Effects of pig slurry treatment with a peroxide-based additive, GasAbate, on the seasonal growth of perennial ryegrass.

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1 Abstract

Livestock manures, such as slurry, are valuable sources of crop nutrients, however during storage they release significant greenhouse gas (GHG) and ammonia (NH₃) emissions. These represent gaseous losses of nitrogen and carbon, thereby lowering the nutrient value of slurry and precluding the closing of nutrient loops. Slurry additives represent a valuable means of mitigating emissions, thereby potentially retaining their maximum fertiliser value. GasAbate, a peroxide-based slurry additive, was employed in a previous trial to reduce emissions from stored pig slurry at 1 m³ scale. However, its effects upon crop uptake following pig slurry application have not been assessed. To this end, a 5-month pot trial was set up to assess perennial ryegrass yields after fertilisation with either untreated (SU) or GasAbate treated (ST) pig slurry post-storage, where two soils were assessed. In both soil types, dry matter yields were consistently higher at all four harvest points: in CD soils across the entire growing season, the cumulative yield from ST (18.5 t DM ha⁻¹) exceeded that from SU (15.3 t DM ha⁻¹; a 21.2% increase; p < 0.05). In the lower OM soil, cumulative yields were lower, but again yields from ST (16.6 t DM ha⁻¹), exceeded those from SU (13.7 t DM ha⁻¹; a 20.3% increase; p < 0.05). SPAD indices also demonstrated that, in one soil type, GasAbate treated slurry resulted in grass with marginally higher (p < 0.05) chlorophyll content which would increase the nutritional value of the pasture. Overall, the consistent performance of treated slurry (ST) in improving both dry matter yield and chlorophyll content highlights its potential for significantly enhancing grass productivity across different soil types.

2 Introduction

Livestock manures are valuable sources of nutrients for food and fodder crops globally, where their correct use improves the circularity of agriculture (Marques-dos-Santos et al., 2023) and offsets the use of chemical fertilizers. However, due to microbial decomposition of livestock manures and slurries during storage, they also emit significant methane (CH₄), carbon dioxide (CO₂) and nitrous oxide (N₂O) and as such are major greenhouse gas (GHG) sources, contributing in excess of 10% of agricultural GHG emissions (Shukla et al., 2019). In addition, livestock manures and wastes contribute the majority (>80%) of ammonia (NH₃) emissions in the EU (Amon et al., 2019; Van Damme et al., 2021). There are significant detrimental impacts

of both GHG (Filonchyk et al., 2024) and NH₃ (Wyer et al., 2022) on the environment and human health. Pig production also results in notable odour emissions which can impact the quality of life of surrounding residents (Blanes-Vidal et al., 2009), where hydrogen sulphide (H₂S) is a major odour contributor and its noxious effects pose risks to both farm workers and pigs (Brglez, 2021).

In addition to these negative impacts, these emissions represent losses of nitrogen, sulphur and carbon, which lowers the resource value of slurry for onward use either as a fertilizer or as a feedstock for renewable biogas production, and hampers closing of nutrient loops (Marques-dos-Santos et al., 2023). Thus, mitigation of emissions from stored manures, through their retention of C and N within the system, can preserve the resource value of these wastes. Among the mitigation strategies for stored manures are slurry additives, which offer promising yet underutilized solutions for reducing emissions and maintaining the nutrient content of stored manure. In addition, they could potentially improve animal health in intensive system through improved ambient air conditions.

Acidification is the most widely adopted slurry additive, and due to its ability to retain the slurry nitrogen value, through mitigation of NH₃ emissions, has been shown to increase the fertilizer value of acidified versus untreated slurries (Kai et al., 2008) and improve the uptake of N and P by plants (Fangueiro et al., 2018). However, due to the hazardous nature of strong acids in addition to issues with onward use of acidified slurry, particularly for AD (Moset et al., 2012), there exist opportunities for other additives. A peroxide-based additive (GasAbate®) has been applied to dairy cattle (Thorn et al., 2022) and pig (Nolan et al., 2023) slurries and shown strong GHG and NH₃ mitigation as well as reducing H₂S. As the oxidation of organic matter that results from the addition of a peroxide could alter the availability of key elements including N, P and S, the impact of treating slurry with this additive requires assessment. To this end, a five-month pot trial was set up to assess yields of perennial ryegrass after four successive harvests, following the application of either untreated or GasAbate treated slurry that had been stored for 30 days prior to the growth trial.

