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The Effect of an Engineered Biostimulant Derived from *Ascophyllum nodosum* on Grass Yield under a Reduced Nitrogen Regime in an Agronomic Setting

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Abstract: Nitrogen (N) is the most important macronutrient used in modern agricultural systems to enhance crop yields; however, a significant amount of applied N is not taken up by the crop and is lost to the environment. Improving the nitrogen use efficiency (NUE) of crops can curb these environmental losses while concurrently delivering economic gains. Plant biostimulants have potential to improve NUE in agronomic settings. In this research, a granular N-containing fertilizer coated with the biostimulant PSI-362, an extract from the brown seaweed *Ascophyllum nodosum*, was applied to grass managed under different production systems to assess its impact on NUE. The role of soil type, pH, phosphorus (P) and potassium (K) on the efficacy of the biostimulant in improving NUE was assessed using lysimeters filled with six different soils. A significant increase in grass yield (29%) was found with PSI-362 addition at a 75% N rate over the 75% N control (8478 kg of dry matter (DM) ha⁻¹ vs. 6772 kg of DM ha⁻¹) over two years of trials under a simulated grazing platform of six rotations. The NUE increased to 96.6% for the PSI-362-treated grass compared to 82.8% for controls. Field-based evaluations demonstrated no decrease in yield and quality from harvested and grazed grass treated with the biostimulant when the N rate was reduced by 20 to 25%. Based on these results, the application of PSI-362 allows a reduction in nitrogen input by up to 25% without losses of grass yield or quality.

Keywords: nitrogen use efficiency; *Ascophyllum nodosum*; nitrogen reduction; grassland productivity; environment; sustainability; biostimulant

1. Introduction

Just over a century ago, the Haber–Bosch process allowed nitrogen (N) to be synthetically produced on an industrial scale, enabling the beginnings of modern agriculture and thus the ability of the planet to support its current population. N is the most significant of the macronutrients in terms of the amount applied to the soil and its influence on crop yield and quality. The N application rate depends on a number of factors such as crop type and agronomic system; for example, in Ireland, the application rate of N to grassland can vary from 40 kg ha⁻¹ to 306 kg ha⁻¹ depending on the stocking rate [1]. It is also an expensive input for farmers, representing up to one fifth of all direct costs in some production systems [2]. N mineral fertilizers have a large carbon footprint due to the intensive energy requirement for their production along with their transportation costs. The carbon footprint

can range from 3.5 kg CO₂eq/kg nitrogen to almost 12 kg CO₂eq/kg nitrogen for calcium ammonia nitrate (CAN) depending on the region of manufacture and energy source [3]. Additional negative environmental impacts of N use include acidification of soils [4] and the degradation of water quality (in the form of nitrate; NO₂⁻) and air quality [5]. The two main air pollutants from N fertilization are NH₃ (ammonia) and N₂O (nitrous oxide). NH₃ contributes to the acidification of soils and watercourses leading to losses in biodiversity [6], while N₂O is a potent greenhouse gas, with almost 300 times more warming ability than CO₂. Globally, N₂O is responsible for 38% of all direct agricultural emissions [7].

Nitrogen use efficiency (NUE), in agronomic terms, is defined as the percentage of N applied to the soil that is taken up by the crop [8,9]. NUE is dependent on many factors, ranging from the amount of N applied, the timing of application, type of N applied, soil and environmental conditions, to the type and variety of crop [10]. Values for NUE will vary from crop to crop, across regions and between agronomic systems. Estimates for NUE can range from 25 to 80%, thus representing a potential significant loss of N to the environment [11]. Zemenchik and Albrecht (2002) investigated N application rates on three varieties of grasses (Kentucky bluegrass, smooth brome grass and orchardgrass) where they found a N recovery of 0.15 to 0.5 (15 to 50% NUE), while [12] reported NUE varying from 63 to 87% across various compounded mineral fertilizers in perennial ryegrass. Additionally, environmental conditions, N levels and split applications were observed to exert a significant effect on NUE on permanent grasslands for first-cut silage [13].

Plant biostimulants are legally defined within the EU fertilizer regulation 2019/1009 as “any product stimulating plant nutrition processes independently of the product’s nutrient content, with the aim of improving one or more of the following characteristics of the plant: nutrient use efficiency, tolerance to abiotic stress, crop quality traits or availability of confined nutrients in the soil and rhizosphere” [14]. Plant biostimulant products can be classified under a number of categories based on the origin of the raw material used in their manufacture, e.g., seaweed extracts and plant/animal protein hydrolysates, etc. [15]. Traditionally, agronomic research involving biostimulants has focused on enhancing the plant’s ability to overcome abiotic stress leading to improved yield and/or quality. In recent years, there has been an increased interest in investigating their effects on nutrient use efficiency [16,17], driven in part through various initiatives such as the European Commission’s Biodiversity Strategy, where a key goal is a 20% reduction in fertilizer use [18]. Goñi et al. [19] previously reported that under a 75% N regime, a biostimulant-coated granular fertilizer increased NUE in barley by 30–60%. The increased NUE may be attributed to an increased uptake of nitrate (17 to 72% over control plants) that was associated with upregulation of the expression of root nitrate transporters (NRT 1.1, NRT 2.1 and NRT 1.5). The aim of this study was to evaluate the effectiveness of a biostimulant, PSI-362, derived from the brown seaweed, *Ascophyllum nodosum*, on a range of grass production systems to maintain yield and quality with a reduced N (20–25% reduction) fertilizer application regime. The co-application of mineral N fertilizer and a plant biostimulant as reported here is suitable for implementation in current agronomic practice and is aligned with best practice, with regard to timing of granular fertilizer application at key growth stages and optimal environmental conditions. Grass systems ranged from open lysimeter trials with different soils to large-scale field trials (pasture grazing trials and silage harvesting trials) at 13 sites conducted in 2020 and 2021, where there was no loss in grass quality or yield when treated with PSI-362 under a reduced nitrogen regime.

