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Monitoring and analysis of the structure, function and biodiversity of soils

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MONITORING AND ANALYSIS OF THE STRUCTURE, FUNCTION AND BIODIVERSITY OF SOILS

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Soils Training And Research Studentships

DECLARATION

I hereby declare that this thesis is the results of my own investigations, except where otherwise stated. All other sources are acknowledged by bibliographic references. This work has not previously been accepted in substance for any degree and is not being concurrently submitted in candidature for any degree unless, as agreed by the University, for approved dual awards.

Yr wyf drwy hyn yn datgan mai canlyniad fy ymchwil fy hun yw'r thesis hwn, ac eithrio lle nodir yn wahanol. Caiff ffynonellau eraill eu cydnabod gan droednodiadau yn rhoi cyfeiriadau eglur. Nid yw sylwedd y gwaith hwn wedi cael ei dderbyn o'r blaen ar gyfer unrhyw radd, ac nid yw'n cael ei gyflwyno ar yr un pryd mewn ymgeisiaeth am unrhyw radd oni bai ei fod, fel y cytunwyd gan y Brifysgol, am gymwysterau deuol cymeradwy.

THESIS SUMMARY

Soil health and function is key to general ecosystem health and human society, yet soils are under ever-increasing pressure from anthropogenic activities. The complexity of the soil system, with physical, chemical and biological factors interacting, make it difficult to understand what underpins soil health. The technical capabilities within soil science are greater than ever before, generating vast amounts of data. Despite this, identifying the key properties and interactions that influence soil health at policy-relevant scales remains an ongoing challenge. Evaluating current soil health and predicting future responses to global change therefore requires soil information at national levels as well as experimental analyses. The objectives of this thesis were (i) to evaluate the state of soils in Wales in regard to their physicochemical properties and biological communities, (ii) to establish the relative roles of physicochemical and biological factors in determining soil biodiversity, (iii) to explore the associations between soil physical properties and biological communities across Wales, and (iv) to evaluate the impact of climate change on soil microbial communities and function. This thesis combined soil physicochemical and microbial community characterisation through DNA sequencing results from a national scale field survey of the Welsh landscape and a long term climate change experiment in order to identify key dynamics and better predict responses to future change. Results from the national scale field survey indicated that soil pH and carbon drive many of the gradients in soil physicochemical and biological properties across Wales, with limited impact of land use. The Welsh soil landscape was largely split into two groupings: that of the near-neutral soils underlying improved and neutral grasslands, and that of the acidic soils that underlie bogs, heathlands and acidic grasslands. Soil microbial diversity was positively driven by soil pH, with soil textural heterogeneity increasing bacterial diversity once the increase with pH and decrease with carbon was accounted for. Soil physical properties were both influencing biological communities and being influenced by them, as shown by soil surface water repellency being associated with plant and microbial community composition. Plant and soil microbial diversity were positively correlated but this was driven entirely by changes in soil pH. However, the composition of above and belowground communities showed associations even after accounting for environmental gradients. In the long term field experiment, soil bacterial and fungal communities responded to experimental drought and warming, yet at a Welsh landscape scale microbial communities were largely unresponsive to climatic variables. Plant communities were an important link between climate and land use drivers and soil biological and functional responses. The combination of soil physicochemical, microbial and aboveground information throughout this thesis provides new understanding of the complex interactions and feedbacks that underpin soil health and function. Consideration of these dynamics is key to evaluating and monitoring soil health and resilience to future change.

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CHAPTER 1

Introduction

Monitoring and analysis of the structure, function and biodiversity of soils

Increasing human population and per capita demand for resources is putting greater strain on ecosystems, both natural and anthropogenic (Millennium Ecosystem Assessment, 2005; Tilman et al., 2011; United Nations, 2015). This is unlikely to cease in the near future, and attempts need to be made to reconcile the demands upon the environment for resources and maintaining the health of the planet (Foley et al., 2011; Rockström et al., 2009). Determining what we believe to be a healthy ecosystem is difficult and controversial, as there are numerous potential definitions which, while superficially similar, can lead to completely different results or viewpoints (Vieweger & Döring, 2015). In order to sustainably manage ecosystems clear goals are needed alongside a deeper understanding of relationships between different ecosystem properties. Much of the work on quantifying ecosystem properties has focused upon the aboveground system (Costanza et al., 1997; Tilman et al., 2013). However, the importance of soils and the belowground ecosystem and interactions between above and the belowground are increasingly being recognised as important for biodiversity and ecosystem service delivery, whilst soil information is integrated increasingly into conservation practice (Banwart, 2011).

Few people consider soils in their day-to-day lives, yet they are of undeniable importance to humanity. Degradation of soils limits functionality and has been important throughout history, with soil salinisation and erosion underpinning the breakup of many ancient civilisations (Evans et al., 2018; Hillel, 1992; Jacobsen & Adams, 1958). The impacts of soil degradation events on ecosystem properties and human society can be observed for hundreds of years, if not thousands, yet practices that degrade soil are still occurring today (Hall et al., 2013). This is particularly concerning due to the wide range of services soils provide to humanity (Robinson et al., 2014). Historically, most soil research has had an agricultural focus, yet soils are not just important to humans for growing crops. They perform many other functions and support the delivery of ecosystem services, especially for earth system regulation, including the storage of 1500-2400 Pg of carbon globally, around four times the amount stored in vegetation (Stocker et al., 2013). Soils act as essential regulators of nutrient cycling, the water cycle and act as a reservoir of many valuable resources

(Blum, 2005). The ability of any given soil to perform these essential functions is often defined as soil quality, or soil health (Bünemann et al., 2018; Kibblewhite et al., 2008).

Determining how to define soil quality, or health, is part of a wider discussion around the best way to present the status of global ecosystems in all of their complexity. The ecosystem services concept attempted to reframe the discussion around ecosystem conservation through determining the benefits, or services, provided to humanity by various ecosystem components (Daily, 1997). These ecosystem components are termed natural capital, an example of this framework as applied to soils is presented by Dominati et al. (2010). This approach has proved popular and underpinned various assessments of ecosystem quality (Millennium Ecosystem Assessment, 2005; UK National Ecosystem Assessment, 2011), as well as more controversial attempts to assign monetary value to global ecosystem services (e.g. Costanza et al., 1997). The use of this approach within the Millennium Ecosystem Assessment found that 60% of the ecosystem services they examined are being degraded or used unsustainably, with future declines and non-linear changes in ecosystem quality expected. However, the ecosystem service approach has also had detractors, with the term proving vague to apply in practice (Danley & Widmark, 2016; Potschin & Haines-Young, 2016), and many objecting to the presentation of ecosystems solely in terms of their extrinsic value to humanity (e.g. Peterson et al., 2010; Puig de la Bellacasa, 2015). All of the frameworks used for presenting ecosystem, and soil, quality require an understanding of how the measurable properties within ecosystems relate to emergent behaviour such as resilience to stress and support of key services. Establishing these linkages between different ecosystem properties remains an ongoing challenge in evaluating all of the different frameworks of ecosystem quality.

Identifying key soil quality indicators is a challenge and has rarely been done in relation to specific threats, functions or ecosystem services in order to evaluate soil quality (Bünemann et al., 2018). In fact, we still have relatively poor understanding of how specific soil properties link to soil functions at a spatial and temporal scale of interest to humanity (Kibblewhite et al., 2016). Soil biodiversity has been suggested as a potential indicator of soil quality and health (Maron et al., 2018; Ritz et al., 2009). Biodiversity within soils is high; soil organisms are estimated to represent 25% of all

described species and the lower effort put into describing soil organisms potentially means that this is an underestimate (Decaëns, 2010). Biodiversity is of interest as it has been found to relate to the resilience of ecosystems, proposed to be the criterion for ecosystem health (Döring et al., 2015; Tilman et al., 2013). Also, soil biodiversity may represent a hitherto little explored resource for medicines and other substances, with promising new antibiotics being recently discovered (Ling et al., 2015). However, soil biodiversity comprises many different domains and trophic levels with each one being relevant to different functions, if any, and all being challenging to measure. Some researchers have used multitrophic diversity to address this issue (Soliveres et al., 2016), but these results are often highly dependent upon the organisms included within the analysis as well as potentially obscuring finer-scale detail.

In this era of ever increasing pressure on natural ecosystems and soil it is essential to monitor ecosystem change over time. Soil is an integral part of natural ecosystems, and is required for biomass production whilst also representing a valuable store of carbon and other resources. The need to produce food for the industrial revolution meant that early work on soils focused on inventory and suitability for crop growth, which evolved into soil surveys in many countries in the 20th Century. If ecosystems are to be managed for long-term sustainability then an understanding of the long-term response of soil to environmental change is essential (Tugel et al., 2005). This requires a shift in the way we observe soils, moving away from inventory towards a monitoring of change and undertaking experiments to understand soil response to potential change.

1.1 Interplay between soil structure and biology

Soil structure is a dynamic soil property, influenced by physical, biological and anthropogenic processes. While it is well understood that processes such as tillage have a strong and lasting impact on soil structure, causing compaction and increasing soil erosion, the impact of certain biological processes on soil structure are still being discovered. We are beginning to establish what kind of feedback processes exist between the soil structure and biological activity. For example, the feedbacks determining soil carbon content are of great interest due to the role of soil carbon in

determining the water storage capability, the predominance of anoxic metabolic processes and thus greenhouse gas emissions and global biogeochemical cycles. There have been recent results suggesting that the physical location of the carbon within the soil is important in controlling its degradation, with more carbon being decomposed within pores of 30-100 μm diameter which constitute an optimal habitat for microbial activity (Kravchenko et al., 2019; Quigley et al., 2018). The migration of carbon between the different pore sizes of soil will strongly influence the eventual fate of said carbon, showing the interplay of soil structure and biology in determining carbon storage. The difficulties involved in determining which processes are relevant and dominate in the field are immense, but through a careful combination of experiments, observations and modelling these questions can be answered.

1.1.1 Influence of soil structure on microbes

The structure of a soil influences the biological communities that can proliferate and function within it. Soil is highly heterogeneous and consists of multiple micro-habitats, which can provide different physicochemical environments and support a variety of different organisms (Vos et al., 2013). The heterogeneity in microhabitats can influence a variety of different taxa, ranging from plants and macrofauna through mesofauna and microbial communities (Burton & Eggleton, 2016; Hu et al., 2014; Stromberger et al., 2012; Vos et al., 2013). Different microbes prefer different chemical and physical environments, ranging from preferences in oxic vs anoxic conditions to preferences for certain minerals (Nishiyama et al., 2012) and particle size fractions (Hemkemeyer et al., 2018; Poll et al., 2003). The soil structure and microhabitats available can strongly influence microbial community assembly and response to external inputs (Neumann et al., 2013).

The heterogeneity of the soil environment can inform the physical niche space available to the microbial community and the connectivity of different microbial communities, with implications for community assembly. The texture of a soil influences the structures that can develop there, with implications for pore space and soil hydraulic properties (van Genuchten, 1980). This then influences the microbial communities found within a soil, as found in experimental results. One study found that manipulation of the particle size distribution within soil mesocosms had a greater

impact on microbial community structure than pH alteration (Sleutel et al., 2012), while another found that low pore connectivity increased bacterial diversity in soil (Carson et al., 2010). Alteration of the physical environment will also change the chemical environment of the soil, and these have to be considered in tandem within semi-natural ecosystems.

The chemical environment of the soil is known to be important in determining the biological communities that can exist there. At the national and global scale, pH is the strongest driver of soil microbial diversity and composition (Bickel et al., 2019; Delgado-Baquerizo et al., 2018; Griffiths et al., 2011; Hendershot et al., 2017; Lauber et al., 2009). Climatic influences also express themselves as apparent drivers of the soil microbial community at large spatial scales, but these may be mediated by changes within the soil physicochemical properties (Bickel et al., 2019; Delgado-Baquerizo et al., 2018; Tedersoo et al., 2014). At finer spatial scales the influence of the variety of soil nutrients, habitat variation and plant community dynamics become greater (Cao et al., 2016; Constancias et al., 2015; Ranjard et al., 2013). However, clearly the biotic communities within soils possess the ability to modify their physicochemical environment which makes it difficult to predict the impact of future change in the environment upon soil ecology.

1.1.2 Influence of biology on soil structure

Soil organisms can influence their environment through the exudation of substances, binding together of particles, preferential breakdown of soil material, or even simply by moving through the soil. Many soil organisms can produce highly water repellent organic matter, which influences the wetting behaviour of the soil and aggregate formation and stability. Fungi have been known for many years to produce hydrophobic surfaces on their hyphae, hypothesised to reduce water loss and increase drought tolerance (Duddridge et al., 1980; Read et al., 1985; Unestam, 1991). Recently however, biofilm production by bacteria has also been found to lead to the creation of extremely water repellent surfaces (Epstein et al., 2011). Mucilage produced by plants and microbial biofilms has been shown to strongly influence rhizosphere rewetting behaviour (Benard et al., 2018). These exudates can influence both water movement through the soil and the creation of soil aggregates.

Soil structure has been described using a recursive model, where particles make up microaggregates which are then themselves bound together into macroaggregates. This perspective on soil, where the patterns of soil structure remain similar across spatial scale have prompted some to describe soil as a fractal system (Grout et al., 1998; Millán et al., 2003; Tyler & Wheatcraft, 1992). There has been much research on the relationships between soil aggregation and microbial communities (Gupta & Germida, 2015). It is clear that microbial communities are both influenced by the presence and structure of soil aggregates and promote their formation (Tecon & Or, 2017; Totsche et al., 2018; Totsche et al., 2010). Plant and fungal communities have been shown to promote soil aggregation both together and independently of each other (Hu et al., 1995; Miller & Jastrow, 1990).

Almost no soils exist with only the microbial part of the food web, and the role of plants and soil macrofauna in determining soil structure and microbial community response cannot be ignored. Plants and macrofauna can change both the soil chemical environment and physical environment through exudation/excretion and bioturbation of the soil. This has implications for the microbial communities that live in the soil, as they may form close interactions with plant roots, be transported by macrofauna through the soil and feed off the deposits from the larger flora and fauna (Read & Perez-Moreno, 2003; Yang & van Elsas, 2018). Interestingly, there are suggestions that the influence of the differing ecological niches within soil may be greater upon bulk soil fungal communities than rhizosphere communities, indicating the importance of the fine scale variation in soil to not only niche creation but the factors driving community assembly and function (Beck et al., 2015). Understanding how the different trophic levels interact to influence soil structure is key to evaluating how future change will impact soil function.

1.1.3 Relevance to future change and stress resilience

The ability to bio-engineer their environment may determine the resilience of organisms to stress. Within both semi-arid and peatland ecosystems the presence of rhizosphere or soil surface hydrophobicity induced by plants can increase tolerance of either drought or fire events (Kettridge et al., 2014, 2017; Robinson et al., 2010; Verboom & Pate, 2006). These processes may not be aimed at directly bio-engineering

the soil environment, as they may occur at the organism scale due to the increased above-ground stress tolerance or competitive ability conferred by the ability to alter the immediate environment. For instance, in plants the ability to alter rhizosphere hydrophobicity has been shown to confer a competitive advantage due to increased acquisition of water (Kroener et al., 2016; Zeppenfeld et al., 2017). In general, the production of extracellular polymeric substances can improve the resilience of organisms to diverse sources of stress (Costa et al., 2018). As environmental sources of stress increase, these abilities could become increasingly important for survival within ecological communities. Resilience has been proposed as a universal criterion of health, therefore the ability of soil organisms to influence their physical environment under this paradigm could be a key determinant of soil health (Döring et al., 2015).

1.2 Making predictions and mitigating issues

The overall goal of soil science is usually to offer solutions to the current challenges facing soils across the globe today, and to predict how future changes will impact soils and their functions. These objectives require an understanding of the mechanisms that underlie soils, and the ability to make causal inferences about how soils work. The aforementioned feedbacks between soil physicochemical properties and the biotic communities that exist in soils pose an issue to establishing the causal mechanisms of relevance to soil management. To address this, we need a combination of experimental, survey and monitoring work to establish which mechanisms plausibly exist and which are relevant at the field and national scale.

1.2.1 Experiments vs field surveys

Soil experiments are useful in that we can manipulate pressures and then analyse the change in response to a certain treatment; while monitoring examines the state and change (Lawrence et al., 2013; Richter et al., 2007). The two approaches provide a powerful combination for understanding drivers of soil change. Long-term monitoring programmes and soil experiments are increasing in number and many useful insights have been gained into long-term behaviour of soil (Richter et al., 2007; Tóth et al., 2013). These range from examining long term impacts of fertiliser application on soil fertility to the impacts of climate change on soil biogeochemical cycling (Edmeades,

2003; Lapenis et al., 2008; Ren et al., 2018). Experiments provide opportunities to explore what exact response will result from a specific intervention, enabling more stringent exploration of mechanisms at the expense of generality across landscapes and spatial and temporal scales.

The real-world response of soil at a national level to global change can be better understood through long-term monitoring programmes. The Countryside Survey in Great Britain was pioneering in this context and has provided evidence of soil change since 1978 (Emmett et al., 2010; Reynolds et al., 2013). Unlike systematic surveys used for inventory, the Countryside Survey is statistically robust, allowing reporting of uncertainty. The robust design has led to the adoption of similar designs at the EU level, for example the Land Use/Land Cover Area Frame Survey (LUCAS) topsoil database covers 25 member states of the European Union and provides a basis for soil-related policies (Orgiazzi et al., 2018; Tóth et al., 2013). However, there is increasing demand from policy-makers for the ability to link these large-scale monitoring approaches to agri-environment policy outcomes in order to evaluate current policy. This requires a combination of monitoring, experimental and modelling approaches, and the implications of this for inferring the potential impacts of interventions need to be considered within the framework of causal inference in order to evaluate its potential strengths and shortcomings.

1.2.2 Causal inference

Established practice when dealing with observational data has been to discuss the results in terms of associations and correlations, but to avoid as much as possible referring to causal linkages. Unfortunately, the careful selection of language will not prevent readers from inferring results in a causal sense, and many observational results fill gaps in the literature due to the impossibility and/or impracticality of doing an equivalent experiment. Therefore there has recently been work on the use of approaches such as directed acyclic graphs (DAGs) to inform causal interpretation of observational research (Pearl & MacKenzie, 2018; Rohrer, 2018). A DAG consists of a graphical model, where there are nodes (representing variables) connected by unidirectional arrows (representing causal links), see example in Figure 1.1. They are acyclic in that there are no feedback loops within the model. These approaches are

designed to enable careful consideration of confounding variables through the building of graphical causal models.

Careful consideration of the causal hypotheses underlying our data, potentially through creating a DAG, informs us which variables need to be controlled within our analysis in order to determine the direct influence of one variable upon another. Often, to identify the direct effect of one variable upon another scientists statistically control for as many other variables as possible. However, this practice will not improve estimates of direct effects, actually distorting the actual effect (Spector & Brannick, 2011). These approaches can be made worse by not including information on measurement error into the model, even at large sample sizes and moderate error rates (Westfall & Yarkoni, 2016).

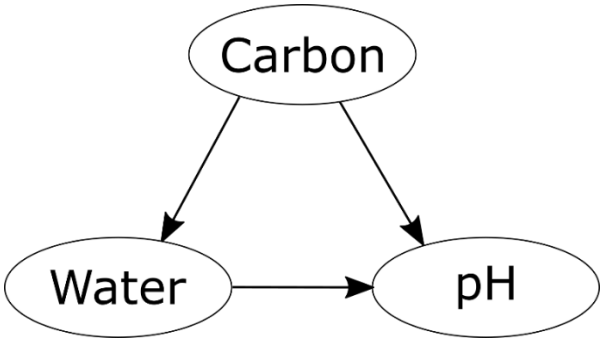


Figure 1.1: A DAG showing the hypothesised relationships between soil carbon, water and pH. The arrows indicate that carbon acts as a causal influence on water and pH, while water causally influences pH.

If we wish to estimate the impact of one variable on another when they both share a common cause then we need to account for the common cause, or confounder. Take the DAG shown in Figure 1.1. If we are to estimate the impact of water upon pH then we need to control for the impact of carbon on pH. This is easily done by including carbon as well as water as predictors of pH within the regression model. This step is necessary if we wish to evaluate the potential impact of changing the water content of the soil on pH. The use of observational data to infer the outcomes of interventions requires the control of confounders.

What if we were to condition upon a variable that is not a common cause, but instead influenced by both the other variables? Variables within DAGs that have arrows pointing at them only in a path are referred to as colliders. For example, in the above

figure pH is a collider between carbon and water. If we wished to discover the impact of carbon on water content we simply need to run a model with carbon as the sole predictor. If we were to condition on pH by adding it to the model we would lose the effect of carbon on water. Figure 1.2 shows a simulated dataset where water is a function of carbon, and pH a function of water and carbon together. Modelling water as a function of carbon leads to a coefficient on carbon of 0.85 (±0.1 S.E.) – close to the true coefficient of 1. If we were to condition on pH, we would estimate the impact of carbon as -0.02 (±0.13 S.E.), while pH would be estimated to have a significant impact of 6.30 (±0.71 S.E.). However, we know that this is not the true model. Widely used approaches such as information criteria would not be able to pick the true causal model, in fact in this case the best model by AICc is the one which includes pH (ΔAICc = 65).

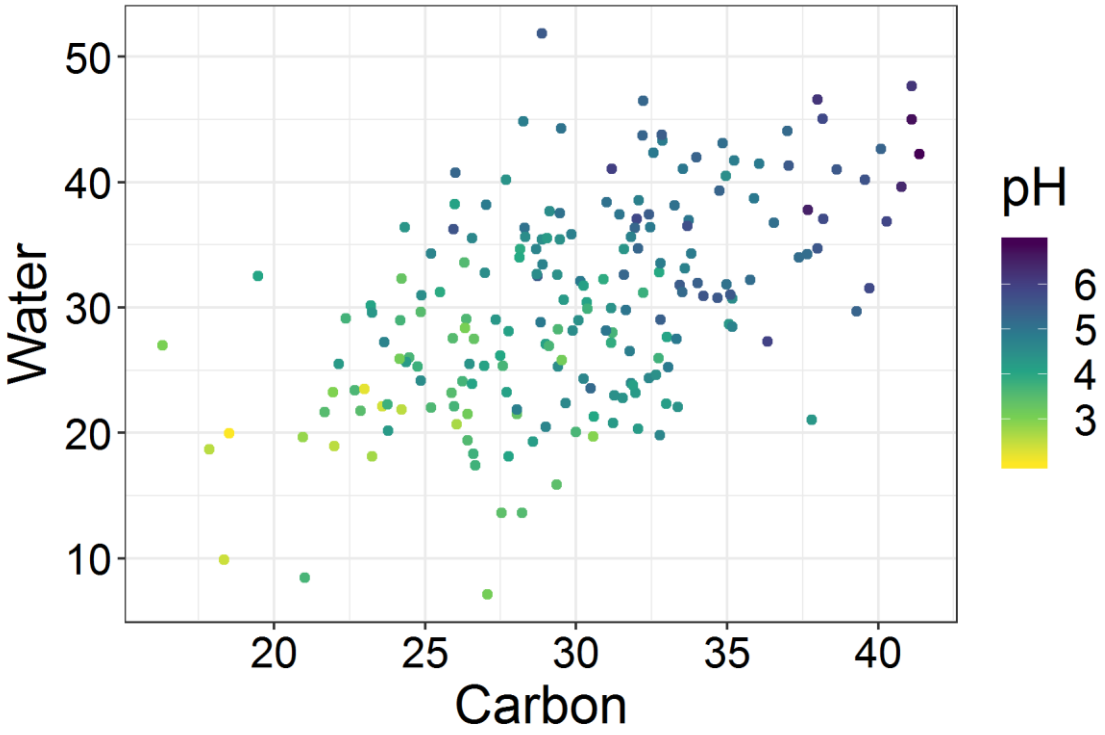


Figure 1.2: The relationship between carbon and water, with points colour coded by pH. This data was simulated based on the DAG in Figure 1.1.

This collider effect can occur without explicitly conditioning on a collider within a model. For example, say you are interested in the relationship between bird abundance and flowers and you are studying arable systems. It is plausible that within the area you are studying that only areas with lower bird and flower abundance are

used for crop production due to nature conservation constraints. Across the entire landscape there may be no relation between bird and flower abundance, yet within arable sites there will be a negative relationship between birds and flowers. Here the arable variable is the collider, and the fact that the study is limited only to arable sites is equivalent to conditioning on this collider. Figure 1.3 shows this for a simulated data set. For the whole landscape there is no link from flowers to birds (-0.01 ±0.03), while in arable sites there is a negative relationship (-0.22 ±0.05).

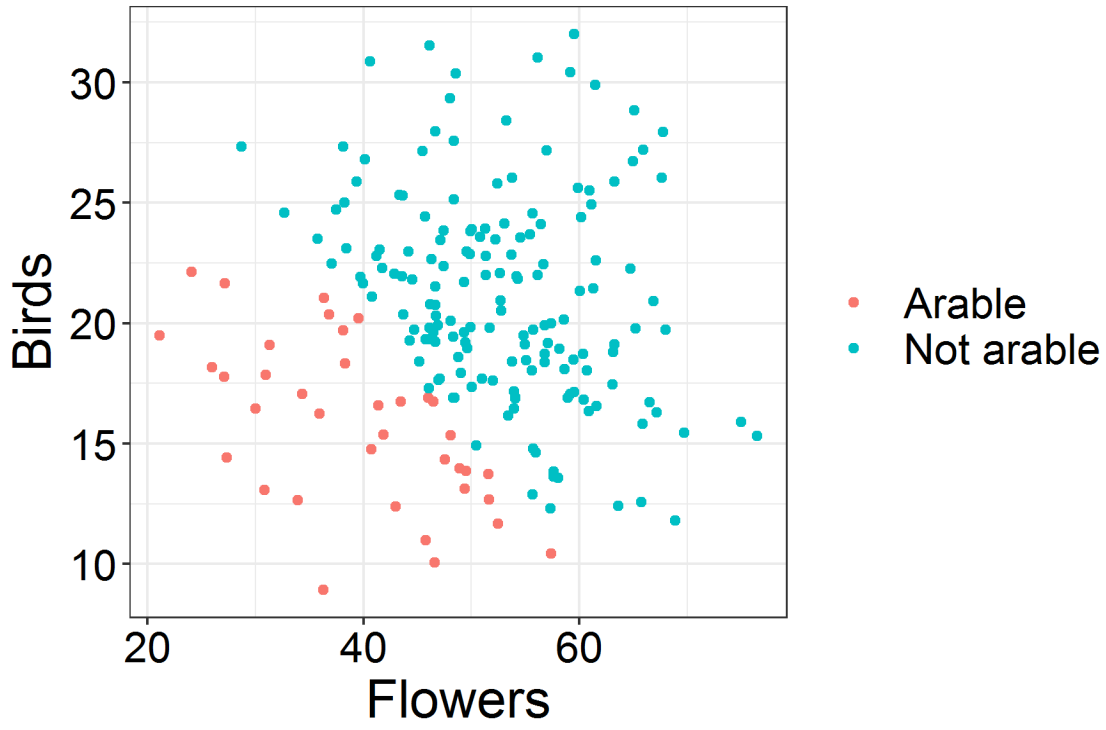


Figure 1.3: The relationship between flower and bird diversity in a simulated dataset. If the combined diversity is above 50 then the site is categorised as not arable. Note the decreasing bird diversity with increasing flower diversity in arable sites, while overall there is no pattern.

It is tempting to consider experimental results to give definitive answers as to the causal effects of a certain treatment. However, interpretation of experimental results can easily fall into certain traps which can lead to erroneous conclusions. For example, in many experiments it is common to control for post treatment variables, however, this can lead to violation of the assumption of random assignment of treatments which can profoundly bias the results (Montgomery et al., 2018). This is equivalent to conditioning on a collider, as discussed above. Careful consideration of the proposed

mechanisms underlying the behaviour of soils is needed for both observational and experimental results.

1.2.3 Information theory

With the use of new technologies in soil science has come an increasing amount of information with a relative decrease in the amount known or hypothesised about this information. Examples of this include the increasing use of laser granulometry in particle size distribution causing a switch from a 3-category system (sand, silt, clay) to over a hundred particle size bins (Bieganowski et al., 2018). On a larger scale within soil microbial studies the advent of next generation sequencing has led to the genetic characterisation of thousands of microbes within a single soil sample, many of which were previously unknown, unculturable, or with limited taxonomic and functional metadata (Knight et al., 2018). This proliferation of data has required more tools and metrics to characterise the soil system. To do so in a way that is both accurate and useful is a work in progress, as we attempt to take that which is incomprehensible in its complexity and boil it down to a set of simple results and rules.

1.2.3.1 Information diversity

Quantifying the information content of a selection of objects is an issue that was addressed by Shannon with respect to communication systems through the creation of his entropy index (Shannon, 1948). This H index has proven to be a key measure of information, choice, and uncertainty, and is given by adding together the probabilities of each event (p_i) multiplied by the logarithm of the probabilities.

$$H = -\sum_{i=1}^{n} p_i \log(p_i) \qquad \text{Equation 1.1}$$

Within ecological research this is used as a metric of biodiversity where the probabilities are represented by the proportions of the different species. Other biodiversity metrics also fall into a similar information theory framework. Hill laid out this framework that involved measuring biodiversity taking into account both species richness and relative abundances (Hill, 1973). If p_i is taken to be the proportion of the total sample represented by the species then a useful metric is the average of p_i across

the sample. This can then be weighted, i.e. the impact of rare species versus common species changes by varying the exponent, q.

$$\bar{p_i} = \left(\sum_{i=1}^{n} {p_i}^q \right)^{1/(q-1)} \qquad \text{Equation 1.2}$$

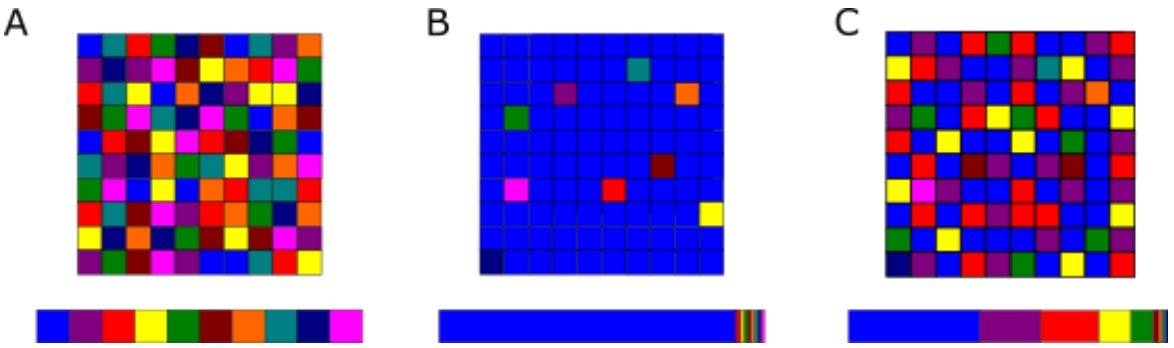
In equation 1 n is the total number of species. True alpha diversity (${}^{q}D_{\alpha}$) can be defined as the inverse of $\bar{p_i}$ and can be understood as the effective number of species in a sample (Hill, 1973; Tuomisto, 2010). In the case that q = 0 then the true diversity will be the same as species richness. As q increases it can be thought of as examining the sample in less detail, so that only the most common species are recorded and the effective number of species declines (Hill, 1973). The Simpson's index occurs at $q = 2$ and calculates the mean proportional abundance of the sample, which can then be transformed into an effective number of species by inversion (Simpson, 1949). At $q = 1$ Equation 1.2 will not work due to division by 0, however it can be shown that as q tends to 1 this become the Shannon information entropy in Equation 1.1. The effective number of species in the sample when $q = 1$ is therefore:

$$ {}^{1}D = \exp\left(-\sum_{i=1}^{n} p_i \ln\left(p_i\right) \right) \qquad \text{Equation 1.3}$$

Within this framework it can be seen that the Shannon-Weaver index of biodiversity places more emphasis on rare species than the Simpson index. This is because the q value is lower (1 < 2), and as discussed above this means the sample is effectively being examined in more detail. Although many studies report the weighted mean proportional abundance (e.g. the Shannon-Weaver index) the effective number of species (e.g. the exponential of the Shannon-Weaver index) is often intuitively easier to understand. The effective number of species also holds useful mathematical properties, such as always being positively correlated with the number of actual species (Hill, 1973; Jost, 2007; Tuomisto, 2010). Alternatively, there are diversity metrics based upon theories on species abundance distributions (Fisher et al., 1943; Hill, 1973; Magurran, 2004), however careful attention must be paid to the underlying assumptions of these models. Box 1.1 represents a few examples of different metrics of

biodiversity, and how they vary as the relative abundance of the different species change.

Box 1.1 A comparison of diversity indices:



Diversity indices for three theoretical sites are shown. Each individual is represented by a square that is colour coded according to species. Despite having the same species number each site has very different patterns of species abundance. This can be summarised using the true diversity at q=1, the inverse of the Shannon-Weaver index, and the true diversity at q=2, the inverse of the Simpson index. This is shown in the table below.

Site	Species Number	q=1		q=2	
		Index	True diversity	Index	True diversity
A	10	2.3	10	0.1	10
B	10	0.5	1.6	0.8	1.3
C	10	1.7	5.5	0.2	4.2

In many cases when dealing with information the scale at which the data is examined will influence the amount of information that is gathered. Within chaos and fractal theory the aforementioned information functions are extended into the Rényi dimensions, modifying by the division of the information function by the logarithm of the scale (Peitgen et al., 1992). First, the information function for a given scale (s) and exponent (q):

$$I_q(s) = \frac{1}{q-1}\log_2 \sum_{i=1}^{N(s)} {p_i}^q \qquad \text{Equation 1.4}$$

The Rényi dimension D_q for the parameter q is given by:

$$D_q = \lim_{s\to 0} \frac{I_q(s)}{\log_2 1/s} \qquad \text{Equation 1.5}$$

At $q = 1$ Equation 1.4 is not directly applicable, so we use the limit of $I_q(s)$ as $q \to 1$:

$$\lim_{q\to 1} I_q(s) = -\sum_{i=1}^{N(s)} p_i \log(p_i) \qquad \text{Equation 1.6}$$

Observe that the information function here is the same as the Shannon entropy as described before, demonstrating that the Rényi information dimension D_1 represents what the information entropy would be at the finest scale of scrutiny. Other special dimensions within the Rényi dimensions are the box-counting dimension (D_0) and the correlation dimension (D_2). The development of these within fractal theory allows description of how much information we expect the system would hold at the finest scale. These dimensions are often applied to characterise the soil structure through analysis of the particle size distribution or pore distribution (Caniego et al., 2003; Grout et al., 1998; Tyler & Wheatcraft, 1992).

1.2.3.2 Composition

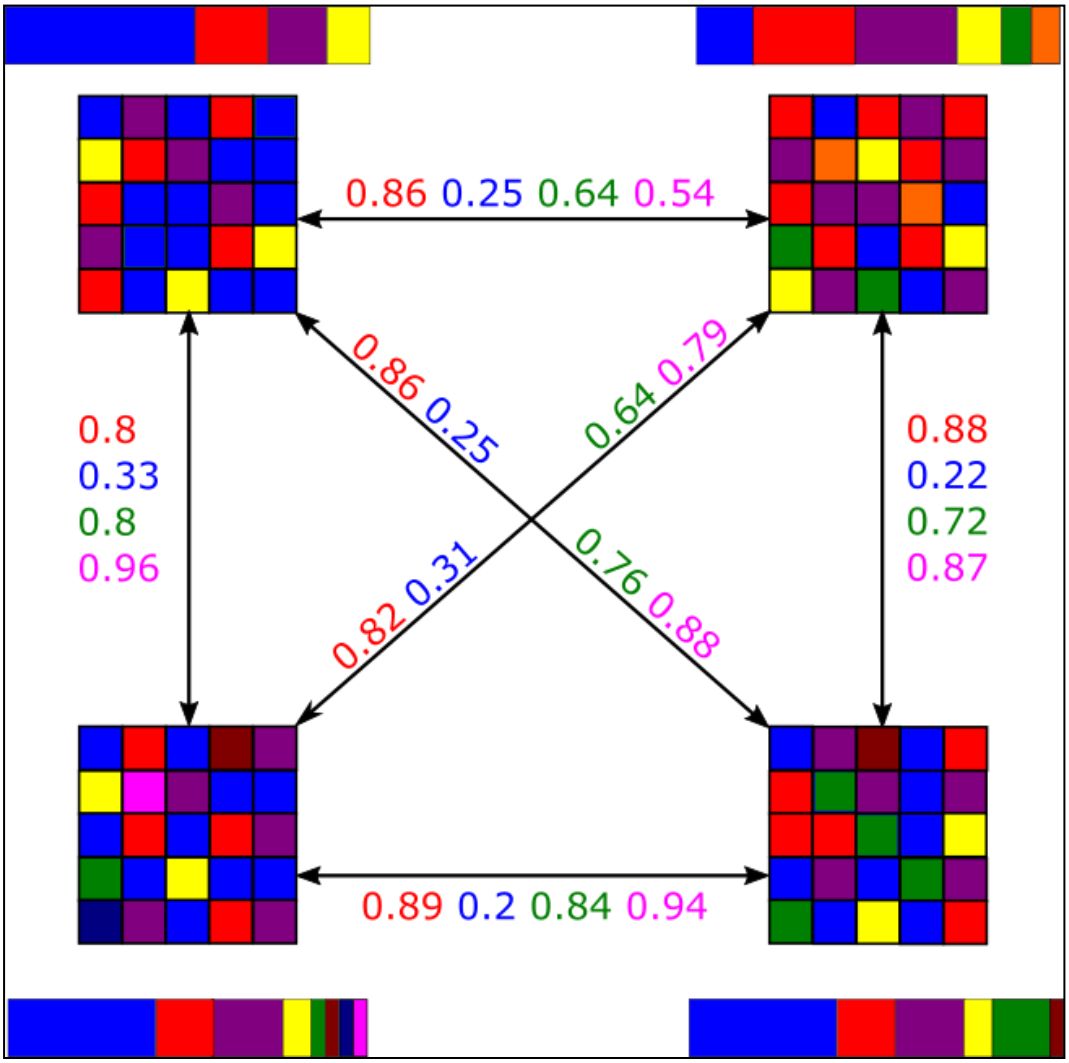
In many cases we are not just interested in the presence or absence of units, such as species, but in their identity and the relative composition of different sites. Some types of information can be encoded into information indices, such as phylogenetic and functional information which has been included in some versions of biodiversity metrics (Chao et al., 2010; Rao, 1982; Scheiner, 2012). These indices have been suggested to be more closely linked to functional diversity than species diversity itself (Milcu et al., 2013). They may also be used to help ameliorate the difficulties in applying the species concept to microbes, and indeed to any genetic data without a lot of information about the inter vs intraspecific variation of that gene within the taxonomic group (Kress et al., 2005; Nilsson et al., 2008; Schoch et al., 2012). If we are

interested in the impact of biodiversity on ecosystem function it would seem logical to measure functional or phylogenetic diversity rather than taxonomic diversity.

The above indices of biodiversity are all concerned with alpha diversity; that is the diversity within a certain area. What may also be of interest is the beta diversity; or how dissimilar two separate ecosystems are. The term beta diversity has been used in many different ways to describe different phenomena (Tuomisto, 2010). "True beta diversity" has been defined as the factor needed to multiply "true alpha diversity" by to get the global (gamma) diversity. Here, true alpha diversity corresponds to the average number of species within the sampling units (Tuomisto, 2010). However, what is more often referred to by beta diversity is the degree to which two communities are dissimilar, which can also be referred to as community overlap or dissimilarity.

Similar to the case for alpha diversity there are community overlap indices that take into account only presence/absence data (qualitative) but also those which use abundance data (quantitative) (Magurran, 2004). One of the most commonly used qualitative indices is the Sørensen index, which is widely regarded as one of the most effective presence/absence similarity measures particularly for molecular data where abundances are less clear (Magurran, 2004). If abundance data is available one of the many quantitative community overlap indices can be used, such as the Bray-Curtis index or the Morisita-Horn index (Wolda, 1981, 1983). There are numerous community overlap indices, of which only the most commonly used are described here, and compared in Box 2. The selection of the best index will depend upon the question of interest, the dataset and the eventual goal of the analysis (Magurran, 2004). Clearly, how to extract the useful information from biodiversity and other data is still a matter of much debate and controversy.

Box 1.2: A Comparison of Beta Diversity Indices:



Four sites are shown, each with 25 individuals represented by squares. The colour of the square indicates the species, and the distance along the bar represents the total proportion of the community that is made up of each species. Community overlap values are given on the arrows connecting the sites, first (in red) is the Sørensen index, second (in blue) is the Marczewski-Steinhaus distance, third (in green) is the Bray-Curtis index and finally in pink is the Morisita-Horn index. The abundance based measures (BC and MH) pick out the site in the top right as being particularly different from the others, whereas the qualitative measures do not. True beta diversity of these sites is 1.50 at q=0, 1.16 at q=1 and 1.09 at q=2.

1.3 Soils in Wales

Wales is varied in terrain and geological history, resulting in a wide variety of habitats and soil types over a relatively small geographic area. Wales also contains a relatively high proportion of high-carbon peatland soils, with 5.6% of the land area covered by peatlands (Vanguelova et al., 2012). Many of these valuable habitats are being degraded by agricultural use and forest plantations. In 2018 around 90% of Welsh land was used for some type of agriculture, with the majority being used for pasture and only 13% being used for arable production (Welsh Government, 2019a). Since 2008 there has been an expansion of the total area of Wales used for agriculture (78% to 90%), although this is at least partly attributable to new registration of pre-existing farmland for access to new government funding, and also the proportion that is used for arable production (10% to 13%) (Welsh Government, 2019a). Land use across Wales is expected to change further in the future as political uncertainty and the impacts of climate change influence the relative sustainability of farming systems, with associated impacts on the Welsh environment.

The climate of Wales is predicted to move towards having warmer, wetter winters with hotter, drier summers (Figure 1.4, Murphy et al., 2018). There are already more frequent and intense extreme events which are projected to increase further in the future, with increasing frequency of drought and flooding across the UK including Wales (Kendon, E. J. et al., 2014; Kendon, M. et al., 2019). This will have major impacts on the types of agricultural and natural landscapes that can persist in Wales (ASC, 2016). The differing abilities of species to adapt and move in response to the new climate conditions, in tandem with other anthropogenic pressures, will lead to changes in the composition and stability of ecosystems (Morecroft & Speakman, 2015). Coastal landscapes will also change, as increasing sea level and potentially increased storm surge lead to increased flooding of coastal ecosystems (Palmer et al., 2018). All of these predictions assume that the models based on current atmospheric and oceanic dynamics are accurate to future dynamics, but there is potential for abrupt climate change with shutdown of major ocean-atmosphere systems which would have dramatic and difficult to predict impacts on the Welsh landscape (Rahmstorf et al.,

2015). This ever increasing threat to Welsh ecosystems is occurring at a time of increased land use pressure, pollution, and political uncertainty.

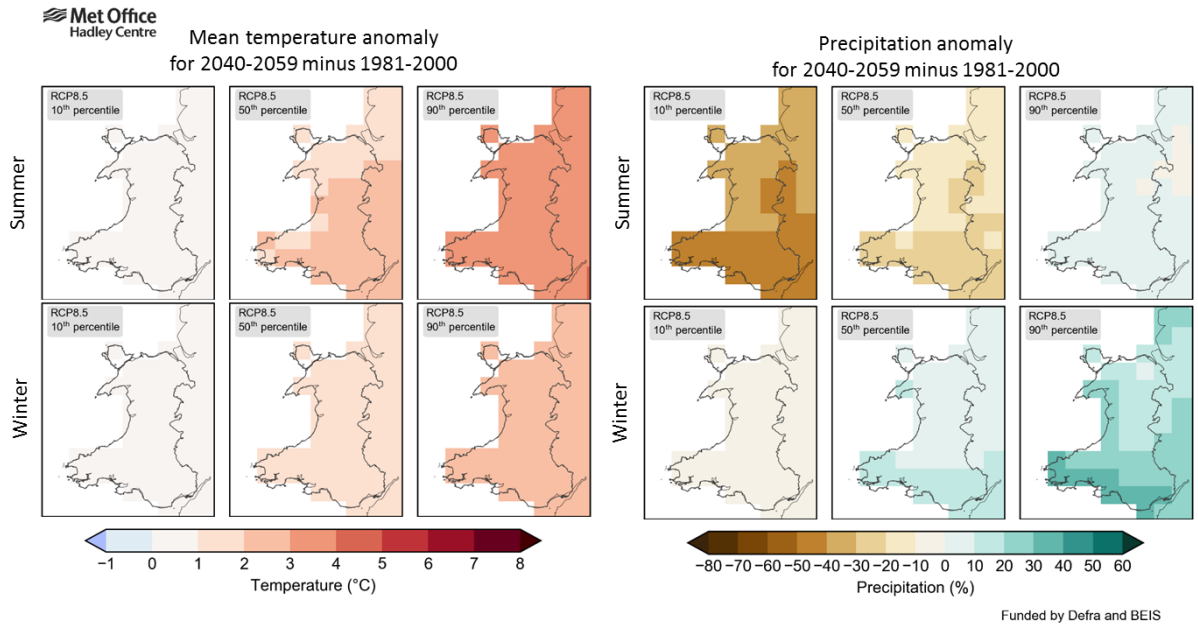


Figure 1.4: Projected future change in summer (top) and winter (bottom) temperature (left) and precipitation (right) for Wales under RCP8.5, the high emissions scenario, for 2040-2059 compared to 1981-2000. Note the increasing temperature for both summer and winter, and the drier summers and wetter winters. Source: UKCP18 website, Met Office © Crown Copyright.

Within the UK, agricultural and environmental policy is devolved to the Welsh and Scottish Governments, with England and Northern Ireland both having their agricultural and environmental policies determined by the UK Government. These separate administrations have different policies and priorities, with overall requirements under the EU Common Agricultural Policy. Uniquely within the UK Welsh Government has recognised the importance of soil by adding soil carbon to their national indicators of well-being alongside social and economic indicators (Wellbeing of Future Generations Act (Wales) 2015). As the political landscape changes within the UK due to Brexit the agri-environment schemes within Wales and the other countries of the UK will change. Scoping of the post-Brexit agricultural policy options is being undertaken by Welsh Government with open consultations (Welsh Government, 2019b). Based upon the Welsh Government's commitment to sustainability within the Wellbeing of Future Generations Act the new agri-environment scheme will be based around the principles of sustainability and

sustainable land management. The details of this scheme are still far from decided but it is clear the transition from current agri-environment schemes to the new scheme will take many years.

In order to improve the Welsh environment and the countryside Welsh Government launched the Glastir agri-environment scheme in 2012 (Rose, 2011). Glastir replaced four existing agri-environment schemes in Wales - Tir Cynnal, Tir Gofal, Tir Mynydd, and the Organic Farming Scheme – as well as the Better Woodland for Wales scheme. Initially, the scheme was composed of five components: the All Wales element, Targeted element, Common Land element, ACRES (the Agricultural Carbon Reduction and Efficiency Scheme), and the stand-alone Woodland Creation scheme. The All Wales element was open to all farmers across Wales and involved farmers choosing from a range of options that promote environmental health. The Targeted element was spatially targeted to certain areas of Wales that were identified as being of concern relating to factors such as soil carbon, water quality, biodiversity, historic environment and land access. The Common Land element was available to farmers who hold rights to common land and where the Commoners' Association agreed to all graziers removing their stock during a winter closed period and managing sward height throughout the year. The ACRES and Woodland Creation scheme was open to farmers who invested in energy efficiency saving equipment and planting woodland respectively. To evaluate the impact of Glastir the Glastir Monitoring and Evaluation Programme (GMEP) was initiated (Emmett et al., 2014). GMEP combined monitoring of the Welsh countryside over a four-year field survey with modelling approaches in order to quantify the condition of the Welsh environment and the impacts of Glastir (Emmett et al., 2017).

The GMEP field survey followed a similar design to the Countryside Survey, with 300 1 km squares surveyed once each across the four years with a variety of measurements including plant surveys, freshwater measurements, pollinator transects, identification of historical features, habitat mapping, and soil sampling. Unlike the Countryside Survey, the square selection in GMEP was done under two separate criteria: (1) the Wider Wales Component which surveyed land classes across Wales in proportion to

their extent; and (2) the Targeted Component which preferentially included areas of Wales that were of special interest to Welsh agri-environment policy.

Evaluating the impact of Glastir requires consideration of the factors influencing scheme uptake and implementation. Glastir does not constitute a randomised controlled trial, therefore as discussed above in section 1.2.2 in order to evaluate the impact of Glastir interventions factors that causally influence both the uptake of Glastir and the environmental property of interest need to be considered and accounted for. Factors that are known to influence uptake of agri-environment schemes include: previous experience of agri-environment schemes (Franzén et al., 2016); the ease of integration of the scheme with the farmer's planned farm development and current resources (Ingram et al., 2013; Karali et al., 2014); the mental health of the farmer (Hounsome et al., 2006); the belief framework held by the farmer (Johansson et al., 2013); and consistently the financial implications of the scheme (Franzén et al., 2016; Karali et al., 2014; Van Rensburg et al., 2009). Agri-environment schemes also often have different options and which the farmers choose to engage in could impact interpretation. Within the Glastir scheme a small number of environmental interventions were disproportionately selected by farmers and access to some types of payments was dependent upon previous engagement with agri-environment schemes (Arnott et al., 2019). Many of these above factors can be assumed to have no impact upon the environmental property of interest, however others such as previous experience of agri-environment schemes may need to be accounted for.

The structure of Glastir, with a targeted component involving certain areas being included due to their environmental characteristics, makes it more likely that there will be potential confounders upon the intervention – response relationship. Future repetition of the field survey within the Environment & Rural Affairs Monitoring and Modelling Programme (ERAMMP) aims to allow more confident attribution of the impacts of Glastir by examining trends over time. While it may be difficult to judge the impact of Glastir based upon GMEP, the field survey offers opportunities to evaluate the current state of the Welsh landscape. The combination of co-located soil physicochemical, soil microbial, plant community and animal community data across

the variety of Welsh land use offers powerful opportunities to examine ecological processes.

1.4 Thesis aims and Objectives

This section details the aims and objectives of this thesis, followed by a brief description of the relevant chapters and experimental work referring to each objective. A list of the experimental chapter titles is presented in section 1.5. Individual hypotheses and objectives are described in the each of the prepared manuscripts.

1.4.1 Thesis aims

The overall aims of this thesis are to explore the linkages between soil structure and microbial communities across a range of Welsh soils.

1.4.2 Objective 1

> *To evaluate the state of soils in Wales in regard to their physicochemical properties and biological communities.*

In Chapter 2 the results from the soil physicochemical properties from GMEP are presented with focus on the range of soil carbon, pH and nitrogen across Welsh habitats. The soil textural characteristics across GMEP are presented initially in Chapter 2, with extension in Chapter 3 with the application of the multifractal concept for soil textural heterogeneity characterisation. Chapter 4 presents the range of soil water repellency. The soil microbial community of GMEP is characterised in Appendix A.

1.4.3 Objective 2

> *To establish the relative roles of different physicochemical and biological factors in determining soil biodiversity.*

The influence of soil heterogeneity upon soil microbial diversity is presented in Chapter 3. The relationships between aboveground and belowground diversity, and evaluation of multitrophic diversity, is presented in Chapter 5.

1.4.4 Objective 3

To explore the associations between soil physical properties and biological communities across Wales.

In Chapter 3, the relationship between soil textural characteristics and soil microbial diversity within GMEP is analysed. The impact of soil microbial and plant community properties upon soil water repellency is analysed and presented in Chapter 4.

1.4.5 Objective 4

To evaluate the impacts of climate change on soil microbial communities and the potential knock-on effects on soil functions.

The impact of climate change on the soil microbial community is evaluated within a long term warming and drought experiment on Welsh heathland in Chapter 6. The impact of these changes upon function are explored in Appendix B where the changes in respiration over the course of the above experiment are explored. The impact of drought on soil function at the Welsh landscape level is explored within the analysis of drivers of soil water repellency in Chapter 4.

1.5 Experimental chapter information

The experimental chapters of the current thesis have been prepared in the style of journal article manuscripts. The title page of each experimental chapter includes details of the authors, author contributions to the manuscript and the current progress of each manuscript (e.g. published / accepted / submitted / not yet submitted). The thesis consists of five experimental chapters, located in Chapters 2-6 of the current document. For continuity and clarity, the experimental chapters will be referred to as they appear in this thesis. The titles of the experimental chapters are as follows:

Chapter 2: Identifying soil functional classes from survey data at a national scale

Chapter 3: Soil textural heterogeneity impacts bacterial but not fungal diversity

Chapter 4: Plant and soil communities are associated with the response of soil water repellency to environmental stress

Chapter 5: A diversity of diversities: evaluating the relationships between below and aboveground biodiversity

Chapter 6: Bacterial and fungal communities respond differently to realistic climate change manipulations over time

Chapter 7: Synthesis and discussion

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CHAPTER 2

Soil health cluster analysis based on national monitoring of soil indicators

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Contribution statement:

Study concept and design: Emmett, Henrys, Robinson, Seaton. Field survey implementation: Williams, Burden, Garbutt, Maskell, Keith, Fitos, Smart. Laboratory analyses: Barrett, Pereira, Keenan, Lebron. Analysis and interpretation of data: Seaton, Reinsch, Smart, Jones, Robinson, Creer. Drafting of the manuscript: Seaton. Critical revision of the manuscript for important intellectual content: Robinson, Reinsch, Jones, Barrett, Smart, Griffiths, Creer. Statistical analysis: Seaton. Obtained funding: Emmett. Study supervision: Robinson, Emmett.

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Abstract

A major challenge in soil science is to monitor and understand the state and change of soils at a national scale, to inform decision making and policy. To address this, there is a need to identify key parameters for soil health and function, and determine how they relate to other parameters and traditional soil surveys. Here we present a national scale data set of topsoil sampled as part of a wider agri-environment monitoring scheme in Wales, UK. Over 1350 topsoils (0-15 cm) were sampled across a very wide range of habitats and a range of physical, chemical, and biological soil quality indicators measured. We show consistent differences in soil physicochemical properties across habitat types, with carbon decreasing and pH increasing across the habitat productivity gradient from bogs through woodlands and grasslands to arable systems. The soils within our dataset are largely within the limits identified as important for supporting habitat function, with the exception of excessive plant available phosphate (Olsen P) levels in mesotrophic grassland. Cluster detection methods identified four soil functional classes based on measured topsoil properties, which were more related to habitat type than the genesis-based soil classification from soil maps. These soil functional classes can be interpreted as phenoforms within the soil genoforms found by traditional soil classification. This shows the importance of land use management in determining the soil health and functional capacity of soils. Our work provides an accounting of the current state of soil health in Wales, their relationship to soil function and a baseline for future monitoring to track changes against agri-environment and other policy targets.

2.1 Introduction

Soils underpin human existence through food, feed, fibre and timber production, as well as through earth system functions that support the delivery of other ecosystem services. Soil degradation affects 33% of all land globally according to the Intergovernmental Technical Panel on Soils (FAO & ITPS, 2015), and '52 % of the land used for agriculture is moderately or severely affected by soil degradation' as reported in Goal 15 of the U.N. sustainable development goals. In 2015, the first U.N. ITPS proposed four urgent actions to tackle and reverse degradation. The fourth was the development of robust soil monitoring systems to determine the current state and trend of soil health. Soil monitoring has become increasingly important in recent years, as nutrient loss, erosion, and land use change have implications not just for agriculture but for human activities as a whole. Land use change impacts heavily upon soil function (FAO & ITPS, 2015), making integrated surveys for both soils and land management particularly opportune to understand the impacts on land use and climate change. The measurements we report here provide both a baseline for the continuing monitoring of soil health and directly align with previous monitoring allowing greater power to detect anthropogenic impacts on soil health.

Traditionally, soil genesis and development studies have focused on processes occurring on the centennial to millennial time scales (Walker & Syers, 1976). However, there is an increasing recognition of the importance of sub-decadal changes in response to land use change, pollution and climate drivers (Varallyay, 1990). This in turn is leading to a greater recognition of the importance of soil change and determining the speed of this change (Richter Jr & Markewitz, 2001; Tugel et al., 2005) and perhaps more importantly its potential impact on earth system function (Amundson et al., 2015; Schmidt et al., 2011). This shift in thinking has led to a difficulty in integrating the traditional methods and results in soil science, emphasising soil development and classification, with more recent needs for measuring and interpreting change in soil function which recognises the more urgent need for evidence and action. In addition to traditional pedogenic-based classification (e.g. taxonomy), several approaches to bring together soil classification based upon genesis trajectories and results based on soil functional properties have been

proposed. These include the FAO topsoil classification (Broll et al., 2006), soil varieties within the Genetic Soil Classification of China (Shi et al., 2010), and the genoform – phenoform concept (Droogers & Bouma, 1997; Rossiter & Bouma, 2018). Under the latter, soil classifications are seen as genoforms, which are time-invariant at human timescales (e.g. climate, long term organisms or land cover, relief & parent material acting through time). Soils that are sufficiently different within a genoform to substantially affect soil function and be persistent over time are classed as phenoforms (e.g. managed properties known to be important in soil function such as pH, and organic carbon). Genoforms act as fundamental controls on soil phenoforms and their function that can develop. This enables linkages between soil maps and function to be clearly expressed.

Soil functions are inherent capabilities of the soil that include biomass and food production, maintaining soil biodiversity, carbon and nutrient sequestration, water filtration and transformation, landscape and heritage, and a source of raw materials (Blum, 2005). In order to track changes in soil functions, functional properties must be defined, which for monitoring at the national scale are required to be scalable to large areas and representative of functions across a variety of landscapes (Bünemann et al., 2018). This set of functional properties together represent a way to assess soil health. Here we define soil functional properties as those which can be managed in a habitat-specific manner and are associated with the above functions. Therefore, we include carbon, pH, bulk density, nitrogen, phosphorus and water repellency (Van Alphen & Stoorvogel, 2000). Soil carbon, pH, water content and bulk density are the most commonly proposed indicators for soil function due to their impacts on a wide range of soil functions (Bünemann et al., 2018). Bulk density, soil texture and associated water related properties have been considered to be key indicators for monitoring of physical soil health (Corstanje et al., 2017). Soil carbon and nitrogen are key determinants of various soil functions, including greenhouse gas emissions, biomass production and influencing biological communities but their exact impacts are often hard to evaluate (Gärdenäs et al., 2011). Other soil properties that have been found to be important in determining soil functions may be system-dependent; for example, electrical conductivity and salinity are highly influential on soil functions

when at high levels more common in arid or intense arable systems but are less important in other soil systems.

Wales, the location of our study, has recognised the role of soil in supporting wider ecosystem functions by inclusion of soil carbon as a key sustainability indicator within domestic legislation (Well-being of Future Generations (Wales) Act 2015). As awareness of the role of soils in supporting key ecosystem functions has increased, programs to monitor and promote soil management have been put in place in various countries (e.g. Orgiazzi *et al.*, 2018), and in Wales this is integrated within the Glastir land management scheme (Rose, 2011). In order to achieve these aims, current soils data is required to monitor changes in soil functions in response to wider ecosystem change and their downstream effects. Data on soil properties that underlie health and function needs to be collected using methods which are transferrable across the range of soils within Wales but also the UK, Europe and globally, so comparisons can be made at large scales (Ribeiro et al., 2015). Frequency of data collection needs to be sufficient to detect changes within a politically relevant time period to allow for adaptive change of current policies as well as slower changes. The Glastir Monitoring and Evaluation Programme (GMEP) scheme meets these criteria in that it collects data on soil as part of an integrated monitoring programme covering vegetation, soil and water properties using a robust soil sampling methodology which has been used successfully across the variety of soils in Wales (Emmett et al., 2014). GMEP uses a methodology used in previous surveys in 1978, 1998 and 2007 to also allow for links to historical datasets. The GMEP soil measurements seek to address the need for data to understand soil state and change at a national scale to inform policy.

Two approaches are commonly used to monitor long-term changes in soil properties: (i) localised monitoring of change in response to modifications of soil treatment often in the form of field-scale manipulation experiments (Jenkinson & Rayner, 1977), and (ii) large-scale "soil quality" surveys designed to inform land use and policy (Tóth et al., 2013). Our approach is unique and differs from these in that national soil change, and change in areas subject to management interventions, are both measured through the same survey design. This enables the evaluation of land management interventions for policy goals. The survey design is based on a stratified random approach developed

for a GB-wide integrated monitoring programme – the UK CEH Countryside Survey (Carey et al., 2008); an example map of site selection is shown in Figure 2.1. The soil monitoring programme also includes measurements not reported here relating to factors not routinely measured in large-scale soil surveys, such as a holistic evaluation of soil biodiversity (George et al., 2019). In addition, the survey allows for direct comparison between soil properties and above-ground factors such as land use change and plant species composition as well as streamwater quality, due to the soil and above-ground surveys being co-located and adjacent streams and ponds being sampled. Here we present results from the first iteration of this monitoring programme, a survey of topsoil (0-15 cm) health across Wales. We use this data to identify clusters of soils with similar topsoil properties and compare these classes with previously mapped soil groups. Our objectives are:

1. To present the topsoil results of a sub-decadal rolling agri-environment monitoring program by habitat type
2. To determine if pH, Olsen phosphorus (Olsen P) and bulk density values are within the nationally determined thresholds for habitat support
3. To evaluate the relationships between topsoil functional properties
4. To classify soils based on topsoil properties and compare these classes to land use and traditional soil classification methods

2.2 Methods

2.2.1 Field measurement programme

Topsoil measurements were conducted through a 4 year field survey of 300 1-km squares across Wales (Figure 2.1), half of which are focused on areas prioritised by the Glastir agri-environment scheme to determine the impact of land management interventions. The 1-km squares were selected at random from 26 land classes in proportion to their extent following the methodology of the UK CEH Countryside Survey (Carey et al., 2008; Reynolds et al., 2013), ensuring good coverage of the Welsh landscape. The initial survey took place over the summers of 2013 to 2016, and it is

these results we present here. Each year, ~75 squares were monitored with each square having 5 soil sampling sites, each randomly located within a segment of the square. The soil sampling locations are centrally located within a 200 m^2 square quadrat that has a corresponding vegetation survey and habitat assigned by the surveyors according to the UK Biodiversity Action Plan broad habitat classification (Jackson, 2000). The soil cores for physicochemical analysis were taken with a corer of 5 cm diameter down to 15 cm depth after removal of vegetation and any loose litter. The major soil group for each site was taken from the UK National Soil Map of England and Wales (Proctor et al., 1998).

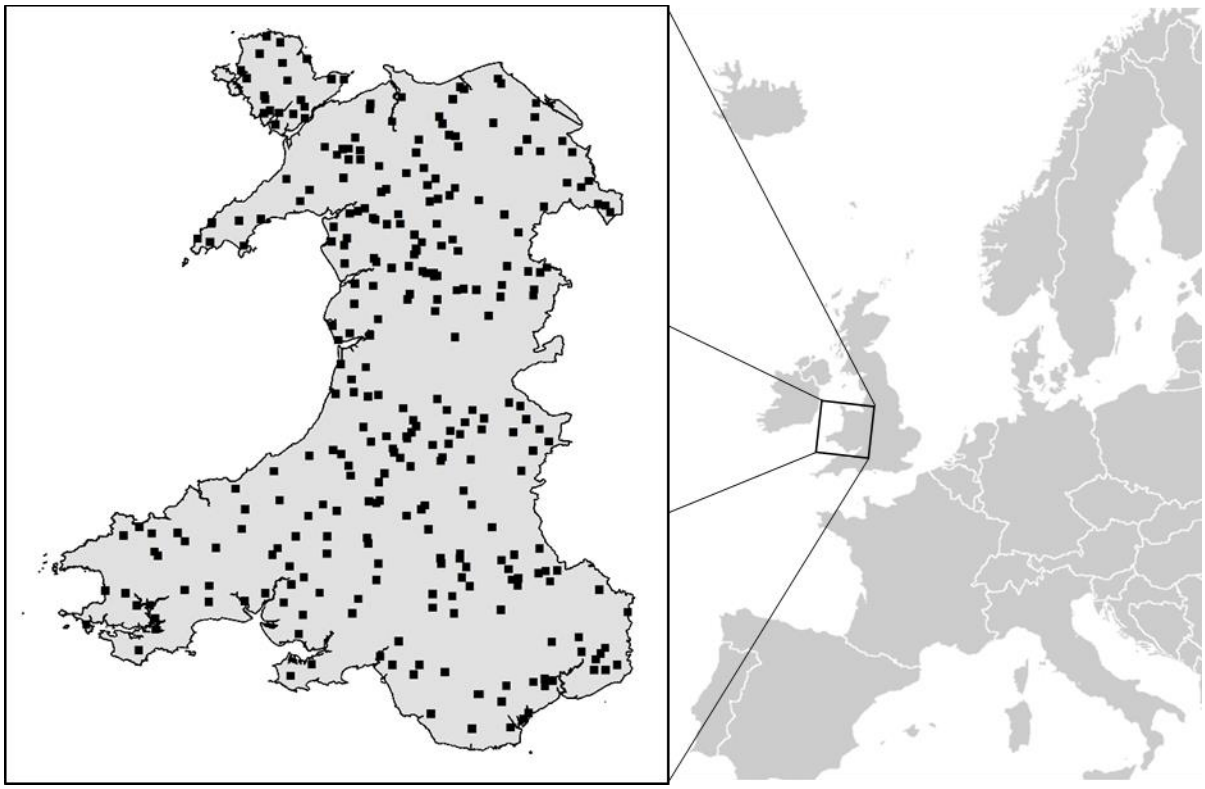

Figure 2.1: Map of Wales and the locations of the 300 individual survey squares. Locations are randomly shifted to any point on land within 10 km of the original location to ensure data confidentiality.

Sites were selected by a random stratified sampling method, with half the squares being selected to provide a representative sample of the major land classes in Wales whilst the remaining half weighted towards habitats of particular interest for farmer payments within the Glastir scheme. For the latter, each 1 km square across Wales had probability of being selected proportional to the score assigned to it under the Glastir

Advanced scheme by the Welsh Government. Models were used to estimate expected future Glastir scheme outcomes so that adjustments can be made to match Welsh Government priorities (climate change mitigation and water resources in years one & two), and scheme impact can be maximised. The national monitoring program in Wales has evolved from the Countryside Survey soil sampling approach and methodology (Emmett et al., 2008). In total there are: 20 supra-littoral sediment sites; 39 arable sites; 388 improved grassland sites; 300 neutral grassland sites; 205 acid grassland sites; 79 broadleaf sites; 84 conifer sites; 86 heathland sites; 41 bracken sites; 53 fen and other sites; and 92 bog sites. Improved grassland is composed of fast-growing grasses typically managed as pasture or for silage production with the addition of fertiliser and/or lime. Neutral grasslands are usually found on soils with pH 4.5 to 6.5 and lack plants with strong preference for base-rich or acid soils. Acid grassland is characterised by plants with strong preference for acidic soils. Of the 1387 sites, 1353 had complete measurements for pH, carbon, nitrogen, total phosphorus, and bulk density.

2.2.2 Laboratory methods

The analysis of soil variables was performed using the methods employed in the Countryside Survey (Emmett et al., 2008). In addition, soil surface water repellency was measured using the water drop penetration time method as described in Seaton et al. (2019). Details of the methodology are presented within the supporting information, and the full dataset is available from the Environmental Information Data Centre (EIDC) (Appendix I, Robinson et al., 2019).

2.2.3 Statistics

The differences in soil physicochemical properties by habitat were examined by providing summary statistics by habitat, counts of number of sites outside nationally determined threshold levels per habitat type and plotting using the ggplot2 and egg packages (Auguie, 2019; Wickham, 2016). The relationships between the different soil properties were examined using Spearman rank correlations. Classification of the soils was undertaken using cluster analysis upon the soil properties considered to affect soil functions, including pH, bulk density, carbon concentration, water content, soil surface water repellency, and total nitrogen. Soil properties such as total phosphorus,

Olsen P and electrical conductivity are considered to affect soil function but had such low variation in our dataset that they were not included in this analysis. Soil water repellency was $\log_{10}$-transformed before inclusion. The clusters were fit using hierarchical clustering with Ward's criterion and four clusters were selected as the most appropriate divide based on the hierarchical tree (Murtagh & Legendre, 2011). The correlation of the clusters with the habitat groups was calculated using the χ^2 test and the strength of the correlation presented using Crámer's V statistic. All statistical analyses and graphing was performed in R version 3.6.1 (R Core Team, 2019).

2.3 Results

2.3.1 Soil properties across habitat types

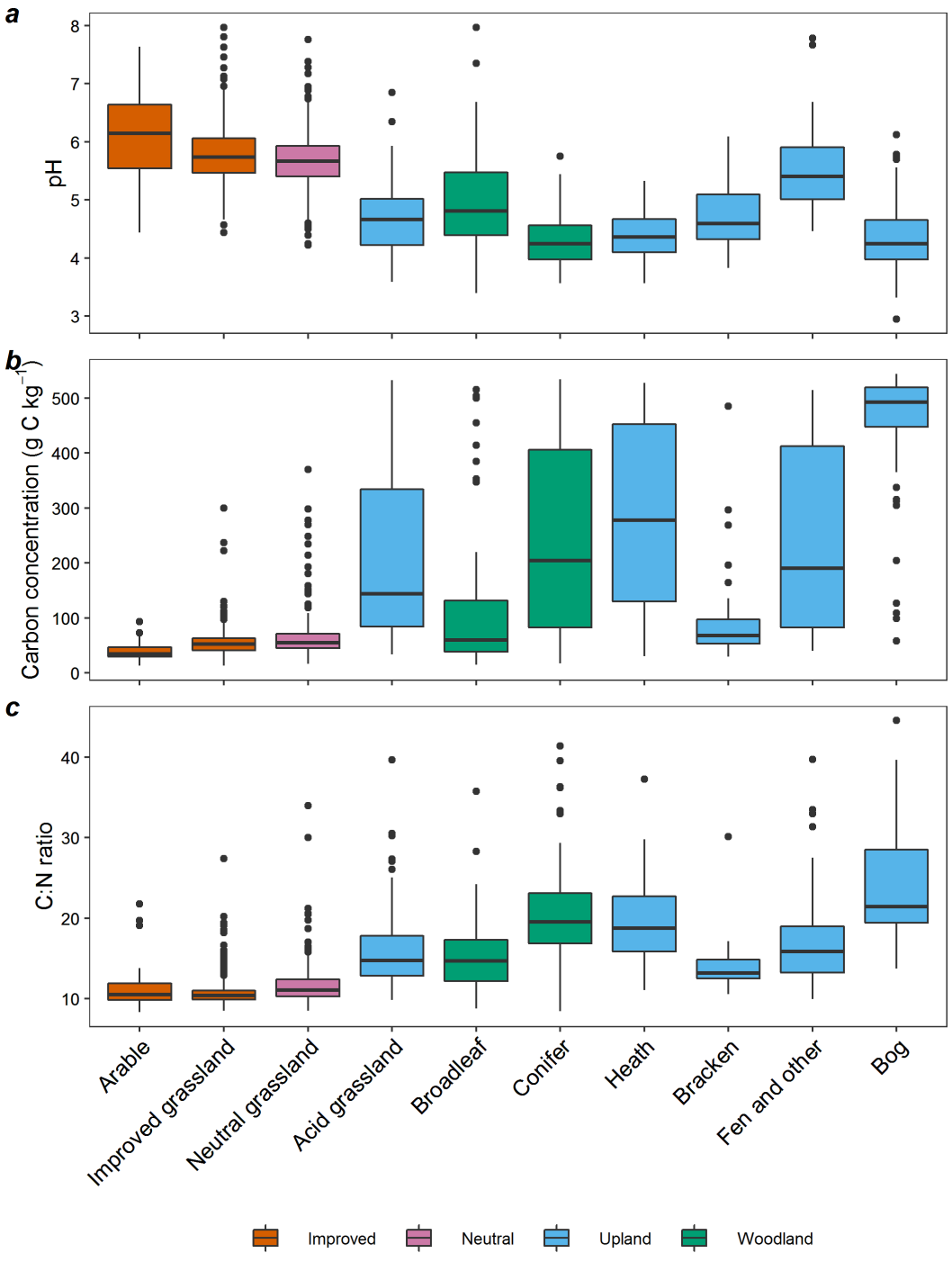

Figure 2.2. Differences in soil pH (a), soil carbon concentration (b) and C:N ratio (c) across the range of habitats found in our study across Wales. Habitats are coloured by which aggregated habitat group they belong to and arranged in decreasing plant productivity order. The line bisecting each box represents the median value, with the box extending to the first and third quartiles of the data. The whiskers extend to the furthest values no more than 1.5 times the inter-quartile range. Outliers are plotted individually.

Topsoil pH, carbon and nitrogen concentration vary across the different habitats found in Wales (Figure 2.2). Arable, improved grassland and neutral grassland tend to have the highest pH, lowest organic carbon concentration and lowest carbon to nitrogen (C:N) ratio of the habitat types. The majority of all other habitat types have acidic soils (Figure 2.2A), with fen habitats having a slightly higher pH than the other habitats. Bog is an important carbon store, with a median loss-on-ignition (LOI) carbon concentration of ~490 g carbon kg^{-1} (carbon stock ~6 kg carbon m^{-2}), with acid grassland, coniferous woodland, heathland and fen having large ranges in carbon concentration and some sites having around 500 g carbon kg^{-1} (carbon stock >15 kg carbon m^{-2}) (Figure 2.2B). C:N ratios are generally high across the different habitats, particularly in the high carbon habitats (Figure 2.2). Topsoil total nitrogen follows a similar variation across habitats to soil carbon, although total phosphorus and Olsen P show limited variation with habitat (Appendix D Figure 1). Bulk density varies considerably across habitat types, being highest in arable followed by improved and neutral grasslands and lowest in bogs (Appendix D Figure 2). Rock volume of soil and electrical conductivity show limited variation across habitat types (Appendix D Figure 2).

The broad habitats identified by the surveyors were aggregated into four habitat groups: improved land, neutral land, upland, and woodland for ease of interpretation. The range of organic carbon concentration, pH, nitrogen, phosphorus and Olsen P for the habitat groups are presented in Table 2.1. We do not present results for Olsen P in upland or woodland sites due to its methodological unreliability within low pH soils (Emmett et al., 2010).

Table 2.1: Topsoil chemical properties: means ± SD, median, min and max, carbon concentration estimated from Loss-On-Ignition (LOI). Phosphorus measured as total phosphorus, Olsen-P results are only presented for improved land and neutral grassland.

HABITAT GROUPS	INDICATOR	UNIT	MEAN	MEDIAN	MIN	MAX
IMPROVED LAND N=419	LOI Carbon	g/kg dry soil	53.8 ± 25.1	51.3	13.2	300
	Carbon concentration	g/kg dry soil	50.1 ± 27.7	46.1	5.50	313
	pH	Unitless	5.83 ± 0.54	5.75	4.44	7.97
	Nitrogen	g/100 g dry soil	0.45 ± 0.18	0.45	0.02	1.77
	Phosphorus (total P)	g/kg dry soil	113.7 ± 50.9	112.1	9.6	398.2
	Olsen-P	g/kg dry soil	25.1 ± 17.4	19.6	2.22	104
NEUTRAL GRASSLAND N=300	LOI Carbon	g/kg dry soil	65.3 ± 41.7	55.2	16.6	370
	Carbon concentration	g/kg dry soil	61.8 ± 43.0	49.6	13.3	370
	pH	Unitless	5.69 ± 0.50	5.67	4.22	7.76
	Nitrogen	g/100 g dry soil	0.52 ± 0.28	0.47	0.11	2.31
	Phosphorus (total P)	g/kg dry soil	100.6 ± 49.7	96.1	16.7	397.0
	Olsen-P	g/kg dry soil	17.2 ± 15.1	12.1	1.11	105
UPLAND GRASS AND HEATHLAND N=467	LOI Carbon	g/kg dry soil	268 ± 181	219	29.6	544
	Carbon concentration	g/kg dry soil	262 ± 1780	217	24.9	545
	pH	Unitless	4.66 ± 0.64	4.58	2.95	7.78
	Nitrogen	g/100 g dry soil	1.35 ± 0.78	1.27	0.16	3.31
	Phosphorus (total P)	g/kg dry soil	100.0 ± 45.6	92.6	11.5	317.2
WOODLAND N=162	LOI Carbon	g/kg dry soil	179 ± 166	101	15.0	534
	Carbon concentration	g/kg dry soil	173 ± 163	95.0	10.0	530
	pH	Unitless	4.63 ± 0.77	4.46	3.40	7.97
	Nitrogen	g/100 g dry soil	0.86 ± 0.67	0.58	0.10	2.66
	Phosphorus (total P)	g/kg dry soil	80.0 ±42.6	72.6	3.0	237.1

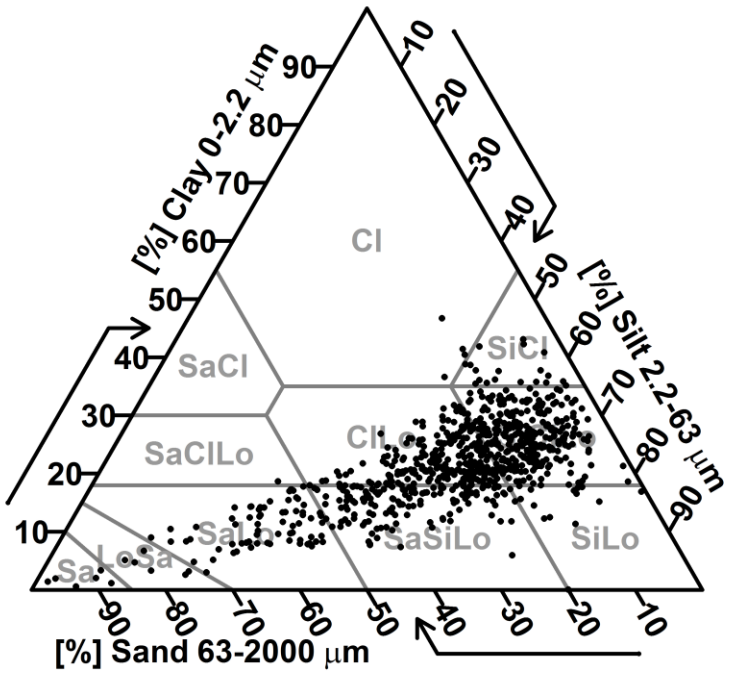

Figure 2.3: The clay, silt and sand percentages of a subset of the soils (n=781) plotted on a ternary diagram.

The soils were generally highest in silt-sized and sand-sized particles (Figure 2.3), with the majority being silty clay loam (n=284) or clay loam (n=232). There were also 145 sandy silty loams, 69 sandy loams, 19 silty clays, 15 silty loams, 6 loamy sand, 6 sands, 4 clays and 1 sandy clay loam. As the soil texture method involves organic matter removal prior to measurement it was only carried out on samples with lower loss-on-ignition (LOI < 50%), so the carbon-rich soils are not included in these statistics.

2.3.2 Thresholds

The majority of our sites are within the pH limits used as a national guideline for representing good support for the ecological habitat and biodiversity within specific habitat types (Bhogal et al., 2008). We compare this to our new analysis of the Countryside Survey topsoil data which compared the Welsh sites to the same thresholds (Appendix D Table 1, Reynolds et al., 2013). Within the sites with mesotrophic grassland plant communities, i.e. improved and neutral grasslands, there are only 6% of sites which are outside the recommended pH range of 5-7 (Table 2.2). Two thirds of these 39 sites are deemed too acidic. This is considerably fewer

mesotrophic grassland sites than have been identified as being too acidic in Wales in previous surveys such as the Countryside Survey (Appendix D Table 1). However, in sites with acid grassland plant communities 26% of our sites have pH above 5, which is considered to reduce their ability to support their distinct ecological communities (Bhogal et al., 2008). The previous Countryside Survey sites located in Wales found that the proportion of acid grasslands with pH above 5 increased over time from 1978 through 1998 to 2007 (Appendix D Table 1). Countryside Survey soils in 2007 showed that 24% of acid grasslands had pH above 5, which is comparable to our result. A negligible proportion of our sites had bulk density above the identified threshold, however the reliability of this threshold of bulk density as an indicator of soil status has yet to be fully tested due to a lack of data (Bhogal et al., 2008). Within mesotrophic grassland Olsen-P was higher than the threshold for habitat support in three quarters of the sites, which is similar to previous surveys (Table 2.2, Appendix D Table 1).

Table 2.2: Number of sites above the UK national guidelines set by the Environment Agency. For Olsen-P this is 10 mg/L for mesotrophic grassland, no results are presented for acid grassland and heathland. For pH this is <5 and >7 for mesotrophic grassland, >5 for acid grassland and heathland. For bulk density this is above 1.3 g/cm^3 for mesotrophic grassland and 1.0 - 1.3 g/cm^3 for acid grassland and heath.

Habitat	Olsen-P	pH	Bulk density
Mesotrophic grassland	**510 sites (75.3%)**	**39 sites (5.7%)**	**8 sites (1.2%)**
Acid grassland	**-**	**51 (25.6%)**	**3 (1.5%)**
Dwarf shrub heath	**-**	**4 (4.7%)**	**0 (0%)**

2.3.3 Relationships between soil variables

Soils across Wales show that soil organic carbon concentration, bulk density and total nitrogen are highly correlated with each other. The relationship between organic carbon concentration and bulk density follows the distinctive curved shape found in

previous studies of UK soils (Figure 2.4a) (Emmett et al., 2010; Howard et al., 1995). Total nitrogen follows a positive linear relationship with LOI carbon at low concentrations of carbon with a gradual levelling off and increasing variance at high carbon concentrations (R^2 = 0.87, Figure 2.4e). High LOI carbon content soils are found solely in conjunction with low pH (Figure 2.4c).

