Perennial species mixtures for multifunctional production of biomass on marginal land

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Abstract

Multifunctional agriculture provides noncommodity functions and services along with food, feed and bioenergy feedstocks, for example by preserving or promoting biodiversity, improving soil fertility, mitigating climate change and environmental degradation, and contributing to the socio-economic viability of rural areas. Producing biomass for bioenergy from low-input perennial species mixtures on marginal land has the potential to support biodiversity and soil carbon sequestration in synergy with greenhouse gas mitigation. We compared biomass production in species-rich mixtures of perennial grasses, legumes and forbs with pure-stand grasses and relatively species-poor mixtures under different nitrogen fertilization regimes. Field experiments were performed on different types of marginal land, that is agricultural field margins and land with poor soil fertility, at four sites in southernmost and western Sweden. Biomass production was measured for three years in perennial grasses grown as pure stands, in legume-grass mixtures, and legume-grass-forb mixtures across a species richness gradient. In unfertilized species-rich mixtures, average biomass yields per experimental site and year were in the range from 3 to 9 metric ton DM ha⁻¹ yr⁻¹. While the most productive pure-stand grasses fertilized with 60–120 kg N ha⁻¹ yr⁻¹ often produced higher biomass yields than unfertilized mixtures, these differences were generally smaller than the variations between years and sites. Calculations of climate impact using the harvested biomass for conversion to biogas as vehicle fuel showed that the average greenhouse gas emissions per energy unit were about 50% lower in unfertilized systems than in treatments fertilized with 100–120 kg N ha⁻¹ yr⁻¹. Our findings thereby show that unfertilized species-rich perennial plant mixtures on marginal land provide resource-efficient biomass production and contribute to the mitigation of climate change. Perennial species mixtures managed with low inputs thus promote synergies between productivity and biodiversity in the perspective of climate-smart and multifunctional biomass production.

Keywords: biodiversity, bioenergy, biogas, ecosystem services, fertilization, grasses, greenhouse gas mitigation, legumes, nitrogen

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Introduction

Perennial herbaceous vegetation such as seminatural grasslands and sown grasses or legume-grass mixtures provide biomass that can be used as a bioenergy source, replacing fossil fuels and thereby mitigating climate change (Tilman et al., 2006; Prochnow et al., 2009). In addition, perennial crops have a higher potential for soil carbon (C) sequestration compared to annual crops, as a result of the continuous ground coverage, reduced soil disturbance and enhanced root biomass production (Glover et al., 2010; Powlson et al., 2011; Jarchow et al., 2015). Beneficial effects of perennial crops on soil structure and fertility can also enhance cropping systems’ capacity to adapt to a changing climate and extreme weather events, for example via enhanced capacity to withstand drought periods or floods (Powlson et al., 2011; Asbjornsen et al., 2013). The inclusion of legumes in perennial legume–grass mixtures (PLGM) adds the contribution of symbiotic N₂ fixation and build-up of soil fertility via large inputs of carbon and nitrogen from grass and legume roots (Dybzinski et al., 2008). Furthermore, PLGMs are able to produce high and stable biomass yields under low inputs and with low risks for nitrogen losses, that is expressing high resource-use efficiency (Falmborg et al., 2005; Tilman et al., 2006).

In addition to climate change-related challenges, agriculture needs to reduce its negative effects on the natural biodiversity as well as its reliance on N inputs (Rockström et al., 2009; Tilman et al., 2009; Steffen et al.,...
The intensive use of N fertilizers in modern agriculture has been shown to cause problems with nutrient imbalances, eutrophication and water pollution (Sutton et al., 2011). It is therefore essential to develop biodiversity-promoting cropping systems which are based on high crop diversity and recycling and efficient N use rather than relying on a few crops grown with high inputs of N from external sources. To meet these challenges and contribute to sustainable development, agriculture needs to become more multifunctional, that is increase its capacity to provide food, feed and biomass sources for bioenergy and material, in synergy with the provisioning of noncommodity functions such as climate change mitigation, efficient nutrient cycling, biodiversity preservation and contribution to rural socio-economic viability (Van Huylenbroeck et al., 2005). Species-rich PLGMs managed with low inputs may serve as multifunctional components of agroecosystems, via resource-efficient and resilient biomass production, symbiotic N2 fixation, build-up of soil fertility, promotion of biodiversity and associated ecosystem services such as pollination and biological pest control (Palmborg et al., 2005; Tilman et al., 2006; Öckinger & Smith, 2007; Dybzinski et al., 2008; Pywell et al., 2011; Finger & Buchmann, 2015).