2. Materials and Methods

2.1. PSI-362-Coated Granular N Fertilizer

Granular fertilizer was coated with the biostimulant PSI-362 (an alkaline liquid seaweed extract biostimulant from the brown seaweed, *Ascophyllum nodosum*, engineered using a proprietary process and supplied by Brandon Bioscience, Ireland) at three different rates: 0.5×, 1.0× and 1.5× (the 1.0× rate refers to the rate for commercially available products). The compositional analysis of PSI-362 was previously reported by [19]. For the purposes of

this study, the N content was found to be 0.4% *w/v* (ranging from 0.8 g ha⁻¹ to 2.4 g ha⁻¹ depending on the application rate) while the carbon content of the applied biostimulant ranged from 40 g to 120 g per hectare depending on the application rate. Target Fertilizers (Enniscorthy, Ireland) supplied all granular fertilizers used in the study.

2.2. Modified Lysimeter Grass Trial Setup

The objective of the lysimeter trials was to assess the effectiveness of three rates of biostimulant coated on mineral N fertilizer applied to a ryegrass sward at a reduced N rate (75% N) versus no biostimulant at a 75% N rate and no biostimulant at a 100% N rate on biomass dry matter (DM) accumulation. The experiment was setup to simulate a rotational pasture grazing system with six different soils classified into two different soil types. The six different soils (2 × loam and 4 × silt-loam) were collected from the Kerry, Cork and Tipperary regions of Ireland (each soil type corresponded to a single location) in 2013. Each treatment had three lysimeter replicates for each of the six soils. Soils were classified in terms of their sand, silt and clay contents (Supplementary Table S1) using the dispersion (sodium hexametaphosphate method) PSD test [20]. The modified lysimeters (Supplementary Figure S1) were filled by placing 30 kg of soil (taken from the A horizon to a max. depth of 45 cm) into a 20-litre lysimeter (surface area of 0.003 m² per lysimeter). To ensure adequate drainage, a grommet was placed at the bottom of the lysimeter, over which a layer of coarse sand and pea gravel was added (about 2 to 3 inches) before the soils were added on top. All soils were kept under permanent pasture until 2019 when reseeding commenced with a commercial grass seed mixture (Diamond Hi Digestibility supplied by Kellihers Feed and Agricultural Supplies, Tralee, Co., Kerry, Ireland) applied at a rate of 34.6 kg ha⁻¹ (or 14 kg acre⁻¹) in September 2019. The grass seed mixture contained perennial rye grass (*Lolium perenne*) varieties: Astonenergy (29%), Meiduno (23%), Oakpark (21%), Astonking (21%) and white clover (*Trifolium repens*): Crusader (6%).

2.3. Lysimeter Soil Nutrient Analysis

Prior to experiments beginning in 2020, all soils were analyzed for pH using the SMP buffer test and P and K contents via Morgan's extraction method using colorimetric and atomic spectroscopy, respectively [21]. This analysis was performed by Southern Scientific Services (Farranfore, Co., Kerry, Ireland; Supplementary Table S1).

2.4. Grass Growth Rate, Yield Measurement and Quality Assessment

The lysimeter trials ran from June to October in 2020 and from March to August in 2021. At the beginning of both years, grass in all lysimeters was cut to a height of 4 to 6 cm from the soil level. Fertilizer was applied according to the rates listed in Supplementary Table S2. Application rates were based on an intensive livestock system stocked at rates of 2.47 livestock units ha⁻¹ [1]. The grass growing period per rotation ranged from 18 to 30 days depending on seasonal growth rates. Grass in lysimeters was harvested at the 3-leaf growth stage (≈1200 to 1600 kg DM ha⁻¹) to simulate maximum utilization efficiency under a pasture grazing system leaving a residual of 4 to 6 cm in height [22]. Grass was collected after each harvest/rotation in a plastic bag, weighed immediately to calculate yield and subsequently reported as kg DM ha⁻¹. Dry weight (DW) was calculated by taking representative samples after each harvest and drying these overnight in a convection oven at 100 °C. The growth rate was reported as kg DM ha⁻¹ day⁻¹ and was calculated by dividing the DM yield per rotation by the number of days since the last harvest. Samples were also taken for the determination of crude protein (CP) and dry matter digestibility (DMD) at two timepoints in 2020 (June and September) and 2021 (April and June). CP was determined using a N analyzer (Leco CNS 928 Analyzer) and scanning via NIRS (near infrared spectroscopy) to predict DMD. This analysis was carried out by Southern Scientific Services (Farranfore, Co., Kerry, Ireland). Subsequent to harvesting, fertilizer was applied to all lysimeters within 48 h according to rates listed in Supplementary Table S2, once again to simulate typical agronomic activity, before the pasture cycle continued once

more. Fertilizer was applied as calcium ammonium nitrate (CAN) (27% N + 4% S); the control received 100% of recommended N, while treatments (with/without biostimulant) received 25% less N (75% N). Nitrogen use efficiency (NUE) was calculated by subtracting the determined N in grass from the unfertilized lysimeters (N_0) from the determined N in the various treated lysimeters (N_x), and dividing this figure by kg of N applied: [(grass yield at N_x – grass yield at N_0)/kg of N applied at N_x] \times 100 [23].

2.5. Silage Harvesting Field Trials

In order to assess the success of PSI-362 under typical agronomic systems, silage harvesting and pasture grazing trials were conducted where areas treated with PSI-362 received up to 25% less N and were compared to a 100% N control for that farm, in terms of grass DM yield, DMD and CP.

