Nutrient supply to Reed Canary Grass as a Bioenergy Crop

Intercropping and Fertilization with Ash or Sewage Sludge

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Licentiate Thesis
Swedish University of Agricultural Sciences
Umeå 2012

Cover: Intercropping experiment at Ås 2009
(photo: C. Palmborg)

ISBN 978-91-576-9068-5

Print: Arkitektkopia AB, Umeå 2012

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Abstract

Production of renewable energy from herbaceous crops on agricultural land is of great interest since fossil fuels need to be replaced with sustainable energy sources. Reed canary grass (RCG), *Phalaris arundinacea* L. is an interesting species for this purpose.

The aim of this thesis was to study different approaches to reduce the requirement of mineral fertilizers in RCG production for bioenergy purposes. Paper I describes a study where fertilization effects and risk of heavy metal enrichment were studied, using annual applications of ash for seven years. Ash from co-combustion of RCG and municipal wastes (mixed ash), pure RCG ash and commercial fertilizers were compared. The experiment was harvested each spring. Paper II describes an ongoing study in which the effects of intercropping RCG in mixture with nitrogen-fixing perennial legumes are examined in two experiments, in combination with various fertilization treatments. Three fertilization treatments were applied: high N, low N (half of the high N) and low N + RCG ash/sewage sludge. A delayed harvest method was used; cutting the biomass in late autumn and harvesting in spring. Besides dry matter yield, the N-fixation rate was estimated.

The results from paper I showed no differences between treatments in the dry matter yields or in the heavy metal concentrations in the biomass. Soil samples, taken when the experiment was finished, showed differences between treatments for Cd, Pb and Zn only in the uppermost soil level, highest levels for the mixed ash treatment. The results in paper II showed that at one site the legume proportion in the mixtures was low and did not affect RCG growth negatively. The high N treatment gave a higher spring yield than the low N treatments. Mean rates of N_2-fixation in the first production year were 12-28, 33-40 and 55 kg N ha^{-1} kg for goat´s rue (*Galega orientalis* Lam.), red clover (*Trifolium pratense* L.), and alsike clover (*Trifolium hybridum* L.), plots, respectively. At the other site, competition with higher proportion of the clovers affected RCG growth and spring yield negatively. The N-fixation rates were 33 - 42 kg N ha^{-1} for red clover and 24 kg N ha^{-1} for alsike clover. As a conclusion, pure RCG ash can be used to complement mineral fertilizers in RCG crops, but it is important to analyse the ash for plant nutrients and heavy metals before use. There was no spring yield benefit of legume/RCG intercropping. Thus, the method cannot be recommended in a spring harvest system, at least not under the tested conditions.

Keywords: Biofuel, reed canary grass, delayed harvest, ash fertilization, heavy metals, intercropping with legumes, nitrogen fixation

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Contents

List of Publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

I Lindvall, E., Gustavsson, A–M., Samuelsson R., Magnusson T. and Palmborg, C. Ash fertilization of reed canary grass: effects of nutrient and heavy metal composition on plant and soil (submitted).

II Lindvall, E. Anne-Maj Gustavsson, A.–M. and Palmborg, C. Establishment of reed canary grass with perennial legumes or barley and different fertilization treatments: Effects on yield, botanical composition and nitrogen fixation (submitted)

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The contribution of Eva Lindvall to the papers included in this thesis was as follows:

I. Planned a final sampling and chemical analyses in an existing long term experiment jointly with the co-authors. Conducted part of the sampling. Analysed the data and wrote the paper.

II. Planned sampling and chemical analyses in two field experiments jointly with the co-authors. Wrote PMs for field staff. Conducted part of the sampling. Analysed the data and wrote the paper jointly with the co-authors.

