

Standard and Non-Standard Measurements of Acidity and the Bacterial Ecology of Northern Temperate Mineral Soils

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Abstract

Databases of soil pH values today guide the decisions of land managers and the experimental designs of microbiologists and biogeochemists. Soil acidity underpins fundamental properties and functions in the soil, such as the solubilities of exchangeable ions and nutrients, or bacterial use of gradients of internal and external acidity to generate ATP and turn flagellar motors. Therefore, it is perhaps unsurprising that soil pH has emerged as the strongest predictor of soil bacterial community composition. However, the measurement of these particular values today does not address whether soil pH accurately represents the *in situ* acidity of soil microhabitats where microorganisms survive and reproduce. This study analyzes and compares soils of a large-scale natural soil pH gradient and a long-term experimental soil pH gradient for the purposes of testing new methods of measuring and interpreting soil acidity when applied to soil ecology. We extracted and prepared soil solutions using laboratory simulation of levels of carbon dioxide and soil moisture more typical of soil conditions while also miniaturizing extraction methods using a centrifuge for extractions. The simulation of *in situ* soil conditions resulted in significantly different estimates of soil pH. Furthermore, for soils from the long-term experimental soil pH gradient trial, the simulated soil pH values substantially improved predictions of bacterial community composition (from $R^2 = 0.09$ to $R^2 = 0.16$).

25 We offer suggestions and cautions for researchers considering how to better represent
26 soil pH as it exists *in situ*.

27 Introduction

28 Soil pH measurements have guided land management and biogeochemical research for
29 over a century (Libohova et al., 2012; Miller and Kissel, 2010), aiding agronomists in op-
30 timizing crop yields from soils across the spectrum of pH values. A number of methods
31 are used to measure soil pH, notably a dilute settled soil suspension, in which a glass pH
32 probe is immersed. The standard soil pH method has produced large databases of soil
33 pH values, which have provided microbial ecologists one of the best existing predictors of
34 the composition of soil bacterial communities worldwide (Bahram et al., 2018; Delgado-
35 Baquerizo et al., 2018; Shen et al., 2013; Wakelin et al., 2016). These measurements
36 of soil acidity hold great potential for the management of the diversity and composition
37 of bacterial communities in target soils (Fierer and Jackson, 2006, p. 627; Lauber et
38 al., 2009, p. 5114; Tripathi et al., 2012). In general, neutral soils (standard soil pH ap-
39 proaching 7) exhibit the largest diversity and abundance of bacteria, with many signals
40 of “acidity specialists” in acidic soils as well as “alkalinity specialists” in alkaline soils
41 (Barberán et al., 2012; Jones and Bennett, 2017; Vieira et al., 2020). However, the ex-
42 act biogeochemical mechanisms underpinning the relatively strong correlation between
43 soil pH and soil bacterial community composition remain unknown or vague, reflecting
44 the methodological challenge of explaining the optimal soil pH of cultivated soil bacteria
45 in classic microbiological studies (Small, 1954, p. 212) as well as more recent studies
46 that have utilized culture-independent molecular methods (Lauber et al., 2008; Rousk,
47 Bååth, et al., 2010; Tecon and Or, 2017).

48 The acidity of soil is an emergent property relying on several interacting biotic and abi-
49 otic protic reservoirs of protons (Supplemental Figure 1). Most bacteria directly depend
50 on their microenvironments to supply the elements and molecules necessary for life, as
51 well as to supply the Nernstian potential for protonmotive force by which cells perform

52 oxidative phosphorylation and many other powerful cellular processes, such as power-
53 ing flagella (Junge and Nelson, 2015; Lerman, 1978). However, precise theories for the
54 responsiveness of bacteria to the acidity of soil microenvironments are diverse and con-
55 tested today (Mikutta et al., 2006; Sinsabaugh et al., 2008), including abiotic factors,
56 such as pH-mediated nutrient availability in bulk soils or the rhizosphere (Song et al.,
57 2015; Stark et al., 2014), biotic factors, such as limitations to microbial cell densities
58 or metabolisms (Dennis et al., 2009; Poole, 1999), or an interaction of both abiotic and
59 biotic factors entwined. Simultaneously, because most molecular methods use solu-
60 tions and substances whose chemical behaviors are highly dependent on pH and ionic
61 strength (Barrow, 1984; Kerndorff and Schnitzer, 1980; Kirk et al., 2004, p. 171; Naidu
62 et al., 1994; Young et al., 2014), we should also be cautious of the risk of soil acidity
63 causing chemical biases within molecular methods themselves, such as DNA extraction
64 and PFLA extraction (Bååth and Anderson, 2003, pp. 958–959; Frostegård et al., 2011,
65 p. 1624; Rousk, Brookes, et al., 2010a, 2010b).

66 Several guides exist for the measurement of pH of concentrated solutions (e.g. Thermo
67 Fisher Scientific Application Note 009, 2014) and invariably provide cautionary notes
68 for the interpretation of the pH values of such solutions: “ion mobility decreases in the
69 high ionic strength samples and the activity differs from the concentration [...] High
70 ionic strength solutions change the liquid junction potential. This may lead to bias [...].
71 (*Measuring pH of concentrated samples*, 2014, p. 1)” However, such guidance offers little
72 by way of insight when solving the underlying chemical problem of the highly narrow
73 thresholds of applicability of pH to systems such as soils as they exist naturally. So-
74 lution extracts from soils of typical moisture constitute “highly concentrated solutions”
75 owing to their greater density of ions, biomolecules, and organic matter, in addition to
76 clays, the smallest of which being highly chemically and catalytically reactive.

77 Given the spatial scale at which soil microbes meaningfully perceive their environments
78 (Vos et al., 2013), in order to effectively investigate why soil pH is such a strong deter-
79 minant of bacterial community composition and to represent the dynamic acidity of soil

80 microhabitats, accurate and precise values of *in situ* soil pH will be required (Bjerrum
81 and Gjaldbæk, 1919, p. 4). The conditions under which standard measurements of soil
82 pH are made in the lab likely do not correspond to conditions in the field. Juxtaposing
83 laboratory and field conditions, we can see that, generally, the chemical properties of
84 solutions in the controlled conditions of the laboratory (“*ex situ*”), further altered with
85 the addition of solutions and processing of extracts, are often highly incommensurable
86 with the same chemical properties of solutions in the field (“*in situ*”). Soil conditions in
87 the field are undisturbed, yet they are challenging to control experimentally as they are
88 unpredictably variable over time. This methodological challenge extends also to gases
89 in soils. The soil atmosphere often has much higher partial pressures of carbon diox-
90 ide than surface conditions, and these partial pressures change with depth (Belnap et
91 al., 2003; Cary and Holder, 1982; Jury and Horton, 2004, p. 215; Vernadsky, 1913)
92 reaching levels as high as 4% to 6% at depths at or below 2 [m] and levels approximat-
93 ing atmospheric carbon dioxide levels (400 [ppm] or 0.04%) at depths of < 5 [cm], and
94 the lowest extreme (0 [ppm]) is not uncommon in photosynthetic biological crusts (Oh
95 et al., 2005). Furthermore, typical laboratory atmospheres are approximately equal to
96 the lower atmosphere, only several hundred parts per million (depending on the human
97 investigators present and the lab’s collection of plants) and would therefore represent
98 the lower bound of typical soil CO₂ concentrations. If a soil sample collected from a soil
99 profile at 1 [m] is moved to the laboratory for measurement of acidity or other chemical
100 characteristics, does the fact that the *in situ* CO₂ levels may be orders of magnitude
101 lower than the *ex situ* conditions affect our measurements of soil properties such as
102 pH?

103 CO₂ in the soil atmosphere will equilibrate with the soil solution, as described by
104 Henry’s law, $K_H = a_i/P_i$, where, for CO₂, K_H signifies Henry’s constant (approximately
105 $3.4 * 10^{-4} [\frac{\text{mol}}{\text{m}^3\text{Pa}}]$ at standard temperature for carbon dioxide in water (Sander, 2015, p.
106 4488)), a_i (unitless) signifies the thermodynamic aqueous activity of CO₂ benchmarked
107 to the standard state, and P_i [Pa] signifies the partial pressure of CO₂. As Strawn et

108 al. (2020, pp. 90–97) explain with caution, in reference to early research (Smith et
109 al. (1937); Whitney and Gardner (1943)) that first demonstrated the linear acidification
110 effect of CO₂ on soil pH of *dilute* suspensions:

111 Several simplifying assumptions [are] required to solve the carbonate system
112 equations that may not be possible or appropriate in other aqueous equilib-
113 rium problems. Additionally, the assumption that activity and concentrations
114 are equal (ideal solution) is fine for showing trends, but activity corrections can
115 cause significant changes in the predicted pH or concentrations of the species.

116 Therefore, although it would be challenging to predict the precise shift in soil pH ex-
117 pected from an increase in CO₂, as Bjerrum curves relate the concentrations of carbonic
118 acid to mono- and di-protic carbonate in dilute solutions (Andersen, 2002), elevated
119 carbon dioxide partial pressures may not increase acidity in concentrated solutions,
120 such as the extracts of solution from soils at typical soil water content. As noted by
121 Šimunek and Suarez (1994) in reference to their previous two-part publication (Suarez
122 and Šimunek, 1993; Šimunek and Suarez, 1993), “existing models also assume either a
123 fixed pH or a fixed CO₂, which are questionable assumptions for soils, which usually ex-
124 hibit fluctuation of both of these variables.” Such “fixed” or non-varying pH and CO₂ are
125 obviously very uncommon in soils across textures, series, depth, and time, warranting
126 fundamental reappraisal.

127 To address the overarching challenge of better representing *in situ* soil conditions in bio-
128 geochemical measurements and instrumentation, two approaches present themselves:
129 (1) to perform direct *in situ* measurements in the field while minimizing the perturba-
130 tion of the original conditions of soil profiles (and the functionality of instruments), or
131 (2) to simulate the original conditions of intact soils during the analysis of soil samples
132 that have been collected from the field and brought to the laboratory. Both of these
133 approaches have complementary advantages and disadvantages, but both approaches
134 are also a significant departure from traditional methods described in standard method-
135 ological references (Jacob et al., 2002, pp. 1481–1509). While most soil scientists rarely

136 measure soil solution pH in the field, due to the numerous challenges of doing so, scien-
137 tists in other fields are acutely aware of the value of *in situ* measurements or maintaining
138 *in situ* conditions, as exemplified by the works of Sasowsky and Dalton (2005) on the
139 importance of such measurements of water chemistry in caves, Parfitt et al. (1995) on
140 the chemistry of aluminum in suspensions of orchard soils, and Matthiesen (2004) in
141 archaeological excavations.

142 The present study expands upon the foundational soil acidity experiments performed
143 by Whitney and Gardner (1943), with application to soil bacterial ecology. Additionally,
144 beyond the improvement of the fundamental understanding of bacterial ecology of soils,
145 the paradigm of “soil pH” itself is explored in terms of metrological interpretation in par-
146 allel with standard and non-standard soil acidity measurement protocols (acidimetry).
147 This study presents a multifactorial chemical and microbial study across both natu-
148 ral and experimental soil pH gradients in temperate mineral soils in Wisconsin, USA.
149 We assess the limitations of soil pH measurements using a non-standard methodol-
150 ogy: extraction of soil solution at moisture levels approximating field capacity and drier,
151 miniaturization of the resulting analyte to allow for high-throughput pH measurement,
152 simulation of soil conditions during pH measurement, and exponentiation of pH values
153 to hydrogen ion activity (a_{H^+}). Non-standard soil pH values are then used to predict soil
154 microbial community composition across said experimental and natural pH gradients in
155 the Wisconsin region of the United States. We hypothesized that these protocols would
156 improve correlations with both chemical properties of soils as well as microbial com-
157 munity features, due to the improved representation of *in situ* soil conditions, with the
158 ultimate goal of better informing the mechanisms by which the acidity of soil microhab-
159 itats influences soil microorganisms.

160 Methods

161 Standard and Non-Standard Soil pH Values

162 Our objective was to determine whether standard soil pH measurements or non-
163 standard soil pH measurements (i.e., soil pH values under conditions simulating *in*
164 *situ* soil conditions of moisture and carbon dioxide levels) were better predictors of
165 bacterial community composition across soil pH gradients. For the purposes of this
166 study, we define “standard soil pH” as the pH value measured at ambient carbon
167 dioxide (approximately 0.04%) and a ratio of solution:soil of 1 : 1 (Thomas, 1996, pp.
168 487–488), where the solution may vary from deionized water (pH_W) to a dilute (0.01
169 [mol/L]) electrolyte solution (pH_{CaCl₂} or pH_{KCl}). For the comparison to standard soil
170 pH in this study, “simulated soil pH” is defined as the multifactorial set of pH values
171 measured at ambient and elevated carbon dioxide (2.2%(±0.05)) and a range of 1 : 2 to
172 1 : 4 solution:soil ratios. All solutions added to soils in this study were the dilute elec-
173 trolyte 0.01 [mol/L] KCl. For each sample, we applied a miniaturized, centrifuge-based
174 soil solution extraction method, manipulating solution:soil ratios and atmospheric CO₂
175 levels during measurement using a glass microprobe to measure pH (specific details
176 follow).

177 Site Descriptions and Sample Collection

178 In order to investigate the effects of these methods on soils with similar underlying
179 mineralogy, we collected and analyzed soils from a 25-year soil pH manipulation trial at
180 the University of Wisconsin-Madison Spooner Agricultural Research Station (Spooner,
181 WI; details of manipulation below). In order to investigate the effects of these methods
182 on a wide range of soil types, we applied these methods to soil spanning a natural soil
183 pH gradient of nine University of Wisconsin-Madison agricultural research stations from
184 across the state. Where noted, “Topsoil” signifies any combination of A horizons, and
185 “Subsoil” signifies all beneath the A horizon to the depth specified.

186 The pH manipulation trial at the Spooner Agricultural Research Station began in 1994

187 (“Long-term pH Trial”). The study soil is of the series Mahtomedi, consisting of very
188 deep, excessively drained, rapidly permeable soils formed in sandy outwash of the Late
189 Wisconsinan Age on glacial moraines and outwash plains. Corn, soy, and alfalfa have
190 been grown at the site. Four replicates of 22 [m] wide by 220 [m] long field plots have
191 been maintained at target soil pH values of 4.7, 5.2, 5.7, 6.2, and 6.7, through annual
192 additions of pell lime or sulfur after annual soil tests (personal correspondence with
193 Superintendent Phil Holman). Samples were collected on November 3, 2017. Three 1-
194 inch diameter cores to 20 [cm] depth were randomly sampled at locations determined by
195 a random number generator using the length of the long rectangular plots, avoiding the
196 plot edges by 5 [ft].

197 The second set of sites (“Wisconsin Soils”) were selected using legacy chemical and phys-
198 ical data for University of Wisconsin Agricultural Research Stations from Web Soil Sur-
199 vey, retrieved on August 7th, 2018. From the database’s graphical user interface, a
200 depth of 0 [cm] to 50 [cm] was selected for the following parameters: calcium carbonate,
201 cation exchange capacity at pH 7 (CEC-7), electrical conductivity (EC), gypsum, soil pH,
202 sodium adsorption ratio, available water capacity and supply, bulk density at $\frac{1}{3}$ bar,
203 liquid limit, percent organic matter, percent clay, percent sand, percent silt, and sat-
204 urated hydraulic conductivity (K_{sat}), parent material, and representative slope. These
205 features were used to select a wide variety of characteristics, namely the widest breadth
206 of textural classes, organic matter content, and soil pH values. The following research
207 stations were selected, listing ID letter and soil pH values according to Web Soil Survey
208 listed in parentheses: Kemp (K, 5.40), Rhinelander (R, 5.50), Marshfield (M, 5.65), Spooner
209 (S or Sp, 5.80), Hancock (H, 6.20), Arlington (A, 6.50), Lancaster (L, 6.60), West-Madison
210 (W, 6.70), and Peninsular (P, 7.20). Supplemental Figure 2 shows a map of the locations
211 of these sites across the soil pH gradient in Wisconsin, while Supplemental Table 1 lists
212 the latitude and longitude of each site (Kartesz, 2015).

213 The Wisconsin soils were collected from each of the two or three most common soil
214 series of each agricultural research station listed above, between August and September,

215 2018. At each site, a soil pit was dug to > 50 [cm] depth and, after excavation, several
216 kilograms of soil were gathered from each horizon evenly spanning the upper to the
217 lower boundary. Horizon boundaries were easily visible, and photos of all soil profiles
218 can be found in the Supplemental Materials. Soil samples were placed in sterile bags
219 and transported within 24 hours of collection to the Department of Soil Science at the
220 University of Wisconsin-Madison and placed in a refrigerator (4 [°C]). Within two days of
221 arrival, each sample was homogenized, subsampled, and stored at -80°C .

222 Soil Chemical Analyses

223 The Spooner Agricultural Research Station performed chemical analyses for the long-
224 term experimental soil pH plots in 2017: organic matter was 2.15% (± 0.24), phosphorus
225 level was 33 (± 6) [ppm], and potassium level was 93 (± 25) [ppm] (personal correspon-
226 dence with Superintendent Phil Holman). All samples of the Wisconsin set were ho-
227 mogenized, subsampled, and submitted to the University of Wisconsin Soil and Forage
228 Laboratory where the samples were dried and sieved to conduct the following analyses:
229 Routine Tests (pH using 1:1 water, P using Bray No 1 extraction test, K also using Bray
230 No 1 extraction test, and OM using loss on ignition), Cation Exchange Capacity (sum-
231 mation, including calcium and magnesium), acidity extracted using ammonium acetate,
232 and total nitrogen and organic carbon (dry combustion) (specific protocols in Burt and
233 Staff (2014)).

234 Soil Solution Extraction

235 The “suspension effect” has long been observed (Gorham, 1960; Jenny et al., 1950;
236 Oman et al., 2007; Ponnampereuma et al., 1966), and describes the apparent decrease
237 in pH when a pH probe is moved between the supernatant and sediment of a settled
238 suspension, although the precise explanation for the problem is somewhat unresolved
239 (Feldman, 1956; Fornasier et al., 2018). Sacchi et al. (2001) have recommended prepar-
240 ing fresh samples using the centrifugation method of extracting solutions from clay-
241 water systems, pertaining to most unsaturated soils, with a risk of incomplete water

242 extraction at extreme dry conditions. In order to minimize the “suspension effect”, we
243 reduced the density of soil particles from solution extracts via centrifugation, and mea-
244 sured the supernatant rather than the sediment.

