Strategic nitrogen management in stockless organic cropping systems

Redistribution of residual biomass for improved energy and nitrogen balance

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Cover: Re-circulation of biomass, nutrients and energy between the farm and the city (Illustration by Christel Lindgren, 2017).

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Abstract

Agriculture faces the challenge of producing high yields to feed a growing world population, while simultaneously addressing environmental problems such as eutrophication, emissions of greenhouse gases, loss of biodiversity and soil degradation. Organic farming can be part of the solution, as it promotes biodiversity, uses less energy for fertiliser production and often has higher inputs of organic matter to soil than conventional farming. However, yields are often lower, partly due to asynchrony in mineralisation of organic nitrogen (N) and crop acquisition. Growing legumes for protein production and input of biological N_2 fixation to supply the cropping system with N is a common practice on organic farms. The addition of reactive N to the agroecosystem via legumes may, just as with synthetic fertilisers, lead to N surpluses and environmentally harmful N losses. It is therefore important to improve N cycling within agricultural cropping systems.

This thesis assessed the effects of strategic redistribution of residual biomass on productivity, crop quality, N balance, N and carbon (C) turnover, eutrophication potential and global warming potential in a stockless organic cropping system. A field experiment was established to test three strategies for recirculating N in residual biomass within a six-year crop rotation; 1) leaving crop residues *in situ* at harvest (IS), 2) biomass redistribution as silage to non-legume crops (BR) or 3) anaerobic digestion of the silage before redistribution (AD). A soil incubation experiment in a controlled environment was also performed, to measure mineralisation of N, soil respiration and greenhouse gas emissions from incorporation of fresh and anaerobically digested grass clover ley. Moreover, energy balance, greenhouse gas emissions and eutrophication potential in BR and AD were compared with those in IS in a life cycle assessment (LCA). Results from the field experiment showed that the BR and AD strategies maintained the same yields as IS, but resulted in higher N_2 fixation in the legumes and consequently a more positive N balance. The soil incubation experiment showed that total C losses during 90 days after soil application of ley were higher than from digested ley. A major energy gain was achieved in AD, and a decrease in global warming potential compared to BR. There was a reduction in eutrophication potential with the strategic redistribution of silage and digestate (BR and AD), compared with IS. In conclusion these results show that strategic redistribution of biomass-based digestate can improve the N balance of crop rotations and produce a surplus of bioenergy, which are key elements for enhancing the sustainability of stockless organic cropping systems.

Keywords: bioenergy, biomass management, crop rotation, ecological intensification, green manure, life cycle assessment, nitrogen cycling, organic agriculture, soil incubation, stockless cropping systems

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Sammanfattning

Jordbruket står inför utmaningen att föda en växande världsbefolkning samtidigt som det behöver göras åtgärder för att minska relaterade miljöproblem som övergödning, utsläpp av växthusgaser, förlust av biologisk mångfald och markförstöring. Ekologiskt jordbruk kan vara en del av lösningen eftersom dess produktionsmetoder främjar biologisk mångfald, använder mindre energi för gödselproduktion och medför högre tillförsel av organiskt material till mark än konventionellt jordbruk. Skördarna är emellertid ofta lägre i ekologisk produktion jämfört med konventionell, vilket delvis beror på att mineralisering av organiskt kväve inte sker samtidigt som grödornas upptag. Odling av baljväxter för proteinproduktion och biologisk kvävefixering är vanligt vid ekologiska gårdar, men tillsatsen av reaktivt kväve via baljväxter kan, liksom vid användning av handelsgödsel, leda till kväveöverskott och miljöskadliga kväveförluster. Det är därför viktigt att förbättra kvävecirkulering inom jordbrukets odlingssystem.

Den här avhandlingen innehåller en utvärdering av effekterna från strategisk omfördelning av restbiomassa i ett ekologiskt odlingssystem utan djur, med avseende på grödornas produktivitet och kvalitet, kvävebalans, kväve och kolomsättning, utlakningsrisk och global uppvärmningspotential. Tre strategier för recirkulering av kväve i restbiomassa testades via ett fältförsök baserat på en sexårig växtföljd; 1) skörderester lämnas *in situ* vid skörd (IS), 2) omfördelning av ensilerade skörderester till andra grödor än baljväxter (BR) eller 3) anaerob rötning av ensilaget före omfördelningen (AD). Mineralisering av kväve, jordrespiration och växthusgasutsläpp undersöktes efter att färsk och anaerobt nedbruten vall blandats med jord i ett laboratorieförsök. Energibalans, växthusgasutsläpp och eutrofieringspotential i de olika strategierna för hantering av restbiomassa jämfördes i en livscykelanalys.

Resultaten visade att BR- och AD-strategierna gav samma skörd som IS i fältförsöket, men resulterade i högre kvävefixering och en mer positiv kvävebalans. Totala C-förluster i laboratorieexperimentet under 90 dagar efter inblandningen av vall i jord var högre än från den iblandade rötresten. Livscykelanalysen visade på en stor energiförbättring och minskning av den globala uppvärmningspotentialen i AD jämfört med BR. Utlakningsrisken minskade med den strategiska omfördelningen av ensilage och rötrest (BR och AD) jämfört med IS.

Slutsatsen var att strategisk omfördelning av rötrest baserad på odlingssystemets restbiomassa kan förbättra kvävebalansen och producera ett överskott av bioenergi, vilka båda är viktiga faktorer för att förbättra hållbarheten i djurlösa ekologiska odlingssystem.

Contents

Abstract 3
Sammanfattning 4

List of publications 8

List of figures 10

Abbreviations 11

List of tables in appendix 12

1 Introduction 13
1.1 Global agricultural challenges 13
1.1.1 Food security 13
1.1.2 Eutrophication 14
1.1.3 Soil fertility 14
1.1.4 Greenhouse gases 15
1.2 Organic stockless agriculture as part of the solution 15
1.2.1 Energy demand 17
1.2.2 Soil organic carbon 17
1.3 The nitrogen cycle in organic stockless farming 18
1.3.1 Nitrogen fixation 18
1.3.2 Nitrogen cycling 20
1.3.3 Nitrogen use efficiency 20
1.3.4 Nitrogen mineralisation and availability affects yield 21
1.4 Potential solutions and unanswered questions 22
1.4.1 Organic nitrogen fertilisers 22
1.4.2 Leaching of nitrate 23

2 Overall aims and hypotheses 24

3 Materials and methods 27
3.1 Field experiment (Papers I & II) 27
3.1.1 Study site and soil 27
3.1.2 The crop rotation 28
3.1.3 Experimental design 29

3.1.4 Sampling 31
3.1.5 Nitrogen balance 31
3.2 Soil incubation (Paper III) 32
3.2.1 Experimental design 32
3.2.2 Sampling 33
3.3 Life cycle assessment 33
3.3.1 System boundaries and limitations 33
3.3.2 Life cycle inventory 35

4 Results 36
4.1 Crop yield and quality influenced by management of residual biomass (Paper I) 36
4.1.1 Yield and nitrogen concentration of rye, cabbage and beetroot 36
4.1.2 Yield and nitrogen concentration of the intercrops lentil/oat and pea/barley 36
4.1.3 Yield of cover crops and green manure ley 37
4.2 Effects of internal recycling with residual biomass on biomass nitrogen acquisition and balance (Paper II) 37
4.2.1 Nitrogen acquisition 37
4.2.2 Nitrogen exported in the edible crop fraction 38
4.2.3 Nitrogen in residual crop biomass, green manure ley and cover crops 38
4.2.4 Nitrogen balance 39
4.3 Mineralisation rate and greenhouse gas emissions from digested and undigested ley (Paper III) 39
4.3.1 Nitrogen mineralisation 39
4.3.2 Gaseous losses 40
4.3.3 Total losses of carbon 41
4.4 Life cycle assessment 41
4.4.1 Life cycle impact assessment 41

5 Discussion 46

6 Conclusions 51

7 Future perspectives 53

8 Critical reflections 55

References 56

Popular science summary **70**

Populärvetenskaplig sammanfattning **71**

Acknowledgements **72**

Appendix 1. Life cycle inventory **74**
Conversion factors 74
Cultivation 74
Emission factors 75
Silage 75
Biogas and digestate production 76
Nordic energy mix 76
Field application 77

List of publications

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

I Råberg, T., Carlsson, G. and Jensen, E.S. (2017). Productivity in an arable and stockless organic cropping system may be enhanced by strategic recycling of biomass. *Renewable Agriculture and Food Systems.* Doi: 10.1017/S1742170517000242.

II Råberg, T., Carlsson, G. and Jensen, E.S. (2017). More efficient use of nitrogen by internal recycling of residual biomass within a stockless organic cropping system? *Nutrient Cycling in Agroecosystems* (submitted).

III Råberg, T., Ernfors, M., Kreuger, E. and Jensen, E.S. Carbon and nitrogen dynamics after addition of anaerobically digested and undigested ley to soil (manuscript)

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The contribution of Tora Råberg to the papers included in this thesis was as follows:

I Developed the research ideas and hypotheses together with the co-authors. Designed, planned and performed the cropping system experiment. Planned and performed most of the sampling and preparation of biomass for ensiling and analysis. Analysed and compiled the results, wrote the article and corresponded with the journal.

II Developed the research ideas and hypotheses together with the co-authors. Designed, planned and performed the cropping system experiment. Collected and prepared samples for isotopic analysis. Performed all calculations and analyses of the data, compiled the results, wrote the article and corresponded with the journal.

III Developed the research ideas and hypotheses together with the co-authors. Designed the soil incubation experiment together with the second author. Planned and performed the incubation, samplings and measurements. Analysed the data, did most of the compilation of results and wrote the article.

List of figures

Figure 1. The crops in the six-year rotation studied in Papers I and II. 29

Figure 2. The field experiment with four blocks, with six crops in rotation, and three biomass treatments. Photo by Joakim Svensson, 2014. 30

Figure 3. Global warming potential from the emissions in treatments with biomass redistribution (BR) and anaerobic digestion (AD), expressed as the difference compared with the reference scenario with biomass left *in situ* (IS), based on emissions from Table A7 and amount of digestate in Table A2 42

Figure 4. Eutrophication potential from cultivation, biogas production and substitution of Nordic energy in treatments with biomass redistribution (BR) and anaerobic digestion (AD), expressed as the difference compared with the reference scenario with biomass left *in situ* (IS). 43

Figure 5. Energy comparison in treatments with biomass redistribution (BR) and anaerobic digestion (AD) between diesel energy usage in cultivation as positive values and surplus net electricity as negative output, expressed as the difference compared with the reference scenario with biomass left *in situ* (IS). 44

Figure 6. Global warming potential (GWP) from the treatments with biomass left *in situ* (IS), biomass redistribution (BR) and anaerobic digestion (AD) when using experimental data compared with the emission factors suggested by IPCC for N_2O and CH_4 emissions at field application. 45

Figure 7. Eutrophication potential from the treatments with biomass left *in situ* (IS), biomass redistribution (BR) and anaerobic digestion (AD) when using the emission factors from experimental data compared with emission factors suggested by IPCC for N_2O emissions. 45

Abbreviations

%Ndfa = proportion (%) of accumulated nitrogen derived from symbiotic nitrogen fixation in a legume
AD = anaerobic digestion
BNF = biological nitrogen fixation
BR = biomass redistribution
CC = cover crop
CHP = heat and power unit
CO_2-eq = carbon dioxide equivalents
CS = cropping system
CSTR = continuous stirred-tank reactor
EP = eutrophication potential
GHG = greenhouse gas
GWP = global warming potential
IC = intercrop
IS = *in situ*
LCA = life cycle assessment
NUE = nitrogen use efficiency
SOC = soil organic carbon
SOM = soil organic matter
WFPS = water-filled pore space

List of tables in appendix

Table A1. Category indicators used for global warming potential (GWP) and eutrophication potential (EP) 74
Table A2. Biomass yield at harvest, after ensiling, after digestion in the reactor and after storage of digestate. FW = fresh weight, DW = dry weight. 74
Table A3. Emissions factors used for the losses from manure storage. 75
Table A4. Emissions from production, distribution and incineration of plastic used for covering the silage. 75
Table A5. Energy use, emissions and energy conversion from running the reactor and generator. 76
Table A6. Average emissions generated from the production of energy in the Nordic countries between 2013 and 2015. 76
Table A7. Nitrogen losses caused by NH_3 emissions during the spreading of biomass. 77
Table A8. Nitrous oxide and CH_4 emissions after shallow incorporation of biomass into the soil. 77
Table A9. Amount of nitrate leached from a reference crop depending on incorporation time. 77
Table A10. Direct energy usage from diesel using the field machinery in scenario BR and AD compared with IS. 78
Table A11. Emissions from diesel production, distribution and incineration. 79

1 Introduction

1.1 Global agricultural challenges

Agriculture faces the challenge of producing high yields to feed a growing world population, while simultaneously addressing a large group of environmental problems such as eutrophication, emissions of greenhouse gases (GHG), loss of biodiversity, soil degradation and the consequences of unpredictable weather due to climate change (Tilman *et al.*, 2001; Lal, 2004; Harvey & Pilgrim, 2011). While dealing with these issues, agriculture also has to meet expectations from governments to provide ecosystem services such as biomass for sustainable bioenergy production and climate change mitigation (Tilman *et al.*, 2009; Harvey & Pilgrim, 2011; Sapp *et al.*, 2015).

1.1.1 Food security

The human population continues to grow and the global population is estimated to reach a peak of approximately nine billion people by the middle of the 21st century. Competition for land, water and energy is thus expected to increase (Godfray *et al.*, 2010). For example, it has been suggested that 50-100% more food will be needed by 2050 compared with 2008 (World Bank, 2007; Godfray *et al.*, 2010). Resolving this challenge requires a paradigm shift in the way food is produced and handled. For example, feeding livestock requires more nutrients than the final animal-based product contains (Rubatzky & Yamaguchi, 2012). Thus global production of animal feed currently accounts for over 50% of the total N input, while the animal sector delivers only 17% of global food calorie production (Liu *et al.*, 2016).

1.1.2 Eutrophication

To obtain high yields, nitrogen (N) must be available in sufficient amounts to support adequate plant growth. Agriculture thus relies on processes to convert atmospheric N_2 to nitrate (NO_3^-) and ammonium (NH_4^+), which can be leached and emitted to the surrounding environment as reactive N. Reactive N is already causing problems such as eutrophication of the Baltic Sea and contributes to climate change via nitrous oxide (N_2O) emissions (Rockström *et al.*, 2009; Steffen *et al.*, 2015).

Intensification of agricultural production has resulted in increasing environmental pollution with reactive N (Van der Werf & Petit, 2002), such as eutrophication of surface water (Baggs *et al.*, 2002; MEA, 2005; Galloway *et al.*, 2008; Foley *et al.*, 2011; Cohen, 2015). One of the main contributors to eutrophication is NO_3^-, which mainly originates from mineral fertilisers and also from mineralisation of organic fertilisers and plant residues left in the field after harvest (Beman *et al.*, 2005; Giles, 2005; Matsunaka *et al.*, 2006). Residues left *in situ* continue to mineralise in late summer and autumn, while crop N acquisition declines (Powlson, 1993; Kirchmann *et al.*, 2002). Nitrate from this and other processes mainly leaches through the soil profile with the drainage water, but also through surface runoff, ending up in the surrounding aquatic environment (Foster *et al.*, 1982). Subsequent environmental enrichment with NO_3^- can lead to undesirable changes in ecosystem structure and function (Smith *et al.*, 1999) and contamination of drinking water (Spalding & Exner, 1993).

1.1.3 Soil fertility

High soil fertility must be maintained in the long term to assure food security. A fertile soil provides essential nutrients for crops and supports a diverse and active biotic community that provides the conditions for well-functioning decomposition (Mäder *et al.*, 2002). However, the soil organic carbon (SOC) that supports this fertility can decline in systems where a large mass of organic matter is removed, such as after conversion of forest or pasture to intensively managed agricultural with annual crops (Cowie *et al.*, 2006; Hellebrand *et al.*, 2010). Many cultivated soils are already showing a steady decline in SOC pools, with negative impacts on soil biota and soil structure (IPCC, 2007; Sommer & de Pauw, 2011).

1.1.4 Greenhouse gases

Agriculture and land use change is responsible for 22-30% of anthropogenic GHG emissions (Tubiello *et al.*, 2013; Knapp *et al.*, 2014). Three of the principal gases emitted are carbon dioxide (CO_2), methane (CH_4) and N_2O (Robertson *et al.*, 2000; Knapp *et al.*, 2014). The addition of CO_2 emissions to the atmosphere comes from the use of fossil fuels and the oxidation of SOC when land is converted for intensive agriculture (Cole *et al.*, 1997). Of the CH_4 emissions in European Union countries (EU-15), approximately two-thirds come from enteric fermentation by ruminants and one-third from livestock manure (Moss *et al.*, 2000). Globally, paddy rice cultivation is another major CH_4 contributor (Smith *et al.*, 2014b), producing 45 Tg CH_4 year^{-1} (2005), but these emissions are decreasing due to improvements in farming practices (Kai *et al.*, 2011). Emissions of N_2O mainly originate from application of N fertiliser or manure under wet conditions and storage of animal manure (Munch & Velthof, 2006; Prosser, 2006; Smith *et al.*, 2014b). Combined, CH_4 and N_2O contributed with 11% (~5.4 Gt CO_2 equivalents year^{-1}) of the total anthropogenic non-CO_2 GHG emissions in 2012 (Tubiello *et al.*, 2015).

1.2 Organic stockless agriculture as part of the solution

Consumers today are often concerned about the environment and/or the chemicals used in food production, and both supply and demand for certified organic production continue to grow (Mueller & Thorup-Kristensen, 2001; Willer & Schaack, 2015). For example, the EU-28 increased its total area cultivated as organic from 5.0 to 11 million hectares between 2002 and 2015 (Eurostat, 2015). This large-scale conversion of production needs to be met with intensified research to ensure that it is efficient and that pollution is minimised.

Organic farming often yields less than conventional farming (Seufert *et al.*, 2012), which calls for a complementary shift in diet to meet the increasing demand for food. Reducing the consumption of meat, dairy products and eggs to half of what it is today in the European Union would achieve 23% per capita less use of cropland for food production (Westhoek *et al.*, 2014). By using crops to feed humans instead of animals, the efficiency of land use can be strongly increased (Rubatzky & Yamaguchi, 2012; Bailey, 2016). The United Nations Environment Programme estimates that the calories lost by using cereals as animal feed instead of using them directly as human food could theoretically feed an extra 3.5 billion people (UNEP, 2015).

The manufacturing of fertiliser, together with the cultivation of leguminous crops, convert more atmospheric N_2 into reactive N than the combined effects of all terrestrial processes (Rockström *et al.*, 2009). Under current levels of total N per unit of food production and without changes in agricultural practices and waste-to-food ratios, it is estimated that an additional amount of 100 Tg N yr^{-1} will be needed by 2030 for a baseline scenario that would meet hunger alleviation targets for over 9 billion people (Liu *et al.*, 2016). Less intensive animal production and increased recirculation of N could reduce the need for N application in 2030 by 8% relative to the level in 2000 (Liu *et al.*, 2016; Shibata *et al.*, 2017). Decreased animal production and consumption would have the largest impact on lowering the need for larger N inputs. For example, the N requirement is 84 g N per 1000 kcal for animal calorie production, compared with only 16 g N per 1000 kcal for vegetable calorie production (Liu *et al.*, 2016). Therefore, using cropland to produce animal feed, no matter how efficient, leads to much higher total N usage.

Greenhouse gas emissions would also be reduced by producing and eating less meat compared with today, if accompanied by a change in crop production to feed humans instead of animals (Stehfest *et al.*, 2009; Nijdam *et al.*, 2012; Nelson *et al.*, 2016). The livestock sector and its by-products account for between 18 and as much as 50% of world-wide emissions of CO_2 equivalents (CO_2eq) per year, depending on the model used in calculations (Steinfeld *et al.*, 2006; Goodland & Anhang, 2009). Of the products assessed by Yue *et al.* (2017), meat had the highest average C footprint (6.21 kg CO_2eq kg^{-1}), and vegetables had the lowest (0.15 kg CO_2eq kg^{-1}), but there are large variations between different species and production methods. Reducing the consumption of meat, dairy and eggs in the European Union to half of what it is today would achieve a 25-40% reduction in GHG emissions (Westhoek *et al.*, 2014).

Developing policies to change consumption patterns towards more resource-efficient plant-based foods would reduce land use, production of reactive N and GHG emissions. However, it would also need to be accompanied by an increase in organic stockless farming.

Farmers of a region often specialise in either crop or animal production, which makes animal manure inaccessible to many stockless organic farms (Mueller and Thorup-Kristensen 2001; Schmidt et al. 1999; Stinner et al. 2008). There has been a prevailing idea that organic arable farming needs to be combined with animal production to be sustainable. However, animal husbandry is one of the main contributors to both GHG emissions and eutrophication (Garnett, 2011). Modern organic arable farms with low or no animal production thus need to find other ways to fertilise the crop. Therefore, there is a need for research on the options and implications for strategic biomass circulation on organic arable farms.

1.2.1 Energy demand

Agriculture is responsible for about 5% of the total energy used on a global basis (Pinstrup-Andersen, 1999) or 2.8% (2014) in EU28 (Eurostat, 2017) and the major energy source is fossil. The use of fossil energy needs to decrease in all sectors, mainly due to the problems with emissions of the greenhouse gas CO_2 (IPCC, 1997). Energy savings or even surplus energy systems can be obtained with farm-scale bio-fuel production that replaces fossil fuel (Pimentel & Pimentel, 2003; Fredriksson *et al.*, 2006; Michel *et al.*, 2010).

Organic farming might provide a possibility to save energy in comparison with conventional farming (Dalgaard *et al.*, 2001; Mäder *et al.*, 2002; Pimentel *et al.*, 2005). On evaluating a long-term field experiment, Pimentel *et al*. (2005) concluded that their animal-based and stockless organic cropping systems used less energy than the conventional systems. Energy use in both cattle and pig production has been observed to be higher in conventional than in organic production (Dalgaard *et al.*, 2001). Although conventional crop production often has higher yields, it uses more energy per hectare and kg produce (Dalgaard *et al.*, 2001; Mäder *et al.*, 2002). The greatest difference in energy use between organic and conventional agriculture stems from the production of synthetic N for fertilisers and the production of pesticides (Pimentel *et al.*, 2005; Gellings & Parmenter, 2016). Inorganic fertiliser accounts for almost one-third of the total energy input to crop production in the United States (Gellings & Parmenter, 2016).

1.2.2 Soil organic carbon

Soil carbon, the content of which correlates with soil organic matter (SOM) levels, is an important part of sustainable farming because it enhances soil fertility mediated by soil organisms. Soil organic carbon generally mitigates soil compaction, reduces soil erosion and surface crusting, increases workability and water-holding capacity and improves pest control (Pimentel *et al.*, 2005). It also provides a continuous nutrient supply, as most plant nutrients are part of, or bound to, soil organic matter (SOM) and become available to the crop when the SOM is mineralised (Bommarco *et al.*, 2013). A decrease in yield variability has been found to be correlated with increased SOM levels (Pan *et al.*, 2009). Soil organic matter is also important for CO_2 sequestration, as around 50% of the organic matter is carbon (Mondelaers *et al.*, 2009).

Meta-analyses indicate significantly higher C content in organically managed topsoil (6.4%) compared with conventional topsoils, but the increase is higher when the initial SOM is initially very low (Mondelaers *et al.*, 2009). In one study, soil C increased significantly more after 22 years of cultivating two organic cropping systems based on either animal manure (27.9%) or stockless legume-based (15.1%) compared with a conventional cropping system (8.6%) (Pimentel *et al.*, 2005). In another study, higher water-holding capacity was cited as the reason for higher yields in five drought years in both stockless and animal-based organic cropping systems, compared with a conventional system (Letter *et al.*, 2003).

1.3 The nitrogen cycle in organic stockless farming

Organic agriculture, compared with conventional, offers benefits such as increased recycling of nutrients and lower energy usage for processing fertilisers of organic origin (Worrell *et al.*, 2000; Vance, 2001; Rockström *et al.*, 2009). Recycling of N is central to reducing the need for production of more reactive N (Bodirsky *et al.*, 2014). However, N is often the most limiting nutrient for crop performance in terms of yield and quality, and is needed in larger quantities than any of the other essential nutrients (Mengel & Kirkby, 1978; Sinclair & Horie, 1989). To obtain high yield and quality, mineralisation of N from organic fertilisers and SOM needs to be in synchrony with crop acquisition. Organic stockless agriculture that simultaneously maximises both yield and N recycling thus needs to consider fixation, cycling, use efficiency and mineralisation of nitrogen.

1.3.1 Nitrogen fixation

Nitrogen fixation by leguminous crops is one of the most fundamental sources of N in organic farming systems, especially in stockless farms. (Watson *et al.*, 2002a; Foyer *et al.*, 2016). The fraction of N derived from N_2 fixation in the legume crop (%Ndfa) is determined not only by the legume and rhizobium genotypes, but also by the interaction between the soil N environment and total legume growth (Unkovich & Pate, 2000; Van Kessel & Hartley, 2000). For example, a high level of mineral N and particularly NO_3^- in the soil will generally depress both nodulation and N_2 fixation (Streeter & Wong, 1988; Waterer & Vessey, 1993) and thereby make the legume more dependent on soil mineral N. Rhizobium genotype is important because absence of the bacterial strain that exhibits symbiosis with the legume species leads to non-existent N_2 fixation. In such cases, N_2 fixation can be significantly improved by seed inoculation with bacterial strains that can form an efficient symbiosis with the legume to be grown (Van Kessel & Hartley, 2000; Galloway *et al.*, 2004).

Nitrogen fixation rates in annual legumes are strongly correlated to dry matter accumulation, which in turn depends on weather and soil conditions (Unkovich & Pate, 2000). The large variation in total N accumulation by individual crop species between years and sites makes it difficult to generalise regarding nitrogen fixation levels. For example, N_2 fixation has been reported to be within the range 4-244 kg N ha^{-1} for pea (*Pisum sativum* L.) (Armstrong *et al.*, 1994; Evans *et al.*, 1995; Jensen, 1997) and 5-191 kg N ha^{-1} for lentil (*Lens culinaris* Moench) (van Kessel, 1994; McNeill *et al.*, 1996; Kurdali *et al.*, 1997). Nitrogen fixation by rhizobium in symbiosis with forage legumes such as lucerne and clover used as green manure can reach 150-350 kg N ha^{-1} (Smil, 1999; Carlsson & Huss-Danell, 2003).

When conditions are optimal and high N_2 fixation is achieved by the legume, the requirement for N fertiliser to the subsequent crop can be strongly reduced. For grain legumes, however, a large proportion of the fixed N is removed with the grain (Jensen & Hauggaard-Nielsen, 2003; Crews & Peoples, 2004; Li *et al.*, 2015). Thus, grain legumes grown as sole crops or intercrops with cereals do not supply as much N as cover crops and green manure ley with forage legumes (Jensen, 1997).

Including green manure ley with legumes in the crop rotation can deliver a large supply of N. On the other hand, dedicating land to green manure production reduces the amount of land that can be used for food production. There may also be a risk of N losses by NH_3 and N_2O volatilisation, and/or NO_3^- leaching, depending on incorporation time and technique (Li, 2015). Growing cover crops inter-sown at the same time as the main crop or after harvest is an important strategy for reducing N losses and improving the N availability for the subsequent crop (Askegaard *et al.*, 2005; Engström *et al.*, 2010). This is the result of two processes: accumulation of N (including N_2 fixation in legumes) by the cover crop during its growth cycle and release of N from the biomass by mineralisation (Thorup-Kristensen, 1994; Thorup-Kristensen & Nielsen, 1998). Another advantage of inter-sown cover crops is that no land needs to be taken out of food production.

Fixation of N_2 also occurs during lightning strikes and this N is deposited on land (Ehhalt *et al.*, 2001). Other non-specific sources that contribute to deposition include combustion of fuel, which emits NO_x, and animal manure and plant residues, which emit NH_3. The deposition rate of total N varies widely, from 1 to 20 kg ha^{-1} $year^{-1}$ (Smil, 1999). The area in southern Sweden that was the geographical context of the studies in this PhD thesis receives approximately 9 kg total N ha^{-1} $year^{-1}$ (SMHI, 2013-2014). Such a contribution is minor in comparison with mineral N production and N_2 fixation by legumes.

1.3.2 Nitrogen cycling

The N_2 fixation by legumes contributes by addition of reactive N that can be lost to the atmosphere, as is also the case with industrial fertiliser production, which is why N cycling is crucial to decrease total levels of N input. Crop rotations are an important part of N cycling, as a large part of the N supply to the crop originates from crop residues, cuttings, and roots that have been left *in situ* from the previous crop. Availability by mineralisation is also influenced by, for example, the amount of N assimilated by the crop, the C:N ratio of the crop residues, subsequent crop N demand, soil type, soil N availability and management practices. The amount of N that can be assimilated by a subsequent cash crop depends largely on temperature, humidity and cash-crop N acquisition dynamics (Jensen, 1992; Ranells & Wagger, 1997; Kramberger *et al.*, 2009). Biomass can be left *in situ* or transported and applied fresh on the soil surface or incorporated into the soil (Coppens *et al.*, 2006). Most of the N in the fresh biomass becomes available already in the first year, but there are large N_2O and CO_2 emissions and a high risk of leaching during the mineralisation process, especially when biomass is left on the soil surface compared with soil incorporation (Baggs *et al.*, 2003).