3 Materials and methods

3.1 Slurry treatments

Slurry from a storage trial undertaken in 1 m^3 storage tanks was used, where the storage methodology and results of GHG and NH₃ emissions during the trial are detailed by Nolan et al. (2024). Briefly, the tanks contained 750 L of weaner slurry and received one dose of 1.86 L

GasAbate (providing $0.87g~H_2O_2/L~slurry$), by injection into the base of the tank, at the beginning of the 30-day trial, with air flow over the surface of the slurry ($0.3~m~s^{-1}$). One untreated control and one additive-treated tank were sampled at the end of the trial following agitation and the 20L sample from each was kept refrigerated until use. A sub-sample of slurry from each tank was sent to an independent, validated laboratory for physico-chemical analyses of pH, total and volatile solids content, total Kjeldahl nitrogen (TKN), Olsen's phosphorous (P) and Magnesium (Mg) and potassium (K) (Table 2).

3.2 Pot trial set up

Two soil types were used to test the effect of the GasAbate slurry additive on plant growth following slurry application, where the soils were chosen to provide a contrast in OM and in P and K index (Table 1). The Carryduff (CD) soil was an organic topsoil with high OM content, while the Maryland (ML) soil was a heavy gley. Subsamples of each soil type were analysed at an independent, accredited laboratory for pH, OM content, total Kjeldahl nitrogen (TKN), Olsen's phosphorous (P) and exchangeable magnesium (Mg) and potassium (K) using ammonium nitrate extraction. The trial was conducted over a full growing season from May to October 2023, at a plant growth facility in Lisburn, Northern Ireland (54° 32' 25.6236" N, 6° 5' 37.356" W). A randomized design, with total of 24 pots (10L volume; 28 cm in diameter and 24 cm in depth) was used, where pots were blocked by soil type allowing four replicate pots per condition tested. Pots were filled with either 12 kg dry weight (DW) of CD topsoil (1.1 g cm⁻³ bulk density), or 8.3 kg DW of ML soil (0.75 g cm⁻³ bulk density). Perennial ryegrass, (Lolium perenne) of the Gosford variety, was sown into each pot at 190 mg per pot.

Table 1. Characteristics of the Carryduff and Maryland soils

	Carryduff soil	Maryland soil
pН	6.3	7.1
OM (%)	24	8
Available P (mg L ⁻¹)	9.8 (P index 1)	19 (P index 2)
Available K (mg L ⁻¹)	59 (K index 0)	195 (P index 2+)
$Mg (mg L^{-1})$	211 (Mg index 4)	213 (Mg index 4)

3.3 Application of soil amendments and grass cuts

After six weeks of grass growth, the first application of soil amendments were applied, where those evaluated were: untreated pig slurry (SU), GasAbate-treated pig slurry (ST), and an

inorganic fertiliser treatment (MinC) applied based on the recommended slurry equivalent (RSE) of typical pig slurry in Ireland (McCutcheon and Quinn 2020). Slurry was applied at a rate equivalent to 33 m³ kg per ha (Humphreys and Lawless 2006) and the mineral RSE was applied to match this and deliver 69 kg N ha⁻¹ (applied as calcium ammonium nitrate); 25.4 kg P per ha⁻¹ (as triple super phosphate) and 72.6 kg K per ha⁻¹ (as Muriate of Potash). During the trial, four treatment applications were made on days 0, 46, 85 and 119, where slurry and mineral fertilizer were surface applied. Additionally, four grass cuts were conducted on days 44, 83, 118 and 154, which took place in the months of June, August, September, and October, respectively. At each cut, the grass from individual pots was cut to 3 cm using battery-powered hand shearers (Makita), equipped with a cutter bar approximately 160 mm wide.

