



Effects of legumes and fertiliser on nitrogen balance and nitrate leaching from intact leys and after tilling for subsequent crop

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ABSTRACT

Grass-legume leys combine multiple agronomic benefits, several of which are associated with symbiotic nitrogen (N) fixation. However, the best combinations of legume abundance and N fertilisation to achieve high productivity at low nitrate leaching are not yet established. Nitrate leaching risk of pure grass swards (Grs), grass-legume mixtures (Mix) and pure legume swards (Leg) at a fertiliser level of either 50, 150 or 450 kg N ha⁻¹ yr⁻¹ (N50, N150 and N450) was studied during two key periods: the intact ley for forage production and after tilling for subsequent winter wheat in crop rotation. The risk of nitrate leaching was determined from (i) monitoring of the nitrate concentration in the soil solution (NCSS) and (ii) the soil mineral N (SMN) content. Furthermore, the soil surface N balance was assessed by measuring N input from fertiliser and symbiosis, and N output with harvested biomass. During the period of intact plant cover, soil surface N balance of Grs swards was strongly negative at N50 and N150 with negligible NCSS and SMN. At N450, the positive N balance of Grs swards resulted in a high leaching risk after three years of cultivation (NCSS ≤ 13 mg NO₃-N L⁻¹ and SMN ≤ 20 kg N ha⁻¹). For Mix swards, the N balance was close to zero at N50 and N150, which led to similarly low NCSS and SMN. At N450, the N balance of Mix swards was beyond zero, resulting in significantly increased NCSS and SMN (≤ 30 mg NO₃-N L⁻¹ and ≤ 35 kg N ha⁻¹). For Leg swards, the N balance was far beyond zero at all fertilisation levels, resulting in a leaching risk even at N50 (≤ 8 mg NO₃-N L⁻¹ and ≤ 23 kg N ha⁻¹). During the period following tilling the leys, SMN was similar or lower for Mix compared to Grs swards (≤ 43 vs. ≤ 62 kg N ha⁻¹ at N50 and N150, 72 vs. 68 kg N ha⁻¹ at N450), while it was significantly higher for Leg swards (SMN ≤ 95 kg N ha⁻¹). We conclude that grass-legume mixtures combine high yields, low fertiliser requirements, and low nitrate leaching better than either pure grass or pure legume swards. This holds true both during the period with intact plant cover and after tillage for the subsequent crop.

1. Introduction

Productive mown grasslands are generally associated with a low risk for nitrate (NO₃) leaching to the environment (Di and Cameron, 2002). A severe risk has however been identified for high N fertiliser applications (Wachendorf et al., 2004; Klaus et al., 2018) and for some pure legume stands (Loiseau et al., 2001; Palmberg et al., 2005). Balanced grass-legume mixtures benefit the production of both forage (Nyfeler et al., 2009) and the follow-on crop (Fox et al., 2020) thanks to large N input from symbiotic N₂ fixation and positive mixing effects (Nyfeler et al., 2011; Finn et al., 2013; Hammelehle et al., 2018). However, it is not clear whether these N inputs from symbiosis are associated with an

increased NO₃ leaching risk.

Grass-legume mixtures combine the N fixing ability of legume species with the dense and efficient rooting system of grass species, which suggest low NO₃ leaching. Nevertheless, results on NO₃ leaching from swards containing both legumes and grasses are conflicting. Some studies observed an increase in NO₃ leaching when compared to swards without legumes (Scherer-Lorenzen et al., 2003; Leimer et al., 2016), while others observed no sizeable increase in leaching due to the presence of legumes in mixed swards (Loiseau et al., 2001, simulated urine and faeces patches; Bracken et al., 2022, with lower N fertilisation on mixed than on pure grass swards). Better quantifying the differences in NO₃ leaching among grass-only, grass-legume and legume-only leys is

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therefore of critical importance for managing potential trade-offs between production and N losses.

The two main N inputs into production-oriented grass-legume leys are by far symbiotic N fixation and fertiliser applications (Ammann et al., 2009; Oberson et al., 2013). With such leys, at least two mechanisms limit the increase of plant-available N in the soil. Firstly, the N input through symbiotic fixation in the system decreases when the level of plant-available N in the soil increases (Soussana and Tallec, 2010; Nyfeler et al., 2011; De Notaris et al., 2021). Secondly, the grass component of the mixture is a strong sink for N and can capture as much fertiliser and soil mineral N as grass monocultures (Nyfeler et al., 2011; Husse et al., 2017). Moderately fertilised grass-legume mixtures might therefore pose less risk of NO₃ leaching than pure grass swards that are heavily fertilised. Studies on the effect of the ratio of the two main N inputs, symbiotic fixation and fertiliser applications, on NO₃ leaching have yielded contradicting results. Some results indicate that the source of N, i.e. symbiotic vs. fertiliser N, might be a better indicator for NO₃ leaching losses than the total N input (Crews and Peoples, 2004), but others indicate the opposite (Bracken et al., 2022). Studying the interactive effects of legume abundance and mineral N fertilisation will help to close this knowledge gap.

Indisputably, the balance between N inputs and outputs in harvested products – i.e. the soil surface N balance – does affect the risk for N losses, among which NO₃ leaching is crucial. However, the direct link between N balance and NO₃ leaching remains unclear (Eriksen et al., 2015; Jensen et al., 2022). Thus, our knowledge about the combined effects of soil surface N balance of the forage crop, the sources of N to the forage crop, and NO₃ leaching is still patchy.

As part of a crop rotation, leys are normally tilled after a few years to establish an arable crop. To evaluate the effects of ley types and management on NO₃ leaching, the focus must be on the period following eradication of plant cover. Leaching occurring during this period could offset any advantage of using grass-legume mixtures, i.e. of partly replacing fertiliser N with symbiotic N during the grassland phase (Hansen et al., 2019). Mineralisation processes are triggered when the plant cover is eradicated by herbicide and/or tilling, sometimes resulting in large amounts of nitrate being leached (Eriksen et al., 2004, 2015). The impact of the botanical composition of the leys during this critical period has nevertheless only rarely been evaluated.

The aim of the study was to assess the soil surface N balance and the risk of nitrate leaching of leys as affected by legumes and N fertilisation during the two key periods, namely the ley phase for forage production and the phase after tilling for the follow-on crop. To achieve this objective, a four-year factorial field experiment combined the three sward types of pure grass, grass-legume mixtures and pure legume with three contrasting levels of mineral fertiliser applications. The soil surface N balance, the nitrate concentration in the soil solution (NCSS) and the amount of soil mineral nitrogen (SMN) were assessed. It is hypothesised that combining the functional diversity of grasses and legumes in the mixture with a low to moderate fertiliser level allows high productivity at a low risk of nitrate leaching.

2. Materials and methods

2.1. Experimental design

The experiment included three types of swards and three levels of N fertilisation arranged in a completely randomized design. The sward types were pure grass swards (Grs), pure legume swards (Leg) and grass-legume mixtures (Mix). Pure grass swards were established using either the grass species *Lolium perenne* L. or *Dactylis glomerata* L., while Leg swards contained either the clover species *Trifolium pratense* L. or *Trifolium repens* L. The grass-legume mixtures were sown with either 20, 50 or 80% legumes, and included all four species. There were four Grs plots, four Leg plots and ten Mix plots per level of N fertilisation, resulting in a total of 54 plots. The three N fertilisation levels

corresponded to application rates of 50, 150 or 450 kg N ha⁻¹ yr⁻¹, and are thereafter referred to as N50, N150 and N450.