1 Introduction

Increasing local production of renewable energy from herbaceous crops on agricultural land is of great interest. Imported fossil fuels need to be replaced with sustainable energy sources to decrease the emissions of greenhouse gasses. Also, in a directive of the use of energy from renewable sources, the European Parliament endorsed a mandatory target of a 20 % share of energy from renewable sources in overall Community energy consumption by 2020. The potential for utilizing a number of plant species for this purpose is being evaluated in several countries, among them reed canary grass (*Phalaris arundinacea* L., hereafter RCG) (Kukk et al., 2010; Kryzeviciene et al., 2008; Tonn et al., 2010; Tahir et al., 2011; Beringer et al., 2011; Xiong et al., 2008). Other examples of crops under evaluation are switchgrass (*Panicum virgatum* L.) (Boehmel et al., 2008; Hallam et al., 2001; Wang et al., 2010), *Miscanthus spp*. (Boehmel et al., 2008), giant reed (*Arundo donax* L.) (Di Nasso et al., 2010), maize (*Zea mays* L.) and hemp (*Cannabis sativa* L.) (Alaru et al., 2009). However, except for RCG and possibly also hemp, the other species are not adapted to the climatic conditions in northern part of Sweden.

Perennial grasses are of particular interest as they have many advantages over annual crops. Successfully established swards of perennial grasses can have lifetimes of at least 8 - 10 years before they need to be reseeded and therefore require less cultivation than annual crops. They also have lower requirements for pesticides and nutrients. The latter is because some nutrients from the shoots are recycled to the roots during the autumn (Heinsoo et al., 2011; Wrobel et al., 2009; Xiong et al., 2009). In addition, grass fields can be harvested using machinery that is readily available on farms. The fields can be easily converted to food production without substantial costs for restoring the land, unlike (for instance) short-rotation *Salix* coppices or tree plantations.

The potential utility of reed canary grass RCG for bioenergy production has been evaluated in Sweden, several other European countries and the USA (Landström et al., 1996; Finell et al., 2011; Lewandowski et al., 2003). A special technique for harvesting of this grass has also been developed, the delayed harvest system. The technique means that the grass is cut in late autumn, but removal of the biomass from the field is delayed until the following spring (Larsson et al., 2006). This technique is less costly than collecting the biomass immediately after cutting since no drying is required. It also results in lower ash contents, partly due to losses through leaching of Cl, Na and K. These elements cause corrosion and ash sintering in boilers. Thereby the quality of the fuel is improved (Landström et al., 1996; Burvall, 1997).

To date, RCG for commercial production of bioenergy has been most extensively used in Finland (Pahkala, 2007; Pahkala et al., 2008). The commercial RCG production in Sweden is limited to a few areas as the market still is uncertain, and its profitability is low. Thus, in order to increase the use of this and other herbaceous crops for bioenergy production it is important to increase their profitability by decreasing the costs. This can, for instance, be done by reducing the use of mineral fertilizers as sources of plant nutrients, provided this can be done without reducing dry matter (DM) yields (or at least without reducing yields so much that the losses outweigh the cost-benefits of reducing fertilizer applications).

A possible way to reduce fertilization costs is to use appropriate waste materials as replacements for mineral fertilizers, for instance RCG ash or sewage sludge. Combustion of RCG leads to the production of relatively large amounts of ash compared to combusting wood fuel ((Burvall, 1997). This has been regarded as a problem, but ash can also be seen as a source of plant nutrients, especially P and, to some extent, K (Dimitriou et al., 2006). Sewage sludge is also rich in P, and could be used as a fertilizer for bioenergy crops, provided it is not contaminated by heavy metals or other undesirable compounds (Eriksson et al., 2008) (Odlare et al., 2011). Upper limits for heavy metal additions with sewage sludge have been issued by the Swedish Environmental Protection Agency (Naturvårdsverket, 1994) (Table 1). A potential way to reduce mineral N requirements for RCG production might be to intercrop RCG with perennial legumes. The legumes fix N_2 from the atmosphere by symbiosis with Rhizobium bacteria. Some of the N is transferred to the soil and can be used by the intercropped grass as reviewed by Fustec et al., (2010). Experiments in Lithuania have shown promising results for such mixtures (Kryzeviciene et al., 2008).

Two papers are included in this thesis. Paper I describes a study in which the effects of annual applications of RCG ash (either pure or from mixed combustion with sorted municipal waste ash) and mineral fertilizer on RCG biomass production were compared in a field experiment. The objectives of the experiment were to study the fertilization effects of those ashes and the effects on concentrations of heavy metals and plant nutrients in soil and plant biomass.

