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Root Rot Affects Red Clover More Than Other Forage Legumes in Mixed Grass-Legume Leys

Nilla Nilsdotter-Linde[1] | Eva Stoltz[2] | Ann-Charlotte Wallenhammar[2]

[1]Swedish University of Agricultural Sciences, Department of Crop Production Ecology, Uppsala, Sweden | [2]Rural Economy and Agricultural Society|HS Konsult AB, Örebro, Sweden

Correspondence: Nilla Nilsdotter-Linde (nilla.nilsdotter-linde@slu.se) | Ann-Charlotte Wallenhammar (ann-charlotte.wallenhammar@hushallningssallskapet.se)

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ABSTRACT

Red clover (RC) (*Trifolium pratense* L.) is the most important forage legume in Swedish mixed grass-legume (MGL) leys. This study evaluated the persistence of two RC cultivars, white clover (WC), birdsfoot trefoil (BT), and lucerne (LU) in mixtures of timothy and meadow fescue associated with root rot (RR). Agronomic performance and nutritive value were measured across 3 years in a field trial conducted across two sites in a 2- or 3-cut regime. Disease progression of RR was assessed each spring and autumn, and symptoms of RR were frequent in the autumn of the seeding year. After two harvest years, all assessed RC roots were severely infected as disease severity index (> 60). The legume proportion (LP) in RC treatments was largest for Years 1 and 2 and significantly decreased by 50% in Year 3. LP in white clover, LU, and BT were lower than in RC, but less affected by RR. LP of WC remained at about 23% for Years 1–3. LP decreased from a low level for BT by 46%, and LP in LU was extremely low. Total annual dry matter (DM) yield was larger for Years 2 and 3 than Year 1 for all treatments and increased in Year 3 for mixtures with WC, BT, and LU. Economically, WC and RC with three cuts were the best options, evaluated as DM yield, digestibility, and protein content. Application of lime, 6 and 9 t ha^{-1}, evaluated in MGL swards in two separate field trials did not affect RC proportion, disease progress, or yield compared to untreated control.

1 | Introduction

Grasslands play an important role within Swedish crop and livestock production. Indeed, temporary grasslands constitute 42% (corresponding to 1,064,000 ha) of the total acreage of arable land in Sweden (SBA 2022). Red clover (RC, *Trifolium pratense* L), white clover (WC, *Trifolium repens* L.), and birdsfoot trefoil (BT, *Lotus corniculatus* L.) are all native plants of the Nordic countries and are successfully cultivated in the northern parts beyond 67° N (Stoddard et al. 2009) and 69° N (Sturludóttir et al. 2013). Mixed swards containing these forage legumes and lucerne (LU, *Medicago sativa* L.) are widely used for livestock feed. In terms of quality and quantity, the grasses timothy (TI; *Phleum pratense* L.) and meadow fescue (MF; *Festuca pratensis* L.) are the most dominant species grown in mixed grass-legume (MGL) swards in temporary grasslands in the northern hemisphere. Red clover is the most important constituent in Swedish MGL production, and RC within the feed ration offers several positive effects, including improved milk yield, milk quality (Bertilsson et al. 2001; Kuoppalas 2010; Halmemies-Beauchet-Filleau et al. 2014), and nitrogen utilisation amongst ruminants (Lee et al. 2006).

Nonetheless, the weak persistence of RC has restricted sustainable MGL production in Europe for decades (Ylimäki 1967; Rufelt 1979; Pokorný et al. 2003; Hejduk 2006; Wallenhammar et al. 2006) and RC proportions have been reported as gradually reduced in mixed MGL swards (Frankow-Lindberg 1989; Nykänen et al. 2000; Nilsdotter-Linde et al. 2002; Mela 2003; Marshall et al. 2017; Parsons et al. 2018). The reduced productivity of RC in forage swards is associated with the destruction of the tap root by a complex of microorganisms that cause root rot (RR). Soil-borne fungi such as *Fusarium avenaceum* and other fungi from the genus *Fusarium*, *Cylindrocarpon destructans*, *Phoma medicaginis*, and *Pythium* spp. (Ylimäki 1967; Rufelt 1986) are most often linked with this complex. The proliferation of RR in RC plants in MGL leys was first described in Finland by Ylimäki (1967) in a national survey and was then later shown throughout the Swedish ley production areas by Rufelt (1979). More recently, organic farms in both south and central Sweden experienced a severe depletion of RC stands during the second winter, and extensive RR infections were identified in the roots of RC plants in MGL leys (Wallenhammar et al. 2006). This complex of fungi affects the establishment, growth, persistence, and overwintering capacity of the RC plant. The early stages of infection are usually confined to lesions and discoloration of the cortical region of the primary and secondary roots, as described by Ylimäki (1967). As the infection progresses, the internal vascular cylinder is damaged, leading to a hollowing of the root, which is ultimately destroyed. The injured tissue is brown, and the colour is dependent on the microorganisms causing the disease. Certain bacteria such as *Pseudomonas viridiflava* can also play an active role in increasing the degradation of cellulose (Leath et al. 1989). Recent molecular studies quantified the root-associated fungi causing RR using real-time PCR (Almquist et al. 2016). *Fusarium avenaceum*, *P. medicaginis*, and *C. destructans* were identified in the roots 2 months after seeding, thereby indicating an early infection. Shifting the environment in the rhizosphere of RC roots through lime application is a reported option to reduce the impact of the *Fusarium* species (Close 1994) since this group of fungi is favoured by acidic conditions. The overall persistence of a RC plant is affected by various abiotic and biotic factors, where adaptation to changing climate conditions such as drought and flooding tolerance, management practises, and access to micronutrients is essential (Rufelt 1986; Stoltz and Wallenhammar 2012; Stoltz and Wallenhammar 2018; Parsons 2024).

Other forage legumes such as WC, LU, and BT are important species for sustainable livestock systems. White clover, the major temperate forage legume, has a stoloniferous growth habit and can therefore spread vegetatively in swards (Marshall et al. 2017). White clover is more persistent than RC when harvested more than twice a year (Nilsdotter-Linde et al. 2002). Predictions of longer droughts (Olesen et al. 2011) mean that adapting to climate change will require enhanced access to drought-tolerant forage legume species such as BT, a forage legume highly adapted for growth under drought conditions (Inostroza et al. 2015) with the ability to persist for at least three years in the sward (Nilsdotter-Linde and Bergkvist 2005). Lucerne, a perennial, high-yielding forage legume with superior drought tolerance and a deep tap root, improves fertility building and structure in the subsoil (Halling et al. 2004; Picasso et al. 2019). Moreover, being a high-producing species, LU requires a 3-cut system for optimal yield (Wolf and Smith 1964) whereas BT is best suited to a 2-cut system (Nilsdotter-Linde 1999). Aside from increasing the diversity between two functional groups of grasses and legumes, tall herbs such as chicory (CI; *Cichorium intybus* L.), a relatively deep tap-rooted, drought-tolerant, mineral-rich (Belesky et al. 2001), and highly palatable broadleaf perennial with positive dietetic properties and good regrowth (Rydberg 1998) can increase biomass production. In legume-intensive cropping systems, i.e., legume stands in long rotations or legume stands in repeated short rotations, there is a risk of a substantial build-up of the abundance of soil-borne inoculum of the pathogenic fungi that cause RR.

The overall aim of this study was to determine disease progress in RC and to evaluate the effects thereof through various management strategies across three harvest years, alongside determining the proportion of legumes and measuring sward DM production and feed quality. The objectives were to evaluate the persistence and severity of RR for RC, WC, BT, and LU throughout three harvest years and to identify the species possessing the least amount of disease for the purpose of alternating with RC in MGL production.

In this work we hypothesised that:

i. A severe infection of RR affects both the botanical composition and quality of the sward.

ii. The crude protein (CP) concentration decreases as the RC content of the sward is reduced.

iii. The susceptibility of RR, which is caused by a range of soil-borne pathogens, varies for legume species (RC > LU > WC > BT) and for the RC cultivars (cv. Fanny > cv. Vivi); RR can be reduced by including the herb CI.

iv. Application of lime prior to establishment of the RC ley reduces the infection of RR.