Marginal land is defined as land that is not suited for cost-efficient production of food and feed crops, that is the harvest-generated incomes do not cover the production costs, due to poor soil conditions or socio-economic or political reasons (Dauber et al., 2012). Marginal land is often suggested as a land reserve potentially suitable for the cultivation of bioenergy crops (Tilman et al., 2009; Gopalakrishnan et al., 2011; Dauber et al., 2012). Using marginal land for producing bioenergy feedstock has the advantage of reducing land-use conflicts between food and bioenergy (Harvey & Pilgrim, 2011). However, many types of marginal land may be valuable for noncommodity functions such as preserving biodiversity, sequestering soil carbon or serving as areas for recreational activities – services that might be lost if marginal land use changes towards intensive cultivation of bioenergy crops (Tilman et al., 2009; Gopalakrishnan et al., 2011; Dauber et al., 2012). Investigating options for low-input production of species-rich PLGMs on marginal land should therefore be considered of high importance, to define land-use systems that promote synergies between bioenergy production, biodiversity and the provisioning of multiple ecosystem services.

The aim of this study was to assess productivity and climate impact (greenhouse gas (GHG) mitigation when producing and converting biomass to biogas as vehicle fuel) of perennial grasses and PLGMs designed for biomass production with low inputs and considering biodiversity preservation/promotion on agricultural field margins and land with low soil fertility. To fulfil this aim, we have compiled measurements from four similar (although not identical) field experiments into a synthesis of productivity and bioenergy conversion of biomass from species-poor and species-rich perennial vegetation grown on marginal land without and with moderate levels of N fertilization. The following two hypotheses were tested: (i) without N fertilizer, species-rich PLGMs produce higher biomass yields than sole-crop grasses; (ii) methane gas obtained from biogas conversion of biomass from unfertilized PLGMs results in a larger GHG mitigation compared to the corresponding production obtained from N-fertilized PLGMs and perennial grasses.

Material and methods
Biomass yields were measured during 3 years in four field experiments, established in 2011 and 2012 at four sites in southernmost and western Sweden (one experiment per site). The four experiments were designed to assess low-input biomass production on marginal land, with low harvest intensity (two cuts per year) at all sites. The field experiments were established and managed independently, thus with some variability in the details of the experimental treatments, but all experiments contained comparisons of different levels of species richness and N fertilization. The first factor in the experimental design consisted of different sown species compositions, which were grouped into three main diversity levels. Diversity level 1, pure grasses, contained only perennial grasses (either as sole crops or a mixture of three grass species); diversity level 2, species-poor PLGMs, contained mixtures of perennial grasses and legumes (up to seven sown species); diversity level 3, species-rich PLGMs, contained diverse (more than 50 species) mixtures of native Swedish meadow species (grasses, legumes and non-leguminous forbs, species are listed in supporting information Tables S1 and S2). The second factor was N fertilization level, where an unfertilized treatment was compared with 60, 100 or 120 kg N (ammonium nitrate) ha⁻¹ yr⁻¹. An overview of the experimental design is provided in Table 1.

Study sites
Experiment 1. The first experiment was located in western Sweden, at a farm in the municipality Falköping in the region Västra Götaland. The experiment was established in spring 2011 on a field margin of a dairy farm where cereals and legume–grass leys are the main crops in the rotation. The previous crop in 2010 (including the field margin where the experiment was to be established) was spring barley. Five main treatments, consisting of sown species composition within diversity levels 1 and 2 (Table 1), were distributed in 3 × 24 m plots in a complete random block design with four replicates. Two N fertilization levels, 0 and 100 kg N (ammonium nitrate) ha⁻¹ yr⁻¹, were added as a split-plot treatment across the species composition treatments. Biomass harvests were performed in late June and early-mid-September each year.
Experiment 2. The second experiment was located in southernmost Sweden, at the SITES Lönntorp field experimental station, Swedish University of Agricultural Science in Alnarp, municipality of Lomma in the region Skåne. The experiment was established in spring 2012 on a field with a light sandy soil. The previous management of the field was pasture (before 2006) followed by cultivation of annual crops (cereals and oilseed rape) 2006–2011. The experiment included species diversity levels 1 and 3, and N fertilization levels 0, 60 and 120 kg N ha$^{-1}$ yr$^{-1}$ (Table 1), and these treatments were distributed in 3 × 6 m plots in a complete random block design with four replicates. The first biomass harvest was performed in mid-June each year, and the second harvest in early October (2013) or early September (2014 and 2015).

