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SUSTAINABLE CROPPING OF REED CANARY GRASS FOR ENERGY USE

DOCTORAL THESIS

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ACADEMIC DISSERTATION

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DEDICATION

To my dear parents; Epie Mathias Enuge and Epie Elah Ntube.
They never lived to see this day.

“Tough times never last but tough people do”
~ Robert H. Schuller

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LIST OF ORIGINAL PUBLICATIONS

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I **Epie, K.E.,** Virtanen, S., Santanen, A., Simojoki, A., Stoddard, F.L. 2014. The effects of a permanently elevated water table in an acid sulphate soil on reed canary grass for combustion. Plant and Soil 375: 149-158.

II **Epie, K.E.,** Saikkonen, L., Santanen, A., Jaakkola, S., Mäkelä, P., Simojoki, A., Stoddard, F.L. 2015. Nitrous oxide emissions from perennial grass–legume intercrop for bioenergy use. Nutrient Cycling in Agroecosystems 101: 211-222.

III **Epie, K.E.,** Cass, S., Stoddard, F.L. 2015. Earthworm population structure under boreal grass and legume bioenergy crops in pure stands and mixtures. Pedobiologia- Journal of Soil Ecology 58: 49-54.

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Publication I

The planning of the study was done by all authors. Kenedy Epie, Seija Virtanen and Asko Simojoki performed the experimental work. Kenedy Epie had the main task of collecting data, analysing them and interpreting the results with guidance from Frederick L. Stoddard. Kenedy Epie wrote the manuscript, incorporated the inputs of other authors and was the corresponding author of the paper.

Publication II

The planning of the study was done by all authors except Seija Jaakkola and Liisa Saikkonen. Gas and soil samples were collected by Kenedy Epie who with guidance from Asko Simojoki did the laboratory and statistical analyses and interpretation of the results. Seija Jaakkola assisted in energy analysis while Liisa Saikkonen contributed in CO_2 gas equivalent calculations of N_2O gas emissions. Kenedy Epie wrote the manuscript, incorporated the inputs of other authors under general guidance of Frederick L. Stoddard, and was the corresponding author of the paper.

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The planning of the study was done by all authors except Susannah Cass. Kenedy Epie did all the experimental work and the main task of collecting data and some of the analyses. Susannah Cass helped in identifying the earthworms and analysing some of the earthworm data. Kenedy Epie interpreted the results and wrote the manuscript, incorporated the inputs of other authors under general guidance of Frederick L. Stoddard, and was the corresponding author of the paper.

ABBREVIATIONS

AEBIOM	European Biomass Association
C	Carbon
Ca	Calcium
CH_4	Methane
Cl	Chlorine
CO	Carbon monoxide
CO_2	Carbon dioxide
DM	Dry matter
EIA	Energy Information Administration
EC	European Commission
EU	European Union
g	gram
GBEP	Global Bioenergy Partnership
GHG	Greenhouse gas
GJ	Giga joule
H	Hydrogen
ha	hectare
IEA	International Energy Agency
IPCC	Intergovernmental Panel on Climate Change
IRGA	Infrared gas analysis
K	Potassium
kg	kilogram
kJ	kilojoule
MJ	Megajoule
N	Nitrogen
N_2O	Nitrous oxide (dintrogen dioxide)
P	Phosphorus
RCG	Reed canary grass
S	Sulphur
Si	Silicon
t	Ton

KEY DEFINITIONS

Bioenergy: a renewable form of energy produced from biomass.

Bioenergy crop: crop grown for use to produce bioenergy (Lemus and Lal 2005).

Biofuel: fuel made directly or indirectly from biomass such as wood, charcoal, biodiesel, bioethanol and biogas.

Biomass: non-fossil materials of biological origin, such as plants, agricultural and forestry wastes and by-products.

Biomass cropping: the cultivation of crops for biomass.

Biomass feedstock: biological materials used as key inputs in production processes to create bioenergy.

Carbon sequestration: the transfer and secure storage of atmospheric CO_2 in long-lived pools (Lal 2004).

Ecosystem services: the benefits obtained from ecosystems. These include provisioning services such as food and water; regulating services such as flood and disease control; cultural services such as spiritual, recreational, and cultural benefits; and supporting services, such as nutrient cycling, that maintain the conditions for life on Earth (UNEP 2005).

Fossil fuel: hydrocarbons, primarily coal, crude oil or natural gas, formed by exposure to heat and pressure in the earth's crust hundreds of millions of years ago.

Renewable energy: energy derived from sources such as sun and natural processes that are replenished constantly.

Sustainability: providing for today’s needs without jeopardising the ability of meeting tomorrow’s needs (Bruntland Report 1987)

ABSTRACT

The increasing use of fossil fuel is plagued with problems leading to interest in alternative sources of energies. Bioenergy or biomass energy remains today's important renewable energy source that can contribute to reducing the overall consumption of fossil fuel and can move energy systems towards sustainability and supply security. However, doubts on sustainability impede the acceptance of bioenergy. Hence, the sustainable cropping of reed canary grass (*Phalaris arundinacea* L., RCG), an established perennial energy grass, was studied. Important sustainability criteria were considered, namely; land use, biomass productivity, emission of greenhouse gas nitrous oxide (N_2O) and biodiversity. The general aim of the study was to develop farming methods that would provide biomass feedstock of RCG in a sustainable manner.

Field and glasshouse experiments were carried out at the University of Helsinki, Finland, during 2008 to 2013. The suitability of problematic acid sulphate soils managed with raised water tables for cropping RCG was investigated in lysimeter experiments. Growth parameters were measured and biomass yield and energy qualities were determined. In field conditions with soils classified as Gleyic Stagnosol, RCG was supplied with N from inorganic fertilizer and N fixed into soil by intercropped legume galega (*Galega orientalis* Lam.) and its biomass yields and mineral element composition and other energy qualities were determined. Gases were collected from these fields using closed chambers and greenhouse gas N_2O emissions were analysed by gas chromatography. The crop and crop mixture effects on earthworm communities were determined by the extraction of earthworms using mustard oil and manual separation from soil.

Reed canary grass grew well in acid sulphate soils and even performed better by producing more biomass with better quality when the water table was raised to reduce acidity and to avoid environmental hazards. Carbon was also sequestered into the soil by RCG root biomass. In the field experiment, RCG–galega mixtures produced equally good biomass yields and of better energy quality than the fertilized RCG counterpart. The annual cumulative emissions of N_2O from mixtures were marginally lower than those from fertilized RCG soils. Although fertilized RCG produced twice as much biomass and correspondingly higher nitrogen and energy yields, its low emission of N_2O per ton of dry matter or per unit of harvestable bioenergy was not significantly different from that of the mixtures. Mixtures also enhanced earthworm abundance and species numbers compared to pure RCG stands. Therefore cropping an RCG–galega mixture for biofuel may supply a good quantity of biomass feedstock, result in lower N_2O gas fluxes, and sustain earthworm biodiversity but requires management to maintain grass as the major component.

Using managed acid sulphate soils for perennial energy cropping will help to reduce the tension between food and energy crop production over arable land and may improve the negative perception of bioenergy as a whole. A 25% Galega-75% RCG mix has the potential to replace N fertilizer input during energy crop

cultivation, meaning reduced cost of production and more income for energy crop farmers. Moreover soil macrofauna diversity will be conserved. With reduced N_2O gas emission, this grass-legume mixture could make a significant contribution in mitigating climate change and its effects. All these will come a long way to help in making bioenergy more sustainable.

1 INTRODUCTION

1.1 Bioenergy, an alternative to fossil fuels

Until late in the nineteenth century, bioenergy was used for heat, cooking and lighting, and was then replaced by fossil fuels in many industrialized countries. Today, it is again one of the important renewable energy sources, alongside solar, wind, geothermal, and hydro-power. Because bioenergy requires large cropping areas, it cannot completely replace fossil fuels, but it can contribute to reducing overall fossil fuel consumption and can move energy systems towards sustainability and supply security (Resch et al. 2008). Bioenergy production can also generate incomes and investment in rural areas where the biomass is produced, thereby contributing to the rural economy and development. The central role bioenergy occupies in the world's three great challenges; energy security, climate change, and poverty reduction (FAO/GBEP 2007), has stimulated extensive research in the past few years.

The use of bioenergy is not only determined by energy demand and biomass feedstock availability, but also depends on the choice of biomass feedstock, the management of land resources when growing the feedstock, the kind of land-use changes induced by cultivation and, finally, on the conversion and processing methods used in the energy production (Fritsche and Wiegmann 2008). Based on these, biofuel is classified into "first" and "second" generation. First generation biofuels are mostly from food crops (starch, sugar and oil crops) that require energy inputs mostly in the form of fertilizers for biomass feedstock production. Second generation biofuels are from lignocellulosic non-food crops or food crop co-products that produce large amounts of biomass and possess high resource use efficiency (Larson 2008) and require further technological and commercial development (Connor and Hernandez 2009).

