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1 Abstract

The rapidly changing climate increasingly impacts on the productivity of grasslands-based livestock, and grasslands provide the optimal source of affordable and healthy feed for livestock while delivering important ecosystem services. New grass options are required for farmers that provide them with some resilience to mitigate climate change and to help safeguard the livestock industry. *Festulolium* are hybrids of fescue (*Festuca)* and ryegrass (*Lolium)* species, and are bred to combine the high forage quality and productivity of ryegrass with the resilience characteristic of the fescues. This study aimed to assess potential of three *Festulolium* cultivars in three areas of grassland production. These were: i) resilience under compacted soil; ii) nitrogen use efficiency; and, iii) protein stability during ensiling. Here, we report on the findings of this research to provide new information on the use of these novel grasses in livestock systems in the UK.

Using triplicated grass plots, above and below ground forage biomass was measured comparatively from compacted and non-compacted soils. Compaction increased soil bulk density and penetration resistance however, water infiltration was not affected. *Festulolium* cultivar Lp x Fg, (Lp: *Lolium perenne* L. x Fg: *Festuca arundinacea var. glaucescens* Roth.) showed a tendency to have a higher root biomass than ryegrass under compacted soils but both grasses had equal annual dry matter yields.

Roots and forage measurements collected over two harvest years from replicated field plots which were treated by two N rates (178 & 356 kg ha^{-1}). Ryegrass showed higher N use efficiency at 178 kg ha^{-1} and produced higher annual yield than *Festulolium* in first harvest year but both measurements were comparable in second year. Interestingly, *Festulolium* produced a higher yield in the second-harvest year summer cut. This second harvest year (2018) was affected by a prolong drought. Root biomass was positively and significantly correlated with both N use and utilization efficiencies of grasses and was more evident in the *Festulolium*, especially Lp x Fg.

In a silage experiment investigating proteolysis during the ensiling process, the key findings was that overall, Lp x Fg silage had a higher crude protein concentration than ryegrass when measured at different time points up to 90d ensiling.

The current research provides evidence that future use of *Festulolium* grasses provides a viable and sustainable alternative to livestock farmers to safeguard forage production and ensuring ecosystem services linked with their higher root biomass.

2 Introduction

The demand for food has increased mainly due to global population rise, but the supply is associated with many constraints, and among these, climate change is a significant component. Livestock are a key element of the available food supply and demand for meat products is increasing globally, largely due to increased income per capita. However, food production from the livestock industry is closely associated to resilience to climate change. Grasslands are multifunctional, providing both the primary feed and the habitat for maintenance of grazing livestock animals while also providing additional ecosystem services and incomes for communities (frequently fragile) associated with such production systems. However, advantages of livestock systems are also distributed to other social strata directly and indirectly. Changing climate has significant impacts on current grasslands. Future meteorological projections indicate that the intensity, frequency and length of agriculturally harmful climatic extremes are expected to increase. More specifically, droughts, floods, heat-waves and potential changes in length of crop growing seasons will be likely to affect grassland productivity and, for an anticipated perennial crop, its long-term persistency. In particular, incidents of either prolonged water deficit or excess have potential to damage grasslands, the soil on which they grow and the animals who depend on the forage they provide.

Ryegrass is regarded as a high quality, highly digestible forage for ruminant livestock in temperate grassland locations where rainfall is well distributed and of sufficient supply. When conditions are suboptimal for ryegrass growth, alternative grass species are frequently employed, such as cocksfoot, timothy and different fescue species. In a UK context, where future expectations are for increased hot dry summers and wetter warmer winter temperatures, it is necessary to find ways of improving the resilience of currently productive grasslands either through improved breeding strategies or refined management practices or better still, a combination of both.

In this context, novel grass hybrids called *Festulolium* (hybrids formed between closely-related ryegrass (*Lolium*) and fescue (*Festuca*) species) are gaining attention due to their increased resilience particularly to cold and hot weather, enhanced disease resistance and potential to safeguard sufficient forage production to satisfy livestock requirements and to also deliver beneficial ecosystem services. Existing studies on the impact of abiotic stresses on grasslands rarely encompass a broader approach that accounts for their impact over the whole grassland ecosystem. The potential for future use of *Festulolium* hybrids in this context is considered here at some depth to determine the potential benefits of incorporating such grasses into animal-based agricultural systems with the overall aim to future safeguard to ensure continued feed supply for use in livestock systems. In the current study, improved root and stress resistance characteristics of *Festulolium* were investigated. Their potential impact on the soil and the feeding animal were considered. Although use of *Festulolium* hybrids has been gaining attention as an option for increased use as forage in pasture based production systems, their potential for ecosystem service, specifically in the areas of resilience against soil compaction, protein protection capabilities in extreme conditions (i.e. during ensiling) and for improved nitrogen efficiencies, has never been previously tested. The current work represents an in depth approach to evaluate more extensively the potential for *Festulolium* use as an effective input in UK ruminant systems and to deliver environmentally solid, productive and multifunctional new grass feed opportunities for the benefit of end user in order to maintain sustainable livestock systems.

This work investigates the efficacy of *Festulolium* as a grass within UK dairy systems in terms of their effects on soil properties, nutrient use efficiency and as conserved forage for livestock. Three research objectives were employed to attain the aim of study. The first and second research objectives were based on the hypothesis that improved rooting characters expected in *Festulolium* may lead to improved stability and resilience of soil and plant biomass under soil compaction and also in soil-plant nitrogen use efficiency. The third objective of this study relates to the resilience reported in certain F*estulolium* hybrids where acquiring fescue parent genomes in combination to ryegrass supports efforts to safeguard plant proteolysis in rumen simulation experiments and to determine if these properties are also evident during the ensilingprocess when these forages are conserved.

In summary the objectives of the research are to evaluate the:

- resilience of *Festulolium* to machinery compaction in terms of productivity and impact on soil characteristics
- nutrient use efficiency of *Festulolium* with reference to nitrogen (N) in order to test capabilities of *Festulolium* to facilitate N absorption and subsequent utilization
- protein stability of *Festulolium* during ensiling to safeguard silage protein

3 Materials and Methods

Three independent research experiments were conducted to accomplish the aim of the study.

