Nutrient supply to Reed Canary Grass as a Bioenergy Crop

Intercropping with Legumes and Fertilisation Strategies for Phosphorus and Potassium

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Abstract

Growing energy demand and global warming are promoting research into potential new sources of renewable energy. Fossil fuels need to be replaced with sustainable energy sources to decrease emissions of greenhouse gases. Production of bioenergy from herbaceous crops on agricultural land is one alternative. In Sweden, reed canary grass (*Phalaris arundinacea* L.) is considered an interesting species for this purpose, as it is perennial and well-adapted to the northern climate. This thesis evaluated different approaches to reduce the mineral fertiliser requirement in reed canary grass (RCG) production for bioenergy purposes in a spring harvesting system.

Fertilisation effects and the risk of heavy metal enrichment were studied in a field experiment involving annual applications of ash for seven years. Three different treatments were applied: ash from co-combustion of RCG and municipal waste (mixed ash), pure RCG ash and, as a control, PK fertilisers. There were no significant differences between treatments in terms of RCG dry matter yield or biomass concentrations of heavy metals. Samples from the uppermost soil layer (0-5 cm) differed between treatments in terms of cadmium, chromium, copper, lead and zinc concentration, with higher concentrations in the mixed ash treatment compared with the control.

The effects of intercropping RCG with different nitrogen (N)-fixing perennial legumes were examined in three field experiments, in combination with various fertilisation treatments. Two levels of N fertilisation combined with RCG ash or sewage sludge were applied in a delayed harvest system, which involved cutting the biomass in late autumn, leaving it on the field during winter and harvesting in spring. The estimated N fixation rate in red clover and alsike clover was high enough to compensate for lower N fertilisation. However, in most cases growth of the RCG in monoculture was not N-limited at half the recommended N fertilisation rate and intercropping with legumes was not beneficial.

In order to determine the P and K requirements of RCG in a delayed harvest system, different levels of phosphorus (P) and potassium (K), supplied by mineral fertilisers or RCG ash are being compared in an ongoing study. No differences in biomass yield between treatments have been found after two harvest years.

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

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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, Palmborg C (2013) Ash as a phosphorus fertilizer to reed canary grass: effects of nutrient and heavy metal composition on plant and soil. *Global Change Biology Bioenergy* (In press).

II Lindvall E, Gustavsson A-M, Palmborg C (2012) Establishment of reed canary grass with perennial legumes or barley and different fertilization treatments: effects on yield, botanical composition and nitrogen fixation. *Global Change Biology Bioenergy*, 4(6), 661-670.

III Lindvall E, Gustavsson A-M, Palmborg C. Intercropping of reed canary grass and legumes as biofuel: Effect of legume species and fertilization treatments on yields, nitrogen fixation and fuel quality (manuscript)

IV Palmborg C, Lindvall, E, Gustavsson A-M. Phosphorus and potassium requirements in reed canary grass grown in a delayed harvest system (manuscript)

Papers I-II are reproduced with the permission of the publishers.

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.

III 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.

IV Planned the experiment jointly with the co-authors. Conducted part of the sampling. Participated in the writing of the manuscript.

1 Introduction

1.1 Background

Growing energy demand, both globally and in a more local perspective, and global warming are stimulating extensive research into potential new, sustainable sources of renewable energy production. Fossil fuels need to be replaced with sustainable energy sources to decrease the emissions of greenhouse gases. In its directive on the use of energy from renewable sources, the European Parliament endorsed a mandatory target of 20% energy from renewable sources in overall EU energy consumption by 2020. Bioenergy has some energy and environmental advantages over many other energy sources (Hoogwijk *et al.*, 2003). By definition, bioenergy is the chemical energy contained in organic materials and bioenergy refers to biological material which can be used as a source to produce heat, electricity or fuel for transportation. Production of heat and electricity is at present mainly achieved by direct combustion of the biomass, a system classed as first generation biofuel. Second generation biofuels, for example ethanol produced from lignocellulose biomass like wood or grasses, requiring more complex and expensive refinement processes, are under development but are not yet available on the market (Bessou *et al.*, 2011). The most common bioenergy source at present, in Sweden and world-wide, is forest-based fuel, but utilisation of other types of biomass for energy purposes is under continuous evaluation. Increasing local and sustainable production of renewable energy from herbaceous crops on agricultural land is of great interest. The potential for utilising a number of plant species for this purpose, among them reed canary grass (*Phalaris arundinacea* L., hereafter RCG), is being evaluated in several countries (Kryzeviciene *et al.*, 2008; Xiong *et al.*, 2008; Kukk *et al.*, 2010; Tonn *et al.*, 2010; Beringer *et al.*, 2011; Tahir *et al.*, 2011). Other examples of crops under evaluation are switchgrass (*Panicum virgatum* L.) (Hallam *et al.*, 2001; Boehmel *et al.*, 2008; 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 climate conditions in northern Sweden.

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

1.2 Reed canary grass

Figure 1. Reed canary grass plant

Reed canary grass is native in temperate regions world-wide, in northern Europe throughout the Nordic countries. Its natural habit is in wet areas, such as lake shores and along rivers. Reed canary grass is used as a forage crop, but nowadays the main interest, at least in Europe, is in using it for heat and electricity production by combustion of the biomass. Reed canary grass is a tall, coarse grass which develops rooting stems (rhizomes) beneath the surface of the soil (Evans and Ely, 1935) (Figure 1). The rhizome and root system is very large, constituting up to half the total biomass of the plant (Kätterer and Andren, 2009), enabling it to absorb nutrients efficiently from the soil and store these in biomass (Xiong *et al.*, 2009). It is a persistent species which grows well on most kinds of soils, but is one of the best grass species for poorly drained soils and tolerates flooding better than many other grass crops. Although in nature it normally grows in wet places, it is more drought-resistant than many other grass species once it is well established, due to its deep root system (Lewandowski *et al.*, 2003). Reed canary grass is adapted to a cool climate and has also good winter hardiness and survives very well in northern Scandinavia.

1.3 The delayed harvest system

The most common harvesting method used for RCG as a biofuel crop in the past was delayed harvest, whereby the previous year's crop is cut and harvested in spring after it has over-wintered in the field. This significantly improves the fuel quality compared with summer harvesting. The concentrations of elements such as alkali metals and chlorine, which can cause fouling and corrosion problems in boilers when incinerated, are reduced during over-wintering due to leaching and loss of some leaf material (Burvall, 1997). The amount of ash formed is also reduced. Furthermore, in late autumn most of the nutrients are relocated from the above-ground parts of the plant to the rhizome and can be utilised by the plant in the following growing season (Wrobel *et al.*, 2009; Xiong *et al.*, 2009). Moreover, the biomass is dry enough to store without any costly artificial drying (Figure 2).

However, over the years harvesting techniques have improved. With the currently most common technique, 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). The advantages with this combination of autumn cutting and spring harvesting are a longer period when cutting is possible in autumn than in spring and no risk of cutting sprouting shoots of RCG, which might occur in spring. There is also less tractor traffic in spring, when the soil is soft from soil frost and snowmelt, and thus less risk of crop and soil damage (Palmborg, 2012).

Figure 2. Reed canary grass bales after spring harvest

1.4 Commercial production costs

To date, RCG for commercial production of bioenergy is most extensively used in Finland (Pahkala, 2007; Pahkala *et al.*, 2008), but use has decreased in recent years, from about 20000 ha in 2007 to 6600 ha in 2013 (Tike, 2014). Commercial RCG production in Sweden is limited to a few areas, as the market is still uncertain and the 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 production costs. This can be done for instance by reducing the use of mineral fertilisers as sources of plant nutrients, provided this does not significantly reduce dry matter (DM) yield (or at least does not reduce yield so much that the losses outweigh the cost benefits of reducing fertiliser rate). There is a lack of information about the requirements for phosphorus (P) and potassium (K) in a delayed harvest system for RCG production, since previous fertilisation experiments in Scandinavia have focused mainly on nitrogen (N) (Landström *et al.*, 1996; Xiong *et al.*, 2009). The existing fertilisation recommendations need to be improved by further fertilisation experiments which include all essential plant nutrients. Some other possible ways to reduce fertilisation costs are to use appropriate waste materials or to intercrop the RCG with a perennial N_2-fixing legume.

1.5 Use of waste materials as fertilisers

Combustion of RCG results in a relatively high amount of ash formation (Burvall and Hedman, 1994; Burvall, 1997). This is regarded as a problem, since dumping large amounts of ash in landfill is costly and can cause environmental problems. Recycling the ash as a fertiliser to agriculture is desirable in a sustainable resource management perspective. The ash contains reasonably high concentrations of plant nutrients, *e.g.* P, K and magnesium (Mg) (Burvall, 1997), but the plant-availability of the nutrients in RCG ash has not been confirmed. High availability of P in ash derived from other agricultural products, *e.g.* straw, rapeseed meal and cereals, has been reported (Eichler-Loebermann *et al.*, 2008). The fertilisation effect (P uptake in a range of agricultural plants) of rapeseed ash, straw ash and cereal ash is comparable to that of triple superphosphate (Schiemenz and Eichler-Lobermann, 2010). Good availability of nutrients in ash has also been demonstrated in a study showing that fertilisation of a willow stand with wood ash increased the extractable P, K, calcium (Ca) and Mg in the soil (Park *et al.*, 2005). However, since heavy metals accumulate in ash, it is important to monitor how these metals are cycled through soil, plant, fuel and ash.

Sewage sludge is also rich in P and could be used as a fertiliser for bioenergy crops, provided it is not contaminated with heavy metals or other undesirable compounds (Andersson and Nilsson K, 1976; Eriksson *et al.*, 2008).

1.6 Intercropping with nitrogen-fixing legumes

An alternative, more sustainable way to reduce the mineral N requirements for RCG production might be to intercrop the grass with perennial legumes. The legumes fix N_2 from the atmosphere by symbiosis with rhizobial bacteria. Some of the N is transferred to the soil and can be used by the intercropped grass, as reviewed by Fustec *et al.* (2011). Nitrogen can be transferred between legumes and grass plants in different ways. Nitrogen compounds released from leaf leakage or root exudate, and from decomposition and decay of nodules and roots, can be utilised by the companion plants (Høgh-Jensen and Schjoerring, 2000; Paynel *et al.*, 2001). Mycorrhizal fungi can transfer N by linking plants (Bethlenfalvay *et al.*, 1991; Mårtensson *et al.*, 1998). Transfer of N between clover and grass under field conditions has been reported in a number of studies (Høgh-Jensen and Schjoerring, 2000; Pirhofer-Walzl *et al.*, 2012; Carlsson and Huss-Danell, 2014). Experiments in Lithuania on intercropping RCG and legumes have shown promising results (Kryzeviciene *et al.*, 2008).

