Dose-response relationships between gypsum and the peat microbial community

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Dose-response relationships to GGR methods

Peatlands that are degraded or drained emit huge amounts of carbon as the plant material they are made of is exposed to oxygen and released as carbon dioxide. Keeping peat wet is a way to avoid these emissions, however wet peat naturally emits methane, a powerful greenhouse gas.

What is needed, then, is a method of managing wet peatlands that both sequesters carbon in the peat soil but that also limits methane production. Adding sulphate (as the mineral gypsum) to the system is one way to do this as it 'nudges' the microbial community away from methane production but we don't know how much we will need to add to the peat to stop methane production.

Our project proposes to assess what dose of sulphate is required to achieve a low methane peat system and how other factors, such as the starting chemistry of the peat, will influence this. This knowledge will allow us to understand how the greenhouse gas removals achievable by peatland management may vary across the country and stop us potentially adding more sulphate than is necessary.

Methane formation post-rewetting is dependent on the availability of labile substrate, as well as the concentration of terminal electron acceptors (TEAs) (Robinson et al., 2023). We therefore hypothesise that the efficacy of the sulphate application may be dependent on peat chemistry (as a control on substrate availability) and the starting concentration of TEAs. To test this hypothesis we propose to characterise the dose-response relationship between sulphate additions (added as gypsum) and the microbial community within peat of different starting chemistries.

Experiment 1: impact of peat humification (therefore labile C availability) on methane production.

Experiment 2: dose-response relationship between gypsum additions and methane production potential.

These experiments will tell us how sulphate, nitrogen and labile carbon control methane production and will therefore allow us to scale the results from the wider GGR-Peat programme to sites of different starting chemistries.

Initial sampling

Figure 1 a 50 cm peat core taken from Featherbed Moss, UK.

Initial core sampling took place at Featherbed Moss in the Peak District in July 2024. Cores were cut into 5 cm section for the first 30 cm and then 10cm sections thereafter. Cores show a much darker top section, likely due to spheroidal carbonaceous particles from the industrial revolution as the site is ~30 miles downwind of Manchester, UK (Figure 1).

Sampling of Featherbed an additional GGR-Peat project sites will happen in autumn 2025.

The core was then analysed for S content to confirm where sulphate pollution from the industrial revolution was present in the peat profile (Figure 2) this confirmed our sampling strategy as the industrial revolution peak is within out high-resolution sampling zone. This also confirms a natural gradient in sulphate is present in our samples to use in later experiments.

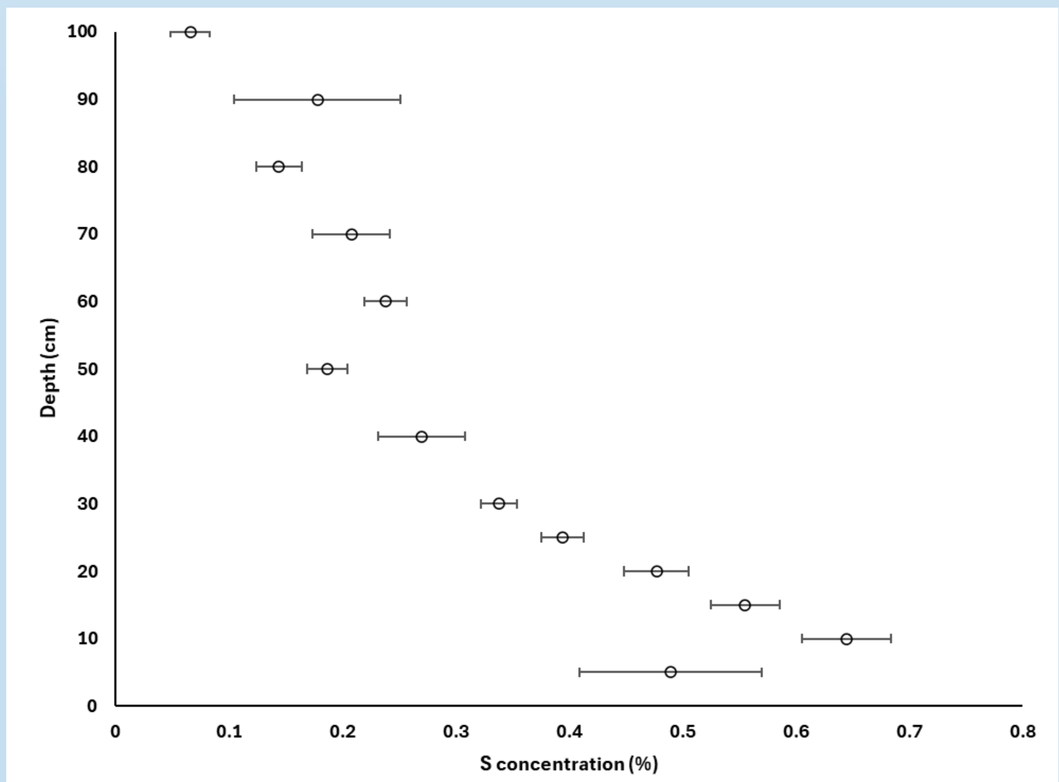

Figure 2 Sulphur content with depth down the core (mean and SE off three replicate cores)

