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Mapping and validating predictions of bacterial biodiversity using European and national scale datasets

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Abstract

Recent research has highlighted strong correlations between soil edaphic parameters and bacterial biodiversity. Here we seek to explore these relationships across the European Union member states with respect to mapping bacterial biodiversity at the continental scale. As part of the EU FP7 EcoFINDERs project, bacterial communities from 76 soil samples taken across Europe were assessed from eleven countries encompassing Arctic to Southern Mediterranean climes, representing a diverse range of soil types and land uses (grassland, forest and arable land). We found predictable relationships between community biodiversity (ordination site scores) and land use factors as well as soil properties such as pH. Based on the modelled relationship between soil pH and bacterial biodiversity found for the surveyed soils, we were able to predict biodiversity in ~1000 soils for which soil pH data had been collected as part of national scale monitoring. We then performed interpolative mapping utilising existing EU wide soil pH data to present the first map of bacterial biodiversity across the EU member states. The predictive accuracy of the map was assessed again using the national scale data, but this time contrasting the EU wide *spatial* predictions with point data on bacterial communities. Generally the maps were useful at predicting broad extremes of biodiversity reflective of low or high pH soils, though predictive accuracy was limited for Britain particularly for organic/acidic soil communities. Spatial accuracy could however be increased by utilising published maps of soil pH calculated using geostatistical approaches at both global and national scales. These findings will contribute to wider efforts to predict and understand the spatial distribution of soil biodiversity at global scales. Further work should focus on enhancing the predictive power of such maps, by harmonising global datasets on soil conditioning parameters, soil properties and biodiversity; and the continued efforts to advance the geostatistical modelling of specific components of soil biodiversity at local to global scales.

1. Introduction

Soil bacteria contribute the largest proportion of the soil genetic resource (Urich et al 2008; Fierer et al, 2012), reflecting their ubiquity and high abundance across all soil systems. Given bacterial importance in the regulation of soil ecosystem services (Comerford et al, 2013), increased understanding of the environmental controls of bacterial biodiversity is required from both scientific and policy perspectives in order to predict biodiversity change, and determine functional consequences of change due to future climatic or land use pressures. Attempts to characterise the bacterial communities in soils and understand ecological drivers have previously been hampered by methodological difficulties in assessing taxonomic diversity due to the limited culturability of many bacterial taxa coupled with vast taxonomic diversity (e.g Janssen et al, 2002). These problems have to some extent been overcome through the development of molecular technologies to assess the diversity of taxonomic marker genes (particularly the 16S rRNA gene) PCR amplified from extracted soil DNA (Hirsch et al, 2010).

The application of molecular methods to wide ranging globally dispersed soil samples has revealed that soil bacterial communities are broadly structured along gradients of soil properties, with strong correlations between measures of bacterial biodiversity and key soil variables such as soil pH and organic matter, which are co-related with broader environmental parameters such as land use, climate, and parent material (Fierer et al, 2006; Lauber et al, 2009; Griffiths et al 2011). Therefore, whilst the causal mechanisms underlying these relationships are complex it is apparent that the same pedogenic factors which determine the nature of soils (e.g Jenny, 1941) also determine the taxonomic characteristics and structure of the soil bacterial community. This new knowledge permits spatial forecasting of bacterial biodiversity at a range of scales and under change scenarios; which together with parallel developments in understanding microbial biodiversity-function relationships, may allow for enhanced prediction of soil processes under future environmental change.

Molecular surveys permit the production of range maps of soil bacterial distributions at various spatial scales. Spatial distribution maps provide a visual representation of the forces shaping populations or communities and therefore provide the foundation for macro ecological understanding (Elton, 1927). Maps can also guide policy decisions with respect to land management, and can be useful visual resources guiding scientific experimentation and enquiry. Importantly, more recently rasterised maps provide georeferenced data which can feed wider ecological, climatic or biogeochemical models. Already there has been several attempts to map soil microbial properties at national and regional scales, using molecular methodologies applied to nationwide soil monitoring schemes (Bru et al, 2011; Griffiths et al 2011, Dequidet et al 2009; Dequidet et al 2011). These studies mapped point sampled microbial data using *interpolative* methods (e.g inverse distance weighting, kriging; see Bivand et al; 2008) to fit surfaces predicting the microbial properties at unsampled locations by weighted averages of surrounding measured values. These methods are useful to show large differences in microbial properties over large areas but local accuracy is limited by the spatial scale of sampling.

