Contrasting environmental preferences of photosynthetic and non-photosynthetic soil cyanobacteria across the globe

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Running title: Global preferences of soil cyanobacteria

Abstract

Aim: Cyanobacteria have shaped the history of life on Earth, and continue to play important roles as carbon and nitrogen fixers in terrestrial ecosystems. However, their global distribution and ecological preferences remain poorly understood, particularly for two recently discovered non-photosynthetic cyanobacterial classes (*Sericytochromatia* and *Melainabacteria*).

Location: 237 locations across six continents encompassing multiple climates (arid, temperate, tropical, continental and polar) and vegetation types (forests, grasslands and shrublands).

Time period: Sampling was carried out between 2003 and 2015.

Major taxa studied: Photosynthetic and non-photosynthetic cyanobacterial taxa

Methods: We conducted a field survey and used co-occurrence network analysis and structural equation modelling to evaluate the distribution and environmental preferences of soil cyanobacteria across the globe. These ecological preferences were used to create a global atlas (predictive distribution maps) of soil cyanobacteria.

Results: Network analyses identified three major groups of cyanobacteria taxa, which resembled the three main cyanobacterial classes: the photosynthetic *Oxyphotobacteria*-dominated cluster, which were prevalent in arid and semiarid areas, and the non-photosynthetic *Sericytochromatia*- and *Melainabacteria*-dominated clusters, which preferred hyperarid oligotrophic and acidic/ humid environments, respectively.

Main conclusions: This study provides novel insights into the environmental preferences of non-photosynthetic cyanobacteria in soils globally. Our findings highlight the contrasting environmental preferences among the three clusters of cyanobacteria and suggest that alterations in environmental conditions linked to climate change may result in important changes in the ecology and biogeography of these functionally important microorganisms.

Keywords: non-photosynthetic Cyanobacteria, Cyanobacteria, global distribution, microbial biogeography, microbial network, 16S amplicon sequencing

1 INTRODUCTION

Cyanobacteria are microorganisms responsible for some of the most important events in Earth's history, including the rise of oxygen levels via oxygenic photosynthesis (Dismukes *et al.*, 2001; Rasmussen *et al.*, 2008) and the formation of plastids through endosymbiosis (Mereschkowsky, 1905; Margulis, 1970). Despite being one of the most studied microbial groups (Castenholz *et al.*, 2001; Garcia-Pichel *et al.*, 2003; Garcia-Pichel, 2009; Whitton & Potts, 2012), there are still major gaps of knowledge associated with the diversity and global distribution of these organisms. Recent studies have revealed the existence of two new bacterial clades closely related to cyanobacteria, 4C0d-2 (*Melainabacteria*) and ML635J-21 (*Sericytochromatia*), recently proposed as new classes of phylum cyanobacteria (Soo *et al.*, 2014, 2017). These non-photosynthetic classes are included in the latest releases of the most commonly used rRNA databases, Silva and Greengenes (DeSantis *et al.*, 2006; Quast *et al.*, 2013). Unlike photosynthetic cyanobacteria (hereafter class *Oxyphotobacteria)*, these clades have no genes associated with photosynthesis, and have provided a new perspective on the phylum, broadening our understanding of the functional capabilities of cyanobacteria and their evolutionary origin.

The construction of metagenome-assembled genomes has enabled the assessment of the metabolic potential of these organisms, suggesting that *Melainabacteria* and *Sericytochromatia* are chemoheterotrophs with metabolisms mostly centered on fermentation (Di Rienzi *et al.*, 2013; Soo *et al.*, 2014, 2017; Soo, 2015). Additionally, no genes for phototrophy or carbon (C) fixation have been found in *Melainabacteria* and *Sericytochromatia* (Soo *et al.*, 2017), indicating that oxygenic photosynthesis could be a trait acquired later in *Oxyphotobacteria* by horizontal gene transfer (Raymond *et al.*, 2002). Such physiological and genetic differences might result in contrasting ecological preferences for these novel cyanobacterial taxa, but empirical evidence for this is lacking.

Soil-borne *Oxyphotobacteria* are widely distributed on the Earth (Garcia-Pichel *et al.*, 2003; Whitton & Potts, 2012; Moreira *et al.*, 2013) but they are specially predominant in hot arid and polar regions with sparse plant cover. They are an important component of biocrusts, soil surface communities dominated by lichens, mosses, cyanobacteria and associated microorganisms (Weber *et al.* 2016) and play key ecological roles in these environments by regulating critical soil processes such as nitrogen (N) and C fixation, soil stabilization and infiltration/runoff (Mager & Thomas, 2011; Sciuto & Moro, 2015). Other terrestrial cyanobacterial communities grow on the surface or inside rocks and soil (endolithic and subsoils forms), and are well adapted to dry conditions and high or low irradiation regimes (Warren-Rhodes *et al.*, 2006; Domínguez & Asencio, 2011; Puente-Sánchez *et al.*, 2018). The capacity of *Oxyphotobacteria* to stay dormant during long periods of time is also a fundamental characteristic of these organisms, which allow them to survive in extreme environments characterized by high or low temperatures, desiccation regimes or high ultraviolet radiation (Garcia-Pichel, 2009; Quesada & Vincent, 2012; Whitton & Potts, 2012).

Local and regional studies show that soil *Oxyphotobacteria* are generally considered to prefer neutral to alkaline pH for optimum growth (Brock, 1973; Whitton & Sinclair, 1975; Nayak & Prasanna, 2007). However, the global biogeography of soil *Oxyphotobacteria* has not been fully resolved due to the concentration of cyanobacterial research in particular regions, e.g. studies in western United States or the Antarctic continent (Garcia-Pichel *et al.* 2001; Namsaraev *et al.* 2010)(Garcia-Pichel *et al.*, 2003; Moreira *et al.*, 2013; Büdel *et al.*, 2016; Williams *et al.*, 2016) and the focus given to key and abundant taxa, such as *Microcoleus vaginatus* or the genus *Chroococcidiopsis* (Bahl *et al.*, 2011; Dvořák *et al.*, 2012), or specific habitats such as cold ecosystems and deserts (Jungblut *et al.* 2010; Bahl *et al.* 2011). There are clear gaps of knowledge of their distribution in certain regions of the world, such as South America (Büdel *et al.*, 2016). Despite their wide dispersal ability due to small size, aeolian transport and tolerance to desiccation and irradiation (Billi *et al.*, 2000; Kellogg & Griffin,

2006), and their often cosmopolitan distribution (Garcia-Pichel *et al.*, 1996; Taton *et al.*, 2006; Flombaum *et al.*, 2013), current knowledge suggests a more complex biogeography of these microorganisms that is likely to be also influenced by their phylogeny and historical legacies (Garcia-Pichel *et al.*, 1996, 2003; Taton *et al.*, 2006; Nayak & Prasanna, 2007; Flombaum *et al.*, 2013).

The ecology and biogeography of the non-photosynthetic cyanobacteria classes (*Melainabacteria* and *Sericytochromatia)* in soils is poorly known. Available information on these organisms comes from genomes from aphotic environments such as animal guts or subsurface groundwater and artificial systems such as water treatment facilities and laboratory bioreactors (Ley *et al.*, 2005; Warnecke *et al.*, 2007; Yagi *et al.*, 2010; Di Rienzi *et al.*, 2013; Soo *et al.*, 2014; Utami *et al.*, 2018) and the scarce environmental studies correspond only to aquatic ecosystems such as lakes and algal biofilms (Monchamp *et al.*, 2018, 2019).

