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ORIGINAL ARTICLE

Nitrogen balance in a stockless organic cropping system with different strategies for internal N cycling via residual biomass

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Abstract A major future challenge in agriculture is to reduce the use of new reactive nitrogen (N) while maintaining or increasing productivity without causing a negative N balance in cropping systems. We investigated if strategic management of internal biomass N resources (green manure ley, crop residues and cover crops) within an organic crop rotation of six main crops, could maintain the N balance. Two years of measurements in the field experiment in southern Sweden were used to compare three biomass management strategies: anaerobic digestion of ensiled biomass 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 content from soil, 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 the second study year. The N balance ranged between − 9.9 and 24 kg N ha^{-1}, with more positive budgets in AD and BR than in IS. The storage of biomass for reallocation in spring led to an increasing accumulation of N in the BR and AD systems from one year to another. These strategies also provide an opportunity to supply the crop with the N when most needed, thereby potentially decreasing the risk of N losses during winter.

Keywords Anaerobic digestion · Arable and horticultural crops · N balance · N_2 fixation · Soil and residue N · Strategic biomass N 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 highlight the importance of reducing global inputs of new reactive nitrogen (N) to ecosystems (Steffen et al. 2015). The amounts of N applied as fertiliser in agriculture have not been sufficiently constrained to prevent

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widespread leakage to freshwaters and the atmosphere, with effects on human health, biodiversity and climate (Fowler et al. 2013). Organic agriculture, compared with conventional, offers benefits such as increased cycling of nutrients and lower energy usage for processing fertilisers of organic origin than for synthetic fertilisers (Worrell et al. 2000; Vance 2001; Rockström et al. 2009). 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 and Thorup-Kristensen 2001; Willer and 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 the methods are optimised for high yields and that pollution is minimised.

Modern agriculture has increasingly led to specialization in either crop or animal production in entire regions, causing limited availability of animal manure for stockless farms (Schmidt et al. 1999; Mueller and Thorup-Kristensen 2001; 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 (Watson et al. 2002; Wivstad 2009; Colomb et al. 2013). To reduce the need for external fertiliser inputs, researchers suggest strategies that could improve soil fertility and internal nutrient cycling at the farm level, such as improved residue management, intercropping and growing cover crops (Tilman et al. 2002; Bommarco et al. 2013). Cycling of N is central to reduce 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 and Kirkby 1978; Sinclair and Horie 1989). To obtain high yield and quality, mineralisation of N from organic fertilisers and SOM needs to be in synchrony with crop acquisition.

Incorporation of residues from legume cover crops and forage legumes, containing symbiotically fixed N, improves N supply substantially and is very important in organic farming systems without livestock, where other options for N input are limited. Incorporating residual biomass (here defined as crop residue, green manure ley and cover crop cuttings) 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 synchronised (Pang and Letey 1998; Möller et al. 2008a; Mohanty et al. 2013). It may be possible to improve the synchrony between application of residual biomass N, N_2 fixation and plant acquisition of N by pre-treating and storing the biomass as silage or as digestate from anaerobic digestion (Gutser et al. 2005; Gunnarsson et al. 2011; Frøseth et al. 2014). Ensiling initiates mineralisation, but also conserves the biomass by lowering the pH and creating an anaerobic environment (Herrmann et al. 2011). Anaerobic digestion of plant material and subsequent use of the residual digestate as a fertiliser is of particular interest to supply N for non-legume crops in the absence of animal manure in stockless organic systems (Gunaseelan 1997). In general, there is a larger proportion of plant available N in the digestate compared with in fresh or ensiled biomass (Weiland 2010). A high level of mineral N in the soil will generally decrease both nodulation and N_2 fixation (Streeter and Wong 1988; Waterer and Vessey 1993) and thereby make the legume more dependent on soil mineral N. Increased yield and N_2 fixation might be possible by redistributing the N rich silage or digestate to non-legume sole crops. It is challenging to balance N inputs in organic farming 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 has negative impacts on the surrounding ecosystem (de Ponti et al. 2012; Colomb et al. 2013; Seufert and Ramankutty 2017).

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 can summarise 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 at crop, cropping system or farm level is also a valuable tool for identifying risks of N depletion or build-up of a N surplus, thereby highlighting the potential need for improved N management. A N balance made for 76 organic arable farms in Sweden showed an average N surplus of 39 kg/ha (Wivstad 2009). The surplus was mainly due to imported nutrients from digestate, yeast liquid and dried slaughter house waste. Horticultural cropping systems tend to import even more N than arable farms, which results in a N balance with higher N surpluses (Watson et al. 2002), and is thus prone to a higher risk of N losses.

