

1     **Conditionally rare taxa contribute but do not account for prokaryotic**  
2     **community changes in soils**

3

4     Rachel Kaminsky <sup>1</sup> and Sergio E. Morales <sup>1\*</sup>

5     <sup>1</sup> *Department of Microbiology and Immunology, Otago School of Medical Sciences,*

6     *University of Otago, Dunedin, New Zealand*

7

8     Running title: Conditionally rare taxa do not modify communities

9     \*Corresponding author: sergio.morales@otago.ac.nz (S. E. Morales)

10

11     Conflict of Interest: We claim a potential Conflict of Interest through funding

12     provided by Mainland Minerals LTD (a fertilizer company based on NZ) to support

13     the submitted research.

14

## 15    **Abstract**

16            Conditionally rare taxa (CRT) are thought to greatly impact microbial  
 17    community turnover across many environments, but little is known about their role in  
 18    soils. Here, we investigate the contribution of CRT to whole community variation  
 19    over space and time in a series of geographically distinct soils dedicated to three  
 20    agricultural practices of differing intensities and sampled over a full seasonal cycle.  
 21    We demonstrate that soil CRT do not account for observed total community changes,  
 22    but that these rare taxa can be modified by spatiotemporal filters.

23           Rare species are pervasive in microbial consortia (Curtis et al., 2002). Known  
24   as the “rare biosphere,” these members are being increasingly recognized for their  
25   importance in microbial communities (Pedrós-Alió, 2007, Lynch and Neufeld, 2015).  
26   There are several potential causes of rarity including transience, competition, niche  
27   breadth and predation of abundant taxa (Jousset et al., 2016). It has been suggested  
28   that the rare biosphere is a dormant “seed bank” wherein members become abundant  
29   after predation events (Lennon and Jones, 2011). However, rare microbes can also be  
30   active (Campbell et al., 2011, Hugoni et al., 2013, Kurm et al., 2017), signifying  
31   several potential ecological roles for the rare biosphere. Recent work identified  
32   conditionally rare taxa (CRT)—microbes that are rare at certain points in time/space  
33   and “bloom” to abundance at other points—as major contributors to community  
34   dynamics in several environments (Shade et al., 2014). This confirms that some rare  
35   microbes are transient and are potentially responsible for changes in community  
36   structure. In soils, studies have found that rare microbes bloom after disturbances  
37   (Aanderud et al., 2015, Fuentes et al., 2016). Despite these important findings, the  
38   literature is still developing with regard to the soil rare biosphere. Given the  
39   importance of soil microbial communities to mediating ecosystem processes,  
40   understanding the contribution of rare soil microbes is of great importance.

41           We investigated the contribution of conditionally rare prokaryotes using  
42   agricultural soils as a model system (see Supplementary Methods). We sampled soils  
43   from 24 sites under three agricultural practices in New Zealand. Sampling occurred  
44   during three time points over a year to capture the most divergent seasonal stages  
45   (summer to winter and return to summer) to assess community changes over space  
46   and time. We tested two hypotheses: (H<sub>1</sub>) CRT contribute disproportionately to whole

community structure and (H<sub>2</sub>) recruitment from the rare biosphere is linked to spatiotemporal filtering.

Time responsive CRT constituted 4-6% of OTUs in each site while space responsive CRT represented 1% of the total community (*b* value, > 0.9, relative abundance > 0.01%, Figure S1-3). To assess the contribution of CRT to whole community structure, we constructed three Bray-Curtis distance matrices from OTU tables for each site: (1) only OTUs identified as CRT, (2) whole communities including CRT and (3) whole communities excluding CRT. Mantel tests between distance matrices for CRT and whole communities including CRT are insignificant (Figure 1A). The correlation between space sensitive CRT and the whole community including CRT is significant, but weak ( $R^2 = 0.02$ ,  $P = 0.002$ ). Mantel tests between communities excluding CRT and whole communities including CRT have  $R^2$  values close to 1 (Table S1). This indicates that CRT do not contribute significantly to community variation, which does not align with previous findings, though it should be noted that these studies used moving windows analysis (Shade et al., 2014) and measurements of activity (Aanderud et al., 2015), which were not employed here. It is possible that soil CRT have a limited role in soil functions, remaining mostly dormant and only blooming to abundance in extreme cases, as it is estimated that a substantial portion of microbes are inactive (Lennon and Jones, 2011). On the other hand, CRT might be K-selected, investing in few members and therefore exhibiting a life strategy that isn't reflected in whole community dynamics. This may be favorable given the heterogeneity of soil, wherein CRT may not overtake dominant taxa, but perform key functions that are costly for those taxa. For example, *Desulfosporosinus* is estimated to perform the majority of sulfate-reduction in peatlands despite its relatively minor contribution to community variance (Pester et al., 2010).