Globally, policies and objectives for bioenergy have been developed by many countries in recent years (IEA 2005). Strict targets and regulations are put in place to increase use of renewable sources in energy production and to reduce greenhouse gas emissions. The EC binding renewable energy target in 2020 (Berndes and Hansson 2007) is one such. The 2009 EU Directive on Renewable Energy is a policy document that requires that 20% of total energy consumption comes from renewables and 10% of transport fuels be delivered by biofuels by 2020 (EC 2009). At national levels in Europe, bioenergy has received supportive policies and measures to promote its production and use in countries such as Finland, Sweden and Austria (McCormick and Kaberger 2007).

Nevertheless, the sustainability of bioenergy production has been questioned. It has been implicated as a cause of increased food prices (Mitchell 2008), disputes over land use leading to poverty in many developing countries (Koh and Wilcove 2008), and emissions of GHGs from deforestation for biofuel plantations (Fargione et al. 2008). Diverting arable land and or food-crops to energy production affects world food security, so the feasibility and ethics of this practice is being debated (Pimental and Patzek 2005).

1.1.1 Bioenergy contribution to energy supply

With the global primary energy consumption set to almost double in 30 years (Bakkes et al. 2008), bioenergy will continue to play an important role as a renewable energy source contributing more than 60% of total renewable energy and about 15% of global primary energy demand (AEBIOM 2010). In many developing countries, bioenergy accounts for 80% of the total primary energy supply used for heating and cooking, and about 15% for modern use (GBEP 2008), whereas in most industrialized countries it provides less than 5% (Keam and McCormick 2008).

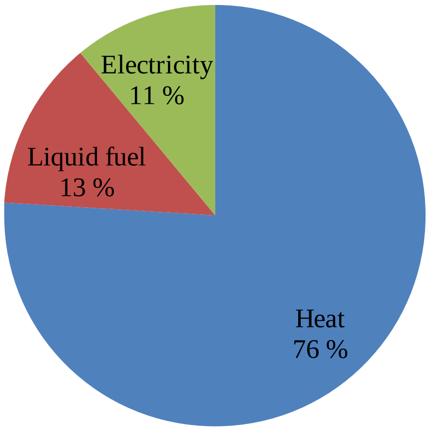

Figure 1. Biomass fuel uses in the EU in 2008 (AEBIOM 2010).

In the EU in 2008, bioenergy contributed 13% liquid fuel, 11% electricity and the lion share of 76% went to the heating sector (Figure 1) (AEBIOM 2010). Finland is one of the leading EU countries for energy from renewable sources, as wood is the main source of bioenergy (Routa et al. 2012), constituting 25% of energy use (Ramo et al. 2009) in 2006. In 2008, its contribution to primary energy consumption dropped to 21% (Statistics Finland 2009).

1.1.2 Why search for alternatives to fossil fuel?

Fossil fuels supply most of the present day energy so there is need to address problems associated with its use and to look for alternative sources of energies.

The world population is expected to reach 9.3 billion in 2050 (World Population Prospects 2011). The demand for food and energy is expected to double in 50 years (IEO 2006), so more socio-economic development is needed and consequently more energy is required. Fossil fuel resources alone will not be able to meet this demand. They are non-renewable, become quickly depleted and are limited in supply from the small number of supplier countries. Its remote location in deep bedrock makes extraction, transportation and processing difficult and costly. The unequal distribution of fossil fuel reserves around the globe creates geopolitical tensions and conflicts over these reserves and production (El Bassam 2012).

The environmentally damaging effects from drilling, refining, and burning fossil fuel include soil, water and air pollutions, GHG emissions and global warming. Global warming is caused by accumulation of GHGs in the atmosphere released from the use of fossil fuel (IPCC 2007). This accumulation was earlier implicated in the average global temperature increases of 1.5-4.5°C (Smith and Almaraz 2004). The above mentioned points and impacts on human health have raised concerns over dependence on fossil fuels (Stern 2006) and hence the need to replace them.

1.1.3 Advantages of bioenergy and biofuels

Justification for use of biofuel is centred on environment and health. During biomass cropping, the constantly replenishing natural process of photosynthesis helps to bind atmospheric CO_2 which is then sequestered and later released when the biomass is used for energy production, so does not shift the C cycle (Kort et al. 1998). During sequestration, atmospheric CO_2 is captured by plants and stored as C in stems and roots of the plants and in the soil, thus reducing the level of CO_2 in the atmosphere (Lemus and Lal 2005).

Biofuels contain less S and emit less GHG than fossil fuels (Zhuang et al. 2010), consequently improving air quality and are mostly used as additives to transport fuels, and have recently been used on their own as transportation fuels in vehicles with specialized engines. Bioethanol contains 1/3 elemental oxygen which improves combustion and reduces car pollution. A blend of maize ethanol with gasoline helps in reducing emissions because the ethanol serves as an oxygenate (Hansen et al. 2006). Mixing 5% ethanol into gasoline removes or replaces 7% dangerous aromatics such as benzene that causes cancer (Caserta et al. 1995). Biodiesel is biodegradable and non-toxic, and when compared to petroleum diesel, it emits less carbon monoxide (CO), soot (particulate) and unburned hydrocarbons, and it is safer to handle and transport because of its high flash point of 150°C (Krawcsyk 1996).

1.1.4 Conversion and use of biofuels

The most common form of biofuel is from lignocellulose biomass comprising herbaceous and woody tissues burnt to produce electricity and heat (Qin et al. 2006). Crop biomass can be fired or co-fired with coal to generate heat and electricity (Powlson et al. 2005). Combined heat and power plants (CHP) are used to burn biomass pellets or bales and the hot gases produced are used either for heating or electricity production (El Bassam 2010).

Biomass feedstock is processed by pyrolysis and gasification to produce biogas. Both processes are thermo-chemical. During pyrolysis, biomass is exposed to different temperatures in the absence of oxygen to produce gases, liquids and solids and during gasification, the biomass undergoes partial oxidation by heat at 1200°C to produce gas (Dumbleton 1997).

Bioethanol can be sugar-based from sugarcane or sugar beet, starch-based from maize or potatoes and cellulosic from herbaceous crops and woody materials. Starch and lignocellulose biomass are first broken down into sugars by acids and hydrolytic enzymes during anaerobic digestion (Mamma et al. 1995). Then the sugars are fermented using yeast and enzymes to produce bioethanol (Sun and Cheng 2002, El Bassam 2010). Ethanol conversion efficiencies on dry matter (DM) basis of grains are comparatively higher than that of biomass (Wallace et al. 2005).

Vegetable oils or animal fats can be treated by transesterification, a conversion process using alcohol and either NaOH or KOH to produce biodiesel (Stephenson et al. 2008). Biodiesel functions efficiently in conventional diesel engines without need for modification, but can be used as an additive to fossil diesel (Al-Zuhair 2007).

Lastly it is also possible to digest biomass anaerobically to produce biogas comprised of 60% methane and 40% carbon dioxide (El Bassam 2010). It is worth noting that these conversion processes produce residues and by-products that are recycled into the bioenergy production chain as fertilizers, feedstocks and animal feed (Lumpkins et al. 2004).

1.2 Cropping for bioenergy

1.2.1 Reed canary grass

Reed canary grass is a perennial, heterogamous, rhizomatous member of the Poaceae. It is a C3 cool-season grass, native to northern Europe. Since it is indigenous in Finland, growing mostly along water bodies, the invasive growing pattern should not be a problem in this country, but it is considered invasive in North America. Besides sexual reproduction by seeds, this grass can reproduce vegetatively. The rhizome and root system make up to half the total biomass (Kättere and Andren 2009) serving as a large reserve for nutrients that may significantly influence plant growth (Conchou and Fustec 1988). Reed canary grass is well adapted to short growing seasons and low temperature, reproduction is mostly by seed, it overwinters well and can be harvested at all times of the year (Lewandowski et al. 2003). The grass grows well in most kinds of soils (Ostrem 1987), including poorly drained soils, and also tolerates drought better than many other grass species (Vose 1959). In the beginning, the growth of RCG is slow and yields are low in the seeding year. Thereafter biomass yield increases into the 3rd year due to the well-developed rhizome system, better recirculation and storage of nutrients, and plantations can be productive for over 10 years (Hadders and Olsson 1997).

In Finland, there was extensive commercial production of RCG for bioenergy (Pahkala et al. 2008), but recently use and cropping area have decreased (Tike 2014). RCG has long been used as forage grass, but different types and concentrations of poisonous alkaloids limit this use (Marten et al. 1976). It has been used in paper and pulp industry (Saijonkari-Pahkala 2001) where it produces high quality fibre pulp (Finell and Nilsson 2004), mostly when leaves and sheaths are removed to reduce the ash and mineral contents (Pahkala and Pihala 2000).

RCG also has environmental importance. Because of its high nutrient requirement, it is used to catch nutrients in land treatments (Geber 2002), thus preventing nutrient leaching (Partala et al. 2001). The large rhizomatous root system can bind the soil, thereby controlling erosion (Antieau 2004). It could be used to improve the quality of waste water as it removes N and other elements (Picard et al. 2005) and can be effective in phytoremediation (Chekol et al. 2002). RCG has been bred in Australia to increase tolerance to acid soils characterized by high concentrations of Al^{3+} and Mn^{2+} and deficient in Ca^{2+} (Jackson 1967, Requis and Culvenor 2004).

