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Suitability of fen plant biomass as biogas substrate

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Summary

The traditional agricultural use of fenlands is drainage-based. However, by lowering the water table, the peat body is aerated and consequently mineralized. This in turn results in high emissions of greenhouse gases (GHG) such as CO_2 and N_2O. Rewetting can markedly reduce these emissions, but conventional land use is then no longer possible. Paludiculture (= productive use of wet peatlands) is an alternative to drainage-based cultivation and combines rewetting with biomass production. The produced biomass might be a suitable substrate for anaerobic digestion in biogas plants.

The aims of this thesis were (1) to identify fen plant species from a pre-selected subgroup, which might be suitable as biogas substrate, (2) to investigate how the biogas potential and the biogas yield per hectare of fen plants changes with increasing plant maturity, (3) to evaluate if models developed for the prediction of the biochemical methane potential (BMP) are also suitable for fen plant species, and (4) to investigate the influence of adding fen plant material to maize silage on long-term process stability and biogas yield.

It was demonstrated that biomass of *Typha* spp., *Phalaris arundinacea*, and *Phragmites australis* harvested in early summer is potentially suitable as biogas substrate. These plant materials reached biogas potentials, which were similar to those of grass silage. Further experiments were conducted with *Typha* spp. and *P. arundinacea.* Their biogas potential and BMP decreased with increasing plant maturity and was negatively correlated with the lignin content. Plant material, which is physiologically older and thus less digestible, can accumulate in the fermenter and lead to process disturbances in the long term. For a high biomass yield with a decent anaerobic digestibility, *Typha* spp. and *P. arundinacea* should be harvested between the development stages of full flowering and shortly after the seed heads turned brown. Published linear regression models, which included the lignin content as main regressor, could also predict the biogas potential and BMP of these fen plant species.

Zusammenfassung

Bei der traditionellen landwirtschaftlichen Nutzung der Niedermoore werden die organischen Böden entwässert. Durch die Absenkung des Grundwasserspiegels wird jedoch der Torfkörper belüftet und folglich mineralisiert. Dies wiederum führt zu hohen Emissionen an Treibhausgasen (THG) wie CO_2 und N_2O. Eine Wiedervernässung kann diese Emissionen deutlich reduzieren, eine konventionelle Landnutzung ist dann aber nicht mehr möglich. Die Paludikultur (= produktive Nutzung nasser Moore) ist eine Alternative zum entwässerungsbasierten Anbau und kombiniert die Wiedervernässung mit der Produktion von Biomasse. Die erzeugte Biomasse könnte ein geeignetes Substrat für die anaerobe Vergärung in Biogasanlagen sein.

Die Ziele dieser Arbeit waren (1) die Identifizierung von Niedermoor-Pflanzenarten aus einer vorselektierten Teilgruppe, die als Biogassubstrat geeignet sein könnten, (2) die Untersuchung, wie sich das Biogaspotential und der Biogasertrag pro Hektar von Niedermoorpflanzen mit zunehmender Pflanzenreife verändern, (3) die Beurteilung, ob Modelle, die für die Vorhersage des biochemischen Methanpotentials (BMP) entwickelt wurden, auch für Niedermoorpflanzenarten geeignet sind, und (4) die Untersuchung der Auswirkung der Zugabe von Niedermoorpflanzenmaterial zu Maissilage auf die langfristige Prozessstabilität und den Biogasertrag.

Es konnte gezeigt werden, dass die im Frühsommer geerntete Biomasse von *Typha* spp., *Phalaris arundinacea* und *Phragmites australis* potenziell als Biogassubstrat geeignet ist. Diese Pflanzenmaterialien erreichten Biogaspotentiale, die mit denen von Grassilage vergleichbar sind. Mit *Typha* spp. und *P. arundinacea* wurden weitere Experimente durchgeführt. Ihr Biogaspotential und BMP nahmen mit zunehmender Pflanzenreife ab und waren negativ mit dem Ligningehalt korreliert. Reiferes Pflanzenmaterial, das physiologisch älter und damit weniger verdaulich ist, kann sich im Fermenter anreichern und langfristig zu Prozessstörungen führen. Um einen hohen Biomasseertrag bei guter anaerober Verdaulichkeit zu erzielen, sollten *Typha* spp. und *P. arundinacea* zwischen den Entwicklungsstadien der Vollblüte und kurz nach dem Braunwerden der Kolben geerntet werden. Veröffentlichte lineare Regressionsmodelle, die den Ligningehalt als Hauptregressor enthielten, konnten auch das Biogaspotential und den BMP dieser Niedermoor-Pflanzenarten vorhersagen.

List of abbreviations

ADF	Acid detergent fiber
ADL	Acid detergent lignin
BMP	Biochemical methane potential
C	Celsius
C. acutiformis	*Carex acutiformis* EHRH.
C. diandra	*Carex diandra* SCHRANK
C. elata	*Carex elata* ALL.
C. lasiocarpa	*Carex lasiocarpa* EHRH.
C. riparia	*Carex riparia* CURTIS
C. rostrata	*Carex rostrata* STOKES
CH_4	Methane
CL	Cellulose
CO_2	Carbon dioxide
GHG	Greenhouse gases
H_2	Hydrogen
H_2S	Hydrogen sulfide
HC	Hemicellulose
K	Kelvin
LfL	Bayerische Landesanstalt für Landwirtschaft (Bavarian State Research Center for Agriculture)
LR	Linear regression
MCC	Microcrystalline cellulose
MGC	MilliGascounter
MLR	Multiple linear regression
N	Nitrogen
n	Sample size
N_2O	Nitrous oxide
NDF	Neutral detergent fiber
OR	Organic residue
p	Pressure
P. arundinacea	*Phalaris arundinacea* L.
P. australis	*Phragmites australis* (CAV.) TRIN. EX STEUD.
RS	Reducing sugars
ST	Starch
T	Temperature
T. angustifolia	*Typha angustifolia* L.
T. latifolia	*Typha latifolia* L.
T. x *glauca*	*Typha* x *glauca* GODR.
TS	Total solids
VDI	Verein Deutscher Ingenieure (Association of German Engineers)
VDLUFA	Verband Deutscher Landwirtschaftlicher Untersuchungs- und Forschungsanstalten (Association of German Agricultural Analytic and Research Institutes)
VFA	Organic volatile fatty acids
VOA/TIC	Total inorganic carbonate buffer
VS	Volatile solids

XA	Crude ash
XF	Crude fiber
XL	Crude fat
XP	Crude protein

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1. Introduction

1.1. Paludiculture

Globally, peatlands cover only 3% of the land surface (Yu et al. 2011). In contrast, they store substantial amounts (about 600 gigatons) of carbon and contain a larger carbon pool than tropical rainforests (Parish et al. 2008, Yu et al. 2011). In Germany, organic soils occur mainly in the north and south (Roßkopf et al. 2015). They cover an area of about 18 000 km², of which 10 000 km² are fen soils, 3 000 km² are bog soils, and 5 000 km² are other organic soils (Roßkopf et al. 2015). This corresponds to approximately 5% of the area of Germany or about 7% of the utilized agricultural area (Geurts et al. 2019, Tiemeyer et al. 2020). Large parts of the peatlands in Germany were drained for agricultural and forestry use. 53% are currently used as grassland, 20% as cropland, and 16% for forestry. The remaining 11 % are shrubland, unutilized land, open water bodies, peat extraction sites and settlement areas (Tiemeyer et al. 2020).

However, the drainage-based use of peatlands provokes a variety of problems. These include management problems, such as increased susceptibility to wind erosion, the formation of a hydrophobic topsoil, and, in the long term, a reduction in soil fertility and biomass yields (Wichtmann et al. 2016). In addition, ecosystem services, such as water purification and storage of water and nutrients, are reduced (Wichtmann et al. 2016). Furthermore, drainage leads to microbial mineralization of the peat body, which in turn results in the release of enormous amounts of greenhouse gases (GHG), such as carbon dioxide (CO_2) and nitrous oxide (N_2O) (Tanneberger et al. 2021). In Germany, GHG emissions from agriculturally used peatlands correspond to 4% of the total national GHG emissions or 37% of the agricultural GHG emissions (Geurts et al. 2019, UBA 2010). A reduction of GHG emissions and a full or partial reestablishment of ecosystem services is possible through rewetting (Tanneberger et al. 2021, Wichtmann and Joosten 2007). However, further conventional peatland use is then no longer feasible (Wichtmann and Schäfer 2007).

Paludiculture (*Latin* "palus" = swamp, "cultivare" = to cultivate) is the productive use of wet peatlands combining rewetting with biomass production (Wichtmann et al. 2016, Wichtmann and Joosten 2007). The peat is preserved due to permanently high water levels. Since typically perennial plants are cultivated, soil tillage is only required to establish the plants. From an

economic perspective, it is not only important that plant species for paludicultural systems cope with wet conditions, but exhibit high biomass production in adequate quality (Wichtmann and Joosten 2007). Typical high-yielding fen plant species, which are cultivated in paludicultures, are *Typha* spp. (cattails), *Phragmites australis* (common reed), *Phalaris arundinacea* (reed canary grass) and *Carex* spp. (sedges, Fig. 1, Abel et al. 2013). Besides high biomass yields, *P. australis* and *Carex* spp. have the advantage that they contribute to peat formation (Succow and Joosten, 2001, Wichtmann et al. 2016). Only above-ground parts of the fen plants are harvested so that the peat body is not disturbed (Abel et al. 2013).

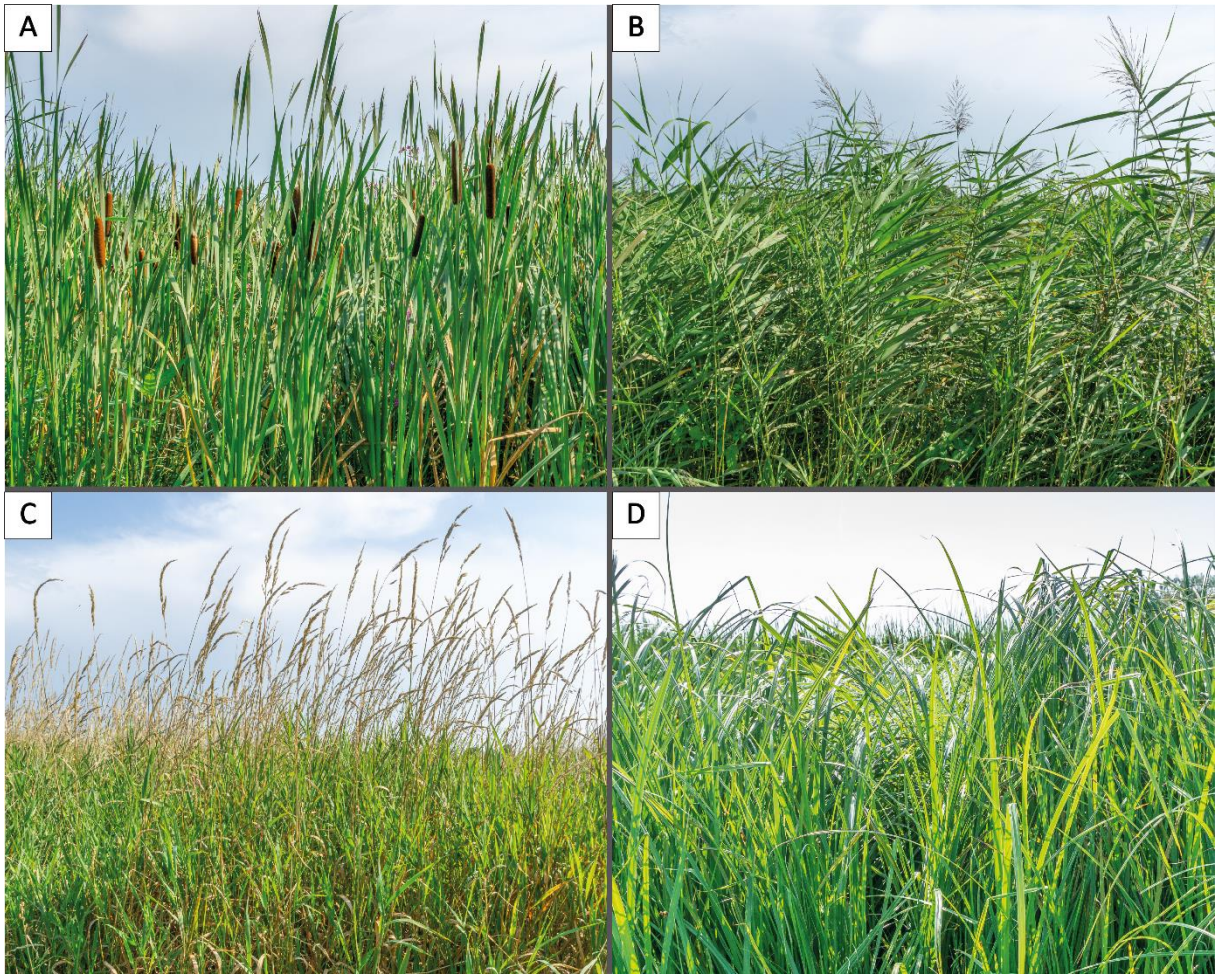

Figure 1: Fen plant species, which are commonly cultivated in paludicultures: *T. latifolia* (A), *P. australis* (B), *P. arundinacea* (C) and *C. acutiformis* (D).

The produced biomass can be used in various ways. Possible material uses are applications as growing media component, insulation and building material and animal fodder (Hartung and Meinken 2021, de Jong et al. 2021, Kamali Moghaddam et al. 2021, Beyzi et al. 2022). Furthermore, the fen plant biomass can be energetically converted via combustion (Giannini et al. 2016, Kuptz et al. 2022). It might also be suitable as substrate for anaerobic digestion in

biogas plants to potentially replace grass and maize, which are currently grown on drained peatlands.

1.2. Structure and anaerobic digestion of lignocellulosic substances

Fen plant biomass consists primarily of lignocellulose (Dragoni et al. 2017, Kandel et al. 2013, Pijlman et al. 2019). The main components of lignocellulose are cellulose, hemicellulose and lignin (Isikgor and Becer 2015). Cellulose is a polymer composed of D-glucose subunits that are linked to each other by β-1-4 glycosidic bonds and form large, unbranched chains (Fig. 2, Monlau et al. 2013, Wei 2016). Hemicellulose is a collective term for branched polymers with short lateral chains built up of different sugar monomers such as xylose, mannose, galactose, rhamnose, and arabinose (Barakat et al. 2013, Monlau et al. 2013). The most common monosaccharide in hemicelluloses is xylose (Lübken et al. 2010, Monlau et al. 2013). Lignin is a complex three-dimensional polymer of aromatic compounds (Čater et al. 2014). It consists of three different phenylpropane alcohols: sinapyl, coniferyl, and p-coumaryl (Monlau et al. 2013). The linkages between the phenylpropane units are very strong, non-hydrolysable carbon-carbon bonds or relatively inert ether bonds (Betts et al. 1991).

The core of the lignocellulose structure is formed by bundles of parallel cellulose chains, which are aggregated together in so-called micro-fibrils (Anwar et al. 2014). Bundles of multiple micro-fibrils aligned in parallel result in a structure that contains highly ordered regions of crystalline cellulose alternating with less ordered amorphous regions (Fig. 2, Zhao et al. 2012). This micro-fibril structure is surrounded by hemicellulose, which connects the cellulose fibers to lignin and thus gives the entire plant matrix more rigidity (Ahmed et al. 2019, Boontian 2014). Lignin polymers fill the space between and around hemicellulose and cellulose (Faisal et al. 2021). This is also referred to as lignin incrustation (Klimiuk et al. 2010).

The anaerobic digestion of, among others, lignocellulosic substances involve four steps, which run spatially and temporally in parallel in the fermenter: hydrolysis, acidogenesis, acetogenesis and methanogenesis (Fig. 3, Agregán et al. 2022).

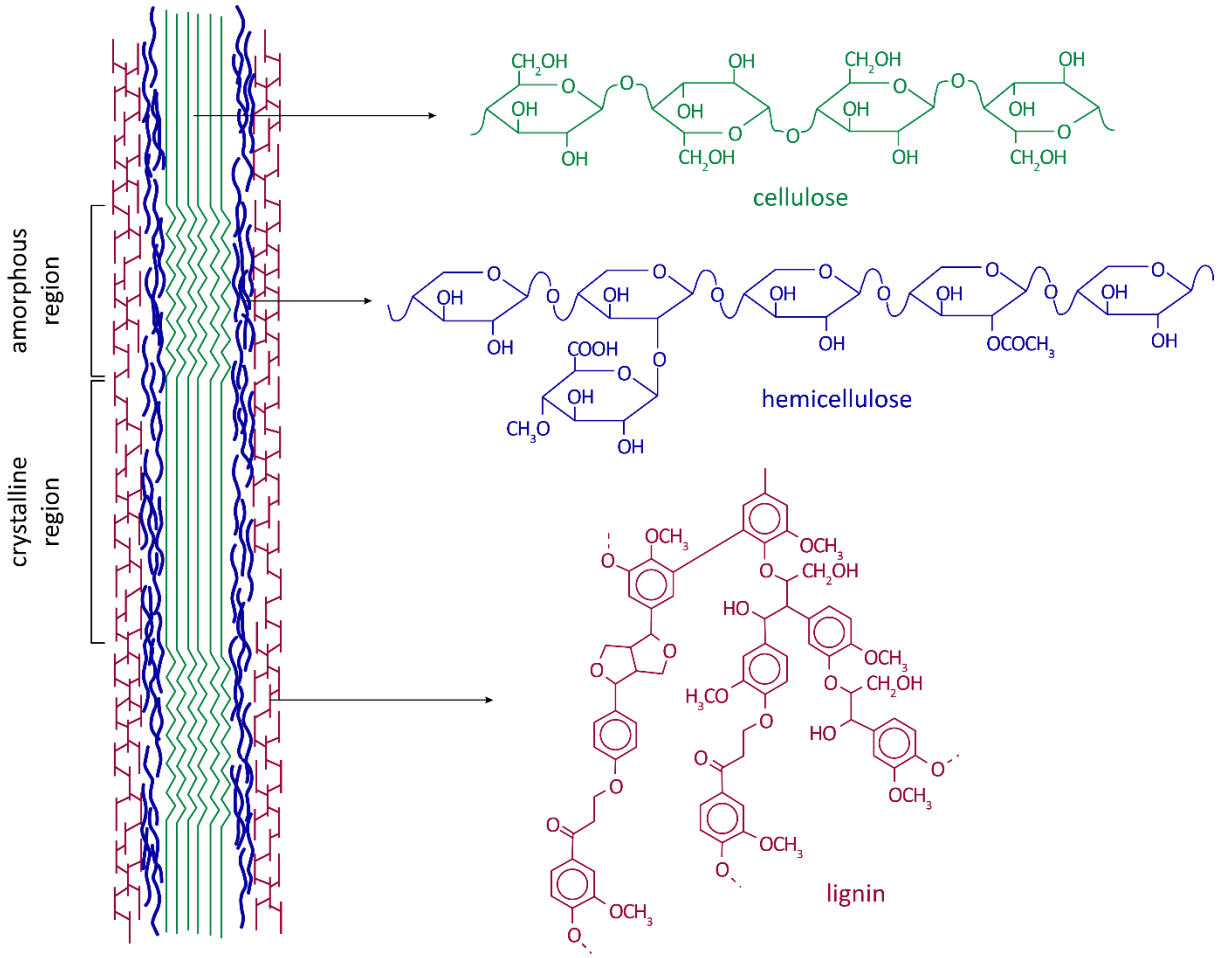

Figure 2: Schematic structure of lignocellulose (modified after Reineke and Schlömann 2007).

During the hydrolysis phase, complex organic molecules such as lignocellulose, carbohydrates, proteins, and fats are broken down into oligo- and monomers by extracellular enzymes, which are excreted by facultative and obligate anaerobic bacteria (Boontian 2014). Hydrolysis is normally the rate-limiting, i.e., slowest step in the anaerobic degradation of lignocellulosic materials (Ahmed et al. 2019). This is primarily related to the poor accessibility of the lignocellulose structure for hydrolytic enzymes. Lignin is relatively resistant to anaerobic degradation and acts as a physical barrier via incrustation (Faisal et al. 2021, Monlau et al. 2013). At sites where the lignocellulose structure is accessible to enzymes, the cellulose and hemicellulose fractions can be hydrolyzed. Hemicelluloses have an amorphous structure and can be easily hydrolyzed (Boontian 2014). Cellulose is in general more resistant to hydrolysis than hemicelluloses (Carrere et al. 2016). The enzymatic hydrolysis of cellulose occurs predominantly at its amorphous regions (Čater et al. 2014).

In the acidogenesis phase, the products from the hydrolysis phase are further degraded into low-molecular organic acids and alcohols by different acidogenic bacteria (Adekunle and

Okolie 2015, Boontian 2014). In addition, acetate, hydrogen, and carbon dioxide, which are base products for methane formation, are already formed during acidogenesis (Adekunle and Okolie 2015).

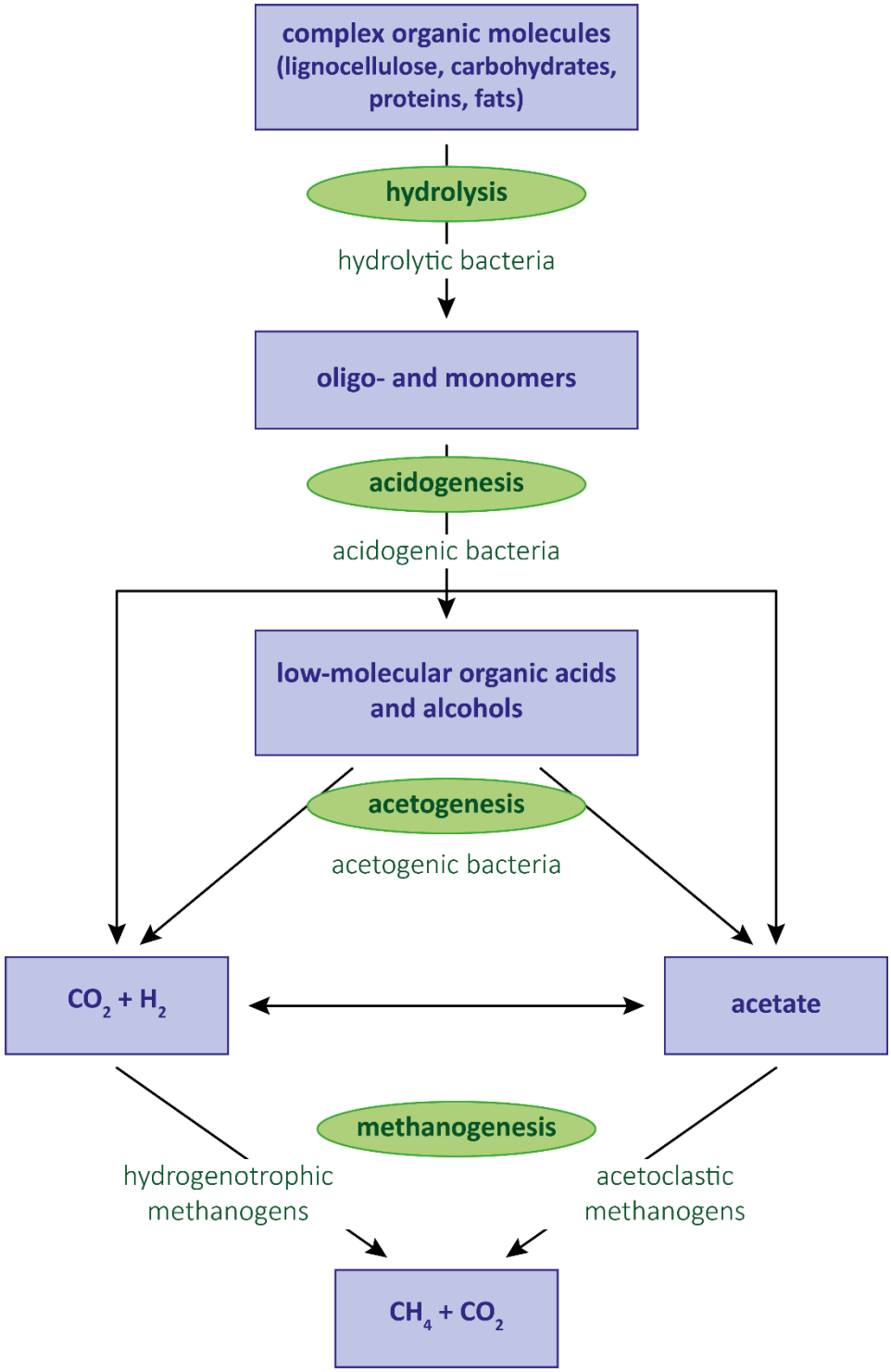

Figure 3: Simplified depiction of the anaerobic digestion of lignocellulosic biomass (modified after Gao et al. 2022).

During the acetogenesis phase, acetogenic bacteria form acetic acid, hydrogen and carbon dioxide from the low-molecular organic acids and the alcohols of the acidogenesis (Boontian 2014). High hydrogen partial pressures inhibit the metabolism of acetogenic bacteria. During methanogenesis, hydrogen is rapidly consumed to form methane. Consequently, acetogenesis and methanogenesis are dependent on each other (Meegoda et al. 2018).

The methane production during the methanogenesis takes place under strict anaerobic conditions and is performed by methanogenic archaea (Boontian 2014, Meegoda et al. 2018). Hydrogenotrophic methanogens produce methane from hydrogen and carbon dioxide, while acetoclastic methanogens produce methane from acetate (Hashemi et al. 2021). Typically, about 70 % of methane is produced via the acetoclastic pathway and about 30 % via the hydrogenotrophic pathway (Čater et al. 2014). Methanogens have a slower growth rate than the bacteria, which are involved in the hydrolysis, acidogenesis and acetogenesis, and are very sensitive to changes in environmental conditions (Agregán et al. 2022, Meegoda et al. 2018).

1.3. Biogas potential and BMP of fen plant biomass

The biogas potential and the biochemical methane potential (BMP) are key parameters to assess the suitability of substrates for anaerobic digestion. While the biogas potential is defined as the total volume of biogas, which is produced per kilogram added volatile solids (VS), the BMP is calculated by multiplying the biogas potential by the methane content in the biogas (VDI 2016). Both the biogas potential and the BMP are determined in standardized batch-tests, which are described in detail in the material and methods section (Holliger et al. 2016).

To date, only a few studies exist that determined the biogas potential or the BMP of fen plant species (supplementary table S1). The data availability is particularly poor for *Typha* spp., *P. australis* and *Carex* spp. (Fig. 4). In addition, mainly older plant material was examined for these plant species. Since the BMP of plants decrease with increasing plant maturity due to a progressing lignin incrustation, only relatively low BMPs were measured (Triolo et al. 2012, Kandel et al. 2013, Roj-Rojewski et al. 2019). The values ranged between 151 and 252 L_N kg^{-1} VS for *Typha* spp., 102 and 253 L_N kg^{-1} VS for *P. australis* and 121 and 275 L_N kg^{-1} VS for *Carex* spp. (Fig. 4). In contrast, the BMP of *P. arundinacea* reached values of up to 426 L_N kg^{-1} VS,

since young plant material, which was harvested in spring, was also included in the analysis. Kandel et al. 2013 divided the aboveground plant parts of *P. arundinacea* into leaves and stems and determined their BMP separately. The authors found that the BMP of leaves and stems was very similar. Only a small difference was found, when *P. arundinacea* was harvested in September.

