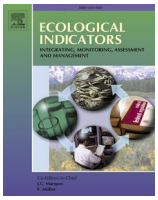
Ecological Indicators 130 (2021) 108047

Contents lists available at ScienceDirect

Ecological Indicators

journal homepage: www.elsevier.com/locate/ecolind



BactoTraits – A functional trait database to evaluate how natural and man-induced changes influence the assembly of bacterial communities

Aurélie Cébron [a,*], Emna Zeghal [a], Philippe Usseglio-Polatera [b], Albin Meyer [b], Pascale Bauda [b], Florian Lemmel [a], Corinne Leyval [a], Florence Maunoury-Danger [b]

[a] Université de Lorraine, CNRS, LIEC, F-54000 Nancy, France
[b] Université de Lorraine, CNRS, LIEC, F-57000 Metz, France

ARTICLE INFO

Keywords:
Bacteria
Database
Ecological diagnostic
Functional traits
Metals
PAH
Soil

ABSTRACT

In the environment, abiotic (climatic conditions, physico-chemical parameters), biotic (interactions between microorganisms, vegetation and fauna), and anthropogenic (stress, pollution) filters are driving the microbial diversity observed locally. A key question in microbial ecology is to understand the impact of these filters on bacterial diversity and ecosystem functioning. To highlight the responses of bacterial assemblages to these ecological filters, a new approach based on bacterial functional traits has been developed. This approach provides a functional picture of bacterial assemblages using morphological, physiological, and genomic traits as proxies of functions, and leads to a generalizable approach over a larger range of ecosystems with different bacterial diversities.

We have created a user-friendly database of bacterial functional traits, thanks to the properties of 19,455 bacterial strains. This database has been called BactoTraits. For example, oxygen preference, size and shape of bacteria, motility, optimum and range of pH and temperature, genome GC percent and trophic type are among the 19 traits included in BactoTraits. Based on the best-informed strains in the database, we identified five functional groups (i.e. mesophiles, competitors, colonizers, stress-sensitives and stress-tolerants) exhibiting a wide strain taxonomic diversity but with quite similar trait profile combinations.

As an example of application, BactoTraits was used to characterize the traits and functional diversity of bacterial assemblages in soil samples from 10 sites with different physico-chemical properties and various levels of metal and polycyclic aromatic hydrocarbon (PAH) contaminations. Inference of functional traits was based on taxonomic diversity information obtained by high-throughput sequencing of 16S rDNA. This trait-based approach has allowed to discriminate soils according to their physico-chemical properties and levels of contamination and to go further into the description of the bacterial assemblages. Several bacterial traits were identified as indicators of specific contaminants such as metals (e.g. filament shape, microaerophile and temperature optimum/range higher than 40 °C) or PAHs (e.g. spherical shape, facultative anaerobe/aerobe, no spore production, pH optimum $\geq$ 8, low temperature optimum but high temperature variation tolerance).

Inferring trait values from a taxonomy-based approach can be extended readily to other microbial systems and contexts such as (i) studies on soils and aquatic ecosystems, (ii) microbial ecology along various environmental gradients, (iii) human, plant and animal microbiotes, as well as (iv) trophic interactions between bacterial communities and their predators.

1. Introduction

Understanding how biotic and abiotic factors govern the spatio-temporal assembly of microbial communities at various nested scales from microhabitats to biogeographical areas, and consequently how ecosystem processes and services are modified, are some of the long-standing goals in microbial ecology (Prosser et al., 2007; Nemergut et al., 2013; Lajoie and Kembel, 2019). More specifically, in the context of global changes and environmental contaminations, a deeper understanding of microbial ecology and adaptations to many biotic and

* Corresponding author at: LIEC, UMR7360 CNRS-Université de Lorraine, Faculté des Sciences et Technologies, Bd des Aiguillettes, BP70239, 54506 Vandoeuvre-les-Nancy Cedex, France.
E-mail address: aurelie.cebron@univ-lorraine.fr (A. Cébron).

https://doi.org/10.1016/j.ecolind.2021.108047
Received 4 December 2020; Received in revised form 11 June 2021; Accepted 27 July 2021
Available online 17 August 2021

abiotic stresses is needed to better evaluate the responses of microbial communities to the main drivers of ecosystem functioning. Similarly, a better understanding of the mechanisms structuring microbial communities will allow a better understanding of biotic interactions among organisms and within holobionts.

Although extensive consideration has been given to the diversity and composition of microbial communities thanks to high throughput sequencing methods (Langenheder et al., 2010), functional aspects of microbial community structuring have received quite little attention (Bewick et al., 2019) even though it would facilitate identifying general rules of species assembly and ecosystem functioning. Examining traits which describe some functional aspects of the microbial community may therefore prove to be valuable for going further in the explanation of community assembly, e.g. via the identification of groups of microorganisms with common combinations of attributes and strategies. Traits are well-defined, quantifiable properties (i.e., morphological, anatomical, physiological, biochemical and phenological characteristics) of organisms, usually measured at the individual level and used comparatively across species. Functional traits are surrogates of organismal performance, i.e. its fitness (McGill et al., 2006; Violle et al., 2007; Reiss et al., 2009). The functional trait approach has been widely used in plant (e.g., Westoby and Wright, 2006; Cornwell et al., 2008; Kattge et al., 2011; Adler et al., 2014), as well as animal ecology (e.g., freshwater fishes: Logez et al., 2013; macrobenthic freshwater invertebrates: Usseglio-Polatera et al., 2000; Usseglio-Polatera et al., 2001; megabenthic marine invertebrates and fishes: Bremner et al., 2006; Bremner, 2008; Beauchard et al., 2017; birds: Ding et al., 2013; terrestrial beetles: Barton et al., 2011; leaf litter ants: Silva and Brandão, 2010), from local to continental scales.

Studying the covariation of traits in different environmental contexts to understand the ecological strategies that underlie community patterns (Green et al., 2008) started to interest only recently the microbial ecologists (McGill et al., 2006; Kraft et al., 2008; Barberán et al., 2012; Barberán et al., 2017; Bewick et al., 2019; Madin et al., 2020). Traits can (i) be analyzed across wide geographical ranges and species pools (Bernhardt-Römermann et al., 2011; Nelson et al., 2016), (ii) support the calculation of a variety of functional diversity indices (Schleuter et al., 2010), and (iii) easily lead to estimation of functional redundancy (Darr et al., 2014) and specialization (Mondy and Usseglio-Polatera, 2014), or inform on ecosystem functioning (Bremner et al., 2006).

Studying microbial diversity from the perspective of functional traits is quite well developed for fungi due to well-established functional guild databases (Nguyen et al., 2016; Zanne et al., 2020) and trait-based framework (Crowther et al., 2014). However, this approach for bacteria is far more complex (Lajoie and Kembel, 2019). Current trends using bacterial community aggregated traits include: (i) shotgun sequencing metagenomic studies (Tringe et al., 2005; Selengut et al., 2007; Kanehisa et al., 2011) reflecting bacterial functions (DeLong et al., 2006; Fierer et al., 2014) or deducing some bacterial traits (Weimann et al., 2016), and (ii) 16S rRNA gene amplicon sequencing that infers bacterial functions based on reference genomes (e.g., PICRUSt: Langille et al., 2013; or Tax4Fun: Aßhauer et al., 2015). Despite on-going improvements in functional reference databases (e.g., AnnoTree; Mendler et al., 2019), and comprehensive web-server to assist in metagenomic analyses (e.g. METAGENassist; Arndt et al., 2012), the gold standard for defining bacterial phenotypic and ecological traits remains cultivation of bacteria (Ernebjerg and Kishony, 2012) to constitute databases easily usable for functional inference.

On-line trait databases have been developed for various plants and animals in both aquatic and terrestrial ecosystems such as *EFI* + or *freshwaterecology.info* for European fishes, invertebrates, macrophytes, diatoms and phytoplankton (Frimpong and Angermeier, 2009; Logez et al., 2013; Schmidt-Kloiber and Hering, 2015), or other databases for North-American lotic invertebrates (Vieira et al., 2006), plants (*TRY*; Kattge et al., 2011), carabids (Homburg et al., 2014), soil invertebrates (Pey et al., 2014), or ants (Bertelsmeier et al., 2013). The best sources of information on bacterial traits are databases describing isolated bacterial strains such as *BacDive* (https://bacdive.dsmz.de) or bacterial known metabolisms such as the *Kyoto Encyclopedia of Genes* and *Genomes* (*KEGG*; Kanehisa et al., 2011). They provide comprehensive datasets on the taxonomy, morphology, physiology, geographic origin, genomic, and application of prokaryote strains. Recently, attempts have been made to link these phenotypic and environmental tolerance traits with taxonomy (Barberán et al., 2017), to aggregate large phenotypic, genomic and environmental characteristics datasets (Madin et al., 2020) and develop prediction tools (*BugBase*; Ward et al., 2017). These studies have laid the foundation for trait-based approaches using morpho-ecological or genomic traits of bacteria. Nevertheless, they still did not explicitly test the potential of this trait-based approach to define bacterial ecological groups or to examine the impact of natural or man-induced changes on the selection of bacterial traits at the community level.

Our first objective was to constitute a user-friendly database called "BactoTraits" aggregating information on 19 bacterial traits. Traits reflect bacterial behaviour and physiological requirements, as well as morphological and genome properties. In this study, we introduced the BactoTraits database workflow based on the inference of individual bacterial traits to the whole bacterial community thanks to taxonomic affiliation obtained from traditional high throughput 16S rRNA gene amplicon sequencing methods. We described how traits were coded, and defined bacterial functional groups sharing similar suites of traits that could be used as indicators of the functional organization of bacterial communities in various environments.

Our second objective was to give an example of application of BactoTraits. We investigated how a trait-based approach can describe the impact of natural and man-induced gradients (e.g. increasing polycyclic aromatic hydrocarbon (PAH) and metal contaminations) on the functional assembly of soil bacterial communities. Using 16S rRNA gene based diversity of a range of soil samples (Lemmel et al., 2019), we tested if the trait-based approach would highlight bacterial community adaptations to pollution, indicating the selection of different functional strategies in bacterial assemblages to cope with environmental constraints.

2. Material and methods

2.1. Source of bacterial strain trait information

BactoTraits was built based on the global BacDive database for pure culture bacteria. Data were downloaded via the online portal in May 2019 (https://bacdive.dsmz.de/). From the 76,998 bacteria strains available on Bacdive, we kept in BactoTraits the 19,455 bacterial strains having at least one trait described (Fig. 1). The taxonomic affiliation of the bacterial strains was updated using Silva 132 taxonomy (https://www.arb-silva.de/). These strains belonged to 34 phyla (including 19 phyla with at least 10 strains). At present BactoTraits (Table 1) covers 19 traits, each described by 2 to 10 attributes (i.e., categories). It holds altogether 97,341 trait entries representing between 101 (for the trait "Pigment") and 15,516 entries (for the trait "Oxygen") (Fig. 1A), i.e., between about 0.5% and 79.5% of the whole strains described. Several traits describe the preference and tolerance of bacterial strains to environmental parameters such as pH, salinity, temperature, and oxygen. For the first three parameters, this tolerance is described by three independent traits: (i) the optimum value for strain growth, (ii) the range of values the strain can grow in (from minimum to maximum value) and (iii) the delta of values the strain can tolerate for growth providing its ecological valence (narrow and wide delta values indicate stenotopic and eurytopic species, respectively). Morphological traits (such as the cell size and shape), the mobility, the Gram staining, and the pigment and spore production are included. A molecular trait describes the Guanine-Cytosine (GC) percent of strain genome. Finally, an ecological trait provides information on the trophic type of the strains.

