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Soil Biology and Biochemistry

DOI:
10.1016/j.soilbio.2020.107766

Published: 01/05/2020

Publisher's PDF, also known as Version of record

Soil Biology and Biochemistry 144 (2020) 107766

Contents lists available at ScienceDirect

Soil Biology and Biochemistry

journal homepage: http://www.elsevier.com/locate/soilbio

Soil textural heterogeneity impacts bacterial but not fungal diversity

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ARTICLE INFO

Keywords:
Microbial communities
Grain size
Land use type
Particle size analysis
Textural class
Climate

ABSTRACT

Soils harbour high levels of microbial diversity, underpinning their ability to provide key soil functions and ecosystem services. The extreme variety of soil microbial life is often explained by reference to the physical and chemical heterogeneity of the soil environment. However, detailed understanding of this link is still lacking, particularly as micro-scale studies are difficult to scale up to the soil profile or landscape level. To address this, we used soil samples collected from a wide range of temperate oceanic habitats (e.g. arable, grassland, coniferous and deciduous woodland, heathland; 335 sites in total) to evaluate the link between soil texture and microbial diversity. Soil particle size distribution was measured in each sample using laser granulometry (i.e. sand, silt, clay), while the diversity of bacterial and fungal communities were determined by metabarcoding with an Illumina MiSeq using16S and ITS1 taxonomy marker gene regions, respectively. Multifractal analysis of the soil particle size distribution was then used to describe the heterogeneity of the soil particle sizes. Overall, our results showed no impact of habitat type upon textural heterogeneity indicating that it is an aspect of soil quality resistant to management decisions. Using a structural equation modelling approach, we show that soil textural heterogeneity positively influences bacterial diversity but had little impact upon fungal diversity. We also find that textural composition impacts both bacterial and fungal composition, with many specific microbial taxa showing co-occurrence relationships with clay and fine-silt sized particles. Our results strongly indicate that soil textural heterogeneity influences microbial community diversity regardless of soil management practices and biophysical activities. The close linkages between different groups of soil organisms can obscure the mechanisms driving the development of biodiversity, however, it is clear that the soil physical environment has differential impacts on organisms with different life history strategies.

1. Introduction

The rich reservoir of microbial diversity within the soil performs many functions and offers many resources, including controlling geochemical cycles, remediating pollution, providing novel pharmaceutical products and more (Ling et al., 2015; van der Heijden et al., 2008). There is often reference to the thousands of soil microbial taxa within a gram of soil, yet the mechanisms leading to the establishment of such diversity are still poorly understood (Bardgett and van der Putten, 2014). The heterogeneity of soil particles and their structural arrangement has been suggested to explain this diversity, as it leads both to an increased variety of environments for organisms and isolates communities promoting differentiation (Or et al., 2007; Tecon and Or, 2017; Vos et al., 2013; Zhou et al., 2002). Heterogeneity of soil structure can also lead to spatial heterogeneity of nutrient availability and other physicochemical properties which has been shown to lead to increased microbial diversity (Curd et al., 2018). However, microbial communities also moderate the heterogeneity of their surroundings, altering not only the chemical environment but also the physical structure of the soil using hydrophobic films and aggregate formation (Totsche et al., 2010). Soil heterogeneity both drives and is driven by microbial diversity and function (Young and Crawford, 2004).

Structural heterogeneity of the soil environment leads to increased physical niche space and spatial isolation which should increase microbial diversity (Wang and Or, 2012). Here we consider the physical niche space to be the dimensions within the multidimensional ecological niche that are determined by the physical environment. There is evidence that soil microbes do show preference for certain physical niches,

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https://doi.org/10.1016/j.soilbio.2020.107766
Received 4 December 2019; Received in revised form 28 February 2020; Accepted 29 February 2020
Available online 4 March 2020

as microbial communities differentiate between different minerals (Nishiyama et al., 2012) and particle size fractions (Gardner et al., 2012; Hemkemeyer et al., 2018; Poll et al., 2003). These preferences could be due to the physical or chemical properties of certain minerals, with microbes showing preference for minerals that provide certain nutrients (Roberts, 2004). Also the surface area to volume ratio of mineral material could influence microbial community assembly and activity, with links between surface area ratio and bacterial communities being found in marine sediments (Wang et al., 2015). Therefore, we should expect that as different particle size fractions are available, differing microbial assemblages should be present. This leads to the expectation of a wider range of communities and increased overall diversity as there is a wider range of particle size fractions available. The identity of the particle size fractions important to individual microbial taxa may not align to the sand, silt and clay boundaries used traditionally in soil science. This, together with the potential importance of the range of soil particles, indicates the importance of emerging technologies and new analytical methods in evaluating the relationship between soil texture and microbial communities. Spatial isolation of communities can lead to increased speciation and reduce competitive pressure leading to increased overall diversity. In soil, spatial isolation of communities is based upon both the texture of the soil and its water content; altering these properties to increase isolation of bacterial communities has been shown to increase overall diversity (Carson et al., 2010). The water content of the soil is also influenced by texture (Rawls et al., 2003), with feedback effects on microbial communities and soil functions (Carson et al., 2010; Rabot et al., 2018).

The impact of soil textural heterogeneity upon microbial activity and diversity is moderated by the motility of those organisms within their environment. Bacterial movement and communities are largely limited to water filled areas. Bacteria also have limited capacity for directed movement, only capable of moving themselves very short distances. However, larger organisms (e.g. earthworms, plant roots) can break up the soil structure and move bacteria long distances, as can the mass flow of water (Yang and van Elsas, 2018). Other organisms such as hydrophobic fungi are much less limited to hydrated areas and can, in some cases, grow across vast distances relative to their size (Ferguson et al., 2003; Tecon and Or, 2017). Land use and management will influence the soil structure both directly and indirectly, with associated impacts on soil organisms and their ability to move through the soil. However, if the extent of the impact of land use upon soil texture is limited, we would infer limited interaction between the joint impacts of soil texture and land use upon microbial communities. The ability of an organism to migrate through the soil, and the interactions between different organisms, completely change the impact of soil structure upon biological activity and diversity.