245 Soil solution was extracted as follows, informed by Gillman (1976) and Wolt (1994, pp.
246 95–120). Empty tubes were labeled and weighed, and masses were recorded. Packed
247 fresh (not dried) soil was added to fill 1.0 [mL] to 1.3 [mL] of the tube, and the exact
248 mass added was recorded. The soil mass was used to estimate the volume of 0.01 [M]
249 KCl solution (specific mass approximately equal to water, or 1.0 [g/mL]) required to
250 reach the target solution:soil ratio (1 : 1, 1 : 2, 1 : 3, or 1 : 4). The addition of a weak
251 electrolyte such as 0.01 [M] KCl minimizes the liquid junction potential of glass probe
252 pH acidimetry (Bates, 1973, pp. 31–58; Kadis and Leito, 2010; Libohova et al., 2014;
253 MacInnes, 1915). This solution produces highly dilute spectator ions without acid-base
254 reactivity that cannot increase ionic strength past the threshold beyond which pH is
255 applicable while minimizing liquid junction potentials. Tubes were then vortexed until
256 well-mixed and let rest 40 minutes to 1 hour. Tubes were centrifuged for 60 seconds
257 at 8000[RPM], which causes a relative centrifugal force ($RCF = RPM^2 \times 1.118 \times 10^{-5} \times$
258 rotational radius) equal to 7,155 g force. 100[μ L] of supernatant was pipetted into a 0.5
259 [mL] tube for measurement. All aliquots were prepared and then frozen at $-20[^\circ C]$ for
260 later thawing and pH measurement. The original soil remaining in the 1.5 [mL] tubes
261 after centrifugation and supernatant extraction was then dried and massed. These dry
262 soil mass values enabled the calculation of the starting gravimetric water content, from
263 which the exact solution:soil ratios were calculated for subsequent analyses.

264 Simulation of Soil Atmospheric Carbon Dioxide

265 For samples measured under elevated CO_2 , we used a vinyl anaerobic airlock chamber
266 (Coy Laboratory Products, Inc., Grass Lake, Michigan, see Supplemental Figures 3 and
267 4) to maintain an atmosphere of 2.2%(± 0.05) CO_2 . The elevated CO_2 level decreased the
268 pH of 1.0[mL] of 0.01[M] $CaCl_2$, which was used as a standard throughout the experiment,

269 from $7.0(\pm 0.05)$ in normal laboratory conditions to $6.0(\pm 0.05)$. CO_2 was produced in the
270 chamber through the initial reaction of 100[g] of NaHCO_3 with excess 5% acetic acid,
271 after which CO_2 levels were adjusted to target levels with a combination of venting and
272 additional reactions. The chamber air was mixed with a small fan and CO_2 was moni-
273 tored with a USB CO_2 Probe Data Logger (CO2Meter.com, K-30 Probe, CM-0040) with a
274 measurement range of 0% (0 [ppm]) to 30% (300,000 [ppm]), with an error not exceeding
275 5% of the quantity measured and logged using the GasLab software (v. 2.2.1.36). Sam-
276 ples measured under ambient CO_2 were measured in the same chamber fully open and
277 vented to the laboratory space.

278 Measurement of pH with a Microprobe

279 pH was measured using an InLab Micro pH glass microelectrode (Mettler-Toledo; Mate-
280 rial No. 51343160; further details on probe can be found in Supplemental Materials). To
281 monitor the quality of measurements throughout the analysis at elevated CO_2 , the pH
282 of identical volumes of several controls were taken alongside the soil extract, including
283 100 [μL] each of 0.01[M] CaCl_2 , 5% (0.833[M]) acetic acid, 0.01 KCl, and deionized water.
284 The 0.01 KCl solution was measured every 50 soil pH measurements to detect probe
285 drift. These control values deviated < 0.15 pH units during each series of measurements
286 across the entire experiment. Exponentiation of the soil pH values did not require fur-
287 ther measurements but rather calculated activity of hydrogen ions (a_{H^+}), which adopts
288 the units of moles per liter to represent effective concentration when the activity coeffi-
289 cient of hydrogen ions (i.e., hydronium and related cationic species of solvated protons)
290 is 1.0 (de Levie, 2014).

291 Statistical Analyses for Chemical Properties

292 To compare the non-standard pH values with standard values, we fit linear regression
293 models to determine their relationships. To determine which other soil chemical prop-
294 erties were the most strongly associated with soil pH as measured by the standard
295 and non-standard methods, linear models correlating soil chemical measurements and

296 all values of pH were analyzed using a Bayesian information criterion (BIC) approach
297 (Kass and Wasserman, 1995). The calculations were performed in R (Team, 2018; Wick-
298 ham, 2009) using the **regsubsets** function from the R package leaps (Lumley and Miller,
299 2020). Interpretation of the results involved assessing which factors, when added to the
300 model, produce the most negative BIC, where more negative BIC values indicate better
301 models when certain factors are incorporated and others excluded. The collection of
302 models with the most negative BIC values in the “BIC dropoff” region offer an assort-
303 ment of models that best predict the factor of interest—in our case, pH. We calculated
304 models and their associated BIC values using the soil chemical analyses as predictors
305 for each of the four sets of soil pH values generated for the extremes of this study’s
306 multifactorial: high and low CO₂ and the highest and lowest soil solution content (1 : 1
307 and 1 : 4 solution-to-soil ratio by mass).

308 Soil DNA Extraction and Bacterial Community Sequencing

309 Total genomic DNA was extracted from frozen soils using the PowerLyzer PowerSoil DNA
310 Isolation Kit (Catalog No. 12888, Qiagen, Germantown, MD, USA). All DNA was stored at
311 or below -20°C from the date of extraction throughout stages of sequencing. Because
312 soil pH can potentially interact with the chemicals used for extracting DNA, we also
313 investigated the predictive value of the pH of solutions along two steps of the DNA ex-
314 traction protocol (see Supplemental Figure 9 and Supplemental Note 2). 16S rRNA genes
315 were amplified from extracted DNA using polymerase chain reaction (PCR), with three
316 replicate reactions per sample. Variable region V4 of the 16S rRNA gene was targeted
317 using forward primer 515F and reverse primer 806R with modification by Walters et
318 al. (2016), which increased degeneracy of bases that have caused detection bias among
319 some bacterial clades. Primers also had barcodes and Illumina sequencing adapters
320 added, following Kozich et al. (2013) (all primers in Supplemental Table 2). The follow-
321 ing reagents were added to each PCR reaction: (1) 12.5[μL] Q5 Hot Start High-Fidelity 2X
322 Master mix (New England BioLabs INC., Ipswich, MA), (2) 1.25[μL] 515f forward primer
323 (10[mM]), (3) 1.25[μL] 806r reverse primer (10[mM]), (4) 1[μL] DNA extract, and (5) 7.75[μL]

324 PCR-grade water. The plate was sealed, gently vortexed, and briefly centrifuged to en-
325 sure all liquids were well mixed. The plate was then run on an Eppendorf Mastercycler
326 nexus gradient thermal cycler (Hamburg, Germany) using the following parameters for
327 30 cycles: 98[°C] for 2 minutes + (98[°C] for 30 seconds + 58[°C] for 15 seconds + 72[°C] for
328 10 seconds) \times (30 + 72) [°C] for 2 minutes and 4[°C] hold.

329 Successful amplification was verified via gel electrophoresis. To purify amplicons and
330 normalize PCR products, we used a SequalPrep Normalization Plate Kit (Invitrogen Cor-
331 poration, Thermo Fisher Scientific, Waltham, MA, USA). The PCR triplicates for each
332 sample were pooled and normalized according to manufacturer's instructions. The Wiz-
333 ard SV Gel and PCR Clean-Up System A9282 (Promega, Madison, WI) was used to
334 extract and purify the combined PCR product library according to manufacturer's in-
335 structions except for the following two deviations: (1) the SV Minicolumn incubation
336 and centrifugation (steps 5.A.2-5.A.3) steps were repeated twice for each sample, and
337 (2) nuclease-free water application was divided into and increments with the incuba-
338 tion step and centrifuge step after each addition (step 5.A.6). DNA was concentrated
339 using a SpeedVac Vacuum Concentrator System (Thermo Fisher Scientific, Waltham,
340 MA, USA) before and after using the Wizard SV Gel and PCR Clean-Up to meet the
341 sequencing requirements of 15[ng/ μ L]. The final library was sequenced at the Univer-
342 sity of Wisconsin-Madison Biotechnology Center on a Illumina MiSeq Sequencer using
343 2×250 [bp] paired-end reads.

344 Microbial Community Analyses

345 Sequencing generated 1.3M reads, with a mean of 104,655 reads per sample (minimum
346 48,207, maximum 257,394 reads per sample). We quality-filtered and trimmed (truncation
347 length 235 bp for forward and 144 bp for reverse reads, left trim of 5 bp for forward and
348 reverse reads with other default settings), learned errors (using all sequences), derepli-
349 cated, determined operational taxonomic units (OTUs) (default settings), and removed
350 chimeras using dada2 (Callahan et al., 2016) as implemented in R, and run on the UW-

351 Madison Center for High-Throughput Computing cluster. This resulted in a final mean
352 of 53,777 reads per sample (minimum 18,610, maximum 152,682 reads per sample). All
353 reads have been deposited at the National Center for Biotechnology Information Short
354 Reads Archive under BioProject ID PRJNA643927.

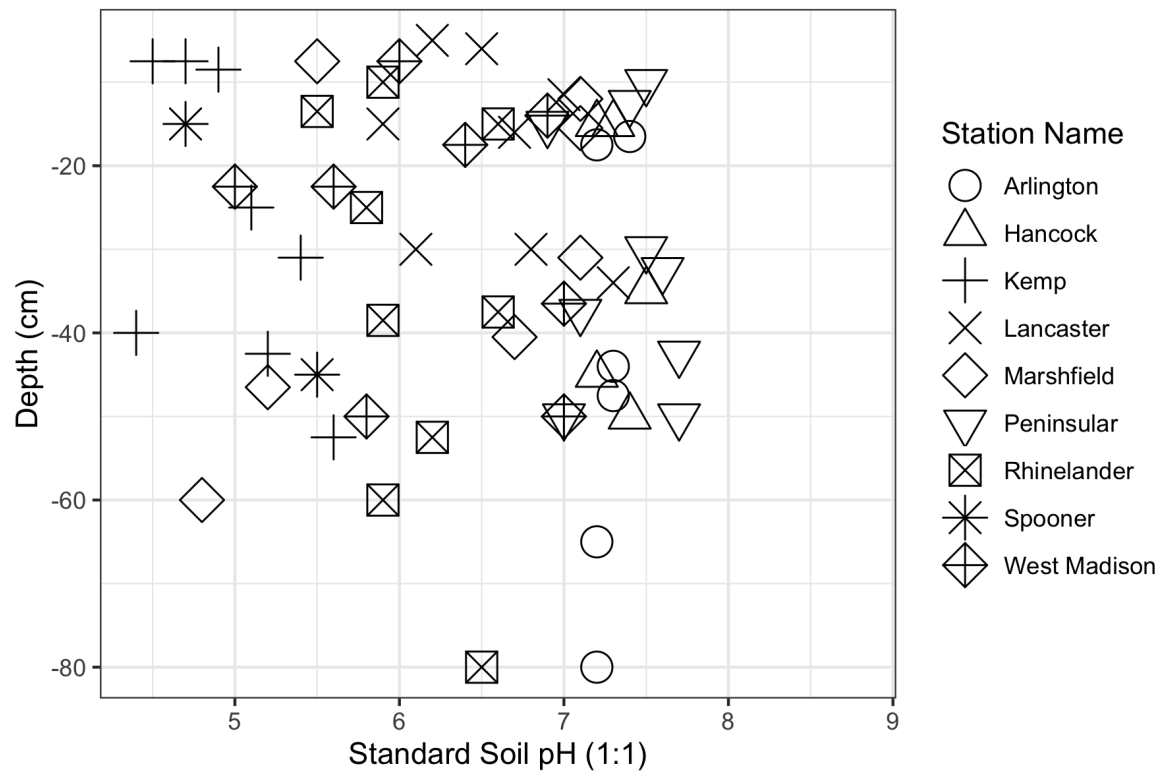
355 We analyzed bacterial communities using the R packages phyloseq (McMurdie and
356 Holmes, 2013) and vegan (Dixon, 2003). OTUs were filtered to remove mitochon-
357 dria and chloroplast sequences and were normalized by relative abundance for each
358 sample. We assessed the influence of pH measurement technique on community
359 composition using permutational multivariate analysis of variance (PERMANOVA)
360 (Anderson, 2014) with Bray-Curtis dissimilarities (Bray and Curtis, 1957) and illus-
361 trated these differences in community composition using non-metric multidimensional
362 scaling (NMDS) plots (Agarwal et al., 2007). Code for all analyses can be found at
363 <https://github.com/michaeljbraus/usda-wisconsin-soil-ph>.

364 Results

365 Non-Standard Soil pH Values at Four Levels of Soil Moisture

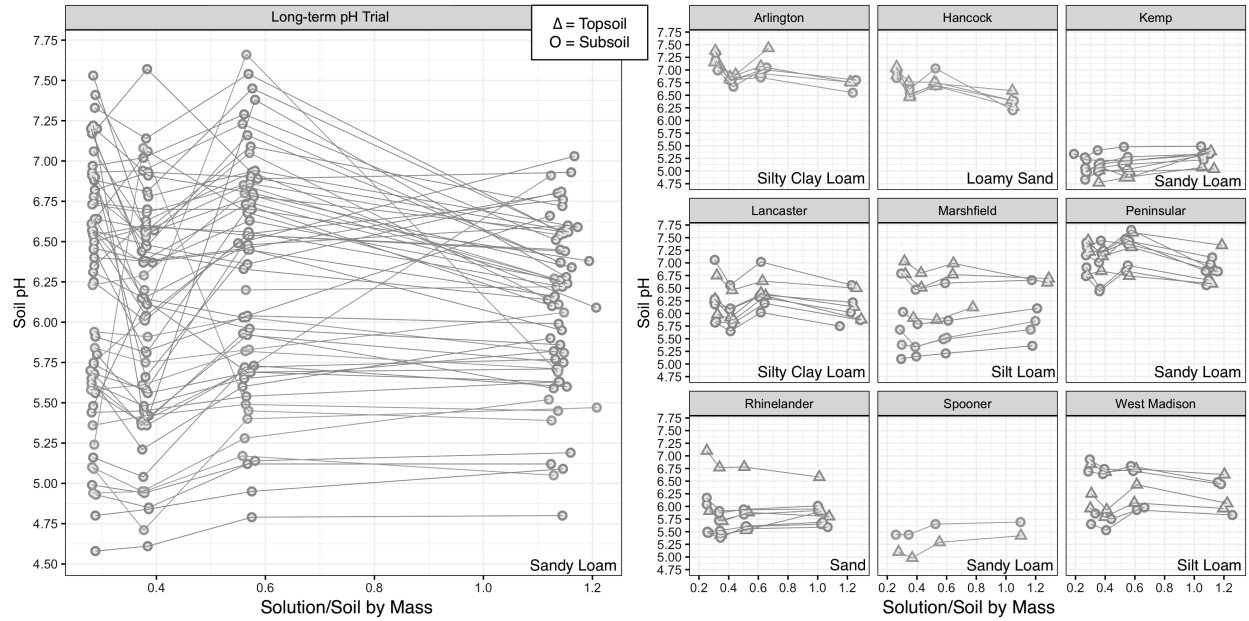
366 Soils from across Wisconsin's natural soil pH gradient spanned a wide range (4.4 to
367 7.8) of standard soil pH values (Figure 1). In the ambient CO₂ atmosphere, decreasing
368 solution:soil ratios resulted in changes in measured pH spanning decreases of more
369 than 1.0 to increases of more than 1.0, with 18% of measured values differing by more
370 than 0.5 units from their standard soil pH measurements (Figure 2). Among the soils
371 from the long-term pH manipulation trial, the more alkaline soil pH values > 6.5 tended
372 to increase, by approximately 0.2 and up to 1.0 when soil moisture was lowered, whereas
373 the soils of pH < 6.5 changed little with decreasing soil moisture and had somewhat lower
374 variability (Figure 2). These trends were similar in the cross-Wisconsin dataset, where
375 soils with pH exceeding approximately 6.0 tended to increase in pH with decreasing water
376 soil moisture, whereas soils of lower pH tended to change less or to decrease. Across
377 both datasets, and for all soils, pH tended to decrease among solution extracts of 3 : 1

378 soil:solution ratio in comparison to a 1 : 1 ratio, and then increase again at the 4 : 1
379 soil:solution ratio (Figure 2).



380

381 Figure 1. Standard soil pH values for all samples as a function of depth from soil samples
382 from agricultural field stations across Wisconsin. See also Supplemental Figure 2 depicting the
383 relative locations in Wisconsin of these stations.



384

385 Figure 2. Soil pH as a function of solution-to-soil ratio for (A) soils from a long-term pH ma-
386 nipulation trial in Spooner, WI and (B) soils from across Wisconsin's natural soil pH gradient.
387 Each point represents a single pH measurement. Triangles indicate topsoil samples, while circles
388 indicate subsoil samples for the Wisconsin dataset. Topsoil and subsoil are not distinguished
389 in the long-term pH manipulation trial dataset. Points from the same soil sample are joined by
390 straight lines for ease of comparison. Soil texture is indicated in the bottom right quadrant of
391 each sub-plot. Note that exact solution:soil ratios are plotted, hence the small variation in the
392 x-axis for a given moisture treatment.

