https://doi.org/10.17221/369/2025-PSE

Effect of fertilisation and utilisation methods of red clover on surface nutrient balance

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Citation: Panakhyd H., Kozak N., Olifir Y., Partyka T., Havryshko O., Konyk H., Stasiv O. (2026): Effect of fertilisation and utilisation methods of red clover on surface nutrient balance. Plant Soil Environ., 72: 28–38.

Abstract: The research was conducted in a long-term stationary experiment established on light grey forest surface-gleyed soil in 1965. Data presented in this study were collected during 2022–2024 growing seasons within the framework of this long-term experiment. The experiment is registered in the NAAS long-term field experiments registry (certificate No. 29) and the Global Long-Term Agricultural Experiments Network (GLTEN). The study examined the effect of growing red clover in a four-field crop rotation on nutrient balance at different fertiliser and lime doses and ratios. Red clover was used for feed and feed-green manure purposes. The research aimed to substantiate optimal methods of utilising this valuable forage crop and optimise fertilisation systems to ensure sustainable agricultural development. Growing the first cut of red clover for feed purposes and the second as green manure with fertilisation ($N_{105}P_{101}K_{101}$ + organic fertilisers + liming) ensures a positive surface balance of 402 kg/ha of nitrogen, 150 kg/ha of phosphorus, and 204 kg/ha of potassium. These data are almost twice higher than indicators under minimal fertilisation doses. Despite the reduction in symbiotic nitrogen fixation from 217 kg/ha to 147 kg/ha when growing red clover in crop rotation with intensive fertilisation, it remains an effective phytobiological ameliorant.

Keywords: biological fixation; symbiotic activity; leguminous crops; fertilisation systems; surface nutrient balance

The main component of all landscapes is perennial forage vegetation, which ensures sustainable ecosystem development and has a general positive impact on society (Huyghe et al. 2014). Modern agriculture faces the dual challenge of maintaining high productivity while preserving soil fertility and environmental quality. Perennial forage legumes play a crucial role in addressing this challenge through their ability to fix atmospheric nitrogen, improve soil structure, provide high-quality feed for livestock production, and protect soil from erosion (Protopish et al. 2012). Due to their capacity for biological nitrogen fixation, legumes can significantly improve soil nitrogen regime and reduce dependency on mineral fertilisers (Herben et al. 2017), making them essential components of sustainable crop rotations and soil fertility management systems.

Red clover (*Trifolium pratense* L.) is one of the most widespread forage legumes in temperate climate zones. According to various scientists (Herben et al. 2017, Marshall et al. 2017), depending on soil-climatic conditions, it can accumulate 45–340 kg/ha of nitrogen per year through biological fixation. This nitrogen fixation capacity makes red clover particularly valuable in crop rotations, especially on soils with low natural fertility. According to some researchers (Nyfeler et al. 2017), the yield of legume-grass mixtures without nitrogen fertiliser application is equivalent to the yield of grasses under which 112–250 kg/ha of nitrogen active ingredient was applied, demonstrating the substantial nitrogen contribution of legumes. Furthermore, N-fixing legumes exhibit high root phosphatase activity, especially

Supported by the National Academy of Agrarian Sciences of Ukraine, Project No. 01.01.03.08.Ф.

https://doi.org/10.17221/369/2025-PSE

under low soil phosphorus availability (Png et al. 2017), thereby enhancing their ability to mobilise soil phosphorus and improve nutrient availability for subsequent crops.

However, the benefits of red clover cultivation extend beyond its role as a nitrogen source. Different methods of red clover utilisation – for green feed, hay, silage, or as a green manure crop – affect the soil nutrient balance and the sustainability of the cropping system differently. Using red clover as green manure allows the replacement of synthetic nitrogen fertilisers (Rasmussen et al. 2012), and this is most realistic if it is based on nitrogen fixation in biomass or immobilisation in soil (Carter et al. 2014). According to Lynge et al. (2022), red clover used as green manure allows obtaining about 500 kg/ha of nitrogen under intensive management with four to five cuts per year. In two-cut mowing systems, which are more common in practical agriculture, the second cut yields 210 kg/ha of nitrogen. Råberg et al. (2018) claim that the amount of nitrogen that red clover can provide as green manure depends on growing conditions and varies from 230 to 470 kg/ha. Understanding these nutrient dynamics is essential for developing sustainable forage production systems that maintain long-term soil fertility while meeting livestock feeding requirements.

A key element in understanding the processes of nutrient transformation and migration is the study of nutrient balance in the soil-plant system. Surface nutrient balance – calculated as the difference between inputs (fertilisers, biological fixation, atmospheric deposition) and outputs (crop removal, gaseous losses) – provides a practical tool for assessing the sustainability of agricultural systems. This issue becomes particularly relevant for light grey forest soils, which are characterised by relatively low natural fertility levels, low buffer capacity, and susceptibility to degradation processes (Olifir et al. 2023). On such soils, inappropriate management can lead to rapid fertility decline, making careful nutrient balance assessment essential for sustainable production.

