Received: 3 December 2019 | Revised: 21 August 2020 | Accepted: 31 August 2020

DOI: 10.1111/ejss.13040

SPECIAL ISSUE ARTICLE

European Journal of Soil Science WILEY

Decoupled richness of generalist anaerobes and sulphate-reducing bacteria is driven by pH across land uses in temperate soils

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Funding information
Biotechnology and Biological Sciences Research Council/NERC, Grant/Award Number: NE/M009106/1; Maranhão Foundation for Research, Science and Technology Development

Abstract

Sulphate-reducing bacteria (SRB) represent a key biological component of the global sulphur (S) cycle and are common in soils, where they reduce SO_4^{2-} to H_2S during the anaerobic degradation of soil organic matter. The factors that regulate their distribution in soil, however, remain poorly understood. We sought to determine the ecological patterns of SRB richness within a nation-wide 16S metabarcoding dataset. Across 436 sites belonging to seven contrasting temperate land uses (e.g., arable, grasslands, woodlands, heathland and bog), SRB richness was relatively low across land uses but greatest in grasslands and lowest in woodlands and peat-rich soils. There was a shift in dominant SRB taxa from *Desulfosporosinus* and *Desulfobulbus* in arable and grassland land uses to *Desulfobacca* in heathland and bog sites. In contrast, richness of other generalist anaerobic bacterial taxa found in our dataset (e.g., *Clostridium*, *Geobacter* and *Pelobacter*) followed a known trend of declining richness linked to land-use productivity. Overall, the richness of SRBs and anaerobes had strong positive correlations with pH and sulphate concentration and strong negative relationships with elevation, soil organic matter, total carbon and carbon-to-nitrogen ratio. It is likely that these results reflect the driving influence of pH and competition for optimal electron acceptors with generalist anaerobic bacteria on SRB richness.

Highlights

- Sulphate-reducing bacteria (SRB) are key but rare soil biota that may compete with other anaerobes

- As UK sulphur deposition rates fall, local populations of SRB may also decline in soils
- Sulphate concentrations were higher in arable and wooded sites, not at higher elevation as expected
- SRB richness was lower than generalist anaerobes, with peaks in grasslands and a drop in lowland woods.

KEYWORDS

anaerobes, atmospheric deposition, dissimilatory sulphate reduction, nutrient cycling, soil acidity

1 | INTRODUCTION

Sulphate-reducing bacteria (SRB) are common soil organisms, which are capable of transforming sulphate (SO_4^{2-}) into hydrogen sulphide (H_2S) under anoxic conditions (Bahr et al., 2005; Hines et al., 1999; Xia et al., 2014). Consequently, these organisms play a fundamental role in global sulphur (S) cycling and also in the iron (Fe) cycle through the formation of FeS_2 (Muyzer & Stams, 2008). After waterlogging, soils are often rich in H_2S, in part due to high local abundances of SRB, leading to changes in plant metabolism (Lamers et al., 2013; Li, Min, & Zhou, 2016; Stubner, 2004). Currently, more than 220 species of SRB have been described, with soils often possessing diverse SRB communities (Barton & Fauque, 2009). For example, the number of known SRB operational taxonomic units (OTUs) has been shown to range from 60 per gram in rice-associated soils (Scheid & Stubner, 2001) to 70 per gram of landfill cover soil (Xia et al., 2014).

These bacteria may form relationships with other S-dependent bacteria, such as green and purple phototrophic S bacteria (Overmann & van Gemerden, 2000). Therefore, despite their namesake, a strict assumption that SRB communities are directly linked to S or SO_4^{2-} availability and that SRB rely on strict anaerobic conditions is overly simplistic, as many SRB taxa are known to utilize nitrogenous (Dalsgaard & Bak, 1994; López-Cortés, Fardeau, Fauque, Joullan, & Olivier, 2006) and S compounds other than SO_4^{2-} as terminal electron acceptors, in addition to a wide range of other compounds (Muyzer & Stams, 2008). Furthermore, certain species are oxygen (O_2) tolerant (Mogensen, Kjeldsen, & Ingvorsen, 2005) or even dependent on O_2 accessibility (Sigalevich, Meshorer, Helman, & Cohen, 2000). Competition between SRB and other anaerobic bacteria for C substrates (e.g., acetate) has also been shown to strongly influence anaerobic community compositions in laboratory experiments and bioreactors (Oude Elferink, Visser, Hulshoff-Pol, & Stams, 1994; Schönheit, Kristjansson, & Thauer, 1982), and has been observed in wetland substrate in a mesocosm experiment (Chen et al., 2014).

It is unclear to what extent different edaphic factors regulate SRB populations in soil, in particular the availability of SO_4^{2-} and major regulators of microbial community structure such as pH. Major SO_4^{2-} inputs to agricultural land include inorganic fertilizer addition (e.g., ammonium and potassium sulphates), soil amendments (e.g., calcium sulphate) and livestock waste (Abdelmseeh, Jofreit, & Hayward, 2008; Allison, Fowler, & Allen, 2001; Carvalho & van Raij, 1997; Pan, Lam, Moiser, Lou, & Chen, 2016). Atmospheric S deposition from anthropogenic and marine sources is a major source of SO_4^{2-}, especially at higher elevations in wetter climates (Stevens, Ormerod, & Reynolds, 1997). Subsequently, one might expect richness and/or relative abundance of SRB to increase with elevation owing to an increase in anaerobic niches in upland sites and SO_4^{2-} availability. Indeed, Drenovsky, Steenwerth, Jackson, and Scow (2010) demonstrated with phospholipid fatty acid analyses that the proportion of *Desulfobacter* biomass increased with soil moisture in California. It is also possible that SRBs may be used as an environmental indicator of ecosystem recovery from acid deposition (Review of Transboundary Air Pollution, 2012), which is now declining in many industrialized countries (Kirk, Bellamy, & Lark, 2010; Reynolds et al., 2013). This, however, requires an understanding of the key factors that regulate SRB communities across a wide range of land uses.

