

1 **Soil classification predicts differences in prokaryotic communities across a range**  
2 **of geographically distant soils once pH is accounted for**

3

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10

11

12 **Abstract**

13           Agricultural land is typically managed based on visible plant life at the  
14 expense of the belowground majority. However, microorganisms mediate processes  
15 sustaining plant life and the soil environment. To understand the role of microbes we  
16 first must understand what controls soil microbial community assembly. We assessed  
17 the distribution and composition of prokaryotic communities from soils representing  
18 four geographic regions on the South Island of New Zealand. These soils are under  
19 three different uses (dairy, sheep and beef, and high country farming) and are  
20 representative of major soil classification groups (brown, pallic, gley and recent). We  
21 hypothesized that pH would account for major community patterns based on 16S  
22 profiles, but that land use and location would be secondary modifiers. Community  
23 diversity and structure was linked to pH, coinciding with land use. Soil classification  
24 correlated with microbial community structure and evenness, but not richness in high  
25 country and sheep and beef communities. The impact of land use and pH remained  
26 significant at the regional scale, but soil classification provided support for  
27 community variability not explained by either of those factors. These results suggest  
28 that several edaphic properties must be examined at multiple spatial scales to robustly  
29 examine soil prokaryotic communities.

## 30 **Introduction**

31

32 Sustained population growth has placed a major strain on food production,  
33 forcing the development of intensive land use practices that maximize yields<sup>1</sup>. This  
34 includes use of heavy machinery and extensive applications of chemical amendments  
35 such as fertilizers and herbicides. This intensification of agricultural production has  
36 drastically altered soil conditions, causing physicochemical changes (e.g. compaction,  
37 decreased organic matter and erosion)<sup>2,3,4</sup> that have led to well-documented losses in  
38 biodiversity, including that of belowground microbial communities<sup>5,6,7</sup>. Microbes are  
39 known to be important to maintaining ecosystem processes<sup>8,9</sup>. As a result,  
40 understanding the consequences of these anthropogenic changes is essential for  
41 sustained soil health.

42 Microorganisms are keystone species that contribute to soil health through  
43 bioremediation of contaminants<sup>10,11,12</sup> and regulation of nutrient cycling<sup>13,14,15</sup>.  
44 Despite this, the factors that control their distribution and composition are highly  
45 contested. Many studies have shown that land use changes influence belowground  
46 communities<sup>16,17,18</sup>, while pH is a consistent and dominant driver of microbial  
47 assemblages on a continental scale and across a range of environments<sup>19,20,21,22</sup>.  
48 However, other edaphic factors like C:N ratio<sup>23</sup> and soil texture<sup>24,25</sup> can affect  
49 microbial communities. The confounding effects of specific soil factors draws  
50 attention to a major gap in prediction and interpretation of microbial community  
51 responses to land use change.

52 Despite the vast number of studies linking individual environmental factors to  
53 changes in microbial community structure, the mechanisms underlying these  
54 relationships have not been resolved. For example, though there is a widely reported

55 relationship between pH and microbial community structure, it is currently not clear  
56 whether pH itself is the most important factor, or if individual chemical and physical  
57 factors that contribute to pH are driving this variation<sup>19</sup>. Additionally, many studies  
58 concerning land use change focus on a single practice at a particular site<sup>24, 26, 27, 28</sup>.  
59 While such analyses provide insight into small-scale microbial community responses  
60 to land use intensification, information regarding the comparative responses of  
61 communities at multiple scales and across land use types is limited. Moreover, while  
62 microbial ecologists seek to capture any and all drivers of belowground communities,  
63 it is nearly impossible to measure all environmental factors in a given soil. Most  
64 studies evaluate physical factors in terms of soil texture, which is limited in its  
65 representation of the complexity of soil. Soil classification provides a more complete  
66 description of soils that takes into account the parent material, particle size and  
67 permeability, as well as major chemical traits<sup>29</sup>. This parameter also relates soil  
68 profiles to climactic and physicochemical features such as weathering, leaching, soil  
69 moisture, metal oxides and clay mineral content<sup>30</sup> and might provide additional  
70 resolution for characterizing prokaryotic communities.