More advanced geostatistical approaches can be used to predict a variable of interest at unsampled locations based on known relationships between the dependant variable and other predictor variables (e.g climate, soil type, land cover). Such approaches are commonly used in wider ecology (sometimes termed environmental-, ecological-, or species- distribution modelling: Elith et al, 2006), and can be used to predict either species or communities at unsampled locations (Chapman and Purse, 2011). These environmental correlational approaches have so far been used to predict historical change in soil bacterial biodiversity due to land use at regional scales (Fierer et al, 2013); and also to improve on the interpolated maps of bacterial biodiversity across Great Britain (Griffiths et al, 2011) by modelling the observed relationships between bacterial communities and environmental variables, and then forecasting communities in unsampled locations using remote sensed land cover information

and parent material maps (Henrys et al, 2015). This paper aside there are few studies which have examined in detail the predictive performance of such maps compared to simple interpolation. More widely, large scale spatial predictions of soil parameters are increasingly being disseminated through downloadable map resources (e.g soilgrids.org, ukso.org), and there is now a need to identify specific predictive limitations in order to further improve accuracy (Hengl et al, 2014).

Here as part of this special issue reporting results from the EU FP7 EcoFINDERs project coordinated soil sampling campaign, we seek to assess the bacterial communities in 76 soils sampled across Europe in order to produce a soil bacterial map at the European scale, which can be validated against national scale datasets. We predict that soil pH will be the strongest correlate with measures of community biodiversity, which will then allow us to predict and spatially interpolate communities based on publicly available European scale point data on soil pH (from the LUCAS survey: Tóth et al, 2013). The predictive accuracy of this map will be assessed by comparing predictions with observed point data on bacterial communities collected with similar methods from over 1000 soils across Great Britain (Griffiths et al, 2011). We will also explore whether the predictions from this simple interpolated map can be improved upon, by spatially predicting communities based on existing soil pH maps produced using more advanced environmental correlation approaches (from soilgrids.org and ukso.org).

2. Materials and Methods

2.1. Sampling

Bacterial communities were examined in soils sampled across the EU member states as part of the EcoFINDERs project “transect” sampling campaign, full details of which are prodived elsewhere in this issue (Stone et al, 2015). Briefly, a range of sites spanning a gradient of soil properties (principally pH, organic matter and texture), climatic zones, and

126 land uses (grassland, arable, forest) were targeted for sampling following examination of EU
127 wide datasets (see supplementary material for site locations, S1). Samples were collected at
128 the end of summer 2012 according to standardised protocols to 5cm depth, and sent to a
129 central processing lab for homogenisation and distributing to various partner labs for further
130 analyses. In total eighty-two soils from 11 countries encompassing Arctic to Southern
131 Mediterranean climes of which 76 are assessed in this study. Soil chemical determinations
132 were also conducted by a single laboratory to provide measures of volumetric moisture
133 content, pH (in water), texture, and total/organic carbon (C) and nitrogen (N) contents.

134 **2.2. DNA extraction and community analyses**

135 Total genomic DNA was extracted from all soil samples using a previously described
136 DNA extraction procedure (Plassart et al., 2012). Briefly, 1g of soil was mixed at 70°C with a
137 extraction buffer containing 100 mM Tris-HCl (pH 8), 100 mM EDTA (pH8), 100 mM NaCl,
138 2% (w/v) polyvinylpyrrolidone (40 g mol-1) and 2% (w/v) sodium dodecyl sulphate. Proteins
139 were precipitated from the supernatant with 1/10 volume of 3 M sodium acetate, before
140 nucleic acid precipitation with isopropanol. DNA was further purified through
141 polyvinylpolypyrrolidone (PVPP) Microbiospin minicolumns (BIORAD, Marnes-la-Coquette,
142 France) and finally using the Geneclean Turbo kit (MP-Biomedicals, NY, USA).

143 Bacterial communities were examined using TRFLP as described by Griffiths et al
144 (2011) using the forward primer 63F (5′-CAGGCCTAACACATGCAAGTC-3′) labelled at the
145 5′ end with D4 blue fluorescent dye and reverse primer 530R (5′-GTA TTA CCGCGG CTG
146 CTG-3′). Amplifications were performed in 50 µl reactions under the following conditions:
147 94^0C for 90 s, followed by 35 cycles of 94^0C for 45 s, 55^0C for 1 min and 72^0C for 3 min,
148 followed by a final extension of 72^0C for 10 min. Amplicons were then purified using the ZR-
149 96 DNA clean-up kit (Zymo research, Freiburg, Germany), prior to enzymatic digestion.
150 Purified bacterial DNA was digested with MspI restriction enzyme (New England Biolabs Inc.,
151 Ipswich, MA, USA) at 37^0C for 3 h. Fragment analysis was performed with a Beckman Coulter

CEQ 2000XL capillary sequencer (Beckman Coulter Corporation, California, USA). Peak height data were analysed using GeneMarker software (Softgenetics, LLC, PA, USA). Relative abundances were calculated as the ratio between the fluorescence of each terminal restriction fragment (T-RF) and the total integrated fluorescence of all T-RFs.