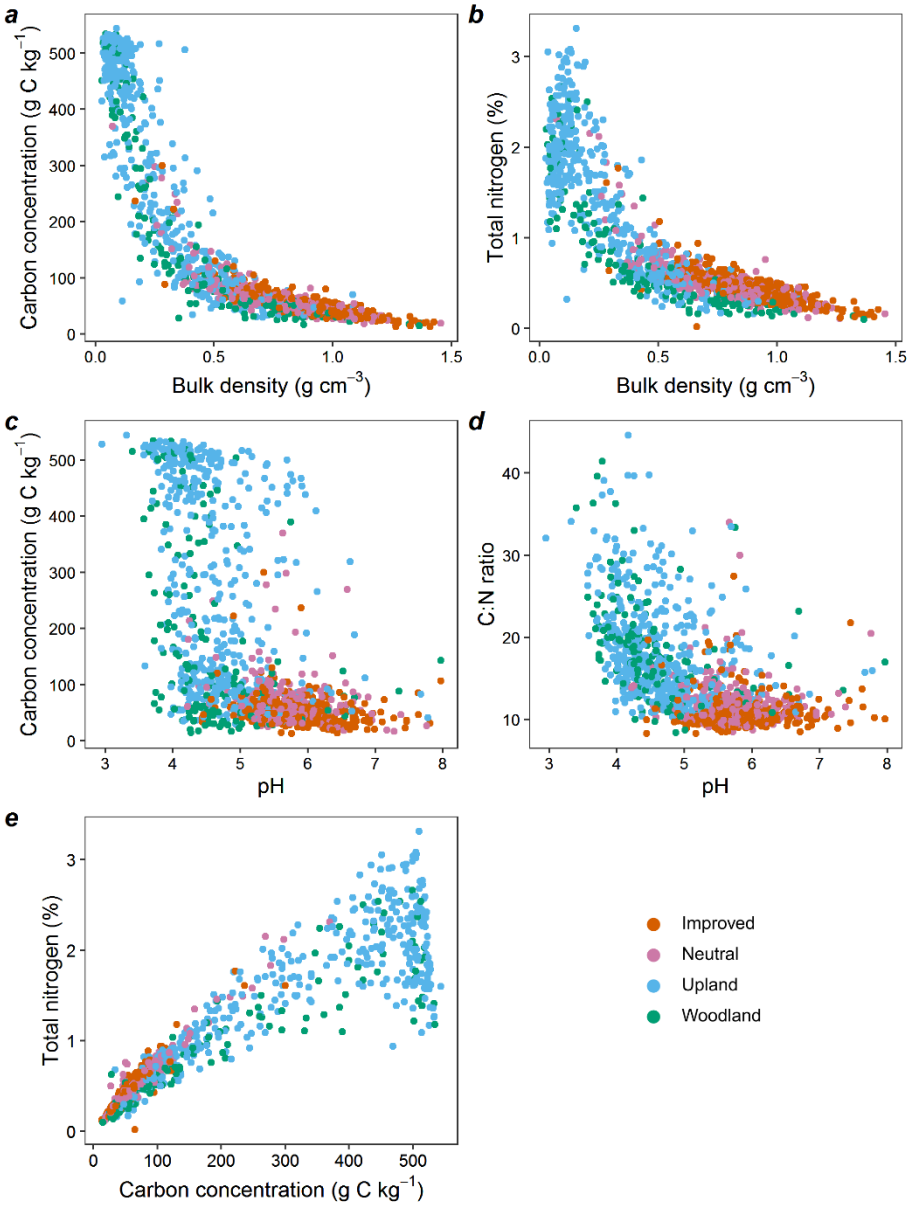

Figure 2.4. The major soil parameters plotted against each other and coloured by habitat group (n = 1367, 1363, 1367, 1362, 1364 for panels a to e respectively).

Plotting the spearman correlations as a network shows the strong inner cluster of inter-correlated total carbon, bulk density and nitrogen that change in tandem across our sites (correlations ~ ±0.9, Figure 2.5). Highly correlated with these three are pH, water content and soil water repellency. The rock content of the soil, electrical conductivity and total phosphorus are poorly correlated with the other soil parameters and situated on the edge of the diagram. For the exact correlation values see Appendix D Table 2.

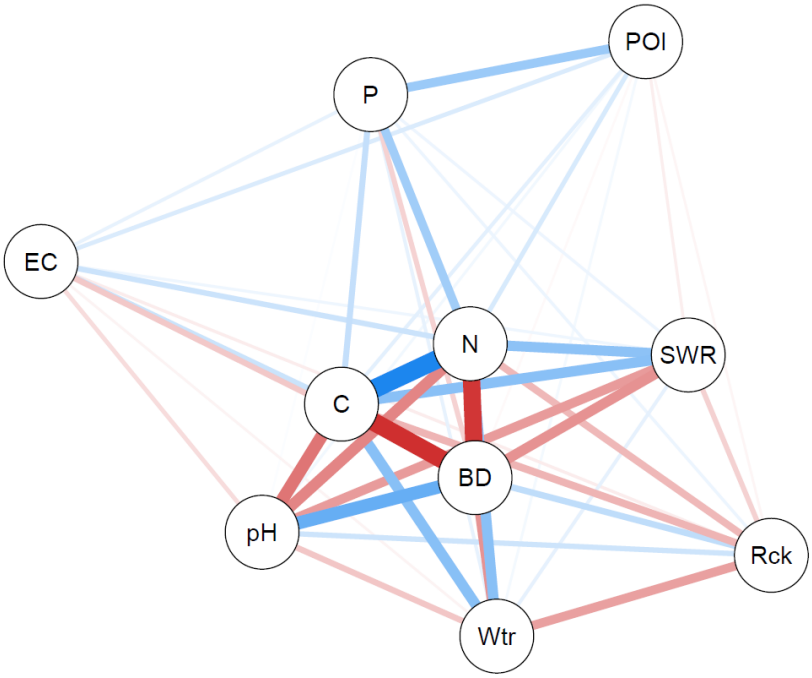

Figure 2.5. The Spearman's rank correlations between the variables plotted as a network. Each circle (node) is a variable, and the lines between circles represent the correlation between those two variables across the entire network. The width of the line is proportional to the strength of the correlation and the lines are coloured with blue for positive correlations and red for negative correlations. The layout of the network is selected by an algorithm that attempts to put strongly related variables closer together. The node labels correspond to BD = bulk density (log), C = total carbon concentration, EC = electrical conductivity (log), N = total nitrogen, P = total phosphorus, pH = pH, POI = Olsen-P, Rck = rock volume in soil, SWR = soil water repellency (log), Wtr = volumetric water content.

2.3.4 Alternative soil classifications

Using the key soil parameters identified in the previous section (total carbon concentration, total nitrogen, bulk density, pH, water content and water repellency) we placed our soils into different categories. The cluster dendrogram from the k-means method of clustering usefully organised the hierarchical patterns of similarity in the dataset. This allowed the most ecologically informative clusters to be identified, striking a compromise between too few with too much internal variance versus too many similar groups, resulting in four approximately equally sized categories (Figure 2.6). We plotted these soil categories against the properties used to create these clusters and named the categories as: organic; organo-mineral; acid mineral; and neutral mineral soils (Appendix D Figure 3, Appendix D Table 3).

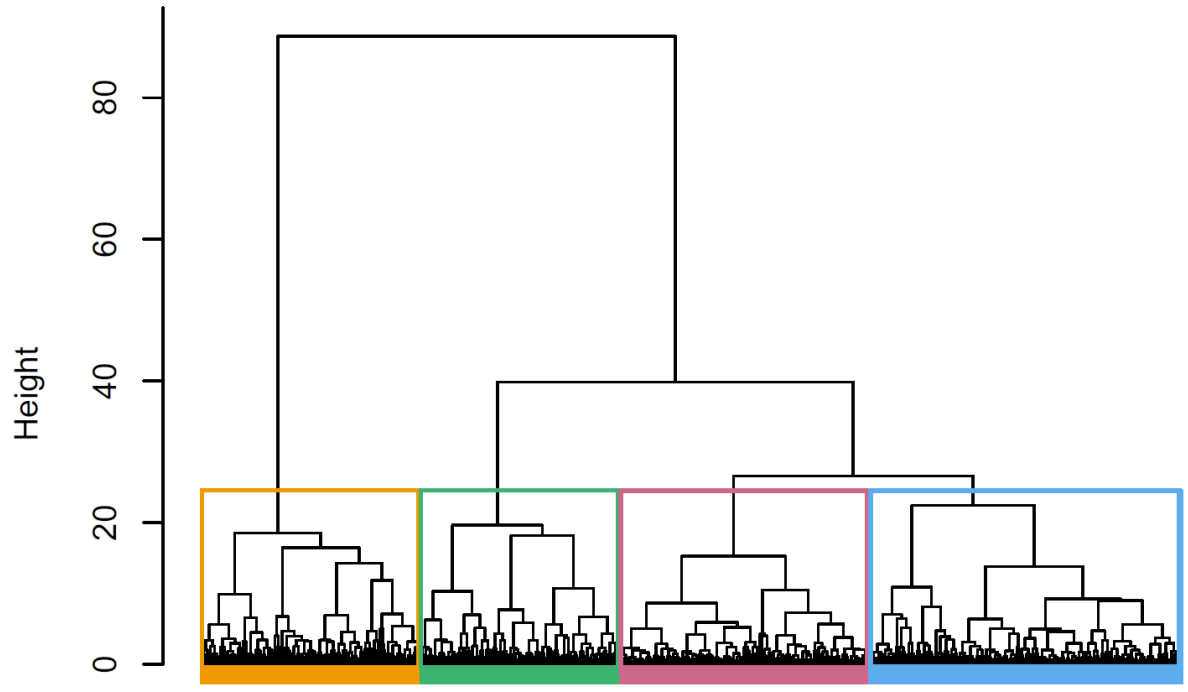

Figure 2.6. The results of the classification algorithm in dendrogram form. The tree diagram is truncated to remove individual data points. The groups identified by visual inspection are surrounded by coloured boxes, with the colours corresponding to the colours used in Figure 7. From left to right: group 1 (308 members, orange: improved grassland), group 2 (280 members, green: woodland), group 3 (350 members, pink: neutral grassland) and group 4 (437 members, blue: upland).

The classification of soils into our categories showed a stronger relationship with the aboveground habitat than the major soil group within the mapped classification by genesis did (Figure 2.7). Both soil classification systems were significantly associated with the aggregated habitat group (χ^2 test, $p < 0.001$), however the relationship between the soil topsoil properties classification and habitat was stronger than the relationship between the soil genesis classification and habitat (Crámer's V were 0.455 and 0.301 respectively). The results for broad habitat were also significant and showed the same pattern of strength. The topsoil properties classification strongly separated out the bog which was found only on the organic class and the arable which was found almost solely on neutral mineral soils. All other habitats showed a definite trend with the topsoil properties classification. Improved and neutral habitats were more likely to be associated with acid and neutral mineral soils, or brown soils in the case of the mapped soil classes. There are differences in the proportions of topsoil properties clusters per each mapped soil unit (χ^2 test, $p < 0.001$, Crámer's V = 0.356), but every mapped classification had at least one example of every topsoil functional cluster with limited differences in proportions across the three most numerous mapped soil classes (Appendix D Figure 4).

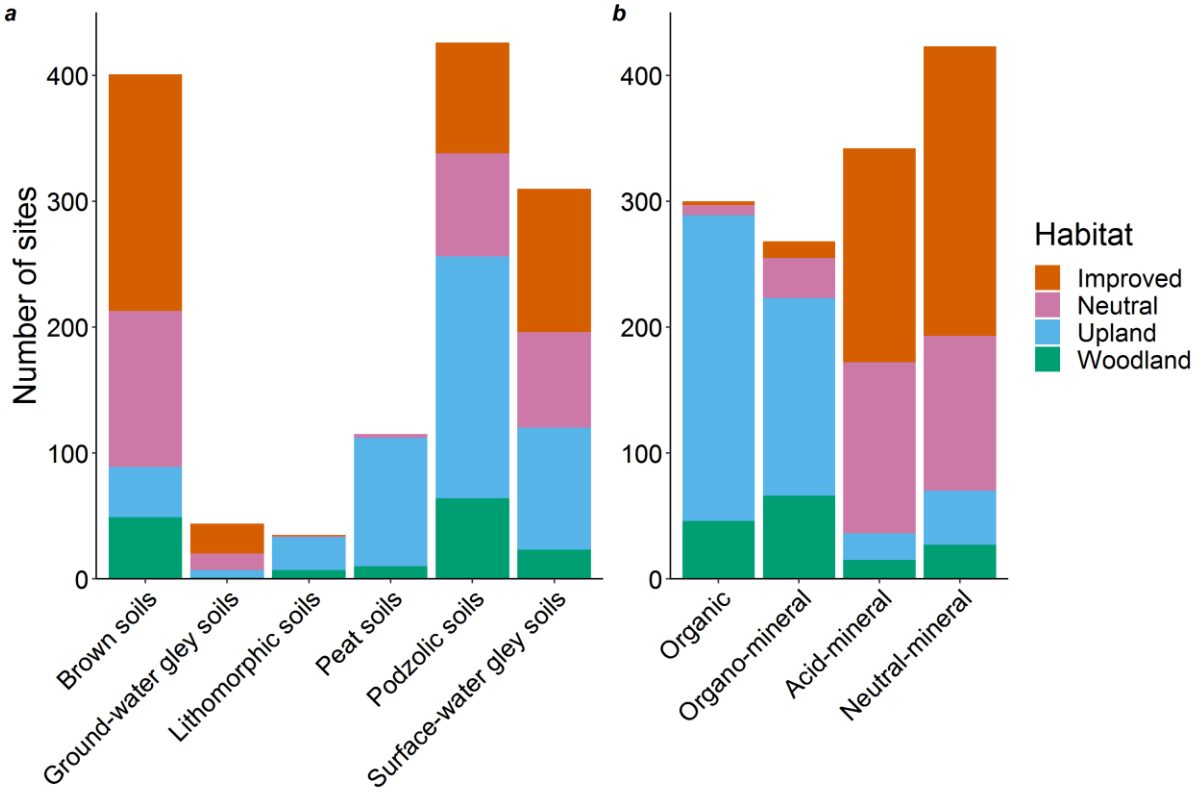

Figure 2.7: The different habitats distributed across the different soil classification types, classification by genesis (a) and topsoil properties (b).

2.4 Discussion

2.4.1 Key soil parameters status and correlations

The range and distribution of soil physicochemical properties found within this survey are in agreement with previous national scale surveys of UK soils (Baxter et al., 2006; Bellamy et al., 2005; Reynolds et al., 2013). The trend in carbon and pH with habitat showed that arable and improved grassland habitats had the lowest carbon concentration and acidity, with these increasing in the lower productivity habitats such as bogs and heathland. Wales contains many carbon-rich, low pH soils which are not always included in other surveys due to their focus on soils of high agricultural production (e.g. Baxter *et al.*, 2006). However the improved lands included within our survey had pH levels consistent with previous studies of agricultural lands within Wales (Baxter et al., 2006; Reynolds et al., 2013). Compared to the rest of Europe Welsh soils have on average higher carbon concentration and lower pH, this is also

true when only comparing them with the soils from the Atlantic climatic region (Tóth et al., 2013). This may be related to the general dominance of an acid geology, and the high precipitation, as globally there is lower pH in areas with greater precipitation which is thought to be linked to acidity and slower decomposition, thus enabling a build-up of soil organic matter (Slessarev et al., 2016). Our soil texture results also support evidence from the National Soil Survey that Wales is lacking in finer-grained, clay mineral, soils compared to the rest of the Atlantic region of Europe (Tóth et al., 2013). All of these properties will contribute to the generally low productivity of many Welsh soils and infrequent presence of arable farming systems.

The relationships between the different soil physicochemical variables are consistent with those found previously in the UK Countryside Survey, especially the strong correlation between carbon concentration and pH and other variables such as total nitrogen and bulk density (Reynolds et al., 2013). The distinctive curved negative association of carbon concentration with bulk density has been found by many studies across a variety of climatic zones (Emmett et al., 2010; Howard et al., 1995; Périé & Ouimet, 2011). There was limited correlation of total phosphorus, Olsen-P phosphate or electrical conductivity with the other variables in our dataset which suggests phosphorus supply is not primarily linked to organic matter formation but rather the composition of the soil parent material and potentially in some cases the external supply of phosphorus from fertilisers.

The comparison of our soils to nationally set thresholds enable us to increase our understanding of soil health across Wales in a domain-specific manner. Some thresholds are relatively well established, such as those for pH and Olsen-P which have been identified for different environmental interactions and habitat support (Bhogal et al., 2008). Other indicators have been proposed, such as bulk density, soil carbon and C:N ratio but often there is limited evidence and/or consistency across ecosystems in the impact of these (Bhogal et al., 2008). Bulk density, together with clay content, has been proposed as a soil quality indicator for British soils in relation to trends over time rather than passing a pre-identified threshold (Corstanje et al., 2017). We have limited sites at the higher levels of bulk density which makes it difficult to evaluate the threshold value and overall there was little correlation

between bulk density and soil biological indicators such as total mesofauna (George et al., 2017). Bulk density within our data strongly correlated with soil carbon, which may suggest that in these types of soil systems bulk density and carbon represent the same aspects of soil health. Instead of a single threshold, it has been suggested that evidence of decreasing soil carbon acts as a trigger value, in particular due to its relevance to carbon storage and biogeochemical cycling. The continuation of this survey in the coming years will allow clear identification of any habitats that may be losing carbon and thus should be targeted for land management interventions. Past surveys for Wales using the same methodology and some common locations in Countryside Survey did not identify any consistent trends in carbon concentration or density between 1978, 1998 and 2007 (Reynolds et al., 2013). Other survey and modelling approaches have suggested soils for the UK are on average losing carbon (Bellamy et al., 2005; Jenkinson et al., 1991; Jones et al., 2005), or are remaining stable (Reynolds et al., 2013; Smith et al., 2005). However, trends appear to be highly specific to land use types e.g. soil carbon loss in arable soils and but gains in woodland soils (Reynolds et al. 2013) and thus country-level trends perhaps mask important trends linked to specific management practices within land use type. It is important to note also that this survey does not measure changes in subsoil carbon, which is critical for carbon storage and likely to be less influenced by land use than topsoil carbon, limiting the inferences that can be made about the overall soil carbon stock and changes (Harrison et al., 2011; Simo et al., 2019).

We have found three quarters of our mesotrophic grassland sites with Olsen-P above the trigger value related to habitat support, which is similar to the 60 % and 90% of mesotrophic sites we found to be above the trigger value in the Countryside Survey in 2007 and 1998 respectively. Globally, phosphate decreases in grasslands have been predicted from model data (Sattari et al., 2016), and have been previously reported in Wales from Countryside Survey data (DeLuca et al., 2015; Reynolds et al., 2013). This is linked to the 60% reduction in use of P fertilisers in the UK from the 1980s to 2010 which has since stabilised (The British Survey of Fertiliser Practice, 2019), which could be expected to impact across the landscape even on unimproved land due to reduced transfer of phosphate by hydrological or atmospheric pathways. Reduced grazing

could be reducing the removal of phosphate by preferential grazing on phosphorus enriched areas and thus reducing its diversion away from grazing sites (Schütz et al., 2006; Statistics for Wales, 2016). High levels of phosphate have been linked to lower plant diversity (Critchley et al., 2002; Michalcová et al., 2011). Moreover, elevated phosphate levels can persist in the soil for long periods, and have lasting impact on the plant communities that can establish at a site (Horrocks et al., 2016).

The current proportion of sites that are outside the pH thresholds are comparable to the most recent surveys of the Welsh countryside by Countryside Survey in 2007, which is markedly less acidic than surveys within 1978 and 1998 (Reynolds et al., 2013). This can be interpreted as stabilisation rather than continued recovery from historic acidification due to atmospheric acid deposition. This does not necessarily mean that Welsh soils are fully recovered from acidification, as there are some indications from model data that recovery from acidification is not yet complete (RoTAP, 2012). The stalling of recovery from acidification could indicate that the soils have entered into a lower pH stable state (Suding et al., 2004), thus to enhance productivity some soils may require active remediation to return to pre-acidification pH. Similar results showing reductions in recovery from acidification are reported for woodland and other organic soils (Evans et al., 2008; Kirk et al., 2010; Reynolds et al., 2013), attributed to vegetation uptake of base cations, nitrogen deposition or capture of acidic pollutants by woodland canopy which offsets SO_2 reductions. This does however raise the issue as to whether the assumption that a pH of less than 5 is required for habitat support in acid grassland and dwarf shrub heath will hold, as the impacts of anthropogenic acidification are reduced and soil pH values increase across the UK. The shifting baseline in soil pH may be altering our perception of what constitutes a good pH value for an acid grassland (Soga & Gaston, 2018). The thresholds for pH were established based largely on data from UK grasslands in the 1990s (Bhogal et al., 2008), which would represent sites that are in the process of recovery from intense acidification and therefore may not actually be similar to a true natural state. The pH trigger values for supporting metal retention and microbial function are actually contradictory with that suggested for supporting acid grassland and heathland habitats (<5 and >5 respectively), and recent results indicate microbial

function may decrease below pH 5.5 rather than 5 exacerbating this difference (Jones et al., 2019). This shows the difficulties in designating appropriate boundaries when multiple functions and services are involved, especially when the different functions show differing responsiveness to change (Bhogal et al., 2008; Bünemann et al., 2018; Jarvis et al., 2019).

2.4.2 Soil classification

The soil physicochemical clusters identified in this work have strong similarities with previous analysis of UK soils. Analysis of the soils collected as part of the Countryside Survey of the UK in 2007 found that there were three main clusters of soil physicochemical properties corresponding to mineral soils, organo-mineral soils and organic soils (Simfukwe et al., 2010). In our data we split the mineral soils into two groups, however in other respects our classifications are similar. These results support the use of soil organic material in categorising soils, as evidenced by the use of carbon classifications within multiple classification systems (Broll et al., 2006; Emmett et al., 2010).

Our analysis has shown that as we hypothesised the traditional soil classification methods, such as that used within the UK soil classification (Avery, 1980), are weakly correlated with differences in habitat type and land use while those based on key topsoil manageable parameters are more strongly related. This is consistent with previous results showing that soil dissolved organic carbon is not well related to soil type in UK soils (Simfukwe et al., 2011). We have also found that there seems to be only limited relationship between our identified topsoil class and the traditional classification, with the exception of peat soils. This suggests that there may be limited constraints from the soil genesis type upon the functional nature of the topsoil, indicating the importance of management decisions in determining soil function. The functional capacity of the subsoil, however, may be more constrained by the soil genesis type than land cover. Therefore soil functions which are dominated by different soil horizons may have influenced more or less strongly by the plant community versus the soil genesis type. One key limitation of this analysis is that the

soil classification by genesis was taken from a map based largely on data collected from 1960-1970 and which fails to capture changes in soil management and land use that have occurred since then. In addition, our survey locations were classified at the soil association rather than series level. Soil surveying to classify soil genesis is time consuming and labour intensive, often making funding of large-scale soil surveys unattractive. In practice, many key survey and modelling results are either based on previous soil mapping efforts or topsoil sampling only, which is what we have compared here to land cover. Many ecosystem service maps use the soil classification maps, when actually soil function is more related to the plant communities and land cover type.

The soil properties we have presented here are a selection of properties that are known to influence soil function and are both manageable and measurable at a national level. The soil properties that are often measured in the scientific literature to represent function, e.g. carbon mineralisation rates (Simfukwe et al., 2011), are usually difficult to scale up to large areas due to factors such as expense and limited generality across landscapes (Sanchez et al., 2003). Many of these properties can also be only measured in a laboratory environment on highly processed soils which means that they can fail to capture the conditions as they really exist, particularly the influence of plants in regulating soil functioning (Carlyle et al., 1998; Oburger & Jones, 2009). Different functions can also respond differently to the soil properties considered here and even soil biodiversity can be represented by different aspects with different responses. For example, in our sites microbial diversity is highest in our habitats with high pH and low carbon (George et al., 2019) while mesofaunal abundance is highest in habitats with more intermediate pH and carbon (George et al., 2017). However, some properties such as soil carbon have been widely accepted to be indicators of soil function, influencing greenhouse gas emission, nutrient cycling, water filtration and biomass production among others (Amundson et al., 2015; Bünemann et al., 2018; Environment Audit Committee, 2016; Rossiter & Bouma, 2018). It is these parameters – pH, carbon, nitrogen, bulk density and water – that we have found to be pivotal in determining the topsoil property classes of our Welsh soils and therefore why we term these functional clusters.

The clusters we have found behave differently in their functional attributes reflecting their different land management regimes. The key functional attributes of soil vary depending upon their pedogenic characteristics and the overlying land use. We see the classes proposed by our analysis as a way of reducing complexity to enable comparison of like-for-like, and consequently, we do not apply the principles of functionality derived from lowland arable soils to upland peatlands. This comparison of appropriate classifications is particularly relevant for determining policy at the national scale, when balancing the need for provision of multiple functions across a heterogeneous landscape. There is no way to tell within our data whether differences in areas targeted for agri-environment interventions are due to the scheme or pre-existing conditions and thus, we have not evaluated that here. However, the dataset presented here offers an understanding of the current state of soil health in Wales that can be used as a baseline for future surveying to evaluate the response of soil health and function to land management interventions. The differences in soil health and function across habitats we have found show the importance of land management to soil function.

There have been objections to the principle of classifying soils into strictly defined categories since the advent of soil classification systems (Webster, 1968). In response, many authors have chosen to use fuzzy mathematical methods to classify soils (Burrough, 1989; Mazaheri et al., 1995; Stevenson et al., 2015). This can allow any given soil to belong to more than one class, potentially better capturing the range of soils between different classes than the artificially abrupt boundaries between classes in a hierarchical classification system. Soils generally exist on a continuum in trait space, exhibiting different characteristics across a variety of landscapes. They can also change over time and under different management practices; particularly those already at the edge of the categorisation boundaries. Results such as ours which find certain categories of soils based on their properties should be interpreted within this context. Whilst categorisation is a useful tool for informing management and monitoring, it cannot represent the full breadth and flexibility of soil types.

The clusters of soils we have identified can be aligned to the phenoform concept, where the phenoforms are the functional clusters which can be nested within the

genoforms, i.e. the mapped soil classes by genesis. However, we have found that the genoform poses no major constraint upon the types of phenoform that can develop there, which suggests the nested nature of the genoform-phenoform concept may be an unnecessary complication in practice at least with respect to topsoil. One issue with the comparison of our results to the genoform-phenoform concept is that the phenoform definition considers only soil properties that are persistent and require substantial management change to alter (Rossiter & Bouma, 2018). The properties often identified as being key to functional classification, such as carbon and pH, are experiencing ongoing change and are the target of key initiatives such as the 4 per mille initiative which aims to increase global soil organic matter stocks by 0.4% per year (Minasny et al., 2017). There is a conflict in the application of the phenoform concept that hinges on the identification of what constitutes "substantial" management. This conflict reaches its peak when considering changes over time. If we were now to find that the 4 per mille initiative was successful then this would constitute enough change to alter the phenoform of all of our soils. But if all change in tandem, as occurred with the recovery from acidification in UK soils (Reynolds et al., 2013), then new attempts to define phenoforms on the basis of cluster analysis of soil properties will not show these changes and find the same phenoforms again. It may be unlikely that different areas will respond in tandem to external changes due to differences in the application of these changes, the inherent differences in responsiveness of different habitats, and the non-linearities of change directions as indicated in the fundamental different direction of soil carbon losses within different land use types reported by Reynolds et al. (2013). However, the direction and magnitude of change within soils is a key constraint on the application of the phenoform concept that requires further investigation. The value of repeated soil monitoring in establishing any trends in health and presence of phenoforms cannot be overstated, as soil health is dynamic at management relevant timescales.

2.5 Conclusions

We present a national dataset which provides a baseline for the survey of Welsh topsoils (0-15 cm), allowing for the quantification of the current health of the soil and enabling future surveys to track trends in these conditions. We show that there are

consistent differences in soil properties across habitats. Few of our soils are outside established thresholds of pH and bulk density for ecosystem health, but high levels of phosphate in improved grasslands remain an issue. Several key soil properties, such as carbon, nitrogen and pH, are strongly correlated across our soils and can be used to create a classification of the soils. We propose that our conceptual classification of the topsoil is related to soil functionality, due to the known relationships between the key soil properties featured here and soil functions. Consequently, the functional classification of the topsoil developed in the present analysis is more related to land use type than soil classes based on traditional methods.

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CHAPTER 3

Soil textural heterogeneity impacts bacterial but not fungal diversity

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Contribution statement:

Study concept and design: Seaton, Robinson. Laboratory analyses: Lebron. Bioinformatic analysis: George. Analysis and interpretation of data: Seaton, Jones, Robinson, Creer. Drafting of the manuscript: Seaton. Critical revision of the manuscript for important intellectual content: Robinson, Jones, Creer, Smart. Statistical analysis: Seaton. Study supervision: Robinson.

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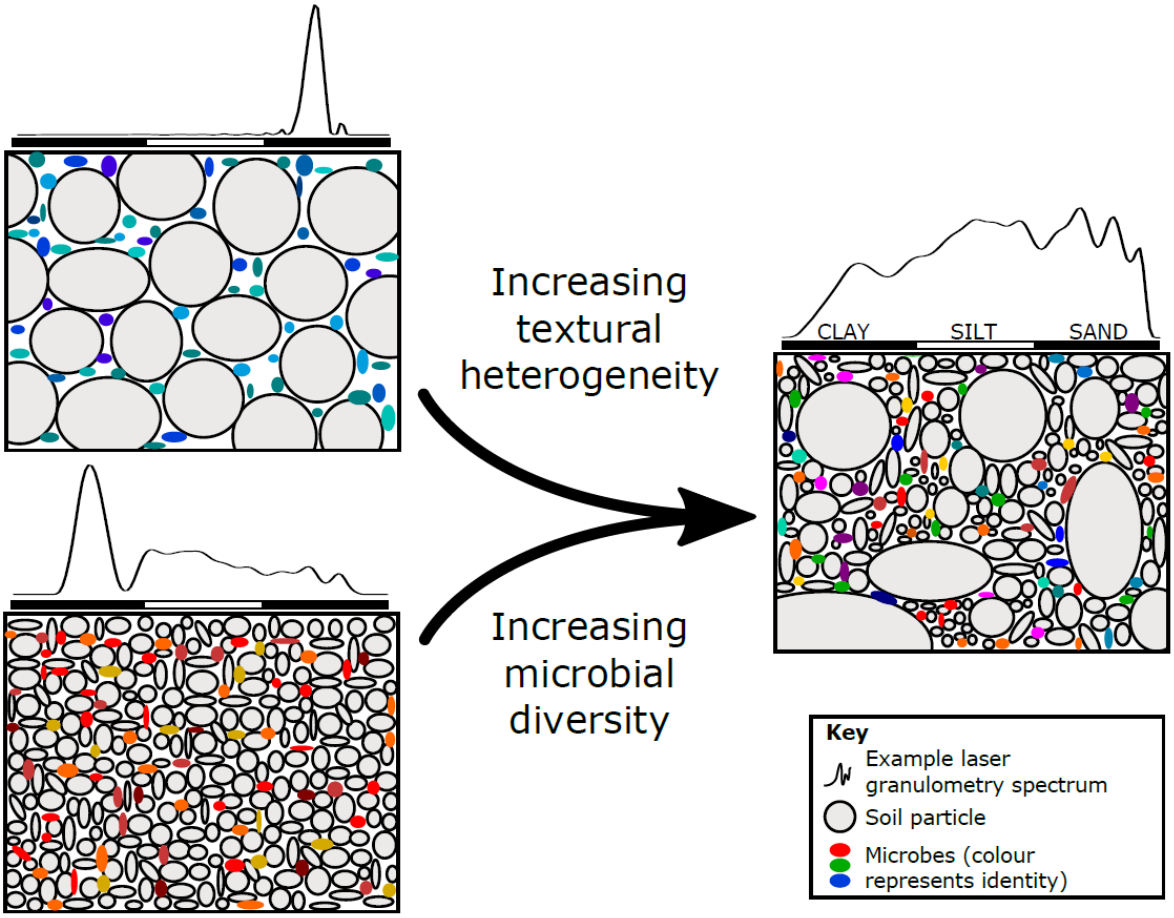
Increasing
textural
heterogeneity
Increasing
microbial
diversity
CLAY
SILT
SAND
Key
Example laser
granulometry spectrum
Soil particle
Microbes (colour
represents identity)

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 sequencing 16S and ITS1 taxonomy marker gene regions respectively using an Illumina MiSeq. Multifractal analysis of the soil particle size distribution was then used to describe the heterogeneity of the soil particles. 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.

3.1 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 billions of soil microbes within a gram of soil, yet the mechanisms leading to the establishment of such diversity are still poorly understood (Bardgett & 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 & 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 & Crawford, 2004).

Structural heterogeneity of the soil environment leads to increased physical niche space and spatial isolation which should increase microbial diversity (Wang & 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, 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. 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 & 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 & Or, 2017). 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. 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, 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.

3.2 Methods

3.2.1 Sample collection

Soil samples were collected as part of the Glastir Monitoring and Evaluation Programme from sites across Wales (Emmett & 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 (Figure 3.1). 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 carbon 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 carbon 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).

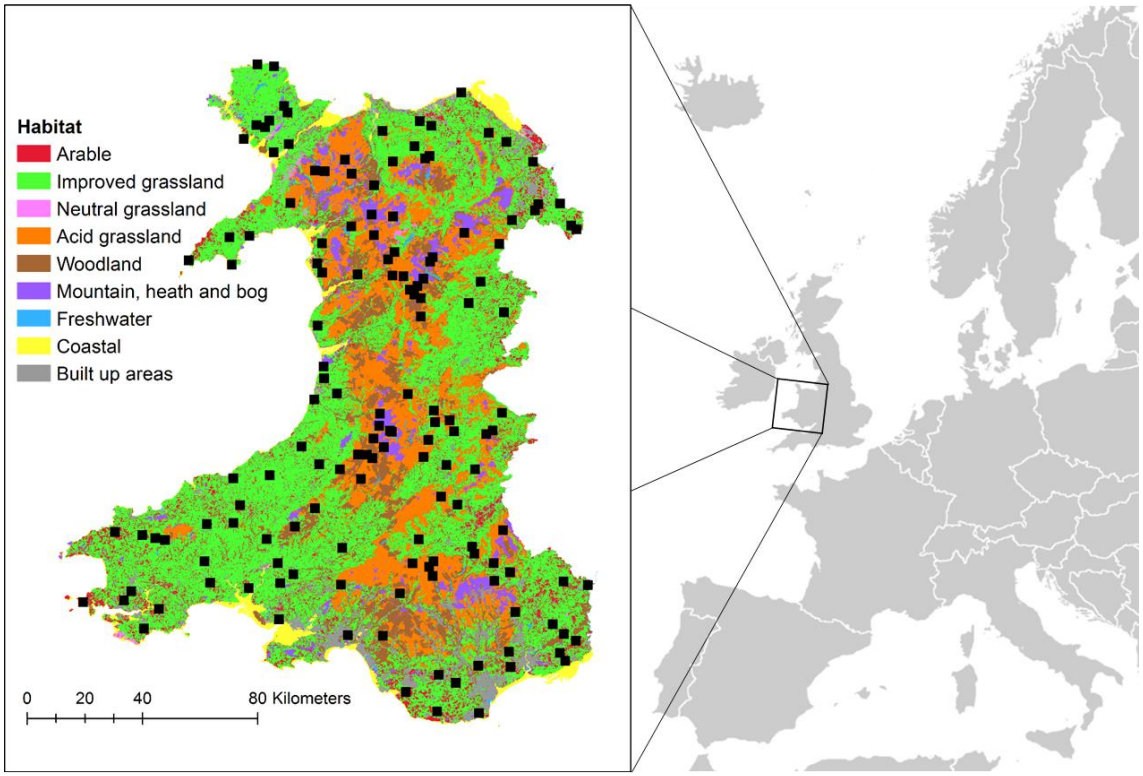

Figure 3.1. Map showing the location of the survey square locations used in this study.

3.2.2 Laser granulometry

Soil samples with less than 50% organic carbon 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 carbon following the method of Gee and Or (2002). Once the organic carbon 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 & 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.

3.2.3 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 & 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 & 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 3.1.

$$D_q = \frac{1}{q-1} \lim_{\varepsilon \to 0} \frac{\log \mu(q, \varepsilon)}{\log \varepsilon} \quad 3.1$$

Where ε is the size of the box and $\mu(q, \varepsilon)$ is defined according to equation 3.2.

$$\mu(q, \varepsilon) = \sum p_i^q \quad 3.2$$

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).

3.2.4 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 (prokaryotes) and ITS1 (fungi) regions were used to create triplicate amplicon libraries using a two-round PCR. 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 ITS data. For full details on the methodology used see George et al. (2019). 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 OTU richness which we use as our measure of alpha diversity.

3.2.5 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 soiltexture package in R (Moeys, 2015). Figure 3 was plotted using the ggplot2 package (Wickham, 2016). 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 ANOVAs, 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, limiting the network to only significant positive links (Csardi & 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. Links that were pre-identified as being potentially nonsignificant 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).

3.3 Results

3.3.1 Soil texture

Our samples showed considerable spread across soil texture categories (Figure 3.2), 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.

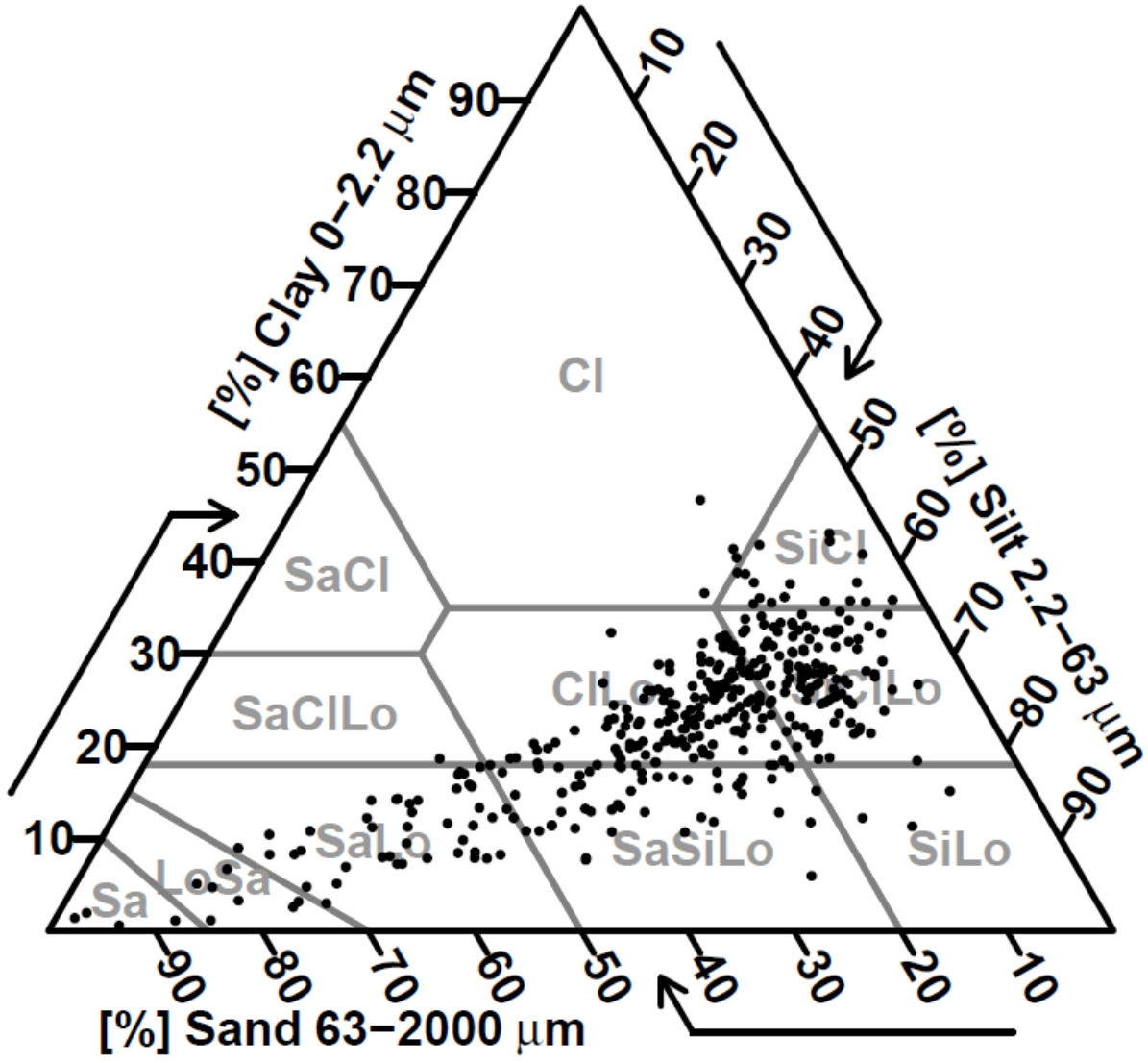

Figure 3.2. Sand, silt and clay percentages of our samples plotted on a ternary diagram to show the range of texture classes examined in this study. Sa, sand; Si, silt; Cl, clay; Lo, loam.

As expected, the amount of particles in a particular size category was strongly positively correlated with the amount of particles in adjoining categories (Figure 3.3). The very smallest size categories, of less than 0.1 µm, were strongly negatively correlated with larger clay sized particles (0.16 to 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).

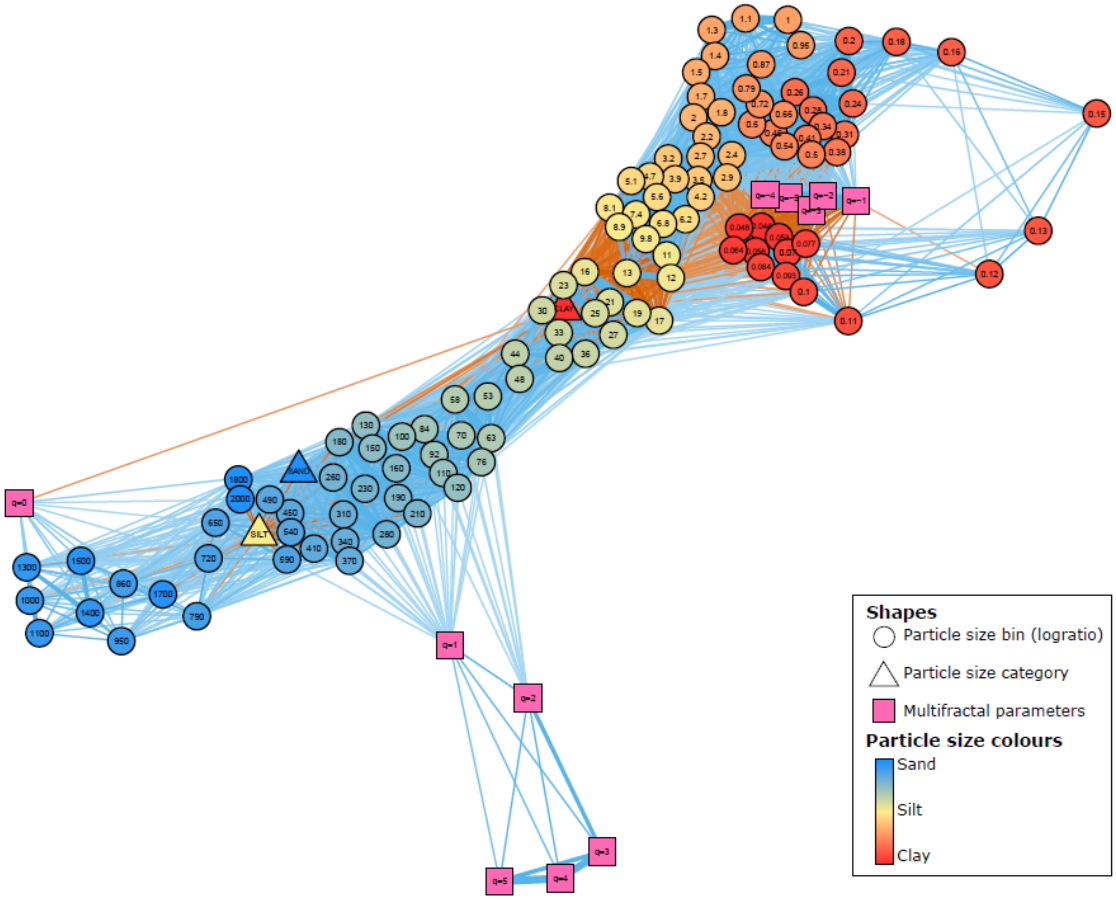

Figure 3.3. Correlation network of soil particle size bins and multifractal parameters. Each circle is a node that represents a variable measured, with lines between nodes representing the correlation between those variables. Nodes are coloured according to identity: with a colour gradient of red for 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.

3.3.2 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 (Appendix E Table 2).

The box counting dimension was positively correlated with sand content (Spearman's rank rho = 0.62; Figure 3.3). 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 Figure 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 $p < 0.01$. Appendix E Figure 2). 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 (Appendix E Figure 3).

3.3.3 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 richness and textural

multifractal parameters (Figure 3.4, Appendix E Figure 3). 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.

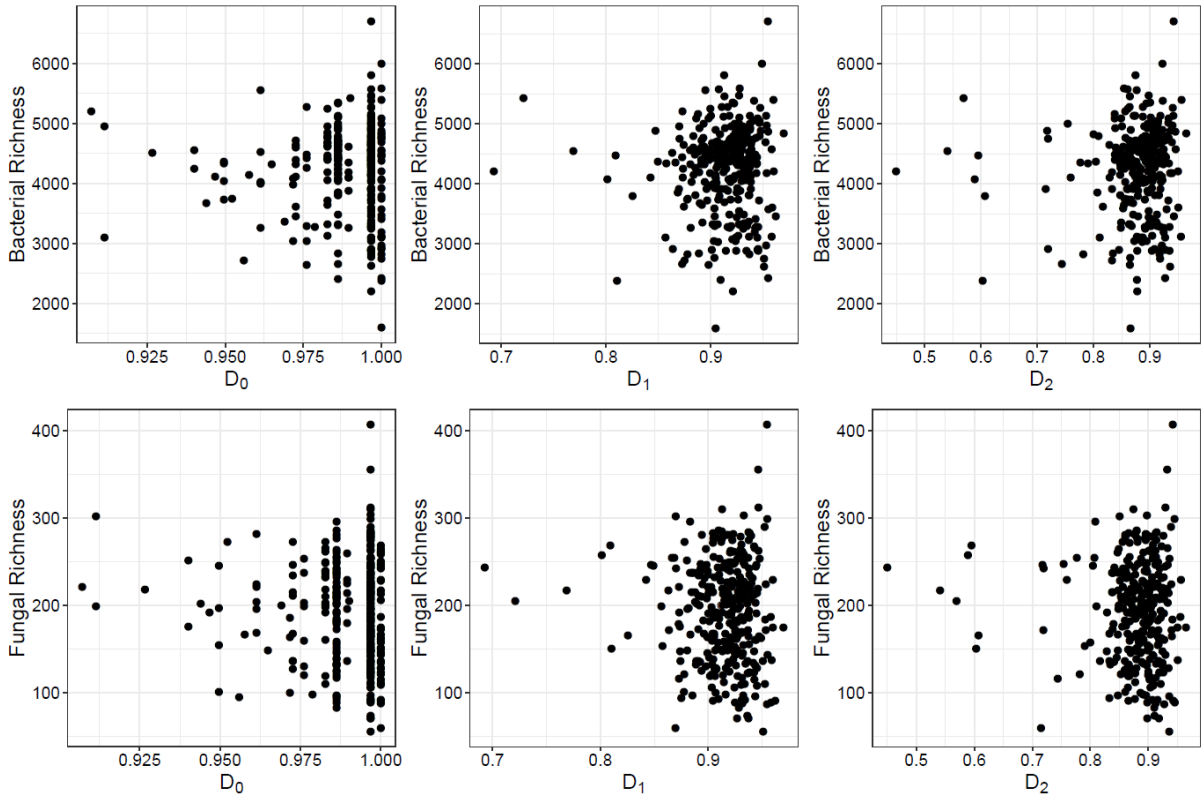

Figure 3.4. Change in bacterial and fungal richness with soil texture multifractal parameters (D_0, D_1 and D_2).

Structural equation modelling revealed a direct effect of textural heterogeneity upon bacterial diversity once changes in soil chemistry were accounted for (Figure 3.5). 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 carbon (full model output in Appendix E Table 2). Within structural equation modelling we can estimate the indirect effects of variables as well as the direct path coefficient (Appendix E Figure 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 (Appendix E Figure 4); consistent with the lack of significant correlation seen in Figure 3.4. 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 (Appendix E Figure 4).

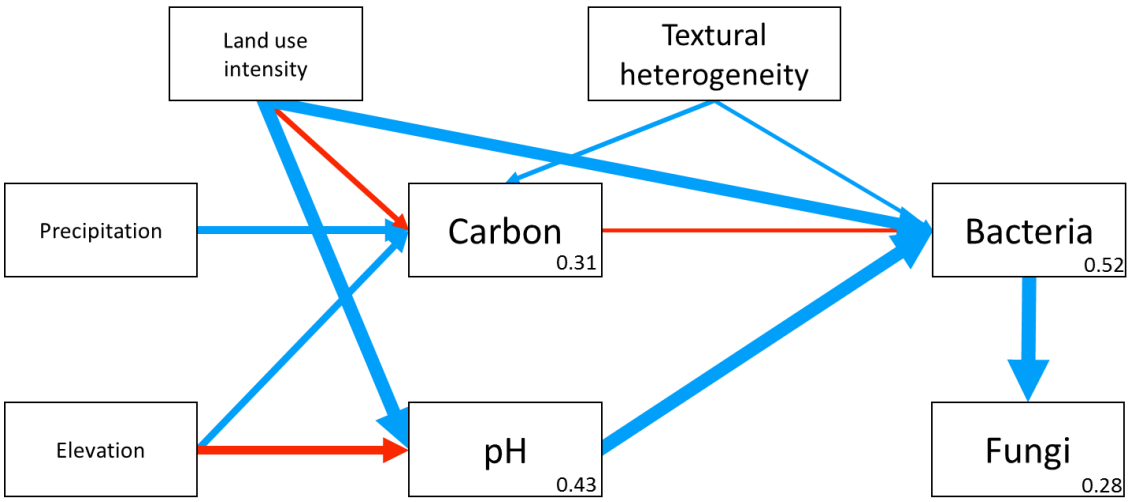

Figure 3.5. Structural equation model showing the significant impact of textural heterogeneity on bacterial diversity after controlling for land use, soil carbon and pH (n=310). Positive links are represented by blue lines, negative by red lines. Insignificant links (at p > 0.05) are not shown. R squared values for endogenous variables are shown in the corner of each box. Model fit was good: robust χ^2 = 2.117 on 7 df, p = 0.95 (scaling correction factor = 6.91). A full model output is presented in Supplementary Table S2.

3.3.4 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 (Appendix E Figures 5 and 6). Both the bacterial and fungal NMDS ordinations were significantly affected by nine aggregated

texture size particle bins; particularly the clay and silt sized particles (Appendix E Tables 3 and 4). The impact of D_1 and D_2 was nonsignificant, 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 (Figure 3.6). The classes of fungi that were correlated with particle size fractions appear to be a proportional subset of the overall common fungal class composition (Appendix E Figure 7a). However the phyla of bacteria that were correlated with particle size fractions are relatively low in Proteobacteria and high in other phyla such as the Chloroflexi compared to the overall composition of the common bacterial taxa (Appendix E Figure 7b).

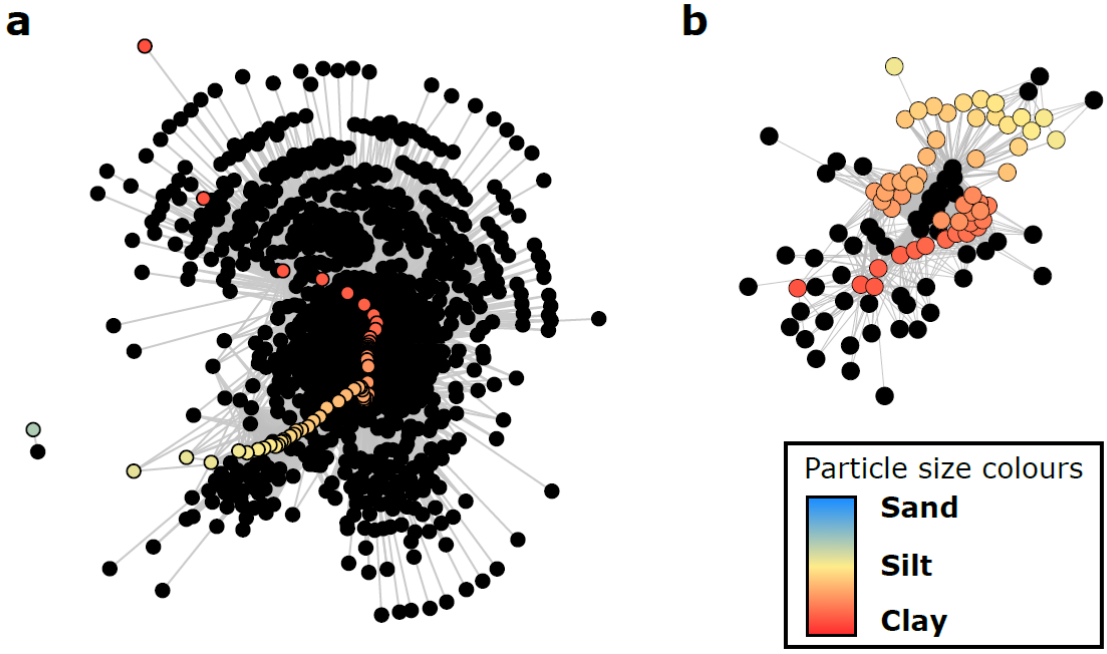

Figure 3.6. The network of spearman rank correlations between particle size bins and bacterial (panel a) and fungal (panel b) OTUs. Only correlations directly between a particle size bin and a microbial OTU are shown for graphical simplicity. The particle size bins are coloured as in Figure 3.3.

3.4 Discussion

3.4.1 Soil texture

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, D. 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.

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. This 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 & 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.

3.4.2 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). 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 & 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). The absence of a consistent relationship between soil particle size heterogeneity and texture size classes may mean these apparently inconsistent results are still driven by the positive relationship between bacterial diversity and soil particle size heterogeneity. 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 also 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 nutrient content of quartz sand relative to clay and silt sized particles. 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 (Nishiyama et al., 2012; Roberts, 2004).

The present work attempts to focus on the aspect of soil structure represented by the particle size distribution. There has been much research on the influence of aggregate type upon microbial communities (Gupta & Germida, 2015). It is clear that microbial communities are both influenced by the presence and structure of soil aggregates and promote their formation (Tecon & Or, 2017; Totsche et al., 2010). Our results are concerned with a more fundamental, relatively unchanging, aspect of soil structure than aggregation. Therefore, 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.

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 & 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 & 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 physicochemical properties, the strong positive link between bacteria and fungi means they appear to respond similarly to external gradients. The ability of the model to describe fungal diversity is limited, which may be due to the lack of inclusion of plant data which 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, Creer, et al., 2019). 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. 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.

3.5 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 drive community assembly processes in different microbial groups. However, despite these differences in biophysical driving factors likely ecological interactions can cause disparate microbial groups to respond similarly to environmental gradients.

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CHAPTER 4

Plant and soil communities are associated with the response of soil water repellency to environmental stress

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Contribution statement:

Study concept and design: Seaton, Robinson. Laboratory analyses: Lebron, Barrett, Seaton. Bioinformatic analysis: George. Analysis and interpretation of data: Seaton, Smart, Jones, Robinson, Creer. Drafting of the manuscript: Seaton. Critical revision of the manuscript for important intellectual content: Robinson, Jones, Creer, Smart. Statistical analysis: Seaton. Obtained funding: Emmett. Study supervision: Robinson, Emmett.

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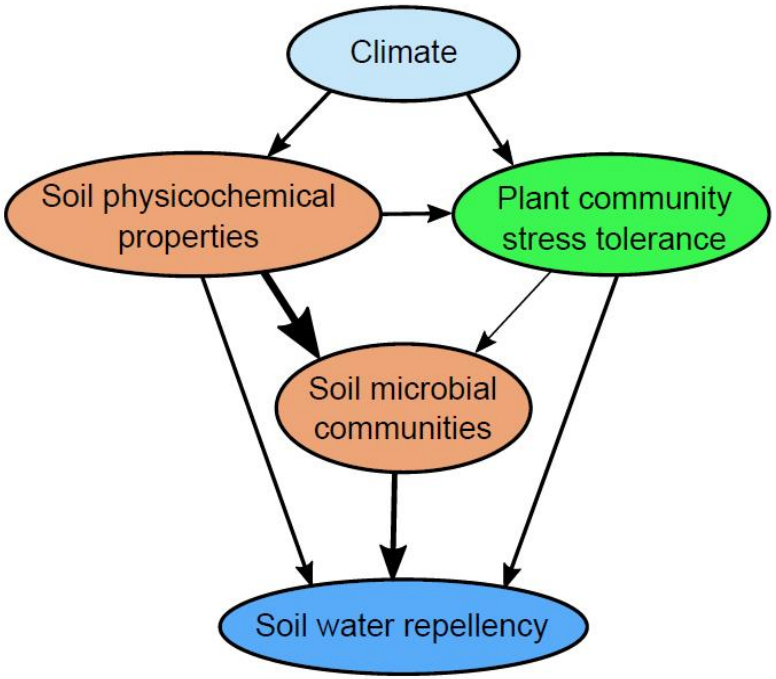
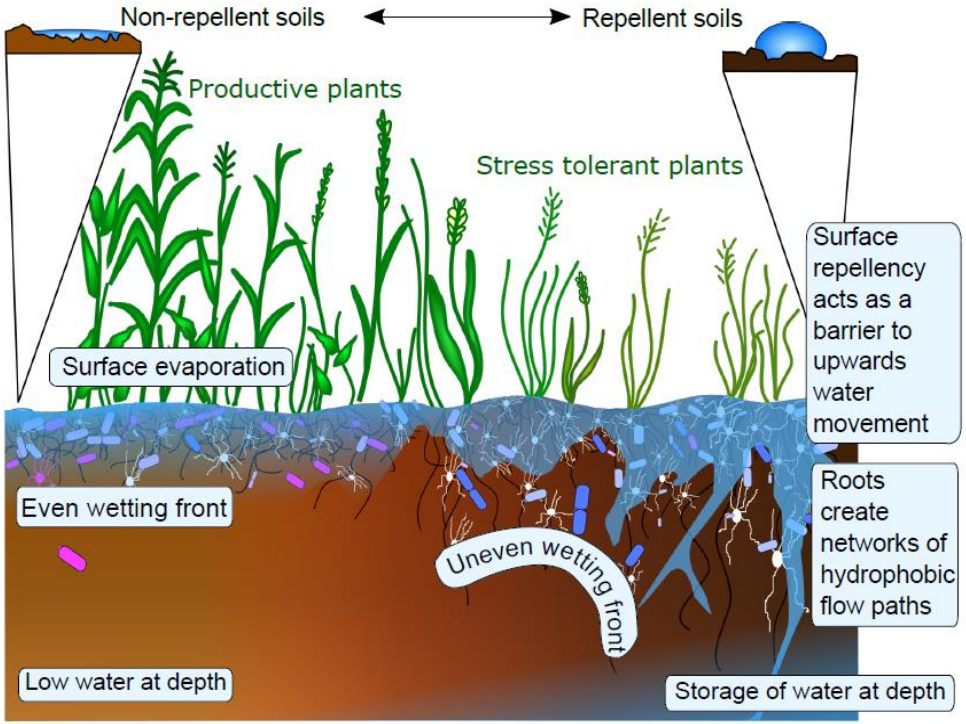

Climate
Soil physicochemical properties
Plant community stress tolerance
Soil microbial communities
Soil water repellency


Non-repellent soils
Repellent soils
Productive plants
Stress tolerant plants
Surface repellency acts as a barrier to upwards water movement
Surface evaporation
Roots create networks of hydrophobic flow paths
Even wetting front
Uneven wetting front
Low water at depth
Storage of water at depth

Abstract

A warming climate and expected changes in average and extreme rainfall emphasise the importance of understanding how the land surface routes and stores surface water. The availability and movement of water within an ecosystem is a fundamental control on biological and geophysical activity, and influences many climatic feedbacks. A key phenomenon influencing water infiltration into the land surface is soil hydrophobicity, or water repellency. Despite repellency dictating the speed, volume and pattern of water infiltration, there is still major uncertainty over whether this critical hydrological process is biologically or physicochemically controlled. Here we show that soil water repellency is likely driven by changes in the plant and soil microbial communities in response to environmental stressors. We carried out a field survey in the summers of 2013 to 2016 in a variety of temperate habitats ranging across arable, grassland, forest and bog sites. We found that moderate to extreme repellency occurs in 68% of soils at a national scale in temperate ecosystems, with 92% showing some repellency. Taking a systems approach, we show that a wetter climate and low nutrient availability alter plant, bacterial and fungal community structure, which in turn are associated with increased soil water repellency across a large-scale gradient of soil, vegetation and land-use. The stress tolerance of the plant community and associated changes in soil microbial communities were more closely linked to changes in repellency than soil physicochemical properties. Our results indicate that there are consistent responses to diverse ecosystem stresses that will impact plant and microbial community composition, soil properties, and hydrological behaviour. We suggest that the ability of a biological community to induce such hydrological responses will influence the resilience of the whole ecosystem to environmental stress. This highlights the crucial role of above-belowground interactions in mediating climatic feedbacks and dictating ecosystem health.

4.1. Introduction

The frequency and intensity of extreme climatic events is predicted to increase over the next century and beyond (IPCC, 2014). Soil moisture has been shown to have major implications for carbon storage and related climatic feedbacks (Green et al., 2019), therefore it is more important than ever to understand how the flow of water interacts with ecosystem health and the mechanisms controlling water fluxes at the land-atmosphere interface. There are still many uncertainties surrounding how water, soil, and vegetation will respond to the escalation of climatic stress in addition to prevailing land use stresses. Resilience to change varies between ecosystems, yet in most cases resilience and recovery only occur within limits and are less likely under multiple stressors (Côté, Darling, & Brown, 2016). Biological communities shift in response to stress, and soil physicochemical properties change in tandem, creating an overall ecosystem response (van der Putten et al., 2013). Further, the ecosystem response to one stressor, such as drought, may change the response to another, such as flood. Many habitat stressor responses and feedbacks are as yet unknown but are globally important if we are to model and predict impacts helping to mitigate ecosystem damage (Robinson et al., 2019).

Soil water repellency fundamentally changes the way water infiltrates and moves through the soil. A water repellent (hydrophobic) soil is defined by the behaviour of liquid on the soil surface, with repellent soils causing water drops to bead and resist capillary absorption. Previous seminal work on water repellency has emphasised its impact on hydrological processes through increasing surface runoff and soil erodibility, predominantly in fire driven systems (Doerr, Shakesby, & Walsh, 2000; Goebel et al., 2011). To date, it is often negative impacts of repellency associated with crop production, flood risk, water quality and biogeochemical cycling that have been the focus of the literature (Dekker & Ritsema, 1994; Doerr et al., 2000). However, an emerging body of work provides evidence for the ecological role of repellency in promoting the resilience of plant communities and soil carbon stock to wildfire and drought stress in various ecosystems (Kettridge et al., 2014; Robinson et al., 2010; Zeppenfeld et al., 2017). Water repellency has been shown to induce unsaturated preferential flow of water into the soil rather than piston flow in many soils (Dekker &

Ritsema, 1994; Rye & Smettem, 2017). Of the 17 ecosystem service categories identified by Costanza *et al.* (1997), twelve benefit from preferential flow and three are affected detrimentally (Clothier, Green, & Deurer, 2008).

Water repellency induces increased runoff if the soil has no macropores and unsaturated preferential flow of water into the soil, rather than piston flow, in the presence of macropores (Dekker & Ritsema, 1994). The partitioning between preferential flow and surface run-off will depend on a number of factors in addition to the degree of repellency, e.g. texture, macropore density the topography of the area and the spatial pattern of repellency, which is often highly spatially heterogeneous (Bodí et al., 2013; Doerr et al., 2000). With preferential flow, water penetrates deeper into the soil profile by following roots or other macropores generating fingered flow, while with piston flow it penetrates evenly down the soil profile (Bogner et al., 2010). In an ecosystem where the spatial pattern of plants can adjust to the heterogeneity of infiltration due to repellency, preferential flow can be an advantage. For example, preferential flow can result in greater storage of water at depth (Rye & Smettem, 2018) which can increase a plant's resilience to drought stress and give an advantage to deep-rooting plants over shallow-rooting plants in drought stressed environments (De Boeck & Verbeeck, 2011; Zeppenfeld et al., 2017). Whereas, in agricultural production systems where the pattern of plants is predetermined and there are limited macropores for the development of preferential flow paths soil moisture spatial heterogeneity and dry spots results in yield loss.

Water repellency is considered to be created by the amount, nature and configuration of soil organic material (Doerr et al., 2000; Mao et al., 2019), yet there is still uncertainty over the origins of the hydrophobic compounds in global soils (Mao et al., 2016; Schaumann et al., 2007; Spohn & Rillig, 2012). Until now, potential mechanisms for inducing water repellency have not been tested at realistic scales, hampering the emergence of a coherent theory across habitat types for the development and persistence of water repellency. In this work we analysed soil repellency across a wide range of habitats (Figure 4.1) within a temperate oceanic climate. This wide range of biota within a limited climatic range enabled us to evaluate the relative role of biotic influence on repellency versus soil physicochemical influences, without confounding

effects of climate. We characterised the plant community and soil physicochemical properties within 1326 sites, including 425 sites in which the belowground communities were measured, allowing an in-depth look at how the whole ecosystem shifts in tandem with soil hydrological shifts. Given the emerging evidence discussed we hypothesise that:

1) Soil water repellency depends on habitat, particularly showing greater persistence in those habitats that experience environmental stress such as drought and high acidity.
2) Persistence of repellency depends on the microbial community composition, as microbes can adapt to water stress by either becoming repellent or producing repellent compounds to aid water conservation.

We test these hypotheses through the following objectives: (i) measure repellency across habitat types and determine its prevalence; (ii) test the relationship between soil, plant and microbial communities and the persistence of soil repellency; and (iii) explore whether our pre-identified physicochemical and biological variables predict the changes in repellency across land use.

4.2. Methods

We used data collected as part of the Glastir Monitoring and Evaluation Programme (GMEP) field measurement program in Wales, a sampling domain of ~2,000,000 ha comprising varied land use and topography and situated on the oceanic Atlantic seaboard of NW Europe (Emmett & the GMEP team, 2017). There were 300 individual 1 km squares randomly selected from within land classification strata and each included 5 vegetation plots (Figure 4.1, Appendix F Figure 1). The sites were selected to be representative of the range of habitat types across Wales; consequently, different grassland habitats were sampled extensively, complemented by substantial numbers of woodland and wetland sites (Appendix F Table 1). Sampling occurred over a five month period across each of the summers of 2013 to 2016, each square was only surveyed once over the four years with different squares being surveyed each year. Every plot had a vegetation survey performed for a 200 m^2 square and where possible soil samples taken at the south corner of an inner 2m square (Appendix F Figure 1). A

soil core for physicochemical analysis was taken with a plastic corer of 5 cm diameter down to 15 cm depth. The squares from the first two years of the survey had soil samples for microbiology taken from three randomly selected plots within the square. Soil samples for microbiology were taken using a gouge auger at 5 points around the physicochemical soil core location down to 15 cm, and then bulking together the samples. The surveyors assigned each plot to a habitat according to the Joint Nature Conservation Committee criteria (Jackson, 2000). The main habitats included in this study were: arable; improved grassland; neutral grassland; acid grassland; broadleaved woodland; coniferous woodland; dwarf shrub heath; fen, marsh and swamp; bog; and bracken.

4.2.1 Field sampling design

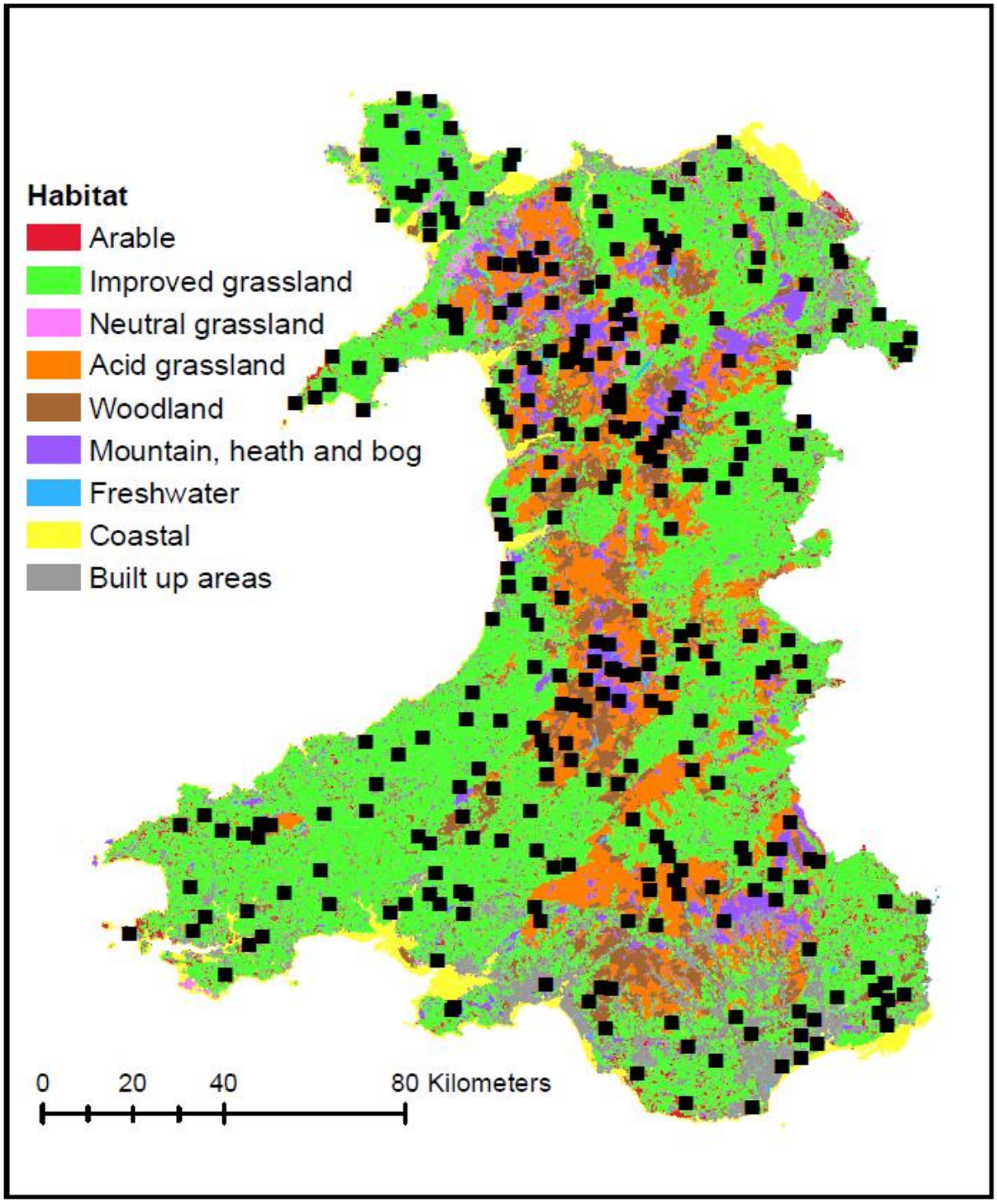


Figure 4.1: A map of the survey square locations and the range of habitats included in the survey. The white circles represent approximate survey square locations. The habitats shown are aggregated from the categories within the Land Cover Map 2015. These aggregated habitat classes were not obtained using the same methods as the field survey assignment so care must be taken in linking the results.

Elevation data was taken from NEXTmap based on the GPS coordinates of the plots. Precipitation is the Standardised Annual Average Rainfall for 1961-1990 calculated on a 1 km grid. Drought is a measure of the annual average number of dry spell events, defined as 14 day events with less than 2 mm rainfall per day, over the previous 30 years to sample collection and calculated on 5 km grid square basis. All precipitation and drought data came from the Met Office © Crown copyright 2017. The Land Cover Map 2015 was used to represent the range of habitats across Wales (Rowland et al., 2017).

4.2.2 Soil physicochemical laboratory analyses

Analysis of soil variables was undertaken using the methods of the Countryside Survey (Emmett et al., 2008). Soil pH was measured by suspending 10 g of fresh soil in 0.01 M $CaCl_2$ in a 1:2.5 (weight/volume) soil suspension (Avery & Bascomb, 1974). The pH used was measured in $CaCl_2$ instead of deionised water as the $CaCl_2$ solution has similar ionic strength to the soil solution in fertilised temperate soils and thus the pH is more representative of field conditions (Schofield & Taylor, 1955).

The surface 2 cm of the air-dry core was removed intact for water repellency measurement using the water drop penetration time method on the soil surface (Doerr, 1998) in the laboratory between 50-60% relative humidity. Six 1 ml droplets of deionised water were dropped on top of the soil surface from a height of 1 cm using a pipette. The absorption of the water droplets was recorded using video recording equipment, enabling measurement of the WDPT at a precision of 1s. This surface section of the soil was recombined with the rest of the core for further processing. The complete soil samples had particles greater than 2 mm size removed and the remaining fine earth fraction ground by a deagglomerator (Pulverisette 8). Soil carbon of the fine earth fraction of the soil was measured by oxidative combustion followed by thermal conductivity detection using an Elementar Vario EL analyser. The soil water content was calculated as the volumetric percentage of the fine earth fraction of the soil, taking into account the volume of particles >2 mm removed.

4.2.3 Biological community data

4.2.3.1 Plant community analysis

Multiple indices of plant community properties were calculated, including both those based on Ellenberg indicator values (Hill, Preston, & Roy, 2004) and those based on Grime's CSR theory. Grime's CSR theory states that species can be categorised into competitors, stress tolerators and ruderals (Grime, 1977; Hodgson, Grime, Hunt, & Thompson, 1995). For these indices the score assigned to each plant species was taken and then a mean score per plot calculated based on species identity. Within this analysis we used Ellenberg fertility and Grime's stress tolerance.

4.2.3.2 Microbial community analysis

DNA was extracted using a mechanical lysis and homogenisation in triplicate from 0.25 g of soil per sample using PowerLyzer PowerSoil DNA Isolation Kits (MO-BIO) after pre-treatment with 750 µl of 1 M $CaCO_3$ (Sagova-Mareckova et al., 2008). Amplicon libraries were created using primers for the 16S (bacteria) and ITS1 (fungi) regions of the rRNA marker gene using a two-round PCR. The primer combinations used for the first round were 515F/806R (V4 16S) for 16S libraries (Caporaso et al., 2011; Walters et al., 2011) and ITS5/5.8S_fungi (ITS1) for ITS1 libraries (Epp et al., 2012). For a full description of the methods used see George et al. (2019). Amplicon libraries of 2013 samples were constructed at Bangor University. Library preparation for 2014 samples and Illumina sequencing for both years were conducted at the Liverpool Centre for Genome Research. Sequences with limited sample metadata have been uploaded to The European Nucleotide Archive with the following primary accession codes: PRJEB27883 (16S) and PRJEB28028 (ITS1).

All bioinformatics were performed on the Supercomputing Wales system. Illumina adapters were trimmed from sequences using Cutadapt (Martin, 2011). The sequences were then de-multiplexed, filtered, quality-checked, and clustered using a combination of USEARCH v. 7.0 (Edgar, 2010) and VSEARCH v. 2.3.2 (Rognes, Flouri, Nichols, Quince, & Mahé, 2016) programmes. Sequences with a maximum error greater than 1 and > 200 basepairs were removed following the merging of forward and reverse reads for all sequences. Operational taxonomic units (OTUs) were clustered using open reference methodology as described in George et al., (2019).

Filtered sequences were matched first against either the GreenGenes v. 13_8 (DeSantis et al., 2006) or UNITE v. 7.2 (Kõljalg et al., 2013) databases. Ten per cent of sequences that failed to match were clustered *de novo* and used as a new reference database for failed sequences. Sequences that failed to match with the *de novo* database were subsequently clustered *de novo*. Chimeric sequences were removed. Taxonomy was assigned to OTUs using QIIME (Caporaso et al., 2010) with RDP methodology (Q. Wang, Garrity, Tiedje, & Cole, 2007) from the GreenGenes database v. 13_8 and UNITE database v. 7.2 for the 16S and ITS1 data, respectively. Singletons and OTUs appearing in only 1 sample were removed from OTU tables following taxonomic assignment. All non-bacterial and non-fungal OTUs were removed from each OTU table.

To account for variation in read depth across samples, fungal data was rarefied to 1750 reads and bacterial data was rarefied to 18800 reads using the vegan package (Oksanen et al., 2018; Weiss et al., 2017). Rarefaction was repeated 100 times for fungi and 50 times for bacteria and the rounded mean used for all analyses. Fungal OTUs were also assigned to trophic mode using FUNGuild (Nguyen et al., 2016). In total 53.2% of the OTUs were assigned to a trophic mode, 82.9% of those assignations being rated probable or highly probable. The FUNGuild data was rarefied to 1500 read depth 100 times and the mean value across the repetitions used to calculate the proportions of OTUs identified to be solely pathotrophic, symbiotrophic or saprotrophic. Due to the low proportion of solely pathotrophic fungi within our samples only the symbiotrophic and saprotrophic proportions were used in the statistical analysis.

4.2.4 Statistical analysis

All statistical analysis was undertaken in R (R Core Team, 2018), and were performed on the natural logarithm of the median WDPT. The WDPT was categorised into the WDPT ratings of Doerr et al. (2006). Figure 4.2 was created using the ggplot2 package (Wickham, 2016). Non-metric multidimensional scaling of the OTUs was performed using the vegan package (Oksanen et al., 2018) using Sørensen community composition distances.

Structural equation modelling was used to evaluate the factors influencing water repellency in our dataset. This approach involves proposing a causative model, taking into account direct and indirect pathways, then fitting to the data and critically evaluating the proposed causative model. A set of climate, soil and plant variables were selected based on previous work constructing hypothesised relationships consistent with mechanisms that could drive repellency. These variables were built into a piecewise structural equation model (SEM) (Shipley, 2000) using Bayesian multilevel models (Bürkner, 2017; Clough, 2012), and evaluated using Shipley's test of d-separation (Shipley, 2009, 2013). Further details on the SEM approach and parameter selection are contained within the supplementary information.

4.3. Results

4.3.1 Soil water repellency at the national scale

Overall, we found that 92% of the soils showed at least slight water repellency with 32% showing severe to extreme water repellency (Appendix F Table 1). We found that water repellency was strongly associated with soil carbon, water content and the composition of the plant and soil microbial communities at a site (Figure 4.2). Soil carbon had the largest impact upon water repellency in both the model across the full dataset (Figure 4.3b, Appendix F Table 2) and the model with microbial data (Figure 4.2b, Appendix F Table 3).

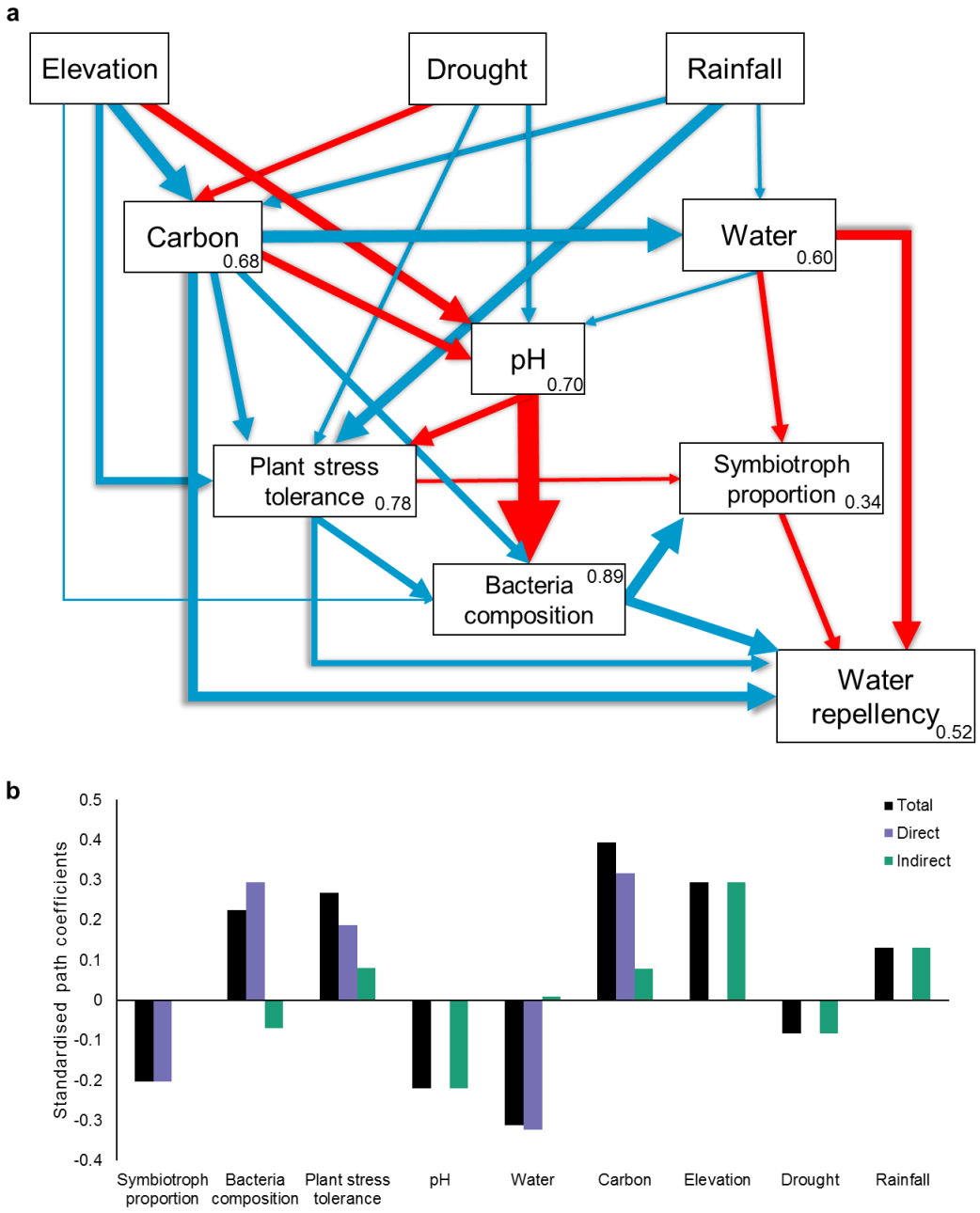


Figure 4.2: Structural equation modelling reveals soil water repellency is strongly influenced by biological community composition. ***a*** *The width of the arrow joining two boxes is proportional to the strength of the relationship, i.e. the parameter estimate. Positive relationships are represented by a blue arrow, negative by red and endogenous variables feature the proportion of variance within the variable explained by the model, the conditional R^2 value, in the corner of the box. The model fitted the data well ($C = 20.22$, $p = 0.68$, $n = 425$) and all other SEMs tested had a ΔAICc score > 2. The full output from the model is in Appendix F Table 2.* ***b*** *The total, direct and indirect effect of each predictor on soil water repellency as estimated from the model parameters.*

4.3.1 Biological influences on water repellency

Plant stress tolerance strongly impacted water repellency, having a direct impact that was over 50% higher than the effects of soil pH, soil water or climatic variables across the entire dataset (Figure 4.3, Appendix F Table 2). Although precipitation and drought were negatively correlated, both significantly increased the Grime stress tolerance score of a site. The stress score as a representative of the plant community was responsive to multiple forms of climatic stress as well as pH stress. A stress tolerant plant community at a site was associated with more repellent soils. The stress tolerance of the plant community impacts repellency directly and indirectly through differences in the soil microbial communities.

