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Scoping Opportunities for Nitrogen Use Efficiency Among Productive Agricultural Forage Grasses With Diverse Rooting Systems

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ABSTRACT

For forage production to be efficient and environmentally sustainable, the extent and timing of nitrogen fertiliser applications should match the uptake and growth capabilities of a grass crop. A two-year field experiment, comprising four diverse grass cultivars, was conducted to assess the impact of two contrasting N-application rates on forage and root biomass (RB) production and nitrogen-use-efficiencies (NUEs). Replicated field plots of perennial ryegrass, tall fescue and two *Festulolium* (ryegrass × fescue hybrid) cultivars were compared at Low N (LN) 178 and High N (HN) 356 kg ha⁻¹ over 2 years. HN applications increased dry matter yield (DMY) in Year 1 ($p < 0.05$) but not in Year 2. Ryegrass outyielded all in Year 1 but in Year 2, fescue had the highest DMY at HN ($p < 0.05$), but cultivars did not differ in yield at LN. *Festulolium* yields were consistently intermediate. Root biomass at LN in Yr1 was highest in the *Festulolium* (*Lolium perenne* × *Festuca arundinacea* var *glaucescens*) ($p < 0.05$). For all grasses, and in both years, mean RB and nitrogen use efficiency (NUE) and nitrogen utilisation efficiency (NutE) were higher under LN, than HN. Nitrogen uptake efficiency (NupE) was similar in all grasses in Year 1, irrespective of N treatment, but in Year 2, excepting tall fescue, was greater in grasses grown under LN. Increasing RB correlated ($p < 0.05$) with improved NUE and NutE, but no association was evident for NupE. Grass cultivars differed in their response to nitrogen applications. Whilst relative forage production of ryegrass and fescue contrasted over the 2 years, forage yields of *Festulolium* cultivars were more consistent. In conclusion, HN application depressed NUE by productive grass cultivars and correlations between RB and NUEs may indicate opportunities to help tailor grass cultivar/fertiliser combinations and achieve sustainable forage and root production.

1 | Introduction

Improving nitrogen use efficiency (NUE) in grasslands is fundamental to the delivery of sustainable agri-food systems. For agriculture to meet targets for zero carbon and environmental sustainability, coupled with food provisions sufficient to sustain an increasing human population, it will require

‘precision-agriculture’ practices tailored to achieve optimal efficiencies. Supplementary N applications have been deemed essential management tools in grassland farming to enhance forage yield, quality and composition, and dependent on these, the subsequent voluntary dry matter (DM) intake by livestock (Ghosh, Mahanta, and Ram 2017), with previous research on forages with higher protein levels shown to increased DM intake and

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livestock productivity (Marley et al. 2007; Thomas et al. 2023). However, the efficiency of plant N uptake is generally poor, and it is estimated that only 6%–50% of applied N fertiliser is taken up by plants, with the surplus an environment hazard, including polluted watercourses, in addition to being a monetary waste (Carranca 2012; Barker and Culman 2020).

Nitrogen mineral fertiliser is the nutrient used most frequently to help encourage growth in agricultural crops (Delaby et al. 2020). Inorganic N production, under high temperatures and pressures (Haber-Bosch process), creates high atmospheric CO₂ emissions, which has serious implications on climate and the environment. Depending on their type and production location, inorganic fertilisers produce a CO₂ equivalence of between 0.6 and 3.5 t CO₂ per tonne N fertiliser product at the plant gate (Brentrup, Hoxha, and Christensen 2016; Rosa and Gabrielli 2022). Leaching and/or runoff of nitrogen fertilisers pollutes groundwater with N losses through surface runoff leading to eutrophication of water bodies, reducing their recreational value and threatening the life of aquatic animals and plants (Cameron, Di, and Moir 2013; Schils et al. 2022). Application of N fertiliser to grasslands has other far-reaching social implications, such as health-related issues associated with human consumption of nitrate-polluted water (Riaz et al. 2024). These health concerns have led to the creation of nitrate vulnerable zones (NVZ) to alleviate the risk of high nitrate levels in water in the UK (Defra 2024) and in other countries (Serra et al. 2024).

Plant root system architecture, and ontogeny, and their responses and interactions to soil water and nutrient availability play a vital role in determining crop persistence and production (Correa et al., 2019). In the face of likely impacts of a changing climate on soils (Karmakar et al. 2016), deep-rooting and stress-tolerant *Festulolium* forage grass cultivars are needed to provide reliable and economic forage for livestock (Marshall et al. 2016; Humphreys and Zwierzykowski 2020). New *Festulolium* cultivars can provide agronomic performance equivalent to current UK National Listed ryegrass varieties (BGS 2024/25), and stress tolerance is more characteristic of their fescue parent species (Humphreys and Zwierzykowski 2020). Significant evidence has also accrued to demonstrate the resilience of *Festulolium* cultivars to the impacts of climate change and related environmental stresses, such as droughts, floods or freezing temperatures (e.g., Babić et al. (2022); Humphreys et al. (2006); MacLeod et al. (2013)). Using a phenomics-based platform, Humphreys et al. (2018) studied the detailed root ontogeny over a consecutive four-month period of two *Festulolium* and a perennial ryegrass cultivar that was used in the current study. They demonstrated that the *Festulolium* cultivars had a higher root biomass and deeper root systems than their ryegrass counterparts. Studies by Muhandiram et al. (2020) showed under compacted soil conditions in the field that one of these *Festulolium* cultivars had greater root production compared to perennial ryegrass. The drought resistance demonstrated by many *Festulolium* cultivars has been shown to be, at least in part, a consequence of their large deep root systems (Durand et al. 2007; Humphreys et al. 2018). Many ecosystem benefits of utilising different forages in grassland systems, including *Festulolium*, derive from their extensive root biomass (RB) and their soil interactions at extents sufficient to help deliver ecosystem

services and mitigate excessive rainfall run-off and compaction (Humphreys et al. 2014; Crotty et al. 2015; Muhandiram et al. 2020, 2023; Kamau et al. 2020; Marley et al. 2024).

Root distribution-related characteristics such as root biomass, depth in the soil (distance from the stem), architecture (special distribution) and morphological characteristics (root hairs, diameter, secondary roots patterns, root cap and cortical senescence) are crucial root characteristics in plant nutrient uptake (Lynch 1995). Research has shown that deep roots facilitate efficient N uptake from deeper soil layers (Wiesler and Horst 1994; Chen et al. 2013; Lynch 1995; Li et al., 2015). Yet, to date, very few studies have compared the efficiency of *Festulolium* and ryegrass in their N uptake and subsequent utilisation (e.g., Harper et al., 2018). The current research describes N efficiency in two amphiploid *Festulolium* cultivars, both reported with large root biomass and forage production (Humphreys et al. 2014, 2018). Their N efficiency was compared to a UK National-Listed tetraploid perennial ryegrass cultivar with the same ploidy as the *Festulolium* cultivars and a tall fescue cultivar.

