

Article

Processing of Legume Green Manures Slowdowns C Release, Reduces N Losses and Increases N Synchronisation Index for Two Years

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Abstract: The number of livestock farms decreased by 40% in Europe over the last 10-year period. Stockless organic cropping systems started to dominate in many intensive agricultural regions in Europe. Developing the sustainable management of an organic stockless agroecosystem is related to guaranteeing self-sufficiency in nitrogen (N) supply, maintaining high grain yields, and promoting carbon (C) sequestration in the soil. The aim of this study was to investigate if the processed legume green manures can be an alternative to granulated cattle manure and direct ploughing of legume biomass in order to develop the sustainability of the stockless organic cropping system. The decomposition rate and C and N release were observed for green manures made of fermented red clover and composted red clover with wheat straw. Fresh red clover biomass and granulated cattle manure were used for the comparison. Results of the 3-year field experiment showed that technologically processed legume biomass had a positive effect on the productivity of crops at least two years in rotation. Fermented red clover and red clover compost increased N use efficiency by 15% and biomass output efficiency by 16% compared with fresh red clover biomass. Processed legume green manures significantly increased the synchronisation index between crop N demand and N supply. In autumn, incorporated fresh red clover biomass lost 65.6% of its initial C and 37.6 kg ha^{−1} (50.1%) of its initial N under decomposition in the first non-growing season. It also increased mineral N losses deeper into the subsoil by 52.7%. Meanwhile, fermented red clover and red clover compost released 43% of its N during the first crop growing season, sustained at least one year slower C release to the soil, promoted ecosystem productivity, prevented mineral N losses to subsoil and gained high N synchrony indexes. The best N synchrony was achieved using fermented red clover, with a higher decomposition rate positively significantly correlated ($r = 0.47\text{--}0.78$, $p < 0.05$) with grain yield, total biomass, protein content and total N accumulated in the plant of spring wheat and spring barley.

Keywords: red clover; silage; compost; N use efficiency; biomass output efficiency



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1. Introduction

Livestock farms are decreasing, with increasing stockless organic cropping systems in many intensive agricultural regions in Europe. Developing an organic arable farming system that is self-sufficient in N supply while maintaining high yields is very important and challenging. The sustainable management of stockless organic agroecosystems is even more complicated, with mounting difficulties in N cycling and acceptable crop productivity achievement while maintaining the health of the environment [1,2]. Stockless organic farming systems legumes are suggested as one of the key elements for N supply, decreasing dependence on external N inputs [3,4].

The temperate broadleaf and mixed forest biome in Europe is characterised by seasonal vegetation, and during the cool season, crop cultivation is limited by low temperatures and short daylight. Incorporation of green matter into the soil, instead of its removal after the crop growing period, has been suggested as one of the options to increase soil organic carbon (SOC) stocks [5], but this could lead to nutrient loss due to leaching and gas emissions from the soil maintained without a cover crop [3].

The decomposition time and potential rate could provide information on whether the material has the potential to increase nutrient loss during the non-growing season, crop growing season or the entire crop rotation. Decomposition is the process during which organic substances are broken down into simpler organic particles. The process is a part of the nutrient cycle and is essential for legume N recycling. During decomposition, the process of mineralisation describes the transfer of organic matter to soluble inorganic forms that increase the nutrient bioavailability to plants [6]. Mineralisation is the opposite of immobilisation [7]. The mineral N is immobilised when the C:N ratio of the decomposing organic matter is above 20:1 [8], and then the decomposing microbes absorb N in mineral forms as ammonium or nitrates [9]. When the C:N ratio is lower, further decomposition causes mineralisation by the simultaneous release of labile inorganic N fractions [10] and, therefore, increases the total mineral N in the soil ($\text{NO}_3\text{-N}$ and $\text{NH}_4\text{-N}$). The mineral N in the soil can be taken up by the plant or lost to the environment [6,11], reducing water quality by N leaching in the groundwater and reducing air quality by boosting greenhouse gas (N_2O) emissions [12]. Therefore, it is important to conduct a quantitative and qualitative investigation of the decomposition of organic matter in specific sites and soils.

Plant material by itself predetermines the decomposition rate. The major biotic factors include the chemical and physical characteristics of the straw, such as the nutrient content, C:N ratio, lignin, cellulose, hemicellulose and polyphenol contents and the lignin to N ratio [13–16]. Additionally, plant chemical traits regulate community functioning and affect decomposers' metabolism [10,17]. Legume residues with lower C:N ratios result in increased dry matter yield and N uptake by the following crop while decomposing faster than the cereal residues [18]. However, a very narrow C:N ratio results in rapid legume mass decomposition. Red clover decomposes very intensively within the first 2.5 months after incorporation into the soil, while other crop decomposition is more intensive in the 2.5–26 months period [19].

To prevent N losses from narrow C:N ratio legumes [20,21], especially in the non-growing season, and to synchronise the nutrient supply with ecosystem needs, new technological approaches suggest legume mass processing [22]. Previously, these processed organic manures were called mobile green manures or cut-and-carry fertilisers [23,24]. Selecting appropriate plant species in silage or compost can control C:N ratios and prevent nutrient losses or excess, and also synchronise nutrient supplementation for two years in the crop rotation with increased humus content for a long-term effect [25]. However, the nutrient composition of these manures is highly dependent on the processing method and chemical compounds of the component legume plants, which may vary due to local environmental and cultivation conditions. This leads to unpredictable value and effect of these processed plant-based manures and should be investigated in terms of specific farming systems.

Our hypothesis states that processed legume manures can maintain the same productivity of crops compared with legume mulch and granulated cattle manure but, at the same time, to increase N use efficiency, biomass output efficiency and synchronisation index between crop N demand and N supply.

