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3 1 Functional trait relationships demonstrate life strategies in terrestrial prokaryotes.
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20 modelling
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23 Abstract

24 Functional, physiological traits are the underlying drivers of niche differentiation. A
25 common framework related to niches occupied by terrestrial prokaryotes is based on
26 copiotrophy or oligotrophy, where resource investment is primarily in either rapid
27 growth or stress tolerance, respectively. A quantitative trait-based approach sought
28 relationships between taxa, traits and niche in terrestrial prokaryotes. With 175 taxa
29 from 11 Phyla and 35 Families ($n = 5$ per Family), traits were considered as discrete
30 counts of shared genome-encoded proteins. Trait composition strongly supported non-
31 random functional distributions as preferential clustering of related taxa via unweighted
32 pair-group method with arithmetic mean. Trait similarity between taxa increased as
33 taxonomic rank decreased. A suite of Random Forest models identified traits
34 significantly enriched or depleted in taxonomic groups. These traits conveyed functions

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3 35 related to rapid growth, nutrient acquisition and stress tolerance consistent with their
4 36 presence in copiotroph-oligotroph niches. Hierarchical clustering of traits identified a
5 37 clade of competitive, copiotrophic Families resilient to oxidative stress *versus*
6 38 glycosyltransferase-enriched oligotrophic Families resistant to antimicrobials and
7 39 environmental stress. However, the formation of five clades suggested a more
8 40 nuanced view to describe niche differentiation in terrestrial systems is necessary. We
9 41 suggest considering traits involved in both resource investment and acquisition when
10 42 predicting niche.
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21 45 1. Introduction

22 46 Niche differentiation, the process of physiologically distinct organisms adapting better
23 47 to certain conditions, is a contributing factor to the high biodiversity inherent in microbial
24 48 communities (Prosser, 2012). Such differentiation is likely an inevitable consequence
25 49 of the principles of competitive exclusion and natural selection working in tandem – no
26 50 two organisms can theoretically occupy the same niche, as the poorer competitor must
27 51 either adapt to a unique niche or be driven to extinction in that system (Gause, 1932,
28 52 Hutchinson, 1957, Leibold, 1995). The physiological traits driving niche differentiation
29 53 must have the capacity to convey an advantage to the organism's ability to survive and
30 54 reproduce (*i.e.* fitness) and be inherited by successive generations (McGill *et al.*, 2006).
31 55 Importantly, this implies that microbial communities are not only diverse in terms of
32 56 individual 16S rRNA gene sequences, commonly used to assess community diversity,
33 57 but also diverse in regard to their physiological traits.

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43 58 Explaining niche differentiation through the functional, physiological traits present in
44 59 ecological community members has a long history in macroecology. For example,
45 60 differences between beak size and shape in Galápagos finches was instrumental in
46 61 Darwin's hypothesis that a common ancestor had differentiated into multiple, island-
47 62 specific species. Within the past century, trait-based analyses have been particularly
48 63 predominant in plant ecology, with seed germination in submerged soil, salt tolerance,
49 64 carbon to nitrogen biomass stoichiometry, and leaf mass per unit area acting as
50 65 examples of traits linked to niche differentiation (Gleason, 1926, Grime, 1979, Keddy,
51 66 1992). In contrast, trait-based approaches to explain microbial ecology have only been
52 67 performed in few instances, such as conceptualizing niches of methanotrophs based
53 68 on abundance in high *versus* low methane environments or disturbed *versus*

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3 69 undisturbed soils (Ho *et al.*, 2013), correlating increasing growth rate with increasing
4
5 70 ribosomal gene and ribosome-associated gene copy number (Vieira-Silva & Rocha,
6
7 71 2010), deterministic modelling of nitrification rate based on ammonia and oxygen
8
9 72 uptake rate, temperature sensitivity and growth rate (Bouskill *et al.*, 2012), defining
10
11 73 distinct niches of 32 marine microorganisms based on clustering of genome-encoded
12
13 74 functional proteins (Lauro *et al.*, 2009), identifying habitat generalists and specialists
14
15 75 based on taxon co-occurrence patterns (Barbéran *et al.*, 2012) and recently
16
17 76 comparisons of 23 'core' traits (e.g. motility, carbon metabolism, optimal pH for growth)
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19 77 across 15 000 diverse host-associated and environmental genomes (Madin *et al.*,
20
21 78 2020).

22 79 A consistent trend noted in macroecology is that traits linked to how carbon and
23
24 80 energy is processed and allocated to biomass can describe separate niches (Brown *et*
25
26 81 *al.*, 2004). The canonical example are *r* and *K* strategists, where carbon and energy
27
28 82 are primarily invested in reproduction, or alternatively invested in tolerating biotic
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30 83 and/or abiotic stressors, respectively (Grime, 1977). These dichotomous strategies
31
32 84 have been observed in microbial ecology: copiotrophs are considered as
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34 85 microorganisms with relatively high growth rates that have relatively poor growth
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36 86 efficiency (as carbon incorporated to biomass per unit resource), relatively high cell
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38 87 maintenance energy costs, dependence on relatively high concentrations of organic
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40 88 carbon in their environment, demonstrate rapid population blooms upon the addition of
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42 89 organic matter and are not overly tolerant of abiotic stress (Semenov, 1991, Koch,
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44 90 2001, Roller & Schmidt, 2015, Ho *et al.*, 2017). Oligotrophs are considered as the
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46 91 inverse – low growth rate, high growth efficiency, low cell maintenance energy
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48 92 requirements, high substrate uptake affinity, slow growth yet at a consistent rate and
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50 93 are resilient to abiotic stress. Although the niche concept in macroecology has a
51
52 94 formalized definition founded on where a taxon can maintain a stable population within
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54 95 multi-dimensional environmental space (Leibold, 1995), in this study, niche is used
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56 96 simply to distinguish between prokaryotes being relatively more copiotrophic *versus*
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58 97 oligotrophic.

59 98 These distinct niches became associated with specific terrestrial taxa at high
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61 99 taxonomic rank based on recent molecular analyses. In complex microbial
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63 100 communities, the relative abundance of Gammaproteobacteria, Bacteroidetes and
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65 101 Actinobacteria were correlated with rapid growth in response to the addition of labile
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67 102 organic matter or nitrogen (copiotrophs) (Fierer *et al.*, 2007, Goldfarb *et al.*, 2011,

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3 103 Fierer *et al.*, 2012, Leff *et al.*, 2015). Conversely, the Deltaproteobacteria,
4 104 Acidobacteria, Verrucomicrobia and Planctomycetes were negatively correlated with
5 105 the addition of organic matter or nitrogen (oligotrophs) (Fierer *et al.*, 2007, Fierer *et al.*,
6 106 2012, Leff *et al.*, 2015, Bastida *et al.*, 2016). Conflicting reports exist of Beta- and
7 107 Alphaproteobacteria, with some studies describing them as copiotrophic and others as
8 108 oligotrophic highlighting that a consistent niche may not necessarily exist across
9 109 species within a large taxonomic group (Ho *et al.*, 2017). A genomic basis for traits
10 110 associated with soils dominated by putative copiotrophs and oligotrophs has been
11 111 expertly reviewed elsewhere, and interested readers are referred to Trivedi *et al.*,
12 112 (2013) and references therein. Importantly, these observations suggest that specific
13 113 traits that allow terrestrial prokaryotes to occupy these two niches should (generally)
14 114 be associated with taxonomy. This is an example of ecological coherence at high
15 115 taxonomic rank, whereby members within a taxon tend to have similar life strategies,
16 116 niches and possess common traits compared to members of other taxa (Philippot *et*
17 117 *al.*, 2010). While ecological coherence of taxa has been considered previously, the
18 118 shared, specific traits that drive niche differentiation in terrestrial prokaryotes remains
19 119 an open question.

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22 120 To identify the traits that differ between terrestrial prokaryote taxonomic groups, and
23 121 whether these traits could describe the niches they occupy, a functional trait-based
24 122 approach was adopted here. We posited that a trait must: a) be associated with a
25 123 physiological process that conveys a fitness advantage under certain environmental
26 124 conditions; b) be measurable in well-defined units; and c) vary more between
27 125 taxonomic groups than within a taxonomic group (McGill *et al.*, 2006, Kearney *et al.*,
28 126 2010). Traits were measured as discrete counts of chromosome-encoded proteins
29 127 shared between at least two of 175 terrestrial prokaryotes. Markov Chain clustering
30 128 (MCL) was used to group proteins as traits based on amino acid sequence similarity
31 129 (%) akin to a previous approach that confirmed close taxonomic relatives tend to share
32 130 functional traits in 1 374 genomes (Zhu *et al.*, 2015). This was necessary to compare
33 131 highly similar (but non-identical) proteins from separate genomes that carry out the
34 132 same biological function. To better identify important, distinguishing traits of terrestrial
35 133 prokaryotes, this study differed from Zhu *et al.*, by: a) comparing 175 publicly available
36 134 terrestrial prokaryote genomes from 35 Families ($n = 5$ each), from 11 Phyla and two
37 135 Kingdoms; b) selecting prokaryotes involved in terrestrial ecosystem processes of
38 136 interest, including organic matter decomposition, nitrogen fixation, nitrification,

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3 137 denitrification, methane oxidation, plant-growth promotion, bioremediation of
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5 138 pollutants, pathogenesis and methanogenesis; c) selecting taxa isolated from a wide
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7 139 range of terrestrial environments, such as nutrient rich decomposing plant material and
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9 140 rhizosphere, submerged wetland and rice paddy soils, polluted soils, and nutrient poor
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11 141 hot and cold arid environments; and d) avoiding the inclusion of multiple subspecies
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13 142 and/or strains of a single species to prevent biases in analyses where highly over-
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15 143 represented species are compared with species that have fewer cultured
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17 144 representatives. The taxonomic system used here is from the NCBI, which is built upon
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19 145 a historical array of culture-dependent, physiological observations and genetic
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21 146 similarity to cultured isolates as average nucleotide identity, DNA-DNA hybridisation or
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23 147 16S rRNA gene homology (ncbi.nlm.nih.gov/Taxonomy/Browser). The use of this
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25 148 classification system and comparison to others is discussed further below.

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27 149 We hypothesised that: 1) traits are non-randomly distributed, with relatively closely
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29 150 related taxa demonstrating greater similarity than unrelated taxa (ecological
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31 151 coherence); 2) traits that are differentially enriched between taxonomic groups would
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33 152 primarily be involved in metabolism, nutrient acquisition and/or tolerating
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35 153 environmental stress; and 3) copiotrophic and oligotrophic taxonomic groups would
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37 154 emerge based on collective trait enrichment.

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155 156 157 2. Methodology

158 2.1 Collection of terrestrial prokaryote genomes

159 A collection of 175 sequenced and annotated genomes was collated (Supplementary
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161 Table 1). Listed are the genome ID, phylogenetic lineage, role in an ecosystem process
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163 if known, and isolation or genome sequencing reference. These genomes were
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165 sourced from the National Centre for Biotechnology Information (NCBI) and Joint
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167 Genome Institute (JGI) databases. Genomes were chosen based on several criteria:
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169 a) five isolates per Family were chosen to have an equal minimum sample size per
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171 group, with this sample size being constrained by sequenced genomes of under-
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173 represented groups in public databases; b) only a single subspecies/strain per species
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175 was included to avoid bias due to over-representation of some species in public
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177 databases; c) an emphasis was placed to include isolates from diverse taxonomic
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179 lineages involved in terrestrial ecosystem processes of interest, such as ammonia
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181 oxidation and methanogenesis; and d) there was an emphasis to include taxonomic

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3 171 groups frequently stated to be either copiotrophic (e.g. Actinobacteria,
4 172 Gammaproteobacteria) or oligotrophic (e.g. Acidobacteria, Planctomycetes) based on
5 173 observations from soil nutrient addition studies (Ho *et al.*, 2017). Taxonomic
6 174 annotations for Phyla, Class, Order etc. were based on NCBI taxonomy as most
7 175 genomes were sourced there. The authors recognise that taxonomy is constantly
8 176 shifting, particularly so with the recent development of the Genome Taxonomy
9 177 Database (GTDB) (Parks *et al.*, 2018). Of note is that the vast majority of taxa here
10 178 have the same taxonomy in NCBI as in GTDB, with the exceptions that GTDB
11 179 considers the Sporomusaceae as split into three separate Families, the
12 180 Leuconostocaceae to be Lactobacillaceae, the Promicromonosporaceae to be
13 181 Cellulomonadaceae, and the Bradyrhizobiaceae and Methylococcaceae have been
14 182 renamed as Xanthobacteraceae and Methylomonadaceae, respectively. Taxon
15 183 selection was constrained by availability of genomes for under-represented groups,
16 184 such as the Chloroflexi, Verrucomicrobia, Planctomycetes, Thaumarchaeota and
17 185 Euryarchaeota. To meet the $n = 5$ requirement for balanced statistical analyses, it was
18 186 necessary to consider these under-represented groups as 'Families'. Furthermore, due
19 187 to the great diversity inherent within Proteobacterial Classes, Gamma-, Alpha-, Beta-
20 188 and Deltaproteobacteria were considered as independent 'Phyla' for statistical
21 189 analyses here. Indeed, GTDB now defines Deltaproteobacteria as its own Phylum,
22 190 while Betaproteobacteria are considered as the Burkholderiales Order within the
23 191 Gammaproteobacteria. The total of 175 genomes analysed here falls within the upper
24 192 range of previous hypothesis-driven trait-based studies which varies from 11 isolates
25 193 (Bouskill *et al.*, 2012) to 214 genomes (Vieira-Silva & Rocha, 2010).

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196 2.2 Functional trait clustering

197 A step-by-step walkthrough of reproducible code to perform the following analyses on
198 a subset of 12 genomes is available at: [https://github.com/DamienFinn/Trait-](https://github.com/DamienFinn/Trait-based_analyses)
199 [based_analyses](https://github.com/DamienFinn/Trait-based_analyses). Firstly, a pairwise similarity comparison of all amino acid sequences
200 (964 951 sequences) across the 175 genomes was performed with the all *versus* all
201 basic local alignment tool function for proteins, BLAST-P (Altschul *et al.*, 1990). Amino
202 acid sequences were subsequently clustered as traits via MCL weighted by pairwise
203 amino acid similarity (Enright *et al.*, 2002). Functional traits were grouped at a cluster
204 value of 90.2, whereby > 65 is considered 'fair' and confidence in accurately separating

clusters cannot be higher than 100. The value of 90.2 is not chosen by the user but rather is a reflection of the quality of clustering in a given dataset. The MCL identified a total of 220 664 traits shared between at least two genomes. A random subset of 1700 amino acid sequences were selected and the similarity of each sequence within its trait group (as determined by MCL) *versus* between other trait groups was visualised as a box and whisker plot (Supplementary Figure 1) in R version 4.0.0 (R Core Team, 2013). 1 700 sequences were chosen to maximise comparisons between trait groups under technical limitations, as increasing sequences led to exponential increases in trait combinations. A Student's T test was applied to determine whether sequences were more similar within their trait group relative to between trait groups in R. Finally, a matrix of genome x functional trait was generated in a two-step process by first associating genome IDs to the MCL output with a novel script 'MCLtoReshape2.py' (available at the above Github address) and secondly by casting the long data format to a wide data matrix with the 'reshape2' package in R (Wickham, 2007). Box and whisker plots comparing counts of proteins per genome (input) and counts of functional traits shared by at least two genomes (output of computational workflow), for the 35 Families, is presented as Figure 1.

2.3 UPGMA dendrogram of trait similarity between genomes

The unweighted pair group method with arithmetic mean (UPGMA) was chosen to compare distance-based similarity between taxa based on discrete counts of individual traits per genome. This method is more robust for comparing similarity between sample units (*i.e.* taxa) based on discrete counts of variables (*i.e.* individual traits per taxon) rather than neighbour joining or maximum likelihood methods better suited for DNA or amino acid sequence comparisons (Weins, 1998). The UPGMA was performed in R with the 'phangorn' package as described (Schliep *et al.*, 2017) on a Bray-Curtis transformed dissimilarity functional trait matrix, generated with the 'vegdist' function in the 'vegan' package (Oksanen *et al.*, 2013). To measure ecological coherence (C) of taxa within shared Super Groups, Phyla and Families, a similarity index was adapted from Levins' Overlap (Finn *et al.*, 2020a), which measures pairwise similarity in distributions of taxa, as the following:

$$C = 1 - \left(\frac{\sum b_{ij}}{n^2} \right) \quad \text{Equation 1.}$$

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Where b_{ij} is the pairwise branch length between taxon i and j in the UPGMA tree, measured here as Bray-Curtis dissimilarity, which is summed for each taxon and its relatives within a shared Super Group, Phylum or Family, and where n is the number of taxa being compared within a shared Super Group, Phylum or Family.

Furthermore, the full length 16S rRNA gene of each taxon was collated from NCBI. Genes were aligned with MUSCLE (Edgar, 2004) and a neighbour joining phylogenetic tree was constructed with the 'phangorn' package in R. Phylogenetic distance present in taxonomic groups (P) was measured as per Equation 1., excepting that branch length was in units of DNA sequence similarity as opposed to Bray-Curtis dissimilarity. Finally, simple linear regression was used to test a relationship between P and C .

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2.4 Functional trait annotation

To inform the biological process a functional trait facilitated, traits were annotated with the Kyoto Encyclopedia of Genes and Genomes (KEGG) database. This was performed in five steps: 1) a representative amino acid sequence from each trait was extracted with the novel script 'IdentifyTraits.py'; 2) these sequences were annotated with KEGG Orthology (KO) terms using the BlastKOALA database algorithm with a bit score cut-off value of 75 (Kanehisa *et al.*, 2016); 3) BRITE functional hierarchies associated with each KO term (e.g. KO1179 gene: endoglucanase, BRITE 1: Metabolism, BRITE 2: Carbohydrate Metabolism, BRITE 3: Starch and Sucrose Metabolism) were collected with the novel script 'GetBRITEinfo.py'; 4) Genome ID, trait ID, KO term and BRITE metadata were all collated with the novel script 'MatchFCs.py'; and 5) the 'reshape2' package in R was used to create matrices of genome x BRITE hierarchy. Where KEGG was unable to annotate a trait, it was considered as 'Uncharacterised'. As above, all novel scripts and a step-by-step walkthrough of reproducible code is available at: https://github.com/DamienFinn/Trait-based_analyses.

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2.5 Identifying traits differentially enriched in taxonomic groups

Random Forest classification was chosen as a non-linear, multivariate cluster-based method capable of identifying numerous predictor variables (*i.e.* traits) that define

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3 273 different classes of a response variable (*i.e.* taxonomic group). This was performed
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5 274 with the 'randomForest' package as described (Liaw & Weiner, 2002). Discrete counts
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7 275 of traits at BRITE level 3 were used (*e.g.* Starch and Sucrose Metabolism) as this level
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9 276 had the most accurate resolution of biological processes facilitated by traits. A total of
10
11 277 six Random Forest models were optimised to classify taxonomic groups at the level of:
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13 278 a) Phylum, with Proteobacteria Classes separated due to their extensive diversity ($n =$
14
15 279 14); b) Family ($n = 35$); c) specifically for Families in the Proteobacteria ($n = 13$); d)
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17 280 Families in the Actinobacteria ($n = 7$); e) Families in the Firmicutes ($n = 5$); and f)
18
19 281 Families from 'Under-represented' groups, which were all other Families ($n = 10$).
20
21 282 Optimal numbers of trees grown for each model were: 300, 400, 320, 300, 300 and
22
23 283 400, respectively. Six traits were randomly selected at each branch. As the Random
24
25 284 Forest only identifies traits that best explain separation of taxonomic groups, and does
26
27 285 not show whether traits have positive or negative associations with groups, box and
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29 286 whisker plots and Fisher's Least Significant Difference (LSD) *post hoc* tests were
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31 287 performed with the 'agricolae' package (de Mendiburu, 2014) to definitively state which
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33 288 taxonomic groups were significantly enriched or depleted in traits identified via
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35 289 Random Forest.

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292 *2.6 Hierarchical clustering of Families by defining traits*

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38 293 Finally, the relationship between Families based on similarity in counts of 60 traits was
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40 294 assessed via hierarchical clustering. Traits were chosen based on being selected via
41
42 295 the above Random Forest models in this study, and from previous studies that
43
44 296 identified traits associated with copiotroph-oligotroph growth strategies in single
45
46 297 species or mixed communities (Lauro *et al.*, 2009, Vieira-Silva & Rocha, 2010, Roller
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48 298 & Schmidt, 2015, Pascual-Garcia & Bell, 2020). The mean of trait discrete counts in
49
50 299 the five Family members was used as representative of each Family. Comparing trait
51
52 300 means between Families was considered acceptable as prior LSD *post hoc* tests had
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54 301 demonstrated significant differences between Families. As traits had highly variable
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56 302 copy numbers per Family (*e.g.* ABC transporter trait copies ranged from 10 – 350,
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58 303 while bacterial chemotaxis traits ranged from 0 – 15 copies) the trait copies were
59
60 304 normalised for more appropriate comparisons. Normalised variance was calculated
61
62 305 across the 35 Families for all traits with the 'decostand' function in the 'vegan' package
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64 306 (Oksanen *et al.*, 2013). Hierarchical clustering of Families based on normalised trait

counts was visualised with the 'heatmap.2' function in the 'gplots' package (Warnes *et al.*, 2019).

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3. Results

3.1 Trait clustering and UPGMA

The 964 951 amino acid sequences encoded by the 175 genomes were clustered as 220 664 traits by MCL. A random subset of 1 700 traits showed that amino acid sequence similarity within traits ranged from 62.4, 82.5 and 100% for the 1st quartile, mean and 3rd quartile, respectively (Supplementary Figure 1). Amino acid sequence similarity between traits ranged from 27.6, 34 and 37.8% for the 1st quartile, mean and 3rd quartile, respectively. A Student's T test found that sequences grouped together as a trait were significantly more similar to each other than to sequences grouped as different traits (t value = 61.3, $p = 2 \times 10^{-16}$). Manual comparisons of amino acid sequences within several traits supported clustering of proteins with identical biological function based on KEGG annotation. Thus, the MCL was considered to perform well. However, the minimum amino acid sequence similarity within traits was 23.27% and maximum similarity between traits was 85.24%, indicating that across the 220 664 traits, a small proportion of dissimilar amino acid sequences were grouped as a trait incorrectly, while some amino acid sequences that were highly similar were considered different traits. This small number of incorrectly clustered sequences can be explained by the MCL clustering efficiency being 90.2, out of a possible 100.

A comparison of the number of proteins per genome at the Family level (mean = 5 533), found that there were fewer functional traits per genome (mean = 4 240) (Figure 1). These represent the input number of proteins per genome before trait clustering and the output number of traits after clustering, respectively. As only functional traits shared between at least two genomes were considered here, the loss of highly genome-specific traits that could not be compared between genomes was expected. Despite this drop in average traits per genome, this initial approach serves as a proof of concept to demonstrate that numbers of traits per genome at the Family level reflect trends in proteins per Family, and thus the MCL was not distorting trait clustering (Figure 1).

The UPGMA dendrogram comparing the 220 664 traits per genome showed that trait compositions were non-randomly distributed (Figure 2a). Specifically,

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3 341 Thaumarchaeota, Euryarchaeota, Acidobacteria, Betaproteobacteria,
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5 342 Gammaproteobacteria, Alphaproteobacteria, Cyanobacteria, Verrucomicrobia,
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7 343 Planctomycetes, Bacteroidetes, Actinobacteria, Firmicutes and Deltaproteobacteria
8
9 344 clustered together preferentially. The Chloroflexi were split into two clusters: one
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11 345 *Dehalococcoides* and one *Ktedonobacter/Herpetosiphon/Anaerolinea* cluster. Several
12
13 346 prokaryotes did not cluster with their high taxonomic rank, including a Planctomycetes
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15 347 bacterium, *Polyangium brachysporum* (Deltaproteobacteria), *Agreia pratensis*
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17 348 (Actinobacteria) and *Sporomusa ovata* (Firmicutes). Also of interest was that, in regard
18
19 349 to distance between terminal nodes (as noted by the scale bar), the Betaproteobacteria
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21 350 and Gammaproteobacteria were more similar to each other than the
22
23 351 Alphaproteobacteria, which formed its own large, diverse clade. A neighbour joining
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25 352 tree of full length 16S rRNA genes showed that all taxa clustered preferentially based
26
27 353 on their taxonomic nomenclature at high taxonomic rank, including the Chloroflexi,
28
29 354 indicating that the discrepancies in the UPGMA were not due to misclassification of the
30
31 355 individual taxa (Supplementary Figure 2a).

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33 356 A simple index to measure trait similarity, as ecological coherence (C), within groups
34
35 357 was devised (Equation 1). C increased as taxonomic rank decreased: Super Group <
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37 358 Phylum < Family (Figure 2b). C was lowest for the larger, more diverse Proteobacteria
38
39 359 and Terrabacteria (Super Group), and Firmicutes and Actinobacteria (Phylum). As the
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41 360 number of taxa being compared at the Super Group (e.g. Terrabacteria = 70 *versus*
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43 361 Acidobacteria = 10) and Phylum (e.g. Actinobacteria = 35 *versus* Thaumarchaeota =
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45 362 5) were variable, the most meaningful comparisons between groups are at the Family
46
47 363 level ($n = 5$ each). With the exception of the highly divergent 'Acidobacteria Lineage',
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49 364 all Families had a C greater than 0.3, with certain groups in the Alphaproteobacteria
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51 365 (Beijerinckiaceae), Firmicutes (Bacillaceae and Leuconostocaceae) and
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53 366 Actinobacteria (Promicromonosporaceae) being highly coherent ($C > 0.55$). Indeed, all
54
55 367 individual Proteobacterial Families had $C > 0.4$, indicating that all five taxa within each
56
57 368 of these Families had similar trait compositions. Despite not truly belonging to the same
58
59 369 Family as per NCBI taxonomy, the $C > 0.33$ of the five Cyanobacteria,
60
370 Thaumarchaeota and the Methanogen Lineage taxa was similar to other Families from
371 the Bacteroidetes and Firmicutes. Thus, the UPGMA demonstrated that taxonomic
372 relatives at the Phylum level tended to cluster with each other preferentially based on
373 trait composition, and secondly that while similarity was broadly highest at low
374 taxonomic rank, some Families were more coherent than others.

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3 375 Phylogenetic distance (P) of each taxonomic group increased with decreasing
4 376 taxonomic rank, and was highest in Proteobacteria, Actinobacteria and Firmicutes
5 377 Families (Supplementary Figure 2b). There was a strong positive linear relationship
6 378 between P and C ($y = 0.86x - 0.25$, $R^2 = 0.39$, $p < 0.001$) supporting the result that
7 379 taxonomic groups of closer related taxa tended to share more similar compositions of
8 380 traits.

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14 382

17 383 *3.2 Random Forest trait identification*

18 384 The KEGG annotated traits belonged to 260 different BRITE 3 categories. The
19 385 percentage of traits that could not be annotated (and were termed 'Uncharacterised')
20 386 ranged from 28 – 65% per genome, being particularly high in the Archaea. On average,
21 387 47% of traits per genome were Uncharacterised with a standard deviation of 9.5%.

22 388 Of the 260 BRITE 3 categories, the 60 most important traits in separating all Phyla
23 389 and Families are ranked by importance measured as Mean Decrease in Accuracy
24 390 (MDA) of the Random Forest models (Figure 3). This is a measure of the average
25 391 increase in classification error during permutation of trees ($n = 300 - 400$) when that
26 392 particular trait is missing from the tree. For example, the accuracy of classifying
27 393 Families was most improved by inclusion of the ABC transporters trait. Based on the
28 394 identified traits, the Phylum model was capable of successfully classifying 81.14% of
29 395 individual taxa. The Family model was capable of successfully classifying 71.43% of
30 396 individual taxa. Confusion matrices for both models are presented as Supplementary
31 397 Tables 2 – 4, and show that classification was particularly difficult for Chloroflexi and
32 398 Planctomycetes (classification error $> 80\%$) in the Phylum model and for
33 399 Cellulomonadaceae and the divergent Acidobacteria Lineage (classification error $>$
34 400 80%) in the Family model. Random Forest models were robust against variation in P
35 401 within Families, for example the nine families with all taxa perfectly classified ranged
36 402 in P from the lowest (0.68) to highest (0.9).

37 403 The important traits in classifying the taxonomic groups were involved in: a)
38 404 metabolism and nutrient acquisition (oxidative phosphorylation, tricarboxylic acid
39 405 (TCA) cycle, glyoxylate/decarboxylate, thermogenesis, propanoate, starch/sucrose,
40 406 nitrogen, methane metabolism, synthesis of antioxidants such as glutathione, ATP-
41 407 binding cassette (ABC) transporters, sugar uptake via phosphotransferase systems
42 408 (PTS)); b) responding to environmental cues and stressors (protein kinases, two-

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3 409 component systems, transcription factors, proteasome, protein chaperones, RNA
4 410 transport, chromosome repair via non-homologous DNA end joining); c) core cell
5 411 physiology (flagella assembly, chemotaxis, sporulation, lipopolysaccharide (LPS),
6 412 peptidoglycan, glycerolipid, sphingolipid and lipoarabinomannan (LAM) biosynthesis);
7 413 and d) cell-cell interactions (beta-Lactam resistance, general secretion systems and
8 414 Type IV secretion systems). Box and whisker plots of discrete counts of identified traits,
9 415 and LSD results, are provided as Supplementary Figures 4 – 7. The Families
10 416 significantly enriched and depleted in these traits are listed in Table 1. The Phyla
11 417 significantly enriched and depleted in identified traits are listed in Supplementary Table
12 418 9.

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15 419 To better identify the more subtle differences between Families in the
16 420 Proteobacteria, Actinobacteria, Firmicutes and the 'Under-represented' Phyla,
17 421 individual Random Forest models were constructed for each of the four groups. The
18 422 successful classification rates were 72.31, 74.29, 76 and 72%, respectively. Confusion
19 423 matrices for each model are presented as Supplementary Tables 5 – 8. The models
20 424 were unable to reliably classify Bradyrhizobiaceae and the divergent Acidobacteria
21 425 Lineage (classification error > 80%).

22 426 Supplementary Figure 8 shows the most important traits in classifying the four
23 427 groups. Unique traits not identified in the prior Phylum and Family models were: a) for
24 428 Proteobacteria, glycosyltransferases, butanoate metabolism, aminotransferases,
25 429 ribosome biogenesis, mRNA biogenesis and degradation; b) for Actinobacteria,
26 430 porphyrin and chlorophyll synthesis, pyruvate metabolism, aminotransferases, fatty
27 431 acid and aliphatic hydrocarbon metabolism, polyketide and Type II polyketide
28 432 biosynthesis, antimicrobial resistance genes; c) for Firmicutes, lysine, folate and varied
29 433 amino acid synthesis, porphyrin and chlorophyll synthesis, DNA replication, bacterial
30 434 toxins, penicillin and cephalosporin synthesis; and d) for the 'Under-represented' taxa,
31 435 glycosyltransferases, peptidases and inhibitors, photosynthesis and AMP-activated
32 436 protein kinases. Box and whisker plots of discrete counts of identified traits, and LSD
33 437 results, are provided as Supplementary Figures 9 – 12. Tables summarising Families
34 438 enriched and depleted in these traits are included as Supplementary Tables 10 and
35 439 11.

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38 442 **3.3 Hierarchical clustering of defining traits**

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3 443 Hierarchical clustering based on 60 traits, identified from Random Forest in this study
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5 444 and by previous copiotroph-oligotroph studies, indicated five general clades. The
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7 445 dendrogram on the y axis of Figure 4 shows clustering of taxa as these five clades.
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9 446 The dendrogram on the x axis shows clustering of co-occurring traits. Clade I consisted
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11 447 of Proteobacteria, specifically the Pseudomonadaceae, Burkholderiaceae,
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13 448 Rhodospirillaceae, Bradyrhizobiaceae and Rhizobiaceae. These Families were
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15 449 uniquely enriched in flagellar assembly, chemotaxis, pyruvate metabolism, glutathione
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17 450 metabolism, ABC transporters, benzoate metabolism, transcription factors, glyoxylate
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19 451 and fatty acid metabolism. Clade II, also Proteobacteria, included Nitrosomonadaceae,
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21 452 Neisseriales Lineage, Methylocystaceae, Beijerinckiaceae, Methylococcaceae and
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23 453 Moraxellaceae. These Families clustered based on being enriched in Clade I traits, but
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25 454 to a lesser degree than the Pseudomonadaceae, Burkholderiaceae,
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27 455 Rhodospirillaceae, Bradyrhizobiaceae and Rhizobiaceae. Exceptions included the
28
29 456 absence of benzoate metabolism and enrichment of methane metabolism in several
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31 457 Clade II Families.

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33 458 Clade III, a diverse collection of Bacteroidetes (Chitinophagaceae, Cytophagaceae),
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35 459 Verrucomicrobia, Planctomycetes, divergent Acidobacteria Lineage and the
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37 460 Deltaproteobacteria (Polyangiaceae, Myxococcaceae), shared enrichment of
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39 461 sphingolipid metabolism, beta-Lactam resistance, penicillin and cephalosporin
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41 462 biosynthesis, LPS biosynthesis, glycosyltransferases and starch/sucrose metabolism.
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43 463 Many of these Families shared Clade I and II traits, including Type IV secretion system,
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45 464 oxidative phosphorylation, TCA cycle, PTS, nitrogen and glycerophospholipid
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47 465 metabolism. The absence of glutathione in non-Deltaproteobacterial Clade III Families
48
49 466 was notable.

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51 467 The three Actinobacteria Families in Clade IV, Mycobacteriaceae, Frankiaceae and
52
53 468 Streptomycetaceae, were highly similar to each other because they were enriched in
54
55 469 Type II polyketide biosynthesis. They also shared some Clade I traits (ABC
56
57 470 transporters, transcription factors, pyruvate, benzoate and fatty acid metabolism) and
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59 471 Clade III traits (membrane trafficking, transcription machinery, polyketide biosynthesis
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472 and starch/sucrose metabolism). Similar to Clade III, these Actinobacteria were also
473 depleted in glutathione traits.

474 Finally, taxa within Clade V were similar to each other due to being depleted in traits
475 shared among the other clades. Cyanobacteria were the only taxa that possessed
476 photosynthesis traits. Lactic acid bacteria (Lactobacillaceae and Leuconostocaceae)

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3 477 were enriched in Unclassified nucleotide metabolism. The Archaea (Thaumarchaeota
4 and Methanogen Lineage) shared eukaryote-like traits, proteasome, basal
5 478 and Methanogen Lineage) shared eukaryote-like traits, proteasome, basal
6 479 transcription factors and RNA transport. The Archaea were enriched in carbon fixation
7 480 traits. Methanogens were also enriched in methane metabolism. Ammonia oxidising
8 481 Thaumarchaeota were not enriched in nitrogen metabolism, however they were
9 482 enriched in traits annotated by KEGG as Global maps only (unclassified metabolism).
10 483 Further analysis found this to be the *fpr* gene, encoding a ferredoxin-flavodoxin NADP⁺
11 484 reductase (K00528). Non-lactic acid bacteria of the Firmicutes (Bacillaceae,
12 485 Sporomusaceae and Clostridiaceae) were enriched in sporulation and motility traits.
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22 488 4. Discussion

23 489 4.1 Non-random trait clustering demonstrates ecological coherence of taxa

24 490 A trait-based approach to investigate taxonomic relationships and potential biological
25 491 function was carried out with a collection of 175 terrestrial prokaryotes. We
26 492 hypothesised that traits would be non-randomly distributed amongst taxonomic groups,
27 493 supported by previous observations that noted closely related taxa are isolated from
28 494 similar habitats (Philippot *et al.*, 2010). Similarity in the composition of 220 664 traits,
29 495 within 175 taxa, demonstrated strong agreement with established taxonomy at high
30 496 (Phyla) and low (Family) rank (Figure 2a). The exceptions to this ecological coherence
31 497 at high rank were the division of the Chloroflexi and an individual from each of the
32 498 Planctomycetes, Deltaproteobacteria, Actinobacteria and Firmicutes clustering with
33 499 unrelated Phyla. These taxa were not mischaracterised, as based on phylogenetics of
34 500 the full length 16S rRNA gene (Supplementary Figure 2a). Trait similarity between
35 501 related taxa, measured as *C*, tended to be highest at low rank (Figure 2b). Uneven
36 502 sample sizes between groups within Super Group and Phyla make comparisons at this
37 503 level difficult – the inclusion of many diverse Firmicutes and Actinobacteria likely drove
38 504 *C* to be lower here than in Thaumarchaeota and Euryarchaeota. However, equal
39 505 comparisons at the Family level demonstrated interesting variability in coherence. All
40 506 Proteobacterial Families had relatively high coherence ($C = 0.4 - 0.6$). The high *C* in
41 507 Beijerinckiaceae is of particular interest as this group contained both specialist
42 508 methanotroph (*Methylocapsa*, *Methylocella*), methylotroph (*Methyloferula*) and
43 509 generalist heterotroph (*Beijerinckia* spp.) taxa. With such varied metabolic traits, one
44 510 could reasonably expect *C* to be relatively low within this Family. The Beijerinckiaceae

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3 511 appear to have evolved from a common methylo-trophic ancestor and still share traits
4 512 for nitrogen fixation and tolerance for low pH soils (Tamas *et al.*, 2014), and the high
5 513 C measured here indicates that many additional shared traits remain. Both the
6 514 relatively recent divergence of Families from a common ancestor and the higher
7 515 number of shared traits are likely causes of the higher C observed at low taxonomic
8 516 rank. The differing values of C for Methanogen and photosynthetic Cyanobacteria
9 517 functional groups (0.33 and 0.44, respectively) is also worthy of note. Despite all five
10 518 taxa in each group performing the same core role in a community, the individual
11 519 isolates came from varying environments. The methanogens were isolated from a
12 520 range of geographically separate wetlands, rice paddy soil and farm slurry and, while
13 521 the Cyanobacteria were also isolated from geographically separate environments, they
14 522 were all from sandy deserts or other nutrient poor, arid soils (Supplementary Table 1
15 523 and references therein). Ultimately a taxon's trait composition will be affected by its
16 524 functional role in a community, its evolutionary life-history (e.g. Beijerinckiaceae
17 525 described above) and its local environment.