Nitrogen-rich residual biomass can be moved between fields to the crops with the highest acquisition rates, or stored for strategic application when the timing is adequate for mineralisation. This technique is sometimes referred to as 'cut and carry' or 'biomass redistribution' and is used to prevent NO_3^- leaching under high effluent N loading rates (Barkle *et al.*, 2000; Dodd *et al.*, 2014). Biomass silage is a storage option to synchronise mineralisation with crop uptake. Ensiling initiates mineralisation, but also conserves the biomass by lowering the pH and creating an anaerobic environment (Herrmann *et al.*, 2011). Anaerobic digestion of organic plant material and subsequent use of the residual digestate as a bio-fertiliser is yet another option and is of particular interest to supply N for non-legume crops in the absence of animal manure in stockless organic systems (Gunaseelan, 1997). Generally, a larger proportion of N is available to the plant as mineral N in the digestate compared with in fresh or ensiled biomass (Weiland, 2010).

1.3.3 Nitrogen use efficiency

Plants that are efficient in acquisition and utilisation of nutrients are said to have high nitrogen use efficiency (NUE), which is a desirable trait as it reduces the need for high inputs of reactive N and decreases the losses of nutrients to ecosystems. High NUE also reduces the cost of fertilisers.

Definitions of NUE differ and depend on whether plants are cultivated to produce biomass or grain yield. However, for most plant species, NUE mainly depends on how plants extract mineralised N from the soil, assimilate NO_3^- and ammonium (NH_4^+), and recycle organic N (Masclaux-Daubresse *et al.*, 2010). Nitrogen use efficiency is defined in this thesis as N fertiliser recovery in aboveground plant biomass (see Paper II). The N which is not recovered in the crop may be immobilised in the soil organic N pool, which comprises both microbial biomass and SOC (Cassman *et al.*, 2002).

1.3.4 Nitrogen mineralisation and availability affects yield

The highest yield that can be obtained depends mainly on the synchronisation of soil N availability with crop N acquisition, which in turn is largely influenced by soil N mineralisation dynamics (Sinclair & Horie, 1989; Godfray *et al.*, 2010; Tuomisto *et al.*, 2012). The time of greatest N acquisition in cereals is normally during the stem elongation phase, when the crop is growing the fastest. For high-protein grain crops, there is an even greater demand around the flowering phase. The yield will be lower than optimum if there is not an adequate amount of mineralised N when the acquisition is peaking (Angus, 2001). Nitrogen supply and demand should match in time and space not only for single crops, but for a crop rotation as an integrated system, in order to achieve high total NUE (Spiertz, 2010).

The use of organic N sources makes the availability of nutrients less controllable compared with the use of mineral fertiliser (Swift *et al.*, 1979), as it involves biological decomposition through mineralisation (Angus, 2001; Agehara & Warncke, 2005). Mineralisation of organic N depends on many factors, such as particle size of the organic fertiliser, available types of microorganisms and their abundance, and access to C of various qualities. Abiotic factors such as soil temperature and moisture are major factors affecting the N availability from organic N sources (Agehara & Warncke, 2005).

Organic fertilisers often have a pool of organic N and C structures that are unavailable to most crops (Kumar & Goh, 2003; Lorenz *et al.*, 2007). To become available, these organic materials need to be processed by bacteria, fungi and other organisms, including microarthropods (Hendrix *et al.*, 1990; Bernal *et al.*, 2009). The mineralisation rate is often limited by N availability, as the decomposers have a lower C/N ratio than most organic amendments (Recous *et al.*, 1995; Henriksen & Breland, 1999; Corbeels *et al.*, 2000).

1.4 Potential solutions and unanswered questions

To meet environmental, economic and social challenges, agriculture needs to become more productive and resilient, while minimising environmental impacts. This can possibly be achieved by circulating N-rich biomass, optimising N mineralisation in combination with crop acquisition and replacing fossil fuel.

1.4.1 Organic nitrogen fertilisers

Organic solid manures used in stockless arable farming systems typically include green manure (Benke *et al.*, 2017). The green manure is often grown on the farm to reduce the cost of handling and transportation compared with other organic inputs such as blood meal ('biofer'), yeast-based fertilisers from breweries ('vinass') or algae compounds ('algomin'). Green manure can be composed of a single legume crop, several legume species or a mixture of legume and grass species. The crop mixture is grown primarily as a soil amendment and a nutrient source for subsequent crops. Some of the specific ecosystem services are provision of biologically fixed N, provision of pollen and nectar for insects and weed control by competition and frequent cutting. Green manure approaches may also drive long-term increases in SOC and microbial biomass, which improves nutrient retention and soil fertility (Cherr *et al.*, 2006). Nitrogen is mainly present in its organic form and if mineralisation occurs when there is low or no crop acquisition, there will be leaching and/or emissions to the air. It may be possible to reduce the risk of N losses by removing the green manure, processing it and then reallocating it to non-legume crops. Composting, ensiling and anaerobic digestion serve as pre-treatments that conserve the biomass. Composting the biomass has the advantage of sanitising the material, due to elevated temperatures. The downside is substantial N losses in the process (Sørensen *et al.*, 2013; Smith *et al.*, 2014a) and at field application (Larsson, 1998). Ensiling is a viable alternative to composting as losses of N are lower (6-8%), than when composting the biomass (18-30%) (Sørensen *et al.*, 2013). Anaerobic digestion of the green manure and crop residues in a biogas reactor results in a digestate with a higher concentration of mineralised N, which is directly available to the crop. In organic fertilisers with low C/N ratio (1-5), such as certain types of digestate, it has been shown that 60-80% of the N is mineralised during the anaerobic digestion process (Delin *et al.*, 2012). As crop N acquisition mainly relies on mineralised N, adapting the time of applying digestate with low C/N ratio can potentially optimise the synchrony between N availability and crop N demand. Anaerobic digestion can also contribute with a surplus of bioenergy. However, concerns have been raised that anaerobic digestion of biomass might decrease the C input to the soil, as CH_4 is extracted in the digestion process (Johansen *et al.*, 2013). A controlled laboratory reactor was set up in this thesis work to measure C extracted as CH_4 and CO_2 from the digestion of ley. The carbon losses were added to the C losses from soil application of the digestate in a soil incubation. The results were compared with those following application of undigested ley (Paper III).

1.4.2 Leaching of nitrate

Balancing the amount of N needed for optimum plant growth while minimising the NO_3^- transported to groundwater and surface waters is a major challenge. Loss of NO_3^- from fields to water resources is caused by a combination of factors, such as amount of mineral N present when crop acquisition is low, tillage, drainage, crop growth, SOC, hydrology, temperature and precipitation patterns (Dinnes *et al.*, 2002). For example, Beaudoin *et al.* (2005) concluded that NO_3^- concentration in drainage water is primarily affected by soil type and soil water-holding capacity. The concentration was three-fold higher in shallow sandy soil compared with deep loamy soil in that study and the use of catch crops enabled a 50% reduction in NO_3^- losses at the annual scale and 23% reduction at the rotation scale, despite moderate biomass accumulation (Beaudoin *et al.*, 2005). Nitrate leaching decreases most when non-legume catch crops are used (Quemada *et al.*, 2013). A positive effect can also be obtained from straw incorporation into the soil, as it slows down mineralisation in autumn after harvest (Beaudoin *et al.*, 2005). Other strategies to reduce nitrate leaching include improved timing of N application at appropriate rates, reducing tillage and optimising N application techniques (Dinnes *et al.*, 2002). In the cropping system established in this thesis work, with the introduction of cover crops and winter crops to retain N, and thus decrease the eutrophication potential, oats and barley were intercropped with lentils and peas, respectively, as the practice of intercropping uses the NO_3^--N from fertiliser in a more efficient way than sole cropping of cereals (Zhang & Li, 2003). Yield and N uptake in the crops were measured as an indication of potential losses of N. The treatments that were compared included leaving crop residues *in situ* (IS) after harvest in late summer, compared with storing the biomass as silage for spring biomass redistribution (BR), or anaerobic digestion (AD) of the biomass, with the digestate redistributed to non-legumes in spring. A soil incubation was performed to study the mineralisation rates of ley compared with digested ley, and thus identify when the N is available for crop acquisition. The treatments in the field experiment were assessed for their leaching potential in a life cycle assessment (LCA), using reference emission data (Papers I-III).

2 Overall aims and hypotheses

The overall aims of this PhD project were to assess effects on cropping system measures from strategic redistribution of residual biomass. The following aspects were assessed: productivity, energy balance, eutrophication potential, N dynamics and crop quality. Three different types of residual biomass were investigated: crop residues, green manure ley and cover crop cuttings. The residual biomass was applied either as silage biomass for redistribution (BR) or biogas digestate from anaerobic digestion (AD) to non-legume sole crops. For comparison, residual biomass was also left *in situ* (IS).

The aim of the study described in Paper I was to determine how crop yield and product quality were influenced by the biomass management strategy. A three-year field experiment was used to test the following hypotheses:

1) Strategic recycling of digestate from anaerobic digestion of residual biomass leads to higher edible crop yield of non-legume crops compared with redistribution of biomass as silage or incorporation *in situ* (no redistribution).
2) The concentration of N in the edible plant parts of non-legume crops is higher with strategic recycling of digestate compared with biomass redistribution or *in situ* incorporation.
3) Strategic recycling of biomass to a main crop increases the biomass production of the following cover crops compared with *in situ* incorporation of biomass.

The aim of the study reported in Paper II was to determine whether anaerobic digestion (AD) of the residual biomass from the cropping system and use of the digestate for N recirculation would improve crop N acquisition, compared with the corresponding biomass redistribution (BR) of undigested silage or just leaving the biomass *in situ* (IS) within the respective field plots.

The hypotheses were:

4) The amount and proportion of N_2 fixed in legume crops is greater with AD and BR than in the IS system.
5) Nitrogen acquisition from soil and residual biomass in non-legume crops is greater in AD than BR and IS.
6) The nitrogen balance ranking at the cropping system level is IS<BR<AD.
7) Total N acquisition originating from soil and added biomass in all crops is on average greater in AD and BR than in IS.

These hypotheses were tested in the same field experiment as in Paper I. The amounts of N acquired from N_2 fixation and soil (including N recirculated from the residual biomass) were assessed by the ^{15}N natural abundance method and from the total N content of the crop. Nitrogen balance calculations were used to investigate how the biomass management strategy influenced the soil pool of N at the cropping system and crop level. The calculations did not include N emissions.

The aim of the study reported in Paper III was to compare the effects of anaerobically digested and undigested ley as a soil amendment on the mineralisation and immobilisation turnover of N and on CO_2, N_2O and CH_4 emissions. Nitrogen and carbon transformations were quantified. The treatments with digested and undigested ley were compared with a control treatment without organic amendments. The hypotheses were:

8) In the treatment with undigested ley, an initial period of immobilisation is followed by a period of mineralisation.
9) Following application of digestate, mineralisation is relatively low.
10) The amount of accumulated mineral N (added and mineralised) after 90 days is higher with digested compared with undigested ley.
11) After 90 days of incubation, more C is left in the soil after application of undigested ley compared with digested ley.
12) Total N_2O emissions over 90 days are in the order undigested ley > digested ley > control soil.

These hypotheses were tested by means of a soil incubation study in a climate chamber, where soil subjected to the three treatments was analysed destructively for total N and mineral N on seven occasions during a 90-day incubation period. The accumulated GHG emissions were sampled with the same frequency in all treatments.

In a fourth study presented in this thesis and not published elsewhere, a life cycle assessment (LCA) comparing the three biomass management methods (AD, BR and IS) was performed. The aim was to summarise the use of resources and the environmental consequences of activities involved in farm-level scenarios, using the same crop rotation and biomass management strategies as in the field experiment. The hypotheses were:

13) The AD scenario uses less total energy than the BR and IS scenarios, after considering the energy from farm-based bioenergy production.
14) The eutrophication potential caused by NO_3^-, NH_3, N_2O and NO_x is larger in IS than in AD and BR.
15) Greenhouse gas emissions are lower in AD than in BR and IS.

These hypotheses were tested in a LCA as a comparative study, with IS as the reference to BR and AD. Aspects considered were energy balance, eutrophication and GHG emissions.

3 Materials and methods

A combination of methods was used to address research questions concerning the effects of redistribution of residual biomass and digestate from anaerobic fermentation to crops grown without legumes. These were: i) a field experiment, ii) a soil incubation study with soil and plant-derived amendments, and iii) a life cycle analysis that compared the three techniques of recirculating plant-based nutrients.

3.1 Field experiment (Papers I & II)

A multifunctional and multipurpose cropping system was established for the study of food and feedstock production for bioenergy, N_2 fixation, nutrient retention with catch crops and winter-growing main crops and the provision of food for beneficial insects to prevent pests and increase resilience (Paper I). The crop responses after leaving residual biomass resources *in situ* were compared with the responses after redistributing the same biomass resources after ensiling or after ensiling plus additional anaerobic digestion. In all treatments, the biomass was rotated within the same cropping system without external biomass input. The rotation was based mainly on food crops, but one-sixth of the rotation was grown with green manure ley to produce additional biomass.

3.1.1 Study site and soil

The experiment was established in 2012 on a sandy loam soil at the SITES (Swedish Infrastructure for Ecosystem Science) field research station Lönnstorp (55°39′21″N, 13°03′30″E), Swedish University of Agricultural Sciences, Alnarp, Sweden. The land was certified for organic farming in 1993 and the preceding crop was a one-year legume-grass ley.

3.1.2 The crop rotation

A six-year crop rotation was used for the study, although the experiment was only performed during the three full seasons in 2012-2015. Within each treatment and block, the crop rotation was established in six separate plots, so that each of the six main crops in the rotation was grown during each year of the experiment.

The rotation consisted of the following food crops: pea/barley (*Pisum sativum* L./*Hordeum vulgare* L.), lentil/oat (*Lens culinaris* Medik/*Avena sativa* L.), white cabbage (*Brassica oleracea* L.), beetroot (*Beta vulgaris* L.), and winter rye (*Secale cereale* L.) (Figure 1). In addition, there was a green manure ley composed of *Dactylis glomerata* L., *Festuca pratensis* L., *Phleum pratense* L., *Medicago sativa* L., *Meliolotus officinalis* L. and *Trifolium pratense* L. The ley was under-sown in the pea/barley intercrop, harvested three times during the year after establishment, and harvested again in early spring the following year, before establishing white cabbage as the next crop. Cover crops were included in the rotation after white cabbage (buckwheat (*Fagopyrum esculentum* Moench)/oilseed radish (*Raphanum sativus* L.)) and rye (buckwheat/lacy phacelia (*Phacelia tanacetifolia* Benth.)) and under-sown in lentil/oat (ryegrass (*Lolium perenne* L.)/red clover (*Trifolium pratense* L.)/white clover (*T. repens* L.)) (Paper I).

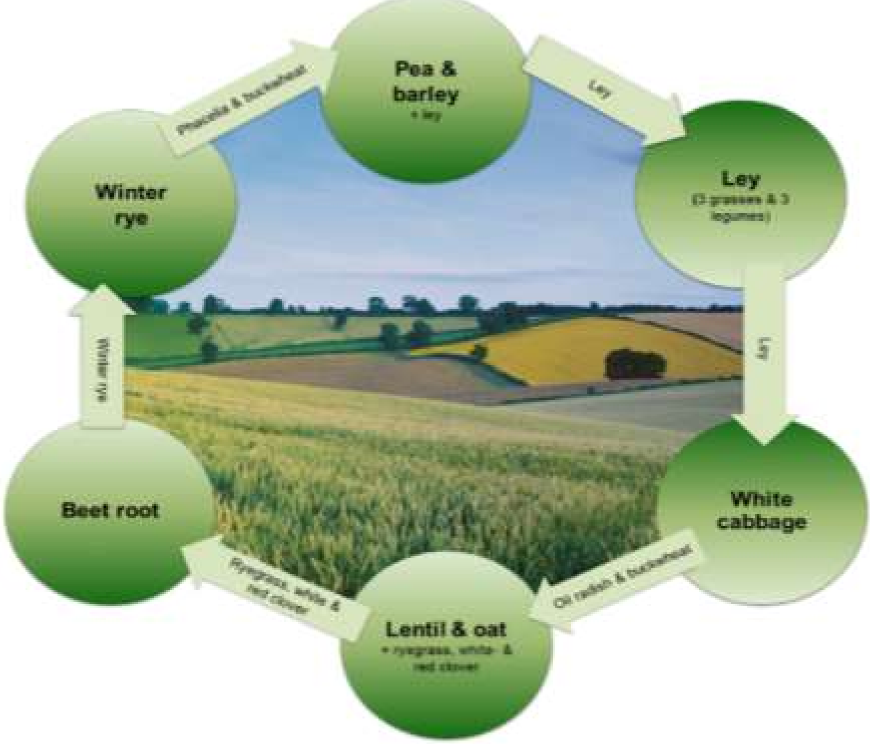

Figure 1. The crops in the six-year rotation studied in Papers I and II.

3.1.3 Experimental design

The field experiment comprised in total 72 experimental plots measuring 3×6 m^2, distributed in four replicate blocks (Figure 2). The experiment started by establishing each of the six main crops, which were followed by cover crops and main crops according to the designed crop rotation. This was performed in the same physical plots during the two following years, thereby providing a three-year crop sequence with all six crops present each year. Within each block, 18 individual plots (six main crops × three treatments) were randomly assigned to one of the three biomass management treatments. The treatments were applied at the cropping system level consistently throughout the three-year crop sequence:

IS – *in situ* incorporation of biomass resources (crop residues, cover crops and green manure ley), *i.e.* leaving the biomass after harvest in the same plot as it was grown.

BR – biomass redistribution: storing the biomass resources as silage and redistributing it to cabbage, beetroot and rye growing in the same system in the following year.
AD – anaerobic digestion of biomass resources (after storing them as silage) and redistribution of the digestate to cabbage, beetroot and rye growing in the same system in the following year.

The residual biomass comprised straw from grain legumes and cereals, leaves from cabbage and beetroot and all aboveground biomass of cover crops. The green manure consisted of ley, from which aboveground biomass was harvested four times. The silage was made in 1 m^3 containers adjacent to the experimental field. Digestion of the biomass for biogas and digestate production was performed in a two-step batch reactor at Anneberg pilot facility, in collaboration with Lund University (Lehtomäki & Björnsson, 2006).

Figure 2. The field experiment with four blocks, with six crops in rotation, and three biomass treatments. Photo by Joakim Svensson, 2014.

3.1.4 Sampling

Samples for analyses of yield from the edible fractions and the N concentration, cover crop and green manure ley yield were obtained from subplots in each plot (Paper I). The residual biomass, cover crops and ley cuttings were subjected to analyses of botanical composition (grouped into legumes and non-legumes), dry matter (DM), N content and natural abundance of the stable isotope ^{15}N (Paper II).

3.1.5 Nitrogen balance

The N balance for the cropping sequences was calculated per crop and as an annual sum of each treatment for 2012-2014. The balance calculations used input data from N_2 fixation measured by the ^{15}N natural abundance method (Unkovich *et al.*, 2008), regional measurements of atmospheric N deposition (SMHI, 2013-2014), N content in seeds and in plants used for establishing the cabbage crop and addition of N via residual ensiled (BR) and digested (AD) biomass from the previous year's crops (Equation 1). In cases where a cover crop was grown after a main crop, the yearly atmospheric N deposition was divided and allocated equally to the main and cover crop in the N balance calculations. The additional supply of 115 kg N from imported digestate at the start of the experiment (2012) was also included in the calculations. The N outputs in the balance consisted of the amounts of N exported in the edible fractions of the food crops (all treatments) and N exported in residual biomass in AD and BR to be redistributed in the next growing season.

$$\text{N balance} = \text{bnf} + \text{dep} + \text{seed} + \text{biomass}_{\text{added}} - \text{food} - \text{biomass}_{\text{removed}} \quad (\textit{Eq. 1})$$

bnf = biological N_2 fixation in the current year
dep = atmospheric N deposition
seed = seed and (cabbage) plant N
$\text{biomass}_{\text{added}}$ = N from added residual biomass and cuttings from the previous year
edible fraction =exported cash crop total N
$\text{biomass}_{\text{removed}}$ = total N from cuttings and residual biomass removed to be circulated in the next year

3.2 Soil incubation (Paper III)

A microcosm experiment was set up, with three treatments: 1) soil receiving grass-clover ley (L), 2) soil receiving anaerobically digested grass-clover ley (DL) and 3) soil without amendment (S). The same ley was used for the L and DL treatments, but half of it was fertilised with ^{15}N-labelled N. The digestate used in the study was produced in a two-step laboratory digestion facility at Lund University (Paper III). Both ley and digestate had been frozen prior to the experiment and were slowly defrosted in gastight containers in a refrigerator. The incubation was performed in 400-mL glass jars (each jar was one microcosm) at 15 °C in darkness and lasted for a period of 90 days, simulating a Nordic spring or autumn (Figure 1 in Paper I). The soil depth for incorporation of the amendments was half of that used for incorporating residues by harrowing in the field experiment described in Paper I.

3.2.1 Experimental design

Eight replicate microcosms were prepared for each sampling time in both the L and DL treatments. The eight replicates were identical except for the isotopic composition of their organic and mineral N pools. In four of the replicates (A), the NH_4^+ pool was labelled with ^{15}N, while the organic N pool was unlabelled. In the other four replicates (B), the organic N was labelled with ^{15}N while the NH_4^+ pool was unlabelled or had only a low atom% excess of ^{15}N. In the S treatment, four replicate microcosms were prepared for each sampling time, all of which were labelled with ^{15}N in the NH_4^+ pool only.

The labelling in the DL treatment was achieved by adding the solid fraction of the unlabelled digestate and the liquid fraction of the ^{15}N-labelled digestate to the (A) microcosms and, conversely, by adding the solid fraction of the ^{15}N-labelled digestate and the liquid fraction of the unlabelled digestate to the (B) microcosms. The ^{15}N labelling of inorganic N in the (A) microcosms was further increased by adding a small amount of NH_4Cl at 98 atom% ^{15}N excess, while the (B) microcosms received a corresponding amount of unlabelled NH_4Cl. The (A) microcosms in the L treatment received unlabelled ley and a small amount of NH_4Cl at 98 atom% ^{15}N, while the (B) microcosms in the L treatment received ^{15}N-labelled ley and a small amount of unlabelled NH_4Cl. The S treatment received a small amount of NH_4Cl at 98 atom% ^{15}N. The NH_4Cl was diluted in deionised water to the amount needed to achieve 66% water-filled pore space (WFPS) in all jars.

3.2.2 Sampling

The soil was sampled destructively for mineral and organic N, and gas samples were collected, at 0, 2, 4, 7, 20, 55 and 90 days (t_{Xd}) after initiation of the experiment. The first sampling was performed one hour after initiation of treatment. All the soil from each microcosm was transferred to a separate 1-L flask and 600 mL of 2 M KCL were added. The flasks were shaken at room temperature for 1 h on a shaking table and then left for sedimentation for at least 12 h at 4 °C.

The soil solution samples were slowly defrosted in a refrigerator prior to analysis. The abundance of ^{15}N in the inorganic N was determined in the soil extract by the micro-diffusion method, where NO_3^- and NH_4^+ were converted into NH_3, which was trapped on an acidified filter paper folded into a Teflon tape, using the method by Stark & Hart (1996) and Sörensen & Jensen (1991), with only minor.

3.3 Life cycle assessment

Life cycle assessment (LCA) is a tool that summarises the use of resources and the environmental consequences of all the activities involved in one or several scenarios being compared (Haas *et al.*, 2000; Höjer *et al.*, 2008). A wide range of impact categories can be used, depending on the scope of the study. The LCA approach was primarily developed in applications to industrial production systems (Audsley *et al.*, 1997), but has been used for assessing a number of agricultural systems. Audsley *et al.* (1997) and Ceuterick (1996, 1998) have compiled examples of complete LCAs for single crops and production processes. Kramer *et al*. (1999) used part of the methodology to assess GHG emissions related to crop production systems in the Netherlands. Flessa *et al*. (2002) similarly evaluated GHG emissions from two farming systems in southern Germany and showed the important contribution of individual gases to climate change. De Boer (2003), Cederberg and Mattsson (2000) and Haas *et al*. (2001) further illustrated the possibilities of using LCA to compare agricultural production systems. The LCA method is internationally standardised according to ISO 14040 guidelines (Finkbeiner *et al.*, 2006).

3.3.1 System boundaries and limitations

The analysis dealt with the life cycle flow of the different biomass management strategies (Paper I), including crop production and power generation. In the case of biogas combustion, electricity and heat were generated from the gas produced. The time frame for crop and electrical energy production in the analysis was one year and followed the average results from the three years of the field experiment (2012-2015). The functional unit was set to 100 ha year^{-1}, to represent a theoretical organic farm of 100 ha. The crop yield was set to be the same regardless of the fertiliser scenario. This assumption was based on results from the field experiment, which showed no significant differences in yield between treatments (Paper I). Energy usage for field operations and processes in the biogas reactor was included in the calculations and based on reference values. The energy needed for heating and electricity to run the biogas reactor was subtracted from the energy produced with a generator. Implementation of systems for making use of excess heat was not considered. Direct energy demand included diesel, electricity and heat used in the biogas reactor (Rehl *et al.*, 2012). Indirect energy usage included production of diesel, plastic, building materials for the biogas plant and machines and a concrete surface for silage storage. In the analysis, some variable emissions and energy demand were included, such as production and distribution of diesel and plastic to cover the silage. Fixed emissions from the use of material and energy embedded in machines and buildings were not included. The timing for conversion of silage to biogas in scenario AD was optimised to produce digestate when there was a demand for fertilising the crop, *i.e.* March-May. As a consequence, GHG emissions from storage of digestate were substantially reduced compared with storing the digestate during the warmest months of the year.

Input data

The LCA was based on yield data from Paper I, with the three biomass management scenarios described in section 3.1.3, where IS was used as the reference scenario designed according to a plausible system in organic farming in Sweden representing best management practices.

Data on emissions of GHG from biomass incorporation into soil were obtained from the soil incubation study described in section 3.2 of this thesis, where emissions from soil mixed with grass clover ley or digested grass clover ley (stored at 8 °C for 12 h before the incubation study) were compared with emissions from bare soil. The emissions from soil amended with grass clover ley were assumed to correspond to both fresh crop residual biomass and silage applied to the field. The emissions during 90 days were used as an estimate for GHG emitted during a year, as most emissions occur shortly after application of biomass to agricultural fields.

Literature data were used in the analysis to calculate losses that were not quantified in the field experiment or incubation study, *i.e.* GHG emissions from ensiling and storage of silage, the anaerobic digestion and the storage of digestate and leaching of NO_3^- from the field experiment. Literature values were also used calculate diesel consumption for field operations, reactor energy consumption and emissions. A sensitivity analysis was made where experimental data were used, comparing the results with literature emission data. Transport of biomass to and from the fields was not included in the analysis due to lack of reference data.

3.3.2 Life cycle inventory

The category indicators from IPCC (2006) were used as conversion factors for calculating the global warming potential (GWP) and eutrophication potential (EP) in CO_2 and PO_4^{3-} equivalents, respectively (see Table A1 in the appendix). The emissions and energy usage were based on mass flows of biomass and N (Table A2). Emission factors for animal manure were used to estimate the emissions from storing silage on a concrete platform (Table A3), covered with plastic in scenario BR and AD (Table A4). The energy used for the production of plastic for ensiling was 16 MJ ton^{-1} (Björnsson *et al.*, 2016).

Modelling data for a conventional continuous stirred-tank reactor (CSTR) for the production of biogas were used for the calculations of energy consumption, emissions and energy conversion in an electricity generator for scenario AD (Table A6). The surplus of energy produced in the reactor and the generator was assumed to be sold to the national grid, where it reduced emissions based on the Nordic energy mix (Table A7). Nitrogen losses emitted at the anaerobic digestion or storage of digestate was allocated to the category "biogas production", presented in the result section. The ammonia emissions from field application of the biomass were calculated using reference data in the National Inventory Report (NIR, 2016), and were based on animal manure being incorporated within four hours (Table A8). The N_2O and CH_4 emissions from the three scenarios were adapted from Paper III and compared with reference data from IPCC (Table A9) in a sensitivity analysis (Figure 6 and 7). The risk of NO_3^- leaching causing eutrophication, depending on autumn or spring incorporation of biomass, was estimated from the mean values from an experiment by Stopes *et al*. (1996) (Table A10). The additional usage of diesel in scenarios BR and AD compared with IS was based on estimates from the rural economy and agricultural society of Sweden (HIR Malmöhus & Maskinkalkylgruppen, 2014) and German reference data (Achilles *et al.*, 2005) (Table A11). The emissions from diesel production, distribution and combustion are presented in Table A12.

4 Results

4.1 Crop yield and quality influenced by management of residual biomass (Paper I)

The aim of the study presented in Paper I was to determine how crop yield and product quality are influenced by biomass management strategy.