After harvesting, the freshly cut samples were weighed then dried overnight at 104°C in an industrial-grade oven (Birmingham and Blackburn Unitherm Drier) to enable determination of dry matter (DM) yield, reported in tons per hectare (t DM ha⁻¹), for each sample. Relative chlorophyll content was measured using the Soil Plant Analysis Development (SPAD) index with a portable chlorophyll meter (SPAD-502, Konica Minolta, Inc., Osaka, Japan). The SPAD value was calculated from the average readings of three of the youngest, fully developed leaves, randomly selected from each pot.

3.4 Data analyses

Statistical analyses were performed in R (v4.4.1; R. Core Team 2021) where for each soil type, differences in DM yield or SPAD value as a function of fertilizer regimen were analysed by fitting a linear mixed effect (LME) model using the restricted maximum likelihood (REML) from the nlme package (v3.1-167; Pinheiro et al., 2021). Treatment and cut number were used as fixed effects and pot identification number as a random effect. Model residuals were verified for normality before analysing the model with an ANOVA. Where a treatment effect was seen (p < 0.05), estimated marginal means (emmeans v4.0-3; Lenth et al., 2018) were used to perform pairwise comparisons between treatments. If an interaction of cut and treatment were seen, then data were analysed and visualised to reflect this, where graphical representation was performed using ggplot2 (v3.5.1; Wickham 2016) and ggprism (v1.0.5; Dawson 2024).

4 Results

4.1 Characterisation of pig slurries

Physicochemical analysis of slurry samples taken from the study of Nolan et al. (2024) revealed that the GasAbate additive had no effect on slurry pH, which was 8.3 in untreated and 8.2 in additive treated slurry (Table 2), solids however were higher in treated than untreated slurry, likely as the peroxide halted decomposition of the organic matter and thus destruction of solids was slower. Nitrogen (TKN) was slightly higher after storage with GasAbate additive 0.57% vs 0.61% in untreated slurry. While available K was very similar between the two slurries, total P was notably higher (1181 mg kg⁻¹) in treated slurry than in untreated slurry (820 mg kg⁻¹), as was Mg at 656 and 445 mg kg⁻¹ in treated and untreated slurry respectively.

Table 2. Characterisation of pig slurry samples used, which were taken from untreated and GasAbate additive treated, dynamic storage tanks from a trial performed by Nolan et al. (2024) where TKN, P, K and Mg data are expressed per unit of slurry (fresh weight).

	Untreated slurry	GasAbate slurry
рН	8.3	8.2
TS (%)	4	5.5
TKN (% w/w)	0.57	0.61
Total P (mg kg ⁻¹)	820	1181
Total K (mg kg ⁻¹)	3256	3216
$Mg (mg kg^{-1})$	445	656
S (mg kg ⁻¹)	419	487

4.2 Dry matter yields

Dry matter yield is crucial in ryegrass cultivation as it provides a comprehensive measure of both productivity and quality. It directly impacts the quantity and nutritional value of fodder available for cattle, influencing animal health and reducing production costs. Furthermore, monitoring dry matter yield is essential for predicting overall yields, optimizing harvesting practices, and promoting sustainable land use. Evaluating dry matter yield is therefore crucial in assessing the effect of slurry additives on grass production post-spreading.

The yield from CD soils receiving the recommended slurry equivalent of fertilizer (MinC), ranged between 2.7 and 4.1 t DM ha⁻¹ across the four growth periods (Figure 1). Higher yields were seen during the warmer end of the season at Cuts 1 and 2, which were performed in

June and August, respectively, while later in the season, at Cuts 3 (September) and 4 (October), growth was reduced. Yields from SU treated pots averaged between 2.95 and 4.38 t DM ha⁻¹ over the four periods. The yields were numerically higher than from the mineral fertilizer equivalent (MinC) pots, but this was only statistically significant in the first and, particularly, the final harvest date. Yields from pots receiving GasAbate treated slurry ranged from 3.44 to 5.75 t DM ha⁻¹ during the trial. When comparing additive-treated slurry with its untreated counterpart, significant increases in yield (p < 0.05) were seen at all four cuts, with a maximum 31% increase at Cut 2 and a minimum 17% increase at Cut 3. Cumulative yields from the four harvests were 13.0, 15.3 and 18.5 t DM ha⁻¹ from the MinC, SU and ST amended pots, respectively, representing a 20.9% increase (p < 0.05) in yield from the GasAbate treated pots over the untreated slurry amended pots.