To determine surface nutrient balance and simultaneously assess the agroecological efficiency of crop production, root mass accumulation in soil is an important indicator. When growing red clover, all accumulated roots remain in the soil, enriching it with nutrients that contribute to the overall nutrient budget. Root biomass and its nutrient content are often overlooked as inputs in simplified balance calculations. Yet, they can significantly affect the overall nutrient budget, particularly in systems where aboveground biomass is removed from the field. Root system ageing after cultivation also contributes to increasing organic matter in soil, providing a set of ecosystem services (McKenna et al. 2018). The amount of root mass depends on many factors, including soil type, cultivated variety, fertilisation regime, and crop yield. In studies by Lithuanian scientists (Skersiene et al. 2024), red clover accumulated up to 290 g/m^2 (2.9 t/ha) of dry root mass. Similar indicators (230 g/m^2) were obtained by Azzaroli Bleken et al. (2025), demonstrating the substantial contribution of belowground biomass to soil organic matter and nutrient pools.

Long-term fertilisation systems create cumulative effects on soil properties that, in turn, influence red clover productivity and nutrient cycling. The interaction between fertilisation history (organic, mineral, or combined systems), liming practices on acidic soils, and utilisation methods determines both immediate productivity and long-term sustainability. However, while numerous studies have examined red clover productivity and nitrogen fixation under various management systems, there is limited information on how different utilisation methods interact with long-term fertilisation regimes to affect surface nutrient balance on light-textured acidic soils. This knowledge gap is significant for the light grey forest soils of Western Ukraine, where nutrient management is challenging and inappropriate practices can lead to rapid fertility decline.

We hypothesised that: (1) feed-green manure utilisation would result in more favourable nutrient balance compared to two-cut feed utilisation due to incorporation of the second cut biomass, which returns nutrients to the soil; (2) long-term application of combined organic and mineral fertilisation systems with adequate liming would significantly affect nutrient accumulation and balance in red clover cultivation, with integrated systems providing more balanced nutrient cycling than single-input approaches.

These studies aimed to determine: (1) the amount of nitrogen contributing to the surface balance as a result of biological fixation by red clover under different long-term fertilisation systems; (2) the volumes of removal of primary nutrients (nitrogen, phosphorus, potassium) with harvested biomass under two utilisation methods; (3) the contribution of root biomass to nutrient input; and (4) the resulting surface nutrient balance for nitrogen, phosphorus, and

https://doi.org/10.17221/369/2025-PSE

potassium as affected by the interaction of fertilisation systems and utilisation methods. This research was conducted within a long-term stationary experiment established in 1965, allowing assessment of fertilisation system effects accumulated over decades of continuous application. The research results will allow optimising red clover utilisation methods and fertilisation systems to ensure sustainable agricultural development, increase the efficiency of utilising the natural potential of leguminous crops, and reduce the agrochemical load on agroecosystems.

MATERIAL AND METHODS

Research location and experimental design. The scientific research work was performed based on a classic long-term stationary experiment established in 1965, registered in the NAAS long-term field experiments registry (certificate No. 29) and the Global Long-Term Agricultural Experiments Network (GLTEN). The experimental plot is located in the western part of the Forest-steppe zone of Ukraine, which is part of the Carpathian region and belongs to the Western European district of the Atlantic-continental climatic region. The experiment is located at 49°49'N, 24°0'E, altitude 317 m a.s.l.

The soil of the experimental plot is light grey forest surface-gleyed coarse dusty-light loamy on loess-like deposits (Albic Pantostagnic Luvisol according to FAO classification).

The research was conducted in a four-field crop rotation with the following crop sequence: corn, spring barley with red clover undersowing, red clover, winter wheat. The experiment was arranged in a split-plot design with fertilisation systems as the main plot factor and utilisation methods as the subplot factor. Each treatment was replicated three times. Each main plot represents one fertilisation treatment and is arranged sequentially within each replication. The total area of each main plot is 168 m^2 (28 m × 6 m), consisting of 4 subplots of 42 m^2 each (one for each crop in rotation). The harvest area for yield accounting within each subplot is 35 m^2 (excluding the 1 m border rows on each side to eliminate edge effects).

The current fertilisation scheme has been applied since 2000 (25 years). During the period 1965–2000, a fertilisation system with higher mineral fertiliser doses was used. All plots had the same previous management history throughout the entire period (1965–2024).

The research was conducted in the third field of rotation, where red clover cultivar Truskavchanka was sown (in the previous year under spring barley undersowing).

Weather conditions. Weather conditions during 2022–2024 were warmer than average, with winter temperatures 2.6–4.5 °C above long-term norms and growing-season temperatures 1.4–3.2 °C above long-term norms. Growing season precipitation varied from 426 mm in 2022 and 425.4 mm in 2024 (both ~145 mm below the 571 mm average) to 590.7 mm in 2023 (near normal). The hydrothermal coefficient ranged from 1.1 to 1.6, indicating sufficient to favourable moisture availability despite precipitation deficits in two of the three years.

