Can plant-based nitrogen replace externally produced animal-based nitrogen?

Designing a self-reliant legume-based organic strip cropping system

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Can Plant-Based Nitrogen Replace Externally Produced Animal-Based Nitrogen? Designing a Self-Reliant Legume-Based Organic Strip Cropping System

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Abstract

Organic agriculture is facing the challenge to provide sufficient crop nutrients, in particular nitrogen, that are based on organic sources. A novel legume-based strip cropping system that uses grass clover as chop and drop fertilizer has great potential to deal with this challenge. This system utilizes genetic, temporal and spatial dimensions of crop diversification. However, design and practical implementation are still in the early days. This study aimed to investigate ways to improve the delivery of nitrogen through the temporal and spatial incorporation of leguminous species (grass clover, pea and fava bean) and use of grass clover as chop and drop fertilizer in a strip organic cropping system. The effect on dry matter yield and nitrogen yield for potato, winter wheat, cabbage and barley in the legume-based cropping system were compared with results of an animal manure-based cropping system. Soil available nitrogen and nitrogen uptake were assessed using various tools, such as soil analysis, chlorophyll meter and the NDICEA model. Results indicate that, when nitrogen delivery is carefully synchronized in time and applied in specific amounts, there were no significant differences between the two systems in terms of nitrogen delivery and effect on yield. To optimize the legume-based cropping system, synergies and trade-offs need to be balanced, in particular with regard to the cereal and legume mixture.

Keywords: chop and drop fertilizer; crop diversification; crop rotation; intercropping; legume-based strip cropping; NDICEA model; nitrogen; organic agriculture; soil fertility

1. Introduction

Organic agriculture in the Netherlands is still relatively modest in terms of land area (occupying 66,623 ha or 3.8% of the total agriculture area) and below average compared to other European Union countries [1]. Between 2010 and 2018, the number of registered organic farms increased by more than 20% from 1658 to 2012. The number is expected to increase by another 7% in 2019 compared to 2018 [1]. Most of the organic farms are highly specialized and intensive, growing high value crops such as potatoes, onions, carrots, cabbages and sugar beets, on a rotation basis with wheat and rye or grass clover. Most commonly, they practice a six-year crop rotation, which meets the requirement for soil health, weed suppression and protection against pest and diseases [2]. Over the last 10 years, demand for organic produce in the country has increased by an average of 8.5% annually in sales volume. Expectations are that the demand will continue to grow driven mostly by the perceived benefits to human health and the safety of organically produced foods. This increasing demand has resulted in both opportunities and challenges for organic farmers and their efforts to keep supply apace with demand under a changing climate.

Good soil fertility in organic farming is fundamental for crop health, productivity and human health. "Feeding the soil, not the plant," is an old mantra of organic practitioners that remains as relevant today as it was 50 years ago [3]. Unlike conventional farming, organic farming practices depend on a long-term, integrated and cyclical approach of nutrient management [4]. An adequate supply of nutrients to crops depends on appropriate soil management (including plant residues) and nutrient release as a function of mineralization processes in soils [5]. In organic farming, the major challenge is to provide enough nutrients based on organic sources, N in particular, for plant growth while sustaining soil quality [6]. Adequate supply of N is critical for organic crop productivity; sufficient nitrogen availability enhances rapid, early establishment and development of healthy crops, which, in turn, suppresses weed infestation [7,8].

To address the N challenge in organic farming, various management options exist to meet plant nutritional needs, such as animal-based manures, green manures, cover crops, composting and crop rotations with inclusion of 25 percent or more legumes [4,9]. However, these options are not optimal. The use of large amounts of animal manure or slurry to leads to nitrogen leaching, denitrification and ammonia volatilization. It also increases the risk of phosphorus movement to surface water, causing eutrophication [10]. Crops that are fertilized using cattle slurry experience a shortage of N due to lower N/P ratios in manure with regard to crop demand [11]. The use of green manure and compost requires more time to decompose and has a slow N release rate influenced by factors such as soil moisture level, temperature, texture, mineralogy and acidity [12,13]. Due to the difficulty to synchronize the application of N and other nutrients according to plant demand, yields are often lower with higher dry matter crop content than in conventional agriculture [6].

Inclusion of legume species in a crop rotation scheme can address some of these problems, but it is not easy to predict adequately the actual amount of nitrogen fixed. Factors that influence N fixation include species/cultivar used, weather conditions and growing stage of the legume crop [4]. A novel fertility building strategy aiming to overcome these constraints, is the legume-based strip cropping system, which increases field level diversity and enables the utilization of genetic, temporal and spatial dimensions of crop diversification [14,15]. In this system, single fields are subdivided in strips with different crops. Legumes, such as grass-clover, can be grown next to field crop(s) and used as chop and drop fertilizer to meet the nitrogen demand of different crops that are not met by rotation and intercropping with legumes. Leguminous species, such as fava bean (also known as broad bean) and pea can be mixed with the field crop(s).

Research findings [16] demonstrate that the introduction of a legume in crop rotations and in an intercropping system improves soil fertility (soil organic carbon, humus content and N and P availability). This is due to the capability of legumes to fix atmospheric nitrogen and make it available to succeeding and companion crops. As a result, external inputs can be reduced, and, consequently, greenhouse gas emissions are lowered [15,16]. The direct use of crops as a fertilizer can increase the nitrogen use efficiency as feeding crops to animals and producing manure incur nitrogen losses of 20 % -30 % [17, 18].

The crucial question strip cropping points to is: can plant-based nitrogen replace externally produced animal-based nitrogen? To contribute to answer this question, a novel research experiment was designed and carried out in Wageningen, the Netherlands. The objectives were to: 1) investigate how temporal (rotation-wise) and spatial incorporation of leguminous crop species in a strip organic cropping system can improve the delivery of nitrogen; 2) identify the effect on the dry matter yield and nitrogen yield of wheat, potatoes, spring barley, cabbage and sugar beet, and 3) design a self-reliant legume-based organic strip cropping system. In order to achieve these objectives, four research questions were formulated: 1) How much nitrogen is produced, where and when? 2) How much nitrogen is available for the plants and when? 3) What is the effect on crop yield of wheat, potatoes, spring barley, sugar beet and cabbage? 4) How can production and consumption of nitrogen be synchronized? It was hypothesized that in order to provide enough N from grass clover, timely application will be required. The second hypothesis was that growing legumes appropriately in time and space in strip cultivation will produce yields for wheat, potato, barley, sugar beet and cabbage comparable to the practices of rotation and use of animal manure.

2. Materials and Methods

2.1. Experimental Site and Design

The field experiment was conducted from March to November 2019 following the crop cycle on the Droevendaal organic experimental farm in Wageningen, the Netherlands (51°59’33.06” N, 5°39’43.56” E). The soil type found on the farm is classified as sandy consisting of 3% clay, 12% silt and 85% sand with an average organic matter content of 3.8%. The experimental set up used the existing incomplete block design established on the Droevendaal farm comprised of three fields. Each field is divided in three blocks, which are subdivided in strips of three treatments; with crops grown in pairs (**Appendix A**). For this study, the three different treatments are:

- Reference-time (referred to as ref_time): a mono reference field with a single crop species. Animal manure (both solid and slurry) was used as a source of nutrients;
- Strip (referred to as strip): a single crop sown in a pair with a second single crop in a strip; animal manure in solid form as well as slurry were used as a source of nutrients for all the crops.
- Strip with additive design (referred to as strip_add): with a main crop variety grown together in a strip and in an alternated strip with a leguminous crop (grass clover, fava bean and pea). Crops included were potato, winter wheat, sugar beet, cabbage, spring barley and grass clover. The crop rotation was based on the dominant crops grown by Dutch organic farmers **(Appendix B).** The sugar beet germinated but failed to grow. It was not replaced by another crop.

The details of the three different treatments, including the different crops and related management practices that were tested and compared, are presented in **Table 1**.

Table 1. Overview of crops, varieties, treatments, companion crops, planting dates and fertilizer applications used in the strip cropping experiment on the Droevendaal farm, Wageningen

Crop	Variety	Treatment	Companion crop	planting date	Planting density	Fertilizer	Application date	N applied (kg/ha)
Potato	Agria	ref_time	potato-ryegrass	May 03, 2019	potato -3000 kg/ha	20 t/ha LM[1],5 t/haFYM[2]	Apr 29,2019	164
	Agria	strip	potato-ryegrass		rye grass -35 kg/ha	20 t/ha LM,5 t/ha FYM		164
	Agria	strip_add	potato-grass clover		rye grass -30 kg/ha red clover- 5kg/ha	3.1 ton/ha DM[3] chop and drop fertilizer		37.2
Wheat	Julius	ref_time	wheat-cabbage	Oct 05, 2018	wheat -200 kg/ha	15 t/ha FYM	Apr 29,2019	60
	Julius	strip	wheat-cabbage		cabbage-3100 plants/ha	15 t/ha FYM		
	Julius	strip_add	wheat+fava-cabbage		fava- 20,000 plants/ha	N/F[4]	N/F	N/F
Cabbage	Rivera	ref_time	cabbage-wheat	May 23, 2019	cabbage - 3100 plants/ha	20 t/ha LM,5 t/ha FYM 2 t/ha OPF[5]	 Apr 29,2019	164 220
	Rivera	strip	cabbage-wheat		wheat -200 kg/ha	20 t/ha LM,5 t/ha FYM 2 t/ha OPF	 Aug 08, 2019	164 220
	Rivera	strip_add	cabbage-wheat+fava			2 t/ha OPF	Aug 08,2019	220
Barley	Irina	ref_time	barley-sugarbeet	May 02, 2019	barley- 200kg/ha	15 t/ha FYM	Apr 29, 2019	60
	Irina	strip	barley-sugarbeet			15 t/ha FYM	Apr 29,2019	60
	Irina	strip_add	barley+pea-sugarbeet		pea - 20,000 plants/ha	N/F	N/F	N/F

[1] LM: liquid manure (cattle slurry),[2] FYM: Farmyard manure, [3] DM: Dry Matter, [4] N/F: No Fertilizer applied, [5] OPF (Organic Plant Fertilizer).

2. 2. Research Approach: the Describe, Explain, Explore, Design Research Cycle

The Describe, Explain, Explore, Design (DEED) research cycle was used to analyze the current organic strip cropping system and design an improved legume-based strip cropping system [19]. This allowed investigating if the legume-based cropping system could provide enough nitrogen without having to add animal manure to reach a targeted yield. The use of DEED allows identifying the uncertainties and levers for change in designing best-fit options to overcome noted constraints in the current strip organic system(s). The four phases of the cycle are briefly described.

In the Description and Explanation phases, the current crop yields and soil mineral N collected from three different treatments were analyzed based on field observations and the outcomes of the Nitrogen Dynamics in Crop rotations in Ecological Agriculture (NDICEA) model [20]. The NDICEA model was used to estimate the amount of available nitrogen and nitrogen uptake by each crop grown in 2019. A two-year crop scenario (2018 and 2019) for three different fields and three different treatments (ref_time, strip and strip_add) for four crops (potato, winter wheat, cabbage and winter barley) was generated. In order to generate nitrogen availability and uptake for potato, leek was planted as a pre-crop in 2018. For winter wheat, the pre-crop was potato; for cabbage, grass clover or rye grass (grown for one and a half year); and cabbage for winter barley.

