Field lab

Sward Improver:

Nitrogen-Free Soil Treatments For Grassland Productivity

Final report

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Summary

The application of soil treatments to augment ecosystem services in extensive grassland swards can recoup substantial environmental gains, including i) aquatic biodiversity benefits (by eutrophication avoided downstream), and ii) reducing fossil fuel-emissions. The first is achieved through reductions in soil leaching of nitrate; the second is achieved by *the use of carbon rich by-products (from fossil-fuel substitutes).* The present IF 'Field Lab' was a pilot to see if these "plant-powered" by-products could also increase the abundance of native nitrogen fixing plants (primarily clover) thus contributing to long-term protein generation in the grazed sward. The original trial was scheduled to complete in 2020, but had to be extended until 2022 due to the pandemic.

**climate positive* is defined as genuine long-term carbon offsets by substitution of fossil-C combustion with biofuel-C combustion.*

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1 Field lab aims (up to 50 words)

Primary: Determine if a very high C:N ratio soil amendment (by-product of biodiesel production) would increase the abundance of native nitrogen fixing plants in this extensively grazed chalk downland sward.

Secondary: Determine if the same treatment would improve drought-tolerance at the site.

Tertiary: Investigate the mechanisms driving the above.

2 Background (up to 250 words)

Increasing the abundance of native nitrogen fixing flora like clover can help ensure the sustainability of protein generation. Previous observations have shown that "biodiesel co-product" (BCP; primarily glycerol) can achieve this through application to soil in a domestic setting (Figure #1) but it had not been tested in an open grassland system. This value-added use for BCP could complement existing climate benefits of local biodiesel production from offsetting the emissions that would otherwise occur from the combustion of fossil fuels. The application of 'climate-positive' soil treatments to increase productivity of extensive grassland swards could represent other environmental gains too: including *i) aquatic biodiversity benefits (downstream), and ii) avoiding dependence on artificial fertilisers for protein generation*. Increased nitrogen fixation from increased clover abundance could increase plant biomass production for several seasons.

Figure 1 – Test of the principle in a domestic setting after 1 year: BCP applied to lawn area to the right.

As a secondary aim we chose to investigate potential for imparting drought-tolerance. Work from Rothamsted Research shows that naturally absorbent biopolymers or 'EPS' ('Extracellular Polymeric Substances') are produced by soil microbes in response to BCP applied to soil in the laboratory (Redmile-Gordon et al., 2015). However, this has not been tested in downland calcareous soils. Increased EPS biopolymers in soil should help to improve plant drought-resistance, bringing obvious economic benefits in the increasing summer droughts we expect in the UK due to climate change.

3 Methodology (up to 800 words)

To test the new sward improvers we selected an extensively grazed pasture at Truleigh Hill which is characteristic of intermediate-diversity parcels on the South Downs of the UK. All the grassland at Truleigh Hill has been managed with zero inputs for 26 years. It is grazed by cattle, sheep, and a small herd of Bagot goats. Red and White Clover was spread quite evenly across the area, indicating capacity for N fixing cover to be improved by the treatments (Figure 2).

Figure 2: mixed grasses and clovers at the study site

The selected area has been managed under HLS prescription HK7 “restoration of species rich semi natural grassland” since January 2010, and was fenced off in December 2019. Two experimental areas were established within the enclosure (‘EXP1’ for crude glycerol, and ‘EXP2’ for BCP; Figure 3). Nitrogen fertiliser (‘nitram’) was included for quality control as N limitation is known to reduce productivity, and affect drought tolerance. Treatment allocations were randomly designated, according to the following plan:

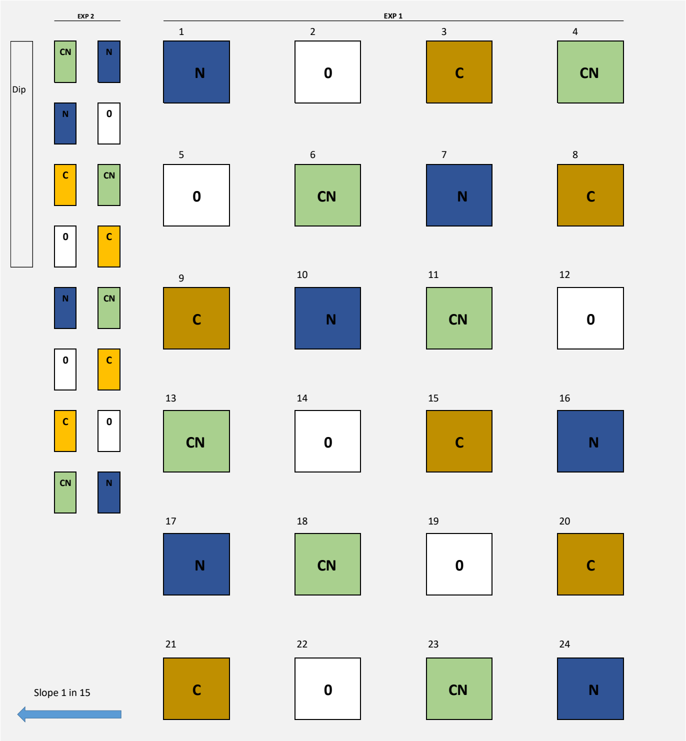
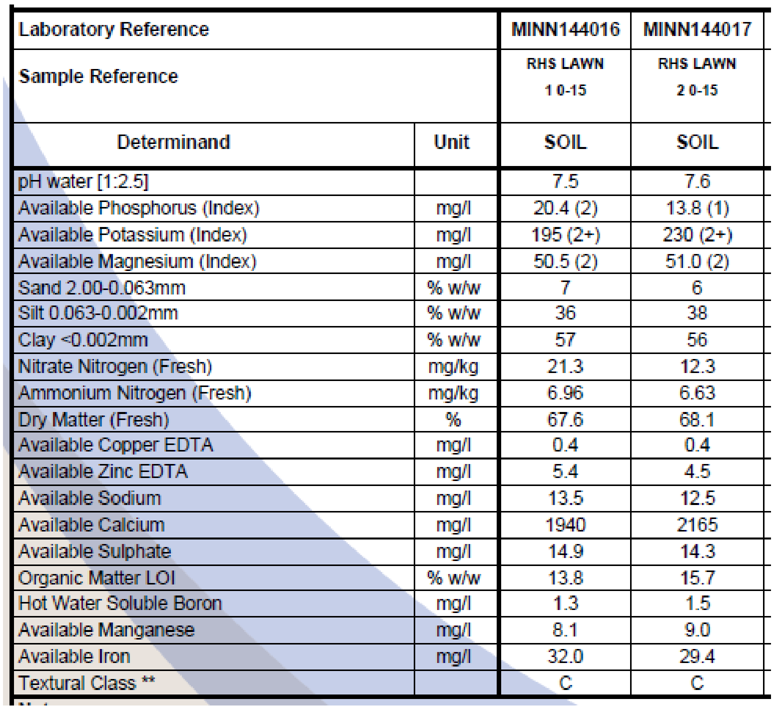


Figure 3: Plots given carbon (C), Nitrogen (N) and water only (0)

Grass was cut (clippings removed) late February 2019 to remove old material in preparation for application. However, a 1-year hiatus followed (pandemic lockdown) with the site thus being ungrazed and unmanaged. In March 2021, the trial recommenced. Two composite soil samples (5 locations; W pattern) were taken on 29th March 2021. Properties of soils before treatment are provided in Table 1. Average “available N” in the soil was 44.3 kg ha^{-1}



Laboratory Reference		MINN144016	MINN144017
Sample Reference		RHS LAWN 1 0-15	RHS LAWN 2 0-15
Determinand	Unit	SOIL	SOIL
pH water [1:2.5]		7.5	7.6
Available Phosphorus (Index)	mg/l	20.4 (2)	13.8 (1)
Available Potassium (Index)	mg/l	195 (2+)	230 (2+)
Available Magnesium (Index)	mg/l	50.5 (2)	51.0 (2)
Sand 2.00-0.063mm	% w/w	7	6
Silt 0.063-0.002mm	% w/w	36	38
Clay <0.002mm	% w/w	57	56
Nitrate Nitrogen (Fresh)	mg/kg	21.3	12.3
Ammonium Nitrogen (Fresh)	mg/kg	6.96	6.63
Dry Matter (Fresh)	%	67.6	68.1
Available Copper EDTA	mg/l	0.4	0.4
Available Zinc EDTA	mg/l	5.4	4.5
Available Sodium	mg/l	13.5	12.5
Available Calcium	mg/l	1940	2165
Available Sulphate	mg/l	14.9	14.3
Organic Matter LOI	% w/w	13.8	15.7
Hot Water Soluble Boron	mg/l	1.3	1.5
Available Manganese	mg/l	8.1	9.0
Available Iron	mg/l	32.0	29.4
Textural Class **		C	C