Table 1 Location and time for establishment, N fertilization treatments (N levels; kg N ha$^{-1}$ yr$^{-1}$) and species composition treatments (grouped according to the three diversity levels) in the four field experiments

| Experiment | Location | Year | N levels | Species composition treatments |
|------------|----------|------|----------|-------------------------------|
| 1. Jättene, Västra Götaland 58°12′N, 13°33′E. Spring 2011 | 0, 100 | DL1: 1-Gr | Sole crop of *Phalaris arundinacea* L. |
| | | | DL1: 3-Gr | Mixture of *Festuca arundinacea* L., *P. arundinacea* and *Phleum pratense* L. |
| | | | DL2: 1-Gr-Leg | *Medicago sativa* L., *Trifolium hybridum* L., *T. repens* L. and *Galega orientalis* L. sown in *P. arundinacea*. |
| | | | DL2: 3-Gr-Leg | *M. sativa*, *T. hybridum*, *T. repens* and *G. orientalis* sown in *Festuca arundinacea* L., *P. arundinacea* and *P. pratense* L. |
| | | | DL2: Cl-Gr | *T. pratense* L., *T. repens*, *F. pratensis* L., *F. arundinacea* and *P. pratense* |
| 2. Alnarp, Skåne 55°38′N, 13°3′E. Spring 2012 | 0, 60, 120 | DL1: 1-Gr | Sole crops of *Dactylis glomerata* L., *Festolium* (hybrid of *F. arundinacea* × *Lolium multiflorum* L.) and *P. arundinacea* |
| | | | DL1: 3-Gr | Mixture of *D. glomerata*, *Festolium* and *P. arundinacea* |
| | | | DL2: 1-Gr-Leg | *Medicago sativa* L., *Trifolium hybridum* L., *T. repens* L. and *Galega orientalis* L. sown in *P. arundinacea*. |
| | | | DL2: 3-Gr-Leg | *M. sativa*, *T. hybridum*, *T. repens* and *G. orientalis* sown in *Festuca arundinacea* L., *P. arundinacea* and *P. pratense* L. |
| | | | DL2: Cl-Gr | *T. pratense* L., *T. repens*, *F. pratensis* L., *F. arundinacea* and *P. pratense* |
| | | | DL3: Div64 | 64 species (supporting information Table S1) |
| 3. Götala, Västra Götaland 58°22′N, 13°28′E. Spring 2012 | 0, 60 | DL1: 1-Gr | Sole crops of *Dactylis glomerata*, *Festolium* and *P. arundinacea* |
| | | | DL1: 3-Gr | Mixture of *D. glomerata*, *Festolium* and *P. arundinacea* |
| | | | DL2: 1-Gr-Leg | *Medicago sativa* L., *Trifolium hybridum* L., *T. repens* L. and *Galega orientalis* L. sown in *P. arundinacea*. |
| | | | DL2: 3-Gr-Leg | *M. sativa*, *T. hybridum*, *T. repens* and *G. orientalis* sown in *Festuca arundinacea* L., *P. arundinacea* and *P. pratense* L. |
| | | | DL2: Cl-Gr | *T. pratense* L., *T. repens*, *F. pratensis* L., *F. arundinacea* and *P. pratense* |
| | | | DL3: Div64 | 64 species (supporting information Table S1) |
| 4. Ellinge, Skåne 55°49′N, 13°22′E. Spring 2011 | 0, 100 | DL2: Cl-Gr | *T. pratense*, *T. repens*, *F. pratensis*, *F. arundinacea* and *P. pratense* |
| | | | DL3: Div57 | 57 species (supporting information Table S2) |

Experiment 3. The third experiment was located on nutrient-poor agricultural soil close to forest, in the municipality of Skara in the region Västra Götaland. The field was managed as green fallow (cutting the vegetation once or twice per year, leaving the biomass on the ground) the year before establishing the experiment in spring 2012. The experiment included the same species composition treatments as in experiment 3 with the N fertilization levels 0 and 60 kg N ha$^{-1}$ yr$^{-1}$ (Table 1). The treatments were distributed in 2 × 12 m plots in a complete random block design with four replicates. Biomass harvests were performed in late June/early July and mid/late September each year.