In this paper, we present findings on the nitrogen use efficiencies, as defined in Congreves et al. (2021) where:

1. Nitrogen Use Efficiency (NUE) is the expression of DM yield per unit of fertiliser N applied (= kg DM yield/kg N applied);
2. Nitrogen uptake Efficiency (NupE) is the effectiveness of plant root in taking up soil N (= kg N yield/kg N applied); and,
3. Nitrogen utilisation Efficiency (NutE) is used to identify plants that produce a high yield relative to plant tissue N (= kg dry matter yield/kg N yield).

Here, we tested the following hypotheses, whilst anticipating that increasing the rate of N fertiliser application will enhance grass forage production:

1. Increasing the rate of N fertiliser application will be detrimental to NUE expression in grass cultivars; and
2. Increasing grass root biomass will enhance nitrogen use efficiencies.

2 | Material and Methods

2.1 | Experimental Site

The field experiment was conducted during 2016–2018 at the Institute of Biological, Environmental and Rural Sciences (IBERS), Wales, Aberystwyth (52° 25' 59" N, 4° 0' 45" W, 40 m of altitude). The experimental site was on an area of stony, well-drained loam of the Rheidol soil series. Meteorological data for the 2 years that incorporated the field experiment is summarised in Table S1. The summer rainfall contrasted over the two-year period. Of particular note, the mean rainfall over months June–August in Year 2 (2018) was 52 mm month⁻¹, just 38% of that recorded over the same period in Year 1 (2017) (136 mm month⁻¹).

2.2 | Treatments and Design

Three randomised field blocks within a split-plot experimental design were established in September 2016. Main plots were treated with two N rates, either 178 or 356 kg N ha⁻¹ year⁻¹ hereafter referred to as, low N (LN) and high N (HN), respectively. Four grass cultivars were randomised at the sub-plot level. Forage treatments consisted of two amphiploid *Festulolium* cultivars each derived from a cross between *L. perenne* L. (4×) and two drought-tolerant tetraploid *Festuca* species; *Festuca mairei* (Hack.) (Atlas fescue) (4×) and *Festuca arundinacea* var. *glaucescens* (Roth.) (4×). *Festulolium* cultivar *L. perenne* L. × *F. mairei* (Hack) is referred to herein as Lp×Fm. The *Festulolium* cultivar, Lp×Fm was described previously as LpFm in Kamau et al. (2020) and subsequent to its inclusion on the Recommended Grass and Clover List for England and Wales (Agriculture and Horticulture Development Board (AHDB) 2023), as *Festulolium* cv AberRoot (also described as AberRoot in Humphreys and Zwierzykowski 2020). The *Festulolium* cultivar *L. perenne* L. × *F. arundinacea* var *glaucescens* (Roth.) is referred to herein as Lp×Fg. The field performance and ecosystem provisions of Lp×Fg have been described elsewhere (Humphreys et al. 2014; Muhandiram et al. 2020, 2023), and referred to as LpFg (Kamau et al. 2020).

Field performance of the *Festulolium* hybrid cultivars was compared with cultivars considered suitable as representative of their parental species. Neither *F. mairei* nor *F. arundinacea* var *glaucescens* has sufficient agronomic value to warrant their cultivar development, and as consequence, no suitable seed was available. As an alternative *Festuca* spp. control, an old UK cultivar Dovey from a genetically related hexaploid agricultural grass Tall fescue (*F. arundinacea* Schreb) was employed. As a *L. perenne* control, the 2023/24 England and Wales Recommended Grass and Clover Variety ryegrass cv. AberBite (4×) was employed. Of relevance to the current study, the detailed root ontogeny of the ryegrass used in this study was compared previously to that of Lp×Fg and Lp×Fm over an equivalent four-month time period in Humphreys et al. (2018), with the *Festulolium* cultivars there referred to as Bx514 (for Lp×Fg) and Bx511 (for Lp×Fm), respectively.

2.3 | Plot Management

Grass sub-plots were hand sown using standard NIAB-based sowing rates of 3.3 gm⁻² for the two *Festulolium* cultivars and for AberBite. For tall fescue cv Dovey, the seed had been maintained > 5 years in the IBERS seed store and a sowing rate of 6.6 gm⁻² was used to compensate for its inferior germination rate (as advised by IBERS Seed Store Manager, Glanmor Thomas, pers. comm.). All grass plots were allowed to establish fully and achieve consistent full ground cover. Each grass plot was 6 × 2 m and was divided into two sub-plots, one 4 × 2 m used for forage measurements, and an adjoining 2 × 2 m sub-plot used for destructive soil sampling to determine root biomass.

Experimental plots were managed in accordance with NIAB's UK National List Trial recommendations and were consistent with IBERS's standard field management procedures. Prior to both growing seasons, plots were cut to a height of approximately 10 cm on 28 March 2017 and 19 March 2018, respectively,

to remove dead plant material and to achieve a consistent initial sward height throughout the field experiment. All plots were then harvested on five occasions year⁻¹ at six-weekly intervals, both in their first (2017; Year 1) and second (2018; Year 2) harvest years, to simulate a conservation management and silage-based grass harvest system.

Five fertiliser rounds, each supplying N, P and K, were applied annually. The first application was within 3 days of the winter cut (i.e., 31 March 2017, 20 March 2018). Thereafter, fertiliser was applied at six-weekly intervals and within 72 h following each grass harvest. N was applied as ammonium nitrate (NH₄NO₃; 34.5% N), P as triple superphosphate (TSP; 46% P₂O₅) and K as muriate of potash (60% K₂O). Nitrogen was applied at either 356 kg N ha⁻¹ year⁻¹ applied as 100, 85, 85, 43 and 43 kg ha⁻¹ for HN plots or 178 kg N ha⁻¹ year⁻¹ as 50, 42.5, 42.5, 21.5 and 21.5 kg ha⁻¹ for LN plots. The quantities of triple superphosphate (TSP) and muriate of potash were based on initial soil analysis and RB209 guidelines (AHDB 2020) for recommended fertiliser application rates for grassland managed for silage, with an allowance for an additional fifth cut. TSP was applied to provide 50 kg P₂O₅ ha⁻¹ year⁻¹ and muriate of potash to provide 450 kg K₂O ha⁻¹ year⁻¹ and applied equally to all plots as 5 aliquots of 10 kg P₂O₅ and 90 kg K₂O ha⁻¹, respectively. All fertilisers were combined and mixed prior to application. Wooden framed quadrats (6 × 2 m), divided into 1 m² sections using nylon threads, were placed on each grass sub-plot prior to manual fertiliser application to ensure even distribution within the plots. Natural N-adding pathways to soil such as mineralisation or aerial depositions and any soil residual N were not quantified in this study but were assumed to be similar for all treatments. Following the post-winter cut, herbicide (Harvest: Bayer Crop Science Ltd., Cambridge, UK) was sprayed to suppress unsown plant species within the plots. Neither insect nor disease damage was observed, and no supplementary irrigation was provided during the two-year experiment.