Both bacterial and fungal community composition explained significant residual variance in soil water repellency once changes in soil carbon, pH and water content were accounted for ($p < 0.001$), indicating a direct link between the soil microbial communities and water repellency. Soil water repellency decreased with increasing proportions of symbiotrophic fungi (Figure 4.2), the majority of which were ectomycorrhizal in this dataset (61%). Bacterial composition had a particularly high direct impact upon repellency (93% of the impact of soil carbon, the source of hydrophobic material; Figure 4.2b, Appendix F Table 3).

4.3.3 Mediation of climate and pH stress

Within our model the impacts of environmental stressors on repellency were completely mediated by changes in the biological communities at a site. Within the model without microbial data there are direct links between precipitation, drought and repellency (Figure 4.3) however these were not present in the model with microbial data (Figure 4.2). Water repellency does increase considerably with elevation, and alters with changing rainfall regime, yet this was entirely mediated by changes in soil properties and the biological community (Figure 4.2b). We also found no further association between soil pH and water repellency once changes in the soil bacterial community composition were accounted for.

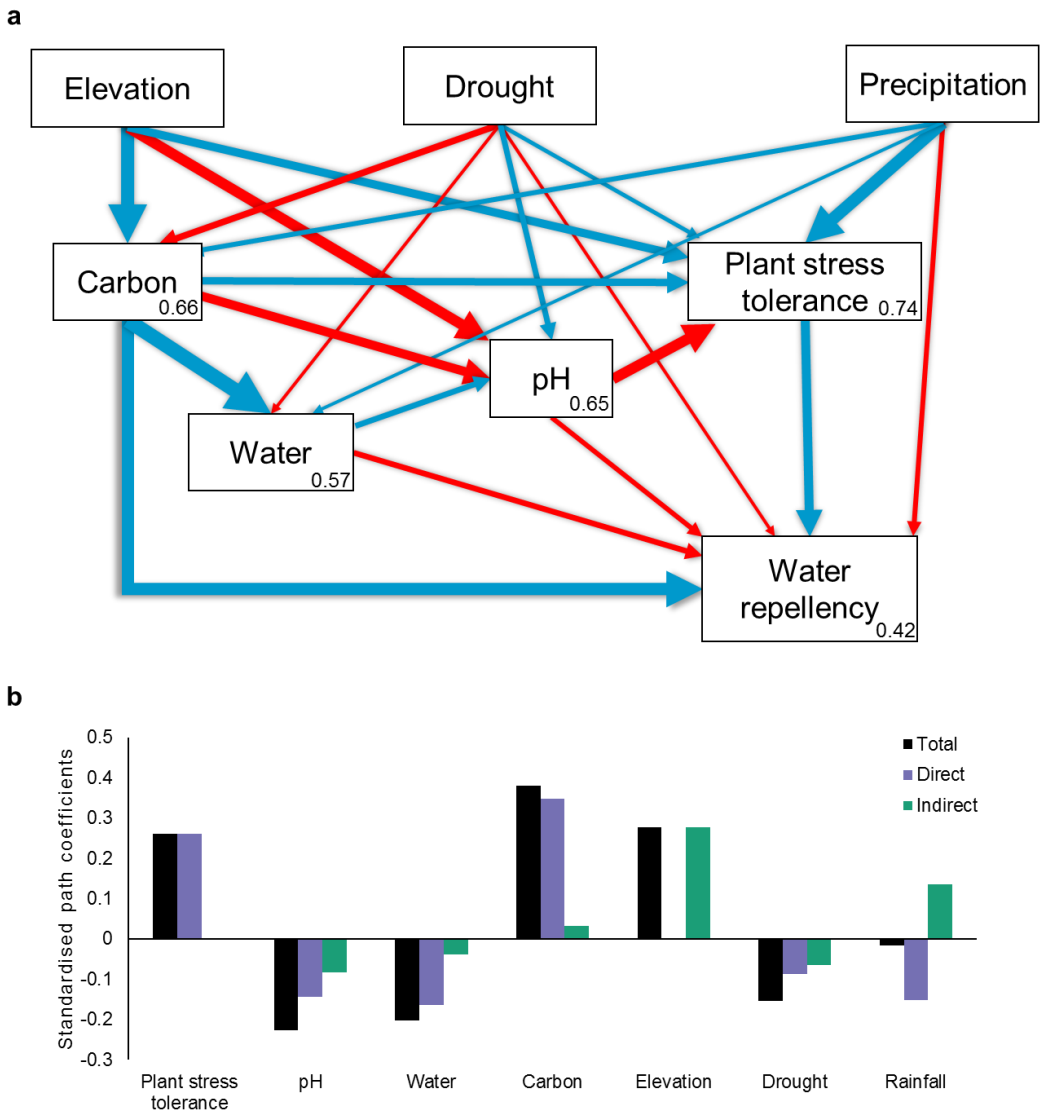


Figure 4.3: Structural equation modelling reveals the drivers of soil water repellency across the entire dataset. a The width of the arrow joining two boxes is proportional to the strength of the relationship, i.e. the parameter estimate. Positive relationships are represented by a blue arrow, negative by red and endogenous variables feature the proportion of variance within the variable explained by the model, the conditional R^2 value, in the corner of the box. The model fitted the data well (C=8.40, p=0.40, n=1326), and all other SEMs tested had ΔAICc > 2. The full output from the model is in Appendix F Table 3. b The total, direct and indirect effect of each predictor on soil water repellency is depicted as estimated from the model parameters.

4.3.4 Influence of land use on soil water repellency

Repellency varied across the different habitat types in our study, with higher repellency in low productivity habitats such as acid grassland and bog compared to

high productivity habitats such as improved grassland. Repellency was highly variable within most habitat types, particularly in broadleaved woodlands and fens (Figure 4.4). Arable systems had significantly lower water repellency than all other habitat types (Figure 4.4, Appendix F Table 1). The low water repellency of arable systems persisted after accounting for their higher pH and lower soil carbon content (ANOVA on impact of habitat on residuals for whole dataset: $F_{9, 1295} = 7.394$; $p < 0.0001$; Appendix F Table 4) and different microbial communities (ANOVA on impact of habitat on residuals: $F_{2, 380}=2.458$; $p = 0.01$; Appendix F Table 5). Arable habitats were the only habitats that were still different from other habitats after accounting for soil physicochemical and biotic variables (Appendix F Table 5).

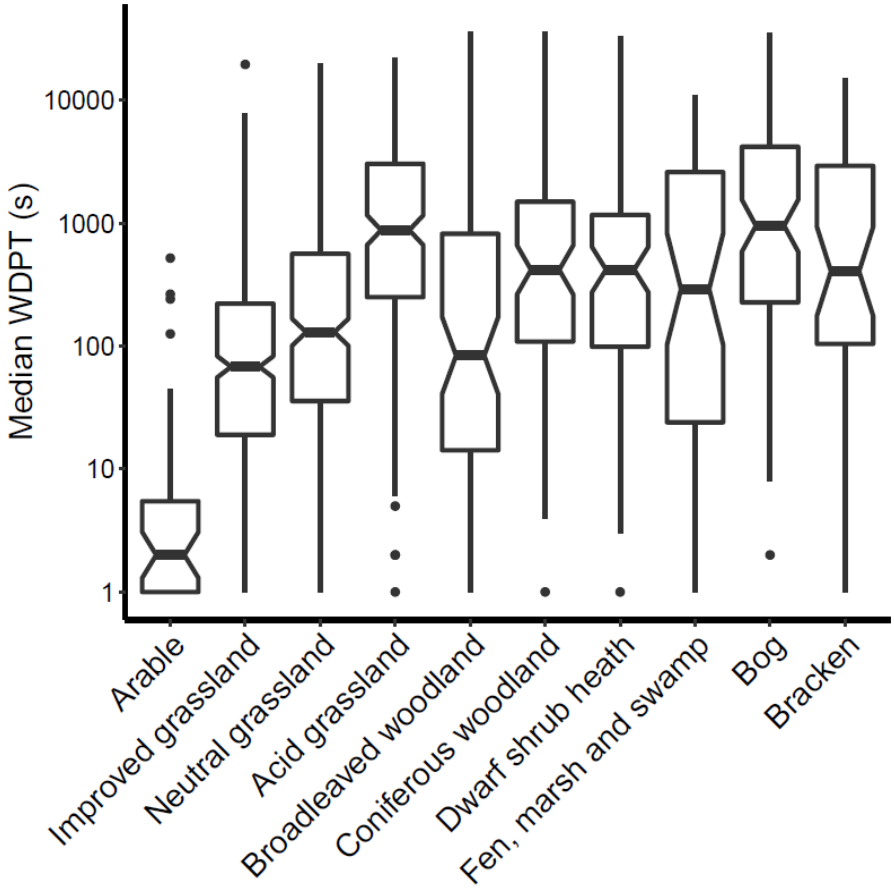


Figure 4.4: Arable systems show lower water repellency than all other habitat types. Water repellency increases with decreasing fertility of grassland (improved to neutral to acid grassland). The non-overlap of notches indicates that their medians are approximately significantly different at a 95% confidence level. Other habitats had lower sample sizes, overlapping notches and it is more difficult to draw strong conclusions.

4.4. Discussion

4.4.1 Biological influences on soil water repellency

We found that repellency is higher in ecosystems with greater soil carbon, higher plant stress tolerance and associated changes in soil pH and microbial communities (Figure 4.5). The strong influence of soil carbon on water repellency is consistent with previous work (Hermansen et al., 2019; Mao et al., 2019; Wang et al., 2016), but the association between plant community stress tolerance, microbial composition and repellency is novel. Our results provide evidence supporting literature conjecture that the ability to induce water repellency could confer a competitive advantage to plants within stressful environments (Robinson et al., 2010; Verboom & Pate, 2006). Multiple types of environmental stressors, including both climatic and acidity related stressors, have been found to be related to repellency. Surface water repellency can divert water deeper into the soil profile through inducing preferential flow of water and preventing water movement upwards by creating a evaporative barrier layer at the soil surface providing dual protection from evaporation (Doerr et al., 2006; Rye & Smettem, 2017). In semi-arid ecosystems the pattern of soil moisture in relation to trees suggests that the trees respond to drought by inducing water repellency to promote water flux down their root systems into deeper soil layers (Robinson et al., 2010; Verboom & Pate, 2006). Rhizosphere hydrophobicity has been found in modelling exercises to give a competitive advantage for plant growth due to greater acquisition of water and mitigating the impacts of drought stress (Kroener et al., 2016; Zeppenfeld et al., 2017).

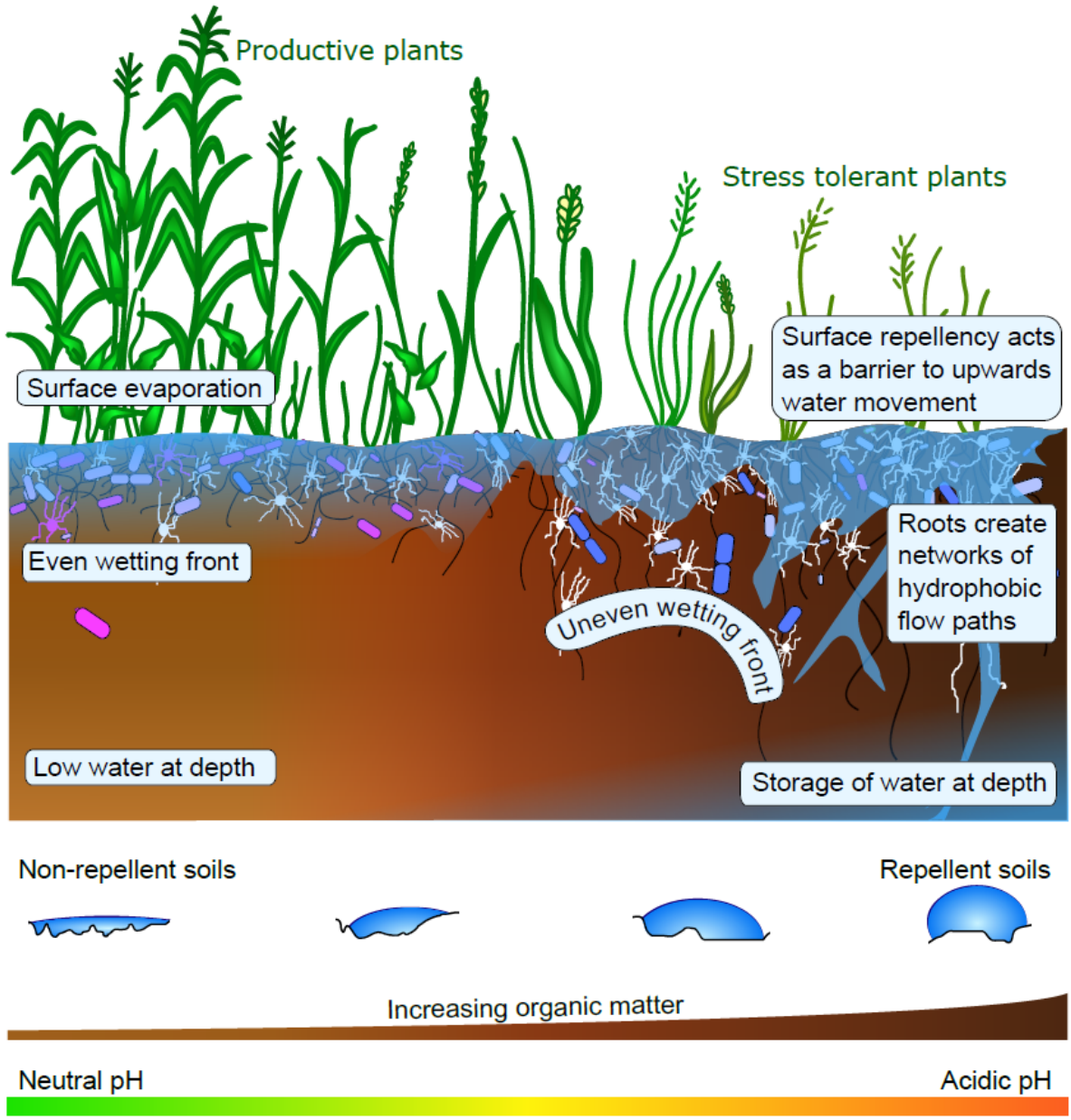


Figure 4.5: A representation of the change in repellency across an environmental stress gradient and its impact upon water fluxes in the soil when dry. Upon the left of the diagram we have a plant community that is adapted to be competitive in low-stress environments, highly productive with a non-repellent soil. Water infiltrates the soil in a piston flow manner. On the right we have a stress tolerant plant community with a repellent soil that alters water infiltration to follow preferential flow paths. This results in greater water next to plant roots and stored at depth within the soil.

We know from different parts of the literature that plant exudates (Svenningsson, Sundin, & Liljenberg, 1990), fungal mats (Spohn & Rillig, 2012), and bacterial

communities (Achtenhagen et al., 2015) can all respond to stress by producing water repellent compounds. For the microbial community the production of water repellent compounds can be an important survival mechanism both in dry and saturated systems. For example, Unestam (1991) argued that the lipoid, hydrophobic fungal surface protected both the fungus and tree roots against desiccation during drought periods. Furthermore, he observed that the mycorrhizal roots withstood a drier soil environment in rhizoscopes than did the hydrophilic non-mycorrhizal roots. Another advantage is that hydrophobic mycorrhizal hyphae may translocate water more efficiently, being less susceptible to water loss (Duddridge, Malibari, & Read, 1980; Read, Francis, & Finlay, 1985). In saturated conditions, Unestam (1991) argued that the fungal mats, particularly the complex hydrophobic structures, such as the mantle, cords, and patches, could produce air pockets. As obligate aerobes, saturation for extended periods would cause death, so the air pockets could provide a lifeline.

Bacteria have been found to produce extremely water repellent biofilms (Epstein et al., 2011). One aspect of this repellency is that it prevents the penetration of antimicrobials into the biofilm. This has been exploited in crop protection where the biofilm development can shield roots from waterborne pathogens. Moreover, it has been argued that both hydrophobic bacterial cell walls and bacterial biofilms protect bacteria from desiccation or bursting in response to cycles of drying and rapid rewetting (Achtenhagen et al., 2015). Water stress was shown to activate a number of processes in microorganisms, (Morales, Parlange, & Steenhuis, 2010; Schimel, Balser, & Wallenstein, 2007). Hence our proposal that the development of water repellency is an ecosystem response to a stressful environment, as a means of protection for microbes and better resource allocation with plants. Our results, covering climatic stress, soil physicochemical properties, plant and soil microbial communities together, support the development of such an ecological theory.

4.4.2 Persistence of repellency

Microbial communities are quicker to respond to change than plants and our results indicate that repellency could be induced by microbes on short timescales in response to environmental stressors. There is still much uncertainty over the persistence of repellency over time and space (Bodí et al., 2013; Leighton-Boyce et al., 2007; K.

Müller et al., 2014; Rye & Smettem, 2015). Our study analysed the air-dry repellency of the soil, which can be interpreted as the ability of the sample to become repellent upon drying and thus would be less variable over time than repellency of the fresh soil surface. The different ways in which repellency is created and maintained may be a critical factor in determining how long repellency will persist. Some studies have found that hydrophobicity can originate from plant material, both litter and root exudates, which clearly indicates a potential for long term maintenance of repellency by plants (Cesarano, Incerti, & Bonanomi, 2016; Hallett et al., 2009; Mao et al., 2016; Naveed et al., 2018). Microbial communities are more changeable than plants yet could still result in the long term ability to induce repellency. Microbes both create and destroy repellent compounds, and changes in the composition of the community help determine water repellency.

4.4.3 Evaluating the directionality of links and mediation in SEM

Within our analysis we assumed that soil repellency was caused by changes in the microbial community, rather than the reverse. We consider that repellency is caused by hydrophobic compounds within the soil (Hermansen et al., 2019; Mainwaring et al., 2013; Mao et al., 2019), however, it is feasible that the physical configuration of soil components could play some role, which remains largely unexplored (Benard et al., 2018). It is these hydrophobic factors that we consider to be altered by biotic communities. It is possible that the hydrophobic compounds within the soil could be altering the microbial communities through changing the suitability of the environment (Barnard, Osborne, & Firestone, 2013; Or et al., 2007; Wang & Or, 2013). However microbial communities are both the source of, and mediator of, the breakdown of hydrophobic compounds (Achtenhagen et al., 2015; Chau et al., 2012; Li et al., 2018; Schaumann et al., 2007). There is likely a feedback mechanism whereby, as the physical environment is altered by the production or degradation of hydrophobic compounds, this then forces changes in microbial communities which are adapted to different situations. We believe that the shorter feedback is in the direction of microbes to repellency, and it is this we have included in our model.

We have found complete mediation of climatic and some physicochemical stressors on repellency. Thus once we know the biotic community composition we do not need

to know the wider environmental conditions to be able to predict repellency. In particular, the complete mediation of pH related influences on repellency by the microbial community is of interest. This suggests that the change in water repellency with pH found in many observational studies (Lebron et al., 2012; Mirbabaei et al., 2013; Zavala et al., 2014) is not likely to be due to chemical modification of particles, which has been found to alter water repellency in pH modification experiments (Amer, Schaumann, & Diehl, 2017; Diehl, 2013). The complete mediation of climatic stressors upon repellency suggests that the influence of climate on soil surface water content will be strongly impacted by the biological community at a site, with implications for earth system modelling (Goebel et al., 2011; Green et al., 2019). The infiltration of water into the soil in these systems is driven by biological factors, not physicochemical, and will therefore change as biological communities are placed under increasing stress.

4.4.4 Influence of land use on soil water repellency

The differing land uses within our study had differing repellency, however the impact of land use on repellency was in most cases explained by the variation in carbon, pH and biotic communities across the land use types. This supports the findings of Doerr et al. (2006), who also found a land cover dependency for soil water repellency in the United Kingdom. Repellency is known to have a strong role in the function of some land use types. For example, within some peatland systems extreme water repellency was created after fire, which lowered evaporation, allowed the maintenance of a high water table, and increased speed of ecosystem recovery compared to systems that did not become repellent after fire (Kettridge et al., 2014). With regard to stress it has been found that, in pasture systems a negative relationship between productivity and repellency has been found (Müller et al., 2014). This suggests that the competitive advantage found by the aforementioned modelling studies (Kroener et al., 2016; Zeppenfeld et al., 2017) are limited to locations that are undergoing stress and are potentially therefore less productive. Our results are consistent with this as stress resilient plant species are found in less productive sites.

There is however one habitat in which knowing the carbon, water and biotic community does not mean that you can predict repellency: arable. Arable systems

have lower than predicted repellency even after taking into account soil physicochemical, above and belowground community composition. There is something qualitatively different about arable systems which results in lower repellency, perhaps due to the mechanical disturbance of the soil through tillage, which has been found to reduce water repellency and infiltration (Müller et al., 2016; Roper et al., 2013). Water repellency is likely to be related to soil biophysical structure, the networks of roots, fungal hyphae and microbial biofilms that permeate the soil and follow, create and maintain preferential flow paths for water infiltration.

4.4.5 Water repellency and biological community response to stress

The concept of water repellency as an adaptive stress response suggests that the ability to induce water repellency promotes ecosystem resilience to drought and other stressors. Access to water stores has been shown to be crucial in determining carbon loss and plant resilience during drought (De Boeck & Verbeeck, 2011). We propose that water repellency indicates a healthy ecosystem response to stress, and the inability of tilled land to induce water repellency can be interpreted as an unhealthy lack of resilience. We have found that multiple different natural stressors: drought; high precipitation and low nutrient status acidic soils had a consistent relationship with our realistic large-scale gradient of soil water repellency. It is the biological communities which are more closely related to soil repellency than physicochemical factors, showing the importance of ecology in modifying hydrological processes through feedbacks that will help conserve water. The homogeneity of response indicates there are consistent mechanisms induced by biological communities across ecosystem types to increase resilience. These mechanisms are those we should be interested in monitoring and influencing to understand, predict and mitigate ecosystem shifts in response to increasing stress from land use and climate change.

4.6. References

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CHAPTER 5

A diversity of diversities: evaluating the relationships between below and aboveground biodiversity

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Contribution statement:

Study concept: Seaton, George, Creer, Jones, George, Robinson. Study design and statistical analysis: Seaton. Analysis and interpretation of data: Seaton, George, Alison, Smart. Drafting of the manuscript: Seaton. Critical revision of the manuscript for important intellectual content: Robinson, Jones, Creer. Study supervision: Robinson.

Abstract

Understanding the diversity and composition of ecological communities is a key component in predicting future biodiversity responses to environmental change and implications for ecosystem health. Linking across the domains of life and trophic levels is essential for understanding whole-ecosystem dynamics but is often difficult and limited in scope. Here we analyse data from an extensive biodiversity dataset gathered across a variety of oceanic temperate habitats comprising 300 sites with co-located soil microbial, plant, bird and pollinator surveys along with climate and soil physicochemical information. Soil microbial groups are analysed using Illumina sequencing of the 16S, ITS1 and 18S DNA regions, allowing in depth characterisation of microbial community composition and diversity. Using Bayesian hierarchical regression models, we show that a positive correlation between plant diversity and soil bacterial and fungal diversity is actually driven by changes in soil pH. However, positive associations between plant diversity and bird, bee and butterfly diversity persisted after accounting for changes in climate. The composition of soil bacteria, fungi, bees, butterflies and birds are all impacted by the plant community in conjunction with edaphic factors. The heterotrophic protistan community strongly tracks the bacterial community in both diversity and composition. Co-occurrence relationships were identified across the different microbial domains by sparse conditional network analysis, resulting in habitat specific clusters of taxa. The residual species associations after accounting for climatic and spatial variation in a hierarchical Bayesian joint species distribution model also revealed cross-domain correlations that largely structured into two clusters of low and high fertility species respectively. Taken together these results indicate the importance of cross-domain interactions in structuring local ecological communities and caution against the concept of a domain specific response to environmental stressors. Overall, our results comprehensively show the differential responses, linkages and divergences of diversity and composition in aboveground-belowground ecological communities to environmental and biotic properties.

5.1 Introduction

The biodiversity of our planet is valuable in both its own right and in the provision of ecosystem resilience, functions and services (IPBES, 2019). Biodiversity is under increasing threat but monitoring change is often challenging, particularly for certain taxonomic groups and habitats. To address these difficulties many authors have attempted to evaluate whether certain taxa can act as indicators of other more difficult to survey taxa (e.g. Ceballos & Ehrlich, 2006; Landeiro et al., 2012; Prober et al., 2015; Wolters et al., 2006). There is a theory that diversity of certain groups can lead to increased diversity of other groups; for example, a more diverse plant community can support a more diverse pollinator community through a proliferation and utilisation of different ecological niches. The generation of biodiversity by biodiversity is somewhat supported by the presence of hotspots across the globe with consistently, geographically coincident high diversity across a multitude of taxonomic groups (Myers et al., 2000), although more detailed investigation indicates that there is limited congruence within taxonomic groups in species distributions (Ceballos & Ehrlich, 2006; Orme et al., 2005). There is still uncertainty over the strength of this diversity effect, as relationships between the diversity of different taxa aboveground have been shown to be variable and weak (Wolters et al., 2006). Results investigating the relationship between above- and below-ground biodiversity have also been variable, with either positive or no correlations commonly observed between plant diversity and soil microbial diversity.

The relationships between the diversity of different taxonomic groups can vary by the identity of those groups, the overlying environmental conditions and the type of diversity considered. Certain taxonomic groups appear to show more positive relationships with plant diversity. For example, fungal diversity, particularly mycorrhizal diversity, is often found to be positively related to plant diversity (Hiiesalu et al., 2014; Milcu et al., 2013; Nguyen et al., 2016; Peay et al., 2013; Ren et al., 2017; Yang et al., 2017). Also, the type of plants that are considered in plant diversity can be important, for example the number of flowering plant species and their abundance has been shown to be important in influencing bee and butterfly abundance and richness (Kearns & Oliveras, 2009; Potts et al., 2009). Some of the

positive correlations that have been found between plant diversity and soil bacterial, fungal and protistan diversity are explained by shared response to environmental variables such as climate or physicochemical soil properties. Soil pH and fertility have been found to drive a positive correlation between plant diversity and bacterial diversity in a variety of ecosystems (Goberna et al., 2016; Yashiro et al., 2018; Yuan et al., 2017). The metric of diversity used is also important, with phylogenetic diversity having been used as an alternative to species richness or diversity, with positive associations being noted in some studies, e.g. plant and butterfly communities (Pellissier et al., 2013), and no improvement in predictive performance for others, e.g. plant and bacterial communities (Barberán et al., 2015; Navrátilová et al., 2018).

While relationships between the biodiversity of different taxonomic groups may be variable, there have been many results demonstrating that the composition of plants may be related to the composition of other taxonomic groups even when biodiversity, i.e. species richness and diversity, is not. Relationships between the composition of plants and the composition of soil bacteria, fungi and protists have been found in both experimental and observational studies (Barberán et al., 2015; Cline et al., 2017; Delgado-Baquerizo et al., 2018; Leff et al., 2018; Prober et al., 2015). These correlations in composition in observational results can persist even after controlling for environmental drivers such as climate or soil physicochemical properties (Delgado-Baquerizo et al., 2018; Prober et al., 2015). At the larger scales, information on the heterogeneity of vegetation and landscape composition from satellite imagery has also been used to predict bird richness and composition (St-Louis et al., 2014), and to a lesser extent bee species (Hofmann et al., 2017).

Correlation between the biodiversity of different taxa could be due to the direct influence of one taxonomic group on another or down to shared response to environmental conditions. These two mechanisms may lead to similar patterns but have differing implications for land use management. Therefore, disentangling the relative influence of abiotic and biotic impacts upon biodiversity is key. There have been some experimental manipulations established to evaluate the impact of plant diversity upon ecosystem functioning, including changes in the biodiversity of the other groups. These have found positive relations between plant diversity and the

diversity of some other organisms, particularly for aboveground herbivores and less so for soil microbial groups (Cline et al., 2017; Dassen et al., 2017; Lind et al., 2015; Weisser et al., 2017). However, by necessity these experiments are limited in scope, covering a limited set of environmental conditions and plant species combinations. The use of field survey information to establish real-world correlations across taxa is essential if we are to cover a wide range of environmental and biotic conditions. Controlling for confounding variables within statistical analysis can further help us consider what might be the direct impact of one taxonomic group upon another.

Here we present the results from a field survey across the entire range of terrestrial habitats in Wales, where plant, bird, pollinator and soil fungal and microbial diversity were each measured at the same sites. Previous analysis has found that plant, bird and butterfly species richness was positively associated with land use intensity and habitat heterogeneity (Maskell et al., 2019), and also that the soil microbial community was strongly driven by land use type and pH (George et al., 2019). Here we extend these analyses to consider the whole ecosystem, both above and below-ground and consider the roles of the plant community and abiotic environment in influencing both the diversity and composition of other taxonomic groups across diverse habitat types. The objectives here are i) to investigate if plant, bird, pollinator and soil microbial diversity are correlated with each other in a temperate ecosystem, not confounded by geographical variation, ii) to investigate if the composition of one taxonomic group is a reliable predictor of another, iii) to evaluate whether any correlations in diversity or composition are due to shared response to environmental drivers, and iv) to identify the presence of any co-occurrence relationships between specific taxa of different domains and identify overarching ecological rationale that defines ecosystem level shared community composition.

5.2 Methods

5.2.1 Field measurement programme

The data was collected as part of the Glastir Monitoring and Evaluation Programme (GMEP) field measurement program in Wales (Emmett et al., 2017). In total, 300 individual 1 km squares were randomly selected from within land classification strata

in proportion to their extent in order to be representative of the range of habitat types across Wales. Sampling occurred over a five month period across each of the summers of 2013 to 2016; each square was only surveyed once over the four years with different squares being surveyed each year. Every square was subjected to a habitat survey, bird survey, two pollinator survey transects and multiple plant survey plots. For each square there were up to five 200 m^2 square plant survey plots that also had soil samples taken. Soil samples were analysed for a variety of soil physicochemical properties, including pH in 1:2.5 $CaCl_2$ suspension, total carbon, nitrogen and phosphorus, bulk density, water content, water repellency and electrical conductivity in 1:2.5 distilled water suspension. The data and full methods are available at doi.org/10.5285/0fa51dc6-1537-4ad6-9d06-e476c137ed09 (Robinson et al., 2019). Within the first two years of the survey soil samples were taken for microbial community composition analysis from three of the 200 m^2 plots, randomly selected per each 1 km square.

The surveyors mapped the habitats of each square and assigned each plot to a habitat according to the UK Joint Nature Conservation Committee criteria (Jackson, 2000). The main habitats included in this study were different grassland habitats, with some woodland and heathland. The plot level measurements of microbial diversity were derived from 31% improved grassland; 23% neutral grassland; 12% acid grassland; 7% broadleaved woodland; 7% coniferous woodland; 7% bog; 4% arable; 3% dwarf shrub heath; 3% fen, marsh and swamp; and 2% bracken. Precipitation was the annual average rainfall and temperature was the annual average daily temperature for 1981-2010 calculated on a 1 km grid. All climate data came from the Met Office © Crown copyright 2019.

5.2.2 Biological data

5.2.2.1 Plant survey

Vegetation surveys were conducted for multiple plots per square. Overall, there were ten types of sampling plots in total. Some plots were targeted to specific landscape features (e.g. hedges, stream banks, priority habitats), meaning the total number and

proportion of plot types varies across squares. For each plot, plant functional properties were created based on reference to literature values for growth form and other indices such as Ellenberg values (Hill et al., 2004). The total number of vascular plant species recorded across the entire 1 km square was used as plant species richness for the square level analyses. Due to the differences in sampling effort across the squares rarefaction curves were constructed to check that the number of plant species found had reached saturation. Plant species that were important to the diet of lowland birds, butterfly larvae and nectar provision were identified and the species richness of those three groups calculated (Baude et al., 2016; Smart et al., 2000). A phylogenetic tree for the plants within the survey was created using the V.PhyloMaker package to match plant species to a phylogenetic tree (Jin & Qian, 2019), with the taxize package having been used to match our plant species identifiers to the NCBI database (Chamberlain & Szocs, 2013).

5.2.2.2 Bird and pollinator surveys

Birds were surveyed based on four morning visits to each square, equally spaced through mid-March to mid-July. Surveyors walked a route that passed within 50 m of all parts of each survey square, varying the start point for each survey in order to visit all parts of the square at least once before 08:00 h. The total number of bird species recorded across all visits per square was used to calculate total bird species richness. Pollinator surveys were split into two independent parts: two 1 km transect routes separated by at least 500 m and where possible 250 m from the edge of the 1 km square; and a 20 minute timed search in a 150 m^2 flower rich area within the 1 km square. The total number of butterfly species and bee groups recorded per square were used to calculate richness. Pollinator surveys were managed by Butterfly Conservation and bird surveys managed by the British Trust for Ornithology.

5.2.2.3 Soil microbial diversity and community composition

Soil samples for microbial biodiversity analyses were taken using a gouge auger at 5 points around the physicochemical soil core location down to 15 cm, and then bulking together the samples. DNA was extracted from these samples using mechanical lysis and homogenisation in triplicate from 0.25 g of soil per sample. The 16S (V4), ITS1 and 18S regions of the rRNA marker gene were targeted for amplicon sequencing to

analyse the bacterial, fungal and general eukaryotic diversity respectively. For a full description of the methods used see George et al. (2019). Amplicon libraries of 2013 samples were constructed at Bangor University. Library preparation for 2014 samples and Illumina sequencing for both years were conducted at the Liverpool Centre for Genome Research. Sequences with associated sample metadata have been uploaded to The European Nucleotide Archive with the following primary accession codes: PRJEB27883 (16S), PRJEB28028 (ITS1) and PRJEB28067 (18S).

All bioinformatics were performed on the Supercomputing Wales system. Illumina adapters were trimmed from sequences using Cutadapt (Martin, 2011). The sequences were then de-multiplexed, filtered, quality-checked, and clustered using a combination of USEARCH v. 7.0 (Edgar, 2010) and VSEARCH v. 2.3.2 (Rognes et al., 2016) programmes. Sequences with a maximum error greater than 1 and < 200 basepairs were removed following the merging of forward and reverse reads for all sequences. Operational taxonomic units (OTUs) were clustered using open reference methodology as described in George et al. (2019). Filtered sequences were matched first against either the GreenGenes v. 13_8 (DeSantis et al., 2006) or UNITE v. 7.2 (Kõljalg et al., 2013) databases. Ten per cent of sequences that failed to match were clustered *de novo* and used as a new reference database for failed sequences. Sequences that failed to match with the *de novo* database were subsequently clustered *de novo*. Chimeric sequences were removed. Taxonomy was assigned to OTUs using QIIME (Caporaso et al., 2010) with RDP methodology (Wang et al., 2007) from the GreenGenes database v. 13_8 and UNITE database v. 7.2 for the 16S and ITS1 data, respectively. Singletons and OTUs appearing in only 1 sample were removed from OTU tables following taxonomic assignment. All non-bacterial and non-fungal OTUs were removed from each OTU table. Fungal OTUs were matched to trophic mode at a genus level with FunGUILD (Nguyen et al., 2016).

5.2.3 Statistics

All statistical analyses were performed within R version 3.6.1 (R Core Team, 2019). To account for the differences in read depth between samples, rarefaction was used as it has been shown to preserve biological relationships (Weiss et al., 2017). The fungal data was rarefied to 1750 reads, bacteria to 30000 reads, and heterotrophs to 1750

reads. Samples below this threshold were discarded; resulting in 432 fungal (ITS) measurements, 430 fungal (18S) measurements, 431 bacterial measurements and 425 heterotrophic protist measurements. Arbuscular mycorrhizal (AM) fungi were identified as the members of the phylum Glomeromycota within the 18S dataset. Biodiversity indices, i.e. richness, Shannon and Simpson diversity, were calculated after rarefaction, which was repeated 100 times and the rounded average result used in further analysis. For the analysis of community composition the rounded average of 20 rarefaction repeats was used. Bray-Curtis distance was calculated on the rarefied matrix. Rarefaction and distance calculation were performed using the vegan package (Oksanen et al., 2018). Unrooted phylogenetic diversity was calculated using the PhyloMeasures package in R for plants, bacteria, heterotrophic protists and AM fungi (Tsirogiannis & Sandel, 2016).

The effect of pH upon the bacterial, fungal and heterotrophic richness was modelled as a sigmoidal non-linear Bayesian model within the brms package in R (Bürkner, 2017). There was a group-level effect on the intercept as square ID in order to account for the spatial element of the data. For heterotrophic protists the fit of this sigmoidal pH model was compared to the fit of a linear model with bacterial richness. The impact of plant richness upon bacteria or fungi was modelled using three models: a no interaction model; a model that allowed the upper threshold of bacterial or fungi to vary with plant richness; or a model that allowed all parameters other than the pH at maximum growth to vary with plant richness. Models that also allowed the pH at maximum growth parameter to vary with plant richness did not converge. Heterotrophs were modelled using both a no interaction model and allowing an interaction between bacterial and plant richness. The effect of plant richness, precipitation and elevation on butterfly richness, bee richness and bird richness were modelled as a multivariate Bayesian regression model with a negative binomial response. Biological recording region (vice county) and year were used as group-level effects to account for the spatial and temporal nature of the data. Plant species richness always had precipitation and elevation as the sole fixed effect predictors. Models were compared using leave-one-out cross-validation (Vehtari et al., 2017).

Comparison of the composition of the different biological communities was done through comparison of the ecological distance matrices. Binary Jaccard distance was used for plant, bird and butterfly species composition and Bray-Curtis distance used for bee and hoverfly groups due to their lower richness. Distance matrices were compared directly using visual inspection, spearman rank correlations and Procrustes analysis using the protest function in the vegan package. To account for the shared environmental drivers and spatial factors that could be driving changes in the aboveground communities the variance was partitioned using the varpart function. The response variables were the NMDS scores of each group based on Jaccard distance and the predictors were climate (represented by temperature and precipitation), spatial distance (represented by principal coordinates of neighbour matrices), plant community composition (represented by NMDS scores in 4 dimensions), and for the plot level data only the first four dimensions of a PCA upon soil physicochemical properties (pH, carbon, nitrogen, total phosphorus, bulk density, electrical conductivity, water content, water repellency).

The co-occurrence of specific taxa was analysed for the microbial and co-located plant data using the SpiecEasi package which examines co-occurrence relationships conditional on all the other taxa present (Kurtz et al., 2015). We analysed cross-domain relationships using an extension to SpiecEasi (Tipton et al., 2018). Only common taxa were included within the analysis to reduce the number of false positive associations (Weiss et al., 2016), which we identified as being present in 75% of sites for bacteria, 50% for heterotrophic protists, 25% for fungi (ITS only) and 10% for plants. In total, there were 1213 bacterial OTUs, 512 heterotrophic OTUs, 180 fungal OTUs and 28 plant species that fulfilled these criteria. The resulting network was analysed for the presence of clusters using the spinglass algorithm to take account of both the positive and negative links in the igraph package (Csardi & Nepusz, 2006; Traag & Bruggeman, 2009). The resulting clusters were laid out without their interlinks using the Fruchterman-Reingold layout algorithm based on positive links only for graphical simplicity, then the layout used for the plots with all edges.

A Bayesian joint species distribution model was used to analyse the response of each plant, bird, butterfly, bee and hoverfly taxa to climate while accounting for spatial

autocorrelation in the Hmsc package (Ovaskainen et al., 2017; Tikhonov et al., 2019). The environmental predictors were average annual precipitation (1981-2010) and daily temperature (1981-2010) which were scaled to have mean zero and standard deviation of one. The original annual means were 1535 mm (standard deviation 521 mm, range 660-3650 mm) and 9.0°C (standard deviation 1.1°C, range 5.8-11°C). Only species that appeared in over 10 sites were included in the model and thirteen of those had to be removed in order to let the model converge. All species were represented as binary for presence-absence and the models run with the default probit distribution. The resulting model fit object had the mean residual association between each species pair extracted and converted into a network which had the clusters identified and plotted as above.

5.3 Results

5.3.1 Alpha diversity

We found that bacterial, fungal, heterotrophic protistan, bird, bee and butterfly richness positively correlated with plant diversity (Appendix G Figures 1 and 2). Diversity of the different groups varied considerably across Wales (Figure 5.1). Within the soil microbial groups, bacteria and arbuscular mycorrhizae (AM) showed the strongest correlation with plants (Spearman rank correlation of both 0.38), with the heterotrophic protists similar (rho = 0.34), and the general fungal (ITS) correlation lower (0.10) (Figure 5.2). Bacterial richness was positively correlated with both fungal (ITS: 0.52, AM: 0.57) and heterotrophic protistan richness (rho = 0.65). Soil microbial richness was positively correlated to the richness of forb plant species rather than to overall plant richness or graminoid richness. Microbial richness was also negatively correlated to woody plant richness, particularly in AM fungi (rho -0.67).

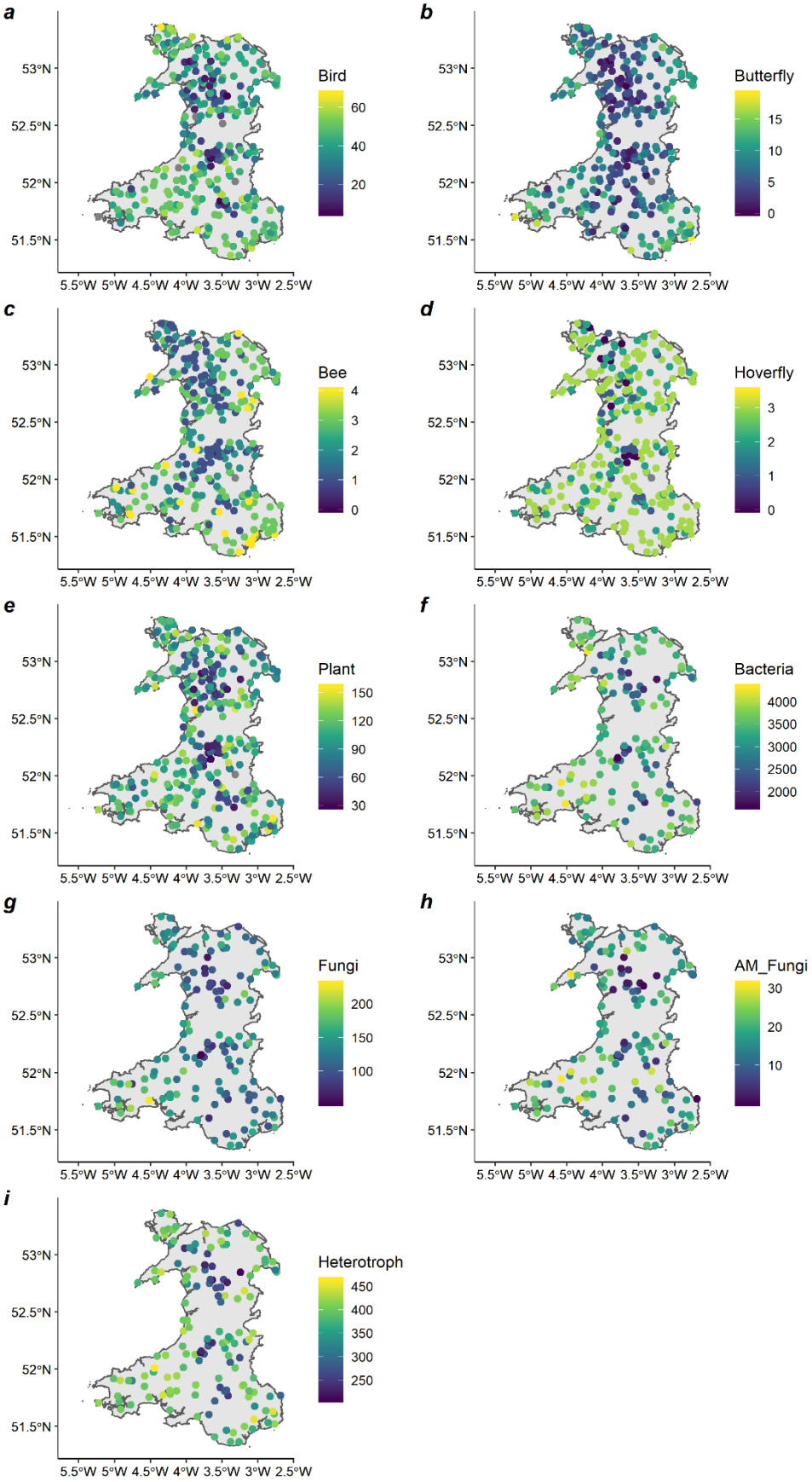

Figure 5.1: The square level richness of birds (a), butterflies (b), bee groups (c), hoverfly groups (d), plants (e), bacteria (f), fungi (g), AM fungi (h) and heterotrophic protists (i) across Wales.

Plant diversity in the 1 km squares positively correlated most strongly with bird species richness (0.50), followed by butterfly species richness (0.43), then bee group (0.36) and hoverfly group (0.34) richness. The correlation with the richness of plant species identified as either nectar producing or important to the diet of birds was comparable: birds 0.58; butterfly 0.49; bee 0.40 and hoverfly 0.38. The different pollinator groups were positively associated with each other with butterflies and bees associated more strongly than hoverflies with the other two groups (rho = 0.56 compared to rho 0.42-0.44). Bird species richness was positively correlated with butterfly (0.48) and bee richness (0.47) and to a lesser extent hoverfly richness (0.29). Average soil microbial diversity per 1 km square also positively correlated with bird, butterfly and hoverfly richness (Figure 5.1). Bacterial richness showed a positive correlation of 0.49 with bird richness, 0.54 with butterfly richness, 0.37 with bee richness and 0.34 with hoverfly richness. Fungal and heterotrophic protistan richness showed correlations of similar magnitude (Appendix G Figure 2).

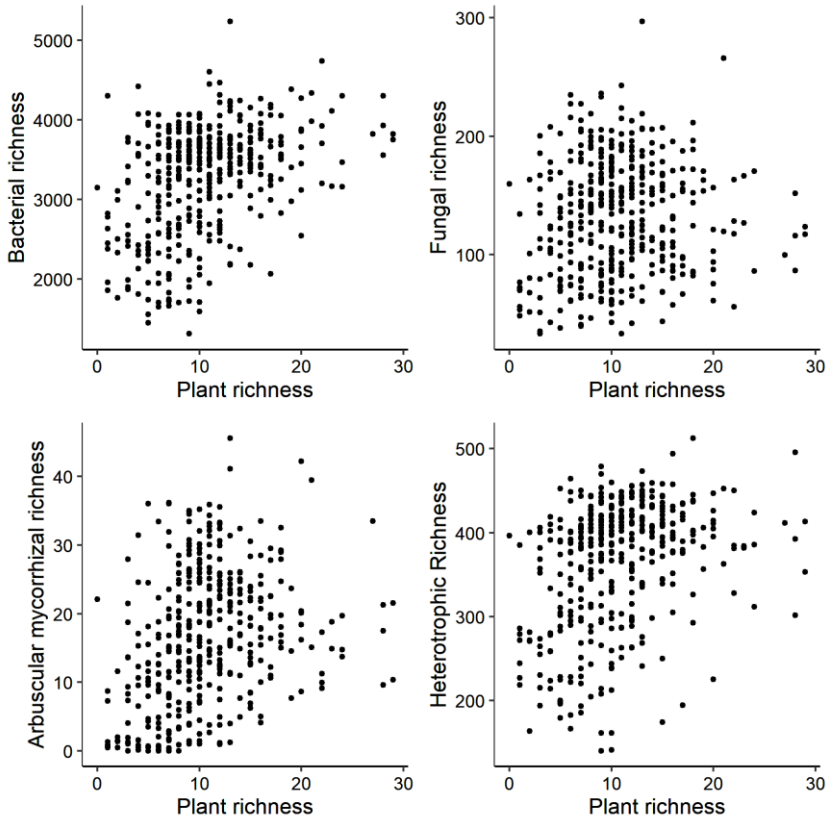

Figure 5.2: Microbial richness is weakly positively related to plant richness across multiple groups, spearman rank correlations were 0.38, 0.10, 0.38, and 0.34 for bacteria, fungi, AM fungi and heterotrophs respectively.

5.3.1.1 Belowground diversity

The impact of plant richness upon bacterial and fungal richness was negligible once the change in soil pH was accounted for (Figure 5.3, Appendix G Figure 3). The inclusion of plant richness as well as pH to predict bacterial richness resulted in a slight improvement in predictive performance but the standard errors of the improvement were of the same magnitude as the difference (elpd difference: 3.2 ± 3.0). This was also the case for overall fungal diversity (elpd difference: 3.8 ± 3.0) and AM fungi (elpd difference: 1.9 ± 1.9). Bacterial richness appears to increase with increasing plant richness in soils with pH > 3.5 but decrease with increasing plant richness in soils with pH < 3.5. The opposite trend is true for fungal ITS richness, and AM fungal richness always increases with increasing plant richness. Using forb richness instead of overall plant richness resulted in equivalent model predictive performance once standard errors were taken into account for bacteria (0.5 ± 2.6) and AM fungi (0.7 ± 7.6), and slightly worse performance for general fungi (3.8 ± 1.9).

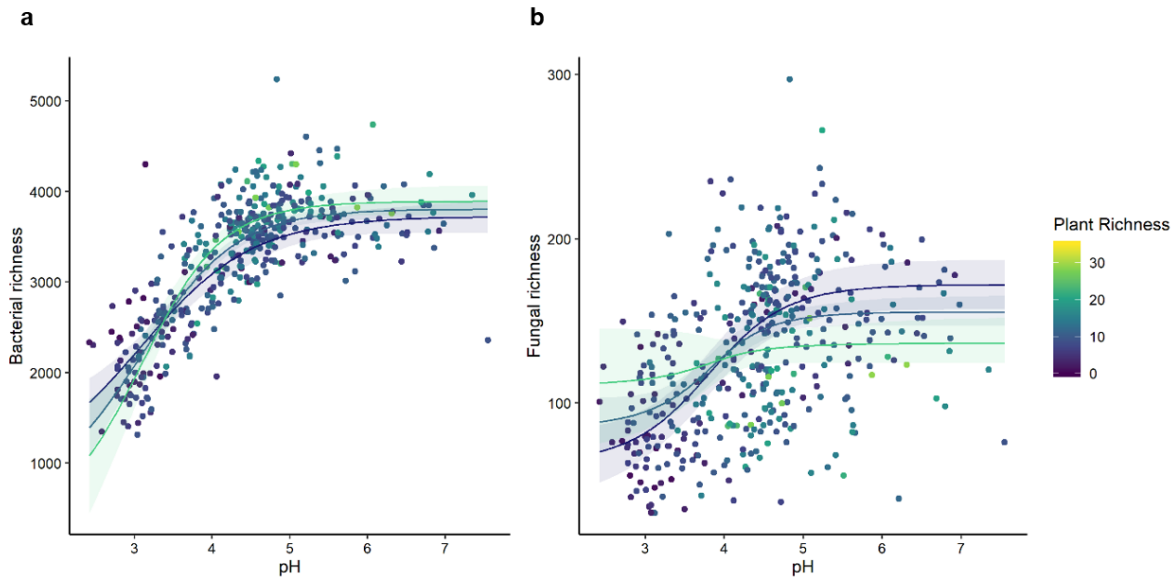

Figure 5.3: The impact of plant diversity upon bacterial and fungal richness once the gradient in pH is accounted for. Panel a shows bacterial richness, panel b fungal (ITS) richness. Points are coloured by the plant richness and fit lines predicted microbial richness for 1 plant species (purple), 10 plant species (blue) and 20 plant species (green) are included. Note that these models showed no substantial improvement in predictive performance compared to pH only.

The richness of the heterotrophic protists was better predicted by changes in bacterial richness than soil pH (Figure 5.4). pH did not predict any residual changes in heterotrophic richness once the positive trend in response to bacteria was accounted for. The impact of plant richness was negligible, with only marginal improvement in model predictive performance with the addition of plant richness (elpd difference: 0.5 ± 1.3). The effect size of the plant richness effect was so small as to be of little relevance to soils in practice (Figure 5.4). There was only a negligible improvement in predictive performance when forb richness was used instead of overall plant richness (elpd difference: 1.1 ± 1.6).

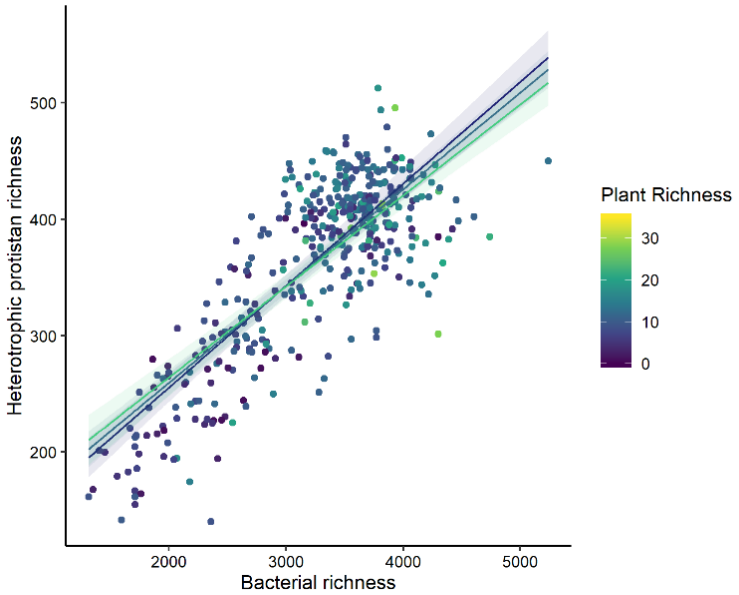

Figure 5.4: Heterotrophic protistan richness plotted against bacterial richness. Points are coloured by plant richness and predicted heterotroph richness for 1 plant species (purple), 10 plant species (blue) and 20 plant species (green) are included.

Phylogenetic diversity (PD) was calculated for plants, bacteria, heterotrophic protists and fungi using the 18S region. Bacterial PD increased with increasing plant PD when pH was above 4 (Appendix G Figure 4, elpd difference compared to pH only 8.5 ± 3.7). Fungal PD was only slightly impacted by plant PD once pH was taken into account (elpd difference 1.0 ± 0.9). Heterotrophic and bacterial PD were less strongly related than their respective richness values, and heterotrophic PD more strongly tracked pH than bacterial PD (Appendix G Figure 5, elpd difference 25.6 ± 13.1).

5.3.1.2 Aboveground diversity

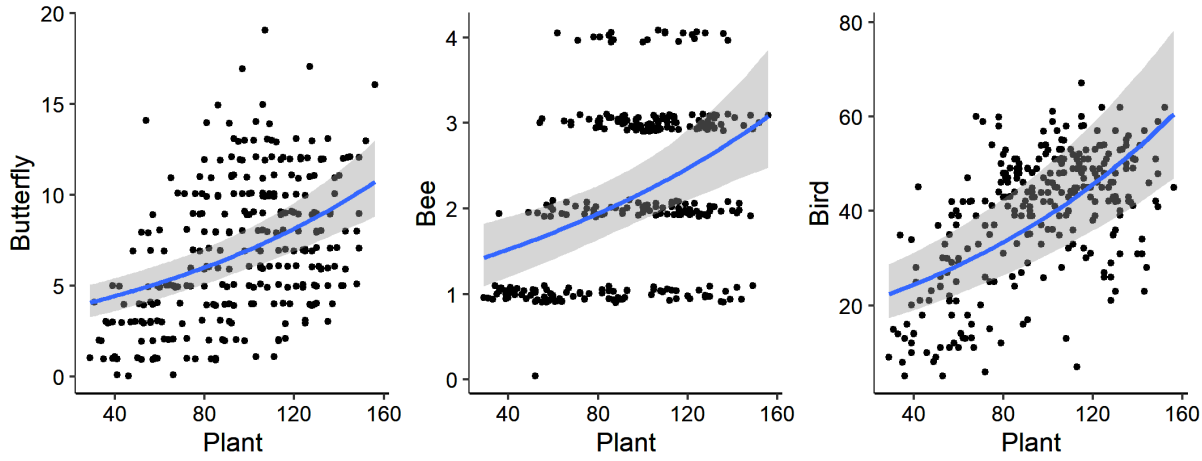

Figure 5.5: The predicted impact of plant diversity on butterfly, bee and bird diversity in our dataset. All diversities are positively correlated with each other. The points are randomly jittered on the vertical axis by 0.1 to reduce data overlap.

Plant diversity positively impacted the diversity of birds, bees and butterflies even after accounting for changes in precipitation and temperature (Figures 5.5, 5.6, Appendix G Figure 6). The best model by leave-one-out cross validation had temperature and precipitation as predictors of plant, butterfly, bee and bird diversity and plant diversity as a predictor of butterfly, bee and bird diversity. Removing plant diversity reduced the predictive capability of the model (elpd difference = 19.2 ±6.7, Appendix G Figure 7), but not as much as removing precipitation and elevation (elpd difference = 100.5 ±14.3). The predicted impact of going from the 53 to 132 plant species (the 10th to 90th quantile) if precipitation and temperature were kept constant (at 1500 mm and 9°C respectively) would be to increase butterfly richness by 1.2 species, bee richness by 0.5 groups and bird diversity by 13.2 species. This is in contrast to an expected increase in butterfly richness by 4 species, bee richness by 1 group and bird diversity by 25.8 species with the same increase in plant richness from the model without keeping constant temperature and precipitation.

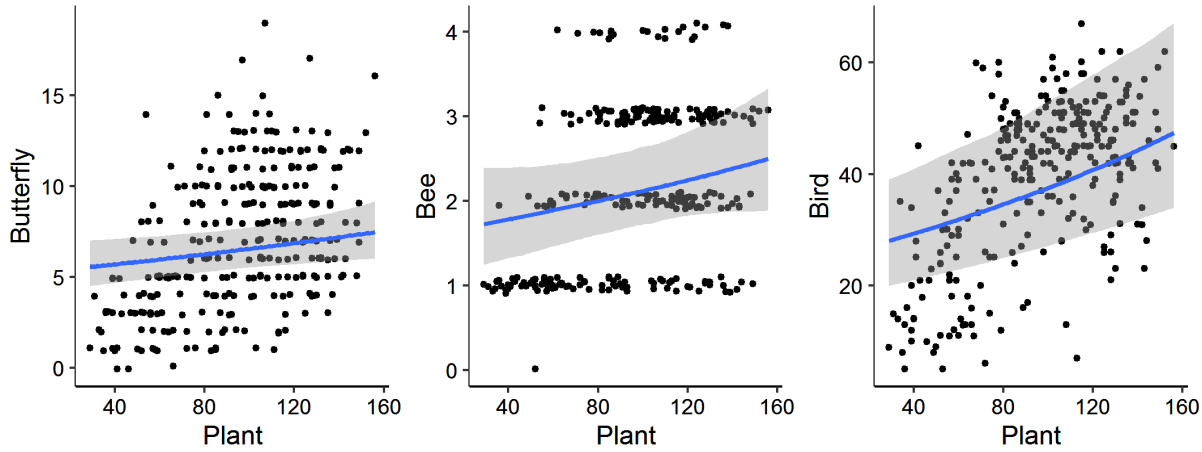

Figure 5.6: The predicted impact of plant diversity on butterfly, bee and bird diversity once changes in precipitation and elevation were included in the model. The points are randomly jittered on the vertical axis by 0.1 to reduce data overlap.

The positive influence of plant species richness upon bee and butterfly richness may be driven by the increased variety of food sources when more plants are present. The diversity of nectar producing plants and plants identified as important in the diets of lowland birds were highly positively correlated with overall plant species richness (spearman rho of 0.96 and 0.87 respectively). Using the species richness of nectar producing plants resulted in marginally better predictive performance for bees and butterflies but improvement was close to the margin of error (elpd difference for butterflies: 1.6 ± 1.5; bees: 0.5 ± 0.3). The richness of nectar producing plants was more closely related to butterfly richness than was the richness of plants important to butterfly larvae, but both performed in a similar way (elpd difference: 1.0 ± 1.5). The species richness of plants identified to be important in the diet of lowland birds did no better than overall plant richness at predicting bird richness (elpd difference: -0.3 ± 3.5).

5.3.2 Composition

5.3.2.1 Correlation

Plant community dissimilarities were positively associated with fungal, bacterial, heterotrophic protistan, bird and butterfly community dissimilarities (Figures 5.7, 5.8). Bacteria and heterotrophic protists showed a stronger correlation to plant composition than fungi did, with the Procrustes correlation statistic being 0.64, 0.65 and 0.55 respectively. All were significant at p = 0.001. Differences in heterotrophic protistan composition strongly tracked changes in bacterial composition, with a Procrustes correlation statistic of 0.93 (Figure 5.7). Fungal composition also correlated with bacterial composition, with a Procrustes correlation statistic of 0.71. Birds and butterfly community dissimilarity were more strongly related to plant community dissimilarity than the link between bee, hoverfly and plant dissimilarity. This was reflected by both the spearman rank correlations (Figure 5.8) and the Procrustes correlation statistic (0.73, 0.59, 0.23 and 0.32 for bird, butterflies, bees and hoverflies respectively). However, all the Procrustes analyses were found to be significant at p = 0.001.

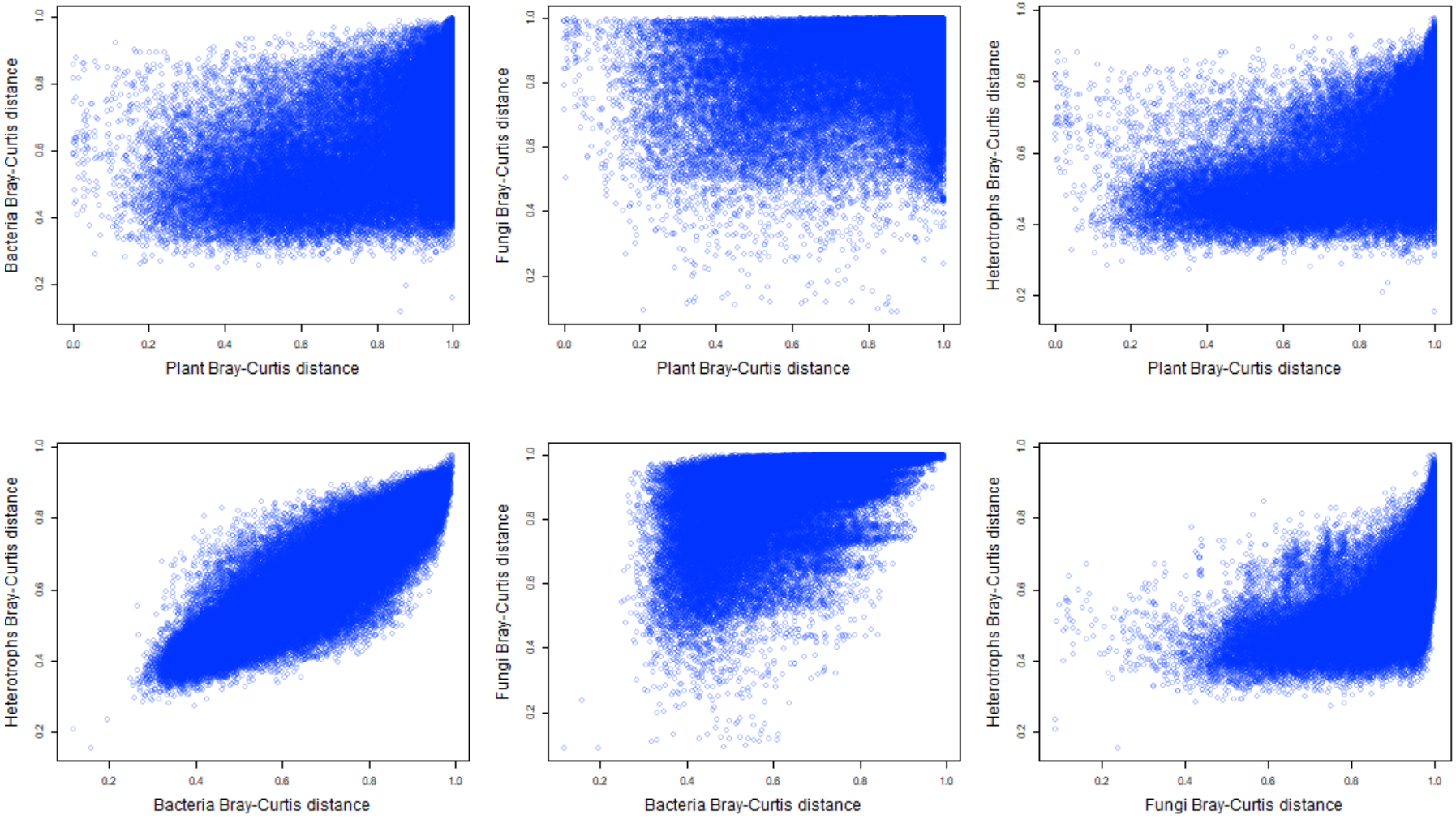

Figure 5.7: Bray-Curtis distance between belowground trophic communities are positively correlated.

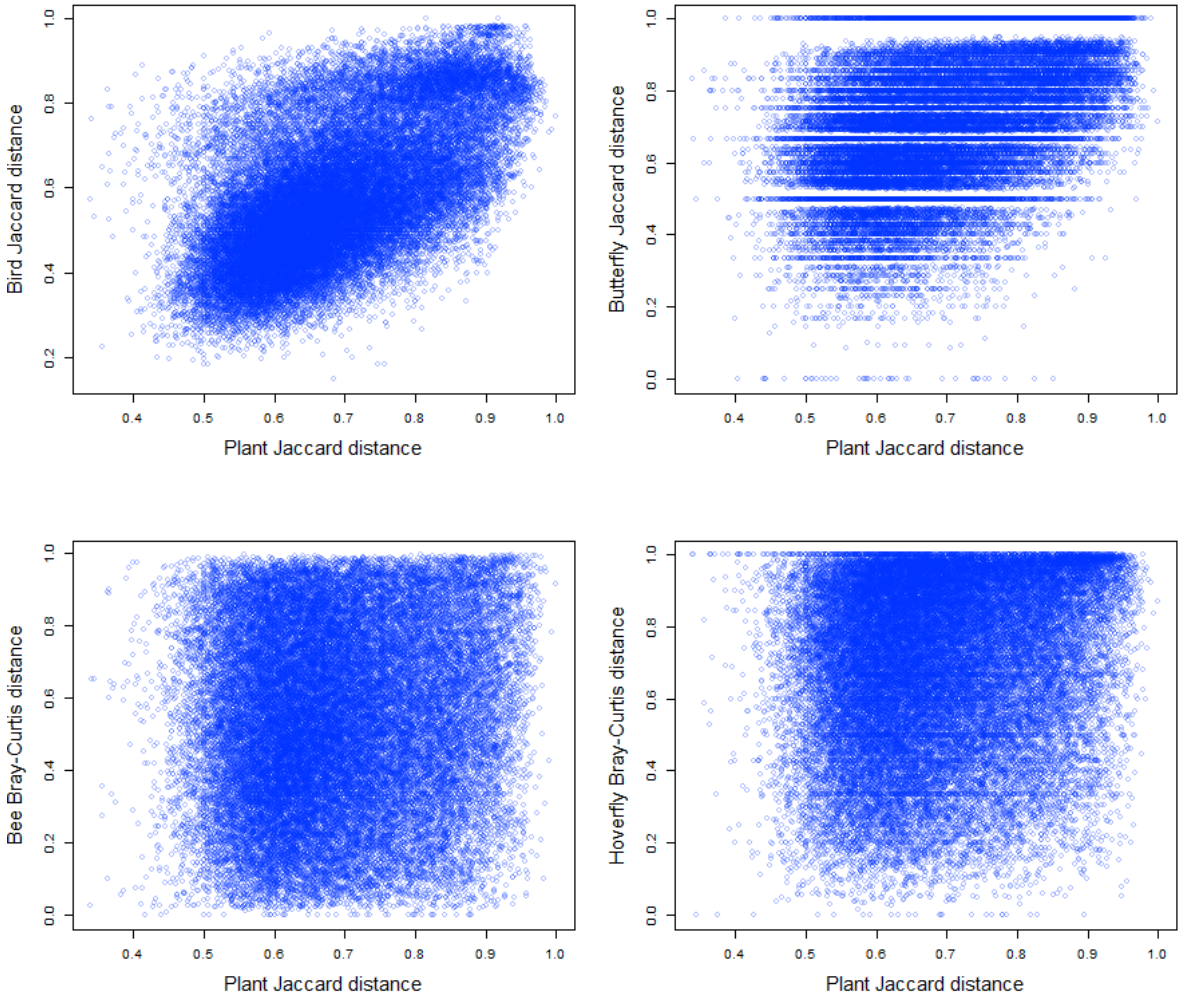

Figure 5.8: Correlation between plant community dissimilarity and bird and pollinator dissimilarity varies by group. Spearman rank correlations are 0.55 (birds ~ plants), 0.45 (butterflies ~ plants), 0.09 (bees ~ plants) and 0.15 (hoverflies ~ plants).

5.3.2.2 Accounting for environmental factors

Partitioning the variance described by the spatial, soil and plant community reveals that the plant community explains unique variance in the bacterial, fungal and heterotrophic protist communities (Figure 5.9). The plant community at the plot scale was partially explained by soil, climate and spatial factors but had considerable unexplained variance. Climate was initially included but did not explain any unique variance in the soil microbial communities so was omitted from this analysis. The pattern of variance explained was similar for bacteria and fungi, with the soil physicochemical properties and plant community explaining more variation than the distance between samples. However, there was a large proportion of variation that was

explained by soil, plant and spatial factors together. The heterotrophic protist community had a large proportion of their variance explained by variation in the bacterial community. Soil properties, spatial factors and the plant community explained limited variation once changes in the bacterial community were accounted for but did explain joint variation with the bacterial community.

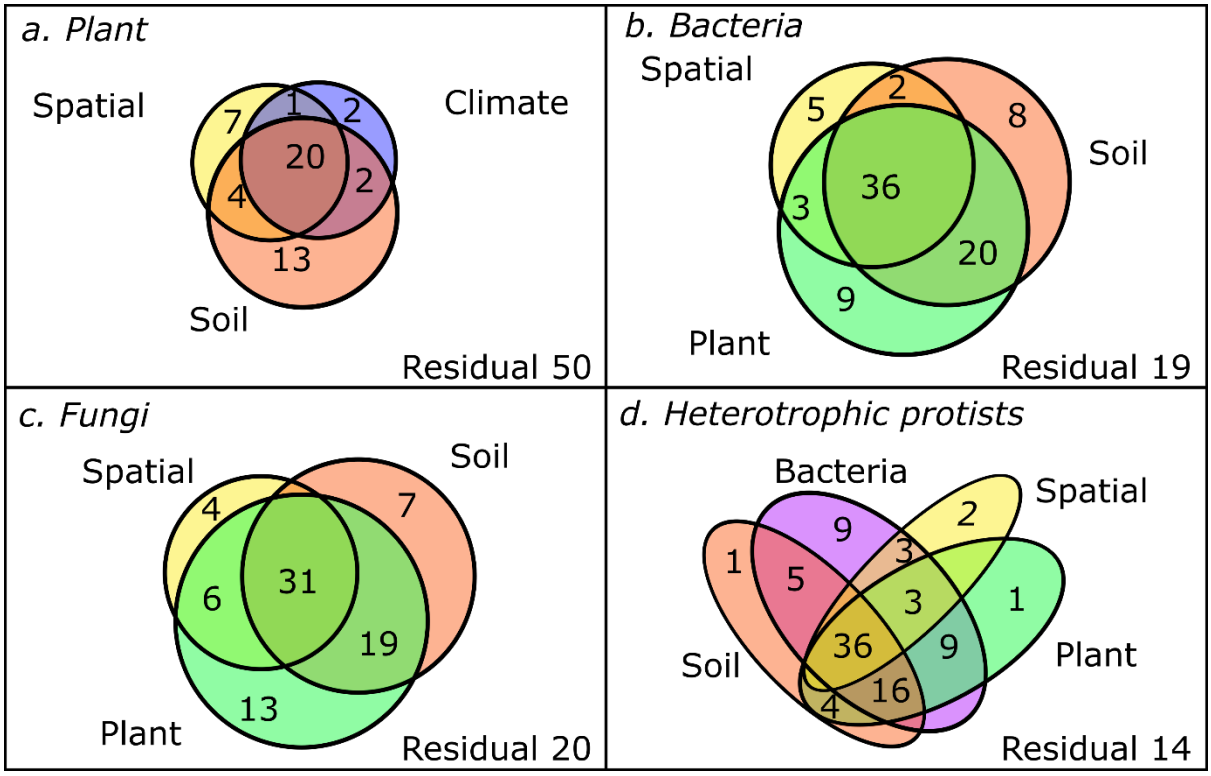

Figure 5.9: Variation partitioning of the effects of space, climate, and soil properties on the composition of the plant community (a), bacteria (b), fungi (c), and heterotrophic protists (d). Plant community composition is included as a predictor for soil microbes, and bacterial composition as a predictor of heterotrophic protists. The circles, or ellipses, are coloured by the identity of the predictor: spatial factors are yellow, soil properties are orange, climate is blue, plant community composition is green, and bacterial composition is purple. Circle areas are proportional to the relative importance of each predictor, with the overlap being positioned by eye to be roughly representative of the relative importance of each segment. The adjusted R^2 are written on or next to the corresponding segment with adjusted $R^2 < 0.01$ being omitted from the diagram.

The plant community explained some of the variance in bird, butterfly and bee and hoverfly community composition (Figure 5.10). The plant community at the 1 km square level was largely explained by variation in precipitation and temperature, with some effect of the distance between squares interacting with climate. The bird community was described fairly well by the plant community and the climate, whilst

also incorporating a spatial interaction between the two. The pollinator communities were explained relatively poorly, the plant community, climate and spatial factors do appear to be important together but overall there is little variation explained. Precipitation and temperature are less important to bees and hoverflies than they are to the other groups, with precipitation and temperature in general being the least important factor for the animal groups but much more important for plants. Spatial factors appear to be relatively more important for the animal groups than they are for the plants.

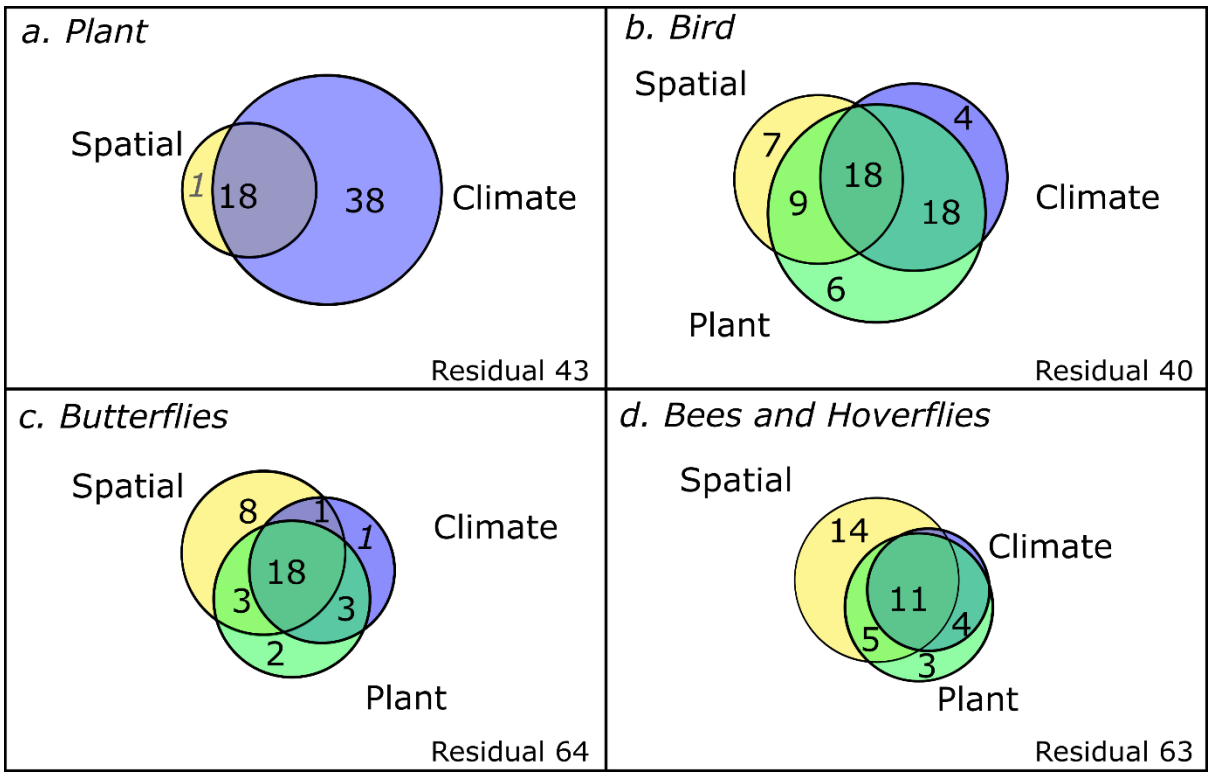

Figure 5.10: Variation partitioning of the effects of space, and climate on the composition of plants at the square level (a), birds (b), butterflies (c), and bees and hoverflies (d). Plant community composition is included as a predictor for bird, butterfly and bee and hoverfly composition. Spatial factors are represented by yellow circles, climate is represented by blue circles and plant community composition is represented by green circles. All other properties as in Figure 5.9.

5.3.3 Co-occurrence relationships

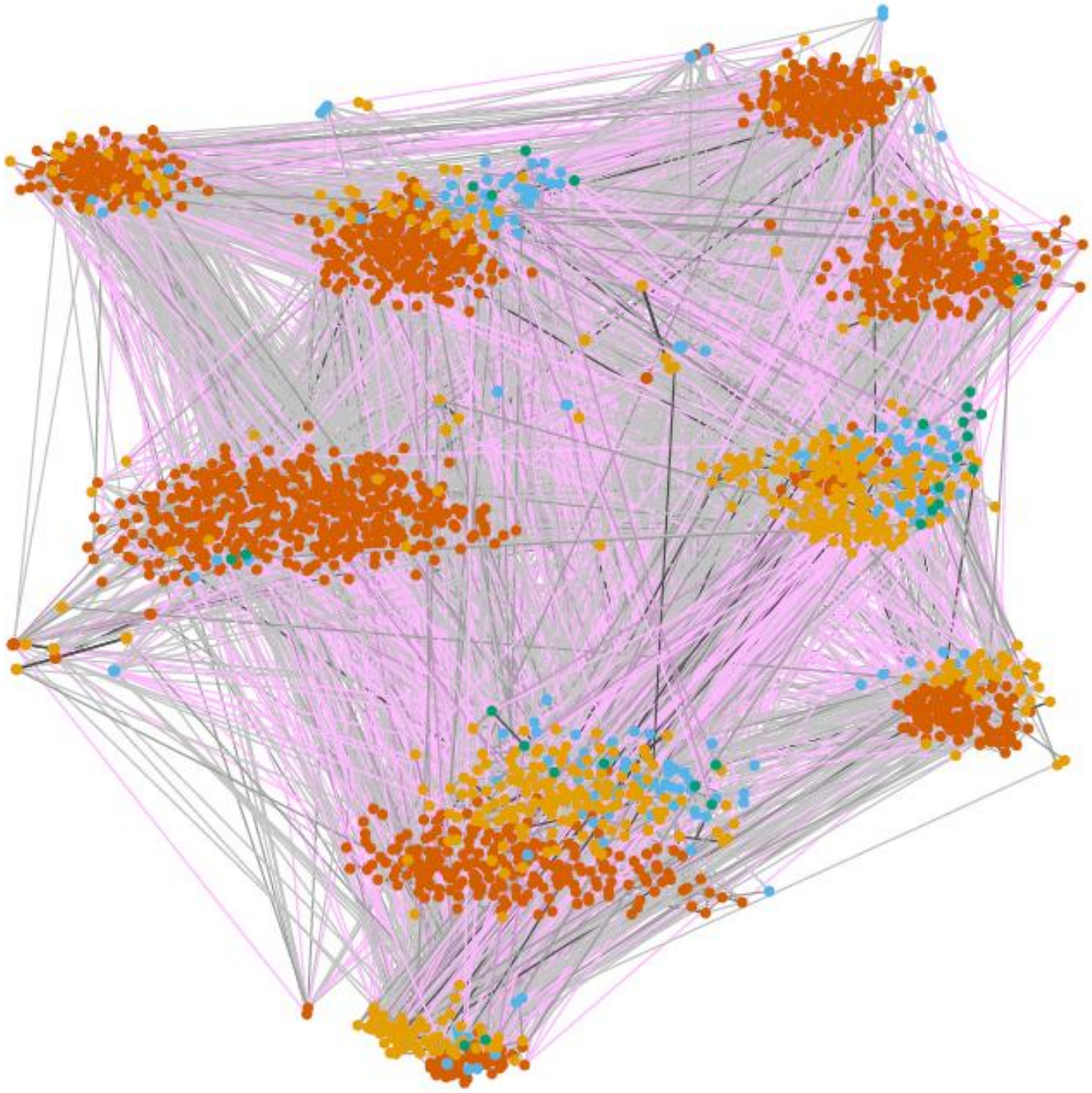

Figure 5.11: The co-occurrence network between bacterial, fungal, heterotrophic protist and plant taxa. The taxa are represented by circles and coloured by whether they are bacteria (red), fungi (blue), heterotrophic protists (orange), or plants (green). Negative links are shown in violet (dark violet if less than -0.1), and positive links are shown in grey (medium dark grey if greater than 0.1, and dark grey if greater than 0.2). Links with absolute value less than 0.05 are not shown for graphical simplicity but are included in all calculations.

Cluster analysis of the microbial and plant co-occurrence network created by SpiecEasi revealed the presence of a few clusters of taxa that occur in specific habitats (Figure 5.11). In total there were 1378 bacterial taxa, 529 heterotrophic protists, 188 fungal taxa (ITS only) and 28 plant taxa included in the analysis. SpiecEasi found 71144 links between the 2123 taxa of which two thirds were positive. Three quarters of the

links had particularly low weights between -0.05 and 0.05. Twenty-three clusters were identified, of which there were nine with over 100 members and 14 with less than 20 members. The largest, bacteria dominated, cluster towards the middle left in Figure 5.11 (cluster 20 in Appendix G Figure 8) contains many taxa specific to acidic environments, including a large proportion of acidobacteria and proteobacteria as well as bilberry (*Vaccinium myrtillus*) and purple moor-grass (*Molinia caerulea*). The second largest cluster (cluster 8, 393 taxa compared to 397 in cluster 20) below and to the right of the largest cluster contains more fungi, heterotrophic protists and several grasses and forbs that prefer more fertile and less acidic environments (Appendix G Figure 8). The third largest cluster is dominated by fungi, heterotrophic protists and plants (cluster 2), with a wide variety of fungal trophic modes and plant environmental preferences. Also of interest are two clusters tentatively identified as being related to waterlogged environments. This includes a cluster of taxa (cluster 12, at the base of Figure 5.11) that contains many obligate anaerobic bacteria such as Clostridia and Geobacter, many Heteromita protists, a limited number of saprotrophic fungi and two common wetland grasses (*Juncus effusus* and *Agrostis stolonifera*). In addition, cluster 17, towards the bottom right of Figure 5.11, also contains obligate anaerobes such as Clostridia but is dominated by the mesophilic spore-forming gram-positive Bacillales, many Heteromita and saprotrophic fungi.