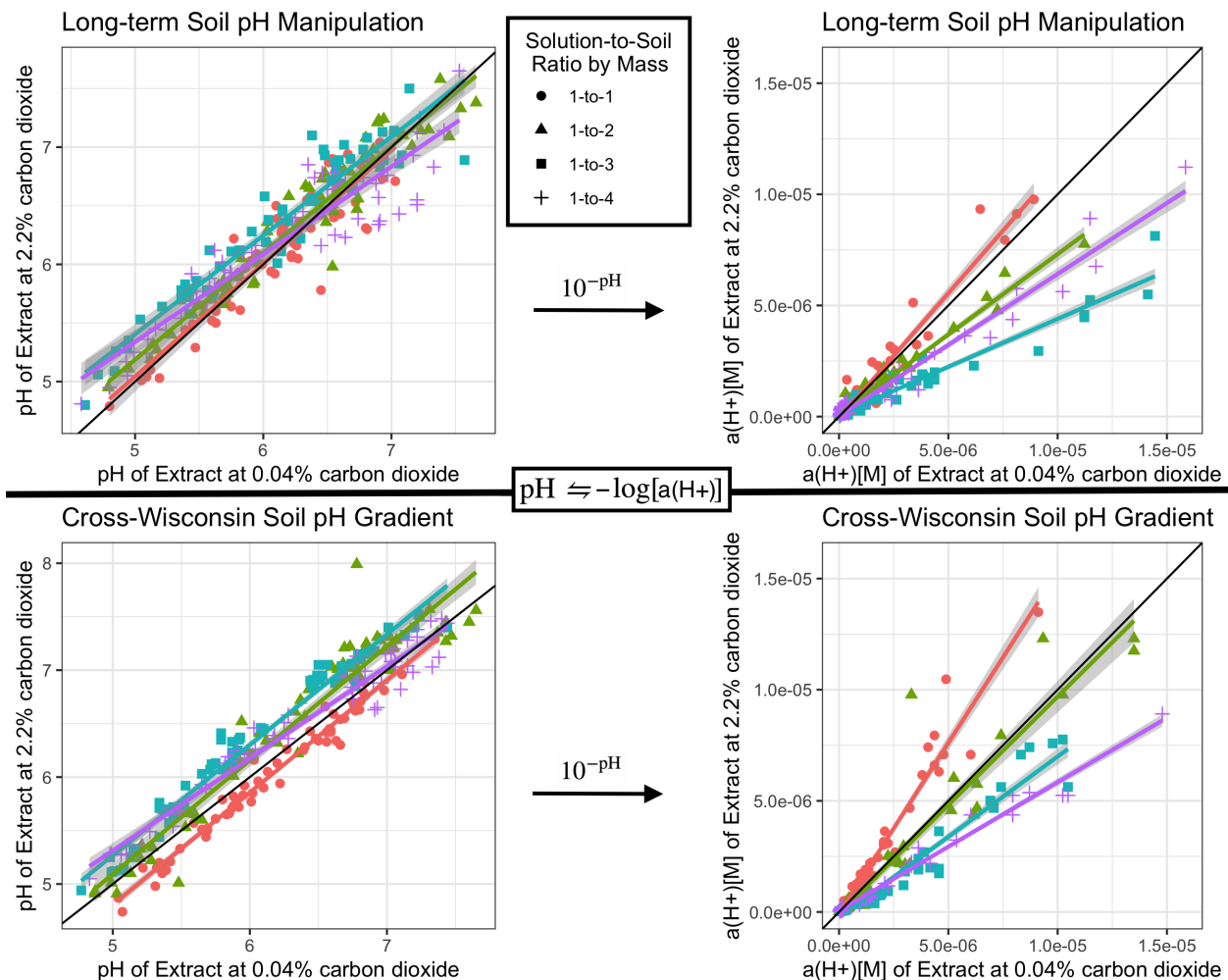
393 Under the 2.2%(±0.05) CO₂ atmosphere, all soils in the cross-Wisconsin dataset tended
 394 to increase in measured pH with decreasing solution:soil ratios, but the same general
 395 trend of higher pH soils being more affected by decreasing solution:soil ratios persisted
 396 (slopes 1.18 – 1.22). In the long-term soil pH manipulation trial, pH of most samples
 397 tended to increase with decreased moisture contents, and the higher pH samples again
 398 had somewhat greater variability (Table 1 and Supplemental Figures 5-8).

399 Table 1. Linear regressions of soil pH and soil activity (a_{H^+}) relating these values at ambient
 400 laboratory CO₂ (0.04%) to values at a typical soil atmospheric CO₂ (2.2%(±0.05)) as a result of
 401 chemical analysis of the Wisconsin Soils set from across a natural soil pH gradient and the
 402 Long-term pH Trial set of samples from an experimental soil pH gradient.

Soil Set	Acidity Metric	Solution:Soil Ratio	Intercept	Slope	R-squared
Wisconsin Soils	pH	1-to-1	-0.442	1.049	0.976
Wisconsin Soils	pH	1-to-2	-0.234	1.065	0.927
Wisconsin Soils	pH	1-to-3	0.099	1.033	0.967
Wisconsin Soils	pH	1-to-4	1.001	0.862	0.959
Wisconsin Soils	a(H ⁺)	1-to-1	0.000	1.687	0.941
Wisconsin Soils	a(H ⁺)	1-to-2	0.000	0.976	0.897
Wisconsin Soils	a(H ⁺)	1-to-3	0.000	0.711	0.959
Wisconsin Soils	a(H ⁺)	1-to-4	0.000	0.580	0.981
Long-term pH Trial	pH	1-to-1	0.218	0.965	0.843
Long-term pH Trial	pH	1-to-2	0.639	0.910	0.928
Long-term pH Trial	pH	1-to-3	1.190	0.842	0.909
Long-term pH Trial	pH	1-to-4	1.600	0.748	0.867
Long-term pH Trial	a(H ⁺)	1-to-1	0.000	1.075	0.963
Long-term pH Trial	a(H ⁺)	1-to-2	0.000	0.707	0.978
Long-term pH Trial	a(H ⁺)	1-to-3	0.000	0.507	0.937
Long-term pH Trial	a(H ⁺)	1-to-4	0.000	0.613	0.976

403 Non-Standard Soil pH Values at Ambient and High CO₂

404 Soil pH values were also affected by the level of carbon dioxide during measurement.
 405 In the long-term pH manipulation trial soils, increasing CO₂ did not markedly change
 406 measured pH values for solution:soil ratios of 1 : 1 to 1 : 3. However, at solution:soil ratios
 407 of 1 : 4, increasing CO₂ decreased measured pH values in the higher pH samples (pH
 408 > 6.5, approximately) (Figure 3A and 3C). In the cross-Wisconsin dataset, only solution
 409 extracts prepared according to the standard (1 : 1) ratio exhibited the expected trend
 410 of acidification at elevated carbon dioxide, with measured pH values decreasing by as
 411 much as 0.6, while in samples with lower solution:soil ratios, increasing CO₂ increased
 412 measured pH slightly (Figure 3B and 3D).

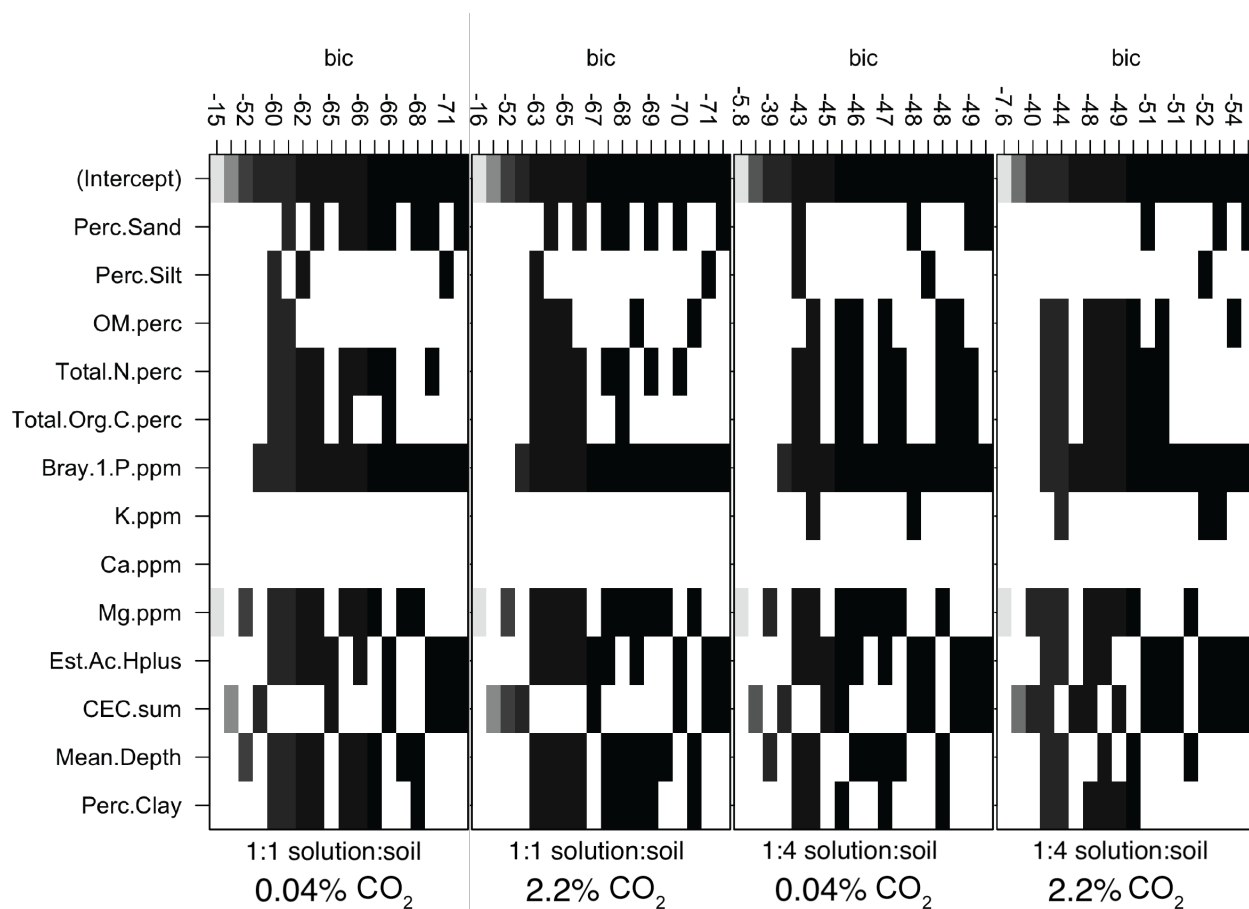


413

414 Figure 3. Soil pH and hydrogen ion activity (a_{H^+}) values, which are interchangeable according to
 415 the definition of pH scale as the negative log of hydrogen ion activity, measured at ambient or
 416 low (0.04%) and high (2.2% (± 0.05)) carbon dioxide levels and soil water content at four levels from
 417 the natural cross-Wisconsin soil acidity gradient and long-term soil pH manipulation gradients.
 418 Grey regions surrounding linear regression lines are standard error, and the solid black line
 419 signifies $y = x$. Points are labelled by color and shape to signify solution:soil ratio, where red
 420 circles = 1 : 1, green triangles = 1 : 2, blue squares = 1 : 3, and purple crosses = 1 : 4.

421 **Correlations of Soil Properties with pH Measurements**

422 For the cross-Wisconsin dataset, the factors significantly correlated with standard and
423 simulated soil pH values fall into the broad categories of textural (sand, silt, and clay
424 content), chemical (SOM, C, N, P, K), and exchangeable (CEC and exchangeable acidity).
425 The most consistently influential correlates for soil pH values were the exchangeable
426 factors and the Bray-extracted phosphorus (Figure 4). The decrease of water content
427 from a solution:soil ratio of 1 : 1 to 1 : 4 generally caused the influence of textural factors
428 to decrease and chemical factors to increase. Calcium was not influential in any model,
429 and changing CO₂ levels had little influence on the model results.

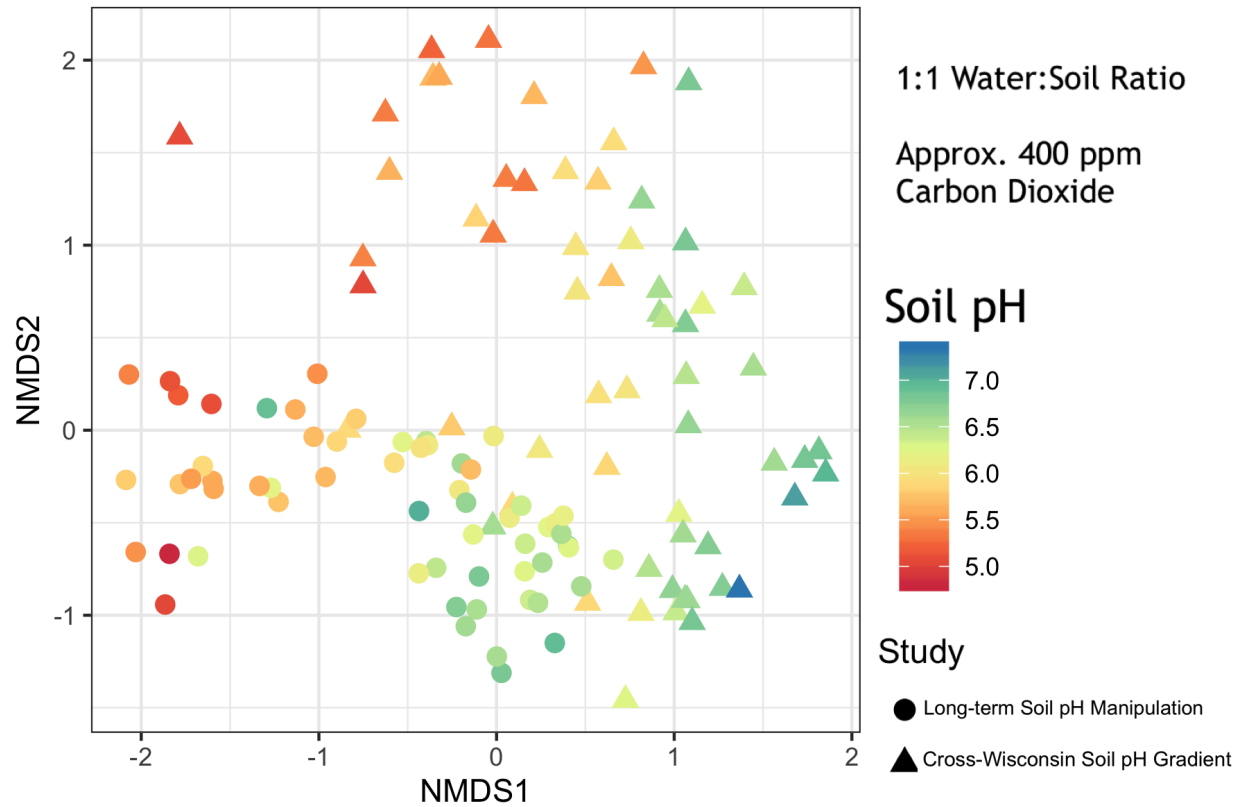


430

431 Figure 4. Bayesian information criterion (BIC) plot for soil properties as possible correlates of soil
 432 pH as determined by a ratio of solution:soil ratio of 1 : 1 compared to 1 : 4 and a soil atmosphere
 433 with approximately 0.04% compared to 2.2%(±0.05) carbon dioxide. Vertical axes are discrete and
 434 not continuous, where each value represents the ranked BIC value of the model using the input
 435 factors indicated by blocks. Shading of blocks indicates the degree to which a proposed model
 436 can be considered relevant, where the darker squares represent good selections to include in a
 437 chosen model.

438 Microbial Communities

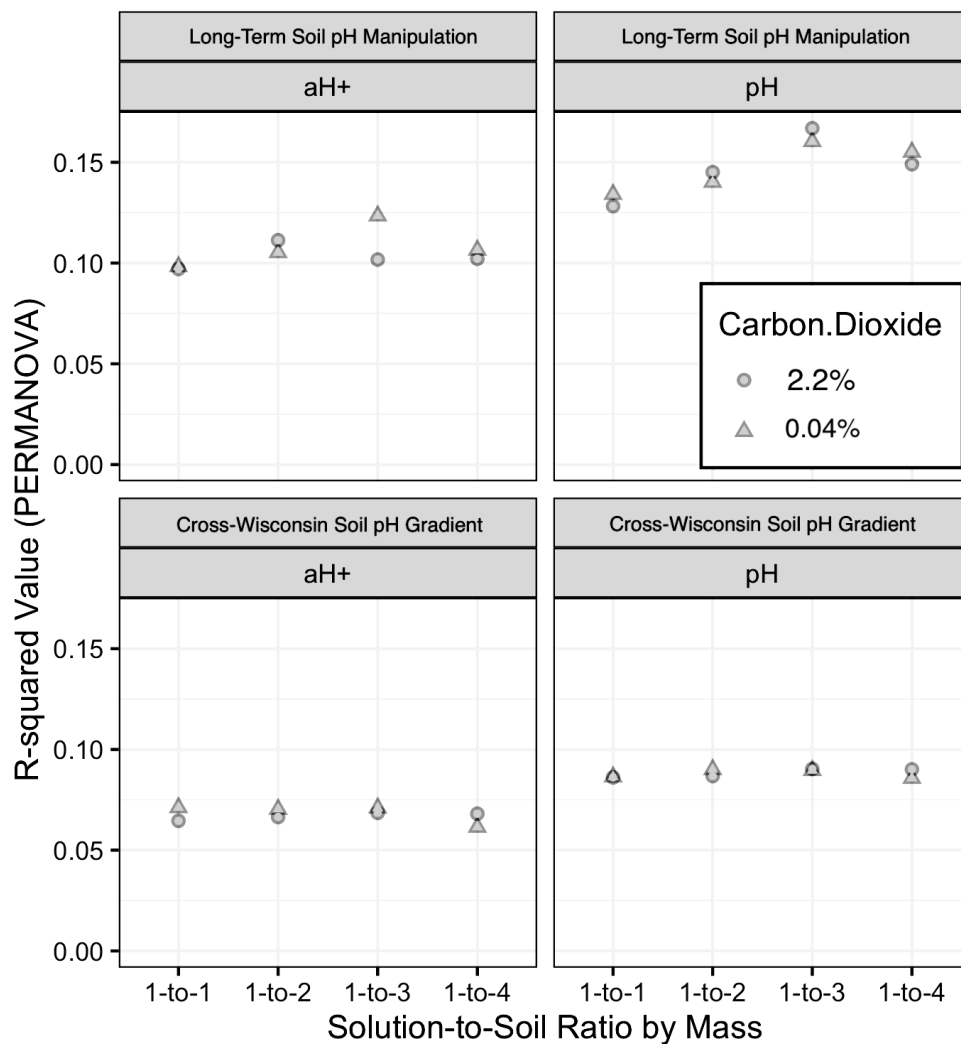
439 In both datasets, out of all tested soil properties (pH, total organic C, total N, percent
440 sand, percent silt, CEC, K, Mg, Ca, Bray P, and soil depth), pH was the best predictor,
441 with stronger effects for the long-term pH manipulation trial (PERMANOVA, $R^2 = 0.1341$,
442 $p = 0.001$; Figure 5), than the cross-Wisconsin soils (PERMANOVA, $R^2 = 0.0864$, $p = 0.001$;
443 also Figure 5). Other factors besides soil pH were also correlated with the micro-
444 bial community dissimilarities found among the Wisconsin soils, but to lesser degrees
445 ($R^2 < 0.086$). In a multivariate model, every soil property that was included added ex-
446 planatory power for community composition (PERMANOVA, $p = 0.001-0.03$, $R^2_{\text{partial}} = 0.02-$
447 0.05 , $R^2_{\text{model}} = 0.40$).



