November *2018*

Final project report (2018)

(Information will be part of the final BioValue reporting to the Innovation Fund Denmark on 31/12-2018. Status should cover specific progress in the period February 1, 2018 – October 1, 2018 and accumulated results over the last 5 years)

Project no.	Project title	Project leader
1	**Innovative biomass production systems, harvest, and conservation technologies**	**Lars Villadsgaard Toft**

For 2018: Milestones and Deliverables for the project in the period Feb 2018 – October 2018
List the status of the tasks engaged in the period based on the last activity plan. If table is not relevant, use minimum 100 words.

Milestones and Deliverables 2018	Status (Done, ongoing, delayed)	Comments to status
KU: Determination of mineral composition of green biomass fractions from biorefinement of green biomasses to soluble fraction, green-protein pellet, and fibrous pulp. (deadline April	Done	The analysis has been done on grass-clover material. The results will be published in paper on fractionation of green biomass (project 2)
KU: Analysing, writing and submission of paper "Pretreatment of wheat straw before enzymatic saccharification affects the detectability of genetic variation in released sugars between cultivars" (deadline March 2018)	Ongoing	The paper was delayed due to maternity leave but is now almost finalised, only figure editing is missing (Submission expected in January 2019)
KU: Analysing, writing and submission of paper "Identification of gene markers associated with high straw yield and quality traits without compromising grain yield and quality" (deadline August 2018)	Ongoing	The paper was delayed due to maternity leave but is now almost finalised, figure editing and discussion are ongoing (Submission expected in December 2018)
KU: Analysing, writing and submission of paper "Identification of genetic differences in straw morphology between winter wheat cultivars" (deadline September 2018)	Delayed	The paper is put on standby
KU: Analysing, writing and submission of paper "Statistical methods for analysing association mapping in data consisting of multiple small crossing populations" (deadline June 2018)	Ongoing	The paper is put on standby Data analysis is ongoing and a first drift is expected to be available in January 2019
KU: Fractionation and processing of green Trilobit biomass into protein and sugars. Analysis and submission of paper (deadline September 2018)	Delayed	All analyses are done and preparation of paper ongoing. Submission December 2018.
AU: Results 2013-2015 on biomass, radiation utilisation, nitrogen balances and protein contents are completed and work published.	Done	
AU: Modelling the field experiments from Flakkebjerg. Kiril is spending half-2018 in China, but it was discussed with Uffe that the work will be conducted there. The aim is throughout 2018, the Flakkebjerg experiments to be modelled, nitrate leaching to be calculated, and publication comparing data for 2016-2018 between Foulum and Flakkebjerg to be published. Kiril and Uffe are in contact with the colleagues in Flakkebjerg for field data supply.	Delayed.	Data from Flakkebjerg has been collected, however it was not achieved to publish yet. Kirils position is running out but other postdocs from AU can start conducting this task soon. We expect, that work can be done throughout 2019.
AU: One of the papers planned is on water balance of the innovative cropping systems for biorefinery at Foulum. Whether this will be achieved in 2018 or not depends much on time, since the previous activity has priority.	Delayed.	The data is obtained, but has not yet been analysed. Same plan as above.
AU: One of the papers planned is on soil carbon development for the innovative cropping systems for biorefinery at Foulum. Whether this will be achieved in 2018 or not depends much on time, since many data are yet to be delivered from the lab (so far only topsoil carbon and nitrogen are delivered). Also, we have to evaluate if two points of data on a time development is enough for a publication	Ongoing	Topsoil data are obtained and these have been presented at a conference (see relevant section for details). A detailed analysis including correction for soil density has been performed and data is discussed for possible publication.
DTI: Protein extraction and protein yield from tall fescue depending on N fertilization: Preparation and submission of paper (June 2018)	Done	Manuscript submitted to Industrial Crops and Products 24/5 2018. No comments received yet.
DTI: Storage trials with ensiling of grass fibre: Analysis of methane potential in grass fibre with and without ensiling,	Ongoing	Data have been analysed and manuscript is nearly ready for submission, expected

analysis of various data, preparation and submission of manuscript (October 2018)		submission to Bioresource Technology December 2018.
SEJET: Implementation of developed DNA markers	Done/ongoing	Selection of wheat populations in the field with potential of high straw yield, was done based on marker data, including markers on the dwarfing genes. Individuals of a population containing different levels of filled straw-pith in cross section between ear and upper node, has been selected. Markers for this trait are currently being developed and will tested on these lines
SEGES: Transfer of knowledge from the BioValue Spir Platform to advisory services for farmers and back from farmers/advisory services to BioValue	Ongoing	A seminar on Green Biorefining was held on the 17th of September at AU, Foulum. More than 100 persons attended the seminar, and among the participants where many farmers and local farm advisors. SEGES has published multiple articles and reports on SEGES project site and Landbrugsinfo.dk. Landbrugsinfo.dk has each month visited by 50.000 people from the agro-industry.
SEGES: Possibilities & barriers for implementation of biorefining scenarios into agriculture	Done	Article has been published on SEGES project site and on Landbrugsinfo.dk
SEGES: Improvement of existing logistic tool to fit the grass protein scenario	Done	A logistics tool for calculation of biomass harvesting and transportation costs in Green Biorefining

5 year status: Overall research results *(The section will be used publicly)*

Go to your project website and look at "preliminary results" – now write a "Final results" to be added to the website too. Please include illustrations and text suitable for reading online. (Minimum 500 words – maximum 800). Please address the objectives of your project according to the original application, which were:

Project	Project objectives
Project 1: Innovative biomass production, harvest and conservation	(i) Increase quantity and quality of biomass for biorefining (multi-purpose production systems, annual and perennial plant species, genotypic differences)
	(ii) Extending the crop growing season and exploring plant complementarity
	(iii) Optimise land management, logistics, biomass harvesting and storage
	(iv) Develop cost-effective management practices for marginal lands to harvest their biomass potential
Project 2: Products from green biomass	To develop process-technologies, enzyme systems and analytical tools for maximizing yield and quality of products from different green biomass sources, demonstrated in a pilot plant (production of animal protein feed fractions and gut-healthy oligosaccharide feed additives from green biomass)
Project 3: Upgraded sugar streams from biomass	(i) To provide improved knowledge and technology base for efficient processing of plant biomass and carbohydrate product streams.
	(ii) Production of a range of biomass mono-saccharide streams, including C6 and C5 sugar streams of specified purities designated for different defined purposes.
	(iii) Dewatering concepts
	(iv) Production of lignin with improved properties for biorefining.
Project 4. Lysine production from a biomass-based sugar platform	Develop and demonstrate a microbial production of the amino acid lysine from C5 and C6 sugar streams based on low cost feedstocks which are competitive with the starch-based process
Project 5. Value-added products from catalytic conversion of carbohydrate feed streams	Develop novel catalytic processes for the chemical conversion of carbohydrate streams into value-added products including methyl lactate
Project 6. Value-added products from lignin	(i) Development and comparative evaluation of new methods for conversion of lignin into value-added products such as phenols
	(ii) Development and comparative evaluation of new methods for conversion of lignin into value-added products such as functional binders

SeSE (Socio-economics, Sustainability and Ethics)	To link the results from Project 1-6 into a production/value chain context based on the assumption that increased production of biomass and biobased products will have economic as well as organisational impact on a number of production sectors