2.2. Field history, establishment, harvest and fertilisation of the experiment

Prior to our study, the experimental site (Zürich-Reckenholz; 47°26'N, 8°32'E, 491 m a. s. l.) was under crop rotation (1997: winter wheat, 1998–2000: leys, 2001: silage maize, 2002: peas) (Fig. 1: (b) Field management). In early August 2002, the field was ploughed at 20 cm depth and the leys were sown on plots of 3 m × 6 m. Starting 2003 (thereafter referred to as year 1), all swards were cut five times annually at 5 cm above ground surface. Annual N fertilisation consisted of five equal applications of ammonium-nitrate, with the first application performed in the middle of April and the following ones 3–15 days after the first four harvests of the year. The first N fertilisation of the experiment in April 2003 was an exception, as all plots were initially fertilised with 30 kg N ha⁻¹, to insure uniform establishment of the swards among N fertilisation levels. All plots received 40 kg P ha⁻¹ yr⁻¹ (as superphosphate) and 220 kg K ha⁻¹ yr⁻¹ (as potassium sulphate) in early spring. All harvesting and fertilisation operations were carried out on the same day for all treatments. In 2006 (thereafter referred to as year 4), glyphosate was applied to the leys on September 28, followed by seedbed preparation using a rotary tiller at 10 cm depth and sowing winter wheat on October 20.

2.3. Site conditions

Based on ten spatially distributed examinations of soil profiles, the soil of the experimental site was classified as a slightly gleyic eutric cambisol as described in IUSS Working Group WRB (2022). Spatial variation of soil properties across the experimental site was small. In the soil layer 0–30 cm, soil texture was 52 g sand, 31 g silt and 16 g clay per 100 g (loam; IUSS Working Group WRB, 2022). Soil organic matter (Allison, 1965) was 2.2 g per 100 g and C:N ratio was 12.9. Mean pH was 6.4 (in H₂O) and cation exchange capacity was 13.9 meq per 100 g. The main rooting zone was no deeper than 60 cm.

Mean annual precipitation from the 20-year average was 1031 mm, with a relatively even distribution across the year. Mean annual temperature was 9.4°C, with a range of monthly averages from 0.2°C in January to 18.6°C in July. Climate conditions were generally in line with these 20-year averages during the period of the experiment, except for an exceptionally warm and dry summer in year 1 (Fig. S1).

2.4. Determination of nitrogen concentration in the soil solution below the main rooting zone

Porous cup tension lysimeters were installed to extract soil water at a depth of 60 cm below ground surface. The 8 cm long suction cups of these lysimeters consisted of a porous ceramic material and were connected to an air-tight polymer tube. The tubes were inserted into the soil at a 45° angle to ensure that the tension cups were placed at the targeted soil depth. In each of the 54 sampling plots, two of these lysimeters were installed at a horizontal distance of 1.4 m. Both lysimeters were connected with flexible tubes to obtain a bulked soil solution sample of each plot. After 24 hours of suction power at 80 kPa, the accumulated soil water was collected from the tension lysimeters. Samples represented therefore a one-day integral, and were deep-frozen within two hours after collecting. Soil solution samples as described were then collected in two-week intervals from early October of year 2 until late May of year 4 (Fig. 1: (a) Field measurements; for precise dates see Table S4). At two sampling events, no soil solution could be collected due to drought during summer or frozen soil during winter (Table S4). Concentrations of nitrate-N (NO₃-N) and ammonium-N (NH₄⁺-N) were determined from defrosted samples by spectrophotometry using a Skalar SANplus Segmented Flow Analyser (Skalar, Tinststraat 12, NL-4823 AA Breda)

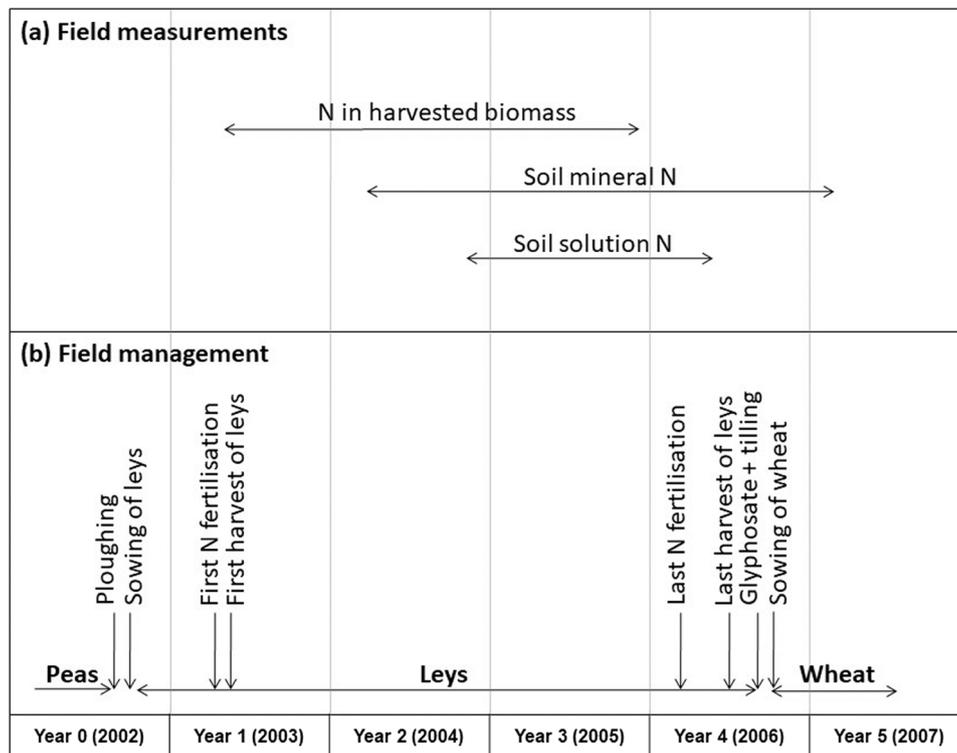


Fig. 1. (a) Overview of the field measurements during the experiment. Horizontal arrows indicate the periods of plant, soil and soil solution sampling. (b) Field management during the relevant period of crop rotation. Vertical arrows indicate the most important interventions at the start and the end of the period with leys.

following the methods described in Kempers and Luft (1988) and Krom (1980). As the amount of $\text{NH}_4^+\text{-N}$ in soil solution samples was negligible in this study ($0.1 \text{ mg NH}_4^+\text{-N L}^{-1}$ in average), N content is presented as the soil solution nitrate concentration (NCSS). NCSS values over the

whole measurement period are shown in Fig. 2. As winter is the critical period for nitrate leaching under grassland in this climate zone, the periods between the latest harvest in autumn until the first fertilisation in spring (denominated as winter) were analysed for the differences

Table 1

Soil surface budget for pure grass swards (Grs, pure stands of either *L. perenne* or *D. glomerata*, $n = 4$), grass-legume mixtures (Mix, $n = 10$) and pure legume swards (Leg, pure stands of either *T. pratense* or *T. repens*, $n = 4$) fertilised at three N levels (N50, N150, N450). Total N input is calculated as the sum of applied fertiliser N and total symbiotic N input from year 1 to the last harvest of year 3. Total N output is fractionated into N derived from fertiliser, symbiosis and other sources in the harvested plant material. In the top section of the table, significance levels of experimental factors resulting from ANOVA are shown (*** = $P < 0.001$, ** = $P < 0.01$, * = $P < 0.05$, and ns = not significant; for details of the ANOVA see Table S1a). In the bottom section, means of each treatment are shown. Within a column, different letters indicate significance of Tukey range test ($P < 0.05$). Latin script is used for comparing sward types within fertilisation levels, and Greek script for comparing fertilisation levels within sward types. Colours are used as visual help for the comparison within sward types, as the values to be compared are not arranged consecutively. Instead of indicating the standard error for each mean, only the average of all group means is specified. The very last column shows the variation of the N balance over the three years (standard error of each year's mean).