3.1 Experiment 1: Resilience of *Festulolium* to machinery compaction in terms of forage productivity and soil characteristics

In this study, three tetraploid *Festulolium* grass cultivars, each comprising a perennial ryegrass, and alternative fescue species' derived genomes (*Lolium perenne* L. x

Festuca mairei Hack., referred to herein as **Lp x Fm**; *Lolium perenne* L. x *Festuca arundinacea var. glaucescens* Roth., referred to as **Lp x Fg**; and; *Lolium perenne* L. x *Festuca pratensis* Huds. cv Prior, referred to as **Lp x Fp** *)*, were compared against each other and against currently used perennial ryegrass (*Lolium perenne* L. cv. AberBite (4x)) and tall fescue (*Festuca arundinacea* Schreb. cv. Kora (6x)) varieties. Three replicated 5 x 1.5m experimental plots on stony, well-drained loam soil of the Rheidol series arranged in randomised blocks had previously been established with grasses in autumn 2012. Prior to the current research, plots were maintained under a five cut year^{-1} regime to simulate conservation management for silage using conventional NIAB field management protocols (Humphreys *et al*., 2014). For the compaction treatment, randomly selected 1.5 x 2.1m halves of each grass subplot were compacted (17th March 2016) using a 2,040kg weight Cambridge ring roller at a rate of six passes plot^{-1}. Soil characteristics and below and above ground growth of the five grasses at both soil conditions (SC: compacted (C) and non-compacted (NC)) were compared by giving attention on soil conditions, grasses and their interactions. Both soil (physical (water infiltration rate (WIR), penetration resistance (PR), bulk density (BD)) & chemical properties (nitrate and ammonium N, pH, P, K, Ca and Mg) and forage characteristics (tiller density (TD), dry matter (DM) yield, botanical composition (BOC), root biomass (RB)) were determined at two time points: a) prior to compaction (baseline); and, b) post compaction. However, DM yield, BOC and RB were collected only as post compaction measurements.

3.2 Experiment 2: Determining the nitrogen use efficiency of *Festulolium* compared to currently used related grass varieties

Triplicate plots (6 x 2m) were established in split-plot design in September 2016 on an area of stony, well-drained loam of the Rheidol soil series to compare *Festulolium* cultivars of Lp x Fm and Lp x Fg with perennial ryegrass (AberBite) and tall fescue (Dovey) cultivars. Experimental plots were managed in accordance with NIAB's UK National List Trial recommendations consistent with IBERS standardized field management procedures. All plots were harvested on a five cuts year^{-1} basis both in their first (2017) and second (2018) harvest year to simulate a conservation management and silage-based grass harvest system. With respect to experimental design (split-plot), the sub plot was represented by the grasses while main plot by N fertilizer application rates. Nitrogen fertilizer treatments comprised two application rates (356 kg N ha^{-1}growing year^{-1} (considered as high N, HN); and 178 kg N ha^{-1}growing year^{-}

^{1} (as low N; LN). Each grass sub-plot (6 x 2m) was divided into two areas of different length, 4 x 2m and 2 x 2m. A 4 x 2 m section of each sub-plot was used for forage measurements and a 2 x 2 m section was utilized for destructive soil sampling to determine root biomass. Forage measurements (in six week intervals) for dry matter yield (DMY), total N concentration and yield were determined for each of the five cuts per harvest year. In the first harvest year (2017), plots were harvested on 8 May (cut 1), 19 June (cut 2), 31 July (cut 3), 11 September (cut 4) and 25 October (cut 5). In the second harvest year (2018), plots were harvested on 30 April, 11 June, 23 July, 4 September and 16 October. The soil samples were collected at approximately 12-week intervals (simultaneously with grass cut 1, 3 and 5) to assess overall and intervening root growth.

Alongside the field-based study, a hydroponic experiment was also carried out to provide an investigation of root and foliar growth involving the four grass cultivars (Lp x Fg, Lp x Fm, AberBite and Dovey) to supplement the analyses undertaken in the field study (experiment 2). This approach was to overcome the limitations of measuring rooting traits under field conditions which make it difficult to capture total root biomass (Pierret *et al.*, 2005) and quantifying of tiller numbers which closely associated with root production (Matthew *et al.*, 2001). Grass cultivars similar to field study were first exposure to a modified traditional Hoagland solution for four weeks to generate baseline data and this provided information as to whether any significant difference in root growth was evident among the grass cultivars prior to exposure to the contrasting N treatments. later, Hoagland solution with two N concentrations (High N (HN‡): 2 mM and Low N (LN‡): 0.02 mM) were applied to create a split plot design where the N treatment represented the main plot/unit and the grass cultivar the sub plot/unit. Both baseline and N treated data were collected by destructively sampling the whole plant and determining the total number of tillers, shoot dry weight (SDW), root length (RL) and root dry weight (RDW) of each plant.

3.3 Experiment 3: Investigating the protein stability of *Festulolium* during early stages of ensiling

Monoculture field plots sown at the Institute of Biological, Environmental and Rural Sciences (IBERS), Wales, Aberystwyth on soil of the Denbigh series, characterised as well drained, fine loamy and silty over rock in August 2014 were utilized for this work. Experimental field plots were harvested 5 times year^{-1} using National List trial procedures followed by IBERS in accordance with NIAB protocols. Grass plots were established in four replicates randomized blocks and for this study, forage materials were taken from *Festulolium* cultivar Lp x Fg and ryegrass cultivar AberMagic. Experimental forage comprised forage from grass cut 2 (harvested on 31st of May 2016) and cut 4 (harvested on 9th of August 2016). Harvested forage of two grass cultivars from 4 replicate field plots were combined grass cultivar wise to ensure homogeneity. Broad leaved and clearly identifiable grass weeds were manually removed. Equal forage samples (circa 14kg and 13kg per cultivar respectively at cut 2 and 4) were given 24h to wilt. Subsequent to the 24h wilt, the two grasses were treated with a silage inoculant (Wynnstay Dominator *Lactobacillus Plantarum*; Wynnstay Group Plc, Powys, UK) as recommended by the manufacturer (1.1kg dissolved in 200l water for 100t forage) and the purpose of this practice was to make sure that the forage undergone efficient fermentation with respect to the size of silos utilized. Forage was subsequently passed through a forage harvester (Lely Storm 130P, Netherlands) to achieve an average theoretical chop length of 30mm. In the laboratory, representative triplicate sub-samples of the two forages were manually (cut 2: 1st of June; cut 4: 10th of August, 2016) transferred into sterile glass tube silos of approximately 100g capacity. The tubes were packed compactly, sealed with airlocks containing liquid paraffin, and kept at a constant room temperature of 21°C. The silos were opened at six time points: 9h, 24h, 48h, 72h, 14d and 90d post ensiling, respectively, and three replicate mini-silos were destructively sampled for each grass cultivar at each time point. Samples were stored at -80°C until required and analysed for their silage dry matter (DM) and chemical composition (pH, total nitrogen (TN), soluble nitrogen (SN) and ammonia nitrogen (NH_3-N).

