscanthus x giganteus* biomass in a continuously operated digester and measured only 100 ml (g oDM)$^{-1}$. These lower yields may be an effect of the fermentation technology applied by Klimiuk *et al.* (2010). It is assumed here that *Miscanthus x giganteus* biomass requires longer retention times in continuously operated digesters, as the biogas production rate of this lignified biomass is comparatively low.

For anaerobic digestion, a harvest before winter is favourable, but the nutrient removal, especially for nitrogen and potassium, is significantly higher than after winter [see also Cadoux *et al.* (2012)]. The maximum removal was obtained in August and decreased until winter harvest. As too early harvest resulted in yield decline, only the late single-cut regime (harvest in October) is discussed here. A higher nitrogen fertilization resulted in a slightly higher yield, but also in a higher nitrogen removal. The nitrogen removal in 2013 was higher than in 2014. This can be explained as oversupply, due to lower removal of nitrogen in the year before the trial started. In 2014, the nitrogen removal was slightly higher than the fertilization in the 80 kg ha^{-1} treatment and slightly lower than that of the 140 kg N ha^{-1} treatment. Both nitrogen levels delivered high yields, and the yield response to the higher nitrogen level was low. Therefore, the ideal fertilization level for long-term yield stability is considered to be between 80 and 140 kg N ha^{-1} a^{-1}. However, deposition from the air and soil fertility should also be taken into account when estimating nutrient requirements. Long-term observations are required to analyse which nitrogen level is sufficient for a steady green harvest of the crop. Low nitrogen fertilization requirements are seen as an important advantage of perennial crops, especially in terms of reducing the environmental impacts of biomass production (McCalmont *et al.*, 2015). Early harvesting of miscanthus decreases this benefit because larger amounts of nutrients are withdrawn and need to be replaced by fertilization. However, in the case of biomass for biogas production, the largest part of these nutrients can be recycled by the application of biogas digestate. This is common practice in commercial biogas production. Direct emissions from nitrogen fertilizer or digestate application increase the global warming potential of crop cultivation, but only low-yield increases of 0.26 to 2.54 t DM ha^{-1} are required to offset these (Roth *et al.*, 2015).

In conclusion, *Miscanthus x giganteus* can be used for anaerobic digestion when harvested in October, but long-term effects of the green harvest on the productivity need to be assessed. The removed nutrients need to be replaced to ensure long-term productivity, but recycling of digestates should be sufficient. The methane yield and behaviour of the biomass in large-scale digesters need to be further researched. Due to the slower rate of biogas production, additional pretreatment or larger digester volumes may be required. Breeding and selection of new miscanthus genotypes for biogas production should focus on development of genotypes with higher substrate-specific methane yield in October (e.g. less lignified) and earlier refilling of rhizome starch stores, to allow a broader harvest window. The replacement of biogas maize by miscanthus offers great potential for reducing the environmental impacts of biogas production without increasing land-use competition.

Acknowledgements

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Chapter 3 - Environmental Performance of Miscanthus, Switchgrass and Maize: Can C4 Perennials Increase the Sustainability of Biogas Production?

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Environmental Performance of Miscanthus, Switchgrass and Maize: Can C4 Perennials Increase the Sustainability of Biogas Production?

Andreas Kiesel *, Moritz Wagner and Iris Lewandowski

Department Biobased Products and Energy Crops, Institute of Crop Science, University of Hohenheim, Fruwirthstrasse 23, 70599 Stuttgart, Germany; moritz.wagner@uni-hohenheim.de (M.W.); Iris_Lewandowski@uni-hohenheim.de (I.L.)

* Correspondence: a.kiesel@uni-hohenheim.de; Tel.: +49-711-459-22379; Fax: +49-711-459-22297

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Abstract: Biogas is considered a promising option for complementing the fluctuating energy supply from other renewable sources. Maize is currently the dominant biogas crop, but its environmental performance is questionable. Through its replacement with high-yielding and nutrient-efficient perennial C4 grasses, the environmental impact of biogas could be considerably improved. The objective of this paper is to assess and compare the environmental performance of the biogas production and utilization of perennial miscanthus and switchgrass and annual maize. An LCA was performed using data from field trials, assessing the impact in the five categories: climate change (CC), fossil fuel depletion (FFD), terrestrial acidification (TA), freshwater eutrophication (FE) and marine eutrophication (ME). A system expansion approach was adopted to include a fossil reference. All three crops showed significantly lower CC and FFD potentials than the fossil reference, but higher TA and FE potentials, with nitrogen fertilizer production and fertilizer-induced emissions identified as hot spots. Miscanthus performed best and changing the input substrate from maize to miscanthus led to average reductions of −66% CC; −74% FFD; −63% FE; −60% ME and −21% TA. These results show that perennial C4 grasses and miscanthus in particular have the potential to improve the sustainability of the biogas sector.

Keywords: anaerobic digestion; *Miscanthus x giganteus*; *Panicum virgatum*; *Zea mays*; LCA; GWP; carbon mitigation; fossil fuel depletion; acidification; eutrophication

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1. Introduction

Biogas is a renewable energy carrier produced by anaerobic digestion of biomass. Various kinds of biomass can be utilized for biogas production, such as sewage sludge, agricultural residues (e.g., manure), biogenic waste and energy crops [1]. Power production based on biogas is more reliable than other renewable energy sources, e.g., wind and solar, and can be used to cover power demand peaks or fluctuations in production due to unfavorable weather conditions. Biogas can be utilized directly in combined heat and power units (CHP) or can be upgraded to biomethane and transported to large gas power stations via the gas grid.

The Renewable Energy Act (EEG) and its amendments have led to a rapid increase in biogas exploration in Germany [1]. Here, approximately 8075 biogas plants with a total installed capacity of 4.1 GW were in operation in 2016 [2]. The latest amendments promote the restructuring of biogas plants to flexible operation, and approximately 31% of the installed capacity [2] have already been modernized. This allows power production to be adapted more to demand. Currently, 182 biogas plants upgrade biogas to biomethane and inject it into the gas grid [2]. These numbers show that, in Germany, there

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is a significant biogas infrastructure in place and the process of adapting it to the needs of a future renewable power supply has already begun. However, to allow an economically and environmentally viable operation, this infrastructure needs a reliable, affordable and sustainable supply of biomass. In 2014, substrate input (based on mass) was composed of 52% energy crops (of which 73% was maize) and 43% manure [2]. However, the proportion of biogas produced from energy crops is considerably higher than their proportion by mass, because they have a higher specific biogas and methane yield than other biogas substrates, e.g., manure. In Germany, about 1.4 million ha energy crops are grown for biogas production, of which 0.9 million ha are biogas maize [2]. This reveals the great importance of energy crops—and in particular energy maize—in Germany. The high economic viability of maize [3] for biogas production is given by its high methane yield, easy digestibility, and well-established, optimized crop production and harvest logistics, including storage as silage.

However, the strong reliance of the biogas sector on maize as substrate crop can lead to environmental problems and a low acceptance in public opinion. The environmental profile of maize cultivation is characterized by a high nitrogen fertilizer input, high risk of erosion and leaching, and negative impact on biodiversity [4–6]. In particular, the regional concentration in areas with high biogas plant densities can lead to environmental problems, such as surface and groundwater pollution through erosion and leaching, and losses in biodiversity and soil organic matter due to the high proportion of maize in crop rotations [7]. Other aspects are also criticized, such as the high concentration of maize in the landscape and the use of good agricultural land for growing energy instead of food crops. For these reasons, the sustainability of the biogas sector is often questioned not only by environmentalists but also by the general public.

The replacement of maize (*Zea mays*) by crops with a more benign environmental profile is seen as one route towards more sustainable biogas production. These crops, however, should have an equally high yield and biomass supply potential as maize. The high-yielding and nutrient-efficient perennial C4 grasses miscanthus and switchgrass are considered promising options.

The miscanthus genotype, *Miscanthus x giganteus*, was introduced into Europe in 1935 and is today still the only commercial genotype available on the market [8]. However, promising breeding efforts have begun in recent years and latest results show the suitability of novel genotypes for marginal lands and the potential contribution of miscanthus to greenhouse gas (GHG) mitigation [9]. Progress in upscaling miscanthus cultivation and crop production has also raised interest in the industrial sector [10]. Miscanthus' beneficial environmental profile is mainly due to its perennial nature and because soil organic carbon tends to increase when arable land is converted to its cultivation [11]. It is a very resource- and land-use efficient crop with efficient nutrient-recycling mechanisms and high net energy yields per unit area [12,13]. For this reason, the global warming potential (GWP) and the resource depletion potential of miscanthus cultivation is low [14,15]. Miscanthus is suitable for biogas production and has a high methane yield potential per unit area [16–18]. For anaerobic digestion, the biomass is harvested before winter, which increases the yield and digestibility [18]. Whittaker et al. [19] proved storage of green miscanthus via ensilaging to be feasible with losses in a similar range as for maize. These losses were significantly reduced by the addition of silage additives [19]. Compared to the conventional harvest of dry biomass in early spring, a green harvest in late autumn prevents leaf fall over winter, which leads to a higher nutrient removal than at spring harvest [13,18]. However, the recycling of fermentation residues is assumed to at least partially compensate for this removal and contribute to the formation of soil organic carbon. Nevertheless, the effects of a green cut on the development of soil carbon and fertility needs to be further investigated and is for this reason not considered in this study.

The crop production and environmental profile of switchgrass (*Panicum virgatum*) is comparable to that of miscanthus, except establishment via seeds and not rhizomes. Switchgrass is native to the US and Canada, where it has been developed as a promising energy grass [20]. It is also suitable for biogas production as harvest of green biomass and even double-cutting is possible [21]. Although yields are generally lower than with *Miscanthus x giganteus* [22], switchgrass can perform equally well under abiotic stress, such as cold and drought [23]. Its major advantage over the miscanthus genotypes presently available (mainly propagated clonally via rhizomes) is its low-cost establishment via seeds. Currently, switchgrass is not commercially cultivated in Germany and miscanthus is grown on an estimated area of 4000 hectares, mainly for combustion purposes [9]. Extending the utilization to anaerobic digestion could contribute to the sustainability and crop diversity (important for biodiversity) of the biogas sector.

The objective of this paper is to assess and compare the environmental performance in biogas production of the perennial C4 grasses miscanthus and switchgrass and the annual C4 crop maize. This was done in a Life Cycle Assessment (LCA) according to ISO standards 14040 and 14044 [24,25], using data from a field trial and laboratory measurements. Wagner and Lewandowski [26] showed that, when analyzing the environmental performance of biobased value chains, it is crucial to consider more impact categories than just global warming potential (GWP). Therefore, the following impact categories were assessed to estimate the environmental performance of the crops and their subsequent utilization: climate change (CC)—which corresponds to the GWP, freshwater eutrophication (FE), marine eutrophication (ME), terrestrial acidification (TA) and fossil fuel depletion (FFD). The impact categories FE, ME and TA were chosen as eutrophication and acidification have been identified as important impact categories for agricultural systems. The category marine eutrophication represents the impact of nitrogen on biomass growth in aquatic ecosystems. Freshwater eutrophication represents the same impact, but caused by phosphorus [27,28].

The data for the LCA were collected from a randomized split-block field trial, where miscanthus, switchgrass and maize were grown under *ceteris paribus* conditions. The field trial was started in 2002 and allows a comparison of annual and perennial crops. Samples and yield measurements for this LCA were taken in 2012 and 2013 and laboratory analyses were performed to estimate biogas and methane yield and biomass quality.

2. Material and Methods

2.1. Scope and Boundaries

The scope of the present study is an assessment of the environmental performance of the cultivation of three dedicated energy crops ((i) miscanthus (*Miscanthus x giganteus*); (ii) switchgrass (*Panicum virgatum* L.) "Kanlow"; and (iii) silage maize (*Zea mays*) "Mikado") and their subsequent fermentation in a biogas plant. The biogas produced is utilized in a CHP unit (Combined Heat and Power) to produce electricity and heat. The cultivation as well as the utilization of the biomass takes place in Germany. One kilowatt hour of electricity ($kWh_{el.}$) was chosen as the functional unit (FU). The environmental impacts of these biobased value chains were compared with the German electricity mix as a fossil reference. In order to do this, a system expansion approach was applied which enables the inclusion of fossil reference system hot spots.

The systems are described in Figure 1. On the right side the maize cultivation is shown, on the left side the cultivation of the perennial crops miscanthus and switchgrass. The system boundaries include the production of the mineral fertilizers and the herbicides used, the production of the propagation material (miscanthus rhizomes as well as switchgrass and maize seeds), and the agricultural management (soil preparation, planting, mulching, fertilizing, spraying of herbicides, harvesting, recultivation resp. stubble cultivation) over the whole cultivation period which is for maize 1 year, for switchgrass 15 years and for miscanthus 20 years. Miscanthus and switchgrass are mulched in the first year and harvested from the second year onwards. All crops are harvested with a self-propelled forage harvester. The biomass is then transported to the biomass plant where it is fermented to biogas which is combusted in a CHP unit to produce electricity and heat. The fermentation residues are rich in nutrients and are used as fertilizer.

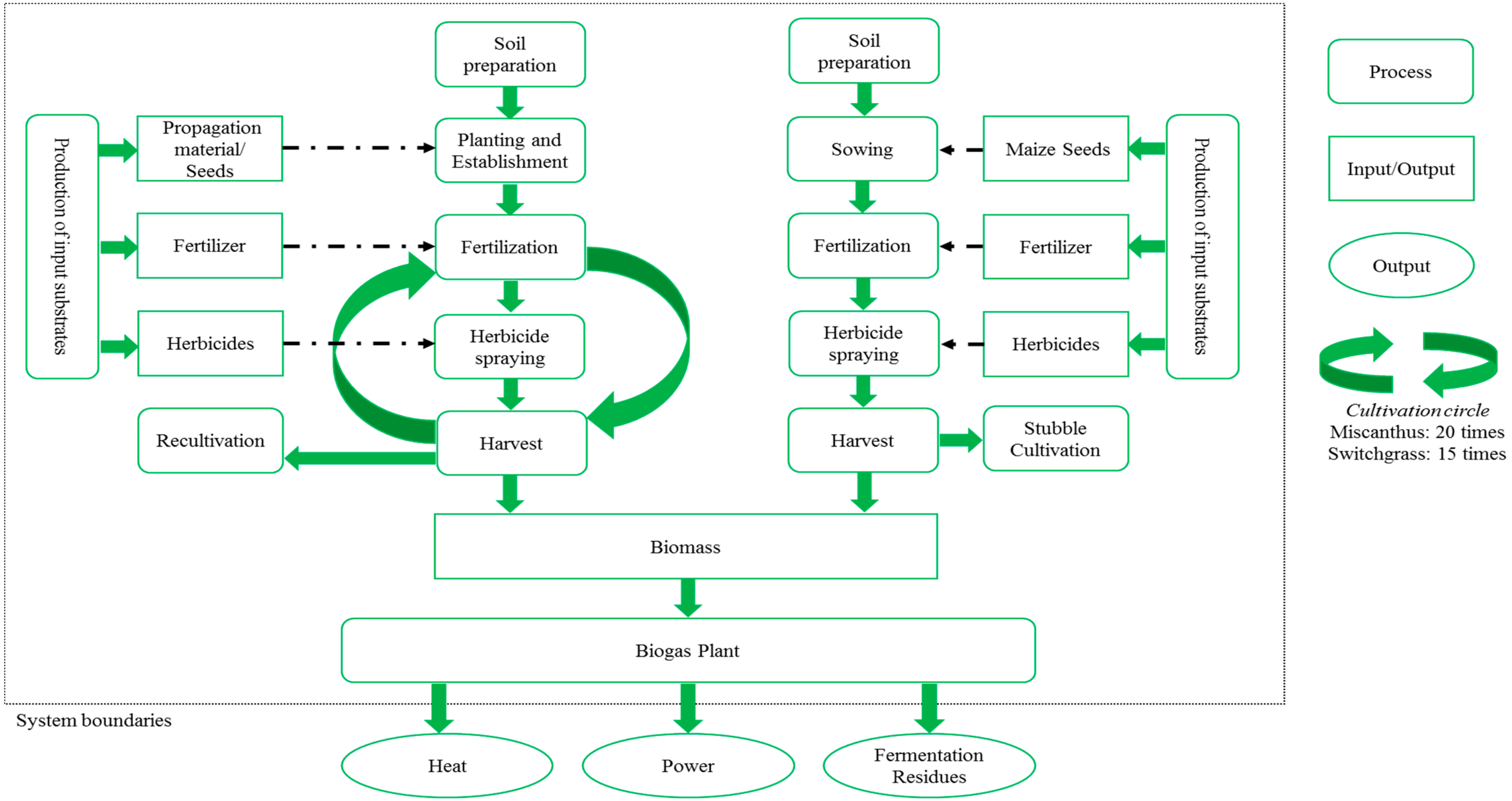

Figure 1. System description and boundaries for miscanthus, switchgrass (**left**) and maize (**right**) biomass cultivation, the fermentation to biogas and the subsequent utilization in a CHP unit.

2.2. Life Cycle Inventory

The data for the cultivation process used in this LCA were obtained from a multiannual field trial at Ihinger Hof. The Ihinger Hof is a research station of the University of Hohenheim and is located in southwest Germany (48.75°N and 8.92°E). The soil belongs to the soil class Haplic Luvisol. The long-term average annual air temperature and precipitation at the research station are 8.3 °C and 689 mm, respectively. The experimental design of the trial is described in Boehmel et al. [29].

Data on cultivation practices such as fertilizer and herbicide inputs were available for an 11-year period from 2002 to 2013. Miscanthus and switchgrass were established in spring 2002 by rhizome planting and sowing, respectively. Maize was sown on 27 April 2012 and 21 May 2013 at a density of 9.5 seeds m^{-2}. Nitrogen was applied as calcium ammonium nitrate (CAN), K_2O as potassium chloride and P_2O_5 as triplesuperphosphat (TSP). The use of herbicides during the miscanthus and switchgrass cultivation is described in Iqbal et al. [30]. For maize cultivation chemical weeding was performed using two conventional herbicides mixtures following good agricultural practice. The first application was a mixture of three herbicides (2.0 $L{\cdot}ha^{-1}$ Stomp Aqua, BASF SE, active ingredient 455 $g{\cdot}L^{-1}$ Pendimethalin; 1.0 $L{\cdot}ha^{-1}$ Spektrum, BASF SE, active ingredient 720 g L^{-1} Dimethenamid-P; and 1.0 $L{\cdot}ha^{-1}$ MaisTer power, Bayer, active ingredient 31.5 $g{\cdot}L^{-1}$ Foramsulfuron + 1.0 $g{\cdot}L^{-1}$ Iodosulfuron + 10.0 $g{\cdot}L^{-1}$ Thiencarbazone + 15.0 $g{\cdot}L^{-1}$ Cyprosulfamide). The second application was a mixture of two herbicides (1.7 $L{\cdot}ha^{-1}$ Laudis, Bayer, active ingredient 44 $g{\cdot}L^{-1}$ Tembotrione + 22 $g{\cdot}L^{-1}$ Isoxadifen-ethyl; and 0.35 $L{\cdot}ha^{-1}$ Buctril, Bayer, active ingredient 225 $g{\cdot}L^{-1}$ Bromoxynil).

The principle data for the cultivation of miscanthus, switchgrass and silage maize used in this analysis are summarized in Table 1. The data are shown for the years 2012 and 2013. In the year 2013 the weather conditions were not ideal for silage maize cultivation in Germany which is an important reason for the significantly lower yield of silage maize in the year 2013 compared to 2012. After a serious frost period in February 2012, the weather conditions in 2012 where quite usual, spring was rather dry, but followed by plenty of rain in June (Figure 2). Weather conditions in 2013 were completely contrary and very challenging for agriculture. The spring and especially May was unusually cool and wet. Due to this challenging weather conditions, maize sowing was delayed to late May. In July, the temperatures were unusually high and the crops faced a serious drought followed by few days of rain from 24 to 29 July. In this period, 168.5 mm of rainfall occurred in 4 major events, which represents 97% of the rain of the complete month.

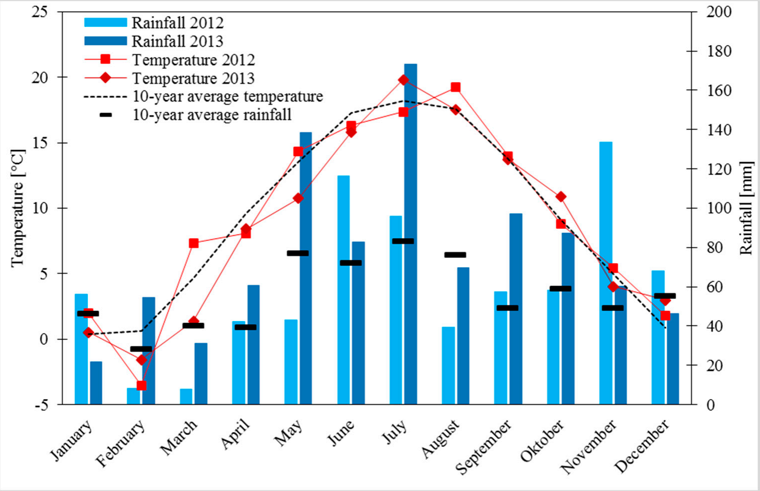

Figure 2. Temperature and rainfall in 2012 and 2013 at the field site on the research station "Ihinger Hof". For comparison the 10-year average temperature and rainfall from 2003 to 2012 is shown.

Maize was harvested at milk-ripe stage (end of September in 2012; late October in 2013) and miscanthus and switchgrass in late October in both years. The years 2012 and 2013 were selected to compare the environmental performance of perennial crops as an alternative to maize under different conditions for silage maize cultivation. The yield of maize, miscanthus and switchgrass is shown for the favorable year 2012 and non-favorable year 2013 in Table 1. However, the yield of the two perennial crops is the average yield over the whole cultivation period (20 years for miscanthus, and 15 for switchgrass) including the establishment phase based on the measured yield of the respective year. In the first year, miscanthus and switchgrass are mulched and not harvested. Full yields are only reached from the third year on. This calculation is exemplarily shown for the yield in 2012 for miscanthus in Equation (1) and for switchgrass in Equation (2) and was performed in the same way for the lower yields in 2013. The variable *yield_year2* describes the yield in the second cultivation year, which, for both crops, is slightly lower than the mean yield achieved in the following years.

$$Mean\ yield\ miscanthus\ \left[t\ DM\ ha^{-1}\cdot yr^{-1}\right] = \frac{yield\_year2\ +\ \text{yield\_}year\text{\_2012} \times 18}{20} \tag{1}$$

$$Mean\ yield\ switchgrass\ \left[t\ DM\ ha^{-1}\cdot yr^{-1}\right] = \frac{yield\_\ year2\ +\ \text{yield\_}year\text{\_2012} \times 13}{15} \tag{2}$$

The methane yield was measured as described in Kiesel and Lewandowski [18]. A biogas batch test was performed for 35 days at mesophilic conditions (39 °C) according to VDI guideline 4630. The approach of the biogas batch test was certified by the KTBL and VDLUFA interlaboratory comparison test 2014 and 2015. Each sample was assessed in four technical replicates.

Table 1. Summary of the in- and outputs of the three energy crops.

Input/Output	Unit	Maize		Switchgrass		Miscanthus	
		2012	**2013**	**2012**	**2013**	**2012**	**2013**
N	$Kg\cdot yr^{-1}\cdot ha^{-1}$	240	240	80	80	80	80
K_2O	$Kg\cdot yr^{-1}\cdot ha^{-1}$	304	204	137	137	128	128
P_2O_5	$Kg\cdot yr^{-1}\cdot ha^{-1}$	100	100	37	37	32	32
Herbicides	$Kg\cdot yr^{-1}\cdot ha^{-1}$	6.05	6.05	1.32	1.32	1.375	1.375
Dry matter yield	$Kg\cdot yr^{-1}\cdot ha^{-1}$	18915	12616	14227	8369	22760	18929
Dry matter content	%	25.4	21.1	38.9	36.2	43.4	41.2
Methane yield	$m^3\ CH_4\ yr^{-1}\cdot ha^{-1}$	5594	3635	3328	2095	5006	4542
Agricultural land required for biogas plant	$ha\cdot yr^{-1}$	173	266	291	461	194	213

The background data for the environmental impacts associated with the production of the input substrates (seeds, propagation material, herbicides and fertilizers) and the cultivation processes were taken from the GaBi database [31]. Direct N_2O and NO emissions from the mineral fertilizers used were calculated according to Bouwman et al. [32]. The estimations of indirect N_2O emissions from mineral fertilizers and N_2O emissions from harvest residues were done in accordance to IPCC [33]. Nitrate leaching to groundwater was calculated according to the SQCB—NO_3 model [34]. Ammonia emissions were calculated using emission factors from the Joint EMEP/CORINAIR Atmospheric Emission Inventory Guidebook [35]. Phosphate emissions were estimated according to van der Werf et al. [36].

In this study a transport distance of 100 km by truck for the input material such as herbicides or fertilizer and of 5 km by tractor for the biomass from the field to the biogas plant was assumed. This assumption is align with literature [37–39] and was done, since no data for the transport distance of the input substrates to the farmer and the biomass to the biogas plant were available. The emission stage for the truck used was assumed to be EUR5. The data for the transportation processes of the input material and the biomass were taken from the GaBi database [31].

After the harvest, the biomass of the different crops is ensiled. During the ensilage process dry matter losses of 12% were assumed [40]. The silage is subsequently fermented in a biogas plant. The methane hectare yield of the different crops is shown in Table 1. In the biogas plant methane losses of 1% were assumed [41]. The biogas is then combusted in a CHP with an electrical capacity of 500 kW to produce heat and power. The technical characteristics of the CHP used in this analysis are shown in Table 2. The inherent power consumption for miscanthus and switchgrass was assumed to be 12% and thus significantly higher than for maize. This is due to the more energy intensive pre-treatment of lignocellulosic biomass before the fermentation process. The emissions associated with the combustions of the biogas were taken from the ecoinvent database [42]. The electricity generated is fed into the grid. Twenty percent of the heat produced is used internally for the heating of the fermenter. In practice the remaining heat is partially used for heating nearby buildings thereby substituting heat produced by fossil sources. In this study, it was assumed that of the remaining heat 50% is used for this purpose.

Table 2. CHP unit—technical characteristics.

Technical Characteristics		Unit
Full load hours	7800	h
Plant output electrical	500	$kWh_{el.}$
Plant output total	1219	kWh
Electrical efficiency	41	% of plant total output
Thermal efficiency	41	% of plant total output
Inherent heat demand	20	% of total heat production
Inherent power consumption—perennial crops	12	% of total power production
Inherent power consumption—silage maize	6.6	% of total power production

The residues of the fermentation process are rich in nutrients. Table 3 shows the plant available nutrients, which can be recycled through the use of fermentation residues as fertilizers (related to the generation of the functional unit of 1 $kWh_{el.}$). The nutrient content is the average of the measured values of year 2012 and 2013. The phosphorus and the potassium content of the biomass fermented remains fully in the fermentation residues. Only 70% of the nitrogen compounds in the fermentation residues are available for the plants. That is why the nitrogen content can therefore not be taken fully into account. The nitrogen (N) content was analyzed according to the DUMAS principle (method EN ISO 16634/1 and VDLUFA Method Book III, method 4.1.2) using a Vario Macro Cube (Elementar Analysensysteme GmbH, Hanau, Germany) element analyzer. The phosphor (P) and potassium (K) contents were analyzed according to DIN EN ISO 15510 and VDLUFA Method Book III, method 10.8.2 [43] using ICP-OES and a ETHOS.lab microwave (MLS GmbH, Leutkirch, Germany).

Table 3. Nutrients in the biomass of the analyzed energy crops and plant available nutrients which can be recycled through the use of fermentation residues per FU.

Nutrient	Miscanthus		Switchgrass		Maize	
	in % of Biomass (d.b.)	in kg/FU	in % of Biomass (d.b.)	in kg/FU	in % of Biomass (d.b.)	in kg/FU
N	0.47	0.0036	0.50	0.0035	1.29	0.0058
P	0.09	0.0010	0.10	0.0010	0.18	0.0011
K	1.11	0.0119	1.03	0.0105	1.29	0.0083

2.3. Choice of Impact Categories

In this LCA study the life cycle impact assessment method ReCiPe was used [44]. The following impact categories were considered: climate change (CC), which corresponds to global warming potential (GWP); terrestrial acidification (TA); freshwater eutrophication (FE); marine eutrophication (ME); and fossil fuel depletion (FFD). Characterization factors were taken from Goedkoop et al. [44]. These impact categories were chosen according to their relevance for perennial biomass production, which was analyzed in the study by Wagner and Lewandowski [26].

3. Results

For each impact category analyzed, data are shown for the two climatically different production years 2012 and 2013 (2012 favorable and 2013 non-favorable for silage maize cultivation) and for two scenarios, one with and one without heat utilization. These are presented both in figures and in tables, depicting the results with (figures) and without (tables) a system expansion approach. The results are presented per functional unit (FU), which is kWh electricity. In the supplementary material (S1–S5), the same results are presented per kg dry biomass.

The value in each impact category shows the net impact or benefit of the substitution of the fossil reference through a biobased alternative. In this study, the German electricity mix was substituted by power generated through the fermentation of dedicated energy crops and the subsequent combustion of the biogas in a CHP unit. A negative value in this case is thus a net benefit while a positive value is a negative impact on the environment.

In contrast, the table shows the environmental impact of the generation of 1 $kWh_{el.}$ in each impact category without this substitution, separated into the main emission sources. In this context, the *recycling of nutrients* represents the emission savings associated with the reduction in fertilizer in other crops through the use of the fermentation residues. The *agricultural management* summarizes all operation steps from soil preparation, planting, mulching, fertilizing, and spraying of herbicides to recultivation. The *fertilizer-induced emissions* are emissions associated with the use of fertilizers, such as N_2O emissions, which occur after the application of nitrogen fertilizer. *Credits heat utilization* are credits given for the substitution of heat produced via a fossil reference (in the present study natural gas) by heat generated via the combustion of biogas in the CHP unit. In the heat utilization scenario, 20% of the heat produced is used internally in the biogas plant. Of the remaining 80%, one half (40% of total heat produced) is used to heat nearby buildings, thus substituting heat from conventional sources.

3.1. Climate Change and Fossil Fuel Depletion

The production and use of the analyzed C4 crops, both perennial and annual, leads to a net GHG emission reduction up to 0.66 kg·CO_2-eqv. $(kWh_{el.})^{-1}$ through the substitution of a fossil reference (Figure 3). Furthermore, all scenarios show a net decrease of the fossil fuel depletion of up to 0.18 kg·oil-eqv. $(kWh_{el.})^{-1}$ (Figure 4). As expected, the scenarios with heat utilization lead to both higher GHG emission and fossil fuel saving (Figures 3 and 4). On average, miscanthus shows the highest GHG emission and fossil fuels saving potentials. Both perennial grasses perform better than maize (Figures 3 and 4). The advantage of miscanthus over switchgrass is larger than the advantage of switchgrass over maize.

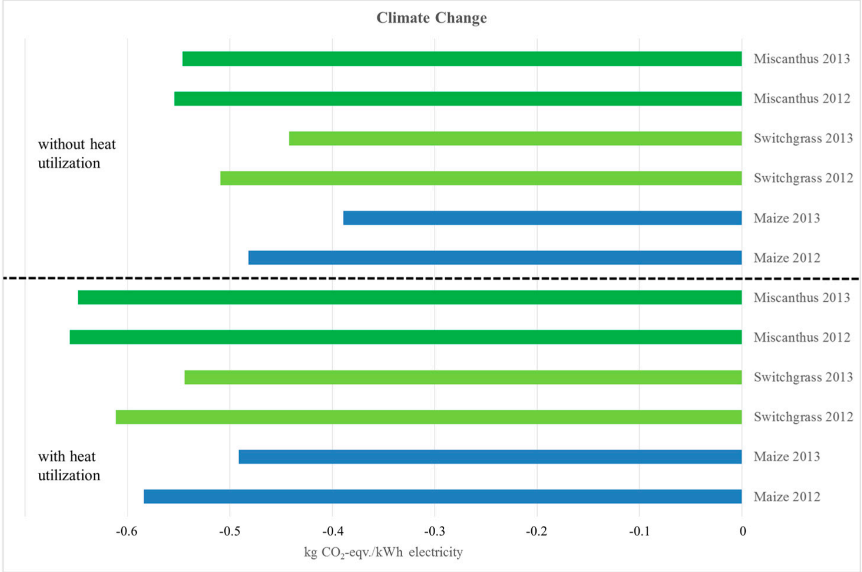

Figure 3. Assessment of the net benefits in kg·CO_2-eqv. of substituting 1 kWh_{el}. of the German electricity mix by power generated via combustion of the biogas in a CHP.

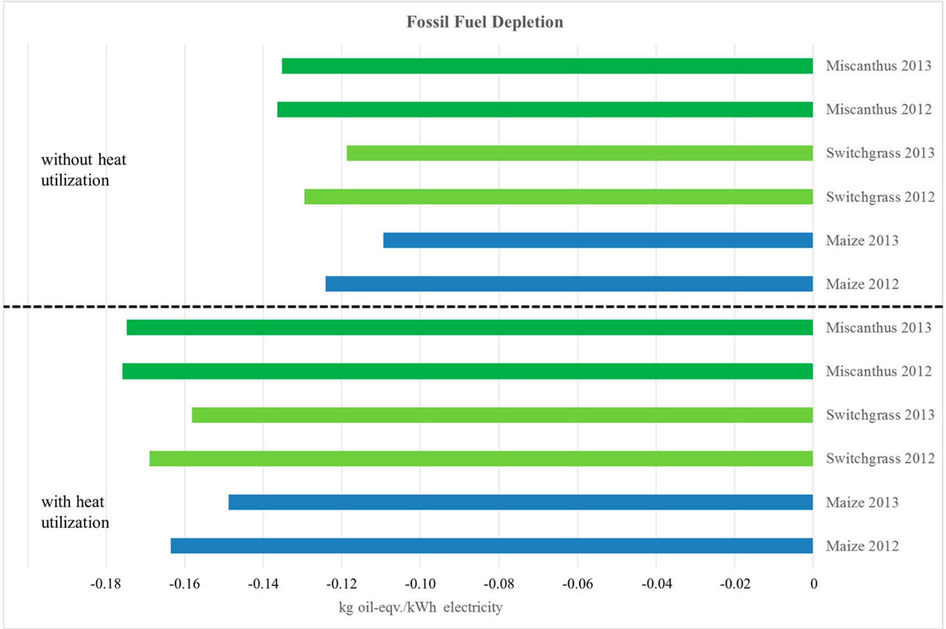

Figure 4. Assessment of the net benefits in kg·oil-eqv. of substituting 1 kWh_{el}. of the German electricity mix by power generated via combustion of the biogas in a CHP.

