



## Metagenomes from Eastern Brazilian Amazonian Floodplains in the Wet and Dry Seasons

[Andressa M. Venturini,](https://orcid.org/0000-0002-5901-1658)<sup>a,b</sup> Júlia B. Gontijo,<sup>a</sup> Aline G. da França,<sup>a</sup> José M. S. Moura,<sup>c</sup> ©[Klaus Nüsslein,](https://orcid.org/0000-0002-0663-4448)<sup>d</sup> Brendan J. M. Bohannan,<sup>e</sup> (D[Jorge L. M. Rodrigues](https://orcid.org/0000-0002-6446-6462),<sup>f</sup> Siu M. Tsai<sup>a</sup>

a Cell and Molecular Biology Laboratory, Center for Nuclear Energy in Agriculture, University of São Paulo, Piracicaba, SP, Brazil bPrinceton Institute for International and Regional Studies, Princeton University, Princeton, New Jersey, USA cCenter for Interdisciplinary Formation, Federal University of Western Pará, Santarém, PA, Brazil dDepartment of Microbiology, University of Massachusetts, Amherst, Massachusetts, USA eInstitute of Ecology and Evolution, University of Oregon, Eugene, Oregon, USA f Department of Land, Air, and Water Resources, University of California - Davis, Davis, California, USA

Andressa M. Venturini and Júlia B. Gontijo contributed equally to this work. The author order was determined alphabetically by first name.

ABSTRACT Here, we report the metagenomes from two Amazonian floodplain sediments in eastern Brazil. Tropical wetlands are well known for their role in the global carbon cycle. Microbial information on this diversified and dynamic landscape will provide further insights into its significance in regional and global biogeochemical cycles.

Floodplains and wetlands constitute 14% of the total area of the Amazon basin ([1](#page-2-0)) and are considered the largest natural geographic source of methane (CH4) in the tropics [\(2\)](#page-3-0). Therefore, several studies have investigated the  $CH<sub>4</sub>$ -producing and -consuming microbial communities in these sediments and their responses to a range of environmental factors using 16S rRNA amplicon sequencing [\(3](#page-3-1)[–](#page-3-2)[5](#page-3-3)). However, their overall microbial taxonomic and functional diversity remains little explored. Here, we report 12 metagenomes from two Amazonian floodplains in the wet and dry seasons.

The samplings were carried out in two floodplains in the State of Pará, Brazil, namely, one located at the Amazon River (FP2, "Maicá", 2°28'11.2"S 54°38'49.9"W) and the other at the intersection between the Amazon and the Tapajós rivers (FP3, "Açu",  $2^{\circ}22'44.8''5$  54° 44'21.1"W). The Amazon and Tapajós are considered whitewater and clearwater rivers, respectively, according to Junk et al. [\(6](#page-3-4)). Sediment samples from a depth of 0 to 10 cm were collected using a corer (5-cm diameter by 10-cm depth) at both sites in the wet and dry seasons (May and October 2016, respectively) in triplicate, totaling 12 samples, and homogenized thoroughly. Total DNA was extracted in duplicate from 0.25 g of sediment using the PowerLyzer PowerSoil DNA Isolation Kit (Qiagen, Hilden, Germany), following an optimized protocol for Amazonian sediments [\(7](#page-3-5)). Metagenomic libraries were constructed using the NEBNext Ultra II DNA Library Prep Kit for Illumina (New England BioLabs, Inc., Ipswich, MA) and pairedend sequenced (2  $\times$  150 bp) on an Illumina HiSeq 2500 instrument (Illumina, Inc., San Diego, CA) at Novogene Co., Ltd. (Beijing, China). Detailed information about the study sites, sampling, sediment physicochemical properties, and DNA extraction and quantification have been described previously ([5\)](#page-3-3).

Metagenomic reads were imported into the KBase platform [\(8\)](#page-3-6), and default parameters were used for all software unless otherwise specified. Reads were evaluated using FastQC v0.11.9 [\(9](#page-3-7)), trimmed and filtered using Trimmomatic v0.36 (adapters, TruSeq3-PE-2; seed mismatches, 5; sliding window size, 5; sliding window minimum quality, 20; head crop length, 10; leading minimum quality, 20; trailing minimum quality, 20; minimum read length, 70) [\(10\)](#page-3-8), and again evaluated using FastQC v0.11.9 ([9\)](#page-3-7). Overlapping paired-end reads were joined with FASTQ-JOIN v2.0.2 [\(8](#page-3-6), [11](#page-3-9)) and taxonomically classified using Kaiju v1.7.3 (taxonomic level, Editor J. Cameron Thrash, University of Southern California

Copyright © 2022 Venturini et al. This is an open-access article distributed under the terms of the [Creative Commons Attribution 4.0](https://creativecommons.org/licenses/by/4.0/) [International license](https://creativecommons.org/licenses/by/4.0/).