In the exploration and design phase, the NDICEA model was used to explore a six-year crop rotation scenario for animal manure-based cropping system and a legume-based cropping system. The simulation was generated aligned with current organic farmers' yields (potato, 35 t/ha; winter wheat, 3.5 t/ha; sugar beet, 50 t/ha; cabbage, 50 t/ha; winter barley, 3.5t/ha) following the current fertilizer regime (**Table 1**). The weather data for 2019 were used for the six years. For the animal manure-based cropping system, animal manure for each crop was provided. Multiple scenarios for legume-based cropping system with targeted yield and different management practices were explored to identify these requirements. The N content of grass clover used in NDICEA model was 1.2 % and 1.8 % respectively. Grass clover production used was 10 DM t/ha based on the grass clover harvests of April and July 2019. The results from different explorations were used to develop a new, improved legume-based design. In order to obtain the scenario that represents strip additive (the mixture of main crop and leguminous crop), planting and harvesting dates for wheat, fava bean, barley and pea were identical, but planting densities differed by crop. Specific quantities and timings of fertilizer applications for each crop were determined based on scientific literature.

2.3. Soil Data

Soil samples were taken from two strips for strip and strip_add excluding the buffer strip and 10 meters from each side of the strips. For ref_time, the samples were taken from the middle rows. In every sample, 12 subsamples were collected from a soil depth of 25 cm according to soil tillage depth in a zig-zag pattern using a soil auger. The 12 subsamples were mixed together to form a composite sample. The composite soil samples were dried at 40^{o}c for 48 hours and sieved at 1mm. The prepared samples were sent to the laboratory for mineral nitrogen analysis. T**able 2** presents the number of soil samples and sampling schedule. The detail sampling procedures for each crop are presented in **Appendix C.**

Table 2. Soil samples and sampling schedule per crop per treatment for five crops (2019)

Crop	**Treatment**	**Total number of samples**	**Dates**
Potato	ref_time	3 fields x 1 composite sample = 3	Apr 25, Jun 11, Sep 18
	strip	3 fields x 2 composite samples = 6	
	strip_add	3 fields x 2 composite samples = 6	
Winter wheat	ref_time	3 fields x 1 composite sample = 3	Apr 11, Jul 29
	strip	3 fields x 2 composite samples = 6	
	strip_add	3 fields x 2 composite samples = 6	
Sugar beet	ref_time	3 fields x 1 composite sample = 3	Jun 25
	strip	3 fields x 2 composite samples = 6	
	strip_add	3 fields x 2 composite samples = 6	
Cabbage	ref_time	3 fields x 1 composite sample = 3	Apr 26, Jun 11, Nov 6
	strip	3 fields x 2 composite samples = 6	
	strip_add	3 fields x 2 composite samples = 6	
Spring barley	ref_time	3 fields x 1 composite sample = 3	Apr 26, Jun11 Aug 22
	strip	3 fields x 2 composite samples = 6	
	strip_add	3 fields x 2 composite samples = 6	

2.4. Plant Data

The SPAD-502 (Soil Plant Analysis Development) meter was used for potato, winter wheat, cabbage and spring barley to measure in season N status following the critical stages of crop development [21,22,23,24,25,26,27]. The plant samples and sampling schedule is presented in **Table 3** and detailed sampling methods are described in **Appendix C**.

Table 3. SPAD measurement and sampling schedule per crop per treatment for four crops (2019)

Crop	Treatment	Total number of samples	Date
Potato	ref_time	3 fields x 30 plants = 90	18 Jun, 2 Jul, 16 Jul, 30 Jul
	strip	3 fields x 2 strips x 15 plants = 90	
	strip_add	3 fields x 2 strips x 15 plants = 90	
Winter wheat	ref_time	3 fields x 30 plants = 90	9 May
	strip	3 fields x 2 strips x 15 plants = 90	
	strip_add	3 fields x 2 strips x 15 plants = 90	
Cabbage	ref_time	3 fields x 30 plants = 90	13 Jun, 8 Jul, 31 Jul
	strip	3 fields x 2 strips x 15 plants = 90	
	strip_add	3 fields x 2 strips x 15 plants = 90	
Spring barley	ref_time	3 fields x 30 plants = 90	14 Jun, 27 Jun
	strip	3 fields x 2 strips x 15 plants = 90	
	strip_add	3 fields x 2 strips x 15 plants = 90	

2.5. Analysis of N Delivery from Grass Clover

As part of the overall experiment, a litterbag experiment to monitor the decomposition of grass clover was carried out guided by the grass clover harvesting regime in the experimental field [28,29]. The litterbags were 30 cm x 20 cm with a mesh size of 1mm to exclude earth worms and avoid excretion contamination. Two batches (12 litterbags/batch) from two harvests with different N content (1.2 % N and 1.8 % N) were buried at different times to evaluate N release from grass clover. The first batch of four litterbags of 30 g was buried randomly at 15 cm depth in the potato ridges in the three fields and monitored from spring to summer. The second batch of litterbags was buried in between the cabbages at 15 cm depth in the three fields and monitored from summer to autumn.

At the beginning of the experiment, the grass clover with leaves and stems was collected and oven dried at 70 ^{o}C for 28 hours, then grounded to 1 mm length and thoroughly mixed to determine total % of N and calculate the remaining N [28]. From each replicate, one litterbag was excavated at 2, 4, 8, 12 weeks [28, 29]. At each sampling moment, the remaining plant material was taken out from the litterbags and oven dried at 70 ^{o}C for 24 hours, then grounded and sieved using a 1 mm sieve to determine remaining N percentage and ash content of the grass clover. The percentage of N released over time from the grass clover was calculated using the following equation (Equation 1) [28,29]:

$$\text{N released (\%)} = (\text{Nt}/\text{No}) \times 100 \qquad (1)$$

Where, Nt is the amount of N remaining at the excavation time, t (mg/g) and No is the initial N content (mg/g) in grass clover.

After collecting the samples, the roots of the plants were removed manually, and soil contamination was corrected using the Equation (2) based on Cusick et al. (2006) [30]:

$$\text{SC} = (\text{ACAR} - \text{ACBP}) / \text{ACS} \qquad (2)$$

Where SC is the dry weight of soil contamination (g), ACAR is the ash content of plant material in litterbag (mg) after removal, ACBP is the ash content of plant material in litterbag (mg) before its placement, and ACS is the ash content of the soil (mg g−1).

The N disappearance at each sampling event was expressed relative to the initial amount. The pattern of N disappearance during the time was further used to calculate the apparent initial age of the grass clover. More details are found in section 2.7.

2.6. Dry Matter Yield and N Yield

For all the crops, the dry matter yield and N yield was determined using fresh weight, dry matter ratio and total N percentage content of the crops.To determine fresh yield for potato, in strip and strip_ add, the fresh yield of potato from each row was measured (strip length x 0.75 m). For ref_time, the potato yield from four middle rows was measured (strip length x 0.75). A subsample of 1 kg each from edge and middle rows was taken for strip and strip_add; for ref_ time, only four middle rows were taken which were cleaned and weighed to determine fresh weight (FW). The cleaned potatoes were then sliced into small pieces and oven dried at 70 °C for 72 hours to determine the dry weight (DW). The dry matter ratio (DMR) was calculated to determine the dry matter yield of the potatoes. The oven-dried samples were grounded and sieved at 1 mm for N analysis.

The wheat harvest was conducted for the edge and middle rows of strip and strip_add (with 1.5 m width) using the combine harvester. For ref_time, only the middle rows (3m) were harvested. In strip_add, the wheat grains were separated from the fava bean. Both grain and bean seed fresh yield were measured. One thousand wheat grains selected using an automated seed counter. The fresh weight and dry weight of the thousand grains were measured. A subsample of fava bean was measured to determine fresh weight. Wheat grains and fava beans were oven dried at 70 °C for 48 hours and dry weight was measured. Then they were milled using a 1.5 sieve and sent for N analysis to the laboratory. The same method was followed for spring barley to determine the fresh yield, dry matter and N content of the grains.

The fresh yield of cabbage was conducted row wise in strip and strip_add. In ref_time, fresh yield was taken from the four middle rows. Two cabbages from each row were sampled. The head leaves and stems were removed, sliced and weighed to determine fresh weight. The processed cabbage was oven dried at 70 °C for 48 hours and then weighed again to obtain dry weight. The dried samples were milled, and N content was analyzed.

2.7. Modelling

The following variables were analyzed using the NDICEA model: available nitrogen and nitrogen uptake by the crops over time. Since the model is target-oriented and supports decision-making processes at tactical and strategic levels [20], the following experimental data were used as inputs for the modelling: yield, dry matter content, nitrogen content of wheat, potato, cabbage and barley, measured soil mineral nitrogen and soil organic matter content (**Appendix B**). Since crop residue and N content of crop residue were not measured, default model values were used. The nitrogen content of rye grass was set to 2% based on the color of the rye grass observed in the field. The sugar beet yield (50 t/ha) was determined based on the available literature [31] and the default values for dry matter content (20%) and nitrogen content (0.55% for the beet) were used. The yearly nitrogen deposition (43 kg/ha/year) of the province of Gelderland (of which Wageningen is part) was determined based on a recent study [32]. Measurements of the ground water level and nitrate content in the groundwater were taken from a previous experiment on the Droevendaal farm [33].

The main parameters, including Saturated Soil Moisture Content or SMO (the volumetric moisture content on saturated soil or pore space, in m/m3), GAM (a texture-specific shape factor that describes the pF curve), Crc and Crx (parameters that describe capillary rise dynamics depending on the distance of the specific soil layer and water table, in cm), were estimated by matching the calculated pF with the pF curves and soil moisture content in the soil layers of 0-30 cm and 30-60 cm of the experimental field, using a formula developed by Wosten et al. (1986) [34] for different soils.

The results from the litterbag experiment carried out over 12 weeks were used to find the best-fit apparent initial age of the grass clover that was incorporated to the soil, using the Equation (3)[35]:

$$yt = x \exp 4.7\{(a+t)-0.6 - a-0.6\} \tag{3}$$

Where yt is remaining organic matter, x is the initial amount of organic matter, a is apparent initial age of the organic material and t is starting time.

In this formula, the value for organic matter was replaced by nitrogen content since only nitrogen was measured in this experiment. The assumption made was that the source of nitrogen does not change strongly. The initial age determined was used as parameter in the NDICEA model for calibration. The weather environment file for the NDICEA model was developed based on the precipitation and temperature data taken from the Droevendaal weather station, while the evapotranspiration data were obtained from the Royal Dutch Metrological Institute weather station of De Bilt (located at 52 km from Wageningen).