Our study's aim was to investigate if processed legume green manures can be an alternative to granulated cattle manure and direct ploughing of legume biomass and improve the N cycling in organic stockless cropping systems. The main objectives were to (i) observe the decomposition rate and C, N turnover of processed legume manures (fermented red clover manure and compost of red clover and straws) and compare it with that of traditional organic manures (red clover mulch and granulated cattle manure) and (ii)

to measure the productivity of crops in rotation, N use efficiency, biomass output efficiency and synchronisation index between crop N demand and N supply.

2. Materials and Methods

2.1. Experimental Site and Conditions

Field experiments were conducted in 20 years of organically managed sites at the Lithuanian Centre for Agriculture and Forestry in Akademija (55.401147 °N, 23.862496 °E) Lithuania in 2015–2017. The experimental site was located in a cool temperate climatic zone, which can also be referred to as the Nemoral environmental zone [26], where the growing season lasts only 169–202 days.

Meteorological conditions. Weather data were collected at the meteorological station located in Akademija. Winter of 2015–2016 was unusually warm (Table 1). Winter wheat vegetation started early, but May and July were cooler compared to SCN. The period after the cereal harvest was warm; the average daily temperature in August and September was 1.8–2.9 °C higher than SCN. In 2016, the temperature of the vegetation period in the experimental site was 0.8–2.6 °C higher than the long-term average temperature (SCN). The months of July and August were very wet, with precipitation of 51.5 and 36.0 mm, respectively, exceeding the SCN. Autumn (except September) was wet. The winter period was relatively mild. Spring of 2017 started early. The air temperature of the 2017 growing season differed from the long-term average nonsignificant. The amount of precipitation was distributed very unevenly. May was dryer (precipitation was only 3.4 mm) compared with the SCN. However, the summer of 2017 was wet during the growing season, so the growth and development conditions for the spring barley were satisfactory.

Table 1. The monthly average air temperature (°C) and sum of precipitation (mm) in the 2015–2017 year period, Akademija.

Year, Month	Temperature, °C				Precipitation, mm			
	2015	2016	2017	SCN	2015	2016	2017	SCN
January	−0.6	−8.0	−3.2	−4.4	71.6	35.9	14.2	31.2
February	0.1	1.5	−1.6	−4.2	4.6	78.5	25.1	25.9
March	4.4	1.9	3.5	−0.6	48.7	37.1	38.9	28.2
April	7.0	7.1	5.6	5.9	54.5	59.5	48.2	37.6
May	11.4	15.0	12.8	12.4	50.4	27.3	3.4	51.4
June	15.1	17.5	15.4	15.7	26.3	57.4	72.1	61.7
July	17.1	18.6	16.7	17.8	57.6	128.2	153.8	76.7
August	19.7	17.1	17.3	16.8	5.6	109.2	53.2	73.2
September	14.0	14.0	13.3	12.2	66.0	8.7	123.1	51.3
October	5.9	5.4	7.4	6.8	6.7	87.9	89.3	49.8
November	4.7	1.3	4.0	1.9	71.3	78.4	51.0	45.0
December	2.3	0.9	1.0	−2.2	41.8	41.1	64.0	38.2
Crop season	Usual	Warmer	Usual	Average	Dry	Wet	Wet	Average

Notes: SCN—standard climate normal.

The soil of the experimental site was a loamy *Endocalcaric Epigleyic Cambisol (Drainic, Loamic) CM-can.glp-dr.lo* according to WRB classification. Characteristics of the soil arable layer (0–25 cm) were: pH 7.5, humus content 3.9%, total N 185 mg kg^{−1}, available phosphorus (P₂O₅) 58 mg kg^{−1} and available potassium (K₂O) 69 mg kg^{−1}. The site has been managed organically since 2003, with no additional irrigation, pesticides, or chemical contamination. The farming type is exceptionally crop production, where N is supplied by a high variety of grain and forage legume plants, green manures and microbial substances. Crops were cultivated using conventional tillage.

2.2. Experimental Design and Treatments

A field experiment with five treatments and four replicates was established in a complete randomised block design. Individual plots were 12 m in length and 3 m in width.

Treatments contained different green manures: (1) control with no fertilisers (C), (2) fresh biomass of red clovers (RC), (3) fermented red clovers (FerRC), (4) composted red clovers and wheat straw (ComRC + S) and (5) granulated cattle manure (GCM). The research was carried out in a field crop rotation: winter wheat—spring wheat—spring barley. In the first year (2015), winter wheat (‘Ada’ variety; 5.5 million seeds ha^{−1}) was cultivated; in the second year (2016), spring wheat (‘Vanek’; 6.0 million seeds ha^{−1}) was sown; and in the third year (2017), spring barley (‘Noja’; 5.0 million seeds ha^{−1}) was sown. In the second treatment, red clover (‘Arimaičiai’, 3.5 million seeds ha^{−1}) was undersown into winter wheat. Each organic green manure was applied to the plots in the spring of the second year (20-04-2016), excluding the second treatment (RC), which was naturally grown, cut and incorporated in the autumn of the first year during ploughing (Table 2).

Table 2. Experimental plants, tillage, manuring, litter bags burial and sampling time.

Year, Main Crop Season	2015, Winter Wheat		2016, Spring Wheat		2017, Spring Barley	
	Spring	Autumn	Spring	Autumn	Spring	Autumn
Undersowing	treatment RC	-	-	-	-	-
Tillage type and depth	-	ploughing 0–24 cm	cultivation 0–15 cm	ploughing 0–24 cm	cultivation 0–15 cm	ploughing 0–24 cm
Green manure application time and treatments	-	13-10-2015 in treatment RC	22-04-2016 in treatments FerRC, ComRC + S, GCM	-	-	-
Litter bags burying depth and treatments	-	0–15 cm in treatment RC	0–15 cm in treatments FerRC, ComRC + S, GCM	-	-	-
Litter bag sampling dates	-	13-10-2015 Initial for RC	20-04-2016 First for RC Initial for others	05-10-2016 Second for RC First for others	13-04-2017 Third for RC Second for others	10-10-2017 Fourth for RC Third for others
Soil mineral N determination	14-04-2015	-	20-04-2016 *	-	21-04-2017	-

Note. *—before organic manure incorporation.