448

449 Figure 5. Non-metric multidimensional scaling plots of Bray-Curtis dissimilarities for soil bac-
450 terial communities from 16S amplicon analysis of two sets of samples: a long-term soil pH
451 manipulation trial and a cross-Wisconsin soil dataset ($k = 3$, stress = 0.109). Points are coloured
452 by standard soil pH (1 : 1 solution:soil and atmospheric CO₂).

453 Low-moisture measurements of soil pH were better predictors of microbial community
454 composition than standard soil pH in the long-term soil pH manipulation trial, explain-
455 ing as much as 16% of bacterial community dissimilarity (Figure 6). However, low-
456 moisture measurements did not substantially improve the predictive value for the Wis-
457 consin soils ($R^2 = 0.086 \pm 0.002$ throughout). Carbon dioxide levels showed little influence
458 on the predictive power of any measurement of soil acidity for both datasets. Activity
459 measurements (a_{H^+}) were poorer predictors of microbial community composition than
460 pH (Figure 6). Our findings also suggested that biases in DNA extraction solutions did
461 not explain the effects of pH on bacterial community composition, although the pH of the
462 extraction solution was significantly and negatively correlated with soil calcium content
463 ($p < 0.001$, $R^2 = 0.41$; Supplementary Note 2).



464

465 Figure 6. R-squared (R^2) values yielded from a PERMANOVA analysis of all soil pH values and
466 activity values (a_{H^+}) as factors predicting bacterial community composition, determined by 16S
467 amplicons for the cross-Wisconsin soils set and long-term pH manipulation soils set analyzed in
468 this investigation.

469 Discussion

470 Relation of Non-Standard Soil pH Values to Standard Soil pH

471 Standard soil pH measurements have underpinned fundamental advances in agronomy,
472 allowing land managers to optimize the acidity of soils to support the production of di-
473 verse and abundant crops. However, while standardized methods allow for strong repli-
474 cability across locations and time, these *ex situ* measurements of first dried and then
475 saturated soil slurries were never designed to attempt to mimic accurately *in situ* soil
476 conditions. The combined methods of extraction via centrifugation and miniaturization
477 of analyte investigated in this study were designed to allow us to more accurately char-
478 acterize the *in situ* acidity of soil microhabitats. However, our finding that standard soil
479 pH values did not consistently correspond to simulated soil pH values as solution:soil
480 ratios decreased (Figure 2) presents a confounding aspect of soil biogeochemistry. This
481 finding echos the works of Bjerrum and Gjaldbæk (1919), review by Jackson (1958, p.
482 43) (Supplemental Figure 10), resurfacing of the issue by Kilian (1961) and Mubarak
483 and Olsen (1976, p. 882), and revisited by a number of other more recent studies,
484 such as the work of Elberling and Matthiesen (2007). We will discuss here two observed
485 patterns when comparing standard and non-standard soil pH values.

486 First, the “zig-zagging” behaviour of measured pH as soil moisture was lowered from
487 a slurry (1 : 1 solution:soil by mass) to a more typical soil moisture content (1 : 4 so-
488 lution:soil by mass) (Figure 2) may be the result of a “chemical competition” between
489 the various acidic and basic buffers present in soil solutions. Multiprotic acids, multi-
490 protic bases, and the liquid junction potential together may compete for dominance in
491 their influence on the solutions’ acidities, causing the oscillation of pH values observed
492 as soil solution extracts grew increasingly concentrated. Because different chemical
493 compounds all interact with each other to determine their respective equilibrium con-
494 centrations, effectively concentrating the soil solution by as much as 4× could certainly
495 have different effects on chemical equilibria (and corresponding emergent pH values) as
496 solution:soil ratios decrease. For example, an initial increase in carbonate dissolution

497 could have caused the pH to rise, but then the effect could become overwhelmed as the
498 strength of the acidity of the soil organic matter in solution was further concentrated.
499 The BIC models support this changing-factor rationale: models predicting pH values
500 for low solution:soil ratios were less influenced by the textural properties of the soils
501 and more influenced by the chemical properties of the soils, as compared to models for
502 standard soil pH (Figure 4).

503 Second, we expected that increasing CO₂ would dissolve as carbonic acid and acidify
504 the solution in all cases, as was outlined by Strawn et al. (2020, pp. 90–97). This
505 effect was observed in the standard soil pH measurements only, and all concentrated
506 soil solution extracts (i.e. typical soil moisture content) exhibited the opposite trend.
507 Considering only the standard soil pH values of this study, Mubarak and Olsen (1976,
508 p. 882) showed a comparable trend where, using standard 1 : 1 soil slurries, “the loss of
509 CO₂ from the soil samples caused the pH to increase from 0-0.3 pH units. In other words,
510 an error of as much as +0.3 pH units can occur simply by allowing loss of CO₂ from the
511 sample by equilibration with the atmosphere.” Kaupenjohann and David (1996) found
512 that degassing carbon dioxide raised soil pH values by as much as +0.3 as well, but
513 these experiments were conducted using contained bottles, which may not correspond
514 to an experiment testing soils exposed to the larger atmosphere or chamber with carbon
515 dioxide. In another study by Dahlgren et al. (1997), degassing carbon dioxide did
516 not significantly affect soil pH, but a large decrease in ionic strength was observed,
517 owing to the loss of the HCO₃⁻ anion. Using a similar methodology to this study,
518 the authors concluded that “failure to recognize this artifact could seriously affect the
519 interpretation of data resulting from collection and analysis of soil solutions extracted
520 by centrifugation.” Thus, if one wants to gain an accurate estimate of soil pH as it exists
521 in the field, one must maintain or otherwise replicate the atmospheric conditions under
522 which soil microhabitats existed *in situ*.

523 In contrast to our expectations, at the lower solution:soil ratios, increasing CO₂ in the
524 atmosphere during pH measurements had minimal effects or even alkalifying effects,

525 instead of the consistent acidifying effect as predicted. This may be a relatively minor
526 effect - for the lines of best fit relating standard pH to non-standard pH in the cross-
527 Wisconsin dataset, the shifts in intercept were not large for 1 : 2 or 1 : 3 solution:soil
528 ratios (Figure 3), and the slopes, although different for each ratio, are still very close to
529 1 in terms of effect size (1.06 and 1.03). While slope and intercept were both significant for
530 the lowest soil:solution ratio (1 : 4), this may be largely driven by the clustering of points
531 at the higher pH levels that responded as would be expected—i.e., decreasing under high
532 CO₂. For the long-term soil pH trial dataset, lines of best fit changed similarly to the
533 cross-Wisconsin dataset with decreasing solution:soil ratios, suggesting that the small
534 shifts in pH for lower solution:soil ratios with increased CO₂ represent complex and
535 unpredictable behaviour of solutions of high ionic strength (> 0.1[M]).

536 Our observations of the effects of solution concentration on pH in this study were gener-
537 ally consistent with the conclusions of Chapman et al. (1941, p. 200)—namely, that soils
538 having a moisture content above approximately 30% gravimetric soil water content ex-
539 hibit a more consistent soil pH value, approaching neutral with further dilution, whereas
540 in soils of lower moisture content (i.e., most soils in the environment), these pH values
541 will diverge in a variable magnitude and sign. Highly diluted solutions, such as those in
542 which we typically measure soil pH, resemble the highly dilute solutions to which aque-
543 ous models apply well, but we must recognize that soils at typical soil moisture levels are
544 considered highly concentrated solutions, and thus intractably violate the “dilute solu-
545 tion assumption” required for most models of aqueous chemistry. Without meeting this
546 key assumption, we cannot accurately apply—without extreme caution—most aqueous
547 chemical models, such as the Debye-Hückel theory (Debye and Hückel, 1923; Ferguson
548 and Vogel, 1927), Sørensen’s acidity function named “pH” (MacInnes, 1948; Sørensen,
549 1909), and mean ionic activity itself (Lewis and Randall, 1921). Drained mineral soils
550 and the sediments of brackish regions, such as the coasts of all oceans and saline seas,
551 therefore have an effective ionic strength surpassing that which permit standard appli-
552 cations of pH measurements altogether. Only soils that are naturally highly saturated

553 and would not require the addition of solution to produce a dilute supernatant for anal-
554 ysis would enable commensurability of soil pH to *in situ* pH, and even these soils risk
555 substantial shifts in pH upon extraction due to degassing of CO₂ and even other gasses,
556 such as NH₃ (Elberling and Matthiesen, 2007, p. 208).

557 Non-Standard Soil pH and Microbial Communities

558 In this study, we have explored standard and non-standard measurements of soil pH
559 for the prediction of soil bacterial community composition. As numerous other studies
560 have found (Bahram et al., 2018; Bartram et al., 2014; Delgado-Baquerizo et al., 2018;
561 Rousk, Bååth, et al., 2010), soil bacterial community composition was strongly corre-
562 lated with pH across both small and large regions (Figure 5). We hypothesized that soil
563 pH values taken during the simulation of soil conditions (elevated carbon dioxide and
564 typical solution:soil ratios) would more closely represent *in situ* conditions of microhab-
565 itats and therefore predict bacterial community composition better than standard soil
566 pH values. This hypothesis was supported in the long-term pH manipulation field trial,
567 but was not meaningfully supported in the cross-Wisconsin dataset (Figure 6). This
568 suggests that, by lowering solution:soil ratios to more typical moisture levels of mineral
569 soils, we were better able to represent the conditions experienced by microbial commu-
570 nities that reflected in their composition as measured by molecular methods. This also
571 suggests that the non-standard *in situ* soil pH method developed here will apply well to
572 soils of similar texture but poorly to soils of diverse texture. Overall, the range of soil pH
573 values grew widely at low moisture whereas the range of soil pH values varied little from
574 neutral at high moisture, namely the standard soil suspension method. This growing
575 range of soil pH values measured under more typical conditions results in improved pre-
576 dictions of microbial community composition, suggesting further that standard soil pH,
577 as it is currently measured, fails to distinguish differences in environmental conditions
578 that are relevant to microbial life in soils.

579 Why, then, did similar changes in non-standard pH in the cross-Wisconsin dataset not

580 result in similarly improved predictions of microbial community composition? While
581 the soils from the pH manipulation trial were controlled and relatively similar in all
582 characteristics except soil pH, the Wisconsin soil dataset was designed to be diverse in
583 texture, organic matter, and other factors. Thus, pH had weaker explanatory power to
584 begin with, due to the presence of other influential differences in the Wisconsin dataset.
585 Furthermore, the mechanisms by which adjusting solution content affects pH may differ
586 in different soils. Additionally, we should recognize that the long-term experimental pH
587 plots had been amended with lime to raise the soil pH and sulfur to lower the soil pH.
588 We cannot rule out that some excess unreacted amendment may have persisted in the
589 samples, whose suspension during preparation for analysis might have dissolved and
590 reacted to affect the analyte. This would potentially help explain why the higher soil pH
591 values increased and the lower ones decreased at lower solution:soil ratios but would
592 *not* explain why the pH values of the improved method were more accurately related to
593 the composition of respective soil microbial communities.

594 Why did increasing CO₂ levels not affect predictive power of pH measurements? The ef-
595 fects of increasing CO₂ levels were more consistent across the range of pH levels, i.e., the
596 intercept changed, but the slope changed less than it did when changing solution:soil
597 ratios (Figures 2 and 3). Thus, it is not surprising that we did not gain predictive power
598 from adjusting CO₂ levels during measurements. If one is concerned about an extremely
599 accurate measurement of pH, then it may be advisable to measure the soil solution un-
600 der CO₂ levels designed to mirror those of the soil. However, if one is interested primarily
601 in predictive values in mineral soils, then these results suggest that such an approach is
602 not essential. The measurement of the effects of CO₂ on *in situ* soil pH, when this effect
603 is measured in the future, may prove more significant. We might also consider whether
604 the causes of high CO₂ levels in a given soil—e.g., optimal moisture, temperature, and
605 organic matter availability for microbial respiration—are more directly influential on mi-
606 crobial composition than their indirect (and perhaps transient) effects of elevating CO₂
607 that shifts the pH of the soil solution.

608 Finally, a comment should be made on the assumptions underpinning the correlations
609 between pH and microbial community composition. A PERMANOVA effectively tests
610 for the presence of a linear relationship between microbial community dissimilarities
611 and the variables of interest. As we are all well aware, pH is logarithmically related
612 to a_{H^+} . Although studies have historically found a significant and large relationship
613 between pH and microbial community composition, there is no reason that the causative
614 factors underpinning the specific effect of pH on soil microbial communities should be
615 specifically proportional to the negative log of a_{H^+} . That is to say, there is not an obvious
616 reason that a 10x increase in a_{H^+} should have half the effect on the microbial community
617 composition that a 100x increase does, nor would we necessarily expect differences in
618 community composition to be linearly related to a_{H^+} itself (Figure 6). It is important
619 to consider this caveat when exponentiating soil pH values and performing statistical
620 analyses with these calculated values in molar units.

621 Recommendations

622 Because the microbial ecology of soil microorganisms, the acidity and acidification of
623 soils, and the mechanisms by which soil bacteria survive are all of great relevance to
624 sustainable crop production and biogeochemical models, non-standard soil pH values
625 may offer both microbiologists and agronomists more targeted metrics to monitor and
626 ultimately improve soil health (Meena, 2019, pp. 113–159). Unfortunately, whether
627 and how to choose an appropriate non-standard protocol can be challenging, even if we
628 recognize the need for alternate approaches. On the one hand, the use of non-standard
629 methods of measuring soil acidity risks violating the commensurability of an investi-
630 gator's pH values to the standard soil pH values found in large databases (Minasny et
631 al., 2011). On the other hand, the large diversity and variability through time of soil
632 environments warrants diversification and customization of methods as well as the sub-
633 sequent interpretation of the values that novel or adapted methods yield. For example,
634 most soils collected at field capacity do not require the addition of excess analytical so-
635 lution to extract soil solution via centrifugation (Geibe et al., 2006; Wolt, 1994, p. 104).

636 A saturated peatland may require neither drying nor addition of solution but simply
637 gentle centrifugation and analysis of the supernatant with a glass pH probe to obtain
638 an informative measurement of pH. On the opposite extreme, a study of saline desert
639 soils inhabited by plants having halotolerant root physiology would require the addition
640 of a solution, almost certainly equal to or in excess of the typical 1 : 1 solution:soil ratio
641 by mass, to create solution extract dilute enough for pH measurement. We must also
642 continue (or begin) to ask what “soil pH” fundamentally means for frozen systems. In
643 many regions of Earth’s surface, the soil solution is in solid phase for all or a large pe-
644 riod of the year, whereby the solution is intractably shifted away from away from states
645 resembling lab conditions.

646 We may reformulate soil pH measurement recommendations for the improved use of
647 such values in microbial ecology, possibly viewing the elevated concentration of solutes
648 and carbonate in the analytes of these sites as a means of both heightening the detection
649 of important acids and bases found in typical soil solution by the glass probe as well as
650 improving the representation of *in situ* conditions of soil microhabitats (Sumner, 1994).
651 However, the further concentration of analyte beyond a 1 : 4 solution:soil ratio may
652 cause the analyte to begin interfering with the functioning of the glass probe, which,
653 as stated above, only functions without error $\leq 5\%$ in analytes of ionic strength ≤ 0.01
654 moles per liter (Anderegg and Kholeif, 1994, p. 1521; Baucke, 2002, p. 774; Butler,
655 1998, pp. 462–463; Covert and Hore, 2016, pp. 235–238; de Levie, 2014, p. 615, 2010;
656 Dobrovolskii et al., 2018, p. 87; Galster, 1991, p. 16; Sparks, 1998, p. 112; Spitzer
657 and Pratt, 2011, p. 75; Volk and Rozen, 1977; Wright, 2007, p. 382; p. 1569; Pourbaix,
658 1974, p. 14; Ashcraft, 1957, p. 3, 1947, p. 29; Bates and Guggenheim, 1960, p. 167;
659 Debye and Hückel, 1923, p. 197; Feldman, 1956, p. 1865, 1956, p. 1865; MacInnes,
660 1939, p. 148; Sena, 1972, Appendix 3).