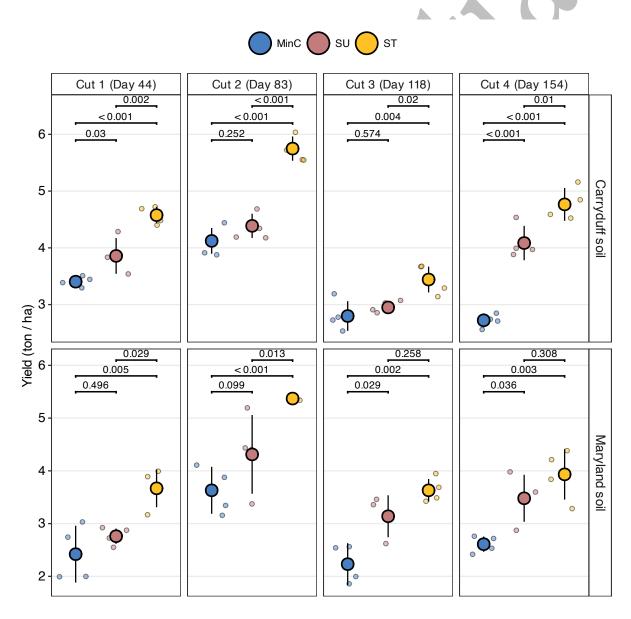


Figure 1. Yields from the A) Carryduff soils and B) Maryland soils per cut during the 154 day trial where p < 0.05 denote statistically significant differences in yield between amendments. MinC = recommended slurry equivalent of inorganic fertiliser; SU = untreated pig slurry; ST = GasAbate treated pig slurry. Smaller points represent yields from each individual pot while larger points with error bars denote the mean and standard deviation (n=4) from each treatment per cut.

In the ML pots, yields varied less between the four harvests (Figure 1B), and as seen in the CD soils, maximum yields were seen at Cut 2, for all three amendments. Compared to the CD pots, yields from the ML soil were slightly lower, where MinC pots yielded between 2.2 and 3.6 t DM ha⁻¹ across four growth periods. DMY from SU pots ranged from 2.8 to 4.3 t DM ha⁻¹, and again yields from the SU pots exceeded those from its MinC counterpart. This difference increased as the trial proceeded and was statistically significant at Cuts 3 and 4. Grass yields from pots receiving GasAbate treated slurry ranged from 3.6 to 5.4 t DM ha⁻¹ over the four cuts. While DM yields from ST pots were numerically higher than those from the untreated counterpart, SU, these were only significant at the first two cuts (p < 0.05). Cumulative yields from the four harvests were 10.9, 13.7 and 16.6 t DM ha⁻¹ from the MinC, SU and ST amended pots respectively, representing a 21.2% increase in yield from the GasAbate treated vs untreated slurry pots (p < 0.05).

4.3 Chlorophyll content

The assessment of relative chlorophyll content, measured in terms of SPAD values, provides valuable insights into the health and vigour of grass crops. Chlorophyll content is a crucial indicator of plant photosynthetic capacity, which directly influences growth and overall productivity. SPAD values were measured across different treatments and growth periods to evaluate the impact of slurry treatments on chlorophyll content and, by extension, on grass productivity.