4.1.1 Yield and nitrogen concentration of rye, cabbage and beetroot

Yield of the edible fraction of rye, cabbage and beetroot was not significantly different after leaving the biomass *in situ* (IS), strategically redistributing ensiled biomass (BR) or strategically redistributing the digestate (AD) (Paper I). Moreover, the redistribution treatments BR and AD did not result in different concentrations of N in the edible fraction of rye, cabbage and beetroot or yield of biomass residues.

4.1.2 Yield and nitrogen concentration of the intercrops lentil/oat and pea/barley

Lentil grain yield was significantly lower in IS compared with BR in 2013 (Paper I). Data on the grain yield of the pea and barley intercrop in 2013 are not available, since the crop was damaged by rabbits and hares in that year. The biomass treatments did not result in any significant difference in the N concentration of grain legume or cereal seeds. The IS treatment resulted in significantly higher yields of oat straw in both years.

4.1.3 Yield of cover crops and green manure ley

The yield of buckwheat/lacy phacelia (grown after rye) was significantly higher in BR compared with IS and AD in both years (Paper I). The clover proportion of the grass clover cover crop was exceptionally low for all treatments at harvest in May 2013. The legume proportion of the green manure ley was significantly higher in the BR and AD treatments compared with IS in 2014.

4.2 Effects of internal recycling with residual biomass on biomass nitrogen acquisition and balance (Paper II)

The aim of the study presented in Paper II was to determine whether anaerobic digestion (AD) of the residual biomass from the cropping system and use of the digestate for N recirculation improves crop N acquisition, compared with the corresponding biomass redistribution (BR) of undigested silage or just leaving the biomass *in situ* (IS) within the respective field plots.

4.2.1 Nitrogen acquisition

Total nitrogen accumulation in aboveground parts of the crops ranged between 140 and 180 kg ha^{-1} year^{-1}, with no significant difference between the biomass strategies (Paper II).

Symbiotic N_2 fixation in legumes

The proportion of N derived from N_2 fixation in the legumes (%Ndfa) was found to be between 68 and 98%, but was not significantly different between treatments (Paper II). The amount of N_2 fixed was higher with the BR and AD crop rotations compared with IS (p=0.002). A large part of the increased N_2 fixation was from the legumes of the green manure ley, with significantly higher (p<0.001) N_2 fixation in BR and AD compared with IS in 2014. The amount of N_2 fixation in lentil and pea varied inconsistently between treatments in the two years. No significant difference between treatments was found for the amount of N_2 fixed in clover grown together with ryegrass in the cover crop, which ranged between 0.24 kg N ha^{-1} year^{-1} for May harvest in 2013 and 62.9 kg N ha^{-1} year^{-1} for May harvest in 2014 (Paper II).

Nitrogen acquisition from soil

The total N accumulation from soil and added biomass varied between 110 and 140 kg N ha^{-1}, calculated as an average for the entire crop rotation, and the total accumulation was significantly higher ($p=0.002$) in 2014 compared with 2013 (Paper II). Differences between the three treatments were small and in most cases non-significant. The BR treatment led to significantly ($p<0.001$) higher accumulation of soil-derived N in the cover crop buckwheat/lacy phacelia in both years compared with the IS and AD treatments (Paper II).

4.2.2 Nitrogen exported in the edible crop fraction

Average N accumulation in the exported edible fraction of the five edible crops varied between 49 and 60 kg ha^{-1}, with the highest amount exported in rye grain (Paper II). The N content of the edible fraction was not affected by treatment, even if the N supply differed substantially (Paper II).

4.2.3 Nitrogen in residual crop biomass, green manure ley and cover crops

The total amount of N in crop residues, cover crops and ley cuttings from six ha varied between 97 and 129 kg N ha^{-1}, without any significant differences between the treatments (Paper II). In 2013, the ley cuttings constituted between 36 and 40% of the total amount of N and in 2014 the contribution increased to between 49 and 54%. There was a significant interaction between treatment and year when the total N accumulation of all crops from the three systems was compared ($p=0.001$). Nitrogen accumulation in the whole cropping system was larger in IS compared with BR and AD in 2012 ($p=0.009$), since N in residual biomass in BR and AD was 'exported' for redistribution in the next growing season without corresponding inputs during the initial year of the experiment. The nitrogen content of all the residual biomass increased in the three years that the experiment was running, regardless of treatment. There was a significant ($p<0.001$) increase in residual biomass N for all treatments, corresponding to an average difference of 19 kg N ha^{-1} between 2013 and 2014 (Paper II).

4.2.4 Nitrogen balance

The three crops that were fertilised with biomass in BR and AD resulted in an N surplus for the N balance in both years, with the highest surplus in cabbage with the BR treatment in 2014 (Paper II). The exception to the surplus results was the winter rye crop with BR treatment in 2014, which resulted in a negative balance. Cabbage, beetroot and rye all had a negative N balance in IS.

The lentil/oat intercrop resulted in a negative result with all treatments, and most negative for AD and BR. The pea/barley intercrop resulted in a surplus for IS in both 2013 and 2014, while the balance for BR and AD resulted in between 5 and -47 kg ha^{-1}. The non-legume catch crops had a negative result in BR and AD, while IS resulted in a positive result due to the absence of exported biomass. Both the cover crop ryegrass/clover and the green manure ley (summer and the following spring yield) resulted in negative results in BR and AD, as biomass was removed and stored for manuring the next year's crop. There was surplus N in IS for both crops (Paper II).

The nitrogen balances at the cropping system level gradually became more positive in the BR and AD treatments, when not considering the residual biomass N that was removed temporarily in the harvest year and used as an input in the next year in BR and AD.

4.3 Mineralisation rate and greenhouse gas emissions from digested and undigested ley (Paper III)

The aim of the study presented in Paper III was to compare the effects of anaerobically digested and undigested ley as a soil amendment on the mineralisation and immobilisation turnover of N, and on CO_2, N_2O and CH_4 emissions.

4.3.1 Nitrogen mineralisation

The concentrations of mineral N (NH_4^++NO_3^-), including the mineral N (N-min) already present at the start of the incubation, were significantly lower in the L treatment compared with the DL treatment throughout the experiment (Figure 2 in Paper III). The N-min concentration did not differ between the L and S treatments initially (t_{0d} and t_{7d}), but was significantly higher in S compared with L from 20 days to 90 days (t_{20d} to t_{90d}). There was no difference between the N-min concentration of DL and S at t_{7d} and between t_{50d}-t_{90d}. However, the concentration changes in mineral N should not be interpreted as absolute mineralisation without correcting for N losses in the form of gaseous emissions (Figure 4 in Paper III).

The apparent net mineralisation values over 90 days, calculated from the change in mineral N pools over time, were -0.57 (SEM 5.68), -12.3 (SEM 17.5) and 34.6 (SEM 7.91) mg N kg^{-1} dw soil for L, DL and S, respectively. When these values were corrected for the estimated N losses, the net mineralisation values were instead 108 (SEM 18.6), 69.0 (SEM 51.0) and 45.7 (SEM 6.58) mg N kg^{-1} dw soil for L, DL and S, respectively (Figure 3). The treatments did not differ significantly from each other before or after the correction of losses, but the mineralisation of ley was significantly higher after correction.

4.3.2 Gaseous losses

The cumulative emissions of CO_2, N_2O and CH_4 over 90 days added up to 255, 267 and 98 mg CO_2eq kg^{-1} dw soil, for the L, DL and S treatments, respectively. Soil with addition of digestate or ley thus emitted similar amounts of GHG, despite the different quality of the added organic material and the different relative amounts of mineral and organic N. The dominating contribution of GHG was from N_2O in all treatments. Emissions ranged from 90 to 251 mg CO_2eq kg^{-1} dw soil, with the lowest emissions from S and the highest from DL.

Nitrous oxide

Nitrous oxide emissions showed a sharp peak at t_{2d} for the L treatment and lower, but longer-lasting emissions for the DL and S treatments (Figure 4a in Paper III). The cumulative N_2O emissions over 90 days were 13.8 (SEM 1.05), 19.2 (SEM 5.32), and 6.87 (SEM 2.24) mg N_2O-N kg^{-1} dw soil for the L, DL and S treatments, respectively.

Carbon dioxide respiration

Carbon dioxide emissions from microbial respiration in the L treatment were significantly higher than those in the DL and S treatments (Figure 4b in Paper III). The cumulative CO_2 emissions over 90 days were 1.87 (SEM 0.01), 0.38 (SEM 0.04) and 0.21 (SEM 0.01) g CO_2-C kg^{-1} dw soil for the L, DL and S treatments, respectively. Carbon dioxide respiration was significantly higher in L compared with the other treatments ($p<0.001$), and DL had higher emissions than the S reference treatment ($p<0.001$).

Methane

Methane emissions were generally low and fluctuated around zero, but there was a peak in the L and DL treatments at 55 days (t_{55d}) (Figure 4c in Paper III). The cumulative CH_4 emissions over 90 days were 0.27 (SEM 0.02), -0.15 (SEM 0.04) and -0.21 (SEM 0.02) mg CH_4-C kg^{-1} dw soil for the L, DL and S treatments, respectively. The emissions from the L treatment were significantly higher than the emissions from the other treatments ($p<0.001$).

4.3.3 Total losses of carbon

Over the 90 days of incubation, 1889 (SEM 57.0), 382 (SEM 34.6) and 214 (SEM 10.2) mg C kg^{-1} dw (soil + amendment) were lost from the L, DL and S treatments, respectively. These carbon losses comprised measured microbial respiration (CO_2), as well as emissions of CH_4. The cumulative C losses were significantly higher from the L treatment compared with DL and S ($p<0.001$). After subtracting the C losses in the S treatment, the average C losses from the amendments in the L and DL treatment were 49 (1.68 mg C kg^{-1} dw (soil + amendment)) and 13% (0.17 mg C kg^{-1} dw (soil + amendment) of the total C added through the amendments. The carbon loss from the L biomass was significantly higher than in the DL treatment also after subtracting the C losses of the soil ($p<0.001$). The total C loss from the digested ley was 42%, after adding the amount lost as CH_4 and CO_2 in the digestion process to the losses during the incubation. In total, the undigested ley added 7% less C to the soil compared with the digested ley after 90 days of incubation, based on equivalent amounts of added total N content as ley and digested ley to the soil.

4.4 Life cycle assessment

The aim of the LCA was to summarise the use of resources and the environmental consequences of activities involved in farm-level scenarios using the same crop rotation and biomass management strategies as in the field experiment.

4.4.1 Life cycle impact assessment

The two treatments BR and AD were compared with IS, which served as a reference scenario to represent how biomass is commonly managed on farms. The assessment resulted in a higher GWP for the BR scenario compared with AD and IS (Figure 3). Eutrophication potential decreased for both BR and AD compared to IS, where the biomass was left in the field mainly in autumn, when the uptake and growth of the crop is low (Figure 4). There was higher energy usage at the cultivation stage in BR compared with AD (Figure 5). A sensitivity analysis compared experimental data with literature data, which showed higher GWP and eutrophication potential based on experimental data (Figure 6).

Global warming potential

The largest contributor to GWP after conversion to CO_2 equivalents (Table A1 in appendix) was direct and indirect N_2O emissions during and after field application of the different types of biomass, with silage application contributing most (35.2, 52.2, and 45.3 Mg CO_{2eq} 100 ha^{-1} for IS, BR and AD, respectively) (Figure 3). The greatest emissions from the biogas production in AD originated mainly from CH_4, emitted from the process of converting biogas to electricity. The negative values presented as "substitution" in AD, represent avoided emissions after substituting the Nordic energy production (Table A6) with biogas-generated electricity (Table A5).

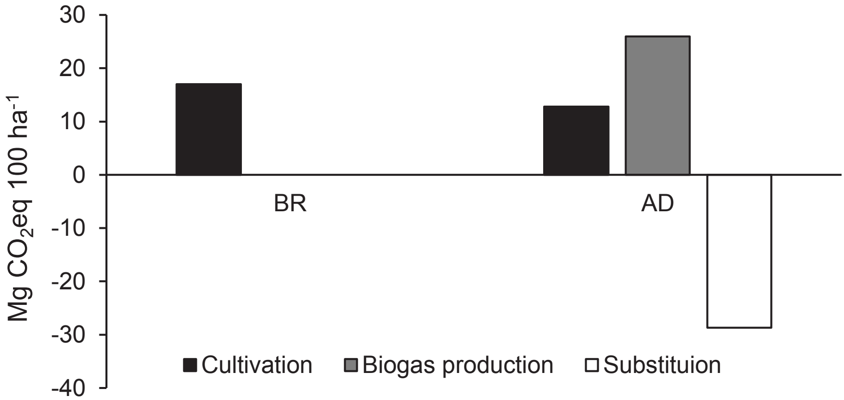

Figure 3. Global warming potential from the emissions in treatments with biomass redistribution (BR) and anaerobic digestion (AD), expressed as the difference compared with the reference scenario with biomass left *in situ* (IS), based on emissions from Table A7 and amount of digestate in Table A2

Eutrophication potential

The eutrophication potential was high after application of the nutrient-rich biomass in all scenarios, especially in IS (Figure 4), due to large emissions of NH_3 (Table A7) and NO_3^- leaching from the fresh biomass left and incorporated *in situ* after harvest during summer, autumn and winter (Table A9). The cumulative eutrophication potential was 2230, 1605 and 673 kg PO_4^{3-}eq 100 ha^{-1} for IS, BR and AD, respectively. The eutrophication potential was lowest for AD, since the digestate mainly contained NH_4^+, which partly adheres to soil colloids and can be taken up by the growing crop in the spring. There was also more biomass N to be leached in IS compared with BR and AD, as a result of N losses during the conversion steps (Table A2).

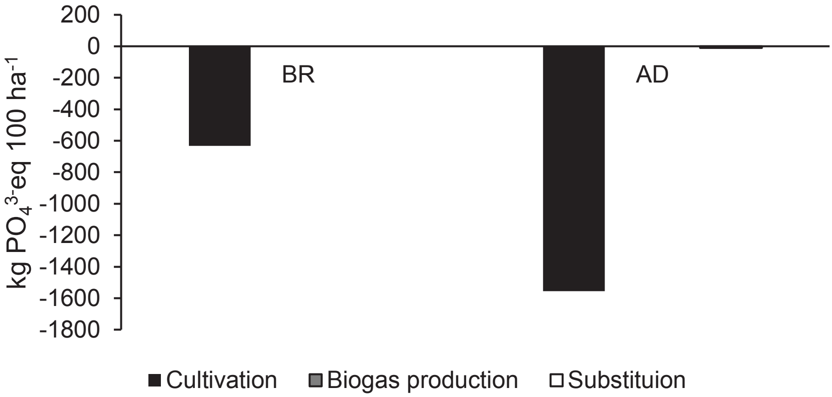

Figure 4. Eutrophication potential from cultivation, biogas production and substitution of Nordic energy in treatments with biomass redistribution (BR) and anaerobic digestion (AD), expressed as the difference compared with the reference scenario with biomass left *in situ* (IS).

Energy balance

There was higher energy usage in BR compared with AD at the crop production stage (Figure 5). This was due to the higher diesel usage when silage was applied to the field with a solid manure spreader, compared with the usage of a trailing hose ramp for application of digestate (Table A10). The electricity and heat produced in the AD scenario resulted in a surplus after deducting the energy consumption (Figure 3).

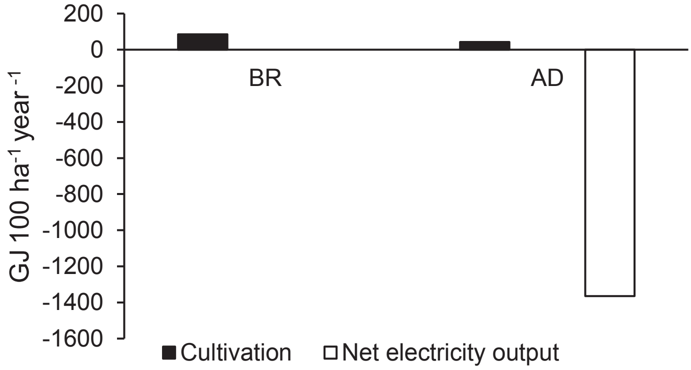

Figure 5. Energy comparison in treatments with biomass redistribution (BR) and anaerobic digestion (AD) between diesel energy usage in cultivation as positive values and surplus net electricity as negative output, expressed as the difference compared with the reference scenario with biomass left *in situ* (IS).

Sensitivity analysis

A sensitivity analysis was made to compare experimental data on N_2O and CH_4 emissions from applying biomass in the soil incubation (Figure 3), with the emission factors given by IPCC (Table A8). All three treatments had similar emissions with the factors from IPCC but, as AD had lost more N during the different handling steps, slightly less GHG were produced (Figure 6). There were major differences when the factors from IPCC and the emission factors obtained from the soil incubation were used. The results indicated that the emission factors from IPCC would result in a higher GWP for all the treatments.

A comparison between emission factors from IPCC on eutrophication potential (Table A9) with experimental data was also performed (Figure 4). The results showed the same trends with higher eutrophication risk from IS compared to the other treatments (Figure 7).

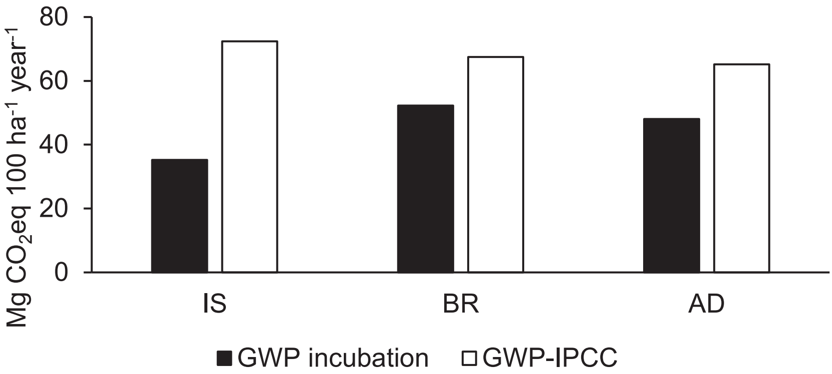

Figure 6. Global warming potential (GWP) from the treatments with biomass left *in situ* (IS), biomass redistribution (BR) and anaerobic digestion (AD) when using experimental data compared with the emission factors suggested by IPCC for N_2O and CH_4 emissions at field application.

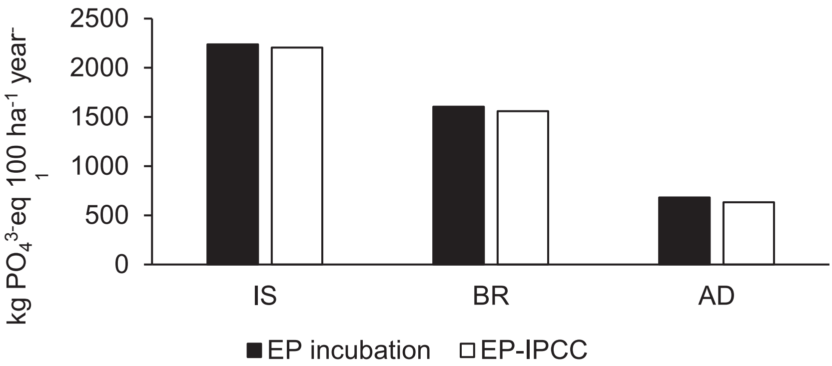

Figure 7. Eutrophication potential from the treatments with biomass left *in situ* (IS), biomass redistribution (BR) and anaerobic digestion (AD) when using the emission factors from experimental data compared with emission factors suggested by IPCC for N_2O emissions.

5 Discussion

Strategic biomass management, here comprised of biomass redistribution (BR) and anaerobic digestion (AD), maintained levels of food crop yields, with increased biomass production potential of cover crops and an increase in legume proportions in intercrops, green manure and ryegrass/clover leys (Paper I). This is important, because an increased proportion of legume biomass in the green manure ley leads to a reduced need for external inputs of N to cover requirements of the following crop. In stockless organic agriculture, this is of particular importance as there are few economically viable options to supply N when there is no access to animal manure. The first and second hypotheses of increased yield and N content in the edible parts of the crops grown with the AD treatment, was not confirmed, but the third hypothesis of increased cover crop yield was confirmed for the BR treatment. The possibility of using AD as a treatment for residual and green manure biomass without losses in yield and quality provides the opportunity of producing bioenergy as an additional source of energy or income for the farmer. Tuomisto and Helenius (2008) even argue that a slightly lower crop yield in a bioenergy scenario would be acceptable in the energy balance compared with leaving the biomass *in situ*.

There was no difference in soil- and biomass-derived N accumulation in the crops in contrast to hypothesis five and hypothesis seven, which could have been caused by the lower than expected NH_4^+ concentration in the digestate (Paper II). There are several possibilities to improve the anaerobic digestion of the feedstock, such as mixing, shredding, alkali pre-treatment and minimising the contact with oxygen at storage prior to digestion (Hjorth *et al.*, 2011; Carrere *et al.*, 2016). There may also have been N losses during handling of the digestate and during field application of the digestate (Banks *et al.*, 2011; Möller & Müller, 2012). Losses of N from digestate in the field could have been decreased by using equipment for shallow direct injection into the soil (Möller & Müller, 2012).

The fourth hypothesis was rejected as the proportion of N_2 fixation (%Ndfa) in the legumes of this study was high, but not significantly influenced by biomass management method. This was probably because the legumes were grown in intercrops/mixtures with cereal/grasses. The competitive ability of cereals and grasses for mineral N results in non-proportional acquisition of soil mineral N between the species, leading to low availability of mineral N for the legumes and high %Ndfa (Carlsson & Huss-Danell, 2003; Hauggaard-Nielsen *et al.*, 2008; Bedoussac *et al.*, 2015). The green manure leys fixed higher amounts of N_2 in BR and AD than in IS in 2014, and a similar tendency could also be seen in 2013. The higher amount of N_2 fixation in legumes, grown as green manure ley with the BR and AD treatment, is most likely a consequence of the removal of N-rich cuttings, reducing N availability and thereby the competitiveness of the grasses, thus promoting growth and N_2 fixation by the legumes.

The N balance that did not consider the temporary removal of residual biomass in BR and AD resulted in a surplus in 2014 of 7.8 and 24 kg N ha^{-1} respectively, with the highest N surplus in the AD treatment (IS<BR<AD), which confirmed the hypothesis. The nitrogen stored in BR and AD and applied to the non-legume crops in the spring was potentially protected from being lost by mineralisation during autumn and winter. This strategy thus offered an important potential improvement for stockless organic farms, where sufficient N supply can be in conflict with minimising the risk of N losses. The N surplus on stockless organic farms can be as high as 194 kg ha^{-1} (Watson *et al.*, 2002b). The increased N accumulation in biomass from 2013 to 2014 described in this thesis originated partly from higher N_2 fixation in BR and AD, but mainly from the applied residual biomass. The fact that the amount of residual biomass N increased over time explains the negative N balances in BR and AD when the temporary storage and redistribution of biomass N was taken into account, since the amount of temporarily exported biomass N was larger than the amount of biomass N redistributed from the previous year. The difference between the key inputs and outputs at the cropping system level, *i.e*. N_2 fixation minus N exports in edible crop fractions, was more negative in IS than in BR and AD (Paper II). This result further highlights the advantage of strategic biomass management in BR and AD. The sustainability of the N management in stockless organic farming systems depends on the balance between nutrient export via the cash crops, nutrient inputs through N_2 fixation, the level of success in internal recycling and reduction of losses (Legg & Meisinger, 1982)). In this perspective, the biomass N management strategies evaluated in this thesis show promising results.

As hypothesised, there appeared to be an initial immobilisation of N in the ley (L) treatment (Figure 2, in Paper III) during the first 20 days (t_{0d}-t_{20d}), followed by mineralisation. However, correcting the data for N losses as gaseous emissions resulted in cumulative mineralisation, which indicates that part of the initial decrease in mineral N concentrations could have been due to gaseous N losses (Figure 4, Paper III). The digestate (DL) treatment contained a large amount of NH_4^+ - N at the start of the incubation, originating from the digestion process (Table 1, Paper III). Contrary to our hypothesis, the concentration of inorganic soil N decreased during the incubation period in DL. However, a large part of this apparent immobilisation was most likely due to gaseous losses of N, as indicated by measured N_2O emissions and qualitative measurements of NH_3, and confirmed by decreasing amounts of ^{15}N during the incubation. Other studies have reported similar results (Wolf, 2014). The hypothesis that the amount of cumulative mineral N would be higher in DL than in the L treatment after 90 days was rejected, as there was no significant difference between the treatments. In a field situation with spring application of digestate, it is likely that mineralised N would be acquired by the growing crop and the emissions would thereby be decreased. Competition between crop root absorption of mineral N and re-absorption by microorganisms has been seen (Jingguo and Bakken, 1997; Bruun et al., 2006). In contrast, leaving crop residues in the field in late summer or autumn, without sowing a winter crop or cover crop, can be associated with large losses through both leakage and gaseous emissions. When calculating the mineralisation and immobilisation with the addition of the N lost as gaseous emissions, there was cumulative mineralisation in all the treatments throughout the experiment. In the absence of plants in the soil incubations, it is likely that mineralised N was immobilised by microorganisms or emitted as artificially high emissions of N_2, N_2O and NH_3. Immobilisation of mineral N, as well as high gaseous emissions, have been observed in other studies when crop acquisition has been absent or low (Janzen and McGinn, 1991; Raun and Johnson, 1999; Baggs et al., 2000). Much of the microbially assimilated N will be re-mineralised, but a significant part will inevitably remain as relatively stable organic N in the soil (Jingguo and Bakken, 1997), which was also observed in this study.

The high CO_2 respiration from L compared with the other two treatments, during the entire incubation period (t_{0d} to t_{90d}), indicated high microbial activity (Figure 3b, Paper III), which was consistent with the generally accepted observation that undigested material is less recalcitrant compared to the corresponding digestate (Sánchez et al., 2008). Other studies have also reported higher soil respiration from undigested feedstock compared with application of digested material (Möller, 2015). The undigested ley had emitted more total C than the digested ley after 90 days of incubation, even after including C emissions during the anaerobic digestion. This result is in accordance with results from other studies, and is related to the extraction of C from easily decomposable C structures in the digestion process, which results in a digestate with a higher biological stability with respect to the feedstock (Marcato et al., 2009; Tambone et al., 2009).

There were high emissions of N_2O between t_{0d} and t_{2d} (Figure 3a, Paper III), which can probably be explained by anaerobic conditions as a result of the high respiration peak. A decrease in total ^{15}N suggests large denitrification emissions during the incubation period. Similar studies with different untreated legume residues have found initial peaks of CO_2 emission rates combined with N_2O emission peaks when the water-filled pore space is higher than 60%, as in the present study (Aulakh *et al.*, 1990). The relatively high water-filled pore space in the jars (66%) could have facilitated the build-up of N_2O emissions (Clayton et al., 1997; Conen et al., 2000) and the emissions in a field situation are likely to be lower. Aulakh et al (1990) saw similar results with N immobilisation combined with high denitrification losses during the first 10 days of a soil incubation with crop residues (Aulakh et al., 1990). When the emissions of CH_4 and N_2O were transformed to CO_2 equivalents based on the 100 year factors presented by IPCC (34 for CH_4 and 298 for N_2O; (Myhre et al., 2013), it was found that the cumulative GHG emissions from ley and digested ley were similar, with N_2O dominating the emissions in all treatments. The CH_4 emissions were negligible in comparison with the magnitude of the other gaseous emissions. The main focus for decreasing greenhouse gas emissions should therefore be on N_2O, in all steps of biomass management.

The results of the LCA confirmed the hypothesis that the AD scenario contributed much more energy than it used, and some could be used to replace emissions from the national electricity production. As the Nordic energy mix used as a reference in this analysis is mainly based on renewable energy sources, the replacement of this energy source with biogas in AD only resulted in a minor decrease in GHG emissions. The GHG decrease in the AD treatment would be much larger if the biogas were to be used to replace *e.g.* fossil vehicle fuel. The energy production peaks coincide well with the highest energy need for heating (winter) and the digestate production coincides well with the crop requirement for nutrients (spring) with the reactor technology chosen for the scenario. Excess heat and digestate from the biogas scenario could theoretically have been sold to a neighbouring farm with greenhouse production, which would improve the energy balance for the AD scenario. It should be kept in mind that only differences in energy consumption were analysed in this thesis. There would, for example, be higher diesel consumption if all the field management activities were included. The BR and AD scenarios would also have had higher energy consumption and GHG emissions if transport of biomass to the storage and biogas reactor would have been included in the assessment.

The removal of N-rich biomass in the autumn decreased the risk of leaching in both the BR and AD scenarios, which confirmed the hypothesis. However, the risk assessment of N leaching was based on only one publication and it would be interesting to use the cropping system programme VERA from the Swedish Board of Agriculture for more accurate calculations (SJV, 2016).

The experimental data resulted in lower emissions compared with more general emissions factors from IPCC for GWP originating mainly from field application of fresh, ensiled or digested biomass. The difference between the emission factors was most pronounced for IS, as the emissions originate from residual biomass in the field, while BR and AD also emitted GHG from silage plastic and machinery redistributing the silage or digestate. The emissions from the three scenarios also included indirect N_2O emissions originating from NH_4^+ and NO_3^-. Some emissions factors from the literature were based on animal manure, which may introduce erroneous results. A more detailed LCA based on results from this thesis is in progress, which will provide more precise comparisons between the three scenarios and a more complete set of factors that may influence emissions, eutrophication and energy use.