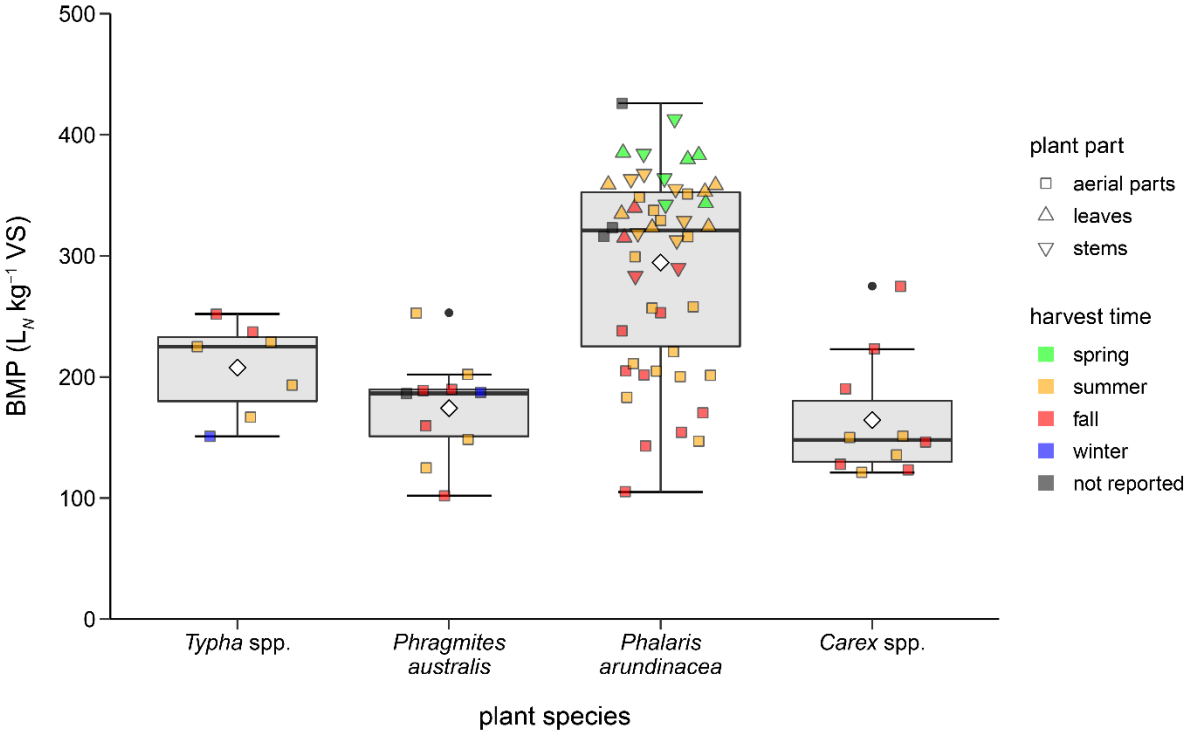

Figure 4: Values from the literature for the BMP of *P. arundinacea*, *P. australis*, *Typha* spp. and *Carex* spp. measured in batch-tests under mesophilic conditions (35 - 39 °C). Harvests of old plant material, which were conducted in March or April, were categorized as winter harvest. Diamonds depict the mean value. The references for the individual values are listed in the supplementary table S1.

1.4. Finding the optimal harvest date for the use as biogas substrate

Besides the biogas potential and the BMP, the biogas yield per hectare is a crucial parameter to determine the optimal harvest date of plants for the use as biogas substrate (Braun et al. 2008, Frigon and Guiot 2010). It is calculated by multiplying the biogas potential by the biomass yield per hectare. As mentioned above, the biogas potential decreases with increasing harvest date. In contrast, the biomass yield per hectare of fen plants is often highest in late summer or fall and only low biomass yield are obtained in spring (Fig. 5). *P. australis* tends to have the highest biomass yields, followed by *Typha* spp., *P. arundinacea* and *Carex*

spp.. Natural stands of *Typha* spp. and *P. australis* usually have wider ranges in their biomass yields than cultivated stands. However, the mean values are often comparable. For *P. arundinacea* and *Carex* spp., it is not feasible to compare the biomass yields of natural stand with those of cultivated stands due to the low number of literature values. The optimal harvest date for the use of plant biomass as biogas substrate is a trade-off between high biogas potentials, and thus a good anaerobic digestibility, and high biomass yields.

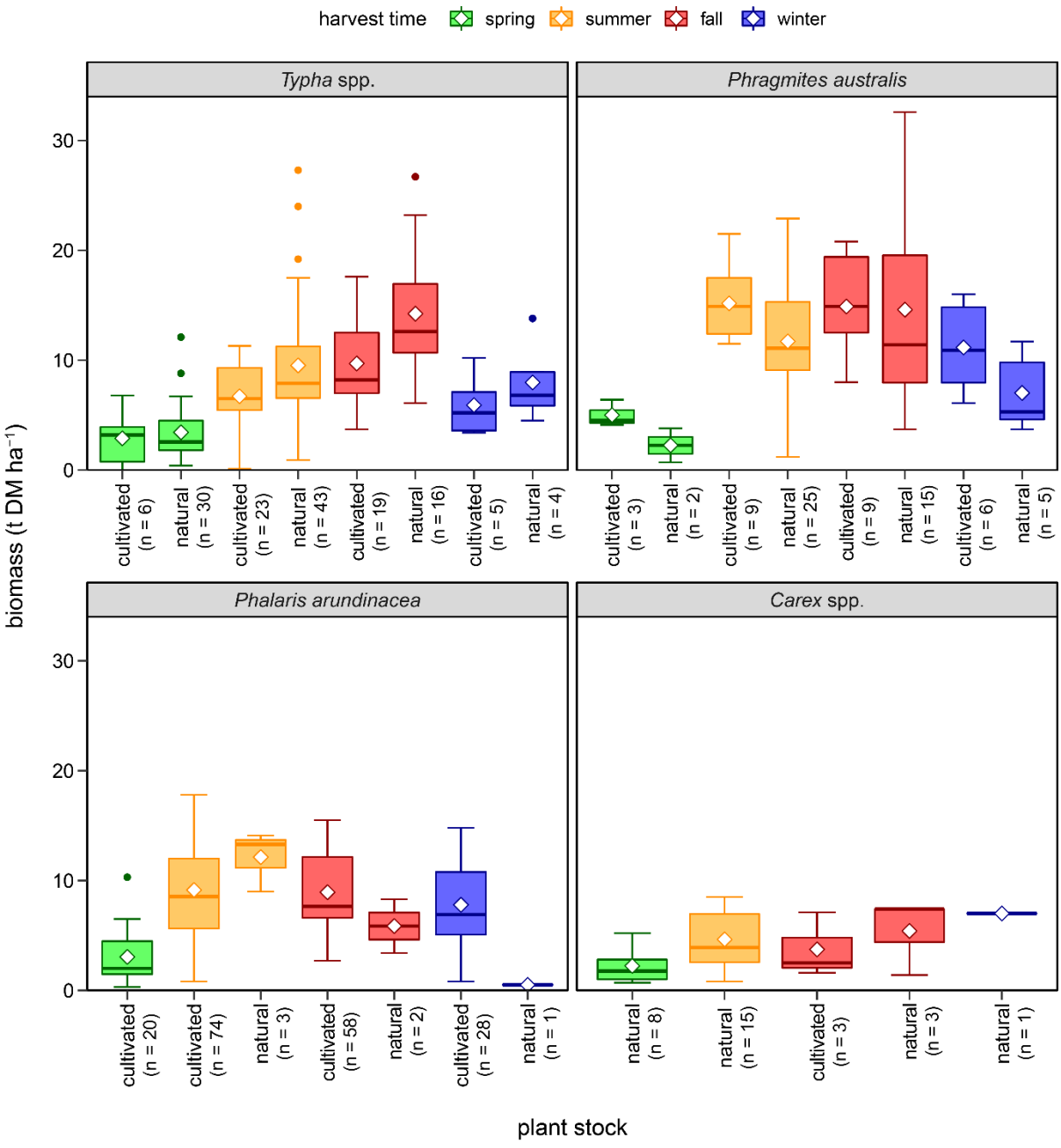

Figure 5: Literature values for biomass yields of cultivated and natural stands of *P. arundinacea*, *P. australis*, *Typha* spp. and *Carex* spp. Harvests of old plant material, which were conducted in March or April, were categorized as winter harvest. Diamonds depict the mean value. The references for the individual values are listed in the supplementary table S2.

1.5. Long-term effects of substrates on the anaerobic digestion process

The biogas potential and the BMP provide no information regarding the long-term biogas yields that can be achieved in biogas plants and the effects of the biogas substrate on the process stability (VDI 2016). In the long term, anaerobic digestion of substrates can cause by-products to accumulate in the fermenter if their digestion is slow. These by-products can destabilize the process by inhibiting the microorganisms, which are involved in the anaerobic digestion (Mizuki et al. 1990). However, it is more common for not degraded fibers to wrap around the stirrer, and thus hindering stirring, or for the digester contents to thicken due to the accumulation of mucilage (Wall et al. 2015)

1.6. Regression models for the prediction of anaerobic digestibility

To some extent, the anaerobic digestibility of plant materials can be predicted based on its chemical composition. This has the advantage that the analysis of the chemical composition via a Weender-Van Soest analysis is cheaper and much faster than the determination of the biogas potential in batch-tests. Several studies have developed regression models to predict the biogas potential and the BMP for traditional biogas substrates such as maize or grass (table 1). Models predicting the biogas potential or BMP of lignocellulosic plant materials usually include acid detergent lignin (ADL) as a key parameter. There are even models with lignin as the only regressor (Dandikas et al. 2014, Thomsen et al. 2014, Triolo et al. 2011). The biogas potential or the BMP always decreases with increasing ADL content, since lignin is hardly degradable under anaerobic conditions (Monlau et al. 2013). However, Dandikas et al. (2015) showed that models developed for lignocellulosic substrates cannot be automatically applied to new plant materials because the nature of lignin incrustation depends on several factors such as the cross-linking of lignin to other matrix components (Monlau et al. 2013).

Table 1*:* Published regression models for predicting the biogas potential and BMP of lignocellulosic biomass via fodder analysis parameters. The biogas potential and BMP are expressed in L_N kg^{-1} VS and the chemical components are expressed in % VS (Triolo et al. 2011, Dandikas et al. 2014, Dandikas et al. 2015) or % DM (Thomsen et al. 2014).

Regression model	Substrates used for model calibration	Reference
Linear regression (LR)		
BMP = 460.6 − 25.8 ADL	energy crops (n = 10)	Triolo et al. 2011
biogas potential = 775 − 39.3 ADL	energy crops (n = 31)	Dandikas et al. 2014
BMP = 395 − 20.0 ADL		
BMP = 347 − 7.85 ADL	lignocellulosic biomass (n = 64)	Thomsen et al. 2014
Multiple linear regression (MLR)		
BMP = 447.1 − 0.7 CL − 27.7 ADL	energy crops (n = 10)	Triolo et al. 2011
biogas potential = 727 + 2.5 HC − 39.3 ADL	energy crops (n = 31)	Dandikas et al. 2014
BMP = 371 + 1.3 HC − 20.0 ADL		
biogas potential = 670 + 4.4 XP + 1.6 HC − 30.2 ADL	grasses and legumes (n = 61)	Dandikas et al. 2015
BMP = 370 + 2.1 XP + 0.5 HC − 16.1 ADL		

1.7. Aims

The main aims of this thesis are

(1) to identify species within four fen plant species, which might be suitable as biogas substrate (publication I),

(2) to investigate how the chemical composition and consequently the biogas potential of fen plants changes with increasing plant maturity (publication I and II),

(3) to evaluate if models developed for the prediction of the biogas potential and the BMP of typical energy crops are also suitable for fen plant species (publication II),

(4) to identify the optimal harvest date of two selected fen plant species in respect to degradability and biogas yield per hectare (publication II), and

(5) to investigate the influence of adding fen plant biomass to maize silage on long-term process stability and biogas yield (publication I).

2. Material and methods

2.1. Substrates and their description

For the identification of suitable paludiculture species for anaerobic digestion (publication I), wild-grown *T. latifolia*, *P. australis*, *P. arundinacea*, and *C. acutiformis* were collected from the fen peatland *Freisinger Moos* in mid-June, mid-August, and mid-October 2016 (Table 2). The fen peatland is part of the *Munich gravel plain* and located 30 km northeast of Munich in Southern Germany (48°22´N, 11°41´E; 445 m above sea level).

To investigate the effect of fen plant biomass addition on the SBY of maize silage (publication I), *P. arundinacea*, which was grown on a rewetted site of the fen peatland *Freisinger Moos*, was cut at the beginning of June 2017. In addition, *T. latifolia* was harvested in a water retention basin located in the fen peatland *Bayerisches Donaumoos* (48°40´N, 11°08´E; 415 m above sea level) in the middle of July 2017. Beside the pure *P. arundinacea* and *T. latifolia* plant material, maize silage and mixtures of maize silage with 10%, 20%, 30%, or 40% *T. latifolia* or *P. arundinacea* were also tested in this experiment. The proportions were based on VS.

In order to determine long-term effects of *T. latifolia* and *P. arundinacea* addition on anaerobic digestion (publication I), mixtures with 20% or 40% *T. latifolia* or *P. arundinacea* were selected for the semi-continuous fermentation experiment. Maize silage and a mixture of maize silage with 20% grass silage, consisting of *Lolium perenne*, were used for comparison.

To identify the optimal harvest time of *T. latifolia*, *T. angustifolia* and *P. arundinacea* for the use as biogas substrate (publication II), these three plant species were cultivated in a paludicultural field trial located in the fen peatland *Freisinger Moos*. In 2018, *T. latifolia* and *P. arundinacea* were harvested on five different dates (5 May, 29 May, 19 June, 19 July, and 12 September) and in 2020, *T. latifolia* and *T. angustifolia* were also cut on five different times (12 May, 3 June, 25 June, 21 July, and 15 September). More details concerning the harvest can be found in publication II.

Table 2: Investigated fen plant materials (proportions based on VS).

Experiment	substrates	harvest dates of the paludicultural plant material
first batch-test	*T. latifolia*	13.06.2016, 15.08.2016, 18.10.2016
(publication I)	*P. australis*	13.06.2016, 15.08.2016, 18.10.2016
	P. arundinacea	13.06.2016, 15.08.2016, 18.10.2016
	C. acutiformis	13.06.2016, 15.08.2016, 18.10.2016
second batch-test	10 % *T. latifolia* + 90 % maize silage	12.07.2017
(publication I)	20 % *T. latifolia* + 80 % maize silage	12.07.2017
	30 % *T. latifolia* + 70 % maize silage	12.07.2017
	40 % *T. latifolia* + 60 % maize silage	12.07.2017
	100 % *T. latifolia*	12.07.2017
	10 % *P. arundinacea* + 90 % maize silage	09.06.2017
	20 % *P. arundinacea* + 80 % maize silage	09.06.2017
	30 % *P. arundinacea* + 70 % maize silage	09.06.2017
	40 % *P. arundinacea* + 60 % maize silage	09.06.2017
	100 % *P. arundinacea*	09.06.2017
semi-continuous fermentation	20 % *T. latifolia* + 80 % maize silage	12.07.2017
(publication I)	40 % *T. latifolia* + 60 % maize silage	12.07.2017
	20 % *P. arundinacea* + 80 % maize silage	09.06.2017
	40 % *P. arundinacea* + 60 % maize silage	09.06.2017
	20 % grass silage + 80 % maize silage	
	100 % maize silage	
third batch-test	*T. latifolia*	09.05.2018, 29.05.2018, 19.06.2018, 19.07.2018, 12.09.2018,
(publication II)		12.05.2020, 03.06.2020, 25.06.2020, 21.07.2020, 15.09.2020
	T. angustifolia	12.05.2020, 03.06.2020, 25.06.2020, 21.07.2020, 15.09.2020
	P. arundinacea	09.05.2018, 29.05.2018, 19.06.2018, 19.07.2018, 12.09.2018

All paludiculture plant material was dried at 60 °C, chopped, and ground with a cutting mill (sieve size < 10 mm, SM 300, Retsch, Haan, Germany). Maize and grass silage was stored at - 18 °C. The chemical composition of the substrates was analyzed via a Weender-Van Soest fodder analysis according to the procedure described in the VDLUFA (Association of German Agricultural Analytic and Research Institutes) book of methods (VDLUFA 1976). Accordingly, the following parameters were measured: total solids (TS), crude ash (XA), nitrogen (N), crude fat (XL), crude fiber (XF), neutral detergent fiber (NDF), acid detergent fiber (ADF), acid detergent lignin (ADL), starch (ST), and reducing sugars (RS). Volatile solids (VS), crude protein (CP), cellulose (CL), hemicellulose (HC) and organic residue (OR) were calculated: VS = 100 − XA; CP = 6.25 * N; CL = ADF − ADL; HC = NDF − ADF; OR = 100 − XA − XP − XL − ST − RS − NDF. ADL is referred to as lignin.

2.2. Batch-test

The batch-tests (publication I and II) were conducted according to VDI 4630 (VDI 2016), VDLUFA (VDLUFA 2011) and Holliger et al. (2016) at the Institute for Agricultural Engineering and Animal Husbandry (ILT) at the Bavarian State Research Center for Agriculture (LfL). Fermenter content from a pilot biogas plant (working volume = 2.5 m^3), which was operated under steady-state conditions, was taken as inoculum. The biogas plant was fed with a mixture consisting of 80% cattle slurry and 20% dairy cattle feed, which was mostly maize and grass silage, and run at an organic loading rate of 3 kg VS m^{-3} d^{-1} (batch-test of publication I) and 2.5 kg VS m^{-3} d^{-1} (batch-test of publication II), respectively, under mesophilic conditions (T = 38 °C).

Before the start of the experiment, the inoculum was stored without feeding at 38 °C for one week to reduce its biogas potential and sieved through a 10 mm sieve to enhance its homogeneity. Thereafter, 1000 g of the inoculum and 20 g of the samples, which are listed in Table 2, were filled in each case in 2 L glass fermenters. Furthermore, 400 - 500 ml distilled water was added since the dry matter content in the fermenter must not exceed 10% to ensure sufficient mass transfer. In order to prevent biogas losses, the fermenters were immediately closed with rubber stoppers after filling. Microcrystalline cellulose (MCC) was used as positive control. At a conversion rate of 100% and taking the new formation of

biomass into consideration, a biogas volume of 745 L_N kg^{-1} VS (at 50% methane content) would be produced from MCC. If the value deviated by less than 10%, it can be assumed that the inoculum has a sufficient biological activity. In addition, a dried whole crop maize with known biogas potential was used as a reference substrate. Furthermore, an unfed control was included as blank to determine the biogas production of the inoculum alone.

The biogas potential was determined six times for MCC and in triplicate for the reference substrate, blank and the other samples tested. For this purpose, the fermenters were incubated in incubation chambers at 38 ± 1 °C (Fig. 6) and swiveled twice a week to promote outgassing of the biogas formed on the one hand and to prevent the formation of dry, inactive floating layers on the other hand. Each fermenter was connected via a tube to a tipping counter (MilliGascounter, Ritter Apparatebau GmbH & Co. KG, Bochum, Germany; accuracy ±3% of each reading). The produced biogas entered the housing of the tipping counter from below through a microcapillary and rose upwards in the form of small gas bubbles into a two-chamber measuring cell. As soon as a volume of approximately 1 ml was collected, the measuring cell tilted by buoyancy into a position in which the filling of the second measuring chamber began and the first was emptied at the same time. Each gas counter was regularly calibrated to determine the exact volume of a tilt. The number of tilts was registered by a laser barrier and the data was stored on an hourly basis. Room temperature and air pressure were also recorded every hour for standardization of biogas yields to normal conditions (T = 273.15 K, p = 1013.25 mbar). After the biogas passed the tipping counter, the gas from the three fermenters per sample was collected in a gas bag. As soon as the gas bag contained 1.5 L, the methane and carbon concentrations of the biogas were analyzed with an infrared sensor and the oxygen concentration was measured with an electrochemical sensor (AWITE Bioenergie GmbH, Langenbach, Germany). The sensor specifications are listed in Table 2 of publication I. The batch-test was terminated for all fermenters at the same time as soon as the daily produced biogas volume was below 0.5% of the total biogas volume produced up to that time. This was the case after 36 - 43 days. After standardization of the recorded gas volume to 273.15 K and 1013.25 mbar and elimination of the water vapor content, the biogas potential and BMP were calculated as standard liter per kilogram volatile solids (VS).

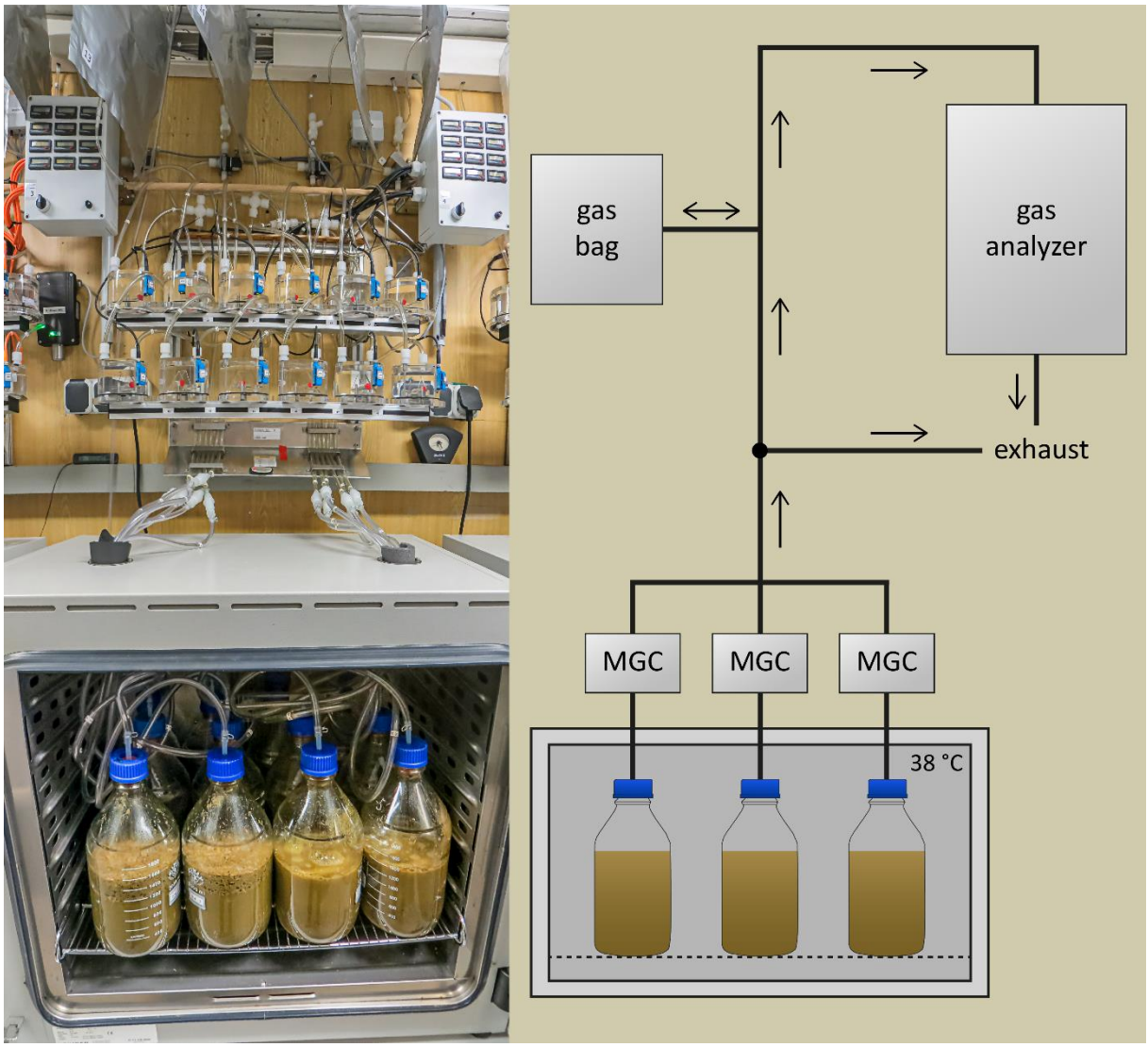

Figure 6: Experimental setup of the batch-tests. MGC: MilliGascounter.

2.3. Semi-continuous fermentation

The semi-continuous fermentation experiment was also performed at the Institute for Agricultural Engineering and Animal Husbandry following VDI 4630 (VDI 2016). Digestate was taken as inoculum from a biogas plant fed with 69% maize silage, 28% grass silage and 3% corn-cob mix and operated at an organic loading rate of 4.6 kg VS m^{-3} d^{-1}. The inoculum was filled into a horizontal fermenter, which had a volume of 243 L (working volume: 190 L), and diluted down with tap water from a TS content of 12.0% to a TS content of 8.5% since the stirrer could not cope with high TS contents. The fermenter was run under mesophilic conditions (T = 38 °C), stirred continuously and not fed for the first 18 days. From day 19, the digester was fed daily with maize silage and operated at an organic loading rate of 1 kg VS m-

^{3} d^{-1}. During Christmas time (day 36 - 53) feeding was carried out only every 3rd to 6th day due to staff shortage. From day 58, the feeding regime was changed to 70% maize silage, 10% grass silage, 10% *T. latifolia*, and 10% *P. arundinacea* (based on VS) to adapt the microbial community to the digestion of these substrates. The organic loading rate was increased to 1.5 kg VS m^{-3} d^{-1} on day 64 and to 2.0 kg VS m^{-3} d^{-1} on day 97.

Directly after the adaptation phase the semi-continuous fermentation experiment was started. Before the experiment, all trial fermenters were checked for tightness. During the tightness test, a pressure of approximately 20 mbar was set. The fermenters were considered technically tight if the pressure drop was less than 0.02 mbar min^{-1}. At the beginning of the experiment, the inoculum was divided evenly among six vertical semi-continuous flow-through fermenters, which had a volume of 36 L (working volume: 28 - 32 L). The fermenters were operated at 38 °C ± 1 °C and continuously stirred.

During the first 11 days of the experiment, the feeding was not changed. Thereafter, the following feeding regimes were applied: 20% *T. latifolia* + 80% maize silage, 40% *T. latifolia* + 60% maize silage, 20% *P. arundinacea* + 80% maize silage, 40% *P. arundinacea* + 60% maize silage, 20% grass silage + 80% maize silage, and 100% maize silage (Table 2; proportions based on VS). The last two feeding regimes were used as a reference, since grass and maize silage are commonly used biogas substrates in Germany and they are currently cultivated on drained peatlands. On day 44 of the experiment, the grass silage was dried at 60 °C and then ground < 10 mm to prevent long fibers from wrapping around the stirrer. One day before feeding, *T. latifolia*, *P. arundinacea* and dried grass silage were moistened with distilled water to adjust the water content comparable to that of maize silage. This should prevent effects that can arise due to different substrate moisture contents. During daily feeding, 1 L of digestate was taken through a withdrawal nozzle attached to the bottom of the fermenter. Each fermenter was connected via a tube to a pressure compensation bag to compensate for the negative pressure, which built up throughout this process. The removed digestate was then mixed with the substrate and fed to the fermenter through a feed auger. 2 L of digestate were then removed and returned to the fermenter to mix the fermenter content vertically. Once or twice a week the level of the fermenters was measured and adjusted to a volume of 28 L. The organic loading rate was gradually increased in steps of 0.5 starting from 2.0 kg VS m^{-3} d^{-1} if the methane production varied by less than 5% for at least five consecutive days. This was the

case on day 33, 68, 117 and 148 of the experiment (see also Fig. 5 of publication I). Before each increase of the organic loading rate and during critical phases in the anaerobic degradation process, digestate samples were taken. The following parameters were determined according to the German standard analysis methods for the analysis of water, wastewater and sludge (DEV 2015): TS, VS, pH, volatile organic acids per total inorganic carbonate buffer (VOA/TIC), organic volatile fatty acids (VFA), and ammonium.

Each fermenter was connected via a tube to a tipping counter (MilliGascounter, Ritter Apparatebau GmbH & Co. KG, Bochum, Germany; Fig. 7) with which the biogas production was measured. The measuring principle of the tipping counters is described in the material and methods part of the batch-test. All tipping counters were calibrated regularly. Room temperature and air pressure were recorded for gas volume standardization on an hourly basis. After the biogas passed the tipping counter, it was collected in a gas bag. As soon as the gas bag contained 4 L of biogas, the gas composition was analyzed. CH_4 and CO_2 were measured with an infrared sensor and O_2, hydrogen (H_2) and hydrogen sulfide (H_2S) were analyzed electrochemically (AWITE Bioenergie GmbH, Langenbach, Germany; see Table 2 of publication I for sensor specifications). Biogas and methane yields of the recorded gas volumes were calculated after standardization at 273.15 K and 1013.5 mbar as standard L per added kg VS. During the experiment, the viscosity in some of the fermenters increased significantly. Each time this happened, the contents of all fermenters were diluted by taking 5 L of digestate and adding 7 L of distilled water per fermenter. Dilution was done on day 106 and 138 of the experiment. Gas yield data collected after the first dilution on day 106 were excluded from data analysis.