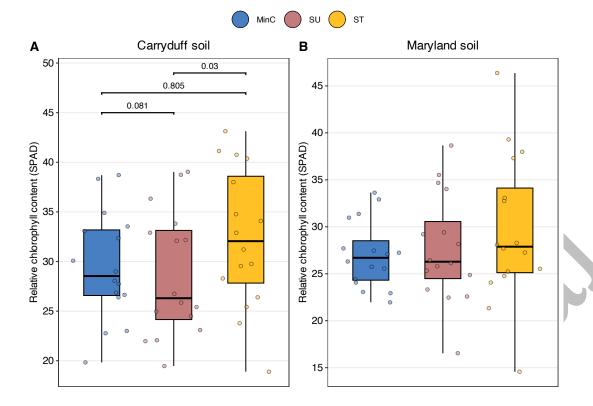


Figure 2. Relative chlorophyll content (SPAD value) from grasses grown in pots with A) Carryduff and B) Maryland soils amended with either MinC (recommended slurry equivalent of inorganic fertiliser); SU (untreated pig slurry) or ST (GasAbate treated pig slurry). Points represent readings from each individual pot while boxplots summarise these data with boxes representing the median and quartiles while whiskers denote the minimum and maximum values. Differences as a function of treatment, for each soil type, are represented by brackets with p values above, where statistically significant differences are considered at p < 0.05.

For the CD soils, SPAD values saw variations between cuts, with higher readings for all treatments seen in the latter two cuts (Supplementary Figure 1). Over the four cuts, MinC treated grasses averaged 29.5 (± 5.42) relative units while that from SU were marginally lower at 28.7 (± 6.29) units. While no significant differences in SPAD values were detected at each individual cut (Supplementary Figure 1), over the course of the whole trial, chlorophyll readings were higher from ST pots at 32.4 (± 7.02) units than from SU pots (p = 0.03; Figure 2A).

The chlorophyll content of grasses grown in the ML soil were fractionally lower than those from the CD soil, with MinC treated pots producing grass of 27.0 (±3.57) units (Figure 2B). Similar chlorophyll contents were seen in SU treated pots, of 27.7 (±5.74) units. While the average SPAD index over the four cuts was higher from the ST pots, at 29.6 (±7.85) units this was not statistically significant. As seen in the CD soils, SPAD content was highest, for all three treatments, at the final cut, which took place in October.



Supplementary Figure 1. Relative chlorophyll content (SPAD value) from grasses grown in pots with A) Carryduff and B) Maryland soils amended with either MinC (recommended slurry equivalent of inorganic fertiliser); SU (untreated pig slurry) or ST (GasAbate treated pig slurry). Smaller points represent readings from each individual pot while larger points with error bars denote the mean and standard deviation (n=4) from each treatment per cut.

5 Discussion

Dry matter yield is a pivotal metric in ryegrass cultivation, providing critical insights into both the productivity and nutritional quality of fodder. This measure is instrumental in assessing the effectiveness of different slurry treatments and optimizing grass production for better animal health and reduced production costs. This study evaluated the impact of three slurry treatments—recommended slurry equivalent (MinC), untreated slurry (SU), and treated slurry (ST)—on dry matter yield across different growth periods for two soil types.

When comparing the CD and ML soils, despite the higher availability of P and K in the ML soils, both yields and SPAD content were slightly lower from this soil type, irrespective of

fertiliser input. The largest physico-chemical difference between the two soils was soil organic matter (SOM) which was notably lower in ML soils (8% vs 24% in CD). While SOM is known to affect crop yields, the levels in the soils employed herein are well above the \sim 2% threshold that would typically limit yields due to SOM alone (Oldfield et al., 2019). However, SOM is also the predominant reservoir of elemental sulphur (Schroth et al., 2007) and perhaps therefore these soils were lower in S than CD soils and this limited growth somewhat.