72           Despite a minor contribution to whole community variance, CRT community  
73   structure is linked to spatiotemporal factors. ANOSIM tests revealed significant  
74   correlations for CRT and whole communities at individual sites with time (Figure 1B,  
75   Figure S4-5). Mantel tests between space responsive CRT, the whole community and  
76   pH were significant, and ANOSIM tests with land use and soil order were also  
77   significant (Figure S6-7, Table S2). These results agree with previous studies which  
78   have found that soil prokaryotic communities exhibit temporal patterns (Lauber et al.,  
79   2013), are sensitive to pH change (Lauber et al., 2009), land use change (Steenwerth  
80   et al., 2003) and soil type (Kaminsky et al., 2017). This indicates that soil CRT follow  
81   the same assembly rules as abundant taxa, thus contributing to community changes,  
82   but are not overwhelmingly represented in these dynamics.

83           Although CRT communities exhibit broad relationships with spatiotemporal  
84   factors, results revealed that only 8% of time responsive CRT and 0.005% of space  
85   responsive CRT are correlated to measured spatiotemporal factors (Figure 2). Key  
86   taxa are represented; for example, *Acidobacteria* is widely known to be sensitive to  
87   pH, and is reflected as such here, where it is rare in high pH soils and abundant in low  
88   pH soils. *Saprospiraceae* vary seasonally, which is consistent with previous findings  
89   (Schauer et al., 2006). These results may show that certain rare members have major  
90   functional roles in soils, but aren't well represented in overall community variance.  
91   Further, it shows that ecological filters not accounted for here, such as neutral  
92   processes or unmeasured niche factors, govern most soil CRT.

93           Results indicate that while soil CRT are sensitive to spatiotemporal filters,  
94   they are not accountable for observed whole community variability across space and  
95   time. This is significant in that it implies an ecological role for soil CRT that is not  
96   related to abundance.

97

## 98 **Conflict of Interest**

99

100 The authors declare a conflict of interest. This work was funded by a grant from  
101 Mainland Minerals Ltd.

102

## 103 **Acknowledgements**

104

105 We thank Mainland Minerals and Mainland Minerals Southern for sampling aid. We  
106 also thank Hill Laboratories and Soiltech for providing physicochemical analyses. RK  
107 was funded through a Callaghan Innovation education fellowship (MMSOU1301).

108

109

## 110 **References**

111

112 Aanderud ZT, Jones SE, Fierer N, Lennon JT. (2015). Resuscitation of the rare  
113 biosphere contributes to pulses of ecosystem activity. *Front Microbiol* **6**: 24.

114

115 Campbell BJ, Yu LY, Heidelberg JF, Kirchman DL. (2011). Activity of abundant and  
116 rare bacteria in a coastal ocean. *Proc Natl Acad Sci USA* **108**: 12776-12781.

117

118 Curtis TP, Sloan WT, Scannell JW. (2002). Estimating prokaryotic diversity and its  
119 limits. *Proc Natl Acad Sci USA* **99**: 10494-10499.