2 | Materials and Methods

2.1 | Field Experiment 1: Effects of Seed Mixture and Harvest Regime on Legume Persistence

2.1.1 | Site Description

The field experiment was farmed organically (KRAV 2025) at two sites: Rådde Field Research Station, Länghem, Tranemo community (57°37′ N, 13°15′ E) in south-west Sweden with climate classification Cfb according to Kottek et al. (2006) and at the field experimental farm Kvinnersta, Örebro community (59°36′ N, 15°25′ E) in central Sweden with climate classification Dfb (Kottek et al. 2006) (Table 1). Soil samples (approximately 750 mL) were collected from each experimental area. Extractable phosphorus, potassium, calcium, and magnesium were determined by extraction with acid ammonium lactate (pH 3.75) (SIS 1993). Base saturation was calculated using methods developed by Eurofins Agro Testing AB. Soil pH values were determined potentiometrically in water (1.0:2.5 wt/wt). The soil analyses were performed at SLU Provcentralen in Uppsala, Sweden. Data on average temperature and precipitation at each field trial site was collected from local weather stations at Länghem (Tranemo) and Säbylund (Örebro) (Lantmet

TABLE 1 | Location of the field experimental sites at Rådde, Kvinnersta, and Åkerby, and the soil characteristics in the two experimental set-ups.

Site	Position	Soil texture	pH	SOM % by weight	Clay % by weight	P-AL[1] mg/100 g	K-AL[1] mg/100 g	Mg-AL[1] mg/100 g	Ca-AL[1] mg/100 g	Base saturation %
Rådde[2]	57°37′ N, 13°15′ E	Loam	5.9	5.4	10	6.9	5.0	4.2	110	40
Kvinnersta[3]	59°36′ N, 15°25′ E	Silty clay loam	6.6	3.4	31	8.5	18	26	210	72
Åkerby[4]	59°36′ N, 15°05′ E	Silt loam	5.7	5.4	23	5.5	8.0	11	150	45

Note: (1) Effects of seed mixture and harvest regime on legume persistence and (2) Response of red clover persistence to lime treatments.
[1]Egnér et al. (1960).
[2]Undersown in spring barley.
[3]Undersown in oats.
[4]Established without cover crop.

TABLE 2 | Experimental design for Field experiment 1, legume species and cultivars, and number of cuts in seed mixtures with timothy cv. Alexander (10 kg ha^{-1}) and meadow fescue cv. Kasper (7 kg ha^{-1}).

Seed mixture		
Species (seed rate, kg ha^{-1})	**Cultivar**	**Number of cuts year^{-1}**
Red clover (RC) (8)	Fanny 4n[1] (medium heading)	2
Red clover (RC) (8)	Vivi 4n[1] (medium heading)	2
Red clover (RC) (8)	Fanny[1]	3
Red clover (RC) (8) + chicory (CI) (1)	Fanny[1] + Grasslands Puna	3
White clover (WC) (4)	Sonja (intermediate leaf size)	3
Birdsfoot trefoil (BT) (11)[2]	Oberhaunstaedter	2
Lucerne (LU) (14)[3]	Pondus	3

[1]4n = tetraploid.
[2]Inoculated with *Rhizobium loti*.
[3]Inoculated with *Rhizobium meliloti*.

Network 2022: http://www.ffe.slu.se/lm/LMHome.cfm) during 2004–2007 and are presented in Figure S1a,b.

2.1.2 | Plant Materials

Four legume species were compared: tetraploid medium flowering RCs, cvs. Fanny and Vivi; WC, cv. Sonja; LU, cv. Pondus; and BT, cv. Oberhaunstaedter (Halling 2002). The selected companion grasses were the robust species TI cv. Alexander and MF cv. Kasper. Red clover 8 kg ha^{-1}, WC 4 kg ha^{-1}, BT 11 kg ha^{-1}, or LU 14 kg ha^{-1} were established with seed mixtures containing 10 kg ha^{-1} TI and 7 kg ha^{-1} MF. In an additional treatment, 1 kg ha^{-1} CI was added to the RC (Table 2). The RC cultivar Vivi was considered tolerant to RR according to the breeders at Lantmännen Seed (former Svalöf Weibull AB).

2.1.3 | Field Experimental Design

The field experiment was arranged in a randomised complete block design (RCBD) with four replicates. Each plot measured 3.5 m × 18 m (63 m^2) at Rådde and 3 m × 18 m (54 m^2) at Kvinnersta. The forage mixtures were undersown in spring barley and oats (200 kg ha^{-1}) on 30 April 2004 at Rådde and 6 May 2004 at Kvinnersta, respectively, with a Wintersteiger seeder (Wintersteiger PlotseedS; Wintersteiger AG., Rein im Innkreis, Austria) and harvested at the beginning of September. Fifteen tonnes per ha of solid cattle manure was applied in spring prior to sowing at Rådde. According to official standard values (Andersson et al. 2024) this corresponds to 90 kg total-N, 22 kg P, and 78 kg K ha^{-1}. No manure was applied at Kvinnersta. In early spring of the second and third harvest years, both field trials received 25 t ha^{-1} of cattle slurry, corresponding to 108 kg total-N, 15 kg P, and 95 kg K ha^{-1} to maintain potassium levels. The plots were managed according to Table 2.

Plant sampling for visual assessment of RR was performed 4–6 November of the year of establishment (2004). During the period 2005–2007, plants from each plot were sampled twice a year, 20–25 April and at the end of October. Additionally, the Rådde field experiment was sampled in mid-April 2008. Ten randomly selected legume plants were dug up to about 25–30 cm and removed with the entire root system. The plants were subsequently stored at +8°C until assessment.

2.1.4 | Sampling of Herbage and Analysis at Harvest

The dry matter herbage yield (DM yield) at each harvest was measured on a 1.5 m wide swath and harvested from the centre of each 3 m wide plot with a Haldrup 1500 plot harvester (J. Haldrup, Løgstør, Denmark) which left a stubble height of 5–7 cm. The fresh weight of the samples was measured on-site and a sub-sample of 1000 g was used for DM analysis from each plot. The sub-samples were pre-dried in a Ropack oven at Rådde (Industrial ovens—Ropack) and an Elektrohelios oven

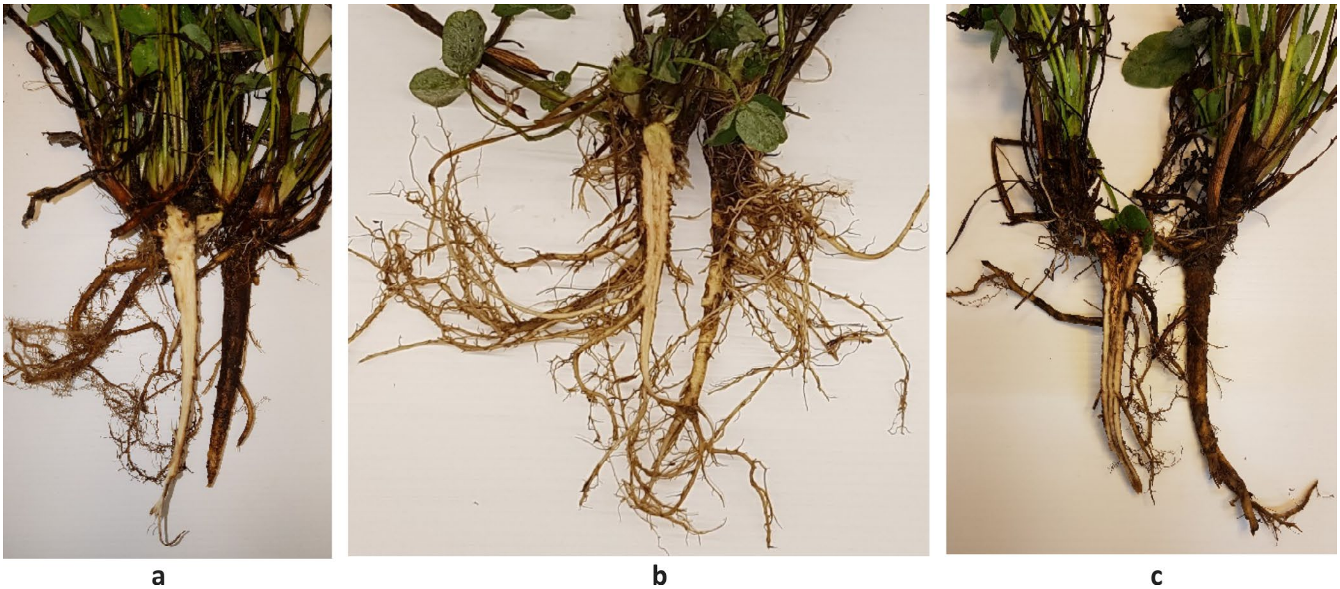

FIGURE 1 | Longitudinal sections of red clover roots showing the invasion of fungi causing root rot along the vascular tissues. (a) Healthy root (b) Mild symptoms of root rot with a light brown discoloration confined to the cortical region (c) Severe infection of root rot. The vascular tissues are invaded and discoloured by both fungal mycelium and decomposition products from the vessel walls. Photo: Eva Edin.