71 To this end, our study used 16S rRNA gene profiles to investigate prokaryotic  
72 community composition and distribution in soils on both landscape and regional  
73 scales. We examined soils across a series of sites comprising three land use types and  
74 four geographic regions. We assess the relationship between prokaryotic communities  
75 in these soils with several abiotic factors including pH, land use and soil  
76 classification. We hypothesized that prokaryotic community structure would be  
77 primarily correlated to pH, while land use would have a secondary relationship with  
78 community structure. Furthermore, we hypothesized that soil classification—  
79 evaluated at the soil order and subgroup levels—would account for much of the

80 variation in prokaryotic communities not described by either land use or pH. Finally,  
81 we sought to understand how individual taxonomic groups responded to these factors.

82

## 83 **Results**

84

### 85 **Soil Characteristics**

86 We sampled soils under three land uses: dairy, sheep and beef, and high  
87 country. These uses differ in stock type as indicated by their names, but also in their  
88 management intensity (i.e. low country = highly managed soils with high stocking  
89 rates) as well as location (high country agriculture is carried out on high altitude  
90 pastures). Soil physicochemical characteristics varied across land uses, soil order and  
91 soil subgroup (Table S1). The sampled soils represented a range of pH values (5.1-  
92 6.3). High country soils had, on average, 1.08-fold lower pH than dairy and sheep and  
93 beef soils, which were similar in this respect. Soil classification varied within land  
94 uses, but most soils are classified within the brown and pallic soil orders, with a few  
95 dairy soils representing the recent and gley orders.

96

### 97 **Prokaryotic community structure varies with pH and land use**

98 We examined prokaryotic communities from sites representing three land uses  
99 and four geographic regions. A total of 115,445 OTUs (at 97% sequence similarity)  
100 were detected within 72 samples representing 24 sites. OTUs per sample ranged  
101 between 2,414 and 3,641. Prokaryotic alpha diversity was estimated across all  
102 samples and correlations with soil parameters were determined using linear  
103 regressions. Richness was correlated with land use (Figure 1A) (Kruskal-Wallis chi-  
104 squared = 11.3,  $p < 0.004$ ), with increasing richness from high-country sites to sheep

105 and beef sites. This trend was related to pH (Figure S1A) (regression  $R^2 = 0.23$ ,  $p <$   
106 0.001) with richness increasing as pH became more neutral. Trends for the Shannon  
107 diversity index were similar to those observed for richness with diversity being  
108 correlated to both land use (Kruskal-Wallis chi-squared = 26.1,  $p < 0.001$ ) and pH  
109 (Figure S1B) (regression  $R^2 = 0.48$ ,  $p < 0.001$ ). The remaining chemical data  
110 measured in this study (Table S1) did not account for as much variability as pH and  
111 land use.

112 Detrended correspondence analysis (DCA) confirmed trends observed using  
113 alpha diversity, with both land use and pH linked to clustering of samples (Figure  
114 1B). Samples from across the three land uses formed a gradient indicating that  
115 differences in prokaryotic communities were primarily correlated with changes in pH  
116 (Mantel  $R^2 = 0.63$ ,  $p < 0.001$ ). While three land uses are included in the study,  
117 analysis of similarity (ANOSIM) testing indicated only two major categories: high  
118 and low country soils (sheep and beef, and dairy) (Figure S2A, B) (ANOSIM  $R^2 =$   
119 0.52,  $p < 0.001$ ). Hierarchical clustering of Bray-Curtis distances (Figure S3)  
120 confirms the strength of high country and low country environments in explaining the  
121 variance in prokaryotic communities (70% confidence). However, sub-clusters  
122 representing individual replicates from a site within the high/low country split are  
123 better supported using these methods (95% confidence), suggesting unaccounted for  
124 factors that are linked to changes in community structure.

125

126 **Variation in community composition within land uses is explained by the**  
127 **underlying soil classification.**

128 To assess relationships between soil properties and community variation, and  
129 observed clustering of samples, within the three land uses data was subset by land use

130 and analyzed independently. Major differences in community structure within the  
131 same land use were correlated with soil order, while soil subgroup resolved only a  
132 few clusters (Figure 2). Soil subgroup has a significant effect on both the observed  
133 species count (Kruskal-Wallis chi-squared = 32.4,  $p < 0.006$ ) and the Shannon  
134 diversity index (Kruskal-Wallis chi-squared = 50.6,  $p < 0.001$ ) (Figure 2A).  
135 Interestingly, samples grouped based on soil order (Figure 2B) do not have  
136 significantly different richness values ( $p > 0.05$ ). However, soil order does correlate  
137 weakly with Shannon diversity (Kruskal-Wallis chi-squared = 8.2,  $p < 0.05$ ).