Silage harvesting field trials were conducted across five sites (five farms) in 2020 and six sites (six farms) in 2021 (trial site coordinates and weather data are available in Supplementary Tables S3 and S4). Soil sampling was carried out at all sites within the previous 12 months with P and K levels being determined as described previously. All trials in 2020 were third-cut grass silage trials, while trials in 2021 were a mixture of first (two trials), second (three trials) and third (one trial) cuts. Nitrogen was applied to treated areas in 2020 via a coated (coated at the 1x concentration of PSI-362) CAN (27% N + 4% S) granular fertilizer, while the control areas received N in the form of CAN (27% N + 4% S) at the appropriate higher rate as stated in Supplementary Table S4 (100% vs. 80% N). For farm 5, N was also applied in the form of organic manure at a rate of 9 kg N ha⁻¹ for both control and treated sites. According to Wall and Plunkett [1], the inorganic nitrogen requirement will depend on the application of organic N, age of the sward, grazing and fertilizer history as well as environmental conditions, and is typically 125 kg ha⁻¹ for first-cut silage and 100 kg ha⁻¹ for subsequent cuts, indicating that the 100% N control did not exceed these rates (Supplementary Table S3).

For the 2021 field trials, a compound fertilizer (a fertilizer that contains nitrogen, phosphorous and potassium (N-P-K)) was coated with the biostimulant PSI-362 (20-2-12 + 3 S; N-P-K + S) and applied to treated areas while control (100% N) areas received either 24-2.5-10 + 3 S or 21-2.2-10 + 3 S (N-P-K + S) at appropriate rates (see Supplementary Table S3). Farms 1 and 2 (both first-cut silage trials) also received N in the form of organic manure at a rate of 12 kg N ha⁻¹, while farm 3 (second cut) received 9 kg N ha⁻¹ from organic manure. All trial sites have been under permanent pasture (perennial ryegrass, *Lolium perenne* L.) for at least three years except farm 11 from the 2021 trial, which was reseeded in 2020. Yield was calculated on the day of harvest via the cut and weigh technique [24]. Samples were also taken and analyzed for % DM, DMD and CP by near infrared spectroscopy at FBA laboratories (Cappoquin, Co., Waterford, Ireland). In first-cut silage trials from 2021 ($n = 2$), samples for DMD and CP were taken and analyzed three weeks prior to harvesting of both sites. Samples for DMD and CP for second-cut silage trials were taken and analyzed two weeks prior to harvesting in 2021. In third-cut silage trials from 2020 ($n = 5$) and 2021 ($n = 1$), samples for DMD and CP were taken and analyzed five days prior to harvesting.

2.6. Grazing Field Trials

Grazing field trials were conducted across two different sites in 2021. Site 1 is located at Shanntallow, Moneymore, Co., Galway, Ireland (53°16'15.9" N 8°50'42.5" W), and site 2 is located at Ahabeg, Co., Kerry, Ireland (52°24'37" N 9°40'14" W). All paddocks at site 1 have been under permanent pasture since 2018, while paddocks at site 2 were reseeded in 2020 and 2021. The pH ranged from 6.2 to 6.6 for site 1 over the 34 individual paddocks used in the study. No paddock at site 1 had a P and K index less than 3 for both nutrients, while pH at site 2 was 6.6 with a P and K index of 3 and 4, respectively. Both farms were growing a perennial ryegrass mixture, including varieties Aston Energy, Oakpark, Meiduno, Alfonso and Glenviegh at site 1 (here all fields had a 5% white clover variety (*Trifolium repens*; Crusader). Site 2 a 50:50 mixture of tetraploid: diploid (Abergain and

Aberchoice). Treated areas received 75% N in the form of PSI-362-coated CAN (22% N + 4% S) while control areas received 100% N rate in the form of CAN (27% N + 4% S) at site 1 and protected urea (46% N containing the urease inhibitors NBPT (N-(n-butyl)thiophosphoric triamide) and NPPT (N-(n-propyl) thiophosphoric triamide)) at site 2. Grazing ran from May until August at site 1 and from May until July at site 2. Rates for the 100% N control were 56 kg ha⁻¹ in May and 24 kg ha⁻¹ for subsequent months, with the 75% N treatment receiving the appropriate reduced amounts. At both farms, grass was measured twice weekly utilizing the cut and weigh technique [24], and daily growth rates were calculated and inputted into Pasturebase Ireland [25]. Samples for analysis of DMD and CP were taken in June and July from crops just prior to grazing (\approx 1200 to 1500 kg DM ha⁻¹) and were analyzed as described previously by FBA laboratories (Cappoquin, Co., Waterford, Ireland) for the pasture at site 1 only.

2.7. Weather Data

Supplementary Table S4 describes the rainfall and mean 10 cm soil temperature for all field sites. Weather information was gathered from the nearest (within 20 miles) Met Eireann (The Irish Meteorological Service) weather station relevant to the individual sites. Where available, the long-term averages (LTAs) over the preceding 30 years were included.

The lysimeter trials ran from June to October in 2020 and from February to August in 2021. The mean soil temperature at 10 cm depth from June to October in 2020 was 14.5 °C, with a total rainfall in this period of 591 mm. In 2021, the mean soil temperature from February to August was 12.2 °C, with a total rainfall during this time of 626 mm.

In 2020, for the silage harvesting trials, sites 1, 2 and 3 received 240 mm of rain over the months of July and August (the LTAs were 157 mm, representing a 53% increase in rainfall over these months), with an average 10 cm soil temperature of 15.7 °C, while sites 4 and 5 received 230 mm of rain (the LTA was 194 mm, representing a 19% increase in rainfall over these months), with a mean 10 cm soil temperature of 15.6 °C over the trial period. In 2021, field sites 6, 7 and 8 received 132.9 mm of rain, a value that is broadly in line with the LTA (135.1 mm), with a mean 10 cm soil temperature of 9.2 °C in May and June; site 10 received 83.5 mm of rain (LTA of 147 mm), with a mean 10 cm soil temperature of 15.6 °C over the months June and July; and site 11 received 217 mm of rain (a 38% decrease relative to the LTA of 157 mm), and a mean 10 cm soil temperature of 15.6 °C in July and August.