661 Standard measurements of soil pH, such as those that populate our national or global
662 soil databases, have been extremely useful for agronomy, and have also correlated
663 strongly with bacterial community composition. However, we recognize that these meth-

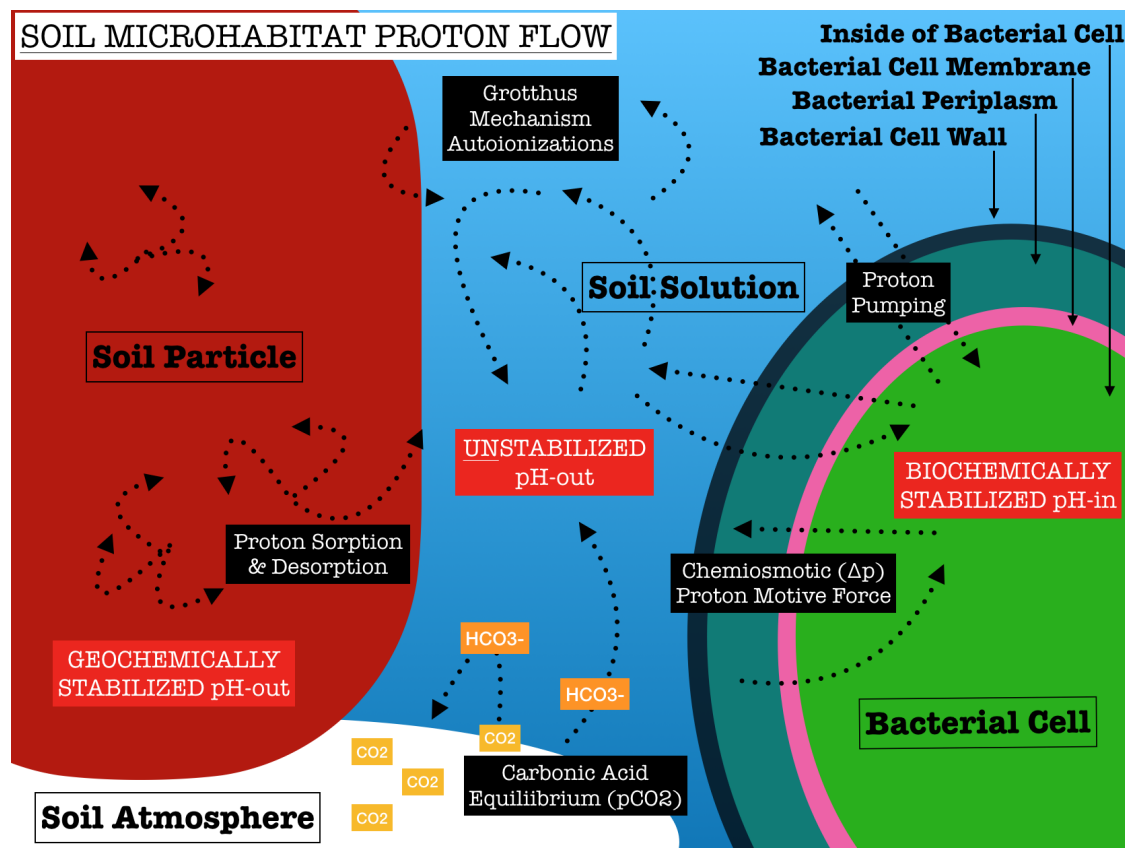
664 ods offer only a limited representation of the acidity of soil microhabitats as they are
665 experienced by microbes. By using methods for measuring soil acidity that simulate the
666 *in situ* conditions of soils, we may improve the predictive models of the ecology of soil
667 bacteria. The tools and equipment used here are all common to a molecular microbiol-
668 ogy laboratory, and as such offer investigators the ability to miniaturize and concentrate
669 the soil-solution suspension. Miniaturization of soil solution preparation also enables
670 the analysis of more measurements at a higher throughput, as well as more readily
671 simulating the conditions of soil microhabitats in the laboratory to measure *in situ* soil
672 pH in a glove box to simulate non-standard atmospheric conditions, if desired. Such
673 non-standard soil pH values have the potential to improve the modeling of temporal
674 variability and enhance the characterization of study systems of both agronomists and
675 microbial ecologists.

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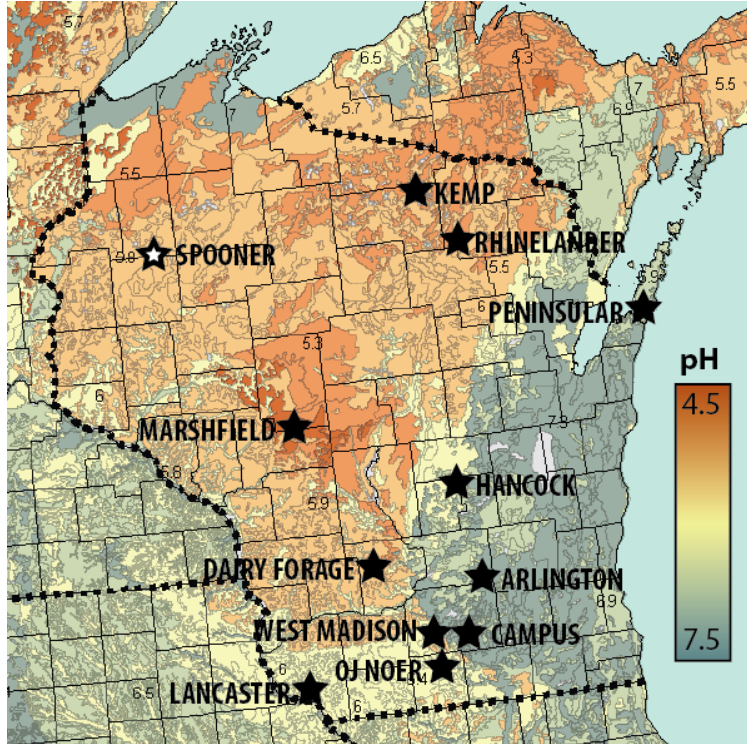
687 **Supplemental Materials**

688 **Supplemental Figures**



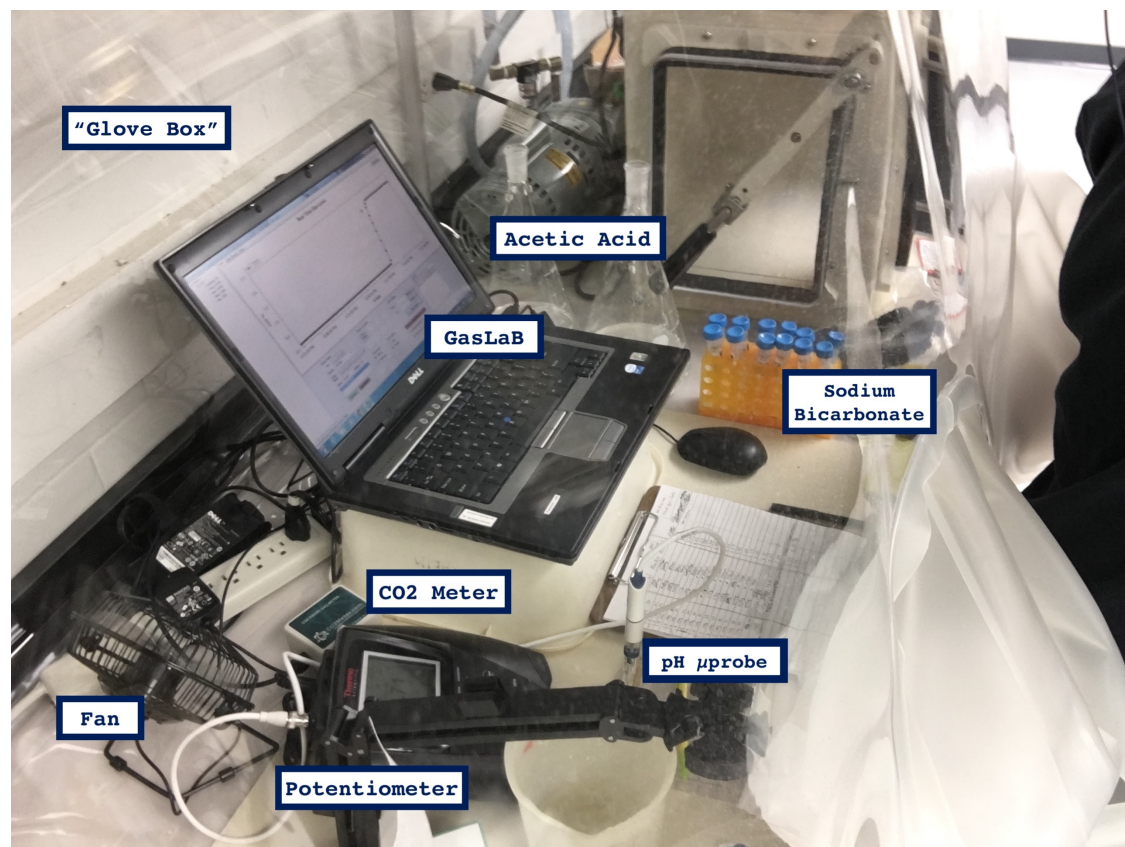
689

690 Supplemental Figure 1. Soil microhabitat proton flow describes the biogeochemical processes
691 connecting abiotic and biotic proton reservoirs.



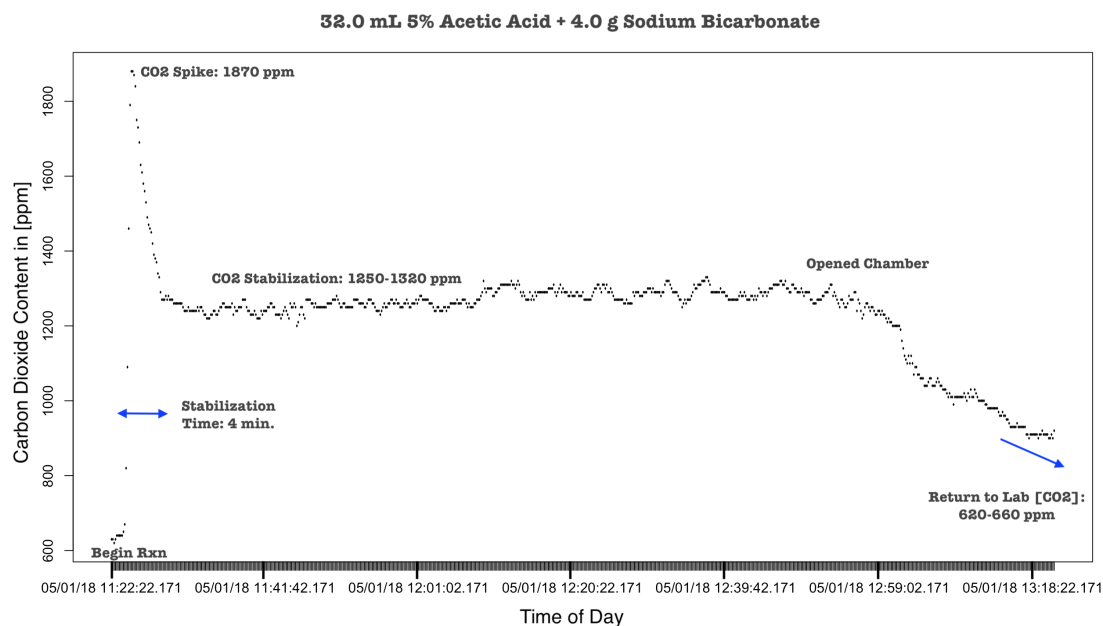
692

693 Supplemental Figure 2. Map of field locations in Wisconsin with reference to the natural soil pH
694 gradient across this region. Modified with permission from bonap.org (Kartesz, 2015).



695

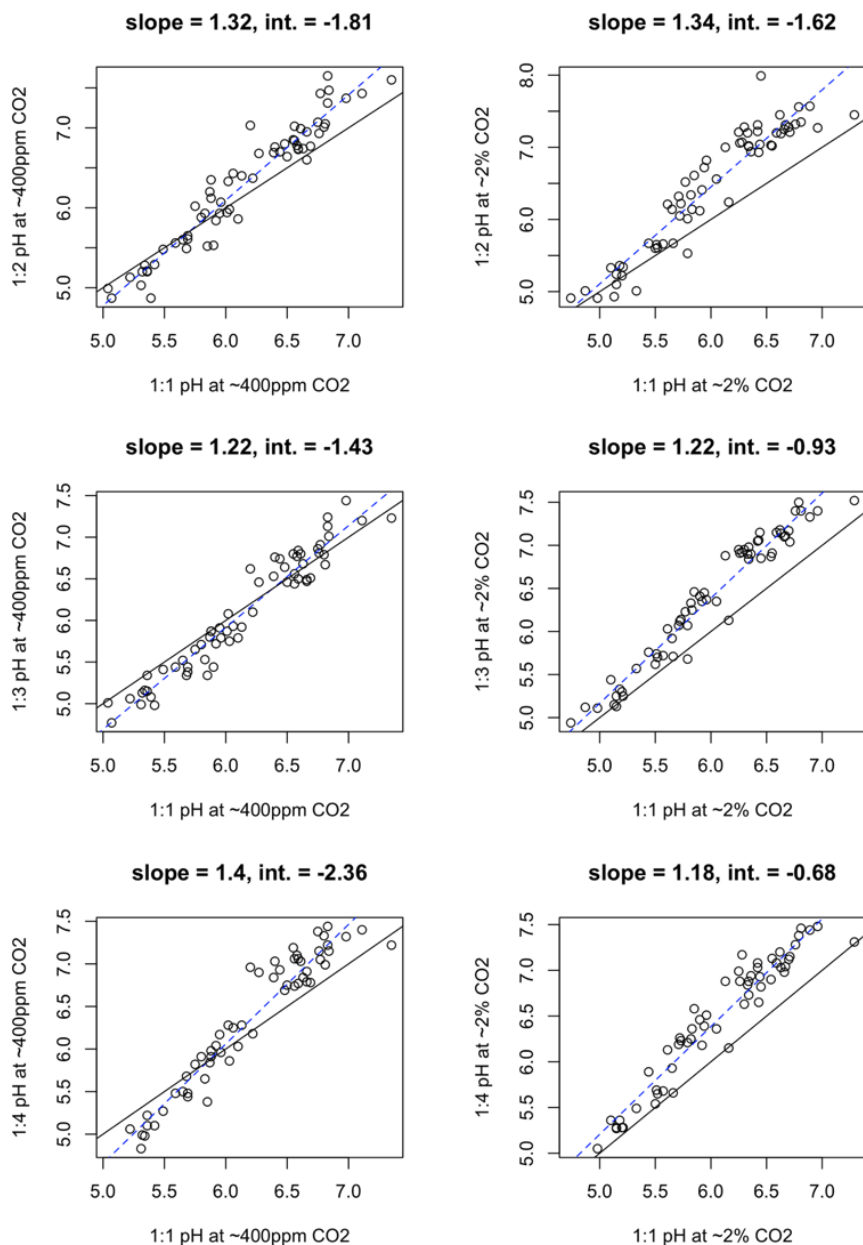
696 Supplemental Figure 3. "Simulated soil pH" experimental rig, equipment, and reagents for
697 acidimetry under elevated carbon dioxide resembling a typical *in situ* soil atmosphere.



698

699 Supplemental Figure 4. Test showing that the chamber (“glove box”) and gas analyzer provide
700 a stable and controllable elevated carbon dioxide atmosphere for sufficient time and elvels to
701 perform chemical analyses such as acidimetry while simulating soil atmospheric conditions. The
702 carbon dioxide content exhibits an initial spike, stabilization, then an extended period whereby
703 the chamber has an elevated carbon dioxide creating a partially simulated soil atmosphere. The
704 chamber can be opened and vented once more to return to laboratory carbon dioxide levels.

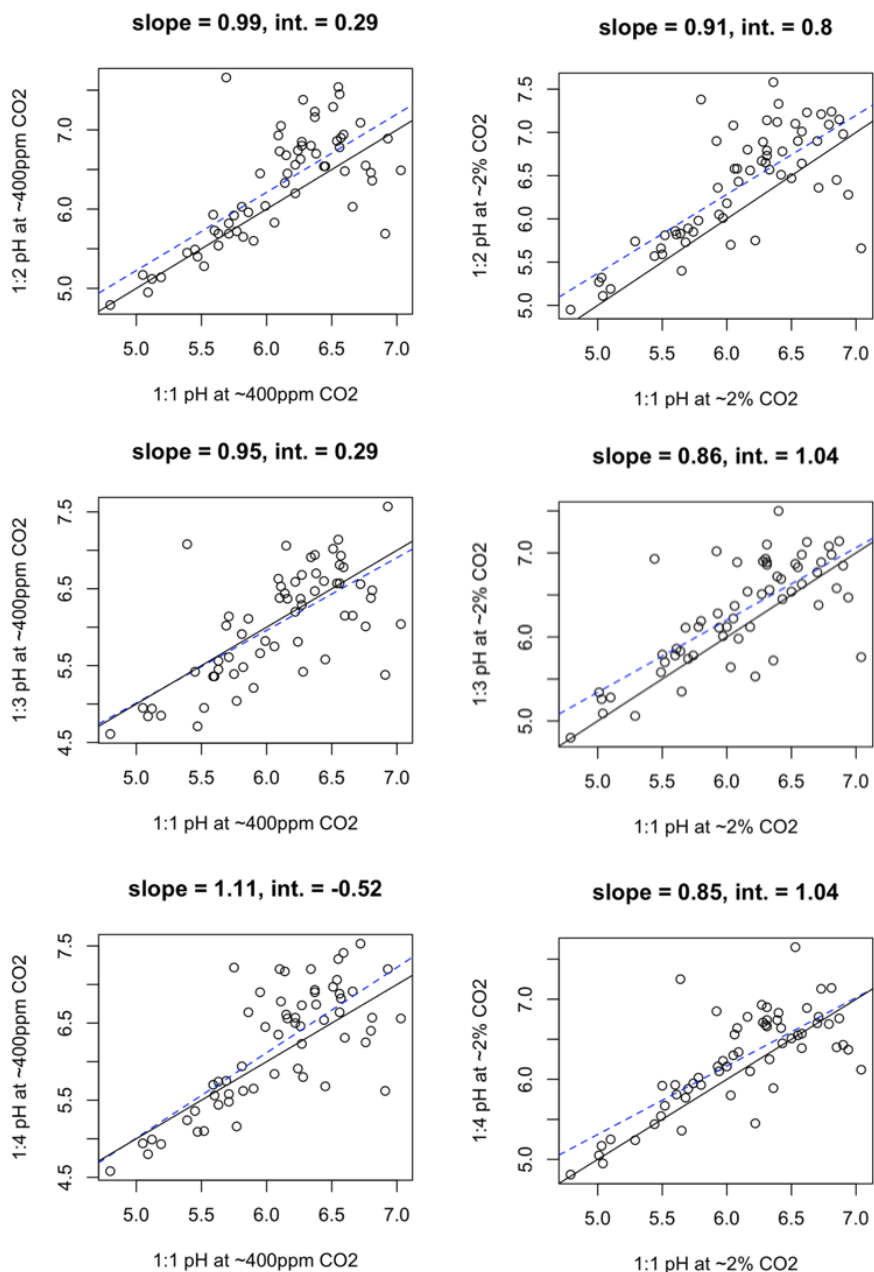
Cross-Wisconsin Soil pH Gradient



705

706 Supplemental Figure 5. Standard soil pH (solution:soil ratio) of cross-Wisconsin soils compared
707 to three other ratios (2 : 1, 3 : 1, 4 : 1) at two levels of carbon dioxide (0.04% ppm and 2.2%(±0.05)).