2.8. Statistical Analysis

Yield, dry matter ratio, dry matter yield, N content, N yield, available soil mineral N and soil organic matter measured in 2018 and 2019 were averaged according to treatments and fields. These averaged data were used by the NDICEA model to generate a two-year crop scenario (a pre-crop grown in 2018 combined with a crop grown in 2019). In total, 36 two-year crop scenarios were generated. For each crop, the available nitrogen from the top 30 cm of the soil and the nitrogen uptake by the crops were calculated treatment wise from each field. These data covered the period between planting and harvesting date of each crop. All the data were assessed for normality with the Shapiro-Wilk Normality test [36]. The crop and soil available mineral nitrogen collected from field experiment and the data extracted from NDICEA model were analyzed with the Linear Mixed-Effects model (LMMs) with lme4 package [37]. In the linear mixed effect model, the treatments were selected as fixed effect. To account for the variation among the fields due to different past management practices, different ground water level and soil organic matter, the field was selected as random effect. To determine the significance of fixed variables on model, linear model testing was done. This was followed by determining the significance level between the treatments using the pairwise comparison using the lsmeans package by Tukey's method as a post-hoc test [38]. Statistical software R [39] was used to analyse all the data of this research project. Different statistical mean values ($p<0.05$) are indicated by different letters.

3. Results

3.1. Description Phase: Current Organic Strip Cropping System

3.1.1. Nitrogen Availability in the Soil and Nitrogen Uptake of Potato

The early season soil available nitrogen level in the potato field was not significantly different between strip-add and ref_time. The result for strip_add (53.34 ± 11.61 kg N/ha) and ref_time (55.88 ±4.92 kg N/ha) was significantly higher than strip (35.05 ± 3.17 kg N/ha). Over time, the available soil nitrogen levels for both ref_time (47.18 ± 6.13 kg N/ha) and strip_add (47.78 ± 4.83 kg N/ha) decreased to a level equivalent to strip (46.02 ± 4.77 kg N/ha). Relative crop uptake measured at two weeks after emergence (18 June) using the SPAD meter indicated that the strip_add uptake was in between ref_time and strip. Throughout the growing season, the SPAD measurements value for strip_add were decreasing, but they remained significantly higher than for ref_time and strip. The above ground plant N analysis carried out during tuberization did not reveal a significant difference between the treatments (**Table 4**).

Table 4. Comparison of soil mineral N, SPAD value and above ground plant N between different treatments of potato on the Droevendaal farm, Wageningen (2019)

Potato	**Treatment**				**Date**
	ref_time	**strip**	**strip_add**	***p*_value**	
T1soil$_{min}$ N(kg/ha)	55.88 ± 4.92^{a}	35.05 ± 3.17^{b}	53.34 ± 11.61^{a}	0.028*	25 Apr
T2soil$_{min}$ N(kg/ha)	30.75 ± 13.53	36.51 ± 6.22	26.79 ± 6.28	0.147 ns	11 Jun
T3soil$_{min}$ N(kg/ha)	47.18 ± 10.61	46.02 ± 4.77	47.78 ± 4.83	0.884 ns	25 Aug
Above ground plant N (%)	2.55 ± 0.19	2.17 ± 0.29	2.48 ± 0.28	0.226 ns	15 Jul
R1 SPAD measurement	44.54 ± 3.32^{b}	45.68 ± 3.37^{a}	45.25 ± 2.99^{ab}	0.055 ns	18 Jun
R2 SPAD measurement	42.35 ± 3.19^{b}	43.99 ± 2.89^{a}	43.47 ± 2.19^{a}	<0.001***	2 Jul
R3 SPAD measurement	39.08 ± 3.52^{b}	40.59 ± 3.15^{a}	40.55 ± 2.87^{a}	0.002**	16 Jul
R4 SPAD measurement	32.78 ± 4.61^{c}	34.32 ± 3.80^{b}	36.66 ± 2.93^{a}	<0.001***	30 Jul

Different mean values (±standard deviation) followed by different letters indicate statistically significant differences according to Tukey HSD test, $p<0.05$ at 0.95 confidence level. *p*-value significance refers to: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘ns’ 1(not significant). T1 soil$_{min}$: First soil mineral measurement, T2 soil$_{min}$: Second soil mineral measurement, T3 soil$_{min}$: Third soil mineral measurement, R1 SPAD measurement: First SPAD measurement, R2 SPAD measurement: Second SPAD measurement, R3 SPAD measurement: Third SPAD measurement, R4 SPAD measurement: Fourth SPAD measurement.

The total available nitrogen calculated by the NDICEA model for strip_add was 54.57 ± 27.41 kg N/ha which was significantly lower than ref_time and strip (83.46 ± 23.48 kg N/ha and 81.42 ± 22.44 kg N/ha) (**Figure 1**). The cumulative nitrogen uptake for ref_time, strip and strip_add were 53.15 ± 50.47 kg N/ha, 49.44 ± 41.56 kg N/ha and 47.69 ± 38.72 kg N/ha respectively. No significant differences were found in cumulative nitrogen uptake between treatments.

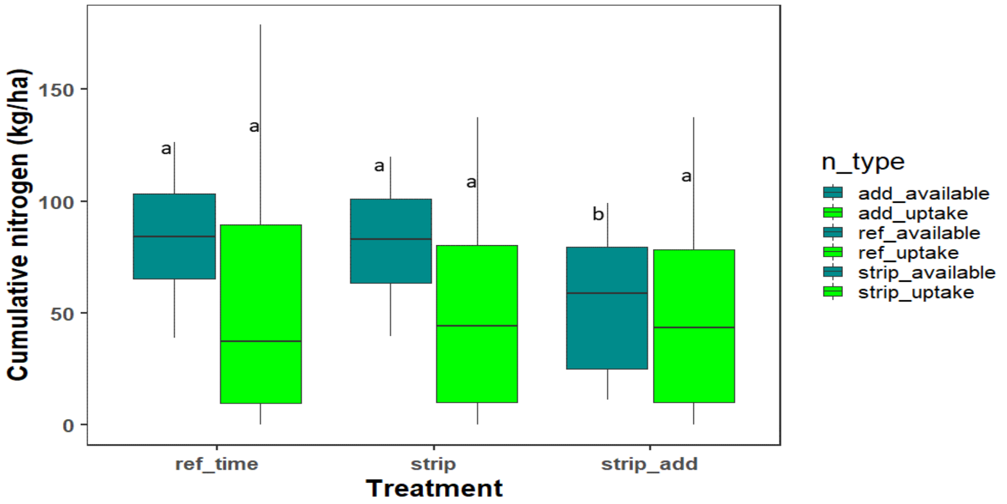

Figure 1. Differences between the three treatments in cumulative available nitrogen and nitrogen uptake for potato. The different letters indicate statistically significant differences according to Tukey HSD test, p<0.05 at 0.95 confidence level.

3.1.2. Nitrogen Availability in the Soil and Nitrogen Uptake of Winter wheat

The soil analysis conducted during the vegetative growth (on 11 April 2019) varied between 8.25 kg N/ha and 10.02 kg N/h and was low for all three treatments without a significant difference. The SPAD measurement taken before booting stage on 9 May 2019, did not reveal a significant difference between the treatments (**Table 5**).

Table 5. Comparison of soil mineral N and SPAD values of winter wheat for different treatments on the Droevendaal farm, Wageningen (2019)

	Treatment				**Date**
Wheat	**ref_time**	**strip**	**strip-add**	***p*_value**	
T1soil$_{min}$ N (kg/ha)	10.02 ± 1.71	8.25 ± 0.82	9.45 ± 2.18	0.288 ns	11 Apr
T2 soil$_{min}$ N (kg/ha)	35.50 ± 8.10	32.83 ± 3.77	35.41 ± 5.36	0.663 ns	29 Jul
R1 SPAD measurement	33.59 ± 4.88	32.47 ± 4.46	33.16 ± 5.44	0.308 ns	9 May

Different mean values (±standard deviation) followed by different letters indicating statistically significant differences according to Tukey HSD test, p<0.05 at 0.95 confidence level. *p*-value significance refers to: 0 '***' 0.001 '**' 0.01 '*' 0.05 'ns' 1 (not significant). T1 soil$_{min}$: First soil mineral measurement, T2 soil$_{min}$: Second soil mineral measurement, R1 SPAD measurement: First SPAD measurement.

The cumulative soil available nitrogen amounts of ref_time (32.56 ± 22.72 kg N/ha) and strip_add (33.83 ± 25.71 kg N/ha) had no significant difference. However, a difference was apparent between strip_add (33.83 ± 25.71kg N/ha) and strip (30.08 ± 19.88 kg N/ha); the latter significantly higher than strip (Figure 2). The total nitrogen uptake for strip_add was 25.59 ± 31.93 kg N/ha and was significantly higher than ref_time (16.59 ± 20.42 kg N/ha) and strip (11.42 ± 13.70 kg N/ha).

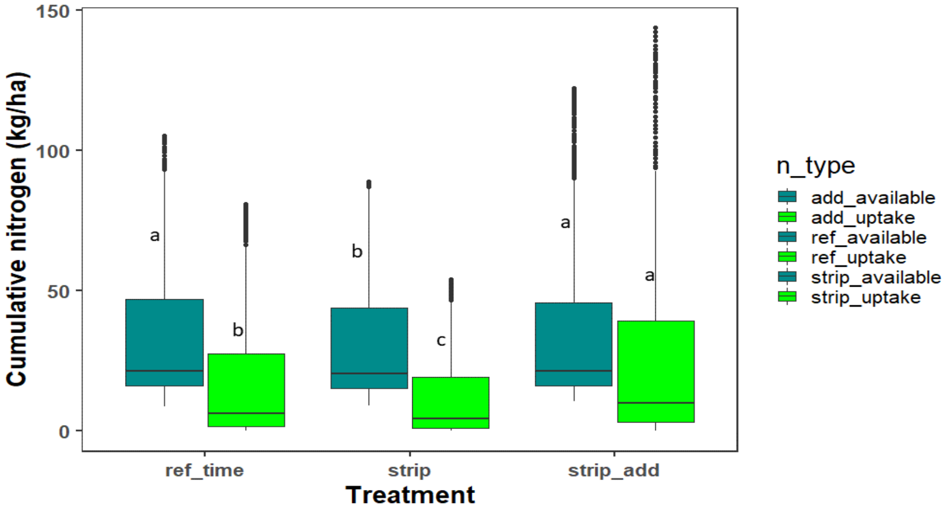

Figure 2. Differences between the three treatments in cumulative available nitrogen and nitrogen uptake for winter wheat. The different letters indicate statistically significant differences according to Tukey HSD test, p<0.05 at 0.95 confidence level.

3.1.3. Nitrogen Availability in the Soil and Nitrogen Uptake of cabbage

No significant differences were observed in soil available nitrogen between treatments at the beginning of the growing season, in-season and at harvest time. The SPAD measurements conducted on 13 June and 8 July revealed significantly higher values for strip_add than ref_time and strip. For the measurement taken on 30 July, the SPAD value for strip_add value dropped down to a level significantly lower than ref_time (63.11 ± 6.08). There was no significant difference between strip-add and strip (**Table 6**).

