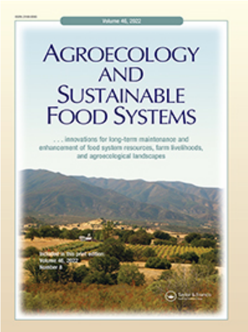

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Sustainable intensification through double-cropping and plant-based fertilization: production and plant-soil nitrogen interactions in a 5-year crop rotation of organic vegetables

Margita Hefner[a], Jorn Nygaard Sorensen[a], Richard de Visser[b], and Hanne Lakkenborg Kristensen[a]

[a]Department of Food Science, Aarhus University, Aarhus N, Denmark; [b]HortiAdvice A/S, Odense SV, Denmark

ABSTRACT
A sustainably intensified (SI) organic vegetable rotation, employing plant-based fertilizers, more crops, reduced tillage, and cover crops was compared to common practice (CP) where plants were fertilized with animal manure, typically one crop was grown per season, soil was plowed and often left bare over winter. Second and third-year results are presented. Nitrogen (N) input obtained within the rotation from N_2 fixed by legume cover crops was higher under SI (34% potential self-sufficiency) than CP (5%). Marketable yields of cabbage, celeriac, leek, lettuce, and onion were similar, and aboveground dry matter increased by 16% under SI (8.6 Mg ha^{-1}) compared to CP (7.5 Mg ha^{-1}). Nitrogen use efficiency (N output/ N input) was 8–16% higher under SI compared to CP, mainly due to the full year clover. Nitrogen surface balance (N input – N output) was higher for SI compared to CP, indicating increased N leaching risk under SI. Short season and shallow-rooted crops under SI left more mineral N to 2.5 m depth in autumn than deeper-rooted crops under CP. Cover crops indicated to mitigate N leaching risk. Vegetable production can be intensified sustainably using more yielding crops, cover crops, reduced tillage, and plant-based fertilizers.

KEYWORDS
Cover crops; green manure; nitrogen use efficiency; nitrate leaching; reduced tillage

Introduction

The 70%-yield gap between organic and conventional agriculture in northern Europe (de Ponti, Rijk, and van Ittersum 2012) indicates the need to improve and intensify organic production to meet global food demand. However, intensive agriculture leads to degraded soils and pollutes water resources when excess nutrients are leached to the environment (Drinkwater and Snapp 2007). Vegetable production, in particular, exhibits a high risk of nitrogen (N) leaching, due to high N inputs and the low N use efficiency

CONTACT Hanne Lakkenborg Kristensen hanne.kristensen@food.au.dk Kristensen Hanne Lakkenborg Department of Food Science, Aarhus University, Agro Food Park 48, 8200 Aarhus N, Denmark
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(NUE) of many short-season vegetables (Tei et al. 2020). Yet, higher production without harmful impacts to the environment could still be obtained by employing sustainable intensification. This term defines a system where yields are increased without adverse environmental impacts and without cultivating more land (Pretty and Bharucha 2014). Possible methods to achieve sustainable intensification include increasing plant diversity in combination with various nutrient sources that have longer mean residence times in the soil (Drinkwater and Snapp 2007), as well as using cover crops for recycling nutrients and increasing yield (West, Ruark, and Shelley 2020).

Plant diversity can be increased by growing two crop species per season. Intercropping (i.e. growing two crop species together at the same spatial scale), can increase yields without needing to increase inputs, thus facilitating sustainable intensification (Brooker et al. 2015). Relay intercropping is defined as planting a second crop before the first crop reaches maturity, while strip intercropping involves the simultaneous growth of two crop species in strips (Brooker et al. 2015). Double cropping (i.e. growing two crop species per season consecutively across time) provides an additional way to increase yields by exploiting an extended growing season.

The aim to phase out the use of animal manure from conventional origin in organic production creates the need for alternative fertilizer sources (Oelofse, Jensen, and Magid 2013). Legumes have the ability to fix atmospheric N_2 and can, therefore, be used as plant-based fertilizers, which can be produced on-farm. However, growing legumes over a full year comes at the price of reduced yield, due to the extra space required for growth, unless they are grown for fodder. Consequently, these crops are often under-represented in the crop rotation of stockless organic farms, especially in countries that allow input of animal manure of conventional origin (Thorup-Kristensen, Dresboll, and Kristensen 2012). For example, in Danish organic vegetable production, it is common practice to rely heavily on external input of nutrients to cropping systems instead of recycling nutrients by cover crops. This does not align with the principle of ecology in organic farming (IFOAM, 2020). Therefore, managers of specialized organic vegetable farms must design cropping systems that have a higher share of N input via biological N_2 fixation, as this approach fosters more balanced nutrient levels (Möller 2018).

If two crops are grown per season, more fertilizers might be required to meet crop nutrient demand, even though total application might be below that of crops grown separately. Thus, a strategy is required to reduce N leaching losses during winter. Cover crops are an effective tool for improving N management in agricultural systems, with crop species having different effects on soil N status. Legumes and non-legumes reduce N leaching by up to 40% and 70%, respectively, compared to bare soil (Tonitto, David, and Drinkwater 2006). Despite the reduced efficiency of legumes in depleting the soil of mineral N, they are beneficial in adding N to agricultural systems through biological N_2 fixation, potentially helping to reduce fertilizer N inputs (e.g. Hefner et al. 2020). The inclusion of cover crops (legumes and non-legumes) as fertility-building crops in crop rotations appears to reduce N leaching losses more than rotations without cover crops (Thorup-Kristensen, Dresboll, and Kristensen 2012). Moreover, cover crops reduce weed pressure and facilitate additional N input via legumes, thereby maintaining yield levels when soil tillage intensity is reduced (Wittwer et al. 2017).

Thus, a more holistic approach is needed to achieve sustainable intensification, whereby crop rotation sequences, soil and crop management must be included (Dore et al. 2011). Reducing N leaching risk in parallel to maintaining vegetable yields could only be achieved when integrating several strategies for N management (Tei et al. 2020). Therefore, we applied a system's approach, in which we compared different fertilizer sources, number of crops, cover crops and tillage practices in parallel to investigate the combined effects of these management strategies on the plant and soil system.

We hypothesized that self-sufficiency in terms of N input, yields, and NUE would be improved under a sustainable intensified (SI) system, where plant-based fertilizers, more crops per season, reduced tillage and winter cover crops are employed compared with common practice (CP), where animal slurry, typically one crop per season, plowing, and few cover crops are employed. Moreover, we hypothesized that N surface balance would be smaller under SI and that N leaching risk would not differ compared to CP.

Material and methods

Field site and experimental design

A field experiment was conducted at Aarhus University Aarslev, Denmark (55°18′N, 10°27′E) over 3 years (2017–2019). The soil was sandy loam (Typic Agrudalf), and the physical and chemical properties are shown in (Table 1). Average monthly temperature and cumulative monthly precipitation during the experimental period are shown in (Figure 1). The field was managed according to the Danish organic farming regulation since 1996.

A split-plot randomized complete block study design with three replicates (blocks) was implemented, with cropping system as the whole-plot factor and field as the sub-plot factor. Several aspects were changed between the two

Table 1. Soil properties of the experimental site.

Soil depth	C	Clay	Silt	Sand	$pHCaCl_2$	P	K
m	g kg^{-1}	g kg^{-1}	g kg^{-1}	g kg^{-1}		mg kg^{-1}	mg kg^{-1}
0–0.5	9	134	151	696	6.8	24	119
0.5–1	2	188	132	676	5.9	19	102
1–2.5	2	181	138	678	7.3	16	105

Note: P was extracted with 0.5 M $NaHCO_3$ and K was extracted with CH_3COONH_4.