For the pasture grazing trials, total rainfall for site 1 in May, June, July and August was 355 mm (LTA over this period was 349 mm), with a mean soil temperature of 14.2 °C, while site 2 received 350 mm of rain in the same period and had a mean soil temperature of 15.3 °C (LTA = 283 mm and 15.0 °C).

2.8. Statistical Analysis

Statistical analysis was carried out using the XLSTAT statistical software for Microsoft Excel. Where appropriate, tests ranged from a t-test for two independent samples (when comparing the means of two independent groups) to multiple (pair-wise) comparisons using Student–Newman–Kuels (SNK) and/or Tukey’s honest significant difference (HSD) test (when comparing three or more independent groups) to the Kruskal–Wallis test (for non-normal distribution of data). Both one- and two-way ANOVAs for qualitative independent variables and linear regression for quantitative independent variables were used to assess the interaction between factors. In the text, the term ‘significant’ refers to $p \leq 0.05$.

3. Results & Discussion

3.1. Nitrogen Response Curve

A nitrogen response experiment in the lysimeter trials was carried out to correlate the effect of additional N applications with yield. Figure 1 shows a linear response ($R^2 = 0.97$) between the three applications of N (% of N is related to amounts described in Supplementary Table S2) and total grass yield. At these rates, the typical levelling-off effect of the N response curve between N application and crop yield was not reached [26]. Between 75%

N and 100% N, there was still a substantial increase in yield of 36%, highlighting the impact of the additional 25% N on grass growth.

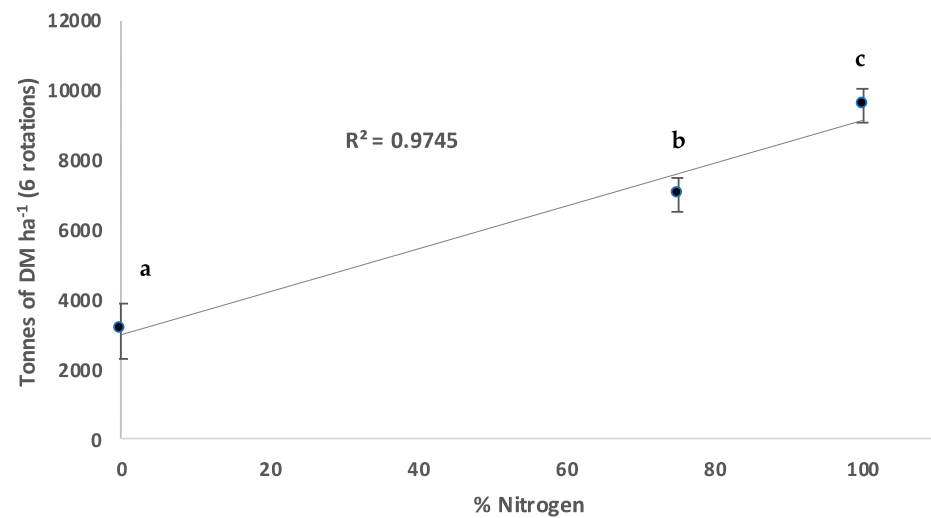


Figure 1. Nitrogen response curve for the modified lysimeter trials. Results are the means \pm SE ($n = 6$). Significance ($p < 0.05$) is indicated by different letters (Tukey HSD).

3.2. Modified Lysimeter Grass Growth Yield and Quality Information

The lysimeter trials ran from June to October in 2020 and from February to August in 2021. Soil fertility (pH, P and K levels), texture and textural class for the six soils are described in Supplementary Table S2. Weather data are described in Supplementary Table S4. The daily grass growth in terms of dry matter (DM) ha⁻¹ for the lysimeter trials is presented in Figure 2, where both the 1 \times and 1.5 \times rates of PSI-362 significantly increased the yield compared to the 75% N control. There was also a concentration effect evident, as the rate of PSI-362 increased as did the effect on the average daily grass growth per hectare (0.5 \times = 55.3 kg, 1 \times = 61.9 kg and 1.5 \times = 66 kg). The 1.5 \times rate of PSI-362 (@ 75% N) out-grew the 75% N control by almost 15.2 kg (66.0 vs. 50.8 kg ha⁻¹) and the 100% N control by 1.2 kg (64.6 kg ha⁻¹).

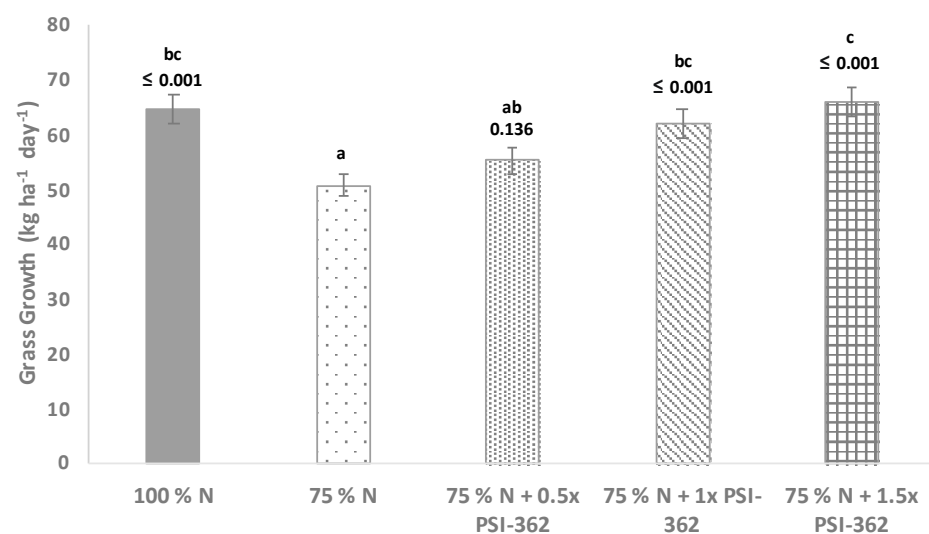


Figure 2. Average grass growth (kg ha⁻¹ day⁻¹) from the lysimeter trials over two years from three replicates of six soils with six rotations per year. $N = 216$. Numbers represent mean \pm SE. Significance is indicated by different letters ($p < 0.05$). p values represent significance levels compared to the 75% N control—Tukey HSD.

