

1 **TerrestrialMetagenomeDB: a public repository of curated and** 2 **standardized metadata for terrestrial metagenomes**

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11

12 **ABSTRACT**

13 Microbiome studies focused on the genetic potential of microbial communities (metagenomics)
14 became standard within microbial ecology. MG-RAST and the Sequence Read Archive (SRA), the
15 two main metagenome repositories, contain over 202 858 public available metagenomes and this
16 number has increased exponentially. However, mining databases can be challenging due to
17 misannotated, misleading and decentralized data. The main goal of TerrestrialMetagenomeDB is to
18 make it easier for scientists to find terrestrial metagenomes of interest that could be compared with
19 novel datasets in meta-analyses. We defined terrestrial metagenomes as those that do not belong to
20 marine environments. Further, we curated the database using text mining to assign potential
21 descriptive keywords that better contextualize environmental aspects of terrestrial metagenomes,
22 such as biomes and materials. TerrestrialMetagenomeDB release 1.0 includes 15 194 terrestrial
23 metagenomes from SRA and MG-RAST. Together, the downloadable data amounts to 68 Tbp. In
24 total, 199 terrestrial terms were divided into 14 categories. These metagenomes span 84 countries,
25 31 biomes and 7 main source materials. The TerrestrialMetagenomeDB is publicly available at
26 <https://webapp.ufz.de/tmdb>.

27 **INTRODUCTION**

28 A metagenome, in microbiome research, encompasses the genetic potential of a microbial community
29 obtained through shotgun sequencing of DNA extracted from a sample (1). A few databases provide
30 permanent storage and public access to DNA sequencing. The major database with these
31 characteristics is the Sequencing Read Archive (SRA) (2). SRA is part of the International Nucleotide
32 Sequence Database Collaboration (3) along with the European Nucleotide Archive (4) and the DNA
33 Data Bank of Japan (5). Another important repository is MG-RAST (6), which also provides analysis
34 services. Due to crescent availability of metagenomes in public databases, scientists are able to
35 revisit publicly available data and to answer new hypothesis and research questions by applying
36 recent or novel bioinformatic techniques. Reanalysis of public available data may lead to novel
37 discoveries and insights, especially when data analyses of multiple studies are combined. For
38 example, a study by Parks and collaborators (7) substantially expands the tree of life with the

39 recovery of nearly 8 000 assembled genomes from 1 550 metagenomes and a meta-analysis study
40 with 9 428 metagenomes defines the core species inhabiting the human gut (8). However, mining
41 metagenomes of interest from databases is often not an easy task. For instance, to retrieve
42 metagenomes from SRA can be very laborious since submitters often mislabel their datasets, making
43 it impossible to distinguish metagenomes from amplicon sequencing data (9). The first initiative to
44 suggest standards for metagenome submission was made in 2008 by the Genomic Standards
45 Consortium (10). Soon after, these standards were implemented by MG-RAST (11). In 2011, SRA
46 integrated BioProject and BioSample in their database (12), so that any submitted metagenomic
47 sample must include the minimum information about a metagenome (13). To date, no studies have
48 been published regarding the number of mislabelled or when no data was added, particularly
49 regarding sample-related attributes. More recently, specialists started to improve and to curate
50 standards within their fields. For example, Bernstein and collaborators (14) and Pasoli and
51 collaborators (15) curated and standardized data from human-specific samples deposited in SRA.
52 Nevertheless, prior TerrestrialMetagenomeDB, a resource focused on metagenomes obtained from
53 terrestrial environments was not available. Here, we define terrestrial metagenomes as any
54 environmental metagenome that belongs to terrestrial biomes as defined by Buttigieg and
55 collaborators (i.e., ENVO:00000446) (16). In 2018, a resource called MGnify, former EBI-
56 Metagenomics (17), was introduced (<https://www.ebi.ac.uk/metagenomics/>). Among its functionalities,
57 MGnify can be used for the discovery of metagenomes from SRA. However, only for metagenomes
58 that were analysed by this platform.