2.4 | Measurements

Forage dry matter yield (DMY), total N (TN) concentration and N yield were determined at each harvest. In the first harvest year plots were harvested on 8 May, 19 June, 31 July, 11 September and 25 October 2017. In the second harvest year, on 30 April, 11 June, 23 July, 4 September and 16 October 2018. Measurements for root biomass obtained from soil-core samples were taken at three time points in both harvest years. These were on the days immediately subsequent to grass cut 1 (spring), 3 (summer) and 5 (autumn). The soil samples were collected at approximately 12-week intervals to compare root biomass within the upper soil layers throughout the forage harvest times (i.e., changes to root development, persistency and turn-over).

2.4.1 | Dry Matter and Nitrogen Yield

Field plots were cut at the height of 5 cm using a Haldrup 1500 plot harvester (J. Haldrup a/s, Løgstør, Denmark). Grass fresh weights were taken promptly following cutting. Immediately after harvesting, sub-samples were collected and weighed prior to storage at -80°C and then freeze drying and milling before TN analysis. TN concentration was determined using a Leco FP

428N analyser (Leco Corporation, St Joseph, MI, USA). A further sub-sample was used to determine dry matter (DM) content by oven drying at 100°C for 24 h to establish DMY.

2.4.2 | Root Biomass

Soil cores (8 cm diameter and 15 cm depth) were extracted using a root auger (Eijkelkamp, Giesbeek, Netherlands). Soil cores were taken following cuts 1, 3 and 5 in both Year 1 and Year 2. Soil samples were collected and placed in zip-lock bags immediately after extraction and stored at 4°C prior to root biomass determination, as described in Muhandiram et al. (2020). The difference in the current study was that all grass foliage present in the uppermost 3 cm of the soil core was removed according to the method described by Cougnon et al. (2014) prior to drying. Following extraction, roots were dried at 80°C for 48 h to determine root dry weights.

2.5 | Statistical Analysis

Effects of nitrogen application rate and grass cultivar and their interaction were examined using analysis of variance according to the split-plot design. When effects of grass cultivar were statistically significant ($p < 0.05$), the Student Newman-Keuls method was used to compare means. Where significant interactions were present in tables of means, the Bonferroni adjustment was used to reduce the comparison-wise type I error rate, and comparisons were made within grass and within NR only. Pearson's product moment correlation coefficients were used to examine the extent of linear association between N efficiency estimators and root biomass. Experimental data were analysed using GenStat (Release 22, Baird et al. 2017).

3 | Results and Discussion

3.1 | Meteorological Data

Perennial productive grasslands suitable for sustainable agricultural systems in maritime temperate climates, and where external stresses may fluctuate wildly, such as those in the UK, require a significant plasticity in their adaptive response. Climate change

scenarios that enhance temperature and rainfall extremes will only add to the need for increased resilience in grassland systems.

In a two-year field experiment, rainfall during the growing season, March–October, at a total of 659 mm was notably lower in Year 2 than over the same months in Year 1 (882 mm) (Figure 1). Rainfall during the months June to August 2018, which included harvest cuts two and three, was only 38% of rainfall recorded in 2017 (Table 1). Mean air and soil temperatures (March–October) over the two harvest years were similar. The mean daily maximum air temperature was 16.4°C in both years, and the mean monthly 10 cm soil temperature at 12.8°C and 12.4°C in Years 1 and 2, respectively (Table S1).

3.2 | Forage Dry Matter Yield

It is common agricultural practice to incorporate N fertiliser applications to fields to enhance growth and forage yield. In accordance with previous studies and standard agricultural practice, the current study did find higher yields over Year 1 in all four grasses when treated with higher N fertiliser (Aavola and Kärner, 2008; Kowalenko and Bittman, 2000; Delevatti et al., 2019). In the current work, cultivars of four forage grasses, comprising two *Festulolium* hybrids and representative *Lolium* and *Festuca* parent species' controls, were selected to compare responses to high and low nitrogen fertiliser applications. From previous investigations, the grasses are known to vary in phenotype, both above and below ground, and in their agronomic performance and abiotic stress resistance (Humphreys et al. 2018, 2014; Babić et al. 2022).

Differences in forage yield between the grasses were confirmed in the field experiment. Total annual dry matter yield (DMY) differed among the four grasses in both years (Tables 1 and 2) ($p < 0.001$ (Year 1); $p = 0.018$ (Year 2)). DMY of all four grasses and at all five harvests in Year 2 was inferior to that of Year 1. One major contributory effect was considered to be the lower rainfall recorded in Year 2 (Figure 1). The higher N application rate also produced a distinct increase in annual DMY in Year 1 ($p < 0.001$), but this was no longer evident in Year 2 ($p = 0.232$). All yields were lower in Year 2 compared to Year 1 but particularly so with HN.

In Year 1, the ryegrass had a higher DMY compared to the other grasses, whilst both *Festulolium* cultivars had higher DMY than

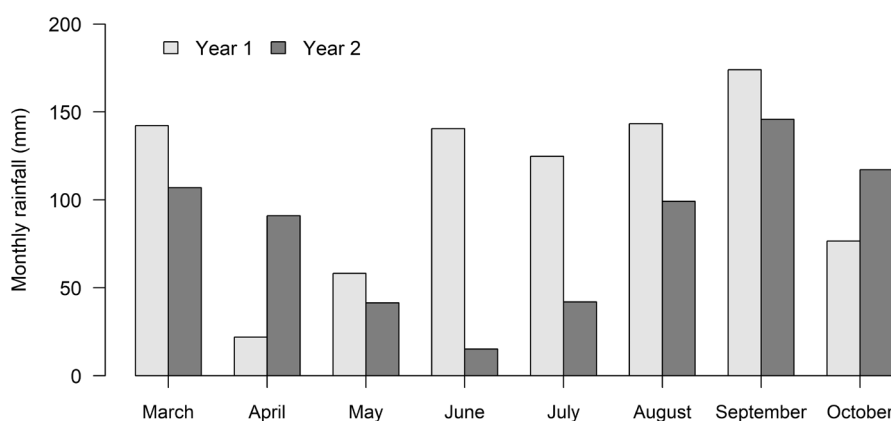


FIGURE 1 | Monthly rainfall (mm) data for the 2 years that incorporated the field experiment comparing four grasses in their first (2017) and second (2018) harvest years.

TABLE 1 | Forage dry matter yield (t ha⁻¹) for four grasses during their first harvest year (2017) under two rates of inorganic nitrogen application either 178 kg (LN) or 356 kg (HN) N ha⁻¹ growing year⁻¹.