4 Results

4.1 Key results of experiment 1

The mean (± SEM) soil pH, prior to compaction was 6.12 (± 0.04) while post compaction pH was 6.27 (± 0.04). Prior to soil compaction, PR through the uppermost 30cm of soil was lower ($P < 0.05$) with the perennial ryegrass control when compared to two of the *Festulolium* cultivars, Lp x Fm and Lp x Fg, with Lp x Fp and tall fescue intermediate. Soil underneath all three *Festulolium* treatments and the ryegrass had higher water infiltration rates than the tall fescue control ($P < 0.05$). Following compaction treatment, In comparison to the non-compacted sub-plots, both BD and PR (0 - 30 cm) were higher in compacted soils ($P = 0.011$ and $P < 0.001$ respectively). The PR data showed that the main effect of compaction treatment was mostly evident at a depth of 5 - 15cm. However, whilst soil BD and PR were both negatively affected by compaction, there was no difference between compacted and non-compacted soil treatments in terms of WIR ($P > 0.05$).

Prior to soil compaction, plant TD (no. of tillers 0.1m^{-2}) of all three *Festulolium* cultivars were higher than the tall fescue ($P < 0.05$), but did not differ significantly from the ryegrass control. After compaction, TD numbers among the grass types did not differ significantly although the tall fescue still had the lowest tiller number. However, TD were significantly lower ($P < 0.05$) in the compacted sub-plots (251 ± 10) compared to those growing in non-compacted soils (271 ± 14.2). Forage dry matter yield DM yield at the first (9 weeks following compaction) harvest cut in the compacted sub-plots was lower than in their non-compacted counterparts (2.97 v 3.28 t DM ha^{-1} respectively ; $P < 0.001$). Conversely, the DM yield of the compacted plots was higher ($P < 0.01$) than the non-compacted at the second harvest cut (15 weeks following compaction). There were no further effects of compaction on DM yield following the second cut ($P > 0.05$) and total DMY for all grass harvests with respect to both soil conditions were not significantly different ($P > 0.05$). The DM yield of the first harvest cut of the tall fescue was higher than all other grasses ($P = 0.01$). Ryegrass produced a higher DM yield in the second harvest cut compared to all others ($P < 0.001$), but in the third and fourth cuts, tall fescue had the highest yields ($P < 0.001$). The *Festulolium* treatments had similar DM yield to ryegrass at all cuts except in second cut (Table 5). Tall fescue produced the highest total annual DM yield of all grass treatments ($P < 0.001$). All three *Festulolium* cultivars produced a total DM yield that was not different significantly from the perennial ryegrass control ($P > 0.05$). The RB within 0 – 15cm, 15 – 30cm soil depths and when combined (0 – 30cm) indicated there was no significant impact of the compaction treatment on root presence. Within 15 – 30cm soil depth, the fescue had higher root numbers ($P < 0.01$) than all the other grasses whilst the ryegrass and the three *Festulolium* cultivars had very similar root numbers. However, for the upper 0 – 15cm soil profile and the overall RB (0 – 30cm), there was a significant grass x soil compaction interaction ($P = 0.04$), which reflected a tendency ($P < 0.05$) for Lp x Fg to have a higher root biomass than ryegrass in compacted soils whilst higher roots than fescue in non-compacted soil.

4.2 Key results of experiment 2

The results presented below are represented by the key findings of field plot experiment and glasshouse hydroponic experiment respectively.

4.2.1 Field experiment

Experimental data were collected over first and second harvest years of treated grass plots. Further, In the second harvest year (2018), the UK experienced the third driest May-July recorded since 1910 (Hannaford, 2018). The natural field dry conditions in summer 2018 provided ideal testing conditions to measure grass yield performance under soil water deficit conditions.

4.2.1.1 DM yield

In the first harvest year, the ryegrass produced higher total annual DM yield compared to the other grass cultivars ($P < 0.001$). The tall fescue produced the lowest yield ($P < 0.001$) with both *Festulolium cultivars* achieving similar yields, intermediate to their parental controls. Overall, in the second harvest year (2018), there was no significant difference in DM yield between the ryegrass and *Festulolium* cultivars ($P > 0.05$) but the tall fescue had the highest yield ($P < 0.05$) compared to the other grasses. By Cut 3 of second harvest year (23rd July 2018), the tall fescue had a higher DM yield compared to all other treatments and the *Festulolium* treatments had a higher yield compared to the ryegrass ($P < 0.001$).

Throughout the first harvest year (2017), grasses treated with 356 kg N ha^{-1} yr^{-1} (HN) had a significantly higher DM yield than plots receiving 178 kg N ha^{-1} yr^{-1} (LN). Overall, the total annual DM yield of grasses treated with high N was similar to that of the low N treatment in the second harvest year. However, with respect to grass and N treatment interactions of annual DM yield, the tall fescue was the only grass to have a significantly higher yield at high N compared to the low N treatment, with all other grasses having a similar DMY at both N application rates.

4.2.1.2 Forage N content

Foliage TN concentrations in 2017 for tall fescue *cv* Dovey were significantly ($P < 0.01$) higher than for ryegrass and both the *Festulolium* cultivars and TN concentrations were not significantly different between ryegrass and *Festulolium* hybrids. In 2018, all grasses had similar TN concentrations in above ground growth.

Grass foliar TN concentrations were compared as per N supply (HN and LN) and higher quantities were observed in HN treated plots regardless of the harvest year (2017: $P < 0.001$; 2018: $P = 0.01$).