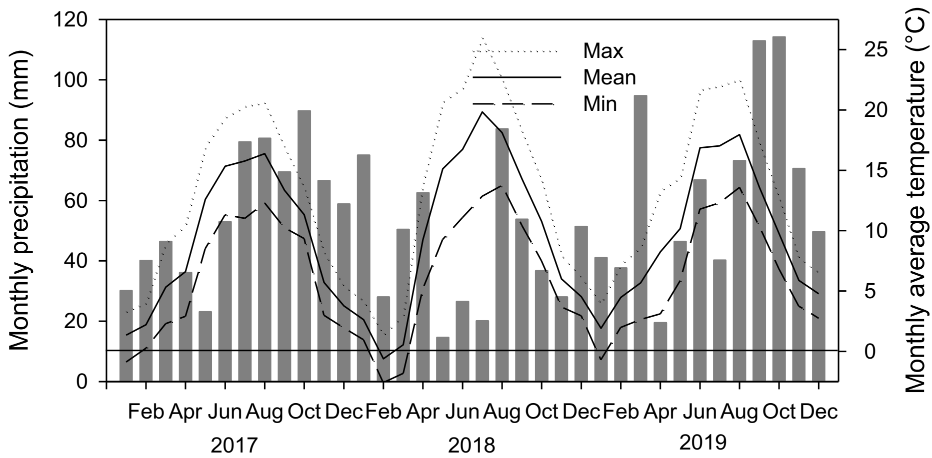

Figure 1. Precipitation and temperature during the experimental period.

cropping systems being compared, making this a system's approach instead of the more commonly used factorial set-up. The two cropping systems differed with respect to soil tillage, fertilizer type, crop species, number of crops per season and cover crops (Table 2). Cover crops were not included (except for the cabbage stubble) and fertilization was mainly import based under CP, as is common practice for organic vegetable farms in Denmark (see also O1 system in Thorup-Kristensen, Dresboll, and Kristensen 2012). The sub-plot factor consisted of five fields: 1) clover (CLO), 2) cabbage (CAB), 3) celeriac (CEL), 4) leek (LEE), and 5) lettuce (LET). These crops were grown at a spatial (fields) and temporal (crop rotation) scale, which meant that a total of 30 plots were managed each year (2 cropping systems × 5 fields × 3 blocks). Sub-plots were 8 × 10 m^2 in size. Vegetable crops were planted at a row distance of 0.5 m. The crops and cover crops grown in each field and system are presented in (Table 2).

Crop management

The timing of all agricultural operations, including fertilization, planting, weeding, harvesting and harrowing, is shown in (Table 3). Seedbeds were prepared with a rotary harrow (Howard, Kongskilde, Denmark) with 0.1-m working depth, and crops were transplanted with a three-row planting machine (Checchi & Magli Wolf, Italy). Lettuce was transplanted in two rows per bed as the second crop between the three rows of onion (first crop), with the same planting machine being adapted to two rows under SI-LET with 13–34 days of overlapping growing period all three years. In 2017, summer white cabbage and leek (second crops) were also transplanted in two rows per bed between pointed cabbage and lettuce under SI-CAB and

Table 2. Different management practices and schematic overview of crops grown in the five fields of the sustainably intensified (SI) and common practise (CP) cropping systems.

Management	Sustainable Intensification	Common Practice
Tillage	Non-inversion	Ploughing
Fertilisation	Plant-based	Animal manure and by-products
Number of crops	Two (in 3 rotations)	One (in 4 rotations)
Cover Crops	In all rotations	In two rotation

Quartal	Rotation	Crop	Species	Latin name	Cultivar	Crop	Species	Latin name	Cultivar
Q1	1) CLO	CC	Red clover (50%)	*Trifolium pratense* L.	Suez (17/18), Callisto (19)	BS			
Q2			White clover (50%)	*Trifolium repens* L.	Klondike (17/18),	C1	Spring barley	*Hordeum vulgare* L.	Invictus (17/18), Flair (19)
					Silvister (19)	CC	Red clover (13%)		
Q3							White clover (27%)		
							Lucerne (27%)	*Medicago sativa* L.	Virgo
Q4							Perennial ryegrass (33%)	*Lolium perenne* L.	Foxtrot

(*Continued*)

Table 2. (Continued).

Q1	2) CAB	BS				BS			
Q2		C1	Pointed cabbage	*Brassica oleracea* L. convar. *capitata* var. *capitata* f. *conica*	Sonsma (17/18), Erstling (19)	C1	Winter cabbage	*B. oleracea* L. convar. *capitata* var. *capitata* f. *alba*	Lennox (17/18), Marner Langerweiss (19)
Q3		C2	Summer cabbage	*B. oleracea* L. convar. *capitata* var. *capitata* f. *alba*	Farao				
Q4		CC	Hairy vetch (75%)	*Vicia villosa* Roth	Villana	CC	Cabbages stubble left as cover crop		
Q1	3) CEL		Black oat (25%)	*Avena sativa* L.					
Q2		C1	Celeriac	*Apium graveolens* var. *rapaceum*	Prinz (18), Rowena (19)	C1	Celeriac	*Apium graveolens* var. *rapaceum*	Prinz (18), Rowena (19)
Q3		CC	Spinach	*Spinacia oleracea* L.	Winterriesen	BS			
Q4									
Q1	4) LEE								
Q2		C1	Lettuce	*Lactuca sativa* L.	Ensamble				
Q3		C2	Leek	*Allium ampeloprasum L.*	Hannibal	C1	Leek	*Allium ampeloprasum L.*	Ensamble
Q4		CC	Winter rye	*Secale cereale* L.	Livado	BS			
Q1	5) LET								
Q2		C1	Onion	*Allium cepa* L.	Forum				
Q3		C2	Lettuce		Design	C1	Lettuce		Diamantinas
						C2	Lettuce		Design
Q4		CC				BS			

Note: The year is divided into four quarters (Q): Q1) Jan–Mar, Q2) Apr–Jun, Q3) Jul–Sep, Q4) Oct–Dec. CLO = clover field, CAB = cabbage field, CEL = celery field, LEE = leek field, LET = lettuce field, CC = cover crop, BS = bare soil, C1 = crop 1, C2 = crop 2.

Table 3. Dates of agricultural management under sustainable intensified (SI) and common practice (CP) cropping systems.

	2018									
	CLO		CAB		CEL		LEE		LET	
	SI	CP	SI	CP	SI	CP	SI	CP	SI	CP
Fertilisation		Apr 19	Apr 11	Apr 19	May 16 vetch incorporation	Apr 19	Apr 11	Apr 19	Apr 11	Apr 19
		Apr 30	Jun 6	June 6	Jul 20	Jul 23	Jun 7	Jul 23	May 31	Jun 7
							Jul 20		Jun 6	
Planting 1st crop		Apr 30	Apr 12	Apr 20	Jun 1	Jun 1	Apr 19	Jun 20	Apr 16	Apr 20
Planting 2nd crop		May 23	Jun 19				Jun 21		Jun 19	Jun 19
Weeding			May 7	May 16	Jun 14	Jun 14	May 14		May 14	May 14
				Jun 13		Jun 27				
			Jul 11		Jul 11	Jul 11	Jul 11	Jul 11	Jul 11	Jul 11
						Jul 23		Jul 23		
						Aug 8	Aug 8	Aug 8	Aug 8	
					Aug 23	Aug 23	Aug 22			
Harvest 1st crop	May 30,	Aug 6	May 31	Aug 31	Oct 17	Oct 17	Jun 7	Oct 15	Jul 2	Jun 4
	Jul 3,									
	Jul 20,									
	Aug 21,									
	Nov 16									
Harvest 2nd crop		Nov 16	Oct 3				Oct 15		Aug 2	Aug 2
Harrowing	Nov 29	Apr 10	Apr 10	Apr 10	Apr 10	Apr 10	Apr 10	Apr 10	Apr 10	Apr 10
			Oct 3	Nov 29			Jun 7		May 31	Jun 7, Jun 19
									Jun 6	Aug 23
Cover crop sowing			Oct 10		Oct 18		Sep 18		Aug 17	

	2019									
	CLO		CAB		CEL		LEE		LET	
	SI	CP	SI	CP	SI	CP	SI	CP	SI	CP
Fertilisation		Apr 9	Apr 5	Apr 9	Jun 4	May 16	Apr 5	May 16	Apr 5	Apr 9
					vetch incorporation					
			Jun 18	May 29	Jul 17	Jul 23	Jun 4?	Jul 23	Jun 4	Jun 18
					Aug 27		Jun 18			
Planting 1st crop		Apr 17	Apr 10	Apr 11	Jun 25	Jun 11	Apr 11	Jun 24	Apr 9	Apr 25
Planting 2nd crop		May 2	Jun 25				Jun 24		Jun 19	Jun 25

(*Continued*)

Table 3. (Continued).