KU

Agricultural residues, such as straw, offers an opportunity to produce biofuels and chemicals in biorefineries without compromising food production. The ideal “dual purpose cultivar” would have high yield of grain and straw. In addition, the straw should be easy to process in a biorefinery, i.e. good degradability, high concentration of carbohydrates and low concentration of ash. Nitrogen (N) is an essential nutrient important for plant growth, crop yield and grain quality. However, N production and application comes with a high cost and high environmental footprint. The N application should consequently be based on an economical optimum. Within the BioValue project, field trials have been conducted over five cropping seasons to investigate the effect of cultivar and N supply on the biorefining potential. Detailed analyses of the straw, including saccharification potential, have been performed. The experiment included 14 commercial wheat cultivars and one triticale cultivar. Among the wheat cultivars, Creator was the cultivar with highest straw yield and average grain yield – total above ground biomass yield 18.7 tonnes per ha. However, the triticale cultivar Trilobit had superior straw yield and also high grain yield – total above ground biomass yield 22.5 tonnes per ha. The N supply directly affected the yield of straw and grain. In addition, the protein concentration in grain and straw increased, but the composition of the straw with respect to carbohydrates and lignin was largely unaffected by N supply. The only significant change was a lower silicon concentration at increasing N application rate, which could be beneficial for lignin valorization in biorefineries. Likely due to the negligible changes in cell wall composition, the effect of N application rate on straw degradability was not significant. N application should therefore primarily be optimized with respect to grain quality and overall yield of grain and straw. Differences between cultivars were also minor with respect to their performance in a biorefinery process. From a breeding and agronomic perspective, focus should therefore be put on maximizing the biomass output from the field, i.e. selecting the cultivar with highest grain and straw yield and optimizing the application of fertilizer to get optimum N use efficiency. In conclusion, the triticale cultivar had the highest potential for release of sugar due to superior straw yield.

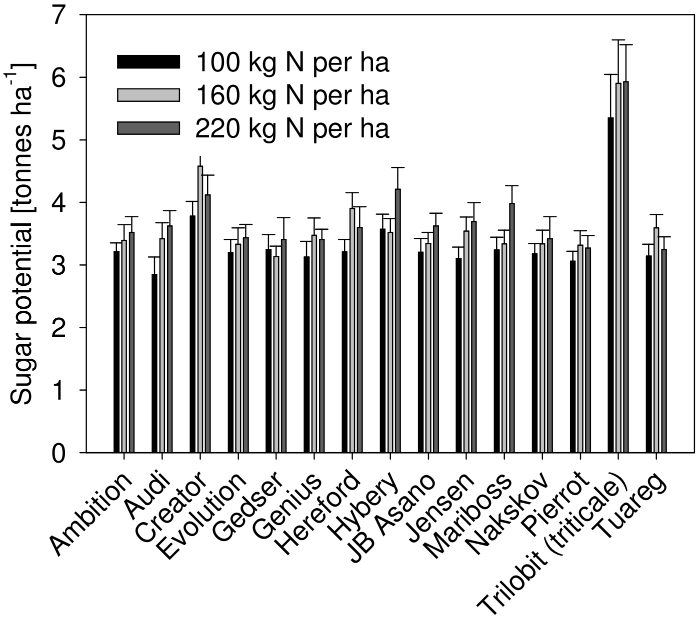


Figure 1: Total sugar (glucose and xylose) production potential on a hectare basis. Results are average of samples harvested 2013, 2014 and 2015 at maturity after application of 100, 160 and 220 kg N ha^{-1} in spring.

The field studies revealed that straw N concentration at harvest was strongly correlated with N application and significant amounts of N remained in the mature straw. To get better understanding of the influence of nitrogen application on the nitrogen uptake and distribution in plant at maturity, a detailed analysis of the nitrogen pools/compounds in wheat organs was conducted on winter wheat samples grown on a test field under 5 different nitrogen regimes (60, 100, 160, 220, 280 kg N/ha). Residual N concentrations were generally the highest in leaf blades followed by leaf sheaths and true stems. Non-protein N (nitrate, DNA, chlorophyll) only contributed minor to the overall pool of residual N and was significantly larger in leaf blades (14 %) than in leaf sheaths (8 %) and true stems (5 %). Generally, the ratio of non-protein N to total N of straw fractions was not significantly influenced by N fertilization. Protein N was determined by amino acid analysis on whole material (total amino acid N) and isolated cell wall material (CWP N). The difference between total amino acid N and CWP N was termed CP N and represents cytosolic protein and loosely bound CWP. In all preparations, the majority of the protein N was found as CWP N. The results revealed that the majority of residual straw N at harvest was in the form of protein that had not been effectively remobilised from the leaves during grain filling and therefore represents a potential loss of protein in the grain.

Early green harvest of Trilobit (in early June and early July) to produce green protein and pulp for biorefining to sugars was investigated. The total above ground dry matter biomass yield was the same if harvested in early July (GS80) as compared to at final harvest middle of

August (GS92). The amount of crude protein (CP) in the juice that could be extracted from the green triticale was higher than the amount of CP in the grain at final harvest, up to 1400 kg CP per ha. However, precipitation yields were only 50-60%, and the final yield of CP in the concentrated protein product was therefore only up to 800 kg CP per ha as compared to 1200 kg CP per ha in the harvested grain. The amino acid profile of the extracted protein thus seems more favorable compared to protein in the grain, e.g. higher content of essential amino acids - lysine (up to 100% higher) and threonine (up to 40% higher). The potential for utilizing the pulp for generation of sugars in a biorefinery setting revealed that the pulp was less recalcitrant compared to straw from the final harvest. Along with high concentrations of readily available sugars in the juice, the implications of this are that green harvest of Trilobit could be attractive in a biorefinery setup producing protein and sugars when also including the potential advantage of earlier establishment of a second crop.

Essential for the developing and screening for new cultivars with improved characteristics with respect to bioconversion or to study the effect of agricultural practices on the recalcitrance of the straw is the availability of a robust and reliable high throughput method for pretreatment and enzymatic hydrolysis. The influence of assay conditions on the ability of the high throughput method available at University of Copenhagen to pick up genetic variance in sugar (glucose, xylose and arabinose) release was investigated.

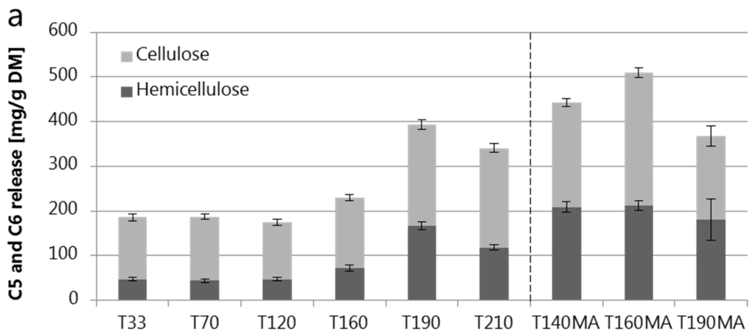


Figure 2. Effect of pretreatment temperature on sugar release. MA indicates addition of malic acid.