When comparing additive treated slurry (ST) with untreated slurry, findings from both CD and ML soils highlight the consistent performance when using additive treated slurry (ST) in improving dry matter yield across various growth periods. In CD soil, ST application resulted in a 20.9% increase in cumulative yields over the course of the trial when compared to the slurry that had been stored without an additive (SU). In the ML soil, a similar increase of 21.2 % was observed. These increased yields were a consequence of the retention of the nutrient value of the slurry through the use of an emissions-mitigating additive during storage, which was reflected in the physico-chemical analysis of the two slurry types. One of the more notable differences between the slurries was the apparent increased content of P and Mg content in the GasAbate treated slurry, despite these not being elements that would be lost through gaseous emissions from untreated slurry. These elements are however closely related in that much of the P found in manures is inorganic, and in pig manures a significant portion of that is often bound with Mg to form struvite (MgNH₄PO₄ • 6H₂0) (Bril and Salomons, 1990; Christensen et al. 2009; Schott et al., 2022). The dissolution of struvite is pH dependent, where it becomes unstable at lower pH and as such Mg and P are found in solution in higher concentrations in acidified than unacidified slurries (Pedersen et al., 2017; Regueiro et al., 2020). While GasAbate does not have an acidifying effect on slurry, perhaps the oxidative effect of the peroxide alters the complexation between organic matter and inorganic ions thus preventing struvite formation and increasing the P and Mg in solution. This however would require further in-depth characterisation to compare the elements in solution versus those in the particulates, for example using the methods described in Schott et al. (2023).

However, despite these P increases, it does not appear to be the principal driver behind increased yields as the CD soil which was a low P index 1 soil, saw a 20.9% increase in yields while the index 2 ML soil saw a very similar, and even marginally higher 21.2% increase in yields, despite being a P index 2 soil. The Mg indices followed the same trend of being higher

in the ML soil, together this suggests other elements were driving this increase over and above the increased P and Mg.

The total N (Kjeldahl) was slightly higher in additive treated slurry (7% higher), and as this was reported on a dry matter basis and the additive treated slurry had higher solids, when reported as fresh weight, this increase becomes more notable and would also serve to increase yields. During the storage trial of Nolan et al. (2024), a significant reduction of hydrogen emissions (H_2S) was observed, and this is confirmed in the physico-chemical analyses, which reveal a marked increase in the S content of the slurry. The uptake of N has been shown to increase significantly with the addition of S, thereby improving yields (Aspel et al., 2022; Zhao et al., 1999).

The assessment of chlorophyll content using SPAD values offers a significant understanding of the health and productivity of grass crops, serving as an indicator of the plants' photosynthetic capacity. Indeed, chlorophyll meters are frequently used to assess the N status of crops and pastures (Fontes and de Araujo, 2006; Errecart et al., 2012; Gáborčík, 2003) which consequently indicates the crude protein content and therefore the nutritional value of pasture grasses.

In the CD soil, SPAD values indicated a clear trend of increased chlorophyll content in response to treated slurry (ST), particularly in the fourth growth period. During the initial growth periods (Cuts 1 and 2) all treatments yielded similar SPAD values, suggesting that early growth stages were not significantly influenced by the type of slurry applied. However, in Cuts 3 and 4, ST treatment resulted in significantly higher SPAD values compared. No significant difference in SPAD values was seen at each individual cut, however, across the growing season, ST showed a significant increase in chlorophyll content compared to SU (p < 0.05). While SPAD values in the ML soil were numerically higher on average, these differences were not consistent or statistically significant. Perhaps the grass grown in the low P index CD soil responded better to the increased P content of the GasAbate slurry. Furthermore, the higher S in additive treated slurry may have contributed, as Zhao et al. (1999) demonstrated that the improved N uptake resulting from S addition served to increase the leaf N content and subsequently the chlorophyll content.

6 Conclusion

This study provides comprehensive insights into the effect of treating pig manure during storage with a GHG mitigating additive, on the growth and quality of perennial ryegrass post-application.

The GasAbate treated slurry retained a nutrient profile more similar to that of fresh slurry, which resulted in a consistent improvement in pasture yields from both soil types tested of around 20% increase in dry matter. Despite differences in their P indices the two soil types responded similarly suggesting that the increased yield was principally due to the increased N and S of the slurry. In terms of chlorophyll content, however, increases due to additive treated slurry were only seen in the lower P index 1 soil (CD), so while it didn't appear to alter the yield response, perhaps the increased P was more important for pasture quality. Future work aims to address the effects of the GasAbate additive on phosphorus forms within the slurry and testing at field scale.

These data serve to demonstrate that by preventing gaseous losses during storage, the nutrient value of the slurry was maintained. This has the potential onward benefit of reducing the need to buy in costly synthetic fertilisers, which would help offset costs associated with applying such an additive to stored manure.

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