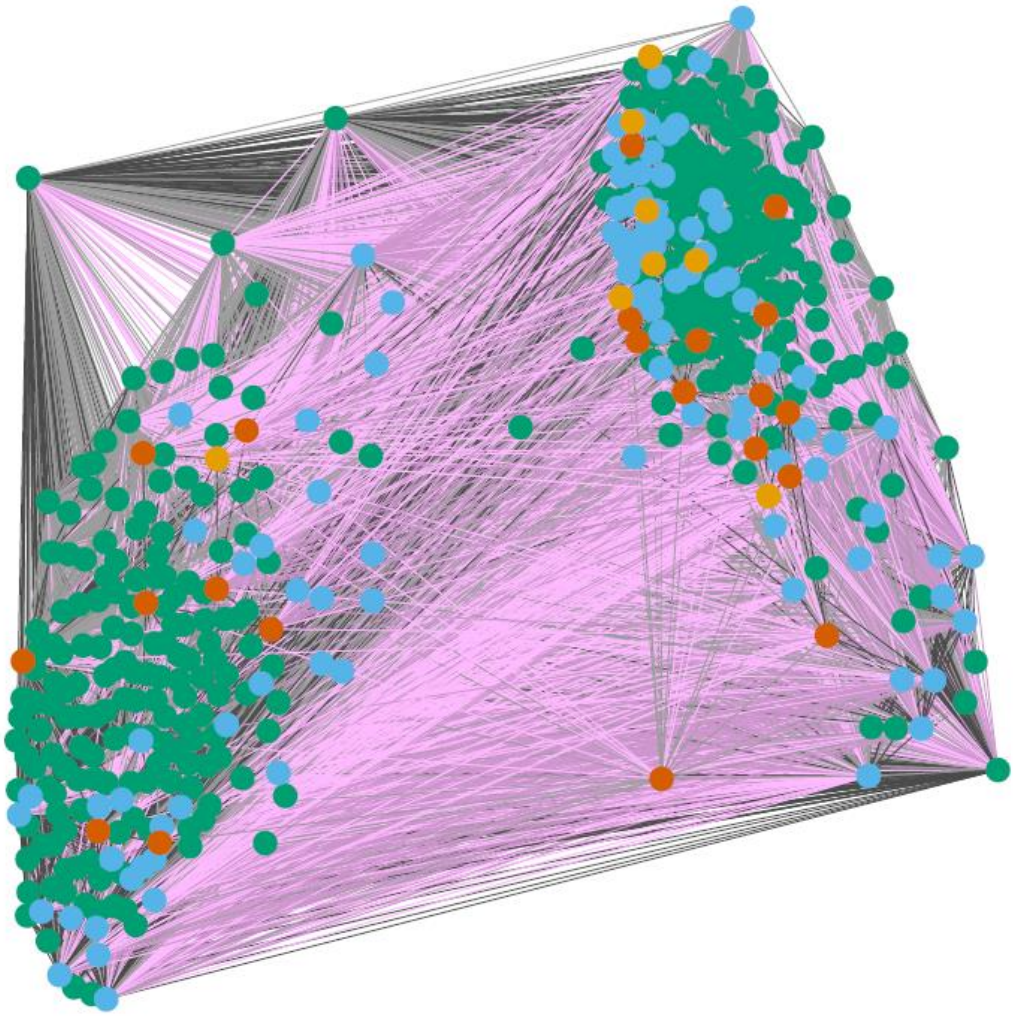

Figure 5.12: The co-occurrence network between plant, bird, butterfly, bee and hoverfly taxa. The taxa are represented by circles and coloured by whether they are plant (green), bird (blue), butterflies (red), or bees and hoverflies (yellow). Negative links are shown in violet (dark violet if less than -0.5), and positive links are shown in grey (medium dark grey if greater than 0.1, and dark grey if greater than 0.5). Links with absolute value less than 0.1 are not shown for graphical simplicity but are included in all calculations.

Joint species distribution modelling of the plant, bird and pollinator species revealed two main clusters of species response (Figure 5.12). In total 464 taxa were included in the analysis as they were present in sufficient sites to warrant robust analysis. The macrobial taxa included 335 plant species, 100 bird species, 22 butterfly species, 4 bee groups and 4 hoverfly groups. Overall, the proportion of the variance in species occurrence explained by rainfall and temperature was low (Appendix G Figures 9, 10). The residual associations between species could be largely split into two clusters

(Figure 5.12, Appendix G Figure 11), including a species cluster associated with more fertile landscapes, on the right of Figure 5.12, with many species that are more regulated by temperature than precipitation. The cluster includes all but one bee and hoverfly group, all the butterflies that are associated with gardens and hedgerows, plants that are more adapted to fertile conditions, and 64 bird species which had a large proportion of woodland species. The other large cluster on the left of Figure 5.12 included plant species that were adapted to less fertile conditions, 36 bird species of various habitat preference, grassland butterflies, and one hoverfly group (thin aphid eaters). The clusters do not separate out into groups containing plants, birds or pollinators only at this scale, and at the finer scale of cluster associations there are only a few small pockets of plant or bird co-occurring clusters.

5.4 Discussion

While we found that the diversities of above and below-ground taxonomic groups positively correlated with plant diversity, the direct effect of plants was much higher above-ground than below-ground. Our results that the positive correlation of plant and soil microbial diversity is driven by the changes in the soil environment are in agreement with previous work showing the importance of soil pH and fertility in jointly influencing plant and microbial diversity (Goberna et al., 2016; Tedersoo et al., 2016; Yashiro et al., 2018; Yuan et al., 2017). However, we did find that plant phylogenetic diversity positively influenced bacterial phylogenetic diversity at pH > 4, which was unexpected as many previous results have found that plant phylogenetic diversity is a poor predictor of soil microbial communities (Barberán et al., 2015; Goberna et al., 2016; Leff et al., 2018; Navrátilová et al., 2018). Both arbuscular and ecto-mycorrhizal fungi have been found to be positively related to plant phylogenetic diversity (Milcu et al., 2013; Nguyen et al., 2016). However, we found no relationship between fungal phylogenetic diversity and plant phylogenetic diversity. We found some indication that the correlation between plant and soil microbial diversity was due to a correlation between forb diversity and soil microbial diversity. Herbs have been found to be more strongly related to prokaryotic diversity in forest ecosystems (Wang et al., 2016), however, in our results it appears that the association is due to shared responses to environmental drivers rather than direct diversity relationships.

Increasing bird and pollinator diversity in conjunction with plant diversity was only partially confounded by the climate gradient, indicating that plant diversity has a direct impact upon bird and pollinator groups. This could be due to the provision of a greater variety of food and habitat sources, through increased landscape heterogeneity or potentially through the influence of an unidentified confounding variable such as anthropogenic activity (Potts et al., 2009; Stein & Kreft, 2014). Previous work upon this dataset has found that higher land-use intensity and habitat heterogeneity were positively associated with the species richness of plants, birds and butterflies (Maskell et al., 2019). Our result that the diversity of food source plants was a slightly better predictor than general plant species richness for bees and butterflies but not for birds suggests that the relative role of food and habitat provision, land-use intensity and habitat heterogeneity may differ by group. For birds it appears that provision of food sources is not the key driver within our data, indicating that the land-use intensity and habitat heterogeneity may be more important. The heterogeneity of the landscape, particularly the heterogeneity of the topography, land cover and vegetation, has previously been found in many areas to be positively associated with the diversity of plants, birds and pollinators (Hofer et al., 2011; Hofmann et al., 2017; St-Louis et al., 2014). Land-use intensity can positively influence the diversity of certain animals when land-use intensity shifts from low to medium. Our data contained few high-intensity farmland sites which we would expect to have lower biodiversity due to the unimodal relationship between land use intensity and biodiversity (Maskell et al., 2019; Maskell et al., 2013).

The very strong relationships between heterotrophic protistan diversity and composition and that of bacteria within our analysis most likely reflect the importance of the bacterial food source to heterotrophic protists. Bacteria and bacterivorous protists have been found to show similar seasonal patterns in grassland habitats, with the implication that they may be following a predator-prey cycle, but the major properties structuring heterotrophic protist communities in that grassland were spatial and soil edaphic properties (Fiore-Donno et al., 2019). The properties that influence protistan community structure do appear to be different across different ecosystems (Tedersoo et al., 2016). The lack of influence of plant species richness upon

heterotrophic diversity was in agreement with previous results on experimental manipulations of plant diversity (Dassen et al., 2017). Functional changes in plant communities have been linked to changes in heterotrophic protists (Dassen et al., 2017) but in at least some cases these appear to be mediated by changes in the bacterial community (Valencia et al., 2018). Our results clearly indicate a strong linkage between bacterial and heterotrophic protistan communities that could represent the dominant role of trophic dynamics over edaphic properties and plant inputs in structuring those communities.

The limited correlation between plant and soil microbial diversity we have found in our analysis may be related to the fact that our soil microbial communities are from the bulk soil and not the rhizosphere. However, even papers looking at the impact of plant species richness on bacterial and fungal richness in the rhizosphere have found no relationship, but have found a relationship between composition (Navrátilová et al., 2018; Singh et al., 2008). The impact of plants upon the soil will decrease as the distance from the root increases but note that the root distribution may cover far more distance than the aboveground plant distribution (Barberán et al., 2015; Hiiesalu et al., 2012). Soil organisms that are more dependent upon plant inputs and that are capable of growing through large volumes of soil, e.g. mycorrhizal fungi, might be expected to be show stronger relations with the plant community (Nguyen, Williams, et al., 2016). We found that there was a weak positive relationship between AM richness and plant diversity. This is consistent with previous results showing positive relationships between plant diversity and AM fungi (Hiiesalu et al., 2014), and may be related to the fact that the majority of the plants in our survey are herbs and grasses which more commonly form AM associations than other types of mycorrhizal associations (Wang & Qiu, 2006). The issue of scale and how this influences trophic interactions is key to understanding how the ecosystem works and the impact of different taxa upon each other and ecosystem function.

Our results indicate that the impact of plant diversity upon bacterial and fungal diversity is both minimal and dependent upon the environmental context. Unexpectedly, we found that the direction of impact of plant diversity upon both fungal and bacterial diversity reversed in low pH soils. The importance of the soil and

climatic environment in influencing the links between plant and soil microbial diversity and composition have been found previously in a variety of ecosystems (Delgado-Baquerizo et al., 2018; Gao et al., 2017; Waldrop et al., 2017). This suggests that plant diversity impacts soils in different ways depending upon the soil environment and particularly that conclusions regarding the impact of plant diversity within more commonly studied neutral soils cannot be extrapolated to other environments. This offers a cautionary note to taking the results of plant biodiversity experiments conducted on neutral grassland soils, e.g. the Jena experiment pH 7.1-8.4 (Weisser et al., 2017), and applying them outside that context. The low pH soils within our study are a reservoir of carbon that is of great relevance to global biogeochemical cycling and thus requires careful study and management (Cavicchioli et al., 2019; Ferretto et al., 2019).

We found that the composition of both above and belowground communities were influenced by plant community composition. The proportion of variance explained by plants was much higher in the bacterial, fungal and heterotrophic protist communities than in the bird, butterfly, bee and hoverfly communities. The finding that plant community composition explains bacterial, fungal and heterotrophic protistan composition is consistent with previous findings at the field (Leff et al., 2018), regional (Barberán et al., 2015; Chen et al., 2017; Yang et al., 2017), and global scales (Delgado-Baquerizo et al., 2018; Prober et al., 2015). The higher proportion of explained variance in bird composition compared to butterfly, bee and hoverfly composition was unexpected as pollinators have in general been found to be closely related to plant composition (Hofmann et al., 2017; Kearns & Oliveras, 2009; Weisser et al., 2017). Overall, very little of the variation in butterfly, bee and hoverfly composition was explained by climate, plant communities or spatial factors. This may be due to the description of the plant community through NMDS scores may not have been in sufficient detail to capture the plant-pollinator interactions. However, within the co-occurrence analysis there were several pollinator species and groups that did not show particularly strong relationships to any other taxa. Alternatively, anthropogenic influences or other factors not incorporated into our model may be highly important in driving pollinator community assembly.

The identification of clusters of co-occurring species within the microbial and the aboveground systems that correspond to particular habitat preferences indicate the importance of cross-domain interactions in structuring ecological communities. The identified clusters largely comprised of a mix of different domains and groups. Such diversity identified within clusters raises into question the concept of a "bacterial response" versus a "fungal response" to environmental stressors, when we can show that certain taxa within a domain are more related to taxa in another domain than they are to the rest of their domain. Some of the identified linkages may represent shared response to environmental drivers while some may be true mutualistic interactions across domains (Carr et al., 2019). We attempted to control for shared response to environmental conditions within the co-occurrence analysis of aboveground communities by modelling the response to temperature and rainfall and examining residual species correlations. However, the presence of any correlation link still must not be taken as the presence of a true mutualistic interaction without independent verification.

Both the different taxonomic groups and the ecosystem properties and functions that occur across the landscape are relevant to different spatial and temporal scales. Previous work has shown that the scale at which biodiversity is measured impacts the strength of the correlation found, with 10 km^2 areas showing the strongest relationship for aboveground diversity (Wolters et al., 2006). The life history traits of the community measure should be expected to impact the scale of interest, and within our dataset we have organisms that are largely limited to very short distances such as soil bacteria (Yang & van Elsas, 2018) ranging up to bird species that can travel vast distances. These differing communities are both intrinsically valuable and underpin different ecosystem services that operate at different scales, ranging from nitrogen mineralisation in a grain of soil through to provision of recreational activities across the landscape. Our results indicate that the different taxonomic groups, and therefore their impact, have limited areas in which they coincide. The decoupling of aboveground and belowground diversity that we have found indicate that interventions targeted to the plant community will have predictable impact upon the bird and pollinator diversity but shift belowground composition rather than diversity.

Soil microbial composition is more responsive to aboveground properties and has been found in some cases to be more relevant to soil functions (Delgado-Baquerizo et al., 2017; Zheng et al., 2019). Clearly our study is observational in nature and interpreting our results in a causal nature requires careful consideration of the potential other mechanisms underlying the biodiversity patterns that we have uncovered. Our results do, however, provide insight into the ecological communities across a wide variety of habitats and environmental conditions with potential implications in land management across the temperate zone.

5.5 Conclusions

The diversity of soil microbial groups are strongly correlated with each other and only weakly correlated with plant and aboveground biodiversity across Wales. Plant diversity influences bird and pollinator diversity even when accounting for shared response to precipitation and temperature. However, plant composition much more strongly influences soil bacterial and fungal composition than it does bird and pollinator composition. Heterotrophic protistan diversity and composition strongly tracks bacterial diversity and composition. Overall, there appear to be ecological communities that have developed across Wales that comprise diverse membership across various domains of life. In particular, the communities in high vs low pH and fertility environments are distinct in their composition and diversity. Collectively, the data provide an insight into aboveground and belowground biodiversity relationships across diverse habitats, revealing clear associations and divergences between the alpha and beta diversity of all domains of life in terrestrial habitats.

5.6 References

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CHAPTER 6

Long term drought and warming alter soil bacterial and fungal communities in an upland heathland

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Contribution statement:

Seaton assisted in designing the sampling scheme, carried out the soil sampling, all statistical analysis and drafted the initial manuscript. Goodall carried out the DNA extraction, primer design, library generation, sequence data generation and the bioinformatics pipelines under the direction of Griffiths. Reinsch and Emmett maintain the Clocaenog experimental field site. White carried out the fungal root colonisation assays under the direction of Smith. Reinsch, Jones, Emmett, and Robinson conceived this project and assisted in designing the sampling scheme. Reinsch, Griffiths, Jones, Robinson and Creer revised the manuscript for important intellectual content.

Abstract

Understanding how climate change could impact ecosystems globally is essential in order to mitigate and adapt to future threats. The response of soil microbial communities to a changing climate will impact global biogeochemical cycles, potentially leading to positive and negative feedbacks as carbon and other greenhouse gases are emitted. However our understanding of how soil microbial communities respond to climate change and the implications of these changes for future soil function is limited. Here we assess the response of soil bacterial and fungal communities to long-term experimental climate change in a heathland organo-mineral soil ecosystem. We assessed samples at two depths, from plots undergoing 4- and 18-years of *in-situ* summer drought or warming treatments and examined the microbial communities using Illumina sequencing of the 16S rRNA gene and ITS2 region. We also assessed the colonisation of *Calluna vulgaris* roots by ericoid and dark septate endophytic (DSE) fungi using microscopy after 16 years of treatment. We found significant changes in both the bacterial and fungal communities in response to drought and warming, partially mediated by changes in soil pH and electrical conductivity. Changes in the bacterial and fungal communities were more pronounced after a longer period of climate manipulation. Additionally, the subsoil communities of the long-term warmed plots became similar to the topsoil, indicating a change in the depth stratification of the microbial community. Ericoid mycorrhizal colonisation decreased with depth while DSEs increased, however these trends with depth were removed by warming. We largely ascribe the observed changes in microbial communities to shifts in plant cover and subsequent feedback on soil physicochemical properties, especially pH. Our results demonstrate the importance of considering changes in soil microbial responses to climate change across different soil depths and after extended time scales in heathland ecosystems. Alterations in root abundance, their associated symbionts, together with general shifts in microbial community structure also implies significant alterations in soil functioning under prolonged warming and drought.

6.1 Introduction

Climate change has and will continue to fundamentally alter global ecosystem functioning, and understanding how ecosystems will respond to future change is essential for societal adaptation as well as mitigation. Soils are a source of major uncertainty in future earth predictions, as we still do not know in sufficient detail how soil biogeochemical cycling, hydrology or biology will change in response to climate change and how these changes will alter climatic feedbacks. The potential for soils to accentuate or mitigate future climate change is in large part due to the vast quantities of carbon that is currently stored in the soil system (~2400 Pg, three times as much as is in the atmosphere) (Batjes, 1996; Stocker et al., 2013). In order to predict how soil systems will change in the future, we have to take into account how climate alters both the soil physicochemical environment as well as changes in the soil biota (e.g. Robinson et al., 2019).

Soil microbial communities have responded to climate change manipulations across various experimental systems (Cavicchioli et al., 2019; Jansson & Hofmockel, 2019). The response of microbial communities to the different climate change related stressors is dependent on the type of climatic stress, the ecosystem type and the identity of the microbial communities. There have been recent suggestions that microbial responses to drought are phylogenetically conserved (Amend et al., 2016); though this has not been evidenced by another analysis of multiple independent global studies (Oliverio et al., 2016). In general, warming has a stronger impact on fungi than bacteria (García-Palacios et al., 2015), and stronger impacts on microbial abundances in colder regions (Chen et al., 2015). Meta-analyses of the impact of altering precipitation on microbial abundance and composition found that the impact on biomass depended on the climate, with high precipitation areas being more responsive to drought and low precipitation areas being more responsive to increased precipitation (Ren et al., 2018, 2017; Zhou et al., 2018). Drought has been found in temperate heath to result in increased fungal dominance (Haugwitz et al., 2014), and in grassland ecosystems fungi showed higher resilience to drought than bacteria (de Vries et al., 2018).

The response of soil microbial communities to climate change has implications for various essential ecosystem functions, including the provision of nutrients to plants through mycorrhizal associations. The response of mycorrhizal associations to experimental drought or warming varies according to the specific type of mycorrhiza (Binet et al., 2017; Olsrud et al., 2009). There is some evidence that changes in mycorrhizal associations in response to climate change may be more related to changes in plant composition than to changes in mycorrhizal interactions (Rudgers et al., 2014). Studies across a variety of climates and ecosystem types have found that altering precipitation can impact the extracellular enzyme activities within soils (Ren et al., 2017), resulting in impacts on soil and root respiration and associated soil carbon loss (Crowther et al., 2016; Ren et al., 2018). The impact of drought upon soil respiration has been found to be dependent on the local climate, with high precipitation areas being more responsive (Reinsch et al., 2017). Drought has been found to affect the microbial community impact on litter decomposition across various studies (Allison et al., 2013; Martiny et al., 2017; Santonja et al., 2017; Tóth et al., 2017). The legacy of global change persists within the microbial community for several years and impacts their ability to carry out key functions in response to new or altered environments (Martiny et al., 2017).

The increasing awareness of the impact of legacy effects upon the ability of an ecosystem to respond to future change makes the use of long term ecological experiments increasingly important. Long term climate change has been found to impact plant communities (Andresen et al., 2016; Fridley et al., 2011), soil respiration (Crowther et al., 2016; Domínguez et al., 2016), hydrological behaviour (Robinson et al., 2016), soil mesofauna (Holmstrup et al., 2013; Petersen, 2011) and soil microbial communities (Rousk et al., 2013; Sayer et al., 2017). Importantly, many of these impacts emerge only after years of experimental treatment (e.g. Andresen et al., 2016), indicating how essential long term experiments are for evaluating future change. Within this study we will look at the climate manipulation experiment in the Clocaenog Forest, NE Wales, UK which has imposed summer drought and warming treatments over an organo-mineral heathland soil since 1999. There has been a rise in respiration in the treatments compared to the controls (Reinsch et al., 2017), which

has not been matched by changes in the plant community (Kröel-Dulay et al., 2015). Previous work on this site has found that drought could be impacting the summer fungal community only (Toberman et al., 2008), and other studies show limited impact of the treatments upon microbial biomass, extracellular enzyme activity, microbial community as measured by PFLA analysis and microbial growth rates (Domínguez et al., 2017; Rousk et al., 2013). Within this study our aims were to characterise the winter bacterial and fungal communities after four versus eighteen years of drought and warming, to see if the microbial communities are altered by the legacy of repeated drought and warming, to assess whether the microbial response can be cleanly split into a bacterial versus a fungal response through identifying co-occurrence patterns; to compare changes in the bulk community composition to changes in mycorrhizal associations, and to see if these changes were associated with changes in the soil physicochemical environment. Our hypotheses were:

1. The bacterial and fungal communities would be different after the legacy of repeated summer drought and warming compared to control.
2. The impact of climate treatments on microbial community differs after eighteen versus four years of treatment, and would differ in the topsoil compared to the subsoil.
3. The bacterial and fungal communities would show differing responses to the treatments, with limited interactions across the Kingdoms identified through network analysis.
4. The root fungal associations would be impacted by treatment in a manner similar to that of the bulk soil.
5. The changes in microbial community composition with treatment would be partially mediated by changes in soil pH and water content.

6.2 Methods

6.2.1 Site description and sampling

Long term climate manipulations were carried out in North Wales on a peaty podzol. The vegetation is dominated by *Calluna vulgaris*, and we sampled ~8 cm of the

carbon-rich topsoil layer and an underlying ~4 cm deep layer of gley subsoil (Table 6.1). Climate manipulations started in 1999, and consisted of a summer drought treatment, a warming treatment as well as un-manipulated control plots. There are three replicate plots per treatment. The treatments are imposed using a retractable roof system, with drought plots having the roofs cover the plots during summer rainfall events (~54% of summer rainfall is excluded) and the warming plots having the roofs cover the plots overnight to keep in the heat. A full description of the experimental set-up is provided in Beier et al. (2004).

Table 6.1: Site characteristics

Coordinates	53°03'N 3°28'W
MAP (mm)	1263
MAP reduction in drought plots (%)	25
MAT (°C)	7.4
MAT increase in warming plots (°C)	0.2
Dominant plant species	*Calluna vulgaris*
Soil type (FAO)	Peaty podzol
Topsoil organic matter (%)	89
Subsoil organic matter (%)	37

Soil samples were collected in February 2003 and 2017 using a stainless steel auger. Samples were collected from both the organic topsoil and gley subsoil for both years, and in 2017 the 2 cm interface between the two soil layers was analysed separately. The samples for each plot were bulked together for topsoil and subsoil in 2003; while three soil cores per plot from 2017 were analysed with the three depths separately. In total, there were 15 samples from 2003 and 78 from 2017, as some samples were not able to be included. Soil pH and EC were measured on frozen 2017 soil samples using a Corning 220 pH meter (VWR combination electrode for pH and EC 662-1805; Jenway 4510). Soil pH was measured from a 1:2.5 (w/v) soil-to-0.01 M $CaCl_2$ suspension after equilibration for 0.5 h.

6.2.2 Molecular analyses of soil microbial communities

6.2.2.1 DNA extraction and sequencing

DNA was extracted from 0.2 g frozen field moist soil using a Powersoil® DNA Isolation Kit (Mo Bio Laboratories Inc., Carlsbad, CA) according to the manufacturer's instructions. Amplicons were generated using a 2-step amplification approach, using Illumina Nextera tagged primers. Bacterial 16S V4 primers 515f GTGYCAGCMGCCGCGGTAA and 806r GGACTACNVGGGTWTCTAAT (Walters et al., 2016), and Fungal ITS primers GTGARTCATCGAATCTTTG and TCCTCCGCTTATTGATATGC (Ihrmark et al., 2012) were each modified at 5' end with the addition of Illumina pre-adapter and Nextera sequencing primer sequences. Amplicons were generated using a high-fidelity DNA polymerase (Q5 Taq, New England Biolabs). After an initial denaturation at 95°C for 2 minutes, PCR conditions were: denaturation at 95°C for 15 seconds; annealing at temperatures 55°C and 52°C for 16S and ITS reactions respectively; annealing times were 30 seconds with extension at 72°C for 30 seconds; repeated for 25 cycles. A final extension of 10 minutes at 72°C was included.

PCR products were cleaned using a ZR-96 DNA Clean-up Kit (Zymo Research Inc., Irvone, CA) following manufacturer's instructions. MiSeq adapters and 8nt dual-indexing barcode sequences were added during a second step of PCR amplification. After an initial denaturation 95°C for 2 minutes, PCR conditions were: denaturation at 95°C for 15 seconds; annealing at temperatures 55°C; annealing times were 30 seconds with extension at 72°C for 30 seconds; repeated for 8 cycles with a final extension of 10 minutes at 72°C.

Amplicon sizes were determined using an Agilent 2200 TapeStation system. Libraries were normalized using SequalPrep Normalization Plate Kit (Thermo Fisher Scientific), quantified using Qubit dsDNA HS kit (Thermo Fisher Scientific) and pooled together at equal concentrations. The pooled library was diluted to achieve 400 pM in a 40 µl volume after denaturation and neutralisation. Denaturation was achieved with 4 µl 2 M NaOH for 5 minutes followed by neutralisation with 4 µl 2 M HCl. The library was then diluted to its load concentration of 14 pM with HT1 Buffer and 5% denatured PhiX control library. A final denaturation was performed by heating to 96°C

for 2 minutes followed by cooling in crushed ice. Sequencing was performed on an Illumina MiSeq using V3 600 cycle reagents. The DNA sequences are available on the European Nucleotide Archive under primary accession reference PRJEB33721 (Appendix J).

6.2.2.2 Molecular Bioinformatics

Illumina demultiplexed sequences for 16S and ITS were processed separately in R using DADA2 (Callahan et al., 2016) to quality filter, merge, denoise and assign taxonomies as follows:

Amplicons reads were trimmed to 270 and 220 bases, forward and reverse respectively. Filtering settings were maximum number of Ns (maxN) = 0, maximum number of expected errors (maxEE) = c(3, 5), and amplicon primer sequences removed using trimLeft = c(20, 20). Sequences were dereplicated and the DADA2 core sequence variant inference algorithm applied. Forward and reverse reads were then merged using mergePairs function to produce actual sequence variants (ASVs). Sequence tables were constructed from the resultant ASVs and chimeric sequences were removed using removeBimeraDenovo default settings. ASVs were subject to taxonomic assignment using assignTaxonomy at default settings; training databases were GreenGenes v13.8 (DeSantis et al., 2006; McDonald et al., 2012) and Unite v7.2 (Kõljalg et al., 2005) for 16S and ITS respectively.

6.2.3 Root fungal colonisation

Soil cores for root assays were extracted on April 2015. Each plot had a single 8 cm diameter core taken to 8 cm depth. The cores were cut into 1 cm subsections, soaked in tap water and *Calluna vulgaris* roots removed by hand and washed to remove soil particles. Root length, diameter and number of tips were measured using WinRHIZO version 3.2 on a flatbed scanner. Proportional colonisation of ericoid mycorrhizae (ErM) and dark septate endophytes (DSE) was estimated using the magnified intersection technique (McGonigle et al., 1990). Roots were bleached in 10% KOH for 20 h and then stained with a 5% vinegar-ink solution (Arndal et al., 2013; Vierheilig et al., 1998). Roots were cut to 1-2 cm in length and 2 mm passes made along each root length. At the end of each pass all cells were examined for ErM colonisation or the

presence of DSE hyphae. ErM colonisation was categorised as 0%, 0-1%, 1-10%, 10-50%, 50-90% and 90-100% colonisation based upon the classification system of Trouvelet et al. (1986). DSE proportional colonisation was calculated as the number of colonised intervals divided by the total number of intervals. Root biomass, length and fungal colonisation data is available online at the NERC Environmental Data Centre (Appendix K, White et al., 2019).

6.2.4 Statistics

All statistics were performed in R version 3.6.0 (R Core Team, 2019). Bacterial taxa were rarefied to 25000 reads 100 times using the vegan R package (Oksanen et al., 2018) and the rounded average used for calculation of richness and diversity indices. The same procedure was used for fungal taxa with rarefaction to 10000 reads. The cut-offs for rarefaction were identified based on evaluation of the read depths of the samples and removal of the samples with considerably lower read depths than the rest of the data (Salter et al., 2014). The effect of climate treatment, depth and year of collection upon diversity was calculated using ANOVA, and the best model chosen based on AICc (Mazerolle, 2016). Two-dimensional NMDS ordination on the Bray-Curtis distances between taxa based on the rarefied data was used to characterise community composition. The response of community composition to treatment was analysed using PERMANOVA, the homogeneity of dispersion assumption was shown to hold at $p > 0.1$. All figures were plotted using ggplot2 (Wickham, 2016)

Co-occurrence networks were constructed using the SpiecEasi package in R (Kurtz et al., 2015). Bacterial and fungal taxa that appeared in over 50% of the 2017 samples were used simultaneously to construct networks that contained intra- and inter-kingdom co-occurrence relationships for each of the control, warming and drought treatments (Tipton et al., 2018). The network was then plotted and its characteristics determined using the igraph package (Csardi & Nepusz, 2006). Node degree and betweenness were used to identify the key taxa within the network.

Indicator taxa for the warming and drought treatments were identified through analysing the differential relative abundance of taxa by treatment using the DESeq2 method (Love et al., 2014) and the apeglm shrinkage estimator (Zhu et al., 2018). Year,

climate treatment and soil depth were included as additive terms within the DESeq2 model. The presence of any interaction terms was tested using the likelihood-ratio test, and the absence of any taxa responding to interaction term compared to the reduced model taken as confirmation that no interaction occurred.

The impact of depth and treatment on DSE colonisation was modelled using a Bayesian regression model with a zero-inflated beta distribution using the brms package (Bürkner, 2017). The impact of depth and treatment upon ErM colonisation was modelled using the brms package as well, as a cumulative ordinal regression model assuming the latent variable to be normally distributed (Bürkner & Vuorre, 2019). Core identity was used as a random effect, and an interaction between depth and treatment was assumed.

The relative impact of soil pH, EC, soil moisture and temperature on the soil microbial community composition was established using multivariate Bayesian regression models within brms. Soil moisture and temperature were taken from in situ sensors on the day of sampling. Soil temperature is measured at 5 cm depth with a T107 sensor from Campbell scientific. Soil moisture is measured as volumetric water content with CS616 sensors from Campbell scientific. The numeric predictors (pH, temperature, moisture) were first transformed by centring the mean at zero and dividing by twice the standard deviation so they were on a similar scale to any binary predictor. Models were compared using leave-one-out cross validation to estimate pointwise out-of-sample prediction accuracy (Vehtari et al., 2017). For the models predicting NMDS scores the models were fit with both NMDS scores as response variables simultaneously so that the residual correlation between the two could be estimated. Data from 2017 only was used within these models due to the absence of pH and EC values from 2003.

6.3 Results

6.3.1 Microbial diversity

There were 8818 unique sequences from a total of 4,514,220 reads returned by the 16S primer, of which 8673 were matched to bacteria and 138 to archaea. There were 2539

unique ITS sequences from a total of 5,711,663 reads, of which 2377 matched to fungi. The bacterial data was rarefied to 25000 reads, and the fungal to 10000 reads. This resulted in 28 samples of the 93 failing to amplify enough fungal DNA for inclusion in the analysis, of which 12 samples had 0 reads and 14 samples had less than 200 reads. Of the failed samples, 15 were from warming plots, compared to 5 and 8 from control and drought plots respectively. The median read depth of successfully amplified samples was 41110 for bacteria (Q1: 35623, Q3:50001), and 87494 for fungi (Q1: 43626, Q3: 112151).

The diversities of the fungal and bacterial communities varied across the different treatments, soil depths and the two sampling periods, however there was limited consistency in the response. There was a significant effect of soil depth, sampling year and climate treatment upon both bacterial and fungal richness ($p = 0.0004$ and $R^2 = 0.30$; $p < 0.0001$; $R^2 = 0.68$ respectively; Figure 6.1). The impact of depth or treatment did not change by year for bacteria but did for fungi ($\Delta AICc < 2$). The response of bacterial and fungal Shannon and inverse Simpson diversity indices to depth, year and treatment followed the same patterns as richness (Appendix H Figures 1 and 2). Overall, while the models found significant impacts there was no conclusive pattern of the effect of treatment, depth or year on microbial diversity.

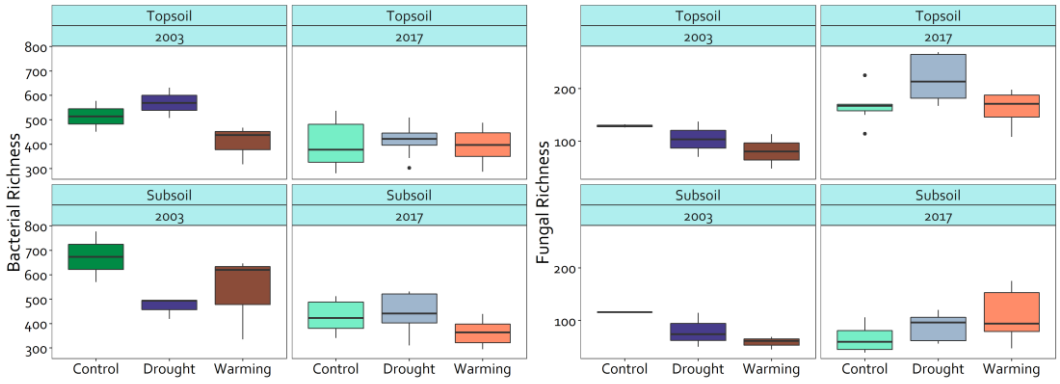

Figure 6.1: Change in bacterial richness per 25000 rarefied reads (a) and fungal richness per 10000 rarefied reads (b) with soil depth (topsoil, subsoil), year (2003, 2017) and treatment (Control, Drought, and Warming).

6.3.2 Microbial community composition

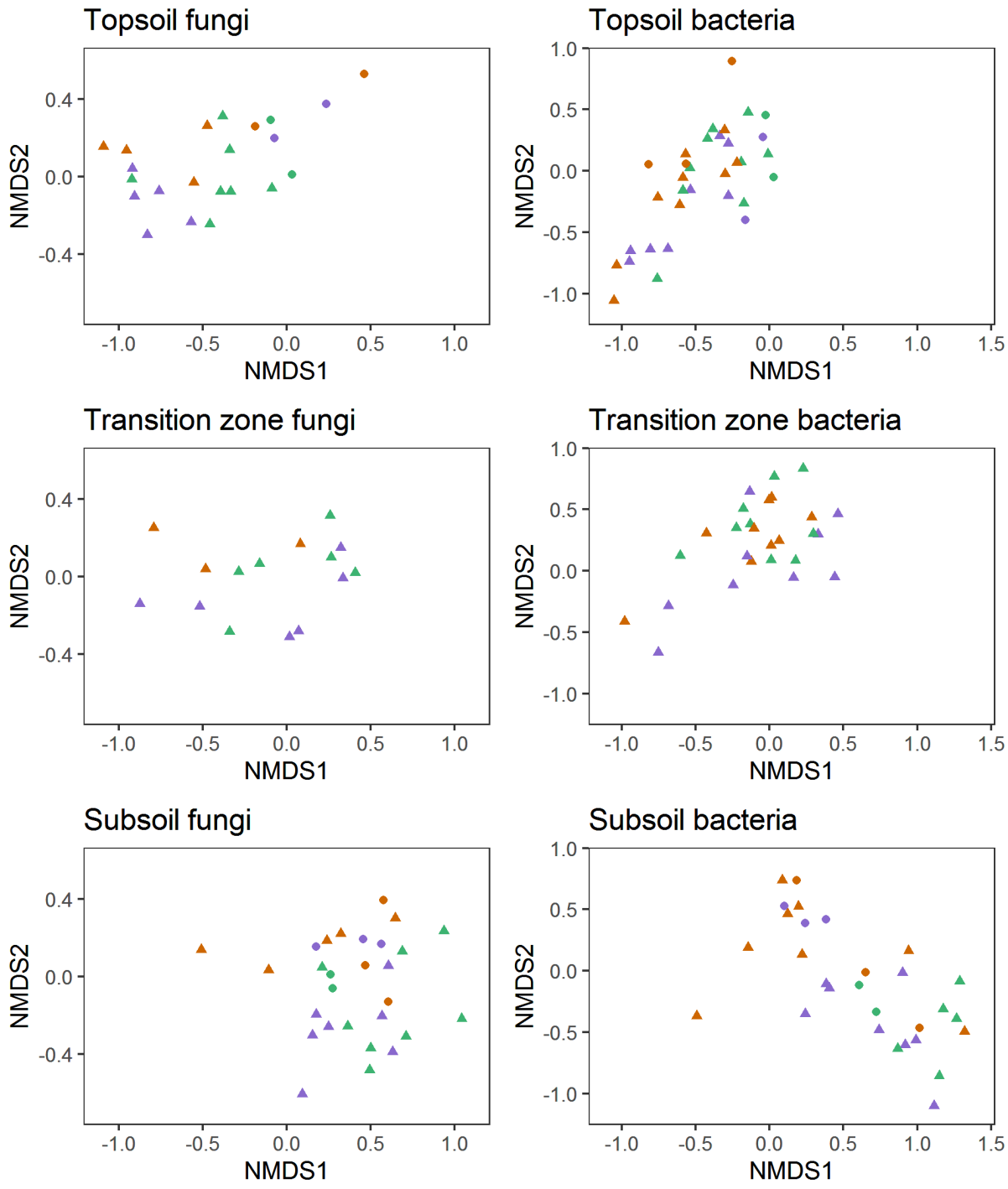

Figure 6.2: NMDS of the fungal (left) and bacterial communities (right) for 2003 (circles) and 2017 (triangles). Note that changes by treatment are more evident for the 2017 samples than the 2003. Results are shown split by depth for graphical clarity only. Topsoil is shown in the upper panels, transition zone in the centre and subsoil in the lower panels. Control = green, drought = purple, warming = orange.

The microbial community composition was impacted by depth and treatment in both sampling periods, with a clear separation by depth and some separation by treatment

(Figure 6.2). The warming subsoil shows greater similarity to the topsoil than to the subsoil of the control and drought plots. There was a significant effect of both soil depth and climate treatment upon both bacterial and fungal composition in 2017, but no significant interaction (Bacterial PERMANOVA: $R^2 = 0.219$, $p = 0.001$ (Depth), $p = 0.002$ (Treatment), $p = 0.12$ (Interaction); Fungal PERMANOVA: $R^2 = 0.186$, $p = 0.001$ (Depth), $p = 0.002$ (Treatment), $p = 0.38$ (Interaction)). The 2003 samples were largely clustered within the 2017 samples, with less visible impact of depth and treatment. However, at the phylum level there was limited change by depth, treatment or year for both bacteria and fungi (Appendix H Figures 3 and 4). The majority of fungi were not able to be assigned to any trophic mode and there was limited evidence of change in trophic modes to treatment (Appendix H Figure 5).

6.3.2.2 Network analysis

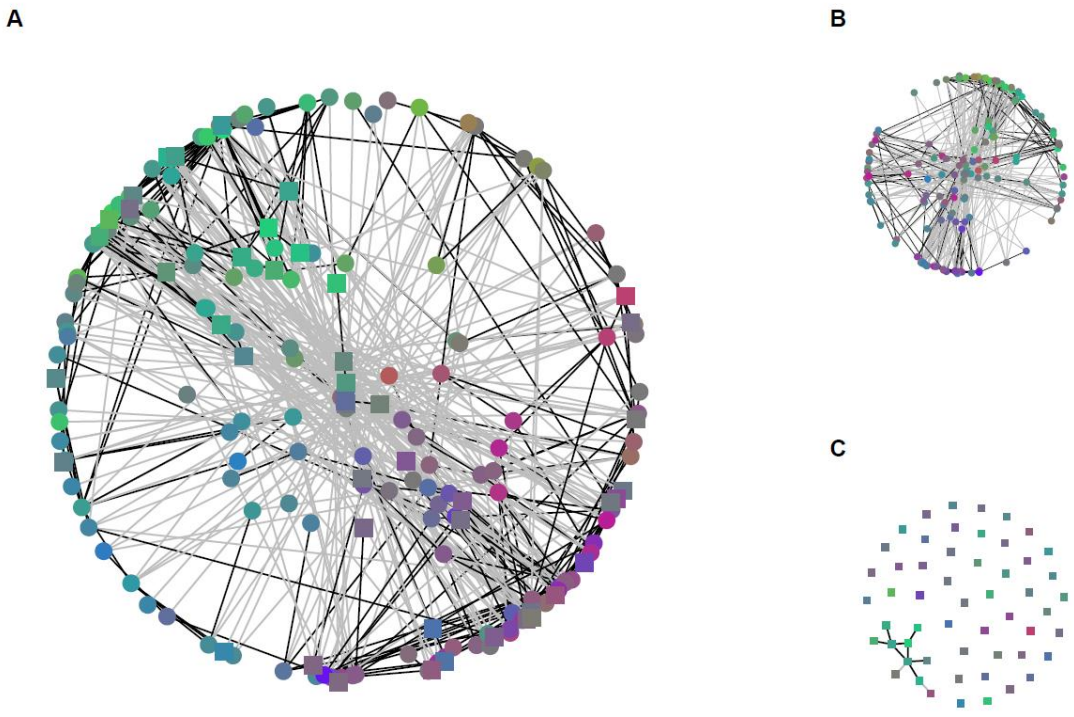

Figure 6.3: Microbial co-occurrence networks for bacteria and fungi together (A), bacteria only (B) and fungi only (C). Black links are positive, grey negative connections. The nodes are clustered together according to the Fruchterman Reingold algorithm (for graphical simplicity the fungal layout is based on the unweighted edges). Nodes are coloured by their preference for different depths: green are topsoil specialists, red subsoil specialists and blue transition zone specialists. Data from 2017 only.

The nature of the co-occurrence patterns across the microbial data is dependent on whether the bacteria and fungi are considered together or separately. There were 161 bacterial taxa and 54 fungal taxa that appeared in over 50% of the samples, and were thus included in the networks (Figure 6.3, Appendix H Figure 6). The network construction was run for bacteria and fungi together, bacteria only and fungi only. The majority of the abundant microbial taxa included within our network showed a distinct depth preference, but no treatment effect of treatment (Appendix H Figure 7). There were many links within the joint network that would not have been found if bacteria and fungi were only considered separately (123 out of a total 428 links, with another 283 within bacteria and 22 within fungi). The bacteria only network had 312 edges and the fungi only network had 10 edges. The interactions between the different microbial communities in our sites act across the kingdom boundaries and ignoring across-kingdom interactions changes specific links and overall network stability (Appendix H Figure 6).

6.3.2.3 Indicator taxa

Analysis of the different relevant abundance of bacterial and fungal taxa in drought and warming compared to control treatments revealed a small number of taxa that responded strongly to the treatments, with no taxa responding differently to the treatment in the different depths or years (Table 6.2, Appendix H Figure 8). There were more bacterial taxa that declined under the climate change treatments than increased, and over half of the taxa that declined did so under both drought and warming. However, in the fungal communities while the warming treatments caused a decline in more taxa than increased, there were far more fungal taxa that increased under the drought treatment than decreased.

Table 6.2: Number of taxa that responded to warming or drought treatment compared to control (adjusted p-value < 0.1)

		Warming	Drought	Both
Bacteria (n=4936)	Increase	104 (2.2%)	213 (4.4%)	48
	Decrease	320 (6.7%)	295 (6.1%)	189
Fungi (n=1704)	Increase	99 (5.8%)	167 (9.8%)	42
	Decrease	169 (9.9%)	99 (5.8%)	46

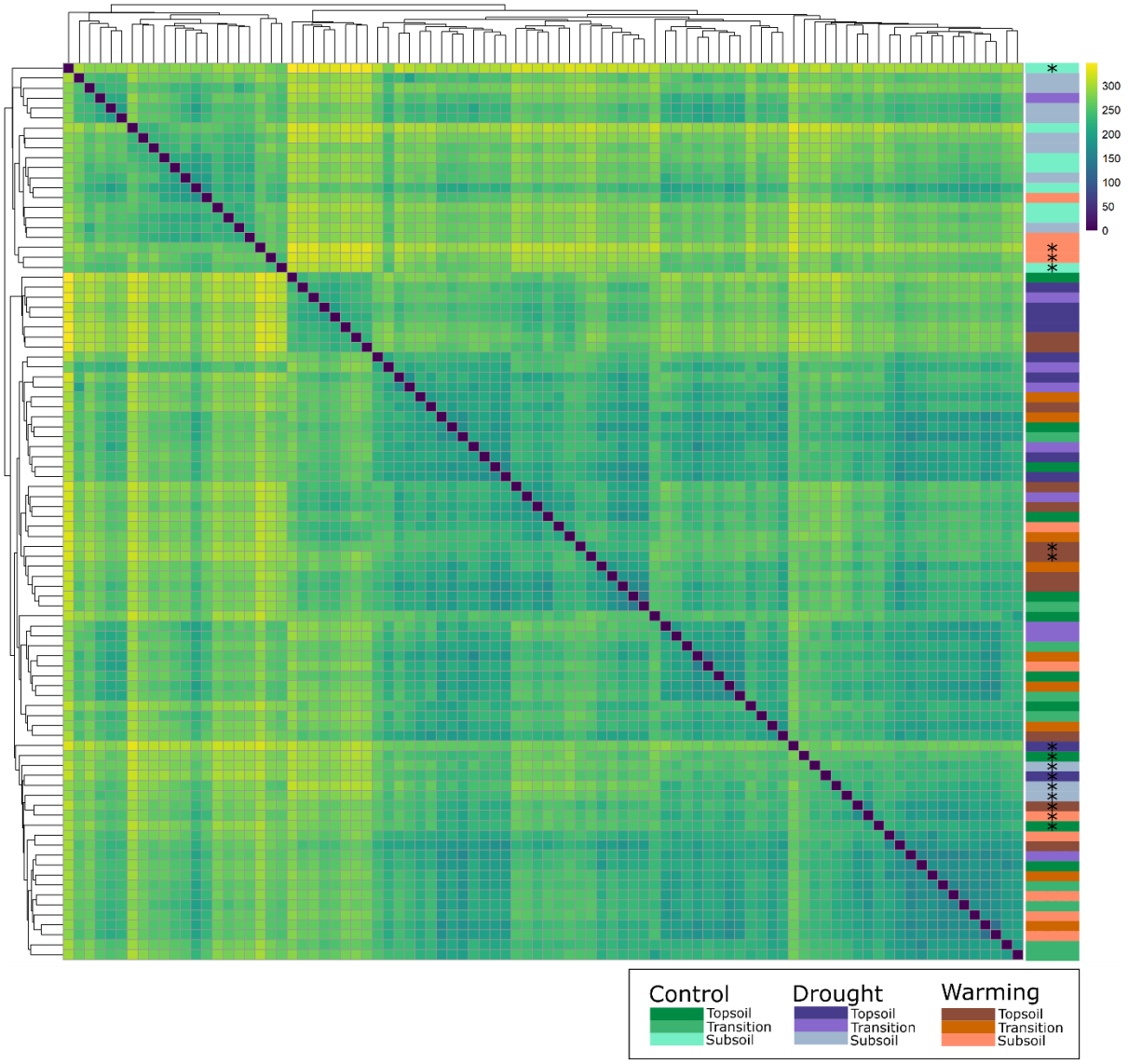

Figure 6.4: Heatmap of samples clustered by their transformed similarity according to DeSEq2 analysis of the bacterial taxa. On the right the samples are colour coded by treatment and soil depth, the key is on the bottom right of the figure. Samples from 2003 are designated by asterisks.

The subsoil bacterial community strongly separated from the topsoil and transition zone communities following the clustering of samples according to the differential relative abundance of taxa (Figure 6.4). However there were communities from the subsoil of the drought and warming plots in 2003 (3/3 and 1/3 respectively) and the subsoil of the warming plots only in 2017 (5/7) that were not within this subsoil cluster and instead nested within the topsoil and intermediate layers from both 2003 and 2017. There was no strong separation of the 2003 and 2017 bacterial community composition, with the 2003 communities not forming their own single cluster but instead tending to form small nested groups within the 2017 samples. There was no

separation of a single subsoil cluster in the fungal communities, however there were two sets of communities that separated out from the rest of the data (Appendix H Figure 9). These were almost entirely composed of topsoil and intermediate samples with the only exception being two samples from the subsoil of a warming treatment included in the larger (13-member) cluster. The microbial communities of the subsoil are more similar to the communities of the topsoil across the site than to other subsoil communities.

6.3.3 Root fungal colonisation

The climate treatments impacted overall root biomass and the change in mycorrhizal colonisation of roots with depth as well as the overall microbial community composition. There was a decrease in root biomass and number of root tips with depth (Appendix H Figure 10). Drought and warming had limited effect upon the number of root tips and fine root biomass once depth was accounted for (elpd of all models within standard errors of each other), but did decrease the overall root biomass (elpd difference 3.7 ± 2.2). The rate of ericoid mycorrhizal colonisation also decreased with depth, however proportionally more roots were colonised by dark septate endophytes at the lower depths. These changes in colonisation with depth were not apparent in the warming plots (Figure 6.5, Appendix H Figure 11). Overall, compared to the control plots the warming plots had lower rates of fungal colonisation, while drought plots had higher rates of ErM colonisation in the topsoil.

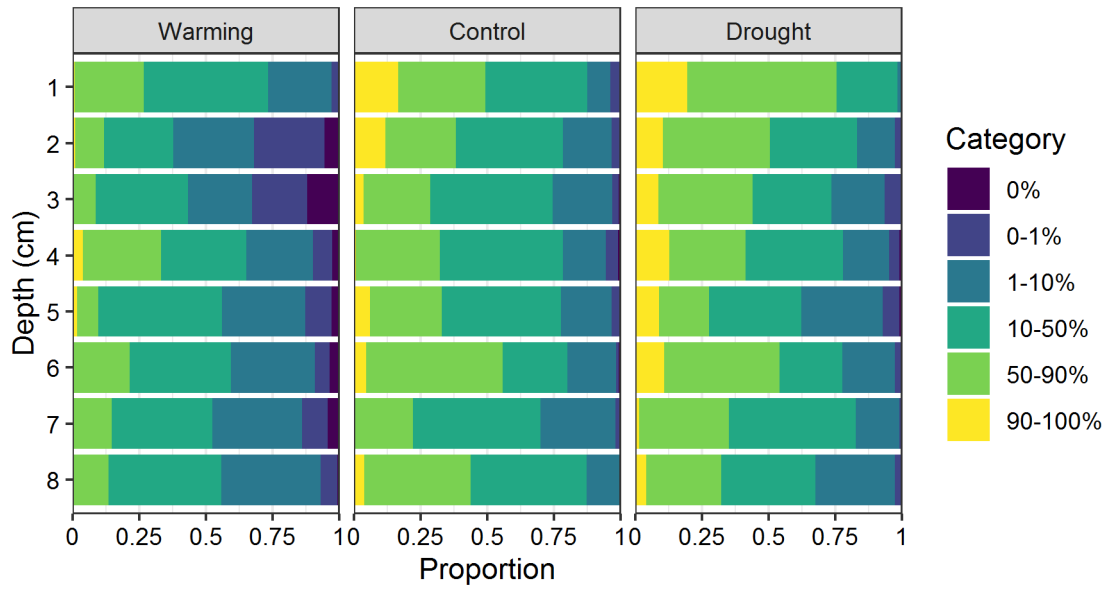

Figure 6.5: Changes in proportion of each ErM colonisation category with depth. Note that there is overall lower colonisation in the warming treatment and higher in the drought treatment. There is also limited change with depth within the warming treatment, a slight decrease in the high proportional colonisation categories in the control treatment and a larger change within the drought plots.

6.3.4 Impact of soil chemical properties on microbial communities

The change in pH and EC with depth was altered by the experimental treatment (Figure 6.6). Under control conditions pH increases with depth and this remained the same in the drought conditions, but within the warming plots there was no change in pH with depth. EC was lowest in the subsoil in the control and drought plots, however in the warming plots there was no change in EC with depth. However, these differences in pH and EC with depth and treatment were limited, with the addition of treatment to the model performing equivalently to the model with depth only (elpd difference of adding treatment for pH +1.0 ± 3.0, for EC -0.7 ± 1.1). The subsoil of the warming plots had similar conditions to the topsoil throughout the site.

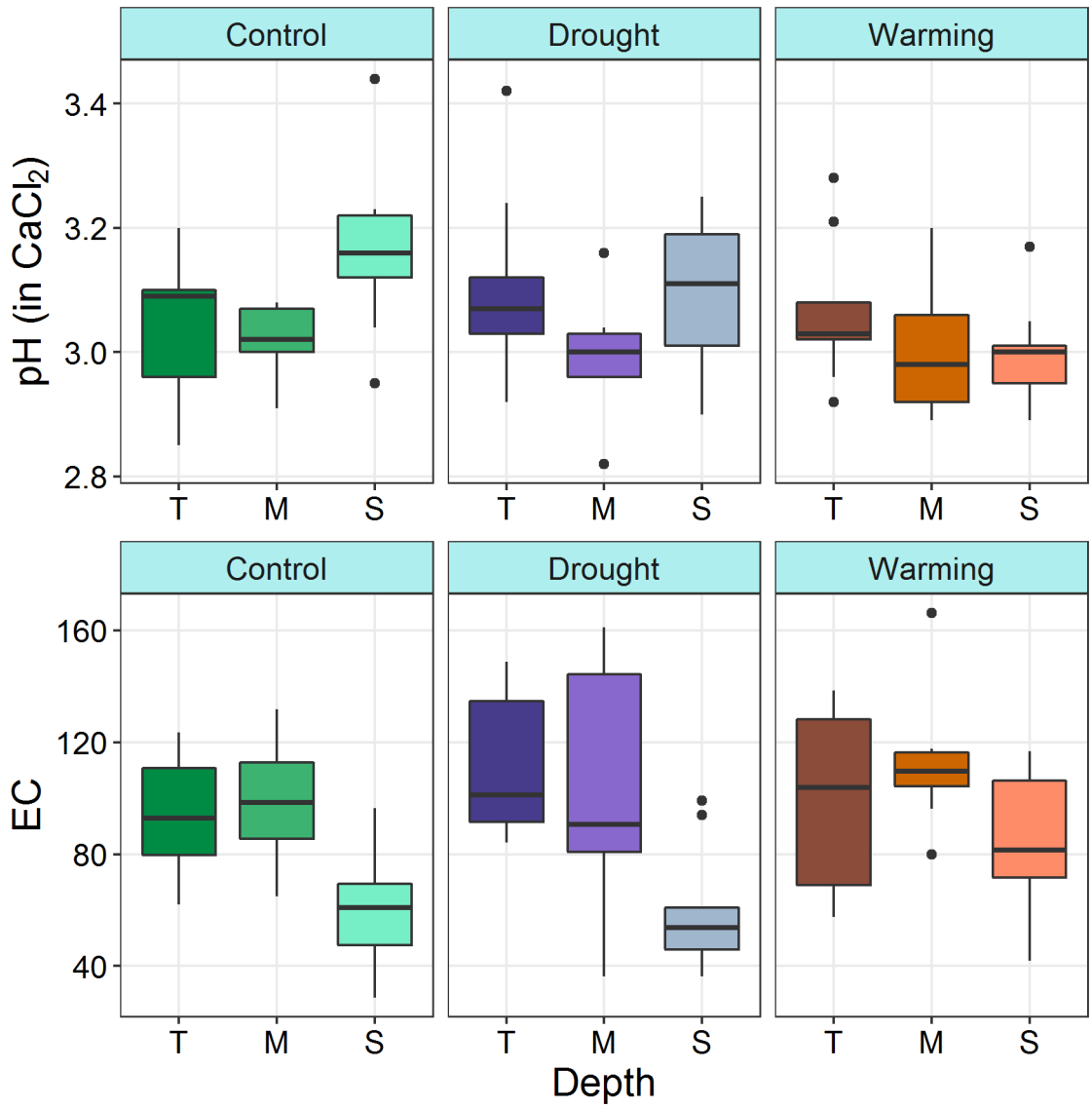

Figure 6.6: The change in pH and EC with soil depth (T = Topsoil, M = intermediate, S = Subsoil) and climate treatments in 2017

The impact of treatment upon fungal richness was fully mediated by the changes in soil physicochemical environment, while bacterial richness was less impacted by the change in soil properties. No models did particularly well at predicting the bacterial richness, but the best model for investigating bacterial richness had solely climate treatment and soil depth as predictors (Bayes $R^2 = 0.227 \pm 0.071$), with three other equivalent models by elpd (taking standard errors into account) adding subsets of pH, EC, moisture and temperature to the predictors. Bacterial richness was lowest in the intermediate depth and highest in the drought plots. Fungal richness was impacted by the physical properties of the site, with the best model including pH, EC, soil depth,

temperature and moisture as predictors (Bayes R^2 = 0.651). The model with only climate treatment and soil depth as predictors was notably worse than all the other models (elpd difference of 3.4 compared to <1 for all other models). Fungal richness decreased with depth and increased with EC and pH. There was also evidence for decreasing richness with moisture and increasing with temperature.

The microbial community composition was still impacted by the treatment after accounting for changes in soil temperature, moisture, pH and EC. The best model had treatment, pH and EC as predictors of bacterial and fungal composition (Figure 6.7). The relative impact of pH on bacterial composition was higher than that of EC, while the reverse was true for fungal composition. The treatments have resulted in changes in the soil physicochemical structure which have impacted the microbial community composition, but the impact of treatment is only partially mediated by the measured changes in soil chemistry.

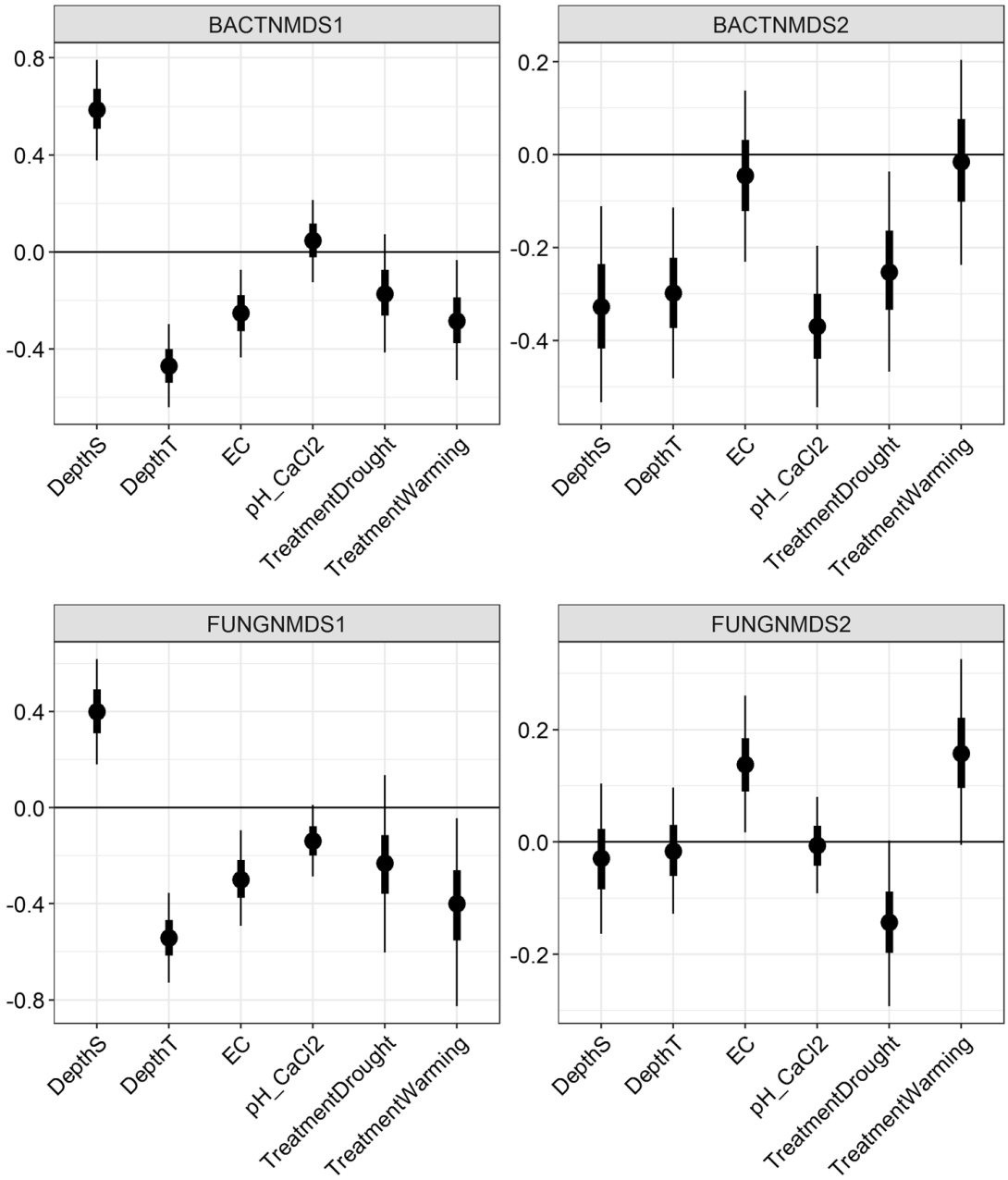

Figure 6.7: The parameter estimates for the impact of soil depth, EC, pH and climate treatment on bacterial (top row) and fungal (bottom row) NMDS scores. The circles are the mean estimate, the thick bars the standard error and the thin bars the 90% CI. Data presented is from 2017 only. The impact of depth is represented as the impact of the subsoil (DepthS) and topsoil (DepthT) relative to the transition zone, and the impact of treatment represented relative to control.

6.4 Discussion

6.4.1 Microbial community response to warming and drought

Our results show that fungal taxa are more responsive to drought and warming than bacterial taxa, with warming altering soil biological and chemical properties throughout the soil profile. These impacts are at least partially moderated by changes in the soil chemical environment which are likely driven by alterations in soil hydrology and the plant community at the site in response to treatment (Domínguez et al., 2015; Robinson et al., 2016). We have observed changes in the fungal community using both DNA sequencing of the soil and microscopic examination of the mycorrhizal colonisation of *C. vulgaris* roots. This indicates that changes in the bulk soil microbial community reflect changes in the functional capability of the soil microbiome to interact with plants which could promote biogeochemical cycling (Read & Perez-Moreno, 2003; Tedersoo & Bahram, 2019).

Previous studies on the microbial response to long term climate change have largely focused on measuring the microbial biomass response, which is highly dependent on the soil type and climate conditions (Ren et al., 2018; Zhou et al., 2018). Drought and warming have been found to impact microbial composition more strongly and persistently than microbial richness in both short and long term experiments (Birnbaum et al., 2019; de Vries et al., 2018; Sayer et al., 2017; Tóth et al., 2017; Yu et al., 2018). While we have found significant changes in microbial richness and diversity in response to experimental climate change, the impact on composition is clearer and more consistent in our results. Changes in composition in response to long term drought have been shown in some cases to be driven by changes in the rarer taxa in a calcareous grassland (Sayer et al., 2017). This supports our finding that the dominant taxa show limited preference for treatment in contrast to their strong depth preference.

6.4.2 The interaction between soil depth and treatment

A variety of observational studies have shown a distinct difference in microbial composition with depth, consistent with our results (Delgado-Baquerizo et al., 2017; Griffiths et al., 2003; Serkebaeva et al., 2013; Seuradge et al., 2017). The subsoil is

generally less biologically active and shows less seasonal variation in its physical conditions and microbial communities (Griffiths et al., 2003). Therefore it is no surprise that many studies have found that the topsoil is more strongly linked to the aboveground land use and plant productivity (Delgado-Baquerizo et al., 2017; Seuradge et al., 2017). However, our results suggest that the impact of climate change can penetrate deeper into the soil, impacting soil physicochemical properties, plant-soil interactions and microbial processes. This change in the stratification with depth of physicochemical properties and microbial communities could be linked to the distinct change in hydrological behaviour and roots over the course of the experiment (Robinson et al., 2016). Our soils are admittedly shallow and the changes with depth we have observed are over relatively short distances. However, the results still hold relevance for a geographically extensive soil type and are likely to occur in many deeper soil types.

The change in stratification with depth of the soil physical, chemical and ecological properties in response to warming may be related to the increased cover of moss in the warming plots (Domínguez et al., 2015). Moss acts as a layer of insulation on the soil surface, buffering changes in soil temperature and moisture (Turetsky et al., 2012). Mosses have been found to reduce evapotranspiration, increase surface infiltration and influence the partitioning of heat fluxes (Beringer et al., 2001; Blok et al., 2011). The thermal and hydrological influences of moss cover can lead to differences in belowground soil microbial communities, changing their biomass and activity (Benavent-González et al., 2018; Gornall et al., 2007), with relevance to global biogeochemical cycling (Porada et al., 2014). The insulative properties of moss could be reducing the magnitude of daily and seasonal heat and moisture fluctuations at the soil surface, therefore reducing subtle temperature and hydrological differences between the topsoil and subsoil. However, our results indicate that the subsoil is becoming slightly more like the topsoil, and not vice versa, which could mean that the changes in water infiltration and heat fluxes are leading to increasing penetration of water and translocation of chemicals within the soil profile.

6.4.3 Evaluating the concept of a bacterial vs a fungal response

The community composition response to drought and warming we have found shows some differences between bacterial and fungal taxa. However the presence of co-occurrence links between specific bacteria and fungi suggest that caution should be used in interpreting these results as a bacterial response vs a fungal response. While the overall abundance and dominance of bacteria and fungi may change in response to climate change this obscures changes in fine-scale dynamics which could be creating specific drought and warming communities that are composed of a combination of both bacteria and fungi. We do find that more fungal taxa respond positively to drought and negatively to warming which indicates that the drought microbial community could consist of relatively more fungi. This is consistent with previous results showing higher tolerance of fungi to drought (Haugwitz et al., 2014). Neglecting the inter-Kingdom links could lead to erroneous assumptions about microbial community stability, as suggested by work in the human microbiome (Tipton et al., 2018). It is possible that the correlations between bacteria and fungi are driven solely by changes in environmental conditions and do not represent a true microbial interaction, as these co-occurrence networks are prone to this kind of error (Carr et al., 2019). However this result does offer an intriguing avenue for future work in establishing whether specific inter-Kingdom interactions exist and can influence microbial community structure and activity in soils.

6.4.4 Fungal root colonisation

Our results from examination of the fungal root colonisation are in agreement with previous results that have indicated that ErM and DSE colonisation respond differently to experimental warming (Binet et al., 2017; Olsrud et al., 2009). However, the treatment responses we found differed from previous results, with Olsrud et al. (2009) finding no effect of warming on ErM colonisation after 6 years of treatment in a subarctic birch forest. Other results from a Danish heathland with similar experimental set-up to our site found that ErM colonisation was lower in the warming treatment at 5-10 cm depth compared to drought and control, and that DSE was lower at depth in the drought treatments (Arndal et al., 2013). Overall, we found lower ErM colonisation in response to warming, however we found higher ErM colonisation in

response to drought which is different from Arndal et al.'s results. These contrasting results could reflect the difference in duration of treatment or potentially the differences in the plant response to treatment. The implications of shifts in mycorrhizal type for plant community resilience and biogeochemical cycling in response to warming are unclear due to the lack of knowledge on the relative role of DSE versus ErM colonisation upon plant nutrient uptake and stress resilience (Newsham et al., 2009). However, there are some suggestions that DSE colonisation may enable the uptake of organic nitrogen compounds and improve resilience to certain stressors (Hill et al., 2019; Newsham, 2011).

6.5 Conclusions

We have found that bacterial and fungal community composition and diversity is altered by long term drought and warming treatments. The impact of simulated climate change is greater after eighteen years of treatment compared to four years. The shift in bulk soil community composition is also reflected by a shift in fungal root colonisation of the dominant *Calluna vulgaris* plant species. These changes are at least partially driven by changes in the distribution of roots throughout the soil profile and changes in the soil chemical environment. In general, fungal taxa appear to be more responsive to the climate change treatments than bacterial taxa but the presence of cross-domain co-occurrence relationships and sensitive taxa in both domains cautions against interpreting our results as a fungal vs a bacterial response. These insights into soil microbial community response to drought and warming can inform our understanding of what drives differences in soil functional response to climate change.

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CHAPTER 7

Discussion and recommendations for future research

7.1. Introduction

In this chapter, the experimental work presented in chapters 2-6 is summarised and discussed in relation to the common themes and initial objectives of the thesis. These were: to evaluate the state of soils in Wales; explore the associations between soil physical properties and biological communities; to establish the relative roles of physicochemical and biological factors in determining soil biodiversity and to evaluate the impact of climate change on soil microbial communities. Detailed discussion of the results from the work in this thesis are described in each chapter. Here, I present a synthesis of the results in terms of the overall aims of the thesis and the wider implications of the findings.

7.2. Synthesis of findings

7.2.1 Evaluating the state of Welsh soils

The state of key soil physicochemical and biological parameters in Wales have been presented across this thesis, including an overview of soil physicochemical properties in Chapter 2 and description of the microbial communities in Appendix A. Some of these results were consistent with expectations, such as the range of soil carbon, pH and nitrogen across Welsh soils which were consistent with previous surveys (Reynolds et al., 2013). Others were more unexpected, such as the high level of soil surface water repellency and increasing soil fungal diversity with land use intensity. Wales is dominated by grassland ecosystems, with the majority of the sites surveyed being improved grassland, neutral grassland or acid grassland. These grasslands showed a clear gradient in soil properties including pH, carbon, nitrogen and microbial diversity. There was also a significant proportion of bogs and heathlands which had very high carbon, low pH and low microbial diversity. We found no majorly concerning trends in soil quality across Wales at a national scale. For example, we saw that the majority of our soils were within nationally accepted pH limits for habitat support (Chapter 2). However, one important caveat is that the measurements of soil properties presented within this thesis are all limited to the topsoil (0-15cm). The topsoil is the most biologically active section of the soil, however it is clear that omitting the subsoil from an evaluation of the state of Welsh soils will majorly impact

conclusions drawn on soil properties that are more related to subsoil properties such as carbon storage and evidence for P saturation (Simo et al., 2019). Other soil properties are more important at the soil surface, such as microbial community activity and soil water repellency.

Throughout this thesis the importance of pH and carbon within Welsh soils has become apparent, as we found how strongly they correlate with other soil properties such as nitrogen and bulk density in Chapter 2, how they drive physical properties such as soil water repellency (Chapter 4) and their importance in determining soil microbial dynamics (Chapter 3 and Appendix A). This sits well with the use of soil pH and carbon as key soil quality indicators that is common across research and monitoring schemes (Bünemann et al., 2018). The importance of these in Welsh soils may also be related to the wide range of carbon and pH in our soils, with some highly acidic and carbon-rich soils present. Other factors such as salinity are consistently low in Wales and could be expected to play a dominant role in determining soil quality and health in more arid environments. Wales appears to be consistent with other temperate environments in which the clear soil quality indicators are pH and carbon. It should be noted, however, that few calcareous (high pH) soils were evaluated in this survey.

In general, we found that pH and carbon showed some trend with land use in Wales, with higher pH and lower carbon in more intensive grasslands. This made it difficult to separate out the impact, if any, of land use upon soil microbial communities and physical properties once changes in pH and carbon were accounted for. In the case of water repellency the impact of land use was nonsignificant once changes in plant, microbial and soil physicochemical properties were accounted for, with the notable exception of arable systems. Wales has proportionally few arable farms, with the vast majority of the Welsh landscape being under pasture (Welsh Government, 2019). The relatively low intensity of farming across Wales limits the comparability of our results to analysis of the high intensity arable farming more common in the South-East of England and other parts of Europe (Maskell et al., 2019). Analysis of the impacts of agriculture and woodland planting upon soil quality specifically is limited due to the smaller sample population of those habitats within the analysis and limited range of

environmental conditions. However, there are indications that arable systems are different to grasslands for at least some soil properties and while our analysis found limited evidence of woodlands being qualitatively different for soil physicochemical or microbial properties there is evidence that mesofaunal communities are different in woodlands (George et al., 2017). Further investigation of the impacts of ongoing arable intensification and woodland planting within Wales could benefit from comparison to the wider UK and increased focus upon these transitions.

7.2.2 Factors driving Welsh biodiversity

Throughout this thesis it has become apparent that soil microbial diversity in Wales is driven largely by physicochemical properties such as pH and carbon. There is a clear non-linear response of bacterial richness to pH, made most apparent in Chapter 5. Below a pH of around 5 there is a dramatic decline in bacterial richness, with levels being fairly stable from pH 5 to 7. There is also evidence for a similar pattern in fungal richness, however this is less pronounced. Previous research has shown that there is a transition point in microbial activity and carbon use efficiency around pH 5.5, which is related to the point at which aluminium becomes soluble (Jones et al., 2019). This strongly suggests that the properties controlling soil dynamics differ at the different pH values. It is of particular interest due to the gradual recovery of acidification occurring across the UK (Reynolds et al., 2013), with a continuing trend of fewer sites at low pH found in our analysis in Chapter 2. The non-linearities in response to pH could result in the presence of thresholds and tipping points, meaning that the response to global change would involve difficult to predict instabilities and transitions. The majority of analyses, including those in this thesis, model microbial and functional response linearly which could result in difficulties in extrapolation and prediction.

The response of soil biodiversity to land use does appear to be largely driven by response to soil pH which varies considerably by habitat. The bacterial, fungal and protistan communities across Wales appeared within our analysis to be different in neutral/improved grassland and arable compared to acid grassland, heathland and woodland. The only analysis within this thesis that found an impact of land use upon the microbial diversity, even after accounting for pH, was in Chapter 3, with a binary

predictor separating the improved and unimproved habitats. However, this could have been driven by the non-linearity in the pH response which was not incorporated into that structural equation model. Overall, the impact of land use upon the soil microbial community appeared to be driven by soil physicochemical parameters for diversity and additionally, potentially plant community dynamics for composition. This could in part be due to the low frequency of arable and forested sites in our data which might be expected to have different factors driving soil biodiversity. The fact that arable sites had lower water repellency, even after accounting for soil physicochemical and biological parameters, indicates that these types of sites may be qualitatively different. Comparing soil biological communities and water repellency is of particular interest as they have both recently been shown to respond to multiple ecosystem multiplicatively rather than to the identity of each stressor, unlike many other soil properties such as respiration and aggregate stability (Rillig et al., 2019). So while pH appears to be driving microbial diversity and composition, rather than land use, we cannot rule out the possibility that extreme land use types will have different microbial communities in a way that is not dependent on pH alone.

Within this thesis the descriptions of the microbial communities have been based upon DNA metabarcoding of regions general to bacteria, fungi or overall eukaryotes, which offers a description of the composition of microbial communities. This does not provide any measurements of microbial biomass and is limited in describing the functional variety of the soil microbial community. The analysis of amplicon sequencing data requires several decisions made within all stages of analysis which could have considerable effects on the downstream results (Pollock et al., 2018). We found it difficult to explain variation in the fungal richness, with maximum R^2 values of ~0.3 for richness in Chapters 3 and 5, which may have been related to the choice of the ITS1 region for sequencing as opposed to the ITS2 or 18S regions (Blaalid et al., 2013; George et al., 2019). Alternatively, the difficulty in explaining fungal richness may be due to it being a largely random variable and that fungal composition, for which we explained a much greater proportion of variation, is more related to actual soil processes. As another example in differences in metabarcoding methods, over the past few years there has been a switch from analysing amplicon data based upon an

OTU approach (usually clustering at 97% similarity) to an ASV approach (identifying sequence variants at 100% similarity accounting for error rates). The ASV approach has been found to improve estimates of microbial richness and is theoretically easier to compare across studies (Callahan et al., 2017, 2016). Within this thesis the GMEP microbial data presented in Chapters 3-5 was analysed using an OTU based approach while the Clocaenog microbial data in Chapter 6 was analysed using an ASV approach. This could impact the comparison of the results, particularly the analysis of microbial richness which are known to be more sensitive than composition to changes in the bioinformatic pipeline.

Throughout this thesis we have seen that the plant community across Wales is both related to the soil system and other environmental variables such as climate. In Chapter 4 we observed that the average stress tolerance of the plant community was closely related to soil carbon and both drought and precipitation. In Chapter 5 we saw that the plant community at our sites was influenced by climate, soil physicochemical parameters and to a lesser extent, spatial distance between sites. We also observed that the plant community correlated with bird, butterfly, bee and hoverfly communities in diversity, composition and specific inter-taxon relationships. These results suggest that the plant community plays a key role in connecting the below- and above-ground ecological communities. Overall ecosystem resilience and service provision is dependent on multiple components of the ecosystem, the majority of which are associated with the plant community ranging from bird diversity to soil stability.

7.2.3 Associations between soil physical properties and microbial communities

Throughout this thesis we have seen that physical, chemical and biological properties in soils are closely interlinked. The bacterial, and to a lesser extent fungal, communities responded to soil textural diversity and composition in the analysis within Chapter 3. The strong relationship found between heterotrophic protistan communities and bacterial communities in Chapter 5 indicates that protistan communities are likely to also be affected by soil texture. Soil texture is a relatively fixed aspect of soil structure making it unlikely to be affected by either the plant community, represented in Chapter 3 by habitat, or the soil microbial communities.

However, biological communities not only respond to changes in the soil physicochemical environment but can influence it themselves. In Chapter 4 we observed that soil water repellency, which controls the movement of water through the soil profile, is affected by plant, bacterial and fungal communities. In particular, the unexpected full mediation of the relationship between pH and water repellency by the bacterial community demonstrates the important role of microbial communities in determining soil physicochemical parameters and not just responding to them.

The work within this thesis is largely observational and thus the causal relationships suggested must be interpreted cautiously. The statistical techniques used in this thesis attempt to rate the likelihood of causal relationships, however issues such as the presence of error in measurements can confound the identification of causal relationships. One particular example is that due to the single time point of measurement and the different levels of variation over time in the different properties, one property may in fact represent an integration over time of another property. For example, as the bacterial community composition is strongly responsive to soil pH and if the bacterial community changed on a longer timescale than pH then bacterial composition would be reflective of not just the current pH but past pH as well. Therefore, any other property that we measured that was responsive to both current and past pH could be more strongly correlated to bacterial composition than pH itself. This particular example is unlikely as bacterial communities change over time more quickly and drastically than pH (Bardgett & van der Putten, 2014). However, without in depth knowledge of the mechanistic relationships or measurements at multiple time points distinguishing which causal direction is the case remains difficult.

Identification of causal linkages within soil science is particularly difficult due to the presence of positive and negative feedbacks. Soil is an incredibly complex medium with many different processes and ecologies occurring at any one time. Plant communities interact with soil microbial communities in a way that is dependent on the soil environment, and then go on to modify the soil environment. Whether or not soil can ever be considered to reach a quasi-steady state can be dependent on the timeframe under consideration and the range of different properties still deemed to be one state. Within the Clocaenog experiment presented in Chapter 6 and Appendix B

there has been evidence put forward for the shift from one state to another following an external drought event (Robinson et al., 2016), however it is now undergoing gradual recovery over years or decades to a hydrology state more similar to that of twenty years ago. This importance of timeframe and inherent variability should be considered when applying results to the wider landscape as the timeframe of ~ 5 years inherent in many policy decisions is both far longer than many soil biological processes and far too short for evaluating soil health trajectories.

7.2.4 The impact of climate change on soil properties and communities

The climate in Wales is predicted to involve hotter, drier summers and warmer, wetter winters (Murphy et al., 2018). The recent exposure of Wales to increased drought is associated with changes in the dominance of stress tolerant plants and altered soil physicochemical properties, which then affected soil microbial communities and water repellency (Chapter 4). Within the Clocaenog experiment examined within this thesis we have shown that summer drought and warming impact microbial communities within heathland ecosystems (Chapter 6). This appeared to have a knock-on effect upon soil respiration, with summer warming in particular resulting in greater loss of soil carbon (Appendix B). The experimental manipulations at Clocaenog were limited in magnitude of treatment but have been ongoing for 20 years. Combining these experimental manipulations with the observational results across Wales gives us greater confidence in our predictions that soil microbial communities within Wales will change in response to climate change and that this will have implications for soil quality.

The response of different organisms and soil physicochemical properties to climate change varies. For example, within Chapters 3 through 5 we saw that the plant community responded to climatic variables while the soil microbial community did not. The impact of climate change upon soil microbial communities would be mediated by soil physicochemical properties and plant communities. However, different aspects of the ecosystem could respond to different aspects of climate, for example the plant community stress tolerance showed positive responses to both precipitation and drought while soil carbon responded positively and negatively respectively in Chapter 4. This divergence in properties and organisms in response to

external forces within an ecosystem can reduce resilience to change and make the overall ecosystem more vulnerable to other external stressors. The differential responses not only destabilise ecosystem connectivity but also leads to issues with establishing mechanistic relationships in semi-natural ecosystems. For the purposes of understanding the mechanistic underpinnings of soil science and the predicted impacts of interventions it is essential to understand if the systems we are basing our assumptions on can be extrapolated to other sites.

Not only is the average climate of Wales changing but so too are the frequency of extreme events. In chapter 4 we saw that the plant communities were responsive not just to the average precipitation but also the frequency of dry spell events. The extreme drought of 2003 had a large impact on the Clocaenog site, causing a transition between states with recovery still ongoing (Robinson et al., 2016). This extreme event, and the differing resilience of the plots undergoing different treatments, may be partially responsible for the changes in respiration and microbial communities between treatments and over time (Appendix B; Chapter 6). Gradual climate change could reduce the resilience of soil and ecosystem properties to extreme events. Exposure to previous stress could reduce ecosystem connectivity as different components vary in response time and adaptability. In particular, the fact that climate change is occurring concurrently with various land use changes, pollution and other anthropogenic pressures could lead to greater impacts upon soil and ecosystem health as multiple stressors often act synergistically or in other hard to predict ways.

