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Increasing legume forage productivity through slurry application – a way to intensify sustainable agriculture?

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Highlights:

- Aim to increase DM and N yield of legumes under frequent cutting by slurry addition
- Dry matter yields were all significantly higher where slurry was applied
- Legume forages had higher DM yields, with lucerne having highest DM yield
- Slurry increased ryegrass yield at first cut and red clover yield at second cut
- Increase in DM yield of legumes treated with slurry but no increase in N yield

Abstract

Legume forages are fundamental in the development of sustainable livestock systems, building soil fertility and providing home-grown protein for ruminant livestock. Legumes derive N from the

atmosphere but defoliation reduces N-fixation, reducing yield. Livestock, particularly dairy, systems generate considerable quantities of slurry. There is a lack of knowledge on whether this resource can be used sustainably to improve the dry matter (DM) yield of regularly defoliated legumes. An experiment investigated whether applying slurry to red clover (*Trifolium pratense*), lucerne (*Medicago sativa*) or hybrid ryegrass (*Lolium hybridicum*), managed by frequent cutting, would increase DM and nitrogen (N) yield compared with plots without slurry. Treatments were compared within a randomised block design. Plots were harvested for silage on four occasions. Soil P and K indices were maintained at sufficiency levels throughout. Forage DM yields were higher on all plots treated with slurry than those with zero slurry. Overall, legume forages had higher DM yields than ryegrass, with lucerne having the highest DM yield. DM yields decreased at each cut for all forages ($P < 0.001$); however forage N content increased after each cut ($P < 0.001$). Slurry addition increased ryegrass yield at first cut and red clover yield at second cut ($P < 0.05$) indicating ryegrass needed the nutrient boost to commence growth, whilst red clover needed it to aid recovery after defoliation stress. N concentration differed between forages, although for ryegrass and red clover there was no difference between slurry treatments ($P > 0.05$) but was higher in lucerne grown without slurry. Applying slurry to legumes under frequent cutting regimes increased total DM yield but not N yield. Overall, findings demonstrate the potential to use slurry as a resource to intensify sustainable agriculture by increasing the DM yields of frequently harvested forage legumes.

Keywords: red clover, *Trifolium*, lucerne, *Medicago sativa*, nutrient budgeting, alternative forage crops, sustainable agriculture.

Increasing legume forage productivity through slurry application – a way to intensify sustainable agriculture?

1. Introduction

In the last 30 years food production has started to plateau, whilst environmental awareness has increased, producing a conflict between the need to intensify agriculture, but without increasing land area, creating the idea of sustainable intensification (Garnett *et al*., 2013). Using legumes as a forage crop will build soil fertility (Shah *et al*., 2003) and provide a source of home-grown protein for ruminant livestock (Peyraud *et al*., 2009). Leguminous species commonly grown as forage crops are white clover (*Trifolium repens*), lucerne (*Medicago sativa*) and red clover (*Trifolium pratense*). In monoculture, clovers are grown over an area of 10 million hectares across Europe (Rochon *et al.*, 2004) and lucerne covers over 28.4 million hectares worldwide (Yuegao and Cash 2009). Legumes generally have higher crude protein levels compared to ryegrass (Butler et al., 2012). These high-protein forage legumes have been found to improve ruminant productivity due to enhanced nutrient

use efficiency compared to grass diets (Marley *et al.*, 2007).