Paper II describes an on-going study in which the effects of intercropping RCG with a number of perennial legumes are examined in two experiments, in combination with various fertilization treatments. The main hypotheses were that N-fertilization rate could be substantially reduced by intercropping with legumes and that use of RCG ash/sewage sludge as P and K fertilizers could reduce the input of mineral fertilizers. In this thesis the establishing phase (the two first growing seasons) of these experiments are covered.

Table 1. Balance between supplied and removed amounts of nutritional elements and heavy metals during the experimental period, seven years of fertilization. The amounts removed per hectare were calculated using the average concentrations in grass samples from 2004 and 2009, and the average dry matter yield per year. Upper limits for heavy metal concentrations in sewage sludge are issued by the Swedish Environmental Protection Agency (Naturvårdsverket) are included.

		kg ha^{-1}				g ha^{-1}					
Treatment		P	K	Mg	Ca	Cd	Cr	Cu	Ni	Pb	Zn
Mixed ash	Supplied	105	560	252	2503	59.03	9286	6999	721	9962	30564
	Removed	34	81	16	60	0.87	32	184	19	25	741
	Balance	71	479	236	2443	58.16	9254	6815	702	9937	29823
RCG ash	Supplied	117	577	81	380	2.44	471	958	195	41	1656
	Removed	32	71	17	54	0.93	35	171	22	26	693
	Balance	85	506	64	327	1.51	436	787	172	14	963
Control	Supplied	105	560	0	140	1.79					
	Removed	33	67	14	49	0.97	27	171	21	24	659
	Balance	72	493	-14	91	0.82	-27	-171	-21	-24	-659
Upper limit per year						0.75	40	300	25	25	600
Upper limit for a period of seven years						5,25	280	2100	175	175	4200

2 Materials and methods

2.1 Paper I

Figure 1. Location of the research fields

In a field experiment at Röbäcksdalen experimental station ($63^{o}48'$N; $20^{o}14'$E), Umeå, Sweden (Figure 1) the effects of using ashes as P fertilizers on RCG biomass production were compared to mineral fertilization. The trial was established in spring 2002, finished in spring 2009. Two kinds of ashes were used, pure RCG ash and mixed ash from co-burning RCG and sorted municipal wastes. The target amounts of nutrients applied per year in all treatments were 100 kg ha^{-1} of nitrogen (N), 15 kg ha^{-1} of phosphorous (P) and 80 kg ha^{-1} of potassium (K). The annually supplied amounts of the ashes were calculated from their total concentrations of P to cover the requirement of 15 kg ha^{-1}. The K supplied with the ashes was supplemented with mineral fertilizer (KCl), and all N was applied as mineral fertilizer (NH_4NO_3). The control treatment was fertilized with mineral fertilizers (NH_4NO_3, KCl, $Ca(H_2PO_4)_2$). The total amounts of plant nutritional elements supplied during the experimental period are given in Table 1.The experiment was designed as a randomized block experiment with three fertilization treatments and four replicates.

The RCG crop was cut and harvested, using a plot harvester (Haldrup), in May each year between 2003 and 2009, and the biomass was weighted. A sample of the plant biomass was taken from each plot from the harvested biomass. The biomass samples from 2004 and 2009 were analysed for concentrations of heavy metals and macronutrients (SS 28311, modified and ASTM D3682-91, modified). The top soil (0-20 cm) and the sub soil (20-40 cm) were sampled in 2003 and 2008. The samples from 2003 were pooled to one composite sample per treatment and soil depth respectively. The samples from 2008 were analysed from each individual plot. The amounts of plant-available soil nutrients in all samples were estimated by extraction with ammonium lactate (AL, Swedish standard, SS 028310). Since differences in heavy metal concentrations in the soil can be hard to detect, a stratified sampling was done in spring 2009 specific for the heavy metal analyses. Soil samples from three levels, 0-5 cm, 5-10 cm and 10-20 cm, were taken from each plot to determine which soil levels contained heavy metals from the ash. Those samples, and the pooled samples from 2003, were analysed for concentrations of heavy metals and plant nutrients (SS 28311, modified and ASTM D3682-91, modified). For further details see the *Materials and Methods* section of Paper I. As this experiment was in the finishing phase when I started my work, I did not have any influence of the design or sampling in the experiment, except for the last soil sampling.