Table 6. Comparison of soil mineral N and SPAD values of cabbage for different treatments on the Droevendaal farm, Wageningen (2019)

Cabbage	**Treatment**				**Date**
	ref_time	**strip**	**strip_add**	***p*_value**	
T1 soil$_{min}$ N (kg/ha)	38.98 ± 13.75	39.88 ± 11.63	32.38 ± 10.22	0.717 ns	26 Apr
T2 soil$_{min}$ N (kg/N)	39.47 ± 11.09	37.69 ± 6.10	39.14 ± 9.99	0.944 ns	11 Jun
T3 soil$_{min}$ N (kg/ha)	17.07 ±1.74	19.7 ± 4.32	21.16 ± 3.55	0.324 ns	29 Oct
R1 SPAD measurement	52.61 ± 6.14^{b}	53.91 ± 4.69^{b}	56.49 ± 5.16^{a}	<0.001***	13 Jun
R2 SPAD measurement	61.29 ± 6.46^{b}	61.53 ± 5.41^{b}	63.43 ± 7.18^{a}	0.049*	8 Jul
R3 SPAD measurement	66.43 ± 5.52^{a}	63.93 ± 5.7^{b}	63.11 ± 6.08^{b}	<0.001***	30 Jul

Different mean values (±standard deviation) followed by different letters indicating statistically significant differences according to Tukey HSD test, p<0.05 at 0.95 confidence level. *p*-value significance refers to: 0 '***' 0.001 '**' 0.01 '*' 0.05 'ns' 1 (not significant). T1 soil$_{min}$: First soil mineral measurement, T2 soil$_{min}$: Second soil mineral measurement, T3 soil$_{min}$: Third soil mineral measurement, R1 SPAD measurement: First SPAD measurement, R2 SPAD measurement: Second SPAD measurement, R3 SPAD measurement: Third SPAD measurement.

Based on the NDICEA model output, cumulative available nitrogen was significantly lower for strip_add (80.55 ± 21.53 kg N/ha) compared to ref_time (111.66 ± 27.98 kg N /ha) and compared to strip (102.95 ± 23.81 kg N/ha) (**Figure 3**). The cumulative N uptake for cabbage was significantly lower for strip_add (55.91 ± 41.21 kg N/ha) than for ref_time (98.88 ± 70.23 kg N /ha) and strip (80.91 ± 55.94 kg N/ha).

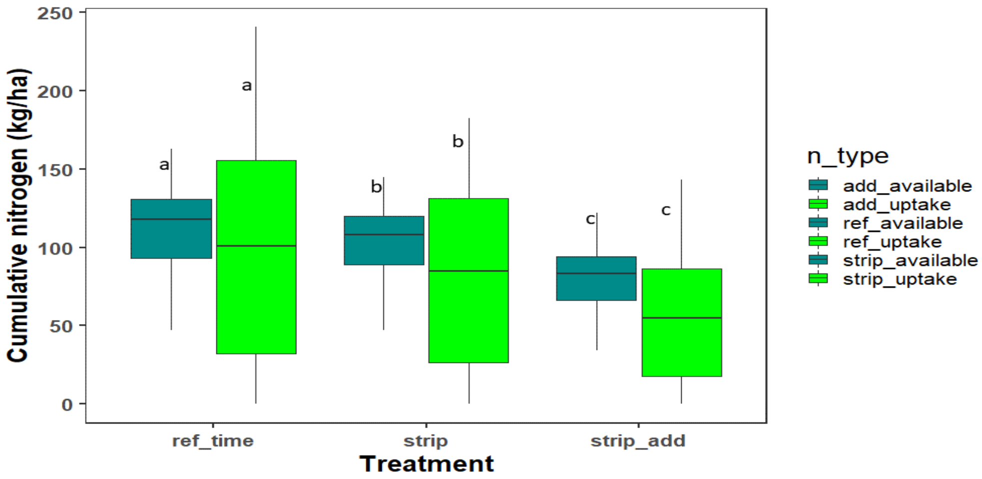

Figure 3. Differences between the three treatments in cumulative available nitrogen and nitrogen uptake for cabbage. The different letters indicate statistically significant differences according to Tukey HSD test, p<0.05 at 0.95 confidence level.

3.1.4. Nitrogen Availability in the Soil and Nitrogen Uptake of Spring Barley

The amounts of soil available N before sowing, during the growing season and after harvest did not indicate a significant difference between the treatments. The first SPAD measurements taken on 14 June, during the tillering stage were significantly higher for strip_add than ref_time and strip (**Table 7**).

Table 7. Comparison of soil mineral N and SPAD values between different treatments of spring barley on the Droevendaal farm (2019)

Barley	**Treatment**				**Date**
	ref_time	**strip**	**strip_add**	***p*_value**	
T1 soil$_{min}$ N (kg/ha)	38.72 ± 28.88	52.97 ± 13.11	40.97 ± 18.78	0.648 ns	26 Apr
T2 soil$_{min}$ N (kg/ha)	26.97 ± 10.77	33.29 ± 10.44	29.77 ± 8.94	0.652 ns	11 Jun
T3 soil$_{min}$ N(kg/ha)	52.45 ± 6.99	50.84 ± 7.73	51.75 ± 5.26	0.652 ns	22Aug
R1 SPAD measurement	39.25 ± 4.80^{b}	39.81 ± 4.18^{b}	41.27 ± 3.87^{a}	0.005**	14 Jun
R2 SPAD measurement	38.75 ± 4.57	39.50 ± 3.77	39.79 ± 3.89	0.212 ns	27 Jun

Different mean values (±standard deviation) followed by different letters indicating statistically significant differences according to Tukey HSD test, p<0.05 at 0.95 confidence level. *p*-value significance refers to: 0 '***' 0.001 '**' 0.01 '*' 0.05 'ns' 1(not significant). T1 soil$_{min}$: First soil mineral measurement, T2 soil$_{min}$: Second soil mineral measurement, T3 soil$_{min}$: Third soil mineral measurement, R1 SPAD measurement: First SPAD measurement, R2 SPAD measurement: Second SPAD measurement.

Based on the analysis of the NDICEA modelling outcomes, the cumulative soil available nitrogen for strip_add was 24.45 ± 17.39 kg N/ha; significantly lower than ref_time (30.03 ± 21.26 kg N/ha) and strip (29.55 ± 20.48 kg N /ha) (**Figure 4**). The total nitrogen uptake for strip_add (8.14 ± 9.71 kg N /ha) was significantly lower than for ref_time (14.28 ± 16.97 kg N/ha) and strip (14.00 ± 16.52 kg N/ha).

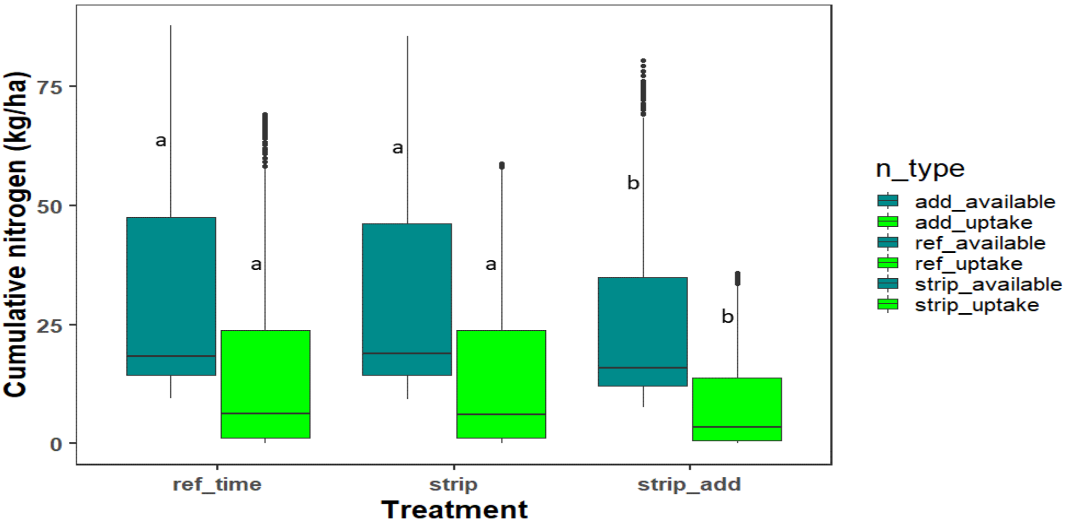

Figure 4. Differences between the three treatments in cumulative available nitrogen and nitrogen uptake for spring barley. Dots represent outliers. The different letters indicate statistically significant differences according to Tukey HSD test, p<0.05 at 0.95 confidence level.

3.1.5. Dry Matter Yield and N Yield of the Crops

There was no significant difference in dry matter yield of potato between the treatments. For winter wheat the dry matter yield for the strip_add (with inclusion of dry matter yield of fava bean) was 0.05 ± 0.03 kg/m^2), which was significantly lower than for ref_time (0.25 ± 0.07 kg/m^2) and for strip (0.19 ± 0.10 kg/m^2). The cabbage dry matter yield for strip_add (0.22 ± 0.07 kg/m^2) was significantly lower compared to ref_time (0.38 ± 0.10 kg/m^2) and strip (0.33 ± 0.05 kg/m^2). For spring barley, dry matter yield for strip_add (0.12 ± 0.03 kg/m^2) was significantly lower than ref_time (0.19 ± 0.07 kg/m^2) and strip (0.18 ± 0.05 kg/m^2) (**Figure 5**).

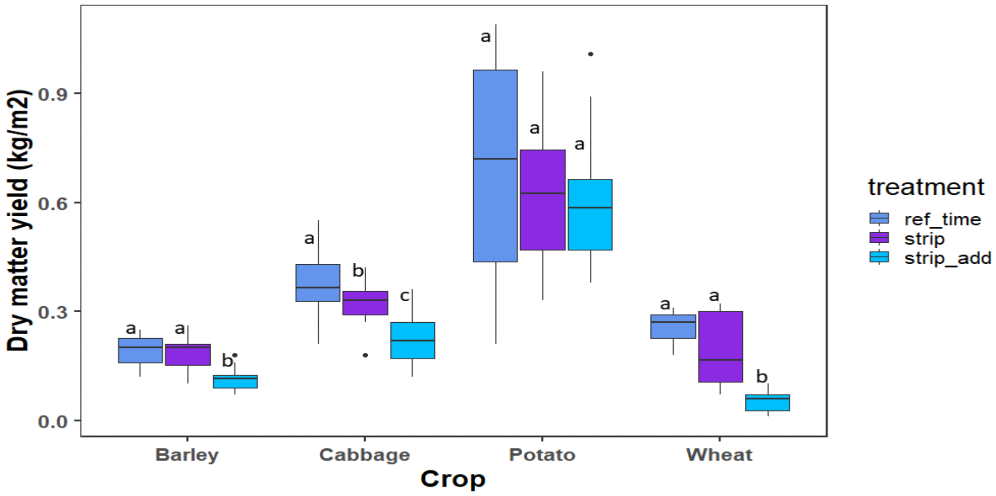

Figure 5. Dry matter yield of spring barley, cabbage, potato and winter wheat for all three treatments. Dry matter yield was calculated by multiplying fresh yield and dry matter ratio for each treatment. For the strip_add the dry matter yield of fava bean is included with wheat dry matter yield. Dots represent outliers. The different letters indicate statistically significant differences according to Tukey HSD test, p<0.05 at 0.95 confidence level.