	2019									
	CLO		CAB		CEL		LEE		LET	
	SI	CP	SI	CP	SI	CP	SI	CP	SI	CP
Weeding			May 6 Aug 6	May 7 May 29 Jul 10	Jul 17 Aug 27 Sep 25	Jul 1 Jul 15 Jul 24 Aug 28 Sep 23	May 8 May 16 Jul 10 Aug 28	Jul 10 Jul 24 Aug 7	May 2 May 14 Jul 10 Jul 17	May 7 May 16 May 20 Jul 16
Harvest 1st crop	Jun 3, Jul 17, Aug 28, Nov 18	Aug 21	June 11	Aug 14	Nov 25	Nov 13	Jun 4	Sep 26	Jul 9	Jun 12
Harvest 2nd crop		Nov 18					Sep 26		Aug 7	Aug 7
Harrowing	Dec 1		Jun 18 Jun 28		Jun 6		Jun 4		Jun 4 Aug 21	Jun 18
Cover crop sowing			Sep 26		None		None		Aug 26	

SI-LEE, respectively, with 6–12 days of overlapping growing period. However, transplanting between the existing rows resulted in damage to cabbage and lettuce (first crops) under SI-CAB and SI-LEE, respectively; thus, the second crop was transplanted to three rows immediately after the first crop was harvested in 2018 and 2019. Crop residues were chopped with a flail mower (Spearhead, UK) and were incorporated in the soil with a cultivator (Kuhn, France) to 0.15-m depth. The plots were harrowed before establishing the second crop. A specialized in-row harrow (Breviglieri, Italy) was used to cultivate the inter-rows between the rows of the first crop under SI-LET (2017–2019) and under SI-CAB and SI-LEE (2017). Weeding was conducted with an inter-row cultivator (Rath Maschinen, Austria), a weed-brush machine (Rath Maschinen, Austria) and by manual hoeing.

Fertilizer type and the amount of N application in all fields and years is shown in Table 4. N_2 fixed by clover was applied as a fertilizer by cutting SI-CLO four times in the growing season and applying one-half and one-third of the cuttings as fresh clover fertilizer in SI-LET during May 2018 and 2019, respectively, all cuttings in SI-CEL during July 2018/2019, and half of the cutting in SI-CEL during August 2019. Clover cuttings under SI-CLO were exported outside the rotation at the start of July and in August 2018, as they did not fit in the fertilization schedule. Considering all clover cuttings, SI reached a potential self-sufficiency of 34% in terms of fertilizer N input obtained within the crop rotation (of which 37% was applied directly). The other fresh clover applications in SI-CAB, SI-CEL and SI-LEE were imported to the rotation from a 300-m nearby organic red clover field and accounted for 46% (2018) and 31% (2019) of the total fertilizer N input. The C/N ratio of all applied clover cuttings was within a range of 10 to 20. Total N input was calculated as the sum of fertilizer N, N_2 fixation from legumes and atmospheric N deposition. Total N input was generally higher under SI compared to CP (Table 4) to supply sufficient N to a greater number of or increased diversity of crops grown under SI in CAB, LEE and LET fields. The lower amount of N applied under CP was justified by a lower N requirement of a smaller number of crops, which aligned with the Danish fertilization regulation (Landbrugsstyrelsen 2020).

In 2017, all plots were irrigated with a lateral moving irrigation system with 133 mm water distributed over 7 dates from 7 April to12 July. In 2018, crops were irrigated with 255 mm water distributed over 10 dates from 17 May to 7 August. Crops were not irrigated in 2019 due to sufficient precipitation. Pest control was conducted in CAB by covering cabbage with a net (2017, 2018 and 2019) and in CEL and LEE by applying a bio pesticide (DiPel; *Bacillus thuringiensis*) on 23 August 2019. The clover mixtures were incorporated by tillage in late November/early December to avoid preemptive competition with the subsequent crop. Winter cabbage stubble was left in the field after harvest to act as a cover crop (nitrogen catch crop) under CP. All CP plots were plowed to 0.2-m depth with a plow (Kverneland, Norway) at the start of December.

Soil and plant sampling

Soil samples were taken three times during the growing season: (1) before fertilization in spring (15 March 2017; 26–27 March and 10 April 2018; and 25–28 March 2019), (2) mid-season (12 June 2017; 10 July 2018; and 11 July 2019), and (3) at harvest (8–13 Nov 2017; 5–9 Nov 2018; and 25–29 Nov 2019). In spring and autumn, 10 sub-samples of soil were randomly taken from each subplot by a machine-driven soil piston auger with a 14-mm inner-diameter. The 0–0.5 m, 0.5–1.5 m and 1.5–2.5 m depth layers of soil were sampled with the auger, and were mixed into one composite sample per soil layer. At mid-season, nine soil sub-samples were taken with a hand-driven soil piston auger (15-mm inner diameter) in the 0–0.25 m depth layer, which was mixed to form one composite sample. Soil samples were frozen (−18°C) until analysis. For mineral N analysis, 100 g fresh weight sub-samples were taken after thawing and were immediately extracted in 1 M KCl for 1 h (1 soil: 2 solution). The soil extract was centrifuged, and the supernatant was subjected to NH_4^+ and NO_3^- analyses by standard colorimetric methods in an AutoAnalyzer 3 (Bran+Luebbe, Germany).

Clover was machine harvested (17 m^2 per plot). An area of 1 m^2, 5.12 m^2, and 4.8 m^2 was harvested by hand for barley/cover crops, celeriac, and all other crops, respectively. Yields were separated into marketable yield and crop residues, where applicable. Marketable yield was evaluated with respect to crop weight and damage by pests or disease according to the market standard. Plant material was chopped, mixed well, weighed, oven-dried at 80°C for 20 h, and weighed again to determine dry matter content. Total plant N content was analyzed by the combustion method according to the VDLUFA (1991), wherein plant material was first combusted at 950°C, and molecular N was then measured by a LECO TruSpec CN (CP. Joseph, MI). Total organic carbon content (C) was determined by Dumas' dry combustion method, wherein plant material was combusted at 1000°C, and total organic carbon content was measured by an ELTRA Helios C/S-analyzer (Haan, Germany).

Data processing and statistical analysis

The results from the first experimental year (2017) were not included in the analyses because this was the establishment year, where management operations were optimized for double cropping, and the pre-crop was the same in all fields.

Crop yields were determined as fresh weight per area. Sales units were registered according to local market standards as the number of individual crops (cabbage, lettuce, and celeriac) or bunches of three leeks and five onions per area. Above ground N accumulation of plant material was calculated by multiplying total plant dry matter per area and N content. Soil mineral N was calculated per unit area from measured N concentrations in 0–0.5 m, 0.5–1.5 m and 1.5–2.5 m soil layers and the corresponding bulk densities, which were obtained by a previous study at the same site (Kristensen & Thorup-Kristensen, 2004).

