

Identifying sulphate-reducing and magnetotactic bacteria in a hyperalkaline cave system

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Abstract: Sulphate-reducing bacteria (SRB) and magnetotactic bacteria (MTB) are two special groups of prokaryotes that emerged early in Earth's history. These extremotolerant bacterial groups have rarely been identified or studied within cave and karst environments, especially hyperalkaline cave systems (pH > 9), such as Poole's Cavern in Derbyshire, England. In this study, we identify the sulphate-reducing MTB *Desulfovibrio magneticus*, within this hyperalkaline cave system. It appears to survive in a diverse range of environments including soil, stalactites and cave sediments. Additionally, we identify various extremotolerant SRB in similar Poole's Cavern environments. We show that these SRB and MTB can move successfully into subsurface environments and adapt concomitantly to the anomalous pH, saline, and relatively nutrient-poor conditions found in Poole's Cavern. These findings are significant to our understanding of microorganisms on early Earth because it is believed, but not proven, that underground environments might have been hot spots for early microbial life. We expect that these early bacteria would have been able to adopt similar adaptation strategies, transferring and acclimatizing to underground environments, in ways comparable to the SRB and MTB identified in this study.

Keywords: Poole's Cavern; sulphate-reducing bacteria; magnetotactic bacteria; 16S rRNA sequencing; extremotolerance

Introduction

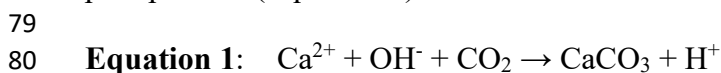
Bacteria capable of performing sulphate reduction have been found in caves across the globe (Guo *et al.*, 2021; Portillo and Gonzalez, 2009). These sulphate-reducing bacteria (SRB) oxidize organic compounds or hydrogen by using sulphate as an electron acceptor. As SRB act to cycle sulphate they perform an important function, ensuring equilibrium of sulphurous sources within their surrounding environment. Within the SRB community, a few members are able to develop magnetotaxis; they are called magnetotactic bacteria (MTB). It is worth noting that not all MTB are SRB. MTB are a special group of microorganisms that form intracellular, membrane-capsulated, magnetite, meaning that these microorganisms align naturally with the Earth's magnetic field lines and can "swim" along them (Blakemore, 1975). In general, MTB are discovered in aqueous bodies or waterlogged soils and occupy the micro-anaerobic and oxic-anoxic transition zone. It is believed that their magnetotaxis allows them to travel appropriate distances to reach regions of optimal oxygen concentration within these transition environments (Frankel and Bazylinski, 1994). Although MTB are postulated

50 to exist in caves (Djerrab and Aïfa, 2010; Ellwood *et al.*, 2001; Ellwood *et al.*, 1998),
51 MTB in cave waters and sediments are rarely identified (Goswami *et al.*, 2022).

52 Regardless of this sparsity of data, the question of SRB and MTB survival within
53 cave systems is an important one. SRB and MTB emerged early (around 3.0–3.5 Ga)
54 within Earth’s history (Barton and Fauque, 2009; Lin *et al.*, 2017; Shen and Buick,
55 2004), at a time when the surface was extremely hostile, with more-frequent meteorite
56 impacts, volcanic activities and high radioactive heat fluxes (Orme, 2013). During that
57 initial period of development, caves were one of the important hot spots for the
58 development of early life, because they sheltered microbes from the far more
59 inhospitable surface environments (Vaccarelli *et al.*, 2021). Whereas caves might well
60 have provided shelter from intense surface irradiation, these would still have been
61 extreme environments, requiring life to develop strategies, such as extreme tolerance to
62 variable pH in both waters and sediments, to resist various inhospitable conditions
63 (Kadoya *et al.*, 2020; Kempe and Kazmierczak, 1997).