Here we investigated how soil textural heterogeneity altered across a variety of temperate habitats and then assessed the impact of soil texture on bacterial and fungal communities. We used laser granulometry to analyse soil texture, enabling detailed characterisation of the soil particle size distribution. We hypothesised that the soil particle size bins measured by laser granulometry would more strongly correlate with other soil particle size bins of a similar size. Due to the highly managed aspect of the landscape, and the fact that textural class is more closely related to parent material than aboveground community, we expected that the habitat type would have minimal impact on soil texture class. We hypothesised that soil textural heterogeneity would increase diversity of both bacteria and fungi, driven by associations of different microbial taxa with certain particle size fractions. In particular, we hypothesised that soil textural heterogeneity would positively impact bacterial and fungal richness after accounting for changes in pH and soil carbon (C), which have been previously identified as strongly related to microbial diversity in UK soils (Griffiths et al., 2011). We also hypothesised that shifts in diversity would be driven by different microbial groups associating with different particle size fractions and consequently, that microbial composition would be more affected by textural composition than textural heterogeneity.

2. Materials and methods

2.1. Sample collection

Soil samples were collected as part of the Glastir Monitoring and Evaluation Programme from sites across Wales (Emmett and the GMEP team, 2017). Sites were randomly selected from land use classes in proportion to their extent in order to be representative of the variety of Welsh habitats (e.g., arable, improved and unimproved grassland, broadleaved and coniferous woodland, heathland), and dominant soil types (e.g., Cambisols, Podzols, Gleysols, Histosols, Lithosols, Rankers). In total, there were 127 individual 1 km squares with up to three sampling sites randomly located within each square (Fig. 1). The majority of these sites were grassland (132 improved grassland, 89 neutral grassland and 37 acid grassland), with 14 arable sites, 22 broadleaved woodland, 18 coniferous woodland, 10 marshland and 13 other. Topsoil samples (0–15 cm) were collected in summer 2013 and 2014 and analysed for multiple soil properties including total organic C and pH. Soil pH was measured by suspending 10 g of fresh field-moist soil in 25 ml of 0.01 M $CaCl_2$. After air-drying the soil samples had particles greater than 2 mm size removed and the remaining fine earth fraction ground by a deagglomerator (Pulverisette 8; Fritsch GmbH, Idar-Oberstein, Germany). Total organic C of the ground fine earth fraction of the soil was measured by oxidative combustion followed by thermal conductivity detection using the Elementar Vario EL (Elementar UK Ltd., Stockport, UK). Methods were consistent with the United Kingdom Countryside Survey; for a full description see Emmett et al. (2008) and George et al. (2017).

2.2. Laser granulometry

Soil samples with less than 50% organic C were selected for analysis ($n = 335$). Prior to analysis, each air-dried sample was subsampled by manual quartering and 0.5 g removed and treated with H_2O_2 to remove organic C following the method of Gee and Or (2002). Once the organic C had been removed, the samples were transferred to 250 ml bottles, and 5 ml of 5% sodium hexametaphosphate (Calgon®) added to promote particle dispersal and the samples were shaken overnight at 240 rev min^{-1}. The particle size distribution in each sample was then determined with a laser diffraction LS320 particle size analyser (Beckman-Coulter Inc., Pasadena, CA). In brief, this involved dispersal of the sample within a bath and subsequent passage of the sample through a measurement cell. Within the analyser there is a change in detector type at small particle sizes, as the higher ratio of particle dimension to light source wavelength lowers the sensitivity of the method and makes it more difficult to obtain accurate size values. To extend the lower size limit to 40 nm the patented Polarization Intensity Differential Scattering (PIDS) technology was used to determine particle sizes below 1 μm. The outflow from the machine was also passed through a 63 μm sieve and the collected sand-sized particles weighed. This allowed the sand content measured by the laser to be verified.

To convert the machine measurements into a particle size distribution an optical model must be used, and we chose to use the Mie theory approach (Bieganowski et al., 2018). The choice of optical model is known to be highly influential on the results, and improper model choice will make any further analysis meaningless (Keck and Müller, 2008). Soil is a composite material, and its components have different refractive indices, which can make model specification challenging. Values of the optical model reported in the literature vary considerably (Bieganowski et al., 2018), and many papers do not mention which parameters they used. For our analysis we used an RI of 1.55 and an AC of 0.1, as in Özer et al. (2010). This best reproduced the known particle size fractions of internal laboratory soil standards representative of our soils.

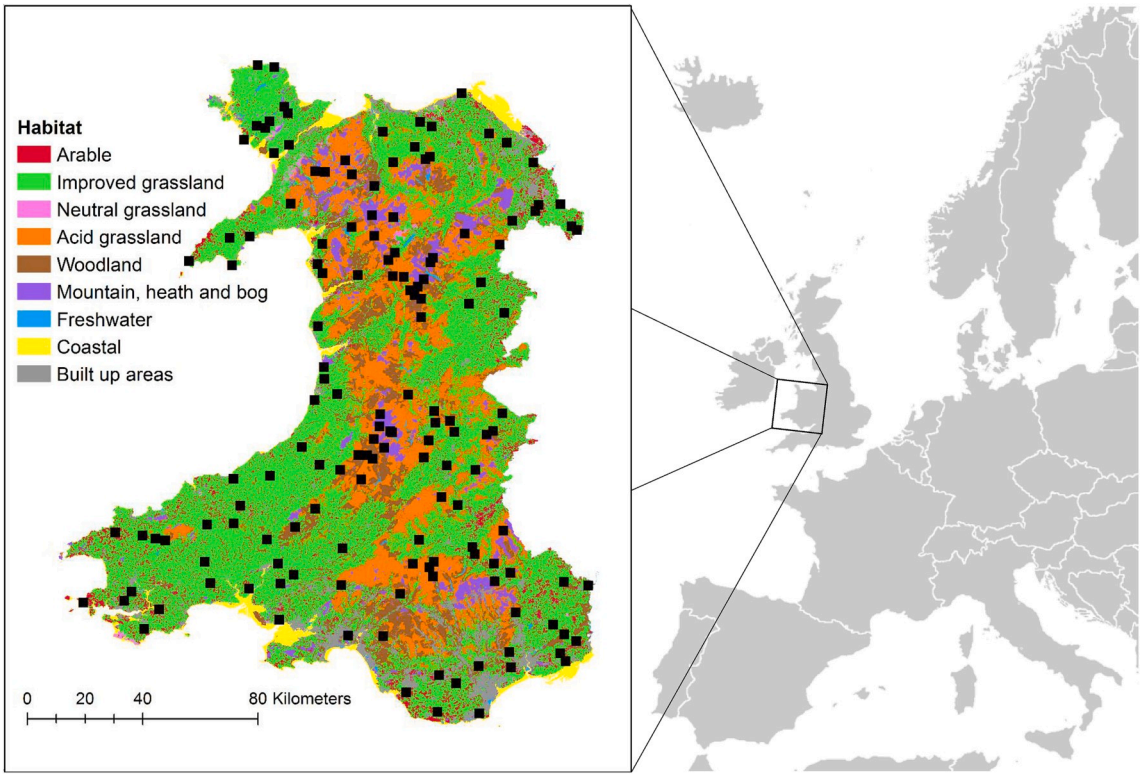

Fig. 1. Map showing the location of the survey square locations used in this study.