Experimental treatments. This paper presents results from the 2022–2024 monitoring period of red clover cultivation. Two utilisation methods were studied: feed utilisation – two cuts for feed; feed-green manure utilisation – first cut for feed, second cut for green manure (incorporation into soil). The first cut was performed at the budding-early flowering stage (typically mid-June), and the second cut at the late flowering stage (late August-early September). These timings corresponded to approximately 60–65 days of growth for the first cut and 70–75 days of regrowth for the second cut.

Soil conditions at the start of monitoring (spring 2022) differed substantially depending on the long-term application of fertilisation and liming systems (Table 1).

Limestone flour was used as the liming material (93.5% $CaCO_3$). Semi-decomposed cattle manure on straw bedding was used with the following characteristics: 25% dry matter, 0.5% N, 0.11% P, 0.5% K (on fresh weight basis), decomposition degree approximately 60%, storage period 5–6 months. Manure was applied under corn at 40 t/ha fresh weight. Phosphorus-potassium fertilisers were applied in autumn, nitrogen and complex fertilisers – before pre-sowing cultivation. Red clover was not fertilised directly; all types of fertilisers were applied to other crops in the rotation according to the scheme shown in Table 1.

Chemical analyses. Aboveground biomass yield accounting from the first and second cuts was determined by the direct harvest method with weighing of fresh mass from each plot from the designated harvest area of 35 m^2 (excluding 1-m border rows on each side of the 7 m × 6 m subplot). Yield was presented as absolute dry mass (DM), with hygro-

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Table 1. Experimental treatments, fertilisation schemes and soil characteristics under red clover in spring 2022 in the 0–20 cm arable layer

Treatment	Fertilisers applied in crop rotation				Liming scheme	Soil characteristics under red clover			
	corn	spring barley + red clover	red clover	winter wheat		pH_{KCl}	N	P	K
							(mg/kg)		
NF	–	–	–	–		4.13	96.3	37.0	47.1
L8	–	–	–	–	L8	5.10	100.8	40.7	48.8
O	manure 40 t/ha	–	–	–		4.88	103.4	51.3	62
NPK	$N_{120}P_{90}K_{90}$	$N_{70}P_{90}K_{90}$	–	$N_{70}P_{90}K_{90}$		4.18	106.5	168.7	113.2
NPK + L8	$N_{120}P_{90}K_{90}$	$N_{70}P_{90}K_{90}$	–	$N_{70}P_{90}K_{90}$	L8	4.57	105.3	175.7	123.2
NPK + L8 + O	manure 40 t/ha + $N_{120}P_{90}K_{90}$	$N_{70}P_{90}K_{90}$	–	$N_{70}P_{90}K_{90}$	L8	4.94	112.0	170.5	182.9
NPK + L4 + O	manure 40 t/ha + $N_{120}P_{90}K_{90}$	$N_{70}P_{90}K_{90}$	–	$N_{70}P_{90}K_{90}$	L4	5.16	113.1	161.8	165.8
0.5NPK + L8 + O	manure 40 t/ha + $N_{60}P_{45}K_{45}$	$N_{30}P_{45}K_{45}$	–	$N_{30}P_{45}K_{45}$	L8	4.89	108.4	161.2	133
2NPK + L8 + O	manure 40 t/ha + $N_{180}P_{135}K_{135}$	$N_{120}P_{135}K_{135}$	–	$N_{120}P_{135}K_{135}$	L8	4.88	113.6	197.3	189.7
2NPK + 1.5L8	$N_{180}P_{135}K_{135}$	$N_{120}P_{135}K_{135}$	–	$N_{120}P_{135}K_{135}$	1.5 8	4.96	108.9	202.2	150.9

*Nutrient doses are given as pure elements (N, P, K). NF – not fertilisers; O – organic manure; L – liming; Liming scheme: L8 – 6 t/ha $CaCO_3$ once every 8 years (rate based on hydrolytic acidity); L4 – 2.5 t/ha $CaCO_3$ once every 4 years (rate based on acid-base buffer capacity); 1.5L8 – 9 t/ha $CaCO_3$ once every 8 years (1.5 rate based on hydrolytic acidity)

scopic moisture preliminarily determined by drying a 0.5 kg sheaf sample at 105 °C to constant weight (DSTU ISO 6497:2005).

Root mass accumulation was determined to a depth of 0–10 cm using the monolith method (soil cores 25 cm × 25 cm). Soil samples were collected in spring 2022 from the 0–20 cm layer using the envelope method (5 subsamples per plot). In soil, aboveground and underground mass, total nitrogen was determined by Kjeldahl method (DSTU ISO 5983-2003); phosphorus was determined by spectrophotometric method after wet digestion (DS/EN ISO 6878:2004) (spectrophotometer Ulab 102 UV, Zhengzhou, China); potassium by flame photometry after the same digestion procedure (flame photometer FLAFO 4, Jena, Germany).

Calculations. Surface soil balance was determined according to the methodology of Oenema et al. (2003) using the average 2022–2023 data. The difference between nitrogen, phosphorus and potassium input and their removal with harvest was calculated:

$$\text{surface balance} = \text{input} - \text{removal}$$

Where: input – mineral fertilisers applied in rotation + organic fertilisers applied in rotation + biological N-fixation by clover + elements in root mass + elements in incorporated green mass (for green manure treatment); removal – elements in harvested biomass

Total phosphorus and potassium input was determined as the sum of the elements applied with fertilisers (distributed across the 4-year rotation), elements accumulated in green mass (if it was incorporated), and root mass.