(No. 36444) at Kvinnersta at 60°C for 24 h. They were each corrected for residual water content by drying for a minimum of 3 h at 105°C.

For each harvest, a 500 g sample of fresh herbage was collected from each replicate by cutting 6 sub-samples at 5–7 cm stubble height, which were then manually separated into the following fractions: sown legume, other legumes, grass, weeds, and stubble (results are not reported here). The fractions were dried in fibre sachets at (max) 60°C for 24 h and weighed to determine the botanical composition. The fractions were pooled prior to determining the concentrations of CP, ME, and NDF (neutral detergent fibre) for each seed mixture and harvest in Years 1 and 2, and plot-wise determination in Year 3. In Year 3, the indigestible neutral detergent fibre (iNDF) fraction was also determined. Crude protein concentration was calculated by multiplying the N concentration by 6.25 (Nordic Committee on Food Analysis 1976). Metabolisable energy concentration was estimated from 96 h in vitro digestibility of organic matter (IVDOM) in 2% rumen liquid by the Swedish standard method, VOS (Lindgren 1979), and the equation for grass was used for samples with $<50\%$ legumes; the equation for legumes was used for samples with $\geq 50\%$ legumes. Ash content was determined by combustion at 550°C for 3 h (EC No. 152/2009). Ash-free content of NDF was analysed by the oven method of Chai and Udén (1998) without amylase and sodium sulphite at SLU, Kungsängen Research Center, Uppsala, Sweden. Indigestible NDF was analysed with Near Infrared Spectroscopy (NIRS) at Agrilab AB, Uppsala, Sweden, using an in-house calibration based on samples analysed according to Åkerlind et al. (2011).

2.1.5 | Disease Severity of Root Rot

In the laboratory, leaves were removed, and the plants were thoroughly washed in running tap water and split longitudinally with a scalpel. Each root was scored for disease severity on the root surface (external symptoms) following a visual five-class scale according to Rufelt (1986): 0 = no visible lesions; 1 = minor changes or discolouring, single spots (most often on the upper parts of the root); 2 = several minor damages or a single more severe/deeper damage; 3 = more than half of the root surface damaged or discoloured; 4 = the entire root discoloured with minor or major damages. The surfaces of the split root (internal symptoms) (Figure 1) were also scored following a visual five-class score: 0 = no discolouring; 1 = the root is discoloured (injuries typically occur in the upper parts of the root, however, the discolouration can also commence from an external damage in the lower part of the root); 2 = minor discolouration in parts of the root; 3 = at least one third of the root discoloured; some of the plant shoots are dead; 4 = at least two thirds of the root is discoloured. The largest root was selected from the WC plants for assessment. Disease severity indices (DSI) were calculated separately for external (DSI_e) and internal (DSI_i) assessments according to the following equation:

$$DSI = \frac{\sum_{class}[(\text{class score}) \times (\text{no. of roots in each class})]}{(\text{total no. of roots})}$$

The proportion of infected plants (disease incidence, DI_e and DI_i) was calculated as the percentage of infected plants per plot.

2.2 | Field Experiment 2: Evaluation of Lime Application for Development of Root Rot in Red Clover

2.2.1 | Site Description

The response of lime application on the persistence of RC was evaluated in a field trial established in 2004 at Kvinnersta, Örebro community (59°36′ N, 15°25′ E) and at Åkerby, Örebro community (59°36′ N, 15°05′ E) where soil pH ranged from 5.7 to 6.6 at the start of the study (Table 1).

2.2.2 | Lime Product

A limestone product, Nordkalk Active ($CaCO_3$), with a lime effect equivalent to 52% of CaO, is provided by Nordkalk AB, Köping, Sweden.

2.2.3 | Field Experimental Design

Field experiment 2 was arranged in a RCBD with three replicates. Each experimental plot measured 3 m × 11 m (33 m^2) at Kvinnersta and 3 m × 16 m (48 m^2) at Åkerby. Lime treatments were spread uniformly by hand across the plots and were immediately incorporated into the soil to a depth of 10–15 cm with a TIVE cultural harrow (Tierp, Sweden) prior to sowing on 11 May and 13 May, respectively. The treatments consisted of Nordkalk Active applied at rates of 6 and 9 t ha^{-1} lime. The untreated control did not receive any lime. Seed mixture, harvest regime, and fertiliser regime for the treatment (RC Fanny, TI, and MF, 2 cuts) in Field experiment 1 were used (Table 2). Twenty-five tonnes per ha of cattle slurry was applied in early spring of Years 2 and 3 to maintain potassium levels. According to official standard values (Andersson et al. 2024) this corresponds to 108 kg total-N, 15 kg P, and 95 kg K ha^{-1}. Observations, determination of herbage and DM yield, botanical analyses, sampling, and RR assessments were performed as described in Sections 2.1.3, 2.1.4 and 2.1.5. Soil pH and $Ca\text{-}_{AL}$ were determined plot-wise at the termination of the field experiment in 2007.

2.3 | Statistical Analysis

Field experiment 1, which involved grass-legume mixtures, was analysed using a linear mixed-effects model with random effects of plots and fixed effects of sites, treatments, harvest years, their two-way interactions, and replicates. These analyses were performed for each harvest. To produce homogeneous variance, legume proportion was square-root transformed prior to analysis. Root rot was analysed using a linear mixed-effects model with random effects of plots and fixed effects of sites, treatments, harvest years, cuts, their two-way interactions (except site × year), and replicates. Field experiment 2, which evaluated the effect of lime on the persistence of RC, was analysed using a linear mixed-effects model with random effects of plots and fixed effects of sites, treatments, years, cuts, their two-way interactions, the three-way interaction site × year × cut, and replicates. Dry matter yield was log-transformed prior to analysis. Tukey's HSD method was used for pair-wise comparisons. All models were fitted using JMP 8.0.1 (SAS Institute Inc. 2009).

3 | Results

3.1 | Effects of Seed Mixture and Harvest Regime on Legume Persistence

3.1.1 | Developmental Stages at Harvest

The timing of the first cut was in accordance with the phenological development of TI in all treatments (Gustavsson 2011) and harvesting occurred at early heading (DC 51) at Kvinnersta (16 June 2005, 14 June 2006), at heading (DC 55) at both Rådde (16 June 2006) and Kvinnersta (8 June 2007), and at inflorescence bearing internode just visible above the leaf blade base (DC 59) at Rådde (23 June 2005, 11 June 2007). In the 2-cut treatments (Table 2) the regrowth was harvested approx. 9 weeks after the first cut corresponding to DC 51 in 2005 and at Kvinnersta in 2006, to DC 31 at Rådde in 2006, and in 2007 to DC 59 at Rådde and to DC 55 at Kvinnersta. For 3-cut treatments, the second cut occurred 6 weeks after the first cut corresponding to DC 31–55 at Rådde and DC 31–51 at Kvinnersta. The third cut was performed after an additional 6 weeks, corresponding to DC 31 with exceptions at Rådde in 2006 and 2007 when the TI was cut at DC 11–19 and DC 51, respectively. The corresponding stages of development for the legumes at harvest are described in Table S1 (Hedlund and Höglund 1983; Ohlsson and Wedin 1989).

3.1.2 | Dry Matter Yield

No significant interaction occurred for site × treatment, thus the herbage DM production is presented as means of sites across treatments (Table S2). The total DM yield for each year was larger in Years 2 and 3 than Year 1 for all treatments (Table 3). The DM yield increased in the third harvest year for treatments with WC, BT, and LU, contrasting to the RC treatments except for RC cv. Vivi which produced the largest total DM yield across all three harvest years. No significant differences in DM yield were found between RC Fanny (2) and RC Vivi (2) swards. The DM production decreased 111 and 356 kg ha^{-1} between the second and third year for RC Fanny (2) and RC Fanny (3), respectively. Overall, there was no significant difference in total DM yield between two and three cuts with RC Fanny. In Year 3, the total DM production for RC Vivi was significantly larger than for RC Fanny + CI.

In the first cut of the first year, the treatment with LU yielded significantly less than RC Fanny (2), RC Vivi (2), and RC Fanny (3); thereafter, no differences were observed (Table 3). The treatment with RC, harvested thrice a year, led to a significantly larger DM production in the third cut than the treatment with LU (first and second year) and the treatment with WC (second year), respectively.