The end results from an LCA are partly based on subjective selection of the category (*e.g.* GWP, eutrophication and energy production) considered to be the highest priority. As we are living in an age where global warming is one of the greatest threats to earth, the greatest attention should be devoted to the category of global warming. The BR scenario contributed most to emissions in this category, with direct and indirect N_2O emissions from field application of silage as the major contributor together with tractor operations. The N_2O emissions data were based on results from the soil incubation without a crop, where a certain water-filled pore space was used and maintained during 90 days. The soil humidity and thus the aerobic bacteria in the soil will vary and probably reduce the total N_2O production as the soil dries up in an agricultural field. Shallow direct injection of fertiliser in the soil has the potential for keeping the emissions low, which could potentially decrease the GHG emissions from the AD scenario even further. The assessment of eutrophication potential resulted in potentially lower emissions and higher energy production for the AD scenario compared with BR and IS, which makes this a scenario of high interest and great potential for enhancing the sustainability of organic stockless cropping systems.

6 Conclusions

There are ways to reduce negative impacts from food production in farming and at the same time increase food security for a growing global population, but this requires a large paradigm shift in diets. Stockless organic agriculture is a challenging but attractive option that not only decreases GHG emissions, but also uses land and N supply to produce protein and calories in a more efficient than intensive livestock production.

The results presented in this thesis show that food, biomass for bioenergy carriers and digestate can be produced within the same cropping system without reductions in yield and N concentration of the food crops, relative to standard organic farming practices, *e.g.* green manuring and crop residue incorporation. Maintenance of food crop yields and increased biomass yields, as was found for one of the cover crops, show that strategic redistribution of residual biomass resources has potential for increasing the overall system productivity and opens up additional biomass uses in synergy with on-farm nutrient recirculation. The allocation of biomass resources for the additional production of CH_4 without yield losses in the AD treatment can enhance on-farm self-sufficiency and potentially also farm profitability, depending on energy pricing.

Strategic management of biomass resources for internal recirculation to non-legume crops has several potential advantages for sustainable N management in arable cropping systems. This thesis shows that positive effects are dominated by the increased N_2 fixation in the legumes, compared with leaving the residues, catch crop biomass and green manure ley cuttings in situ (Paper II). Strategically choosing where and when to add biomass N resources in the crop rotation has great potential to improve the N use efficiency of the cropping system. Nevertheless, care needs to be taken when applying residual biomass to selected crops in the cropping system, since high application rates might also lead to N losses depending on timing and incorporation technique of the silage/digestate into the soil. These aspects require further research about how strategic biomass

N management influences N losses within different processes and at the entire cropping system level.

The losses of N as gaseous emissions were high in the experiment, as there was no crop taking up mineralised N, which would simulate the conditions in autumn when untreated crop residues are left *in situ* and no winter crop or catch crop are sown. The gaseous emissions would possibly be reduced by crop acquisition of mineralised N if the amendments were applied in spring. Gaseous losses of N play an important part in determining the availability of mineralised N for plant acquisition. Studies of mineralisation-immobilisation turnover may be misleading if not all gaseous losses of N are measured and taken into account.

The cumulative GHG emissions from ley and digested ley were similar, with N_2O dominating the emissions in all treatments. As N_2O is a potent greenhouse gas it is of importance to aim for reductions of N_2O emissions in all steps of biomass management. More research is needed on application techniques and pH manipulation to prevent N_2O emissions from field application of digestate to improve the strategy even further. The carbon emissions during anaerobic digestion of crop residues do not necessarily lead to a reduced contribution to SOC after applying digestate to the soil compared with the application of untreated crop residue. The results presented in this thesis indicate an actual increase in soil C after addition of digested ley.

Generalisation of the results obtained in the LCA using experimental data from the soil incubation study to a 100 ha farm indicated that major electricity gain could be achieved if stored silage were used to produce energy in a farm-based anaerobic reactor.

The conclusion of the results achieved in this thesis is that it is possible to improve the environmental sustainability on organic stockless farms with strategic biomass management that involves a farm-based biogas reactor, without a decrease in food production.

7 Future perspectives

Organic production and consumption is growing, especially in Sweden and Denmark where the organic share of food consumption is now the highest in the world (Ryegård, 2017). Future agriculture needs to deliver more food with less external resources, which can be partly achieved by dietary choices facilitated by long-term strategic governance (Foley *et al.*, 2011; Garnett, 2011; Verburg *et al.*, 2013). The choice of reducing or excluding animal products from the diet would make a great decrease in greenhouse gas production if adopted by many persons (Garnett *et al.*, 2017). Farms have several important functions and other potential income sources than producing and exporting food (Haberl *et al.*, 2007; Foley *et al.*, 2011). Rural societies can buy electricity, heating and biogas as a car fuel from the local farm with a biogas reactor as well as buying the food produced, and thereby decrease transport (Bernstad & la Cour Jansen, 2011). Problems resulting in low gas production and low revenue from farm-based reactors have been identified in a survey made in Sweden (2015). The main conclusion of the Rural Economy and Agricultural Society was that some cheap and simple adjustments could increase farmer economy substantially and reduce greenhouse gas emissions. The guidelines could hopefully give an incentive that results in establishment of reactors on more farms than today (Bergström Nilsson *et al.*, 2015). Household food waste can be collected by the local municipality or by the farmers to be used as feedstock for biogas production, together with the residues produced on the farm. This is becoming an increasingly common practice in north European countries (Wulf *et al.*, 2002). A study has shown that farmers who interact with consumers are encouraged to diversify their production, leading to an improvement in ecosystem services, while selling a large diversity of products at a local market can also lead to better income for farmers (Björklund *et al.*, 2009). Life cycle analysis has concluded that anaerobic digestion of household waste and recycling of the digestate on the farm reduces global warming potential, acidification (Bernstad & la Cour Jansen, 2011) and possibly even eutrophication.

More research and implementation is needed with the goal of phasing out fossil fuel not only in the transport sector, but also in agriculture. There are many field operations that do not require the high amount of energy contained in diesel. It could be possible to pay a machine contractor to do the heavier work, such as ploughing, and for farms to have tractors running on methane or electricity.

The Ministerial Communiqué (OECD) has declared that beyond its primary function of supplying food and fibre, agriculture can also shape the landscape and provide benefits such as land conservation, sustainable management of renewable natural resources, preservation of biodiversity and improved socio-economic viability of many rural areas (OECD, 2001). However, reaching such goals calls for a focus on increased efficiency of natural resource use, improved nutrient cycling techniques and agro-ecological methods for protecting and possibly enhancing biodiversity (Halberg *et al.*, 2015; Jensen *et al.*, 2015). These topics can be addressed by a well-planned production system based on functional diversity of crops within the field and over the cropping season (Drinkwater & Snapp, 2007; Niggli *et al.*, 2008; Doré, 2011).

Functional diversification was an aim in the field experiment presented in Paper I, but further improvement could probably be achieved by partial replacement of annual crops with perennial or semi-perennial, as their inclusion often results in an increase in SOC (Paustian *et al.*, 1997; Reeves, 1997). A dynamic agricultural landscape that hosts a diversity of species is claimed to be more resilient (has the capacity to reorganise after disturbance) (Tscharntke *et al.*, 2005) and thereby enhance ecosystem services. A change in our view on the use of natural resources and consumption pattern is needed to increase the resilience of civic society and reduce the exploitation of nature. A large-scale change that really makes a difference on a global scale needs to be supported by intergovernmental policies that reward sustainable lifestyle decisions.

8 Critical reflections

There are always many details that could have been improved in retrospect. There are many advantages in the use of established cropping systems, where field personnel are accustomed to the crop and the management. The machinery and irrigation system used for the management is optimised, and long-term effects of established treatments can be studied. The downside is that it might be difficult to re-design the field experiment to fit the research questions. A detail that has the potential to decrease variation and 'edge effects' would be to establish larger experimental plots in the field than the 18 m^2 used in Paper I and II. This could give more robust results that better reflect the situation on commercial farms. Harvesting two sub-samples instead of one from each plot could give a more solid average value. A better plan for primarily mechanical weed management would have decreased the timely manual work considerably. Fencing off the experimental area from the surrounding field could have avoided some of the damage caused by mammals to the crops. Another major improvement that could have decreased potential NH_3 emissions would be direct soil incorporation of digestate. If it had been possible to use digestate with the same composition in all three years, it would have been easier to interpret the results and exclude variation and unknown losses of NH_4^+ from somewhere in the process.

Several of the references in the LCA present emission factors based on animal manure, which has a different composition than ley and plant-based digestate. Those factors are planned to be used in a future publication, but with a profound analysis of all available references on plant-based redistribution of biomass and including transport of biomass from and to the field as diesel.

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Popular science summary

The projected population growth requires that more high quality food is produced in a way that is sustainable. Organically produced food is becoming more popular and organic agriculture has the potential of meeting challenges with loss of biodiversity and declining soil carbon in agricultural soils. However, yields are often lower than in conventional farming, which is partly due to insufficient nitrogen supply. Finding ways to the use harvest residues for efficient recirculation of nitrogen within the cropping system might enhance yields and reduce risks for nitrogen losses in organic crop production without animals.

A field experiment with an organic crop rotation included in this thesis, have shown promising results from using ensiled or anaerobically digested biomass, compared to leaving residual biomass in the field after the harvest. The digestion increases the concentration of stable carbon compounds, which can potentially increase the soil carbon. The digestion and storage of the digestate opens up for a possibility of improving the timing of N supply with crop uptake. The result is less risk of leaching, compared to leaving the residues in the field in late summer and autumn. The digestion of crop residue in a reactor produces more energy than needed on the arable farm used as an example in the LCA in this thesis. This opens up for an alternative income on a farm, depending on the energy politics. This research shows that strategic redistribution of biomass-based digestate can improve the N balance of crop rotations and produce a surplus of bioenergy, which are key elements for enhancing the sustainability of stockless organic cropping systems.

Populärvetenskaplig sammanfattning

Jordens befolkning ökar fortfarande, och det för med sig ett större behov mat. Denna måste produceras hållbart för att möta utmaningar så som en utarmad biologisk mångfald, minskat kol i marken, och övergödning av vattendrag och hav. Ekologiskt jordbruk kan möta dessa utmaningar, men ger ofta lägre skördar än konventionellt jordbruk på grund av att kväve tillförs i otillräckliga mängder och ur fas med växtens behov. Genom att använda kväverika skörderester strategiskt finns flera möjliga vinster att göra. Till exempel kan resterna rötas och restprodukten lagras för att sedan gödsla grödan när behovet är som störst.

Resultat från fältförsöket som var en del av denna avhandling visade att ökad kvävefixering och bättre kvävebalans kan uppnås genom att ensilera eller röta skörderester som sedan återförs som gödning, jämfört med dagens teknik där skörderesterna lämnas i fält efter skörden. Experiment i klimatkammare visade även att rötning av skörderester (vall) kan minska utsläpp av koldioxid samt bidra till att öka innehållet av kol i marken eftersom rötning ökar innehållet av stabila kolföreningar. Livscykelanalyser tyder dessutom på att tekniken med rötning minskar risken för utlakning. Alternativet, där skörderester lämnas i fält på sensommaren, leder annars till lättrörligt kväve som inte tas upp utan lakas ut på hösten när grödornas upptag sjunker. Att röta skörderester i en reaktor ger även biogas, vilket öppnar upp för en alternativ inkomst.

Sammantaget visar resultaten i avhandlingen att strategisk omfördelning av skörderester kan förbättra kvävebalansen i odlingssystemet och producera ett överskott av bioenergi. Detta kan bidra till att förbättra hållbarheten i ekologiskt jordbruk.

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Appendix 1. Life cycle inventory

Conversion factors

Table A1. Category indicators used for global warming potential (GWP) and eutrophication potential (EP)

Gas	Global Warming Potential$_{100}$ (kg CO_2 per kg)	Reference
Carbon dioxide (CO_2e)	1.00	(IPCC, 2006)
Methane bionic (CH_4)	23.0	
Dinitrogen (N_2O)	296	
Element	**Eutrophication Potential (kg PO_4^{3-} kg^{-1})**	**Reference**
Phosphorus (P)	3.06	(Guinée *et al.*, 1992)
Ammonia (NH_3)	0.35	
Nitrogen oxides, NO_X other than N_2O	0.13	
Dinitrogen (N_2O)	0.27	
Nitrate (NO_3^-)	0.10	
Nitrogen (N)	0.42	

Cultivation

Table A2. Biomass yield at harvest, after ensiling, after digestion in the reactor and after storage of digestate. FW = fresh weight, DW = dry weight.

100 ha^{-1}	Yield of residual biomass	After ensiling	Digestate after digestion	Digestate after storage
Total biomass (Mg FW)	2649	2119	1843	1839
Total biomass (Mg DW)	640	512	236	232
Dry substance (%)	24	24	13	13
N content (Mg)	10.8	8.62	8.62	8.54
N (%)	1.68	1.68	3.65	3.68

Emission factors

Silage

Table A3. Emissions factors used for the losses from manure storage.

Emission	Emission factor	Reference
NH_3-N (kg NH_3-N/kg N)	0.2*	(NIR, 2016)
N_2O (kg N_2O-N/kg N)	0.005*	(IPCC, 2006)
Indirect N_2O emissions (kg N_2O-N/kg NH_3-N)	0.01	(IPCC, 2006)

*Based on animal manure

**Based on data from solid animal manure.

Table A4. Emissions from production, distribution and incineration of plastic used for covering the silage.

Production & distribution (g/MJ)	Emission factor - air	Emission factor - water	Reference
CO_2	5.31		(Gode *et al.*, 2011)
NO_x	0.019		
SO_2	0.013		
CH_4	0.0291		
N_2O	5.26E-05		
NH_3	1.26E-05	1.42E-08	
NH_4^+		1.99E-05	
NO_3^-		2.72E-05	
PO_4^{3-}		3.21E-07	
Incineration (g/MJ)	**Emission factor - air**	**Emission factor - water**	**Reference**
CO_2	5.31		(Gode *et al.*, 2011)
NO_x	0.019		
SO_2	0.013		
CH_4	0.0291		
N_2O	5.26E-05		
NH_3	1.26E-05	1.42E-08	
NH_4^+		1.99E-05	
NO_3^-		2.72E-05	
PO_4^{3-}		3.21E-07	

Biogas and digestate production

Table A5. Energy use, emissions and energy conversion from running the reactor and generator.

Activity	% of gas produced	Reference
Heating of the reactor	14	(Tufvesson *et al.*, 2013)
Electricity needed in the reactor (CSTR)	4.0	
Losses of methane (heating)	1.0	
Methane losses in digestion process of total methane production	0.005	
Energy conversion in generator	Emission factor	Reference
CH_4	1.56	(Nielsen *et al.*, 2010)
N_2O	0.006	
NO_x	0.727	
Digestate produced (m^3 CH_4/t VS)	271	
VS in digestate (t/yr)	188	
Methane production capacity factor (%)	3.5	
Methane production in digestate storage (m^3/yr)	1785	
Methane losses from storage (kg CH_4/yr)	1277	

Nordic energy mix

Table A6. Average emissions generated from the production of energy in the Nordic countries between 2013 and 2015.

CO_2	NO_x	SO_2	CH_4	N_2O	NH_3	Reference
18.97	0.04	0.038	0.067	0.0018	0.0040	(Ecoinvent, 2013-2015)

Field application

Table A7. Nitrogen losses caused by NH_3 emissions during the spreading of biomass.

Biomass	NH_3-N (% of total N)	Conditions
Fresh biomass (IS)	35	Broadcast, solid manure, mulching within 4 h, early autumn
Ensiled (BR)	33	Broadcast, solid manure, mulching within 4 h, spring
Digested (AD)	8	Trailing hoses, mulching within 4 h, spring

(NIR, 2016)

Table A8. Nitrous oxide and CH_4 emissions after shallow incorporation of biomass into the soil.

Biomass	N_2O (% of total N) (Paper III)	N_2O (% of total N) (IPCC, 2006)
Digested ley	7	1
Fresh ley	4	1
	CH_4 (% of total C) (Paper III)	CH_4 ref. IPCC (% of total C) (IPCC, 2006)
Digested ley	0.017	0
Fresh ley	0.718	0

Table A9. Amount of nitrate leached from a reference crop depending on incorporation time.

Scenario	Application time	NO_3^- (kg/ha/year)	Reference
IS	Late summer/autumn	60	(Stopes *et al.*, 1996).
BR	Spring	15	
AD	Spring	15	

Table A10. Direct energy usage from diesel using the field machinery in scenario BR and AD compared with IS.

Equipment-BR	Diesel (MJ/100 ha)
Tractor 4WD, 100 kW	0
Solid manure spreader, 12 m^3, 6 m wide	49000
Loader	1764
Loading wagon 25-30 m^3 DIN	0
Field hack, 6 m wide	17640
Pickup	3920
Sum	**72 324**
Equipment-AD	Diesel (MJ/100 ha)
Tractor 4WD, 100 kW	
Loader	1764
Loading wagon 25-30 m^3 DIN	0
Trailing hose ramp 24 m	12250
Tank wagon 15 m^3	0
Digestate pump	0
Field hack, 6 m wide	17640
Pickup	3920
Sum	**35 574**

(Achilles *et al.*, 2005; HIR Malmöhus & Maskinkalkylgruppen, 2014)

Table A11. Emissions from diesel production, distribution and incineration.

Diesel production & distribution (g/MJ)	Emission factor - air	Emission factor - water	Reference
CO_2	6.32E+00		(Gode *et al.*, 2011)
NO_X	1.84E-02		
SO_2	1.68E-02		
CH_4	3.28E-02		
N_2O	1.04E-03		
NH_3	2.84E-04	2.56E-02	
NH_4^+		2.42E-05	
NO_3^-		2.58E-05	
PO_4^{3-}		3.04E-07	
Diesel-incineration (g/MJ)	**Emission factor - air**	**Emission factor - water**	**Reference**
CO_2	6.96E+01		(Börjesson *et al.*, 2010)
NO_X	0.800		
SO_2	0.002		
CH_4	8,30E-04		
N_2O	1.00E-03		
NH_3	3.80E-04		

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Productivity in an arable and stockless organic cropping system may be enhanced by strategic recycling of biomass

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Abstract

Recirculation of nitrogen (N) from crop residue and green-manure biomass resources may reduce the need to add new reactive N to maintain crop yield and quality. The aim of this study was to determine how different strategies for recycling residual and green-manure biomass influence yield and N concentration of the edible parts of food crops in a stockless organic cropping system. For this purpose, three biomass distribution treatments were investigated in a field experiment, based on a cropping system designed to produce both high-quality food crops and biomass resources from crop residues, cover crops and a green-manure ley. The three treatments, applied at the cropping system level, were: (1) incorporating the aboveground biomass resources *in situ* (IS); (2) harvesting, ensiling and redistributing the same biomass resources to the non-legume crops (*biomass redistribution*, BR); and (3) harvesting, ensiling and using the biomass resources as substrate for production of bio-methane via *anaerobic digestion* (AD) followed by distribution of the digestate as bio-fertilizer to the non-legume crops. The redistribution of ensiled (BR) and digested (AD) biomass did not increase the yield of the edible parts in winter rye (*Secale cereal* L.), white cabbage (*Brassica oleracea* L.) or red beet (*Beta vulgaris* L.) compared with leaving the biomass on the ground at harvest (IS). The BR treatment increased the yield of lentil intercropped with oat, compared with IS treatment in one of the two studied years. The total biomass yield of the cover crop following winter rye was significantly higher in the BR treatment than in IS in both years. The legume proportion in the green-manure ley was significantly higher in the AD and BR treatments as compared with IS in one of the experimental years. This study showed that strategic biomass redistribution has the potential to enhance biomass productivity while maintaining food crop yields, thereby enhancing whole system productivity. Biomass redistribution systems both with and without biogas digestion offer a new strategy for the development of multifunctional arable cropping systems that rely on internal nutrient cycling.

Key words: anaerobic digestion, cover crop, digestate, diversity, green-manure biomass, intercropping, agronomy, horticulture, arable, stockless, strategic recycling

Introduction

Agriculture faces the challenge of producing more food with fewer inputs, while simultaneously addressing problems such as soil degradation, loss of biodiversity and unpredictable weather due to climate change (Harvey and Pilgrim, 2011). Governments also have elevated expectations that agriculture should provide additional ecosystem services such as biomass for sustainable bioenergy production and climate change mitigation (Tilman et al., 2009; Harvey and Pilgrim, 2011; Sapp et al., 2015). These challenges call for a focus on eco-functional intensification and multifunctionality, i.e., increased efficiency of natural resource use, improved nutrient-cycling techniques and agro-ecological methods for protecting and possibly enhancing biodiversity (Halberg et al., 2015; Jensen et al., 2015). A well-planned production system with functional diversity of crops within the field and over the cropping season has the potential to improve the outcome of several of these challenges (Drinkwater and Snapp, 2007; Niggli et al., 2008; Doré et al., 2011).

Nitrogen (N) is often the most limiting nutrient for crop performance in terms of yield and quality, but can also be a major contributor to pollution of drinking water, eutrophication of surface water and pollution of the atmosphere with the potent greenhouse gas nitrous oxide (N_2O) (Baggs et al., 2002; MEA, 2005; Galloway et al., 2008; Foley et al., 2011; Cohen, 2015). Increased levels of N in natural or semi-natural ecosystems also lead to a reduction in biodiversity (Zillén et al., 2008; Sutton et al., 2011). Regardless of whether the N is fixed industrially or biologically by legumes, the fixation contributes to the availability of reactive N. Excessive inputs of reactive N lead to disequilibrium of the planetary N cycle and thereby to detrimental effects on ecosystems (Rockström et al., 2009). Improved retention and recycling of N is, and should continue to be, a highly prioritized goal of policy makers, advisors and farmers (Steffen et al., 2015). It is common that farmers supply N in stockless organic systems by including green-manure crops based on N_2-fixing legumes (Watson et al., 2002). A disadvantage is that growing green manures reduces the amount of land available for food crops. There may also be a high risk of N losses through ammonia (NH_3) and N_2O volatilization, and/or nitrate (NO_3^-) leaching, depending on incorporation time and technique (Li, 2015). Another N supply option is to grow grain legumes for food production, but the organic N left in the field after grain harvest is often not sufficient to cover the needs of the succeeding non-legume crop (Beck et al., 1991; Jensen, 1997). Roots with nodules left in the field or additional residual biomass may nevertheless be a valuable addition to soil N.

The harvest of ensiled or anaerobically digested biomass permits target-oriented application of organic nutrients, to fertilize crops with the highest nutrient requirements (Möller and Müller, 2012). The biogas (bio-methane) produced via anaerobic digestion can be used on the farm, or sold to the market. Generally, a larger proportion of the total N is present as mineral N and the C/N ratio is lower in the digestate obtained after anaerobic digestion compared with in fresh or ensiled biomass (Gutser et al., 2005). This is because the bacterial digestion of organic matter results in release of C, mainly as methane (CH_4) but also CO_2, while most of the organic N is converted to ammonium (NH_4^+), which remains in the digestate (Möller and Müller, 2012). Several studies have observed an increased yield of cereals fertilized with plant-based digestate compared with un-digested feedstock (Stinner et al., 2008; Frøseth et al., 2014). On the other hand, Gunnarsson (2012) reports a lack of yield increase or even a decreased vegetable yield in response to fertilization with digestate, as compared with undigested biomass harvested from a green-manure ley (Gunnarsson, 2012). The availability of N in biomass and digestate for crop N acquisition also depends on mineralization and immobilization dynamics, which in turn are influenced by many factors such as C/N ratio, temperature and moisture (Trinsoutrot et al., 2000; Nicolardot et al., 2001; Cabrera et al., 2005). If the mineralization is delayed, the application of biomass or digestate to a few crops in the cropping system can also be expected to increase the biomass yield and N accumulation in cover crops growing after the fertilized main crops (Kumar and Goh, 2002; Peoples et al., 2009).

The aim of this study was to compare three methods for strategic recycling and application of residual and green-manure biomass N in terms of yield and N concentration of the edible fraction of food crops in an organic stockless cropping system. The crop response after leaving residual biomass resources *in situ* compared with redistributing the same biomass resources after ensiling or ensiling plus anaerobic digestion was evaluated in a crop rotation. Our main hypotheses were that (1) strategic recycling of the digestate from anaerobic digestion of biomass leads to higher yield of winter rye, white cabbage and red beet, due to a higher concentration of plant-available N in the digestate compared with strategic redistribution of ensiled biomass or *in situ* incorporation; (2) concentration of N in the edible plant parts of winter rye, white cabbage and red beet increases with strategic recycling of digestate, due to a higher concentration of plant-available N in the digestate compared with biomass redistribution and *in situ* incorporation; and (3) strategic recycling of ensiled or digestate biomass increases the biomass production of the cover crops following a main crop receiving biomass, compared with after *in situ* incorporation of biomass. The reason for the third hypothesis is that the targeted addition of a large quantity of silage or digestate will increase the N availability also for the cover crops following the fertilized crops.

Materials and Methods

Study site and soil

The experiment was established in 2012 at the Swedish University of Agricultural Sciences in Alnarp, Sweden (55°39′21″N, 13°03′30″E), on the SITES (Swedish Infrastructure for Ecosystem Science) field research station in Lönnstorp on a sandy loam soil (Table 1) characterized as an Arenosol (Deckers et al., 1998). The land has been organically certified since 1993 and the preceding crop was a 1-yr legume-grass ley. Soil nutrient availability and particle distribution was analyzed at the start of the experiment (Table 1) by a commercial soil analysis laboratory (LMI, Helsingborg, Sweden) using the modified Spurway Lawton method (extraction in 0.1% acetic acid) (Spurway and Lawton, 1949).

Climatic data

The region has a typical northern-European maritime climate with mild winter and summer temperatures. Lowest and highest monthly mean temperature and monthly precipitation data from the 3 yr of the field experiment are presented in Figure 1. The 30-yr (1961–1990) average for annual temperature and total annual precipitation were 7.9°C and 666 mm, respectively, measured at the weather station in Lund (55°43′N, 13°12′E).

Table 1. Soil characteristics in the upper 0–30-cm soil layer and the lower 30–60-cm in March 2012.

Soil characteristic	Soil depth (cm)	
	0–30	30–60
pH	6.4	6.9
NO_3^-N (kg ha^{-1})	42	0
NH_4^+N (kg ha^{-1})	63	24
P (kg ha^{-1})	72	27
K (kg ha^{-1})	255	60
Gravel > 2 mm (%)	4.21	0.93
Sand 63–2 mm (%)	66.1	62.9
Silt 0.063–0.002 µm (%)	14.8	22.4
Clay < 0.002 µm (%)	14.9	13.8
Loss on ignition (%)	3.22	1.56

The temperature and precipitation in 2012–2015 were close to the average for the region, except for unusually high temperatures during November to February in 2013–2014 and high rainfall in August 2014 (Fig. 1).

Crop rotation

A 6-yr crop rotation was used for the study (Fig. 2), although the experiment was only performed during the three full seasons in 2012–2015 (Fig. 3). Within each treatment and block, the crop rotation was established in six separate plots, so that each of the six main crops in the rotation was grown during each year of the experiment. Since the experiment started in spring 2012 without any autumn-sown crop from the previous year, winter rye (*Secale cereale* L.) was replaced by spring barley (*Hordeum vulgare* L.) during the first year.

The crops included in the rotation (Table 2) were chosen to optimize several functions, namely the production of food crops, provision of biomass resources for internal recycling of nutrients, biological N_2 fixation, weed suppression and enhancing the presence of beneficial insects. The rotation therefore included crops with different functional traits, such as fast stem elongation, variation of leaf architecture, nectar-rich flowers, rapid root growth and efficient nutrient acquisition. The cropping system also included several different crop-management strategies in accordance with the principles of organic agriculture, i.e., hoeing in row crops and frequent cutting of the ley to reduce pest and weed pressures.

Intercrops contained legumes to provide symbiotic N_2 fixation, promote soil N availability and produce food crops with high-protein concentration. The pea (*Pisum sativum* L.)/barley and lentil (*Lens culinaris* Medik)/oat (*Avena sativa* L.) intercrops were selected, since mixtures with legumes and cereals have been shown to enhance resource use efficiency and reduce weed abundance compared with legume sole crops (Hauggaard-Nielsen et al., 2008; Bedoussac et al., 2014). A replacement design (De Wit and Van den Bergh, 1965) was employed with the ratio 80/20 for pea/barley and 90/10 for lentil/oat. Winter rye was included in the rotation since it competes well with weeds, retains N and reduces the risk of soil erosion. Row crops [red beet (*Beta vulgaris* L.) and cabbage (*Brassica oleracea* L.)] were included during two of 6 yr in the rotation, as examples of high-value food crops that also enable efficient mechanical reduction of weeds between the rows. The six species included in the ley were chosen to add diversity for resilience of biomass production, N_2 fixation and provide a food source for beneficial insects. The composition followed a replacement design with 16.7% of recommended sowing density for each species. Each main crop was followed by an autumn- or winter-growing main or cover crop in order to reduce N leaching, reduce weeds and produce biomass during the autumn or winter season. Oilseed radish (*Raphanus sativus* L.) and lacy phacelia (*Phacelia tanacetifolia* Beneth) were selected as cover crops for three reasons: they have a high NO_3^- uptake (Thorup-Kristensen, 2001), oilseed radish has shown partial resistance to clubroot (*Plasmodiophora brassicae*) (Diederichsen et al., 2009), and lacy phacelia is a valuable food source for beneficial insects such as parasitic wasps and bees (Araj and Wratten, 2015; Barbir et al., 2015). Both cover crops were grown in combination with buckwheat (*Fagopyrum esculentum* Moench) (50% of each species' recommended sowing density) in order to further provide resources for beneficial insects, and since it has been indicated that buckwheat produces compounds that can limit the growth of weeds (Kalinova et al., 2007). The mixture of perennial ryegrass (*Lolium perenne* L.), red clover (*Trifolium pratense* L.) and white clover (*Trifolium repens* L.) was used as a cover crop growing during autumn, winter and spring since these crops can improve soil structure (Breland, 1995) and retain NO_3^- (Askegaard et al., 2011). The sowing densities of ryegrass, red clover and white clover in this mixture were 73/15/12% of the recommended density for each species as sole crop.