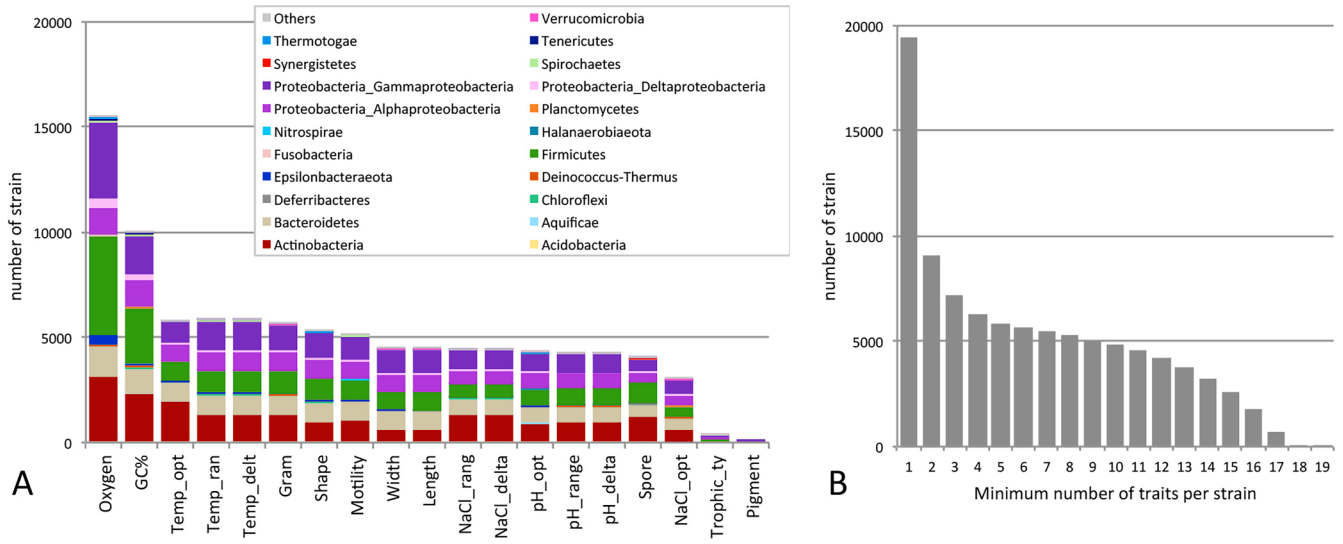


Fig. 1. Description of the 19 traits and the number of strains described in BactoTraits (a total of 19,455 strains are informed). A) Number of strains with available trait profile (per trait) and their taxonomic affiliation at phylum level. B) Number of strains having at least 1 to 19 traits entered. The group "Others" comprises: Armatimonadetes (2 strains), Caldiserica (1), Calditrichaeota (3), Chlamydiae (7), Chrysiogenetes (4), Coprothermobacteraeota (3), Dictyoglomi (2), Elusimicrobia (1), Fibrobacteres (3), Gemmatimonadetes (2), Kiritimatiellaeota (1), Lentisphaerae (4), Epsilon-Proteobacteria (6), Zeta-Proteobacteria (1), Magnetococcia-Proteobacteria (1), Rhodothermaeota (3), Thermodesulfobacteria (1), Thermosulfidibacteraeota (1).

Traits were expressed in categorical, nominal or ordinal categories (continuous quantitative traits were classified in quantitative categories). They could be mutually exclusive (for example, the pH_optimum of a given strain is only in one of the four categories defined, e.g., "< 6" or "≥ 6–7" or "≥ 7–8", or "≥ 8" pH units) or potentially co-occurring (for example, for its trophic type a strain can be "heterotroph" and also "organotroph", cf. part 2.2). Table 1 presents the standardized traits taken into account in BactoTraits. The relationships between the traits and the bacterial community functions or responses have been given (Table 1 and Table S1) via examples and underlying literature sources have been displayed.

2.2. Coding of traits

Trait categories were defined taking into account differences in ecological preferences and morphological differences and avoiding – or gathering (when possible) - categories with too few representative strains. Raw trait-based information was coded using a full disjunctive system for 13 traits (i.e., selection of a single attribute among 2 to 8 categories according to traits, Table 1). For example, if a strain has a rod shape, a value of 1 was allocated to the "rod" attribute of the trait "Shape" and a value of 0 to all the other attributes of this trait. For the last six traits, "Gram", "Pigment", "pH_range", "NaCl_range", "Temp_range" and "Trophic_type", the fuzzy coding procedure described by Chevene et al. (1994) was adapted to describe the relative affinity of a given strain for the different categories of the trait. We used a multiple binary code (one score per strain and trait category) with a final transformation of each series of scores (i.e. each "strain × trait") as the relative frequency distribution of the strain affinity scores for the corresponding trait. When the strain attributes belonged to a single trait category, the strain was coded 1 for this attribute. If the strain attributes belonged to two categories, both categories were coded 0.5. If the strain attributes belonged to three categories, each of them was coded 0.33, etc. In any cases, the sum of scores of a strain for a given trait was equal to 1. The number of attributes a strain can exhibit is presented in the Table 1. Potential functions and responses to environmental conditions conferred by these traits are detailed in Table 1 and Table S1. Note that: (i) strains described as Gram variable were coded 0.5 in Gram-negative and 0.5 in Gram-positive, because this pattern of response represents only 84 strains, (ii) strains categorized as facultative anaerobe or facultative aerobe were gathered in one unique attribute category, (iii) mobile strains were gathered into the category "mobile" whatever their motility type (i.e., amphitrichous, gliding, lophotrichous, monotrichous, peritrichous, and polar). The encoded BactoTraits database is available at the https://doi.org/10.24396/ORDAR-53.

2.3. Trait inference from taxonomic data

Interoperability ensures that information from different datasets can seamlessly be merged for subsequent integrated analyses. This interoperability has been performed from the taxonomic affiliation of the strains present in BactoTraits that was linked to the taxonomic affiliation of the Operational Taxonomic Units (OTUs) obtained from high throughput sequencing to infer traits to bacterial OTUs identified from environmental samples. This interoperability can be queried at any level of the taxonomy from genus to phylum. This process was automated thanks to a script programmed in R (v3.6.0; R Core Team, 2016). From a matrix with OTU numbers and their taxonomic affiliation (phylum, class, order, family, genus, species; referred as *Taxonomy_table* below and in Fig. S1) the script successively looked if each OTU corresponded to a single strain or several strains of the same species in BactoTraits. If only one strain was found belonging to a given species in the database, its trait profile was directly allocated to the OTU. If this OTU corresponded to more than one strain of the same species in the database, the mean of the strain trait profiles was allocated to the OTU. For example, for the "Width" trait, if four strains, belonging to the same species, were recognized in BactoTraits with one belonging to the 0.50–0.65 μm category and three belonging to the 0.65–0.90 μm category, the trait profile allocated to the OTU was the mean of these four strain profiles with an affinity score of 0.25 in the 0.50–0.65 μm category and an affinity score of 0.75 in the 0.65–0.90 μm category. If no strain belonging to this species was found in BactoTraits, the script allowed to look for the strains belonging to the superior taxonomic rank (in order: genus, family, order, class, phylum), and the mean of the found trait profiles (for the genus, family, order, class or phylum) was allocated to the OTU as previously described. OTUs having the taxonomic affiliation "unclassified bacteria" were removed from analyses. As many OTUs get similar taxonomic affiliations giving similar trait profiles, these OTUs were grouped to simplify the datasets (*OTUgroups_table* in Fig. S1).

Table 1
Description of the 19 traits of the BactoTraits database. Description of the trait categories and the related functions or responses to environmental conditions these trait categories could be linked with (see Table S1 for more complete description and references).

Trait label	Trait description	Unit	Data expression	Number and description of the trait attributes	Number of trait attributes a strain can be assigned to	Related functions or responses to environmental conditions
Gram	Gram staining	–	Nominal	2 categories (negative, positive)	1 to 2	Resistance to desiccation, antibiotics and toxics Copiotrophs or r-strategists (Gram -) *vs.* oligotrophs or K-strategists (Gram +) Different sensitivity to bacteriophage attack or nematode grazing
Width	Width of the cell	µm	Quantitative, categorial	4 categories (<0.50, ≥0.50–0.65, ≥0.65–0.90, ≥0.90)	1	Resistance to predation, viral lysis, hydrodynamic variation
Length	Length of the cell	µm	Quantitative, categorial	4 categories (<1.3, ≥1.3–2.0, ≥2.0–3.0, ≥3.0)	1	Niche differentiation Transport and dispersion
Shape	Shape of the cell	–	Nominal	6 categories (rod, sphere, curved_spiral, filament, ovoid, other_star_dumbbell,_pleomorphic)	1	Nutrient access, surface attachment, dispersion Resistance to predation
Pigment	Colour of the colonies	–	Nominal	10 categories (pink, yellow, brown, black, orange, white, cream, red, green, carotenoid)	1 to 2	Protection against grazing, ultraviolet radiation, desiccation stress, reactive oxygen species Converts energy from sunlight
Spore	Sporulation ability	–	Nominal	2 categories (yes, no)	1	Resistance to unfavorable environmental conditions, increase the resilience to perturbation Pathogenicity
Motility	If a strain is motile or not	–	Nominal	2 categories (non-motile, motile)	1	Taxis mechanisms Versatile *vs.* specificity Dispersion, biofilm formation, protection against grazing
pH_opt	pH for optimal strain growth	pH unit	Quantitative, categorial	4 categories (0–6, ≥6-7, ≥7-8, ≥8-14)	1	Niche differentiation, adaptation, tolerance to variations, plasticity Link with salt and temperature tolerance Link with metal exposure Link with plant colonization
pH_range	Range of pH where a strain can grow (from min to max values)	pH unit	Quantitative, categorial	6 categories (0–4, ≥4-6, ≥6-7, ≥7-<8, ≥8-10, ≥10-14)	1 to 5	
pH_delta	Extend of pH units that a strain can grow (calculated from pH_Range trait: max–min value)	pH unit	Quantitative, categorial	6 categories (<1, ≥1-2, ≥2-3, ≥3-4, ≥4-5, ≥5-9)	1	
NaCl_opt	NaCl concentration for optimal strain growth	%	Quantitative, categorial	4 categories (<1, ≥1-3, ≥3-8, ≥8)	1	Niche differentiation, adaptation, tolerance to variations, plasticity Link with pH tolerance
NaCl_range	Range of NaCl concentrations where a strain can grow	%	Quantitative, categorial	4 categories (<1, ≥1-3, ≥3-8, ≥8)	1 to 4	
NaCl_delta	Extend of NaCl concentrations that a strain can grow (calculated from NaCl_Range trait)	%	Quantitative, categorial	4 categories (<1, ≥1-3, ≥3-8, ≥8)	1	
Temp_opt	Temperature for optimal strain growth	°C	Quantitative, categorial	7 categories (<10, ≥10-22, ≥22-27, ≥27-30, ≥30-34, ≥34-40, ≥40)	1	Niche differentiation, adaptation, tolerance to variations, plasticity Link with pH tolerance
Temp_range	Range of temperature where a strain can grow	°C	Quantitative, categorial	7 categories (<10, ≥10-22, ≥22-27, ≥27-30, ≥30-34, ≥34-40, ≥40)	1 to 6	
Temp_delta	Extend of the temperature range in which a strain can grow (calculated from temp_Range trait)	°C	Quantitative, categorial	5 categories (≥1-5, ≥5-10, ≥10-20, ≥20-30, ≥30)	1	
Oxygen	Oxygen demand of the strain	–	Nominal	4 categories (anaerobe, aerobe, facultative anaerobe and aerobe, microaerophile)	1	Niche differentiation, adaptation, tolerance to variations, plasticity
Trophic_type	Conditions for growth: source of energy, source carbon, and electron donor	–	Nominal	9 categories (hetero-, auto-, organo-, litho-, chemo-, photo-, copio-/diazo-, methyl-, oligo-trophic)	1 to 4	Niche differentiation thanks to carbon and energy sources and available nutrients Trophic position and role
GC%	GC content of strain genome	mol %	Quantitative, categorial	4 categories (<42.6, ≥42.6–57.0, ≥57.0–66.3, ≥66.3)	1	Genome size, adaptation to environmental conditions, energy cost Higher horizontal gene transfer for high GC% strains Link with aerobic lifestyle, temperature or cadmium resistance

2.4. Environmental data used

Bacterial diversity data from Lemmel et al. (2019) were reanalyzed to get OTU taxonomic affiliation through Silva 132 database, and then used to illustrate the ecological interest and evaluate the performance of BactoTraits. We worked on 30 top-soil samples (0 to 30 cm depth) from 10 sites (3 replicates per site, collected 1 m apart, in November 2015) from Northeastern France (Fig. S2) presenting both PAH and metal contamination gradients (Lemmel et al., 2019) due to former steel activities during the 20th century. These soils were distributed into three types (controls, slag heaps, and settling ponds) based on their physico-chemical properties and pollution level (Lemmel et al., 2019). Three uncontaminated soils, considered as the control soils (*ctrl*), were sampled at Hémilly (*He*), Montiers-sur-Saulx (*Mo*), and Dieulouard (*Di*). Four soils from former slag heap (*sh*) sites were sampled at Homécourt (*Ho*), Terville (*Te*), Uckange (*Uc*), and Neuves-Maisons (*NM*). Three soils from former settling pond (*sp*) sites were sampled at Pompey (*Po*), Mont-St-Martin (*MsM*), and Russange-Micheville (*RM*). Soil characteristics, presented in the Table S2, highlight that slag heap sites were mainly characterized by a high PAH contamination and moderate level of metal contamination, while settling pond sites were characterized by high metal contamination and moderate level of PAH contamination.