Table 4 shows the contribution of different processes to the GHG emissions and Table 5 the use of fossil fuels in these processes. The production of nitrogen fertilizer is responsible for the largest impact in both impact categories and for all crops. This is also the reason for the high credit—in terms of fossil energy savings—given for the recycling of nutrients from the fermentation residues (Table 5). Other processes with high impacts on GHG emissions and fossil energy consumption are harvest operation and biomass transport to the biogas plant (Tables 4 and 5).

Table 4. Assessment of the climate change in kg·CO_2-eqv. of 1 kWh_{el}. generated via the production and fermentation of dedicated energy crops and combustion of the biogas in CHP.

	Processes/Flows	**Maize per FU**		**Switchgrass per FU**		**Miscanthus per FU**		**Unit**
		2012	**2013**	**2012**	**2013**	**2012**	**2013**	
Input substrates	Production of nitrogen fertilizer	0.077	0.1185	0.0504	0.0800	0.0335	0.0369	kg·CO_2-eqv.
	Production of potassium fertilizer	0.0048	0.0075	0.0043	0.0068	0.0027	0.0029	kg·CO_2-eqv.
	Production of phosphate fertilizer	0.0064	0.0099	0.0047	0.0074	0.0027	0.0030	kg·CO_2-eqv.
	Recycling of nutrients	−0.0415	−0.0415	−0.0279	−0.0279	−0.0288	−0.0288	kg·CO_2-eqv.
	Herbicides	0.0028	0.0044	0.0012	0.0019	0.0008	0.0009	kg·CO_2-eqv.
	Seeds/Rhizomes	0.0002	0.0003	0.0001	0.0002	0.0003	0.0003	kg·CO_2-eqv.
Agricultural operations	Agricultural management	0.0075	0.0115	0.002	0.0032	0.0012	0.0013	kg·CO_2-eqv.
	Harvest	0.0038	0.0058	0.007	0.0111	0.0045	0.0049	kg·CO_2-eqv.
	Transport input substrates	0.0012	0.0018	0.0008	0.0013	0.0006	0.0006	kg·CO_2-eqv.
	Transport biomass	0.0049	0.0061	0.0047	0.0047	0.0045	0.0044	kg·CO_2-eqv.
	Ensilage	0.0003	0.0004	0.0005	0.0009	0.0004	0.0004	kg·CO_2-eqv.
	Fertilizer-induced emissions	0.0549	0.0906	0.0472	0.0725	0.0281	0.0311	kg·CO_2-eqv.
CHP	Biomass production system	0.1223	0.2154	0.0950	0.1622	0.0504	0.0580	kg·CO_2-eqv.
	CHP—Direct emissions	0	0	0	0	0	0	kg·CO_2-eqv.
	Credits heat utilization	−0.1021	−0.1021	−0.1021	−0.1021	−0.1021	−0.1021	kg·CO_2-eqv.
Total	**Total with credits**	**0.0202**	**0.1132**	**−0.0071**	**0.0600**	**−0.0518**	**−0.0441**	**kg·CO_2-eqv.**
	Total without credits	**0.1223**	**0.2154**	**0.0950**	**0.1622**	**0.0504**	**0.0580**	**kg·CO_2-eqv.**

Table 5. Assessment of the fossil fuel depletion in kg·oil-eqv. of 1 kWh$_{el}$. generated via the production and fermentation of dedicated energy crops and combustion of the biogas in CHP.

	Processes/Flows	**Maize per FU**		**Switchgrass per FU**		**Miscanthus per FU**		**Unit**
		2012	**2013**	**2012**	**2013**	**2012**	**2013**	
Input substrates	Production of nitrogen fertilizer	0.01598	0.02460	0.01046	0.01661	0.00695	0.00766	kg·oil-eqv.
	Production of potassium fertilizer	0.00206	0.00317	0.00182	0.00289	0.00113	0.00125	kg·oil-eqv.
	Production of phosphate fertilizer	0.00323	0.00497	0.00234	0.00372	0.00135	0.00148	kg·oil-eqv.
	Recycling of nutrients	−0.01020	−0.01020	−0.00742	−0.00742	−0.00774	−0.00774	kg·oil-eqv.
	Herbicides	0.00128	0.00196	0.00054	0.00087	0.00038	0.00042	kg·oil-eqv.
	Seeds/Rhizomes	0.00004	0.00005	0.00002	0.00003	0.00007	0.00008	kg·oil-eqv.
Agricultural operations	Agricultural management	0.00238	0.00367	0.00064	0.00101	0.00038	0.00042	kg·oil-eqv.
	Harvest	0.00121	0.00187	0.00222	0.00353	0.00143	0.00157	kg·oil-eqv.
	Transport input substrates	0.00037	0.00057	0.00027	0.00043	0.00019	0.00021	kg·oil-eqv.
	Transport biomass	0.00157	0.00194	0.00151	0.00152	0.00144	0.00139	kg·oil-eqv.
	Ensilage	0.00009	0.00014	0.00017	0.00028	0.00012	0.00013	kg·oil-eqv.
	Fertilizer-induced emissions	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	kg·oil-eqv.
CHP	Biomass production system	0.01801	0.03274	0.01258	0.02346	0.00569	0.00687	kg·oil-eqv.
	CHP—Direct emissions	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	kg·oil-eqv.
	Credits heat utilization	−0.03948	−0.03948	−0.03948	−0.03948	−0.03948	−0.03948	kg·oil-eqv.
Total	**Total with credits**	**−0.02147**	**−0.00674**	**−0.02691**	**−0.01602**	**−0.03379**	**−0.03262**	**kg·oil-eqv.**
	Total without credits	**0.01801**	**0.03274**	**0.01258**	**0.02346**	**0.00569**	**0.00687**	**kg·oil-eqv.**

3.2. Freshwater Eutrophication and Marine Eutrophication

The substitution of the fossil reference lead to a net increase in freshwater eutrophication of up to 3.5×10^{-5} kg·P-eqv. (kWh$_{el.}$)$^{-1}$ in all scenarios (Figure 5). On average, the freshwater eutrophication potentials are lowest for miscanthus, followed by switchgrass and then maize (Figure 5).

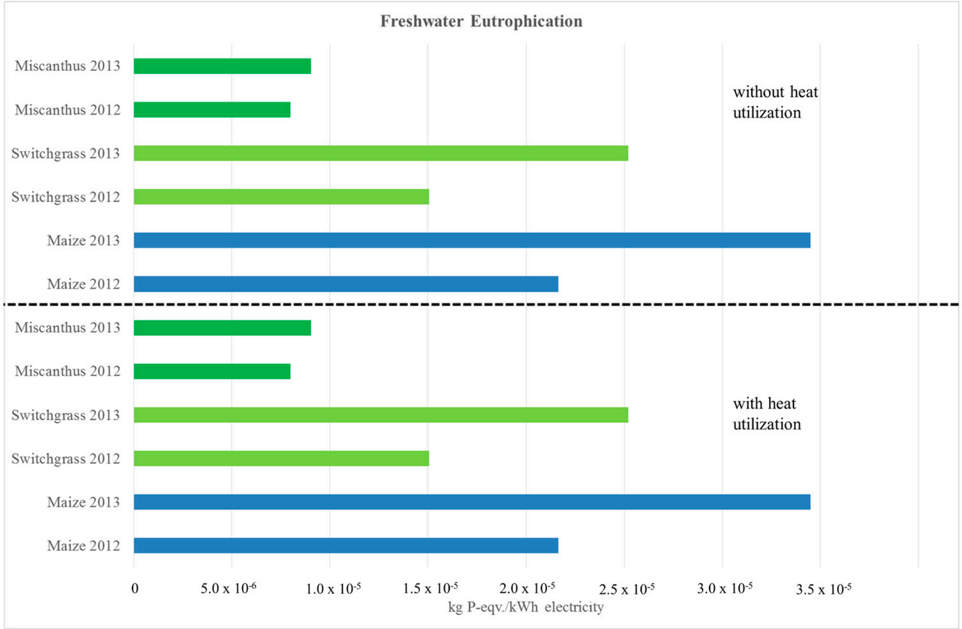

Figure 5. Assessment of the net impacts in kg·P-eqv. of substituting 1 kWh$_{el.}$ of the German electricity mix by power generated via combustion of the biogas in a CHP.

The recycling of nutrients leads to a high credit, which has a positive impact on the freshwater eutrophication (Table 6). In all scenarios, fertilizer-induced emissions account for the largest share of freshwater eutrophication. These are phosphate emissions associated with the use of phosphorus fertilizer, which are highest in maize and lowest in miscanthus (Table 6). The second-largest share comes from nitrogen fertilizer production, followed by the production of phosphate fertilizers (Table 6).

Table 6. Assessment of the freshwater eutrophication in kg·P-eqv. of 1 $kWh_{el.}$ generated via the production and fermentation of dedicated energy crops and combustion of the biogas in CHP.

	Processes/Flows	Maize per FU		Switchgrass per FU		Miscanthus per FU		Unit
		2012	2013	2012	2013	2012	2013	
Input substrates	Production of nitrogen fertilizer	1.18×10^{-7}	1.82×10^{-7}	7.74×10^{-8}	1.23×10^{-7}	5.14×10^{-8}	5.67×10^{-8}	kg·P-eqv.
	Production of potassium fertilizer	7.21×10^{-9}	1.11×10^{-8}	6.38×10^{-9}	1.01×10^{-8}	3.96×10^{-9}	4.36×10^{-9}	kg·P-eqv.
	Production of phosphate fertilizer	7.56×10^{-8}	1.16×10^{-7}	5.49×10^{-8}	8.72×10^{-8}	3.16×10^{-8}	3.48×10^{-8}	kg·P-eqv.
	Recycling of nutrients	-9.63×10^{-8}	-9.63×10^{-8}	-7.09×10^{-8}	-7.09×10^{-8}	-7.30×10^{-8}	-7.30×10^{-8}	kg·P-eqv.
	Herbicides	1.47×10^{-8}	2.26×10^{-8}	6.28×10^{-9}	9.97×10^{-9}	4.36×10^{-9}	4.80×10^{-9}	kg·P-eqv.
	Seeds/Rhizomes	1.34×10^{-7}	2.07×10^{-7}	2.88×10^{-8}	4.58×10^{-8}	2.76×10^{-7}	3.04×10^{-7}	kg·P-eqv.
Agricultural operations	Agricultural management	4.91×10^{-8}	7.56×10^{-8}	1.31×10^{-8}	2.09×10^{-8}	7.91×10^{-9}	8.72×10^{-9}	kg·P-eqv.
	Harvest	2.50×10^{-8}	3.85×10^{-8}	4.58×10^{-8}	7.28×10^{-8}	2.94×10^{-8}	3.24×10^{-8}	kg·P-eqv.
	Transport input substrates	7.63×10^{-9}	1.17×10^{-8}	5.55×10^{-9}	8.82×10^{-9}	3.87×10^{-9}	4.27×10^{-9}	kg·P-eqv.
	Transport biomass	3.23×10^{-8}	4.00×10^{-8}	3.12×10^{-8}	3.13×10^{-8}	2.97×10^{-8}	2.87×10^{-8}	kg·P-eqv.
	Ensilage	2.80×10^{-9}	2.80×10^{-9}	5.67×10^{-9}	5.67×10^{-9}	2.62×10^{-9}	2.62×10^{-9}	kg·P-eqv.
	Fertilizer-induced emissions	2.34×10^{-5}	3.60×10^{-5}	1.70×10^{-5}	2.70×10^{-5}	9.78×10^{-6}	1.08×10^{-5}	kg·P-eqv.
CHP	Biomass production system	2.38×10^{-5}	3.67×10^{-5}	1.72×10^{-5}	2.74×10^{-5}	1.01×10^{-5}	1.12×10^{-5}	kg·P-eqv.
	CHP—Direct emissions	0	0	0	0	0	0	kg·P-eqv.
	Credits heat utilization	-4.46×10^{-9}	-4.46×10^{-9}	-4.46×10^{-9}	-4.46×10^{-9}	-4.46×10^{-9}	-4.46×10^{-9}	kg·P-eqv.
Total	**Total with credits**	$\mathbf{2.38 \times 10^{-5}}$	$\mathbf{3.66 \times 10^{-5}}$	$\mathbf{1.72 \times 10^{-5}}$	$\mathbf{2.73 \times 10^{-5}}$	$\mathbf{1.01 \times 10^{-5}}$	$\mathbf{1.12 \times 10^{-5}}$	**kg·P-eqv.**
	Total without credits	$\mathbf{2.38 \times 10^{-5}}$	$\mathbf{3.67 \times 10^{-5}}$	$\mathbf{1.72 \times 10^{-5}}$	$\mathbf{2.74 \times 10^{-5}}$	$\mathbf{1.01 \times 10^{-5}}$	$\mathbf{1.12 \times 10^{-5}}$	**kg·P-eqv.**

A net benefit in the impact category marine eutrophication was achieved for the utilization of switchgrass and maize only in the year 2012—where the yield was significantly higher than in 2013—and when the heat utilization was accounted for (Figure 6). Miscanthus was the only crop that led to a reduction of marine eutrophication in comparison to the fossil reference in all years and scenarios. The maximum reduction was—4.6×10^{-5} kg·N-eqv. $(kWh_{el.})^{-1}$ (Figure 6).

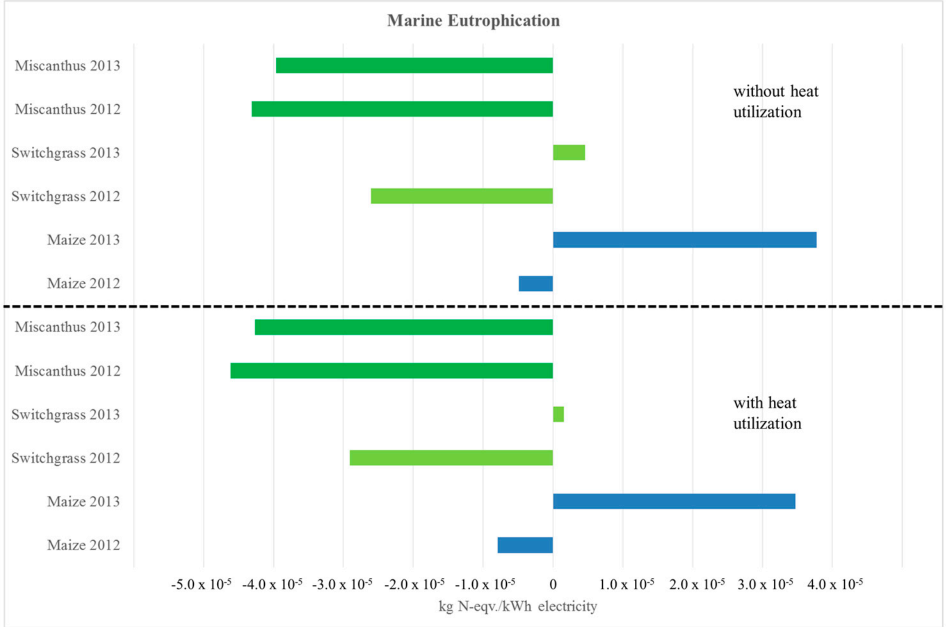

Figure 6. Assessment of the net benefits and impacts in kg·N-eqv. of substituting 1 kWh_{el}. of the German electricity mix by power generated via combustion of the biogas in a CHP.

The production of nitrogen fertilizer had the strongest impact on marine eutrophication for all crops, followed by fertilizer-induced emissions. Ammonia emissions and nitrate leaching due to the use of nitrogen fertilizer play a particularly important role here. Both impacts were highest for maize and lowest for miscanthus (Table 7). The recycling of nutrients results in a significant credit (Table 7).

Table 7. Assessment of the marine eutrophication in kg·N-eqv. of 1 kWh$_{el}$. generated via the production and fermentation of dedicated energy crops and combustion of the biogas in CHP.

	Processes/Flows	Maize per FU		Switchgrass per FU		Miscanthus per FU		Unit
		2012	2013	2012	2013	2012	2013	
Input substrates	Production of nitrogen fertilizer	2.60×10^{-5}	4.01×10^{-5}	1.70×10^{-5}	2.70×10^{-5}	1.13×10^{-5}	1.25×10^{-5}	kg·N-eqv.
	Production of potassium fertilizer	5.80×10^{-7}	8.92×10^{-7}	5.13×10^{-7}	8.14×10^{-7}	3.18×10^{-7}	3.51×10^{-7}	kg·N-eqv.
	Production of phosphate fertilizer	1.22×10^{-6}	1.87×10^{-6}	8.83×10^{-7}	1.40×10^{-6}	5.08×10^{-7}	5.60×10^{-7}	kg·N-eqv.
	Recycling of nutrients	-1.29×10^{-5}	-1.29×10^{-5}	-8.18×10^{-6}	-8.18×10^{-6}	-8.36×10^{-6}	-8.36×10^{-6}	kg·N-eqv.
	Herbicides	3.75×10^{-7}	5.76×10^{-7}	1.60×10^{-7}	2.54×10^{-7}	1.11×10^{-7}	1.22×10^{-7}	kg·N-eqv.
	Seeds/Rhizomes	1.89×10^{-6}	2.91×10^{-6}	1.06×10^{-6}	1.69×10^{-6}	1.64×10^{-6}	1.80×10^{-6}	kg·N-eqv.
Agricultural operations	Agricultural management	4.20×10^{-6}	6.46×10^{-6}	1.18×10^{-6}	1.87×10^{-6}	7.05×10^{-7}	7.77×10^{-7}	kg·N-eqv.
	Harvest	2.10×10^{-6}	3.23×10^{-6}	3.85×10^{-6}	6.11×10^{-6}	2.47×10^{-6}	2.72×10^{-6}	kg·N-eqv.
	Transport input substrates	2.97×10^{-7}	4.56×10^{-7}	2.16×10^{-7}	3.43×10^{-7}	1.50×10^{-7}	1.66×10^{-7}	kg·N-eqv.
	Transport biomass	2.97×10^{-6}	3.67×10^{-6}	2.86×10^{-6}	2.87×10^{-6}	2.72×10^{-6}	2.64×10^{-6}	kg·N-eqv.
	Ensilage	1.94×10^{-7}	2.98×10^{-7}	3.80×10^{-7}	6.03×10^{-7}	2.52×10^{-7}	2.78×10^{-7}	kg·N-eqv.
	Fertilizer-induced emissions	4.09×10^{-5}	6.29×10^{-5}	2.67×10^{-5}	4.25×10^{-5}	1.78×10^{-5}	1.96×10^{-5}	kg·N-eqv.
CHP	Biomass production system	6.78×10^{-5}	1.10×10^{-4}	4.67×10^{-5}	7.73×10^{-5}	2.96×10^{-5}	3.31×10^{-5}	kg·N-eqv.
	CHP-Direct emissions	4.58×10^{-6}	4.58×10^{-6}	4.58×10^{-6}	4.58×10^{-6}	4.58×10^{-6}	4.58×10^{-6}	kg·N-eqv.
	Credits heat utilization	-3.04×10^{-6}	-3.04×10^{-6}	-3.04×10^{-6}	-3.04×10^{-6}	-3.04×10^{-6}	-3.04×10^{-6}	kg·N-eqv.
Total	**Total with credits**	$\mathbf{6.94 \times 10^{-5}}$	$\mathbf{11.2 \times 10^{-5}}$	$\mathbf{4.82 \times 10^{-5}}$	$\mathbf{7.88 \times 10^{-5}}$	$\mathbf{3.11 \times 10^{-5}}$	$\mathbf{3.47 \times 10^{-5}}$	**kg·N-eqv.**
	Total without credits	$\mathbf{7.24 \times 10^{-5}}$	$\mathbf{11.5 \times 10^{-5}}$	$\mathbf{5.13 \times 10^{-5}}$	$\mathbf{8.19 \times 10^{-5}}$	$\mathbf{3.42 \times 10^{-5}}$	$\mathbf{3.77 \times 10^{-5}}$	**kg·N-eqv.**

3.3. Terrestrial Acidification

All scenarios led to higher terrestrial acidification than the fossil references. Maize without heat utilization performed worst and led to emissions of 3.5×10^{-3} kg·SO$_2$-eqv. (kWh$_{el.}$)$^{-1}$ (Figure 7). Miscanthus performed best with the lowest terrestrial acidification potential (Figure 7).

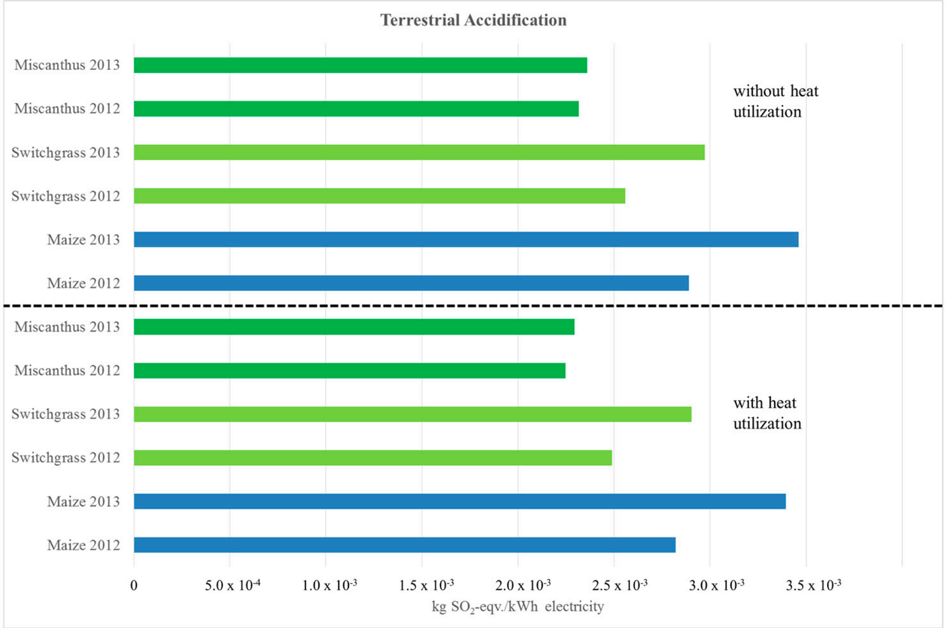

Figure 7. Assessment of the net benefits and impacts in kg·SO$_2$-eqv. of substituting 1 kWh$_{el.}$ of the German electricity mix by power generated via combustion of the biogas in a CHP.

Fertilizer-induced emissions—especially ammonia—had the highest impact on terrestrial acidification for all crops and accounted on an average for around 20% of total emissions (Table 8). The second largest source of emissions responsible for terrestrial acidification was production of nitrogen fertilizer, followed by transport of the biomass (Table 8).

Table 8. Assessment of the terrestrial acidification in kg·SO_2-eqv. of 1 kWh_{el}. generated via the production and fermentation of dedicated energy crops and combustion of the biogas in CHP.

	Processes/Flows	**Maize per FU**		**Switchgrass per FU**		**Miscanthus per FU**		**Unit**
		2012	**2013**	**2012**	**2013**	**2012**	**2013**	
Input substrates	Production of nitrogen fertilizer	7.34×10^{-5}	1.13×10^{-4}	4.80×10^{-5}	7.63×10^{-5}	3.19×10^{-5}	3.52×10^{-5}	kg·SO_2-eqv.
	Production of potassium fertilizer	8.25×10^{-6}	1.27×10^{-5}	7.30×10^{-6}	1.16×10^{-5}	4.53×10^{-6}	5.00×10^{-6}	kg·SO_2-eqv.
	Production of phosphate fertilizer	4.73×10^{-5}	7.28×10^{-5}	3.43×10^{-5}	5.45×10^{-5}	1.97×10^{-5}	2.18×10^{-5}	kg·SO_2-eqv.
	Recycling of nutrients	-6.22×10^{-5}	-6.22×10^{-5}	-4.71×10^{-5}	-4.71×10^{-5}	-4.88×10^{-5}	-4.88×10^{-5}	kg·SO_2-eqv.
	Herbicides	6.35×10^{-6}	9.77×10^{-6}	2.71×10^{-6}	4.31×10^{-6}	1.88×10^{-6}	2.08×10^{-6}	kg·SO_2-eqv.
	Seeds/Rhizomes	2.19×10^{-6}	3.36×10^{-6}	8.04×10^{-7}	1.28×10^{-6}	1.98×10^{-6}	2.18×10^{-6}	kg·SO_2-eqv.
Agricultural operations	Agricultural management	5.16×10^{-5}	7.95×10^{-5}	1.46×10^{-5}	2.32×10^{-5}	8.73×10^{-6}	9.62×10^{-6}	kg·SO_2-eqv.
	Harvest	2.58×10^{-5}	3.97×10^{-5}	4.72×10^{-5}	7.50×10^{-5}	3.03×10^{-5}	3.34×10^{-5}	kg·SO_2-eqv.
	Transport input substrates	8.90×10^{-7}	1.37×10^{-6}	6.47×10^{-7}	1.03×10^{-6}	4.51×10^{-7}	4.97×10^{-7}	kg·SO_2-eqv.
	Transport biomass	3.69×10^{-5}	4.57×10^{-5}	3.56×10^{-5}	3.57×10^{-5}	3.39×10^{-5}	3.28×10^{-5}	kg·SO_2-eqv.
	Ensilage	2.46×10^{-6}	3.78×10^{-6}	4.83×10^{-6}	7.67×10^{-6}	3.21×10^{-6}	3.54×10^{-6}	kg·SO2-eqv.
	Fertilizer-induced emissions	8.29×10^{-4}	1.28×10^{-3}	5.42×10^{-4}	8.61×10^{-4}	3.61×10^{-4}	3.97×10^{-4}	kg·SO_2-eqv.
CHP	Biomass production system	1.02×10^{-3}	1.60×10^{-3}	6.91×10^{-4}	1.10×10^{-3}	4.48×10^{-4}	4.95×10^{-4}	kg·SO_2-eqv.
	CHP - Direct emissions	2.61×10^{-3}	2.61×10^{-3}	2.61×10^{-3}	2.61×10^{-3}	2.61×10^{-3}	2.61×10^{-3}	kg SO_2-eqv.
	Credits heat utilization	-6.82×10^{-5}	-6.82×10^{-5}	-6.82×10^{-5}	-6.82×10^{-5}	-6.82×10^{-5}	-6.82×10^{-5}	kg·SO_2-eqv.
Total	**Total with credits**	$\mathbf{3.57 \times 10^{-3}}$	$\mathbf{4.14 \times 10^{-3}}$	$\mathbf{3.24 \times 10^{-3}}$	$\mathbf{3.65 \times 10^{-3}}$	$\mathbf{2.99 \times 10^{-3}}$	$\mathbf{3.04 \times 10^{-3}}$	**kg·SO_2-eqv.**
	Total without credits	$\mathbf{3.64 \times 10^{-3}}$	$\mathbf{4.21 \times 10^{-3}}$	$\mathbf{3.31 \times 10^{-3}}$	$\mathbf{3.72 \times 10^{-3}}$	$\mathbf{3.06 \times 10^{-3}}$	$\mathbf{3.11 \times 10^{-3}}$	**kg·SO_2-eqv.**

4. Discussion

Here the results of this study are considered in a broader context, also including other environmental aspects not modeled in the LCA. The discussion concludes with opportunities and challenges of the introduction of novel perennial C4 crops in the biogas sector.

4.1. Environmental Performance in Impact Categories Modelled in the LCA

The results of this study show that, as soon as more impact categories are assessed than climate change and fossil fuel depletion, the environmental performance of the bioenergy conversion route "biogas" is not so clear-cut. All three energy crops have a significantly better environmental profile than the fossil reference (German electricity mix) in the impact categories climate change (CC) and fossil fuel depletion (FFD). Similar findings have been reported in the literature [45,46]. However, all three energy crops showed significantly higher impacts than the fossil reference in the impact categories freshwater eutrophication (FE) and terrestrial acidification (TA). The results for marine eutrophication (ME) were more variable. Here, miscanthus (both years) and switchgrass (2012 only) had a significantly lower impact than the fossil reference, whereas maize had a significantly higher impact in 2013 due to the low yield. High biomass yields have been shown to be a crucial factor for favorable environmental performance [47]. Again, these results correspond to findings of other studies, which mainly also found a higher impact of energy-crop-derived biogas than the fossil reference in acidification and eutrophication potential [48–50].

4.1.1. Overall Impact of Process Steps in Impact Categories

The production of nitrogen fertilizer was identified as the most relevant process step in the impact categories FFD and CC and the second most relevant in ME. Fertilizer-induced emissions were identified as the most important flow in the categories FE and ME and second most important in CC and TA. Similar results have been reported in the literature and numerous studies have already described the strong impact of nitrogen fertilizer production and related direct and indirect emissions on FFD and CC (e.g., [39,46,50,51]). The present study also showed a strong impact of mineral nitrogen fertilizer application on eutrophication (FE and ME) and acidification potential of crop production. This seems logical, since nitrate is one of the major contributors to eutrophication and the nitrification process a major contributor to soil acidification [27].

In TA, direct CHP emissions were the most important flow. Rehl et al. [49] identified sulfur dioxide from the CHP as one of the most important contributors to the acidification potential. One possibility to reduce these emissions could be the upgrading of biogas to biomethane, because sulfur dioxide is almost completely removed during this process. In addition, new techniques for biomethane production (e.g., pressurized anaerobic digestion) could help reduce the carbon footprint of biomethane production in the near future, because the demand for energy-intensive compression is reduced in such approaches [52]. Lijó et al. [53] reported production of nitrogen fertilizer, fertilizer-induced emissions and emissions of agricultural management as important factors for the environmental performance of energy crops. In this study, emissions from agricultural management were found to be the third most relevant process in CC, FFD and ME for maize cultivation, but considerably less important for miscanthus and switchgrass.

4.1.2. Impact of the Process Steps for Each Crop

Emissions and fossil fuel depletion from production of nitrogen fertilizer and agricultural management and fertilizer-induced emissions were highest for maize in each of the considered impact categories. This is because maize production consumes more energy for soil cultivation and requires higher nitrogen fertilizer levels for high yields than the C4 perennial grasses. For maize, data from the treatment with the highest nitrogen fertilization (240 $\text{kg}\cdot\text{N}\cdot\text{ha}^{-1}$) were used, which on long-term average yielded significantly higher than the medium fertilization rate (120 $\text{kg}\cdot\text{N}\cdot\text{ha}^{-1}$). However, the high nitrogen fertilization is probably above the marginal revenue and a lower fertilization rate could reduce the environmental impact of maize. Nevertheless, the nitrogen demand of miscanthus and switchgrass are still lower than that of maize. In addition, for miscanthus and switchgrass, data from the treatment with the highest nitrogen fertilization rate (80 $\text{kg}\cdot\text{N}\cdot\text{ha}^{-1}$) were used, in order to consider the higher nutrient removal by the green harvested biomass. Although green harvest increases the withdrawal of nitrogen compared to a spring harvest, the biomass of miscanthus and switchgrass contained approximately 60% less nitrogen than maize biomass (Table 3).

The annual cultivation of maize led also to significantly higher emissions and fossil fuel depletion for agricultural management in CC, ME and FFD. For this reasons, changing the crop production system from annual crops with a high nitrogen demand to perennial C4 crops with improved nutrient efficiency seems to be a very promising option for increasing the environmental sustainability of the biogas sector and the bioeconomy, as already described by Lewandowski [54]. Compared to maize, miscanthus and switchgrass showed in the scenarios without heat utilization 59%–73% and 25%–28% lower CC potential, 68%–79% and 28%–30% lower FFD potential, 57%–69% and 25%–28% lower FE potential, 53%–67% and 29% lower ME potential and 16%–26% and 9%–12% lower TA potential, respectively.

Considering all impact categories, miscanthus performed best amongst the three assessed crops. Especially in 2013, the yield and thereby the environmental performance of miscanthus was much more stable compared to maize and switchgrass. Both crops reacted more sensitively to the unfavorable weather conditions in 2013. This resulted in lower yields and is also reflected by the performance in the environmental impact categories. The higher stress tolerance and yield stability of miscanthus is therefore not only favorable for the farmer, but also from an environmental point of view.

The nutrient recycling via fermentation residues led to a significant credit for all crops, especially in the impact categories CC, FFD and ME. However, fermentation residue application on the perennial grasses miscanthus and switchgrass and resulting emissions need to be further investigated. Since the fermentation residues cannot be incorporated into the soil in such perennials, higher ammonia emissions could occur, which could lead to higher eutrophication and acidification potentials [48]. This needs to be further investigated to allow consideration of such an effect in future assessments of the environmental performance.

4.2. Other Environmental Aspects

In the section above, the environmental performance was analyzed in five impact categories and it was shown that the perennial grasses, especially miscanthus, performed better than the annual crop maize. However, the five considered impact categories are not sufficient for a holistic assessment of the environmental performance. Therefore, other aspects relevant to environmental performance are discussed in the following section.

Intensive soil cultivation in annual maize is accompanied by an increased risk of soil erosion, due to the slow youth development of the crop [6]. For annual maize, there is also a low to medium risk for soil compaction [55]. However, for green-harvested miscanthus and switchgrass the risk of soil compaction may be lower due to its perennial nature, but needs to be assessed to allow comparison. The combination of intensive soil cultivation and low amount of crop residues in silage maize has a negative impact on content of soil organic carbon. Both environmental aspects could be improved by changing substrate supply of biogas plants from maize to perennial C4 grasses, since miscanthus and switchgrass generally lead to an increase in soil organic carbon compared to annual cropping systems [11,56,57]. Under miscanthus, the largest proportion of the soil organic carbon is found in the topsoil, which can be explained by the high proportion of roots in the top 0.35 m [58]. The sequestration of carbon in the soil can increase the GHG mitigation potential significantly, especially if the cropping system is changed from annual to perennial [56,59]. In this study, the sequestration effect was not considered, because the effect of the green harvest on the root and rhizome development and on the soil carbon sequestration potential is not yet known. Therefore, the development of the soil organic carbon under green harvested miscanthus and switchgrass needs to be further investigated to determine the sequestration potential of this harvest regime.