Nitrogen input to the surface layer was calculated using the empirical model of Høgh-Jensen et al. (2004). For nitrogen input, symbiotic nitrogen was considered without accounting for atmospheric nitrogen deposition. The amount of harvested nitrogen obtained from symbiosis was calculated by multiplying the harvested nitrogen content of each legume species by its corresponding symbiosis fraction. The fraction obtained from symbiosis was calculated according to the nitrogen difference method based on McAuliffe et al. (1958), where the proportion of nitrogen derived from atmospheric fixation was estimated by comparing nitrogen content in nodulated legumes with non-nodulated control plants or with nitrogen uptake by non-legume reference crops grown under identical conditions. This method assumes that the difference in N uptake between legumes and reference plants represents atmospheri-

https://doi.org/10.17221/369/2025-PSE

cally fixed N. For red clover in our study, we used a coefficient of 0.75 as the baseline value (meaning approximately 75% of nitrogen in the plant comes from biological fixation, 25% from soil), consistent with values reported by Carlsson and Huss-Danell (2003) for perennial forage legumes. However, this coefficient is not constant and varies between treatments depending on soil nitrogen availability. In our experiment, the proportion of N derived from fixation ranged from 0.68 to 0.82 across treatments, with lower values observed in high-N fertilisation treatments (2NPK) and higher values in unfertilised or organically fertilised treatments. These coefficients were adopted from the literature (McAuliffe et al. 1958) and empirically adjusted. This variation reflects the well-documented phenomenon that legumes reduce their reliance on symbiotic fixation when soil mineral N is readily available, as soil N uptake is energetically less costly than N_2 fixation (Nyfeler et al. 2011). For treatments with high mineral N availability (> 150 kg N/ha in soil), we adjusted the coefficient downward to 0.68–0.70; for low-N treatments, we used 0.78–0.82. These treatment-specific coefficients were validated by comparing our results with published data from similar soil types and fertilisation regimes. We acknowledge that the N difference method has limitations, including the assumption that legumes and reference plants have similar rooting patterns and N uptake efficiency from soil. More precise quantification could be achieved using ^{15}N natural abundance or enrichment techniques. Nevertheless, this approach provides reasonable estimates for comparing the relative effects of fertilisation treatments on symbiotic activity, which is the primary objective of our balance calculations.

Statistical analysis. Statistical analysis was performed using Microsoft Excel (Redmond, USA) and Statistica 10 software(Tulsa, USA). Data were processed by analysis of variance (ANOVA) for a split-plot design with three factors: year (2022–2024), fertilisation treatment (10 levels as main plots), and utilisation method (2 levels as subplots). The significance of differences between mean values was assessed by the least significant difference (*LSD*) at $P \leq 0.05$. Correlation analysis was performed only between continuous quantitative variables: DM yield, symbiotic nitrogen fixation, and root mass accumulation. Pearson correlation coefficients were calculated, and significance was tested at $P < 0.05$. Categorical factors (fertilisation systems, utilisation methods) were analysed using ANOVA and post-hoc tests as described above.

ANOVA results showed no significant effect of year on any of the studied parameters (*F*-values ranged from 0.87 to 1.45, all $P > 0.25$) and no significant year × treatment interaction ($F = 0.87$, $P = 0.621$). The effects of fertilisation treatment and utilisation method were highly significant for all parameters ($P < 0.001$). Therefore, data are presented as three-year means across years, separated by fertilisation system and utilisation method.

RESULTS AND DISCUSSION

Aboveground mass yield and root accumulation. The highest dry matter (DM) yield of red clover, 12.6 t/ha, was obtained in the organic-mineral fertilisation and liming system treatment with application in crop rotation of $N_{105}P_{101}K_{101}$, 10 t/ha manure and 6 t/ha $CaCO_3$ (Table 2).

Statistical analysis of the data showed that, on average over three years of research, dry matter yield depended 99% on the fertilisation system in the crop rotation area. According to the correlation analysis, the most significant influence was from the application of organic-mineral fertilisation systems combined with liming, with a correlation coefficient of 0.892 ($P < 0.001$, $n = 30$).

Fertilisation systems in crop rotation had a noticeable effect on red clover root mass accumulation. In particular, a decrease in root mass was noted with increasing nitrogen fertiliser doses. These data coincide with studies by Kobyrenko et al. (2015), according to which the application of complete mineral fertilisers negatively affected root mass growth by slowing the activity of nitrogenase, one of the main enzymes responsible for biological nitrogen fixation.

A large amount of roots accumulated in unfertilised red clover stands (7.3 t/ha), with organic fertiliser application (7.5 t/ha), and the highest (7.6 t/ha) was with $N_{30}P_{34}K_{34}$ combined with liming and organic fertilisers.