Address correspondence to Andressa M. Venturini, andressa.venturini@alumni.usp.br.

The authors declare no conflict of interest.

Received 23 May 2022 Accepted 26 June 2022 Published 19 July 2022

<span id="page-1-0"></span>

TABLE 1 Results of 12 metagenomic samples

TABLE 1 Results of 12 metagenomic samples



<span id="page-2-1"></span>FIG 1 Taxonomic classification of the sequence reads at the phylum level. (A) Most abundant bacterial phyla (mean relative abundance of > 1% across samples). (B) Archaeal phyla. (C) Fungal phyla. Relative abundance calculated based on the classified reads. WS, wet season; DS, dry season.

phylum/class; reference database, NCBI BLAST nr+euk; low abundance filter, 0.01%; subsample percent, 100%) [\(12](#page-3-10)). The results were plotted using ggplot2 3.3.5 [\(13](#page-3-11)) in R 4.1.2 [\(14](#page-3-12)).

The metagenomic samples had between 22 and 31 million 150-bp long paired-end reads [\(Table 1\)](#page-1-0). After quality control, between 19 and 29 million paired-end reads remained, ranging from 70 to 140 bp. The joining of the overlapping paired-end reads resulted in samples with between 9 and 15 million reads, ranging from 76 to 274 bp. A considerable part of the reads (mean of 42% across samples) was not classified. Most of the classified reads were assigned to Bacteria, but also Archaea, Fungi, and viruses [\(Fig. 1](#page-2-1)). The most dominant phyla (mean relative abundance of  $> 10\%$  across samples), among the 90 microbial phyla found, were Proteobacteria, Actinobacteria, and Acidobacteria.

Data availability. The raw metagenomic sequences are available in the NCBI Sequence Read Archive (SRA) under the umbrella project [PRJNA782633.](https://www.ncbi.nlm.nih.gov/sra/PRJNA782633) The raw sequences, apps, and all the outputs of the analyses described here are also available on the KBase platform at [https://www.doi.org/10.25982/113717.182/1864845.](https://www.doi.org/10.25982/113717.182/1864845)

## ACKNOWLEDGMENTS

This work was supported by the São Paulo Research Foundation (FAPESP; grant numbers 2014/50320-4, 2015/19979-2, 2018/14974-0, 2019/25924-7, and 2019/25931-3), the National Council for Scientific and Technological Development (CNPq; grant numbers 133769/2015-1, 311008/2016-0, and 314806/2021-0), the Coordination for the Improvement of Higher Education Personnel - Brasil (CAPES) - Finance Code 001, and the National Science Foundation - Dimensions of Biodiversity (DEB 1442214). A.M.V.'s research is currently funded by the Fung Global Fellows Program of the Princeton Institute for International and Regional Studies (PIIRS; Princeton University). This publication was supported by the Princeton University Library Open Access Fund.

We thank Wagner Piccinini for the assistance in the field and the Large-Scale Biosphere-Atmosphere Program (LBA), coordinated by the National Institute for Amazon Researchers (INPA), for the logistical support and infrastructure during field activities.

We declare no conflict of interest.

J.B.G. and S.M.T. designed the research with contributions from A.M.V., J.M.S.M., K.N., B.J.M.B., and J.L.M.R. J.B.G. collected the samples with A.M.V., J.M.S.M., and K.N. and conducted the molecular analyses with the help of A.M.V. and A.G.F. A.M.V. and J.B.G. analyzed the microbial data. S.M.T. contributed with field sampling logistics, reagents, materials, and analytic tools. A.M.V. wrote the article with the help of J.B.G. All authors critically revised the manuscript.

## **REFERENCES**

<span id="page-2-0"></span>1. Hess LL, Melack JM, Affonso AG, Barbosa C, Gastil-Buhl M, Novo EMLM. 2015. Wetlands of the lowland Amazon Basin: extent, vegetative cover, and dual-season inundated area as mapped with JERS-1 synthetic aperture radar. Wetlands 35:745–756. <https://doi.org/10.1007/s13157-015-0666-y>.