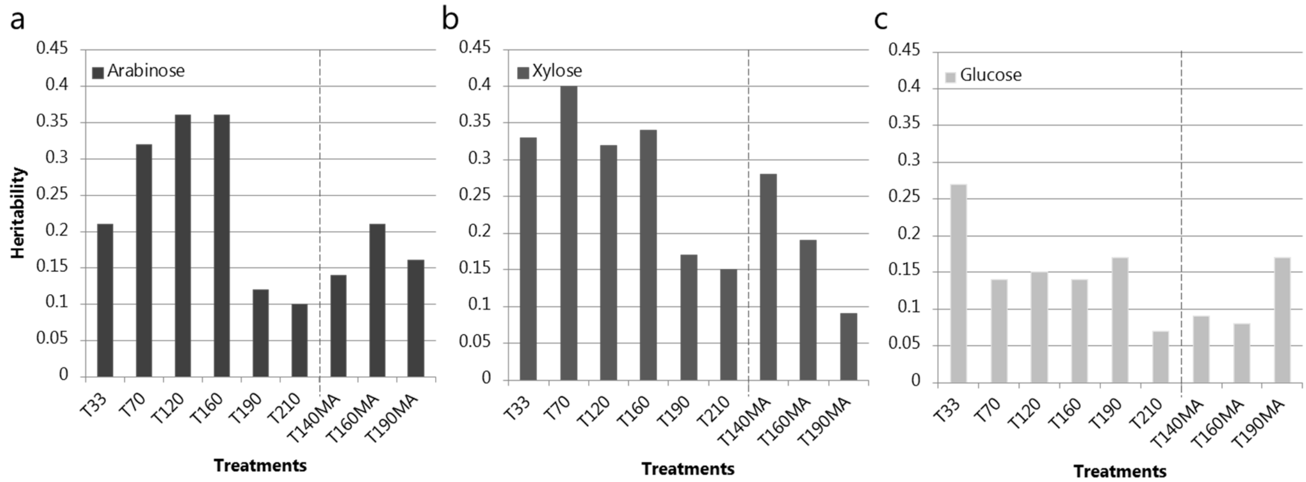


Figure 3. Effect of pretreatment temperature on heritability.

The effect of pretreatment conditions on release of sugars after enzymatic saccharification was tested at 6 different pretreatment temperatures and with/without the addition of malic acid for 2 biological replicates of 10 different winter wheat cultivars. The yield of sugars showed that the optimum temperature for maximum sugar release was around 190°C for a hydrothermal pretreatment, which is consistent with previous findings in other studies (Figure 2). Addition of acid did at all tested temperatures increase the yield of monosaccharides released after enzymatic saccharification compared to hydrothermal pretreatment (Figure 2). The optimum temperature for acidic pretreatment was 160°C. The choice of pretreatment conditions did affect the detection of heritability (how much of the detected variance that is caused by genetic variance) in sugar release between wheat lines. Milder pretreatment conditions increased the chance of detecting genetic variance in sugar release between winter wheat cultivars. The optimum temperature to detect genetic variance was between 33-120°C depending on monosaccharide type (Figure 3). The choice of temperature affected the heritability of arabinose and xylose more than glucose. The results of the study shows that assay conditions are depending on the purpose of the study. For testing sugar potential of a given biomass, optimum conditions will be around 190°C (Figure 2), whereas conditions around 120°C are more suitable if the assay is to be used in screening for genetic variance and selection of new genotypes (Figure 3).

Identification of genetic markers (SNP – single nucleotide polymorphism) for straw yield and quality properties was based on two studies. In the first study a three year breading programme with 190 lines was used for an association study. The heritability was determined for all traits and identification of significant markers was obtained for traits shown in Table 1. The heritability was relatively low for many of the traits (0.01-0.12) and especially for the biorefinery traits; straw yield, glucose, xylose and arabinose release (Table 1). However, despite the low heritability it was still possible to detect significant markers for most of the traits including lodging, plant height, *Septoria tritici* blotch (STB), harvest index, glucose, xylose and arabinose release. Table 1 list the SNP effect for the different traits and show how large an effect the SNP has on the trait. For instance, one SNP affected the degree of early lodging by 2.19 scores on a scale going from 0 to 9. The only traits that were not possible to detect significant markers for were straw yield and grain yield, which are both highly qualitative traits. The study showed that it was possible to use a real breeding programme for identification of SNP markers for agronomic traits and biorefinery traits and that breeding for dual-purpose crops is a possibility in the future.

Table 1: The heritability and the SNP effect for a number of traits associated with yield or biorefining quality

Phenotype	H^2	SNP Effect
Straw yield	0.10	
Grain yield	0.21	
Plant height	0.32	2.27 to 3.83 cm
Harvest index	0.07	2.06 to 2.48 %
Early lodging	0.05	0.10 to 2.19 score
Intermediate lodging	0.12	
Late lodging	0.18	1.18 to 1.85 score
STB	0.41	0.34 to 0.58 score
Arabinose	0.02	0.25 to 0.74 mg/g DM
Xylose	0.04	3.17 to 5.57 mg/g DM
Glucose	0.01	4.81 to 7.25 mg/g DM

The purpose of the second study was to analyse whether multi-crossing populations consisting of a few off springs can be used as material for identification of genetic markers straw yield and quality traits. Here we tested the effect of using genome-wide association studies of multi-crosses to detect markers. Data was based on 74 crossing populations consisting of 1-16 off springs grown for 1 year. The preliminary GWAS results indicated that it was possible to detect markers, if the proper correct for family relatedness (STRUCTURE and kinship matrix) was included in the mixed linear models in the genome-wide association study. The data analysis is still ongoing and final results are expected in January 2019.

AU 2015-2018

Data for three years, 2012/2013 to 2016, from field trials at Foulum and Jyndevad are available and include biomass and protein productivity, canopy reflectances and soil water and nitrate contents. The data have been published and the results from these studies show that fertilized perennial grasses and novel rotations with annual crops could be an alternative to traditional crops in terms of increasing biomass- (figure 4A) and protein yield per hectare (figure 4B), and also for diversification of current agroecosystems. Unpublished results for 2016/2017 further support the findings by showing the superiority of miscanthus for high biomass productivity once the crop has established, as well as the biomass yields of willow after the first three years (figure 4C). The main mechanism behind these finds is mainly the extended crop growing season, plant complementarity for crop rotations, as well as quality of the biomass, tailored by careful field management practices. The radiation use efficiencies (RUE, efficiency for conversion of photosynthetic light to biomass) for the perennial grasses was, however, rather low (figure 5), which indicate a breeding potential to improve their radiation conversion into more above ground biomass.

Annual nitrogen (N) balances for all systems have also been computed for 2013-2015 and published. These results show that the fertilised perennial grasses outperformed all other systems by doubling biomass N and reducing nitrate leaching by 70–80% compared to the traditional systems (figure 6). Compared to continuous maize monoculture, the optimised rotation supplied 70% more biomass N and 40% less nitrate leaching on coarse sandy soil, whereas on sandy loam soil it yielded about 10% less biomass N but with 50% less nitrate leaching. The nitrate leaching results are updated for 2016 and 2017 (unpublished) and show high soil nitrate concentrations for some systems (e.g. festulolium and triticale), which resulted in high leaching, due to lower harvested crop N and increased soil N mineralisation. Soil total N stocks, determined by soil mass N balances, were estimated to decline for the majority of the systems, although soil total carbon (C) stocks measured for the topsoil (2017 versus 2017) tend to increase for the perennial grasses. However, this data is scrutinized for possible artifacts of changes in soil density before they will be published. In addition during 2018 the flux of N_2O from grasses under different treatments (+/-plowing, +/- N-fertiliser) has been measured using both automatic and manual chamber technologies.