The objective of this study was to assess how different strategies for internal N cycling via residual biomass influenced the N balance of a stockless organic cropping system, where the input of N was limited to biological N_2 fixation, N contained in seeds/plantlets for crop establishment and atmospheric N deposition. The specific aims were (1) to determine whether anaerobic digestion (AD) of the residual biomass from the cropping system, and use of its digestate for N recirculation, would improve the N acquisition in the following crop, compared to the corresponding biomass redistribution (BR) of silage or just leaving the biomass in situ (IS) and (2) to test if strategic management of residual biomass (AD and BR) could improve the N balance of the cropping system. Six main crops, including both arable and horticultural crops, were combined in a field experiment with the purpose to study how the soil N acquisition and symbiotic 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 a biomass management strategy influenced the risk of depleting or creating surplus of 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 the green manure ley, lentil (*Lens culinaris* Medik), pea (*Pisum sativum* L.), clover (*Trifolium pratense* L. and *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 ranks 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.

Materials and methods

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 2013–2014, in the region where the field experiment was situated (SMHI 2016).

Climatic data

The region has a typical northern-European maritime climate with mild winter and summer temperatures (annual average of 9.3 °C and 664 mm precipitation) (Råberg et al. 2017).

Crop rotation

Six different crops in a rotation including different legume species, over-wintering cash and cover crops (Table 1) were studied during 2 years (2013 and 2014). 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.). The sixth main crop was a green manure ley composed of the following six species in equal proportions: orchard grass (*Dactylis glomerata* L.), meadow fescue (*Festuca pratensis* L.), timothy grass (*Phleum pratense* L.), lucerne (*Medicago sativa* L.), yellow sweet clover (*Melilotus officinalis* L.) and red clover (*Trifolium pratense* L.) (Råberg et al. 2017). The green manure ley was under-sown in pea/barley, cut three times during the year after establishment, and cut again in early spring the subsequent year, before establishing white cabbage as the next crop. The herbage was removed in BR and AD and left in situ in IS. 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 was

Table 1 The main crops and cover crops in the rotation

Main crop	Cover and winter crops
White cabbage	Buckwheat/oilseed radish
Lentil/oat + english ryegrass/red and white clover	English ryegrass/red and white clover
Beet root	Winter rye
Winter rye	Buckwheat/phacelia
Pea/barley + green manure ley	Green manure ley
Green manure ley	Green manure ley

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 during each year of the experiment. Winter rye was replaced by spring barley during the establishment year (2012), since the experiment started in spring without any autumn-sown crop from the previous year. The choice of crops in the rotation was based on maximising the cash crop yield and improve the functional diversity to strengthen ecosystem services (Råberg et al. 2017).

Field management

The ley pre-crop was incorporated by inversion tillage (tillage depth 25 cm) before the experiment started. During the field experiment, a non-inversion rotary cultivator was used mixing the crop residue with soil to a maximum depth of 15 cm. The experimental area received an initial supply of 115 kg N ha^{-1} of plant-based digestate applied with trailing hoses in spring 2012. No other external fertiliser was added during the field experiment. Crop protection followed the national organic regulations. The weeding was made by hand in the row crops (cabbage and beetroot) in the same way and around the same dates in all treatments. Seed bed preparations was made by harrowing approximately a week before sowing to allow for weeds to germinate, and then weeds were removed by a second harrowing. The cabbage was grown under an insect mesh and hand-sprayed with *Bacillus thuringiensis* every second week after spotting *Lepidoptera* larvae.

Experimental design

The field experiment comprised in total 72 experimental plots measuring 3 × 6 m, distributed in four blocks. All six crops and the three treatments were randomly distributed within each block (18 plots). The reference treatment was a system where all the residual biomass (crop residues, green manure ley and cover crops cuttings) was incorporated fresh in situ (IS) in the experimental plot (Fig. 1). The IS treatment was used as a reference, as it is common practice to leave most of the crop residue in the field in organic arable farming (Ögren 1992; Ascard and Bunnvik 2008). Two additional biomass management treatments were: (1) ensiling and redistributing all residual biomass (BR) to experimental plots with cabbage, winter rye and beetroot; and (2) all of the residual biomass was ensiled and later anaerobically digested (AD) in a biogas reactor, and the digestate was 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 the supplementary material. This design allowed for