4.2.1.3 Forage total N yield

Taken over all five cuts in 2017, the overall total annual TN yield of all grasses was similar ($P > 0.05$). Further, overall annual TN yield in 2018 was highest in tall fescue ($P < 0.01$) with TN in the two *Festulolium* similar both to each other and to the ryegrass control. The ranking among the grasses for TN yields remained generally consistent throughout the whole second growing season.

Grass plots treated with HN had a higher annual forage TN yield than LN plots ($P < 0.001$) over the first harvest season which corresponded to the higher foliar DMY found in the grass plots provided with HN. As in 2017, the highest annual TN yield was found in the grasses grown in the HN plots ($P = 0.01$) in second harvest year.

4.2.1.4 Root biomass

Root biomass collected from soil cores at Cut 1 in the first harvest year (2017) showed Lp x Fg had significantly ($P < 0.01$) higher root biomass than both the tall fescue and Lp x Fm. Although, root biomass was numerically highest in *Festulolium* cv Lp x Fg, the differences in root biomass among grasses were not statistically different for the root biomass collected from the remainder of 2017 and throughout 2018. However, during late Autumn, 2017 (cut 5: 25th October), Lp x Fg (mean ± SEM 1.60 ± 0.25) had a tendency ($P = 0.065$) for higher root biomass than ryegrass (mean ± SEM 1.19 ± 0.16).

Over both growing seasons, N fertilizer rate applications had no measurable effect on root biomass production and this was illustrated by similar root recoveries from the HN and LN plots ($P > 0.05$). However, late Autumn in 2018 (cut 5: 16th October), Lp x Fg showed tendencies ($P = 0.06$) for higher root biomass in LN (mean ± SEM; 1.70 ± 0.16) plots compared to their HN (mean ± SEM; 1.46 ± 0.15) equivalents.

4.2.1.5 N use efficiency (NUE)

Significant interactions between grass types and N application rates were observed in 2017. At LN the ryegrass had a higher ($P < 0.05$) NUE compared to all other grasses. However, the two *Festulolium* cultivars had similar NUE, and were both higher than tall fescue. At HN, NUE for both *Festulolium* cultivars and ryegrass was similar, but tall fescue was significantly lower ($P < 0.05$). All grass plots grown under LN conditions had significantly ($P < 0.01$) higher NUE compared to those growing with HN.

In 2018, the second growing year, at LN, all four grasses had similar NUE. However, contrary to 2017, in HN the tall fescue had the highest ($P < 0.01$) NUE compared to all the other grasses. The other three grasses had similar NUE under HN applications. As in 2017, but to a lesser extent, higher NUE was achieved at LN compared to HN ($P < 0.05$) for all grass cultivars.

4.2.1.6 N uptake efficiency (NupE)

In 2017, all grasses had similar NupE ($P > 0.05$). Contrary to the overall NUE results, NupE, was higher in HN than in the LN plots ($P < 0.05$) showing the grasses were all potentially benefiting from having higher N uptake.

In 2018, and contrary to 2017, apart from *Festulolium* cv Lp x Fg, all the other grasses had generally similar NupE, irrespective of the rates of N applied. However, Lp x Fg was different under the two N application rates and was significantly higher in NupE ($P < 0.01$) in LN treated plots compared to its HN.

4.2.1.7 N utilization efficiency (NutE)

In 2017 NutE of ryegrass, Lp x Fm and Lp x Fg was similar and was higher than ($P < 0.01$) in the tall fescue. LN plots had significantly higher NutE than at HN ($P < 0.01$).

Year 2018 data illustrated that at LN, both ryegrass and Lp x Fm were similar, but both were significantly higher than the tall fescue and Lp x Fg ($P < 0.05$) which were themselves similar (Figure 3.7). At HN, all grass treatments had similar N utilization. As shown in the 2017 results, LN grass plots had higher ($P < 0.01$) NutE than HN in the second harvest year .

4.2.1.8 Root biomass (RB) and nitrogen efficiencies

A possible correlation between grass underground growth and different aspects of NUE was investigated. In 2017, both NUE ($P = 0.001$) and NutE ($P < 0.05$) were significantly correlated with RB, but no such relationship was evident for NupE ($P > 0.05$, $r = 0.09$). The root biomass when treated as an independent variable was positively correlated with both NUE and NutE ($r = 0.62$ and 0.45, respectively). The square of the correlation coefficient (R^2) in 2017 described 39% of the variation in NUE of the grasses and was related to the variation of root biomass. However, root biomass explained only 20% of the variation for NutE.

In the second harvest year (2018), there were also significant and positive correlations between RB and NUE ($P < 0.001$) and NutE ($P = 0.001$) with respective r values of 0.65 and 0.62. As with data in 2017, no correlation in 2018 between RB and NupE was detected ($P > 0.05$, $r = 0.34$). Furthermore, 43% of variation in NUE in the second harvest year can be explained by variation of root biomass and 39% for NutE.

4.2.2 Hydroponic experiment

As per baseline data, there were no significant differences between the grass cultivars for the variables measured other than for grass tiller numbers, where two *Festulolium* cultivars and perennial ryegrass *cv* AberBite had similar numbers, all statistically higher ($P < 0.05$) than the tall fescue *cv* Dovey. There was a tendency ($P = 0.06$) for a positive correlation ($r = 0.57$) between plant tiller number and root biomass. This was obtained from the baseline data where tiller number was featured as an independent variable. Furthermore, 32% of root biomass variation is explained by the plant tiller number variations ($R^2 = 0.3212$).

Following N treatment, perennial ryegrass and the *Festulolium* cultivars had statistically similar tiller numbers, higher ($P < 0.01$) than the tall fescue cultivar. More tillers were obtained in grasses grown in HN[‡] than in LN[‡]. The root to shoot (R/S) ratio of the ryegrass *cv* AberBite was higher ($P < 0.05$) than Lp x Fm but comparable to Lp x Fg and the tall fescue. There was no notable difference of R/S ratio between the two N rates ($P > 0.05$). Other experimental variables such as SDW, RL, RDW and RGR were found to be statistically the same among the grasses and between the two N rates ($P > 0.05$). However, grass root biomass was positively correlated with plant tiller numbers ($P <$

0.001, r = 0.7). Tiller number variation explained 48% of root biomass variations ($R^2 = 0.4831$).