Table 1: laboratory analysis of soils; textural class C = clay

We obtained both 1) a tried-and-tested BCP (from community-scale production), and 2) a mass-produced similar co-product (crude glycerol) to ensure UK-wide availability (kindly donated by Argent Energy). BCP is produced as a the 'whole' by-product of biofuel production (methanol removed) as is proven to reduce nitrate (N) pollution from agricultural soils (Redmile-Gordon et al., 2014). Crude glycerol (the main constituent of BCP) is also C-rich and N poor - meaning it is likely to have similar impacts to BCP (after standardising the pH).

Material prep for EXP2

Biodiesel co-product for EXP2 was produced using the methods described by Redmile-Gordon et al., (2014). The resulting alkaline material has a 'syrup-like' consistency and was dissolved in water at the approximate ratio 9:1 (water:BCP) and adjusted to pH 7.5 by adding malt vinegar while stirring (Figures 4 a-c).

Figures 4a-c: "Organic" pH-balancing of homemade biodiesel co-product

Application of C and N to the soil

Sufficient BCP and crude glycerol was prepared to provide 200 g C per metre (2 tonnes per hectare equivalent). Initial treatments were applied 22nd – 24th April 2021. Application to the area "EXP2" was given as a split dose (100 g C per metre in April and remaining 100g C per m on 5th June). At 30% C, crude glycerol was applied as a single dose (200 g C per metre), requiring 671 g per M. Accordingly, 1611 mL of crude glycerol (density 1.25) was made up to 7 litres and applied an area of 3 metres within each 9M plot. This was repeated twice to complete the application for each plot. Sufficient ammonium nitrate was subsequently dissolved in 7L cans and applied similarly to provide 18 g N per M to plots designated N (figure 3; giving an 'easily available' C/N ratio of 11.11 on plots marked 'CN'). Finally, water was applied to make up any differences in water applied (example = 42 litres per 'zero-input' control plots of EXP1).

Data collection

- **Soil samples** were taken as above and sent to NRM (Cawood Scientific) for analysis.
- **BCP and Crude Glycerol** C and N contents were analysed by NRM (Cawood Scientific).
- **Sward growth** was determined by mowing, and clippings were weighed**.** The sensitivity of this approach was not found to exceed that by eye but enabled statistical analysis. Sward growth was compared statistically using a 2-way ANOVA.
- **Clover abundance and drought** was compared and corroborated by eye (Researcher, Landowner, Farmer, Visitors from South Downs National Park Authority and representatives from the local water company (Southern Water)).

Adaptation:

To determine the sward's capacity for N Fixation we focussed on identifying any changes in the abundance of clover (and other N fixing species). As a secondary goal, we were to monitor for drought-effects. Preliminary observations in 2021 indicated that the 1 year delay (2020 lockdown: preventing mowing) had reduced the abundance of clover over the entire enclosed area. Since i) our *primary objective* depended on a clover effect, we redirected resources from tertiary objectives (mechanisms; analytical) into extended mowing period to maximise the chances of us capturing the emergence of a primary treatment effect (on clover abundance) in 2021-2022.

