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Integrating glycerol and straw into digestate applications: Microbial biomass and crop responses under field conditions

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Abstract

Global food production relies on inorganic nitrogen to meet population consumption demands. However, synthetic fertilizer production currently relies heavily on fossil fuels. Anaerobic digestate offers an alternative source of nutrients because it is produced from organic matter. Yet digestate application results in similar problems of environmental pollution that synthetic fertilizer use incurs, such as nitrate leaching and N_2O emissions. Co-amendment of digestate with a high carbon amendment may result in higher nitrogen use efficiency and fewer losses by adjusting the C:N ratio to encourage microbial assimilation of nitrogen. Therefore, we conducted a field plot experiment to determine the effects of adding carbon with digestate on the nitrogen uptake, yield, and quality of sugar beet (*Beta vulgaris*) grown on a calcareous sandy-loam soil. N-rich liquid digestate was applied at 100 kg-N ha^{-1}, with carbon added as either glycerol or straw at 24 kg-C m^{-3} and compared against farm standard application of inorganic nitrogen. Sugar beet yield and quality under liquid digestate application performed comparatively with synthetic fertilizer, indicating that digestate can be used to fertilize sugar beet. The addition of glycerol resulted in higher microbial biomass carbon than digestate alone in the first month after application. However, no benefit to crop or nitrogen retention in soil was recorded by adding carbon to digestate, which could be due to the low carbon addition rate. Future studies need to focus on determining the optimal amount of carbon to add with digestate to positively impact yield and reduce nitrogen losses.

Plain Language Summary

Anaerobic digestate contains nitrogen that is important for crop growth. However, methods are needed to increase the amount of digestate supplied nitrogen that is taken up by crops, as otherwise the nitrogen can cause environmental pollution. Soil microorganisms can help keep the nitrogen in the soil, since they need nitrogen for

Abbreviations: ANOVA, analysis of variance; LD, liquid digestate; MBC, microbial biomass carbon; TN, total nitrogen.

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cellular development. Therefore, we added glycerol and straw to the digestate, which contain carbon that microorganisms feed on, to investigate if it would make sugar beet use nitrogen more efficiently. The results showed that adding glycerol increased microbial growth within the first month after application; however, this did not affect soil nitrogen concentration or nitrogen uptake in the sugar beet and yield. This could be due to the low quantity of carbon that was added. Therefore, future studies are needed to find the optimal amount of carbon to add with digestate to positively impact yield and reduce nitrogen losses.

1 | INTRODUCTION

Nearly half of the global population relies on food produced using synthesized inorganic nitrogen fertilizers (Rosa & Gabrielli, 2022). However, there are negative consequences to its intensive use. The Haber-Bosch process that synthezes ammonium nitrogen by fixation of atmospheric dinitrogen is an energy-intensive process, currently reliant largely on fossil fuels (Smith et al., 2020). Using materials rich in already fixed nitrogen, such as anaerobic digestate, can reduce our reliance on fossil fuels. Digestate is a by-product of the biogas industry and is produced from the anaerobic decomposition of organic matter such as food waste, animal wastes, and energy crops. In the UK, over 70% of AD plants utilize feedstocks sourced directly from agricultural activities such as purpose grown energy crops, manures, and crop residues (NNFCC, 2021). Agriculture represents the primary sink for digestate, with over 90% of the material applied to farmland as a locally available and cost-effective source of crop nutrients (WRAP, 2020). Due to its nutrient-rich properties, digestate is considered a biofertilizer; however its high liquid content causes biogas plants to commonly separate digestate into two fractions to reduce cost of transport and storage (Al-Seadi & Lukehurst, 2012). Both the unseparated digestate and the liquid fraction have been found to produce yields comparable to inorganic nitrogen fertilizers through extensive field trials on cereals such as maize, wheat, and rice (Buligon et al., 2023; Doyeni et al., 2021; Du et al., 2019; Grillo et al., 2021; Pastorelli et al., 2021; Ran et al., 2022; Šimon et al., 2015; Zicker et al., 2020) and fodder or energy crops such as grasses and maize (Głowacka et al., 2020; Montemurro et al., 2015; Saunders et al., 2012; Tilvikienė, Šlepetienė & Kadžiulienė, 2018; Tsachidou et al., 2019; Vanden Nest et al., 2015; Vaneeckhaute et al., 2013; Walsh et al., 2018).