To advance our understanding of the biogeography and ecological preferences of soil photosynthetic and non-photosynthetic cyanobacteria, we used data from a global soil survey covering a wide diversity of climate, soil and vegetation types (Delgado-Baquerizo *et al.*, 2018). We expected the distinct ecological attributes of photosynthetic and non-photosynthetic cyanobacteria to be associated with very different environmental preferences. For example, we know that some *Oxyphotobacteria* have developed highly competitive adaptations to thrive in arid soils with low soil organic C and plant productivity (Lund, 1967; Whitton & Sinclair, 1975; Maestre *et al.*, 2015). In these environments, we expect *Oxyphotobacteria* to dominate due to their capacity to build protective sheath pigments and to fix atmospheric C and N, which can be an important ecological advantage. However, *Oxyphotobacteria* are also expected to appear in a wide variety of environmental conditions, including low light, low oxygen or even anoxygenic environments due to their enormous functional diversity (Stal & Moezelaar, 1997; Adams & Duggan, 1999; Garcia-Pichel, 2009; Puente-Sánchez *et al.*, 2018). Conversely, non-photosynthetic cyanobacteria rely on soil organic C pools to grow, which

147 could translate into contrasting preferences related to soil nutrient availability. We expect to
148 find groups of taxa co-occurring and sharing similar environmental preferences (hereafter
149 *ecological clusters*) related to photosynthetic capability, habitat preferences and historical
150 legacies.

151 2 MATERIALS AND METHODS

152 2.1 Global survey: Sites, soil collection, soil and molecular analyses

153 We used 16S rRNA gene amplicon sequencing data from a global survey of 237 locations (Fig.
154 S1) across six continents encompassing multiple climates (arid, temperate, tropical,
155 continental and polar) and vegetation types (forests, grasslands and shrublands) (Delgado-
156 Baquerizo *et al.*, 2018). A composite soil sample (0-7.5 cm depth) was collected under the
157 dominant vegetation at each surveyed location. A fraction of each sample was immediately
158 frozen at -20^{o}C for molecular analyses; the other fraction was air-dried for chemical analyses.
159 Sample collection of soils took place between 2003 and 2015. We do not expect differences in
160 the timing of sample collection to largely affect our results for two main reasons. First, at the
161 global scale seasonal variability is expected to be largely overcome by cross-biome variability
162 (e.g., see Carini *et al.*, 2020 on the importance of spatial vs. temporal scales when analyzing
163 soil microbial communities). To put it simple, a dryland and a boreal forest are so different that
164 usually harbor distinct microbial communities regardless of their seasonal variability. Second,
165 we are using amplicon sequencing DNA-based analyses (see below), which characterize not
166 only the active portion of cyanobacterial communities but also the dormant one at the
167 moment of sampling (Li *et al.*, 2017). The soils sampled comprise a wide variety of physico-
168 chemical properties, pH ranged from 4.04 to 9.21, texture of the fine fraction (%clay+silt)
169 ranged from 1.4 to 92.0%, soil total organic carbon (OC) from 0.15 to 34.77%, soil total
170 nitrogen (TN) from 0.02 to 1.57, C:N ratio (CN) ranged from 2.12 to 67.52 and soil total

phosphorus (TP) from 75.10 to 4111.04 mg P kg^{-1} soil. These analyses were done using standard laboratory methods described in Delgado-Baquerizo *et al.* (2018).

Climatic variables (maximum and minimum temperature [MAXT, MINT], precipitation seasonality [inter-annual coefficient of variation in precipitation, PSEA] and mean diurnal temperature range [MDR]) were obtained for each site from the WorldClim database (Hijmans *et al.*, 2005). Aridity Index (precipitation/potential evapotranspiration) was obtained from the Global Potential Evapotranspiration database (Zomer *et al.*, 2008), which uses interpolations from WorldClim. The annual ultraviolet index (UV Index), a measure of the risk of UV exposition ranging from 0 (minimal risk) to 16 (extreme risk), was obtained for each site using data from the Aura satellite (Newman & McKenzie, 2011). Net aboveground primary productivity [ANPP] was estimated with satellite imagery using the Normalized Difference Vegetation Index (NDVI) from the Moderate Resolution Imaging Spectroradiometer (MODIS) aboard NASA's Terra satellites (Justice *et al.*, 1998). This index provides a global measure of the greenness of the Earth for a given period (Pettorelli *et al.*, 2005). Here, we used monthly averaged values for NDVI for the sampling period between 2003 and 2015 (10 km resolution).

Microbial DNA was extracted using the PowerSoil DNA Isolation Kit (MoBio Laboratories, Carlsbad, CA, USA) following manufacturer's instructions. DNA extracts were sequenced targeting the bacterial V3-V4 region using 16S rRNA gene primers 341F (CCTACGGGNGGCWGCAG) and 805R (GACTACHVGGGTATCTAATCC) and the Illumina Miseq platform of the Next Generation Genome Sequencing Facility at Western Sydney University (Australia). Bioinformatic analyses were performed with a combination of QIIME (Caporaso *et al.*, 2010), USEARCH (Edgar, 2010) and UPARSE (Edgar, 2013). After merging of the reads, the primers were trimmed and sequences of low quality (expected error rate > 1) were discarded. Phylotypes were defined with UCLUST (Edgar, 2010) at an identity level of 97% and taxonomy was assigned using Silva Incremental Alligner *Search and classify* with Silva database (complementing not identified phylotypes with Greengenes database) (DeSantis *et al.*, 2006;

Quast *et al.*, 2013). Phylotypes represented by only a single read (singletons) were removed. The final dataset of phylotypes was filtered for phylum Cyanobacteria (excluding Chloroplast) and the relative abundance each of cyanobacterial phylotype in relation to total bacteria (all 16S rRNA reads) was calculated.

2.2 Structure of the community: Network analyses

To explore the different patterns of cyanobacterial co-occurrence across our samples, we conducted a network analysis with the CoNet software (Faust & Raes, 2016). This tool detects significant non-random patterns of co-occurrence using multiple correlation and dissimilarity measures. Two correlation coefficients (Pearson and Spearman) and dissimilarity distances (Bray-Curtis and Kullback Leiber) were used to obtain a more reliable network (Faust & Raes, 2012). When links were detected by more than one correlation/dissimilarity measure, they were considered as a single link. Samples were standardized prior to network analyses with the "col_norm" function, which divides each column by its sum, converting abundances in column-wise proportions. We computed the network with the top 1000 links for each measure and tested the statistical significance of each link with 1000 permutations and the function "shuffle rows" as the resampling strategy. Multiple testing was corrected by using Benjamini-Hochberg's procedure (Benjamini & Hochberg, 1995), keeping links with an adjusted merged p-value below 0.05. The final network was visualized with the interactive platform gephi (Bastian *et al.*, 2009). We obtained the ecological clusters with the function "fastgreedy" from the igraph package (Csárdi & Nepusz, 2006) in R version 3.4.0 (Team, 2013), and tested the statistical significance of modularity using 10000 random networks. Network analysis allowed us to divide the community between ecological clusters, that we used for further analysis. The relative abundance of each ecological cluster per sample was calculated by averaging the standardized (z-score) relative abundance of the phylotypes present within each ecological cluster. Thus, we obtained a balanced contribution of each cyanobacterial phylotype to the

relative abundance of its ecological cluster. Note that the use of z-score standardization transforms relative abundances, and therefore negative values can be obtained.