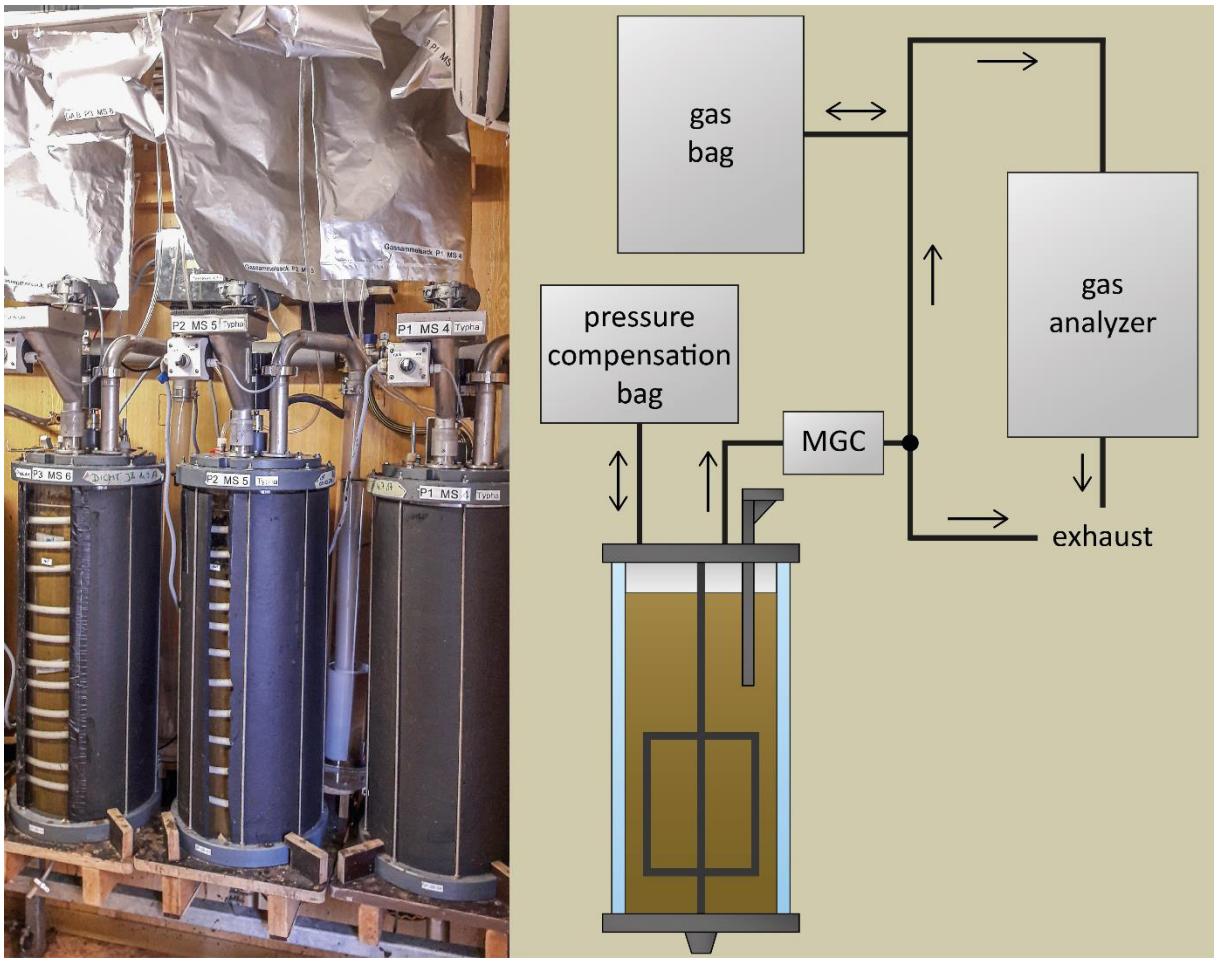

Figure 7: Experimental setup of the semi-continuous fermentation experiment. MGC: MilliGascounter.

2.4. Statistical analysis

All statistical analyses were conducted using R programming language version 3.4.3 (R Core Team 2017, publication I) and version 4.0.2 (R Core Team 2020, publication II). The standard deviation of the calculated variables was calculated following the rules of the Gaussian error propagation. One-way analysis of variance was performed to test for significant differences between the biogas potentials, biomass yields or biogas yields of different harvest dates within one species (publication I and II). This test was also used to compare the biogas potentials between different organic loading rates within one feeding regime in the semi-continuous fermentation experiment (publication I). In case of significant differences, Tukey's test was performed. The assumption of the normality of residuals was tested using the Shapiro-Wilk normality test. Furthermore, the Fligner-Killeen test was used to check for homogeneity of variances in residuals. Biogas potentials of maize silage in mixture with *T.*

latifolia or *P. arundinacea* were compared to the biogas potential of pure maize silage using Dunnett's test (publication I). Linear regressions were performed for *T. latifolia*, *T. angustifolia* and *P. arundinacea* to identify correlations between the biogas potential and the chemical composition of these plants (publication II). They were also used to evaluate and compare the performance of different models for the prediction of the biogas potential (publication II).

3. Publications

3.1. Publication I

Hartung, C., Andrade, D., Dandikas, V., Eickenscheidt, T., Drösler, M., Zollfrank, C., & Heuwinkel, H. (2020). Suitability of paludiculture biomass as biogas substrate − biogas yield and long-term effects on anaerobic digestion. *Renewable Energy*, *159*, 64-71. https://doi.org/10.1016/j.renene.2020.05.156

Background: Drainage-based agriculture on peatlands results in peat mineralization, which in turn leads to high emissions of greenhouse gases. Rewetting will at least diminish this effect and re-establish peatland ecosystem services. However, rewetting is often accompanied by loss of arable land. Paludiculture (i.e. productive use of wet and rewetted peatlands) is an alternative to combine rewetting with biomass production. Biomass from paludicultures might be used as substrate for anaerobic digestion in biogas plants.

Aims: The aims of this study were (1) to identify fen plant species, which might be suitable as biogas substrate, (2) to evaluate the biogas potential of pure maize silage compared to mixtures of maize silage with various proportions of fen plant biomass, (3) to investigate the influence of adding fen plant biomass to maize silage on long-term process stability and biogas yield.

Material and methods: Wild-grown *T. latifolia*, *P. australis*, *P. arundinacea*, and *C. acutiformis* were harvested in the middle of June, August, and October 2016. The biogas potential and BMP of these plant materials were determined via batch-tests. In a second batch-test, the anaerobic digestibility of *T. latifolia* and *P. arundinacea*, which were both harvested in June/July 2017, maize silage, and mixtures of maize silage with 10%, 20%, 30%, and 40% *T. latifolia* or *P. arundinacea* was analyzed. The long-term effects on the anaerobic digestion of maize silage in mixture with 20% and 40% *T. latifolia* or *P. arundinacea* was investigated in a semi-continuous fermentation experiment.

Results: The biogas potential was highest for *T. latifolia*, *P. australis* and *P. arundinacea* from the mid-June harvest reaching an average value of 538 $L_N\ kg^{-1}$ VS. Mixtures with equal or more than 10% *T. latifolia* and 40% *P. arundinacea* had a significantly reduced biogas potential compared to maize silage in the batch-test. In the semi-continuous long-term experiment, the poor degradability of *T. latifolia* led to an accumulation of non-degraded material causing mechanical and biological process problems.

Conclusion: *T. latifolia*, *P. australis* and *P. arundinacea* are suitable as biogas substrate, whereby the harvest date had a greater influence on the anaerobic digestibility than the fen plant species. A maximum of 20% fen plant biomass (based on volatile solids) should be used in biogas substrate mixtures.

Contribution: Christina Hartung planned the experiments together with Hauke Heuwinkel, Vasilis Dandikas and Diana Andrade. She conducted the batch-tests and the semi-continuous fermentation experimentation with the help of Vasilis Dandikas, Anke Aschmann, Natascha Siddiqui, Diana Andrade, Ellen Redderberg, Sebastian Hüttl, Johanna Barth and Claudia Bieloch. Furthermore, she carried out the statistical analyses, interpreted the results together with the co-authors and wrote the original draft of the manuscript. Vasilis Dandikas, Diana Andrade, Tim Eickenscheidt, Cordt Zollfrank and Hauke Heuwinkel revised the manuscript.

3.2. Publication II

Hartung, C., Dandikas, V., Eickenscheidt, T., Zollfrank, C., & Heuwinkel, H. (2023). Optimal harvest time for high biogas and biomass yield of *Typha latifolia*, *Typha angustifolia* and *Phalaris arundinacea*. *Biomass and Bioenergy*, *175*, 106847. https://doi.org/10.1016/j.biombioe.2023.106847

Background: In publication I, *Typha* spp. and *P. arundinacea* were identified as promising fen plant species for biogas production. The biogas potential depends on the chemical composition of plants, which changes with advancing plant age. Various studies developed regression models that predict the anaerobic digestibility of traditional lignocellulosic biogas substrates based on its chemical composition. It is unclear if these models are also suitable for fen plant species. Besides the biogas potential, the biogas yield per hectare is essential to determine the optimal harvest time.

Aims: The aims of this study were (1) to identify which chemical components of *T. latifolia*, *T. angustifolia* and *P. arundinacea* determine the biogas potential of these fen plant species, (2) to evaluate if models developed for the prediction of the biogas potential and the BMP of classical energy crops are also suitable for fen plant species and (3) to identify the optimal harvest date of these plants in respect to degradability and biogas yield per hectare.

Material and methods: *T. latifolia*, *T. angustifolia* and *P. arundinacea* were cultivated as monocultures on a rewetted fen peatland. Four plots of *T. latifolia* and *P. arundinacea* were cut on five different dates in 2018 and three plots of *T. angustifolia* and *T. latifolia* were harvested on five different dates in 2020, respectively. At each harvest, the dry matter yield and the developmental stage of the plants were determined and the chemical composition was analyzed. Additionally, the biogas potential and BMP were measured in a batch-test.

Results: The biogas potential of *T. latifolia*, *T. angustifolia* and *P. arundinacea* dropped with increasing plant age and ranged from 315 to 647 L_N kg^{-1} VS, 405 to 596 L_N kg^{-1} VS and 361 to 597 L_N kg^{-1} VS, respectively. It was negatively correlated with the lignin content and could be predicted using published regression models that included the lignin content as main regressor. The optimal harvest date ranged between the development stage full flowering and the time shortly after the seed heads turned brown.

Conclusion: It is concluded that high biomass yields at early development stages are necessary to realize fen plants as suitable substrate for biogas plants. This might be achieved by the selection of high-yielding wildtypes or cultivars.

Contribution: Christina Hartung planned the experiments together with Hauke Heuwinkel. She conducted the field experiments and the sample preparation with the help of Tim Eickenscheidt, Carina Lemke Fabio Mathony, Valentin Schürger, Fehmi Eroglu, Felix Lipp and Moritz Then. The chemical composition of the plant samples was analyzed at the LUFA Nord-West. Vasilis Dandikas, Anke Aschmann and Natascha Siddiqui conducted the batch-tests. Christina Hartung carried out the statistical analyses, interpreted the results together with the co-authors and wrote the original draft of the manuscript. Vasilis Dandikas, Tim Eickenscheidt, Cordt Zollfrank and Hauke Heuwinkel revised the manuscript.

4. General discussion

The major aim of this thesis was to examine the suitability of fen plant biomass as biogas substrate. Of the four fen plant species tested, *Typha latifolia*, *P. australis* and *P. arundinacea* were proven to be potentially suitable as biogas substrates (publication I). Their biogas potentials ranged between 507 and 581 L_N kg^{-1} VS when harvested in early summer and were comparable to those of grass silage (publication I). In contrast, *C. acutiformis* had a relatively low anaerobic digestibility and is therefore only moderately suitable as biogas substrate (publication I). After the first determination of the biogas potentials of the different fen plant species, *Typha* spp. and *P. arundinacea* were selected for all further experiments due to their high biogas potentials and biomass yields and other favorable characteristics like the high tolerance to cutting of *P. arundinacea*. Therefore, the general suitability of these species as biogas substrates will be discussed in more detail below.

4.1. Advantages and disadvantages of *Typha* spp. and *P. arundinacea* regarding their cultivation

Before *Typha* spp. and *P. arundinacea* can be used as biogas substrate, it is necessary to establish plant stands. In the following, it will be discussed how easily these fen plant species can be cultivated.

Both *Typha* spp. and *P. arundinacea* can be established by sowing, which is less expensive and labor-intensive than planting (Carlson et al. 1996, Dubbe et al. 1988, Venendaal et al. 1997). *Typha* spp. has the restriction that the establishment by sowing will only work if the water level is raised to the soil surface within one to seven days (Eickenscheidt et al. 2023, Wichtmann et al. 2016). Prior to sowing, seeds need to be obtained. Seed collection is relatively easy for *Typha* spp., because one single seed head contains about 200 000 seeds (Dubbe et al. 1988). In contrast, individual seeds of *P. arundinacea* fall off as soon as they are fully ripe. This phenomenon is known as seed shattering (Carlson et al. 1996, Lewandowski et al. 2003). Consequently, the harvest date needs to be timed to maximize the yield of mature seeds.

Typha spp. and *P. arundinacea* have different requirements regarding the water level. The latter species grows under a wide range of water levels. It tolerates dry periods as well as

occasionally flooding (Venendaal et al. 1997). *Typha* spp. need higher water levels for proper growth than *P. arundinacea* (Eickenscheidt et al. 2023). They have a well-developed aerenchyma and can tolerate water level of up to 1.5 m above ground (Wichtmann et al. 2016). Fen plant species, which are typically cultivated in paludicultures, have generally a high competiveness towards weeds and form monodominant stands. This competiveness is especially high for *P. arundinacea* (Lewandowski et al. 2003, Venendaal et al. 1997).

A main goal of paludiculture is to reduce GHG emissions released from organic soils. To achieve this, the global warming potential of the cultivated *Typha* spp. and *P. arundinacea* stands needs to be close to zero. Eickenscheidt et al. (2023) showed that both *T. latifolia* and *P. arundinacea* even act as a small sink for CO_2. In this study a global warming potential of - 1.6 and - 0.9 t $CO_{2\text{-eq.}}$ ha^{-1} yr^{-1} was measured for *P. arundinacea* and *Typha* spp., respectively.

4.2. Conservation of *Typha* spp. and *P. arundinacea* biomass

After harvesting, conservation of the fen plant material is necessary in order to feed it continuously in biogas plants. Plant materials can be either stored as hay or silage (Franco et al. 2016). Field drying is not possible for paludicultures, since the soil surface is wet. Alternatively, the biomass might be dried in drying facilities.

For ensiling, the water content of the plant material should not exceed 70%, as too much moisture allows the growth of undesirable bacteria before the lactic acid bacteria have reduced the pH (Bochmann et al. 2013, Coblentz and Atkin 2018). *T. latifolia*, *T. angustifolia* and *P. arundinacea* had water contents of 79 - 85%, 81 - 82%, and 65 - 76%, respectively, in early summer harvests (publication II, data not shown). Consequently, a reduction in water content by wilting – especially when ensiling *Typha* spp. – will be often required. In addition, the content of fermentable sugars also plays an important role for successful ensiling (Bochmann et al. 2013). This content is relatively low for both *Typha* spp. and *P. arundinacea*. In publication II, the sugar contents of *T. latifolia*, *T. angustifolia* and *P. arundinacea* harvested in early summer ranged between 6.3 and 10.9% DM, 2.9 and 4.6% DM, and 4.0 and 8.8% DM, respectively. However, Bélanger et al. (2016), Chen et al. (2019) and Cherney et al. (2006) showed that *P. arundinacea* could be ensiled without the addition of sugar-rich biomass or sugary additives. In all of these studies, *P. arundinacea* was inoculated with lactic acid bacteria.

Chen et al. (2019) also tested the ensiling of *P. arundinacea* without an inoculation prior to ensiling. However, this untreated silage underwent a secondary fermentation by clostridia, characterized by high pH values and low lactic acid and acetic acid contents.

So far, only a few more recent studies investigated the ensilability of *Typha* spp. (Bestman et al. 2019, Musa et al. 2020). *Typha* spp. can be ensiled, but it appears more difficult to successfully produce silage than it is the case with grass or maize silage (Bestman et al. 2019). In addition, additives are necessary to ensure that the pH value drops during ensiling, thus preventing secondary fermentation by clostridia. Additives that resulted in good silage quality in *T. latifolia* were molasses (Bestman et al. 2019) and formic acid (Musa et al. 2020).

4.3. Achievable biogas potentials, biomass yields, and biogas yields per hectare of *Typha* spp. and *P. arundinacea*

The measured biogas potentials of *Typha* spp. und *P. arundinacea* were between 400 and 650 L_N kg^{-1} VS and 450 and 650 L_N kg^{-1} VS (Fig. 8A), respectively, when harvested at the optimal harvest time determined in publication II. They are thus comparable to those of grass silage, which has biogas potentials of about 600 L_N kg^{-1} VS, but lower than those of maize silage (Dandikas et al. 2021, Döhler et al. 2013). For maize silage, typical values for the biogas potential are about 660 L_N kg^{-1} VS.

The biomass yield for both *Typha* spp. and *P. arundinacea* was about 7.2 to 8.4 t DM ha^{-1} on average (Fig. 8B), which is comparable to the biomass yields of low- to medium-yielding permanent grassland (Döhler et al. 2013), and could reach values of up to 12.9 t DM ha^{-1} (*Typha* spp.) and 17.8 t DM ha^{-1} (*P. arundinacea*).

Biogas yields per hectare were on average 3600 Nm^3 ha^{-1} for *Typha* spp. (Fig. 8C) and thus in the same magnitude as the typical yields for low-yielding permanent grassland, which are around 3800 Nm^3 ha^{-1} (Döhler et al. 2013). For *P. arundinacea*, the mean value was 4700 Nm^3 ha^{-1} and therefore comparable to the biogas yields per hectare of permanent grassland with a medium yield level, which reaches yields of about 4800 Nm^3 ha^{-1} (Döhler et al. 2013). However, the values for both *Typha* spp. and *P. arundinacea* were much lower than those for

silage maize, which has biogas yields per hectare ranging from 7600 to 11400 $Nm^3\ ha^{-1}$ (Döhler et al. 2013).

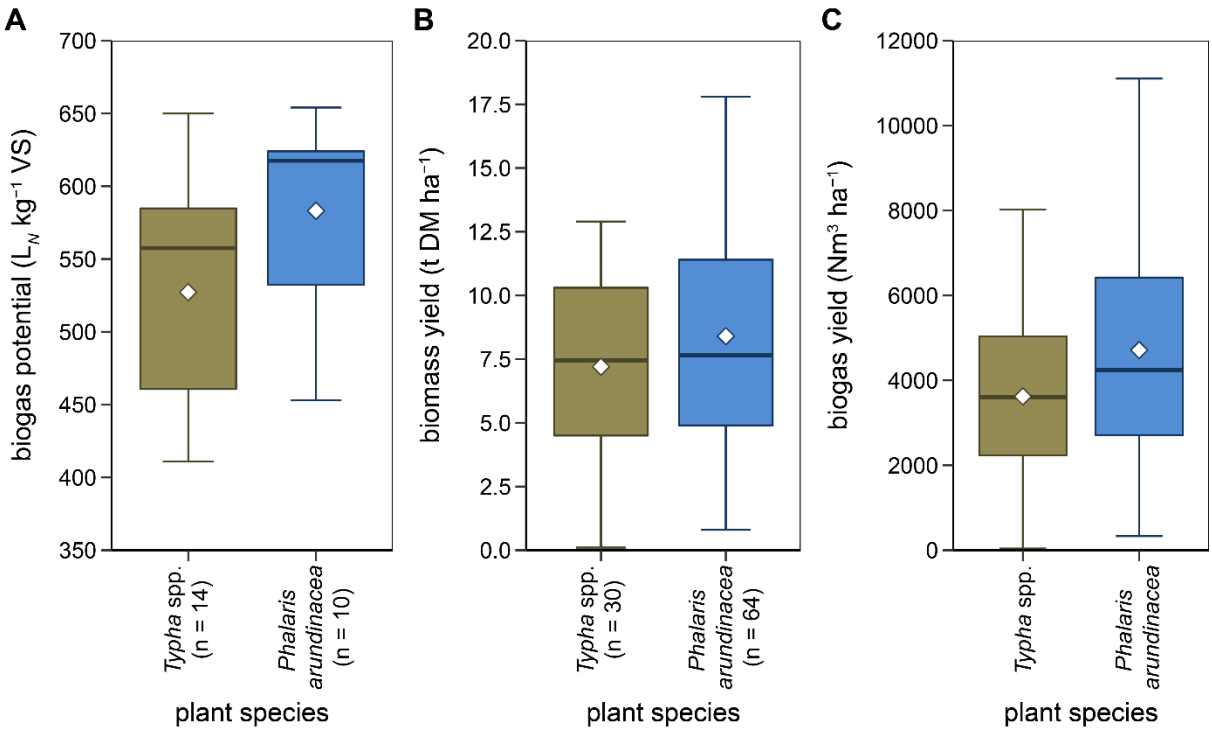

Figure 8: Biogas potentials (A), biomass yields (B), and biogas yields per hectare (C) of *Typha* spp. and *P. arundinacea* harvested in June or July. Values for biogas potentials and biomass yields were taken from publication I and II and from further experiments, which were not included in this thesis (Eickenscheidt et al. 2023). Additional data for biomass yields of cultivated *Typha* spp. and *P. arundinacea* stands was taken from other studies and is listed in the supplementary table S2. For the estimation of possible biogas yields per hectare, every biogas potential value was multiplied with every biomass yield value. Diamonds depict the mean value.

4.4. Tolerance of *Typha* spp. and *P. arundinacea* to multiple cuts per year

The biogas yield per hectare might be enhanced by harvesting biomass of *Typha* spp. and *P. arundinacea* twice or more times per year. However, long-term effects of multiple harvests on the plant stand must also be considered.

P. arundinacea is relatively tolerant to cutting (Casler 2010). In the study of Geber (2002) and Nielsen et al. (2021), the highest biomass yield of *P. arundinacea* was obtained when the plants were harvested twice a year (harvest in June and August/September). Čížková et al. (2015) showed that the harvest frequency can be further increased to three harvests per year, when the plants were adequately fertilized. More than three cuts seems not to be suitable for

P. arundinacea. This was observed in the study by Tilvikienė et al. (2012). Here, *P. arundinacea* was harvested four times per year, with the first cut either at heading or at flowering. In the latter variant, very little biomass grew back after the second cut. In contrast, the biomass yield of the first variant was still relatively high at the third cut and negligible at the fourth cut. Overall, only slightly more biomass was harvested with three cuts per year, if the first cut was conducted at heading and not at flowering (9 t DM ha^{-1} versus 8 t DM ha^{-1}). Harvesting *P. arundinacea* three or four times per year, can also have negative effects on the plant stand. In the study of Geber (2002), less biomass grew up in the following year and also the weed content in the harvested biomass increased. Further research is needed that investigates the long-term effects of different cutting regimes on *P. arundinacea*.

In the study by Kandel et al. (2013), the methane yield per ha of *P. arundinacea* could be increased by about 45% by increasing the cutting frequency from one to two cuts per year (harvest in June and September) when being fertilized after the first cut. The methane yield per ha of the non-fertilized plants harvested twice yearly was lower than the one of the plants harvested only once per year. In further experiments, which were not included in this thesis, the biogas yield per ha could be increased with a second cut in September by approximately 25 - 30% (Eickenscheidt et al. 2023). A fertilization after the first cut led to a slightly higher biomass yield in the following year. However, the effect of the fertilization was not as pronounced as in Kandel et al. (2013). Seppälä et al. (2009), obtained results similar to the ones of Kandel et al. (2013) and Eickenscheidt et al. (2023). In this study, the methane yield per ha could be increased by about 30 - 50% by a second cut (harvest in June and August).

In the case of *T. latifolia*, Pijlman et al. (2019) showed that multiple cuts per year can increase the biomass yield of physiologically young plant material. After the third harvest, the cumulated biomass yield reached a plateau and only a small amount of biomass was harvested at the fourth harvest. However, an increased cutting frequency was also associated with a decrease in shoot density. In the study by Jeke et al. (2019) with *Typha* spp., two harvests per year (harvest in July and September) already had negative effects on the plant stand. In the two subsequent years, the biomass yield was reduced by more than 50% compared to the previous year. This was not a year-dependent effect, as the control plots, which were harvested for the first time in each year, always had similar biomass yields. This is in agreement with the results of further experiments, which were published in Eickenscheidt et

al. (2023). In this study, a second harvest in late summer also had a negative effect on the biomass development of *T. angustifolia* in the following year. In addition, it was shown that the second cutting increased the biogas yield per ha by only about 10%. The low cutting tolerance of *Typha* spp. is probably due to the fact that multiple harvests of physiologically young aboveground plant material reduce the downward translocation of photosynthates from the shoot to rhizome (Sharma and Kushwaha 1990). This leads to a decrease in belowground biomass and thus to a reduced regrowth of shoots.

4.5. Potential of different *P. arundinacea* varieties

In addition to increasing the frequency of cutting, the biogas yield per hectare of *P. arundinacea* might be enhanced by selecting high-yielding varieties and wild-types.

P. arundinacea shows a high genetic variation for various traits (Carlson et al. 1996, Cheng et al. 2013, Gyulai et al. 2003). In the 1920s, *P. arundinacea* breeding programs started in the USA (Carlson et al. 1996). In addition, there were also breeding programs in Canada and northern Europe (Casler 2010). The main objective of these programs was to improve *P. arundinacea* for forage production (Casler 2010). However, breeding focused also on traits that are interesting when cultivating *P. arundinacea* for the use as biogas substrate. These include high biomass yields, reduced lignification or reduced cross-linking between lignin and cell-wall polysaccharides, late maturity, disease resistance and improved seed retention (Butkutė et al. 2014, Carlson et al. 1996, Casler 2010, Gyulai et al. 2003, Klebesadel and Dofing 1991).

Butkutė et al. (2014) compared the biomass yields of different *P. arundinacea* varieties and wild-types. The highest yield was achieved by the variety “Palaton”. However, some wild-types had biomass yields that were almost as high. Besides “Palaton”, other high-yielding *P. arundinacea* varieties are “Vantage” and “Bamse” (Carlson et al. 1996, Sahramaa 2004). Oleszek et al. (2014) measured the biogas potential of the latter one and compared it to the biogas potential of a *P. arundinacea* wild-type. The authors found that the biogas potential of “Bamse” was more than three times higher than the one of the wild-type (406 L_N kg^{-1} VS versus 120 L_N kg^{-1} VS). A possible cause for this might be the lower lignin content and higher hemicellulose and cellulose content of “Bamse”. There are various studies that suggest that

the hemicellulose, cellulose and lignin contents of *P. arundinacea* can be altered by breeding (Carlson et al. 1996, Casler 2010, Gyulai et al. 2003, Marum et al. 1979). Since the biogas potential of *P. arundinacea* is negatively correlated to its lignin and cellulose content (publication II), a reduction in these contents could result in an increased anaerobic digestibility. Further studies are needed that tests this assumption.

In contrast to *P. arundinacea*, no cultivars have been bred for *T. latifolia* and *T. angustifolia*. Various studies showed that the genetic diversity within these two *Typha* species is extremely low (e.g. Keane et al. 1999, Lamote et al. 2005). Consequently, breeding programs with *T. latifolia* and *T. angustifolia* will have only low prospects of success.