Across Europe, S deposition rates have declined significantly following a shift away from coal-powered energy generation (Grennfelt & Hov, 2005; Review of Transboundary Air Pollution, 2012). There has been a steady decrease in S deposition over the past 40 years across the UK (Review of Transboundary Air Pollution, 2012; Stevens et al., 1997), driven by a shift away from unabated coal-fired electrical power, which is expected to be eliminated by 2025 (DEFRA, 2018). In Wales, historical S-deposition levels were higher at greater elevations (Stevens et al., 1997), suggesting that upland and alpine habitats may have previously supported robust SRB populations. However, it is unclear whether these areas remain hotspots for SRB following these changes in

emissions or if increasing local SO_4^{2-} inputs from point sources or agriculture have created other SRB-favourable habitats.

Recent studies have highlighted a range of inconsistencies between the scales required for soil biodiversity sampling and those used for mapping (Hendershot, Read, Henning, Sanders, & Classen, 2017; George et al., 2019; Seaton et al., 2020). Thus lately, research has focused on soil properties, aboveground habitat (Seaton et al., 2020) and anthropogenic land uses (George et al., 2019) as determinants of microbial community composition, rather than soil type defined from soil classification (e.g., Avery, 1980). As a result, land-use categories have proven a better determinant of microbial richness in national-level soil surveys (George et al., 2019). In particular, the Aggregate Vegetation Class (AVC) system (Bunce et al., 1999) has proven to be an effective method of assessing soil biota in British soils (Black et al., 2003; George et al., 2019; Griffiths et al., 2011). This method involves creating high-level aggregations of plant communities based on plant species data at the plot level (Bunce et al., 1999). These land uses are further clustered in order of soil productivity, from highest in arable and grassland sites to lowest in upland heathlands and uplands (Bunce et al., 1999). Therefore, we expect that assessments of SRB richness and distribution across land uses will be more informative as they incorporate both soil type and aboveground factors.

Here, we use a national-level metabarcoding dataset to determine the distribution of SRB richness in soil. We hypothesized that land use would be a major driver of SRB richness and therefore we expected richness and relative abundance to increase in acidic and anoxic soils from upland low-productivity areas (i.e., wetlands and heathlands) as compared to agricultural areas (i.e., arable and grasslands). Because other anaerobic microbes can directly compete with SRBs (Muyzer & Stams, 2008), we also investigated this same relationship between some common anaerobic bacterial taxa and the aforementioned land uses. In addition, we assessed proportional abundances of SRB and common anaerobic taxa to look for shifts in community compositions across land uses. We finally hypothesized that richness of both SRB and other anaerobic bacteria would be positively correlated with increasing acidity, SO_4^{2-} concentration and elevation, because these variables are expected to increase in wetland/heathland areas.

2 | MATERIALS AND METHODS

2.1 | GMEP topsoil survey

This work was undertaken by analysing the metabarcoding dataset of soil biodiversity across Wales, UK, collected as part of the Glastir Monitoring and Evaluation Programme (GMEP) presented in George et al. (2019) (Supplementary Material). Soil samples were collected across Wales ($n = 436$) between late spring and early autumn in 2013 and 2014 (Figure S1). Sampling protocols followed the UK Countryside Survey (Emmett et al., 2010), whereby samples were collected from randomly selected 1 km^2 squares. Within each 1 km^2 square, up to three samples were collected; for further details see Emmett et al. (2010). Soil physicochemical properties, including pH (measured in 0.01 M $CaCl_2$), organic matter (% loss-on-ignition), total C and nitrogen (N) (%), C:N ratio, phosphorus (P) (mg kg^{-1}), bulk density (g cm^{-3}) and moisture content (g water g^{-1}), were measured from 4-cm-diameter soil cores at 15-cm depth. Geographic coordinates and elevation (m) were also collected. Mean annual precipitation (ml) at each site was extracted from the CHESS dataset (E. L. Robinson et al., 2017). Sulphate concentrations (mg kg^{-1}) were determined using 1:5 (w/v) distilled water extracts (Tabatabai, 1996), followed by analysis by ion chromatography (Metrohm Ltd, Herisau, Switzerland).

At each sample site, land use was classified using plant species assemblages into one of seven AVCs as described by Bunce et al. (1999). Briefly, samples were grouped into AVCs based on clustering of aboveground plant community composition based on detrended correspondence analysis. There were seven AVCs present in our dataset, namely: Crops/weeds ($n = 9$), Fertile grassland ($n = 98$), Infertile grassland ($n = 162$), Lowland wood ($n = 17$), Upland wood ($n = 44$), Moorland-grass mosaic ($n = 54$) and Heath/bog ($n = 52$) (Supplementary Material; Table S1). The clustering of AVCs follows a gradient of soil nutrients (highest in Crops/weeds; lowest in Heath/bog), from which land-use intensity and productivity can also be inferred (Supplementary Material). Maps of S deposition from non-marine (2013–2015; Figure 1a) and marine sources (Figure 1b) were made by the UK Centre for Ecology and Hydrology using annual monitoring data (Smith, Dore, Tang, & Stedman, 2018). Summarized environmental and soil property data across AVCs from George et al. (2019) are presented in Table S2.