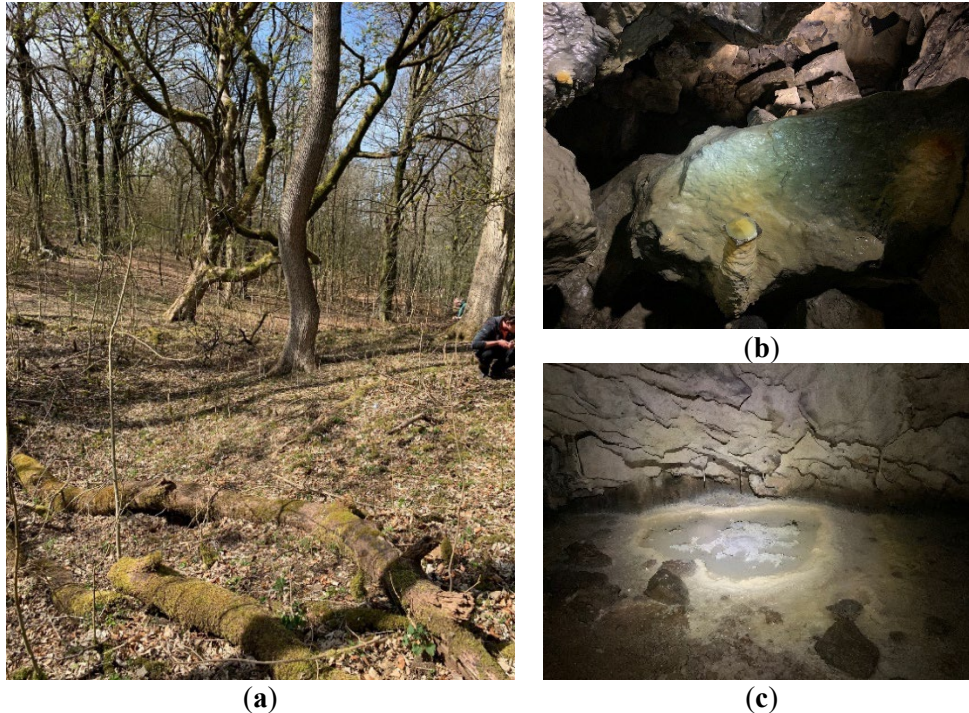
64 SRB and MTB can inhabit extreme pH environments on the surface of the Earth,
65 including the saline Mono Lake, California, with a pH of 9–10 (Lefevre *et al.*, 2011;
66 Pikuta *et al.*, 2003). It is believed that they developed physiological mechanisms to
67 tolerate such high pH, including the development of intracellular granule formations,
68 or cytoplasmic buffering for the neutralization of environmental alkalinity (Abreu *et al.*,
69 2018; Krulwich *et al.*, 2011). Whilst there is evidence of SRB and MTB surviving
70 within high-pH environments and evidence of SRB within caves, currently there are no
71 studies that consider high-pH cave environments as a possible refuge for SRB and MTB.
72 Here we analyze SRB and MTB species in the sediments and hyperalkaline drip waters
73 (pH > 9) of Poole’s Cavern using 16S rRNA sequencing.

74 Poole’s Cavern is an unusually hyperalkaline (pH 9–13) cave system located in
75 Buxton, Derbyshire, England. Its high-alkalinity drip water is sourced from industrial
76 (19th century) limekiln waste (Hartland *et al.*, 2010). In contrast to circumneutral or
77 acidic settings, alkaline conditions cause marked acceleration of calcium carbonate
78 precipitation (Equation 1).



81
82 This rapid precipitation results in the fast growth of cave speleothems. Accordingly, the
83 speleothems in Poole’s Cavern display a very different morphology, such as poached
84 egg-like shapes (Figure 1b), from those in other caves.