Experiment 4. The fourth experiment was a demonstration trial located in southernmost Sweden, at a farm in the
municipality Eslov in the region Skane, included in the study to provide biomass yield estimates for some of the species composition treatments at an additional site. The experiment was established in spring 2011 on a field margin (set aside in 1990 and managed as a green fallow until 2010) in an agricultural landscape dominated by intensively managed arable crops (mainly cereals, oilseed rape and sugar beet). The experiment included species diversity levels 2 and 3 (Table 1) as main treatments which were pseudoreplicated in four 20 × 25 m subplots. Two N fertilization levels (0 and 100 kg N ha⁻¹ yr⁻¹) were applied as a split-plot treatment in diversity level 2, while the plots in diversity level 3 did not receive any N fertilization. Biomass harvests were performed in late June and late September/early October each year.

**Sampling and analyses**

At each harvest occasion, samples for biomass yield measurements were collected either by cutting the vegetation by hand in 0.25 or 0.5 m² squares within each experimental plot (this method was applied in experiment 2 and 4), or by harvesting the biomass with a Haldrup forage harvester that recorded total biomass fresh weight per plot. Directly after harvesting with the forage harvester (experiments 1 and 3), subsamples from each plot were collected for analyses of botanical composition and dry matter (DM) content. Botanical composition was analysed by hand-sorting each sample into legumes, grasses and other species. Sorted samples were dried at 65 °C for 48 h before measuring the DM weight of each fraction. In each experiment, the effects of diversity level of the sown species and N fertilization on biomass yield were analysed using univariate general linear model (GLM) with Tukey’s post hoc test (with significance level P < 0.05) in the SPSS software (IBM SPSS Statistics for Windows version 22.0; IBM Corp, Armonk, NY, USA) after the fulfillment of the normality assumption using Blom’s normality test with Q–Q plots was confirmed.

**Assessment of climate impact**

To investigate how sown species composition and management intensity influence climate impact when using the harvested biomass as substrate for biogas vehicle fuel (replacing natural gas), a production process model was developed and used for calculating the greenhouse gas (GHG) emissions from cultivating, harvesting, transporting and converting biomass to biogas. Greenhouse gas emissions were calculated based on measured biomass yields in the field experiments and related to the energy outcome of the production process as g carbon dioxide (CO₂) equivalents per mega joule (MJ) of fuel, g CO₂-eq. MJ⁻¹. These data were then related to the GHG mitigation targets that need to be met according to the EU sustainability criteria for biofuels (European Union, 2009).

**Production process modelling**

For the calculation of GHG emissions, it was assumed that no field management operations other than sowing and, where applicable, fertilizer spreading according to nitrogen (N) fertilization level were applied. Phosphorous (P) and potassium (K) fertilization was assumed to balance nutrients removed with the harvested biomass according to typical content of these two nutrients (supporting information Table S3). Seeds for establishing the perennial species and liming requirements were found to have only minimal impact on the outcome and therefore omitted. No pesticides were used.

The perennial grasses and PLGMs were assumed to be grown for 10 years without reseeding and harvested twice per growing season, maximizing energy yield by having a good balance between harvested biomass yield and methane potential (Prade et al., 2015). Field losses are known to be higher when harvesting biomass with full-scale machinery compared to hand-harvested samples (Prade et al., 2015). Measured biomass yields were therefore converted to harvestable biomass yields according to the regression equation for the recovery coefficient calculated by Prade et al. (2015; see also supporting information Table S4). The same recovery coefficient slope was used for hand- and Haldrup-harvested samples, but the intercept was set at a 10% units higher recovery coefficient for Haldrup-harvested samples to reflect the higher stubble left by the Haldrup compared to hand harvest.