2 Objectives

The overall objective of this work was to evaluate the possibilities to decrease the input of industrially manufactured mineral fertilisers in production of RCG as a bioenergy crop and thereby make it more profitable.

In the first study, the effects of annual applications of RCG ash (either pure or from mixed combustion with sorted municipal waste) and mineral fertiliser on RCG biomass production were compared in a field experiment (Paper I). The aims were to determine whether replacing mineral P fertiliser with ash of different composition resulted in changes in the concentrations of heavy metals, nutrients and trace elements in soil and in plant biomass, and to measure the impact on the biomass yield.

In the next study, the effects of intercropping RCG with a number of perennial legumes were examined in three experiments at different sites, in combination with various fertilisation treatments (Papers II and III). The main hypotheses tested were that N fertilisation rate could be substantially reduced by intercropping RCG with legumes and that use of RCG ash or sewage sludge to supply P and K could reduce the input of mineral fertilisers.

Finally, an ongoing study is aiming to determine the P and K requirements of RCG in a delayed harvest system (Paper IV). Different levels of P and K fertilisation, in the form of mineral fertiliser and RCG ash, are being compared. The results from two harvest years are reported in this thesis.

3 Materials and methods

3.1 Paper I

In a field experiment at Röbäcksdalen research station (63^{o}48'N; 20^{o}14'E) near Umeå, Sweden, the effects of using ash as a P fertiliser in RCG biomass production were compared with those of mineral fertiliser. The experiment was established in a field sown in 2001 with the RCG cultivar SW Bamse. The experiment was laid out in a randomised block design, with three treatments and four replicates. The three different fertiliser treatments which were applied yearly, from 2002 on, after spring harvesting of biomass:

- A mixed ash treatment, using ash produced from combustion of RCG together with separated municipal waste (mainly paper, plastic and leather), supplemented with mineral fertiliser (N and K).
- A treatment using pure RCG ash obtained from combustion of pure RCG, supplemented with mineral fertiliser (N and K).
- A control treatment involving fertilisation (N, P and K) solely with mineral fertiliser. The fertiliser compounds used were ammonium nitrate (NH_4NO_3), potassium chloride (KCl) and triple superphosphate ($Ca(H_2PO_4)_2$).

The target amounts of nutrients applied per year in all treatments were 100 kg N ha^{-1}, 15 kg P ha^{-1} and 80 kg K ha^{-1}. The amounts of ash supplied annually were based on the total P concentration of the ash to meet the requirement of 15 kg P ha^{-1}.

The RCG crop was cut and harvested using a Haldrup plot harvester in May each year between 2003 and 2009, and the biomass was weighed. A sample of the plant biomass was taken from each plot on each harvesting occasion. 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 topsoil (0-20 cm) and subsoil (40-60 cm) were sampled in 2003 and 2008. The samples from 2003 were pooled to one composite sample per treatment and soil layer. The samples from 2008 were analysed on an individual plot basis. The amounts of plant-available soil nutrients in all samples were estimated by extraction with ammonium lactate (AL; SS 028310). Since differences in heavy metal concentrations in the soil can be difficult to detect, stratified sampling was carried out in spring 2009 to obtain specific samples for heavy metal analyses. Soil samples from three layers (0-5 cm, 5-10 cm and 10-20 cm depth) were taken from each plot to determine which soil layers 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).

3.2 Papers II and III

The intercropping experiments were performed at three sites (Figure 3): Röbäcksdalen research station, Umeå (established 2009), Ås research station (63°15’N; 14°34’E) near Östersund (established 2008) and Kyrkeby farm (59°11′N; 15°8′E) near Örebro (established 2010). The year of establishment and the first harvest year at Röbäcksdalen and Ås are described in Paper II, while the experiment at Kyrkeby and harvest years two and three at Röbäcksdalen and Ås are reported in Paper III. The term ‘harvest year’ is used as follows: the ‘first harvest year’ refers to the biomass produced in the year after sowing and harvested in autumn of that year or in the following spring, while ‘second harvest year’ and ‘third harvest year’ are the corresponding biomass for the following years. Reed canary grass was intercropped with different perennial legumes: alsike clover (*Trifolium hybridum* L.), red clover (*T. pratense* L.), lucerne (*Medicago sativa* L.), goat’s rue (*Galega orientalis* Lam.) or kura clover (*T. ambiguum* M. Bieb). As an extra treatment to the legume intercrops, RCG in monoculture was undersown in barley (*Hordeum vulgare* L.) as a nurse crop in the establishment year at Ås (Table 1). A split plot design in four blocks was used, with fertilisation as main treatment and species mixture as sub-treatment. In total, there were 84 plots (3 m x 10 m) at each site. The grass/legume mixtures and the control RCG monoculture were sown without a nurse crop.

Figure 3. Intercropping experiment sites

At each site, three fertilisation treatments were applied for each species mixture:

- The treatment ‘Normal N + PK’ followed current recommendations for northern Sweden for N, P and K.
- The ‘Half N + PK’ treatment comprised fertilisation with 50% of the recommended N rate and the normal rate of P and K.
- In the ‘Half N + sludge + K’ and ‘Half N + ash’ treatments, P and K were supplied with sewage sludge at Ås in the establishment year and the third harvest year. At Röbäcksdalen and Kyrkeby, P and K were supplied as RCG ash in the establishment year and in some of the harvest years. In years when ash or sewage sludge was not applied or when the concentration of K was low, the fertilization was complemented with mineral K.

Ammonium nitrate (NH_4NO_3) was used to supply N, triple superphosphate ($Ca(H_2PO_4)_2$) to supply P and potassium chloride (KCl) to supply K.

In August each year, biomass samples from small, manually cut subplots (50 cm x 50 cm) were sorted into sown species and weeds, to determine the botanical composition resulting from each treatment. The samples were 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 two different methods:

1. The ^{15}N natural abundance method: The proportion of N_2 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 factor was set to -1 ‰, a common value for perennial legumes (Unkovich *et al.*, 2008). The Ndfa% value was calculated using the equation:

$$\text{Ndfa\%} = \frac{100 * (\delta^{15}\text{N}_{RCG} - \delta^{15}\text{N}_{legume})}{(\delta^{15}\text{N}_{RCG} - \text{B})}$$

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

2. The nitrogen difference method: The proportion of N derived from the atmosphere in the legumes was calculated as: Nitrogen difference = kg N in intercrop total biomass - kg N in RCG monoculture biomass from the same fertilisation treatment plot.

Table 1. *Crop species and seed rates used in the experiments*

Site	**Abbreviation**	**Species**	**Seed rate, kg ha^{-1} ****
Röbäcksdalen	RCG mono	Reed canary grass monoculture	11
	RCG + RC autumn	RCG + Red clover*	11 + 4.4
	RCG + RC	RCG + Red clover	11 + 4.4
	RCG + GR	RCG + Goat's rue	11 + 8
	RCG + KC	RCG + Kura clover	11 + 4.7
	RCG + AC	RCG + Alsike clover	11 + 3.8
	RCG + RC + GR	RCG + Red clover + Goat's rue	11 + 2.2 + 8
Ås	RCG mono	Reed canary grass monoculture	10
	RCG + barley	RCG undersown in barley*	10 + 128
	RCG + RC	RCG + Red clover	10 + 4
	RCG + GR 8	RCG + Goat's rue	10 + 8
	RCG + GR 16	RCG + Goat's rue	10 + 16
	RCG + AC	RCG + Alsike clover	10 + 3.2
	RCG + RC + GR	RCG + Red clover + Goat's rue	10 + 2 + 8
Kyrkeby	RCG mono	Reed canary grass monoculture	18
	RCG + RC	RCG + Red clover*	18 + 6
	RCG + RC	RCG + Red clover	18 + 6
	RCG + GR	RCG + Goat's rue	18 + 13.4
	RCG + LU	RCG + Lucerne	18 + 6
	RCG + AC	RCG + Alsike clover	18 + 5
	RCG + RC + GR	RCG + Red clover + Goat's rue	18 + 3 + 13.4

*) Harvested in autumn in the sowing year
**) RCG + barley/legume 1 + legume 2

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 the isotope ^{15}N to be enriched in the soil compared with the atmosphere, which results in a higher $\delta^{15}N$ value in the non-fixing reference plant than in the legume. In several plots at Ås in the first harvest year, the $\delta^{15}N$ value obtained for RCG was lower 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 in Paper II. In harvest years two and three, the $\delta^{15}N$ value was high enough to use the natural abundance method.

Large subplots (1.5 m x 7.5 m) were cut with a Haldrup plot harvester (Figure 4). To imitate delayed harvest as applied in practice, the biomass was cut with the plot harvester in late autumn and spread out manually on each plot. The biomass was then harvested (removed from the field) in the following spring with the plot harvester, as soon as the field was sufficiently dry to support traffic. On all occasions, the biomass was weighed and sampled (a minimum of three subsamples from each plot pooled to one sample).

To determine the fuel quality, spring-harvested biomass was analysed for elements important for fuel quality (Ca, K, Mg, sodium (Na), silicon (Si)). Normalised mean values for samples of RCG and RCG + legumes, from each site, for the system $CaO+MgO – K_2O+Na_2O – SiO_2$ were compared against ash melting isotherms (Boström *et al.*, 2012) in a ternary plot.

In the statistical analyses, data for species mixtures and fertilisation treatments were evaluated separately for each site and year. In Paper II, the statistical software NCSS 8 (General Linear Model) was used (Hintze, 2012). In Paper III, the MIXED procedure in SAS software (SAS, 2013) was used. Effects of treatments on botanical composition, biomass yield, N concentration, $\delta^{15}N$ value, concentrations of mineral elements and N_2 fixation rate were evaluated by analysis of variance as a split-plot design with four replicates. Comparisons were also made between biomass yield in treatments with and without (denoted pure RCG) a significant amount of legumes. Subsequently, a comparison was made between yields for pure RCG in the normal and the Half N + PK fertilisation treatments and mixtures with legumes in the Half N + PK treatment, in order to test whether half the N fertiliser could be replaced by N fixed by the legume intercrop.

3.3 Paper IV

Fertilisation experiments were started in spring 2012 at two sites that were selected because of the low concentrations of plant-available P and K in both topsoil and subsoil. These were Runtorp, close to Kalmar, in southern Sweden and Röbäcksmyran, close to Umeå, in northern Sweden. The soil at Runtorp is a sandy loam and that at Röbäcksmyran a humus-rich loamy sand.