138 DCA reveals that prokaryotic communities form distinct clusters based on soil  
139 order (Figure 2C, D), though all land use sub-communities have statistically  
140 significant relationships with both soil subgroup and soil order (ANOSIM  $p < 0.001$ ).  
141 Soil order has a slightly stronger correlation with high country soils ( $R^2 = 0.91$ )  
142 (Figure S4A), while sheep and beef communities ( $R^2 = 0.58$ ) (Figure S4C) have a  
143 slightly stronger relationship with soil subgroup. Hierarchical clustering confirms  
144 these results, where high country communities form two clusters (Figure S5), and  
145 sheep and beef communities form two (Figure S6). On the other hand, dairy  
146 communities do not separate according to soil classification, despite significant  
147 correlations with soil order and subgroup ( $R^2 = 0.30, 0.67$ ) (Figure S7). These  
148 communities remain stable across a wide geographic range, forming one large cluster  
149 indicating that an unknown factor reduces variation in dairy soils.

150

151 **Influences of pH and land use are stable across multiple spatial scales, but soil**  
152 **classification provides additional support**

153 To determine the impact of geographic scale on observed patterns (based on  
154 pH, land use and soil classification), we individually examined the communities from

155 the four geographic regions (Figure S8 and S9). Prokaryotic community changes  
156 within regions confirm that pH and land use are the most significant predictors of  
157 community structure at multiple scales, while soil classification accounts for the  
158 remaining variation (Figure S10-13, Table S2). ). Interestingly, land use has the most  
159 significant relationships with regional communities where pH was the most  
160 significant variable at the multi-region scale.

161

### 162 **Prokaryotic indicators of pH, land use and soil order**

163 Prokaryotic taxa (OTUs) significantly correlated ( $p < 0.001$ ) to changes in pH,  
164 land use, or soil order were identified using Spearman's correlations, the Wald test or  
165 the Kruskal-Wallis test respectively. The taxa were then mapped onto cladograms  
166 (Figure 3; taxa with correlations are provided in Supplementary Table S3).

167 Overall, we found 678 OTUs (0.6% of total OTUs) that were correlated with  
168 one or more edaphic properties. 34% of these OTUs correlated with pH, 27%  
169 correlated with land use and 40% correlated with soil order. The most represented  
170 phyla were the *Proteobacteria* (31% of significant OTUs), *Acidobacteria* (22%),  
171 *Actinobacteria* (17%), *Bacteroidetes* (6%) and *Planctomycetes* (5%). A consistent  
172 response to specific edaphic properties was not observed at the phylum level.

173 At the genus level, there was significant overlap between OTU's identified  
174 based on soils classification, pH and land use. Generally, high pH, low country soils,  
175 pallic, gley and recent soils shared correlated OTUs (e.g. *Adhaeribacter* and  
176 *Revranelia*) while low pH, high country soils and brown soils had significantly  
177 correlated OTUs in common (e.g. *Bryobacter*, *Acidotherrnus*, *Koribacter*,  
178 *Telmatobacter*, *Mycobacterium* and *Candidatus Methylacidiphilum*).



179           However, the relative abundances of several genera correlated with only one  
180 edaphic property. *Anaeromyxobacter*, *Singulisphaera* and *Rhodanobacter* had  
181 positive correlations with pH, while *Rhizobium*, *Variovorax* and *Flavobacterium* were  
182 negatively correlated to pH. High country soils were correlated with  
183 *Frigoribacterium*, *Jatrophihabitans* and *Massilia*, while low country soils had  
184 correlations with *Janibacter*, *Pseudonocardia* and *Pelobacter*. Lastly, *Rubroacter*,  
185 *Defluviicoccus* and *Parasegetibacter* were most strongly correlated with brown soils  
186 while *Marmoricola*, *Nocardiodes* and *Gemmatimonas* had significant correlations  
187 with the other three soil orders.