Nitrogen rate (NR)	Grass (G)				Mean		s.e.m.	Within G s.e.m	Prob
	Ryegrass	Fescue	Lp×Fm	Lp×Fg					
Cut 1									
LN	1.28	0.94	1.55	1.43	1.30	NR	0.009		<0.001
HN	2.07	1.61	2.50	2.15	2.08	G	0.057		<0.001
Mean	1.68 ^b	1.28 ^a	2.02 ^c	1.79 ^b		NR×G	0.071 (12.4)	0.081	0.380
Cut 2									
LN	4.27	2.30	3.60	4.22	3.60	NR	0.032		<0.001
HN	6.15	3.57	5.18	5.99	5.22	G	0.093		<0.001
Mean	5.21 ^c	2.94 ^a	4.39 ^b	5.10 ^c		NR×G	0.118 (13.5)	0.131	0.165
Cut 3									
LN	2.24	^A 2.27	^A 2.10	^A 2.05	2.17	NR	0.079		0.011
HN	3.04 ^a	^B 3.45 ^b	^B 3.28 ^{ab}	^B 3.08 ^{ab}	3.21	G	0.046		0.005
Mean	2.64	2.86	2.69	2.57		NR×G	0.097 (4.4)	0.065	0.037
Cut 4									
LN	1.10	1.12	0.76	0.75	0.93	NR	0.045		0.003
HN	2.24	2.33	1.99	1.85	2.10	G	0.047		<0.001
Mean	1.67 ^b	1.72 ^b	1.38 ^a	1.30 ^a		NR×G	0.073 (9.50)	0.066	0.768
Cut 5									
LN	0.62	0.46	0.39	0.40	0.47	NR	0.112		0.001
HN	1.00	0.81	0.89	0.86	0.89	G	0.041		0.024
Mean	0.81 ^b	0.63 ^a	0.64 ^a	0.63 ^a		NR×G	0.052 (13.1)	0.058	0.545
Annual									
LN	9.52	7.09	8.41	8.85	8.47	NR	0.021		<0.001
HN	14.51	11.77	13.84	13.92	13.51	G	0.173		<0.001
Mean	12.02 ^c	9.43 ^a	11.12 ^b	11.39 ^b		NR×G	0.213 (12.2)	0.245	0.524

Note: Value in parentheses after s.e.m. denotes degrees of freedom. Grass treatment means with differing lowercase superscripts (^{a,b,c}) differ ($p < 0.05$) based on a Student Newman-Keuls test. Interaction means with differing lowercase superscripts (^{a,b}) within rows or with differing uppercase superscripts (^{A,B}) within columns differ ($p < 0.05$) based on Bonferroni adjusted LSD's.

the tall fescue cultivar ($p < 0.05$). However, in Year 2 due to a positive response by only tall fescue to HN application, fescue showed a higher DMY ($p < 0.05$) than was achieved by the other grasses under HN. The perennial ryegrass and the two *Festulolium* cultivars in Year 2 all had equivalent yields, irrespective of their N treatment. Tall fescue is recognised as having drought resistance (Carrow and Duncan 2003; Loka et al., 2018), superior to that of ryegrass, and also to *Festulolium* cultivars, at a lesser extent, in part due to its deeper root system (e.g., Durand et al. 2007). The substantially lower rainfall recorded in Year 2, compared to that in Year 1, could potentially have conveyed tall fescue a growth and N uptake advantage that led to its superior forage production. However, this was deemed unlikely as despite their differences in morphology and physiology, all four grass cultivars in Year 2 had similar DMY when grown under LN and with no significant differences in root biomass evident (Table 3).

A difference in ontogeny between the grass cultivars was evident and reflected in their forage growth patterns at different harvest times throughout the growth seasons. At all harvests, except harvest 5 in 2018, the grass cultivars varied in their DMY. However, their ranking positions changed at different harvest dates. Initially (Cut 1) in Year 1, *Festulolium* cv Lp×Fm had highest DMY, with tall fescue the lowest, and with *Festulolium* Lp×Fg and perennial ryegrass intermediate for DMY ($p < 0.05$). At cut 1 in Year 2 (Table 2), tall fescue had the highest DMY, and ryegrass the lowest, whilst the *Festulolium* cultivars were both intermediate. At harvest cut 2 in Year 2, early into the dry summer months (Table 2), perennial ryegrass outyielded the three other grasses, with the fescue the lowest in forage production, and the *Festulolium* cultivars, intermediate ($p < 0.05$). At cut 3 (6 weeks after cut 2), the fescue had highest DMY, and the ryegrass the lowest. The *Festulolium* cultivars remained

TABLE 2 | Forage dry matter yield (t ha⁻¹) for four grasses during their second harvest year (2018) under two rates of inorganic nitrogen application either 178 kg (LN) or 356 kg (HN) N ha⁻¹ growing year⁻¹.

Nitrogen rate (NR)	Grass (G)				Mean		s.e.m.	Within G s.e.m.	Prob
	Ryegrass	Fescue	Lp×Fm	Lp×Fg					
Cut 1									
LN	0.77 ^a	1.20 ^b	0.99 ^{ab}	0.99 ^{ab}	0.99	NR	0.076		0.200
HN	0.84 ^a	1.72 ^b	1.02 ^a	1.18 ^a	1.19	G	0.057		<0.001
Mean	0.80	1.46	1.08	1.00		NR×G	0.104 (6.07)	0.081	0.038
Cut 2									
LN	3.28	2.02	2.78	3.01	2.77	NR	0.0132		0.407
HN	3.41	2.76	2.76	2.93	2.96	G	0.116		<0.001
Mean	3.34 ^c	2.39 ^a	2.77 ^b	2.97 ^b		NR×G	0.197 (7.6)	0.164	0.101
Cut 3									
LN	0.15	0.65	0.31	0.35	0.36	NR	0.014		0.021
HN	0.17	1.11	0.45	0.29	0.50	G	0.068		<0.001
Mean	0.16 ^a	0.88 ^b	0.38 ^a	0.32 ^a		NR×G	0.085 (12.6)	0.097	0.092
Cut 4									
LN	1.35	^A 1.28	1.19	1.31	1.28	NR	0.046		0.505
HN	1.36 ^{ab}	^B 1.75 ^b	1.21 ^a	1.04 ^a	1.34	G	0.060		0.006
Mean	1.35	1.51	1.20	1.17		NR×G	0.086 (12.0)	0.084	0.007
Cut 5									
LN	0.84	0.87	0.77	0.82	0.82	NR	0.083		0.252
HN	0.86	1.31	1.02	0.86	1.01	G	0.069		0.078
Mean	0.85	1.09	0.90	0.84		NR×G	0.118 (7.0)	0.097	0.149
Annual									
LN	6.39	6.02	6.03	6.47	6.23	NR	0.324		0.232
HN	6.62 ^a	8.64 ^b	6.62 ^a	6.14 ^a	7.01	G	0.218		0.018
Mean	6.50	7.33	6.33	6.31		NR×G	0.420 (5.2)	0.308	0.002

Note: Grass treatment means with differing lowercase superscripts differ ($p < 0.05$) based on SNK test. Interaction means with differing lowercase superscripts within rows and with differing uppercase superscripts within columns differ ($p < 0.05$). Value in parentheses after s.e.m. denotes degrees of freedom.

intermediate in forage production but not higher than the ryegrass ($p > 0.05$).