In the spring of 2016, the crop seedbed preparation was performed with a soil finisher/field cultivator shortly before seeding. Organic manure (3–5 treatments) was spread manually on the soil surface and incorporated into the soil (0–15 cm) before the sowing of spring wheat. The fertiliser rate was calculated based on the N content so that 1 ha of land would receive 50 kg N ha^{−1}, except for fresh red clover biomass, which naturally accumulated 75 kg N ha^{−1} (Table 3).

Table 3. The amount of nutrients accumulated in organic fertiliser and incorporated into the soil.

Legume Manure	Incorporated into the Soil during Fertilisation, kg ha ^{−1} DM			
	Biomass	N	P	K
Control	0	0	0	0
Fresh red clover mass	2269	75	6.0	64.9
Fermented red clover mass	2632	50	5.0	45.3
Composted red clover and straw mass	1592	50	10.8	47.9
Granulated cattle manure	1812	50	10.6	81.8

Legume green manures were produced from red clover biomass (cut at the beginning of the flowering stage) by ensiling or composting their biomass with winter wheat straw. The compost was piled on 22 June 2015 from two main components: aboveground biomass of red clover (3 parts) and winter wheat straw (1 part). Aerobic composting was used to stimulate decomposition, and the pile was mixed five times. After 10 months, the compost

was used as manure. The fermentation/ensiling of red clover aboveground biomass was performed as follows: the biomass was cut, chopped, piled into a special trench and then pressed well to minimise the access to air as much as possible. Having completed the piling, the mass was sealed with a special film as hermetically as possible.

For the determination of the decomposition intensity of each green manure, the litterbag method was used [27]. Samples of all legume and granulated cattle manures (fresh weight of 25 g) were packed in poly-chloric-vinyl mesh bags of 20×15 cm (mesh size of 1.0 mm) and buried in the same treatment plots, where the green manures were applied on the surface also. Each treatment received five bags per plot for each four bag removal times (total 20 bags per plot). The decomposition was followed for two years, and the bags were removed four times (Table 2). The dry matter, C and N content were determined each time for each bag. The organic matter weight was recalculated depending on ash content.

2.3. Data Collection

Chemical analyses of organic materials. Before the incorporation of green manure, their chemical composition was determined: C, N, phosphorus (P) and potassium (K). The biomass of fresh red clover (treatment RC) was determined in autumn 2015 after harvesting, and for other green manure, they were determined in 2016 before incorporation. The concentrations of C and N in the manure were investigated 6, 12, 18, and 24 months after the incorporation of manure and litterbags into the soil (Table 2). N content in plant biomass, legume-based organic manure, grains and straws of cereals were determined. Samples from each plot were ground to flour, and N content was measured at the Chemical Research Laboratory of the Lithuanian Research Centre for Agriculture and Forestry. Samples for total N determination were analysed using the Kjeldahl method with a Kjelttec system 1002 (Foss Tecator, Sweden). The content of C was determined using a spectrophotometric measurement method at a wavelength of 590 nm using glucose as a standard after wet combustion. Concentrations of P were quantified spectrophotometrically using a coloured reaction with ammonium molybdate vanadate at a wavelength of 430 nm on a spectrophotometer Cary 50 UV-Vis (Varian Inc., Palo Alto, CA, USA). Respective K concentrations were evaluated using atomic absorption spectrometry with an Analyst 200 (Perkin Elmer, Waltham, MA, USA) in accordance with the manufacturer's instructions. At the hard dough stage (BBCH 87), spring wheat and spring barley were harvested. Cereal straw and grain yield were measured by weighting. Grain samples (1 kg) were taken from each plot for the determination of thousand kernel weight (TKW), DM content and chemical composition. Grain and straw samples were dried and ground using a ZM200 ultra-centrifugal mill (Retsch GmbH, Haan, Germany) with 1 mm mesh sieves and analysed for N content. Grain N content was recalculated for crude protein by the conventional factor 5.7 (ISO 20483). Starch content was analysed using polarimetry according to ICC 123/1, with modification using ADP 410 Polarimeter (Bellingham and Stanley Ltd., Tunbridge Wells, UK). The data on the chemical composition (C, N, P, K, protein, starch content) were recalculated on a DM basis.

Chemical analyses of the soil. Soil samples for agrochemical characterisation were taken from the 0–25 cm soil layer prior to the experiment installation. The pH was measured using the potentiometric method (C5020, Consort, Belgium). The content of humus was calculated using an organic carbon conversion factor of 1.72, while after wet combustion, organic carbon was determined using a spectrophotometric measurement at 590 nm (UV/Vis Cary 50, Varian Inc., Palo Alto, CA, USA) with glucose as a standard. The content of total N was determined after the wet digestion process with sulfuric acid (H_2SO_4) using the Kjeldahl method, using a spectrophotometric measuring procedure (UV/Vis Cary 50, Varian Inc., Palo Alto, CA, USA) at the 655 nm wavelength. The mobile phosphorus (P_2O_5) and potassium (K_2O) were determined using the A-L method. Soil samples for the analysis of soil nitrate (N-NO_3) and ammonium (N-NH_4) nitrogen concentrations (mg kg^{-1} of soil) were collected from the 0–30 and 30–60 cm soil layers three times during the experimental period: in 2015 spring before winter wheat growth resumed (BBCH 25), in 2016 before

fertiliser incorporation and in 2017 spring before cereals sowing. The concentrations of nitrate (N-NO₃) nitrogen were determined using the potentiometric method (CyberScan 2100, Eutech Instruments, Vernon Hills, IL, USA) in a 1% extract of KAl(SO₄)₂ × 12H₂O (1:2.5, *w:v*), and ammonium (N-NH₄) nitrogen using a spectrophotometric measurement (UV/Vis Cary 50, Varian Inc., Palo Alto, CA, USA) procedure at a wavelength of 655 nm in a 1 M KCl extract (1:2.5, *w:v*). Soil mineral N content was calculated as the sum of N-NH₄ and N-NO₃.