2.3. Statistical Analyses

A site by taxon (TRF) relative abundance table derived from the TRFLP analyses was used to explore community relationships with environmental variables, and calculate community scores (ordination site scores and diversity estimates) using standard routines in the vegan library within the R package (R Core Development Team, 2005). Geostatistical calculations, manipulations and plots were also performed within R using the maptools, gstat, raster, and RColorBrewer libraries. Specifically, to produce the bacterial map we used the inverse distance distance weighted (IDW) interpolation method, on account of it's simplicity and widespread application (Lam, 1983). The IDW method predicts a value at an *unsampled* location based on the weighted average of values at *sampled* point locations, with weights decreasing linearly with distance from that location. We used the idw function of the R library gstat to perform the interpolation, using leave one out cross validation to establish the optimum power parameter value (determining how much the weightings decrease with distance) and evaluate the overall performance of the interpolation with respect to predictive power. For both the IDW interpolative mapping, and the prediction contrasts with observed data from a national scale dataset, predictive power was evaluated by assessing the coefficient of determination (R2) and root mean square error (RMSE) between observed and predicted values.

3. Results and Discussion

3.1 Continental scale patterns of soil microbial communities

NMDS ordinations revealed distinct clustering of sampled communities according to land use type (Figure 1). This was further confirmed following multivariate permutation tests using the anosim statistic ($R = 0.28$, $P = 0.0001$). Pairwise comparisons further revealed that bacterial communities in forest soils were most distinct from to arable and grass communities ($R=0.54$, and $R=0.41$ respectively, $p<0.0001$) with the largest differences in community structure consistently observed between forest and arable soils. Arable and grass communities were more similar, yet significant differences were still apparent despite the wide dispersion at the continental scale of sampling units ($R=0.08$, $p<0.05$). Bacterial communities were found to differ between countries ($R = 0.13$, $P < 0.01$). However, this effect could be predominantly attributed to the Swedish soil communities which were all sampled from forest sites and formed a distinct outgroup in the ordination (Figure 1b). When Swedish samples were excluded country of origin had no significant effect (bacteria $R = 0.001$, $P = 0.46$).

Fitting of environmental variables to the ordination scores also confirmed that microbial communities sampled across Europe were strongly correlated with environmental gradients. The dominant five environmental conditions most strongly associated with microbial community structure differences are presented in Table 1. Generally, bacterial community differences were highly correlated with change in soil chemistry and nutrient status, with soil pH showing the strongest relationship, confirming that across large spatial scales the structuring of soil bacterial communities is largely predictable by common soil physicochemical parameters.

These findings further highlight the difficulty in separating direct effects of land use on soil biota versus indirect effects, mediated by changes in soil abiotic properties. It is becoming increasingly apparent that none of these parameters are independent. Human land use is generally influenced by the local pedo-climatic context which determines the economic suitability of different land management options. The baseline pedo-climatic state will naturally create topsoils of distinct properties, which can be further modified by land use depending on

the specific intervention. Different land uses are therefore often accompanied by distinct abiotic soil properties and consequently bacterial biodiversity, given the strong relationships between edaphic properties and soil bacterial communities. For instance, Scandinavian regions are characterised by cold conditions and acidic soils giving rise to more forest and less arable suitability. This in itself does not mean that soil bacterial communities are inherently geographically structured, nor that they are fundamentally driven by the land use of forestry, but is more a reflection of the natural pedo-climatic state which determines both the human land use and the soil biotic and abiotic properties. With respect to contrasts between arable and grassland habitats; whilst arable soils are generally defined by a relatively narrower set of soil properties (e.g high pH and low organic matter) it is possible for grasslands to possess similar properties, particularly if the grassland is part of a arable rotation. Such historical data is not available in this study and such specific contrasts are better addressed in locally focused long term experimental contrasts.

3.2 Predictability and mapping of soil bacterial communities

The site scores for bacterial communities were clearly strongly aligned along the first axis of the NMDS ordination which corresponds with a gradient of soil pH. This afforded the opportunity to extrapolate and predict communities over larger spatial scales using wider soil pH datasets. Such datasets are available across 23 EU member states from the LUCAS topsoil survey (Toth et al, 2013), which provides data on the percentage of coarse fragments, particle size distribution, pH, organic carbon, carbonate content, phosphorous content, total nitrogen content, extractable potassium content, cation exchange capacity and multispectral properties from approximately 20000 soils. We therefore sought to model the relationships between soil pH and bacterial communities from the present survey, and then predict community NMDS scores for the 20000 data points across the EU of soil pH to enable the production of EU wide maps of predicted soil biodiversity using simple interpolative approaches.