7.2.5 The implications of the results

Specific recommendations from this thesis to policy and land management include clear separation of policy goals for high carbon upland soils vs lowland agricultural soils, the re-evaluation of the soils trigger-values used by the Environment Agency (Bhogal et al., 2008), and reconsideration of the best soil microbial indicators for soil health. Priorities in managing soil and ecosystem health vary over time and between different stakeholders. Even assuming we can agree on which functions and services we wish our soils to provide, there are still major gaps in our knowledge of which soil properties underlie these at a policy relevant landscape scale (Kibblewhite et al., 2016). Across this thesis we have found that pH and carbon are important to many other soil

physicochemical and microbial properties, yet there is an underlying assumption that the other soil properties we have measured are useful. Discussion of the important functions within soils must consider not only what kind of functions we are interested in but also what can be measured at regional or national scale.

In general, land management policy is concerned with the ecosystem services that soils provide to humanity and the natural capital that underpins the delivery of these services. Appendix C reviews the soil ecosystem services concept: exploring how soil properties and processes underpin ecosystem services; how to measure and model them; and to identify the wider benefits they provide to society. There have been many attempts to define the wide range of ecosystem services that soils provide, as well as identify the properties that are relevant to those services (Dominati et al., 2010; Maseyk et al., 2017; Robinson et al., 2013). However, some have criticised the ecosystem services and related concepts for their anthropogenic focus with alternatives including the concept of soil care having been suggested (Appendix C; Puig de la Bellacasa, 2015). Translating scientific results into specific policy and outreach initiatives must involve careful consideration of the intended and potential unintended consequences. The language that is used as well as the results that are presented can impact the eventual outcome.

Soil quality, health and function are often used terms but surprisingly difficult to define (Bünemann et al., 2018; Döring et al., 2015). All, however, are concerned with ranking soils and providing an indication of what is bad and what is good. Soils provide a wide range of functions and services that vary considerably between soil types. We have seen in Chapter 2 how the upland bogs and heathlands of Wales are very different from the lowland arable and improved grasslands in their characteristics and the functions they can support. Combining these very different soils into one index of good vs bad, or as in many cases bad vs worse, is a dangerous oversimplification of the state of soil health. What constitutes a healthy or good quality soil varies depending on the metric considered and the presence of trade-offs in soil functions making it impossible to optimise all soils in the same way. For example, in Chapter 4 we have proposed that soil water repellency may increase ecosystem resilience to stress, yet in tilled agricultural systems water repellency has

been viewed for decades as an issue for efficient crop growth. There is a requirement to maintain a range of soil types, each of which should be managed in a way to optimise the provision of the functions it is best qualified to provide. Soil health should be defined in the context of the potential soil functionality in any given environmental and land use situation. This is particularly important due to the inherent trade-offs between different ecosystem services. Management of soil resources and health needs to consider the optimal mosaic of soil types at the landscape scale and then offer appropriate interventions to maintain each element of the landscape at maximum health.

National monitoring of soil quality requires not only trajectory information but information on the impacts of changes in soil properties, which can easily be inferred by the use of threshold values. The current pH threshold for sites being too acidic in mesotrophic grassland and too neutral in acid grassland is 5 (Bhogal et al., 2008). Our research indicates that this may need to be raised to 5.5 on the basis on the shift in microbial community, in particular in combination with other research on soil functions in response to pH and the backdrop of reducing acidity across the UK (Evans et al., 2008; Jones et al., 2019; Reynolds et al., 2013). The increasing extent of arable cropping in Wales raises concerns about the use of fertilisers on Welsh soils, as around 75% of mesotrophic grasslands are already over the appropriate limit for available phosphorus. Careful controls of fertiliser use are required to prevent the worsening of nutrient status in Welsh soils. The results from Chapter 4 also suggest that the increasing agricultural intensification of Welsh soils could have negative impacts on soil hydrological behaviour. Our interpretation of the link between plant stress tolerance and water repellency is that water repellency could positively impact ecosystem resilience, and that in this era of ever-increasing stress the balance between ecosystem productivity and resilience needs to be managed carefully.

Despite recent initiatives promoting soil microbial diversity as a key indicator for soil monitoring programmes we did not find good evidence that microbial richness specifically should be a management priority (Stone et al., 2016). We did however find evidence that microbial composition can impact soil functions and suggest that we should refocus the discussion away from diversity when discussing the importance of

soil microbiota. Soil biodiversity is itself a soil function due to the potential for isolation of useful antibiotics and other substances (Ling et al., 2015). However, the majority of soil management is focused upon managing for other services such as carbon storage, water filtration and crop production. Within our analyses bacterial, fungal and heterotrophic protistan richness was strongly affected by soil pH. This makes it difficult to manage separately from the many other soil functions determined by soil pH and we found a negative relationship between soil carbon and bacterial richness once pH was accounted for (Chapter 3). Managing soils in order to increase microbial diversity may mean reducing carbon storage. Also, the measurements of microbial richness are dependent on the laboratory methodology and bioinformatic pipeline to a much greater extent than microbial composition (Callahan et al., 2017; Xue et al., 2018). Therefore, measured microbial diversity may pose little relation to the true soil diversity and is difficult to compare across studies. In this thesis we did find that microbial composition was related to soil functions such as water repellency while microbial richness was not (Chapter 4) and that microbial composition responded more consistently than richness to experimental drought and warming (Chapter 6). We also identified indicator taxa for specific ecosystems, plant communities and climate change responses (Chapters 5 and 6). Therefore, we suggest that biodiversity is an inappropriate metric and term for describing the importance of soil microbial communities due to its focus on the number of taxa rather than their identity. We propose that soil microbial composition should be the focus of future monitoring schemes and evaluation of soil health.

7.3. Future work

Defining soil function, health and quality is difficult due to the inherent uncertainties in the concepts, differences in interpretation and use, and the multidimensional nature of the soil system. Some authors have recently been applying the multifunctionality approach to measuring ecosystem and soil health, whereby each measured function is combined into a single score. This can then be compared to land use or biodiversity to infer the impacts of these on ecosystem quality, and in some cases biodiversity of different trophic levels has been combined in a similar manner to create a multitrophic diversity index (Soliveres et al., 2016). The breadth of the data

presented through Chapters 2 through 5 could allow us to perform a similar analysis; in particular evaluating whether the combination of functional properties and trophic levels obscures important relationships and trade-offs between functions. The wide range of habitats within this dataset would allow us to evaluate whether the same ecosystem properties drive multifunctionality in grasslands vs forests vs heathlands. This is particularly important for predicting future response to change and informing policy.

The aim of national monitoring schemes is to repeatedly measure sites to estimate national-level change. The data presented here in Chapters 2 through 5 represents a starting point for future surveys which will be able to evaluate if national soil health follows the trajectory required for commitments to sustainable growth. Following the future surveys, we will be able to evaluate if more soils are within the thresholds for soil health shown in Chapter 2 and whether the overall trends in soil carbon across Wales are going to meet the targets of the 4 per mille initiative. We will also be able to evaluate specifically if the soils in the uplands are losing carbon in response to climate change as predicted from the long-term climate change experiment (Appendix B). Changes in land use are occurring in conjunction with climate change, and the design of this survey could mean identification of whether the agri-environment scheme in Wales is helping to improve ecosystem health.

The resurveying of the sites within the GMEP monitoring scheme also allows us to identify sites that have changed with respect to their land use or plant community and measure the impact this has on soil biodiversity and health. There have been repeated land use surveys of the UK, which gives great opportunities for evaluating the impact of historical land use upon soil health. Legacy effects of agriculture and urban areas could be limiting the functionality of soils at various sites, although with the trend for increasing urbanisation and expansion of agriculture, the areas which have been left to rewild are few which may mean monitoring data will be unable to yield meaningful trends. Evaluating more detailed information on the plant community could allow demonstration of any potential lag effect as soils and plants change along the land use intensity spectrum. This national scale data could be used in conjunction with

experimental and more detailed approaches to predict future responses to agricultural intensification or rewilding.

Future change in climate will not only impact overall average properties but also the frequency of extreme events and it is the impacts of the combination of these that are particularly difficult to predict. The importance of drought events in determining soil microbial response and quality was shown in this thesis. In Chapter 3 the impacts of dry spell events upon the plant community and soil properties was demonstrated even once overall precipitation changes were accounted for. The shift over time in Clocaenog may also have been related to an extreme drought event in the early 2000's (Robinson et al., 2016). We require further investigation of the impact of extreme events upon soil quality and biodiversity, examining the role of flood and drought events upon national scale monitoring data as well as experimental approaches. Changes in the frequency of other extreme events, such as the reduction in frost days and snow cover should also be investigated for their potential impact upon soil health. Across the UK we have detailed information upon the extreme weather events of the past decades along with repeated ecological monitoring data which could be combined to analyse the ecosystem response to extreme events and how it varies by habitat. The variation in resilience and resistance to extreme events between the different components of the ecosystem must be examined and accounted for in predictions of the stability of future ecosystem health in the short and long term.

Stability of the current soil system is not only related to how responsive each component of the system is to external drivers but also the presence of non-linearities and potential alternative states. Within Chapter 5 we saw that the response of soil microbial diversity to pH is non-linear. The presence of this transition point at ~ pH 5 could make ecosystems change suddenly in functionality and health in ways that are more difficult to predict. These transition points in biodiversity and key soil functions such as nitrification and carbon use efficiency need to be fully verified and tested in order to inform monitoring and policy. In Chapter 2 we saw that there are national level trigger values for pH and other soil properties which are related to soil health (Bhogal et al., 2008). The exact response of soil functioning to moving across these thresholds is still unclear, particularly at field and regional scales. Therefore, more

experimental and modelling approaches are required to analyse the potential impacts of such transitions to ecosystem health particularly in relation to the stability of any such transition.

Throughout this thesis we have used many different assumptions on the mechanisms underlying the soil processes we have measured to constrain our analysis. Many of these have been tested either only at a qualitative level or not at all. There have recently been attempts to inoculate soil microcosms with different microbial communities to directly measure the impact of different microbial communities upon soil processes (Maron et al., 2018; Sergaki et al., 2018). This offers intriguing possibilities in answering various questions, e.g. the relative role of plants vs soil microbes in determining community assembly. This could help inform interpretation of Chapter 5, in which it was assumed that plants determine soil microbial communities due to the relatively long-lived nature of plant communities. However, despite their relatively short lifespans (<1 year) differences in soil microbial communities can persist for hundreds of years due to persistent differences in soil physicochemical properties (Diedhiou et al., 2009). These persistent differences in soil microbial, and physicochemical, properties could be determining plant community dynamics and experimental approaches could help disentangle these interrelated drivers.

The nature and direction of the relationships between plant, microbial and physicochemical processes were analysed only simplistically within this thesis. For example, the assumptions in Chapter 4 were that the plant and microbial community produce hydrophobic compounds that determine water repellency and not that water repellency influences the plant and microbial communities. This requires testing, in particular the finding that stress tolerance of the plant community may be related to water repellency. The exudates produced by plants of differing stress tolerance under different stresses should be examined for their hydrophobic and nutrient containing properties. These types of complicated interactions between biological, chemical and physical properties in response to external and internal drivers are both difficult and essential to understand. The potential role of chaotic, unstable dynamics in determining soil properties and health requires further investigation. Microbial and

physical structure of the soil underpins soil health but is continually changing and dynamic. Repeated measurements of soil microbial community and physical properties at a site like Clocaenog would increase our understanding of the role of seasonal and shorter-term processes in driving soil dynamics. Ideally these analyses should be repeated across a range of ecosystem types, in order to understand the differences in dynamics between grasslands, woodlands and heathlands. Understanding changes over time in conjunction with mechanistic understanding of the processes is necessary for predicting future soil stability and health.

Soil physical structure is dynamic and difficult to measure. Within this thesis we have presented broad brush, sample scale measurements such as bulk density and water content (Chapter 2), as well as more detailed information on the textural composition of the soil (Chapter 3). There are other methods of analysis of soil structure which can offer more detailed information on soil mineral composition, pore connectivity and aggregate structure, including water release curves and X-ray CT scanning (Helliwell et al., 2013; Rabot et al., 2018). These could be used to analyse the impact of plant and microbial exudates upon soil water movement and aggregate stability. This analysis could also be used to establish whether the correlations we observed in Chapter 3 between different sizes of soil particles and microbial taxa relate to those particles being associated with different mineralogies, aggregate sizes or physical areas of the soil. They could also be used to better link microbial properties to soil function, as soil structure underpins essential functions such as water filtration and carbon storage. Within particularly organic soil similar analyses could be undertaken linking microbial taxa with specific types of organic material using compound-specific analysis or similar methods (e.g. pyrolysis-mass spectrometry, Fourier-transform ion cyclotron resonance mass spectrometry). Understanding the role of soil structure as a dynamic property in determining microbial diversity and function is an essential link in evaluating the impacts of future change on soil health.

The methods used to describe the soil microbial community are ever improving and understanding the strengths and limitations of each method still developing. Even within the DNA metabarcoding methods used within this thesis there is still uncertainty of the importance of the bioinformatic pipeline in determining the results

(Callahan et al., 2017). The difference between the use of an ASV vs an OTU approach within national-scale monitoring and particularly under the range of abiotic conditions which affect DNA recovery and quality requires further evaluation. Also, the ability to link to functional microbial processes is limited which is why techniques such as shotgun metagenomics are increasing in use (Knight et al., 2018). Metagenomic techniques allow analysis of specific genes or taxa that are important to key functions and can incorporate all organisms within the soil for a more comprehensive evaluation of the relative importance of prokaryotes, protists, fungi, animals and viruses in soil health. Application of metagenomic, metatranscriptomic, metaproteomic, and metabolomic techniques in evaluating soil microbial function could lead to much greater understanding of soil function across Wales and the significance of the microbial response to experimental warming presented in Chapter 6.

The methods applied to the analysis of the microbial data across this thesis are in no way comprehensive and there is further information to be gained from the application of different methods, such as phylogenetic analysis. Phylogenetic information on the relatedness of different microbial taxa can be combined with species diversity approaches based upon Hill numbers to create new metrics that provide a better characterisation of the shifts in microbial composition in response to environmental change (Chao, Chiu & Jost, 2010, 2014; McCoy & Matsen, 2013). However, applying these approaches to metabarcoding studies requires consideration of the fact that differences in sequence abundance between taxa are unlikely to relate to differences in the actual abundance of the taxa (Amend, Seifert & Bruns, 2010; Nguyen et al., 2014). There are an ever-increasing number of statistical methods for analysing microbial metabarcoding data that can take into account differences in read depth across samples and various sample meta-information which could provide new insight into microbial communities (e.g. Harrison et al., 2020; Ovaskainen et al., 2017; Sankaran & Holmes, 2019). Further work is required to evaluate the most effective way to bring together all of the information gained from metabarcoding studies of soil microbial communities in order to evaluate their response and relevance to global environmental change.

Evaluation of the relevance of this work to the wider world requires comparison to other sites and regions. The site of our long term climate change experiment analysed in Chapter 6 and Appendix B is unusual in the amount of carbon it has lost in response to the warming treatment (Crowther et al., 2016; van Gestel et al., 2018). Currently we do not understand what drives these differences in soil respiration response to warming, although suggestions have been made that soil biological communities could be important (Ren et al., 2018; van Gestel et al., 2018). Comparison with other sites which have lost far less carbon could provide valuable insights into the mechanisms, biological or otherwise, which are driving these differences. Increasingly, there have been more studies evaluating change in soil microbial communities in response to long term climate change (e.g. Birnbaum et al., 2019; Sayer et al., 2017) which offer more opportunities for meta-analyses.

The work within this thesis has focused on the soils of Wales and there are opportunities to compare the results with the soils of different regions to evaluate the wider validity of the results. Within the UK, the Countryside Survey has multiple years of field survey data with similar spatial structure, most recently from 2007, which could offer insight as to whether the results we have found also occur in the more intense arable soils of the South-East of England and the extensive peatlands in the North of Scotland (Reynolds et al., 2013). Across the EU the LUCAS field survey follows a similar structure to that of the Countryside Survey and GMEP and the results could be used in a similar manner to evaluate soil health across Europe (Orgiazzi et al., 2018). All of these surveys have also begun to analyse the soil microbial communities from their sites, offering new opportunities in understanding biological dynamics and quality across Europe.

The examples provided above are of comparable data sources to those within the thesis, however there are great gains to be made by the combining of different types of data to further soil and ecological understanding. The increasing use of citizen science to gather data on species occurrence, land use and even soils data provides a high density of data that is often difficult to quality control and analyse. Even within comparison of different scientific projects it can be difficult to compare results from different methods of analysing ecosystem properties, hence why there have been

recent efforts to standardise measurement methodology on climate experiments (Halbritter et al., 2019). However, there are recent advances in developing statistical techniques to combine data sources in ways that greatly increase the power of detecting ecological change (Isaac et al., 2019). While it may be easier to motivate citizens to gather data on the presence of birds than the quality of the soil these principles hold in combining a variety of data sources.

7.4. Concluding remarks

Throughout this thesis, in addition to soil forming factors such as parent material, climate and relief, we have seen the importance of soil structure, microbial activity and plant communities in determining overall soil characteristics and ecosystem functioning. We have seen how the range of soil types across Wales vary in their physicochemical and biological characteristics and linked these to the functions and services they provide. The strong correlation between pH and many soil functions including carbon storage, microbial composition, and hydrological behaviour shows how key soil indicators can be used as a crude measure of soil health. However, we have also shown that not all soil health indicators correlate with each other and therefore that management decisions must prioritise certain functions over others. We have found that soil microbial community composition and diversity is responsive to both soil structure and plant communities, and that greater understanding of the nature of these relationships is essential to understanding soil function and predicting future change.

Soils are under increasing stress, as demand for food and infrastructure increase in tandem with climate change and pollution. Within this thesis we have seen that changes in climate can impact soil biology and key functions such as hydrological behaviour and respiration. More importantly, however, we have shown the interconnectedness of the soil system, as biological taxa show strong relationships with both each other and soil physicochemical properties. Understanding this interconnectedness and its influence on ecosystem resilience to stress is the key challenge facing soil science. Integrating different sources of information, including monitoring, experimentation and modelling, allows us to address questions key to the

maintenance and improvement of soil health at a national scale. The future of soil science lies in combining the variety of effort and data collection to address the fundamental questions around climate change, sustainable land use and food production facing us as a society.

7.5. References

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APPENDIX A

Divergent national-scale trends of microbial and animal biodiversity revealed across diverse temperate soil ecosystems

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Contribution statement:

Jones, Robinson, Creer, and Emmett conceived this project. Lallias, Jones, and Lebron processed the soil samples and collected data with quality assurance by Seaton. Lallias and Creer led the DNA extraction, primer design, library generation, and established the bioinformatics pipelines. Kenny and Eccles led sequence data generation and assisted with library preparations. Bioinformatics and statistical analyses were led by George with assistance from Creer, Griffiths, Lallias, and Seaton. George wrote the first draft of the manuscript and Seaton, Creer, Lallias, Griffiths, Robinson, and Jones contributed to subsequent revisions. All authors read and approved the final draft of the manuscript.

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Abstract

Soil biota accounts for ~25% of global biodiversity and is vital to nutrient cycling and primary production. There is growing momentum to study total belowground biodiversity across large ecological scales to understand how habitat and soil properties shape belowground communities. Microbial and animal components of belowground communities follow divergent responses to soil properties and land use intensification; however, it is unclear whether this extends across heterogeneous ecosystems. Here, a national-scale metabarcoding analysis of 436 locations across 7 different temperate ecosystems shows that belowground animal and microbial (bacteria, archaea, fungi, and protists) richness follow divergent trends, whereas β-diversity does not. Animal richness is governed by intensive land use and unaffected by soil properties, while microbial richness was driven by environmental properties across land uses. Our findings demonstrate that established divergent patterns of belowground microbial and animal diversity are consistent across heterogeneous land uses and are detectable using a standardised metabarcoding approach.

Introduction

Soil biota, including bacteria, archaea, protists, fungi and animals underpin globally important ecosystem functions. Fundamental functions of soil communities include nutrient and hydrological cycling, decomposition, pollution mitigation, and supporting terrestrial primary production, which are inextricably linked to global food security, climate regulation and other ecosystem services[1-2]. Nevertheless, until recently, characterising soil biodiversity (popularly referred to as a "black box") has been constrained by our inability to identify typically intractable levels of diversity using either traditional or molecular approaches. High-throughput sequencing has however resulted in a step change, facilitating the characterisation of bacteria[3-7], archaea[6-8], fungi[9-10], protists[11-13], and animals[14] within the belowground biosphere. Increasingly, efforts have been made to investigate the total biodiversity of the soil biosphere across large ecological[15-17] and taxonomic scales[15-16,18-19].

Understanding the response of the total soil biosphere to changes in land use and environmental drivers has become an important research focus in regional soil monitoring programmes[15-16,19] and in small-scale field[20-21] and mesocosm experiments[18,20]. Yet despite the move towards unified study of soil biota, fundamental challenges of technique and scale remain. Often such studies require the comparison of soil biota metrics captured through both traditional and modern molecular techniques[15,19-21]. To our knowledge, relatively few studies have attempted to assess all components of belowground communities using a multi-marker metabarcoding approach[22].

There is mounting evidence that the microbial and animal fractions of soil communities may respond differentially to land use change. Microbial richness increases[15], whereas richness of soil fauna declines in response to more intense land use[15,23-24]. However, these findings come from relatively homogenous landscapes, such as grasslands[15]. It is unclear whether the differential responses of soil microbes and fauna extend across heterogeneous land uses. For example, across heterogeneous landscapes of Wales, UK, α-diversity of mesofauna is both lowest in agricultural and bog systems, which are the most- and least-intensively managed systems in the country, respectively[23]. Changes in soil properties may further dictate declines of

common soil fauna in low-intensity land uses. Therefore, it is critical to assess whether the positive effect of increasing land use intensity on microbial richness is consistent across regions made up of markedly diverse ecosystems and land uses. Similarly, the importance of individual soil properties in shaping belowground communities has also proven difficult to disentangle. Many studies have demonstrated the consistent dominance of pH in shaping belowground community composition at national [23,25-28] and global scales[4-5,9,29]. However, climatic factors[9,30] and other soil properties, including organic matter, nitrogen (N) availability, and the carbon (C)-to-N ratio[9] are also recognised as important drivers of belowground community composition yet consistent trends remain elusive[30]. Therefore it is unclear whether the total soil biosphere responds to changes in land use and soil properties in the same manner across heterogeneous landscapes.

Here, we sought to assess whether divergent responses to land use and soil properties in the microbial and animal fractions of soil communities persist across heterogeneous systems at the national-scale using a standardised metabarcoding approach. We present a national-scale analysis of soil biodiversity across Wales, UK, from the micro-to-macro scale including all major groups of soil microbes in addition to animals, from 436 sites over 2 years across a diverse array of oceanic-temperate ecosystems, including grasslands, forests, bogs, and managed systems. Biotic metrics come from high-throughput sequencing of prokaryotic, fungal, microbial eukaryotic and soil animal communities using 16S, ITS, and 18S rRNA marker genes; these are complemented by an extensive suite of co-located abiotic soil properties and vegetation cover data. Specifically, we investigate how richness and β-diversity of all major fractions of subterranean life respond to land use type and prevailing soil properties (e.g. organic matter, pH, and N) to explore which lineages play a demonstrable role in determining belowground community structures across large and complex ecological gradients. Our results demonstrate that across a gradient of heterogeneous land uses, richness of soil animals is governed more by land use regime rather than intrinsic soil properties. In contrast, microbial richness is driven by soil properties and demonstrates a largely linear trend of decreasing richness along a productivity gradient of land use based on decreasing soil nutrient availability.

Results

Sequencing results

Illumina sequencing and environmental data was collected from across Wales as part of the Glastir Monitoring and Evaluation Programme (GMEP)[31]. Sample sites were categorised into Aggregate Vegetation Classes (AVCs) based on plant species assessments using established criteria (see Supplementary Note 1). An explanation of the composition of AVCs is described in Supplementary Table 1. Briefly, the 7 AVCs used in the current study were established by clustering samples based on an assessment of vegetation data using a detrended correspondence analysis[32]. The ordination of the detrended correspondence analysis has shown that the land use categories follow a gradient of soil nutrient content[32] from which soil productivity and management intensity can also be inferred (see Supplementary Note 1 and Supplementary Table 1). The AVCs in descending order of productivity are: Crops/weeds, Fertile grassland, Infertile grassland, Lowland wood, Upland wood, Moorland grass-mosaic, and Heath/bog.

In total, 29,690 bacterial and 156 archaeal operational taxonomic units (OTUs) were identified from 16S reads. Overall, the most abundant class was Alphaproteobacteria (Fig. 1a). Proportional abundances (OTU n/total x 100) of Acidobacteria increased in less-productive land use types from its lowest in Crops/weeds to its highest in Heath/bog AVCs. In contrast, abundances of Actinobacteria followed the exact opposite trend, as did Spartobacteria and Bacilli (Fig. 2a). For archaea, Nitrososphaeria was the most abundant class overall (Fig. 1d); however, the proportion of Thermoplasmata became dominant in less productive AVCs (Fig. 2d).

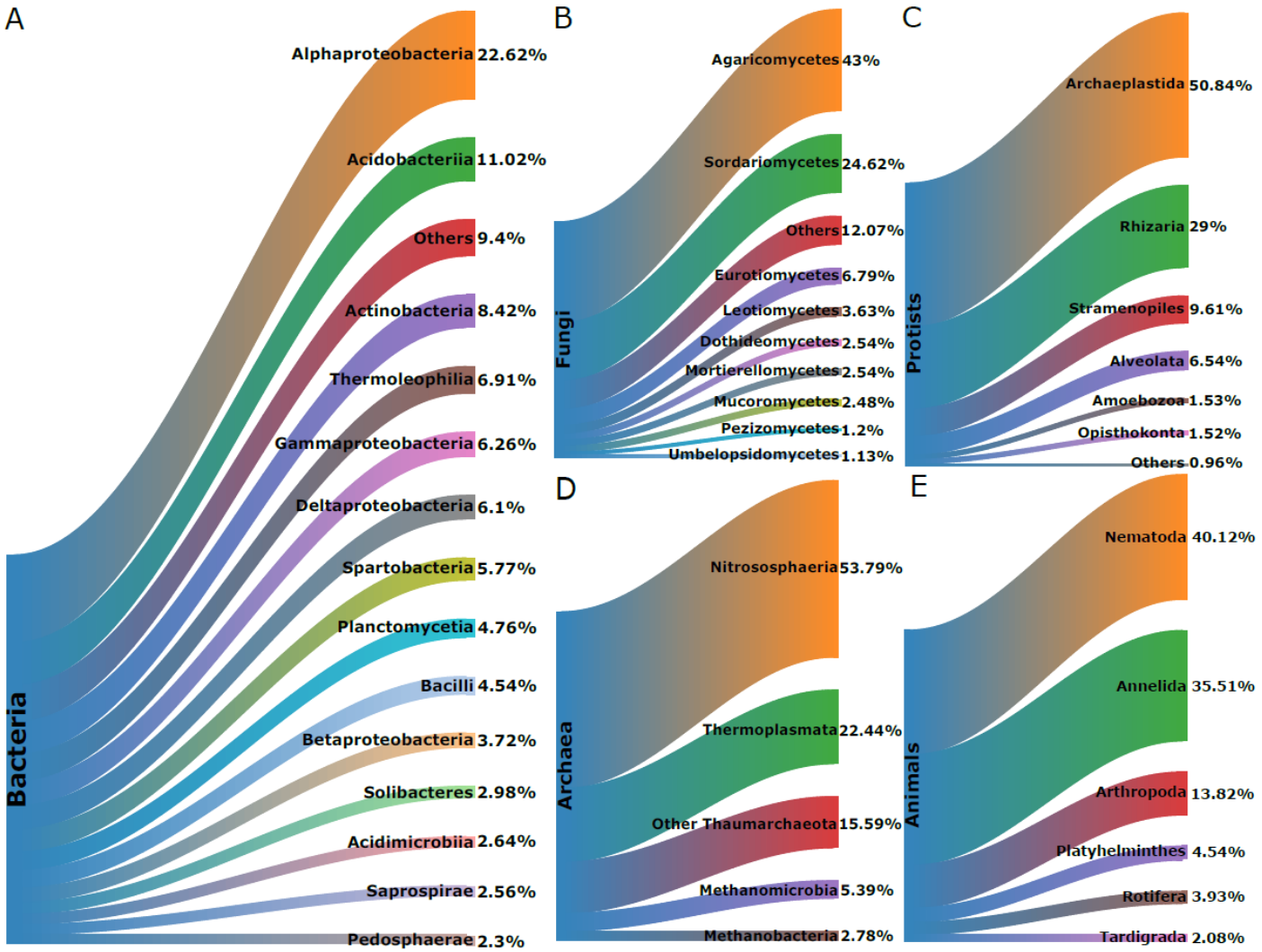

*Fig. 1. Sankey diagrams of proportional abundances of OTUs from all samples for major soil biota groups. Arms denote proportions of OTUs at the class-level for **a)** bacteria; **b)** fungi; of major lineages of **c)** protists; class-level for **d)** archaea; and at the phylum-level for **e)** animals. For information on how this figure was created, please see the Supplementary Methods.*

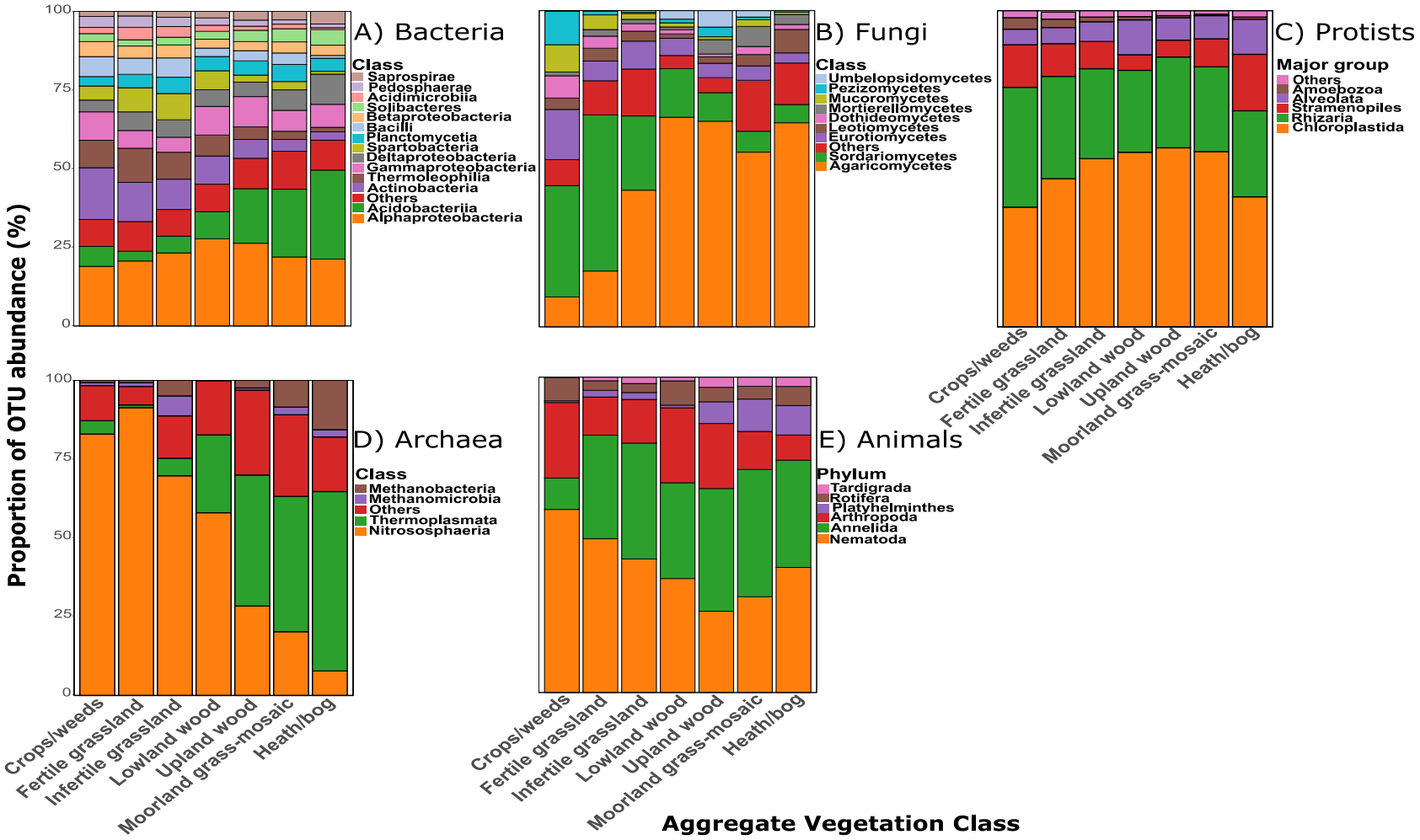

*Fig. 2. Proportionate abundances of OTUs for major soil biota groups within each Aggregate Vegetation Class ordered from most (Crops/weeds) to least (Heath/bog) using the same divisions as Fig. 1 for **a)** bacteria; **b)** fungi; **c)** protists; **d)** archaea; and **e)** animals.*

There were 7,582 OTUs recovered from ITS1 sequences. Agaricomycetes were the most abundant class of fungi overall. There were also a large proportion of Sordariomycetes (Fig. 1b). Proportionate abundances of Sordariomycetes and Agaricomycetes followed contrasting trends, with the dominance of the former replaced by the later in lower productivity AVCs (Fig. 2b).

In total, 8,683 protist OTUs were recovered from the 18S reads. Chloroplastida (green algae) was by far the most abundant protist group, followed by Rhizaria, Stramenopiles, and then Alveolates (Fig. 1c). Green algae, largely comprised of unidentified sequences (Supplementary Fig. 1a), were least abundant in Crops/weed and Heath/bog sites (Fig. 2c). Proportions of Rhizaria were relatively constant across AVCs (Fig. 2c) and entirely comprised of Cercozoa (Supplementary Fig. 1b). Among Stramenopiles proportions of Ochrophyta were also largely consistent, while those of Oomycetes and Bicosoecida followed contrasting trends across the productivity gradient of AVCs, declining and increasing, respectively (Supplementary Fig. 1c). Ciliates were the most common Alveolates in most AVCs; however, the proportion of Apicomplexa was greater in the Lowland wood and grassland AVCs (Supplementary Fig. 1d). The proportion of Amoebozoa was surprisingly low (Fig. 1c), potentially due to primer bias in our study when compared to other studies[12,15]. Across AVCs Tublulinea was consistently dominant among the Amoebozoa, though divergent trends in Gracilipodida and Discosea can be seen along the productivity/intensity gradient (Supplementary Fig. 1e).

In the animal dataset, 1,138 OTUs were recovered. Nematode OTUs were the most abundant animal group across all samples (Fig. 1e). Annelids and arthropods followed opposing trends in proportionate abundance, increasing and decreasing respectively, across the productivity gradient. Proportions of platyhelminthes and tardigrades also increased in less-productive AVCs (Fig. 2e).

Effect of land use on belowground richness

We found significant differences in biodiversity trends across land use types. There was a marked shift along the productivity gradient of Crops/weeds-to-Heath/bog in all organismal groups, except animals (Fig. 3). Significant differences in

the mean richness of bacterial OTUs were prominent ($F_{6, 264}$ = 78.47, p < 0.0001) following ANOVA. Bacterial richness decreased in AVCs across the productivity gradient with highest values in the most productive Crops/weeds and grasslands and lowest in the low productivity land uses (i.e. Moorland grass-mosaic, Heath/bog) (Fig. 3a). The same trend was also observed in fungi ($F_{6, 248}$ = 48.98, p < 0.001; Fig. 3b), and protists ($F_{6, 249}$ = 59.86, p < 0.001; Fig. 3c). For individual pair-wise comparisons see Supplementary Note 4. Richness of archaeal OTUs had an opposing trend to that of other microbial groups. Archaeal OTU richness was significantly lower ($F_{6, 185}$ = 24.37, p < 0.001) in higher-productivity AVCs and highest in the least-productive land-use types (Fig. 3d). In the Crops/weeds AVC richness of archaeal OTUs was significantly lower than Upland wood (p = 0.01), Moorland grass-mosaic (p = 0.005), and Heath/bog sites (p < 0.001) based on Tukey's *post hoc* tests, with the remaining land uses displaying intermediate OTU richness values.

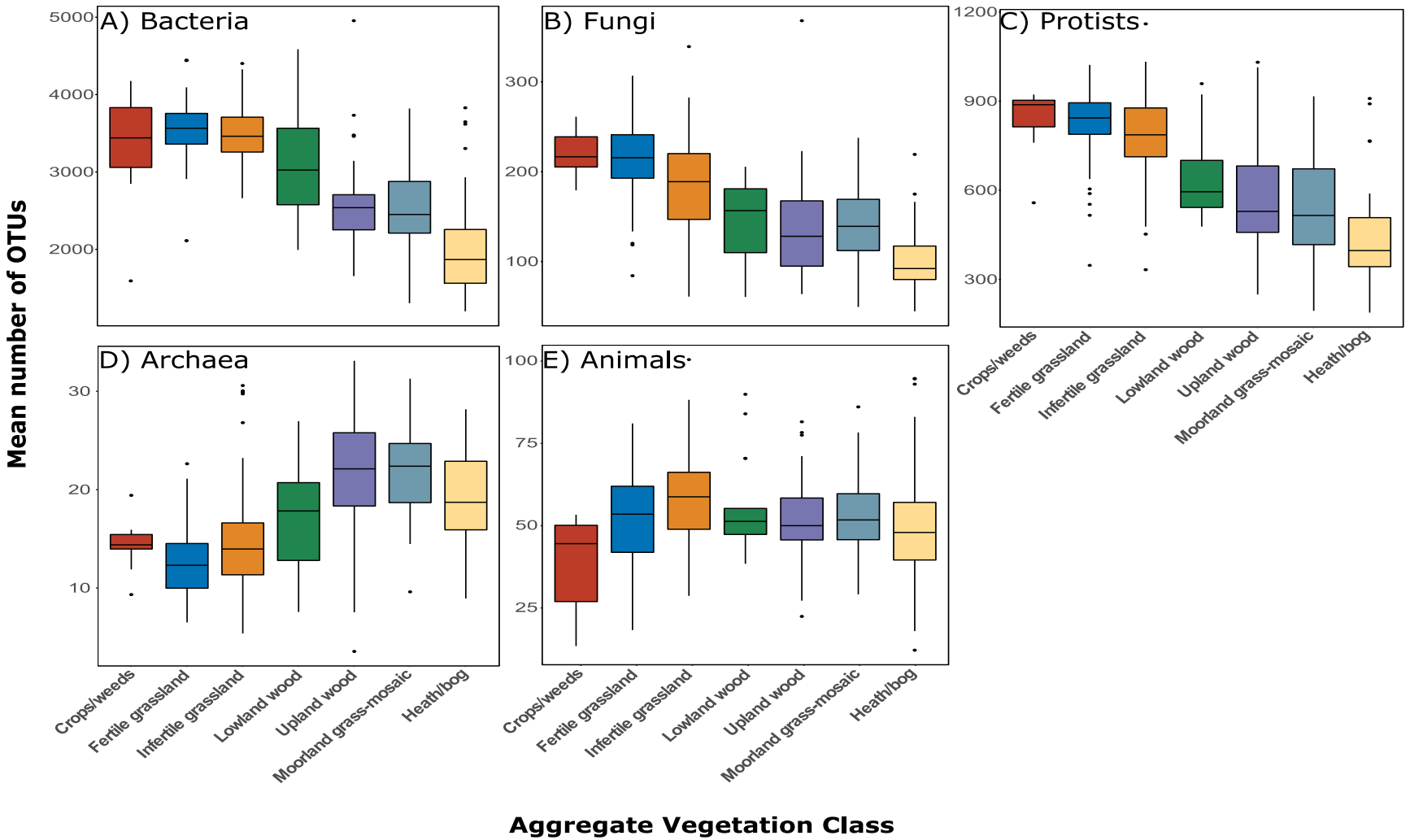

*Fig. 3. Boxplots of OTU richness for a) bacteria; **b**) fungi; **c**) protists; **d**) archaea; **e**) animals plotted against Aggregate Vegetation Class ordered from most (Crops/weeds) to least (Heath/bog) productive. Boxes are bounded on the first and third quartiles; horizontal lines denote medians. Black dots are outliers beyond the whiskers, which denote 1.5X the interquartile range. Source data are provided as a Source Data file.*

Animal OTU richness did not follow the trends observed in microbial communities. Differences observed with ANOVA were significant ($F_{6, 244} = 6.25$, $p < 0.001$) but plateaued after the grassland AVCs, as opposed to the sloped trend of microbial groups across the productivity gradient (Fig. 3e). Richness in the Infertile grasslands was significantly greater than in Crops/weeds ($p = 0.008$), Heath/bog ($p = 0.003$), and Upland wood ($p = 0.02$) based on Tukey's *post hoc* tests. Richness was lowest in the most intensively management Crops/weeds sites and was shown to be significantly lower than richness of Lowland woods ($p = 0.04$) with Tukey's test. Collectively the results demonstrate a strong divergence between the richness of animal and microbial communities across all AVCs.

Relationships of richness between organismal groups

Bacterial richness from the total data set was significantly correlated with all other organismal groups (Supplementary Table 2). Such relationships were positive between bacterial richness and richness of fungi, protists, and animals. Similarly there was a positive relationship between protistan richness and both fungal and animal richness. However, archaeal richness demonstrated significant, but negative correlations with all organisms except animals. Indeed animal richness (measured by metabarcoding) was only significantly correlated with animals (measured by taxonomic assessment; Table 1) and protists (Supplementary Table 2).

*Table 1 Results of partial least squares regressions for soil biota against soil properties for richness. Positive relationships are underlined; negative relationships are written in italics. *** indicates p < 0.001, ** 0.001 > p < 0.01, * 0.01 > p < 0.05, blank indicates p > 0.05.*

Soil and environmental variables	Taxon				
	Bacteria	Archaea	Fungi	Protists	Animals
Total C^{L}	*1.14 (R^2 = 0.44***)*	1.21 (R^2 =0.13***)	0.44	*1.3 (R^2 = 0.35***)*	0.9
Total N^{L}	0.93	0.89	0.93	0.8	1.18
C:N ratio^{S}	*1.45 (R^2 = 0.41***)*	1.31 (R^2 = 0.09***)	*1.64 (R^2 = 0.28***)*	*1.67 (R^2 = 0.35***)*	0.1
Total P (mg kg ^-1)^{S}	0.35	0.59	0.7	0.85	0.67
Organic matter (% LOI)^{L}	*1.47 (R^2 = 0.5***)*	1.27 (R^2 = 0.14***)	*1.13 (R^2 = 0.29***)*	1.27 *(R^2 = 0.35***)*	1.08
pH ($CaCl_2$)	1.98 (R^2 = 0.51***)	*1.68 (R^2 = 0.25***)*	1.52 (R^2 = 0.23***)	1.56 (R^2 = 0.33***)	0.9
Soil water repellency^{L*}	*1.31 (R^2 = 0.2***)*	0.9	*1.23 (R^2 = 0.13***)*	0.93	0.98
Volumetric water content (m^3 m^3 ^-1)	0.36	1.33 (R^2 = 0.13***)	0.6	0.41	0.4
Soil bound water (g water g dry soil ^-1)	*1.25 (R^2 = 0.41***)*	0.83	*1.08 (R^2 = 0.26***)*	*1.23 (R^2 = 0.31***)*	0.63
Rock volume (mL)	0.25	0.61	0.64	0.27	1.3
Bulk density (g cm^3 ^-1)	1.39 (R^2 = 0.44***)	*1.43 (R^2 = 0.18***)*	1.41 (R^2 = 0.29***)	1.5 (R^2 = 0.35***)	1.39
Clay content (%)^{A}	0.85	*1.19 (R^2 = 0.1***)*	0.84	1.14 (R^2 = 0.09***)	0.05
Sand content (%)^{A}	0.45	0.16	0.6	0.51	0.78
Elevation (m)	*1.66 (R^2 = 0.42***)*	1.7 (R^2 = 0.27***)	*1.68 (R^2 = 0.22***)*	*1.65 (R^2 = 0.36***)*	0.57
Mean annual precipitation (mL)	*1.08 (R^2 = 0.25***)*	1.75 (R^2 = 0.3***)	*1.44 (R^2 = 0.18***)*	*1.48 (R^2 = 0.27***)*	0.46
Temperature (°C)	0.51	0.5	0.56	0.58	0.35
$Collembola^{L1}$	0.34	0.06	0.41	0.17	1.14 (R^2 = 0.03***)
$Mites^{L1}$	0.49	0.2	*1.17 (R^2 = 0.03***)*	0.23	1.74 (R^2 0.08***)
Total $mesofauna^{L1}$	0.44	0.1	*1.03 (R^2 = 0.01*)*	0.15	1.71 (R^2 0.08***)

*Note: ^{A} denotes Aitchison's log-ratio transformation; ^{L} denotes log_{10}-transformation; ^{L1} denotes log_{10} plus 1 transformation ^{S} denotes square-root-transformation; * soil water repellency was derived from median water drop penetration times (s)*

Relationships between richness and environmental variables

Partial least squares (PLS) regressions demonstrated that the divergence observed between animal and microbial communities may be due to the effects of soil properties. No soil properties were significantly correlated with richness of soil animal OTUs (Table 1). Conversely, there were strong relationships between microbial richness and a range of soil properties. However, although microbes were influenced by the same environmental variables, there were distinct patterns within each group. For example, while pH was the best predictor of bacterial richness, it was ranked as second for fungi and protists and third for archaea. Bulk density and C:N ratio were also major drivers of richness across all microbial groups. Elevation (here closely linked with precipitation and organic matter content) was the most important environmental variable in relation to archaea and protist richness. Organic matter and bulk density were strong predictors of fungal OTU richness. All environmental properties that had positive relationships with OTU richness of bacteria, fungi, and protists had negative relationships with archaea.

Community structure (β-diversity) across land uses

Non-metric multidimensional scaling (NMDS) using Bray-Curtis distances showed consistent differences in β-diversity between AVCs across all organismal groups. Plots show tight clustering of the Crops/Weeds, Fertile Grassland, and Infertile Grassland AVCs, whereas the other AVCs form a more dispersed organismal assemblage (Fig. 4 for bacteria and Supplementary Figs 2-5). Results of PERMANOVAs were significant across all groups and analyses of dispersion were also significant (Fig. 4 for bacteria and Supplementary Figs 2-5) for all groups except for the dispersion of animals ($F_{6,\ 401} = 0.67$, $p = 0.68$) owing to the wide range of sample numbers within each AVC (Supplementary Fig. 5). We also found that this clustering was present using constrained canonical analyses of principle components (CAP) ordinations for each organismal group (Supplementary Figs 6-10).

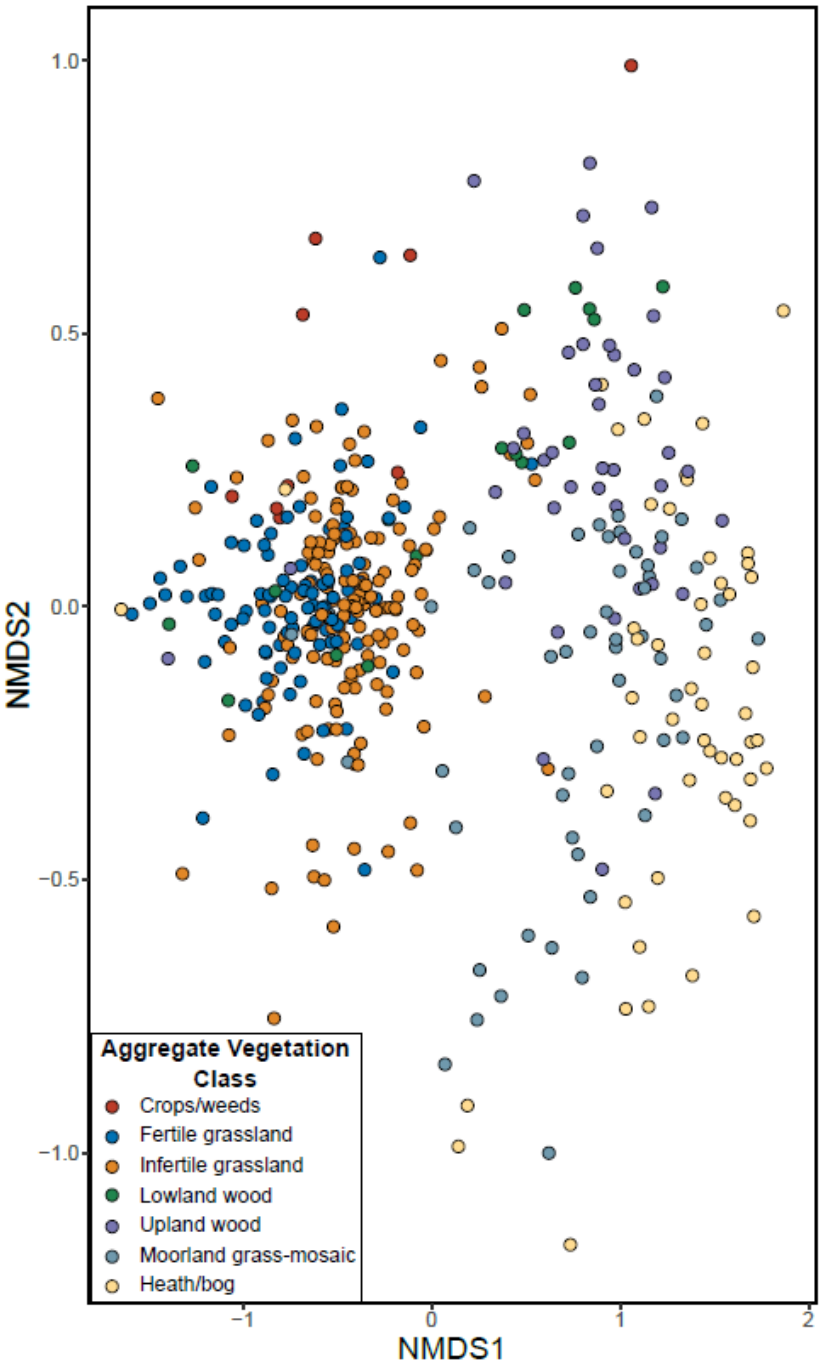

Fig. 4. Plot of the non-metric dimensional scaling ordination (stress = 0.06) of bacterial community composition across GMEP sites. Samples are coloured by Aggregate Vegetation Class. Results of PERMANOVA ($F_{6,427}$ = 30.76, p = 0.001) and dispersion of variances of groups ($F_{6,427}$ = 10.97, p = 0.001) were significant.

pH was the best predictor of β-diversity from linear fitting for all soil organisms (Table 2 and Supplementary Tables 3-6). The carbon-to-nitrogen (C:N) ratio was the second most important variable in all major groups except animals. Mean C:N values were

higher in the Crops/weeds and grassland AVCs and lower in the remaining land use types (Supplementary Table 6). Mean pH values and C:N ratios (Supplementary Table 6) reflect the distribution of points in NMDS plots, with tight groupings observed in the Crops/Weeds and grasslands AVCs and increasingly more spread out groupings in all other AVCs as pH values decreased and became more varied (Fig. 4 for bacteria and Supplementary Figs 2-5). Across all groups, all or nearly all variables were significant following linear fitting; however, most were only weakly correlated with β-diversity values. Other important variables varied in their ranked importance including: elevation, mean annual precipitation, organic matter content, total C, bulk density, volumetric water content, and clay content of soil (Table 2 and Supplementary Tables 3-6). The results of linear model fitting for CAP ordinations, though not identical (Supplementary Tables 7-11), were highly related to those of the NMDS ordinations (Supplementary Fig. 11).

Table 2 Summary of relationships amongst environmental factors and bacteria communities.

Soil and environmental variables		Correlation		
	R^2	Axis1	Axis2	Axis3
pH ($CaCl_2$)	0.71^{***}	-	-	+
C:N ratio^{S}	0.52^{***}	+	-	+
Volumetric water content (m^3 m^3 ^-1)	0.49^{***}	+	-	+
Bulk density (g cm^3 ^-1)	0.47^{***}	-	+	-
Organic matter (% LOI)^{L}	0.46^{***}	+	-	+
Elevation (m)	0.45^{***}	+	-	-
Mean annual precipitation (mL)	0.43^{***}	+	-	-
Total Carbon^{L}	0.39^{***}	+	-	+
Clay content (%)^{A}	0.33^{***}	-	+	-
Soil bound water (g water g dry soil ^-1)	0.31^{***}	+	-	+
Soil water repellency^{L*}	0.27^{***}	+	-	-
Total Nitrogen (%)^{L}	0.26^{***}	+	-	+
Sand content (%)^{A}	0.21^{***}	+	+	+
Collembola^{L1}	0.09^{***}	-	+	-
Mites^{L1}	0.06^{***}	+	+	-
Total Phosphorus (mg kg ^-1)^{S}	0.06^{***}	-	-	-
Total mesofauna^{L1}	0.06^{***}	+	+	-
Rock volume (mL)	0.05^{**}	-	+	-
Temperature (°C)	0.03^{*}	+	+	-

*Note: +/- signify the direction of association between each variable and respective NMDS axes. ^{A} denotes Aitchison's log-ratio transformation; ^{L} denotes log_{10}-transformation; ^{L1} denotes log_{10} plus 1 transformation ^{S} denotes square-root-transformation; * soil water repellency was derived from median water drop penetration times (s)*

Discussion

High-throughput sequencing the biosphere amongst heterogeneous soils revealed both expected and novel relationships between soil organisms and environmental drivers. The richness of microbes and animals had notable contrasting trends across land use types. The richness of microbial communities was strongly influenced by both land use and environmental variables, especially pH, C:N ratio, elevation, organic matter, and annual precipitation. Conversely, we found no significant associations between measured environmental variables and animal richness, which was negatively impacted by higher intensity land use, suggesting that richness patterns of microbial and macrobial life fractions adhere to different ecological determinants. For β-diversity, pH was by far the most important environmental variable in shaping community composition of all organismal groups, yet other drivers were attributable for influencing patterns of α-diversity.

Our findings demonstrate that diverging trends between soil microbes and fauna extend across distinct, heterogeneous land uses. Furthermore, we build on the work of Gossner et al.[15] by demonstrating that microbial richness, with the exception of archaea, increases with greater land use intensity across heterogeneous ecosystems at the national-scale. The divergence between microbes and animals at this scale is supported by previous findings from French soils[17,25]. Across France, bacterial richness[17] and biomass[25] were strongly linked to belowground environmental properties but largely unaffected by aboveground climatic variables, which commonly influence animal and plant biogeography[25,30]. Our findings show that richness of archaea, fungi, and protists also follow this trend – whereas archaea follow an opposing trend to all other groups.

There are several mechanisms that may explain the relationship between higher microbial richness and intensifying anthropogenic disturbance. One explanation is that consistent nutrient inputs from fertilizers and disturbance under tillage stimulate high α-diversity in these areas[16]. Indeed higher α-diversity has been observed in cropping systems than in forest or grassland sites for both bacteria[16-17], and fungi[16]. Interestingly, high microbial richness in more productive land use types (e.g. arable) may illustrate the intermediate disturbance hypothesis (IDH) within soil ecosystems.

Under the IDH, as outlined by Connell[33], diversity reaches its highest levels where succession has been interrupted by intermittent disturbance events. In our sites, microbial richness was highest in AVCs concurrent to disturbances (augmented by nutrient inputs) from agricultural interventions such as fertilisation, tilling, clearing, and the cultivation of livestock. However, it is also possible that the high diversity observed in the grassland and especially in agricultural land uses stems from organisms that have entered a dormant state after disturbance-induced changes to their environment[13,34]. Disturbance pressures can also lead to high bacterial diversity through the reduction in dominant OTUs, which are replaced by a wide range of weaker competitors. It has been demonstrated that α-bacterial diversity is greater in the phyllosphere of ivy in urban habitats associated with more anthropogenic stressors than in less disturbed sites[35]. Our findings suggest that the phenomenon of greater species richness resulting from the addition of nutrients and non-equilibrium dynamics induced by disturbance may extend to across all microbial groups, with the possible exception of archaea.

Richness of all microbial groups, except archaea, followed the land use productivity/management intensity gradient[32] with higher richness in the highly productive and more disturbed grasslands and arable sites and lower richness in the least productive, relatively undisturbed upland Heath/bog sites. Changes within bacterial and fungal communities reflected expected within-community changes following the shift in soil nutrient quality across land uses. Actinobacteria[36] and Sordariomycetes[37] are known to dominate bacterial and fungal communities in high productivity grasslands as witnessed here. In contrast, Acidobacteria increased in proportion in low productivity, highly acidic AVCs as expected based on previous studies from the UK[27] and across the globe[7]. Likewise, the greater proportion of Agaricomycetes OTUs in low productivity AVCs is intuitive as many Agaricomycete fungi are common in bogs and related low-productivity habitats across Wales[38].

Protists have been chronically overlooked in European soil monitoring programmes (but see[28]), as extracting trends of protist diversity across land uses is difficult. For example, Gossner et al.[15] were not able to show changes in richness across all protists with land use intensification. We demonstrate that protistan richness follows the

trends of bacteria and fungi across land uses, with the highest richness levels in arable land. As with other microbes, there is evidence of increased protist richness at the mesocosm[39] and field[40] level, in response to fertiliser addition. Furthermore, in German grassland soils, protist richness has been shown to increase with land use intensity[41]. Our results show that an association between intensification and protistan richness extends across the national-scale over multiple land uses.

Unlike other microbes, archaeal richness was greatest in low productivity AVCs and lowest in highly productive sites (Fig. 3d). Furthermore, our understanding of the extent of soil archaeal diversity and its functional capabilities is continually increasing[6-8]. Recent research has revealed many lineages of Thaumarchaeota are crucial links in the N cycle and methanogenesis in soils[7-8]. Archaeal richness was highest in the Moorland grass-mosaic and Heath/bog AVCs, likely due to the specialised nature of acidophilic lineages. In particular, the Thaumarchaeota[42] and Thermoplasmata[43] are known to proliferate (Fig. 2d) under reduced competition from bacteria.

Animal richness did not change linearly with land use and was not strongly influenced by environmental variables. Our molecular analysis of soil eDNA support recent findings by George et al.[23] based on morphological assessments of coincident soil mesofauna. Both the present work and George et al.[23] demonstrated that animal richness and abundance were lowest in land uses associated with more intensive management. Animal richness peaked in Infertile grasslands and was lowest in Crops/weeds sites (Fig. 3e). Agricultural disturbance negatively affects soil faunal richness and diversity across large geographic scales[14,23-24]. However, in the low-productivity land uses, although proportional abundances of arthropod taxa declined similarly to the findings of George et al.[23], overall richness was not as strongly affected due to an increase in fractions of annelids, platyhelminthes, and tardigrades. Such an increase in the peat-rich, low-disturbance, higher elevation sites is rather intuitive since annelids, platyhelminthes, and tardigrades are susceptible to desiccation and require moist habitats to be active components of the soil community[44-45]. As soil animals still exhibited expected lower diversity trends in more intensively managed land uses[15,23-24], there are further opportunities for research into understanding the

mechanisms underlying the divergent richness trends between microscopic animals and the rest of soil communities.

Soil pH, as evidenced by ordination results, was the most important environmental variable in our study for β-diversity and in most cases richness as has been previously observed across the UK[27-28] and at larger national[25-26] and continental scales[4-6]. pH has been implicated with driving richness of soil Archaea[42-43] and is the most important driver of protist communities in the UK[28]. However, pH only plays a marginal role in shaping soil protist communities globally[11]. Likewise pH is a poor predictor of global fungal biogeography, yet is a good predictor of ectomycorrhizal fungal richness[9], which may contribute to the Agaricomycetes OTUs observed in the present study. Nevertheless, it is important to acknowledge the inconsistent nature of correlations between microbial biodiversity and pH, potentially due to variations in soil properties occurring at scales that do not align with large-scale soil surveys[30].

We also observed a strong effect of C:N ratio in determining richness of microbes and β-diversity of all organismal groups, as has been observed in bacterial[27] and protistan[28] β-diversity across Britain and some fungi globally[9]. Yet C:N ratio is often co-correlated with other soil properties including bulk density, total C, organic matter, elevation, and mean annual precipitation. Disentangling such related variables is difficult; despite using PLS analyses[46] we could not disentangle co-correlated soil properties. For example, AVCs such as Moorland grass-mosaic and Heath/bog generally had higher elevation, mean annual precipitation, C:N ratio, and both total C and N (Supplementary Table 12) owing to their less-disturbed, upland location and often peat-rich soils. Higher C:N ratios are indicative of lower-quality soils[47] and have historically been associated with a shift in microbial biomass from bacterial to fungal dominance[48]. Our results suggest that, with the exception of archaea, microbial richness is equally susceptible to the effect of soil quality degradation. According to our results, archaea, on the contrary, appear to be well adapted to habitats with lower nutrient quality.

We observed strong relationships between soil properties and microbial, but not animal richness. We suspect this is due to the direct effects of soil properties on microbes. For example, shifts in pH towards either a more alkaline or acidic condition

inhibit the ability of most non-specialised bacteria to uptake nutrients from their environment[26]. In addition the quality of soil nutrients, as discussed previously, was likely a strong determinant of available nutrient resources and therefore total richness of microbes. We also found strong relationships between soil properties and β-diversity and across all organismal groups. These relationships between Bray-Curtis dissimilarities of and soil properties demonstrate that more dissimilar belowground communities correlate positively with indicators of better quality soils across the breadth of soil biota (Supplementary Table 6). However, associations between nutrient quality and animal community composition are likely the result of nutrients influencing the composition of the aboveground plant community[49] rather than direct interactions with animals. Furthermore, animals are more vagile than microbes and can actively seek out microhabitats with better resources[50], limiting the direct impact of soil properties on animal richness.

Using an extensive soil sampling programme and metabarcoding, we present perhaps the most comprehensive assessment of the belowground diversity in Europe. Despite uncertainties on the ability of environmental DNA methods using small soil volumes to accurately characterise communities of larger organisms[51], we were still able to detect key differences in larger organisms (i.e. animals) across land uses. Our results highlight the complexity of belowground ecology by demonstrating a divergence of patterns of richness between soil fauna and microorganisms at a national-level. We show that microbial richness is strongly influenced by soil properties in a near-uniform manner, whereas animal richness is not. Rather, animal richness is likely driven by changes in aboveground communities that stem from intensive land use management, while microbial richness was affected by soil properties in addition to land use. A particularly interesting outcome of our analyses is the near-uniform trend of declining microbial richness along a gradient of decreasing land use productivity/management intensity. The data therefore suggest that soil properties strongly affect bacteria, fungi, and protists in a similar manner, whereby richness decreases with soil quality; whereas archaea showed an opposing trend with increasing richness as productivity declined. The richness of animal OTUs, on the contrary, was not affected by soil properties although β-diversity was. Although often considered as

ecological 'black boxes', soils continue to provide unique and coherent insights into the differences between interconnected microbial and macrobial assemblages. Our findings also highlight the importance of the dynamics between biotic and abiotic processes that drive the organization of belowground biological diversity.

Methods

Sampling

Soil samples were collected between late spring and early autumn in 2013 and 2014 as part of GMEP (Supplementary Note 2), established to monitor the Welsh Government's agri-environment scheme, Glastir. The scheme covered an area of 3,263 km^2 with 4,911 landowners[31]. Briefly, surveyors collected samples from randomly selected 1 km^2 squares with up to 3 locations within squares, following protocols established by the UK Countryside Survey[52]. As described previously, habitat within plots was classified using plant species assessments into one of seven AVCs[32]: 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 Note 1; Supplementary Table 1). Soil type was derived from the National Soil Map[53] (Supplementary Note 3; Supplementary Table 13). Organic matter content was classified by loss-on-ignition (LOI) following the protocols of the 2007 Countryside Survey[51].

A total of 436 cores were collected from 1 km^2 squares, with up to 3 samples coming from an individual square based on a randomised sampling design. Cores were transported to the Centre for Ecology and Hydrology, Bangor, United Kingdom, and stored at -80 °C until DNA extraction. Soil physical and chemical properties were taken from 4 cm diameter by 15 cm deep cores co-located with the high-throughput sequencing cores. These included total C (%), N (%), P (mg kg ^-1), organic matter (% LOI), pH (measured in 0.01 M $CaCl_2$), mean soil water repellency (median water drop penetration time in seconds), bulk density (g cm^3 ^-1), volume of rocks (cm^3), soil bound water (g water g dry soil ^-1), volumetric water content (m^3 m^3 ^-1), as well as clay and sand content (%) of soil. Abundances of mesofauna collected as part of GMEP

were taken from George et al.[23] and geographic data including grid eastings, northings, and elevation were also included in our analyses. For complete details on chemical analyses see Emmett et al.[51]. Temperature (°C) and mean annual precipitation (mL) were extracted from the Climate Hydrology and Ecology research Support System dataset[54]. Mean values for each variable were recorded for each AVC (Supplementary Table 12) and soil properties were normalised where appropriate.

Soil texture data were measured by laser granulometry with a LS320 13 analyser (Beckman-Coulter). We subsampled approximately 0.5 g of soil taken from 15 cm cores by manual quartering and removed organic C using H_2O_2 and then transferred the sample into 250 mL bottles, added 5 mL of 5 % Calgon ® and shook overnight at 240 rpm. Bottles were emptied manually into the laser diffraction instrument for measuring particle size distribution. Full Mie theory was used to obtain a particle size distribution from the raw measurement data, with the real refractive index set to 1.55 and the absorption coefficient at 0.1 as in Özer et al.[55]. The cut-off points for clay, silt, and sand were: 2.2 µm, 63 µm and 2000 µm respectively. Clay and sand percentages were selected for subsequent analyses and normalised using Aitchison's log-ratio transformation.

DNA extraction

Soils were homogenised by passing through a sterilised 2 mm stainless steel sieve. Sieves were sterilised between samples by rinsing under the tap water using high flow, applying Vircon laboratory disinfectant and UV-treating each side for 5 minutes. DNA was extracted by mechanical lysis and the homogenisation step performed in triplicate from 0.25 g of soil per sample using a PowerLyzer PowerSoil DNA Isolation Kit (MO-BIO). Pre-treatment with 750 µL of 1 M $CaCO_3$ following Sagova-Mareckova et al.[56] was performed as it was shown to improve PCR performances, especially for acidic soils. Extracted DNA was stored at -20 °C until amplicon library preparation began. To check for contamination in sieves 3 negative control DNA extractions were completed and an additional 2 negative control kit extractions were performed using the same technique but without the $CaCO_3$ solution.

Primer selection and PCR protocols for library preparation

Amplicon libraries were created using primers for rRNA marker genes, specifically for the V4 region of the 16S rDNA gene targeting bacteria and archaea (515F/806R)[57], ITS1 targeting fungi (ITS5/5.8S_fungi)[58], and the V4 region of the 18S rDNA gene (TAReuk454FWD1/TAReukREV3)[59] targeting a wide range of, but not all, eukaryotic organisms. We used a two-step PCR following protocols devised in conjunction with the Liverpool Centre for Genome Research. Amplification of amplicon libraries was run in triplicate on DNA Engine Tetrad® 2 Peltier Thermal Cycler (BIO-RAD Laboratories) and thermocycling parameters for each PCR started with 98 °C for 30 s and terminated with 72 °C for 10 min for final extension and held at 4 °C for a final 10 min. For the 16S locus, first-round PCR amplification followed 10 cycles of 98 °C for 10 s; 50 °C for 30 s; 72 °C for 30 s. For ITS1, there were 15 cycles of 98 °C for 10 s; 58 °C for 30 s; 72 °C for 30 s. For 18S there were 15 cycles at 98 °C for 10 s; 50 °C for 30 s; 72 °C for 30 s. Twelve µL of each first-round PCR product were mixed with 0.1 µL of exonuclease I, 0.2 of 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 74 °C for 15 min and held at 4 °C. Addition of Illumina Nextera XT 384-way indexing primers to the cleaned first round PCR products were amplified following a single protocol which started with initial denaturation at 98 °C for 3 min; 15 cycles of 95 °C for 30 s; 55°C for 30 s; 72 °C for 30s; final extension at 72 °C for 5 min and held at 4 °C. Twenty-five µL of second-round PCR products were purified with an equal amount of AMPure XP beads (Beckman Coulter). Library preparation for 2013 samples was conducted at Bangor University. Illumina sequencing for both years and library preparation for 2014 samples were conducted at the Liverpool Centre for Genome Research.

Bioinformatics

Bioinformatics analyses were performed on the Supercomputing Wales cluster. A total of 130,219260, 104,276,828, and 98,999,009 raw reads were recovered from the 16S, ITS1, and 18S sequences, respectively. Illumina adapters were trimmed from sequences using Cutadapt[60] with 10% level mismatch for removal. Sequences were then de-multiplexed, filtered, quality-checked, and clustered using a combination of USEARCH v. 7.0[61] and VSEARCH v. 2.3.2[62]. Open-reference clustering (97% sequence

similarity) of operational taxonomic units (OTUs) was performed using VSEARCH; all other steps were conducted with USEARCH. Sequences with a maximum error greater than 1 and shorter than 200 bp were removed following the merging of forward and reverse reads for 16S and ITS1 sequences. A cut-off of 250 bp was used for 18S sequences, according to higher quality scores. There were 15,202,313 (16S), 7,242,508 (ITS1), and 9,163,754 (18S) cleaned reads left at the end of these steps. Sequences were sorted and those that only appeared once in the dataset were removed. Briefly, filtered sequences were matched first against a number of different reference databases: Greengenes 13.8[63], UNITE 7.2[64], and SILVA 128[65] for 16S, ITS1, 18S, respectively. Ten per cent of sequences that failed to match were clustered *de novo* and used as a new reference database for failed sequences. Sequences that failed to match with the *de novo* database were subsequently also clustered *de novo*. All clusters were collated and chimeras were removed using the uchime_ref command in VSEARCH.

Chimera-free clusters and taxonomy assignment were used to create an OTU table with QIIME v. 1.9.1[66] using RDP[67] methodology with the GreenGenes database for 16S and UNITE database for ITS1 data. Taxonomy was assigned to the 18S OTU table using BLAST[68] against the SILVA database and OTUs appearing only once or in only 1 sample were removed from each OTU table.

Newick trees were constructed for the 16S and 18S tables using 80% identity thresholds. The trees were combined with their respective OTU tables as part of analyses using the R package phyloseq[69], removing OTUs that did not appear in both the tree and OTU table. OTUs identified as eukaryotes in the 16S OTU table, non-fungi OTUs in the ITS OTU table, as well as OTUs identified as fungi, plants, and non-soil animals were removed from the 18S OTU table. Read counts from each group were normalised using rarefaction. The OTU tables were rarefied 100 times using phyloseq[69] (as justified by Weiss et al.[70]) and the resulting mean richness was calculated for each sample. The read depth used for rarefaction varied for each group (Supplementary Table 14). Samples with lower read counts than this cut-off were removed before rarefaction. A summary of number of replicates per AVC is included in Supplementary Table 1.

Statistical analyses

All statistical analyses were run using R v. 3.3.3[71] using the rarefied data sets for each organismal group. The vegan package[72] was used to assess β-diversity via NMDS and CAP ordinations based on Bray-Curtis dissimilarities. A linear model for each environmental variable was fit separately to the ordination using the envfit function, the results are presented ranked according to goodness-of-fit. Results of goodness-of-fit for each variable from both ordination methods were compared using regression analyses to look for congruence. The values of all variables were plotted against NMDS scores to determine if there were positive or negative relationships with each NMDS axis. Differences in β-diversity amongst AVCs were calculated with PERMANOV. The assumption of homogeneity of dispersion was verified using the betadisper function.

Linear mixed models were constructed using package nlme[73] to test the differences in α-diversity amongst AVCs for each organismal group. Model selection was performed using AVC, soil type, LOI classification, and sample year as fixed factors; sample square identity was the random factor. To determine the best possible model, predictors other than AVC were dropped to find the lowest AIC scores using the AICcmodavg package[74]. For each model, significant differences were assessed by ANOVA and pairwise differences were identified with Tukey's *post-hoc* tests from the multcomp package[75].

Partial least squares regressions found in package pls[76] were used to identify the most important environmental variables for richness. Such analysis is ideal for data where there are many more explanatory variables than sample numbers or where extreme multicollinearity is present[46]. As in Lallias et al.[46], we used the variable importance in projection (VIP) approach[77] to sort the original explanatory variables by order of importance; variables with VIP values > 1 were considered most important. Relationships between important variables and richness values for each group of organisms were investigated by linear regression. Richness was normalised before regression when necessary. Pearson's correlation coefficient was used to directly compare richness of organismal groups.

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APPENDIX B

Microbial community composition is associated with soil respiration in a long term climate change experiment

This appendix presents a piece of work linking the soil microbial community characterisation presented in Chapter 6 with concurrent measurements of soil respiration at the same site. It is currently being compiled into a manuscript on soil respiration at Clocaenog, lead author Sabine Reinsch. Within this work winter respiration for the 2016/17 season was modelled in response to treatment and soil physicochemical variables. We found that adding bacterial and fungal community composition improved the ability of the model to predict winter respiration.

Introduction

Predicting the future response of soil carbon to climate change is essential for understanding global climate feedbacks due to the size of the soil carbon pool (Batjes, 1996; Stocker et al., 2013). However, the soil respiration response to climate change is highly variable and we still have limited understanding of what drives this variation (Crowther et al., 2016; van Gestel et al., 2018). One of the factors that is often suggested to influence respiration is microbial community composition. The microbial community is known to respond to experimental drought and warming, with greater response to drought in wetter climates and warming in colder climates (Chen et al., 2015; Ren et al., 2018; Zhou et al., 2018). This can be compared to the non-linear changes in respiration with temperature globally, where respiration follows a Gaussian response to temperature resulting in greater sensitivity to temperature increases in colder climates (Carey et al., 2016).

The Clocaenog long-term drought and warming experiment has shown an increase in soil respiration for both the drought and the warming treatment compared to the control over the 20 years of the experiment (Reinsch et al., in prep). The ever-increasing technical capability to study the microbial communities in soil allow us to begin to establish whether the soil ecology could be playing a role in determining the whole-ecosystem response. Previous studies on the soil mesofauna in Clocaenog and similar sites have found no change in mesofaunal communities with drought or warming, indicating that the soil meso-ecology cannot always act as a reliable indicator of processes occurring at smaller scales (Holmstrup et al., 2012, 2013; Petersen, 2011). Establishing what within our site is driving the increase in respiration will inform the relevance of our results to the wider landscape.

Methods

Average Rs from November to February 2016-17 was modelled in a Bayesian framework using the brms package (Bürkner, 2017) in R version 3.6.1 (R Core Team, 2019). Predictor variables included in the base model were climate treatment, average soil moisture at time of Rs measurement and EC of the topsoil. Plot identity was used as a random effect. The bacterial and fungal NMDS scores from the topsoil sample

closest to the location of the Rs collar in the field were added to the base model. For a description of the methods used to characterise the soil microbial community see Chapter 6. Missing values from failed DNA amplification were imputed within the multivariate model using the identity of the corresponding microbial community in the transition zone, climate treatment, EC and pH as predictors.

Results

Inclusion of the bacterial and fungal composition improved the ability of the model to predict average respiration for winter 2016/17 (Figure 1). The best model by R^2 of respiration had experimental treatment, moisture, electrical conductivity, and topsoil bacterial and fungal NMDS scores (Figure 2). Addition of pH did not improve the model's ability to predict respiration. The model with fungal data and not bacterial data performed slightly better than the model with bacterial data only, however the standard errors of this are large due to the high level of missing fungal data which had to be imputed from topsoil physicochemical properties and fungal communities deeper in the soil profile. Including both bacterial and fungal data improved the model explanatory power to above 50%.

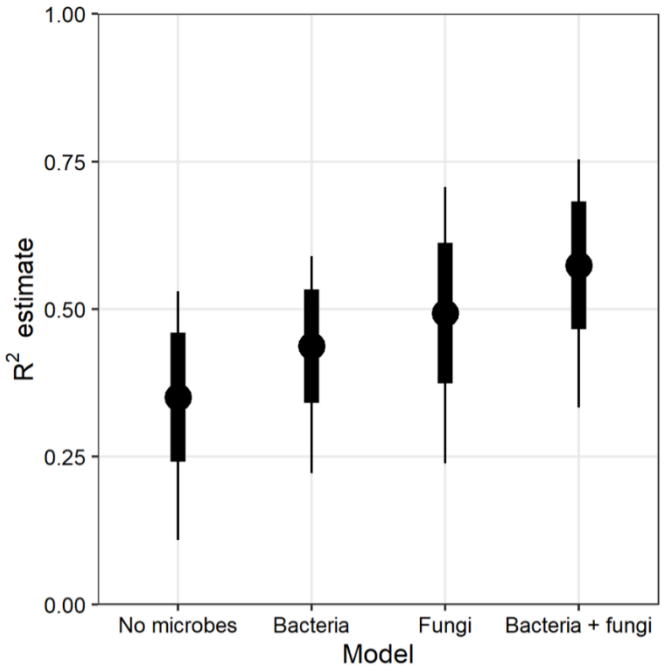

Figure 1: Development of model fits for Rs models with and without considering the microbial community composition (bacteria, fungi, and bacteria + fungi) when climate treatment, soil moisture and electrical conductivity were accounted for.

The standard errors of all the predictors were large and overlapped zero (Figure 2). However, it can be seen in general that the drought treatment positively influenced respiration, while warming had little impact once the soil physicochemical and biological parameters were accounted for. Increasing electrical conductivity increased respiration, and to a lesser extent this is true for moisture. The fungal NMDS scores both had negative impacts on respiration, if you compare to Figure 6.2 this indicates that the drought plot fungal communities are associated with higher respiration. The bacterial NMDS scores had the opposite effects on soil respiration, comparison to Figure 6.2 shows that respiration increases as you go towards the top left of the NMDS plot towards the control and warming associated bacterial communities.

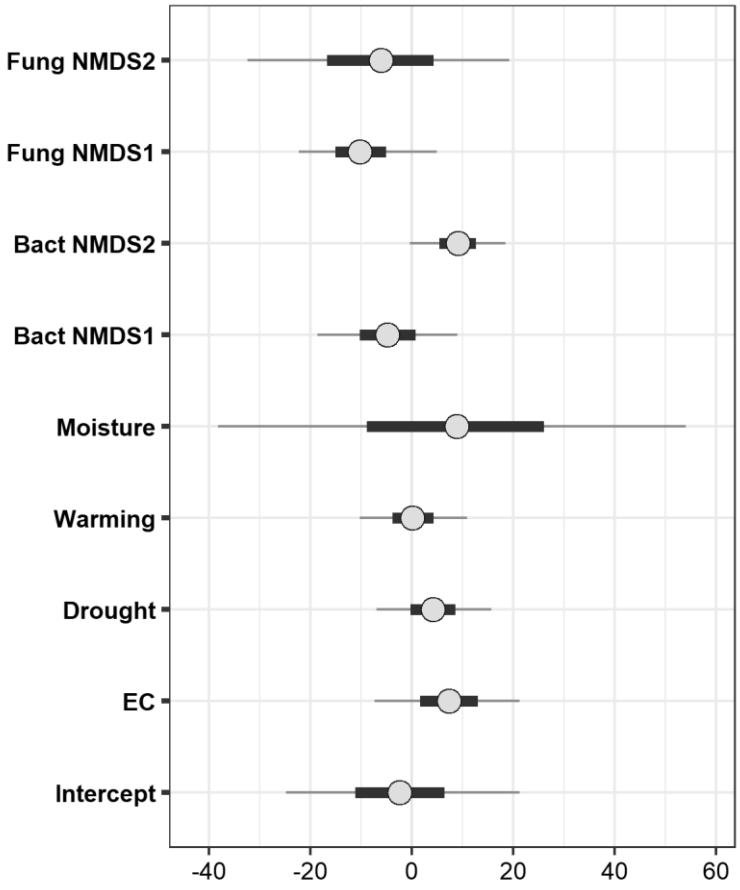

Figure 2: The predictors of the average respiration for the model with bacterial and fungal community composition included.

Discussion

Our result that soil microbial community composition improves ability to predict soil respiration is in agreement with previous work showing the importance of microbial

communities in determining decomposition (Allison et al., 2013; Martiny et al., 2017). Microbial communities drive soil processes and functions (Bardgett & van der Putten, 2014) and are particularly important to soil respiration (Davidson & Janssens, 2006). The direct impact of warming and drought upon respiration was minimal once changes in topsoil moisture, EC and microbial communities were accounted for, but drought still had a small positive influence on respiration. This indicates that while the impact of drought was mostly mediated by changes in these properties there are still potential undescribed pathways through which drought impacts soil respiration. The effect of warming, however, was completely mediated by changes in the soil physicochemical properties and microbial community.

Within this analysis we have used only microbial community data from the topsoil as this is the most carbon-rich, biologically active layer of our soil. However, it is possible that dynamics lower down in the soil profile could be influencing soil respiration. We saw changes in the subsoil microbial community in response to warming (Chapter 6), but it is the topsoil layer that has seen the reduction in root biomass in response to drought (Appendix H Figure 10). Roots can be important in influencing respiration both directly and through their influence on soil microbial communities (Bond-Lamberty et al., 2004), so this could contribute to the unexplained variation in respiration rates. Overall, it appears that even small changes in soil bacterial and fungal community composition can be associated with changes in critical soil functions such as soil respiration.

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APPENDIX C

Soil Resources, the Delivery of Ecosystem Services and Value

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Contribution statement:
Robinson led the writing and co-ordinated all the authors. Parry Roberts wrote the sections on "Soils and ecosystem services" and the "United Nations, Sustainable Development Goals and the System of Environmental Economic Accounting". Robinson wrote the section on "Soil Security". Seaton and Puig de la Bellacasa wrote the section on "Valuing nature or caring for our natural resources?". Seaton wrote the section titled "The psychology of value". Puig de la Bellacasa wrote the section on "Caring for soils". Stolte wrote the section on "Threats to soil natural capital". Sharps, Thomas, and Jones wrote the section titled "Modelling: InVEST, LUCI and ARIES". Van der Ploeg wrote the section on "Cross cutting and dealing with complexity". All authors saw and approved the final version of the manuscript.

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Abstract

Soils provide important functions, which according to the European Commission include: biomass production (e.g. agriculture and forestry); storing, filtering and transforming nutrients, substances and water; harbouring biodiversity, (habitats, species and genes); forming the physical and cultural environment for humans and their activities; providing raw materials; acting as a carbon pool, and forming an archive of geological and archaeological heritage, all of which support human society and planetary life. The basis of these functions is the soil natural capital, the stocks of soil material. Soil functions feed into a range of ecosystem services which in turn contribute to the United Nations sustainable development goals (SDG's). This overarching framework hides a range of complex, often non-linear, biophysical interactions with feedbacks and perhaps yet to be discovered tipping points. Moreover, interwoven with this biophysical complexity are the interactions with human society and the socio-economic system which often drives our attitudes toward, and the management and exploitation of, our environment.