4.3 Key results of experiment 3

4.3.1 Dry matter

For both harvest cuts, ryegrass-based silage had higher DM compared to the *Festulolium* cultivar Lp x Fg ($P < 0.001$). With the progression of ensiling time dry matter content of silage were significantly changed irrespective of harvest cut and DM reductions observed in 90d ryegrass silage when compared to original 9h silage were 8.1 g kg^{-1} (2.3%) and 12.7 g kg^{-1} (2.9%) respectively in cut 2 and 4, however, this reductions for Lp x Fg were only 4.9 g kg^{-1} (1.5%) and 12.8 g kg^{-1} (3.9%) subsequently ($P < 0.05$).

4.3.2 pH

Although forages of both grasses had similar pH (mean ± SEM; 6.0 ± 0.01), the pH in silage of Lp x Fg was significantly lower than that of the ryegrass ($P < 0.001$). There was a significant interaction of grass and ensiling time ($P < 0.001$) where both grass silage pH was decreased significantly with increasing ensiling time. Generally, regardless of forage harvest cut, the fall in pH between 9h – 14d of ensiling was apparent however, commonly for both harvest cuts at 14d and 90d both grasses produced silage with equivalent pH.

4.3.3 Crude protein (CP)

There were significant statistical differences in CP between the silage grass treatments at both harvest cuts where Lp x Fg based silage found with higher CP (cut 2: $P < 0.05$; cut 4: $P < 0.001$) than ryegrass silage. Though, there was not any significant interaction of grass and ensiling time, generally CP content of silages was increased over ensiling time irrespective of harvest cut at least at $P = 0.001$. At 90d, comparatively, there was 2.1% and 5.5% higher CP found present within the Lp x Fg silage respectively at cut 2 and 4 than ryegrass.

4.3.4 Soluble N (SN)

With respective to cut 2, ryegrass silage had a higher SN fraction than the *Festulolium* ($P < 0.001$) but in cut 4, both grass treatments were comparable ($P > 0.05$). Commonly, the SN content of the silages increased with ensiling time regardless of grass type or harvest cut ($P < 0.001$). Interactions of grass and ensiling time were tended to significant ($P = 0.05$) for harvest cut 2 based silage where both grasses found comparable SN at 9h, but at 24h, ryegrass had significantly higher SN than Lp x Fg. However, at each following time points, silage SN content were similar between both grasses. For harvest cut 4 silage, there was not significant interaction between grass and ensiling time which led equivalent SN of grass treatments at each time points eventually to made grass silage with similar SN content between grasses.

4.3.5 Ammonia N (NH_3-N)

The ryegrass silage had a comparable NH_3-N concentration as with *Festulolium* silage made by forages harvest at both cut 2 and 4 ($P > 0.05$). With increasing ensiling time, NH_3-N generally increased ($P < 0.001$) for both grasses at two harvest cuts.

5 General Discussion

Experimental specific discussions have been presented in the thesis. This discussion cover the general aspects of each experiment comparatively giving attention to the key findings.

5.1 Forage productivity of *Festulolium*

Throughout this study, above ground productivity of *Festulolium* was investigated and compared to the two control varieties in terms of their tiller density, dry matter yield (experiment 1 & 2); their foliar N content as a measure of forage quality and protein yield (experiment 2); and, the extent of proteolysis during ensilaging (experiment 3).

Grass tiller density was quantified in the current work as this is one of the determinant factors of DMY (Wilman and Mohamed, 1980). Tiller density also represents root potential as every tiller is expected to produce an individual root (Matthew *et al*., 2001). The findings of both the field compaction experiment (experiment 1) and the hydroponic experiment (experiment 2) illustrated the comparable tiller numbers found in three *Festulolium* cultivars (Lp x Fm, Lp x Fg, Lp x Fp). Furthermore, no significant differences were found in tiller density to the current National Listed perennial ryegrass variety, AberBite.

From the study of the NUE, the findings provided the total annual DMY of the *Festulolium* cultivars (Lp x Fm and Lp x Fg), perennial ryegrass, and tall fescue over two consecutive years. In the first harvest year (2017) where the field plots had been previously sown and established in autumn 2016, the perennial ryegrass had the highest DMY, while the tall fescue had the lowest. The yield difference between the two control grass varieties can be most-likely explained by the comparatively rapid establishment of the ryegrass, as previously shown by Casler and Duncan (2003) and slower field establishment of the fescue (Raeside *et al*., 2012) and lower tiller density as confirmed in the experiments here and described in compaction experiment (experiment 1) and N use experiment (experiment 2). The intermediate DMY of the *Festulolium* compared to the ryegrass and fescue controls when grown under similar conditions maybe the compound effect of their hybridization. However, the following harvest year (2018), where extreme summer drought was encountered, produced a different outcome for the rankings for the grass DMYs. The total annual DMY of the ryegrass and the *Festulolium* (Lp x Fm & Lp x Fg) were similar, but interestingly, the summer cut DMY (equivalent to cut 3) showed a higher above ground growth for the both *Festulolium* than their ryegrass counterpart. In recent years, occurrences of drought in the UK summer have led to livestock-based industries having to find alternative feed sources to grass to feed farm animals;

highlighting a feed security threat to UK livestock systems (Unsworth *et al*., 1993; Agriculture and Horticulture Development Board: AHDB, 2014; Morison and Matthews, 2016). Thus, indications that *Festulolium* cultivars, such as those used here, may help safeguards forage production during water stress, are noteworthy. Further supporting these conclusions, the total annual DMY findings presented in compaction experiment (experiment 1) using mature field plots (>4 years) growing under non-drought stress conditions, showed the persistency and potential of *Festulolium* to produce above ground growth which was equivalent to that found from perennial ryegrass and this was previously reported by Humphreys., *et al*. (2014) and Macleod *et al*. (2013). Hence, under favourable conditions the *Festulolium* populations described here ensure efficient forage production, and may indeed provide some additional resilience to grasslands as they encounter climatic related abiotic stresses.