The experiments are laid out in a randomised block design with seven fertilisation treatments and four replicate blocks (plot size 3 m x 10 m). All treatments receive a basic fertilisation of 100 kg N ha^{-1} as Axan NS 27-4™ each year. In addition, in the first year, RCG ash (3000 kg ha^{-1}, supplying 34 kg P and 104 kg K ha^{-1}) was spread in one treatment. The other treatments are two levels of P (34 and 17 kg ha^{-1}), two levels of K (100 and 50 kg ha^{-1}), a control fertilised with the recommended level of P and K for forage grass (20 kg P and 130 kg K kg ha^{-1}), and another control without P and K fertilisation. In the second year, the ash and P treatments received only N, while all the other treatments were the same as in the first year. All fertilisers are applied at the start of the growing season.

Soil samples were taken from the topsoil (0-20 cm) and subsoil (40-60 cm) at the start of the experiment and after harvest in 2013. Morphological development of the plants was assessed in both summers at approximately the time of flowering of the RCG, using samples from small plots (50 cm x 50 cm) hand-cut at 2 cm height. The samples were sorted into flowering shoots, healthy vegetative shoots, shoots with damaged tops, dead shoots and weeds. The plots were harvested with Haldrup plot harvesters in late autumn and samples were taken for determination of dry matter (DM), and in the second year also for chemical analysis. The harvested plots were 1.5 m x 6 m and were located in the middle of the plots. Concentrations of plant-available nutrients (Ca, K, Mg, P) were analysed in the soil samples and concentrations of minerals in the biomass samples (Ca, K, Mg, P, copper (Cu), manganese (Mn), sulphur (S), zinc (Zn)).

Figure 4. Spring harvest at Ås. (photo: P-E Nemby)

4 Results

4.1 Paper I

On average for all fertilisation treatments, DM yield in the ash fertilisation experiment varied between 1500 and 6800 kg DM ha^{-1} in the different harvest years and did not differ between treatments (Figure 5).

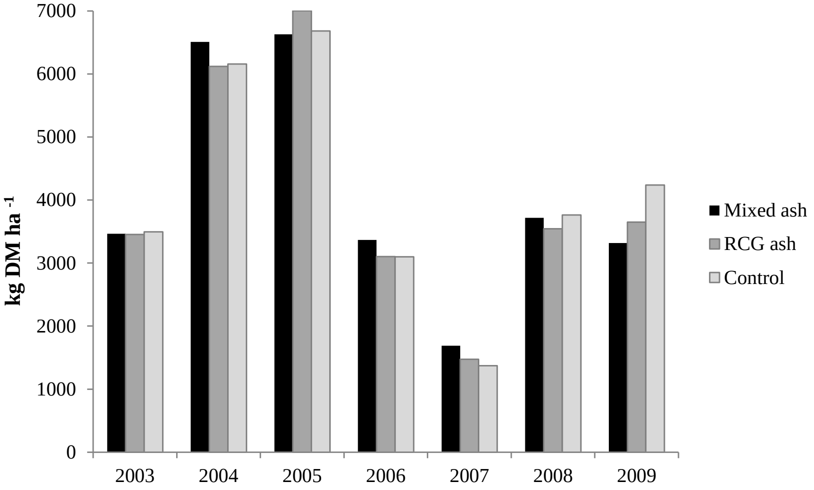

Figure 5. Dry matter yield in the ash fertilization experiment (Paper I), kg ha^{-1}, of RCG biomass in the different fertilization treatments and harvest years

The heavy metal concentrations in the spring-harvested RCG biomass were not affected by the ash fertilisation treatments. However, on average for all treatments, the concentrations of cadmium (Cd), nickel (Ni) and lead (Pb) were significantly lower in biomass samples collected in 2009 than in samples from 2004, while the concentration of only one heavy metal (Zn) was higher.

The only significant between-treatment differences in soil concentrations of heavy metals were observed in the uppermost layer of the soil (0-5 cm) at the

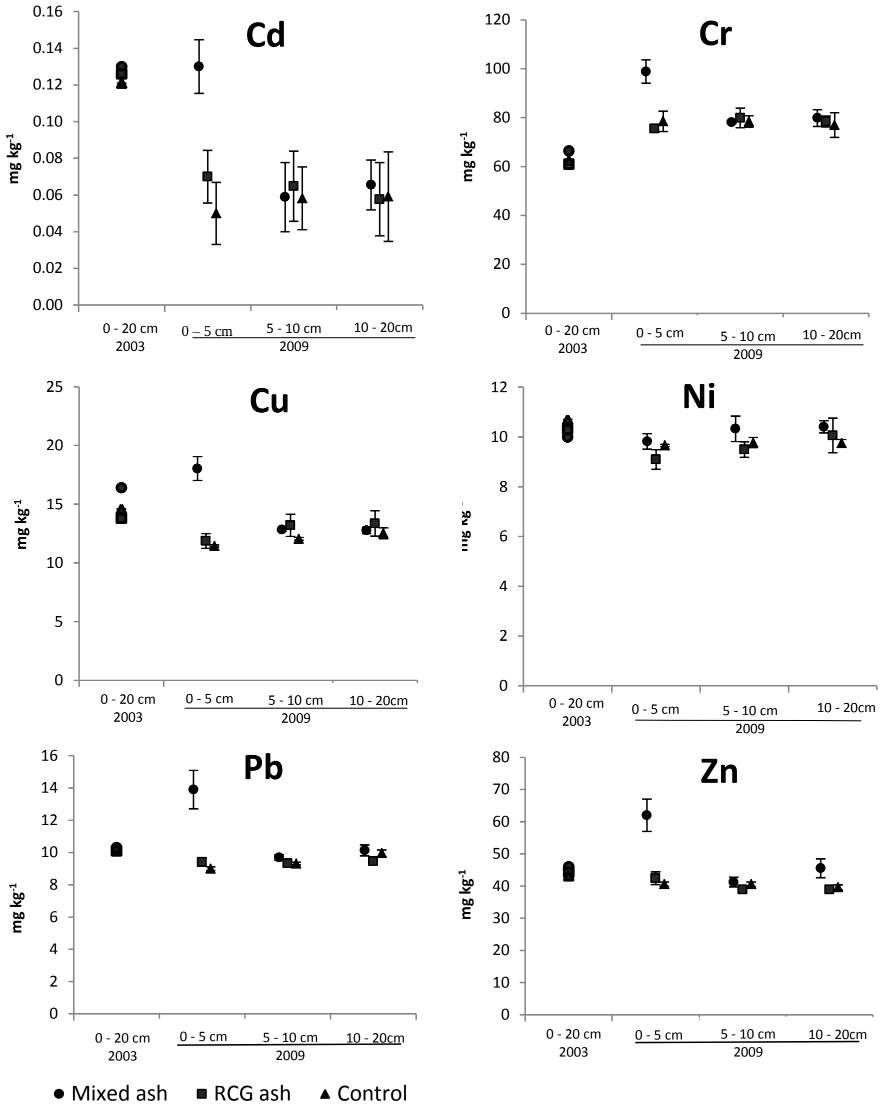

Figure 6. Heavy metal content in different topsoil layers sampled in spring 2009. The error bars show the standard error (*n*=4). The samples from 2003 were pooled to form one sample per treatment. The upper limits (mg kg^{-1} dry soil) set by the Swedish Environmental Protection Agency for sewage sludge application are: Cd 0.4, Cr 60, Cu 40, Ni 30, Pb 40, Zn 100.

end of the experimental period (2009). The concentrations of all heavy metals, except Ni, were higher in plots supplied with the mixed ash compared with the pure RCG and mineral fertiliser treatments (Figure 6). For all treatments, the annual amounts of most of the added macronutrients and trace elements analysed in the soil were higher than the amounts removed in the harvested biomass. In both ash treatments, and most strongly in the mixed ash treatment, the inputs of heavy metals greatly exceeded the outputs.

Regarding the macronutrients, there was a tendency for lower K-AL concentration in the topsoil in 2008 for the mixed ash treatment compared with the NPK-fertilised control, but the difference was not significant (p=0.06). The P-AL concentration did not differ between the treatments.

4.2 Papers II and III

4.2.1 Botanical composition

Analyses of the botanical composition of samples collected in subplots (50 cm x 50 cm) at Röbäcksdalen in 2010 (the first harvest 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. The biomass yield of kura clover and goat's rue was very low, since most plants did not survive the winter. There were no significant between-fertilisation treatment differences in amounts of biomass for any species (Paper II). From the second harvest year on, almost no legumes remained at Röbäcksdalen and the proportion of weeds (7%) was about the same as in the first year (Paper III).

At Ås, the legumes were not very abundant and did not negatively affect RCG biomass yield. The total biomass yield from the small plots in autumn in the year after sowing (2009) was significantly lower where RCG was undersown in barley. There were no differences in total yield between the fertilisation treatments. The amount of alsike clover was significantly higher than the amount of red clover and goat's rue. However, there were no differences in botanical composition between the fertilisation treatments within any of the legume mixtures (Paper II). Red clover and alsike clover had almost disappeared by the second harvest year, whereas the mean proportion of goat's rue increased from 5% in the first harvest year to 20% in the third year. Botanical composition differed between fertilisation treatments only in the third year, with a lower proportion of goat's rue in the normal N treatment than in the treatment Half N + ash. The proportion of weeds in the second and third harvest years was low, 8.3% and 5.5%, respectively (Paper III).

At Kyrkeby, the mean proportion of red clover was 36% and of alsike clover 40% in the first harvest year, while the proportion of goat's rue and lucerne was very low, 0.5 and 3%, respectively. By the second year the legume proportion had declined to less than 1% in all mixtures. The proportion of weeds was generally low, on average 4% in the first year and 2% in the second.

There were no significant differences in proportion of weeds between seed mixtures or fertilisation treatments at any of the sites (Paper III).

4.2.2 Biomass yield

Röbäcksdalen

The total mean autumn yield from the large (1.5 m x 7.5 m), machine-harvested plots in the first harvest year at Röbäcksdalen was 7000 kg DM ha^{-1} and spring yield was 2600 kg ha^{-1}, with no significant between-treatment differences in the autumn. 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 than the RCG monoculture (Paper II). The winter losses were 63% on average and were higher for plots with legumes (66%) than plots without (58%). Comparing all fertiliser treatments, DM yield of pure RCG was higher than DM yield of RCG in mixtures with red clover or alsike clover; 7500 kg ha^{-1} and 6600 kg ha^{-1}, respectively, in autumn and 3200 kg ha^{-1} and 2100 kg ha^{-1}, respectively, in spring. The comparison between pure RCG in the Normal and the Half N + PK fertilisation treatment and RCG in mixtures with red clover or alsike clover in the Half N + PK treatment showed a significant difference only for spring yield in the first harvest year (pure RCG 3160 kg ha^{-1}, clover mixtures 2020 kg ha^{-1}) (Figure 7).