The total DM yield across all treatments was almost 1.3 t ha^{-1} larger on average per year at Kvinnersta compared to Rådde, with a significant difference in the first and third harvest years (Table S2). There was no significant difference in DM production between sites for Year 2.

3.1.3 | Legume Proportion at Harvest

The proportion of legumes was lower in the first cut than in the second and third cuts (Table 4). There was a significantly higher legume proportion in the RC treatments compared to other legumes on average per year in the first cut in Years 1 and 2, and in the second cut in Year 1. The RC proportion (RCP) progressively decreased, and the differences between the RCP and WC proportion (WCP) had balanced out (Table 4) by Year 3. The LU proportion (LUP) was significantly lower compared to all other

TABLE 3 | DM yield (kg ha^{-1}) in 1st, 2nd, and 3rd cut and total annual yield for three consecutive harvest years; 2005, 2006, and 2007 for Field experiment 1.

Harvest year	Treatment (number of cuts year^{-1})	1st cut	2nd cut	3rd cut	Total yield
2005	RC Fanny (2)	5211*cde*[1]	3383*ab*		8595*cdefgh*
	RC Vivi (2)	5122*cde*	3241*bc*		8364*fgh*
	RC Fanny (3)	5122*cde*	2110*d*	1277*cde*	8510*efgh*
	RC Fanny + CI Grasslands Puna (3)	4830*def*	1988*def*	1218*de*	8038*hi*
	WC Sonja (3)	4530*ef*	1470*efg*	1051*ef*	7051*ij*
	BT Oberhaunstaedter (2)	4901*def*	2186*d*		7088*ij*
	LU Pondus (3)	4313*f*	1265*g*	821*f*	6399*j*
2006	RC Fanny (2)	6195*ab*	3490*ab*		9685*abcde*
	RC Vivi (2)	6440*a*	3774*ab*		9714*abcde*
	RC Fanny (3)	6338*a*	1343*g*	2076*a*	9758*abcd*
	RC Fanny + CI Grasslands Puna (3)	5825*abc*	1482*efg*	1994*a*	9301*abcdefg*
	WC Sonja (3)	5808*abc*	1412*fg*	1318*bcde*	8538*cdefgh*
	BT Oberhaunstaedter (2)	5746*abc*	2496*d*		8242*ghi*
	LU Pondus (3)	5781*abcd*	838*g*	1220*bcdef*	7975*dhij*
2007	RC Fanny (2)	6147*ab*	3426*ab*		9574*abcdef*
	RC Vivi (2)	6372a	3912*a*		10,285*a*
	RC Fanny (3)	5856*abc*	2045*de*	1501*bcd*	9402*abcdefg*
	RC Fanny + CI Grasslands Puna (3)	5536*bcd*	2030*de*	1606*b*	8950*bcdefgh*
	WC Sonja (3)	6090*ab*	2402*d*	1508*bc*	10,000*ab*
	BT Oberhaunstaedter (2)	6372*a*	3313*b*		9686*abcde*
	LU Pondus (3)	5959*abcd*	2412*cd*	1483*bcd*	9949*abcefg*
	p (ley year × treatment)	$p < 0.0077$	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$

Note: Red clover (RC), RC with chicory (CI), white clover (WC), birdsfoot trefoil (BT), and lucerne (LU) grown in mixed swards with timothy and meadow fescue, cut two or three times a year. Least square means across the experimental sites Rådde and Kvinnersta.
[1]Means followed by a common letter within columns across all years are not significantly different by the Tukey test at the 5% level of significance.

treatments on average each year except for the first cut during Years 1 and 3, respectively. Regarding the second cut in Year 3, significant differences were observed wherein the highest legume proportion was recorded for RC cv. Vivi (Table 4). This is also reflected in the mean across all harvests in the third year where the RCP of cv. Vivi was significantly higher than that of RC cv. Fanny mixed with CI (35% and 21%, respectively).

The WCP was consistent throughout the three harvest years (Table 4). No significant differences were found between harvest years, except for the second cut in Year 2 when the WCP was 63% compared to 31% in Year 1 (Table 4). The BT proportion (BTP) was significantly lower than the RCP, and often lower than the WCP. The CI proportion (CIP) was low and ranged from 2% to 26% with the highest proportion reported in the second cut.

The legume proportion in Year 1, as a mean across all treatments in the first and second cuts, was higher at Kvinnersta with 36% and 53% for first and second cuts, respectively, compared to Rådde, with 12% and 38% (Table 4). The proportion of legumes was significantly higher at Kvinnersta than at Rådde for RC Vivi, RC Fanny + CI, and WC treatments. The RCP of cv. Vivi was significantly higher at Kvinnersta (56%) than at Rådde (37%) as a mean across the three harvest years. Contrastingly, the LUP was lower at Kvinnersta than at Rådde. Harvest of the LU treatment was omitted at Kvinnersta after Year 1 due to an extremely low LUP.

The weed proportion was very low; 0%–6%, 0%–4%, and 0%–3% in Years 1–3, respectively, dominated by *Taraxacum* sect. *ruderalia* (Kirschner, H. Øllg. & Stepanek) and *Cirsium arvense* (L.) for Kvinnersta. The corresponding values for Rådde were 0%–2%, 0%–1%, and 0%–1%.

3.1.4 | Nutritive Value

Analyses from the first and second harvest years for each treatment showed that the concentration of CP was highest in the RC treatments in Year 1, notably in the first cut in Year 2

TABLE 4 | DM proportion of legumes (×10 g kg^{-1}) in 1st, 2nd, and 3rd cut and as an average per harvest year for three consecutive harvest years: 2005–2007 for Field experiment 1.

		Legume proportion (%)			
Harvest year	**Legume (number of cuts year^{-1})**	**1st cut**	**2nd cut**	**3rd cut**	**Average per year**
2005	RC Fanny (2)	44*a*[1]	74*a*		56*a*
	RC Vivi (2)	36*ab*	65*ab*		48*ab*
	RC Fanny (3)	39*ab*	71*a*	58*ab*	50*ab*
	RC Fanny + CI Grasslands Puna (3)[2]	42*a*	64*ab*	64*a*	50*ab*
	WC Sonja (3)	11*cdef*	31*c*	44*abc*	21*def*
	BT Oberhaunstaedter (2)	9*def*	32*c*		16*ef*
	LU Pondus (3)	4*fgh*	8*de*	4*d*	3*hi*
2006	RC Fanny (2)	39*a*	76*a*		53*a*
	RC Vivi (2)	44*a*	77*a*		56*a*
	RC Fanny (3)	40*a*	64*ab*	66*a*	46*ab*
	RC Fanny + CI Grasslands Puna (3)[2]	44*a*	57*ab*	59*ab*	49*ab*
	WC Sonja (3)	16*cde*	63*ab*	41*bc*	28*cd*
	BT Oberhaunstaedter (2)	7*efg*	27*c*		13*fg*
	LU Pondus (3)	1*i*	1*ef*	1*d*	0*j*
2007	RC Fanny (2)	16*cde*	32*c*		23*cdef*
	RC Vivi (2)	22*bc*	56*ab*		35*bc*
	RC Fanny (3)	18*cd*	30*c*	30*c*	22*cdef*
	RC Fanny + CI Grasslands Puna (3)[2]	16*cde*	29*c*	33*c*	21*def*
	WC Sonja (3)	13*cde*	45*bc*	41*bc*	26*cde*
	BT Oberhaunstaedter (2)	4*fgh*	13*d*		7*gh*
	LU Pondus (3)	< 0*hi*	< 0*f*	6*d*	1*ij*
	p (ley year × treatment)	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$

Note: Red clover (RC), RC with chicory (CI), white clover (WC), birdsfoot trefoil (BT), and lucerne (LU) grown in mixed swards with timothy and meadow fescue, cut two or three times a year. Least square means across the experimental sites Rådde and Kvinnersta.
[1]Means followed by a common letter within columns are not significantly different by the Tukey test at the 5% level of significance.
[2]Percentage of chicory ranged from 1 to 15 Year 1, 6 to 26 Year 2, and 1 to 22 Year 3.