1.2.2 Perennial grasses versus forest bioenergy crops

Bioenergy crops should be highly adaptable to marginal land (uncultivated, acidic or alkaline soils or otherwise not suitable for food crops), fast growing, easy to harvest, contain large energy reserves and not cause damage to the environment (Li et al. 2010). They should have the capacity to sequester C into the soil, produce large volumes of biomass, and hence high energy potentials (Lemus and Lal 2005). Perennial herbaceous crops or woody forest crops are more often considered to have bioenergy potential, because they possess more of the above characteristics, than annuals. The cultivation and harvesting of annuals for bioenergy requires large amounts of energy in the form of annual sowing and fertilization (Hulsbergen et al. 2001), putting into doubt their ability to reduce GHG emission (Farrell et al. 2006).

Grasses including RCG are usually perennial herbaceous field crops with little or no woody tissue. In the autumn, they take up nutrients, hold them in tissues and later translocate them to roots before harvesting takes place at the end of the growing season (Lemus 2004). By recycling nutrients into the deep rooting systems (Sommer et al. 2000), they require low nutrient inputs (Christian et al., 1997) and often little or no pesticide use (Lewandowski et al. 2000), so they have a positive net energy balance (Hill, 2007) and also increase soil organic carbon (SOC) in deeper soil layers (Tolbert et al. 2002). They do not need fossil fuel consumption for sowing and tillage except for the first year, thus reducing the cost of cultivation and the risk of erosion (Ma et al. 1999). When further compared to annuals such as maize and soybean (*Glycine max* (L). Merr.), perennial crops for energy generation reduce GHG emissions (Adler et al. 2007), 11 times less CO_2 emission than when fossil fuel was used (Ma et al. 2000).

Generally, lignocellulosic perennial biomass plantations can last as long as 10 to 15 years (Lemus and Lal 2005). The lignin-rich feedstocks they supply have high C contents that give a high heating value. Cropping lignocellulosic perennials decreases soil erosion, improves water quality and protects natural biodiversity (Semere and Slater 2007), so providing more environmental benefits (Robertson et al. 2008). Elephant grass (*Pennisetum purpureum* Schum.), switch grass (*Panicum virgatum* L.), miscanthus (*Miscanthus* spp.), RCG and tall fescue

(*Festuca arundinacea* Schreb.) are examples of perennial grasses identified as promising energy crops (Madakadze et al. 1999, Lewandowski et al. 2003).

Woody species are grown for their high density biomass. The biomass is easy to store and handle, has low ash content, and mixes well with other woody residues or waste (Johnson et al. 2007). In comparison with perennial grasses, they require more years to mature (Lemus and Lal 2005) and are harvested only once. Meanwhile perennial field energy crops are harvested annually, giving annual income, and the land can easily be returned to arable crop cultivation. The greatest concern with using woody plants for bioenergy is that it will lead to harvesting of native forests and thus exacerbate biodiversity loss. Although still used mostly for heating, wood plays a very important role as a renewable energy source. In the EU, projections reveal that wood harvest rates will increase by about 30% in 2020 compared to 2010 (EC 2013). In the USA, short rotation woody species that re-sprout from the stump, such as poplar (*Populus* ssp.), willow (*Salix* spp.), cottonwood (*Populus fremontii* L.), and *Eucalyptus* species are being developed for biomass energy production (Ruark 2006).

1.2.3 Nitrogen sources for bioenergy cropping

1.2.3.1 Synthetic N fertilizer use in bioenergy cropping

Plants need a constant supply of N, P, K and other plant nutrients (Mengel and Kirkby 2001). Atmospheric N_2 is used by plants only when it is converted into reactive forms, mainly nitrates, urea and ammonium produced by soil microorganisms, from manure or other animal wastes, or as synthetic N fertilizers (Havlin et al. 2005). During the production of synthetic fertilizers, energy input is required to reduce N_2 to ammonia in the Haber-Bosch process (Helsel 1992). Most of the global energy used in agriculture is to produce N fertilizers (Isherwood 2000). Therefore energy input is one of the indicators of the environmental impact of fertilizer use in conventional crop production and also for bioenergy crops (Lewandowski and Schmidt 2006). Moreover, the production and use of synthetic fertilizer is accompanied by the release of CO_2 (Nemecek and Kägi 2007) and N_2O (Mäkinen et al. 2006), both GHGs.

Global fertilizer consumption has increased steadily since 1961, and more N fertilizer is used than other fertilizers (FAO 2008). The application of synthetic fertilizers to agricultural crops has increased over 7 fold since then, while crop yield has increased by only 2.4 fold (Tilman et al. 2002). Bioenergy crop fertilization is estimated to use 1 to 8% of global synthetic fertilizer produced in 2015, and this consumption is expected to double by 2030 (Smeets and Faaji 2005).

Bioenergy production systems, until recently, were dominated by annuals that require input of N fertilizers in feedstock production. Besides the already mentioned fact that N fertilizer production is energy and C-intensive (Worrell and Block 1994), its application into the soil may also lead to nutrient leaching and volatilisation (Goolsby and Battaglin 2000). As with all crop production, N and P fertilizers can cause eutrophication if in contact with surface and ground waters, changing ecosystem structure and function (Smith et al 1999), loss of biodiversity (Suding et al. 2005), and water quality degradation (Dodds 2006). To become more accepted, bioenergy cropping must use little N fertilizer so as to lower most importantly emissions of GHGs from fossil fuel use (Börjesson and Tufvesson 2011).

1.2.3.2 Synthetic N fertilizer use in reed canary grass cropping

RCG may fail to give any financially viable yield on low productivity soils (Shield et al. 2012). Because of its perennial nature, it is easy to crop, but for full yield potential to be achieved there is need for N fertilizer. Its biomass increases linearly with N fertilizer application (Cherney et al. 1991) and without mineral N application, the biomass potential is not achieved in soils poor in mineralizable organic N (Kukk et al. 2011). Site and year significantly influence biomass yields (Saijonkari-Pahkala 2001, Strasil et al. 2005) due to differences in soil and climatic factors.

In Finland, Pahkala et al (2005) suggested an application rate of 60-90 kg ha^{-1} of N fertilizer in RCG production. At 150 kg ha^{-1} of N in some trials, no yield effect was measured (Lewandowski et al. 2003). In the Czech Republic on sandy loam soils, N fertilizer rate of 80 kg ha^{-1} increased yields by 29% over unfertilized values (Strasil 2012). The use of synthetic fertilizers can be reduced in bioenergy cropping when symbiotic N-fixing legumes are used (Stoddard 2007).

1.2.3.3 Fodder galega

Fodder galega (*Galega orientalis* Lam.) is a rhizomatous perennial in the family Fabaceae, used mainly for production of forage. It fixes nitrogen, enriches the soil with organic matter and increases the amount of humus and decreases erosion potential (Egamberdieva et al. 2010). Galega is persistent in different soils, can fix 300-400 kg ha^{-1} of N annually (Laidna, 1993), and produce up to 14.7 t ha^{-1} DM (Lillak and Laidna, 2000). It has also proven to be suitable and productive for growing in mixtures with other grasses (Vosa and Meripold 2008). Generally, legumes grow well in the absence of other sources of N, thereby reducing N fertilizer use (Jensen 1996). They can contribute directly or indirectly to the nutrition of associated crops and minimise herbicide application due to improved competition with weeds (Hauggaard-Nielsen et al. 2001).

1.2.3.4 Grass-legume intercrops

In monocultures, crop diversity is reduced to one species usually planted in a uniform layout, and external inputs are often applied in large amounts that can cause damage to the environment. In mixed or intercropping systems, two or more species are cultivated at the same time on the same area. Environmentalists prefer multispecies cropping systems to monocultures, as they are often considered as a practical application of ecological principles of increased biodiversity and plant interactions. These principles are assumed to have potential advantages in productivity and ecological sustainability (Hauggaard-Nielsen et al. 2008), although sometimes considered hard to manage (Vandermeer 1989).

Crop diversity involving legumes may contribute to sustainability, as they fix N_2, release soil phosphorus, and supply pollen and nectar to bees (Stoddard et al., 2011). Environmental awareness has increased the use of legumes as sources of N for potential use in cropping systems. Tilman et al. (2006) reported an increase of 25% of total soil nitrogen by legumes. Some of the fixed N can be used by the intercropped grass (Fustec et al. 2010) through root exudates and after decomposition and decay of roots and nodules (Paynel et al. 2001). Although some of the N was leached, grass-legume mixtures produced the same DM yields as grasses fertilized with 200 kg ha^{-1} of N (Scholefield et al. 2002) and allowed a decrease from the 300 kg ha^{-1} of N fertilizer that would have been applied to pure grass pastures. Yields are not significantly compromised in mixtures compared to N-fertilised grasses. Kryževiciene (2006) reported promising yields of 4.8-7.4 t ha^{-1} DM for RCG mixed with galega in Lithuania. In mixtures, the proportion of legumes changes in biomass DM over time. The galega content increased from 10% in the first year to 26% in the second year and 56% in the third year (Kryzeviciene 2005).