		legumes			N _{input} (kg N ha ⁻¹ yr ⁻¹)			N _{output} (kg N ha ⁻¹ yr ⁻¹)			N balance (kg N ha ⁻¹ yr ⁻¹)	
		N _{fert in} ¹⁾	N _{sym in} ²⁾	Total	N _{fert out}	N _{sym out} ³⁾	N _{other out} ⁴⁾	Total				
Fertilisation	-	-	***	***	***	***	***	ns	***	***	***	***
Sward	-	-	***	***	***	**	***	***	***	***	***	***
Interaction	-	-	*	***	*	*	ns	***	***	***	***	***
N50	Grs	0.01	57	2.7 - -	59.3 a α	21.3 a α	1.7 - -	89.9 a α	113.0 a α	-53.6 a α	3.6	
	Mix	0.42	57	314.9 a γ	371.6 b α	31.3 b α	188.4 a γ	163.6 b α	383.3 b α	-11.7 ab α	47.7	
	Leg	0.71	57	328.8 a β	385.5 b α	23.2 a α	193.4 a β	145.0 b α	361.6 b α	23.9 b α	64.0	
N150	Grs	<0.01	150	0.2 - -	150.2 a β	75.6 a β	0.1 - -	106.8 a α	182.5 a α	-32.3 a α	8.8	
	Mix	0.32	150	233.5 a β	383.5 b α	94.5 b β	141.1 a β	161.0 b α	396.6 b α	-13.1 a α	55.8	
	Leg	0.73	150	299.1 a β	449.1 c β	65.9 a β	176.7 a β	145.3 b α	387.8 b α	61.2 b α	66.5	
N450	Grs	<0.01	430	0.0 - -	430.0 a γ	278.6 b γ	0.0 - -	114.7 a α	393.3 a β	36.7 a β	35.5	
	Mix	0.21	430	115.1 a α	545.1 b β	272.0 b γ	70.3 a α	152.7 b α	495.1 b β	50.0 a β	43.8	
	Leg	0.80	430	201.9 b α	631.9 c γ	168.6 a γ	119.4 b α	136.5 ab α	424.4 ab α	207.4 b β	50.8	
	SE	0.02	-	19.98	13.58	7.16	10.62	9.58	15.26	12.82		

¹⁾ As all swards were fertilised at the same rate at the first N application (i.e. 30 kg N ha^{-1}) to establish all swards similarly, the N amounts of the first year were 70, 150 and $390 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ for N50, N150 and N450, respectively.

²⁾ Total symbiotic N input was estimated following the model of Høgh-Jensen et al. (2004), using the measured N fraction in the harvested legume biomass derived from symbiotic fixation (¹⁵N dilution technique).

³⁾ Grs-swards were not included in the respective ANOVA because of a lack of, or very low proportions of legumes so that preconditions for ANOVA would not be fulfilled.

⁴⁾ N output from other sources could originate from soil N (from soil solution N and mineralisation of soil organic matter) and N from atmospheric deposition.

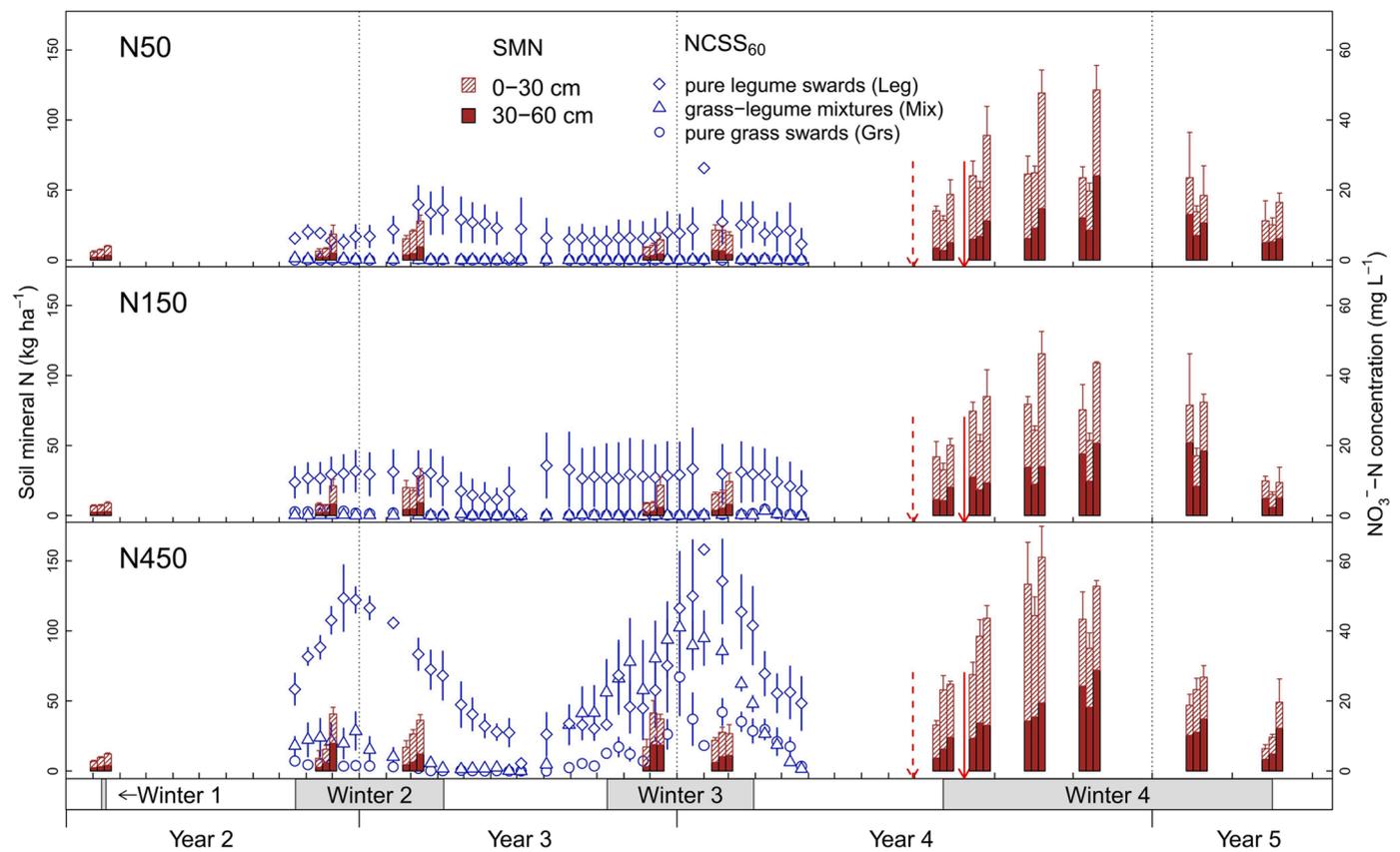


Fig. 2. Time course of soil mineral N (SMN, bars) and nitrate concentrations in the soil solution at 60 cm depth (NCSS₆₀, symbols) from year 2 to 5 of the experiment. Tripartite sets of columns represent mean soil mineral N for (from left to right) pure grass swards (Grs; pure stands of either *L. perenne* or *D. glomerata*, $n = 4$ before tilling and $n = 2$ after tilling), grass-legume mixtures (Mix; $n = 10$ respectively 6) and pure legume swards (Leg; pure stands of either *T. pratense* or *T. repens*, $n = 4$ respectively 2). The hatched part of the columns indicates SMN in the upper soil layer, the solid part indicates SMN in the lower soil layer. Mean nitrate concentrations in the soil solution samples are represented by circles (pure grass swards), triangles (mixtures) and diamonds (pure legume swards). Bright grey bars below the figure indicate the time period over which values were averaged for statistical analyses in Tables 2 and 3. Vertical lines indicate standard errors. The red arrows in September and October of year 4 indicate the application of glyphosate (dashed) and rotary tiller (continuous). Note that the time scale after September of year 4 is expanded (by a factor of three) for better visibility.

among treatments.