Whilst anaerobic digestate is a suitable substitute for inorganic nitrogen, crop nitrogen use efficiency is reduced due to nitrogen losses from ammonia volatilization, nitrate leaching, and nitrous oxide (N_2O) emissions. Ammonia losses can be reduced by 45%–95% by changing spreading techniques to precision applicators such as injectors or band spreaders (Nicholson et al., 2018), acidifying the digestate by adding sulfuric acid (Sánchez-Rodríguez et al., 2018; Wagner et al., 2021), or through plasma treatment (Graves et al., 2019). However, these techniques come with concerns of pollution swapping by increasing leaching and N_2O emissions (Fangueiro et al., 2015). The use of nitrification inhibitors is demonstrating positive reductions in N_2O emissions (Hegewald et al., 2021) and leaching (Giacometti et al., 2020); however uptake is limited due to poor cost-effectiveness (Freeman, 2020). Applying nitrogen in one bulk application results in lower nitrogen use efficiency compared to a split application of multiple smaller doses to match nitrogen demanding crop growth stages (Hu et al., 2021), as the single application incurs greater nitrogen losses via the aforementioned pathways. Yet, applying digestate in split applications is difficult as Wilson et al. (2021) recorded mechanical damage to maize at later crop-growing stages from an application system using an umbilical cord. As such, it is useful to identify strategies that could slow the rate of nitrogen released from digestate once it is in the soil to maximize the amount of supplied nitrogen taken up by the plant and improve productivity.

Previous research (Pittaway et al., 2018; Reichel et al., 2018) suggests that co-applying organic carbon-rich materials with inorganic nitrogen fertilizers can retain nitrogen in the soil through a microbially driven process. As soil microorganisms assimilate carbon for population growth, they also immobilize nitrogen for cellular development, a proportion of which persists as necromass after cell death, which is subsequently remineralized through necromass turnover (Kindler et al., 2006). In planted systems, nitrogen mineralization from necromass may be enhanced by priming effects induced by plant root exudates (Meier et al., 2017). In an incubation experiment (20°C and soil moisture at 40% of the water holding capacity), van Midden et al. (2024) identified that combining glycerol or straw with the liquid digestate (LD) increased microbial biomass nitrogen, thus demonstrating that carbon additives stimulated nitrogen immobilization. However, this work was done in the absence of a plant, and the effects of adding these materials with digestate on plant growth and yield require investigation.

The aim of this experiment was to evaluate how the addition of carbon additives to the liquid fraction of anaerobic digestate influences microbial nitrogen immobilization and the subsequent effects on field-grown crop uptake of nitrogen and yield. This experiment was undertaken in field conditions and compared anaerobic digestate with and without high carbon amendments (glycerol and straw) to the standard practice of using inorganic nitrogen fertilization on sugar beet as a tuber-producing crop. Sugar beet was selected as it is a high-value UK cash crop, providing over 55% of sugar produced to meet the UK's total sugar demand (Short et al., 2014), and it has not been studied in relation to fertilization with LD. We hypothesized that the combination of a high-carbon organic material with LD would increase the yield and quality of sugar beet. This is based on our premise that soil microbial nitrogen would increase due to the microbial utilization of amendments for growth, which would then act as a slow-release mechanism to supply sugar beet with nitrogen later in the growing season.

Core Ideas

- The addition of glycerol to liquid digestate increases soil microbial biomass within the first month of application.
- Applying glycerol with a nitrogen source such as digestate is a potential management strategy to increase soil organic carbon concentrations.
- Liquid digestate is a suitable fertilizer substitute for synthetic nitrogen when growing sugar beet.

2 | METHODS

2.1 | Experimental site, materials, and treatments

A field experiment was established growing sugar beet (*Beta vulgaris*, variety KWS Rixta) in 2023 on a commercial farm near Feltwell, UK (52° 29′ 59.68″ N, 0° 32′ 20.74″ E) on a calcareous sandy loam soil over chalk (Table 1). Average daily air temperature and precipitation data were collected from an on-farm weather station between February and November 2023 (Figure 1). The digestate (Table 1) used was generated by Future Biogas Ltd. in a plant that utilized a mixture of maize and rye silage, with seasonal addition of sugar beet pulp and horse manure as its feedstock. The digester has two primary and one secondary digester tanks and ran at a temperature of 47°C with a retention time of 82 days. Post digestion, the digestate was separated by screw press into liquid and solid fractions. Wheat straw (40% total carbon content), sourced on-farm, was used, and glycerol (38% total carbon content) was bought from Monarch Chemicals Ltd. (UK).

The experiment was laid out in a randomized complete block design with three replicates. The four treatments were (1) LD, (2) digestate with glycerol (LD + glycerol) at 24 kg-C per m^3 of digestate, (3) digestate with straw (LD + straw) at 24 kg-C per m^3 of digestate, and (4) inorganic nitrogen. The carbon rate was selected from earlier work determining the optimal carbon addition rate to stimulate microbial immobilization of digestate supplied nitrogen (van Midden et al., 2025). This gave a total of 12 experimental plots, each 26 m long and 32 m wide to fit within the tramlines. A combination of a historic yield map, a soil map provided by Landis.org.uk, and normalized difference vegetation index images was used to identify the area within the field with the most homogenous conditions for siting the experimental area (Supporting Information).