Challenges abound, both social and environmental, in terms of how we feed an increasingly populous and material world, while maintaining some semblance of thriving ecosystems to pass on to future generations. How do we best steward the resources we have, prevent them from degradation and restore them where necessary as soils underpin life. How do we measure and quantify the soil resources we have, how are they changing in time and space, what can we predict about their future use and function? What is the value of soil resources and how should we express this? This chapter explores how soil properties and processes underpin ecosystem services, how to measure and model them and to identify the wider benefits they provide to society. Furthermore, we consider value frameworks including caring for our resources.

Introduction

Humanity has had an indelible impact on the earth's surface, so much so that it has been proposed that the planet has entered a new geological epoch, the anthropocene (Crutzen 2002). A population of about 7 billion people that will likely grow to 9.6 billion by 2050 is stressing Earth's resources. Maintaining the planet in an equitable state for human life is perhaps our greatest challenge. Currently, humans have transformed 38% of the earth's ice-free land surface to agriculture, crops and pasture (Foley et al., 2011). Production agriculture, and the necessity of producing food for a growing population, has had a tremendous impact on our ecosystems and resources, especially through the abstraction of water, and by leaving residues. Rockstrom et al. (2009) propose that we need a 'safe operating space for humanity with respect to the Earth system,' and that there exist biophysical planetary boundaries (or thresholds) which it is inadvisable to cross if we want to maintain the equitable state. However, consideration of a "safe operating space for humanity" should also take into account the needs of human society (Raworth, 2012). The natural capital and ecosystem services approach is seen as one way of bridging the science/policy divide, improving communication, and working toward an aim of living within sustainable boundaries.

The marriage of ecosystems with the notion of goods and services emerged from the economic and social cultural conditions after the Second World War. Of particular note was the work by Schumacher which led to the book Small is Beautiful (Schumacher, 1973) and the ecological economic perspectives proposed by the stock-flow, fund-service framework of Georgescu-Roegen (1971); and more recently reviewed by Daly and Farley (2011). Eventually, researchers felt it necessary to co-opt the language of the dominant ideology of the day in an attempt to increase awareness of the value of natural systems to human society. Westman (1977) suggested that society could make more informed decisions and policy by incorporating the idea that ecosystems offered benefits of social value. The term 'ecosystem services' then began to emerge in the early 1980s (Mooney, Ehrlich, & Daily, 1997) being used, 'to describe a framework for structuring and synthesizing biophysical understanding of ecosystem processes in terms of human well-being'. Since then, an increasing body of

interdisciplinary work has developed that embodies ecology, earth science, economics and social science.

Riding on the back of the mounting wave of both economic globalisation and global environmental concern, ecosystem services broke into more widespread awareness with the publication of Daily's classic text Nature's Services (Daily, 1997). She offered a broad definition of ecosystem services - including the 'conditions and processes' of ecosystems as services (Daily, 1997). She also expressly called the total-use value of ecosystems 'infinite', but argued for the need to assess the 'marginal value' of nature (Daily, 1997). Whilst agreeing with Daily on the total welfare value of nature (i.e. infinite), Costanza et al. (1997) identified 17 broad ecosystem services categories and estimated their value at $33 trillion US dollars annually, much to the chagrin of some economists (Spash, 2013). Although not the first, it is probably the most famous attempt to place a financial value on ecosystem services, and has shaped subsequent attitudes to valuation. It must be noted that they regarded their approximation as a minimum estimate, and one which has subsequently been updated to $125 trillion per annum; with a yearly loss of services value from land-use change since 1997 placed at between $4.3 – $20.2 trillion (Costanza et al., 2014).

A landmark in the uptake of the ecosystem services concept came with the United Nations' (UN) Millennium Ecosystem Assessment (MEA, 2005). Whilst not an attempt to attribute directly financial value to ecosystem services, the Millennium Ecosystem Assessment (MEA) was, rather 'the first attempt by the scientific community to describe and evaluate, on a global scale, the full range of services people derive from nature' (MEA, 2005). Shockingly, its evaluation concluded that, of the ecosystem services they could reasonably assess, some 60% were in decline (MEA, 2005). With a more comprehensive list than other previous definitions, the MEA attempted to bring some structure and clarity to the concept by devising a four-part classification scheme – ecosystem services could now be meaningfully described as either provisioning, regulating, supporting or cultural services.

Part one: Soils, challenges and the delivery of ecosystem services and their value

Central to the challenge facing humanity and managing the environment is the fact that the increasing human population is projected to grow to 9 billion by 2050. This combined with changes in life style, is increasing demand for food and other resources, especially water. Ensuring food security, whilst maintaining the planet in an equitable state for a diversity of life is one of our greatest societal challenges. The ecosystem services approach has been heralded as offering a conceptual framework that accounts for the provisioning of goods from nature, without neglecting the regulating and cultural services that ecosystems provide, by attempting to link ecosystems with economic value for policy making. In so doing, it provides a potential way to examine trade-offs and impacts between ecosystem goods and services that may inform policy.

Representing the value of the environment to policy makers and the public is often difficult, something ecosystem service frameworks seek to address by representing how the environment contributes to human wellbeing. Within this approach the many benefits humans gain from ecosystems are referred to as ecosystem services (Costanza et al., 1997; MEA, 2005). The stocks within the ecosystem that lead to these services are referred to as natural capital (Costanza et al., 1997). The underlying theory is that as humans see what they gain from ecosystems they will then decide to conserve natural capital, thereby leading to better protection of the natural world. The increasing popularity of ecosystem services is apparent in the conservation literature. A literature search shows that the proportion of papers on ecosystem conservation that include the word “service” or “services” increased from 0.4% in 1984-5 through 7.7% in 2000-01 to 28.1% in 2014-15 (Web of Science search, 15/01/16). A similar analysis using Scopus and Google Scholar, found an exponential increase of the number of articles on ecosystem services, and an increase in the number of subject areas discussing the concept (Chaudhary, McGregor, Houston, & Chettri, 2015). Governments and institutions are now using the concept of ecosystem services, and natural capital, to shape policy frameworks (Chaudhary et al., 2015; Schaefer et al., 2015; United-Nations, 2014) However this method of measuring environmental value

still has many shortcomings, which may have long-term impacts on decision making and the health of the planet (Crompton & Kasser, 2010). And this is not without vehement critics, both within academia (Spash, 2008) and the popular press (Monbiot, 2012).

Soils and natural capital

The idea of natural capital can be traced back to the 1830's or earlier (Robinson et al., 2013), whereas ecosystem services is more recent. Costanza and Daly (1992) broadly define natural capital as "a stock that yields a flow of valuable goods or services into the future". In more recent work Costanza et al. (1997) define it as, 'the stock of materials or information contained within an ecosystem'. Attempts to define soil natural capital can be found in (Palm et al., 2007), who focused on texture, mineralogy and organic carbon. Robinson, Lebron, and Vereecken (2009) considered soil in a more fundamental sense to be mass, energy and organisation. Both teams considered natural capital to underpin processes and functions for the delivery of ecosystem services.

Soil natural-capital may be thought of as a stock, yet it is very much more varied, and resistant to quantification, than that simple word may suggest. Even the relatively well characterised mineral element of soil has considerable uncertainties attached to it, particularly regarding the rate of pedogenesis. Although some estimates suggest, alarmingly, that rates of erosion are 1-2 orders of magnitude greater than soil formation in much of the world's agricultural land (Montgomery, 2007). As if problems of quantifying the physical constituents of soil natural capital were not enough, the fact that its structure must be considered when assessing its condition, and therefore capital value, adds an extra dimension of complexity. Indeed, the size and distribution of the porous voids within a given area of soil are an integral part of its natural capital value. Pore architecture, as much as, and in conjunction with, the elemental aspects of soil, controls processes and functions, and thus the services and benefits, that arise from the soils beneath our feet (de Jonge, Moldrup, & Schjønning, 2009; Lavelle, 2002). Often thought of as separate spheres, the hydrosphere and pedosphere in fact interpenetrate one another, confined by the porosity of the soil, and it is at this interface, mediated by flows of water, from which originate many of

the ecosystem services which we associate with soils (Clothier, Green, & Deurer, 2008; Coates et al., 2013).

Soils and ecosystem services

Although Daily (1997) was perhaps the first to attempt to classify the ecosystem services of soils, this has been followed by other classifications (Andrews, Karlen, & Cambardella, 2004; Wall, 2004), especially a number for the purposes of agriculture (Swinton et al., 2007). It was Dominati, Patterson, and Mackay (2010) who attempted to pull together a combined soil natural-capital and ecosystem-services framework (Figure 1). This acknowledged the important cycles of soil formation and degradation altering the stock of soil natural capital, which in turn affects the delivery of ecosystem services which fulfil human needs. The proposed framework has served as a benchmark in soil science.

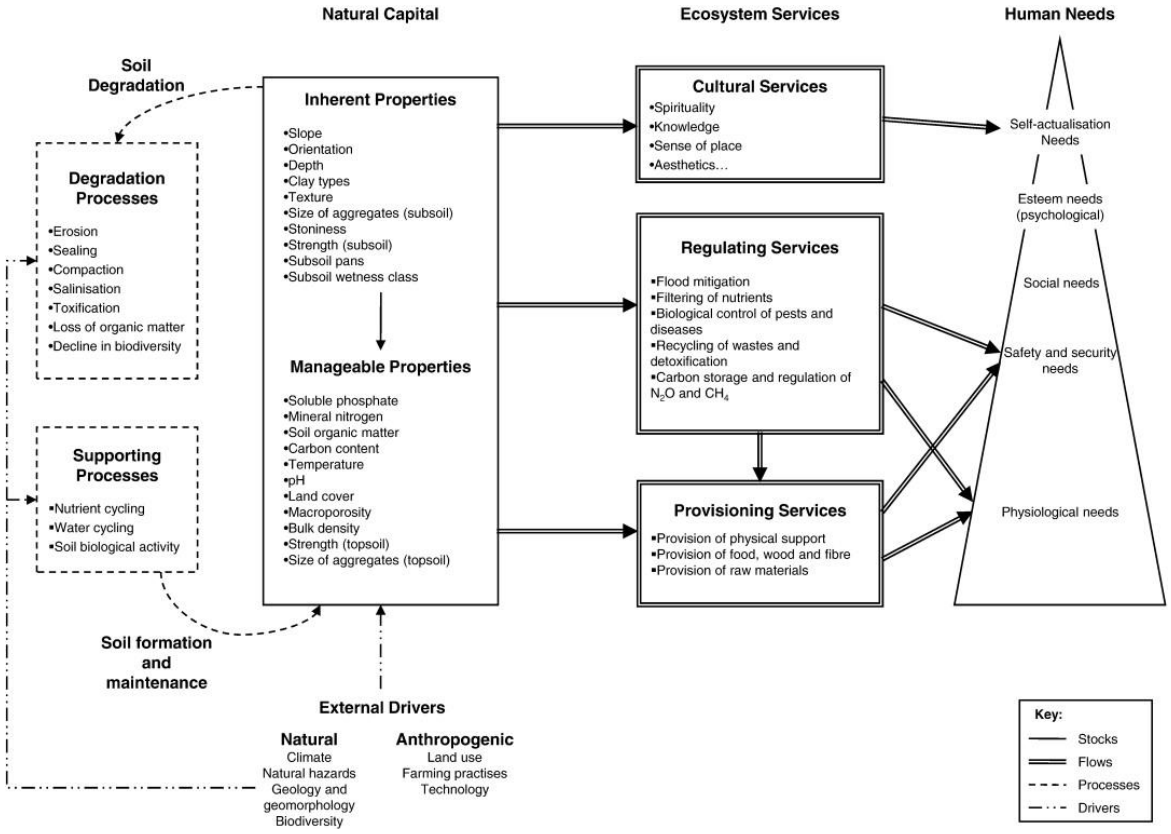

Fig. 1. Framework for the provision of ecosystem services from soil natural capital. From Dominati et al. (2010).

While progress has been made identifying the ecosystem services that soil delivers, or helps deliver (Dominati et al., 2014; Robinson et al., 2013), it has proved challenging to decide where they contribute in overarching Ecosystem Service typologies. The MEA (2005), The Economics of Ecosystems and Biodiversity, or TEEB (Sukhdev et al., 2010), and The Common International Classification of Ecosystem Services (CICES) (Haines-Young & Potschin, 2012) represent the major ecosystem service classifications. The MEA placed soils in the supporting services, whereas the TEEB emphasises the role of soils in regulating services through erosion prevention and the maintenance of soil fertility. The CICES also focuses on the regulating services provided by soil, with these contributing to a number of groups in the CICES classification, as highlighted in yellow in Figure 2. In their report Haines-Young and Potschin (2012) stated that:

> *Clarification of the ways soils provide services was a further area identified where the structure of CICES might be looked at: The system does not currently take account of the services provided by soil very well. [Our] soils scientists identified that the services provided by soil extend beyond the soil formation and composition service identified in the classification.*

Section	Division	Group
Provisioning	Nutrition	Biomass
		Water
	Materials	Biomass, Fibre
		Water
	Energy	Biomass-based energy sources
		Mechanical energy
Regulation & Maintenance	Mediation of waste, toxics and other nuisances	Mediation by biota
		Mediation by ecosystems
	Mediation of flows	Mass flows
		Liquid flows
		Gaseous / air flows
	Maintenance of physical, chemical, biological conditions	Lifecycle maintenance, habitat and gene pool protection
		Pest and disease control
		Soil formation and composition
		Water conditions
		Atmospheric composition and climate regulation
Cultural	Physical and intellectual interactions with ecosystems and land-/seascapes [environmental settings]	Physical and experiential interactions
		Intellectual and representational interactions
	Spiritual, symbolic and other interactions with ecosystems and land-/seascapes [environmental settings]	Spiritual and/or emblematic
		Other cultural outputs

Fig. 2. The section, division and group sections of the CICES classification of ecosystem services. The boxes marked in yellow in the group section represent those with soils contributing. From Haines-Young & Potschin (2012).

They concluded there was a need to reflect better the status of soil and that there was a need to revise the classification. In Figure 3 we attempt to synthesise the goods and services identified by Robinson et al. (2013) with the CICES classification. In addition to groups and classes existing in the classification where soils contribute (yellow), we have added a soil group in provisioning. This highlights that soil resources are widely extracted and used for topsoil, peat, turf grass and as a building material, e.g. for bricks. We have also brought out the biological resources extracted from soils like earth worms and microbes used for biomedical resources. Soils play an important role in life cycle maintenance with an estimated quarter of global biodiversity residing in soils, and in the regulation of pathogens and diseases. Within soil formation we propose focusing on soil production and the release of nutrients, as decomposition is dealt with in waste mediation. Soils play a crucial role in climate regulation, both through soil moisture, temperature changes, and carbon storage. With regard to

cultural services, in addition to the preservation of heritage, they are also relevant as burial grounds and potentially in terms of bequest value.

Group	class	classtype
Biomass	Soil biota	Earth worms, biomedical organisms
Water		
Biomass, Fibre		
Soil	Mineral soil	Manufactured mineral soil, sand, loam, clay
	Organic soil	Manufactured organic soil, peat
	Support function for activities	Turf grass
Water		
Biomass-based energy sources		
Mechanical energy		
Mediation by biota	Bio-remediation by micro-organisms, algae, plants, and animals, soils	
	Filtration/sequestration/storage/accumulation by micro-organisms, algae, plants, and animals, soils	
Mediation by ecosystems	Filtration/sequestration/storage/accumulation by ecosystems, including the soil ecosystem	
	Dilution by atmosphere, freshwater, marine and soil ecosystems	
	Mediation of smell/noise/visual impacts	
Mass flows	Mass stabilisation and control of erosion rates	
Liquid flows		
Gaseous / air flows		
Lifecycle maintenance, habitat and gene pool protection	Soil gene pool	
Pest and disease control	Soil pathogen reservior and vector	
Soil formation and composition	Weathering processes (Soil production)	
	Weathering processes (Nutrient release)	
	Decomposition and fixing processes (Remove, dealt with under mediation of waste)	
Water conditions		
Atmospheric composition and climate regulation	Soil moisture store buffering heat and cold waves	
	Carbon store buffering atmospheric carbon levels	
Physical and experiential interactions		
Intellectual and representational interactions	Heritage and cultural	Preservation of heritage by soils
Spiritual and/or emblematic	Sacred and or religious	By use, soils in burial grounds
Other cultural outputs	Bequest	Farmland

Fig. 3. A focus in on the group sections of the CICES classification of ecosystem services, Haines-Young & Potschin (2012), containing soils, with suggestions for incorporating more soils information in the green boxes based on the ecosystem services soils contribute to identified in Robinson et al. (2013).

One of the challenges regarding the classification of soils is that the direct use e.g. topsoil or peat, is small in comparison to their role as a 'means' to underpin the delivery of other ecosystem services, e.g. biomass production. This is a concern regarding the ecosystem service approach in that, for reasons of seeking methodological parity with GDP accounting (Boyd & Banzhaf, 2007), the focus has

shifted to the delivery of final goods and services. This then overlooks the significant potential degradation of soil ecosystems in delivering other services, such as the provisioning of biomass.

United Nations, Sustainable Development Goals (SDG's) and the System of Environmental Economic Accounting (SEEA)

Natural-capital accounting may offer a solution to soil degradation being overlooked due to the dual problem of simply focusing on final goods and services, and the fact that soils are often subsumed into larger ecosystem or biome categories when considering ecosystem services (Baveye, Baveye, & Gowdy, 2016). Natural-capital accounting may offer a more complete approach to environmental economic-assessment, one which includes ecosystem services, but also monitors the state of natural-capital resources. In 2014, the UN launched the System of Environmental Economic Accounts (SEEA) which addresses the fact that GDP, often used as a welfare indicator, does not consider degradation. Perversely, degradation such as soil erosion, actually stimulates economic activity when remediated and it is therefore counted as a gain under GDP. The foundation of the SEEA is the identification of 7 environmental asset classes (Mineral & energy resources, Timber, Aquatic, Other biological, Water resources, Land cover and Soil). The aim is to assess the extent, volume/mass and condition of the resources, and capture the biophysical and economic flows to correct GDP through satellite accounts. The SEEA, in combination with the ecosystem services approach, has the potential to provide a monitoring and reporting socio-economic, environmental framework that with the use of biophysical monitoring could provide a global monitoring tool.

If we are to address the United Nations Sustainable Development Goals (SDG's) and attempt to measure their success, then it is only through the use of combined social, economic and environmental monitoring tools that we will be able to make biophysical and economic assessments, and measure the trade-offs between development and degradation. We now have an important conceptual framework developing that demonstrates how soils provide functions that deliver ecosystem

services that contribute to us achieving the sustainable development goals (Figure 4) from (Keesstra et al., 2016).We have modified this figure to include soil natural-capital in the central circle, as it is the soil natural-capital that supports soil functioning and which is vulnerable to degradation. Obst (2015) pointed out that soils remains an under-developed component of the SEEA and there is a job to be done developing a natural capital framework for soils that describes soil assets. This should acknowledge the important soil cycles, through quantitative assessment of carbon gain and loss, nutrient release and soil production and erosion as called for by Amundson et al. (2015). Moreover, it must account for the 11 soil threats, considered in Part 2, that can degrade our soils and reduce their capacity to deliver earth-system functions and ecosystem services.

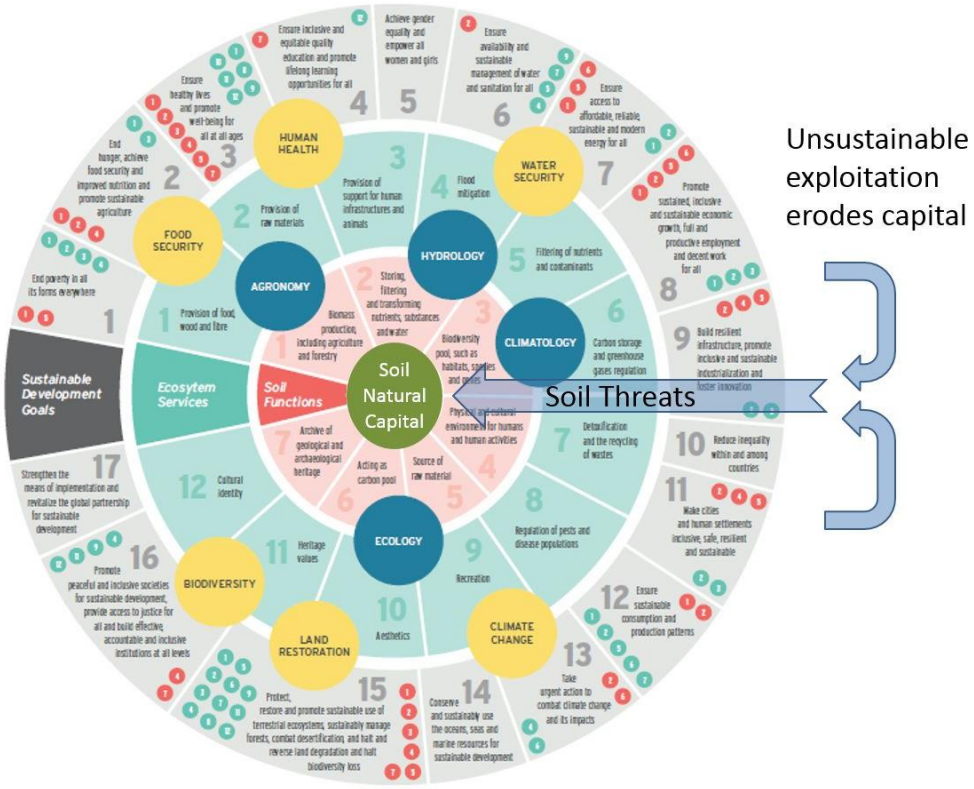

Fig. 4. The link between soil natural capital, soil functions, ecosystem services and the sustainable development goals. Soil threats act to degrade soil natural capital and this limit the delivery of functions and services. Adapted from Keesstra et al. (2016).

Soil Security

Concurrent to these efforts to link soils and ecosystem services, McBratney, Field, and Koch (2014) argue that soil security must be at the heart of this effort because soils underpin the delivery of so many services. 'Soil security' is defined in McBratney et al. (2014) as maintaining and improving the world's soil resource to produce food, fibre and freshwater, contribute to energy and climate sustainability, and maintain the biodiversity and the overall protection of the ecosystem. Soils perform important functions for humanity as identified earlier. Soils can support the provision of ecosystem goods and services both directly and indirectly; whilst some soil processes can have a major adverse impact on the delivery of ecosystem goods and services. The ability of soils to function can be threatened by human activity as identified in The Thematic Strategy for Soil Protection (European Commission, 2006). But something all the ecosystem approaches share is the sense that soils are valuable and this needs to be articulated.

Valuing nature or caring for our natural resources?

The default position of the ecosystem-services approach is to link the environment and economy through monetary valuation. This remains a controversial topic (Peterson et al., 2010). Soils present an interesting case study given they are an economic resource in their own right, as well as supporting major economic activities such as food production. Amundson et al. (2015) argued that agricultural soil systems are one of Earth's most valuable commodities. They referenced FAOSTAT, which for 2012 estimates that the global production of agricultural products was worth nearly $3816 billion U.S. dollars. However, agriculture is competing with increasing urban and suburban soil demands. Within soil science a number of papers have attempted to either value soils and their contribution to the delivery of ecosystem services (Clothier et al., 2008; Dominati et al., 2014) (Clothier et al., 2008; Clothier, Green, & Deurer, 2008; Dominati et al., 2014), or reviewed economic valuation of soils (Robinson et al., 2014). With regard to linking to the economy, two approaches appear to be emerging. One deals with the local scale, for example helping farmers make management decisions; often relying on cost benefit approaches. While at the national scale there is continued development of the SEEA initiative (United-Nations, 2014).

Regardless of the valuation approach, ecosystem-service evaluation relies on the first step of biophysical assessment, followed by some form of valuation. With regard to valuation, especially economic, we have yet to determine the exact goals and ways in which it will help us look after, and manage soil resources. We know soils are a valuable resource. Life as we know it would not exist without them, but is monetary valuation the best way to express this value?

The psychology of value

Due to the way in which human-perception functions, it is possible that financial valuation of ecosystems will corrupt their actual value. The relationships between values are not random. They can be more, or less, compatible with each other. Ten distinct value types have been identified across cultures and countries (Schwartz, 2006,), within which all values can be placed (Figure 5). This also shows a close correspondence to the structure of individuals' goals (Grouzet et al., 2005). Correspondence between these main value-types has been found to be consistent across countries, cultures and economic background. Whilst individuals differ in the importance they assign to each value (Gollan & Witte, 2014; Grouzet et al., 2005; Schwartz, 2006). Invoking one set of values will suppress opposing values, and prime values that correspond to the same value-type (Maio et al., 2009). Understanding how values correspond with each other is thus essential when discussing ecosystem value, as it can have impacts on the behaviour of those listening. Therefore, a long-term strategy for environmental protection should appeal to those values which are most likely to engender a positive long-term relationship with nature.

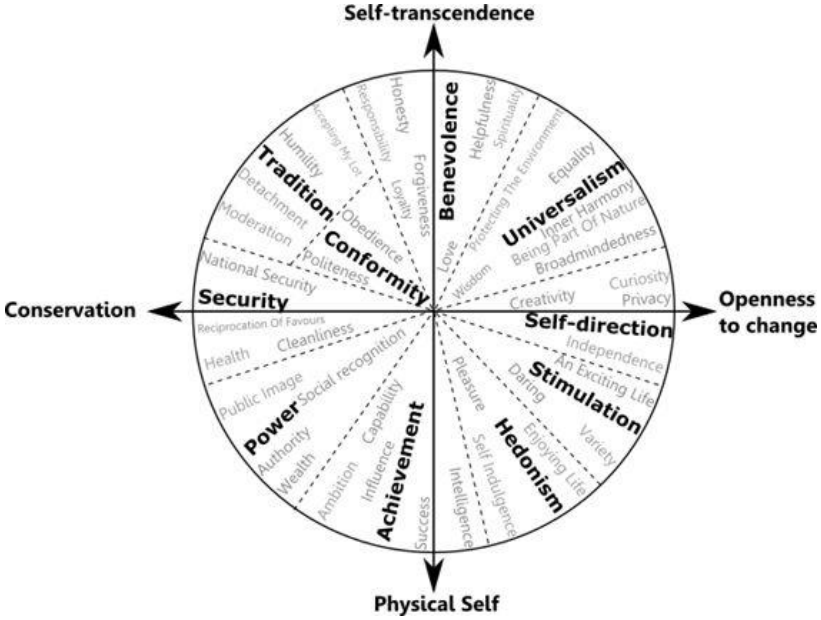

Fig. 5. Value Systems: Adapted from Schwartz (1992), values are plotted around a circle where the closer they are the more compatible they are. Segment titles are in bold and represent a distinct value type. In grey are examples of values for each distinct value type. The layout of values is related to two main axes, the conflict between openness to change and conservation, and also the conflict between prioritizing the physical self and things outside your physicality.

Humans struggle to hold contradictory values in their mind, within their value system, at the same time. Therefore discussion of the environment in financial terms will suppress intrinsic motivation to help the environment. This can be seen in Figure 5 where Wealth, situated in Power, is diametrically opposed to protecting the Environment, situated in Universalism (Schwartz, 1992). Placing a higher emphasis on intrinsic values, situated near Self-transcendence in Figure 5, as opposed to extrinsic values, situated near Physical Self, has been related to a higher willingness to pay to protect the environment (Ku & Zaroff, 2014). However it must be noted that this study measured self-reported behaviour, which only coincides with actual behaviour 20% of the time (Kormos & Gifford, 2014). Furthermore, when confronted with actual demands for payment, people are potentially more powerfully primed to think in terms of extrinsic values, which will reduce willingness to pay (Ku & Zaroff, 2014).

In the discussion of the value of the environment we have to think carefully about what kinds of value we wish to place on it. If we appeal to one value we also appeal to

the values corresponding to the same main distinct value type. This could be powerful in bringing about change. For example appealing to environmental concerns arguing for car-sharing, also increased observed rates of recycling (Evans et al., 2013). However appealing to a set of values that opposes those already held can reduce the power of the pre-existing values. For example, providing monetary incentives to behave in an environmentally friendly way can 'crowd out' other motivations to be environmentally friendly, particularly when positive incentives are too small (Rode, Gómez-Baggethun, & Krause, 2014). In order to convey the value of ecosystems we have to be aware of pre-existing values, which may vary by demography, and incorporate tools such as goal setting, social modelling and prompts (Osbaldiston & Schott, 2012).

Therefore in order to promote the environment we need to appeal to pre-existing values, and preferably intrinsic values. But what about soil? Few people have an understanding of the intrinsic value of soil, therefore it may make sense to appeal to pre-existing extrinsic values. However this may still have undesirable side effects, making people less likely to engage in other pro-environmental behaviour and reducing altruism towards other members of society both present and future. Soil conservation usually involves landowners, who may have different motivations than the students so beloved of psychology experiments. In Sweden, landowners had value structures that tended towards conservation and self enhancement, and usually engaged in biodiversity conservation projects for altruistic concerns (Johansson, Rahm, & Gyllin, 2013). In order to get people with these types of values involved in conservation they need to be operating within a context that is supportive of such behaviour, making monetary incentives a sensible option (Johansson et al., 2013). However if the group targeted already has strong intrinsic motivation to help the environment then building upon that would be more effective.

It will obviously be difficult to target the information provided according to the value system of the beholder. However, the current trend of defaulting to a financial, extrinsic perspective can devalue the environment and remove existing intrinsic motivation to protect the environment. Already we see some undesirable side-effects. When the Pope announced that it was a moral obligation to combat climate change, a Republican presidential candidate responded: 'I don't get economic policy from my

Bishops, or my Cardinal or my Pope' ("'I won't take the Pope's advice on climate change,' says Jeb Bush," 2014) That may be true, but when did caring for the environment become purely an economic concern?

This was clearly not the intention in the 1970s with environmentalists discussing ecosystem services who, rather, sought a pedagogical tool to help society appreciate the value of ecosystems, and highlight the imperative of protecting them (Gómez-Baggethun et al., 2010). Yet, as the notion of ecosystem services has been inexorably drawn into, and redefined, by the wider societal shift towards neoliberal economics, we have been left with a framework which has commodified ecosystem services and ignores the work done by the supporting services and ecosystem functions which produce them (Peterson et al., 2010)

Society often seeks a 'silver bullet' solution to environmental problems. Ecosystem accounting is not a silver bullet in decision making, especially because there may be lack of care engendered if the environment is represented solely in financial terms. However, it forms one lens through which to consider environmental challenges.

The value that an individual places on the environment will vary across people and cultures, yet the choice to represent the environment solely in financial terms could consistently engender a lack of care for nature.

The use of the ecosystem services concept shifts perceptions away from the inherent value of nature, particularly in those individuals used to thinking in terms of commodities. This risks engendering a lack of care towards to the environment.

A Republican presidential candidate may not represent the average view point, but this should serve as a reminder of the lack of care which may be engendered if the environment is represented solely in financial terms.

There may be lack of care engendered if the environment is represented solely in financial terms.

Quantification of value may be important to convince people of the value of the environment, yet too often a quantified value is taken to mean a monetary value. Indeed, the very notion of being able to reduce the pluralistic value of the

environment into simple monetary terms without distortion is questionable (Spash, 2008). Alternatives do exist: such as promoting an attitude of care towards the environment, which may foster a more powerful long-lasting relationship when considering conserving and protecting resources such as soil (Bellacasa, 2017).

Caring for soils

In human-environment relations, care has been mostly thought of as delivered by humans to the environment. But humans need everyday care to survive, and it is not difficult to see that much of this care would not be possible without the biophysical world, including living soils. Care is however based on very different values to those notions normally associated with appraising soils' contribution to human well-being such as: economic worth, natural capital, or provision of services. More than any of these notions, care denotes a necessary relation for the basic survival of living beings. In that sense, thinking about human-environment relations as care has different consequences. Care is a multi-layered notion. It involves work and practice, as in 'taking care' of things. It is also affective and emotional, as when we care about something. And also it has ethical value implying responsibility (Tronto, 1993). It is also an ecological obligation (Bellacasa, 2017). Humans and soils are involved in relations that involve all of those dimensions. In a world so affected by human activity, soils cannot live without human care at all these different levels. But the work of care that living soils effectively perform for the web of life is also essential for survival and subsistence. And while the ethical responsibility of soil care is a human affair, its concrete realisation depends on how different soils respond. The care we put into the soils, or the absence of care, its neglect, will inevitably affect the capacity of soils to care for all the living beings and processes depending on it.

Linking human-soil relations with care emphasises human interdependency with the environment. Looking at soil value from the perspective of the functions or services which produce human well-being represents an important attempt to change the parameters of a purely economic valuation of natural resources and limited to extraction, production, and consumption. But it has not proved enough to alter the anthropocentric and devastating reduction of non-human entities to 'resources'. Whether we speak of the services, care, or functions that soils provide in contribution

to human well-being, we should consider the possibility of having an obligation to "give back" to soils as much as we receive from them. Relations of care emphasise this condition of liveable interdependency. They invite us to confront the split between nature and culture that posits the human as a consumer of natural resources (S. Jackson & Palmer, 2015). Emphasising that we live in an interdependent web of care with biophysical entities of all kinds urges an ethical, emotional and practical shift towards a more eco-centric relationship with soils.

Part Two: Biophysical assessment of soil change and the delivery of ecosystem services

Threats to soil natural capital and function

The protection of soil is of significance for human well-being and social and economic development (Schwilch et al., 2016). Within the 17 UN Sustainable Development Goals (SDG), soils have a strong relation with SDG 2, 3, 6, 13 and 15 (Bouma & Montanarella, 2016). Keesstra et al. (2016), present steps on how the soil science community can meet these goals (Figure 4). The European Commission (2006) identified eight main threats to soil. These threats were erosion, local and diffuse contamination, loss of organic matter, loss of biodiversity, compaction and other physical soil deterioration, salinisation, floods and landslides, and sealing (European Commission, 2006). In some estimates, erosion, organic matter decline, salinization, landslides and soil contamination alone might cost the EU up to €38 billion annually (European Commission, 2006) and the majority of these costs are borne by society. Recognizing the soil degradation and its transboundary nature, the EC declared in 2006 that for sustainable development, soils need to be protected from degradation. Climatic factors and human actions both threaten soil functioning and natural capital. These threats should not be regarded as distinct. They are interlinked in the sense that threats to soil from human activity can contribute to climate change, and, in turn, climate change causes or intensifies threats to soil.

Soils are under increasing pressure from a wide range of human activities, which undermine their long-term sustainable use. Policy directly or indirectly affects soil threats by enabling and incentivising, or by prohibiting and limiting, a particular

human activity, thereby making activities more or less attractive to land users. Policy regulations therefore, are a strong instrument in providing opportunities for soil protection. But they can conversely put significant pressure on soils if they are wrongly targeted, and induce overexploitation of resources. From a wider perspective, policy can also affect soil threats by driving changes in land use. *The Thematic Strategy for Soil Protection* (European Commission, 2006), being the main soil-focused EU instrument, aims to protect soils while using them sustainably, through the prevention of further degradation, the preservation of soil functions and the restoration of degraded soils (European Commission, 2006; Jones et al., 2012). There is a need to promote the four pillars of *The Soil Thematic Strategy*. These are i) more awareness raising campaigns; ii) supporting soil research projects; iii) integration of soil science in policy making, and iv) improved legislation (European Commission, 2006). Much progress has been made since 2006 on the first three pillars, but no initiatives have been implemented for the legislation at the European scale.

Socio-economic and cultural drivers directly or indirectly affect soil threats, having a strong link with policy. Feeding an increased population puts pressure on food production through agricultural intensification. Additionally population growth contributes to the pressures on land resource use through urban growth, mining, and tourism, thereby potentially degrading soils and increasing instances of soil sealing, contamination and salinization.

A major challenge in understanding and describing the relationship between the various threats to soil and the soil functioning, is how to quantify the interactions between the various threats, and how these interactions in turn, affect soil functions. Clarifying these relationships is essential in order to gain a holistic view of the status of threatened soils and the interactions between the different threats and functions of the soil. Table 1 presents an overview of the main challenges threatening soil function. Analysis of the effects of these threats to soil functions is beginning to emerge. By linking the status of soil, in terms of the degree to which it is threatened, with soil functions and ecosystem services, the relationships between those processes driving threats to soil, and thus to the societal benefits derived from soil, may become clearer.

Table 1. The main threats to soil functions.

Soil functions	Main threat	References
Biomass production (in agriculture & forestry)	Soil erosion by water, soil sealing, salinization, compaction	Gardi, Panagos, Van Liedekerke, Bosco, & De Brogniez, 2015; Li, Pu, Zhu, & Zhang, 2012; Boardman & Poesen, 2007; Håkansson & Reeder, 1994
Storage, filtering and transformations of nutrients, substances and water	Soil sealing, contamination, compaction	Etana et al., 2013; Reeves & Baker, 2000
Biodiversity (such as habitats, species, and genes)	Decline in OM and biodiversity loss	Jeffery et al., 2010; Primavesi, 2006
Physical base for construction	Desertification, soil erosion by water, floods and landslides	Morgan, 2006; Van-Camp et al., 2004
Source of raw materials	Floods and landslides	Stankoviansky, Minár, Barka, Bonk, & Trizna, 2010
Acting as carbon pool (store and sink)	Decline in OM and biodiversity	Jeffery et al., 2010; Primavesi, 2006
Archaeological & geological heritage	Soil erosion, floods and landslides, compaction	Camera, Apuani, & Masetti, 2015; Sdao & Simeone, 2007

Monitoring of soil natural capital, state and change:

In this era of ever-increasing pressure on natural ecosystems and soil it is essential to monitor ecosystem changes over time. Soil is an integral part of natural ecosystems, and is required for biomass production whilst also representing a valuable store of carbon and other resources. The need to produce food for the industrial revolution meant that early work on soils focused on inventory and the suitability for crop growth, which evolved into soil surveys in many countries in the 20th C. If ecosystems are to be managed for long-term sustainability then an understanding of the long-term response of soil to environmental change is essential. This requires a shift in the way we observe soils, moving from inventory to monitoring of change, and to experiments to understand the long-term response of soil to change. The need for this is identified in the first report by the UN Food and Agriculture Organisation (FAO), Intergovernmental Technical Panel on Soils (ITPS, 2015). This proposed that the following four actions are the greatest priorities to stabilize or reverse over exploitation of global soil resources:

1. Minimize further degradation of soils, and restore the productivity of soils that are already degraded in those regions where people are most vulnerable.

2. The global stores of soil organic matter (i.e. soil organic carbon (SOC) and soil organisms) should be stabilized or increased.

3. Act to stabilize or reduce global N and P fertilizer use while simultaneously increasing fertilizer use in regions of nutrient deficiency.

4. Develop monitoring systems to determine the current **state and trend** of soil condition. Regional assessments used in the report often predate the 1990s using observations predating the 1980s.

This fourth recommendation requires that we not only consider 'state', but that we must monitor 'change'. How else will we know if interventions are effective? An increasing number of monitoring programs around the world are beginning to factor this in. In the UK, the Countryside Survey (CS) was pioneering in this context and has provided evidence of soil change since 1978 (Emmett et al., 2010; Reynolds et al., 2013). Unlike systematic surveys used for inventories, CS is statistically robust, allowing reporting of uncertainty. The robust design has led to the adoption of similar designs at the EU level, for example the Land Use/Land Cover Area Frame Survey (LUCAS) topsoil database which covers 25 member states of the European Union. This provides a basis for soil-related policies (Tóth, Jones, & Montanarella, 2013).

Quantifying the state and change at global scales is challenging, as we simply do not have the data for most countries. Amundson et al. (2015) made an important contribution by attempting to quantify state and change at a global scale for soil carbon, phosphorous and degradation. They argued that the expansion of urban centres, often termed 'soil sealing', is removing soil from other uses. In Europe, for example, this is now considered to cover on average 9 % of the land surface (Scalenghe & Marsan, 2009). Land-use change in the form of creating new cropland is one of the major drivers of imbalances in the soil carbon cycle (Gottschalk et al., 2012), along with accelerated rates of soil erosion (Oldeman, 1994). Whilst according to Amundson et al. (2015), phosphorus, critical to plant growth, is unevenly distributed and supplies depend on dwindling geological sources. This global picture of soil degradation is

borne out by the national data we do have. Bellamy, Loveland, Bradley, Lark, and Kirk (2005) and Reynolds et al. (2013) both reported a decline in arable-soil carbon in the UK. Declines were also observed in Belgium (Goidts & van Wesemael, 2007) between 1955–2005; in Flanders (Sleutel et al., 2003) between 1989-2000, and in arable and pasture systems in the French mountains (Saby et al., 2008) between 1990–2004. Whilst we focus mostly on soil organic carbon, it is important to recognise that inorganic carbon is also an important constituent of many soils (Rawlins et al., 2009), and it has also been observed to be in decline for instance in China (Yang et al., 2012).

The importance of 'state and change' monitoring, such as the Countryside Survey (Emmett et al., 2010) is that it shows we cannot have everything, and that we need to make choices. This is made clear by the analysis from Maskell et al. (2013)(Figure 6). The ecosystem service indicators alter, often in a non-linear way with proportion of intensive land use (Fig 6a). All decline, other than production, with intensification. Figure 6b&c goes on to show that changes in moisture inputs, moisture regime, or that alteration of soil pH would change the service delivery balance. At no point do we get everything. In order to make choices we therefore need decision support, which requires models to help predict the outcomes of interventions.

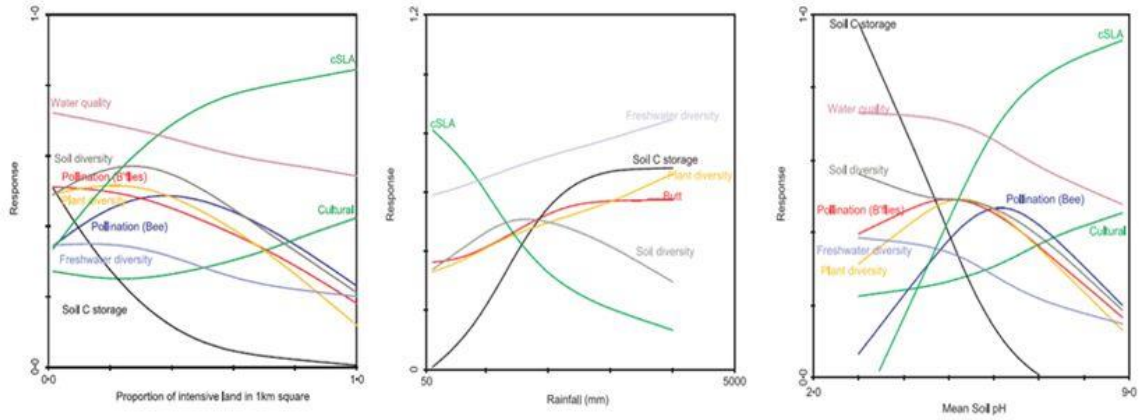

Fig. 6. Response curves of mean ecosystem service indicators per 1-km2 across Great Britain, fitted using generalized additive models to ordination axes constrained by; (a) proportion of intensive land (arable and improved grassland habitats) within each 1-km square from CS field survey data; (b) mean long-term annual average rainfall (1978–2005); and (c) mean soil pH from five random sampling locations in each 1-km square. All X axes are scaled to the units of each constraining variable. Adapted from Maskell et al. (2013)

Modelling: InVEST, LUCI and ARIES

Soil attributes are a critical component required as input data to model many ecosystem services. Carbon stocks can be directly modelled from soil type using look-up tables and assumptions about soil depth. This is the most commonly applied approach for soil C. However, since soil properties play a role in governing the ecosystem functions which underpin many of the regulating services, soil type is often used as a proxy to model these services on a spatial basis. For example, soil properties dictate how water moves through landscapes (infiltration rates, water storage, runoff), what that water takes with it (soil erosion, nutrient and pathogen transport into rivers), and the fluxes of the principle greenhouse gases (CO_2, N_2O, CH_4).

There are a wide variety of ecosystem service (ES) modelling tools available, ranging from basic spreadsheets such as the Ecosystem Services Review (Landsberg et al., 2011), to more complex spatial models, which can highlight the key areas within a study site contributing to each service. Three of the main spatially explicit ES modelling tools are InVEST (Sharp et al., 2016), LUCI (Sharps et al., 2017) derived from the Polyscape framework described in (Jackson et al., 2013), and ARIES (Villa et al., 2014). These models can be used to investigate the potential impacts of different management scenarios, for example, demonstrating how changes in soil properties can affect service provision, or how soil type mediates the effects of land-cover change. The models can produce maps of service provision and quantitative outputs, including biophysical and (for some services) economic values. In the following paragraphs we briefly review these models and show how soil information contributes to model outputs.

ARIES - ARtificial Intelligence for Ecosystem Services

ARIES can be more accurately described as a modelling framework, which contains a number of ecosystem service modelling applications. A basic principle underlying the ARIES framework is its consideration of multiple aspects of ecosystem service delivery: source, sink, use and flow. **Sources** are the aspects of the environment that produce the service, **sinks** are aspects of the environment which detract from, or reduce the amount of service. **Use** is the amount of service used by people, and **flows** are the physical flow-paths or other quantification of how the service reaches the users. The

fundamentals of the approach are outlined in Villa et al. (2014) as an extension of ecosystem-services science with a stated aim to renew its focus on beneficiaries and the spatial and temporal dynamics of flows. Numerous individual service models have been developed within ARIES and it has been applied worldwide in many case studies, and in multi-service comparisons e.g. Balbi et al. (2015). Many of the original service models use a spatial Bayesian modelling framework. However other process models can be incorporated by model wrapping and some, such as the dynamic global vegetation model LPJ-GUESS, which models carbon and water fluxes, have been hard-coded into the software.

Key features of ARIES include the ability to model service flow, flexibility of model development, the ability to incorporate expert knowledge into quantitative models, use in data-poor situations, and representation of uncertainty in model outputs.

Service flow is poorly captured in many other ecosystem service models at present, but there is an increasing focus on improving calculation of service flows in ecosystem service assessments (Bagstad et al., 2013). The flexibility of the modelling approach can be considered both an advantage due to the ability to write new models or adapt existing models to new applications, but can also be considered a disadvantage since there is a certain time investment required to be able to run and use the models. However, there are plans to release an on-line version with a library of models which could be run with existing (usually global) datasets, but with the capability to load one's own datasets for bespoke applications. Use of the Bayesian modelling approach confers three advantages. The probabilistic nature of Bayesian approaches means that expert input can be used to generate models for certain services that are otherwise difficult to model, like cultural services (Balbi et al., 2015). It also means that once models have been developed, they can be applied in areas where data is missing or patchy. Lastly, the probabilistic approach allows explicit quantification and mapping of uncertainty in the model outputs, which can be published alongside maps of the service itself (e.g. Sharps et al., 2017).

InVEST – Integrated Valuation of Ecosystem Services and Tradeoffs

InVEST is a freely available suite of ecosystem services models, developed by the Natural Capital Project, a partnership between Stanford University and the University

of Minnesota, The Nature Conservancy, and the World Wildlife Fund. (http://www.naturalcapitalproject.org). InVEST combines spatial data on land use and land cover (LULC) patterns with information on the biophysical processes supplying the services and service demand to provide outputs in biophysical or economic terms. The models can be used as stand-alone tools, or within ArcGIS, and can be run individually for each service. The spatial resolution of the models is flexible and models can be run at local, regional or global scales, depending on available input data. InVEST also has a detailed and comprehensive user guide, with default data available for a number of model inputs (Sharp et al., 2016).

This modelling tool is widely used. Posner et al. (2016) reported that 19 different InVEST models were run 43,363 times in 104 countries over a 25-month period (June 2012 - June 2014). There are many published studies available, including determining how changes in climate may affect hydrological service delivery in a semi-arid basin in NE Spain (Terrado et al., 2014), investigating how agricultural expansion may impact on biodiversity and carbon storage in Brazil (Chaplin-Kramer et al., 2015), and demonstrating how catchment water quantity and quality vary under a number of land-use change scenarios in China (Zheng et al., 2016).

There are currently 18 InVEST ecosystem services models,plus a number of 'helper tools', available for terrestrial, marine, freshwater and coastal environments, including examples of cultural, provisioning, supporting and regulating services. Of these, there are 8 models that require information on soil properties (for example, soil erodibility; soil fertility; soil depth; soil carbon; soil type) as a data input (see Table 2). InVEST is an open-source tool and uses Python scripting. Therefore while the current models can be freely downloaded by users, there is potential for adapting models further for individual use.

Table 2. Soil data-sets used in the InVEST ecosystem service model, the ecosystem service it assesses and the potential for future development.

Main soil data it uses	**Main ecosystem service assessment that the soil layer contributes to**	**Potential for development**
Carbon density in soil (Tonnes/ha) per land use/land cover class.	Carbon storage in soils and biomass (tonnes per grid cell).	Results are as detailed as the land classification used. Classes could be further split by soil type, elevation or management.
Carbon storage in soil (Mt of CO_2 e/ha) per land use class; accumulation rate (Mt of CO_2 e/ha-yr), % disturbance and half-life of carbon emitted within soil per land use/land cover class.	Coastal blue carbon: carbon stock, carbon accumulation, carbon emissions, net carbon sequestration (all Mt CO_2 e/ha) and net present value (currency/ha).	Currently uses a simplified approach for modelling dynamics of the carbon cycle and is based on a number of assumptions, e.g. carbon is assumed to be stored and accumulated linearly through time.
Current global model driven mostly by climate data. Next steps will be to incorporate soil (fertility and depth) and topographic data.	Crop production: crop yield per grid cell, financial analysis (yield, costs, returns and revenues per crop), nutritional contents (based on values entered by the user for 1 tonne of crop biomass).	Model currently under active development. Further field-level data is required to run a more fine-scale model.
Carbon density in soil (Tonnes/ha) per land use/land cover class.	Forest carbon edge effect: Carbon storage in soils and biomass (tonnes per grid cell).	This model is an update of the carbon storage model, allowing for the degradation of carbon that occurs in tropical forests due to edge effects.

Nutrient retention due to biochemical degradation in soils (value between 0 and 1); distance (m) after which soil is assumed to retain nutrient at maximum capacity.	Nutrient delivery ratio (nitrogen or phosphorus): total nutrient load in the watershed (kg yr^{-1}); total nutrient export from the water shed (kg yr^{-1}).	Requirement for more accurate export coefficients, from local studies if possible. Sensitivity analyses are recommended to investigate how changes in input data affect final outputs.
Soil erodibility (K) (t ha h ha^{-1} MJ^{-1} mm^{-1}); fraction of topsoil particles finer than coarse sand (calibration parameter).	Sediment delivery ratio: total sediment exported to the stream (tonnes per grid cell); Sediment retention (tons per watershed).	Currently based on the revised USLE (universal soil loss equation) (Renard, Foster, Weesies, McCool, & Yoder, 1997), which is widely used but has limitations (doesn't represent all possible erosion processes).
Root restricting layer depth of soil (mm); plant available water content of soil (fraction 0-1).	Water yield: total annual water supply per watershed (m^3).	The current model simplifies water consumption (one value per land class).
Soil hydrologic groups (based on hydraulic conductivity and soil depth); Curve number (CN) for each soil group.	Seasonal water yield: outputs are indices for the relative contribution of each grid cell to base flow (occurs during dry weather) and quick flow (present during or just after rain events).	The model does not currently provide quantitative estimates of flow.

LUCI - Land Utilisation and Capability Indicator

The LUCI (Land Utilisation and Capability Indicator) tool was developed to synthesise biophysical data to inform on ecosystem service delivery. The model is designed to be able to run with a limited number of data inputs, with a user-friendly GIS interface. Public release of the model and documentation is planned for 2017. It is designed as a planning tool and is specifically tailored to investigate the impact of farm scale interventions on catchment scale function. To do this, LUCI explicitly tracks the lateral as well as vertical movement of mass (water, sediment and nutrients) through the landscape at spatial resolutions on the order of meters. Soils data is important in underpinning the outputs from LUCI along with topography, landcover and climate data. At present soil data must be supplied to match country specific classifications for England and Wales or New Zealand. There are plans to support global data using the World Reference Base (WRB) soils format. The main ecosystem services modelled by LUCI are agricultural productivity, carbon storage and sequestration, flood risk mitigation, nutrient runoff mitigation, and habitat suitability and connectivity. Of these, the tools for agriculture, carbon, and habitat suitability depend significantly on soil functioning. Table 3 is based on the version using the England and Wales soil-survey data, and shows which ecosystem services the soil data currently contributes to. In New Zealand, the tool has been developed to incorporate influence of soil on N and P exports, and soil hydrological function. These enhancements have not yet been implemented for UK soil (Trodahl, Deslippe, & Jackson, 2016). Trade-offs, impacts, and synergies between individual service provisions can be mapped spatially. The model has been applied at national scale for Wales to inform implementation of agri-environment activities under the Glastir Monitoring and Evaluation Program (Emmett et al., 2014). It can therefore be seen that input data related to soil properties are required for many models, from agricultural production to annual water yield, highlighting the important contribution of soil to a wide variety of ecosystem services.

Table 3. Soil data sets used in the LUCI ecosystem service model, the ecosystem service it assesses and the potential for future development.

Model and soil data it can use	**Main soil data it uses**	**Main ecosystem service assessment that the soil layer contributes to**	**Potential for development**
LUCI, soil survey of England and Wales	Soil water holding characteristics and fertility (by soil association, classified based on expert opinion and literature), slope and aspect	Agricultural productivity potential: category high-low	Bring in effects of slope position (areas accumulating flow more at risk of waterlogging) Currently based on dominant soil series in association-could add error bounds for other series components
LUCI	Landcover and soil association combination (classified based on average for that combination from national datasets)	Carbon storage in soils and biomass: kg m^{-2}, category high-low	Include data on slope impacts on soil depth, and estimated effects of different management within the landcover classes. Metamodeling of expected influence of climate change
LUCI	Landcover and soil association combination (classified based on average for that combination from national datasets)	Carbon sequestration potential: kg m^{-2}, category high-low	
LUCI	Soil type, spatial units for calculation (Simpson's and Shannon's indices are calculated based on the chosen level of disaggregation of soil type)	Soil diversity: values for all selected indices in each landscape unit.	

Comparison of models

As the range and complexity of ecosystem-services modelling tools increases, it is important that intercomparisons between models are made, ideally for the same site and services, to help users choose the most-suitable tool for their needs. Vigerstol and Aukema (2011) and Bagstad, Johnson, et al. (2013) provide useful overviews of modelling tools, covering model inputs, outputs and how the models can be applied. The InVEST and ARIES tools were compared for three services (carbon storage, water supply and scenic viewsheds) in a semi-arid river basin in Arizona, USA (Bagstad, Semmens, & Winthrop, 2013), with similar overall conclusions reached for each service using both tools.

Sharps et al. (2017) also compared modelling tools, running ARIES, LUCI and InVEST for three services (water supply, carbon storage and nutrient retention) in a temperate North Wales catchment with a wide variety of land-use types. This study focused on the range of different outputs that each modelling tool can produce per service, and on validating the model outputs against observed data. Using carbon as an example, the mapped outputs from the ARIES, InVEST and LUCI carbon models can be seen in Figure 7. InVEST and LUCI provide directly comparable maps of carbon stock for the study site (Fig 7 a & d, and b & e). There were some differences between the output maps, both in terms of spatial pattern and in quantities of carbon stocks, particularly for carbon stocks in biomass and soil at a depth of 30cm. This is thought to be due to differences in the approaches used between the two models, but highlights that not all models will give the same answer, even for a relatively simple service to model such as carbon. The LUCI carbon model is based on soil type and (aggregated) land use combinations, whereas the InVEST model uses only land-use data. Despite the differences in spatial distribution of carbon from the two models, the quantitative outputs for InVEST and LUCI (total carbon stocks aggregated to catchment level) were similar and within <10% of each other. LUCI can also provide a map of carbon sequestration potential (Figure 7), highlighting areas where existing carbon stock is high (red), and therefore with less potential for change, and areas where there is potential for increased carbon if the land-use changed (green). The ARIES model predicted carbon density (g/kg) in the top 15cm of soil (Fig 7g), rather than carbon

stocks. Both InVEST and ARIES can produce maps of uncertainty (Fig 7c & h respectively), based on the model inputs used for carbon values per land use type.

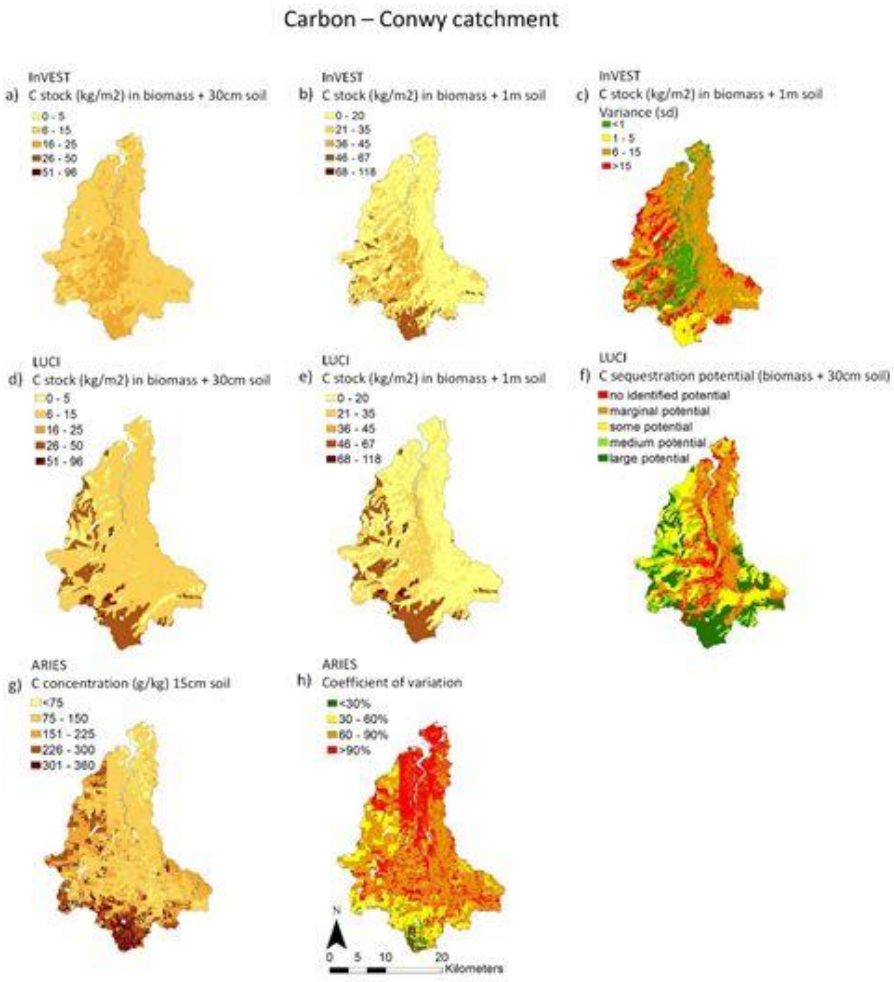

Fig. 7. Carbon stocks for the Conwy catchment, including soil and above-ground, below-ground, and dead vegetation for: a) InVEST, carbon stocks (kg m− 2) to 30 cm soil depth; b) InVEST, carbon stocks (kg m− 2) to 1 m soil depth; c) InVEST, variance associated with carbon stock estimates to 1 m depth (kg m− 2); d) LUCI, carbon stocks (kg m− 2) to 30 cm soil depth; e) LUCI, carbon stocks (kg m− 2) to 1 m soil depth; f) LUCI, carbon sequestration potential (30 cm depth). Carbon concentration: g) ARIES, expected carbon concentration in topsoil, 15 cm depth; h) ARIES, uncertainty measured as coefficient of variation (%).

ARIES, InVEST and LUCI each have different strengths. Customised models can be developed using ARIES, if the user has the technical skills. This tool is also a good option if data are scarce, and it can explicitly model the flow of services such as service use relating to water-based services through the catchment. InVEST can model a wide

variety of services, and the manual with default input data is very helpful for new users. This is one of the few tools that currently models some cultural services. And it can also provide economic valuations as an output. LUCI produces traffic-light maps, which allow easy interpretation of model outputs for decision makers. LUCI also has a unique trade-off tool that can demonstrate the potential impact of different scenarios on multiple services, highlighting areas where they may be "win-wins," with multiple services benefitting, or trade-offs, where one service is improved while another is reduced. There are also some differences in modeling approaches, for example in the scale of model outputs. The InVEST water supply and nutrient-delivery ratio models run at the grid-cell level, with outputs given per watershed, whereas ARIES and LUCI can provide an output for every point in the landscape.

Overall, the choice of modelling tool depends on the question, available input data and the scale of outputs required. Sharps et al. (2017) recommend further model comparison studies, running tools for a wide range of services, including cultural services (such as recreation or viewsheds), and also testing models across multiple scales, for example from sub-catchment to sub-continental. There is increasing interest in running ensemble suites of ecosystem service models. This is similar to the approach used in running global circulation models (GCM) for climate simulations, to capture some aspects of the variability in outputs between models and to start to address issues of uncertainty in model outputs.

Joint environmental and socio-economic modelling at national to global scales

The ecosystem services approach recognises that we live in a coupled socio-economic – environmental system. Large-scale ecosystem service models take an integrated, systemic approach to understand better the linkages and feedbacks between different biophysical and human systems. An example of a model attempting this is the Global Unified Meta-model of the BiOsphere (GUMBO). The model divides the earth's surface into the 11 biomes for assessment. The pedosphere is not dealt with as an explicit module, but it is included in the lithosphere. Although predictions for soil formation, carbon and nutrient fluxes and weathering and erosion processes are included (Boumans et al., 2002). It provides a bold attempt to model the earth system

in an integrated way, incorporating biophysical characteristics of the earth system and socio-economic aspects of humanity's activities.

The importance of this integrated approach is further demonstrated by the CLIMSAVE Integrated Assessment Model (IAP) for Europe (Harrison, Dunford, Holman, & Rounsevell, 2016). The IAP integrates models of agriculture, forestry, urban growth, land use, water resources, flooding and biodiversity within a spatially-explicit software environment that operates on 10 x 10 minute spatial grid for the countries of the European Union, plus Norway and Switzerland. It can be used to simulate the impacts of different climate and socio-economic scenarios on a wide range of sectoral and ecosystem-service output indicators. The linking of the different sectoral models enables analysis of cross-sectoral interactions and assessment of potential trade-offs between ecosystem services. It is designed as an interactive web-based tool that researchers and stakeholders can use to explore and understand cross-sectoral vulnerability to climate and socio-economic change and how it might be reduced by various adaptation options.

Cross cutting and Dealing with Complexity

In a recent article in Nature, Schmidt et al. (2011) wrote that

> *'soils are now in the 'front line' of global environmental change—we need to be able to predict how they will respond to changing climate, vegetation, erosion and pollution so that we can better understand their role in the Earth system and ensure that they continue to provide for humanity and the natural world'.*

They recognized that although only a thin layer of material at the earth's surface, soils like many interfaces play a pivotal role in regulating the flow and transfer of mass and energy between the atmosphere, biosphere, hydrosphere and lithosphere. Moreover, the structure and organization of soils leaves an important imprint on the earth's surface in terms of how land is used, and how ecosystems develop. Soils help regulate the Earth's physical processes, such as water and energy balances, and act as the biogeochemical engine at the heart of many earth-system cycles and processes on which life depends. Some soil processes contribute to the delivery of ecosystem goods

and services directly, whilst other soil processes impact the delivery of goods and services. Soils may actually be 'used up' through topsoil or peat extraction, or they may serve as a 'means' to the delivery of an ecosystem service as with filtration, and subsequently becoming degraded. This section examines how soil processes impact soil and ecosystem function and the production of goods and services of benefit to humanity.

According to FAO soils provide the following eleven functions: 1. Water purification and soil contaminant reduction, 2. Climate regulation, 3. Nutrient cycling, 4. Habitat for organisms, 5. Flood regulation, 6. Source of pharmaceuticals and genetic resources, 7. Foundation for human infrastructure, 8. Provision of construction materials, 9. Cultural heritage, 10. Provision of food, fibre and fuel, 11. Carbon sequestration.

How well a soil is suited for any of these functions depends on the feedback with the climate and thus location on the globe, and also on its composition. While all soils contain a different mixture of sand, silt, clay and organic matter, it is the heterogeneity of that mixture, its chemical composition in combination with growing plant roots and the activity of soil organisms, effectively a soil's structure, that make up the complexity of soil processes. For example one of the soil's ecosystem goods and delivery services is the storage and filtering of water. Soils play an essential role in regulating how much water infiltrates the soil, how much will become surface runoff, how much is available for plant growth, and what quantity will flow towards groundwater. Research on the variability of soil moisture crosses a range of scientific fields such as agriculture; biochemistry; remote sensing; ecology and hydrology, including its control on nitrification rates; satellite radar interferometry and climate change science (Brake et al., 2013; Lawrence & Hornberger, 2007; Robinson et al., 2016). When it comes to biophysical interactions in landscapes — those biotic and abiotic processes in a landscape that have an influence on the developments within and evolution of a landscape, including anthropogenic influences — dealing with the complexity of interacting processes demands collaboration between multiple disciplines. Wassen et al. (2013) have discussed how anthropogenic loading of nitrogen (N) and phosphorus (P) has changed nutrient availability in many ecosystems, leading to shifts in plant productivity between species, and potentially

impacting carbon, N, P and water cycles The interactions between these are only beginning to be understood.

Another example illustrating the complexity of soil ecosystem function and services is the process of desertification and restoration. Desertification, defined as land degradation in drylands by the UNCCD, is the result of an interplay between resource exploitation, population increase, and environmental change. The occurrence of desertification in itself can be a matter of debate (Kaptué, Prihodko, & Hanan, 2015), but even more is re-greening (either spontaneous or by restoration efforts) in land-atmosphere interactions and possible feedbacks with precipitation (Giannini, Biasutti, & Verstraete, 2008). The effect of adding water to an ecosystem on the regional climate has been practically applied on large scale in the form of production enhancement by irrigation, yet quantification of its effects are inconclusive. Douglas et al. (2009) used RAMS (Regional Atmospheric Modelling System) to simulate the effects of irrigation in India and found that it increased the regional moisture flux, which in turn increased the convective available potential energy (CAPE). This led to a reduction in surface temperature, modified regional circulation patterns and led to changes in mesoscale precipitation. The magnitude and direction of the effect of adding water seem to depend on the extent of an area (Im & Eltahir, 2014), the geographic location (Barnston & Schickedanz, 1984; Chase et al., 1999; Sen, Bin, & Yuqing, 2004) , and the existing weather patterns (Ozdogan et al., 2010). Some of these studies report an increase in precipitation downwind of an irrigated area (Eddy et al., 1975), while others just give the direction of the increase in their specific case (Moore & Rojstaczer, 2002). DeAngelis et al. (2010) concluded that the evapotranspiration of irrigated areas contributes to downwind precipitation with a larger contribution when evapotranspiration rates are higher, Chase et al. (1999) reported that irrigation in the Colorado Plains has an impact on the climate in the foothills of the Rocky Mountains and even influence the mountains themselves; cloud cover and precipitation being substantially affected. On the other hand, Sen et al. (2004) and Im and Eltahir (2014) both reported rainfall increases and decreases in the vicinity of the irrigated areas. This demonstrates the importance of including feedbacks in our models if we want to truly understand ecosystem services.

Notwithstanding the debate surrounding the complexity in soil processes, disciplines involved in environmental research unite in acknowledging spatial structure and heterogeneity of environmental systems (e.g. Schröder & Seppelt, 2006). Much research is focused on quantifying spatial structure and heterogeneity, such as climate variability, urban sprawl, deforestation and habitat loss (Ahlqvist & Shortridge, 2010). To be able to understand the emerging patterns resulting from spatial structure and heterogeneity, connectivity has been acknowledged as a useful theoretical concept, from ecology (Pringle, 2003), biology (Taylor et al., 1993), hydrology (Gomi et al., 2008), soil science (Vogel, 2000) and geomorphology (Baartman et al., 2013; Bracken & Croke, 2007). The key aspect of the connectivity concept is that it can create pathways for feedbacks which are so often missing in the contemporary context of soil processes.

Traditionally, the centre of gravity for many studies on soil processes has been physically, chemically and biologically related (Vereecken et al., 2016). Studies considering ecosystem services are often focused on production related, or biodiversity indicators, while links to non-traditional areas such as construction or cultural heritage are somewhat limited. For example, a common framework between soil knowledge and urban planning is missing and generally not considered in urban expansions. Many of the world's global deltas are urbanized, and further expected urbanization (Heilig, 2012) will result in large parts of the landscape being covered in concrete or asphalt. Especially in deltas, such as for example the Netherlands, soil sealing may increase flood risks if rain water cannot infiltrate into the soil, and sewage systems may not be adequate for extreme rainfall events. While flood and drought risks have obvious economic consequences, environmental indicators and socio-economic indicators are often perceived as unrelated, especially in urbanized areas. It is assumed that by implementing nature-based solutions in the rural areas, this will also reduce risks in the cities. Built-up areas are all but ignored in the water and soil system, yet are an integral part of it. Those most affected by flood events tend to live in urban centres rather than the countryside, which calls for better integration of soil related research and urban planning. Couplings developed between humans and natural systems (Liu et al., 2007), necessitate a bridge between the question-driven

ecology-centred spatial view and solution-driven society-centred holistic view (van der Ploeg, 2016; Wu, 2006). Hence the better integration of soil research with other disciplines, and demonstrating how soils and their connectivity underpins the delivery of ecosystem services is the growing challenge of soils research.

Conclusion

This chapter offers an assessment of the current natural capital and ecosystem service approaches. While these have often stood as two separate ways of looking at nature, efforts like the United Nations System of Environmental and Economic Accounts seek to unify them in an operational framework. Soils are the least developed of the natural resources in terms of framework development and work is needed to correct this. Clearly this needs to include monitoring of 'state and change in condition' as called for by the United Nations World Soil Resources Report in 2015. A monitoring framework in itself is a powerful tool to inform policy, and to date little progress has been made on how to value soils, which is the next step of the accounting approach. While we recognise that valuation is an integral part of the ecosystem services approach, we must take a step back and consider what it means to value soil, and emphasise the need to care for our resources.

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APPENDIX D

Supporting Information for Chapter 2

Supporting methods

Sample Collection Methods

Within each of the 300 x 1 km sample squares, a set of soil samples were taken from 5 pre-determined randomly dispersed locations (where this was physically possible). Soil was sampled after removal of vegetation and any loose litter using a black plastic core (5.1 cm diameter, 15 cm long) from a location centrally located within a 200m^2 plot surveyed for vegetation. After collection, the soil cores were refrigerated and stored until posted, usually within 1-2 days to laboratories located at the Centre for Ecology & Hydrology (CEH), Bangor and Lancaster.

Laboratory Protocols and Analytical Methods

Soil organic matter, Loss-on-Ignition (LOI) and derived carbon concentration

The soil was sieved to pass 2 mm and air dried. Subsequently, LOI was measured on a 10 g sub-sample. For samples where less than 10 g was available, as close as possible mass of soil was used and records of the exact quantity were recorded. The sub-sample was oven dried at 105°C for 16 hours to remove moisture, weighed, then combusted at 375°C for 16 hours. The cooled sample was then weighed, and the loss-on-ignition (%) calculated (Emmett et al., 2008, 2010). Carbon concentration is a derived unit from the LOI measurement and is determined by multiplying the LOI by 5.5.

Total soil organic carbon (SOC) and nitrogen

After treatment of the sample to remove inorganic carbon, the remaining carbon (SOC) was measured using a UKAS accredited (17025:2017) method SOP3102, at CEH Lancaster. Samples were analysed using an Elementar Vario-EL elemental analyser (Elementaranalysensysteme GmbH, Hanau, Germany). The Vario EL is a fully automated analytical instrument working on the principle of oxidative combustion followed by thermal conductivity detection. Following combustion in the presence of

excess oxygen, the oxides of nitrogen and carbon flow through a reduction column which removes excess oxygen. Carbon is trapped on a column whilst nitrogen is carried to a detector. Carbon is then released from the trap and detected separately. Sample weights are usually 15 mg for peat and 15-60 mg for mineral soil samples (Emmett et al., 2010).

Total soil phosphorous

Air-dried and ground to 2 mm soils were digested with hydrogen peroxide (100 Volumes) and sulphuric acid in a 5:6 ratio. Selenium powder and lithium sulphate were added to raise the boiling point of the acid.

Samples were then placed at 250°C for 15 minutes and then at 400°C where the temperature was maintained for 2 hours to complete the digestion. After digestion the samples were diluted with ultrapure water and allowed to settle overnight. The supernatant was then further diluted and P measured colourimetrically using a SEAL AQ2 discrete analyser. Phosphorus was determined using ammonium molybdenum blue chemistry with the addition of ascorbic acid to control the colour production.

Olsen-phosphorous

Two grams of air-dried soil samples are extracted in 40 ml Olsens reagent (0.5 M $NaHCO_3$ at pH 8.5) for 30 minutes in a mechanical end-over-end shaker. The sample was then filtered through a Whatman 44 filter paper to separate the soil and the filtrate; the filtrate was kept for analysis.

The analysis was performed on a Seal Analytical AA3 segmented flow. The samples are mixed in the flow channel with an acidic ammonium molybdate and potassium antimony tartrate to form a complex with phosphate. This complex is reduced with ascorbic acid to develop a molybdenum blue colour. The reaction is temperature controlled to 40°C using a water bath to ensure uniform colour development. The developed colour is measured at 880 nm.

Soil pH in deionized water

Soil pH was carried out on a suspension of fresh field-moist soil in deionised water. The ratio of soil to water is 1:2.5 by weight. The method described here is based upon that employed by the Soil Survey of England and Wales (Avery & Bascomb, 1974).

Soil solution electrical conductivity

Field moist soil (10 g) was placed in a beaker and 25 mL of deionised water (DIW) added. This was then stirred with a rod to produce a homogeneous suspension. After 30 min, the samples were stirred again with the rod and EC measured using an Pt electrode and a conductivity meter (Jenway 4510).

Soil bulk density of fine earth and volumetric water content of fine earth

Bulk density was determined from the soil core, which was 15 cm long with a radius of 2.55 cm. Dry bulk density is calculated using the following equation:

$$Dry\ bulk\ density\ (g\ cm^{-3}) = \frac{Dry\ weight\ core\ (105°C)(g) - stone\ weight\ (g)}{Core\ volume\ (cm^{-3}) - stone\ volume\ (cm^{-3})}$$

Fine earth volumetric water content when sampled

Once the bulk density has been calculated, the volumetric water content of the fine earth fraction was determined by multiplying the bulk density and the gravimetric water content of the fine earth.

Soil water repellency, water drop penetration time

Soil water repellency (surface) measurement is carried out by measuring the time for a fixed volume droplet of deionised water (100 µL) to be fully absorbed into the soil surface (Water Drop Penetration Time (WDPT)). Six drops of water were applied to an air-dried undisturbed soil surface. The entire process was filmed using a digital video camera so that the timing could be determined accurately. The samples were maintained in a laboratory at a relatively constant temperature ~20°C. Some soils, especially arable, were not consolidated so measurements were taken on surface unconsolidated soil or aggregates using 20 g soil added to a tin lid, and procedure followed as described above.

Soil texture

Soil texture was measured by subsampling the air-dried sample by manual quartering, removing 0.5 g and treating with H_2O_2 to remove the organic C (Gee & Or, 2002). The samples were transferred to 250 ml bottles, 5 ml of 5% sodium hexametaphosphate added and the samples shaken overnight at 240 rev min^{-1} to disperse the samples. The particle size distribution was measured using a laser diffraction LS320 particle size analyser (Beckman-Coulter Inc, Pasadena, CA). The outflow from the machine was also passed through a 63 µm sieve and the collected sand-sized particles weighed for quality control of the sand content measured by the laser. The particle size distributions were calculated using the Mie theory approach, with an RI of 1.55 and an AC of 0.1 (Bieganowski et al., 2018; Özer, Orhan, & Işik, 2010). Particles within the range 0.04 to 2.2 µm were categorised as clay, 2.2 to 63 µm as silt and 63 to 2000 µm as sand.

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Supporting tables

Table 1: Number of Welsh sites in the Countryside Survey outside the Environment Agency prompt values. For Olsen P this is 10mg/l for mesotrophic grassland, no results presented for acid grassland and heathland. For pH this is <5 and >7 for mesotrophic grassland, >5 for acid grassland and heathland. For bulk density this is above 1.3 for mesotrophic grassland and 1.0 for acid grassland and heath.

	Olsen P		pH			Bulk density
Habitat	1998	2007	1978	1998	2007	2007
Mesotrophic grassland	36 (88%)	34 (60%)	33 (55%)	8 (14%)	30 (10%)	15 (6%)
Acid grassland	-	-	0 (0%)	1 (14%)	12 (24%)	1 (2%)
Dwarf shrub heath	-	-	0 (0%)	1 (25%)	1 (4%)	0 (0%)

Table 2: Spearman rank correlations between pH, carbon (total C), nitrogen, bulk density, water (volumetric water content), soil water repellency, total phosphorus, electrical conductivity and rock volume of the soil. Cells are coloured according to the strength and direction of the correlation, blue being positive and red being negative.

	pH	Carbon	Nitrogen	Bulk density	Water	Repellency	Total P	EC	Rock
pH	1	-0.609	-0.537	0.641	-0.252	-0.437	0.019	-0.154	0.21
Carbon	-0.609	1	0.952	-0.913	0.499	0.495	0.247	0.222	-0.33
Nitrogen	-0.537	0.952	1	-0.881	0.495	0.481	0.391	0.217	-0.314
Bulk density	0.641	-0.913	-0.881	1	-0.474	-0.481	-0.195	-0.242	0.269
Water	-0.252	0.499	0.495	-0.474	1	0.109	0.1	-0.054	-0.416
Repellency	-0.437	0.495	0.481	-0.481	0.109	1	0.076	0.072	-0.202
Total P	0.019	0.247	0.391	-0.195	0.1	0.076	1	0.102	0.08
EC	-0.154	0.222	0.217	-0.242	-0.054	0.072	0.102	1	-0.083
Rock	0.21	-0.33	-0.314	0.269	-0.416	-0.202	0.08	-0.083	1

Table 3: The mean and standard deviations of the different soil properties per cluster. All properties are significantly different between the clusters at $p < 0.0001$.

Cluster	pH	Bulk density (g cm^{-3})	Total carbon (g/100g dry soil)	Total nitrogen (g/100g dry soil)	Water content (%)	Water repellency (log(s))
1	4.45 (±0.64)	0.140 (±0.089)	40.4 (±10.8)	1.920 (±0.499)	57.1 (±15.4)	6.53 (±1.84)
2	4.72 (±0.52)	0.487 (±0.167)	10.3 (±5.15)	0.693 (±0.299)	35.6 (±16.3)	6.38 (±2.42)
3	5.67 (±0.51)	0.842 (±0.162)	4.96 (±2.15)	0.434 (±0.129)	26.4 (±8.5)	5.52 (±1.53)
4	5.87 (±0.64)	0.852 (±0.228)	5.08 (±2.30)	0.441 (±0.166)	41.0 (±11.7)	3.17 (±1.64)

Supporting figures

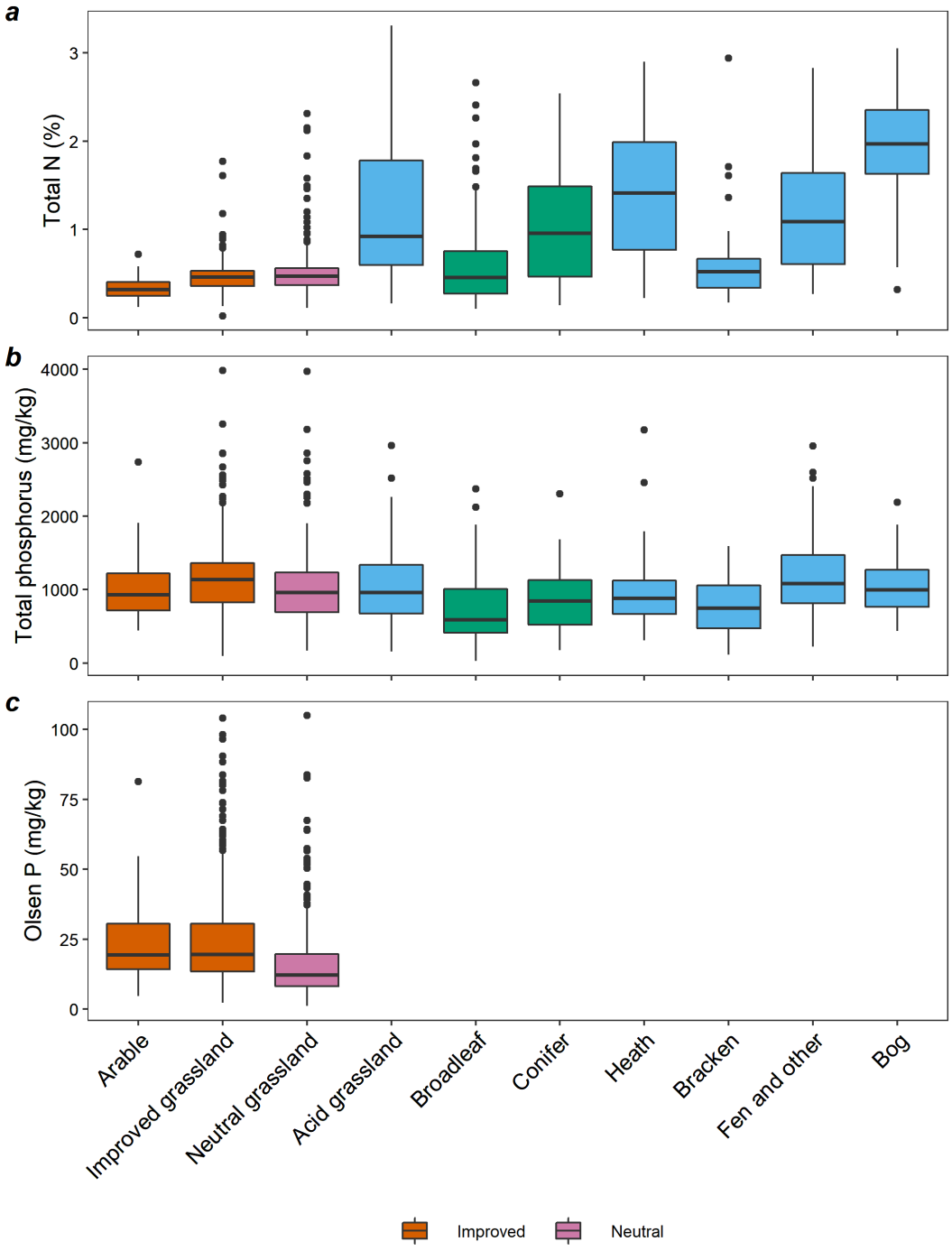


Figure 1: Differences in soil total nitrogen (a), soil total phosphorus (b) and soil Olsen P (c) across the range of habitats found in our study across Wales. Layout and colours as in Figure 2.2. Olsen P is not presented for upland or wooded sites due to its unreliability in low pH soils.