2.5. Determination of soil mineral nitrogen

Content of mineral N in the soil (soil mineral nitrogen, SMN), i.e. the sum of $\text{NO}_3\text{-N}$ and $\text{NH}_4^+\text{-N}$ was determined in soil layers 0–30 cm and 30–60 cm in winter 2 (1 sampling event), and winters 3 and 4 (2 sampling events each) (Fig. 1: (a) Field measurements). At each sampling event, five soil cores in each plot were taken (Table S4) and separated into samples from 0 to 30 and 30–60 cm soil depth. Furthermore, several additional samples were taken in autumn of year 4 and the subsequent winter after applying glyphosate (1 sampling event), as well as after seedbed preparation by a rotary tiller and sowing winter wheat (5 sampling events) (for dates see Table S4). For the sampling events following tilling of the leys, samples were taken from a reduced set of plots (two Grs-, two Leg- and six Mix-plots per level of N fertilisation). This was to insure enough resources for monitoring SMN evolution (six points in time). Selected were the Grs- and Leg-swards that had been least invaded by unsown species, and for the six Mix-swards representing the average legume proportion at the final stage of the experiment.

The five soil cores per sampling event, plot and soil layer were bulked to analyse soil mineral N ($\text{NO}_3\text{-N} + \text{NH}_4^+\text{-N}$) by spectrophotometry from moist soil samples using a 0.01 M CaCl_2 extraction. The N content in the samples (as mg kg^{-1} soil) was then determined based on concentrations in soil extraction. SMN (as kg N ha^{-1}) was calculated from these concentrations taking into account average bulk density and skeleton

content of the soil at the experimental site (1.33 g cm^{-3} and 10% respectively).

2.6. Determination of soil surface budget

Soil surface N balance (Oenema et al., 2003) was calculated as the difference between N input and N output. The respective parameters were determined from year 1 to 3 (Fig. 1: (a) Field measurements). Total N input was defined as the sum of applied fertiliser N ($N_{\text{fert in}}$) and N input from symbiosis ($N_{\text{sym in}}$), without considering atmospheric N deposition. N output was the amount of N harvested with the forage biomass.

$$N \text{ balance}(\text{kg N ha}^{-1}) = N_{\text{fert in}} + N_{\text{sym in}} - N_{\text{output}} \quad (1)$$

To gain further information on the fate of applied fertiliser N and N input from symbiosis within the plant-soil system, N_{output} was segregated into N derived from fertiliser ($N_{\text{fert out}}$), from symbiosis ($N_{\text{sym out}}$) and from other sources ($N_{\text{other out}}$). To determine the percentage of N derived from symbiosis and fertiliser in harvested plant material, ^{15}N -labelled fertiliser (as $^{15}\text{NH}_4^{15}\text{NO}_3$) was applied at each fertiliser application on a permanently defined central part of each plot (the method is described in detail in Nyfeler et al., 2011).

$$N_{\text{output}}(\text{kg N ha}^{-1}) = N_{\text{fert out}} + N_{\text{sym out}} + N_{\text{other out}} \quad (2)$$

To calculate N_{output} , plant material from the central $1.5 \text{ m} \times 6 \text{ m}$ of each plot was harvested and weighed. Total dry matter yield was then

determined by air-drying subsamples to a constant weight (65°C, 48 h) for all five harvests. At the first, third and fifth harvest of each year, the biomass proportions of the four sown species and the bulk of unsown species (hereafter referred to as ‘unsown species’) were measured on a dry weight basis by manually separating the harvested biomass from permanent sub-plots (0.8 m × 0.3 m) within the ¹⁵N labelled area (1.4 m × 1.5 m) and drying the separated plant material to constant weight (65°C, 48 h). At the second and fourth harvest of each year, a bulked sample of each plot harvest area was taken. Plant samples were then ground and analysed for their total N content by thermal conductometry (Dumas) with an automatic system (vario MAX CN, Elementar). To calculate N output of each harvest, dry matter yield of each species was multiplied by its N content and this was then summed up for all species (for first, third and fifth harvest) or dry matter yield of the entire plot was multiplied by N content of the bulked sample (for second and fourth harvest). Total over three years was then calculated by adding together all harvests from year 1 to 3 (for the correlation between SMN respectively NCSS in winter 2, only harvests from year 1 to 2 were taken into account).

The content of N output deriving from fertiliser was estimated based on the percentage from fertiliser (%N_{fert}) which was calculated following Vose and Victoria (1983):

$$\%N_{fert(species)} = \left(\frac{atom\%^{15}N - excess_{species}}{atom\%^{15}N - excess_{fertiliser}} \right) \quad (3)$$

where ‘species’ is representing *L. perenne*, *D. glomerata*, *T. pratense*, *T. repens* or the bulk of unsown species, and ¹⁵N was determined by gas isotope ratio mass spectrometry.

Because ¹⁵N was repeatedly applied at each fertilisation and at the same enrichment across the three years of the experiment, %N_{fert} represents the percentage of N derived from all fertiliser applications since the very first application in spring of year 1.

The amount of harvested N deriving from symbiosis was calculated by multiplying the harvested N content in legume species by their respective fraction from symbiosis (for details see Nyfeler et al., 2011). %N_{sym} was calculated following McAuliffe et al. (1958):

$$\%N_{sym} = \left(1 - \frac{atom\%^{15}N - excess_{legume}}{atom\%^{15}N - excess_{reference}} \right) \times 100 \quad (4)$$

where ‘legume’ is representing *T. pratense* or *T. repens*. *L. perenne* grown in the same plot served as reference plant (Boller and Nösberger, 1988).

The amount of harvested N derived from other sources was calculated as the difference between total N output and its fraction from fertiliser and symbiosis. N_{other out} originated from soil N (from available soil mineral N and mineralisation of soil organic matter) and N from atmospheric deposition.

To calculate the total N input from symbiosis into the soil-plant system from our measurements of N from symbiosis in the harvested legume biomass, we used the empirical model by Høgh-Jensen et al. (2004). This model estimates N from symbiosis in legume roots and stubbles, N transferred to companion grasses, and N ‘immobilised’ in the organic soil pool at the end of the growing period, each as a proportion of the amount of symbiotic N in harvested legume biomass. For the coefficient estimating ‘immobilised’ symbiotic N, the model differentiates between clay and sandy soils. Because the soil of our experiment was a loam closer to a sandy soil than to a clay, we weighted the coefficient for ‘immobilised’ symbiotic N by 0.75·(Coef_{sandy soil}) + 0.25·(Coef_{clayey soil}). Based on this model, harvested legume N from symbiosis was multiplied by 1.6 for *T. pratense* and by 1.8 for *T. repens* in year 1 and 2, and by 1.4 and 1.5 in year 3, respectively, to calculate total N input from symbiotic fixation (for factor’s discussion see Table S1a).

2.7. Data analysis

Soil surface budget was calculated for each year separately, for years 1–2 and for years 1–3. Data of individual components of the budget over the 3-year period was analysed by two-way ANOVAs (type II as the number of plots differed among the three sward types) with the level of N fertilisation and the sward type (and their interaction) as factors (Fox and Weisberg, 2023). To fulfil preconditions for ANOVA (homoscedasticity), N_{input} and N_{output} values were transformed by decimal logarithm, which was not necessary for N balance values. Total N input was analysed by a generalised least squares procedure, segregating swards with legumes from swards without legumes because of a bias on residuals due to the nil to very low symbiotic N input in the Grs swards. Based on significant effects in the global analysis, significant differences were revealed following the Tukey range test within each factor level (i.e. among different sward types at the same fertilisation level or vice versa). The full model was used at any one time for these pairwise comparisons. We refrained from a multiple comparison of all nine means because of the excessive loss of statistical power when comparing a large number of means.

For the statistical analysis of SMN during the period of intact plant cover, early and late winter values were averaged for each plot. For the period after tilling, all six samples taken during winter 4 were averaged (Table S4). Statistical analysis was performed according to soil surface budget, using only decimal logarithm of values.

For the statistical analysis of NCSS, data collected during winter 2 and during winter 3 was averaged and analysed identically to above described parameters.