2.3. Fractal analysis

The increasing use of laser granulometry to describe soil particle size distributions has led to a need to find more descriptive measures of the shape of the particle size distribution (Bieganowski et al., 2018). One increasingly popular method is the use of fractal geometry to describe the heterogeneity of the soil particle size distribution (Millán et al., 2003; Miranda et al., 2006; Rodríguez-Lado and Lado, 2017; Yu et al., 2015). Tyler and Wheatcraft (1992) used a single fractal model to describe fractal scaling of soil particle size, but found that many soils did not exhibit simple fractal scaling. Instead of the simple power law of fractal scaling, soils can be analysed in terms of multifractal scaling as first shown by Grout et al. (1998). Multifractal analysis uses a spectrum of fractal dimensions to describe systems that have different fractal properties at different scales or regions (Stanley and Meakin, 1988).

Within this paper multifractal analysis was undertaken according to the moment method as described in Salat et al. (2017). The Rényi dimension Dq for the parameter q is defined according to equation (1).

$$D_q = \frac{1}{q-1}\lim_{\varepsilon \to 0}\frac{\log \mu(q,\varepsilon)}{\log \varepsilon} \quad (1)$$

Where ε is the size of the box and $\mu(q, \varepsilon)$ is defined according to equation (2).

$$\mu(q,\varepsilon) = \sum p_i^q \quad (2)$$

And p_i is the proportion of mass in the ith box of size ε.

A single fractal is characterized by the equality of the values of D_0, D_1 and D_2 (Posadas et al., 2001). If Dq decreases strictly for increasing parameter $q \geq 0$, then the measure is called multifractal (Peitgen et al., 1992). The various multifractal parameters give different types of information about the distribution. D_0 is known as the box-counting dimension and is equal to 1 when all subintervals are occupied at all scales and declines with increasing empty subintervals. D_1 is known as the entropy dimension and quantifies the degree of disorder present in the system – most heterogeneous gives $D_1 \approx 1$, most homogenous gives $D_1 \approx 0$. D_2 is known as the correlation dimension as it computes the correlation of measures contained in size ε (Posadas et al., 2001).

2.4. Microbial community characterisation

Soils were homogenised by sieving with a sterilised 2 mm stainless steel sieve. Sterilisation was achieved using high-level laboratory disinfectant and 5 min UV-treatments on each side. DNA was extracted in triplicate using PowerLyzer PowerSoil DNA Isolation Kits (MO-BIO) upon 0.25 g of soil per sample. Primers for the 16S rRNA gene (prokaryotes: 515F/806R) and ITS1 (fungi: ITS5/5.8S_fungi) regions were used to create triplicate amplicon libraries using a two-round PCR, for full details see George et al. (2019b). Taxonomy was assigned through QIIME using the GreenGenes database v. 13_8 and RDP methodology (Wang et al., 2007) for 16S data. Taxonomy was assigned to the ITS1 OTU table using the UNITE database v. 7.2 (Quast et al., 2013). Singletons and OTUs appearing in only 1 sample were removed from OTU tables. Archaeal, mitochondrial and chloroplast OTUs were removed from the 16S data and non-fungi OTUs from the ITS1 data. For full details on the methodology used see George et al. (2019b). Fungal trophic modes were identified using FunGUILD (Nguyen et al., 2016). To account for differences in read depth across samples, the bacterial and fungal OTU tables were rarefied to 18800 and 1500 reads respectively (Oksanen et al., 2018; Weiss et al., 2017). Rarefaction was repeated 50 times for bacteria and 100 times for fungi and the rounded mean used for the calculation of richness. Richness was represented using the number of observed OTUs per sample, which we use as our measure of alpha diversity.

2.5. Statistical analysis

All statistical analysis, including the calculation of multifractal parameters, were performed in R version 3.5.0 (R Core Team, 2019). The sand, silt, clay percentages of the samples were assigned to texture

classes from the UK Soil Survey of England and Wales and plotted on a ternary diagram using the soil texture package in R (Moeys, 2015). Fig. 3 was plotted using the ggplot2 package (Wickham, 2009). The impact of habitat on the fractal parameters D_0, D_1, D_2, D_1/D_0 and D_2/D_1 was tested using separate ANOVAs for each fractal parameter with habitat as the only predictor, with significance assessed using the Bonferroni correction (i.e. $p < 0.05/5$ for significance).

A correlation network was created from Spearman's rank correlation of the log-ratio transformed particle size bins (i.e. size fractions), and plotted using the qgraph package (Epskamp et al., 2012). Significant correlations were identified by the asymptomatic t approximation, with the p value required for significance lowered using a Bonferroni correction. The walktrap algorithm within the igraph package was used to detect the presence of clusters within the network to compare these clusters to the traditional clay, silt and sand boundaries, limiting the network to only significant positive links (Csardi and Nepusz, 2006).

We used structural equation modelling (SEM) to evaluate the relative influence of soil texture on bacterial and fungal diversity. SEM was chosen due to its ability to evaluate multiple processes at once and thus offer a more complete picture of the complex network of processes affecting soil microbial ecology (Grace et al., 2010). A SEM model was built using the lavaan package in R (Rosseel, 2012), and using the lavaan.survey package to account for the spatial structure of the data by incorporating square identity (Oberski, 2014). Land use intensity was encoded as a binary predictor, with arable, improved grassland and neutral grassland being set to 1 (intensive land use) and all other habitats being set to 0 (extensive land use). In total there were 310 samples with both texture and microbial data, with 221 samples being coded as intense land use. Summary statistics for the data included in the SEM can be found in Supplementary Table S1. We assumed no direct effect of precipitation or elevation upon bacterial or fungal diversity based upon preliminary graphical investigations, and confirmed this by checking if adding direct links improved the fit of the best model. Links that were pre-identified as being potentially insignificant and also having a p-value greater than 0.2 were removed in a stepwise manner until the best model according to AICc was found.