The nitrogen yields for potato and barley were similar between the treatments. No significant differences between the treatments were observed. However, for winter wheat the nitrogen yield for strip _add (9.16 ± 5.79 kg/m^2) was significantly lower compared to ref_time (42.58 ± 12.56 kg N/ha) and strip (31.90 ± 15.05 kg N/ha). For the cabbage, the strip_add (60.57 ± 17.69 kg N/ha) was significantly lower than for ref_time (107.37 ± 31.61 kg N /ha) and strip (89.79 ± 14.45 kg N/ha). (**Figure 6**).

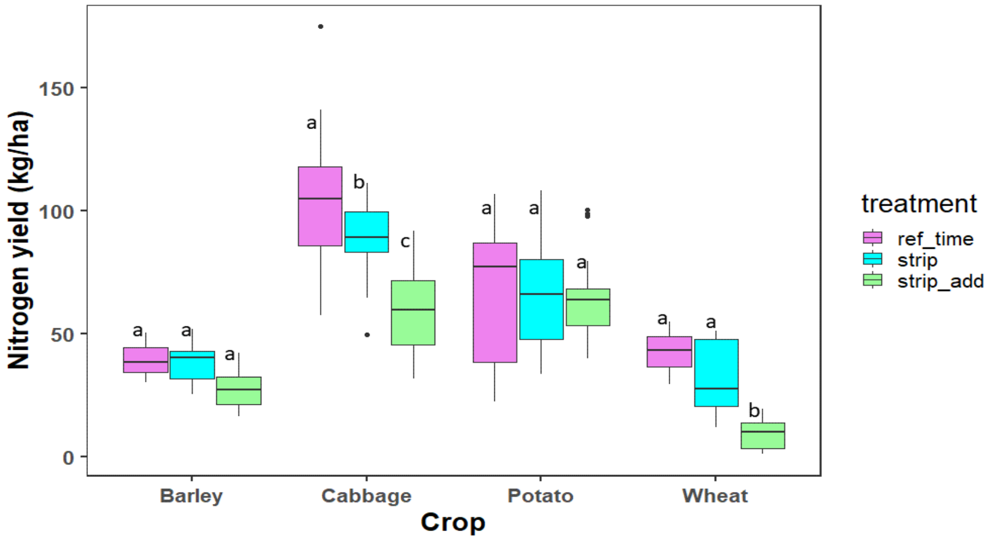

Figure 6. Nitrogen yield of barley, cabbage, potato and wheat for all three treatments. Nitrogen yield was calculated by multiplying dry matter yield and nitrogen content. For the strip_add the nitrogen yield of fava bean is included in the wheat nitrogen yield. Dots represent outliers. The different letters indicate statistically significant differences according to Tukey HSD test, p<0.05 at 0.95 confidence level.

3.1.6. Analysis of N Delivery from Grass Clover

The amounts of N released from the grass clover in the litterbags were different over time and between the seasons. The litterbags that were buried on 29 April 2019 were monitored until 22 July 2019; the original nitrogen content of 1.2 % reached 72 % ± 8.89 after two weeks. The maximum amount of N released was after 12 weeks, with a total release of 92 % with a deviation of ±2.89 (**Figure 7**). The second batch with grass clover containing 1.8 % of nitrogen, which was buried on 29 July 2019 and monitored until 21 October 2019, after two weeks reached 49 % with a deviation of ± 8.96. The maximum amount of N released was after 12 weeks with total N released of about 85 % ± 2.31.

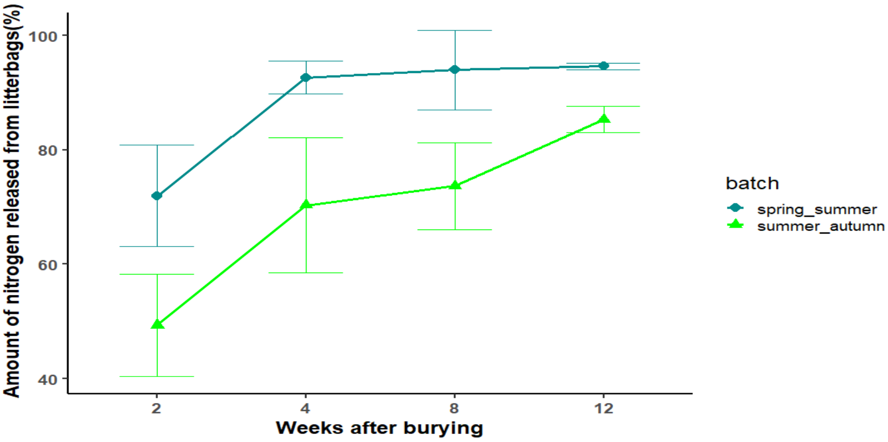

Figure 7. Nitrogen released over time from the decomposition of grass clover in the litterbag experiment with measurements carried out at different times during the growing seasons (2019)

3.2 Exploration Phase: Current Organic Strip Cropping System

3.2.1. Scenario 1

The first scenario of the legume-based cropping system (**Figure 8**), when compared with the animal manure-based cropping system (**Figure 10**), indicated N shortage for potato, which was fertilized with one-time chop and drop fertilizer of 3.1 DM t/ha (1.2 % N content). For winter wheat, which was grown with fava bean, the N amount was not enough to reach the targeted yield of wheat when fava bean yield was set higher than 1.2 t/ha. Sugar beet grown after the mixture of cover crops did not show any N shortage, although no fertilizer was supplied. The cabbage grown after one and half year of grass clover growth with an application of 2 t/ha of plant-based fertilizer (OPF) (220 kg N/ha) in August at head formation, demonstrated N shortage. The winter barley grown with pea in a strip after cabbage, demonstrated N shortage when pea production was set higher than 3.8 t/ha. In this scenario, the overall legume-based cropping system resulted in low N leaching (56 kg N/ha) compared to animal manure-based cropping system (95 kg N/ha) (**Figure 9**).

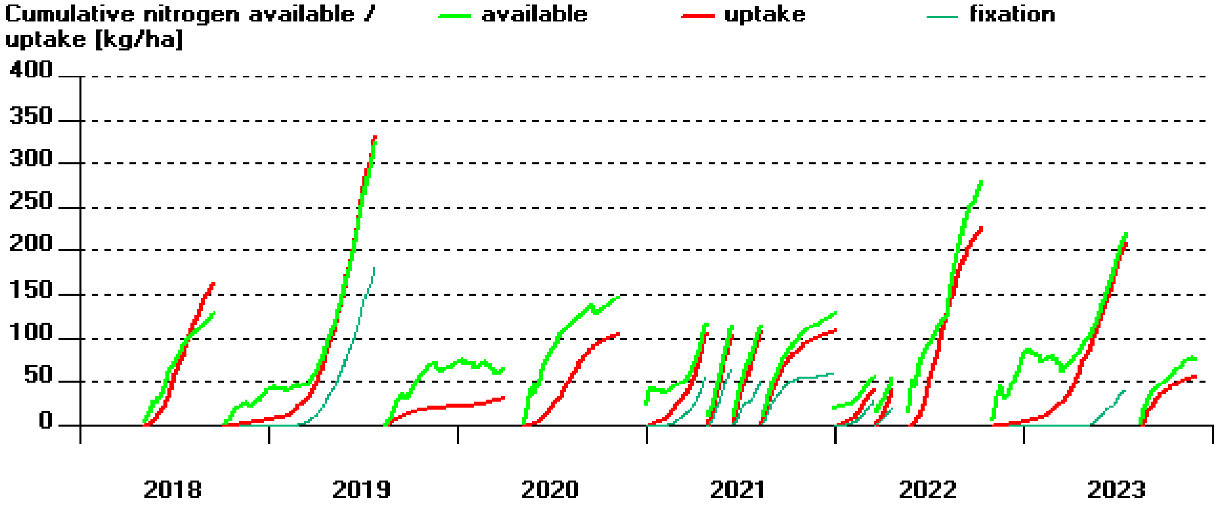

Figure 8. Scenario 1 Cumulative nitrogen available and nitrogen uptake for a six-year crop rotation generated using targeted yields with the current fertilizer scheme (**Table 1**) for legume-based organic strip cropping system.

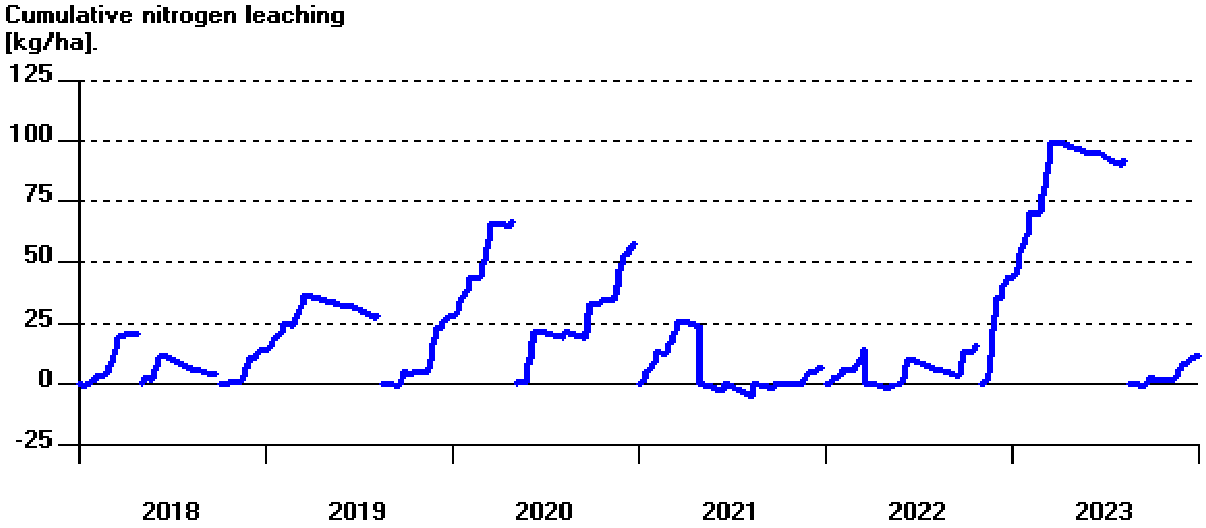

Figure 9. Scenario 1 Cumulative nitrogen leaching for a six-year crop rotation for legume-based organic strip cropping system.