All data was analysed with the statistics software R, version 4.3.1 (R Core Team, 2023), using the nlme package for generalized linear models (Pinheiro et al., 2022), the car package for type II ANOVA (Fox and Weisberg, 2023) and the multcomp package for Tukey range tests (Hothorn et al., 2008).

3. Results

3.1. Soil surface budget

3.1.1. Input

Total N input of the treatments differed largely from 59 kg N ha⁻¹ yr⁻¹ (Grs-N50) to 632 kg N ha⁻¹ yr⁻¹ (Leg-450) over the three years (Table 1). At N50 and N150, symbiotically derived N was by far the major N input for the Leg- and Mix-swards, amounting up to 329 kg N ha⁻¹ yr⁻¹ (Leg-N50) and 315 kg N ha⁻¹ yr⁻¹ (Mix-N50). As a result, total N input was only slightly lower in Mix-N50 and Leg-N50 than in Grs-N450 (−58 kg N for Mix and −45 kg N for Leg). In particular, N input from symbiosis did not differ significantly between Leg- and Mix-swards (N50 and N150). The very small amounts of symbiotic N in Grs-N50 and Grs-N150 was derived from a few clover plants that invaded these swards during the experiment.

Increased N fertilisation significantly reduced symbiotic fixation from 315 to 115 kg N ha⁻¹ yr⁻¹ for Mix and from 329 to 202 kg N ha⁻¹ yr⁻¹ for Leg, as it reduced the average clover proportion of the mixtures from 42% to 21% (Table 1) and down-regulated the symbiotic N fixation activity of the clover plants from 89% to 62% (calculated from Table S1b). Despite this significant reduction of symbiotic N, both Mix- and Leg-swards at N450 still had a remarkably high N input from symbiosis.

3.2. Output

Total N output from the plots with the harvested biomass differed substantially from 113 (Grs-N50) to 495 kg N ha⁻¹ yr⁻¹ (Mix-N450) (Table 1). At all N fertiliser levels, N outputs of Mix and Leg swards did not differ significantly from each other and tended to be larger for Mix than for Leg. At N50 and N150, total N_{output} from mixtures and pure

legume swards was significantly larger compared to pure grass swards. At N450, only the total N_{output} of Mix was significantly larger than the total N_{output} of Grs. Mixtures at N50 achieved a similar N output as Grs-N450. With a 9-fold increase in N fertilisation (N50 to N450), N output inflated to a factor of 3.5 for the Grs-swards but only to a factor of 1.3 and 1.1 for the Mix- and the Leg-swards, respectively.

At N50 and N150 the N amount in the harvested plant material deriving from fertiliser (N_{fert}) was markedly larger in the mixtures than in the Grs-swards (+47% and +25%, respectively, Table 1). The amount of applied fertiliser N that was not recovered in the harvested biomass (and thus potentially prone to loss) increased for all swards with increased fertiliser application. For the Grs swards, for instance, the values were 36, 74 and 151 kg N ha⁻¹ yr⁻¹ for N50, N150 and N450, respectively ($N_{\text{fert in}} - N_{\text{fert out}}$ in Table 1).

Symbiotic N accounted for the largest part of harvested N for Mix- and Leg-swards at N50 and N150, but was exceeded by N derived from fertiliser at N450.

The N content deriving from soil and atmospheric deposition in the harvested plant material was superior in Mix- than in Grs-swards at all fertilisation levels (from $N_{\text{other out}}$ in Table 1, considering that atmospheric N deposition was the same for all plots). The level of N fertilisation did not significantly affect the amount of $N_{\text{other out}}$.

3.3. Balance

The annual soil surface N balance, calculated as the mean over 3 years, ranged from -54 (Grs-N50) to +207 kg N ha⁻¹ per year (Leg-N450). It was strongly negative for Grs-swards and slightly negative for Mix-swards fertilised at N50 and N150, but positive for all other treatments (Table 1). As a general pattern, it shifted towards more positive values in the order Grs-, Mix- and Leg-swards. The balance was virtually identical for Mix-N50 and Mix-N150.

4. Nitrate concentration in the soil solution

Averaged over each winter period, NCSS was significantly affected by both fertilisation and sward type (Table 2 & S2). Mean NCSS did not differ significantly between N50 and N150 but was significantly elevated at N450 (Table 2). At a given fertilisation level, NCSS of Mix-

and Grs-swards generally did not differ significantly, while NCSS in Leg-swards was significantly increased. TreatmentLeg-N450 in winter 3 was an exception, because NCSS of mixed swards at N450 and the variation of all sward types at N450 had strongly increased during winter 3. The magnitude of this increase was a factor of 54, when both N50 and N150 as well as both winter periods were considered.

Nitrate concentration in the soil solution at 60 cm depth (NCSS) ranged from 0 to 63 mg NO₃-N L⁻¹ for different sward types and fertilisation levels at individual samplings (Fig. 2). The average across all sward types and fertilisation levels during the whole measurement period, however, was only 6.5 mg NO₃-N L⁻¹. At N50 and N150, NCSS below the pure grass swards, the mixtures and the pure legume swards was only marginally fluctuating across the entire measurement period (Fig. 2). In contrast, NCSS at N450 was clearly elevated in winter 2 and 3 compared to the growing season (spring to autumn of year 3 and spring of year 4). For the pure legume swards, these 'winter peaks' were similarly high in both winter periods (up to 49 mg NO₃-N L⁻¹ during winter 2 and 63 mg NO₃-N L⁻¹ during winter 3). For pure grass swards and mixtures fertilised at N450 however, NCSS was clearly elevated in winter 3 compared to winter 2 (up to 27 vs. 3 mg NO₃-N L⁻¹ for Grs-N450 and up to 41 vs. 11 mg NO₃-N L⁻¹ for Mix-N450).

5. Soil mineral N content

5.1. During period of intact plant cover

Total SMN for both soil layers (SMN₀₋₆₀) did not exceed 40 kg N ha⁻¹ at individual sampling events (treatment means; Fig. 2). Although the larger part of SMN was usually in the form of NO₃-N, large contributions were sometimes derived from NH₄⁺-N (which also could be prone to leaching after nitrification). Because SMN did not vary substantially between the samples taken at the beginning and at the end of a winter period, we focus on averaged data of both samplings of each winter. Maximal SMN₀₋₆₀ was found at N450 and for Leg-swards (Table 3: 39 kg N ha⁻¹ in winter 2). SMN₀₋₆₀ did not exceed 20 kg N ha⁻¹ for Grs-swards at N450. At N50 and N150, SMN₀₋₆₀ was 25 kg N ha⁻¹ at maximum over all types of leys. Importantly, within these two fertilisation levels, SMN₀₋₆₀ for the Mix was never significantly higher than for the Grs-swards.

Table 2

Nitrate concentration in the soil solution (NCSS as mg NO₃-N L⁻¹) at 60 cm depth averaged over winter 2 or winter 3, respectively, for pure grass swards (Grs, pure stands of either *L. perenne* or *D. glomerata*, n = 4), grass-legume mixtures (Mix, n = 10) or pure legume swards (Leg, pure stands of either *T. pratense* or *T. repens*, n = 4) fertilised at three N levels. In the top section of the table significance levels of experimental factors resulting from ANOVA are shown (*** = $P < 0.001$, ** = $P < 0.01$, * = $P < 0.05$, and ns = not significant, for further parameters of the ANOVA output see Table S2). In the bottom section, means of each treatment are shown. Within a column, different letters indicate significance of Tukey range test ($P < 0.05$). Latin script is used for comparing sward types within fertilisation levels, and Greek script for comparing fertilisation levels within sward types. Colours are used as visual help for the comparison within sward types, as the values to be compared are not arranged consecutively.