Agricultural land occupation is another important environmental aspect, due to limited expansion potential for agricultural land and negative impacts from the transformation of natural land. In this paper, agricultural land occupation was not directly assessed, but the data in Table 1 show that maize required the smallest area (173 ha) of agricultural land in 2012 to supply the biogas plant with the required biomass. Changing the input substrate from maize to miscanthus or switchgrass increased the agricultural land demand in 2012 by 12% or 68%, respectively. Under unfavorable weather conditions in 2013, the agricultural land demand for miscanthus cropping was 20% lower and for switchgrass 73% higher than for maize cultivation. Agricultural land occupation for biogas production can lead to indirect land-use change (iLUC), which can significantly reduce the GHG mitigation potential and even lead to higher GWP than the fossil reference [14]. For this reason, the comparatively high agricultural land demand of switchgrass to deliver the required biomass substrate is a clear disadvantage compared to the other crops. In contrast, the area demand of miscanthus was only slightly higher and even lower when unfavorable weather conditions occurred for maize production. Again, the higher abiotic stress tolerance and yield stability of miscanthus can be seen as environmental advantage. However, both perennial C4 crops could be grown in future mainly on marginal or contaminated land [9,23]. This could reduce the pressure on agricultural land and expand the area available for biomass production.

Biodiversity is difficult to assess just by the crop itself, because it strongly depends on other factors, e.g., the distribution of fields in a landscape and structural elements such as hedges. However, modern agriculture is assumed to have a negative impact on the biodiversity by simplification of agricultural landscapes, e.g., large field sizes, and small amount of crop varieties which are grown in monoculture [4]. An increased number of crop species and a higher proportion of perennial cropping systems in modern agriculture is seen as one option to promote biodiversity [4]. For this reason, replacing biogas maize with miscanthus or switchgrass could positively affect the biodiversity by adding novel, perennial crops to the agricultural landscapes. However, it should be noted that the impact on soil biodiversity may be influenced by the choice of the perennial biomass crop [60]. Furthermore, both perennials can be characterized by their comparatively low-input crop management, after their successful establishment in year one. For miscanthus, a higher abundance of insects, spiders and earthworms than in arable land is reported, as well as additional niches for birds and, provided a spring harvest is performed, over winter cover for small mammals in intensive arable regions [11,61]. For switchgrass, similar positive effects can be expected, which leads to the assumption, that both could increase the biodiversity and structure-richness of agricultural landscapes. Again, the effect of the pre-winter harvest, which clearly removes the winter cover for small mammals and reduces the mulch layer, is not yet known and needs to be investigated. However, both crops also induce risks for biodiversity because they are not native to Europe and could potentially appear as invasive species. *Miscanthus x giganteus* has a very low invasiveness risk, because it does not produce fertile seeds and no escapes were observed over more than two decades of *M. x giganteus* production in Europe. Current miscanthus breeding efforts aim to produce fertile genotypes that can be propagated by seeds [10], but several mechanisms to avoid seed escape are incorporated, including preferring candidates which require a very long vegetation period for seed production to avoid viable seeds being produced in regions of biomass cultivation [9]. It is also necessary to mention that miscanthus as well as switchgrass seedlings have a very low competitiveness compared to weeds and a slow youth development. For this reason it is quite unlikely that they become invasive species in Europe. Nonetheless, the invasiveness potential of novel miscanthus genotypes and switchgrass needs to be investigated and monitored.

Finally, the socioeconomic aspects of landscape appearance need to be considered. Crops such as maize are often criticized in the public, due to their height and monotony. The same could appear for miscanthus, due to its height and density in well-established commercial fields. Smaller and nicely flowering miscanthus genotypes or switchgrass could be experienced more favorably and might influence the appearance of landscapes more positively. However, this could compromise the yield and lead to a trade-off between yield and public acceptance. Public acceptance could also be positively influenced by using smaller fields or strip cropping instead large monoculture fields.

4.3. Implementation—Chances and Challenges

In this study, it is shown that implementation of perennial C4 grasses for biogas production can have significant environmental benefits. From an environmental point of view, miscanthus in particular would be a desirable crop for biogas production. The main weak point of switchgrass is clearly its lower yield potential than miscanthus and related to that its higher area demand, fossil fuel consumption and emissions. For the farmer, the implementation of miscanthus and switchgrass as biogas crops is accompanied by opportunities and challenges, which are discussed in the following section but require further research.

This study is based on methane yields measured in a batch test using milled biomass. In order to transfer these values to a full-scale biogas plant, a pre-treatment of the biomass was considered for miscanthus and switchgrass, which leads to a higher electricity demand for plant operation. For this reason, the electricity demand for miscanthus and switchgrass was assumed to be almost twice as high as that for maize. Before implementation, the methane yield, the necessity of a pre-treatment and the energy consumption of such a pre-treatment should be verified under more realistic conditions. Ensiling of miscanthus biomass, and presumably also switchgrass, appears possible [19], but also needs to be demonstrated in practice.

The long-term performance of green-harvested miscanthus is one of the major uncertainties for its biogas utilization, because miscanthus reacts sensitively to very early mid-season harvest, but tolerates green harvest in late autumn [18]. However, it is not yet known if green-harvested miscanthus is productive for as long as a spring-harvested crop (more than 20 years) and if recycling of fermentation residues is sufficient to maintain its productivity. In addition, the farmer has to dedicate arable land to miscanthus for several years to achieve return on investment, due to the high establishment costs. However, current research focuses on reducing establishment costs by developing seed-based genotypes, which may allow direct sowing in future [10]. Further, most biogas plants are designed for a minimum of 20 years' operation, which would fit in very well with the expected productive lifetime of miscanthus. Cost-effective miscanthus establishment offers the chance of significantly reducing biomass costs. As shown in this paper, the yield of miscanthus is not as sensitive as annual maize to unfavorable weather conditions, which may become more common in future due to climate change. One of the main reasons for the low maize yield was the very late sowing date and the early summer drought stress. In miscanthus, planting is only required once in 20–30 years and the established crop benefits from winter soil moisture. Therefore, miscanthus seems very suitable for risk mitigation of such weather conditions.

In contrast to miscanthus, switchgrass can be established cheaply via direct sowing of seeds. However, the establishment of switchgrass is difficult due to an often low germination rate, low competitiveness of seedlings and limited availability of herbicides. Current research focuses on the optimization of the establishment method and herbicide testing [62]. Nevertheless, early green harvest of switchgrass seems less problematic than in miscanthus and even a double cut is possible [21]. The shorter productive life of approximately 15 years, lower investment costs and the ability of direct sowing may increase farmers' willingness to adopt this crop. However, the lower yield potential limits its implementation to very poor and shallow soils, where it is likely to perform better than miscanthus [23].

From an environmental point of view, miscanthus cultivation for biogas production is generally recommended if the biogas plant technology is suitable for the digestion of fibrous substrates or adequate pre-treatment options are available.

Supplementary Materials: The following are available online at www.mdpi.com/2071-1050/9/1/5/s1, Table S1: Climate change in kg CO_2-eqv. per kg DM biomass, Table S2: Freshwater eutrophication in kg P-eqv. per kg DM biomass, Table S3: Fossil fuel depletion potential in kg oil-eqv. per kg DM biomass, Table S4: Marine eutrophication potential in kg N-eqv. per kg DM biomass, Table S5: Terrestrial acidification potential in kg SO_2-eqv. per kg DM biomass.

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Chapter 4 - Site-Specific Management of Miscanthus Genotypes for Combustion and Anaerobic Digestion: A Comparison of Energy Yields

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Site-Specific Management of Miscanthus Genotypes for Combustion and Anaerobic Digestion: A Comparison of Energy Yields

Andreas Kiesel[1]*, Christopher Nunn[2], Yasir Iqbal[1], Tim Van der Weijde[3], Moritz Wagner[1], Mensure Özgüven[4], Ivan Tarakanov[5], Olena Kalinina[1], Luisa M. Trindade[3], John Clifton-Brown[2] and Iris Lewandowski[1]

[1] Department Biobased Products and Energy Crops, Institute of Crop Science, University of Hohenheim, Stuttgart, Germany, [2] Institute of Biological, Environmental and Rural Sciences, Aberystwyth University, Aberystwyth, UK, [3] Department of Plant Breeding, Wageningen University, Wageningen, Netherlands, [4] Faculty of Agriculture and Natural Sciences, Konya Food and Agriculture University, Konya, Turkey, [5] Russian State Agrarian University–Moscow Timiryazev Agricultural Academy, Moscow, Russia

Edited by:
Soren K. Rasmussen,
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Reviewed by:
Davide Fissore,
Politecnico di Torino, Italy
Hinrich Uellendahl,
Aalborg University, Denmark

***Correspondence:**
Andreas Kiesel
a.kiesel@uni-hohenheim.de

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In Europe, the perennial C_4 grass miscanthus is currently mainly cultivated for energy generation via combustion. In recent years, anaerobic digestion has been identified as a promising alternative utilization pathway. Anaerobic digestion produces a higher-value intermediate (biogas), which can be upgraded to biomethane, stored in the existing natural gas infrastructure and further utilized as a transport fuel or in combined heat and power plants. However, the upgrading of the solid biomass into gaseous fuel leads to conversion-related energy losses, the level of which depends on the cultivation parameters genotype, location, and harvest date. Thus, site-specific crop management needs to be adapted to the intended utilization pathway. The objectives of this paper are to quantify (i) the impact of genotype, location and harvest date on energy yields of anaerobic digestion and combustion and (ii) the conversion losses of upgrading solid biomass into biogas. For this purpose, five miscanthus genotypes (OPM 3, 6, 9, 11, 14), three cultivation locations (Adana, Moscow, Stuttgart), and up to six harvest dates (August–March) were assessed. Anaerobic digestion yielded, on average, 35% less energy than combustion. Genotype, location, and harvest date all had significant impacts on the energy yield. For both, this is determined by dry matter yield and ash content and additionally by substrate-specific methane yield for anaerobic digestion and moisture content for combustion. Averaged over all locations and genotypes, an early harvest in August led to 25% and a late harvest to 45% conversion losses. However, each utilization option has its own optimal harvest date, determined by biomass yield, biomass quality, and cutting tolerance. By applying an autumn green harvest for anaerobic digestion and a delayed harvest for combustion, the conversion-related energy loss was reduced to an average of 18%. This clearly shows that the delayed harvest required to maintain biomass quality for combustion is accompanied by high energy losses through yield reduction over winter. The pre-winter harvest applied in the biogas utilization pathway avoids these yield losses and largely compensates for the conversion-related energy losses of anaerobic digestion.

Keywords: biogas, harvest time, biomass, yield, energy yield, substrate-specific methane yield, moisture content

INTRODUCTION

Miscanthus is a resource-use efficient, high-yielding perennial C4 grass species native to East Asia, including China, Korea, Taiwan, and Japan (Lewandowski and Schmidt, 2006; Clifton-Brown et al., 2015). The cultivation of miscanthus is characterized by its perennial nature and low nitrogen-fertilization demand, due to its effective nutrient recycling system (Christian et al., 2008; Strullu et al., 2011; Cadoux et al., 2012). This leads to a generally benign environmental profile, often associated with soil carbon sequestration (McCalmont et al., 2017). For these reasons, miscanthus biomass utilization generally shows a low global-warming and resource-depletion potential (Felten et al., 2013; Styles et al., 2015; Meyer et al., 2016). Despite these positive aspects, the miscanthus cultivation area is still rather small in Europe, mainly due to its high establishment costs and the current lack of valorisation options.

The only cultivar presently commercially available is *Miscanthus x giganteus* (Mxg), a natural, sterile hybrid of *Miscanthus sacchariflorus* and *Miscanthus sinensis*, which was introduced into Europe in 1935 (Greef et al., 1997; Clifton-Brown et al., 2015). As Mxg is sterile, only clonal propagation is possible. This is costly and does not allow for crop development by conventional breeding. Therefore, miscanthus breeding for European conditions is mainly focussing on the groups *M. sinensis*, *M. sacchariflorus,* and *Miscanthus floridulus*, which offer broad genetic variability and the possibility of reducing establishment costs through economical, seed-based propagation (van der Weijde et al., 2013; Clifton-Brown et al., 2017). In the EU project OPTIMISC (FP7 No. 289159), early stage crossings from the ongoing miscanthus breeding programmes of Aberystwyth (IBERS) and Wageningen University (WUR) were tested at several locations, under different stress conditions and for various utilization options (Lewandowski et al., 2016).

Combustion is one of the most common utilization options for miscanthus biomass, but production of cellulosic ethanol and anaerobic digestion were identified as promising alternatives (van der Weijde et al., 2013, 2017b; Mayer et al., 2014; Wahid et al., 2015; Kiesel and Lewandowski, 2017). For each utilization option, ideal harvest time is of crucial importance to maintain high quality and yield. For combustion, the harvest time is delayed to reduce the contents of moisture, ash, and critical elements (Iqbal and Lewandowski, 2014). However, there is a trade-off here between yield and quality, as leaf losses occur over winter and lead to a decrease in biomass yield (Iqbal et al., under review). For biogas, an early green harvest delivers a higher quality, since the substrate-specific methane yield decreases with ongoing lignification (Kiesel and Lewandowski, 2017). Here again there is a trade-off, as a very early green harvest delivers a lower yield, due to insufficient utilization of the vegetation period, and also impairs the crop growth the next season due to insufficient relocation of carbohydrates (Purdy et al., 2015; Kiesel and Lewandowski, 2017). The latter is referred to as “cutting tolerance,” which has been defined for miscanthus as the ability of the crop to recover from an early green harvest without yield reductions in the following year (Kiesel and Lewandowski, 2017). As the ideal harvest time is a compromise between yield, quality, and cutting tolerance in both utilization options, the development of the energy yield (which includes biomass yield and quality) needs to be quantified throughout the year. In addition, a comparison of energy yield between combustion and anaerobic digestion is required to establish the loss associated with the generation of the higher-value product. In this case, biomethane—which is upgraded solid biomass—is seen as a higher-value product. As a gaseous fuel, it has a broader range of applications, including transport fuel, and its application in combined heat and power generation is easier, including transport, storage, and utilization of biomethane in existing natural gas infrastructure.

In addition to harvest time, the genotype also affects biomass quality. For combustion, genotypes with low contents of moisture, ash and critical elements at harvest are optimal, while for anaerobic digestion a low degree of lignification and ease of digestibility is preferred. Iqbal and Lewandowski (2014) found notable genotypic differences in contents of ash and critical elements, which can be partly attributed to genotypic differences in nutrient relocation and leaching of soluble elements. For biogas and ethanol utilization, van der Weijde et al. (2017b) observed both a higher saccharification potential and substrate-specific methane yield in less lignified genotypes. Location may also play a crucial role. For example, drought conditions can increase the saccharification potential of miscanthus biomass (van der Weijde et al., 2017a).

The objective of this paper is (i) to identify the effect of genotype, environment and harvest time on yield and biomass quality for anaerobic digestion and combustion and (ii) to compare the energy yield of both pathways throughout the year. For this purpose, five miscanthus genotypes from the OPTIMISC multi-location field trials were sampled at monthly intervals throughout the end of the vegetation period until final harvest in spring at the locations in Adana (Turkey), Moscow (Russia), and Stuttgart (Germany). Energy yield, biomass yield, and a number of quality parameters (including substrate-specific methane yield) were assessed and compared for each sampling date. This allows identification of site-specific optimization potentials for each utilization option. This paper focuses on biomass quality for anaerobic digestion, but also includes some basic quality criteria relevant for the energy yield via combustion, such as moisture and ash content. A detailed combustion quality analysis, including the content of critical elements, and a quantification of the trade-off between yield and biomass quality can be found in Iqbal et al. (under review). Further the net energy yield via anaerobic digestion and combustion, which considers moisture and ash content, was assessed and compared, to allow site-specific identification of the best suited harvest date for each utilization option.

MATERIALS AND METHODS

Field Trial

The field trial was established in 2012 as part of the EU-financed project OPTIMISC (FP7 No. 289159) to compare 15 miscanthus genotypes at 6 sites across Europe and Russia: at Aberystwyth (UK), Adana (Turkey), Moscow (Russia), Potash (Ukraine), Stuttgart (Germany), and Wageningen (Netherlands). It was set up in a randomized block design with three biological replications at each location. A detailed description of the field trial including genotypes used, soil and climatic conditions can be found in Kalinina et al. (under review) and Lewandowski et al. (2016). For this paper, five genotypes (best yields) and three locations (contrasting climates) were selected, where at least one representative from each miscanthus group (species) was included. The selected genotypes are shown in **Table 1** and the chosen locations were Adana, Moscow, and Stuttgart.

The genotypes were sampled at intervals of 1–2 months from the end of vegetation period until the final harvest in spring (**Table 2**). In Moscow and Stuttgart, the final harvest was performed in March. In Adana, it took place in January, because the plants had already started to regrow. In Moscow, sampling was interrupted after September to the final harvest, because the aboveground parts of the crop were completely killed by a harsh frost a few days before the sampling date in September.

Figure 1 depicts rainfall and temperature data for the three locations Adana, Moscow, and Stuttgart. In Adana, a seasonal drought period occurred in July and August. There was only little frost in January 2015 (**Figure 1A**). In Moscow, July was particularly dry and the plants faced a serious drought (**Figure 1B**). The winter started very abruptly at the end of September with harsh frosts and the crop was frozen most of the time until March. In Stuttgart, June was abnormally dry, but in the following 2 months the rainfall was higher than usual (**Figure 1C**). Overall, the winter 2014/2015 was mild, but there was a frost period in January and February 2015.

Biomass Yield Estimation

On each sampling date, eight tillers were collected randomly from each genotype. The samples were taken from the second outer row to avoid damaging the core plot, which was used for final harvest biomass yield estimation. To ensure the samples were taken randomly, a bar with marks every 60 cm was used. The tiller closest to each 60-cm mark was collected. The central four m^2 of each plot were used for biomass yield estimation at final harvest in January (Adana) or March (Moscow, Stuttgart) and harvested manually using a hedge trimmer or sickle bar mower. Before the final harvest, another eight tillers were collected randomly. All samples were dried to constant weight at 60°C in a cabinet dryer and fresh and dry weight was recorded. Dry matter content and reciprocal value moisture content were calculated according to weight loss. Based on the weight of the eight tillers at each sampling date and the biomass yield at final harvest, the dry and fresh matter yield at each sampling date was calculated (Equation 1). The dry matter yield at each sampling date was calculated using a ratio of the stem weights at the sampling date and the final harvest. The details of this calculation are described by Nunn et al. (under review).

TABLE 1 | Miscanthus "genotypes" used in this investigation (Lewandowski et al., 2016).

Genotype ID	Provider	Species
OPM 3	IBERS	*Miscanthus sacchariflorus*
OPM 6	IBERS	*Miscanthus sinensis* x *Miscanthus sacchariflorus* hybrid
OPM 9	IBERS	*Miscanthus* x *giganteus*
OPM 11	IBERS	*Miscanthus sinensis* "Goliath"
OPM 14*	WUR	*Miscanthus sinensis*

**strictly speaking, OPM 14 is a "within species" hybrid rather than a true genotype, but for convenience is referred to throughout as a "genotype."*

$$Yield_n = \frac{Weight\ 8\ tillers_n}{Weight\ 8\ tillers_m} * Yield_m \tag{1}$$

where

$Yield_n$ = Biomass yield at sampling date n

Weight 8 $tillers_n$ = Weight of eight tillers at sampling date n

Weight 8 $tillers_m$ = Weight of eight tillers at final harvest in March (January at Adana)

$Yield_m$ = Biomass yield at final harvest in March (January at Adana), estimated at central 4 m^2.

Laboratory Analysis

All dried samples were send to University of Hohenheim, where all further analysis have been performed. The biomass samples were milled in a cutting mill SM 200 (Retsch, Haan) using a 1 mm sieve before further laboratory analysis. The ash content of all samples was assessed by incineration in a muffle kiln at 550°C for 4 h according to VDLUFA book III method 8.1 (Naumann and Bassler, 1976/2012).

Content of neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) was estimated by near infrared spectroscopy (NIRS). Calibration and validation samples were analyzed using an ANKOM^{2000} Fiber Analyzer and Daisy II Incubator (ANKOM Technology, Macedon, USA) according to VDLUFA book III method 6.5.1 (NDF), 6.5.2 (ADF), and 6.5.3 (ADL) (Naumann and Bassler, 1976/2012). The standard error of the NIRS calibration (SEC) and prediction (SEP) and the R^2 of the NIRS calibration and validation are shown in **Table 3**. The ADL content is considered lignin. Cellulose content was calculated by subtracting ADL from ADF, and hemicellulose by subtracting ADF from NDF.

The specific methane yield (SMY) was measured in a biogas batch test at 39°C according to VDI guideline 4630. The biogas

TABLE 2 | Sampling dates and location characteristics. na = not applicable/no sampling performed.

Location	Latitude Longitude Altitude (m)	Sampling date					
		1 August (A)	2 September (S)	3 October (O)	4 November (N)	5 January (J)	6 March (M)
Adana	37.00 35.00 27	20.8.14	20.9.14	20.10.14	20.11.14	20.01.15	na
Moscow	55.50 37.33 140	20.8.14	20.9.14	na	na	na	13.03.15
Stuttgart	48.74 8.93 463	28.8.14	25.9.14	23.10.14	27.11.14	22.01.15	18.03.15

batch method was certified by the KTBL and VDLUFA inter-laboratory comparison test in 2014 and 2015 and is described in detail in Kiesel and Lewandowski (2017). The SMY was analyzed by using 200 mg oDM of the dried and milled biomass samples and 30 g of inoculum, which contained various macro- and micronutrients according to Angelidaki et al. (2009). The fermentation was performed for 35 days in gastight fermentation flasks and the biogas production was measured by the pressure increase using a HND-P pressure meter (Kobold Messring GmbH, Hofheim). The methane content of the biogas was measured by using a GC 2014 gas chromatograph (Shimadzu, Kyoto). However, for capacity reasons it was not possible to analyse all samples. Therefore, a minimum of one field replication of each genotype from each sampling date and each location was selected randomly to be analyzed. All samples were analyzed in one run of the biogas batch test to assure statistical soundness. A randomized block design with four technical replicates was applied. For capacity reasons, the batch test had to be split into two water baths. Replicates 1 and 2 were analyzed in one and replicates 3 and 4 in the other.

The methane yield per hectare was calculated based on estimated dry matter yield (DMY), ash content and SMY. As the SMY was mostly analyzed for only one of the three field replications, this value (or the average of all field replications analyzed) was assumed for all three field replications.

The net energy yield of anaerobic digestion was calculated by multiplying the methane yield per hectare by the calorific value of methane (35.883 MJ m^{-3}) as shown in Equation (2). The net energy yield of combustion was calculated according to Equation (3), in which an average calorific value of 18 MJ kg^{-1} for dry miscanthus biomass (Kołodziej et al., 2016) and 2.443 MJ kg^{-1} enthalpy of water vaporization was assumed. The net energy yield is considering not only ash and moisture content of the biomass, but also the energy required to evaporate the incorporated water.

$$Net\ Energy\ Yield_{Anaerobic\ digestion} = CV_{Methane} * SMY * DMY * (1 - AC) \quad (2)$$

$$Net\ Energy\ Yield_{Combustion} = CV_{Miscanthus} * DMY * (1 - AC) - EE_{Water} * FMY * MC \quad (3)$$

where

$CV_{Methane}$ = calorific value of methane (35.883 MJ m^{-3})
SMY = substrate-specific methane yield
DMY = dry matter yield of miscanthus
AC = ash content of the miscanthus biomass
$CV_{Miscanthus}$ = calorific value of dry miscanthus biomass (18 MJ kg^{-1})
EE_{Water} = evaporation enthalpy water (2.443 MJ kg^{-1})
FMY = fresh matter yield of miscanthus
MC = moisture content of the miscanthus biomass.

Statistical Analysis

Statistical analysis was performed using the software SAS version 9.4 (SAS Institute Inc., Cary, North Carolina). The program "Procmixed" was used and a mixed model applied (Equation 4). A test on homogeneity of variance and normal probability of residues was performed. The effects were tested at a level of probability of $\alpha = 0.05$.

$$y = \mu + \text{Loc} + \text{Geno} + \text{Loc} * \text{Geno} + \text{HD}(\text{Loc}) + \text{Geno} * \text{HD}(\text{Loc}) + e \quad (4)$$

where

μ = general mean effect
Loc = effect of location (Adana, Moscow, Stuttgart)
Geno = effect of genotype (OPM 3, 6, 9, 11, 14)
Loc∗Geno = effect of interaction of location and genotype
HD(Loc) = effect of location specific sampling date
Geno ∗ HD(Loc) = effect of interaction of genotype and location specific sampling date
e = residual error.

RESULTS

In the following chapter, the results of each genotype at each harvest date and location are shown in figures, but for clarity

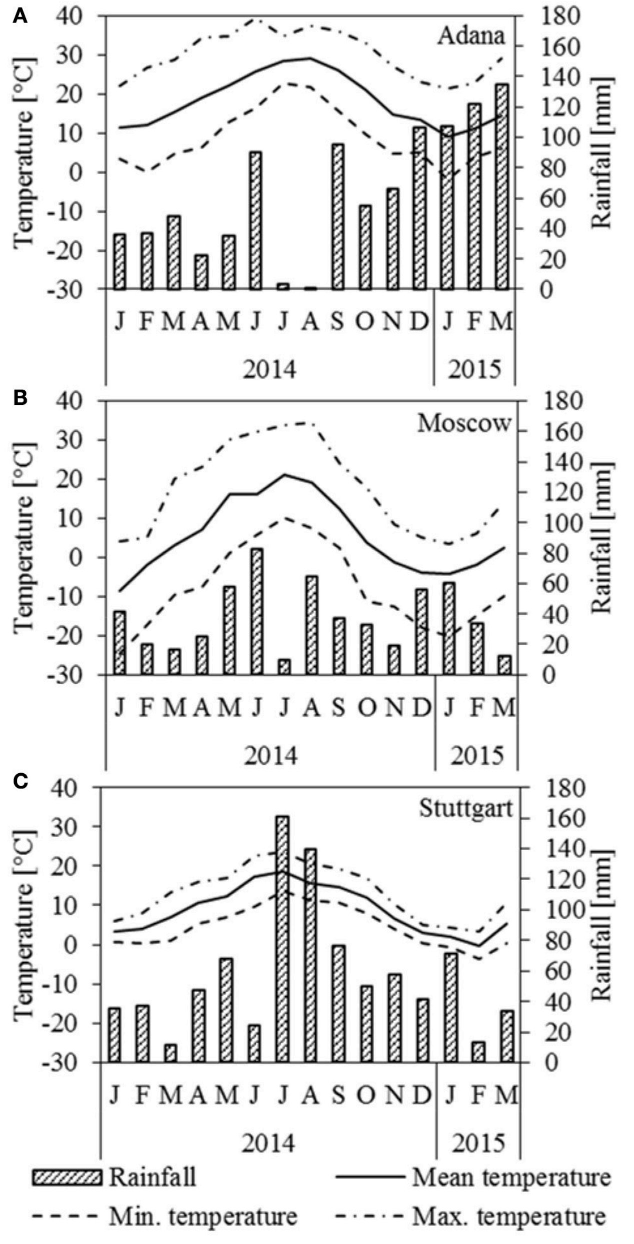


FIGURE 1 | Temperature and rainfall at the location (A) Adana, **(B)** Moscow, and **(C)** Stuttgart for 2014 and the first 3 months of 2015.

reasons letters are displayed only for the sampling dates per location [HD(Loc)]. Tables with means for genotype and location at each harvest date and the respective letter displays are given in the supplementary material.

TABLE 3 | NIRS calibration and validation statistics.

	Calibration			Validation		
	Number of samples	Standard error of calibration	R^2	Number of samples	Standard error of prediction	R^2
NDF	160	1.2672	0.953	20	2.345	0.858
ADF	160	1.3331	0.959	20	2.699	0.834
ADL	160	0.6492	0.888	20	0.773	0.706

Fixed Effects

Location (Loc) and sampling date per location [HD(Loc)] showed highly significant impacts on all traits analyzed (**Table 4**). Genotype (Geno) and interaction of location and genotype (Loc∗Geno) had a highly significant impact on quality parameters and a still significant impact on yield-related parameters, such as methane yield per hectare and net energy yield of biogas and combustion (**Table 4**). This may be influenced by the high variance in yield, caused by the fairly rough yield estimation using eight tillers. The interaction of genotype and sampling date per location [Geno∗HD(Loc)] showed a significant impact only on dry matter, hemicellulose and lignin content. Again, the variance due to the small sampling size of eight tillers may have been too high. However, larger sampling size was not feasible to avoid impact on the field trial.

Biomass Yield and Dry Matter Content

There was a large difference in biomass yield development throughout the year between the Adana location (the warmest in this study) and the other two locations (**Figure 2**).

In Adana, the biomass yield was significantly highest in August and then declined steadily until final harvest in March (**Figure 2A**). The highest biomass yields at each sampling date were found for OPM 9, which declined from 22.6 t DM ha^{-1} in August to 13.0 t DM ha^{-1} in March. Significantly lower biomass yields were found in OPM 3. The biomass yields of all the other genotypes showed no significant differences.

In Moscow, significantly higher biomass yields were found in September (**Figure 2B**) and OPM 3 (11.2 t DM ha^{-1}) was the highest-yielding genotype in this month (**Figure 2B**). At final harvest in March, OPM 6 and 9 had the highest DM yields (10.3 and 7.7 t DM ha^{-1}). These had stayed quite stable over winter, while the yield of OPM 3 had declined severely to 4.7 t DM ha^{-1}.

In Stuttgart, the biomass yield behavior was similar to that in Moscow. Significantly higher biomass yields were found in September and October and all genotypes showed significant yield losses over winter (**Figure 2C**). The highest DM yields were found for OPM 6, which increased to 25.0 t DM ha^{-1} in September and then decreased to 16.2 t DM ha^{-1} in March. However, the biomass yields of OPM 6 were only significantly different from OPM 14. Interestingly, OPM 9 (Mxg) showed comparatively low biomass yields in the course of the year but an increase from January to March (10.2–13.4 t DM ha^{-1}). Yield measurement in OPM 9 was difficult due to the shape of the crop (center of the plot was considerably higher than the border rows), which may have led to an underestimation of yield, especially in January. However, the final harvest in March was performed at the center of the plot and therefore delivered reasonable biomass yields.

The dry matter content (DMC) increased steadily at all locations throughout the year and the significantly highest DMC was recorded at final harvest in March/January (**Figure 2**). In

TABLE 4 | *P*-values of fixed effects.

	Yield	Dry matter content	Ash content	Cellulose content	Hemicellulose content	Lignin content	SMY	Methane yield per hectare	Net energy yield biogas	Net energy yield combustion
Loc	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Geno	0.010	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.037	0.039	0.006
Loc*Geno	0.006	0.001	<0.001	<0.001	<0.001	<0.001	0.015	0.029	0.030	0.036
HD(Loc)	<0.001	<0.001	<0.001	<0.001	0.007	<0.001	<0.001	<0.001	<0.001	<0.001
Geno* HD(Loc)	ns	<0.001	ns	ns	0.001	0.037	ns	ns	ns	ns

Adana, OPM 6 showed the highest DMC throughout the year and at final harvest in January (**Figure 2A**). It was also the only genotype in Adana that achieved a DMC of above 80% FM at final harvest, which is crucial for safe storage of the biomass. In Moscow, no significant differences in DMC were detected between the genotypes, but OPM 9 was the only genotype with a DMC of below 80% FM at final harvest (**Figure 2B**). In Stuttgart, OPM 6 showed the highest DMC from August to November, but further drying was hindered by lodging of the crop (**Figure 2C**). In January, OPM 11 and 14 showed the highest DMC. However, the differences in DMC at final harvest in March were very small, due to good weather conditions (frost in winter, dry before harvest).

Methane Yield and SMY

In Moscow, the substrate-specific methane yield (SMY) did not change significantly throughout the year (**Figure 3B**). In Adana and Stuttgart, it decreased significantly from August to final harvest in March (**Figures 3A,C**). However, the impact of the SMY on methane yield was only slight compared to that of biomass yield. It can be clearly seen that MY follows the same trend as dry matter yield and is therefore not described separately here.

The SMY of OPM 9 was the significantly lowest of all assessed genotypes at all locations. That of OPM 14 was very similar at all three locations, while that of OPM 9 and 11 was significantly higher in Stuttgart than in Adana and Moscow. The SMY of OPM 3 and OPM 6 was significantly lower in Adana than in Stuttgart, but there was no significant difference between Stuttgart and Moscow.

Fibre and Ash Contents

Ash content was strongly influenced by location and Adana showed the significantly highest ash contents at each sampling date (**Figure 4**). In Adana, the ash content only decreased significantly from November to January. In Stuttgart, a significant decrease was also observed from November to January and the biomass sampled in January and March had the significantly lowest ash content. In contrast, the ash content in Moscow increased slightly, but significantly, from August to March. Genotype OPM 11 showed the significantly highest ash content at Adana and OPM 14 at Stuttgart. In Moscow, no significant genotypic differences were recorded.