No harvest was assumed to be carried out at harvestable biomass yields below 1 metric ton (Mg, hereafter: t) DM ha⁻¹. Dry matter content at cutting was set to 20%, followed by chopping and harvesting after field-drying to 35% DM. Tractor-drawn field wagons were assumed to be used for transport to the biogas plant (8 km average distance), where the harvested biomass was unloaded into a concrete bunker silo, compacted by front loaders and covered by a plastic sheet for ensiling. Dry matter losses during ensiling and feeding into the biogas plant were set to 5% (Gissén et al., 2014). The biogas yield was calculated according to methane potential and volatile solids (VS) content typical for grass biomass (Prade et al., 2015; supporting information Table S3). The biogas was further upgraded to vehicle fuel quality (98% methane) and compressed. Further details are listed in supporting information Tables S3 and S4.

**Diesel consumption**

Based on data on diesel consumption (L h⁻¹) and capacity (the actual input data were inverted capacity, h ha⁻¹) of typical machinery (Algerbo et al., 2014), diesel consumption for sowing, spreading of fertilizers and rolling was set to 2,3, 4,7 and 2.3 L ha⁻¹. Diesel consumption for cutting, chopping, transport, compaction in the silo and feeding biomass to the biogas plant were calculated individually for each harvest occasion using linear regressions based on literature data on machinery capacity (h ha⁻¹) and diesel consumption (L ha⁻¹) (Algerbo et al., 2014; Prade et al., 2015; supporting information Table S4). The regression equations for harvest-related field operations (cutting, chopping and transport) have different validity ranges depending on harvestable biomass yield. The lower biomass yield range reflects a situation where full machinery capacity cannot be reached, that is the machine will not be able drive faster to process more biomass per time unit. In the higher
range, only machinery capacity limits the harvest. This differentiation was applied to better reflect inefficient harvest situations at low biomass yields.

**Emissions**

Greenhouse gas emission from mineral fertilizer applications and diesel consumption were calculated based on data on fertilizer and diesel use multiplied by corresponding GHG emission factors (supporting information Table S3). The requirement for storage \([\text{m}^3 \text{ ha}^{-1}]\) was calculated according to biomass yield and compacting density and translated into energy requirements using typical energy coefficients for the silo and a plastic silo cover, and the emissions for storage were calculated using corresponding emission factors. For the calculation of emissions from biogas production, an aggregated emission factor was used that included emissions for heat and electricity requirements, methane losses from the biogas reactor, the upgrading unit and the flare for excess biogas as well as direct and indirect emissions of nitrous oxide \((\text{N}_2\text{O})\) from the digestate storage tank. Crop residues (aboveground, belowground) contributing to emissions on \(\text{N}_2\text{O}\) were calculated according to IPCC (2006), where the amount of belowground residues was estimated as an average for the 10-year cultivation period. The annual soil carbon (C) sequestration effect of the perennial biomass crops was calculated from a linear increase with 111 kg C ha\(^{-1}\) t DM\(^{-1}\) up to a biomass yield of 2.7 t DM ha\(^{-1}\) (Börjesson et al., 2015), thereafter levelling out at approximately 340 kg C ha\(^{-1}\) yr\(^{-1}\) (supporting information Table S4). This increase pattern is assumed to be typical for soils with a soil organic C (SOC) content of 2.0% (Börjesson et al., 2013). The SOC effect itself is not accounted for in the GHG balance according to the EU renewable energy directive (RED; European Union, 2009), but is used to account for calculating the amount of N from crop residues that is immobilized in the soil organic matter and therefore does not contribute to indirect \(\text{N}_2\text{O}\) emissions. Further details are listed in supporting information Tables S3 and S4.

**Results**

**Effects of diversity level and N fertilization on biomass yields**

In experiment 1, diversity of sown species (diversity level 1 vs. level 2) influenced the biomass yield only in 2014 \((P < 0.01)\), while fertilization with 100 kg N ha\(^{-1}\) yr\(^{-1}\) increased the biomass yield in all years (Fig. 1; 2012: \(P < 0.01\), 2013: \(P < 0.001\), 2014: \(P < 0.001\)). An interaction between diversity and fertilization levels was detected in all years (2012: \(P < 0.05\), 2013: \(P < 0.01\), 2014: \(P < 0.001\)). This interaction, together with the stronger significance of fertilization effects, motivated separate analyses within fertilization levels which revealed significantly higher biomass yield in clover-grass compared to the other species compositions under zero N fertilization in 2012 \((P < 0.001)\) and 2014 \((P < 0.001)\) (Fig. 1a,c).

