

The pH optimum of soil exoenzymes adapt to long term changes in soil pH

Article

Accepted Version

Creative Commons: Attribution-Noncommercial-No Derivative Works 4.0

Puissant, J., Jones, B., Goodall, T., Mang, D., Blaud, A., Gweon, H. S. ORCID: <https://orcid.org/0000-0002-6218-6301>, Malik, A., Jones, D. L., Clark, I. M., Hirsch, P. R. and Griffiths, R. (2019) The pH optimum of soil exoenzymes adapt to long term changes in soil pH. *Soil Biology and Biochemistry*, 138. 107601. ISSN 0038-0717 doi: <https://doi.org/10.1016/j.soilbio.2019.107601> Available at <https://centaur.reading.ac.uk/86780/>

It is advisable to refer to the publisher's version if you intend to cite from the work. See [Guidance on citing](#).

To link to this article DOI: <http://dx.doi.org/10.1016/j.soilbio.2019.107601>

Publisher: Elsevier

All outputs in CentAUR are protected by Intellectual Property Rights law, including copyright law. Copyright and IPR is retained by the creators or other copyright holders. Terms and conditions for use of this material are defined in the [End User Agreement](#).

www.reading.ac.uk/centaur

CentAUR

Central Archive at the University of Reading

Reading's research outputs online

1 Soil extracellular enzymes are locally adapted
2 toward soil pH

3

4 Jérémy Puissant^a, Briony Jones^{b,c}, Tim Goodall^a, Dana Mang^a, Aimeric Blaud^{b1}, Hyun Soon
5 Gweon^{a,e}, Ashish Malik^a, Davey L. Jones^c, Ian M. Clark^d, Penny R Hirsch^d, Robert Griffiths^b

6

7 ^a Centre for Ecology & Hydrology, Maclean Building, Benson Lane, Crowmarsh Gifford,
8 Wallingford, Oxfordshire OX10 8BB, United Kingdom

9 ^b Centre for Ecology & Hydrology, Environment Centre Wales, Deiniol Road, Bangor,
10 Gwynedd, LL57 2UW, United Kingdom

11 ^c School of Natural Sciences, Bangor University, Deiniol Road, Bangor, Gwynedd, LL57 2UW,
12 United Kingdom

13 ^d Dept. Sustainable Agriculture Sciences, Rothamsted Research, Harpenden, AL5 2JQ, United
14 Kingdom

15 ^e School of Biological Sciences, University of Reading, RG6 6AS, United Kingdom

16

17 Corresponding author: Tel.: +44 1491692547; E-mail address: jeremy.puissant@gmail.com

18 ¹ Current address: School of Applied Sciences, Edinburgh Napier University, Sighthill campus,
19 Edinburgh, EH11 4BN, United Kingdom.

20

21

22

23

24 **Abstract**

25 Soil extracellular enzymes released by microorganisms break down organic matter and are crucial
26 in regulating C, N and P cycling. Soil pH is known to influence enzyme activity, and is also a
27 strong driver of microbial community composition; but little is known about how alterations in
28 soil pH affect enzymatic activity and how this is mediated by microbial communities. To assess
29 long term enzymatic adaptation to soil pH, we conducted enzyme assays at buffered pH levels (2.5
30 to 10, 0.5 interval) on two historically managed soils maintained at either pH 5 or 7 from the
31 Rothamsted's Park Grass Long-term experiment). The pH optima for a range of enzymes was
32 found to differ between the two soils, the direction of the shift being toward the source soil pH,
33 indicating the production of pH adapted isoenzymes by the soil microbial community. Soil
34 bacterial and fungal communities determined by amplicon sequencing were found to be clearly
35 distinct between pH 5 and soil pH 7 soils, possibly explaining differences in enzymatic responses.
36 Furthermore, β -glucosidase sequences extracted from metagenomes revealed an increased
37 abundance of Acidobacteria in the pH 5 soils, and increased abundance of Actinobacteria in pH 7
38 soils; these taxonomic shifts were more pronounced for enzymatic sequences when compared with
39 a number of housekeeping gene sequences. Particularly for the Acidobacteria, this indicates that
40 broad taxonomic groups at phylum level may possess enzymatic adaptations which underpin
41 competitiveness in different pH soils. More generally our findings have implications for modelling
42 the efficiency of different microbial enzymatic processes under changing environmental
43 conditions; and future work is required to identify trade-offs with pH adaptations, which could
44 result in different activity responses to other environmental perturbations.

45

46

47 Keywords: Extracellular enzyme, soil pH, liming, adaptation, Park Grass

48 **1. Introduction**

49 Soil microbes produce exoenzymes to degrade complex plant and soil organic matter (OM)
50 into smaller compounds, which are then assimilated for growth and metabolism (Allison, 2005).
51 These proteins break down large OM compounds through hydrolytic and oxidative processes
52 (Burns et al., 2013; German et al., 2011; Sinsabaugh, 2010) and their activity rates have been
53 hypothesized to be a rate-limiting step in OM decomposition (Bengtson and Bengtsson, 2007).
54 Enzyme activity is predominantly controlled by temperature and pH which affect enzyme kinetics
55 through change in substrate binding and stability. In contrast to intracellular enzymes, the physico-
56 chemical conditions in which exoenzymes operate are poorly controlled by microorganisms and
57 activity rates are thus influenced by local conditions (e.g pH). Thus, to cope with their local
58 environment, microorganisms evolve to produce different types of enzyme (isoenzyme), resulting
59 in equivalent functionality but with altered thermodynamic and kinetic properties. For example,
60 cold adapted enzymes, are believed to exhibit higher conformational flexibility within their active
61 site or protein surface to become more efficient at lower temperatures due to a decrease in the
62 enzyme activation energy (E_a) (Åqvist et al., 2017). However exoenzymes adaptation results in
63 various trade-offs between efficiency and enzyme stability (Åqvist et al., 2017; Zanphorlin et al.,
64 2016); meaning both specific exoenzymes catalyzed processes as well as other non-specific
65 microbial processes may be affected by a changing environment. Though it is known that microbes
66 can tune the properties of EE they produce to adapt to new conditions, little is known about the
67 drivers, mechanisms and timescale of such adaptations in natural habitats such as soil.

68 To date, in soil systems much research has focused on enzyme adaptation to cold
69 temperatures and extreme environmental conditions (Åqvist et al., 2017) with little reporting of
70 adaption to other edaphic properties. Soil pH is one of the main variables affected by global change
71 through agricultural intensification, climate change and other polluting events such acid rain. In
72 addition, pH is known to be one of the main factors affecting soil microbial diversity and function
73 (Fierer et al, 2017; Griffiths et al., 2011, Malik et al, 2018). How changes in soil pH affect
74 microbial life constraints is poorly understood, but should be addressed to better understand
75 microbial ecophysiology, competition and efficiency in degrading substrates across different soil
76 systems. This is especially true when considering pH constraints on enzyme catalytic efficiency in
77 cycling essential nutrients (C, N, and P) from organic matter compounds, and determining how
78 that may impact soil microbial function and decomposition rates. Moreover, recent C
79 decomposition models now explicitly integrate enzyme kinetics (Allison, 2012; Davidson et al.,
80 2012; Wang et al., 2013) but little empirical data on enzyme kinetic parameters under changing
81 environmental conditions are available. Currently, there is little understanding in the degree to
82 which microbial extracellular enzymes can be or are adapted to their local soil pH, a factor which
83 could help explain different functional responses across different soil systems.