3.2.1 | Nitrogen Use Efficiency (NUE)

In Year 1, significant interactions were observed between the grass cultivars and N application rate ($p = 0.025$) (Figure 2a). All grasses had higher NUE when grown under LN, rather than HN application ($p = 0.002$). However, the grasses varied significantly in the extent of their NUEs under LN. Perennial ryegrass had the highest NUE at LN, ($p < 0.05$) but not significantly greater than Lp×Fg. The two *Festulolium* cultivars were similar for NUE at LN, with all the grasses superior in NUE than the tall fescue ($p < 0.05$). At HN, the NUE for the ryegrass and the two *Festulolium* cultivars were similar, but NUE for tall fescue was lower ($p < 0.05$), as it was under LN. In Year 2 (Figure 2b),

and overall, all four grasses had higher NUE when grown under LN rather than HN ($p = 0.021$). However, contrary to Year 1, the NUE of all the grasses under LN was similar. Under HN, the tall fescue cultivar had the highest ($p < 0.05$) NUE of all the grasses, with the other three grasses having similar NUEs. Variables such as soil type, fertiliser (Abassi et al., 2005), grass species (Ju et al., 2015) and the climatic conditions that prevail (Kanter et al., 2016) will all factor as major components that determine crop NUE.

3.2.2 | Nitrogen Uptake Efficiency (NupE)

In Year 1 (Figure 3a), the grasses showed no difference in their NupE with either HN or LN fertiliser applications ($p = 0.138$). However, the amount of N application did have an impact, with grasses grown under HN registering higher NupE than those

TABLE 3 | Root biomass (t DM ha⁻¹) at 0–15 cm soil depth of four grasses during their first (2017) and second harvest (2018) years under two rates of inorganic nitrogen application either 178 kg (LN) or 356 kg (HN) N ha⁻¹ growing year⁻¹.

Year	Nitrogen rate (NR)	Grass (G)				<i>Mean</i>		s.e.m.	Within G s.e.m.	Prob
		Ryegrass	Fescue	Lp×Fm	Lp×Fg					
2017	Cut 1									
	LN	1.38	0.78	1.05	1.49	1.17	NR	0.054		0.306
	HN	1.18	0.88	0.90	1.31	1.07	G	0.102		0.007
	<i>Mean</i>	1.28 ^{bc}	0.83 ^a	0.97 ^{ab}	1.40 ^c		NR×G	0.137 (14.0)	0.145	0.709
	Cut 3									
	LN	1.70	1.20	1.61	1.62	1.51	NR	0.060		0.355
	HN	1.47	1.29	1.25	1.52	1.41	G	0.104		0.125
	<i>Mean</i>	1.59	1.24	1.57	1.43		NR×G	0.141 (13.8)	0.147	0.346
	Cut 5									
	LN	1.55	1.56	1.66	1.98	1.69	NR	0.230		0.598
	HN	1.30	1.43	1.34	1.87	1.48	G	0.130		0.065
	<i>Mean</i>	1.42	1.49	1.50	1.93		NR×G	0.279 (4.2)	0.183	0.928
	Mean									
	LN	1.54	1.18	1.41	1.69	1.46	NR	0.074		0.322
	HN	1.32	1.20	1.29	1.48	1.32	G	0.059		0.004
	<i>Mean</i>	1.43 ^{bc}	1.19 ^a	1.35 ^{ab}	1.58 ^c		NR×G	0.103 (6.74)	0.084	0.453
2018	Cut 1									
	LN	1.53	1.49	1.45	1.64	1.53	NR	0.105		0.594
	HN	1.21	1.53	1.37	1.63	1.43	G	0.091		0.218
	<i>Mean</i>	1.37	1.51	1.41	1.63		NR×G	0.153 (7.4)	0.128	0.522
	Cut 3									
	LN	2.18	2.25	2.59	2.44	2.36	NR	0.140		0.100
	HN	1.60	1.66	2.23	1.65	1.78	G	0.154		0.127
	<i>Mean</i>	1.89	1.95	2.05	2.41		NR×G	0.235 (10.2)	0.217	0.801
	Cut 5									
	LN	1.95	1.94	2.27	1.98	2.04	NR	0.051		0.058
	HN	1.52	1.77	2.06	1.65	1.75	G	0.131		0.158
	<i>Mean</i>	1.74	1.85	2.17	1.81		NR×G	0.169 (13.7)	0.186	0.884
	Mean									
	LN	1.89	1.89	2.07	2.05	1.98	NR	0.008		0.001
	HN	1.44	1.65	1.84	1.70	1.66	G	0.080		0.112
	<i>Mean</i>	1.66	1.77	1.87	1.95		NR×G	0.098 (12.2)	0.113	0.744

Note: Grass treatment means with differing lowercase superscripts differ ($p < 0.05$) based on SNK test. Interaction means with differing lowercase superscripts within rows and with differing uppercase superscripts within columns differ ($p < 0.05$). Value in parentheses after s.e.m. denotes degrees of freedom.

under LN ($p = 0.031$). In Year 2 (Figure 3b), and contrary to Year 1, the four grasses differed in NupE ($p = 0.001$), with an interaction between N application rate and grass cultivar ($p = 0.009$). However, the main effect of N level was not statistically significant ($p = 0.276$). The fescue registered the highest NupE

under HN ($p < 0.05$), but the differences between the cultivars were smaller and not statistically significant when under LN ($p > 0.05$). This disparity is evident in other studies: Olf and Werner (1994), Abassi et al. (2005) and Lü et al. (2014) have also shown that plant N uptake was positively affected by rates of N