2.3 Factors determining cyanobacterial global distribution

Environmental effects: We conducted Structural Equation Modelling (SEM, Grace 2006) to evaluate the direct and indirect effects of spatial, climatic, vegetation and soil variables as predictors of the abundance of the main cyanobacterial ecological clusters (See Fig. S2 for our *a priori* model). This approach is useful for simultaneously testing the influence of multiple variables and the separation of direct and indirect effects of the predictors included in the model (Grace, 2006). These included spatial (Latitude, sine Longitude, cosine Longitude), climatic (MDR, MAXT, MINT, PSEA and Aridity [1-Aridity Index]) and vegetation (Grassland, Forest and ANPP) variables, as well as soil properties (CN, soil OC, pH and percentage of clay and silt). Prior to modelling, we transformed them to improve normality: Aridity, OC, PSEA and CN were log-transformed and both ANPP and the percentages of clay and silt were square root transformed. We used the chi-square fit test, supplemented with root mean square error of approximation (RMSEA) to test the overall fit of the model. We analysed path coefficients of the model and their associated P values and the total effects of each variable. As some of the variables were not normally distributed despite transforming them, we used 5000 bootstraps to simultaneously test the significance of each path. SEM analyses were conducted using AMOS 24.0.0 (IBM SPSS, Chicago, IL, USA).

To obtain a prediction of the potential distribution of the main cyanobacterial ecological clusters, we used the regression model Cubist (Quinlan, 2014) as implemented in the R package Cubist (Kuhn *et al.*, 2016). This model uses a linear regression tree analysis that predicts the most important factors affecting the abundance of each ecological cluster based on environmental covariates. Covariates in our models included the same variables used in our SEMs. Global predictions of the distribution of major clusters were done on a 25 km resolution grid. Soil properties for this grid were obtained from SoilGrids (Hengl *et al.*, 2017). Major

248 vegetation types (grasslands and forests) were obtained using Globcover2009 map from the
249 European Space Agency (Bontemps *et al.*, 2013). Information on climate, UV index and net
250 primary productivity were obtained from the WorldClim database and NASA satellites as
251 described above.

252 We conducted multiple analyses to support the validity of our global prediction maps.
253 First, we used kernel density estimations to compare the distribution of key soil and climate
254 variables of our dataset with those from high resolution global maps: SoilGrids (Hengl *et al.*,
255 2017) and Worldclim (Hijmans *et al.*, 2005). Our dataset comprises a large percentage of their
256 global variability (Fig. S3): 78.51% for OC, 94% for pH, 58.25% for Aridity, 45.98% for PSEA,
257 71.63% for MINT, 47.03% for MAXT and 96.43% for ANPP. These results indicate that our
258 sampling covers a large proportion of the environmental variability found on Earth. Second, we
259 found a strong correlation between the relative abundance of our cyanobacterial ecological
260 clusters and key microbial environmental factors at the global scale (see results below), which
261 suggests that environmental data can be used to predict their distribution. Finally, predictive
262 maps were cross-validated with an independent dataset obtained from the Earth Microbiome
263 Project (EMP, Thompson *et al.*, 2017), which contains data on soil cyanobacteria from 403 sites
264 worldwide (see Fig. S1). For doing so, we estimated the relative abundance of the three main
265 cyanobacterial clusters for the EMP dataset using the 97% similar EMP phylotypes. We first
266 calculated relative abundance of each cyanobacterial phylotype in relation to total bacteria (all
267 16S rRNA reads of the EMP dataset). Then, the relative abundance of each ecological cluster
268 per sample was computed by averaging the standardized (z-score) relative abundance of the
269 phylotypes of each ecological cluster, as explained above for our dataset. We then used our
270 predictive maps to extract the predicted relative abundance of each cluster for the EMP
271 locations. These predictive abundances were then compared with the independent results of
272 relative abundance of each cluster calculated with the EMP dataset using Pearson correlations.

273 We also conducted a Permanova analysis with Bray Curtis distances to evaluate the

effect of vegetation type on the abundance of each cyanobacterial cluster with the *adonis* function and 1000 permutations. To test for the differences in the relative abundance of each cluster across vegetation types we first tested the homogeneity of groups dispersions (variances) with *betadisper* function and from the result of that call we performed the post hoc analysis Tukey Honest Significant Differences with *TukeyHSD* function. All these analysis were done with vegan v2.4-2 (Oksanen, 2015) and R version 3.6.0 (Team, 2013).

Phylogenetic tree**:** The phylogenetic tree of cyanobacteria was constructed using the SILVA Alignment, Classification and Tree (ACT) Service (www.arb-silva.de/ act). Multiple sequence alignment of the 343 rRNA gene sequences was performed using SINA v1.2.11 (Pruesse *et al.*, 2012). A phylogenetic tree was obtained with their built-in tree computation tool FastTree (Price *et al.*, 2009) using the General Time Reversible Model of nucleotide evolution (Nei & Kumar, 2000) and keeping the default parameters. The display and annotation of phylogenetic tree were made with iTol v5.5 (Letunic & Bork, 2019).

3 RESULTS

3.1 Global cyanobacterial co-occurrence patterns

Despite the common and widespread occurrence of soil cyanobacterial taxa on Earth, we did not find any of the 343 phylotypes present in all samples. The most ubiquitous cyanobacterial phylotype, *Microcoleus vaginatus*, was detected in 113 of the 237 sites surveyed. Moreover, the relative abundance of cyanobacterial phylotypes in our soils ranged from 0.01% to 4.35% of all bacterial 16S rRNA gene sequences (see Table S1). The cyanobacterial orders with the highest relative abundances included Oscillatoriales (*Oxyphotobacteria*), followed by Obscuribacterales (*Melainabacteria*) and Nostocales (*Oxyphotobacteria*) (Fig. 1). Non-photosynthetic phylotypes appeared almost in all samples (235/237 samples 99.2%). Photosynthetic cyanobacteria phylotypes appeared in the majority of them (185/237, 78.1%).

Our final network had 281 phylotypes and was arranged in 10 ecological clusters. Among these clusters, we identified three major groups of taxa co-occurring and comprising 65% of the cyanobacterial phylotypes identified (Fig. 2a). The remaining seven clusters were minor, encompassing from 8% to 1% of phylotypes. The three main ecological clusters were dominated by either *Oxyphotobacteria* (82% of 76 phylotypes), *Sericytochromatia* (52% of 31 phylotypes) or *Melainabacteria* (83% of 76 phylotypes; see Table S1). We focused on these main ecological clusters for the downstream analyses. Our correlation network showed a contrasting node distribution for cyanobacterial phylotypes characterized by photosynthetic and non-photosynthetic capabilities (Fig. 2b). Overall, the three ecological clusters identified were strongly dominated by the three extant cyanobacterial classes (Fig. 2c, 2d).

3.2 Environmental preferences of photosynthetic and non-photosynthetic soil cyanobacteria

Vegetation type significantly affected the abundance of each of the main cyanobacterial clusters identified (Permanova R^2=0.28, 0.24 and 0.15 for *Melainabacteria*, *Sericytochromatia* and *Oxyphotobacteria*-dominated clusters, respectively, $p<0.05$ in all cases).