Legumes fix nitrogen symbiotically deriving N from the atmosphere, reducing reliance on N fertiliser required for ryegrass forages when grown together in mixtures (Butler *et al.*, 2012). Nitrogen fixation in above-ground plant tissues is approximately 20 kg of shoot N fixed for every tonne of herbage dry matter (although this varies if legumes are grown in a pure stand or mixed sward) (Peoples *et al.*, 2012). As well as variation dependent on legume species, management and soil N supply which can all alter the amount of soil N fixed by legumes (Mallarino *et al.*, 1990). Forage dry matter yield is greatly influenced by nitrogen acquisition through fixation, with research showing relationships between growth rate, dry matter yield and companion cropping (Carlsson and Huss-Danell, 2003). In particular, N_2 fixation is reduced by plant defoliation (Butler, 1987), due to a reduction in energy provision from photosynthesis (Farnham and George, 1994). Under regular simulated defoliation in pot trials, pure stands of white clover have been shown to respond to N fertiliser treatment (Marriott and Haystead, 1992). Lucerne has also been shown to benefit from N fertiliser additions during the establishment phase (Hannaway and Shuler, 1993) but the addition of slurry to legume-grass mixtures did not reduce N_2 fixation in lucerne (Rasmussen 2012). Typically, the persistency of legumes in grassland systems have been found to be affected by N applications and competition with non-N-fixing plants (Schipanski and Drinkwater, 2012), with a negative relationship found between repetitive N applications over the growing season and clover persistence and production (Frame and Boyd, 1987).

The efficient use of slurry and manure is central to the development of sustainable livestock systems, notably dairy systems. Slurry and manure are valuable sources of farm nutrients which can be used to reduce reliance on bought-in inorganic fertilisers (Crotty *et al.*, 2014) but its value will depend on farmers having suitable storage facilities and good management practices at spreading, to reduce the potential environmental risks. Dairy cows will produce between 69 – 103 kilograms of manure per 1000 kilograms of live weight (Wilkerson et al., 1997), which can create imbalances in supply compared to the amount of land available for its efficient utilisation. Improvements in slurry utilisation across Europe have focused on developing slurry treatment methods to enhance N efficiency, e.g. anaerobic digestion, manure composting, slurry separation, acidification, aeration mixing etc and manure application e.g. injection, trailing shoe / hose, rapid incorporation etc (Webb et al., 2013). These factors highlight the need for research to understand the implications of the use of this resource as a fertiliser when legume forages are grown as sustainable protein feed on livestock farms in the pursuit of sustainable agronomic practices for forage-based livestock systems. The aim of this study was to test whether the application of slurry to plots of red clover or lucerne, when frequently cut for silage production would, overall increase dry matter (DM) yield, and thereby N yield, when compared with plots without slurry and in comparison to ryegrass.

2. Materials and methods

2.1 Experimental site, plot establishment and maintenance

Red clover *(Trifolium pratense)* (cv. Merviot), lucerne *(Medicago sativa)* (cv. Vertus) or hybrid ryegrass (*Lolium hybridicum*) (cv. AberExcel), were sown as pure stands in early September at 14.5, 19 and 35 kg ha^{-1}, respectively in 12 x 2.5 m plots, in a randomised block design (Figure 1). The treatments were the three forage crops, either with or without slurry application, with four replicates of each treatment creating 24 plots. The soil within the site was of the Rheidol series and was a stony, well-drained loam located at the Institute of Biological, Environmental and Rural Sciences (IBERS) site, University of Aberystwyth, Wales (52° 26' 55" N, 4° 1' 27" W) (see Crotty *et al.*, 2014 for full site description). Ground limestone was applied at a rate of 5 t ha^{-1} to achieve a target soil pH of 6.0. Soil P and K indices were amended using a muriate of potash applied at the rate of 140 kg K_2O ha^{-1} and triple super phosphate at the rate of 100 kg P_2O_5 ha^{-1} (indices 2+ as specified in UK government fertiliser manual RB209 (DEFRA, 2010)). Prior to this, the experimental site was part of a grass-cereal rotation.