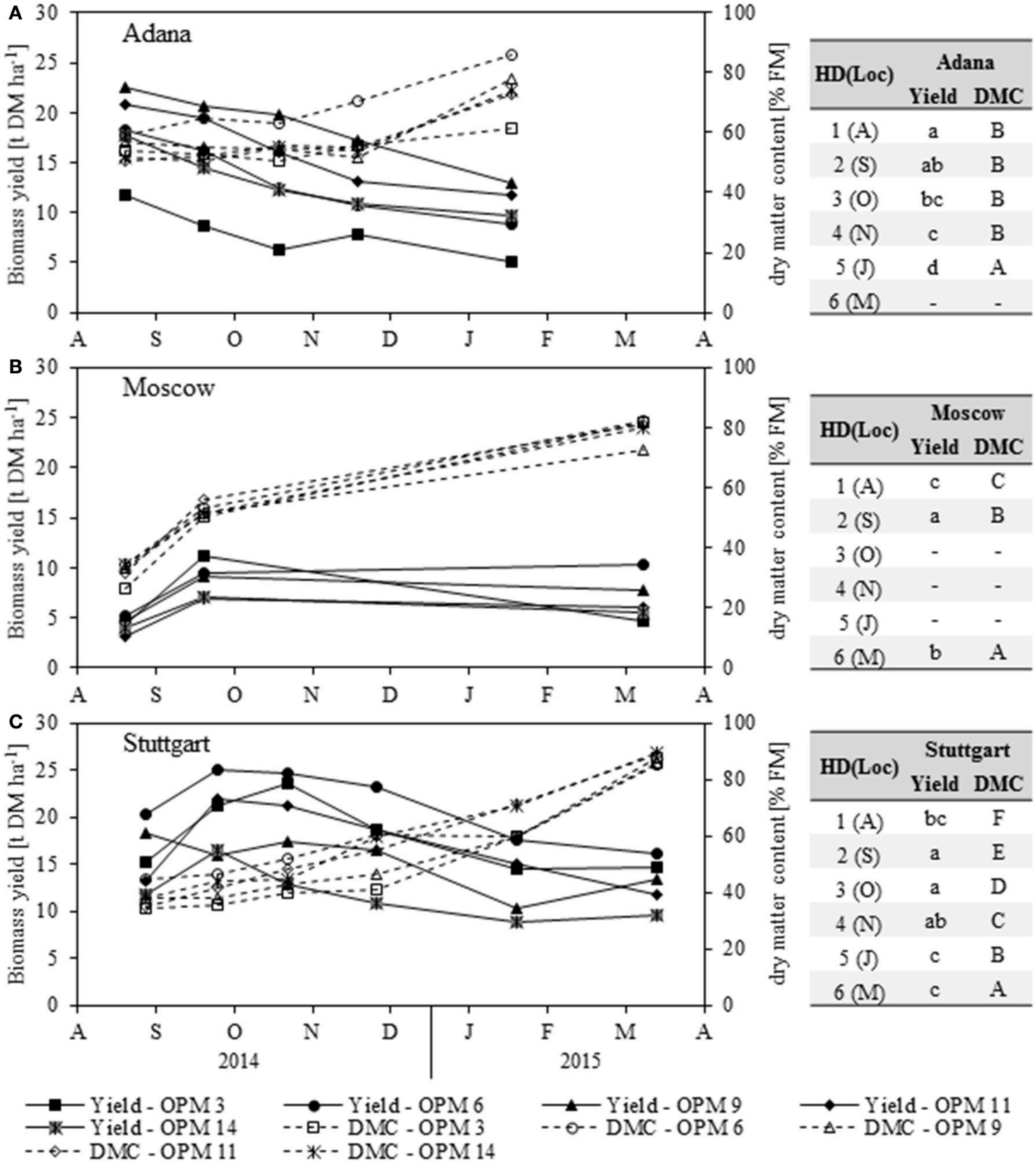
The cellulose content increased steadily at Adana and Stuttgart, where the significantly highest contents were recorded for sampling dates January and March (**Figure 5**). All genotypes showed the significantly highest cellulose contents at Stuttgart, but those at Adana and Moscow were mostly not significantly different. Here, OPM 9 showed the significantly highest cellulose content of all genotypes (not significantly higher than OPM 11 in Adana). In Stuttgart, the significantly highest cellulose contents were found with OPM 6 and OPM 9. In Moscow, both cellulose and hemicellulose contents did not significantly change over the year; only a slight, but significant decrease in lignin was recorded.

In Adana, the hemicellulose content increased slightly with later sampling dates and the significantly highest hemicellulose content was found in January, but it was not significantly different from November and October (**Figure 5A**). In Stuttgart, the hemicellulose content increased slightly until November (significantly highest) and then decreased at the same rate (**Figure 5C**). At all locations, OPM 9 had the significantly lowest hemicellulose content, except OPM 3 at Stuttgart. The hemicellulose content of all genotypes was highest (mostly significantly) at the Moscow location.

The lignin content increased steadily with later sampling dates at the Adana and Stuttgart locations, where the significantly highest lignin contents were recorded in January and March (**Figure 5**). At all locations, OPM 9 showed the significantly highest lignin content, however it was not significantly higher than that of OPM 3 at Stuttgart.

Net Energy Yields

The net energy yield of anaerobic digestion is influenced by dry matter yield, SMY, and ash content, whereas the net energy yield of combustion is influenced by dry matter yield, moisture content and ash content. For both, dry matter yield has the largest impact. As the development of both net energy yields clearly follows that of dry matter yield, it is not described separately here (**Figure 6**). In Adana, the highest net energy yield of combustion and anaerobic digestion was recorded for OPM 9 in August at 344 and 203 GJ ha^{-1}, respectively. At this location, the net energy yield of both combustion and anaerobic digestion decreased steadily, by 37 and 49% respectively, until final harvest in January. In Moscow, the genotypes with the highest net energy yield of combustion and anaerobic digestion in September were OPM 3 at 168 and 113 GJ ha^{-1} and OPM 6 at 143 and 92 GJ ha^{-1}, respectively. While the net energy yield of OPM 3 decreased noticeably (−53% for combustion and −60% for anaerobic digestion), OPM 6 showed a net energy



HD(Loc)	Adana	
	Yield	DMC
1 (A)	a	B
2 (S)	ab	B
3 (O)	bc	B
4 (N)	c	B
5 (J)	d	A
6 (M)	-	-

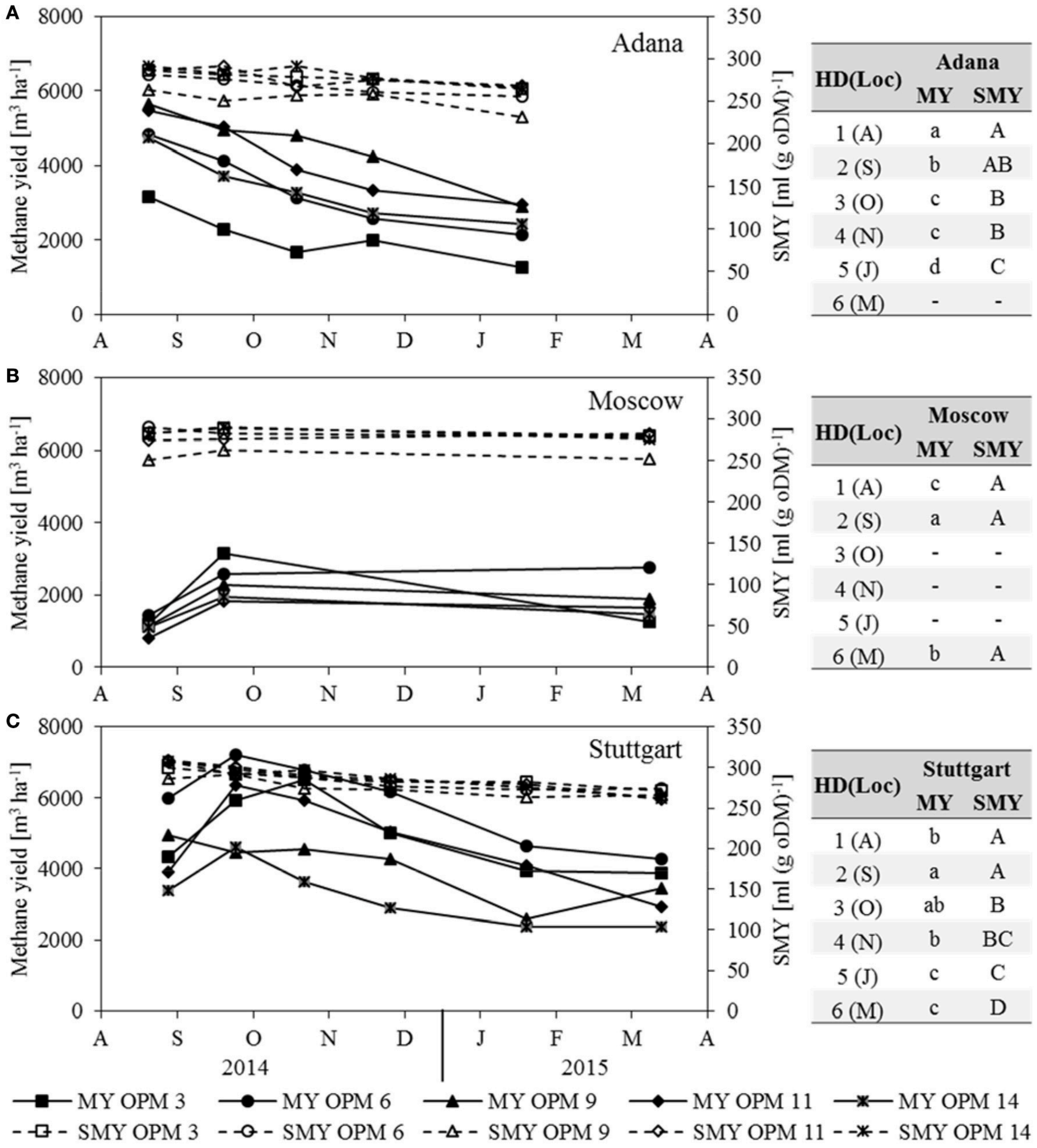
HD(Loc)	Moscow	
	Yield	DMC
1 (A)	c	C
2 (S)	a	B
3 (O)	-	-
4 (N)	-	-
5 (J)	-	-
6 (M)	b	A

HD(Loc)	Stuttgart	
	Yield	DMC
1 (A)	bc	F
2 (S)	a	E
3 (O)	a	D
4 (N)	ab	C
5 (J)	c	B
6 (M)	c	A

FIGURE 2 | Biomass dry matter yield (Yield) and dry matter content (DMC) of each genotype [OPM 3, 6, 9, 11, 14) for each sampling date (1 = August (A), 2 = September (S), 3 = October (O), 4 = November (N), 5 = January (J), 6 = March (M)] at the locations (A) Adana, **(B)** Moscow and **(C)** Stuttgart. Tables include the letter display for the sampling date per location [HD(Loc)] for the traits yield and DMC. Different lower- (Yield) and upper-case (DMC) letters indicate significant differences at a probability level of α = 0.05 for sampling dates at a specific location.

yield of combustion and anaerobic digestion of 172 and 99 GJ ha^{-1}, respectively. In Stuttgart, the highest net energy yield of combustion was observed in October and of anaerobic digestion in September for OPM 6 at 370 and 259 GJ ha^{-1}, respectively. Here, at final harvest in March, the energy yield of combustion and anaerobic digestion of OPM 6 was 275 and 154 GJ ha^{-1}, respectively.

A comparison of the two energy yields shows that, on average over all locations, genotypes and sampling dates, anaerobic digestion delivers 65% of the energy yield of combustion. However, there are noteworthy differences between location, genotypes and harvest dates. Early sampling in August improves the net energy yield of anaerobic digestion through an increase in SMY, but impairs the net energy yield of combustion through a



HD(Loc)	Adana MY	Adana SMY
1 (A)	a	A
2 (S)	b	AB
3 (O)	c	B
4 (N)	c	B
5 (J)	d	C
6 (M)	-	-

HD(Loc)	Moscow MY	Moscow SMY
1 (A)	c	A
2 (S)	a	A
3 (O)	-	-
4 (N)	-	-
5 (J)	-	-
6 (M)	b	A

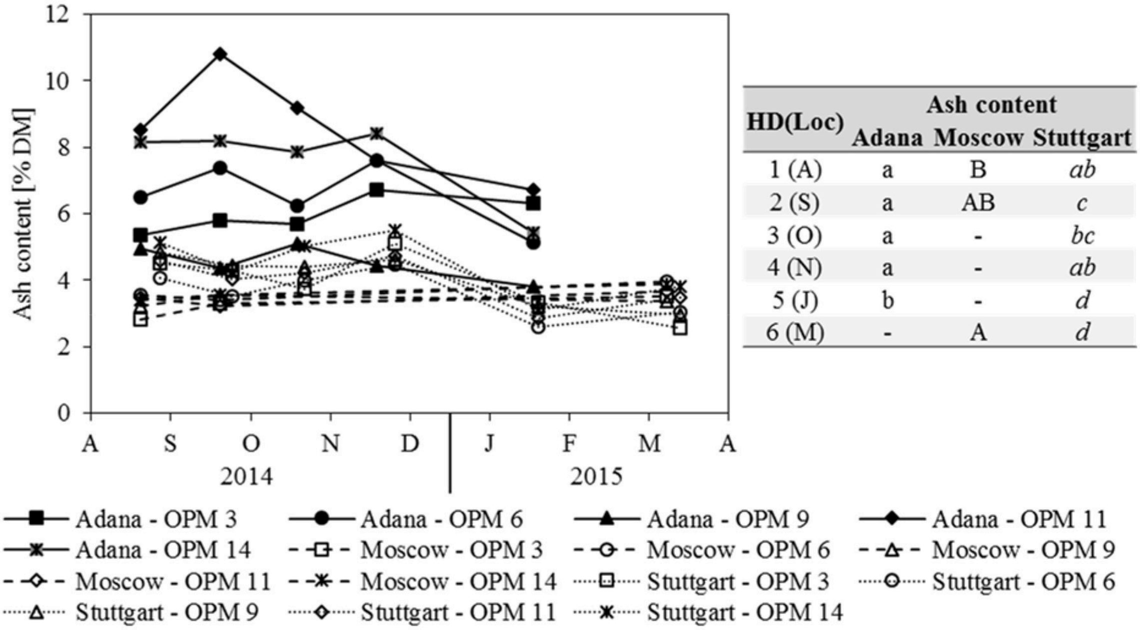
HD(Loc)	Stuttgart MY	Stuttgart SMY
1 (A)	b	A
2 (S)	a	A
3 (O)	ab	B
4 (N)	b	BC
5 (J)	c	C
6 (M)	c	D

FIGURE 3 | Methane yield (MY) and substrate-specific methane yield (SMY) for each genotype [OPM 3, 6, 9, 11, 14) and sampling date (1 = August (A), 2 = September (S), 3 = October (O), 4 = November (N), 5 = January (J), 6 = March (M)] at the locations (A) Adana, **(B)** Moscow and **(C)** Stuttgart. Tables include the letter display for the sampling date per location [HD(Loc)] for the traits methane yield (MY) and substrate-specific methane yield (SMY). Different lower- (MY) and upper-case (SMY) letters indicate significant differences at a probability level of α = 0.05 for sampling dates at a specific location.

higher moisture content. In August, the average net energy yield of anaerobic digestion for all locations and genotypes was 75% that of combustion; in Stuttgart and Moscow even 79 and 83%, respectively. Late harvest in January or March leads to a decrease in SMY and improved quality for combustion (lower moisture content). At final harvest, the net energy yield of anaerobic digestion, averaged over all locations and genotypes, was 55% of that of combustion; for OPM 9 even as low as 52%.

DISCUSSION

The energy yields (used here synonymously with "net energy yield") per hectare of combustion and anaerobic digestion are mainly influenced by the harvestable biomass yield per hectare, but are differentially sensitive to content of organic and inorganic compounds in the biomass. The different biomass fractions, e.g., moisture, ash, and lignin content, interact to



HD(Loc)	Ash content		
	Adana	Moscow	Stuttgart
1 (A)	a	B	*ab*
2 (S)	a	AB	*c*
3 (O)	a	-	*bc*
4 (N)	a	-	*ab*
5 (J)	b	-	*d*
6 (M)	-	A	*d*

FIGURE 4 | Ash content for each genotype (OPM 3, 6, 9, 11, 14) and sampling date [1 = August (A), 2 = September (S), 3 = October (O), 4 = November (N), 5 = January (J), 6 = March (M)] at the three locations Adana, Moscow, and Stuttgart. Tables include the letter display for the sampling date per location [HD(Loc)] for the traits ash content. Different lower- (Adana) and upper-case (Moscow) and italic (Stuttgart) letters indicate significant differences at a probability level of $\alpha = 0.05$ for sampling dates at a specific location.

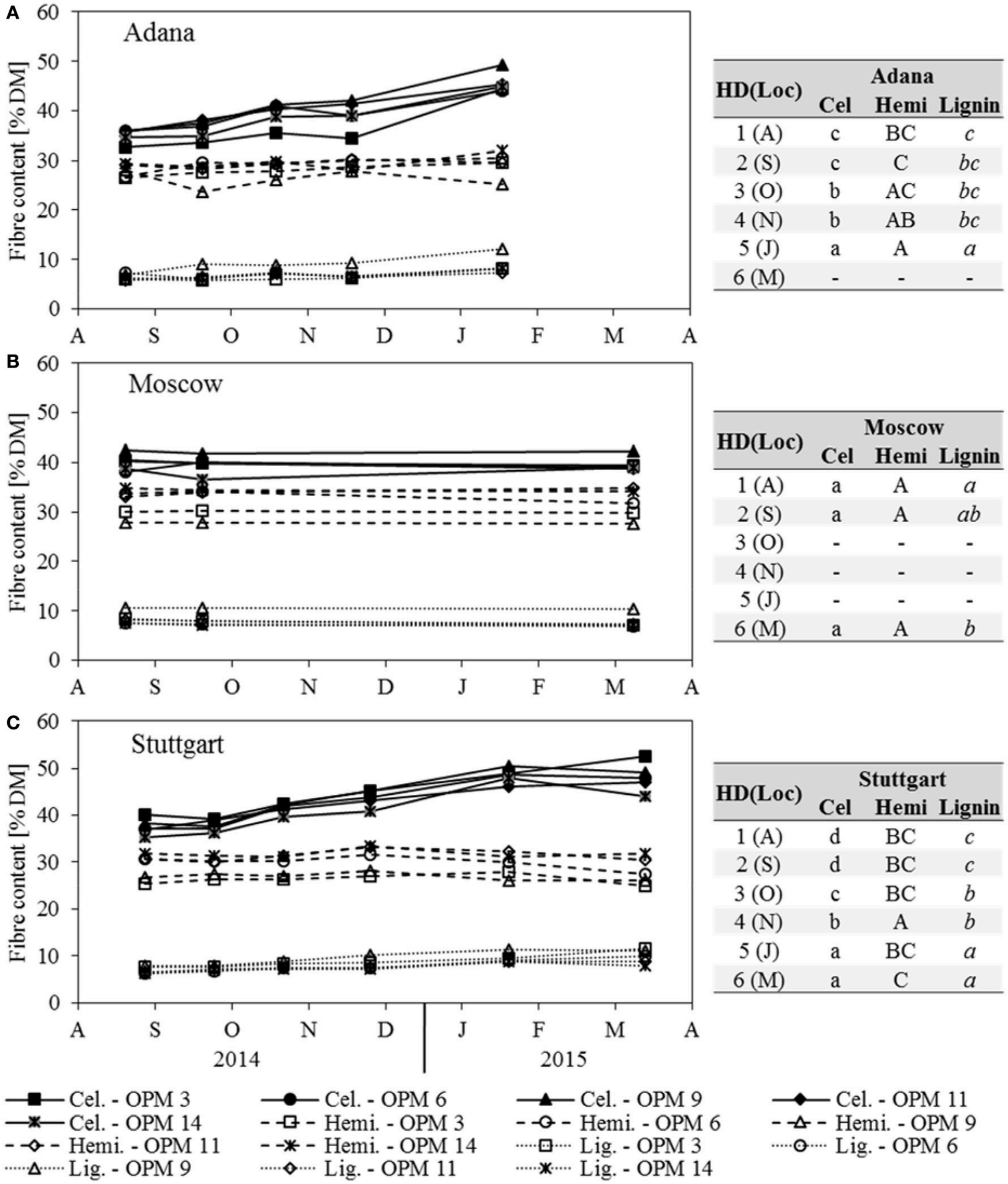
produce a thermal calorific value (combustion) or substrate-specific methane yield (anaerobic digestion). In combustion, inorganics such as ash mainly reduce the combustible proportion of the yield, whereas vaporization of water consumes additional energy and reduces the calorific value. For this reason, moisture content has the strongest quality-related impact on the energy yield of combustion. Biomass quality for anaerobic digestion is mainly related to the organic composition, in particular the lignin content. Here the energy yield is directly measured by the substrate-specific methane yield (SMY) in a biogas batch test, which is therefore the sole determining quality factor. Other biomass quality characteristics, such as lignin content, are only used to explain differences in SMY. The moisture content is not relevant for the energy yield of anaerobic digestion, since it is already considered during estimation of dry matter yield. In both conversion pathways, ash content reduces the amount of combustible and digestible biomass to the same extent (SMY is also calculated on the basis of organic dry matter), therefore it is not discussed in the following section.

All these yield and quality traits are influenced by genotype, location, harvest date and interaction of genotype and location. The following sections first discuss the impacts of the above determinants on energy yields of combustion and anaerobic digestion and then the energy yields are compared.

Factors Influencing Energy Yield

In both utilization pathways, harvestable yield (standardized by calculating dry matter at the different harvest times) had the largest impact on energy yield. Since location, genotype, and harvest date all have an influence on harvestable dry matter yield, these also had a considerable impact on energy yield. In Adana, the maximum biomass yield was recorded before the first sampling date of this investigation (Nunn et al., under review), after which the yield declined steadily because drought in July and August ended the growth season. Interestingly, the standard genotype Mxg (OPM 9) performed best in terms of energy yield under the water-limited conditions in 2014 in Adana. The low irrigation levels applied to ensure survival of the crop will have influenced the performance of the genotypes. Indeed, Mxg is well known for sensitivity to drought (Clifton-Brown et al., 2002). However, from these observations, we conclude that while none of the genotypes tested here are optimally adapted to the climatic conditions of the Mediterranean area, *M. sinensis* coped better than the others.

In Moscow, the yield was comparatively low due to the short growing season determined by the more extreme continental climate (**Figure 1B**). This clearly shows that cold-tolerant genotypes, which start growing at lower temperatures, are required for such locations in order to make best use of the available vegetation period. However, Fonteyne et al. (2016) found that, for a C4 plant, miscanthus shows a comparatively high chilling tolerance. In Stuttgart, the mild continental climate with high water availability (**Figure 1C**) supported active growth for a longer period, resulting in higher autumn yields than in Moscow and Adana. Considerable genotypic differences were observed in Stuttgart, where the novel genotype OPM 6 performed best. This was mainly influenced by its high shoot density (Kalinina et al., under review). The effect of plant



HD(Loc)	Adana Cel	 Hemi	 Lignin
1 (A)	c	BC	*c*
2 (S)	c	C	*bc*
3 (O)	b	AC	*bc*
4 (N)	b	AB	*bc*
5 (J)	a	A	*a*
6 (M)	-	-	-

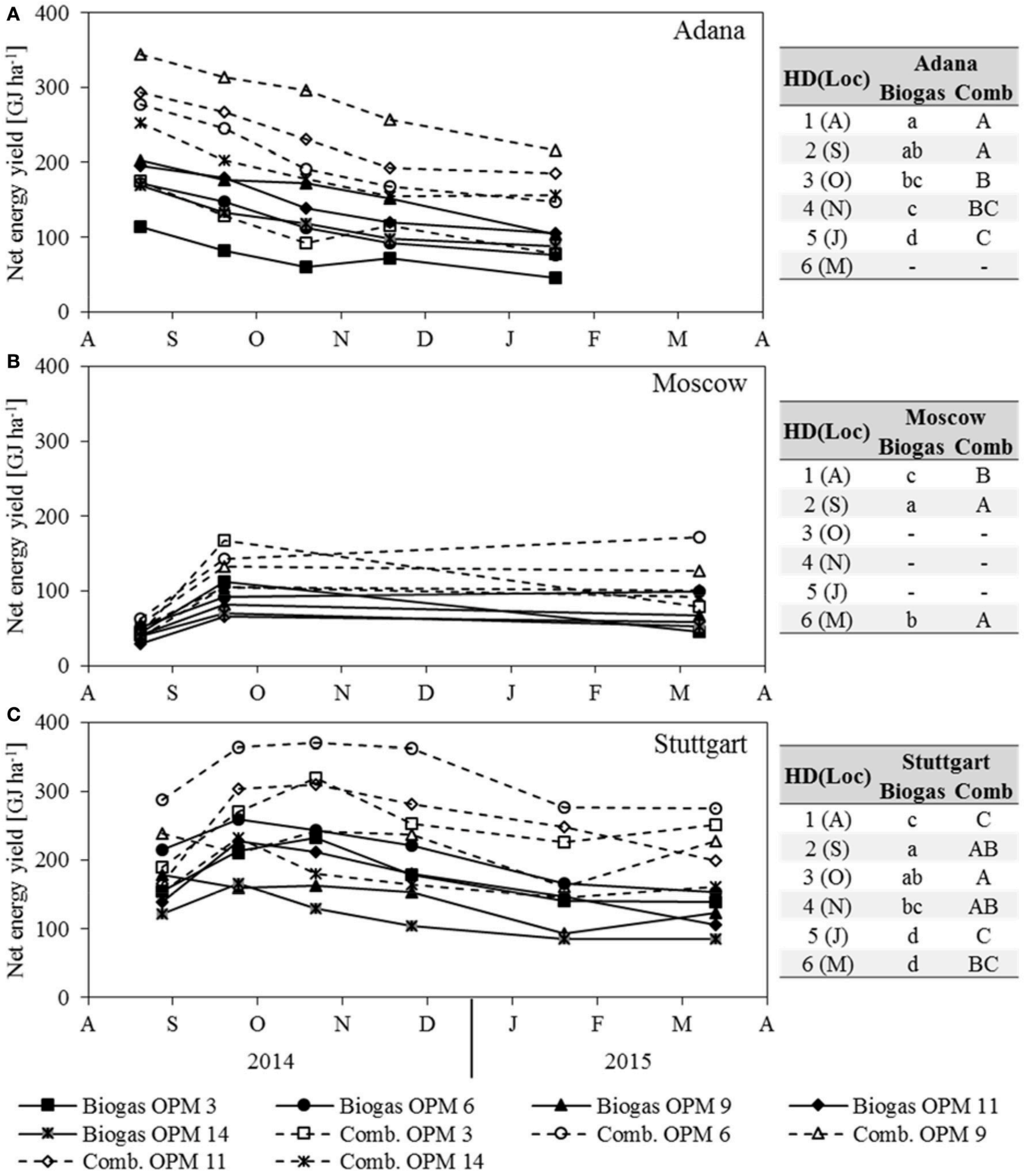
HD(Loc)	Moscow Cel	 Hemi	 Lignin
1 (A)	a	A	*a*
2 (S)	a	A	*ab*
3 (O)	-	-	-
4 (N)	-	-	-
5 (J)	-	-	-
6 (M)	a	A	*b*

HD(Loc)	Stuttgart Cel	 Hemi	 Lignin
1 (A)	d	BC	*c*
2 (S)	d	BC	*c*
3 (O)	c	BC	*b*
4 (N)	b	A	*b*
5 (J)	a	BC	*a*
6 (M)	a	C	*a*

FIGURE 5 | Cellulose (Cel), hemicellulose (Hemi) and lignin content of each genotype (OPM 3, 6, 9, 11, 14) and sampling date [1 = August (A), 2 = September (S), 3 = October (O), 4 = November (N), 5 = January (J), 6 = March (M)] at the three locations (A) Adana, **(B)** Moscow, and **(C)** Stuttgart. Tables include the letter display for the sampling date per location [HD(Loc)] for the traits cellulose, hemicellulose and lignin content. Different lower- (Cel) and upper-case (Hemi) and italic (Lignin) letters indicate significant differences at a probability level of α = 0.05 for sampling dates at a specific location.

morphology on biomass yield demonstrates the opportunities of breeding high-yielding hybrids.

Earlier studies have found that moisture content is not only influenced by harvest date, but also determined by complex interactions between genotype and growth location environment (Iqbal and Lewandowski, 2014). Obviously, moisture content impacts the energy yield of combustion, since it directly reduces the heating value. However, the moisture content at final harvest is not only crucial for combustion quality, but also for safe storage of the biomass.



HD(Loc)	Adana Biogas	 Comb
1 (A)	a	A
2 (S)	ab	A
3 (O)	bc	B
4 (N)	c	BC
5 (J)	d	C
6 (M)	-	-

HD(Loc)	Moscow Biogas	 Comb
1 (A)	c	B
2 (S)	a	A
3 (O)	-	-
4 (N)	-	-
5 (J)	-	-
6 (M)	b	A

HD(Loc)	Stuttgart Biogas	 Comb
1 (A)	c	C
2 (S)	a	AB
3 (O)	ab	A
4 (N)	bc	AB
5 (J)	d	C
6 (M)	d	BC

FIGURE 6 | Net energy yield of anaerobic digestion (Biogas) and combustion (Comb) of each genotype (OPM 3, 6, 9, 11, 14) and each sampling date [1 = August (A), 2 = September (S), 3 = October (O), 4 = November (N), 5 = January (J), 6 = March (M)] at the three locations (A) Adana, **(B)** Moscow, and **(C)** Stuttgart. Tables include the letter display for the sampling date per location [HD(Loc)] for the net energy yield of anaerobic digestion (Biogas) and combustion (Comb). Different lower- (Biogas) and upper-case (Comb) letters indicate significant differences at a probability level of α = 0.05 for sampling dates at a specific location.

Genotypes with active senescence could help maintain sufficiently low moisture content at final harvest (Nunn et al., under review). This is especially relevant for locations with mild winters, as frost kills the aboveground biomass, thus accelerating senescence, initiating ripening, and drying the biomass (Robson et al., 2012). The largest genotypic differences in moisture content at final harvest were recorded in Adana, where almost no frost occurred over winter. At the other locations, only small differences in moisture content between genotypes were recorded, because there were sufficiently harsh frosts (below −3°C daily mean temperature). In Adana, only OPM 6, a *M. sinensis* x *M. sacchariflorus* hybrid, showed a sufficiently low moisture content of below 20% FM, while OPM 3, a pure *M. sacchariflorus* genotype, showed a particularly high moisture content. Genotypes with active senescence could also be useful at the Stuttgart location, because sufficient frosts to dry the crop below a moisture content of 20% do not occur every year. Iqbal and Lewandowski (2014) reported high differences in moisture content between single years at this location. Here, OPM 11 and 14 showed favorable development of moisture content until January, but after the February frost period, all genotypes had the same low moisture content at final harvest in March. In Adana, OPM 6 showed a gradual reduction in moisture content from autumn to spring. In Stuttgart, a similar decrease in moisture content from August until November was observed, but lodging hindered further drying. Genotypes with active senescence not only offer the potential to ensure sufficient drying even at locations with mild winters, but additionally allow optimization of harvest time for combustion (Iqbal et al., under review).

Moisture contents of above 60% have a greater impact on energy yield (Equation 3). Such high moisture contents were only recorded in August at Moscow and in August and September at Stuttgart. Drying over winter positively influenced the energy yield of combustion, but the improved biomass quality did not compensate for the yield losses e.g., due to leaf fall. This "trade-off" between biomass yield and quality is well known (Lewandowski et al., 2003; Cadoux et al., 2012) but has rarely been quantified due to the lack of serial harvests through the winter months. This paper quantifies the energy yield losses of delayed harvest in late winter compared to harvest at peak yield for the first time. Average energy yield losses were found to be 43% in Adana, 20% in Stuttgart and only 11% in Moscow. Some genotypes showed high energy yield losses over winter, such as OPM 3 in Adana (56%) and Moscow (53%), and OPM 11 in Stuttgart (36%). Genotype OPM 9 showed comparatively low losses at all locations (37% in Adana, 6% in Stuttgart and 4% in Moscow). However, as mentioned earlier, the biomass yield measurement of OPM 9 in Stuttgart was subject to technical variation, which could have negatively influenced these results from August to January. Other genotypes also showed contrasting results at the three locations, e.g., OPM 11 had high losses in Stuttgart (36%), but low losses in Moscow (4%) and Adana (36%). The yield losses could be associated with the leaf shares and OPM 9 showed the lowest leaf-to-stem ratio (Iqbal et al., under review). From an energy point of view, an earlier harvest would be theoretically advantageous for combustion, but is in conflict with biomass quality (see also Iqbal et al., under review).

The energy yield of anaerobic digestion is influenced more by DM yield than SMY, because SMY variations in the serial harvests were lower than initially expected. Similar findings have recently also been reported from other experiments (Wahid et al., 2015; Kiesel and Lewandowski, 2017). The biomass analyzed in the present study was milled (1 mm), which can affect the SMY. Frydendal-Nielsen et al. (2016) used a larger particle size than in our study and measured a lower SMY for miscanthus. In their study, pre-treatment increased the SMY of miscanthus significantly due to size reduction of the biomass particles. The SMY values in our paper show more the technical potential than the biogas yield, which would be obtained in full-scale biogas plants using chopped biomass. The current standard chip format for anaerobic digestion was developed for maize. Thus, presumably a pre-treatment would be required for miscanthus to achieve a similar SMY in full-scale biogas plants to that measured in our study. Various pre-treatment methods, including physical (e.g., milling, ultrasonic, steam-explosion), chemical (acid or alkaline), and biological methods (white and brown rot fungi, enzymes), to improve digestibility and methane yield of difficult and lignocellulosic substrates in anaerobic digestion are described in literature (Patinvoh et al., 2017). In recent years, suitable pre-treatment technology has become more available and is increasingly utilized in practice.

At the Adana and Stuttgart locations, the SMY decreased significantly with later harvest dates as the lignin content increased. Under anaerobic conditions, lignin is generally not digested and also inhibits the digestibility of other compounds (den Camp et al., 1988). Of all genotypes, OPM 9 had significantly lower SMY's, which correlates with the highest lignin content across all locations. Again, it is worth mentioning that the biomass was milled (1 mm) prior to the biogas batch test. This milling can be considered pre-treatment, which is known to increase digestibility of lignocellulosic biomass (Menardo et al., 2013; Frydendal-Nielsen et al., 2016). The SMY could have been positively affected by milling, especially for later harvest dates and genotypes with a higher degree of lignification. The effect of location on SMY is not clear. In the present study, Adana often had a significantly lower SMY, but also the lowest lignin content. Generally, drought conditions are expected to increase the lignin content (Le Gall et al., 2015). However, van der Weijde et al. (2017a) reported that drought conditions decreased lignin contents of miscanthus and increased the proportion of cellulose converted to ethanol. In our study, the drought conditions in Adana seemed to decrease the lignin content, but no positive effect on the SMY was observed.

Since biomass yield is more relevant than SMY for the energy yield of anaerobic digestion, the priority should be placed on harvesting at biomass peak yield. However, sufficient green-cutting tolerance is a prerequisite for this (Kiesel and Lewandowski, 2017). Green-cutting tolerance is assumed to be determined by relocation of carbohydrates from the aboveground biomass to the rhizome in late summer and early autumn (Purdy et al., 2015). By contrast, an increased nitrogen fertilizer application had almost no impact on the regrowth the following year of a 5-year-old Mxg crop in Stuttgart (Kiesel and Lewandowski, 2017). Green cuts also result in larger nutrient offtakes (Kiesel and Lewandowski, 2017), which need to be replaced, e.g., by digestate, to maintain long-term productivity of the crop.