The field had received a base fertilizer (from Law Fertilisers Ltd) supplying 75 kg potassium ha^{-1}, 200 kg sodium ha^{-1} and 80 kg magnesium ha^{-1} in November 2022 to maintain the soil at target index for growing sugar beet (British Beet & Research Organisation) and an application of farmyard manure at 40 tonne ha^{-1} to add organic matter to the soil, which supplied approximately 24 kg-N ha^{-1}. In February 2023, preceding seedbed preparation, the straw was chopped and applied to plots of treatment 3 using a straw blower at 1.1 tonne ha^{-1}. The whole field was deep tilled (30-40 cm), disced, and rolled with a Cousins Patriot subsoiler in February, followed by shallow tillage (top 5 cm of soil) using a Dalbo Rollermaximum directly before sowing to prepare the seedbed. LD (without or with glycerol) was applied to the plots of treatments 1, 2, and 3 by A&R Cramphorns (Rutland, UK) using a band-spreader at the recommended rate for sugar beet (AHDB, 2023), equivalent to 100 kg-N ha^{-1} on March 30, 2023. The field was sown on April 4 at a rate of 115,000 seeds (variety KWS Rixta) per ha. Inorganic nitrogen fertilizer (Law Fertilisers Ltd) was applied to the plots of treatment four according to farm standard practices with a split dose of nitrogen-phosphorus-sulfur fertilizer to deliver 50 kg-N ha^{-1} on April 18 and calcium ammonium nitrate at 52 kg-N ha^{-1} on May 3. All other necessary crop husbandry was done following farm standard practices equally across all plots.

2.2 | Plant and soil sampling

Soil sampling was carried out six times throughout the year. The first occurred before the straw was added by way of pre-experimental characterization (January 31, 2023). The following five sampling events occurred between April and November to coincide with sugar beet emergence, the start of canopy growth, 85% canopy cover, the beginning of senescence, and harvest. To measure nitrogen uptake in the sugar

TABLE 1 Soil and liquid digestate characterization.

Parameter	Soil	Liquid digestate
Dry matter (%)	84	6.4
Organic matter (% w/w)	3.7	4.8
pH	8.5	8.1
Total carbon (% w/w)	5.5	2.7
Nitrogen		
Total nitrogen (% w/w)	0.2	0.53
Available nitrogen (mg N kg^{-1})	6.0	3106
Phosphorous		
Total (mg P kg^{-1})	–	564
Available (mg P kg^{-1})	48.6	–
Potassium		
Total (mg K kg^{-1})	–	4759
Available (mg K kg^{-1})	561	–
Cation exchange capacity (meq 100 g^{-1})	14.1	–
Bulk density (g cm^{-3})	1.5	–
Microbial biomass C (mg C kg^{-1})	314	–
Microbial biomass N (mg N kg^{-1})	46	–

Note: Results for digestate are on fresh weight basis and soil on dry weight basis.

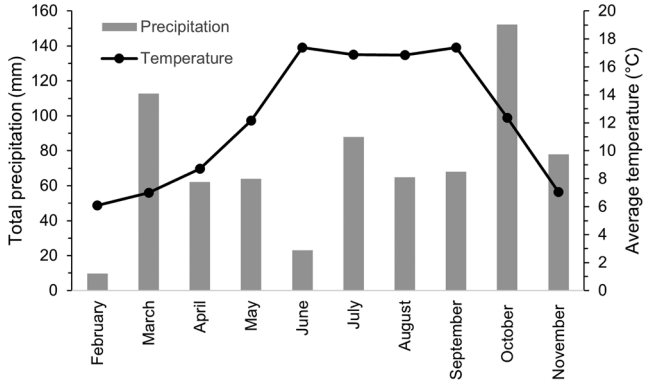


FIGURE 1 Monthly total precipitation and average temperature during the experiment.

beet, a total of 20 youngest and fully expanded leaves were collected from five points per plot following a "W" pattern across each plot. At the same locations, topsoil samples (0–15 cm) were collected using an auger and combined to make a 250 g bulk sample. At three points per plot along the "W," undisturbed soil samples were collected using stainless steel cylindrical cores (57 mm internal diameter and 45 mm in height), oven dried at 105°C for 48 h, and bulk density was calculated as g per cm^3 using the formula of soil dry weight divided by the core volume. Bulk density cores were not collected in July and September due to the hardness of the soil surface after a prolonged dry period.

Soil (sieved to 2 mm) was analyzed for microbial biomass carbon (MBC) and nitrogen following the chloroform-fumigation method (Vance et al., 1987). In brief, soil portions weighing 12.50 g dry weight equivalent were fumigated for 24 h and extracted using 0.5 M potassium sulfate. The extracts were filtered then analyzed on Shimadzu total organic carbon with a total nitrogen (TN) module, and the MBC and nitrogen contents were calculated from the difference between the fumigated and non-fumigated samples and converted using KEC (extractable part of microbial biomass C after fumigation) and KEN (extractable part of microbial biomass N after fumigation) values of 0.45 and 0.54, respectively (Brooks et al., 1985; Vance et al., 1987). Three analytical replicates per plot were processed to account for inherent within-plot variation. Soil pH was measured in a 1:5 v/v air-dry soil samples in a 1 M potassium chloride (KCl) solution following the British Standard BS ISO 10390:2005 method. Soil available nitrogen was analyzed at the last two timepoints in samples taken at 30–50 cm deep to estimate the amount of potentially leachable nitrogen. Although the depth of sugar beet roots can exceed 2 m (Windt & Märländer, 1994), samples were taken at this depth due to reaching the chalk layer underlying the soil. Soil samples were sieved to 2 mm, extracted with a 2 M KCl solution (20 g soil and 100 mL of solution), and analyzed on a segmented flow analyzer (Seal AA3) for total oxidized nitrogen (nitrate plus nitrite-N; total oxides of nitrogen) and ammonium-N following MAFF (1986).