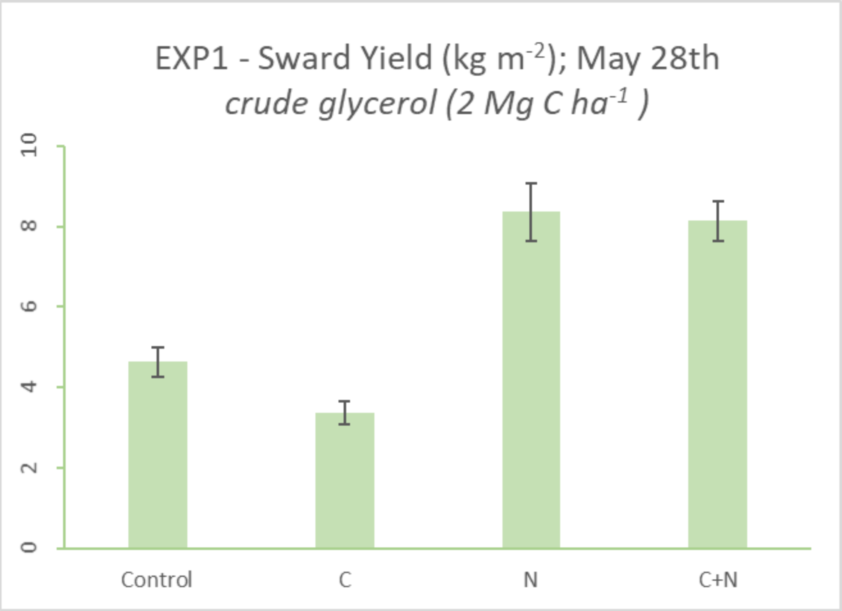
4 Results and discussions (total 1,500 to 2,000 words not including graphs, tables etc)

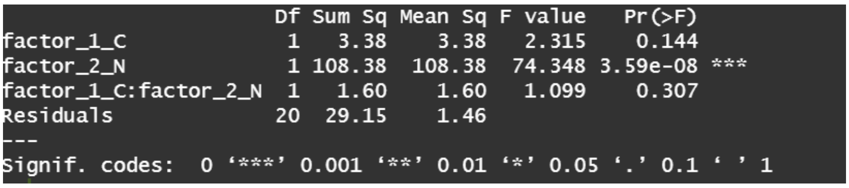
The experimental area was prepared to determine early differences in yield (Figs. 5 & 6). A suppressive effect on the growth of grass was expected, because BCP/glycerol application (hypothesised here to cause an increase in clover abundance) is known to cause microbial immobilisation of N in soil.

Figure 5: before yield-cut and measurement (ground)

Figure 6: before yield-cut and measurement (overhead view)

Differences in growth had become clearly evident within 2 weeks of application. Below are the results for differences in yield of grass clippings taken at the mowers' maximum cut-height (approximately 2-3 inches) 4 weeks after application (Figure 7).





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                      Df Sum Sq Mean Sq F value   Pr(>F)    
factor_1_C             1   3.38    3.38   2.315    0.144    
factor_2_N             1 108.38  108.38  74.348 3.59e-08 ***
factor_1_C:factor_2_N  1   1.60    1.60   1.099    0.307    
Residuals             20  29.15    1.46                     
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
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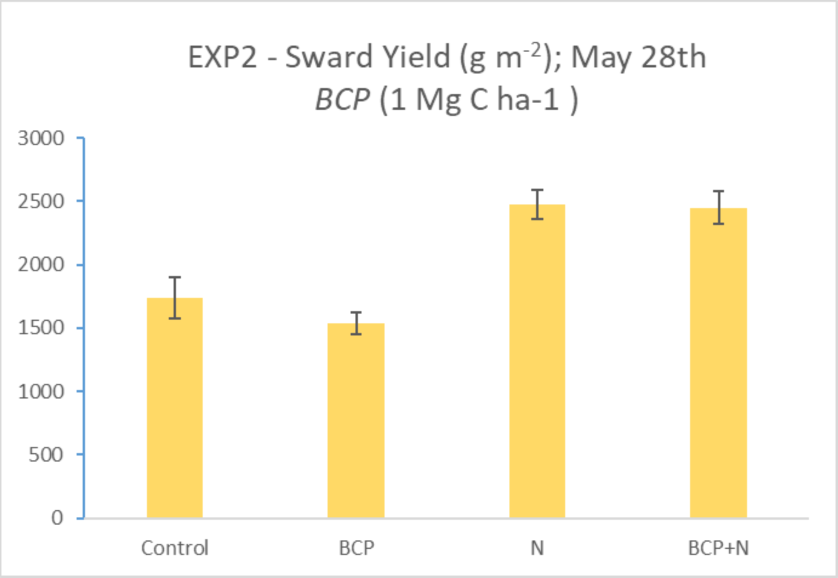
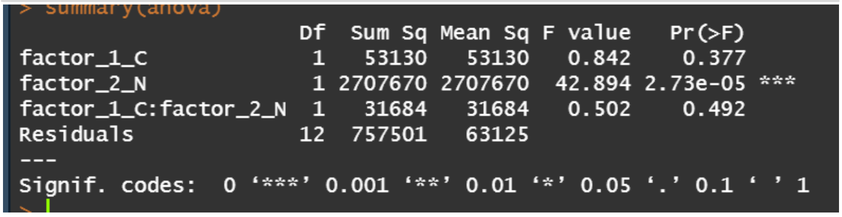
Figure 7: average sward yields, one month after glycerol application (full dose) with summary statistics (ANOVA)