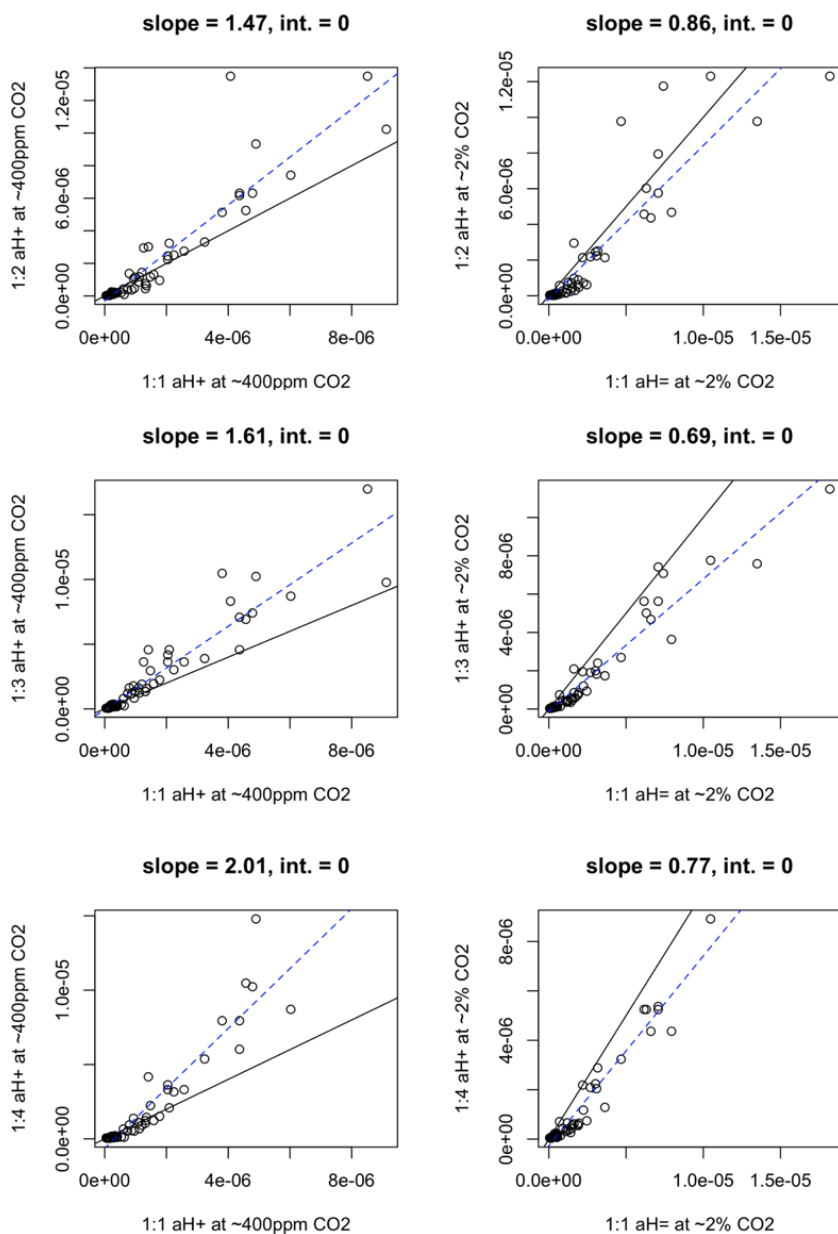
Long-term Soil pH Manipulation



708

709 Supplemental Figure 6. Standard soil pH (1 : 1 solution:soil ratio) of long-term pH manipulation
710 soils compared to three other ratios (2 : 1, 3 : 1, 4 : 1) at two levels of carbon dioxide (0.04% and
711 2.2%(±0.05)).

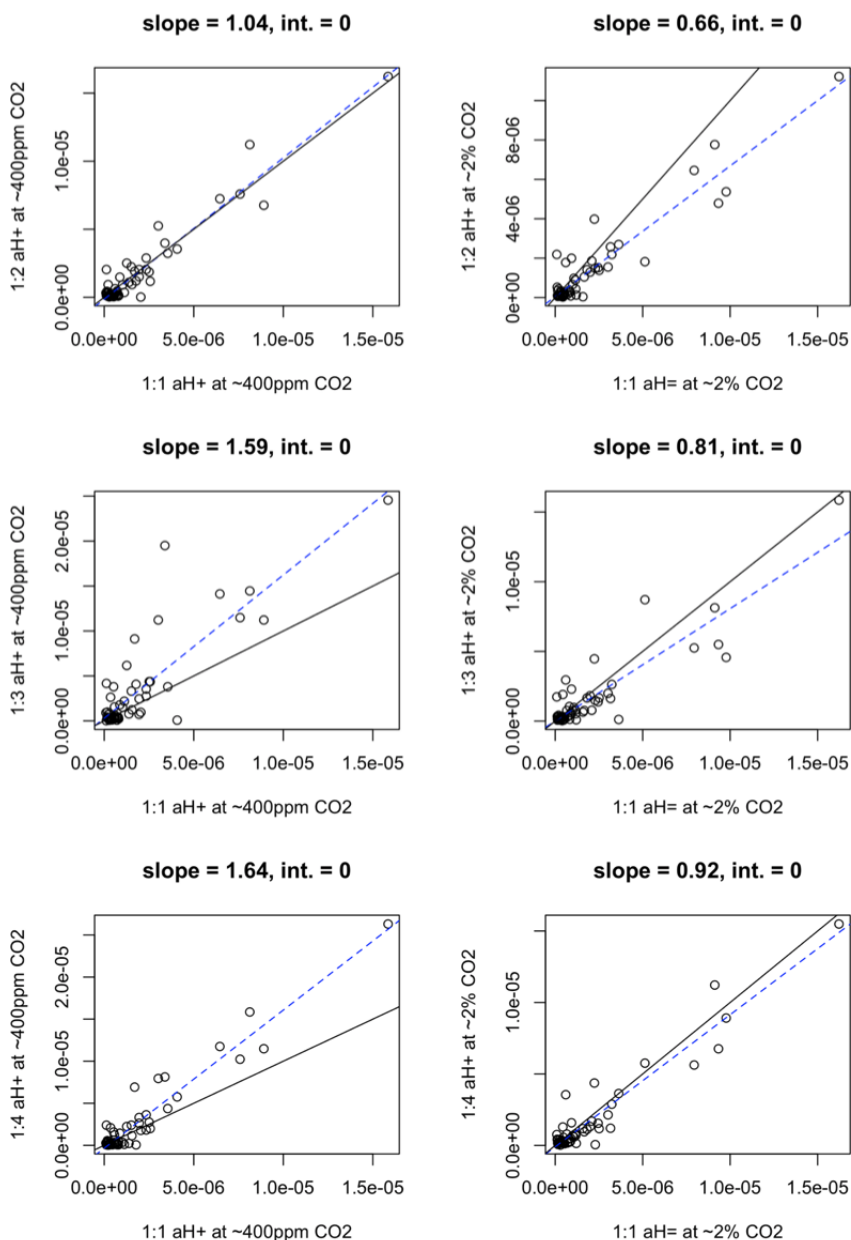
Cross-Wisconsin Soil pH Gradient



712

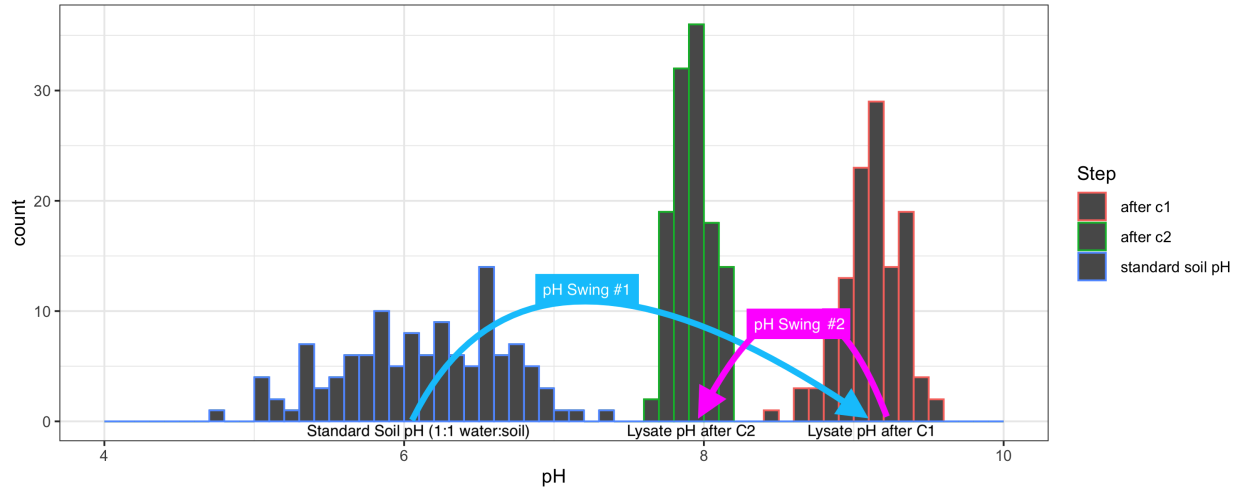
713 Supplemental Figure 7. Soil a_{H^+} (1 : 1 solution:soil ratio) of cross-Wisconsin soils compared to
 714 three other ratios (2 : 1, 3 : 1, 4 : 1) at two levels of carbon dioxide (0.04% and 2.2%(±0.05)).

Long-term Soil pH Manipulation



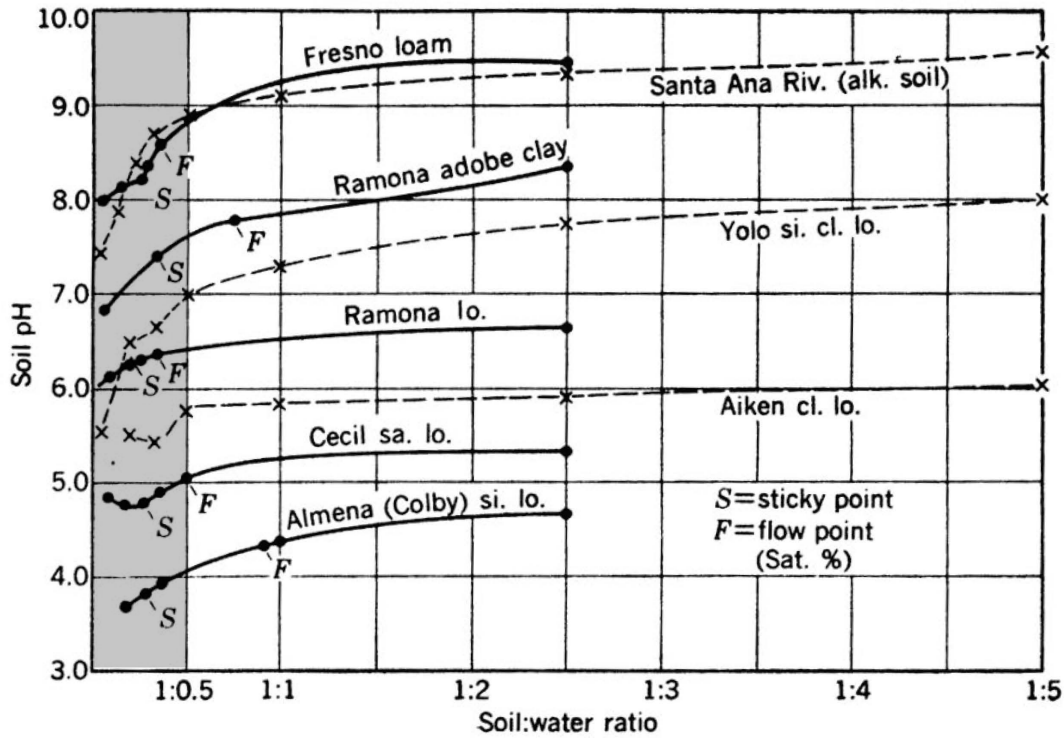
715

716 Supplemental Figure 8. Soil a_{H^+} (1 : 1 solution:soil ratio) of long-term pH manipulation soils
717 compared to three other ratios (2 : 1, 3 : 1, 4 : 1) at two levels of carbon dioxide (0.04% and
718 2.2% (± 0.05)).



719

720 Supplemental Figure 9. Histograms of standard soil pH and the pH of the lysate supernatants
721 after treatment with buffers “C1” and “C2”, respectively, of the first two steps (“pH swings”) of
722 the soil DNA extraction protocol.



723

724 Supplemental Figure 10. The slurry-to-paste dilution pH differentiation trend, adapted from
725 Jackson (1958, p. 43). Solid lines were derived from Chapman et al. (1941) and dashed lines
726 were derived from Huberty and Haas (1940). The moisture levels considered typical and repre-
727 sentative of *in situ* conditions that are much less diluted than standard soil pH (1 : 1 solution:soil
728 by mass) in this study are greyed.

729 Supplemental Notes

730 Supplemental Note 1: pH Probe Details

731 The pH electrode had a shaft length of 60 [mm] and a diameter of 3 [mm] with a built-in ARGEN-
732 THAL reference system of 3.0 [M] KCl reference electrolyte. The probe was stored in either 3.0 [M]
733 KCl saturated electrolyte solution or InLab storage solution (Material No. 30111142). The probe's
734 glass was made from U Glass with a membrane resistance of 600 [Mohm]. The probe, owing to
735 the sorption of solution to its surface, will remove approximately 5 [μ L] per measurement, and
736 the accuracy during this study was $< \%15$ while performing ≤ 3 repeated measurements of the
737 same extracts in different simulated conditions.

738 Supplemental Note 2: "pH Swings" of Soil DNA Lysate During Extraction

739 Refer to Supplemental Figure 9. The pH values of miniaturized analytes of the first two steps of a
740 standard soil DNA extraction protocol were measured. Two sets of DNA extraction kits with bead-
741 beating tubes and solutions C1 and C2, which are identical to the solutions and materials used
742 in the PowerLyzer PowerSoil DNA Isolation Kit used for 16S amplicon sequencing in this study,
743 were used to generate lysates of the first two steps of the soil DNA extraction. Excess addition of
744 C1 and C2 solutions allowed for the removal of small aliquots of solution without disrupting the
745 chemical events and buffers of the first steps of DNA extraction. 100 [μ L] was removed from the
746 lysate after the addition and bead-beating with solution C1, and another 100 [μ L] was removed
747 from the lysate after the addition of solution C2. The pH values of these solutions ("after C1" and
748 "after C2") were compared to the standard soil pH values (i.e., 1 : 1 solution:soil ratio at ambient
749 carbon dioxide levels).

750 The first pH swings to from the the more variable and acidic standard soil pH values (1 : 1
751 solution:soil), then the second pH swings down to approximately , narrowing the range of pH
752 values as the DNA extraction progresses (Supplemental Figure 11). The acidic soils (< 5.5) were
753 nearly $100\times$ more acidic than the neutral-to-basic soils (< 7.0) according to their standard soil pH
754 measurement, but the DNA extraction kit treated these soils with an identical alkaline buffer in
755 the first step.

756 Although solution C1 pH and solution C2 pH were both significant predictors of community
757 composition on their own, after controlling for other soil properties, neither was a significant
758 predictor, nor were they correlated with soil pH measurements ($p_{C1} = 0.46$ and $p_{C2} = 0.69$). How-
759 ever, they were significantly negatively correlated with total Ca ($p < 0.001$, $R^2 = 0.41$).

760 **Supplemental Tables**

761 **Supplemental Table 1. Latitude, longitude, soil series, and soil pH of field sites according to the**
 762 **Web Soil Survey database.**

Pit.ID	Research.Station	Latitude	Longitude	Soil.Series	Soil.pH..WSS.
K1	Kemp	45.84073	-89.67555	Sayner loamy sand, 15 to 45 percent slopes	5.4
K3	Kemp	45.83834	-89.67427	Sayner loamy sand, 15 to 45 percent slopes	5.4
K4	Kemp	45.85040	-89.65060	Vilas loamy sand, 6 to 15 percent slopes	5.5
R1	Rhineland	45.66480	-89.26794	Vilas loamy sand, 0 to 6 percent slopes	5.5
R2	Rhineland	45.65433	-89.26533	Vilas loamy sand, 0 to 6 percent slopes	5.5
R3	Rhineland	45.66651	-89.21747	Padus-Pence sandy loams, 0 to 6 percent slopes	5.4
M1	Marshfield	44.76046	-90.09719	Withee silt loam, 0 to 3 percent slopes	5.6
M2	Marshfield	44.76225	-90.09930	Loyal silt loam, 1 to 6 percent slopes	5.7
M3	Marshfield	44.76370	-90.11234	Marshfield silt loam, 0 to 2 percent slopes	5.1
Sp	Spooner	45.82540	-91.86877	Mahtomedi-Cress complex, 2 to 6 percent slopes	5.8
H1	Hancock	44.12066	-89.53984	Sparta loamy sand, 0 to 2 percent slopes	6.2
H2	Hancock	44.11900	-89.54606	Plainfield sand, 0 to 2 percent slopes	5.3
A249	Arlington	43.30450	-89.36342	Channahon silt loam, 12 to 30 percent slopes, eroded	7.3
A341	Arlington	43.30205	-89.35450	Saybrook silt loam, 6 to 12 percent slopes, eroded	6.5
L2	Lancaster	42.83506	-90.79082	Fayette silt loam, uplands, 6 to 10 percent slopes, moderately eroded	6.0
L3	Lancaster	42.82901	-90.79458	Palsgrove silt loam, 6 to 12 percent slopes, moderately eroded	6.4
L4	Lancaster	42.84232	-90.79415	Dubuque soils, deep, 10 to 15 percent slopes, moderately eroded	6.2
W4	West Madison	43.05465	-89.53524	Griswold loam, 12 to 20 percent slopes, eroded	6.9
W5	West Madison	43.06537	-89.54614	Plano silt loam, gravelly substratum, 2 to 6 percent slopes	6.6
W7	West Madison	43.07023	-89.54216	Dresden silt loam, 6 to 12 percent slopes, eroded	6.6
P1	Peninsular	44.87988	-87.33316	Onaway-Ossineke fine sandy loams, moraine, 1 to 6 percent slopes	6.6
P2	Peninsular	44.88135	-87.33140	Longrie Loam, 2 to 6 percent slopes	6.7
P4	Peninsular	44.88060	-87.32387	Summerville loam, 0 to 2 percent slopes	7.3