120

121 Fuentes S, Barra B, Caporaso JG, Seeger M. (2016). From rare to dominant: a fine-  
122 tuned soil bacterial bloom during petroleum hydrocarbon bioremediation. *Appl*  
123 *Environ Microbiol* **82**: 888-896.  
124  
125 Hugoni M, Taib N, Deboras D, Domaizon I, Isabelle JD, Bronner G, *et al* (2013).  
126 Structure of the rare archaeal biosphere and seasonal dynamics of active ecotypes in  
127 surface coastal waters. *Proc Natl Acad Sci USA* **110**: 6004-6009.  
128  
129 Jousset A, Bienhold C, Chatzinotas A, Gallien L, Gobet A, Kurm V, *et al* (2017).  
130 Where less may be more: how the rare biosphere pulls ecosystem strings. *ISME J* **11**:  
131 853-862.  
132  
133 Kaminsky R, Trouche B, Morales SE. (2017). Soil classification predicts differences  
134 in prokaryotic communities across a range of geographically distant soils once pH is  
135 accounted for. *Sci Rep* **7**: 45369.  
136  
137 Kurm V, Putten WH, Boer W, Naus-Wiezer S, Hol WH. (2017). Low abundant soil  
138 bacteria can be metabolically versatile and fast growing. *Ecology* **98**: 555-564  
139  
140 Lauber CL, Hamady M, Knight R, Fierer N. (2009). Pyrosequencing-based  
141 assessment of soil pH as a predictor of soil bacterial community structure at the  
142 continental scale. *Appl Environ Microbiol* **75**: 5111-5120.  
143  
144 Lauber CL, Ramirez KS, Aanderud Z, Lennon J, Fierer N. (2013). Temporal  
145 variability in soil microbial communities across land-use types. *ISME J* **7**: 1641-1650.

146

147 Lennon JT, Jones SE. (2011). Microbial seed banks: the ecological and evolutionary  
148 implications of dormancy. *Nat Rev Microbiol* **9**: 119-130.

149

150 Lynch MDJ, Neufeld JD. (2015). Ecology and exploration of the rare biosphere. *Nat*  
151 *Rev Microbiol* **13**: 217-229.

152

153 Pedrós-Alió C. (2007). Dipping into the rare biosphere. *Science* **315**: 192-193.

154

155 Pester M, Bittner N, Deevong P, Wagner M, Loy A. (2010). A ‘rare biosphere’  
156 microorganism contributes to sulfate reduction in a peatland. *ISME J* **4**: 1591-1602.

157

158 Schauer M, Jiang J, Hahn MW. (2006). Recurrent seasonal variations in abundance  
159 and composition of filamentous SOL cluster bacteria (*Saprospiraceae*, *Bacteroidetes*)  
160 in oligomesotrophic Lake Mondsee (Austria). *Appl Environ Microbiol* **72**: 4704-4712.

161

162 Shade A, Jones SE, Caporaso JG, Handelsman J, Knight R, Fierer N, Gilbert JA.  
163 (2014). Conditionally rare taxa disproportionately contribute to changes in microbial  
164 diversity. *MBio* **5**:e01371-14.

165

## 166 **Figure legends**

167

168 **Figure 1** Contribution of CRT to community variance. Summary of Mantel  
169 correlations between Bray Curtis distances for site-level time responsive CRT  
170 communities and whole communities for each site (A) and summary of ANOSIM



171 results for correlation between time and either site-level CRT or site-level whole  
 172 community changes (B). pH and land use are shown to discount confounding effects  
 173 by a dominant soil driver.  
 174  
 175  
 176 **Figure 2** Relationships between individual CRT and key spatiotemporal factors.  
 177 OTUs identified as CRT and significantly correlated to changes in pH (A), land use  
 178 (B), soil order (C) and time (D). The OTU from each site that varied most  
 179 significantly with time was plotted. A full list of OTUs correlated with time is  
 180 reported in Table S3. Taxa were chosen based on a Kruskal Wallis  $P < 0.05$  (land use,  
 181 soil order and time), and a Spearman's  $P < 0.05$  for pH. Taxa are presented at the  
 182 lowest classification level available, and colored at the phylum level.  
 183  
 184