18 526 However, these results are dependent on accurate taxonomic classification, and the
19 527 C of Sporomusaceae, relatively low compared to other Families here, supports splitting
20 528 this group into Sporomusaceae, Anaeromusaceae and Pelosinaceae by GTDB (Parks
21 529 *et al.*, 2018). Finally worth noting, some groups at high rank were considered as
22 530 'Families' here due to the number of available terrestrial genomes, e.g. Cyanobacteria
23 531 and Chloroflexi. Even so, Cyanobacteria demonstrated a higher C than many
24 532 taxonomically-defined Families, perhaps due to their common role as primary
25 533 colonisers of nutrient-poor soils (Garcia-Pichel *et al.*, 2001). The number of Families
26 534 are too numerous to discuss each at length here, but C was an effective means of
27 535 measuring and comparing coherence between groups in the UPGMA tree.

28 536 While the method of comparing taxa here differs from other studies, the results were
29 537 not surprising as many 16S rRNA gene surveys of terrestrial systems consistently
30 538 demonstrate ecological coherence at high rank. For example, independent studies of
31 539 increasing agricultural intensity in soils show reductions in Actinobacteria abundance
32 540 (Philippot *et al.*, 2009, Jangid *et al.*, 2011). Nitrogen addition to soils frequently enriches
33 541 numerous taxa within the Actinobacteria and Proteobacterial Classes while negatively
34 542 affecting taxa within the Verrucomicrobia and Planctomycetes (Wessen *et al.*, 2010,
35 543 Fierer *et al.*, 2012, Leff *et al.*, 2015, Bastida *et al.*, 2016). Arid, nutrient poor
36 544 environments select for Actinobacteria-dominated communities (Cary *et al.*, 2010,

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3 545 Crits-Christoph *et al.*, 2013) and, in the absence of other primary producers, allow
4 546 biological soil crust forming Cyanobacterial taxa to establish (Garcia-Pichel *et al.*,
5 547 2001). Anoxic wetland and rice paddy environments support diverse communities of
6 548 anaerobic Firmicutes, Chloroflexi and methanogenic Archaea (He *et al.*, 2019, Finn *et*
7 549 *al.*, 2020b). These trends were noted prior to bioinformatic advances of metagenome
8 550 assembled genomes (MAGs) that allow for the specific comparison of individual traits
9 551 between uncultured environmental prokaryote genomes (Hug *et al.*, 2013). The
10 552 generation of MAGs has emerged as a useful tool for identifying traits necessary for
11 553 life in such environments, and particularly for expanding knowledge of severely under-
12 554 represented, difficult to culture taxonomic groups. For example, the recent
13 555 reconstruction of 52 515 MAGs from a wide range of host-associated and
14 556 environmental metagenomes was able to increase genomic information of
15 557 Planctomycetes and Verrucomicrobia by 79% and 68%, respectively (Nayfach *et al.*,
16 558 2021). Importantly, both 16S rRNA gene surveys and MAGs demonstrate that some
17 559 functional traits that facilitate life under certain environmental conditions are
18 560 intrinsically linked to taxonomy.

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31 561 The ecological coherence observed in Figure 2 does not imply that closely related
32 562 taxa (*e.g. Bacillus velezensis* LS69 and *Bacillus amyloliquefaciens plantarum* FZB42)
33 563 have identical phenotypes. Close relatives possess a combination of core and
34 564 accessory genes (traits) and the presence of even a single accessory gene is sufficient
35 565 to dramatically alter a strain's phenotype (van Rossum *et al.*, 2020). Rather, our results
36 566 (Figure 2) demonstrate that the composition of core and accessory traits in
37 567 alphaproteobacterial Beijerinckiaceae are most similar to each other, relative to
38 568 alphaproteobacterial Rhizobiaceae or to Actinobacteria, Firmicutes etc.

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571 4.2 What can the Random Forests tell us?

572 The Random Forest works by identifying the traits that are most reliable in classifying
573 individual Phyla and Families. It selects traits that tend to be: a) of equal copy number
574 per genome within a taxonomic group; and b) that differ markedly in copy number
575 between taxonomic groups, since distinct separation of copies will maximise
576 successful classification. A clear example of this is the consistent identification of
577 eukaryote-like basal transcription factors, proteasome and RNA transport present in
578 the Thaumarchaeota and Euryarchaeota, since they are absent from the majority of

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3 579 Bacterial Phyla. The binary nature of these traits (yes Archaea, no Bacteria) make
4
5 580 them strong indicators. The presence of these and more eukaryote-like vesicular
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7 581 trafficking and actin traits have been noted in the Archaeal TACK super-phylum
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9 582 previously, and lend credence to the hypothesis that eukaryotes are descended from
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11 583 Archaea (Embley & Martin, 2006, Spang *et al.*, 2015). However, the Random Forest
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13 584 will not identify a trait unique to *Can. Nitrosotalea devanterra* that is absent from other
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15 585 Thaumarchaeota, as this single trait will not improve classification of the group as a
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17 586 whole. Consequently, the traits identified via Random Forest all tended to be core,
18
19 587 fundamental traits shared by other members of a taxon's Phylum/Family.

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21 588 Many of the best traits for distinguishing taxa have been historically used by
22
23 589 microbiologists to do exactly that. These included fundamental cell physiology traits,
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25 590 such as oxidative phosphorylation, LPS biosynthesis, sporulation, flagellar assembly
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27 591 and chemotaxis. The Phylum model separated Betaproteobacteria, Actinobacteria and
28
29 592 Bacteroidetes as taxa with the highest copies of oxidative phosphorylation traits.
30
31 593 Firmicutes, Chloroflexi and Methanogens were identified as anaerobes depleted in
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33 594 oxidative phosphorylation, and all other taxa as sitting in between (Supplementary
34
35 595 Figure 4 and Supplementary Table 9). Some Gram-negative Families were
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37 596 significantly enriched in LPS biosynthesis compared to others. These were the
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39 597 Pseudomonadaceae, Burkholderiaceae, Chitinophagaceae, divergent Acidobacteria
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41 598 and Verrucomicrobia (Table 1). This has been noted in soil communities previously.
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43 599 The extensive repertoire of LPS-associated genes in Chitinophagaceae, Acidobacteria
44
45 600 and Verrucomicrobia likely play a critical role in enhancing soil aggregation (Cania *et*
46
47 601 *al.*, 2019) potentially through high LPS production and/or biofilm formation (discussed
48
49 602 further below). In a demonstration of the robustness of the methods used here, the
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51 603 highly unusual Firmicute Sporomusaceae were shown to possess similar counts of
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53 604 LPS biosynthesis traits relative to most Gram-negative Families (Supplementary Table
54
55 605 4) in addition to sharing heat-resistant spore formation with Bacillaceae and
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57 606 Clostridiaceae (Table 1). The presence of both traits in a single Family have been
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59 607 remarked upon previously and used to conceptualise the evolution of Gram-negative
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61 608 *versus* Gram-positive lineages (Stackebrandt *et al.*, 1985). The Sporomusaceae were
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63 609 also shown to have high Porphyrin and Chlorophyll metabolism traits in the Firmicutes
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65 610 model (Supplementary Table 10). The capacity to dechlorinate the soil pollutant
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67 611 perchloroethene to trichloroethylene via a porphyrin-based corrinoid is yet another
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69 612 interesting trait of this Family (Terzenbach & Blaut, 1994).

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3 613 Finally, flagella assembly and chemotaxis traits identified Alphaproteobacteria,
4 614 Betaproteobacteria and Acidobacteria as Phyla that were particularly enriched with this
5 615 mechanism of motility, while bacterial Actinobacteria, Bacteroidetes, Cyanobacteria,
6 616 Chloroflexi and Verrucomicrobia were depleted (Supplementary Table 9). The
7 617 Proteobacteria, Firmicutes and Under-represented models were better suited for
8 618 identifying specific Families homogenously enriched or depleted in bacterial flagella
9 619 and chemotaxis (Supplementary Tables 10 and 11). Enriched Families included the
10 620 Rhodospirillaceae, Nitrosomonadaceae, Neisseriales lineage, divergent Acidobacteria
11 621 lineage, Sporomusaceae, Bacillaceae, Clostridiaceae and Planctomycetes. Other
12 622 forms of motility such as twitching and gliding have been noted in the
13 623 Pseudomonadaceae, Myxococcaceae and Cyanobacteria (McBride, 2001) but these
14 624 traits were not identified by the Random Forest as being homogenously enriched in
15 625 any Families. Furthermore, while Thaumarchaeota and Methanogens were both
16 626 identified as being depleted in bacterial flagella assembly and chemotaxis traits
17 627 (Supplementary Table 9 and 11), Archaea possess a structurally distinct flagellum
18 628 more similar to the Type IV bacterial pilus (Jarrell and Albers, 2012). These taxa were
19 629 not enriched with Type IV pilus, either, and it is possible that archaeal flagella may
20 630 have failed proper characterisation by KEGG. Thus, while some Families were
21 631 relatively enriched/depleted in bacterial flagella and chemotaxis traits, specific taxa
22 632 depleted in these are not necessarily non-motile.

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37 633 In summary, while the Random Forests may overlook certain traits in individual taxa,
38 634 the models were highly robust in detecting conserved, shared traits within a
39 635 Phylum/Family. Here the 'depth' of shared traits is limited by the number of taxa that
40 636 could be considered as Phylum or Family. In future, if five (or more) taxa belonging to
41 637 the same Genus or even Species could be compared, unique traits would be observed
42 638 to explain how these subgroups have evolved from their respective Families to occupy
43 639 distinct niches. Ideally the selection of individual taxa within groups for such future
44 640 comparative analyses would also be standardised based on phylogenetic distance,
45 641 either with *P* or a similar method, that would improve the robustness of trait-based
46 642 comparisons at such a fine taxonomic level.

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49 645 *4.3 Plant-derived carbon metabolism and nutrient acquisition*

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3 646 Secondly, we hypothesised that the traits differentially enriched between taxonomic
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5 647 groups would largely reflect those associated with copiotrophs or oligotrophs, namely
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7 648 metabolism, nutrient acquisition and environmental stress response and tolerance. Of
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9 649 fundamental interest to soil microbiologists is the decomposition of plant biomass. This
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11 650 is the primary source of organic carbon to non-arid terrestrial systems (Kögel-Knabner,
12
13 651 2002) and the transformation of plant material to substrates bioavailable for
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15 652 microorganisms is essential for community growth and activity. The traits involved in
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17 653 plant material catabolism belonged to the BRITE categories 'Starch and Sucrose
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19 654 Metabolism' (e.g. extracellular cellobiosidases, endoglucanases, glucosidases,
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21 655 trehalases, amylases) and 'Glycosyltransferases', all of which are carbohydrate
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23 656 activated enzymes (CAZy). The Families particularly enriched in these traits were the
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25 657 Polyangiaceae, Myxococcaceae, Rhizobiaceae, Streptomycetaceae,
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27 658 Mycobacteriaceae, Frankiaceae and Verrucomicrobia (Table 1, Supplementary Table
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29 659 11). Genomic and culture-dependent analyses support *Sorangium cellulosum*
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31 660 (Polyangiaceae), *Streptomyces coelicolor* A3(2) (Streptomycetaceae) and
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33 661 *Chthoniobacter flavus* (Verrucomicrobia) as having particularly large genomes with
34
35 662 extensive repertoires for cellulose, hemicellulose, pectin and lignin degradation
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37 663 (Bentley *et al.*, 2002, Sangwan *et al.*, 2004, Schneiker *et al.*, 2007). Comparative
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39 664 genomics analyses have also identified Actinobacteria, Acidobacteria and
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41 665 Verrucomicrobia as being enriched in numerous enzymes for cellulose, hemicellulose
42
43 666 and starch catabolism (Trivedi *et al.*, 2013). *In situ* these Families likely play a critical
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45 667 role in making organic carbon bioavailable as di- and monosaccharides for the
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47 668 microbial community.

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49 669 The Families equipped with many copies of high-affinity sugar uptake
50
51 670 'Phosphotransferase systems' (3 – 14 copies) did not necessarily correspond to those
52
53 671 enriched with CAZy – only the Myxococcaceae and Verrucomicrobia were enriched in
54
55 672 both. Pseudomonadaceae, Rhodospirillaceae, Neisseriales lineage and
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57 673 Clostridiaceae were only enriched in PTS. Despite being enriched in CAZy, the
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59 674 Frankiaceae were simultaneously depleted in PTS. The complex interplay between
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675 taxa capable of producing extracellular CAZy and competitors that rapidly scavenge
676 available di- and monosaccharides has been well described by models that predict
677 such competitive interactions exert important controls on the growth rate of the
678 community as a whole (Freilich *et al.*, 2011) and may even act to aid terrestrial carbon
679 storage and limit carbon dioxide emissions from microbial respiration (Kaiser *et al.*,

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2
3 680 2015). Here, we identified the ‘specialist’ Families enriched in CAZy traits *versus* the
4
5 681 ‘opportunists’ scavenging for sugars via PTS (Table 1, Supplementary Table 11).

6 682 ABC transporters facilitate the ATP-dependent uptake of soluble compounds across
7
8 683 membranes or export waste metabolites, extracellular enzymes and toxins (Young &
9
10 684 Holland, 1999, Higgins, 2001). This means of active transport allows microorganisms
11
12 685 to acquire nutrients with high affinity at concentrations of 5 – 500 $\mu\text{g carbon L}^{-1}$ *versus*
13
14 686 the less efficient diffusion of nutrients across membranes, dependent on extracellular
15
16 687 concentrations of 0.5 – 5 mg carbon L^{-1} (Kuznetsov *et al.*, 1979). In the spatially
17
18 688 heterogenous soil environment where the concentration of bioavailable carbon
19
20 689 substrate often limits growth (Blagodatsky & Richter, 1998), possession of high affinity
21
22 690 transporters likely provides a competitive advantage. The rhizosphere-associated
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24 691 Rhodospirillaceae, Rhizobiaceae and Burkholderiaceae tended to have the highest
25
26 692 trait copies of ABC transporters (100 – 350 copies per genome, Supplementary Figure
27
28 693 4). The diverse, non-rhizospheric Deltaproteobacteria, Actinobacteria, Firmicutes,
29
30 694 Cyanobacteria and Verrucomicrobia all had greater than 50 copies per genome,
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32 695 highlighting the importance of these traits in soil. The particularly high gene copy
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34 696 number in rhizosphere-associated taxa from presumably nutrient-rich environments
35
36 697 contrasts the assumption that ABC transporters are considered to play a greater role
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38 700 in nutrient-poor environments (Lauro *et al.*, 2009). Comparative genomics analyses of
39
40 701 soil bacteria have also found putatively copiotrophic Proteobacteria and Firmicutes to
41
42 702 be particularly enriched in PTS and ABC transporters (Trivedi *et al.*, 2013). In this
43
44 703 study, the transporters enriched in rhizosphere-associated taxa were primarily aimed
45
46 704 at scavenging maltose, phosphate, amino acids, oligopeptides and export of LPS, and
47
48 705 these results suggest that these traits are not only for survival in nutrient-poor
49
50 706 environments but also likely confer a competitive advantage in the rhizosphere. As
51
52 707 prokaryotes compete simultaneously with other prokaryotes and plants for nitrogen
53
54 708 and phosphorus in the rhizosphere, the high affinity acquisition of such nutrients is
55
56 709 likely critical for growth.

57 710 *4.4 Nitrogen and methane metabolism*

58 711 The BRITE category ‘Nitrogen metabolism’ encompasses nitrogen fixation,
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60 712 denitrification, ammonia oxidation and synthesis of glutamate/glutamine which are
713 critical amino acids for peptide synthesis. Since nitrogen limitation acts as an important

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3 714 control on soil microbial activity, these traits are also of interest to soil microbiologists.
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5 715 Three Proteobacterial Families, Rhodospirillaceae, Bradyrhizobiaceae and
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7 716 Burkholderiaceae, were particularly enriched in these traits. Genomic and culture-
8
9 717 dependent analyses show these Families to be free-living or symbiotic diazotrophs in
10
11 718 soil and freshwater environments (Madigan *et al.*, 1984, Itakura *et al.*, 2009, de los
12
13 719 Santos *et al.*, 2018). Given their significantly greater copies of nitrogen-fixing genes,
14
15 720 these Families may be a particularly important source of organic nitrogen for soil
16
17 721 communities. Saprotrophic Mycobacteriaceae genomes, also identified as nitrogen
18
19 722 cyclers, tend to have many copies of genes involved in ammonia uptake and glutamate
20
21 723 synthesis (Amon *et al.*, 2010). This taxon may play an alternative role in converting
22
23 724 mineral nitrogen to biomass where organic nitrogen as protein in excreted products or
24
25 725 necromass can undergo proteolysis and uptake between other community members.
26
27 726 The identification of Sporomusaceae as enriched in 'Nitrogen metabolism' traits is
28
29 727 unusual as these obligate anaerobic fermenters cannot use nitrate as an electron
30
31 728 acceptor (Möller *et al.*, 1984). Nor were the Sporomusaceae enriched in ammonia
32
33 729 uptake or glutamate synthesis genes (data not shown), and so it is uncertain what role
34
35 730 this Family plays in nitrogen cycling. Thaumarchaeota and Nitrosomonadaceae, known
36
37 731 ammonia oxidisers, were not enriched in 'Nitrogen metabolism' traits relative to other
38
39 732 Families (Supplementary Figure 5) despite Nitrosomonadaceae possessing multiple
40
41 733 copies of the operon responsible for ammonia oxidation (Klotz & Norton, 1998).
42
43 734 Specific traits may be overlooked here if the BRITE category includes many diverse
44
45 735 KOs (e.g. ammonia oxidation, nitrogen fixation, glutamate synthesis etc).

46
47 736 Another specialised metabolic pathway of interest involves 'Methane metabolism'
48
49 737 that includes production and oxidation of a potent greenhouse gas. Unsurprisingly, the
50
51 738 Methanogens and methanotrophic Methylococcaceae, Beijerinckiaceae,
52
53 739 Methylocystaceae were all enriched in traits involved in methane metabolism. While
54
55 740 methane oxidation can be present in some taxa from the Verrucomicrobia (Op den
56
57 741 Camp *et al.*, 2009) the above proteobacterial representatives act as the primary
58
59 742 terrestrial methane sink (Dunfield, 2007, Conrad, 2009).

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745 *4.5 Sensing, responding and tolerating the environment*

746 A particularly interesting divergence of traits were involved in how taxa detect and
747 respond to environmental stimuli. Gram-negative Pseudomonadaceae,

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3 748 Rhodospirillaceae, Bradyrhizobiaceae and Myxococcaceae were enriched in two-
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5 749 component systems. These membrane-bound histidine kinases respond rapidly to
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7 750 extracellular stimuli (Galperin, 2005) and these traits were primarily involved in
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9 751 nitrogen, potassium, initiating chemotaxis and C₄-dicarboxylate responses. Families
10
11 752 enriched in transcription factors were the Myxococcaceae, Polyangiaceae,
12
13 753 Streptomycetaceae, Mycobacteriaceae and Frankiaceae. These factors regulate
14
15 754 transcription in response to intracellular cues and here these factors were primarily
16
17 755 *rpoD* (housekeeping), *rpoH* (heat-shock/protein damage), *rpoE* (extra-cellular
18
19 756 cytoplasmic stress) and *rpoS* (starvation) responses (Shimada *et al.*, 2017). The
20
21 757 genomes of these taxa are also heavily enriched in regulatory genes for complex
22
23 758 developmental stages, fruiting bodies and/or filamentous branching growth in soils
24
25 759 (Bentley *et al.*, 2002, Gao *et al.*, 2006, Schneiker *et al.*, 2007). Thus, certain taxa may
26
27 760 respond primarily to extracellular cues while others strictly monitor and respond to
28
29 761 changes in cell homeostasis. This trend has been noted previously – in 167 genomes
30
31 762 across various Bacteria and Archaea, Proteobacteria had a higher ratio of sensors for
32
33 763 external *versus* internal stimuli and were considered ‘extroverts’, while Cyanobacteria
34
35 764 were considered strong ‘introverts’ focussed on responding to internal stimuli
36
37 765 (Galperin, 2005).

34 766 As mentioned above, Archaea exhibited unique traits in basal transcription and
35
36 767 protein regulation via proteasome. These transcription factors were primarily involved
37
38 768 in identifying DNA damage and excision repair: TFII-B, TFII-D, ERCC-2 and ERCC-3.
39
40 769 DNA repair differs markedly between Bacteria and Archaea/eukaryotes. Specifically,
41
42 770 Bacteria excise 12 nucleotides around a damaged site with a 3 polypeptide system
43
44 771 whereas Archaea excise 24 – 32 nucleotides with a 13 – 16 polypeptide system
45
46 772 (Sancar, 1996). The use of ubiquitin-labelling and proteasome degradation of
47
48 773 misfolded or ‘old’ proteins is arguably a more efficient system for recycling amino acids
49
50 774 and regulating the ‘lifespan’ of a protein in eukaryotes, however, Bacteria are still fully
51
52 775 capable of regulating protein misfolding or proteolysis with RpoH (and others) induced
53
54 776 upon environmental stress (Goldberg, 2003). From an ecological perspective, it is
55
56 777 difficult to discern if these eukaryote-like traits confer any sort of competitive advantage
57
58 778 to Archaea. They may simply be examples of convergent evolution for dealing with
59
60 779 environmental stress.

58 780 Finally, most microbial cells likely exist within complex biofilms and/or assemblages
59
60 781 adhered to surfaces with excreted exopolysaccharides, DNA and protein that serve to

1
2
3 782 protect from adverse environmental factors (Flemming & Wingender, 2010). Families
4
5 783 with high copy numbers of exopolysaccharide biosynthesis and secretion systems may
6
7 784 act as integral members of soil communities by predominantly contributing to
8
9 785 biofilm/aggregate formation. The 'LPS biosynthesis' and 'Starch and sucrose
10
11 786 metabolism' BRITE categories can synthesise N-acetyl glucosamine-based and
12
13 787 cellulose-based exopolysaccharides, respectively. Taxa enriched in both these
14
15 788 categories and secretion systems were the Polyangiaceae and Burkholderiaceae
16
17 789 (Table 1), and in the refined 'Under-represented' model, Acidobacteria and
18
19 790 Verrucomicrobia (Supplementary Table 11).
20
21 791
22 792

22 793 4.6 Direct cell-cell interactions

23
24 794 Type IV secretion systems were another important trait identified in the Random Forest
25
26 795 models. These were enriched in Pseudomonadaceae and Myxococcaceae (Table 1)
27
28 796 and Acidobacteria, Planctomycetes and Verrucomicrobia (Supplementary Table 11).
29
30 797 These are highly specialised exporters that deliver DNA and/or toxins directly to other
31
32 798 bacterial or plant cells, however, their role in ecology is poorly understood beyond root
33
34 800 galls induced by *Agrobacterium tumefaciens* (Christie & Vogel, 2000). These taxa
35
36 801 should be explored for whether they utilise these traits for horizontal gene transfer or
37
38 802 to inject toxins directly into other prokaryotes, and thus potentially provide a selective
39
40 803 advantage for colonisation and competition.

41 804 Another archetypal trait for interactions between community members are
42
43 805 production of antimicrobials and antimicrobial resistance genes. Penicillin and
44
45 806 cephalosporin synthesis were enriched in the Sporomusaceae and Bacillaceae relative
46
47 807 to other Firmicutes. Polyketide and Type II polyketide syntheses were important for
48
49 808 separating Frankiaceae and Streptomycetaceae from other Actinobacteria
50
51 809 (Supplementary Table 10). The Streptomycetaceae have a long history of use in
52
53 810 biotechnology as prolific antimicrobial producers (Bentley *et al.*, 2002). Bacillaceae (in
54
55 811 particular *Bacillus subtilis* species) are also well known producers of a wide variety of
56
57 812 antimicrobials (Caulier *et al.*, 2019), but we noted that Sporomusaceae have an even
58
59 813 greater number of these traits (Supplementary Figure 9). To the authors' knowledge,
60
814 antibiotic production in Sporomusaceae has not been investigated thoroughly and this
815 may be a consequence of its obligate anaerobic nature and difficulties in culturing. In
addition to prolific Type II polyketide producers, Streptomycetaceae were also enriched

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2
3 816 in antimicrobial resistance genes, while Bacteroidetes, Planctomycetes and
4 817 Verrucomicrobia were specifically enriched in beta-Lactam resistance (Supplementary
5 818 Tables 9 and 10).

8 819

10 820

11 821 *4.7 Life strategies emerge from differentially enriched traits*

12 822 We hypothesised that taxa would emerge as being inherently copiotrophic or
13 823 oligotrophic based on trends in their enriched traits. Traits were chosen based on
14 824 identification via Random Forest and identification as associated with copiotroph-
15 825 oligotroph species or in mixed communities as described previously (Lauro *et al.*, 2009,
16 826 Vieira-Silva & Rocha, 2010, Roller & Schmidt, 2015, Pascual-Garcia & Bell, 2020).
17 827 Rhizosphere-associated Gamma-, Alpha- and Betaproteobacteria in Clade I fit the
18 828 assumptions of a copiotrophic niche that invests in high metabolic rate – these taxa
19 829 were uniquely enriched in competing for nutrient uptake via high-affinity ABC
20 830 transporters, and energy generation from pyruvate, fatty acids, benzoate and
21 831 glyoxylate carbon sources. Clade I was also enriched in glutathione metabolism, which
22 832 acts as the major antioxidant for reducing intracellular free radicals produced during
23 833 central carbon metabolism (Smirnova & Oktyabrsky, 2005). Antioxidants have been
24 834 hypothesised as an essential function for copiotrophs to survive their high metabolic
25 835 rates (Koch, 2001). All five Clade I Families were enriched in oxidative phosphorylation.
26 836 The oxidative phosphorylation traits encompass a wide variety of electron transport
27 837 chain proteins (oxidoreductases, dehydrogenases, cytochromes and ATPases) and
28 838 are crucial for efficient energy production (Brochier-Armanet *et al.*, 2009). All five
29 839 Families were also enriched in nitrogen metabolism, which included both nitrogen
30 840 fixation and glutamate (*i.e.* protein) synthesis. Nitrogen fixation is an energy intensive
31 841 process requiring 20 – 30 ATP per reduced N₂ (Burris & Roberts, 1993) and may be
32 842 intrinsically linked to taxa with high oxidative phosphorylation. Finally, Clade I also
33 843 shared motility and chemotaxis, which are also energy intensive traits. Clade II
34 844 consisted of the remaining Gamma-, Alpha- and Betaproteobacteria, yet these were
35 845 relatively less enriched in Clade I 'copiotroph' traits. These particular taxa may be
36 846 responsible for the lack of a consistent copiotrophic response upon nutrient addition in
37 847 Proteobacteria (Ho *et al.*, 2017).

38 848 Clade III was comprised of taxa generally considered as oligotrophs (Ho *et al.*, 2017)
39 849 with the exception of Bacteroidetes (Fierer *et al.*, 2007). These taxa possessed high

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3 850 LPS and sphingolipid synthesis that can defend against desiccation and antimicrobials
4
5 851 through biofilm and capsule/slime production (Flemming & Wingender, 2010), beta-
6
7 852 Lactam resistance, penicillin biosynthesis and several members had high pentose
8
9 853 phosphate pathway for efficient carbon metabolism under starvation (Hodgson, 2000).
10
11 854 Clade III also possessed high CAZy traits, which Clade I largely lacked, and is
12
13 855 consistent with observations of oligotrophs being primarily responsible for catabolising
14
15 856 relatively recalcitrant plant material (Goldfarb *et al.*, 2011). While Clade III were equally
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17 857 enriched in oxidative phosphorylation as Clade I, with the exception of the
18
19 858 Deltaproteobacteria, these taxa were depleted in glutathione metabolism. The low
20
21 859 copies per genome of this trait would explain why the abundance of oligotrophs drop
22
23 860 rapidly in nutrient addition studies as they would be either out-competed by
24
25 861 glutathione-rich taxa capable of exploiting plentiful nutrients or will lyse if their
26
27 862 metabolic rate exceeds capacity to reduce free radicals (Koch, 2001). Taken together,
28
29 863 all of these traits indicate Clade III lead oligotrophic lifestyles whereby they are tolerant
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31 864 to adverse environmental conditions, can acquire carbon from recalcitrant plant
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33 865 material, and are incapable of rapid growth rates.

34
35 866 These results support previous observations that Rhodospirillaceae,
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37 867 Bradyrhizobiaceae, Burkholderiaceae, Pseudomonadaceae and Rhizobiaceae are
38
39 868 copiotrophic, while Planctomycetes, Verrucomicrobia, Myxococcaceae,
40
41 869 Polyangiaceae and Acidobacteria are oligotrophic (Ho *et al.*, 2017 and references
42
43 870 therein). As has been proposed previously, the dominance of these groups in certain
44
45 871 soils can provide inferences for ecosystem processes in that system, for example soils
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47 872 dominated by Verrucomicrobia, Planctomycetes and Acidobacteria will have greater
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49 873 capacity to degrade complex plant material while retaining most catabolised carbon in
50
51 874 biomass (*i.e.* high growth or carbon use efficiency) or excreted byproducts that assist
52
53 875 in soil aggregation (*e.g.* high LPS production) (Trivedi *et al.*, 2013). Conversely, soils
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55 876 dominated by copiotrophic Proteobacteria Families will be systems primarily
56
57 877 dependent on labile di- and monosaccharides that demonstrate low carbon use
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59 878 efficiency.

60
61 879 Streptomycetaceae, Mycobacteriaceae and Frankiaceae in Clade IV shared
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63 880 enrichment of several Clade I copiotroph traits. As mentioned above in Section 4.5,
64
65 881 these Actinobacteria invest carbon and energy into complex filamentous growth and
66
67 882 developmental cycles. They demonstrate classic copiotrophic responses to nutrient
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69 883 addition (Goldfarb *et al.*, 2011, Leff *et al.*, 2015) and their enriched ABC transporters,

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3 884 pyruvate, glyoxylate, benzoate and fatty acid metabolism all likely contribute to
4
5 885 generating energy for complex lifecycles. Simultaneously, their enrichment of Clade III
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7 886 oligotroph traits for CAZy metabolism in addition to many traits for producing and
8
9 887 resisting antimicrobials indicate a unique niche for these Actinobacteria that does not
10
11 888 necessarily fall within the classical copiotroph-oligotroph framework.

12 889 Clade V differed markedly from all other clades and mostly consisted of 'specialist'
13
14 890 metabolic functional groups involved in photosynthesis, ammonia oxidation,
15
16 891 methanogenesis, lactic acid production and other fermentation. Similar to Clade III,
17
18 892 these taxa would also be expected to have relatively low metabolic rates due to
19
20 893 depletion of copiotroph traits associated with rapid metabolism and energy generation.
21
22 894 Unlike Clade III, these taxa seemed to lack consistent mechanisms for stress
23
24 895 tolerance. Thus, while certain taxa did invest in traits for rapid metabolic rate (Clades I
25
26 896 and IV) and others primarily in stress tolerance (Clades III and IV), some taxa lacked
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28 897 these approaches altogether and pursued entirely distinct niches (Clade V). As an
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30 898 average of 47% of traits within each genome were Uncharacterised, Clade V is an
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32 899 over-simplification and that if novel, currently uncharacterised proteins and the traits
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34 900 they fulfil were incorporated into hierarchical clustering, this clade would separate more
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36 901 meaningfully.

37
38 902 If one were to consider taxa within the one-dimensional copiotroph-oligotroph
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40 903 spectrum, Clade I would represent one extreme, Clades III and V another, with Clades
41
42 904 II and IV falling in between. Figure 5a is a conceptual diagram where these Clades
43
44 905 have been placed on a singular axis of 'resource investment', with the niche space of
45
46 906 Clades enriched in traits associated with rapid growth (e.g. glutathione) toward the
47
48 907 'copiotrophy' pole while Clades enriched in stress tolerance traits (e.g. LPS production)
49
50 908 are placed toward the 'oligotrophy' pole. However, this approach overlooks the diverse
51
52 909 functional potentials (and distinct niches) for carbon and energy metabolism
53
54 910 associated with the various Clades. Furthermore, the large overlaps in niche space
55
56 911 between Clades would suggest taxa from each group could not co-exist if 'resource
57
58 912 investment' was the only important consideration (Gause, 1932, Hutchinson, 1957,
59
60 913 Leibold, 1995). A more meaningful perspective would be to consider the additional role
61
62 914 of 'resource acquisition' that incorporates multiple axes for the life strategies identified
63
64 915 via hierarchical clustering of traits. Figure 5b is a conceptual diagram of niche space
65
66 916 where clades have been further separated along additional dimensions based on their
67
68 917 enrichment of traits involved in competition, degradation or specialised metabolic

1
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3 918 pathways. The BRITE categories listed on the various axes are chosen to be useful
4
5 919 markers in predicting the niche of a taxon. This expanded niche space would suggest
6
7 920 taxa in Clade I are well equipped for nutrient acquisition (primarily, but not limited to,
8
9 921 di- and monosaccharides), rapid growth and oxidative stress regulation as
10
11 922 'copiotrophic competitors'. Clade II, which may not be capable of competing directly
12
13 923 with Clade I for carbon and energy, may thus occupy the niche space of specialist
14
15 924 Proteobacterial methylotrophs, methane and ammonia oxidisers so as to be
16
17 925 'copiotrophic metabolic specialists'. The strategy of Clade III would be to decompose
18
19 926 plant material via diverse CAZy and possess a variety of environmental stress
20
21 927 tolerance traits as 'oligotrophic degraders'. Clade IV, which include for example
22
23 928 Actinobacteria that share traits for competition, degradation and oligotrophy, could thus
24
25 929 occupy niche space between Clades I and III. Finally, the strategy of Clade V would
26
27 930 be to fill highly specialised, unrelated metabolic niches reliant on completely distinct
28
29 931 carbon sources to other taxa. The large space conceptualised for Clade V in Figure
30
31 932 5b, which does not imply either copiotrophic or oligotrophic resource investment, is an
32
33 933 oversimplification and with improved understanding of traits in these taxa it may be
34
35 934 possible to fracture and further separate them into more detailed groups. This would
36
37 935 prove particularly beneficial for the poorly characterised Archaea.

34 936 Moving beyond a one-dimensional *r-K* spectrum to accommodate additional trait-
35
36 937 driven life strategies has been proposed in plant ecology (Grime, 1977). Specifically,
37
38 938 Grime argued that plant taxa fall within a multi-dimensional space defined by extremes
39
40 939 on three axes: 'competitors' that acquire nutrients, light, water etc. more effectively
41
42 940 than neighbouring taxa in the same environment, 'stress tolerators' that are long-lived,
43
44 941 slow growing taxa that resist desiccation, alkaline soils etc., and 'ruderals' that have
45
46 942 very brief lifecycles between periods of disturbance and invest in environmentally
47
48 943 hardy seeds. Despite these varied strategies for resource investment, plants are
49
50 944 unified in that photosynthesis is their primary form of acquiring carbon and energy. The
51
52 945 diversity of microbial strategies for acquiring carbon and energy enables them to
53
54 946 explore a greater range of potential niche space, and in addition to growth traits that
55
56 947 allow for a relatively more copiotrophic or oligotrophic investment of those resources,
57
58 948 likely contributes to the high diversity of co-existing taxa observed in soil microbial
59
60 949 communities.

58 950 However, to truly unravel differentiated niches and general microbial life strategies,
59
60 951 two limitations must be overcome. Firstly, a better understanding of the many

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2
3 952 'Uncharacterised' traits in environmental isolates is required. For example, the recent
4
5 953 large-scale MAG study by Nayfach *et al.*, (2021) identified 5.8 million protein clusters
6
7 954 (traits), of which over 75% could not be annotated meaningfully by current protein
8
9 955 databases. Secondly, robust trait-based analyses down to the finer scale of distinct
10
11 956 genomes will likely be necessary to consider how individual taxonomic members of a
12
13 957 community have either differentiated in order to co-exist or are in the throes of
14
15 958 competition that will ultimately exclude one of the competitors.

15 959

16 960

17 961 5. Conclusion

18
19 962 In a collection of 175 terrestrial prokaryotes that possess 220 664 traits shared
20
21 963 between at least two taxa, concepts in niche differentiation were explored. Non-random
22
23 964 trait distributions were shown as preferential clustering of related taxa within most
24
25 965 Phyla with a general trend of highest similarity at the level of Family. This strongly
26
27 966 supported ecological coherence of shared traits within close relatives. Random Forest
28
29 967 models successfully identified BRITE 3 categories that best explained differing traits
30
31 968 between taxonomic groups. These traits were involved in a wide range of biological
32
33 969 functions, including core physiological traits used historically to categorise taxa. Many
34
35 970 traits were also involved in functions often associated with copiotrophs and oligotrophs,
36
37 971 namely metabolism, nutrient acquisition and environmental stress tolerance.
38
39 972 Hierarchical clustering of differential traits formed five distinct clusters, with Clade I
40
41 973 representing the classical copiotrophic niche, Clades III and V as oligotrophic, and
42
43 974 Clades II and IV in between. A more refined perspective would be to consider each
44
45 975 Clade as its own life strategy in a niche space that considers both resource investment
46
47 976 and acquisition simultaneously; for example, the strategy of Clade I is to invest in
48
49 977 competition and rapid growth, while Clade V pursue highly distinct, specialised
50
51 978 metabolic functions. The trait-based analyses here were effective in identifying general
52
53 979 trends in potential function of terrestrial microbial taxa at the Phylum and Family level.
54
55 980 Further investigation will be necessary to identify traits that give rise to niche
56
57 981 differentiation at lower taxonomic ranks and, ultimately, the importance of this for
58
59 982 ecosystem processes of interest.

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57 984

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12
13 991 here; it is hoped the majority of those involved are cited within the Supplementary
14
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16
17 993 theoretical ecology performed here would not be possible.

18 994

19 995 Conflict of Interest

20 996 The authors declare no conflict of interest regarding this study or its outcomes.