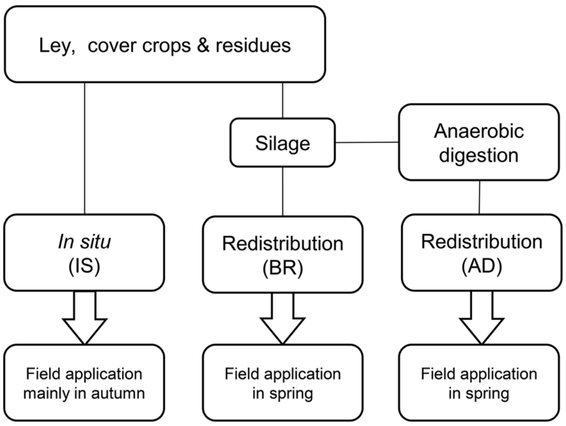


Fig. 1 Residue management within the crop rotation: (1) IS = In situ incorporation, (2) BR = biomass redistributed to the non-leguminous crops grown in pure stand and (3) AD = digested biomass distributed to the non-leguminous crops grown in pure stand. The residual biomass in IS was applied fresh, in BR it was ensiled prior to field application and in AD it was ensiled and anaerobically digested as a pre-treatment

sampling and harvesting of each crop with the three different management strategies in every year.

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 1-year delay for the use of the May harvest of green manure ley and ryegrass/clover in the BR and AD treatments. Measurements of yield and N content started in 2012 and the last samples were collected in 2015 for two over-wintering crops, green manure ley and ryegrass/clover cover crop. Samples from overwintering crops harvested in May, were allocated to the biomass production of the previous year. Since no effects of the biomass management treatments could be expected during the establishment year, the study is based on samples and measurements from 2013 to 2014. Average yields and N content in harvests during 2012 are listed in supplementary material, Table S2.

All residual biomass (crop residues, green manure ley and cover crops) was weighed before returning it to the field plot (IS) or ensiled for later redistribution (BR and AD), as described in Råberg et al. (2017). Subsamples from a 0.25 m^2 surface per plot were taken for analyses of botanical composition (grouped into legumes and non-legumes), N concentration and natural abundance of the stable isotope ^{15}N. After drying and milling the edible and residual biomass fractions of each subsample (see Råberg et al. 2017 for details), the N concentration and $^{15}N/^{14}N$ ratio in each fraction was measured with an elemental analyser coupled to an isotope ratio mass spectrometer (PDZ Europe 20-20, at UC Davies in USA) in legume-containing crop mixtures. A Flash 2000 Thermo Scientific elemental analyser (at SLU, Alnarp, Sweden) was used for determination of N concentration in each fraction of sole crops. The analyses of these subsamples were then used for calculating N_2 fixation, N export in edible fractions and N circulation via residual biomass (see below).

Calculations and statistics

N_2 fixation and N acquisition from soil and added biomass

The N inputs from N_2 fixation was assessed according to the ^{15}N natural abundance method (Unkovich et al. 1997, 2008), using the lowest observed legume $\delta^{15}N$-value as β-value in Eq. (1), 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 ($\delta^{15}N_{ref}$).

$$\%N_{dfa} = \frac{(\delta^{15}N_{ref} - \delta^{15}N_L)}{\delta^{15}N_{ref} - \beta} \times 100, \quad (1)$$

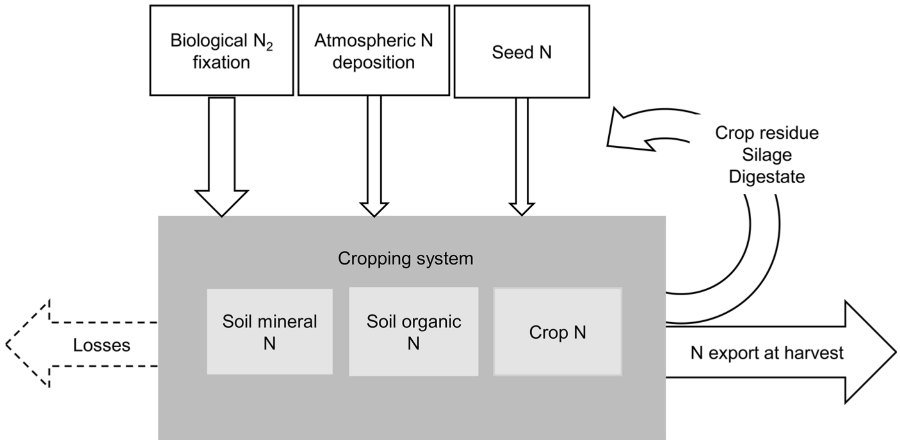
% N_{dfa} = the proportion of the total N uptake originating from N_2 fixation; $\delta^{15}N_{ref}$ = the ^{15}N signature of the grasses and weeds grown together with the legumes; $\delta^{15}N_L$ = the ^{15}N signature of the legume; β = the ^{15}N signature of the target legume ($\delta^{15}N_L$) when fully dependent on N_2 fixation for its N acquisition.