The correlation between the bacterial and fungal compositions and textural composition was first evaluated by repeated calculation of the Procrustes statistic using the protest function in the vegan R package. To examine if the particle size impacted the microbial community, the particle size bins were aggregated into 9 categories (three per sand, silt and clay respectively) and these were fitted as vectors to a non-metric dimensional scaling (NMDS) ordination in vegan. The common taxa for bacteria and fungi were tested for co-occurrence relationships with specific particle size bins by calculating the spearman rank correlations between the microbes and the particle size bins and limiting to those that were significant with Bonferroni correction (Harrell, 2017).

3. Results

3.1. Soil texture

Our samples showed considerable spread across soil texture categories (Fig. 2), consistent with the previously measured range of soil types across Wales (Proctor et al., 1998). Many of our samples were classified as clay loam ($n = 125$) with silty clay loam also constituting a significant proportion of the samples ($n = 97$). The next most abundant categories were sandy loam and sandy silt loam with 36 and 38 samples respectively. All other categories had fewer than 15 samples.

As expected, the amount of particles in a particular size category was strongly positively correlated with the amount of particles in adjoining categories (Fig. 3). The very smallest size categories, of less than 0.1 μm, were strongly negatively correlated with larger clay sized particles (0.16–2.2 μm). Two clusters of related nodes within the network were detected with overall modularity 0.48: a fine silt and coarse clay-sized particle cluster (0.13–13 μm); and a sand, coarse silt and very fine clay-sized particle cluster (0.04–0.13 μm and 15–2000 μm).

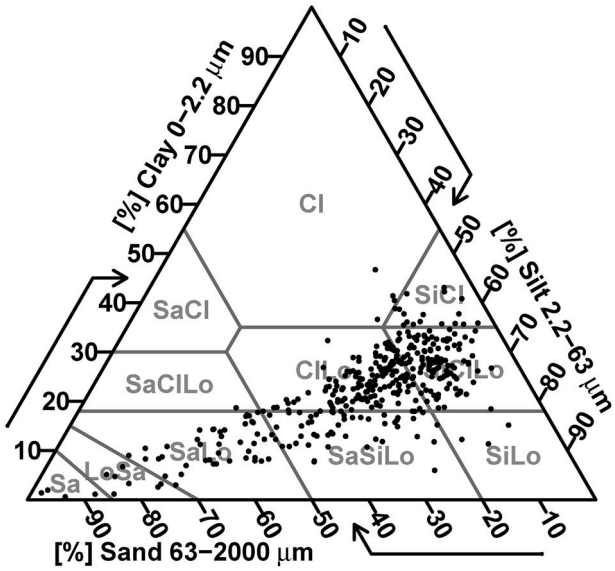

Fig. 2. Sand, silt and clay percentages of our samples plotted on a ternary diagram to show the range of texture classes examined in this study. Sa, sand; Si, silt; Cl, clay; Lo, loam.

3.2. Multifractal parameters

D declined with increasing q, showing that the soil particle size distribution does not follow a power law distribution. This indicated that a single fractal model would be inappropriate for our data. The box counting dimension (D_0) varied from 0.907 to 1, the entropy dimension (D_1) from 0.693 to 0.97, and the correlation dimension (D_2) from 0.45 to 965, with medians of 0.997, 0.920 and 0.890 respectively (Supplementary Table S2).

The box counting dimension was positively correlated with sand content (Spearman's rank rho = 0.62; Fig. 3). Sand content was significantly negatively correlated with all Dq values when q was negative (rho −0.31 to −0.33) and positively correlated with all D_q values when q was positive (rho = 0.54, 0.44, 0.37, 0.32 and 0.28 for D_1, D_2, D_3, D_4 and D_5, respectively). Many of our samples had no coarse sand present, while smaller size categories were ubiquitous, even if they were present at very low percentages. Therefore, the box counting dimension decreased in low sand content samples as these contained the only empty boxes. Clay content was positively correlated with all negative q D_qs (rho ranging from 0.59 to 0.63) and negatively correlated with both D_0 and D_1 (−0.61 and −0.36, respectively). Silt content was negatively correlated with D_0, D_1, D_2, D_3 and D_4 (rho = −0.40, −0.40, −0.34, −0.29 and −0.26, respectively). Note that while all these correlations were below the Bonferroni-corrected level for significance, many had a rho of less than 0.5 and were thus excluded from Fig. 3.

There was no significant difference by habitat for D_0, D_1, D_2, D_1/D_0 or D_2/D_1 (ANOVA on 6, 323 d.f. $p = 0.039$, $p = 0.22$, $p = 0.98$, $p = 0.84$ and $p = 0.97$, respectively. Significance assessed as $p < 0.01$. Supplementary Fig. S2). The change in D_0 with habitat was on the margin of being significant and it may be that woodland habitats have higher D_0 values. A higher value of this box-counting dimension indicates that these habitats have soil particle size distributions with few missing values. In our dataset this most likely means there are fewer woodlands on clayey or silty soils, as across the entire dataset the only missing values for texture occurred in the coarse sand fraction. There was no clear pattern of change in the $D_q \sim q$ spectra by habitat (Supplementary Fig. S3).