In compliance with the OECD (2001) model, N surface balance was calculated as the difference between N input (N added with fertilizer and by N_2 fixation of legumes grown in the fields, as well as atmospheric N deposition) and N output (N removed from field in marketable products and all clover cuttings). An empirical model (Hogh-Jensen et al. 2004) was used to quantify N_2 fixation by clover, vetch, and grass-clover:

$$N_2 \text{ fixation} = N_{shoot} * P_{fix} * (1 + P_{root} + P_{immobile} + P_{trans\ soil})$$

where N_{shoot} is the amount of N in the shoot, which was calculated based on the plant sample dry matter and respective N content. P_{fix} is the proportion of fixed N of total shoot N, and was set to 0.74 for clover and vetch, and to 0.95 for grass-clover according to Hogh-Jensen et al. (2004). Nitrogen fixed in the shoot was then corrected with N parameters in the root (P_{root}), N immobilized in soil in partly decomposed organic matter ($P_{immobile}$), and N transferred to other species via soil ($P_{trans\ soil}$); specifically P_{root} was set to 0.25, $P_{immobile}$ was set to 0.3 for clover and vetch and 0.38 for grass-clover, and $P_{trans\ soil}$ was set to 0.1. Of note, $P_{trans\ soil}$ was only considered in the grass-clover mixture (Hogh-Jensen et al. 2004). Nitrogen fixation was added as input from all cuttings in the CLO field and from vetch before incorporation in the CEL field. In 2018, data on grass-clover biomass, and consequently N_2 fixation, is missing. Atmospheric N deposition was set to 12 kg N ha^{-1} yr^{-1}, according to Ellermann et al. (2015). Nitrogen accumulation of all other cover crops was not included, due to low cover crop biomass. Nitrogen use efficiency (NUE) was calculated as the ratio between N output and N input.

Results of crop yield, sales units, above ground biomass and N accumulation were analyzed for each field separately across years. A Gaussian linear mixed model was used containing two fixed effects (cropping system and year), an interaction between these effects, and a random component representing block within year to account for spatial variation between blocks each year. Soil mineral N results were analyzed for each soil layer and year separately. Nitrogen balance and NUE were analyzed for years separately. A Gaussian linear mixed model was set up with two fixed effects (cropping system and field), an interaction between these effects, and a random component accounting for the split-plot design (representing the block and the whole-plot). Data were logarithmically transformed when assumptions of homogeneity of variance and normal distribution of residuals were not met.

Table 4. Nitrogen input (kg ha^{-1}), including N added through N_2 fixation from legumes and with fertilizers, under sustainable intensified (SI) and common practice (CP) cropping systems.

	2018					2019				
Field	CLO	CAB	CEL	LEE	LET	CLO	CAB	CEL	LEE	LET
SI										
N_2 fixation from legumes	277	0	103	0	0	300	0	0	0	0
Lupine seeds	0	0	0	0	0	0	0	0	0	0
Clover silage	0	82	0	61	61	0	165	0	132	132
Fresh clovera	0	152	39	207	156	0	68	163	135	66
Total	277	234	142	269	217	300	232	163	267	198
CP										
Pig slurry	74	74	74	74	74	75	75	94	94	75
Chicken manure	18	81	51	51	81	0	80	31	31	80
N_2 fixation from legumes	n.a.	0	0	0	0	33	0	0	0	0
Total	92	155	125	125	155	108	155	125	125	155
$SI_{N\ input} - CP_{N\ input}$	185	79	18	144	62	192	77	38	142	43

afrom the transferred clover cuttings of CLO and from a nearby field; n.a. = not available.

The statistical analyses were performed in R software, version 3.4.2 (R Core Team, 2017). The mixed models were defined with the R-package lme4 (Bates et al., 2015). Nested models (e.g., a large model containing the interaction and a reduced model containing additive main effects) were compared with likelihood ratio tests for generalized linear mixed models implemented in the 'anova()' R-function to detect significant interactions between fixed factors. Post-hoc analyses were conducted using the correction for multiple comparisons based on the method of control of False Discovery Rate (FDR, see Benjamini and Yekutieli, 2001) with the R-package 'pairwiseComparisons.' Mean values are reported followed by standard errors

Results

Climate

Cumulative precipitation was lower in 2018 (531 mm) compared to 2019 (767 mm), with particularly low values in May–July 2018 (Figure 1). Average daily temperature in April–October was 14.9°C in 2018 and 13.4°C in 2019.

Clover productivity and N_2 fixation

Clover biomass was higher when full-year clover was cut four times per season under SI (15 Mg ha^{-1} dry weight) in contrast to under-sown grass-clover under CP (1 Mg ha^{-1} dry weight) (Table 5). Likewise, N_2 fixation was higher under SI (300 kg N ha^{-1} yr^{-1}) compared to CP (33 kg N ha^{-1} yr^{-1}). The sum of N input in CAB, CEL, LEE and LET fields was 863 and 860 kg N ha^{-1} in 2018 and 2019 (Table 4). Averaged for both years, 34% of this N input was obtained by N_2 fixation of full year clover under SI. However, only 107 kg N ha^{-1} (averaged for

Table 5. Clover biomass (dry matter in Mg ha^{-1}), N accumulation in biomass and N_2 fixation (kg ha^{-1}) from four clover cuttings and a final cut before termination of clover in November in the CLO field under sustainably intensified (SI) and common practice (CP) cropping systems.

	2018		2019	
	SI	CP	SI	CP
Biomass	14.8 ± 0.9	n.a.	15.7 ± 2.2	1 ± 0.1
N accumulation	366 ± 19	n.a.	397 ± 64	33 ± 5
N_2 fixation	277 ± 14	n.a.	300 ± 47	33 ± 5

n.a. = not available. Mean values are presented with standard error (n = 3).

both years; accounting for 37% of N_2 fixed by all clover cuttings in CLO) was applied within SI, due to poor timing of N availability and crop demand. Consequently, fertilization was supplemented with clover from a nearby field. Self-sufficiency, in terms of N input by N_2 fixation from clover cuttings applied within the crop rotation, was 12%, and increased to 19% when including the incorporated legume cover crops in the CLO and CEL fields. In CP, only 5% of the 635 kg N ha^{-1} N input was supplied by N_2 fixation of under-sown grass-clover in 2019.

Crop yield, sales units and N accumulation

Above ground dry matter biomass averaged across all fields increased by 16% under SI (8.6 Mg ha^{-1}) when compared with CP (7.5 Mg ha^{-1}). In particular, the above ground biomass of CLO, LEE and LET (2018/2019) increased under SI compared with CP; however, the aboveground biomass of CAB (2018/2019) and CEL (2019) decreased (Figure 2).

Marketable yields averaged across all four vegetable fields (CAB, CEL, LEE, LET) were maintained under SI when compared with CP. Marketable yields of LEE (2018/2019) and LET (2019) were 157–191% and 34% higher, respectively, under SI compared to CP. However, the marketable yield of CEL (2019) was 30% lower under SI compared to CP (Figure 3). In all other cases, marketable yields were comparable between systems.

Sales units averaged across fields (excluding CLO) increased under SI compared to CP. In CAB and LEE fields, two crops were grown per season under SI in contrast to one crop under CP, increasing sales units under SI (Figure 2). Sales units of CEL (2018/2019) and LET (2019) were comparable between systems, whereas sales units of LET (2018) were lower under SI (bunches of five onions followed by lettuce) compared to CP (lettuce followed by lettuce).

Above ground N accumulation averaged across all fields was 63% higher under SI (168 kg N ha^{-1}) compared to CP (114 kg N ha^{-1}). In particular, N accumulation of CLO (2018/2019), LEE (2018/2019), and LET (2019) increased under SI. In contrast, N accumulation of CEL (2019) and LET (2018) decreased under SI by 19% and 17%, respectively (Figure 3).