Our SEM model indicated that the cluster dominated by *Oxyphotobacteria* was positively and negatively related to aridity and net aboveground productivity, respectively (Figs. 3, 4 and S4a), which explains their high relative abundance in dry grasslands (Fig. 6). We also observed a positive association between the relative abundance of the *Oxyphotobacteria* dominated cluster and both soil pH and minimum temperature (Fig. 3, 4, and S4a). We predicted the distribution of this cluster in a wide range of arid and semiarid areas worldwide (e.g., southern Sahara, southern Africa, northern Australia, India, Arabian Peninsula, areas surrounding the Amazon Basin, southwestern US and northwestern Mexico; Fig. 5a).

The cluster dominated by *Sericytochromatia* had a strong preference for arid environments with low soil C content (Fig. 3, 4, 6 and S4b). Taxa within this ecological cluster

were also positively associated with locations characterized by high inter-annual rainfall variability (Figs. 3, 4 and S4b). Our global atlas predicts that taxa within this ecological cluster can be found in hyper-arid areas such as the Saharan Desert, central Australia, the Atacama, Gobi and Taklamakan Deserts and the Arabian Peninsula, with almost no areas of intermediate relative abundance (Fig. 5b).

Unlike the other two ecological clusters identified, the *Melainabacteria*-dominated cluster showed a preference for humid and acidic soils, as indicated by the reduced relative abundance of this cluster with increases in aridity and pH (Figs. 3, 4 and. S4c). The vast majority of phylotypes found in our study corresponded to the order Obscuribacterales (1, 2d). This ecological cluster is found mainly in tropical and cold forests and grasslands (which are mostly temperate; see Fig. 6). Prediction maps show high relative abundance values of this cluster in humid areas of the Amazon Basin, central Africa, west Asian coast and Pacific Islands (Fig 5c). Despite the methodological differences between our dataset and the EMP dataset (primer sets used here 341F/805R vs. 515F/806R for the EMP; read lengths here 400bp/sequence vs. <150bp for the EMP and the lack of standardization in the EMP soil sampling protocols and metadata collection) we obtained positive and significant correlations between both results: Melainabacteria dominated cluster Pearson's $r=0.28$ ($P<0.001$), Sericytochromatia dominated cluster Pearson's $r=0.53$ ($P<0.001$), Oxyphotobacteria dominated cluster Pearson's $r=0.35$ ($P<0.001$). These results support the validity of our maps as representative of the distribution of the main ecological clusters of cyanobacteria across the globe.

4 DISCUSSION

The discovery of non-photosynthetic cyanobacteria has expanded one of the currently most diverse bacterial phylum (Castenholz *et al.*, 2001; Garcia-Pichel, 2009; Whitton & Potts, 2012; Dvořák *et al.*, 2017). There is a large body of knowledge about photosynthetic cyanobacteria

348 showing their importance in terrestrial ecosystems, as they are key components of
349 cryptogamic covers, which are estimated to fix 3.9 Pg carbon per year (Elbert *et al.*, 2012).
350 They increase soil fertility by fixing atmospheric N (Cleveland *et al.*, 1999), stabilize soils by
351 producing extracellular polysaccharides (Mazor *et al.*, 1996; Mager & Thomas, 2011),
352 protecting it from erosion and creating suitable habitats for the colonization of mosses and
353 lichens (Zhang, 2005; Lan *et al.*, 2015). However we know relatively little about the distribution
354 and environmental drivers of the newly described non-photosynthetic cyanobacteria in soils.
355 Our work provides novel insights into the ecology and biogeography of these key organisms,
356 and advances our understanding of on the potential vulnerabilities of photosynthetic and non-
357 photosynthetic cyanobacteria to changing environmental conditions.

358 Photosynthetic taxa represented by the *Oxyphotobacteria*-dominated cluster prefer
359 areas with sparse vegetation cover, and therefore greater accessibility to light, such as dry
360 grasslands (Figs. 3,4, 6 and S4a). Accordingly, they are reported as key components of biocrust
361 communities in low productivity ecosystems such as arid environments (Garcia-Pichel, 2009;
362 Belnap *et al.*, 2016), where the ability to fix atmospheric C and N can be an important
363 ecological advantage. As with the remaining bacterial communities (Fierer & Jackson, 2006)
364 soil acidity is a key factor shaping the global distribution of *Oxyphotobacteria* (Fig. 4).
365 Consistentwith previous studies (Baas-Becking *et al.*, 1960; Brock, 1973; Nayak & Prasanna,
366 2007) we found that photosynthetic cyanobacteria have a preference for neutral to alkaline
367 soils (Figs. 3,4 and S4a), which are characteristic of drylands (Schlesinger & Bernhardt, 2013).
368 Our analyses further indicate a wide distribution of this cluster in drylands worldwide (Fig. 5),
369 as previously reported for members of this taxa in continental-scale distribution studies (Bahl
370 *et al.*, 2011; Garcia-Pichel *et al.*, 2013). Together with temperature, soil moisture plays a key
371 role driving the physiology, small-scale distribution and behaviour of soil photosynthetic
372 cyanobacteria (Garcia-Pichel & Pringault, 2001; Rajeev *et al.*, 2013). The high tolerance and
373 photosynthetic performance of *Oxyphotobacteria* at high temperatures is one of the reasons

why cyanobacterial-dominated biocrusts are so abundant in hyper-arid and arid environments (Grote *et al.*, 2010; Wang *et al.*, 2012). Thus, we observed a positive influence of high minimum temperatures and aridity on this cyanobacterial cluster (Figs. 3. and S4a). By moving from local/regional to the global scale, including samples from poorly-studied regions of South America (Garcia-Pichel *et al.*, 2003; Büdel *et al.*, 2016), and considering multiple terrestrial global biomes, our results provide novel predictions of the global distribution of *Oxyphotobacteria* in global soils.

Unlike *Oxyphotobacteria*, non-photosynthetic cyanobacteria require relatively large soil organic C pools for growth. We observed contrasting environmental preferences for each of the non-photosynthetic clusters across the oligotrophic-copiotrophic continuum, such as those reported for other soil heterotrophic organisms (e.g., methanotrophs in Nazaries *et al.* 2018). A key finding of our study is that the *Melainabacteria*-dominated cluster was especially abundant in mesic forests (tropical and cold forests, Fig. 6) and temperate grasslands, while the *Sericytochromatia*-dominated cluster is associated with locations with reduced plant cover and high temperatures (e.g., hyperarid deserts in Fig. 5, dry grasslands in Fig. 6). We found very little overlap between the predicted distributions of non-photosynthetic clusters of cyanobacteria (Figs. 5b, 5c) and a negative relationship between the relative abundances of these two non-photosynthetic clusters (Spearman correlation $r = -0.31$, $p<0.05$). Interestingly, a sizable percentage of members of *Melainabacteria* appears in the *Sericytochromatia* dominated-cluster (38%). We know that members of class *Melainabacteria* are capable of aerobic respiration because they contain respiratory components of the complex III-IV operon, which is adapted to low oxygen conditions, a C-family oxygen reductase and two cytochrome bc oxydases (Soo *et al.*, 2017). However, the *Melainabacteria*-dominated cluster is dominated by members of the order Obscuribacterales (Fig. 2d), for which there is little functional information available in the literature. Genomic analyses of the *Candidatus Obscuribacter phosphatis* suggest that this particular species is adapted to dynamic environments involving

400 feast-famine nutrient cycles, and has the capacity for aerobic or anaerobic respiration and
401 fermentation (Soo *et al.*, 2014). These features allow it to survive in both oxic and anoxic
402 environments. To our knowledge there is no information available of the contribution of this
403 cyanobacterium to the structure and function of forest ecosystems. However, our results
404 suggest that molecular ecologists and taxonomists targeting taxa in *Melainabacteria*-
405 dominated cluster should focus mainly on mesic forests across the globe. We also expect non-
406 photosynthetic cyanobacteria to play a significant role in soil biogeochemical cycles in both
407 high and low productive soils through C degradation and/or H_2 production, as reported for
408 *Melainabacteria* in an alluvial aquifer (Wrighton *et al.*, 2014). However, studies linking non-
409 photosynthetic soil cyanobacteria to carbon degradation in terrestrial environments are still
410 lacking. Future studies are thus needed to identify the relative contributions of non-
411 photosynthetic cyanobacteria to organic matter decomposition and C cycling in soils from
412 contrasting biomes.