The bacterial taxonomic diversity of these 30 top-soil samples was assessed by 16S rDNA Illumina MiSeq 2x250 paired-end sequencing of the V3/V4 region (Lemmel et al., 2019). After read treatment and rarefaction to 34,191 reads per sample, we classified OTUs with 97% similarity in their sequences. Taxonomy affiliation of the OTUs was incremented using Silva 132 version. From an *OTU_table* with 14,149 OTUs (all samples combined), we generated an *OTUgroups_table* with 676 unique OTU groups by regrouping OTUs (addition of read numbers) with exactly similar taxonomic affiliation and eliminating OTU having unclassified bacteria affiliation.

Based on these data, we used four tables (Fig. S1): (i) *OTUgroups_table* with the abundance (read number on a total of 29,689 to 33,839 reads per sample) of each OTU for the 30 samples (10 sites in triplicates), (ii) *Taxonomy_table* with phylum, class, order, family, genus and species affiliations of each OTU based on Silva 132 database, (iii) *Traits_OTUgroups_table* (generated from *Taxonomy_table* and BactoTraits, thanks to the procedure described in the section 2.3) with the trait attributes of the 676 OTU groups, and (iv) *SampleMetadata_table* with the available soil characteristics (soil_history: control, slag heap and settling pond; C, N, C/N, organic matter OM, dissolved organic carbon DOC, P_2O_5, carbonate, pH, CEC, texture: silt and sand content in percentage, organic acids, carbohydrates, sum of the 16 US-EPA PAHs, total Cr, Cu, Ni, Zn, Co, Pb, Cd, Tl, Mo, Al, Ca, Fe, K, Mg, Mn, Na and extractible Cd, Cr, Cu, Ni, Pb, Zn).

2.5. Statistical analyses of BactoTraits data and environmental data

A Fuzzy Correspondence Analysis (FCA; Chevene et al., 1994) was used to analyze the relationships among trait categories and to ordinate the bacterial strains of BactoTraits on the basis of their trait profile combinations. FCA also allowed identification of the traits that were the more contributive to the ordination of bacterial taxa (Chevene et al., 1994). Two traits ("Pigment" and "Trophic type") exhibiting a very low percentage of information in the database were discarded at this step of the analysis. Similarly, to avoid potential bias in analysis related to strains with low trait information in the database, the FCA was performed only on a subset of 5826 strains having at least five trait profiles available in the database. This minimum of five traits was determined as a trade-off between maximizing the number of traits and maximizing the robustness of trait structure, based on the results of successive FCAs applied to trait profiles of strains having at least 1, 2, 3, until 17 traits addressed. Functional groups (FGr) of taxa with similar suites of traits were then defined using the K-means clustering method on the coordinates of the strains on the F1xF2 factorial plane of the FCA (Borcard et al., 2011). The Simple Structure Index criterion (Borcard et al., 2011) was used to identify the optimal minimal number of FGr. Being delineated regardless of their roles in ecosystem processes, these groups of taxa can be considered as 'emergent groups' reflecting correlated traits resulting from adaptive responses and evolutionary constraints (Lavorel et al., 1997; Hooper et al., 2002). The 13,629 other strains - having one to four traits available in the database - were projected onto the first factorial plane of the FCA as "supplementary individuals" and assigned to one of the FGs depending on the distance of each strain to the nearest FGr centroid. Using the environmental trait dataset, we calculated the relative abundance of the OTU groups from each of the 30 top-soil samples, belonging to the FGr groups defined by FCA and clustering analysis.

From log-transformed *OTUgroups_table (*i.e. *pooled read data),* we calculated the Community Weighted Mean profile of traits (CWM; [0;1]), the Community Specialization Index (CSI; [0;1]), the Overlap index (OL; [0;1]) and the Rao quadratic entropy index (RAO) for each trait and for each soil sample. CWM profiles give the relative proportion of each trait category in the community. CSI is the average of the species specialization index values calculated over all the OTUs present in this sample weighted by their log-transformed abundances (Julliard et al., 2006; Devictor et al., 2008; Mondy and Usseglio-Polatera, 2014). CSI values vary between 0 and 1, with values close to 1 when all the species (or OTUs, here) are "specialized" in one trait category for a given trait. OL is the community averaged degree to which taxa have similar trait profiles/niches for each trait (Pianka, 1974). The Rao quadratic entropy is a measure of the diversity of ecological communities proposed by Rao (1982) and is based on the relative abundances of species present in a community and a measure of the trait-based dissimilarity between them. In this study, the dissimilarity ranges from 0 to 1 and is based on the selected set of functional traits selected in Bactotraits. The Rao quadratic diversity index is a biodiversity metric widely applied to studies on functional and phylogenetic ecology (Rao, 1982; Mouchet et al., 2010).

The package *FD* was used to compute different multidimensional Functional Diversity (FD) indices from the taxon-by-trait matrix (Laliberté et al., 2014). FD indices include the functional richness (FRic; here, standardized between 0 and 1), functional evenness (FEve), and functional divergence (FDiv) (Villéger et al., 2008), the functional dispersion (FDis) (Laliberté and Legendre, 2010), and the Rao quadratic entropy (RaoQ) (Botta-Dukát, 2005).

Kruskal-Wallis tests ($p < 0.05$) followed by Dunn's multiple comparison tests and p-value corrections (Bonferroni) were applied to compare functional indicator values among the ten studied sites and among the three soil types.

Variations in the bacterial community composition of sites, based on the trait community weighted mean profiles, wer visualized by nonmetric multidimensional scaling (NMDS) using the function '*metaMDS*' (from the package *vegan* for R; Oksanen et al., 2013) applied to a square root-transformed Bray-Curtis dissimilarity matrix. To determine how soil types (control, slag heap and settling pond) could explain bacterial community composition, permutation-based multivariate analyses of variance (PERMANOVA) were done (Anderson, 2001) through the '*adonis2*' function of the *vegan* R-package, yielding 999-permutations based F-tests of significance. To validate PERMANOVA we examined the homogeneity of variances using permutational tests of multivariate group dispersions ('*betadisper*' function).

Pearson's correlation tests were performed for each pair of trait categories (CWM) and a subset of soil parameters of interest (total Zn, Fe, Na and PAH concentrations, pH, DOC, total C and N, C/N ratio, and silt and sand percentage were selected as representatives when many soil parameters were correlated and gave similar correlations with traits). P-values of the correlation tests were corrected to consider the false discovery rate (Benjamini and Hochberg, 1995). The correlation matrix between both datasets was then used to build dendrograms, one for each dataset, to identify clusters of trait categories or soil parameters exhibiting similar "correlation profiles". The "correlation profiles" were

hierarchically clustered using Euclidean distances and the UPGMA linkage algorithm (Unweighted Pair-Group Method using Arithmetic averages; Borcard et al., 2011) proposed by the *hclust* function in R. A heatmap was used to illustrate the existing correlations between functional trait diversity descriptors and the soil characteristics (*SampleMetadata_table*). The final heatmap, including both dendrograms, was drawn using the function *heatmap2* from the R package *gplots* (Warnes et al., 2020).

All statistical analyses were performed in R version 3.6.0 (R Core Team, 2016).

3. Results and discussion

3.1. Description of the strains of the BactoTraits database

BactoTraits includes 19 bacterial traits defined at the strain level, and gathers information from 19,455 bacterial strains. These strains belong to 34 phyla, with 19 phyla having at least 10 representative strains (Fig. 1A). BactoTraits consists of 33.3% Proteobacteria (21.7% gamma- and 8.9% alpha-Proteobacteria), 27.8% Firmicutes, 24.0% Actinobacteria and 8.4% Bacteroidetes. Members of these phyla are dominating diverse marine environments (Aravindraja et al., 2013) and represent in average ca. 36%, 1%, 31% and 2%, respectively, of bacterial strains observed in soils (Delgado-Baquerizo et al., 2018) and respectively 16–36%, 0.4–37%, 10–26% and 5–14% of the bacterial strains in the soils from the 10 sites used as examples in the present study (Lemmel et al., 2019) and Firmicutes and Bacteroidetes are the dominant phyla in human gut microbiota (Brooks et al., 2018). Compared to the proportions of the different bacteria phyla in the environment, we are aware that some phyla were under-represented; e.g., the Acidobacteria, Verrucomicrobia, Chloroflexi, Planctomycetes, and Gemmatimonadetes representing only 0.27%, 0.23%, 0.21%, 0.11% and 0.01% of the strains in the database (53, 45, 41, 22 and 2 strains, respectively). These 5 phyla respectively represent in average ca. 14%, 4%, 5%, 7% and 2% in soil communities (Delgado-Baquerizo et al., 2018) and 4–31%, 0.6–22%, 0.8–16%, 0%, and 0.3–5% in the bacterial communities of the 10 sites illustrating the present study (Lemmel et al., 2019) while the three first phyla could be abundant and widespread in marine environments (Yilmaz et al., 2016). As a consequence, trait profiles currently allocated to bacterial OTUs of these phyla cannot be precise (because allocated at a