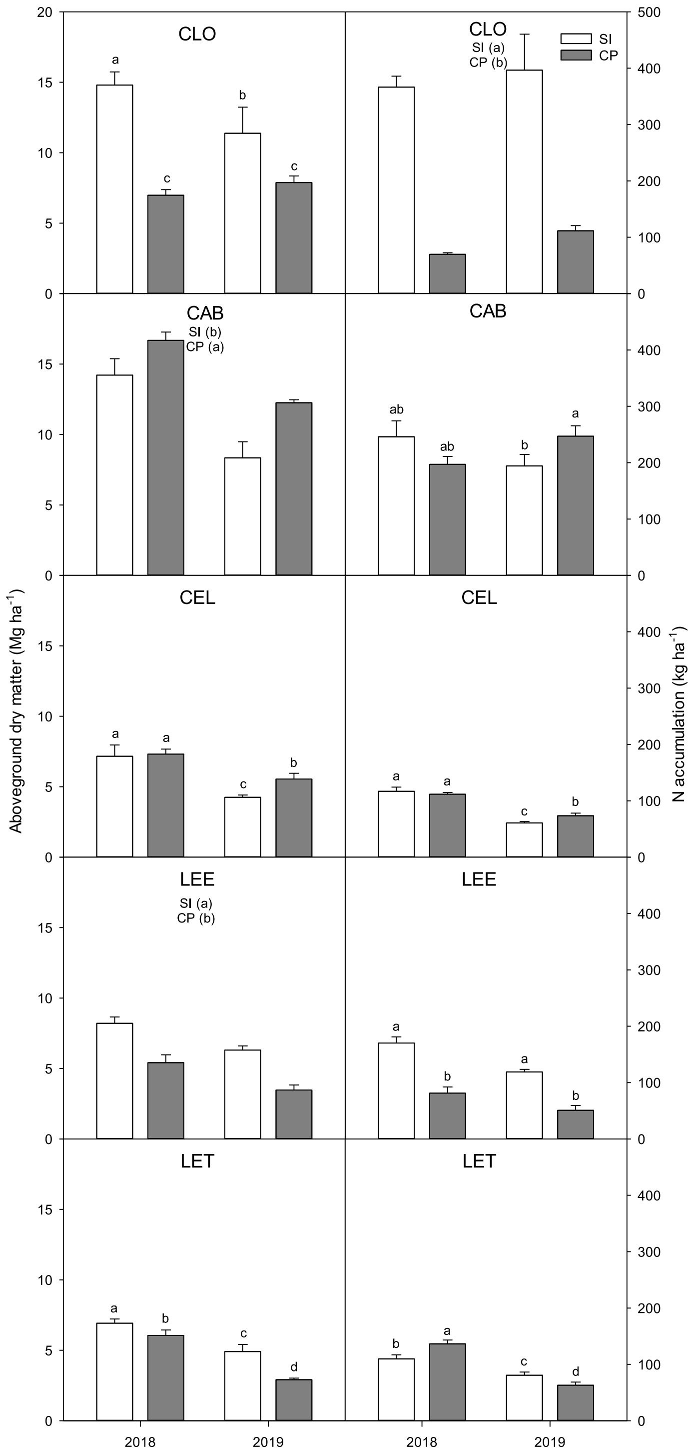

Figure 2. Above ground biomass (dry matter; left column) and N accumulation (right column) of five crop fields across two years. SI = sustainable intensified system, CP = common practice. CLO = clover (SI) and spring barley under-sown with clover (CP); CAB: pointed cabbage followed by white cabbage (SI) and white cabbage (CP); CEL: celeriac (SI and CP); LEE: lettuce followed by leek (SI) and leek (CP); LET: green onion intercropped with lettuce (SI) and lettuce followed by lettuce (CP). Bars indicate standard error (n = 3). Different lower-case letters indicate significant differences between cropping systems for each field separately (P < .05).

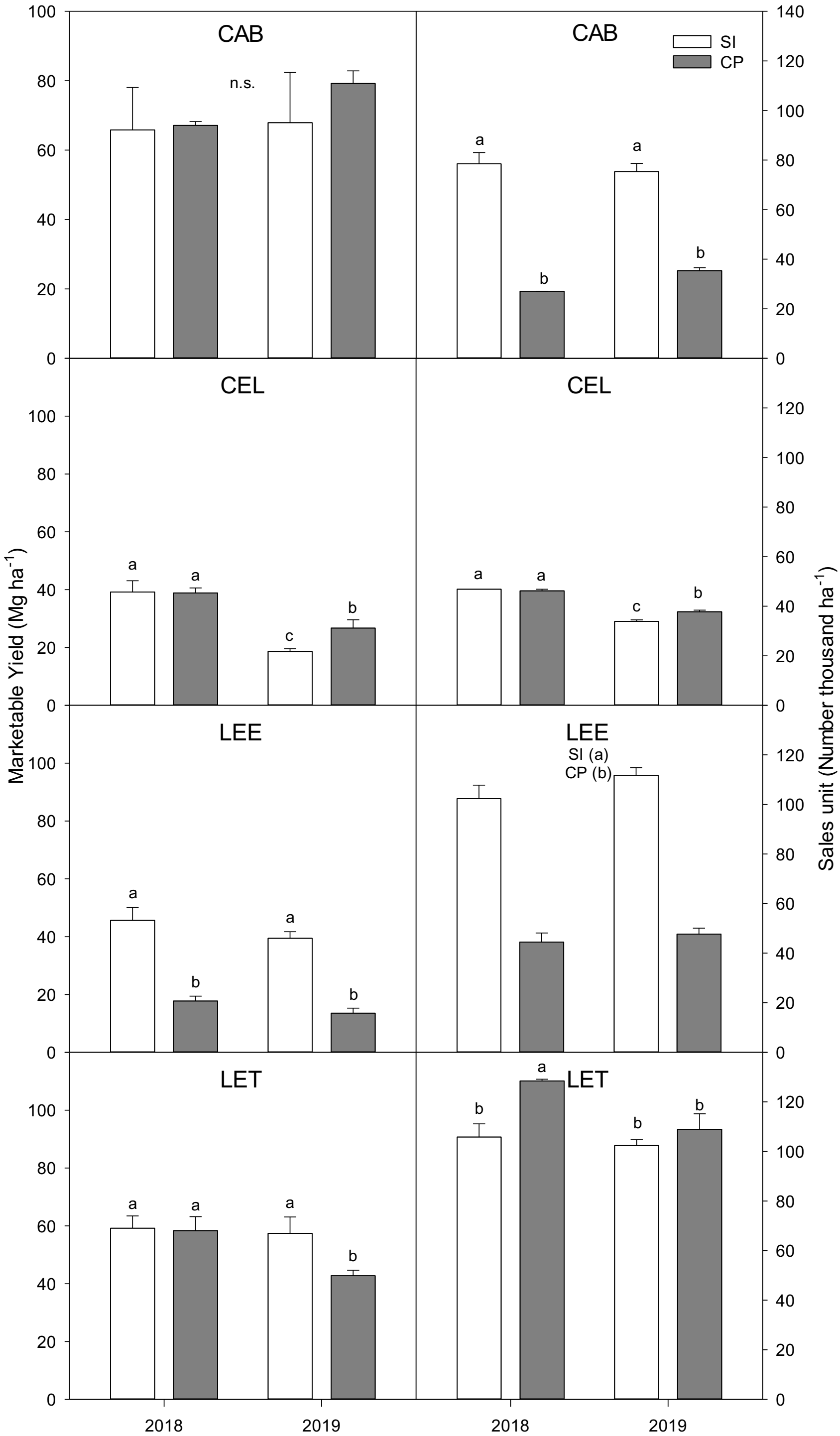

Figure 3. Marketable yield (left column) and sales units (right column) of four fields over two years. SI = sustainable intensified system, CP = common practice. CAB: pointed cabbage followed by summer white cabbage (SI) and autumn white cabbage (CP); CEL: celeriac (SI and CP); LEE: lettuce followed by leek (SI) and leek (CP); LET: green onion intercropped with lettuce (SI) and lettuce followed by lettuce (CP). Bars indicate standard error (n = 3). Different lower-case letters indicate significant differences between cropping systems for each field separately ($P < .05$).