Calculations. For each vegetation period, N use efficiency (NUE) was calculated as a ratio of N_{output}/N_{input} as defined by the EU N Expert Panel [28]:

$$\text{NUE} = \text{N}_{\text{output}} / \text{N}_{\text{input}} \quad (1)$$

N output was calculated for grains, N input with residues and green manure. The biomass output efficiency (BOE) was calculated as the biomass of crop output (B_{output}), including grain and straw, achieved for each kilogram of N input (N_{input}), as described by Iannetta et al. [29]:

$$\text{BOE} = \text{B}_{\text{output}} / \text{N}_{\text{input}} \quad (2)$$

The N synchrony index (SI) assessing the synchronism between changing soil N concentrations (N_{release}) and crop N uptake (N_{total}) was calculated for the vegetation period after legume green manure incorporation. SIN represents the additive soil N content in 0–30 cm layer (difference from control), calculated from soil mineral N content before the vegetation period.

$$\text{SI} = \text{SIN} + \text{N}_{\text{release}} - \text{N}_{\text{total}} \quad (3)$$

2.4. Data Analysis

All statistical analyses were performed using SAS software version 9.4 (SAS Institute Inc., Copyright© 2002–2010). In the beginning, homogeneity and normality were verified using Bartlett's test. Experimental data were analysed using one-way analysis of variance (ANOVA), and mean comparisons between treatments were performed using Tukey's mean separation test. The smallest significant difference, R₀₅ was calculated using a probability level of $p < 0.05$. Additionally, correlation coefficients (*r*) were calculated to test the linear relationship between the production of cereal in two types of rotation and N estimates of residues and manures.

3. Results

3.1. N and C in Legume Green Manures

After processing legumes, green manures are distinguished by different chemical compositions and C:N ratios (Figure 1). Fresh red clover biomass had the narrowest C:N ratio. Fresh red clover biomass had a similar N content to granulated cattle manure and compost of red clover with straw, but these two had higher amounts of phosphorus and potassium. Fresh red clover biomass had a lower C:N ratio because it was grown as an intercrop with wheat and did not produce high biomass and C amounts. Red clovers used for the fermentation were cultivated separately and had a higher C content before the fermentation. Fermentation using silage preserved a higher content of C and widened the C:N ratio of ensiled red clovers. The composting of red clover and straw biomass gave a reduction in the C:N ratio, so this biomass, after processing, did not differ significantly from the sole fresh red clover.

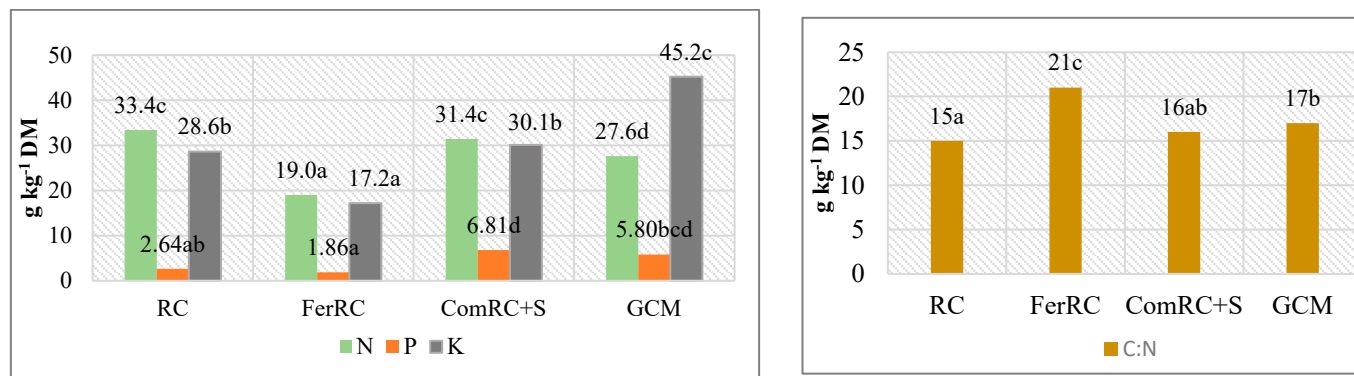


Figure 1. Chemical composition and C:N ratio of green manures used. Notes. RC—fresh red clover biomass; FerRC—fermented red clover; ComRC + S—composted red clover and straw; GCM—granulated cattle manure. Means of the same elements followed by the same letters in the same section do not differ from one another ($p \leq 0.05$).

3.2. C Release during the Green Manure Decomposition

Legume green manures, after processing, were used as organic fertiliser at different times of the season. Fresh red clover biomass was ploughed down in the autumn, while other green manures were incorporated into the soil in spring. It was found that during the first non-growing season (October–April), fresh red clover biomass lost 65.6% of its initial C (Figure 2). After the first crop growing season, green manures retained significantly different amounts of undecomposed C (from 19.2 to 67.4% DM). Fermented and composted red clovers released similar amounts of C during the first growing season, while granulated cattle manure released the least. Granulated cattle manure and compost of clover and straw had slow C release to the soil in all treated 2-year periods, while fresh red clover biomass released 91% of C quickly and stabilised in a short one-and-a-half-year period. Its stabilised C value was 8.5% of the total incorporated C.