Figure 3 presents data on DM, CP and DMD yields of the lysimeter trials across the two years (six rotations/year). The effect of a 25% reduction in N was evident in the yields for the two controls (100% N vs. 75% N), with a 25% decrease in DM yield (kg DM ha^{-1}), a 29% decrease in CP (kg ha^{-1}) per hectare and a 26% reduction in DMD (kg ha^{-1} ; Figure 3A–C). All PSI-362 rates increased DM, CP and DMD yield over the 75% N control. In addition, these increases were correlated with increasing concentrations of the biostimulant PSI-362 on the coated fertilizer. Increasing the PSI-362 concentration from 0.5 \times to 1.0 \times to 1.5 \times resulted in an increase in DM yield of 9%, 24% and 32%, respectively, over the 75% N control (6772 kg of dry matter ha^{-1} ; with the 1 \times and 1.5 \times concentration being significantly different). The 1.5 \times rate exceeded the 100% N control by 4% (Figure 3A): The total dry matter (kg ha^{-1}) of six rotations according to year and soil type from the lysimeter trials is presented in Supplementary Table S5.

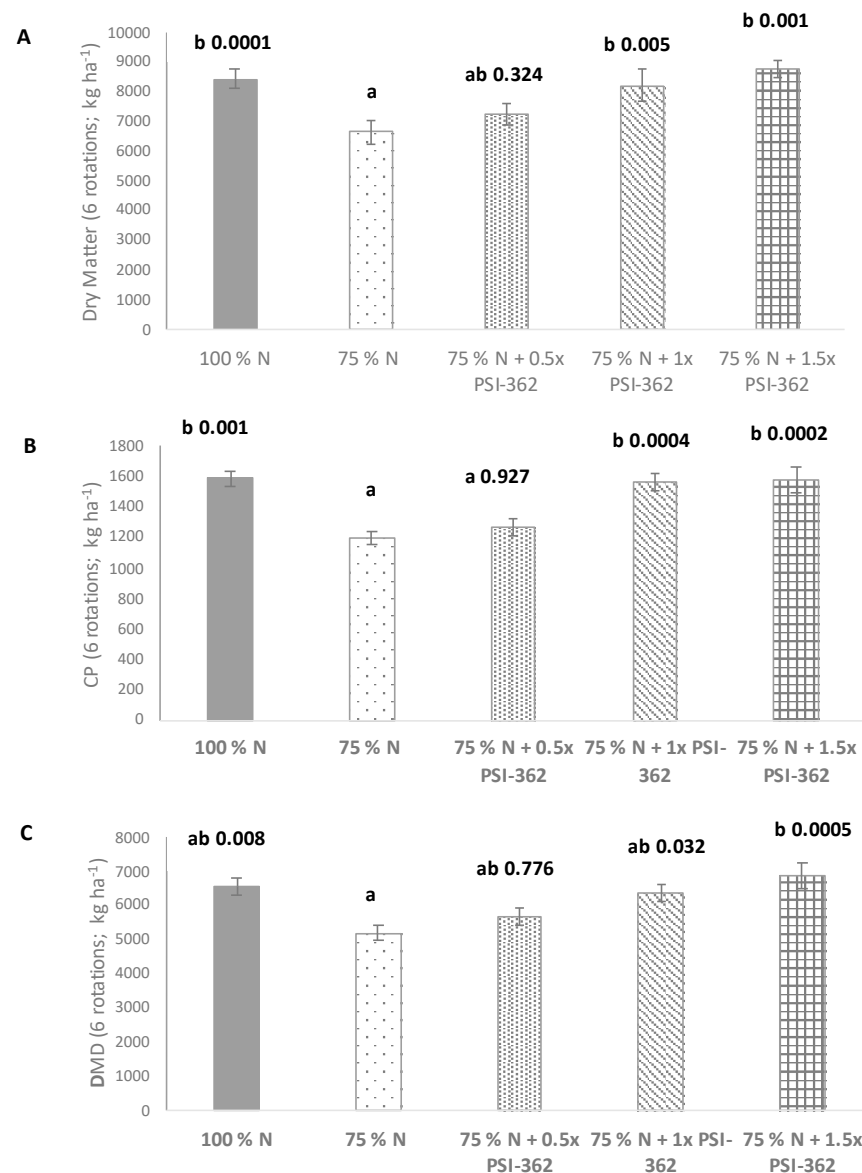


Figure 3. Effects of biostimulant application on the agronomic traits of perennial ryegrass. (A) Results for total yield (DM ha^{-1}), which is the average of the sum of three replicates over six rotations per year across two years for six different soils, (B) CP ha^{-1} and (C) DMD ha^{-1} . Significance is indicated by different letters, while p values represent significance levels compared to the 75% N control (Kruskal–Wallis). Values represent mean \pm SE ($n = 36$).