Based on recent cutting trials with Mxg, a harvest in late October does not affect biomass yield the following year in Stuttgart, but earlier harvest can reduce DM yields by 40–60% (Kiesel and Lewandowski, 2017). Due to the harsh frost just before the sampling date in September in Moscow, it can be assumed that green harvest in late September or early October is feasible. In Adana, the season end was not defined by frost, but by drought in July and August. For this reason, it is questionable which harvest date would be tolerated by the crop here. Due to the favorable growing conditions before the drought period, the plants flowered very early, which may have induced senescence and carbohydrate relocation (Jensen et al., 2016). However, Purdy et al. (2015) observed no influence of flowering on carbohydrate relocation, but the growing conditions at their locations in UK were completely different from Adana. The steady biomass yield decrease in Adana shows there was no biomass growth after the drought period. This can be seen as an indication that an August green harvest could be tolerated by the crop here. Should this be the case, biomass yield losses and the necessary irrigation for crop survival during the drought period could be avoided. Cutting tolerance presumably also depends on genotype and location but this needs to be assessed for further genotypes and locations. A more detailed assessment of possible harvest dates in autumn (from September to late October) would be required to identify the feasibility of a harvest at biomass peak yield. For this reason, multi-location cutting tolerance studies should be performed for new leading genotypes such as OPM-6.

Combustion vs. Anaerobic Digestion

Combustion has many advantages over anaerobic digestion. In this paper, the energy yield of anaerobic digestion, averaged over all harvest dates, was 35% lower than that of combustion. In addition, dry-harvested biomass can be stored easily for combustion, if the moisture is below 20%. Green-harvest could still be problematic for combustion due to content of critical elements and low ash melting temperature (Iqbal et al., under review). The identification of optimum harvest date requires a number of factors to be considered, including combustion technology applied, biomass yield, moisture content and various biomass quality aspects (Iqbal et al., under review). Therefore, it may not always be possible to harvest miscanthus at biomass peak yield for combustion and the state-of-the-art for most combustion applications is to delay harvest until March to improve biomass quality and moisture content. For this reason, it is perhaps less useful to compare energy yields for anaerobic digestion and combustion on the same harvest dates. If it is assumed that the crop tolerates green harvest in late August in Adana, anaerobic digestion delivers, on average, a 14% higher energy yield than combustion at final harvest in January. Harvest in late September for anaerobic digestion in Moscow and Stuttgart supplies only a 19 and 7% lower energy yield, respectively, than harvest for combustion in March. Even with delaying the harvest in Adana (September) and Stuttgart (October) to improve the cutting tolerance, the energy yield of anaerobic digestion is, on average, only 18% lower than that of combustion at final harvest.

Recommendations for Site-Specific Genotype Choice

For both utilization options, genotypes with a high dry matter yield are required. Whereas, for anaerobic digestion the autumn biomass yield (often equal to peak yield) is crucial, for combustion a high biomass yield in late winter or spring is necessary. For this reason, genotypes such as OPM 9 with lower losses over winter (e.g., due to lower leaf share) are better suited for combustion. However, senescence of OPM 9 can be insufficient when winters are too mild, which leads to higher moisture content of the biomass accompanied by difficulties for harvest, storage and combustion. At such locations, high-yielding *M. sinensis* (e.g., OPM 11) or *M. sinensis* x *M. sacchariflorus* hybrids (such as OPM 6) could help ensure low moisture content at spring harvest. Since lodging occurred in OPM 6, this genotype cannot be recommended for combustion, because lodging makes the harvest more difficult and hinders drying of the biomass over winter. For anaerobic digestion, the impact of lodging is less critical, but still renders the harvest more difficult. Although OPM 6 lodged in Stuttgart, its utilization for anaerobic digestion still seems promising, because this genotype had a combination of high yield potential in autumn, high SMY and low lignin content. In Adana, OPM 11 appears promising due to its high yield in late summer and high SMY, but the cutting tolerance remains to be assessed. In Moscow, the *M. sacchariflorus* genotype OPM 3 performed best for anaerobic digestion, but cannot be recommended due to its creeping rhizome. For this reason, the second best-performing genotype OPM 6 is recommended for anaerobic digestion at this location.

Anaerobic digestion is a promising utilization option for miscanthus biomass, as the energy losses from conversion into gaseous fuel can be largely compensated for by avoiding biomass losses over winter. A short summary of the main findings is shown in **Box 1**. The storage of green miscanthus biomass via ensiling also appears feasible and can be further improved through the use of additives (Whittaker et al., 2016). To optimize the harvest date for anaerobic digestion, the cutting tolerance should be assessed at several locations and for multiple genotypes. Further, biogas plant technology needs

BOX 1 | Short Summary of the main outcomes:

- Anaerobic digestion is a promising novel utilization pathway for miscanthus biomass, which provides both a higher value product and a high productivity per hectare
- Higher biomass yields due to harvest in autumn/at peak yield compensates largely for the conversion losses of anaerobic digestion. However, cutting tolerance of such novel genotypes needs to be assessed for a broad spectrum of locations.
- Biomass and energy losses due to delayed harvest for combustion, are the costs of quality improvements to meet the quality and storage requirements. Pre-winter harvest could increase energy yield of combustion, because higher moisture content is overcompensated by higher biomass yields. However, adapted and suitable technology for storage and combustion of wet biomass are required.
- Environmental impacts (soil organic carbon, biodiversity) of pre-winter harvest needs to be assessed, since mulch layer is likely to decrease due to reduced leaf fall and reduced winter-cover.
- Combustion and anaerobic digestion both require genotypes with a high biomass production. However, for combustion low yield losses over winter and a high stability of the crop (no lodging) are of importance, while for anaerobic digestion cutting tolerance and easier digestibility (low lignin content) are important.

to be adapted to process lignocellulosic miscanthus biomass or extended by suitable pre-treatment facilities. Encouraging practical experience has been gained using a MeWa Bio-QZ (ANDRITZ MeWa GmbH,Gechingen) at the full-scale research biogas plant of the University of Hohenheim. Anaerobic digestion of miscanthus has the potential to produce biogas more cheaply than other feedstocks and offers the co-benefit of easier nutrient recycling via digestate than via ash from combustion.

AUTHOR CONTRIBUTIONS

AK and IL were leading the preparation and writing of this paper. JC, LT, and all other co-authors contributed to the writing of the manuscript and in discussing the results. CN collected and provided data of the multilocation trials relevant for the preparation of this paper. YI, MÖ, IT, and OK supported and conducted sampling of the field trials.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: http://journal.frontiersin.org/article/10.3389/fpls.2017.00347/full#supplementary-material

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Chapter 5 - Evaluation of *Miscanthus sinensis* biomass quality as feedstock for conversion into different bioenergy products

Picture: University of Hohenheim/Astrid Untermann

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Evaluation of *Miscanthus sinensis* biomass quality as feedstock for conversion into different bioenergy products

TIM VAN DER WEIJDE[1,2], ANDREAS KIESEL[3], YASIR IQBAL[3], HILDE MUYLLE[4], OENE DOLSTRA[1], RICHARD G. F. VISSER[1], IRIS LEWANDOWSKI[3] and LUISA M. TRINDADE[1]

[1]*Wageningen UR Plant Breeding, Wageningen University and Research Center, PO Box 386, 6700 AJ Wageningen, The Netherlands,* [2]*Graduate School Experimental Plant Sciences, Wageningen University, Droevendaalsesteeg 1, 6708 PB Wageningen, The Netherlands,* [3]*Biobased Products and Energy Crops, University of Hohenheim, 70599 Stuttgart, Germany,* [4]*Institute for Agricultural and Fisheries Research (ILVO), 9090 Melle, Belgium*

Abstract

Miscanthus is a promising fiber crop with high potential for sustainable biomass production for a biobased economy. The effect of biomass composition on the processing efficiency of miscanthus biomass for different biorefinery value chains was evaluated, including combustion, anaerobic digestion and enzymatic saccharification for the production of bioethanol. Biomass quality and composition was analyzed in detail using stem and leaf fractions of summer (July) and winter (March) harvested biomass of eight compositionally diverse *Miscanthus sinensis* genotypes. Genotype performance in tests for enzymatic saccharification, anaerobic digestion and combustion differed extensively. The variation between the best and the worst performing genotype was 18% for biogas yield (ml g^{-1} dm) and 42% for saccharification efficiency (glucose release as %dm). The ash content of the best performing genotype was 62% lower than that of the genotype with the highest ash content and showed a considerably high ash melting temperature during combustion. Variation between genotypes in biomass quality for the different thermochemical bioconversion processes was shown to be strongly correlated to differences in biomass composition. The most important traits that contributed favorably to biogas yields and saccharification efficiency were a high content of *trans*-ferulic acid, a high ratio of *para*-coumaric acid to lignin and a low lignin content. Additionally, a high content of hemicellulosic polysaccharides positively affected saccharification efficiency. Low contents of ash and inorganic elements positively affect biomass quality for combustion and low potassium and chloride contents contributed to a higher ash melting temperature. These results demonstrate the potential for optimizing and exploiting *M. sinensis* as a multipurpose lignocellulosic feedstock, particularly for bioenergy applications.

Keywords: anaerobic digestion, bioethanol, biogas, biomass quality, cell wall composition, combustion, enzymatic saccharification, lignin, *Miscanthus sinensis*

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Introduction

Miscanthus is a promising fiber crop with high potential for sustainable biomass production in temperate climates (Heaton *et al*., 2010). It is a perennial C4 grass characterized by high annual biomass yields and a high resource-use efficiency (Long *et al*., 2001; Lewandowski *et al*., 2003b; Heaton *et al*., 2004, 2008; Van Der Weijde *et al*., 2013). Given its potential as a high yielding, low-input lignocellulosic feedstock, there is growing interest in the use of miscanthus biomass for a plethora of applications, in particular the production of bioenergy and biofuels (Brosse *et al*., 2012). Applications of lignocellulosic biomass are manifold, and three important bioenergy conversion routes include direct combustion, anaerobic digestion to produce biomethane and enzymatic saccharification and fermentation to produce bioethanol. The chemical composition and structure of cell walls play an important role in biomass quality for each of the aforementioned processes. Therefore, optimization of chemical composition and physical structure are envisioned to improve the process efficiency, which will subsequently contribute to the feasibility and economic success of bioenergy conversion technologies (Wyman, 2007; Torres *et al*., 2016).

There are different options to optimize and improve the biomass quality to facilitate the respective thermochemical conversion processes. Improved biomass quality can be achieved through breeding for

Correspondence: Luisa M. Trindade, tel. +31 317 482127, fax +31 317 483457, e-mail: luisa.trindade@wur.nl

quality traits. Currently, biomass quality is an important breeding objective in bioenergy crops such as miscanthus (Hodgson *et al*., 2010; Van Der Weijde *et al*., 2013). Another option is to improve biomass quality through 'on field quality management practices' such as fertilization and harvest time (Lewandowski & Kicherer, 1997; Lewandowski & Heinz, 2003; Lewandowski *et al*., 2003a; Iqbal & Lewandowski, 2014). However, intrinsic differences between the distinct conversion routes result in route-specific requirements on biomass quality, either because they target other plant components or use another process to convert them into products. Lignin, for example, negatively affects the efficiency of biological conversion routes, such as fermentation or anaerobic digestion (Jørgensen *et al*., 2007; Wyman, 2007; Zhao *et al*., 2012), but has a favorable influence on the heating value of biomass for direct combustion (Lewandowski & Kicherer, 1997). Furthermore, for most bioconversion processes, the route-specific biomass quality requirements are not yet clearly defined due to a number of reasons. First of all, most bioconversion processes are not yet mature technologies and still need to be optimized. A second reason is that we do not yet fully understand the complex structure of lignocellulose and how it affects different bioconversion processes. The final reason is that biomass recalcitrance factors have evolved over a long time to protect the plant against environmental threats and we are now challenged to find ways to manipulate biomass recalcitrance without adversely affecting plant performance (Himmel *et al*., 2007; Zhao *et al*., 2012).

Improving biomass quality in miscanthus through plant breeding is plausible, as the genus *Miscanthus* harbors extensive genetic diversity that may be exploited for the development of new varieties (e.g., Clifton-Brown *et al*., 2008; Heaton *et al*., 2010). *Miscanthus sinensis* is one of the most promising species of miscanthus for biomass production in different environments, as it naturally occurs over a large geographical range in terms of latitude, longitude and altitude (Lewandowski *et al*., 2000; Farrell *et al*., 2006; Clifton-Brown *et al*., 2008). Moreover, extensive variation in cell wall composition has been reported in *M. sinensis*, with cellulose content ranging from ~26 to 47%, hemicellulose content from ~25 to 43% and lignin content from ~5 to 15% of dry matter (Allison *et al*., 2011; Qin *et al*., 2012; Zhao *et al*., 2014). Due to this extensive variation, the development of varieties with optimized biomass quality seems to be promising for various bioconversion routes.

The aim of this study was to understand how variation in lignocellulose composition affects the efficiency of different bioconversion processes and to explore the potential of miscanthus as a multipurpose crop that can be bred for a variety of different biobased applications. In this study, a diverse set of eight *M. sinensis* genotypes was selected from the miscanthus breeding program of Wageningen University and evaluated to gain insight in their potential for different biobased applications, including combustion, anaerobic digestion and enzymatic saccharification for ethanol production. The chemical composition of the stem and leaf fractions of biomass of these genotypes harvested in summer and winter was investigated to get insight in the effects of harvest time and genotype on traits considered to be relevant to the different bioenergy conversion technologies. The aim was to demonstrate the potential options for the use of miscanthus biomass as a feedstock for generating different types of bioenergy and to further define the selection criteria that will allow breeders to develop new varieties that are compositionally tailored to different value chains.

Materials and methods

Plant materials

Eight *M. sinensis* genotypes with a diverse cell wall composition profile were selected from the miscanthus breeding program of Wageningen University and used to establish a replicated field trial on a sandy soil in Wageningen, the Netherlands, in June 2013. A more detailed description and background information of the evaluated genotypes are given in Table S1. The genotypes were propagated *in vitro* to generate enough plantlets for setting up the trial. The trial was managed without irrigation, fertilization, pest, or weed control. The field trial had a design with four randomized blocks of eight plots. Plots had a size of 9 m^2 and contained 16 plants. All plots were surrounded by two rows with medium-sized *M. sinensis* plants to minimize possible border effects. Plant spacing between and within rows was 75 cm. In the establishment year, the trial was harvested (March 2014), but no samples were taken for analysis. After the establishment year, two different harvest regimes were imposed on the trial: Two of the four blocks were randomly assigned to be subjected to a double-cut harvest regime and the other two blocks were subjected to a single-cut harvest regime. The single-cut harvesting regime involved a cut in March 2015, referred to as 'winter cut'. The double-cut harvesting regime involved a green cut in mid-July 2014, referred to as 'summer cut' and a harvest of the regrowth in March 2015, referred to as 'regrowth cut', which coincided with the winter cut of the single-cut harvesting regime. At the time of the summer cut, genotypes OPM-42, 49 and 87 were already flowering, whereas the other genotypes were still in the vegetative phase.

For each of the three cuts, the leaf, stem and total dry matter yield were determined per plot after chopping the samples into ~2 cm chips and subsequent air drying at 60 °C for 72 h in a forced-air oven. The leaf fraction consisted only of leaf blades, with leaf sheets remaining in the stem fraction. The samples from the summer and the winter cut were subsequently used for laboratory analysis: The separated leaf and stem fractions were used for biochemical analysis of the different cell wall components in both tissues, while a subsample in which stems and leaves were kept together was used for biomass quality assessment, including analyses of biogas yield, saccharification efficiency and combustion quality. All samples were ground using a hammer mill with a 1-mm screen.

Cell wall polymer composition

Neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin contents (ADL) of stem dry matter were determined according to protocols developed by Ankom Technology (ANKOM Technology Corporation, Fairpoint, NY), which are essentially based on the work of Van Soest and coworkers (Van Soest, 1967; Goering & Van Soest, 1970). Neutral and acid detergent extractions were performed using an ANKOM 2000 Fiber Analyzer (ANKOM Technology Corporation). Acid detergent lignin was determined after 3-h hydrolysis of the ADF residue in 72% H_2SO_4 with continuous shaking. All fiber analyses were performed in triplicate. The weight fractions of detergent fiber residues in dry matter were subsequently used as estimate for the content of cell wall in dry matter (NDF%dm) and to obtain the contents of cellulose ([ADF%dm-ADL%dm]/NDF%dm × 100%), hemicellulosic polysaccharides ([NDF%dm-ADF%dm]/NDF%dm × 100%) and lignin (ADL%dm/NDF%dm × 100%) relative to the cell wall content.

Cell wall monosaccharide composition

The residual NDF material of the replicated fiber analyses was pooled per sample and used as a basis for determination of neutral sugar contents as described previously by Van Der Weijde *et al.* (2016). Measurements were taken in three replications. Briefly, 30 mg of NDF material was hydrolyzed in 0.3 ml 72% H_2SO_4 in a 10-ml glass pressure tube for 1 h at 30 °C with constant shaking (160 rpm). After 1 h, the acid concentration was diluted to 4% by adding 8.4 ml deionized water, after which samples were hydrolyzed for 3 h in a heating block set at 100 °C with a rotation speed of 160 rpm. After cooling down, the samples were centrifuged and a subsample of the supernatant was purified using a 0.45 μm filter. Contents of glucose (Glu), xylose (Xyl), arabinose (Ara) and galactose (Gal) in the purified supernatant were determined by high-performance anion exchange chromatography (HPAEC) analysis on a Dionex system equipped with a CarboPac PA1 column and a pulsed amperometric detector (Dionex, Sunnydale, CA). The degree of hemicellulose substitution (DHS) is the weight ratio of arabinose to xylose expressed as a percentage.

Monosaccharide acetylation

The amount of acetyl groups on monosaccharides was estimated by quantifying acetic acid in the undiluted, purified neutral sugar hydrolysate using an acetate dehydrogenase assay kit (Megazyme International Ireland Ltd., Bray, Ireland) adapted to a 96-well microplate format. The increase in sample absorbance at 340 nm following enzymatic dehydrogenase reactions was quantified using a Bio-Rad Microplate reader (Bio-Rad, Richmond, CA, USA).Acetic acid concentration in the sample was calculated from the increase in sample absorbance by interpolation from a six point standard curve of acetic acid (Megazyme International Ireland Ltd.). The degree of hemicellulose acetylation (DHA) is the dry weight of acetic acid expressed as a percentage of the dry weight of xylose on a sample basis.

Hydroxycinnamic acids

Hydroxycinnamic acids, specifically *p*-coumaric acid (*p*CA) and *trans*-ferulic acid (TFA), were quantified after extraction as described previously (Buanafina *et al.*, 2006). Briefly, an Eppendorf tube was filled with 10 mg NDF material of the samples and for the reference tests with 10 mg cellulose (Cellulose type 101; Sigma-Aldrich, Diegem, Belgium). The latter are also spiked with 100 μg TFA (Sigma-Aldrich) and *p*CA (Sigma-Aldrich). The tubes were subsequently incubated overnight in 750 μl 2 M NaOH at 25 °C and under constant shaking. Trimethoxycinnamic acid (TMCA, Sigma-Aldrich) was added as internal standard, and the pH of all samples was adjusted to two with HCL. A liquid–liquid extraction with diethyl ether was performed twice, after which the residue was dried for 1 h at 40 °C and resuspended in 1 ml 5% acetonitrile (MeCN) and vortexed for 15 s. Subsequently samples were 10 times diluted with 5% MeCN and stored at −20 °C before analysis. For each sample, 10 μl was injected into a liquid chromatography–high-resolution mass spectrometry (LC-HRMS) system. Chromatographic separation was performed with an Acquity Ultra Performance system (Waters, Milford, MA, USA) using a Waters BEH Shield C18 column (2.1 × 150 mm, 1.7 μm) held at 40 °C and equipped with an Shield C18 VanGuard precolumn (Waters). The mobile phase consisted of H_2O + 0.1% TFA (solvent A) and MeCN + 0.1% TFA (solvent B) at a flow rate of 0.35 ml min^{-1}. Gradient separation was performed as follows: linear increase from 5% to 50% B in 30 min, subsequent linear increase to 100% B in 1 min, held at 100% B for 6 min, followed by immediate decrease to 5% B and finally re-equilibration at 5% B for 5 min. Mass spectrometric detection and quantification were performed using a Synapt G2-S high-resolution mass spectrometer (Waters) acquiring full scan HRMS data (50–1200 Da) in resolution mode negative (20 000 FWHM). Source temperature and desolvation temperature were set 120 and 500 °C, respectively. Prior to analysis, the HRMS was calibrated (50–1200 Da) using a sodium formate solution. During analysis, leucine-enkephalin (200 pg μl^{-1}) was constantly infused as lock mass. Data were analyzed using the MassLynx software version 4.1 (Waters). The ratio of *p*CA to ADL (*p*CA/ADL) was calculated by expressing the dry weight of *p*CA as a percentage of the dry weight of ADL on a sample basis. Similarly, the ratio of TFA to xylose (DHF, for degree of hemicellulose feruloylation) was calculated by expressing the dry weight of TFA as a percentage of the dry weight of xylose on a sample basis.

Contents of ash and inorganic elements

Dried biomass samples were analyzed in the laboratory for N, Na, K, Ca, Mg, P, Cl, Si and ash content. Analysis of N was carried out following the Dumus principle using a Vario Macro cube (Elementaranalysensysteme GmbH, Hanau, Germany). For determination of Na, K and Ca, 500 mg dried biomass samples were dissolved in 8 ml HNO_3 (65%), to which 4 ml H_2O_2 was added to remove color. Samples were then digested in a microwave (Ethos.Lab, MLS GmbH, Leutkirch, Germany) at 120–180 °C and a pressure of 24.16 bar for 40 min. Digested samples were then filtered through Whatman filter paper and contents of P, K, Mg, Ca and Na in the extracts were determined using inductively coupled plasma-optical emission spectrometry (ICP-OES; Vista Pro, Varian Inc., Palo Alto, CA, USA). For determination of Cl, extractions were performed with hot deionized water and treated with a clarifying agent (Carrez I, containing 15 g $K_4Fe(CN)_6{\cdot}3H_2O$ in 100 ml deionized water and Carrez II, containing 30 g $Zn(CH_6COO)_2{\cdot}2H_20$ in 100 ml deionized water). The extracts were measured by ion chromatography (ICS 2000; Dionex Corporation, Sunnyvale, CA, USA). For the determination of Si content, samples were digested with HNO_3 and HF and measured with help of using ICP-OES (Vista Pro, Varian). Ash content was quantified gravimetrically after 4-h incineration in an electric muffle furnace at 550 °C.

Ash melting behavior during combustion

To assess the ash melting behavior during combustion process, the method was adopted from Tonn *et al.* (2012). Briefly, 100 mg ash samples were transferred to ceramic combustion boats (Lab Logistics group GmbH, Meckenheim, Germany) and subjected to four different combustion temperature treatments (800, 900, 1000 and 1100 °C) for 2 h in an electric muffle furnace. The electric muffle furnace was heated at an average rate of 10 °C min^{-1} until the required heating temperature was achieved. After 2 h, the combustion boats were transferred into an exicator to allow them to cool down before microscopic analysis. Each sample was analyzed under a stereo microscope (Zeiss Stemi 2000-C; Carl Zeiss AG, Oberkochen, Germany) at magnifications up to 40× and classified into one of four ash fusion classes (AFC) (Table 1) as described by Tonn *et al.* (2012).

Table 1 Ash fusion classes and ash fusion temperature along with microscopic observations (source: Tonn *et al.*, 2012)

Ash-fusion classes	Microscopic observations
(1) Loosening	Particles are arranged in loose layers, spatula can move through without any resistance, shiny surfaces with tiny molten vesicles
(2) Partially sintered	Particles start becoming compact through strong adhesive forces, still easy to disintegrate, produces crispy sound when spatula passes through, larger molten vesicles on the surface
(3) Highly sintered	Difficult to disintegrate, most of the area covered with larger molten vesicles. Organogenic material also visible in some parts
(4) Molten	Particles are completely molten, manual disintegration is not possible, no organogenic material visible

Biogas yield upon anaerobic digestion

The substrate-specific biogas (SSBY) and methane yields (SSMY) were measured in a biogas batch test under mesophilic conditions at 39 °C according to VDI guideline 4630. The biogas batch method was described by Kiesel & Lewandowski (2016) and certified after KTBL and VDLUFA inter-laboratory comparison test 2014. The fermentation period was 35 days. Four replicates of each sample were analyzed. A maize standard was analyzed alongside the miscanthus samples to monitor the activity of the inoculum. The inoculum originated from the fermenter of a commercial mesophilic biogas plant which uses the following substrates: maize silage, grass silage, cereal whole crop silage, liquid and solid cattle manure and small quantities of horse manure. The inoculum was sieved and diluted to 4% (w/w) dry matter with deionized water. Various macro- and micronutrients were added as described by Angelidaki *et al.* (2009). Afterward, the inoculum was incubated at 39 °C under anaerobic conditions for 6 days.

For the biogas batch analysis, 200 mg miscanthus samples were transferred into a 100-ml fermentation flask, 30 g inoculum was added, and the gas-containing headspace was flushed with nitrogen to attain anaerobic conditions. The fermentation flasks were closed gastight by a butyl rubber stopper and an aluminum cap. The pressure increase in the fermentation flasks was measured by puncturing the butyl rubber stopper with a cannula attached to a HND-P pressure meter (Kobold Messring GmbH, Hofheim, Germany). The biogas production was calculated as dry gas (water vapor pressure was considered) from the pressure increase and was standardized to 0 °C and 1013 hPa using Eqns (1) and (2). Equation (1) was used for the first measurement and considers the pressure increase due to warming from laboratory temperature to 39 °C and the water vapor partial pressure. Equation (2) was used for the subsequent 17 measurements, which were taken on regular basis.

$$V_{\mathrm{biogas}} = V_{\mathrm{HS}} * T_{\mathrm{S}}/T_{\mathrm{F}} * ((P_{\mathrm{A1}} + P_{\mathrm{F1}}) - (P_{\mathrm{A0}} * T_{\mathrm{F}}/T_{\mathrm{Lab}}) - P_{\mathrm{WP}})/P_{\mathrm{S}} \tag{1}$$

where V_{biogas} = volume of produced biogas, V_{HS} = volume of gas-containing headspace, T_{S} = standard temperature of 273.15 °K (= 0 °C), T_{F} = fermentation temperature of 312.15 °K (= 39 C), P_{A1} = ambient pressure at first measurement, P_{F1} = overpressure in fermentation flasks at first measurement, P_{A0} = ambient pressure at sealing of the fermentation flasks (batch test start), T_{Lab} = laboratory temperature at sealing of the fermentation flasks (batch test start), P_{WP} = water vapor partial pressure at 39 C, P_{S} = standard pressure (1013 hPa)

$$V_{\mathrm{biogas}} = V_{\mathrm{HS}} * T_{\mathrm{S}}/T_{\mathrm{F}} * ((P_{\mathrm{An}} + P_{\mathrm{Fn}}) - (P_{\mathrm{A}(n-1)} + P_{\mathrm{F}(n-1)}))/P_{\mathrm{S}} \tag{2}$$

where P_{An} = ambient pressure at the actual measurement, P_{Fn} = overpressure in fermentation flask at the actual measurement, $P_{\mathrm{A}(n\text{-}1)}$ = ambient pressure at the previous time-point, $P_{F(n-1)}$ = overpressure in the fermentation flasks at the previous time-point.

During the course of the biogas batch test, it was occasionally necessary to remove the produced biogas from the fermentation flasks. The overpressure in the fermentation flasks was removed using a gastight syringe once it had reached an approximate value of 500 mbar. The biogas was transferred to a gastight storage flask where it was kept until the end of the batch test. After each gas collection, the remaining overpressure in the fermentation flasks was allowed to level off to ambient pressure by injecting a blank cannula. For the subsequent measurement, $P_{F(n-1)}$ was then set to zero in Eqn (2). At the end of the batch test, the remaining biogas in the headspace of the fermentation flasks was removed by active extraction with a syringe and also transferred into the storage flask. An aliquot of the collected biogas was used for analyzing the methane content using gas chromatography (GC-2014, Shimadzu, Kyoto, Japan). The gas chromatograph was equipped with a thermal conductivity detector, and the detection temperature was set to 120 °C. Two columns (HayeSep and Molsieve) were used for separation, with system temperature set at 50 °C and argon as carrier gas. The gas samples were injected using a Combi-xt PAL auto-sampler (CTC Analytics AG, Zwingen, Germany).

Saccharification efficiency for bioethanol production

Saccharification reactions were carried out as described previously by Van Der Weijde *et al.* (2016). Briefly, 500 mg biomass samples was briefly treated with α-amylase and repeatedly washed with deionized water (3×, 5 min, ~60°C) to remove all interfering soluble sugars. Remaining biomass was subjected to alkaline pretreatment with 15 ml 2% NaOH at 50°C and constant shaking (160 rpm) for 2 h in a shaker incubator (Innova 42; New Brunswick Scientific, Enfield, CT, USA). Pretreated samples were then washed to neutral pH with deionized water (2×, 5 min, 50°C) and with 0.1 M sodium citrate buffer (pH 4.6, 5 min, 50°C).

Saccharification reactions were subsequently carried out according to the NREL Laboratory Analytical Procedure ‘Enzymatic saccharification of lignocellulosic biomass’ (Selig *et al.*, 2008). Pretreated samples were hydrolyzed for 48 h with 300 μl of the commercial enzyme cocktail Accellerase 1500 (DuPont Industrial Biosciences, Leiden, the Netherlands) supplemented with 15 μl endo-1,4-β-xylanase M1 (Megazyme, Bray, IE, USA) in a shaker incubator (Innova 42; New Brunswick Scientific) set at 50°C and constant shaking (160 rpm). These enzymes combined have the following specific activities: endoglucanase 2200–2800 CMC U g^{-1}, beta-glucosidase 450–775 pNPG U g^{-1} and endoxylanase 230 U mg^{-1}. Reactions were carried out in 44 ml 0.1 M sodium citrate buffer (pH 4.6), containing 0.4 ml 2% sodium azide to prevent microbial contamination.

Enzymatic saccharification liquors were analyzed for glucose and xylose content by HPAEC as described previously for neutral sugars. The potential of a genotype for bioethanol production was assessed by expressing the total fermentable sugar yield in two ways. The first is the absolute yield of glucose and xylose as a percentage of dry matter (glucose release %dm and xylose release %dm). The second way is to express the yield of glucose and xylose as a percentage of the respective total available cell wall glucan (glucose conversion %) and xylan (xylose conversion %), as measures of saccharification efficiency.

Statistical analyses

General analyses of variance (ANOVA) were performed to determine the significance of genotype differences ($P < 0.05$) in compositional traits and quantitative route-specific quality parameters. Friedman’s nonparametric ANOVA was performed to determine the significance of genotype differences in ash fusion classes. Variance analyses were performed following the standard procedure of a mixed effect model with a random genetic effect and a fixed block effect, following the model (3):

$$Y_{ij} = \mu + G_{ij} + B_j + e_{ij} \tag{3}$$

where Y_{ij} is the response variable, μ is the grand mean, G_{ij} is the genotype effect, B_j is the block effect, and e_{ij} is the residual error.

Correlation analyses were performed to identify the significance, strength and direction of interrelationships between traits using Pearson’s correlation coefficients. Multiple linear regression analyses were performed for the development of simple regression equations for biogas yield and saccharification efficiency. All statistical analyses were performed using Genstat for Windows, 18th edition software package (VSN International, Hemel Hempstead, UK).

Results

Large differences in field performance between genotypes and harvest regimes

The field performance of the eight miscanthus genotypes was evaluated by assessing dry stem, leaf and total biomass yields of the genotypes from a single- and a double-cut harvest regime (Table 2). Biomass yields from the double-cut harvest regime were significantly lower than from the single-cut harvest regime. Averaged over all genotypes, the summer cut yielded 1803 kg dm ha^{-1}, and the regrowth cut yielded an additional 630 kg dm ha^{-1}. The winter cut, however, yielded on average 6314 kg dm ha^{-1}. The highest yielding genotype (OPM-69) in the winter cut had an average total biomass yield as high as 10 583 kg dm ha^{-1}. Furthermore, roughly 60% of the summer cut and roughly 45% of the regrowth cut consisted of stem material, whereas the biomass of the winter cut consisted almost completely of stem material. The genotypic variation for dry biomass yield and stem fraction of total yield, respectively, as realized during the first whole growing season was extensive (Table 2).

Genotypes show highly diverse cell wall composition

The summer cut and winter cut biomass samples of the eight miscanthus genotypes were analyzed

Table 2 Means of a diverse set of eight *Miscanthus sinensis* accessions for total dry matter yield and the weight distribution of total dry matter among stem and leaf fractions evaluated in a single cut and double cut harvest regime following the first complete growing season

			Accession										
Harvest regime	Trait	Unit	OPM 42	OPM 48	OPM 49	OPM 65	OPM 69	OPM 73	OPM 77	OPM 87	Average	Range	*F*-prob.
Double-cut	Yield	kg dm ha^{-1}	747	490	423	614	994	1099	661	568	700	675	<0.001
(Summer cut)	Stem	%	60	53	63	52	70	66	58	58	60	18	<0.001
	Leaf	%	40	47	37	48	30	34	42	42	40	18	<0.001
Double-cut	Yield	kg dm ha^{-1}	1866	2649	1102	1664	2206	2427	1873	2329	2015	1548	0.006
(Regrowth cut)	Stem	%	44	54	56	43	46	55	70	44	52	27	0.022
	Leaf	%	56	46	44	57	54	45	30	56	48	27	0.022
Single-cut	Yield	kg dm ha^{-1}	5788	5948	4975	5422	11 759	7925	7494	6809	7015	6783	0.005
(Winter cut)	Stem	%	93	96	92	97	94	91	94	88	93	9	0.056
	Leaf	%	7	4	8	3	6	9	6	12	7	9	0.056

Table 3 Means of a diverse set of eight *Miscanthus sinensis* genotypes for stem biomass and cell wall components of the summer cut

		Genotype										
Trait	Unit	OPM 42	OPM 48	OPM 49	OPM 65	OPM 69	OPM 73	OPM 77	OPM 87	Average	Range	*F*-prob.
NDF	%dm	83.92	83.10	80.81	80.00	80.33	82.06	83.54	81.47	81.90	3.91	0.005
ADF	%dm	49.22	50.73	48.44	47.51	55.39	51.34	50.72	48.76	50.26	7.88	0.003
CEL	%cw	51.11	54.85	53.51	53.40	58.84	55.63	53.82	52.45	54.20	7.73	<0.001
HEM	%cw	41.34	38.95	40.06	40.63	31.04	37.43	39.29	40.14	38.61	10.31	<0.001
ADL	%cw	7.55	6.20	6.42	5.97	10.12	6.94	6.89	7.41	7.19	4.15	0.003
Glu	%cw	51.82	52.29	53.93	53.00	53.37	55.26	51.69	51.33	52.84	3.93	0.195
Xyl	%cw	32.02	31.30	33.07	30.84	27.54	30.24	31.62	30.89	30.94	5.53	0.005
Ara	%cw	3.43	3.52	3.78	3.36	2.57	2.73	3.44	3.28	3.26	1.21	0.002
Gal	%cw	0.38	0.27	0.33	0.31	0.24	0.20	0.37	0.23	0.29	0.19	<0.001

NDF, neutral detergent fiber; ADF, Acid detergent fiber; Cel, Cellulose; Hem, Hemicellulose; ADL, Acid detergent lignin; Glu, Glucose; Xyl, Xylose; Ara, Arabinose.

biochemically (Tables 3 and 4, respectively). The tables show the mean performance of each genotype for a wide set of stem biomass and cell wall traits, such as the content, chemical composition and structural complexity of various cell wall polymers. Significant differences between genotypes were found for nearly all cell wall components. Stem samples of the winter cut were analyzed in most detail, as they represent the largest weight fraction of all the harvested biomass.