During the first harvest year, plots were maintained by cutting to a height of 6 cm on 1 April and then 10 cm on 26 May, 13 July, 3 September, and 18 October. Ryegrass plots were also cut at a height of 6 cm on 10 December (Figure 1). At each harvest, the forage material was removed. Inorganic N was applied to ryegrass plots as 34.5 % ammonium nitrate, on four occasions, at a target rate of 200 kg N ha^{-1} annum^{-1} using a Gandy plot fertiliser (BLEC Landscaping Equipment Ltd., Spalding, Lincolnshire). P and K were added to maintain indices of 2+ to 3 on 28 May and 8 September. Slurry collected from 12-month-old dairy heifers fed on ryegrass / clover silage was stored in 1 m^3 plastic vessels at 4°C prior to use. During the second harvest year, slurry was applied to half of each plot of each treatment (Figure 1) in a randomised block design. The slurry was applied manually using calibrated watering cans with a spoon attachment to simulate a splash-plate (surface broadcast) application. At application, the slurry was diluted so that all was applied at a ratio of 1:1 slurry to water. To avoid any effects of weather conditions or time of day, the slurry was kept well mixed and applied within a set time on the same day. Slurry was applied at a rate of 35 t ha^{-1} twice, firstly on 30 March and then secondly on 24 May (Figure 1). This provided a total of 114 kg N ha^{-1} annum^{-1}.

2.2 Soil and slurry analysis

Experimental soil samples were taken to a depth of 0 – 30 cm and 30 – 60 cm for N analysis (or to bedrock if soil depth was less than 60 cm from the surface). Soil analysis was determined from samples obtained in the autumn of the first harvest year (prior to slurry application) and the spring and autumn of the second harvest year (after slurry application); from bulked cores taken in a W-formation across each individual plot. Soil N was determined as nitrate (NO_3-N) and ammonium-N

(NH$_4$-N). P and K analysis was also performed to check sufficiency levels were maintained throughout the harvest years. Sub-samples of each slurry type were collected at the same time as spreading and were analysed for pH, dry matter (DM) content, total N, nitrate-N and ammonium-N. Ammonium-N and nitrate-N concentrations were determined as described in Crotty et al., (2014). Briefly, ammonium-N and nitrate N were extracted using a 2M KCl solution; nitrate was reduced to nitrite using a cadmium column followed by colorimetric measurement at 520 nm and ammonium-N at 660 nm.

There were no significant differences between the composition of the slurry applied at the first application (30 March) and the second application (24 May) for all parameters measured. Organic C content of the slurry was 14.5 ($\pm$ 0.88) g kg^{-1} and total N content was 1.6 ($\pm$ 0.05) g kg^{-1} (with ammonia at 72.9 ($\pm$ 1.92) and nitrate at 2.1 ($\pm$ 0.60) mg 100g^{-1}) and had a dry matter percentage of 9.5% ($\pm$ 0.25). The slurry was relatively alkaline at pH 8.4 ($\pm$ 0.05) and K content was 5.9% ($\pm$ 0.35); Ca 1.7% ($\pm$ 0.05); P 0.8% ($\pm$ 0.03); and Mg 0.7% ($\pm$ 0.04).

2.3 Dry matter yield and nitrogen offtake

Plots were harvested by cutting to a height of 10 cm on four occasions: 23 May, 12 July, 5 Sept and 25 October (Figure 1) using a Haldrup 1500 plot harvester (J. Haldrup a/s, Løgstør, Denmark). Forage material cut from an area of 12 m x 1.5 m within each plot was used to determine fresh yield. Representative sub-samples of harvested forage were taken to determine dry matter (DM) yield and N offtake and the botanical composition of the sward. Sown and non-sown components (i.e. legume or grass and non-legume/non-ryegrass depending on treatment) were hand separated to determine annual DM yield, as represented by the accumulated yield of each single harvest, for each of the forages. All sample material was stored at - 20ºC prior to subsequent chemical analysis. Forage DM content was determined by drying to constant weight at 80 °C in a forced-draught oven, and the DM content of the samples taken for chemical analysis after freeze-drying. Forage and slurry total N concentrations were determined using a Leco FP 428 nitrogen analyser (Leco Corporation, St. Joseph, MI, US). Total N input was calculated as the sum of slurry N applied, N$_2$ biological N fixation by the legumes (Cuttle *et al.*, 2003) and atmospheric N deposition at a rate of 25 kg ha^{-1} yr^{-1} (Kirkham, 2001). N balance was calculated by subtracting total offtakes, summed over the entire period (four cuts) from total N input (Vos and van der Putten, 2000).