The amount of N_2 fixed (kg N ha^{-1}) in each legume crop was calculated by multiplying %Ndfa with total crop N content (N concentration × crop biomass). Soil N acquisition in legumes, 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. For non-legume crops, the amount of N acquired from soil and added biomass was the same as the total aboveground crop N content.

Nitrogen balance

The balance of N for the cropping sequences was calculated per crop and as an annual sum of each treatment for the years 2013 and 2014. The N balance was calculated from data on N input and output from the cropping system (Eq. 2, Fig. 2).

Fig. 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)



$$\text{N balance} = \text{bnf} + \text{dep} + \text{seed} + \text{biomass}_{\text{added}} - \text{edible fraction} - \text{biomass}_{\text{removed}} \quad (2)$$

bnf = biological N_2 fixation in current year, calculated as described above; dep = atmospheric N deposition; seed = seed N and plantlet N; $\text{biomass}_{\text{added}}$ = N from added residual biomass from previous year; edible fraction = exported N in the edible fraction of cash crops; $\text{biomass}_{\text{removed}}$ = total N from residual biomass removed to be circulated succeeding year.

The regional measurements of atmospheric N deposition during the time of the field experiments added 9.4 kg total N ha^{-1} $year^{-1}$ (SMHI 2016), which was divided and allocated on two crops when there was more than one crop in the same field and in the same year (i.e. main crop and cover crop). The N contribution from seeds was obtained from measured seed N content for the cereals and grain legumes (Table 3), and was calculated from literature for the non-food seeds and plantlets (Schroeder et al. 1974). The amounts of N added via residual biomass corresponded to the redistribution of ensiled (BR) and digested (AD) biomass from the previous year (supplementary material, Table S1). The N outputs in the balance consisted of the amounts of N exported in the harvested edible fraction of the cash crops and N exported in residual biomass in AD and BR to be redistributed in the next growing season.

Analyses of variance (ANOVA) were conducted to test the significance in differences between years (2013 and 2014) and effects of block and treatment (IS, BR and AD) on the response variables (%Ndfa, amounts of N derived from N_2 fixation and from soil acquisition, amounts of N in export of edible crop fractions, amounts of N in residual biomass), both on individual crop and on cropping system level (except for %Ndfa which was only tested at crop level). These ANOVAs were performed using the general linear model (GLM) in the Minitab software, assuming block and treatment as fixed factors. Whenever a significant interaction between year and treatment was found, treatment effects were again tested for significance separately for each year.

Results

Nitrogen acquisition

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

Nitrogen fixation

The total N_2 fixation in leguminous crops constituted 14–26% of total N content in the aboveground plant parts of all crops, which corresponded to an average of 23–40 kg ha^{-1} $year^{-1}$ (Fig. 3). The %Ndfa was found to be in the range 68–98% across all legume species in the cropping system, and 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 ($p = 0.002$) as the years were analysed together. The effect was only significant in 2014 ($p = 0.021$) (Fig. 3), when the years were analysed separately. A large part of the

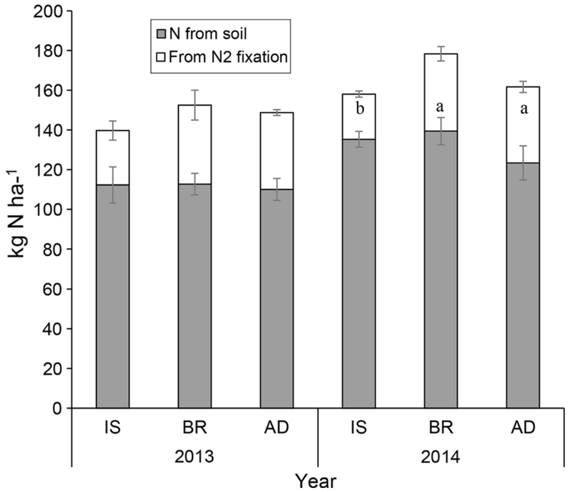


Fig. 3 The total mean N content of the crop biomass from the entire cropping systems in 2013 and 2014 in kg ha^{-1}. Total N is presented as a sum of N acquired from the soil and through N_2 fixation. The letters show significant differences between treatments in N_2 fixation. The error bars represent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

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 (Fig. 4b). The amount of N_2 fixation in lentil and pea varied inconsistently between treatments in the 2 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.