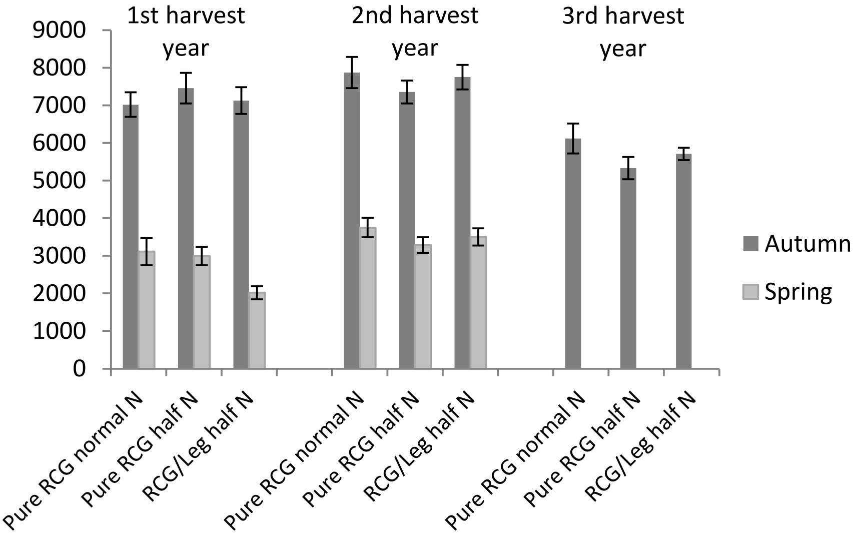

Figure 7. Dry matter yield (kg ha^{-1}) in autumn and spring for the different harvest years at Röbäcksdalen. The error bars show the standard error for pure RCG in the normal and the half N treatments (n=12) and RCG + legumes (red clover or alsike clover) in the half N treatment (N=16)

In the second harvest year, total mean yield was higher than in the first year in autumn and in the following spring (7900 and 3600 kg ha^{-1}, respectively). There were no differences between the species mixtures or fertilisation treatments (Paper III). The average winter losses were 54%. Comparing treatments with or without successful legume establishment over all fertiliser treatments, the treatments with legumes yielded higher in autumn than pure RCG (8100 kg ha^{-1} compared with 7600 kg ha^{-1}), but this difference did not persist until spring harvest. Mean autumn yield in the third harvest year was 6000 kg ha^{-1}, with no differences between the mixtures or fertilisation treatments. The experiment was not harvested in spring in the third harvest year. No differences were found between pure RCG in the Normal or Half N + PK treatment, and RCG in mixtures with red clover or alsike clover in the Half N + PK treatment in the second and third harvest years.

Ås

The total mean autumn yield for all plots in the first harvest year at Ås was 10800 kg DM ha^{-1}. Mean spring yield was 6700 kg, but yield was significantly higher in the normal N treatment (Paper II). The winter losses were 38%. Comparing pure RCG in the Normal N + PK treatment with RCG in mixtures with legumes in the Half N + PK treatment, the spring yield for pure RCG was higher (8000 kg ha^{-1}) than for the legume mixtures (6600 kg ha^{-1}) (Figure 8). When the RCG was undersown in barley, the yield was significantly lower, in autumn and in spring, than in the RCG monoculture.

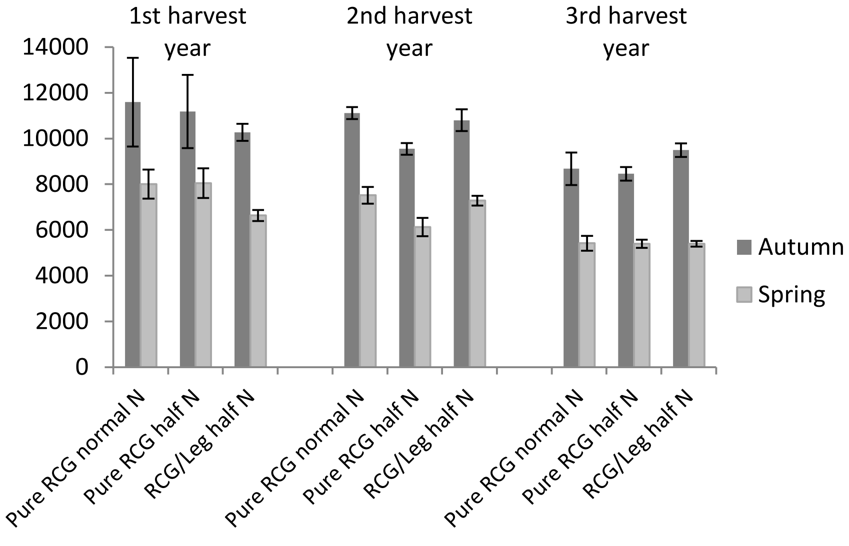

Figure 8. Dry matter yield (kg ha^{-1}) in autumn and spring for the different harvest years at Ås. The error bars show the standard error for pure RCG in the normal and the half N treatments (n=4) and RCG + legumes (red clover or alsike clover) in the half N treatment (N=8)

In the second year, total mean yield in autumn and spring was 10200 and 6900 kg ha^{-1}, respectively, and winter losses were 31%. There were no significant differences between any of the treatments (Paper III). Total mean yield in the third harvest year was 9000 kg ha^{-1} in autumn and 5400 kg ha^{-1} in spring. Winter losses were on average 39%, and were higher in mixtures with goat's rue (44%) than those without (35%). Except for higher autumn yield in one mixture with goat's rue, there were no differences between legume mixtures compared with RCG in monoculture. No differences were found between pure RCG in the Normal or Half N + PK treatment, and RCG in mixtures with legumes in the Half N + PK treatment in the second and third harvest years. Furthermore, the treatment with sewage sludge did not differ from the mineral PK fertilisation treatment.

Kyrkeby

At Kyrkeby, total mean yield in autumn in the first harvest year was 8200 kg DM ha^{-1} and in spring 6800 kg ha^{-1} (Paper III). The mean winter losses were 17%. In the second year the mean yield in autumn and spring was 9800 and 6900 kg ha^{-1}, respectively, and the winter losses were on average 28%. No significant differences between seed mixtures or fertilisation treatments were detected in either year (Figure 9).

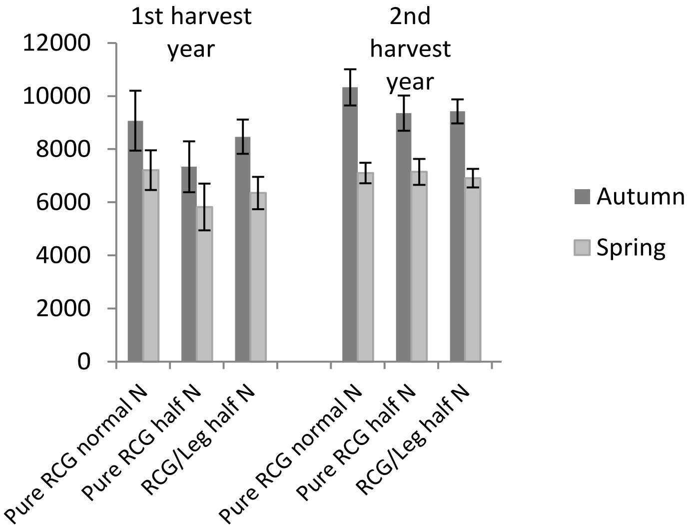

Figure 9. Dry matter yield (kg ha^{-1}) in autumn and spring for the different harvest years at Kyrkeby. The error bars show the standard error for pure RCG in the normal and the half N treatments (n=12) and RCG + legumes (red clover or alsike clover) in the half N treatment (N=16)

4.2.3 Nitrogen concentrations, δ^{15}N values and nitrogen fixation

The concentration of N in RCG was significantly higher in samples from Röbäcksdalen and the δ^{15}N value was lower when RCG was grown in mixtures with red clover or alsike clover compared with RCG monoculture. The differences between fertilisation treatments in this respect were not significant. Nitrogen fixation rate, 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 (Paper II). Only a few small differences in N concentration and δ^{15}N value were found between samples of RCG in the second harvest year. In the third year, RCG in the normal N fertilisation treatment had a higher N concentration than that in the half N treatment with only mineral fertilisers. Since almost no legumes remained after the first harvest year, N fixation was negligible (0-4 kg ha^{-1}) in the second and third year (Paper III).

In samples from Ås the concentration of N in the RCG biomass was significantly higher in plots undersown with barley than in biomass from the monoculture and from all mixtures except that with alsike clover. On average for all mixtures, the N concentration in RCG biomass was also higher in plots subjected to the high N fertilisation treatment. The N fixation rate, as determined by the N difference method, for the goat's rue, red clover and alsike clover plots was 12-28, 33-40 and 55 kg N ha^{-1}, respectively, in the first harvest year. No significant species- or fertilisation-associated differences in this variable were detected (Paper II). No differences in N concentration or δ^{15}N between the mixture treatments were seen in the second harvest year. In the third year, the N concentration in RCG in one of the goat's rue mixtures was higher than in RCG in monoculture. The mean N concentration in RCG was higher in the normal N fertilisation treatment than the half N treatment in all three harvest years. In the second year, the N concentration in goat's rue was on average 2.57% and the δ^{15}N value -0.32‰, while the corresponding values for the third harvest year were 2.46% N and δ^{15}N -0.18‰. The N fixation rate in goat's rue mixtures, estimated using the natural abundance method, varied from 13 to 21 kg ha^{-1} in the second year and from 49 to 53 kg ha^{-1} in the third year (Paper III).

At Kyrkeby, the monoculture RCG had a lower N concentration (0.88%) in the first year than the RCG in one of the red clover mixtures and in the alsike clover mixture (1.16%). The concentration of N in legume biomass was significantly lower in lucerne (1.60%) than in red clover (1.99%) and alsike clover (1.99%). The N fixation rate in the first harvest year was low for lucerne (1.9 kg ha^{-1}) and did not differ between the clover species (64 kg ha^{-1}).

Nitrogen fixation was negligible in the second harvest year, since most of the legume plants did not survive the winter (Paper III).

4.2.4 Fuel quality in spring-harvested biomass

There were few significant differences in concentrations of the main ash-forming elements in the samples. The mean ash concentration in the biomass samples varied from 3.1% at Röbäcksdalen to 9.8% at Kyrkeby. Estimated ash melting temperature in samples from Röbäcksdalen and Kyrkeby was most often above 1300°C, while in samples from Ås it was between 1100 and 1300 °C (Paper III).