2.2 | Soil microbial community analysis

Soil cores were collected for metabarcoding analyses. The sampling strategy, DNA extraction and bioinformatics analyses are described in George et al. (2019). Briefly, DNA was extracted in triplicate from 0.25 g of soil via mechanical lysis using MO-BIO PowerLyser PowerSoil DNA Isolation Kits (Qiagen, Hilden, Germany), following

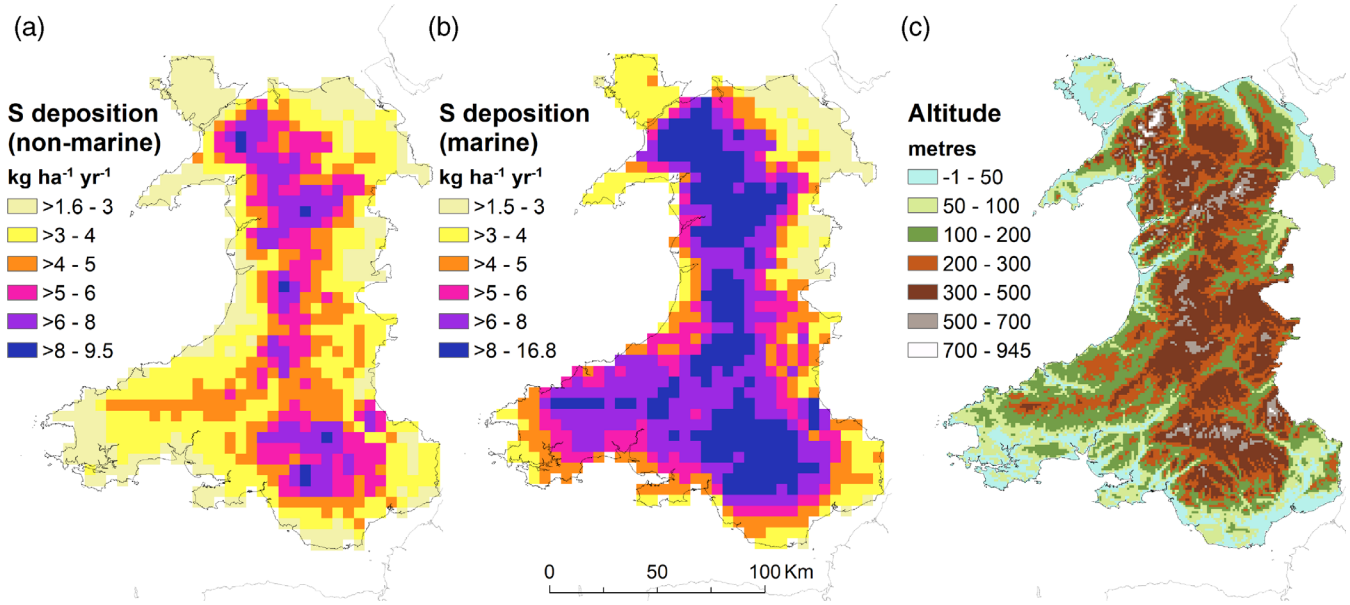

FIGURE 1 Maps of Wales showing sulphur deposition from (a) non-marine (2013–2015) and (b) marine (2014–2016) sources as well as (c) elevation

homogenization from being passed through sterilized sieves. A pre-treatment of 750 µl of 1 M $CaCO_3$ (Sagova-Mareckova et al., 2008) was used for all DNA extractions as this has been shown to improve PCR performances of DNA extracted from acidic soils. Extracted DNA was pooled and sequenced using a two-step Illumina Mi-Seq (San Diego, CA, USA) amplicon sequencing protocol. Amplicon libraries were created in triplicate on a DNA Engine Tetrad® 2 Peltier Thermal Cycler (BIO-RAD Laboratories, Hercules, CA, USA) using the V4 region of the 16S rDNA gene with the 515F/806R universal primers (Caporaso et al., 2011) at Bangor University and the Liverpool Centre for Genomic Research in 2013 and 2014, respectively. First-round PCR amplification began at 98°C for 30 s, followed by 10 cycles of 98°C for 10 s; 50°C for 30 s; 72°C for 30s; with a final extension stage of 72°C for 10 min and held at 4°C for a further 10 min. For the second-round PCR, 12 µl of first-round product was mixed with 0.1 µl exonucleaseI, 0.2 µl thermosensitive alkaline phosphatase and 0.7 µl of water and cleaned in the thermocycler with a programme of 37°C for 15 min and then 74°C for 15 min, followed by a hold at 4°C. Next, Illumina Netera XT 384-way indexing primers were added and amplified with an initial denaturation at 98°C for 3 min; followed by 15 cycles of 95°C for 30 s; 55°C for 30 s; 72°C for 30 s; and a final extension at 72°C for 5 min and then held at 4°C. These products were subsequently purified using an equal volume of AMPure XP beads (Beckman Coulter, Brea, CA, USA).