2.4 Statistical analysis

All data were analysed using GenStat (14th Edition, (Payne *et al.*, 2011)). Effect of fertiliser application on DM yield, N offtake and botanical composition were assessed by an analysis of variance (ANOVA) according to the randomised block design. A repeated measures ANOVA was

used to compare differences in DM yield, N offtake and botanical composition at each harvest period (cut). Soil N composition on two sampling dates and at two depths was also compared by ANOVA. Where applicable, the Student Newman Keuls test was used for multiple comparisons within treatments, when crop/treatment as a factor was significantly different ($P < 0.05$). Apparent N recovery (ANR) was calculated according to the method of Kanneganti and Klausner (1994). Using the calculation that N offtake ((N offtake with slurry – N offtake without slurry) / slurry N applied) x 100, expressed as a percentage of the difference in total N applied.

3. Results

3.1 Slurry and soil analysis

The ammonium content of soils was not significantly affected by crop ($P = 0.367$) or fertiliser treatment ($P = 0.321$) (Table 1). There were differences in soil chemistry between the two soil depths across treatments ($P < 0.001$), with the lower depth (30-60cm) always significantly lower in ammonium content than the upper soil layer (0-30cm) (Table 1). There was also a greater accumulation of ammonium in the soil over time ($P = 0.018$), with a significant increase from the spring to the autumn (Table 1). However, there were no significant interactions between the treatments, time and depth of sampling for soil ammonium content. Significant differences were found between forage treatments for nitrate content ($P < 0.001$; Table 1) with both ryegrass treatments (with and without slurry application) having significantly lower soil nitrate than the two legume treatments (with and without slurry application). There were also significant differences between the two depths (across treatments) ($P < 0.001$; Table 1) with the lower depth (30-60cm) always having a lower content than the upper soil layer (0-30cm). There was also a higher amount of nitrate found in the autumn sampling (after both slurry applications) across all forage treatments than the previous spring ($P = 0.004$; Table 1; after one slurry application). However, there were no interaction effects between time, crop and fertiliser treatment ($P = 0.416$) or time, crop and depth ($P = 0.443$) (Table 1).

3.2 Sward density, herbage yield – totals and individual cuts

DM yields were significantly different among treatments ($P < 0.001$), in the second harvest year (post slurry application), with both ryegrass treatments (with and without slurry) having a significantly lower yield compared to all legume treatments (Table 2). Red clover with slurry had 161% higher DM yield than ryegrass with slurry applied; however red clover with slurry had only obtained 7.6% higher yields than red clover without slurry; Lucerne with slurry obtained a similar yield increase of 7.8% compared to lucerne without slurry. The lucerne treatment obtained the highest yields, lucerne with slurry was significantly higher than both the lucerne and red clover without slurry treatments (Figure 2). A similar pattern was seen when considering the yield of only the sown species ($P < 0.001$). There was no significant difference between total dry matter yield and the total sown yield (T-test: T-

statistic = 0.45 on 190 d.f; P = 0.652); and the amount of unsown (weed) species growing across treatments was negligible. There were no differences across treatments for the yield of unsown (weed) species (P = 0.452; Table 2). There were however, significant differences in yields between cuts for unsown yield, with the highest yield of unsown species found in the first cut. The low yields of unsown species, shown in the botanical composition, confirm that the treatment effects studied were due to the forage treatments sown. Regular cutting did not increase the unsown species yield, as it was highest after the first cut and reduced over time (Table 2). There were also no differences among forage treatments, with or without slurry application, for unsown species yield.

Overall, dry matter yields decreased at each cut across all forage treatments, consequently $1^{st} > 2^{nd} > 3^{rd} > 4^{th}$ (P < 0.001), with significant differences also found between each of the forage treatments (P < 0.001) (Table 2). Ryegrass had significant differences in yield for the first cut (54 days after slurry addition) with higher yields for the 'with slurry' treatment. Red clover had significant differences in yield when comparing with or without slurry for the second cut only (50 days after slurry addition). Lucerne obtained relatively similar yields for crops grown with or without slurry for each of the cuts.