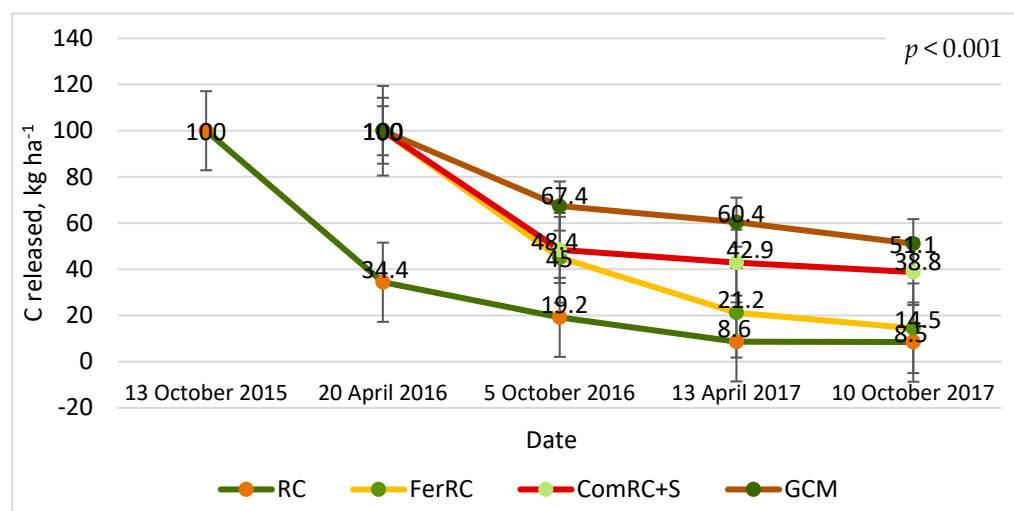


Figure 2. Dynamic of total C left in green manures over a 2-year period of the decomposition. Notes. RC—fresh red clover biomass; FerRC—fermented red clover; ComRC + S—composted red clover and straw; GCM—granulated cattle manure. Whiskers show the variation of data.

3.3. N Release during the Decomposition

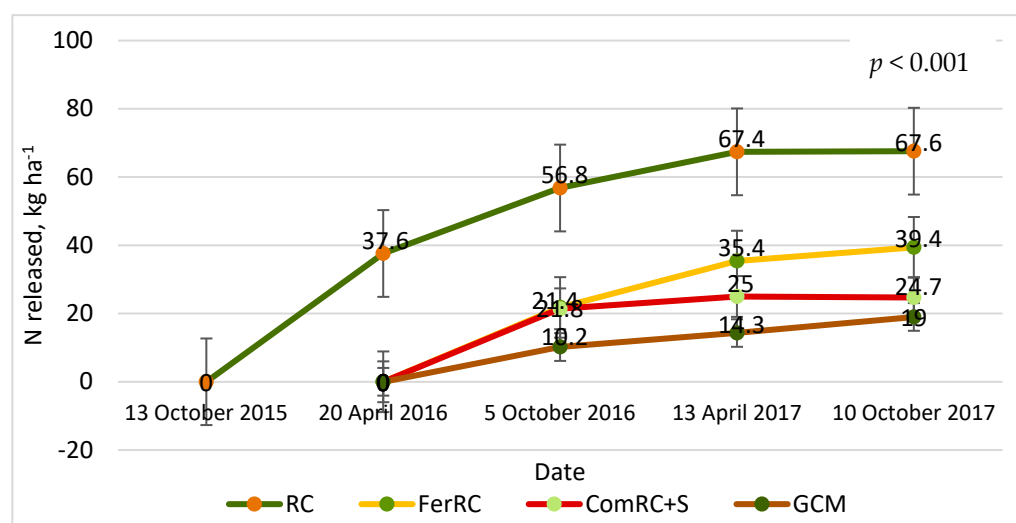
N content, during the 2-year decomposition period, was the highest and most varied between 2.5–4.8% DM for fresh red clover biomass (Table 4). The C:N ratio during different decomposition periods varied between 10.3 and 25.4 but did not differ significantly between treatments.

Table 4. N content (%) and C:N ratio of undecomposed legume green manures, measured with the litterbag method, during a 2-year period, every 6 months after manure incorporation in the soil.

Legume Green Manure	N of Undecomposed Green Manure, %					C:N Ratio of Undecomposed Green Manure				
	Litterbag Sampling Time									
	0	6/0	12/6*	18/12	24/18	0	6/0	12/6	18/12	24/18
	13 October 2015	20 April 2016	5 October 2016	13 April 2017	10 October 2017	13 October 2015	20 April 2016	5 October 2016	13 April 2017	10 October 2017
RC	3.3	4.8 a	3.3 a	2.8 a	2.5 a	15.6	10.3 b	11.9 b	12.4 b	12.8 b
FerRC		1.9 c	3.0 a	2.1 ab	2.3 ab		20.6 a	16.8 b	15.3 b	14.5 b
ComRC + S		3.1 b	2.0 b	2.2 ab	2.2 ab		16.4 ab	13.7 b	11.1 b	13.4 b
GCM		2.8 b	2.8 a	2.7 a	2.8 a		17.0 ab	14.4 b	14.4 b	14.0 b

Notes. 0—initial data before burying litterbags, 12/6* data during litterbag sampling (the numbers show months after burying, first one (etc. 12) for RC, second (etc. 6) for FerRC, ComRC + S and GCM). RC—fresh red clover biomass; FerRC—fermented red clover; ComRC + S—composted red clover and straw; GCM—granulated cattle manure. Means followed by the same letters in the same column and section do not differ from one another ($p \leq 0.05$).