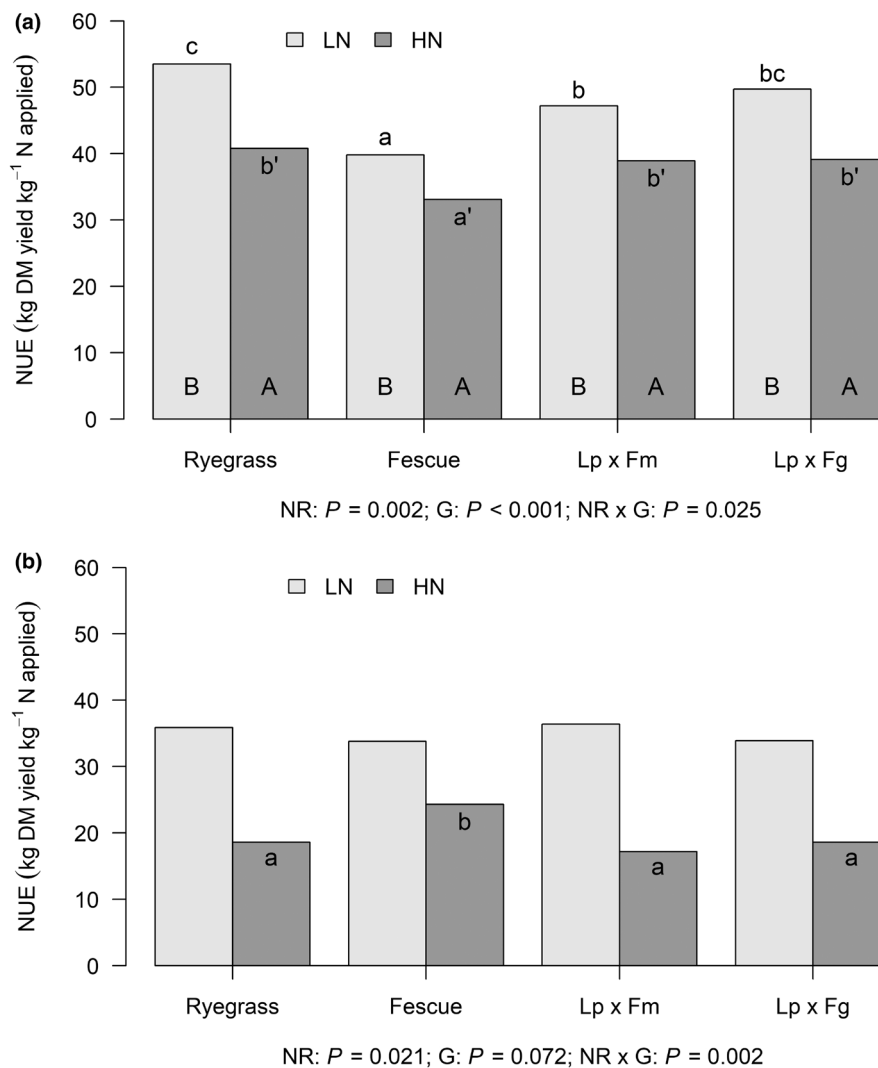


FIGURE 2 | (a) Nitrogen Use Efficiency (NUE) (kg DM yield kg⁻¹ N applied) for four grasses (G) during their first harvest year (2017) under two rates of inorganic N application (NR) either 178 kg (LN) or 356 kg (HN) N ha⁻¹ growing year⁻¹. Lp x Fm, *L. perenne* x *F. mairei*. Lp x Fg, *L. perenne* x *F. arundinacea* var *glaucescens*. Differing lower case letters indicate differences ($p < 0.05$) between species under LN. Differing lower case letters (a', b') indicate differences ($p < 0.05$) between species under HN. Uppercase letters indicate differences ($p < 0.05$) within species under LN and HN. (b) Nitrogen Use Efficiency (NUE) (kg DM yield kg⁻¹ N applied) for four grasses (G) during their second harvest year (2018) under two rates of inorganic N application (NR) either 178 kg (LN) or 356 kg (HN) N ha⁻¹ growing year⁻¹. Lp x Fm, *L. perenne* x *F. mairei*. Lp x Fg, *L. perenne* x *F. arundinacea* var *glaucescens*. Differing lower case letters indicate differences ($p < 0.05$) between species under HN.

fertiliser application, whereas, conversely, Aavola and Kärner, (2008) in their studies, did not encounter such distinction of grass N uptake with N supply variations.

3.2.3 | Nitrogen Utilisation Efficiency (NutE)

In both years, HN applications reduced NutE across all grass cultivars ($p = 0.002$ and 0.003), respectively. In Year 1 (Figure 4a), the tall fescue cultivar had the lowest NutE ($p < 0.05$), whilst the other grasses were similar. In Year 2 (Figure 4b), NutE under both LN and HN was lower than in Year 1. There was an interaction between grass cultivar and N application rate ($p = 0.025$). At LN, the ryegrass had higher NutE than the fescue ($p < 0.05$) with the *Festuloliums* intermediate, but not significantly different, from either the ryegrass or fescue ($p > 0.05$). At HN, all grasses had similar NutE but lower than at LN ($p < 0.05$). The high DMY

of the tall fescue and superior NutE when compared to the other grasses in Year 2 were not reflected in its NutE measures.

3.2.4 | Root Biomass (RB) and NUE

The mean RB collected from 15 cm deep soil cores at the time of forage cuts 1, 3 and 5 representing root development in spring, summer and autumn in each year is presented in Table 3. The RB measures of the grasses differed significantly in Year 1 ($p = 0.004$). This was primarily due to grass RB differences measured in the young swards in the spring. At that time, the *Festulolium* Lp x Fg had significantly greater RB than the fescue (which had the lowest RB) and also the *Festulolium* Lp x Fm ($p < 0.05$). However, the RB of Lp x Fg was not different to that of the ryegrass ($p > 0.05$). Subsequent RB measures taken later in Year 1 and throughout Year 2 showed no differences between