N acquisition from soil

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

Nitrogen exported in the edible crop fraction

The average N content in the exported edible fractions of the five food crops varied between 49 and 60 kg ha^{-1}, with the highest amount exported in rye grain and the lowest in pea/barley. The N content in the edible fraction was not affected by the three treatments (Table 3), even if the N supply differed substantially (table in supplementary material).

Nitrogen in residual biomass

The total amount of N in residual biomass varied between 97 and 129 kg N ha^{-1} (Table 4). There was a significant interaction between treatment and year when the total N content of all the crops from the three systems were compared ($p = 0.001$), but when each year was analysed individually there was no significant difference between the three treatments. In 2013, the green manure ley cuttings constituted 36–40% of the total amount of residual biomass N, and in 2014 the

Table 2 The proportion of nitrogen acquired through N_2 fixation (%Ndfa) in legumes at different biomass treatments within the crop rotation: (1) IS = In situ incorporation, (2) BR = biomass redistributed to the non-leguminous crops grown in pure stand and (3) AD = digested biomass distributed to the non-leguminous crops grown in pure stand

Crops	Ndfa (%)					
	2013			2014		
	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

Presented as mean ± standard error (N = 4, except for green manure ley 2013 with N = 3)

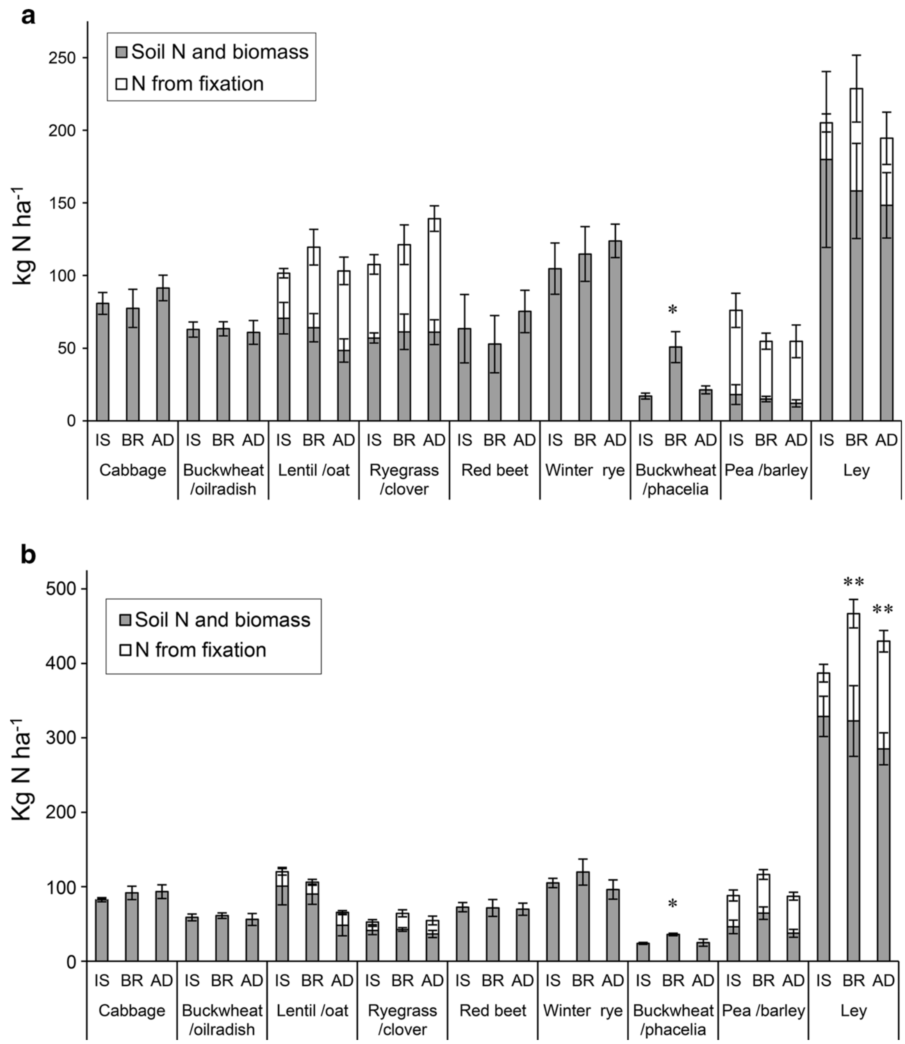


Fig. 4 Nitrogen content of the aboveground biomass of individual crops (kg N ha^{-1}) in 2013 (**a**) and 2014 (**b**), presented as mean ± standard error (N = 4 except for ley with N = 3 in 2013). The grey bars represent N acquisition from soil and residual crop biomass, and the white bars represent N_2 fixation of the 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. The error bars represent standard error for each fraction. * = Significance according to ANOVA at $p < 0.05$. ** = Significance according to ANOVA at $p < 0.01$

part increased to between 49 and 54%. When summed for all biomass resources in the cropping system, the total N content of the residual biomass increased over time, regardless of treatment, with an average difference of 19 kg N ha^{-1} between 2013 and 2014 (Table 4).

Nitrogen balance

The N balances at the cropping system level was more positive in 2014 than in 2013 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 (Table 5; Stored biomass not considered as export).