The total N release was significantly the highest for fresh red clover biomass and, during the 2-year period, reached 67.6 kg ha^{-1} with the highest effect in the first non-growing season, 37.6 kg ha^{-1} and in the first crop growing season, 19.2 kg ha^{-1} (Figure 3). During the first crop growing season, a significantly similar ($p < 0.001$) amount of N was released by fermented red clover and compost of red clover with straw ($21.4\text{--}21.8 \text{ kg ha}^{-1}$), also by granulated cattle manure ($7.9\text{--}10.2 \text{ kg ha}^{-1}$). During the second non-growing season, a high loss of N was recorded for fresh and fermented red clover biomass ($10.6\text{--}14.0 \text{ kg ha}^{-1}$). Positive N release during the second crop season was observed for fermented red clovers and granulated cattle manure ($3.4\text{--}4.7 \text{ kg ha}^{-1}$).

**Figure 3.** Dynamic of total N release from legume green manures over a 2-year period of the crop rotation. Notes. RC—fresh red clover biomass; FerRC—fermented red clover; ComRC + S—composted red clover and straw; GCM—granulated cattle manure. Whiskers show the variation of data.

3.4. Changes in Mineral N in the Soil

Legume green manures incorporated in the soil were mineralised, and legume N was transferred into the soil at various rates in its mineral forms of N-NH_4 and N-NO_3 . One year after amendment with legume green manures, the amount of N-NH_4 was four times lower than N-NO_3 and did not vary significantly among the treatments. The higher amounts of N-NH_4 were observed for two amendments—compost of red clover and straw (+144%) and granulated cattle manure (+89%) in 0–30 cm depth of soil. Conversely, the remaining two amendments—fresh red clover biomass and fermented red clover—had

160% and 298% higher amounts of N-NO₃ than the control, respectively. Among the applied green manures, lower N-NH₄ and N-NO₃ leaching to the deeper soil layer (30–60 cm) was observed for fresh red clover biomass and granulated cattle manure in the second year after amendment incorporation. However, unlike other green manures, fresh red clover biomass was incorporated in the autumn of 2015, not in the spring of 2016; therefore, higher N_{min} leaching to the 30–60 cm layer was noticed during the non-growing season in 2016 (Figure 4).

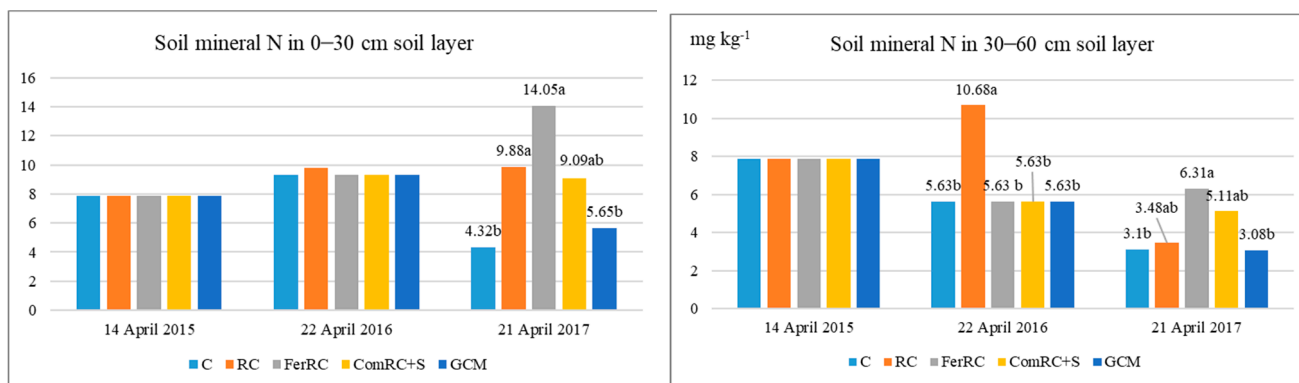


Figure 4. Mineral N content in the soil depths 0–30 and 30–60 cm in 3-year crop rotation, before and after legume green manure incorporation. Notes: RC—fresh red clover biomass; FerRC—fermented red clover; ComRC + S—composted red clover and straw; GCM—granulated cattle manure. Means followed by the same letters in the same section do not differ from one another ($p \leq 0.05$) according to Tukey's test. Means followed by the same letters in the same particular time lag do not differ from one another ($p \leq 0.05$). Columns without letters represent no significant differences between the treatments in the particular time lag.

3.5. The Effect of Legume Green Manures on Crop Productivity

Analysing the duration of the effect of legume green manures, a correlation was noticed between green manure features and the productivity/quality of cereals two years after green manure application (Table 5). In the first year after green manure application in the spring, the yield, total biomass, protein content in grain, total N accumulated in straw, grain, and total plant of spring wheat positively significantly correlated ($r = 0.47$ – 0.78 , $p < 0.05$) with total N amount incorporated into the soil with legume green manures. Not only was the total N of green manures important, but also the spring wheat indexes positively correlated ($r = 0.55$ – 0.71 , $p < 0.01$) with the decomposition rate of green manures during the first year of vegetation. The higher decomposition rate of green manures (especially for fresh red clover and fermented red clover) in a linear relationship increased the biomass, protein content, total N of straw and aboveground biomass of spring wheat.

The decomposition rate also adjusted the N amount released by green manures in a specific time lag of a specific crop vegetation. Therefore, the N amount released during the first year of vegetation was correlated with spring wheat indexes more strongly than the total N incorporated with green manures in the beginning. An unexpectedly strong positive correlation was noticed between mineral N in the deeper layer of the soil (30–60 cm depth) and the same mentioned variables of spring wheat. This could be explained by a strong positive correlation of total N, decomposition rate and seasonal N released by legume green manures with mineral N in the 30–60 cm depth of soil, meaning the higher N leaching possibility was conditioned by the increased rate of decomposition and higher N release.