2.2 Paper II

Reed canary grass was intercropped with the perennial legumes alsike clover (*Trifolium hybridum* L.), red clover (*Trifolium pratense* L.), goat´s rue (*Galega orientalis* Lam.) or kura clover (*Trifolium ambiguum* M. Bieb) at two field sites in northern Sweden, one at Röbäcksdalen, Umeå, (established 2009) and the other at Ås, near Östersund, (established 2008) (Figure 1). A complete split plot design, in four blocks, with fertilization as main treatments and species mixtures as sub-treatments was used. In total, there were 84 plots (3 x 10 m) at each site. As an extra treatment beside the legume intercrops, RCG in monoculture was undersown in barley (*Hordeum vulgare* L.) as a nurse crop in the establishment year at Ås. The grass/legume mixtures and the control RCG monoculture were sown without a nurse crop. At each site, three fertilization treatments were also applied to plots sown with RCG alone and each species mixture. At Ås, these treatments comprised: (i) high N + K, (ii) low N (half as much as in the high N treatment) + K, and (iii) low N + K + sewage sludge. At Röbäcksdalen, the fertilization treatments comprised: (i) high N + K + mineral P, (ii) low N + K + P, and (iii) low N + ash from RCG combustion. In High N, 40 kg N ha^{-1} was applied in the establishment year and 100 kg ha^{-1} the first production year. In Low N, 20 and 50 kg N ha^{-1} were applied in the two years respectively. According to the plan, the experiments will continue for three harvest years after the sowing year.

In autumn, biomass samples from small, manually cut subplots (50 x 50 cm), were sorted into each sown species and weeds at the end of the growing season, to determine the botanical composition resulting from each treatment.

The samples where then analysed for N concentration (N%) and the abundance of ^{15}N relative to ^{14}N ($\delta^{15}N$‰). The amount of N_2 derived from the atmosphere in the aboveground biomass of each legume species was calculated using (1) the ^{15}N natural abundance method and (2) the N difference methods.

(1) The proportion of N derived from the atmosphere (Ndfa%) in the legumes was calculated using the difference between their respective $\delta^{15}N$ values and that of the non N-fixing RCG (Högberg, 1997). Published B factors (their respective $\delta^{15}N$ values when acquiring N_2 solely from the atmosphere) were used for red clover and alsike clover (Carlsson et al., 2006). For Kura clover and goat's rue the B-values were set to -1 ‰, a common value for perennial legumes (Unkovich *et al.*, 2008). The Ndfa % value was calculated using the formula:

$$\mathrm{Ndfa\%} = \frac{100 * (\delta^{15}N_{RCG} - \delta^{15}N_{legume})}{\delta^{15}N_{RCG} - B}$$

The non-fixing plants were from the same plots as the legumes.

(2) Nitrogen difference = kg N in intercrop total aboveground biomass – kg N in RCG monoculture biomass from the same fertilization treatment plot.

The ^{15}N natural abundance method is the preferred method for measurement of N-fixation in intercrops of legumes and non-N-fixing plants (Unkovich *et al.*, 2008). The method requires that the isotope ^{15}N is enriched in the soil compared to the atmosphere, which results in a higher $\delta^{15}N$ value in the non-fixing reference plant than in the legume. At Ås, the $\delta^{15}N$ of RCG was lower in several plots than the 2 ‰ lower limit recommended for reference species by Unkovich *et al.* (2008) for use of the ^{15}N natural abundance method. Because of this we also calculated the N fixation rate by the N difference method.

Larger sub-plots (1.5 x 7.5 m) were cut at Ås with a plot harvester (Haldrup) in 28 October 2009 and Röbäcksdalen (1.5 x 6.0 m) in 22 September 2010. To imitate delayed harvest as applied in practise, the biomass was cut with the plot harvester in autumn and then, after weighing and sampling, replaced manually on each plot. The biomass was then harvested (removed from the field) in May the following spring with the plot harvester, as soon as the field was sufficiently dry to support it. For further details see the *Materials and Methods* section of Paper II.