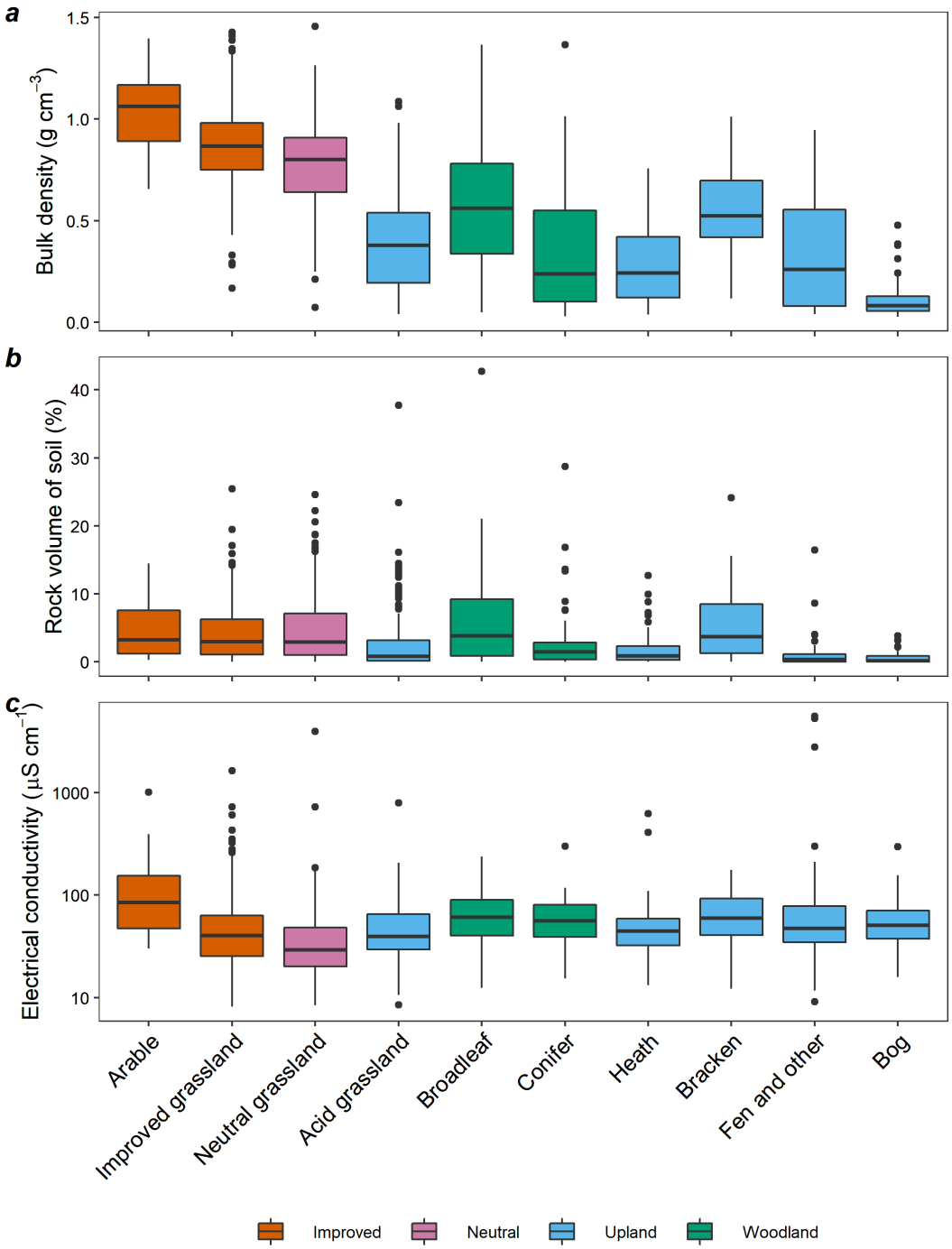


Figure 2: Differences in soil bulk density (a), soil rock volume (b) and soil electrical conductivity (c) across the range of habitats found in our study across Wales. Layout and colours as in Figure 2.2.

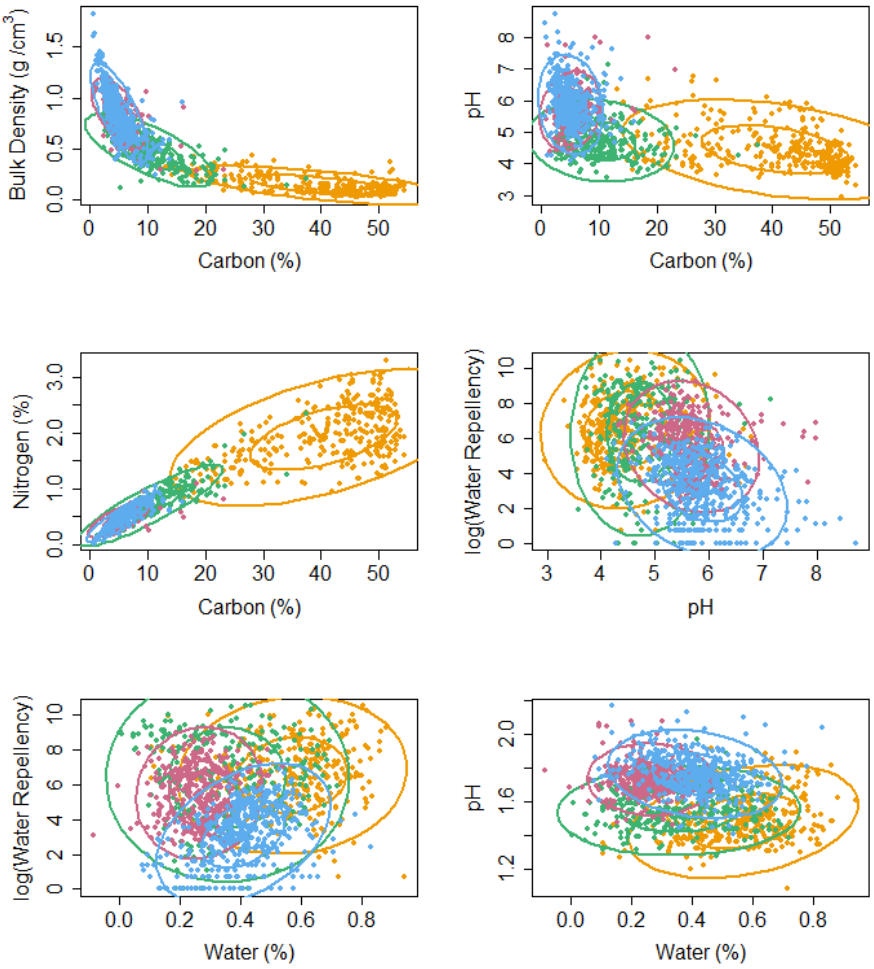


Figure 3: Parameters used within the classification process are plotted against each other. Points are coloured by the group identity: yellow is group 1 (organic); green is group 2 (organo-mineral); red is group 3 (acid mineral); and blue is group 4 (neutral mineral). These names are approximations of their positions on these graphs. The inner ellipse on each graph contains 50% of the points of the corresponding colour and the outer ellipse contains 90% of the points.

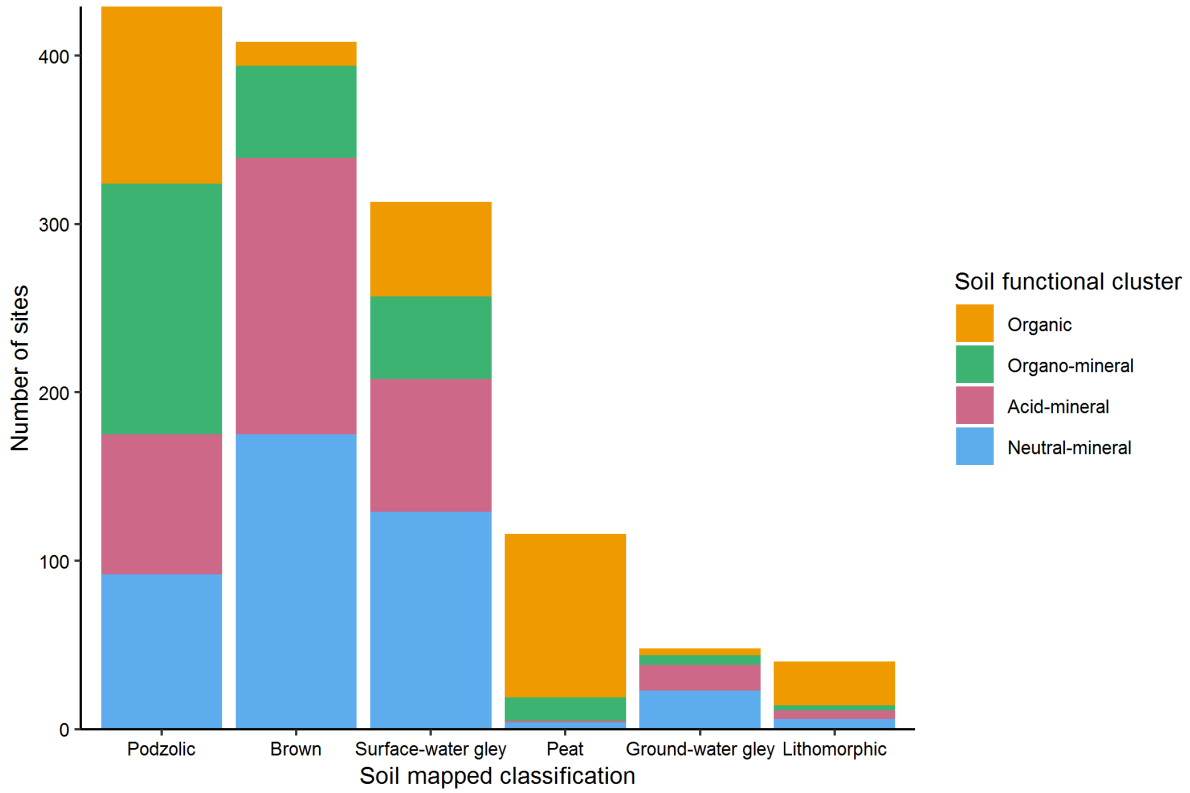


Figure 4: The count of sites per major soil group, coloured by the topsoil property cluster as found within our analysis.

APPENDIX E

Supporting information for Chapter 3

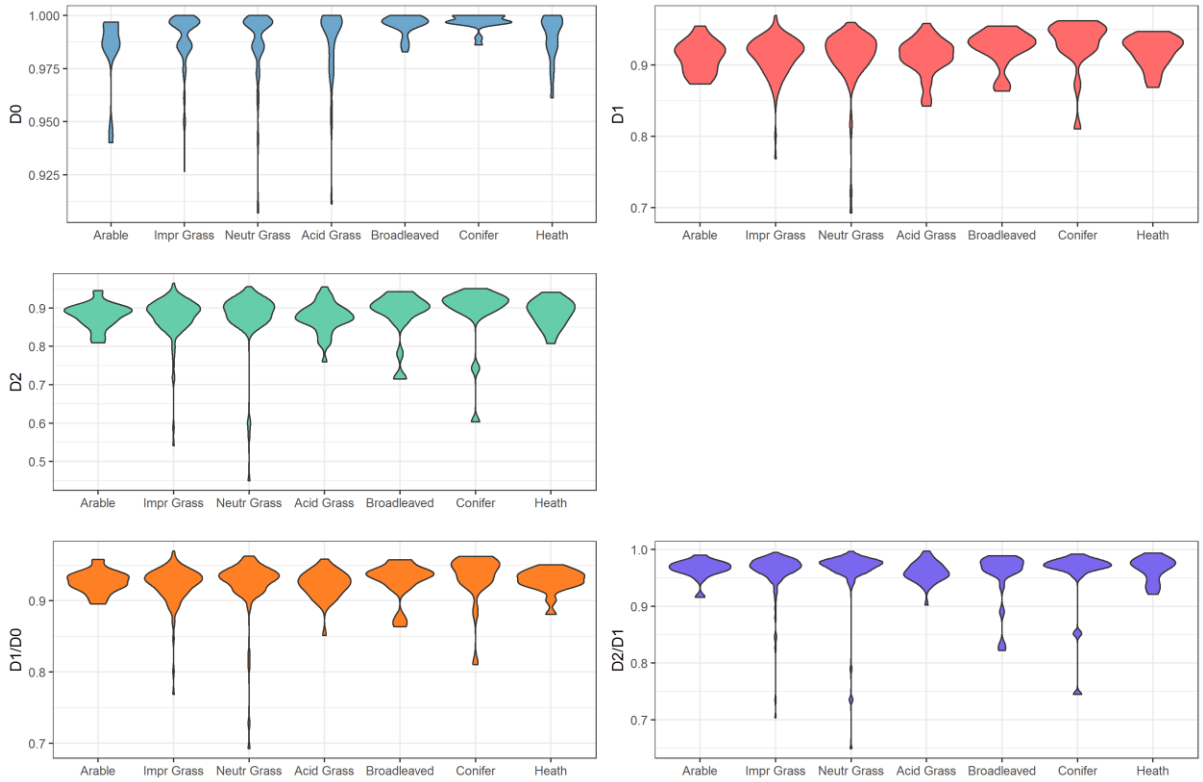


Figure 1. Impact of habitat on multifractal parameters. Overall, the violin plots show no significant change in multifractal parameters with habitat. Impr Grass = Improved Grassland; Neutr Grass = Neutral Grassland; Acid Grass = Acid Grassland; Broadleaved = Broadleaved, Mixed and Yew Woodland; Conifer = Coniferous Woodland; Heath = Dwarf Shrub Heath, Fen, Marsh, Swamp or Bracken.

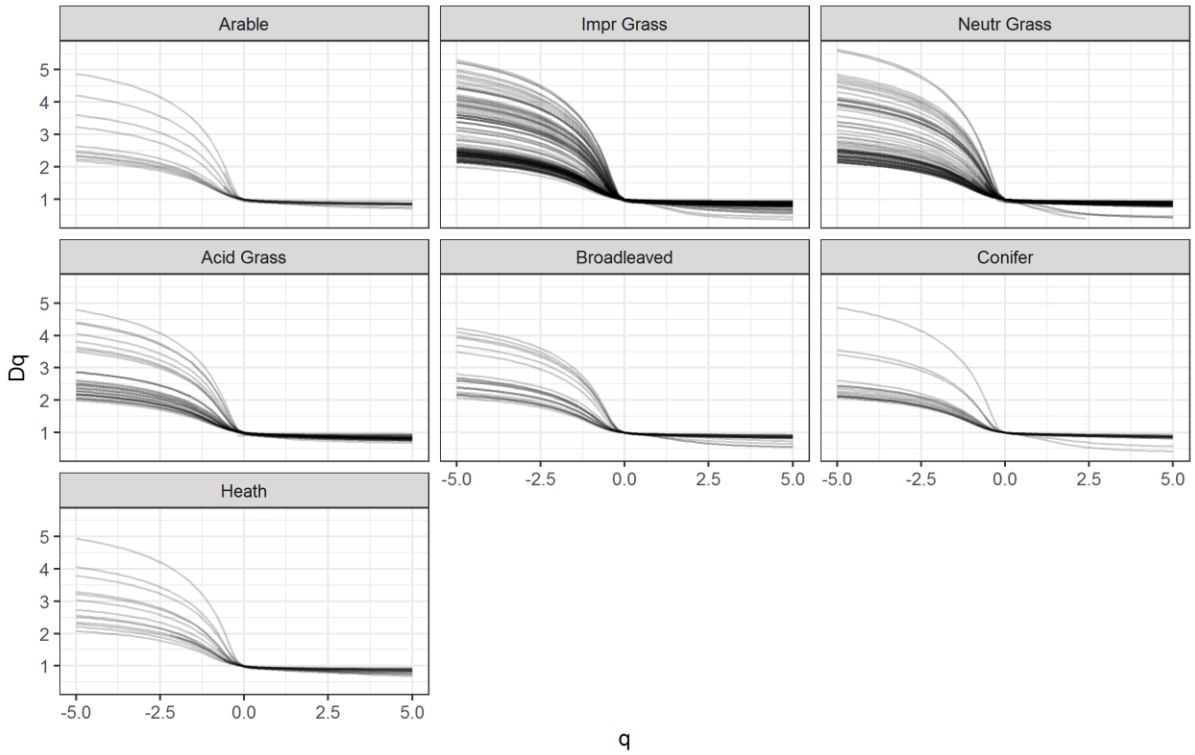


Figure 2. Impact of habitat on multifractal spectra. While there is considerable variation in the shape of the Dq spectra across samples, there appears to be no pattern with habitat.

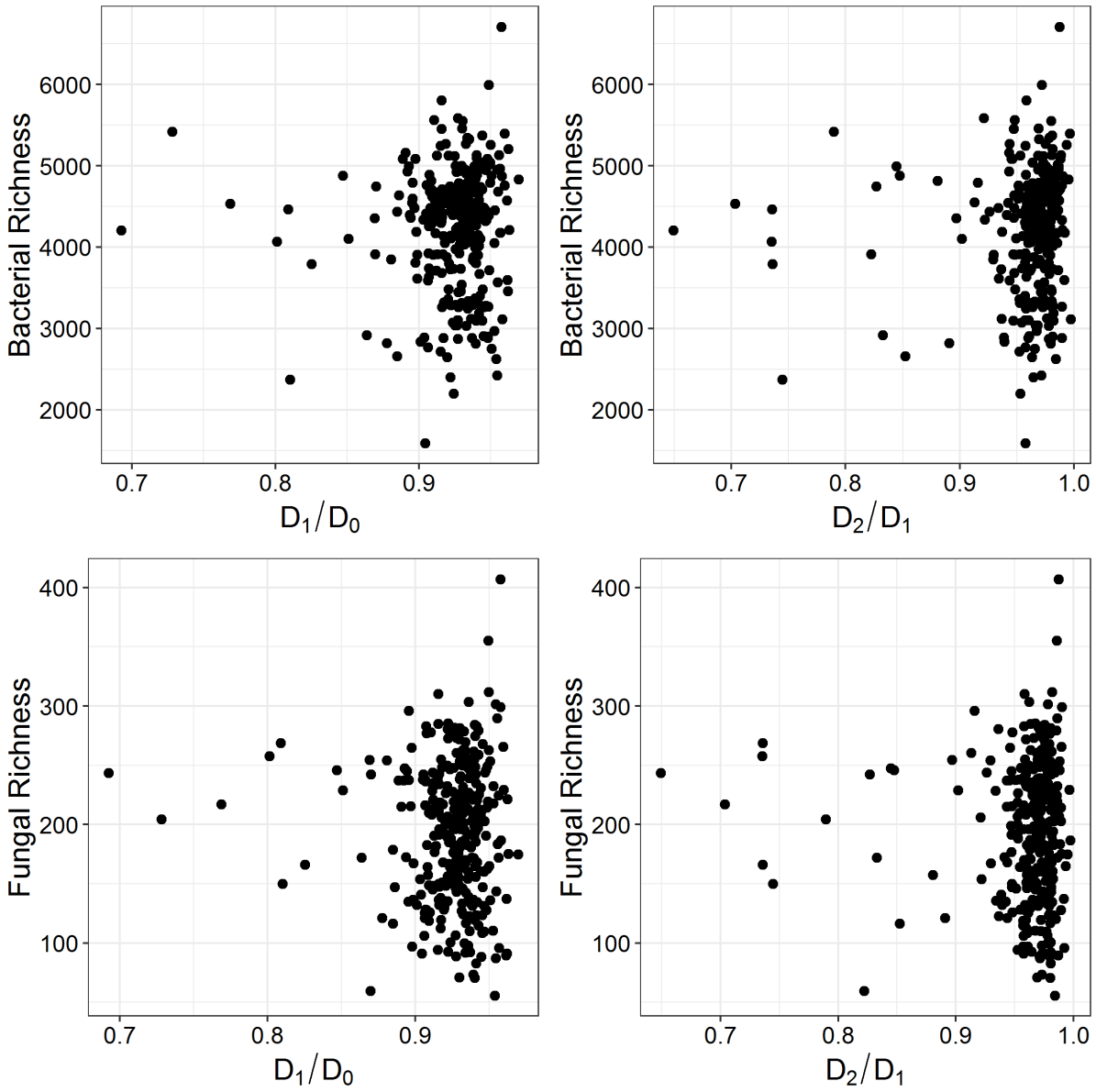


Figure 3. Impact of Multifractal parameter ratios on microbial richness. No change in bacterial or fungal richness was observed with changing ratios of multifractal parameters. The Spearman's rank correlations between fungal richness and D1/D0 and D2/D1 were -0.009 and 0.052 respectively. For bacteria they were 0.051 and 0.138.

Table 1. Summary statistics for the soil and climate data used in the structural equation model

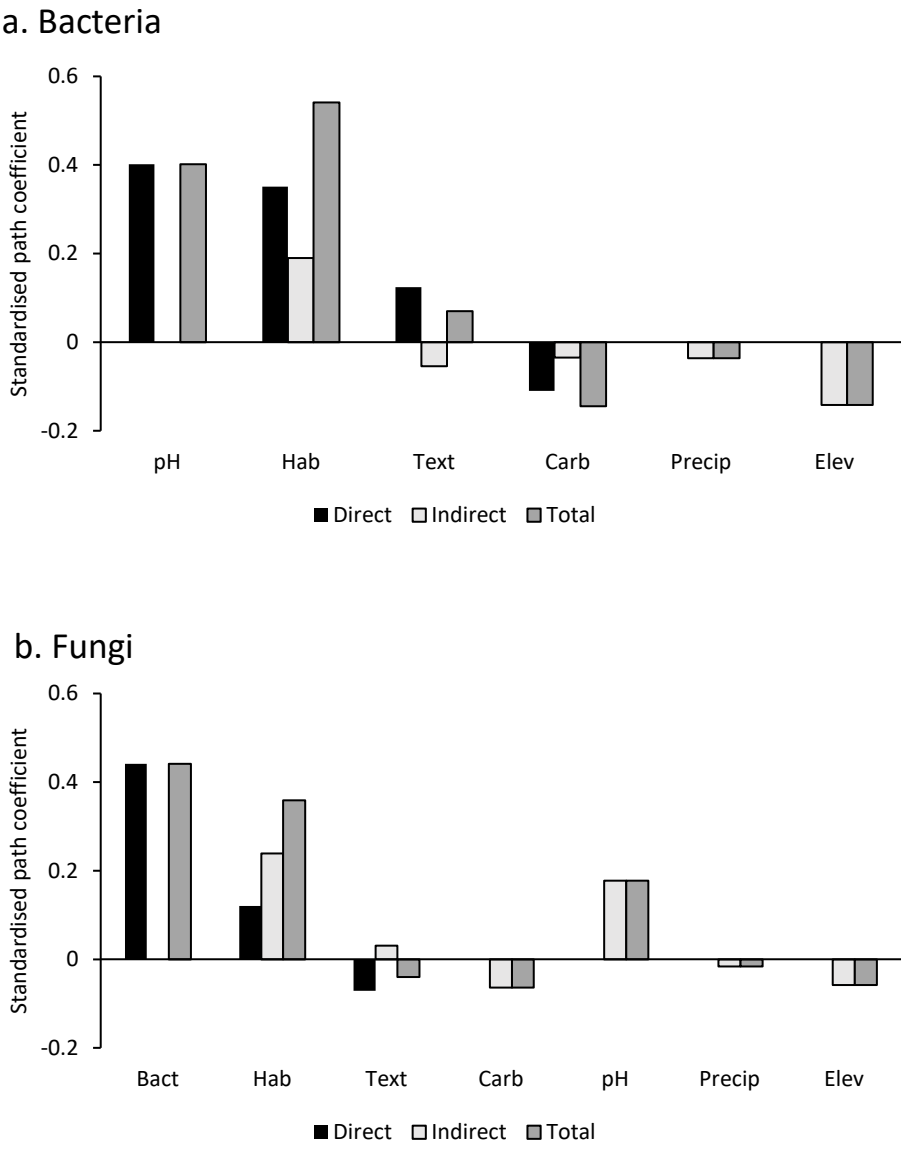
Variable	Mean	Median	Min	Max
Carbon	6.96	5.11	1	48.2
pH	4.58	4.56	2.42	7.35
Fungal richness	193.7	198	56	237
Bacterial richness	4245	4402	1591	6710
Elevation	190	157	3	653
Precipitation	1278	1144	671	3627
D_0	0.989	0.997	0.907	1
D_1	0.915	0.920	0.693	0.970
D_2	0.879	0.892	0.450	0.965
D_1/D_0	0.925	0.930	0.693	0.970
D_2/D_1	0.960	0.971	0.649	0.997

Table 2. Summary of the structural equation model output

Regressions:						
	Estimate	Std.Err	z-value	P (>|z|)	Std.all	Std.se
Carbon~						
Precipitation	0.333	0.126	2.644	0.008	0.250	0.092
Elevation	0.091	0.029	3.134	0.002	0.220	0.070
Texture	2.747	1.084	2.535	0.011	0.148	0.055
Habitat_Intensity	-0.223	0.091	-2.441	0.015	-0.169	0.071
pH~						
Carbon	-0.128	0.099	-1.286	0.198	-0.087	0.070
Elevation	-0.166	0.054	-3.063	0.002	-0.274	0.083
Texture	-2.205	1.452	-1.519	0.129	-0.081	0.053
Habitat_Intensity	0.789	0.147	5.363	0.000	0.412	0.079
Bacteria ~						
Carbon	-0.135	0.069	-1.966	0.049	-0.11	0.056
pH	0.337	0.058	5.783	0.000	0.402	0.055
Texture	2.832	1.315	2.154	0.031	0.124	0.056
Habitat_Intensity	0.565	0.114	4.938	0.000	0.351	0.071
Fungi ~						
Bacteria	0.349	0.063	5.580	0.000	0.441	0.077
Texture	-1.273	0.785	-1.621	0.105	-0.071	0.044
Habitat_Intensity	0.152	0.091	1.680	0.093	0.120	0.072
Intercepts:						
	Estimate	Std.Err	z-value	P(>|z|)	Std.all	Std.se
.Carbon	-1.223	1.009	-1.212	0.226	-2.058	1.647
.pH	6.563	1.372	4.784	0.000	7.569	1.598
.Bacteria	-0.057	1.285	-0.045	0.964	-0.079	1.765
.Fungi	1.510	0.762	1.982	0.047	2.623	1.348
Variances:						
	Estimate	Std.Err	z-value	P(>|z|)	Std.all	Std.se
.Carbon	0.243	0.033	7.317	0.000	0.687	0.062
.pH	0.431	0.07	6.082	0.000	0.574	0.058
.Bacteria	0.253	0.03	8.446	0.000	0.478	0.048
.Fungi	0.239	0.022	10.89	0.000	0.721	0.048

Table columns correspond to: Estimate, the estimated value of the path coefficients, intercepts and variances; Std.Err, the standard error of the aforementioned value; z-value, the test statistic; P (>|z|), the p-value; Std.all, the standardised value (standardised by the z-score method); and Std.se the standard error of the standardised value.

Figure 4. Indirect and direct effects upon bacterial and fungal richness derived from the structural equation model.



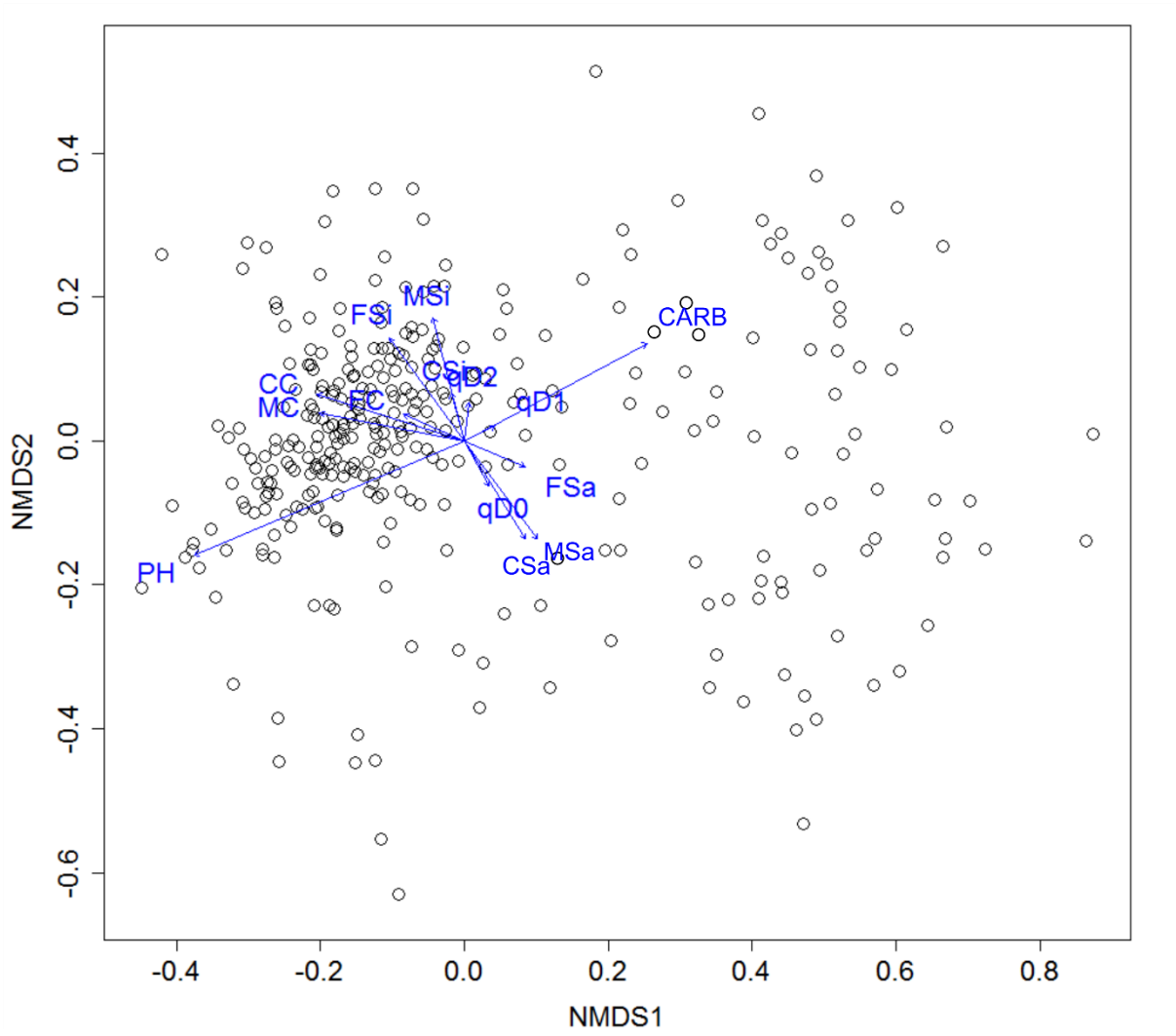


Figure 5 The results of fitting environmental vectors onto the fungal NMDS using 9 particle size classes, pH and carbon. Variables are labelled as pH, CARB is carbon, FC is fine clay (0.04 – 0.13 µm), MC is medium clay (0.13 – 0.45 µm), CC is coarse clay (0.45 – 1.5 µm), FSi is fine silt (1.5 – 5.1 µm), MSi is medium silt (5.1 - 17 µm), CSi is coarse silt (17 - 58 µm), FSa is fine sand (58 - 194 µm), MSa is medium sand (194 - 653 µm) and CSa is coarse sand (653-2000 µm). The particle size boundaries here do not correspond to classification systems used elsewhere as they are based upon grouping together nine laser granulometry bins for each new grouping.

Table 3. Envfit results for fungal NMDS

Variable	NMDS1	NMDS2	R^2	Pr(>r)
PH	-0.921	-0.389	0.623	0.001
CTOT	0.882	0.472	0.311	0.001
CC	-0.953	0.302	0.174	0.001
MC	-0.982	0.187	0.159	0.001
FSi	-0.587	0.809	0.118	0.001
MSi	-0.247	0.969	0.117	0.001
MSa	0.596	-0.803	0.108	0.001
CSa	0.527	-0.850	0.097	0.001
FC	-0.917	0.400	0.032	0.006
FSa	0.921	-0.390	0.031	0.007
qD0	0.483	-0.876	0.019	0.049
CSi	-0.272	0.962	0.018	0.056
qD2	0.123	0.992	0.011	0.159
qD1	0.900	0.437	0.009	0.248

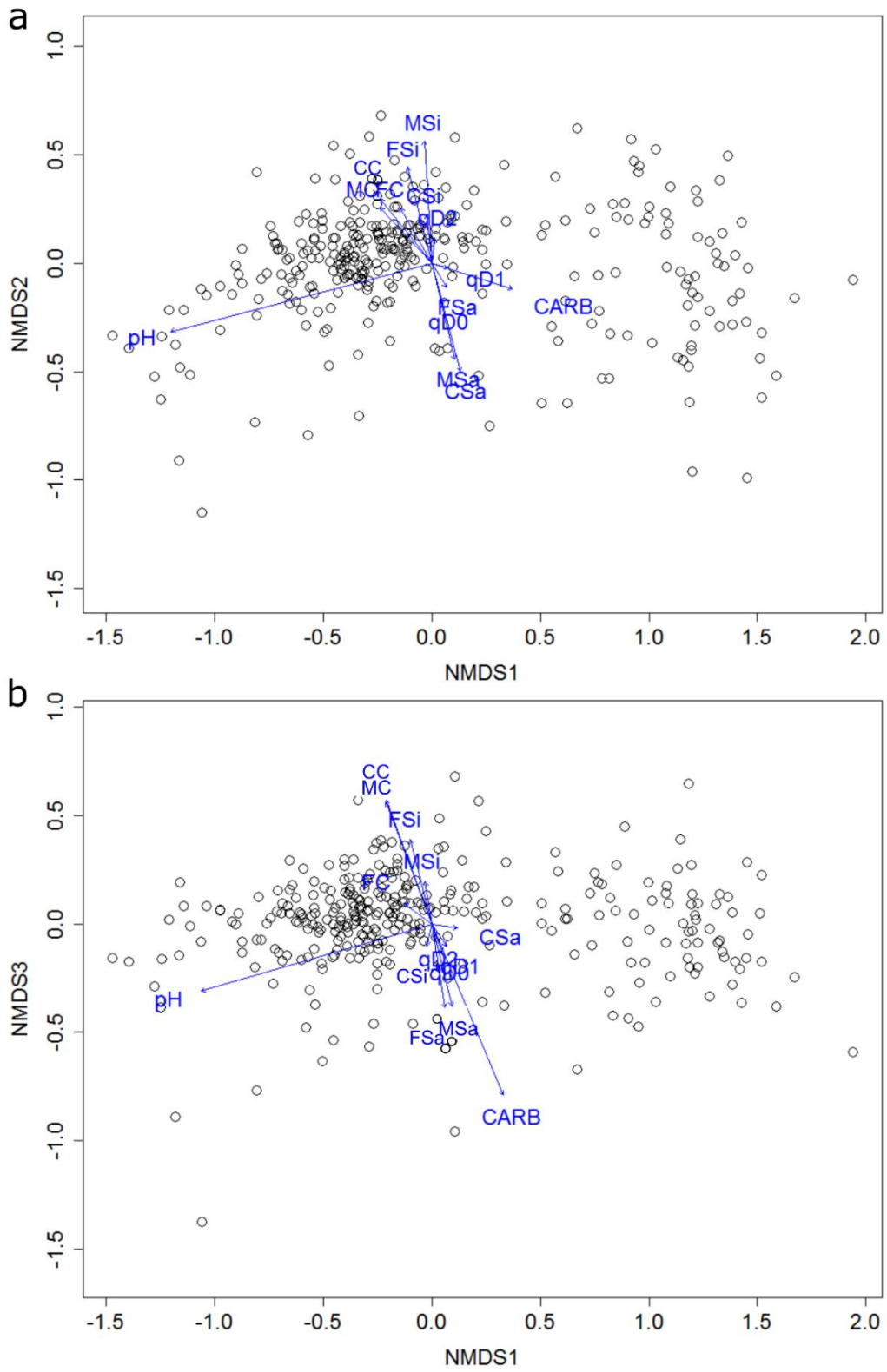


Fig. 6. The results of fitting environmental vectors onto the bacterial NMDS using 9 particle size classes, pH and carbon. Panel a shows the first and second axis of the ordination, panel b shows the first and third of the ordination. Variables are labelled as in Fig. S5.

Table 4. Envfit results for the bacterial ordination, with variables ordered by decreasing R^2 value.

Variable	NMDS1	NMDS2	NMDS3	R^2	Pr(>r)
pH	-0.931	-0.244	-0.271	0.709	0.001
CARB	0.381	-0.121	-0.916	0.402	0.001
CC	-0.318	0.395	0.862	0.239	0.001
MC	-0.330	0.357	0.874	0.222	0.001
FSi	-0.178	0.699	0.692	0.171	0.001
MSa	0.166	-0.709	-0.685	0.167	0.001
MSi	-0.058	0.929	0.365	0.157	0.001
CSa	0.256	-0.966	-0.045	0.113	0.001
FSa	0.145	-0.245	-0.958	0.088	0.001
FC	-0.463	0.815	0.350	0.042	0.006
CSi	-0.104	0.886	-0.451	0.027	0.034
qD0	0.211	-0.768	-0.604	0.026	0.035
qD1	0.533	-0.165	-0.830	0.009	0.424
qD2	0.103	0.823	-0.559	0.008	0.448

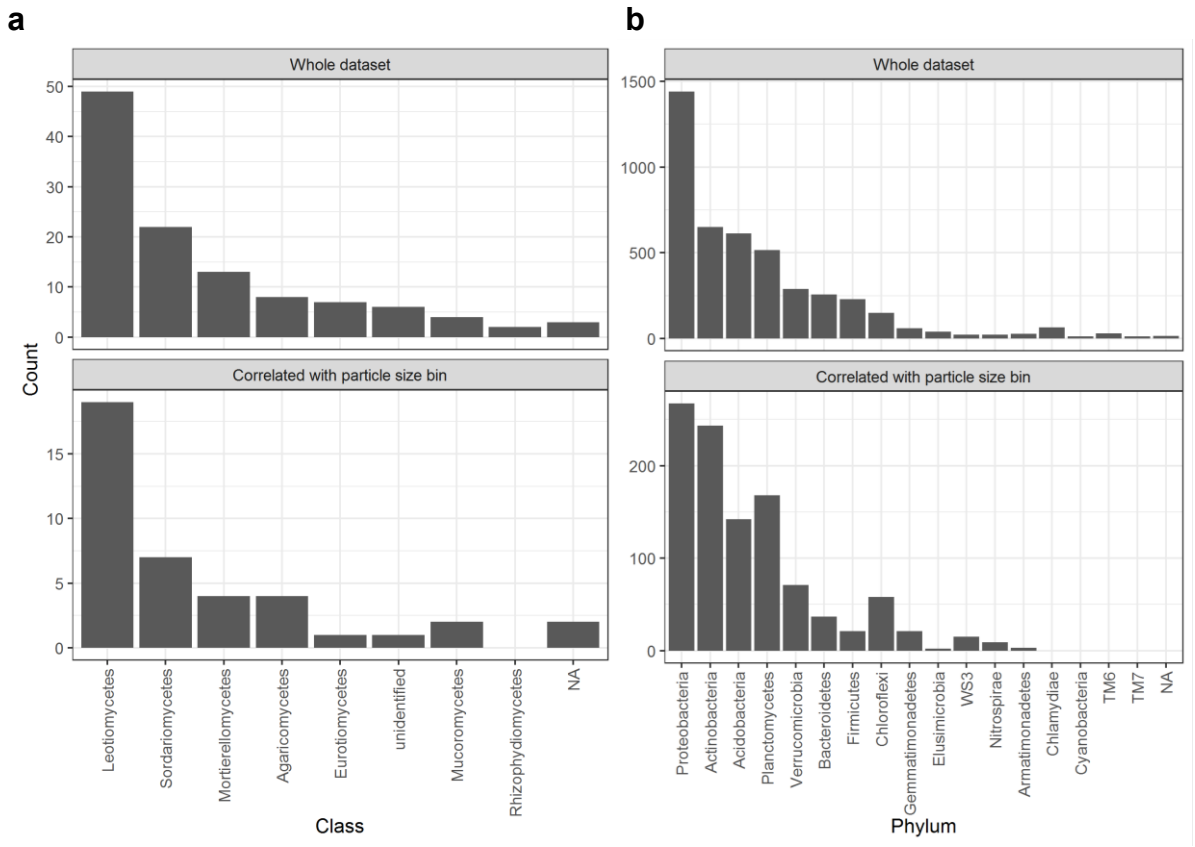


Figure 7. Relative compositions of common microbial taxa across whole dataset and those that are correlated with particle size bins. Panel a is the fungal classes and panel b is the bacterial phyla. The top panel for each is the composition in the common taxa (defined as appearing in >50% of sites for bacteria and >25% of sites for fungi) that are present in the entire dataset, and the bottom panel is the composition in the common taxa that are significantly correlated to any particle size bin.

APPENDIX F

Supporting information for Chapter 4

Supplementary methods: Structural Equation Modelling (SEM)

SEM and its role in estimating causal mechanisms

Identifying the mechanisms responsible for ecosystem change is a research imperative but a major analytical challenge. Experimental manipulations provide strong evidence of causation. However careful design is needed to ensure homogeneity of starting conditions across controls and treatments, which often means that manipulated areas are relatively small. This often means that only a limited number of treatment effects and levels can be replicated and randomised over a constrained area and time horizon relative to the realistic spatial and temporal scales at which phenomena play out across landscapes and ecosystems. Hence, the inferential strength of experimental results trade-off against their scale-dependent ecological relevance. An alternative is to tackle the challenge of estimating causal mechanisms head on by analysis of large-scale observations either with or without a temporal component. Typical approaches have relied on forms of multiple regression. These however, ignore and can indeed conceal the contribution of mediating variables in propagating effects along a mechanistic pathway. This is because in multiple regression, all the explanatory variables compete for fractions of the same variance in the response variable. For example, if precipitation results in conditions that favour particular plant traits and water repellency changes in response to incorporation of material from these plants into the soil then a more accurate analysis of the mechanisms involved in driving soil water repellency would include the mediating effect of plant species traits in conveying the climatic conditioning effect of rainfall. In a multiple regression, strong positive correlation between rainfall and plant traits would lead to one or at worst neither variable being considered 'significant'. An advantage of SEM is that it explicitly allows the user to estimate parameters for the sequential effects of one variable on another and then on another. In this respect SEM allows for more realistic

representations of possible causal pathways. Clearly, if considering mediating relationships where A drives C drives D, there is a much larger set of possible paired relationships between any two of a total set of variables than in a multiple regression. Partly for this reason but mainly because we have prior knowledge, our initial SEM is set up to test only those relationships that are consistent with our causal model. After fitting the model, diagnostic testing alerts us to additional possible relationships that we might consider including in the model. Because this test detects correlated residuals it can also highlight the possibility of missing variables and therefore components of the mechanistic pathway that we have not measured. For example, having fitted some mediating predictor (A) to a response (B) we find that the residuals from the fitted predictor correlate with the residuals from the fitted response. In this case a more complete, and accurate, representation of the causal mechanism would need to consider another variable or variables conveying a causal influence on both A and B. This message from the model would also be likely to arise if we tried using the model for predicting some new cases because a wrong prediction would be likely to occur where the missing variable exerted an effect. It is also worth noting that an experimental set up would be very unlikely to have included replicated, randomised and orthogonal main effects and interactions for the large number of variables that can be included in a realistic SEM, thus reducing the understanding gained about causal mechanisms. Moreover, unless experiments were also replicated along gradients that accidentally coincided with the missing variable above, we would gain no insight into its operation or even existence and so our experimental result would lack realism and transferability despite the strong inference we could draw about the effects tested in the experiment. Therefore, in terms of causation we would argue that experimentation is not a clear analytical winner nor a gold standard to aim at if a full and realistic understanding of mechanisms is to be achieved. Both SEM and experimentation work together to build causal understanding. The advantage of the SEM approach is precisely that it encourages us to think about mechanisms and to test these claims against new cases by modelling them. This goes beyond extracting associations (Grace, 2006, 2008).

Statistical method

Piecewise SEM involves fitting multiple regression models together to form one large overall model. The overall model is evaluated according to whether it is missing informative paths, which can then be used to compare models. This comparison based on missing paths is known as Shipley's d-separation test (Shipley, 2000, 2009). Shipley's d-separation test was performed by adding each missing path into the model and examining the posterior distribution of the parameter estimate for the missing path. If the posterior distribution of the path substantially crosses zero then the path is considered less important and is likely to be dropped from the model. To systematically establish which paths would be dropped from the model for each path the credible interval that crossed zero was used to create a p-value (Clough, 2012). The p-values from all the missing paths within the overall model were combined using Fisher's C, and then this statistic used to construct an AICc score for each overall model (Shipley, 2013). The best overall model was selected as the one with the lowest AICc score.

The parameter estimates were used to evaluate the relative impact of the predictors on the response variable. The direct impact is given by the parameter estimate in the tables below, and the indirect impact found by adding all the indirect paths within the causal model from the predictor to response together. The total impact is calculated by adding together the direct and indirect impact.

Proposed causative model

The causal model used within the SEM analysis was established before beginning the analysis, based on expert knowledge and the literature. SEM requires that all causal links be unidirectional and there be no feedback loops. Where there was a potential influence in both directions the link that acts on the shortest timescale was chosen. For example, carbon causes water content by influencing the water holding capacity but water also causes carbon content by changing biomass production. As biomass production takes longer to occur than water holding capacity the directionality of the link was chosen from carbon to water content.

For climatic variables precipitation and drought were chosen to represent the rainfall overall and also the level of variability in rainfall as both drought and water inputs in

general are known to be important in establishing soil water repellency (Goebel et al., 2011). Elevation was included as a predictor due to the strong altitudinal gradient in soils across Wales, in particular to check if the changes in temperature regime (both changes in the average and range with altitude) may be affecting repellency.

Soil carbon, water content and pH have all been previously shown to strongly influence soil water repellency so were included in the model (Amer et al., 2017; Diehl, 2013; Doerr et al., 2000; Lebron et al., 2012; Mainwaring et al., 2013; Mirbabaei et al., 2013; Zheng et al., 2016). Bulk density was considered as another possible predictor of repellency and was tested to see if it explained any further variation in soil water repellency once the other predictors were accounted for.

Previous work in the literature had suggested that plant drought stress tolerance may be positively related to repellency, and plant productivity inversely (Müller et al., 2014; Robinson et al., 2010; Verboom & Pate, 2006). We had sufficient data to include either the Ellenberg fertility score as a proxy for productivity, or the Grime stress tolerance score as a proxy for stress tolerance. We posited that soil microbial communities would also be important to soil water repellency, based on literature evidence that microbial communities both create and destroy repellent organic material (Achtenhagen et al., 2015; Chau et al., 2012; Li et al., 2018; Schaumann et al., 2007). The bacterial community composition was represented by the NMDS axis score. Ordination scores are difficult to compare across studies so we chose to represent the fungal community by the proportion of fungi that fall into different trophic modes as identified by FUNGuild (Nguyen et al., 2016). Fungal community composition was assumed to be responding to changes in bacterial community composition rather than vice versa due to the differences in their life history strategies. Microbial community composition were modelled as responding to all other variables (other than repellency), as their relatively quick lifespans and adaptability to environmental change should mean that a change in the environment causes a change in microbial community composition before a change in microbial communities change the environment. All these biological variables were tested to see if they explained any more variation once changes in climate and soil physicochemical properties were

accounted for, and a p value of less than 0.05 for the link between the variable and repellency taken as indicative that they were important to repellency.

Model fitting

Of the two SEMs, 1326 plots had sufficient data to be included in the SEM without microbial data and 425 had sufficient data to be included in the microbial SEM. Over the 300 squares from the full survey many did not have five plots surveyed due to access restrictions, and other plots that were surveyed had ground too rocky for soil sampling or insufficient plant data to construct the Grime stress tolerance score. For the SEM without microbial data only the plant community from the central 2 m-by-2 m of the plot was used to construct community indices due to changes in the vegetation survey procedure in the latter two years of the survey. Soil carbon and water repellency were log-transformed before analysis. All data were standardised so that 0 was the minimum value and 1 was the average of the top 2% of values. Square identity was used as a random effect, year of sample collection was found to have an nonsignificant impact on model fit when included as a random effect so was excluded from analysis. Bulk density, Grime Stress and Ellenberg Fertility were all initially excluded from the model, then tested to see if they had a clear impact on the residuals of the fully specified model for the four years. The residuals of water content, pH and water repellency were all tested to see if they were impacted by bulk density. Only the residuals of water repellency were tested against Grime Stress and Ellenberg Fertility. Of these tests only residuals(repellency) ~ Grime Stress was significant ($p = 0.0124$), hence Grime Stress was included in the final model while bulk density and Ellenberg Fertility were not. Microbial community composition was represented by proportion of symbiotrophic fungi and bacteria NMDS scores from axis 1.

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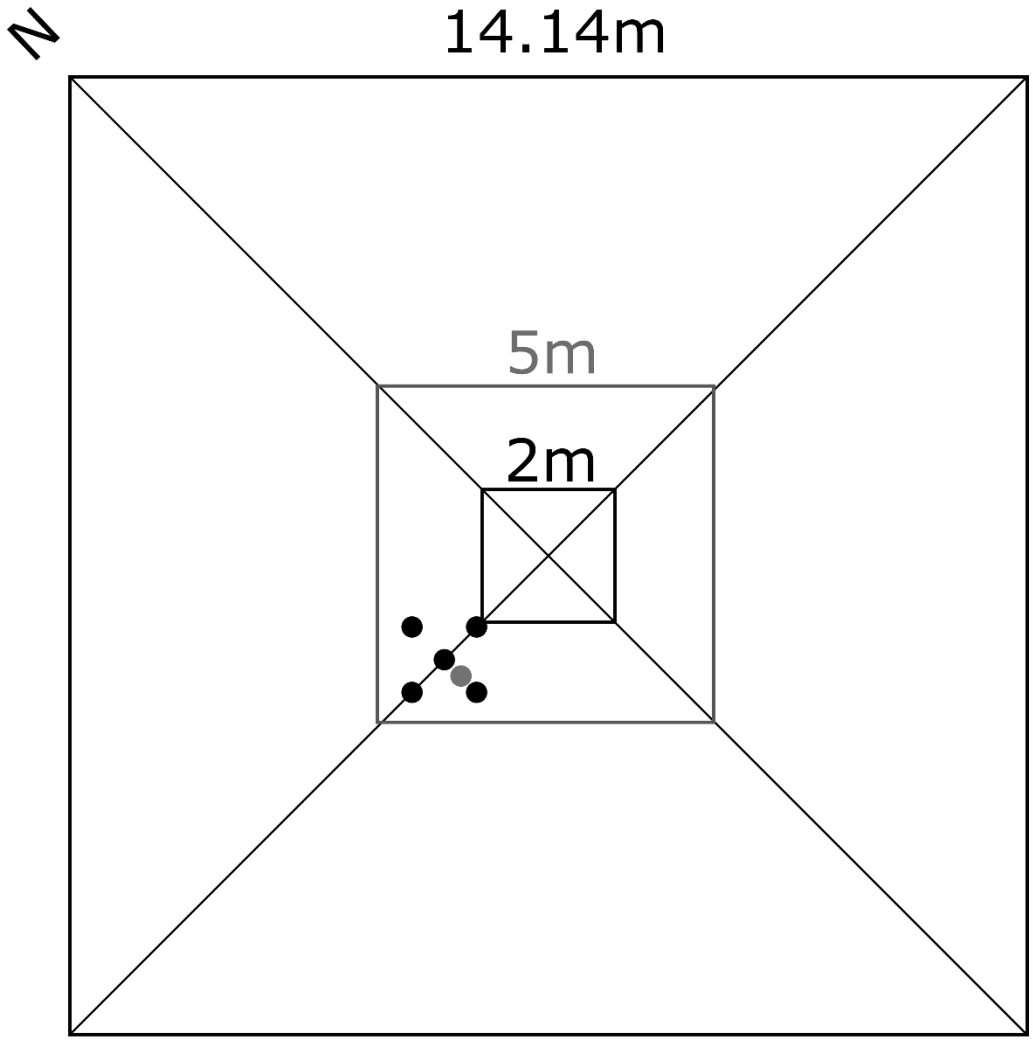
Figure 1: Plot layout



- Soil physicochemical sample
- Locations of gauge samples for DNA analysis

Table 1: Repellency of different habitats. The number of samples in each repellency (WDPT) rating, by broad habitat.

WDPT rating	Acid Grassland	Arable	Bog	Bracken	Broadleaved Woodland	Coniferous Woodland	Dwarf Shrub Heath	Fen, Marsh, Swamp	Improved Grassland	Neutral Grassland	Other	Total
None (0-5s)	3	30	2	2	13	2	1	7	29	20	4	113 (8.2%)
Slight (6-60s)	15	6	4	4	26	13	8	12	146	84	15	333 (24.1%)
Moderate (61-600s)	51	1	34	20	28	28	44	14	135	124	20	499 (36.1%)
Severe (601-3600s)	74	0	28	11	15	14	25	15	41	61	11	295 (21.3%)
Extreme (>3600s)	44	0	22	10	7	14	10	12	5	11	8	143 (10.3%)
Total	187	37	90	47	89	71	88	60	356	300	56	1383

Table 2: Bayesian SEM coefficients full dataset

Response	Marg. R-sq	Cond. R-sq	Predictor	Estimate	Est. Error	lower 95% C.I.	upper 95% C.I.	Eff. Sample	Rhat	p-value from C.I.
Repellency	0.29 (±0.02)	0.42 (±0.02)	Intercept	0.412	0.044	0.326	0.499	14070	1.000	<0.0001
Repellency			Plant stress tolerance	0.261	0.038	0.188	0.336	16833	1.000	<0.0001
Repellency			pH	-0.145	0.039	-0.220	-0.068	16883	1.000	<0.0001
Repellency			Water	-0.165	0.034	-0.231	-0.099	20000	1.000	<0.0001
Repellency			Carbon	0.348	0.039	0.272	0.424	17761	1.000	<0.0001
Repellency			Precipitation	-0.153	0.057	-0.264	-0.041	11324	1.001	0.0081
Repellency			Drought	-0.088	0.047	-0.181	0.003	10197	1.001	0.0588
Plant stress tolerance	0.64 (±0.01)	0.74 (±0.01)	Intercept	0.276	0.033	0.212	0.339	9193	1.001	<0.0001
Plant stress tolerance			pH	-0.317	0.027	-0.369	-0.265	14201	1.000	<0.0001
Plant stress tolerance			Carbon	0.210	0.026	0.159	0.260	14616	1.000	<0.0001
Plant stress tolerance			Precipitation	0.354	0.046	0.263	0.444	7002	1.001	<0.0001
Plant stress tolerance			Elevation	0.239	0.030	0.179	0.298	8479	1.000	<0.0001
Plant stress tolerance			Drought	0.121	0.037	0.047	0.194	7106	1.001	0.0011
pH	0.44 (±0.01)	0.65 (±0.01)	Intercept	0.552	0.024	0.504	0.599	10564	1.000	<0.0001
pH			Water	0.168	0.024	0.120	0.215	20000	1.000	<0.0001
pH			Carbon	-0.269	0.028	-0.323	-0.215	16713	1.000	<0.0001
pH			Elevation	-0.306	0.028	-0.361	-0.250	9524	1.001	<0.0001
pH			Drought	0.168	0.032	0.105	0.231	8670	1.000	<0.0001

Water	0.32 (±0.02)	0.57 (±0.01)	Intercept	0.263	0.034	0.197	0.330	6746	1.001	<0.0001
Water			Carbon	0.410	0.027	0.356	0.463	13126	1.001	<0.0001
Water			Precipitation	0.078	0.051	-0.022	0.179	6140	1.001	0.1256
Water			Drought	-0.094	0.046	-0.185	-0.005	6164	1.001	0.0381
Carbon	0.46 (±0.02)	0.66 (±0.01)	Intercept	0.457	0.030	0.397	0.517	5850	1.001	<0.0001
Carbon			Precipitation	0.155	0.056	0.044	0.262	5796	1.002	0.0056
Carbon			Elevation	0.379	0.033	0.316	0.445	6782	1.001	<0.0001
Carbon			Drought	-0.204	0.045	-0.292	-0.117	5679	1.001	<0.0001

Table 3: Bayesian SEM coefficients microbial data

Response	Marg. R-sq	Cond. R-sq	Predictor	Estimate	Est. Error	lower 95% C.I.	upper 95% C.I.	Eff. Sample	Rhat	p-value from C.I.
Repellency	0.38 (±0.03)	0.52 (±0.03)	Intercept	0.314	0.034	0.249	0.382	20000	1.000	<0.0001
Repellency			Plant stress tolerance	0.187	0.067	0.056	0.322	20000	1.000	0.0045
Repellency			Water	-0.322	0.061	-0.441	-0.202	20000	1.000	<0.0001
Repellency			Carbon	0.316	0.069	0.178	0.452	20000	1.000	<0.0001
Repellency			Symbiotroph	-0.203	0.058	-0.315	-0.088	20000	1.000	0.0004
Repellency			Bacteria	0.295	0.067	0.161	0.427	17587	1.000	<0.0001
Symbiotroph	0.34 (±0.05)	0.34 (±0.05)	Intercept	0.167	0.027	0.114	0.220	20000	1.000	<0.0001
Symbiotroph			Water	-0.187	0.047	-0.278	-0.097	20000	1.000	0.0002
Symbiotroph			Plant stress tolerance	-0.116	0.054	-0.223	-0.011	18126	1.000	0.0320
Symbiotroph			Bacteria	0.346	0.049	0.251	0.443	18720	1.000	<0.0001
Bacteria	0.86 (±0.01)	0.89 (±0.01)	Intercept	0.504	0.029	0.447	0.560	20000	1.000	<0.0001
Bacteria			Plant stress tolerance	0.252	0.035	0.181	0.320	20000	1.000	<0.0001
Bacteria			pH	-0.675	0.033	-0.740	-0.610	20000	1.000	<0.0001
Bacteria			Carbon	0.235	0.031	0.174	0.295	20000	1.000	<0.0001
Bacteria			Elevation	0.072	0.031	0.011	0.133	20000	1.000	0.0216
Plant stress tolerance	0.67 (±0.02)	0.78 (±0.01)	Intercept	0.238	0.050	0.139	0.336	9892	1.000	<0.0001
Plant stress tolerance			pH	-0.253	0.044	-0.339	-0.167	14193	1.000	<0.0001

Plant stress tolerance			Carbon	0.244	0.042	0.160	0.325	13502	1.000	<0.0001
Plant stress tolerance			Precipitation	0.344	0.066	0.216	0.476	7457	1.000	<0.0001
Plant stress tolerance			Elevation	0.239	0.047	0.147	0.330	8916	1.000	<0.0001
Plant stress tolerance			Drought	0.137	0.056	0.025	0.246	7808	1.000	0.0156
pH	0.48 (±0.03)	0.69 (±0.02)	Intercept	0.556	0.042	0.474	0.638	11435	1.000	<0.0001
pH			Water	0.127	0.048	0.036	0.221	18127	1.000	0.0070
pH			Carbon	-0.264	0.048	-0.357	-0.172	17225	1.000	<0.0001
pH			Elevation	-0.324	0.046	-0.415	-0.234	9913	1.001	<0.0001
pH			Drought	0.164	0.052	0.062	0.268	10497	1.000	0.0014
Water	0.32 (±0.04)	0.60 (±0.03)	Intercept	0.287	0.023	0.243	0.331	20000	1.000	<0.0001
Water			Carbon	0.384	0.043	0.299	0.469	20000	1.000	<0.0001
Water			Precipitation	0.117	0.052	0.015	0.221	17594	1.000	0.0265
Carbon	0.50 (±0.03)	0.68 (±0.02)	Intercept	0.418	0.046	0.328	0.509	9261	1.000	<0.0001
Carbon			Precipitation	0.191	0.079	0.033	0.347	9040	1.000	0.0169
Carbon			Elevation	0.364	0.052	0.262	0.467	9979	1.000	<0.0001
Carbon			Drought	-0.210	0.067	-0.341	-0.080	9319	1.000	0.0018

Table 4: Impact of habitat on SWR residuals for full dataset (Tukey HSD)

Habitat comparison	diff	lower	upper	p adj
Arable and horticultural-Acid Grassland	-1.766	-2.727	-0.805	0.000
Bog-Acid Grassland	0.089	-0.557	0.736	1.000
Bracken-Acid Grassland	0.188	-0.686	1.063	1.000
Broadleaved, mixed and yew woodland-Acid Grassland	-0.542	-1.217	0.132	0.244
Coniferous Woodland-Acid Grassland	-0.306	-1.031	0.420	0.945
Dwarf Shrub Heath-Acid Grassland	-0.850	-1.505	-0.196	0.002
Fen, Marsh and Swamp-Acid Grassland	-0.184	-0.995	0.628	0.999
Improved Grassland-Acid Grassland	-0.070	-0.514	0.374	1.000
Neutral Grassland-Acid Grassland	0.059	-0.403	0.522	1.000
Bog-Arable and horticultural	1.855	0.814	2.896	0.000
Bracken-Arable and horticultural	1.955	0.759	3.150	0.000
Broadleaved, mixed and yew woodland-Arable and horticultural	1.224	0.165	2.282	0.010
Coniferous Woodland-Arable and horticultural	1.460	0.369	2.552	0.001
Dwarf Shrub Heath-Arable and horticultural	0.916	-0.130	1.962	0.146
Fen, Marsh and Swamp-Arable and horticultural	1.583	0.432	2.733	0.001
Improved Grassland-Arable and horticultural	1.697	0.768	2.625	0.000
Neutral Grassland-Arable and horticultural	1.826	0.888	2.763	0.000
Bracken-Bog	0.099	-0.862	1.061	1.000
Broadleaved, mixed and yew woodland-Bog	-0.631	-1.415	0.153	0.242
Coniferous Woodland-Bog	-0.395	-1.223	0.434	0.888
Dwarf Shrub Heath-Bog	-0.939	-1.706	-0.172	0.004
Fen, Marsh and Swamp-Bog	-0.273	-1.177	0.632	0.995
Improved Grassland-Bog	-0.159	-0.756	0.439	0.998
Neutral Grassland-Bog	-0.030	-0.641	0.582	1.000
Broadleaved, mixed and yew woodland-Bracken	-0.731	-1.711	0.250	0.350
Coniferous Woodland-Bracken	-0.494	-1.510	0.522	0.875
Dwarf Shrub Heath-Bracken	-1.039	-2.005	-0.072	0.024
Fen, Marsh and Swamp-Bracken	-0.372	-1.451	0.707	0.985
Improved Grassland-Bracken	-0.258	-1.097	0.581	0.994
Neutral Grassland-Bracken	-0.129	-0.978	0.720	1.000
Coniferous Woodland-Broadleaved, mixed and yew woodland	0.237	-0.614	1.087	0.997
Dwarf Shrub Heath-Broadleaved, mixed and yew woodland	-0.308	-1.098	0.483	0.967
Fen, Marsh and Swamp-Broadleaved, mixed and yew woodland	0.359	-0.566	1.284	0.968
Improved Grassland-Broadleaved, mixed and yew woodland	0.473	-0.155	1.100	0.335
Neutral Grassland-Broadleaved, mixed and yew woodland	0.602	-0.039	1.243	0.087
Dwarf Shrub Heath-Coniferous Woodland	-0.544	-1.379	0.290	0.551

Table 5: Impact of habitat on SWR residuals for microbial subset (Tukey HSD)

Habitat comparison	diff	lower	upper	p adj
Arable and horticultural-Acid Grassland	-1.436	-2.850	-0.023	0.043
Bog-Acid Grassland	-0.574	-1.749	0.602	0.845
Broadleaved, mixed and yew woodland-Acid Grassland	-0.218	-1.357	0.920	1.000
Coniferous Woodland-Acid Grassland	0.090	-1.038	1.217	1.000
Dwarf Shrub Heath-Acid Grassland	-0.077	-1.728	1.573	1.000
Fen, Marsh and Swamp-Acid Grassland	-0.155	-1.604	1.295	1.000
Improved Grassland-Acid Grassland	0.079	-0.763	0.922	1.000
Neutral Grassland-Acid Grassland	0.162	-0.720	1.045	1.000
Bog-Arable and horticultural	0.863	-0.651	2.376	0.696
Broadleaved, mixed and yew woodland-Arable and horticultural	1.218	-0.267	2.702	0.207
Coniferous Woodland-Arable and horticultural	1.526	0.050	3.002	0.037
Dwarf Shrub Heath-Arable and horticultural	1.359	-0.547	3.265	0.392
Fen, Marsh and Swamp-Arable and horticultural	1.282	-0.453	3.016	0.341
Improved Grassland-Arable and horticultural	1.516	0.244	2.788	0.007
Neutral Grassland-Arable and horticultural	1.599	0.300	2.898	0.005
Broadleaved, mixed and yew woodland-Bog	0.355	-0.905	1.616	0.994
Coniferous Woodland-Bog	0.663	-0.587	1.914	0.774
Dwarf Shrub Heath-Bog	0.496	-1.241	2.233	0.993
Fen, Marsh and Swamp-Bog	0.419	-1.128	1.966	0.995
Improved Grassland-Bog	0.653	-0.349	1.654	0.521
Neutral Grassland-Bog	0.736	-0.299	1.772	0.396
Coniferous Woodland-Broadleaved, mixed and yew woodland	0.308	-0.907	1.524	0.997
Dwarf Shrub Heath-Broadleaved, mixed and yew woodland	0.141	-1.571	1.853	1.000
Fen, Marsh and Swamp-Broadleaved, mixed and yew woodland	0.064	-1.455	1.583	1.000
Improved Grassland-Broadleaved, mixed and yew woodland	0.298	-0.659	1.255	0.988
Neutral Grassland-Broadleaved, mixed and yew woodland	0.381	-0.612	1.374	0.957
Dwarf Shrub Heath-Coniferous Woodland	-0.167	-1.871	1.537	1.000
Fen, Marsh and Swamp-Coniferous Woodland	-0.244	-1.755	1.267	1.000
Improved Grassland-Coniferous Woodland	-0.010	-0.954	0.934	1.000
Neutral Grassland-Coniferous Woodland	0.073	-0.907	1.053	1.000
Fen, Marsh and Swamp-Dwarf Shrub Heath	-0.077	-2.010	1.855	1.000
Improved Grassland-Dwarf Shrub Heath	0.157	-1.374	1.688	1.000
Neutral Grassland-Dwarf Shrub Heath	0.240	-1.314	1.793	1.000
Improved Grassland-Fen, Marsh and Swamp	0.234	-1.078	1.546	1.000
Neutral Grassland-Fen, Marsh and Swamp	0.317	-1.021	1.655	0.998
Neutral Grassland-Improved Grassland	0.083	-0.549	0.715	1.000

APPENDIX G

Supplementary information for Chapter 5

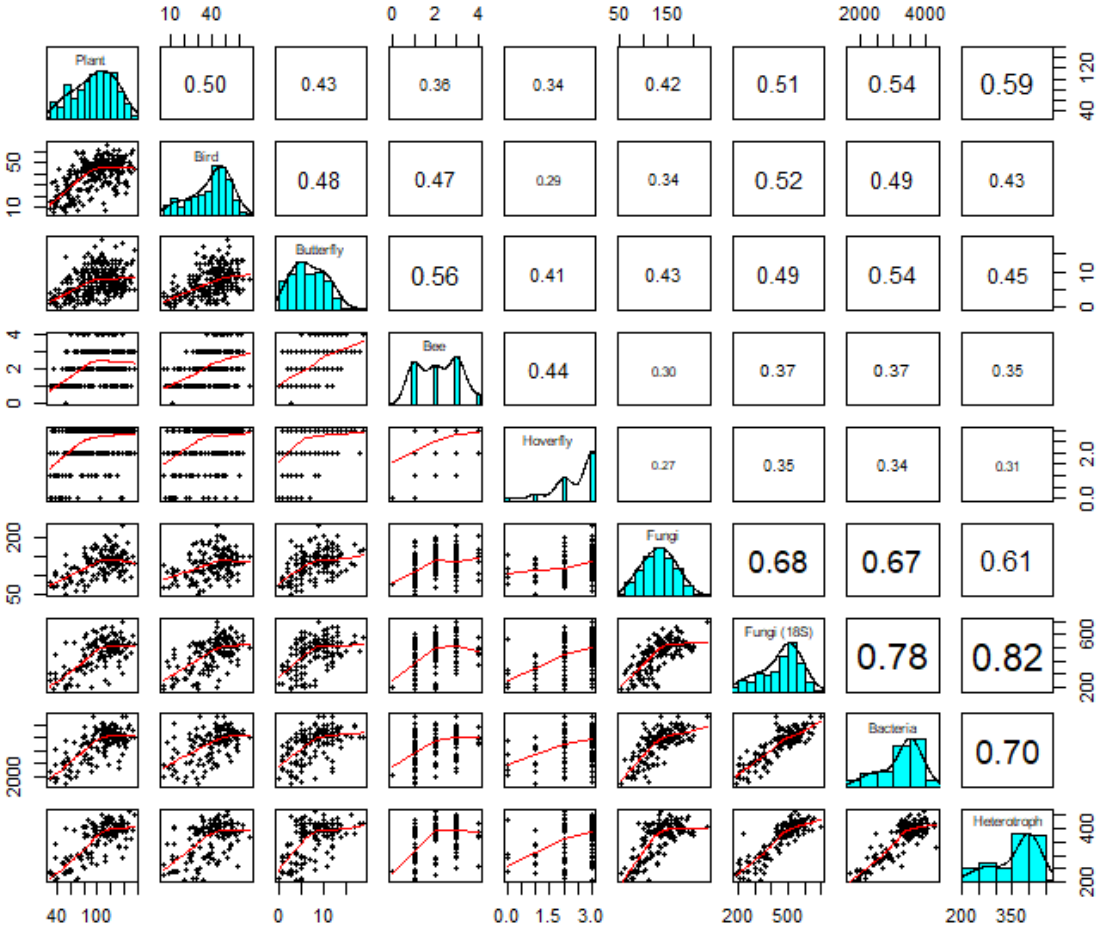


Figure 1: A pairs panel plot of the taxonomic group richness for plants, birds, butterflies, bee groups, hoverfly groups, fungi (ITS), fungi (18S), bacteria and heterotrophs. The diagonal contains the names of the variables, their histogram and density plot. The upper triangle shows the Spearman rank correlation proportional to their size and the lower triangle the variables plotted against each other with loess fit line. Microbial richness values are mean for the sampling square. In total, there are 298 sampling squares with aboveground information including 148 with soil microbial information. Plotted using the psych package in R.

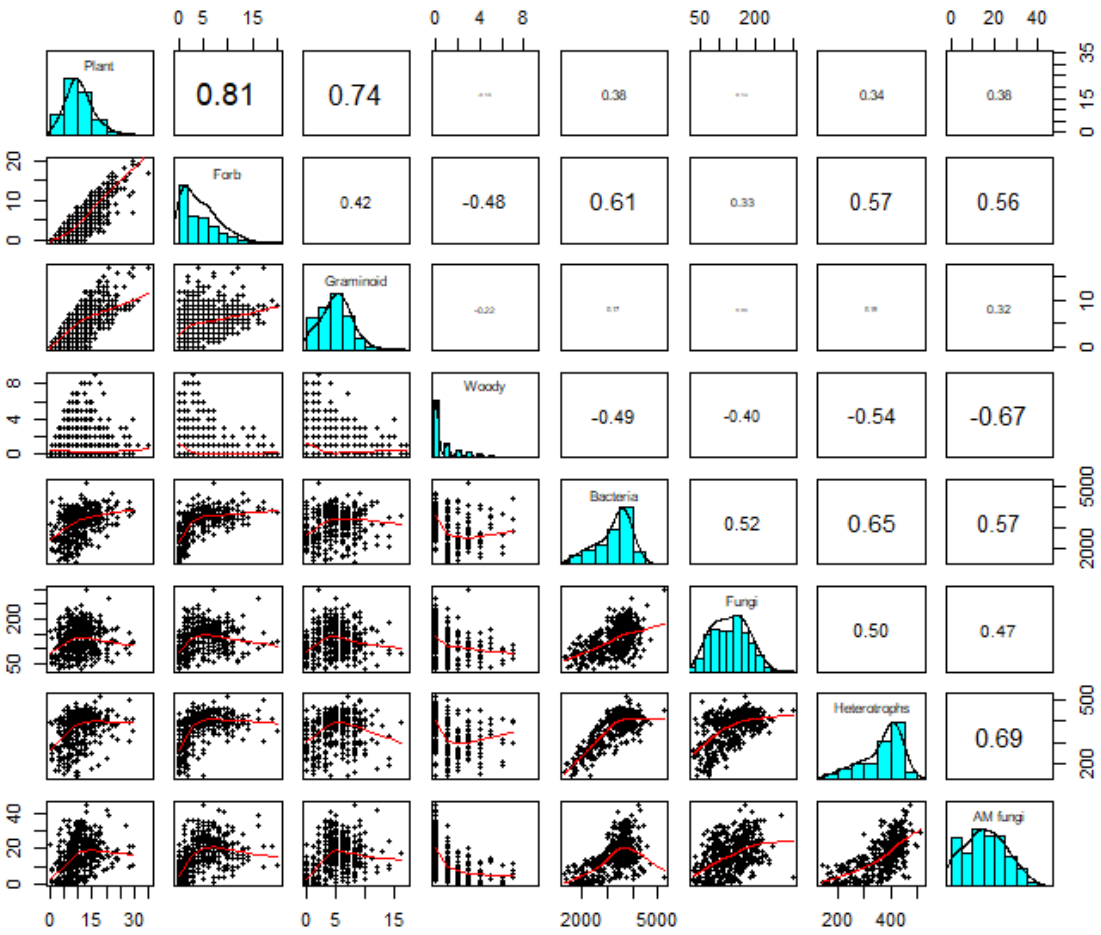


Figure 2: A pairs panel plot of the taxonomic group richness for all vascular plants, forb plants, graminoid plants, woody plants, bacteria, fungi (ITS), heterotrophic protists and AM fungi. The diagonal contains the names of the variables, their histogram and density plot. The upper triangle shows the Spearman rank correlation proportional to their size and the lower triangle the variables plotted against each other with loess fit line. Microbial richness values are mean for the sampling square. In total there are 298 sampling squares with aboveground information including 148 with soil microbial information.

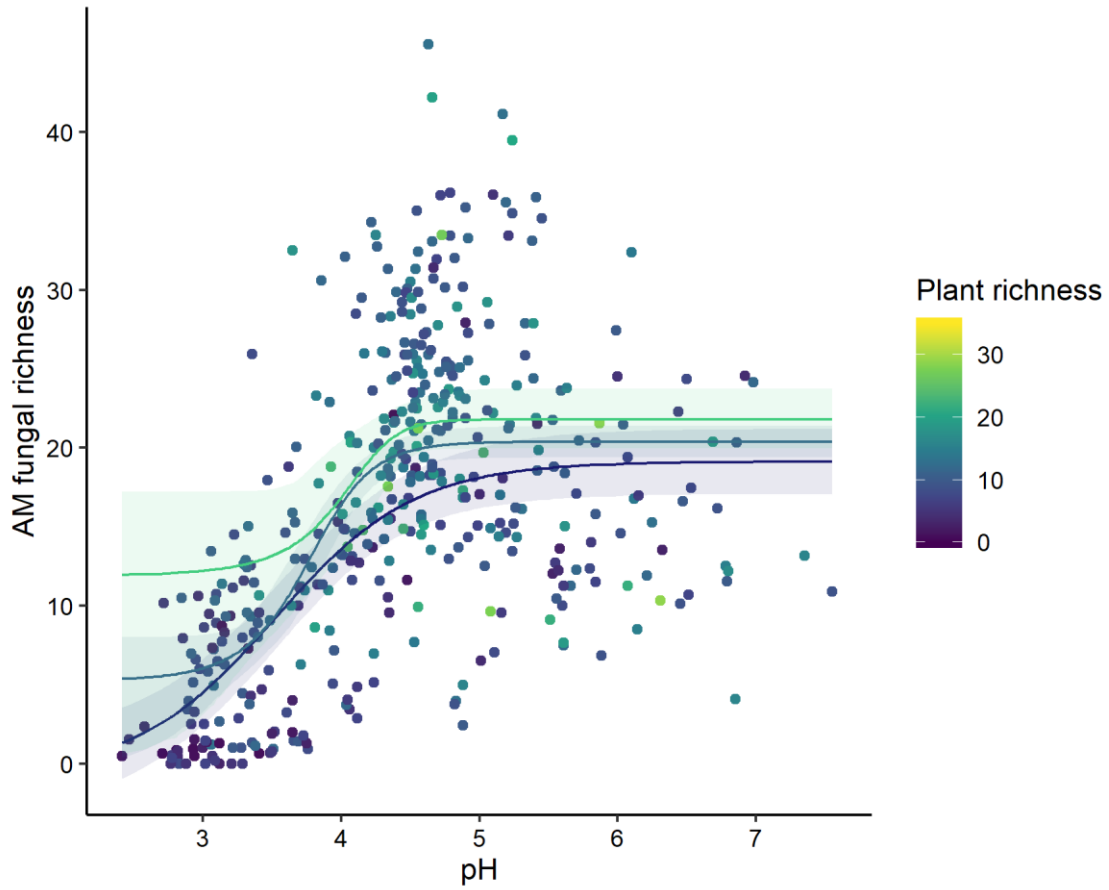


Figure 3: Plant richness positively influences AM fungal richness at all pH values, with overall AM fungal richness being higher at pH values greater than 4 (n = 429).

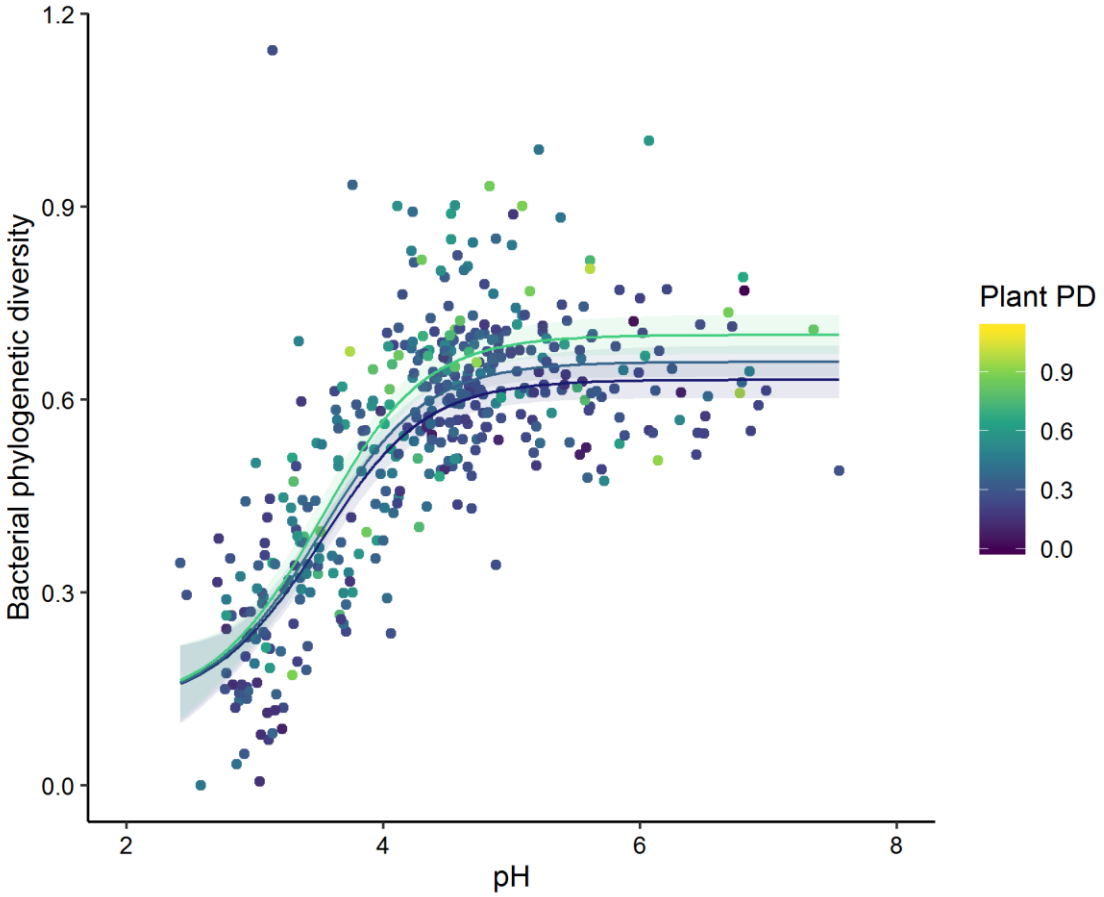


Figure 4: Plant phylogenetic diversity has a positive effect on bacterial phylogenetic diversity at pH values greater than 4 (n = 431).

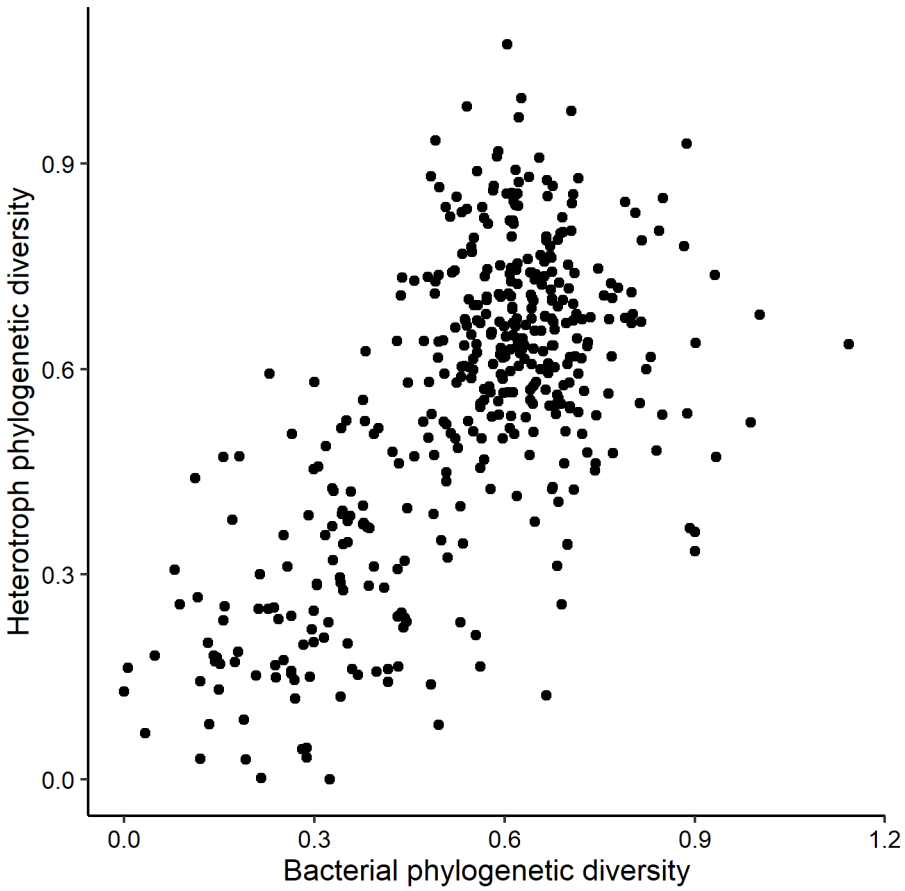


Figure 5: Correlation between heterotrophic phylogenetic diversity and bacterial phylogenetic diversity (n = 423).