84
85 In order to evaluate potential exoenzymatic adaptation to local soil pH, we conducted enzymatic
86 assays at a range of buffered pH levels (from 2.5 to 10, 0.5 interval) on soil of the Park Grass long-
87 term experiment (Rothamsted) which had been maintained at either pH 5 or 7 for over 100 years.
88 Hydrolytic exoenzymes studied were selected to correspond to enzymes involved in organic
89 carbon, nitrogen and phosphorus cycling. We hypothesise that enzyme pH optimum will be
90 affected by ancestral soil pH treatment, with soil exoenzymes from soil pH 5 being more adapted

91 towards acidic conditions and exoenzymes from soil pH 7 more adapted towards more neutral or
92 alkaline conditions. To better understand the microbial community relationships underpinning EE
93 pH adaptation, we investigated the change in microbial community composition (bacteria and
94 fungi) with amplicon sequencing, and functional genes using a metagenomics sequencing
95 approach. Specifically, we wished to determine whether change in enzyme activity is associated
96 with change in specific microbial enzyme producers or adaptation of exoenzymes to environmental
97 conditions.

98

99 **2. Method**

100 **2.1 Soil sampling**

101 The Park Grass Long-term experiment (Rothamsted, UK, McDonald 2018), originally started in
102 1856 on permanent pasture to investigate ways of improving hay yields, is managed with a range
103 of fertilisers and pHs with the hay cut twice a year. Soils cores (0-15cm, 4 cm Ø) were sampled
104 on the 27th of November 2015 in subplots 'a' (pH ~ 7) and 'c' (pH ~ 5) of the Nil plot 12, which
105 has never received any fertilisers (Storkey et al., 2016). The soil pH is regularly monitored and
106 controlled by liming, in subplot 'a' to reach pH~7 since 1903 (every 4 yr and then every 3 yr from
107 1976), in subplot 'c' to reach pH~5 since 1965 (every 3 yr). However, because the natural soil pH
108 was 5.4-5.6, the Nil plot received little liming. Five samples were taken in a straight line in each
109 plot.

110

111 **2.2 Basic characterization of bulk soil samples**

112 Gravimetric soil moisture content was determined by drying 15g of soil at 105 °C for 48 h. All
113 other chemical analyses were performed using sieved soil (2mm) and dried (40 °C). Soil pH was

114 measured in H₂O (1:5 weight:vol) according to the protocol NF ISO 10390 (2005). Soil organic
115 carbon C, total nitrogen (N) and total phosphorus (P) were measured according to CS Technical
116 report No. 3/07 (Emmett et al., 2008). The chemical fingerprint of soil samples was assessed using
117 mid-infrared (MIR) spectroscopy. Dried soil samples were ball-milled and further dried overnight
118 at 40 °C to limit interferences with water, without altering OM chemistry. Milled samples were
119 analyzed using a Nicolet iS10 FT-IR spectrometer (Thermo Fisher Scientific Inc., Madison, WI,
120 USA). Spectral acquisition was performed by diamond attenuated total reflectance (MIR-ATR)
121 spectroscopy over the spectral range 4,000–650 cm⁻¹, with spectral resolution of 8 cm⁻¹ and 16
122 scans per replicate.

123

127 **2.3 Enzyme assays**

128 Hydrolytic soil extracellular enzyme activities of β-glucosidase (GLU, EC number: 3.2.1.21,
129 substrate: 4-MUB-β-D-glucopyranoside), acetyl esterase (ACE, EC number: 3.1.1.6, substrate: 4-
130 MUB-acetate), phosphatase (PHO, EC number: 3.1.3.1, substrate: 4-MUB-phosphate), and
131 leucine-aminopeptidase (LEU, EC number : 3.4.11.1, substrate: L-Leucine-7-AMC) were
132 measured by fluorogenic methods using methylumbelliferyl (MUB) and 7-amino-4-
133 methylcoumarin (AMC). PHO, GLU, ACE and LEU are involved in phosphorus mineralization,
134 release of glucose from cellulose, deacetylation of plant compound and degradation of protein into
135 amino acids, respectively. Enzyme assays were performed according to Turner et al. (2010) with
136 modifications. A range of buffered pH solutions (from 2.5 to 10, in increments of 0.5) was prepared
137 by adjusting 50mL of modified universal buffer with 1.0M HCl and 1.0M NaOH, at 20°C, then
138 diluting to 100mL with deionized water. For each sample, a soil slurry was prepared by adding
139 20mL deionized water to 0.5g of soil, then rotary shaking on a magnetic plate for 20min at 28°C.

140 10mL of this soil solution was diluted in 25mL of deionized water to give a 1:100 soil-to-water
141 ratio. Enzyme reactions were measured in 96-well microplates containing 50 μ L of the specific
142 buffer, 50 μ L of soil slurry and 100 μ L of substrate solution (saturated concentration, 200 μ M).
143 Microplates were then incubated in the dark for 4 hours at 28 °C, with one fluorometric
144 measurement every 30 minutes (BioSpa 8 Automated Incubator) to follow the kinetic of the
145 reaction.

146 For each sample, three methodological replicates (sample + buffer + substrate) and a quenched
147 standard (sample + buffer + 4-MUB or 7-AMC) were used. Quenching curves were prepared with
148 a serial dilution of 4-MUB solution for different amount of fluorophore in well (3000, 2000, 1000
149 pmol). For each substrate, a control including the 4-MUB- or 7-AMC-linked substrate and the
150 buffer solution alone were used to check the evolution of fluorescence without enzyme degradation
151 over the duration of assay. The fluorescence intensity was measured using a Cytation 5
152 spectrophotometer (Biotek) linked to the automated incubator (Biospa 8, Biotek) and set to 330
153 and 342 nm for excitation and 450 and 440 nm for emission for the 4-MUB and the 7-AMC
154 substrate, respectively. All enzyme activities were calculated in nmol of product per minute per g
155 of dry soil and normalized per the highest enzyme activity value measured at the pH optimum in
156 order to express enzyme activity as relative activity in percentage.

157

158 **2.4 Soil microbial community composition**

159

160 For sequencing analyses of bacterial and fungal communities, DNA was extracted from 5 replicate
161 soil samples per treatment using 0.25 g of soil and the PowerSoil-htp 96 Well DNA Isolation kit
162 (Qiagen) according to manufacturer's protocols. The dual indexing protocol of Kozich et al (2013),
163 was used for Illumina MiSeq sequencing of the V3-V4 hypervariable regions of the bacterial 16S

164 rRNA gene using primers 341F (Muyzer et al., 1993) and 806R (Youngseob et al., 2005); and the
165 ITS2 region for fungi using primer ITS7f and ITS4r, (Ihrmark et al., 2012). Amplicon
166 concentrations were normalized using SequalPrep Normalization Plate Kit (Thermo Fisher
167 Scientific) prior to sequencing on the Illumina MiSeq using V3 chemistry. Fungal ITS sequences
168 were analysed using PIPITS (Gweon et al., 2015) with default parameters as outlined in the
169 citation. A similar approach was used for analyses of bacterial sequences, using PEAR (sco.h-
170 its.org/exelixis/web/software/pear) for merging forward and reverse reads, quality filtering using
171 FASTX tools (hannonlab.cshl.edu), chimera removal with VSEARCH_UCHIME_REF and
172 clustering to 97% OTUs with VSEARCH_CLUSTER (github.com/torognes/vsearch). The
173 Illumina MiSeq sequencing generated in average per sample 28205 reads for 16S rRNA gene and
174 40406 for ITS2 region.

