

# **Metagenomic insights for antimicrobial resistance surveillance in soils with different land uses in Brazil**

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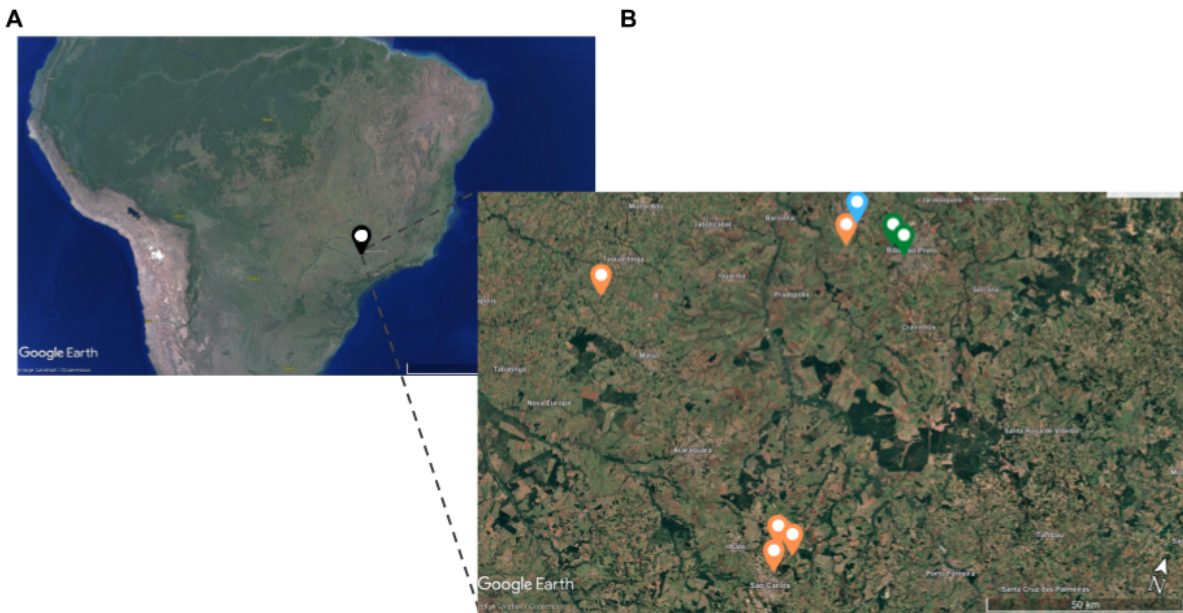
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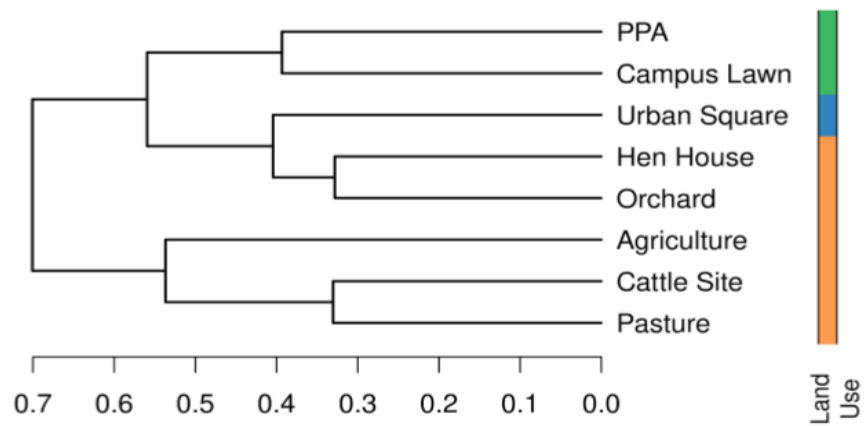
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## **Supporting Materials**



**Figure S1.** Map of soil sample locations. **(A)** Brazil map, with Ribeirão Preto marked in green. **(B)** Part of the São Paulo northeastern region, in which collection sites, along with their land use classification, are shown with green (forest), blue (urban) and orange (farming soils) markers. The map was taken using Google Maps and Google Earth Pro (<https://www.google.cl/maps>; accessed on 29 July 2022).