4.3 Paper IV

The mean yield at Runtorp, harvested in November, was 10 000 kg ha^{-1} in 2012 and 9700 kg ha^{-1} in 2013. The relative amount of fertile shoots was 0.84 in 2012 and 0.66 in 2013. At Röbäcksmyran, the mean yield in October 2012 was 6100 kg ha^{-1} in 2012 and 7500 kg ha^{-1} in 2013. The relative amount of fertile shoots was 0.47 in 2012 and 0.70 in 2013. There were no differences in yield, amount of weeds or proportion of fertile shoots between the treatments at any of the sites. The concentration of plant-available K in the topsoil in both experiments was higher in the full PK-fertilised control than in the unfertilised control. At Röbäcksmyran, the concentration of P was also higher. The only significant differences in concentrations of minerals in the biomass at Runtorp were lower Mg and Ca concentrations in the full PK treatment compared to the control. At Röbäcksmyran the concentrations of P and K were higher and the concentrations of Ca, S and Cu were lower in the full PK-fertilised control than in the unfertilised control.

5 Discussion

5.1 Use of ash as a fertiliser

Recycling of waste products containing sufficient concentrations of plant nutrients is desirable in a sustainable agricultural production system. In particular, P is a limited and non-renewable resource, as existing rock phosphate resources could be exhausted within the next 50-100 years (Cordell *et al.*, 2009). Municipal sewage sludge and ash from agricultural crops such as RCG contain enough P to be used as fertilisers in agriculture. However, despite the benefits of recycling plant nutrients, there are concerns about using these waste products on arable land. Since undesirable elements such as heavy metals are concentrated in ash and sludge, there may be a risk of heavy metal enrichment in the soil and subsequently increased uptake by crops grown on the fields (Odlare *et al.*, 2011; Seleiman *et al.*, 2012; Börjesson *et al.*, 2014).

5.2 Heavy metals

In the ash fertilisation experiment in Paper I, the amounts of heavy metals added annually with the mixed ash (RCG co-combusted with household waste) exceeded several-fold the upper limits for heavy metals in sewage sludge set by the Swedish Environmental Protection Agency (SEPA) (Naturvårdsverket, 2004). The limits for sewage sludge were used as a guide, since there are currently no recommendations on the use of ash as a fertiliser on agricultural land.

After seven years of fertilisation, the mixed ash treatment led to significantly higher levels for all heavy metals except Ni in the uppermost soil layer (0-5 cm) compared with the pure RCG ash and the control (commercial mineral fertiliser) (Figure 6). Indications of increases compared with 2003 were seen for Cr, Pb and Zn, but could not be statistically verified as the analyses were not replicated in 2003. The mobility of the heavy metals downwards in the soil profile appeared to be low, as no differences in concentrations between the treatments were found in samples from deeper soil layers. To the best of our knowledge, comparable data on heavy metals in agricultural soil after fertilisation with ash have not been reported. However, it has been shown previously that ash fertilisation in peatland forests, involving single doses of 2.5-6.6 t ha^{-1} of different wood ash products, does not significantly increase heavy metal concentrations in the soil (Sikström, 2009). The concentrations of heavy metals in the soil in Paper I at the end of the study period were generally similar to typical values reported for the Umeå area (Mark- och grödoinventeringen, 2011). The only exception was the Cr concentration, which was approximately three-fold higher than the local average. Additional sampling of other neighbouring fields at the research station confirmed that the Cr concentration is generally high at the site (69.9-89.3 mg kg^{-1} dry soil). The reason for this is unknown, but the site is close to the local airport and to an industrial area.

None of the ash treatments resulted in any significant increase in heavy metal concentrations in plant biomass compared with the control treatment. The reason for this could be low metal solubility due to low soil acidity. The availability of heavy metals generally increases with decreasing pH (Magnusson, 2000). Plant uptake of ammonium and other cations can cause the pH in the rhizosphere to decrease due to excretion of hydrogen ions (Whitehead, 1995). However, reed canary grass does not prefer ammonium over nitrate (Neuschutz and Greger, 2010), and nitrate uptake counteracts acidification by excretion of OH^- ions. Thus in Paper I, pH was only slightly lowered, from 5.9 to 5.7-5.8, in the RCG ash and control treatments, while in the mixed ash treatment the Ca in the ash buffered the soil pH and prevented a decrease in pH. This might have counteracted any rise in heavy metal availability following heavy metal addition with the ash. The concentrations reported in Paper I (except for Zn) were lower or similar to those reported in studies of RCG on constructed wetlands for treatment of municipal sewage in the Czech Republic (Vymazal *et al.*, 2007; Vymazal *et al.*, 2010). However, since those studies did not report the concentrations of heavy metals in the soil or the input with the sewage, a closer comparison cannot be made. In Sweden, a study by Tyler and Olsson (2001) determined uptake of elements by common bentgrass (*Agrostis capillaris* L.) at different soil pH values, while another study in southern Sweden compared mineral uptake in perennial ryegrass (*Lolium perenne* L.) and cocksfoot (*Dactylis glomerata* L.) (Frankow-Lindberg *et al.*, 2009). Compared with their findings, the heavy metal concentrations in the RCG biomass reported in Paper I were similar or lower, except for Cr and Pb where the concentrations in RCG were higher.

In Paper IV, where fertilisation with RCG ash and different levels of P and K applied as mineral fertiliser were compared, only the concentrations of Cu and Zn in biomass in the second harvest year were analysed. That was done because Cu and Zn are also essential micronutrients for plants. Neither Cu nor Zn concentrations were elevated in the biomass after ash fertilisation; on the contrary the concentration of Cu was lower at Röbäcksmyran compared to the unfertilised control. This could have been related to the K applied with the ash, since the concentration of Cu was lower also in the full PK fertilisation treatment and K fertilisation can cause decreases in plant uptake of cations (Marschner, 1995).

5.3 Plant nutrients and biomass yield

The yield level of the spring-harvested biomass in the ash fertilisation experiment (Paper I) was generally low and the variation between years was large (Figure 5). The variation may have been due to abiotic factors such as temperature and precipitation during the growing season. Biotic factors such as insect pests, *e.g.* gall midges or aphids (Hellqvist *et al.*, 2003), or plant diseases might have affected the yields, although no obvious incidence of insect damage or plant pathogens was observed during the study period. The recorded yields were the actual harvested DM yields in spring. Since there were no measurements of plant growth in autumn, possible differences in winter losses could not be estimated. Field losses owing to degradation of biomass depend on winter conditions and mechanical losses might have differed depending on biomass conditions at the time of harvest, for instance moisture content.

The biomass yields in Paper I did not differ significantly between the control treatment, in which all P and K were supplied by mineral fertilisers, and the ash treatments, in which all P and some K were supplied by the ash and the rest of the K by mineral fertiliser (Figure 5). As the treatments were applied for seven consecutive years, the availability of the P in the ash appears to have been at least adequate to supply the crop. The finding that the P-AL concentration was not reduced in the topsoil confirms this.

Good availability of P in ash from other agricultural products (straw, rapeseed meal and cereals) has also been reported, *e.g.* by Eichler-Loebermann *et al.* (2008) and Schiemenz and Eichler-Lobermann (2010). Element balances compiled by comparing the amounts of P and K added to the plots and the amounts removed in the biomass clearly indicate that the added amounts of those elements exceeded crop requirements, which may partly explain why no differences between the treatments were observed.

The biomass yield of RCG was not limited by P or K availability even in the unfertilised control in Paper IV. However, two years is a short period when examining P and K fertilisation levels and deficiencies that give significant yield reductions take time to develop (Øgaard *et al.*, 2002; Saarela *et al.*, 2006). The concentrations of P-AL were considerably lower at Runtorp and Röbäcksmyran compared with soil analysis from the other experiments reported in this thesis (Table 2). That could suggest that the amount of plant available P in the soil would have been sufficient for the growth even without any P fertilisation also in the experiments reported in Papers I, II and III.

Table 2. *Concentrations (mg 100 g^{-1} dry soil) of plant available (AL) P and K in the topsoil at start of the experiments reported in the thesis. The classes are according to the Swedish classification system; class I low, class III medium and class V high*

		pH	P-AL	P-AL class	K-AL	K-AL class
Paper I	Röbäcksdalen	5.9	10.0	IV A	13.0	III
Paper II & III	Röbäcksdalen	6.0	8.5	IV A	7.3	II
	Ås	6.4	15.0	IV B	11.1	III
	Kyrkeby	6.4	13.0	IV B	22	IV
Paper IV	Runtorp	6.4	3.7	II	8.8	III
	Röbäcksmyran	5.4	2.8	II	6.4	II

5.4 Intercropping of reed canary grass and legumes

5.4.1 Establishment and botanical composition

Red clover and alsike clover in the mixtures with RCG established very well at all experimental sites. Data on botanical composition in the experiments at Röbäcksdalen and Kyrkeby showed that competition caused by a large proportion of clover restricted the establishment of RCG (Papers II and III). Similarly, in an American study where different legume species were intercropped with switchgrass, legume species with large biomass production restricted growth of switchgrass more than less productive legumes (George *et al.*, 1995). Some of the legume species did not establish well at Röbäcksdalen (goat's rue and kura clover) and Kyrkeby (goat's rue and lucerne), presumably partly due to unsuccessful or lacking inoculation. Since these species have never been cultivated previously on the experimental fields, successful inoculation is presumably crucial in order to establish an effective N_2-fixing symbiosis between legume plants and the N-fixing *Rhizobium* bacteria. Most plants of these species did not survive the first winter. Goat’s rue has been tested at Röbäcksdalen previously, without success. At Ås, where the proportion of clovers was lower, the legumes did not significantly affect the growth of RCG (Paper II). The goat’s rue also established well at Ås and contributed to the total biomass yield to approximately the same degree as red clover in the first harvest year. The good establishment of goat’s rue at Ås could be explained by higher soil pH at that site, since goat’s rue prefers neutral to acidic soil (Raig *et al.*, 2001). As goat’s rue established slowly compared with red clover and alsike clover, the competition with RCG during the first years was lower. In similar intercropping studies in Lithuania, RCG also competed well with goat’s rue during the first two years (Jasinskas *et al.*, 2008; Kryzeviciene *et al.*, 2008).

In forage production, the level of N fertilisation can be used to control the proportion of the legume in grass-legume leys, as higher amounts of N favour the grass in competition with the legumes (Carlsson and Huss-Danell, 2003). However, in Papers II and III a higher N fertilisation level did not significantly affect the clover proportion, which could have been expected due to enhanced competitiveness from the RCG for water, nutrients and light. Lower amounts of N applied in all treatments might have resulted in more pronounced differences.