In the summer cut, approximately 82% of the stem dry matter consisted of cell wall material, which increased to approximately 92% in the winter cut (Tables 3 and 4). In the winter cut, very little variation in stem cell wall content existed and genotypes were not found to be significantly different from each other (Table 4). The composition of the cell wall material also differed markedly between the summer and winter cut samples, with the summer cut samples generally being lower in cellulose and lignin contents, but higher in contents of hemicelluloses. In both cuts, particularly, large genotypic variation was found for Hem and ADL. Hemicellulose content in stem cell walls varied among genotypes ~31–41% in the summer cut and from ~29 to 37% in the winter cut. For stem cell wall, lignin content variation among genotypes ranged from ~6 to 10% in the summer cut and from ~8 to 13% in the winter cut (Tables 3 and 4).

In both cuts, also large variation was found in the neutral sugar composition of the stem cell wall material, particularly for arabinose and galactose, which are sugars that are present in side chains on the xylan backbone of grass hemicelluloses (Tables 3 and 4). For the winter cut stem samples, additional measurements were taken to investigate minor components of the cell wall matrix, such as hydroxycinnamic acids (TFA and *p*CA) and acetic acid. The ratios of arabinose to xylose (DHS), TFA to xylose (DHF), acetic acid to xylose (DHA) and *p*CA to ADL were investigated, as these provide

Table 4 Means of a diverse set of eight *Miscanthus sinensis* genotypes for stem biomass and cell wall components of the winter cut

		Genotype										
Trait	Unit	OPM 42	OPM 48	OPM 49	OPM 65	OPM 69	OPM 73	OPM 77	OPM 87	Average	Range	*F*-prob.
NDF	% dm	92.01	90.51	90.91	91.56	93.12	91.18	90.83	91.77	91.49	2.62	0.193
ADF	% dm	58.39	58.61	59.20	57.68	66.55	61.75	58.83	59.39	60.05	8.87	0.002
CEL	% cw	53.99	55.71	56.44	55.03	58.31	58.87	55.12	54.35	55.98	4.88	0.006
HEM	% cw	36.53	35.24	34.88	37.01	28.54	32.28	35.23	35.29	34.38	8.47	<0.001
ADL	% cw	9.47	9.05	8.68	7.96	13.15	8.86	9.65	10.36	9.65	5.19	<0.001
PCA	μg mg^{-1} cw	14.98	16.48	16.16	15.45	15.15	13.75	15.96	15.51	15.43	2.74	<0.001
PCA/ADL	% ADL	15.81	18.21	18.62	19.40	11.53	15.52	16.56	14.97	16.33	7.87	<0.001
Glu	% cw	46.20	46.33	45.32	45.30	48.30	49.09	45.03	46.65	46.53	4.07	0.040
Xyl	% cw	32.06	30.66	30.49	31.13	26.28	30.64	31.35	31.42	30.50	5.77	<0.001
Ara	% cw	3.18	3.21	3.08	3.00	2.41	2.44	3.11	2.81	2.91	0.80	0.001
Gal	% cw	0.38	0.25	0.26	0.28	0.22	0.16	0.34	0.23	0.26	0.22	<0.001
TFA	μg mg^{-1} cw	4.97	5.60	5.98	5.68	3.70	5.03	5.65	4.98	5.20	2.28	<0.001
Acetic acid	μg mg^{-1} cw	0.28	0.27	0.26	0.25	0.25	0.26	0.25	0.28	0.26	0.03	0.069
DHS	% Xyl	9.94	10.46	10.10	9.65	9.18	7.98	9.93	8.94	9.52	2.48	0.016
DHA	% Xyl	0.09	0.09	0.09	0.08	0.10	0.08	0.08	0.09	0.09	0.02	0.062
DHF	% Xyl	1.43	1.65	1.78	1.67	1.31	1.50	1.64	1.45	1.55	0.47	<0.001

NDF, neutral detergent fiber; ADF, Acid detergent fiber; Cel, Cellulose; Hem, Hemicellulose; ADL, Acid detergent lignin; PCA, para-coumaric acid; Glu, Glucose; Xyl, Xylose; Ara, Arabinose; TFA, trans-ferulic acid; DHS, Degree of hemicellulose substitution (ratio of arabinose to xylose); DHA, Degree of hemicellulose acetylation (ratio of acetic acid to xylose); DHF, Degree of hemicellulose feruloyation (ratio of TFA to xylose).

indications of the complexity and level of substitutions/side groups on xylose and lignin residues. Significant genotypic differences were found for all these ratios, with the exception of DHA, indicating that genetic variation for these trait ratios is available in the species (Table 4).

Leaf samples of the summer and winter cuts were also analyzed biochemically and the results are summarized in boxplots that display the variation in cell wall, cellulose, hemicellulose and lignin contents, respectively (Fig. 1a–d). Compared to the stem samples, leaf samples generally contained lower contents of NDF and cellulose, but higher contents of hemicellulosic polysaccharides. In the summer cut samples, stem tissues were higher in lignin content than leaf tissues, while in the winter cut samples, the opposite trend was observed (Fig. 1d).

Large variation in genotype performance in different bioenergy conversion processes

The potential as feedstock of eight *M. sinensis* genotypes for three different types of bioenergy conversion processes, that is, anaerobic digestion, enzymatic saccharification and combustion, was evaluated in this study. Genotype means of specific quality characteristics relevant to the different types of bioenergy conversion route are presented in Tables 5 and 6, for biomass samples harvested in the summer and winter cut, respectively. Genotypes showed significant differences for many specific quality traits relating to the different bioenergy applications. Anaerobic digestion of samples from the summer cut resulted in higher biogas yields compared to biomass samples from the winter cut, with genotype means for substrate-specific biogas yields ranging from 539 to 591 ml g^{-1} dry matter for the summer cut and 441 to 520 ml g^{-1} dry matter for the winter cut. Methane content in the produced biogas was approximately 52%, regardless of the time of harvest. The highest biogas yields were achieved by OPM-65 in the summer cut and OPM-73 in the winter cut, while in both cuts OPM-69 consistently had the lowest biogas yields.

To assess the quality of the biomass samples from the summer and the winter cut for fermentation of structural sugars into bioethanol, the samples were pretreated and incubated with a commercial enzyme cocktail to study the yield and efficiency of the release of fermentable sugars. Significant differences among genotypes were found for glucose release and glucose conversion in both harvests and for xylose release in the green cut, but not for xylose conversion (Tables 5 and 6), despite large differences between genotypes in hemicellulose content (Tables 3 and 4). Similar to the results for biogas yield, higher sugar release and saccharification efficiency were found using the biomass samples of

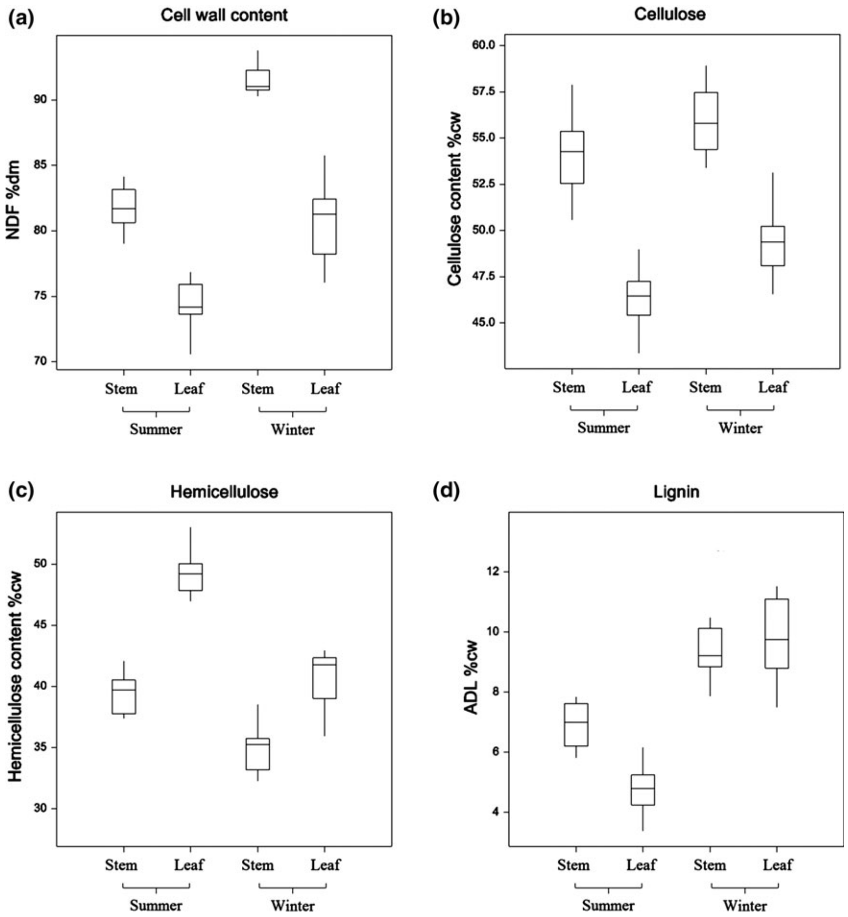


Fig. 1 Boxplots depicting variation in the cell wall content (a), cellulose (b), hemicellulose (c) and lignin (d) contents of miscanthus stem and leaf fractions of eight *Miscanthus sinensis* accessions harvested in a summer cut (July) or a winter cut (March).

the summer cut compared to the samples of the winter cut. Variation among genotypes in glucose conversion was extremely large, especially for biomass samples from the winter cut, ranging from 33% (for OPM-69) to 50% (for OPM-65).

For combustion quality, ash content is an important biomass quality determinant. The average ash content of the samples was 1.54% of dry matter in biomass from the winter cut compared to 3.28% of dry matter in biomass from the summer cut, when the plants had not yet senesced. As a result of the lower ash content, the quality of the biomass samples for combustion was higher in the winter cut than in the summer cut. Significant differences between genotypes for ash content were only found for biomass samples from the summer cut. Genotypes also showed significant differences in the contents of silicon and potassium in the summer cut and chloride and potassium in the winter cut. Furthermore, microscopic observations of ash melting behavior at different combustion temperatures were performed to make a classification of the genotypes into different ash fusion classes. Although samples could be assigned to distinct classes at each of the different temperatures, the classification for none of the tested temperatures has proven to lead to significant differences among genotypes (Tables 5 and 6).

Influence of biomass composition on genotype performance in different types of bioenergy conversion processes

The interrelations between compositional characteristics and specific quality traits for the different bioconversion processes were assessed using correlation analysis. Some of the most important correlations were highlighted in Fig. 2a–d, while the full correlation matrix is presented in Fig. 3. Similarities were found in the traits affecting the efficiency of enzymatic saccharification and anaerobic digestion. Both were negatively correlated to ADL and positively correlated to *p*CA/ADL and TFA (Fig. 2a–c). Additionally, both traits were negatively correlated to NDF and positively correlated to DHF (Fig. 3). No significant correlations were found between

Table 5 Mean performance of biomass of eight *Miscanthus sinensis* genotypes from a summer cut for quality traits relevant for specific bioenergy conversion routes

Route-specific quality characteristics	Genotype OPM 42	OPM 48	OPM 49	OPM 65	OPM 69	OPM 73	OPM 77	OPM 87	Average	Range	*F*-prob.
Anaerobic digestion											
SSBY (ml g^{-1} dm)	562.90	572.87	575.40	591.78	538.84	572.20	561.01	560.33	566.92	52.94	<0.001
SSMY (ml g^{-1} dm)	290.30	296.50	296.64	305.34	278.19	293.57	288.39	290.37	292.41	27.15	<0.001
Methane (% SSBY)	52.13	52.25	52.11	52.13	52.18	51.94	52.02	52.30	52.13	0.36	0.203
Relative quality rating*	–	+	+	++	––	+	–	–			
Fermentation											
Glucose release (%dm)	23.70	24.17	26.25	25.84	25.64	24.96	25.55	25.43	25.19	2.55	0.018
Xylose release (%dm)	6.70	6.85	7.28	7.27	7.71	7.25	7.09	6.82	7.12	1.01	0.175
Glucose conversion (%)	62.96	64.82	66.62	67.59	64.92	64.17	67.95	66.38	65.68	4.99	0.014
Xylose conversion (%)	30.84	32.24	32.48	32.68	33.13	30.95	32.65	31.38	32.04	2.30	0.409
Relative quality rating*	–	++	+	++	––	+	–	–			
Combustion											
Ash (%dm)	2.59	5.05	3.27	3.38	3.42	2.38	3.00	3.13	3.28	2.67	0.006
Silicon (Si) (%dm)	0.31	0.44	0.26	0.33	0.29	0.18	0.26	0.34	0.30	0.26	0.046
Chloride (Cl) (%dm)	0.14	0.24	0.18	0.15	0.19	0.12	0.16	0.14	0.16	0.12	0.065
Potassium (K) (%dm)	0.84	1.90	1.28	1.15	1.42	0.95	1.13	1.17	1.23	1.07	<0.001
Calcium (Ca) (%dm)	0.18	0.21	0.15	0.16	0.09	0.28	0.18	0.18	0.18	0.19	0.670
AFC – 800 °C	1.00	3.00	3.00	1.00	1.75	1.00	1.50	1.75	1.75	2.00	0.066†
AFC – 900 °C	2.00	3.25	4.25	2.00	3.00	1.50	2.00	3.75	2.72	2.75	0.130†
AFC – 1000 °C	3.50	4.25	4.50	4.50	3.50	2.00	4.50	4.25	3.88	2.50	0.088†
AFC – 1100 °C	5.00	4.50	5.00	5.00	4.50	3.75	5.00	5.00	4.72	1.25	0.195†
Relative quality rating*	+	––	–	–	–	++	+	+			

SSBY, substrate-specific biogas yield; SSMY, substrate-specific methane yield.

*Rating based on ranking genotypes by SSBY for anaerobic digestion, by glucose yield for fermentation and by HHV for combustion route. Rank 1 scored '++', rank 2–4 scored '+', rank 5–7 scored '–' and rank 8 scored '––'.

†*P*-value using chi-square approximation resulting from Friedman's nonparametric ANOVA test.

biogas yield and saccharification efficiency, but a weak correlation was found between biogas yield and glucose release. Some cell wall compositional traits were not correlated to biogas yield, but did show correlations to the release and yield of glucose and xylose. Such correlations included positive correlations with Hem, Xyl, Ara and Gal, and negative correlations with ADF, Cel and Glu (Figs 2d and 3).

Multiple regression analysis was performed to develop regression models for glucose conversion and biogas yield based on cell wall compositional characteristics. A simple regression model was found for glucose conversion including only two traits, *p*CA/ADL and galactose, which cumulatively explained 83.2% of the variation for glucose conversion among these genotypes. Two simple regression models were found for SSBY, one which included ADL and galactose, and a second which included *p*CA/ADL and arabinose. Both models were able to account for 83.4% of the variation for SSBY among these genotypes.

Only two cell wall compositional characteristics were found to be correlated to combustion specific quality traits, that is, *p*CA content (r = 0.68) and DHS (r = 0.54), which both showed a positive correlation to the classification of samples to ash fusion classes at a combustion temperature of 800 °C (Fig. 3). However, inorganic elements silicon, potassium and calcium were strongly positively correlated to ash formation during combustion. Moreover, potassium and chloride were shown to be significantly correlated to classification of the genotypes in different ash fusion classes at all tested combustion temperatures (Table 7).

Discussion

Large genetic diversity in biomass composition and quality

The extensive genetic diversity in cell wall compositional traits found in the eight *M. sinensis* genotypes analyzed in this study indicate that there is a large potential in this species for the improvement of biomass quality for different applications. Particularly large variation between genotypes was found for the contents of

Table 6 Mean performance of biomass of eight *Miscanthus sinensis* genotypes from a winter cut for quality traits relevant for specific bioenergy conversion routes

Route-specific quality characteristics	Genotype OPM 42	OPM 48	OPM 49	OPM 65	OPM 69	OPM 73	OPM 77	OPM 87	Average	Range	*F*-prob.
Anaerobic digestion											
SSBY (ml g^{-1} dm)	457.89	500.75	502.08	507.83	441.15	520.08	473.11	462.90	483.22	78.93	0.01
SSMY (ml g^{-1} dm)	236.61	256.40	258.59	260.77	228.70	266.57	244.52	238.11	248.78	37.86	0.018
Methane (% SSBY)	52.27	51.94	52.13	52.02	52.39	51.95	52.27	52.12	52.14	0.46	0.013
Relative quality rating*	–	+	+	+	–	++	–	–			
Fermentation											
Glucose release (%dm)	18.63	18.66	19.68	20.78	14.64	18.25	18.67	17.00	18.29	6.14	0.007
Xylose release (%dm)	8.41	7.74	7.88	8.25	5.78	7.37	8.12	7.66	7.65	2.62	0.005
Glucose conversion (%)	43.83	44.48	47.74	50.11	32.55	40.77	45.65	39.77	43.11	17.57	0.003
Xylose conversion (%)	29.29	28.59	29.12	29.62	24.35	27.22	29.31	27.44	28.12	5.27	0.077
Relative quality rating*	+	–	+	++	–	–	+	–			
Combustion											
Ash (%dm)	1.67	1.77	1.54	1.09	1.64	1.45	1.62	1.56	1.54	0.68	0.358
Silicon (Si) (%dm)	0.37	0.35	0.36	0.30	0.36	0.34	0.35	0.37	0.35	0.07	0.993
Chloride (Cl) (%dm)	0.02	0.06	0.00	0.01	0.05	0.03	0.03	0.02	0.03	0.06	0.002
Potassium (K) (%dm)	0.07	0.28	0.06	0.04	0.18	0.10	0.11	0.04	0.11	0.24	0.001
Calcium (Ca) (%dm)	0.10	0.10	0.11	0.08	0.08	0.09	0.10	0.11	0.10	0.03	0.531
AFC – 800 °C	1.00	2.75	1.50	1.00	1.50	1.00	1.75	1.25	1.47	1.75	0.076†
AFC – 900 °C	1.50	3.25	1.50	1.25	2.00	1.75	2.00	1.50	1.84	2.00	0.076†
AFC – 1000 °C	1.75	4.25	2.00	2.00	2.25	2.25	2.50	2.25	2.41	2.50	0.254†
AFC – 1100 °C	2.25	5.00	2.50	2.25	2.50	2.75	3.00	2.75	2.88	2.75	0.277†
Relative quality rating*	–	–	+	++	–	+	–	+			

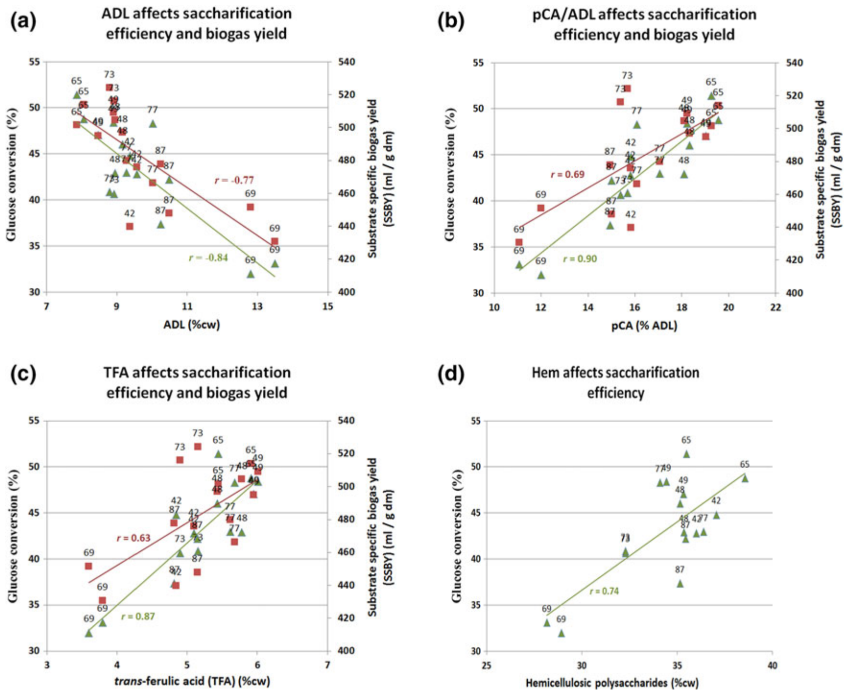
SSBY, substrate-specific biogas yield; SSMY, substrate-specific methane yield.

*Rating based on ranking genotypes by SSBY for anaerobic digestion, by glucose yield for fermentation and by ash content for combustion route. Rank 1 scored '++', rank 2–4 scored '+', rank 5–7 scored '–' and rank 8 scored '–'.

†*P*-value using chi-square approximation resulting from Friedman's nonparametric ANOVA test.

Hem and ADL, which are the key factors determining lignocellulose recalcitrance (Xu *et al.*, 2012; Torres *et al.*, 2014; Van Der Weijde *et al.*, 2016). Additionally, significant genotypic variation was found for specific traits that relate to the degree of cross-linking between hemicelluloses or between hemicelluloses and lignin, and more specifically to the degree of substitution of the xylan backbone of hemicellulosic polysaccharides by arabinose (DHS), the degree of xylan acetylation (DHA) and feruloyation (DHF), and the ratio of *para*-coumaric acid to lignin (*p*CA/ADL). This is an important observation, as there is strong evidence that cell wall cross-links play important roles in cell wall degradability (e.g., Hatfield *et al.*, 1999; Grabber *et al.*, 2004; Torres *et al.*, 2014; De Souza *et al.*, 2015).

The performance of the genotypes in different bioenergy conversion processes was evaluated. These tests showed significant genotypic differences for many specific quality traits relating to anaerobic digestion and enzymatic saccharification. This finding indicates that considerable improvements in the techno-economic efficiency of bioconversion processes can be achieved by selecting a more suitable feedstock, as for example suggested for maize stover (Torres *et al.*, 2016). For enzymatic saccharification of winter harvested biomass, for example, the best performing genotype released 42% more glucose and 45% more xylose per gram dry matter than the worst performing genotype (Table 6). Similarly, for anaerobic digestion, the best performing genotype achieved 18% higher biogas yield than the worst performing genotype (Table 6). These findings indicate that major improvements in final product yield are possible, which will probably have a favorable effect on process economics. Also processing conditions may become less severe with a more suitable feedstock. The mild pretreatment reactions in saccharification experiments were not only chosen because they are optimal for the detection of genotypic differences in conversion efficiency, but also because they give information on the potential to reduce the severity of pretreatment conditions, while maintaining high yields of fermentable sugars. Savings with respect to energy and chemical consumption can be realized in this way,


(a) ADL affects saccharification efficiency and biogas yield

(b) pCA/ADL affects saccharification efficiency and biogas yield

(c) TFA affects saccharification efficiency and biogas yield

(d) Hem affects saccharification efficiency

Fig. 2 The effects of cell wall compositional traits ADL (a), pCA/ADL (b), TFA (c) and Hem (d) on saccharification efficiency and biogas yield in stem samples of the winter cut. Saccharification efficiency was plotted as glucose conversion as a percentage of total cell wall glucan (green triangles), and biogas yield was plotted as substrate-specific biogas yield expressed in ml g^{-1} dm (red squares). Number labels represent accession numbers (OPM).

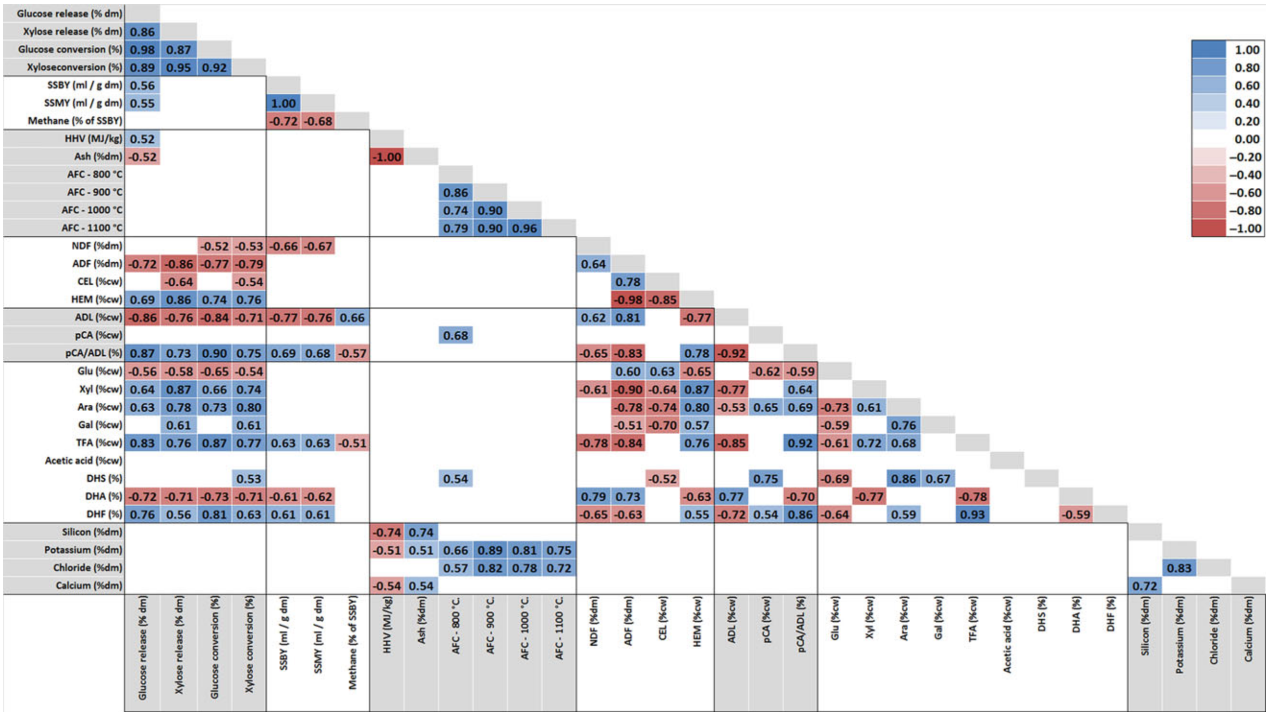
which will be a major cost reduction for the production of bioethanol.

Similarly, significant genotypic variation in contents of ash and inorganic elements was found, which can be exploited to improve the techno-economic performance of biomass combustion processes. Ash and certain inorganic elements are known to cause corrosion, slagging and fouling of the combustion chamber, thereby decreasing the quality of the biomass for combustion. Good combustion quality pertains to low ash content and a high ash melting point. Considerable genotypic variation in potassium and chlorine contents was found (Tables 5 and 6). This is in agreement with the large genotypic variation for elemental composition reported for *M. sinensis* (Atienza *et al*., 2003a,b). The classification of genotypes in ash fusion classes showed that the ashes of some genotypes (OPM-49 and OPM-65) were still only partly sintered at a combustion temperature of 1000 °C, whereas ashes of another genotype (OPM-48) at the same temperature were already completely molten (Tables 5 and 6). OPM-65 was shown to have a 62% lower ash content and was consistently classified in a lower ash fusion class during combustion than OPM-48, which is indicative of a higher ash melting point. For many important biochemical components and biomass quality traits, significant genotypic differences were found in this diverse set of *M. sinensis* genotypes that can potentially be exploited to optimize the feedstock for different applications.

Improving bioconversion efficiency by optimization of biomass composition

To show that genotype performance in bioconversion processes can be improved by optimizing biomass composition, correlation analyses were performed between compositional traits and biomass quality characteristics. It was shown that the efficiency of anaerobic digestion and saccharification is affected by biomass composition in a similar way. Lignin content had a negative impact on both conversion technologies, as anticipated and as is well established in literature (Campbell & Sederoff, 1996; Akin, 2008; Dandikas *et al*., 2014; Van Der Weijde *et al*., 2016). A high content of hemicellulosic polysaccharides was furthermore shown to be favorable for saccharification efficiency ($r = 0.74$, Figs 2d and 3).

Hemicellulosic polysaccharides and lignin both provide structural rigidity to the cell wall and are often negatively correlated (in this study $r = -0.77$, Fig. 3) (Qin *et al*., 2012; Torres *et al*., 2014; Van Der Weijde *et al*., 2016). Reductions in lignin content may be compensated for by an increase in hemicellulosic



	Glucose release (% dm)	Xylose release (% dm)	Glucose conversion (%)	Xylose conversion (%)	SSBY (ml / g dm)	SSMY (ml / g dm)	Methane (% of SSBY)	HHV (MJ/kg)	Ash (%dm)	AFC - 800 °C	AFC - 900 °C	AFC - 1000 °C	AFC - 1100 °C
Glucose release (% dm)													
Xylose release (% dm)	0.86												
Glucose conversion (%)	0.98	0.87											
Xyloseconversion (%)	0.89	0.95	0.92										
SSBY (ml / g dm)	0.56												
SSMY (ml / g dm)	0.55				1.00								
Methane (% of SSBY)					-0.72	-0.68							
HHV (MJ/kg)	0.52												
Ash (%dm)	-0.52							-1.00					
AFC - 800 °C													
AFC - 900 °C										0.86			
AFC - 1000 °C										0.74	0.90		
AFC - 1100 °C										0.79	0.90	0.96	
NDF (%dm)			-0.52	-0.53	-0.66	-0.67							
ADF (%dm)	-0.72	-0.86	-0.77	-0.79									
CEL (%cw)		-0.64		-0.54									
HEM (%cw)	0.69	0.86	0.74	0.76									
ADL (%cw)	-0.86	-0.76	-0.84	-0.71	-0.77	-0.76	0.66						
pCA (%cw)										0.68			
pCA/ADL (%)	0.87	0.73	0.90	0.75	0.69	0.68	-0.57						
Glu (%cw)	-0.56	-0.58	-0.65	-0.54									
Xyl (%cw)	0.64	0.87	0.66	0.74									
Ara (%cw)	0.63	0.78	0.73	0.80									
Gal (%cw)		0.61		0.61									
TFA (%cw)	0.83	0.76	0.87	0.77	0.63	0.63	-0.51						
Acetic acid (%cw)													
DHS (%)				0.53						0.54			
DHA (%)	-0.72	-0.71	-0.73	-0.71	-0.61	-0.62							
DHF (%)	0.76	0.56	0.81	0.63	0.61	0.61							
Silicon (%dm)								-0.74	0.74				
Potassium (%dm)								-0.51	0.51	0.66	0.89	0.81	0.75
Chloride (%dm)										0.57	0.82	0.78	0.72
Calcium (%dm)								-0.54	0.54				

	NDF (%dm)	ADF (%dm)	CEL (%cw)	HEM (%cw)	ADL (%cw)	pCA (%cw)	pCA/ADL (%)	Glu (%cw)	Xyl (%cw)	Ara (%cw)	Gal (%cw)	TFA (%cw)	Acetic acid (%cw)	DHS (%)	DHA (%)	DHF (%)	Silicon (%dm)	Potassium (%dm)	Chloride (%dm)	Calcium (%dm)
NDF (%dm)																				
ADF (%dm)	0.64																			
CEL (%cw)		0.78																		
HEM (%cw)		-0.98	-0.85																	
ADL (%cw)	0.62	0.81		-0.77																
pCA (%cw)																				
pCA/ADL (%)	-0.65	-0.83		0.78	-0.92															
Glu (%cw)		0.60	0.63	-0.65		-0.62	-0.59													
Xyl (%cw)	-0.61	-0.90	-0.64	0.87	-0.77		0.64													
Ara (%cw)		-0.78	-0.74	0.80	-0.53	0.65	0.69	-0.73	0.61											
Gal (%cw)		-0.51	-0.70	0.57				-0.59		0.76										
TFA (%cw)	-0.78	-0.84		0.76	-0.85		0.92	-0.61	0.72	0.68										
Acetic acid (%cw)																				
DHS (%)			-0.52			0.75		-0.69		0.86	0.67									
DHA (%)	0.79	0.73		-0.63	0.77		-0.70		-0.77			-0.78								
DHF (%)	-0.65	-0.63		0.55	-0.72	0.54	0.86	-0.64		0.59		0.93			-0.59					
Silicon (%dm)																				
Potassium (%dm)																				
Chloride (%dm)																		0.83		
Calcium (%dm)																	0.72			

Fig. 3 Heat map depicting the extent and the direction of correlations among biomass compositional and biomass quality traits. Only Pearson correlation coefficients that differed significantly from zero (*P* > 0.05) are reported. Blue values indicate positive correlation coefficients, and red values indicate negative correlation coefficients.