85

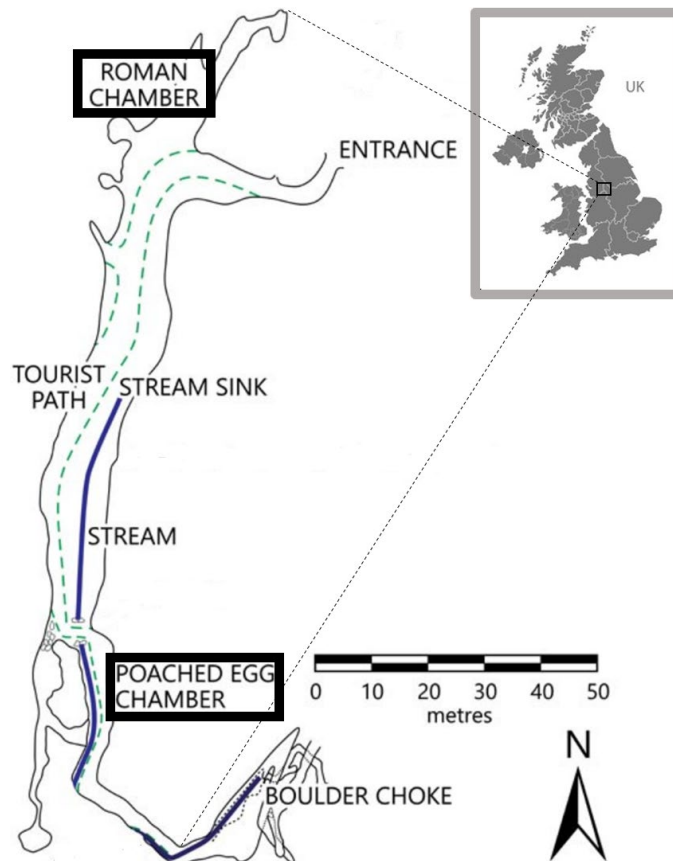


86 **Figure 1.** The surface and cave environments from which microbiological samples
87 were taken: (a) above-cave woods, (b) Poached Egg Chamber, showing classic
88 speleothem morphology and (c) Roman Chamber pool, showing surrounding
89 sediments. [BGS © UKRI]

91 **Materials and Methods**

92 **Sample collection**

93 Sample locations and methods for this study have previously been reported in detail in
94 Shen *et al.* (2022). In brief, six samples were collected from Poole’s Cavern (Figure 2),
95 using aseptic sampling techniques. In the Poached Egg Chamber (PE) (pH ~12.2,
96 temperature ~7.3°C), samples were collected from a straw stalactite (PE Straw) and
97 drip waters (PE Water – Figure 1b). In the Roman Chamber (RC) (pH ~9.3, temperature
98 ~9.3 °C), samples were collected from calcite (RC Calcite) and muds (RC Muds)
99 (Figure 1c). Additionally, soil samples were acquired directly above Poole’s Cavern
100 (Soils above Poole’s – Figure 1a) and from a nearby alkaline brook (Brook Bottom soil).
101 Samples were transported to the laboratory immediately, and frozen at –20°C, pending
102 DNA extraction one week later.



103
104 **Figure 2.** Plan view of Poole's Cavern and internal chamber locations of this study
105 (Shen *et al.*, 2022).
106

107 **Isolation of nucleic acids and sequencing**

108 As described in the previous article (Shen *et al.*, 2022), DNA was extracted from six
109 samples and one blank using FastDNA Spin Kit for Soil DNA Isolation (MP
110 Biomedicals, CA, USA). Subsequently, DNA was sequenced with the 16S rRNA primer
111 pair of 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-
112 GGTTACCTTGTTACGACTT-3') (Reysenbach *et al.*, 2000) via Oxford Nanopore
113 Technologies (ONT) MinION 16S metagenomic sequencing. Basecalling was carried
114 out using Guppy (v 5.0.11) under the supervision of MinKNOW (v20.10.6). Passed
115 reads were analyzed using the EPI2ME pipeline (server v1.1.6) with the following
116 options: minimum identity (accuracy) of 100%, minimum coverage of 30%, minimum
117 quality score of 7, BLAST E-value filter of 0.01, minimum length filter of 200 and
118 without maximum length filter. Since this study employed the 16S rRNA sequencing
119 technique instead of whole genome sequencing for species characterization, the
120 minimum identity (accuracy) for the output of the EPI2ME pipeline was set to 100% to
121 constrain the taxonomic classification as strictly as possible.
122

123 **Results**

124 In the sequencing results filtered by 100% identity, a total of 347 reads were specified
125 with certain bacterial species, whereas other reads did not pass either the quality control
126 or percentage identity threshold. Qualified reads were analyzed and discussed in detail
127 in Shen *et al.* (2022). Thus, in this study, only sulphate-reducing species were examined.
128 Among them, 34 reads were identified in PE Straw; 81 reads were identified in PE
129 Water; 62 reads in RC Calcite; 58 reads in RC Mud; 20 reads in Soils above Poole's;

130 and 92 reads in Brook Bottom soils. Out of the 347 reads, 19 were SRB reads and 8
 131 MTB reads (Table 1). These SRB and MTB were not found in the blank.

132

133 **Table 1.** Reads of identified SRB and MTB species (all belong to Desulfobacterota)
 134 from Poole's Cavern samples with minimum identity of 100%.