The three crops that were fertilised 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 for 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 21–47 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 cover crops had a negative result for BR and AD, − 15 to − 57 kg ha^{-1},

Table 3 Nitrogen exported in edible fractions of crops (kg N ha^{-1}) at different biomass treatments within the crop rotation: (1) IS = In situ incorporation, (2) BR = biomass redistributed to the non-leguminous crops grown in pure stand and (3) AD = digested biomass distributed to the non-leguminous crops grown in pure stand

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
Mean	43 ± 3.2	43 ± 3.5	47 ± 3.0	42 ± 1.5	50 ± 3.8	41 ± 4.0

Presented as mean ± standard error (n = 4). The mean value for the entire cropping system (bottom line) was calculated from 6 ha, even if ley is excluded in the sum, but nevertheless crucial for the production of edible produce in the cropping system

Table 4 Nitrogen in residual biomass (kg N ha^{-1}) at different biomass treatments within the crop rotation: (1) IS = In situ incorporation, (2) BR = biomass redistributed to the non-leguminous crops grown in pure stand and (3) AD = digested biomass distributed to the non-leguminous crops grown in pure stand

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
Mean	97 ± 7.0	110 ± 5.5	102 ± 1.8	117 ± 3.5	129 ± 8.1	118 ± 5.7

Superscript letters and numbers in bold mark significant differences. Presented as mean ± standard error (n = 4, ley n = 3 in 2013). The mean value for the entire cropping system (bottom line) was calculated from 6 ha

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 to 57 kg N ha^{-1} in the ryegrass/clover cover crop and 39–74 kg N ha^{-1} in the green manure ley (Fig. 5).

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 internal redistribution and reduction of losses (Legg and Meisinger 1982). Stockless organic systems often depend on growing green manure leys, which occupy land for one or more growing seasons. We designed a cropping system with 1/6 of the land allocated for green manure ley and the

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 (Stored biomass considering export and addition next year), and without considering the temporary stored N in residual biomass or the N addition from biomass (Stored biomass not considered as export) (kg N ha^{-1})