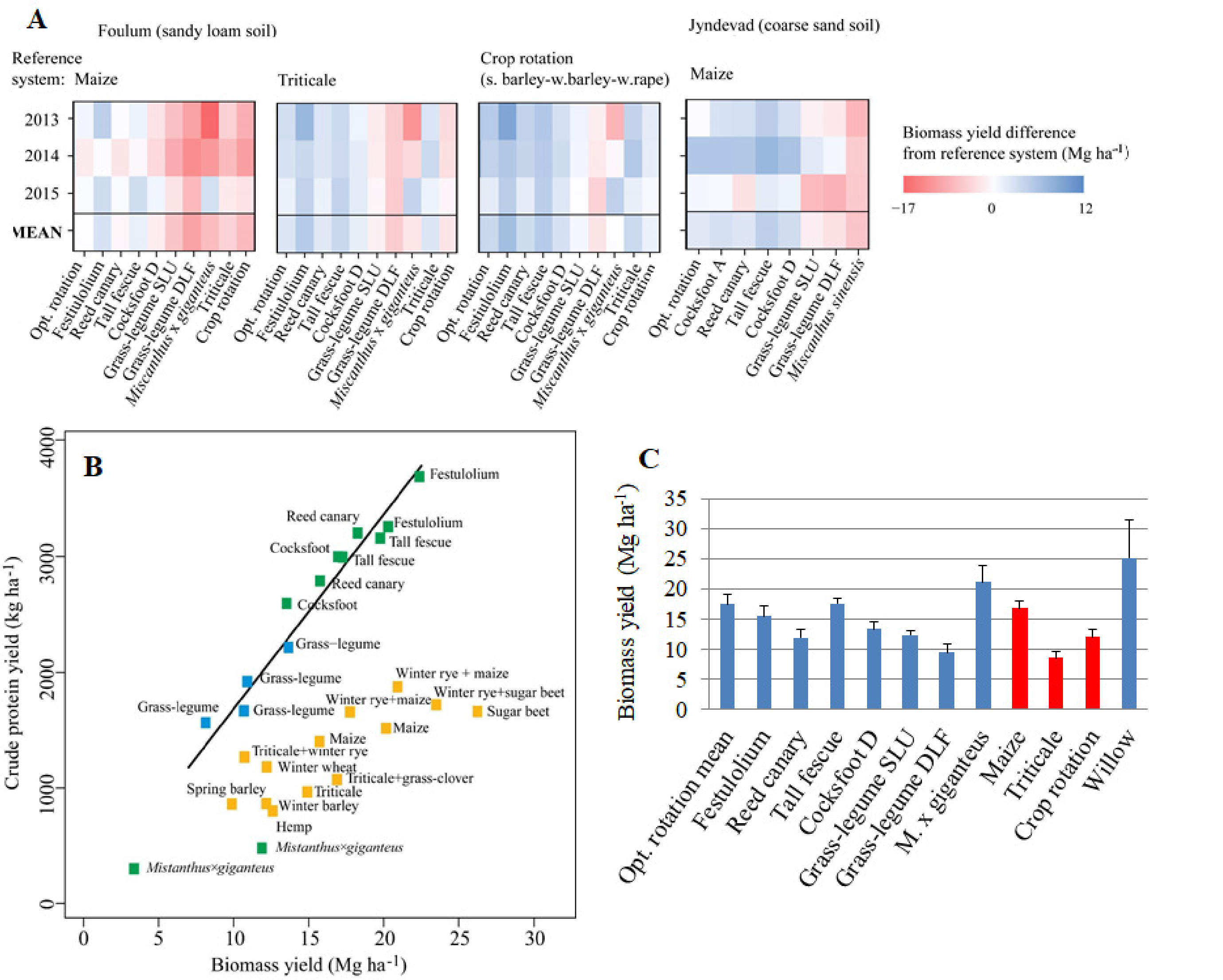


Figure 4: **Mean annual (2013-2015) differences in biomass yield (A) and crude protein yield (B) for novel cropping systems optimised for biomass productivity relative to traditional systems as references, and biomass yields for 2016 (C; traditional systems denoted with red colour bars). Figure adopted from Manevski et al. (2017) and Solati et al. (2018). The exact composition of the systems can be found in the papers.**

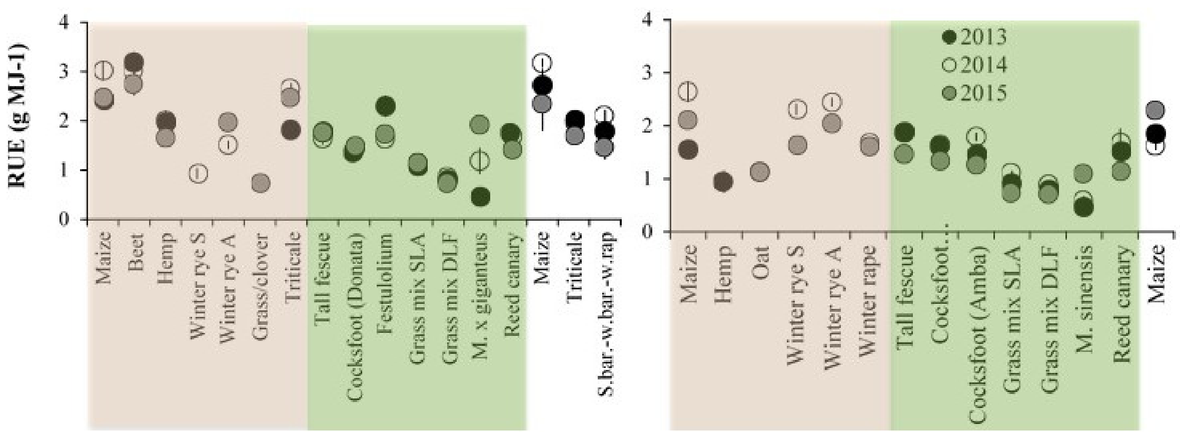


Figure 5: **Mean radiation use efficiency (RUE) for the crops in the novel rotation (shaded reddish) and perennial grasses (shaded green), relative to traditional systems (unshaded) as references at sandy loam soil (left plot) and coarse sand soil (right plot). Data from Manevski et al. (2017).**