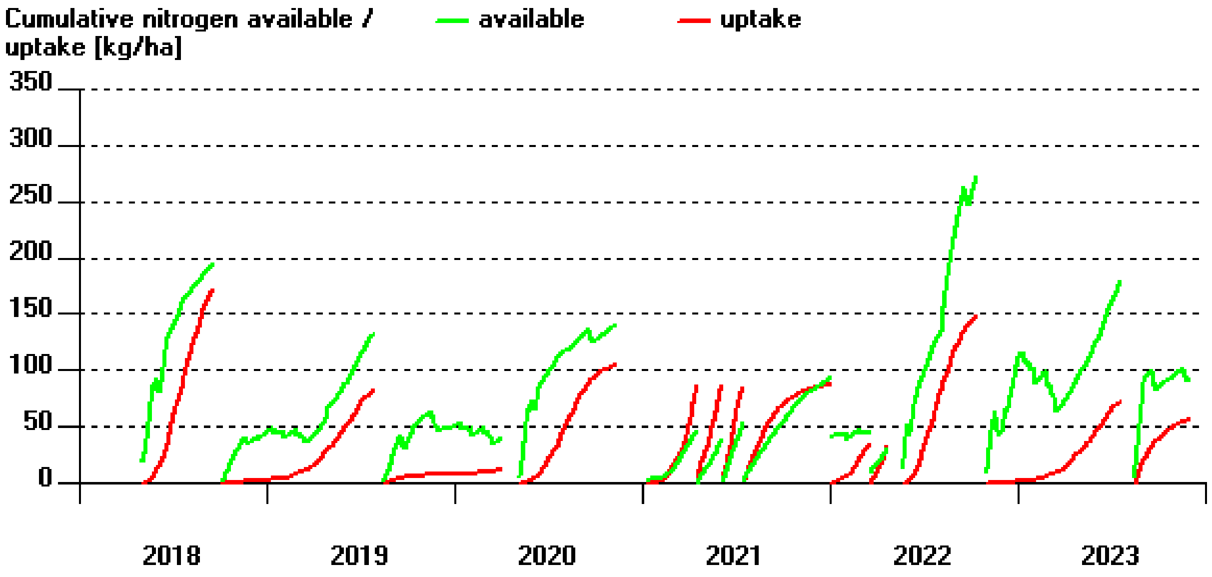

Figure 10. Scenario 1 Cumulative nitrogen available and nitrogen uptake for a six-year crop rotation generated using targeted yields with the current fertilizer scheme (**Table 1**) for animal manure-based cropping system.

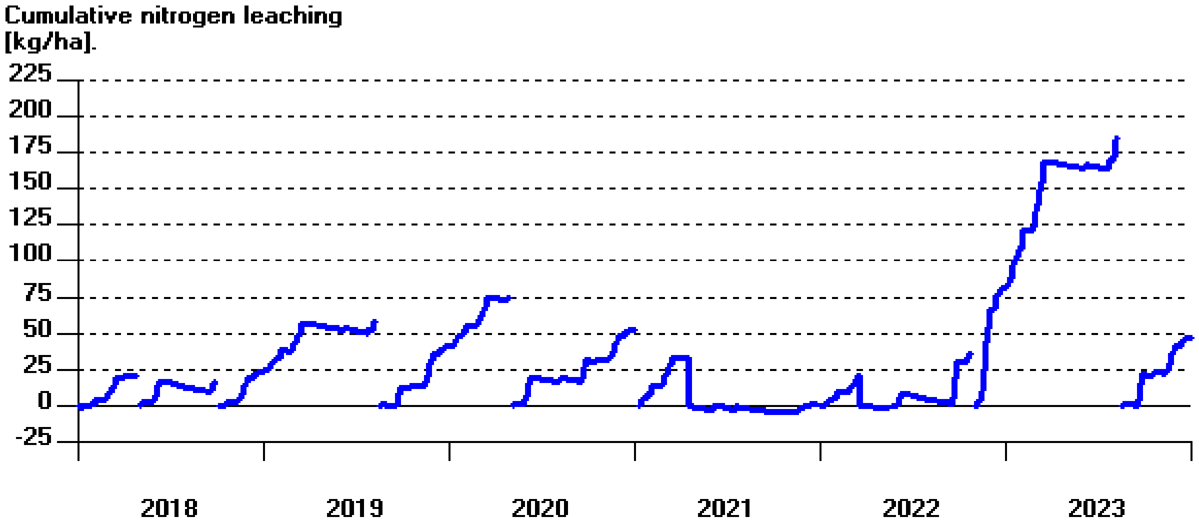

Figure 11. Scenario 1 Cumulative nitrogen leaching for a six-year crop rotation for animal manure-based cropping system

3.2.2. Scenario 2

The second scenario (Figure 12), with an improved fertilizer scheme with two times chop and drop fertilizers applied (3.1 DM t/ha and 4.5 DM t/ha) for potato and two times chop and drop fertilizers (8 DM t/ha and 3 DM t/ha) applied for cabbage did not show N shortage, and was predicted to reach the targeted yields. However, the NDICEA model predicted that the amount of grass clover produced by the current cropping system was not enough to satisfy crop requirements.

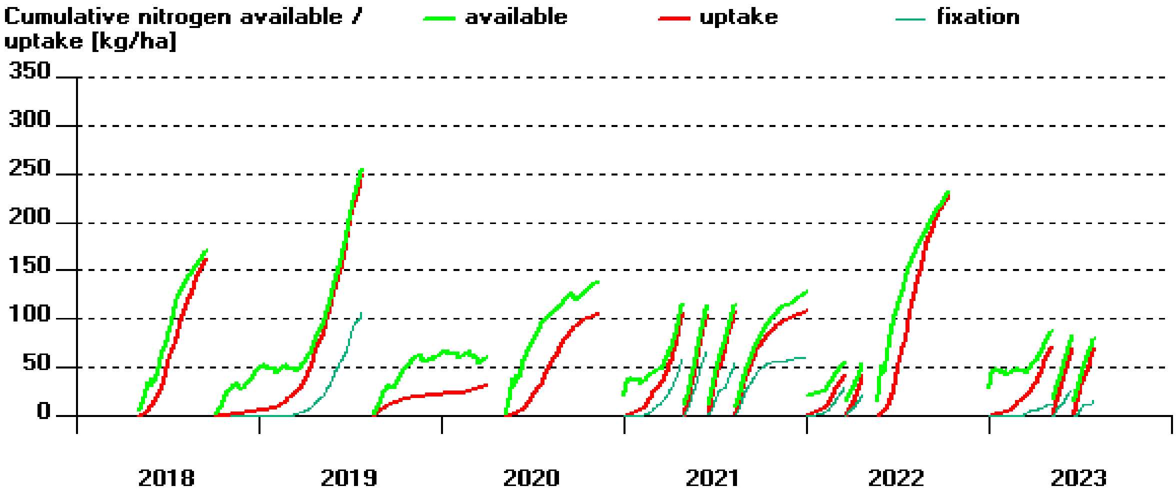

Figure 12. Scenario Cumulative nitrogen and nitrogen uptake for a six-year crop rotation of a legume-based cropping system based on targeted yields and with an improved fertilizer scheme. The winter barley and pea mixture (02/11/2022 -20/07/2023) was replaced by grass clover used as a chop and drop fertilizer.

The overall cropping system nitrogen leaching over six year was lower (51 kg N/ha) than the animal manure-based cropping system (95 kg N/ha) **(Figure 13)**.

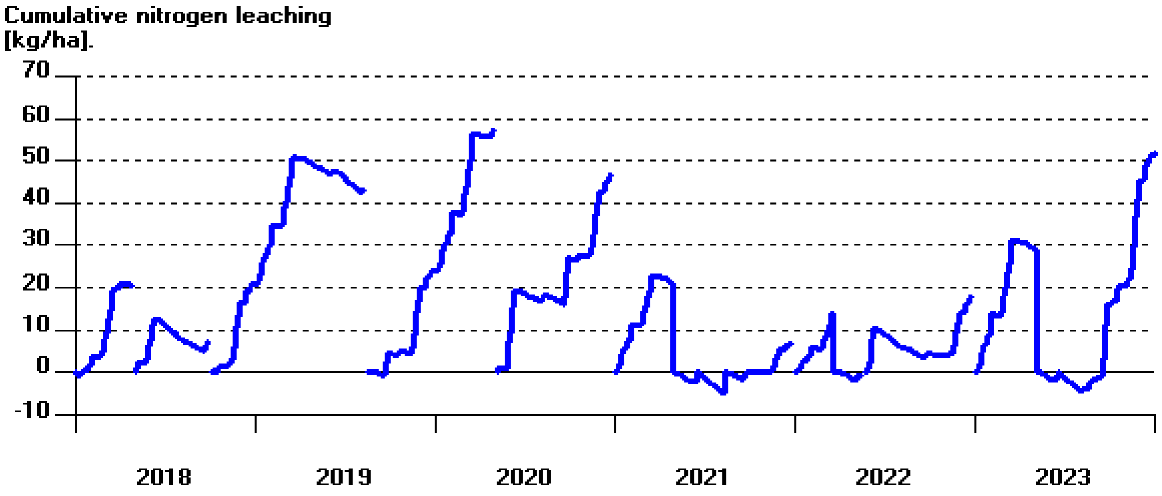

Figure 13. Scenario 2 Cumulative nitrogen leaching for improved legume-based cropping system.

4. Discussion

4.1 Explanation phase: Current Organic Strip Cropping System

4.1.1 Soil Available N, N Uptake and Yield for Potato

The results based on the field observations indicate that potato did not had a shortage of soil available nitrogen for any of the treatments. However, in the NDICEA model, the cumulative available nitrogen over the growing seasons was significantly lower in strip-add than ref_time and strip. There are two plausible reasons for this difference between field and model. First, a lower amount N in grass clover (3.1 dm t/ha, 37.2 kg N/ha) was applied to strip_add compared to ref_time and strip, which both received animal manure (25 t/ha, 164 kg N/ha). The nitrogen content of the grass clover of the first and second harvests was low (1.2 % and 1.8 % respectively) compared to N content reported in other studies conducted in the Netherlands (2.5%) (12,40,41]. This low percentage could be due to a low clover content in the mixture and the timing of harvest of the grass clover [40,41,42,43]. A second reason could be the timing and frequency of the measuring of the soil available nitrogen in the field, in particular to capture the fluctuation of nitrogen availability over time [44].

The total N uptake in the model was the same for all three treatments with all the input parameters having more or less the same value (yield, dry matter content, seeding densities and nitrogen content). In the strip-add, despite a lower amount of grass clover applied than in ref_time and strip, there was no significant difference in dry matter yield and nitrogen yield. This suggests that there was timely release of nitrogen from the grass clover. This was confirmed by results of the litterbag experiment, which indicated a maximum release of N at 12 weeks, during initial tuber formation. This N release result was similar to results obtained in previous studies [45,46].