1.3 Sustainable bioenergy crop production

1.3.1 Sustainability

In a basic definition, Bruntland Report (1987) stated that sustainable development is meeting the needs of the present without compromising the ability of the future generations to meet their own needs. With growing evidence that humanity is not using resources in a sustainable manner (Wilbanks 2010), sustainability could mean conserving and managing scarce resources and ecosystems which include land, air and water. Hansen (1996) described sustainability as the ability to continue making improvements over time under changing conditions. It is also used to describe practices that are environmentally sound, economically profitable, and socially just (Dale et al. 2013). These practices reflect society’s priorities, so they may change with time and circumstances. It is therefore important to clearly state the goals and priorities for sustainability so there can be a strong relationship with what is measured (Davidson 2011). Therefore, assessing sustainability is a challenging task due to the lack of a unique, objective, and commonly agreed methodology.

1.3.2 Sustainability in agriculture

Studying the interaction between nature and society has contributed much to agriculture (Wu 2006). Sustainability in agriculture means providing adequate food, fibre, and fuel supply to meet present needs without jeopardizing the capacity to providing the same services to future generations (Dale et al. 2013). A key reason for sustaining agriculture is to meet the growing demand for primary products while retaining or even enhancing the services of ecosystems (Mueller et al. 2012). Monteith (1990) described sustainability in agriculture as farming systems that produce higher outputs with fewer inputs on a unit production basis. These can be achieved by reduced tillage (Mitchell, 2009) and it is a further challenge to develop low-polluting, high-yielding cropping systems that use minimal fertilizer and pesticide inputs.

1.3.3. Sustainability of bioenergy cropping systems

For bioenergy to be successful, its production and use must be sustainable. This means that options for future generations should be considered when making important decisions about what, where and how bioenergy crops are grown. The focus on sustainability provides an opportunity to decide how biofuels might be "done right" (Kline et al. 2009).

Sustainability of bioenergy cropping systems is currently viewed in terms of energy security, food production, land use and productivity, soil quality and erosion, greenhouse gas emissions, biodiversity enhancement, and air and water quality. It can be achieved by designing appropriate cropping systems. First and most important, these systems must take into consideration the effects of changing land use from a pre-existing cropping system from an unmanaged ecosystem to bioenergy cropping. This is usually accompanied by changes in land management that will include altered fertilization, irrigation, cultivation, and harvesting regimes (Dale et al. 2010). Increasing biomass yield per hectare without increasing cost of production is a way of making bioenergy crops compete with other fuels.

1.3.4 Land use and acid sulphate soils in bioenergy

Land use is a key factor in bioenergy sustainability. Increases in food cost and insecurity worldwide have been partly attributed to the use of food crops for energy purposes and the conversion of land previously used for food and feed production into biomass feedstock production (Johansson and Azar 2007). To minimize these aspects of competition and conflict, food and feed could be cultivated on established and productive agricultural land while bioenergy crops, especially cellulosic perennial crops (Tilman et al. 2006), could be cultivated on so-called "surplus" land. Surplus land is used to describe fallow, set aside, abandoned, marginal, degraded, reclaimed and waste lands (Dauber et al. 2012). Campbell et al. (2008) estimated that about 450 million ha of such land exist that could produce over 2 billion tons of biomass. Problematic soils, as well as polluted and contaminated land (Jadia and Fulekar 2009) could also be considered "surplus" or marginal land well suited for energy crops.

Acid sulphate soils were deposited as sediments thousands of years ago and occur mainly in coastal and floodplain areas (Andriesse and van Mensvoort, 2006). They contain highly acidic horizons, with a pH of 4 or less. When artificially drained for efficient cultivation, the sulfidic materials in the subsoil undergo oxidation and this gives rise to an acid load, which has been shown to negatively affect the aquatic environment of some watercourses by impairing the ecosystems in recipient rivers and estuaries (Dent and Pons 1995, Boman et al. 2010). The acidification is accompanied by leaching of heavy metals, which sometimes causes sudden mass deaths among fish (Erixon 2009). Massive fish mortality on the western coast of Finland (Vehanen et al., 2012) was a recent example of the environmental hazards caused by draining these soils. Liming of these soils can make them productive for agricultural purposes but does not mitigate the environmental hazards. Different methods for improving the quality of water discharging from cultivated AS fields are being tested (Uusi-Kämppä et al., 2011). These include raised water tables in lysimeters (Virtanen et al. 2013).

1.3.5 Productivity of reed canary grass

1.3.5.1 Biomass yields

Soil N content and weather conditions are important determinants of RCG biomass yield. With high soil N and suitable weather conditions for growth, unfertilised fields can yield 6-7 t ha^{-1} of DM (Kukk et al. 2011). With good management and N fertilizer, yields as high as 12-17 t ha^{-1} can be obtained (Sahramaa, 2003) at spring harvest. On clay soils in Finland, yields of 7.5 t ha^{-1} of DM (Landström 1999) and 7-8 t ha^{-1} (Saijonkari-Pahkala 2001) were reported. Using high doses of fertilizers on soils with low humus content yielded the same 7-8 t ha^{-1} of RCG biomass (Kukk et al. 2011). Meanwhile, Seppälä et al. (2009) using different fertilization levels reported 13.7 t ha^{-1}. Low rainfall during a growing period may reduce yield significantly (Saijonkari-Pahkala 2001). Winter yield losses are common in perennial bioenergy cropping systems (Pahkala and Pihala 2000) and this affects RCG biomass yields as well, where losses of 15-30% have been estimated (Strasil et al. 2005) and may exceed 50% (Lindh et al. 2009). Harvesting in autumn gave 10-12 t ha^{-1} but storage could not be guaranteed, and quality was unacceptable (Landström et al. 1996), and durability of the plantation was reduced (Pahkala et al. 2005).

1.3.5.2 Feedstock quality of biomass

Crop and soil type, fertilization and harvest time determine the desirable features of energy crops intended for combustion, including high biomass yield, low moisture content, and low levels of ash and of alkali elements (K, Ca, Mg), Cl, S and Si. Plants take up these elements from the soil and high concentrations are undesirable as they remain in the ash causing slagging, fouling and corrosion during burning (Jenkins et al. 1998, Brummer et al. 2002). Alkali elements are water soluble and react with silica to produce a sticky liquid that ends up blocking air passages in furnaces and boilers (McKendry 2002). These problems, including relatively high ash contents (Burvall 1997), are common when using stalky biomass from RCG and other grasses during the combustion process in combined heat and power (CHP) plants (Cuiping et al. 2004). High concentrations of K, S, Na, and Cl in the crop feedstock affect ash quality, boilers and heat exchangers, require more frequent maintenance and ash removal, and thereby result in higher operating costs of CHP (Nordin 1994).

Application of fertilizers increases concentrations of N, P, K, and S in plants at harvest (Katterer et al. 1998). N concentrates in leaves and sheaths rather than in stems (Lanning and Eleuterius 1989), and when the crop is allowed to overwinter, the N is translocated to rhizomes where it enhances growth at the beginning of the next season (Partala et al. 2001). It is recommended that the N content of biomass be less than 0.6% (Obernberger et al. 2006). N, S, and Cl content of biomass for use in combustion needs to be minimized because they cause emissions of undesirable gases such as nitrous oxides and sulphur oxides (Burvall 1997, Clarke and Preto 2011). The lignocellulosic content determines the C:N ratio and confers the energy value of biomass (Obernberger and Thek 2004).

The harvesting period of RCG and other energy crops depends on their use. For the purpose of burning, RCG is mostly harvested in spring of the following year as a dry crop after it has over-wintered in the field. At this time, the moisture content of the harvested biomass is below 15%, significantly reducing the need for heating and drying. This delay also reduces the mineral concentration and yield (Adler et al. 2006). Spring harvest or delayed harvest also allows time for nutrient resources to be translocated to rhizomes from shoots for use during the next growing season thereby reducing the removal of nutrients from farms (Hadders and Olsson 1997). Some of the minerals leach from the plants as the crop over-winters, so fuel quality is improved (Burvall 1997). Landström et al. (1996) reported that delayed harvest to spring lowered by about 6 times the concentrations of the undesired elements Cl and K and the ash content declined from 5.9% in August to 4.7% in spring. In the autumn, RCG has higher than desirable levels of chlorine, and nitrogen (Burvall 1997). The most important disadvantage of spring harvest is that yields are reduced (Tahir et al. 2010), mostly due to loss of leaves constituting about 10% of dry matter (Hadders 1994). Substantial loss is also in the form of stubble not possible to reach with cutting machine.

Net calorific values and energy potentials in GJ ha^{-1} are also influenced by soil and harvest regimes. Net calorific value of RCG grown in clay soils was 16.6 MJ kg^{-1}, with 17.6 MJ kg^{-1} reported for spring harvest (Burvall 1997). In spring, the average net calorific value of RCG biomass is 16.7 MJ kg^{-1} and this is higher compared to other seasons (Strasil 2012).

Harvesting RCG in spring is also ideal for cellulosic bioethanol production (Pahkala et al. 2007) because the mature RCG has a high concentration of cellulose at that time. Using ethanol from RCG reduced GHG emissions by 85% in comparison with gasoline (Adler et al. 2007). For biogas production, it is best to harvest it either at the vegetative stage in summer or at a more mature stage during autumn (Boateng et al. 2006).