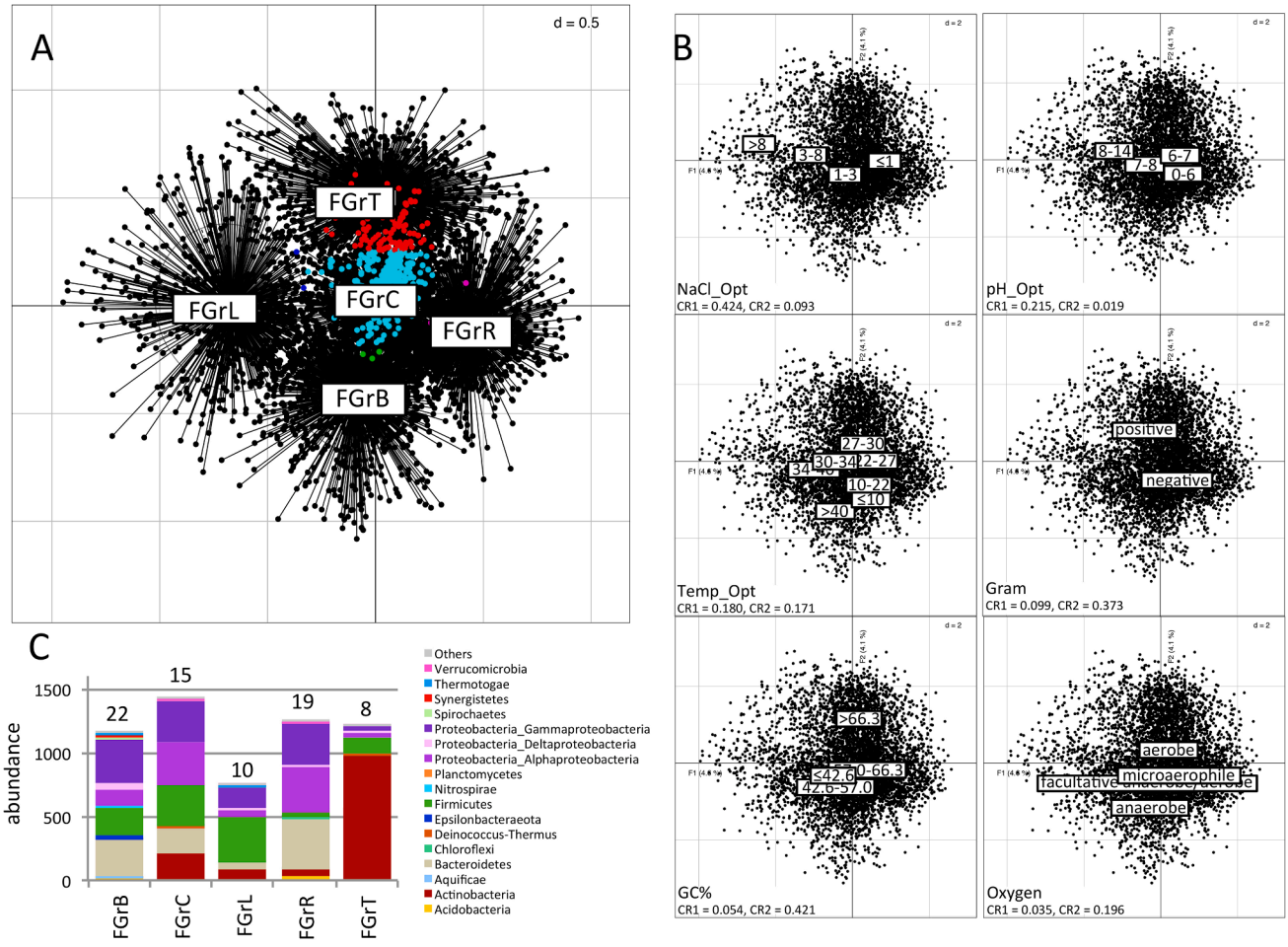


Fig. 2. Trait-based ordination of the strains of BactoTraits by Fuzzy Correspondence Analysis (FCA) and identification of trait-based functional groups (FGr) by cluster analysis. A) Distribution of strains on the FCA first factorial plane, clustered in 5 functional groups (FGrC, FGrR, FGrL, FGrT and FGrB) based on the K-means method. Ordination was generated from 5790 strains (solid black circles) having at least 5 trait profiles described. The 13,665 other strains (with 1 to 4 traits described; coloured circles) were plotted on the first FCA plane and assigned to one functional group (FGrL blue, FGrT red, FGrC cyan, FGrR purple, FGrB green) based on their lowest distance to the five functional group centroids. B) Location of trait categories on the first factorial plane for the six most discriminating traits (i.e. with the higher correlation ratio CR1 or CR2 values; for other traits see Fig. S3). Trait category labels are located at the weighted average of the locations of strains using the corresponding trait categories. CR1 and CR2 are correlation ratios of traits along the F1 and F2 axes. C) Taxonomic distribution at the phylum level for the 5790 core strains belonging to the 5 functional groups. Numbers over the bar plots indicate the numbers of phyla present in each group. See the legend of Fig. 1 for further details on the taxonomic composition of the group "Others".

high taxonomic level) due to a deficit of precise trait information, only known from few cultivable isolates (Madin et al., 2020). Delgado-Baquerizo et al. (2018) showed indeed that only 45% of the dominant soil bacterial phylotypes were related to cultivated isolates. However, our approach is in its first step, and is bound to evolve with additional information on new cultivable strains. This point highlights the importance of continuing to isolate, characterize and describe traits of new taxa that are not currently cultivated. BactoTraits includes between 3017 and 5873 strains for most of the traits (Fig. 1A). 'Oxygen preference' and 'GC%' are the best-informed traits with 15,516 and 10,065 strains, while 'Pigment' of bacterial colonies and 'Trophic_type' are the two least-informed traits with only 101 and 339 strains described, respectively (Fig. 1A). Only three strains have been fully described for the 19 traits, but >5000 strains have at least nine traits described (Fig. 1B). In a similar approach conducted in parallel to our study, Madin et al. (2020) released an aggregated dataset of 23 phenotypic, genomic and environmental characteristics of 14,884 species-aggregated records, but also highlighted the same limits in terms of taxonomy coverage and lack of information for some bacterial traits.

3.2. Functional groups of strains based on similar suites of trait attributes

A functional ordination of the strains included in BactoTraits was established through a fuzzy correspondence analysis (FCA; Fig. 2) based on the 5790 strains having at least 5 traits entered in the database (Fig. 1B). The FCA of the strain-by-trait array allowed a description of both trait-based similarities among strains and the relationships among trait categories. FCA was already used for analysing trait-based functional diversity (Ilg and Castella, 2006; Beauchard et al., 2017). The distances between strains on the first factorial plane were used to cluster them into five functional groups (FGr; Fig. 2A, functional group labels were defined based on their location on the FCA first factorial plan, FGrC at the centre, FGrR on the right, FGrL on the left, FGrT on the top and FGrB on the bottom). Correlation ratios (CR; Chevene et al., 1994) allowed the identification of traits that were best explained by the first two axes. The categories of the traits 'NaCl_delta', 'NaCl_opt', 'NaCl_range', 'pH_Optimum' were well separated along the F1 axis ($0.21 < CR1 < 0.59$), and those of the traits 'GC%', 'Gram', 'Oxygen', 'Length', 'Shape', 'Temperature_opt' and 'Motility' were rather well separated along the F2 axis ($0.17 < CR2 < 0.42$; Fig. 2B and Fig. S3). The categories of other traits were not clearly discriminated on the first factorial plane (Fig. S3).

The five functional groups of strains corresponded to specific combinations of traits (for some examples see Fig. 2B or Fig. S3) that provide adaptive solutions to particular habitats. FGrB and FGrT were separated along the second axis (Fig. 2A). FGrB strains are mostly Gram negative mobile bacteria, anaerobe, having long shape (curved, spiral or filament), with extreme temperature optimum for growth (below 10 °C and above 40 °C). FGrT strains are mostly Gram positive non-mobile bacteria with high GC percent (>66.3%), being aerobe, small-sized, and having spheric or ovoid shape. FGrL and FGrR were separated along the first axis (Fig. 2A). FGrL strains are spore-forming bacteria with high tolerance to salinity (>8% NaCl), high pH (pH 8–14) and temperature (34–40 °C) optima and high tolerance to temperature variation (delta > 30 °C) for growing. FGrR strains have low tolerance to salinity (≤1% NaCl), and low pH optimum (0–6 and 6–7). Finally, strains of FGrC, located close to the origin of the first factorial plane, could exhibit i) trait profiles without particular specialization, ii) a wide distribution of their trait attributes in the first factorial plane or iii) two sets of trait attributes with opposed locations along the same axis, these different patterns resulting in a mean location of the group at the centre of the FCA first factorial plane.

The five functional groups consisted of 741 to 1428 strains belonging to 10 to 22 phyla, with a relatively homogeneous taxonomic distribution of strains across functional groups at the phylum level, except for FGrT gathering most of the Actinobacteria (Fig. 2C). As explained by Naeem and Wright (2003), a functional classification scheme in which taxonomic diversity and functional diversity are highly correlated would be of little utility. Even if strains are taxonomically distinct and belong to various phyla, our study has highlighted that strains of different phyla can share similar suites of functional traits and lumped into the same group, highlighting rather low phylogenetic constraints on the composition of functional groups.

3.3. Ecological classification of BactoTraits functional groups

Our approach gave us the opportunity to classify known bacterial strains in five trait-based functional groups. We have hypothesized that these five groups with clearly different suites of traits could correspond to major ecological strategies acquired by bacteria to cope with various environmental conditions. In the literature, several ecological classifications have been already proposed to help interpreting bacteria community data. The copiotroph-oligotroph continuum was proposed as analogous to the r- and K-selection theory for plants and animals (Koch, 2001; Fierer et al., 2007). Plants, animals as well as fungi have been also classified through the C-S-R model (competitors, stress tolerators and colonizers/ruderals; Grime, 1977) to describe their distribution and influence on ecosystem processes. Several recent efforts have tried also to apply the C-S-R life history strategies to microbial assemblages, especially in the context of environmental changes (Ho et al., 2013; Krause et al., 2014; Fierer, 2017), and through the description of an additional group of traits (Wood et al., 2018). Interestingly, Malik et al. (2020) proposed the microbial Y-A-S (high yield-resource acquisition-stress tolerance) life history framework, which suggests that trade-offs in resource allocation among traits select microbes under different environmental conditions. When resources are limited, stress tolerance traits can trade-off against traits linked to resource acquisition and growth yield (Schimel et al., 2007; Berlemont et al., 2014). Given the vast metabolic diversity of microorganisms and their ability to inhabit extreme environments that are both stressful and frequently disturbed, we propose a trait-based functional classification of bacteria along three axes (Fig. 3). The first two axes describe niche colonisation strategy and stress adaptation, with one axis opposing colonizers to competitors (FGrT *vs.* FGrB) and the second opposing stress-tolerators to stress-sensitives (FGrL *vs.* FGrR), with in the middle the mesophilic species (FGrC), i.e. strains preferring intermediate ranges of values for most of the studied gradients (O_2, pH, temperature, salinity, etc), and including generalist species. One other axis separates bacteria according to their resource acquisition ability and their growth yield (r- *vs.* K-strategists).

The FGrB gathers colonizers or invaders considering their specific suite of trait attributes. Colonizers or invasive bacteria should be r-strategists with high growth rate, dispersal capacities, phenotypic plasticity and resource use efficiency that allow access to resources unavailable to other species, and could facilitate colonisation and adaptation to novel environments (Litchman, 2010). Gram negative bacteria are often described as copiotroph r-strategists using simple C-compounds (Kramer and Gleixner, 2008; Fanin et al., 2019). Long shape (e.g. filaments) is an advantage for nutrient access (by increasing the surface/volume ratio) and surface attachment (by increasing the specific surface area in direct contact with solid particles) (Young, 2006). Anaerobe growth and ability to grow at extreme temperature insure colonization potential and survival in various fluctuating environmental conditions (e.g. in terms of redox, temperature or even pH), because thermophilic and psychrophilic bacteria have often the ability to tolerate wide pH range (Dhakar and Pandey, 2016). Colonizers have been also characterized as ruderal species (Grime, 1977) favored in disturbed environments.

At the opposite, small-sized, spherical and/or Gram-positive bacteria of the FGrT (Fig. 3) have been considered as competitors, with traits improving the access to resources even when limited ("high resource acquisition" in the Y-A-S model (Wood et al., 2018) or K-strategy), and promoting strategies against mortality. Oligotrophic environments with

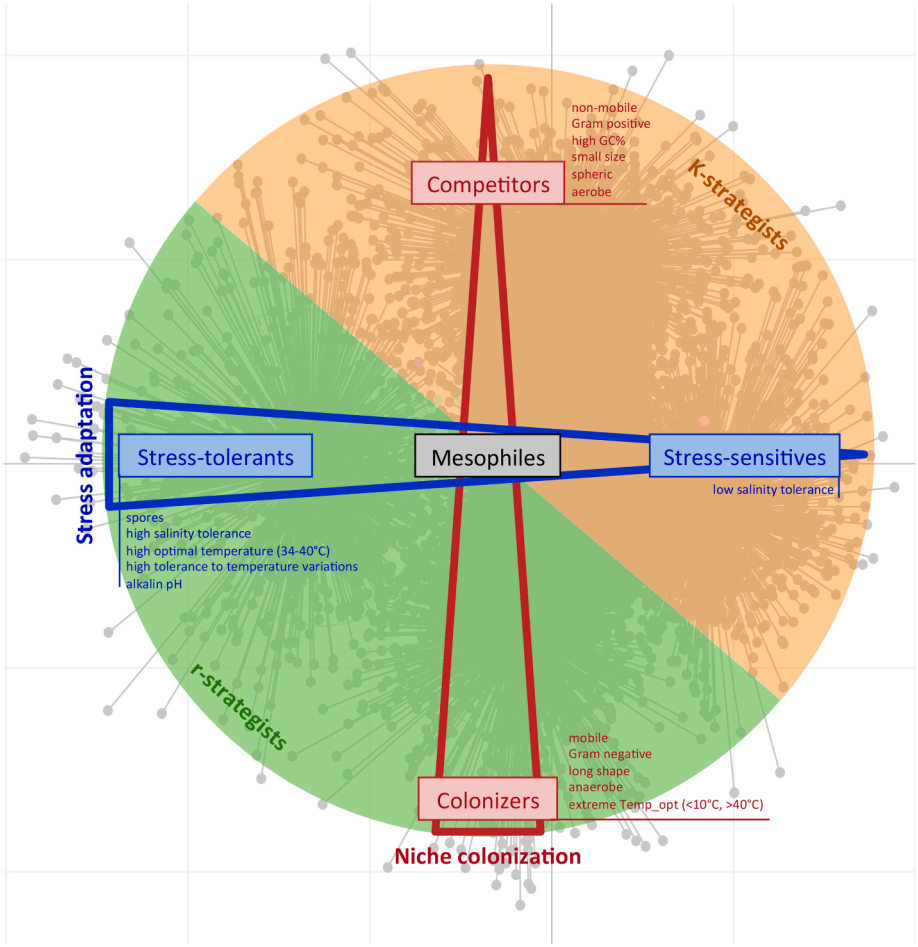


Fig. 3. Ecological classification of the five functional groups (mesophiles, stress-tolerants, stress-sensitives, colonizers and competitors) defined through FCA analysis and their major trait attributes. Groups are defined based on their niche colonization (red), stress adaptation (blue), resource acquisition and growth yield (r- and K strategists in green and orange, respectively) properties.

low substrate concentrations in the environment would favor smaller bacteria since they consume less energy for their survival (Schulz and Jørgensen, 2001). Being small may be also advantageous for life in inner-microaggregate environments not colonized by other microorganisms, giving access to low concentrated unexploited resources (Mummey and Stahl, 2004). Furthermore, Gram-positive bacteria are recognized as oligotrophs capable of using complex carbon forms (Kramer and Gleixner, 2008; Fanin et al., 2019) justifying their advantage as competitors. One general hypothesis would be that bacteria from the FGrT could be luxury consumers (i.e. organisms acquiring an excess of non-limiting resources) of certain nutrients (C, N, P; Goberna et al., 2014) giving them a competitive advantage through carbon and energy storage materials when resources become scarcer (de Mazancourt and Schwartz, 2012). Traits of the FGrT group also confer protection to bacteria by avoiding excess mortality (ruderal species; Grime, 1977). Small bacteria could escape viral lysis (Weinbauer and Höfle, 1998) or predation by bacterivorous flagellates, protozoa or nematodes (Andersson et al., 1986; Hahn and Höfle, 2001). Gram-positive bacteria are known for production of toxins (Hibbing et al., 2010) or antibiotics (Hussein et al., 2018) having allelopathic action against other species, as in *Streptomyces* species (Barazani and Friedman, 2001).