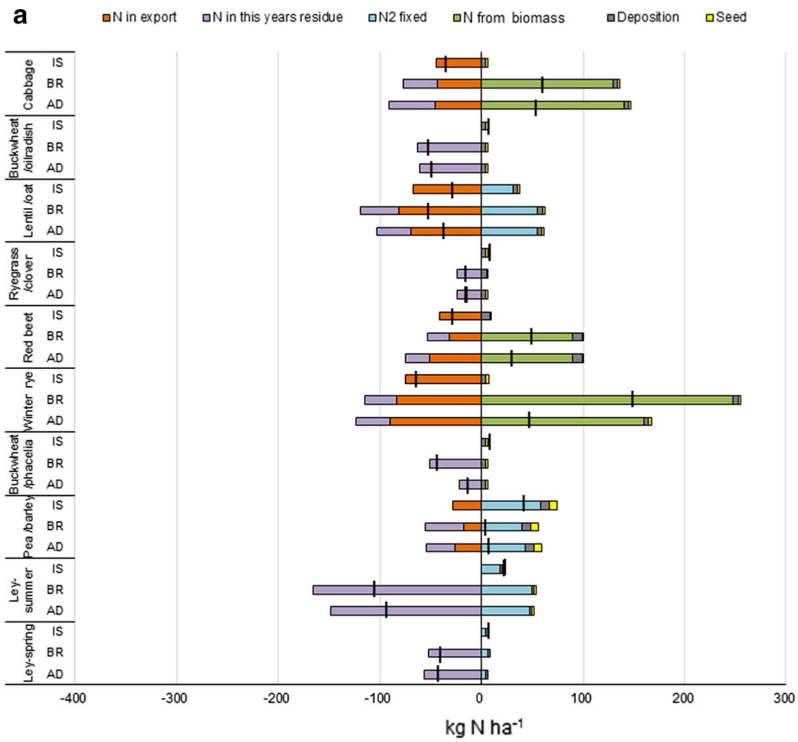
Treatment	Year	Stored biomass considering export and addition next year	Stored biomass not considered as export
IS	2013	− 9.9	− 9.9
	2014	1.1	1.1
BR	2013	− 12	− 3.3
	2014	− 43	7.8
AD	2013	− 22	− 7.9
	2014	− 60	24

remaining land used for food crops, and studied how different strategies for managing residual biomass affected internal N cycling and the N balance. The composition of the rotation was based on a large variation of species from different plant families, to avoid the risk of multiplying soil-borne diseases and the choice of varieties had partial resistance to the most common diseases.

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 uptake of 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 first hypothesis of higher amount of N_2 fixation in AD and BR, compared to IS was confirmed for the green manure ley in 2014, and a similar tendency could also be seen in 2013. The higher amount of N_2 fixation 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 (Unkovich et al. 1998; Möller et al. 2008b; Dahlin and Stenberg 2010).

According to the second hypothesis, the N acquisition from soil and redistributed biomass N resources in non-legumes would be higher in AD than the other treatments, as the mineral N concentration was expected to be higher in the digestate than in the biomass/silage in IS and BR. However, this hypothesis was not confirmed for any of the non-legume main crops or cover crops. On the contrary, one of the cover crops (buckwheat/phacelia, grown after winter rye) showed significantly higher N acquisition in BR than in AD and IS. A likely reason why AD did not result in an increased non-legume N acquisition is that the NH_4^+ concentration in the digestate was lower than expected. The digestate obtained in this study contained 0.18–0.27 kg NH_4–N Mg^{-1} fresh weight (Råberg et al. 2017), which is relatively low compared to similar studies using plant-based digestates (Möller et al. 2008a; Gunnarsson et al. 2011). The total amount of N in the digestate was considerably lower than in the biomass resources in IS and BR (supplementary material, Table S1), indicating that there were significant N losses during the handling of the silage before digestion and/or during the handling of the digestate. As discussed in Råberg et al. (2017), the lack of pre-treatment before the anaerobic digestion might also have contributed to the low NH_4^+ concentration in our study. There are several options for improved management of the biogas feedstock to optimize both the methane yield and the NH_4^+ concentration of the digestate, i.e. mixing, shredding, alkali pre-treatment and minimising the contact with oxygen at storage prior to digestion (Hjorth et al. 2011; Carrere et al. 2016). Furthermore, there may also have been N losses at the handling and during field application of the digestate (Wulf et al. 2002; Banks et al. 2011; Möller and Müller 2012). Using shallow direct injection of the digestate into the soil would have reduced the risk of N losses at application (Möller and Müller 2012), but this technology was not possible to apply in our experimental plots.

Our third hypothesis suggested a lower ranking of IS N balance compared to BR and AD. The N balance