3 Results

3.1 Paper I

Dry matter yield and heavy metal concentration in the spring harvested RCG biomass were not affected by the ash fertilization treatments. However, as an average over all treatments, concentrations of Cd, Ni and Pb were significantly lower (while concentrations of only one heavy metal, Zn, were higher) in biomass samples collected in 2009 than in samples from 2004.

The only significant between-treatment differences in soil concentrations of heavy metals were observed in the uppermost layer of the soil (0 to 5 cm) at the end of the experiment period (2009). Concentrations of Cd, Pb and Zn were higher in plots supplied with the mixed ash compared to the pure RCG and the mineral fertilizer treatments (Figure 2). The annual amounts of most of the added macronutrients and analysed trace elements in the soil were higher than the amounts removed in the harvested biomass, for all treatments (Table 1). In both ash treatments, and most strongly in the mixed ash treatment, the inputs of heavy metals widely exceeded the outputs.

Regarding the macro nutrients, there was a tendency to lower K-AL concentration in the top soil 2008 for the mixed ash treatment compared to the control, however the difference was not significant (p=0.06). The P-AL concentration did not differ between the treatments.

3.2 Paper II

Analyses of the botanical composition of samples collected in sub plots (50x50 cm) at Röbäcksdalen in 2010, the first production year, showed that there were significantly lower amounts of RCG in plots sown with mixtures containing red clover or alsike clover in comparison with the RCG monoculture (Figure 3). The biomass yields of kura clover and goat´s rue were very small since most plants did not survive the winter. There were no significant between-