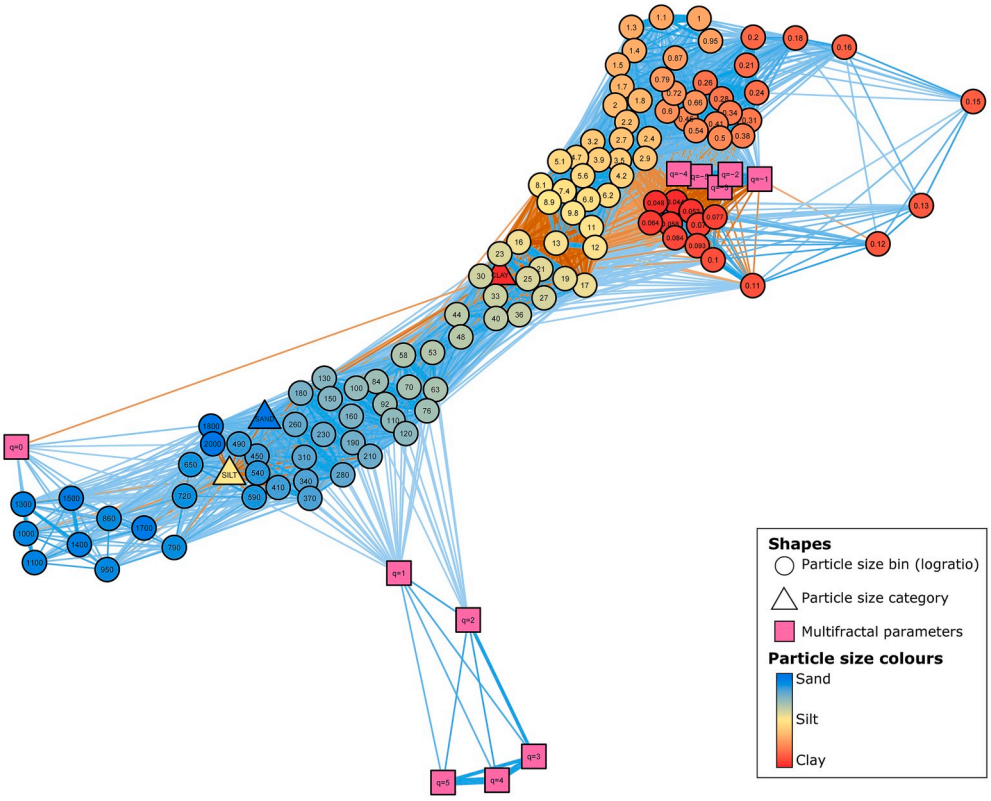

Fig. 3. Correlation network of soil particle size bins and multifractal parameters. Each circle is a node that represents a variable measured, with lines between nodes representing the correlation between those variables. Nodes are coloured according to identity: with a colour gradient of red for clay through yellow for silt and blue for sand. Triangular nodes represent the summed proportions of clay, silt and sand. Rectangular pink nodes represent the D_q values for q through −5 to 5. Red lines indicate negative correlation and blue positive, with the width of the line proportional to the strength of the correlation. Only correlations with an absolute value of rho >0.5 are shown. More closely related nodes are clustered closer together as much as possible. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

3.3. Relationship between textural heterogeneity and microbial diversity

We found no relationship between microbial alpha diversity and soil textural heterogeneity (D_1) when no other parameters were taken into account. Overall, there was no significant correlation between bacterial or fungal OTU richness and textural multifractal parameters (Fig. 4, Supplementary Fig. S3). The Spearman's rank correlations between fungal richness and D_0, D_1, and D_2 were −0.068, −0.046, and −0.024, respectively while for bacteria, they were 0.007, 0.064 and 0.084, respectively.

Structural equation modelling revealed a direct effect of textural heterogeneity upon bacterial diversity once changes in soil chemistry

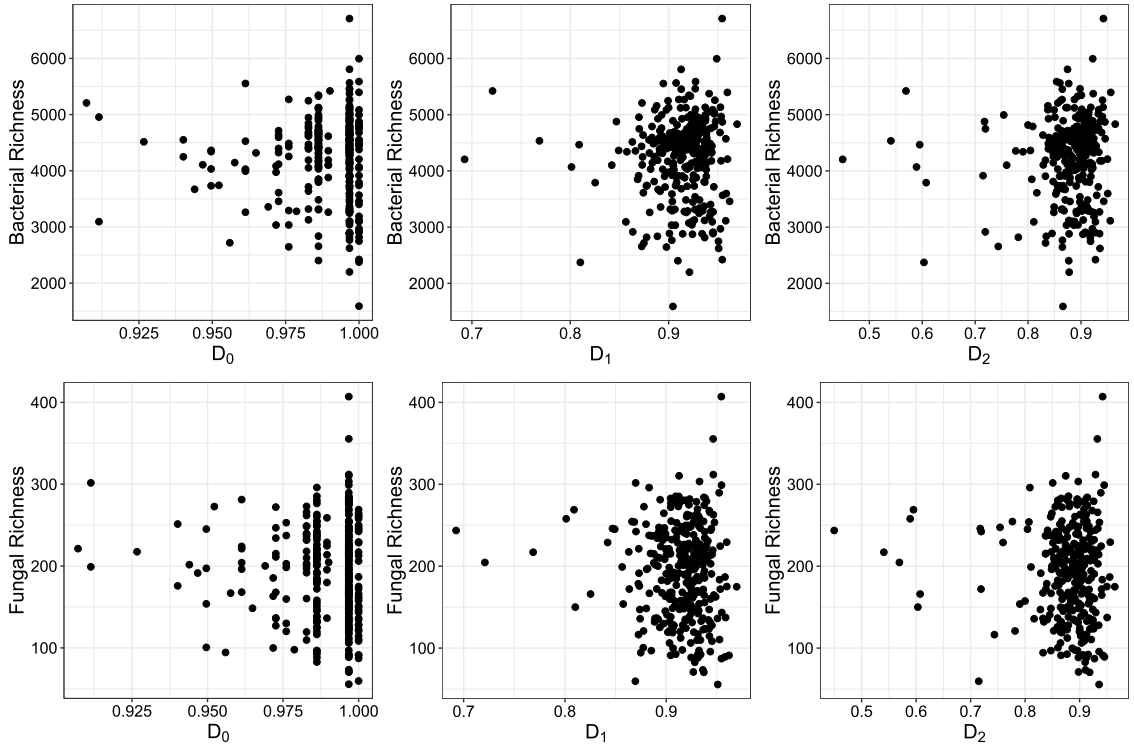

Fig. 4. Change in bacterial and fungal OTU richness with soil texture multifractal parameters (D_0, D_1 and D_2).

were accounted for (Fig. 5). Adding median grain size as a measurement of the difference between clay-rich and sandy soils did not improve model fit and the model with median grain size instead of textural heterogeneity performed considerably worse (ΔAIC > 900 and > 2000 respectively). Bacterial OTU richness increased with textural heterogeneity (represented by D_1), while fungal OTU richness did not. The direct impact of texture on bacterial diversity was low compared to soil pH and land use intensity but comparable to that of total soil C (full model output in Supplementary Table S2). Within structural equation modelling we can estimate the indirect effects of variables as well as the direct path coefficient (Supplementary Fig. 4). For example, the indirect effect of pH on fungi in our SEM can be calculated by multiplying together the standardised impact of pH upon bacteria and the standardised impact of bacteria upon fungi. We found that the positive impact of soil textural heterogeneity upon bacterial diversity is partially counteracted by changes in soil chemistry associated with the different soil textures (Supplementary Fig. S4); consistent with the lack of significant correlation seen in Fig. 4. We also found that while we identified few direct drivers of fungal richness the indirect effects of many of the soil physicochemical and climatic effects mirrored the response of bacteria richness due to the strong link between bacterial and fungal richness (Supplementary Fig. 4).