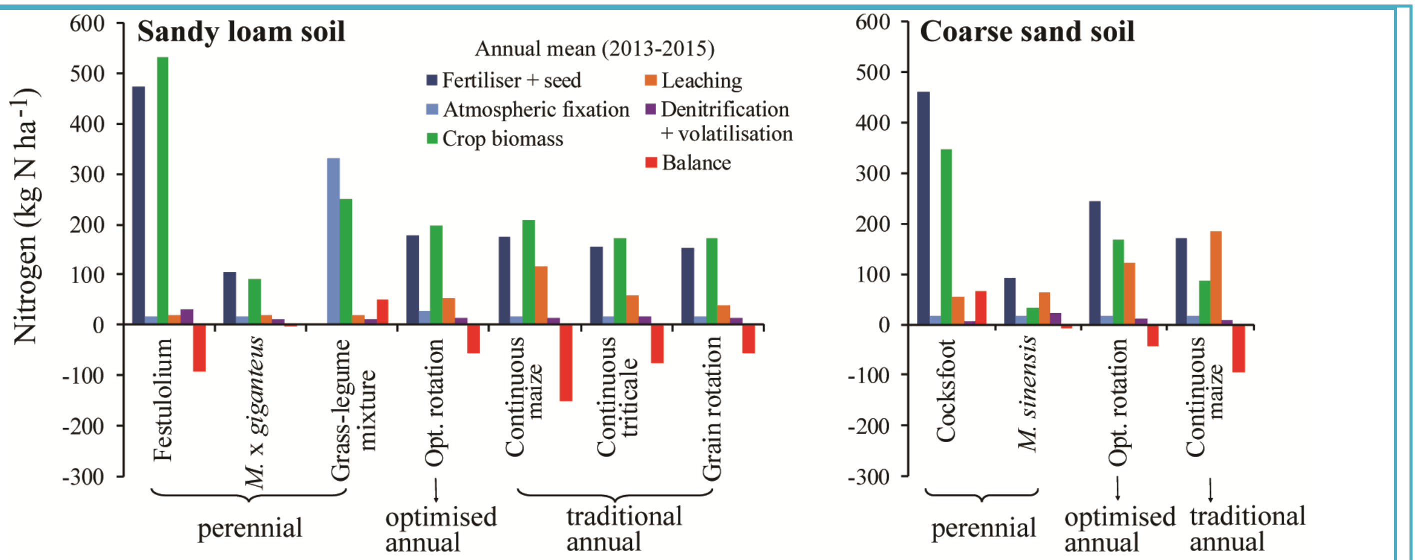
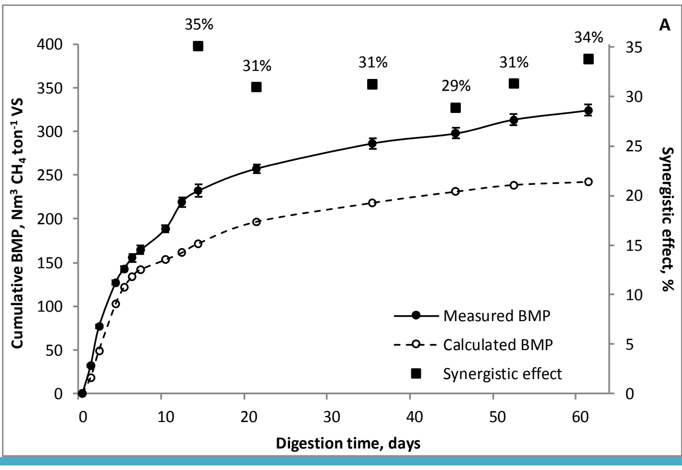


Figure 6: **Nitrogen (N) inputs, outputs and balance for the studied systems in Denmark. Festulolium and cocksfoot are proxy for fertilised perennial grasses at their respective sites. Grass-legume mix on sandy loam soil is proxy for coarse sand soil. All fertiliser is mineral (N-P-K) Detailed description of all systems can be found in Manevski et al. (2017, 2018).**

Danish Technological Institute

Co-ensiling of straw+sugar beet leaves

Straw generally has a low biochemical methane potential (BMP) when used for biogas production. Sugar beet leaves, on the other hand, has high BMP but also a low dry matter content which results in excessive effluent run-off when ensiled alone. Co-ensiling of wheat straw and sugar beet leaves has been studied in lab-scale and pilot-scale ensiling experiments. The experiments showed significant increases in BMP of biomass mixtures after ensiling ('measured BMP') compared to the expected BMP for un-ensiled mixtures ('Calculated BMP') (figure 7A and 7B). The increase in BMP ranged between 19 to 34 % after 9 months of co-ensiling in lab-scale and between 18 and 32 % after 6 months of co-ensiling in pilot-scale. There was no effluent run-off when ensiling a mixture of 24 % straw and 76 % sugar beet leaves (fresh matter basis) with a dry matter content of 29.2 % in the mixture. Hence, co-ensiling of straw and sugar beet top has proved to be a biological pretreatment with a dual function: 1) The straw absorbs excess juice from the sugar beet leaves and prevents effluent run-off during ensiling. 2) The juice from the sugar beet leaves provides moisture and water-soluble carbohydrates for the ensiling process which results in an increased methane potential of the straw.



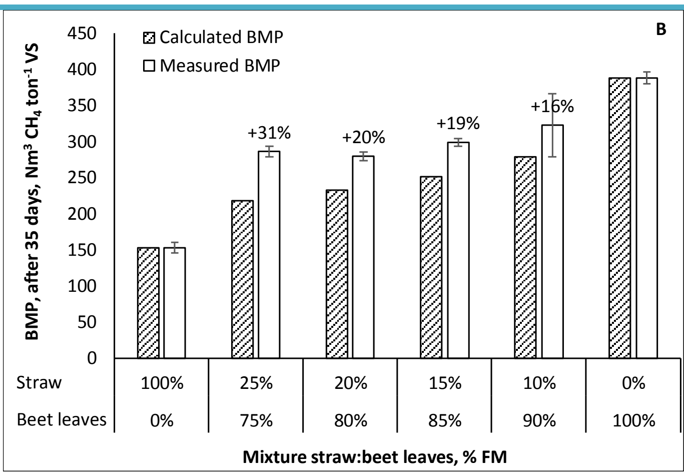


Figure 7. Measured and calculated biochemical methane potential (BMP) after co-ensiling of mixtures of wheat straw and sugar beet leaves. The difference between measured and calculated BMP indicates the synergistic effect of the ensiling on the BMP. A: Cumulative BMP for a mixture with 25 % straw and 75 % beet leaves (fresh matter percentages) ensiled for 9 months. B: BMP in various mixtures of straw and sugar beet leaves. (From Larsen et al. (2017). Bioresource Technology, 245, 106-115).

Combined harvest and co-ensiling of straw and catch crops
Straw and catch crops are both interesting resources of residual biomass for biogas production. However, straw has a relatively low methane potential without any pretreatment. Catch crops have a relatively high methane potential but often a low dry matter content, leading to a risk of considerable effluent run-off when ensiled alone. Besides, the biomass yield of catch crops is often too low to make harvest feasible. Straw from strip harvesting or from conventional combined harvesting with a tall stubble may be harvested together with catch crops, resulting in a relatively high total biomass yield, and the biomass mixture may be ensiled for biogas production. The straw fraction of the mixture will absorb excess juice from the catch crop fraction, and the ensiling process may increase the methane potential of the straw.

Field trials in 2014-2015 with strip-harvesting of spring barley have indicated that combined harvest of straw+catch crops in October may offer a way of harvesting a considerable biomass yield. The composition of the mixture varies largely, primarily depending on the establishment and growth of the catch crop. Catch crop biomass generally has larger methane potential than pure straw biomass and, consequently, the methane potential may differ considerably between various mixtures (figure 8). Lab-scale ensiling experiments have shown that these biomass mixtures can ensile well as indicated by low pH value (pH 4.1-4.2). Also, the trials have shown that the methane potential increases gradually during the process of ensiling (figure 8). After 300 days of ensiling, the methane potential had increased by 9 to 12 % compared to the level of fresh, un-ensiled biomass. The results demonstrate that ensiling may both provide a way of preserving wet biomass as well as improving the quality, i.e. the co-ensiling of straw and catch crops may serve as a biological pretreatment of straw for biogas production.

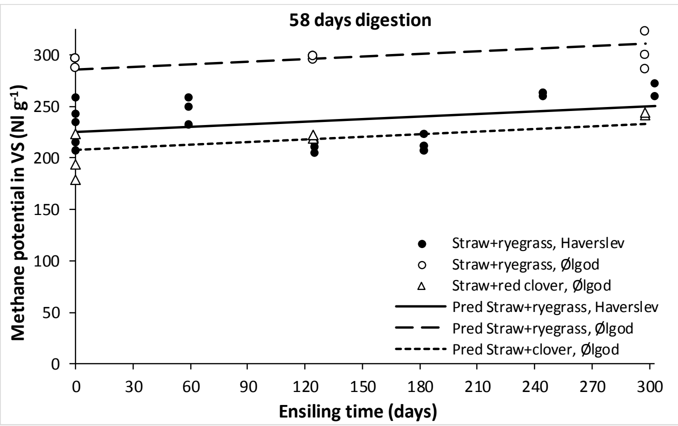


Figure 8. Methane potential in mixtures of straw and catch crops (Italian ryegrass or red clover) after ensiling for various durations. The figure shows results for 58 days of digestion in the analysis of methane potential.