763

764 Supplemental Table 2. Primers used to amplify 16S gene.

Sample.Name	Fwd_Primer_ID	Fwd_Primer_Barcode	Rev_Primer_ID	Rev_Primer_Barcode
001.K1.0.17	515f_SA501	ATCGTACG	806r_SA701	AACTCTCG
002.K1.17.45	515f_SB501	CTACTATA	806r_SA709	GTCGTAGT
003.K1.45.60	515f_SC501	ACGACGTG	806r_SB705	CGTAGATC
004.K2.Muck	515f_SA502	ACTATCTG	806r_SA702	ACTATGTC
005.K3.0.15	515f_SB502	CGTTACTA	806r_SA710	TAGCAGAC
006.K3.15.35	515f_SC502	ATATACAC	806r_SB706	CTCGTTAC
007.K3.35.50	515f_SA503	TAGCGAGT	806r_SA703	AGTAGCGT
008.K4.0.15	515f_SB503	AGAGTCAC	806r_SA711	TCATAGAC
009.K4.15.30	515f_SC503	CGTCGCTA	806r_SB707	GCGCACGT
010.K4.30.50	515f_SA504	CTGCGTGT	806r_SA704	CAGTGAGT
011.R1.0.27	515f_SB504	TACGAGAC	806r_SA712	TCGCTATA
012.R1.27.50	515f_SC504	CTAGAGCT	806r_SB708	GGTACTAT
013.R1.50.70	515f_SA505	TCATCGAG	806r_SA705	CGTACTCA
014.R2.0.30	515f_SB505	ACGTCTCG	806r_SB701	AAGTCGAG
015.R2.30.45	515f_SC505	GCTCTAGT	806r_SB709	GTATACGC
016.R2.45.60	515f_SA506	CGTGAGTG	806r_SA706	CTACGCAG
017.R2.60.100	515f_SB506	TCGACGAG	806r_SB702	ATACTTCG
018.R3.0.20	515f_SC506	GACACTGA	806r_SB710	TACGAGCA
019.R3.20.30	515f_SA507	GGATATCT	806r_SA707	GGAGACTA
020.M1.0.31	515f_SB507	GATCGTGT	806r_SB703	AGCTGCTA
021.M1.31.50	515f_SC507	TGCGTACG	806r_SB711	TCAGCGTT
022.M1.50.70	515f_SA508	GACACCGT	806r_SA708	GTCGCTCG
023.M2.0.24	515f_SB508	GTCAGATA	806r_SB704	CATAGAGA
024.M2.24.38	515f_SC508	TAGTGTAG	806r_SB712	TCGCTACG
025.M2.38.55	515f_SA501	ATCGTACG	806r_SB712	TCGCTACG
026.M3.0.15	515f_SB501	CTACTATA	806r_SA701	AACTCTCG
027.M3.15.30	515f_SC501	ACGACGTG	806r_SA709	GTCGTAGT
028.S.0.30	515f_SA502	ACTATCTG	806r_SB705	CGTAGATC
029.S.30.60	515f_SB502	CGTTACTA	806r_SA702	ACTATGTC
030.H1.0.30	515f_SC502	ATATACAC	806r_SA710	TAGCAGAC
031.H1.30.40	515f_SA503	TAGCGAGT	806r_SB706	CTCGTTAC
032.H1.40.60	515f_SB503	AGAGTCAC	806r_SA703	AGTAGCGT
033.H2.0.30	515f_SC503	CGTCGCTA	806r_SA711	TCATAGAC
034.H2.30.60	515f_SA504	CTGCGTGT	806r_SB707	GCGCACGT
035.A249.0.35	515f_SB504	TACGAGAC	806r_SA704	CAGTGAGT
036.A249.35.60	515f_SC504	CTAGAGCT	806r_SA712	TCGCTATA
037.A341.0.33	515f_SA505	TCATCGAG	806r_SB708	GGTACTAT
038.A341.33.55	515f_SB505	ACGTCTCG	806r_SA705	CGTACTCA
039.A341.55.75	515f_SC505	GCTCTAGT	806r_SB701	AAGTCGAG
040.A341.75.85	515f_SA506	CGTGAGTG	806r_SB709	GTATACGC
041.L2.0.23	515f_SB506	TCGACGAG	806r_SA706	CTACGCAG
042.L2.23.45	515f_SC506	GACACTGA	806r_SB702	ATACTTCG
043.L3.0.12	515f_SA507	GGATATCT	806r_SB710	TACGAGCA
044.L3.12.20	515f_SB507	GATCGTGT	806r_SA707	GGAGACTA
045.L3.20.40	515f_SC507	TGCGTACG	806r_SB703	AGCTGCTA
046.L4.0.10	515f_SA508	GACACCGT	806r_SB711	TCAGCGTT
047.L4.10.20	515f_SB508	GTCAGATA	806r_SA708	GTCGCTCG
048.L4.20.40	515f_SC508	TAGTGTAG	806r_SB704	CATAGAGA
049.W3.Compost	515f_SA501	ATCGTACG	806r_SB704	CATAGAGA
050.W4.0.28	515f_SB501	CTACTATA	806r_SB712	TCGCTACG
051.W4.28.45	515f_SC501	ACGACGTG	806r_SA701	AACTCTCG
052.W4.45.55	515f_SA502	ACTATCTG	806r_SA709	GTCGTAGT
053.W5.0.35	515f_SB502	CGTTACTA	806r_SB705	CGTAGATC
054.W5.35.65	515f_SC502	ATATACAC	806r_SA702	ACTATGTC
055.W7.0.15	515f_SA503	TAGCGAGT	806r_SA710	TAGCAGAC

Sample.Name	Fwd_Primer_ID	Fwd_Primer_Barcode	Rev_Primer_ID	Rev_Primer_Barcode
056.W7.15.30	515f_SB503	AGAGTCAC	806r_SB706	CTCGTTAC
057.P1.0.30	515f_SC503	CGTCGCTA	806r_SA703	AGTAGCGT
058.P1.30.45	515f_SA504	CTGCGTGT	806r_SA711	TCATAGAC
059.P1.45.55	515f_SB504	TACGAGAC	806r_SB707	GCGCACGT
060.P2.0.20	515f_SC504	CTAGAGCT	806r_SA704	CAGTGAGT
061.P2.20.45	515f_SA505	TCATCGAG	806r_SA712	TCGCTATA
062.P2.45.55	515f_SB505	ACGTCTCG	806r_SB708	GGTACTAT
063.P4.0.25	515f_SC505	GCTCTAGT	806r_SA705	CGTACTCA
064.P4.25.35	515f_SA506	CGTGAGTG	806r_SB701	AAGTCGAG
065.P4.35.50	515f_SB506	TCGACGAG	806r_SB709	GTATACGC
181.Sp11.Cr31.0.20	515f_SC506	GACACTGA	806r_SA706	CTACGCAG
182.Sp11.Cr32.0.16	515f_SA507	GGATATCT	806r_SB702	ATACTTCG
183.Sp11.Cr33.0.20	515f_SB507	GATCGTGT	806r_SB710	TACGAGCA
184.Sp12.Cr34.0.16	515f_SC507	TGCGTACG	806r_SA707	GGAGACTA
185.Sp12.Cr35.0.18	515f_SA508	GACACCGT	806r_SB703	AGCTGCTA
186.Sp12.Cr36.0.20	515f_SB508	GTCAGATA	806r_SB711	TCAGCGTT
187.Sp13.Cr37.0.20	515f_SC508	TAGTGTAG	806r_SA708	GTCGCTCG
188.Sp13.Cr38.0.20	515f_SA501	ATCGTACG	806r_SA708	GTCGCTCG
189.Sp13.Cr39.0.19	515f_SB501	CTACTATA	806r_SB704	CATAGAGA
190.Sp14.Cr40.0.17	515f_SC501	ACGACGTG	806r_SB712	TCGCTACG
191.Sp14.Cr41.0.18	515f_SA502	ACTATCTG	806r_SA701	AACTCTCG
192.Sp14.Cr42.0.20	515f_SB502	CGTTRACTA	806r_SA709	GTCGTAGT
193.Sp15.Cr43.0.18	515f_SC502	ATATACAC	806r_SB705	CGTAGATC
194.Sp15.Cr44.0.17	515f_SA503	TAGCGAGT	806r_SA702	ACTATGTC
195.Sp15.Cr45.0.20	515f_SB503	AGAGTCAC	806r_SA710	TAGCAGAC
196.Sp16.Cr46.0.20	515f_SC503	CGTCGCTA	806r_SB706	CTCGTTAC
197.Sp16.Cr47.0.17	515f_SA504	CTGCGTGT	806r_SA703	AGTAGCGT
198.Sp16.Cr48.0.20	515f_SB504	TACGAGAC	806r_SA711	TCATAGAC
199.Sp17.Cr49.0.20	515f_SC504	CTAGAGCT	806r_SB707	GCGCACGT
200.Sp17.Cr50.0.18	515f_SA505	TCATCGAG	806r_SA704	CAGTGAGT
201.Sp17.Cr51.0.18	515f_SB505	ACGTCTCG	806r_SA712	TCGCTATA
202.Sp18.Cr52.0.18	515f_SC505	GCTCTAGT	806r_SB708	GGTACTAT
203.Sp18.Cr53.0.19	515f_SA506	CGTGAGTG	806r_SA705	CGTACTCA
204.Sp18.Cr54.0.20	515f_SB506	TCGACGAG	806r_SB701	AAGTCGAG
205.Sp19.Cr55.0.20	515f_SC506	GACACTGA	806r_SB709	GTATACGC
206.Sp19.Cr56.0.18	515f_SA507	GGATATCT	806r_SA706	CTACGCAG
207.Sp19.Cr57.0.18	515f_SB507	GATCGTGT	806r_SB702	ATACTTCG
208.Sp20.Cr58.0.20	515f_SC507	TGCGTACG	806r_SB710	TACGAGCA
209.Sp20.Cr59.0.19	515f_SA508	GACACCGT	806r_SA707	GGAGACTA
210.Sp20.Cr60.0.20	515f_SB508	GTCAGATA	806r_SB703	AGCTGCTA
211.Sp1.Cr1.0.20	515f_SC508	TAGTGTAG	806r_SB711	TCAGCGTT
212.Sp1.Cr2.0.20	515f_SA501	ATCGTACG	806r_SB711	TCAGCGTT
213.Sp1.Cr3.0.20	515f_SB501	CTACTATA	806r_SA708	GTCGCTCG
214.Sp2.Cr4.0.20	515f_SC501	ACGACGTG	806r_SB704	CATAGAGA
215.Sp2.Cr5.0.20	515f_SA502	ACTATCTG	806r_SB712	TCGCTACG
216.Sp2.Cr6.0.20	515f_SB502	CGTTRACTA	806r_SA701	AACTCTCG
217.Sp3.Cr7.0.20	515f_SC502	ATATACAC	806r_SA709	GTCGTAGT
218.Sp3.Cr8.0.20	515f_SA503	TAGCGAGT	806r_SB705	CGTAGATC
219.Sp3.Cr9.0.20	515f_SB503	AGAGTCAC	806r_SA702	ACTATGTC
220.Sp4.Cr10.0.20	515f_SC503	CGTCGCTA	806r_SA710	TAGCAGAC
221.Sp4.Cr11.0.20	515f_SA504	CTGCGTGT	806r_SB706	CTCGTTAC
222.Sp4.Cr12.0.20	515f_SB504	TACGAGAC	806r_SA703	AGTAGCGT
223.Sp5.Cr13.0.20	515f_SC504	CTAGAGCT	806r_SA711	TCATAGAC
224.Sp5.Cr14.0.20	515f_SA505	TCATCGAG	806r_SB707	GCGCACGT
225.Sp5.Cr15.0.20	515f_SB505	ACGTCTCG	806r_SA704	CAGTGAGT
226.Sp6.Cr16.0.15	515f_SC505	GCTCTAGT	806r_SA712	TCGCTATA
227.Sp6.Cr17.0.20	515f_SA506	CGTGAGTG	806r_SB708	GGTACTAT

Sample.Name	Fwd_Primer_ID	Fwd_Primer_Barcode	Rev_Primer_ID	Rev_Primer_Barcode
228.Sp6.Cr18.0.20	515f_SB506	TCGACGAG	806r_SA705	CGTACTCA
229.Sp7.Cr19.0.20	515f_SC506	GACACTGA	806r_SB701	AAGTCGAG
230.Sp7.Cr20.0.20	515f_SA507	GGATATCT	806r_SB709	GTATACGC
231.Sp7.Cr21.0.13	515f_SB507	GATCGTGT	806r_SA706	CTACGCAG
232.Sp8.Cr22.0.20	515f_SC507	TGCGTACG	806r_SB702	ATACTTCG
233.Sp8.Cr23.0.20	515f_SA508	GACACCGT	806r_SB710	TACGAGCA
234.Sp8.Cr24.0.20	515f_SB508	GTCAGATA	806r_SA707	GGAGACTA
235.Sp9.Cr25.0.15	515f_SC508	TAGTGTAG	806r_SB703	AGCTGCTA
236.Sp9.Cr26.0.20	515f_SA501	ATCGTACG	806r_SB703	AGCTGCTA
237.Sp9.Cr27.0.15	515f_SB501	CTACTATA	806r_SB711	TCAGCGTT
238.Sp10.Cr28.0.20	515f_SC501	ACGACGTG	806r_SA708	GTCGCTCG
239.Sp10.Cr29.0.15	515f_SA502	ACTATCTG	806r_SB704	CATAGAGA
240.Sp10.Cr30.0.20	515f_SB502	CGTTACTA	806r_SB712	TCGCTACG

References

- 765
- 766 Agarwal, S., Wills, J., Cayton, L., Lanckriet, G., Kriegman, D., Belongie, S., 2007. Gen-
767 eralized non-metric multidimensional scaling, in: *Artificial Intelligence and Statistics*.
768 pp. 11–18.
- 769 Anderegg, G., Kholeif, S., 1994. The extrapolation of experimental equilibrium constant
770 data to zero ionic strength: Critical review and new approach. *Talanta* 41, 1507–1522.
- 771 Andersen, C.B., 2002. Understanding carbonate equilibria by measuring alkalinity in
772 experimental and natural systems. *Journal of Geoscience Education* 50, 389–403.
- 773 Anderson, M.J., 2014. Permutational multivariate analysis of variance (permanova).
774 *Wiley Statsref: Statistics Reference Online* 1–15.
- 775 Ashcraft, E.B. (Ed.), 1957. *Symposium on pH measurement: Fifty-ninth annual meeting*
776 (1956). American Society for Testing Materials, Philadelphia.
- 777 Ashcraft, E.B. (Ed.), 1947. *Symposium on pH measurement: Forty-ninth annual meet-*
778 *ing* (1946). American Society for Testing Materials, Philadelphia.
- 779 Bahram, M., Hildebrand, F., Forslund, S.K., Anderson, J.L., Soudzilovskaia, N.A., Bode-
780 gom, P.M., Bengtsson-Palme, J., Anslan, S., Coelho, L.P., Harend, H., others, 2018.
781 *Structure and function of the global topsoil microbiome*. *Nature* 560, 233–237.
- 782 Barberán, A., Bates, S.T., Casamayor, E.O., Fierer, N., 2012. Using network analysis
783 to explore co-occurrence patterns in soil microbial communities. *The ISME Journal* 6,
784 343–351.
- 785 Barrow, N.J., 1984. Modelling the effects of pH on phosphate sorption by soils. *Journal*
786 *of Soil Science* 35, 283–297.
- 787 Bartram, A.K., Jiang, X., Lynch, M.D., Masella, A.P., Nicol, G.W., Dushoff, J., Neufeld,
788 J.D., 2014. Exploring links between pH and bacterial community composition in soils
789 from the craibstone experimental farm. *FEMS Microbiology Ecology* 87, 403–415.
- 790 Bates, R., 1973. *Determination of pH; theory and practice*. Wiley, New York.
- 791 Bates, R.G., Guggenheim, E.A., 1960. Report on the standardization of pH and related
792 terminology. *Pure and Applied Chemistry* 1, 163–168.
- 793 Baucke, F.G., 2002. New iupac recommendations on the measurement of pH-
794 background and essentials. *Analytical and Bioanalytical Chemistry* 374, 772–777.
- 795 Bååth, E., Anderson, T.H., 2003. Comparison of soil fungal/bacterial ratios in a pH
796 gradient using physiological and PLFA-based techniques. *Soil Biology and Biochemistry*
797 35, 955–963.
- 798 Belnap, J., Hawkes, C.V., Firestone, M.K., 2003. Boundaries in miniature: Two exam-
799 ples from soil. *BioScience* 53, 739–749.
- 800 Bjerrum, N.J., Gjaldbæk, J.K., 1919. Undersøgelser over de faktorer som bestemmer
801 jordbundens reaktion. *Den Kongelige Veterinær-Og Landbohøjskole Aarsskrift* 48–91.
- 802 Bray, J.R., Curtis, J.T., 1957. An ordination of the upland forest communities of south-
803 ern wisconsin. *Ecological Monographs* 27, 325–349.

- 804 Burt, R., Staff, S.S. (Eds.), 2014. Kellogg soil survey laboratory methods manual, Soil
805 survey investigations report. United States Department of Agriculture, Natural Re-
806 sources Conservation Service, National Soil Survey Center, Kellogg Soil Survey Labo-
807 ratory.
- 808 Butler, J.N., 1998. Ionic equilibrium: Solubility and pH calculations. Wiley.
- 809 Callahan, B.J., McMurdie, P.J., Rosen, M.J., Han, A.W., Johnson, A.J.A., Holmes, S.P.,
810 2016. DADA2: High-resolution sample inference from illumina amplicon data. *Nature*
811 *Methods* 13, 581–583.
- 812 Cary, J.W., Holder, C., 1982. A method for measuring oxygen and carbon dioxide in soil
813 1. *Soil Science Society of America Journal* 46, 1345–1347.
- 814 Chapman, H.D., Axley, J.H., Curtis, D.S., 1941. The determination of pH at soil mois-
815 ture contents approximating field conditions. *Soil Science Society of America Journal* 5,
816 191–200.
- 817 Covert, P.A., Hore, D.K., 2016. Geochemical insight from nonlinear optical studies of
818 mineral–water interfaces. *Annual Review of Physical Chemistry* 67, 233–257.
- 819 Dahlgren, R.A., Percival, H.J., Parfitt, R.L., 1997. Carbon dioxide degassing effects on
820 soil solutions collected by centrifugation. *Soil Science* 162, 648–655.
- 821 de Levie, R., 2014. A pH centenary. *Electrochimica Acta* 135, 604–639.
- 822 de Levie, R., 2010. Potentiometric pH measurements of acidity are approximations,
823 some more useful than others. *Journal of Chemical Education* 87, 1188–1194.
- 824 Debye, P., Hückel, E., 1923. Zur theorie der elektrolyte. I. Gefrierpunktserniedrigung
825 und verwandte erscheinungen. *Physikalische Zeitschrift* 24, 185–206.
- 826 Delgado-Baquerizo, M., Oliverio, A.M., Brewer, T.E., Benavent-González, A., Eldridge,
827 D.J., Bardgett, R.D., Maestre, F.T., Singh, B.K., Fierer, N., 2018. A global atlas of the
828 dominant bacteria found in soil. *Science* 359, 320–325.
- 829 Dennis, P.G., Hirsch, P.R., Smith, S.J., Taylor, R.G., Valsami-Jones, E., Miller, A.J.,
830 2009. Linking rhizoplane pH and bacterial density at the microhabitat scale. *Journal of*
831 *Microbiological Methods* 76, 101–104.
- 832 Dixon, P., 2003. VEGAN, a package of r functions for community ecology. *Journal of*
833 *Vegetation Science* 14, 927–930.
- 834 Dobrovolskii, V., Zvezdina, V., Prokunin, S., Shchipunov, A., 2018. Investigation of
835 metrological characteristics of buffer solutions in pH measurements. *Measurement*
836 *Techniques* 61, 85–89.
- 837 Elberling, B., Matthiesen, H., 2007. Methodologically controlled variations in laboratory
838 and field pH measurements in waterlogged soils. *European Journal of Soil Science* 58,
839 207–214.
- 840 Feldman, I., 1956. Use and abuse of pH measurements. *Analytical Chemistry* 28,
841 1859–1866.