Sugar beet was harvested on the November 1, 2023: 20 sugar beet tubers per plot were collected from three rows in the plot, the leafy tops were removed, and the tubers were stored in breathable hessian sacks. Upon return to the laboratory, five tubers were cleaned, chopped, weighed, and put into an oven at 80°C for 2 weeks until no further weight loss was recorded to determine dry matter content. The remaining 15 tubers were sent to the British Beet Research Organisation (Bexwell, UK) for analysis of sugar content, amino nitrogen, potassium, and sodium analysis. They were analyzed using techniques from the International Commission for Uniform Methods of Sugar Analysis (ICUMSA, 1994, 2007), which are standard to the UK sugar industry. The sugar beet leaves collected over the experimental duration were dried at 80°C for 48 h when no further weight loss was recorded, then finely milled and passed through a 0.75-mm sieve to be analyzed for TN content by dry combustion using an elemental analyzer (Elementar, Vario EL III).

2.3 | Statistical analysis

A repeated measures analysis of variance (ANOVA) was used to analyze data from measurements taken at multiple time points, and a one-way ANOVA was used for harvest data. In the case of the microbial biomass and available nitrogen

TABLE 2 Changes in the nitrogen content (mg-N g^{-1}) of sugar beet leaves (mean ± SE, $n = 3$) over time.

	Treatment			
Days after sowing	**Liquid digestate (LD)**	**LD + glycerol**	**LD + straw**	**Inorganic nitrogen**
49[A]	52.4 ± 0.8	49.7 ± 1.4	48.4 ± 1.7	49.7 ± 2.7
100[A]	49.9 ± 1.6	47.1 ± 2.3	50.3 ± 1.1	50.6 ± 1.2
155[AB]	46.9 ± 1.0	48.6 ± 1.6	49.3 ± 1.89	46.1 ± 2.6
209[B]	46.8 ± 2.3	44.4 ± 1.6	46.5 ± 0.8	47.8 ± 0.1

Note: Different superscript capital letters indicate significant differences between sampling times according to Tukey's test at 5% probability. There were no significant differences between treatments.

TABLE 3 Effect of treatments on various sugar beet tuber parameters (mean ± SE, $n = 3$) at harvest.

	Treatments			
Parameter	**Liquid digestate (LD)**	**LD + glyercol**	**LD + straw**	**Inorganic nitrogen**
Individual beet mass (kg)	0.89 ± 0.11	0.97 ± 0.10	0.91 ± 0.11	0.95 ± 0.13
Beet yield (t ha^{-1})	77.8 ± 3.6	84.9 ± 6.1	79.5 ± 1.8	83.0 ± 2.8
Sugar content (%)	17.4 ± 0.2	17.1 ± 0.3	17.2 ± 0.0	17.4 ± 0.2
Dry matter (%)	21.0 ± 0.3	21.3 ± 0.3	22.0 ± 0.3	21.4 ± 0.3
Amino N (mg 100 g^{-1} beet)	26.7 ± 0.3	29.0 ± 3.1	26.3 ± 0.9	28.3 ± 0.3
Sodium (mg 100 g^{-1} beet)	9.9 ± 0.4	10.2 ± 0.8	9.3 ± 0.5	10.0 ± 0.4
Potassium (mg 100 g^{-1} beet)	151 ± 10	159 ± 10	156 ± 7	163 ± 8

datasets where three analytical replicates were conducted per plot, the average value was used in the ANOVA model. Data were first tested for normality using Kolmogorov–Smirnov & Lilliefors test and homoscedasticity using Levene's test. Significant differences were determined using Tukey's post hoc test with significance determined at $p < 0.05$. All statistical analysis was performed on Statistica Tibco version 14.

3 | RESULTS

3.1 | Plant analysis

Very few seedlings had emerged at the first post-application sampling, so the first leaf sampling for TN occurred in the second post-application sampling time. There was no treatment effect ($p = 0.75$) on the TN content in sugar beet leaves at any timepoint (Table 2). However, leaf nitrogen concentration had significantly ($p < 0.001$) decreased by 7%, 209 days after sowing compared to May and July sampling dates. The sugar beet tubers were harvested 210 days after planting. There were no treatment effects on yield, sugar content, or impurities (Table 3).