188

## 189 **Discussion**

190

191           Results revealed that: prokaryotic assemblages differed significantly between  
192 land uses and across a pH gradient, however much of the variation within land uses  
193 and regions was better accounted for by soil order. Additionally, taxonomic profiles  
194 revealed that while overlap exists between OTUs identified as being correlated with  
195 pH, land use and soil classification, each parameter identified specific populations not  
196 correlated with either of the remaining two.

197           The studied soils harbored distinct prokaryotic communities, revealing  
198 consistent impacts of pH and, to a lesser extent, land use across spatial scales. Our  
199 results also confirm the notion that acidic soils support a smaller breadth of diversity.  
200 These results are in agreement with many previous studies that have established the  
201 role of pH and land use on prokaryotic communities<sup>19, 20, 21</sup>. It has been previously  
202 suggested that soil texture is an important predictor of prokaryotic community  
203 structure<sup>24, 31, 32</sup>. To build on this relationship, we evaluated the potential link between

204 soil classification (soil order and subgroup) and prokaryotic communities. This  
205 allowed us to investigate the extent to which agricultural intensification impacts the  
206 relationship between inherent soil properties, like soil texture, and prokaryotic  
207 communities. The rationale was that soil classification provides a more thorough  
208 representation of the soils' physical and chemical factors including those not  
209 measured (e.g. metal oxides), as well as the geological origins of the soils.

210 We observed strong relationships between soil classification and prokaryotic  
211 community diversity and structure. Brown soils had the lowest diversity, while pallic  
212 soils had the highest. The low pH values of the sampled brown soils, combined with  
213 the wet climate where some of the brown soils are commonly found<sup>30</sup>, results in low  
214 nutrient levels compared to other NZ soils leading to conditions that select for a less  
215 diverse community of microbes. In contrast, pallic soils have higher pH values and  
216 are only weakly leached, retaining more nutrients allowing for a more diverse  
217 community. While richness levels between the two soils were comparable, Shannon  
218 diversity differed, indicating changes in evenness. As exemplified by the evenly high  
219 levels of iron oxides in brown soils, depleting nutrient stocks and low pH lead to  
220 uniform conditions favoring a smaller subset of taxa as shown in our study.

221 The analysis of sub-communities within each of the four regions suggests that  
222 both land use and soil classification have strong relationships with prokaryotic  
223 communities. Southland soils had the strongest relationship with land use, but soil  
224 order resolved some differences between clusters along the second axis, where  
225 communities from a recent soil clustered away from the brown soils. Recent soils are  
226 unique in that they are weakly developed, meaning the soil has fewer horizons than  
227 the moderately or well-developed soils comprising the other soil orders in this study<sup>33</sup>.  
228 Prokaryotic communities from Otago soils were most strongly correlated with soil

229 subgroup. This is especially interesting, as in this region, one of the low country sites  
230 grouped with the high country soils on the first DCA axis, but formed their own  
231 cluster on the second axis. This cluster happens to contain communities from the only  
232 brown soils in this particular region, providing further evidence for soil order as a  
233 strong predictor of prokaryotic community structure. In Otago, the two pallic soils  
234 clustered quite distantly from one another, explained by the distinction in soil  
235 structure between laminar and fragic pallics; laminar soils have layers of clay in the  
236 subsoil, while fragic soils are brittle, hard and contain a compacted pan in the  
237 subsoil<sup>33</sup>.

238         Our finding that prokaryotic communities within land uses and regions  
239 correlated with soil order indicates that soil classification is a good predictor for  
240 prokaryotic communities that are geographically distant from one another. However,  
241 we found that dairy communities do not separate clearly based on soil classification. It  
242 is possible that the high stocking rates that are characteristic of dairy farms<sup>34, 35</sup> cause  
243 heightened deposition of manure and urine, creating a new soil layer that is  
244 fundamentally disconnected from the parent material. It has been shown previously  
245 that dairying does impact soil ecosystems in ways that high country, and sheep and  
246 beef management does not. For example, Barkle and colleagues observed that  
247 application of dairy farm effluent (a mixture of water, urine and manure) onto pasture  
248 leads to the accumulation of nutrients and increased prokaryotic biomass<sup>36</sup>. Haynes  
249 and colleagues found similar results in camp areas (where livestock tends to  
250 congregate) when compared to non-camp soils, which provides further insight into the  
251 discrepancy in stocking rate as it affects prokaryotic communities<sup>37</sup>. As a result, the  
252 inherent properties expected for soils subjected to dairy management wouldn't have a  
253 relationship with prokaryotic communities. This also gives insight into pH, since soil