Table 7 Impact of elemental composition on ash formation and ash melting behavior during combustion assessed using correlation analysis. Only Pearson correlation coefficients that differed significantly from zero (*P* > 0.05) are reported

Combustion specific quality traits	Silicon (% dm)	Potassium (% dm)	Chloride (% dm)	Calcium (% dm)
Ash (% dm)	0.74	0.51		0.54
AFC – 800 °C		0.66	0.57	
AFC – 900 °C		0.89	0.82	
AFC – 1000 °C		0.81	0.78	
AFC – 1100 °C		0.75	0.72	

polysaccharides, as well as in hemicellulose–hemicellulose and hemicellulose–lignin cross-links, so that lowering lignin content not necessarily leads to concomitant detrimental reductions in plant cell wall rigidity and associated negative effects on plant fitness. The accompanying changes in the cell wall matrix, however, while still imparting strength to the cell wall, might make the cell wall less recalcitrant to biological conversion processes, such as anaerobic digestion or enzymatic saccharification. This theory is supported by the fact that hemicelluloses are often found to be positively associated to cell wall degradability and saccharification efficiency (Xu *et al*., 2012; Li *et al*., 2013; Torres *et al*., 2013).

Detailed profiling of the samples for minor cell wall components, such as acetic acid, *trans*-ferulic acid and *para*-coumaric acid, as well as hemicellulose monomeric constitution, was also proven to be important for understanding the effects of composition on biomass quality. The content of *trans*-ferulic acid was found to have a strong positive effect on the efficiency of both anaerobic digestion and enzymatic saccharification (Fig. 2c). In the literature, ferulate content is often considered to be negatively associated with cell wall degradability, as it is a key component that mediates cross-links between hemicelluloses and lignin (Hatfield *et al*., 1999; Grabber, 2005; Yu *et al*., 2005) and because feruloylated arabinose side chains of hemicelluloses are implicated as an initiation/nucleation site for lignin polymerization and deposition (Ralph *et al*., 1995). However, it has also been reported that lignins that extensively incorporate hydroxycinnamic esters can be easily depolymerized using alkaline pretreatments (Ralph, 2010), which may help to explain the positive associations found in this study. Moreover, TFA content had a strong negative correlation (*r* = −0.85) to lignin content. Therefore, TFA content may be indirectly positively associated to biogas yield and saccharification efficiency.

In addition, ratios between the different cell wall components were also found to be important, such as the ratio of *p*CA to ADL and the ratio of arabinose to xylose (DHS), which both positively affected biogas yield and saccharification efficiency (Figs 2b and 3). The positive effect of a higher ratio of arabinose to xylose is implicated to be due to a reduction in cellulose crystallinity associated with increase hemicellulose–cellulose cross-linking (Xu *et al.*, 2012; Li *et al.*, 2013). *p*CA is a phenolic compound that is ester-bound mainly to the S-subunit of the lignin polymer. A higher ratio of *p*CA to ADL might thus reflect a higher fraction of the lignin polymer to be comprised of the S-subunit. A higher S/G ratio is in literature sometimes associated with a higher saccharification efficiency (Li *et al.*, 2010; Studer *et al.*, 2011), especially with no or mild pretreatment (Chen & Dixon, 2007). It is also suggested that acylation of lignin with *p*CA impairs the copolymerizing of ferulates with monolignols (Grabber, 2005), which may also contribute to increased cell wall degradability. A high content of TFA, a high ratio of *p*CA to ADL and a low content of lignin are thus potentially interesting breeding targets for miscanthus for improving biomass quality for both saccharification and anaerobic digestion.

Although anaerobic digestion and enzymatic saccharification shared similar correlation patterns to compositional characteristics, the strength of these correlations was higher for saccharification efficiency, which indicates that this conversion process was more dependent on cell wall composition than biogas production. Moreover, biogas yield and saccharification efficiency were not significantly correlated to each other, suggesting that there are biomass quality traits that influence these conversion processes differently. One such trait was found to be Hem, which positively contributed to saccharification efficiency ($r = 0.74$, Figs 2d and 3), but not to biogas yield. Torres *et al.* (2014) showed that digestibility in rumen liquid (an anaerobic digestion process) and saccharification efficiency have many communalities, but a critical difference was that degree of hemicellulose substitution was relevant for saccharification efficiency, but not a major determinant for rumen liquid digestibility; a digestion process that resembles the process of anaerobic digestion for biogas production. This is also shown by the fact that the relative quality rating of the genotypes differed for anaerobic digestion and saccharification processes, with the best genotype for biogas production (OPM-73) being one of the worst for saccharification (Table 4). However, there were also genotypes that performed well in both platforms (for example, OPM-65), which indicates that it might be possible to improve biomass quality for both anaerobic digestion and enzymatic saccharification simultaneously.

For both conversion routes, it was clear that the summer cut had a better biomass quality than the winter cut, which is partly explained by the fact that lignin contents in the summer cut were much lower than in the winter cut. Lignin is mainly deposited after plant cells stop growing, when cell walls no longer need to accommodate cell expansion and become rigidified by lignification (Lam *et al.*, 2013; Da Costa *et al.*, 2014). Other factors that contributed to the higher conversion efficiencies of biomass of the summer cut are the facts that the relative weight ratio of leaves to total biomass was higher in the summer cut (Table 2), and that leaves were shown to have lower lignin contents in the summer cut then stem fractions (Fig. 1d). Despite higher conversion efficiencies, summer harvesting of miscanthus was shown to have a considerable and negative impact on total annual harvestable biomass yields, as the accumulated yield of the summer cut and the regrowth cut achieved only ±40% of the yield achieved in the winter cut (Table 2). Like for the genotypes evaluated in this study, a low tolerance to early green cuttings in July and August was also reported for *M. x giganteus*. However, a green harvest in October was shown to have less detrimental effects on crop yield, while beneficially affecting biomass quality for biogas production compared to winter harvesting (Kiesel & Lewandowski, 2016).

Combustion efficiency is known to be heavily dependent on the elemental composition of the feedstock, as such elements form ash in the combustion chamber, can be corrosive and cause slagging and fouling (Lewandowski & Kicherer, 1997; Atienza *et al.*, 2003a,b). Not surprisingly, contents of inorganic elements and ash were much lower in samples from the winter cut than from the summer cut, as these elements are translocated into the roots during winter and removed from the plant by leaf shed (Lewandowski & Heinz, 2003; Lewandowski *et al.*, 2003a). In addition, due to natural drying on the field during winter, the dried stems and leafs are more easily fractured by wind, which facilitates the leaching of inorganic elements in periods of rain. The low ash contents in samples of biomass from the winter cut compared to the corresponding samples from the summer cut favorable affect combustion quality (Tables 3 and 4). Moreover, it is known that lignin has a higher caloric value than cellulose and hemicellulose (Lewandowski & Kicherer, 1997) and samples harvested from the winter cut were shown to have higher lignin contents (Tables 3 and 4, Fig. 1d). Ash melting behavior could also be optimized. It was shown that potassium and chlorine were associated with lowering the ash melting point and that low contents of these elements positively affect combustion quality (Table 7). The relative quality rating of genotypes for combustion quality differed for some genotypes from that for biogas or for saccharification, but notably there were as well genotypes that performed well in all conversion platforms, such as OPM-49 and OPM-65. However, these were not the highest yielding genotypes. The highest yielding genotype (OPM-69) on the other hand unfortunately tended to slag and had higher contents of Cl and K, resulting in a low quality for combustion. These results show that it is possible to optimize biomass quality for different utilization options simultaneously and develop multipurpose genotypes, but that several quality traits need to be cross-bred. Extensive genetic variation for many biomass quality traits was found in the eight *M. sinensis* genotypes evaluated in this study, but it is likely that the full extent of variation for these traits within the species is even broader. The exploitation of such variation through breeding will greatly accelerate the realization of biomass derived energy and fuel production, as well as many other biobased applications, generating many market options for the use of miscanthus biomass.

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Supporting Information

Additional Supporting Information may be found online in the supporting information tab for this article:

Table S1. Information on genotype backgrounds.

Chapter 6 - General Discussion

Picture: University of Hohenheim/Wolfram Scheible

The General Discussion aims to increase the understanding of the potential of miscanthus for biogas production, potential environmental benefits and integration into the biogas supply chain. The former focusses on mechanisms influencing green-cut tolerance, substrate-specific methane yield (SMY) and methane yield potential of green-harvested miscanthus for biogas production. The potential integration of miscanthus into the biogas value chain is discussed based on the suitability for ensilaging, requirements in fermentation technology and economic performance of miscanthus compared to annual crops. Different environmental categories are considered to discuss the potential environmental impacts and benefits from replacing maize for biogas production by miscanthus. The energy efficiency of different miscanthus utilization routes are compared and discussed and an outlook on the potential role of miscanthus in the agriculture and the bioeconomy is provided.

6.1. The potential of miscanthus for biogas production

The development of miscanthus as a feedstock for biogas plants can follow two approaches: 1st pre-treating the biomass harvested after winter to improve accessibility for the microorganisms in the digester; and 2nd an earlier harvest with a green cut to improve digestibility and potentially reduce the need for pre-treatment. The advantage of the first route is that the miscanthus harvest is performed after winter and the crop has enough time to relocate nutrients and carbohydrates to the rhizome (Cadoux *et al.* 2012). Spring harvest of miscanthus is an established cultivation method, which provides a high yield potential, low nutrient off-takes and high long-term productivity (Lewandowski *et al.* 2000; Lewandowski *et al.* 2003; Angelini *et al.* 2009; Alexopoulou *et al.* 2015). In addition, the biomass is harvested dry with a moisture content below 18% and can be stored easily at low loss rates and costs. However, the dry harvested biomass is highly lignified and requires pre-treatment, such as steam explosion, to increase the substrate-specific biogas yield and suitability for wet fermentation biogas plants (Menardo *et al.* 2013). Intensive pre-treatment is energy consuming and costly and will decrease the economic advantage of the lower crop production costs of perennial miscanthus compared to annual crops. The yield of *Miscanthus x giganteus* peaks in autumn and then declines over winter until harvest, generally by about 35% mainly due to leaf losses (Lewandowski *et al.* 2003). Harvesting before winter can prevent such losses and also has the potential to increase the SMY as the biomass is less lignified.

For this reason, the present work focused on the 2nd approach and aimed to improve the suitability of miscanthus for biogas production by performing a green harvest before winter. The suitability of a crop for biogas production can be defined by a number of criteria, including SMY, biomass yield per hectare and methane yield per hectare. Since miscanthus is a perennial crop, the long-term yield stability is also a crucial criterion.

6.1.1. Influence of crop management on suitability for biogas production

Nitrogen fertilization had no impact on the SMY, although the nitrogen content of the biomass in the higher fertilization treatment was higher (Kiesel and Lewandowski 2017). The highest SMY of 281 ml_N (g oDM)$^{-1}$ was found for the earliest harvest date (July) and it decreased with later harvest dates down to 233 ml_N (g oDM)$^{-1}$ after winter (Kiesel and Lewandowski 2017). The negative impact of a later harvest on SMY is also known from other crops and similar findings have been reported for miscanthus in the literature (Wahid *et al.* 2015; Mangold *et al.* 2018; Mangold *et al.* 2019b). The SMY identified in this work is within the range of that reported elsewhere (Mayer *et al.* 2014; Wahid *et al.* 2015; Mangold *et al.* 2018; Schmidt *et al.* 2018; Mangold *et al.* 2019b). The SMY of miscanthus is lower than a typical SMY of maize, indicating the influence of the different substrate composition: miscanthus has a higher content of the cell wall components cellulose, hemicellulose and lignin and a lower content of non-fibre carbohydrates than maize (Kiesel *et al.* 2017a; Mangold *et al.* 2018). The lignocellulosic biomass composition of miscanthus also leads to slower methane production than in maize, as has been reported in the literature (Mangold *et al.* 2018). Especially the lignin content was identified to negatively affect the SMY (van der Weijde *et al.* 2017). Both the measured and reported SMY of green-harvested miscanthus indicate its general suitability for biogas production. However, the slower digestibility compared to maize suggests that longer retention times and thus larger digester volumes are required for miscanthus. Should digester volume be limited, pre-treatment could help to improve digestibility (Menardo *et al.* 2013).

The methane yield strongly depends on the biomass yield and was less affected by a lower SMY. About 78-85% of the methane yield decline from peak yield to spring yield can be explained by the biomass yield decline, while the lower SMY only explained 15-22% of the overall methane yield decline in that period (Kiesel and Lewandowski 2017; Kiesel *et al.* 2017a). This is also supported by the literature and indicates that the harvest date mainly needs to consider the biomass yield (Mangold *et al.* 2019b). Over two years, harvest in late October delivered the highest dry matter (DMY) and methane yields (MY) of on average 26 t dry matter (DM) ha^{-1} and approx. 6000 m^3 ha^{-1}, respectively (Kiesel and Lewandowski 2017). Similar methane yield potentials have been reported in the literature for miscanthus harvested in October or late autumn (Wahid *et al.* 2015; Schmidt *et al.* 2018; Mangold *et al.* 2019b). Preponing the harvest before winter avoided losses in dry matter yield of about 28-35% and in SMY of 4-7% (Kiesel and Lewandowski 2017; Kiesel *et al.* 2017a). This shows that harvesting in autumn improves both biomass yield and quality for biogas production by avoiding over winter losses (Kiesel and Lewandowski 2017; Kiesel *et al.* 2017a). Earlier green harvest in August increased the SMY by in average 7% compared to harvest in late October, but led to a severe yield decline in the second year (Kiesel and Lewandowski 2017). In literature similar findings are reported for harvest in August, which confirms that harvest in August is highly unsuitable for *Miscanthus x giganteus*, since it strongly damages the crop (Fritz and Formowitz 2010). Also a double-cut regime (harvest in July and again in October) was identified to be unsuitable for *Miscanthus x giganteus*, since the regrowth was too little to allow for an economically viable second harvest (Kiesel and Lewandowski 2017). Consequently, this study identified late October as a suitable green-harvest date for *Miscanthus x giganteus* for biogas production.

In this study, late October was the only harvest date identified as suitable, and late August as unsuitable under the climate conditions in south-west Germany. However, harvest in late October can be challenging due to wet soil conditions at harvest and especially on heavy clay soils. For practical implementation, the possible harvest window needs to be defined in more detail and it is crucial for the farmer to know if a harvest could already be performed in September or if late October is the only possible harvest date. A recent study found only a moderate yield decline for harvest in mid-September and no negative effect on the yield in the following year for harvest in early October (Mangold *et al.* 2019b). Other studies observed no negative effect of a green harvest in late September on the biomass yield the following year in a five-year-old crop, but a moderate negative effect on the yield of a 18-year-old crop (Ruf *et al.* 2017; Schmidt *et al.* 2018). This shows that the possible harvest window for *Miscanthus x giganteus* seems to be quite flexible in October and harvest time can be harmonized with that of other crops, e.g. maize. If necessary, a harvest could even be performed in mid-September, but with a higher risk of a moderate yield decline the following year. The trial used for this work has meanwhile been continued for another 3 years and the plots harvested in October are still producing high and stable yields, which leads to the assumption that the productive lifetime of a green-harvested crop is not necessarily shorter than that of a conventionally harvested crop (data not published). However, long-term studies are needed to confirm the productive life of a green-harvested miscanthus crop.

Novel genotypes showed the potential to improve the SMY by 3-8% compared to *Miscanthus x giganteus* and, due to the higher biomass yield on the more challenging soil conditions of the site in south-west Germany also the methane yield per hectare (Kiesel *et al.* 2017a). However, in this study, the crop was only three years old and *Miscanthus x giganteus* is known to often achieve maximum biomass yield in year five (Clifton-Brown *et al.* 2007). Lignin content was found to have a significant negative effect on the SMY and genotypes with a high lignin content generally had a lower SMY (van der Weijde *et al.* 2017). Interestingly, the genotypes with the highest SMY also had the highest leaf proportion. In the literature, lignin content of leaves is reported to be significantly lower than that of stems and the SMY of leaves is reported to be higher than that of stems (Wahid *et al.* 2015; Mangold *et al.* 2019b). This shows optimization potential for novel varieties by breeding, and breeding targets for biogas production should be green cut tolerant, high-yielding varieties with low lignin content. Low lignin content could be achieved by selecting genotypes with a high leaf proportion and low lignin content in the stems.

6.1.2. Influence of crop management on long-term productivity

Understanding the mechanisms which influence green-cut tolerance also helps to estimate if long-term productivity of the crop can be achieved. Since miscanthus is a perennial crop, the green-cut tolerance is mainly influenced by internal relocation processes. These can be either relocation of nutrients, such as the macro-nutrients N, P and K, or of carbohydrates to refill the starch reserves in the rhizomes (Masuzawa and Hogetsu 1977). The most important carbohydrate reserve is starch, although grasses from the family *Poaceae* are also able to store other soluble sugars (Zeeman *et al.* 2010; Purdy *et al.* 2015; Angelini *et al.* 2009). In this study, two nitrogen fertilization levels (80 and 140 kg N ha^{-1}) were tested under different green-harvest regimes, but no significant effect was observed on the DMY in the first or second year (Kiesel and Lewandowski 2017). Even though the lower N-fertilization level was not sufficient to completely replace the nutrient offtake by the harvested biomass in the double-cut regime and the early single-cut regime in August, no significant difference in dry matter yield between the two fertilization levels were observed in either regimes. Fertilization only had a positive impact on the regrowth after a green cut in a pot trial under very nutrient-limited conditions (Kiesel and Lewandowski 2017). Based on these results, it is hypothesized that fertilization and relocation of mineral nutrients has only a limited impact on the green-cut tolerance and the green-cut tolerance is largely related to relocation of carbohydrates to the rhizome. This hypothesis is supported by findings from other studies which identified that starch content in *Miscanthus x giganteus* rhizomes increases continuously until late autumn (Souza *et al.* 2013; Purdy *et al.* 2015). Carbohydrate relocation largely takes place in late summer when maximum aboveground biomass has been achieved (Purdy *et al.* 2015). Starch content or the extent to which starch reserves in the rhizomes are filled seems to be a reasonable explanation for the crop response to different green-cut regimes, since this reserve carbohydrate fuels the sprouting in spring. Early green-cut regimes showed much lower shoot heights in spring and early summer than the control harvested in winter or the late green-cut regime with harvest in October (Kiesel and Lewandowski 2017). Genotype and site conditions, e.g. low daily minimum temperatures, can also influence the relocation of carbohydrates to the rhizomes (Purdy *et al.* 2015). In addition, crop age could influence starch relocation and thus green-cut tolerance, due to potential acclimatization of the crop to the site conditions (Ruf *et al.* 2017; Schmidt *et al.* 2018). Senescence and flowering play an important role in relocation processes in miscanthus and genotypes showed different senescence behaviour (Jensen *et al.* 2017). Senescence could be also relevant for relocation of carbohydrates, but there is a potential trade-off if senescence induces lower SMY due to less soluble sugars in the biomass. This shows that selection of genotypes with earlier relocation of carbohydrates has the potential to improve green-cut tolerance and allow for a wider harvest window.

Although fertilization seems to have only limited direct impact on green-cut tolerance, it is absolutely necessary to maintain availability of (macro-) nutrients for the crop to ensure long-term productivity. Harvest in October leads to a significantly higher nutrient off-take than harvest after winter (Kiesel and Lewandowski 2017). The nutrient off-take needs to be replaced through fertilization and, for biogas production, application of biogas digestate seems to be a suitable approach to closing the nutrient cycle. However, more research is required to identify a suitable application technique and time. In the present study, the yield of the lower fertilization level of 80 kg N ha^{-1} was not significantly lower than that of the higher fertilization level of 140 kg N ha^{-1} (Kiesel and Lewandowski 2017). The nutrient removal in this treatment was 100 kg N ha^{-1}, 53 kg P_2O_5 ha^{-1} and 195 kg K_2O ha^{-1} (Kiesel and Lewandowski 2017), which is recommended as the maximum level for nutrient availability. Considering typical average nutrient contents of biogas digestate (Möller and Müller 2012), approx. 19 m^3 would be sufficient to fulfil the nutrient demand of green-harvested miscanthus. Technically, the application of digestate is state-of-the-art, however further research is needed to identify suitable application strategies and technologies to minimize nitrogen losses and emissions. For example, it is unclear if low-emission application technologies, such as slurry injection (Webb *et al.* 2010), would damage the miscanthus rhizomes and thus the crop. If slurry injection is not possible in miscanthus, diluted digestate or the liquid fraction after separation could be used to minimize nitrogen losses (Chadwick *et al.* 2000; Holly *et al.* 2017). In contrast to other crops, miscanthus typically forms a mulch layer, which could lead to increased ammonia losses after digestate application on topsoil (Bless *et al.* 1991). However, it has been observed that mulch layers are largely reduced in green-harvested miscanthus compared to harvest after winter, as leaf loss over winter is avoided. A mulch layer might be critical for digestate application, but provides several functions for the crop, including insulation against harsh winter frosts, a deceleration of soil warming in spring and weed suppression (Ruf and Emmerling 2017). In the field trials performed for this study, earlier shoot emergence was observed for plots without mulch layer than for those with. A missing mulch layer can therefore increase the risk of frost damage over winter and of late frost damage in spring. To date, no overwintering problems, damage from late frosts or weed problems have been observed for *Miscanthus x giganteus* harvested green in October, however frost and late frost risk also depends on the location and frost tolerance of the genotype. This needs to be further monitored and considered in breeding of miscanthus varieties for biogas production.

The work performed for this study contributed to the identification and optimization of the suitability of miscanthus for biogas production. It was demonstrated that green harvest in October is suitable for *Miscanthus x giganteus* under climatic conditions in south-west Germany and biomass harvested in October has a high potential for use as biogas substrate. October harvest was identified as the optimum date for the temperate climate in south-west Germany due to the very high methane yield potential, yield stability over two years and increased SMY compared to harvest after winter. This study also contributed to the identification of mechanisms influencing the green-cut tolerance and it was concluded that the relocation of carbohydrates is of major relevance here. This is a very important finding which needs to be considered in the breeding of varieties with improved green-cut tolerance, e.g. earlier senescing genotypes. This study showed that miscanthus can be utilized for biogas production; however, there are a number of questions that remain unanswered regarding the practical implementation and integration of miscanthus in the biogas supply chain. These are discussed in the following section.

6.2. The potential of integrating miscanthus into the biogas supply chain

6.2.1. Ensiling and optimization of germplasm

Miscanthus harvested after winter is easily storable under roof or plastic cover, due to its high dry matter content (Huisman and Kortleve 1994). Green-harvested miscanthus contains more water and has an average dry matter content of 44% of fresh matter (FM) (Kiesel and Lewandowski 2017). Such high water contents require more advanced storage methods to avoid losses through microorganism activity. The biogas supply chain is designed to cope with substrates of higher water contents and ensiling is generally used for feedstock conservation. The optimum dry matter (DM) content range for ensiling is considered between 28 and 35% of FM. Lower DM contents lead to the formation of silage effluents, causing energy losses; higher DM contents often lead to higher pH values, which can decrease aerobic stability after the silo is opened (McDonald *et al.* 1968; McGechan 1990). The suitability of green-harvested miscanthus for ensilaging remains unclear. This and the optimization of the integration of miscanthus into the biogas value chain are discussed in the following section.

Several studies have analysed ensiled miscanthus and have not reported difficulties during ensiling (Mayer *et al.* 2014; Schmidt *et al.* 2018; Ruf *et al.* 2017). However, in these studies, the silage quality was not assessed in detail. A recent study tested ensiling of biomass collected from three different harvest dates in autumn and reported successful ensiling for all three harvest dates (Mangold *et al.* 2018). Interestingly, biomass from the latest harvest date showed lowest pH value and mass losses and therefore the highest suitability for ensiling. The mass losses during ensiling were compensated by improved SMY of the ensiled biomass compared to not ensiled biomass (Mangold *et al.* 2018). Baldini *et al.* also observed improved silage quality of autumn harvested miscanthus compared to summer harvest (Baldini *et al.* 2017). This finding is striking, since the content of soluble sugars generally declines from summer to autumn (Purdy *et al.* 2015), which are required for formation of acids during ensiling and to reach a low pH value. Improved suitability in autumn than in summer is therefore somewhat contrary to expectations. The higher dry matter content of *Miscanthus x giganteus* observed by Mangold *et al.* may indicate that different microorganisms, e.g. yeasts, played a more important role during ensilaging (Mangold *et al.* 2018). This has also been observed for ensilaging of drier biomass in the past (McDonald *et al.* 1968). This aspect requires further research to understand the interaction of sugars, dry matter content and buffer capacity for miscanthus ensilaging. In any event, the silage quality can be improved by the use of silage additives, which increase lactic and acetic acid content and reduce mass losses during ensiling (Whittaker *et al.* 2016). Higher content of lactic and acetic acid is important, since both stabilize the silage during storage and after the silo is opened (McGechan 1990).

These first results at laboratory scale show that green-harvested miscanthus can be ensiled. However, ensiling on a larger scale could be more challenging and needs to be assessed, including suitability for compaction by tractor wheels. Compaction in the bunker silos is essential to minimize respiration losses and remove oxygen from the silage heap (Weinberg and Ashbell 2003). Crop physiology, such as hollow stems containing air, and very high DM contents can influence the consolidation of the biomass and removal of oxygen in the bunker silo (Muck and Holmes 2000; Weinberg and Ashbell 2003). A recent study observed difficulties during compaction of the biomass harvested in late October due to the high DM content and reported a lower packaging density than that of the earlier harvest treatments (Mangold *et al.* 2018). This shows that compaction of miscanthus in the silo could be more challenging and research is required to assess the suitability of miscanthus for large-scale ensiling and the need for silage additives. Mixed ensilaging of miscanthus with other crops, including catch crops with low DM content, could be an alternative approach to improve suitability for ensilaging and should be considered.

In the work performed for this study, *Miscanthus x giganteus* was identified as suitable for green-harvest in October and for biogas production. However, breeding is required to develop novel varieties with extended and earlier harvest window, improved suitability for ensilaging and SMY. Extended and earlier harvest window is very important on challenging sites, including temporarily waterlogged and clay-rich soils, to avoid soil compaction and damaging the crop. Harvest in early September would compromise yield of *Miscanthus x giganteus* in the following year and novel miscanthus varieties with improved green-cut tolerance are needed to allow the farmers more flexibility in harvest time with reduced risks for yield decline in the following year. The research performed for this study indicates that varieties with improved green-cut tolerance need to refill their starch stores in the rhizome earlier. Senescence and flowering were identified as drivers for relocation processes of mineral nutrients and might also influence relocation of carbohydrates (Jensen *et al.* 2017). Refilling of starch stores in the rhizomes follows seasonal dynamics with genotypic variations, but is accompanied with a decline of soluble sugars and starch in the aerial biomass (Purdy *et al.* 2015). Improving green-cut tolerance could be therefore accompanied by the trade-off of reduced suitability for ensiling or SMY, because both could benefit from higher non-structural carbohydrate contents in the aerial biomass. Increased digestibility of cellulose and hemicellulose could help to overcome this trade-off and increase green-cut tolerance, suitability for ensilaging and SMY simultaneously (Purdy *et al.* 2017). Greater flexibility in harvest time would further increase the possibilities to harmonize miscanthus harvest with that of other crops, including the novel perennial biomass crop cup plant (*Silphium perfoliatum*), which is generally harvested in early September (Gansberger *et al.* 2015). This might also help to increase farmers' acceptance of miscanthus for biogas production.

6.2.2. Fermentation technology

Lignocellulosic and fibrous biomass such as miscanthus can cause problems in wet fermentation biogas plants by forming floating layers (Weiland 2010; Baldini *et al.* 2017). In the experiments performed for this study, no specific problems were observed for anaerobic digestion of green-and brown harvested miscanthus. However, these experiments were performed at laboratory-scale only and the samples were milled using a cutting mill equipped with a 1 mm sieve. Further it is expected that green-harvested miscanthus might be less problematic compared to brown-harvested biomass, due to the lower dry matter content and the higher SMY. Other authors also observed that green harvested biomass was more easily dispersed than the dry biomass harvested after winter (Schmidt *et al.* 2018). However, the need for stirring not only depends on substrate, but also on organic loading rate and higher loading rate requires shorter stirring intervals (Tian *et al.* 2015). More research and experiments at full scale are needed to establish the stirring demand and risk of forming floating layers of higher miscanthus proportions in the overall feeding mix.

Full-scale biogas plants often have quite a short retention time of several weeks in the digester (Weiland 2010). During the experiments it was observed, that biogas production from green-harvested miscanthus is much slower than that of conventional biogas crops such as maize. This was also observed and reported elsewhere (Mangold *et al.* 2018). Slower biogas production can be a limiting factor for methane production in biogas plants, because of limited digester volumes. To achieve the same methane production rate as for maize, larger digester volumes would be needed with increasing miscanthus proportions in the feeding mix, which is very expensive. Another strategy to increase the biogas production rate could be pre-treatment of the substrate before digestion. Suitable full-scale pre-treatment technologies have shown positive effects on methane yield and velocity of methane production for other challenging substrates, such as horse manure (Mönch-Tegeder *et al.* 2014a; Mönch-Tegeder *et al.* 2014b). Cross-flow pre-treatment also reduced the proportion of large particles in the biogas plant and showed a clear positive effect on viscosity and the avoidance of floating layers in a full-scale plant (Naegele *et al.* 2014). To avoid costly larger digester volumes, pre-treatment of miscanthus would be a promising approach. The energy demand for stirring during anaerobic digestion and methane yield of green-harvested miscanthus needs to be assessed in full-scale biogas plants to identify the technological and economic demand for pre-treatment.

6.2.3. Economic performance of miscanthus for biogas production

Perennial crops such as miscanthus require less field work after successful establishment compared to annual crops, since no soil cultivation, seedbed preparation and chemical crop protection is required anymore. Less field work during the productive life of a perennial crop can offer the potential to reduce costs for biomass production. Biomass production costs are often the most important cost factor for biogas plants and can reach up to 60% of total costs (FNR 2016). Typical costs for biomass production including transport to the silo are 0.28-0.34 € per m^3 methane for maize

(LWK NRW 2019). Biomass production costs of green-harvested miscanthus for biogas production can be lower and are between 0.16-0.23 € per m^3 methane (Winkler *et al.* 2019). This would offer the potential to reduce the biomass production costs by 28-53% and further reduction would be possible if establishment or harvest costs could be reduced. Upscaling of rhizome propagation and novel establishment options via seeds could help to further decrease establishment costs. However, an assessment of the total costs is required, due to potentially additional costs for pre-treatment of miscanthus biomass.

The perennial miscanthus can be also used as a risk mitigation measure and to manage work load peaks. Annual crops such as maize require annual soil cultivation, seedbed preparation and sowing. Since maize is the most important biogas crop with about 73% mass related input proportion of all energy crops (FNR 2018), a high acreage performance is required for annual crop establishment. However, in case of very wet conditions in spring or on soils with high clay content, establishment can be delayed, which negatively affects yield. In such cases, the perennial crop miscanthus can be seen as risk mitigation measure, since it is already established on the field and no field work in spring is required, except fertilization with digestate. Avoiding soil cultivation in spring also helps miscanthus to use the soil moisture more efficiently and to be less affected by droughts. Soil cultivation generally leads to significant soil moisture losses, while unworked soils help to use the water more efficiently and can help to avoid drought stress in crops (Blevins *et al.* 1971). Combined with the high water-use efficiency of miscanthus, this can help to mitigate drought risks in biogas crop production, which are likely to increase with ongoing climate change (IPCC 2018). Breeding is developing novel miscanthus varieties with improved drought tolerance than the standard cultivar *Miscanthus x giganteus*, which would further reduce the risk of yield losses due to drought stress (Clifton-Brown *et al.* 2019; Lewandowski *et al.* 2016).

It is concluded that miscanthus can contribute to reducing feedstock costs and increasing feedstock diversification of biogas plants. Although miscanthus is a more challenging substrate than maize, solutions for ensiling and anaerobic digestion are available and, if required, need to be applied on large scale. Miscanthus harvested brown after winter has a benign environmental profile, which also renders it attractive for the reduction of the biogas sector's environmental impact. The following section discusses whether, or to which extent, green-harvested miscanthus also has the potential to increase the environmental performance of the biogas sector.

6.3. Potential environmental benefits from miscanthus for biogas production

Miscanthus harvested dry after winter is a low-input crop, which often requires no fertilization and chemical crop protection after successful establishment, and has the potential to provide low-carbon footprint energy (McCalmont *et al.* 2015; Wagner *et al.* 2017). However, green harvest of miscanthus before winter and biogas production might influence carbon mitigation and environmental profile of miscanthus cultivation. This will be discussed in the following chapter and is linked to the second objective of this work.

6.3.1. Global warming potential

Several studies have identified combustion of miscanthus biomass as a potential route to produce electricity and heat with lower greenhouse gas (GHG) emissions than the fossil reference systems (Hastings *et al.* 2008; Hastings *et al.* 2009; Wagner *et al.* 2017). In the present study, green-harvested miscanthus for biogas production improved the environmental performance of biogas production compared to the utilization of maize in the impact categories climate change (CC), fossil fuel depletion (FFD), terrestrial acidification (TA), freshwater eutrophication (FE) and marine eutrophication (ME) (Kiesel *et al.* 2017b). This was confirmed by a later study, which found considerable lower impacts of biogas from green-harvested miscanthus compared to the fossil reference in most of the considered impact categories (Wagner *et al.* 2019). Compared to the German electricity mix, a GHG mitigation potential of up to 256 kg CO_{2eq} GJ_{el}^{-1}. (= 0.922 kg CO_{2e} kWh^{-1}) was identified for electricity from green-harvested miscanthus (Wagner *et al.* 2019). This shows that green-harvested miscanthus for biogas production is suitable to reduce the environmental impact, especially GHG emissions, of the electricity production. Considering normalized values in 18 impact categories, biogas production was identified to have an overall lower environmental impact than different scenarios of direct biomass combustion (Wagner *et al.* 2017). However, material use for insulation purposes seems to have the lowest environmental impacts, due to considered energy production at the end of life (Wagner *et al.* 2017). Biogas production from miscanthus can be considered as a low environmental impact energetic biomass valorisation route, however emissions from digestate application and removal of the miscanthus crop need to be considered, which could be especially relevant for CC, FE and ME (McCalmont *et al.* 2018). Nevertheless, miscanthus provides the potential to strongly reduce the environmental impact of the biogas crop production compared to annual maize. However, for a holistic evaluation of the environmental performance also other factors, which are generally not included in a life cycle assessment, are important and are therefore discussed in the following paragraphs.