- <span id="page-3-0"></span>2. Pangala SR, Enrich-Prast A, Basso LS, Peixoto RB, Bastviken D, Hornibrook ERC, Gatti LV, Marotta H, Calazans LSB, Sakuragui CM, Bastos WR, Malm O, Gloor E, Miller JB, Gauci V. 2017. Large emissions from floodplain trees close the Amazon methane budget. Nature 552:230–234. <https://doi.org/10.1038/nature24639>.
- <span id="page-3-1"></span>3. Gabriel GVM, Oliveira LC, Barros DJ, Bento MS, Neu V, Toppa RH, Carmo JB, Navarrete AA. 2020. Methane emission suppression in flooded soil from Amazonia. Chemosphere 250:126263. [https://doi.org/10.1016/j.chemosphere](https://doi.org/10.1016/j.chemosphere.2020.126263) [.2020.126263](https://doi.org/10.1016/j.chemosphere.2020.126263).
- <span id="page-3-2"></span>4. Bento MS, Barros DJ, Araújo MGS, Da Róz R, Carvalho GA, do Carmo JB, Toppa RH, Neu V, Forsberg BR, Bodelier PLE, Tsai SM, Navarrete AA. 2021. Active methane processing microbes and the disproportionate role of NC10 phylum in methane mitigation in Amazonian floodplains. Biogeochemistry 156:293–317. [https://doi.org/10.1007/s10533-021-00846-z.](https://doi.org/10.1007/s10533-021-00846-z)
- <span id="page-3-3"></span>5. Gontijo JB, Paula FS, Venturini AM, Yoshiura CA, Borges CD, Moura JMS, Bohannan BJM, Nüsslein K, Rodrigues JLM, Tsai SM. 2021. Not just a methane source: Amazonian floodplain sediments harbour a high diversity of methanotrophs with different metabolic capabilities. Mol Ecol 30:2560–2572. [https://](https://doi.org/10.1111/mec.15912) [doi.org/10.1111/mec.15912.](https://doi.org/10.1111/mec.15912)
- <span id="page-3-4"></span>6. Junk WJ, Piedade MTF, Schöngart J, Cohn-Haft M, Adeney JM, Wittmann F. 2011. A classification of major naturally-occurring Amazonian lowland wetlands. Wetlands 31:623–640. <https://doi.org/10.1007/s13157-011-0190-7>.
- <span id="page-3-5"></span>7. Venturini AM, Nakamura FM, Gontijo JB, da França AG, Yoshiura CA, Mandro JA, Tsai SM. 2020. Robust DNA protocols for tropical soils. Heliyon 6:e03830. <https://doi.org/10.1016/j.heliyon.2020.e03830>.
- <span id="page-3-6"></span>8. Arkin AP, Cottingham RW, Henry CS, Harris NL, Stevens RL, Maslov S, Dehal P, Ware D, Perez F, Canon S, Sneddon MW, Henderson ML, Riehl WJ, Murphy-Olson D, Chan SY, Kamimura RT, Kumari S, Drake MM, Brettin TS, Glass EM, Chivian D, Gunter D, Weston DJ, Allen BH, Baumohl J, Best AA, Bowen B, Brenner SE, Bun CC, Chandonia J-M, Chia J-M, Colasanti R, Conrad N, Davis JJ, Davison BH, DeJongh M, Devoid S, Dietrich E, Dubchak I, Edirisinghe JN, Fang G, Faria JP, Frybarger PM, Gerlach W, Gerstein M, Greiner A, Gurtowski J, Haun HL, He F, Jain R, Joachimiak MP, Keegan KP, Kondo S, et al. 2018. KBase: the United States Department of Energy Systems Biology Knowledgebase. Nat Biotechnol 36:566–569. <https://doi.org/10.1038/nbt.4163>.
- <span id="page-3-7"></span>9. Andrews S. 2010. FastQC: a quality control tool for high throughput sequence data. [https://www.bioinformatics.babraham.ac.uk/projects/fastqc.](https://www.bioinformatics.babraham.ac.uk/projects/fastqc)
- <span id="page-3-8"></span>10. Bolger AM, Lohse M, Usadel B. 2014. Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics 30:2114–2120. [https://doi.org/10](https://doi.org/10.1093/bioinformatics/btu170) [.1093/bioinformatics/btu170](https://doi.org/10.1093/bioinformatics/btu170).
- <span id="page-3-9"></span>11. Aronesty E. 2011. ea-utils: Command-line tools for processing biological sequencing data. [https://github.com/ExpressionAnalysis/ea-utils.](https://github.com/ExpressionAnalysis/ea-utils)
- <span id="page-3-10"></span>12. Menzel P, Ng KL, Krogh A. 2016. Fast and sensitive taxonomic classification for metagenomics with Kaiju. Nat Commun 7:11257. [https://doi.org/](https://doi.org/10.1038/ncomms11257) [10.1038/ncomms11257.](https://doi.org/10.1038/ncomms11257)
- <span id="page-3-11"></span>13. Wickham H. 2016. ggplot2: elegant graphics for data analysis. 2nd ed. Springer-Verlag, New York, NY.
- <span id="page-3-12"></span>14. R Core Team. 2021. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. <https://www.R-project.org/>.