Nitrogen is a fundamental element of living organisms and for intensively managed grasslands have to regular N fertilizer applications, considered an important agronomic practice necessary to maintain enhance productivity hence, it is the most utilized mineral nutrient (Li *et al.*, 2015). The soil available N content has great impact on plant nutrient profile (King *et al*., 2012) and hence is linked with amount of animal protein ingestion. The experiment described here on the NUE revealed that a higher rate of N application increased the N concentration of forage, as confirmed by other studies (Aavola & Kärner, 2008; Martin *et al*., 2017; Delevatti *et al.,* 2019). Climate predictions (Jenkins *et al.,* 2009; Gornall *et al.,* 2010), indicate a propensity for prolonged drought and flood events, which are expected to increase in their severity in future. It is important to optimise N supply to support a healthy soil and optimal forage and, thus, livestock production under UK climatic conditions (Goulding *et al*., 2008). However, it is also essential to avoid an excess in N supply, which may lead to gaseous losses (Chadwick *et al*., 2018) and may not be compatible with grass uptake capabilities during a drought period and may result in a surplus of N in the soil (Stark & Firestone, 1995; Weltzin *et al*., 2006). Accumulations of soil N, topped up by further applications are likely to lead to leaching (Decau *et al*., 2004) and losses in run-off during wet winter periods and those are detrimental to the environment. Leaching of plant available N has been the main cause of impede plant N recovery (Lynch, 2013). Comparable foliar N concentrations and annual N yields were observed in *Festulolium* cultivars Lp x Fm and Lp x Fg and in perennial ryegrass in both harvest years respective to experiment 2. However, as per experiment 1, 49% and 32% respectively, higher soil nitrate content was found in soils under Lp x Fg and Lp x Fm than under ryegrass at the end of growing season and may be indicative a of their lower requirement for supplementary N during the next growing year. However, the differences were not significant statistically, as stated, and were not confirmed by differences in NUE in the experiment reported here (N use experiment: experiment 2). Further, perhaps longer-term, studies on this may be warranted. In the current study, it is possible that the drought during the second growing year (experiment 2) may have had an impact on the NUE findings.

The findings overall in the current studies, provide evidence that at the very least the N requirements for *Festulolium* do not exceed those for perennial ryegrass in order to achieve equivalent yields and forage quality. These results can be added to other independent studies involving both the current *Festulolium* cultivars e.g. Humphreys., *et al.* (2014), Macleod *et al*. (2013) and outcomes from alternative *Festulolium* cultivars that demonstrate similar excellent field performance such as the variety AberNiche, the first *Festulolium* variety to gain entry onto the UK's Nationally Recommended Variety List as stated by Humphreys., *et al.,* 2014. For the successful adoption of *Festulolium* by UK farmers as an alternative to perennial ryegrass, it should require N application rates that are at least equivalent, in order to achieve a comparable forage productivity when assessed as monocultures in accordance with standardized NIAB field trial protocols used for impartial assessments prior to entry onto the UK National Recommended Variety lists (Humphreys., *et al*., 2014) and fit-for-purpose for wide-scale commercial use.

Onsets of multiple stresses both climatic and edaphic encountered by grasslands will undoubtedly compromise on annual yields. These abiotic factors interact with grass species natural growth cycles, differences in ontogeny and resilience and persistency and will result in uneven availability of high-quality fodder for livestock use throughout the year. In particular suppressed grass winter growth will result in very limited access to fresh forage for farm animals in the UK. Conserved forage, harvested when grass is in plentiful supply earlier in the year, has an important role as fodder for livestock during the winter when live forage supplies are curtailed. Silage is conserved as a major winter feed in UK and its protein and energy content can affect NUE within ruminants (Böttger *et al.,* 2019). The fresh foliar N concentrations were found to be similar for ryegrass and *Festulolium* (values here for each, respectively; as shown in experiment 2) but did not reflect what was found in the N fractions for these forages during ensilage. Experiment 3 showed that in grass comparison, silage from *Festulolium* cultivar Lp x Fg had a higher CP concentration than in ryegrass, regardless of the forage harvesting date (whether spring (cut 2) or summer (cut 4)). Furthermore, the findings reported here for the first time, provide some evidence that Lp x Fg based silage will, compared to perennial ryegrass, comprise lower SN fractions in forage harvested early in the season specifically at 24h of ensiling. Later, finding suggests there was a reduced early phase protein breakdown (proteolysis) in *Festulolium cv* based silage, when use cut 2 forage. A difference between the two grasses in their protein stability (favouring Lp x Fg) when subjected to ingestion by ruminants has been suggested (Shaw, 2006). The hypothesis of that study was that this reduce proteolysis was derived from the presence of heat shock proteins which were active in Lp x Fg hybrids but not in ryegrass during the early hours following grass ingestion. The hypothesis suggested the stress tolerance in the

Festulolium derived from its tall fescue parent which regularly would encounter high temperatures in its natural environment. The tall fescue parent had a Mediterranean origin where high temperatures were encountered regularly. Shaw (2006), O'Donovan (2015), Kamau (2017), and Kamau *et al.* (2018) through a series of PhD studentships developed the hypothesis and demonstrated the enhanced protein stability expressed and inherited in Lp x Fg (and *L. multiflorum x F. arundinacea var glaucescens*) hybrids. The current study results are the first to extend the previous findings from the *in-vitro* rumen digestion simulations to investigate these properties of *Festuloliums* during ensiling. The findings of silage experiment (experiment 3) presented in the current study demonstrated that the use of spring cut Lp x Fg as silage would, compared to a UK National Listed ryegrass, may shield against protein breakdown particularly at early stage. The outcome of this silage experiment showed that overall, this *Festulolium* produced a silage with a higher average crude protein content over 90 days of ensiling (typical storage time before silage if fed to livestock) than the ryegrass control, highlighting the potential for *Festulolium* to help provide feed security in livestock systems.