3.3 Nitrogen content, offtake and apparent N recovery

The N content of the forages were significantly different (P < 0.001), with ryegrass having a significantly lower N concentration than both red clover and lucerne (with and without slurry). Whilst lucerne without slurry had the greatest N concentration, significantly greater than lucerne grown with slurry, and both red clover treatments (Table 2). The N offtake yield differed among the three forages (P < 0.001) but there was no difference for N offtake yield between the two treatments within the same crop (Table 2). The legumes obtained over six times the amount of N offtake (kg) as the ryegrass (with and without slurry addition) forages; at the same time the red clover without slurry had significantly lower N offtake compared to both lucerne treatments which had the highest offtake, whilst the red clover with slurry treatment was intermediate (Table 2).

Considering the effect of frequent cutting on N concentration for each forage, there were significant differences between forage and cut (both P < 0.001 respectively). However, the N concentration of the forages all increased over time ($4^{th} > 3^{rd} > 2^{nd} = 1^{st}$). Lucerne showed significant differences between slurry applications for N concentration, with N concentration being greater in the lucerne grown without slurry treatment. The total DM yield was positively correlated with the total N the crops received (through fixation and slurry) (using a simple linear regression P < 0.001). The N balance calculated found significant differences between forages and slurry treatments (P < 0.001); however, this relies on accurate estimation of total N input (including biological N fixation by the legumes). The apparent N recovery (the amount of slurry N utilised by the crops) there was no significant differences between crop (legume or ryegrass, P = 0.556; Table 2). The apparent N recovery from

slurry was lowest for lucerne (-1.9%) whilst the red clover and ryegrass were both numerically higher and similar (16.1% and 16.5% respectively). The N balance was significantly different between treatments, with the most negative balance for both lucerne and red clover without slurry treatments. All forages with slurry applied had positive N balances (Table 2).

4. Discussion

The aim of this experiment was to improve our understanding of nutrient utilisation by leguminous forages after repeated defoliation from harvesting, through the application of slurry to red clover or lucerne monocultures in comparison to ryegrass. Where these leguminous monocultures are cut for silage production, this frequent defoliation is known to reduce N_2 fixation (Butler, 1987). Therefore, supplementation of N through slurry addition was used to minimise the effect of this reduction in N_2 fixation and to increase DM and, potentially, N yields, compared to plots without slurry application. The optimisation of nutrient use and supply has the potential to sustainable intensify agriculture, through a greater understanding of the performance of forage legumes - increasing production, whilst utilising slurry, thereby reducing slurry storage costs. When stored and utilised correctly, farmyard manure and slurry are a source of essential plant nutrients (Bradford, 1999) that reduce the need for expensive inorganic fertilisers and help to improve soil quality.

Leguminous crops have the potential to build soil fertility over time through the transfer of surplus N to the soil (Marley *et al.*, 2013). This was evident from the higher soil nitrate concentrations found in the legume treatments compared to ryegrass (Table 1). Fields with a history of legume-based management have been found to have larger labile soil N pools, although intercropped legumes tend to have higher biological N fixation rates than pure stands because non-legumes out compete legumes for available soil N (Schipanski and Drinkwater, 2011). DM yields of the forage treatments were within the expected range for yields, given the N applications received and exemplify the high yields of leguminous forage crops that can be achieved without any requirement for additional N fertiliser. For example, the lucerne yield with slurry was very similar to expected yields (14.0 - 14.9 t DM ha^{-1} for the vertus cultivar) (NIAB, 2002). The study did show that DM yields were increased for all forages when slurry was applied compared to the non-slurry treatment – including the leguminous forages (Figure 2). The higher DM yields of the leguminous crops compared to ryegrass confirmed there was a deficiency of N to sustain high yields of ryegrass. This was expected as the slurry application only provided 114 kg N ha^{-1} annum[-1] whereas, on average, N applications to ryegrass is normally in the range of 200 - 400 kg N ha^{-1} annum[-1] (NIAB-TAG, 2012). However, this was the experimental approach required to allow for a direct comparison of the effects of the slurry between the ryegrass and the legume treatments.