- 842 Ferguson, A., Vogel, I., 1927. On the agreement with experiment of the dilution formula
843 deduced from the debye-hückel theory. *Transactions of the Faraday Society* 23, 404–
844 408.
- 845 Fierer, N., Jackson, R.B., 2006. The diversity and biogeography of soil bacterial com-
846 munities. *Proceedings of the National Academy of Sciences* 103, 626–631.
- 847 Fornasier, E., Fornasier, F., Di Marco, V., 2018. Spectrophotometric methods for the
848 measurement of soil pH: A reappraisal. *Spectrochimica Acta Part A: Molecular and*
849 *Biomolecular Spectroscopy* 204, 113–118.
- 850 Frostegård, Å., Tunlid, A., Bååth, E., 2011. Use and misuse of PLFA measurements in
851 soils. *Soil Biology and Biochemistry* 43, 1621–1625.
- 852 Galster, H., 1991. pH Measurement: Fundamentals, methods, applications, instrumen-
853 tation. VCH.
- 854 Geibe, C.E., Danielsson, R., van Hees, P.A.W., Lundström, U.S., 2006. Comparison of
855 soil solution chemistry sampled by centrifugation, two types of suction lysimeters and
856 zero-tension lysimeters. *Applied Geochemistry* 21, 2096–2111.
- 857 Gillman, G.P., 1976. A centrifuge method for obtaining soil solution.
- 858 Gorham, E., 1960. The pH of fresh soils and soil solutions. *Ecology* 41.
- 859 Huberty, R., Haas, R., 1940. The pH of soil as affected by soil moisture and other
860 factors. *Soil Science* 49, 455–478.
- 861 Jackson, M.L., 1958. *Soil chemical analysis*. Prentice-Hall, Inc., Englewood Cliffs, N.J.
- 862 Jacob, H.D., Clarke, G.T., Dick, W.A., 2002. *Methods of soil analysis part - 4 physical*
863 *methods: SSSA book series - 5*. Soil Science Society of America.
- 864 Jenny, H., Nielsen, T.R., Coleman, N.T., Williams, D.E., 1950. Concerning the measure-
865 ment of pH, ion activities, and membrane potentials in colloidal systems. *Science* 112,
866 164–167.
- 867 Jones, A.A., Bennett, P.C., 2017. Mineral ecology: Surface specific colonization and
868 geochemical drivers of biofilm accumulation, composition, and phylogeny. *Frontiers in*
869 *Microbiology* 8, 491.
- 870 Junge, W., Nelson, N., 2015. ATP synthase. *Annual Review of Biochemistry* 84, 631–
871 657.
- 872 Jury, W.A., Horton, R., 2004. *Soil physics*. John Wiley & Sons, Inc.
- 873 Kadis, R., Leito, I., 2010. Evaluation of the residual liquid junction potential contribu-
874 tion to the uncertainty in pH measurement: A case study on low ionic strength natural
875 waters. *Analytica Chimica Acta* 664, 129–135.
- 876 Kartesz, J.T., 2015. *The biota of north america program (BONAP)*. Taxonomic Data
877 Center, Chapel Hill, N.C.
- 878 Kass, R.E., Wasserman, L., 1995. A reference bayesian test for nested hypotheses and
879 its relationship to the schwarz criterion. *Journal of the American Statistical Association*
880 90, 928–934.

- 881 Kaupenjohann, M., David, M.B., 1996. Evidence for effects of CO₂ on soil solution
882 chemistry in spodosols by a simple in-field extractor. *Zeitschrift Für Pflanzenernährung
883 Und Bodenkunde* 159, 195–198.
- 884 Kerndorff, H., Schnitzer, M., 1980. Sorption of metals on humic acid. *Geochimica et
885 Cosmochimica Acta* 44, 1701–1708.
- 886 Kilian, K.C., 1961. Effect of various factors on soil pH measurement. University of
887 Wisconsin–Madison.
- 888 Kirk, J.L., Beaudette, L.A., Hart, M., Moutoglis, P., Klironomos, J.N., Lee, H., Trevors,
889 J.T., 2004. Methods of studying soil microbial diversity. *Journal of Microbiological
890 Methods* 58, 169–188.
- 891 Kozich, J.J., Westcott, S.L., Baxter, N.T., Highlander, S.K., Schloss, P.D., 2013. Develop-
892 ment of a dual-index sequencing strategy and curation pipeline for analyzing amplicon
893 sequence data on the miseq illumina sequencing platform. *Applied and Environmental
894 Microbiology* 79, 5112–5120.
- 895 Lauber, C.L., Hamady, M., Knight, R., Fierer, N., 2009. Pyrosequencing-based assess-
896 ment of soil pH as a predictor of soil bacterial community structure at the continental
897 scale. *Applied and Environmental Microbiology* 75, 5111–5120.
- 898 Lauber, C.L., Strickland, M.S., Bradford, M.A., Fierer, N., 2008. The influence of soil
899 properties on the structure of bacterial and fungal communities across land-use types.
900 *Soil Biology and Biochemistry* 40, 2407–2415.
- 901 Lerman, A., 1978. Chemical exchange across sediment-water interface. *Annual Review
902 of Earth and Planetary Sciences* 6, 281.
- 903 Lewis, G.N., Randall, M., 1921. The activity coefficient of strong electrolytes. *Journal of
904 the American Chemical Society* 43, 1112–1154.
- 905 Libohova, Z., Wills, S., Hempel, J.W., Odgers, N.P., Thompson, J.A., 2012. Using pedo-
906 transfer functions for estimating soil pH and bulk density at regional scale, in: *Digital
907 Soil Assessments and Beyond: Proceedings of the 5th Global Workshop on Digital Soil
908 Mapping 2012*, Sydney, Australia. CRC Press, p. 313.
- 909 Libohova, Z., Wills, S., Odgers, N.P., Ferguson, R., Nesser, R., Thompson, J.A., West,
910 L.T., Hempel, J.W., 2014. Converting pH 1:1 h₂o and 1:2 cacl₂ to 1:5 h₂o to contribute
911 to a harmonized global soil database. *Geoderma* 213, 544–550.
- 912 Lumley, T., Miller, A., 2020. Leaps: Regression subset selection.
- 913 MacInnes, D.A., 1948. Criticism of a definition of pH. *Science* 108, 693–693.
- 914 MacInnes, D.A., 1939. The principles of electrochemistry. Reinhold Publishing Corpo-
915 ration.
- 916 MacInnes, D.A., 1915. Liquid junction potentials. *Journal of the American Chemical
917 Society* 37, 2301–2307.
- 918 Matthiesen, H., 2004. In situ measurement of soil pH. *Journal of Archaeological Science*
919 31, 1373–1381.

- 920 McMurdie, P.J., Holmes, S., 2013. Phyloseq: An r package for reproducible interactive
921 analysis and graphics of microbiome census data. *PloS One* 8.
- 922 Measuring pH of concentrated samples (Application Note No. 009), 2014.. Thermo
923 Fisher Scientific.
- 924 Meena, R.S., 2019. Soil health restoration and management. Springer.
- 925 Mikutta, R., Kleber, M., Torn, M.S., Jahn, R., 2006. Stabilization of soil organic matter:
926 Association with minerals or chemical recalcitrance? *Biogeochemistry* 77, 25–56.
- 927 Miller, R.O., Kissel, D.E., 2010. Comparison of soil pH methods on soils of north amer-
928 ica. *Soil Science Society of America Journal* 74, 310–316.
- 929 Minasny, B., McBratney, A.B., Brough, D.M., Jacquier, D., 2011. Models relating soil pH
930 measurements in water and calcium chloride that incorporate electrolyte concentration.
931 *European Journal of Soil Science* 62, 728–732.
- 932 Mubarak, A., Olsen, R.A., 1976. An improved technique for measuring soil pH 1. *Soil
933 Science Society of America Journal* 40, 880–882.
- 934 Naidu, R., Bolan, N.S., Kookana, R.S., Tiller, K.G., 1994. Ionic-strength and pH effects
935 on the sorption of cadmium and the surface charge of soils. *European Journal of Soil
936 Science* 45, 419–429.
- 937 Oh, N.-H., Kim, H.-S., Richter Jr, D.D., 2005. What regulates soil CO₂ concentrations?
938 A modeling approach to CO₂ diffusion in deep soil profiles. *Environmental Engineering
939 Science* 22, 38–45.
- 940 Oman, S.F., Camões, M.F., Powell, K.J., Rajagopalan, R., Spitzer, P., 2007. Guidelines
941 for potentiometric measurements in suspensions part a. The suspension effect (iupac
942 technical report). *Pure and Applied Chemistry* 79, 67–79.
- 943 Parfitt, R.L., Percival, H.J., Van Der Lee, G., 1995. Aluminium species and pH in soil
944 solution under different crops, in: *Plant-Soil Interactions at Low pH: Principles and
945 Management*. Springer, pp. 817–822.
- 946 Ponnampertuma, F.N., Martinez, E., Loy, T., 1966. Influence of redox potential and
947 partial pressure of carbon dioxide on pH values and the suspension effect of flooded
948 soils. *Soil Science* 101, 421–431.
- 949 Poole, R.K., 1999. Bacterial responses to pH. John Wiley & Sons.
- 950 Pourbaix, M., 1974. Atlas of electrochemical equilibria in aqueous solutions. National
951 Association of Corrosion Engineers.
- 952 Rousk, J., Bååth, E., Brookes, P.C., Lauber, C.L., Lozupone, C., Caporaso, J.G., Knight,
953 R., Fierer, N., 2010. Soil bacterial and fungal communities across a pH gradient in an
954 arable soil. *The ISME Journal* 4, 1340–1351.
- 955 Rousk, J., Brookes, P.C., Bååth, E., 2010a. Investigating the mechanisms for the oppos-
956 ing pH relationships of fungal and bacterial growth in soil. *Soil Biology and Biochemistry*
957 42, 926–934.

- 958 Rousk, J., Brookes, P.C., Bååth, E., 2010b. The microbial PLFA composition as affected
959 by pH in an arable soil. *Soil Biology and Biochemistry* 42, 516–520.
- 960 Sacchi, E., Michelot, J.-L., Pitsch, H., Lalieux, P., Aranyossy, J.-F., 2001. Extraction of
961 water and solutes from argillaceous rocks for geochemical characterisation: Methods,
962 processes and current understanding. *Hydrogeology Journal* 9, 17–33.
- 963 Sander, R., 2015. Compilation of henry's law constants (version 4.0) for water as solvent.
964 *Atmos. Chem. Phys* 15, 4399–4981.
- 965 Sasowsky, I.D., Dalton, C.T., 2005. Measurement of pH for field studies in karst areas.
966 *Journal of Cave and Karst Studies* 67, 127–132.
- 967 Sena, L.A., 1972. Units of physical quantities and their dimensions. Mir Publishers,
968 Moscow.
- 969 Shen, C., Xiong, J., Zhang, H., Feng, Y., Lin, X., Li, X., Liang, W., Chu, H., 2013. Soil
970 pH drives the spatial distribution of bacterial communities along elevation on changbai
971 mountain. *Soil Biology and Biochemistry* 57, 204–211.
- 972 Sinsabaugh, R.L., Lauber, C.L., Weintraub, M.N., Ahmed, B., Allison, S.D., Crenshaw,
973 C., Contosta, A.R., Cusack, D., Frey, S., Gallo, M.E., others, 2008. Stoichiometry of soil
974 enzyme activity at global scale. *Ecology Letters* 11, 1252–1264.
- 975 Small, J., 1954. Modern aspects of pH with special reference to plants and soils. D. Van
976 Nostrand Co. Inc., New York.
- 977 Smith, F.B., Brown, P.E., Millar, H.C., Bodily, H.L., 1937. The effect of carbon dioxide
978 on soil reaction and on the solubility of phosphorus in soils. *Soil Science* 43, 93–104.
- 979 Song, N., Ma, Y., Zhao, Y., Tang, S., 2015. Elevated ambient carbon dioxide and tri-
980 choderma inoculum could enhance cadmium uptake of lolium perenne explained by
981 changes of soil pH, cadmium availability and microbial biomass. *Applied Soil Ecology*
982 85, 56–64.
- 983 Sparks, D.L., 1998. *Soil physical chemistry*, second edition. Taylor & Francis.
- 984 Spitzer, P., Pratt, K.W., 2011. The history and development of a rigorous metrological
985 basis for pH measurements. *Journal of Solid State Electrochemistry* 15, 69–76.
- 986 Stark, S., Männistö, M.K., Eskelinen, A., 2014. Nutrient availability and pH jointly
987 constrain microbial extracellular enzyme activities in nutrient-poor tundra soils. *Plant
988 and Soil* 383, 373–385.
- 989 Strawn, D.G., Bohn, H.L., O'Connor, G.A., 2020. *Soil chemistry*. Wiley Blackwell.
- 990 Suarez, D.L., Šimunek, J., 1993. Modeling of carbon dioxide transport and production
991 in soil: 2. Parameter selection, sensitivity analysis, and comparison of model predictions
992 to field data. *Water Resources Research* 29, 499–513.
- 993 Sumner, M.E., 1994. Measurement of soil pH: Problems and solutions. *Communica-
994 tions in Soil Science and Plant Analysis* 25, 859–879.
- 995 Sørensen, S., 1909. Enzymstudien ii: über die messung und die bedeutung der
996 wasserstoffionenkonzentration bei enzymatischen prozessen, *Biochemische zeitschrift*.

997 Springer-Verlag.

998 Šimunek, J., Suarez, D.L., 1994. Two-dimensional transport model for variably sat-
999-urated porous media with major ion chemistry. *Water Resources Research* 30, 1115-
1000-1133.

1001 Šimunek, J., Suarez, D.L., 1993. Modeling of carbon dioxide transport and production
1002 in soil: 1. Model development. *Water Resources Research* 29, 487-497.

1003 Team, R.C., 2018. R: A language and environment for statistical computing. R Founda-
1004-tion for Statistical Computing.

1005 Tecon, R., Or, D., 2017. Biophysical processes supporting the diversity of microbial life
1006 in soil. *FEMS Microbiology Reviews* 41, 599-623.

1007 Thomas, G.W., 1996. Soil pH and soil acidity. *Methods of Soil Analysis: Part 3 Chemical*
1008 *Methods* 5, 475-490.

1009 Tripathi, B.M., Kim, M., Singh, D., Lee-Cruz, L., Lai-Hoe, A., Ainuddin, A.N., Go, R.,
1010-Rahim, R.A., Husni, M.H.A., Chun, J., others, 2012. Tropical soil bacterial communities
1011 in malaysia: pH Dominates in the equatorial tropics too. *Microbial Ecology* 64, 474-484.

1012 Vernadsky, V.I., 1913. On the chemical composition of soils. *Pochvovedenie* 1-21.

1013 Vieira, S., Sikorski, J., Gebala, A., Boeddinghaus, R.S., Marhan, S., Rennert, T., Kan-
1014-deler, E., Overmann, J., 2020. Bacterial colonization of minerals in grassland soils is
1015 selective and highly dynamic. *Environmental Microbiology* 22, 917-933.

1016 Volk, V.I., Rozen, A.M., 1977. Interpretation of pH measurements in salt solutions. I.
1017 The influence of the salting-out agent on the determination of the activity of hydrogen
1018 ions from pH measurements (parts i, ii, and iii). *Russian Journal of Physical Chemistry*
1019 51.

1020 Vos, M., Wolf, A.B., Jennings, S.J., Kowalchuk, G.A., 2013. Micro-scale determinants
1021 of bacterial diversity in soil. *FEMS Microbiology Reviews* 37, 936-954.

1022 Wakelin, S.A., Gerard, E., Koten, C. van, Banabas, M., O'Callaghan, M., Nelson, P.N.,
1023-2016. Soil physicochemical properties impact more strongly on bacteria and fungi than
1024 conversion of grassland to oil palm. *Pedobiologia* 59, 83-91.

1025 Walters, W., Hyde, E.R., Berg-Lyons, D., Ackermann, G., Humphrey, G., Parada, A.,
1026-Gilbert, J.A., Jansson, J.K., Caporaso, J.G., Fuhrman, J.A., others, 2016. Improved
1027 bacterial 16S rRNA gene (v4 and v4-5) and fungal internal transcribed spacer marker
1028 gene primers for microbial community surveys. *Msystems* 1, e00009-15.

1029 Whitney, R.S., Gardner, R., 1943. The effect of carbon dioxide on soil reaction. *Soil*
1030 *Science* 55, 127-142.

1031 Wickham, H., 2009. *Elegant graphics for data analysis (ggplot2)*.

1032 Wolt, J.D., 1994. *Soil solution chemistry: Applications to environmental science and*
1033 *agriculture*. Wiley.

1034 Wright, M.R., 2007. *An introduction to aqueous electrolyte solutions*. John Wiley &
1035 Sons.

1036 Young, J.M., Rawlence, N.J., Weyrich, L.S., Cooper, A., 2014. Limitations and recom-
1037 mendations for successful DNA extraction from forensic soil samples: A review. *Science*
1038 & *Justice* 54, 238–244.