Experimental design

The field experiment comprised in total 72 experimental plots measuring 3 m × 6 m, distributed in four replicate blocks. The experiment started by establishing each of the six main crops, which were followed by cover crops and main crops according to the designed crop rotation (Fig. 2) in the same physical plots during the two subsequent years, thereby providing a 3-yr crop sequence with all six crops present each year (Fig. 3). Within each block, 18 individual plots (six main crops × three treatments) were randomly assigned to one of the following biomass-management treatments applied at the cropping system level, i.e., consistently throughout the 3-yr crop sequence:

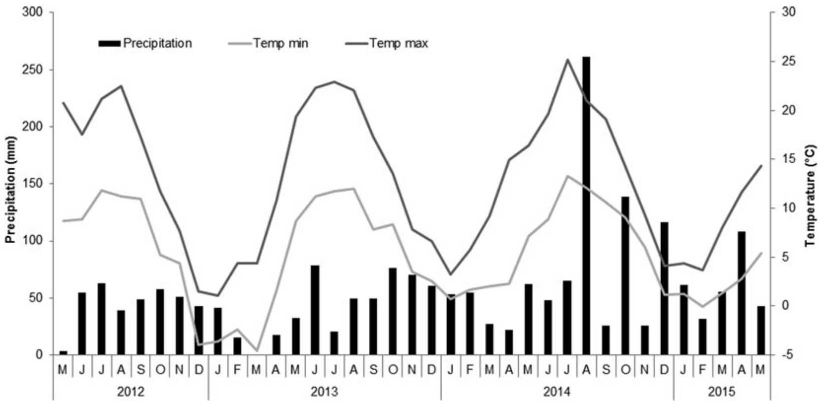

Figure 1. Mean of minimum (light gray line) and maximum (dark gray line) monthly temperatures and monthly accumulated precipitation (histogram) during the field experiment. The data were retrieved from a weather station LantMet, Alnarp (55°40′N, 13°6′E).

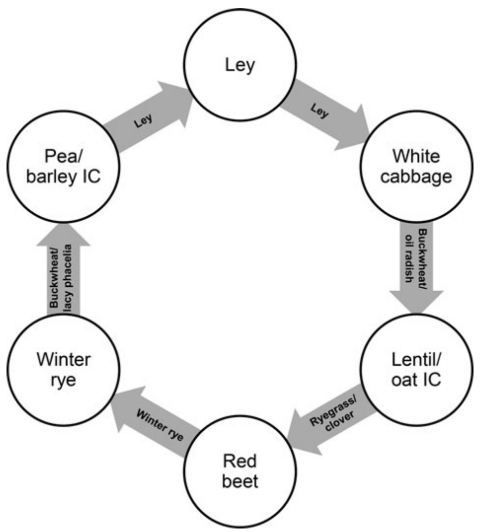

Figure 2. Crop rotation that was used for the 3 yr crop sequence. The main crops are marked with a circle and the cover crops or overwintering crops as an arrow.

IS—*in situ* incorporation of biomass resources (crop residues, cover crops and green-manure ley), i.e., leaving the biomass after harvest in the same plot as they were grown.

BR—biomass redistribution: storing the biomass resources as silage and redistributing them to cabbage, red beet and rye growing in the same system in the following year.

AD—anaerobic digestion of the biomass resources (after storing them as silage) and redistributing the digestate to cabbage, red beet and rye growing in the same system in the following year.

The residual biomass comprised straw from grain legumes and cereals, leaves from cabbage and red beets, and all aboveground biomass of cover crops. The green manure consisted of ley, from which aboveground biomass was harvested four times. The IS treatment differed from BR and AD already during the first year (2012), since biomass resources were left *in situ* instead of being removed from the plot, and redistributed in the next year as silage in BR and digested silage in AD. In contrast, the distinction between BR and AD did not start until the second year (2013), when the non-legume crops were fertilized either with silage (BR) or digestate (AD). The May cuttings of the green-manure ley and the ryegrass/clover were stored together with the other residual biomass sources harvested later in the growing season, and redistributed in the following year.

The distribution of N in BR and AD was based on the strategy to use all available biomass resources for redistributing N to the non-legume main crops within the cropping system, in proportions that reflected national recommendations for N fertilization of rye, cabbage and red beet, respectively. Total N content of biomass was measured in subplot samples for each treatment and used to estimate total N in the residual and green-manure biomass (Table 3). The total N content, i.e., the sum of all biomass resources, was similar for the three treatments in 2013, while in 2014, the AD treatment resulted in a lower amount of N applied than in the IS and BR treatments. The differences in total N between AD biomass and AD digestate mean that there have been losses of N during handling of biomass, silage and digestate in the AD treatment. Losses of N from the IS and BR systems were not quantified.

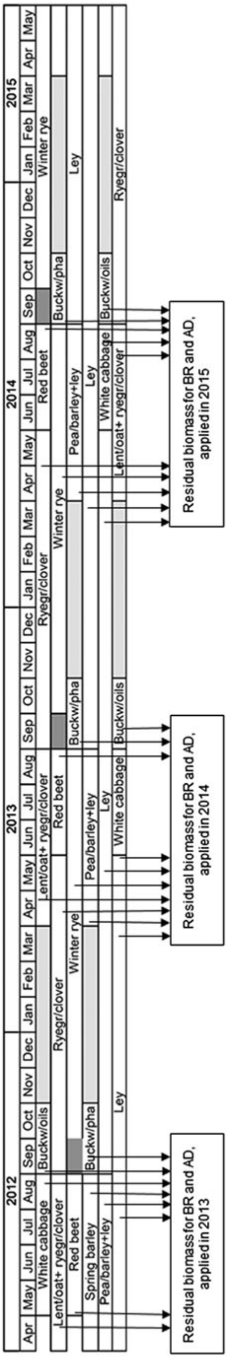

Figure 3. Three-year sequence of the crop rotation investigated in the study, with all six main crops present each year from 2012 to 2014. The light grey sections represent uncultivated stubble after cover crop harvest and the dark grey sections represent a short black fallow after red beet harvest. The vertical arrows show the harvests of residual (food crops) and total aboveground (cover crops and green-manure ley) biomass for redistribution in the subsequent year in the BR and AD treatments. In IS, the same biomass resources were left *in situ* in the same field plot as they had been growing.

Crop management

All crops were sown with a density based on national recommendations in organic farming (Table 2). The row spacing for winter rye was 12.5 cm in 2012 and doubled to 25 cm in 2013 to facilitate spreading the biomass and digestate in the rows. Red beet and cabbage were sown and planted with a row spacing of 50 cm. The variety of red beet was changed from the monogerm type 'Alvro mono' in 2012 and 2013 to the multigerm variety 'Kestrel' in 2014. The cabbage plants were mechanically transplanted in rows with 50 cm apart and irrigated to assure the establishment of the plants, in order to simulate a large-scale production farm. In 2012, six rows were sown and planted in each plot of red beet and cabbage. They were reduced to five rows in 2013 and 2014, since plants in the border rows were severely stunted in 2012. The green-manure ley and the clover/ryegrass catch crop were undersown in their respective main crops (Table 2) at the same time as the main crop.

At the start of the experiment in spring 2012, the previous crop (ley) was ploughed, and the soil was harrowed twice over two consecutive weeks to control weeds. Subsequent soil management was made with non-inversion tillage (2013 and 2014). At the time of establishment in 2012 (not repeated in the following years), the entire field was fertilized with digestate from a stockless organic farm with biogas production. The digestate (containing 7.1 kg total-N Mg^{-1} digestate, 5.4 kg NH_4^+-N Mg^{-1}, 1.3 kg P Mg^{-1} and 1.7 kg K Mg^{-1}) was applied at a rate of approximately 16 Mg digestate ha^{-1}, to achieve 115 kg N ha^{-1}. The digestate was applied with a 20-m wide boom that had trailing hoses.

The weeds in the row crops were controlled by hand hoeing during each growing season. Winter rye was sown in late September/early October, after red beet harvest in late August. During this short fallow period, the soil was tilled when the weeds emerged and again a few weeks later. No weed control was used in the intercrops or cover crops. The cabbage was covered with an insect net (0.8 mm × 0.8 mm mesh). Hand spraying of *Bacillus thuringiensis* with knapsack spraying equipment occurred in 2013 and 2014 as an organic pest control measurement of *Lepidoptera* species. The spraying started at the observation of the larvae on the crop and was repeated two times with an interval of 2 weeks.

Anaerobic digestion and application of biomass resources

The anaerobic digestion of biomass resources in the AD treatment was made using a mesophilic leach bed reactor at the Annenberg research facility (Biotechnology, Lund University, Sweden). In this type of batch reactor, solids are hydrolyzed by adding and circulating water over the biomass (Lehtomäki et al., 2008). Recirculation

Table 2. The components of the crop rotation with main and cover crops.

No in sequence	Main crop, sowing/planting density	Cover crop/winter crop (and sowing density when not listed as main crop)
1	Green-manure ley: Orchard grass *Dactylus glomerata* L. 'Luxor', 3.3 kg ha^{-1} Meadow fescue *Festuca pratensis* L. 'Sigmund', 3.3 kg ha^{-1} Timothy *Phleum pratense* L. 'Ragnar', 2.0 kg ha^{-1} Yellow sweet clover *Melilotus officinalis* Lam. 'Unknown', 3.3 kg ha^{-1} Lucerne *Medicago sativa* L. 'Creno', 2.5 kg ha^{-1} Red clover *Trifolium pratense* L. 'Titus', 2.0 kg ha^{-1}	Green-manure ley
2	Cabbage (white cabbage) *Brassica oleracea* L. 'Sir', 40,000 plants ha^{-1}	Buckwheat/oil radish: Buckwheat *Fagopyrum esculentum* Moench 'Hanelka', 30 kg ha^{-1} Oilseed radish *Raphanus sativus* L. 'Unknown', 13 kg ha^{-1}
3	Lentil/oat intercrop: Lentil *Lens culinaris* Medik. 'Le May', 45 kg ha^{-1} Oat *Avena sativa* L. 'Kerstin', 21 kg ha^{-1} Undersown with ryegrass/clover cover crop	Ryegrass/clover: Perennial ryegrass *Lolium perenne* L. 'Birger', 22 kg ha^{-1} White clover *Trifolium repens* L. 'Hebe', 0.6 kg ha^{-1} Red clover *T. pratense* L. 'Titus', 0.6 kg ha^{-1}
4	Red beet *Beta vulgaris* L. *var. conditiva*, 'Alvro mono', 850 kg ha^{-1} 'Kestrel', 1920 kg ha^{-1}	Rye (main crop no. 5)
5	Rye (winter rye) *Secale cereale* L. 'Amilo', 180 kg ha^{-1}	Buckwheat/lacy phacelia: Buckwheat *F. esculentum* Moench 'Hanelka', 30 kg ha^{-1} Lacy phacelia *Phacelia tanacetifolia* Benth (unknown), 12.5 kg ha^{-1}
6	Pea/barley intercrop Pea *Pisum sativum* L. 'Clara', 212 kg ha^{-1} Barley (spring barley) *Hordeum vulgare* L. 'Tipple', 21 kg ha^{-1} Undersown with green-manure ley	Green-manure ley (main crop no. 1)

of the liquid stimulates the microbial digestion of the biomass, due to the continuous redistribution of inoculum, nutrients and dissolved organic matter (Chanakya et al., 1997; Lissens et al., 2001). The silage feedstock in our study had a dry matter content of 24% in both years and was not pre-treated in any other way than mixing the pile of silage with a tractor-carried shovel before loading it into the reactor. The digestion was allowed to run for 2 months in early spring in both 2012–2013 and 2013–2014. The resulting digestate was delivered in a liquid and solid phase (Table 4). The mean C/N ratio of the pooled digestate (liquid + solid) was 12 and 14, in 2013 and 2014, respectively (Table 4). The total N concentration in the pooled digestate was 1.1 kg Mg^{-1} (fresh weight) in both years, and the NH_4^+-N concentration of total N was 25% in 2013 and 16% in 2014.

The aim of the study was to measure the effect of redistributing the entire residual and green-manure biomass resource, and thus the total amount of biomass or digestate was divided in specific ratios to the non-legume crops in BR and AD, respectively. The

Table 3. Total N content in the residual and green-manure biomass from the previous year, redistributed to rye, cabbage and red beet in the BR and AD treatments and applied *in situ* at harvest in the IS treatment (kg ha^{-1}).

	2013				2014			
Crop	**IS biomass**	**BR biomass**	**AD biomass**	**AD digestate**	**IS biomass**	**BR biomass**	**AD biomass**	**AD digestate**
Cabbage	70	130	–	140	220	260	–	180
Buckwheat/oilseed radish	55	0	–	0	35	0	–	0
Lentil/oat	60	0	–	0	60	0	–	0
Ryegrass/clover	80	0	–	0	35	0	–	0
Red beet	20	90	–	90	110	150	–	70
Rye	60	248	–	160	20	100	–	130
Buckwheat/lacy phacelia	35	0	–	0	30	0	–	0
Pea/barley	15	0	–	0	20	0	–	0
Ley	90	0	–	0	50	0	–	0
Total N in biomass[1]	485	465	455	390	580	510	480	380

[1] Refers to yield from 6 ha.

Table 4. Composition of digestate produced from residual and green-manure biomass in the studied cropping system in 2013 (from anaerobic digestion of biomass resources harvested in 2012) and 2014 (from anaerobic digestion of biomass resources harvested in 2013).

	2013		2014	
Digestate characteristics	**Liquid**	**Solid**	**Liquid**	**Solid**
pH	7.4	–	7.2	–
Amount (kg)	2110	449	1800	585
C/N	3.83	16.2	3.90	16.7
NH_4^+-N (kg Mg^{-1})	0.26 (0.03)	0.31 (0.01)	0.15 (0.10)	0.27 (0.03)
Total N (kg Mg^{-1})	0.42 (0.16)	4.22 (0.49)	0.30 (0.00)	3.86 (0.25)
P (kg Mg^{-1})	0.01 (0.00)	0.65 (0.18)	0.01 (0.00)	0.40 (0.13)
K (kg Mg^{-1})	1.35 (0.07)	1.90 (0.42)	1.20 (0.00)	1.40 (0.42)

Standard deviation of 2–3 samples is presented within brackets. Data are based on fresh weight analyses.

application rate to the different crops (Table 3) was based on a discussion with advisors in organic farming. There was a delay in N analysis of some crops, which made it necessary to make estimates of concentration of N in the BR silage based on the previous year, with the aim of providing the same ratio in total N supply in both BR and AD. The solid phase of the digestate was mixed on a tarpaulin and weighed to achieve the right amount per crop according to defined proportions. The liquid phase was carefully stirred and then measured by volume in watering cans, according to the same proportions as the solid fraction, adding liquid on top of the distributed solid digestate. In the red beet and cabbage plots, applied digestate was incorporated into the soil by non-inversion tillage machinery before planting and sowing. The plants of winter rye had grown too tall to incorporate the digestate with machinery, and it was therefore banded on the soil surface between the rows.

Sampling and harvest

Immediately before crop harvest, samples for analyses of yield and crop quality were obtained by sampling subplots in each main crop. The samples of cereals, legumes and grasses were harvested from an area of 0.25 m^2 at a position approximately 1 m from the northern side of each plot. The crops were cut 5 cm above the soil surface and divided in legumes and non-legumes before drying and milling. Samples were dried at 70°C for 24–72 h, depending on water content. The grain legumes and cereal grains were hand-separated from straw. The red beet was sampled by harvesting all the plants from 2 m in a centrally located row, followed by separation of beet roots from leaves by hand. The beet roots were rinsed with water and allowed to dry in room temperature for 30 min before being counted and weighed. A subsample consisting of two small, two large and one medium beet root, each cut in half (discarding one-half of each beet), was dried and milled. This sampling method was chosen to get a representative nutrient subsample from the core to the skin from beets of different sizes. Four adjacent cabbages in a central row were harvested for analysis of the weight of the residue and edible fraction. The edible fraction was defined as a tight smooth head, and the rest of the plant was defined as residue. A 1-cm thick slice was cut all the way into the core as a subsample from all four heads. The sample was weighed, dried and milled.

The crops and biomass resources used for digestion and redistribution were harvested on the entire area of each plot (after subsampling for analyses, as described above) with methods that mimicked commercial farming practices as far as possible. Ley and cover crops were cut with a large-scale lawn mower and the harvest from each plot was collected and weighed in bags. Grain legume/cereal intercrops were harvested with a Sampo Rosenlew plot combine harvester with a bag collecting the straw from each plot for weighing. Red beet leaves and cabbage residues (the outermost layer of leaves) were separated from the beets and heads, and weighed in the field prior to ensiling the residues. The biomass in the BR and AD treatments was collected in separate 1-m^3 plastic containers, where it was compressed and covered with a tarpaulin and four 15-kg sandbags. The first biomass was collected in May and the last in October. The cuttings from the May harvest of the green-manure ley and ryegrass/clover cover crop were ensiled, and also digested in AD, in preparation for application in the next growing season (Fig. 3).

The green-manure ley was harvested once in August in 2012, as it was established the same spring, and the yield was expected to be low compared with if the ley is established the previous year by undersowing in a main crop. The second harvest was in May 2013 before tilling and establishing the next crop. The green-manure ley undersown in pea/barley in 2012 was harvested at three consecutive occasions in 2013: in June, July and September, with an additional harvest occasion in May 2014 before soil tilling. Similarly, the green-manure ley undersown to pea/barley in 2013 was harvested at three occasions in 2014 (June, August and September) plus a fourth occasion in May 2015. The grain legumes and cereals were harvested when they were mature, while the harvest of cabbage and red beet was based on optimal timing for yield and quality, but also so that there was sufficient time for establishment and growth of cover and winter crops before the onset of winter. All biomass resources were weighed (total fresh weight per plot) before ensiling, and subsamples were used for analyses of dry matter concentration.

Calculations and statistics

The effect of the different biomass-management systems was measured in terms of yield (food fraction and straw/residual leaves), with the intercrops separated into legumes and non-legumes. Nitrogen concentration in the edible fraction of the crops was measured as a quality parameter, using an elemental analyzer (PDZ Europe ANCA-GSL for the intercrops and Flash 2000, Thermo Scientific for rye, cabbage and red beet). The data were analyzed with a general linear model and Tukey's *post hoc* analysis at a 5% significance level using the software Minitab 16.

Results

Yield and N concentration of rye, cabbage and red beet

The yield of the edible fraction of rye, cabbage and red beet neither show any statistically significant difference in yield between treatments (Table 5), nor did the treatments result in different concentrations of N in the edible fraction of rye, cabbage and red beet (Table 6) or yield of biomass residue (Table 7).

Yield and N concentration of the intercrops lentil/oat and pea/barley

The lentil grain yield was significantly lower in IS compared with BR in 2013 (Table 5). Data are not available for the grain yield of pea and barley intercrop in 2013, since the crop was severely damaged by rabbits and hares that year. The biomass treatments did not result in any significant difference in the N concentration of grain legume or cereal seeds. The IS treatment resulted in significantly higher yields of oat straw in both years (Table 7).

Yield of cover crops and green-manure ley

The yield of buckwheat/lacy phacelia (grown after rye) was significantly higher in BR compared with IS and AD in both years (Table 7). The redistributed biomass (BR and AD) had no carry-over effect on the other cover crops. The clover proportion of the ryegrass/clover cover crop was exceptionally low in general for all the treatments at harvest in 2013. The legume proportion of the green-manure ley was significantly higher in the BR and AD treatments compared with IS in 2014.

Discussion

As compared with the IS treatment, removal of biomass (AD and BR) resulted in a shift in legume/non-legume proportions in several of the crop mixtures, i.e., higher lentil grain yield in 2013, lower oat straw biomass in both years and higher legume yields in the green-manure ley in 2014. This shift is most likely a result of the removal of N-rich biomass in treatments BR and AD compared with IS, leading to reduced N availability and thereby a lower competitive ability of the oat in the intercrop and

Table 5. Yield of edible fraction (dry weight), presented as average ± standard error of the mean ($n = 4$).

	Yield (Mg ha^{-1})					
	2013			2014		
Crop	**IS**	**BR**	**AD**	**IS**	**BR**	**AD**
Rye	$5.10^{a} \pm 0.55$	$5.54^{a} \pm 0.96$	$6.07^{a} \pm 0.62$	$4.94^{a} \pm 0.35$	$5.36^{a} \pm 0.63$	$4.38^{a} \pm 0.51$
Cabbage	$3.38^{a} \pm 0.55$	$2.60^{a} \pm 0.53$	$3.02^{a} \pm 0.21$	$2.76^{a} \pm 0.48$	$3.22^{a} \pm 0.51$	$3.70^{a} \pm 0.73$
Red beet	$1.89^{a} \pm 0.76$	$1.41^{a} \pm 0.58$	$2.42^{a} \pm 0.55$	$2.54^{a} \pm 0.21$	$2.83^{a} \pm 0.44$	$2.45^{a} \pm 0.40$
Lentil/oat IC	$2.86^{a} \pm 0.45$	$3.36^{a} \pm 0.46$	$2.92^{a} \pm 0.53$	$2.12^{a} \pm 0.66$	$2.56^{a} \pm 0.51$	$2.22^{a} \pm 0.36$
Lentil	$\mathbf{0.46^{b} \pm 0.08}$	$\mathbf{0.88^{a} \pm 0.13}$	$\mathbf{0.81^{ab} \pm 0.06}$	$0.34^{a} \pm 0.08$	$0.35^{a} \pm 0.10$	$0.28^{a} \pm 0.06$
Oat	$2.40^{a} \pm 0.52$	$2.48^{a} \pm 0.52$	$2.12^{a} \pm 0.50$	$1.78^{a} \pm 0.72$	$2.21^{a} \pm 0.58$	$1.94^{a} \pm 0.35$
Pea/barley IC	NA	NA	NA	$2.00^{a} \pm 0.69$	$2.82^{a} \pm 0.17$	$1.38^{a} \pm 0.53$
Pea	NA	NA	NA	$0.99^{a} \pm 0.26$	$1.25^{a} \pm 0.14$	$0.94^{a} \pm 0.35$
Barley	NA	NA	NA	$1.01^{a} \pm 0.52$	$1.57^{a} \pm 0.29$	$0.44^{a} \pm 0.22$

IS, *in situ* incorporation; BR, biomass redistributed to the non-leguminous crops; AD, digested biomass distributed to the non-leguminous crops; NA, data not available.
Intercrops are shown both as total and separate as IC component yields. Means that do not share a letter within a row and year are significantly different. Bold indicates year and crop with significant effect of biomass treatment.

Table 6. Nitrogen concentration (%) of the edible fraction of the crops, presented as average ± standard error of the mean ($n = 4$).

	Nitrogen concentration (%)					
	2013			2014		
Crop	**IS**	**BR**	**AD**	**IS**	**BR**	**AD**
Rye	1.45 ± 0.09	1.55 ± 0.07	1.46 ± 0.06	0.86 ± 0.05	0.91 ± 0.05	0.92 ± 0.04
Cabbage	1.35 ± 0.10	1.72 ± 0.16	1.48 ± 0.05	2.13 ± 0.51	1.87 ± 0.33	1.66 ± 0.16
Red beet	2.50 ± 0.27	2.26 ± 0.13	2.16 ± 0.22	1.65 ± 0.15	1.42 ± 0.11	1.68 ± 0.16
Lentil IC	4.54 ± 0.10	4.14 ± 0.09	4.35 ± 0.12	4.10 ± 0.05	4.29 ± 0.09	4.16 ± 0.09
Oat IC	1.95 ± 0.05	1.82 ± 0.15	1.68 ± 0.10	2.18 ± 0.06	2.11 ± 0.07	2.13 ± 0.10
Pea IC	3.46 ± 0.03	3.64 ± 0.16	3.63 ± 0.13	3.28 ± 0.14	3.17 ± 0.22	3.17 ± 0.21
Barley IC	1.73 ± 0.18	1.72 ± 0.07	1.64 ± 0.09	1.77 ± 0.27	1.72 ± 0.21	1.91 ± 0.26

IS, *in situ* incorporation; BR, biomass redistributed to the non-leguminous crops grown in pure stand; AD, digested biomass distributed to the non-leguminous crops grown in pure stand.

the grasses in the ley. Our results thereby confirm previous findings about the effect of N availability on legume/non-legume proportions in crop mixtures (Ledgard and Steele, 1992; Jensen, 1996; Hejcman et al., 2010).

We did not observe a significant effect of the biomass management on yields of rye, cabbage and red beet or the other crops, indicating that biomass removal and extraction of CH_4 could be performed without a decrease in yields or N concentration of the food crops. However, the hypotheses that redistributing the digestate from anaerobic digestion of the biomass resources would have a positive effect on crop yields and crop N concentration had to be rejected. It is possible that the higher N availability in the digestate also led to higher losses of N through NH_3 volatilization during handling and after field application of the digestate (pH value of digestate in this study: 7.2–7.4). In particular, it is likely that NH_3 losses occurred in winter rye in the AD treatment, as it was not possible to inject the digestate into the soil or till after application (Möller and Stinner, 2009; Möller and Müller, 2012), due to the advanced growth stage of the established crop. There may also have been losses of NH_4^+ via seepage from silage and when the silage was mixed prior to digestion. Potential N losses in the AD system might thus have counteracted the expected benefits of a higher N availability for crop N acquisition.

Our third hypothesis was supported by the result that the buckwheat/lacy phacelia following winter rye produced higher biomass yields in the BR than in the IS treatment, which could be explained by the higher addition of biomass N to the preceding main crop in BR than in IS. The fact that the corresponding yield effect was not observed in the main crop (rye) receiving the biomass N in BR indicates that the mineralization was delayed, and not in synchrony with the requirements of the main

Table 7. Yield (dry weight) from crop residues, green-manure ley and cover crops presented as average ± standard error of the mean (*n* = 4, * = *n* = 3).