**Figure S2.** Soil sample clusterization based on microbial composition. The cladogram was constructed using Ward's algorithm for hierarchical clustering on a distance matrix obtained with Bray-Curtis dissimilarity method. Land use is indicated in orange (Farming), blue (Urban) and green (Forest).

**Table S1.** Shotgun sequencing - Illumina NovaSeq 6000- data quality summary.

<b>Sample ID*</b>	<b>Raw Reads</b>	<b>Raw data (G)</b>	<b>Effective (%)</b>	<b>Error (%)</b>	<b>Q20 (%)</b>	<b>Q30 (%)</b>	<b>GC (%)</b>
SOrch	117562592	17.6	99.87	0.03	97.36	92.70	62.79
SLawn	95499316	14.3	99.84	0.03	97.73	93.48	61.67
SPas	107762684	16.2	99.86	0.03	97.53	93.22	66.09
SUrbSq	120088348	18.0	99.84	0.03	97.57	93.23	65.32
SCatt	124484298	18.7	99.84	0.03	97.58	93.22	66.34
SHen	104840520	15.7	99.82	0.03	97.44	92.89	65.26
SPpa	83080300	12.5	99.82	0.02	98.04	94.51	63.29
SAgri	81025724	12.2	99.80	0.03	97.90	94.13	64.14

\* SOrch: Orchard; SLawn: Campus Lawn; SPas: Pasture; SUrbSq: Urban Square; SCatt: Cattle Site; SHen: Hen House; SPpa: Permanent Protected Area; SAgri: Agriculture.

**Table S2.** Relative abundances of most abundant genera (above 2.5%) identified in sequenced soils.

<b>Genus</b>	<b>Soil Sample</b>	<b>Read Count</b>	<b>Relative Abundance</b>
<i>Occallatibacter</i>	PPA	1667	0.056
<i>Pseudolabrys</i>	PPA	1483	0.050
<i>Rhodoplanes</i>	PPA	1302	0.044
<i>Edaphobacter</i>	PPA	1224	0.041
<i>Tumebacillus</i>	PPA	1152	0.039
<i>Terrimonas</i>	PPA	1083	0.037
<i>Paraburkholderia</i>	PPA	890	0.030
<i>Bradyrhizobium</i>	PPA	888	0.030
<i>Terriglobus</i>	PPA	881	0.030
<i>Vicinamibacter</i>	PPA	848	0.029
<i>Lysobacter</i>	PPA	764	0.026
<i>Terrimonas</i>	Campus Lawn	758	0.122
<i>Lysobacter</i>	Campus Lawn	406	0.066
<i>Pseudolabrys</i>	Campus Lawn	274	0.044
<i>Occallatibacter</i>	Campus Lawn	259	0.042
<i>Haliangium</i>	Campus Lawn	229	0.037
<i>Gemmatimonas</i>	Campus Lawn	216	0.035
<i>Rhodoplanes</i>	Campus Lawn	212	0.034
<i>Vicinamibacter</i>	Campus Lawn	207	0.033
<i>Chitinophaga</i>	Campus Lawn	192	0.031
<i>Flavisolibacter</i>	Urban Square	1684	0.048
<i>Massilia</i>	Urban Square	1260	0.036
<i>Gemmatimonas</i>	Urban Square	1157	0.033
<i>Haliangium</i>	Urban Square	1154	0.033
<i>Vicinamibacter</i>	Urban Square	1021	0.029
<i>Rhodoplanes</i>	Urban Square	914	0.026
<i>Bacillus</i>	Agriculture	335	0.312
<i>Staphylococcus</i>	Agriculture	62	0.058
<i>Rhodoplanes</i>	Agriculture	33	0.031