The results of the litterbag experiment should be analyzed with some caution [47,48]. The grass clover applied before planting was subject to the same climatic conditions as the grass clover in the litterbags that were buried. The litterbags were buried on the same day of applying grass clover to the potato. However, there could be a slightly different pattern of N release from the grass clover in the litterbags when compared to the field due to a difference between dry and fresh grass clover as drying might alter the chemical composition [49]. The grass clover applied for potato confirmed the first hypothesis that grass clover can provide enough nitrogen given a timely application.

4.1.2. Soil Available N, N Uptake and Yield for Winter Wheat

Figures 2 indicate a difference in cumulative soil available nitrogen between strip_add and strip, but no difference between ref_time and strip_add. The difference in cumulative soil available nitrogen could be due to the nitrogen fixed by the fava bean. Such influence has been reported by other studies about nitrogen fixation due to the high N-sharing effect of biological nitrogen fixation in cereal-legume intercropping [50]. The strip_add treatment indicates a higher cumulative nitrogen uptake than the other two treatments. This is probably related to the total amount of nitrogen taken up by the mixture of wheat and fava bean. However, the dry matter yield and nitrogen yield for strip_add were low compared to ref_time and strip. This could be due to an error that occurred in planting density of the fava bean which was half as higher as normal density. Based on visual observations from the early growth period of both crops (February to April), the above ground competitive effect of fava bean over wheat was evident. The wheat plants could hardly be seen (**Appendix D**). This kind of crop behavior has been reported by previous studies conducted by Zhang et al. (2006) [49] and Guiducci et al. (2018) [51]. They found negative interspecific interaction between wheat and fava bean leading to lower dry matter yield compared to monoculture. Other factors such as low soil available nitrogen, rainfall amount and low temperature were found to favor the legume's early establishment, thus increasing the competitive effect against wheat [51]. Other studies have demonstrated that often the yields in mixtures of a cereal and a legume had similar or lower yields than the monocropping yield due to the interspecific competition [52,53,54].

In this study, the roots of the crops were not studied. Root growth and distribution are essential for N acquisition and influence underground competition between companion crops [54,55]. Such competition could also have had an impact on the wheat-fava bean mixture.

4.1.3. Soil Available N, N Uptake and Yield for Cabbage

The cumulative available nitrogen and total nitrogen uptake for cabbage were lower for strip_add than for ref_time and strip. This was confirmed by the SPAD measurements taken on 30 July (**Table 6**) just before cupping stage. This result was expected as no fertilizer was applied in strip _add while in ref_time and strip, 25 t/ha of animal manure was applied (164 kg N/ha). In August, at head formation, 2 t/ha of OPF with 220 kg N/ha was applied to all three treatments. The dry matter yield and nitrogen yield for strip_add were significantly lower compared to the other two treatments. This indicates that the late season N application did not satisfy the early requirement of N to contribute to high dry matter yield and nitrogen yield, despite the normally relatively fast N release rate of OPF [56,57,58]. It also could be that the OPF dose of 220 kg N/ha was not adequate and/or applied at the wrong time [59]. One factor that likely contributed to lower dry matter yield and nitrogen yield in strip-add was calcium deficiency, dry weather conditions and soil preparations which disturbed the soil layers. Animal manure, which contains "fresh" calcium was not supplied to strip_add, thus strip_add treatment was suffered from calcium deficiency. Based on visual assessment, it affected about 25 % of the strip-add crop causing irregular growth and premature head formation (**Appendix D**). Other factors contributing to the lower than expected yield could be the nutrient status of other key macronutrients and the timing of their respective application for the good growth of cabbage [57,59].

4.1.4. Soil Available N, N Uptake and Yield for Spring Barley

The soil available nitrogen fluctuated over time, but there was no significant difference between the treatments. The SPAD measurement did also not show a significant difference. Based on the NDICEA model, both cumulative soil available nitrogen and cumulative nitrogen uptake were significantly lower for strip_add. This is most likely due to the zero-fertilization coupled with the failure of pea to grow with the barley. During the summer, the soil available nitrogen was low in strip_add and considerable weed development took place (especially Chenopodium album L). Jørnsgård et al. (1996) [60] observed that densities of Chenopodium album L were highest when soil available nitrogen levels were low since Chenopodium album L has a lower nitrogen optimum than barley. This could explain the poor establishment of barley, giving advantage to the development of

Chenopodium album L. Due to the poor barley performance, the nitrogen uptake was low for strip_add compared to ref_time and strip (**Figure 4**). After the harvest, a high soil mineral N level was found, despite no fertilizer being applied. The poor growth development was easily observed in the field due to late sowing, further aggravated by dry weather and hasty tillage (**Appendix D).**

4. 2. Design and Explanation Phase: Designing Improved Legume-Based Cropping System

Given the limitations of the first scenario (**Figure 8**)**,** in the second scenario (**Figure 12**) alternative chop and drop grass clover amounts and timing were analyzed aligned with the grass clover harvest. Potato was supplied with two times chop and drop fertilizer. The first amount of 3.1 DM t/ha of grass clover from the first harvest containing 1.2 % of nitrogen (37.2 kg N/ha) was applied before planting (29 April). The second application coincided with the second grass clover harvest (29 June). The total amount of 4.5 DM t/ha containing 1.8 % of nitrogen (81 kg N/ha) was applied. The grass clover production and N content estimations were based on the two harvests of April and June 2019. Simulation of the grass clover production and N content based on these two applications predicted that these amounts would be not enough to reach targeted yields. The predicted shortage would be 49.3 kg N/ha or approximately 4.2 DM t/ha.

For the wheat and fava bean mixture, in order to reach the targeted yield for wheat, the fava bean yield was set at 1.2 t/ha. For sugar beet, which was grown after the mixture of cover crops and did not show an N shortage, no fertilizer was applied. For cabbage, the OPF was replaced with two times chop and drop fertilizer. The first with an amount of 8 DM t/ha containing 1.2 % of nitrogen with the total nitrogen 96 kg N/ha was applied in 16 May; and the second, 3 DM t/ha containing 1.8 % of nitrogen (54 kg N/ha) applied in 30 July did not show N shortage [58]. The chop and drop fertilizer for cabbage was applied from the preceding one and half year grass clover which produced 12 DM t/ha with 180 kg N/ha. This amount fulfills the cabbage N requirement. The remaining grass clover of 30 kg N/ha was applied to potato, but it was predicted that this remaining amount was not enough. There was still a shortage of 19.3 kg N/ha. To remedy this shortage, the barley with pea was removed, given that the barley and pea mixture had a high degree of leaching. Growing grass clover in a strip alternated with sugar beet will also benefit the latter. The simulation indicates that it will produce 5 DM t/ha of grass clover with varying N content (1.2 % and 1.8 % N content), sufficient to reach the targeted yield. According to previous research, two cuts of grass clover (in April and June) are feasible [12, 41].

5. Conclusions

This study investigated if in-situ produced plant-based nitrogen can replace externally produced animal-based nitrogen. The results obtained from an analysis for a 6 years rotation organic strip cropping system indicate that it is feasible. The combined field observations and the improved scenarios provide the evidence. For potato, to obtain yields comparable to those of an animal manure-based cropping system, two times chop and drop fertilizer should be applied. The first amount of 3.1 DM t/ha (37.2 kg N/ha) should be applied at least one month before planting (29 April); followed by a second amount of 4.5 DM t/ha (81 kg N/ha in 29 June) before the canopy closures. For cabbage, it requires 8 DM t/ha (96 kg N /ha) of chop and drop fertilizer to be applied before planting and another 3 DM t/ha (54 kg N /ha) before head formation. To produce these amounts of grass clover, it would require an area of 3.72 ha. The precise amounts depend on the N content of the grass clover used. The NDICEA model can be used to calculate the appropriate amounts. The rate and timing of availability of N from the grass clover is determined by the rate of decomposition and immobilization influenced by soil organisms, which will differ based on soil conditions, previous management practices, C:N ratio, temperature and soil moisture. Thus, timing of chop and drop fertilizer application is very important. Wheat intercropped with fava bean, and barley with pea can produce the same dry matter yield and N yield without application of chop and drop fertilizer, but the two mixtures have a trade-off in terms of reaching the targeted yield for both crops in the mixture. For sugar beet, no field data could be collected and analyzed due to crop failure. However, the NDICEA simulation indicated that sugar beet, when planted following a mixture of cover crops, does produce good results without additional supply of chop and drop fertilizer.

The results are supportive of both hypotheses suggesting that is feasible to use legume species efficiently and effectively in time and space in organic strip cultivation. When N is supplied as in this research, it can enable yields for wheat, potato, barley and cabbage (for the latter the field results were not satisfactory, but the NDICEA simulation produced positive results) that are comparable to yields used in an animal manure-based cropping system. To optimize the legume-based cropping system, synergies and trade-offs need to be carefully balanced, in particular with regard to the cereal and legume mixture. Recommended are timely and effective crop management (weeding, pest and disease monitoring, monitoring of macronutrients and micronutrient deficiencies), appropriate choice of legume species and the use of compatible plant densities to obtain good yield and quality in cereal and legume intercropping.

Given the complexity and dynamic nature of N in agricultural production, a system's approach, such as the DEED framework, can support knowledge-based decision making and guide farmers to plan management practices over a multi-year cropping cycle. Such an approach can benefit from detailed and long-term field observations and (scenario) modelling. The findings can be translated to practical recommendations for farmers. The research findings and model-based scenarios developed are based on a one-year experiment and should be considered as a contribution to a longer-term assessment of the utility of alternative agricultural management systems. The experiment at the Droevendaal farm in Wageningen could serve as the basis for additional, on farm experiments in diverse agro-ecological conditions in the Netherlands and beyond. This would allow validation of the legume-based strip cropping system in actual, diverse farming contexts and the integration of farmers' own observations about the feasibility of this new system and the generation of new ideas about how to improve it further, e.g. which cereal-legume mixtures work best under what agro-ecological conditions and appropriate timing of harvesting grass clover.

Author Contributions

The author designed, implemented and evaluated the experiment, analyzed the data and wrote the article.

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Conflict of Interest

The author declares that she has no conflict of interest.

Appendices

Appendix A. Experimental Design and Layouts

Figure A1. The incomplete block experimental design with three replicates in 6.18ha field in Droevendaal – representation of fields, blocks and strips of crops with their respective treatments (indicated by strip_add, strip_var, strip, ref_time, rotation, ref_space,LER and strip6)

Appendix B. Crop and Soil Data of Droevendaal Experimental Field, Wageningen

Table B1. The crop sequence for non-legume and legume-based strip organic cropping system at Droevendaal experimental field, Wageningen.