According to statistical analysis, the application of an organic-mineral fertilisation system with a fertiliser dose of $N_{65}P_{68}K_{68}$ or $N_{105}P_{101}K_{101}$, with liming, had a significant effect on root mass accumulation in red clover ($P < 0.05$). Application of individual types of fertilisation, liming, and organic-mineral fertilisation system with reduced rates of mineral fertilisers ($N_{30}P_{34}K_{34}$) had no significant effect on root quantity in the upper soil layer ($P < 0.05$). However, statistical analysis indicates complete dependence of nutrient accumulation in root mass on fertilisation and liming, both separately and in combination ($R^2 = 0.95$, $P < 0.001$).

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Table 2. Dry aboveground mass yield and accumulation of roots and nutrients by red clover depending on fertilisation and liming in four-field crop rotation (average for 2022–2024)

Treatment	DM yield (t/ha)	Nutrient removal with harvest (first cut + second cut) (kg/ha)			Root DM (t/ha)
		N	P	K	
NF	4.6 ± 0.4^{a}	$49 \pm 5 + 49 \pm 5^{a}$	$4 \pm 0.4 + 5 \pm 0.4^{a}$	$60 \pm 6 + 45 \pm 3^{a}$	7.3 ± 0.7^{cd}
L	6.6 ± 0.5^{b}	$82 \pm 8 + 82 \pm 8^{c}$	$7 \pm 0.6 + 7 \pm 0.6^{b}$	$55 \pm 4 + 56 \pm 7^{a}$	6.6 ± 0.5^{bc}
O	$8.0 \pm 0.^{c}$	$111 \pm 10 + 91 \pm 10^{d}$	$9 \pm 0.8 + 7 \pm 0.7^{b}$	$117 \pm 10 + 76 \pm 6^{b}$	7.5 ± 0.4^{cd}
NPK	6.9 ± 0.5^{d}	$83 \pm 7 + 64 \pm 7^{b}$	$9 \pm 0.8 + 6 \pm 0.7^{b}$	$152 \pm 12 + 82 \pm 7^{c}$	5.9 ± 0.3^{b}
NPK + L8	10.3 ± 0.8^{d}	$150 \pm 12 + 120 \pm 11^{e}$	$14 \pm 1.0 + 11 \pm 0.9^{c}$	$195 \pm 13 + 127 \pm 11^{d}$	4.6 ± 0.2^{a}
NPK + L8 + O	11.3 ± 0.8^{de}	$153 \pm 12 + 142 \pm 11^{f}$	$14 \pm 1.1 + 13 \pm 0.9^{c}$	$214 \pm 15 + 177 \pm 14^{f}$	5.3 ± 0.3^{ab}
NPK + L4 + O	11.9 ± 0.9^{e}	$161 \pm 14 + 143 \pm 12^{f}$	$16 \pm 1.2 + 18 \pm 1.1^{d}$	$280 \pm 16 + 178 \pm 14^{g}$	5.2 ± 0.2^{a}
0.5NPK + L8 + O	10.6 ± 0.8^{d}	$163 \pm 15 + 122 \pm 10^{ef}$	$14 \pm 1.2 + 11 \pm 0.9^{c}$	$190 \pm 14 + 134 \pm 11^{d}$	7.6 ± 0.6^{a}
2NPK + L8 + O	12.6 ± 0.9^{e}	$163 \pm 14 + 142 \pm 13^{f}$	$16 \pm 1.3 + 13 \pm 0.9^{c}$	$275 \pm 18 + 192 \pm 16^{g}$	5.0 ± 0.4^{a}
2NPK + 1.5L8	11.3 ± 0.9^{de}	$175 \pm 15 + 130 \pm 11^{f}$	$15 \pm 1.3 + 12 \pm 0.9^{c}$	$183 \pm 18 + 171 \pm 16^{e}$	4.9 ± 0.2^{a}
$LSD_{0.05}$	0.470	2.351	1.987	2.056	1.458

Values are mean ± SD (n = 3 years). Different letters in the first column (see the Experimental Design) indicate different fertiliser applications, for each parameter (DM yield, N, P, K removal, and root DM), different lowercase letters (a, b, c, etc.) indicate statistically significant differences among fertilisation treatments according to the least significant difference (*LSD*) test at $P \leq 0.05$. Means within the same column sharing the same letter are not significantly different

Root mass of red clover in unfertilised stands had the highest nitrogen content; accordingly, 234 kg/ha of nitrogen contributed to the surface balance from root mass. The largest amount of phosphorus in root mass (37 kg/ha) was recorded with organic fertiliser application, and potassium (167 kg/ha) with organic-mineral fertilisation system ($N_{65}P_{68}K_{68}$ + organic fertilisers) with limestone application at a dose of 2.5 t/ha every four years.

Atmospheric nitrogen uptake. Red clover, like other leguminous crops, can fix atmospheric nitrogen through a symbiotic relationship with Rhizobium bacteria and thereby contribute to the surface nutrient balance. Therefore, to calculate the nutrient balance, the amount of nitrogen fixed by red clover was estimated. In our studies, the amount of nitrogen fixed by red clover depended significantly on crop rotation fertilisation systems (F = 34.56, P < 0.001) (Table 3).