<i>Vicinamibacter</i>	Agriculture	29	0.027
<i>Tumebacillus</i>	Agriculture	28	0.026
<i>Bacillus</i>	Hen House	1833	0.044
<i>Massilia</i>	Hen House	1808	0.044
<i>Vicinamibacter</i>	Hen House	1352	0.033
<i>Fimbriiglobus</i>	Hen House	1260	0.030
<i>Occallatibacter</i>	Hen House	1074	0.026
<i>Bacillus</i>	Pasture	7529	0.177
<i>Vicinamibacter</i>	Pasture	2643	0.062
<i>Rhodoplanes</i>	Pasture	1618	0.038
<i>Fimbriiglobus</i>	Pasture	1288	0.030
<i>Gaiella</i>	Pasture	1119	0.026
<i>Bacillus</i>	Cattle Site	3017	0.118
<i>Vicinamibacter</i>	Cattle Site	1304	0.051
<i>Gaiella</i>	Cattle Site	907	0.035
<i>Rhodoplanes</i>	Cattle Site	805	0.031
<i>Occallatibacter</i>	Cattle Site	701	0.027
<i>Paludibaculum</i>	Cattle Site	653	0.025
<i>Fimbriiglobus</i>	Cattle Site	648	0.025
<i>Fimbriiglobus</i>	Orchard	996	0.051
<i>Occallatibacter</i>	Orchard	949	0.048
<i>Rhodoplanes</i>	Orchard	666	0.034
<i>Bryobacter</i>	Orchard	614	0.031
<i>Paludibaculum</i>	Orchard	612	0.031
<i>Flavisolibacter</i>	Orchard	553	0.028
<i>Terrimonas</i>	Orchard	522	0.027
<i>Terriglobus</i>	Orchard	520	0.026
<i>Edaphobacter</i>	Orchard	503	0.026

**Table S3.** Alpha diversity indexes of sampled soils.

<b>Soil</b>	<b>Shannon Index</b>	<b>Simpson Index</b>
Hen House	5.087	0.9867
Urban Square	5.056	0.9866
Orchard	4.859	0.9835
Cattle Site	4.791	0.9746
Pasture	4.543	0.9570
PPA	4.504	0.9783
Campus Lawn	4.343	0.9670
Agriculture	3.781	0.8932

**Table S4.** Data availability. The trimmed sequences, along with their quality scores, resulting from shotgun sequencing are publicly available under the BioProject PRJNA900430.

<b>BioSample Accession</b>	<b>Sample Name/SPUID</b>	<b>Organism</b>	<b>Tax ID</b>	<b>BioProject</b>	<b>SRA Accession</b>
SAMN31691838	SHen	soil metagenome	410658	PRJNA900430	SRR22278232
SAMN31691839	SCatt	soil metagenome	410658	PRJNA900430	SRR22278231
SAMN31691840	SLawn	soil metagenome	410658	PRJNA900430	SRR22278230
SAMN31691841	SURbSq	soil metagenome	410658	PRJNA900430	SRR22278229
SAMN31691842	SPast	soil metagenome	410658	PRJNA900430	SRR22278228
SAMN31691843	SOrch	soil metagenome	410658	PRJNA900430	SRR22278227
SAMN31691844	SAgri	soil metagenome	410658	PRJNA900430	SRR22278226
SAMN31691845	SPpa	soil metagenome	410658	PRJNA900430	SRR22278225

**Table S5.** Summary of the identified ARGs by CARD in sequenced metagenomes of soils under different land use systems.

<b>ARG</b>	<b>Count</b>	<b>Resistance</b>	<b>Soil</b>	<b>Land Use</b>
<i>vanRO</i>	15	Glycopeptide	Campus Lawn	Forest
<i>rbpA</i>	2	Rifamycin	Campus Lawn	Forest
<i>mtrA</i>	2	Macrolide;Penam	Campus Lawn	Forest
<i>vanRO</i>	13	Glycopeptide	PPA	Forest
<i>dfrB7_1</i>	1	Trimethoprim	PPA	Forest
<i>dfrB3</i>	1	Trimethoprim	PPA	Forest
<i>rbpA</i>	2	Rifamycin	PPA	Forest
<b>Total Forest</b>	<b>36</b>			
<i>cpt</i>	1	Phenicol	Urban Square	Urban
<i>aac2-Ib</i>	1	Aminoglycoside	Urban Square	Urban
<i>blaF</i>	1	Beta-lactamase	Urban Square	Urban
<i>vanRO</i>	26	Glycopeptide	Urban Square	Urban
<i>rbpA</i>	5	Rifamycin	Urban Square	Urban
<i>mtrA</i>	3	Macrolide;Penam	Urban Square	Urban
<b>Total Urban</b>	<b>37</b>			
<i>mtrA</i>	5	Macrolide;Penam	Hen House	Farming
<i>rbpA</i>	11	Rifamycin	Hen House	Farming
<i>vanRO</i>	17	Glycopeptide	Hen House	Farming
<i>efpA</i>	1	Isoniazid;Rifamycin	Hen House	Farming
<b>Total</b>	<b>34</b>			
<i>blaLRA-9</i>	1	Beta-lactamase	Orchard	Farming
<i>blaBJP-1</i>	1	Beta-lactamase	Orchard	Farming
<i>vanRO</i>	15	Glycopeptide	Orchard	Farming
<i>rbpA</i>	4	Rifamycin	Orchard	Farming
<i>mtrA</i>	1	Macrolide;Penam	Orchard	Farming