3.2 | Soil analysis

In the first month following application, soils from the LD + glycerol treatment had a significantly higher ($p = 0.04$) microbial biomass C than digestate alone, but no statistically significant treatment effect on microbial biomass N ($p = 0.06$) nor MBC:N ratio ($p = 0.85$) was measured. Thereafter, no significant treatment effects on either microbial biomass C or N were observed (Figure 2). Time of sampling significantly ($p < 0.001$) affected both microbial biomass C and N similarly, with lower concentrations in samples collected in the 16 and 155 days after sowing compared to 209 days. MBC:N ratio was also significantly affected by time ($p = 0.01$), where MBC:N ratio was significantly higher at 100 days after application compared to 49 and 209 days (Table 4).

Soil available nitrogen was not significantly different between treatments ($p = 0.47$) at either 155 or 209 days after sowing (Table 5). However, there was a significant decrease ($p < 0.001$) in soil available nitrogen by 61% between the two sampling dates. Neither soil pH nor bulk density was affected by treatments ($p = 0.10$ and 0.41, respectively) at any timepoint (Table 5). However, both significantly ($p < 0.001$) increased over time irrespective of treatment, with a rise in soil pH of 0.1 unit and an increase in bulk density by 85% (from 0.76 g cm^3 to 1.4 g cm^3) between 16 and 209 days after sowing.

4 | DISCUSSION

For crops to reach their yield potential, it is important that both the amount and timing of nitrogen fertilizer applied match plant demand. Sugar beet requires an early dose of

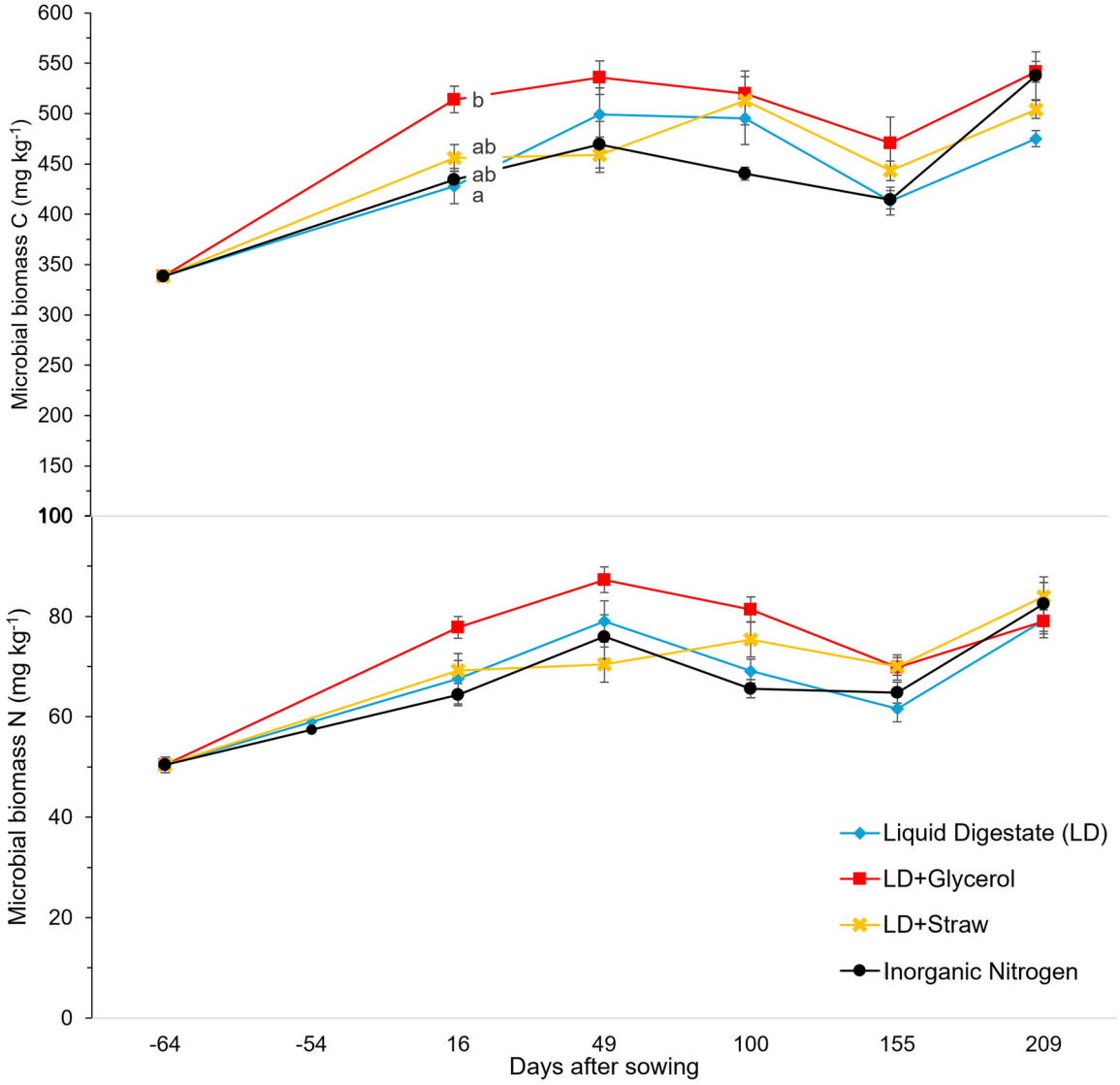


FIGURE 2 Changes in microbial biomass carbon (A) and microbial biomass nitrogen (B) in soils on a fresh weight basis over time. Different letters at each sampling time indicate significant differences between treatments according to Tukey's test at 5% probability. Error bars denote the standard error of the mean, $n = 3$.