ANOVA indicated the mean yield response to crude glycerol addition was 5.75 kg per 9 m plot, without C, this was 6.50 kg per 9 m plot. Accordingly, 200 g of glycerol-C application had supressed the growth of grass by <u>83 g per metre</u>. However, variability means this effect was not statistically significant (F = 2.315; P = 0.144). Nonetheless, the effect was to be expected, as clear by reference to related literature (presented in subsequent section).





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> summary(anova)
                      Df  Sum Sq Mean Sq F value   Pr(>F)    
factor_1_C             1   53130   53130   0.842    0.377    
factor_2_N             1 2707670 2707670  42.894 2.73e-05 ***
factor_1_C:factor_2_N  1   31684   31684   0.502    0.492    
Residuals             12  757501   63125                     
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Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

Figure 8: average sward yields, one month after BCP application (half dose) with summary statistics

ANOVA indicated the mean yield response to BCP addition (half dose at this stage) was 1992 g per 2-metre plot, and without BCP was 2107 g per 9-metre plot (Figure 8). Accordingly, 100 g BCP-C application had supressed the growth of grass by 57 g per metre. However, due to variability, this effect was not statistically significant (F = 0.842; P = 0.377). Nonetheless, the effect was to be expected, as clear by reference to related literature (presented in subsequent section).

Primary objective (clover abundance)

No differences in the abundance of clover were observed. However, there was a crash in the clover population over the entire site. The reasons for this are not known.

It seems likely that the absence of grazing and cutting in 2020 (pandemic and lockdown preventing access) caused this population crash by competition with excessive grass growth. Observations in the summers of 2021 and 2022 (Researcher, Landowner, Stock manager, visitors from South Downs National Park authority and the local water company (Southern Water)) concurred that while clover was still abundant outside of the enclosure (grazed as normal) there remained almost no clover within the enclosure besides the occasional randomly distributed plant. Accordingly, no correlation between treatment and clover occurrence could be made, with most plots being completely free from any clover. Of all possible explanations, it seems most likely that that the hiatus in management or grazing caused this crash by grasses (of higher stature) competitively excluding the clover.

A crash in clover (or other N fixing plants) was not noticed at the time of application (April 2021) because plants had not begun to grow significantly. April 2021 was exceptionally dry in the UK, with the most air frosts recorded for at least 60 years. Clover plants either side of the fence at this very exposed site were accordingly wind- and frost-damaged and exceedingly small.

Following in a similar vein of luck, the summer of 2021 was wet, and dull in South East England, with 200% average rainfall falling in June (source: MetOffice). Accordingly, water was not limiting to growth and the secondary aim -to drive improvements in drought tolerance- could not be tested. Given the absence of emerging differences we put our unused resources into continued mowing and clipping removal (thanks to Stock Manager; Tristan Driver). This was to give time for any differences in clover abundance to emerge (either from vestigial plants or seedbank) until May 2022. However, in hindsight, it seems that the damage had already been done, and we were unable to make up for the year of the pandemic.

5 Conclusions (up to 500 words)

The present study was unable to confirm or deny whether BCP application can be a practical farming strategy to increase the abundance of native nitrogen fixing plants in a sward, or improve resistance against drought. However, the applications of carbon (C) (as BCP or glycerol) almost certainly decreased nitrate leaching into the chalk aquifer below the study site. This is of interest to water companies seeking to protect groundwater quality and reduce climate-impacts of their operations.