		Winter 2				Winter 3			
Fertilisation		***				***			
Sward		***				***			
Interaction		ns				***			
		mean	SE			mean	SE		
N50	Grs	0.03	0.02	a	α	0.01	0.00	a	α
	Mix	0.19	0.05	b	α	0.06	0.01	a	α
	Leg	8.16	3.43	c	α	7.96	5.48	b	α
N150	Grs	0.78	0.74	a	αβ	0.19	0.18	a	α
	Mix	0.11	0.03	a	α	0.07	0.02	a	α
	Leg	11.42	5.50	b	α	11.60	9.52	b	αβ
N450	Grs	1.29	0.76	a	β	13.07	3.76	a	β
	Mix	6.55	3.03	a	β	30.03	7.28	a	β
	Leg	37.41	0.85	b	α	39.14	10.10	a	β

Table 3

Amount of soil mineral N in the soil depth 0–60 cm (SMN, as kg N ha⁻¹) averaged over all samplings of the respective winter period (one in winter 1, two in winter 2 and 3, respectively, and six in winter 4). SMN is shown for pure grass swards (Grs, pure stands of either *L. perenne* or *D. glomerata*, n = 4, except winter 4 with n = 2), grass-legume mixtures (Mix, n = 10, respectively n = 6 for winter 4) or pure legume swards (Leg, pure stands of either *T. pratense* or *T. repens*, n = 4 respectively n = 2) fertilised at three N levels. The table shows the sum of the upper and the lower soil layer (SMN₀₋₆₀, values for each soil layer are given in Table S3c). In the top section of the table significance levels of experimental factors resulting from ANOVA are shown (*** = P < 0.001, ** = P < 0.01, * = P < 0.05, and ns = not significant, for further ANOVA output parameters see Table S3a). In the bottom section, means of each treatment are shown. Within a column, different letters indicate significance of Tukey range test (P < 0.05). Latin script is used for comparing sward types within fertilisation levels, and Greek script for comparing fertilisation levels within sward types. Colours are used as visual help for the comparison within sward types, as the values to be compared are not arranged consecutively. Instead of indicating the standard error for each mean, only the average of all group means is specified.

		Intact plant cover			After tilling
		Winter 1	Winter 2	Winter 3	Winter 4
0-60 cm	Fertilisation	*	**	***	***
	Sward	***	***	ns	***
	Interaction	ns	ns	ns	ns
N50	Grs	5.6 a α	10.8 a α	15.2 a α	50.38 ab α
	Mix	7.4 ab αβ	14.4 ab αβ	16.0 a α	43.35 a α
	Leg	9.9 b α	23.2 b α	16.2 a α	77.27 b α
N150	Grs	7.0 a α	14.0 ab α	11.9 a α	62.5 b α
	Mix	7.1 a α	12.6 a α	12.8 a α	43.0 a α
	Leg	9.0 a α	24.8 b αβ	23.1 a αβ	77.39 b α
N450	Grs	7.0 a α	13.6 a α	19.6 a α	67.8 a α
	Mix	9.5 ab β	21.2 a β	34.6 a β	72.49 a β
	Leg	12.2 b α	38.6 b β	32.0 a β	95.35 a α
	SE	0.82	2.61	2.79	5.08

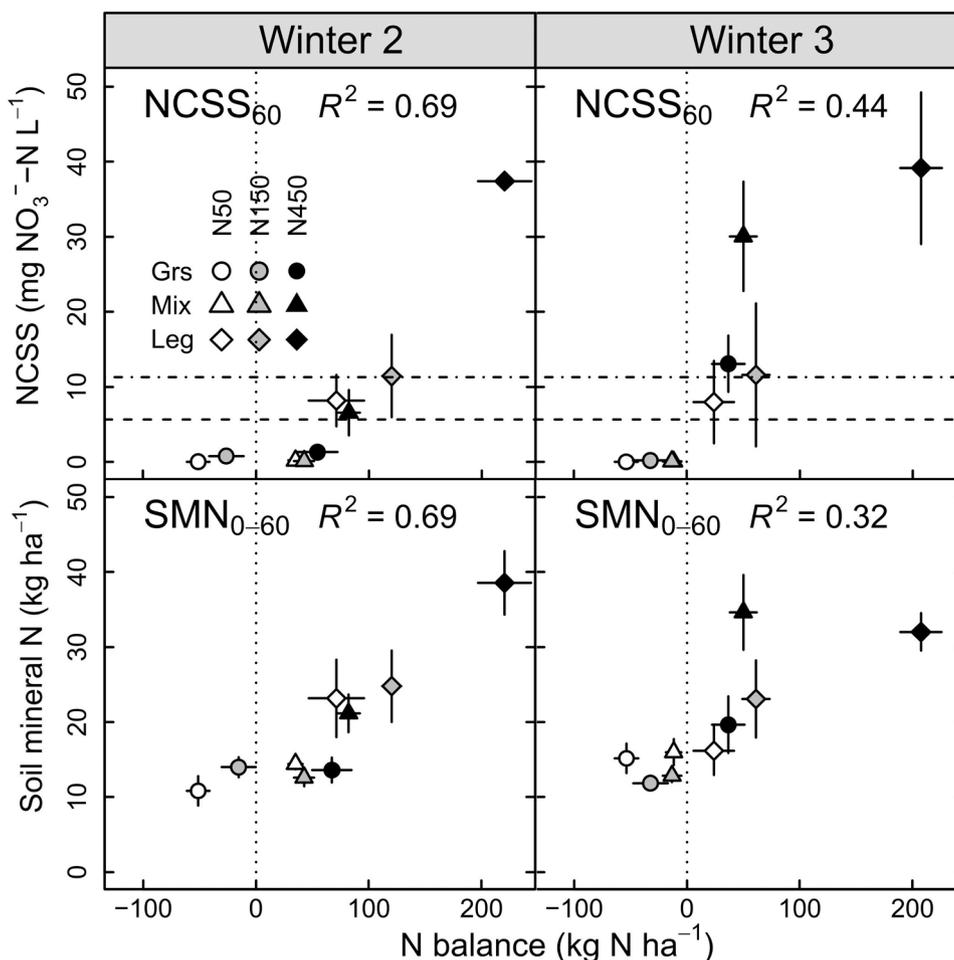


Fig. 3. Relationship between N balance and nitrate concentration in the soil solution at 60 cm depth (NCSS₆₀) and soil mineral N in the soil layer 0–60 cm (SMN₀₋₆₀). For winter 2, N balance is calculated from year 1 to 2, and for winter 3 from year 1 to 3 (average per year). Symbols indicate means of different sward types at N50, N150 or N450. Standard errors of the means are indicated by continuous horizontal lines (N balance) and continuous vertical lines (SMN and NCSS). The dotted vertical lines indicate an N balance of 0. The nitrate limit for groundwater is indicated by the dash-dotted horizontal (European Union) and dashed horizontal line (Switzerland) respectively. Coefficients of determination (adjusted) are derived from quadratic regression (winter 2) and linear regression (winter 3) respectively.

At all sampling events during the period with intact plant cover, the content of soil mineral N was higher in the upper soil layer (SMN₀₋₃₀) than in the lower soil layer (SMN₃₀₋₆₀) (Fig. 2, Table S3b). SMN₃₀₋₆₀ was positively correlated to SMN₀₋₃₀ ($R^2 = 0.46, 0.69, 0.71$ and 0.79 in Winter 1, 2, 3 and 4, respectively).

5.2. Period after tilling

N uptake by plants was stopped by herbicide application. This effect, combined with tilling of the swards, resulted in a strong increase of SMN for all treatments compared to the period with intact plant cover (Table 3 & Fig. 2). During the months after tilling, SMN increased until a maximum in November (0–30 cm) respectively in December (30–60 cm). Mean values at individual sampling events of up to 153 kg N ha^{-1} were observed in the total within both soil layers. After reaching these peaks, SMN levelled off by February, to nearly the magnitude observed before tilling. From December to February, SMN in the lower soil layer was at least as high as in the upper layer, in contrast to all preceding samplings.