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3 1 Figure Legends:
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5 2 Figure 1. Box and whisker plots of the raw counts of proteins per genome beside the
6 3 counts of shared functional traits per genome derived from MCL. Taxa $n = 5$ per
7 4 Family.
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11 6 Figure 2. a) Unweighted pair group method with arithmetic mean (UPGMA)
12 7 dendrogram comparing similarity in composition of 220 664 traits across the 175
13 8 terrestrial microbial taxa. The Phylum of each taxon is highlighted. The scale bar units
14 9 represent Bray-Curtis dissimilarity. b) Comparisons of ecological coherence (C)
15 10 between taxa belonging to the same Super Group, Phylum and Family. C , which varies
16 11 between 0 and 1, was measured from branch lengths in a). Values of C approaching
17 12 1 indicate an ecologically coherent group with a similar composition of traits. Super
18 13 groups were as follows: Proteobacteria, all Gamma-, Alpha-, Beta- and
19 14 Deltaproteobacteria; Terrabacteria, all Actinobacteria, Firmicutes, Chloroflexi and
20 15 Cyanobacteria; FCB were Bacteroidetes; Acidobacteria, all Acidobacteria; PVC,
21 16 Verrucomicrobia and Planctomycetes; TACK were Thaumarchaeota; Euryarchaeota
22 17 were Euryarchaeota.
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36 20 Figure 3. Random Forest identified traits that best explain separation of taxa groups
37 21 at Phylum and Family taxonomic rank. Mean Decrease in Accuracy (MDA) is a
38 22 measure of the average increase in classification error during permutation of trees if
39 23 the trait is not included in the model. For example, the Family Random Forest was
40 24 most accurate when ABC transporters were included in the model.
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48 27 Figure 4. Hierarchical clustering of Families based on 60 traits selected from Random
49 28 Forest and previously identified copiotroph-oligotroph traits. Families are clustered
50 29 along the y axis, and specific co-occurring traits are clustered on the x axis. Five
51 30 general Clades were identified based on Family clustering. Units are normalised
52 31 variance in mean counts of traits per Family.
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3 34 Figure 5: A conceptual diagram of overlapping life strategies between the five
4 35 functional clades identified in this study. a) the classical copiotroph-oligotroph
5 36 dichotomy whereby the five clades are placed on a singular axis of 'resource
6 37 investment', where growth strategies are either relatively more targeted toward rapid
7 38 growth (copiotroph) or toward stress tolerance (oligotroph). BRITE categories are
8 39 given as examples of functional traits contributing to either axis pole. The space
9 40 occupied by clades along this axis is dependent on enrichment or depletion of these
10 41 traits. All clades exhibit a great deal of overlap and certain clades, such as IV
11 42 (including, for example, Actinobacteria), are hard to identify as either copiotroph or
12 43 oligotroph. b) a multidimensional concept where three axes for 'resource acquisition'
13 44 are added, further separating taxa as either 'competitors', 'degraders' or 'metabolic
14 45 specialists'. Again, BRITE categories are provided as examples of traits contributing
15 46 to the additional axes and space occupied by clades is dependent on enrichment or
16 47 depletion of these traits. These extra dimensions would suggest the niche space of
17 48 clade IV exists between 'copiotrophic competitors' (I) and 'oligotrophic degraders' (III),
18 49 potentially allowing IV to co-exist alongside both.
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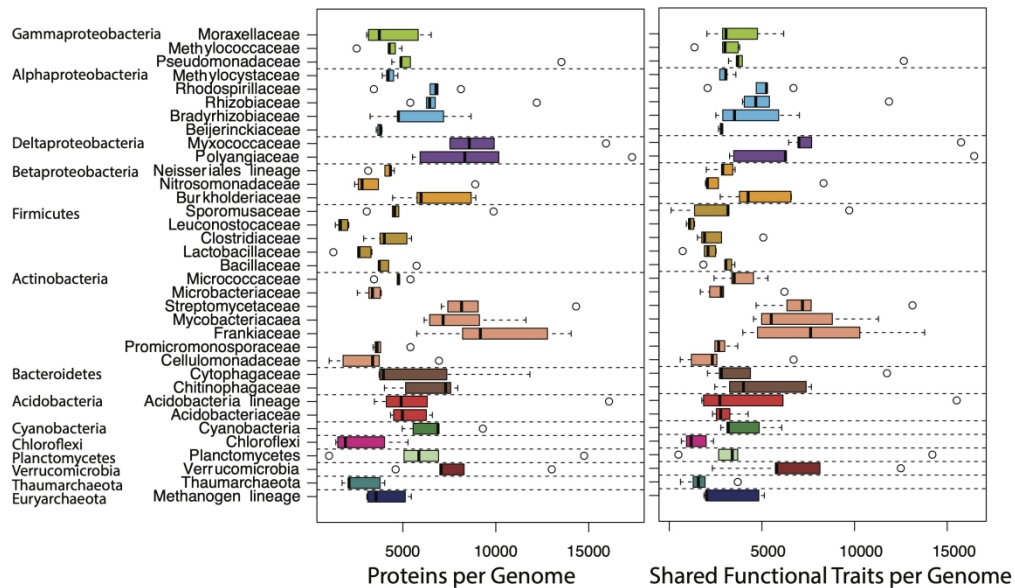


Figure 1

268x157mm (300 x 300 DPI)

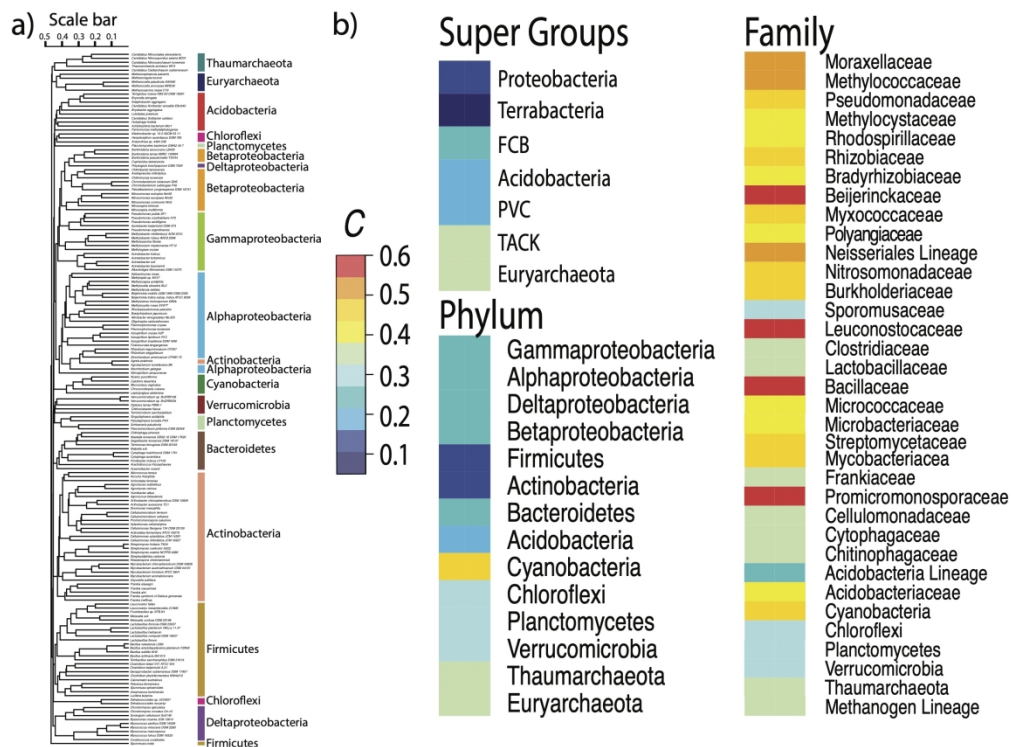


Figure 2

232x172mm (300 x 300 DPI)

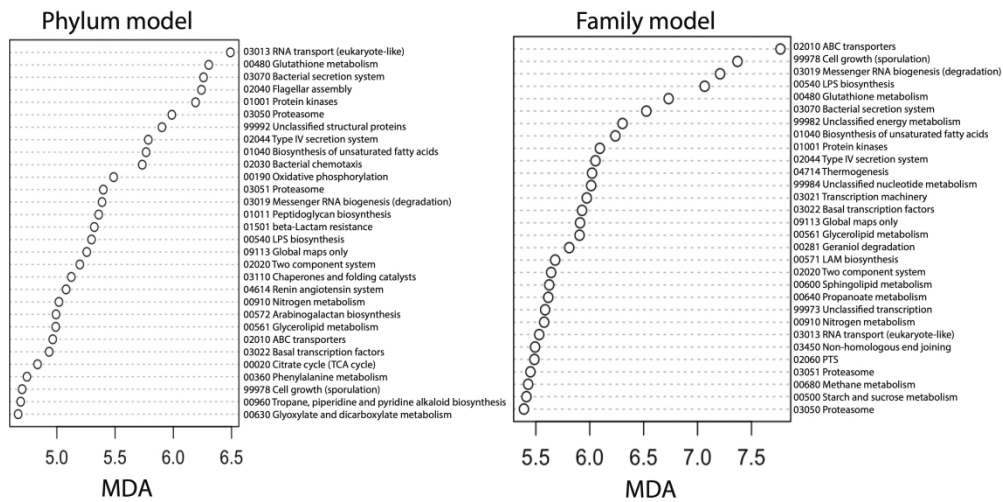


Figure 3

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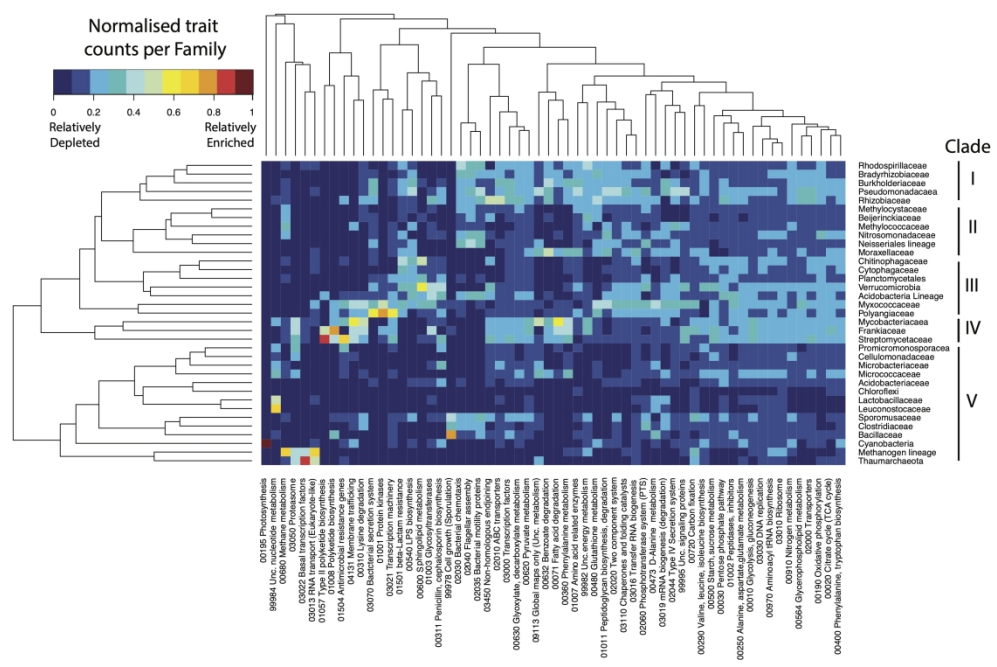


Figure 4

327x212mm (300 x 300 DPI)

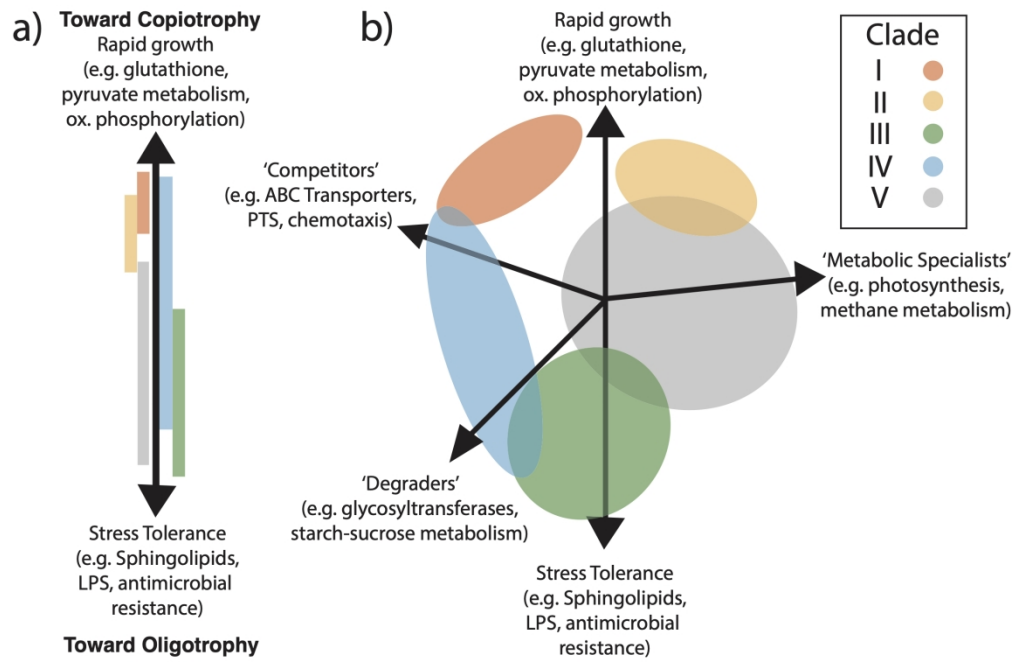


Figure 5

203x134mm (300 x 300 DPI)

Table 1: Summary of traits significantly enriched and depleted in the Family Random Forest model.

BRITE 1	BRITE 3	Significantly enriched	Significantly depleted
Metabolism	00540 LPS biosynthesis	Pseudomonadaceae, Burkholderiaceae, Chitinophagaceae, Acidobacteria Lineage, Verrucomicrobia	Leuconostocaceae, Clostridiaceae, Lactobacillaceae, Bacillaceae, all Actinobacteria, Chloroflexi, Thaumarchaeota, Methanogen Lineage
	01040 Biosynthesis of unsaturated fatty acids	Mycobacteriaceae, Frankiaceae, Pseudomonadaceae	Leuconostocaceae, Clostridiaceae, Lactobacillaceae, Bacillaceae, Cyanobacteria, Chloroflexi, Thaumarchaeota, Methanogen lineage
	00561 Glycerolipid metabolism	Myxococcaceae, Mycobacteriaceae, Frankiaceae	Thaumarchaeota, Methanogen Lineage
	00571 LAM biosynthesis	Streptomycetaceae, Mycobacteriaceae, Frankiaceae	All other Families
	00600 Sphingolipid metabolism	Myxococcaceae, Chitinophagaceae, Planctomycetaceae, Verrucomicrobia	All Gammaproteobacteria, Rhodospirillaceae, Bradyrhizobiaceae, Neisseriales Lineage, Nitrosomonadaceae, Sporomusaceae, Leuconostocaceae, Lactobacillaceae, Cellulomonadaceae, Chloroflexi, Thaumarchaeota, Methanogen Lineage
	00500 Starch and sucrose metabolism	Polyangiaceae, Streptomycetaceae, Mycobacteriaceae, Frankiaceae, Verrucomicrobia	Moraxcellaceae, Thaumarchaeota, Methanogen Lineage
	04714 Thermogenesis	Methylocystaceae, Rhodospirillaceae, Bradyrhizobiaceae, Beijerinckaceae, Mycobacteriaceae	All other Families
	00640 Propanoate metabolism	Burkholderiaceae, Mycobacteriaceae, Frankiaceae	Methylococcaceae, Leuconostocaceae, Lactobacillaceae, Thaumarchaeota
	00910 Nitrogen metabolism	Rhodospirillaceae, Bradyrhizobiaceae, Burkholderiaceae, Sporomusaceae, Mycobacteriaceae	Leuconostocaceae, Lactobacillaceae, Chloroflexi
	00680 Methane metabolism	Methylococcaceae, Methylocystaceae, Methanogen Lineage	Leuconostocaceae, Lactobacillaceae
Environmental Information Processing	99982 Unclassified energy metabolism	Rhodospirillaceae, Beijerinckiaceae, Mycobacteriaceae, Frankiaceae	Leuconostocaceae, Lactobacillaceae, Microbacteriaceae, Promicromonosporaceae, Cellulomonadaceae, Chloroflexi, Planctomycetales, Thaumarchaeota
	09113 Global maps only (unclassified metabolism)	Pseudomonadaceae, Mycobacteriaceae	Methylococcaceae, Beijerinckiaceae, Nitrosomonadaceae, Leuconostocaceae, Bacillaceae, Cytophagaceae, Acidobacteriaceae, Cyanobacteria, Verrucomicrobia
	00480 Glutathione metabolism	Rhizobiaceae, Bradyrhizobiaceae, Myxococcaceae, Polyangiaceae, Burkholderiaceae	All Firmicutes, Acidobacteriaceae, Chloroflexi, Planctomycetales, Verrucomicrobia, Thaumarchaeota, Methanogen Lineage
	00281 Geraniol degradation	Moraxellaceae, Myxococcaceae, Mycobacteriaceae	Methylococcaceae, Pseudomonadaceae, Rhizobiaceae, Beijerinckiaceae, Neisseriales Lineage, Nitrosomonadaceae, all Firmicutes, Streptomycetaceae, Promicromonosporaceae, Cellulomonadaceae, Acidobacteriaceae, Cyanobacteria, Chloroflexi, Planctomycetaceae, Verrucomicrobia, Thaumarchaeota, Methanogen Lineage
	01001 Protein kinases	Myxococcaceae, Polyangiaceae, Frankiaceae	Lactobacillaceae
	02010 ABC transporters	Rhodospirillaceae, Rhizobiaceae, Burkholderiaceae	Acidobacteriaceae, Thaumarchaeota, Methanogen Lineage
	02020 Two component system	Pseudomonadaceae, Rhodospirillaceae, Bradyrhizobiaceae, Myxococcaceae	Leuconostocaceae, Lactobacillaceae, Bacillaceae, Promicromonosporaceae, Cellulomonadaceae, Chloroflexi, Thaumarchaeota
	02060 Phosphotransferase system (PTS)	Pseudomonadaceae, Rhodospirillaceae, Myxococcaceae, Neisseriales lineage, Clostridiaceae, Verrucomicrobia	Methylococcaceae, Frankiaceae, Acidobacteria Lineage, Thaumarchaeota, Methanogen Lineage

	03070 Bacterial secretion system	Polyangiaceae, Myxococcaceae, Burkholderiaceae	Leuconostocaceae, Lactobacillaceae, Bacillaceae, Streptomyetaceae, Mycobacteriaceae, Chloroflexi, Thaumarchaeota, Methanogen lineage
	02044 Type IV secretion system	Pseudomonadaceae, Myxococcaceae	Leuconostocaceae, all Bacteroidetes, Thaumarchaeota
Genetic Information Processing	03021 Transcription machinery	Myxococcaceae, Polyangiaceae, Streptomyetaceae, Mycobacteriaceae, Frankiaceae	Methylocystaceae, Rhizobiaceae, Bradyrhizobiaceae, Beijerinckiaceae, Leuconostocaceae, Lactobacillaceae, Thaumarchaeota, Methanogen lineage
	99973 Unclassified transcription factors	Pseudomonadaceae, Rhizobiaceae, Myxococcaceae, Streptomyetaceae	Chitinophagaceae, Cyanobacteria, Chloroflexi, Planctomyetaceae, Verrucomicrobia
	03022 Basal transcription factors	Thaumarchaeota, Methanogen Lineage	All other Families
	03013 RNA transport (eukaryote-like)	Myxococcaceae, Thaumarchaeota, Methanogen Lineage	All other Families
	03019 Messenger RNA biogenesis (degradation)	Myxococcaceae, Burkholderiaceae	Methylocystaceae, Rhizobiaceae, Bradyrhizobiaceae, Beijerinckiaceae, Cyanobacteria, Chloroflexi, Thaumarchaeota
	03450 Non-homologous end joining	Rhizobiaceae, Streptomyetaceae, Verrucomicrobia	Moraxellaceae, Methylococcaceae, Neisseriales Lineage, Leuconostocaceae, Clostridaceae, Lactobacillaceae, Cyanobacteria
	03050 Proteasome	Micrococcaceae, Streptomyetaceae, Mycobacteriaceae, Frankiaceae, Promicromonosporaceae, Cellulomonadaceae, Thaumarchaeota, Methanogen Lineage	All other families
	03051 Prokaryote 20S Proteasome	Micrococcaceae, Streptomyetaceae, Mycobacteriaceae, Frankiaceae	All other Families
Signalling and Cellular Processes	99978 Cell growth (sporulation)	Sporomusaceae, Clostridiaceae, Bacillaceae	All other families
Not included in pathway or BRITE	99984 Unclassified nucleotide metabolism	Leuconostocaceae, Lactobacillaceae, Micrococcaceae	Moraxellaceae, Methylococcaceae, Methylocystaceae, Rhodospirillaceae, Bradyrhizobiaceae, Beijerinckiaceae, all Deltaproteobacteria, all Betaproteobacteria, Sporomusaceae, Clostridiaceae, Streptomyetaceae, Frankiaceae, all Bacteroidetes, all Acidobacteria, Chloroflexi, Planctomyetes, Thaumarchaeota, Methanogen Lineage

Supplementary Material

Supplementary Table 1: List of microorganisms utilised in this study.

ID	Phylum	Class	Order	Family	Genus	Species	Strain	Ecosystem Process ^a	Reference
Ga0074846 _101	Firmicutes	Bacilli	Bacillales	Bacillaceae	<i>Terribacillus</i>	<i>saccharophilus</i>	DSM21619	ND	(An et al 2007b)
Ga0067102 _11	Firmicutes	Bacilli	Bacillales	Bacillaceae	<i>Bacillus</i>	<i>amyloliquefaciens</i>	Plantarum FZB42	Rhizosphere	(Chen et al 2007)
Ga0077930 _11	Firmicutes	Bacilli	Bacillales	Bacillaceae	<i>Bacillus</i>	<i>anthracis</i>	BA1015	Pathogen	(Helgason et al 2000)
Ga0175433 _11	Firmicutes	Bacilli	Bacillales	Bacillaceae	<i>Bacillus</i>	<i>subtilis</i>	KH2	ND	(Graham and Istock 1978)
CP015911 LAC_LBA_D RAFT__cont	Firmicutes	Bacilli	Bacillales	Bacillaceae	<i>Bacillus</i>	<i>velezensis</i>	LS69	Rhizosphere	(Liu et al 2017)
ig00001 Ga0106160 _101	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	<i>Lactobacillus</i>	<i>plantarum</i>		Rhizosphere	(Chen et al 2005)
Ga0106062 _101	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	<i>Lactobacillus</i>	<i>composti</i>	DSM18527	Rhizosphere	(Endo and Okada 2007)
1423745	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	<i>Lactobacillus</i>	<i>floricola</i>	DSM23037	Rhizosphere	(Kawasaki et al 2011)
1670446 Ga0126468 _101	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	<i>Lactobacillus</i>	<i>florum</i>	F91	Rhizosphere	(Endo et al 2010)
Ga0106620 _101	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	<i>Lactobacillus</i>	<i>herbarum</i>		ND	(Mao et al 2015)
Ga0114202 _101	Firmicutes	Bacilli	Lactobacillales	Leuconostocaceae	<i>Leuconostoc</i>	<i>mesenteroides</i>	213MO	Rhizosphere	(Chen et al 2005)
155866	Firmicutes	Bacilli	Lactobacillales	Leuconostocaceae	<i>Weissella</i>	<i>confusa</i>	DSM20196	Rhizosphere	(Chen et al 2005)
907931 BO10DRAF T_scf71800	Firmicutes	Bacilli	Lactobacillales	Leuconostocaceae	<i>Fructobacillus</i>	<i>sp</i>	EFBN1	Decomposition	(Endo et al 2009)
00000004_q uiver.1 CLOBL_conti g000001	Firmicutes	Clostridia	Clostridiales	Leuconostocaceae	<i>Weissella</i>	<i>solii</i>		Rhizosphere	(Magnusson et al 2002)
	Firmicutes	Clostridia	Clostridiales	Clostridiaceae	<i>Leuconostoc</i>	<i>fallax</i>	KCTC3537	Rhizosphere	(Barrangou et al 2002)
	Firmicutes	Clostridia	Clostridiales	Clostridiaceae	<i>Clostridium</i>	<i>phytofermentans</i>	KNHs212	Decomposition	(Warnick et al 2002)
	Firmicutes	Clostridia	Clostridiales	Clostridiaceae	<i>Clostridium</i>	<i>beijerinckii</i>	A21	Rhizosphere	(Johnson et al 1997)