	Yield (Mg ha^{-1})					
	2013			2014		
Crop	**IS**	**BR**	**AD**	**IS**	**BR**	**AD**
Rye	$5.03^{a} \pm 0.53$	$5.32^{a} \pm 0.71$	$5.80^{a} \pm 0.22$	$7.76^{a} \pm 0.64$	$7.95^{a} \pm 0.66$	$6.75^{a} \pm 0.56$
Cabbage	$2.31^{a} \pm 0.27$	$2.16^{a} \pm 0.23$	$3.03^{a} \pm 0.26$	$1.54^{a} \pm 0.20$	$1.71^{a} \pm 0.22$	$1.82^{a} \pm 0.19$
Red beet	$0.65^{a} \pm 0.29$	$0.71^{a} \pm 0.24$	$0.78^{a} \pm 0.10$	$1.23^{a} \pm 0.09$	$1.30^{a} \pm 0.20$	$1.12^{a} \pm 0.11$
Buckwheat/oilseed radish	$1.97^{a} \pm 0.16$	$2.05^{a} \pm 0.10$	$2.03^{a} \pm 0.25$	$1.50^{a} \pm 0.13$	$1.56^{a} \pm 0.08$	$1.55^{a} \pm 0.18$
Buckwheat/lacy phacelia	**$0.67^{b} \pm 0.18$**	**$1.96^{a} \pm 0.32$**	**$0.92^{b} \pm 0.10$**	**$1.18^{b} \pm 0.10$**	**$2.15^{a} \pm 0.08$**	**$1.26^{b} \pm 0.12$**
Lentil/oat IC	$4.97^{a} \pm 0.31$	$4.74^{a} \pm 0.30$	$4.78^{a} \pm 0.42$	$3.93^{a} \pm 0.13$	$3.47^{a} \pm 0.25$	$3.55^{a} \pm 0.26$
Lentil	*$0.92^{a} \pm 0.11$*	*$1.46^{a} \pm 0.36$*	*$1.29^{a} \pm 0.33$*	*$0.56^{a} \pm 0.16$*	*$0.60^{a} \pm 0.09$*	*$0.55^{a} \pm 0.14$*
Oat	***$4.05^{a} \pm 0.28$***	***$3.28^{b} \pm 0.10$***	***$3.49^{b} \pm 0.28$***	***$3.36^{a} \pm 0.18$***	***$2.88^{b} \pm 0.31$***	***$2.93^{b} \pm 0.22$***
Pea/barley IC	$4.61^{a} \pm 0.15$	$4.26^{a} \pm 0.45$	$4.16^{a} \pm 0.43$	$5.26^{a} \pm 0.54$	$4.94^{a} \pm 0.36$	$4.47^{a} \pm 0.39$
Pea	*$1.77^{a} \pm 0.25$*	*$1.71^{a} \pm 0.17$*	*$1.49^{a} \pm 0.17$*	*$2.47^{a} \pm 0.33$*	*$1.82^{a} \pm 0.17$*	*$2.37^{a} \pm 0.33$*
Barley	*$2.84^{a} \pm 0.28$*	*$2.54^{a} \pm 0.48$*	*$2.67^{a} \pm 0.32$*	*$2.79^{a} \pm 0.29$*	*$3.12^{a} \pm 0.28$*	*$2.10^{a} \pm 0.29$*
Green-manure ley	$12.1^{a} \pm 3.30$*	$12.7^{a} \pm 0.81$*	$12.7^{a} \pm 0.91$*	$17.5^{a} \pm 0.75$	$20.9^{a} \pm 1.72$	$18.3^{a} \pm 0.98$
Ley—legume	*$0.88^{a} \pm 0.25$*	*$3.07^{a} \pm 0.67$*	*$2.23^{a} \pm 0.59$*	***$3.01^{b} \pm 0.68$***	***$6.94^{a} \pm 0.99$***	***$6.78^{a} \pm 0.94$***
Ley—non-legume	*$11.3^{a} \pm 3.75$*	*$9.63^{a} \pm 1.46$*	*$10.4^{a} \pm 0.79$*	*$14.5^{a} \pm 1.16$*	*$13.9^{a} \pm 0.86$*	*$12.2^{a} \pm 0.80$*
Ryegrass/clover	$0.55^{a} \pm 0.12$	$0.74^{a} \pm 0.07$	$0.73^{a} \pm 0.08$	$4.12^{a} \pm 0.21$	$4.22^{a} \pm 0.40$	$5.00^{a} \pm 0.61$
Ryegrass	*$0.55^{a} \pm 0.12$*	*$0.73^{a} \pm 0.07$*	*$0.73^{a} \pm 0.08$*	*$3.22^{a} \pm 0.24$*	*$3.05^{a} \pm 0.60$*	*$3.26^{a} \pm 0.56$*
Clover	*$0.00^{a} \pm 0.00$*	*$0.01^{a} \pm 0.00$*	*$0.00^{a} \pm 0.00$*	*$0.89^{a} \pm 0.23$*	*$1.17^{a} \pm 0.38$*	*$1.74^{a} \pm 0.25$*
	Sum of biomass (Mg 6 ha^{-1})					
	32.9	34.6	34.9	44.0	48.1	43.8

IS, *in situ* incorporation; BR, biomass redistributed to the non-leguminous crops grown in pure stand; AD, digested biomass distributed to the non-leguminous crops grown in pure stand.
Italic numbers represent fractions in intercrops (IC) and species mixtures (green-manure ley and ryegrass/clover). Means that do not share a letter within the same row and year are significantly different. Bold indicates year and crop with significant effect of biomass treatment. The sum of biomass presented at the bottom of the table represent the total amount of biomass resources that would be available if all main crops and associated cover crops were cultivated on 1 ha each.

crop, but leading to an increased N availability for the subsequent cover crop. Moreover, the lack of a corresponding increase of the same cover crop biomass yield in the AD treatment, even in 2014 when rye in AD received more N than rye in BR (Table 3), implies that the N dynamics differ if the residual biomass is applied as silage or digestate. As discussed above, potentially higher NH_3 emissions after field application of the digestate (Wulf et al., 2002) compared with silage may explain the lack of yield increase of cover crops in AD.

Since this study was based on recycling, all biomass resources obtained within the cropping system inclusion of N-poor biomasses such as cereal straw contributed to a relatively low total N concentration in the digestate. The digestate also contained water added during the digestion process, which diluted the nutrient concentration expressed on a fresh weight basis (1.1 kg N Mg^{-1}). The C/N ratio (12–14) of our digestate was within the range (7–39) of other plant-based digestate (Möller et al., 2008; Gunnarsson et al., 2010; Gunnarsson et al., 2011; Frøseth et al., 2014). The NH_4^+-N proportion of total N (16–25%) and concentration of NH_4^+-N (0.18–0.27 kg NH_4^+-N Mg^{-1} fresh weight) in our digestate were also within, but at the lower end of the range of plant-based digestate from other studies (6–55% NH_4^+-N of total N; 0.18–1.52 kg NH_4^+-N Mg^{-1} fresh weight) (Möller et al., 2008; Gunnarsson et al., 2010; Gunnarsson et al., 2011). The relatively low concentration of NH_4^+-N in the digestate in our study indicates that the digestion of the biomass has not been efficient, which might in turn lead to a slow mineralization of the organic N in the soil. The chemical composition of the digestate depends both on the composition and pre-treatment of the feedstock, and the lack of pre-treatment (e.g., shredding) might also have contributed to a low concentration of NH_4^+-N in the digestate.

The biomass treatments did not result in different N concentrations in the food fraction of cereals or legumes. There was a trend of normal to high N concentrations in oat in this study, as compared with the mean concentration for the variety when it is grown in similar climate (Hagman et al., 2014). The barley grain N concentration was on average in line with the critical optimum for desirable malting quality, i.e., <1.84% N (Bertholdsson, 1999). The mean N concentration of the winter rye variety 'Amilo' in variety tests (Hagman et al., 2014) is similar to results from our experiment in 2013, while all treatments resulted in lower N concentrations in 2014, indicating suboptimal N supply for rye in the second year of the experiment.

Strategic biomass management (BR and AD) maintained levels of food crop yields, with increased biomass production potential of cover crops and an increase in legume proportions in intercrops, green-manure and ryegrass/clover leys. An increased proportion of legume biomass in the green-manure ley is correlated with increased N inputs via N_2 fixation (Evans et al., 1989; Carlsson and Huss-Danell, 2003), which leads to a reduced need for external input of N to cover requirements of the following crop. This is essential in stockless organic agriculture as there are few economically viable options to supply N, when there is no access to animal manure.

The possibility of using AD as a treatment of residual and green-manure biomass without losses in yield and quality provides the opportunity of producing bioenergy as an additional source of energy or income for the farmer. Tuomisto and Helenius (2008) even argue that a slightly lower crop yield in a bioenergy scenario would be beneficial for the systems energy balance compared with leaving the biomass *in situ*.

Conclusions

Our results show that food, biomass for bioenergy carriers and digestate can be produced within the same cropping system without reductions in yield and N concentration of the food crops, relative to standard organic farming practices, e.g., green manuring and crop residue incorporation. Maintenance of food crop yields and increased biomass yields, as was found for one of the cover crops, show that strategic redistribution of residual biomass resources has a potential for increasing the overall system productivity and opens up for additional biomass uses in synergy with on-farm nutrient recirculation. The allocation of biomass resources for the additional production of CH_4 without yield losses in the AD treatment enhances on-farm self-sufficiency and potentially also farm profitability depending on the energy pricing.

Our results indicate that the anaerobic digestion of biomass resources and field applications of the digestate might be associated with larger N losses than the biomass management in BR and IS. More detailed studies of N losses at each step of the management of biomass resources and digestate as well as at the entire cropping system level are therefore important in order to develop N-efficient cropping systems that provide bioenergy extraction in synergy with food production. An analysis of nutrient balances, energy and economics is also required to gain more knowledge for further developments of biomass resource-management systems for enhanced farm sustainability.

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II

More efficient use of nitrogen by internal recycling of residual biomass within a stockless organic cropping system?

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Abstract

A major future challenge in agricultural systems is to reduce the requirement for inputs of new reactive nitrogen in cropping systems. We have investigated if strategic management of internal biomass N resources (green manure ley, crop residues and cover crops) in a six-year organic crop rotation could maintain soil N balance without creating a large N surplus. Three biomass management strategies were compared; anaerobic digestion of the biomass silage and application of the digestate to the non-legume crops (AD), biomass redistribution as silage to non-legume crops (BR), and leaving the biomass *in situ* (IS). Neither aboveground crop N accumulation from soil and nor the proportion of N derived from N_2 fixation in legumes were influenced by biomass management treatment. On the other hand, the allocation of N-rich silage and digestate to non-legume crops resulted in higher N_2 fixation in AD and BR (57 and 58 kg ha^{-1} year^{-1}), compared to IS (33 kg ha^{-1} year^{-1}) in 2014. The N balance for 2013-2014 ranged between -9.9 and 24 kg N ha^{-1}, with more positive numbers in AD and BR than in IS, when the temporary removal of biomass in AD and BR was not considered. The storage of biomass for reallocation in spring led to an increasing accumulation of N in BR and AD over the years, at the same time as it provides an opportunity to supply the crop with the nutrient when most needed and thereby potentially decreases the risk of N losses during winter.

Keywords: Anaerobic digestion, arable and horticultural crops, nitrogen balance, nitrogen fixation, soil and residue nitrogen, strategic biomass management

Abbreviations

AD = anaerobic digestion

BNF = biological nitrogen fixation

BR = biomass redistribution

IS = in situ

%Ndfa= proportion (%) of accumulated nitrogen derived from nitrogen fixation

Introduction

The planetary boundary research (Steffen et al. 2015) highlight the importance of reducing global inputs of new reactive nitrogen (N) to ecosystems. The amounts of N applied as fertilizer in agriculture have not been sufficiently constrained to prevent widespread leakage to freshwaters and the atmosphere, with effects on human health, biodiversity and climate (Fowler et al. 2013). It is challenging to balance N inputs to ensure long-term soil fertility with high and stable yields, avoiding depletion of the soil N pool and at the same time avoid a surplus that have negative impacts on the surrounding ecosystem (Colomb et al. 2013; de Ponti 2012; Seufert and Ramankutty 2017). Farmers of a region often specialize in either crop or animal production, which adds serious costs of transporting animal manure over long distances (Baggs et al. 2000; Stinner et al. 2008). Thus, many arable and horticultural organic farms choose to import a considerable amount of concentrated fertiliser made from by-products of the food industry (Colomb et al. 2013; Watson et al. 2002; Wivstad 2009). To reduce the need for external fertiliser inputs, researchers suggest strategies that could improve soil formation and internal nutrient cycling at the farm level (Bommarco et al. 2013; Foley et al. 2011; Tilman et al. 2002). The basic N input in organic farming systems to maintain soil N fertility is biological N_2 fixation by legume crops (Foyer et al. 2016). Grain legumes can fix substantial amounts of atmospheric N_2, which also reduces the requirement for applying N to subsequent crops and improve soil fertility through inputs of legume residues and rhizodeposition. However, a large proportion of the fixed N is removed with the grain (Crews and Peoples 2004; Jensen and Hauggaard-Nielsen 2003; Li et al. 2015). Thus, grain legumes grown as sole or intercrops with cereals are not supplying as much N to the agroecosystem, as cover crops and green manure ley with forage legumes (Jensen 1997). Legume cover crops and forage legumes improves N supply from soil substantially after incorporation of their residues, containing symbiotically fixed N, and are thus very important in the organic farming system without livestock, where other options for N input are limited. Incorporating residual biomass (crop residue, cover crop, etc.) *in situ* is a common practice in agriculture, but it may result in substantial losses of N, if mineralisation and acquisition of the following crop is not well synchronized (Mohanty et al. 2013; Möller 2008; Pang and Letey 1998). It may be possible to improve the synchrony between application of crop biomass N and plant acquisition of N by pre-treating and storing the residual biomass as silage or as digestate from anaerobic digestion (Frøseth et al. 2014; Gunnarsson et al. 2011; Gutser et al. 2005).

Calculation of N balance is a tool for expanding the understanding of the N cycle and evaluate the effect of different management practices on the soil-crop N cycle and the sustainability of N management methods (Watson et al. 2002). A N balance summarizes the complex agricultural N cycle by documenting the major flow paths as N enters and emerges from various pools and leaves the system for various fates (Meisinger et al. 2008). Calculating the N balance is also a valuable tool for identifying risks of N depletion or build-up of N surplus at the crop, cropping system or farm level, thereby highlighting the potential need for improved N management. A nitrogen balance made for 76 organic arable farms in Sweden showed an average N surplus of 39 kg/ha. The surplus was mainly due to imported nutrients such as digestate, yeast liquid and dried slaughter house waste (Wivstad 2009). Horticultural cropping systems tend to import even more N than arable farms, which results in an N budget with higher N surpluses (Watson et al. 2002), and is thus prone to a higher risk of N losses. Comparing N balances of different cropping systems may also indicate possibilities to decrease the input of new reactive N into agroecosystems (Galloway et al. 2008).

The aim of this study was to determine whether anaerobic digestion (AD) of the residual biomass from the cropping system, and use of the digestate for N recirculation, would improve the N acquisition in the following crop, compared to the corresponding biomass redistribution (BR) of undigested silage or just leaving the biomass *in situ* (IS) within the respective field plots. Several arable and horticultural crops were combined in the cropping systems, with the purpose to study how the soil N accumulation and N_2 fixation of the different crops respond to the biomass management strategies. We used the N balance method as a tool to determine how biomass strategy influenced the loss or increase in soil N at both individual crop and at the cropping system level, but without considering N emissions to the environment in the calculation. The hypotheses were: I) the amount and proportion of N_2 fixed in legume crops (legumes in ley, lentil (*Lens culinaris* Medik), pea (*Pisum sativum* L.), clover (*Trifolium pratense* L. & *T. repens* L.) in cover crop) is greater with AD and BR than in the IS management, II) N acquisition from soil and residual biomass in non-legume crops is greater in AD than BR and IS, III) the N balance at the cropping system level rank IS<BR<AD, and IV) the total N acquisition originating from soil and added biomass in all crops is on average larger in AD and BR than in IS.

Material & methods

2.1. Study site and soil

The experiment was established in 2012 at the Swedish University of Agricultural Sciences in Alnarp, southern Sweden (55° 39′ 21″N, 13° 03′ 30″E), on a sandy loam soil of Arenosol type (Deckers et al. 1998). The field experiment was conducted on organically certified agricultural land within the SITES Lönnstorp field research station, with grass-clover ley as the pre-crop. The annual mean atmospheric deposition of N contributed with a total of 9.4 kg ha^{-1} $year^{-1}$ during 2013 to 2014, in the region where the field experiment was situated (SMHI 2013-2014).

2.2. Climatic data

The region has a typical northern-European maritime climate with mild winter and summer temperatures. The temperature and precipitation in 2012–2015 were close to the average for the region (1961-1990) (Råberg et al. 2017).

2.3. Crop rotation

A six-year crop rotation including different legume species, several over-wintering cash and cover crops was studied during three years (2012-2014, with two overwintering crops harvested in May 2015). The rotation consisted of the following food crops: pea/barley (*Pisum sativum* L./*Hordeum vulgare* L.) and lentil/oat (*Lens culinaris* Medik/*Avena sativa* L.), white cabbage (*Brassica oleracea* L.), beetroot (*Beta vulgaris* L.) and winter rye (*Secale cereale* L.). There was a green manure ley composed of *Dactylis glomerata* L., *Festuca pratensis* L., *Phleum pratense* L., *Medicago sativa* L., *Meliolotus officinalis* L. and *Trifolium pratense* L. (Råberg *et* al. 2017). The ley was under-sown in the pea/barley intercrop, harvested three times during the year after establishment, and harvested again in early spring the subsequent year, before establishing white cabbage as the next crop. Cover crops were included in the rotation after white cabbage (buckwheat, *Fagopyrum esculentum* Moench/oilseed radish, *Raphanum sativus* L.), rye (buckwheat/lacy phacelia, *Phacelia tanacetifolia* Benth.) and under-sown in lentil/oat (ryegrass, *Lolium perenne* L. / red clover, *Trifolium pratense* L. /white clover *T. repens* L.). All six main crops in the rotation were grown in separate plots during each year of the experiment. Winter rye was replaced by spring barley during the first year, since the experiment started in spring 2012 without any autumn-sown crop from the previous year.

2.4. Field management

The soil was managed with non-inverting tillage equipment after the ley pre-crop was incorporated with a conventional inverting plow before the rotation was established. The experimental area received an initial supply of 115 kg N ha^{-1} through import of plant-based digestate applied with trailing hose in spring 2012. Crop protection followed the national organic regulations.

2.5. Experimental design

The field experiment comprised in total 72 experimental plots measuring 3×6 m, distributed in four replicate blocks. The reference biomass management treatment was a system where the residual biomass (crop residues, cover crops and ley cuttings) was incorporated fresh *in situ* (IS) in the experimental plot (Figure 1).

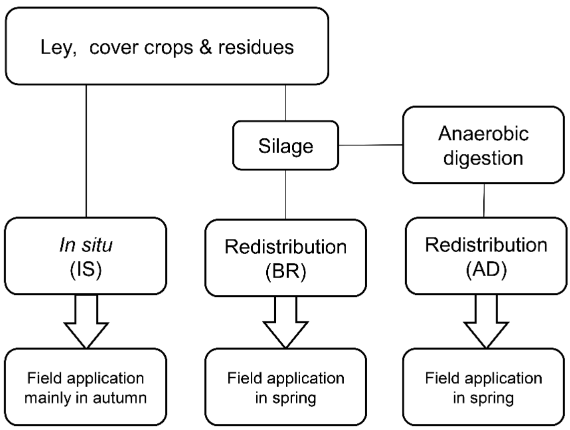


Figure 1. Residue management in the IS, BR and AD treatments. The residual biomass in IS was applied fresh, in BR it was ensiled prior to field application and in AD it was ensiled and anaerobically digested as a pre-treatment.

Two additional biomass management treatments were: 1) ensiling and redistributing the biomass resources (BR) to experimental plots with cabbage, winter rye and beetroot; and 2) the biomass was ensiled and later anaerobically digested (AD) in a biogas reactor, and the digestate applied to cabbage, winter rye and beetroot, as described in Råberg *et* al. (2017). The N supplied to the crops in each treatment are presented in Table 1.

Table 1. Total N content in the residual and green manure biomass from the previous year, redistributed to rye, cabbage and beetroot (on 3 x 1 ha) in the BR and AD treatments. Left *in situ* at harvest in the IS treatment (kg ha^{-1}).

Crop	2013				2014			
	IS biomass	BR biomass	AD biomass	AD digestate	IS biomass	BR biomass	AD biomass	AD digestate
Cabbage	70	130	-	140	220	260	-	180
Buckwheat/oilseed radish	55	0	-	0	35	0	-	0
Lentil/oat	60	0	-	0	60	0	-	0
Ryegrass/clover	80	0	-	0	35	0	-	0
Beetroot	15	90	-	90	110	150	-	70
Rye	60	240	-	160	20	100	-	130
Buckwheat/lacy phacelia	35	0	-	0	30	0	-	0
Pea/barley	15	0	-	0	20	0	-	0
Ley	90	0	-	0	50	0	-	0
Total N in biomass*	480	460	455	390	580	520	480	380

*Sum of N from 6 ha

2.6. Sampling and harvest

The residual biomass was collected and ensiled separately in BR and AD, with harvest from spring until October each year, to allow time for digestion in AD. The same strategy was used for the collection of biomass in BR to make it comparable to AD. The method resulted in a one-year delay for the use of the May harvest of green manure ley and ryegrass/clover in the BR and AD treatments. Measurements started in 2012 and the last samples were collected in 2015 for two over-wintering crops i.e. green manure ley and ryegrass/clover cover crop.

All above-ground residues, cover crops and ley cuttings were weighed before returning or redistributing the biomass, and subsamples from a 0.25 m^2 surface per plot were taken for analyses of botanical composition (grouped into legumes and non-legumes) dry matter (DM), N concentration and natural abundance of the stable isotope ^{15}N. The biomass yield and N concentration presented in the result section was based on the subsamples.

2.7. Calculations and statistics

The balance of N for the cropping sequences was calculated per crop and as an annual sum of each treatment for 2012-2014. The balance calculations used input data from N_2 fixation measured by the ^{15}N natural abundance method (Unkovich *et al.*, 2008), regional measurements of atmospheric N deposition, N content in seeds (estimation for lacy phacelia, oilseed radish, ryegrass and clover) and plants used for establishing cabbage (estimation), addition of N via residual ensiled (BR) and digested (AD) biomass from previous year's crops (Eq. 1, Figure 2). In cases when there was a cover crop grown after a main crop, the yearly atmospheric N deposition was divided and allocated equally to the main and cover crop in the N balance calculations. The additional supply of 115 kg N from imported digestate at the start of the experiment (2012) was also included in the calculations. The N outputs in the balance consisted of the amounts of N exported in the edible fractions of the food crops (all treatments) and N exported in residual biomass in AD and BR to be redistributed in the next growing season.

$$\text{N balance} = \text{bnf} + \text{dep} + \text{seed} + \text{biomass}_{\text{added}} - \text{food} - \text{biomass}_{\text{removed}} \qquad (\textit{Eq. 1})$$

bnf =biological N_2 fixation in current year

dep=atmospheric N deposition

seed=seed N and plantlet N

$\text{biomass}_{\text{added}}$=N from added residual biomass and cuttings from previous year

edible fraction=exported cash crop total N

$\text{biomass}_{\text{removed}}$= total N from cuttings and residual biomass removed to be circulated succeeding year

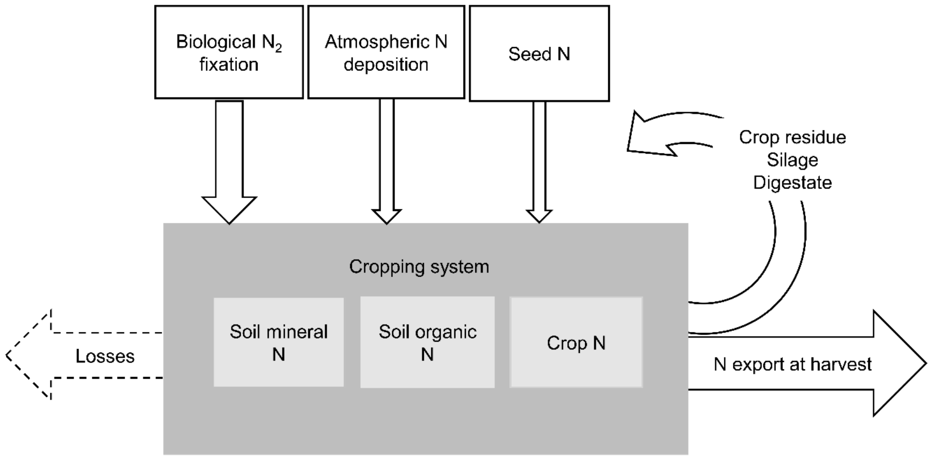


Figure 2. Input and output components of the N balance. The N coming in and leaving from the crop-soil system was quantified, except for the losses of nitrogen (ammonia volatilization, denitrification and N leaching) (dashed arrow).

Soil N acquisition, representing N from the soil N pool as well as from added residual biomass, was calculated by subtracting the amount of N_2 fixed from the total crop N content in the aboveground plant parts. The N_2 fixation was assessed according to the ^{15}N natural abundance method (Unkovich et al. 1997) using the lowest observed legume $\delta^{15}N$-value as β-value in equation 2, as recommended by *e.g.* Hansen and Vinther (2001) and (Huss-Danell et al. 2007). The β-value is defined as a measure of the ^{15}N content of the target legume ($\delta^{15}N_L$) when fully dependent on N_2 fixation for its N acquisition (Unkovich et al. 2008). In the present study, the samples used as β-value were also included in the calculations of the average N_2 fixation per treatment. The ^{15}N signature of the grasses and weeds grown together with the legumes in the green manure ley, intercrops and cover crops were used as reference plants ($\delta^{15}N_{ref}$).

$$\% N_{dfa} = \frac{(\delta^{15}N_{ref} - \delta^{15}N_L)}{\delta^{15}N_{ref} - \beta} \times 100, \qquad (Eq.\ 2)$$

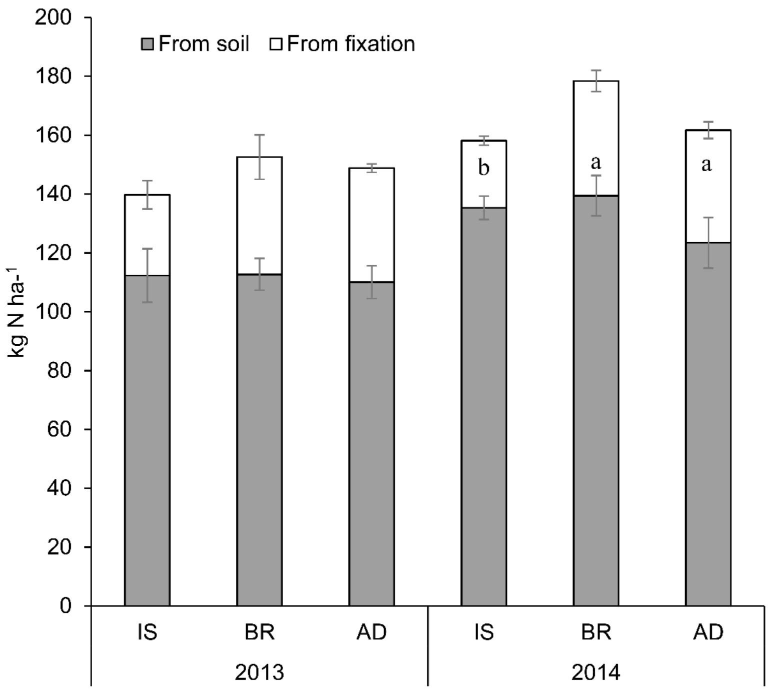
The total concentration of N and $^{15}N/^{14}N$ ratio was measured with an elemental analyzer coupled to an isotope ratio mass spectrometer (PDZ Europe 20-20, UC Davies in U.S.A) in legume-containing crops mixtures. A Flash 2000 Thermo Scientific elemental analyzer (at SLU, Alnarp, Sweden) was used for determination of N concentration in sole crops. Treatment effects were tested by analyses of variance using the general linear model (GLM) procedure in the Minitab software.

Results

Nitrogen acquisition

Total N accumulation in the aboveground parts of the crops ranged between 140 and 180 kg ha^{-1} year^{-1} (Figure 3), with no significant difference between the biomass strategies.

Figure 3. The total mean N content of the crop biomass from the entire cropping systems in 2013 and 2014 in kg ha^{-1}. Total N is presented as a sum of N acquired from the soil and through N_2 fixation. The letters show significant differences between treatments in N_2 fixation. The error bars presented as average ± standard error of the mean (N=4 except for ley with N=3 in 2013).



Nitrogen fixation

The total N_2 fixation in leguminous crops constituted 14-26 % of total N acquisition in the aboveground plant parts of the crops, which corresponded to an average of 23-40 kg ha^{-1} year^{-1} (Figure 3). The proportion of N derived from N_2 fixation in the legumes (%Ndfa) was found to be between 68 and 98%, but was not significantly different between biomass management treatments (Table 2). The amount of N_2 fixed was higher with BR and AD treatments, compared to IS (p=0.002). The effect was significant in 2014 (p=0.021) (Figure 3). A large part of the increased N_2 fixation was derived from the legumes of the green manure ley, with a significantly higher (p<0.001) N_2 fixation in BR and AD compared to IS in 2014 (Figure 4b). The amount of N_2 fixation in lentil and pea varied inconsistently between treatments in the two years. No significant difference between treatments was found for the amount of N_2 fixed in clover grown together with ryegrass in the cover crop, which ranged between 12 and 78 kg N ha^{-1} year^{-1} and was higher in 2013 than in 2014.

Table 2. The proportion of nitrogen acquired through N_2 fixation (%Ndfa) in legumes. IS = *In situ* incorporation. BR = biomass redistributed to the non-leguminous crops grown in pure stand. AD = digested biomass distributed to the non-leguminous crops grown in pure stand. Presented as average ± standard error of the mean (N=4, except for ley 2013 with N=3).

	Ndfa (%)					
	2013			**2014**		
Crops	**IS**	**BR**	**AD**	**IS**	**BR**	**AD**
Lentil	83±3.8	87±7.7	98±1.7	73±3.8	68±11	80±11
Clover	96±0.5	95±2.9	95±1.3	93±3.0	92±1.6	94±0.9
Pea	94±2.1	86±2.1	88±3.7	89±3.5	87±1.6	89±4.5
Green manure ley	74±8.3	85±3.3	83±3.6	76±2.1	81±2.2	81±1.2

N acquisition from soil

The total N accumulation from soil varied between 110 and 140 kg N ha^{-1} calculated as an average for the entire crop rotation, and the total accumulation was significantly higher (p=0.002) in 2014, compared to 2013 (Figure 3). Differences between the three biomass residue management methods were small and in most cases non-significant (Figures. 4a and 4b). The BR treatment led to significantly (P<0.001) higher soil N accumulation in the cover crop buckwheat/lacy phacelia in both years as compared to IS and AD treatments (Figure 4).