254 orders differ in this regard. While it is well established that soil pH is linked to  
255 prokaryotic communities on a continental scale, the factors that contribute to pH  
256 changes are unresolved<sup>19</sup>. We can hypothesize that the pH of sheep and beef, and  
257 high country soils is connected to inherent soil properties, represented by soil  
258 classification, while the pH of dairy soils has been modified by increased agricultural  
259 intensification, impacting prokaryotic communities accordingly. Furthermore, while  
260 we can be confident in the predictive power of soil order for other land uses, there is  
261 less resolution when using soil subgroup. Current methods (charting latitude and  
262 longitude onto LRIS soil maps) may not be precise enough to accurately classify soils  
263 at this level.

264         While we have established that pH, land use and soil order are good predictors  
265 of prokaryotic community structures, little is known about the mechanisms that  
266 account for these relationships. It is possible that pH, land use and soil order serve as  
267 integrative variables for multiple chemical and physical characteristics that  
268 individually impact prokaryotic communities. Our results suggest that land use, pH  
269 and soil order each exert direct pressure on certain prokaryotic taxa, but also contain  
270 some overlap in their taxonomic profiles, indicating that they may also integrate some  
271 of the same soil properties.

272         Members of both *Firmicutes* (*Bacillus*) and *Thaumarchaeota* (uncultured  
273 representative) are significantly represented in low country soils, but not at high pH  
274 levels. This is interesting, as many members of these phyla are thought to thrive at  
275 high pH levels<sup>38,39</sup>, suggesting that the members detected here have different life  
276 strategies that are selected for by land use. Additionally, DCA plotting showed that  
277 high country soils are strongly correlated with low pH, which is supported by their  
278 shared relationship with several *Acidobacteria* groups. However, there were several

279 members from the *Proteobacteria* (e.g. *Massilia*), *Actinobacteria* (e.g.  
280 *Frigoribacterium*), and *Chloroflexi* (e.g. *Ktedonobacter*) that were significantly  
281 represented in high country soils but not at low pH levels. Little is known about the  
282 ecophysiology of many of these genera. However, *Massilia* are copiotrophs, and are  
283 sensitive to nutrient availability. It is established that high country rangelands are  
284 subjected to less rigorous management regimes compared to their low country  
285 counterparts<sup>40</sup>. This management strategy may give rise to a nutrient profile that is  
286 preferable for the maintenance of *Massilia* populations<sup>41</sup>. Selection by land use is  
287 further evidenced by the strong correlation between high country soils and the  
288 verrucomicrobial phylotype Da101 and, contrastingly, a positive correlation with pH.  
289 As high country soils tend to have lower pH values, and *Verrucomicrobia* are thought  
290 to persist in low-nutrient environments<sup>42, 43</sup>, it can be inferred that the stable nutrient  
291 status of high country soils explains the abundance of this phylotype rather than pH.  
292 Other taxa, like *Gaiella* (originally isolated from an aquifer<sup>44</sup>) and *Nitrospira*, which  
293 are normally found in wet environments<sup>45</sup>, were most significantly correlated with  
294 gley soils. These soils are known to have high water tables<sup>30</sup>, which would likely  
295 provide preferable conditions for these microbes to thrive.

296 Our results confirm soil pH is the strongest predictor of community structure,  
297 diversity and composition across multiple spatial scales, but we also show strong  
298 relationships with land use and soil order. We propose that soil order may serve as an  
299 integrative factor that accounts for physical and chemical properties and can be used  
300 when direct assessment of specific edaphic factors is not possible. Further, the  
301 identification of specific OTUs correlated to more than one factor suggests that  
302 spurious correlations are highly likely and other factors besides pH might better  
303 explain observed patterns.