ANOVA showed that fertilisation systems significantly affected symbiotic nitrogen fixation (P < 0.001, Table 3). Pairwise comparisons revealed

Table 3. Symbiotic nitrogen assimilation by red clover depending on fertilisation and liming in four-field crop rotation, kg/ha (average for 2022–2024)

Fertilisation treatment	1st cut	2nd cut	Total
NF	32 ± 0.2	44 ± 0.3	76 ± 0.5
L	53 ± 0.3	75 ± 0.6	128 ± 0.8
O	72 ± 0.6	83 ± 0.6	155 ± 0.9
NPK	35 ± 0.1	56 ± 0.4	91 ± 0.5
NPK + L8	63 ± 0.6	104 ± 0.8	167 ± 0.9
NPK + L8 + O	64 ± 0.5	124 ± 0.7	188 ± 1.1
NPK + L4 + O	68 ± 0.5	125 ± 0.6	192 ± 1.2
0.5NPK + L8 + O	106 ± 0.8	111 ± 0.8	217 ± 1.8
2NPK + L8 + O	41 ± 0.3	107 ± 0.7	147 ± 0.9
2NPK + 1.5L8	44 ± 0.3	97 ± 0.5	141 ± 0.8

Values are mean ± SD (n = 3 years). Different letters in the first column (see the Experimental Design) indicate differe ent fertiliser applications

https://doi.org/10.17221/369/2025-PSE

that the unfertilised control had significantly higher N fixation per unit area than the fertilised treatments ($P < 0.05$), which was associated with both a higher Ndfa coefficient and greater biomass production. We also examined the relationship between continuous variables: root biomass accumulation showed a strong positive correlation with total N input from fixation ($r = 0.78$, $P < 0.01$), suggesting that both aboveground and belowground productivity were similarly affected by long-term fertilisation history. Thus, plants receiving higher doses of mineral nitrogen reduce their dependence on atmospheric N_2 fixation, as soil mineral N is energetically more favourable for plant uptake than symbiotic fixation, which requires significant carbohydrate investment (Nyfeler et al. 2011).

The highest rate of nitrogen assimilation by red clover (217 kg/ha) was recorded when using mineral fertilisers at a dose of $N_{30}P_{34}K_{34}$ combined with organic fertilisers and liming at a dose of 2.5 t/ha once every four years – conditions that provided optimal soil pH and phosphorus availability while limiting soil mineral nitrogen, thereby encouraging symbiotic activity.

The smallest amount of assimilated nitrogen was recorded in the unfertilised variant – 76 kg/ha. These data are consistent with studies by Karbivska (2019), which showed that red clover without fertiliser application accumulated 84 kg/ha of symbiotic nitrogen. The limited fixation in unfertilised conditions likely reflects nutrient limitations (notably P and Mo) that restrict both plant growth and nitrogenase enzyme activity.

In the second cut, an increase in fixed nitrogen was observed across all treatments. A significant increase in fixed nitrogen in the second cut was also observed in studies by Riesinger and Herzon (2010), who attributed this to more favourable weather conditions (higher temperatures promoting nodule activity) and a compensatory growth response after the first cutting. Thus, accounting for symbiotic nitrogen accumulation in red clover in the second cut is an important component of nutrient balance calculations.

Surface balance under feed utilisation. The highest nitrogen input from growing red clover in a four-field crop rotation when used for feed (477 kg/ha) was observed with $N_{30}P_{34}K_{34}$ with L8 and organic fertilisers, but in this red clover stand, only 80 kg/ha phosphorus and 204 kg/ha potassium contributed to the surface balance. The highest accumulated phosphorus indicator was noted with the application of $N_{105}P_{101}K_{101}$ + L8 + organic fertilisers. At the same rate of mineral fertilisers, with a 1.5-fold increase in lime dose and without organic matter, the highest potassium content was recorded – 326 kg/ha (Table 4).

Growing red clover in the four-field crop rotation without fertilisation and with application of any type of fertilisation resulted in a positive gross surface balance of nitrogen and phosphorus, as evidenced by the positive balance of these elements. Without

Table 4. Surface balance when using two cuts for feed, kg/ha (average for 2022–2024)

Fertilisation treatment	Nutrient input includes			Nutrient removal includes			Total balance		
	N	P	K	N	P	K	N	P	K
NF	319	30	74	98	9	105	222	21	–31
L	285	22	79	164	13	111	121	9	–32
O	342	53	172	202	16	192	140	37	–20
NPK	286	100	202	147	16	234	139	85	–33
NPK + L8	416	112	222	270	25	322	146	87	–100
NPK + L8 + O	411	110	256	296	27	390	115	83	–135
NPK + L4 + O	423	113	285	304	34	458	118	79	–173
0.5NPK + L8 + O	477	80	204	285	25	325	192	55	–120
2NPK + L8 + O	423	152	287	305	29	466	118	123	–179
2NPK + 1.5L8	412	147	326	305	27	354	107	120	–28

Different letters in the first column (see the Experimental Design) indicate different fertiliser applications. Nutrient input includes fertilisers applied across the 4-year rotation + biological N-fixation + nutrients in root mass. Nutrient removal includes nutrients in harvested biomass (both cuts)

https://doi.org/10.17221/369/2025-PSE

fertiliser application, the positive surface balance was +222 kg N and +22 kg P per hectare.