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8	01	Firmicutes	Clostridia	Clostridiales	Clostridiaceae	<i>Clostridium</i>	<i>tetani</i>	ATCC454	Pathogen	(Cohen et al 2017)
9	1121919	Firmicutes	Clostridia	Clostridiales	Clostridiaceae	<i>Geosporobacter</i>	<i>subterraneus</i>	DSM17957	ND	(Klouche et al 2007)
10	857293	Firmicutes	Clostridia	Clostridiales	Clostridiaceae	<i>Caloramator</i>	<i>australicus</i>	RC3	ND	(Ogg and Patel 2009)
11	GCA_90047									
12	6375.1	Firmicutes	Negativicutes	Selenomonadales	Sporomusaceae	<i>Lucifera</i>	<i>butyrica</i>		ND	(Sanchez-Andrea et al 2018)
13	NZ_CP0109									
14	78.1	Firmicutes	Negativicutes	Selenomonadales	Sporomusaceae	<i>Pelosinus</i>	<i>fermentans</i>	JBW45	Decomposition	(de Leon et al 2012)
15	16187	Firmicutes	Negativicutes	Selenomonadales	Sporomusaceae	<i>Sporomusa</i>	<i>ovata</i>	DSM2662	ND	(Möller et al 1984)
16	51809	Firmicutes	Negativicutes	Selenomonadales	Sporomusaceae	<i>Sporomusa</i>	<i>sphaeroides</i>	DSM2875	ND	(Möller et al 1984)
17	GCA_00043									
18	0605.1	Firmicutes	Negativicutes	Selenomonadales	Sporomusaceae	<i>Anaeroarcus</i>	<i>burkinensis</i>	DSM6283	Rhizosphere	(Strömpl et al 1999)
19	Ga0074713									
20	_11	Actinobacteria	Actinobacteria	Actinomycetales	Micrococcaceae	<i>Arthrobacter</i>	<i>chlorophenolicus</i>	DSM12829	Bioremediation	(Westerberg et al 2000)
21	Ga0067230									
22	_11	Actinobacteria	Actinobacteria	Actinomycetales	Micrococcaceae	<i>Arthrobacter</i>	<i>aurescens</i>	TC1	Bioremediation	(Mongodin et al 2006)
23	1531955	Actinobacteria	Actinobacteria	Actinomycetales	Micrococcaceae	<i>Sinomonas</i>	<i>mesophila</i>	MPKL26	ND	(Prabhu et al 2015)
24	378753	Actinobacteria	Actinobacteria	Actinomycetales	Micrococcaceae	<i>Kocuria</i>	<i>rhizophila</i>	DC2201	Rhizosphere	(Takarada et al 2008)
25	574650	Actinobacteria	Actinobacteria	Actinomycetales	Micrococcaceae	<i>Micrococcus</i>	<i>terreus</i>		ND	(Zhang et al 2010)
26	Ga0080924									
27	_101	Actinobacteria	Actinobacteria	Actinomycetales	Mycobacteriaceae	<i>Mycobacterium</i>	<i>chlorophenolicum</i>	DSM43826	Bioremediation	(Das et al 2015)
28	Ga0124153									
29	_1001	Actinobacteria	Actinobacteria	Actinomycetales	Mycobacteriaceae	<i>Mycobacterium</i>	<i>fortuitum</i>	ATCC6841	Bioremediation	(Nohynek et al 1993)
30	Ga0056865									
31	_101	Actinobacteria	Actinobacteria	Actinomycetales	Mycobacteriaceae	<i>Mycobacterium</i>	<i>austroafricanum</i>	DSM44191	Bioremediation	(Leys et al 2005)
32	443218	Actinobacteria	Actinobacteria	Actinomycetales	Mycobacteriaceae	<i>Hoyosella</i>	<i>subflava</i>	DQS39A1	Bioremediation	(Hamada et al 2016)
33	1440774	Actinobacteria	Actinobacteria	Actinomycetales	Mycobacteriaceae	<i>Mycobacterium</i>	<i>aromaticivorans</i>	JCM16368	Bioremediation	(Hennessee et al 2009)
34	NC_014151									
35	Ga0128466	Actinobacteria	Actinobacteria	Actinomycetales	Cellulomonadaceae	<i>Cellulomonas</i>	<i>flavigena</i>	DSM20109	Decomposition	(Abt et al 2010)
36	_10001	Actinobacteria	Actinobacteria	Actinomycetales	Cellulomonadaceae	<i>Cellulomonas</i>	<i>chitinilytica</i>	JCM16927	Decomposition	(Yoon et al 2008)
37	Ga0128457									
38	_10001	Actinobacteria	Actinobacteria	Actinomycetales	Cellulomonadaceae	<i>Cellulomonas</i>	<i>xylanilytica</i>	JCM14281	Decomposition	(Rivas et al 2004 ^a)
39	862422	Actinobacteria	Actinobacteria	Actinomycetales	Cellulomonadaceae	<i>Actinotalea</i>	<i>fermentans</i>	DSM3133	Decomposition	(Bagnara et al 1985)
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7	948458	Actinobacteria	Actinobacteria	Actinomycetales	Cellulomonadaceae	<i>Actinotalea</i>	<i>ferrariae</i>	CF54	ND	(Li et al 2013)
8	Ga0057460	Actinobacteria	Actinobacteria	Actinomycetales	Streptomycetaceae	<i>Streptomyces</i>	<i>scabiei</i>	NCPFB4086	Pathogen	(Dees et al 2013)
9	_1001	Actinobacteria	Actinobacteria	Actinomycetales	Streptomycetaceae	<i>Streptomyces</i>	<i>lividans</i>	TK24	Decomposition	(Ruckert et al 2015)
10	Ga0057466	Actinobacteria	Actinobacteria	Actinomycetales	Streptomycetaceae	<i>Streptomyces</i>	<i>coelicolor</i>	A3(2)	Decomposition	(Bentley et al 2002)
11	_gi6723671	Actinobacteria	Actinobacteria	Actinomycetales	Streptomycetaceae	<i>Streptomyces</i>	<i>coelicolor</i>	A3(2)	Decomposition	(Bentley et al 2002)
12	50.1	Actinobacteria	Actinobacteria	Actinomycetales	Streptomycetaceae	<i>Streptomyces</i>	<i>coelicolor</i>	A3(2)	Decomposition	(Bentley et al 2002)
13	Ga0076575	Actinobacteria	Actinobacteria	Actinomycetales	Streptomycetaceae	<i>Streptomyces</i>	<i>coelicolor</i>	A3(2)	Decomposition	(Bentley et al 2002)
14	_11	Actinobacteria	Actinobacteria	Actinomycetales	Streptomycetaceae	<i>Streptomyces</i>	<i>coelicolor</i>	A3(2)	Decomposition	(Bentley et al 2002)
15	105422	Actinobacteria	Actinobacteria	Actinomycetales	Streptomycetaceae	<i>Streptacidiphilus</i>	<i>carbonis</i>	DSM41754	Decomposition	(Kim et al 2003)
16	1348663	Actinobacteria	Actinobacteria	Actinomycetales	Streptomycetaceae	<i>Kitasatospora</i>	<i>cheerisanensis</i>	KCTC2395	ND	(Hwang et al 2015)
17	326424	Actinobacteria	Actinobacteria	Frankiales	Frankiaceae	<i>Frankia</i>	<i>alni</i>	ACN14a	Nitrogen fixation	(Normand and Lalonde 1982)
18	106370	Actinobacteria	Actinobacteria	Frankiales	Frankiaceae	<i>Frankia</i>	<i>casuarinae</i>	Ccl3	Nitrogen fixation	(Normand et al 2007)
19	298654	Actinobacteria	Actinobacteria	Frankiales	Frankiaceae	<i>Frankia</i>	<i>inefficax</i>	Eul1c	ND	(Nouioui et al 2017)
20	656024	Actinobacteria	Actinobacteria	Frankiales	Frankiaceae	<i>Frankia</i>	<i>sp</i>	Datisca symbiont	Nitrogen fixation	(Persson et al 2015)
21	222534	Actinobacteria	Actinobacteria	Frankiales	Frankiaceae	<i>Frankia</i>	<i>elaeagni</i>	BMG512	Nitrogen fixation	(Nouioui et al 2016)
22	15565	Actinobacteria	Actinobacteria	Micrococcales	Promicromonosporaceae	<i>Cellulosimicrobium</i>	<i>cellulans</i>	J36	Decomposition	(Schumann et al 2001)
23	75317	Actinobacteria	Actinobacteria	Micrococcales	Promicromonosporaceae	<i>Cellulosimicrobium</i>	<i>terreum</i>		Decomposition	(Yoon et al 2007)
24	NC_013530.	Actinobacteria	Actinobacteria	Micrococcales	Promicromonosporaceae	<i>Xylanimonas</i>	<i>cellulosilytica</i>	DSM15894	Decomposition	(Rivas et al 2003)
25	_1	Actinobacteria	Actinobacteria	Micrococcales	Promicromonosporaceae	<i>Xylanimonas</i>	<i>cellulosilytica</i>	DSM15894	Decomposition	(Takahashi et al 1987)
26	16475	Actinobacteria	Actinobacteria	Micrococcales	Promicromonosporaceae	<i>Promicromonospora</i>	<i>sukumoe</i>	327MFSHa3.1	Decomposition	(Rivas et al 2004 ^b)
27	76147	Actinobacteria	Actinobacteria	Micrococcales	Promicromonosporaceae	<i>Xylanibacterium</i>	<i>ulmi</i>		Decomposition	(Zgurskaya et al 1992)
28	50964	Actinobacteria	Actinobacteria	Micrococcales	Microbacteriaceae	<i>Agromyces</i>	<i>cerinus</i>		ND	(Jurado et al 2005)
29	16552	Actinobacteria	Actinobacteria	Micrococcales	Microbacteriaceae	<i>Agromyces</i>	<i>subbeticus</i>	DSM16689	ND	(Schumann et al 2003)
30	54323	Actinobacteria	Actinobacteria	Micrococcales	Microbacteriaceae	<i>Agreia</i>	<i>pratensis</i>		Rhizosphere	(Schumann et al 2003)
31	16541	Actinobacteria	Actinobacteria	Micrococcales	Microbacteriaceae	<i>Agrococcus</i>	<i>lahaulensis</i>	DSM17612	ND	(Mayilraj et al 2006)
32	16623	Actinobacteria	Actinobacteria	Micrococcales	Microbacteriaceae	<i>Humibacter</i>	<i>albus</i>	DSM18994	Decomposition	(Vaz-Moreira et al 2008)
33	Ga0065637	Actinobacteria	Actinobacteria	Micrococcales	Microbacteriaceae	<i>Humibacter</i>	<i>albus</i>	DSM18994	Decomposition	(Vaz-Moreira et al 2008)
34	_11	Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae	<i>Koribacter</i>	<i>versatilis</i>	Ellin345	Decomposition	(Ward et al 2009)
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7	Terro_Conti g51.1	Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae	<i>Terriglobus</i>	<i>roseus</i>	DSM18391	Decomposition	(Eichorst et al 2007)
8	Ga0133452 _11	Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae	<i>Luteitalea</i>	<i>pratensis</i>	DSM100886	Decomposition	(Vieira et al 2017)
9	863522	Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae	<i>Bryocella</i>	<i>elongata</i>	DSM22489	Decomposition	(Dedysh et al 2012)
10	1121860	Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae	<i>Edaphobacter</i>	<i>aggregans</i>	DSM19364	Decomposition	(Koch et al 2008)
11					Divergent					
12					Acidobacteria	<i>Candidatus</i>				
13	234267	Acidobacteria	Acidobacteria	Solibacteriales	lineage	<i>Solibacter</i>	<i>usitatus</i>	Ellin6076	Decomposition	(Ward et al 2009)
14					Divergent					
15	1340493	Acidobacteria	Acidobacteria	Solibacteriales	Acidobacteria	<i>Bryobacter</i>	<i>aggregatus</i>	MPL3	Decomposition	(Kulichevskaya et al 2010)
16					lineage					
17	454194	Acidobacteria	Acidobacteria	Blastocatellales	Acidobacteria	<i>Pyrinomonas</i>	<i>methyhaliphatogenes</i>	K22	Decomposition	(Crowe et al 2014)
18					Divergent					
19					Acidobacteria					
20	903818	Acidobacteria	Acidobacteria	Holophagales	lineage	<i>Holophaga</i>	<i>foetida</i>	DSM6591	ND	(Liesack et al 1994)
21					Divergent					
22	1660251	Acidobacteria	Acidobacteria	Unclassified	Acidobacteria	<i>Acidobacteria</i>	<i>bacterium</i>	Mor1	Rhizosphere	(Tanaka et al 2017)
23	Ga0077356 _1001	Proteobacteria	Beta	Burkholderiales	Burkholderiaceae	<i>Burkholderia</i>	<i>pseudomallei</i>	TSV44	Pathogen	(Brook et al 1997)
24	Ga0065534 _11	Proteobacteria	Beta	Burkholderiales	Burkholderiaceae	<i>Burkholderia</i>	<i>xenovorans</i>	LB400	Rhizosphere	(Chain et al 2006)
25	Ga0056711 _1001	Proteobacteria	Beta	Burkholderiales	Burkholderiaceae	<i>Burkholderia</i>	<i>terrae</i>	NBRC100964	Rhizosphere	(Yang et al 2006)
26	1121278	Proteobacteria	Beta	Burkholderiales	Burkholderiaceae	<i>Chitinimonas</i>	<i>koreensis</i>	DSM17726	ND	(Kim et al 2006)
27	1040978	Proteobacteria	Beta	Burkholderiales	Burkholderiaceae	<i>Cupriavidus</i>	<i>taiwanensis</i>	STM6018	Nitrogen fixation	(Chen et al 2001)
28	Ga0105865 _101	Proteobacteria	Beta	Nitrosomonadales	Nitrosomonadaceae	<i>Nitrosomonas</i>	<i>europaea</i>	Nm50	Nitrification	(Koops et al 1991)
29	Ga0105862 _1001	Proteobacteria	Beta	Nitrosomonadales	Nitrosomonadaceae	<i>Nitrosomonas</i>	<i>communis</i>	Nm2	Nitrification	(Koops et al 1991)
30	Ga0105869 _101	Proteobacteria	Beta	Nitrosomonadales	Nitrosomonadaceae	<i>Nitrosomonas</i>	<i>eutropha</i>	Nm56	Nitrification	(Koops et al 1991)
31	1266925	Proteobacteria	Beta	Nitrosomonadales	Nitrosomonadaceae	<i>Nitrospira</i>	<i>briensis</i>	C128	Nitrification	(Rice et al 2016)
32	323848	Proteobacteria	Beta	Nitrosomonadales	Nitrosomonadaceae	<i>Nitrospira</i>	<i>multiformis</i>	ATCC25196	Nitrification	(Norton et al 2008)
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6	G520DRAF									
7	T_scaffold00									
8	001.1	Proteobacteria	Beta	Neisseriales	Neisseriales_lineage	<i>Paludibacterium</i>	<i>yongneupense</i>	DSM19731	ND	(Kwon et al 2008)
9	Ga0077161									
10	_101	Proteobacteria	Beta	Neisseriales	Neisseriales_lineage	<i>Chromobacterium</i>	<i>subtsugae</i>	F49	ND	(Soby et al 2013)
11	Ga0080895									(Koburger and May 1982)
12	_101	Proteobacteria	Beta	Neisseriales	Neisseriales_lineage	<i>Chromobacterium</i>	<i>violaceum</i>	GN5	Pathogen	
13	1121274	Proteobacteria	Beta	Neisseriales	Neisseriales_lineage	<i>Chitinibacter</i>	<i>tainanensis</i>	DSM15459	Decomposition	(Chern et al 2004)
14	1120999	Proteobacteria	Beta	Neisseriales	Neisseriales_lineage	<i>Andreprevotia</i>	<i>chitinilytica</i>	DSM18519	Decomposition	(Weon et al 2007)
15	Ga0077767									
16	_1001	Proteobacteria	Gamma	Pseudomonadales	Pseudomonadaceae	<i>Pseudomonas</i>	<i>putida</i>	SF1	Rhizosphere	(Tikariha et al 2016)
17	Ga0061119									
18	_scaffold000									
19	01.1	Proteobacteria	Gamma	Pseudomonadales	Pseudomonadaceae	<i>Azotobacter</i>	<i>beijerinckii</i>	DSM373	Nitrogen fixation	(Lipman 1904)
20	Ga0129289									(Lymeropoulou et al 2017)
21	_101	Proteobacteria	Gamma	Pseudomonadales	Pseudomonadaceae	<i>Pseudomonas</i>	<i>oryzihabitans</i>	H72	Rhizosphere	
22	289370	Proteobacteria	Gamma	Pseudomonadales	Pseudomonadaceae	<i>Pseudomonas</i>	<i>argentinensis</i>	CECT7010	Rhizosphere	(Peix et al 2005)
23	1292027	Proteobacteria	Gamma	Pseudomonadales	Pseudomonadaceae	<i>Pseudomonas</i>	<i>azotifigens</i>	DSM17556	Nitrogen fixation	(Hatayama et al 2005)
24	Ga0044516									
25	_101	Proteobacteria	Gamma	Pseudomonadales	Moraxellaceae	<i>Acinetobacter</i>	<i>solii</i>		ND	(Kim et al 2008)
26	Ga0080829									(Davenport et al 2014)
27	_1001	Proteobacteria	Gamma	Pseudomonadales	Moraxellaceae	<i>Acinetobacter</i>	<i>baumannii</i>		Pathogen	
28	Q337DRAF									
29	T_scaffold00									
30	010.10	Proteobacteria	Gamma	Pseudomonadales	Moraxellaceae	<i>Alkanindiges</i>	<i>illinoisensis</i>	DSM15370	Bioremediation	(Bogan et al 2003)
31	1217715	Proteobacteria	Gamma	Pseudomonadales	Moraxellaceae	<i>Acinetobacter</i>	<i>bohemicus</i>	ANC3994	ND	(Krizova et al 2014)
32	1217660	Proteobacteria	Gamma	Pseudomonadales	Moraxellaceae	<i>Acinetobacter</i>	<i>indicus</i>	ANC4215	Bioremediation	(Malhotra et al 2012)
33	GY38DRAF									
34	T_scf71800									
35	00000021_q									
36	uiver.1	Proteobacteria	Gamma	Methylococcales	Methylococcaceae	<i>Methylobacter</i>	<i>whittenburyi</i>	ACM3310	Methane oxidation	(Whittenbury et al 1970)
37	MetmiDRAF									
38	T_scaffold1.									
39	1	Proteobacteria	Gamma	Methylococcales	Methylococcaceae	<i>Methylovulum</i>	<i>miyakonense</i>	HT12	Methane oxidation	(Iguchi et al 2011)
40	MetluDRAF									
41	T_scaffold1.									
42	1	Proteobacteria	Gamma	Methylococcales	Methylococcaceae	<i>Methylobacter</i>	<i>luteus</i>	IMVB3098	Methane oxidation	(Whittenbury et al 1970)
43	1132443	Proteobacteria	Gamma	Methylococcales	Methylococcaceae	<i>Methylosarcina</i>	<i>fibrata</i>	AMLC10	Methane oxidation	(Wise et al 2001)
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7	493385	Proteobacteria	Gamma	Methylococcales	Methylococcaceae	<i>Methylogaea</i>	<i>oryzae</i>	JCM16910	Methane oxidation	(Geymonat et al 2011)
8	Ga0061138									
9	_scaffold000									
10	01.1	Proteobacteria	Alpha	Rhodospirillales	Rhodospirillaceae	<i>Azospirillum</i>	<i>brasiliense</i>	DSM1690	Nitrogen fixation	(Kuklinsky-Sobral et al 2004)
11	Ga0048939	Proteobacteria	Alpha	Rhodospirillales	Rhodospirillaceae	<i>Azospirillum</i>	<i>oryzae</i>	A2P	Nitrogen fixation	(Xie and Yokota 2005a)
12	_110									
13	Ga0048941	Proteobacteria	Alpha	Rhodospirillales	Rhodospirillaceae	<i>Azospirillum</i>	<i>lipoferum</i>	R1C	Nitrogen fixation	(Van et al 1997)
14	_11									
15	570967	Proteobacteria	Alpha	Rhodospirillales	Rhodospirillaceae	<i>Fodinicurvata</i>	<i>fenggangensis</i>	DSM21160	ND	(Wang et al 2009)
16										(Magalhaes et al 1983)
17	1003237	Proteobacteria	Alpha	Rhodospirillales	Rhodospirillaceae	<i>Nitrospirillum</i>	<i>amazonense</i>	Y2	Nitrogen fixation	
18	DL88DRAFT									
19	_scaffold000									
20	01.1	Proteobacteria	Alpha	Rhizobiales	Beijerinckiaceae	<i>Beijerinckia</i>	<i>mobilis</i>	DSM2326	Nitrogen fixation	(Derx 1950)
21	182677003	Proteobacteria	Alpha	Rhizobiales	Beijerinckiaceae	<i>Beijerinckia</i>	<i>indica</i>	ATCC9039	Nitrogen fixation	(Derx 1950)
22	217976201	Proteobacteria	Alpha	Rhizobiales	Beijerinckiaceae	<i>Methylocella</i>	<i>silvestris</i>	BL2	Methane oxidation	(Dunfield et al 2003)
23	395964	Proteobacteria	Alpha	Rhizobiales	Beijerinckiaceae	<i>Methylocapsa</i>	<i>acidiphila</i>	B2	Methane oxidation	(Dedysh et al 2002)
24	876269	Proteobacteria	Alpha	Rhizobiales	Beijerinckiaceae	<i>Methyloferula</i>	<i>stellata</i>	AR4	Methane oxidation	(Vorobev et al 2011)
25	A3OODRAF									
26	T_scaffold1.									
27	1	Proteobacteria	Alpha	Rhizobiales	Methylocystaceae	<i>Methylocystis</i>	<i>rosea</i>	SV97T	Methane oxidation	(Wartiainen et al 2006)
28	MettrDRAFT									
29	_Contig106	Proteobacteria	Alpha	Rhizobiales	Methylocystaceae	<i>Methylosinus</i>	<i>trichosporium</i>	OB3b	Methane oxidation	(Whittenbury et al 1970)
30	A3OUDRAF									
31	T_chromosome1.	Proteobacteria	Alpha	Rhizobiales	Methylocystaceae	<i>Methylopila</i>	<i>sp</i>	M107	Methane oxidation	(Chistoserdova 2011)
32	1122963	Proteobacteria	Alpha	Rhizobiales	Methylocystaceae	<i>Pleomorphomonas</i>	<i>oryzae</i>	DSM16300	Nitrogen fixation	(Xie and Yokota 2005b)
33	1122962	Proteobacteria	Alpha	Rhizobiales	Methylocystaceae	<i>Pleomorphomonas</i>	<i>korensis</i>	DSM23070	Nitrogen fixation	(Im et al 2006)
34	Ga0065594	Proteobacteria	Alpha	Rhizobiales	Bradyrhizobiaceae	<i>Nitrobacter</i>	<i>winogradskyi</i>	Nb255	Nitrification	(Starkenbourg et al 2006)
35	_11									
36	Ga0098245	Proteobacteria	Alpha	Rhizobiales	Bradyrhizobiaceae	<i>Bradyrhizobium</i>	<i>japonicum</i>	E109	Nitrogen fixation	(Brunel et al 1988)
37	_11									
38	Ga0077765	Proteobacteria	Alpha	Rhizobiales	Bradyrhizobiaceae	<i>Rhodopseudomonas</i>	<i>palustris</i>	42OL	Bioremediation	(Larimer et al 2004)
39	_1001									
40	1031710	Proteobacteria	Alpha	Rhizobiales	Bradyrhizobiaceae	<i>Oligotropha</i>	<i>carboxidovorans</i>	OM4	Decomposition	(Volland et al 2011)
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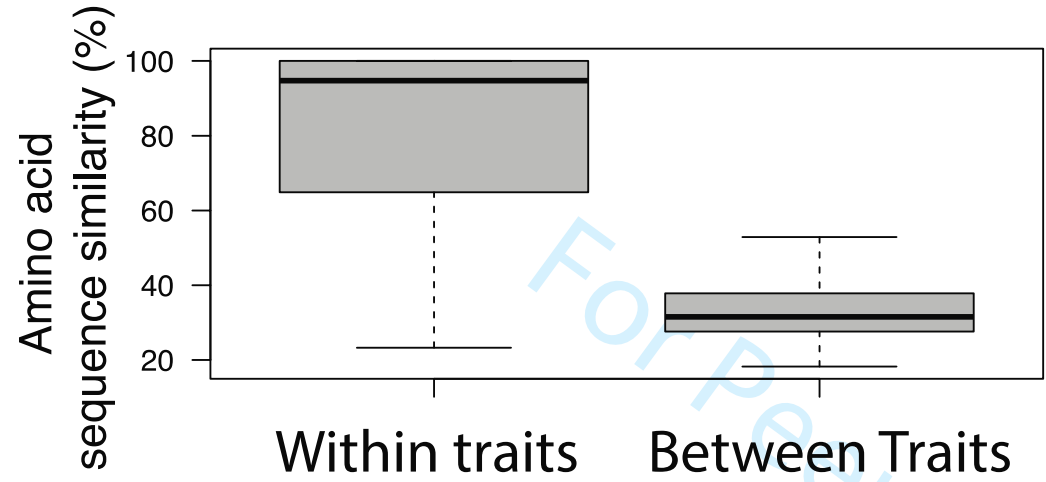
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7	1123229	Proteobacteria	Alpha	Rhizobiales	Bradyrhizobiaceae	<i>Salinarimonas</i>	<i>rosea</i>	DSM21201	ND	(Liu et al 2010)
8	Ga0127029	Proteobacteria	Alpha	Rhizobiales	Rhizobiaceae	<i>Agrobacterium</i>	<i>tumefaciens</i>	B6	Rhizosphere	(Levanon 1978)
9	EW91DRAFT									
10	T_scaffold00									
11	_001.1	Proteobacteria	Alpha	Rhizobiales	Rhizobiaceae	<i>Rhizobium</i>	<i>leguminosarum</i>	CF307	Nitrogen fixation	(Naeem et al 2004)
12	Ga0174993	Proteobacteria	Alpha	Rhizobiales	Rhizobiaceae	<i>Sinorhizobium</i>	<i>americanum</i>	CFNEI73	Rhizosphere	(Toledo et al 2003)
13	_11									(Osterman et al
14	1028800	Proteobacteria	Alpha	Rhizobiales	Rhizobiaceae	<i>Neorhizobium</i>	<i>galegae</i>	HAMBI540	Nitrogen fixation	2014)
15	1764550	Proteobacteria	Alpha	Rhizobiales	Rhizobiaceae	<i>Rhizobium</i>	<i>aegyptiacum</i>	1010	Nitrogen fixation	(Shamseldin et al
16	Ga0128794	Proteobacteria	Delta	Myxococcales	Polyangiaceae	<i>Sorangium</i>	<i>cellulosum</i>	So0149	Decomposition	2016)
17	_10470									(Li et al 2014)
18	Ga0098230	Proteobacteria	Delta	Myxococcales	Polyangiaceae	<i>Chondromyces</i>	<i>crocatus</i>	Cmc5	ND	(Zaburannyi et al
19	Ga0081738	Proteobacteria	Delta	Myxococcales	Polyangiaceae	<i>Polyangium</i>	<i>brachysporum</i>	DSM7029	ND	2016)
20	_11									(Tang et al 2015)
21	1192034	Proteobacteria	Delta	Myxococcales	Polyangiaceae	<i>Chondromyces</i>	<i>apiculatus</i>	DSM436	ND	(Kleinig and
22	Ga0128396	Proteobacteria	Delta	Myxococcales	Polyangiaceae	<i>Byssovorax</i>	<i>cruenta</i>		ND	Reichenbach 1973)
23	_10001									(Reichenbach et al
24	Ga0131205	Proteobacteria	Delta	Myxococcales	Myxococcaceae	<i>Myxococcus</i>	<i>xanthus</i>	DSM16526	ND	2006)
25	_101									(Voelz and Dworkin
26	Ga0070493	Proteobacteria	Delta	Myxococcales	Myxococcaceae	<i>Myxococcus</i>	<i>virescens</i>	DSM2260	ND	1962)
27	Ga0131203	Proteobacteria	Delta	Myxococcales	Myxococcaceae	<i>Myxococcus</i>	<i>fulvus</i>	DSM16525	ND	(Lang et al 2008)
28	_101									(Jahn 1911)
29	1144275	Proteobacteria	Delta	Myxococcales	Myxococcaceae	<i>Corallococcus</i>	<i>coralloides</i>	DSM2259	ND	(Huntley et al 2012)
30	1189310	Proteobacteria	Delta	Myxococcales	Myxococcaceae	<i>Myxococcus</i>	<i>macrosporus</i>	DSM14697	ND	(Lang and
31	NC_013132	Bacteroidetes	Bacteroidetes	Chitinophagales	Chitinophagaceae	<i>Chitinophaga</i>	<i>pinensis</i>	DSM2588	Decomposition	Stackebrandt 2009)
32	B154DRAFT									(Sangkhobol and
33	_scaffold_0.									Skerman 1981)
34	1	Bacteroidetes	Bacteroidetes	Chitinophagales	Chitinophagaceae	<i>Segetibacter</i>	<i>koreensis</i>	DSM18137	ND	(An et al 2007a)
35	H608DRAFT									
36	_scaffold000									
37	01.1	Bacteroidetes	Bacteroidetes	Chitinophagales	Chitinophagaceae	<i>Terrimonas</i>	<i>ferruginea</i>	DSM30193	ND	(Xie and Yokota
38	551991	Bacteroidetes	Bacteroidetes	Chitinophagales	Chitinophagaceae	<i>Arachidicoccus</i>	<i>rhizosphaerae</i>	Vu144	Rhizosphere	2006)
39	929713	Bacteroidetes	Bacteroidetes	Chitinophagales	Chitinophagaceae	<i>Niabella</i>	<i>solii</i>	DSM19437	ND	(Madhaiyan et al
40										2015)
41										(Weon et al 2008)
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7	2506615776 Ga0066359	Bacteroidetes	Sphingobacteria	Sphingobacteriales	Cytophagaceae	<i>Niastella</i>	<i>koreensis</i>	GR2010	ND	(Weon et al 2006)
8	_101	Bacteroidetes	Sphingobacteria	Sphingobacteriales	Cytophagaceae	<i>Cytophaga</i>	<i>hutchinsonii</i>	DSM1761	Decomposition	(Xie et al 2007)
9	Ga0116983	Bacteroidetes	Sphingobacteria	Sphingobacteriales	Cytophagaceae	<i>Pontibacter</i>	<i>indicus</i>	LP100	Bioremediation	(Singh et al 2014)
10	_11	Bacteroidetes	Sphingobacteria	Sphingobacteriales	Cytophagaceae	<i>Arsenicibacter</i>	<i>rosenii</i>	SM1	Bioremediation	(Huang et al 2017)
11	1750698	Bacteroidetes	Sphingobacteria	Sphingobacteriales	Cytophagaceae	<i>Arsenicibacter</i>	<i>rosenii</i>	SM1	Bioremediation	(Huang et al 2017)
12	1121373	Bacteroidetes	Sphingobacteria	Sphingobacteriales	Cytophagaceae	<i>Cytophaga</i>	<i>aurantiaca</i>	DSM3654	Decomposition	(Winogradsky 1929)
13	Ga0056857	Verrucomicrobia	Verrucomicrobiae	Verrucomicrobiales	Verrucomicrobia lineage	<i>Verrucomicrobium</i>	<i>sp</i>	BvORR106	Rhizosphere	Unpublished
14	_1001	Verrucomicrobia	Verrucomicrobiae	Verrucomicrobiales	Verrucomicrobia lineage	<i>Verrucomicrobium</i>	<i>sp</i>	BvORR034	Rhizosphere	Unpublished
15	Ga0056855	Verrucomicrobia	Verrucomicrobiae	Verrucomicrobiales	Verrucomicrobia lineage	<i>Verrucomicrobium</i>	<i>sp</i>	BvORR034	Rhizosphere	Unpublished
16	_1001	Verrucomicrobia	Verrucomicrobiae	Verrucomicrobiales	Verrucomicrobia lineage	<i>Verrucomicrobium</i>	<i>sp</i>	BvORR034	Rhizosphere	Unpublished
17	182411827	Verrucomicrobia	Verrucomicrobiae	Verrucomicrobiales	Verrucomicrobia lineage	<i>Opitutus</i>	<i>terrae</i>	PB90	ND	(Chin et al 2001)
18	690879	Verrucomicrobia	Verrucomicrobiae	Verrucomicrobiales	Verrucomicrobia lineage	<i>Terrimicrobium</i>	<i>sacchariphilum</i>	NM5	ND	(Qiu et al 2014)
19	497964	Verrucomicrobia	Verrucomicrobiae	Verrucomicrobiales	Verrucomicrobia lineage	<i>Chthoniobacter</i>	<i>flavus</i>	Ellin428	ND	(Sangwan et al 2004)
20	Ga0154327	Planctomycetes	Planctomycetia	Planctomycetales	Planctomycetales lineage	<i>Planctomyces</i>	<i>sp</i>	GWA2407	ND	Unpublished
21	_1001	Planctomycetes	Planctomycetia	Planctomycetales	Planctomycetales lineage	<i>Planctomyces</i>	<i>sp</i>	GWA2407	ND	Unpublished
22	Ga0111584	Planctomycetes	Planctomycetia	Planctomycetales	Planctomycetales lineage	<i>Planctomicrobium</i>	<i>piriforme</i>	DSM26348	ND	(Kulichevskaya et al 2015)
23	_101	Planctomycetes	Planctomycetia	Planctomycetales	Planctomycetales lineage	<i>Planctomicrobium</i>	<i>piriforme</i>	DSM26348	ND	(Kulichevskaya et al 2015)
24	Ga0198709	Planctomycetes	Planctomycetia	Planctomycetales	Planctomycetales lineage	<i>Paludisphaera</i>	<i>borealis</i>	PX4	ND	(Kulichevskaya et al 2016)
25	_11	Planctomycetes	Planctomycetia	Planctomycetales	Planctomycetales lineage	<i>Paludisphaera</i>	<i>borealis</i>	PX4	ND	(Kulichevskaya et al 2016)
26	1123242	Planctomycetes	Planctomycetia	Planctomycetales	Planctomycetales lineage	<i>Schlesneria</i>	<i>paludicola</i>	DSM18645	ND	(Kulichevskaya et al 2007)
27	886293	Planctomycetes	Planctomycetia	Planctomycetales	Planctomycetales lineage	<i>Singulisphaera</i>	<i>acidiphila</i>	DSM18658	ND	(Kulichevskaya et al 2008)
28	GCA_00021	Cyanobacteria	Cyanobacteria	Oscillatoriales	Microcoleaceae	<i>Microcoleus</i>	<i>vaginatus</i>	FGP-2	Phototroph	(Starkenburger et al 2011)
29	4075.2	Cyanobacteria	Cyanobacteria	Oscillatoriales	Microcoleaceae	<i>Microcoleus</i>	<i>vaginatus</i>	FGP-2	Phototroph	(Starkenburger et al 2011)
30		Cyanobacteria	Cyanobacteria	Nostocales	Nostocaceae	<i>Nostoc</i>	<i>punctiforme</i>	PCC73102	Phototroph	(Rippka and Herdman, 1992)
31	67533	Cyanobacteria	Cyanobacteria	Chroococcidiopsidales	Chroococcidiopsidaceae	<i>Chroococcidiopsis</i>	<i>cubana</i>		Phototroph	(Komarek and Hindak, 1975)
32		Cyanobacteria	Cyanobacteria	Nostocales	Calotrichaceae	<i>Calothrix</i>	<i>desertica</i>		Phototroph	(Schwabe, 1960)
33	74975	Cyanobacteria	Cyanobacteria	Nostocales	Calotrichaceae	<i>Calothrix</i>	<i>desertica</i>		Phototroph	(Schwabe, 1960)
34	GCA_00163	Cyanobacteria	Cyanobacteria	Synechococcales	Leptolyngbyaceae	<i>Leptolyngbya</i>	<i>valderiana</i>	BDU20041	Phototroph	(Anagnostidis and Komarek, 1988)
35	7395.1	Cyanobacteria	Cyanobacteria	Synechococcales	Leptolyngbyaceae	<i>Leptolyngbya</i>	<i>valderiana</i>	BDU20041	Phototroph	(Anagnostidis and Komarek, 1988)
36	NC-002936.3	Chloroflexi	Dehalococcoidia	Dehalococcoidales	Dehalococcoidaceae	<i>Dehalococcoides</i>	<i>mccartyi</i>	CG5	Bioremediation	(He et al 2003)
37	50999	Chloroflexi	Ktedonobacteria	Ktedonobacteriales	Ktedonobacteraceae	<i>Ktedonobacter</i>	<i>Sp.</i>		ND	Unpublished
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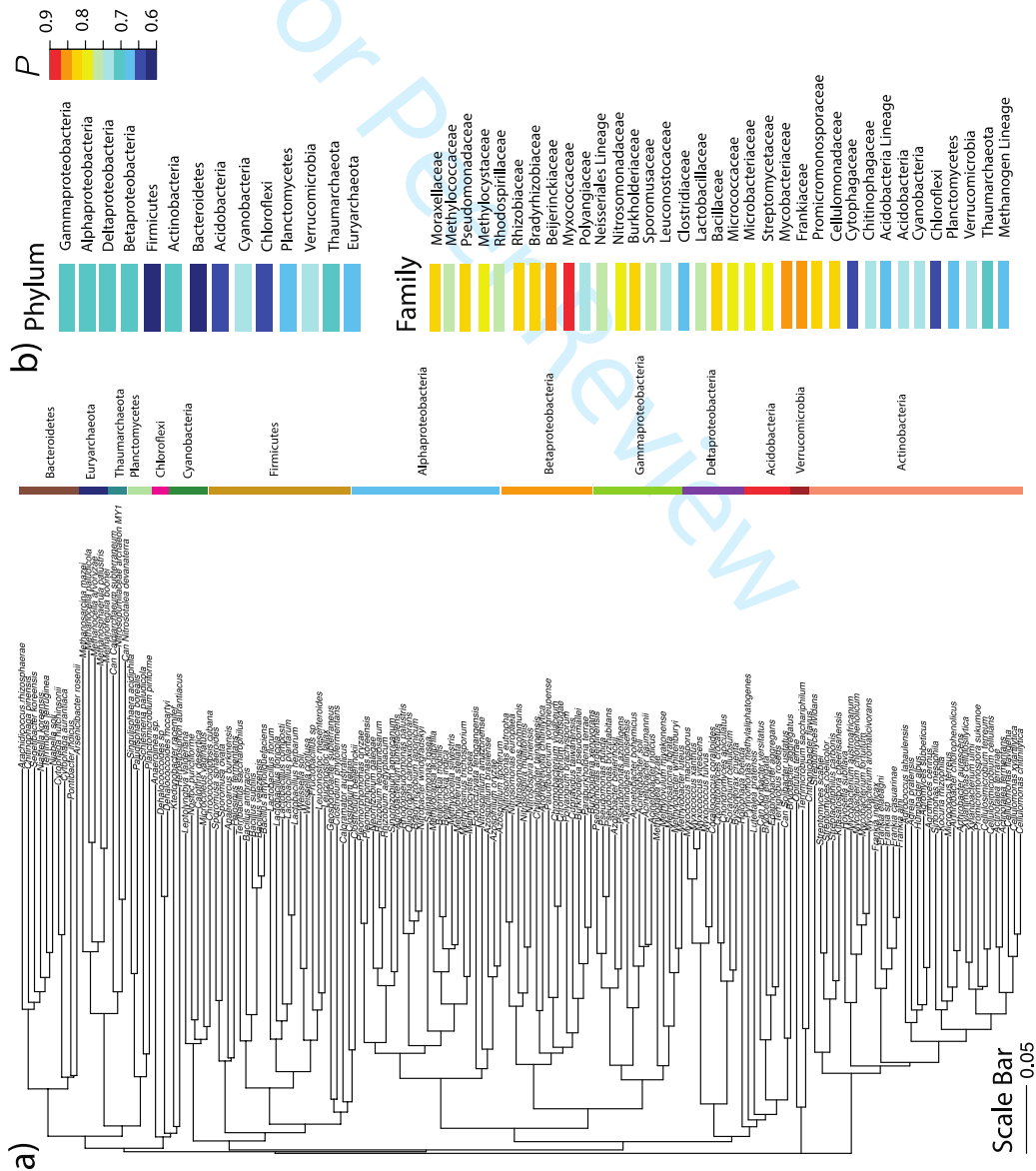
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46725	Chloroflexi	Anaerolineae	Anaerolineales	Anaerolineaceae	<i>Anaerolinea</i>	<i>Sp.</i>		Decomposition	Unpublished
13657	Chloroflexi	Dehalococcoidia	Dehalococcoidales	Dehalococcoidaceae	<i>Dehalococcoides</i>	<i>Sp.</i>		Bioremediation	Unpublished
GCA_000018565.1	Chloroflexi	Chloroflexia	Herpetosiphonales	Herpetosiphonaceae	<i>Herpetosiphon</i>	<i>aurantiacus</i>		ND	(Kiss et al 2011)
LRC50LV_640427159s	Euryarchaeota	Methanomicrobia	Methanocellales	Methanogen lineage	<i>Methanocella</i>	<i>arvoryzae</i>	MRE50	Methanogenesis	(Sakai et al 2010)
MCPLV_646311984standard	Euryarchaeota	Methanomicrobia	Methanocellales	Methanogen lineage	<i>Methanocella</i>	<i>paludicola</i>	SANAE	Methanogenesis	(Sakai et al 2008)
Ga0072472_11	Euryarchaeota	Methanomicrobia	Methanosarcinales	Methanogen lineage	<i>Methanosarcina</i>	<i>mazei</i>	C16	Methanogenesis	(Maestrojuán et al 1992)
456442	Euryarchaeota	Methanomicrobia	Methanosarcinales	Methanogen lineage	<i>Methanoregula</i>	<i>boonei</i>	6A8	Methanogenesis	(Brauer et al 2011)
521011	Euryarchaeota	Methanomicrobia	Methanosarcinales	Methanogen lineage	<i>Methanosphaerula</i>	<i>palustris</i>	E19c	Methanogenesis	(Cadillo-Quiroz et al 2009)
Ga0073019_101	Thaumarchaeota	Nitrososphaeria	Nitrososphaerales	Thaumarchaeota lineage	<i>Nitrocosmicus</i>	<i>oleophilus</i>	MY3	Nitrification	(Jung et al 2016)
CCsub_BA00048_1	Thaumarchaeota	Nitrososphaeria	Nitrososphaerales	Thaumarchaeota lineage	<i>Caldiarchoaeum</i>	<i>subterraneum</i>		Nitrification	(Takami et al 2015)
bd31_gi386807657.1	Thaumarchaeota	Nitrososphaeria	Nitrososphaerales	Thaumarchaeota lineage	<i>Nitrosopumilus</i>	<i>salaria</i>	BD31	Nitrification	(Mosier et al 2012)
1078905	Thaumarchaeota	Nitrososphaeria	Nitrososphaerales	Thaumarchaeota lineage	<i>Nitrosotalea</i>	<i>devanterra</i>		Nitrification	(Lehtovirta-Morley et al 2014)
1001994	Thaumarchaeota	Nitrososphaeria	Nitrososphaerales	Thaumarchaeota lineage	<i>Nitrosoarchaeum</i>	<i>koreensis</i>	MY1	Nitrification	(Kim et al 2011)

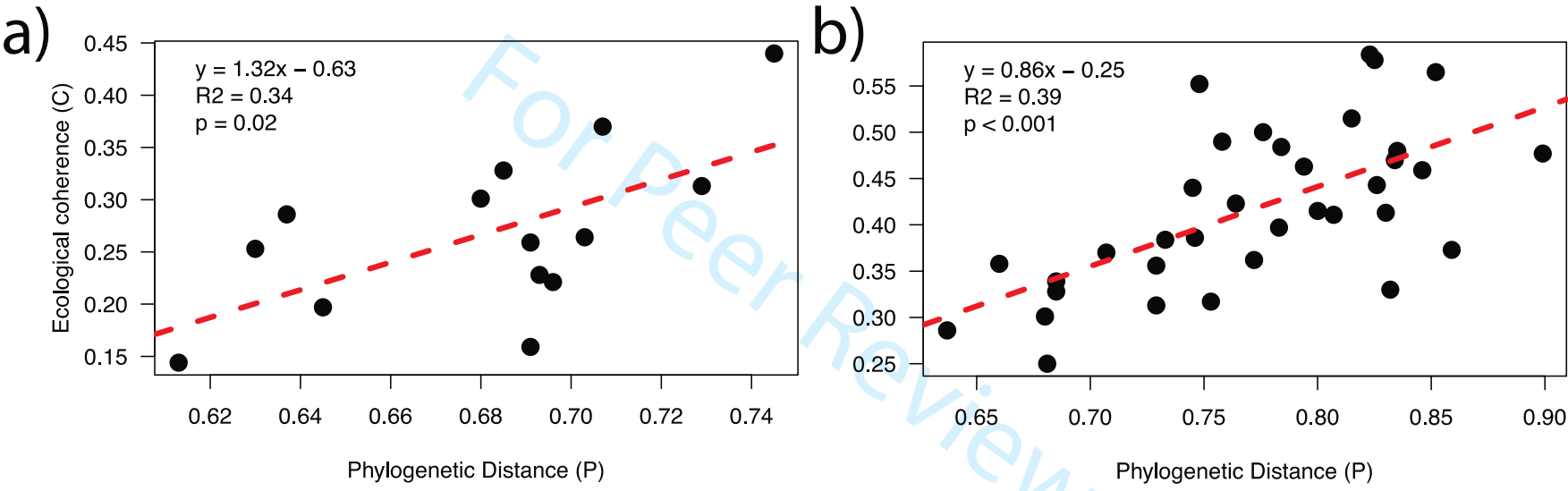
^aND: Not defined.



Supplementary Figure 1: Box and whisker plot comparing amino acid sequence similarity (%) within traits versus between traits, as determined by MCL. Amino acid sequences grouped together as traits were significantly more similar than those in different traits (Student's T test, $p = 2 \times 10^{-16}$).



Supplementary Figure 2: a) Neighbour joining tree of full length 16S rRNA genes of taxa analysed in this study. b) Phylogenetic distance (*P*) of each taxonomic group, ranging between 0.6 (dissimilar) and 0.9 (similar) determined from branch lengths of 16S rRNA gene analysis in a). *P* was calculated as ecological coherence (Eq 1.) excepting that branch lengths are in units of gene sequence similarity and not Bray-Curtis distance (*C*).



Supplementary Figure 3: a) Linear correlation between Phylogenetic Distance (*P*) derived from 16S rRNA genes and ecological coherence (*C*) of functional traits present in Phyla analysed in this study. b) Linear correlation at the level of Family.

Supplementary Table 2: Confusion matrix of Phylum level predictions based on Random Forest selection of important traits.

Successful classifications

	Acid.	Actino.	Alpha.	Bacter.	Beta.	Chloro.	Cyano.	Delta.	Eury.	Firm.	Gamma.	Planc.	Thaum.	Verruc.	Success rate (%)
Acidobacteria	6			1				1		2					60
Actinobacteria		33	1							1					95
Alphaproteobacteria			25												100
Bacteroidetes				9						1					90
Betaproteobacteria			2		11						2				74
Chloroflexi		1				0				3			1		0
Cyanobacteria		1					3			1					60
Deltaproteobacteria	1				1			8							80
Euryarchaeota									5						100
Firmicutes						2				23					92
Gammaproteobacteria			1		1					1	12				80
Planctomycetes	2							1		1		1			20
Thaumarchaeota										1			4		80
Verrucomicrobia	1			2										2	40

Supplementary Table 3: Confusion matrix of Family level predictions.

Phylum	Family	Mor.	Methyloc.	Pseu.	Methylocy.	Rhod.	Rhiz.	Brad.	Beij.	Myxo.	Poly.	Neis.	Nitr.	Burk.	Spor.	Leuc.	Clos.	Lact.	Bac.	Micrococ.	Microbac.	Success Rate (%)	
Gammaproteobacteria	Moraxellaceae	5																				100	
	Methylococcaceae		4																				80
Alphaproteobacteria	Pseudomonadaceae			4									1										80
	Methylocystaceae				3				2														60
	Rhodospirillaceae				1	4																	80
	Rhizobiaceae						4	1															80
	Bradyrhizobiaceae				2		1	2															40
	Beijerinckiaceae				1					4													80
Deltaproteobacteria	Myxococcaceae									5													100
	Polyangiaceae									1	3			1									60
Betaproteobacteria	Neisseriales Lineage											5											100
	Nitrosomonadaceae			1									4										80
Firmicutes	Burkholderiaceae			1								1		3									60
	Sporomusaceae														2								40
	Leuconostocaceae															5							100
	Clostridiaceae																4		1				80
	Lactobacillaceae															2		3					60
	Bacillaceae																1		4				80
Actinobacteria	Micrococcaceae																			3			60
	Microbacteriaceae						1														4		80
	Streptomycetaceae																			1			60
	Mycobacteriaceae																					1	100
	Frankiaceae																						60
Promicromonosporaceae																						100	

For Peer Review

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6		Cellulomonada																	
7		ceae															1		20
8	Bacteroidetes	Cytophagaceae																	40
9		Chitinophagaceae																	40
10		Divergent																	0
11	Acidobacteria	Acidobacteria							1		1								100
12		Acidobacteriaceae																	100
13	Cyanobacteria	Cyanobacteria																	100
14	Chloroflexi	Chloroflexi										1							80
15		Planctomycetales Lineage										1							40
16	Planctomycetes	Verrucomicrobia Lineage																	80
17	Verrucomicrobia	Thaumarchaeota Lineage																	80
18	Thaumarchaeota	Methanogen Lineage																	100
19	Euryarchaeota																		100

Supplementary Table 4: Confusion matrix of Family level predictions.

Phylum	Family	Strep.	Myco.	Fran.	Prom.	Cell.	Cyto.	Chitin.	Acido.Lin.	Acido.	Cyano.	Chloro.	Planc.	Verru.	Thaum.	Methan.	Success Rate (%)
Gammaproteobacteria	Moraxellaceae																100
	Methylococcaceae											1					80
	Pseudomonadaceae																80
Alphaproteobacteria	Methylocystaceae																60
	Rhodospirillaceae																80
	Rhizobiaceae																80
	Bradyrhizobiaceae																40
	Beijerinckiaceae																80
Deltaproteobacteria	Myxococcaceae																100
	Polyangiaceae																60
Betaproteobacteria	Neisseriales Lineage																100

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2										
3										
4										
5										
6										
7		Nitrosomonadaceae								80
8		Burkholderiaceae								60
9	Firmicutes	Sporomusaceae			1			1	1	40
10		Leuconostocaceae								100
11		Clostridiaceae								80
12		Lactobacillaceae								60
13		Bacillaceae								80
14		Micrococcaceae	1	1						60
15	Actinobacteria	Microbacteriaceae								80
16		Streptomycetaceae	3		1					60
17		Mycobacteriaceae		5						100
18		Frankiaceae	1	1	3					60
19		Promicromonosporaceae			5					100
20		Cellulomonadaceae			2	1			1	20
21	Bacteroidetes	Cytophagaceae					2	3		40
22		Chitinophagaceae					3	2		40
23	Acidobacteria	Divergent Acidobacteria					0	1	1	0
24		Acidobacteriaceae						5		100
25	Cyanobacteria	Cyanobacteria							5	100
26	Chloroflexi	Chloroflexi							4	80
27	Planctomycetes	Planctomycetales Lineage				1	1		2	40
28	Verrucomicrobia	Verrucomicrobia Lineage						1		80
29	Thaumarchaeota	Thaumarchaeota Lineage							4	80
30	Euryarchaeota	Methanogen Lineage								80
31										5
32										100
33										
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Supplementary Table 5: Confusion matrix of Proteobacterial Family predictions.