Fewer plant residues remained in the field under SI (2.8 and 1.6 Mg ha^{-1}) compared to CP (3.4 and 1.9 Mg ha^{-1}) in 2018 and 2019, respectively, mainly due to lower cabbage and onion/lettuce residues (results not shown). Nitrogen accumulation in residues was equal between systems in 2018 (53 kg N ha^{-1}), but was lower under SI (37 kg N ha^{-1}) compared to CP (48 kg N ha^{-1}) in 2019 (results not shown).

Nitrogen input, output, surface balance and use efficiency

Nitrogen input was 77–79 kg N ha^{-1}, 142–144 kg N ha^{-1}, and 43–62 kg N ha^{-1} higher under SI compared to CP in CAB, LEE and LET fields, respectively (Table 4). Higher N input was paralleled by higher organic matter input, as plant-based fertilizers were applied. In 2018, SI-CAB had higher N output compared to CP-CAB; however, this difference was not observed in 2019 (Table 6). In contrast, N surface balance (N input – N output) for CAB did not differ between SI and CP in 2018, but was higher under SI in 2019. In LEE, N output and N surface balance were higher under SI compared to CP in both years. Nitrogen output and NUE (N output per N input) were highest for SI-CLO compared to all other fields and cropping systems, whereas N surface balance (N input – N output) was lowest (Table 6).

Among fields, NUE was generally lower for LEE and LET (16%–38%), intermediate for CAB (45–77%) in both years and CEL in 2018 (41–42%), and highest for SI-CLO (120–127%) in both years (Table 6). Nitrogen use efficiency was higher under SI compared to CP for CLO in both years, lower under SI compared to CP for CAB in 2019, but comparable between cropping systems in all other fields and years. Mean NUE across fields was 8–16% higher under SI compared to CP (Table 6).

Soil mineral N

Growing clover over winter under SI-CLO reduced soil mineral N content at 1.5–2.5 m soil depth in the spring of 2018 and at 0.5–1.5 m soil depth in the spring of 2019 compared with bare soil under CP-CLO (Figure 4). In contrast, soil mineral N was higher following two short-season cabbages under SI compared to one long-season cabbage under CP in CAB at 0–1.5 m soil depth in autumn 2018 and at 1.5–2.5 m soil depth in autumn 2019 (Figure 4). Soil mineral N content was similar between systems in LEE fields (supplementary material), but differences appeared in the following spring under LET, where soil mineral N content was higher under CP compared to SI at 1.5–2.5 m depth in 2018 and 0.5–2.5 m depth in 2019 (Figure 4). In contrast, higher soil mineral N was recorded in autumn in LET under SI compared to CP at 0.5–1.5 m soil depth (2018/2019) (Figure 4).

Table 6. Nitrogen (N) output (kg ha^{-1}), N surface balance (kg ha^{-1}), and N use efficiency (NUE) (%).

	2018						2019					
Field	CLO	CAB	CEL	LEE	LET	Mean	CLO	CAB	CEL	LEE	LET	Mean
N output												
SI	$366^{a} \pm 19$	$119^{b} \pm 18$	$63^{cd} \pm 5$	$72^{c} \pm 9$	$78^{c} \pm 8$	140 ± 10	$376^{a} \pm 63$	$111^{b} \pm 14$	$24^{fg} \pm 2$	$51^{cd} \pm 2$	$73^{c} \pm 5$	127 ± 15
CP	$58^{cd} \pm 2$	$75^{c} \pm 3$	$57^{cd} \pm 1$	$35^{d} \pm 4$	$63^{c} \pm 6$	58 ± 1	$58^{c} \pm 4$	$129^{b} \pm 14$	$34^{ef} \pm 3$	$22^{g} \pm 3$	$39^{de} \pm 3$	57 ± 2
N surface balance												
SI	$-77^{g} \pm 5$	$127^{c} \pm 18$	$91^{de} \pm 12$	$209^{a} \pm 9$	$151^{b} \pm 8$	100 ± 10	$-64^{f} \pm 16$	$133^{bd} \pm 14$	$151^{b} \pm 2$	$228^{a} \pm 2$	$137^{bc} \pm 5$	117 ± 6
CP	$48^{f} \pm 4$	$92^{de} \pm 3$	$80^{e} \pm 1$	$102^{cd} \pm 4$	$104^{cd} \pm 6$	85 ± 2	$62^{e} \pm 4$	$38^{e} \pm 14$	$103^{d} \pm 3$	$115^{cd} \pm 3$	$128^{bd} \pm 3$	89 ± 3
NUE												
SI	$127^{a} \pm 0$	$48^{bc} \pm 7$	$41^{cd} \pm 5$	$26^{ef} \pm 3$	$34^{df} \pm 4$	55 ± 4	$120^{a} \pm 2$	$45^{cd} \pm 6$	$14^{f} \pm 1$	$18^{f} \pm 1$	$35^{de} \pm 2$	46 ± 2
CP	$54^{b} \pm 4$	$45^{bd} \pm 2$	$42^{cd} \pm 1$	$25^{f} \pm 3$	$38^{cde} \pm 3$	41 ± 1	$48^{c} \pm 3$	$77^{b} \pm 8$	$25^{ef} \pm 3$	$16^{f} \pm 2$	$23^{ef} \pm 2$	38 ± 1