(Table S3a,b). The concentration of CP in Year 2 for WC and BT treatments was 178 and 141 g kg^{-1} DM, respectively, as a mean for all cuts despite the relatively low legume proportion which was 28% and 13%, respectively (Tables 4, S3b, and S4). The 3-cut system resulted in higher digestibility of organic matter (IVDOM) compared to the 2-cut system. The average digestibility of Fanny (2) decreased from 81% in the first cut to 77% in the second cut, whilst the digestibility of Fanny (3) increased from 81% in the first cut to 85% and 86% in the second and third cut, respectively (Table S3a,c). This is further reflected in the protein concentration averaging over all cuts at 148 g CP kg^{-1} DM in the 2-cut system and 176 g CP kg^{-1} DM in the 3-cut system (Tables S3a,c and S4). The corresponding energy content was 10.1 and 10.6 MJ ME kg^{-1} DM.

For the third harvest year, plot-wise samples were analysed; and because statistical interaction occurred for site × treatment, the results are presented by site (Table S3c). No statistical differences for CP were recorded in the first cut between treatments. The concentration of CP ranged from 110 to 126 g kg^{-1} DM at Rådde when harvested at DC 59, according to Gustavsson (2011), and from 126 to 139 g kg^{-1} DM at Kvinnersta when harvested at DC 55. In the second cut at Rådde, the RC Fanny and BT treatments with a 2-cut regime contained approximately 40 g kg^{-1} DM less CP and were significantly lower ($p = 0.0045$) compared to the 3-cut regime of RC Fanny, RC Fanny and CI, and WC. In pairwise comparisons, the RC Fanny + CI treatment contained 0.4–0.6 MJ kg^{-1} DM more ME than all other treatments ($p < 0.05$) except for WC in the first cut. Regarding energy content, significant differences were observed at Rådde in Year 3 between treatments with two cuts and three cuts of RC Fanny, with higher ME obtained in the 3-cut system. Concerning fibre concentration, significant differences were shown at Rådde, where the RC and WC treatments with three cuts per year contained significantly less NDF than the other swards in the second cut ($p < 0.05$).

3.1.5 | Effects of Seed Mixture and Harvest Regime on Disease Incidence and Disease Severity of Root Rot in Forage Legume Crops

Disease progress of external assessments of the root surface is illustrated in Figure S2a,b and was most severe for RC treatments. In the seeding year (2004) disease incidence ranged from 71% to 98% infected RC roots in the four different RC treatments, with a corresponding DSI_e ranging from 20 to 29. Visible internal symptoms assessed during the year of establishment (2004) ranged from 60% to 87% infected roots. For BT, LU, and WC, DI_i was lower than for RC and ranged from 18% to 55% infected roots. For severity, internal disease severity index (DSI_i) ranged from 16.5 to 24.0 for RC and was significantly higher ($p < 0.0001$) for RC Fanny (3) and RC Fanny + CI than WC, where DSI_i ranged from 6.8 to 8.0 and for BT, where DSI_i ranged from 5.0 to 8.8. Lucerne exhibited an intermediate disease severity and DSI_i ranged from 5.8 to 16 at Kvinnersta and Rådde, respectively.

Assessment of internal symptoms during 2005–2007 showed that DI_i for RC treatments was significantly higher than for WC, BT, and LU for all years ($p < 0.0001$) (Figure 2a) as an average for both sites and all occasions of assessment. When comparing DSI_i for RC treatments, no differences were found between RC cv. Fanny and RC cv. Vivi or for different harvest regimes or impact of admixture of CI. The largest increase in DI_i and DSI_i for RC occurred during Year 2 (Figure 2b) when infection progressed rapidly and stabilised at a high level as virtually all plants showed disease symptoms. Root sampling in spring demonstrated consistently lower infection compared to the assessment the previous autumn (Figures 3 and S3). For RC treatments, these differences were significant ($p < 0.0001$).

3.2 | Response of Red Clover Persistence to Lime Treatments

3.2.1 | Dry Matter Yield and Red Clover Proportion at Harvest

No significant differences in DM yield were observed when lime was applied, but significant interactions were observed between site, harvest year, and harvest time ($p < 0.0001$). The first cut was significantly larger than the second cut at both sites for all years (Table 5). Dry matter yield in the second cut was significantly larger at Kvinnersta than at Åkerby: 2895 and 2065 kg DM ha^{-1}, respectively, on average for three harvest years. The difference between the first and second cut on average for the two sites was highest in Year 1.

Although the RC proportion was not affected by lime application, there were significant interactions (site × year × harvest) ($p < 0.0001$) (Table 6). The RC proportion was, on average, 35% and 60% in the first and second cut, respectively ($p < 0.0001$). The RC proportion was significantly lower in Year 3 (34%) compared to both Year 1 (52%) and Year 2 (57%), respectively, as an average for location, harvest time, and lime treatment ($p < 0.0001$). Physiochemical soil properties such as soil pH and $Ca\text{-}_{AL}$ at the end of the field experiments are displayed in Table 7 and a significant increase for $Ca\text{-}_{AL}$ was shown. The largest increase occurred at the Åkerby site, where $Ca\text{-}_{AL}$ increased by 90%.

3.2.2 | Effects of Lime on Disease Incidence and Disease Severity of Root Rot in Red Clover

Disease symptoms were observed on the root surface and in the cross-section of most of the assessed roots during the year of establishment. DI_e and DSI_e are displayed in Table S5a,b. Results from the first assessment in the spring of Year 1 showed low infection, 23% infected plants, but increased during the growing season as 99% of the roots were infected in October. DSI_i on average for the locations ranged from 28 to 33 for the different treatments, and the average DSI_i for each location was 30. No significant differences between lime treatments and the untreated control or locations were found for the establishment year or for the consecutive harvest years (Table 8). Disease progress followed the same tendency as the MGL field experiment, with a higher infection of RR occurring in autumn compared to the following spring for all treatments ($p < 0.0147$) (Figure 3), although the difference decreased with time.

3.3 | Relationship Between Disease Severity in Autumn and Red Clover DM Yield the Following Year

The correlation between DSI_i assessed in autumn in Years 1 and 2, and the total RC yield the consecutive harvest year (Years 2 and 3) for all RC treatments on average, included in two MGL field experiments at Rådde and Kvinnersta and for two lime field experiments at Kvinnersta and Åkerby, is displayed in Figure 4. The yield of RC grown in MGL swards the following year is predicted by the equation $y = -66.224x + 7419.3$ where $x = DSI_i$ in autumn of Years 1 and 2 ($R^2 = 0.54$).

4 | Discussion

Legumes are important in sustainable agriculture, notably when they are intercropped with other species (Ergon and Bakken 2022) yet the persistence of RC is often lower than that of its companion species (Ergon et al. 2016; Brophy et al. 2017). In this study, agronomic properties with a specific emphasis on the persistence of the forage legumes RC, WC, BT, and LU, grown in mixed swards with TI and MF, were evaluated.

4.1 | Forage Yield

The RC treatments produced the largest total DM herbage yield of the investigated forage legumes across all three harvest years. The legume proportion was lower in the first cut than in the consecutive cuts, which is in accordance with previous studies e.g., Svanäng and Frankow-Lindberg (1994) and Bergqvist (2021). Red clover cv. Vivi was distinguished amongst the other RC treatments