Effect of nitrogen fertilization on protein yield, protein extractability and amino acid composition when biorefining tall fescue biomass
Experiments with protein extraction from tall fescue at various N fertilization levels showed that DM yield, crude protein (CP) concentration and CP yield per hectare increases significantly with increased N fertilization. The CP allocation in the concentrate fraction after protein extraction did not appear to increase with increasing N fertilization. However, the CP concentration in both the concentrate fraction and the pulp fraction increased significantly with increasing N fertilization which may prove advantageous in the biorefining process. In general, there was only little effect of N fertilization on the amino acid profile of both the concentrate and the pulp fraction.

Ensiling of the pulp fraction after biorefining of grass into pulp and protein juice
When extracting protein juice from green biomass, water soluble carbohydrates (WSC) are also removed with the juice. This may potentially hamper a subsequent ensiling of the fibre fraction, since WSCs are driving the ensiling process. The ensiling of grass fibre has been studied in trials with addition of sugar and with varying time from protein extraction to ensiling. Addition of sucrose decreased the pH of the grass fibre silage but pH was relatively low (3.8) even without sugar addition as long as the fibre was ensiled soon after juice extraction (figure 9). However, if ensiling was delayed up to 53 hours after juice extraction, pH of the silage gradually increased to 4.8 (figure 10). The delay in ensiling also caused a decrease in the concentration of dry matter, lactic acid as well as total amino acids and free amino acids in the silage. This indicates, that the grass fibre must be ensiled as soon as possible after the extraction process in order to achieve a good silage quality of low pH and, hence, smooth logistics should reduce the time from extraction to ensiling.

Pilot-scale ensiling experiments for 7 to 11 months with the pulp fraction from two types of grass biomass demonstrated a limited effluent run-off (0-1.8 %) and a high recovery of volatile solids (97-98 %) as well as the methane production potential (98-116 %) after ensiling compared to fresh pulp. However, the recovery was lower for true protein (67-85 %) and total amino acids (84-90 %) after ensiling. The methane production potential was shown to be higher in pulp silage than in fresh pulp at digestion times up to 20 days but not at longer digestion times.

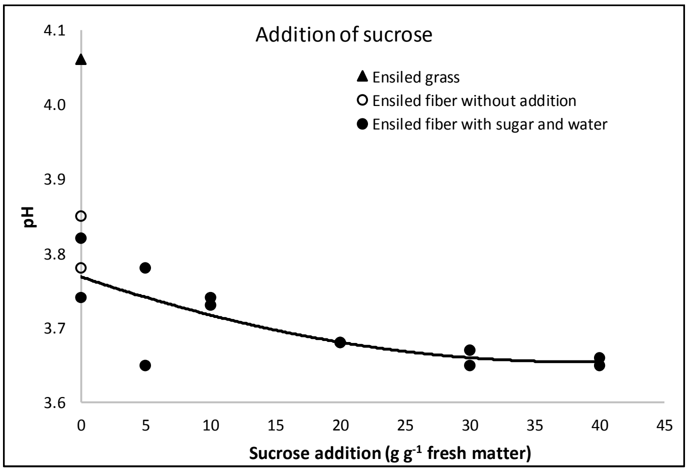


Figure 9. Effect of addition of sucrose on pH in silage of grass fibre after extraction of protein juice. Fibre and grass was ensiled within 2.5 to 4.5 hours after juice extraction. pH was measured after 89 days of ensiling.

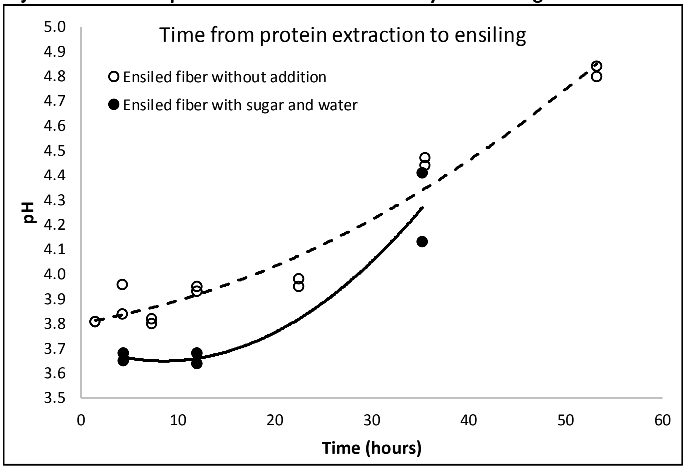


Figure 10. Effect of time from extraction of protein juice to ensiling and addition of sucrose on pH in silage of grass fibre. Just prior to vacuum packing, sucrose was added to certain samples as an aqueous solution with 40 g of sucrose and 40 g of water per kg fresh matter. pH was measured after 89 days of ensiling.

Survey on potential protein production based on grass from meadows

A desk study on potential protein production based on grass from meadows indicated that there are considerable resources of meadow grass that could potentially be utilized for protein production. However, the feasibility depends on various factors such as the production costs, the market prize of protein and the alternative value of the meadow grass. With the current conditions, the business case of meadow grass for protein production is not very likely to be feasible; this would, for instance, require improvements of the protein extraction process and reductions in the costs.

SEGES

The main focus of SEGES work in the BioValue project has been on dissemination and identification of barriers for bringing the results from the BioValue-project at the Danish farms.

A Farmer Survey has been made where 10 organic pig producers were interviewed on the subject of Green Biorefininng. The main conclusions were:

- The farmers know of green biorefining and are interested
- Their main driver is the possibility of being self-sufficient and for having and increasing the amount of clover grass in their crop rotation
- The farmers would prefer to use a dry product, and most of the farmers would prefer to have it delivered from a farm supply company
- Their main concerns are increased cost of the product and amino acid composition
- The farmers do not expect to own and operate the biorefining plants themselves

SEGES has evaluated the biomass and protein yields that can be expected in practice when delivering biomass to a green biorefinery. The evaluation is based on analysis of many years of plant production made as a part of Landsfosøgene™. The biomass and protein yield are depicted below on Figure 11 for conventional (a) and organic farming (b) from 3000 ha. The production is based upon 7 blocks of clover grass each of 429 ha, that are cut in rotation 3 times during the year in order to ensure continuous provision of biomass during the harvest season.

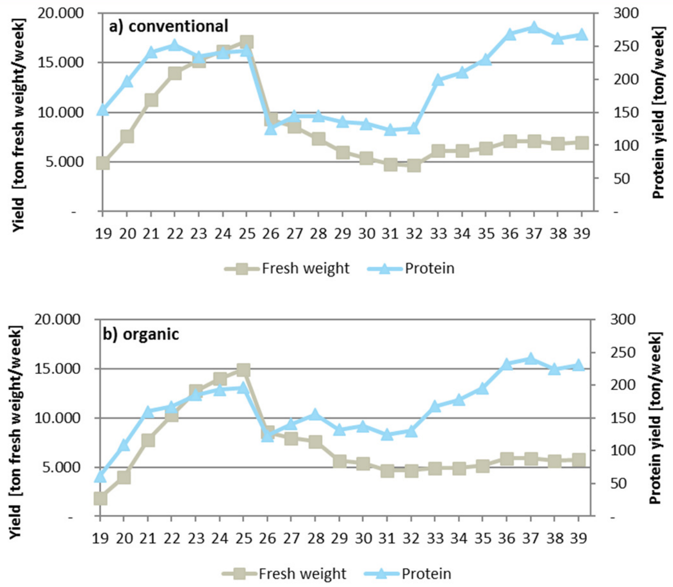


Figure 11. Weekly biomass production for a green biorefinery

SEGES has within the BioValue project made detailed calculations of the cost for various biomass supply systems. The costs include biomass production, harvesting, logistics and storage, and the data were used as basis for the calculations made in the SESE-platform.