413 The topology of our phylogenetic tree (Fig. 2c) reflects the expected evolutionary
414 relationships from previous research with separation of three main clades (Soo *et al.*, 2017);
415 the basal deep branched *Sericytochromatia*, *Melainabacteria* and photosynthetic
416 *Oxyphotobacteria*. As the ecological clusters are related to these classes, their global
417 distribution is likely to be related to past evolutionary events within this ancient phylum (Bahl
418 *et al.*, 2011; Moreira *et al.*, 2013). The ecological diversification observed in the non-
419 photosynthetic clades is particularly noteworthy. We found a niche-differentiation between
420 the basal cyanobacterial clade, *Sericytochromatia,* which occupies extremely dry
421 environments, and *Melainabacteria*, which is mostly found in humid forests. Interestingly, the
422 presence of phylotypes from *Melainabacteria* in the *Sericytochromatia*-dominated cluster may
423 point to the existence of common ancestral traits between both classes and the later
424 expansion of *Melainabacteria* into new "humid" niches. Photosynthetic cyanobacteria
425 (*Oxyphotobacteria*) are known for being extraordinarily ecologically versatile, mostly living in

environments with at least some exposure to sunlight, and capable of inactivating their photosynthetic apparatus (Harel *et al.*, 2004) or performing light-independent energy generation (Stal, 2012) when needed. There is still no consensus about the date the acquisition of oxygenic photosynthesis by *Oxyphotobacteria*; this could have happened either after divergence from other non-photosynthetic clades (Soo *et al.*, 2017) or before, sharing a photosynthetic common ancestor (Harel *et al.*, 2015). Regardless, the acquisition of oxygenic photosynthesis was a revolutionary event that allowed cyanobacteria to expand into diverse niches, and also the evolution of algae and terrestrial plants through endosymbiosis (Mereschkowsky, 1905; Margulis, 1970).

Our findings represent a starting point towards the understanding of the ecological preferences and global distributions of non-photosynthetic soil cyanobacteria. They highlight the fact that major photosynthetic and non- photosynthetic groups of soil cyanobacteria have contrasting ecological preferences across the globe. However, and given the difficulty of predicting microorganisms at a global scale, conclusions should be viewed as preliminary. The potential distribution maps presented here and the identification of the main environmental drivers of soil cyanobacterial distribution also illustrate how different cyanobacterial lineages might respond to ongoing climate and land use change. For example, the positive influence of aridity on the *Sericytochromatia*- and *Oxyphotobacteria*-dominated clusters suggests that the distribution of these taxa could expand under future climate change scenarios (Huang *et al.*, 2016). Consequently, our findings advance our understanding of the ecological distributions of these functionally important microbial communities and provide a basis for predicting possible future shifts of cyanobacterial terrestrial communities in a human-dominated, warmer and more arid world. To complement and expand our findings, future studies should further investigate the temporal dynamics of photosynthetic and non-photosynthetic cyanobacteria in terrestrial ecosystems, particularly along multiple temporal scales.

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DATA ACCESSIBILITY STATEMENT

Raw data related with this manuscript are available in Figshare, https://figshare.com/s/82a2d3f5d38ace925492