1.3.6 Bioenergy, greenhouse gases and climate change

There is a very strong link between GHG emissions into the atmosphere and climate change (IPCC 2007). As world economies continue to rely on fossil energy, fossil fuels will also continue to contribute to greenhouse gas emissions (Le Quere et al. 2010). The concentrations of N_2O (Figure 2) and CO_2 have continued to increase in the atmosphere. That of CO_2 already exceeded 400 ppm in 2015 (Dlugokencky and Tans 2015), an increase from 280 ppm in 1750 in the pre-industrial (IPCC 2001). As the concentrations of these GHGs increase, the earth becomes warmer. Since 1900, the average annual global temperature has increased by 0.8°C (IPCC 2007) and presently a warming rate of 0.2°C is recorded every ten years (Hansen et al. 2006).

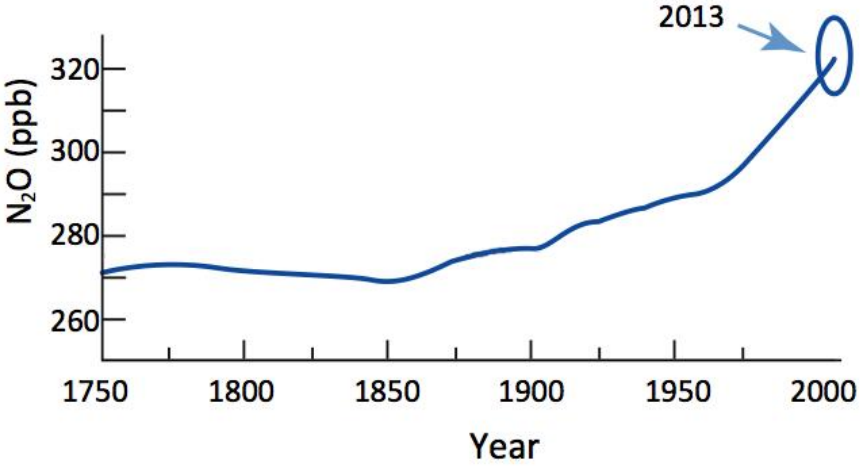

Figure 2. Atmospheric concentrations of N_2O from 1750 to 2013 (www.carbonbrief.org)

Climate change is one of the reasons for interest in alternative energy sources such as biofuels that can address this global threat by reducing greenhouse gas emissions and sequestering atmospheric carbon (Farrell et al. 2006). It is important to note that not every form of bioenergy use contributes to the mitigation of climate change. Biomass cropping may increase GHG emissions. N_2O gas is the most significant GHG emitted in bioenergy cropping systems (Adler et al. 2007), resulting from N fertilizer application and fixation, residue decomposition, and mineralization of soil organic matter (Del Grosso et al., 2005). Reducing farm operations such as tillage and N fertilizer use may reduce N_2O gas emissions (Kim and Dale 2004). Therefore there is a need to encourage energy cropping methods that will require less N fertilizer use.

Climate change may benefit agriculture in northern Europe, where crops adapted to warmer climates such as maize and soybean may be grown in the future. In the present northern cooler areas, average temperatures will increase and longer growing seasons will favour biomass production (Fronzek and Carter, 2007) and yields will increase (IPCC 2007). However, extreme weather events such as heat waves will increase the risk of crop failure in these areas (Porter and Semenov 1999).

1.3.7 Bioenergy and earthworm biodiversity

According to the Convention on Biological Diversity (CBD), biological diversity means the variability among living organisms and the ecological complexes of which they are part; this includes diversity within species, between species and of ecosystems. Biodiversity contributes to human well-being directly (through provisioning, regulating and cultural ecosystem services) and indirectly (through supporting ecosystem services) and is fundamental to ecosystem functioning

(CBD 2010). Agricultural intensification and expansion can cause losses in biodiversity and reduce habitats for organisms beneficial to man (Klein et al. 2007). Depending on the type of production system and geographical region among others, bioenergy crop production can increase or decrease biodiversity. This influence could be through land-use change, overexploitation, pollution, invasive species and climate change (MEA 2005, Dornburg et al. 2010). Converting natural forest for bioenergy cropping and introduction of invasive species have been major concerns and the impacts of large scale cultivation on biodiversity is a key sustainability concern (Wicke et al. 2012).

Food-based biofuel systems have been implicated to biodiversity loss through deforestation and other environmental degradation processes associated with them. On the other hand, bioenergy perennial mixtures support biodiversity (Hoffman et al. 1995). There is a need to preserve soil fauna, as these organisms provide ecosystem services critical to crop production and society at large (Power 2010). Earthworms are examples of such organisms. Their burrowing and casting activities in the soil influence the physical and chemical properties of soil and hence soil fertility (Edwards and Bohlen 1996). In a study to investigate the effects of bioenergy crop cultivation on earthworm communities, it was revealed that the diversity was especially enhanced and showed a more balanced species composition in extensively managed soils under *Miscanthus* (Felten and Emmerling 2011). Large populations of earthworms were found in soils treated with synthetic N fertilizer (Edwards and Lofty 2002, Iordache and Borza 2010), contradicting earlier results by Ma et al (1990) that inorganic N fertilizers drastically decreased earthworm numbers. In intercrops involving legumes, the N-rich high quality litter may attract more earthworms (Eisenhauer et al. 2009, Manhaes et al. 2013).

2 AIMS OF THE STUDY

As a result of a growing need to increase energy production from biomass sources, quality biomass feedstocks are becoming increasingly insufficient in quantity for direct use or for conversion into biofuels. Food crops and energy crops are competing for land and the use of food crops for energy purpose is questioned. Conventional cropping methods have been used to grow energy crops characterised by large inorganic fertiliser and pesticide inputs, all implicated for causing environmental degradation and climate change. Therefore there is need to develop cropping practices that will balance the need to increase production while maintaining critical ecosystem functions. The impact of bioenergy production on biodiversity is often not taken seriously, and direct and indirect effects such as loss of species and reduced abundance are rarely assessed. Insufficient information exists on how energy grasses, can be grown on marginal soils, how its biomass yield and chemical composition are influenced by legume intercropping, and how this affects N_2O emissions from soils and the macro faunal communities in soil. The overall purpose of the study was to produce new knowledge about growing RCG for bioenergy in a sustainable way in AS soils and by legume intercropping.

This research aimed to address the following questions:

1. can problematic soils such as acid sulphate soils be used to grow reed canary grass as an energy crop instead of using food and feed crop land? (I)
2. can a perennial RCG – galega intercrop grown for energy use lower emission of the greenhouse gas, N_2O? (II)
3. does this intercrop affect earthworm species diversity and abundance in the soil? (III)
4. is the intercrop biomass yield of good quantity and quality compared to fertilized RCG? (II, III)

3 MATERIALS AND METHODS

In this section, the experimental part of the work is described as a general outline. Detailed descriptions are presented in the original publications (I – III).

3.1 Experimental sites and soils

The field experiment was established in April 2008 at the Viikki Experimental Farm, University of Helsinki, Finland (60°13′ N, 25°0′ E). The field experiment ran from 2008 to 2013 (II and III) and the off-field part of the experiment (I) was conducted in lysimeters under the glass-roofed and wire-netted compartment of the glass house. Soils were Gleyic Stagnosols (drainic) (II and III) and a Sulfic Cryaquept (Yli-Halla et al. 2008) with B horizon of actual acid sulphate soil, and C horizon of potential acid sulphate soil (Virtanen et al. 2013) (I).

The year 2008 was generally warmer and 16% wetter than the long term average (Table 1). The year 2010 was generally colder than the long term averages. The mean rainfall of June and July 2009 was more than twofold those of 2010. The wettest year was 2012.

Table 1. Mean air temperature (^{o}C) and total monthly precipitation (mm) in Helsinki 2008-2013 (FMI 2008 to 2013), compared with long term average (1971-2000) at Kaisaniemi, Helsinki.