The correlation between increasing rates of PSI-362 and 25% less N was also evident in the results of the quality parameters measured. The Cp values were dependent of the level of PSI-362 applied, as there were increases of 6%, 31% and 32% with 0.5×, 1.0× and 1.5× PSI-362, respectively, over the 75% control (1197 kg of CP ha⁻¹). This suggests that the optimal dose is between the 1× and 1.5× concentration due to the levelling off of the response (Figure 3B). Additionally, the DMD ha⁻¹ values increased according to the application rate of PSI-362, with 9%, 23% and 32% increases in yield for 0.5×, 1× and 1.5× over the 75% N control (5312 kg of DMD ha⁻¹; Figure 3C).

The effect of soil type (loam vs. silt-loam), soil pH, soil P and soil K level on the performance (DM, CP and DMD yield) with the PSI-362-coated fertilizer is shown in Table 1, while Supplementary Figure S2 highlights the significant difference between the 75% N and 100% N control in terms of DM yield according to soil texture. Analysis of the biomass DM yield showed that only treatment had a significant effect ($p < 0.001$). One-way ANOVA and linear regression analysis of soil type and soil quantitative variables (pH, P and K levels) showed no statistically significant effect on biomass DM yield. A similar result for CP and DMD yield was observed, with the treatments having a significant effect on these quality parameters ($p \leq 0.001$).

Table 1. Relationship between treatments (PSI-362-coated fertilizer), soil type (loam or silt-loam), soil pH, soil P and soil K levels on DM (biomass dry matter), CP (crude protein) and DMD (digestible dry matter) yield.

Factor	DM kg ha ⁻¹	CP kg ha ⁻¹	DMD kg ha ⁻¹
Treatment (T)	***	***	***
Soil pH	ns	ns	ns
Soil phosphorus (P)	ns	ns	ns
Soil potassium (K)	ns	ns	ns
Soil type (ST)	ns	ns	ns
Treatment (T)			
75% N	6611a ± 296	1193a ± 45	5299a ± 264
75% N + 0.5×	7239ab ± 377	1269a ± 58	5760ab ± 328
75% N + 1×	8193bc ± 383	1560b ± 61	6459b ± 328
75% N + 1.5×	8738c ± 531	1575b ± 84	6946b ± 443
100% N	8412bc ± 340	1587b ± 50	6602b ± 296

ns, *** Non-significant or significant at $p \leq 0.001$, respectively. Different letters indicate statistical differences with $p \leq 0.05$ based on one-way ANOVA for qualitative independent variables and linear regression for quantitative independent variables. Data are the means ± SE. Number of biological replicates ($n = 12$).

Soil texture is not readily subject to change and is thus considered a basic property [27] in comparison to pH or P and K contents that can be rectified through appropriate agronomic practices. Soil texture will play a significant role in terms of a soil's ability to hold nutrients, with the clay fraction being particularly important as it can impact the colloidal and electrostatic properties of the soil and thus increase the surface area to hold nutrients. Additionally, the water-holding capacity will also vary according to texture, as soils with a lower clay content tend to be more free draining due to the tendency to have a larger pore size, resulting in a greater tendency for nutrients to be washed away and lost as leachate [28]. Soil texture can specifically affect nitrogen loss as nitrate leaching. Van Es et al. found that nitrate leaching was more prevalent in loamy sand soil rather than in clay loam soil (up to 2.5 times greater on maize cropped land) due to higher hydraulic conductivity and lower retentivity [29]. However, the lack of a significant effect in the one-way ANOVA suggests that PSI-362 will perform the same in either soil type.

3.3. PSI-362 Application Enhanced NUE in Lysimeter Trials

Application of PSI-362 at 1× and 1.5× significantly improved NUE when compared to the 75% N control (Figure 4). There was also improved NUE when compared to the 100% N control, which is perhaps unsurprising given the results in CP ha⁻¹ yield from Figure 3B

where the differences between the 1× and 1.5× rate and the 100% N control were negligible, despite the PSI-362 treatment receiving 25% less N. Biostimulants of many different classes have been reported to improve NUE (and indeed other nutrient use efficiencies). Previous authors have reported enhanced NUE in baby lettuce and spinach with a seaweed extract from *Ecklonia maxima*, but no specific data related to N metabolism were reported [16]. In baby lettuce, increases in yield of between 6% and 14% were observed depending on the nitrogen regime. This increased NUE may be attributed to the phytochemical efficiency and a better activation of photosystem 2 (as seen by the increase in chlorophyll for baby lettuce), while in spinach, ascorbic acid and polyphenol levels were significantly increased. Augmentation of these secondary metabolites has been implicated in key enzyme activities such as the enzyme chalcone isomerase (associated with phytochemical homeostasis through the synthesis of flavanone precursors). Roupheal et al. [16] also found increases in polyphenols, ascorbic acid and the SPAD index in spinach grown under glasshouse conditions using seaweed extracts from *Ecklonia maxima* and *Ascophyllum nodosum* combined with vegetal oils and herbal extracts. This report also found that the nitrate content was significantly enhanced in seaweed extract-treated plants, a finding similar to that reported by Goñi et al. [19] using PSI-362-coated fertilizer under both high and low nitrogen regimes in barley. This increased nitrate content may be associated with enhanced nitrogen uptake as seen with the significantly increased expression of *NRT1.1*, *NRT2.1* and *NRT1.5* genes, while the enhanced amino acid profile may be due to increased enzymatic activity in the form of nitrate reductase and glutamate synthase, both key enzymes in the plant N cycle. Siwik-Ziomek and Szczepanek found that a commercial seaweed biostimulant increased nitrogen concentration in the whole plant of oilseed rape at all development stages; however, at the fruit development stage, this effect was linked to the application of sulphur [30]. In the same crop, an *Ascophyllum nodosum* extract was found to increase the expression of genes encoding N metabolism in oilseed rape that resulted in a 115% and 21% increase in nitrogen uptake in the roots and shoots, respectively [31].