In experiment 2, biomass yields were similar between species diversity levels (Fig. 2a–c), and there was no significant interaction between diversity and N fertilization levels. N fertilization increased the biomass yields in 2014 (Fig. 2b: \(P < 0.05\)) and 2015 (Fig. 2c: \(P < 0.01\)).

Also in experiment 3, the different diversity levels resulted in similar biomass yields (Fig. 3), and the production increased by N fertilization (2013: \(P < 0.01\), 2014: \(P < 0.001\) and 2015: \(P < 0.001\)). A significant interaction between diversity level and N fertilization was found only in 2015 (\(P < 0.05\)).

In experiment 4, biomass yields did not differ significantly between species diversity levels (Fig. 4), and there was no significant interaction between diversity...
and N fertilization levels. The fertilized clover–grass yielded significantly more biomass than the unfertilized treatments in 2013 (Fig. 4b; $P < 0.001$) and in 2014 (Fig. 4c; $P < 0.05$).

**Climate impact of unfertilized and fertilized grasses and PLGMs used for production of biogas as vehicle fuel**

The accumulated GHG emissions from production of biogas vehicle fuel obtained using biomass from perennial grasses and PLGMs showed an asymptotically decreasing relationship with increasing biomass yields for all N-fertilized treatments (Fig. 5). On the other hand, GHG emissions from unfertilized treatments varied less across biomass yield levels, compared to fertilized treatments (Fig. 5). This was mainly due to the fact that nearly all GHG emissions were proportional to the biomass yield and therefore also to the energy yield. GHG emissions from biogas obtained from fertilized biomass were, on average, twice as high as in the unfertilized treatments (Fig. 5), as a result of additional emissions from mineral fertilizer production and distribution as well as nitrogen losses, notably as nitrous oxide, contributing with high climate impacts.

According to these calculated GHG emissions, all yield levels in unfertilized treatments fulfilled the current sustainability criteria of 60% emission reduction compared to fossil diesel (Fig. 5). To fulfil sustainability criteria, perennial grasses and PLGMs fertilized with 60, 100 and 120 kg N ha$^{-1}$ would need to produce approximately 6, 8 and 10 t DM ha$^{-1}$, respectively, which is higher than the average yields for those N fertilization levels in the field experiments studied here. Cases where low yields would lead to only one harvest being recovered were found at all N fertilization levels. If the first harvest was also low, this would lead to increased energy-specific GHG emissions, a situation which was more frequent in unfertilized grasses and PLGMs (Fig. 5; data points showing higher GHG emissions at the lowest end of the biomass yield range). Nevertheless, the EU sustainability criteria were met also for the lowest yielding unfertilized grasses and PLGMs (Fig. 5).

**Discussion**

The results presented in this study show that N fertilization often led to a significant increase in biomass yields while the level of sown species diversity most often did not influence the biomass yield. An implication of this result is that growing diversified PLGMs will not penalize the farmer with lower yields compared to growing only productive perennial grasses. Based on our calculations of climate impact, we show that unfertilized PLGMs, which are known to promote biodiversity and associated ecosystem services (e.g. Pywell et al., 2011; Bommarco et al., 2013), provide resource-efficient biomass production with a strong GHG mitigation potential if the biomass is used as biogas feedstock.

**Nitrogen use and environmental benefits of unfertilized PLGMs**

The results did not provide general support for the first hypothesis that unfertilized PLGMs would produce higher biomass yields than unfertilized sole-crop grasses. This hypothesis was supported only by a few observations from experiment 1, where the clover–grass...
mixture in diversity level 2 produced significantly higher biomass yields than the sole grasses in diversity level 1 in two of the three years. Legumes, mainly red clover, occurred as volunteer weeds in the sole grass treatments of experiment 3. The legume weeds likely contributed to increased biomass yields in unfertilized grasses, thereby reducing the potential yield advantage of unfertilized PLGMs over sole grasses. While N fertilization often led to increased biomass yields, our investigations across several sites and years resulted in several observations of unfertilized PLGMs being as productive as perennial grasses or PLGMs fertilized with up to 100 kg N ha$^{-1}$ yr$^{-1}$. Similar evidence for the high resource efficiency in biomass production from species-rich perennial herbaceous plant mixtures is provided also by several other studies (e.g. Palmberg et al., 2005; Bullock et al., 2007; Picasso et al., 2011; Tilman et al., 2012; Finn et al., 2013; Jarchow et al., 2015; Jungers et al., 2015). In addition to producing biomass with very low climate impact, unfertilized PLGMs also have a high capacity to take up excess nutrients and thereby reduce the risks of harmful N losses, for example if established as buffer zones along field margins close to waterways or other sensitive environments (Asbjornsen et al., 2013; Zhou et al., 2014).