The bacteria from the FGrL and FGrR were respectively defined as stress-tolerants and stress-sensitives. Stress could be a change in environmental conditions (changes in humidity, nutrients, temperature, pollutant content) that has an adverse impact on organism growth and survival. Goberna et al. (2014) determined bacterial traits enabling tolerance to abiotic factors and environmental stresses such as traits of survival and adaptation to environmental fluctuations (Odum, 1959). Salinity (e.g. halotolerant or halophilic organisms; Oren, 2006), temperature and alkaline pH tolerance but also resistant structures (e.g. spores) formed to overcome environmental stress and germinate under more favorable conditions (Dworkin, 2006) are stress-tolerant traits characterizing the FGrL bacteria (Fig. 3).

BactoTraits is bound to evolve and it will be interesting to see in the future how the addition of new traits or new strains would modify the trait-based ordination of strains. The functional group description would benefit from new inputs likely to provide more adequate and robust definition of their phenotypic, genomic and ecological preferences. For example, (i) genomic traits such as the genome size, the number of 16S rRNA gene copies, the number of coding genes and the functional categories of genes, (ii) metabolic traits such as the carbon substrate used (Madin et al., 2020), biofilm formation, pathogenicity, mobile element content and oxidative stress tolerance (BugBase; Ward et al., 2017), the production of specific enzymes or their involvement in C, N, P, Fe cycle steps, and (iii) tolerance traits such as antibiotic or metal resistance, would be of great interest to complete the description of the adaptive potential of bacterial communities to perturbations.

3.4. Example of BactoTraits application: Case study on bacterial communities of PAH and metal contaminated soils

We examined if taxa colonizing different soils with similar contamination types and levels would harbor similar suites of trait attributes. We questioned if PAH or metal contamination would favor – or impair –

bacterial functional traits or functional groups or would modify the functional diversity observed at the single trait (CSI, OL, Rao index) and multi-traits levels (functional richness, evenness, diversity, dispersion and RaoQ). Significant differences in functional diversity could indicate post-contamination adaptation of bacterial communities insofar as these trait-based metrics have been already linked to community assembly processes (Mouchet et al., 2010; Spasojevic and Suding, 2012; Mason et al., 2013) or ecosystem functioning (Petchey et al., 2004; Mouillot et al., 2011).

Based on their physico-chemical characteristics, the soils were gathered into 3 types (uncontaminated controls, slag heaps with high PAH and low metal contamination, and settling ponds with high metal and low PAH contamination) that were significantly different based on the trait attributes of bacteria (OTUs) present in the communities (PERMANOVA, $F = 5.81$, $p < 0.001$), as illustrated by the NMDS analysis (Fig. 4). Bacterial assemblages of slag heap sites have lower dispersion in their trait attributes than the assemblages of the other two groups of soils (Permutation test, $F = 7.31$, $p = 0.006$). Based on Kruskal-Wallis tests ($p < 0.05$) and Dunn's multiple comparisons we determined which trait attributes and functional groups were significantly favored in the three soil types (Fig. 4).

Uncontaminated control soils, contaminated settling pond soils and slag heap soils exhibited a significantly higher relative proportion of bacteria belonging to FGrC ("Mesophiles"), FGrB ("Colonizers") and FGrR ("Stress-sensitives"), respectively (Fig. S4). These results suggest that bacterial communities adapted to their environment, with different functional groups being favored according to their habitat physico-chemical characteristics and PAH or metal contamination levels.

Significant differences have been identified in functional trait-based index values among the three soil groups (Figs. S5A, B and C). Overlap index (OL) values of several traits (e.g. OL_pH_opt, OL_GC%, OL_spore) were always lower for uncontaminated control soils than for both types of contaminated soils, indicating higher between-species trait variability in "reference" conditions. For traits such as specialization CSI (CSI_NaCl_delta) and Rao diversity (Rao_Oxygen, Rao_Length), values were respectively lower and higher for settling pond soils than for both other soil types, indicating a higher functional diversity in settling pond soils with more generalist species. The opposite pattern was observed for slag heap soils (higher CSI_Gram and CSI_Temp_delta; lower Rao_Shape and Rao_spore), indicating more specialized species but a lower diversity of trait attributes within bacterial assemblages. This finding could indicate rather lower niche complementarity in slag heap sites (Mason et al., 2013). This convergence of functional traits in bacterial communities highlighted the selection of one major preferential functional strategy in constrained habitats. Macrobenthic assemblages have already exhibited similar patterns, with higher functional divergence and trait dispersion in non-contaminated areas than in sewage contaminated areas (Gusmao et al., 2016). However, no significant differences among the three soil types (Fig. S5D) were found for functional richness (FRic), diversity (FDiv), evenness (FEv), dispersion (FDis) and RaoQ indices, taking into account all the functional traits together. But we must remain cautious in the interpretation of the observed responses of these indices, taking into account the potential bias in calculated values due to the lot of missing data for some traits in BactoTraits.

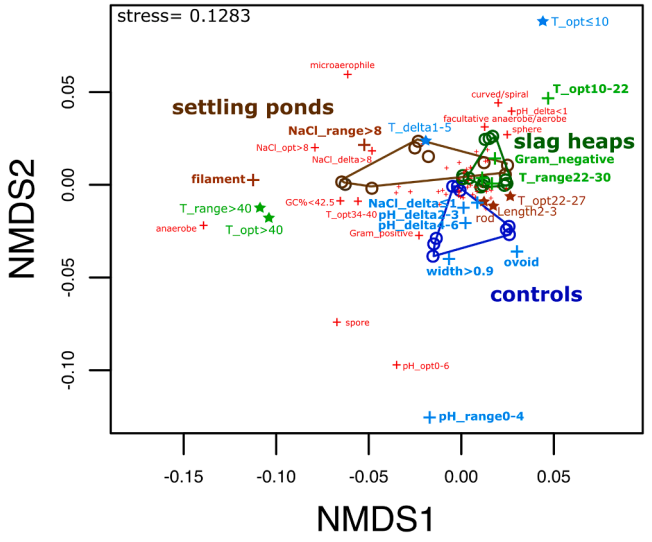


Fig. 4. Nonmetric multidimensional scaling (NMDS) based on the Bray-Curtis distance representing the dissimilarity among the three soil types (controls in blue, slag heaps in green and settling ponds in brown) based on the weighted mean of trait attributes. Trait attributes significantly more represented in controls, slag heaps and settling ponds are represented by blue, green and brown crosses and bold types, respectively. Trait attributes significantly less represented in controls, slag heaps and settling ponds are represented by blue, green and brown stars, respectively. Other trait attributes appear as red crosses, but for simplification, names of all the trait attributes do not appear.

To identify links between environmental condition and trait-based metrics, correlations between trait attributes (or functional indices calculated at the trait level) and soil parameters were established. They should help to distinguish the suites of trait attributes best linked to soil physico-chemical parameters and to metal and/or PAH contamination descriptors (Fig. 5).

Soil pH is one of the main parameters shaping bacterial community structure (Rinnan et al., 2009; Rousk et al., 2012). A soil pH increase seemed to favor a higher proportion of bacteria with a growth pH optimum of 7–8, a tolerance to alkaline pH (pH_ranges 7–8, 8–10 and 10–14) and high values of OL indices for "pH_opt" and "pH_range", as shown through positive correlations (Fig. 5). Acidic soil pH values seemed to favor a higher proportion of bacteria with pH ranges of 0–4 and 4–6, pH optimum at 0–6, pH delta of ≥ 1–2 units and high values of CSI index for "pH_range" (Fig. 5). These intuitively predictable results have emphasized the consistence of our trait-based approach and validated the inference of functional trait through BactoTraits. However, other non-intuitive results have been obtained, e.g. bacteria without spore formation ability, with optimal temperature below 10 °C and NaCl optimum of 1–3% were favored with a pH increase, and the proportion of non-motile, ovoid and anaerobic bacteria increased with a pH decline (Fig. 5). These suites of traits inform on bacteria lifestyle depending on soil pH. Moreover, pH was positively correlated with two CSI indices and three OL indices, indicating an increased specialization for "Gram" and "Width" traits and a more even representation of trait attributes for "Temp_range", "GC%" and "Oxygen", when pH increases.

Several physico-chemical characteristics seemed to influence the trait profiles of the bacterial communities (soil texture, COD, Na, N, and P_2O_5 contents) while others (Fe, C and C/N) only showed few correlations with the studied traits. For example, soil texture (percentage of silt or sand) was correlated with a wide variety of functional diversity descriptors (Fig. 5). Sand content was positively correlated with five CSI and three OL indices, with small, Gram-negative bacteria exhibiting temperature optimum between 10 and 22 °C, slightly alkalin pH optimum, low pH delta tolerance and/or intermediate salt tolerance. The percentage of silt was positively correlated with bacteria capable of anaerobic metabolism, having filament shape and with high (above 40 °C) temperature optimum and range. Moreover, an increase in soil DOC content favored Gram positive, spore forming, ovoid, non-motile and large (width > 0.9 μm) bacteria, having acidic pH optimum and range (Fig. 5).