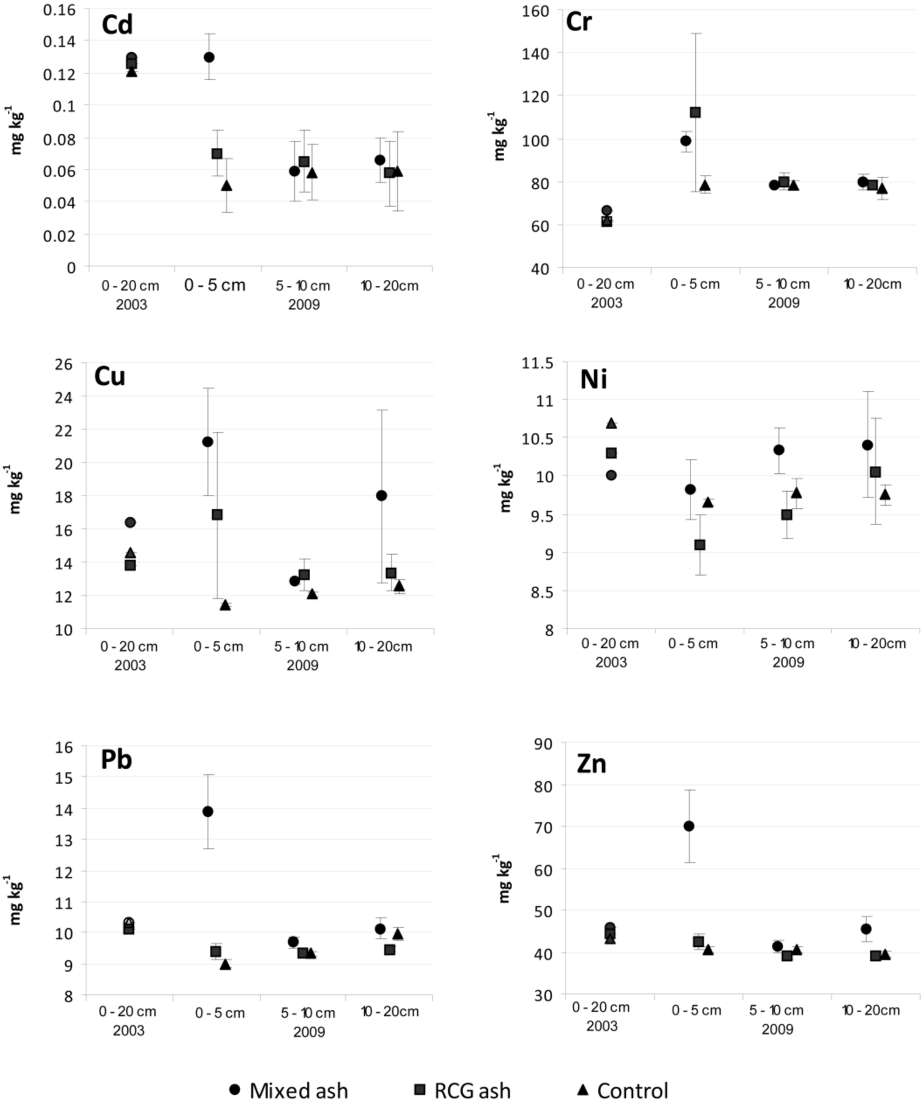

Figure 2. Heavy metal content in different levels of the top soil, sampled in spring 2009. The error bars show the standard error (n=4). The samples from 2003 were pooled together, forming one sample per treatment. Upper limits (in mg kg^{-1} of dry soil) issued by the Swedish Environmental Protection Agency for sewage-sludge applications are as follows: Cd 0.4, Cr 60, Cu 40, Ni 30, Pb 40, Zn 100.

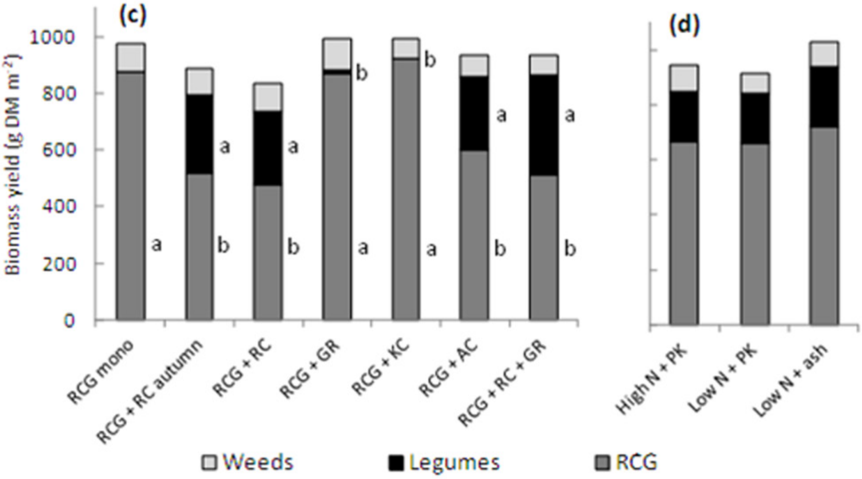

Figure 3. Amount of biomass of each of the sown species and weeds from plots representing each of the seed mixture (a) and fertilization treatments (b) at Röbäcksdalen in autumn 2010. Different letters denote indicate significant differences between means for different components (beside the columns). RC = red clover, GR = goat's rue, KC = kura clover and AC = alsike clover.

fertilization treatment differences in amounts of biomass of any of the species (Figure 3). The concentration of N in RCG was significantly higher, and the δ^{15}N value was lower in mixtures with red clover and alsike clover compared to RCG monoculture, No differences between fertilization treatments were significant in this respect. Nitrogen fixation rates, determined by the ^{15}N natural abundance method, ranged from 33 to 42 kg N ha^{-1} year^{-1} for red clover and amounted to 24 kg N ha^{-1} year^{-1} for alsike clover.

At the other site, Ås, the legumes were not very abundant and did not negatively affect RCG biomass yield. The total biomass yield from the small plots (50 x 50 cm) cut in autumn in the year after sowing (2009) was significantly lower where RCG was undersown in barley (Figure 4). There were no differences in total yield between the fertilization treatments. The amount of alsike clover was significantly higher than the amounts of red clover and goat´s rue. However, there were no differences in botanical composition between the fertilization treatments within any of the legume mixtures. The concentration of N in the RCG biomass was significantly higher in plots undersown with barley than the monoculture and the mixtures, except the alsike clover mixture. On average, over all mixtures, the N concentration in RCG biomass was also higher in plots subjected to the high N fertilization treatment. The nitrogen fixation rates, as determined by the nitrogen difference method, for goat's rue, red clover and alsike clover plots were 12-28, 33-40 and 55 kg N ha^{-1}, respectively, in the first production year. No significant