5.2 Root productivity of *Festulolium* and relations to above ground growth

The *Festulolium* root ontogeny and architecture, as explained by previous studies (Humphreys., *et al.,* 2013; MacLeod *et al.,* 2013; Humphreys., *et al.,* 2018), was given due consideration in the compaction and NUE experiments presented here (experiment 1 and 2). The root productivity of *Festulolium* was compared with ryegrass and tall fescue under two different conditions firstly, under soil compaction (experiment 1), secondly, at different N application rates (experiment 2). Soil compaction of UK grasslands is known to be a limiting factor in grassland productivity (AHDB, 2015). Diminished soil physical (Hodgson, 1997; Food and Agriculture Organization: FAO, 2008; Bullock and Gregory, 2009; Mossadeghi *et al.*, 2016), chemical (Drew and Sisworo, 1979; Abbasi & Adams, 1998; Głąb and Gondek, 2014; Kuncoro *et al*., 2014; Neira *et. al*., 2015) and biological (Maraun *at al*., 1998; Larsen *et al*., 2004; Chan & Barchia, 2007; Van Klink *et al.,* 2015) characteristics were reported as a consequence of compaction; with a reduction in grass forage productivity (Edmond, 1964; Singleton and Addison, 1999; Menneer *et al.*, 2005). Furthermore, degraded soil due to compaction is likely to reduce the ecosystem services provided by grasslands, which covers >70% of the agricultural land in the UK (Department for Environment, Food and Rural Affairs: DEFRA, 2017). Soil compaction, when it occurs alongside excessive rainfall can further increase the degree of damage to soil health (Patto *et al*., 1978; Buliski and Sergiel, 2014). Any evidence for potential new grass swards that are able to withstand and be more resilient to such compaction events are very welcome and support strategies to aide future sustainability of livestock production systems.

Grass tillers provide a degree of 'cushioning' against the impacts of soil compressed by animal hooves or machinery wheels. At least comparable tiller productivity of *Festulolium* compared to ryegrass may capable of providing equal physical soil safeguard to animal hooves and wheels however, such benefit of *Festulolium* may prominent under repetitive compaction conditions (as found in grazing grasslands) when consider tiller reduction (Ryegrass; 12.8%, Lp x Fg; 0.3%) subsequent to compaction compared to non-compaction soil condition. Further, as mentioned earlier, abundant tillers may provide more bases to generate roots (Matthew *et al*., 2001) and this relationship was proved by significant and positive correlation found between root biomass and tiller density of plants as per the results of hydroponic study of experiment 2 in current work.

However, from the root studies the most notable finding was that of the *Festulolium* cultivar Lp x Fg, tended to have a higher root biomass in the top 0 – 15 cm of soil compared to perennial ryegrass in compacted soil (Compaction experiment: experiment 1). Roots available in soil profile increase soil shear strength to improve resistance against external forces (Tengbeh, 1993) which eventually may minimize level of soil compaction damage under repetitive compaction events and in long term, may aid post compaction soil recovery. Furthermore, a higher root biomass in soil would add numerous soil benefits from a wider perspective, particularly the ecosystem value associated with improved soil structure (Bardgett *et al*. 2014; Bardgett, 2017) in addition to carbon sequestration potentials (Kell, 2011). The research presented in this thesis formed part of a five-year BBSRC-Linked Sureroot Project (IBERS). Parallel studies were conducted to evaluate the potential of *Festulolium* aligned with elite clover cultivars under both monoculture and mix grass/clover swards. Further work tested *Festulolium* on participatory farm sites to study the effects of differing conditions, notably differing soil types. The findings of the on-farm studies, as reported by Powell *et al*. (2018), showed a mean higher WIR under monoculture *Festulolium cv* AberNiche (an Italian ryegrass *Festulolium* hybrid) compared to a control hybrid ryegrass (*cv* AberEve) during the autumn. In contrast, it is recognised that the current work presented here is limited to one specific location and one soil type (based on clay loam) but it did show a similar effect for the perennial ryegrass and *Festulolium* types studied prior to compaction, with all three *Festulolium* (Lp x Fm, Lp x Fg and Lp x Fp) and ryegrass *cv* AberBite having a higher WIR compare to tall fescue *cv* Kora in spring 2016. However, following soil compaction, these differences in soil WIR were no longer significant although the soil under *Festulolium cv* Lp x Fp (Prior), Lp x Fg and Lp xFm showed 25%, 56% and 40% higher infiltration than ryegrass respectively. In a separate study conducted as part of the Sureroot project, Collins *et al*. (2019) conducted at IBERS Gogerddan and in close proximity to the present work (on loamy and silty soil attributed to Denbigh series) found a higher water infiltration under a novel white clover hybrid (*cv* AberLasting) compared to that of under the red clover (*cv* AberClaret), white clover (*cv* AberDai), perennial ryegrass (*cv* AberMagic) and *Festulolium* (Lp x Fm). Li *et al.* (2017) on the "Farm-Platform" at North Wyke research Station, Rothamsted Research on hydrologically isolated fields demonstrated how a mixture of *Festulolium cv* Prior (equivalent to Lp x Fp used in current study) and a white clover variety in comparisons to permanent grasslands, enhanced soil water retention and thereby its immediate release following heavy rainfall. Increased soil water infiltration may delay the starting time of water run off following heavy rain hence, possible soil safeguard and improved soil hydrology are noteworthy. Further, selecting *Festulolium* and clover cultivars which facilitate infiltration to be in a sward mix may enhance the effect and may a better solution for grassland flooding. It is important to note that increased below ground growth of *Festulolium* under compaction not diminish the above ground growth compared to ryegrass agreeing with Turner *et al*., 2008 hence, overall, the above and below ground growth attributes of *Festulolium* together may provide higher resilience than perennial grasslands to mitigate compaction.