Order of read	Sampling site	Quality score	Sequence length (bp)	Species	Category
1	PE Straw	11.3	281	<i>Desulforhopalus vacuolatus</i>	SRB
2	RC Calcite	10.0	264	<i>Desulfovibrio magneticus</i>	SRB/MTB
3	RC Mud	11.1	260	<i>Desulfovibrio magneticus</i>	SRB/MTB
4	RC Mud	7.9	283	<i>Desulfacinum infernum</i>	SRB
5	RC Mud	9.7	267	<i>Desulfovibrio magneticus</i>	SRB/MTB
6	RC Mud	9.9	260	<i>Desulfovibrio magneticus</i>	SRB/MTB
7	RC Mud	9.2	252	<i>Desulfovibrio magneticus</i>	SRB/MTB
8	RC Mud	9.1	266	<i>Thermodesulfovibrio hydrogeniphilus</i>	SRB
9	RC Mud	10.9	256	<i>Desulfovibrio magneticus</i>	SRB/MTB
10	RC Mud	10.6	202	<i>Thermodesulforhabdus norvegica</i>	SRB
11	Brook Bottom soils	10.3	247	<i>Desulfovibrio idahonensis</i>	SRB
12	Brook Bottom soils	9.2	280	<i>Desulfacinum infernum</i>	SRB
13	Brook Bottom soils	10.5	274	<i>Desulfovibrio psychrotolerans</i>	SRB
14	Brook Bottom soils	10.2	208	<i>Desulfacinum infernum</i>	SRB
15	Brook Bottom soils	9.9	285	<i>Desulfovibrio magneticus</i>	SRB/MTB
16	Brook Bottom soils	13.3	225	<i>Desulfoglaeba alkanexedens</i>	SRB
17	Brook Bottom soils	11.0	213	<i>Desulfocurvus vexinensis</i>	SRB
18	Brook Bottom soils	11.7	238	<i>Desulfatiglans parachlorophenolica</i>	SRB
19	Brook Bottom soils	10.3	253	<i>Desulfovibrio magneticus</i>	SRB/MTB

135

136 These species were classified within Deltaproteobacteria or Nitrospirae in the classical
 137 phylogenetic system. Lately, they have been reclassified to Desulfobacterota (Table 1),
 138 a phylum that comprises primarily sulphate-reducing and fermentative or syntrophic
 139 lineages (Murphy *et al.*, 2021). Specifically, *Desulforhopalus vacuolatus* was found in
 140 PE Straw; *Desulfovibrio magneticus* was found in RC Calcite, RC Mud and Brook
 141 Bottom soils; *Desulfacinum infernum* was found in RC Mud and Brook Bottom soils;
 142 *Thermodesulfovibrio hydrogeniphilus* and *Thermodesulforhabdus norvegica* were
 143 found exclusively in RC Mud; *Desulfovibrio idahonensis*, *Desulfovibrio*
 144 *psychrotolerans*, *Desulfoglaeba alkanexedens*, *Desulfocurvus vexinensis* and
 145 *Desulfatiglans parachlorophenolica* were all found exclusively in Brook Bottom soils
 146 (Figure 3).