FIGURES

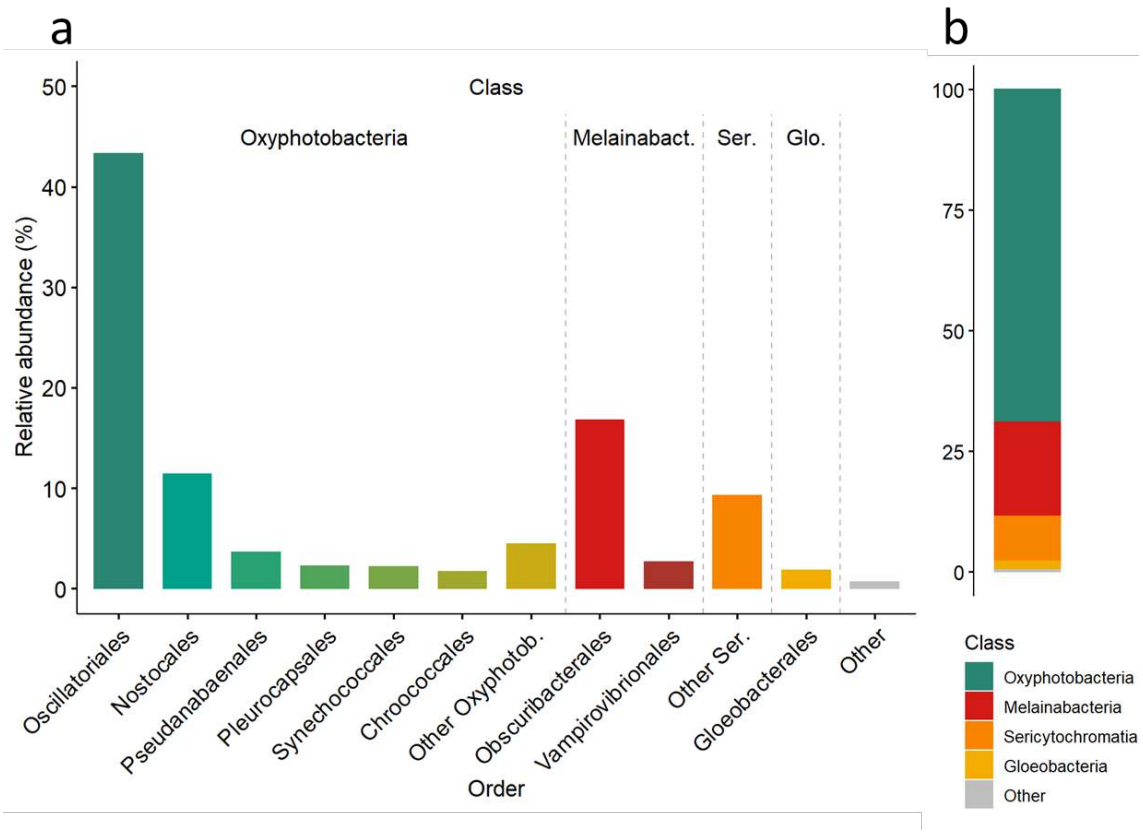
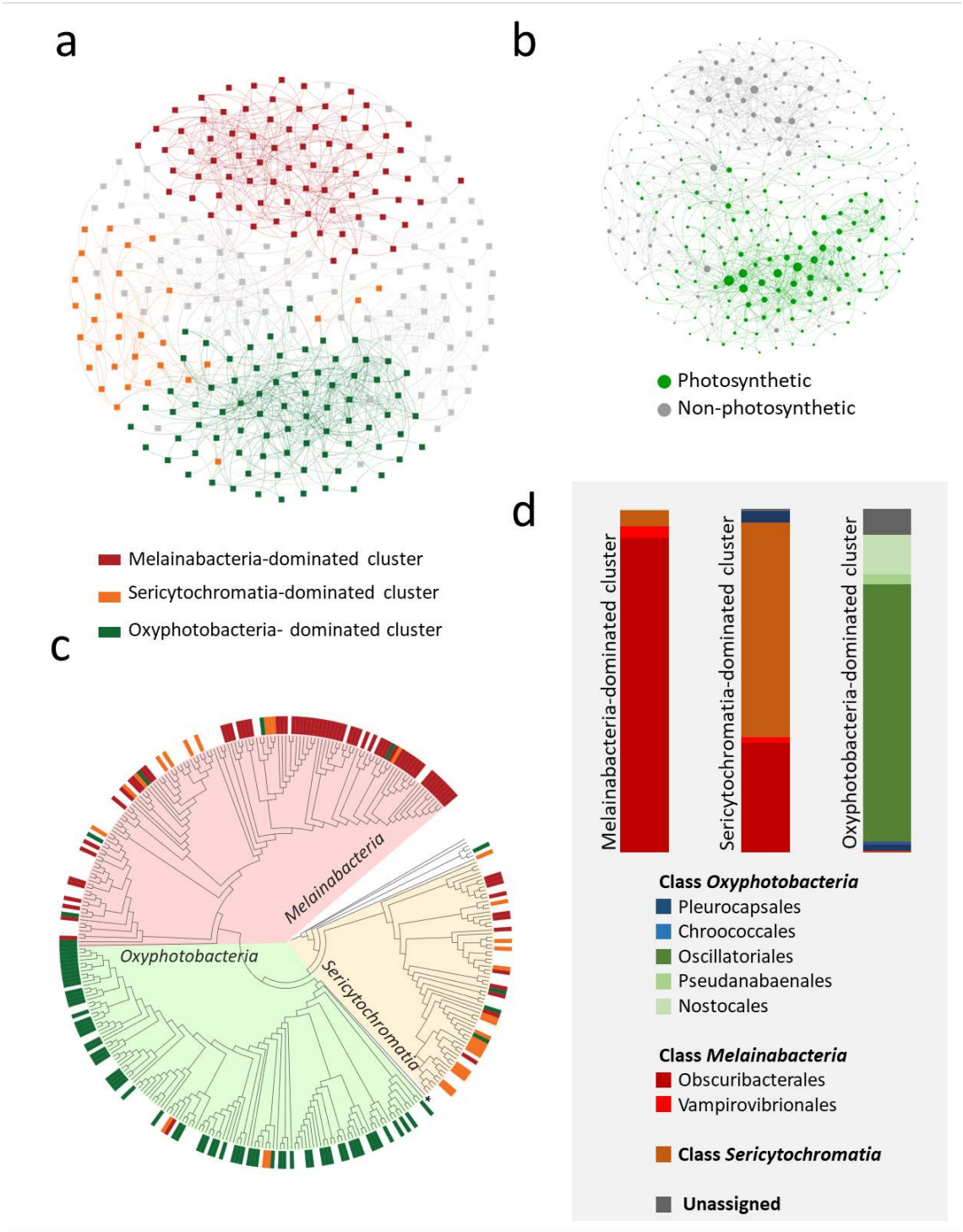
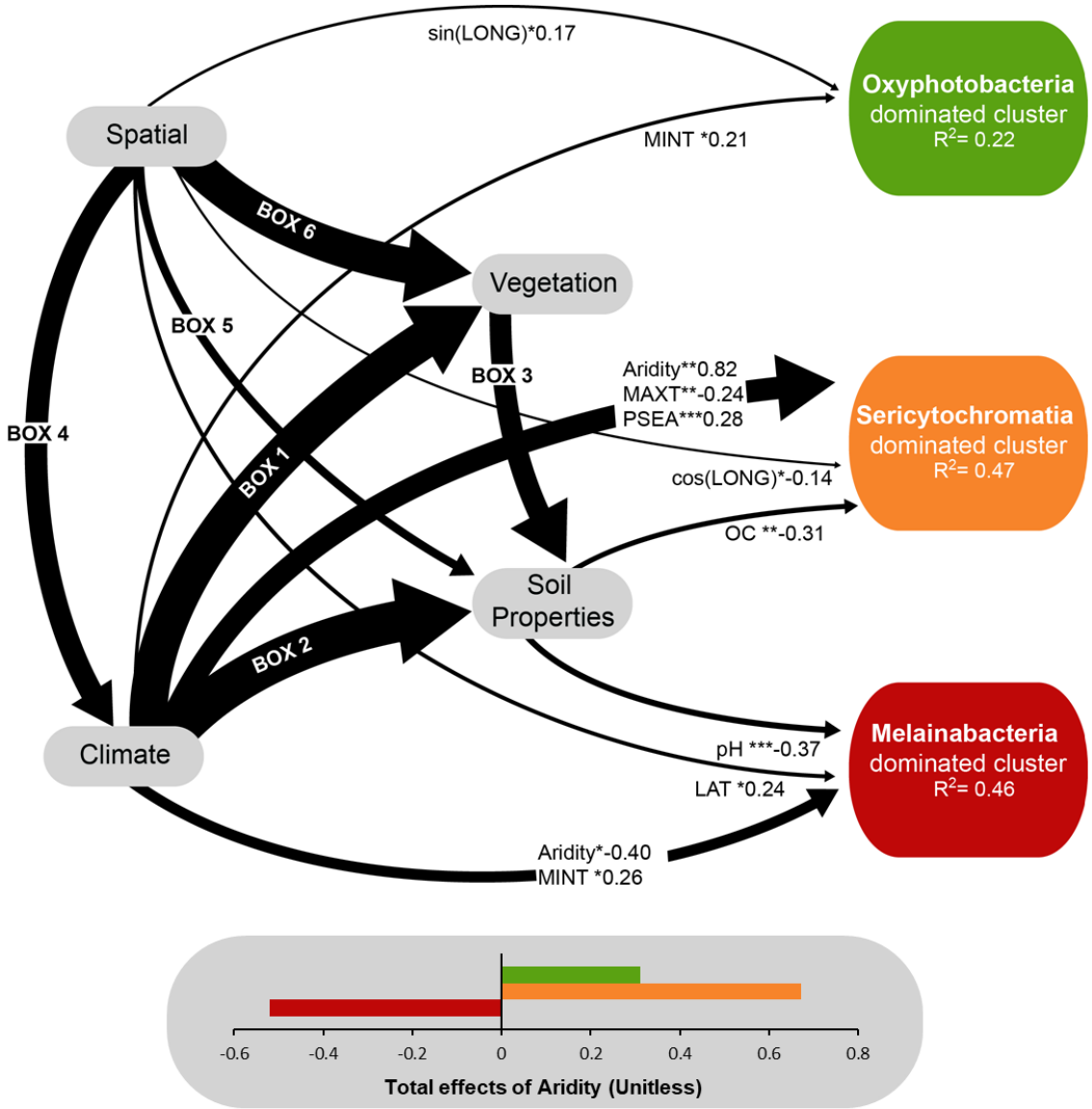


Fig. 1. Taxonomic information on the relative abundance of cyanobacterial orders (a) and classes (b) across all sites. Ser.= Sericytochromatia (no orders described yet) and Glo. = Gloeobacteria.