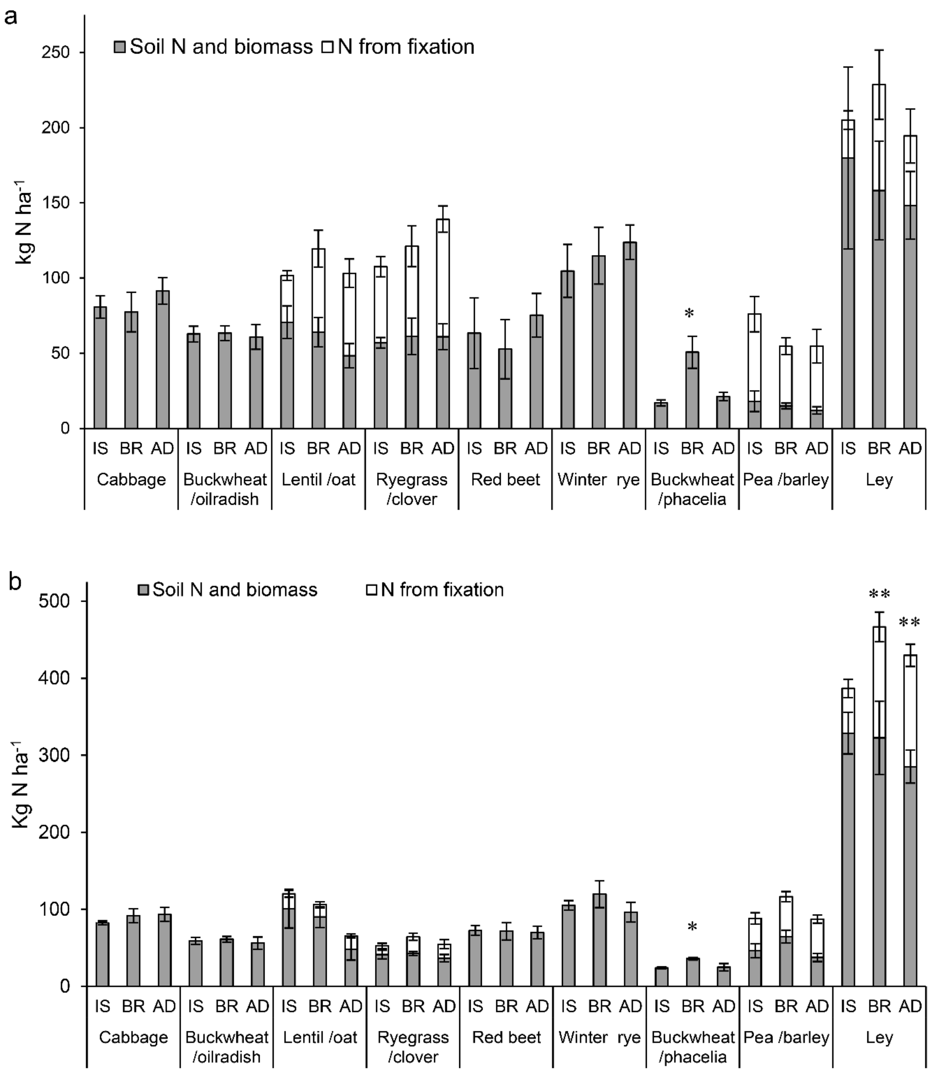


Figure 4. Nitrogen content of the crops (kg N ha^{-1}), presented as average ± standard error of the mean (N=4 except for ley with N=3 in 2013). The grey bars represent N acquisition from soil and residual crop biomass, and the white bars represent N_2 fixation of the crop. IS = *In situ* incorporation. BR = biomass redistributed to the non-leguminous crops grown in pure stand. AD = digested biomass distributed to the non-leguminous crops grown in pure stand. *=significance with 95% confidence interval refers to soil N uptake in phacelia and buckwheat. **=significance with 99% confidence interval refers to N_2 fixation in ley. (a) Nitrogen content of the aboveground part of individual crop in 2013, (b) Nitrogen content of the aboveground part of individual crop in 2014.

Nitrogen exported in the edible crop fraction

The mean (of five edible crops) N accumulation in the exported edible fractions varied between 49 to 60 kg ha^{-1}, with the highest amount exported in rye grain. The nitrogen content in the edible fraction was not affected by the three treatments (Table 3), even if the N supply differed substantially (Table 1).

Table 3. Nitrogen exported in edible fractions of crops (kg N ha^{-1}). IS= In situ incorporation, BR = biomass redistributed to the non-leguminous crops grown in pure stand, AD = digested biomass distributed to the non-leguminous crops grown in pure stand. Presented as average ± standard error of the mean (n = 4). The mean is calculated from 6 ha, even if ley is excluded in the sum, but never the less crucial for the production of edible produce in the cropping system.

Crop	Nitrogen export in edible fraction					
	2013			2014		
	IS	BR	AD	IS	BR	AD
Cabbage	44±5.1	44±8.9	45±4.6	52±2.9	56±5.8	58±6.6
Lentil /oat	67±8.1	81±8.3	70±9.2	53±14	62±9.7	52±7.6
Beetroot	41±15	31±13	51±12	42±4.4	40±6.5	39±4.7
Rye	75±12	84±15	90±12	63±4.0	75±12	60±7.5
Pea /barley	28±11	17±3.8	26±7.9	44±13	67±6.4	35±12
Sum (kg N from 5 ha)	256±19	257±21	282±18	254±9.0	299±23	246±24
Mean (kg N from 1 ha)	43±3.2	43±3.5	47±3.0	42±1.5	50±3.8	41±4.0

Nitrogen in residual crop biomass, green manure ley and cover crops

The total amount of N in crop residues, cover crops and ley cutting from six ha varied between 97 and 129 kg N ha^{-1} (Table 4), without any significant difference between the three treatments. In 2013 the ley cuttings constituted 36 to 40% of the total amount of N and in 2014 the part increased to between 49 and 54%. There was a significant interaction between treatment and year when the total N accumulation of all the crops from the three systems were compared (p=0.001,). The N accumulation from the whole cropping system was larger in IS compared to BR and AD in 2012 (p=0.009), since N in residual biomass in BR and AD was "exported" for redistribution in the next growing season without corresponding inputs during the initiation year of the experiment (Table 5). The N content of all the residual biomass increased in the three years that the experiment was running, regardless of the treatments. There was a significant ($p<0.001$) increase of residual biomass N, corresponding to an average difference of 19 kg N ha^{-1} between 2013 and 2014 (Table 4).

Table 4. Nitrogen in residual biomass and cuttings (kg N ha^{-1}). IS= In situ incorporation, BR = biomass redistributed to the non-leguminous crops grown in pure stand, AD = digested biomass distributed to the non-leguminous crops grown in pure stand. Superscript letters mark significant differences as well as bold numbers. Presented as average ± standard error of the mean (n = 4, ley n=3 in 2013)

Crop	N in residual biomass (kg ha^{-1})					
	2013			2014		
	IS	BR	AD	IS	BR	AD
Cabbage	36.3±3.29	33.7±4.36	46.3±4.50	30.2±2.14	35.3±5.33	35.2±3.02
Buckwheat/oilseed radish	62.9±5,20	63.3±4.82	60.8±8.17	58.8±4.54	61.2±3.69	56.1±8.01
Lentil /oat	34.2±2.70	38.2±4.06	33.3±6.51	66.7±9.49	44.4±6.59	35.2±10.7
Ryegrass/clover	108±4.34	121±9.82	139±10.4	52.4±5.99	64.3±6.46	54.7±3.65
Beetroot	22.0±9.24	21.7±7.10	24.7±3.67	31.0±2.39	31.6±4.87	30.4±4.81
Rye	29.8±5.77	31.0±3.59	33.6±1.84	42.0±3.40	45.1±5.69	35.9±5.69
Buckwheat/phacelia	**16.9^{b}±2.03**	**50.7^{a}±10.6**	**21.3^{b}±2.71**	**23.9^{b}±1.30**	**35.9^{a}±1.68**	**24.9^{b}±4.75**
Pea /barley	47.9±8.34	37.8±5.53	29.0±8.57	56.6±9.57	49.8±6.37	51.9±8.49
Ley	222±64.0	262±9.52	221±15.2	342±19.2	404±42.8	384±23.2
Sum (kg N from 6 ha)	584±41.8	659±33.1	609±10.8	704±20.8	774±48.7	708±33.9
Mean (kg N from 1 ha)	97±7.0	110±5.5	102±1.8	117±3.5	129±8.1	118±5.7

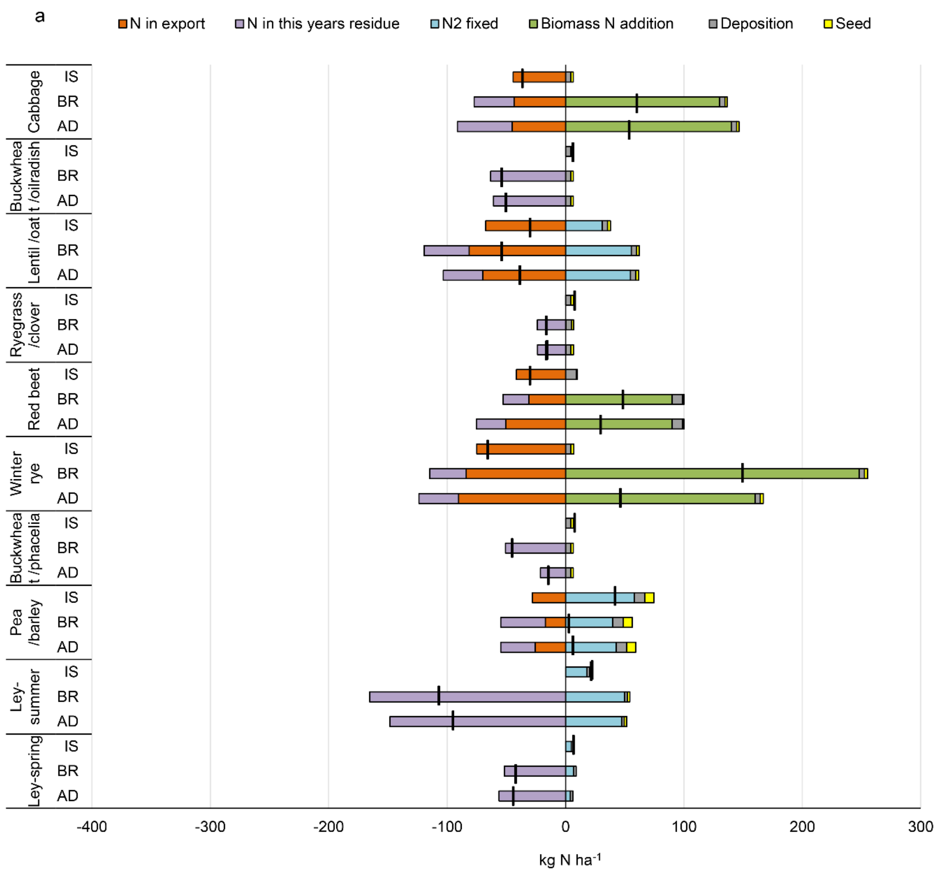
Table 5. Nitrogen balance calculated by taking into account the storage and redistribution of residual biomass as silage/digestate in the subsequent year in BR and AD (N balance), and without considering the temporary stored N in residual biomass (No stored residual biomass) (kg ha^{-1}).

Treatment	Year	N balance	No stored residual biomass
IS	2013	-9.9	-9.9
	2014	1.1	1.1
BR	2013	-12	-3.3
	2014	-43	7.8
AD	2013	-22	-7.9
	2014	-60	24

Nitrogen balance

The three crops that were fertilized with biomass in BR and AD resulted in N surplus for the N balance of both years, with the highest surplus in cabbage with the BR treatment in 2014 (178 kg ha^{-1}). The exception from the surplus results was the winter rye crop with BR treatment in 2014, which resulted in -8 kg ha^{-1} (fig. 5b). Cabbage, red beet and rye all had a negative N balance in IS, ranging from -36 to -68 kg ha^{-1}. The lentil/oat intercrop resulted in a negative result with all treatments, and most negative for AD and BR, from -37 to -79 kg ha^{-1}. The pea/barley intercrop resulted in a surplus of 21 to 47 kg ha^{-1} for IS (2014 and 2013 respectively), while the balance for BR and AD resulted in 5 to -47 kg ha^{-1}. The non-legume catch crops had a negative result for BR and AD, -15 to -57 kg ha^{-1}, while IS resulted in a positive result (7 kg ha^{-1}) due to the absence of exported biomass. Both the cover crop ryegrass/clover and the green manure ley (summer and spring yield) resulted in negative results in BR and AD (-17 to -284 kg ha^{-1}), as biomass was removed and stored for manuring the next year's crop. There was surplus N in IS for both crops, from 7 and 57 kg N ha^{-1} in the ryegrass/clover catch crop and 39 to 74 kg N ha^{-1} in the green manure ley (fig. 5).

The N balances at the cropping system level gradually became more positive in the BR and AD treatments, when not considering the residual biomass N as a temporary export in the harvest year and input in the subsequent year in BR and AD (table 5; "No stored residual biomass").


a
N in export
N in this years residue
N2 fixed
Biomass N addition
Deposition
Seed
Cabbage
Buckwheat /oilradish
Lentil /oat
Ryegrass /clover
Red beet
Winter rye
Buckwheat /phacelia
Pea /barley
Ley-summer
Ley-spring
IS
BR
AD
-400
-300
-200
-100
0
100
200
300
kg N ha-1

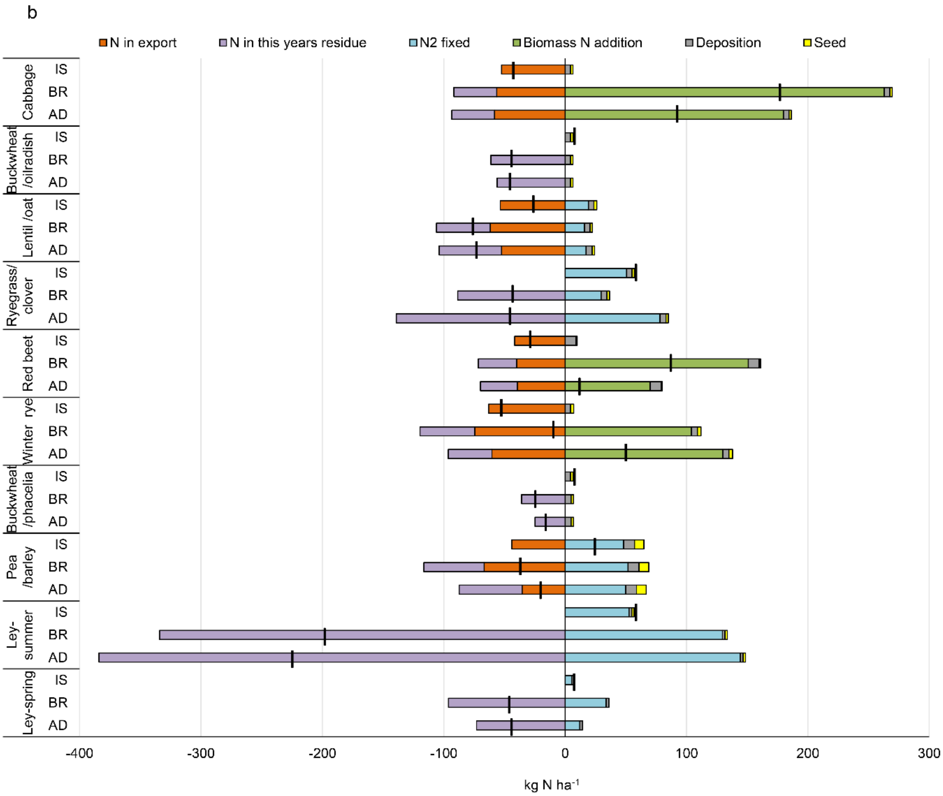


Figure 5. The N balance per crop x treatment. The negative N posts are export of N in edible plant parts and biomass N exported for redistribution the following year. The positive posts are N_2 fixed, biomass addition, deposition and seed contribution (kg ha^{-1}). The black bar shows the balance between import and export of N per crop and for each treatment. a shows 2013 & b 2014.

Discussion

The sustainability of the N management in stockless organic farming systems depends on the balance between nutrient export via cash crops, nutrient inputs through N_2 fixation, the level of success in internal recycling and reduction of losses (Legg and Meisinger 1982). Stockless organic systems often depend on growing green manures, which occupy land for one or more growing seasons. We designed a cropping system with 1/6 of the land allocated for green manure and the remaining land used for food crops, and studied how different strategies for managing residual biomass affected internal N cycling and the N balance.

It was hypothesised that the AD treatment would result in higher N accumulation from soil and recirculated biomass N resources that the other treatments, as the mineral N content was expected to be higher in the digestate than in the biomass/silage in IS and BR. However, there was no difference in soil- and biomass-derived N accumulation in the crops, which could have been caused by a lower than expected NH_4 concentration in the digestate. There are several possibilities to optimise the management of the feedstock i.e. mixing, shredding, alkali pre-treatment and minimising the contact with oxygen at storage prior to digestion (Carrere et al. 2016; Hjorth et al. 2011). There may also have been N losses at the handling of the digestate and during field application of the digestate (Banks et al. 2011; Möller and Müller 2012). Losses of N from digestate in the field could have been decreased by using shallow direct injection into the soil (Möller and Müller 2012).

The proportion of N_2 fixation (%Ndfa) in the legumes of this study was high and not significantly influenced by biomass management method. This was probably because the legumes were grown in intercrops/mixtures with cereal/grasses. The competitive ability of cereals and grasses for mineral N results in a non-proportional acquisition of soil mineral N between the species, leading to a low availability of mineral N for the legumes and a high %Ndfa (Carlsson and Huss-Danell, 2003; Hauggaard-Nielsen et al., 2008; Bedoussac et al. 2015). The green manure leys fixed higher amounts of N_2 in BR and AD than in IS in 2014, and a similar tendency could also be seen in 2013. The higher amount of N_2 fixation in legumes, grown as green manure ley with the BR and AD treatment, is most likely a consequence of the removal of N-rich cuttings, reducing the N availability and thereby the competitiveness of the grasses, thus promoting the growth and N_2 fixation of the legumes.

Our third hypothesis suggested a lower ranking of IS N balance compared to BR and AD. The N balance that did not consider the temporary removal of residual biomass in BR and AD resulted in a surplus in 2014 of 7.8 and 24 kg N ha^{-1} respectively, with the highest N surplus in the AD treatment (IS<BR<AD). The N stored in BR and AD and applied to the non-legume crops in the spring was potentially protected from being lost by mineralization during autumn and winter. This method that temporary stores residual biomass and thus decreases the risk of N losses from large N surplus could provide an improvement to stockless organic farms, where the N surplus can be as high as 194 kg ha^{-1} (Watson et al. 2002). The increased N accumulation in the biomass from 2013 to 2014 of the current study originated partly from a higher N_2 fixation in BR and AD, but mainly from the soil N pool and applied residual biomass. The fact that the amount of residual biomass N increased over time explains the negative N balances in BR and AD when the storage and redistribution of biomass N was taken into account (Table 5), since the temporarily exported biomass N was larger than the biomass N redistributed from the previous year. The difference between the key inputs and outputs at the cropping system level, *i.e.* N_2 fixation minus N export in edible crop fractions, was more negative in IS than in BR and AD. This result further highlights the advantage of strategic biomass management in BR and AD.

Conclusion

The strategic management of the biomass resources for internal recirculation to non-legume crops has several potential advantages for sustainable N management in arable cropping systems. The positive effects are dominated by the increased N_2 fixation in the legumes, compared to leaving the residues, catch crop biomass and green manure ley cuttings *in situ*. The N balance that did not account for the temporary storage of residual biomass N for application in the subsequent season also resulted in a more positive N balance in BR and AD than in IS. Additionally, the risk for N losses was potentially decreased due to the over winter storage of the biomass recycled to non-legumes in the subsequent growth season. Strategically choosing where and when to add biomass N resources in the crop rotation thus has large potential to improve the N use efficiency of the cropping system. Nevertheless, care needs to be taken when applying residual biomass to selected crops in the cropping system, since high application rates might also lead to N losses depending on timing and incorporation technique of the silage/digestate into the soil. These aspects require further research about how strategic biomass N management influences N losses at different processes and at the entire cropping system level.

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III

Carbon and nitrogen dynamics after addition of anaerobically digested and undigested ley to soil

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Abstract

The release pattern of nitrogen (N) from anaerobically digested and undigested organic material over time needs to be known to synchronise N release with plant uptake, and thereby improve N use efficiency. While N supply is often in focus when discussing the application of digestate to soil, there has also been concern that the use of anaerobically digested biomass would decrease the organic matter content and microbial activity of the soil, since part of the organic material is decomposed and carbon (C) is released already in the anaerobic process. One of the main purposes of producing biogas is to replace fossil fuels and thus decrease greenhouse gas emissions. Any emissions of greenhouse gases associated with the use of the resulting digestate therefore need to be quantified. The aim of this study was to examine the effects of grass-clover ley (L) and anaerobically digested grass-clover ley (DL) as amendments to soil, in terms of microbial respiration, mineralisation of organic nitrogen (N) and emissions of N_2O and CH_4. Measurements were made at seven time points during 90 days.

There was more mineral N available in the DL treatment compared to L during the entire incubation period, although from day 55 and onwards it was not more than in the control treatment with no residue addition (S). In the L treatment, there was less mineral N than in the S treatment from day 20 and onwards. The cumulative increases in mineral N over 90 days were -0.57 (SEM 5.68), -12.3 (SEM 17.5) and 34.6 (SEM 7.91) mg kg^{-1} dw soil, for the L, DL and S treatments, respectively. The change in the concentrations of mineral N could not be attributed to low net mineralisation or immobilisation rates in a strict sense, since estimates based on isotopic labelling of the N suggested that large amounts of N were in fact mineralised and subsequently lost as gaseous emissions. After a correction using a conservative estimate of gaseous losses, assumed as denitrification losses only, the cumulative net N mineralisation rates over 90 days were 108 (SEM 18.6), 69.0 (SEM 51.0) and 45.7 (SEM 6.58) mg kg^{-1} dw soil, for the L, DL and S treatments, respectively. The impact on apparent net mineralisation rates by the correction for gaseous losses illustrates the importance of measuring and taking into account all

gaseous N losses in a laboratory incubation. When using the same amendments in a field situation, gaseous losses may or may not occur, depending on the fate of the mineralised N. The N_2O emissions over 90 days did not differ significantly between the treatments, but the emission peak after amendments was higher and shorter in the L treatment compared to DL. The CO_2 respiration was higher in L compared to the other treatments, and DL had higher emissions than S. CH_4 emissions were generally low and fluctuated around zero, but there was a peak in the L and DL treatments on the 55th day. The cumulative CH_4 emissions over 90 days were higher from L than from the other treatments.

The cumulative C losses over 90 days of incubation were significantly higher from the L treatment compared to DL and S, also higher from L than from DL after subtracting the C emissions originating from the soil. The total C loss from L was 49% and 42% from DL, after adding the amount lost as CH_4 and CO_2 in the digestion process to the losses from the incubation. Using digested ley could thus be regarded as an improvement from an organic matter addition perspective, compared to the addition of untreated ley, in stockless organic cropping systems.

Introduction

The high inputs of reactive nitrogen (Nr) in current agriculture affect the climate and the functions of terrestrial and aquatic ecosystems (Galloway et al., 2008; Rockström et al., 2009; Bobbink et al., 2010). Since crop production for food and other ecosystem services is dependent on the input of N, one of the main challenges for the future is to find better ways to manage N cycling, that maximize the benefits of anthropogenic Nr while minimizing its unwanted consequences (Vitousek et al., 1997; Galloway et al., 2008).European organic farmers mainly use animal manure, green manure ley, legume N_2 fixation and digestate from biogas processes as sources of N. In stockless organic farming systems there are fewer options available for accessing organic nutrients, and proper management of all available organic material is thus essential for balancing export and losses of N from the cropping system (Watson et al., 2002; Wivstad, 2009). Several processes in the N cycle are performed by organotrophic microorganisms and thus carbon (C) and N transformations are closely linked (Van Veen et al., 1985). This means that the turnover of organic N after application of crop residues, animal manures and digestate can be more successfully predicted if the decomposability and the relative amounts of C and N in the organic material are known (Christensen, 1987; Janssen, 1996; Kumar and Goh, 2003). If mineralisation and acquisition is synchronised, yield stability will be improved and the risk of Nr losses will be decreased (Gutser et al., 2005; Delin and Engström, 2010).

Anaerobic digestion for the production of biogas is an option for modifying organic material before applying it to agricultural land. The physical and chemical properties of the digestate produced differ from those of the original material (Holm-Nielsen et al., 2009). Freshly harvested biomass does not contain significant amounts of mineral N, but digestate will contain elevated levels of NH_4^+ and a larger relative amount of recalcitrant C structures such as lignin (Chynoweth et al., 2001; Gutser et al., 2005). The short-term N availability of digestate varies from 40 to 80% of total N (Gutser et al., 2005; Delin et al., 2012), depending on the composition of the feedstock and the degree of mineralisation during the digestion. Mineralisation rate of organic N depends on many factors such as particle size of the organic fertiliser, available types of microorganisms and abundance, and the relative amounts of various C compounds. The lignin/N ratio has been seen as an important factor in the determination of mineralisation rate (Melillo et al., 1982; Constantinides and Fownes, 1994; Kumar and Goh, 2003). The polyphenol content of the material has also been seen to affect mineralisation, mainly during the initial stages of decomposition (Vanlauwe et al., 1996; Trinsoutrot et al., 2000). The changes in C quality and quantity and in the relative amounts of mineralized and organic N that occur during anaerobic digestion profoundly affect the supply of N to the crop when the digestate is applied to an agricultural field, compared to applying the same material in its undigested form (Stinner et al., 2008; Möller and Müller, 2012; Nkoa, 2014).

Benke et al. (2017) conducted a greenhouse experiment with both fresh and digested grass-clover ley and concluded that the lower the C/N ratio and the higher the NH_4^+ to total N ratio of the amendment, the higher was the short term effect as N-fertilizer. The early phase of N release from the first ley cut of the season was regulated by the C/N ratio and the NH_4^+-N/total N ratio. However, the digested grass clover ley, which had a higher NH_4^+-N content than untreated ley, induced immobilisation of soil N in the short term. Frøseth et al (2014) on the other hand, concluded that digestate appeared to contribute more to the nutrient supply during early growth than N mineralisation from green manure. For the second and the third ley cut there was no correlation between the C_{org}/N_{total} ratio or the NH_4^+-N/total N ratio on the above-ground biomass N uptake. It was thus assumed that the composition of the remaining organic N is much more recalcitrant with very low N mineralisation rates, after removal of the easily available N compounds (Benke et al., 2017).The easily degradable C and N structures are degraded in the digestion process (Molinuevo-Salces et al., 2013; Möller, 2015), which leads to a reduction of total C in the digestate and at the same time relative increase of the biological recalcitrance in the digestate compared to the feedstock (Sánchez et al., 2008). Hence, if the prediction of mineralisation is based on the C:N ratio of the more easily decomposable plant constituents it might be more accurate (Luxhøi et al., 2006). Luxhöi et al. (2006) studied the mineralisation rate of eight different plant residues with a very wide range in C to N ratios. They concluded that gross N immobilisation rates, for all crops, were correlated with the corresponding respiration rates of the microbes. In contrast, gross N mineralisation rates were less well correlated to the corresponding respiration rates.

While N supply is often in focus when discussing anaerobic digestion, there has also been concern that the use of anaerobically digested biomass would decrease the organic matter content and microbial activity of the soil (Johansen et al., 2013), since easily degradable C compounds is decomposed and C is released in the digester (Stinner et al., 2008). The degree of organic matter (OM) degradation in the digestion process has varied between 11.1%. and 53% depending on the composition of the feedstock, and digestion process (Marcato et al., 2008; Menardo et al., 2011). The organic matter (OM) content of digestate is more recalcitrant than the feedstock and it might result in a decreased microbial degradation in the soil (Kirchmann and Bernal, 1997), compared to undigested biomass.

The use of fossil energy need to decrease mainly due to the problems with emissions of the greenhouse gas CO_2 to the atmosphere (IPCC, 1997). Agriculture is responsible for about 5% of the total energy used on a global basis (Pinstrup-Andersen, 1999) and the major part is fossil fuel. The production of biogas from agricultural residues has a considerable potential for mitigation of CO_2 emissions when it substitute fossil fuels (Cole et al., 1997; Hill et al., 2006; Tilman et al., 2006; Smith et al., 2008).

The aim of this study was to compare the effects of anaerobically digested and undigested grass/clover ley as a soil amendment on the mineralisation and immobilisation turnover of N and on CO_2, N_2O and CH_4 emissions. Nitrogen and carbon transformations were quantified. The treatments with digested and undigested ley were compared with a control treatment without organic amendments. The hypotheses were:

1) In the treatment with undigested ley, an initial period of immobilisation is followed by a period of mineralisation.
2) Following application of digestate, mineralisation is relatively low.
3) The amount of accumulated mineral N (added and mineralised) after 90 days of incubation is higher with digested compared with undigested ley
4) After 90 days, more C is left in the soil after application of undigested ley compared with digested ley.
5) Total N_2O emissions over 90 days are in the order undigested ley > digested ley > control.

Materials and methods

A microcosm experiment was set up, with three treatments: 1) soil receiving grass-clover ley (L), 2) soil receiving anaerobically digested grass-clover ley (DL) and 3) soil without amendment (S). The same ley was used for the L and DL treatments, but half of it was fertilised with ^{15}N labelled N. The use of ^{15}N in the experiment, in the form of labelled ley, digestate and NH_4Cl, was primarily to allow for the modelling of gross N transformations, results that are not presented here, but also to detect losses of N from the microcosms.