Month	Mean air temperature (°C)							Total monthly precipitation (mm)						
	1981-2010	2008	2009	2010	2011	2012	2013	1981-2010	2008	2009	2010	2011	2012	2013
Jan	-3.9	0.6	-2.8	-0.0	-4.4	-3.4	-4.9	52	68	33	30	70	90	30
Feb	-4.7	1.1	-3.6	-8.1	-9.9	-6.8	-1.8	36	56	20	45	24	61	45
Mar	-1.3	0.2	-0.9	-1.8	-1.0	0.8	-5.2	38	56	34	54	15	36	16
Apr	3.9	6.1	4.5	4.6	5.6	3.8	3.1	32	45	7	42	29	58	32
May	10.2	10.9	11.0	11.5	9.9	10.9	12.6	37	9	45	59	27	65	33
Jun	17.8	14.4	14.1	14.6	16.7	17.7	_	63	85	75	33	49	54	_
Jul	16.3	17.6	17.2	21.7	20.6	16.0	_	80	16	131	49	56	39	_
Aug	15.8	15.5	16.7	18.1	17.5	15.5	_	79	94	49	97	173	71	_
Sep	11.5	10.4	13.5	12.2	13.6	12.5	_	56	71	40	50	88	160	_
Oct	6.6	9.3	4.2	6.0	8.5	6.7	_	76	135	90	29	69	93	_
Nov	1.6	3.7	3.6	-0.5	5.3	4.2	_	70	91	86	89	27	80	_
Dec	-2.0	1.3	-3.6	-7.5	3.4	-5.3	_	58	62	51	87	121	85	_

3.2 Plant materials and experimental designs

Crop choices were perennial RCG, cultivar ‘Rival’ (I-III) and galega, cultivar ‘Gale’ (II and III). Seeds were supplied by Naturcom. RCG was the sole species used in the lysimeter experiment (I) where the experimental treatments were high and low water levels in the acid sulphate soil monoliths. In II and III, experimental treatments used for the purposes of N_2O and earthworm determinations were RCG, galega, their 75-25% mixture as well as bare fallow. The galega seeds were inoculated with *Rhizobium galegae* (Elomestari Oy, Finland) before sowing. The mixture was sown at 75% and 25% of the 15 kg ha^{-1} and 12 kg ha^{-1} sowing densities of RCG and galega, respectively. The fertilizer used was N-P-K 27-0-1 (Yara Bela Suomensalpietari, Yara Oy, Finland) and yearly N application rates were 60 kg ha^{-1} (II and III) or 90 kg ha^{-1} (I). Weeds were manually removed when necessary. The experiments were arranged in randomized complete block designs with four replicates (I-III).

3.3 Measurements and analyses

A summary of treatments, measurements and methods used is shown in Table 2.

Table 2. Treatments, measurements and methods used in the original publications I-III

Publication	Treatment	Variable	Method
I	High water table	Total tillers	
	Low water table	Flowering tillers	
		Shattered panicles	
		Tiller height	
		Photosynthesis	IRGA
		Transpiration	IRGA
		Water use efficiency	
		Biomass yield	Oven drying
		Ash content	Loss on ignition (Muffle furnace)
		Elemental composition	Microwave-assisted digestion, ICP-OES
			Chloride analysis
		C:N ratio	Dumas dry combustion
II	Fertlized RCG	Soil moisture content	
	Galega	Soil NH_4^+-N and NO_3^--N	2 M KCl extraction
	RCG-galega mixture		FIA colorimetry
	Bare fallow	Biomass yield	Oven drying
		Energy content	Bomb calorimetry
		Nitrogen yield	Dumas dry combustion
		N_2O flux	Closed chambers, GC-ECD
		Ash content	Loss on ignition (Muffle furnace)
		Elemental composition	Microwave-assisted digestion, ICP-OES
			Chloride analysis
		C:N ratio	Dumas dry combustion
III	Fertlized RCG	Earthworm number	Hand sorting, Mustard oil
	Galega	Earthworm live weights	
	RCG-galega mixture	Earthworm species diversity	
	Bare fallow		

3.3.1 Soil analysis (II, III)

Soil samples for analyses were collected from the topsoil (0-20 cm depth) in periods mentioned in II and III. From each plot, 3 samples were taken and pooled. Samples were then stored in a freezer at -20°C until analysis. Mineral N was determined after extraction of fresh soil with 2 M KCl for 2 h in an orbital shaker and the extracts were analyzed for NH_4^+ and NO_3^- with an autoanalyzer (QuikChem 8000, Lachat Instruments, USA). Gravimetric moisture content was determined from 10 g of moist soil. Samples for total soil C and N were collected in July 2009 and May 2013 and analysed with a Vario MAX CN (Elementar Analysensysteme GmbH, Germany).

3.3.2 Plant growth measurements (I)

The total numbers of tillers, flowering tillers and intact and shattered panicles were counted. Plant height was measured. From the youngest fully expanded leaf, photosynthesis and transpiration were measured using a portable LI 6400 Photosynthesis system (LI-COR Inc. USA). The ratio of the rate of photosynthesis to transpiration was calculated as water use efficiency according to Paez and Gonzalez (1995).

3.3.3 Gas emission measurement (II)

N_2O flux from the soil was measured using a static closed chamber of known volume over the soil surface for a certain time, as described by Jaakkola and Simojoki (1998) and Penttilä et al. (2013). Two steel cylinders, 16 cm in diameter and 25 cm high, were pushed 10 cm into the soil in each plot. The cylinders were closed with neoprene lids during collection periods. The gas was collected with a 10 ml syringe through a 3-way valve system, pierced through the centre of the lid. A gas chromatograph (Hewlett-Packard 5890 Series II GC) was used to determine the N_2O concentration of the gas samples and the rate of change of N_2O concentration was estimated.

3.3.4 Earthworm studies (III)

Earthworms were extracted from 25 x 25 x 25 cm soil blocks by hand sorting (Lee 1985) and from soil underlying the block by a mustard-oil method (Gunn 1992, Gundale et al. 2005). The worms were washed in fresh water, dried with absorbent paper, counted, weighed and stored in 4% formalin solution. Adults and juveniles were separated and identified to species level and key ecological groups (epigeic, endogeic and anecic) following Bouche (1972).

3.3.5 Crop biomass (I, II)

In spring, 1 m^2 samples were collected from each plot (II), and all plants from each monolith (I), sorted into species when necessary, then dried at 65°C for 72 h and weighed. The summer harvest was sorted into species before drying to determine the dry matter ratio of RCG and galega in mixtures (II). A Retsch Rotor Beater (Retsch GmbH, Germany) was used to grind the dried samples through a 0.25 mm sieve. The ground samples were then stored at room temperature for feedstock quality analyses. Root DM was determined after soil was washed off the roots with a root washer (Gillison 714, Gillison’s Variety Fabrics, USA) and organic debris was manually removed (I).

3.3.6 Biomass feedstock quality

3.3.6.1 Ash content (I)

About 1.0 g of the ground plant samples was dried in an oven at 105 °C overnight and precisely weighed (W1). Dried samples were then burnt in a muffle furnace (LV 15/11/P320, Nabertherm GmbH, Germany) for 12 h at 575 °C, cooled in a desiccator, and weighed (W2). Ash content was calculated as follows:

$$Ash\ content\ (\%) = \frac{w2}{w1} \times 100$$

3.3.6.2 Mineral element composition (I)

Concentrations of Ca, K, Mg, S, were determined in ground plant samples. About 300 mg of plant material was weighed into PTFE Teflon tubes (CEM, Matthews, North Carolina, USA), then 6 mL of nitric acid (67-69%, VWR International BVBA, Geldenaaksebaan, Belgium) and 1 mL of hydrogen peroxide (30%, Merck KGaA, Germany) were added and the samples were digested in a microwave oven (MARSXpress, MARS 240/50, CEM, Matthews, USA). Digested samples were filtered through Hartman paper (Grade No. 4, pore size 2.5 µm, GE Healthcare Companies, UK), and diluted in distilled water up to 50 mL, then stored at -20°C overnight. An Inductively Coupled Plasma-Optical Emission Spectrometer (iCAP 6200, Thermo Fisher Scientific, Cambridge, UK) was used to analyze the elemental composition of the digestate. Cl^- was analysed from 0.5 g ground plant samples according to Mäkelä et al. (2003) using a Corning M926 chloride analyser (Corning Ltd., Halstead, Essex, UK). The total C and N contents were analysed from 200 mg ground plant samples by the Dumas combustion method using a Vario MAX CN (Elementar Analysensysteme GmbH, Germany).

3.3.6.3 Energy content

Ground plant samples were compressed into pellets using a Pellet Press (Parr Instrument Co., Moline, IL, USA) and weighed. Benzoic acid pellets (1.0 g, Parr Instrument Co., Moline, USA) were used as standards. The higher heating value (MJ kg^{-1}) was determined by complete combustion of 0.5 g subsamples with excess O_2 at 3.04 MPa in a sealed steel adiabatic bomb calorimeter (Parr 1241EA, Parr Instrument Co., Moline, USA). The gross energy yield in GJ ha^{-1} was calculated as follows:

$$Gross\ energy\ yield = \frac{Biomass\ yield\ \times Energy\ content}{1000}$$

Biomass yield in kg ha^{-1} , and energy content in MJ kg^{-1}.

3.3.6.4 Nitrogen yield

N yield in kg ha^{-1} was calculated as follows:

$$N\ yield = \frac{Biomass\ yield\ \times Biomass\ N\ content}{1000}$$

Biomass yield in kg ha^{-1} , and biomass N content in g kg^{-1}.

3.4 Statistical analyses

Data of different variables measured were subjected to ANOVA using software packages SPSS 15 (I) and later PASW (II and III) statistics 20.0 (IBM, Chicago, USA) to compare 1) the effects of water levels on biomass yield and elemental composition of RCG (I), 2) the effects of different crop species composition on earthworm species and abundance (III), and on biomass energy and nitrogen yields, and nitrous oxide emissions (II). Significant differences between means of treatments were compared Tukey's b test (I-III). Simple correlation coefficient was calculated in order to study the relationship between soil factors and nitrous oxide emission (III). Means were values of four replicates. Statistical significance was recognized at $P < 0.05$.