Raw sequences were de-multiplexed, filtered, quality-checked and clustered using the USEARCH v. 7.0 (Edgar, 2010) and VSEARCH v. 2.3.2 (Rognes, Flouri, Nichols, Quine, & Mahé, 2016) software. Operational taxonomic units (OTUs) were made using open-reference clustering at 97% similarity (George et al., 2019). Sequences with a maximum error >1 and shorter than 200 bp were removed from analysis. The subsequent OTU table was generated using QIIME 1.9.1 (Caporaso et al., 2010) and analysed using the phyloseq package (McMurdie & Holmes, 2013) in R v. 3.5.1 (R Core Team, 2018), removing all OTUs identified as chimeras or non-bacterial taxa using the GreenGenes 13.8 database (DeSantis et al., 2006), as well as singletons. Read counts were normalized through rarefaction. The OTU table was rarefied 100 times at 40,000 read depth and mean richness recorded. Next, we compared SRB taxa from the literature to our dataset and found OTUs identified as 17 SRB genera (*Desulfatiferula*, *Desulfarculus*, *Desulfofacinum*, *Desulfitobacter*, *Desulfobacca*, *Desulfobotulus*, *Desulfobulbus*, *Desulfocapsa*, *Desulfococcus*, *Desulfomonile*, *Desulforhabdus*, *Desulfosarcina*, *Desulfosporosinus*, *Desulfotomaculum*, *Desulfovibrio*, *Desulfovirga* and *Desulfuromonas*), and three generalist anaerobic bacteria (*Clostridium*, *Geobacter*, and *Pelobacter*) were selected for further analysis. Sequences can be accessed at the European Nucleotide Archive (primary accession code: PRJEB27883).

2.3 | Statistical analyses

Linear mixed models were created using the package nlme (Pinheiro et al., 2019) and tested with ANOVAs and Tukey's HSD post hoc tests from the multcomp package (Hothorn,

Bretz, & Westfall, 2008), and effect sizes (ES) based on Cohen's *d* from the emmeans package (Lenth, 2020) were used to assess differences between richness of SRB and previously mentioned anaerobic bacteria across AVCs. For richness models of both SRB and anaerobic taxa, AVC was the independent variable. Identities of 1 km^2 squares (the 1 km^2 square in which samples were located) were used as a random factor. Effect sizes (*ES*) between land uses are also reported. Relationships between environmental variables and the richness of both SRB and anaerobic bacteria were assessed using linear mixed models, which again used 1 km^2 square identity as a random factor. The final models were selected based on stepwise reduction in terms based on best AIC values. Soil properties and environmental variables were normalized via logarithmic or square root transformations where appropriate.

3 | RESULTS

3.1 | Proportional abundance of SRB in soil microbial communities

Of the 29,690 OTUs recorded in the complete dataset, 179, comprising 47,141 sequences, were assigned to SRB taxa (<0.01% of total) and 629, comprising 155,560 sequences, to the generalist anaerobic bacteria of interest (0.02% of total). Although absolute numbers of OTUs were low, we were still able to detect 17 SRB genera. Of these, *Desulfosporosinus* had the highest proportional abundance across most AVCs (Figure 2). This was especially true in the Lowland wood category, where *Desulfosporosinus* made up 71.5% of SRB OTUs. *Desulfobublus* was also a major component of grassland and arable sites. In contrast, *Desulfobacca* replaced *Desulfosporosinus* as the dominant SRB taxa as productivity of land uses fell. *Desulfomonile* also exhibited this pattern to a lesser degree. Other taxa, such as *Desulfacinum*, *Desflovirga* and *Desulfuromonas*, although present, had minor contributions to SRB populations across land uses (Figure 2).

3.2 | Proportional abundance of anaerobic organisms

Geobacter dominated generalist anaerobe populations in all AVCs, with the exception of Upland woods, where *Clostridium* was dominant (Figure S2). When the SRB and anaerobic taxa were studied as a whole, we found an

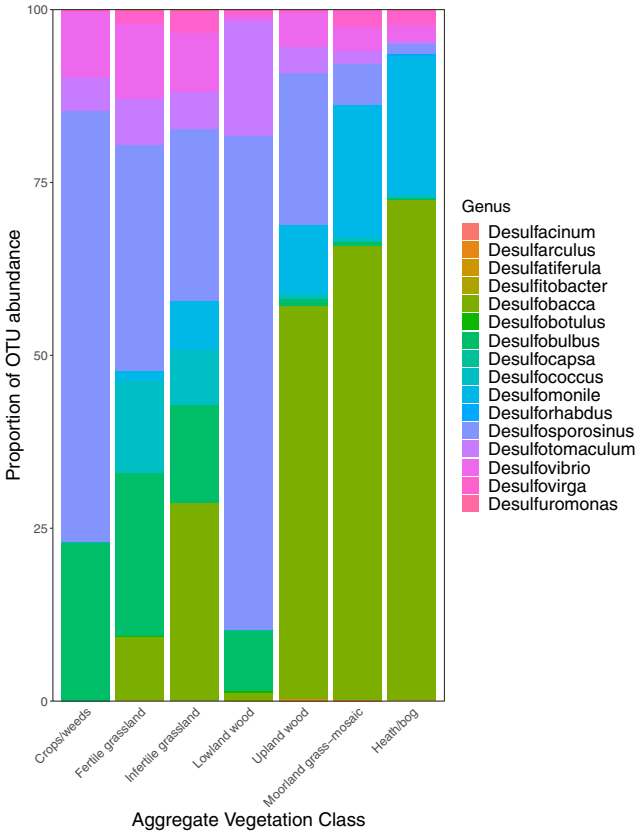

FIGURE 2 Proportional abundance of sulphate-reducing bacteria (SRB) genera across land uses. OTU, operational taxonomic unit