59 We created the TerrestrialMetagenomeDB, the first metadata database focused on terrestrial
60 metagenomes, to help scientists researching terrestrial environments find metagenomes of interest
61 that could be compared with novel datasets in meta-analysis studies. Our database consists of
62 metadata related to biological samples and metadata describing technical aspects of the sequencing
63 data. While the sample metadata supports biological questions in meta-analysis, the sequencing
64 metadata can be crucial for bioinformatics. TerrestrialMetagenomeDB is not meant to replace recent
65 efforts of BioSamples database to standardize and curate data (18), but to promote the exploratory
66 possibilities of terrestrial metagenomes in a user-friendly interface, and to encourage comparison of
67 public available data. Our resource combines the two current main databases (SRA and MG-RAST)
68 and provides manually curated metadata that could be useful in meta-analysis studies.

69 **MATERIAL AND METHODS**

70 **Database construction**

71 The TerrestrialMetagenomeDB was constructed as follows. Briefly, we retrieved metadata of
72 metagenomes from the source databases and parsed and standardized sample attributes. Next, we
73 identified terrestrial metagenomes and removed marine samples. Finally, we combined the metadata
74 of terrestrial metagenomes of SRA and MG-RAST, removed potentially entries that were non-
75 metagenomes (targeted approaches and genome sequencing) and did not belong to terrestrial
76 biomes and implemented the web application. A summarized graphic representation of the
77 construction and availability of the TerrestrialMetagenomeDB is depicted in Figure 1.

78 *Data retrieval.* We retrieved the data for TerrestrialMetagenomeDB from SRA and MG-RAST as they
79 are the largest repositories of publicly available metagenomes. For SRA, we selected identifiers of
80 libraries (SRA Runs) assigned as metagenomes by PARTIE (9), a tool that periodically checks if
81 libraries are correctly annotated as metagenomes. The complete list of identifiers is available at
82 PARTIE's Github (<https://github.com/linsalrob/partie>) as 'SRA_Metagenome_Types.tsv'. After, we
83 retrieved the respective metadata using the SRADB R package (19), which provides local access to all
84 metadata entries from SRA. Additionally, we retrieved quality scores of the sequences with the tool
85 SRA-Tinder (https://github.com/NCBI-Hackathons/SRA_Tinder). We also retrieved the date of
86 creation of the libraries using Entrez Direct (<https://www.ncbi.nlm.nih.gov/books/NBK179288>),
87 because these dates are not available through SRADB. To avoid potential non-WGS datasets we
88 filtered out entries where 'library_selection' was filled with 'PCR' or 'library_strategy' was filled
89 "AMPLICON". For MG-RAST, all entries were requested to its application program interface (API). To
90 select only metagenomes at MG-RAST, we filtered the entries' metadata where values of
91 'investigation_type' and 'seq_meth' were equal to 'metagenome' and 'WGS' for whole genome
92 sequencing.

93 *Standardization of attributes.* In SRADB, all the sample attributes are available in a single field and the
94 attribute names are written in many different ways. For these reasons, we standardized synonyms
95 and screened attribute names and their respective values. For SRA, we standardized nine different
96 attributes: sample latitude, sample longitude, sample depth, sample elevation, sample altitude,
97 sample temperature, sample pH, sample location and sample collection date. Those attributes were
98 parsed from the SRADB field named 'sample_attribute'. We removed parsed attributes with less than
99 10 occurrences. Further, we grouped the remaining attributes by synonyms (Supplementary Table
100 S1). Coordinates were standardized to the format of Decimal Degrees (round to 6 decimal digits,
101 resolving up to 0.11 m). Dates were standardized to the international standard according to ISO 8601
102 (YYYY-MM-DD) and dates that were not between 1950 and the current year were marked as "NA".
103 Countries where the samples were collected were manually labelled according to country names of
104 standard ISO 3166-1. For MG-RAST, the equivalent attributes were already available (except sample
105 pH) from the API retrieval and, when necessary, they were adapted to the above mentioned formats.
106 Additionally, we distinguished between datasets containing assemblies and those containing
107 sequencing reads by adding an attribute named "average_length" (basepairs count / sequences
108 count) per metagenome. From the average length, we inferred assembled Illumina and 454 datasets
109 and added annotation in the attribute named "assembled". We annotated as "Yes" (i.e., assembled
110 data) when the average length was greater than 600 bp.