4.6. Possible issues that may arise during the anaerobic digestion of *Typha* spp. and *P. arundinacea*

In the semi-continuous fermentation experiment, slowly degradable fen plant material accumulated in the fermenter, resulting in a fast increase in TS of the fermenter contents (publication I). The higher TS contents resulted in a higher viscosity of the fermenter contents, which in turn led to greater stress on the stirrers. In real biogas plants this would cause a higher power consumption. The increase in TS was particularly pronounced in the fermenter fed with a substrate mixture containing 40% *T. latifolia*. *T. latifolia* was physiologically older than the *P. arundinacea* used in this experiment. These results show that for anaerobic digestibility, the harvest date is more important than the fen plant species. In the further course of the experiment, a biological process disturbance also occurred in the fermenter fed with 40% *T. latifolia* starting from an organic loading rate of 3.5 kg VS m^{-3} d^{-1}. This was reflected by a decrease in methane content to values below 50% and a steep increase in VFA and VOA/TIC, indicating inhibition of methanogenesis. At higher organic loading rates and thus shorter retention times, the number of syntrophic bacteria and methanogenic archaea was strongly reduced due to a wash-out of these microorganisms and the microbial degradation capacity was exceeded.

To avoid these problems, the time of harvest should not be too late in the year. The optimal harvest time for *T. latifolia*, which represents a compromise between good anaerobic digestibility and high biomass yield, has proven to be between the end of heading and when

the seed heads begin to turn brown. *P. arundinacea* should be harvested between full flowering and the stage of late lactic to early dough ripeness. In the case of *T. angustifolia*, the time shortly after the seed heads turned brown is considered optimal for harvest (publication II). Furthermore, only a maximum of 20% fen plant material (based on VS) should be used in substrate mixtures (publication I).

The anaerobic degradability can possibly be enhanced by pretreatment techniques, as they are already applied to various lignocellulosic materials. Commonly used are mechanical pretreatment methods. These include the mechanical comminution with different mills, which generates new surface areas (Amin et al. 2017). The first batch-test of publication I, which was conducted with fen plant biomass harvested in the middle of June, August and October 2016, also included samples which were finely milled using a vibratory disc mill (data not shown). The finer grinding had no effect on the biogas potential of *T. latifolia* harvested in June. This could be due to the fact that *T. latifolia* already had a relatively large surface area due to its well-developed aerenchyma. For *P. arundinacea* (June harvest), the biogas potential was increased by only 66 $L_N\,kg^{-1}$ VS. The finer grinding had a greater effect on *T. latifolia* and *P. arundinacea* harvested in August or October. Here, the biogas potential was increased by 94 to 131 $L_N\,kg^{-1}$ VS.

Besides mechanical pretreatment, which belongs to the physical pretreatment technologies, other commonly used pretreatment methods for lignocellulosic materials might also be suitable for fen plant biomass. These include chemical pretreatments (e.g. addition of acids or alkalis), biological pretreatments (e.g. usage of microbes or enzymes), other physical pretreatments (e.g. thermal methods) or combined technologies (e.g. steam explosion or physicochemical methods; Cai et al. 2021, Bochmann et al. 2013). The effect of different pretreatment techniques on the anaerobic digestibility of fen plant biomass should be tested in future research.

5. Conclusion

Biomass of *Typha* spp., *P. arundinacea*, and *P. australis* harvested in early summer has the potential to be used as biogas substrates. The biogas potentials of these fen plant species are similar to that of grass silage. In general, the harvest date, i.e. the plant maturity, is more decisive for anaerobic digestibility than the tested fen plant species. The decrease in digestibility with increasing plant age was mainly related to an increasing lignin content. Ideally, *Typha* spp. and *P. arundinacea* should be harvested between the development stages of full flowering and shortly after the seed heads turned brown. Fen plant material, which is physiologically older and thus less digestible, can accumulate in the fermenter and lead to process disturbances. To avoid this, a maximum of 20% fen plant material (based on VS) should be used in substrate mixtures. The anaerobic digestibility of fen plant biomass might be enhanced by pretreatment techniques, which are commonly applied for lignocellulosic plant materials. *Typha* spp. and *P. arundinacea* have comparable biogas potentials and biogas yields per hectare. However, *P. arundinacea* has several advantages as compared to *Typha* spp. These include the comparatively high tolerance to cutting. In addition, it is highly competitive to weeds and growths under a wide range of water levels. *P. arundinacea* can be ensiled more easily than *Typha* spp. and its high genetic variabilities makes it possible to select for beneficial traits such as high biomass yields or good anaerobic digestibility. For these reasons, *P. arundinacea* is more attractive for the use as a biogas substrate.

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Appendix

Publication I

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Suitability of paludiculture biomass as biogas substrate – biogas yield and long-term effects on anaerobic digestion

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ABSTRACT

Fen plants cultivated on wet peatlands might be an environmentally friendly alternative biogas substrate to maize and grass grown on drained peatlands. This study demonstrates that if *Typha latifolia*, *Phragmites australis*, and *Phalaris arundinacea* were harvested in mid-June, then their specific biogas yields (SBY) reached values of up to 581 L_N kg^{-1} volatile solids (VS), which is similar to the SBY of grass, but lower than the SBY, of 670 L_N kg^{-1} VS, for maize. Mixtures with equal or more than 10% *T. latifolia* or 40% *P. arundinacea* (VS-base) exhibited a reduced SBY compared to 100% maize silage in a batch-test. From the composition of the substrates, it remains unclear why fen plants degraded that poorly. However, during the semi-continuous long-term experiment, this effect led to an accumulation of non-degraded material, which destabilized the degradation process at loading rates above 3 kg VS m^{-3} d^{-1}. Destabilization became apparent with substantial increases in the viscosity of the fermenter content, enrichment of acids and a worsened methane formation. Our findings suggest that only small proportions of maize could be replaced by fen plants as substrate for biogas plants.

1. Introduction

Peatlands cover 3% (4 million km^2) of the global land surface. Despite their small area, about 20% of the organic carbon in terrestrial ecosystems is stored in peatlands [1–3]. Drainage-based cultivation systems cause substantial losses of aerated peat through mineralization, which causes the release of substantial amounts of greenhouse gases (GHG) such as CO_2 and N_2O [4]. In the northern center of Europe, pristine peatland is rare. For example, in Germany, only 1% of peatlands are close to their natural condition. Of the remaining 99%, about 70% are used agriculturally (20% as arable land and 50% as grassland) [5]. In 2010, ~6% of the total agricultural land was peatlands, which accounted for 40% of the German agricultural GHG emissions, including the Intergovernmental Panel on Climate Change category 5: land use, land-use change, and forestry [6].

Power and heat from biogas plants is one strategy to mitigate GHG emissions [7]. For example, Eickenscheidt [8] and Rösch et al. [9] reported that anaerobic digestion of grass silage could substitute ~19–21 MWh primary energy per hectare, which corresponds to 6.1–6.7 t CO_2 ha^{-1} yr^{-1}. However, grass silage production on drained peatlands can lead to GHG emissions of about 70 t $CO_{2\ eq.}$ ha^{-1} yr^{-1}, which completely exceeds the CO_2 savings of substituted fossil fuels [7,8].

Therefore, the rewetting of drained peatlands and the introduction of paludiculture, which is the agricultural use of wet or rewetted peatlands under conditions in which peat is conserved, is a preferred alternative [3]. For paludiculture to be established, different plants need to be cultivated, and their management and use needs to be studied to identify economically valuable cultures [10]. However, several uses of paludicultural biomass are conceivable. Here, their potential to replace classical agricultural plants like maize and grass as a substrate for biogas plants is in focus.

The suitability of substrates for anaerobic digestion is determined using two parameters: the biochemical methane potential

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Abbreviations

ADF	Acid detergent fiber
ADL	Acid detergent lignin
BMP	Biochemical methane potential
CL	Cellulose
HC	Hemicellulose
NDF	Neutral detergent fiber
OLR	Organic loading rate
RS	Reducing sugars
SBY	Specific biogas yield
ST	Starch
TS	Total solids
VFA	Organic volatile fatty acids
VOA/TIC	Volatile organic acids per total inorganic carbonate buffer
VS	Volatile solids
XA	Crude ash
XF	Crude fiber
XL	Crude fat
XP	Crude protein

(BMP), which is composed of the specific biogas yield (SBY) and methane content, and the methane yield per hectare, which is the multiplication of the BMP by the biomass yield. Due to their high biomass yield of up to 20, 15, 16, and 8 t total solids (TS) ha^{-1} yr^{-1}, respectively, *Typha latifolia* L. (broadleaf cattail), *Phalaris arundinacea* L. (reed canary grass), *Phragmites australis* (Cav.) Trin. ex Steud. (common reed), and *Carex acutiformis* Ehrh. (marsh sedge) are promising species [3,11]. However, to date, little is known about their BMP. Typically, the BMP of a plant material changes with plant age [12,13] because of lignification, which usually increases with plant age and is typically accompanied by increases in yield [14]. Therefore, the maximum biogas yield per hectare is a compromise between BMP and biomass yield. In contrast, Meserszmit et al. [15] reported only minor changes of BMP or SBY for Molinia stands between May and September. Only a minimal number of studies exist about the SBY of pure stands of *T. latifolia*, *P. australis,* and *C. acutiformis*, as stated by Avellán and Gremillion [16]. Furthermore, the reported values are often not comparable with each other due to different sample preparation and the varying methods used to determine the yields of biogas production. However, more studies are available for *P. arundinacea*. For instance, Kandel et al. [17] examined the development of the BMP of *P. arundinacea* harvested at twelve harvest dates from a cultivated peatland in the Nørreå river valley of Denmark. The BMP of the separately harvested leaves and stems dropped with increasing plant maturity from 515 to 384 L_N CH_4 kg^{-1} VS and 412 to 283 L_N CH_4 kg^{-1} VS, respectively, over a growth period of five months. BMP tests of *P. arundinacea* harvested at the plant development stages of flowering, late flowering, and seed ripening were also performed by Roj-Rojewski et al. [18]. Similarly, this study reported decreasing methane yields with increasing plant age, ranging between 154 and 200 L_N CH_4 kg^{-1} VS for the aboveground biomass. However, BMP tests do not allow conclusions to be drawn about long-term biogas yields under practical conditions, and the impact of certain biogas substrates on process stability [19,20]. The long-term anaerobic degradation of a substrate could enrich detrimental side products in the fermenter because their anaerobic degradation is slower or even worse, impossible. These side products can destabilize the process directly because they are harmful to microbes [21], and more frequently, stirring is hampered because fibers coil up around the stirrer or the fermenter content thickens because of the enrichment of mucilage [22].

The aims of this study were (1) to identify suitable paludicultural plant species for anaerobic digestion within four tested species, (2) to determine the proportion of paludicultural biomass in a maize silage mixture that will not significantly reduce the SBY compared to maize mono-fermentation, and (3) to investigate the impact of the addition of paludicultural substrates to maize silage on long-term process stability and biogas yield.

2. Material and methods

2.1. Substrates and their description

Wild-grown *T. latifolia*, *P. australis*, *P. arundinacea*, and *C. acutiformis* were sampled from the fen peatland *Freisinger Moos*, which is part of the *Munich gravel plain* region in southern Germany, in the middle of June, August, and October 2016 (Table 1). The following year, *P. arundinacea*, which was cultivated in a paludicultural field trial located in the *Freisinger Moos*, was harvested at the beginning of June, while wild-grown *T. latifolia* was sampled at a water retention basin in the fen peatland *Bayerisches Donaumoos*, in mid-July 2017. All plants were air-dried, chopped, and ground with a cutting mill with a sieve size of <10 mm (SM 300, Retsch, Haan, Germany). Grass silage, consisting of *Lolium perenne*, and maize silage, were used for comparison. A Weender and van Soest fodder analysis of the substrates was conducted following the procedure described in the Association of German Agricultural Analytic and Research Institute's book of methods [23]. Accordingly, TS, crude ash (XA), nitrogen (N), crude fat (XL), crude fiber (XF), neutral detergent fiber (NDF), acid detergent fiber (ADF), acid detergent lignin (ADL), starch (ST), and reducing sugars (RS) were measured. Volatile solids (VS = TS − (XA * TS/100)), crude protein (CP = 6.25 * N), cellulose (CL = ADF − ADL) and hemicellulose (HC = NDF − ADF) were calculated. For the rest of the manuscript, ADL is referred to as lignin.

2.2. Batch-test

The SBY was determined in triplicate using batch-tests following Holliger et al. [24]. The first batch-test included samples of *T. latifolia*, *P. australis*, *P. arundinacea*, and *C. acutiformis* harvested at three different dates in 2016. The second batch-test contained samples of *T. latifolia*, *P. arundinacea*, which were both harvested in 2017, maize silage, and mixtures of maize silage with 10%, 20%, 30%, and 40% *T. latifolia* or *P. arundinacea*, based on volatile solids (VS). In 2 L glass fermenters, 20 g of the substrate was mixed with 400 mL of distilled water and 1000 g inoculum, respectively. The inoculum was obtained from a biogas plant that was fed with 80% cattle slurry and 20% dairy cattle feed of mostly maize and grass silage and operated at an organic loading rate of 3 kg VS m^{-3} d^{-1} at a temperature of 38 °C. One week before the experiment, the inoculum was starved out. Microcrystalline cellulose and dried whole-crop maize were used as control and reference substrates, respectively, in both tests. In addition, an unfed control provided the baseline value, which was the biogas production from the inoculum alone.

The fermenters were incubated at 38 °C and swung manually twice a week, and the biogas production of each fermenter was measured using a tipping counter (MilliGascounter, Ritter Apparatebau GmbH & Co. KG, Bochum, Germany) with an accuracy of ±3%, and it was recorded at hourly intervals. Room temperature and air pressure were logged to standardize the gas volume. After passing the tipping counter, the biogas of the three fermenters per sample was collected in a gasbag. Gas analysis was conducted for

Table 1
Overview of the paludiculture biomass used in the batch-tests and the semi-continuous fermentation experiment.

Experiments	Plant species	Harvest dates	Location of harvest
First batch-test	*T. latifolia*	June 2016	*Freisinger Moos*
	P. australis	August 2016	
	P. arundinacea	October 2016	
	C. acutiformis		
Second batch-test and semi-continuous fermentation experiment	*P. arundinacea*	June 2017	*Freisinger Moos*
	T. latifolia	July 2017	*Bayerisches Donaumoos*

each 1.5 L of gas that was obtained. The proportions of methane (CH_4) and carbon dioxide (CO_2) were determined using an infrared sensor, and oxygen (O_2) content was measured using an electrochemical sensor (AWITE Bioenergie GmbH, Langenbach, Germany; see Table 2 for sensor specifications). The batch-test was terminated after 35 days. After standardization of the recorded gas volumes to 273.15 K and 1013.25 mbar, specific biogas and methane yields were calculated as standard L kg^{-1} VS. Gas yields of the unfed control were subtracted from gas yields of the samples. More information about the experimental setup can be found in Dandikas [14].

2.3. Semi-continuous fermentation

The inoculum for the long-term feeding study was obtained from a biogas plant, which was fed with 69% maize silage, 28% grass silage, and 3% corn-cob mix and operated at an organic loading rate of 4.6 kg VS m^{-3} d^{-1}. Pre-adaptation of the inoculum took place in a 243 L fermenter with a working volume of 190 L at mesophilic conditions, of 38 °C, for 111 days. The fermenter was fed daily with maize silage at an organic loading rate of 1.0 kg VS m^{-3} d^{-1}. After 57 days, feeding was changed to a mixture of 70% maize silage, 10% grass silage, 10% *T. latifolia*, and 10% *P. arundinacea* based on VS, to adapt the microbial community to the upcoming substrates. During this period, the organic loading rate was raised from 1.0 to 1.5 kg VS m^{-3} d^{-1} at day 64 and from 1.5 to 2.0 kg VS m^{-3} d^{-1} at day 97 of the adaptation phase.

Subsequent experiments were conducted according to VDI 4630 [20]. Directly after the adaptation phase, the inoculum was equally divided between six semi-continuous flow-through bioreactors, with a volume of 36 L and working volume of 28–32 L, which were stirred continuously. Feeding remained unchanged for the first 11 days of the experiment. Then specific feeding regimes were applied, using proportions calculated based on VS: 20% *T. latifolia* + 80% maize silage, 40% *T. latifolia* + 60% maize silage, 20% *P. arundinacea* + 80% maize silage, 40% *P. arundinacea* + 60% maize silage, 20% grass silage +80% maize silage, and 100% maize silage. The latter two feeding regimes were used as a reference, because grass and maize are commonly used biogas substrates in Germany, and they are currently cultivated on drained peatlands. Grass silage was dried and milled by < 10 mm at day 44 of the experiment to prevent any wrapping of long fibers around the stirrer. One day before feeding, *T. latifolia*, *P. arundinacea*, and dried grass silage was mixed with distilled water to adjust the water content to one that corresponds to that of the maize silage to avoid any effects resulting from varying substrate moisture contents. Bioreactors were operated at a temperature of 38 °C and an organic loading rate of 2.0 kg VS m^{-3} d^{-1}, which was gradually increased by steps of 0.5 if the methane production fluctuated by less than 5% for at least five days (see Fig. 5). The filling level of the fermenters was measured one to two times per week and kept at a volume of 28 L. Samples were taken before increasing the organic loading rate and during critical periods of the anaerobic degradation process. Chemical parameters of the digested contents were determined following the German standard analytical methods for the analysis of water, wastewater, and sludges [25]: TS, VS, pH, volatile organic acids per total inorganic carbonate buffer (VOA/TIC), organic volatile fatty acids (VFA), and ammonium. The biogas production of each fermenter was measured continuously using a tipping counter (MilliGascounter, Ritter Apparatebau GmbH & Co. KG, Bochum, Germany). Room temperature and air pressure were logged every hour to standardize the gas volume. The produced biogas was collected in a gasbag. CH_4 and CO_2 were analyzed using an infrared sensor and O_2, hydrogen (H_2), and hydrogen sulfide (H_2S) were measured electrochemically (AWITE Bioenergie GmbH, Langenbach, Germany; see Table 2 for sensor specifications) for each 4 L of gas. During the experiment, the viscosity of the digestate increased significantly in some of the experimental fermenters, and it consequently led to the failure of the stirrers. Anytime this happened, all fermenter contents were diluted by removing 5 L of the digestate and adding 7 L of distilled water per fermenter. This happened on day 106 and 138 of the experiment. The biogas and methane yields of the recorded gas volumes were calculated after standardization, at 273.15 K and 1013.5 mbar, as standard L kg^{-1} VS added. Gas yield data that were collected after the first dilution of all the fermenters on day 106 of the experiment were excluded.

Table 2
Specifications of the sensors used in the batch-tests and semi-continuous fermentation experiment for gas analyses.

Sensor	Measuring principle	Measuring range	Accuracy
CH_4	Infrared	0 - 100 vol.-%	±2%
CO_2	Infrared	0 - 100 vol.-%	±2%
O_2	Electrochemical	0 - 25 vol.-%	±1%
H_2	Electrochemical	0–500 ppm	±2%
H_2S	Electrochemical	0–5000 ppm	±2%

2.4. Statistical analysis

All statistical analyses were performed using R programming language version 3.4.3 [26]. Standard deviations of the calculated variables were calculated from those of the input variables. Testing for differences between the SBYs at different harvest dates within a species was done using a one-factorial analysis of variance [27]. When there were significant differences between means, Tukey's test was performed [28]. The assumption of the normality of residuals was checked using the Shapiro-Wilk normality test [29], and the homogeneity of variances in residuals was tested using the Fligner-Killeen test [30]. The same tests were applied to compare the SBYs between organic loading rates within one feeding regime in the semi-continuous fermentation experiment. SBYs of maize silage and mixtures with maize silage, which were measured in the second batch-test, were compared in pairs using Dunnett's test [31].

3. Results and discussion

3.1. Identification of suitable paludiculture species for anaerobic digestion depending on harvest date

According to the batch-test, the SBY of the paludicultural biomass sampled at sequential harvest dates in 2016 ranged between 311 and 581 L_N kg^{-1} VS and was always significantly lower than the reference maize substrate, which had a SBY of 670 L_N kg^{-1} VS. The determined average methane concentration was 54–60%. These values are surprisingly high because these plants contain carbon almost only as carbohydrates, which results in ~50% methane according to their degradation [32]. The SBY of the paludicultures was highest for *T. latifolia*, followed by *P. australis*, *P. arundinacea*, and *C. acutiformis*. With advancing plant maturity during the year, SBY significantly decreased (Fig. 1), which was accompanied by a decline in easily fermentable substances, such as hemicellulose, and/or an increase in cellulose and lignin (Fig. 2). In principle, these findings confirmed the data of Kandel et al. [17] and Seppälä et al. [33], who point out the relevance of an adequate harvest date. However, Kandel et al. [17] reported methane yields at a much higher level than in this study: their yield in September was comparable to the yield in June in this study. Regarding the chemical composition of the plants, higher cellulose and lignin contents were reported by Kandel et al. [17] for the September samples than for the June samples in this study. Lignin is supposed to be non-degradable under anaerobic conditions, and it may form lignocellulose complexes, which reduce the digestibility of fibers [34,35]. Hence, a lower methane yield would be expected for Kandel's samples as compared to ours. A partial explanation for the different results is a batch fermentation time that is double that in this study. Additionally, a comparison of absolute values for specific methane yields is difficult due to further factors, such as inoculum properties or particle size of the substrate, which influence the biogas and/or methane production [24,36]. Therefore, the use of a well-known substrate facilitates data comparison. Ruf and Emmerling [37] conducted a batch-test using *P. arundinacea* and maize as a benchmark. Biogas yields of maize were comparable to those of the present study. In this study, *P. arundinacea* was cut twice and produced similar SBY, at both harvest dates, to those measured in June in the present study.

In the present study, all species reached the highest SBYs in June, with no significant differences between *T. latifolia*, *P. australis*, and *P. arundinacea*. Therefore, other factors besides SBY were considered to select the two species *P. arundinacea* and *T. latifolia* for further experiments. *P. arundinacea* is highly competitive to weeds, can be cut multiple times per vegetation period, and exhibits high biomass yields by the end of June [38–40]. Furthermore, it tolerates temporary flooding, as well as drought periods, and can also be used as a forage crop [40,41]. In addition, *T. latifolia* and *P. arundinacea* stands, unlike *P. australis*, can be established by sowing, which is by far less expensive and labor-intensive. Seed collection is particularly simple for *T. latifolia* as about 200 000 seeds can be obtained per flowering head [41,42].

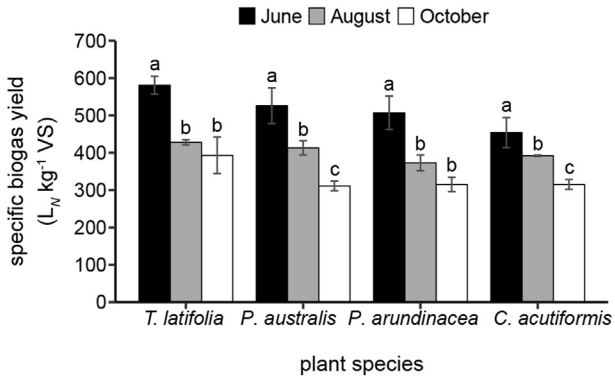

Fig. 1. Specific biogas yields of *T. latifolia*, *P. australis*, *P. arundinacea*, and *C. acutiformis* from three different harvest dates (June, August and October 2016) in the batch-test; mean ± standard deviation (n = 3). Different letters denote significant differences between harvest dates within the same species (Tukey's test, $p < 0.05$).

3.2. Influence of paludicultural biomass addition on the specific biogas yield of maize silage

The *T. latifolia* and *P. arundinacea* biomass for the second batch-test and the semi-continuous fermentation experiments differed from the biomass used in the first batch-test because of different harvest dates and sites. Fodder analysis showed that the values for crude fiber, lignin, cellulose, hemicellulose, and nitrogen of the *T. latifolia* biomass harvested in July 2017 lay between the values of the biomass harvested in June and October 2016 (Table 3, Fig. 2). The composition of *P. arundinacea* harvested in June 2017 was similar to that harvested in June 2016 concerning crude fiber, lignin, and hemicellulose contents, but it had slightly lower cellulose and nitrogen contents. However, the content of reducing sugars was three times higher.

In the second batch-test, the SBY of pure *T. latifolia* was 418 L_N per kg VS (Fig. 3), which is comparable to that of *T. latifolia*, which was harvested in August 2016 (first batch-test, Fig. 1)). Due to the partly different ingredient composition, pure *P. arundinacea* from June 2017 had a distinctly higher SBY (624 L_N kg^{-1} VS) than in June 2016. In line with this, proportions of up to 30% *P. arundinacea* did not significantly reduce the SBY compared to 100% maize silage (684 L_N kg^{-1} VS). However, the SBY was already significantly reduced with 10% *T. latifolia* in the substrate mixture. Therefore, it is expected that a higher proportion of undegraded substrate could affect the process during long-term semi-continuous fermentation.

Mixtures of maize silage, with 20% and 40% paludicultural biomass based on VS, were chosen as the substrate for the semi-continuous fermentation trials to cover a broader measuring range and due to there being only marginal differences in biogas yield, if one compares 10%–20% or 30%–40% of paludiculture plant material in the batch mixture.

3.3. Influence of Typha latifolia and Phalaris arundinacea on the long-term performance of anaerobic degradation

In the semi-continuous fermentation experiment, the SBY of pure maize silage and the mixtures of maize silage with 20% *T. latifolia*, 40% *T. latifolia*, 20% *P. arundinacea*, 40% *P. arundinacea*, or 20% grass silage based on VS at continuous biogas production were 733–785, 673–686, 605–637, 688–741, 652–683, and 682–736 L_N kg^{-1}, respectively (Fig. 4A). The SBY of pure maize silage and mixtures with 20% *P. arundinacea* or grass silage significantly decreased with increasing organic loading, which is reflected by the broader range of SBY values. The other mixtures experienced a considerable reduction of SBY at low organic loading rates, which led to a minor differentiation of the SBY values with increasing organic loading rate. The observed reduction of the SBY as compared to maize at each organic loading rate (Fig. 4B) confirms this observation because the addition of 20% *P. arundinacea* or grass silage resulted in a steady reduction of the SBY by about 7%. With the other mixtures reduced, the reduction was most prominent at low organic loading rates. Comparisons of these results with other studies are difficult because long-term fermentation experiments with paludicultural biomass are still rare in the literature. Riggio et al. [43]

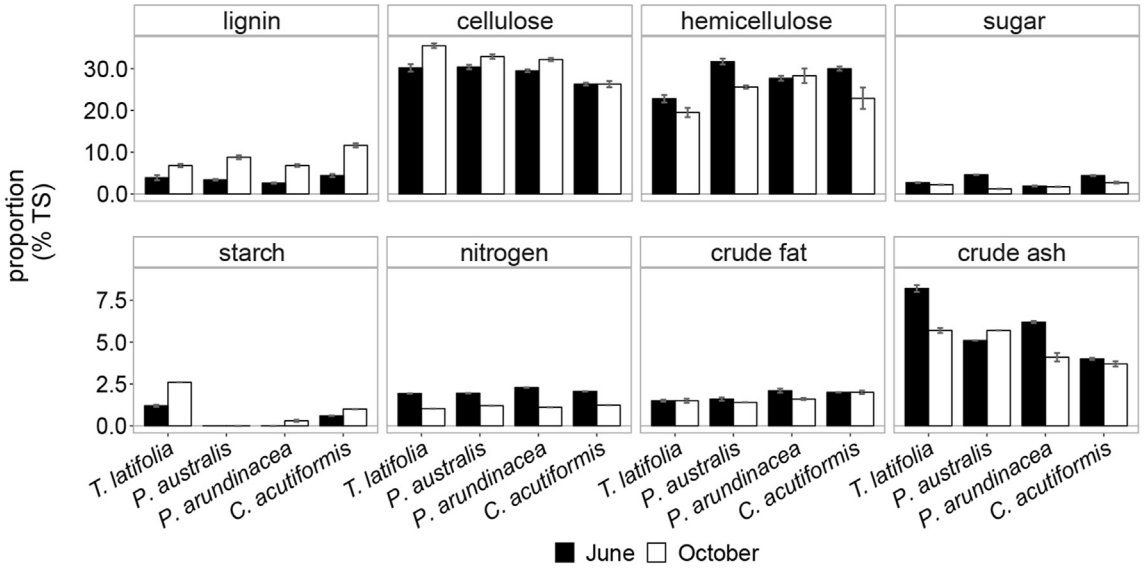

Fig. 2. Composition of the samples of the four species sampled in June and October in 2016 according to the fodder analysis (mean ± standard deviation, n = 3).