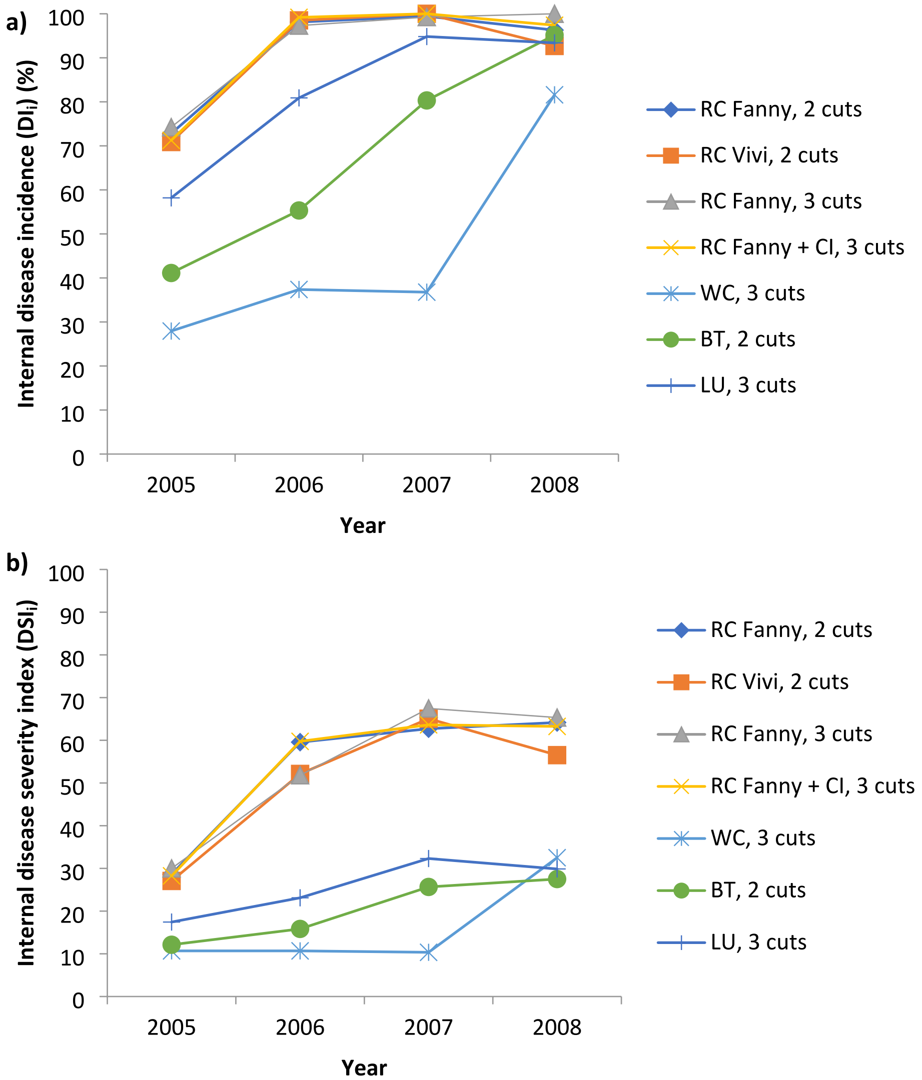

FIGURE 2 | Interaction plot for Field experiment 1 showing internal disease assessment of root rot in roots of red clover (RC), RC + chicory (CI), white clover (WC), birdsfoot trefoil (BT), and lucerne (LU). (a) Disease incidence (DI_i) expressed as percentage infected roots (b) Internal disease severity index (DSI_i) for root symptoms. Each point represents the average for two assessments (April and October) for each treatment, and an average for the experimental sites Rådde and Kvinnersta for the duration of the field experiment 2005–2007 ($n = 16$) and for Rådde in 2008 ($n = 8$).

in Year 3 by a larger total DM herbage yield and a higher proportion of legume than RC cv. Fanny (3) mixed with CI. This was likely caused by interspecific competition in this low input production system as CI is highly responsive to N-fertilisation (Belesky et al. 2000). There was no significant difference in total DM yield of RC and grasses in RC cv. Fanny in the 3-cut regime compared to the 2-cut regime. Our results diverge from previous meta-analyses conducted in Sweden by Gunnarsson et al. (2014) and in Norway by Steinshamn et al. (2016) where mixed grass-legume leys subjected to 2- or 3-cut systems produced higher yields in 2-cut systems and higher nutritive value in 3-cut systems. It is, however, important to consider both the date of the last cut and if the harvest season has been extended when harvesting three times instead of two. In our study, the season was extended, but not in the meta-analyses. Within commercial farming practise, selecting the correct harvest frequency (schedule) depends on the purpose of the harvested forage, and over the years it has become increasingly common to have three or more harvests, even in northern Sweden (Halling et al. 2021; Micke 2024).

4.2 | Root Rot

Of the legumes, red clover was the most infected by RR. A large proportion of RC plants were found infected on the root surface and in the root when assessed at the end of the growing season of the seeding year; DSI_i of RC ranged from 35 to 65, which is consistent with earlier studies and surveys in Sweden (Rufelt 1986; Wallenhammar et al. 2006) and Finland (Ylimäki 1967). Disease severity for RC was significantly higher ($p < 0.0001$) compared to BT, WC, and LU where DSI_i ranged from 6 to 33. This finding demonstrates a faster disease progression compared to previous studies where disease symptoms were assessed in approx. 10% of the surveyed RC plants (Rufelt 1986). After Year 2, all the investigated RC plants were symptomatic and exhibited extensive symptoms of root decay (Figure 1b,c). The average DSI_i for all experimental years (2005–2008) shows that disease severity significantly increased in RC treatments from spring to autumn, in line with the findings of Ylimäki (1967), whilst the increase in disease severity was smaller and not significant for

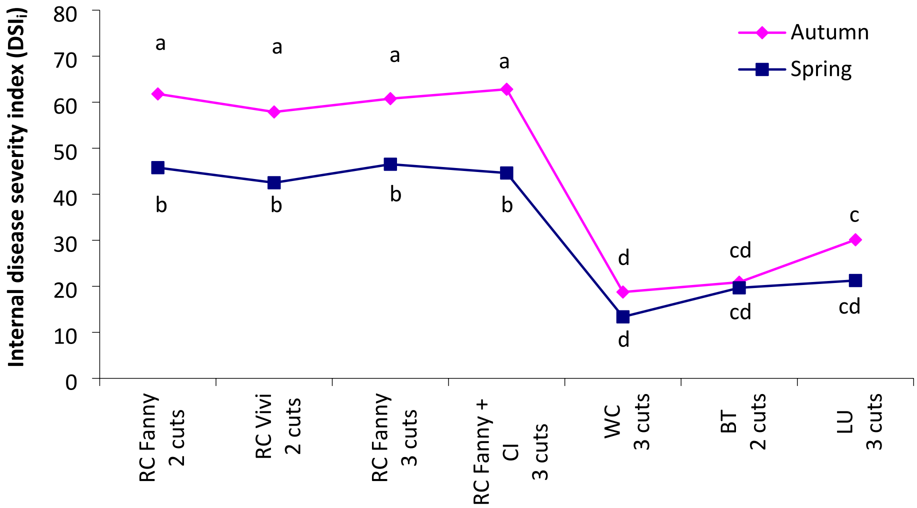

FIGURE 3 | Interaction plot for Field experiment 1 showing internal disease assessment of root rot on roots of red clover (RC), RC + chicory (CI), white clover (WC), birdsfoot trefoil (BT), and lucerne (LU) sampled in April and October of the same year expressed as an average for each treatment for the two experimental sites Rådde and Kvinnersta for the period 2005–2007 ($n = 8$) and for Rådde in 2008 ($n = 4$). Disease progress indicated by increasing internal disease severity index (DSI_i) for root symptoms during the growing season.

TABLE 5 | DM yield (kg ha^{-1}) in 1st and 2nd cut for three consecutive harvest years: 2005, 2006, and 2007 for Field experiment 2.

	2005		2006		2007	
Site	**1st cut**	**2nd cut**	**1st cut**	**2nd cut**	**1st cut**	**2nd cut**
Kvinnersta[1]	6322*c*[2]	3243*e*	5902*cd*	2346*f*	7625*b*	3190*e*
Åkerby	9791*a*	1258*h*	5445*cd*	1866*g*	5284*d*	3753*e*

Note: Red clover grown in mixed swards with timothy and meadow fescue, cut twice a year at the experimental sites Kvinnersta and Åkerby. Least square means across lime treatments 6, and 9 t ha^{-1} lime (52% CaO) and untreated control.
[1]Kvinnersta 1st cut 15 June, 14 June, 8 June and 2nd cut 12 August, 15 August, 13 August. Åkerby 1st cut 22 June, 14 June, 11 June and 2nd cut 16 August, 15 August, 13 August.
[2]Means followed by a common letter are not significantly different by the Tukey test at the 5% level of significance.

TABLE 6 | DM proportion of legumes (×10 g kg^{-1}) in 1st and 2nd cut for three consecutive harvest years: 2005, 2006, and 2007 for Field experiment 2.

	2005		2006		2007	
Site	**1st cut**	**2nd cut**	**1st cut**	**2nd cut**	**1st cut**	**2nd cut**
Kvinnersta	26*ef*[1]	77*a*	38*de*	74*ab*	15*f*	36*de*
Åkerby	57*bc*	50*cd*	38*de*	77*a*	35*de*	50*cd*

Note: Red clover grown in mixed swards with timothy and meadow fescue, cut twice a year at the experimental sites Kvinnersta and Åkerby. Least square means across lime treatments 6, and 9 t ha^{-1} lime (52% CaO) and untreated control.
[1]Means followed by a common letter are not significantly different by the Tukey test at the 5% level of significance.