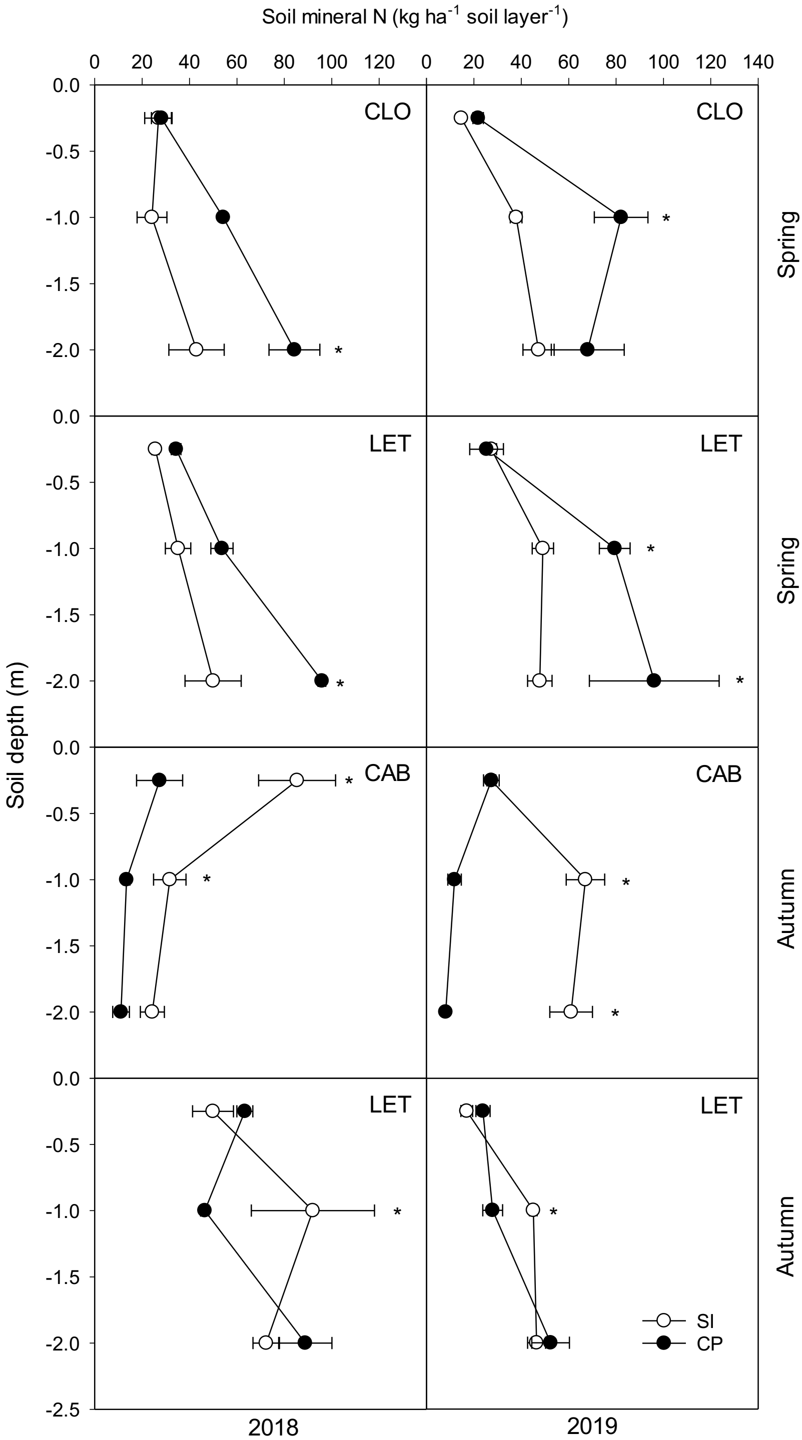

Figure 4. Soil mineral N in three soil layers (0–0.5 m, 0.5–1.5 m, 1.5–2.5 m) of selected fields in the spring and autumn of 2018 and 2019. SI = sustainable intensified system, CP = common practice. Bars indicate standard error (n = 3). * indicates significant differences for sampling time and soil depth separately (P < .05). Soil mineral N of all fields is provided as supplementary material (S1).

Discussion

Nitrogen input and self-sufficiency

Despite N input being higher under SI, 34% of N input within the crop rotation was obtained from N_2 fixed by clover and legume cover crops (of which just 37% was applied). In contrast, CP mainly depended on external N input from animal manure (Table 4). Nitrogen accumulated by red and white clover under SI (366–397 kg N ha^{-1}) was slightly lower than the N accumulated by pure red clover (500 kg N ha^{-1}) or white clover (400 kg N ha^{-1}) cut four times in a previous study at the same site (Sorensen and Grevsen 2016). N_2 fixed under CLO (277–300 kg N ha^{-1} yr^{-1}) that was cut and removed four times during the growing season was slightly lower than the N_2 fixed by a mixture of perennial ryegrass and red and white clover (336–376 kg N ha^{-1} yr^{-1}) cut three times and left on the soil in another study conducted in Denmark (Pandey et al. 2017). The lower N_2 fixation of full year clover documented in our study could be explained by lower N_2 fixation of legumes grown in pure stands compared to legumes grown in mixtures with non-legumes, because the competition for soil mineral N in mixtures stimulates the N_2 fixation of legumes (Nyfeler et al. 2011).

Nitrogen input was generally higher in SI compared to CP (Table 4), because a greater number or variety of crops in CAB, LEE and LET required higher N input. Nitrogen input also increased under SI, because the N transfer rate from fertilizer to crops is lower for leguminous green manure crops (0.6) compared to liquid animal manure (0.8) (Benke et al. 2017), justifying the need for a higher N application rate of plant-based fertilizers under SI.

Thirty-four percent of the total N input was obtained through N_2 fixation by clover under SI (Table 4). Only 37% of this N (accounting for 12% of total N input) was applied within the crop rotation, due to the poor timing of N availability in relation to N demand. However, the potential of storing this N source via ensiling could facilitate N application at times when crop demand is high, as proposed by Möller (2018). The redistribution of N from one year to the next via ensiling increased N accumulation in the cropping system, as N can be applied when crop need is high (Raberg, Carlsson, and Jensen 2018). We showed that replacing one-fifth of the crop rotation with full year clover under SI potentially decreased N import from external sources by one-third if unused cuttings are preserved as silage. Therefore, we supported our hypothesis that self-sufficiency, in terms of N input, increased to a greater extend under SI (34%) compared to CP (5%). Similarly, the strategic use of fertility-building crops, such as legumes, in a crop rotation reduced the dependence on imported nutrients (Thorup-Kristensen, Dresboll, and Kristensen 2012).

The self-sufficiency of the SI system could have been improved further by including more legumes in the crop rotation as cover crops or intercrops. Iannetta et al. (2016) showed that if legume crops are grown for half the year of a crop rotation, N input through N_2 fixation is maximized, reducing the need for external N input. Moreover, using leguminous green manure contributes to a more balanced overall input of nutrients by importing just N without any other elements (Möller 2018). However, increased risks of rotational diseases in legumes need to be taken into account.

Yield response to cropping system

We hypothesized that above ground biomass and marketable yield would be higher under SI compared to CP, due to double- and intercropping crops under SI. We confirmed that the overall above ground biomass under SI was 16% higher than that under CP. This phenomenon was partly attributed to the biological N_2 fixation of full year clover grown in the CLO field, which provided an additional source of N input and biomass production. Full-year grass-clover adds considerable amounts of N to the system through N_2 fixation (Pandey et al. 2017). Moreover, growing two crops instead of one crop in the LEE field increased above ground biomass by 52–82%. This rise was mainly attributed to the extended growing season (175 vs. 107 days) with a higher temperature sum (2668 °Cd vs. 1770 °Cd). It is particularly important to utilize temperature during the extended growing season in Nordic climates, where low temperatures limit plant growth. Average daily temperature from April to October was higher in 2018 (14.9°C) compared to 2019 (13.4°C), and positively affected vegetable yields. Furthermore, diversifying crops grown under LET also increased above ground biomass by 14–68% under SI, in which onion and lettuce were intercropped compared with double cropped lettuce under CP. The increased above ground biomass under LET was an effect of higher onion biomass (4–5 Mg ha^{-1}) compared with lettuce biomass (1–3 Mg ha^{-1}).

Although overall above ground biomass was higher under SI, overall marketable yield was similar between SI and CP, due to the absence of marketable yield under SI-CLO. Marketable yield under SI-LEE sufficiently increased to counterbalance the reduced marketable yield under SI-CLO. Thus, we partly supported our hypothesis, because SI increased above ground biomass and maintained marketable yield compared with CP. The ability to maintain overall marketable yield under SI despite the introduction of a full year clover in the crop rotation showed the potential of this system for application in practice where full year legumes are often replaced by part year legumes in vegetable crop rotations (Thorup-Kristensen, Dresboll, and Kristensen 2012). In addition, overall increased sales units, in particular in CAB and LEE, demonstrated the advantage of the SI system. Similarly, marketable yields of an organic crop rotation based on fertility-building crops with reduced import of animal manure were similar to that obtained for organic rotation based on the common practice of using animal manure and no cover crops in another study conducted at the same location (Thorup-Kristensen, Dresboll, and Kristensen 2012).

Interestingly, the marketable yield of CAB was similar between cropping systems, regardless of whether two short-season cabbage crops or one long-season cabbage crop were grown. However, above ground biomass declined by 15–32% and N accumulation by 25% under SI in 2019. This finding indicates that double cropping two crops incurs slower nutrient uptake during the early growing phase, which contrasted with higher biomass production and nutrient uptake of a long-season crop under CP. Likewise, deep roots of a long-season white cabbage increased access to soil mineral N in deep layers (Kristensen and Thorup-Kristensen 2007), explaining the higher N accumulation of CAB under CP found in our study. Reduced celeriac yield under SI-CEL compared to CP-CEL in 2019 was attributed to delayed plant development due to delayed transplanting under SI by 14 days caused by high precipitation, which prevented machinery traffic. In 2018, transplanting conditions and yields were similar.