3.4. Relationship between microbial and textural composition

Bacterial and fungal composition both showed significant correlation with the texture data, however the correlation between bacteria and texture was greater than the correlation between fungi and texture (correlation in a symmetric Procrustes rotation 0.30 compared to 0.18). Microbial composition was more related to the texture composition as represented by particle size bins than to the textural heterogeneity as represented by the multifractal parameters (Supplementary Figs. S5 and S6). Both the bacterial and fungal NMDS ordinations were significantly affected by nine aggregated texture size particle bins; particularly the clay and silt sized particles (Supplementary Tables S3 and S4). The impact of D_1 and D_2 was insignificant, and D_0 explained only 2–3% of the variation in the data. The proportion of variance explained by the texture data was higher for bacteria with the medium and coarse clay sized particles explaining ~24% of the variation as opposed to fungi where they explained ~16% of the variation. pH and organic carbon were the strongest predictors of both bacterial and fungal composition, with pH explaining 70 and 60% of variation respectively and carbon explaining 40 and 30%. The impact of the textural composition was orthogonal to the pH-carbon primary axis of variation for fungi and to a lesser extent, bacteria.

Co-occurrence analyses were used to identify specific taxa that were more likely to occur in soils that had larger proportions of any given particle size range. OTUs that appeared in at least 50% of sites for bacteria and 25% of site for fungi were used in calculating spearman rank correlations with particle size bins, in total 4279 bacterial OTUs and 175 fungal OTUs were used. Of these 1106 bacterial OTUs were significantly positively correlated with at least one particle size bin, and 53 fungal OTUs. These correlations were mainly with the clay sized particles (above 0.12 μm diameter), with a limited number of correlations with fine to medium silt sized particles and no correlations with sand sized particles (Fig. 6). Of the 53 fungal OTUs, 21 were identified as being likely saprotrophs with 27 OTUs being unmatched to any potential trophic group. The classes of fungi that were correlated with particle size fractions appear to be a proportional subset of the overall common fungal class composition (Supplementary Fig. 7a). However, the bacteria that were correlated with particle size fractions were relatively low in Proteobacteria and high in other phyla such as the Chloroflexi compared to the overall composition of the common bacterial taxa (Supplementary Fig. 7b).

4. Discussion

4.1. Soil texture and biodiversity

We have found the first evidence for a positive relationship between soil textural heterogeneity and bacterial diversity. Textural heterogeneity performed better than median grain size at predicting microbial diversity indicating that in our range of soil textures the variety of soil particle size fractions is more important to microbial diversity than the size of the dominating particle size fractions. Multiple studies have found evidence of a relationship between soil texture and microbial communities, however, the nature, strength and direction of this relationship differs by study. Some experimental results indicate that texture is the most important driver of microbial community structure. For instance, one mesocosm experiment showed a greater impact on microbial community structure from particle size distribution manipulation than from pH alteration or compaction (Sleutel et al., 2012).

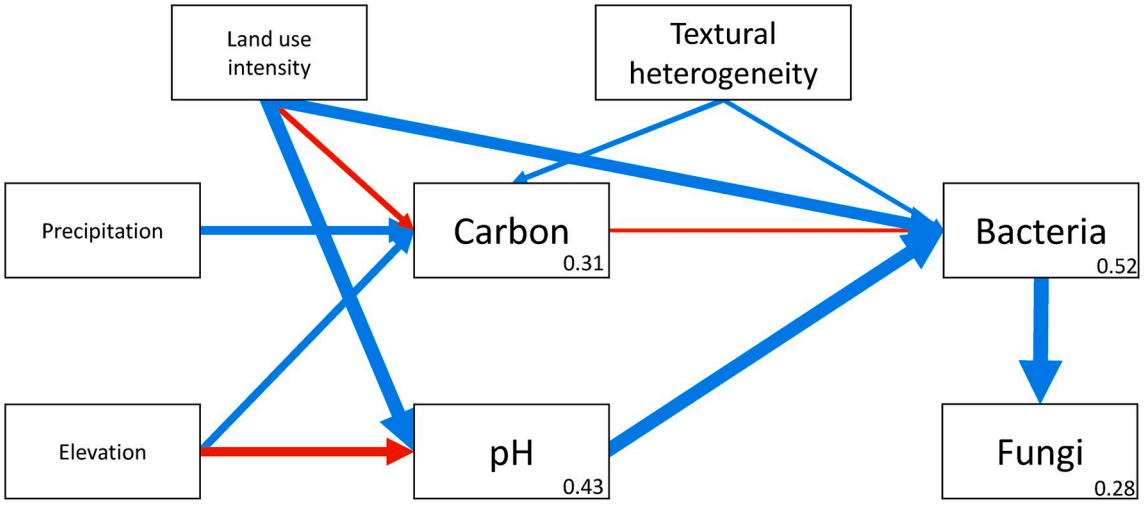

Fig. 5. Structural equation model showing the significant impact of textural heterogeneity on bacterial and fungal OTU richness after controlling for land use, soil carbon and pH (n = 310). Positive links are represented by blue lines, negative by red lines. Insignificant links (at $p > 0.05$) are not shown. R squared values for endogenous variables are shown in the corner of each box. Model fit was good: robust $\chi^2 = 2.117$ on 7 df, $p = 0.95$ (scaling correction factor = 6.91). A full model output is presented in Supplementary Table S2. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