6.3.2. Soil nitrogen

Green harvest in autumn removes approx. twice the amount of nitrogen and potassium with the biomass from the field than brown harvest after winter (Christian *et al.* 2008; Kiesel and Lewandowski 2017). This higher nutrient offtake needs to be replaced by fertilizer. In case of biogas production digestate seems to be suitable to close the nutrient loops and recycle the nutrients. A higher nitrogen fertilization could also increase the risk of nitrate leaching and is more critical for green-harvested miscanthus than for brown-harvested miscanthus, due to the higher fertilizer demand (Christian and Riche 1998). However, the fertilizer demand of green-harvested miscanthus is still lower than for other annual biogas crops such as for maize (Kiesel *et al.* 2017b). Due to perennial nature and the deep rooting system of miscanthus (Neukirchen *et al.* 1999), nitrate leaching risk of green-harvested miscanthus should be lower than that of annual crops and should be more in the range of grassland (Christian and Riche 1998; Davis *et al.* 2012; Lesur *et al.* 2014). In this study, the soil nitrate content was assessed under green-harvested miscanthus and plots harvested in October showed very low values even at 140 kg N ha^{-1} fertilization level and where not significantly different to plots harvested after winter at 80 kg N ha^{-1} fertilization level (data not published). This indicates that nitrate leaching risk for both green- and brown-harvested miscanthus is low with optimised fertilizer regimes. Nevertheless, nitrate leaching risk under green-harvested crop including organic fertilization with digestate needs to be assessed and further researched.

6.3.3. Soil organic carbon

Harvest after winter leads to the forming of a mulch layer through leaf fall over winter. This is largely hindered by harvesting earlier so that the biomass including leaves is removed before winter. A less expressed mulch layer could lead to an increasing weed pressure and consequently an increased demand in herbicide applications. This was not observed in the trials leading to this study for miscanthus harvested in October, however earlier harvest dates (August or July) reduced the competitiveness of the crop, increased the weed pressure and required additional herbicide applications. The mulch layer is also important for soil life and a higher earthworm abundancy was observed under miscanthus compared to annual crops (Felten and Emmerling 2011). In a recent study, slightly negative effects of green harvest on earthworm abundance and soil ecosystem services of miscanthus was observed, but the authors speculated that this effect might increase with continued green harvesting (Ruf and Emmerling 2017). Mulch layer is largely formed by pre-harvest and harvest losses and is one of the most important carbon sources for formation of soil organic matter (Felten and Emmerling 2012). Harvesting before winter will strongly reduce pre-harvest losses and could reduce the potential for carbon sequestration in soil under green-harvested miscanthus. In literature so far no trends towards soil organic carbon depletion were observed in two miscanthus stands over two years of green harvest (Ruf *et al.* 2017). However, since soil processes are rather slow this observation period might have been too short to identify such changes and further research is required to assess the effect of green harvest on soil organic carbon. Ruf *et al* also calculated that the application of digestate could not compensate for the additional amount of carbon removed by green harvested due to avoided losses over winter (Ruf *et al.* 2017). However, organic carbon in digestate is more recalcitrant and decomposes slower in soil than the original biomass and is therefore assumed to contribute similarly to the formation of soil organic matter as the original feedstock (Marcato *et al.* 2009; Möller 2009; Chen *et al.* 2012; Thomsen *et al.* 2013; Bachmann *et al.* 2014; Möller 2015). Considering digestate application, green-harvest of miscanthus for biogas production should therefore lead to a similar soil organic carbon content as under brown-harvested miscanthus.

In practice, green-harvested miscanthus is likely to replace mainly annual biogas crops, such as maize. Long-term miscanthus cultivation on former intensive arable land generally leads to an increase in soil organic carbon stocks compared to continued annual cropping such as maize and an average carbon sequestration of 0.98 t carbon ha^{-1} a^{-1} has been observed (McCalmont *et al.* 2015; Gauder *et al.* 2016). Due to avoided soil cultivation and application of digestate, it can be expected that also green-harvested miscanthus for biogas production will lead to increasing soil organic carbon stocks compared to conventional annual farming. This assumption needs to be confirmed by future assessments quantifying the carbon sequestration of a green-harvested miscanthus crop including digestate application and comparing soil organic carbon content with conventional annual crops and brown-harvested miscanthus.

6.3.4. Biodiversity

Miscanthus cultivation often shows positive impacts on biodiversity compared to intensive arable crops, including birds, mammals, insects and spiders, however the biodiversity richness is negatively correlated with field size and crop density (Semere and Slater 2007; Bellamy *et al.* 2009; Dauber *et al.* 2010; Sage *et al.* 2010; Haughton *et al.* 2016). McCalmont *et al.* concluded that miscanthus could add structure and habitats in intensive arable regions (McCalmont *et al.* 2015) and Emmerling & Pude suggested that miscanthus strips could at least partly re-fulfill the functions of hedges and trees, which were removed in the past decades during reallocation of agricultural land (Emmerling and Pude 2017). The management of green- and brown-harvested miscanthus is quite similar during the vegetation period, except a higher fertilization with digestate. The biggest difference between both utilization pathways is the difference in harvest time. Harvest in October is not during the breeding time of birds and mammals, while harvest after winter can be in early breeding time if harvest takes place in late March or early April. For this reason, October harvest could be positive for birds and mammals, since there is no disturbance in spring except for digestate application. However, harvest before winter removes shelter over winter, which is a great advantage of miscanthus harvested after winter. Overall, it can be hypothesized that a harvest before winter will have quite comparable effects on biodiversity to harvest after winter, with the trade-off of losing overwinter shelter for mammals. For that reason, harvest after winter seems more preferable for biodiversity, but also green-harvested miscanthus should provide positive effects on biodiversity compared to intensively-managed arable land, including maize cultivation. However, the impact of green-harvested miscanthus on biodiversity needs to be further researched and compared with conventional annual crops.

In conclusion, green-harvested miscanthus was shown to provide biomass for biogas production with lower environmental impacts than annual maize. Electricity produced from green-harvested miscanthus was shown to reduce GHG emissions compared to the German electricity mix. This shows miscanthus is a suitable crop to provide sustainably produced feedstock for the biobased industry and the bioeconomy, while providing additional ecosystem services compared to annual crops. These ecosystem services are resulting from the surface and groundwater protection due to the low nitrate leaching and erosion risks, the protection of soil fertility due to the facilitation of soil organic carbon content and the provision of additional habitat conditions for soil organisms, insects, spiders, mammals and birds in intensive agricultural landscapes. Although the crop production of miscanthus is more environmentally benign than other crops, the utilization pathway should be as efficient as possible to maximize the benefits and minimize the impacts. This is discussed in the following section.

6.4. The potential energy efficiency of different miscanthus utilization routes

As the availability of biomass is not unlimited, a highly efficient energy conversion route needs to be chosen to maximize the impact. The state of the art of energy conversion is using brown-harvested miscanthus via direct combustion and this can be used as a benchmark for the overall efficiency. The energy efficiency of different energetic miscanthus utilization pathways is discussed in the following section. The energy efficiency is here compared based on the energy yield per hectare, which is calculated using the lower calorific value of the biomass, methane or bioethanol multiplied with the biomass, methane or bioethanol yield per hectare. In case of combustion, the energy required to evaporate the moisture from the wet biomass is being considered and for this reason the resulting figure is deemed as net energy yield (Kiesel *et al.* 2017a). Energy required for the crop production is not considered in this calculation.

While combustion is a thermochemical process and theoretically 100% of the energy in the biomass can be converted into heat (assuming complete combustion of dry biomass), biotechnological processes such as anaerobic digestion and ethanol fermentation are accompanied by energy losses due to microbial activity. For this reason, biogas from carbohydrate-rich feedstock for example consists of only 52% methane and the rest is mainly carbon dioxide (FNR 2016). In ethanol fermentation, each glucose molecule is also converted into two ethanol and two CO_2 molecules. This simple consideration might lead to the impression that combustion is much more efficient than biotechnological conversion routes. However, the overall efficiency needs to consider more factors, including harvest time, yield, and conversion efficiencies.

In the present study, Kiesel *et al.* showed that harvest date had a strong impact on the biomass yield per hectare and biomass yield in turn strongly influenced the net energy yield of combustion and biogas production (Kiesel *et al.* 2017a). Even though the dry matter (DM) content was only around 50% of fresh matter in autumn and energy was required to evaporate the moisture in the biomass, the peak net energy yield for combustion was generally in autumn when peak yield was achieved. The same applied for biogas production, where the impact of DM yield on net energy yield was more important than the SMY (Kiesel *et al.* 2017a). The best-performing genotype OPM 6 achieved a peak yield of 24.6 t DM ha^{-1} in October in Germany, which corresponds to a 52% higher net energy yield in combustion (370 GJ ha^{-1}) than in biogas production (243.4 GJ ha^{-1}) (Kiesel *et al.* 2017a). However, the DM content in October was 51.9% of fresh matter and increased to 84.9% by the conventional harvest date for combustion in March, when OPM 6 yielded 16.1 t DM ha^{-1} corresponding to a net energy yield of 274.5 GJ ha^{-1} (Table 1) (Kiesel *et al.* 2017a). The yield decline of 34.5% from peak yield to harvest after winter caused that the net energy yield of combustion at conventional harvest in March was only 12.7% higher than that of biogas production at peak yield in October. A similar DM yield decline over winter and energy yield per hectare can be found in the literature (Lewandowski and Heinz 2003).

However, to allow comparison of electricity generation from direct combustion and biogas production also the conversion to electricity needs to be considered, which might include different conversion efficiencies. The conversion efficiency in direct combustion of solid biomass is in the range of 39-44% in large scale applications (100-250 $MW_{el.}$) (van den Broek *et al.* 1996), while electric efficiency of biogas engines is up to 42% also in smaller scale applications (INNIO 2019). This shows the electric efficiency of both conversion routes are in a similar range and for this reason for both a conversion efficiency to electricity of 40% is assumed in the further calculation. At this efficiency, 110 $GJ_{el.}$ ha^{-1} electricity can be generated by direct combustion of miscanthus biomass, while electricity production via biogas conversion route produces 97.2 $GJ_{el.}$ ha^{-1}. This shows, combustion is the most efficient pathway for electricity production from miscanthus biomass. However, the electricity yield gap to biogas production is only 12.8 GJ ha^{-1} and biogas allows provision of peak load power and demand-driven electricity generation (Theuerl *et al.* 2019). This leads to the conclusion that base load power should be produced by direct combustion of miscanthus biomass, while biogas production should be used to provide system services, which are increasingly required due to increasing proportion of fluctuating wind and solar power in the overall electricity mix. Earlier harvest or additional harvest of biomass lost over winter could further increase the electricity yield from direct combustion, however storage issues need to be overcome and combustion technology needs to cope with critical elements in miscanthus biomass (Iqbal and Lewandowski 2016).

Energy yield of bioethanol utilization route depends on yield potential of C6 (hexoses) and C5 (pentoses) sugars per ha. The saccharification potential of different miscanthus genotypes was assessed in Chapter 5 and based on the average yield of 7015 kg DM ha^{-1}, the theoretical glucose and xylose content after complete hydrolysis of cell wall components and the molar mass of glucose, xylose and ethanol, a theoretical maximum ethanol yield of 2526.8 kg ethanol ha^{-1} can be calculated (van der Weijde *et al.* 2017). However, the conversion efficiency from sugars to ethanol is not 100%, but improved in recent years and meanwhile achieved 92% (Kang *et al.* 2014). Considering this sugar conversion efficiency, the theoretical total ethanol yield is 2324.7 kg ha^{-1}, which means theoretically 331 kg ethanol $(t\ DM)^{-1}$ could be produced from the assessed miscanthus genotypes. However, this theoretical calculation does not consider efficiency of pre-treatment and hydrolysis. In literature, ethanol yield of pre-treated wheat straw was assessed in the range of 0.17-0.24 g (g DM)$^{-1}$, which means 170-240 kg ethanol where produce per tonne of wheat straw (Saha *et al.* 2005). Similar ethanol yields from wheat straw of 0.22-0.25 t (t DM)$^{-1}$ are also communicated for full scale bioethanol refineries (Clariant 2014). Since ethanol yield from miscanthus might be slightly lower than that from wheat straw, an ethanol yield of 0.2 g (g DM)$^{-1}$ is assumed for the further considerations, which means 5 t dry miscanthus biomass are required to produce 1 t bioethanol.

In the abovementioned example, this bioethanol yield would lead to an energy yield of 86.3 GJ ha^{-1} for miscanthus harvested in March (Table 1). Utilization of October harvested biomass (24.6 t DM ha^{-1}) could increase the energy yield to 131.9 GJ ha^{-1}, however this is not considered in this example, since utilization of green biomass is not state of the art in bioethanol production until today. In contrast to direct combustion, bioethanol can be directly used as transportation fuel. Assuming a gasoline engine energy efficiency of 40%, this means 34.5 GJ$_{\text{mech.}}$ ha^{-1} mechanical useful energy can be generated by bioethanol utilization pathway.

Table 1 – Exemplary calculation of energy efficiency of different miscanthus biomass utilization routes for electricity generation and as transportation fuel. Yield data (OPM 6, location Stuttgart) are taken from Kiesel *et al.* (Kiesel *et al.* 2017a). Data for overall efficiency of battery electric vehicles refer to Helms *et al.* and include losses during battery loading, storage losses and energy efficiency of electric engines (Helms *et al.* 2010). Electricity generation is considered via direct combustion of miscanthus biomass and biogas production from miscanthus biomass. Transportation fuel includes direct use of biomethane and bioethanol in vehicles with a combustion engine and use of electricity to recharge battery electric vehicles. DM = dry matter, FM = fresh matter, na = not available.

	Unit	**Direct Combustion** *(March harvest)*	**Biogas production** *(October harvest)*	**Bioethanol production** *(March harvest)*
Biomass Yield	t DM ha^{-1}	16.1	24.6	16.1
Biomass moisture content	% FM	84.9	51.9	84.9
Biomethane yield	m^3 methane ha^{-1}	na	6782.5	na
Bioethanol yield	t bioethanol ha^{-1}	na	na	3.22
Energy yield	GJ ha^{-1}	**274.5**	**243.4**	**86.3**
Conversion efficiency of electricity generation	%	40	40	na
Electricity yield	GJ$_{\text{el.}}$ ha^{-1}	**110**	**97.2**	na
Overall efficiency of battery electric vehicles	%	73	73	na
Mechanical useful energy of battery electric vehicles	GJ$_{\text{mech.}}$ ha^{-1}	**80.3**	**71.0**	na
Efficiency of gas/ gasoline engine	%	na	40	40
Mechanical useful energy of direct use of biomethane/ bioethanol	GJ$_{\text{mech.}}$ ha^{-1}	na	**97.4**	**34.5**

Utilization of electricity as transportation fuel can be achieved by battery electric vehicles. Overall efficiency of battery electric cars is identified as 73%, including losses during battery loading, storage losses and energy efficiency of electric engine (Helms *et al.* 2010). Using electricity generated by direct combustion of brown-harvested miscanthus and biogas production from green-harvested miscanthus to fuel a battery electric vehicle generates mechanical useful energy of 80.3 and 71.0 $GJ_{mech.}$ ha^{-1}, respectively. Instead of using biogas for electricity production, biomethane could be also used directly as transportation fuel. Assuming an energy efficiency of the gas engine of 40% this would generate mechanical useful energy of 97.4 $GJ_{mech.}$ ha^{-1}, due to avoided electricity conversion losses. This shows direct utilization of biomethane as transportation fuel would provide the highest efficiency amongst the considered examples, while bioethanol showed the lowest overall efficiency. Similar results were obtained for pre-treated wheat straw, where biogas production was the most efficient and bioethanol the least efficient conversion route (Kaparaju *et al.* 2009). However, energy demand for biogas upgrading to biomethane, losses during biogas upgrading and compaction were not included in this example. Possible energy generation from bioethanol by-products was also not included in this considerations.

The exemplary calculation above shows that combustion provides the highest overall efficiency for power production and biomethane for production of transport fuels. For this reason biogas should be upgraded to biomethane and used as biofuel. For the transportation sector, biomethane from miscanthus seems to be a very suitable option especially for long-distance cars and heavy duty or bus traffic (Börjesson and Mattiasson 2008). However, the small yield gap to combustion also allows the utilization of biogas for electricity generation if system services or peak load power is provided. Bioethanol showed the lowest efficiency as transportation fuel and even battery electric cars fuelled by power produced from miscanthus biomass showed a higher overall efficiency. Increasing the harvestable yield for combustion has the potential to further increase the performance of combustion, prohibited the combustion process is robust enough for higher contents of critical elements or lower ash melting temperatures. The overall efficiency of bioethanol production could be increased by utilization of by-products for energy generation, increasing the bioethanol conversion efficiency and utilization of green-harvested biomass for bioethanol production. Also chemical use of bioethanol should be considered and is anyhow of societal and economic relevance.

6.5. The potential role of miscanthus in European agriculture and bioeconomy

Due to its more environmentally benign profile compared to annual crops, miscanthus is a very promising crop to provide low-impact feedstock for biogas production and to help to reduce the environmental impact of the biogas sector. However, the question is what role could miscanthus play in the future European agriculture and bioeconomy to maximize the benefits for the farmer and the society as a whole? This is addressed in the following section.

6.5.1. Potential role of miscanthus in European agriculture

As discussed in section 6.3, implementation of miscanthus in small field sizes and ideally as strips would help to maximize the environmental benefits and minimize potential risks for biodiversity. Miscanthus strips could thus at least partly re-fulfil the functions of hedges and trees removed in the past decades during reallocation of agricultural land (Emmerling and Pude 2017). Establishment in strips and smaller field sizes also help to promote biodiversity, since these are more favourable for landscape heterogeneity than very large field sizes and minimize the risks to biodiversity caused by too high regional concentration (Semere and Slater 2007; Bellamy *et al.* 2009; McCalmont *et al.* 2015; Haughton *et al.* 2016). The implementation should thus focus on water-sensitive and water protection areas and barrier strips should be established alongside water bodies or fields with a slope. Here, the deep rooting system, low nitrate leaching and erosion risk of an established miscanthus crop could contribute to the protection of water and groundwater quality (Neukirchen *et al.* 1999; Lesur *et al.* 2014; Ferrarini *et al.* 2017b). Due to the deep rooting system, buffer strips with miscanthus can even remove nitrate from subsurface water flow originating from adjacent agricultural fields (Ferrarini *et al.* 2017a). Miscanthus buffer strips could provide both feedstock production for the biogas sector or for other bioeconomy applications and contribute to the protection of water resources and the avoidance of additional societal costs for the cleaning of drinking water. The above-mentioned benefits are especially interesting for regions (=red regions) where groundwater nitrate content exceeds the EU threshold of 50 mg l^{-1}. These are often regions with a high proportion of livestock farming (Council of the European Communities 1991; European Commission 2018). Here miscanthus crop production and utilization, e.g. biogas production, could provide an alternative income for livestock farmers, help to reduce regional concentrations of animal farming, and contribute to restoring groundwater quality.

Miscanthus cultivation will only be adopted by farmers, if the biomass production costs are economically competitive and an attractive revenue can be generated. Production costs are generally decreasing with larger field sizes, which could lead to a trade-off between economy and biodiversity (Winkler *et al.* 2019). Substantial progress has been made in recent years in miscanthus breeding and upscaling, which is the basis for decreasing crop production costs and increasing implementation of miscanthus cultivation (Clifton-Brown *et al.* 2016; Clifton-Brown *et al.* 2019). However, increasing implementation of miscanthus will only be achieved, if a biomass market or economically sound business models are developed. This is discussed in the next section. Biogas is an interesting business model and market, but the future is rather uncertain because feed-in tariffs have been substantially reduced and from 2020 onwards the feed-in tariffs for existing biogas plants guaranteed for 20 years will start to expire (Theuerl *et al.* 2019). Policy incentives are needed to avoid a decrease in biogas production, including approving biomethane from perennial crops as biofuel for the transportation sector and stimulating flexible and demand-driven power production.

For the farmer, miscanthus offers additional benefits, including the potential to utilize underutilized or marginal land, such as sites with awkward field shapes, high stone content, shallow topsoil layer or high clay content, which tend to be temporarily waterlogged (Clifton-Brown *et al.* 2016; Lewandowski *et al.* 2016; Mangold *et al.* 2019b). Using miscanthus as a 15-20 year break crop in conventional annual arable crop rotations offers the potential to improve the soil fertility by increasing soil organic matter and avoiding soil erosion and to break-up herbicide tolerant weed infestations, such as blackgrass (*Alopercurus myosuroides*) (McCalmont *et al.* 2015; Clifton-Brown *et al.* 2016). Breeding of miscanthus aims to improve stress tolerances of new varieties, which improves the crop resilience under increasing stress conditions due to climate change (Lewandowski *et al.* 2016; Clifton-Brown *et al.* 2019). This allows farmers not only to diversify their income by cultivation of a perennial biomass crop, but also to use such a crop as risk mitigation measure and to adapt their cropping systems to climate change, e.g. by increased drought tolerance of perennial crops. Miscanthus can be also used to utilize contaminated sites for biomass production and preventing introduction of contaminants into the food chain (Pidlisnyuk *et al.* 2019; Rusinowski *et al.* 2019). Utilization of marginal land, contaminated soils and protection or even promotion of soil fertility are thus key drivers to avoid competition with other land use options, including food production. To introduce miscanthus as a break-crop in conventional agriculture, efficient integration into crop rotations is important. In greenhouse studies no positive impact of miscanthus on subsequent wheat growth was identified compared to maize, but a potential risk for promoting pathogen infections (Schrama *et al.* 2016). However, in practice miscanthus will be removed in spring after last harvest and ideally followed by a spring crop. Maize was identified as a suitable crop to suppress regrowth of miscanthus and gain a high yield (Mangold *et al.* 2019a).

This thesis showed, that miscanthus is a suitable perennial crop to reduce the environmental impact of the biogas sector due to its environmental benign profile compared to annual biomass crops. By utilization of miscanthus, additional benefits can be achieved which help to tackle environmental problems of the Agricultural sector, including nitrate leaching, water and groundwater protection, management of herbicide-tolerant weed infestations and soil organic carbon content. For the farmer and the agriculture, miscanthus could play a key role to improve the environmental performance of the biomass production for the biogas sector. Additionally, miscanthus is an option for income diversification for the farmer, provided a market for the biomass exists, while contributing to solve abovementioned environmental problems of the agricultural sector. The farmer can achieve this by integration of miscanthus into the crop rotation as break crop, strip cultivation or conversion of marginal land areas. At the same time, miscanthus can provide sustainably produced feedstock for a growing European bioeconomy, as discussed in the following section.

6.5.2. Potential role of miscanthus in a growing European bioeconomy

Providing sustainably produced feedstock for a growing bioeconomy is an important contribution to increase decarbonisation of the economy. However, has miscanthus the potential to contribute substantial quantities of biomass to help to cover the increasing demand of the growing bioeconomy?

In 2017, energy and industrial crops were cultivated on a total area of 2.65 million ha in Germany which is 22.6% of the total 11.7 million ha arable land in Germany (FNR 2018; Statistisches Bundesamt 2018). Largest part of this area was used for energy crops, including 0.9 million ha maize for biogas production and 0.7 million ha rape seed for biodiesel production, while miscanthus cultivation in Germany is estimated on approx. 4000 ha (Lewandowski 2016; FNR 2018). However, approx. 4 million ha of arable land where identified as potential miscanthus cultivation area in Germany (Schorling *et al.* 2015). On EU level, Clifton-Brown *et al.* estimated in a conservative approach that miscanthus cultivation for combustion on 10% of the suitable land in the EU-15 could produce up to 9% of the gross electricity production in the year 2000 (Clifton-Brown *et al.* 2004). The total arable land in the EU-28 is expected to be 104 million ha in 2030 (European Commission 2017). In a theoretical example, the conversion of 10% into miscanthus would lead to a cultivation area of 10.4 million ha within the EU and to a miscanthus biomass production of approx. 156 million t DM per year, assuming an average yield of 15 t DM ha^{-1}. For comparison, in 2017 approx. 15 million t DM of maize were used in Germany for biogas production (FNR 2018; Statistisches Bundesamt 2019). Biogas production could be a driver to unlock the potential of miscanthus and create a market pull for the biomass on short term. The advantage of biogas production compared to other utilization options, including combustion, is the already existing infrastructure which could be used. Biogas production could help to overcome the hen-and-egg problem of miscanthus, which is characterized by unavailability of miscanthus biomass hindering development of industrial processes for the biomass and a missing market to sell the biomass hindering farmers to increase biomass production (McCalmont *et al.* 2015).

To achieve the long-term aim of net zero greenhouse gas (GHG) emissions, lower value applications such as combustion and biogas, are the starting point to establish a market for miscanthus biomass. In the longer term, perennial crops such as miscanthus are required to sustainably secure the feedstock demand of a growing European bioeconomy and provide a renewable carbon source for the chemical industry (Lewandowski 2015; Carus 2018). High-value applications including platform chemicals for chemical industry, biofuels and biobased materials, are currently under development, for example in the EU-funded BBI Demonstration Project GRACE (grant agreement No 745012; website: www.grace-bbi.eu). Biomass is forecasted as one major feedstock for the growing chemical industry to achieve carbon neutrality in this sector by 2050 (Carus 2018), and miscanthus could play a major role by providing sustainably-produced feedstock for the chemical industry. Miscanthus genotypes offer a considerable compositional variation which allows for development of optimized varieties according to the needs of the different utilization pathways (van der Weijde *et al.* 2017).

In a future bioeconomy, farmers should not only deliver low-value biomass to industry, but improve on-farm value creation to increase the overall farm profit (Lewandowski 2015). This can range from decentralised biomass storage to processing, including on-farm refineries e.g. connected to existing biogas plants. Such modular on-farm biorefineries could make use of single fractions of the biomass, e.g. sugars, and deliver intermediate products, such as a lignin-rich fraction, to large-scale refineries, which could perform secondary refinery steps, product separation and conditioning (Dahmen *et al.* 2018). Modular small-scale biorefineries thus offer the potential to also make use of crop residues which are uneconomic to transport to centralized large-scale refineries and to reduce transportation costs and emissions, especially where wet biomass or biomass with low density such as chipped miscanthus is being used (Kolfschoten *et al.* 2014).

This study showed that miscanthus can play a crucial role to provide sustainably produced feedstock in short term for the biogas sector and in longer term for the bioeconomy. Perennial biomass crops, such as miscanthus, are thereby key to achieve net zero GHG emissions, since these can provide sustainably produced feedstock and a renewable carbon source for the chemical industry, which requires carbon in most of their products. Also sustainably produced biogas can play an important role to achieve net zero GHG emissions. This study showed, that miscanthus is suitable for biogas production, more environmental-benign than annual crops, such as maize and can thereby help to reduce the environmental impact of the biogas sector. Sustainably-produced biomethane, which is produced by upgrading biogas, could help to replace fossil natural gas in the gas grid and could be used for peak-load power production or as transportation fuel for long-distance vehicles. A market pull for the miscanthus biomass by utilization for biogas production could lead to further investments into the crop and to the above-described development. Development of integrated on-farm biorefineries for utilization of the feedstock miscanthus and biogas production from the residue streams of such biorefineries could be a sustainable alternative business model for farmers and the agriculture and could help to reduce environmental impacts of the agriculture by reduced cultivation of annual crops. This study showed that miscanthus is a suitable and sustainable feedstock for biogas production and the large compositional variation of the different miscanthus genotypes also allows utilization of the biomass for various products. To improve the environmental performance of the biogas sector, miscanthus should be implemented for feedstock provision and breeding should focus on developing specific varieties for different utilization pathways and growing conditions.

6.6. Publication bibliography

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Curriculum Vitae

Andreas Kiesel

Schorndorfer Straße 61
73061 Ebersbach a.d. Fils
Email: a.kiesel@uni-hohenheim.de
Phone: +49 711 22379
Mobil: +49 151 56366727

Personal details

Date of Birth:	11 August 1986
Place of Birth:	Schwäbisch Gmünd
Nationality:	German

Professional Experience

Since 10/2014 **University of Hohenheim, Institute of Crop Science, Department of Biobased Products and Energy Crops, Stuttgart**
Research Associate and Lecturer

- Lectures in crop production of bioenergy crops and life cycle assessment.
- Assistant supervision of student’s and project thesis
- Scientific work, including field and laboratory trials, scientific publications and writing of project proposals
- Work package leader in FACCE Surplus projects “*BioC4 - New integrative sustainable system from C4 photosyntetic miscanthus to biological synthesis of valuable C4 compounds*” (BMBF, Förderkennzeichen 031B0162B) and “*MISCOMAR – Miscanthus biomass options for contaminated and marginal land: quality, quantity and soil interactions*” (BMBF, Förderkennzeichen 031B0163) under the EU-ERA-NET Cofund framework in Horizon 2020
- Coordination of BBI Demonstration Project “*GRACE – Growing Advanced Industrial Crops on Marginal Lands for Biorefineries*“ (GA No 745012) in the EU Horizon 2020 research framework programme

07/2012 – 09/2014 **University of Hohenheim, Institute of Crop Science, Department of Biobased Products and Energy Crops, Stuttgart**
Doctoral Student

- PhD-Thesis: “The potential of miscanthus as biogas feedstock”
- Research and collaboration performed in the EU-financed project “OPTIMISC – Optimizing Miscanthus Biomass Production” (Grant Agreement ID 289159)

Education

10/2010 – 10/2012 **University of Hohenheim, Stuttgart**
Master Programme “Biobased Products and Bioenergy”

- Master thesis: „Non-conventional biogas substrates and hydrolytic enzymes in anaerobic digestion“
- Master thesis was conducted in cooperation with DSM Biopract GmbH, Berlin, including an internship of 16 weeks at Biopract laboratory in Berlin
- Degree: Master of Science. Grade “Very Good”

10/2007 – 09/2010 **University of Hohenheim, Stuttgart**
Bachelor Programme “Biobased Products and Bioenergy”

- Bachelor's thesis: „Nachhaltige Anbausysteme zur Bereitstellung von Biomasse zur Biogasproduktion“
- Bachelor thesis was conducted in cooperation with EIFER, Karlsruhe, including an internship of 4.5 months at EIFER
- Degree: Bachelor of Science. Grade “Good”

08/1997 – 06/2006 **Limes Gymnasium Welzheim**

- Degree “Abitur” (= General qualification for university entrance)

Skills

Language	German: Native English: fluently
Computing	Good knowledge in MS office and basic knowledge in GaBi and AutoCAD

Conference contributions

09/2019	**Oral presentation** „Miscanthus für die Biogasnutzung: Anbau und Ertragspotential“ at “C.A.R.M.E.N. Fachgespräch 2019 – Das Greening als Booster für Energiepflanzen. Biogas aus Miscanthus – eine Alternative?“. Reutlingen, Germany. 17 September 2019
03/2019	**Oral presentation** „European Bioeconomy: growth targets and timelines – Contribution of GRACE” at “The MISCOMAR Project International Scientific Conference. Multiple benefits of biomass crops on marginal land” in Katowice, Poland. 20-12 March 2019
09/2018	**Oral presentation** “GRACE - GRowing Advanced industrial Crops on marginal lands for biorEfineries” at “1st GRACE Industry Panel Meeting” in Turin, Italy. 26 September 2018
09/2018	**Poster presentation** “GRACE - GRowing Advanced industrial Crops on marginal lands for biorEfineries” at International Forum on Industrial Biotechnology and Bioeconomy (IFIB) in Turin, Italy. 27-28 September 2018
05/2018	**Poster presentation** “Development of a sustainable European Bioeconomy - Contribution of BBI demo project GRACE” at 26th European Biomass Conference & Exhibition in Kopenhagen, Denmark. 14-17 May 2018
05/2018	**Oral presentation** “GRACE - GRowing Advanced industrial Crops on marginal lands for biorEfineries” at 26th European Biomass Conference & Exhibition during the 2nd SEEMLA Symposium in Kopenhagen, Denmark. 15 May 2018
12/2017	**Oral and poster presentation** “GRACE - GRowing Advanced industrial Crops on marginal lands for biorEfineries” at BBI project days and Stakeholder Forum 2017 in Brussels, Belgium. 6-7 December 2017
11/2017	**Oral presentation** “Novel miscanthus-based value chains” at 9. Internationale MEG e.V. Tagung - Bioökonomische Anwendungs- und Forschungsfelder bei Miscanthus in Bonn, Germany. 20-21 November 2017
11/2017	**Oral presentation and workshop moderation** “Workshop Teil 2: Organisation von miscanthus-basierten Wertschöpfungsketten” at 9. Internationale MEG e.V. Tagung - Bioökonomische Anwendungs- und Forschungsfelder bei Miscanthus in Bonn, Germany. 20-21 November 2017

10/2017 **Oral presentation**

“BBI DEMO project GRACE – Scope, aims and challenges” at Italian Forum on Industrial Biotechnology and Bioeconomy in Rome, Italy. 5-6 October 2017

06/2016 **Poster presentation**

“Biogas Potential and Cutting Tolerance of *Miscanthus x giganteus*” at 24th European Biomass Conference & Exhibition in Amsterdam, Netherlands. 6-9 June 2016

10/2015 **Poster presentation**

“Developing *Miscanthus x giganteus* as a crop for biogas production” at IEA Bioenergy Conference 2015 in Berlin, Germany. 27-29 October 2015

10/2015 **Oral presentation**

“Anaerobic digestion of miscanthus” at Perennial Biomass Crops for a Resource-Constrained World in Hohenheim, Germany. 7-10 September 2015

06/2015 **Poster presentation**

“Optimization of Miscanthus x giganteus pretreatment for biogas production” at 23rd European Biomass Conference & Exhibition in Vienna, Austria. 1-4 June 2015

01/2015 **Oral presentation**

“The effect of cutting regime and nitrogen fertilization on the quality of *Miscanthus x giganteus* biomass as biogas substrate” at 8. Internationale MEG e.V. Tagung - *Quo vadis* Miscanthus in Chartres, France. 19-21 January 2015

11/2014 **Oral presentation**

“LCA of biomass value chains: Options and Energy ratios” at Miscanthus Safari and Symposium in Aberystwyth, United Kingdom. 26 November 2014

09/2014 **Poster presentation**

“The effect of different cutting regimes on the quality of miscanthus biomass as biogas substrate” at Progress in Biogas III - Biogas production from agricultural biomass and organic residues in Stuttgart, Germany. 10-11 September 2014

06/2014 **Oral presentation**

“Miscanthus as biogas substrate” at 22nd European Biomass Conference & Exhibition in Hamburg, Germany. 23-26 June 2014

09/2013 **Oral presentation**

“Optimaler Erntezeitpunkt von Miscanthus- Genotypen zur Biogasnutzung” at 56. Tagung der Gesellschaft für Pflanzenbauwissenschaften e.V. – Nachhaltige Erzeugung von Nachwachsenden Rohstoffen in Weihenstephan, Germany. 4-6 September 2013

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