In the second year after legume green manures application, the yield of spring barley positively and medium strongly correlated ($r = 0.42$, $p < 0.05$) with the total N amount incorporated into the soil with legume green manures. Meanwhile, total biomass reflected more the C:N ratio of legume green manures ($r = 0.40$, $p < 0.05$). The N amount released

during the second year of vegetation correlated with the thousand kernel weight and protein content of spring barley. It was observed that the effect of legume green manure was often of moderate strength ($r = 0.40\text{--}0.65$, $p < 0.05$) in the second year, while in the first year, it was often strong ($r = 0.62\text{--}0.88$, $p < 0.05$). The residual effect of the first-year grown spring wheat was low in the third year because no significant correlation was found between the total N accumulated in the straw of spring wheat in 2016 and the variables of spring barley in 2017.

Table 5. The correlation between the variables of legume green manures and cereal traits in the first year (spring wheat) and second year (spring barley) after application.

	Variables of Legume Manures, Soil and Residues						
	N _{tot} kg ha ^{−1}	C:N	D rate % kg year ^{−1}	N _{season} kg ha ^{−1} year ^{−1}	N _{min} 0–30 mg kg ^{−1} soil	N _{min} 30–60 mg kg ^{−1} soil	N straw 2016
Variables in first year ↓							
Yield	0.76 **	−0.27	0.23	0.75 **	0.13	0.64 **	0.58 **
Biomass	0.50 *	0.006	0.62 **	0.75 **	0.07	0.68 **	0.92 **
TKW	0.24	0.24	−0.18	−0.20	0.32	−0.26	−0.31
Protein	0.47 *	0.22	0.66 **	0.52 **	0.37	0.56 **	0.64 **
Starch	0.02	−0.03	0.40	−0.20	0.01	−0.39	−0.34
Total N straw	0.59 **	0.12	0.71 **	0.85 **	0.12	0.78 **	1.00
Total N grain	0.80 **	−0.15	0.39	0.79 **	0.22	0.71 **	0.69 **
Sum N plant	0.78 **	−0.05	0.55 **	0.88 **	0.20	0.80 **	0.87 **
Variables in second year ↓							
Yield	0.42 *	0.16	0.28	0.32	0.02	−0.16	0.23
Biomass	−0.08	0.40 *	0.33	0.08	−0.18	0.04	−0.07
TKW	0.64 **	0.46 *	0.81 **	0.65 **	0.38	0.31	−0.12
Protein	0.20	0.32	0.29	0.43 *	0.26	−0.10	0.08
Starch	0.07	−0.28	−0.05	−0.19	−0.33	−0.16	−0.10

Notes. * Significant at $p \leq 0.05$; ** Significant at $p \leq 0.01$ level; without stars—not significant. D rate—decomposition rate of green manures, N_{season}—N released from manure during the crop growing season, N_{tot}—total N accumulated by grain and straw.

3.6. NUE, BOE and N Synchrony Index

In both experimental years, NUE was lowest for crops amended with red clover fresh biomass and the highest for granulated cattle manure, but only in the first year (Table 6). Biomass output efficiency was higher in the first year than the second and was lowest for fresh red clover treatment. The N synchrony index defined the differences between N utilisation in crop biomass and additional N gained from the management regime (Table 6). SI in all treatments and two years showed negative values, defining the various N deficit amounts, which should be taken from N stocks from the soil. During the first year, fresh red clover biomass and granulated cattle manure showed the most negative SI, while all processed legume manures showed the highest synchronisation. The best synchronisation was for fermented red clover and composted red clover plus straw treatments. During the second year, the best synchrony was achieved for fermented red clover mass and the lowest for granulated cattle manure.

Table 6. N use efficiency, biomass output efficiency and N synchrony index in spring wheat in the first year and spring barley in the second year after green manure incorporation.

Legume Green Manure	Spring Wheat			Spring Barley		
	NUE	BOE	SI	NUE	BOE	SI
C	–	–	−36	–	–	−40
RC	0.52	75	−33	0.43	49	−20
FerRC	0.60	90	−17	0.71	73	−1
ComRC + S	0.60	87	−17	0.70	73	−23
GCM	0.68	89	−32	0.70	75	−34

Notes. RC—fresh red clover biomass; FerRC—fermented red clover; ComRC + S—composted red clover and straw; GCM—granulated cattle manure.

4. Discussion

4.1. C and N Role in the Decomposition

The C:N ratio is one of the main factors which defines the decomposition rate of different organic materials in the soil [30] and also the N mineralisation–immobilisation processes [7]. Legume residues are characterised by lower C:N ratios than non-legume crops [22,31,32]. In our research, red clover as a forage legume was distinguished by the narrowest C:N ratio (14.8–22.0) of straw. This is consistent with other scientific data, showing red clover to have a C:N ratio of 12 [33], 20.5 [22], and 15.4–27.4 [34]. The fresh red clover biomass accumulates a high N amount, but the process of N release is difficult to control [35,36]; therefore, processing of legume mass was used to prevent N losses and to synchronise the nutrient supply [20,21]. Our study showed that after processing, the C:N ratios for all legume-based green manures were narrow 15–21.