SMN values averaged over all samplings of winter 4 did not differ significantly between Grs- and Mix-swards at the same fertilisation level, except for Mix-N150, for which SMN was lower compared to Grs-N150. In contrast to the period with intact plant cover, SMN of Leg-swards after tilling was significantly higher than of Mix-swards (except at N450) but did not differ from Grs-swards.

5.3. Relationship between N balance, SMN and NCSS

N balance was positively correlated with both SMN and NCSS (Fig. 3). For NCSS, the coefficients of determination were 0.69 and 0.44 for winter 2 and 3, respectively. For SMN₀₋₆₀, they were 0.69 and 0.32. For Mix-N450, NCSS was much higher in winter 3 than in winter 2 in spite of a similar N balance. For Grs- and Mix-swards fertilised at N50 or N150, N balances for the period of year 1 to year 2 were between -51 and $+43 \text{ kg N ha}^{-1}$, N balances were clearly negative for both sward types when calculated from year 1 to year 3 (-54 to $-12 \text{ kg N ha}^{-1} \text{ yr}^{-1}$) (Table S1b). Accordingly, both SMN and NCSS values remained very low for these Grs- and Mix-swards. In contrast, SMN and NCSS were clearly elevated for Grs- and Mix-swards at N450 and for pure legume swards at all fertilisation levels, which correlated well with their more positive N balances. SMN correlated well with NCSS in winter 2 and 3 (Fig. S2: $R^2 = 0.67$ for SMN₀₋₆₀).

6. Discussion

6.1. Pure grass swards: No risk for nitrate leaching at low to moderate fertiliser levels, but time-lagged risk at high fertiliser levels

Pure grass swards at low and moderate fertilisation (Grs-N50 and Grs-N150) had much lower N outputs compared to Grs-N450, due to much lower yields and shoot N concentrations. Grass growth was thus clearly N limited in Grs-N50 and Grs-N150, although soil N pools provided approximately $100 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ to the growth of the harvested grass biomass (Table 1: $N_{\text{other out}}$). At our experimental site, N losses through denitrification and N inputs through atmospheric deposition were probably in a similar range (both fluxes in the range of $20\text{--}25 \text{ kg N ha}^{-1} \text{ yr}^{-1}$; Ammann et al., 2009). The substantially negative N balance for Grs-N50 and Grs-N150 must thus have corresponded to soil N depletion, which explains the very low SMN and NCSS values in these plots during the whole period of intact plant cover. At high fertilisation (N450), soil surface N balance of the pure grass swards was on average only slightly positive ($+37 \text{ kg N ha}^{-1} \text{ yr}^{-1}$), because of the large amount of N removed from the field with the harvested biomass. This demonstrates the great capacity of the grass species *Lolium perenne* L. and *Dactylis glomerata* L. with their dense root systems (Hebeisen et al., 1997) to efficiently deplete mineral N from the soil (Daepf et al., 2000;

Schneider et al., 2004). Consequently, the increase in SMN and NCSS at the high N fertiliser application of N450 was only minor, although the amount of fertiliser N not recovered in the harvested plant material was high for Grs-N450 ($= 151 \text{ kg N ha}^{-1} \text{ yr}^{-1}$). With Grs-N450, N balance was $+108 \text{ kg N ha}^{-1}$ in year 1 but close to zero in year 2 and 3 (Table S1). Surprisingly, SMN and NCSS barely increased in the first and second winter in spite of the very high N fertiliser applications. However, SMN and NCSS were elevated in winter 3 compared to Grs-N50 and Grs-N150 (although significantly only for NCSS). Such a time lag between a highly positive N balance over the first year of ley cultivation and increased N leaching was also observed by Eriksen et al. (2015). In their experiment analysing N leaching under leys from the first to the fourth year of cultivation, N leaching was lowest during the first year, although the N balance of this period was higher than in the third or fourth year. The authors argued that this could be explained by a build-up of soil organic N during the first year of ley cultivation (Jensen et al., 2022; Guillaume et al., 2021), which later on might be partly mineralized. The potential role of changes in soil N stocks for the observed poor correlation between soil surface N balance and short-term (a few years) leaching measurements has also been highlighted by Rashid et al. (2022). Besides corroborating that temporal mismatches between strongly positive soil surface N balance and elevated nitrate leaching may occur, the results from the Grs-N450 treatment indicate the necessity of having a few experimental years to assess the risk of nitrate leaching from leys.

6.2. Pure legume swards: Increased risk for nitrate leaching, also at low fertiliser levels

For the pure legume swards, fertilisation rates of 150 and 450 $\text{kg N ha}^{-1} \text{ yr}^{-1}$ were applied for comparison purposes, although such fertilisation rates were clearly overshooting fertilisation recommendations for optimal yields. Besides the observation of no additional yield from Leg-N50 compared to Leg-N450, both the low fertiliser recovery rate in the harvested plant material and the strongly positive N balance indicate over-fertilisation beyond N50 (Table 1). This over-fertilisation provoked elevated NCSS and occasionally SMN values, and therefore an increased risk of N leaching, when compared to the Mix-swards at the same fertilisation level or for Leg-N450 compared to Leg-N50. N input from symbiosis was only reduced by about 30 and $100 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ from Leg-N50 to Leg-N150 and from Leg-N150 to Leg-N450 respectively (average over the three years). Thus, down-regulation of symbiotic fixation only buffered one third of the additional N fertiliser applications in the pure legume swards. At N50, N balance of the Leg-swards was only moderately positive ($+24 \text{ kg N ha}^{-1} \text{ yr}^{-1}$) and fertiliser recovery in the harvested material was in the range of the Grs- and Mix-swards, which indicates that Leg-N50 had not been markedly over-fertilised. Nevertheless, NCSS was elevated by an impressively high factor in Leg-N50 compared to Mix-N50 (43 and 458 times in winter 2 and 3, respectively) and even compared to Mix-N150 (72 and 226 times). This may be related to the much lower root mass of legumes as compared to the grass species (Hebeisen et al., 1997; Hofer et al., 2017) and underlines the crucial role of grasses in the swards – even at low N fertilisation – for maintaining NCSS below the Swiss threshold for drinking water ($5.6 \text{ mg NO}_3\text{-N l}^{-1}$, Annex 2 of the Water Protection Ordinance, 2023) due to their efficient capture of soil N. However, these results gained with white and red clover pure stands might not hold true for pure stands of other species as e.g. lucerne (*Medicago sativa* L.), for which some studies observed low risks for N leaching with pure stands (Benoit et al., 2014).

6.3. Grass-legume mixtures: No increased risk for nitrate leaching despite large N input from symbiosis

In contrast to pure grass swards, plant growth was not or only marginally limited by N availability in Mix-N50 and Mix-N150. This was shown by (i) a significantly enhanced N input and as a direct

consequence, N output in the Mix- compared to the Grs-swards fertilised at the same level, (ii) by an increase in shoot N concentration beyond the deficiency threshold in grass plants (Nyfeler et al., 2011), and (iii) by the very modest yield effect of increased fertiliser application in the Mix-swards (only +29% total N output in Mix-N450 compared to Mix-N50, while N output in Grs-N450 was 3.5 times that in Grs-N50; Table 1).