Soil and preparation of microcosms

A sandy loam soil of Arenosol type (Deckers et al., 1998) from the SITES (Swedish Infrastructure for Ecosystem Science) field research station Lönnstorp (55°39′21″N, 13°03′30″E), was collected in December 2014. The soil was sampled from the top 20 cm in an organically farmed field trial, passed through a 5.5 mm sieve, thoroughly mixed and stored at 10-15 °C for 65 days. Glass jars of 400 mL were filled with 330 +/- 0.25 g of soil (corresponding to 294 g dry weight), which was compacted to 200 ml to achieve a pore space of 43%. The jars were covered with Parafilm© and pierced 10 times with a 1.2 mm syringe to allow for gas exchange with the ambient air and to simultaneously avoid evaporation. The jars were pre-incubated in darkness for three days at 15 °C before initiation of the experiment.

Ley crop production, harvest and storage

A grass-clover ley grown in a farmer's field was fertilized with ammonium nitrate at a concentration of 45 kg/ha on the 15th of August in 2012. One plot of 10 m^2 received isotopically enriched ammonium nitrate (5 atom% ^{15}N) (^{15}N-ley) and another plot of the same size received ammonium nitrate without ^{15}N enrichment (unlabelled ley). Both ley crops were harvested on the 18th of September 2012 and the material was frozen for storage.

Anaerobic digestion of ley

Anaerobic digestion of ^{15}N-ley and unlabelled ley was performed in duplicate reactors in a feed batch anaerobic two-stage process for production of digestate. Each reactor system consisted of two 1 L leach-bed reactors and one 1 L up-flow anaerobic sludge bed (UASB) reactor (Nkemka and Murto, 2013). The leach beds were operated at 37 ºC and the UASBs at 20 ºC. An operation temperature of around 37 ºC is common in the digestion of agricultural substrates. The inoculum for the UASBs was collected from a UASB at Sjöstadsverket, Stockholm (owned by IVL, Swedish Environmental Research Institute and KTH, Royal Institute of Technology). The dry matter (dm) content of the granular sludge was 23.5% and the volatile solids (VS) content was 15.2% of the wet weight (ww). The dm content of the unlabelled ley was 23.2% and VS was 20.7%. The dm of the ^{15}N-ley was 22.3% and VS was 20.0%. The ley was defrosted and cut into 1–1.5 cm pieces before digestion.

The ^{15}N-ley and unlabelled ley was digested in four batches. For the first batch four leach bed reactors (L1–L4) were filled each with 200 g ley and 200 g deionized water (2 with ^{15}N-ley and unlabelled ley). Four UASBs (U1–U4) were filled with 200 g granules and 800 mL buffer medium (KH_2PO_4 400 mg/l, Na_2HPO_4 0.42 mg/L, $NaHCO_3$ 3.20 g/l and NaCl 600 mg/L). Liquid was exchanged between L1–L4 and U1–U4 in pairs. When neutral conditions (pH 6.5–7.5) were reached and methane production was initiated in the leach bed reactors, liquid exchange with the UASBs was stopped. In the second batch four other leach-bed reactors (L5–L8) were started (two with ^{15}N-ley and two with unlabelled-ley) and connected to U1–U4 in pairs. For the first and fourth batch another 200 g of ley and 200 g of water was added to L5–L8 and L1–L4, respectively, with material from batch one and two left in the reactors. The digestion time for the first, second, third and fourth batch was 149, 131, 103 and 93 days, respectively. The applied organic loading rate to the UASBs ranged from about 0.90–2.20 g chemical oxygen demand L^{-1} and day for the first and second rounds and 2.50–5.00 and to 5.30–9.90 in the third and fourth rounds, respectively. One UASB was operated as a control with the same amount of granular sludge and buffer medium as the other UASBs but without addition of ley leachate.

The gas volume produced from the control was subtracted from the other reactors. The digestate was frozen at -20 ºC, in one liquid and one solid fraction from each hydrolytic reactor, directly after the termination of the digestion. The gas was collected in bags. Gas volume was determined with a syringe and composition was determined by gas chromatography (Nkemka and Murto, 2010). The temperature around the gas bags was registered when measuring gas production and CH_4 volumes were normalized to 0 °C, dry gas at 1 atmosphere, assuming a constant pressure of 1 atmosphere.

Characteristics of input materials

The digestate and ley were slowly defrosted in gastight containers during 12 h in a refrigerator to minimise N losses. The ley was cut into 1 cm pieces by hand. The solid fractions of the digestates were centrifuged at 10 000 rpm for 10 minutes at 10°C with a Sorvall RC 6+ Centrifuge Thermo Scientific, with the program SLC 3000, and the supernatant of each solid fraction was added to the corresponding liquid fraction (Figure 1). This procedure created a better separation of solids and liquids, with mostly mineral N in the liquid fraction and mostly organic N in the solid fraction. The composition of the different fractions and final amendments are presented in table 1.

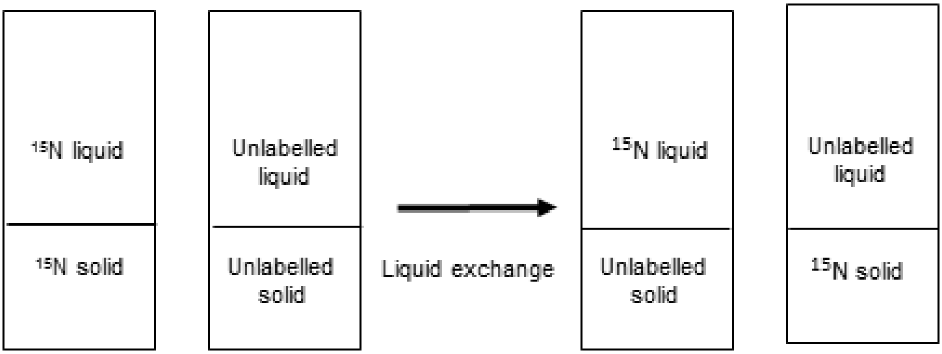

Figure 1. The liquid and solid fractions of the digestates were separated by centrifugation and the ^{15}N labelled and unlabelled liquid fractions were swapped before they were added to the microcosms.

Table 1. The properties of the soil, ley and digested ley used in the incubation

	DW	N-org	NH_4^+ - N	NO_3^- - N	C-tot
	g/jar	mg/jar	mg/jar	mg/g jar	mg/jar
Soil	294	385	0.24	3.51	3610
Ley	2.18	65.9	0	0	1004
Digestate	0.61	26.7	3.08	0	297
NH_4Cl (L & S)	0	0	2.00	0	0
NH_4Cl (DL)	0	0	4.00	0	0

Experimental design

In the L and DL treatments, respectively, there were eight replicate 400 mL glass jars, serving as microcosms, prepared for each sampling time. The eight replicates were identical except for the isotopic composition of their organic and mineral N pools. In four of the replicates (A), the NH_4^+ pool was labelled with ^{15}N, while the organic N pool was unlabelled. In the other four replicates (B), the organic N was labelled with ^{15}N while the NH_4^+ pool was unlabelled or had only a low at% excess of ^{15}N. In the S treatment, there were four replicate microcosms prepared for each sampling time, which were all labelled with ^{15}N on the NH_4^+ only. The labelling of the DL treatment was achieved by adding the solid fraction of the unlabelled digestate with the liquid fraction of the ^{15}N labelled digestate to the (A) microcosms and, conversely, adding the solid fraction of the ^{15}N labelled digestate with the liquid fraction of the unlabelled digestate to the (B) microcosms. The (A) microcosms were further enriched with a small amount of NH_4Cl at 98 atom% ^{15}N while the (B) microcosms received a corresponding amount of unlabelled NH_4Cl. The L (A) received unlabelled ley and a small amount of NH_4Cl at 98 atom% ^{15}N, while the L (B) received ^{15}N labelled ley and a small amount of unlabelled NH_4Cl. The S treatment received a small amount of NH_4Cl at 98 atom% ^{15}N. The NH_4Cl was diluted in deionized water of the amount needed to achieve a 66% water filled pore space (WFPS) in all jars. The amounts and concentrations of N and C in the amendments are presented in Table 1.

The five treatments were applied simultaneously during two-three hours, in a climate chamber, by adding solids, immediately followed by the liquid (liquid fraction of digestate and/or NH_4Cl solution) and mixing with a fork to simulate incorporation in the top soil. Subsequently, the soil was compacted to 43% porosity and covered with Parafilm©, which was pierced 10 times with a 1.2 mm syringe to allow for gas exchange with the ambient air but avoid evaporation. (A) and (B) microcosms were paired and positioned adjacent to each other within each block to provide similar conditions, and positions were randomized within each block. The moisture content of the soil with amendments was regulated by adding deionized water to compensate for the water lost through evaporation during the experiment. The temperature for the incubation was set to 15° C and the incubation lasted for a period of 90 days, simulating a Nordic spring or autumn.

Sampling

The soil was sampled destructively for mineral and organic N, and gas samples were collected, at 0, 2, 4, 7, 20, 55, 90 days (t_{Xd}) after initiation of the experiment. The first sampling was done one hour after the application of treatments. All the soil from each microcosm was transferred to a 1 L flask and 600 mL of 2 M KCL were added. The flasks were shaken at room temperature for 1 h on a shaking table (Edmund Bühler, Hechingen, Germany) at 4.5 units and then left for sedimentation for at least 12 h at 4 +/- 2 °C.

Inorganic N

A subsample of 50 ml of the extract was centrifuged for four minutes at 4000 rpm on a Rotofix 32A. The centrifuged extracts from each of the seven sampling occasions were frozen in -18 °C for later analysis of inorganic N on an auto analyser (Seal analytical AA3) and to determine ^{15}N abundance through diffusion of inorganic N at the end of the experiment.

Organic N

After decanting as much as possible of the KCl extract, 600 ml of deionized water was added to each flask with soil, the flasks were shaken for 1 h and left to sediment for 12 h. The process was repeated three times to remove inorganic N. The rest of the soil was dried at 70° C to constant weight, and a sub-sample was ground using a ball mill (Retsch MM400), for ^{15}N analysis of insoluble organic N on an elemental analyser (Flash 2000). The method was developed from Cheng *et al*. (2013).

^{15}N abundance through diffusion of NH_3

The soil solution samples were slowly defrosted in a refrigerator prior to analysis. The abundance of ^{15}N in the inorganic N was determined in the soil extract by the micro-diffusion method, where NO_3^- and NH_4^+ were converted into NH_3, which was trapped on an acidified filter paper folded into a Teflon tape, using the method by Stark & Hart (1996) and Sörensen & Jensen (1991), with only minor modifications. The total C and N contents and isotopic ratios of $^{15}N/^{14}N$ were measured by Dumas combustion (1020 ºC) on an elemental analyser (Flash 2000, Thermo Scientific, Bremen, Germany) coupled in continuous flow mode to a Thermo Delta V Advantage isotope ratio mass spectrometer (Thermo Scientific, Bremen, Germany), at University of Copenhagen, Denmark.

Gas sampling

Gas samples were collected at each time point, from the same four replicate jars in each treatment. Glass vials (Exetainer©) sealed with silicon septa were used for collecting gas samples for the calculation of CO_2, CH_4 and N_2O fluxes. All vials were evacuated to < 1 mbar prior to the sampling. Each microcosm jar was closed with glass clip top lid and a rubber gasket, to ensure an air-tight seal. Gas samples were collected through a 10.7 mm silicon stopper (Fischer Scientific) in the lid of the jar, using a 20 ml ⌀ 20 mm syringe (Braun Medical Inc.) equipped with a stopcock and a 0.8*25 mm needle (Terumo, Leuven, Belgium). After piercing the membrane with the needle, the syringe was flushed with headspace air three times before withdrawing the sample, closing the stopcock, moving the syringe to the vial, opening the stopcock and injecting the sample into the vial. The sample volumes were chosen to always create a slight overpressure in the vial. Immediately after closing the lid ($t_{initial,}$ t_i), duplicate samples were collected in 6 ml vials, followed by one 12 ml vial. At the end of 60 minutes of gas accumulation ($t_{final,}$

t_f), duplicate samples were collected in 6 ml vials, followed by one 12 ml vial. The 6 ml vials were analysed for CO_2, CH_4 and N_2O concentrations on a gas chromatograph (Agilent 7890); the N_2O concentration on an electron capture detector, CH_4 and CO_2 concentrations on a Flame Ionizing detector.

Calculations and statistics

Net nitrogen mineralisation was calculated as the sum of the NH_4^+ and NO_3^- concentrations at the end of the period, subtracted by the sum of the NH_4^+ and NO_3^- concentrations at the beginning of the period and corrected by adding the estimated amount of mineralised N lost through denitrification. The production of CO_2, CH_4 and N_2O in the microcosm was calculated from the difference in concentration between the t_i and t_f gas samples, taking into account temperature, ambient air pressure and the decrease in air pressure in the jar caused by collecting several subsequent samples. The total amounts of C lost from the jars during the incubation were calculated from the combined CO_2-C and CH_4-C emissions and related to the input of C from undigested or digested ley.

The total losses of N during the incubation were estimated based on decreases in total recovered ^{15}N over time, in the (A) microcosms. N could only escape from the microcosms as gas and the total estimated N losses were thus interpreted as gaseous losses. Decreases in recovered ^{15}N were observed starting from t_{2d} for the L and S treatments and from t_{4d} for the DL treatment. It was assumed that NH_3 emissions were negligible after these time points and the estimated losses of N were interpreted as the combined losses of N_2O and N_2. For each time interval, the loss of N was calculated from the loss of ^{15}N and the measured at% of ^{15}N in the NO_3^- pool. The total amount of mineral ^{15}N, at each time point, was calculated from the concentrations and the at% values of NH_4^+ and NO_3^-, respectively, in the soil extracts. The total amount of organic ^{15}N, at each time point, was calculated from the amount of organic N and the at% value in the washed material. The amount of organic N was calculated from the C:N ratio in the washed material and the amount of remaining C derived from the input and the measured losses of CO_2-C and CH_4-C. Cumulative gas emissions and net mineralisation was analysed using ANOVA with a general linear model. The total recovered ^{15}N data sets from the L (A), DL (A) and S treatments were analysed together using a two-way ANOVA with blocks, treatment and time point as fixed factors and block as a random factor, and separately using one-way ANOVA with block and time point as a fixed factor and block as a random factor. One outlier in the DL (A) treatment was removed since it generated extreme residuals, skewing the data, which was otherwise normally distributed. The decision to remove outliers was guided by the Anderson-Darling normality test. All statistical analyses were performed in Minitab 17, with the significance level α=0.05 and using Tukey's post-hoc test.

Results

Nitrogen mineralisation

The concentrations of mineral N ($NH_4^+ + NO_3^-$), including the mineral N (N-min) already present at the initiation of the incubation, were significantly lower in the L treatment, compared with the DL treatment throughout the experiment (Figure 2). The N-min concentration did not differ between L and S treatment initially (t_{0d} and t_{7d}), but was significantly higher in S compared to L from 20 days to 90 days (t_{20d} to t_{90d}). There was no difference between the N-min concentration of DL and S at t_{7d} and between t_{50d}-t_{90d}. However, the concentration changes in mineral N should not be interpreted as net mineralisation in a strict sense without correcting for N losses in the form of gaseous emissions.

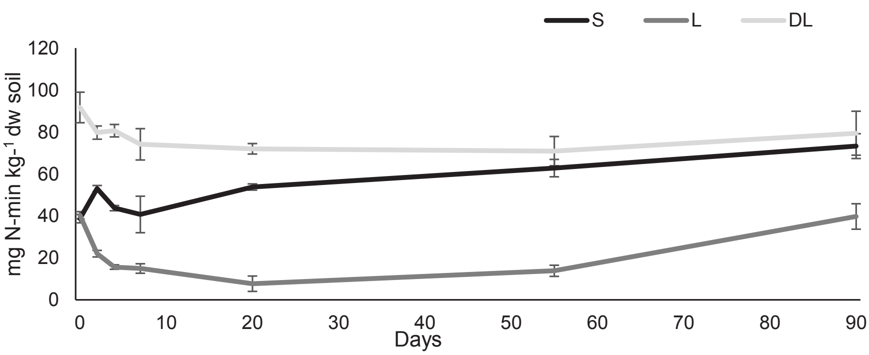

Figure 2. Mineral N concentrations, including initial N-min addition from amendments, S = soil, L = soil + ley, and DL = soil + digested ley.

The apparent net mineralisation values over 90 days, calculated from the change in mineral N pools over time, were -0.57 (SEM 5.68), -12.3 (SEM 17.5) and 34.6 (SEM 7.91) mg N kg^{-1} dw soil for L, DL and S, respectively. When these values were corrected for the estimated N losses, the net mineralisation values were instead 108 (SEM 18.6), 69.0 (SEM 51.0) and 45.7 (SEM 6.58) mg N kg^{-1} dw soil for L, DL and S, respectively (Figure 3). The treatments did not differ significantly from each other before or after the correction of losses, but the mineralisation of ley was significantly higher after correction.

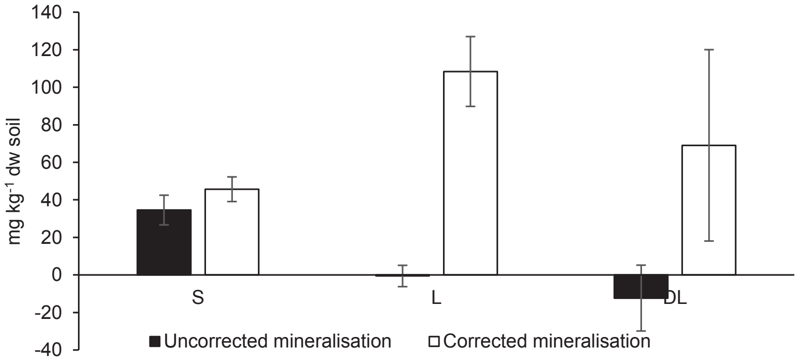

Fig. 3. Cumulative N-mineralisation over 90 days including initial N-min addition from amendments (black). Also presenting the cumulative mineralisation after correcting for gaseous emissions (white). The amendments were S = soil, L = soil + ley, and DL = soil + digested ley.

Gaseous emissions

The cumulative emissions of CO_2, N_2O and CH_4 over 90 days added up to 255, 267 and 98 mg CO_2eq kg^{-1} dw soil, for the L, DL and S treatments, respectively. Soil with addition of digestate or ley thus emitted similar amounts of GHG, despite the different quality of the added organic material and the different relative amounts of mineral and organic N. The dominating contribution of GHG was from N_2O in all treatments. Emissions ranged from 90 to 251 mg CO_2eq kg^{-1} dw soil, with the lowest emissions from S and the highest from DL.

Nitrous oxide

Nitrous oxide emissions showed a sharp peak at t_{2d} for the L treatment and lower but longer lasting emissions for the DL and S treatments (Figure 4a). The cumulative N_2O emissions over 90 days were 13.8 (SEM 1.05), 19.2 (SEM 5.32), and 6.87 (SEM 2.24) mg N_2O-N kg^{-1} dw soil for the L, DL and S treatments, respectively.

Carbon dioxide

Carbon dioxide emissions from microbial respiration in the L treatment were significantly higher than those in the DL and S treatments (Figure 4b). The cumulative CO_2 emissions over 90 days were 1.87 (SEM 0.01), 0.38 (SEM 0.04) and 0.21 (SEM 0.01) g CO_2-C kg^{-1} dw soil for the L, DL and S treatments, respectively. Carbon dioxide respiration was significantly higher in L compared with the other treatments ($p<0.001$), and DL had higher emissions than the S reference scenario ($p<0.001$).

Methane

Methane emissions were generally low and fluctuated around zero, but there was a peak in the L and DL treatments at 55 days (t_{55d}) (Figure 4c). The cumulative CH_4 emissions over 90 days were 0.27 (SEM 0.02), -0.15 (SEM 0.04), and -0.21 (SEM 0.02) mg CH_4-C kg^{-1} dw soil for the L, DL and S treatments, respectively. The emissions from the L treatment were significantly higher than the emissions from the other treatments ($p<0.001$).

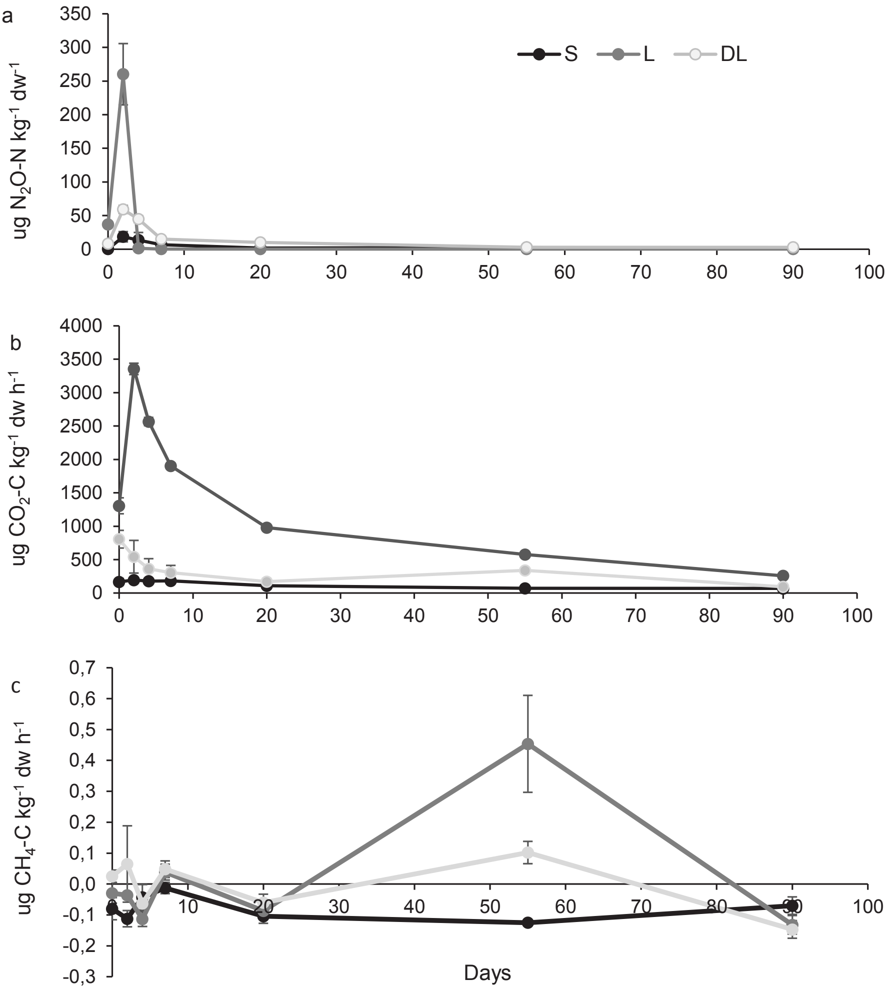

Fig 4. a) Nitrous oxide emissions from S = soil, L = soil + ley, and DL = soil + digested ley. b) Carbon dioxide emissions developed during 90 days of incubation with the three treatments. c) Methane emissions developed during 90 days of incubation with the three treatments.

Cumulative loss of carbon

Over the 90 days of incubation, 1889 (SEM 57.0), 382 (SEM 34.6) and 214 (SEM 10.2) mg C kg^{-1} dw (soil + amendment) were lost from the L, DL and S treatments, respectively. These carbon losses comprised measured microbial respiration (CO_2), as well as emissions of CH_4. The cumulative C losses were significantly higher from the L treatment compared with DL and S ($p<0.001$). After subtracting the C losses in the S treatment, the average C losses from the amendments in the L and DL treatment were 49 (1.68 mg C kg^{-1} dw (soil + amendment)) and 13% (0.17 g C kg^{-1} dw soil) of the total C added through the amendments. The carbon loss from the L biomass was significantly higher than in the DL treatment also after subtracting the C losses of the soil ($p<0.001$). The total C loss from the digested ley was 42%, after adding the amount lost as CH_4 and CO_2 in the digestion process to the losses during the incubation. In total, the undigested ley added 7% less C to the soil compared with the digested ley after 90 days of incubation, based on equivalent amounts of added total N content as ley and digested ley to the soil.

Discussion

As hypothesised, there appeared to be an initial immobilisation of N in the ley (L) treatment (Figure 2) during the first 20 days (t_{0d}-t_{20d}), followed by mineralisation. However, correcting the data for N losses as gaseous emissions resulted in cumulative mineralisation, which indicates that part of the initial decrease in mineral N concentrations could have been due to gaseous N losses (Figure 4). The digestate (DL) treatment contained a large amount of NH_4^+ - N at the start of the incubation, originating from the digestion process (Table 1). Contrary to our hypothesis, the concentration of inorganic soil N decreased during the incubation period in DL. However, a large part of this apparent immobilisation was most likely due to gaseous losses of N, as indicated by measured N_2O emissions and qualitative measurements of NH_3, and confirmed by decreasing amounts of ^{15}N during the incubation. Other studies have reported similar results (Wolf, 2014). The hypothesis that the amount of cumulative mineral N would be higher in DL than in the L treatment after 90 days was rejected, as there was no significant difference between the treatments. In a field situation with spring application of digestate, it is likely that mineralised N would be acquired by the growing crop and the emissions would thereby be decreased. Competition between crop root absorption of mineral N and re-absorption by microorganisms has been seen (Jingguo and Bakken, 1997; Bruun et al., 2006). In contrast, leaving crop residues in the field in late summer or autumn, without sowing a winter crop or cover crop, can be associated with large losses through both leakage and gaseous emissions. When calculating the mineralisation and immobilisation with the addition of the N lost as gaseous emissions, there was cumulative mineralisation in all the treatments throughout the experiment. In the absence of plants in the soil incubations, it is likely that mineralised N was immobilised by microorganisms or emitted as artificially high emissions of N_2, N_2O and NH_3. Immobilisation of mineral N, as well as high gaseous emissions, have been observed in other studies when crop acquisition has been absent or low (Janzen and McGinn, 1991; Raun and Johnson, 1999; Baggs et al., 2000). Much of the microbially assimilated N will be re-mineralised, but a significant part will inevitably remain as relatively stable organic N in the soil (Jingguo and Bakken, 1997), which was also observed in this study.

The high CO_2 respiration from L compared with the other two treatments, during the entire incubation period (t_{0d} to t_{90d}), indicated high microbial activity (Figure 3b), which was consistent with the generally accepted observation that undigested material is less recalcitrant compared to the corresponding digestate (Sánchez et al., 2008). Other studies have also reported higher soil respiration from undigested feedstock compared with application of digested material (Möller, 2015). The undigested ley had emitted more total C than the digested ley after 90 days of incubation, even after including C emissions during the anaerobic digestion. This result is in accordance with results from other studies, and is related to the extraction of C from easily decomposable C structures in the digestion process, which results in a digestate with a higher biological stability with respect to the feedstock (Marcato et al., 2009; Tambone et al., 2009).

There were high emissions of N_2O between t_{0d} and t_{2d} (Figure 3a), which can probably be explained by anaerobic conditions as a result of the high respiration peak. A decrease in total ^{15}N suggests large denitrification emissions during the incubation period. Similar studies with different untreated legume residues have found initial peaks of CO_2 emission rates combined with N_2O emission peaks when the water-filled pore space is higher than 60%, as in the present study (Aulakh *et al.*, 1990). The relatively high water-filled pore space in the jars (66%) could have facilitated the build-up of N_2O emissions (Clayton et al., 1997; Conen et al., 2000) and the emissions in a field situation are likely to be lower. Aulakh et al (1990) saw similar results with N immobilisation combined with high denitrification losses during the first 10 days of a soil incubation with crop residues (Aulakh et al., 1990). When the emissions of CH_4 and N_2O were transformed to CO_2 equivalents based on the 100 year factors presented by IPCC (34 for CH_4 and 298 for N_2O; (Myhre et al., 2013), it was found that the cumulative GHG emissions from ley and digested ley were similar, with N_2O dominating the emissions in all treatments. The CH_4 emissions were negligible in comparison with the magnitude of the other gaseous emissions. The main focus for decreasing greenhouse gas emissions should therefore be on N_2O, in all steps of biomass management.

Conclusions

The losses of N as gaseous emissions were high in the experiment, as there was no crop taking up mineralised N, which would simulate the conditions in autumn when untreated crop residues are left *in situ* and no winter crop or catch crop are sown. The gaseous emissions would possibly be reduced by crop acquisition of mineralised N if the amendments were applied in spring. Gaseous losses of N play an important part in determining the availability of mineralised N for plant acquisition. Studies of mineralisation-immobilisation turnover may be misleading if not all gaseous losses of N are measured and taken into account.

The C stored in the soil after 90 days was slightly increased with the use of digestate compared to undigested ley, which means that carbon emissions during anaerobic digestion of crop residues does not necessarily lead to a reduced contribution to soil organic carbon after applying digestate to the soil, compared to the application of untreated crop residue. The cumulative GHG emissions from ley and digested ley were similar, with N_2O dominating the emissions in all treatments. As N_2O is a potent greenhouse gas it is of importance to aim for reductions of N_2O emissions in all steps of biomass management.

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