The first step was to reliably model relationships between soil pH and the bacterial NMDS scores. Figure 2 shows the relationship observed between soil pH and the first axis bacterial community NMDS scores for the 76 soils assessed in this study. The relationship was visually assessed to be curvilinear and could be modelled with a simple second-degree polynomial (R^2 = 0.93) of the equation:

$$\text{bacterial NMDS}^{\text{axis1}} = -3.748 + 0.9188\text{pH} - 0.04954\text{pH}^2$$

To test the predictive power of the regression equation, we predicted the bacterial community NMDS axis 1 scores from a nationwide survey of over 1000 point measurements conducted across Great Britain using only the measured pH values as predictors (countrysidesurvey.org.uk/). Uniquely, this dataset also comprises bacterial TRFLP profiles (Griffiths et al 2011) therefore allowing us to independently test the predictive power of the regression equation on a different dataset. Despite several differences in methodologies (soil sampling depth, DNA extraction, taxonomic binning) the modelled relationships between soil pH and bacterial community ordination scores from less than 100 soils across Europe provided a reasonable prediction of the bacterial scores in over 1000 soils across Britain (Figure 3). It is noteworthy that the ordination axis scores are themselves arbitrary, and only denote the (dis)similarity between samples analysed at any one time. This fact makes the strong correlations between the EU wide and national scale datasets all the remarkable, and is perhaps testament to the strength and global ubiquity of the relationships between soil pH and bacterial communities, provided a sufficient range of soils are sampled.

To map bacterial communities across the EU member states we then predicted the NMDS axis 1 ordination scores for the ~20000 soils sampled in the LUCAS survey based on pH measurements and the equation outlined above. The LUCAS datasets were downloaded subject to agreements from the JRC European Soil Portal (http://eusoils.jrc.ec.europa.eu/) and a map showing the sampled locations is provided in the supplementary material (S2). Predicted community scores were then mapped using inverse distance weighting

interpolation. We firstly compared interpolative performance using different integer powers parameters (1-5), and the accuracy of predictions assessed by comparing the deviation from the measured data using a leave one out cross-validation procedure. The best performing interpolated map is shown in Figure 4. This was made using an IDW power parameter of 2 which yielded the lowest root mean square error (RMSE) for predicting bacterial NMDS1 scores (0.28), together with the highest precision with respect to the relationship between observed and predicted values (R^2=0.58).

3.3 Features of the map

The map reveals the broad types of bacterial communities found across Europe based on the strong relationships between soil bacterial biodiversity and soil pH. The low (negative) NMDS axis 1 scores reflect communities found in areas such as Scandinavia where acidic and organic rich soils develop due to climatic factors; whereas high values indicate communities found in more productive Southern circum-neutral pH soils, typically with lower organic matter. Areas of contrasting local variability can also be seen in certain regions, where geological factors such as differences in underlying parent material or topography cause local change in communities.

To taxonomically interpret the features of the map we must firstly consider the "meaning" of the first axis ordination scores. The axis 1 ordination scores summarise differences in the broad taxonomic composition and relative abundance of taxa between samples. Additionally, in this study, the scores correlated positively with indices of diversity (Figure 5), with lower scores reflecting low taxonomic diversity. The specific change in abundance of different bacterial taxa across soil pH/diversity gradients has been well studied using sequencing (e.g see Lauber et al 2009; Rousk et al 2010; Griffiths et al, 2011) and can also be inferred to some extent from TRFLP analyses (some illustrative responses of dominant TRFLP peaks are shown the supplementary material, S3). To summarise these responses briefly, acidic and organic rich soils are notably dominated by distinct lineages of

282 acidophilic acidobacteria, as well as alphaproteobacterial taxa. As pH increases over soil
283 environmental gradients, the alphaproteobacteria become dominant, followed by other broad
284 taxonomic groups such as other proteobacteria and the actinobacteria as pH nears neutrality.

285 Neutral soils typically comprise a wider variety of different bacterial taxonomic groups
286 of higher evenness (Griffiths et al, 2011); a phenomena which is at odds with the notion that
287 agricultural soils (often neutral pH) are depauperate with respect to biodiversity (Spurgeon et
288 al, 2013). Potential explanations for higher soil bacterial biodiversity in agricultural soils could
289 be: i) more bacterial taxa exist at neutral pH, due to the requirements of intracellular pH
290 homeostasis (Booth, 1985); ii) soil physical properties in mineral agricultural soils provide
291 more microhabitats promoting evenness (e.g spatial isolation theories, Zhou et al, 2002); and
292 (iii) mineral agricultural soils have less active populations meaning a high diversity of
293 sensescent cells, or even extracellular DNA are being detected. We note also that several
294 studies have reported increased dominance of certain lineages, despite higher phylogenetic
295 diversity, in neutral soils resulting in declines in indices of diversity at higher pH (e.g Fierer et
296 al, 2006). This is apparent to some extent in certain arable and grassland soils in Figure 5
297 which appear to have a marked dominance of alphaproteobacterial TRF peaks, though the
298 underlying causes of this have yet to be fully elucidated. Given the importance of these soils
299 for agricultural production together with recent concerns over soil and food security, the
300 specific controls of neutral-soil taxon abundances, and functional consequences of alterations
301 in abundance, represents a key current knowledge gap.