5.4.2 Biomass production

The biomass yields differed greatly between the three experimental sites in Papers II and III. Total autumn yield for three harvest years was on average about 10000 kg ha^{-1} higher at Ås than at Röbäcksdalen. Comparing the two first harvest years, mean autumn yield at Kyrkeby was similar to that at Ås.

High proportions of red clover and alsike clover in the establishment phase obviously restricted the growth of RCG during the first production year at Röbäcksdalen. This resulted in lower autumn and spring yields of RCG in mixtures with red clover and alsike clover (mean yield) compared with pure RCG (including mixtures with kura clover and goat’s rue where the legumes did not survive the winter). The lower yields might be explained by competition for water from the legumes, causing limitation of RCG growth. Other studies have shown that growth of RCG can be more restricted by water availability than nitrogen availability (Kätterer *et al.*, 1998; Ge *et al.*, 2012). At Röbäcksdalen, precipitation in June and July, when the growth of RCG is most intense, was lower than at the other sites in all harvest years. Moreover, the sandy topsoil at Röbäcksdalen has a lower water-holding capacity than the soils at the other sites, which contain high proportions of clay and silt.

Furthermore, high temperatures at Röbäcksdalen in July in the first harvest year might have forced the growth of RCG to cease earlier in the season. Such a temperature effect was observed in a greenhouse experiment by Zhou *et al.* (2011). The water stress probably decreases with increasing age of the RCG crop as the root depth increases over time.

Larger winter losses in the experiment at Röbäcksdalen resulted in even larger differences in spring yield compared with the other sites. Such high winter losses have not been observed in earlier studies. In a number of Swedish RCG experiments during the 1990s, winter losses of biomass were estimated to be about 15% in northern Sweden and about 26% in southern Sweden (Landström *et al.*, 1996). An Estonian study on RCG production in farmers' fields compared biological yield (measured by hand-cutting of small plots) with practical yield and found winter losses in these small plots to be 5-25%, while they were 37-50% in the surrounding fields (Heinsoo *et al.*, 2011). Field losses occur due to degradation of the biomass during winter and early spring, and due to mechanical losses at harvest when the dry biomass is brittle. The plot harvesters used are not designed to lift cut biomass from the ground and were not identical at the different sites, so their performance might have differed. Thus, mechanical losses may be of varying importance at the different sites, but were not determined in this study. At Röbäcksdalen in particular, it was difficult to recover all of the biomass and the losses were most probably overestimated. Spring harvesting was carried out later at Röbäcksdalen than at the other sites because of slower drying of the soil due to high capillarity in the subsoil. This might have led to increased degradation before harvest. Winter biomass losses for RCG with legumes were higher than for pure RCG biomass. Herbaceous legumes are generally more easily degraded than RCG because of their higher N content (Wagger *et al.*, 1998). A study comparing summer and spring harvest in three grasses and goat's rue in Finland found that the goat's rue lost much more leaves during winter than RCG (Pahkala and Pihala, 2000).

The yield of pure RCG, fertilised with the higher or lower N rate, was compared with mixtures with significant proportions of legumes and given the lower N rate. The aim of this was to find out whether the N fertilisation rate can be lowered by intercropping. The only significant result from this comparison was lower yield for the legume mixtures at spring harvesting in the first harvest year at Röbäcksdalen and Ås. Furthermore, the yield of pure RCG did not differ between the N treatments at any of the sites or in any of the harvest years (Figures 7-9). Previous experiments in Sweden with RCG in monoculture did not show any differences in spring-harvested biomass in comparison between 100 and 200 kg N ha^{-1} (Landström *et al.*, 1996). In another study where application of 0, 50 and 100 kg N ha^{-1} was compared, no significant difference in production of aboveground biomass was found between 50 and 100 kg N ha^{-1}. However, the belowground production of rhizomes and shoot bases was higher when 100 kg ha^{-1} N was applied (Xiong and Kätterer, 2010). The RCG ash and the sewage sludge fertilisation treatments did not affect the biomass yield in any of the experiments. This was not unexpected since the concentrations of plant available P in soil, in particular, were high at all sites.

5.4.3 Nitrogen fixation

The estimated amounts of fixed N in the first harvest year were similar at Röbäcksdalen and Ås, despite the higher proportions of legumes at Röbäcksdalen. The δ^{15}N value for RCG was lower when intercropped with legumes than when grown in monoculture. This is an indication that some atmospheric N_2 might have been transferred from legume to grass via the soil already during the growing season (Høgh–Jensen, 2006; Fustec *et al.*, 2010). Thus, the amount of N_2 fixation might have been 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 as the method assumes that all of the N from the reference species is derived from the soil (Carlsson and Huss-Danell, 2014).

The N_2 fixation rate in red clover and alsike clover was high enough to compensate for lower N fertilisation rates in the mixtures in the first harvest year. The N_2 fixation rate in clovers was generally lower than that reported at comparable latitudes for red clover in two-cut systems for forage production (Carlsson and Huss-Danell, 2003). At Ås, the goat's rue had a lower N_2 fixation rate than the reduction in N fertilisation in the first two production years and similar rates in the third year. In the third year, there also was a small yield benefit from the goat's rue at the autumn cut. The N_2 fixation in goat's rue was higher in the half N fertilisation treatments in the second and third harvest years. We were unable to find any previous studies of N_2 fixation in goat's rue with which to compare our data. The N_2 fixation rate in lucerne at Kyrkeby was very low, probably caused by lack of efficient rhizobia since the seed was not inoculated. In other studies, lucerne derived more N from N_2 fixation than red clover (Carlsson and Huss-Danell, 2003; Pirhofer-Walzl *et al.*, 2012). Nitrogen fixation was not significantly affected by N fertilisation level, but since N fertilisers are manufactured using atmospheric $N_{2,}$ the method used might have had difficulty in distinguishing between the N sources.

5.4.4 Nitrogen concentration in reed canary grass

At all sites, in the first harvest year there were differences in N concentration and at Röbäcksdalen also in $\delta^{15}N$ values, between RCG grown in mixtures with well-established legumes and RCG without or with very sparse legume inclusions. The higher N concentration and lower $\delta^{15}N$ value in RCG intercropped with legumes indicate that transfer of N from legumes to grass had occurred. Transfer of N between clovers and grasses under field conditions has been demonstrated in several previous studies (*e.g.* (Høgh-Jensen and Schjoerring, 2000; Pirhofer-Walzl *et al.*, 2012). Greater N availability for ryegrass in a mixture with red clover has been demonstrated, with considerably higher N concentrations in the grass shoots in the mixture than in the shoots in a pure ryegrass stand (Dahlin and Stenberg, 2010). A study in northern Sweden using ^{15}N-labelled plants of legumes and non-legumes demonstrated that N was transferred in four different directions; from legume to legume, from legume to non-legume, from non-legume to legume and from non-legume to non-legume (Carlsson and Huss-Danell, 2014). In plots with perennial species (timothy and red clover), no evidence of N transfer was detected during the establishment year, but in the following year transfer occurred in all directions (Carlsson and Huss-Danell, 2014).

Another explanation for the higher N concentration in RCG in mixtures could be that since the amount of RCG was lower in the mixtures, more N was available for each RCG plant, as intercropped legumes rely mostly on N_2 fixation. However, this does not explain the differences in $\delta^{15}N$.

5.4.5 Fuel quality and ash characteristics

The ash content in the spring-harvested samples from Röbäcksdalen and Ås was lower (3-4%) than in the corresponding samples from Kyrkeby (9-10%). Silica was the dominant ash constituent at all sites. The uptake of ash components, mainly Si, in RCG is highly affected by soil type (Burvall, 1997; Heinsoo *et al.*, 2011). Results from another Swedish study show that plants grown on heavy clay soils have a higher ash content (10.1%) than plants grown on humus-rich sand soil (2.2%) (Burvall, 1997). This is consistent with our results, since the heavy clay soil at Kyrkeby gave the highest ash content. High ash content lowers the energy yield and increases the ash handling costs. However, when the high ash content is due to high silica content, there are also advantages. When the biomass is incinerated, Si binds to K and Na at high temperatures and forms silicates with a high melting point. Therefore the ash melting temperature in fuels is high when Si is the dominant constituent, as it is in RCG, and formation of the corrosive compounds KCl and K_2SO_4 is counteracted (Boström *et al.*, 2012). Higher Ca and Mg concentrations also lead to higher melting temperatures, but the interaction between Ca, Mg, K and Si is complicated and in RCG a higher Ca concentration can lead to lower ash melting temperature, as seen here in the biomass with legumes sampled at Ås (Paper III). However, the practical use of a particular biofuel is also dependent on the equipment available, such as adapted boilers and fuel handling chain, so this study provides a preliminary indication of fuel quality.

6 Conclusions

The main conclusion from the results to date is that the current fertiliser recommendations in Sweden for production of RCG as a spring-harvested bioenergy crop are too high, since there were no significant differences in biomass yield between the fertilisation levels of N, P or K in most cases studied in this thesis.

Reed canary grass ash or sewage sludge can be used to complement mineral fertilisers in RCG crops. It can prevent long-term depletion of P in the soil, but RCG ash contains small amounts of K and in sandy soils that are prone to K leaching this might not be sufficient. However, it is important to analyse the ash and the sewage sludge for plant nutrients and heavy metals when planning large-scale fertilisation schemes. The ash composition varies depending on factors such as growing site and type of boiler used for combustion. Ash from contaminated sources, such as ash from co-combustion of RCG with household waste, is obviously not sustainable in a longer term perspective because of the high content of heavy metals.

In the short term and on soils fertilised with organic P and K fertilisers at RCG establishment, P and K fertilisation might not be necessary. However, this needs to be confirmed in further experiments on soils with very low availability of P and K before it can be applied in practice.

Intercropping of RCG with legumes in a spring harvesting system is not beneficial, at least not at the N fertilisation levels applied in this thesis. The winter losses were larger when the proportion of legumes was significant. Red clover and alsike clover were obviously unable to survive more than one harvest year in a spring harvesting system. We expected the dead clover biomass to fertilise the RCG in subsequent years, giving an overall benefit, but there was little evidence of this.

Additional long-term experiments to determine the optimal N, P and K fertilisation rates for different soil types are essential to optimise the fertiliser recommendations and thereby enable profitable production of RCG for bioenergy purposes.

7 Svensk sammanfattning

Den ökande efterfrågan på energi, både globalt och i ett mer lokalt perspektiv, samt den globala uppvärmningen är drivkraften för en omfattande forskning och utveckling av nya energikällor. De fossila bränslena måste på sikt ersättas med förnybara råvaror för att minska utsläppen av växthusgaser. Inom EU finns ett direktiv att år 2020 ska 20 % av medlemsländernas totala energikonsumtion komma från förnyelsebara energikällor. Sol-, vind-, vatten- och bioenergi räknas som förnybara energislag.

