bioRxiv preprint doi: https://doi.org/10.1101/165001; this version posted July 18, 2017. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

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

Rachel Kaminsky [1] and Sergio E. Morales [1*]

[1] *Department of Microbiology and Immunology, Otago School of Medical Sciences, University of Otago, Dunedin, New Zealand*

Running title: Conditionally rare taxa do not modify communities

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

Conflict of Interest: We claim a potential Conflict of Interest through funding provided by Mainland Minerals LTD (a fertilizer company based on NZ) to support the submitted research.

bioRxiv preprint doi: https://doi.org/10.1101/165001; this version posted July 18, 2017. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

Abstract

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

bioRxiv preprint doi: https://doi.org/10.1101/165001; this version posted July 18, 2017. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

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

We investigated the contribution of conditionally rare prokaryotes using agricultural soils as a model system (see Supplementary Methods). We sampled soils from 24 sites under three agricultural practices in New Zealand. Sampling occurred during three time points over a year to capture the most divergent seasonal stages (summer to winter and return to summer) to assess community changes over space and time. We tested two hypotheses: (H_1) CRT contribute disproportionately to whole

bioRxiv preprint doi: https://doi.org/10.1101/165001; this version posted July 18, 2017. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

community structure and (H_2) 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).

bioRxiv preprint doi: https://doi.org/10.1101/165001; this version posted July 18, 2017. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

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

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

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

bioRxiv preprint doi: https://doi.org/10.1101/165001; this version posted July 18, 2017. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

Conflict of Interest

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

Acknowledgements

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

References

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

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

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

bioRxiv preprint doi: https://doi.org/10.1101/165001; this version posted July 18, 2017. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

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.

bioRxiv preprint doi: https://doi.org/10.1101/165001; this version posted July 18, 2017. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