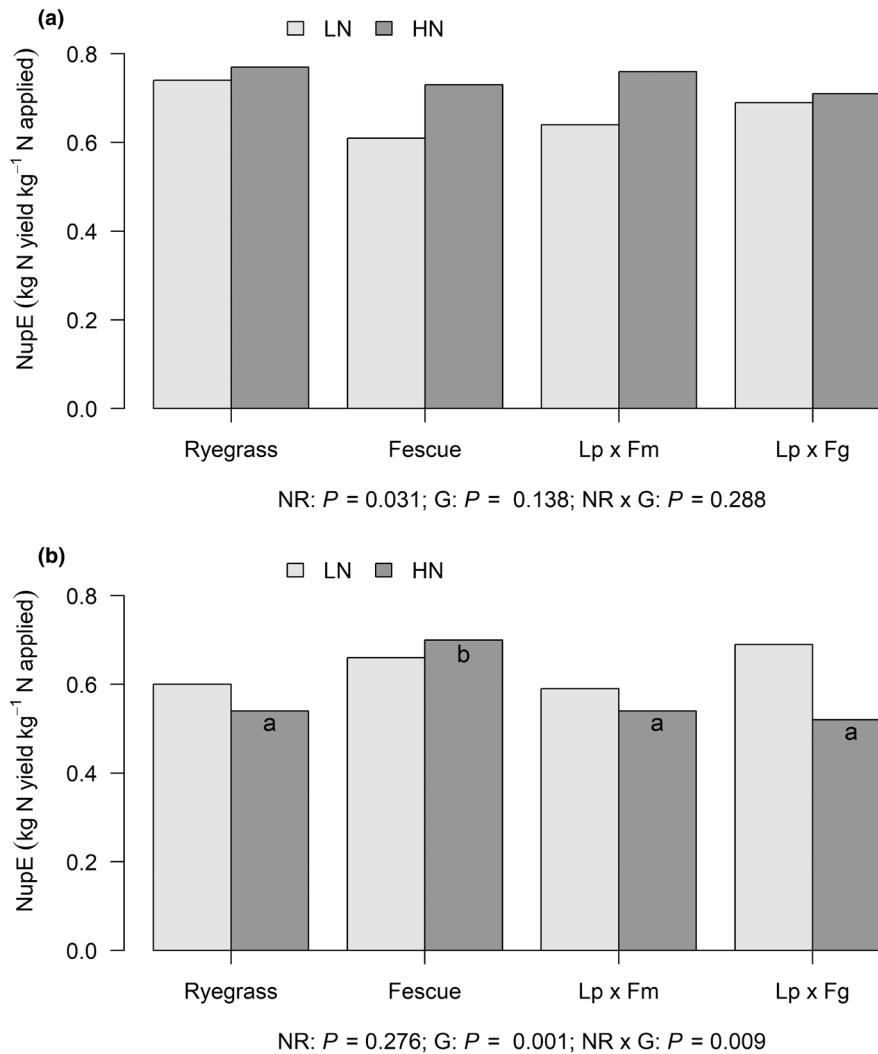


FIGURE 3 | (a) Nitrogen uptake Efficiency (NupE) (kg N yield kg⁻¹ N applied) for four grasses (G) during their first harvest year (2017) under two rates of inorganic N application (NR) either 178 kg (LN) or 356 kg (HN) N ha⁻¹ growing year⁻¹. Lp x Fm, *L. perenne* x *F. mairei*. Lp x Fg, *L. perenne* x *F. arundinacea* var *glaucescens*. (b) Nitrogen uptake Efficiency (NupE) (kg N yield kg⁻¹ N applied) for four grasses (G) during their second harvest year (2018) under two rates of inorganic N application (NR) either 178 kg (LN) or 356 kg (HN) N ha⁻¹ growing year⁻¹. Lp x Fm, *L. perenne* x *F. mairei*. Lp x Fg, *L. perenne* x *F. arundinacea* var *glaucescens*. Differing lower case letters indicate differences ($p < 0.05$) between species under HN.

the four grasses ($p > 0.05$). RB measures of all grasses showed an increase in the second year with fescue showing the greatest proportional increase and ryegrass the lowest.

The general similarities in RB of the four grasses were unexpected given the outcomes of their root ontogeny determined previously in Humphreys et al. (2018). However, it should be noted that the earlier research focused on detailed image-capture data obtained from 40 cm soil profiles using rooting-compost maintained in transparent Perspex-rooting-columns. RB data here was determined from roots extracted from shallower 15 cm soil cores and from soil under field experimental plots. Similar RB findings using alternative high sugar perennial ryegrass and *Festulolium* cultivars where measures taken from soil cores from the field failed to meet the expectations from RB data taken from rooting-columns have been reported elsewhere (Gregory et al. 2022). The total measures for soil-based RB of the two grass cultivars despite their anticipated different root architecture proved similar.

The N fertiliser rate in the current experiment had no effect on RB in Year 1 ($p > 0.05$), but averaged across grasses in Year 2, the annual mean RB was higher in grasses maintained under LN rather than HN ($p = 0.001$), and this effect appeared to develop mid to late season.

As described previously, NUE was consistently higher in grasses when grown under LN (Figure 2). The relationship between RB and NUE, and its component parts NutE and NupE, was examined. As the extent of RB of the individual grass cultivars did not differ significantly in either year of the field experiment, their data were combined. NUE showed a positive association with RB in both harvest years ($r = 0.622$, $p = 0.001$ and $r = 0.654$, $p < 0.001$ for Years 1 and 2, respectively). A positive correlation was also apparent between NutE and RB ($r = 0.448$, $p = 0.028$ (Year 1); $r = 0.625$, $p = 0.001$ (Year 2)). However, no clear relationship was evident between RB and NupE in either year ($r = 0.096$, $p = 0.655$; $r = 0.339$, $p = 0.105$) (Years 1 and 2, respectively).