Avverkningsrester från skogsproduktion, snabbväxande salix, halm och så kallade energigräs är exempel på råvaror för bioenergiproduktion. De används idag främst för produktion av värme och el genom direkt förbränning av biomassan. Rörflen är en gräsart som provats i Sverige för bioenergiproduktion sedan 1990-talet inom olika forskningsprojekt. En skördemetod som innebär att gräset (biomassan) lämnas kvar på fältet under vinter, och skördas påföljande vår, har utvecklats. Rörflen växer med kraftiga jordstammar, rhizomer, och transporterar ned växtnäring från de ovanjordiska växtdelarna till rhizomerna på hösten när växten vissnar. Näringsämnena kan växten sedan utnyttja under nästa års tillväxt. Biomassans kvalitet som bränsle förbättras också eftersom vissa av ämnena kan orsaka problem i värmepannorna vid förbränning.

Den praktiska odlingen av rörflen har dock av olika anledningar hittills varit ganska begränsad i Sverige. Produktionskostnaderna är i nuläget för höga för att kunna konkurrera med restprodukter från skogen. Jämfört med förbränning av skogsbränslen blir mängden aska mycket större när rörflen förbränns. De stora askmängderna anses vara ett problem och medför extrakostnader, och även miljöproblem, om askan ska deponeras. Kostnaden för gödsling av rörflen utgör en betydande del av produktionskostnaderna. En möjlighet att minska användningen av inköpt handelsgödsel kan vara att använda rörflensaska som gödselmedel vid odlingen eftersom askan innehåller växtnäringsämnen som grödan behöver, främst fosfor och kalium. Det finns dock vissa invändningar mot spridning av aska på jordbruksmark därför att skadliga ämnen, främst olika tungmetaller, på längre sikt kan anrikas i jorden. Om marken senare används för produktion av djurfoder eller livsmedel kan det medföra hälsorisker för människor och djur.

I ett fältförsök på Röbäcksdalens forskningsstation, i närheten av Umeå, har vi jämfört gödsling med aska och handelsgödsel. Två olika typer av aska användes, den ena var aska efter förbränning av enbart rörflen och den andra efter förbränning av rörflen blandat med sorterade hushållssopor. Samma mängd fosfor tillfördes i de tre olika gödslingsbehandlingarna. Resultatet efter en period av sju år visade att den årliga avkastningen av biomassa inte skiljde sig mellan de olika gödslingsbehandlingarna. Analyser av jordprov tagna när försöket avslutades visade att det skett en förhöjning av mängden tungmetaller endast i det översta jordskiktet (0 – 5 cm) på de ytor som gödslats med aska efter förbränningen med inblandning av sopor. Denna aska hade mycket höga halter av tungmetaller jämfört med den rena rörflensaskan. Vår slutsats är därför att ren rörflensaska kan återanvändas som ersättning för inköpta fosforgödselmedel i rörflensodlingar, medan aska efter inblandning av olika avfallsprodukter vid förbränningen ska undvikas.

Alla gräs behöver tillförsel av kväve för att ge en hög produktion av biomassa. I fältförsök på tre olika platser i landet har vi undersökt möjligheten att samodla rörflen med olika kvävefixerande baljväxter, främst rödklöver, alsikeklöver och getärt. Dessa växter kan med hjälp av vissa bakterier (*Rhizobium*-bakterier) använda kväve ur luften för sitt näringsbehov. Vår teori var att det kväve som baljväxterna tagit upp från luften skulle komma även gräset tillgodo och därigenom minska behovet av handelsgödselkväve. Resultaten från försöken gav dock inga säkra belägg för detta. En jämförelse mellan ren rörflen som gödslats med den för närvarande rekommenderade mängden kväve och rörflen samodlad med baljväxter med halverad kvävegödsling visade på lägre skördar vid samodling i några fall eller inga påvisbara skillnader. En jämförelse mellan ren rörflen vid de två kvävenivåerna gav heller inga påtagliga skillnader i skördeutbyte.

De gödslingsrekommendationer som finns tillgängliga idag för odling av rörflen som råvara för bioenergiproduktion bygger till stor del på vad som tillämpas vid odling av andra gräsarter för produktion av djurfoder. Eftersom växtsättet hos rörflen och även skördemetoden skiljer sig från de vanligen odlade fodergräsen behövs mer specifika rekommendationer. I ett pågående projekt jämförs gödsling med olika mängder av fosfor och kalium i form av handelsgödsel. Även gödsling med rörflensaska samt ingen fosfor- och kaliumgödsling ingår i försöket. Hela försöket har gödslats med samma mängd kväve. Försöken har än så länge endast skördats två år, och inga skillnader mellan de olika gödslingsbehandlingarna har kunnat påvisas. Växtnäringsförsök behöver följas under betydligt längre tid innan några säkra resultat kan påvisas. Vår slutsats är att de rekommendationer för gödsling av rörflen, som ska skördas på våren för användning som bioenergiråvara, är för höga för såväl kväve som för fosfor och kalium. Fortsatt forskning med syfte att optimera växtnäringsbehovet för olika markförhållanden och klimat är nödvändigt för att odlingen ska kunna bli lönsam.

References

Alaru M, Noormets M, Raave H, Lauk R, Selge A, Viiralt R (2009) Farming systems and environmental impacts. *Agronomy Research*, 7(1), 3-10.

Andersson A, Nilsson K O (1976) Influence on the levels of heavy metals in soil and plant from sewage sludge used as fertilizer. *Swedish Journal of Agricultural Research*, 6(2), 151-159.

Beringer T, Lucht W, Schaphoff S (2011) Bioenergy production potential of global biomass plantations under environmental and agricultural constraints. *Global Change Biology Bioenergy*, 3(4), 299-312.

Bessou C, Ferchaud F, Gabrielle B, Mary B (2011) Biofuels, greenhouse gases and climate change. A review. *Agronomy for Sustainable Development*, 31(1), 1-79.

Bethlenfalvay GJ, Reyessolis MG, Camel SB, Ferreracerrato R (1991) Nutrient transfer between the root zones of soyabean and maize plants connected by a common mycorrhizal mycelium. *Physiologia Plantarum*, 82(3), 423-432.

Boehmel C, Lewandowski I, Claupein W (2008) Comparing annual and perennial energy cropping systems with different management intensities. *Agricultural Systems*, 96(1–3), 224-236.

Boström D, Skoglund N, Grimm A, Boman C, Öhman M, Broström M, Backman R (2012) Ash Transformation Chemistry during Combustion of Biomass. *Energy & Fuels*, 26(1), 85-93.

Burvall J (1997) Influence of harvest time and soil type on fuel quality in reed canary grass (Phalaris arundinacea L). *Biomass and Bioenergy*, 12(3), 149-154.

Burvall J, Hedman B (1994) Bränslekaraktärisering av rörflen - resultat från första- och andraårs vallar. *Röbäcksdalen meddelar*, (5), 1-27.

Börjesson G, Kirchmann H, Kätterer T (2014) Four Swedish long-term field experiments with sewage sludge reveal a limited effect on soil microbes and on metal uptake by crops. *Journal of Soils and Sediments*, 14(1), 164-177.

Carlsson G, Huss-Danell K (2003) Nitrogen fixation in perennial forage legumes in the field. *Plant and Soil*, 253(2), 353-372.

Carlsson G, Huss-Danell K (2014) Does nitrogen transfer between plants confound N-15-based quantifications of N-2 fixation? *Plant and Soil*, 374(1-2), 345-358.

Carlsson G, Palmborg C, Huss-Danell K (2006) Discrimination against N-15 in three N-2-fixing Trifolium species as influenced by Rhizobium strain and plant age. *Acta Agriculturae Scandinavica Section B–Soil and Plant Science,* 56(1), 31-38.

Cordell D, Drangert JO, White S (2009) The story of phosphorus: Global food security and food for thought. *Global Environmental Change-Human and Policy Dimensions,* 19(2), 292-305.

Dahlin AS, Stenberg M (2010) Transfer of N from red clover to perennial ryegrass in mixed stands under different cutting strategies. *European Journal of Agronomy,* 33(3), 149-156.

Di Nasso NNO, Angelini LG, Bonari E (2010) Influence of fertilisation and harvest time on fuel quality of giant reed (Arundo donax L.) in central Italy. *European Journal of Agronomy*, 32(3), 219-227.

Eichler-Loebermann B, Schiemenz K, Makadi M, Vago I, Koeppen D (2008) Nutrient cycling by using residues of bio-energy production - effects of biomass ashes on plant and soil parameters. *Cereal Research Communications*, 36, 1259-1262.

Eriksson E, Christensen N, Schmidt JE, Ledin A (2008) Potential priority pollutants in sewage sludge. *Desalination*, 226(1-3), 371-388.

Evans MW, Ely JE (1935) The rhizomes of certain species of grasses. *Journal of the American Society of Agronomy*, 27, 791-797.

Frankow-Lindberg BE, Lindström B, Öborn I (2009). Annual and seasonal effects on micro-mineral concentrations in four grassland species. *Alternative functions of grassland*. Proceedings of the 15th European Grassland Federation Symposium, Brno, Czech Republic, 7-9 September 2009

Fustec J, Lesuffleur F, Mahieu S, Cliquet J-B (2011) Nitrogen Rhizodeposition of Legumes. *Agronomy for Sustainable Agriculture*, 30, 57-66.

Fustec J, Lesuffleur F, Mahieu S, Cliquet JB (2010) Nitrogen rhizodeposition of legumes. A review. *Agronomy for Sustainable Development*, 30(1), 57-66.

Ge ZM, Kellomäki S, Zhou X, Peltola H, Wang KY, Martikainen PJ (2012) Seasonal physiological responses and biomass growth in a bioenergy crop (Phalaris arundinacea L.) under elevated temperature and CO2, subjected to different water regimes in boreal conditions. *Bioenergy Research,* 5(3), 637-648.

George JR, Blanchet KM, Gettle RM, Buxton DR, Moore KJ (1995) Yield and botanical composition of legume-interseeded vs nitrogen-fertilized switchgrass. *Agronomy Journal*, 87(6), 1147-1153.

Hallam A, Anderson IC, Buxton DR (2001) Comparative economic analysis of perennial, annual, and intercrops for biomass production. *Biomass and Bioenergy*, 21(6), 407-424.

Heinsoo K, Hein K, Melts I, Holm B, Ivask M (2011) Reed canary grass yield and fuel quality in Estonian farmers' fields. *Biomass and Bioenergy*, 35(1), 617-625.