Organic fertiliser is mineralised slowly and directly benefits the crop and ecosystem, with a low risk of leaching, as long as an actively growing crop is present that can take up the nutrients supplied (van Eekeren *et al.*, 2009). Slurry was applied twice, 54 days prior to the first cut, and then immediately after the first cut - 50 days prior to the second cut according to standard farm practice (Figure 1), therefore soil nutrient availability was at its highest around the first two cuts compared to the latter cuts. Ryegrass yield had significant differences between slurry treatments after the first cut, but not after any of the other cuts. This implies that ryegrass needed the initial nutrient boost, more than the supplementary nutrient addition after the second slurry application. Red clover had a significant difference in yield between the slurry treatments after the second cut only, suggesting the addition of slurry at that time aided recovery after defoliation (Culvenor *et al.*, 1989), with concentrations of N within the crop across forage and slurry application increasing over the growing season.

Legumes generally have higher crude protein levels compared to ryegrass (Butler et al., 2012), making them a more profitable forage. After defoliation / harvesting, photosynthesis is low, thus reducing legume N production from the symbiosis, and so supplying legumes with fertiliser can allow for a more rapid regrowth (Barber et al., 1996; Dear et al., 1999; Haby et al., 2006). Overall, the lucerne treatment obtained the highest yields, particularly lucerne with slurry. Although lucerne can obtain most of the N required for growth via symbiotic N_2 fixation, it has been found to be very effective in removing inorganic N from the soil (Yang *et al*. 2011), under natural conditions lucerne was obtaining 35% of its N from the soil rather than symbiotic fixation, therefore additional N supplementation may help to maximise yield potential. It is easier and less energy-consuming for a plant to absorb N from the soil than for it to fix it from the air (Warembourg and Roumet, 1989). The calculation used for total N input was likely to have underestimated the amount of N fixed by the legumes (Anglade et al., 2015), leading to negative N balances for the control forage treatments, whilst studies have consistently found legumes to increase soil fertility (e.g. Cuttle *et al.,* 2003; Shah *et al.,* 2003; Nyfeler *et al.,* 2011; Marley *et al.,* 2013 and Zhao *et al.,* 2014). Lucerne was the only forage that showed significant differences between its N composition dependent on treatment (with / without slurry applied), and N concentrations were higher in the without slurry application, indicating that the mechanism of N_2 fixation was more effective at accumulating N in the plant tissues than slurry N absorption. This difference could also be due to the increased growth of lucerne with slurry but without the necessary improvement in the uptake of N to also increase the N concentration of the plant and the total N yield. . For red clover, findings show there was an increase in productivity for the red clover treatments with the addition of slurry and yet, despite there being a similar N forage concentration for red clover treated with slurry compared to without slurry, this did not result in a higher total N yield. One possible explanation may be that although these legumes have similar seasonal growth patterns (relative to ryegrass) (Marley *et al*., 2013), there may be differences in their seasonal patterns of root N reserves under frequent cutting regimes (Teixeira *et al.*, 2007). For

example, it is not well understood how legumes alter the formation of lateral roots and N-fixing nodules in response to environmental (e.g. soil N availability) and microbial signals (e.g. symbiotic rhizobia) (Goh *et al*., 2016). Further research is needed to understand the differences in the underlying mechanisms altering N uptake and N fixation in these different legumes when harvested under frequent cutting regimes.

Ideally N balances should be positive to compensate for unavoidable losses to the environment (Vos and van der Putten, 2000). However in this study, the total DM yield was positively correlated with the total N the crops received (through fixation and slurry). The N balance findings suggest that our estimates for the fixation of legumes (from Cuttle *et al*., 2003) may have been too low. As all forages with slurry applied had positive N balances (Table 2). Overall our results show an increase in productivity for the treatments with the added slurry across the different forages, but not a significant increase in N yield. As we were cutting for silage, there was still a large proportion of the forage left remaining in the field (cutting height 6 cm and 10 cm for ryegrass and legumes, respectively), which could account for the low apparent N recoveries (Dalal *et al.*, 2011) and could have resulted in differences among the treatments. However, there was no significant difference found between the ryegrass and legume treatments for apparent N recovery, implying that further work is needed to measure differences in slurry application to assess whether greater applications would affect overall N yields.