111 *Identification of terrestrial metagenomes.* A set of words of 'Environmental Material' and 'Terrestrial
112 Environment Biome' was adapted from The Environment Ontology (ENVO) (16). To select the most
113 relevant words, every word was queried against the collected metadata, and the relevant words were
114 grouped. After, we added the prefix 'TMDB' to tag our terrestrial groups (Supplementary Table S2).
115 Further, we queried the words in the complete metadata and assigned them to each terrestrial group.

116 Metagenomes were classified as terrestrial when at least one terrestrial word was present in the
117 metadata.

118 *Removal of marine samples.* We used two different approaches to remove marine samples. These
119 different approaches were based on the presence or absence of coordinates for each metagenome.
120 For metagenomes with coordinates, we removed entries with coordinates outside land boundaries
121 (i.e., in the sea). To that end we used 'is-sea' (<https://github.com/simonepri/is-sea>). For metagenomes
122 without coordinates available, we searched in the metadata for terms that indicated the given
123 metagenomes are potentially from the marine environment. The terms were respectively 'sea',
124 'marine' and 'ocean'.

125 *Combining SRA and MG-RAST metadata.* We selected a collection of equivalent and comparable
126 attributes present in both databases and combined those into the metadata found in the
127 TerrestrialMetagenomeDB (Supplementary Table S3). Only three attributes related to library
128 sequencing quality scores were unique and specific to SRA or MG-RAST; respectively,
129 'quality_above_30_SRA', 'mean_quality_SRA', and 'drisee_score_raw_MGRAST'.

130 *Removal of non-terrestrial and non-metagenomic datasets.* From the final combined metadata, we
131 filtered out datasets related to human-derived samples by searching for terms like "human" and
132 "homo-sapiens". Likewise, for filtering out non-metagenomic datasets we used keywords related to
133 amplicon sequencing, genome sequencing and genome assembly. A list with the regular expressions
134 used to perform the filtering is listed in the Supplementary Table S4.

135 *Web app implementation.* TerrestrialMetagenomeDB web-interface was implemented using Shiny
136 (version 1.3.2) for R (version 3.4.2). The map in the 'Interactive map' tab was added using the *leaflet*
137 package (version 2.0.2), and the selection toolbox was created with the *leaflet.extras* package
138 (version 1.0.0). The function for selecting points on the map was built using the *geoshaper* package
139 (version 0.1.0) and the *sp* package (version 1.3-1). The 'Interactive map' and 'Complete dataset' data
140 tables were set using the *DT* package (version 0.7). The 'Help' and 'Contact' R markdown texts were
141 created with the *markdown* package (version 1.0) and the *knitr* package (version 1.23). The modal
142 that opens with the 'Interactive map' was generated with the package *shinyBS* (version 0.61). The
143 mouse over tooltips were generated with the package *shinyBS* and the numeric range inputs were
144 implemented with the package *shinyWidgets* (version 0.4.8).

145 **RESULTS**

146 **Database content**

147 The current manuscript describes the TerrestrialMetagenomeDB release 1.0, where 15 194
148 metagenomes from the terrestrial environment are available. Those cover 11 years of experiments,
149 since the first terrestrial metagenome was submitted to the SRA on May 2008 and the latest
150 registered at MR-RAST on May 2019. Among those, 6 845 metagenomes are derived from SRA
151 (45%) and 8 349 from MG-RAST (55%). Since SRA and MG-RAST are independent from each other,

152 identical datasets can exist in both databases. In addition, metadata provided by submitters was
153 insufficient to determine which datasets overlap. According to the location where the samples were
154 collected, metagenomes span all 7 continents and 84 countries. Most of the libraries available were
155 sequenced with Illumina sequencing technologies (86%), followed by LS454 (6%), Ion Torrent (2%)
156 and others (6%) (Figure 2).