175

176 **2.5 Metagenome Sequencing**

177 DNA was extracted from 2g of soil from 4 field replicates for the two pH treatments using the
178 PowerMax Soil DNA Isolation kit (Qiagen), and subsequently concentrated and purified using
179 Amicon® Ultra filters. Illumina libraries were constructed using the Illumina TruSeq library
180 preparation kit (insert size < 500- 600 bp) and paired-end sequencing (2 x150 bp) was conducted
181 using the Illumina HiSeq 4000 platform. Prior to annotation, Illumina adapters were removed from
182 raw fastq files using Cutadapt 1.2.1 (Martin, 2011), reads were trimmed using Sickle (Joshi and
183 Fass, 2011) with a minimum window quality score of 20 and short reads were removed (<20bp).
184 Preliminary analysis was conducted using MGRAST to functionally annotate with SEED
185 subsystems and taxonomically annotate with refseq. For more detailed analyses of β -glucosidase
186 sequences, all reads from the 8 samples were co-assembled using MEGAHIT (Li et al., 2015) with

187 a minimum contig length of 1000. Sequences were translated and open reading frames were
188 predicted using FragGeneScan (Rho et al., 2010). Contigs were assigned CAZY (Carbohydrate-
189 Active enZYmes) subfamilies (Lombard et al., 2014) using a hmmer search (Finn et al., 2011)
190 against dbCan2 profiles with an e-value of 1e-15 (Zhang et al., 2018). Contigs were taxonomically
191 annotated against the NCBI Blast non-redundant protein database using Kaiju, a fast translated
192 method, which identifies protein-level maximum exact matches (MEM's) (Menzel et al., 2016).
193 Regions of contigs annotated as relevant β -glucosidase CAZY domains (GH1, GH2, GH3, GH5,
194 GH9, GH30, GH39, GH116) were extracted. To identify pH associations of these sequences, DNA
195 reads were mapped back to assembled domain protein sequences using BlastX, mappings with an
196 identity percentage of < 97% and/or an e-value of > 0.001 were discarded. Mapping outputs were
197 used to identify the relative abundance of assembled domain sequences across pH5 and pH7
198 samples, multinomial species classification method (CLAM) (Chazdon et al., 2011) was used to
199 classify pH generalists and specialists and to discount sequences that were too rare for meaningful
200 categorisation.

201

202 **2.7 Statistical analysis**

203 The effects of assay pH, soil field pH treatment and their interactions were assessed by repeated
204 measures ANOVA. Fixed factors were sampling “assay pH” and “soil field pH”, while soil field
205 replicate was added as a random factor. One-way ANOVA was used to test the effects of enzymatic
206 pH reaction on soil enzyme relative at each pH step (from 2.5 to 10). Differences in relative
207 abundances of microbial taxa between soil pH 5 and soil pH 7 was assessed with one-way
208 ANOVA. Assumptions of normality and homoscedasticity of the residuals were verified visually
209 using diagnostic plots and a Shapiro-Wilk test. To identify soil bacterial and fungal community

210 composition patterns, a principal component analysis (PCA) based on Hellinger-transformed OTU
211 data was performed (Legendre and Gallagher, 2001). Permutational multivariate ANOVA
212 (PERMANOVA) was used to test the effect of soil pH field treatment on soil microbial community
213 composition. All statistical analyses were performed under the R environment software (R
214 Development Core Team 2011), using the R packages vegan (Oksanen et al., 2013) and ade4 (Dray
215 and Dufour, 2007). Fourier-transform infrared spectroscopy (FTIR) spectral data were further
216 processed and analyzed using the hyperSpec package (Beleites and Sergo, 2011),

217

218 **3. Results**

219 **3.1 Soil characteristics**

220 The pH values of the two soils were confirmed to be consistent with the treatments applied, with
221 pH measured at 5.5 and 7.5 for the pH 5 and pH 7 plots, respectively (supplementary figure).
222 Liming soil from pH 5 to pH 7 significantly increased by ~20% soil carbon content and soil total
223 nitrogen (Table 1). Soil moisture, total P and C:N were not significantly different between soil pH
224 5 and soil pH 7 (Table 1). Soil infrared mid-infrared spectroscopy was used to fingerprint soil
225 mineralogy and to assess heterogeneity within and between the two soil pH field treatments. The
226 fingerprints confirm that soil mineralogy is consistent within and between pH field treatments
227 (supplementary figure). The most prominent feature of the FTIR spectra corresponded to peaks
228 indicative of phyllosilicate mineral compound absorption (kaolinite) with peaks at 3696, 3621,
229 1003, 912, 692 cm^{-1} (Dontsova et al., 2004). The 774 cm^{-1} peak is likely to be an indicator of quartz
230 content and the 1642 cm^{-1} peak corresponds to the H–O–H bending band of water (Stuart, 2004,
231 Dontsova et al., 2004). Small differences in peak amplitude between pH 5 and pH 7 soils are the
232 result of small changes in the relative concentrations of compounds but overall the two soils

233 presented very similar mineralogy profiles (according to the peak wavelength positions) which
234 indicates a shared ancestral origin.

235

236 **3.2 Soil microbial community composition**

237 The composition of soil bacterial and fungal community determined by amplicon sequencing (16S
238 rRNA genes and ITS region, respectively) were clearly distinct between soil pH 5 and pH 7 for
239 both communities (Fig.1; PERMANOVA: $R^2 = 0.82$, p-value: <0.001 for fungal community and,
240 $R^2 = 0.51$, p-value: <0.01 for bacterial community). As observed on the PCA (Fig.1) and
241 PERMANOVA results, fungal community structure was more affected than bacterial community
242 by the liming treatment. Stacked bar plots representing the relative proportions of microbial phyla
243 demonstrated relatively greater changes in the fungal compared to the bacterial community from
244 pH 5 to pH 7 (Fig.1). Basidiomycota was significantly more abundant at soil pH 5 (83%, p-value:
245 <0.001 , Fig.1) whereas their relative abundance decreased at soil pH 7 (36%) to the advantage of
246 Ascomycota and Zygomycota taxa (30% and 24% at soil pH 7 compared to 4.5% and 4% at soil
247 pH 5, p-value: <0.01 , respectively, Fig.1). Concerning the bacterial community, higher relative
248 abundance of the phyla Acidobacteria and Verrucomicrobia was observed at pH 5 versus pH 7
249 (22% vs 16%, p-value: 0.02; 26% vs 18%, p-value: <0.01 , respectively Fig.1). In contrast, higher
250 relative abundance of Proteobacteria and Actinobacteria phylum was observed at pH 7 versus pH
251 5 (33% vs 27%, p-value: 0.01; 11% vs 7%, p-value: <0.01 , respectively Fig.1).

252

253 **3.3 Extracellular enzyme pH optimum assays**

254 The pH of the enzymatic reaction had a highly significant impact on the catalytic efficiency of all
255 enzymes examined (Fig.2, Table 2). At extremely low pH (2.5), activity was low or could not be

256 detected for leucine aminopeptidase and acetate esterase. For each enzyme, changes in the assay
257 pH strongly impacted the relative enzyme activity with a 15-fold increase between lowest and
258 highest activity at the pH optimum (Fig.2). After reaching the optima, the activity decreased more
259 or less rapidly depending on the assay. Regardless of the initial pH of the soil, pH optima appeared
260 to be specific to the enzyme studied (Fig.2). The pH optimum of leucine aminopeptidase and acetyl
261 esterase enzymes were close to neutrality, with an average pH optimum at 7.2 and 6.7, respectively
262 (Fig.2). The pH optima for β -glucosidase enzyme was acidic with an average of pH 4.3 (Fig.2).
263 Two pH optima were observed for phosphomonoesterase, one acidic (pH 5.7) and the other
264 alkaline (pH 10), although the alkaline optima may not have been fully reached.