302

303 **3.4 Map validation and contrasts with other mapping approaches**

304 In order to assess the accuracy of the EU bacterial map wide we contrasted the spatial
305 predictions with observed national scale data from the British Survey. The interpolated map
306 was firstly converted to a raster, and then the predicted NMDS1 scores extracted using the
307 sample locations of the British dataset, prior to correlation with the observed scores (figure

6a). Despite a lack of a good linear fit and a tendency for the spatial predictions to cluster near the overall mean, the interpolation performed reasonably well at predicting the NMDS community scores across Britain (RMSE=0.41, R^2 = 0.29). Despite a lack of strong correlative relationships for lower pH communities, there was evidence that higher scoring (higher pH) community scores could be predicted to some extent. This map of bacterial biodiversity therefore gives a very broad overview of the extreme types of communities likely to be found in different geographic locations across Europe, but for Britain it is of limited use in spatially predicting more subtle differences in communities. Its predictive power is limited by its reliance on the locations of the sampled LUCAS topsoil data points, the design of which has an inherent bias towards agricultural lands (Toth et al 2013). Few samples were taken from large areas of Scottish uplands in the LUCAS survey which may be explain the poor relationships between predicted and observed community scores across Britain. The lack of comparable national scale "test" datasets comprising bacterial data, means we are unable to assess the predictive accuracy of the map for other countries.

To assess whether the predictive accuracy could be enhanced by drawing on more advanced geostatistical predictions of soil pH, we next applied the pH-biodiversity transfer function to two existing soil pH maps: a recently published predictive map at the global scale (SoilGrids: soilgrids.org, Hengl et al 2014) and freely available maps of soil pH at the national scale from Britain (Countryside Survey data from ukso.org). Both these maps were constructed using geostatistical models applied to surveyed pH data to predict unknown values using wider landscape level datasets, using information such as land cover, parent material, climate etc. Maps were downloaded and rasterized where necessary, prior to extracting of pH values based on the GB survey coordinates.

Using the detailed 1km resolution SoilGrids soil pH map offered some small improvements in predicting the national scale bacterial data (RMSE=0.39, R^2 = 0.36) particularly with respect to the acidic habitat scores (Figure 6b). However the predictions

again were focused around the mean, and extreme scores were not particularly well estimated. It was notable that in inspecting the range of soil pH values predicted across the UK and comparing with known UK level data that the extreme values were particularly underestimated in the SoilGrids predictions (e.g predictions of pH 4 or pH 8 soils were over or underestimated respectively). Possible explanations could be related to i) difference in pH determination between the Countryside survey and EU wide LUCAS datasets; ii) undersampling of certain habitats at the EU scale; and iii) geo-statistical artefacts. It is impossible to entirely discount (i) as comparable samples are not available from both surveys, but a cursory inspection of the range of pH values for both datasets indicated there were no systemic differences in the range of pH measurements. With respect to ii) as already discussed, part of the reason for the poor fit on the negative side of the interpolated map is the lack of upland areas sampled in the Lucas survey – meaning the predicted pH for under sampled upland areas such as in Scotland would be higher than in reality. Whilst the SoilGrids predictions utilise global soil pH data in the model, the LUCAS dataset is also a large contributory dataset which could have a significant influence on the predictions. Finally with respect to geostatistical artefacts (iii), the predictive maps of pH provide a mean prediction based on a global model, which will always under or overestimate the higher or lower extremities of the pH range respectively. This could explain why the full range of NMDS1 scores was not adequately reflected in the model predictions.

The 1km resolution national scale pH map performed considerably better in predicting the range of community scores across the Britain (Figure 6c), with better coverage of the extreme ends of the scale, and a much better fit overall (RMSE=0.31, R^2=0.60). However the entire gradient of scores was not well reflected, since here the predictive map was calculated based on modelled relationships between soil pH and categorical variables denoting land cover, along with continuous variables related to parent material. Therefore only a limited number of predicted pH categories are available in this map, constrained by the number of

land cover classes. Again we observed larger predictive error for lower pH soil communities, which could relate to potentially weaker relationships between pH and available land cover classes in these habitats, or less influence of parent material in these generally more organic soils. Another possible reason for the larger relative error for lower scoring communities form acidic habitats could relate to landscape patchiness and the resolution of the maps. For instance, intensively managed parcels of land in the Britain are likely to comprise areas of greater than 1km^2 which is the scale of the UKSO pH map. Human intensified landscapes will typically be more homogenous and of approximately neutral soil pH to favour plant production (either "naturally" or agriculturally driven). This enhances the probability that a mean value per km^2 will reflect a pH measurement at any given point within that square. Conversely, marginal 1km^2 land patches unfavourable for intensive agriculture will have a greater variety of habitats and so the predictive accuracy with respect to point measures is likely to be reduced.