inverse relationship between proportional abundances of SRB and generalist anaerobic taxa across AVCs (Figure 3). The proportion of SRB taxa increased with decreasing land-use productivity. However, the proportion of anaerobes did not fall below 25% even in Heath/bog sites dominated by SRB, whereas anaerobes outnumber SRB by ~90% in high productivity AVCs (Figure 3).

3.3 | Relationships of SRB and anaerobes with environmental variables

Contrary to our expectations, based on S-deposition data (Figure 1), SO_4^{2-} concentrations were highest in the Crops/weeds and woodland AVCs, rather than in high elevation Moorland grass-mosaic and Heath/bog sites (Table S2). Indeed, mean SO_4^{2-} levels of 124.6 mg kg^{-1} were observed in Crops/weeds sites, whereas mean values for Moorland grass-mosaic and Heath/bog sites were 49.4 and 55.2 mg kg^{-1}, respectively (Table S2). Linear mixed models were constructed to investigate the relationships between environmental variables and both SRB and anaerobe OTU richness. Stepwise model selection produced a model of SRB richness where moisture content, pH, mean annual precipitation, total N, elevation, SO_4^{2-} concentration and C:N ratio were retained as independent variables. For anaerobe richness, the same variables were selected, except SO_4^{2-} was dropped during stepwise model selection. For these analyses, total N was normalized by log-transformation, whereas C:N ratio and SO_4^{2-} concentration were square root transformed (Table 1). Again, both models used 1 km^2 square identities as random effect variables.

Model outputs demonstrated that SRB OTU richness was significantly related to all variables (Table 1). The strongest interactions were with total N ($F_{1,193} = 9.35, p = .003$), pH ($F_{1,193} = 11.46$; $p = .001$) and moisture content ($F_{1,\ 193} = 33.61$; $p < .001$). There was also a significant positive interaction between SRB richness and sulphate concentration ($F_{1,193} = 5.5$; $p = .02$). Richness of anaerobic taxa was strongly positively correlated with pH ($F_{1,268} = 123.36$; $p < .001$) and strongly negatively correlated with C:N ratio ($F_{1,268} =;29.76$; $p \leq .001$). Full accounts of both models are summarized in Table 1.

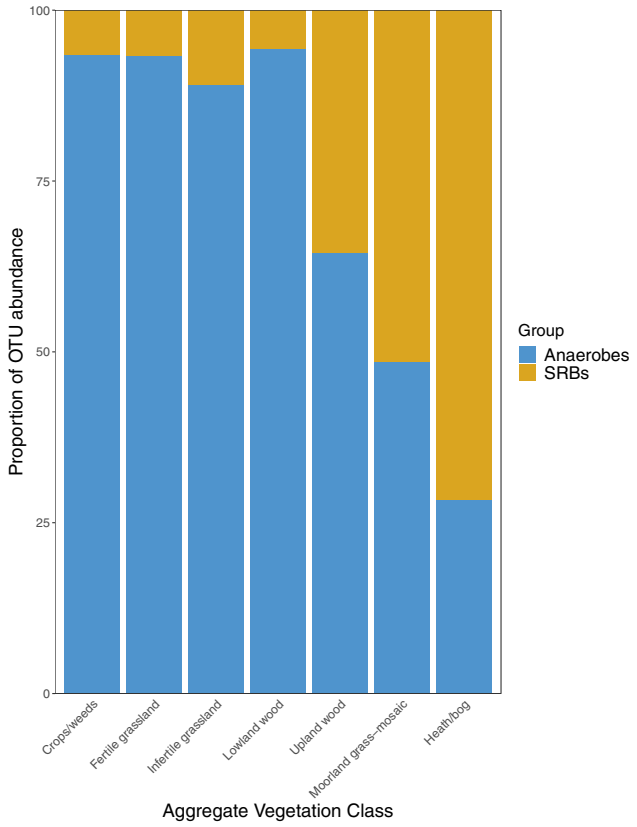

FIGURE 3 Proportional abundance of sulphate-reducing bacteria (SRB) and anaerobic taxa across land uses. OTU, operational taxonomic unit

3.4 | Relationships of SRB and anaerobes with vegetation cover

Richness of SRB OTUs was greater ($F_{6,272} = 5.44$, $p < .001$) in Fertile grasslands, Infertile grasslands and Moorland grass-mosaic than in both Lowland and Upland woods (Figure 4a). Richness was significantly lower in Upland wood than in Fertile grasslands ($ES = -0.76$, $p < .001$), Infertile grasslands ($ES = -0.64$, $p = .005$) and Moorland grass-mosaic ($ES = 0.86$, $p = .002$). Similarly, SRB richness in Lowland wood was lower than in Fertile grasslands ($ES = -0.92$, $p = .007$), Infertile grasslands ($ES = -0.80$, $p = .03$) and Moorland grass-mosaic ($ES = -1.02$, $p = .01$).