265
266 Maintaining field soil at either pH 5 or pH 7 for over 100 years had a strong significant impact on
267 the pH optimum of all enzymes (Table 2). Enzyme pH optima shifted between acidic and alkaline
268 soil whatever the enzyme considered, though this was more pronounced for phosphatase, β -
269 glucosidase and acetate esterase compared to leucine-aminopeptidase. The interaction between
270 enzymatic assay pH and field soil pH was significant for each enzyme assayed, indicating that the
271 magnitude of the difference in enzyme activity between pH 5 and pH 7 soil is dependent upon
272 enzymatic assay reaction pH (Table 2). For each enzyme, optimum activity differed between the
273 two soils by 0.5 pH units. Similar optimal activities were found for acetate esterase and leucine
274 aminopeptidase, while the activity of β -glucosidase and phosphatase was reduced by 4-6% in a pH
275 7 soil. A second optimum at pH 10 was observed for phosphatase and acetyl esterase from pH 7
276 soil, in contrast to little or no activity of these enzymes from pH 5 soil (Fig. 2D). Additionally, the
277 relative activity of enzymes from pH 5 soil was always higher in acidic assay conditions ($<$ pH

278 5.5), while the relative activity of enzymes from pH 7 soil was always higher in more alkaline
279 conditions (> pH 7).

280

281 **3.4 Soil metagenomics**

282 The amplicon sequencing results revealed large shifts in broad taxa between the two soils.,To
283 determine whether similar shifts were also observed in associated enzymatic gene production
284 shotgun metagenomes generated from the same soils was utilized. We focussed our analyses on
285 bacterial β -glucosidases, since the bacteria dominate soil metagenomics gene libraries (Malik et,
286 2017) and the β -glucosidases are genetically well characterized enzymes, known to be important
287 for soil carbon transformations. Analyses of the functional and taxonomic annotations of β -
288 glucosidase related genes using MGRAST revealed they were relatively more abundant
289 insequences from the Acidobacteria in the pH 5 compared to pH 7 soils (15.9% vs 1.9%, p-value:
290 7.4×10^{-5} ; Fig.3 A), while this was reversed in sequences from Actinobacteria in pH 7 compared
291 to pH 5 soils (34.6% vs 43.4%, p-value: 6×10^{-3} ; Fig.3 A). When normalized by housekeeping
292 genes abundances, Acidobacteria β -glucosidase gene abundance were significantly enriched in pH
293 5 soil compared with pH 7 soil (Fig. 3 B) being on average twice as abundant.

294 It is clear that Acidobacterial β -glucosidases are a unique feature of the more acid soils and
295 therefore can be highly implicated as responsible for the pH related differences in enzyme activity.

296 However, this does not rule out that other phyla may have distinct pH-selected sub clades which
297 could also be responsible. To address this, we sought to classify individual taxa according to pH
298 association, by assembling contigs based on the pooled metagenomics sequence reads (all samples
299 from pH 5 and pH 7 soils); extracting β -glucosidase sequences using a hmmer search against
300 dbCan2 profiles; and then mapping back individual reads to these sequences. Sequences were then

301 classified as pH specialists, generalists or too rare to categorise using multinomial species
302 classification method (CLAM). The majority of Acidobacteria sequences were classed as pH 5
303 specialists, this suggests that not only is there a higher relative abundance of Acidobacteria β -
304 glucosidase sequences at pH 5 but that the majority of these sequences appear to be unique to pH
305 5 soils (Fig. 4). Sequences annotated as other dominant phyla such as Actinobacteria and
306 Proteobacteria appeared to have a higher proportion of pH 5 specialist and generalist sequences
307 (supplementary table), whilst Verrucomicrobia included a clear sub-clade of pH 7 specialist
308 sequences (Fig. 4).

309

310 **4. Discussion**

311 The activity of enzymes involved in C, N and P cycles were all found to be strongly
312 dependent on the pH of the assay. Beta-glucosidase had an acidic pH optimum (pH=4.3), which is
313 generally observed for glycosidase enzymes (Niemi and Vepsäläinen, 2005; Sinsabaugh et al.,
314 2008; Turner, 2010), whereas leucine aminopeptidase had an alkaline pH optimum (7.2) as is
315 commonly reported for proteases (Niemi and Vepsäläinen, 2005; Sinsabaugh et al., 2008). Acetyl
316 esterase pH optima were at pH 7 for both soils studied, also in line with previous findings (Degrassi
317 et al., 1999 and Humberstone and Briggs, 2000 respectively). However, source soil pH had a
318 significant and strong impact on soil exoenzyme pH optimum response curves. For each enzyme
319 studied, extracellular enzymes originally from pH 5 soil were more adapted towards acidic pH
320 conditions, whereas pH 7 soil possessed enzymes adapted towards more alkaline conditions
321 (Fig.2). Interestingly, the enzymatic pH optima observed in this study did not correspond exactly
322 to the local soil pH, presumably due to constraints within the active sites that enable
323 physicochemical function to be maintained. It is possible that the responses observed are due to

324 the presence of isoenzymes, which have different kinetic properties adapted toward the local soil
325 pH. Alkaline and acid phosphatases are the most studied example of soil isoenzymes (Nannipieri
326 and al, 2011), and our phosphatase pH response curves illustrate this with a marked bimodal
327 distribution, and extremely low activity for the pH 7 soil compared to the pH 5 soil, at acidic assay
328 pH. Acetyl esterase also exhibited a bimodal response but only in the pH 7 soil, which also
329 exhibited a second pH optimum developing at pH 10.

330 Previous studies have observed different pH optima for the same enzyme across different
331 soil types (Niemi and Vepsäläinen, 2005; Turner, 2010), though the specific causes were not
332 empirically assed. Mechanisms proposed include either abiotic stabilization by soil chemical
333 properties which alter the conformation of the enzyme and thus kinetics; or differences in the
334 microbes that produce the enzymes. Our experiment, conducted on the same soil type, provides
335 strong evidence for microbial control, mediated through altered soil pH. Shifts in enzyme pH
336 optima due to enzyme sorption to different clay types (Leprince and Quiquampoix, 1996; Ramirez-
337 Martinez and McLaren, 1966; Skujins and al., 1974) was discounted as IR based soil chemistry
338 fingerprints (incorporating information on clay content) were very similar between the pH 5 and
339 pH 7 soils (Supplementary Fig.2). Moreover, the dilution factor used to perform enzyme assays
340 1:100 soil-to-water ratio helped to reduce potential effect of small increases in soil organic matter
341 content and total nitrogen observed between the pH 5 and pH 7 soils. Further strong evidence for
342 biotic mechanisms is provided by the consistent non-random shift in optima towards the source
343 soil pH and the presence of bi-modal pH optimum curve indicating clearly the presence of
344 isoenzyme.

345 Our data suggest that differences in microbial communities underpin the observed
346 functional responses. Bacterial and fungal communities were found to be clearly distinct between

347 the two pH soils investigated, as anticipated from previous work in the Park Grass long-term
348 experiment (Zhalnian et al., 2015; Liang et al., 2015). Such differences in microbial community
349 composition are likely to be responsible for the production of different versions of the same
350 enzyme (Fig.2). For example, the Acidobacteria phylum has been reported to possess more diverse
351 and abundant genes encoding for carbohydrate-decomposing enzymes than Proteobacteria (Lladó
352 et al., 2019; Lladó et al., 2016) which could be responsible for shift observed in bulk soil beta-
353 glucosidase pH optimum between pH 5 and pH 7 soils. The metagenomics results clearly showed
354 that different proportions of bacterial phyla produced β -glucosidases across the two soils. Notably,
355 the Acidobacteria contributed more to the β -glucosidase gene pool in the acid soil, and this
356 contribution was more marked than would be expected from examining abundances based on
357 housekeeping genes alone. Furthermore, sub clades of acidobacterial glucosidase were unique in
358 being exclusively found in acid soils, with other broad taxa possessing both generalist enzymes,
359 and a mix of pH specialized genes for either acid or neutral pH. This indicates that acidophilic
360 acidobacterial lineages may possess enzymatic adaptations which underpin their demonstrated
361 competitiveness in acidic soils (Griffiths et al, 2011), and confirms recent genomic studies which
362 have identified enzyme production for carbohydrate degradation as a key feature of these
363 organisms (Eichorst et al, 2018).