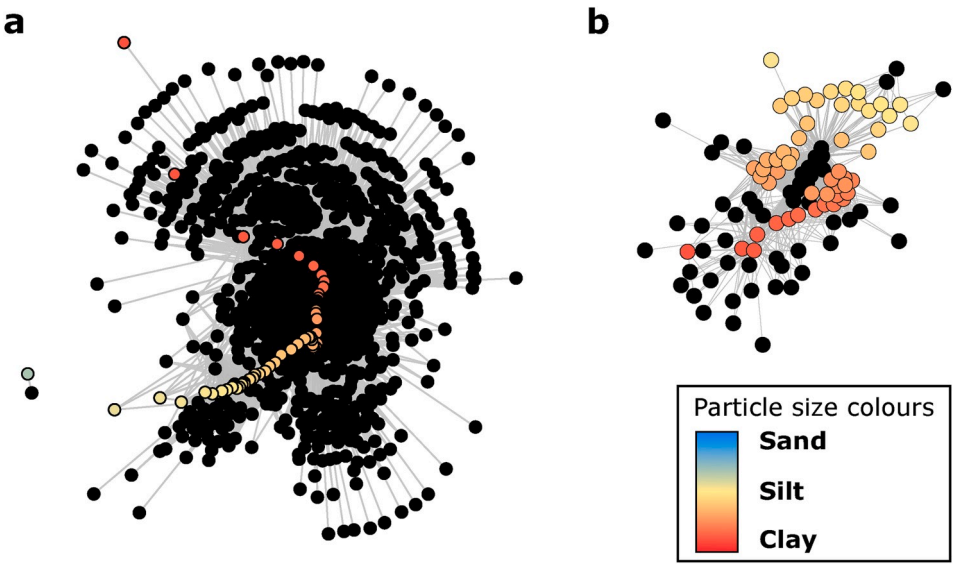

Fig. 6. The network of spearman rank correlations between particle size bins and bacterial (panel a) and fungal (panel b) OTUs. Only correlations directly between a particle size bin and a microbial OTU are shown for graphical simplicity. The particle size bins are coloured as in Fig. 3.

Previous work has found a positive link between microbial biomass and soil textural heterogeneity as described by a single fractal model (Hu et al., 2014). However, most field surveys have found a significant but lesser impact of texture upon microbial communities, in part due to the strong influence of pH on microbial diversity in natural ecosystems (Griffiths et al., 2011; Tecon and Or, 2017). More nuanced impacts of texture upon microbial diversity have been found across landscape types, across agricultural (Constancias et al., 2015; Naveed et al., 2016), grassland (Hu et al., 2014; Yao et al., 2018), forest (Chau et al., 2011), and arid sand (Pasternak et al., 2013). However, these apparently inconsistent results could still be driven by a positive relationship between bacterial diversity and soil particle size heterogeneity as there is no consistent relationship between soil particle size heterogeneity and texture size classes across landscapes. Of particular note is that our results show that just examining the clay-to-sand transition through incorporating median grain size provides less predictive information than the use of textural heterogeneity.

Our results revealed that changes in microbial composition were affected more by changes in particle size composition rather than textural heterogeneity. This is consistent with our hypothesis that different microbes show preference for different particle size bins, as evidenced in previous studies (Hemkemeyer et al., 2018; Poll et al., 2003). We found that clay and silt particle size bins were more important for microbial taxa than sand particles, similar to the results of Poll et al. (2003). The lack of a demonstrable link between microbial diversity and sand particles could be due to the low cation exchange capacity and nutrient content of quartz sand relative to clay and silt sized particles (Carson et al., 2009; Roberts, 2004). Microbial diversity associations with the clay fraction are also consistent with previous results that clay content has been found to be significantly positively related to bacterial diversity at field, regional and national scales in Europe (Constancias et al., 2015; Dequiedt et al., 2011; Naveed et al., 2016). However, a study in forested landscapes in the USA found a positive relationship between sand content and bacterial richness (Chau et al., 2011). There are also other studies which have found that sand sized particles are important for certain microbial taxa (Gardner et al., 2012; Hemkemeyer et al., 2018). These inconsistent results may be driven by the different mineral composition of soil particle size fractions in different regions, which is known to impact microbial association with particles (Carson et al., 2009; Nishiyama et al., 2012; Roberts, 2004). The mineralogy of soil particles in each particle size class varies across Wales and is often poorly related to the underlying geology due to the glacially derived nature of many Welsh soils, which makes it difficult to speculate upon the extent of the mineralogical influence upon the relationship between soil texture and microbial communities (Loveland, 1984; Smithson, 1953).

4.2. Soil texture

There was no impact of habitat upon the multifractal parameters of soil texture indicating that the texture of the soil is unaltered by the plant community and common local management practices and as such represents a difficult to manage constraint on the local microbial communities. This absence of a relationship between soil texture and habitat is contrary to previous results from the literature (Qi et al., 2018; Wang et al., 2008; Yu et al., 2015). Wang et al. (2008) interpreted their results as relating to the impact of vegetation upon soil erosion, with decreasing canopy cover leading to increased soil erosion and decreasing soil particle size heterogeneity. In a temperate oceanic climate more similar to our study area, soil textural heterogeneity has been found to be higher in grasslands and vineyards, which was suggested to be due to ploughing mixing weathered with less weathered soils (Rodríguez-Lado and Lado, 2017). While the above results are often interpreted as land use causing changes in soil texture, there is also the possibility that certain soils are preferentially chosen for certain land uses, e.g. more heterogeneously textured soils may be used more frequently for vineyards. The soils in our study area are less prone to erosion so this could reduce the impact of habitat upon soil texture (Borrelli et al., 2017). Low intensity management within our study area could also be reducing the impact of habitat, as only a small number of sites are within arable cropping systems. Any relationship between soil texture and habitat is dependent on the intensity of land use and the relative rates of soil erosion and disturbance. The lack of relationship between soil texture and habitat in our study also indicates that the influence of soil texture upon soil microbial communities is not due to texture acting as a proxy for a specific land use or other management or climatic gradient.