The amount of BCP/glycerol added was consistent with that used by De, Sawyer, & McDaniel (2022). These researchers corroborated the early findings of Redmile-Gordon et al., (2014), showing

BCP substantially reduces nitrate leaching (up to 99%), via increased growth of the soil microbial biomass (Redmile-Gordon et al., 2014). This effect is also coupled with the associated release of extracellular polymeric substances (EPS) into the soil (Redmile-Gordon et al., 2015), with EPS potentially exceeding the weight of the total microbial biomass under mixed grassland (Wu et al., 2012; Redmile-Gordon et al., 2020).

The scaling up of application methods could be achieved through the use of tractor mounted trailing hose. Alternatively, BCP application around nitrogen hotspots (eg drinking troughs), or karst features, is an interesting prospect as targeted areas that pose greatest risk of N pollution to underlying aquifers (and water supply). Additional advantages (such as reduced phosphate loadings from runoff) may be achieved by the stabilisation of soil that accompanies increased microbial production of EPS from exogenous carbon sources (Redmile-Gordon et al., 2020).

Agricultural/Horticultural support of the biofuel industry (through value-added applications) can prevent long-term negative climate effects i) by substituting fossil-fuel C, ii) leveraging other climate-positive outcomes: from soil structure to improved nitrogen dynamics. These and related ecosystem service enhancements deserve further investigation. One such interesting ecosystem service was the cessation of N_2O emissions (298 times more climate forcing than CO_2 over a 100-year period) when BCP was applied at similar rates to acid soils (Shen et al., 2021).

The present study revealed that the growth of grass can be temporarily slowed by application of 'climate-positive' materials such as glycerol or BCP. This application is also of interest to gardeners (many of whom happen to be farmers) wanting to reduce the number of times a lawn needs mowing. Reduced mowing reduces fossil-fuel carbon emissions, which once released, become part of the unstable carbon pool cycling throughout the biosphere. The application of BCP and/or glycerol to soil thus represents a doubly 'climate-positive' practice, with various gardening, horticultural, and agricultural applications deserving our attention.

6 Tips and recommendations

- Co-ordinators can be very useful in identifying other applications for products and/or techniques.
- Building in flexibility in case of unfavourable weather conditions or unforeseen events is an advantage.
- Frequent communication with the Innovative Farmers Manager (Becky Swinn) helped to ensure decisions remained within farmers' interests and mutually beneficial targets were being pursued.

7 Further reading

De, Sawyer, J. E., & McDaniel, M. D. (2022). Crude glycerol, a biodiesel byproduct, used as a soil amendment to temporarily immobilise and then release nitrogen. European Journal of Soil Science, 73(3), n/a–n/a. https://doi.org/10.1111/ejss.13241

Redmile-Gordon, Armenise, E., Hirsch, P. R., & Brookes, P. C. (2014). Biodiesel Co-Product (BCP) Decreases Soil Nitrogen (N) Losses to Groundwater. Water, Air, and Soil Pollution, 225(2), 1831–15. https://doi.org/10.1007/s11270-013-1831-7

Redmile-Gordon, Evershed, R. P., Kuhl, A., Armenise, E., White, R. P., Hirsch, P. R., Goulding, K. W. T., & Brookes, P. C. (2015). Engineering soil organic matter quality: Biodiesel Co-Product (BCP) stimulates exudation of nitrogenous microbial biopolymers. Geoderma, 259-260, 205–212. https://doi.org/10.1016/j.geoderma.2015.06.006

Redmile-Gordon, Gregory, A. S., White, R. P., & Watts, C. W. (2020). Soil organic carbon, extracellular polymeric substances (EPS), and soil structural stability as affected by previous and current land-use. Geoderma, 363, 114143–114143. https://doi.org/10.1016/j.geoderma.2019.114143

Shen, Redmile-Gordon, M., Song, J., Li, J., Zhang, K., Voroney, P., Xu, J., & Brookes, P. C. (2021). Amendment with biodiesel co-product modifies genes for N cycling (nirK, nirS, nosZ) and greenhouse gas emissions (N2O, CH4, CO2) from an acid soil. Biology and Fertility of Soils, 57(5), 629–642. https://doi.org/10.1007/s00374-021-01546-4

Wu, Y., Kemmitt, S., White, R. P., Xu, J., & Brookes, P. C. (2012). Carbon dynamics in a 60 year fallowed loamy-sand soil compared to that in a 60-year permanent arable or permanent grassland UK soil. Plant and Soil, 352(1-2), 51–63. https://doi.org/10.1007/s11104-011-0979-4

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