364 As soil microorganisms can depend upon proximate decomposition agents for acquiring resource
365 and energy, the efficiency of (costly) extracellular enzymes may represents a fundamental
366 competitive trait (Wallenstein et al. (2011). Here we provide evidence that beta-glucosidase
367 enzymes from pH 5 soil are half as efficient as those from pH 7 soil relative to their total activity
368 (Fig.3, 4% vs 8% of total activity at a pH 7 assay, soil pH5 vs soil pH7 respectively) at neutral
369 assay pH; and these functional changes are accompanied by large changes in the relative

370 abundance of enzyme producing bacteria. We note however that our data does not empirically
371 prove that the taxa detected through metagenomics are directly responsible for altered efficiency.
372 Further support could be achieved through new computational approaches predicting pH optima
373 based on amino acid sequence composition (Yan and Wu, 2012; Lin et al, 2013), or in vitro enzyme
374 testing on novel isolates or expressed metagenomic sequences. We also cannot discount
375 evolutionary processes acting within populations contribute to the observed soil pH optima, e.g.
376 through mutations affecting enzyme active sites (Ohara et al., 2014). Whilst a number of
377 evolutionary adaptations to pH have been documented for bacterial strains (Harden et al, 2015),
378 we found only one study addressing experimentally evolved enzymatic adaptations, which was
379 refuting (Gale & Epps, 1945). Comparatively more is known about the adaptation of microbial
380 enzymes to temperature (Åqvist et al, 2017), and local temperature adaptation has been, in
381 comparison, extensively studied in bulk soil enzyme assays across thermal gradients
382 (Blagodatskaya et al, 2016; Alvarez et al, 2018; Nottingham et al 2016 ; Allison et al, 2018a).
383 However, few studies have examined adaptive capacities of individual populations (Allison et al,
384 2018b). Clearly more detailed testing of community selection versus evolutionary processes in
385 governing enzymatic adaptation to environmental factors is required, but our data strongly
386 implicate a role for the Acidobacteria in carbohydrate-degrading processes in acidic soils. Linking
387 (meta)genetic information to explicit enzymatic functional potential is an exciting new area, where
388 advances could allow prediction of soil function from microbial biodiversity data. More
389 specifically, we feel it is of utmost importance to determine whether the enzymatic adaptations to
390 soil pH observed here, give rise to other functional outcomes or trade-offs, such as alteration of
391 temperature optima. Such knowledge will allow better prediction of decomposition processes in
392 response to changing climate, across global pH-defined soil systems.

393

394 **Acknowledgements**

395 This work has been funded by the UK Natural Environment Research Council under the Soil
396 Security Programme grant “U-GRASS” (NE/M017125/1) as well as the UK Biotechnology and
397 Biological Sciences Research Council S2N - Soil to Nutrition BBS/E/C/000I0310 programme and
398 the National Capabilities programme grant for Rothamsted Long-term Experiments
399 BBS/E/C/000J0300, the Lawes Agricultural Trust.

400

401

402

403

404

405

406

407 **References**

408 Allison, S.D., 2012. A trait-based approach for modelling microbial litter decomposition. *Ecology*
409 *Letters* 15, 1058–1070. doi:10.1111/j.1461-0248.2012.01807.x

410 Allison, S.D., 2005. Cheaters, diffusion and nutrients constrain decomposition by microbial
411 enzymes in spatially structured environments. *Ecology Letters* 8, 626–635.
412 doi:10.1111/j.1461-0248.2005.00756.x

413 Allison, S.D., Romero-Olivares, AL., Lu, Y., Taylor, JW., Treseder, KK., 2018a. Temperature
414 sensitivities of extracellular enzyme Vmax and Km across thermal environments. *Glob*
415 *Change Biology*. doi: 10.1111/gcb.14045

416 Allison, S. D., Romero-Olivares, AL., Lu, L., Taylor, JW., Treseder, K.K., 2018b. Temperature
417 acclimation and adaptation of enzyme physi-ology in *Neurospora discreta*. *Fungal*

418 Ecology,35,78–86. <https://doi.org/10.1016/j.funeco.2018.07.005>

419 Alvarez, G., Shahzad, T., Andanson, L., Bahn, M., Wallenstein, M. D., & Fontaine, S. (2018).
420 Catalytic power of enzymes decreases with temperature: New insights for understanding soil
421 C cycling and microbial ecology under warming. *Global Change Biology*, 24(9), 4238–4250.

422 Åqvist, J., Isaksen, G.V., Brandsdal, B.O., 2017. Computation of enzyme cold adaptation. *Nature*
423 *Reviews Chemistry* 1. doi:10.1038/s41570-017-0051

424 Beleites, C. and Sergio, V., 2012. HyperSpec: a package to handle hyperspectral data sets in R. R
425 package v. 0.98-20110927. <http://hyperspec.r-forge.r-project.org>

426 Bengtson, P., Bengtsson, G., 2007. Rapid turnover of DOC in temperate forests accounts for
427 increased CO₂ production at elevated temperatures. *Ecology Letters* 10, 783–90.
428 doi:10.1111/j.1461-0248.2007.01072.x

429 Biely, P., MacKenzie, C.R., Puls, J., Schneider, H., 1986. Cooperativity of Esterases and
430 Xylanases in the Enzymatic Degradation of Acetyl Xylan. *Bio/Technology* 4, 731–733.
431 doi:10.1038/nbt0886-731

432 Blagodatskaya, E., et al. (2016). "Température sensitivity and enzymatic mechanisms of soil
433 organic matter decomposition along an altitudinal gradient on Mount Kilimanjaro." *Scientiste*
434 *Reports* 6: 22240.

435 Burns, R.G., DeForest, J.L., Marxsen, J., Sinsabaugh, R.L., Stromberger, M.E., Wallenstein, M.D.,
436 Weintraub, M.N., Zoppini, A., 2013. Soil enzymes in a changing environment: Current
437 knowledge and future directions. *Soil Biology and Biochemistry* 58, 216–234.
438 doi:10.1016/j.soilbio.2012.11.009

439

440 Chazdon, R.L., Chao, A., Colwell, R.K., Lin, S.-Y., Norden, N., Letcher, S.G., Clark, D.B.,
441 Finegan, B., Arroyo, J.P., 2011. A novel statistical method for classifying habitat generalists
442 and specialists. *Ecological Society of America* 92, 1332–1343.

443 Davidson, E.A., Samanta, S., Caramori, S.S., Savage, K., 2012. The Dual Arrhenius and
444 Michaelis-Menten kinetics model for decomposition of soil organic matter at hourly to
445 seasonal time scales. *Global Change Biology* 18, 371–384. doi:10.1111/j.1365-

446 2486.2011.02546.x

447 Degrassi, G., Uotila, L., Klima, R., Venturi, V., 1999. Purification and properties of an Esterase
448 from the Yeast *Saccharomyces cerevisiae* and Identification of the Encoding Gene These
449 include : Purification and Properties of an Esterase from the Yeast *Saccharomyces cerevisiae*
450 and Identification of the Encodin. *Applied and Environmental Microbiology* 65, 8–11.

451 Dontsova, K.M., Norton, L.D., Johnston, C.T., Bigham, J.M., 2004. Influence of Exchangeable
452 Cations on Water Adsorption by Soil Clays. *Soil Science Society of America Journal* 68,
453 1218. doi:10.2136/sssaj2004.1218

454 Dray, S., Dufour, A.B., 2007. The ade4 Package: Implementing the Duality Diagram for
455 Ecologists. *Journal of Statistical Software* 22, 1 – 20.

456 Eichorst SA, Trojan D, Roux S, Herbold C, Rattei T, Woebken D. Genomic insights into the
457 Acidobacteria reveal strategies for their success in terrestrial environments. *Environ*
458 *Microbiol.* 2018;20(3):1041–1063. doi:10.1111/1462-2920.14043

459 Emmett, BA, ZL Frogbrook, PM Chamberlain, R Griffiths, R Pickup, J Poskitt, B Reynolds, E
460 Rowe, P Rowland, D Spurgeon, J Wilson, CM Wood. Countryside Survey Technical Report
461 No.03/07.