	Successful classifications													Success rate (%)
	Beij.	Brady.	Burkh.	Methylococ.	Methylocys.	Morax.	Myxo.	Neiss.	Nitroso.	Polyan.	Pseudo.	Rhizo.	Rhodo.	
Beijerinckiaceae	5													100
Bradyrhizobiaceae		1			2						1	1		20
Burkholderiaceae			4					1						80
Methylococcaceae				4					1					80
Methylocystaceae	3				2									40
Moraxellaceae						4		1						80
Myxococcaceae							5							100
Neisseriales lineage								4	1					80
Nitrosomonadaceae									4		1			80
Polyangiaceae			1				1			3				60
Pseudomonadaceae						1					3	1		60
Rhizobiaceae											1	4		80
Rhodospirillaceae					1								4	80

Supplementary Table 6: Confusion matrix of Actinobacterial Family predictions.

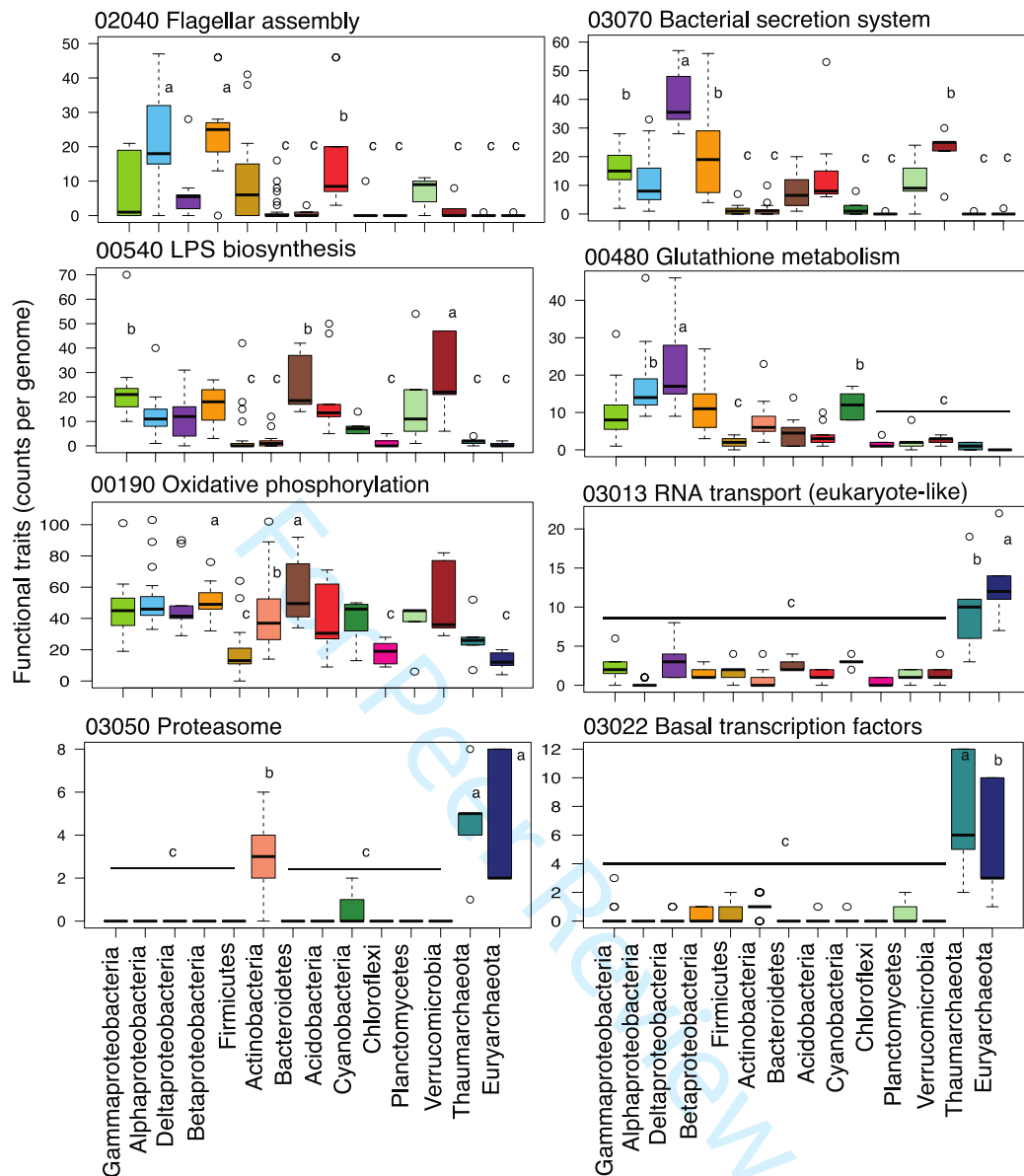
	Successful classifications						Success rate (%)	
	Cellu.	Frank.	Microbac.	Micrococ.	Mycobac.	Promicro.	Strepto.	
Cellulomonadaceae	2		1	1		1		40
Frankiaceae		3		1	1			60
Microbacteriaceae	1		3				1	60
Micrococcaceae				4			1	80
Mycobacteriaceae					5			100
Promicromonosporaceae						5		100
Streptomycetaceae				1			4	80

Supplementary Table 7: Confusion matrix of Firmicutes Family predictions.

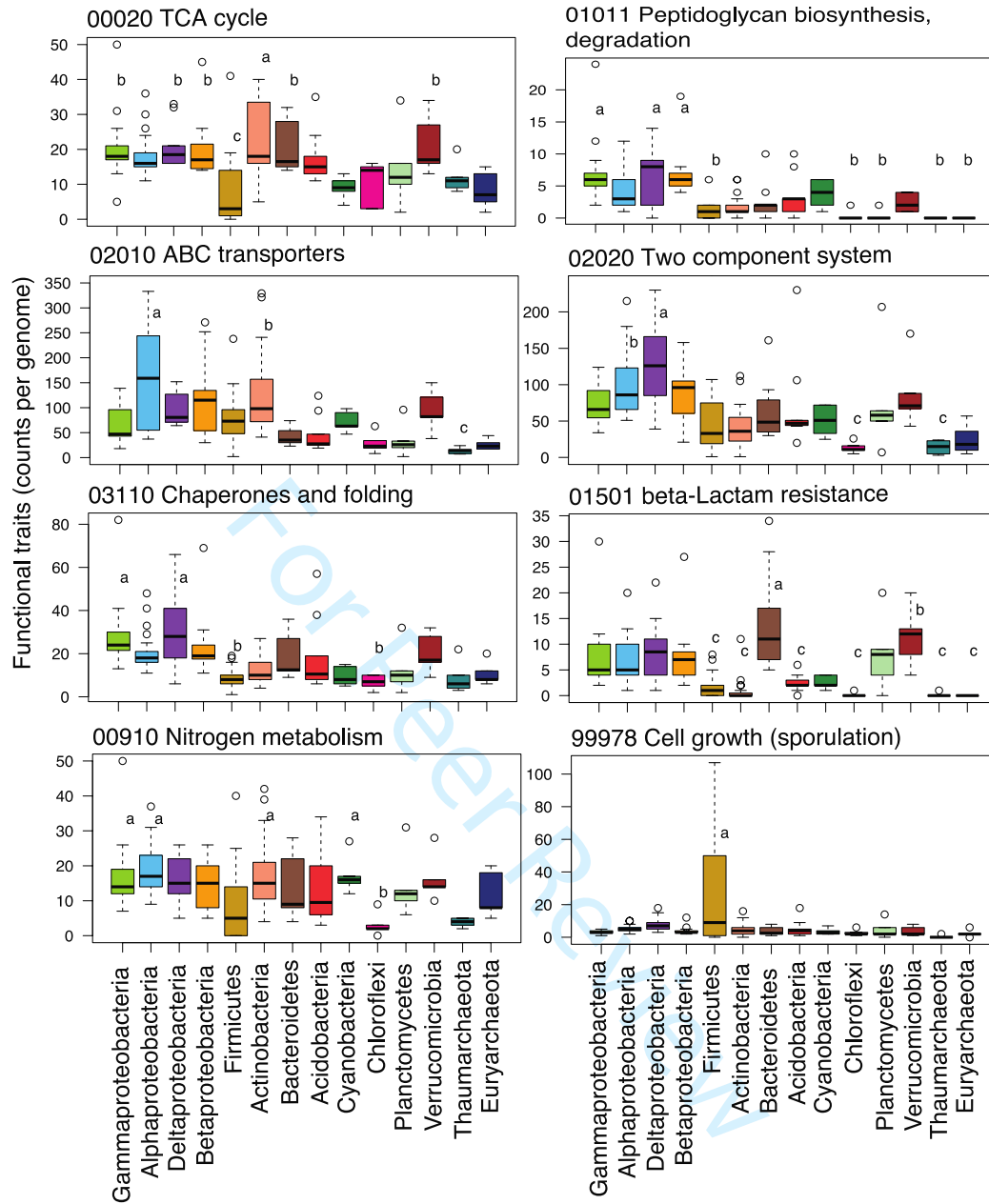
	Successful classifications					Success rate (%)
	Bacill.	Clostrid.	Lacto.	Leuco.	Sporo.	
Bacillaceae	4	1				80
Clostridiaceae	1	3	1			60
Lactobacillaceae			4	1		80
Leuconostocaceae				5		100
Sporomusaceae		1		1	3	60

Supplementary Table 8: Confusion matrix of 'Under-represented' Family predictions.
Successful classifications

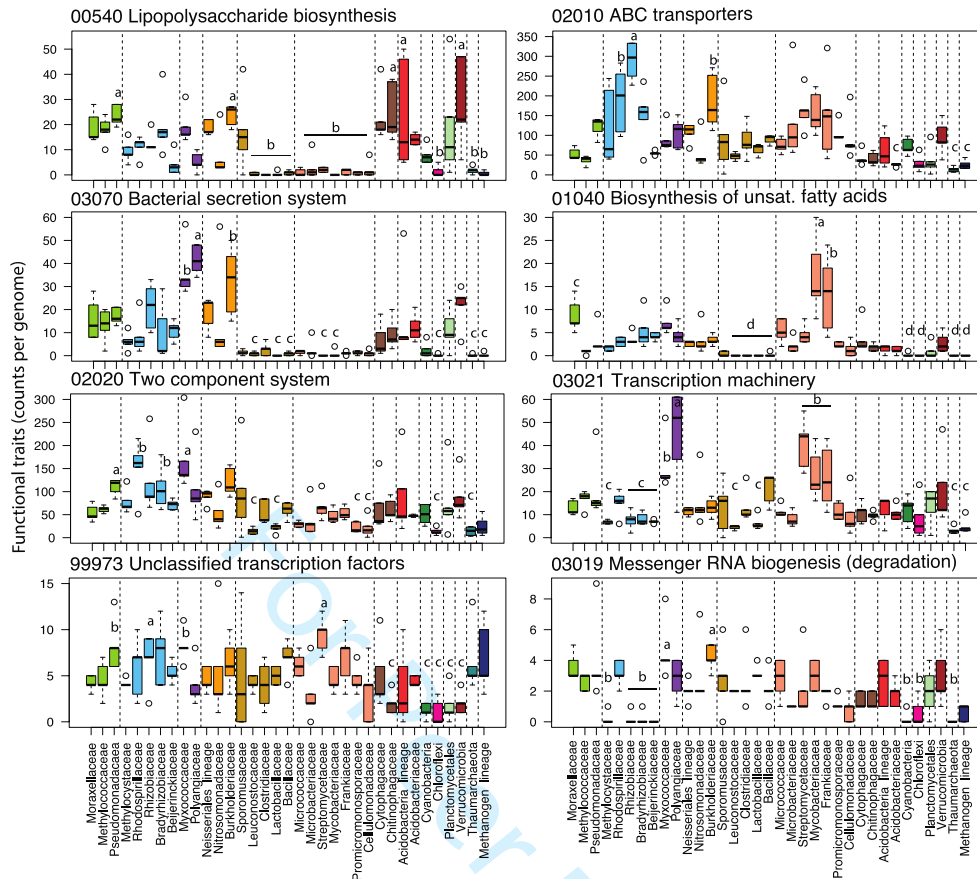
	Acid.	Chitin.	Chloro.	Cyano.	Cytoph.	Acido. Lin.	Methan.	Plancto.	Thaum.	Verruc.	Success rate (%)
Acidobacteriaceae	5										100
Chitinophagaceae		3			2						60
Chloroflexi			4					1			80
Cyanobacteria				5							100
Cytophagaceae		2			3						60
Acidobacteria Lineage	1		2							2	0
Methanogen Lineage							5				100
Planctomycetales			1			1		3			60
Thaumarchaeota			1						4		80
Verrucomicrobia	1									4	80



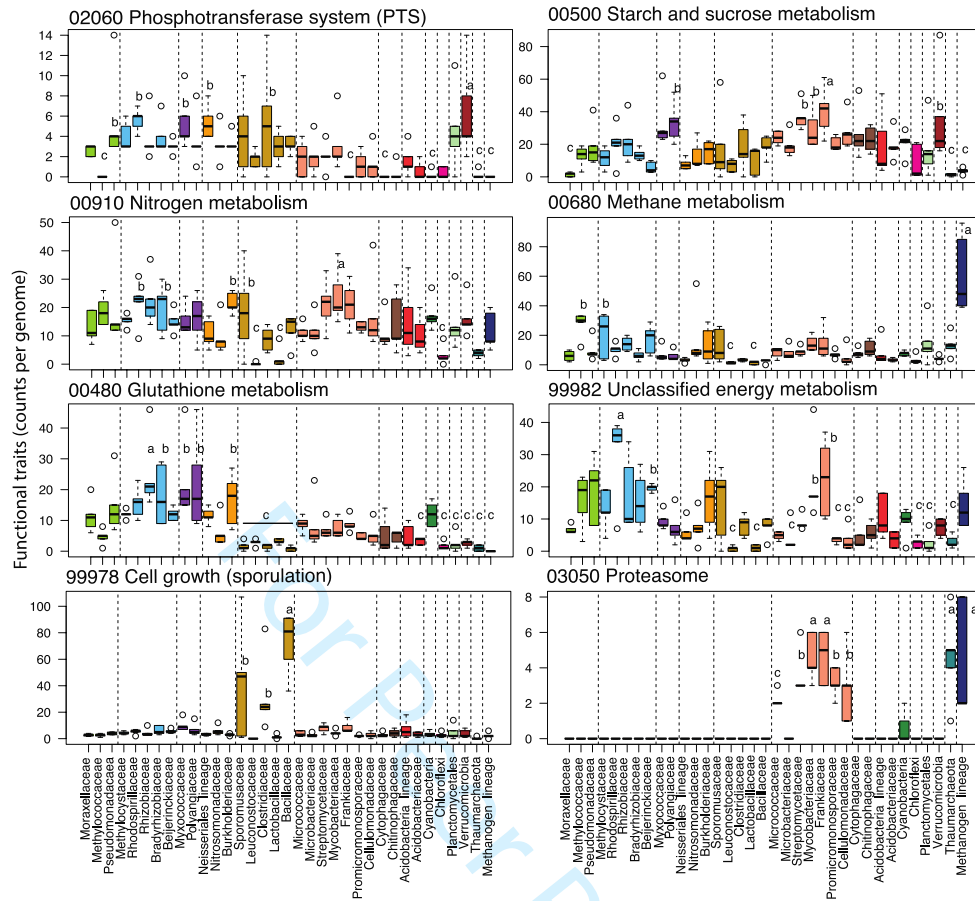
Supplementary Figure 4: Box and whisker plots of functional traits present in Phyla. Traits were selected based on being selected by the Phylum Random Forest model. Letters indicate significantly high or low ($p < 0.05$) counts based on LSD *post hoc* tests.



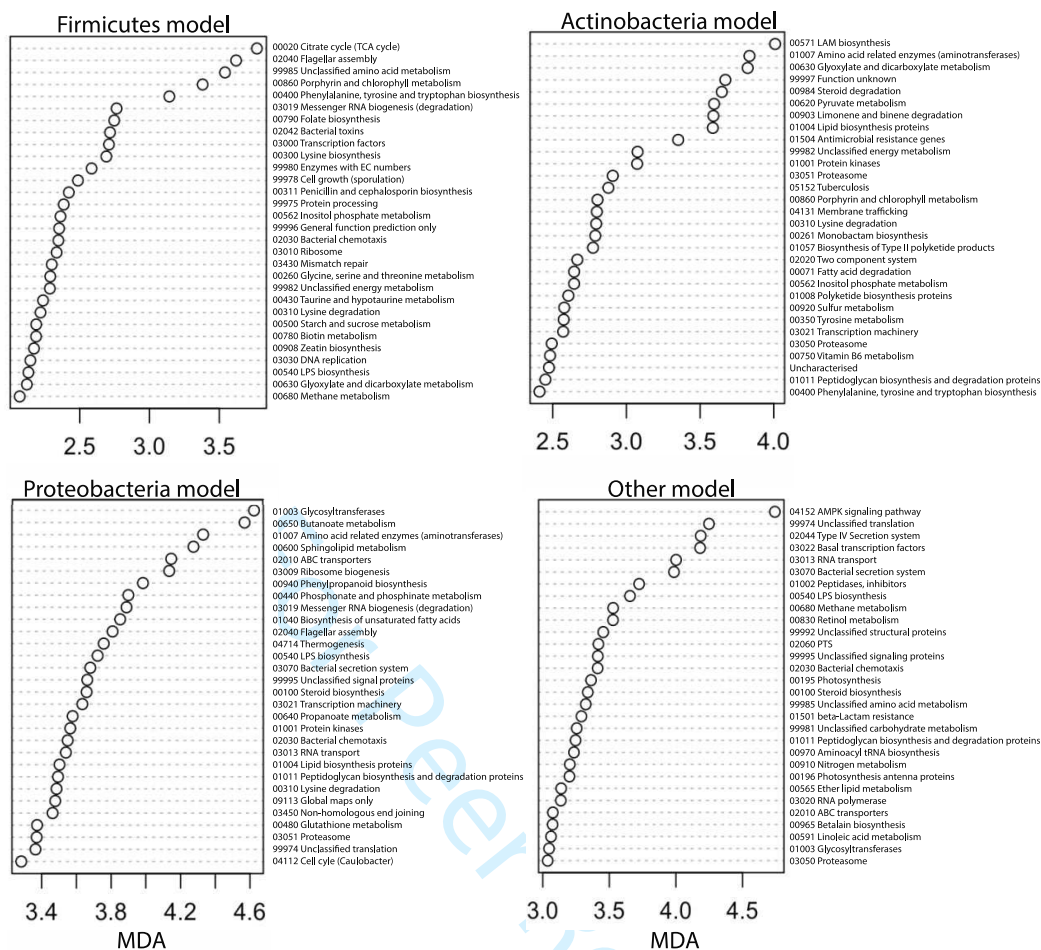
Supplementary Figure 5: Box and whisker plots of functional traits present in Phyla. Traits were selected based on being selected by the Phylum Random Forest model. Letters indicate significantly high or low ($p < 0.05$) counts based on LSD *post hoc* tests.



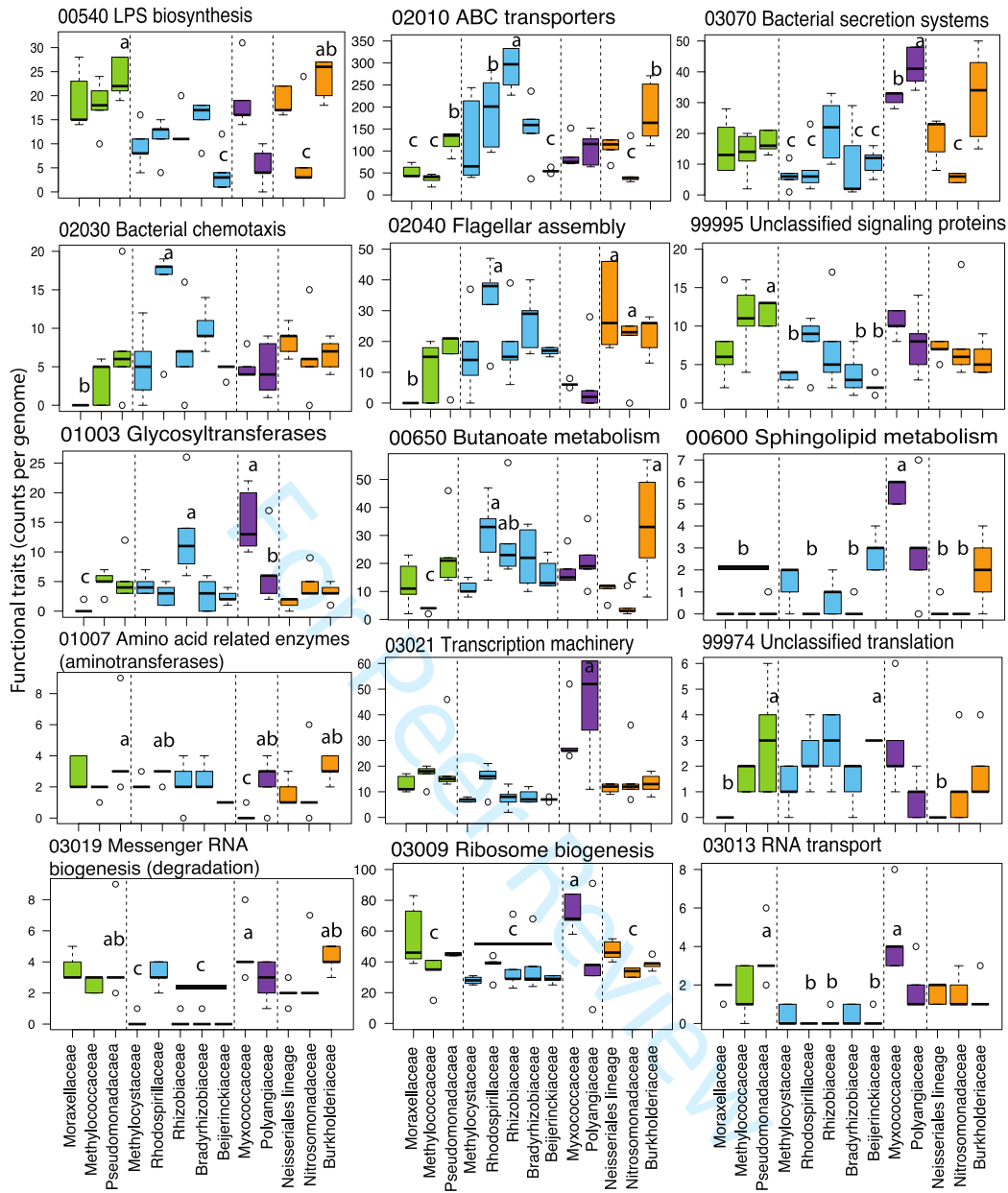
Supplementary Figure 6: Box and whisker plots of functional traits present in Families. Traits were selected based on being selected by the Family Random Forest model. Letters indicate significantly high or low ($p < 0.05$) counts based on LSD *post hoc* tests.



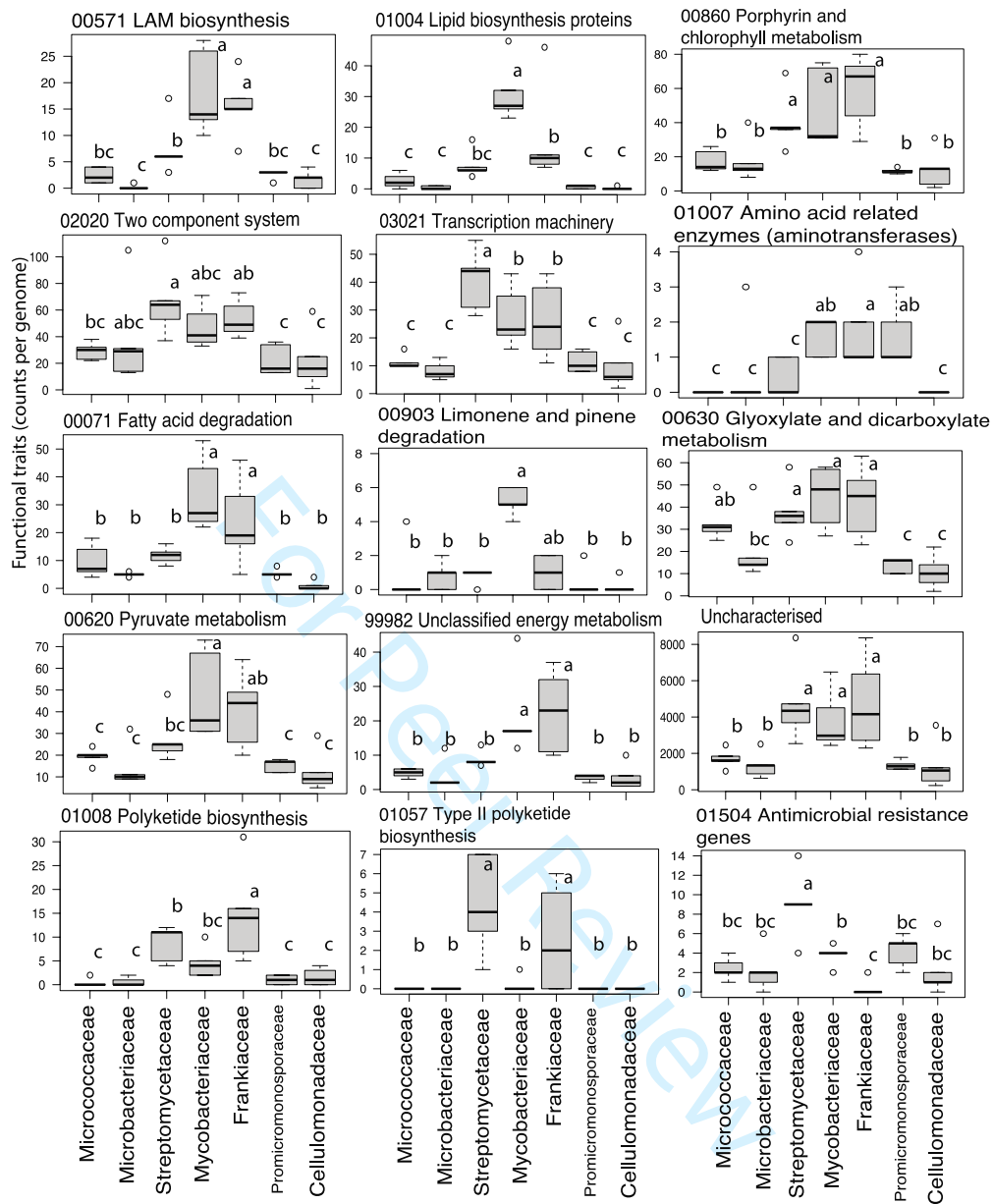
Supplementary Figure 7: Box and whisker plots of functional traits present in Families. Traits were selected based on being selected by the Family Random Forest model. Letters indicate significantly high or low ($p < 0.05$) counts based on LSD *post hoc* tests.



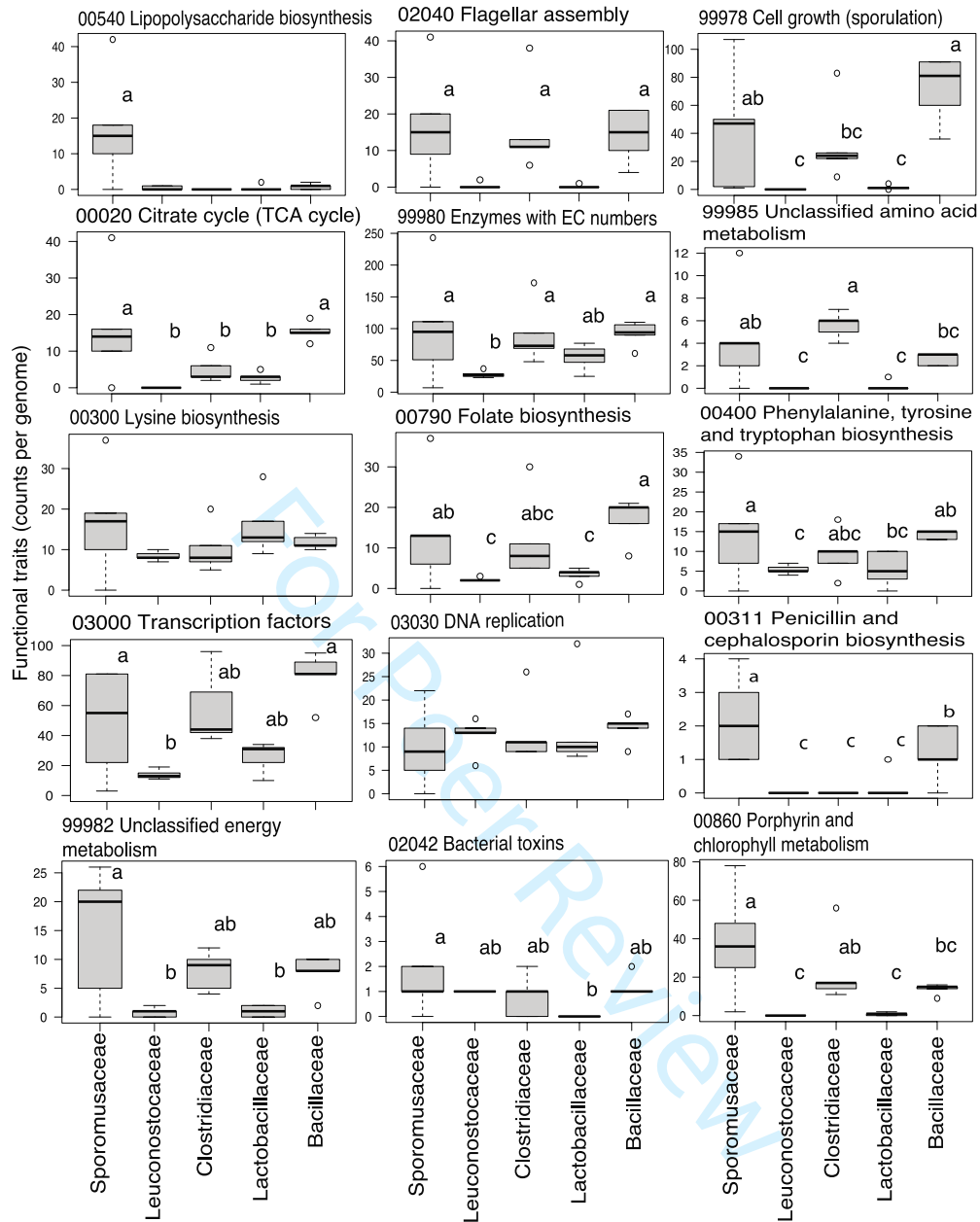
Supplementary Figure 8: Important predictor traits in classifying subgroups of Families as selected by the Proteobacteria, Actinobacteria, Firmicutes and Others model. Traits are ranked in order of decreasing Mean Decrease in Accuracy (MDA) of the four models.



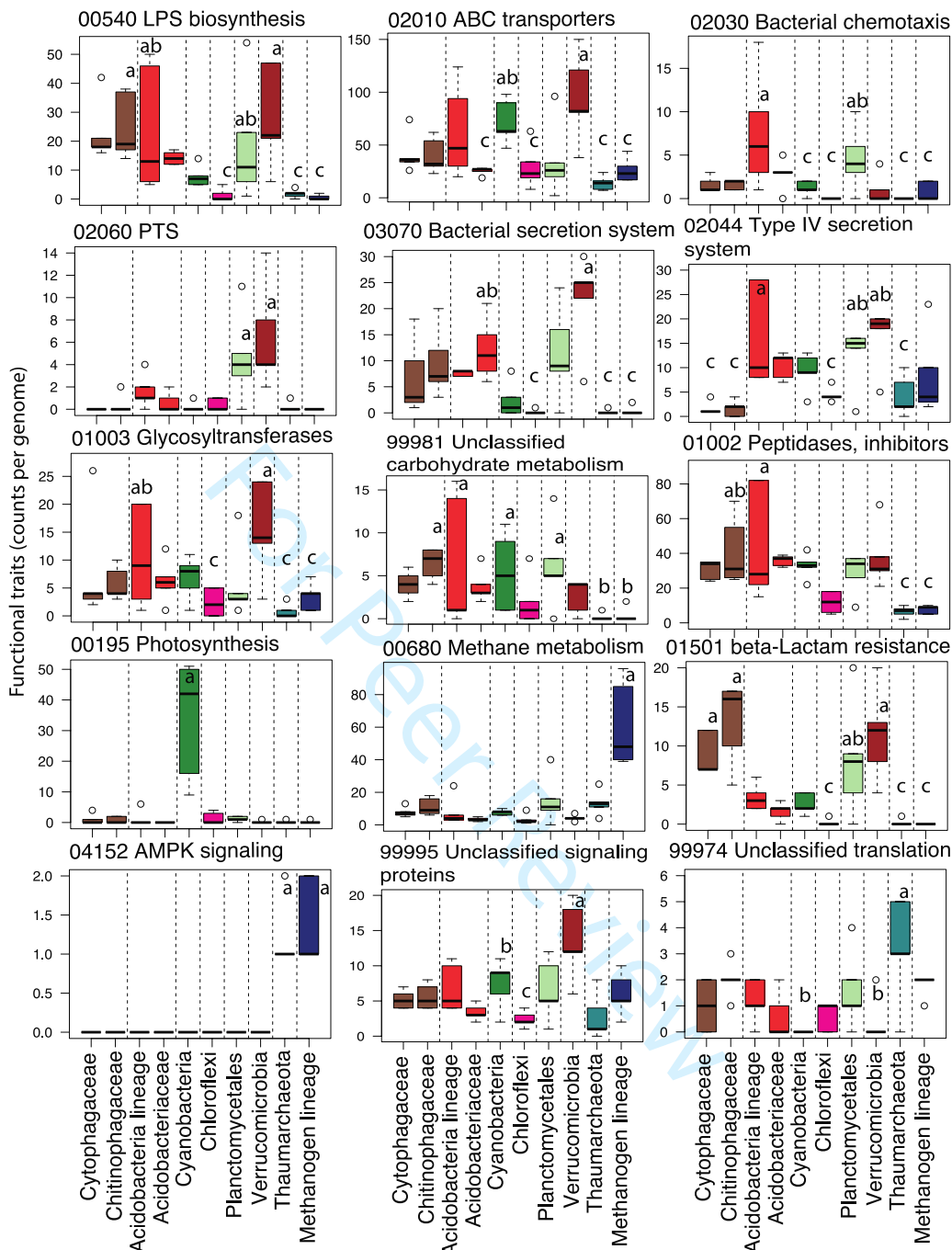
Supplementary Figure 9: Box and whisker plots of functional traits present in Proteobacterial Families. Traits were selected based on being selected by the Proteobacteria Family Random Forest model. Letters indicate significantly high or low ($p < 0.05$) counts based on LSD *post hoc* tests.



Supplementary Figure 10: Box and whisker plots of functional traits present in Actinobacterial Families. Traits were selected based on being selected by the Actinobacterial Family Random Forest model. Letters indicate significantly high or low ($p < 0.05$) counts based on LSD *post hoc* tests.



Supplementary Figure 11: Box and whisker plots of functional traits present in Firmicutes Families. Traits were selected based on being selected by the Firmicutes Family Random Forest model. Letters indicate significantly high or low ($p < 0.05$) counts based on LSD *post hoc* tests.



Supplementary Figure 12: Box and whisker plots of functional traits present in Under-represented Families. Traits were selected based on being selected by the Under-represented Family Random Forest model. Letters indicate significantly high or low ($p < 0.05$) counts based on LSD *post hoc* tests.

Supplementary Table 9: Summary of traits significantly enriched and depleted in the Phylum Random Forest model.