157 Regarding the quality of the library reads, 99% of the SRA metagenomes have quality scores. For
158 MG-RAST metagenomes, another measurement of quality named DRISSE (13) is available, where
159 67% of the metagenomes have this attribute annotated.

160 *Terrestrial metagenomes.* The most populated 'TMDB terrestrial attributes' were 'TMDB material' with
161 76% of present values followed by 'TMDB biome' with 41% of values annotated with our pipeline. The
162 top terms in 'TMDB material' were soil (6209), water (2655), sediment (1561), sludge (1244) and
163 organic material (574). The top terms in 'TMDB biome' were urban (1881), forest (1623), grassland
164 (1039), temperate grassland (475) and shrubland (319). Many terrestrial terms identified in the
165 metadata co-occurred for the same metagenome, for example: the 'TMDB Biome' terms identified in
166 the metagenomic library 'mgm4819186.3' were 'forest' and 'urban'. The frequency and co-occurrence
167 of terrestrial terms of 'TMDB biome' and 'TMDB material' present in the database can be visualized in
168 Figure 2 A-B. The other 12 terrestrial categories defined in this work appeared in a lower frequency.
169 From those, the top populated attributes were 'TMDB organic material' (12%) and 'TMDB soil location'
170 (10%), and all the others were available in less than 5% of the metagenomes metadata.
171 Supplementary Figure S1 depicts the percentage of missing values per attribute in the current TMDB
172 data.

173 **Usage and functionalities**

174 The TerrestrialMetagenomeDB user interface is divided in two main sections so users can choose the
175 section that better fits their needs. In summary, the first section "Complete dataset" holds the full
176 content of the databases' current version. On the other hand, the "Interactive map" section provides a
177 more intuitive way of selecting metagenomes directly from the world map, although being limited by
178 the metagenomes with a pair of valid geographic coordinates available. To provide practical
179 examples, we made 3 video tutorials about the usage of the web application. A link to the tutorials can
180 be found in the item 1 of the "Help" tab in the web application.

181 *Complete dataset.* The 'Complete dataset' tab contains the sum of all entries with and without
182 coordinates (15 194 in total). In this tab the initial data table is displayed with all the entries, what
183 allows filtering and searching in the complete database. For filtering, a set of 6 filters is placed on top
184 of the datatable for the most important attributes. By pushing the button "More filters", the filters
185 dashboard is expanded downwards to show all 33 filters. A panel is fixed on the bottom displaying the
186 current number of filtered metagenomes, so users can keep track of how each filtering step is shaping
187 the data. If no filter is applied, the whole dataset (the full TerrestrialMetagenomeDB data) can be
188 downloaded as a CSV-file.

189 *Interactive map.* The 'Interactive map' tab allows users to interactively explore the world map and
190 select metagenomes from all around the globe (Figure 3). Two drawing tools (rectangle and polygon)
191 are available and allow easy selection of plotted points in the map by marking everything that is inside
192 the selected boundaries. Individual points in the map may indicate several samples collected in the
193 same coordinates. Therefore, we opted to only allow the selection of samples by using the drawing
194 tool. This selection step can be performed multiple times in various regions of the map or re-started
195 by clearing the drawn selection layers. Once the selection step is finished, all the marked points are
196 displayed in the interactive data table below the map. This tab has exactly the same functionalities as
197 described above, but without the filter for geographical coordinates, since this can be done directly on
198 the map. Also, all 31 filters are hidden and can be shown by pushing the "Show filters" button. The
199 columns 'library id', 'project id' and 'sample id' (when valid) are hyperlinked to the original source
200 databases. A search box on the top-right of the data table allows the search for any text inside the
201 selected metagenomes metadata. Reactively, the selected points in the map will be redrawn
202 according to any filtering. The selected metadata (filtered or not) can be downloaded as a CSV-file.