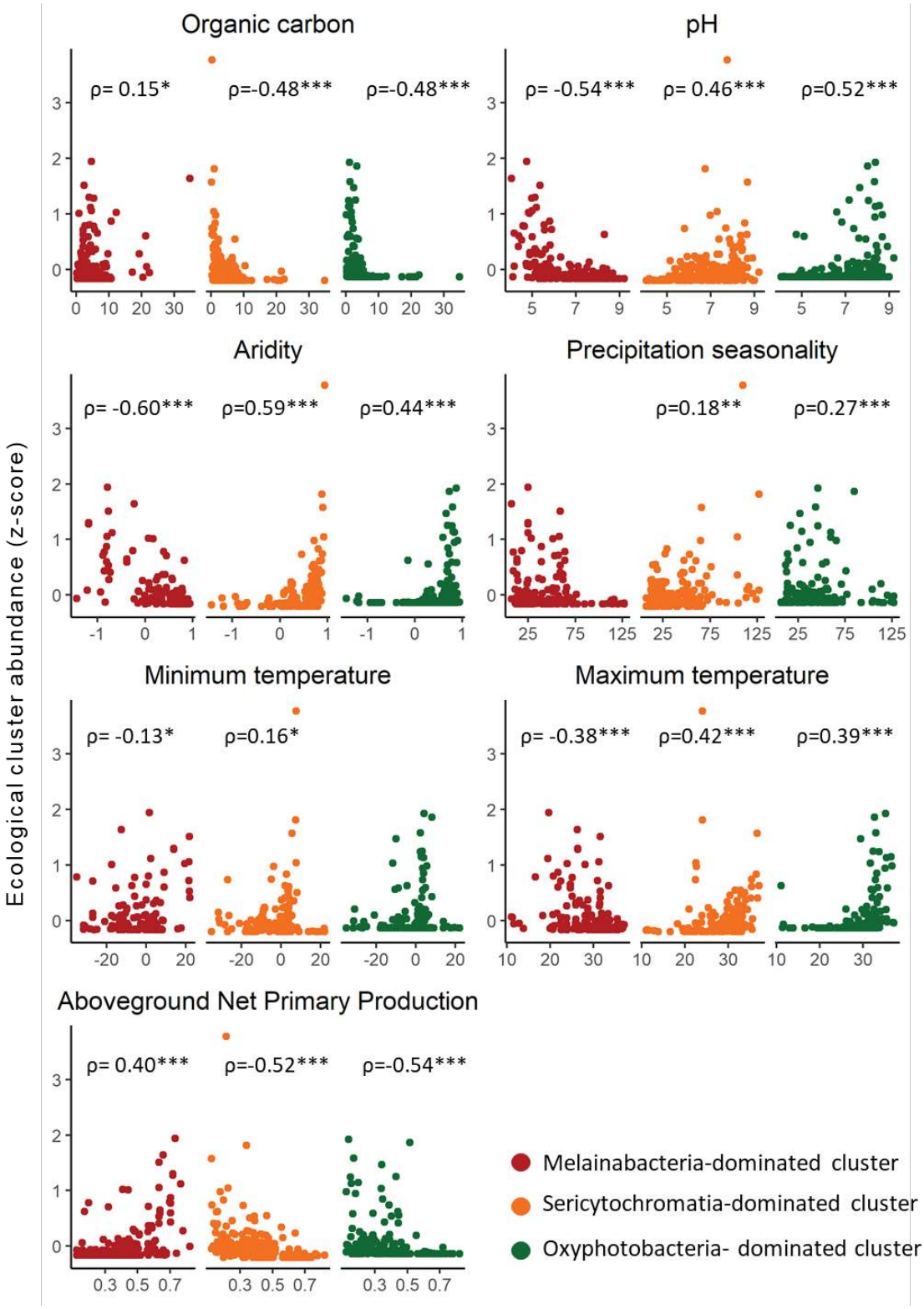
773

774 **Fig. 2** Global network of co-occurrences within soil cyanobacteria, colored by either main
775 ecological clusters (a) or the photosynthetic capability of taxa (b). The size of the nodes is
776 related to the number of links they contain. The network had 282 nodes (cyanobacterial
777 phylotypes) and 986 significant links (potential ecological interactions between phylotypes) (c)
778 Phylogenetic tree obtained with the main ecological clusters located at the end of the branch.
779 Background colored by cyanobacterial class, * for Gloeobacteria class. (d) Taxonomic
780 composition in relation to total 16S reads.



781

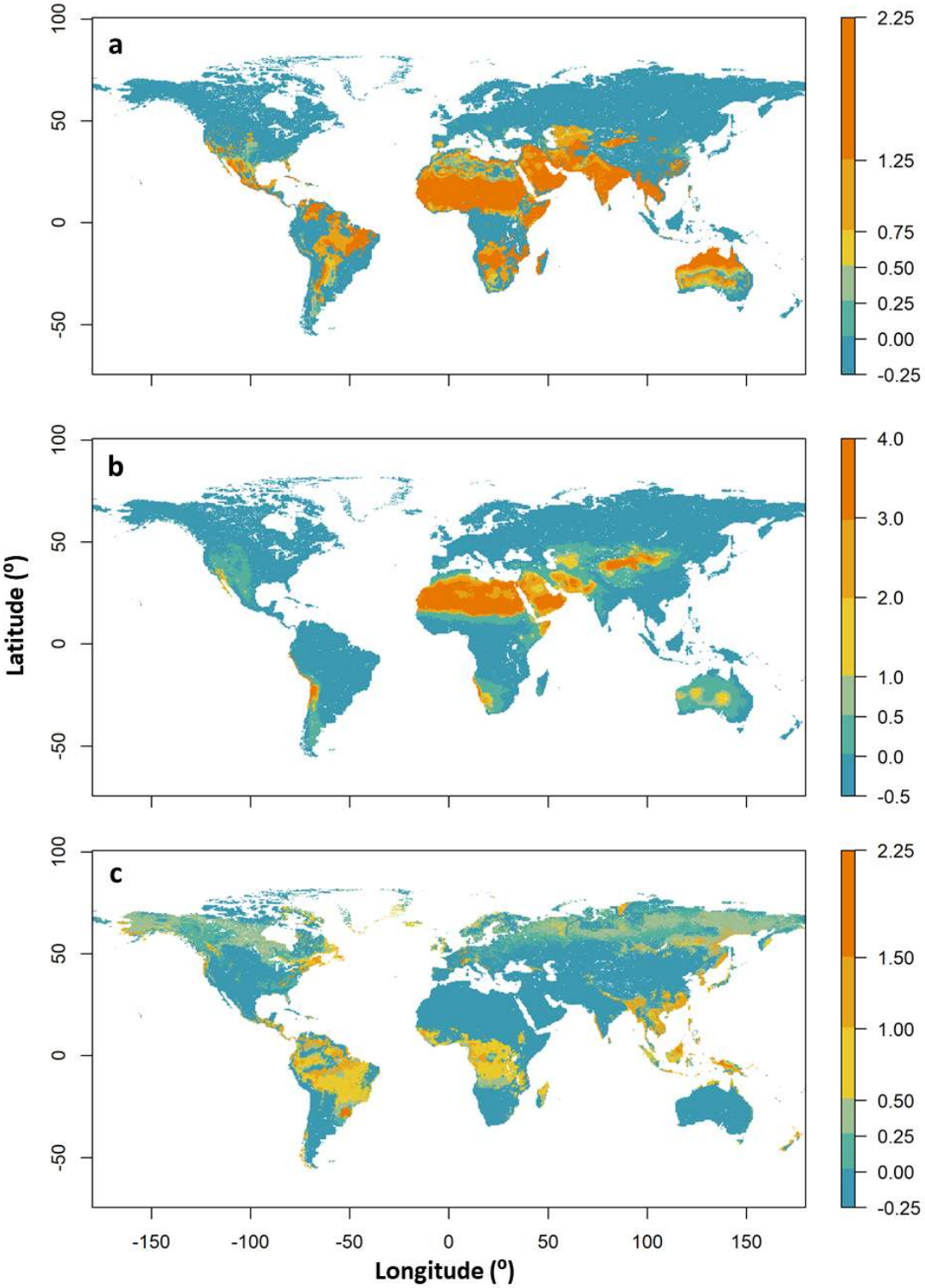
782 **Fig. 3** Structural equation modelling (SEM) showing the direct effects of spatial (Latitude [LAT],
783 Sine Longitude [sin(LONG)] and Cosine Longitude [cos(LONG)]) , climatic (maximum
784 temperature [MAXT], minimum temperature [MINT], precipitation seasonality [PSEA] and
785 aridity, calculated as 1-aridity index) and soil (soil organic carbon [OC] and pH) variables on the
786 abundance of each ecological cluster. Numbers in arrows indicate standardized path
787 coefficients, and their width is proportional to the strength of path coefficients. The proportion
788 of variance explained (R^2) appears below every response variable in the model. Significance
789 levels are as follows *P<0.05, **P<0.01, and ***P<0.001. Model X^2 =2.567, P= 0.463 df= 3,
790 Bootstrap p= 0.254. Information on boxes 1-6 is shown in Fig. S2.