TABLE 7 | Response of different lime treatments (Nordkalk Aktiv) on selected physiochemical soil properties in 2007 (3rd harvest year) for Field experiment 2 at the experimental sites Kvinnersta and Åkerby.

	pH		Ca_{AL}[1] mg 100 g^{-1} of soil	
Treatment	**Åkerby**	**Kvinnersta**	**Åkerby**	**Kvinnersta**
0 t ha^{-1} lime	6.0*b*[2]	6.4*b*	167*b*	239*b*
6 t ha^{-1} lime, (52% CaO)	6.5*a*	6.7*a*	291*a*	299*a*
9 t ha^{-1} lime, (52% CaO)	6.6*a*	6.8*a*	319*a*	316*a*

[1]Egnér et al. (1960).
[2]The letters refer to pairwise comparisons within soil properties. Means followed by a common letter are not significantly different by the Tukey test at the 5% level of significance.

TABLE 8 | Internal disease assessment of root rot in roots of red clover expressed as disease severity index (DSI_i) for Field experiment 2.

	2005		2006		2007	
Site	**April**	**October**	**April**	**October**	**April**	**October**
Kvinnersta	18*d*[1]	50*c*	44*c*	74*ab*	65*ab*	68*ab*
Åkerby	24*d*	45*c*	47*c*	66*ab*	63*b*	75*a*

Note: Roots were assessed for disease symptoms in April and October the 1st, 2nd, and 3rd harvest years (2005–2007) at the experimental sites Kvinnersta and Åkerby. Least square means across lime treatments 6, and 9 t ha^{-1} (52% CaO) and untreated control.
[1]Means followed by a common letter are not significantly different by the Tukey test at the 5% level of significance.

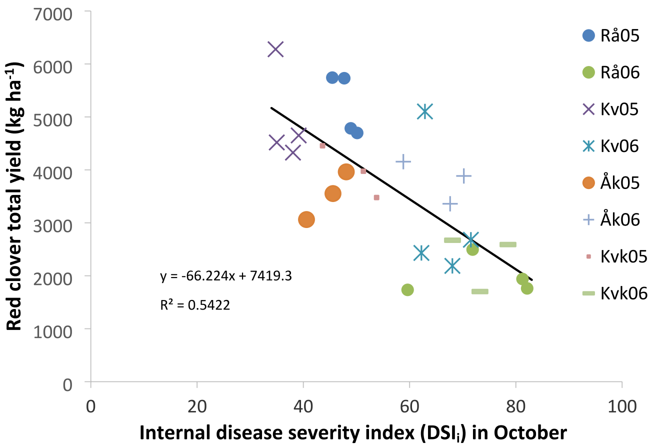

FIGURE 4 | Correlation between internal disease severity index (DSI_i) of root rot assessed in red clover (RC) roots in October of the first harvest year (2005) and the second harvest year (2006) and corresponding total yield of RC in all the RC treatments the following years (second and third harvest years) for two mixed grass-legume ley experiments at Rådde (Rå) and Kvinnersta (Kv) (Field experiment 1) and for two lime mixed grass-legume ley experiments at Kvinnersta (Kvk) and Åkerby (Åk) (Field experiment 2).

WC, BT, and LU (Figure 3). Ylimäki characterised the injuries in RC roots to develop throughout the entire growing season and claimed that RR is not primarily a winter disease, since most of the damage occurs during the growing season. Thus, the plants become weaker and disappear after harsh climatic conditions during the following winter. Frost injuries in spring are particularly detrimental for the further survival of RC plants (Almquist et al. 2016) and in extremely dry conditions, as was the case in 2018, RC plants weakened by disease were unable to produce biomass for a third cut the third harvest year (Wallenhammar et al. 2020). The outcome of external injuries assessed on the root surface is likely to weaken the plant in later stages of development (Rufelt 1986) whereas it is uncertain if the plant is hampered in early stages of infection.

This study demonstrates that RR has a significant ($p < 0.001$) negative influence on the proportion of RC (Table 4) in mixed swards. The results obtained from the assessment of RR in LU are in line with the findings reported by Rufelt (1986) and by Wallenhammar et al. (2020) as LU was more tolerant to RR than RC. Birdsfoot trefoil is, to the best of our knowledge, not assessed for RR at an earlier stage in Sweden, and our assessments reveal a tolerance like that of LU. Studies from other parts of the world, however, have reported injuries from RR and crown rot (Altier and Kinkel 2005). WC was only sparsely surveyed by Rufelt (1986); whereas in this study, WC was the legume (Figure 3) least affected by RR severity during the prevailing climate (Figure S1a) without drought conditions. White clover maintained persistence, as the legume proportion was stable throughout the three harvest years. Aside from infection of RR in the vascular tissues, the persistence of a legume crop is dependent on the site-specific conditions such as soil pH, adjustment of nutrients, and crop rotation, which is indicated by the extent of external infection and overall climatic conditions. At the experimental sites, the management differs from on-farm management, where soil compaction caused by several passings further challenges the persistence of a ley.

The results partially support hypothesis i that a significant infection of RR affected the botanical composition; however, the total herbage DM yield and forage quality were not significantly changed due to compensatory grass growth. The results are not in line with hypothesis iii as no significant difference in susceptibility between the two RC cultivars was found, nor any difference with the inclusion of CI. However, disease severity for LU, BT, and WC was significantly lower than for RC treatments. Lucerne exhibited significantly more disease than WC in autumn (Figure 3) throughout all of the years.

4.3 | Legume Proportion

The proportion of legumes was significantly higher in RC treatments on average in the first two harvest years compared to WC, BT, and LU despite a high infection of RR. In certain developmental stages, secondary roots were observed which can compensate for a damaged taproot (SaWai et al. 1986). Thereafter, RCP significantly decreased in Year 3 from on average 46%–56% to 21%–35%, with the highest RCP observed for cv. Vivi. Simultaneously, the difference in RCP compared to WCP was levelled out, ranging from 21% to 28% on average for all harvest years. Studies have reported an increased RC proportion and a reduced score for RR by applying the mycoparasite *Pythium oligandrum* (Pisarčik et al. 2020), whilst Zn application was found to substantially increase RC yield (Stoltz and Wallenhammar 2018). The BTP was considerably lower than RCP and was also lower than WCP in both the second and third harvest year. It is likely that BT was outcompeted by the grass species during the prevailing climatic conditions where no periods of drought occurred (Figure S1b). A poor establishment due to incomplete inoculation with appropriate symbiont cannot be ruled out as BT was not grown earlier at the experimental sites. Birdsfoot trefoil is considered to be an appropriate legume for infertile, dry, heavy, or acidic soils and for low input, and it outperforms LU and WC in extensive grassland management systems (Carter et al. 1997). In a previous study performed in dry areas of Sweden, the BT proportion was 50% in Year 3 in a 2-cut regime as a mean across the companion grasses TI, MF, and perennial ryegrass (*Lolium perenne* L.) (Nilsdotter-Linde and Bergkvist 2005). The low BTP and LUP in this study was likely due to a poor establishment as well as issues with the inoculant bacteria (Jauregui et al. 2019; Tang et al. 2025) and was not caused by disease (Table 4). Recent studies on identifying management practises for improving establishment of LU have demonstrated the importance of proper inoculants in field soils where LU was not grown earlier, whereas no effects of the treatments were shown at locations where LU had been previously grown (Tang et al. 2025). Besides, the effect of sowing rate on root morphology is an important trait to consider, providing long-living swards (Hakl et al. 2021).

4.4 | Nutritive Value

The economic output in terms of feed costs for milk production depends on the intake of energy and protein. A highly digestible forage with a high protein content favours the intake and is associated with a high economic value. Thus, management based on three harvests over two harvests provides higher forage quality but increases the harvest cost and often decreases the total DM yield. In this study, the CP concentration decreases as the RC content of the sward is reduced in accordance with hypothesis ii. The RC treatments with three harvests typically resulted in the highest content of CP and ME, and the lowest NDF content. Since no significant differences were found for RC yield, RC proportion, or RR infection between two and three harvests in MLG swards, the quality aspect provides a good reason to harvest RC leys three times a year. Newly bred cultivars are better adapted to more intensive harvest systems than older cultivars (Riday and Krohn 2010; Halling et al. 2021). In addition, the correct time for the first harvest according to the developmental stage is essential. In our study, we found lower CP values and higher NDF values in the first cut in Year 1, and also lower CP in Year 3 in the more mature swards at Rådde than at Kvinnersta.