One of our objectives was also to study the impact of metals and PAHs on the selection of bacterial traits. Total zinc concentration, a proxy of metal contamination in these post-industrial brownfield soils (Lemmel et al., 2019), was positively correlated with the proportion of microaerophile bacteria, with filament shape, high temperature optimum (≥40 °C) and tolerance range and temperature delta of 5–10 °C, (Fig. 5). The soils having the highest zinc contamination were ancient

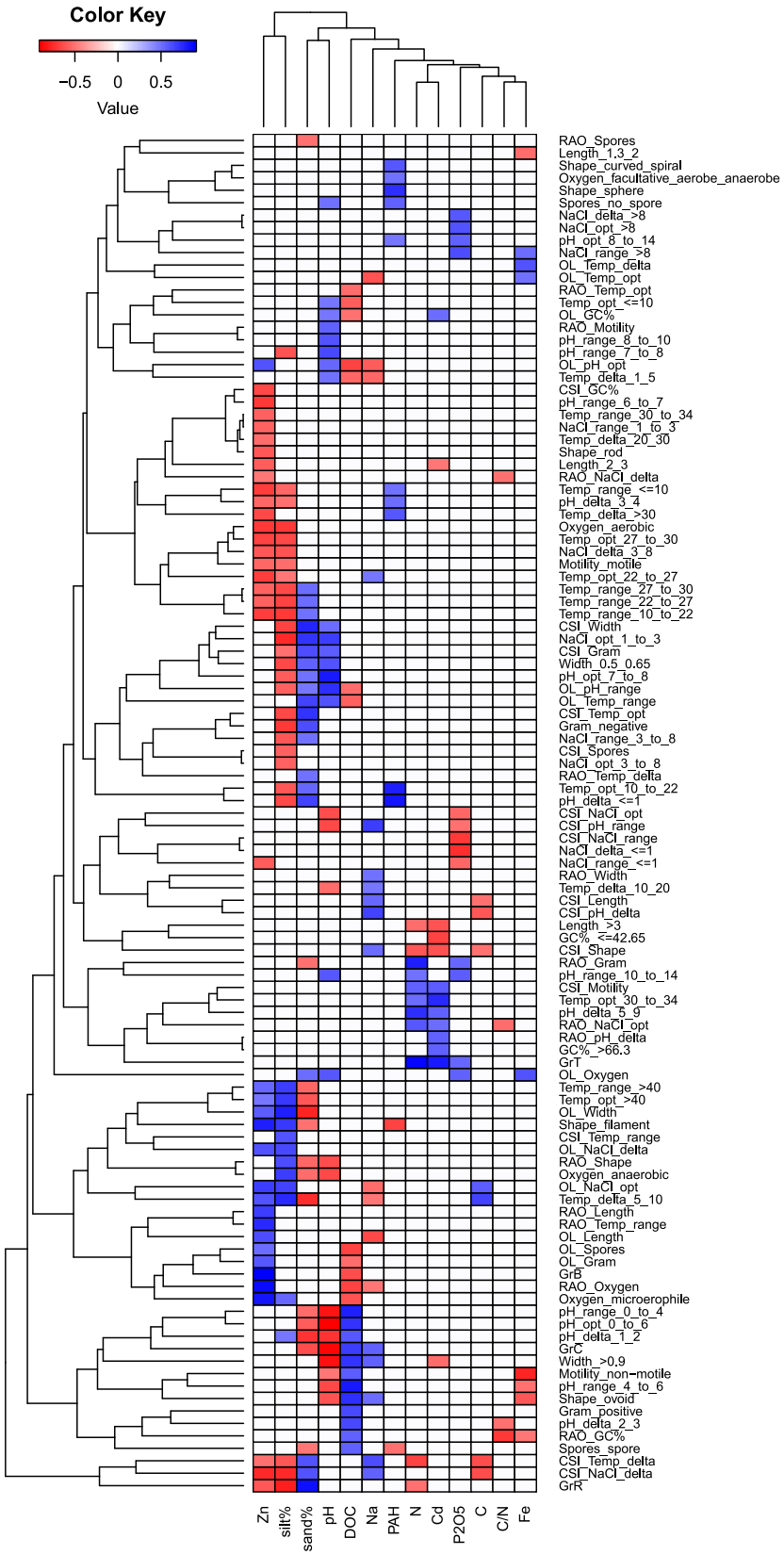


Fig. 5. Heatmap showing the correlations between soil characteristics of the 10 studied sites and functional trait diversity parameters (weighted mean values of each trait attribute, overlap OL and CSI indicator of each trait and functional group GrT "competitors", GrB "colonizers", GrC "mesophiles", GrL "stress-tolerants", GrR "stress-sensitives"). See Table 1 for the full description of traits and trait attributes.

settling ponds. The observed selection of bacterial traits could probably be explained by the anoxic conditions and higher temperatures found in settling ponds few decades ago. Bacterial adaptation to high temperature could be an advantage to cope with high metal contamination. Babich and Stotzky (1982) observed an increase in the resistance of *Aspergillus flavus* to Ni by increasing the temperature from 23 to 33 °C. Atlas et al. (1991) found a different growth response to temperature in microbial populations of undisturbed control versus chemically contaminated environments. Finally, Díaz-Raviña and Bååth (2001) indicated that heavy metal pollution led to metal-tolerant communities with temperature responses (e.g. an increase in temperature tolerance) differing from those of communities in unpolluted soils.

Cadmium and zinc were correlated to different traits (Fig. 5). An increase in Cd concentration induced an increase in the proportion of bacteria having high GC percentage, pH range up to 10–14 and tolerating high pH variation (delta of 5 to 9 pH units). The relationship between high GC content and cadmium resistance was recently shown (Chen et al., 2020). In soil the availability and toxicity of Cd increase when soil pH decreases. Then we could hypothesize that bacteria inhabiting soil highly polluted with Cd would have developed mechanisms to locally increase pH in their surrounding environment to decrease Cd negative impact. Moreover, industrial Cd-contaminated soils often harbor alkaline pH, favoring the development of bacteria with high pH value tolerance.

PAH total concentration in soils was positively correlated with three clusters of trait attributes (Fig. 5). The more soils were PAH-contaminated, the more bacterial community was composed of facultative anaerobes/aerobes with spherical or curved/spiral shapes, without the ability to produce spores and having alkaline pH optimum for growth (pH $\geq$ 8–14), low pH delta tolerance ($\leq$1 pH unit) and medium temperature optimum ($\geq$10-22 °C) but high temperature variation tolerance (delta $\geq$ 30 °C). As PAHs are hydrophobic compounds, high PAH content could induce the formation of almost anoxic microniches, favoring bacteria capable of growth with limited oxygen content. Aged PAH-contaminated brownfield soils, such as slag heaps, often exhibit alkaline pH (Joimel et al., 2016).

It is interesting to note that the occurrences of bacteria belonging to our functional groups were also correlated with some soil parameters. Stress-sensitive strains of FGrR were positively correlated with soil sand content (Fig. 5), probably explaining why they were more represented in slag heap soils (Fig. S4) with a more sandy texture than the other soil groups. The occurrence of FGrC mesophiles were negatively correlated with soil pH and sand content but positively correlated with soil nutrient content (e.g. Na and DOC contents). Higher carbon and mineral nutrient availabilities could explain the higher proportion of bacteria belonging to FGrC in uncontaminated control soils. The occurrence of bacteria belonging to FGrT (competitors) was positively correlated with soil nitrogen and phosphorus (N and P_2O_5) contents. Sarathchandra et al. (2001) and Zhang et al. (2007) previously demonstrated that the functional diversity of soil microflora decreased after many years of N and P soil fertilisation, and that NPK fertilizer application stimulated Gram-positive bacteria and increased the proportion of Actinomycetes belonging to Actinobacteria shown to be dominant in FGrT (Fig. 2). FGrT was also characterized by bacteria with high GC%. GC content is positively correlated with bacterial genome size (Nishida, 2012), and larger genomes allow the microorganisms to adapt to a higher variety of environmental conditions (Litchman, 2010; Bentkowski et al., 2015; Cobo-Simón and Tamames, 2017). Thus FGrT probably consists of bacteria with high resistance to environmental fluctuations. Finally, the occurrence of bacteria belonging to FGrB (colonizers) was positively correlated to soil zinc content and negatively correlated to DOC content. Colonizers are indeed often autotrophic bacteria with the ability to colonize oligotrophic and mineral environments.

4. Conclusions

Although trait-based approaches had been rarely applied to microbial communities, we have demonstrated how such approach can be used to more deeply understand bacterial community assembly and have started explaining the shifts in microbial community composition across environmental gradients based on trait-related functional criteria. Through this approach we could have a better understanding of the functional role of bacteria in disturbed environments. We could also establish links between bacteria community diversity and certain trait assembly, and better understand adaptation or tolerance mechanisms. We will need to test this approach on a higher number of environmental sites and at larger scale, but our trait-based approach is likely to help to define good bioindicators of environmental changes. BactoTraits could be widely used by the whole microbial ecologist community working on various ecosystems from soil and aquatic environments to human or animal microbiotes and studying many different environmental gradients and anthropogenic stress filters. Drawbacks of this functional trait inference from data acquired on cultivated bacterial strains have been previously highlighted (Barberán et al., 2017). Effort is still needed for completing BactoTraits with trait preferences from new isolates as well as new ecological, physiological and genomic traits (Madin et al., 2020). Compiling phenotypic information from cultivated bacterial strains and integrating this information with genomic or marker gene data are critical for advancing the field of microbial ecology. Even though this study gives us fundamental aspects about the ecology of bacteria in their environment, the next important step would be also to formulate hypotheses on the links between specific traits and ecosystem functions that will need to be tested.

CRediT authorship contribution statement

Aurélie Cébron: Conceptualization, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Visualization, Writing – original draft, Writing - review & editing. **Emna Zeghal:** Data curation, Formal analysis, Methodology, Software. **Philippe Usseglio-Polatera:** Conceptualization, Formal analysis, Methodology, Validation, Visualization, Writing - review & editing. **Albin Meyer:** Data curation, Formal analysis, Methodology, Software, Validation, Visualization, Writing - review & editing. **Pascale Bauda:** Supervision, Validation, Writing - review & editing. **Florian Lemmel:** Resources. **Corinne Leyval:** Resources. **Florence Maunoury-Danger:** Conceptualization, Formal analysis, Funding acquisition, Methodology, Resources, Validation, Visualization, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This work was supported by the LIEC laboratory EMMA team, and funded by OSU-OteLo (TraiMic project).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ecolind.2021.108047.

References

Aßhauer, K.P., Wemheuer, B., Daniel, R., Meinicke, P., 2015. Tax4Fun: predicting functional profiles from metagenomic 16S rRNA data. Bioinformatics 31 (17), 2882–2884.

Adler, P.B., Salguero-Gomez, R., Compagnoni, A., Hsu, J.S., Ray-Mukherjee, J., Mbeau-Ache, C., Franco, M., 2014. Functional traits explain variation in plant life history strategies. Proc. Natl. Acad. Sci. 111 (2), 740–745.

Andersson, A., Larsson, U., Hagström, Å., 1986. Size-selective grazing by a microflagellate on pelagic bacteria. Mar. Ecol. Prog. Ser. 33, 51–57.

Anderson, M.J., 2001. A new method for non-parametric multivariate analysis of variance. Austral. Ecol. 26, 32–46.

Aravindraja, C., Viszwapriya, D., Karutha Pandian, S., Badger, J.H., 2013. Ultradeep 16S rRNA sequencing analysis of geographically similar but diverse unexplored marine samples reveal varied bacterial community composition. PLoS ONE 8 (10), e76724. https://doi.org/10.1371/journal.pone.0076724.

Arndt, D., Xia, J., Liu, Y., Zhou, Y., Guo, A.C., Cruz, J.A., Sinelnikov, I., Budwill, K., Nesbo, C.L., Wishart, D.S., 2012. METAGENassist: a comprehensive web server for comparative metagenomics. Nucleic Acids Res. 40 (W1), W88–W95.

Atlas, R.M., Horowitz, A., Krichevsky, M., 1991. Response of microbial populations to environmental disturbance. Microb. Ecol. 22, 249–256.

Babich, H., Stotzky, G., 1982. Nickel toxicity to fungi: influence of environmental factors. Ecotoxicol. Environ. Saf. 6 (6), 577–589.

Barazani, O.z., Friedman, J., 2001. Allelopathic bacteria and their impact on higher plants. Crit. Rev. Microbiol. 27 (1), 41–55.

Barberán, A., Fernandez-Guerra, A., Bohannan, B.J., Casamayor, E.O., 2012. Exploration of community traits as ecological markers in microbial metagenomes. Mol. Ecol. 21 (8), 1909–1917.

Barberán, A., Caceres Velazquez, H., Jones, S., Fierer, N., Hallam, S.J., 2017. Hiding in plain sight: mining bacterial species records for phenotypic trait information. MSphere 2 (4). https://doi.org/10.1128/mSphere.00237-17.

Barton, P.S., Gibb, H., Manning, A.D., Lindenmayer, D.B., Cunningham, S.A., 2011. Morphological traits as predictors of diet and microhabitat use in a diverse beetle assemblage. Biol. J. Linn. Soc. 102 (2), 301–310.

Beauchard, O., Veríssimo, H., Queirós, A.M., Herman, P.M.J., 2017. The use of multiple biological traits in marine community ecology and its potential in ecological indicator development. Ecol. Ind. 76, 81–96.

Benjamini, Y., Hochberg, Y., 1995. Controlling the false discovery rate: a practical and powerful approach to multiple testing. J. Roy. Stat. Soc. B 57 (1), 289–300.

Bentkowski, P., Van Oosterhout, C., Mock, T., 2015. A model of genome size evolution for prokaryotes in stable and fluctuating environments. Genom. Biol. Evol. 7, 2344–2351.

Berlemont, R., Allison, S.D., Weihe, C., Lu, Y., Brodie, E.L., Martiny, J.B.H., et al., 2014. Cellulolytic potential under environmental changes in microbial communities from grassland litter. Front. Microbiol. 5, 1–10.