Since red clover has a high potassium content (typically 2.5–3.5% K in dry matter, compared to 1.5–2.5% N and 0.2–0.4% P), the potassium balance when using both cuts for feed was negative in all treatments. Similar negative potassium balance results in red clover systems were reported by Poliovyi et al. (2022). According to their data, with increasing fertilisation level, potassium removal with red clover harvest increased, as higher biomass production leads to proportionally greater K removal, and unlike N (which can be replenished through fixation), K must be supplied entirely through soil reserves or fertilisation.

Surface balance under feed-green manure utilisation. When using the second cut as green manure (incorporating into soil), the gross surface balance improved substantially, as all accumulated elements in the second cut remained in the surface soil layer. Accordingly, nutrient removal decreased significantly. According to studies by Moyo et al. (2015), when clover was completely removed from the field, the amount of nitrogen accumulated in aboveground mass was 330–360 kg/ha, whereas with one cut as mulch, the amount removed was only 113 kg/ha. These results align with ours: nutrient removal decreased by approximately 50% when using the second cut for green manure compared to two cuts for feed (Table 5).

Using the second cut as green manure resulted in a positive surface nitrogen balance of 351–599 kg/ha. The largest positive nitrogen balance was recorded with the application of $N_{30}P_{34}K_{34}$ combined with liming and organic fertilisers, and with application of only mineral fertilisers ($N_{65}P_{68}K_{68}$), the positive surface balance was the smallest, amounting to 351 kg/ha.

Growing red clover in a four-field crop rotation has significant potential for improving the surface nutrient balance with respect to nitrogen, phosphorus, and potassium, especially due to symbiotic nitrogen fixation and root mass accumulation. Application of organic and organic-mineral fertilisers combined with liming increases symbiotic activity of clover, contributing to the assimilation of up to 217 kg/ha of atmospheric nitrogen, while potassium balance often remains negative due to significant removal at harvest.

The highest nitrogen removal rate (175 kg/ha) was observed with fertilisation 2NPK + 1.5L8. Phosphorus removal was low at 4–15 kg/ha, and potassium removal, as with the previous red clover utilisation system, was the highest, ranging from 55 to 280 kg/ha.

Using the second cut of red clover for green manure ensures a positive surface balance of nitrogen, phosphorus, and potassium. The largest positive surface balance of nitrogen (436 kg/ha) was provided by fertilisation $N_{30}P_{34}K_{34}$ + 6 t/ha $CaCO_3$ once every eight years + organic fertilisers, phosphorus (150 kg/ha) – $N_{105}P_{101}K_{101}$ + 6 t/ha $CaCO_3$ once every eight years + organic fertilisers, and potassium (315 kg/ha) – $N_{105}P_{101}K_{101}$ + 9 t/ha $CaCO_3$ once every eight years.

Table 5. Nutrient balance when using the first cut for feed and the second as green manure, kg/ha (average for 2022–2024)

Fertilisation treatment	Nutrient input includes			Nutrient removal includes			Total balance		
	N	P	K	N	P	K	N	P	K
NF	368	35	119	49	4	60	319	30	60
L	367	29	135	82	7	55	285	22	81
O	432	60	248	111	9	117	322	52	131
NPK	351	107	284	83	9	152	268	97	132
NPK + L8	536	123	349	150	14	195	386	109	154
NPK + L8 + O	553	122	433	153	14	214	400	108	219
NPK + L4 + O	566	131	462	161	16	280	405	115	182
0.5NPK + L8 + O	599	90	339	163	14	190	436	76	148
2NPK + L8 + O	565	165	479	163	16	275	402	150	204
2NPK + 1.5L8	542	159	498	175	15	183	367	144	315

Different letters in the first column (see the Experimental Design) indicate different fertiliser applications. Nutrient input includes fertilisers applied across the 4-year rotation + biological N-fixation + nutrients in root mass. Nutrient removal includes nutrients in harvested biomass (first cut only)

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Comparing red clover utilisation systems, it should be noted that when using the second cut for green manure, the surface nutrient balance, particularly nitrogen, improved dramatically. Thus, the positive surface balance increased by 97–287 kg/ha (Figure 1).

Nitrogen surface balance data from red clover grown under different utilisation systems show increased nutrient surplus with increasing fertiliser doses. Thus, without fertiliser application, the positive surface balance from using the second cut for green manure increased by only 97 kg/ha with L8, 164 kg/ha with organic fertilisers, and 182 kg/ha with NPK. When combining different types of fertilisation, the positive surface balance increased by 239–287 kg/ha, almost twice as much.

Increased doses of mineral fertilisers in short-rotation crop rotation ($N_{105}P_{101}K_{101}$) combined with 10 t/ha organic fertilisers and 6 t/ha lime once in two rotations reduce biological nitrogen fixation, but 402 kg/ha nitrogen, 150 kg/ha phosphorus, and 204 kg/ha potassium create a positive surface balance, which allows using red clover as a phytobiological ameliorant.