462 Fierer, N. Embracing the unknown: disentangling the complexities of the soil microbiome. *Nat.*
463 *Rev. Microbiol.* (2017). doi:10.1038/nrmicro.2017.87

464 German, D.P., Weintraub, M.N., Grandy, A.S., Lauber, C.L., Rinkes, Z.L., Allison, S.D., 2011.
465 Optimization of hydrolytic and oxidative enzyme methods for ecosystem studies. *Soil*
466 *Biology and Biochemistry* 43, 1387–1397. doi:10.1016/j.soilbio.2011.03.017

467 Griffiths, R.I., Thomson, B.C., James, P., Bell, T., Bailey, M., Whiteley, A.S., 2011. The bacterial
468 biogeography of British soils. *Environmental Microbiology* 13, 1642–1654.
469 doi:10.1111/j.1462-2920.2011.02480.x

470 Legendre, P., Gallagher, E., 2001. Ecologically meaningful transformations for ordination of
471 species data. *Oecologia* 129, 271–280. doi:10.1007/s004420100716

472 Leprince, F., and H. Quiquampoix. 1996. Extracellular enzyme activity in soil: effect of pH and
473 ionic strength on the interaction with montmorillonite of two acid phosphatases secreted by

474 the ectomycorrhizal fungus *Hebeloma cylindrosporum*. *Eur. J. Soil Sci.* 47:511–522.

475 Lladó, S., Větrovský, T., Baldrian, P., .Tracking of the activity of individual bacteria in temperate
476 forest soils shows guild-specific responses to seasonality, 2019. *Soil Biology and*
477 *Biochemistry*, <https://doi.org/10.1016/j.soilbio.2019.05.010>.

478 Lladó, S., Žifčáková, L., Větrovský, T. et al. *Biol Fertil Soils* (2016) 52: 251.
479 <https://doi.org/10.1007/s00374-015-1072-6>

480 Harden, M. M., He, A., Creamer, K., Clark, M. W., Hamdallah, I., Martinez, K. A., 2nd, ...
481 Slonczewski, J. L. 2015. Acid-adapted strains of *Escherichia coli* K-12 obtained by
482 experimental evolution. *Applied and environmental microbiology*, 81(6), 1932–1941.
483 doi:10.1128/AEM.03494-14

484 Humberstone, B.F.J., Briggs, D.E., 2000. Extraction and Assay of Ferulic Acid Esterase From
485 Malted Barley *. *Journal Of The Institute Of Brewing* 106, 21–29.

486 Liang Y, Wu L, Clark IM, Xue K, Yang Y, Van Nostrand JD, Deng Y, He Z, McGrath S, Storkey
487 J, Hirsch PR, Sun B, Zhou J. 2015. Over 150 years of long-term fertilization
488 alters spatial scaling of microbial biodiversity. *mBio* 6(2):e00240-15.
489 doi:10.1128/mBio.00240-15.

490 Lin H., Chen, W., Ding H., 2013. AcalPred: A Sequence-Based Tool for Discriminating between
491 Acidic and Alkaline Enzymes. *PLoS ONE*. <https://doi.org/10.1371/journal.pone.0075726>

492 Malik AA, Puissant J, Buckeridge KM, Goodall T, Jehmlich N, Chowdhury S et al. Land use
493 driven change in soil pH affects microbial carbon cycling processes. *Nature Communications*.
494 2018 Sep 4;9(1). 3591. <https://doi.org/10.1038/s41467-018-05980-1>

495 Marx, M., Wood, M., Jarvis, S., 2001. A microplate fluorimetric assay for the study of enzyme
496 diversity in soils. *Soil Biology and Biochemistry* 33, 1633–1640.

497 Nannipieri P, Giagnoni L, Landi L. 2011. Role of phosphatase enzymes in soil. In: Bunemann E,
498 Oberson A, Frossard E, eds. *Soil Biology* 100: 215–243.

499 Niemi, R.M., Vepsäläinen, M., 2005. Stability of the fluorogenic enzyme substrates and pH optima
500 of enzyme activities in different Finnish soils. *Journal of Microbiological Methods* 60, 195–
501 205. doi:10.1016/j.mimet.2004.09.010

502 NF ISO 10390, Soil quality., 2005. Determination of pH. AFNOR.

503 Nottingham, A.T., Turner, B.L., Whitaker, J. et al. *Biogeochemistry* (2016) 127: 217.
504 <https://doi.org/10.1007/s10533-015-0176-2>

505 Ohara, K., Unno, H., Oshima, Y., Hosoya, M., Fujino, N., Hirooka, K., Takahashi, S., Yamashita,
506 S., Kusunoki, M., Nakayama, T., 2014. Structural insights into the low pH adaptation of a
507 unique carboxylesterase from *Ferroplasma*: Altering the pH optima of two carboxylesterases.
508 *Journal of Biological Chemistry* 289, 24499–24510. doi:10.1074/jbc.M113.521856

509 Oksanen, J., Blanchet, F.G., Kindt, R., Legendre, P., O’Hara, R.B., Simpson, G.L., Solymos, P.,
510 Stevens, M.H.H., Wagner, H., 2012. *vegan: Community Ecology*

511 Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D., Core, T.R., 2014. *nlme: Linear and Nonlinear*
512 *Mixed Effects Models. R package version 3.1-117*, [http://CRAN.R-](http://CRAN.R-project.org/package=nlme)
513 [project.org/package=nlme](http://CRAN.R-project.org/package=nlme).

514 Ramírez-Martínez, J. R., and A. D. McLaren. 1966. Some factors influencing the determination of
515 phosphatase activity in native soils and in soils sterilized by irradiation. *Enzymologia* 31:23–
516 38.

517 Sinsabaugh, R.L., Lauber, C.L., Weintraub, M.N., Ahmed, B., Allison, S.D., Crenshaw, C.,
518 Contosta, A.R., Cusack, D., Frey, S., Gallo, M.E., Gartner, T.B., Hobbie, S.E., Holland, K.,
519 Keeler, B.L., Powers, J.S., Stursova, M., Takacs-Vesbach, C., Waldrop, M.P., Wallenstein,
520 M.D., Zak, D.R., Zeglin, L.H., 2008. Stoichiometry of soil enzyme activity at global scale.
521 *Ecology Letters* 11, 1252–64. doi:10.1111/j.1461-0248.2008.01245.x

522 Sinsabaugh, R.L., 2010. Phenol oxidase, peroxidase and organic matter dynamics of soil. *Soil*
523 *Biology and Biochemistry* 42, 391–404. doi:10.1016/j.soilbio.2009.10.014

524 Storkey, J., Macdonald, A.J., Bell, J.R., Clark, I.M., Gregory, A.S., Hawkins, N.J., Hirsch, P.R.,
525 Todman, L.C., Whitmore, A.P., 2016. The unique contribution of Rothamsted to ecological
526 research at large temporal scales. *Adv. Ecol. Res.* 55, 3–42

527 Skujins, J., A. Puksite, and A. D. McLaren. 1974. Adsorption and activity of chitinase on kaolinite.
528 *Soil Biol. Biochem.* 6:179–182.

529 Stuart, B.H., 2004. *Infrared Spectroscopy: Fundamentals and Applications, Methods.*

530 doi:10.1002/0470011149

531 Turner, B.L., 2010. Variation in ph optima of hydrolytic enzyme activities in tropical rain forest
532 soils. *Applied and Environmental Microbiology* 76, 6485–6493. doi:10.1128/AEM.00560-10

533 Wallenstein, M., S. D. Allison, J. Ernakovich, J. M. Steinweg, and R. Sinsabaugh (2011), Controls
534 on the temperature sensitivity of soil enzymes: A key driver of in situ enzyme activity rates,
535 *Soil Enzymol.*22, 245–258.