Bernhardt-Römermann, M., Gray, A., Vanbergen, A.J., Bergès, L., Bohner, A., Brooker, R. W., et al., 2011. Functional traits and local environment predict vegetation responses to disturbance: a pan-European multi-site experiment. J. Ecol. 99 (3), 777–787.

Bertelsmeier, C., Guénard, B., Courchamp, F., Gordon, D.M., 2013. Climate change may boost the invasion of the Asian needle ant. PLoS ONE 8 (10), e75438. https://doi.org/10.1371/journal.pone.0075438.

Bewick, S., Gurarie, E., Weissman, J.L., Beattie, J., Davati, C., Flint, R., Thielen, P., Breitwieser, F., Karig, D., Fagan, W.F., 2019. Trait-based analysis of the human skin microbiome. Microbiome 7 (1). https://doi.org/10.1186/s40168-019-0698-2.

Borcard, D., Gillet, F., Legendre, P., 2011. Numerical Ecology with R. Springer, New York. https://doi.org/10.1007/978-1-4419-7976-6.

Botta-Dukát, Z., 2005. Rao's quadratic entropy as a measure of functional diversity based on multiple traits. J. Veg. Sci. 16, 533–540.

Bremner, J., Rogers, S., Frid, C., 2006. Methods for describing ecological functioning of marine benthic assemblages using biological traits analysis (BTA). Ecol. Ind. 6 (3), 609–622.

Bremner, J., 2008. Species' traits and ecological functioning in marine conservation and management. J. Exp. Mar. Biol. Ecol. 366 (1-2), 37–47.

Brooks, A.W., Priya, S., Blekhman, R., Bordenstein, S.R., Cadwell, K., 2018. Gut microbiota diversity across ethnicities in the United States. PLoS Biol. 16 (12), e2006842. https://doi.org/10.1371/journal.pbio.2006842.

Chen, J., Xing, C., Zheng, X., Li, X., 2020. Functional Genomic Identification of Cadmium Resistance Genes from a High GC Clone Library by Coupling the Sanger and PacBio Sequencing Strategies. Genes 11 (1), 7.

Chevene, Francois, Doleadec, Sylvain, Chessel, Daniel, 1994. A fuzzy coding approach for the analysis of long-term ecological data. Freshw. Biol. 31 (3), 295–309.

Cobo-Simón, M., Tamames, J., 2017. Relating genomic characteristics to environmental preferences and ubiquity in different microbial taxa. BMC Genom. 18 (1), 1–11.

Cornwell, W.K., Cornelissen, J.H., Amatangelo, K., Dorrepaal, E., Eviner, V.T., Godoy, O., et al., 2008. Plant species traits are the predominant control on litter decomposition rates within biomes worldwide. Ecol. Lett. 11 (10), 1065–1071.

Crowther, T.W., Maynard, D.S., Crowther, T.R., Peccia, J., Smith, J.R., Bradford, M.A., 2014. Untangling the fungal niche: the trait-based approach. Front. Microbiol. 5, 579.

Darr, A., Gogina, M., Zettler, M.L., 2014. Functional changes in benthic communities along a salinity gradient–a western Baltic case study. J. Sea Res. 85, 315–324.

Delgado-Baquerizo, M., Oliverio, A.M., Brewer, T.E., Benavent-González, A., Eldridge, D. J., Bardgett, R.D., Fierer, N., 2018. A global atlas of the dominant bacteria found in soil. Science 359 (6373), 320–325.

DeLong, E.F., Preston, C.M., Mincer, T., Rich, V., Hallam, S.J., Frigaard, N.U., Chisholm, S.W., 2006. Community genomics among stratified microbial assemblages in the ocean's interior. Science 311 (5760), 496–503.

de Mazancourt, C., Schwartz, M.W., 2012. Starve a competitor: evolution of luxury consumption as a competitive strategy. Theor. Ecol. 5 (1), 37–49.

Devictor, V., Julliard, R., Jiguet, F., 2008. Distribution of specialist and generalist species along spatial gradients of habitat disturbance and fragmentation. Oikos 117 (4), 507–514.

Dhakar, K., Pandey, A., 2016. Wide pH range tolerance in extremophiles: towards understanding an important phenomenon for future biotechnology. Appl. Microbiol. Biotechnol. 100 (6), 2499–2510.

Díaz-Raviña, M., Bååth, E., 2001. Response of soil bacterial communities pre-exposed to different metals and reinoculated in an unpolluted soil. Soil Biol. Biochem. 33 (2), 241–248.

Ding, Z., Feeley, K.J., Wang, Y., Pakeman, R.J., Ding, P., Webb, T., 2013. Patterns of bird functional diversity on land-bridge island fragments. J. Anim. Ecol. 82 (4), 781–790.

Dworkin, M., 2006. Prokaryotic life cycles. In: Dworkin, M., Falkow, S., Rosenberg, E., Schleifer, K.-H., Stackebrandt, E. (Eds.), The Prokaryotes, Vol. 2. Springer Science + Business Media B.V, New York, pp. 140–166.

Ernebjerg, M., Kishony, R., 2012. Distinct growth strategies of soil bacteria as revealed by large-scale colony tracking. Appl. Environ. Microbiol. 78 (5), 1345–1352.

Fanin, N., Kardol, P., Farrell, M., Nilsson, M.-C., Gundale, M.J., Wardle, D.A., 2019. The ratio of Gram-positive to Gram-negative bacterial PLFA markers as an indicator of carbon availability in organic soils. Soil Biol. Biochem. 128, 111–114.

Fierer, N., Bradford, M.A., Jackson, R.B., 2007. Toward an ecological classification of soil bacteria. Ecology 88 (6), 1354–1364.

Fierer, N., Barberán, A., Laughlin, D.C., 2014. Seeing the forest for the genes: using metagenomics to infer the aggregated traits of microbial communities. Front. Microbiol. 5, 614.

Fierer, N., 2017. Embracing the unknown: disentangling the complexities of the soil microbiome. Nat. Rev. Microbiol. 15 (10), 579–590.

Frimpong, E.A., Angermeier, P.L., 2009. FishTraits: A database of ecological and life-history traits of freshwater fishes of the United States. Fisheries 34 (10), 487–495.

Goberna, M., Navarro-Cano, J.A., Valiente-Banuet, A., García, C., Verdú, M., Morlon, H., 2014. Abiotic stress tolerance and competition-related traits underlie phylogenetic clustering in soil bacterial communities. Ecol. Lett. 17 (10), 1191–1201.

Green, J.L., Bohannan, B.J., Whitaker, R.J., 2008. Microbial biogeography: from taxonomy to traits. Science 320 (5879), 1039–1043.

Grime, J.P., 1977. Evidence for the existence of three primary strategies in plants and its relevance to ecological and evolutionary theory. Am. Nat. 111 (982), 1169–1194.

Gusmao, J.B., Brauko, K.M., Eriksson, B.K., Lana, P.C., 2016. Functional diversity of macrobenthic assemblages decreases in response to sewage discharges. Ecol. Ind. 66, 65–75.

Hahn, M.W., Höfle, M.G., 2001. Grazing of protozoa and its effect on populations of aquatic bacteria. FEMS Microbiol. Ecol. 35 (2), 113–121.

Hibbing, M.E., Fuqua, C., Parsek, M.R., Peterson, S.B., 2010. Bacterial competition: surviving and thriving in the microbial jungle. Nat. Rev. Microbiol. 8 (1), 15–25.

Ho, A., Kerckhof, F.-M., Luke, C., Reim, A., Krause, S., Boon, N., Bodelier, P.L.E., 2013. Conceptualizing functional traits and ecological characteristics of methane-oxidizing bacteria as life strategies. Environ. Microbiol. Rep. 5 (3), 335–345.

Homburg, K., Homburg, N., Schäfer, F., Schuldt, A., Assmann, T., 2014. Carabids.org – a dynamic online database of ground beetle species traits (Coleoptera, Carabidae). Insect Conserv. Divers. 7 (3), 195–205.

Hooper, D.U., Solan, M., Symstad, A., et al., 2002. In: Species diversity, functional diversity, and ecosystem functioning. Oxford University Press, pp. 195–208.

Hussein, E.I., Jacob, J.H., Shakhatreh, M.A.K., Al-Razaq, M.A.A., Juhmani, A.-S., Cornelison, C.T., 2018. Detection of antibiotic-producing Actinobacteria in the sediment and water of Ma'in thermal springs (Jordan). Germs 8 (4), 191–198.

Ilg, C., Castella, E., 2006. Patterns of macroinvertebrate traits along three glacial stream continuums. Freshw. Biol. 51 (5), 840–853.

Joimel, S., Cortet, J., Jolivet, C.C., Saby, N.P.A., Chenot, E.D., Branchu, P., Consalès, J.N., Lefort, C., Morel, J.L., Schwartz, C., 2016. Physico-chemical characteristics of topsoil for contrasted forest, agricultural, urban and industrial land uses in France. Sci. Total Environ. 545-546, 40–47.

Julliard, R., Clavel, J., Devictor, V., Jiguet, F., Couvet, D., 2006. Spatial segregation of specialists and generalists in bird communities. Ecol. Lett. 9 (11), 1237–1244.

Kanehisa, M., Goto, S., Sato, Y., Furumichi, M., Tanabe, M., 2011. KEGG for integration and interpretation of large-scale molecular data sets. Nucleic Acids Res. 40 (D1), 109–114.

Kattge, J., Diaz, S., Lavorel, S., Prentice, I.C., Leadley, P., Bönisch, G., et al., 2011. TRY–a global database of plant traits. Global Change Biol. 17 (9), 2905–2935.

Koch, A.L., 2001. Oligotrophs versus copiotrophs. BioEssays 23 (7), 657–661.

Kraft, N.J.B., Valencia, R., Ackerly, D.D., 2008. Functional traits and niche-based tree community assembly in an Amazonian forest. Science 322 (5901), 580–582.

Kramer, C., Gleixner, G., 2008. Soil organic matter in soil depth profiles: distinct carbon preferences of microbial groups during carbon transformation. Soil Biol. Biochem. 40 (2), 425–433.

Krause, S., Le Roux, X., Niklaus, P.A., Van Bodegom, P.M., Lennon, J.T., Bertilsson, S., Grossart, H.-P., Philippot, L., Bodelier, P.L.E., 2014. Trait-based approaches for understanding microbial biodiversity and ecosystem functioning. Front. Microbiol. 5 https://doi.org/10.3389/fmicb.2014.00251.

Lajoie, G., Kembel, S.W., 2019. Making the most of trait-based approaches for microbial ecology. Trends Microbiol. 27 (10), 814–823.

Laliberté, E., Legendre, P., 2010. A distance-based framework for measuring functional diversity from multiple traits. Ecology 91 (1), 299–305.