Table 3
Composition of the substrates fed in the second batch-test and the semi-continuous fermentation experiment, according to the Weender-van Soest analysis.

Parameter	Unit	*T. latifolia* (harvested in July 2017)	*P. arundinacea* (harvested in June 2017)	Grass silage	Maize silage
Total solids (TS)	% FM	97.4	96.5	41.3 (day 1 – 43) 90.8 (day 44 – 154)	34.1
Volatile solids (VS)	% TS	93.1	95.6	88.3	97.1
Crude protein (XP)	% TS	9.4	13.1	20.1	8.3
Crude fat (XL)	% TS	1.8	2.2	2.2	3.7
Crude fiber (XF)	% TS	29.9	28.3	21.0	14.8
Neutral detergent fiber (NDF)	% TS	60.1	58.0	52.5	36.3
Acid detergent fiber (ADF)	% TS	38.0	30.5	32.0	19.0
Acid detergent lignin (ADL)	% TS	5.4	2.9	5.3	2.3
Cellulose (CL)	% TS	32.6	27.6	26.7	16.7
Hemicellulose (HC)	% TS	22.1	27.5	20.5	17.3
Starch (ST)	% TS	3.6	<0.2	0.3	39.9
Reducing sugars (RS)	% TS	3.7	6.4	1.2	0.8
Crude ash (XA)	% TS	6.9	4.4	11.7	2.9

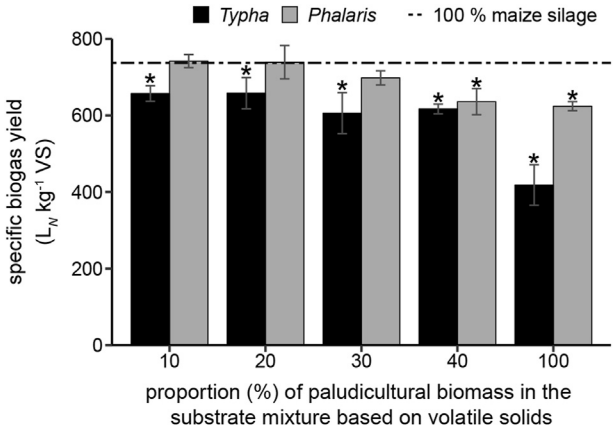

Fig. 3. Specific biogas yield of maize silage in mixture with different proportions of *T. latifolia* or *P. arundinacea* in the batch-test; mean ± standard deviation (n = 3). Asterisks indicate significant differences, at $p < 0.05$, to the specific biogas yield of maize silage, which was tested using Dunnett's test.

performed a fed-batch experiment with a substrate mixture consisting of 50% cattle slurry, 40% cheese whey, and 10% *P. australis*, with proportions based on fresh matter, achieving a methane yield of 241 l CH_4 kg^{-1} VS. However, comparisons are not possible because in their study, among other aspects, the harvest date of *P. australis* was not specified, and no standard feed was tested for comparison.

In the present study, the smaller biogas yields of mixtures with *T. latifolia* in relation to those with grass silage are not in accordance with the chemical composition (Table 3). The lignin content of *T. latifolia* was similar and the contents of anaerobically degradable substances, like hemicellulose, cellulose or reducing sugars, were equal to or even higher than those of grass silage. Linkages between different components of the lignocellulose, the crystallinity and degree of polymerization of cellulose, lignin composition, structural surface area, and structure of hemicelluloses influence the digestibility of lignocellulose complexes [35,44] and may explain the observed difference between *T. latifolia* and grass silage as well as *P. arundinacea*. However, in view of the results of the first batch-test, it is assumed that *T. latifolia* harvested at early dates would also be easier to digest in semi-continuous fermentation trials, since its SBY was the same as for *P. arundinacea*.

The lower degradability of *T. latifolia* and, to a lesser extent, *P. arundinacea* as compared to maize resulted in a faster increase in TS of the flow-through fermenter content. This increase was especially pronounced for the mixture with 40% *T. latifolia* at organic loading rates of 3.0 kg VS m^{-3} d^{-1} or higher (Fig. 5). Higher

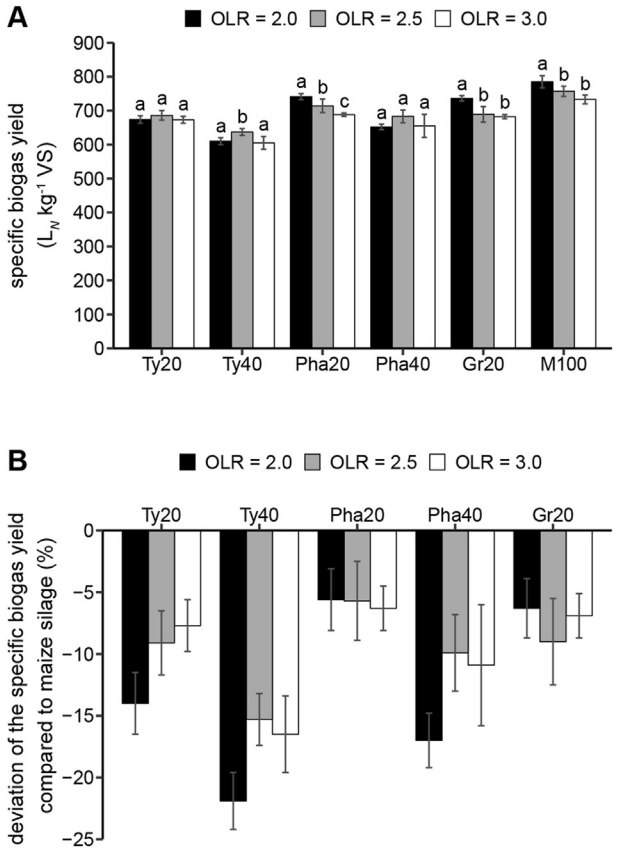

Fig. 4. Specific biogas yield (A) and deviation of the specific biogas yield from the values for maize silage (B) at three organic loading rates (OLR = 2.0, 2.5, and 3.0 kg VS m^{-3} d^{-1}) in semi-continuous fermentation. Fermenters were fed with maize silage (M100) and mixtures of maize silage with 20% *T. latifolia* (Ty20), 40% *T. latifolia* (Ty40), 20% *P. arundinacea* (Pha20), 40% *P. arundinacea* (Pha40), and 20% grass silage (Gr20), respectively, based on VS. The mean value of five days of the experiment at stable biogas production is depicted. Error bars represent the standard deviation during this time, calculated using daily data. Different letters indicate significant differences ($p < 0.05$) between organic loading rates within the same feeding regime using Tukey's test.

total solid contents resulted in a higher viscosity of the fermenter contents. This finally caused stirrer failure at an organic loading rate of 3.0 kg VS m^{-3} d^{-1}. Therefore, for safety reasons and for comparability, all fermenters were diluted at day 106. Until this point in time, the methane concentration of the produced biogas remained unchanged, and only some minor decreases in biogas production were observed. Hence, the degradation process was not inhibited. After dilution of the fermenter contents, TS increased again and very rapidly, and consequently, they had to be diluted a second time on day 138 of the experiment. An increase in the TS of the fermenter content is also reported for grass fermentation [45]. Koch et al. [46] conducted a mono-digestion experiment using grass silage. Here, the TS content in the fermenter reached 16% when 50% dried grass silage mixed with 50% water was used for feeding. Increases in TS content were still observed after adjusting the ratio of dried grass silage to water in the feed to 1:2. Finally, the enrichment of slowly degradable fibers led to a drop in methane concentration and biogas production, indicating a disturbance to the biological process. In the present study, a decline in methane concentration to values less than 50% was also observed for the fermenter fed with 40% *T. latifolia* at an organic loading rate of 4.0 kg VS m^{-3} d^{-1} (data not shown). In addition, this fermenter showed a steep increase in VFA and VOA/TIC starting at an organic loading rate of 3.5 kg VS m^{-3} d^{-1} and reaching values of 4.5 g acetic acid equivalents kg^{-1} FM for VFA and 1.16 for VOA/TIC at the end of the experiment (Fig. 5). According to Döhler et al. [47], the VOA/TIC limiting value for fermenter content should be below 0.6, even though the stability of this ratio over time is more important than the absolute value. Therefore, both criteria were fulfilled for the fermenter fed with 40% *T. latifolia*. The decreasing methane concentrations further suggest inhibition of methanogenesis. Volatile fatty acids accumulated because their further conversion by syntrophic bacteria and methanogenic archaea was reduced [48]. Inhibition of methanogenesis could be caused by an overload of the microbial degradation capacity. Higher organic loading rates result in lower retention times and can consequently lead to wash-out of microorganisms because syntrophic bacteria and methanogens, unlike hydrolytic bacteria, only have low growth rates [48]. Here, the effect was very much increased by the dilution of the fermenter content, which was primarily done for technical reasons: to keep

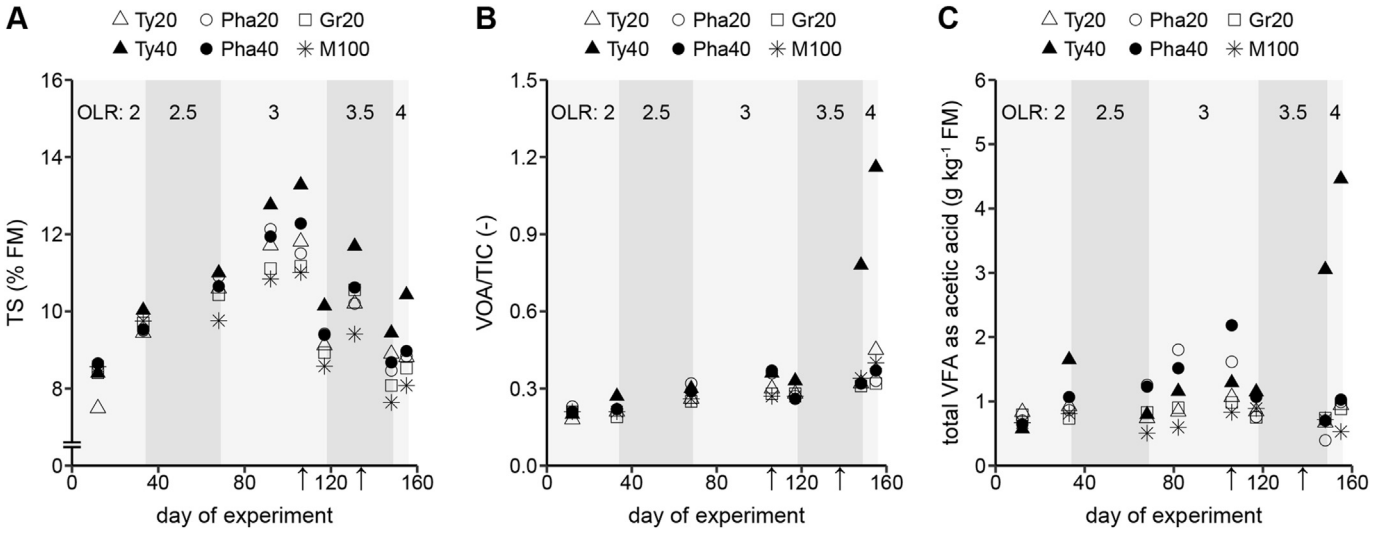

Fig. 5. Total solids (A), volatile organic acids per total inorganic carbonate buffer (B), and total volatile fatty acids (C) of the fermenter contents over time in semi-continuous fermentation. Fermenters were fed with maize silage (M100) or mixtures of maize silage with 20% *T. latifolia* (Ty20), 40% *T. latifolia* (Ty40), 20% *P. arundinacea* (Pha20), 40% *P. arundinacea* (Pha40), and 20% grass silage (Gr20) based on VS, respectively. Periods with the same organic loading rate (OLR = 2.0, 2.5, 3.0, 3.5, and 4.0 kg VS m^{-3} d^{-1}) are separated by gray shading. Arrows at the x-axis mark days 106 and 138 of the experiment when all fermenters were diluted.

the stirrer working in the highly viscose fermenter content. Presumably, the remaining syntrophic bacteria and methanogenic archaea were not present in sufficient numbers to maintain the balance between acid production and consumption.

Further experiments with paludicultural plant material are required to determine whether the instability of the process can be reproduced, especially with fermenter set-ups that can work with very viscose fermenter content. Moreover, studies are needed to evaluate whether the anaerobic degradability of paludicultural biomass could be enhanced by different physical, chemical, or biological (pre)treatment methods, which increase the anaerobic degradability by processes, such as the disintegration of the lignocellulose complex. These techniques are currently applied for grass substrates and include extrusion, acid/alkaline pretreatment, or the addition of enzymes [49–52]. However, the costs and benefits of these techniques should be kept in mind [51].

4. Conclusion

This study shows that paludicultural plants could replace maize or grass as substrates in biogas plants. As with grass, the harvest date of the tested fen plants highly determined the level of SBY. More mature plant biomass was less degraded, which destabilized the degradation process in the long-term test of mixtures with maize. That is why only small proportions (<20%) of these plants are recommended in biogas feed. Future research needs to determine (1) which components might be responsible for the observed effect, (2) and whether (pre)treatment of the substrate or mixtures with other substrates could mitigate these detrimental effects. As long as early harvest dates are preferred, further studies need to test the capability of repeated harvests, which in the long run, may affect the biomass formation of the fen plants as well as the nutrient cycle.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Christina Hartung: Investigation, Conceptualization, Writing - original draft. **Diana Andrade:** Investigation, Conceptualization, Resources, Writing - review & editing. **Vasilis Dandikas:** Investigation, Conceptualization, Resources, Writing - review & editing. **Tim Eickenscheidt:** Project administration, Writing - review & editing. **Matthias Drösler:** Supervision, Funding acquisition. **Cordt Zollfrank:** Writing - review & editing. **Hauke Heuwinkel:** Investigation, Conceptualization, Writing - review & editing.

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Publication II

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Optimal harvest time for high biogas and biomass yield of *Typha latifolia*, *Typha angustifolia* and *Phalaris arundinacea*

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ABSTRACT

Rewetting of peatland is commonly accepted as a useful measure for counteracting climate change. To increase the acceptance, an agricultural use of fen plants is needed. In this study, the optimal harvest date of *Typha latifolia*, *Typha angustifolia* and *Phalaris arundinacea* regarding their biogas potential and biogas yield per hectare was identified. Furthermore, the influence of the chemical composition of *Typha* spp. and *P. arundinacea* on the biogas and biochemical methane potential was determined. Finally, the predictability of the biochemical methane potential (BMP) of *Typha* spp. and *P. arundinacea* by their composition with published regression models was examined. The three fen plant species were harvested on five different dates in 2018 and/or 2020. For each harvest, the biomass yield, biogas potential and BMP were determined, the chemical composition of the biomass was analyzed, and the biogas yield per hectare was calculated. The biogas potential of *T. latifolia*, *T. angustifolia* and *P. arundinacea* decreased with increasing plant maturity and ranged between 315 and 647 L_N kg^{-1} VS, 405 and 596 L_N kg^{-1} VS and 361 and 597 L_N kg^{-1} VS, respectively. The biogas and BMP of all three plant species investigated were negatively correlated with the lignin content and could be predicted with published regression models, which included the lignin content as main regressor. The derived optimal harvest dates, which were a compromise between biomass yield and biogas potential, for all three fen plants ranged between the development stages of full flowering and shortly after the seed heads turned brown.

1. Introduction

In Europe, the traditional use of fen peatlands for agriculture, forestry or peat extraction requires extensive drainage [1]. By lowering the water table, large parts of the peat body are aerated and consequently mineralized. This in turn results in high emissions of greenhouse gases (GHGs), such as CO_2 and sometimes N_2O [2,3]. Negative impacts of drainage include increased vulnerability to wind erosion, formation of hydrophobic topsoil, reduced water storage capacity and, in the long term, a decline in soil fertility and biomass yields [1,4]. Rewetting of drained peatlands can reduce peat mineralization and GHG emissions [5]. In this case, however, conventional agricultural land use will no longer be possible [6]. Paludiculture (*Latin* "palus" = swamp) is an alternative to traditional drainage-based agriculture and combines rewetting with cultivated or naturally grown plants for biomass production [1,7]. Plants cultivated in paludicultures need to cope with high water levels and include typical peatland plants such as *Typha* spp. (cattail), *Phragmites australis* (common reed), *Phalaris arundinacea* (reed canary grass) and *Carex* spp. (sedges) [8]. The produced biomass can be utilized for many purposes, including energy production [9–14]. To this end, biogas production through anaerobic digestion of organic matter is one of the most promising technologies.

In previous studies, *Typha latifolia*, *Typha angustifolia* and *Phalaris arundinacea* were identified as promising species for biogas production [15–18]. Listed plant species showed biogas potentials with values of up to 600 L_N kg^{-1} VS similar to that of grass silage [19] if harvested at an early stage. They could thus replace part of the grass biomass, which is commonly grown for biogas production on drained peatlands, e.g., in Germany. Harvest time is crucial when using plants as biogas substrates. Advancing plant maturity leads to higher proportions of lignin, which is considered to be anaerobically nondegradable [20]. As a consequence,

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Abbreviations

ADF	Acid detergent fiber
ADL	Acid detergent lignin
BMP	Biochemical methane potential
CH_4	Methane
CL	Cellulose
CO_2	Carbon dioxide
CP	Crude protein
DM	Dry matter
GHG	Greenhouse gases
HC	Hemicellulose
LR	Linear regression
MLR	Multiple linear regression
N	Nitrogen
NDF	Neutral detergent fiber
O_2	Oxygen
OR	Organic residue
RS	Reducing sugars
ST	Starch
VS	Volatile solids
XA	Crude ash
XL	Crude fat

the biogas potential drops with increasing plant maturity [17,21,22]. Conversely, less degradable plant material can accumulate and cause technical problems or process disturbances in biogas plants [16]. There are currently only a few studies that investigate the impact of the harvest date on the biogas potential of *Typha* spp. or *P. arundinacea* [16,17,22]. Moreover, the developmental stage of the analyzed plants is often not described in published studies. A simple notification of the harvest date is not sufficient since the development of fen plants depends on various factors, such as weather conditions and water levels, which vary from year to year [23]. Therefore, a classification of the plant developmental stage according to a standard procedure, such as the BBCH scale, is crucial [24]. To find the optimal harvest time, it is essential not only to determine the biogas potential (L per kg volatile solids (VS)) but also to calculate the biogas yield per unit area (L per hectare) [25,26]. This area-specific measure is calculated from the biogas potential and the biomass yield per hectare. The biogas potential of fen plants decreases to a minimal level with increasing plant maturity, whereas the biomass yield per hectare reaches an optimum value [16,17,22,27]. As a consequence, the optimal harvest date is a trade-off between these two parameters. From an economical perspective, the biomass should be harvested when the biogas yield per hectare reaches its maximum.

To a certain extent, the anaerobic digestibility of plant material can be predicted based on its chemical composition. This provides the advantage of the analysis of the chemical composition via feed analysis being less expensive and much faster than the determination of the biogas potential via batch-tests. Various studies have developed regression models to predict biogas potential, mainly for traditional biogas substrates such as maize and grass [19,28–30]. Available models for the prediction of the biogas potential or the biochemical methane potential (BMP) of lignocellulosic plant material usually include acid detergent lignin (ADL) as a key parameter. There are even various models described with lignin as a single regressor [19,30,31]. They have in common that the biogas potential or the BMP decreases with increasing lignin content since lignin is anaerobically not degradable [20]. However, as Dandikas et al. [19] showed, the extent of this effect may not be transferable to new plant types because the kind of lignin incrustation depends on several factors, such as lignin composition and cross-linking of lignin to other matrix components [32].

The aims of this study were to identify which chemical components of *T. latifolia*, *T. angustifolia* and *P. arundinacea* determine the biogas potential of these plants and to identify their optimal harvest date regarding degradability and biogas yield per hectare. Moreover, it was evaluated whether models developed for the prediction of the biogas potential and the BMP of classical energy crops are also suitable for fen plant species.

2. Material and methods

2.1. Substrate production

T. latifolia, *T. angustifolia* and *P. arundinacea* were cultivated as monocultures on a rewetted fen peatland located 30 km northeast of Munich (Freisinger Moos; 48°22′N, 11°41′E; 445 m above sea level). The species were planted on one plot (10 m × 100 m) per species on 11 July 2016 (*T. latifolia* and *T. angustifolia*) and 24 June 2016 (*P. arundinacea*). Water levels were adjusted to average annual values of approximately 10–15 cm below the soil surface via subsurface irrigation. After planting, weeds were controlled mechanically and chemically. The dead aboveground biomass was cut every winter to support the emergence of new shoots in spring.

In 2018, *T. latifolia* and *P. arundinacea* were harvested from four subplots on five different dates (5 May, 29 May, 19 June, 19 July, and 12 September). Additionally, *T. latifolia* and *T. angustifolia* were cut from three subplots on five different dates (12 May, 3 June, 25 June, 21 July, and 15 September) in 2020. The plant developmental stage was determined at each harvest following the BBCH system. According to Meier et al. [24], the growth stages are defined via a uniform code in ten principle phenological development stages (numbered 0 to 9) and described by specific external morphological characteristics (germination to senescence). As no BBCH code for *Typha* spp. and *P. arundinacea* is currently available, the specific morphological descriptions for the respective species were developed by the authors and not taken from the supplementary literature. On each harvest day, plants from a subplot (0.5 m x 2.5 m) were manually cut to 10 cm above the ground. The biomass was dried at 60 °C, and a subsample was used to determine the residual moisture content at 105 °C. The dry weight was determined for each subplot. The plant material of subplots with the same plant species and harvest date was mixed, chopped and ground with a cutting mill (sieve size <10 mm, SM 300, Retsch, Haan, Germany). Subsamples were taken for fodder analysis and finely ground to less than 1 mm. Weender and van Soest fodder analysis was performed according to the procedure described by the VDLUFA (Association of German Agricultural Analytic and Research Institutes) book of methods [33]. The percentages of the following parameters were determined: crude ash (XA), crude fat (XL), nitrogen (N), neutral detergent fiber (NDF), acid detergent fiber (ADF), acid detergent lignin (ADL), starch (ST) and reducing sugars (RS). Volatile solids (VS), crude protein (CP), cellulose (CL), hemicellulose (HC) and organic residue (OR) contents were calculated as follows: VS = 100 − XA; CP = 6.25 * N; CL = ADF − ADL; HC = NDF − ADF; OR = 100 − XA − CP − XL − ST − RS − NDF. VS and XA were expressed as a percentage of dry matter (DM), which was determined at 105 °C, and all other parameters were expressed as a percentage of volatile solids. Below, ADL is referred to as lignin.

2.2. Batch-test

The biogas potential of <10 mm ground *T. latifolia*, *T. angustifolia* and *P. arundinacea* samples from all harvest dates was determined by a batch-test according to VDI 4630 [34]. 2 L glass fermenters were filled with 20 g plant sample, 1000 g inoculum and 500 ml distilled water. The inoculum was obtained from a fermenter fed with 80% cattle manure and 20% cattle feed mainly containing maize and grass silage. The fermenter had a working volume of 2.5 m^3 and was operated at an organic loading rate of 2.5 kg VS m^{-3} d^{-1} at 38 °C. In the batch-test, microcrystalline cellulose served as a control, and dried whole crop maize was used as a reference substrate, which served to control the adequate course of the anaerobic digestion process and thus reference the data. The biogas production from the inoculum was recorded by using an unfed control as a blank. Biogas potentials for each sample were determined in triplicate, with the exception of biogas potential for microcrystalline cellulose, which was measured six times. After filling the batch-fermenters, they were incubated at 38 °C and swung manually twice a week. The batch-test was terminated when the daily biogas production was at least below 0.5% of the total biogas produced after three consecutive days. Biogas production was measured with a tipping counter (MilliGascounter, Ritter Apparatebau GmbH & Co. KG, Bochum, Germany; accuracy ±3% of each reading) per fermenter and recorded every hour. The room temperature and air pressure were also recorded on an hourly basis for standardization of the biogas yields to normal conditions (T = 273.15 K, p = 1013.25 mbar). The produced biogas of the three replicates per sample was collected in a gas bag. As soon as 1.5 L of biogas were captured, the gas composition was analyzed by an infrared sensor (accuracy ±2% of each reading), which measured the methane (CH_4) and carbon dioxide (CO_2) concentrations, and an electrochemical sensor (accuracy ±1% of each reading) was used for oxygen (O_2) detection (AWITE Bioenergie GmbH, Langenbach, Germany). Biogas potentials and BMPs were calculated as standard liters per kilogram volatile solids. Gas yields of the blank were subtracted from the gas yields of each sample.

2.3. Statistical analyses

All statistical analyses were conducted using R version 4.0.2 [35]. Testing for differences between the biogas potentials, biomass yields or biogas yields of different harvest dates within one species was carried out using a one-factorial analysis of variance. In the case of significant differences between means, Tukey's test was performed. The Shapiro-Wilk normality test was used to check the assumption of the normality of residuals, and the Fligner-Killeen test was applied to test the homogeneity of variances in residuals. Linear regressions were performed for each plant species to identify correlations between the biogas potential and the chemical composition. They were also used to evaluate and compare the prediction models.

3. Results and discussion

3.1. Influence of plant development stage on the chemical composition of *T. latifolia, T. angustifolia and P. arundinacea*

All plant development stages between vegetative growth and senescence were covered with the conducted harvests of *T. latifolia*, *T. angustifolia* and *P. arundinacea* (Table 1). Although *T. latifolia* plants were harvested at similar calendrical dates in 2018 and 2020, their development was faster in 2018. This must be considered when evaluating their chemical composition, which is depicted in Fig. 1. The nitrogen contents of all tested plant species decreased with advancing plant maturity until the third or fourth harvest date in 2018 or 2020, respectively. The nitrogen content then remained unchanged for the following harvest dates. In contrast, lignin contents increased over time. Similar observations were made by Pijlman et al. [36] for *T. latifolia* and by Kandel et al. [17] for *P. arundinacea*. The latter also noticed that the increase in the lignin content was more pronounced in the stems than in the leaves of *P. arundinacea*. In addition to nitrogen content, hemicellulose content also decreased with advancing maturity in the case of the *Typha* species. The starch content of *T. latifolia* steeply increased after the second or third harvest date in 2018 and 2020, respectively. This is in accordance with the results of Pijlman et al. (2019) [36], who showed a higher starch content for *T. latifolia* harvested at the beginning of July (3.2% DM) than for *T. latifolia* harvested at the end of May (1.0% DM). Starch is commonly an energy storage of lignocellulosic plants [37]. The faster development of *T. latifolia* in 2018 compared to that in 2020 may explain the steeper increase in starch content in 2018.

Table 1
Stage of development of *T. latifolia*, *P. arundinacea* and *T. angustifolia* at the different harvest dates.

Harvest date	Description of phenological development stages		
	T. latifolia	P. arundinacea	T. angustifolia
09.05.2018	Vegetative (plant height: 95 cm; seven leaves unfolded)	Vegetative (plant height: 70 cm; four leaves unfolded)	–
29.05.2018	End of heading	Full flowering	–
19.06.2018	Damp, brown seed heads with a greenish undertone	Late milk to early dough ripeness	–
19.07.2018	Dry, brown seed heads	Seed shattering	–
12.09.2018	Seed heads begin to disintegrate	Senescence	–
12.05.2020	Vegetative (plant height: 75 cm; seven expanded leaves)	–	Vegetative (plant height: 75 cm; six leaves unfolded)
03.06.2020	Beginning of heading	–	Vegetative (plant height: 130 cm; eight leaves unfolded)
25.06.2020	Seed heads start to turn brown	–	Damp, light brown seed heads
21.07.2020	Dry, brown seed heads	–	Dry, brown seed heads
15.09.2020	Dry, brown seed heads; incipient senescence	–	Dry, brown seed heads; incipient senescence

Only slight differences were observed between the chemical compositions of *T. latifolia*, *T. angustifolia* and *P. arundinacea*. In terms of lignin content, the values for *T. latifolia* were lower than those for *T. angustifolia*. The lignin content of *T. latifolia* was similar to that of *P. arundinacea* during the first three harvest dates in 2018 but showed higher contents on the following sampling dates. In contrast to *T. latifolia*, *T. angustifolia* had a constant starch content, whereas no starch was detected in *P. arundinacea*. The crude fat content of *P. arundinacea* decreased over time, whereas that of *T. latifolia* increased. Fat is usually located in the seeds of fen plants [38,39]. *P. arundinacea* dispersed their seeds in the middle of July. However, the seeds of *T. latifolia*, which have an oil content of 10–20% per weight [38], remained in the female flower spike. This may explain the different changes in the crude fat content.