Model	BRITE 1	BRITE 3	Significantly enriched	Significantly depleted
Phylum	Metabolism	00020 TCA cycle	Gammaproteobacteria, Deltaproteobacteria, Betaproteobacteria, Actinobacteria, Bacteroidetes, Verrucomicrobia	Firmicutes, Euryarchaeota
		00190 Oxidative phosphorylation	Betaproteobacteria, Actinobacteria, Bacteroidetes	Firmicutes, Chloroflexi, Euryarchaeota
		00480 Glutathione metabolism	Alphaproteobacteria, Deltaproteobacteria, Cyanobacteria	Firmicutes, Chloroflexi, Planctomycetales, Verrucomicrobia, Thaumarchaeota, Euryarchaeota
		00910 Nitrogen metabolism	Gammaproteobacteria, Alphaproteobacteria, Actinobacteria, Cyanobacteria	Chloroflexi
		00540 LPS biosynthesis	Gammaproteobacteria, Bacteroidetes, Verrucomicrobia	Firmicutes, Actinobacteria, Chloroflexi, Thaumarchaeota, Euryarchaeota
		01011 Peptidoglycan biosynthesis, degradation	Gammaproteobacteria, Deltaproteobacteria, Betaproteobacteria	Firmicutes, Chloroflexi, Planctomycetales, Thaumarchaeota, Euryarchaeota
		01001 Protein kinases	Deltaproteobacteria, Planctomycetales	Alphaproteobacteria, Firmicutes, Thaumarchaeota, Euryarchaeota
		01040 Biosynthesis of unsaturated fatty acids	Deltaproteobacteria, Actinobacteria	Firmicutes, Chloroflexi, Thaumarchaeota, Euryarchaeota
		09113 Global maps only (unclassified metabolism)	Gammaproteobacteria, Actinobacteria, Thaumarchaeota	Firmicutes, Bacteroidetes, Acidobacteria, Cyanobacteria, Chloroflexi, Planctomycetales, Verrucomicrobia
		04614 Renin-angiotensin system	Deltaproteobacteria, Bacteroidetes	Gammaproteobacteria, Firmicutes, Thaumarchaeota
		00572 Arabinogalactan biosynthesis	Actinobacteria, Planctomycetales	Gammaproteobacteria, Alphaproteobacteria, Chloroflexi, Euryarchaeota
		00561 Glycerolipid metabolism	Deltaproteobacteria, Actinobacteria, Verrucomicrobia	Thaumarchaeota, Euryarchaeota
		00360 Phenylalanine metabolism	Gammaproteobacteria, Actinobacteria	Firmicutes, Acidobacteria, Cyanobacteria, Chloroflexi
		00960 Tropane, piperidine and pyridine alkaloid biosynthesis	Alphaproteobacteria, Betaproteobacteria, Euryarchaeota	Firmicutes, Planctomycetales
		00630 Glyoxylate and dicarboxylate metabolism	Alphaproteobacteria, Acidobacteria	Firmicutes, Chloroflexi, Thaumarchaeota, Euryarchaeota
	Environmental Information Processing	02010 ABC transporters	Alphaproteobacteria, Actinobacteria	Thaumarchaeota
		02020 Two component system	Alphaproteobacteria, Deltaproteobacteria	Chloroflexi, Thaumarchaeota
		03070 Bacterial secretion system	Gammaproteobacteria, Deltaproteobacteria, Betaproteobacteria, Verrucomicrobia	Firmicutes, Actinobacteria, Cyanobacteria, Chloroflexi, Thaumarchaeota, Euryarchaeota
		02044 Type IV secretion system	Gammaproteobacteria, Deltaproteobacteria, Betaproteobacteria, Verrucomicrobia	Firmicutes, Bacteroidetes, Chloroflexi, Thaumarchaeota
	Genetic Information Processing	03013 RNA transport (eukaryote-like)	Thaumarchaeota, Euryarchaeota	All other phyla

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	03022 Basal transcription factors	Thaumarchaeota, Euryarchaeota	All other phyla
	03050 Proteasome	Actinobacteria, Thaumarchaeota, Euryarchaeota	All other phyla
	03110 Chaperones and folding	Gammaproteobacteria, Deltaproteobacteria	Firmicutes, Chloroflexi
	03051 proteasome 20S subunit	Deltaproteobacteria, Actinobacteria	All other phyla
	03019 mRNA biogenesis (degradation)	Gammaproteobacteria, Deltaproteobacteria	Alphaproteobacteria, Cyanobacteria, Chloroflexi, Thaumarchaeota, Euryarchaeota
Signaling and Cellular Processes	99978 Cell growth (sporulation)	Firmicutes	All other phyla
	01501 beta-Lactam resistance	Bacteroidetes, Verrucomicrobia	Firmicutes, Actinobacteria, Acidobacteria, Chloroflexi, Thaumarchaeota, Euryarchaeota
	02040 Flagellar assembly	Alphaproteobacteria, Betaproteobacteria, Acidobacteria	Actinobacteria, Bacteroidetes, Cyanobacteria, Chloroflexi, Verrucomicrobia, Thaumarchaeota, Euryarchaeota
	02030 bacterial chemotaxis	Alphaproteobacteria, Betaproteobacteria	Actinobacteria, Chloroflexi, Thaumarchaeota
Not included in pathway or BRITE	99992 Unclassified structural proteins	Gammaproteobacteria, Betaproteobacteria	Firmicutes, Actinobacteria, Chloroflexi, Thaumarchaeota, Euryarchaeota

Supplementary Table 10: Summary of traits significantly enriched and depleted in Firmicutes and Actinobacteria models.

Model	BRITE 1	BRITE 3	Significantly high	Significantly low	
Firmicutes	Metabolism	00540 LPS biosynthesis	Sporomusaceae	All other families	
		00020 Citrate cycle (TCA cycle)	Sporomusaceae, Bacillaceae	All other families	
		99985 Unclassified amino acid metabolism	Clostridiaceae	Leuconostocaceae, Lactobacillaceae	
		00300 Lysine biosynthesis	All equal	All equal	
		00790 Folate biosynthesis	Bacillaceae	Leuconostocaceae, Lactobacillaceae	
		00400 Phenylalanine, tyrosine and tryptophan biosynthesis	Sporomusaceae	Leuconostocaceae	
		99982 Unclassified energy metabolism	Sporomusaceae	Leuconostocaceae, Lactobacillaceae	
		00860 Porphyrin and chlorophyll metabolism	Sporomusaceae	Leuconostocaceae, Lactobacillaceae	
		Genetic Information Processing	03000 Transcription factors	Sporomusaceae, Bacillaceae	Leuconostocaceae
		Signaling and Cellular Processes	03030 DNA replication	All equal	All equal
			02040 Flagellar assembly	Sporomusaceae, Clostridiaceae, Bacillaceae	Leuconostocaceae, Lactobacillaceae
			99978 Cell growth (sporulation)	Bacillaceae	Leuconostocaceae, Lactobacillaceae
			02042 Bacterial toxins	Sporomusaceae	Lactobacillaceae
00311 Penicillin and cephalosporin biosynthesis	Sporomusaceae, Bacillaceae		Leuconostocaceae, Lactobacillaceae, Clostridiaceae		
Enzymes	99980 Enzymes with EC numbers	Sporomusaceae, Clostridiaceae, Bacillaceae	Leuconostocaceae		
Actinobacteria	Metabolism	00571 LAM biosynthesis	Mycobacteriaceae, Frankiaceae	Microbacteriaceae, Cellulomonadaceae	
		01004 Lipid biosynthesis proteins	Mycobacteriaceae	Micrococcaceae, Microbacteriaceae, Promicromonosporaceae, Cellulomonadaceae	
		00860 Porphyrin and chlorophyll metabolism	Streptomycetaceae, Mycobacteriaceae, Frankiaceae	All other families	
		01007 Amino acid related enzymes (aminotransferases)	Frankiaceae	Micrococcaceae, Microbacteriaceae, Streptomycetaceae, Cellulomonadaceae	
		00071 Fatty acid degradation	Mycobacteriaceae, Frankiaceae	All other families	
		00903 Limonene and pinene degradation	Mycobacteriaceae	Micrococcaceae, Microbacteriaceae, Streptomycetaceae, Promicromonosporaceae, Cellulomonadaceae	
		00630 Glyoxylate and dicarboxylate metabolism	Streptomycetaceae, Mycobacteriaceae, Frankiaceae	Promicromonosporaceae, Cellulomonadaceae	

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		00620 Pyruvate metabolism	Mycobacteriaceae	Micrococcaceae, Microbacteriaceae, Promicromonosporaceae, Cellulomonadaceae
		99982 Unclassified energy metabolism	Mycobacteriaceae, Frankiaceae	All other families
		01008 Polyketide biosynthesis	Frankiaceae	Micrococcaceae, Microbacteriaceae, Promicromonosporaceae, Cellulomonadaceae
		01057 Type II polyketide biosynthesis	Streptomycetaceae, Frankiaceae	All other families
Environmental Information Processing		02020 Two component system	Streptomycetaceae	Promicromonosporaceae, Cellulomonadaceae
Genetic Information Processing		03021 Transcription machinery	Streptomycetaceae	Micrococcaceae, Microbacteriaceae, Promicromonosporaceae, Cellulomonadaceae
Signaling and Cellular Processes		01504 Antimicrobial resistance genes	Streptomycetaceae	Frankiaceae
	Uncharacterised	Uncharacterised	Streptomycetaceae, Mycobacteriaceae, Frankiaceae	All other families

Supplementary Table 11: Summary of traits significantly enriched and depleted in Proteobacteria and Others models.

Model	BRITE 1	BRITE 3	Significantly high	Significantly low	
Proteobacteria	Metabolism	00540 LPS biosynthesis	Pseudomonadaceae, Burkholderiaceae	Beijerinckiaceae, Nitrosomonadaceae	
		01003 Glycosyltransferases	Rhizobiaceae, Myxococcaceae, Polyangiaceae	Moraxellaceae	
		00650 Butanoate metabolism	Rhodospirillaceae, Rhizobiaceae, Burkholderiaceae	Methylococcaceae, Nitrosomonadaceae	
		00600 Sphingolipid metabolism	Myxococcaceae	All Gammaproteobacteria, Rhodospirillaceae, Bradyrhizobiaceae, Neisseriales Lineage, Nitrosomonadaceae	
		01007 Amino acid related enzymes (aminotransferases)	Pseudomonadaceae, Rhodospirillaceae, Polyangiaceae, Burkholderiaceae	Myxococcaceae	
		Environmental Information Processing	02010 ABC transporters	Pseudomonadaceae, Rhizobiaceae, Burkholderiaceae	Moraxellaceae, Methylococcaceae, Beijerinckiaceae, Nitrosomonadaceae
			03070 Bacterial secretion systems	Myxococcaceae, Polyangiaceae	Methylocystaceae, Rhodospirillaceae, Bradyrhizobiaceae, Beijerinckiaceae, Nitrosomonadaceae
		Genetic Information Processing	03021 Transcription machinery	Polyangiaceae	All other families
			99974 Unclassified translation	Pseudomonadaceae, Beijerinckiaceae	Moraxellaceae, Neisseriales Lineage
			03019 Messenger RNA biogenesis (degradation)	Pseudomonadaceae, Myxococcaceae, Burkholderiaceae	Methylocystaceae, Rhizobiaceae, Bradyrhizobiaceae, Beijerinckiaceae
			03009 Ribosome biogenesis	Myxococcaceae	Methylococcaceae, all Alphaproteobacteria, Nitrosomonadaceae
		Signaling and Cellular Processes	03013 RNA transport	Pseudomonadaceae, Myxococcaceae	Rhodospirillaceae, Rhizobiaceae, Beijerinckiaceae
			02030 Bacterial chemotaxis	Rhodospirillaceae	Moraxellaceae
			02040 Flagellar assembly	Rhodospirillaceae, Neisseriales Lineage, Nitrosomonadaceae	
99995 Unclassified signaling proteins	Pseudomonadaceae		Methylocystaceae, Bradyrhizobiaceae, Beijerinckiaceae		
Other	Metabolism	00540 LPS biosynthesis	Chitinophagaceae, Acidobacteria Lineage, Planctomycetales, Verrucomicrobia	Chloroflexi, Thaumarchaeota, Methanogen Lineage	
		01003 Glycosyltransferases	Acidobacteria Lineage, Verrucomicrobia	Chloroflexi, Thaumarchaeota, Methanogen Lineage	
		99981 Unclassified carbohydrate metabolism	Chitinophagaceae, Acidobacteria Lineage, Planctomycetales	Thaumarchaeota, Methanogen Lineage	
		01002 Peptidases, inhibitors	Chitinophagaceae, Acidobacteria Lineage	Thaumarchaeota, Methanogen Lineage	
		00195 Photosynthesis	Cyanobacteria	All other families	
		00680 Methane metabolism	Methanogen Lineage	All other families	
Environmental Information Processing	02010 ABC transporters	Cyanobacteria, Verrucomicrobia	Acidobacteriaceae, Chloroflexi, Thaumarchaeota, Methanogen Lineage		

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	02060 PTS	Planctomycetales, Verrucomicrobia	All other families
	04152 AMPK signaling	Thaumarchaeota, Methanogen Lineage	All other families
	03070 Bacterial secretion system	Acidobacteriaceae, Verrucomicrobia	Chloroflexi, Thaumarchaeota, Methanogen Lineage
	02044 Type IV Secretion system	Acidobacteria Lineage, Planctomycetales, Verrucomicrobia	Cytophagaceae, Chitinophagaceae, Chloroflexi, Thaumarchaeota
Genetic Information Processing	99974 Unclassified translation	Thaumarchaeota	Cyanobacteria, Verrucomicrobia
Signaling and Cellular Processes	02030 Bacterial chemotaxis	Acidobacteria Lineage, Planctomycetales	Cyanobacteria, Chloroflexi, Thaumarchaeota, Methanogen Lineage
	01501 beta-Lactam resistance	Cytophagaceae, Chitinophagaceae, Planctomycetales, Verrucomicrobia	Chloroflexi, Thaumarchaeota, Methanogen Lineage
	99995 Unclassified signaling proteins	Cyanobacteria	Chloroflexi

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3 Manuscript id: FEMSEC-21-01-0026

4 Title: Functional trait relationships demonstrate generalisable life strategies in
5 terrestrial prokaryotes.

6 Authors: Finn, Damien; Bergk-Pinto, Benoit; Hazard, Christina; Nicol, Graeme;
7 Tebbe, Christoph; Vogel, Tim
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11 Reviewers' Comments to Authors:
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13

14 **Reviewer: 1**

15 Comments to the Author

16 Review of the paper: Functional trait relationships demonstrate generalisable life
17 strategies in terrestrial prokaryotes.

18 This paper aims at identifying copiotrophic vs oligotrophic strategies among 170
19 species of bacteria and archaea by comparing their similarity in their traits (based on
20 clustering of functionally similar amino acid sequences). Instead of finding two clear
21 cluster reflecting copiotrophic-oligotrophic framework, they identify 5 clusters. Only
22 one of these clusters can be fit as copiotrophic. All other ones, reflect, as the author
23 suggest, unique life-history strategies on their own.

24 I really like the paper and I think it is worth publication for two reasons: 1) It
25 challenges simplistic views that entire kingdoms (or other higher taxa) of
26 microorganisms can be placed along a unique axis of energy use. 2) the method they
27 use will surely be used by other researchers. I also send my kudos to the authors to
28 develop a step-by-step repo explaining their method. Doing so, not only ensures
29 reproducibility but also encourage other scientist to test similar questions with their
30 approach.
31

32 I like it as it is. However, I want to acknowledge that I cannot judge on the
33 information of the natural history and ecology of many of the taxa included.
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40 Comment:

41 My only major suggestion is to make more explicit the way they test hypothesis 3:
42 "copiotrophic and oligotrophic groups would emerge based on trait enrichment".
43 (L149.150)
44

45 From what I understood in the method section (283-287) that hypothesis was tested
46 by subsetting 60 traits that emerge from their random forest clustering that are
47 "associated with copiotroph-oligotroph growth strategies". This association is based
48 "previous studies". Since this is a major hypothesis in the paper, I suggest to
49 summarize the traits as a table (at least in the supplementary) where it is indicated
50 which ones are expected to belong in copiotroph-oligotroph axis. Even better, it would
51 be great to have them grouped as a figure. A way to simplify this large number of traits
52 in a figure would be to group them according the Brite categories. Ultimately, I am
53 talking about some conceptual figure that shows the idealized clustering of copiotroph-
54 oligotroph based exactly on the 60 traits you use.
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60 Response:

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3 The authors would like to thank the reviewer for their incredibly generous comments
4 and the suggestion to create a conceptual figure summarising the Brite category
5 clustering per Family. Indeed, by trying to consider the best way to communicate our
6 results it has helped us to further synthesise them. Figure 5 is now a conceptual
7 diagram that attempts to show how the five identified clades overlap based on their
8 trait composition. This is either as a) a one-dimensional axis of 'resource investment',
9 analogous to the classical dichotomy of copiotroph-oligotroph, and b) a multi-
10 dimensional space where clades are further separated based on traits for both
11 'resource investment' and different means of 'resource acquisition'. By considering
12 both investment and acquisition together, we hope to show that the clades show less
13 overlap in space, which would theoretically allow them to occupy separate niches and
14 co-exist. We do not include all 60 Brite categories in this figure, but rather have chosen
15 some select traits (14 in total) that may be useful in demonstrating our point e.g. if a
16 taxon possesses many glycosyltransferases in addition to spore formation or
17 antimicrobial resistance genes it could be considered an 'oligotrophic degrader'. The
18 14 traits were partially chosen based on a comment from Reviewer 2, which suggested
19 to name major functional markers that could be used to predict a taxon's niche. Section
20 4.7 has been updated to incorporate and explain Figure 5 in the context of our results.

21 We are acutely aware that this conceptual figure may be met with criticism from the
22 scientific community, and we are more than happy to modify, adjust or otherwise take
23 on board any further constructive comments from the reviewer.
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34 Minor comment

35 L40-42. I think you can be bolder. I think your study shows that the copio-oligotroph
36 view is too simplistic and that more life-history strategies emerge once you compare
37 species based on their traits and not pre-conceived ideas of how microorganisms
38 should behave. I think that stating that the study "warrants a more nuanced view of
39 life-histories" is too soft and vague.
40
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43 Response:

44 The concluding sentences of the abstract have been amended to emphasise that the
45 copiotroph-oligotroph framework is insufficient to describe prokaryote niche, and that
46 we suggest considering traits involved in growth and resource acquisition in tandem
47 when predicting niche:
48
49

50 "However, the formation of five clades suggested a more nuanced view to describe
51 niche differentiation in terrestrial systems is necessary. We suggest considering traits
52 involved both in resource investment and acquisition when predicting niche."
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57 **Reviewer: 2**

58 Comments to the Author

59 I reviewed the manuscript "Functional trait relationships demonstrate generalisable
60

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3 life strategies in terrestrial prokaryotes” by Finn et al. The objective of the manuscript
4 is to identify the traits within soil bacterial and archaeal species that can distinguish
5 their lifestyle and niche occupancy. Genome sequence of 175 taxa from 11 Phyla
6 and 35 Families were used for analysis. The findings strongly suggested a non-
7 random distribution of traits across phylogenetic lineages. Random forest analysis
8 further identified traits that were directly related to niche colonization, substrate use
9 efficiency and metabolism within phylogenetic lineages.

10
11 I enjoyed reading the manuscript. The manuscript fits very well within the scope of
12 journal. Proper statistical tests have been performed to reach the conclusions.

13
14 Manuscript is very well written and presented. I have few suggestions that will further
15 strengthen the manuscript.
16
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20 Comment:

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22 1. There has been some work that has explored the genomic basis of tropic lifestyle
23 in soil bacteria. This work should be cited and discussed appropriately.
24 e.g. Trivedi et al. 2013. Trends in Microbiology.
25

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27 Response:

28
29 The authors would like to thank the reviewer for their generous comments. In regard
30 to this specific comment, references and discussions of Trivedi et al., 2013 have been
31 incorporated several times throughout the manuscript, for example:

32
33 Ln 121: “A genomic basis for traits associated with soils dominated by putative
34 copiotrophs and oligotrophs has been expertly reviewed elsewhere, and interested
35 readers are referred to Trivedi *et al.*, 2013 and references therein.”
36

37
38 Ln 639: “Comparative genomics analyses have also identified Actinobacteria,
39 Acidobacteria and Verrucomicrobia as being enriched in numerous enzymes for
40 cellulose, hemicellulose and starch catabolism (Trivedi *et al.*, 2013).”
41

42
43 And Ln 674: “Comparative genomics analyses of soil bacteria have also found
44 putatively copiotrophic Proteobacteria and Firmicutes to be particularly enriched in
45 PTS and ABC transporters (Trivedi *et al.*, 2013).”
46

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48 Furthermore, the study by Barbéran et al., 2012 to define ‘generalist’ and ‘specialist’
49 soil taxa based on co-occurrence has been incorporated in the Introduction:
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51
52 Ln 86: “... identifying habitat generalists and specialists based on taxon co-occurrence
53 patterns (Barbéran *et al.*, 2012) ...”
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57 Comment:

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59 2. Only five strains from family level classification were selected. Authors have
60 mentioned multiple times that some families will have a significant phylogenetic

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3 diversity within strains, while for others, it will be restricted. How do these differences
4 impact overall results? What was the criteria of selecting only 5 and not more?
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7 Response:

8 A minimum of five genomes per taxonomic group were selected to accommodate
9 Thaumarchaeota, Verrucomicrobia Chloroflexi and some others, which had the least
10 number of publicly available sequenced genomes of soil taxa. We specifically wanted
11 to avoid generating a dataset which favoured frequently isolated and sequenced soil
12 organisms (e.g. Bacilli, Rhizobia) and thus five genomes per group were used to
13 balance statistical analyses. This is mentioned at line 177 in the Methods section,
14 under Collection of terrestrial prokaryote genomes.
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19 Questions 2 and 3 from the reviewer have prompted additional analyses to quantify
20 the phylogenetic (dis)similarity of taxonomic groups based on full length 16S rRNA
21 genes, and to compare this to trait similarity (C). The 16S genes were aligned with
22 MUSCLE, simple Neighbour Joining trees were constructed in R with ape/phangorn
23 packages, and phylogenetic distance (P) calculated as per Equation 1, excepting that
24 P is in units of gene sequence similarity as opposed to Bray-Curtis distance for trait
25 similarity (C). Important considerations between P and C are as follows:
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30 1) Taxa were originally chosen in the hope of maximising P (and presumably C) per
31 Family by choosing different Genera where possible. This was not always possible,
32 and ultimately choices were constrained by what genomes were publicly available.
33 However, looking at values of P per Phylum and Family (Supplementary Figure 2),
34 there does not appear to be a consistent relationship between Families where different
35 Genera were included and P . For example, Lactobacillaceae consisted of 5
36 *Lactobacillus* spp. (P of 0.772) whereas the Beijerinckiaceae consisted of 4 different
37 genera that appear to be actually more similar to each other in regard to the 16S rRNA
38 gene (P of 0.852). In retrospect this lack of consistent variation in phylogenetic
39 distance between taxa based on pre-existing nomenclature should not be a surprise
40 at it is the reason for the efforts of the researchers involved with the Genome
41 Taxonomy Database who seek to standardise the current state of microbial taxonomy.
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47 2) There is a very nice linear relationship between P and C when considering taxa at
48 both the Phyla and Family levels (Supplementary Figure 3). As implied by the
49 reviewer, this will mean any Families that consist of more closely related taxa (*i.e.* high
50 P) will be more likely to be similar in regard to their traits.
51
52

53 3) The importance of this underlying relationship between the variation in phylogenetic
54 distance between taxa in a group and the results of the Random Forest models is,
55 however, uncertain. Supplementary Table 3 shows the Confusion Matrix for the Family
56 level Random Forest. Some Families with the poorest predictive capacity (< 50% taxa
57 correctly predicted) had quite high P (> 0.8), e.g. Bradyrhizobiaceae,
58 Cellulomonadaceae. Some Families with low P (< 0.7) had poor predictive capacity,
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3 e.g. Divergent Acidobacteria. Conversely, Families that had the highest predictive
4 capacity (100%) ranged in P from 0.68 – 0.9. It is the opinion of the authors that the
5 presence of specific, unique and consistent traits in a taxonomic group is far more
6 important for the RF results than the underlying relationship between phylogenetic
7 distance and trait composition. For example, the five Methanogen taxa had very low
8 phylogenetic distance (0.68) yet the RF was perfectly accurate in classifying them,
9 probably due to their methanogen metabolism and Eukaryote-like genetic traits.
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14 Nevertheless, we feel the reviewer raises a very important point when considering
15 taxon selection for these types of comparative genomic analyses, and in future studies
16 we shall place emphasis on standardising phylogenetic distance within groups. The
17 following has been added to the manuscript to highlight this:
18
19

20 In the Methods section 2.3 discussing UPGMA of trait similarity analysis:

21 Ln 291: “Furthermore, the full length 16S rRNA gene of each taxon was collated from
22 NCBI. Genes were aligned with MUSCLE (Edgar, 2004) and a Neighbour-Joining
23 phylogenetic tree was constructed with the ‘phangorn’ package in R. Phylogenetic
24 distance present in taxonomic groups (P) was measured as per Equation 1., excepting
25 that branch length was in units of DNA sequence similarity as opposed to Bray-Curtis
26 dissimilarity. Finally, simple linear regression was used to test a relationship between
27 P and C .”
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32 In the Results section discussing the UPGMA trait tree and RF models:

33 Ln 364: “A Neighbour-Joining tree of full length 16S rRNA genes showed that all taxa
34 clustered preferentially based on their taxonomic nomenclature at high taxonomic
35 rank, including the Chloroflexi, indicating that the discrepancies in the UPGMA were
36 not due to misclassification of the individual taxa (Supplementary Figure 2a).”
37
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40 Ln 388: “Phylogenetic distance (P) of each taxonomic group increased with
41 decreasing taxonomic rank, and was highest in Proteobacteria, Actinobacteria and
42 Firmicutes Families (Supplementary Figure 2b). There was a strong positive linear
43 relationship between P and C ($y = 0.86x - 0.25$, $R^2 = 0.39$, $p < 0.001$) demonstrating
44 that taxonomic groups of closer related taxa tended to share more similar
45 compositions of traits.”
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49 Ln 412: “Random Forest models were robust against variation in P within Families, for
50 example the nine families with 0% classification error ranged in P from the lowest
51 (0.68) to highest (0.9).”
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55 And finally in the Discussion section in regard to the robustness and interpretation of
56 the Random Forest approach:
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59 Ln 643: “Ideally the selection of individual taxa within groups for such future
60 comparative analyses would also be standardised based on phylogenetic distance,
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3 either with P or a similar method, which would improve the robustness of trait-based
4 comparisons at such a fine taxonomic level.”
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6

7 Comment:

8 **3. What were the criteria of selection of 5 species within a family e.g., phylogenetic**
9 **distance?**
10
11

12 Response:

13 As mentioned above, the selection criteria of the five taxa per Family was based
14 primarily on public availability of genomes. However, the authors acknowledge and
15 fully agree with the reviewers point that phylogenetic distance should be considered
16 as a means to standardise selection of taxa. While we are confident that our methods
17 are robust to the underlying relationship between P and C in the coarse Phyla and
18 Family taxonomic groups analysed here, we will incorporate a method for
19 standardising taxon selection in future studies that consider a finer detail of niche
20 differentiation between closely related Species and Strains.
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27 Comment:

28 **4. Lines 165-166: I like the idea of choosing strains that perform particular**
29 **functions. However, these functions are performed by specific bacterial lineages. My**
30 **question is whether these biased selections for traits inflate the closeness within a**
31 **group. Or in other words, if the other members within the families of the selected**
32 **functional groups were selected, do the results would have been the same?**
33
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36 Response:

37 This is an interesting question, and fortunately our results have several functional
38 groups that can be compared to address it. For example, to our knowledge, all
39 characterised members of Nitrosomonadaceae are chemolithoautotrophic nitrifiers
40 while the Cyanobacteria perform photosynthesis. Thus, the relatively high value of C
41 measured here for these groups that share common functions is not surprising.
42 However, trait variation certainly exists within other functional groups, for example the
43 five Methanogens (isolated from diverse anaerobic habitats including rice rhizosphere,
44 tropical and boreal wetlands) had an intermediate value of C . Perhaps the best
45 example is that the inclusion of functionally distinct methanotrophs, methylotrophs and
46 heterotrophs within the Beijerinckiaceae did not have a negative effect on its C , which
47 yielded one of the highest values (discussed at Ln 524). Ultimately trait composition
48 will be influenced by a taxon's functional group (e.g. ammonia oxidiser, heterotroph,
49 etc), its evolutionary life-history (as a very general example Bacteria, Archaea) and
50 the environment from where the taxon was isolated.
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57 We have included a statement this effect in the Discussion, which is an interesting and
58 important concept to consider:
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3 Ln 569: “The differing values of C for Methanogen and photosynthetic Cyanobacteria
4 functional groups (0.33 and 0.44, respectively) is also worthy of note. Despite all five
5 taxa in each group performing the same core role in a community, the individual
6 isolates came from varying environments. The methanogens were isolated from a
7 range of geographically separate wetlands, rice paddy soil and farm slurry and, while
8 the Cyanobacteria were also isolated from geographically separate environments,
9 they were all from sandy deserts or other nutrient poor, arid soils (Supplementary
10 Table 1 and references therein). Ultimately a taxon’s trait composition will be affected
11 by its functional role in a community, its evolutionary life-history (e.g. Beijerinckiaceae
12 described above) and its local environment.”
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19 Comment:

20 5. Like Lauro et al. 2009, is there a way to do a self-organizing map or a constrained
21 analysis showing the separation of different phylogenetic groups according to the
22 traits?
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24
25

26 Response:

27 The constrained analyses of Lauro *et al.*, 2009 revolved around the *a priori*
28 classification of one genome as ‘copiotrophic’ and one genome as ‘oligotrophic’.
29 Unknown genomes were then placed somewhere between these two based on
30 similarity of shared traits. In this study we specifically sought to avoid any
31 preconceived *a priori* assumptions of ‘taxon A is a copiotroph’, and instead opted for
32 unconstrained, exploratory cluster-based statistical techniques (e.g. dendrograms,
33 Random Forest) that would let the data speak for itself. We hoped that the UPGMA
34 dendrogram (Figure 2a) would be the simplest and most flexible way to demonstrate
35 relationships between different phylogenetic groups and their trait composition, while
36 the clustering of taxonomic groups based on the counts of specific copiotroph-
37 oligotroph traits would validate whether certain taxa could be considered copiotrophic
38 (Figure 4).
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45 Comment:

46 6. Based on the results, can you substantiate the phylogenetic grouping of
47 copiotroph/oligotroph groups? I think yes, there is much information, but I would like
48 to have a strong statement suggesting that based on the results, the inclusion of X, Y,
49 and Z phylum as copiotroph was validated. Then can you resolve the debate about
50 the inclusion of groups such as Alpha and betaproteobacteria as copiotroph/oligotroph
51 (Lines 104-105). See 5 as the analysis suggested there can help you to address this
52 question. This information will help the researchers that want to link microbial
53 community composition to soil processes, particularly soil C cycling.
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59 Response:
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3 A definitive statement outlining which groups were confirmed as copiotrophs and
4 oligotrophs has been included in the Discussion. This also explicitly links to how these
5 results can give insight into soil processes such as C cycling:
6
7

8
9 Ln 872: "These results support previous observations that Rhodospirillaceae,
10 Bradyrhizobiaceae, Burkholderiaceae, Pseudomonadaceae and Rhizobiaceae are
11 copiotrophic, while Planctomycetes, Verrucomicrobia, Myxococcaceae,
12 Polyangiaceae and Acidobacteria are oligotrophic (Ho *et al.*, 2017 and references
13 therein). As has been proposed previously, the dominance of these groups in certain
14 soils can provide inferences for ecosystem processes in that system, for example soils
15 dominated by Verrucomicrobia, Planctomycetes and Acidobacteria will have greater
16 capacity to degrade complex plant material while retaining most catabolised carbon in
17 biomass (*i.e.* high growth or carbon use efficiency) or excreted byproducts that assist
18 in soil aggregation (*e.g.* high LPS production) (Trivedi *et al.*, 2013). Conversely, soils
19 dominated by copiotrophic Proteobacteria Families will be systems primarily
20 dependent on labile di- and monosaccharides that demonstrate low carbon use
21 efficiency."
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27 Resolving the outstanding question of taxonomic groups that do not fit into the
28 classical copiotroph-oligotroph framework is addressed further below as a conceptual
29 figure (Comment 11).
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33 Comment:

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35 7. One important trait that distinguish copiotrophs/oligotrophs is the carbon use
36 efficiency (CUE). There have been some efforts to incorporate microbial CUE to
37 parameterize models for soil carbon. While CUE's topic is touched briefly in
38 Introduction, it has not been explicitly discussed in later sections.
39
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41 Response:

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43 Carbon use efficiency has now been mentioned in regard to the specific
44 copiotrophic/oligotrophic taxonomic groups that will have a high CUE (please see the
45 above response to Comment 6).
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49 Comment:

50
51 8. Authors talk about MAGs in the discussion section. It will be worth discussing
52 how MAGs will help to elaborate similar studies that is reported. Where we are and
53 what needs to be done further?
54
55

56 Response:

57
58 The authors agree that this is worth expanding upon. The following two statements
59 have been added to the Discussion to better incorporate the value of MAGs and
60 current gaps for trait-based analyses:

Ln 560: “The generation of MAGs has emerged as a useful tool for identifying traits necessary for life in such environments, and particularly for expanding knowledge of severely under-represented, difficult to culture taxonomic groups. For example, the recent reconstruction of 52, 515 MAGs from a wide range of host-associated and environmental metagenomes was able to increase genomic information of Planctomycetes and Verrucomicrobia by 79% and 68%, respectively (Nayfach et al., 2021). Importantly, both 16S rRNA gene surveys and MAGs demonstrate that some functional traits that facilitate life under certain environmental conditions are intrinsically linked to taxonomy.”

Ln 970: “However, to truly unravel differentiated niches and general microbial life strategies, two limitations must be overcome. Firstly, a better understanding of the many ‘Uncharacterised’ traits in environmental isolates is required. For example, the recent large-scale MAG study by Nayfach *et al.*, (2021) identified 5.8 million protein clusters (traits), of which over 75% could not be annotated meaningfully by current protein databases. Secondly, robust trait-based analyses down to the finer scale of distinct genomes will likely be necessary to consider how individual members of a community have either differentiated in order to co-exist or are in the throes of competition that will ultimately exclude one of the competitors.”

Comment:

9. Based on the results, where will you fit archaea (copiotrophs or oligotrophs)?

Response:

The authors are hesitant to place Archaea as either copiotrophs or oligotrophs as we consider the one-dimensional framework to be insufficient for describing niche. Please see further below for a detailed response (Comment 11).

Comment:

10. How this type of data will be used to distill the complexity within the soil microbiome functions? Can we use the functional signatures to determine the metabolic potential of microbial communities from metagenome data? How can this information help us to incorporate microbial activities into process models? I suggest touching on these in conclusions.

Response:

Again, this will be addressed further in Comment 11.

Comment:

11. One of the hallmarks of impactful papers is to move beyond information to

1
2
3 knowledge. Can you think of making a conceptual figure showing different genes and
4 how they are responsible for different lifestyles or niche colonization? This is just a
5 thought as I understand how difficult it can be.
6
7

8 Response:

9
10 The authors particularly appreciated this comment, and in particular the expression
11 'moving beyond information to knowledge'! Indeed, our attempts to consider a
12 conceptual figure that conveys our results in a simple manner has been extremely
13 helpful in further synthesising them.
14
15

16 Figure 5 is now a conceptual diagram that attempts to show how the five identified
17 clades overlap based on their trait composition. This is either as a) a one-dimensional
18 axis of 'resource investment', analogous to the classical dichotomy of copiotroph-
19 oligotroph, and b) a multi-dimensional space where clades are further separated
20 based on traits for both 'resource investment' and different means of 'resource
21 acquisition'. By considering both investment and acquisition together, we hope to
22 show that the clades show less overlap in space, which would theoretically allow them
23 to occupy separate niches and co-exist. The final section of the Discussion, from Ln
24 916, has been expanded to discuss this figure and its significance for interpreting
25 microbial niche.
26
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31 In regard to Comment 10, which asks how our results may provide signatures for
32 process-based models or linking to community function, we have chosen the BRITE
33 categories in Figure 5 to act as signatures for defining, for example, Rhizobiaceae as
34 'copiotrophic competitors'. This is explicitly stated:
35
36
37

38 Ln 929: "Figure 5b is a conceptual diagram of niche space where clades have been
39 further separated along additional axes based on their enrichment of traits involved in
40 competition, degradation or specialised metabolic pathways. The BRITE categories
41 listed on the various axes are chosen to be useful markers in predicting the niche of a
42 taxon. This expanded niche space would suggest taxa in Clade I are well equipped for
43 nutrient acquisition (primarily, but not limited to, di- and monosaccharides), rapid
44 growth and oxidative stress regulation as 'copiotrophic competitors'. ..."
45
46
47

48 And finally, specifically in regard to whether Archaea should be copiotrophs or
49 oligotrophs:
50
51

52 Ln 956: "The large space conceptualised for Clade V in Figure 5b, which does not
53 imply either copiotrophic or oligotrophic resource investment, is an oversimplification
54 and with improved understanding of traits in these taxa it may be possible to fracture
55 and further separate them into more detailed groups. This would prove particularly
56 beneficial for the poorly characterised Archaea."
57
58
59
60

1
2
3 Comment:

4 12. I appreciate the authors of acknowledging the tedious work of other researchers
5 on culturing. This is very important.
6
7

8 Response:

9 Of course we are happy to do so, as it is essential work.
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For Peer Review

1
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3 1 Functional trait relationships demonstrate ~~generalisable~~-life strategies in terrestrial
4
5 2 prokaryotes.

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7 3
8 4 Damien R. Finn^{1,2,3}, Benoît Bergk-Pinto², Christina Hazard², Graeme W. Nicol²,
9
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32
33 18
34 19 Keywords:

35
36 20 Theoretical ecology, niche differentiation, copiotroph-oligotroph, Random Forest
37
38 21 modelling

39
40 22
41 23
42 24 Abstract

43
44 25 Functional, physiological traits are the underlying drivers of niche differentiation. A
45
46 26 common framework related to niches occupied by terrestrial prokaryotes is based on
47
48 27 copiotrophy or oligotrophy, where ~~genomic-resource~~ investment is primarily in either
49
50 28 ~~metabolism-rapid growth~~ or stress tolerance, respectively. A quantitative trait-based
51
52 29 approach ~~was employed to demonstrate quantitativesought~~ relationships between
53
54 30 taxa, traits and niche in terrestrial prokaryotes. With 175 taxa from 11 Phyla and 35
55
56 31 Families ($n = 5$ per Family), traits were considered as discrete counts of shared
57
58 32 genome-encoded proteins. ~~The composition of 220 664 traits~~ Trait composition strongly
59
60 33 supported non-random trait-functional distributions as preferential clustering of related
34
34 34 taxa via unweighted pair-group method with arithmetic mean. Trait similarity between

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3 35 taxa increased as taxonomic rank decreased. A suite of Random Forest models
4 identified traits significantly enriched or depleted in taxonomic groups. Many
5 traits These traits conveyed functions related to metabolism rapid growth, nutrient
6 acquisition and stress tolerance consistent with their presence in copiotroph-oligotroph
7 niches. Finally, h Hierarchical clustering of these traits identified a clade of competitive,
8 copiotrophic Families resilient to oxidative stress *versus* glycosyltransferase-enriched
9 oligotrophic Families resistant to antimicrobials and environmental stress. However,
10 the formation of five clades, including an Actinobacteria and 'specialised metabolic
11 function' clade, warrants suggested a more nuanced view of 'life strategies' to describe
12 niche differentiation in terrestrial systems is necessary. We suggest considering traits
13 involved in both resource investment and acquisition when predicting niche.
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27 48 1. Introduction

28 49 Niche differentiation, the process of physiologically distinct organisms adapting better
29 50 to certain conditions, is a contributing factor to the high biodiversity inherent in microbial
30 51 communities (Prosser, 2012). Such differentiation is likely an inevitable consequence
31 52 of the principles of competitive exclusion and natural selection working in tandem – no
32 53 two organisms can theoretically occupy the same niche, as the poorer competitor must
33 54 either adapt to a unique niche or be driven to extinction in that system (Gause, 1932,
34 55 Hutchinson, 1957, Leibold, 1995). The physiological traits driving niche differentiation
35 56 must have the capacity to convey an advantage to the organism's ability to survive and
36 57 reproduce (*i.e.* fitness) and be inherited by successive generations (McGill *et al.*, 2006).
37 58 Importantly, this implies that microbial communities are not only diverse in terms of
38 59 individual 16S rRNA gene sequences, commonly used to assess community diversity,
39 60 but also diverse in regard to their physiological traits.