Laliberté E., Legendre P., Shipley B., Laliberté M.E., 2014. Package 'FD'. Measuring functional diversity from multiple traits, and other tools for functional ecology.
Langenheder, S., Bulling, M.T., Solan, M., Prosser, J.I., Bell, T., 2010. Bacterial biodiversity-ecosystem functioning relations are modified by environmental complexity. PLoS ONE 5 (5), e10834. https://doi.org/10.1371/journal.pone.0010834.
Langille, M.G.I., Zaneveld, J., Caporaso, J.G., McDonald, D., Knights, D., Reyes, J.A., Clemente, J.C., Burkepile, D.E., Vega Thurber, R.L., Knight, R., Beiko, R.G., Huttenhower, C., 2013. Predictive functional profiling of microbial communities using 16S rRNA marker gene sequences. Nat. Biotechnol. 31 (9), 814–821.
Lavorel, S., McIntyre, S., Landsberg, J., Forbes, T.D.A., 1997. Plant functional classifications: from general groups to specific groups based on response to disturbance. Trends Ecol. Evol. 12 (12), 474–478.
Lemmel, F., Maunoury-Danger, F., Fanesi, A., Leyval, C., Cébron, A., 2019. Soil properties and multi-pollution affect taxonomic and functional bacterial diversity in a range of French soils displaying an anthropisation gradient. Microb. Ecol. 77 (4), 993–1013.
Litchman, E., 2010. Invisible invaders: non-pathogenic invasive microbes in aquatic and terrestrial ecosystems. Ecol. Lett. 13 (12), 1560–1572.
Logez, M., Bady, P., Melcher, A., Pont, D., 2013. A continental-scale analysis of fish assemblage functional structure in European rivers. Ecography 36 (1), 80–91.
Madin, J.S., Nielsen, D.A., Brbic, M., Corkrey, R., Danko, D., Edwards, K., Engqvist, M.K. M., Fierer, N., Geoghegan, J.L., Gillings, M., Kyrpides, N.C., Litchman, E., Mason, C. E., Moore, L., Nielsen, S.L., Paulsen, I.T., Price, N.D., Reddy, T.B.K., Richards, M.A., Rocha, E.P.C., Schmidt, T.M., Shaaban, H., Shukla, M., Supek, F., Tetu, S.G., Vieira-Silva, S., Wattam, A.R., Westfall, D.A., Westoby, M., 2020. A synthesis of bacterial and archaeal phenotypic trait data. Sci. Data 7 (1). https://doi.org/10.1038/s41597-020-0497-4.
Malik, A.A., Martiny, J.B.H., Brodie, E.L., Martiny, A.C., Treseder, K.K., Allison, S.D., 2020. Defining trait-based microbial strategies with consequences for soil carbon cycling under climate change. ISME J. 14 (1), 1–9.
Mason, N.W.H., de Bello, F., Mouillot, D., Pavoine, S., Dray, S., Zobel, M., 2013. A guide for using functional diversity indices to reveal changes in assembly processes along ecological gradients. J. Veg. Sci. 24 (5), 794–806.
McGill, B., Enquist, B., Weiher, E., Westoby, M., 2006. Rebuilding community ecology from functional traits. Trends Ecol. Evol. 21 (4), 178–185.
Mendler, K., Chen, H., Parks, D.H., Lobb, B., Hug, L.A., Doxey, A.C., 2019. AnnoTree: visualization and exploration of a functionally annotated microbial tree of life. Nucleic Acids Res. 47 (9), 4442–4448.
Mondy, C.P., Usseglio-Polatera, P., 2014. Using fuzzy-coded traits to elucidate the non-random role of anthropogenic stress in the functional homogenisation of invertebrate assemblages. Freshw. Biol. 59 (3), 584–600.
Mouchet, M.A., Villéger, S., Mason, N.W., Mouillot, D., 2010. Functional diversity measures: an overview of their redundancy and their ability to discriminate community assembly rules. Funct. Ecol. 24 (4), 867–876.
Mouillot, D., Villéger, S., Scherer-Lorenzen, M., Mason, N.W.H., Romanuk, T., 2011. Functional structure of biological communities predicts ecosystem multifunctionality. PLoS ONE 6 (3), e17476. https://doi.org/10.1371/journal.pone.0017476.
Mummey, D.L., Stahl, P.D., 2004. Analysis of soil whole-and inner-microaggregate bacterial communities. Microb. Ecol. 48 (1), 41–50.
Naeem, S., Wright, J.P., 2003. Disentangling biodiversity effects on ecosystem functioning: deriving solutions to a seemingly insurmountable problem. Ecol. Lett. 6 (6), 567–579.
Nemergut, D.R., Schmidt, S.K., Fukami, T., O'Neill, S.P., Bilinski, T.M., Stanish, L.F., Knelman, J.E., Darcy, J.L., Lynch, R.C., Wickey, P., Ferrenberg, S., 2013. Patterns and processes of microbial community assembly. Microbiol. Mol. Biol. Rev. 77 (3), 342–356.
Nelson, M.B., Martiny, A.C., Martiny, J.B.H., 2016. Global biogeography of microbial nitrogen-cycling traits in soil. Proc. Natl. Acad. Sci. 113 (29), 8033–8040.
Nguyen, N.H., Song, Z., Bates, S.T., Branco, S., Tedersoo, L., Menke, J., Schilling, J.S., Kennedy, P.G., 2016. FUNGuild: an open annotation tool for parsing fungal community datasets by ecological guild. Fungal Ecol. 20, 241–248.
Nishida, H., 2012. Evolution of genome base composition and genome size in bacteria. Front. Microbiol. 3, 420.
Odum, E.P., 1959. Fundamentals of Ecology, 2nd ed. Saunders, Philadelphia, p. 546.
Oksanen, J., Blanchet, F.G., Kindt, R., Legendre, P., Minchin, P.R., O'Hara, R.B., Oksanen, M.J., 2013. Package 'vegan'. Community Ecology Package, Version 2 (9), 1–295.
Oren, A., 2006. Life at high salt concentrations. In: Dworkin, M., Falkow, S., Rosenberg, E., Schleifer, K.-H., Stackebrandt, E. (Eds.), The Prokaryotes, Vol. 2. Springer Science + Business Media B.V, New York, pp. 263–282.
Petchey, O.L., Downing, A.L., Mittelbach, G.G., Persson, L., Steiner, C.F., Warren, P.H., Woodward, G., 2004. Species loss and the structure and functioning of multitrophic aquatic systems. Oikos 104 (3), 467–478.
Pey, B., Laporte, M.-A., Nahmani, J., Auclerc, A., Capowiez, Y., Caro, G., Cluzeau, D., Cortet, J., Decaëns, T., Dubs, F., Joimel, S., Guernion, M., Briard, C., Grumiaux, F., Laporte, B., Pasquet, A., Pelosi, C., Pernin, C., Ponge, J.-F., Salmon, S., Santorufo, L., Hedde, M., Schuch, R., 2014. A thesaurus for soil invertebrate trait-based approaches. PLoS ONE 9 (10), e108985. https://doi.org/10.1371/journal.pone.0108985.
Pianka, E.R., 1974. Niche overlap and diffuse competition. PNAS 71 (5), 2141–2145.
Prosser, J.I., Bohannan, B.J.M., Curtis, T.P., Ellis, R.J., Firestone, M.K., Freckleton, R.P., Green, J.L., Green, L.E., Killham, K., Lennon, J.J., Osborn, A.M., Solan, M., van der Gast, C.J., Young, J.P.W., 2007. The role of ecological theory in microbial ecology. Nat. Rev. Microbiol. 5 (5), 384–392.
Rao, C.R., 1982. Diversity and dissimilarity coefficients - a unified approach. Theor. Popul Biol. 21 (1), 24–43.
R Core Team, 2016. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria http://www.R-project.org/.
Reiss, J., Bridle, J.R., Montoya, J.M., Woodward, G., 2009. Emerging horizons in biodiversity and ecosystem functioning research. Trends Ecol. Evol. 24 (9), 505–514.
Rinnan, R., Rousk, J., Yergeau, E., Kowalchuk, G.A., Bååth, E., 2009. Temperature adaptation of soil bacterial communities along an Antarctic climate gradient: predicting responses to climate warming. Glob. Change Biol. 15 (11), 2615–2625.
Rousk, J., Frey, S.D., Bååth, E., 2012. Temperature adaptation of bacterial communities in experimentally warmed forest soils. Glob. Change Biol. 18 (10), 3252–3258.
Sarathchandra, S.U., Ghani, A., Yeates, G.W., Burch, G., Cox, N.R., 2001. Effect of nitrogen and phosphate fertilisers on microbial and nematode diversity in pasture soils. Soil Biol. Biochem. 33 (7-8), 953–964.
Schimel, J., Balser, T.C., Wallenstein, M., 2007. Microbial stress-response physiology and its implications for ecosystem function. Ecology 88 (6), 1386–1394.
Schleuter, D., Daufresne, M., Massol, F., Argillier, C., 2010. A user's guide to functional diversity indices. Ecol. Monogr. 80 (3), 469–484.
Schmidt-Kloiber, A., Hering, D., 2015. *www.freshwaterecology.info* – An online tool that unifies, standardises and codifies more than 20,000 European freshwater organisms and their ecological preferences. Ecol. Ind. 53, 271–282.
Schulz, H.N., Jørgensen, B.B., 2001. Big bacteria. Ann. Rev. Microbiol. 55 (1), 105–137.
Selengut, J.D., Haft, D.H., Davidsen, T., Ganapathy, A., Gwinn-Giglio, M., Nelson, W.C., Richter, A.R., White, O., 2007. TIGRFAMs and genome properties: tools for the assignment of molecular function and biological process in prokaryotic genomes. Nucleic Acids Res. 35 (Database), D260–D264.
Silva, R.R., Brandão, C.R.F., 2010. Morphological patterns and community organization in leaf-litter ant assemblages. Ecol. Monogr. 80 (1), 107–124.
Spasojevic, M.J., Suding, K.N., 2012. Inferring community assembly mechanisms from functional diversity patterns: the importance of multiple assembly processes. J. Ecol. 100 (3), 652–661.
Tringe, S.G., Von Mering, C., Kobayashi, A., Salamov, A.A., Chen, K., Chang, H.W., et al., 2005. Comparative metagenomics of microbial communities. Science 308 (5721), 554–557.
Usseglio-Polatera, P., Bournaud, M., Richoux, P., Tachet, H., 2000. Biological and ecological traits of benthic freshwater macroinvertebrates: relationship and definition of groups with similar traits. Freshw. Biol. 43 (175), 205.
Usseglio-Polatera, P., Richoux, P., Bournaud, M., Tachet, H., 2001. A functional classification of benthic macroinvertebrates based on biological and ecological traits: application to river condition assessment and stream management. Archiv für Hydrobiologie 139 (Suppl. 1), 53–83.
Villéger, S., Mason, N.W.H., Mouillot, D., 2008. New multidimensional functional diversity indices for a multifaceted framework in functional ecology. Ecology 89 (8), 2290–2301.
Vieira, N.K.M., Poff, N.L., Carlisle, D.M., Moulton II, S.R., Koski, M.L., Kondratieff, B.C., 2006. A database of lotic invertebrate traits for North America. USGS Numbered Series No. 187, 19.
Violle, C., Navas, M.L., Vile, D., Kazakou, E., Fortunel, C., Hummel, I., Garnier, E., 2007. Let the concept of trait be functional! Oikos 116 (5), 882–892.
Ward, T., Larson, J., Meulemans, J., Hillmann, B., Lynch, J., Sidiropoulos, D., Spear, J.R., Caporaso, G., Blekhman, R., Knight, R., Fink, R., Knights, D., 2017. BugBase predicts organism-level microbiome phenotypes. BioRxiv 133462. https://doi.org/10.1101/133462.
Warnes, G.R., Bolker, B., Bonebakker, L., Gentleman, R., Huber, W., Liaw, A., Lumley, T., Maechler, M., Magnusson, A., Moeller, S., Schwartz, M., Venables, B., 2020. gplots: various R programming tools for plotting data. URL https://CRAN.R-project.org/package=gplots.
Weimann, A., Mooren, K., Frank, J., Pope, P.B., Bremges, A., McHardy, A.C., Segata, N., 2016. From genomes to phenotypes: Traitar, the microbial trait analyzer. MSystems 1 (6). https://doi.org/10.1128/mSystems.00101-16.
Weinbauer, M.G., Höfle, M.G., 1998. Size-specific mortality of lake bacterioplankton by natural virus communities. Aquat. Microb. Ecol. 15 (2), 103–113.
Westoby, M., Wright, I.J., 2006. Land-plant ecology on the basis of functional traits. Trends Ecol. Evol. 21 (5), 261–268.
Wood, J.L., Tang, C., Franks, A.E., 2018. Competitive traits are more important than stress-tolerance traits in a cadmium-contaminated rhizosphere: a role for trait theory in microbial ecology. Front. Microbiol. 9, 1–12.
Yilmaz, P., Yarza, P., Rapp, J.Z., Glöckner, F.O., 2016. Expanding the world of marine bacterial and archaeal clades. Front. Microbiol. 6, 1524.
Young, K.D., 2006. The selective value of bacterial shape. Microbiol. Mol. Biol. Rev. 70 (3), 660–703.
Zanne, A.E., Abarenkov, K., Afkhami, M.E., Aguilar-Trigueros, C.A., Bates, S., Bhatnagar, J.M., Busby, P.E., Christian, N., Cornwell, W.K., Crowther, T.W., Flores-Moreno, H., Floudas, D., Gazis, R., Hibbett, D., Kennedy, P., Lindner, D.L., Maynard, D.S., Milo, A.M., Nilsson, R.H., Powell, J., Schildhauer, M., Schilling, J., Treseder, K.K., 2020. Fungal functional ecology: bringing a trait-based approach to plant-associated fungi. Biol. Rev. 95 (2), 409–433.
Zhang, Q.-C., Wang, G.-H., Yao, H.-Y., 2007. Phospholipid fatty acid patterns of microbial communities in paddy soil under different fertilizer treatments. J. Environ. Sci. 19 (1), 55–59.