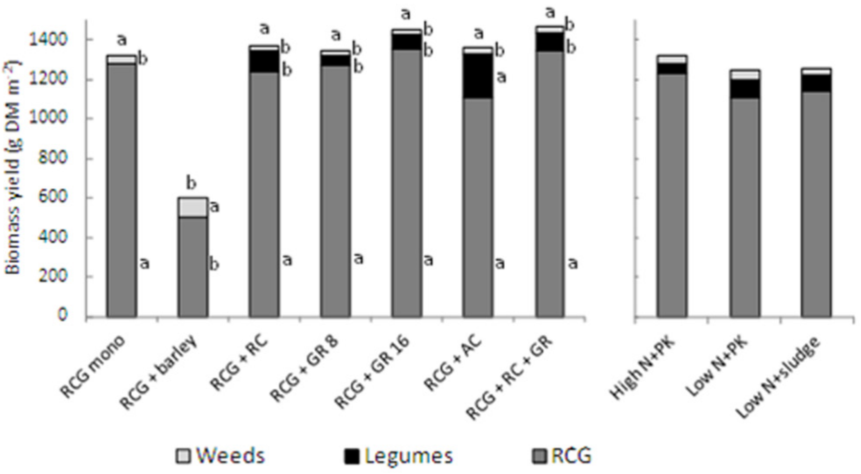

Figure 4. Amount of biomass of each of the sown species and weeds from small plots at Ås in autumn 2009, representing each of the seed mixture (a) and fertilization treatments (b). Different letters indicate significant differences between means for different components (beside the columns) and total amount of biomass (above the columns). = red clover, GR = goat's rue, KC = kura clover and AC = alsike clover.

species or fertilization treatment associated differences in this variable were detected.

The DM yields from the large, machine harvested plots in the late autumn cut for the RCG monocultures at Röbäcksdalen were 7200 kg ha^{-1} with no significant between-treatment differences. None of the legume mixtures differed from the RCG monoculture when Bonferroni test was applied. At harvest (removal from the field) in mid-May in the following spring the mixtures with red clover as the only legume gave a lower DM yield of the remaining biomass than the RCG monoculture (3200 kg ha^{-1}).

The RCG monoculture yield in autumn at Ås was 11000 kg DM ha^{-1}, and in spring 7300 kg ha^{-1}. The yield was significant lower, in autumn as well as in spring, when the RCG was undersown in barley than the RCG monoculture. Also, yields in autumn tended to be higher following the high N fertilizer treatment (p = 0.09). However, no significant differences between treatments with P from different sources (mineral fertiliser or RCG ash/sewage sludge) were seen at either site.

4 Discussion and conclusion

4.1 Use of ash and sewage sludge as fertilizers

In the ash fertilization experiment (Paper I) no differences in yield were observed between the control treatment, in which all P and K were supplied by mineral fertilizers, and the ash treatments (in which all P and some K were supplied by the ashes, and the rest of the K by mineral fertilizer). The rate of N fertilizer supply was the same for all treatments, and as the treatments were applied for a number of years the availability of the P in the ashes appears to be adequate, at least, to supply the crop. The finding that the P-AL concentration was not reduced in the top soil also indicates that, as there were not differences in biomass yield between treatments. Similarly, good availability of P in ashes from other agricultural products (straw, rape meal and cereals) has been reported by Eichler-Loebermann et al., (2008) and Schiemenz and Eichler-Lobermann, (2010).

Element balances compiled by comparing amounts of P and K added to the plots and amounts removed in the biomass (Paper I) clearly indicate that the added amounts of those elements exceeded the crops' requirements, which may partially explain why no differences between the treatments were observed. In the establishment experiments (Paper II) the biomass has only been examined in the first production year to date, which is probably too soon for any detectable effects of P supplied by ash or sludge versus mineral P to appear. The P fertilization level is lower than in the experiment in paper I and thus fertilization effects might become evident with time. Further, the effects of the ash/sewage sludge treatments cannot be compared to those of supplying no P fertilization, since no unfertilized control treatment was applied.