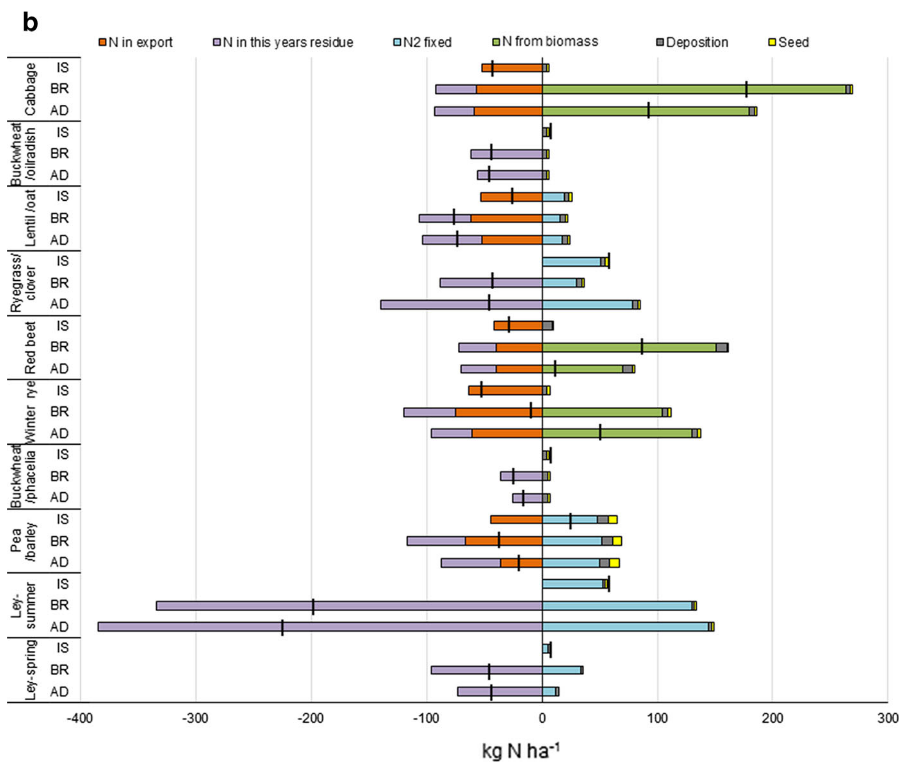


Fig. 5 The N balance per crop x treatment, **a** 2013, **b** 2014. The negative side of the bars illustrates export of N in edible plant parts and biomass N exported for redistribution the following year. The positive side illustrates N_2 fixed, biomass addition, deposition and seed contribution (kg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

that did not consider the temporary removal and delayed addition 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 after mineralisation during autumn and winter (Möller and Müller 2012; Frøseth et al. 2014). 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). It is highly relevant to maintain a low level of N in soils like Arenosol, which have high infiltration and drainage rate (De Paz and Ramos 2004). The increased N content 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 in all three treatments. Consequently, the fourth hypothesis of higher total N acquisition from soil and added biomass in AD and BR compared to IS was not confirmed (Fig. 3).

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 4), 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.

If the field experiment would have continued for a full 6-year cycle or more, it is possible that the N balances in BR and AD would become increasingly larger than in IS, due to an accumulated effect of higher quantities of N_2 fixation and targeted application of silage/digestate to N-demanding non-legume crops. The strategic management of residual biomass in BR and AD would thus sustain crop yields with low risk of long-term depletion of soil N fertility, which might be the case in IS where the N balance is less positive. In addition, BR and AD can also be expected to reduce the risks for NO_3^- leakage and gaseous N emissions compared to the in situ application of residual biomass in IS, where more N would be mineralised in autumn and exposed to losses during times of low crop N acquisition (Hansen et al. 2004; Thomsen 2005; De Ruijter et al. 2010). On the other hand, an increasing N surplus over time in the BR and AD treatments could also lead to larger risks for N losses in these systems in the long term. An interesting option in this case would be to sell parts of the digestate or silage. This possibility further highlights the advantage of strategic biomass management in stockless organic cropping systems.

Conclusion

Our objective was to assess how different strategies for internal N cycling via residual biomass influence the N balance of a stockless organic cropping systems. The result of the assessment was that the AD and BR scenarios showed more positive N balances than IS. Strategically choosing where and when to add biomass N resources in the crop rotation thus has large potential to sustain crop yields and soil fertility, i.e. avoiding the risk of soil N depletion at the cropping system level. The positive effects are dominated by the increased N_2 fixation in the legumes, compared to leaving the residues, cover crop biomass and green manure ley cuttings in situ. Additionally, the risk for N losses was potentially decreased due to the over winter storage of the biomass returned to non-legumes in the subsequent growth season. 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. The conclusion is that organic stockless farms could improve circulation of N by collecting the residual biomass after harvest and thereby reduce the potential risk for N leakage and N emissions. These aspects require further research about how strategic biomass N management influences N losses at different processes and at the entire cropping system level. A comparison between the management systems in terms of the energy use and greenhouse gas emissions related to transportation and storage of the biomass resources would also be relevant for a full assessment of the environmental benefits.

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