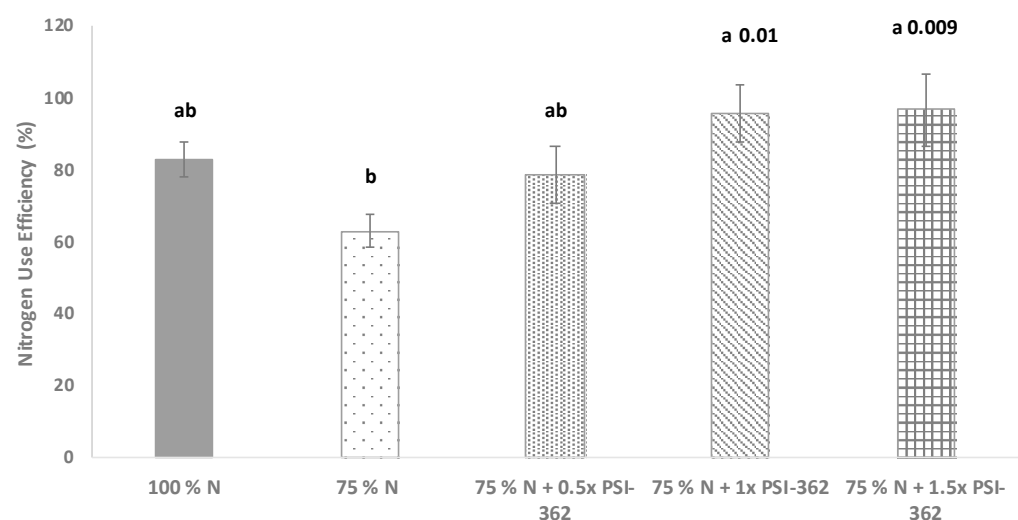


Figure 4. Calculated nitrogen use efficiency (NUE) values from the lysimeter trials. *p* values represent significance levels compared to the 75% N control (Tukey HSD). Values represent mean \pm SE ($n = 6$).

3.4. Silage Harvesting Trials

The silage harvesting field trials took place in 2020 and 2021 across 11 sites. For trials in 2020, PSI-362 was coated on a commercial CAN product (27% N + 4% S), while for 2021 trials, PSI-362 was coated on compound fertilizer (N-P-K; 20-2-12 + 3% S), with the relative amount described in Supplementary Table S3. The results of DM, CP and DMD yield from all 11 trials are shown in Table 2, while Table 3 categorizes the trials as either first (May/June), second (July) or third harvests (August). Supplementary Table S6 provides the dry matter (kg ha^{-1}) values for the individual farms from the silage trials. Results

from all 11 sites showed that there was no significant loss in DM yield between 100% N and 80% N + PSI-362 (4508 kg ha⁻¹ vs. 4594 kg ha⁻¹), CP content (840 kg ha⁻¹ vs. 847 kg ha⁻¹) or DMD (3352 kg ha⁻¹ vs. 3426 kg ha⁻¹) despite a 20% reduction in N for the PSI-362-coated fertilizer regime (Table 2). The dry matter digestibility is a key quality trait of all forages [32]; it is the proportion of forage that can be digested by the ruminant and comprises the crude protein, carbohydrates and lipids. Digestibility is linked to the energy of the forage and ultimately the performance of the animal; increases in DMD lead to increased milk yield or carcass gain in bovines. Variations in digestibility in grass and ultimately silage can range from sward type to harvesting date and fertilizer application. CP is another important quality trait for forages and is often the most expensive component of livestock concentrate rations [33]. Similar to DMD, CP levels in grass and silage will depend on sward type, N application and harvesting/grazing date. The total % DMD and CP was found to be 74% and 18.6%, respectively, in the 100% N control and 75% and 18.4%, respectively, in the 80% N + PSI-362 treated sites (these results are the average DMD and CP as a %, for all silage harvesting trials; $n = 11$). The PSI-362-treated grass silage crop showed no DM yield penalties or quality loss with a significant reduction in N fertilizer. The DMD and Cp values would be considered excellent at harvesting and will likely result in good quality silage.

Table 2. Summary of results for dry matter (DM), crude protein (CP) and digestible dry matter (DMD) yields from the 11 grass silage field trials over two years, 2020 and 2021.

Regime	Yield (kg DM ha ⁻¹)	<i>p</i> Value	CP (kg DM ha ⁻¹)	<i>p</i> Value	DMD (kg DM ha ⁻¹)	<i>p</i> Value
100% N	4508 ± 192		840 ± 80		3352 ± 300	
80% N + PSI-362	4594 ± 225	0.775	847 ± 68	0.948	3426 ± 359	0.878

Values represent the mean ± SE ($n = 11$). *p* values were determined using a two-sample *t*-test.

Table 3. DM, DMD and CP yield from 1st, 2nd and 3rd harvests of silage trials.

Harvest	Regime	DM Yield (kg DM ha ⁻¹)	<i>p</i> Value	CP (kg DM ha ⁻¹)	<i>p</i> Value	DMD (kg DM ha ⁻¹)	<i>p</i> Value
1st	100% N	5325 ± 275		1031 ± 86		4142 ± 259	
	80% N + PSI-362	5485 ± 365	0.760	1013 ± 61	0.884	4387 ± 235	0.557
2nd	100% N	3986 ± 500		738 ± 65		2941 ± 381	
	80% N + PSI-362	4182 ± 321	0.767	800 ± 68	0.580	3154 ± 243	0.672
3rd	100% N	4217 ± 631	a	797 ± 49	a	3217 ± 196	a
	80% N + PSI-362	4124 ± 595	a	746 ± 44	a	3134 ± 185	a
	100% N + PSI-362	4727 ± 873	a	1064 ± 98	b	3328 ± 307	a

1st-cut silage trials from 2021 ($n = 2$). 2nd-cut silage trials from 2021 from three separate sites ($n = 5$). 3rd-cut silage trials from 2020 ($n = 5$) and 2021 ($n = 1$). Note for the 100% control, $n = 6$; 80% N + PSI-362, $n = 6$; 100% N + PSI-362, $n = 4$. Significance by multiple comparison analysis is indicated by different letters for 3rd-cut silage trials; Tukey HSD—significance $p < 0.05$. Values represent the mean ± SE.