Larger differences were observed in anaerobe richness across AVCs (Figure 4b). Unexpectedly, richness of anaerobes was significantly ($F_{6,272} = 27.31$, $p < .001$) greater in the high-productivity AVCs, including Crops/weeds and both Fertile and Infertile grasslands, than in low-productivity AVCs. Anaerobe richness was greater in Crops/weeds than in both types of woodland (Lowland wood, $ES = 1.67$; Upland wood, $ES = 1.49$; both $p < .001$), Heath/bog ($ES = 1.56$, $p < .001$) and Moorland grass-mosaic AVCs ($ES = 0.99$, $p = .03$). Anaerobe richness was also greater in Fertile grasslands than in these same four AVCs (Lowland wood, $ES = 1.81$; Upland wood, $ES = 1.63$; Heath/bog, $ES = 1.70$; Moorland grass-mosaic, $ES = 1.14$; all $p < .001$). This was also true of Infertile grasslands (Lowland wood, $ES = 1.50$; Upland wood,

ES = 1.32; Heath/bog, *ES* = 1.40; Moorland grass-mosaic, *ES* = 0.83; all $p < .001$). There was also significantly greater anaerobe richness in Moorland grass-mosaic sites than in Heath/bog (*ES* = 0.57, $p = .01$) samples (Figure 4b).

4 | DISCUSSION

4.1 | Relationships between atmospheric S deposition and soil SO_4^{2-} content

Within Wales, the areas of greatest atmospheric-derived S-deposition are consistently at high elevation, where dry and wet deposition is greatest (Smith et al., 2018), including the Snowdonia and Brecon Beacons National Parks (Figure 1). Within our soils, however, SO_4^{2-} concentrations were highest in Crops/weeds (arable) and woodland AVCs, which tend to be associated with low altitudes. This was surprising, because SO_4^{2-} deposition rates are known to increase with elevation (Lovett, Thompson, Anderson, & Bowser, 1999; Stevens et al., 1997). Decomposition releases SO_4^{2-} from organic matter into the soil (Muyzer & Stams, 2008). Although arable and woodland AVCs had lower levels of organic matter, this could be a reflection of high-processing rates resulting in higher than expected SO_4^{2-} concentrations. Previous assessments of mesofauna at these sites showed that mesofaunal abundance was highest in woodlands; however, it was lowest in arable sites (George et al., 2017) and so the higher SO_4^{2-} concentrations in arable soils require further explanation. It is possible that arable sites were subjected to amendment with fertilizers containing SO_4^{2-} (Allison et al., 2001; Pan et al., 2016); however, without detailed land-management histories we cannot be confident in this explanation.

4.2 | Relationship between SRB and anaerobic microbial communities

Our analyses showed that grasslands supported greater SRB OTU richness than woodlands and did not follow our prediction of increasing richness in more acidic and moist land uses. Greater richness in grasslands may reflect the presence of dormant SRB (e.g., Gandy & Yoch, 1988), which can be detected by metabarcoding analyses (Wang, Mayes, Gu, & Schadt, 2014). Similarly, this could reflect taxa-specific specialization. *Desulfobulbus*, for instance, was present across a wider range of land uses than other SRB taxa. We ascribe this to its ability to ferment ethanol and lactate in the absence of sulphates (Biswas, Taylor, & Turner, 2014). Indeed, *Desulfobulbus* can ferment exogenous ethanol and lactate into propionate and acetate, which can be further utilized for energy (Widdel & Pfennig, 1982). These compounds may prove to be more available in some soil environments than SO_4^{2-}, although we were unable to assess the prevalence of these compounds in the present study. However, as expected based on our assumptions of increasing SRB

TABLE 1 Linear mixed model outputs for relationships between richness of both sulphate-reducing bacteria (SRB) and anaerobes and soil properties and environmental variables, ranked in order of greatest to least *F* value

SRB (*df* = 193)			
Environmental variable	**Slope (effect size)**	***F***	***p***
Moisture content (g g^{-1})	1.53	33.61	<.001
pH ($CaCl_2$)	2.06	11.46	<.001
Mean annual precipitation (ml)	0.01	10.20	.002
Total N (%)^{L}	5.42	9.35	.003
Elevation (m)	−0.03	6.78	.01
SO_4^{2-} (mg kg^{-1})^{S}	0.27	5.51	.02
C:N ratio^{S}	−5.16	5.04	.03
Anaerobes (*df* = 268)			
Environmental variable	**Slope (effect size)**	***F***	***p***
pH ($CaCl_2$)	16.65	123.36	<.001
C:N ratio^{S}	−25.69	29.76	<.001
Elevation (m)	−0.11	13.96	<.001
Moisture content (g g^{-1})	2.65	9.96	<.001
Mean annual precipitation (ml)	0.02	3.37	.07

Note: ^{L} denotes log-transformed variables; ^{S} denotes square root transformed variables.