The GHG emissions from N-fertilized biomass production are to a large extent due to the large primary energy use in fertilizer production and distribution as well as to higher N$_2$O emissions from soils fertilized with mineral N compared to unfertilized systems.
including legumes (Jensen et al., 2012). Apart from the negative climate impact, agriculture’s inputs of synthetic N fertilizers to cultivated land pose additional environmental problems and costs for societies (Sutton et al., 2011). The large additions of N fertilizers in intensive agriculture have created and continue to increase N surpluses in agricultural systems, which increases the risks for N losses with severe consequences for water quality and the environment in freshwater, coastal and marine ecosystems as well as air quality and GHG emissions (Rockström et al., 2009; Sutton et al., 2011; Steffen et al., 2015). Promoting the management of cropping systems that produce food or biomass with zero or low N fertilizer inputs is therefore of uttermost importance for the transition towards more sustainable agricultural systems. In this context, our findings of the resource efficiency and GHG mitigation potential of biomass from unfertilized PLGMs on marginal land provide further arguments for the increased development of low-input perennial crops as valuable components in sustainable cropping systems.

Climate impact of biofuel from perennial biomass

Our findings provide new evidence that biomass from unfertilized PLGMs as biogas feedstock has a very large GHG mitigation potential, which supports findings from previous research (e.g. Tilman et al., 2006, 2009). Even though the biomass yields of unfertilized PLGMs are often lower than in fertilized perennial grasses and PLGMs, our results show that using the biomass from unfertilized perennial grasses or PLGMs as biogas feedstock strongly mitigates GHG emissions also at low biomass yields.

Perennial biomass from marginal lands has been suggested as source of biomass for subsequent production of renewable energy carriers such as biogas vehicle fuel. The European Commission has set up sustainability criteria for renewable vehicle fuels (European Union, 2009), which need to be fulfilled for a biofuel to be granted tax reductions (Swedish Tax Agency, 2015), which are often needed for economic feasibility. Results of this study show that biomass yields obtained after fertilization with 60, 100 or 120 kg N ha$^{-1}$ would need to be above 6, 8 and 10 t DM ha$^{-1}$, respectively, to meet the 60% GHG emission reduction requirement. In contrast, biomass from unfertilized grasses and PLGMs fulfil the 60% GHG emission reduction requirement at all biomass yield levels obtained in this study. Meeting the EU sustainability criteria for biofuels can therefore be an important incentive for developing biomass and biofuel production systems based on low-input biodiversity-promoting PLGMs.

Choice of diversity level (sole grasses and grass mixtures, species-poor PLGMs or species-rich PLGMs) had only a minor impact on the GHG emissions from the production and conversion of PLGM-derived biomass to biogas vehicle fuel. This means that low-diversity perennial biomass would have as strong GHG mitigation potential as high-diversity PLGMs. However, species-rich PLGMs provide additional ecosystem services via promotion of biodiversity (see below).

Other perennial biomass production systems have also been found to provide an important GHG mitigation potential when converting harvested biomass to biogas, as in the case of unfertilized roadside grass (Pierie et al., 2015), as well as additional benefits using the digestate from the biogas process as an alternative fertilizer. In a study by Börjesson et al. (2015), an
intensively fertilized perennial grass crop yielded 9.1 t DM ha\(^{-1}\), and the conversion of this biomass to biogas vehicle fuel resulted in GHG emissions of 50 g CO\(_2\) MJ\(^{-1}\) (a reduction of approximately 40% compared to fossil fuel). The same study also presented an alternative where mineral fertilizer was replaced by digestate from the biogas process, which also contributes to an increase in SOC, resulting in a stronger GHG mitigation, from 50 to 33 g CO\(_2\) MJ\(^{-1}\) (a reduction of 60% compared to fossil fuel). Using digestate for nutrient circulation and soil C sequestration was not investigated in the present study, but would have a similar effect on the GHG emissions and thus lower the threshold biomass yield required to fulfil the 60% GHG emission reduction criteria.