T. latifolia, which was sampled in 2018 and 2020, showed a similar chemical composition in both years. However, there were a few marked differences. The different decreases in the hemicellulose content and the development of starch, sugar and lignin content may reflect the faster development of the plants in 2018 compared to that in 2020.

3.2. Influence of plant development stage on the biogas potential of *T. latifolia, T. angustifolia and P. arundinacea*

The determined biogas potential of *T. latifolia*, *T. angustifolia* and *P. arundinacea* decreased with increasing plant maturity and ranged between 315 and 647 L_N kg^{-1} VS, 405 and 596 L_N kg^{-1} VS and 361 and

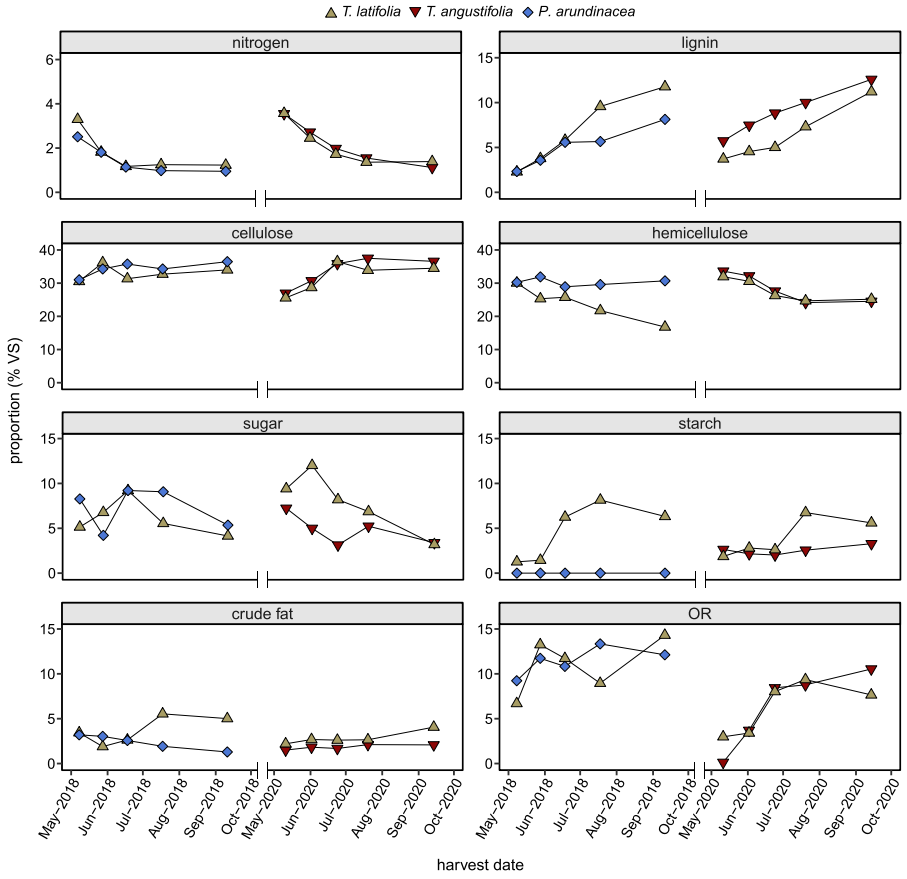

Fig. 1. Influence of harvest date on the chemical composition of *T. latifolia*, *T. angustifolia* and *P. arundinacea*.

597 L_N kg^{-1} VS, respectively (Fig. 2). All observed biogas potentials were significantly lower than the biogas potential of the reference maize, which was 725 L_N kg^{-1} VS. The methane content of the biogas, which was produced by the different fen plant species, ranged between 52.1 and 54.5%. These values agree with those reported by Roj-Rojewski et al. [22] and were as expected considering that fen plants are mainly composed of carbohydrates, which have a theoretical methane yield of 50% [40].

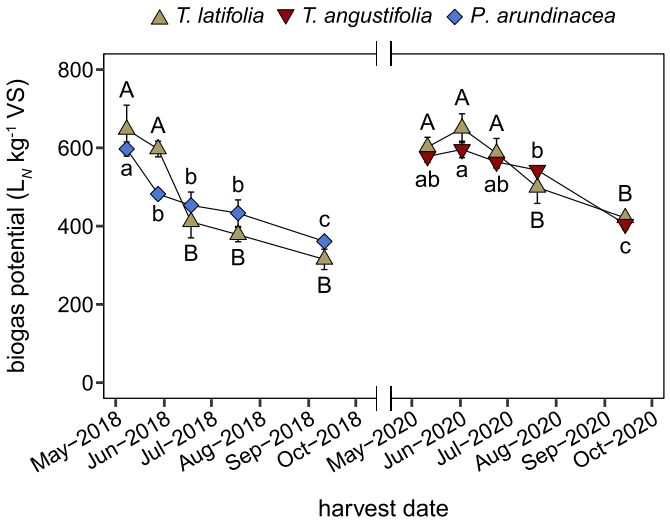

Fig. 2. Determined biogas potential of *T. latifolia*, *T. angustifolia* and *P. arundinacea* at different harvest dates (mean ± standard deviation). Different letters indicate significant differences between harvest dates within the same plant species and within one year (Tukey's test, $p < 0.05$). Upper case letters indicate significant differences of one species and lower case letters for the other species.

In 2018, there was a sharp decline in the biogas potential by nearly 200 L_N kg^{-1} VS between the second and third harvest date of *T. latifolia*. In comparison, the decrease in the biogas potential was smoother for *P. arundinacea* and for *T. latifolia* harvested in 2020. Moreover, the biogas potentials of *T. latifolia* and *T. angustifolia* were higher in 2020 than those of *T. latifolia* and *P. arundinacea* in 2018. For *T. latifolia,* this can be explained by its faster development, i.e., advanced maturity in 2018 compared to 2020.

To date, only a few studies have addressed the biogas potential of *T. latifolia* and *T. angustifolia*. In a previous study [16], the biogas potentials of *T. latifolia*, harvested in June or August had values of 581 and 428 L_N kg^{-1} VS, respectively, and were thus comparable to the reported data in the present study (378–650 L_N kg^{-1} VS for the summer harvests). The biogas potential of *T. latifolia* harvested in fall in the study of Hartung et al. [16] was 393 L_N kg^{-1} VS and therefore in the same range as that of the fall samples of the present study. However, *T. latifolia* in the study of Hartung et al. [16] had a lower lignin content (7% DM vs. 11% DM), indicating a better anaerobic digestibility, which was not measured. It once again makes clear that the biogas potential cannot be derived from the lignin content alone. Unfortunately, no development stages were recorded in the previous study. Kandel et al. [17] conducted batch-tests with *P. arundinacea*, which was harvested between the middle of April and the middle of September. Despite distinct differences in their chemical composition, e.g., lignin content, leaves and stems of *P. arundinacea* showed a very similar methane yield. In the study of Butkutė et al. [15], the biogas potential of *P. arundinacea* in the full flowering stage was 537 L_N kg^{-1} VS and therefore slightly higher than the biogas potential determined in the present study, which reached 482

L_N kg^{-1} VS. Roj-Rojewski et al. [22] harvested *P. arundinacea* between the flowering and seed ripening stages and the values for the biogas potential were between 272 and 385 L_N kg^{-1} VS, i.e., approximately 100–150 L_N kg^{-1} VS lower than in the present study. This might be caused by the determination criterion of the batch-test. Roj-Rojewski et al. [22] stopped taking measurements after 35 days and regardless of the actual development of biogas production, as in this study. The figure of the cumulative methane yields of Roj-Rojewski et al. [22] supports this argument because the plateau phase of gas production had not been reached when their batch-test was terminated.

3.3. Relationship between the biogas potential and the chemical composition

The relationship between the biogas potential and the chemical composition differed among the plant species. For all species, an increase in lignin content clearly decreased the biogas potential (Fig. 3). Numerous studies also found a significant negative correlation between the biogas potential or the BMP and the lignin content for lignocellulosic materials [19,21,28,30,31,41]. This observation is in line with the fact that lignin is considered to be anaerobically nondegradable [20]. Furthermore, it acts as a barrier via incrustation of hemicelluloses and celluloses and thus reduces their digestibility [32]. Differences in the kind or degree of incrustation are possibly responsible for the species-dependent regressions (Fig. 3). Other components varied in their relevance for biogas potential and need a more species-specific interpretation. For example, the biogas potential of *T. latifolia* was also strongly negatively correlated with the starch content ($p < 0.001$). However, the correlation only differentiated between young and mature plant materials, i.e., there are two groups of samples. There was a correlation between the biogas potential and the nitrogen content only for plant material collected during the first three harvest dates. However, a further decrease in biogas potential occurred for the following two harvest dates. An essential result was the negative correlation of biogas potential and cellulose of *P. arundinacea,* which is in line with Kandel et al. [17]. They noted a negative correlation between the biogas potential of leaves or stems of *P. arundinacea* and their lignin or cellulose content, respectively.

The biogas potential and the BMP were perfectly correlated (R^2: 1.00, $p < 0.001$), and consequently, the relationship between the biogas potential and the chemical composition was the same as that for the BMP and the chemical composition.

3.4. Prediction of the BMP with published models

Linear regression (LR) models by Triolo et al. [28], Dandikas et al. [30] and Thomsen et al. [31] were initially developed to predict the BMP of energy crops and other lignocellulosic materials. All of them

Table 2
Published regression models for predicting BMP of lignocellulosic biomass via fodder analysis parameters.

Regression model	Substrates used for model calibration	Reference
Linear regression (LR)		
BMP (L_N kg^{-1} VS) = 460.6–25.8 ADL (% VS)	energy crops (n = 10)	Triolo et al., 2011
BMP (L_N kg^{-1} VS) = 395–20.0 ADL (% VS)	energy crops (n = 31)	Dandikas et al., 2014
BMP (L_N kg^{-1} VS) = 347–7.85 ADL (% DM)	lignocellulosic biomass (n = 64)	Thomsen et al., 2014
Multiple linear regression (MLR)		
BMP (L_N kg^{-1} VS) = 447.1–0.7 CL (% VS) - 27.7 ADL (% VS)	energy crops (n = 10)	Triolo et al., 2011
BMP (L_N kg^{-1} VS) = 371 + 1.3 HC (% VS) - 20.0 ADL (% VS)	energy crops (n = 31)	Dandikas et al., 2014
BMP (L_N kg^{-1} VS) = 370 + 2.1 XP (% VS) + 0.5 HC (% VS) - 16.1 ADL (% VS)	grasses and legumes (n = 61)	Dandikas et al., 2015

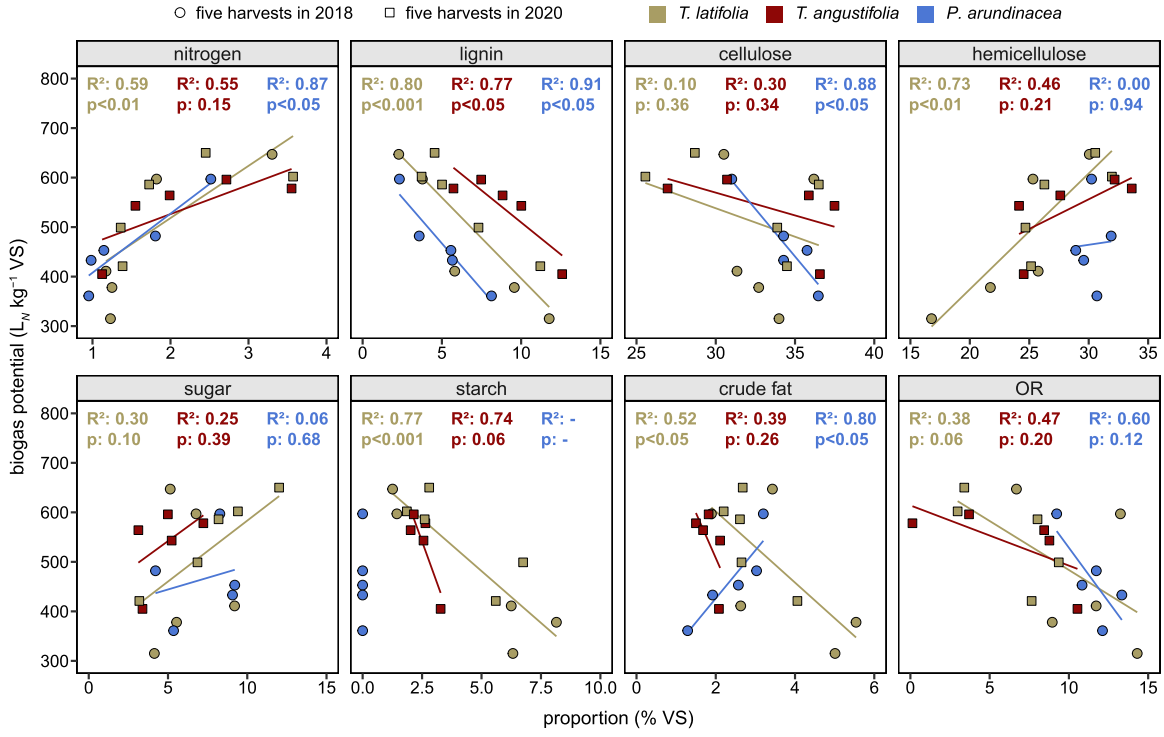

Fig. 3. Relationship between the parameters of the Weender-Van Soest fodder analysis (expressed as a proportion of potential fermentable matter) and the determined biogas potential. Linear regression lines are depicted for each plant species. Coefficients of determination and the p values are noted for each linear regression.

include the lignin content as a single regressor (Table 2). Lignin as a regressor was also suitable for *T. latifolia*, *T. angustifolia* and *P. arundinacea* since the BMP of all three plant species was closely correlated with the lignin content (Fig. 3). For all models, a more or less reasonable correlation between observed and predicted values was found (Fig. 4). The species differentiated from each other systematically, which can be explained by the relevance of lignin in each model (Table 2) and its dominant effect, which can be explained by the biogas potential of the samples, as shown in this study (Fig. 3). Samples from *T. angustifolia* were underestimated, while those from *P. arundinacea* were overestimated. Data for *T. latifolia* were better predicted than those for the other species, as shown by their trend in the graphs in Fig. 4. In detail, the LR model of Triolo et al. [28], which was calibrated with samples that had a lower lignin content than some of the samples of this study, resulted in regression with a slope steadily >1, i.e., the more mature the samples, the greater they were overestimated. In contrast, the LR model of Thomsen et al. [31] was calibrated with samples that covered a much larger range of lignin contents (4.5–37.2% lignin by dry weight) than the samples in our study. Consequently, this model was less sensitive to lignin and resulted in the lowest regression coefficients between the predicted and observed data and a slope « 1; finally, the range of the predicted values was too small. Dandikas et al. [30] developed a LR model, which seemed to be suitable for *T. latifolia* (R^2: 0.81, slope: 0.95), and even the regressions for the other species investigated showed only a bias.

Multiple linear regression (MLR) models of Triolo et al. [28], Dandikas et al. [30] and Dandikas et al. [19] also included the lignin content as an important regressor (Table 2). Other important regressors were cellulose, hemicellulose and/or crude protein. Finally, the consistent high weight of ADL in the model of Triolo et al. [28] (Table 2) led to the same systematic deviations as with the simple linear regression: slope was clearly >1. In contrast, the MLR and LR models of Dandikas et al. [30] performed very similarly for *T. latifolia*, with the MLR being slightly better than the LR (R^2: 0.83 to 0.81 and slope: 1.03 to 0.95, respectively). The MLR model of Dandikas et al. [19] was the most suitable model for *T. angustifolia* within the tested range of this study. However, deviations of predictions for *T. latifolia* and *P. arundinacea* from observed data can be corrected by a bias, i.e., in principle, the differentiation of the species was reflected by the model.

3.5. Optimal harvest date regarding biogas potential and biogas yield per hectare

In addition to the anaerobic digestibility, the biogas yield per hectare, i.e., the product of biogas potential and the biomass yield, is crucial for determining the optimal harvest date for biogas substrates. *T. latifolia*, *T. angustifolia* and *P. arundinacea* reached biomass yields of up to 15, 14 and 11 t DM ha^{-1}, respectively (Fig. 5). The biomass yields of *T. latifolia* and *T. angustifolia* were comparable to those observed in other studies [36,42]. For *P. arundinacea*, the measured biomass yields at the different harvest times were in the same range as those determined by Kandel et al. [43].

T. latifolia, *T. angustifolia* and *P. arundinacea* achieved the highest biogas yield per hectare at the third and fourth harvest date (Fig. 5). Later harvests did not result in higher biogas yields because older plant material has a reduced anaerobic digestibility (Fig. 2), which counteracts the higher biomass yields. Additionally, such an older plant material may destabilize the degradation process [16]. According to the third and fourth harvest date, the optimal time of harvest for *T. latifolia* is between the end of heading and when the seed heads begin to turn brown. *P. arundinacea* should be harvested between the full flowering stage and the stage of late milk to early dough ripeness. In the case of

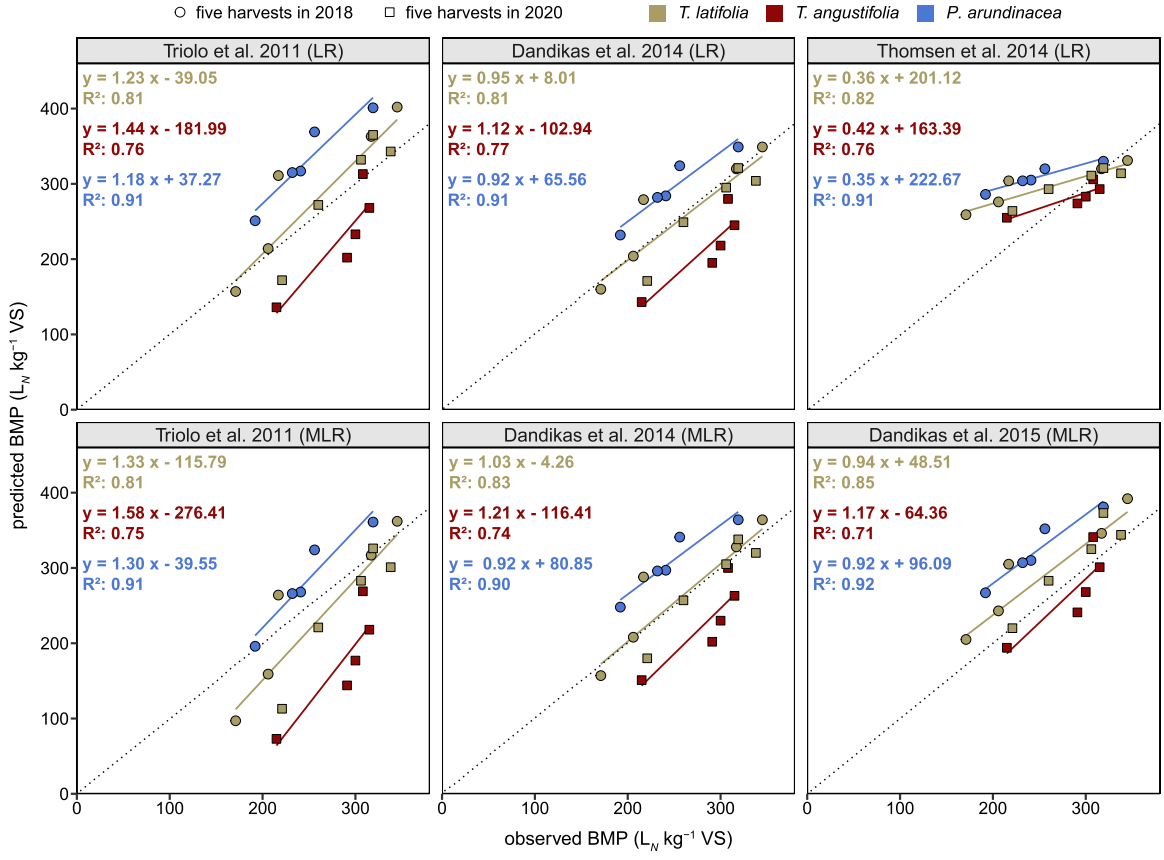

Fig. 4. Predicted versus observed values for the BMP of *T. latifolia*, *T. angustifolia* and *P. arundinacea*. Linear regression lines, equations and coefficients of determination are depicted for each plant species. The dotted lines represents the angle bisector.

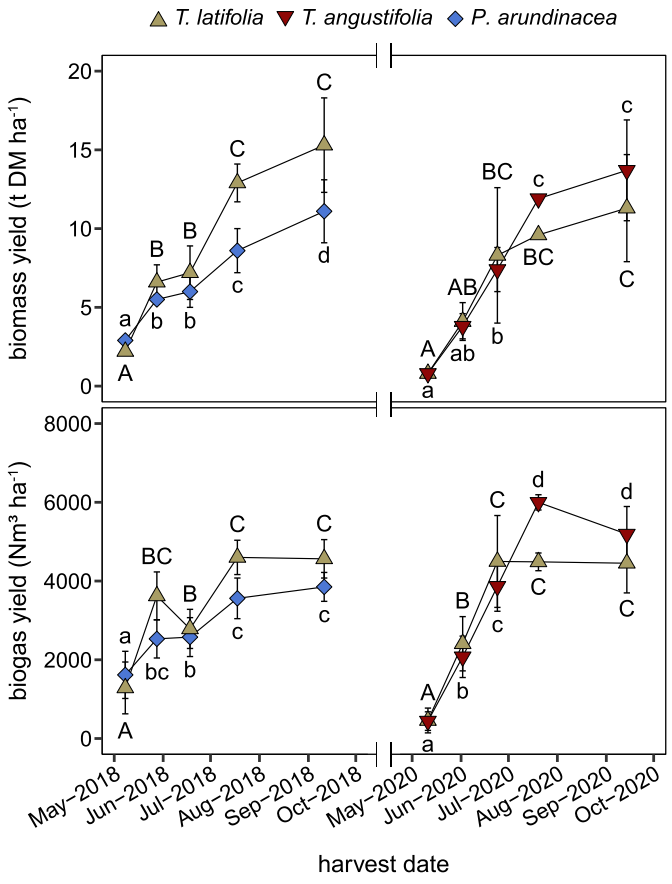

Fig. 5. Biomass and biogas yield of *T. latifolia*, *T. angustifolia* and *P. arundinacea* at different harvest dates (mean ± standard deviation). Different letters indicate significant differences between harvest dates within the same plant species and within one year (Tukey's test, $p < 0.05$). Upper case letters indicate significant differences of one species and lower case letters for the other species.

T. angustifolia, the period shortly after the seed heads turn brown is considered optimal for harvest. When adhering to the recommended harvest dates, biogas yields of 4500, 4000 and 2500 $Nm^3\ ha^{-1}$ can be achieved by one harvest for *T. latifolia*, *T. angustifolia* and *P. arundinacea*, respectively. These values are comparable to the biogas yields per hectare of permanent grassland, which range between 3700 and 7200 $Nm^3\ ha^{-1}$ [44]. However, these grassland data are for two to three harvests per year. If the fen plants are harvested a second time – which seems to be possible according to the actual harvest date – a significantly higher biogas yield than reported seems to be achievable.

4. Conclusion

The highest biogas yield per hectare was achieved earlier than that of the highest biomass yield because the anaerobic digestibility decreased with advancing plant maturity. The reduction in digestibility of the plant material was shown to be primarily related to an increasing lignin content. A prediction of the biogas potential of the samples with published models worked quite well if lignin content was used as the main regressor. However, systematic differences occurred between the predicted and measured values depending on the plant species. Therefore, further investigation is needed on this matter.

Data availability

Data will be made available on request.