203 *Downloading metagenomes of interest.* TerrestrialMetagenomeDB is not a repository of DNA
204 sequence data. Once metagenomes are selected, they have to be downloaded from their respective
205 repositories. A short guide on how to download the actual sequencing data from the original
206 repositories can be found in the database user interfaces' tab named 'Help'. To facilitate the
207 download, we provided a script called "tmdb_downloader.py" that takes as input the downloaded
208 CSV-file from our database. Also, a video tutorial on how to use the script is available in the tutorials
209 playlist.

210 *Suggestion for good practices.* To help scientists analyse their first metagenomes, a guide for 'good
211 practices' when preparing the metadata for metagenomic studies can be found in the database user
212 interfaces' tab named 'Help', at the item 6 'What should I do to include my metagenomes in TMDB?'.
213

213 **CONCLUSION**

214 TerrestrialMetagenomeDB is the first database to centralize and standardize metadata present at the
215 Sequence Read Archive and MG-RAST for terrestrial metagenomes. We arranged terrestrial terms
216 derived from the environment ontology ENVO with the help of scientists from different fields of
217 terrestrial research and identified those terms both in MG-RAST and SRA.

218 TerrestrialMetagenomeDB is in its release 1.0 and it will get two updates per year, due to the
219 exponential number of novel metagenomes added to public repositories. We believe that our
220 database improves the current necessity of adequately described metadata (or contextual data) that
221 will make possible querying and interpretation across projects and meta-analyses.

222

223 **AVAILABILITY**

224 The TerrestrialMetagenomeDB is available at <https://webapp.ufz.de/tmdb>.

225

226 **ACKNOWLEDGEMENT**

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231

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235

236 **CONFLICT OF INTEREST**

237 None declared.

238

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- 296

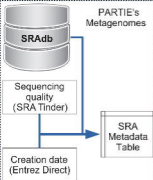
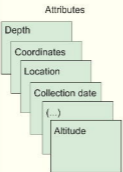
297 **FIGURES LEGENDS**

298

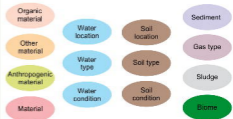
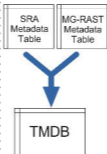
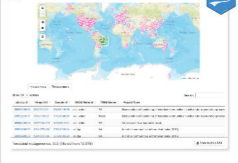
299 Figure 1. Overview of the TerrestrialMetagenomeDB (TMDB) construction and availability. The
300 construction of TMDB comprises: (A) Metadata retrieval for metagenomes present in SRA and MG-
301 RAST; (B) Standardization of attributes; (C) Identification of terrestrial metagenomes; and (D) merging
302 of SRA and MG-RAST metadata. (E) The TMDB was made available through a user friendly Shiny
303 web application.

304 Figure 2. Descriptive statistics of the TerrestrialMetagenomeDB content. (A) Network representation
305 of the frequencies of 'biome'-related terms in the database (polygon shape). The frequencies of pairs
306 of 'biome' terms found in the database are represented by coloured arrows. (B) Network
307 representation of the frequencies of 'material'-related terms in the database (ellipse shape). The
308 frequencies of pairs of 'material' terms found in the database are represented by coloured arrows. (C)
309 Bar plot showing the distribution of the country of origin of the metagenomic samples (Sample
310 location), in this plot the not assigned values (NA's) were omitted. (D) Bar plot of the distribution of
311 sequencing technologies (Sequencing platform) per database of origin (Source database).