In addition to high protein and energy values, there are certain special qualities contributed by legumes. Red clover contains high levels of the enzyme polyphenoloxidase (PPO) and therefore improves nitrogen utilisation in ruminants (Lee et al. 2006). Further recorded positive effects include protecting lipids from degradation, which leads to a higher output of polyunsaturated fatty acids (PUFA) in ruminant products (Van Ranst et al. 2011). Certain forage legumes, such as BT, contain other secondary metabolites such as condensed tannins (CT) (Mueller-Harvey 2006), and the mode of proteolysis inhibition by CT is the formation of tannin–protein complexes in the rumen that increase milk (Eriksson et al. 2012) and beef (Nilsdotter-Linde et al. 2004) production performance. A breeding programme for improved BT agronomic performance correlated to its tannin chemistry is needed (Marley et al. 2006).

4.5 | Cropping System Perspective

The RR causing fungi are prevalent in the soil and the majority of them can survive for long periods on different host plants. Wheat is a host for *F. avenaceum* (Kollmorgen 1974; Lager and Gerhardson 2002) and can cross infect between pasture legumes and wheat (Sivasithamparam 1993). Moreover, some fungal species can survive saprophytically without host plants. If host-mediated selection occurs in the pathogen isolates, the root diseases can be limited to a certain extent. Birdsfoot trefoil and LU were less infected than RC, and these legume crops have not been grown intensively on the experimental sites nor in practical farming. From a Nordic perspective, there is a need for diversity in crop rotations, crops, ley mixtures, species, and cultivars (Parsons 2024). Crop rotation is part of the solution to control RR and clover rot, along with continued investment in plant breeding to develop more resistant cultivars. Thus, site-specific conditions, e.g., crop rotation, soil type, nutrient status, and drainage, have a significant impact on RC persistence, as demonstrated in Field experiment 1 where the yield was 1.3 $t\,ha^{-1}$ larger each harvest year at Kvinnersta compared to at Rådde, with the same ranking occurring in Field experiment 2. There was a difference in crop rotations and soil properties (Table 1) between the experimental sites, wherein MGL leys including RC were grown in shorter rotations at Rådde than at Kvinnersta.

In northern Sweden, the soil-borne pathogen *Sclerotinia trifoliorum* (Erikss.) causes clover rot (Öhberg et al. 2008) which results in the death of young plants usually during the first winter. Although clover rot was not investigated in this study, the disease has recently been observed in fields in southern Sweden. Moreover, clover rot is the primary clover disease in Lithuania (Mikaliūnienė et al. 2015) and is considered a key fungal pathogen threatening European RC production. Progress in genome-assisted breeding will facilitate the development of novel cultivars with increased resistance (Frey et al. 2022). When growing RC in a field without any previous clover-based swards, persistence is longer, as a UK study reported RC persisting for six harvest years (Clavin et al. 2016), although a decline in RC proportion was also reported. However, in Swedish fields where RC is grown in short rotations, RR has a severe impact on persistence (Wallenhammar et al. 2006).

4.6 | Effect of Liming

An addition of lime did not suppress the infection of RR, as no significant differences in DSI_e (Table S5) or DSI_i between treatments were observed for either the establishing year or the consecutive harvest years (Table 8). This contrasts with the experience reported in the USA (Close 1994) and shows that hypothesis iv could not be confirmed. Moreover, we could not demonstrate any differences in yield in the limed treatments. However, both lime applications showed significant ($p < 0.05$) effects on soil chemical parameters such as an increased pH value and an increased calcium level (Table 7). The base saturation at Kvinnersta was 72% at the start of the experiment and at the Åkerby experimental site base saturation was 40%. Red clover showed a yield response to lime application in a study where the initial soil pH ranged from 4.1 to 4.3 (Caddel et al. 2004). In our study, the initial soil pH was close to the optimum pH range for RC, which has generally been reported to be between 6.6 and 7.5 (Taylor and Smith 1995) and in this range no positive effects were observed for yield or infection of RR.

4.7 | Increasing Diversity Paves the Way for Sustainable Mixed Grass-Legume Leys

Here we report on new findings that the weak persistence of RC at a particular field site expressed as a decline in RCP, as reported in earlier studies, is correlated with an increasing prevalence of RR. In addition, the evaluation of disease progress in RC with disease progress in BT, LU, and WC constitutes a basis for increasing diversity in MGL leys at the farm scale with a durable persistence of legume crops for different demands for fodder quality and harvested yield. Further, there are RC cultivars available in Europe of Swiss breed that are selected for improved persistence, as shown by Hoekstra et al. (2018) and Hejduk and Knot (2010). The agronomic properties of these 'mattenkle' cultivars are currently under investigation in Swedish climatic conditions and preliminary results suggest that they are an option in south and central Sweden (Edin et al. 2024). Red clover cultivars with resistance to the complex of soil-borne pathogens are a high-priority breeding objective. As a result of this study, RC cultivars are evaluated for three harvest years instead of two for an appropriate evaluation of sustainability in the Value for Cultivation and Use (VCU) test (Halling et al. 2021). It is essential that new forage legume cultivars are developed and field tested in appropriate environments (Picasso 2024). The correlation displayed in Figure 4 between the internal disease severity index (DSI_i) of RR assessed in RC roots during the autumn of Years 1 and 2 and the corresponding yield of RC predicts the expected RCP in this study, providing data for estimating RCP the following year.

The results obtained in this study also indicate that the frequency of infected plants in the seeding year has increased compared to the study performed 30 years earlier with the same method but with other cultivars (Rufelt 1986). Growers must also know the seedlings are subject to plant death. A new finding in this study is the lesser amount of disease for BT, LU, and WC compared to RC in mixed swards. These legume species can serve as a viable option to improve the sustainability of grass-legume leys.

Regarding economy, environment, and ethics, the importation of protein feed must be replaced by local farm production. Following the poor persistence of RC, feed rations must be planned to ensure that the most demanding milking cows receive the required quality. In this study, WC has demonstrated outstanding traits that enable it to persist for 3 years with an even proportion of legumes. Recent findings on the microbial diversity in RC fields sampled throughout Sweden (Jambagi et al. 2023) show that the microbial community is predominantly shaped by both the geographical location and management procedures. These new insights can pave the way for understanding the challenges associated with improving the persistence of RC.

5 | Conclusions

The proportion of red clover (RC) significantly declined in the third harvest year and was associated with an increasing proportion of diseased roots. RC yield was not affected by disease severity in harvest Year 2; the shift occurred in harvest Year 3 when a compensatory effect from the companion grasses is accounted for. The cutting regime did not affect disease severity or annual RC DM yield. Regarding the economic performance, attributed to dry matter yield, digestibility, and protein content, white clover (WC) and RC in the 3-cut regime were the best options. The disease incidence and disease severity of WC, birdsfoot trefoil (BT), and lucerne (LU) were less compared to the RC cultivars, and no difference was found between the RC cultivars Fanny and Vivi. In this study, no pronounced difference in persistence was, however, found between RC and BT. There is a need for diversity in crop rotations to increase resilience and improve yield quality and stability. As more extreme weather, with longer periods of drought and flooding, is predicted, growers are encouraged to evaluate legume species on farms for different soil types to adapt to abiotic stress. The results from this study will support their decisions. Breeders are urged to include the drought tolerant BT, which possesses specific traits for growth in dry conditions, in the breeding schemes and to evaluate a wide range of cultivars available worldwide.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Figure S1a:** Daily mean air temperature (°C) at Rångedala (57°37′ N, 13°15′ E) and Örebro (59°36′ N, 15°25′ E) weather stations from 1 May 2004 to 30 September 2007. **Figure S1b:** Daily precipitation (mm) at Rångedala (57°37′ N, 13°15′ E) and Örebro (59°36′ N, 15°25′ E) weather stations from 1 May 2004 to 30 September 2007. **Figure S2:** Interaction plot for Field experiment 1 showing external disease assessment of root rot in roots of red clover (RC), RC + chicory (CI), white clover (WC), birdsfoot trefoil (BT), and lucerne (LU). (a) Disease incidence (DI_e) expressed as percentage infected roots. (b) External disease severity index (DSI_e) for root symptoms. Each point represents the average for two assessments (April and October) for each treatment, and an average for the experimental sites Rådde and Kvinnersta for the duration of the field experiment 2005–2007 ($n = 16$) and for Rådde in 2008 ($n = 8$). **Figure S3:** Interaction plot for Field experiment 1 showing external disease assessment of root rot on roots of red clover (RC), RC + chicory (CI), white clover (WC), birdsfoot trefoil (BT), and lucerne (LU) sampled in April and October of the same year expressed as an average for each treatment for two experimental sites Rådde and Kvinnersta for the period 2005–2007 ($n = 8$) and for Rådde in 2008 ($n = 4$). Disease progress is indicated by increasing external disease severity index (DSI_e) for root symptoms during the growing season. **Data S1:** Supplementary tables.