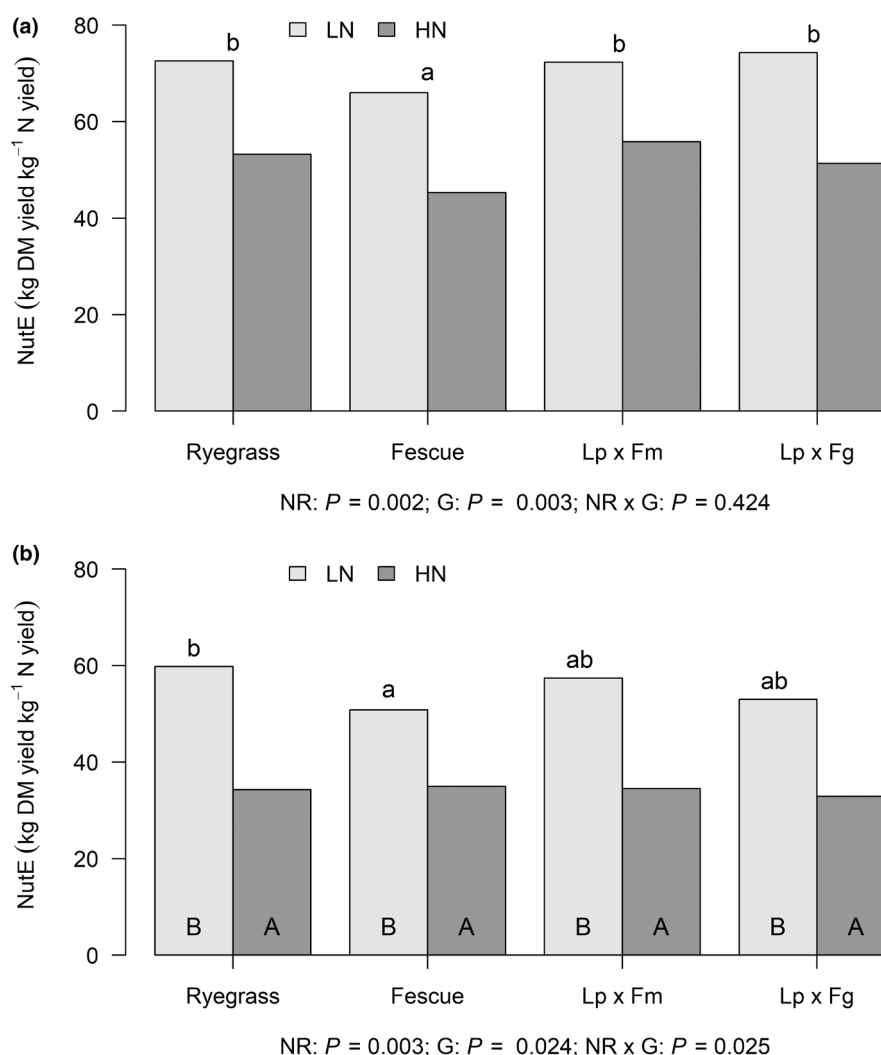


FIGURE 4 | (a) Nitrogen utilisation Efficiency (NutE) (kg DM yield kg⁻¹ N yield) for four grasses (G) during their first harvest year (2017) under two rates of inorganic N application (NR) either 178 kg (LN) or 356 kg (HN) N ha⁻¹ growing year⁻¹. Lp x Fm, *L. perenne* x *F. mairei*. Lp x Fg, *L. perenne* x *F. arundinacea* var *glaucescens*. Differing lower case letters indicate differences ($p < 0.05$) between grass (G) species. (b) Nitrogen utilisation Efficiency (NutE) (kg DM yield kg⁻¹ N yield) for four grasses (G) during their second harvest year (2018) under two rates of inorganic N application (NR) either 178 kg (LN) or 356 kg (HN) N ha⁻¹ growing year⁻¹. Lp x Fm, *L. perenne* x *F. mairei*. Lp x Fg, *L. perenne* x *F. arundinacea* var *glaucescens*. Differing lower case letters indicate differences ($p < 0.05$) between species under HN. Uppercase letters indicate differences ($p < 0.05$) within species under LN and HN.

Hirel et al. (2007) commented that whilst breeding aimed at genetic improvement of NupE, and that improving capabilities for growth at low N was a feasible breeding objective, a significant yield reduction may be difficult to avoid. Research and agronomic studies (e.g., Humphreys et al. (2014); Kamau et al. (2018)) and independent NIAB field trials used to determine the England and Wales Recommended Grass and Clover Lists (BGS 2024/25) have previously demonstrated equivalent agronomic performance between the two *Festulolium* cultivars used here and current UK National Listed perennial ryegrass cultivars. *Festulolium* Lp x Fm was recently included in the UK National Recommended Lists, where it is listed as the cultivar AberRoot. In multi-site UK field trials it was shown to have comparable digestibility (D) values compared with intermediate flowering tetraploid perennial ryegrass cultivars (BGS 2024/25), confirming its high forage quality.

Despite the higher DMY of the tall fescue cultivar in the second year, its known low forage quality and palatability,

when compared to its ryegrass and *Festulolium* alternatives (Mertens 1993; Chen et al. 2002; Østrem et al. 2015), makes use of tall fescue cultivars unattractive in a UK agricultural context. Furthermore, compared to the perennial ryegrass and *Festulolium* cultivars described here, the tall fescue, although the most responsive cultivar to HN fertiliser applications in the second year, had lower NutE than the ryegrass and the *Festulolium* Lp x Fm under LN and, as such, was less suitable for use in systems aimed at sustainable grassland practices capable of providing environmental safeguards.

A changing climate is predicted to have considerable impacts on the availability of certain nutrients (Jansson and Hofmockel 2020). Incidents of more extreme rainfall events in the UK due to climate change have the potential to increase N losses through leaching and surface runoff. Drier summers will limit soil water availability to plants and decrease their N uptake capabilities (Bista et al. 2018) and hence their N use efficiency

(NUE). Factors that cause N losses from soils will inevitably reduce plant available N in the soil profile and thereby reduce plant NUE. Forage yield response to N fertiliser relies heavily on soil water availability, which is becoming problematic due to the impacts of climate change (Errecart et al. 2020). The design and management of economically and environmentally sustainable agro-ecosystems require a better understanding of the meaningful interactions between N and water supply (Sadras et al. 2016). This requires incorporation of productive and resilient grass cultivars capable of providing efficient growth responses to N applications and achieving sustainable forage yields even under stress conditions. Gregory et al. (2022) describe a high sugar perennial ryegrass and a *Festulolium* cultivar that significantly increased the soil organic C and N stocks of the soils they supported, when compared to alternative neighbouring mixed-species permanent pastures, grown in equivalent conditions and similar soils. Their results demonstrate the complex dynamic nutrient interactions between plant roots and soils and therefore the potential for cultivar designs offering efficiencies in nutrient use.

Nutrients tend to accumulate largely near the soil surface (e.g., Errecart et al. 2020). In other studies, Crush, Waller, and Care (2005) concluded that root system architecture was less important than plant growth rate in influencing nitrate interception, suggesting that a finely divided root system with a large surface area might enhance nitrate uptake. Root measures taken in the current study focus on those soil profile areas considered most likely to be nutrient-rich and therefore those most implicated in N uptake. In the soil cores taken across the growing seasons over the two-year field experiment, generally no significant difference in root biomass in the uppermost 15 cm of soil profile was observed between the grasses. The one exception was the initial measure taken during the Spring of Year 1 which showed higher root biomass in Lp×Fg roots compared to the three other cultivars. Therefore, whilst it was impossible to directly relate root architecture of each grass cultivar to N uptake, more general conclusions were evident. Treated collectively, the extent of root biomass of the grasses in the soil cores correlated with grass cultivar NUE. The greater the root biomass, the higher the NUE. Furthermore, the greater the root biomass, the greater the NutE. However, no equivalent association was found between root biomass and NupE.

4 | Conclusion

Specific attributes of grass cultivars should be employed, in seed mixtures aimed at delivery of more sustainable and climate-smart mixed grassland systems. Other reports have described benefits to accrue through use of the *Festulolium* cultivars in relation to improved protein provisions for livestock both from freshly grazed (e.g., Kamau et al. 2018) and ensiled forage (Muhandiram et al. 2023) and other environmental services (e.g., to counter soil compaction (Muhandiram et al. 2020), or to mitigate flooding (MacLeod et al. 2013; Powell et al. 2018)). In considering the future sustainability of grasslands, the introduction of resilient and sustainable grass varieties capable of maintaining yields under different nitrogen applications is becoming an increasingly important plant breeding objective, needed to support future livestock agricultural systems. Increases in root biomass showed a positive association with NUE and NutE in both harvest years, but no clear relationship was evident between RB and NupE in either

year. Overall, findings demonstrated opportunities to incorporate high-quality agricultural grasses with more extensive root biomass into agricultural livestock systems, to improve N efficiency and thereby enhance economic and environmental sustainability. To meet future challenges, it will be essential to assemble cultivar and species mixtures that can incrementally incorporate all available advantageous and complementary measures that in unison can deliver both climate resilience and environmental safeguards for future sustainable UK livestock systems.

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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.