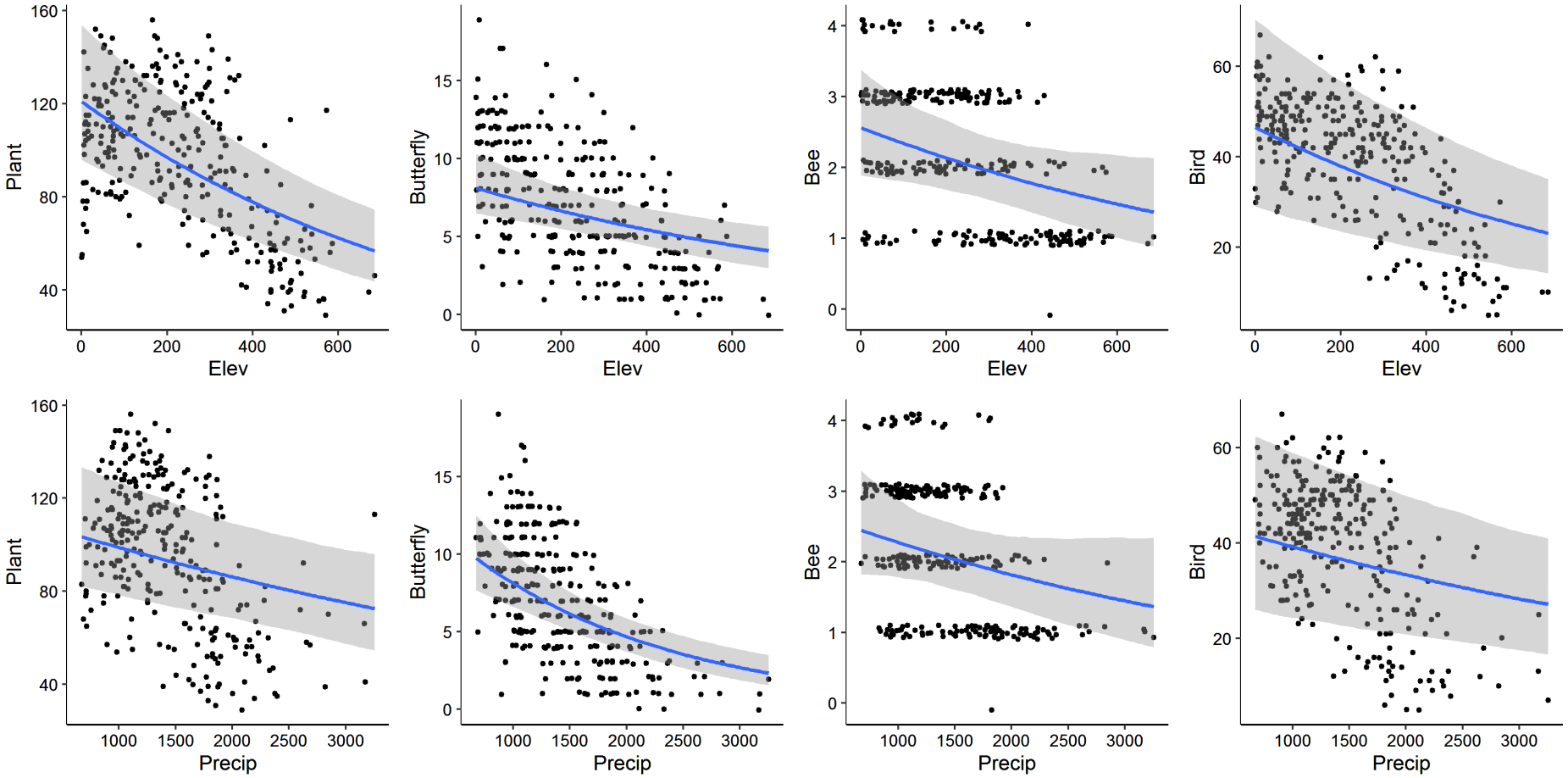


Figure 6: Predicted impacts of elevation (m a.s.l) and precipitation (mm y^{-1}) on aboveground diversity in model with elevation, precipitation and plant diversity as predictors and year and vice county as group-level effects. A vice-county is a geographical division of the British Isles used for the purposes of biological recording and other scientific data-gathering.

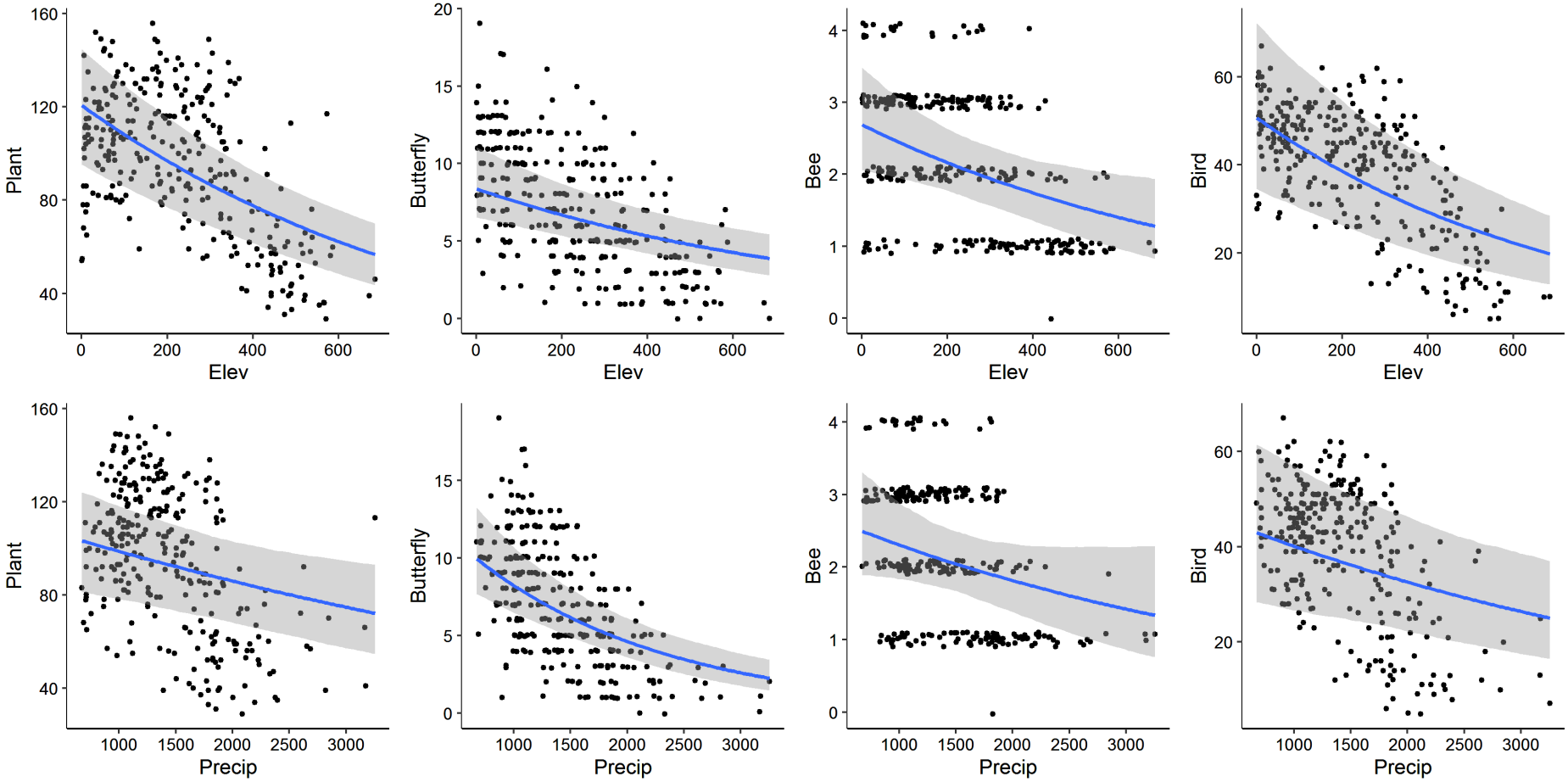


Figure 7: Predicted impacts of elevation (m a.s.l) and precipitation (mm y^{-1}) on aboveground diversity in model with elevation, precipitation as predictors and year and vice county as group-level effects. A vice-county is a geographical division of the British Isles used for the purposes of biological recording and other scientific data-gathering.

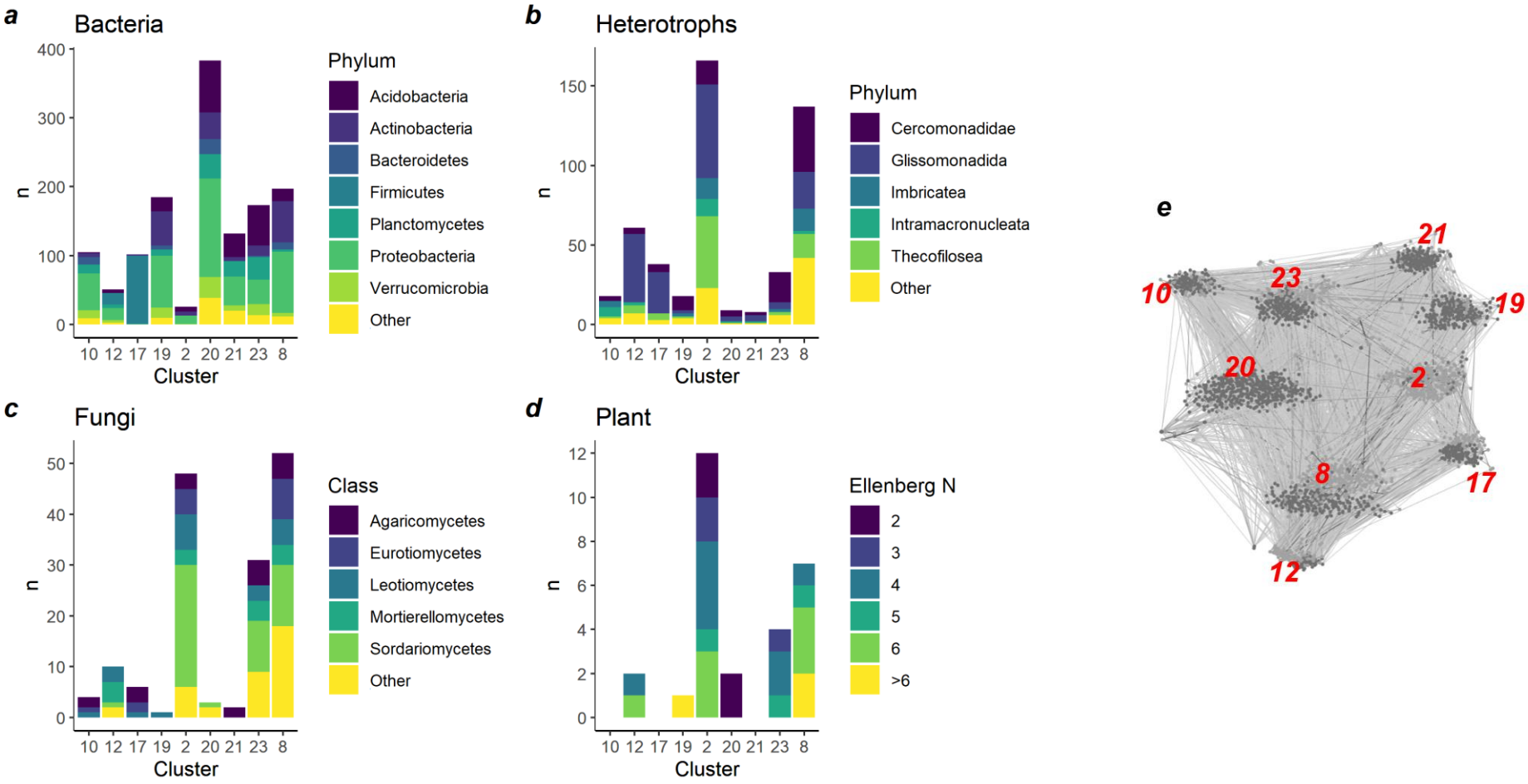


Figure 8: The breakdown of the network cluster membership by phylum for bacteria (a), by group for heterotrophs (b), by class for fungi (c), and by Ellenberg N score for plants (d). Only clusters with over 100 members are shown; the other 14 clusters all had less than 20 members each. Panel e shows the location of each of these large clusters on the network diagram in Figure 5.11.

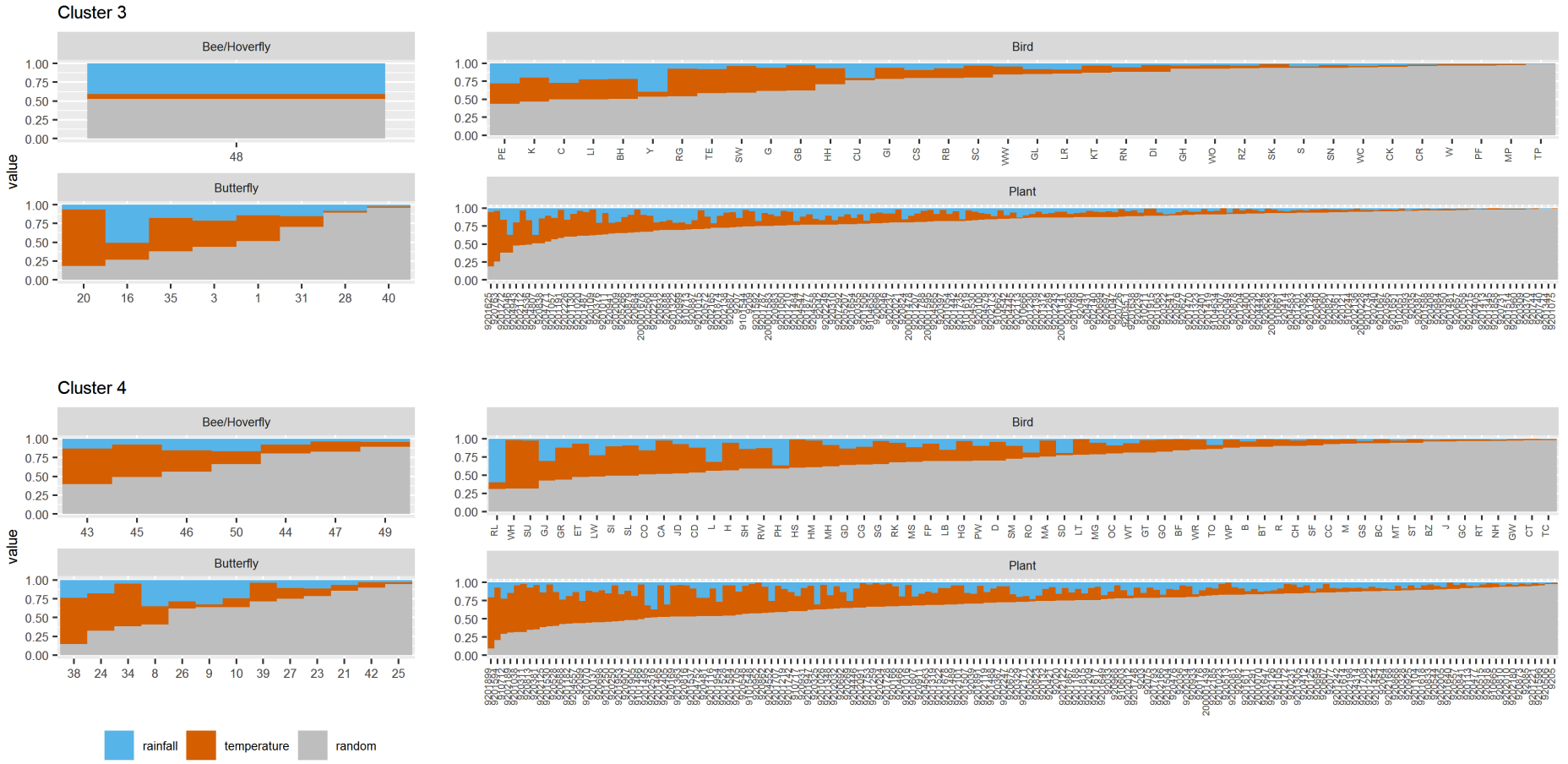


Figure 9: The variance partitioning for every bee, bird, butterfly and plant species split by cluster. Proportion of variance explained by rainfall is shown in blue, proportion of variance explained by temperature is shown in orange and proportion of variance explained by the random component which incorporates spatial variation is shown in grey.

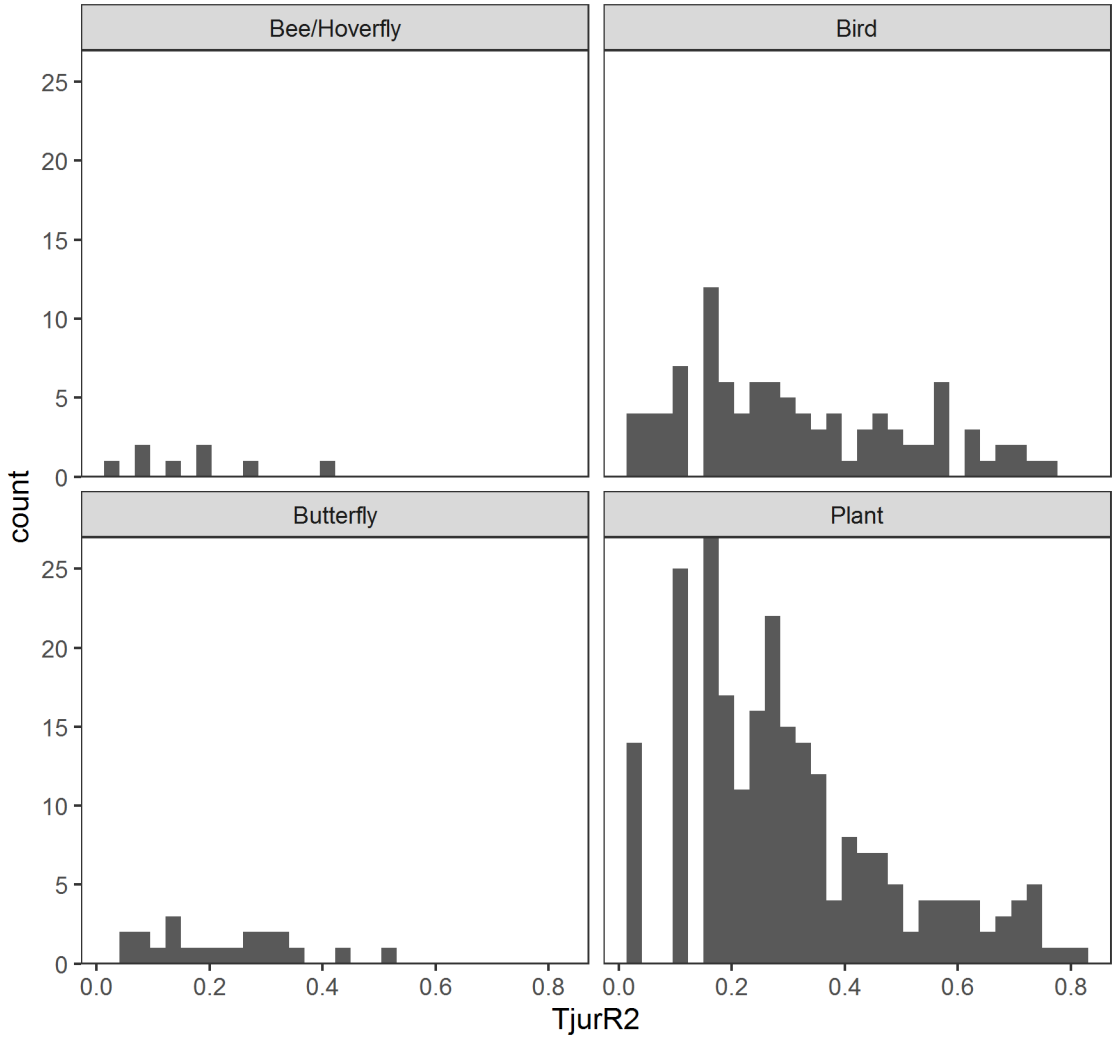


Figure 10: A histogram showing the proportion of variance explained by the model (Tjur's R^2) for each species.

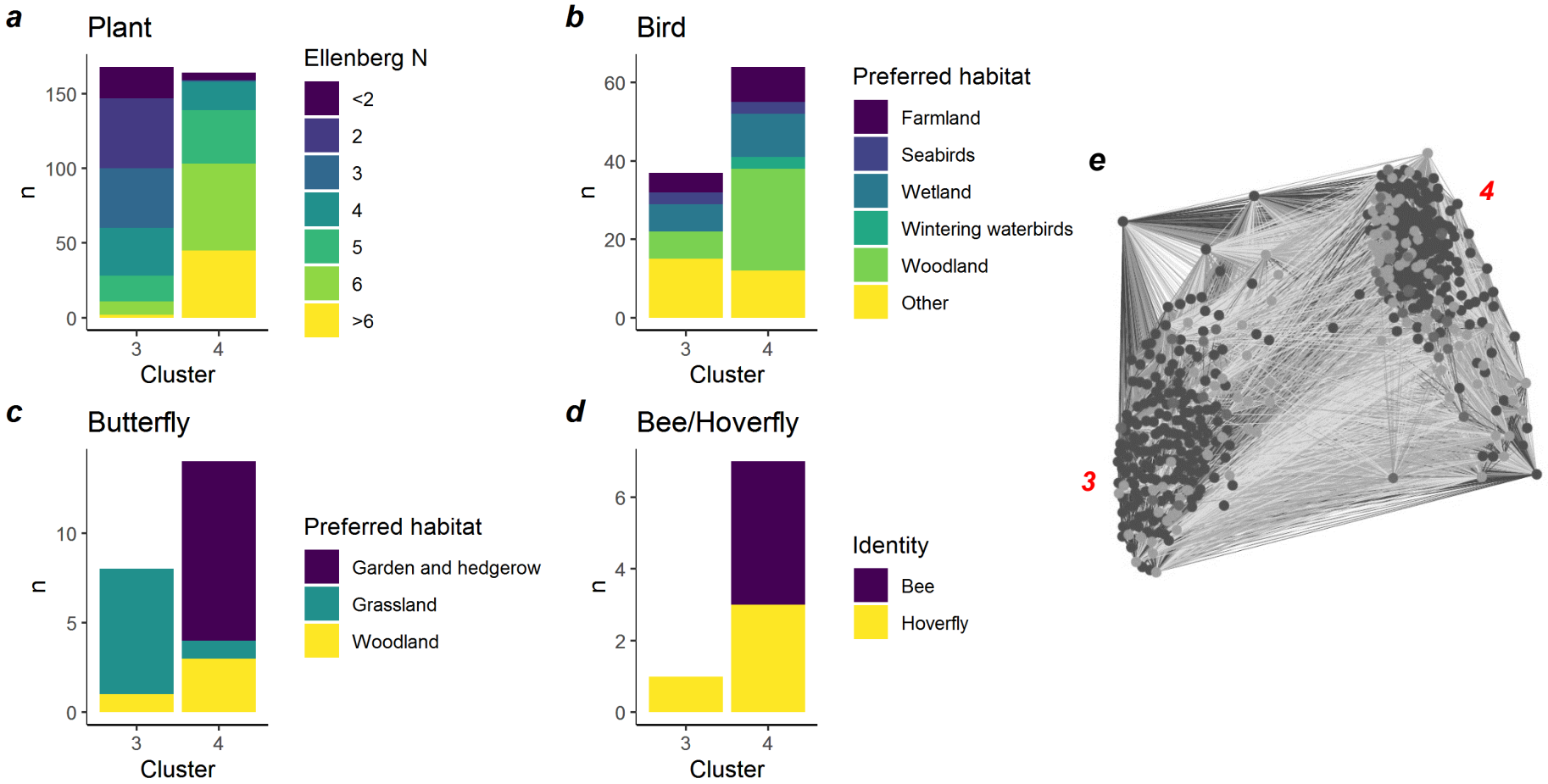


Figure 11: The breakdown of the network cluster membership by Ellenberg N score for plants (a), preferred habitat for birds (b) and butterflies (c), and bee/hoverfly identity (d). Only clusters with over 100 members are shown; the other three clusters all had one member each. Panel e shows the location of each of these large clusters on the network diagram in Figure 5.12.

APPENDIX H

Supplementary information for Chapter 6

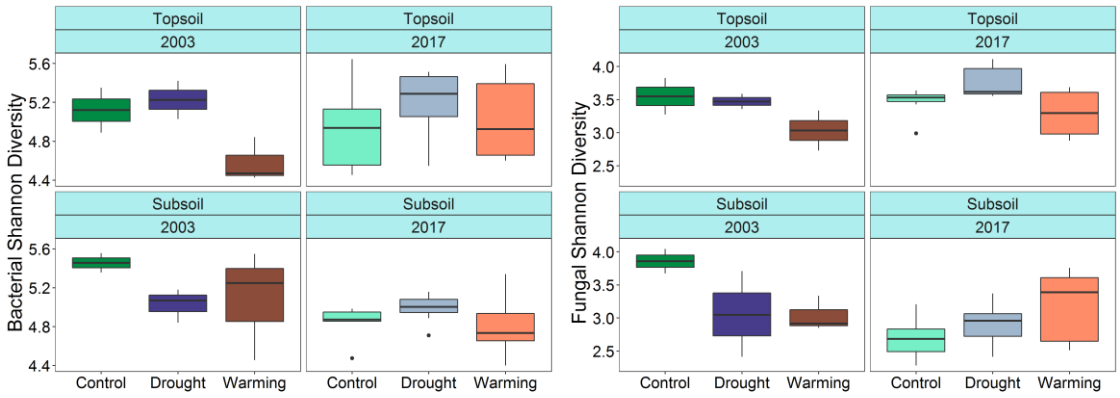


Figure 1: Bacterial (left) and fungal (right) Shannon diversity by year, treatment and depth

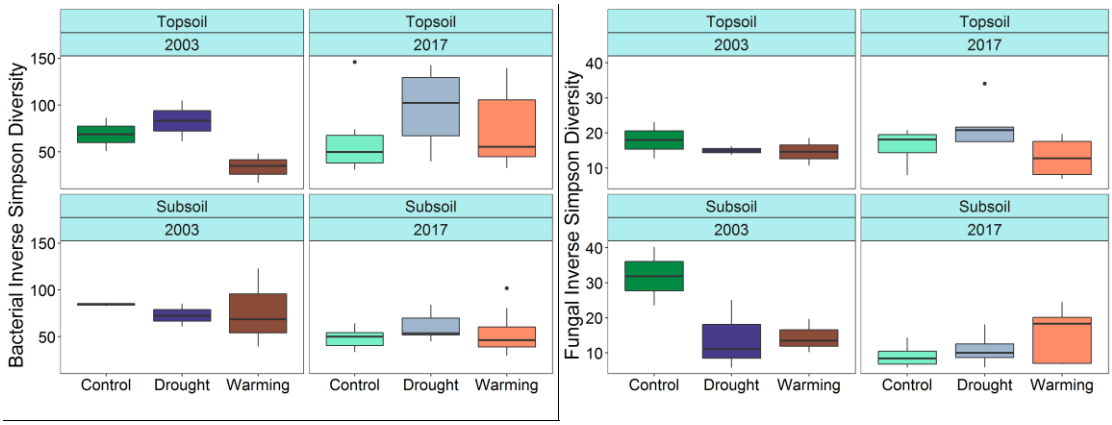


Figure 2: Bacterial (left) and fungal (right) inverse Simpson diversity by year, treatment and depth

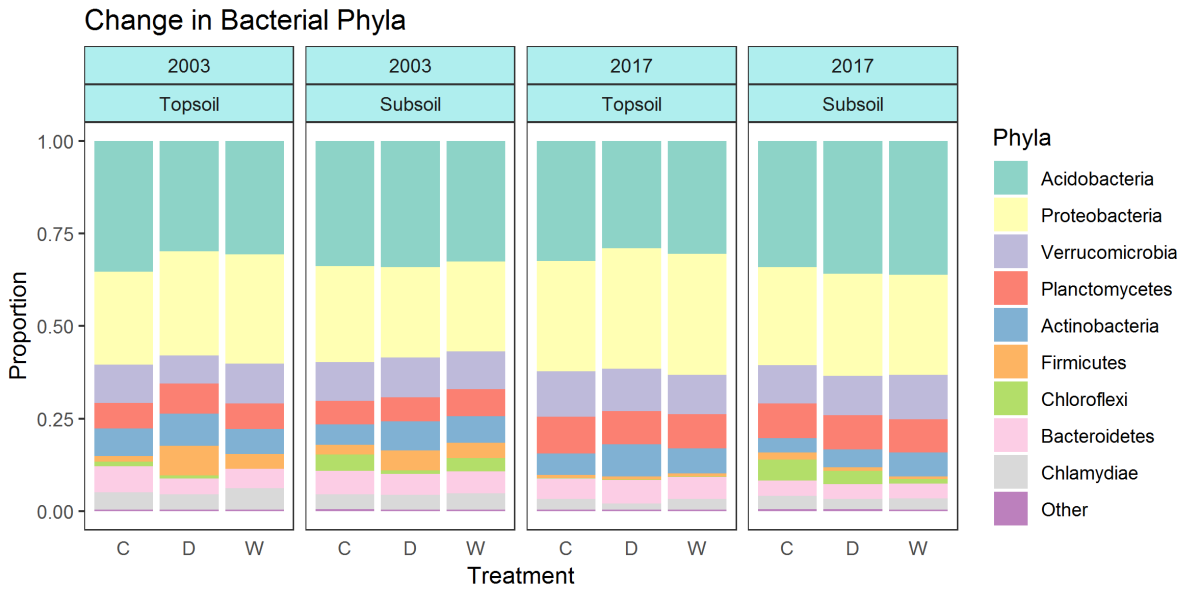


Figure 3: Limited change in bacterial phyla composition with treatment in the different years and depths.

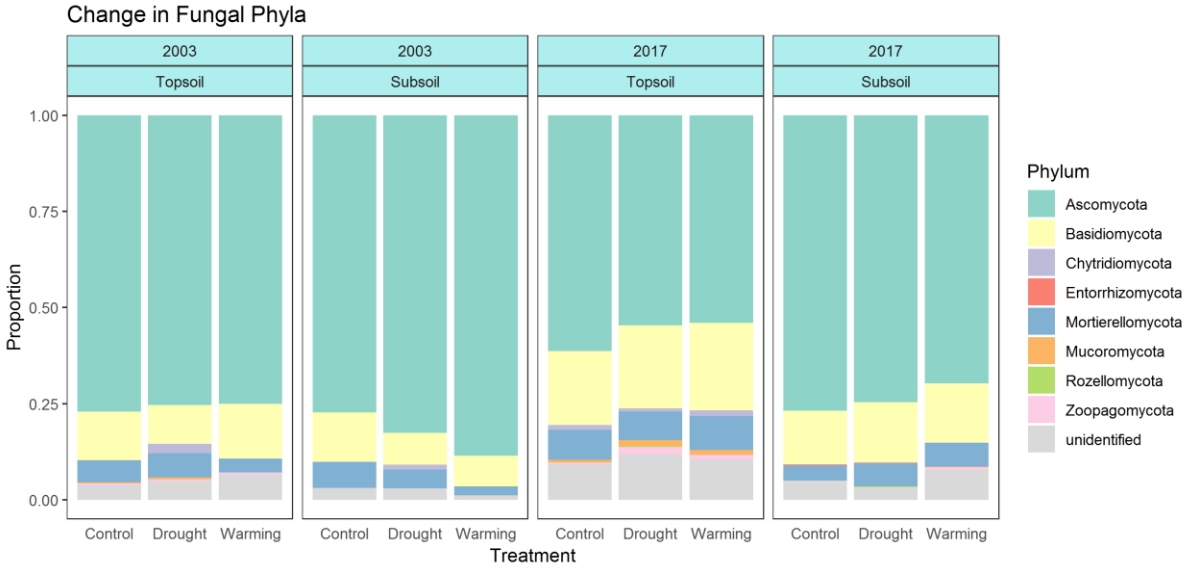


Figure 4: Limited change in fungal phyla composition with treatment in the different years and depths.

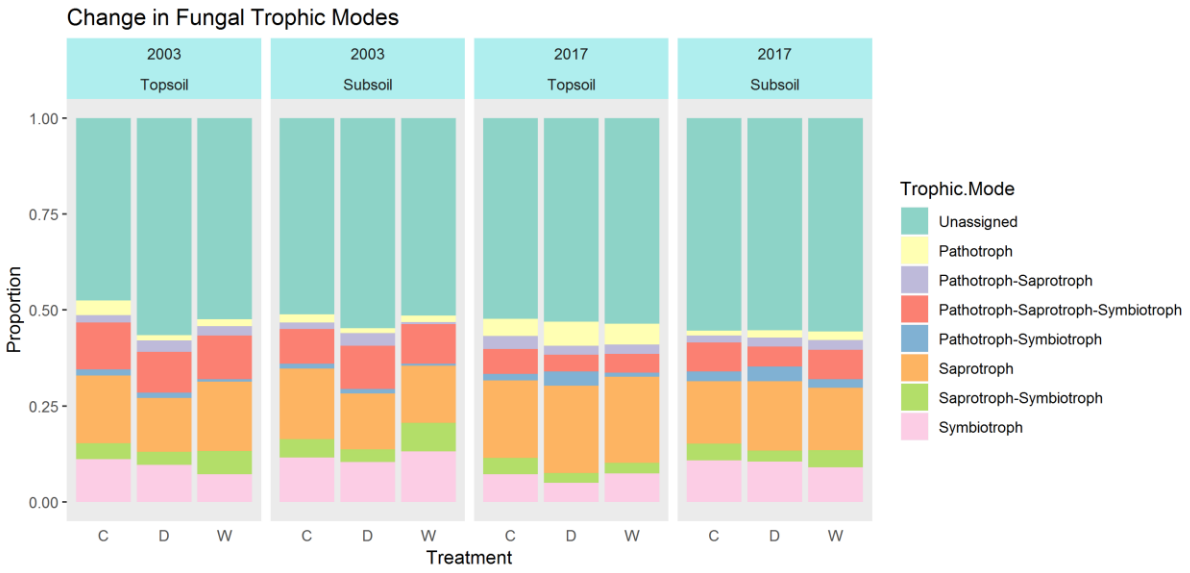


Figure 5: The majority of fungal taxa were unassigned to trophic modes, with limited changes in the relative frequency of the trophic modes by treatment, year and depth.

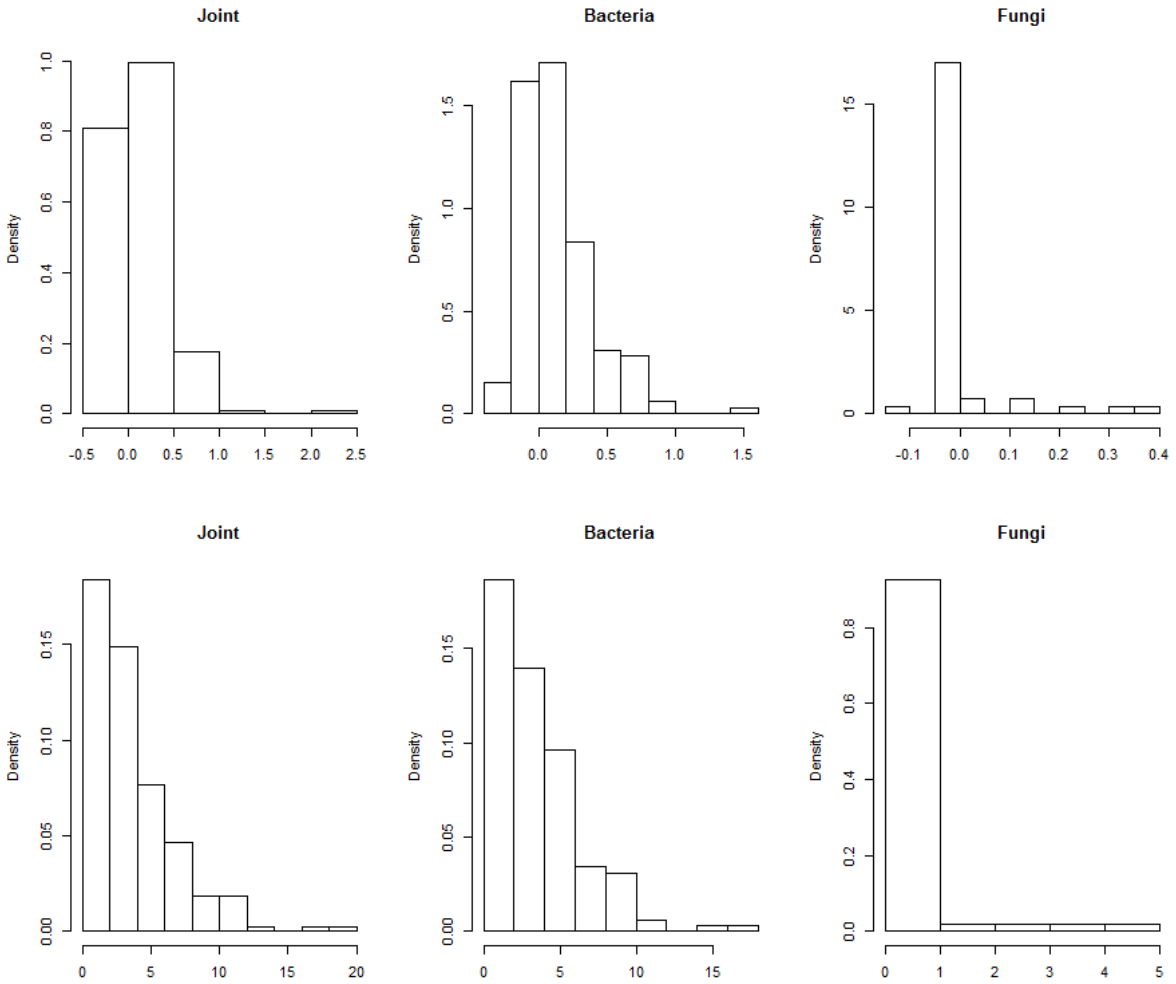


Figure 6: Strength (top row) and degree distribution (bottom row) of the three networks: joint (both bacteria and fungi), bacteria only and fungi only. The transitivity of the networks, or clustering coefficients, were 0.106 for the joint network, 0.131 for the bacteria network and 0.150 for the fungi network.

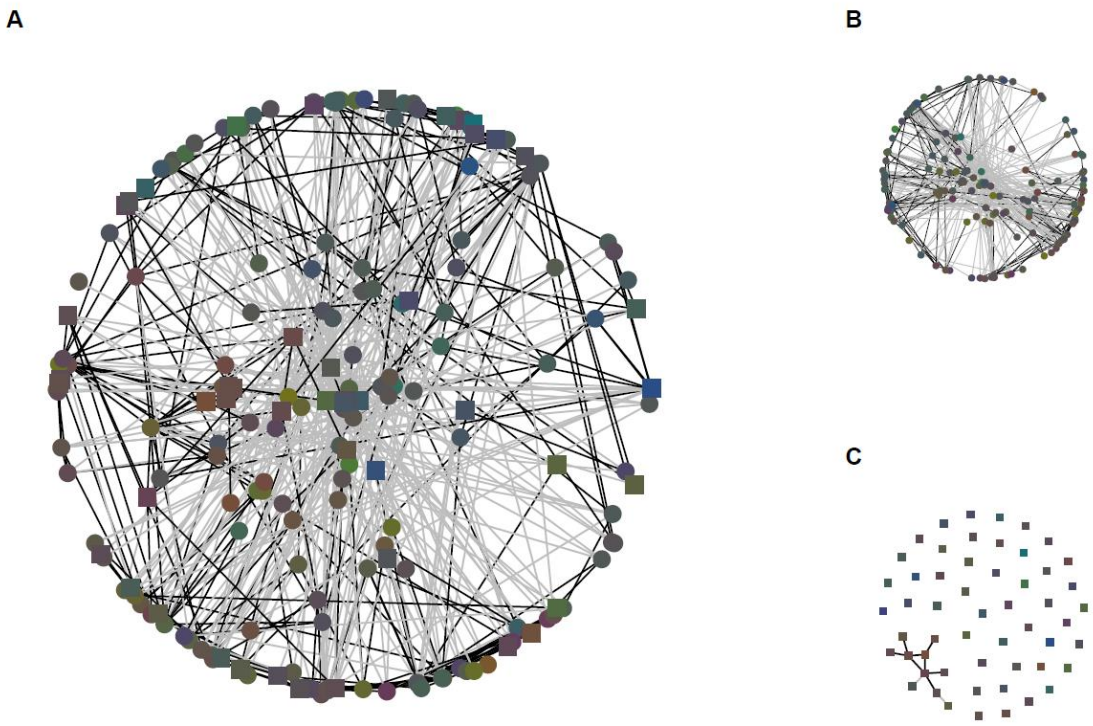


Figure 7: Microbial co-occurrence networks for bacteria and fungi together (A), bacteria only (B) and fungi only (C). Black links are positive, grey negative. The nodes are clustered together according to the Fruchterman Reingold algorithm (for graphical simplicity the fungal layout is based on the unweighted edges). Nodes are coloured by their preference for different treatments: green are control specialists, red warming specialists and blue drought specialists. A brown colour indicates no specialism. Data from 2017 only.

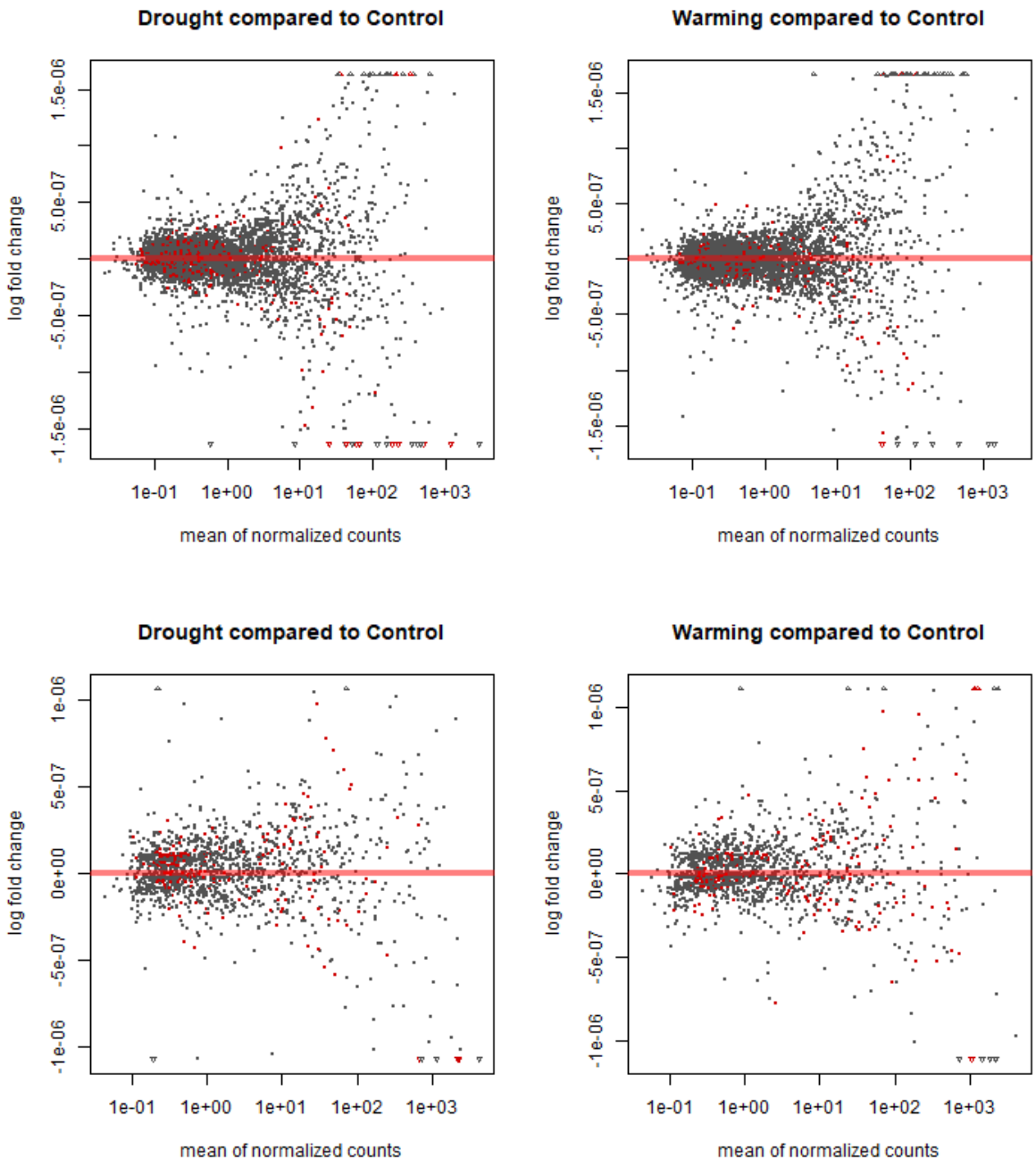


Figure 8: The change in bacterial (top row) and fungal (bottom row) taxa with the treatments compared to control. Taxa that had an adjusted p-value of less than 0.1 are marked in red, all others are marked in black.

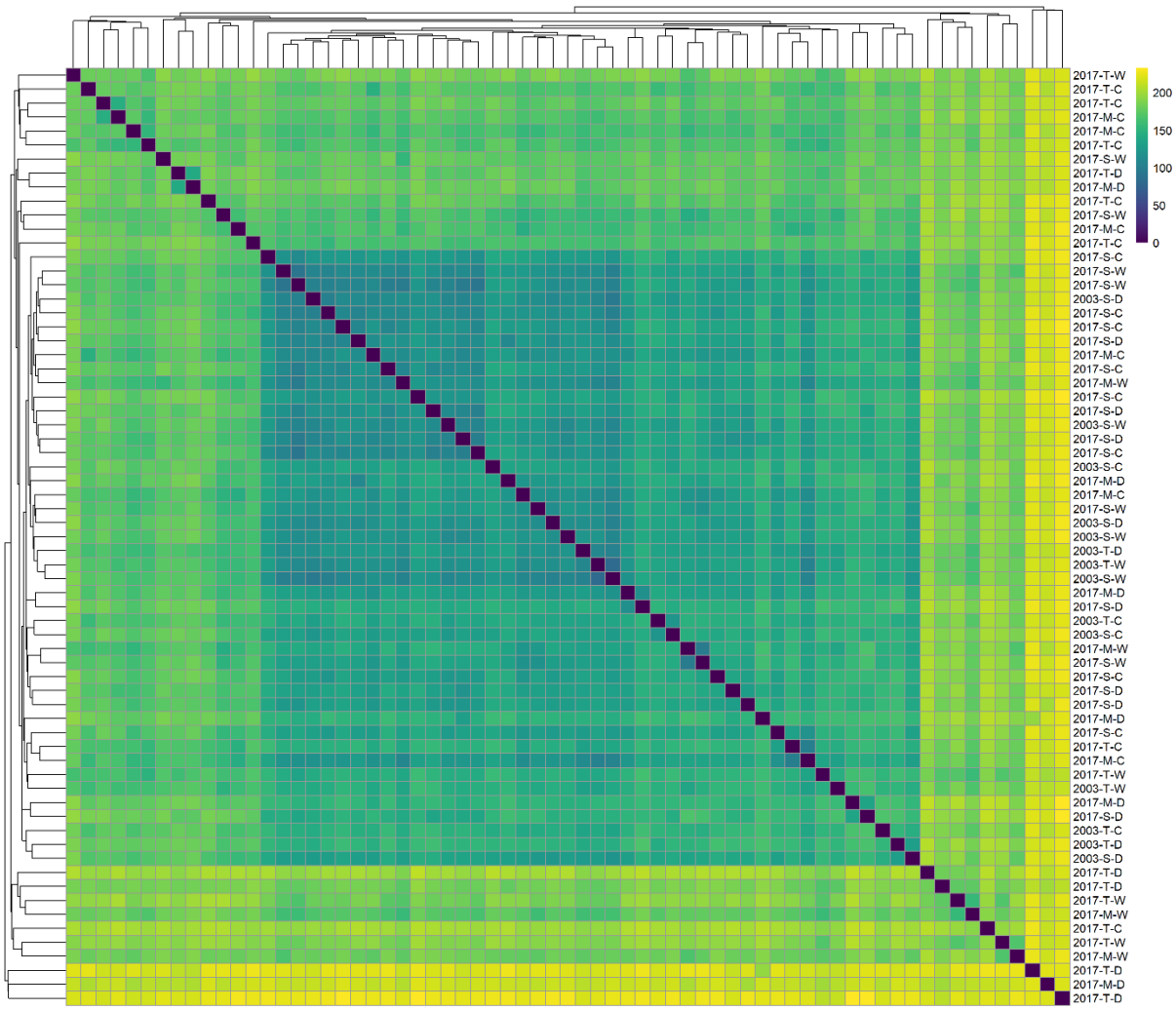


Figure 9: Heatmap of samples clustered by their transformed similarity according to DeSEq2 analysis of the fungal taxa.

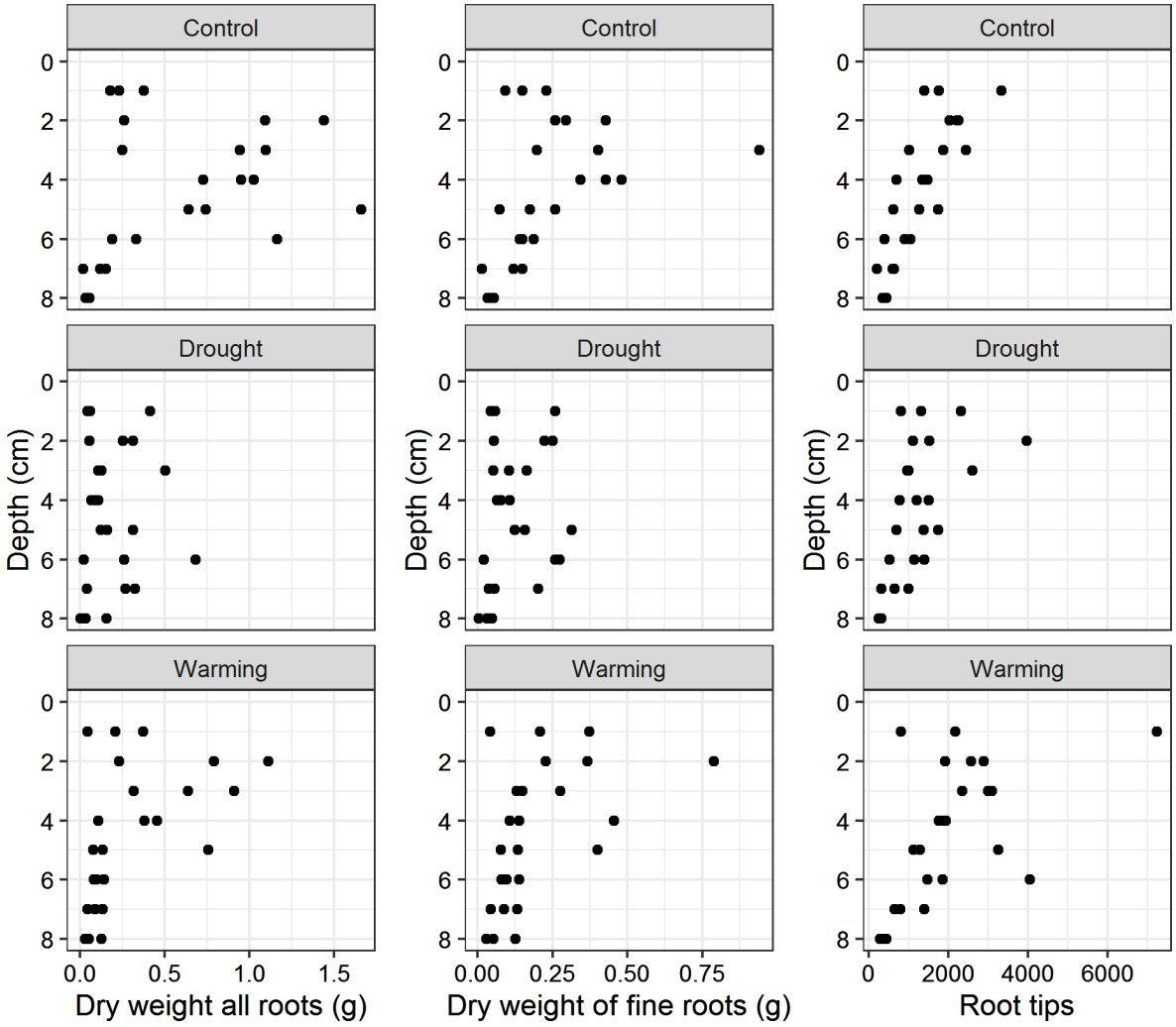


Figure 10: The change in root distribution with depth across the treatments. The left shows the dry weight of all roots, the centre the dry weight of roots under 2 mm in diameter and the right the number of root tips.

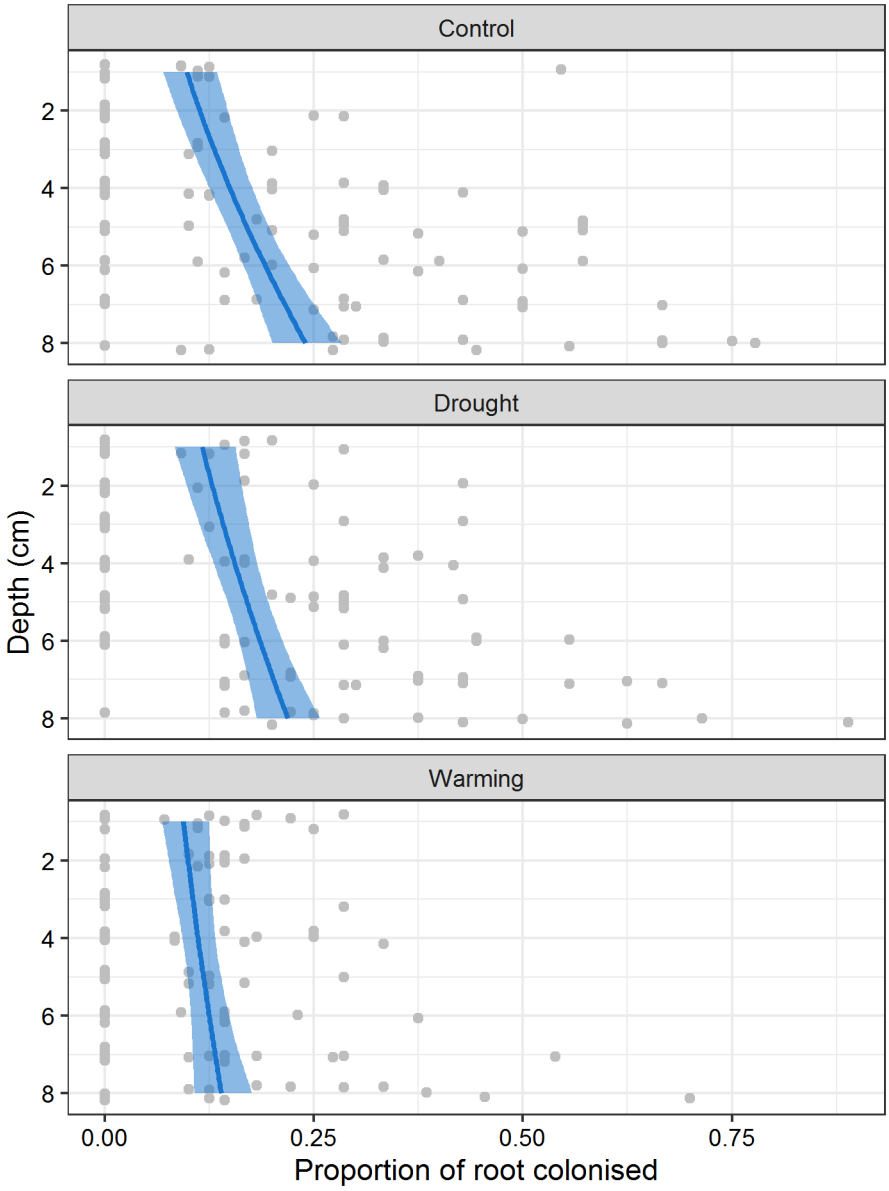


Figure 11: The proportion of root that is colonised by DSE increases with depth in the control and drought treatments but not in the warming treatments. The blue lines represent the model estimated effects plus their error as a blue band.

Table 1: The most connected taxa within the networks and their taxonomic identification. Degree is the number of other taxa they are connected to, strength is the sum of all of the weights of the links connected to the taxa. The taxa with the top 10 degree, top 20 strength and top 10 negative strength are included here.

Taxonomy	**Degree**	**Strength**	**Notes**
k_Bacteria;p_Proteobacteria;c_Alphaproteobacteria;o_Rhodospirillales;f_ ;g_;s_	19	2.397	
k_Bacteria;p_Proteobacteria;c_Gammaproteobacteria;o_Xanthomonadales;f_Sinobacteraceae;g_;s_	9	1.039	
k_Bacteria;p_Acidobacteria;c_Acidobacteriia;o_Acidobacteriales;f_Koribacteraceae;g_;s_	6	0.966	
k_Bacteria;p_Acidobacteria;c_Solibacteres;o_Solibacterales;f_Solibacteraceae;g_Candidatus Solibacter;s_	11	0.859	
k_Bacteria;p_Verrucomicrobia;c_[Pedosphaerae];o_[Pedosphaerales];f_auto67_4W;g_;s_	18	0.802	
k_Fungi;p_Mortierellomycota;c_Mortierellomycetes;o_Mortierellales;f_Mortierellaceae;g_Mortierella;s_humilis	7	0.777	Undefined saprotroph
k_Bacteria;p_Acidobacteria;c_Acidobacteriia;o_Acidobacteriales;f_Koribacteraceae;g_;s_	11	0.681	
k_Fungi;p_Ascomycota;c_Leotiomycetes;o_Helotiales;f_Hyaloscyphaceae;g_Hyaloscypha;s_fuckelii	5	0.642	Undefined saprotroph
k_Bacteria;p_Proteobacteria;c_Alphaproteobacteria;o_Rhizobiales;f_Methylocystaceae;g_;s_	11	0.639	
k_Fungi;p_Ascomycota;c_Eurotiomycetes;o_Chaetothyriales;f_unidentified;g_unidentified;s_unidentified	8	0.625	
k_Bacteria;p_Acidobacteria;c_DA052;o_Ellin6513;f_;g_;s_	9	0.614	
k_Bacteria;p_Proteobacteria;c_Gammaproteobacteria;o_Xanthomonadales;f_Sinobacteraceae;g_;s_	5	0.609	
k_Bacteria;p_Proteobacteria;c_Alphaproteobacteria;o_Rhizobiales;f_Methylocystaceae;g_;s_	11	0.595	
k_Bacteria;p_Acidobacteria;c_Acidobacteriia;o_Acidobacteriales;f_Koribacteraceae;g_;s_	8	0.587	
k_Bacteria;p_Acidobacteria;c_Acidobacteriia;o_Acidobacteriales;f_Acidobacteriaceae;g_;s_	4	0.584	
k_Bacteria;p_Proteobacteria;c_Deltaproteobacteria;o_Syntrophobacterales;f_Syntrophobacteraceae;g_;s_	5	0.574	
k_Bacteria;p_Acidobacteria;c_TM1;o_;f_;g_;s_	10	0.561	
k_Bacteria;p_Acidobacteria;c_Solibacteres;o_Solibacterales;f_;g_;s_	7	0.554	
k_Bacteria;p_Acidobacteria;c_Acidobacteriia;o_Acidobacteriales;f_Koribacteraceae;g_;s_	5	0.552	
k_Bacteria;p_Acidobacteria;c_DA052;o_Ellin6513;f_;g_;s_	6	0.550	
k_Bacteria;p_Acidobacteria;c_Acidobacteriia;o_Acidobacteriales;f_Acidobacteriaceae;g_;s_	9	0.516	
k_Bacteria;p_Proteobacteria;c_Alphaproteobacteria;o_Rhizobiales;f_Methylocystaceae;g_;s_	11	0.457	

k_Bacteria;p_Proteobacteria;c_Alphaproteobacteria;o_Rhizobiales;f_Bradyrhizobiaceae;g_Bradyrhizobium;s_	11	0.340	Nitrogen fixing
k_Bacteria;p_Acidobacteria;c_Acidobacteriia;o_Acidobacteriales;f_Koribacteraceae;g_Candidatus Koribacter;s_	10	0.323	
k_Bacteria;p_Verrucomicrobia;c_[Pedosphaerae];o_[Pedosphaerales];f_[Pedosphaeraceae];g_Pedosphaera;s_	13	0.159	
k_Bacteria;p_Proteobacteria;c_Deltaproteobacteria;o_Syntrophobacterales;f_Syntrophobacteraceae;g_;s_	10	0.158	
k_Bacteria;p_Actinobacteria;c_Actinobacteria;o_Actinomycetales;f_;g_;s_	12	0.015	
k_Bacteria;p_Proteobacteria;c_Gammaproteobacteria;o_Enterobacteriales;f_Enterobacteriaceae;g_Escherichia;s_coli	12	-0.032	
k_Fungi;p_Mortierellomycota;c_Mortierellomycetes;o_Mortierellales;f_Mortierellaceae;g_Mortierella;s_humilis	9	-0.145	Saprotroph
k_Bacteria;p_Acidobacteria;c_Solibacteres;o_Solibacterales;f_;g_;s_	4	-0.188	
k_Fungi;p_Ascomycota;c_Leotiomycetes;o_Helotiales;f_Helotiaceae;g_Meliniomyces;s_unidentified	3	-0.201	Saprotroph-Symbiotroph
k_Bacteria;p_Acidobacteria;c_DA052;o_Ellin6513;f_;g_;s_	6	-0.216	
k_Fungi;p_Ascomycota;c_Lecanoromycetes;o_Lecanorales;f_Ramalinaceae;g_Toninia;s_physaroides	3	-0.221	Symbiotroph (lichenised)
k_Bacteria;p_Proteobacteria;c_Deltaproteobacteria;o_Syntrophobacterales;f_Syntrophobacteraceae;g_;s_	4	-0.226	
k_Bacteria;p_Acidobacteria;c_Acidobacteriia;o_Acidobacteriales;f_Acidobacteriaceae;g_;s_	4	-0.244	
k_Bacteria;p_Verrucomicrobia;c_[Methylacidiphilae];o_S-BQ2-57;f_;g_;s_	3	-0.258	
k_Fungi;p_Ascomycota;c_unidentified;o_unidentified;f_unidentified;g_unidentified;s_unidentified	4	-0.279	
k_Bacteria;p_Verrucomicrobia;c_[Pedosphaerae];o_[Pedosphaerales];f_auto67_4W;g_;s_	6	-0.284	
k_Bacteria;p_Acidobacteria;c_Acidobacteriia;o_Acidobacteriales;f_Acidobacteriaceae;g_;s_	10	-0.462	

Table 2: The microbial diversity and NMDS scores for the different samples. Columns are: Plot (plot number); Quad. (quadrat of plot that was sampled); Depth (T = topsoil, M = middle, S = subsoil); Year (2003 or 2017 sampling); Treatment (Control, drought or warming plot); BACT RICH (bacterial richness); BACT SHAN (bacterial Shannon diversity); BACT SIMP (bacterial Simpson diversity); BACT NMDS1 (score of first axis in bacterial NMDS ordination); BACT NMDS2 (score of second axis in bacterial NMDS ordination); FUNG RICH (fungal richness); FUNG SHAN (fungal Shannon diversity); FUNG SIMP (fungal Simpson diversity); FUNG NMDS1 (score of first axis in fungal NMDS ordination); FUNG NMDS2 (score of second axis in fungal NMDS ordination).

Plot	Quad.	Depth	Year	Treatment	BACT RICH	BACT SHAN	BACT SIMP	BACT NMDS1	BACT NMDS2	FUNG RICH	FUNG SHAN	FUNG SIMP	FUNG NMDS1	FUNG NMDS2
9	G9	T	2017	Control	536	5.64	0.007	-0.762	-0.877	224	3.51	0.07	-0.921	-0.014
6	E7	S	2017	Control	512	4.95	0.018	1.148	-0.855	38	2.28	0.152	1.037	-0.219
3	G9	T	2017	Control	296	4.48	0.033	-0.419	0.263	NA	NA	NA	NA	NA
3	G2	T	2017	Control	481	4.94	0.02	-0.193	0.076	167	3.58	0.048	-0.336	-0.07
3	B3	T	2017	Control	372	4.77	0.024	-0.542	0.025	170	3.56	0.054	-0.396	-0.076
5	G2	M	2017	Drought	256	4.35	0.036	-0.136	0.647	NA	NA	NA	NA	NA
1	F8	M	2017	Warming	301	4.56	0.024	0.283	0.439	NA	NA	NA	NA	NA
3	G2	S	2017	Control	340	4.47	0.03	1.288	-0.083	42	2.44	0.126	0.931	0.229
4	E4	T	2017	Drought	414	5.39	0.008	-0.809	-0.638	269	4.11	0.029	-0.828	-0.301
7	B7	S	2017	Warming	439	5.34	0.01	-0.494	-0.366	175	3.61	0.049	-0.507	0.131
1	NA	T	2003	Warming	469	4.84	0.021	-0.565	0.058	NA	NA	NA	NA	NA
2	D8	T	2017	Warming	426	5.43	0.008	-1.056	-1.053	158	2.88	0.145	-1.09	0.158
7	C4	M	2017	Warming	341	4.6	0.022	0.014	0.598	NA	NA	NA	NA	NA
2	G5	S	2017	Warming	296	4.4	0.034	0.085	0.739	NA	NA	NA	NA	NA
4	E4	M	2017	Drought	482	5.08	0.015	0.442	-0.05	136	3.43	0.053	0.069	-0.281
7	F5	M	2017	Warming	426	5.13	0.013	0.01	0.207	NA	NA	NA	NA	NA
5	B6	M	2017	Drought	359	4.68	0.024	0.463	0.464	97	3.38	0.054	0.321	0.15
7	C4	S	2017	Warming	408	5.09	0.012	-0.146	0.19	153	3.76	0.041	-0.109	0.037
2	C2	T	2017	Warming	299	4.66	0.022	-0.569	0.136	NA	NA	NA	NA	NA
8	C8	M	2017	Drought	526	5.55	0.007	-0.754	-0.663	253	3.87	0.043	-0.877	-0.138
9	NA	T	2003	Control	577	5.35	0.012	0.03	-0.05	132	3.83	0.043	0.034	0.015

8	F5	T	2017	Drought	415	5.19	0.012	-0.279	-0.202	NA	NA	NA	NA	NA
9	NA	S	2003	Control	776	5.56	0.012	0.722	-0.335	116	3.68	0.043	0.27	-0.065
2	C2	M	2017	Warming	303	4.69	0.019	0.066	0.246	NA	NA	NA	NA	NA
8	B7	M	2017	Drought	354	4.69	0.023	0.327	0.296	91	3.07	0.076	0.333	-0.008
4	F8	T	2017	Drought	430	5.14	0.014	-0.536	-0.153	NA	NA	NA	NA	NA
6	D5	M	2017	Control	381	5.14	0.011	0.012	0.09	157	3.5	0.054	-0.283	0.028
1	F8	T	2017	Warming	350	4.92	0.018	-0.756	-0.213	108	3.01	0.116	-0.47	0.264
8	C8	T	2017	Drought	302	4.55	0.025	-0.337	0.287	NA	NA	NA	NA	NA
6	E7	T	2017	Control	377	4.94	0.018	-0.01	0.136	166	3.64	0.049	-0.34	0.137
5	B6	S	2017	Drought	405	5.01	0.015	0.899	-0.015	64	3.03	0.074	0.598	0.052
2	NA	S	2003	Warming	647	5.55	0.008	1.017	-0.463	61	2.85	0.098	0.6	-0.132
7	B7	M	2017	Warming	402	5	0.017	-0.124	0.076	NA	NA	NA	NA	NA
7	NA	T	2003	Warming	318	4.47	0.029	-0.256	0.894	48	2.73	0.094	0.461	0.529
6	C2	T	2017	Control	281	4.45	0.031	-0.385	0.341	114	3.43	0.055	-0.087	-0.066
9	D6	M	2017	Control	360	4.9	0.018	-0.226	0.352	147	3.8	0.034	-0.165	0.066
3	G2	M	2017	Control	242	4.49	0.023	0.226	0.833	NA	NA	NA	NA	NA
7	F5	T	2017	Warming	397	4.97	0.018	-0.221	0.067	NA	NA	NA	NA	NA
4	D2	M	2017	Drought	423	5.2	0.013	-0.686	-0.286	184	3.88	0.036	-0.514	-0.158
7	C4	T	2017	Warming	287	4.64	0.023	-0.305	0.333	NA	NA	NA	NA	NA
5	F8	S	2017	Drought	390	5.14	0.012	0.384	-0.106	113	3.37	0.055	0.174	-0.193
2	D8	M	2017	Warming	398	5.23	0.009	-0.98	-0.413	139	3.06	0.089	-0.793	0.25
4	NA	S	2003	Drought	419	4.84	0.016	0.243	0.389	50	2.41	0.172	0.563	0.169
7	NA	S	2003	Warming	335	4.45	0.025	0.183	0.739	45	2.92	0.074	0.579	0.393
8	C8	S	2017	Drought	425	4.89	0.022	0.404	-0.143	95	2.96	0.119	0.245	-0.261
4	E4	S	2017	Drought	519	5	0.019	0.74	-0.482	56	2.75	0.099	0.563	-0.207
9	G9	S	2017	Control	423	4.98	0.016	1.174	-0.309	64	2.67	0.112	0.495	-0.479
2	G5	M	2017	Warming	385	4.1	0.065	-0.429	0.31	NA	NA	NA	NA	NA
9	D6	T	2017	Control	325	4.55	0.026	-0.147	0.477	NA	NA	NA	NA	NA
5	B6	T	2017	Drought	509	5.46	0.008	-0.691	-0.633	182	3.58	0.057	-0.76	-0.084

5	F8	M	2017	Drought	318	4.56	0.023	-0.15	0.119	NA	NA	NA	NA	NA
1	B4	S	2017	Warming	359	4.74	0.022	0.942	0.164	47	2.52	0.143	0.647	0.301
4	F8	M	2017	Drought	396	4.73	0.031	-0.245	-0.117	NA	NA	NA	NA	NA
8	F5	M	2017	Drought	403	4.87	0.022	0.161	-0.056	139	3.66	0.043	0.011	-0.313
5	NA	T	2003	Drought	507	5.03	0.016	-0.044	0.274	70	3.36	0.062	0.235	0.373
2	NA	T	2003	Warming	437	4.42	0.059	-0.818	0.055	113	3.34	0.054	-0.19	0.253
2	C2	S	2017	Warming	395	4.88	0.019	0.219	0.131	NA	NA	NA	NA	NA
9	E2	T	2017	Control	536	5.28	0.013	-0.176	-0.265	170	3.53	0.07	-0.385	0.314
9	E2	M	2017	Control	411	5.03	0.016	-0.13	0.382	NA	NA	NA	NA	NA
1	B4	T	2017	Warming	471	5.59	0.007	-1.041	-0.761	198	3.58	0.059	-0.958	0.131
3	B3	M	2017	Control	348	4.74	0.024	-0.605	0.122	159	3.14	0.112	-0.341	-0.281
6	D5	T	2017	Control	451	5.13	0.015	-0.587	-0.157	149	2.99	0.126	-0.461	-0.231
3	G9	M	2017	Control	268	4.39	0.025	0.033	0.771	83	2.74	0.151	0.261	0.101
5	F8	T	2017	Drought	427	5.47	0.008	-0.943	-0.652	265	3.97	0.046	-0.903	-0.097
2	D8	S	2017	Warming	329	4.69	0.025	0.196	0.525	79	2.64	0.15	0.324	0.222
5	NA	S	2003	Drought	497	5.18	0.012	0.382	0.421	74	3.05	0.089	0.455	0.196
1	NA	S	2003	Warming	621	5.25	0.015	0.65	-0.014	69	3.34	0.051	0.467	0.06
8	B7	T	2017	Drought	343	4.8	0.018	-0.28	0.224	NA	NA	NA	NA	NA
8	B7	S	2017	Drought	531	5.16	0.012	0.991	-0.566	120	3.1	0.086	0.152	-0.298
4	D2	S	2017	Drought	457	5.06	0.019	0.918	-0.603	58	2.7	0.113	0.626	-0.382
3	B3	S	2017	Control	488	4.87	0.025	0.866	-0.636	46	2.94	0.07	0.707	-0.309
1	D7	T	2017	Warming	488	5.39	0.009	-0.609	-0.277	184	3.69	0.051	-0.553	-0.031
1	D7	M	2017	Warming	275	4.48	0.03	-0.003	0.576	107	3.06	0.091	0.083	0.168
5	G2	T	2017	Drought	493	5.51	0.007	-0.949	-0.74	214	3.62	0.048	-0.92	0.045
6	C2	M	2017	Control	286	4.41	0.042	-0.179	0.507	68	3.02	0.076	0.26	0.312
6	E7	M	2017	Control	358	4.61	0.029	0.295	0.301	77	3.1	0.077	0.405	0.019
7	B7	T	2017	Warming	447	4.92	0.022	-0.3	-0.024	NA	NA	NA	NA	NA
6	NA	T	2003	Control	451	4.89	0.02	-0.026	0.456	126	3.27	0.078	-0.096	0.294
8	NA	T	2003	Drought	632	5.42	0.01	-0.164	-0.4	136	3.58	0.073	-0.075	0.209

1	D7	S	2017	Warming	295	4.55	0.028	0.123	0.465	94	3.39	0.054	0.238	0.181
2	G5	T	2017	Warming	378	4.6	0.031	-0.586	-0.054	NA	NA	NA	NA	NA
9	D6	S	2017	Control	380	4.85	0.02	1.264	-0.39	77	2.51	0.171	0.491	-0.375
1	F8	S	2017	Warming	369	4.73	0.021	1.321	-0.493	NA	NA	NA	NA	NA
4	F8	S	2017	Drought	530	4.97	0.019	0.24	-0.351	NA	NA	NA	NA	NA
1	B4	M	2017	Warming	319	4.73	0.024	-0.107	0.347	191	3.56	0.051	-0.482	0.041
9	G9	M	2017	Control	424	4.93	0.017	0.175	0.084	NA	NA	NA	NA	NA
8	F5	S	2017	Drought	310	4.71	0.02	1.113	-1.101	99	2.42	0.171	0.086	-0.604
6	NA	S	2003	Control	570	5.36	0.012	0.606	-0.116	116	4.04	0.025	0.256	0.008
8	NA	S	2003	Drought	494	5.07	0.014	0.099	0.529	115	3.71	0.04	0.175	0.158
3	G9	S	2017	Control	NA	NA	NA	NA	NA	92	2.8	0.103	0.363	-0.255
4	D2	T	2017	Drought	NA	NA	NA	NA	NA	166	3.56	0.057	-0.568	-0.242
6	C2	S	2017	Control	NA	NA	NA	NA	NA	106	3.21	0.078	0.21	0.047
9	E2	S	2017	Control	NA	NA	NA	NA	NA	55	2.69	0.145	0.682	0.13

APPENDIX I

Topsoil physico-chemical properties from the Glastir Monitoring and Evaluation Programme, Wales 2013-2016

Robinson, D. A.; Astbury, S.; Barrett, G.; Burden, A.; Carter, H.; Emmett, B. A.; Garbutt, A.; Giamperi, C.; Hall, J.; Henrys, P.; Hughes, S.; Hunt, A.; Jarvis, S.; Jones, D. L.; Keenan, P.; Lebron, I.; Nunez, D.; Owen, A.; Patel, M.; Pereira, M. G.; Seaton, F.; Sharps, K. Tanna, B.; Thompson, N.; Williams, B.; Wood, C. M.

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Contribution statement:

DAR led the soil work package and EIDC data preparation. SA and KS were database managers and provided quality assurance. GB, SH and IL led the soil preparation, and the pH, EC and LOI analyses, managed by JH. IL also led the archiving of soil samples. AB and AG managed the field teams, with co-ordination by AO. HC, AH, PK, MP, BT, and NT were laboratory analysts that performed the Olsen P, Total N&C and Total P analyses, managed by MGP. BAE was the GMEP lead scientist. CG and DN performed soil processing. PH, SJ and FS produced the R scripts for derived units and performed data quality control. BW managed the project. CMW performed data management and sample tracking. DLJ was the biodiversity science lead and work package co-supervisor.

Abstract

This data set includes a range of physico-chemical properties measured from topsoil within a wide range of land use types across Wales, collected as part of the Glastir Monitoring and Evaluation Programme (GMEP). The properties included are: soil organic matter (loss on ignition (LOI)), derived carbon concentration, total soil organic carbon (SOC), nitrogen, total soil phosphorous, Olsen-phosphorous (within improved land only), pH, electrical conductivity, soil bulk density of fine earth, fine earth volumetric water content when sampled and soil water repellency - water drop penetration time.

The monitoring programme was set up by the Welsh Government in 2013 to monitor the effects of the Glastir agri-environment scheme on the environment and ran from 2013 to 2016. The field survey element was based on a stratified random sampling design of 300 x 1km square sites across Wales, and was managed by the Centre for Ecology & Hydrology.

Provenance & quality

Within each of 300 x 1km sample squares, a set of soil samples were taken from 5 pre-determined randomly dispersed locations (where practically possible). The soil sample analysed for soil metrics was taken using a black plastic core ('C-core'), 15cm long, from a location co-incident with vegetation surveys.

After collection, the soil cores were refrigerated and stored until posted, usually within a couple of days, to laboratories at the Centre for Ecology & Hydrology (CEH), Bangor and Lancaster. Analysed data were transferred to secure databases at CEH following sample analysis.

APPENDIX J

Dataset title: Soil bacterial and fungal communities from the Climoor long term climate change experiment in Clocaenog forest

Seaton, F.M.; Goodall, T.; Reinsch, S.; Griffiths, R.I.; Robinson, D.A.; Jones, D.L; Brooks, M.R., Emmett, B.A.

Published online in the European Nucleotide Archive under primary accession reference PRJEB33721.

Contribution statement:

FMS led the sampling design, carried out soil sampling, data quality control and uploaded the sequences to the archive. TG performed all laboratory analyses under the supervision of RIG. SR, BAE, DAR and DLJ provided input into the sampling design. SR, MRB and BAE support the Climoor experiment.

Abstract

This dataset is the DNA sequences from Illumina MiSeq sequencing of the bacterial 16S and fungal ITS2 genes in Clocaenog soils. Soil samples were collected from the climate change field site Climoor that is located in Clocaenog forest, Northeast Wales during 2003 and 2017.

The experimental field site consists of three untreated control plots, three plots where the plant canopy air is artificially warmed during night time hours and three plots where rainfall is excluded from the plots at least during the plants growing season (March to September). The Climoor field experiment intends to answer questions regarding the effects of warming and drought on ecosystem processes and has been running since 1999. The microbial community data aims to understand how changes in soil hydrological and chemical properties have influenced the soil microbial composition and the implications of this for biogeochemical cycling.

Provenance & quality

Soil samples were collected in February 2003 and February 2017 respectively by students and staff at the Climoor field site. They were stored at -20°C in CEH Bangor until transported to and sequenced at CEH Wallingford. A small portion of soil (~0.2g) was taken from each sample and the DNA extracted using a MOBIO PowerSoil® extraction kit. The 16S genes were amplified using primers for the V4 region (primers 515F-806R, as in the Earth Microbiome Project) and the ITS2 genes amplified using primers from Ihrmark et al (FEMS Microbiol Ecol 82 (2012) 666–677). The DNA was sequenced upon an Illumina MiSeq system.

APPENDIX K

Dataset title: *Calluna vulgaris* root length and fungal colonisation data from the Climoor long term climate change experiment in Clocaenog forest

White, N.; Seaton, F.M.; Reinsch, S.; Smith, A.R.; Brooks, M.R., Emmett, B.A.

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Contribution statement:

NW performed all soil sampling and laboratory analyses under the supervision of ARS. FMS performed data quality control and prepared the data submission. SR, MRB and BAE support the Climoor experiment.

Abstract

This dataset contains root length, biomass and fungal colonisation data for *Calluna vulgaris* from control, drought and warming treated soils from the long term climate change experiment in Clocaenog forest. Soil samples were collected from the climate change experiment in Northeast Wales during April 2015. Roots were separated from the soil, their length and biomass measured and then analysed using microscopy for Ericoid mycorrhizae (ErM) and dark septate endophyte (DSE) colonisation of *Calluna vulgaris*.

The experimental field site consists of three untreated control plots, three plots where the plant canopy air is artificially warmed during night time hours and three plots where rainfall is excluded from the plots at least during the plants growing season (March to September). The Climoor field experiment intends to answer questions regarding the effects of warming and drought on ecosystem processes and has been running since 1999. The root length and fungal colonisation data aims to understand how changes in soil hydrological and chemical properties have influenced *Calluna vulgaris* rooting behaviour and interactions with the soil microbiome.

Provenance & quality

Soil cores of 8 cm diameter and depth were collected from each plot near the base of *Calluna vulgaris* on the 1st April 2015, then transported back to Bangor and stored at 4°C until further processing. Cores were cut from the top into 1 cm deep subsections. Each subsection was soaked in deionised water and agitated to break up the root/soil clumps. Roots confidently identified as *C. vulgaris* were removed using forceps and thoroughly washed in tap water. Necrotic or rotting roots were discarded.

WinRHIZO version 3.2 was used to measure the length and diameter of cleaned subsection roots on a flatbed scanner. Roots were positioned without overlapping, submerged in 5 mm tap water to improve scanning accuracy. Acquisition parameters were set using the TWAIN interface in professional mode: positive film, 24 bit and 300 dpi. Post scanning, ten of the finest roots were manually selected from each

subsection for microscopic investigation. The remaining roots were oven dried at 70°C for 24 hours, producing dry weight data for those < and > 2mm in diameter.

All core fragments for microscopic assessment were soaked over 20 hours in 10% KOH. Roots were thoroughly rinsed in deionised water and heated in a water bath at 90°C for 15 minutes in 5% vinegar-ink solution. Roots were rinsed, acidified and de-stained by soaking in tap water with a few drops of vinegar for a further 20 minutes. A compound microscope was used to estimate proportional colonisation using the magnified intersection technique, with a scale bar cuticle instead of cross-hair and at a 40x magnification. Roots were cut approximately 1-2 cm in length, with 2 mm passes made along each root length. All cortical cells were examined for colonization by Ericoid mycorrhiza and dark septic endophytes, working through the plane of focus. Each interval was categorised based upon Ericoid mycorrhiza colonisation into 0 %, < 1 %, < 10 %, < 50 %, > 50 % and > 90 % colonisation.