Conclusions

This study characterised bacterial biodiversity and explored environmental correlates in a range of soils sampled across continental Europe. In agreement with previous global studies land use, climate and soil abiotic properties were strongly associated with changes in bacterial communities, with soil pH being the best single correlate. Ultimately these findings point to the general conclusion that broad characteristics of soil bacterial communities can be considered as a dependent soil state variable related to other soil properties (and to some extent human land use); which are ultimately controlled by the independent soil forming factors of climate, relief, parent material, and time (Jenny, 1941). These relationships therefore allow the global prediction of soil bacterial community features over large scales, and we present the first attempt to map bacterial communities across Europe along with a detailed evaluation of the predictions against observed data from national scale surveys of

one of the EU member states. The map performed adequately in predicting community characteristics (ordination axis scores) albeit for opposing ends of the soil biotic/abiotic gradient, and we further demonstrate how the map can be improved by making use of available predictive maps of soil pH, previously calculated using correlations with georeferenced data on wider soil forming factors. In doing so we highlight the current limitations in soil property maps at different geographic scales, with national scale predictions outperforming global scale maps.

To avoid misinterpretation there are some notable caveats which we must stress with respect to the findings of this study and other large surveys of bacterial taxa utilising 16S rRNA amplicon approaches. Firstly when conducting such large scale studies, one necessarily focusses on broad patterns and, particularly in this study using a community profiling technique, broad taxonomic resolution. We therefore do not propose that the map in any way represents similarities between soils in terms of clonal or even species level composition, which may be more governed by local ecological or evolutionary processes (Cho and Tiedje, 2000). Additionally, it is be stressed that soil pH is not the sole driver of differences in bacterial communities, nor should any form of causation be inferred. For instance the role of plant inputs can also affect the relative abundances of taxa over relatively short timescales, with potentially important functional consequences for processes such as carbon cycling (Thomson et al, 2013).

Ultimately these taxonomic limitations will be overcome with wider global adoption of sequencing approaches in soil monitoring networks which will likely enable environmentally driven predictive models of individual taxon abundances (Fierer et al, 2013), rather than using multivariate community estimates and relationships with well characterised soil biotic variables. Whilst field-scale resolution was not the purpose of this mapping exercise, we feel this should be a future ambition of global efforts to characterise soil biodiversity. Our study highlights the benefits of using advances geostatistical approaches for soil biodiversity

mapping using high resolution remote sensed data. It is possible that similar approaches can be applied to other elements of soil biodiversity, including eukaryotes, given enhanced understanding of controlling environmental parameters. Such knowledge can be gained both by efforts to harmonise existing soil biodiversity datasets, but also by increased eukaryotic sampling in national surveys - a realistic possibility now with the availability of rapid molecular tools for eukaryotes (e.g Ramirez et al, 2014). Finally to conclude, our map identifies that predictions are only as good as the surveyed "real" data used to build models. Predictive accuracy will vary depending on the scale of the surveyed data (model inputs) and the spatial extent of the area we seek to predict. Global predictions at high spatial accuracy should therefore be the ultimate goal, which requires increased efforts to standardise and conduct soil biotic and abiotic surveillance at global scales. These advances will be facilitated by better spatial integration of distributed datasets (e.g. global harmonisation of localised climate, geological, remote sensed land cover, and soil datasets) and continued development and validation of mapping predictions against local surveyed data.

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Soil Property	r^2	p	
pH	0.9054	0.001	***
Clay (%)	0.4173	0.001	***
organic C_N_ratio	0.3596	0.001	***
Bulk density (g/cm3)	0.3501	0.001	***
moisture (ml/g)	0.2681	0.001	***
Organic C (%)	0.2624	0.001	***
WHC (ml/g)	0.2521	0.001	***
C (%)	0.2316	0.001	***
Total C_N ratio	0.1961	0.003	**
Silt (%)	0.1372	0.004	**
N (%)	0.1255	0.009	**
Sand (%)	0.0405	0.202	

Table 1. Relationships between soil microbial community ordination axis scores and soil physicochemical properties. Correlations between the NMDS ordination and environmental variables are denoted by r^2 values. Significance (*p*) was determined by 999 permutations.

Figure 1. NMDS ordination of soil bacterial communities sampled across Europe. Soil pH was found to be the best linear fit to the NMDS ordination scores, and is identified in the plots by a colour gradient denoting the pH for each sample. Centroids are also shown representing the mean score per land use type.

Figure 2. Relationship between soil pH and bacterial community NMDS first axis ordination scores from the 76 sampled soils. The second-degree polynomial fit is also displayed together with 95% prediction intervals.

Figure 3. Using the pH-NMDS1 model determined from 76 soils across Europe to predict NMDS1 scores from >1000 soil pH measurements across Britain. The value for the predictions is shown on the y axis, whereas the x axis denotes the actual observed bacterial community scores from the study of Griffiths et al, 2011. The line shows the fitted least squares regression between the observed and predicted values (R^2 = 0.8).