Method development and next stages

To assess how the fungal, bacterial and archaeal communities vary along the vertical strata of the core, DNA extraction and amplification methods were optimised (Table 1). Peat soils contain high concentrations of inhibitors and a low density of microbes, so finding an optimal balance between concentrating the DNA sample and avoiding the co-concentration of inhibitors is paramount. The methods were optimised for the ITS1F/ ITS4 primers targeting the fungal ITS region (Figure 3), and for the 505F/806R primers targeting the bacterial and archaeal 16s region. DNA extraction was carried out with a DNeasy PowerSoil Pro Kit using a Fast-Prep 96 bead beater. Extracts were cleaned and concentrated with Zymo DNA Clean & Concentrator-5 kit.

The 16s primers used thermocycler conditions from the Earth Microbiome Project. The ITS primers used the thermocycler conditions: Stage 1, 95°C for 3 mins; Stage 2, 98°C for 20 secs, 64°C for 15 secs, 72°C for 40 secs; Stage 3, 72°C for 60 secs. Stage 2 was repeated for 40 cycles for both ITS and 16s, and the annealing temperature was reduced by 5°C when 10% DMSO was included in the master mix.

With DNA extraction and amplification optimised, the microbial community down the cores can be sequenced and assigned taxonomic classification. Variation in the microbial communities down the cores is predicted, and variation in the community will be correlated with the variation in soil chemistry and the gypsum addition.

Table 1: *The variation in methods for DNA extraction and amplification, with the optimal methods indicated for ITS and 16s*

Step	Method Variations	ITS Optimal	16s Optimal
Extraction weight	0.2–0.25 g or 0.3–0.35 g	0.2–0.25 g	0.2–0.25 g
Dilution	50 µl or 100 µl	50 µl	50 µl
Clean&Concentrate	With or Without	With	With
DMSO	None, 1%, 10% (−5 °C annealing)	1%	10%
BSA	None, 10µg/ml, 100µg/ml	10µg/ml	100µg/ml

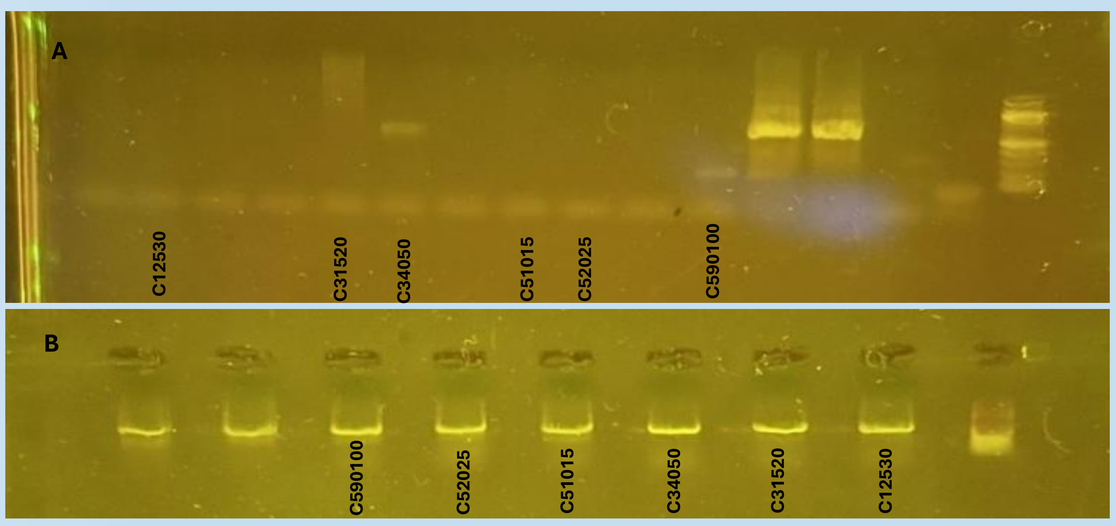

Figure 3: Comparison for ITS PCR amplification. Samples were extracted from 0.25g peat soil and had a final extraction dilution of 50 µl dilution. (A) Without 'Clean & Concentrate', DMSO or BSA (B) With 'Clean & Concentrate', 1% DMSO and 10µg/ml BSA. Sample naming convention: 'C#' indicates core number, followed by depth (cm) eg C12530 = Core 1, 25-30cm depth