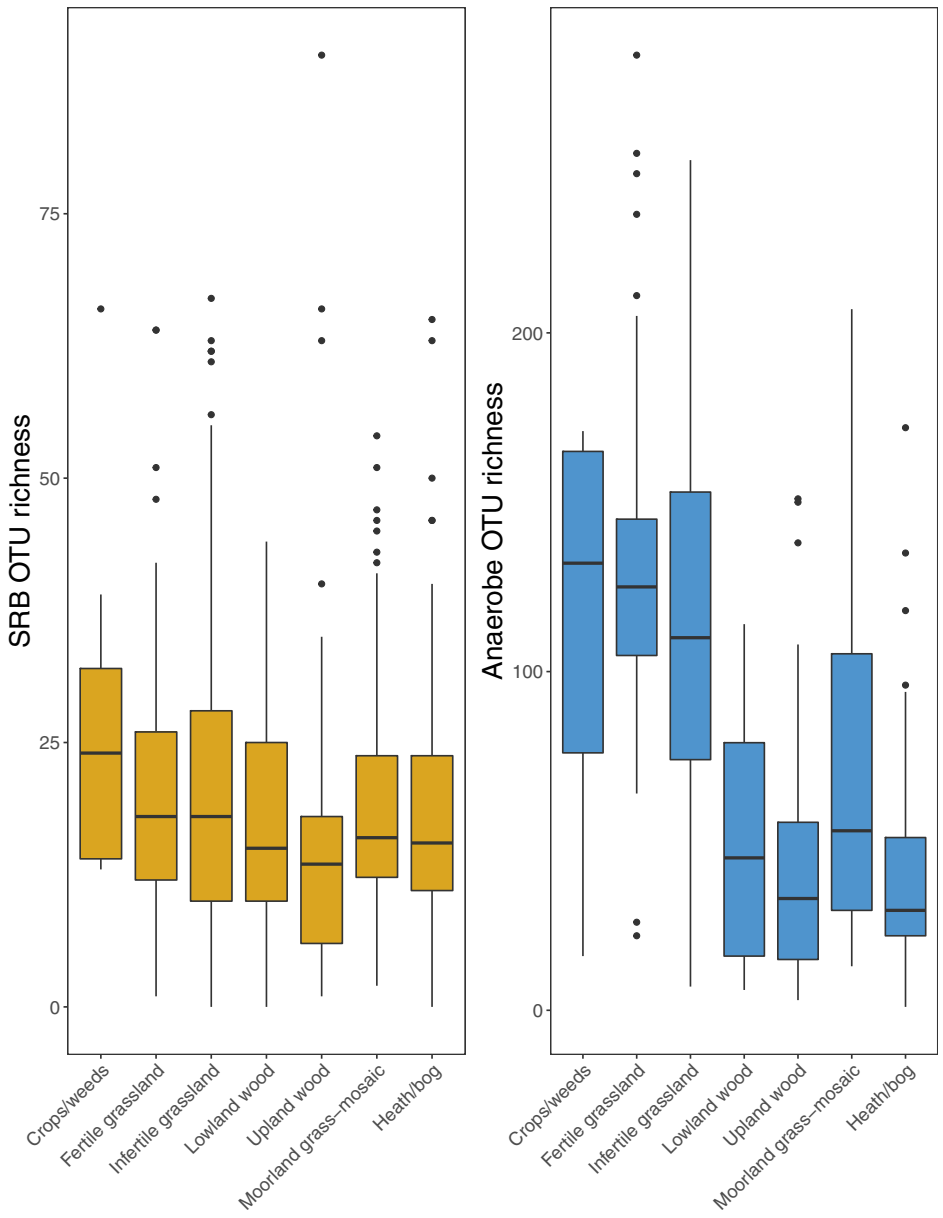

FIGURE 4 Richness of sulphate-reducing bacteria (SRB) (a) and selected generalist anaerobe operational taxonomic units (OTUs) (b) across land uses. Boxes represent the first and third quartiles, whereas horizontal lines denote medians. Black dots are outliers beyond the whiskers, which display 1.5× the interquartile range

richness in upland water-logged sites, there was a marked increase in relative abundance of SRB OTUs in wet Moorland grass-mosaic and Heath/bog areas, which is supported by the positive correlation between SRB and moisture content.

Unexpectedly, richness of the anaerobic bacteria highlighted in this study did not increase in stereotypically anaerobic sites, such as Heath/bog. This is especially surprising given the strong relationships observed between anaerobic richness and pH and elevation, as was expected. This is likely to be due to the generalist nature of these anaerobic bacteria. For example, *Geobacter* sp. are a ubiquitous component of soil bacterial communities, as they are able to utilize a wide range of alternative electron acceptors (Lovley et al., 2011). Similarly, *Clostridium* sp. are common constituents of soil communities (Jeong et al., 2004). Although Hausmann et al. (2016) have previously highlighted the importance of SRB in the rare biosphere in peatlands and associated sites, we found a surprising amount of SRB taxa in our grassland sites.

Overall, richness of anaerobic taxa followed the overarching trend of microbial richness declining with soil productivity across Wales, as found by George et al. (2019). As expected, there were significant relationships between SRB and anaerobic taxa with pH and elevation. However, the directions of these relationships did not conform to our expectations that richness would increase with acidity in higher elevation sites. Furthermore, we observed significant relationships between SO_4^{2-} and SRB richness only. Previous analysis has confirmed the driving influence of pH on bacterial richness across

Wales (George et al., 2019) and the globe (Delgado-Baquerizo et al., 2018; Lauber, Hamady, Knight, & Fierer, 2009). This relationship is evident in the distribution of selected anaerobes in the present study that have a strong negative relationship between richness and pH, which is translated into low abundances in more acidic sites. Both anaerobes and SRB demonstrated the significant positive relationships, although with differing strengths, with pH. The relatively constant richness of SRB across agricultural and grassland AVCs, including in the uplands, however, may indicate that the AVC system may obfuscate sulphate-rich areas, such as waterlogged rhizosphere communities (Lin et al., 2010). It must be recognized that the low contributions of SRB taxa (<0.01%) and generalist anaerobes (0.02% of total) means that both groups belong to the rare biosphere as they contribute <0.1% relative abundance of the total bacterial community (Pedrós-Alió, 2012), exemplifying how components of the rare biospheres can drive important ecosystem functions (Hausmann et al., 2016). It is possible that despite making up a low proportion of species diversity, SRB were highly abundant, although as we were not able to calculate abundance we will not speculate on this possibility.