Figure 4. Interpolated map showing predicted bacterial community ordination scores across EU member states. Colour scale indicates predicted first axis NMDS scores, with negative

scores indicating acidic soils (bogs, acid grassland, upland woods etc) and positive scores indicating communities from more neutral pH soils (productive grassland, arable etc)

Figure 5. Relationship between NMDS first axis scores for bacterial communities and univariate indices of diversity (line denotes a loess fit). Increases in NMDS scores are generally indicative of an increase in bacterial diversity.

Figure 6. Validating spatially mapped predictions of bacterial NMDS scores against national scale survey data for Britain. In all plots the observed data are the actual community scores reported from over 1000 soils sampled across Great Britain (Griffiths et al 2011). Predicted community scores are based on the modelled relationships between bacterial communities and pH from the EcoFINDERs transect sampling fitted to different spatial estimates of soil pH: a) spatial interpolation of community scores predicted from EU wide point data on soil pH (this study); b) geostatistical predictions of topsoil pH values at the global scale (soilgrids.org, Hengl et al, 2014); c) geostatistical predictions of topsoil pH values at the national scale (ukso.org, and see Henrys et al; 2014). Solid red lines show the fitted least squares regression between the observed and predicted values (with associated R^2 displayed in the top right of each plot); and dashed lines display loess fits to illustrate deviations from the linear fit.

Figure 1. NMDS ordination of soil bacterial communities sampled across Europe.

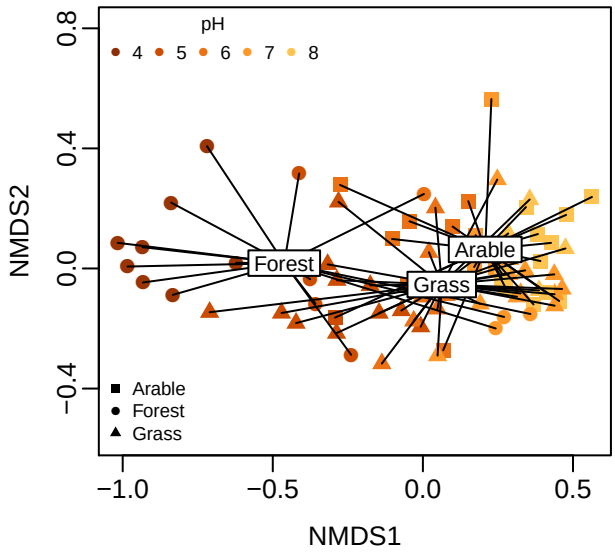

Figure 2. Relationship between soil pH and bacterial community NMDS first axis ordination scores from the 77 sampled soils.

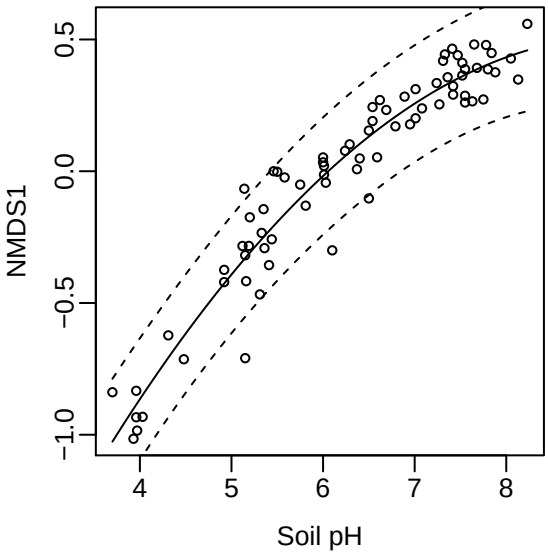

Figure 3. Using the pH-NMDS1 model determined from 77 soils across Europe to predict NMDS1 scores from >1000 soil pH measurements across Britain.

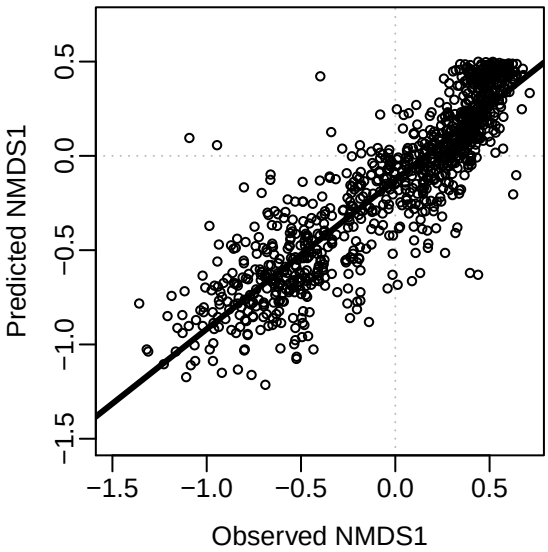

Figure 4. Interpolated map showing predicted bacterial community ordination scores across EU member states.