The root ontogeny of *Festulolium* over the growing season and with the age of sward when grow in stony, well-drained loam soil (Rheidol series) was interesting. For example, as shown by experiment 2 findings, when root cores harvested (from 0 – 15cm depth) simultaneous to first cut of initial harvest year (2017), the *Festulolium cv* Lp x Fg showed at least similar root biomass of ryegrass and higher roots than tall fescue and Lp x Fm. However, when roots were analysed for their biomass in autumn (in same harvest year) simultaneous to fifth harvest cut, the tendency of having higher roots in Lp x Fg compared to ryegrass was revealed. This root ontogeny may be compromised by severe drought during second harvest year (2018) of same experiment and resulted similar roots biomasses among the grass cultivars. In compaction experiment (experiment 1), when root cores collected from four years old grass swards (0 – 30cm depth) in late autumn, 2016 from compacted and non-compacted soils, the *Festulolium cv* Lp x Fg showed a tendency for a higher root productivity than ryegrass and tall fescue respectively. Generally, year 2016 and 2017 represented favourable weather conditions for grass growth and may allowed *Festulolium cv* Lp x Fg to perform well in terms of root growth compared to ryegrass specifically in later in growing season (autumn) regardless sward age. This root ontogeny of *Festulolium* showed and explained previously by Humphreys., *et al*., 2018. Roots aid nutrient uptake by plants and the efficiency depends on plant above ground traits and root system architecture related traits (Rao *et al*., 2016) such as root biomass (Li *et al.,* 2015), number of axial roots, root area, length, branching, penetration ability, rooting depth (Rao *et al*., 2016). Further, De Vries and Bardgett (2016) emphasised that root and forage biomass were the main plant dynamics that influence soil N retention.

Evaluating for any potential root growth advantages by *Festulolium* when presented with contrasting N supply was considered worthy of study in experiment 2. In the field experiment, the NupE of *Festulolium* cultivar Lp x Fg under low N supply (178 kg N ha^{-1} $year^{-1}$) was found to be higher than that of the ryegrass during the second harvest year (2018) which included an extremely dry summer.

This indicated a possible higher uptake of applied N by the *Festulolium* cultivar even at low supply when under potential drought stress. Furthermore, in the same harvest year, N utilization of ryegrass and *Festulolium* cultivar Lp x Fm were comparable both at low and high (356 kg N ha^{-1} $year^{-1}$) N supply. Both grasses were better than Lp x Fg at low N. Therefore, having higher N uptake of Lp x Fg at low N does not guarantee it better utilization. To confirm the economic value of increasing N uptake and utilization, Kant *et al.* (2011) estimated that annually 1.1 billion US$ can be saved globally by increasing those N efficiencies by just 1 per cent. Overall, the forage and root productivity of *Festulolium* may improve N utilisation in plant-soil system.

These main findings of the N use experiment showed not only the potential of *Festulolium* cultivars to address the extreme conditions but also to reveal more opportunities of producing different cultivars for a specific target as they shown cultivars specific characteristics (e.g. differing root behaviours at compaction and N responses). Therefore, plant breeders and geneticist have the challenge to design grasses equipped against climate distresses and its related other severe outcomes like compaction and N use.

6 Industry messages

Festuca-Lolium complex has been gaining special attention in the regions of adverse climatic conditions for grass growth and development often encountered due to its higher resistance and adaptability to such environments. As interspecific hybrids *Lolium* and *Festuca* species (*Festulolium*) share some complementary traits bringing the potential benefits of both parents to the fore (Thomas and Humphreys, 1991). Although the focus of the current work was the suitability for *Festulolium* to be used within a UK livestock system context, it is also widely discussed with respect to the global challenges facing agriculture, with respect to climate change derived abiotic stresses (e.g. Ghesquiere *et al*., 2010). The current study revealed that first harvest year total forage DM yield productivity of a UK National Listed ryegrass was higher when the weather conditions were optimal for grass growth (experiment 2) but findings on the total forage yield (when sward >4 years) in experiment 1 showed there was a comparable yield of *Festulolium*. Under drought stress and very early in the growing seasons (latter indicative of winter grass growth), this study found a higher forage yield for *Festulolium* hybrids compared to ryegrass (experiment 2). Beyond this, the reduced proteolysis in certain *Festulolium* cultivars, shown in related studies under rumen like conditions, was supported by the findings presented here (experiment 3) for reduced proteolysis during early phase of ensiling particularly when use spring cut forages. Resistance to proteolytic activities for *Festulolium* cultivar Lp x Fg was observed during ensiling also proved stress tolerance of hybrids but making silage with a higher crude protein concentration when considering average over 90 days of ensiling.

The tendency for a higher below ground root biomass of *Festulolium* cultivar Lp x Fg under compaction (experiment 1) illustrated various possible soil benefits and related ecosystem services that confirms earlier research. Positive correlations of grass root biomass and N use and utilization indicate *Festulolium* may aid above ground growth and resilience of *Festulolium*. The higher N uptake of certain *Festulolium* both at low N supply and water deficit also worth.

Though, equality among *Festulolium* cultivars experimented in current study for certain measurements were detected, they were also distinct in some cases. The use of different related fescues as parent material offers the potential for “designer-breeding” for alternative target traits. Further investigations of *Festulolium* progenies and alternative fescue species’ genomes to complement with related ryegrass may provide further opportunities for farmers to use these novel grasses, depending on their key attributes and the requirements of the end user. Plant phenotypic traits are the compound effects of interactions between genotype and environments hence, future field trials of *Festulolium* under various soil and climatic conditions may generate broader agronomical and ecosystem benefits. For example, exploring *Festulolium* rooting at greater depth alongside the potential for C sequestration by deeper rooting under various field conditions (the current study was limited to 30cm maximum soil depth and a particular soil type). Future investigation of flood resilience of *Festulolium* compared to ryegrass would also be the interest of industry.

The compaction treatment employed here was a “one time only” and not a repetitive process, as generally found in grasslands which may be subjected to repeated compaction during throughout the season. Hence, future work to evaluate integrated effect of soil compaction under real grassland compaction conditions and to study its interaction with variable N application rates (exploring full N response curve) both warrant further investigation. Broadening the approach, looking at other nutrient supply like P and respective grass use efficiencies alongside of other forage quality characteristics such as fibre content, dry matter digestibility and metabolizable energy might also be interesting in terms of future perspectives. Further advances in the methodologies to aid the detection of the root architecture and ontogeny, including branching, number of lateral roots, strength, root surface area, number of root tips and diameter at various soil types, of *Festulolium* cultivars would enable a greater assessment of their wider employability within livestock systems. Furthermore, it is recognised that the research undertaken here, whilst fully considering livestock systems did not include any animal-based experiments. These will be required to determine the effects of *Festuloliums* on livestock productivity and their environmental impact through evaluation of enteric methane emission and ammonia and nitrous oxide release from excreta, which are essensially linked with ruminant N use efficiencies. Finally, economic assessment of adopting *Festulolium* in place of ryegrass together with their environmental footprints will ease their selection.

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