Comparison of the correlations between different soil particle size bins and the traditional sand, silt and clay boundaries reveal an overall separation of particle size bins into three categories roughly equivalent to the traditional boundaries, with the exception of very fine clay-sized particles. The network of soil particle size classes revealed that the abundance of different sand-sized particle fractions correlate well with each other. However, despite there being three clusters of nodes overall, there was no clear silt-sized or clay-sized cluster. Instead, the very fine

clay-sized particles clustered with the silt and sand-sized particles. This may have been due to the strong negative relationship between the fine sized clay particles and larger sized clay particles which was unexpected. Due to the patented nature of the laser granulometry equipment it is very difficult to ascertain whether it is related to the transition from the PIDS detector to particle size distribution. However, if this is a true description of Welsh soils it could indicate that soils with a greater proportion of coarse fractions have more nanoscale particles than clayey soils. This has implications for the increasing issues surrounding nano-scale pollutants as it may mean silty and sandy soils are more prone to retaining nano-scale particles without binding them to the soil matrix than clayey soils which are known to bind to certain nano-pollutants (Tourinho et al., 2012).

Our finding that the single fractal model was inappropriate for our data was consistent with previous work showing that the single fractal model failed to work for samples with greater than 10% clay (Posadas et al., 2001). The positive correlation of coarse particles with D_0 was consistent with previous results from Yu et al. (2015) but inconsistent with Millán et al. (2003). The negative correlation between clay sized particles and D_0 and D_1 was inconsistent with some previous literature which has found a positive correlation (Millán et al., 2003; Wang et al., 2008; Yu et al., 2015). Liu et al. (2009) reported a positive correlation between fine sized particles and particle heterogeneity but did not distinguish between clay and silt-sized particles. Our result is consistent with results from Miranda et al. (2006) and Posadas et al. (2001) who had similar loam type soils to our study, indicating that the relationship between multifractal parameters and soil texture classes is highly dependent on soil textural class.

The present work attempts to focus on the aspect of soil structure represented by the particle size distribution, which omits many other important structural influences upon soil biological communities. There has been much research on the influence of aggregate type upon microbial communities (Gupta and Germida, 2015). It is clear that microbial communities are both influenced by the presence and structure of soil aggregates and promote their formation (Tecon and Or, 2017; Totsche et al., 2010). The interplay between soil structure and biological communities is complex and likely to be dynamically responding to changes in environmental conditions. Our results are concerned with a more fundamental, relatively unchanging, aspect of soil structure than aggregation. Therefore, our results indicate that regardless of biological activity and typical management actions there will always be a physical control from the soil structure upon the biophysical interactions that can occur.

4.3. Bacterial vs fungal relationships with soil texture

The differential response of bacteria and fungi to soil texture is consistent with previous evidence and the different life history strategies of different microbial groups. Previous evidence has shown that clay content is related to bacterial diversity but not fungal diversity (Naveed et al., 2016). Soil textural heterogeneity provides a diversity of niche space and increased physical separation of communities promoting speciation within microbial communities. Low pore connectivity, as changed by both altering soil texture and lowering water potential, has been found to increase bacterial diversity in soil (Carson et al., 2010). In some respects bacteria are more constrained by their physical environment than fungi, limited to water-filled pore spaces and with low capacity for targeted movement (Yang and van Elsas, 2018). Bacteria dominate the microbial community within the smallest pores due to their smaller size and the lack of migration between these pores, particularly under drier conditions, that could lead to increasing segregation of bacterial communities and the maintenance of high diversity. Fungi are capable of moving through dry pore space and less likely to become restricted to specific soil microenvironments as they can transfer resources through the hyphal network to compensate for changes in the local environment (Tecon and Or, 2017; Whiteside et al., 2019). In combination with our results this suggests that soil texture is less of a constraint on fungal activities and diversities. Both bacteria and fungi show some changing of communities by particle size fraction which would seem to indicate that preference for particle size is not driving the difference between bacteria and fungi (Chiu et al., 2006; Neumann et al., 2013). However, it is possible that the changing fungal communities with particle size is largely dependent on autocorrelated bacterial community changes.

Despite the different direct responses to changes in single physicochemical properties, the strong positive link between bacteria and fungi means they respond similarly, directly or indirectly, to external gradients in those properties. The ability of the model to describe fungal diversity is limited, which may be due to the lack of inclusion of plant data that are known to be important in fungal community processes. Alternatively the choice of ITS1 for fungal community description could be impacting our results, as the DNA metabarcoding region is known to impact the variety of fungi identified (Blaalid et al., 2013; George et al., 2019a). For example, the 18S rRNA gene region is better able to describe the arbuscular mycorrhizal community, which we might expect to be influenced by soil texture indirectly by the plant community (Öpik et al., 2014). The strong relationship between bacteria and fungi offers a cautionary note in interpreting results from organismal groups separately, as the different taxonomic levels interact to such a degree as to make measuring responses to abiotic properties without the confounding biological variables misleading. Specific taxa may also respond directly to changes in soil particle size fractions relatively independently of other members of the microbial community, as suggested by the presence of correlations between fungal and bacterial OTUs and clay-sized particle bins in our results. Establishing the nature of the relationships between biological communities and then how they respond to external factors is essential in order to fully characterise the soil ecosystem.

5. Conclusions

We analysed a broad range of temperate habitats for their soil particle size distribution and microbial community characterisation. For the first time we show that soil textural heterogeneity is positively linked to bacterial richness. Conversely, we found that fungal richness was not directly impacted by soil texture but that there is an indirect effect of texture mediated through the bacterial community. Both bacterial and fungal community composition is impacted by the textural composition of the soil, with certain microbial taxa co-occurring with clay and fine-silt sized particles. Our research shows how different physicochemical factors directly influence community composition and diversity in different microbial groups. However, despite these differences in biophysical driving factors it is likely that ecological interactions can cause disparate microbial groups to respond similarly to environmental gradients.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

We thank all the GMEP team who contributed to collecting all the data. The research was funded by the Welsh Government through the Glastir Monitoring and Evaluation Program (GMEP). Contract reference: C147/2010/11. NERC/Centre for Ecology & Hydrology (CEH Projects: NEC04780/NEC05371/NEC05782). F.M.S. is supported by the award of a studentship grant by the NERC and BBSRC funded STARS (Soils Training And Research Studentships) Centre for Doctoral Training and Research Programme; a consortium consisting of Bangor University,

British Geological Survey, Centre for Ecology and Hydrology, Cranfield University, James Hutton Institute, Lancaster University, Rothamsted Research and the University of Nottingham. Grant Ref: NE/M009106/1. Illumina amplicon sequencing was performed by Delphine Lallias, in collaboration with John Kenny and Richie Eccles of the Liverpool Centre for Genome Research.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.soilbio.2020.107766.

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