4. RESULTS AND DISCUSSION

4.1 Cropping for bioenergy in acid sulphate soils with controlled ground water

RCG was studied in AS soils subjected to raised water table (I), a management option to reduce acidity and avoid environmental hazards. Biomass yields and combustion quality were higher in RCG grown in AS soil with a high water table than that grown in drained, oxidized soil. Keeping the sulphidic materials submerged in the lysimeters maintained a reduced condition (Virtanen et al. 2013) and prevented oxidation to sulphuric acid (Ward et al. 2004). The raised water table caused saturation in the soil horizons, pH was higher and other soil conditions were favourable for RCG growth (Virtanen et al. 2013). Therefore raising the water table in these soils could serve as a solution to the environmental problems caused when the soils are drained and acidic leachates are released into the environment. Water quality could improve and aquatic life could be safer. Downstream acidification affects health of aquatic organisms (Hudd and Kjellman 2002). Moreover, water for transpiration was always available, leading to higher transpiration rates, greater nutrient uptake and consequently high biomass production. RCG biomass from lysimeters with a raised water table had better combustion quality than the low water table counterpart. The generally high yields obtained in both low and raised water table treatments confirm the ability of this grass to adapt to different water tables (Epie 2010).

This study comes at a time when concerns are growing about the land where bioenergy crops are grown and when the profitability and expansion of RCG production has halted in some Nordic countries. The good yields and quality biomass shown in this study are an attractive combination. Using AS soil to grow perennial bioenergy crops such as RCG could help to reduce the tension between food and bioenergy crop production on arable land. In the EU about 5.5 million ha of land (Dworak et al. 2008) is used to grow biomass for bioenergy and about 500 000 ha of arable land exist in Finland (Vainio-Mattila et al. 2005). The 130 000 ha of acid sulphate soils in Finland (Yli-Halla et al. 1999) could be put to use to serve as extra income source.

The Gleyic Stagnosol soils in II and III, as many other soils in Finland and around the world, are used for arable crop farming to supply food to humans and feed to animals. A shift in the use of this land from continuous cereals to perennial energy cultivation is conflicting and seen as contributing to increased food cost and insecurity (Johansson and Azar 2007). Study (I) makes an important contribution to reducing the food/energy crop land conflict by showing that problematic AS soils can be used for bioenergy crops. Land use is one of the important sustainability criteria in bioenergy crop production used in concluding if bioenergy production is done right. Other sustainability criteria such as GHG emissions, productivity and biodiversity all depend on land use (Dale et al. 2011).

4.2 Productivity and energy qualities

In the field study (II), biomass yields were influenced by crop species. Fertilized RCG yielded most biomass, followed by mixtures, and then galega. However, the fertilized RCG yields did not exceed 7.6 DM t ha^{-1} (Table 3), and were in the range reported from the fields by Pahkala et al. (2008). Studies have shown that RCG yields are significantly influenced by site, year and N fertilisation (Saijonkari-Pahkala 2001, Strasil et al. 2005). This study supports the conclusions by Cherney et al. (1991) that RCG responds well to N fertilizer application. Biomass yields of 11.3 to 20.1 t ha^{-1} obtained in lysimeters (I) were more than twice those previously reported (Pahkala et al. 2008) for higher latitudes in field conditions. The lysimeters and their surrounding conditions were very conducive for RCG growth. The plants in the lysimeters were probably responding to the naturally high mineral N supply typical for AS soils and to the synthetic fertilizer supplied at the relatively high rate of 90 kg ha^{-1} of N. However, there was need for this principle to be tested in field conditions. Besides differences in soil types, temperature, rainfall and snow cover in field conditions (II) might have been responsible for the low yields in RCG compared to glass-roofed shelter conditions in (I). However, in both study (I) and (II), RCG biomass yields were high and this is a requirement for a species to be accepted as an energy crop (Larson 2008).

Mixture yields were generally higher than galega yields. In 2010, unfertilized mixture yields of 6.5 t ha^{-1} of dry matter were not significantly different from the 7.6 t ha^{-1} produced by fertilized RCG yields. In 2011, 2012 and 2013, yields decreased as a result of galega dominating RCG in the mixture as presented in study (II). The non-fertilized mixture yields in 2010 showed that if the 75% RCG and 25% galega composition is maintained, the mixture can yield as much biomass as fertilized RCG. Biomass from RCG-galega mixtures has recorded high yields in similar studies in Lithuania (Kryzeviciene, 2005). Farmers could prefer growing RCG in mixtures as it will mean little or no fertilizer cost for the same biomass and energy yield as with fertilized RCG. In bioenergy production, energy yields are strongly related to biomass yields. Higher yields will produce more energy (Mikkola and Ahokas 2009). Energy yields were highest from fertilized RCG harvest (Table 3) and not different from those of mixtures in 2010.

Galega yields did not exceed 3.0 t DM ha^{-1}((II) and Table 3). Winter loss of galega biomass was apparently substantial compared to RCG and mixture. Galega biomass had low ash content, low C:N ratio and relatively high N yield (Table 4). The low ash confers good quality for burning as every increase in its value will decrease the heating value of fuel (Jenkins et al. 1998). With low yields and high N yields obtained in sole crop galega, however, this legume is not a good energy crop for combustion purposes. The N content of biomass is responsible for nitrogen oxide formation (Prochnow et al. 2009), so burning galega may emit more N_2O gas into the atmosphere. However, as most legumes, it fixes atmospheric N into the soil and studies have shown that it has a high potential for biogas production (Vosa and Meripold 2008). It is therefore not a waste of resources to take advantage of galega’s ability to fix atmospheric N by intercropping with grass. Grass-legume intercrop can serve as a promising way of combining productivity and sustainable agriculture.

Table 3. Biomass, energy and nitrogen yields of reed canary grass (RCG), galega and their mixtures grown in field experiment (II) for the period 2010 to 2013.

Year/Treatment	Biomass yield DM t ha^{-1}	Energy yield GJ ha^{-1}	Nitrogen yield kg ha^{-1}
2010			
RCG	7.6b	133b	59a
Galega	2.9a	55a	50a
Mixture	6.5b	116b	52a
2011			
RCG	7.1c	123c	66b
Galega	2.5a	47a	40a
Mixture	4.0b	71b	40a
2012			
RCG	7.0b	122b	100b
Galega	3.0a	58a	60a
Mixture	4.0a	73a	68a
2013			
RCG	5.5b	96b	64a
Galega	2.8a	54a	46a
Mixture	3.1a	59a	55a
P values			
Treatment	***	***	*
Year	***	***	***
Treatment x Year	**	**	*

Means within each column and year with different letters are significantly different.
* $P < 0.05$; *** $P < 0.001$

The high water table management option for the AS soils in (I) produced biomass with generally high C:N ratio and low mineral elements. A high C:N ratio value is an indication that the harvested biomass has more lignocellulose, so it has higher calorific value (Obernbeger and Thek 2004). Less mineral content and consequently less ash content are preferred in biomass intended for burning (Monti et al. 2004, Campbell 2007). High contents of alkali metals and Cl^- can cause deposition and corrosion problems in CHP systems. Although the shift in species composition affected the mineral element composition in (II), mixtures were generally of similar fuel quality as the fertilized RCG (Table 4). Therefore farmers may tend to grow RCG as an energy crop in mixtures with galega than enduring the cost of synthetic fertilizer application. It is worth emphasizing the need to maintain the grass as a major component in order to gain benefits from growing mixtures. Comparing the mineral element contents for RCG in (I) and those in the field (II) (Table 4), values from (II) were lower probably due to washing by precipitation and snow, and better nutrient translocation to the rhizome system of the grass.

Table 4. Ash and element composition of reed canary grass (RCG), galega and their mixtures grown in a field experiment (II) for a 4-year period (II). Data shown are means, n = 4.

Year/Treatment	Ash (%)	Ca (g kg^{-1})	K (g kg^{-1})	Mg (g kg^{-1})	S (g kg^{-1})	Cl (g kg^{-1})	C:N
2010							
RCG	8.3b	1.31a	0.54a	0.38a	1.50a	0.24ab	57b
Galega	3.0a	8.48b	0.68a	0.53a	2.18a	0.18a	27a
Mixture	7.7b	3.30a	0.52a	0.37a	1.40a	0.30b	53b
2011							
RCG	7.8b	1.59a	2.32b	0.42a	1.18a	0.91b	46b
Galega	3.0a	5.93b	1.40a	0.50a	1.12a	0.28a	28a
Mixture	7.2b	2.26a	2.15b	0.40a	1.07a	0.88b	44b
2012							
RCG	8.5c	1.29a	1.94b	0.39a	1.56a	0.44b	29b
Galega	3.4a	4.06b	1.52a	0.66b	1.53a	0.27a	23a
Mixture	7.8b	1.61a	1.94b	0.39a	1.35a	0.47b	25a
2013							
RCG	8.9c	1.49a	2.55a	0.44a	1.13a	0.78a	37b
Galega	3.8a	6.45a	1.29a	0.80b	1.18a	1.16a	28a
Mixture	7.5b	6.48a	2.60a	0.80b	1.26a	0.86a	25a
P-value							
Treatment	***	***	ns	***	ns	ns	***
Year	***	*	ns	***	***	**	***
Treatment x Year	**	ns	ns	ns	ns	ns	***

Means within each column and year with different letters are significantly different.
ns, non significant
*P < 0.05; ** P < 0.01; *** P < 0.001

In spring harvest, biomass yields are lower than in autumn, due to losses during winter as most leaves and some weaker stems break up due to rainfall, frost action and wind. When biomass is harvested during summer and autumn as in (III), these losses are avoided. Losses between autumn 2012 and spring 2013 were about 40% for RCG and 43% for galega-dominated mixtures (data not shown). The losses for RCG were similar to those reported by Lindvall (2014) and high compared to losses of 15-30% reported by Hadders and Olsson (1997). The biomass loss was probably due to the snow cover and heavy rains in 2012. Although the spring harvest is smaller than other seasons, the harvested biomass is drier, easy to store, and carries less ash-forming elements, so it is of better energy quality and more profitable.