In terms of silage harvesting, total DM yields were marginally improved for both 1st- and 2nd-cut trials with PSI-362 treatment (3% and 5%, respectively), with this improvement under reduced N treated sites continuing with total DMD ha⁻¹ (6% and 7%). Both yield and DMD were slightly below those for the 100% N control for the 3rd harvest (−2% and −3%, respectively) although this reduction was not significant ($p = 0.96$ and 0.94 , respectively). For 1st, 2nd and 3rd cuts, total CP ha⁻¹ showed variable results (−2%, +8% and −6%) across the harvests when compared to the 100% N control. A positive control was also included for the 3rd-cut silage trials in 2020 (100% N and PSI-362) where there were increases in yield and DMD along with a significant increase in CP ha⁻¹ of 12%, 3% and 34%, respectively (Table 3).

3.5. Pasture Grazing Field Trials

The pasture grazing trials took place at two sites in 2021. There were 18 replicates per treatment with five grazings per replicate for site 1 and two replicates with five grazings per replicate for site 2. There were no significant differences in daily grass growth and DMD for grazing site 1 between PSI-362-coated fertilizer (75% N) and the 100% N control (Table 4). However, the results showed a 12% increase in CP with the application of PSI-362-coated fertilizer (13.2 kg ha⁻¹ vs. 14.8 kg ha⁻¹), although this increase was not statistically significant. At grazing site 2, the PSI-362-coated CAN fertilizer with 25% less N achieved an 8% increase in daily growth of grass versus the 100% N control, with daily growth figures of 92.4 kg DM ha⁻¹ vs. 99.4 kg DM ha⁻¹. Individual site, rotation and plot data is presented in Supplementary Table S7.

Table 4. Daily grass growth (DGG), DMD and CP for the pasture grazing trials.

Site	Regime	DGG (kg DM ha ⁻¹ Day ⁻¹)	<i>p</i> Value	DMD (kg ha ⁻¹ Day ⁻¹)	<i>p</i> Value	CP (kg ha ⁻¹ Day ⁻¹)	<i>p</i> Value
1	100% N	60.9 ± 2.5	0.629	47.2 ± 1.9	0.132	13.2 ± 0.5	0.45
	75% N + PSI-362	59.3 ± 2.4		46.3 ± 1.9		14.8 ± 0.6	
2	100% N (PU)	92.4 ± 11.8	0.679	ND	N/A	ND	N/A
	75% N + PSI-362	99.4 ± 11.6		ND		ND	

n = 17 for pasture site 1 with five grazings per replicate, *n* = 2 for pasture site 2 with five grazings per replicate, PU = protected urea, Values represent the mean ± SE, *p* values were determined using the two sample *t*-test, ND = not determined, N/A = not applicable.

Maintaining or even improving grass yield or quality under a reduced N regime has many benefits in terms of animal performance, farm economics and a reduced environmental impact. Efficiencies in a grass-based production system for beef and dairy are underpinned by producing quality grass, thus negating the need to supplement feeding with expensive concentrates [34,35]. These efficiencies not only lead to economic benefits but also environmental benefits. Nationally in Ireland, where grass-based systems are the predominant type of system for ruminants, the farm gate NUE is 36.7% [33]. The potential to reduce nitrogen fertilizer input while maintaining yields would enhance these farm gate efficiencies, along with the agronomic NUE.

4. Conclusions

Improved NUE of crops will result in reduced fertilizer input while maintaining crop yield and farm wide productivity. Through the application of a granular fertilizer coated with a biostimulant (PSI-362), specifically developed to enhance NUE, it was possible to reduce N input in grassland by 20 to 25% while maintaining yield and/or quality (in terms of dry matter digestibility and crude protein) in comparison to the 100% N control across different soil types and relevant agronomic systems. Overall, PSI-362 displayed an improved NUE of 17% over the 100% N control. Further studies to evaluate the performance of biostimulant technologies for improving NUE are required in order to demonstrate the robustness of the performance across different geographies, climates and pasture production systems. The European Commission's Biodiversity Strategy (as described by the European Green Deal) for 2030 sets out specific targets with regard to fertilizer, including a 20% reduction in fertilizer use by reducing fertilizer losses by 50% [36]. By incorporating biostimulant technologies, such as that reported here, into mainstream agricultural practice, a 20% reduction in N fertilizer can be achieved without losses in crop yield, quality and overall agricultural productivity.

Supplementary Materials: The following are available online at <https://www.mdpi.com/article/10.3390/agronomy12020463/s1>, Supplementary Figure S1: Modified lysimeter set-up, Supplementary Figure S2: Performance of PSI-362-coated fertilizer sorted according to soil type. Supplementary Table S1: Soil attributes from the six different soils used for the modified lysimeter trials; Supplementary Table S2: The fertilizer regime applied to the modified lysimeter trials; Supplemen-

tary Table S3: Fertilizer regime, soil fertility (P and K) index and coordinates of the 2020 and 2021 field trials; Supplementary Table S4: Weather Data for lysimeter and field trials; Supplementary Table S5: Total dry matter (kg ha⁻¹) of six rotations according to year and soil type from the lysimeter trials; Supplementary Table S6: Dry matter (kg ha⁻¹) values for the individual farms from the silage trials; Supplementary Table S7: Daily grass growth (kg DM ha⁻¹ day⁻¹) for the two pasture grazing sites.

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