4.3 | Relationship between SRB and anaerobic microbial communities and land use

There was a shift in SRB communities across land uses. *Desulfosporosinus* and *Desulfobulbus* dominated high-productivity land uses, especially arable and grassland sites. *Desulfosporosinus* is commonly found in soil-water interfaces, such as rice root systems and soils contaminated by industrial processes (Vatsurina, Badrutdinova, Schumann, Spring, & Vainshtein, 2008). *Desulfobulbus* can utilize lactate and ethanol in the absence of sulphate (Biswas et al., 2014), which may facilitate their prevalence across so many different land uses. In low-productivity land uses, specifically Moorland grass-mosaic and Heath/bog sites, *Desulfobacca* and *Desulfomonile* supplanted these as the dominant taxa. Both of these genera are important members of the rare biosphere in peatlands, where they contribute to SO_4^{2-} reduction, especially at greater depths (Hausmann et al., 2016; Tsitko et al., 2014).

To our knowledge, there has not been a metabarcoding survey targeting SRB in soils. We recognize that the use of general prokaryote primers may have not detected some SRB taxa from our sites. Furthermore, we recognize that our inability to quantify the amount of SRB present at each site limits our analyses to measures of diversity (Bouchez et al., 2015). Nevertheless, we present our findings as preliminary analyses, from which future quantitative work can be derived.

There are qPCR protocols that target dissimilatory sulphite reductase genes for the study of SRB populations (e.g., Agrawal & Lal, 2009; Biswas et al., 2014). Targeting this gene region may provide more clarity on the number of SRB taxa in Wales, although it would make comparisons with other taxa more difficult. Similarly, we did not detect any sulphate-reducing archaea, which are currently known from thermophilic taxa (Jay et al., 2016; Stetter, 1988), although an ever-increasing number of archaeal taxa with a wide range of functional diversity are being described from soils (Timonen & Bomberg, 2009).

4.4 | Conclusions

Our findings demonstrate a relatively constant richness of SRB across diverse temperate land uses. In addition, we found that the distribution of anaerobic bacteria followed previously described trends within our study area. The integration of real-time PCR techniques targeting dissimilatory sulphite reductase genes in future analyses could help elucidate the discrepancies between SRB richness, abundance, activity and S supply. Nonetheless this work highlights the use of national-scale environmental DNA biodiversity inventories in investigating localized microbial populations.

ACKNOWLEDGEMENTS

We thank Sam Tomlinson (CEH) for his assistance in generating sulphate deposition maps. Thanks to Professor Barrie Johnson, Dr Ana Araújo and Dr Olga Golyshina for guidance in selecting our taxa of interest and contextualizing our findings. We also thank the two anonymous reviewers for their comments and suggestions, which helped improve and clarify the manuscript.

This research was supported under the Glastir Monitoring & Evaluation Programme (Contract reference: C147/2010/11) and with underpinning funding from the UK Natural Environment Research Council (NERC), award number NE/R016429/1, as part of the UK – ScaPE Programme Delivering National Capability. P.B.L.G. is supported by a Soils Training and Research Studentship (STARS) grant from the Biotechnology and Biological Sciences Research Council and NERC (grant number NE/M009106/1). KPC is funded by the Maranhão Foundation for Research, Science and Technology Development (FAPEMA).

AUTHOR CONTRIBUTIONS

Paul George: Conceptualization; data curation; formal analysis; investigation; writing-original draft; writing-

review and editing. **Katia Coelho:** Conceptualization; investigation; writing-review and editing. **Simon Creer:** Methodology; supervision; visualization; writing-review and editing. **Inma Lebron:** Data curation; investigation; writing-review and editing. **David Robinson:** Methodology; resources; supervision; writing-review and editing. **Davey Jones:** Conceptualization; funding acquisition; resources; supervision; writing-review and editing.

CONFLICT OF INTEREST

The authors have no competing interests to declare.

DATA AVAILABILITY STATEMENT

Data associated with this paper has been published in the UK National Environment Research Council (NERC) Environmental Information Data Centre (EIDC). Currently, pH, bulk density, C, N, P, moisture, and water repellency data are available (Robinson et al., 2019). Data are also available from the authors upon reasonable request with permission from the Welsh Government. Sequencing data have been uploaded to the European Nucleotide Archive and can be accessed with the primary accession code: PRJEB27883.

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SUPPORTING INFORMATION

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How to cite this article: George PBL, Coelho KP, Creer S, Lebron I, Robinson DA, Jones DL. Decoupled richness of generalist anaerobes and sulphate-reducing bacteria is driven by pH across land uses in temperate soils. *Eur J Soil Sci*. 2021;72: 2445–2456. https://doi.org/10.1111/ejss.13040