791

792 **Fig. 4** Relationships between main environmental predictors and the relative abundance (z-
793 score) of each one of the cyanobacterial clusters. Significant (P<0.05) spearman correlation
794 coefficients are shown on the upper part of each panel.

795



796

797 **Fig. 5** Predicted global distribution of the relative abundance of the main ecological clusters of
798 soil cyanobacteria. Percentage of variation explained by the models as follows: (a)
799 *Oxyphotobacteria*-dominated cluster R^2 = 0.28; P < 0.001, (b) *Sericytochromatia*-dominated
800 cluster R^2 =0.66; P < 0.001, (c) *Melainabacteria*-dominated cluster R^2 = 0.35; P < 0.001. The
801 scale bar represents the standardized abundance (z-score) of each ecological cluster. An
802 independent cross-validation for these maps using data from the Earth Microbiome Project
803 (Thompson *et al.*, 2017) is described in the Methods section.

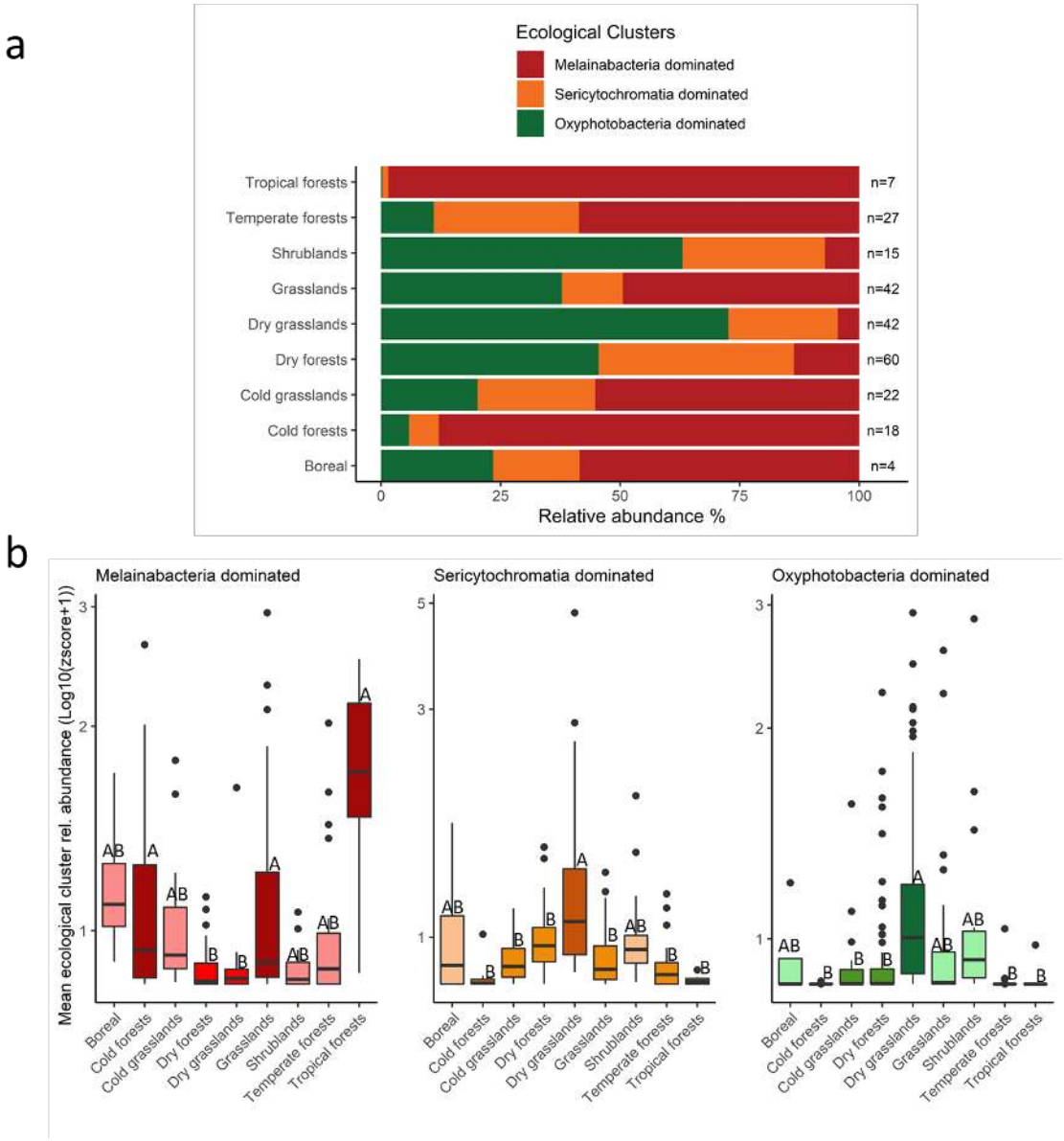


Fig. 6 Relative abundance of cyanobacterial clusters across major vegetation types. A) Stacked bars showing the percentage of phylotypes of each ecological cluster per vegetation type. n=Number of sites per each vegetation type B) Tukey HSD results testing the differences (letters and colour hues) in the relative abundances of each ecological cluster across vegetation types.