Since heavy metals are concentrated in ashes and other waste products, one concern which restricts their use in agricultural production is the risk of heavy metal enrichment. However, in the ash fertilization experiment (Paper I) the ash treatments did not result in any significant increase in heavy metal concentrations in the plant biomass over time. In the soil, the mixed ash treatment caused significantly higher levels of some heavy metals (Cd, Pb and Zn) in the uppermost soil layer (0-5 cm) compared to the control. However, no effects of the pure RCG ash treatment on heavy metal concentrations in soil were detected (Figure 2). The increase in the mixed ash treatment reflects a very high net addition of these elements compared to the upper limits set by the Swedish Environmental Protection Agency (Naturvårdsverket, 1994) (Table 1). The influence of sludge applications in the establishing experiment on soil heavy metal and nutrient contents will be examined later.

The main conclusion from the results to date is that pure RCG ash can be used to complement mineral fertilizers in RCG crops, but with less frequent applications than in the experiment reported in Paper I. However, it is important to analyse the ash for plant nutrients and heavy metals when planning large-scale ash fertilization schemes as the composition of ashes varies depending on factors like growing site and type of boiler used for combustion.

4.2 Establishment of RCG intercropped with legumes

In the experiment at Röbäcksdalen competition from red clover and alsike clover clearly restricted the growth of RCG during the first production year (Paper II), but not at Ås, where the proportions of clover were lower. The goat´s rue and kura clover did not establish well at Röbäcksdalen, possibly due to failure of the inoculation with *Rhizobium* cultures, and most plants of these species did not survive the winter. At Ås, the goat´s rue established well and contributed to the total biomass yield to approximately the same degree as red clover. Goat's rue also performed well in two previous, promising evaluations of mixtures containing RCG and goat´s rue for bioenergy purposes in Lithuania (Jasinskas et al., 2008; Kryzeviciene et al., 2008).

When growing grass-legume mixtures for forage production, the level of N fertilizer can be used to control the proportion of the legume, as higher amounts of N favour the grass and suppress N fixation (Carlsson and Huss-Danell, 2003). In Experiment II, there have been marginal differences in botanical composition and N-fixation between the nitrogen fertilization treatments (high N (40/100 kg ha and year^{-1} and low N 20/50 kg ha and year^{-1} in the establishment year and first production year respectively). Reducing the amount of N applied in all treatments (or including a third, lower N treatment) might have resulted in more pronounced differences. The estimated amounts of fixed N were similar at both sites despite the higher proportions of legumes at Röbäcksdalen. The δ^{15}N value in RCG was lower when intercropped with legumes than when grown in monoculture. This is an indication that some atmospheric N might have been transferred from legume to grass via the soil already during the growing season (Fustec et al., 2010; Høgh–Jensen, 2006). Thus, the amount of N fixation might be underestimated as the calculations of Ndfa% were made using the δ^{15}N value of RCG from plants in the mixtures, not from a monoculture and the method assumes that all the N from the reference species is derived from the soil.

The conclusion, after the establishment phase, is that N-fixing legumes can contribute sufficient N during the establishment years to replace half of the recommended N fertilization, but the proportion of legumes has to be controlled. This was not achieved by N-fertilization as we had expected. There was no spring yield benefit of legume/RCG intercropping and thus the method cannot be recommended in a spring harvest system.

The fuel quality of the biomass, when legumes are mixed with RCG, has to be evaluated. Samples from the spring harvests will further on be analysed in this respect. Further fertilization experiments with ash or sewage sludge in comparison with mineral fertilizers have to be conducted. Control treatments without any supply of plant nutrients have to be included in such experiments. In addition to biomass yield measurements the soil concentrations of plant available macro- and micro-nutrients have to be analysed.

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Acknowledgements

The studies that make up this thesis were financed by the Swedish Thermal Engineering Research Association, Kempestiftelserna, the European Regional Development Fund, the Swedish Research Council Formas and the Swedish Farmers´ Foundation for Agricultural Research.

I would like to thank my supervisors and co-authors Cecilia Palmborg, Anne-Maj Gustavsson, Tord Magnusson and Robert Samuelsson for all your useful support and contributions during the work. Thanks also to all staff at Röbäcksdalen and Ås for performing the field work with competence and patience. And last but not least, thank you to all my other colleagues at NJV.