Non-legume	**Legume- based**	**Latin name**
ref_time & strip	**strip_add**	
Potato	Potato	*Solanum tuberosum L. var. Agria*
Wheat	Wheat+Faba bean	*Triticum aestivum L. var.Julius* *Vicia faba cultivar pyramid*
Buckwheat	Buckwheat+sunflower+ Phacelia+Borage+ Serradelle +Flax + Persian clover +Niger	*Fagopyrum esculentum, Helianthus annuus L.* *Phacelia tanacetifolia,Borago officinalis* *Ornithopus sativus, Linum usitatissimum* *Trifolium resupinatum, Guizotia abyssinica*
Sugar beet	Sugarbeet	*Beta vulgaris var. Anarosa*
Rye grass	Grass + clover	*Lolium multiforum L., Trifolium pratense L.* *Trifolium repens*
Cabbage	Cabbage	*Brassica oleracea L. var. capitata Riveria L.*
Barley	Barley +pea	*Hordeum vulgare var. Irina, Pisum sativum*
Fodder radish	Fodder radish	*Raphanus sativus var. Oleiformis*

Table B2. The field wise (F1, F2 and F3) and treatment wise (ref_time, strip and strip_add) crop and soil data used for the NDICEA modelling

Field 1: Potato

	Treatment		
Variables	**ref_time**	**strip**	**strip_add**
1st soil mineral N (kg/ha)	50.38	38.19	41
2nd soil mineral(kg/ha)	22.4	34.7	24.2
3rd soil mineral N (kg/ha)	33.5	27.6	29.6
Fresh yield (t/ha)	44.67	31.17	30.26
Dry matter ratio (%)	23.14	24.22	24.97
Dry matter yield (t/ha)	10.32	7.56	7.57
N content (%)	0.94	1.07	1.07

Field 2: Potato

Variables	Treatment		
	Ref_time	**Strip**	**Strip_add**
1st soil mineral N (kg/ha)	59.85	35.1	54.95
2nd soil mineral N (kg/ha)	23.4	30.9	23.1
3rd soil mineral N (kg/ha)	22.1	26.5	30.6
Fresh yield (t/ha)	13.36	16.27	18.97
Dry matter ratio (%)	24.22	25.68	25.8
Dry matter yield (t/ha)	3.25	4.17	4.89
N content (%)	0.96	1.06	1.14

Field 3: Potato

Variables	Treatment		
	ref_time	**strip**	**strip_add**
1st soil mineral N (kg/ha)	57.4	31.9	64.05
2nd soil mineral N (kg/ha)	46.36	43.92	33.14
3rd soil mineral N (kg/ha)	36.3	29.6	34.4
Fresh yield (t/ha)	29.11	29.83	22.63
Dry matter ratio (%)	24.75	23.52	25.14
Dry matter yield (t/ha)	7.19	6.97	5.68
N content (%)	1.1	1.09	1.04

Field 1: Wheat

Variables	Treatment			
	ref_time	**strip**	**strip_add**	
			wheat	**fava**
1st soil mineral N (kg/ha)	11.89	8.5	7.24	
2nd soil mineral(kg/ha)	43.68	34.22	33.41	
Fresh yield (t/ha)	2.89	2.3	0.84	0.23
Dry matter ratio (%)	91.98	91.71	91.43	91.07
Dry matter yield (t/ha)	2.66	2.1	0.77	0.21
N content (%)	1.63	1.74	2	4.87

Field 2: Wheat

Variables	ref_time	strip	strip_add	
			wheat	fava
1st soil mineral N (kg/ha)	9.62	7.7	9.29	
2nd soil mineral N (kg/ha)	27.48	32.27	40.39	
Fresh yield (t/ha)	1.93	1.7	0.69	0.67
Dry matter ratio (%)	91.9	90.7	91.6	90.1
Dry matter yield (t/ha)	1.78	1.55	0.63	0.61
N content (%)	1.67	1.71	2.06	5.03

Field 3: Wheat

Variables	ref_time	strip	strip_add	
			wheat	fava
1st soil mineral N (kg/ha)	8.54	8.54	11.83	
2nd soil mineral N (kg/ha)	35.35	32	32.43	
Fresh yield (t/ha)	3.32	2.28	0.74	0.25
Dry matter ratio (%)	92.1	91.37	91.14	91.16
Dry matter yield (t/ha)	3.06	2.09	0.67	0.23
N content (%)	1.79	1.75	2.12	5.19

Field 1: Cabbage

Variables	ref_time	strip	strip_add
1st soil mineral N (kg/ha)	25.68	37.44	20.74
2nd soil mineral(kg/ha)	42.13	30.39±3.09	30.77±4.93
3rd soil mineral N (kg/ha)	15.07	23.24	18.87
Fresh yield (t/ha)	54.80±4.59	43.04±1.63	35.89±1.76
Dry matter ratio (%)	8.63±0.12	8.53±0.19	8.39±0.15
Dry matter yield (t/ha)	4.74±0.44	3.66±0.12	3.01±3.02
N content (%)	2.92±0.09	2.80±0.08	2.56±0.20

Field 2: Cabbage

Variables	Treatment		
	ref_time	strip	strip_add
1st soil mineral N (kg/ha)	38.15	29.67	36.5
2nd soil mineral N (kg/ha)	27.3	40.25±0.35	43.07±10.95
3rd soil mineral N (kg/ha)	17.85	17.68	25.55
Fresh yield (t/ha)	35.18±5.12	35.06±2.51	17.99±1.28
Dry matter ratio (%)	8.77±0.12	8.82±0.09	8.76±0.12
Dry matter yield (t/ha)	3.08±0.43	3.10±0.23	1.58±0.11
N content (%)	2.73±0.10	2.66±0.07	2.87±0.08

Field 3: Cabbage

Variables	Treatment		
	Ref_time	Strip	Strip_add
1st soil mineral N (kg/ha)	53.13	52.54	39.9
2nd soil mineral N (kg/ha)	48.99	42.44±1.03	43.58±0.18
3rd soil mineral N (kg/ha)	18.29	18.28	19.08
Fresh yield (t/ha)	40.67±2.96	35.64±1.04	25.06±1.82
Dry matter ratio (%)	8.88±0.13	8.49±0.03	8.63±0.07
Dry matter yield (t/ha)	3.61±0.25	3.03±0.08	2.16±0.15
N content (%)	2.77±0.06	2.82±0.05	2.81±0.05

Field 1: Spring barley

Variables	Treatment		
	ref_time	strip	strip_add
1st soil mineral N (kg/ha)	19.6	38.5	34.12
2nd soil mineral(kg/ha)	14.55	32.31	23.8
3rd soil mineral(kg/ha)	53.96	55.91	53.21
Fresh yield (t/ha)	2.22	2.23	1.38
Dry matter ratio (%)	90.36	92.27	92.91
Dry matter yield (t/ha)	2.01	2.08	1.28
N content (%)	1.93	2.05	2.15

Field 2: Spring barley

Variables	Treatment		
	ref_time	strip	strip_add
1st soil mineral N (kg/ha)	66.85	56.35	61.99
2nd soil mineral N (kg/ha)	33.81	26.6	33.15
3rd soil mineral(kg/ha)	44.84	49	53.21
Fresh yield (t/ha)	2.71	2.24	1.44
Dry matter ratio (%)	93.13	93.38	92.92
Dry matter yield (t/ha)	2.52	2.09	1.34
N content (%)	2.01	2.12	2.34

Field 3: Spring barley

Variables	Treatment		
	ref_time	strip	strip_add
1st soil mineral N (kg/ha)	29.7	64.05	26.25
2nd soil mineral N (kg/ha)	32.54	40.95	32.38
3rd soil mineral N (kg/ha)	58.57	47.6	48.83
Fresh yield (t/ha)	1.31	1.39	0.96
Dry matter ratio (%)	92.94	91.37	91.48
Dry matter yield (t/ha)	1.22	1.27	0.88
N content (%)	2.5	2.29	2.22

Table B3. General soil variables used in the NDICEA model for all three treatments

Soil variable	
SMO topsoil	0.49
GAM topsoil	0.033
SMO subsoil	0.41
GAM subsoil	0.033
Soil organic matter (%)	3.7
pH	5.5
Topsoil thickness (cm)	25
N deposition (kg/ha/y)	43.4
Summer deepest groundwater level (cm)	150
Winter maximum ground water level(cm)	80
Groundwater N content (mg/l)	6

Appendix C. Soil and crop sampling procedures and timing

Appendix C1.Soil sampling and timing

To determine the availability of soil mineral nitrogen (N) at the beginning of the growing season, soil samples were taken on 26 April 2019 prior to planting and fertilizer applications for potato, cabbage and barley. Since wheat was already sown in October 2018, the in-season soil samples and analysis were done during the tillering stage (on 11 April 2019) before fertilization. The in-season soil sampling and analysis for cabbage, potatoes and barley were carried out during the growing season on 11 June 2019 (one month after the fertilization) to check the availability of soil mineral N and gain insight in additional N requirements needed to supply during the growing season. The third soil sampling and analysis were done at the end of the cropping season after harvest to determine the remaining mineral nitrogen in the soil available for the following crops. Soil samples were taken from two strips per treatment excluding the buffer strip and excluding 10 meters from each side of the strips.

Appendix C2: Crop sampling procedures and timing

In-season crop N status was monitored using SPAD-502 (Soil Plant Analysis Development) meter, a hand-held device that enables quick, easy and accurate measurement of leaf chlorophyll content without damaging the leaf (Follet et al., 1992; Ling et al., 2011). These measurements were taken following the critical stages of each crop development. Based on a methodology used in previous studies (Denuit et al., 2002; Minotti et al., 1994; Vos and Bom 1993), the SPAD measurement for potatoes was taken four times at the interval of two weeks (i.e. at 21 days, 35 days, 49 days and 63 days after emergence). For consistency, measurements were always taken from three terminal leaves (i.e. fourth leaf) from each plant and the average value was recorded. In order to correct the variability, 30 random plants selected by R from each treatment was measured and compared.

For wheat only one SPAD measurement was taken as it was already reached to tillering stage. It was taken one month later after fertilizer application, during the booting stage as indicated by Peltonen et al. (1995), one of the critical stages for N supplement. Following the methodology described by Islam et al., (2014), from each leaf, three readings were taken which was averaged. In total 30 random plants generated by R per treatment were measured. The measurement was taken from uppermost fully expanded leaves. Two SPAD measurement for barley was conducted, one in the early tillering stage and second during the booting stage, exactly at four weeks after emergence as stated by Peltonen et al. (1995). The plant selection and measurements were done following the same method described under wheat.The N status of cabbage was monitored at 21 days after planting, in season monitoring at 46 days after planting when four to six leaves were developed, and last measurement was taken at 69 days during the early head formation. The measurement was taken by clapping onto a single matured leaf by avoiding the midribs. From each plant 3 leaves were measured, and the values were averaged. In total 30 plants were selected randomly from each treatment using R based on plant density (R core Team 2018).

Appendix D. Field observations (pictures)

Figures D1-3. Wheat and fava bean intercropped (strip_add) in February and April 2019 showing the above ground dominance of fava bean over the cereal. Credit: R. Vernooy

Figures D4-6. Premature development of cabbage and cabbage affected by disease (yellowing leaves) in strip_add in the first week of September 2019. Credit: R. Vernooy

Figures D7-9. Barley infested by Chenopodium album L in mid-August 2019 (left: ref_time, middle: strip, and right: strip_add). Credit: R. Vernooy

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