Forage production and the maintenance of soil structure, water regulation and nutrient supply are intricately linked (Murray et al., 2012). Utilising slurry as an N source is preferential than inorganic fertiliser as the benefits to leguminous growth are not vast, although this experiment has shown that productivity could be increased with little impact on cost. Studies of companion cropping, have found mutual stimulation of N uptake leading to increased yield from grass-legume mixtures compared to monoculture alone (Nyfeler *et al.*, 2011). The ability of legumes to fix N and accumulate it in the soil for the benefit of companion or following crops is also well known (Nyfeler *et al.*, 2011; Marley *et al.*, 2013). The accumulation and release of N from legumes is dependent on many factors including harvest management and N mineralisation of the legume residue (Janzen *et al*., 1990). In the UK, white clover is included in at least 75% of pasture seed mixes which are regularly fertilised, however, the level of clover persistence over time could be affected (Frame and Boyd, 1987). The application of slurry to forage legumes within livestock systems has the potential to increase forage DM yield, whilst also maintaining sustainable farming systems.

5. Conclusions

The experiment investigated the effects of slurry addition on nutrient utilisation by leguminous forages, red clover and lucerne under a frequent cutting regime in comparison to ryegrass. Dry matter yield was significantly greater in treatments with slurry across crop type, but noticeably in the legumes treatments. Our results show an increase in productivity with the added slurry addition but not a significant increase in N yield. The lack of slurry effect on total N yield was due to lower N concentrations of lucerne when treated with slurry. Further work is now needed to understand the factors affecting the optimum amount of N that can be supplied via slurry to leguminous crops managed under frequent cutting regimes, ideally using multiple sites and slurries to further validate the findings. Findings show the benefits of slurry as a valuable fertiliser, which has the potential to support the development of sustainable agricultural systems based on legume forages, by increasing forage production whilst converting farm waste into a benefit, via the utilisation of slurry. Overall, this research exemplifies how the optimisation of nutrient use and supply has the potential to sustainable intensify agriculture, sustainably increasing forage production whilst effectively utilising slurry.

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Table 1. Mean N content of soil (mg kg DM^{-1}) from plots (0-30 cm and 30-60 cm) of hybrid ryegrass, lucerne and red clover in the autumn and spring prior to and following autumn post slurry application. Analysis of variance was used to compare treatments (crop and fertiliser) in the same harvest year (n = 4).

Depth	Sampling	NO$_3$ Ryegrass Slurry	Control	Lucerne Slurry	Control	Red Clover Slurry	Control	NH$_4$ Ryegrass Slurry	Control	Lucerne Slurry	Control	Red Clover Slurry	Control
0-30	*Autumn (Harvest year 1)*	4.1	5.7	8.4	9.5	6.8	9.6	5.4	4.4	4.5	5.0	5.1	4.3
30-60		0.1	0.4	2.0	2.5	1.3	2.0	3.7	2.9	3.4	3.4	3.5	2.9
0-30	*Spring (Harvest year 2)*	3.1	3.0	6.0	5.6	6.4	6.5	7.2	6.7	6.1	6.2	6.0	5.1
30-60		0.2	0.2	2.7	2.9	3.4	3.2	4.1	4.2	4.4	3.7	4.3	4.3
0-30	*Autumn (after slurry)*	6.2	5.1	8.0	7.5	8.0	7.3	8.4	8.0	7.6	6.0	8.1	7.1
30-60	*(Harvest year 2)*	0.9	0.8	2.3	2.0	3.1	2.3	4.4	4.7	5.1	4.2	4.0	5.1

ANOVA		**s.e.d**	**Prob**	**Significance**		**s.e.d**	**Prob**	**Significance**
	Crop	0.489	<0.001	HRG < Lucerne = Red Clover		0.429	0.367	No difference
	Fertiliser	0.399	0.954	No difference		0.350	0.321	No difference
	Depth	0.399	<0.001	30-60cm < 0-30cm		0.350	<0.001	30-60cm < 0-30cm
	Time	0.399	0.004	Spring < Autumn		0.350	0.018	Spring < Autumn

Table 2. Annual total forage dry matter yield, nitrogen concentration, mean total N input, N offtake and N balance (kg ha^{-1} year^{-1}), apparent N recovery for plots of hybrid ryegrass, lucerne and red clover either treated either with or without slurry during the second harvest year.