40 61 Explaining niche differentiation through the functional, physiological traits present in
41 62 ecological community members has a long history in macroecology. For example,
42 63 differences between beak size and shape differed between in Galápagos finches was
43 64 instrumental in Darwin's hypothesis that a common ancestor had differentiated into
44 65 multiple, island-specific species. Within the past century, trait-based analyses have
45 66 been particularly predominant in plant ecology, with seed germination in submerged
46 67 soil, salt tolerance, carbon to nitrogen biomass stoichiometry, and leaf mass per unit
47 68 area acting as examples of traits linked to niche differentiation (Gleason, 1926, Grime,

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3 69 1979, Keddy, 1992). In contrast, trait-based approaches to explain microbial ecology
4
5 70 have only been performed in few instances, such as conceptualizing niches of
6
7 71 methanotrophs based on abundance in high *versus* low methane environments or
8
9 72 disturbed *versus* undisturbed soils (Ho *et al.*, 2013), correlating increasing growth rate
10
11 73 with increasing ribosomal gene and ribosome-associated gene copy number (Vieira-
12
13 74 Silva & Rocha, 2010), deterministic modelling of nitrification rate based on ammonia
14
15 75 and oxygen uptake rate, temperature sensitivity and growth rate (Bouskill *et al.*, 2012),
16
17 76 defining distinct niches of 32 marine microorganisms based on clustering of genome-
18
19 77 encoded functional proteins (Lauro *et al.*, 2009), [identifying habitat generalists and](#)
20
21 78 [specialists based on taxon co-occurrence patterns \(Barb eran *et al.*, 2012\)](#) and recently
22
23 79 comparisons of 23 'core' traits (e.g. motility, carbon metabolism, optimal pH for growth)
24
25 80 across 15 000 diverse host-associated and environmental genomes (Madin *et al.*,
26
27 81 2020).

28
29 82 A consistent trend noted in macroecology is that traits linked to how carbon and
30
31 83 energy is processed and allocated to biomass can describe separate niches (Brown *et*
32
33 84 *al.*, 2004). The canonical example are *r* and *K* strategists, where carbon and energy
34
35 85 are primarily invested in reproduction, or alternatively invested in tolerating biotic
36
37 86 and/or abiotic stressors, respectively (Grime, 1977). These dichotomous strategies
38
39 87 have been observed in microbial ecology: copiotrophs are considered as
40
41 88 microorganisms with relatively high growth rates that have relatively poor growth
42
43 89 efficiency (as carbon incorporated to biomass per unit resource), relatively high cell
44
45 90 maintenance energy costs, dependence on relatively high concentrations of organic
46
47 91 carbon in their environment, demonstrate rapid population blooms upon the addition of
48
49 92 organic matter and are not overly tolerant of abiotic stress (Semenov, 1991, Koch,
50
51 93 2001, Roller & Schmidt, 2015, Ho *et al.*, 2017). Oligotrophs are considered as the
52
53 94 inverse – low growth rate, high growth efficiency, low cell maintenance energy
54
55 95 requirements, high substrate uptake affinity, [slow](#) growth ~~at a slow~~ yet [at a](#) consistent
56
57 96 rate and are resilient to abiotic stress. Although the niche concept in macroecology has
58
59 97 a formalized definition founded on where a taxon can maintain a stable population
60
61 98 within multi-dimensional environmental space (Leibold, 1995), in this study, niche is
62
63 99 used simply to distinguish between prokaryotes being relatively more copiotrophic
64
65 100 *versus* oligotrophic.

66
67 101 These distinct niches became associated with specific terrestrial taxa at high
68
69 102 taxonomic rank based on recent molecular analyses. In complex microbial

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2
3 103 communities, the relative abundance of Gammaproteobacteria, Bacteroidetes and
4 104 Actinobacteria were correlated with rapid growth in response to the addition of labile
5 105 organic matter or nitrogen (copiotrophs) (Fierer *et al.*, 2007, Goldfarb *et al.*, 2011,
6 106 Fierer *et al.*, 2012, Leff *et al.*, 2015). Conversely, the Deltaproteobacteria,
7 107 Acidobacteria, Verrucomicrobia and Planctomycetes were negatively correlated with
8 108 the addition of organic matter or nitrogen (oligotrophs) (Fierer *et al.*, 2007, Fierer *et al.*,
9 109 2012, Leff *et al.*, 2015, Bastida *et al.*, 2016). Conflicting reports exist of Beta- and
10 110 Alphaproteobacteria, with some studies describing them as copiotrophic and others as
11 111 oligotrophic highlighting that a consistent niche may not necessarily exist across
12 112 species within a large taxonomic group (Ho *et al.*, 2017). A genomic basis for traits
13 113 associated with soils dominated by putative copiotrophs and oligotrophs has been
14 114 expertly reviewed elsewhere, and interested readers are referred to Trivedi *et al.*,
15 115 (2013) and references therein. Importantly, these observations suggest that specific
16 116 traits that allow terrestrial prokaryotes to occupy these two niches should (generally)
17 117 be associated with taxonomy. This is an example of ecological coherence at high
18 118 taxonomic rank, whereby members within a taxon tend to have similar life strategies,
19 119 niches and possess common traits compared to members of other taxa (Philippot *et*
20 120 *al.*, 2010). While ecological coherence of taxa has been considered previously, the
21 121 shared, specific traits that drive niche differentiation in terrestrial prokaryotes remains
22 122 an open question.

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37 123 To identify the traits that differ between terrestrial prokaryote taxonomic groups, and
38 124 whether these traits could describe the niches they occupy, a functional trait-based
39 125 approach was adopted here. We posited that a trait must: a) be associated with a
40 126 physiological process that conveys a fitness advantage under certain environmental
41 127 conditions; b) be measurable in well-defined units; and c) vary more between
42 128 taxonomic groups than within a taxonomic group (McGill *et al.*, 2006, Kearney *et al.*,
43 129 2010). Traits were measured as discrete counts of chromosome-encoded proteins
44 130 shared between at least two of 175 terrestrial prokaryotes. Markov Chain clustering
45 131 (MCL) was used to group proteins as traits based on amino acid sequence similarity
46 132 (%) akin to a previous approach that confirmed close taxonomic relatives tend to share
47 133 functional traits in 1,374 genomes (Zhu *et al.*, 2015). This was necessary to compare
48 134 highly similar (but non-identical) proteins from separate genomes that carry out the
49 135 same biological function. To better identify important, distinguishing traits of terrestrial
50 136 prokaryotes, this study differed from Zhu *et al.*, by: a) comparing 175 publicly available

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3 137 terrestrial prokaryote genomes from 35 Families ($n = 5$ each), from 11 Phyla and two
4
5 138 Kingdoms; b) ~~the selecting~~ prokaryotes ~~selected are all~~ involved in terrestrial
6
7 139 ecosystem processes of interest, including organic matter decomposition, nitrogen
8
9 140 fixation, nitrification, denitrification, methane oxidation, plant-growth promotion,
10
11 141 bioremediation of pollutants, pathogenesis and methanogenesis; c) ~~selected selecting~~
12
13 142 taxa ~~were~~ isolated from a wide range of terrestrial environments, such as nutrient rich
14
15 143 decomposing plant material and rhizosphere, submerged wetland and rice paddy soils,
16
17 144 polluted soils, and nutrient poor hot and cold arid environments; and d) ~~avoiding~~ the
18
19 145 inclusion of multiple subspecies and/or strains of a single species ~~was specifically~~
20
21 146 ~~avoided~~ to prevent biases in analyses where highly over-represented species are
22
23 147 compared with species that have fewer cultured representatives. The taxonomic
24
25 148 system used here is from the NCBI, which is built upon a historical array of culture-
26
27 149 dependent, physiological observations and genetic similarity to cultured isolates as
28
29 150 average nucleotide identity, DNA-DNA hybridisation or 16S rRNA gene homology
30
31 151 (ncbi.nlm.nih.gov/Taxonomy/Browser). The use of this classification system and
32
33 152 comparison to others is discussed further below.

34
35 153 We hypothesised that: 1) traits are non-randomly distributed, with relatively closely
36
37 154 related taxa demonstrating greater similarity than unrelated taxa (ecological
38
39 155 coherence); 2) traits that are differentially enriched between taxonomic groups would
40
41 156 primarily be involved in metabolism, nutrient acquisition and/or tolerating
42
43 157 environmental stress; and 3) copiotrophic and oligotrophic taxonomic groups would
44
45 158 emerge based on collective trait enrichment.

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161 2. Methodology

162 2.1 Collection of terrestrial prokaryote genomes

163 A collection of 175 sequenced and annotated genomes was collated (Supplementary
164 Table 1). Listed are the genome ID, phylogenetic lineage, role in an ecosystem process
165 if known, and isolation or genome sequencing reference. These genomes were
166 sourced from the National Centre for Biotechnology Information (NCBI) and Joint
167 Genome Institute (JGI) databases. Genomes were chosen based on several criteria:
168 a) five isolates per Family were chosen to have an equal minimum sample size per
169 group, with this sample size being constrained by sequenced genomes of under-
170 represented groups in public databases; b) only a single subspecies/strain per species

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2
3 171 was included to avoid bias due to over-representation of some species in public
4
5 172 databases; c) an emphasis was placed to include isolates from diverse taxonomic
6
7 173 lineages involved in terrestrial ecosystem processes of interest, such as ammonia
8
9 174 oxidation and methanogenesis; and d) there was an emphasis to include taxonomic
10
11 175 groups frequently stated to be either copiotrophic (e.g. Actinobacteria,
12
13 176 Gammaproteobacteria) or oligotrophic (e.g. Acidobacteria, Planctomycetes) based on
14
15 177 observations from soil nutrient addition studies (Ho *et al.*, 2017). Taxonomic
16
17 178 annotations for Phyla, Class, Order etc. were based on NCBI taxonomy as most
18
19 179 genomes were sourced there. The authors recognise that taxonomy is constantly
20
21 180 shifting, particularly so with the recent development of the Genome Taxonomy
22
23 181 Database (GTDB) (Parks *et al.*, 2018). Of note is that the vast majority of taxa here
24
25 182 have the same taxonomy in NCBI as in GTDB, with the exceptions that GTDB
26
27 183 considers the Sporomusaceae as split into three separate Families, the
28
29 184 Leuconostocaceae to be Lactobacillaceae, the Promicromonosporaceae to be
30
31 185 Cellulomonadaceae, and the Bradyrhizobiaceae and Methylococcaceae have been
32
33 186 renamed as Xanthobacteraceae and Methylomonadaceae, respectively. Taxon
34
35 187 selection was constrained by availability of genomes for under-represented groups,
36
37 188 such as the Chloroflexi, Verrucomicrobia, Planctomycetes, Thaumarchaeota and
38
39 189 Euryarchaeota. To meet the $n = 5$ requirement for balanced statistical analyses, it was
40
41 190 necessary to consider these under-represented groups as 'Families'. Furthermore, due
42
43 191 to the great diversity inherent within Proteobacterial Classes, Gamma-, Alpha-, Beta-
44
45 192 and Deltaproteobacteria were considered as independent 'Phyla' for statistical
46
47 193 analyses here. Indeed, GTDB now defines Deltaproteobacteria as its own Phylum,
48
49 194 while Betaproteobacteria are considered as the Burkholderiales Order within the
50
51 195 Gammaproteobacteria. The total of 175 genomes analysed here falls within the upper
52
53 196 range of previous hypothesis-driven trait-based studies which varies from 11 isolates
54
55 197 (Bouskill *et al.*, 2012) to 214 genomes (Vieira-Silva & Rocha, 2010).

56 198

57 199

58 200 *2.2 Functional trait clustering*

59 201 A step-by-step walkthrough of reproducible code to perform the following analyses on
60 202 a subset of 12 genomes is available at: [https://github.com/DamienFinn/Trait-](https://github.com/DamienFinn/Trait-based_analyses)
61 203 [based_analyses](https://github.com/DamienFinn/Trait-based_analyses). Firstly, a pairwise similarity comparison of all amino acid sequences
62 204 (964 951 sequences) across the 175 genomes was performed with the all *versus* all

1
2
3 205 basic local alignment tool function for proteins, BLAST-P (Altschul *et al.*, 1990). Amino
4
5 206 acid sequences were subsequently clustered as traits via MCL weighted by pairwise
6
7 207 amino acid similarity (Enright *et al.*, 2002). Functional traits were grouped at a cluster
8
9 208 value of 90.2, whereby > 65 is considered 'fair' and confidence in accurately separating
10
11 209 clusters cannot be higher than 100. The value of 90.2 is not chosen by the user but
12
13 210 rather is a reflection of the quality of clustering in a given dataset. The MCL identified
14
15 211 a total of 220 664 traits shared between at least two genomes. A random subset of
16
17 212 1700 amino acid sequences were selected and the similarity of each sequence within
18
19 213 its trait group (as determined by MCL) *versus* between other trait groups was visualised
20
21 214 as a box and whisker plot (Supplementary Figure 1) in R version 4.0.0 (R Core Team,
22
23 215 2013). 1_700 sequences were chosen to maximise comparisons between trait groups
24
25 216 under technical limitations, as increasing sequences led to exponential increases in
26
27 217 trait combinations. A Student's T test was applied to determine whether sequences
28
29 218 were more similar within their trait group relative to between trait groups in R. Finally,
30
31 219 a matrix of genome x functional trait was generated in a two-step process by first
32
33 220 associating genome IDs to the MCL output with a novel script 'MCLtoReshape2.py'
34
35 221 (available at the above Github address) and secondly by casting the long data format
36
37 222 to a wide data matrix with the 'reshape2' package in R (Wickham, 2007). Box and
38
39 223 whisker plots comparing counts of proteins per genome (input) and counts of functional
40
41 224 traits shared by at least two genomes (output of computational workflow), for the 35
42
43 225 Families, is presented as Figure 1.

226

227

228 2.3 UPGMA dendrogram of trait similarity between genomes

229 The unweighted pair group method with arithmetic mean (UPGMA) was chosen to
230
231 compare distance-based similarity between taxa based on discrete counts of individual
232
233 traits per genome. This method is more robust for comparing similarity between sample
234
235 units (*i.e.* taxa) based on discrete counts of variables (*i.e.* individual traits per taxon)
236
237 rather than neighbour joining or maximum likelihood methods better suited for DNA or
238
239 amino acid sequence comparisons (Weins, 1998). The UPGMA was performed in R
240
241 with the 'phangorn' package as described (Schliep *et al.*, 2017) on a Bray-Curtis
242
243 transformed dissimilarity functional trait matrix, generated with the 'vegdist' function in
244
245 the 'vegan' package (Oksanen *et al.*, 2013). To measure ecological coherence (C) of
246
247 taxa within shared Super Groups, Phyla and Families, a similarity index was adapted

239 from Levins' Overlap (Finn *et al.*, 2020a), which measures pairwise similarity in
240 distributions of taxa, as the following:

$$241$$
$$242 \quad C = 1 - \left(\frac{\sum b_{ij}}{n^2} \right) \quad \text{Equation 1.}$$
$$243$$

244 Where b_{ij} is the pairwise branch length between taxon i and j in the UPGMA tree,
245 measured here as Bray-Curtis dissimilarity, which is summed for each taxon and its
246 relatives within a shared Super Group, Phylum or Family, and where n is the number
247 of taxa being compared within a shared Super Group, Phylum or Family.

248 Furthermore, the full length 16S rRNA gene of each taxon was collated from NCBI.
249 Genes were aligned with MUSCLE (Edgar, 2004) and a neighbour joining phylogenetic
250 tree was constructed with the 'phangorn' package in R. Phylogenetic distance present
251 in taxonomic groups (P) was measured as per Equation 1., excepting that branch
252 length was in units of DNA sequence similarity as opposed to Bray-Curtis dissimilarity.
253 Finally, simple linear regression was used to test a relationship between P and C .

256 2.4 Functional trait annotation

257 To inform the biological process a functional trait facilitated, traits were annotated with
258 the Kyoto Encyclopedia of Genes and Genomes (KEGG) database. This was
259 performed in five steps: 1) a representative amino acid sequence from each trait was
260 extracted with the novel script 'IdentifyTraits.py'; 2) these sequences were annotated
261 with KEGG Orthology (KO) terms using the BlastKOALA database algorithm with a bit
262 score cut-off value of 75 (Kanehisa *et al.*, 2016); 3) BRITE functional hierarchies
263 associated with each KO term (e.g. KO1179 gene: endoglucanase, BRITE 1:
264 Metabolism, BRITE 2: Carbohydrate Metabolism, BRITE 3: Starch and Sucrose
265 Metabolism) were collected with the novel script 'GetBRITEinfo.py'; 4) Genome ID, trait
266 ID, KO term and BRITE metadata were all collated with the novel script 'MatchFCs.py';
267 and 5) the 'reshape2' package in R was used to create matrices of genome x BRITE
268 hierarchy. Where KEGG was unable to annotate a trait, it was considered as
269 'Uncharacterised'. As above, all novel scripts and a step-by-step walkthrough of
270 reproducible code is available at: [https://github.com/DamienFinn/Trait-](https://github.com/DamienFinn/Trait-based_analyses)
271 [based_analyses](https://github.com/DamienFinn/Trait-based_analyses).

273

2.5 Identifying traits differentially enriched in taxonomic groups

Random Forest classification was chosen as a non-linear, multivariate cluster-based method capable of identifying numerous predictor variables (*i.e.* traits) that define different classes of a response variable (*i.e.* taxonomic group). This was performed with the 'randomForest' package as described (Liaw & Weiner, 2002). Discrete counts of traits at BRITE level 3 were used (*e.g.* Starch and Sucrose Metabolism) as this level had the most accurate resolution of biological processes facilitated by traits. A total of six Random Forest models were optimised to classify taxonomic groups at the level of: a) Phylum, with Proteobacteria Classes separated due to their extensive diversity ($n = 14$); b) Family ($n = 35$); c) specifically for Families in the Proteobacteria ($n = 13$); d) Families in the Actinobacteria ($n = 7$); e) Families in the Firmicutes ($n = 5$); and f) Families from 'Under-represented' groups, which were all other Families ($n = 10$). Optimal numbers of trees grown for each model were: 300, 400, 320, 300, 300 and 400, respectively. Six traits were randomly selected at each branch. As the Random Forest only identifies traits that best explain separation of taxonomic groups, and does not show whether traits have positive or negative associations with groups, box and whisker plots and Fisher's Least Significant Difference (LSD) *post hoc* tests were performed with the 'agricolae' package (de Mendiburu, 2014) to definitively state which taxonomic groups were significantly enriched or depleted in traits identified via Random Forest.

294

295

2.6 Hierarchical clustering of Families by defining traits

Finally, the relationship between Families based on similarity in counts of 60 traits was assessed via hierarchical clustering. Traits were chosen based on being selected via the above Random Forest models in this study, and from previous studies that identified traits associated with copiotroph-oligotroph growth strategies in single species or mixed communities (Lauro *et al.*, 2009, Vieira-Silva & Rocha, 2010, Roller & Schmidt, 2015, Pascual-Garcia & Bell, 2020). The mean of trait discrete counts in the five Family members was used as representative of each Family. Comparing trait means between Families was considered acceptable as prior LSD *post hoc* tests had demonstrated significant differences between Families. As traits had highly variable copy numbers per Family (*e.g.* ABC transporter trait copies ranged from 10 – 350,

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2
3 307 while bacterial chemotaxis traits ranged from 0 – 15 copies) the trait copies were
4
5 308 normalised for more appropriate comparisons. Normalised variance was calculated
6
7 309 across the 35 Families for all traits with the 'decostand' function in the 'vegan' package
8
9 310 (Oksanen *et al.*, 2013). Hierarchical clustering of Families based on normalised trait
10
11 311 counts was visualised with the 'heatmap.2' function in the 'gplots' package (Warnes *et*
12 312 *al.*, 2019).

13 313

14 314

15 315

3. Results

16 316

3.1 Trait clustering and UPGMA

17 317 The 964 951 amino acid sequences encoded by the 175 genomes were clustered as
18
19 318 220 664 traits by MCL. A random subset of 1_700 traits showed that amino acid
20
21
22 319 sequence similarity within traits ranged from 62.4, 82.5 and 100% for the 1st quartile,
23
24 320 mean and 3rd quartile, respectively (Supplementary Figure 1). Amino acid sequence
25
26 321 similarity between traits ranged from 27.6, 34 and 37.8% for the 1st quartile, mean and
27
28 322 3rd quartile, respectively. A Student's T test found that sequences grouped together as
29
30 323 a trait were significantly more similar to each other than to sequences grouped as
31
32 324 different traits (t value = 61.3, $p = 2 \times 10^{-16}$). Manual comparisons of amino acid
33
34 325 sequences within several traits supported clustering of proteins with identical biological
35
36 326 function based on KEGG annotation. Thus, the MCL was considered to perform well.
37
38 327 However, the minimum amino acid sequence similarity within traits was 23.27% and
39
40 328 maximum similarity between traits was 85.24%, indicating that across the 220 664
41
42 329 traits, a small proportion of dissimilar amino acid sequences were grouped as a trait
43
44 330 incorrectly, while some amino acid sequences that were highly similar were considered
45
46 331 different traits. This small number of incorrectly clustered sequences can be explained
47
48 332 by the MCL clustering efficiency being 90.2, out of a possible 100.

49 333 A comparison of the number of proteins per genome, per Family at the Family level
50 334 (mean = 5_533), found that there were fewer functional traits per genome (mean = 4
51 335 240) (Figure 1). These represent the input number of proteins per genome before trait
52 336 clustering and the output number of traits after clustering, respectively. As only
53 337 functional traits shared between at least two genomes were considered here, the loss
54 338 of highly genome-specific traits that could not be compared between genomes was
55 339 expected. Despite this drop in average traits per genome, this initial approach serves
56 340 as a proof of concept to demonstrate that numbers of traits per genome at the Family

341 level reflect trends in proteins per Family, and thus the MCL was not distorting trait
342 clustering (Figure 1).

343 The UPGMA dendrogram comparing the 220 664 traits per genome showed that
344 trait compositions were non-randomly distributed (Figure 2a). Specifically,
345 Thaumarchaeota, Euryarchaeota, Acidobacteria, Betaproteobacteria,
346 Gammaproteobacteria, Alphaproteobacteria, Cyanobacteria, Verrucomicrobia,
347 Planctomycetes, Bacteroidetes, Actinobacteria, Firmicutes and Deltaproteobacteria
348 clustered together preferentially. The Chloroflexi were split into two clusters: one
349 *Dehalococcoides* and one *Ktedonobacter/Herpetosiphon/Anaerolinea* cluster. Several
350 prokaryotes did not cluster with their high taxonomic rank, including a Planctomycetes
351 bacterium, *Polyangium brachysporum* (Deltaproteobacteria), *Agreia pratensis*
352 (Actinobacteria) and *Sporomusa ovata* (Firmicutes). Also of interest was that, in regard
353 to distance between terminal nodes (as noted by the scale bar), the Betaproteobacteria
354 and Gammaproteobacteria were more similar to each other than the
355 Alphaproteobacteria, which formed its own large, diverse clade. A neighbour joining
356 tree of full length 16S rRNA genes showed that all taxa clustered preferentially based
357 on their taxonomic nomenclature at high taxonomic rank, including the Chloroflexi,
358 indicating that the discrepancies in the UPGMA were not due to misclassification of the
359 individual taxa (Supplementary Figure 2a).

360 A simple index to measure trait similarity, as ecological coherence (C), within groups
361 was devised (Equation 1). C increased as taxonomic rank decreased: Super Group <
362 Phylum < Family (Figure 2b). C was lowest for the larger, more diverse Proteobacteria
363 and Terrabacteria (Super Group), and Firmicutes and Actinobacteria (Phylum). As the
364 number of taxa being compared at the Super Group (e.g. Terrabacteria = 70 *versus*
365 Acidobacteria = 10) and Phylum (e.g. Actinobacteria = 35 *versus* Thaumarchaeota =
366 5) were variable, the most meaningful comparisons between groups are at the Family
367 level ($n = 5$ each). With the exception of the highly divergent 'Acidobacteria Lineage',
368 all Families had a C greater than 0.3, with certain groups in the Alphaproteobacteria
369 (Beijerinckiaceae), Firmicutes (Bacillaceae and Leuconostocaceae) and
370 Actinobacteria (Promicromonosporaceae) being highly coherent ($C > 0.55$). Indeed, all
371 individual Proteobacterial Families had $C > 0.4$, indicating that all five taxa within each
372 of these Families had similar trait compositions. Despite not truly belonging to the same
373 Family as per NCBI taxonomy, the $C > 0.335$ of the five Cyanobacteria,
374 Thaumarchaeota and the Methanogen Lineage taxa was similar to other Families from

1
2
3 375 the Bacteroidetes and Firmicutes. Thus, the UPGMA demonstrated that taxonomic
4 376 relatives at the Phylum level tended to cluster with each other preferentially based on
5 377 trait composition, and secondly that while similarity was broadly highest at low
6 378 taxonomic rank, some Families were more coherent than others.

7
8
9
10 379 Phylogenetic distance (P) of each taxonomic group increased with decreasing
11 380 taxonomic rank, and was highest in Proteobacteria, Actinobacteria and Firmicutes
12 381 Families (Supplementary Figure 2b). There was a strong positive linear relationship
13 382 between P and C ($y = 0.86x - 0.25$, $R^2 = 0.39$, $p < 0.001$) supporting the result that
14 383 taxonomic groups of closer related taxa tended to share more similar compositions of
15 384 traits.

16 385

17 386

18 387 *3.2 Random Forest trait identification*

19 388 The KEGG annotated traits belonged to 260 different BRITE 3 categories. The
20 389 percentage of traits that could not be annotated (and were termed 'Uncharacterised')
21 390 ranged from 28 – 65% per genome, being particularly high in the Archaea. On average,
22 391 47% of traits per genome were Uncharacterised with a standard deviation of 9.5%.

23 392 Of the 260 BRITE 3 categories, the 60 most important traits in separating all Phyla
24 393 and Families are ranked by importance measured as Mean Decrease in Accuracy
25 394 (MDA) of the Random Forest models (Figure 3). This is a measure of the average
26 395 increase in classification error during permutation of trees ($n = 300 - 400$) when that
27 396 particular trait is missing from the tree. For example, the accuracy of classifying
28 397 Families was most improved by inclusion of the ABC transporters trait. Based on the
29 398 identified traits, the Phylum model was capable of successfully classifying 81.14% of
30 399 individual taxa. The Family model was capable of successfully classifying 71.43% of
31 400 individual taxa. Confusion matrices for both models are presented as Supplementary
32 401 Tables 2 – 4, and show that classification was particularly difficult for Chloroflexi and
33 402 Planctomycetes (classification error > 80%) in the Phylum model and for
34 403 Cellulomonadaceae and the divergent Acidobacteria Lineage (classification error >
35 404 80%) in the Family model. Random Forest models were robust against variation in P
36 405 within Families, for example the nine families with all taxa perfectly classified ranged
37 406 in P from the lowest (0.68) to highest (0.9).

38 407 The important traits in classifying the taxonomic groups were involved in: a)
39 408 metabolism and nutrient acquisition (oxidative phosphorylation, tricarboxylic acid

1
2
3 409 (TCA) cycle, glyoxylate/decarboxylate, thermogenesis, propanoate, starch/sucrose,
4
5 410 nitrogen, methane metabolism, synthesis of antioxidants such as glutathione, ATP-
6
7 411 binding cassette (ABC) transporters, sugar uptake via phosphotransferase systems
8
9 412 (PTS)); b) responding to environmental cues and stressors (protein kinases, two-
10
11 413 component systems, transcription factors, proteasome, protein chaperones, RNA
12
13 414 transport, chromosome repair via non-homologous DNA end joining); c) core cell
14
15 415 physiology (flagella assembly, chemotaxis, sporulation, lipopolysaccharide (LPS),
16
17 416 peptidoglycan, glycerolipid, sphingolipid and lipoarabinomannan (LAM) biosynthesis);
18
19 417 and d) cell-cell interactions (beta-Lactam resistance, general secretion systems and
20
21 418 Type IV secretion systems). Box and whisker plots of discrete counts of identified traits,
22
23 419 and LSD results, are provided as Supplementary Figures [42](#) – [75](#). The Families
24
25 420 significantly enriched and depleted in these traits are listed in Table 1. The Phyla
26
27 421 significantly enriched and depleted in identified traits are listed in Supplementary Table
28
29 422 9.

30
31 423 To better identify the more subtle differences between Families in the
32
33 424 Proteobacteria, Actinobacteria, Firmicutes and the 'Under-represented' Phyla,
34
35 425 individual Random Forest models were constructed for each of the four groups. The
36
37 426 successful classification rates were 72.31, 74.29, 76 and 72%, respectively. Confusion
38
39 427 matrices for each model are presented as Supplementary Tables 5 – 8. The models
40
41 428 were unable to reliably classify Bradyrhizobiaceae and the divergent Acidobacteria
42
43 429 Lineage (classification error > 80%).

44
45 430 Supplementary Figure [86](#) shows the most important traits in classifying the four
46
47 431 groups. Unique traits not identified in the prior Phylum and Family models were: a) for
48
49 432 Proteobacteria, glycosyltransferases, butanoate metabolism, aminotransferases,
50
51 433 ribosome biogenesis, mRNA biogenesis and degradation; b) for Actinobacteria,
52
53 434 porphyrin and chlorophyll synthesis, pyruvate metabolism, aminotransferases, fatty
54
55 435 acid and aliphatic hydrocarbon metabolism, polyketide and Type II polyketide
56
57 436 biosynthesis, antimicrobial resistance genes; c) for Firmicutes, lysine, folate and varied
58
59 437 amino acid synthesis, porphyrin and chlorophyll synthesis, DNA replication, bacterial
60
438 toxins, penicillin and cephalosporin synthesis; and d) for the 'Under-represented' taxa,
439
440 glycosyltransferases, peptidases and inhibitors, photosynthesis and AMP-activated
441
442 protein kinases. Box and whisker plots of discrete counts of identified traits, and LSD
443
444 results, are provided as Supplementary Figures [97](#) – [120](#). Tables summarising

1
2
3 442 Families enriched and depleted in these traits are included as Supplementary Tables
4 443 10 and 11.

5 444

6 445

7 446 *3.3 Hierarchical clustering of defining traits*

8 447 Hierarchical clustering based on 60 traits, identified from Random Forest in this study
9 448 and by previous copiotroph-oligotroph studies, indicated five general clades. The
10 449 dendrogram on the y axis of Figure 4 shows clustering of taxa as these five clades.
11 450 The dendrogram on the x axis shows clustering of co-occurring traits. Clade I consisted
12 451 of Proteobacteria, specifically the Pseudomonadaceae, Burkholderiaceae,
13 452 Rhodospirillaceae, Bradyrhizobiaceae and Rhizobiaceae. These Families were
14 453 uniquely enriched in flagellar assembly, chemotaxis, pyruvate metabolism, glutathione
15 454 metabolism, ABC transporters, benzoate metabolism, transcription factors, glyoxylate
16 455 and fatty acid metabolism. Clade II, also Proteobacteria, included Nitrosomonadaceae,
17 456 Neisseriales Lineage, Methylocystaceae, Beijerinckiaceae, Methylococcaceae and
18 457 Moraxellaceae. These Families clustered based on being enriched in Clade I traits, but
19 458 to a lesser degree than the Pseudomonadaceae, Burkholderiaceae,
20 459 Rhodospirillaceae, Bradyrhizobiaceae and Rhizobiaceae. Exceptions included the
21 460 absence of benzoate metabolism and enrichment of methane metabolism in several
22 461 Clade II Families.

23 462 Clade III, a diverse collection of Bacteroidetes (Chitinophagaceae, Cytophagaceae),
24 463 Verrucomicrobia, Planctomycetes, divergent Acidobacteria Lineage and the
25 464 Deltaproteobacteria (Polyangiaceae, Myxococcaceae), shared enrichment of
26 465 sphingolipid metabolism, beta-Lactam resistance, penicillin and cephalosporin
27 466 biosynthesis, LPS biosynthesis, glycosyltransferases and starch/sucrose metabolism.
28 467 Many of these Families shared Clade I and II traits, including Type IV secretion system,
29 468 oxidative phosphorylation, TCA cycle, PTS, nitrogen and glycerophospholipid
30 469 metabolism. The absence of glutathione in non-Deltaproteobacterial Clade III Families
31 470 was notable.

32 471 The three Actinobacteria Families in Clade IV, Mycobacteriaceae, Frankiaceae and
33 472 Streptomycetaceae, were highly similar to each other because they were enriched in
34 473 Type II polyketide biosynthesis. They also shared some Clade I traits (ABC
35 474 transporters, transcription factors, pyruvate, benzoate and fatty acid metabolism) and
36 475 Clade III traits (membrane trafficking, transcription machinery, polyketide biosynthesis

1
2
3 476 and starch/sucrose metabolism). Similar to Clade III, these Actinobacteria were also
4
5 477 depleted in glutathione traits.

6 478 Finally, taxa within Clade V were similar to each other due to being depleted in traits
7
8 479 shared among the other clades. Cyanobacteria were the only taxa that possessed
9
10 480 photosynthesis traits. Lactic acid bacteria (Lactobacillaceae and Leuconostocaceae)
11
12 481 were enriched in Unclassified nucleotide metabolism. The Archaea (Thaumarchaeota
13
14 482 and Methanogen Lineage) shared eukaryote-like traits, proteasome, basal
15
16 483 transcription factors and RNA transport. The Archaea were enriched in carbon fixation
17
18 484 traits. Methanogens were also enriched in methane metabolism. Ammonia oxidising
19
20 485 Thaumarchaeota were not enriched in nitrogen metabolism, however they were
21
22 486 enriched in traits annotated by KEGG as Global maps only (unclassified metabolism).
23
24 487 Further analysis found this to be the *fpr* gene, encoding a ferredoxin-flavodoxin NADP⁺
25
26 488 reductase (K00528). Non-lactic acid bacteria of the Firmicutes (Bacillaceae,
27
28 489 Sporomusaceae and Clostridiaceae) were enriched in sporulation and motility traits.

29

30

31 492 4. Discussion

32 493 4.1 Non-random trait clustering demonstrates ecological coherence of taxa

34 494 A trait-based approach to investigate taxonomic relationships and potential biological
35
36 495 function was carried out with a collection of 175 terrestrial prokaryotes. We
37
38 496 hypothesised that traits would be non-randomly distributed amongst taxonomic groups,
39
40 497 ~~supportive of~~ supported by previous observations that noted closely related taxa are
41
42 498 isolated from similar habitats (Philippot *et al.*, 2010). Similarity in the composition of
43
44 499 220 664 traits, within 175 taxa, demonstrated strong agreement with established
45
46 500 taxonomy at high (Phyla) and low (Family) rank (Figure 2a). The exceptions to this
47
48 501 ecological coherence at high rank were the division of the Chloroflexi and an individual
49
50 502 from each of the Planctomycetes, Deltaproteobacteria, Actinobacteria and Firmicutes
51
52 503 clustering with unrelated Phyla. ~~It is possible that these four individual taxa have~~
53
54 504 ~~been~~ These taxa were not mischaracterised, as based on phylogenetics of the full
55
56 505 length 16S rRNA gene (Supplementary Figure 2a). Trait similarity between related
57
58 506 taxa, measured as C, tended to be highest at low rank (Figure 2b). Uneven sample
59
60 507 sizes between groups within Super Group and Phyla make comparisons at this level
508
509 508 difficult – the inclusion of many diverse Firmicutes and Actinobacteria likely drove C to
509
509 509 be lower here than in Thaumarchaeota and Euryarchaeota. However, equal

1
2
3 510 comparisons at the Family level demonstrated interesting variability in coherence. All
4
5 511 Proteobacterial Families had relatively high coherence ($C = 0.4 - 0.6$). The high C in
6
7 512 Beijerinckiaceae is of particular interest as this group contained both specialist
8
9 513 methanotroph (*Methylocapsa*, *Methylocella*), methylotroph (*Methyloferula*) and
10
11 514 generalist heterotroph (*Beijerinckia* spp.) taxa. With such varied metabolic traits, one
12
13 515 could reasonably expect C to be relatively low within this Family. The Beijerinckiaceae
14
15 516 appear to have evolved from a common methylotrophic ancestor and still share traits
16
17 517 for nitrogen fixation and tolerance for low pH soils (Tamas *et al.*, 2014), and the high
18
19 518 C measured here indicates that many additional shared traits remain. Both the
20
21 519 relatively recent divergence of Families from a common ancestor and the higher
22
23 520 number of shared traits are likely causes of the higher C observed at low taxonomic
24
25 521 rank. The differing values of C for Methanogen and photosynthetic Cyanobacteria
26
27 522 functional groups (0.33 and 0.44, respectively) is also worthy of note. Despite all five
28
29 523 taxa in each group performing the same core role in a community, the individual
30
31 524 isolates came from varying environments. The methanogens were isolated from a
32
33 525 range of geographically separate wetlands, rice paddy soil and farm slurry and, while
34
35 526 the Cyanobacteria were also isolated from geographically separate environments, they
36
37 527 were all from sandy deserts or other nutrient poor, arid soils (Supplementary Table 1
38
39 528 and references therein). Ultimately a taxon's trait composition will be affected by its
40
41 529 functional role in a community, its evolutionary life-history (e.g. Beijerinckiaceae
42
43 530 described above) and its local environment.