In the grass-legume mixtures, total N input, N output and therefore soil surface balance were very similar at N50 and N150. Total N input was more or less equal to total N output in these two treatments. This indicates that the decrease in symbiotic N fixation from Mix-N50 to Mix-N150 (−0.87 kg symbiotic N input per additional kg fertiliser N) fully compensated for the increased fertilisation in terms of input-output balance ('buffering effect' from symbiotic N fixation; Nyfeler et al., 2011; De Notaris et al., 2021). This down-regulation of symbiosis derived from a reduction of both legume proportions and symbiotic activity by legume plants with increased fertilisation (Table S1b). Burchill et al. (2014) observed a linear decrease in N from symbiosis across 0–280 kg N ha^{−1} yr^{−1} fertiliser N applied, and this effect might therefore buffer high soil N availability over a wide range of N fertiliser applications in grass-legume mixtures. Correspondingly, both SMN and NCSS were not significantly increased in Mix-N150 as compared to Mix-N50 and Grs-N150, and even to the highly N-limited Grs-N50. The Mix-N50 and Mix-N150 swards showed a very low risk of N leaching across the entire period of intact plant cover. The advantages of such mixtures with an average clover proportion of 42% (N50) and 32% (N150) over the three years are therefore numerous: they achieve high biomass and crude protein yields at low N fertiliser rates (Nyfeler et al., 2009; Finn et al., 2023), while keeping the risk for N leaching low during the entire period of intact plant cover. For grass-legume mixtures, total N inputs of up to 380 kg N ha^{−1} yr^{−1} with a fertiliser N:symbiotic N ratio of 40:60 did not pose any serious risk of N leaching at our experimental site during the period of intact plant cover.

In contrast to N50 and N150 however, symbiotic N fixation was only incompletely down-regulated in the Mix-N450 plots. In spite of the high N fertilisation, N input from fixation was still beyond 100 kg N ha^{−1} yr^{−1} at N450 (average over the three years) and a notably positive N balance ensued in excessively fertilised mixtures (N450). This corresponded to an elevated risk for N leaching during the entire measurement period under intact plant cover as shown by significantly increased SMN and NCSS values. However, such a high fertiliser level (N450) is far beyond the linear range of down-regulation observed by Burchill et al. (2014). Indeed, even an immediate and total cessation of symbiotic N fixation, which accounted for an N input at N50 of 315 kg ha^{−1} yr^{−1}, would not be able to fully compensate an increase in fertiliser N input of 373 kg N ha^{−1} yr^{−1} (from N50 to N450, Table 1).

Besides the capacity of the sward (legume proportion) and the legume plant (proportion of N derived from symbiosis) to regulate N input through symbiosis according to their need for growth, we suggest another process to be of importance for the low risk for nitrate leaching from grass-legume mixtures. Indeed, the grass component of the mixture with its dense root system and its great N uptake capacity seems to result in functional diversity benefits resulting in a steady depletion of available N from the soil. This is impressively demonstrated by the fact that in our study, uptake of fertilizer N ($N_{\text{fert out}}$) and of mineral N from other sources ($N_{\text{other out}}$) was significantly increased in Mix-swards as compared to Grs-swards.

6.4. N balance is a main driver of the risk of nitrate leaching

Both Leg-N50 and Grs-N450 had a positive N balance in the range of 25–35 kg N ha^{−1} yr^{−1} (Table 1). From total N input in Leg-N50, 87% was derived from symbiotic fixation, whereas it was 100% from fertiliser application in Grs-N450. Considering both NCSS and SMN measurements (Tables 2 and 3, Fig. 2), it appears that the risk of N leaching was on average similar for Leg-N50 and Grs-N450. Thus, in our experiment,

pure grass and pure legume swards showed a similar risk of N leaching at a similar level of N excess, independently of the main N source ($N_{\text{fert in}}$ or $N_{\text{sym in}}$). Moreover, the observed relationship between N balance and NCSS and SMN across all treatments (Fig. 3) also indicates that N balance is a stronger driver of the risk of N leaching than the type of sward, and correspondingly, the main N source. Nevertheless, further studies would be necessary to clarify the effect of fertiliser vs. symbiotic N inputs on N leaching, as some studies indicate that replacing fertiliser N with symbiotically fixed N may reduce N leaching (Harris et al., 1994) while others conclude that it would not (Bracken et al., 2022).

6.5. After tilling of leys: no increased risk of nitrate leaching from grass-legume mixtures compared to pure grass swards

The magnitude and temporal evolution of SMN following application of glyphosate, seedbed preparation by rotary tiller and wheat sowing (Table 3, Fig. 2) confirms that disrupting the plant cover of leys increases the risk of nitrate leaching. This well-known effect has been explained by elevated mineralisation of soil organic matter following ley destruction (Seidel et al., 2009; Velthof et al., 2010; Guillaume et al., 2021), causing a rise in SMN. The destruction of leys after several years of cultivation followed by the sowing of a winter crop in autumn is a widespread cultivation procedure in Switzerland and many other countries (Soane et al., 2012; Reumaux et al., preprint, Guillaume et al., 2021). Thus, the pattern of SMN that was observed in our experiment during winter 4 may occur frequently in crop rotations containing leys, which stresses the need for careful choice of forage species associations that do not further increase the risk of N leaching after the eradication of the ley. Tilling could increase SMN by as much as almost 20-fold compared to the earlier measurements (Grs-N450), and by more than ten-fold compared to the system with the lowest N inputs (Grs-N50) (Fig. 2; highest measured values).

After a peak in November to December, SMN sharply decreased during the second half of the winter. This, together with the increased proportion of SMN in the lower soil layer during the second half of the winter (SMN_{30-60} about one third of SMN_{0-60} in October to November and about one half in December to February) indicates that a large part of this N might have been lost through leaching. Tilling of pure legume swards resulted in elevated SMN compared to equally fertilised stands containing grasses, which is in line with results from previous studies (Francis et al., 1994; Kumar and Goh, 2002; Bergstrom and Kirchmann, 2004). This was expected as (i) SMN for pure legume swards was already highest during winter 2 and (ii) legume residues are rapidly decomposed in a first phase after tilling due to their high N content and low C:N ratio (Fillery, 2001). However, the reduced SMN for antecedent grass-legume mixtures compared to pure grass swards was unexpected (tendency at N50, significance at N150, however reverse tendency at N450). Indeed, in addition to the less negative 3-year N balance during the period with intact plant cover, grass-legume mixtures are expected to have a root system at least as dense as that of the pure grass swards (Hebeisen et al., 1997), and with a C:N ratio more favourable to N mineralisation than that of the pure grass swards (Li et al., 2020). The low relative legume abundance in the Mix-N150 swards from year 3 onwards (10%; Table S1b) probably contributed to SMN not being higher in the mixtures than in the pure grass swards following tillage in autumn of year 4. Because of the higher N uptake from soil N pools and fertilisation by the mixtures as compared to the grass pure swards ($N_{\text{other out}}$ and $N_{\text{fert out}}$ in Table 1), soil N availability to soil microorganisms might have been less favourable to residue decomposition (Chen et al., 2014) after the mixtures than after the pure grass swards. This might have counterbalanced the lower C:N ratio in the residues of the mixtures and might partly explain the even lower increase in SMN after tilling mixtures than after tilling pure grass swards.

7. Conclusions

Grass-legume leys pose a very limited risk of nitrate leaching during the period of intact plant cover, as long as a substantial grass fraction is provided and the soil surface N balance does not sizably exceed zero (without accounting for atmospheric N deposition). Low to moderately fertilised grass-legume mixtures with a 30–40% clover proportion and a total N input of up to 380 kg N ha⁻¹ yr⁻¹ did not pose any increased risk of nitrate leaching compared to pure grass swards receiving the same amount of fertiliser, but produced much more forage than the latter. Pure swards of red or white clover had a N balance exceeding zero and created a greater risk of nitrate leaching, at even the lowest N fertilisation level. The increase of soil mineral N during the sensitive period following ley eradication and tillage was also highest after the pure legume swards, and equal or even smaller after the grass-legume mixtures than after the pure grass stands. We conclude that grass-legume mixtures combine high yields, low fertiliser requirements, and low nitrate leaching better than either pure grass or pure legume swards.

Declaration of Competing Interest

Daniel Nyfeler reports financial support and statistical analysis were provided by Swiss State Secretariat for Education Research and Innovation.

Data Availability

Data will be made available on request.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.agee.2023.108776](https://doi.org/10.1016/j.agee.2023.108776).

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