Dissemination of results

Oversigten er udarbejdet i overensstemmelse med universiteternes registreringssystem for publikationer og følger samme definitioner af publikationstyper. Kun de publikationstyper, som er væsentligst for Det Strategiske Forskningsråd, er omfattet. For hver publikationstype skal angives forfatter, titel, sideantal, evt. tidsskrift og årstal. Desuden skal der for hver publikation angives evt. publicering i Open Acces (OA), samt hvor stor andel af publikationen, der skønsmæssigt er finansieret af bevillingen i procent (X %).

Artikler, peer reviewed - (tjek website, hvis de står under "publications" på website, så har jeg dem registreret, ingen grund til at skrive dem igen.)
Artikler, ikke peer reviewed (f.eks. fagmagasiner)
Doktordisputatser
Ph.d.-afhandlinger
Videnskabelige bøger/antologier
Videnskabelige rapporter
Bidrag til bøger/antologier/rapporter
Working papers/arbejdspapirer/preprints
Konferenceartikler, peer reviewed – (kun hvis ikke allerede angivet i en tidligere årsrapport)
Konferenceartikler, ikke peer reviewed
Patenter, patentansøgninger
Datasæt (x hvis afleveret til Dansk Data Arkiv)
Planlagte publikationer (angiv type)
Anden formidling Fx forskningsformidlende bøger/antologier, kronikker, tv, mm. - (kun hvis ikke allerede angivet i en tidligere årsrapport)
Rådgivning Fx artikler, videnskabelige rapporter, mm.
Undervisning Fx lærebøger, kompendier, kurser, kandidatuddannelser

Research education and publications

List Ph.D.-stipends/degrees and postdoc stipends working for the project in the period (name, place of employment, number of months in the period financed by the grant).

Pernille Louise Malik, Dep. Plant & Environmental Sciences, KU, Ph.D.- student, 12 (maternity leave 10.5 months)
Jan van Hecke, Dep. Plant & Environmental Sciences, KU, Ph.D.- student, 10 months
Zeinab Solati, PhD, AU-Foulum, finished
Kiril Manevski, Postdoc, AU-Foulum, ongoing

Khagendra Raj Baral, AU-Foulum, postdoc,

List scientific publications published by the project in 2017 and any completed Ph.D. thesis (Name, title, defense date). If the publication is not 100% financed by BioValue, note the estimated percentage of BioValue financial contribution.

Scientific publications:

Jørgensen, H., van Hecke, J., Zhang, H., Malik, P., Felby, C., Schjoerring, J.K. (2018). Wheat as a dual crop for biorefining: Straw quality parameters and their interactions with nitrogen supply in modern elite cultivars. Global Change Biology Bioenergy, Doi:10.1111/gcbb.12560
Głazowska, S., Baldwin, L., Mravec, J., Bukh, C., Hansen, T.H., Jensen, M.M., Fangel, J.U., Willats, W.G.T., Glasius, M., Felby, C. and Schjoerring, J.K. 2018. The impact of silicon on cell wall composition and enzymatic saccharification of *Brachypodium distachyon*. Biotechnology for Biofuels 11, 171.
Dahl-Lassen, R., van Hecke, J., Jorgensen, H., Bukh, C., Andersen, B. and Schjoerring, J.K. 2018. High-throughput analysis of amino acids in plant materials by single quadrupole mass spectrometry. Plant Methods 14, 8 .
Głazowska, S., Murozuka, E., Persson, D.P., Castro, P.H. and Schjoerring, J.K. 2018. Silicon affects seed development and leaf macrohair formation in *Brachypodium distachyon*. Physiologia Plantarum 163, 231-246.
Manevski, K., Lærke, P.E., Olesen, J.E., Jørgensen, U. Nitrogen balances of innovative cropping systems for feedstock production to future biorefineries. Science of The Total Environment, Volume 633, August 2018, Pages 372-390
Solati, Z., Manevski, K., Jørgensen, U., Labouriau, R., Shahbazi, S., Lærke, P.E. 2018: Crude protein yield and theoretical extractable true protein of potential biorefinery feedstocks. Industrial Crops and Products, Volume 115, May 2018, Pages 214-226.
Solati, Z., Jørgensen, U., Eriksen, J., Søegaard,K. 2017. Estimation of extractable protein in botanical fractions of legume and grass species. Grass Forage Sci., 2017;00:1–10.
Larsen, S.U., Hjort-Gregersen, K., Vazifehkhoran, A.H., Triolo, J.M., Co-ensiling of straw with sugar beet leaves increases the methane yield from straw. Bioresource Technology, Volume 245, Part A, 2017, Pages 106-115.
Solati, Z., Jørgensen, U., Eriksen, J. and Søegaard, K. (2017), Dry matter yield, chemical composition and estimated extractable protein of legume and grass species during the spring growth. J. Sci. Food Agric. doi:10.1002/jsfa.8258
Larsen S, Bentsen NS, Dalgaard T, Jørgensen U, Olesen JE, Felby C. 2017. Possibilities for Near-term Bioenergy Production and GHG-Mitigation through Sustainable Intensification of Agriculture and Forestry in Denmark. Environmental Research Letters. 12(11). DOI: 10.1088/1748-9326/aa9001
Solati, Zeinab 2017. Production of protein in grassland species and innovative cropping systems: quantity and quality for extraction. PhD dissertation, p. 121.
Jørgensen U., Lærke P.E. (2016) Perennial Grasses for Sustainable European Protein Production. In: Barth S., Murphy-Bokern D., Kalinina O., Taylor G., Jones M. (eds) Perennial Biomass Crops for a Resource-Constrained World. Springer, Cham
Christensen, B. T., Lærke, P. E., Jørgensen, U., Kandel, T., & Thomsen, I. K. (2016). Storage of Miscanthus-derived carbon in rhizomes, roots, and soil.Canadian Journal of Soil Science, 96(4), 354-360.
Molinuevo-Salcesa, B.; Larsen, S. U.; Ahring, B. K.; Uellendahl, H.: Biogas production from catch crops: Increased yield by combined harvest of catch crops and straw and preservation by ensiling. Biomass and Bioenergy, May 2015, DOI: 10.1016/j.biombioe.2015.04.040

Popular papers:

Larsen, S.U. (2017). Pænt gasudbytte fra halm og efterafgrøder. Forskning i Bioenergi, 60, juni 2017, 10-12.
Jørgensen, U.; Lærke, P.E.: Merproduktion og mindre udledning i græsmarken – Omlægning til flerårige græsser kan sikre opfyldelse af en række miljømålsætninger i landbruget og understøtte en lokal produktion af protein. Tidsskrift for Landøkonomi, 2016/3. 202. årgang. side 193-202
DCA communication 2017. Behov for mere viden om produktionen af protein fra grøn biomasse
DCA communication 2018. Græs er godt på flere måder
Toft, Lars V. – Nøgletal for grøn bioraffinering, 2018, Landbrugsinfo.dk
Toft, Lars V. – Begrænsninger og muligheder for at implementere bioraffineringsscenarier fra BioValue i landbruget Landbrugsinfo.dk
Hammer, K. – Praktisk erfaring med forbrug, transport og logistik af halm Landbrugsinfo.dk

Reports:

Hinge, J., Larsen, S.U. & Pedersen, J. (2017). Desk study on potential protein production based on grass from meadows. Report, Danish Technological Institute, Maj 2017
SEGES-produced report by Erik Fog, 2017/18: “Farmer-survey om at indgå i grøn bioraffinering”
Harvest methods, capacities, and costs. SEGES rapport: Lyngvig, H. S.; Højholdt, M.
Feasibility study of the profitability of new bioenergy harvesting machinery from a farmer and agricultural machine company perspective.
BioValue rapport: Scovill, A. L:; Abildgaard, L.; Sørensen, C. G.; Støckler, M.; Green, O.