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

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

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

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

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

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

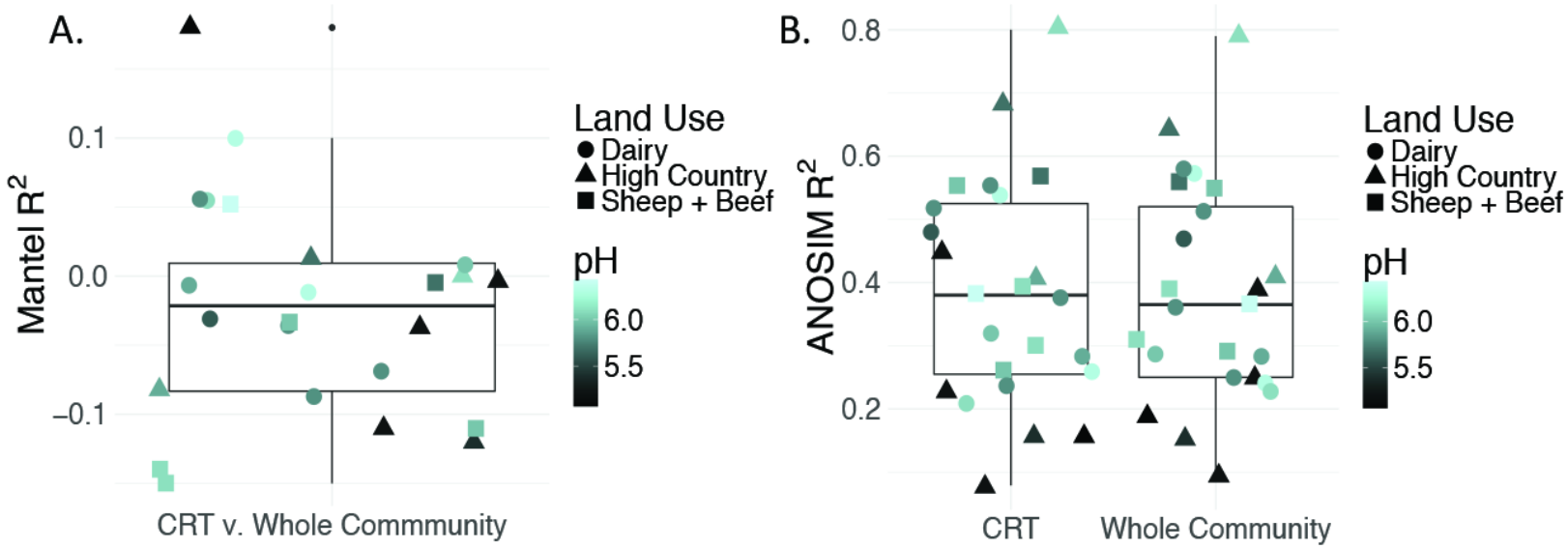
Figure legends

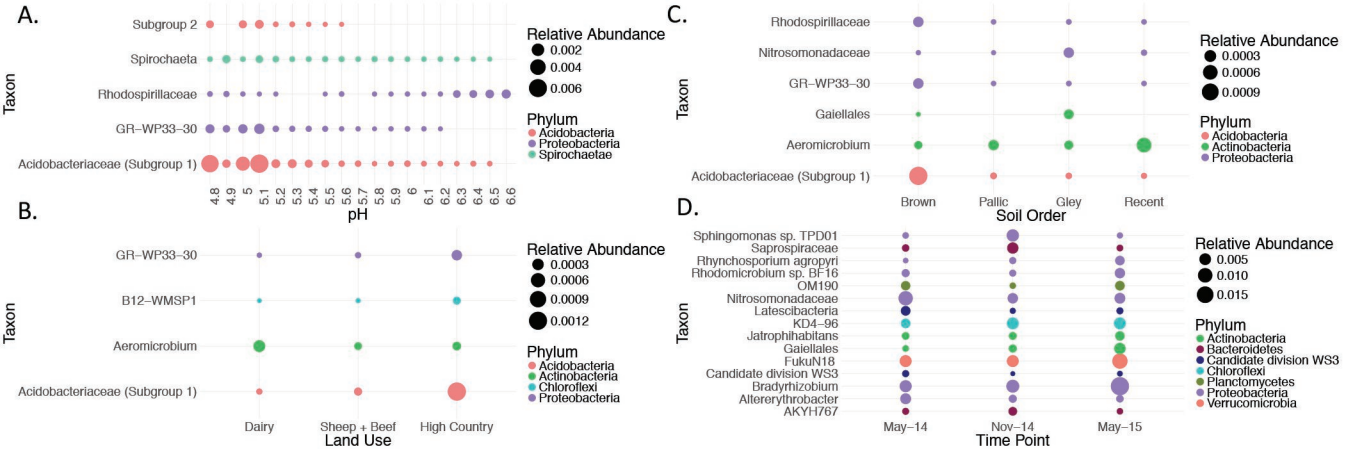
Figure 1 Contribution of CRT to community variance. Summary of Mantel correlations between Bray Curtis distances for site-level time responsive CRT communities and whole communities for each site (A) and summary of ANOSIM

bioRxiv preprint doi: https://doi.org/10.1101/165001; this version posted July 18, 2017. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

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

bioRxiv preprint doi: https://doi.org/10.1101/165001; this version posted July 18, 2017. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.




A.
Subgroup 2
Spirochaeta
Rhodospirillaceae
GR−WP33−30
Acidobacteriaceae (Subgroup 1)
Taxon
4.8 4.9 5 5.1 5.2 5.3 5.4 5.5 5.6 5.7 5.8 5.9 6 6.1 6.2 6.3 6.4 6.5 6.6
pH
Relative Abundance
0.002
0.004
0.006
Phylum
Acidobacteria
Proteobacteria
Spirochaetae
B.
GR−WP33−30
B12−WMSP1
Aeromicrobium
Acidobacteriaceae (Subgroup 1)
Taxon
Dairy
Sheep + Beef
High Country
Land Use
Relative Abundance
0.0003
0.0006
0.0009
0.0012
Phylum
Acidobacteria
Actinobacteria
Chloroflexi
Proteobacteria
C.
Rhodospirillaceae
Nitrosomonadaceae
GR−WP33−30
Gaiellales
Aeromicrobium
Acidobacteriaceae (Subgroup 1)
Taxon
Brown
Pallic
Gley
Recent
Soil Order
Relative Abundance
0.0003
0.0006
0.0009
Phylum
Acidobacteria
Actinobacteria
Proteobacteria
D.
Sphingomonas sp. TPD01
Saprospiraceae
Rhynchosporium agropyri
Rhodomicrobium sp. BF16
OM190
Nitrosomonadaceae
Latescibacteria
KD4−96
Jatrophihabitans
Gaiellales
FukuN18
Candidate division WS3
Bradyrhizobium
Altererythrobacter
AKYH767
Taxon
May−14
Nov−14
May−15
Time Point
Relative Abundance
0.005
0.010
0.015
Phylum
Actinobacteria
Bacteroidetes
Candidate division WS3
Chloroflexi
Planctomycetes
Proteobacteria
Verrucomicrobia