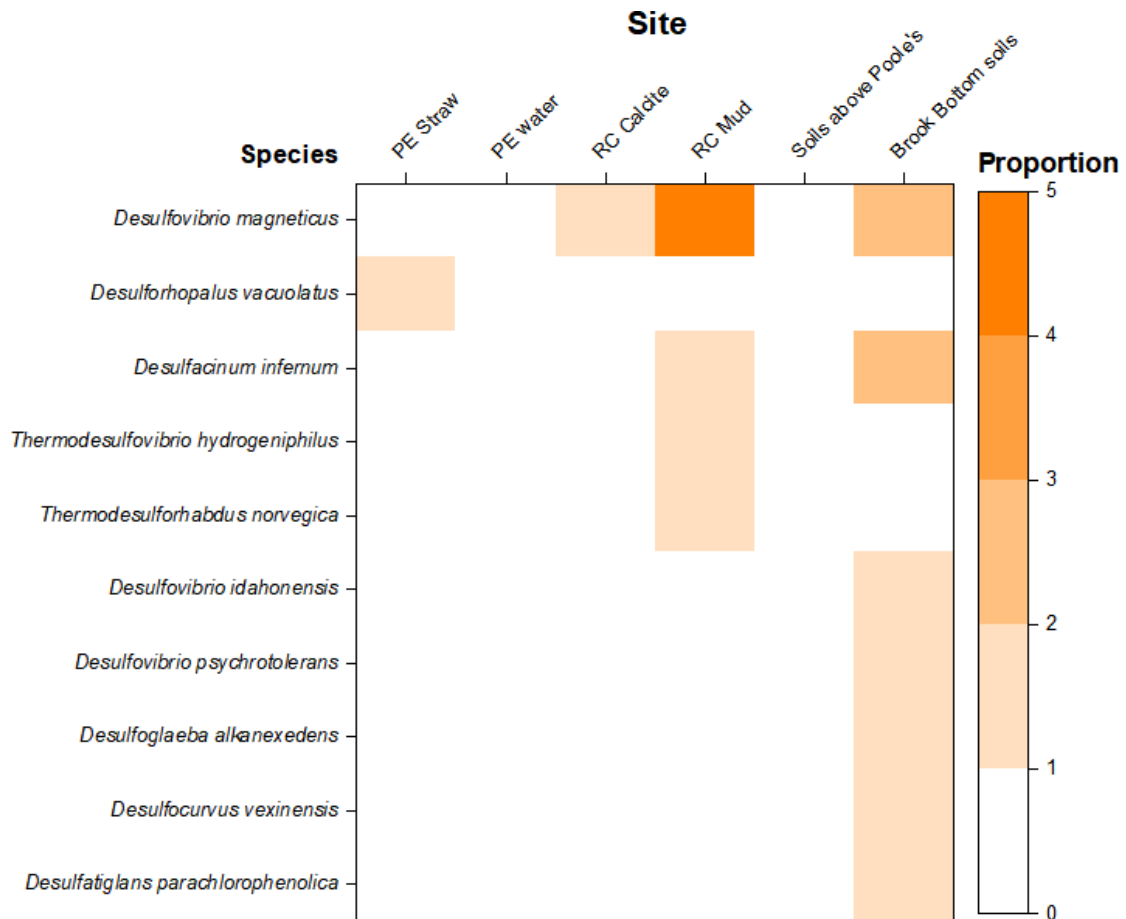


Figure 3: Abundances of identified reads (minimum identity of 100%) related to SRB and MTB in Poole's Cavern samples.

Discussion

In this study, we identify numerous SRB and MTB in or around Poole's Cavern. Previous studies have found sulphate and iron oxide colloids in Poole's Cavern (Fairchild and Hartland, 2010; Hartland *et al.*, 2010; Morgan, 2022) as potential nutrient sources for these microorganisms. Intriguingly, the sulphate-reducing magnetotactic bacterium *Desulfovibrio magneticus* is identified in RC Calcite, RC Mud and Brook Bottom soils (Table 1 and Figure 3). *Desulfovibrio magneticus* is the sole MTB member found within the Poole's cave system. *Desulfovibrio magneticus* contains iron-phosphorus-rich granules in addition to magnetosomes (Byrne *et al.*, 2010). Generally, magnetosomes of MTB are encapsulated by membranous compartments. However, the magnetosome in *Desulfovibrio magneticus* is found to be free of membranes (Byrne *et al.*, 2010). Through a process of exaptation (Lin *et al.*, 2020), MTB *Desulfovibrio magneticus* in both the calcite and sediments of the Roman Chamber might indicate that this MTB is able to be transported via karst waters or air to the deeper subsurface and spread to stalactites and muddy sediments. Interestingly, this MTB is not identified within the above-cave surface soils. This is either because its content is not sufficiently abundant to be extracted from this environment, or that our sampling did not reach the oxic-anoxic transition zone preferred by the bacterium.

The finding of this MTB within different components of the cave environment is an important analogue for the early Earth. MTB are postulated to survive extreme environments through the strategy of exaptation (Lin *et al.*, 2020). Due to the harsh surface environments on the primordial Earth, caves shielded by karst aquifers could have been of great importance (Çolak and Dođruöz Gungör, 2022). Compared with the

174 Poached Egg Chamber (pH ~12.2), the Roman Chamber features a milder pH condition
175 (~9.3) (Shen *et al.*, 2022), which likely occurred on early Earth (Amend and McCollom,
176 2009; Krissansen-Totton *et al.*, 2018). Therefore, the presence of MTB in the Roman
177 Chamber implies a potential strategy of these bacteria to escape from subaerial
178 irradiation to more favourable subsurface environments.