304

## 305 **Materials and methods**

306

### 307 **Soil sampling**

308           A total of 24 field sites across four regions on the south island of New Zealand  
309 were sampled in this study (Figure 1). Sites were chosen to represent: the three main  
310 land uses in New Zealand agriculture (dairy, sheep and beef, high country farming), a  
311 wide range of edaphic parameters (Table S1), and four major regions of New Zealand  
312 (North Canterbury, South Canterbury, Otago, Southland). Samples were collected at  
313 the beginning of the growing season, between May 5 and May 16, 2014. Sites were  
314 delineated in the field by twelve replicate plots (1m<sup>2</sup> each) within a gridded area  
315 enclosed by a 6.5 by 5 m fence. Biological replicates from each site were collected by  
316 sampling three random plots for a total of 72 samples in the study (24 sites x 3 plots at  
317 each site). Each sample comprised a composite of four cores (7.5 cm depth and 2.5  
318 cm diameter) that were taken 0.4 m apart diagonally across the 1m<sup>2</sup> plot. Cores were  
319 screened prior to compositing to remove roots, worms and rocks. Samples were kept  
320 on ice while in the field and stored at -20 degrees until returning to the lab for final  
321 storage at -80 degrees.

322           Chemical analyses were performed by R.J. Hill Laboratories (Hamilton, NZ).  
323 For soil pH determination, a 1:2 soil: water slurry was prepared followed by  
324 potentiometric titration (CITE). Data for soil physical properties were obtained from  
325 the New Zealand Land Resource Information Systems Portal  
326 (<https://lris.scinfo.org.nz/>).

327

### 328 **DNA extraction and sequencing**

329 Genomic DNA was extracted from 0.25 g of soil using the Mo Bio PowerSoil-  
330 htp 96-well soil DNA isolation kit (Carlsbad, CA, USA) according to the  
331 manufacturer's instructions, but with a modification at the lysing step. Samples were  
332 placed on a Geno/Grinder homogenizer (SPEX Sample Prep, LLC, Metuchen, NJ,  
333 USA) for two rounds of fifteen seconds at 1750 strokes/minute. One extraction was  
334 performed on each sample. DNA concentration and purity was determined using a  
335 Nanodrop 1000 Spectrophotometer (Thermo Scientific, Wilmington, DE, USA).  
336 Absorbance was observed at 230, 260, 280 and 320 nm.

337 The V4 region of the 16S rRNA gene was amplified using the universal  
338 primer pair 515F (5'-NNNNNNNNGTGTGCCAGCMGCCGCGGTAA-3') and 806R  
339 (5'-GGACTACHVGGGTWTCTAAT-3') following the Earth Microbiome Project  
340 barcoded conditions<sup>46</sup>. Each sample was given a barcode sequence on the 5' end of  
341 the forward primer for multiplexed sequencing and loaded onto a single Illumina  
342 MiSeq 2 × 151 bp run (Illumina, Inc., CA, USA). Sequences were deposited at the  
343 Sequence Read Archive (NCBI) with the accession numbers: 5902515-5902586 under  
344 the BioProject ID: PRJNA348131.

345

### 346 **Sequence processing**

347 All sequences were initially processed using a QIIME 1.9.0 open-reference  
348 OTU-picking workflow<sup>47</sup>. In brief, raw sequences were first demultiplexed. Forward  
349 sequences were then clustered into OTUs (97% similarity) against the SILVA  
350 database release 119<sup>48</sup> using UCLUST<sup>49</sup>. Reads that failed to hit the reference  
351 database were clustered *de novo*. Taxonomy assignments were determined using  
352 BLAST<sup>50</sup> with a maximum e-value of 0.001 against the SILVA database. The  
353 resulting OTU table was then subsampled to an even depth of 12,000 sequences per

354 sample ten times followed by merging of the resulting ten OTU tables to reduce  
355 biases that arise from unequal library sizes. All data was then exported as a biom file.