TABLE 4 Changes in soil microbial carbon:nitrogen ratio (mean ± SE, $n = 3$) at different sampling times.

	Treatment			
Days after sowing	**Liquid digestate (LD)**	**LD + glycerol**	**LD + straw**	**Inorganic nitrogen**
16^{AB}	6.4 ± 0.2	6.6 ± 0.0	6.6 ± 0.1	6.8 ± 0.2
49^{B}	6.3 ± 0.1	6.1 ± 0.1	6.6 ± 0.2	6.2 ± 0.1
100^{A}	7.2 ± 0.2	6.4 ± 0.2	6.8 ± 0.2	6.7 ± 0.2
155^{AB}	6.7 ± 0.0	6.7 ± 0.3	6.3 ± 0.1	6.4 ± 0.2
209^{B}	6.0 ± 0.2	6.9 ± 0.2	6.0 ± 0.2	6.5 ± 0.4

Note: Different superscript uppercase letters indicate significant difference between sampling times according to Tukey's post hoc test at 5% probability. Treatment effects were non-significant.

TABLE 5 Changes in soil pH, bulk density, and available nitrogen (mean ± SE, $n = 3$) at different sampling times.

		Treatments			
Parameter	**Days after sowing**	**Liquid digestate (LD)**	**LD + glycerol**	**LD + straw**	**Inorganic nitrogen**
Soil pH	16^{B}	7.98 ± 0.01	8.02 ± 0.03	8.02 ± 0.02	7.98 ± 0.01
	49^{B}	7.98 ± 0.02	8.06 ± 0.03	8.00 ± 0.01	7.96 ± 0.02
	100^{A}	7.91 ± 0.03	7.99 ± 0.02	7.96 ± 0.01	7.91 ± 0.04
	155^{A}	7.92 ± 0.01	7.98 ± 0.04	7.96 ± 0.02	7.92 ± 0.02
	209^{C}	8.08 ± 0.01	8.11 ± 0.04	8.12 ± 0.01	8.09 ± 0.02
Bulk density ($g\ cm^3$)	16^{C}	0.79 ± 0.02	0.74 ± 0.03	0.74 ± 0.02	0.77 ± 0.02
	49^{B}	1.25 ± 0.03	1.26 ± 0.03	1.32 ± 0.03	1.31 ± 0.03
	209^{A}	1.41 ± 0.04	1.35 ± 0.04	1.45 ± 0.03	1.45 ± 0.05
Available nitrogen ($mg\text{-}N\ kg^{-1}$ dry soil)	155^{A}	17.6 ± 1.3	16.5 ± 1.0	15.5 ± 1.1	15.0 ± 1.4
	209^{B}	5.8 ± 0.6	6.9 ± 0.7	5.7 ± 0.6	6.2 ± 0.6

Note: Different superscript uppercase letters indicate significant differences between sampling times according to Tukey's post hoc test at 5% probability. Treatment effects were non-significant.

nitrogen to help it to quickly establish a full canopy, which is vital for it to develop high sugar-yielding tubers (Draycott & Christenson, 2003). Inorganic nitrogen fertilizers and anaerobic digestate contain a high proportion of readily plant-available nitrogen. In this study, digestate produced similar sugar beet yields and sugar content compared to applying mineral fertilizer. Additionally, the concentrations of the impurities (α-amino nitrogen, sodium, and potassium), which affect the amount and purity of the sugar that can be extracted from the sugar beet, were also not different between these two fertilizer sources (Table 3). Baryga et al. (2020) observed similar results with no difference in yield over a 3-year period between synthetic nitrogen and whole digestate. Therefore, our study corroborates their work to demonstrate that inorganic nitrogen can be substituted with anaerobic digestate, in our case the liquid fraction, without impacting either the yield, sugar content, or quality of the sugar beet. However, because the nutrient composition of digestate is variable, applying it to meet nitrogen requirements may result in imbalances in other essential nutrients, such as phosphorus, potassium, magnesium, and boron, which may need to be corrected with additional inputs to maintain soil fertility and crop yield (Baryga et al., 2021).