4.3 Impacts of reed canary grass cropping on N_2O emission and earthworm community

4.3.1 Decrease of N_2O emission by RCG cropping

In the field study (II), crop species compositions and fertilization influenced N_2O emissions. Emissions increased following fertilization of RCG in line with Simojoki et al. (2012) and previous studies (Bouwman 1990, Inselsbacher et al. 2011). One of the reasons for bioenergy crop production is to reduce emission of GHGs (Farrell et al. 2006) and mitigate the effects of climate change (IPCC 1997). In study (II), the unfertilized mixture cumulatively emitted marginally lower N_2O gas annually and can be a favourable option if bioenergy cropping must use little or no N fertilizers so as to lower emissions of GHGs (Börjesson and Tufvesson 2011). Similarly, lower emissions have been reported from grass/clover pastures than from pure grass (Ledgard et al. 2009). The cumulative emissions per DM in study (II) were highest for galega and roughly the same for RCG and mixture. This study supports the view that RCG-galega mixtures have the potential to replace N fertilizer input in energy cropping and result in lower N_2O fluxes, but this requires proper management to maintain grass as the major component. Although the pure stand of fertilized grass yielded more biomass than the mixed plots, there was a small and non-significant difference between crops in the release of N_2O per ton of dry matter or per unit of harvestable bioenergy. Also in study (II), emissions increased with temperature during summer. Therefore if average global temperature continues to increase (IPCC 2007), more N_2O gas will be emitted from soils into the atmosphere and climate change scenarios will exacerbate.

When N_2O gas emission from the lysimeters was measured in study (I), high volumes were emitted immediately after fertilization as found on other soil types, and cropping reduced emissions from the soils (Simojoki et al. 2012). Cumulative emissions were relatively high irrespective of water table depth, and the results did not support the view of MacDonald et al. (2010) that high ground water increases emission of this gas from the soil. In study (I), large quantities of roots were found in both soil treatments, which can be taken as an indication of C sequestration. Large amounts of C are sequestered by plants roots into soil every year (Smith and Almaraz 2004). This research also recorded the low N content of harvested biomass from the lysimeters with a raised water table. When the low N content biomass is used for bioenergy, less N_2O gas will be emitted, since high N content of biomass increases N_2O emission during burning (IPCC 1997). This will contribute to reducing the atmospheric concentrations of CO_2 and N_2O implicated in climate change.

4.3.2 Enhanced earthworm species and abundance with crop mixture

Crop species can influence earthworm species number, abundance and biomass. Fertilized RCG soils recorded the lowest earthworm species number and abundance in all the seasons. This was in line with Hole et al. (2005), that cropping grasses in monocultures for energy use usually require synthetic N fertilizer application that can reduce earthworm communities. However, the strong negative response of earthworms to fertilizers and other chemical inputs (Dinter et al., 2013) was not shown here, probably because the sampling days after fertilization in the study were either too short or long for significant effects to be observed. Reports on the effect of synthetic fertilizer application on earthworms are contradictory. Edwards and Lofty (2002) recorded more earthworms in soils treated with synthetic N fertilizers than in untreated plots. Iordache and Borza (2010) concluded that the negative influence of chemical fertilization on earthworm abundance was attributable to changes in soil pH. *Aporrectodea* spp. were dominant in all the soils and these species are affected by synthetic N fertilizers (Ma et al. 1990). This might have explained the lower numbers of earthworms in the soils of fertilized RCG.

Soils under galega significantly sustained more species and larger populations of earthworms than fertilized RCG soils in summer. Since earthworms have a tendency to congregate around N-rich litter in the soil (Gastine et al. 2003), the litter quantity and quality (Eisenhauer et al. 2010) from galega of previous seasons is the possible mechanism involved. These plots were established in 2008 and would have accumulated N in their litter over time. Plant species diversity has long been known to benefit earthworm communities (Spehn et al. 2000), but the relationship was not strong in this study. It is noteworthy that this is not the first time studies have shown a weak linkage between plant species diversity and soil fauna diversity, the relationship not always being significant (Laossi et al. 2008). In this study, the weak relationship could possibly be related to soil organic matter (OM) and C:N ratio, which were not significantly different in the treatments in the course of the study (Table 5). The effects of plant species mixtures on soil physical properties have been studied (Niklaus et al. 2007) and the impacts on soil macro fauna communities remain unclear (Velasquez et al. 2012). Temperature and moisture conditions were generally favourable for earthworm growth and development.

Table 5. Soil organic matter (OM) percentage and C:N ratio under grass-legume crops after one year (2009) and five years (2013) of cropping. Data shown are means, n = 4.

Treatment	Soil OM (%)		Soil C:N	
	2009	2013	2009	2013
RCG	8.1a	7.8a	12.1a	11.5a
Galega	8.0a	8.3a	12.1a	11.0a
Mixture	7.9a	8.2a	11.9a	11.3a
S.E.	0.2	0.7	0.2	0.2

Means within each column and period with same letters were not significantly different at 5% level. S.E. is standard error.

Soils from RCG-galega mixture plots supported 20% more individuals, weighing 28% more than fertilized RCG. Thus this bioenergy crop mixture could sustain more soil macro fauna community. Although other soil fauna communities were not studied, this effect on earthworms could be significant over time to achieve same results as Schmidt et al. (2003) who showed larger earthworm communities in grass-legume mixtures. Study (III) also supported previous reports that *Aporrectodea caliginosa* Sav. remains the dominant species in northern latitudes (Dinter et al. 2013) and in Finland (Nieminen et al. 2011). Plant species, either alone for example galega or in mixtures with grasses as in this study, can support higher earthworm communities. This in turn may sustain energy crop cultivation, because earthworm activities affect soil physical and chemical properties (Brown 1995; Edwards 2004). The abundance of earthworms in soil is considered an indication of good soil quality (Doube and Schmidt 1997).

Earthworm communities were not studied in AS soils managed with raised water table (I). Although a reduced abundance of earthworms is associated with acidic soil conditions (Mele and Carter 1999), there is very little or no empirical data on earthworms in AS soils under raised water table management. This remains a subject for further research.

4.4 Achievement of sustainability criteria

Table 6. Summary of extent of achievement of bioenergy sustainability criteria in lysimeter (High, HWT and Low LWT water table) and field experiments.

Sustainability criteria	Lysimeter		Field	
	HWT	LWT	Fertilized RCG	RCG/Galega Mixture
Land use	++	+	na	na
Productivity (quantity and quality)	++	+	++	++
Greenhouse N_2O emission reduction	na	na	0	+
Earthworm biodiversity	na	na	0	+

Scale: ++ strong, + moderately strong, 0 medium, - moderately weak, -- weak.
na not applicable

The extent of sustainability achievement in Table 6 was concluded based on the following considerations:

1. RCG grown in AS soil with a high water table grew taller and produced more biomass with better quality for use in energy production than that grown in drained, oxidized soil (Table 6).
2. In Gleyic Stagnosol soils, non-fertilized low energy input RCG-galega mixture yielded as much biomass as fertilized RCG in 2010 when crop composition was near 75% RCG and 25% galega. Although crop composition later shifted in favour of galega, mixtures were generally of similar fuel quality as fertilized RCG.
3. The annual cumulative emissions of N_2O gas from mixtures were marginally lower than those from fertilized RCG soils.
4. RCG–galega mixture enhanced earthworm abundance and species numbers compared to pure RCG stands.

5 CONCLUSIONS

Reed canary grass grew well in acid sulphate soils and even performed better when the water table was raised. Acid sulphate soils with groundwater control can make a very important contribution to reducing the food/energy crop land conflict if cultivated with a bioenergy grass. However, this principle remains to be tested in field conditions.

In non-acid sulphate soils, RCG-galega mixture can yield as much biomass of similar fuel quality as fertilized RCG when crop composition of 75% RCG and 25% galega is maintained.

The annual cumulative emissions of N_2O from mixtures were marginally lower than those from fertilized RCG soils. Fertilized RCG produced twice as much biomass and correspondingly higher nitrogen and energy yields, and its low emission of N_2O per ton of dry matter was not significantly different from that of the mixtures. This was an encouraging result and supported the overall objective of the study to lower N_2O gas emission from soils cropped with RCG.

Above ground plant species composition can influence macro faunal communities in the soil. RCG–galega mixture sustained more earthworm biodiversity than the pure grass.

Cropping an RCG–galega mixture for biofuel may replace N fertilizer input in perennial energy grass cropping, but requires management to maintain grass as the major component over time.

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