4.2. Decomposition Rates of Legume Fertilisers

Organic matter quality explains up to 64% of the variation in decomposition rates [37]. One of the most popular plants, which is ploughed down in the autumn to enhance soil fertility, is red clover [38,39]. Red clover manure has a high amount of labile fractions and a narrow C:N ratio of 15. Readily degradable labile fractions of organic compounds in plant material start to decompose first [40]. Incorporating fresh red clover mass before the non-growing season increased the possibility of N losses [20]. During the first non-growing season, fresh red clover mass lost a high amount—65.6% DM of its initial mass and 37.6 kg ha^{−1} of N. This led to a 90% increase in mineral N amounts in the soil's deeper layer of 30–60 cm, indicating the leaching process in this treatment. Froseth and Bleken [36] found that low temperatures of the non-growing season, 0–4 °C maintain the same decomposition patterns as in 8.5–15 °C. During the second non-growing season, a higher loss of N was incurred only by fresh and ensiled red clover mass (10.6–14.0 kg ha^{−1}). Composted red clover and straw matter were characterised by a lower decomposition rate during the non-growing season. Faster decomposable material responds more sensitively to the management factors [40]. To synchronise the nutrient supply with ecosystem needs, processed legume material was incorporated into the soil in the spring. During the first crop growing season, significantly similar ($p < 0.001$) amounts of N were released by fermented red clover mass and compost (21.4–21.8 kg ha^{−1}), as well as by granulated cattle manure (7.9–10.2 kg ha^{−1}). This shows the unique feature of ensiling to preserve legume N for the vegetative season [41,42] and that it competes in value with livestock-based fertilisers [24]. In agreement with this idea, positive N release during the second crop season was observed for both fermented masses and granulated cattle manure (3.4–4.7 kg ha^{−1}). This indicates the second positive feature of ensiled material to attain the prolonged positive effect to two subsequent crop growing seasons in the rotation.

4.3. Mineralisation of Organic Fertilisers

The concentrations of soil N_{min} depended on the weather conditions, the quality of organic fertilisers used, decomposition rate, incorporation depth and soil fauna [43]. The N_{min} content in the soil varied from 3 to 14 mg N kg^{−1} DM, applying differently processed legume mass. All manures increased N_{min} content in the plough layer (0–0 cm) one year after incorporation compared with the control. However, the control treatment (with spring barley straw) and granulated cattle manure were characterised by decreased N mineralisation and could be assigned to promoting immobilisation. Other manures—fresh red clover mass, compost of red clover and straw maintained N_{min} level and could promote a balanced mineralisation-immobilisation process. Significantly higher mineralisation was observed only for fermented red clover mass in the second year of the manure effect. In agreement with our results, Van Opheusden et al. [44] indicate that organic fertilisation with a very high mineralisation rate leads to a significant increase in N_{min} content in the soil over the course of the year. However, the use of fertilisers with medium (manure) or low (plant compost) mineralisation inhibits immobilisation [7] and prolongs organic

matter mineralisation over time (4–5 years) [44]. There is a lack of research on fermented legume mineralisation processes in the soil. It is claimed that it contains more available N for plants [41,45], but the risk of N losses is weak due to low ammonium and low pH [42]. Granulated cattle manure incorporated into the soil becomes altered in its chemical composition, meaning that easily degradable organic substances are fragmented and form more stable organic compounds compared to fermented and green organic fertilisers. The compost decomposes slowly in the soil: a small fraction (10–15%) of its mass decomposes per year [44].

4.4. Correlation with Crop Productivity

Processed manures have not been comprehensively investigated and compared in the literature since processing practices such as ensiling are more common in cattle feeding [46]. There are also many reports on the chemical composition of processed legume green manure, but the effects on cultivated cereal yield, especially two years after fertilisation, are low or limited to pot experimental design [42].

The productivity of crops also decreased when the C:N ratio of applied green legume manure increased [47]. Van Opheusden et al. [44] present a 12-year study in Germany, where fresh green manure was frequently linked with higher vegetable yield than fermented green manure or compost. This increase was explained by initial N content, decomposition rate and yearly N mineralisation level of manures [44]. Our research suggests that the same factors affect cereal productivity. In the first year after manure application, the yield, total biomass, protein content in grains, total N accumulated in straw, grains, and total plant of spring wheat positively significantly correlated ($r = 0.47\text{--}0.78$, $p < 0.05$) with total N amount incorporated into the soil with legume green manures, decomposition rate and periodically released N. Moreover, the N amount released from green manure during the particular year of vegetation was correlated with cereal productivity indexes more strongly than total N incorporated with manures in the beginning, showing the high importance of the synchrony of nutrients' release. For example, the legume compost treatment performance was relatively weak over the entire crop rotation in our study and in some other studies [44,48]. Composting results in fundamental physical and chemical changes, causing a significant reduction in N availability in the soil [49]. Furthermore, cattle manure was related to a lower increase in the grain yield, protein content and accumulated N in winter wheat, compared with red clover fresh manure, due to different timing of N availability in the soil [50].

5. Conclusions

Fresh red clover biomass was distinguished by a high N and the narrowest C:N ratio, and during the first non-growing season (4 months after incorporation), it lost 65.6% DM of its initial C and 37.6 kg ha^{−1} of N. This was consistent with a 90% higher mineral N amount in the 30–60 cm deep subsoil, showing high mineralisation and increased potential for N leaching.

Fermentation preserved a higher C content and widened the C:N ratio of red clovers. The processing of legume biomass by ensilage slowdown C release to the soil in all treated 2-year periods. Meanwhile, fresh red clover biomass released quickly 91% of C. Technologically processed legume biomass increased N use efficiency by 15%, biomass output efficiency by 16% and significantly increased synchronisation index between crop N demand and N supply. The highest synchrony index was achieved by fermented red clover, while fresh red clover biomass was characterised by low synchronisation between N supply and N requirements for crops in the rotation.

Processed legume green manures did not reach the NUE and succeeded in lowering the positive effect on the productivity of spring wheat in the first year compared with granulated cattle manure. However, fermented red clover significantly increased the grain yield of spring barley—in the second year and achieved a much higher total synchronisation

index than granulated cattle manure. This identifies that using fermented red clover is more sustainable for the ecosystem than using granulated cattle manure.

The productivity of cereals significantly correlated with the total N incorporated into the soil, decomposition rate and periodically released N. Additionally, the total biomass, protein content in grain, total N accumulated in straw, grain, and total plant of spring wheat positively significantly correlated ($r = 0.47\text{--}0.78$, $p < 0.05$) with total N amount incorporated into the soil with legume manures and the decomposition rate.

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