Critical evaluation of methodology and limitations. While our gross surface balance approach provides valuable insights into nutrient dynamics, several methodological limitations warrant discussion. First, the nitrogen difference method used to estimate symbiotic fixation (based on McAuliffe et al. 1958) assumes a constant 75% derivation from atmospheric N_2, though we acknowledge variation between treatments (68–82%). More sophisticated approaches using ^{15}N natural abundance or enrichment techniques, as employed by Carlsson and Huss-Danell (2003) in Swedish trials, could provide more precise quantification of N sources. Second, our balance calculations do not account for gaseous losses (NH_3 volatilisation, N_2O emissions) or leaching, which can be substantial in fertilised systems. Studies by Høgh-Jensen et al. (2004) suggest that actual N retention may be 20–30% lower than gross balance indicates. Third, the temporal dynamics of nutrient release from incorporated clover residues remain uncertain. At the same time, we credit the full nutrient content to the year of incorporation; mineralisation patterns may extend over 2–3 years (Carter et al. 2014).

The 60-year duration of our experiment provides unique insights into legacy effects of management on soil properties and clover performance. The substantial differences in initial soil conditions between treatments (Table 1) reflect cumulative impacts of long-term fertilisation strategies. However, this also means that observed treatment effects integrate both current-year inputs and historical soil modification, making it challenging to predict responses in newly established systems. Furthermore, our focus on a single cultivar (Truskavchanka) limits generalisability, as modern breeding has produced cultivars with enhanced N-fixation capacity and stress tolerance (Marshall et al. 2017). Future research should evaluate whether newer germplasm can maintain

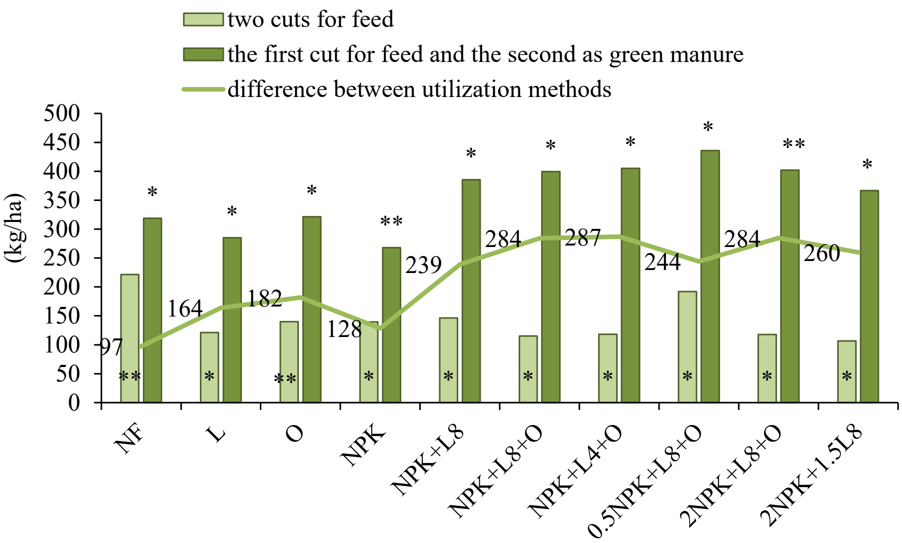

Figure 1. Nitrogen surface balance under different red clover utilisation systems, average for 2022–2024. Different letters in each line (see the Experimental Design) indicate different fertiliser applications. *LSD* *significant at $P < 0.05$, ** – not significant

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higher symbiotic activity even under elevated soil N conditions.

The three-year duration of our monitoring period limits our ability to fully characterise the long-term dynamics of nutrient balance across different red clover utilisation systems. While significant treatment effects were detected, year-to-year variability in weather conditions, pest pressure, and stand persistence could alter nutrient balance patterns over longer timeframes. Future research should extend monitoring to 5–10 years to capture the full range of conditions and confirm the stability of the observed trends. Additionally, longer monitoring would allow assessment of cumulative effects on soil nutrient status and pH, which was beyond the scope of this study.

Practical implications and recommendations. Based on our three-year study, preliminary recommendations for red clover management on acidic, light-grey forest soils can be formulated, though they require validation through longer-term monitoring. Our findings suggest that sustainable production may benefit from a strategy combining moderate mineral fertilisation, regular liming (maintaining pH 5.0–5.5), and strategic utilisation of red clover. The feed-green manure approach (first cut harvested, second cut incorporated) showed a more favourable nutrient balance than full feed utilisation in our three-year dataset, generating a positive nitrogen balance while reducing negative balances for phosphorus and potassium. However, these patterns need confirmation over more extended periods to account for year-to-year variability in weather conditions and potential cumulative effects.

The persistent negative potassium balance observed under two-cut feed utilisation across all fertilisation systems indicates that supplemental K inputs may be necessary when both cuts are removed, particularly on soils with naturally low K reserves. This observation aligns with findings from long-term trials in Denmark (Askegaard and Eriksen 2002), which concluded that K fertilisation becomes critical in intensive forage systems to prevent gradual soil K depletion and subsequent yield decline.

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Received: August 22, 2025
Accepted: January 8, 2026
Published online: January 27, 2026