536 Wang, G., Post, W.M., Mayes, M.A., 2013. Development of microbial-enzyme-mediated
537 decomposition model parameters through steady-state and dynamic analyses. *Ecological*
538 *Applications* 23, 255–272. doi:10.1890/12-0681.1

539 Yan, SM., Wu, G., Prediction of Optimal pH and Temperature of Cellulases Using Neural
540 Network. 2012. *Protein & Peptide Letters*,DOI: 10.2174/092986612798472794

541 Zanphorlin, L.M., De Giuseppe, P.O., Honorato, R.V., Tonoli, C.C.C., Fattori, J., Crespim, E., De
542 Oliveira, P.S.L., Ruller, R., Murakami, M.T., 2016. Oligomerization as a strategy for cold
543 adaptation: Structure and dynamics of the GH1 β -glucosidase from *Exiguobacterium*
544 *antarcticum* B7. *Scientific Reports* 6, 1–14. doi:10.1038/srep23776

545 Zhalnina, K., Dias, R., de Quadros, P.D. et al. *Microb Ecol* (2015) 69:
546 395.https://doi.org/10.1007/s00248-014-0530-2

547 Zhang, J., Siika-aho, M., Tenkanen, M., Viikari, L., 2011. The role of acetyl xylan esterase in the
548 solubilization of xylan and enzymatic hydrolysis of wheat straw and giant reed.
549 *Biotechnology for Biofuels* 4, 60. doi:10.1186/1754-6834-4-60

550

551

552

553

554

555

556

557
558
559
560
561
562
563
564
565
566
567
568
569
570
571
572
573

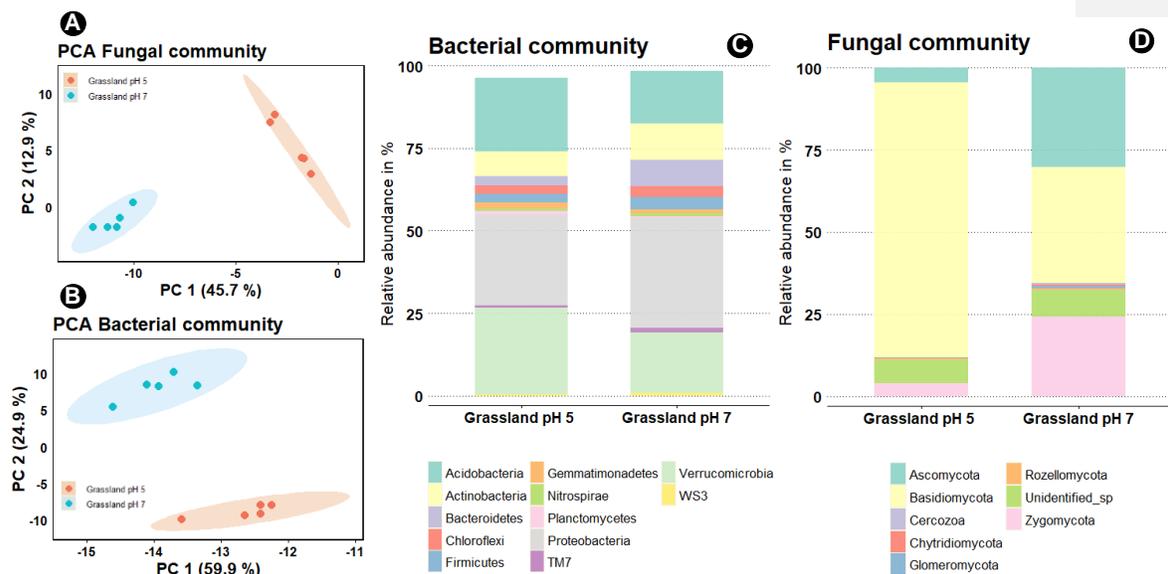
TABLES

Table 1. Effect of soil field pH treatment (soil pH 5 vs soil pH 7) on soil properties. Values represent the mean (n=5) with the associated standard error (SE). Bold letters indicate significant differences ($p < 0.05$).

		Low pH (5)	High pH (7)
	units		
pH (H₂O)	-	5.5 ± 0 a	7.3 ± 0.1 b
Soil moisture	%	30.2 ± 1.1	31.5 ± 1.2
Carbon content	%	3 ± 0.1 b	3.9 ± 0.3 a
CN ratio	-	10.7 ± 0.1	11 ± 0.1
Total Nitrogen	%	2.8 ± 0.1 b	3.5 ± 0.2 a
Total phosphorus	mg/Kg	54 ± 12.9	59.3 ± 2.5

574

575



576 **Table 2. Effects of pH, soil treatment and interactions of both factors on relative enzyme**
 577 **activity at different assay pH** (mixed model, overall repeated measures ANOVA tests).

578

	Assay pH		Field soil pH		Assay pH x field soil pH	
	F-value	P-value	F-value	P-value	F-value	P-value
Leucine amino-peptidase	190.1	<0.001	6.9	0.03	3.42	<0.001
Phosphatase	89.1	<0.001	51.4	<0.001	44.2	<0.001
β-glucosidase	88.4	<0.001	23.4	<0.01	33.7	<0.001
Acetate esterase	397.2	<0.001	30.9	<0.001	38.4	<0.001

579

580

581

582 **FIGURES:**

583

584

585 **Fig 1. Principal component analysis (PCA) ordination of soil bacterial (A) and fungal (B)**

586 **communities. Stacked bar plots show the relative proportion of the main abundant phyla**

587 **(>0.5 %) for C) bacterial and D) fungal community.**

588

589

590

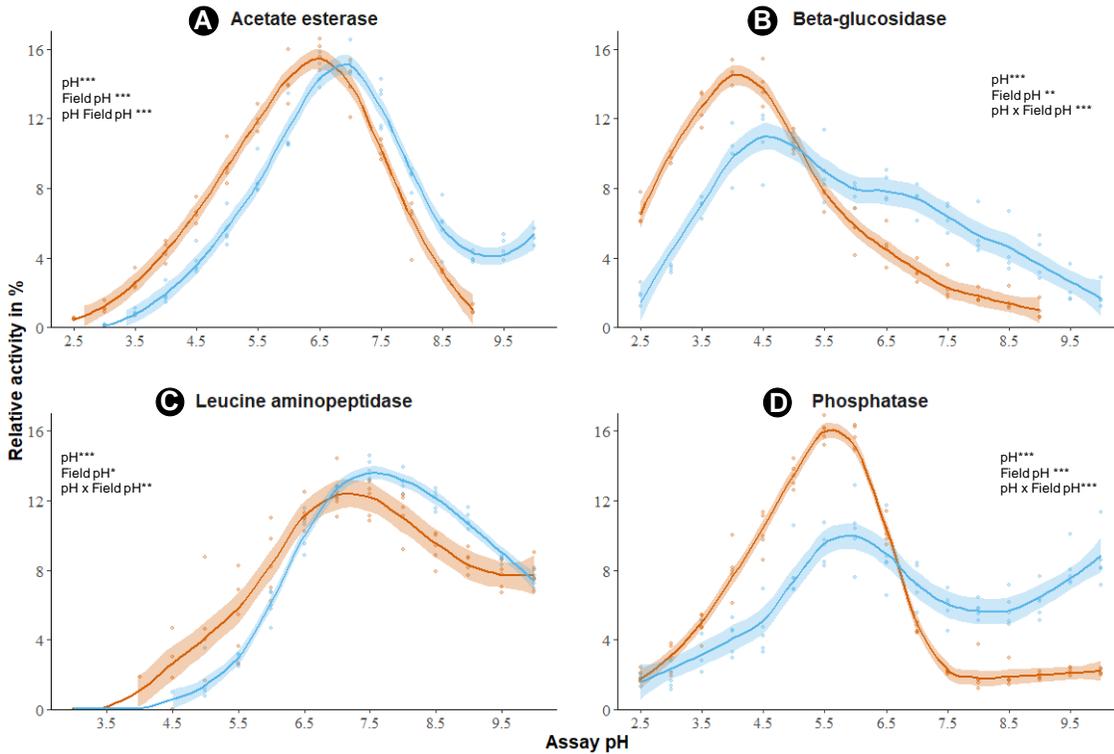
591

592

593

594

Commented [IC1]: Check Labeling of figures as PCA A is fungal and PCA B is labeled bacterial. Also Fig1. C should be in the same order as A and B