Multifunctional land use

Obtaining bioenergy feedstock with low environmental impact and simultaneously enhancing the infrastructure of biodiversity-promoting landscape elements is a major achievement for sustainable development of agricultural systems (IAASTD, 2009). The research presented here shows that low-input species-rich PLGMs harvested twice per year are truly multifunctional components of diversified cropping systems. By establishing and managing such PLGMs on marginal land, for example fallow or abandoned agricultural land, sustainable biomass production will be achieved without competing with food or feed production for land availability, and with synergistic benefits for the natural biodiversity. Establishing and managing species-rich PLGMs on field margins will impose minor land competition with food or feed crops, but on the other hand enhance the cropping system diversity and the potential delivery of multiple ecosystem services: increased biodiversity, pollination and biological pest control (Reitalu et al., 2009; Pywell et al., 2011; Bommarco et al., 2013; Gari-baldi et al., 2013), enhanced soil C sequestration (Glover et al., 2010; Asbjornsen et al., 2013), reduced nutrient losses to the surrounding environment (Asbjornsen et al., 2013; Zhou et al., 2014) as well as landscape connectivity for wildlife and recreational purposes. Multi-functional management of PLGMs on, for example, field margins would also imply that maximum biomass output is not the only objective. For example, more heterogeneous harvest regimes could be applied in time and space, for example by leaving unharvested patches or strips that further enhance the abundance of resources for biodiversity.

Establishing PLGMs on field margins, as buffer zones, or on fallow land is in accordance with current policy within the European Union, that is the ‘greening’ component of direct payments to farmers (European Union, 2013). One of the conditions for this subsidy is that conventional farms larger than 15 ha in the EU should allocate 5% of the land as ecological focus area (EFA), which include management options such as buffer zones, uncultivated field margins, flower strips, fallow land and green cover during periods where no crop is growing on the land (European Union, 2013). Even stronger multifunctionality will be achieved if such EFAs can also be harvested and the biomass used as, for example, bioenergy feedstock.

Our investigations have not included economic assessments, but previous research has shown that increased plant diversity in perennial biomass production systems increases yield stability and reduces economic risks related to the production (Finger & Buchmann, 2015). It is possible that an economic assessment would imply a modification of the assumed threshold for harvesting the biomass (1 t DM ha\(^{-1}\)) used in our calculation of GHG emissions, that is the biomass yield would need to be higher to be profitable. However, costs for the establishment and management of PLGMs on, for example, field margins and fallow land might be motivated by benefits such as reduced nutrient losses and enhanced biodiversity and associated ecosystem services, and partly covered by subsidies aiming to promote these functions. If so, the value of PLGM biomass as feedstock for, for example, biogas production, including a high fertilizer value of the biogas digestate, would only need to compensate the costs for harvesting and transporting the biomass to a biogas plant, and for distributing the digestate to arable crops – thereby also contributing to efficient resource use by redistributing nutrients from marginal to arable land. Another potential economic constraint is the cost for establishing species-rich PLGMs, as the high-diversity seed mixtures used in our experiments are much more expensive than simpler, species-poor PLGMs (e.g. commercially available clover–grass mixtures). An option for less costly establishment could be to distribute hay from local species-rich grasslands, which has been evaluated with good results in grassland restoration experiments (e.g. Kiehl et al., 2010). In conclusion, our results show that unfertilized perennial grasses and PLGMs provide resource-efficient biomass with large GHG mitigation potential if converted to biogas. The finding that species-rich PLGMs provided similar biomass yield levels as productive grasses can be used as an argument for stimulating the establishment and management of such biodiversity-promoting elements in the agricultural landscape, which would strengthen agriculture’s capacity to provide multiple ecosystem services.
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Supporting Information

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

**Table S1.** List of species in DL3: Div64, included in experiments 2 and 3.
**Table S2.** List of species in DL3: Div57, included in experiment 4.
**Table S3.** Parameters and assumed values used in the calculation of the GHG emissions of biogas production based on biomass from perennial grasses or PLGMs.
**Table S4.** Slopes and intercepts for the regression equations used to calculate diesel consumption [L ha\(^{-1}\)] (Prade et al., 2015), annual soil organic carbon (SOC) sequestration effect [kg ha\(^{-1}\)] (Börjesson et al., 2015) and share of harvestable biomass [%] (Prade et al., 2015) according to the biomass yield [t DM ha\(^{-1}\)].