Papers in preparation:

Pernille L. Malik, Luc Janss, Linda K. Nielsen, Finn Borum, Henning Jørgensen, Christian Bukh, Claus Felby, Birger Eriksen, Jan K. Schjørring, Søren K. Rasmussen. Identification of SNP markers associated with high straw yield and quality traits without compromising grain yield. In preparation
Pernille L. Malik, Henning Jørgensen, Christian Bukh, Heng Zhang, Linda K. Nielsen, Finn Borum, Birger Eriksen, Jan K. Schjørring, Søren K. Rasmussen. Pretreatment of wheat straw before enzymatic saccharification affects the detectability of genetic variation in released sugars between cultivars. In preparation
Jan Van Hecke, Henning Jørgensen, Rasmus Dahl-Lassen and Jan K. Schjoerring. Residual nitrogen pools in mature winter wheat straw as affected by nitrogen application. In preparation

Henning Jørgensen, Sune Tjalfe Thomsen, Jan K. Schjoerring. Influence of nitrogen supply and harvesting time on the potential for biorefining of triticale to protein and sugar. In preparation.

List activities presenting project results at conferences during 2018 (papers, proceeding, posters)

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Manevski, K., Lærke, P.E., Jørgensen, U. 2018. A comparative nitrogen balance of novel cropping systems for feedstock production to future biorefineries: the role of perennial grasses and grass-legumes. In: Durand P. et al. (Eds.): Proceedings of the 20th Nitrogen Workshop, "Coupling C - N - P - S cycles", June 25-27, Rennes, France.

Henning Jørgensen, Jan Van Hecke, Laetitia Baldwin, Jan K. Schjoerring. Nitrogen affects the quality of biomass for biorefining. XVIII International Plant Nutrition Colloquium 21 - 24 August 2017, Copenhagen – Denmark (Poster, proceeding)

Schjoerring JK, Jørgensen H and Baldwin L 2017 Effect of nitrogen supply on biomass quality for biorefining. In Innovative solutions for sustainable management of nitrogen. Proceedings from International Conference, Aarhus, Denmark, 25-28 June 2017 (T Dalgaard et al., eds), p. 112. Aarhus University, Denmark. ISBN 978-87-93398-82-5.

Jørgensen, Uffe; Manevski, Kiril; Lærke, Poul Erik, 2017. Sustainable intensification of Northwestern European cropping systems to increase nitrogen and carbon yields, while reducing losses. Conference abstract for the 2nd International Bioeconomy Congress, Stuttgart, Germany.

Manevski, Kiril; Lærke, Poul Erik; Jørgensen, Uffe, 2017. Sustainable intensification and extensification of cropping system for biorefinery in Denmark-what does the nitrogen balance say? Conference abstract for "Innovative Solutions for Sustainable Management of Nitrogen", Aarhus, Denmark.

Manevski, Kiril; Lærke, Poul Erik; Jørgensen, Uffe, 2017. Novel annual and perennial biomass systems produce large quantities for biorefinery and reduce nitrogen losses to the environment. Poster presented at the Annual BioValue meeting, Aarhus, Denmark.

Larsen, S.U., Hjort-Gregersen, K., Triolo, J.M. & Vazifehkhoran, A.H. (2017). Co-ensiling of straw with sugar beet leaves increases the methane yield from straw. Progress in Biogas IV, Science meets Practice. Abstract booklet of the International Conference, 8th – 11th March 2017. University of Hohenheim, Stuttgart. P.63

Larsen, S.U., Hjort-Gregersen, K., Triolo, J.M. & Vazifehkhoran, A.H. (2017). Co-ensiling of straw with sugar beet leaves increases the methane yield from straw. Progress in Biogas IV, Science meets Practice. International Conference, Stuttgart-Hohenheim University, Tyskland, 9/3 2017.

Larsen, S.U. (2017). Danish examples of agricultural biomass for biogas production. Biogas workshop, University of Sao Paulo, Brazil, 23-25 august 2017.

Larsen, S.U. (2017). Sam-ensilering af halm og roetoppe (og andre grønne biomasser) til biogasproduktion. Seminar on straw, Agro Business Park, 27/9 2017.

Abildgaard, L. (2017). Green biorefinery in Denmark. International conference on Biofuels, Bioenergy and Bioeconomy Sao Paulo, Brazil, 4-5 December.

Larsen, S.U., Ambye-Jensen, M. & Jørgensen, H. (2018). Ensiling of the pulp fraction after biorefining of grass into pulp and protein juice. P1.21 ECO-BIO 2018, Royal Dublin Society, Dublin, Irland. 4-7 marts 2018. Arrangeret af Elsevier og BE-Basic Foundation. https://www.elsevier.com/events/conferences/eco-bio

Larsen, S.U., Jørgensen, H., Bukh, C. & Schjørring, J.K. (2018). Effect of nitrogen fertilization on protein yield, protein extractability and amino acid composition when biorefining tall fescue. P1.22 ECO-BIO 2018, Royal Dublin Society, Dublin, Irland. 4-7 marts 2018. Arrangeret af Elsevier og BE-Basic Foundation. https://www.elsevier.com/events/conferences/eco-bio

Larsen, S.U., Ambye-Jensen, M. & Jørgensen, H. (2018). Ensiling of the pulp fraction after biorefining of grass into pulp and protein juice. Poster 1DV.3.5, 26th European Biomass Conference and Exhibition, Bella Center, Copenhagen, Denmark, 14-17th May 2018.

List patents, teaching (e.g. books, compendiums, courses, master programs), advisory service (e.g. articles, databases, reports), etc.

Hansen EM, Thomsen IK, Petersen SO, Lærke PE, Pedersen BF, Rasmussen J, Christensen BT, Jørgensen U, Eriksen J. 2018. Muligheder for reduktion af næringsstoftab i græsrige sædskifter. Notat nr. 2018-760-000776. 38 s. maj 15, 2018.

Grøn Biomasse, DCA rapport nr. 068, September 2015: Termansen et al

Hermansen, John Erik; Jørgensen, Uffe; Lærke, Poul Erik; Manevski, Kiril; Boelt, Birte; Jensen, Søren Krogh; Weisbjerg, Martin Riis; Dalsgaard, Trine Kastrup; Danielsen, Marianne; Asp, Torben; Ambye-Jensen, Morten; Sørensen, Claus Aage Grøn; Jensen, Mikkel Vestby; Gylling, Morten; Lindedam, Jane; Lübeck, Mette; Fog, Erik, 2017. Green biomass - protein producion through bio_refining. DCA - Nationalt Center for Fødevarer og Jordbrug. 72 p.