Once sugar beet reaches a full canopy, it begins to develop its energy storing tuber, which requires nitrogen. To meet this nitrogen demand, it redistributes nitrogen taken up in the leaves from early growth into its root (Draycott & Christenson, 2003) as well as taking up nitrogen from the soil (Armstrong & Milford, 1985). The combination of glycerol with digestate, or synthetic N with straw, immobilizes nitrogen into the soil within the first 2 months of application (Reichel et al., 2018; van Midden et al., 2024). This is the period of time when losses of nitrogen via volatilization and N_2O emissions from the applied digestate are greatest (Nicholson et al., 2017). Therefore, we expected treatments receiving digestate with either glycerol or straw to have had greater nitrogen concentrations in the soil. This would subsequently be remineralized following plant demand at this stage of crop growth to promote higher sugar beet yield. As this was not the case, we must reject our hypothesis and consider reasons why.

The rate and magnitude of microbial nitrogen immobilization are influenced by the bioavailability of the carbon in the amendment. Redmile-Gordon et al. (2014) and van Midden et al. (2024) both observed that the bioavailable glycerol-derived carbon had a quicker and greater effect on microbial uptake of nitrogen than the addition of the more recalcitrant straw-derived carbon. The addition of glycerol to digestate in our study did significantly increase MBC by 20% compared to digestate alone. However, microbial biomass nitrogen, which, whilst on average was higher by 15% under glycerol addition compared to without, was not statistically significantly different. This may have been because the magnitude of change was not great enough to be statistically detectable above background microbial biomass nitrogen, as the microbial C:N ratio did not change, indicating that nitrogen immobilization did occur. As a consequence of this marginal difference in microbial biomass nitrogen early in the season, there was no significant amount of additional nitrogen to be remineralized later in the growing season. This lack of increased nitrogen supply is reflected in the lack of difference in leaf TN content of sugar beet between treatments at any time point.

A challenge with stimulating microbial uptake of nitrogen is to ensure it does not immobilize nitrogen in quantities high enough to negatively affect plant growth, as demonstrated by Alotaibi and Schoenau (2016). They applied glycerol with urea at a ratio of 20:1 (compared to the C:N ratio of 10:1 of digestate with glycerol we used) and measured higher microbial nitrogen compared to urea without glycerol and a decrease in both plant nitrogen concentration and yield

within the first year of application. In contrast, the microbial nitrogen immobilization in our study, whilst too low to benefit crop nitrogen uptake during tuber development, did not deprive the sugar beet of the nitrogen it required during its early growth period. This implies that more carbon is required to stimulate significant microbial biomass nitrogen, but the balance needs to be found between the amount immobilized without negatively affecting yield.

Compared to glycerol, we had expected that nitrogen immobilization and remineralization from the addition of straw with digestate would occur over a longer period, as the depolymerization of lignin, cellulose, and hemicellulose structures in the straw into monomers needs to occur for its carbon to become bioavailable for microbial uptake and metabolism. However, in our study, the addition of straw to digestate did not significantly increase either MBC or nitrogen compared to digestate alone. This could be because, unlike glycerol, not all the carbon in the straw was available at the time of digestate application for microbes to utilize. Another reason for the lack of effect could be that straw was applied 7 weeks before fertilizer application. Gao et al. (2016) measured a 37% loss of straw mass within the first month of burial, suggesting that the easily accessible carbon in the straw may have already been utilized before the digestate was applied, although cool temperatures of late winter in the UK will have limited microbial activity. However, the straw and digestate could not be applied at the same time in the field, as straw needed to be incorporated into the soil, as this increases microbial growth due to having greater contact with the straw (Li et al., 2021). In addition, applying nitrogen onto crop residues lying on the soil surface increases N losses via ammonia volatilization (Rochette et al., 2001). However, wet weather conditions delayed sowing and fertilizer application following the straw application and incorporation operations.

Two soil physiochemical parameters, bulk density and pH, that influence nitrogen mobility in the soil (Nawaz et al., 2013; Truog, 1946) were measured in this study, but neither were significantly affected by treatment, although both did change over time. Changes in soil pH varied by 0.1 unit, which is within the seasonal range around the mean reported by Wuest (2015) in a 3-year study. As such, the change in soil pH measured over the growing season falls within normal parameters. Soil bulk density was lower in spring following cultivation and increased back to pre-cultivation level as the growing season progressed. It was thought that straw addition would reduce bulk density, as demonstrated by Guo and Wang (2013), Wang et al. (2018), Han et al. (2020), and Cui et al. (2022). However, this may be due to the difference in application rates, as we applied straw at 1.5 t ha^{-1} compared to the ~9 t ha^{-1} applied by Cui et al. (2022).