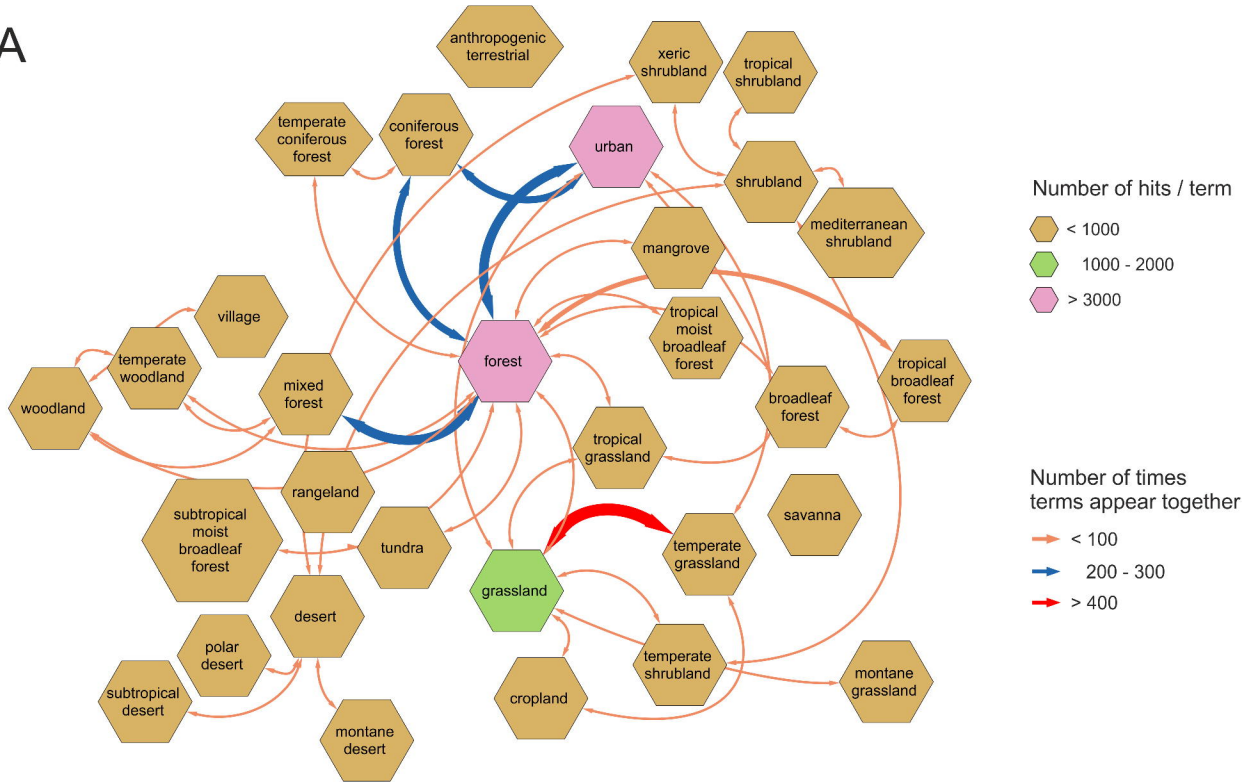
312 Figure 3. Overview of the TerrestrialMetagenomeDB user-interface. (A) Metagenomes can be
313 selected in the 'Interactive Map' using a selection tool. (B) Metadata related to the selected entries is
314 shown in the data table and can be further filtered and exported. For illustrative purposes, only the set
315 of "Quick filters" is depicted.

A Metadata retrievalAPI Call
Metagenomes**B** Standardization**C** Terrestrial metagenomes identification

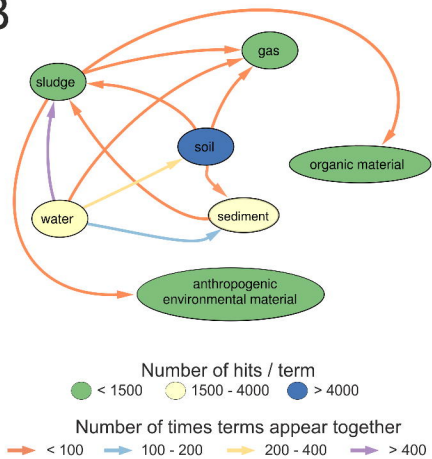
Collection of terms subdivided in 14 groups