Hellqvist S, Finell M, Landström S (2003) Reed canary grass - observations of effects on crop stand and fibre quality caused by infestation of Epicalamus phalaridis. *Agricultural and Food Science in Finland*, 12(1), 49-56.

Hintze J (2012). NCSS 8. Kaysville, Utah, USA. www.ncss.com.

Hoogwijk M, Faaija A, van den Broek R, Berndes G, Gielen D, Turkenburg W (2003) Exploration of the ranges of the global potential of biomass for energy. *Biomass & Bioenergy*, 25(2), 119-133.

Högberg P (1997) Tansley review No 95 - N-15 natural abundance in soil-plant systems. *New Phytologist*, 137(2), 179-203.

Høgh-Jensen H, Schjoerring JK (2000) Below-ground nitrogen transfer between different grassland species: Direct quantification by N-15 leaf feeding compared with indirect dilution of soil N-15. *Plant and Soil*, 227(1-2), 171-183.

Høgh–Jensen H (2006) The nitrogen transfer between plants: An important but difficult flux to quantify. *Plant and Soil*, 282(1–2), 1-5.

Jasinskas A, Zaltauskas A, Kryzeuiciene A (2008) The investigation of growing and using of tall perennial grasses as energy crops. *Biomass and Bioenergy*, 32(11), 981-987.

Kryzeviciene A, Jasinskas A, Gulbinas A (2008) Perennial grasses as a source of bioenergy in Lithuania. *Agronomy Research*, 6(Sp. Iss. SI), 229-239.

Kukk L, Astover A, Muiste P, Noormets M, Roostalu H, Sepp K, Suuster E (2010) Assessment of abandoned agricultural land resource for bio-energy production in Estonia. *Acta Agriculturae Scandinavica Section B-Soil and Plant Science*, 60(2), 166-173.

Kätterer T, Andren O (2009) Predicting daily soil temperature profiles in arable soils in cold temperate regions from air temperature and leaf area index. *Acta Agriculturae Scandinavica Section B–Soil and Plant Science*, 59(1), 77-86.

Kätterer T, Andren O, Pettersson R (1998) Growth and nitrogen dynamics of reed canarygrass (Phalaris arundinacea L.) subjected to daily fertilization and irrigation in the field. *Field Crops Research*, 55(1-2), 153-164.

Landström S, Lomakka L, Andersson S (1996) Harvest in spring improves yield and quality of reed canary grass as a bioenergy crop. *Biomass and Bioenergy*, 11(4), 333-341.

Larsson S, Örberg H, Kalén G, Thyrel M (2006). Rörflen som energigröda. BTK-rapport. Umeå, SLU, Enheten för biomassateknologi och kemi. 11.

Lewandowski I, Scurlock JMO, Lindvall E, Christou M (2003) The development and current status of perennial rhizomatous grasses as energy crops in the US and Europe. *Biomass and Bioenergy*, 25(4), 335-361.

Magnusson M (2000). Soil pH and Nutrient Uptake in Cauliflower (*Brassica oleracea* L. var. *botrytis*) and Broccoli (*Brassica oleracea* L. var. *Italica*) in Northern Sweden. Umeå, Swedish University of Agricultural Science.

Mark- och grödoinventeringen (2011). Swedish University of Agricultural Sciences.

http://www-jordbruksmark.slu.se/AkerWebb/MgiPub/Index.jsp.

Marschner H (1995). *Mineral nutrition of higher plants*. London, UK, Academic Press.

Mårtensson AM, Rydberg I, Vestberg M (1998) Potential to improve transfer of N in intercropped systems by optimising host-endophyte combinations. *Plant and Soil*, 205(1), 57-66.

Naturvårdsverket (Swedish Environmental Protection Agency, SEPA) (1994). Statens naturvårdsverks författningssamling 1994;1994:2.

Neuschutz C, Greger M (2010) Ability of Various Plant Species to Prevent Leakage of N, P, and Metals from Sewage Sludge. *International Journal of Phytoremediation*, 12(1), 67-84.

Odlare M, Arthurson V, Pell M, Svensson K, Nehrenheim E, Abubaker J (2011) Land application of organic waste - Effects on the soil ecosystem. *Applied Energy*, 88(6), 2210-2218.

Pahkala K (2007). Reed canary grass cultivation for large scale energy production in Finland. NJF Seminar 405 Production and utilization of crops for energy, Vilnius, Lithuania.

Pahkala K, Aalto M, Isolahti M, Poikola J, Jauhiainen L (2008) Large-scale energy grass farming for power plants - A case study from Ostrobothnia, Finland. *Biomass and Bioenergy*, 32(11), 1009-1015.

Pahkala K, Pihala M (2000) Different plant parts as raw material for fuel and pulp production. *Industrial Crops and Products*, 11(2-3), 119-128.

Palmborg C (2012). Effects of harvest time (early winter or spring) of reed canary grass on track depth, penetration resistace and plant groth and development. NJF seminar 448 Soil Compaction - effects on soil functions and strategies for prevention. Helsinki, Nordic Association of Agricultural Scientists: 43-45.

Park BB, Yanai RD, Sahm JM, Lee DK, Abrahamson LP (2005) Wood ash effects on plant and soil in a willow bioenergy plantation. *Biomass and Bioenergy*, 28(4), 355-365.

Paynel F, Murray PJ, Cliquet JB (2001) Root exudates: a pathway for short-term N transfer from clover and ryegrass. *Plant and Soil*, 229(2), 235-243.

Pirhofer-Walzl K, Rasmussen J, Høgh-Jensen H, Eriksen J, Soegaard K, Rasmussen J (2012) Nitrogen transfer from forage legumes to nine neighbouring plants in a multi-species grassland. *Plant and Soil*, 350(1-2), 71-84.

Raig H, Nömmsalu H, Meropöld H, Metlitskaja J (2001). *Fodder galega*. Saku, Estonian Research Institute of Agriculture.

Saarela I, Huhta H, Virkajärvi P (2006) Effects of repeated phosphorus fertilisation on field crops in Finland - 2. Sufficient phosphorus application rates on silty and sandy soils. *Agricultural and Food Science*, 15(4), 423-443.

SAS (2013). *SAS Institute Inc*. Version: 9.4. Cary, NC, USA.

Schiemenz K, Eichler-Lobermann B (2010) Biomass ashes and their phosphorus fertilizing effect on different crops. *Nutrient Cycling in Agroecosystems*, 87(3), 471-482.

Seleiman MF, Santanen A, Stoddard FL, Makela P (2012) Feedstock quality and growth of bioenergy crops fertilized with sewage sludge. *Chemosphere*, 89(10), 1211-1217.

Sikström U, R. G.Björk, E. Ring, M. Ernfors, S. Jacobson, M. Nilsson, L. Klemedtsson (2009). Tillförsel av aska i skog på dikad torvmark i södra Sverige ? effekter på skogsproduktion, flöden av växthusgaser, torvegenskaper, markvegetation och grundvattenkemi. Värmeforsk Rapport 1109.

Tahir MHN, Casler MD, Moore KJ, Brummer EC (2011) Biomass Yield and Quality of Reed Canarygrass under Five Harvest Management Systems for Bioenergy Production. *Bioenergy Research*, 4(2), 111-119.

Tike. (2014). *Agricultural statistics*, http://www.maataloustilastot.fi/en/utilised-agricultural-area, (retrieved 05-20, 2014).

Tonn B, Thumm U, Claupein W (2010) Semi-natural grassland biomass for combustion: influence of botanical composition, harvest date and site conditions on fuel composition. *Grass and Forage Science*, 65(4), 383-397.

Tyler G, Olsson T (2001) Plant uptake of major and minor mineral elements as influenced by soil acidity and liming. *Plant and Soil*, 230(2), 307-321.

Unkovich M, Herridge D, Peoples M, Cadisch G, Boddey B, Giller K, Alves B, Chalk P (2008). *Measuring plant-assosiated nitrogen fixation in agricultural systems*. Canberra, Australian Center for International Agricultural Research.

Wagger MG, Cabrera ML, Ranells NN (1998) Nitrogen and carbon cycling in relation to cover crop residue quality. *Journal of Soil and Water Conservation*, 53(3), 214-218.

Wang D, Lebauer DS, Dietze MC (2010) A quantitative review comparing the yield of switchgrass in monocultures and mixtures in relation to climate and management factors. *Global Change Biology Bioenergy*, 2(1), 16-25.

Whitehead DC (1995). Influence of Fertilizer Nitrogen on the Composition and Nutritional Quality of Grassland Herbage. Grassland Nitrogen. D. C. Whitehead, CAB International: 264-282.

Wrobel C, Coulman BE, Smith DL (2009) The potential use of reed canarygrass (Phalaris arundinacea L.) as a biofuel crop. *Acta Agriculturae Scandinavica Section B–Soil and Plant Science*, 59(1), 1-18.

Vymazal J, Kropfelova L, Svehla J, Stichova J (2010) Can multiple harvest of aboveground biomass enhance removal of trace elements in constructed wetlands receiving municipal sewage? *Ecological Engineering*, 36(7), 939-945.

Vymazal J, Svehla J, Kropfelova L, Chrastny V (2007) Trace metals in Phragmites australis and Phalaris arundinacea growing in constructed and natural wetlands. *Science of the Total Environment*, 380(1–3, Sp. Iss. SI and 185VE and 185VE), 154-162.

Xiong S, Burvall J, Örberg H, Kalen G, Thyrel M, Ohman M, Bostrom D (2008) Slagging characteristics during combustion of corn stovers with and without kaolin and calcite. *Energy & Fuels*, 22(5), 3465-3470.

Xiong S, Kätterer T (2010) Carbon-allocation dynamics in reed canary grass as affected by soil type and fertilization rates in northern Sweden. *Acta Agriculturae Scandinavica Section B–Soil and Plant Science*, 60(1), 24-32.

Xiong S, Landström S, Olsson R (2009) Delayed harvest of reed canary grass translocates more nutrients in rhizomes. *Acta Agriculturae Scandinavica Section B–Soil and Plant Science*, 59(4), 306-316.

Zhou X, Ge ZM, Kellomaki S, Wang KY, Peltola H, Martikainen P (2011) Effects of elevated CO_2 and temperature on leaf characteristics, photosynthesis and carbon storage in aboveground biomass of a boreal bioenergy crop (Phalaris arundinacea L.) under varying water regimes. *Global Change Biology Bioenergy*, 3(3), 223-234.

Øgaard AF, Krogstad T, Lunnan T (2002) Ability of some Norwegian soils to supply grass with potassium (K) - soil analyses as predictors of K supply from soil. *Soil Use and Management*, 18(4), 412-420.

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