44
45 531 __ However, these results are dependent on accurate taxonomic classification, and the
46
47 532 C of Sporomusaceae, relatively low compared to other Families here, supports splitting
48
49 533 this group into Sporomusaceae, Anaeromusaceae and Pelosinaceae by GTDB (Parks
50
51 534 *et al.*, 2018). Finally worth noting, some groups at high rank were considered as
52
53 535 'Families' here due to the number of available terrestrial genomes, e.g. Cyanobacteria
54
55 536 and Chloroflexi. Even so, Cyanobacteria demonstrated a higher C than many
56
57 537 taxonomically-defined Families, perhaps due to their common role as primary
58
59 538 colonisers of nutrient-poor soils (Garcia-Pichel *et al.*, 2001). The number of Families
60
61 539 are too numerous to discuss each at length here, but C was an effective means of
62
63 540 measuring and comparing coherence between groups in the UPGMA tree.

64
65 541 While the method of comparing taxa here differs from other studies, the results were
66
67 542 not surprising as many 16S rRNA gene surveys of terrestrial systems consistently
68
69 543 demonstrate ecological coherence at high rank. For example, independent studies of

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2
3 544 increasing agricultural intensity in soils show reductions in Actinobacteria abundance
4 545 (Philippot *et al.*, 2009, Jangid *et al.*, 2011). Nitrogen addition to soils frequently enriches
5 546 numerous taxa within the Actinobacteria and Proteobacterial Classes while negatively
6 547 affecting taxa within the Verrucomicrobia and Planctomycetes (Wessen *et al.*, 2010,
7 548 Fierer *et al.*, 2012, Leff *et al.*, 2015, Bastida *et al.*, 2016). Arid, nutrient poor
8 549 environments select for Actinobacteria-dominated communities (Cary *et al.*, 2010,
9 550 Crits-Christoph *et al.*, 2013) and, in the absence of other primary producers, allow
10 551 biological soil crust forming Cyanobacterial taxa to establish (Garcia-Pichel *et al.*,
11 552 2001). Anoxic wetland and rice paddy environments support diverse communities of
12 553 anaerobic Firmicutes, Chloroflexi and methanogenic Archaea (He *et al.*, 2019, Finn *et*
13 554 *al.*, 2020**b**). These trends were noted prior to bioinformatic advances of metagenome
14 555 assembled genomes (MAGs) that allow for the specific comparison of individual traits
15 556 between uncultured environmental prokaryote genomes (Hug *et al.*, 2013). The
16 557 generation of MAGs has emerged as a useful tool for identifying traits necessary for
17 558 life in such environments, and particularly for expanding knowledge of severely under-
18 559 represented, difficult to culture taxonomic groups. For example, the recent
19 560 reconstruction of 52 515 MAGs from a wide range of host-associated and
20 561 environmental metagenomes was able to increase genomic information of
21 562 Planctomycetes and Verrucomicrobia by 79% and 68%, respectively (Nayfach *et al.*,
22 563 2021). ~~I-but~~ importantly, both 16S rRNA gene surveys and MAGs ~~the earlier studies~~
23 564 demonstrated that some functional traits that facilitate life under certain environmental
24 565 conditions are intrinsically linked to taxonomy.

25 566 The ecological coherence observed in Figure 2 does not imply that closely related
26 567 taxa (e.g. *Bacillus velezensis* LS69 and *Bacillus amyloliquefaciens plantarum* FZB42)
27 568 have identical phenotypes. Close relatives possess a combination of core and
28 569 accessory genes (traits) and the presence of even a single accessory gene is sufficient
29 570 to dramatically alter a strain's phenotype (van Rossum *et al.*, 2020). Rather, our results
30 571 (Figure 2) demonstrate that the composition of core and accessory traits in
31 572 alphaproteobacterial Beijerinckiaceae are most similar to each other, relative to
32 573 alphaproteobacterial Rhizobiaceae or to Actinobacteria, Firmicutes etc.

33 574

34 575

35 576 *4.2 What can the Random Forests tell us?*

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2
3 577 The Random Forest works by identifying the traits that are most reliable in classifying
4 578 individual Phyla and Families. It selects traits that tend to be: a) of equal copy number
5 579 per genome within a taxonomic group; and b) that differ markedly in copy number
6 580 between taxonomic groups, since distinct separation of copies will maximise
7 581 successful classification. A clear example of this is the consistent identification of
8 582 eukaryote-like basal transcription factors, proteasome and RNA transport present in
9 583 the Thaumarchaeota and Euryarchaeota, since they are absent from the majority of
10 584 Bacterial Phyla. The binary nature of these traits (yes Archaea, no Bacteria) make
11 585 them strong indicators. The presence of these and more eukaryote-like vesicular
12 586 trafficking and actin traits have been noted in the Archaeal TACK super-phylum
13 587 previously, and lend credence to the hypothesis that eukaryotes are descended from
14 588 Archaea (Embley & Martin, 2006, Spang *et al.*, 2015). However, ~~it must be noted that~~
15 589 the Random Forest will not identify a trait unique to *Can. Nitrosotalea devanterra*, ~~and~~
16 590 ~~that is~~ absent from other Thaumarchaeota, as this single trait will not improve
17 591 classification of the group as a whole. Consequently, the traits identified via Random
18 592 Forest all tended to be core, fundamental traits shared by other members of a taxon's
19 593 Phylum/Family.

20
21
22 594 Many of the best traits for distinguishing taxa have been historically used by
23 595 microbiologists to do exactly that. These included fundamental cell physiology traits,
24 596 such as oxidative phosphorylation, LPS biosynthesis, sporulation, flagellar assembly
25 597 and chemotaxis. The Phylum model separated Betaproteobacteria, Actinobacteria and
26 598 Bacteroidetes as taxa with the highest copies of oxidative phosphorylation traits.
27 599 Firmicutes, Chloroflexi and Methanogens were identified as anaerobes depleted in
28 600 oxidative phosphorylation, and all other taxa as sitting in between (Supplementary
29 601 Figure 42 and Supplementary Table 9). Some Gram-negative Families were
30 602 significantly enriched in LPS biosynthesis compared to others. These were the
31 603 Pseudomonadaceae, Burkholderiaceae, Chitinophagaceae, divergent Acidobacteria
32 604 and Verrucomicrobia (Table 1). This has been noted in soil communities previously.
33 605 The extensive repertoire of LPS-associated genes in Chitinophagaceae, Acidobacteria
34 606 and Verrucomicrobia likely play a critical role in enhancing soil aggregation (Cania *et*
35 607 *al.*, 2019) potentially through high LPS production and/or biofilm formation (discussed
36 608 further below). In a demonstration of the robustness of the methods used here, the
37 609 highly unusual Firmicute Sporomusaceae were shown to possess similar counts of
38 610 LPS biosynthesis traits relative to most Gram-negative Families (Supplementary Table

1
2
3 611 4) in addition to sharing heat-resistant spore formation with Bacillaceae and
4 612 Clostridiaceae (Table 1). The presence of both traits in a single Family have been
5 613 remarked upon previously and used to conceptualise the evolution of Gram-negative
6 614 *versus* Gram-positive lineages (Stackebrandt *et al.*, 1985). The Sporomusaceae were
7 615 also shown to have high Porphyrin and Chlorophyll metabolism traits in the Firmicutes
8 616 model (Supplementary Table 10). The capacity to dechlorinate the soil pollutant
9 617 perchloroethene to trichloroethylene via a porphyrin-based corrinoid is yet another
10 618 interesting trait of this Family (Terzenbach & Blaut, 1994).

11 619 Finally, flagella assembly and chemotaxis traits identified Alphaproteobacteria,
12 620 Betaproteobacteria and Acidobacteria as Phyla that were particularly enriched with this
13 621 mechanism of motility, while bacterial Actinobacteria, Bacteroidetes, Cyanobacteria,
14 622 Chloroflexi and Verrucomicrobia were depleted (Supplementary Table 9). The
15 623 Proteobacteria, Firmicutes and Under-represented models were better suited for
16 624 identifying specific Families homogenously enriched or depleted in bacterial flagella
17 625 and chemotaxis (Supplementary Tables 10 and 11). Enriched Families included the
18 626 Rhodospirillaceae, Nitrosomonadaceae, Neisseriales lineage, divergent Acidobacteria
19 627 lineage, Sporomusaceae, Bacillaceae, Clostridiaceae and Planctomycetes. Other
20 628 forms of motility such as twitching and gliding have been noted in the
21 629 Pseudomonadaceae, Myxococcaceae and Cyanobacteria (McBride, 2001) but these
22 630 traits were not identified by the Random Forest as being homogenously enriched in
23 631 any Families. Furthermore, while Thaumarchaeota and Methanogens were both
24 632 identified as being depleted in bacterial flagella assembly and chemotaxis traits
25 633 (Supplementary Table 9 and 11), Archaea possess a structurally distinct flagellum
26 634 more similar to the Type IV bacterial pilus (Jarrell and Albers, 2012). These taxa were
27 635 not enriched with Type IV pilus, either, and it is possible that archaeal flagella may
28 636 have failed proper characterisation by KEGG. Thus, while some Families were
29 637 relatively enriched/depleted in bacterial flagella and chemotaxis traits, specific taxa
30 638 depleted in these are not necessarily non-motile.

31 639 In summary, while the Random Forests may overlook certain traits in individual taxa,
32 640 the models were highly robust in detecting conserved, shared traits within a
33 641 Phylum/Family. Here the 'depth' of shared traits is limited by the number of taxa that
34 642 could be considered as Phylum or Family. In future, if five (or more) taxa belonging to
35 643 the same Genus or even Species could be compared, unique traits would be observed
36 644 to explain how these subgroups have evolved from their respective Families to occupy

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3 645 distinct niches. Ideally the selection of individual taxa within groups for such future
4 comparative analyses would also be standardised based on phylogenetic distance,
5 646 either with *P* or a similar method, that would improve the robustness of trait-based
6 comparisons at such a fine taxonomic level.
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13 651 4.3 Plant-derived carbon metabolism and nutrient acquisition

15 652 Secondly, we hypothesised that the traits differentially enriched between taxonomic
16 653 groups would largely reflect those associated with copiotrophs or oligotrophs, namely
17 654 metabolism, nutrient acquisition and environmental stress response and tolerance. Of
18 655 fundamental interest to soil microbiologists is the decomposition of plant biomass. This
19 656 is the primary source of organic carbon to non-arid terrestrial systems (Kögel-Knabner,
20 657 2002) and the transformation of plant material to substrates bioavailable for
21 658 microorganisms is essential for community growth and activity. The traits involved in
22 659 plant material catabolism belonged to the BRITE categories 'Starch and Sucrose
23 660 Metabolism' (e.g. extracellular cellobiosidases, endoglucanases, glucosidases,
24 661 trehalases, amylases) and 'Glycosyltransferases', all of which are carbohydrate
25 662 activated enzymes (CAZy). The Families particularly enriched in these traits were the
26 663 Polyangiaceae, Myxococcaceae, Rhizobiaceae, Streptomycetaceae,
27 664 Mycobacteriaceae, Frankiaceae and Verrucomicrobia (Table 1, Supplementary Table
28 665 11). Genomic and culture-dependent analyses support *Sorangium cellulosum*
29 666 (Polyangiaceae), *Streptomyces coelicolor* A3(2) (Streptomycetaceae) and
30 667 *Chthoniobacter flavus* (Verrucomicrobia) as having particularly large genomes with
31 668 extensive repertoires for cellulose, hemicellulose, pectin and lignin degradation
32 669 (Bentley *et al.*, 2002, Sangwan *et al.*, 2004, Schneiker *et al.*, 2007). Comparative
33 670 genomics analyses have also identified Actinobacteria, Acidobacteria and
34 671 Verrucomicrobia as being enriched in numerous enzymes for cellulose, hemicellulose
35 672 and starch catabolism (Trivedi *et al.*, 2013). *In situ* these Families likely play a critical
36 673 role in making organic carbon bioavailable as di- and monosaccharides for the
37 674 microbial community.

38 675 The Families equipped with many copies of high-affinity sugar uptake
39 676 'Phosphotransferase systems' (3 – 14 copies) did not necessarily correspond to those
40 677 enriched with CAZy – only the Myxococcaceae and Verrucomicrobia were enriched in
41 678 both. Pseudomonadaceae, Rhodospirillaceae, Neisseriales lineage and

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3 679 Clostridiaceae were only enriched in PTS. Despite being enriched in CAZy, the
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5 680 Frankiaceae were simultaneously depleted in PTS. The complex interplay between
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7 681 taxa capable of producing extracellular CAZy and competitors that rapidly scavenge
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9 682 available di- and monosaccharides has been well described by models that predict
10
11 683 such competitive interactions exert important controls on the growth rate of the
12
13 684 community as a whole (Freilich *et al.*, 2011) and may even act to aid terrestrial carbon
14
15 685 storage and limit carbon dioxide emissions from microbial respiration (Kaiser *et al.*,
16
17 686 2015). Here, we identified the ‘specialist’ Families enriched in CAZy traits *versus* the
18
19 687 ‘opportunists’ scavenging for sugars via PTS (Table 1, Supplementary Table 11).

20
21 688 ABC transporters facilitate the ATP-dependent uptake of soluble compounds across
22
23 689 membranes or export waste metabolites, extracellular enzymes and toxins (Young &
24
25 690 Holland, 1999, Higgins, 2001). This means of active transport allows microorganisms
26
27 691 to acquire nutrients with high affinity at concentrations of 5 – 500 $\mu\text{g carbon L}^{-1}$ *versus*
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29 692 the less efficient diffusion of nutrients across membranes, dependent on extracellular
30
31 693 concentrations of 0.5 – 5 mg carbon L^{-1} (Kuznetsov *et al.*, 1979). In the spatially
32
33 694 heterogenous soil environment where the concentration of bioavailable carbon
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35 695 substrate often limits growth (Blagodatsky & Richter, 1998), possession of high affinity
36
37 696 transporters likely provides a competitive advantage. The rhizosphere-associated
38
39 697 Rhodospirillaceae, Rhizobiaceae and Burkholderiaceae tended to have the highest
40
41 698 trait copies of ABC transporters (100 – 350 copies per genome, Supplementary Figure
42
43 699 4). The diverse, non-rhizospheric Deltaproteobacteria, Actinobacteria, Firmicutes,
44
45 700 Cyanobacteria and Verrucomicrobia all had greater than 50 copies per genome,
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47 701 highlighting the importance of these traits in soil. The particularly high gene copy
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49 702 number in rhizosphere-associated taxa from presumably nutrient-rich environments
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51 703 contrasts the assumption that ABC transporters are considered to play a greater role
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53 704 in nutrient-poor environments (Lauro *et al.*, 2009). Comparative genomics analyses of
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55 705 soil bacteria have also found putatively copiotrophic Proteobacteria and Firmicutes to
56
57 706 be particularly enriched in PTS and ABC transporters (Trivedi *et al.*, 2013). In this
58
59 707 study, tThe transporters enriched in ~~these~~ rhizosphere-associated taxa were primarily
60
708 aimed at scavenging maltose, phosphate, amino acids, oligopeptides and export of
709 LPS, and these results suggest that these traits are not only for survival in nutrient-
710 poor environments but also likely confer a competitive advantage in the rhizosphere.
711 As prokaryotes compete simultaneously with other prokaryotes and plants for nitrogen

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3 712 and phosphorus in the rhizosphere, the high affinity acquisition of such nutrients is
4 713 likely critical for growth.

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7 716 *4.4 Nitrogen and methane metabolism*

8 717 The BRITE category 'Nitrogen metabolism' encompasses nitrogen fixation,
9 718 denitrification, ammonia oxidation and synthesis of glutamate/glutamine which are,
10 719 critical amino acids for peptide synthesis. Since nitrogen limitation acts as an important
11 720 control on soil microbial activity, these traits are also of interest to soil microbiologists.
12 721 Three Proteobacterial Families, Rhodospirillaceae, Bradyrhizobiaceae and
13 722 Burkholderiaceae, were particularly enriched in these traits. Genomic and culture-
14 723 dependent analyses show these Families to be free-living or symbiotic diazotrophs in
15 724 soil and freshwater environments (Madigan *et al.*, 1984, Itakura *et al.*, 2009, de los
16 725 Santos *et al.*, 2018). Given their significantly greater copies of nitrogen-fixing genes,
17 726 these Families may be a particularly important source of organic nitrogen for soil
18 727 communities. Saprotrophic Mycobacteriaceae genomes, also identified as nitrogen
19 728 cyclers, tend to have many copies of genes involved in ammonia uptake and glutamate
20 729 synthesis (Amon *et al.*, 2010). This taxon may play an alternative role in converting
21 730 mineral nitrogen to biomass where organic nitrogen as protein in excreted products or
22 731 necromass can undergo proteolysis and uptake between other community members.
23 732 The identification of Sporomusaceae as enriched in 'Nitrogen metabolism' traits is
24 733 unusual as these obligate anaerobic fermenters cannot use nitrate as an electron
25 734 acceptor (Möller *et al.*, 1984). Nor were the Sporomusaceae enriched in ammonia
26 735 uptake or glutamate synthesis genes (data not shown), and so it is uncertain what role
27 736 this Family plays in nitrogen cycling. Thaumarchaeota and Nitrosomonadaceae, known
28 737 ammonia oxidisers, were not enriched in 'Nitrogen metabolism' traits relative to other
29 738 Families (Supplementary Figure 5) despite Nitrosomonadaceae possessing multiple
30 739 copies of the operon responsible for ammonia oxidation (Klotz & Norton, 1998).
31 740 Specific traits may be overlooked here if the BRITE category includes many diverse
32 741 KOs (e.g. ammonia oxidation, nitrogen fixation, glutamate synthesis etc).

33 742 Another specialised metabolic pathway of interest involves 'Methane metabolism'
34 743 that includes production and oxidation of a potent greenhouse gas. Unsurprisingly, the
35 744 Methanogens and methanotrophic Methylococcaceae, Beijerinckiaceae,
36 745 Methylocystaceae were all enriched in traits involved in methane metabolism. While

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3 746 methane oxidation can be present in some taxa from the Verrucomicrobia (Op den
4 747 Camp *et al.*, 2009) the above proteobacterial representatives act as the primary
5 748 terrestrial methane sink (Dunfield, 2007, Conrad, 2009).
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751 *4.5 Sensing, responding and tolerating the environment*

752 A particularly interesting divergence of traits were involved in how taxa detect and
753 respond to environmental stimuli. Gram-negative Pseudomonadaceae,
754 Rhodospirillaceae, Bradyrhizobiaceae and Myxococcaceae were enriched in two-
755 component systems. These membrane-bound histidine kinases respond rapidly to
756 extracellular stimuli (Galperin, 2005) and these traits were primarily involved in
757 nitrogen, potassium, initiating chemotaxis and C₄-dicarboxylate responses. Families
758 enriched in transcription factors were the Myxococcaceae, Polyangiaceae,
759 Streptomycetaceae, Mycobacteriaceae and Frankiaceae. These factors regulate
760 transcription in response to intracellular cues and here these factors were primarily
761 *rpoD* (housekeeping), *rpoH* (heat-shock/protein damage), *rpoE* (extra-cellular
762 cytoplasmic stress) and *rpoS* (starvation) responses (Shimada *et al.*, 2017). The
763 genomes of these taxa are also heavily enriched in regulatory genes for complex
764 developmental stages, fruiting bodies and/or filamentous branching growth in soils
765 (Bentley *et al.*, 2002, Gao *et al.*, 2006, Schneiker *et al.*, 2007). Thus, certain taxa may
766 respond primarily to extracellular cues while others strictly monitor and respond to
767 changes in cell homeostasis. This trend has been noted previously – in 167 genomes
768 across various Bacteria and Archaea, Proteobacteria had a higher ratio of sensors for
769 external *versus* internal stimuli and were considered ‘extroverts’, while Cyanobacteria
770 were considered strong ‘introverts’ focussed on responding to internal stimuli
771 (Galperin, 2005).

772 As mentioned above, Archaea exhibited unique traits in basal transcription and
773 protein regulation via proteasome. These transcription factors were primarily involved
774 in identifying DNA damage and excision repair: TFII-B, TFII-D, ERCC-2 and ERCC-3.
775 DNA repair differs markedly between Bacteria and Archaea/eukaryotes. Specifically,
776 Bacteria excise 12 nucleotides around a damaged site with a 3 polypeptide system
777 whereas Archaea excise 24 – 32 nucleotides with a 13 – 16 polypeptide system
778 (Sancar, 1996). The use of ubiquitin-labelling and proteasome degradation of
779 misfolded or ‘old’ proteins is arguably a more efficient system for recycling amino acids

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3 780 and regulating the 'lifespan' of a protein in eukaryotes, however, Bacteria are still fully
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5 781 capable of regulating protein misfolding or proteolysis with RpoH (and others) induced
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7 782 upon environmental stress (Goldberg, 2003). From an ecological perspective, it is
8
9 783 difficult to discern if these eukaryote-like traits confer any sort of competitive advantage
10
11 784 to Archaea. They may simply be examples of convergent evolution for dealing with
12
13 785 environmental stress.

14 786 Finally, most microbial cells likely exist within complex biofilms and/or assemblages
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16 787 adhered to surfaces with excreted exopolysaccharides, DNA and protein that serve to
17
18 788 protect from adverse environmental factors (Flemming & Wingender, 2010). Families
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20 789 with high copy numbers of exopolysaccharide biosynthesis and secretion systems may
21
22 790 act as integral members of soil communities by predominantly contributing to
23
24 791 biofilm/aggregate formation. The 'LPS biosynthesis' and 'Starch and sucrose
25
26 792 metabolism' BRITE categories can synthesise N-acetyl glucosamine-based and
27
28 793 cellulose-based exopolysaccharides, respectively. Taxa enriched in both these
29
30 794 categories and secretion systems were the Polyangiaceae and Burkholderiaceae
31
32 795 (Table 1), and in the refined 'Under-represented' model, Acidobacteria and
33
34 796 Verrucomicrobia (Supplementary Table 11).

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37 799 *4.6 Direct cell-cell interactions*

38 800 Type IV secretion systems were another important trait identified in the Random Forest
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40 801 models. These were enriched in Pseudomonadaceae and Myxococcaceae (Table 1)
41
42 802 and Acidobacteria, Planctomycetes and Verrucomicrobia (Supplementary Table 11).
43
44 803 These are highly specialised exporters that deliver DNA and/or toxins directly to other
45
46 804 bacterial or plant cells, however, their role in ecology is poorly understood beyond root
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48 805 galls induced by *Agrobacterium tumefaciens* (Christie & Vogel, 2000). These taxa
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50 806 should be explored for whether they utilise these traits for horizontal gene transfer or
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52 807 to inject toxins directly into other prokaryotes, and thus potentially provide a selective
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54 808 advantage for colonisation and competition.

55 809 Another archetypal trait for interactions between community members are
56
57 810 production of antimicrobials and antimicrobial resistance genes. Penicillin and
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59 811 cephalosporin synthesis were enriched in the Sporomusaceae and Bacillaceae relative
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812 to other Firmicutes. Polyketide and Type II polyketide syntheses were important for
813 separating Frankiaceae and Streptomycetaceae from other Actinobacteria

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3 814 (Supplementary Table 10). The Streptomycetaceae have a long history of use in
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5 815 biotechnology as prolific antimicrobial producers (Bentley *et al.*, 2002). Bacillaceae (in
6
7 816 particular *Bacillus subtilis* species) are also well known producers of a wide variety of
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9 817 antimicrobials (Caulier *et al.*, 2019), but we noted that Sporomusaceae have an even
10
11 818 greater number of these traits (Supplementary Figure 9). To the authors' knowledge,
12
13 819 antibiotic production in Sporomusaceae has not been investigated thoroughly and this
14
15 820 may be a consequence of its obligate anaerobic nature and difficulties in culturing. In
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17 821 addition to prolific Type II polyketide producers, Streptomycetaceae were also enriched
18
19 822 in antimicrobial resistance genes, while Bacteroidetes, Planctomycetes and
20
21 823 Verrucomicrobia were specifically enriched in beta-Lactam resistance (Supplementary
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23 824 Tables 9 and 10).

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827 4.7 Generalisable life strategies emerge from differentially enriched traits

828 We hypothesised that taxa would emerge as being inherently copiotrophic or
829 oligotrophic based on trends in their enriched traits. Traits were chosen based on
830 identification via Random Forest and identification as associated with copiotroph-
831 oligotroph species or in mixed communities as described previously (Lauro *et al.*, 2009,
832 Vieira-Silva & Rocha, 2010, Roller & Schmidt, 2015, Pascual-Garcia & Bell, 2020).
833 Rhizosphere-associated Gamma-, Alpha- and Betaproteobacteria in Clade I fit the
834 assumptions of a copiotrophic niche that invests in high metabolic rate – these taxa
835 were uniquely enriched in competing for nutrient uptake via high-affinity ABC
836 transporters, and energy generation from pyruvate, fatty acids, benzoate and
837 glyoxylate carbon sources. Clade I was also enriched in glutathione metabolism, which
838 acts as the major antioxidant for reducing intracellular free radicals produced during
839 central carbon metabolism (Smirnova & Oktyabrsky, 2005). Antioxidants have been
840 hypothesised as an essential function for copiotrophs to survive their high metabolic
841 rates (Koch, 2001). All five Clade I Families were enriched in oxidative phosphorylation.
842 The oxidative phosphorylation traits encompass a wide variety of electron transport
843 chain proteins (oxidoreductases, dehydrogenases, cytochromes and ATPases) and
844 are crucial for efficient energy production (Brochier-Armanet *et al.*, 2009). All five
845 Families were also enriched in nitrogen metabolism, which included both nitrogen
846 fixation and glutamate (*i.e.* protein) synthesis. Nitrogen fixation is an energy intensive
847 process requiring 20 – 30 ATP per reduced N₂ (Burriss & Roberts, 1993) and may be

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3 848 intrinsically linked to taxa with high oxidative phosphorylation. Finally, Clade I also
4
5 849 shared motility and chemotaxis, which are also energy intensive traits. Clade II
6
7 850 consisted of the remaining Gamma-, Alpha- and Betaproteobacteria, yet these were
8
9 851 relatively less enriched in Clade I 'copiotroph' traits. These particular taxa may be
10
11 852 responsible for the lack of a consistent copiotrophic response upon nutrient addition in
12
13 853 Proteobacteria (Ho *et al.*, 2017).

14 854 Clade III was comprised of taxa generally considered as oligotrophs (Ho *et al.*, 2017)
15
16 855 with the exception of Bacteroidetes (Fierer *et al.*, 2007). These taxa possessed high
17
18 856 LPS and sphingolipid synthesis that can defend against desiccation and antimicrobials
19
20 857 through biofilm and capsule/slime production (Flemming & Wingender, 2010), beta-
21
22 858 Lactam resistance, penicillin biosynthesis and several members had high pentose
23
24 859 phosphate pathway for efficient carbon metabolism under starvation (Hodgson, 2000).
25
26 860 Clade III also possessed high CAZy traits, which Clade I largely lacked, and is
27
28 861 consistent with observations of oligotrophs being primarily responsible for catabolising
29
30 862 relatively recalcitrant plant material (Goldfarb *et al.*, 2011). While Clade III were equally
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32 863 enriched in oxidative phosphorylation as Clade I, with the exception of the
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34 864 Deltaproteobacteria, these taxa were depleted in glutathione metabolism. The low
35
36 865 copies per genome of this trait would explain why the abundance of oligotrophs drop
37
38 866 rapidly in nutrient addition studies as they would be either out-competed by
39
40 867 glutathione-rich taxa capable of exploiting plentiful nutrients or will lyse if their
41
42 868 metabolic rate exceeds capacity to reduce free radicals (Koch, 2001). Taken together,
43
44 869 all of these traits indicate Clade III lead oligotrophic lifestyles whereby they are tolerant
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46 870 to adverse environmental conditions, can acquire carbon from recalcitrant plant
47
48 871 material, and are incapable of rapid growth rates.

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50 872 These results support previous observations that Rhodospirillaceae,
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52 873 Bradyrhizobiaceae, Burkholderiaceae, Pseudomonadaceae and Rhizobiaceae are
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54 874 copiotrophic, while Planctomycetes, Verrucomicrobia, Myxococcaceae,
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56 875 Polyangiaceae and Acidobacteria are oligotrophic (Ho *et al.*, 2017 and references
57
58 876 therein). As has been proposed previously, the dominance of these groups in certain
59
60 877 soils can provide inferences for ecosystem processes in that system, for example soils
878 dominated by Verrucomicrobia, Planctomycetes and Acidobacteria will have greater
879 capacity to degrade complex plant material while retaining most catabolised carbon in
880 biomass (*i.e.* high growth or carbon use efficiency) or excreted byproducts that assist
881 in soil aggregation (*e.g.* high LPS production) (Trivedi *et al.*, 2013). Conversely, soils

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3 882 dominated by copiotrophic Proteobacteria Families will be systems primarily
4 dependent on labile di- and monosaccharides that demonstrate low carbon use
5 883 efficiency.
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8 885 Streptomycetaceae, Mycobacteriaceae and Frankiaceae in Clade IV shared
9 enrichment of several Clade I copiotroph traits. As mentioned above in Section 4.5,
10 886 these Actinobacteria invest carbon and energy into complex filamentous growth and
11 887 these Actinobacteria invest carbon and energy into complex filamentous growth and
12 888 developmental cycles. They demonstrate classic copiotrophic responses to nutrient
13 889 addition (Goldfarb *et al.*, 2011, Leff *et al.*, 2015) and their enriched ABC transporters,
14 890 pyruvate, glyoxylate, benzoate and fatty acid metabolism all likely contribute to
15 891 generating energy for complex lifecycles. Simultaneously, their enrichment of Clade III
16 892 oligotroph traits for CAZy metabolism in addition to many traits for producing and
17 893 resisting antimicrobials indicate a unique niche for these Actinobacteria that does not
18 894 necessarily fall within the classical copiotroph-oligotroph framework.

19 895 Clade V differed markedly from all other clades and mostly consisted of 'specialist'
20 896 metabolic functional groups involved in photosynthesis, ammonia oxidation,
21 897 methanogenesis, lactic acid production and other fermentation. Similar to Clade III,
22 898 these taxa would also be expected to have relatively low metabolic rates due to
23 899 depletion of copiotroph traits associated with rapid metabolism and energy generation.
24 900 Unlike Clade III, these taxa seemed to lack consistent mechanisms for stress
25 901 tolerance. Thus, while certain taxa did invest in traits for rapid metabolic rate (Clades I
26 902 and IV) and others primarily in stress tolerance (Clades III and IV), some taxa lacked
27 903 these approaches altogether and pursued entirely distinct niches (Clade V). As an
28 904 average of 47% of traits within each genome were Uncharacterised, Clade V is an
29 905 over-simplification and that if novel, currently uncharacterised proteins and the traits
30 906 they fulfil were incorporated into hierarchical clustering, this clade would separate more
31 907 meaningfully.

32 908 If one were to consider taxa within the one-dimensional copiotroph-oligotroph
33 909 spectrum, Clade I would represent one extreme, Clades III and V another, with Clades
34 910 II and IV falling in between. Figure 5a is a conceptual diagram where these Clades
35 911 have been placed on a singular axis of 'resource investment', with the niche space of
36 912 Clades enriched in traits associated with rapid growth (e.g. glutathione) toward the
37 913 'copiotrophy' pole while Clades enriched in stress tolerance traits (e.g. LPS production)
38 914 are placed toward the 'oligotrophy' pole. However, this approach overlooks the diverse
39 915 functional potentials (and distinct niches) for carbon and energy metabolism in Clade

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2
3 916 ~~V~~associated with the various Clades. Furthermore, the large overlaps in niche space
4
5 917 between Clades would suggest taxa from each group could not co-exist if 'resource
6
7 918 investment' was the only important consideration (Gause, 1932, Hutchinson, 1957,
8
9 919 Leibold, 1995). A more meaningful perspective would be to consider the additional role
10 920 of 'resource acquisition' that incorporates multiple axes for the life strategies identified
11 921 via hierarchical clustering of traits. Figure 5b is a conceptual diagram of niche space
12 922 where clades have been further separated along additional dimensions based on their
13 923 enrichment of traits involved in competition, degradation or specialised metabolic
14 924 pathways. The BRITE categories listed on the various axes are chosen to be useful
15 925 markers in predicting the niche of a taxon. -This expanded niche space would suggest
16 926 hierarchical clustering in Figure 4 with each clade as its own 'life strategy'. The strategy
17 927 of taxa in Clade I follow that of classical 'copiotrophs' and are well equipped for nutrient
18 928 acquisition (primarily, but not limited to, di- and monosaccharides), rapid growth and
19 929 oxidative stress regulation as 'copiotrophic competitors'. Clade II, which may not be
20 930 capable of competing directly with Clade I for carbon and energy, ~~and perhaps the~~
21 931 ~~evolution of~~may thus occupy the niche space of specialist Proteobacterial
22 932 methylotrophs, methane and ammonia oxidisers ~~may be a responseso as~~ to ~~avoid~~
23 933 ~~competition over saccharides~~be 'copiotrophic metabolic specialists'. The strategy of
24 934 Clade III ~~is~~would be to ~~specialise in decomposing~~decompose plant material via diverse
25 935 CAZy and possess a variety of environmental stress tolerance traits as 'oligotrophic
26 936 degraders'. Clade IV, which include for example ~~are unique~~ Actinobacteria that share
27 937 traits for competition, degradation and oligotrophy, could thus occupy niche space
28 938 between Clades I and III ~~traits, yet likely fill a distinct niche due to complex~~
29 939 ~~developmental cycles and filamentous growth~~. Finally, the strategy of Clade V ~~is~~would
30 940 be to fill highly specialised, unrelated metabolic niches reliant on completely distinct
31 941 carbon sources to other taxa. The large space conceptualised for Clade V in Figure
32 942 5b, which does not imply either copiotrophic or oligotrophic resource investment, is an
33 943 oversimplification and with improved understanding of traits in these taxa it may be
34 944 possible to fracture and further separate them into more detailed groups. This would
35 945 prove particularly beneficial for the poorly characterised Archaea. -and are unified by
36 946 an absence of classical copiotroph and oligotroph traits.

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57 947 __ Moving beyond a one-dimensional *r-K* spectrum to accommodate additional trait-
58 948 driven life strategies has been proposed in plant ecology (Grime, 1977). Specifically,
59 949 Grime argued that plant taxa fall within a multi-dimensional space defined by extremes

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3 950 on three axes: 'competitors' that acquire nutrients, light, water etc. more effectively
4 951 than neighbouring taxa in the same environment, 'stress tolerators' that are long-lived,
5 952 slow growing taxa that resist desiccation, alkaline soils etc., and 'ruderals' that have
6 953 very brief lifecycles between periods of disturbance and invest in environmentally
7 954 hardy seeds. Despite these varied strategies for resource investment, plants are
8 955 unified in that photosynthesis is their primary form of acquiring carbon and energy. The
9 956 diversity of microbial strategies for acquiring carbon and energy enables them to
10 957 explore a greater range of potential niche space, and in addition to growth traits that
11 958 allow for a relatively more copiotrophic or oligotrophic investment of those resources,
12 959 likely contributes to the high diversity of co-existing taxa observed in soil microbial
13 960 communities. ~~complexity of co-occurring traits across microbial Families indicate more~~
14 961 ~~generalisable life strategies than the copiotroph-oligotroph spectrum are needed.~~
15 962 However, to truly unravel differentiated niches— and general microbial life
16 963 strategies within these generalisable life strategies, two limitations must be overcome.
17 964 Firstly, will require a better understanding of the many 'Uncharacterised' traits in
18 965 environmental isolates, ~~in addition to robust trait-based analyses at the Species or~~
19 966 ~~even Strain taxonomic levels~~ is required. For example, the recent large-scale MAG study
20 967 by Nayfach *et al.*, (2021) identified 5.8 million protein clusters (traits), of which over
21 968 75% could not be annotated meaningfully by current protein databases. Secondly,
22 969 robust trait-based analyses down to the finer scale of distinct genomes will likely be
23 970 necessary to consider how individual taxonomic members of a community have either
24 971 differentiated in order to co-exist or are in the throes of competition that will ultimately
25 972 exclude one of the competitors.

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975 5. Conclusion

976 In a collection of 175 terrestrial prokaryotes that possess 220 664 traits shared
977 between at least two taxa, concepts in niche differentiation were explored. Non-random
978 trait distributions were shown as preferential clustering of related taxa within most
979 Phyla with a general trend of highest similarity at the level of Family. This strongly
980 supported ecological coherence of shared traits within close relatives. Random Forest
981 models successfully identified BRIT 3 categories that best explained differing traits
982 between taxonomic groups. These traits were involved in a wide range of biological
983 functions, including core physiological traits used historically to categorise taxa. Many

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3 984 traits were also involved in functions often associated with copiotrophs and oligotrophs,
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5 985 namely metabolism, nutrient acquisition and environmental stress tolerance.
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7 986 Hierarchical clustering of differential traits formed five distinct clusters, with Clade I
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9 987 representing the classical copiotrophic niche, Clades III and V as oligotrophic, and
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11 988 Clades II and IV in between. A more refined perspective would be to consider each
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13 989 Clade as its own ~~generalisable~~ life strategy in a niche space that considers both
14
15 990 resource investment and acquisition simultaneously; for example, the strategy of Clade
16
17 991 I is to invest in competition and rapid growth, while Clade V pursue highly distinct,
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19 992 specialised metabolic functions. The trait-based analyses here were effective in
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21 993 identifying general trends in potential function of terrestrial microbial taxa at the Phylum
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23 994 and Family level. Further investigation will be necessary to identify traits that give rise
24
25 995 to niche differentiation at lower taxonomic ranks and, ultimately, the importance of this
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27 996 for ecosystem processes of interest.

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1007 theoretical ecology performed here would not be possible.

1008

1009 Conflict of Interest

1010 The authors declare no conflict of interest regarding this study or its outcomes.

1011

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