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Table S1: Literature values for biogas potential and BMP of *P. arundinacea*, *P. australis*, *Typha* spp. and *Carex* spp. measured in batch-tests under mesophilic conditions (35 - 39 °C).

plant species	harvest time	growth stage	biogas potential (L_N kg^{-1} VS)	CH_4 in biogas (%)	BMP (L_N kg^{-1} VS)	batch-test (days)	particle size (mm)	prepar-ation	reference
P. arundinacea[A]	n.r.	EH	708	60	426	n.r.	3 - 5	n.r.	Butkutė et al. 2014
P. arundinacea[A]	n.r.	EnH	523	62	323	n.r.	3 - 5	n.r.	Butkutė et al. 2014
P. arundinacea[A]	n.r.	FF	537	59	316	n.r.	3 - 5	n.r.	Butkutė et al. 2014
P. arundinacea[A]	late September	n.r.	418[b]	57[a]	238	59	20 - 40	ensiled	Czubaszek et al. 2021
P. arundinacea[L]	mid-April	n.r.	n.r.	n.r.	383[a]	69	<1	dried	Kandel et al. 2013
P. arundinacea[S]	mid-April	n.r.	n.r.	n.r.	413[a]	69	<1	dried	Kandel et al. 2013
P. arundinacea[L]	early May	n.r.	n.r.	n.r.	385[a]	69	<1	dried	Kandel et al. 2013
P. arundinacea[S]	early May	n.r.	n.r.	n.r.	384[a]	69	<1	dried	Kandel et al. 2013
P. arundinacea[L]	mid-May	n.r.	n.r.	n.r.	380[a]	69	<1	dried	Kandel et al. 2013
P. arundinacea[S]	mid-May	n.r.	n.r.	n.r.	342[a]	69	<1	dried	Kandel et al. 2013
P. arundinacea[L]	late May	n.r.	n.r.	n.r.	344[a]	69	<1	dried	Kandel et al. 2013
P. arundinacea[S]	late May	n.r.	n.r.	n.r.	364[a]	69	<1	dried	Kandel et al. 2013
P. arundinacea[L]	mid-June	n.r.	n.r.	n.r.	359[a]	69	<1	dried	Kandel et al. 2013
P. arundinacea[S]	mid-June	n.r.	n.r.	n.r.	368[a]	69	<1	dried	Kandel et al. 2013
P. arundinacea[L]	late June	n.r.	n.r.	n.r.	353[a]	69	<1	dried	Kandel et al. 2013
P. arundinacea[S]	late June	n.r.	n.r.	n.r.	355[a]	69	<1	dried	Kandel et al. 2013
P. arundinacea[L]	early July	n.r.	n.r.	n.r.	358[a]	69	<1	dried	Kandel et al. 2013
P. arundinacea[S]	early July	n.r.	n.r.	n.r.	363[a]	69	<1	dried	Kandel et al. 2013
P. arundinacea[L]	late July	n.r.	n.r.	n.r.	324[a]	69	<1	dried	Kandel et al. 2013
P. arundinacea[S]	late July	n.r.	n.r.	n.r.	329[a]	69	<1	dried	Kandel et al. 2013
P. arundinacea[L]	early August	n.r.	n.r.	n.r.	335[a]	69	<1	dried	Kandel et al. 2013
P. arundinacea[S]	early August	n.r.	n.r.	n.r.	319[a]	69	<1	dried	Kandel et al. 2013
P. arundinacea[L]	late August	n.r.	n.r.	n.r.	323[a]	69	<1	dried	Kandel et al. 2013
P. arundinacea[S]	late August	n.r.	n.r.	n.r.	313[a]	69	<1	dried	Kandel et al. 2013

Table S1: Continued.

plant species	harvest time	growth stage	biogas potential (L_N kg^{-1} VS)	CH_4 in biogas (%)	BMP (L_N kg^{-1} VS)	batch-test (days)	particle size (mm)	prepar-ation	reference
P. arundinacea^{L}	early September	n.r.	n.r.	n.r.	340[a]	69	<1	dried	Kandel et al. 2013
P. arundinacea^{S}	early September	n.r.	n.r.	n.r.	283[a]	69	<1	dried	Kandel et al. 2013
P. arundinacea^{L}	late September	n.r.	n.r.	n.r.	315[a]	69	<1	dried	Kandel et al. 2013
P. arundinacea^{S}	late September	n.r.	n.r.	n.r.	290[a]	69	<1	dried	Kandel et al. 2013
P. arundinacea^{A}	mid-June	n.r.	n.r.	n.r.	338	n.r.	20	none	Laasasenaho et al. 2020
P. arundinacea^{A}	mid-June	n.r.	n.r.	n.r.	348	n.r.	20	none	Laasasenaho et al. 2020
P. arundinacea^{A}	fall	n.r.	n.r.	n.r.	205[a]	90	1	dried	Marchetti et al. 2016
P. arundinacea^{A}	mid-summer	n.r.	n.r.	n.r.	205	n.r.	25 - 50	ensiled	Massé et al. 2011
P. arundinacea^{A}	late summer	n.r.	n.r.	n.r.	201	n.r.	25 - 50	ensiled	Massé et al. 2011
P. arundinacea^{A}	early fall	n.r.	n.r.	n.r.	202	n.r.	25 - 50	ensiled	Massé et al. 2011
P. arundinacea^{A}	mid-summer	n.r.	n.r.	n.r.	211	n.r.	25 - 50	ensiled	Massé et al. 2011
P. arundinacea^{A}	late summer	n.r.	n.r.	n.r.	183	n.r.	25 - 50	ensiled	Massé et al. 2011
P. arundinacea^{A}	early fall	n.r.	n.r.	n.r.	170	n.r.	25 - 50	ensiled	Massé et al. 2011
P. arundinacea^{A}	late July	F	385	53	200	35	20	ensiled	Roj-Rojewski et al. 2019
P. arundinacea^{A}	late August	LF	272	51	147	35	20	ensiled	Roj-Rojewski et al. 2019
P. arundinacea^{A}	early October	SR	286	50	154	35	20	ensiled	Roj-Rojewski et al. 2019
P. arundinacea^{A}	late June	n.r.	n.r.	n.r.	329	75 - 95	<10	none	Seppälä et al. 2009
P. arundinacea^{A}	late June	EF	n.r.	n.r.	316	75 - 95	<10	none	Seppälä et al. 2009
P. arundinacea^{A}	early July	F	n.r.	n.r.	258	75 - 95	<10	none	Seppälä et al. 2009
P. arundinacea^{A}	mid-June	EF	n.r.	n.r.	351	75 - 95	<10	none	Seppälä et al. 2009
P. arundinacea^{A}	late September	n.r.	n.r.	n.r.	253	75 - 95	<10	none	Seppälä et al. 2009
P. arundinacea^{A}	July	n.r.	n.r.	n.r.	299[a]	60	<1	dried	Triolo et al. 2012
P. arundinacea^{A}	August	n.r.	n.r.	n.r.	257[a]	60	<1	dried	Triolo et al. 2012
P. arundinacea^{A}	August	n.r.	n.r.	n.r.	221[a]	60	<1	dried	Triolo et al. 2012
P. arundinacea^{A}	October	n.r.	n.r.	n.r.	143[a]	60	<1	dried	Triolo et al. 2012

Table S1: Continued.

plant species	harvest time	growth stage	biogas potential (L_N kg^{-1} VS)	CH_4 in biogas (%)	BMP (L_N kg^{-1} VS)	batch-test (days)	particle size (mm)	prepar-ation	reference
P. arundinacea[A]	October	n.r.	n.r.	n.r.	105[a]	60	<1	dried	Triolo et al. 2012
P. australis[A]	late September	n.r.	291[b]	55[a]	160	59	20 - 40	ensiled	Czubaszek et al. 2021
P. australis[A]	August	n.r.	n.r.	n.r.	202[a]	15 - 34	5 - 10	dried	Eller et al. 2020
P. australis[A]	August	n.r.	n.r.	n.r.	253[a]	15 - 34	<1	dried	Eller et al. 2020
P. australis[A]	December	n.r.	301[a]	62[b]	187[a]	63	n.r.	dried	Lizasoain et al. 2016
P. australis[A]	late July	EF	277	51	148	35	20	ensiled	Roj-Rojewski et al. 2019
P. australis[A]	late August	F	235	50	125	35	20	ensiled	Roj-Rojewski et al. 2019
P. australis[A]	early October	SR	181	51	102	35	20	ensiled	Roj-Rojewski et al. 2019
P. australis[A]	October	n.r.	n.r.	n.r.	190[a]	60	<1	dried	Triolo et al. 2012
P. australis[A]	October	n.r.	n.r.	n.r.	189[a]	60	<1	dried	Triolo et al. 2012
P. australis[A]	n.r.	n.r.	n.r.	n.r.	186[a]	31	<2	n.r.	Vakalis et al. 2022
T. latifolia[A]	late September	n.r.	431[b]	55[a]	237	59	20 - 40	ensiled	Czubaszek et al. 2021
T. angustifolia[A]	August	n.r.	n.r.	n.r.	167[a]	15 - 34	5 - 10	dried	Eller et al. 2020
T. angustifolia[A]	August	n.r.	n.r.	n.r.	225[a]	15 - 34	<1	dried	Eller et al. 2020
T. latifolia[A]	August	n.r.	n.r.	n.r.	193[a]	15 - 34	5 - 10	dried	Eller et al. 2020
T. latifolia[A]	August	n.r.	n.r.	n.r.	229[a]	15 - 34	<1	dried	Eller et al. 2020
T. latifolia[A]	fall	n.r.	n.r.	n.r.	252[a]	90	1	dried	Marchetti et al. 2016
T. latifolia[A]	April	n.r.	n.r.	n.r.	151	60	n.r.	n.r.	Nkemka et al. 2015
C. elata[A]	late September	n.r.	345[b]	55[a]	190	59	20 - 40	ensiled	Czubaszek et al. 2021
C. elata[A] + *C. acutiformis*[A]	late September	n.r.	491[b]	56[a]	275	60	21 - 40	ensiled	Czubaszek et al. 2021
C. acutiformis[A]	fall	n.r.	n.r.	n.r.	146[a]	90	1	dried	Marchetti et al. 2016
C. riparia[A]	fall	n.r.	n.r.	n.r.	223[a]	90	1	dried	Marchetti et al. 2016
C. elata[A]	late July	SR	289	50	151	35	20	ensiled	Roj-Rojewski et al. 2019
C. elata[A]	late August	SR	259	48	136	35	20	ensiled	Roj-Rojewski et al. 2019

Table S1: Continued.

plant species	harvest time	growth stage	biogas potential (L_N kg^{-1} VS)	CH_4 in biogas (%)	BMP (L_N kg^{-1} VS)	batch-test (days)	particle size (mm)	prepar-ation	reference
C. elata[A]	early October	SR	230	52	123	35	20	ensiled	Roj-Rojewski et al. 2019
C. lasiocarpa[A]	late July	SR	291	49	150	35	20	ensiled	Roj-Rojewski et al. 2019
C. lasiocarpa[A]	late August	SR	230	48	121	35	20	ensiled	Roj-Rojewski et al. 2019
C. lasiocarpa[A]	early October	SR	246	50	128	35	20	ensiled	Roj-Rojewski et al. 2019

[A] aerial plant parts, [L] leaves, [S] stems, [a] value extracted from graph with WebPlotDigitizer (v4.6; Rohatgi, 2022), [b] calculated value, n.r. (not reported)
EH (early heading), EnH (end of heading), F (flowering), EF (early flowering), FF (full flowering), LF (late flowering), SR (seed ripening)

Table S2: Literature values for biomass yields of *P. arundinacea*, *P. australis*, *Typha* spp. and *Carex* spp.

plant species	country	harvest time	growth stage	biomass yield (t DM ha^{-1})	cultivated (c), natural stand (n)	reference
P. arundinacea	Canada	late July	n.r.	7.9	c	Bélanger et al. 2016
P. arundinacea	Canada	early September	n.r.	7.6	c	Bélanger et al. 2016
P. arundinacea	Canada	mid-October	n.r.	7.1	c	Bélanger et al. 2016
P. arundinacea	USA	winter	n.r.	0.8[a]	c	Bernard and Lauve 1995
P. arundinacea	USA	June	n.r.	10.9[a]	c	Bernard and Lauve 1995
P. arundinacea	USA	July	n.r.	13.1[a]	c	Bernard and Lauve 1995
P. arundinacea	USA	August	n.r.	17.1[a]	c	Bernard and Lauve 1995
P. arundinacea	USA	September	n.r.	12.9[a]	c	Bernard and Lauve 1995
P. arundinacea	USA	October	n.r.	7.3[a]	c	Bernard and Lauve 1995
P. arundinacea	USA	winter	n.r.	0.5[a]	n	Bernard and Lauve 1995
P. arundinacea	USA	June	n.r.	9.0[a]	n	Bernard and Lauve 1995
P. arundinacea	USA	July	n.r.	14.1[a]	n	Bernard and Lauve 1995
P. arundinacea	USA	August	n.r.	13.3[a]	n	Bernard and Lauve 1995
P. arundinacea	USA	September	n.r.	8.3[a]	n	Bernard and Lauve 1995
P. arundinacea	USA	October	n.r.	3.4[a]	n	Bernard and Lauve 1995
P. arundinacea	Czech Republic	May	n.r.	10.3[a]	c	Březinová and Vymazal 2015
P. arundinacea	Czech Republic	June	n.r.	14.8[a]	c	Březinová and Vymazal 2015
P. arundinacea	Czech Republic	July	n.r.	16.1[a]	c	Březinová and Vymazal 2015
P. arundinacea	Czech Republic	August	n.r.	13.4[a]	c	Březinová and Vymazal 2015
P. arundinacea	Czech Republic	September	n.r.	13.6[a]	c	Březinová and Vymazal 2015
P. arundinacea	Czech Republic	November	n.r.	12.2[a]	c	Březinová and Vymazal 2015
P. arundinacea	Czech Republic	January	n.r.	11.7[a]	c	Březinová and Vymazal 2015
P. arundinacea	Czech Republic	March	n.r.	6.9[a]	c	Březinová and Vymazal 2015
P. arundinacea	Czech Republic	May	n.r.	1.4[a]	c	Březinová and Vymazal 2015
P. arundinacea	Czech Republic	June	n.r.	5.0[a]	c	Březinová and Vymazal 2015

Table S2: Continued.

plant species	country	harvest time	growth stage	biomass yield (t DM ha^{-1})	cultivated (c), natural stand (n)	reference
P. arundinacea	Czech Republic	July	n.r.	6.8[a]	c	Březinová and Vymazal 2015
P. arundinacea	Czech Republic	August	n.r.	7.2[a]	c	Březinová and Vymazal 2015
P. arundinacea	Czech Republic	September	n.r.	9.7[a]	c	Březinová and Vymazal 2015
P. arundinacea	Czech Republic	November	n.r.	8.7[a]	c	Březinová and Vymazal 2015
P. arundinacea	Czech Republic	January	n.r.	6.9[a]	c	Březinová and Vymazal 2015
P. arundinacea	Czech Republic	March	n.r.	6.4[a]	c	Březinová and Vymazal 2015
P. arundinacea	Czech Republic	April	n.r.	2.0[a]	c	Březinová and Vymazal 2015
P. arundinacea	Czech Republic	May	n.r.	6.5[a]	c	Březinová and Vymazal 2015
P. arundinacea	Czech Republic	June	n.r.	17.3[a]	c	Březinová and Vymazal 2015
P. arundinacea	Czech Republic	July	n.r.	17.3[a]	c	Březinová and Vymazal 2015
P. arundinacea	Czech Republic	August	n.r.	17.1[a]	c	Březinová and Vymazal 2015
P. arundinacea	Czech Republic	September	n.r.	15.5[a]	c	Březinová and Vymazal 2015
P. arundinacea	Czech Republic	October	n.r.	14.6[a]	c	Březinová and Vymazal 2015
P. arundinacea	Czech Republic	December	n.r.	12.9[a]	c	Březinová and Vymazal 2015
P. arundinacea	Czech Republic	January	n.r.	11.4[a]	c	Březinová and Vymazal 2015
P. arundinacea	Czech Republic	March	n.r.	11.0[a]	c	Březinová and Vymazal 2015
P. arundinacea	Czech Republic	April	n.r.	1.5[a]	c	Březinová and Vymazal 2015
P. arundinacea	Czech Republic	May	n.r.	4.7[a]	c	Březinová and Vymazal 2015
P. arundinacea	Czech Republic	June	n.r.	9.4[a]	c	Březinová and Vymazal 2015
P. arundinacea	Czech Republic	July	n.r.	12.0[a]	c	Březinová and Vymazal 2015
P. arundinacea	Czech Republic	August	n.r.	13.6[a]	c	Březinová and Vymazal 2015
P. arundinacea	Czech Republic	September	n.r.	12.9[a]	c	Březinová and Vymazal 2015
P. arundinacea	Czech Republic	October	n.r.	12.8[a]	c	Březinová and Vymazal 2015
P. arundinacea	Czech Republic	December	n.r.	11.4[a]	c	Březinová and Vymazal 2015
P. arundinacea	Czech Republic	January	n.r.	10.7[a]	c	Březinová and Vymazal 2015

Table S2: Continued.

plant species	country	harvest time	growth stage	biomass yield (t DM ha^{-1})	cultivated (c), natural stand (n)	reference
P. arundinacea	Czech Republic	March	n.r.	9.8[a]	c	Březinová and Vymazal 2015
P. arundinacea	Lithuania	n.r.	EH	4.6	c	Butkutė et al. 2014
P. arundinacea	Sweden	mid-June	n.r.	2.5	c	Geber 2002
P. arundinacea	Estonia	July	n.r.	5.1[a]	c	Heinsoo et al. 2011
P. arundinacea	Estonia	October	n.r.	6.7[a]	c	Heinsoo et al. 2011
P. arundinacea	Estonia	April	n.r.	4.1[a]	c	Heinsoo et al. 2011
P. arundinacea	Estonia	July	n.r.	2.2[a]	c	Heinsoo et al. 2011
P. arundinacea	Estonia	October	n.r.	6.8[a]	c	Heinsoo et al. 2011
P. arundinacea	Estonia	April	n.r.	4.7[a]	c	Heinsoo et al. 2011
P. arundinacea	Estonia	July	n.r.	3.8[a]	c	Heinsoo et al. 2011
P. arundinacea	Estonia	October	n.r.	7.1[a]	c	Heinsoo et al. 2011
P. arundinacea	Estonia	April	n.r.	5.0[a]	c	Heinsoo et al. 2011
P. arundinacea	Estonia	July	n.r.	6.8[a]	c	Heinsoo et al. 2011
P. arundinacea	Estonia	October	n.r.	6.6[a]	c	Heinsoo et al. 2011
P. arundinacea	Estonia	April	n.r.	6.6[a]	c	Heinsoo et al. 2011
P. arundinacea	Estonia	July	n.r.	9.5[a]	c	Heinsoo et al. 2011
P. arundinacea	Estonia	October	n.r.	6.3[a]	c	Heinsoo et al. 2011
P. arundinacea	Estonia	April	n.r.	7.1[a]	c	Heinsoo et al. 2011
P. arundinacea	Estonia	July	n.r.	3.2[a]	c	Heinsoo et al. 2011
P. arundinacea	Estonia	October	n.r.	9.7[a]	c	Heinsoo et al. 2011
P. arundinacea	Estonia	April	n.r.	8.2[a]	c	Heinsoo et al. 2011
P. arundinacea	Estonia	July	n.r.	5.7[a]	c	Heinsoo et al. 2011
P. arundinacea	Estonia	October	n.r.	9.1[a]	c	Heinsoo et al. 2011
P. arundinacea	Estonia	April	n.r.	4.9[a]	c	Heinsoo et al. 2011
P. arundinacea	Estonia	July	n.r.	0.8[a]	c	Heinsoo et al. 2011

Table S2: Continued.

plant species	country	harvest time	growth stage	biomass yield (t DM ha^{-1})	cultivated (c), natural stand (n)	reference
P. arundinacea	Estonia	October	n.r.	5.4[a]	c	Heinsoo et al. 2011
P. arundinacea	Estonia	April	n.r.	4.2[a]	c	Heinsoo et al. 2011
P. arundinacea	Estonia	July	n.r.	2.3[a]	c	Heinsoo et al. 2011
P. arundinacea	Estonia	October	n.r.	4.9[a]	c	Heinsoo et al. 2011
P. arundinacea	Estonia	April	n.r.	3.3[a]	c	Heinsoo et al. 2011
P. arundinacea	Estonia	July	n.r.	5.0[a]	c	Heinsoo et al. 2011
P. arundinacea	Estonia	October	n.r.	10.0[a]	c	Heinsoo et al. 2011
P. arundinacea	Estonia	April	n.r.	5.6[a]	c	Heinsoo et al. 2011
P. arundinacea	Estonia	July	n.r.	5.6[a]	c	Heinsoo et al. 2011
P. arundinacea	Estonia	October	n.r.	5.8[a]	c	Heinsoo et al. 2011
P. arundinacea	Estonia	April	n.r.	5.4[a]	c	Heinsoo et al. 2011
P. arundinacea	Estonia	July	n.r.	3.0[a]	c	Heinsoo et al. 2011
P. arundinacea	Estonia	October	n.r.	8.6[a]	c	Heinsoo et al. 2011
P. arundinacea	Estonia	April	n.r.	6.7[a]	c	Heinsoo et al. 2011
P. arundinacea	Denmark	mid-April	n.r.	0.5[a]	c	Kandel et al. 2013
P. arundinacea	Denmark	early May	n.r.	1.0[a]	c	Kandel et al. 2013
P. arundinacea	Denmark	mid-May	n.r.	3.1[a]	c	Kandel et al. 2013
P. arundinacea	Denmark	late May	n.r.	4.4[a]	c	Kandel et al. 2013
P. arundinacea	Denmark	mid-June	n.r.	7.6[a]	c	Kandel et al. 2013
P. arundinacea	Denmark	late June	n.r.	9.1[a]	c	Kandel et al. 2013
P. arundinacea	Denmark	early July	n.r.	8.8[a]	c	Kandel et al. 2013
P. arundinacea	Denmark	late July	n.r.	11.7[a]	c	Kandel et al. 2013
P. arundinacea	Denmark	early August	n.r.	11.6[a]	c	Kandel et al. 2013
P. arundinacea	Denmark	late August	n.r.	12.1[a]	c	Kandel et al. 2013
P. arundinacea	Denmark	early September	n.r.	11.5[a]	c	Kandel et al. 2013

Table S2: Continued.

plant species	country	harvest time	growth stage	biomass yield (t DM ha^{-1})	cultivated (c), natural stand (n)	reference
P. arundinacea	Denmark	late September	n.r.	11.7[a]	c	Kandel et al. 2013
P. arundinacea	Sweden	early June	n.r.	7.5[a]	c	Kätterer et al. 1998
P. arundinacea	Sweden	late June	n.r.	9.1[a]	c	Kätterer et al. 1998
P. arundinacea	Sweden	early July	n.r.	12.0[a]	c	Kätterer et al. 1998
P. arundinacea	Sweden	late July	n.r.	14.6[a]	c	Kätterer et al. 1998
P. arundinacea	Sweden	mid-August	n.r.	13.3[a]	c	Kätterer et al. 1998
P. arundinacea	Sweden	early September	n.r.	14.7[a]	c	Kätterer et al. 1998
P. arundinacea	Sweden	late September	n.r.	13.3[a]	c	Kätterer et al. 1998
P. arundinacea	Sweden	late October	n.r.	12.6[a]	c	Kätterer et al. 1998
P. arundinacea	Sweden	early May	n.r.	0.3[a]	c	Kätterer et al. 1998
P. arundinacea	Sweden	late May	n.r.	2.1[a]	c	Kätterer et al. 1998
P. arundinacea	Sweden	early June	n.r.	4.4[a]	c	Kätterer et al. 1998
P. arundinacea	Sweden	late June	n.r.	9.1[a]	c	Kätterer et al. 1998
P. arundinacea	Sweden	early July	n.r.	10.6[a]	c	Kätterer et al. 1998
P. arundinacea	Sweden	mid-July	n.r.	10.7[a]	c	Kätterer et al. 1998
P. arundinacea	Sweden	early August	n.r.	11.0[a]	c	Kätterer et al. 1998
P. arundinacea	Sweden	mid-August	n.r.	16.7[a]	c	Kätterer et al. 1998
P. arundinacea	Sweden	early September	n.r.	14.0[a]	c	Kätterer et al. 1998
P. arundinacea	Sweden	late September	n.r.	13.5[a]	c	Kätterer et al. 1998
P. arundinacea	Sweden	mid-October	n.r.	12.0[a]	c	Kätterer et al. 1998
P. arundinacea	Estonia	September	n.r.	7.2	c	Mander et al. 2012
P. arundinacea	Estonia	September	n.r.	9.3	c	Mander et al. 2012
P. arundinacea	Estonia	April	n.r.	9.2	c	Mander et al. 2012
P. arundinacea	Estonia	April	n.r.	5.1	c	Mander et al. 2012
P. arundinacea	Canada	mid-summer	n.r.	8.2	c	Massé et al. 2011

Table S2: Continued.

plant species	country	harvest time	growth stage	biomass yield (t DM ha^{-1})	cultivated (c), natural stand (n)	reference
P. arundinacea	Canada	mid-summer	n.r.	10.7	c	Massé et al. 2011
P. arundinacea	Canada	late summer	n.r.	8.2	c	Massé et al. 2011
P. arundinacea	Canada	late summer	n.r.	9.2	c	Massé et al. 2011
P. arundinacea	Canada	early fall	n.r.	7.5	c	Massé et al. 2011
P. arundinacea	Canada	early fall	n.r.	8.8	c	Massé et al. 2011
P. arundinacea	Canada	mid-summer	n.r.	6.4	c	Massé et al. 2011
P. arundinacea	Canada	mid-summer	n.r.	9.9	c	Massé et al. 2011
P. arundinacea	Canada	late summer	n.r.	6.1	c	Massé et al. 2011
P. arundinacea	Canada	late summer	n.r.	9.3	c	Massé et al. 2011
P. arundinacea	Canada	early fall	n.r.	6.0	c	Massé et al. 2011
P. arundinacea	Canada	early fall	n.r.	7.7	c	Massé et al. 2011
P. arundinacea	Denmark	early August	n.r.	8.3	c	Nielsen et al. 2021
P. arundinacea	Denmark	mid-June	n.r.	8.8	c	Nielsen et al. 2021
P. arundinacea	Denmark	mid-May	n.r.	1.6	c	Nielsen et al. 2021
P. arundinacea	Denmark	mid-May	n.r.	2.0	c	Nielsen et al. 2021
P. arundinacea	Denmark	mid-May	n.r.	1.7	c	Nielsen et al. 2021
P. arundinacea	Lithuania	July	n.r.	4.3[a]	c	Pocienė and Kadžiulienė 2016
P. arundinacea	Lithuania	July	n.r.	2.5[a]	c	Pocienė and Kadžiulienė 2016
P. arundinacea	Lithuania	October	n.r.	3.6[a]	c	Pocienė and Kadžiulienė 2016
P. arundinacea	Lithuania	October	n.r.	3.5[a]	c	Pocienė and Kadžiulienė 2016
P. arundinacea	Lithuania	July	n.r.	7.0[a]	c	Pocienė and Kadžiulienė 2016
P. arundinacea	Lithuania	July	n.r.	7.9[a]	c	Pocienė and Kadžiulienė 2016
P. arundinacea	Lithuania	October	n.r.	5.8[a]	c	Pocienė and Kadžiulienė 2016
P. arundinacea	Lithuania	October	n.r.	6.6[a]	c	Pocienė and Kadžiulienė 2016
P. arundinacea	Lithuania	July	n.r.	5.8[a]	c	Pocienė and Kadžiulienė 2016

Table S2: Continued.

plant species	country	harvest time	growth stage	biomass yield (t DM ha^{-1})	cultivated (c), natural stand (n)	reference
P. arundinacea	Lithuania	July	n.r.	5.5[a]	c	Pocienė and Kadžiulienė 2016
P. arundinacea	Lithuania	October	n.r.	5.2[a]	c	Pocienė and Kadžiulienė 2016
P. arundinacea	Lithuania	October	n.r.	6.1[a]	c	Pocienė and Kadžiulienė 2016
P. arundinacea	Latvia	September/October	S	6.4[a]	c	Rancane et al. 2016
P. arundinacea	Latvia	September/October	S	8.6[a]	c	Rancane et al. 2016
P. arundinacea	Latvia	September/October	S	7.4[a]	c	Rancane et al. 2016
P. arundinacea	Latvia	September/October	S	4.7[a]	c	Rancane et al. 2016
P. arundinacea	Latvia	September/October	S	6.8[a]	c	Rancane et al. 2016
P. arundinacea	Latvia	September/October	S	6.9[a]	c	Rancane et al. 2016
P. arundinacea	Latvia	September/October	S	2.7[a]	c	Rancane et al. 2016
P. arundinacea	Latvia	September/October	S	9.0[a]	c	Rancane et al. 2016
P. arundinacea	Latvia	September/October	S	4.4[a]	c	Rancane et al. 2016
P. arundinacea	Poland	late July	F	4.6	c	Roj-Rojewski et al. 2019
P. arundinacea	Poland	late August	LF	7.0	c	Roj-Rojewski et al. 2019
P. arundinacea	Poland	early October	SR	7.0	c	Roj-Rojewski et al. 2019
P. arundinacea	Germany	May	n.r.	1.8[a]	c	Schulz et al. 2011
P. arundinacea	Germany	July	n.r.	6.0[a]	c	Schulz et al. 2011
P. arundinacea	Germany	September	n.r.	7.1[a]	c	Schulz et al. 2011
P. arundinacea	Finland	late June	n.r.	5.2	c	Seppälä et al. 2009
P. arundinacea	Finland	late June	EF	4.2	c	Seppälä et al. 2009
P. arundinacea	Finland	early July	F	7.5	c	Seppälä et al. 2009
P. arundinacea	Finland	mid-June	EF	3.4	c	Seppälä et al. 2009
P. arundinacea	Finland	late September	n.r.	13.7	c	Seppälä et al. 2009
P. arundinacea	Czech Republic	April	n.r.	2.0[a]	c	Vymazal and Krőpfelová 2005
P. arundinacea	Czech Republic	May	n.r.	6.5[a]	c	Vymazal and Krőpfelová 2005

Table S2: Continued.

plant species	country	harvest time	growth stage	biomass yield (t DM ha^{-1})	cultivated (c), natural stand (n)	reference
P. arundinacea	Czech Republic	June	n.r.	17.3[a]	c	Vymazal and Krőpfelová 2005
P. arundinacea	Czech Republic	July	n.r.	17.8[a]	c	Vymazal and Krőpfelová 2005
P. arundinacea	Czech Republic	September	n.r.	15.4[a]	c	Vymazal and Krőpfelová 2005
P. arundinacea	Czech Republic	October	n.r.	15.2[a]	c	Vymazal and Krőpfelová 2005
P. arundinacea	Czech Republic	December	n.r.	14.8[a]	c	Vymazal and Krőpfelová 2005
P. arundinacea	Czech Republic	April	n.r.	1.0[a]	c	Vymazal and Krőpfelová 2005
P. arundinacea	Czech Republic	May	n.r.	6.5[a]	c	Vymazal and Krőpfelová 2005
P. arundinacea	Czech Republic	June	n.r.	17.7[a]	c	Vymazal and Krőpfelová 2005
P. arundinacea	Czech Republic	July	n.r.	17.1[a]	c	Vymazal and Krőpfelová 2005
P. arundinacea	Czech Republic	August	n.r.	15.6[a]	c	Vymazal and Krőpfelová 2005
P. arundinacea	Czech Republic	December	n.r.	13.8[a]	c	Vymazal and Krőpfelová 2005
P. arundinacea	Belarus	March/April	n.r.	9.6	c	Wichtmann et al. 2014
P. australis	Italy	mid-May	n.r.	6.4[a]	c	Dragoni et al. 2017
P. australis	Italy	mid-June	n.r.	11.5[a]	c	Dragoni et al. 2017
P. australis	Italy	mid-July	n.r.	12.4[a]	c	Dragoni et al. 2017
P. australis	Italy	late August	n.r.	14.9[a]	c	Dragoni et al. 2017
P. australis	Italy	late September	n.r.	19.4[a]	c	Dragoni et al. 2017
P. australis	Czech Republic	mid-July	n.r.	11.1	n	Dykyjová and Hradecká 1976
P. australis	Czech Republic	late September	n.r.	15.4	n	Dykyjová and Hradecká 1976
P. australis	Czech Republic	early September	n.r.	19.2	n	Dykyjová and Hradecká 1976
P. australis	Czech Republic	mid-August	n.r.	16.1	n	Dykyjová and Hradecká 1976
P. australis	Czech Republic	early August	n.r.	13.4	n	Dykyjová and Hradecká 1976
P. australis	Czech Republic	early August	n.r.	13.7	n	Dykyjová and Hradecká 1976
P. australis	Czech Republic	mid-August	n.r.	13.3	n	Dykyjová and Hradecká 1976
P. australis	Czech Republic	mid-August	n.r.	14.6	n	Dykyjová and Hradecká 1976