Potential leachable nitrogen, as inferred from soil available nitrogen taken at 30- to 50-cm soil depth, was not significantly different between treatments. This lack of effect is aligned with no difference observed in the MBC and nitrogen at the same sampling time. This indicates no extra nitrogen from the digestate with either straw or glycerol was being held in the microbial biomass compared to digestate alone in the autumn. Petraityte et al. (2022) measured lower concentration of soil nitrate taken from 30- to 60-cm soil depth in digestate-treated plots compared to synthetic fertilizer following a dry spring, whereas under normal conditions they observed no difference. Similarly, when comparing digestate with synthetic nitrogen at comparative rates of nitrogen addition, neither Du et al. (2019) nor Zilio et al. (2022) recorded significant differences in potential leaching as inferred from residual soil nitrate at harvest. Therefore, in combination with our results, research suggests that there is no additional risk of residual nitrate leaching from digestate application versus synthetic fertilizer at the end of the growing season. However, the effect of adding carbon into digestate on potential leachable nitrogen cannot be inferred from our results, as no measurable microbial nitrogen immobilization occurred earlier in the season. Yet, the greater microbial biomass measured under the digestate with glycerol treatment compared to digestate alone does indicate the potential of this treatment to reduce leaching losses in the early months following application, a period of time when nitrogen is vulnerable to leaching (Nicholson et al., 2017).

The greater microbial biomass measured in spring indicates a further benefit, as it can be used as a precursor to indicate changes in soil organic carbon concentrations (Brooks, 2001). Microbial biomass, which is not ingested by other soil organisms, transforms into necromass upon cell death (Kindler et al., 2006), which can make up more than half of soil organic carbon in soils (Liang et al., 2019). As such, mechanisms to increase necromass are an important strategy to sequester carbon (Liang et al., 2020), which in turn supports initiatives and policies regarding climate and food security (Minasny et al., 2017). The addition of glycerol, or a similar labile carbon source, to digestate could provide a mechanism for farmers to increase soil organic carbon concentrations without compromising food production. However, more work is required to determine whether this is the case, including quantifying the trade-off between necromass accumulation and greenhouse gas emissions resulting from microbial activity stimulated by the additional carbon and life-cycle analysis to evaluate the full environmental implications of utilizing a labile-carbon industrial by-product in this way.

5 | CONCLUSION

This experiment demonstrated that the addition of glycerol, but not straw, with digestate did increase MBC under field conditions, with no detrimental effect to the crop. Yet the increase in microbial biomass did not result in significantly greater quantities of nitrogen immobilized in the microbial

biomass, which in turn did not affect sugar beet nitrogen uptake, yield, or quality. To stimulate significant microbial nitrogen immobilization, more bioavailable carbon may be needed. However, care needs to be taken to ensure that the carbon does not cause nitrogen immobilization to the detriment of early crop growth. Therefore, this work contributes to developing the underlying principles regarding the amount of organic carbon materials to add with a nitrogen source to reduce nitrogen losses with the objectives to reduce environmental pollution and benefit crop growth (Chaves et al., 2005; De et al., 2022; Pittaway et al., 2018). However, as this was a single-year experiment on one soil type, it does limit the generalizations about treatment responses that can be made, due to the challenges of extrapolating results to soils with different characteristics. Furthermore, digestate characteristics vary due to feedstock and processing parameters (Risberg et al., 2017), which may influence how it affects soil microorganisms when co-applied with an organic carbon-rich material. Future studies are recommended to focus on two directions. First, to determine the amount of carbon to add to digestate to stimulate microbial nitrogen immobilization to benefit crop nitrogen utilization through a combination of pot experiments verified by field trials. The second direction is to explore both the direct and legacy effects of glycerol addition with fertilizer nitrogen on soil organic carbon, nitrogen, and the microbial community, which are fundamental to the nitrogen and carbon cycles in the soil. Furthermore, it is necessary to understand how these effects are influenced by different soil types, digestates made from different feedstocks, and crop nitrogen relationships such as variable timing of nitrogen demanding growth stages and differing acquisition strategies, including dependence on mycorrhizal associations, as these factors could influence the amount of carbon to add with the digestate. Answering these research gaps will provide confidence in the viability and sustainability of using organic carbon amended digestate to in the agricultural sector to reduce reliance on synthetic nitrogen fertilizers and improve the nitrogen use efficiency of the digestate.

AUTHOR CONTRIBUTIONS

Christina van Midden: Conceptualization; formal analysis; investigation; methodology; project administration; writing—original draft; writing—review and editing. **Tom Sizmur**: Conceptualization; methodology; supervision; writing—review and editing. **Liz Jane Shaw**: Conceptualization; methodology; supervision; writing—review and editing. **Jim Harris**: Conceptualization; methodology; supervision; writing—review and editing. **Hayden Morgan**: Conceptualization; funding acquisition; methodology; resources; writing—review and editing. **Mark Pawlett**: Conceptualization; funding acquisition; methodology; supervision; writing—review and editing.

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CONFLICT OF INTEREST STATEMENT

Hayden Morgan is employed by Future Biogas Ltd., from whom this study received funding and digestate used in this study. The remaining authors declare that the research was conducted in the absence of any other commercial or financial relationships that could be construed as a potential conflict of interest.

DATA AVAILABILITY STATEMENT

The datasets generated during this study are available in the Cranfield University research data (CORD) repository at: https://doi.org/10.57996/cran.ceres-2769.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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