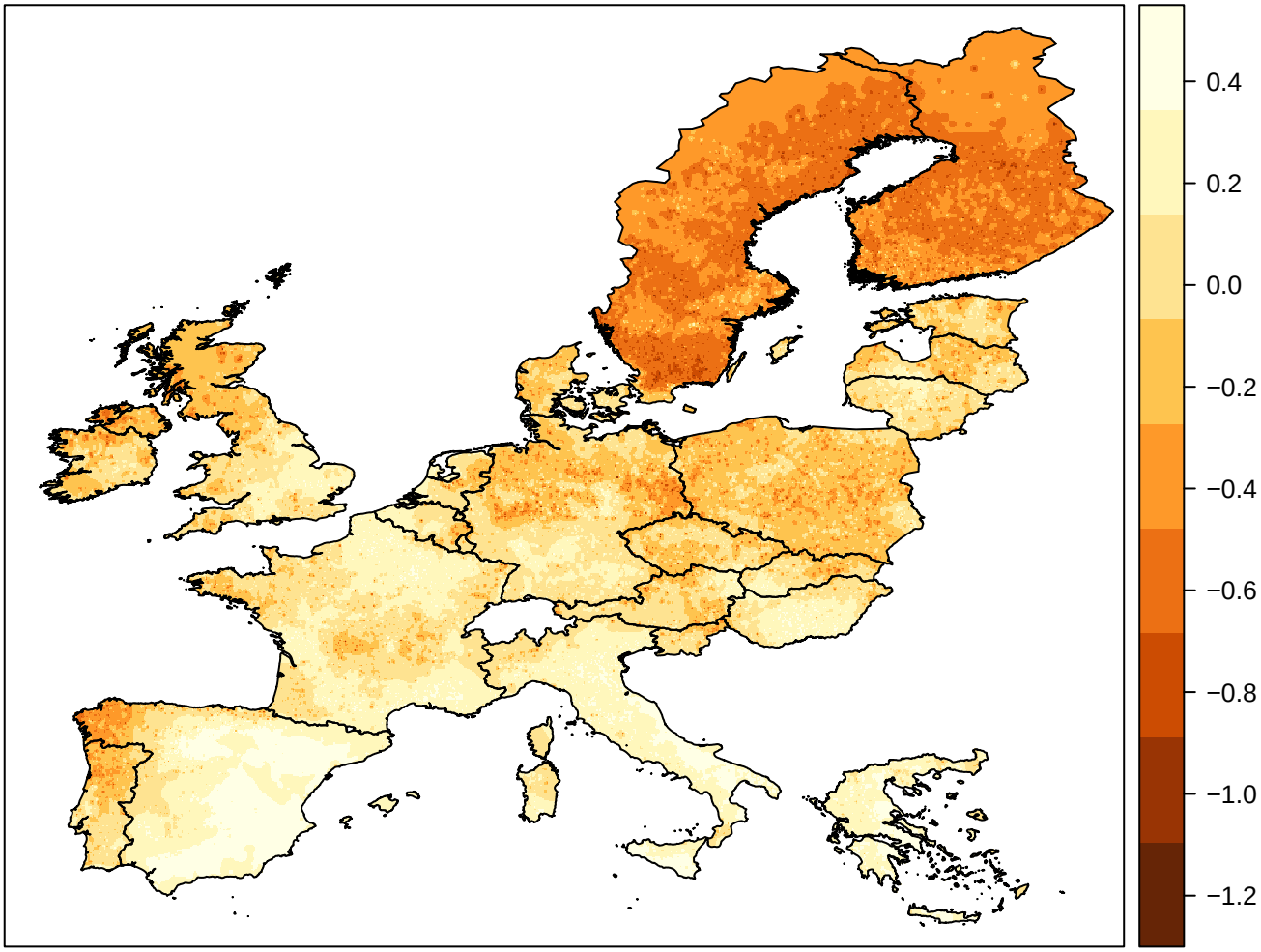

Figure 5. Relationship between NMDS first axis scores for bacterial communities and univariate indices of diversity

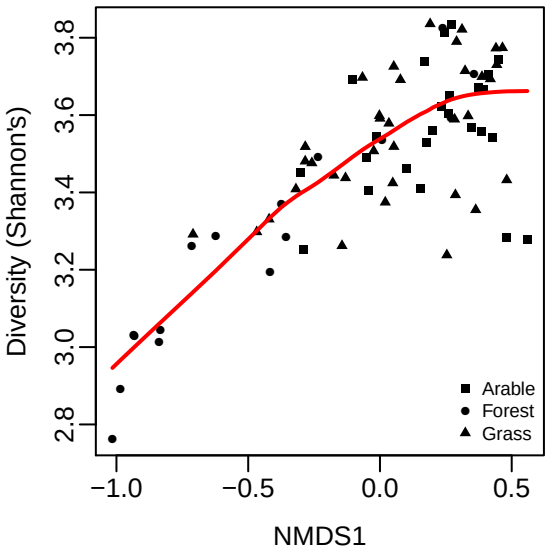

Figure 6. Validating the spatially mapped predictions of NMDS scores against national scale survey data for Britain.