	Cuts	Ryegrass Slurry	Ryegrass Control	Lucerne Slurry	Lucerne Control	Red Clover Slurry	Red Clover Control	ANOVA	Treatment	Cut	Cut x Treatment
Dry Matter Yields (kg)	1 [d]	3238	2235	5128	4989	4998	5136				
	2 [c]	1146	739	4183	3769	4363	3814	SED	84	122	272.1
	3 [b]	407	385	3380	2937	3373	2890	Prob	**<0.001**	**<0.001**	**<0.001**
	4 [a]	206	221	1062	1058	333	305				
	Total	*4997*[b]	*3580*[a]	*13753*[e]	*12753*[cd]	*13068*[de]	*12145*[c]				
Unsown Yield (kg)	1 [b]	252	98	533	630	15	91				
	2 [a]	56	44	15	69	1	112	SED	101.6	78.2	194.5
	3 [ab]	130	129	0	1	0	593	Prob	0.452	**0.036**	0.131
	4 [a]	16	22	4	7	4	13				
	Total	*454*[a]	*293*[a]	*552*[a]	*707*[a]	*20*[a]	*809*[a]				
%N	1 [a]	1.15	1.15	2.91	3.20	2.93	2.94				
	2 [a]	1.51	1.38	2.98	3.30	2.65	2.82	SED	0.093	0.055	0.149
	3 [b]	2.44	2.40	3.17	3.36	3.04	3.12	Prob	**<0.001**	**<0.001**	**<0.001**
	4 [c]	3.53	3.50	5.03	5.08	5.07	5.03				
	Mean	*2.16*[a]	*2.10*[a]	*3.52*[b]	*3.73*[c]	*3.42*[b]	*3.48*[b]				
N offtake (kg)	1 [d]	37	25	150	159	146	151				
	2 [c]	17	10	124	124	116	108	SED	3.6	3.4	8.0
	3 [b]	10	9	104	97	102	89	Prob	**<0.001**	**<0.001**	**<0.001**
	4 [a]	7	8	53	54	17	15				
	Total	*71*[a]	*52*[a]	*432*[c]	*434*[c]	*381*[bc]	*362*[b]				
									SED	Prob	
Total N input[1]		139	25	439	325	384	270		0.093	< **0.001**	
N balance[2]		67.8[c]	-27.4[b]	109.7[b]	-0[a]	3.3[b]	-92.3[a]		14.31	< **0.001**	
Apparent N recovery[3]		16.5		-1.9		16.1			18.42	0.556	

Different superscript letters denote significant differences between treatments (within row) or between cuts (within columns) (P < 0.05).

[1] um of slurry N applied, N$_2$ fixation (Cuttle *et al*., 2003) and atmospheric N deposition at a rate of 25 kg ha^{-1} yr^{-1} (Kirkham, 2001).

[2] N balance calculated by subtracting total offtakes[#], summed over the entire period (four cuts) from total N input (Vos and van der Putten, 2000).

[3] Apparent N recovery (ANR) calculated as ((N offtake with slurry – N offtake without slurry) / slurry N applied) x 100 (Kanneganti and Klausner, 1994).

Figure 1: Schematic representation of ryegrass and legume plots within the experiment, showing the timings of key main management actions. [RC = red clover; G = ryegrass; L = lucerne; C = control; S = slurry treated plots].

Figure 2: Total annual dry matter yield of each forage (with and without fertiliser treatment) (data presented as mean ± se; n = 4). Different letters indicate significant differences (P < 0.05) between treatments as assigned using a Student Newman Keuls test.