Nitrogen dynamics

Nitrogen use efficiency was comparable between systems for all fields, except CLO 2018/2019 and SI-CAB 2019 (Table 6). This finding indicated that the generally higher N outputs (obtained for CAB 2018, LEE 2018/2019, and LET 2019) justified higher N inputs under SI (Table 4). When including CLO in the average NUE across all fields, NUE was higher under SI compared to CP (Table 6). Full -ear clover improved the NUE of the whole crop rotation under SI, because of high N output (366–376 kg N ha^{-1}, Table 6) per N input via N_2 fixation (300–357 kg N ha^{-1}, Table 4). The overall higher NUE under SI showed that the intensification of organic vegetable production could be achieved sustainably, when full year clover is included in the crop rotation. We confirmed part of our hypothesis that SI improved NUE due to higher N outputs compared with CP.

A generally higher N surface balance under SI than CP was found for LET 2019, CAB 2018, CEL 2019, and LEE 2018/2019 (Table 8). This phenomenon was attributed to higher N inputs under SI (Table 4), which was necessary to supply the greater number of crops grown under SI. Average N surface balance across fields was also higher under SI than CP, which was explained by the high N input through N_2 fixation under SI-CLO (Table 4). Similarly, De Notaris et al. (2018) found that crop rotations including full year green manure had the highest N surplus, which was attributed to the N_2 fixation of legumes. Thus, we rejected our hypothesis that N surface balance would be lower under SI compared to CP.

Excess N under SI presents a risk for higher N losses through denitrification, volatilization, and leaching compared with CP, and requires the implementation of measures to reduce N losses. Tei et al. (2020) suggested well-designed crop rotations, growing cover crops and employing inter-cropping as useful management tools to reduce N losses. The calculation of N surface balance in our study did not include the contribution of cover crops grown over winter, because they were considered to recycle N internally. Including cover crops in crop rotations was effective to reduce N leaching (Hefner et al. 2020; Thorup-Kristensen, Dresboll, and Kristensen 2012). Thus, N taken up by cover crops during autumn might have compensated for the higher N surface balance under SI, since cover crops were only grown under SI but not CP. However, growing two vegetable crops per season requires early sowing in spring and late harvesting in autumn, which might not coincide with timely cover crop management and efficiency of cover crops to reduce N leaching risk. Dabney, Delgado, and Reeves (2001) showed that biomass and N accumulation of cover crops declined with delayed sowing date.

Nitrogen leaching risk

Vegetable residues present a high risk of N leaching because of the high quantities of N in their residues and low C/N ratios (Congreves and Eerd 2015; De Neve et al. 2000). The amount of crop residues (mainly cabbage and onion/lettuce) left in the field at harvest was smaller under SI compared to CP in both years, with less N accumulated in residues during 2019. This phenomenon indicated a smaller risk of N leaching from vegetable residues over the winter under SI.

However, increased risk of N leaching under SI was detected under CAB (Figure 4), in which two short-season cabbages were grown compared with one long-season cabbage under CP. The ca. 78 kg ha^{-1} higher fertilizer N application under SI compared to CP (Table 4) contributed to the increased risk of N leaching. Moreover, the deeper and more extensive root system of long-season cabbage, reaching 2.4 m depth (Kristensen and Thorup-Kristensen 2007) under CP might have improved soil N uptake, especially from deeper soil layers, potentially reducing the risk of N leaching. This phenomenon was detected in a previous study in Denmark where deep rooted white cabbage (2 m) was better at reducing soil mineral N in soil layers of 1–2.5 m depth by more than 100 kg N ha^{-1} compared with shallow rooted leek (0.5 m) (Thorup-Kristensen 2006). Similarly, N leaching risk was higher under SI compared to CP in LET, probably due to 24–63 kg ha^{-1} higher N application and more shallow onion roots compared to lettuce roots. Still, the inclusion of hairy vetch/black oat and red clover/white clover cover crops in SI probably were effective tools to reduce N leaching risk as indicated by lower soil mineral N in spring in CLO-SI. Tonitto, David, and Drinkwater (2006) also showed that legumes can reduce N leaching risk by 40% compared with bare soil.

From a crop rotation perspective, focus is required on the low NUEs of LEE and LET (16%–38%), as they present a particularly high risk for N leaching during winter, whereas the NUEs of CAB and CLO were higher (Table 6). Interestingly, soil mineral N content in autumn was similar between SI and CP under LEE, but was lower in deeper soil layers under SI under LET the following spring (Figure 4). This result indicates the positive effect of reducing N leaching by growing lettuce before leek, and of using winter rye cover crops after the leek harvest. In particular, Kristensen and Thorup-Kristensen (2007) suggested that knowledge about differences in root growth between species is required to design crop rotations with high NUEs.

Nitrogen output included N removed in harvested products and legume cuttings. Nitrogen surface balance was calculated as the difference between N input (N in fertilizer + N_2 fixed by legumes (Table 4) + atmospheric N deposition) and N output. NUE was calculated as the percentage of N output per N input. SI = sustainably intensified system, CP = common practice. Mean values are presented with standard error (n = 3). Different superscript letters indicate significant differences for years separately ($P < .05$).

Growing clover over winter under SI reduced the risk of N leaching compared with bare soil under CP in CLO. This phenomenon was evidenced by the lower soil mineral N content the following spring (Figure 4). Reduced N leaching losses in cover-cropped systems are mainly the effect of increased root exploitation by cover crops in contrast to bare soil, which improve N retention (Thorup-Kristensen, Dresboll, and Kristensen 2012).

Our findings support our hypothesis that N leaching risk was comparable between systems, because N taken up by cover crops grown over winter compensated for higher N surplus under SI. This phenomenon was recorded in all fields, except for CAB and LET, possibly due to the shallower root depth of short-season cabbage and onion compared with long-season cabbage and lettuce under CP. Therefore, the crop rotation of the SI system could be improved by incorporating knowledge about differences in root growth between species, cropping duration and the earlier establishment of cover crops (e.g. by under-sowing).

Conclusion

Designing a cropping system that has an increased number of crops, using plant-based fertilizers, cover crops, and reducing tillage facilitated the sustainable intensification of organic vegetable production. Even though N input was higher under SI compared to CP, 19% of N input was obtained within the crop rotation from N_2 fixed by clover and legume cover crops. Potentially 34% self-sufficiency could have been obtained if all clover cuttings had been applied. In contrast, CP mainly depended on external N input from animal manure. Above ground biomass was 16% higher under SI compared to CP, due to high clover production and growing a greater number of crops. Marketable yield was generally comparable between systems, despite the absence of marketable yield in full-year clover under SI. This was because the higher marketable yield of double cropped lettuce and leek under SI counterbalanced the yield disadvantage of full year clover. Nitrogen use efficiency was comparable between systems for all vegetable fields, and was, even, improved, when including full year clover, indicating an advantage of the SI system. However, N surface balance was higher for SI compared to CP, mainly due to higher N inputs under SI, which increased the risk of N leaching. In particular, N leaching risk was higher following two short-season cabbages under SI compared to long-season cabbage under CP, as well as onion under SI compared to lettuce under CP. This phenomenon might be attributed to lower N uptake from the deeper soil layers of shallower rooted crops under SI. But the inclusion of cover crops in the rotation indicated a reduced N leaching risk under SI. In conclusion, the SI system could be implemented to realize the sustainable intensification of vegetable production; however, focus is required on managing N surplus in the system, e.g. by including cover crops for N recycling.

Abbreviations

C	carbon
CAB	cabbage field
CC	cover crop
CEL	celeriac field
CLO	clover field
CP	common practice
cv	cultivar
LEE	leek field
LET	lettuce field
N	nitrogen
NUE	N use efficiency
SI	sustainable intensification

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Disclosure statement

A potential competing interest exists, as Richard de Visser is employed as an advisor at the company HortiAdvice A/S, where he advises organic farmers on how to improve vegetable production.

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