596 **Fig 2. Enzyme pH optima of acetylesterase (A), beta-glucosidase (B), leucine aminopeptidase**
 597 **(C), phosphomonoesterase (D).** Activity is expressed as a percentage of the total activity
 598 measured across the entire pH range (from pH 2.5 to pH 10). The orange and blue lines correspond
 599 to pH 5 and soil pH 7 soils respectively. Shaded area represents 95% confidence intervals around
 600 the trend line using a t-based approximation (LOESS smoothing). Stars indicate result of the
 601 mixed model used to evaluate the effects of assay pH, soil field pH treatment and their interactions
 602 were assessed by repeated measures ANOVA. Fixed factors were sampling “assay pH” and “soil

Commented [IC2]: Check that legend is the same for A, B, C, D. as A should be labeled: pH x Field pH

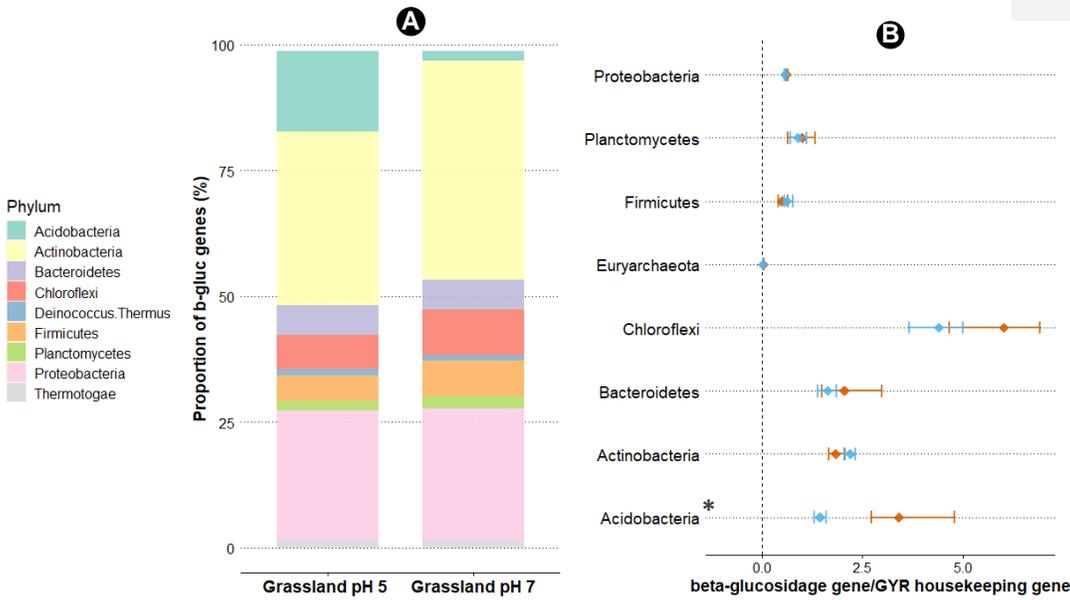
603 field pH”, while soil field replicate was added as a random factor. Significance codes for
 604 ANOVA’s are (***) $p < 0.001$; (**) $p < 0.01$; (*) $p < 0.05$.

605

606

607

608 **Fig 3. Abundances of beta-glucosidase genes from different microbial taxa, from MG-RAST**



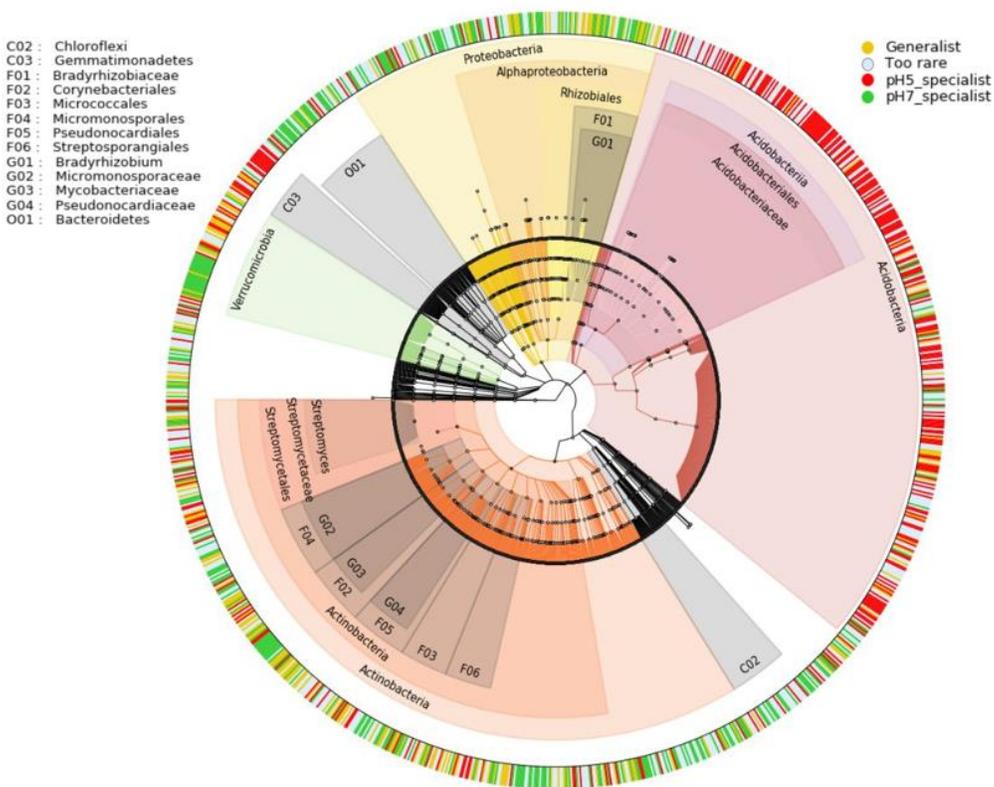
609 **annotated metagenomes (SEED Subsystems).** A: Stacked plot representing the total proportion
 610 of beta-glucosidase genes from dominant bacterial phyla. B: The proportional change of beta-
 611 glucosidase gene abundance compared to the abundance of the DNA gyrase subunit B gene.
 612 Orange and blue colors correspond to pH 5 and pH 7 soil respectively. The x-axis shows the
 613 relative fold change on log2 scale. Error bars indicate +/- standard deviation and the means are

614 indicated by filled diamond shape. Asterisks indicate significance difference between pH 5 and
 615 pH 7 soil (ANOVA $p < 0.05$).

616

617

618



621 **Fig 4. Taxonomy and pH associations of β -glucosidase sequences assembled from**
 622 **metagenomes. Inner tree and labels depict the taxonomy of β -glucosidase gene assemblies**

623 constructed from pooled metagenomes from the pH 5 and pH 7 soils (n=4). Outer ring shows
624 putative pH associations of each assembled gene, following tabulation of reads mapped to the
625 contigs from each of the 8 soil metagenomes, and statistical classification using a multinomial
626 model based on relative abundance across the two soils (CLAM).

627

628

629

630

631

632