179 The absence of MTB in the Poached Egg Chamber with its higher-pH drip waters
180 (Table 1 and Figure 3) does not rule out that MTB are able to inhabit some areas of the
181 most hyperalkaline inner cave environment, albeit it is possible that our sampling
182 missed these MTB within this higher-pH chamber. Further investigations of microbial
183 communities in the Poached Egg Chamber would help address the question of whether
184 MTB can survive pH values as high as 12–13. Because we know that these MTB are
185 found in other niches of the cave, it seems a sensible site at which to investigate the
186 potential survival of MTB at these extreme pH levels.

187 Other than MTB, SRB had previously been found to inhabit hyperalkaline water
188 bodies (Ryzhmanova *et al.*, 2013), although SRB do not show preference for high pH
189 conditions. The sulphate reduction activity of SRB in Poole's Cavern seems minimal
190 based on the generally consistent concentration profile of sulphate (Burke *et al.*, 2012),
191 and their metabolism, if active, can be fermentation (Lee *et al.*, 2014). One moderately
192 psychrophilic SRB species, *Desulforhopalus vacuolatus* (Isaksen and Teske, 1996),
193 was discovered in the high pH of the Poached Egg Chamber (Fig.3). Whereas this is
194 the only species discovered at the highest pH levels it is not the only SRB identified
195 within the cave. As mentioned above, the only determined SRB in RC Calcite is
196 *Desulfovibrio magneticus*. Within RC mud we identify several SRB species that have
197 specific adaptations for survival in extreme conditions. *Desulfacinum infernum* can
198 ingest various carboxylates and tolerate high salinity (Rees *et al.*, 1995);
199 *Thermodesulfovibrio hydrogeniphilus* can oxidize geothermal hydrogen while reducing
200 sulphate (Haouari *et al.*, 2008) and *Thermodesulforhabdus norvegica* is an acetate-
201 oxidizing SRB (Beeder *et al.*, 1995).

202 Some other SRB members are present uniquely in the adjacent Brook Bottom soils
203 (Table 1 and Figure 3). *Desulfovibrio idahonensis* can consume sulphonate,
204 thiosulphate and fumarate and tolerate aqueous environments contaminated by heavy
205 metals (Sass *et al.*, 2009); *Desulfovibrio psychrotolerans* is a psychrotolerant and
206 moderately alkaliphilic SRB (Jyothsna *et al.*, 2008); *Desulfoglaeba alkanexedens* can
207 ingest n-alkanes as energy sources (Davidova *et al.*, 2006); *Desulfocurvus vexinensis*
208 can tolerate saline subsurface aquifers (Klouche *et al.*, 2009); *Desulfatiglans*
209 *parachlorophenolica* is able to consume 4-chlorophenol (Suzuki *et al.*, 2014). When
210 compared to the SRB/MTB compositions within Poole's Cavern and the surface soils
211 directly above the cave, it becomes clear that there is diversity within the local
212 environment. The more diverse SRB/MTB community found in Brook Bottom soils
213 indicates that these species have thrived within this less-harsh environmental niche.

214

215

Conclusion

216 Several extremophilic or extremotolerant SRB and MTB are identified in Poole's
217 Cavern, and a spatial dispersal of these bacteria between surface and subsurface is
218 observed. The findings of this article are important, because they highlight that SRB
219 and MTB are capable of inhabiting the high-pH, low-nutrient environment of Poole's
220 Cavern. It also sheds light on the compositions of SRB and MTB in hyperalkaline cave
221 microbial communities and implies that ancient microbes might have been able to adapt
222 to hostile early-Earth conditions through downward transportation to comparatively
223 more habitable underground voids, including caves.

224

225 **Acknowledgments**

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230 study. The paper has benefitted from constructive reviews by Simon Bottrell and Simon
231 Rout.

232 **Data availability**

233 The 16S rRNA gene sequences used for microbial identification in this study are
234 available in the NCBI BioProject database via the accession number PRJNA807843.
235 [BGS © UKRI]

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