356

### 357 **Statistical analyses**

358 Sample counts were transformed by dividing the individual OTU abundances  
359 by the number of rarefactions (10) followed by rounding prior to downstream analysis  
360 using the phyloseq package<sup>51</sup> in R<sup>52, 53</sup>. Diversity estimates were determined using  
361 observed richness and the Shannon index, as calculated and plotted in phyloseq and  
362 ggplot2<sup>54</sup>. Regression analyses and Kruskal-Wallis tests were performed in R to  
363 assess the relationships between environmental variables and richness and diversity.  
364 Prokaryotic community differences were represented on a two-dimensional ordination  
365 plot using Detrended Correspondence Analysis (DCA) with the Bray-Curtis distance  
366 between samples in phyloseq and ggplot2. Analysis of Similarity (ANOSIM) was  
367 used to quantify the relationships between significant differences in community  
368 structure and categorical variables (land use and soil classification) within the vegan  
369 package<sup>55</sup>. The Mantel test was performed in vegan with 999 permutations to assess  
370 relationships between continuous variables (pH) and community structure. To identify  
371 consistent clustering patterns in the data, hierarchical clustering was performed in the  
372 pvclust package<sup>56</sup> using Ward's method and Bray-Curtis distances. To examine  
373 significant differences in the abundance and distribution of taxa between land uses,  
374 the data were transformed to relative abundance in phyloseq. The Wald chi-squared  
375 test was applied to the data using the DESeq2 package<sup>57</sup>. Spearman's rank  
376 correlations were used to test differences in taxa distributions along the pH gradient.  
377 The Kruskal-Wallis test was used to observe differences in OTU abundances of  
378 significance between the soil orders, and was performed in QIIME. Cladograms were



379 generated in GraPhlAn<sup>58</sup>. Mapping was done using GADM<sup>59</sup> in RStudio with  
380 packages: ggplot2, sp<sup>60, 61</sup>, raster<sup>62</sup>, rgdal<sup>63</sup> and ggsn<sup>64</sup>.

381

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624

#### 625 **Author Contributions**

626           S.E.M. designed the experiment. R.K collected and processed samples. R.K.,  
627 S.E.M and B.T. analyzed data. All authors were involved in the writing process.

628

#### 629 **Additional Information**

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#### 631 **Accession codes**

632           Sequences were deposited at the Sequence Read Archive (NCBI) with the  
633 accession numbers: 5902515-5902586 under the BioProject ID: PRJNA348131.

634

#### 635 **Competing Financial Interests**

636           The authors declare a conflict of interest. This work was funded in part by a  
637 grant from Mainland Minerals Ltd, a fertilizer company in New Zealand.

638

639 **Figure legends**

640

641 **Figure 1** Relationships between bacterial communities under different land uses and  
642 pH. Changes in Alpha (Richness and Shannon Diversity) (A) and Beta (Detrended  
643 correspondence analysis based on Bray-Curtis dissimilarity) diversity metrics in  
644 response to land use and pH (B).

645

646 **Figure 2** Soil classification predicts prokaryotic community structuring within each  
647 land use. Comparison of diversity metrics for each soil subgroup (A) and (B) soil  
648 order. High country (C), Sheep and Beef (D), and dairy (E) soil communities  
649 evaluated using DCA ordination based on Bray-Curtis dissimilarity with color  
650 representing soil subgroup and shape representing soil order.

651

652 **Figure 3** Cladograms showing relationships between key taxa and edaphic properties.  
653 (A) OTUs (97% sequence similarity) significantly correlated with high or low country  
654 soils and are strongly correlated with changes in pH. Significance for land use  
655 preference was determined using the Ward method with a Z lower-limit of 6 and a *p*-  
656 value of <0.001. Correlation with pH was determined by a Spearman's correlation  
657 with a Rho lower-limit of 0.5/-0.5 and a *p*-value of <0.001. Light blue indicates a  
658 negative correlation with pH, and dark blue is positive (B) OTUs significantly  
659 correlated with specific soil orders. Significance was determined using the Kruskal-  
660 Wallis test with a chi-squared lower-limit of 27 and a *p*-value of <0.001. Brown soils  
661 are indicated by yellow, pallic by red, gley by green and recent by blue. A gradient of  
662 8 shades for each color was generated to indicate abundance, where white indicates an  
663 abundance of 0 and the darkest shades indicate an abundance >100.

664

665 **Figure 4** Map of sampling sites throughout the South Island of New Zealand. High  
666 country, dairy, and sheep and beef sites are indicated by triangles, circles and squares,  
667 respectively. 1-North Canterbury, 2-South Canterbury, 3-Otago, 4-Southland. The  
668 map was generated using shapefiles from GADM (v. 2.8, <https://www.gadm.org>) in  
669 RStudio (v. 0.99.903, <https://www.rstudio.com/>) using ggplot2 (v. 2.1.0), sp (v. 1.2-  
670 3), raster (v. 2.5-8), rgdal (v. 1.2-4) and ggsn (v. 0.3.1).

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