Table S2: Continued.

plant species	country	harvest time	growth stage	biomass yield (t DM ha^{-1})	cultivated (c), natural stand (n)	reference
P. australis	Czech Republic	late August	n.r.	16.6	n	Dykyjová and Hradecká 1976
P. australis	Czech Republic	mid-September	n.r.	19.9	n	Dykyjová and Hradecká 1976
P. australis	Czech Republic	late September	n.r.	17.5	n	Dykyjová and Hradecká 1976
P. australis	Czech Republic	early September	n.r.	30.0	n	Dykyjová and Hradecká 1976
P. australis	Czech Republic	early October	n.r.	24.4	n	Dykyjová and Hradecká 1976
P. australis	Czech Republic	mid-August	n.r.	18.6	n	Dykyjová and Hradecká 1976
P. australis	Czech Republic	mid-August	n.r.	15.2	n	Dykyjová and Hradecká 1976
P. australis	Czech Republic	mid-August	n.r.	22.9	n	Dykyjová and Hradecká 1976
P. australis	Czech Republic	late October	n.r.	32.6	n	Dykyjová and Hradecká 1976
P. australis	Italy	June	n.r.	12.8[a]	c	Giannini et al. 2016
P. australis	Italy	September	n.r.	15.0[a]	c	Giannini et al. 2016
P. australis	Italy	February	n.r.	11.6[a]	c	Giannini et al. 2016
P. australis	Italy	September	n.r.	8.0[a]	c	Giannini et al. 2017
P. australis	Italy	September	n.r.	14.9[a]	c	Giannini et al. 2017
P. australis	Sweden	August	n.r.	10.0	n	Granéli 1984
P. australis	Sweden	February	n.r.	5.3	n	Granéli 1984
P. australis	Sweden	early September	n.r.	9.8	n	Granéli 1984
P. australis	Sweden	late October	n.r.	7.2	n	Granéli 1984
P. australis	Sweden	late July	n.r.	17.8	n	Granéli 1984
P. australis	Rumania	August/September	n.r.	9.8	n	Hanganu et al. 1999
P. australis	Rumania	August/September	n.r.	16.3	n	Hanganu et al. 1999
P. australis	Estonia	September	n.r.	13.2[a]	c	Maddison et al. 2009
P. australis	Estonia	January	n.r.	6.1[a]	c	Maddison et al. 2009
P. australis	Estonia	September	n.r.	12.5[a]	c	Maddison et al. 2009
P. australis	Estonia	January	n.r.	7.2[a]	c	Maddison et al. 2009

Table S2: Continued.

plant species	country	harvest time	growth stage	biomass yield (t DM ha^{-1})	cultivated (c), natural stand (n)	reference
P. australis	Estonia	September	n.r.	10.5[a]	c	Maddison et al. 2009
P. australis	Estonia	January	n.r.	10.2[a]	c	Maddison et al. 2009
P. australis	England	mid-May	n.r.	0.7	n	Mason and Bryant 1975
P. australis	England	early June	n.r.	3.3	n	Mason and Bryant 1975
P. australis	England	early July	n.r.	7.3	n	Mason and Bryant 1975
P. australis	England	early August	n.r.	9.4	n	Mason and Bryant 1975
P. australis	England	late August	n.r.	9.1	n	Mason and Bryant 1975
P. australis	England	late September	n.r.	8.7	n	Mason and Bryant 1975
P. australis	England	late October	n.r.	5.9	n	Mason and Bryant 1975
P. australis	England	early June	n.r.	1.2	n	Mason and Bryant 1975
P. australis	England	early July	n.r.	5.2	n	Mason and Bryant 1975
P. australis	England	early August	n.r.	5.0	n	Mason and Bryant 1975
P. australis	England	early September	n.r.	3.9	n	Mason and Bryant 1975
P. australis	England	late September	n.r.	3.7	n	Mason and Bryant 1975
P. australis	Poland	late July	EF	8.5	n	Roj-Rojewski et al. 2019
P. australis	Poland	late August	F	9.3	n	Roj-Rojewski et al. 2019
P. australis	Poland	early October	SR	9.3	n	Roj-Rojewski et al. 2019
P. australis	Germany	May	n.r.	3.8[a]	n	Schulz et al. 2011
P. australis	Germany	July	n.r.	15.3[a]	n	Schulz et al. 2011
P. australis	Germany	September	n.r.	11.4[a]	n	Schulz et al. 2011
P. australis	USA	summer	n.r.	9.8	n	Templer et al. 1998
P. australis	Czech Republic	May	n.r.	4.1[a]	c	Vymazal and Krőpfelová 2005
P. australis	Czech Republic	June	n.r.	12.3[a]	c	Vymazal and Krőpfelová 2005
P. australis	Czech Republic	July	n.r.	18.6[a]	c	Vymazal and Krőpfelová 2005
P. australis	Czech Republic	September	n.r.	20.8[a]	c	Vymazal and Krőpfelová 2005

Table S2: Continued.

plant species	country	harvest time	growth stage	biomass yield (t DM ha^{-1})	cultivated (c), natural stand (n)	reference
P. australis	Czech Republic	October	n.r.	19.7[a]	c	Vymazal and Krőpfelová 2005
P. australis	Czech Republic	December	n.r.	15.9[a]	c	Vymazal and Krőpfelová 2005
P. australis	Czech Republic	May	n.r.	4.5[a]	c	Vymazal and Krőpfelová 2005
P. australis	Czech Republic	June	n.r.	15.0[a]	c	Vymazal and Krőpfelová 2005
P. australis	Czech Republic	July	n.r.	17.5[a]	c	Vymazal and Krőpfelová 2005
P. australis	Czech Republic	August	n.r.	21.5[a]	c	Vymazal and Krőpfelová 2005
P. australis	Czech Republic	December	n.r.	16.0[a]	c	Vymazal and Krőpfelová 2005
P. australis	Belarus	March/April	n.r.	11.7	n	Wichtmann et al. 2014
P. australis	Belarus	March	n.r.	4.6	n	Wichtmann et al. 2014
P. australis	Belarus	March/April	n.r.	9.8	n	Wichtmann et al. 2014
P. australis	Belarus	March	n.r.	3.7	n	Wichtmann et al. 2014
T. latifolia	USA	mid-April	n.r.	0.5[a]	n	Boyd 1970
T. latifolia	USA	early May	n.r.	1.8[a]	n	Boyd 1970
T. latifolia	USA	mid-May	n.r.	3.6[a]	n	Boyd 1970
T. latifolia	USA	late May	n.r.	6.0[a]	n	Boyd 1970
T. latifolia	USA	mid-June	n.r.	6.8[a]	n	Boyd 1970
T. latifolia	USA	mid-July	n.r.	6.6[a]	n	Boyd 1970
T. latifolia	USA	late April	n.r.	2.2[a]	n	Boyd 1971
T. latifolia	USA	early May	n.r.	4.5[a]	n	Boyd 1971
T. latifolia	USA	mid-May	n.r.	6.7[a]	n	Boyd 1971
T. latifolia	USA	early June	n.r.	7.9[a]	n	Boyd 1971
T. latifolia	USA	late June	n.r.	10.3[a]	n	Boyd 1971
T. latifolia	USA	late July	n.r.	11.3[a]	n	Boyd 1971
T. latifolia	USA	late August	n.r.	8.5[a]	n	Boyd 1971
T. latifolia	USA	late April	n.r.	1.3[a]	n	Boyd 1971

Table S2: Continued.

plant species	country	harvest time	growth stage	biomass yield (t DM ha^{-1})	cultivated (c), natural stand (n)	reference
T. latifolia	USA	early May	n.r.	3.4[a]	n	Boyd 1971
T. latifolia	USA	mid-May	n.r.	4.9[a]	n	Boyd 1971
T. latifolia	USA	early June	n.r.	7.3[a]	n	Boyd 1971
T. latifolia	USA	late June	n.r.	9.6[a]	n	Boyd 1971
T. latifolia	USA	late July	n.r.	9.2[a]	n	Boyd 1971
T. latifolia	USA	late August	n.r.	7.1[a]	n	Boyd 1971
T. latifolia	USA	late April	n.r.	1.8[a]	n	Boyd 1971
T. latifolia	USA	early May	n.r.	2.5[a]	n	Boyd 1971
T. latifolia	USA	mid-May	n.r.	3.6[a]	n	Boyd 1971
T. latifolia	USA	early June	n.r.	5.5[a]	n	Boyd 1971
T. latifolia	USA	late June	n.r.	6.8[a]	n	Boyd 1971
T. latifolia	USA	late July	n.r.	7.5[a]	n	Boyd 1971
T. latifolia	USA	late August	n.r.	7.3[a]	n	Boyd 1971
T. latifolia	USA	late April	n.r.	1.5[a]	n	Boyd 1971
T. latifolia	USA	early May	n.r.	2.1[a]	n	Boyd 1971
T. latifolia	USA	mid-May	n.r.	2.9[a]	n	Boyd 1971
T. latifolia	USA	early June	n.r.	4.0[a]	n	Boyd 1971
T. latifolia	USA	late June	n.r.	6.2[a]	n	Boyd 1971
T. latifolia	USA	late July	n.r.	7.9[a]	n	Boyd 1971
T. latifolia	USA	late August	n.r.	6.5[a]	n	Boyd 1971
T. latifolia	USA	late April	n.r.	1.1[a]	n	Boyd 1971
T. latifolia	USA	early May	n.r.	1.9[a]	n	Boyd 1971
T. latifolia	USA	mid-May	n.r.	2.3[a]	n	Boyd 1971
T. latifolia	USA	early June	n.r.	3.3[a]	n	Boyd 1971
T. latifolia	USA	late June	n.r.	4.9[a]	n	Boyd 1971

Table S2: Continued.

plant species	country	harvest time	growth stage	biomass yield (t DM ha^{-1})	cultivated (c), natural stand (n)	reference
T. latifolia	USA	late July	n.r.	5.3[a]	n	Boyd 1971
T. latifolia	USA	late August	n.r.	2.7[a]	n	Boyd 1971
T. latifolia	USA	mid-May	n.r.	0.1	c	Garver et al. 1988
T. latifolia	USA	early June	n.r.	0.6	c	Garver et al. 1988
T. latifolia	USA	early July	n.r.	4.3	c	Garver et al. 1988
T. latifolia	USA	early August	n.r.	7.3	c	Garver et al. 1988
T. latifolia	USA	early September	n.r.	8.2	c	Garver et al. 1988
T. latifolia	USA	late September	n.r.	7.2	c	Garver et al. 1988
T. latifolia	USA	late October	n.r.	8.2	c	Garver et al. 1988
T. angustifolia	USA	mid-May	n.r.	0.0	c	Garver et al. 1988
T. angustifolia	USA	early June	n.r.	0.1	c	Garver et al. 1988
T. angustifolia	USA	early July	n.r.	2.1	c	Garver et al. 1988
T. angustifolia	USA	early August	n.r.	2.7	c	Garver et al. 1988
T. angustifolia	USA	early September	n.r.	4.0	c	Garver et al. 1988
T. angustifolia	USA	late September	n.r.	5.2	c	Garver et al. 1988
T. angustifolia	USA	late October	n.r.	4.8	c	Garver et al. 1988
T. latifolia + *T.* x *glauca*	Canada	August	n.r.	6.0[a]	c	Jeke et al. 2018
T. latifolia + *T.* x *glauca*	Canada	August	n.r.	5.8[a]	c	Jeke et al. 2018
T. latifolia + *T.* x *glauca*	Canada	August	n.r.	5.9[a]	c	Jeke et al. 2018
T. x *glauca*	USA	late May	n.r.	0.4[a]	n	Kim 1985
T. x *glauca*	USA	early June	n.r.	0.9[a]	n	Kim 1985
T. x *glauca*	USA	late June	n.r.	4.2[a]	n	Kim 1985
T. x *glauca*	USA	mid-July	n.r.	14.0[a]	n	Kim 1985
T. x *glauca*	USA	late August	n.r.	17.1[a]	n	Kim 1985
T. x *glauca*	USA	mid-September	n.r.	13.5[a]	n	Kim 1985

Table S2: Continued.

plant species	country	harvest time	growth stage	biomass yield (t DM ha^{-1})	cultivated (c), natural stand (n)	reference
T. latifolia	Estonia	September	n.r.	15.4[a]	n	Maddison et al. 2009
T. latifolia	Estonia	September	n.r.	14.4[a]	n	Maddison et al. 2009
T. latifolia	Estonia	January	n.r.	13.8[a]	n	Maddison et al. 2009
T. latifolia	Estonia	September	n.r.	6.3[a]	n	Maddison et al. 2009
T. latifolia	Estonia	January	n.r.	4.5[a]	n	Maddison et al. 2009
T. latifolia	Estonia	September	n.r.	11.8[a]	n	Maddison et al. 2009
T. latifolia	Estonia	January	n.r.	6.3[a]	n	Maddison et al. 2009
T. latifolia	Estonia	September	n.r.	12.0[a]	n	Maddison et al. 2009
T. latifolia	Estonia	January	n.r.	7.3[a]	n	Maddison et al. 2009
T. latifolia	Estonia	September	n.r.	14.2[a]	c	Maddison et al. 2009
T. latifolia	Estonia	September	n.r.	14.9[a]	c	Maddison et al. 2009
T. latifolia	Estonia	January	n.r.	5.2[a]	c	Maddison et al. 2009
T. latifolia	Estonia	September	n.r.	17.6[a]	c	Maddison et al. 2009
T. latifolia	Estonia	January	n.r.	7.1[a]	c	Maddison et al. 2009
T. latifolia	Estonia	September	n.r.	12.7[a]	c	Maddison et al. 2009
T. latifolia	Estonia	January	n.r.	3.4[a]	c	Maddison et al. 2009
T. latifolia	Estonia	September	n.r.	3.7[a]	c	Maddison et al. 2009
T. latifolia	Estonia	January	n.r.	3.6[a]	c	Maddison et al. 2009
T. latifolia	Estonia	September	n.r.	12.3[a]	c	Maddison et al. 2009
T. latifolia	Estonia	September	n.r.	17.3[a]	c	Maddison et al. 2009
T. latifolia	Estonia	January	n.r.	10.2[a]	c	Maddison et al. 2009
T. angustifolia	England	early May	n.r.	2.6	n	Mason and Bryant 1975
T. angustifolia	England	early June	n.r.	7.6	n	Mason and Bryant 1975
T. angustifolia	England	early July	n.r.	10.1	n	Mason and Bryant 1975
T. angustifolia	England	early August	n.r.	11.2	n	Mason and Bryant 1975

Table S2: Continued.

plant species	country	harvest time	growth stage	biomass yield (t DM ha^{-1})	cultivated (c), natural stand (n)	reference
T. angustifolia	England	early September	n.r.	9.1	n	Mason and Bryant 1975
T. angustifolia	England	late September	n.r.	8.9	n	Mason and Bryant 1975
T. latifolia	Netherlands	late May	n.r.	3.7[a]	c	Pijlman et al. 2019
T. latifolia	Netherlands	late May	n.r.	6.8[a]	c	Pijlman et al. 2019
T. latifolia	Netherlands	early July	n.r.	5.6[a]	c	Pijlman et al. 2019
T. latifolia	Netherlands	early July	n.r.	6.3[a]	c	Pijlman et al. 2019
T. latifolia	Netherlands	early August	n.r.	6.5[a]	c	Pijlman et al. 2019
T. latifolia	Netherlands	early August	n.r.	11.1[a]	c	Pijlman et al. 2019
T. latifolia	Netherlands	mid-September	n.r.	9.8[a]	c	Pijlman et al. 2019
T. latifolia	Netherlands	late October	n.r.	7.4[a]	c	Pijlman et al. 2019
T. latifolia	Netherlands	mid-May	n.r.	2.7[a]	c	Pijlman et al. 2019
T. latifolia	Netherlands	mid-May	n.r.	4.0[a]	c	Pijlman et al. 2019
T. latifolia	Netherlands	early June	n.r.	5.3[a]	c	Pijlman et al. 2019
T. latifolia	Netherlands	early June	n.r.	7.6[a]	c	Pijlman et al. 2019
T. latifolia	Netherlands	mid-June	n.r.	7.5[a]	c	Pijlman et al. 2019
T. latifolia	Netherlands	mid-June	n.r.	8.2[a]	c	Pijlman et al. 2019
T. latifolia	Netherlands	late June	n.r.	10.4[a]	c	Pijlman et al. 2019
T. latifolia	Netherlands	late June	n.r.	11.3[a]	c	Pijlman et al. 2019
T. latifolia	Netherlands	late July	n.r.	10.0[a]	c	Pijlman et al. 2019
T. latifolia	Netherlands	late July	n.r.	11.1[a]	c	Pijlman et al. 2019
T. latifolia	Netherlands	mid-August	n.r.	8.7[a]	c	Pijlman et al. 2019
T. latifolia	Netherlands	mid-August	n.r.	9.9[a]	c	Pijlman et al. 2019
T. latifolia	Netherlands	early September	n.r.	10.9[a]	c	Pijlman et al. 2019
T. latifolia	Netherlands	early September	n.r.	11.3[a]	c	Pijlman et al. 2019
T. latifolia	Netherlands	late September	n.r.	6.8[a]	c	Pijlman et al. 2019

Table S2: Continued.

plant species	country	harvest time	growth stage	biomass yield (t DM ha^{-1})	cultivated (c), natural stand (n)	reference
T. latifolia	Netherlands	late September	n.r.	7.8[a]	c	Pijlman et al. 2019
T. latifolia	Germany	May	n.r.	4.5[a]	n	Schulz et al. 2011
T. latifolia	Germany	July	n.r.	9.3[a]	n	Schulz et al. 2011
T. latifolia	Germany	September	n.r.	6.1[a]	n	Schulz et al. 2011
T. angustifolia	Japan	early May	n.r.	6.1[a]	n	Sharma et al. 2006
T. angustifolia	Japan	early June	n.r.	14.3[a]	n	Sharma et al. 2006
T. angustifolia	Japan	early July	n.r.	17.5[a]	n	Sharma et al. 2006
T. angustifolia	Japan	mid-August	n.r.	19.2[a]	n	Sharma et al. 2006
T. angustifolia	Japan	mid-September	n.r.	23.0[a]	n	Sharma et al. 2006
T. angustifolia	Japan	early October	n.r.	21.6[a]	n	Sharma et al. 2006
T. angustifolia	Japan	early November	n.r.	13.2[a]	n	Sharma et al. 2006
T. angustifolia	Japan	early April	n.r.	4.3[a]	n	Sharma et al. 2006
T. angustifolia	Japan	late April	n.r.	6.0[a]	n	Sharma et al. 2006
T. angustifolia	Japan	mid-May	n.r.	8.8[a]	n	Sharma et al. 2006
T. angustifolia	Japan	late May	n.r.	12.1[a]	n	Sharma et al. 2006
T. angustifolia	Japan	mid-June	n.r.	16.7[a]	n	Sharma et al. 2006
T. angustifolia	Japan	late July	n.r.	24.0[a]	n	Sharma et al. 2006
T. angustifolia	Japan	late August	n.r.	27.3[a]	n	Sharma et al. 2006
T. angustifolia	Japan	late September	n.r.	26.7[a]	n	Sharma et al. 2006
T. angustifolia	Japan	late October	n.r.	23.2[a]	n	Sharma et al. 2006
T. latifolia	USA	late April	n.r.	0.4[a]	n	Smith et al. 1988
T. latifolia	USA	mid-May	n.r.	0.9[a]	n	Smith et al. 1988
T. latifolia	USA	late May	n.r.	2.3[a]	n	Smith et al. 1988
T. latifolia	USA	mid-June	n.r.	6.6[a]	n	Smith et al. 1988
T. latifolia	USA	late June	n.r.	7.8[a]	n	Smith et al. 1988

Table S2: Continued.

plant species	country	harvest time	growth stage	biomass yield (t DM ha^{-1})	cultivated (c), natural stand (n)	reference
T. latifolia	USA	mid-July	n.r.	8.5[a]	n	Smith et al. 1988
T. latifolia	USA	late July	n.r.	9.4[a]	n	Smith et al. 1988
T. latifolia	USA	mid-August	n.r.	12.1[a]	n	Smith et al. 1988
T. latifolia	USA	late August	n.r.	14.0[a]	n	Smith et al. 1988
T. latifolia	USA	mid-September	n.r.	11.3[a]	n	Smith et al. 1988
T. latifolia	USA	late September	n.r.	11.2[a]	n	Smith et al. 1988
T. angustifolia	USA	summer	n.r.	5.1	n	Templer et al. 1998
C. diandra	Netherlands	mid-September	n.r.	1.6[a]	c	Aerts and de Caluwe 1994
C. rostrata	Netherlands	mid-September	n.r.	2.5[a]	c	Aerts and de Caluwe 1994
C. acutiformis	Netherlands	mid-September	n.r.	7.1[a]	c	Aerts and de Caluwe 1994
C. acutiformis	Germany	July	n.r.	8.5	n	Günther et al. 2015
C. acutiformis	Netherlands	early May	n.r.	1.1	n	Hirose et al. 1989
C. acutiformis	Netherlands	mid-June	n.r.	2.5	n	Hirose et al. 1989
C. acutiformis	Netherlands	late July	n.r.	3.9	n	Hirose et al. 1989
C. elata	Poland	late July	SR	5.8	n	Roj-Rojewski et al. 2019
C. elata	Poland	late August	SR	7.4	n	Roj-Rojewski et al. 2019
C. elata	Poland	early October	SR	7.4	n	Roj-Rojewski et al. 2019
C. lasiocarpa	Poland	late July	SR	0.8	n	Roj-Rojewski et al. 2019
C. lasiocarpa	Poland	late August	SR	1.4	n	Roj-Rojewski et al. 2019
C. lasiocarpa	Poland	early October	SR	1.4	n	Roj-Rojewski et al. 2019
C. riparia	Germany	May	n.r.	5.2[a]	n	Schulz et al. 2011
C. riparia	Germany	July	n.r.	5.9[a]	n	Schulz et al. 2011
C. riparia	Germany	September	n.r.	7.4[a]	n	Schulz et al. 2011
C. acutiformis	Netherlands	April	n.r.	2.4[a]	n	Verhoeven et al. 1983
C. acutiformis	Netherlands	May	n.r.	4.0[a]	n	Verhoeven et al. 1983

Table S2: Continued.

plant species	country	harvest time	growth stage	biomass yield (t DM ha^{-1})	cultivated (c), natural stand (n)	reference
C. acutiformis	Netherlands	June	n.r.	6.5[a]	n	Verhoeven et al. 1983
C. acutiformis	Netherlands	July	n.r.	7.9[a]	n	Verhoeven et al. 1983
C. acutiformis	Netherlands	August	n.r.	7.4[a]	n	Verhoeven et al. 1983
C. rostrata	Netherlands	April	n.r.	1.0[a]	n	Verhoeven et al. 1983
C. rostrata	Netherlands	May	n.r.	2.4[a]	n	Verhoeven et al. 1983
C. rostrata	Netherlands	June	n.r.	2.4[a]	n	Verhoeven et al. 1983
C. rostrata	Netherlands	July	n.r.	3.0[a]	n	Verhoeven et al. 1983
C. diandra	Netherlands	April	n.r.	0.7[a]	n	Verhoeven et al. 1983
C. diandra	Netherlands	May	n.r.	1.0[a]	n	Verhoeven et al. 1983
C. diandra	Netherlands	June	n.r.	2.6[a]	n	Verhoeven et al. 1983
C. diandra	Netherlands	July	n.r.	3.5[a]	n	Verhoeven et al. 1983
Carex spp.	Belarus	March/April	n.r.	7.0	n	Wichtmann et al. 2014

[a] value extracted from graph with WebPlotDigitizer (v4.6; Rohatgi, 2022), n.r. (not reported)

EH (early heading), F (flowering), EF (early flowering), LF (late flowering), SR (seed ripening), S (senescence)

List of scientific contributions

Peer-reviewed publications

Hartung, C., Dandikas, V., Eickenscheidt, T., Zollfrank, C., & Heuwinkel, H. (2023). Optimal harvest time for high biogas and biomass yield of *Typha latifolia*, *Typha angustifolia* and *Phalaris arundinacea*. *Biomass and Bioenergy*, *175*, 106847. https://doi.org/10.1016/j.biombioe.2023.106847

Hartung, C., Andrade, D., Dandikas, V., Eickenscheidt, T., Drösler, M., Zollfrank, C., & Heuwinkel, H. (2020). Suitability of paludiculture biomass as biogas substrate − biogas yield and long-term effects on anaerobic digestion. *Renewable Energy*, *159*, 64-71. https://doi.org/10.1016/j.renene.2020.05.156

Other publications

Hartung, C., & Heuwinkel, H. (2022). Wie lassen sich Moore klimaschonend nutzen? In *DLG-Mitteilungen, 3,* 94-96.

Hartung, C., Heuwinkel, H., Dandikas, V., & Eickenscheidt, T. (2021). MOORuse - Paludikulturen als Biogassubstrat. In KTBL-Schrift 524, Biogas in der Landwirtschaft - Stand und Perspektiven, 163-170.

Oral presentations

Hartung, C., Heuwinkel, H., Dandikas, V., & Eickenscheidt, T. (2021). MOORuse - Paludikulturen als Biogassubstrat. In 7. FNR/KTBL-Kongress Biogas in der Landwirtschaft - Stand und Perspektiven, 29.-30.09.2021, online.

Hartung, C., Heuwinkel, H., Dandikas, V. Eickenscheidt, T., & Zollfrank, C. (2021). The optimal harvest date of *Typha latifolia* and *Phalaris arundinacea* for the use as biogas substrates. In RRR2021 - Renewable resources from wet and rewetted peatlands, 09. - 11.03.2021, online.

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Poster presentations

Hartung, C., Meinken, E., Heuwinkel, H. & Drösler, M. (2022). Potential der Nutzung von Niedermoor-Paludikulturen als Biogassubstrat und als Torfersatzstoff (Projekt MOORuse). In Symposium Moorschutz: Forschung und Praxis verbinden, 19.-20.09.2022, Rosenheim.

Hartung, C., Dandikas, V., Heuwinkel, H., Eickenscheidt, T., Zollfrank, C. & Drösler, M. (2020). Influence of the harvest date on the specific biogas production from *Typha latifolia* and

Phalaris arundinacea. In Venice2020 - 8th international symposium on energy from biomass and waste, 16.-19.11.2020, online.

Hartung, C., Zollfrank, C., Dandikas, V., Eickenscheidt, T. & Heuwinkel, H. (2018). Biomass from paludiculture as substrate for biogas formation. In Venice2018 - 7th international symposium on energy from biomass and waste, 15.-18.10.2018, Venice, Italy.

Hartung, C., Heuwinkel, H., Lichti, F., & Dandikas, V. (2017). Biogasertragspotential von Paludikultur-Pflanzen. In Klimaschutz und Moornutzung: Potentiale in Deutschland, 25.-26.09.2017, Greifswald, Germany.