**D** Merging datasets**E** Web application

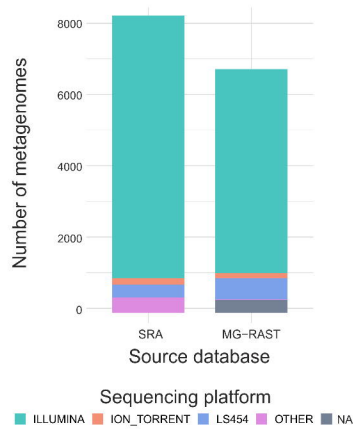
A



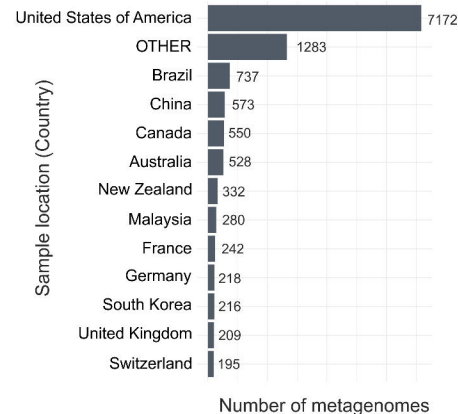
B

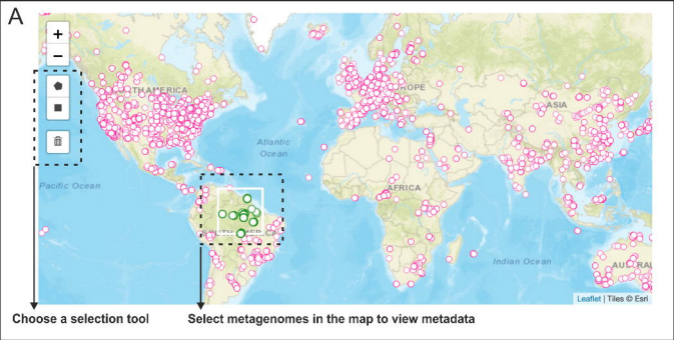


C



D





B

Quick filters

<p>Source Database</p> <p>Nothing selected ▾</p>	<p>TMDB Material</p> <p>Nothing selected ▾</p>	<p>Number of sequences (millions)</p> <p>0 ▾ to 1210 ▾</p>
<p>Sequencing Platform</p> <p>Nothing selected ▾</p>	<p>TMDB Biome</p> <p>Nothing selected ▾</p>	<p>Assembled</p> <p>Nothing selected ▾</p>

⤴ Hide filters ⤴ Reset filters

Filter by attributes

Show **10** entries

Library ID	Project ID	Sample ID	TMDB Material	TMDB Biome	Project Name
SRR2125895	SRX1117184	SRS1014699	soil, water	NA	Biodiversity and functioning of microbial communities in soils under regeneration process from a
SRR2163617	SRX1150037	SRS1034628	soil, water	forest	Biodiversity and functioning of microbial communities in soils under regeneration process from a
SRR2885876	SRX1406314	SRS1143130	soil, water	NA	Cerrado soil Raw sequence reads
SRR3498952	SRX1757435	SRS1433624	sludge	NA	Microbial communities in bromeliad tanks (2015)
SRR3498953	SRX1757436	SRS1433623	sludge	NA	Microbial communities in bromeliad tanks (2015)
SRR3498958	SRX1757441	SRS1433629	sludge	NA	Microbial communities in bromeliad tanks (2015)

Showing 1 to 10 of 553 entries

Previous 1 2 3 4 5 ... 56 Next

Drag the horizontal scroll bar to view all the columns.

Search:

Access the original data source

Search by text

Terrestrial metagenomes: 553 (filtered from 13,378)

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Track how filters shape the selected metadata

Export the selected content as a CSV-file