<i>vanSO</i>	1	Glycopeptide	Orchard	Farming
<b>Total</b>	<b>23</b>			
<i>vanRO</i>	37	Glycopeptide	Pasture	Farming
<i>rbpA</i>	3	Rifamycin	Pasture	Farming
<i>vanSO</i>	1	Glycopeptide	Pasture	Farming
<i>mtrA</i>	3	Macrolide;Penam	Pasture	Farming
<b>Total</b>	<b>44</b>			
<i>cpt</i>	1	Phenicol	Cattle Site	Farming
<i>vanRO</i>	54	Glycopeptide	Cattle Site	Farming
<i>rbpA</i>	2	Rifamycin	Cattle Site	Farming
<i>vanSO</i>	2	Glycopeptide	Cattle Site	Farming
<i>mtrA</i>	1	Macrolide;Penam	Cattle Site	Farming
<b>Total</b>	<b>60</b>			
<i>dfrB3</i>	1	Trimethoprim	Agriculture	Farming
<i>rbpA</i>	2	Rifamycin	Agriculture	Farming
<i>vanRO</i>	17	Glycopeptide	Agriculture	Farming
<b>Total</b>	<b>20</b>			
<b>Total Farming</b>	<b>181</b>			

**Table S6.** Summary of the identified VFs by VFDB in sequenced metagenomes of soils under different land use systems.

<b>VF</b>	<b>Count</b>	<b>Soil</b>	<b>Land Use</b>
<i>acpXL</i>	8	Campus Lawn	Forest
<i>pilG</i>	1	Campus Lawn	Forest
<i>hsiB1/vipA</i>	1	Campus Lawn	Forest
<i>icl</i>	1	Campus Lawn	Forest
<b>Total</b>	<b>11</b>		
<i>acpXL</i>	6	PPA	Forest
<i>hsiB1/vipA</i>	4	PPA	Forest
<i>pilG</i>	3	PPA	Forest
<b>Total</b>	<b>13</b>		

**Table S7.** Summary of the identified VFs by VFDB in sequenced soil metagenomes on urban land use system.

<b>VF</b>	<b>Count</b>	<b>Soil</b>	<b>Land Use</b>
<i>algW</i>	1	Urban Square	Urban
<i>flgC</i>	2	Urban Square	Urban
<i>esxH</i>	1	Urban Square	Urban
<i>fliE</i>	1	Urban Square	Urban
<i>acpXL</i>	9	Urban Square	Urban
<i>hsiB1/vipA</i>	1	Urban Square	Urban
<i>esxN</i>	2	Urban Square	Urban
<i>waaG</i>	1	Urban Square	Urban
<i>mucD</i>	1	Urban Square	Urban
<i>pilT</i>	1	Urban Square	Urban
<i>pilM</i>	1	Urban Square	Urban
<i>fliQ</i>	1	Urban Square	Urban
<i>fliP</i>	1	Urban Square	Urban
<i>fliN</i>	1	Urban Square	Urban
<i>esxM</i>	2	Urban Square	Urban
<i>phoP</i>	1	Urban Square	Urban
<i>icl</i>	1	Urban Square	Urban
<i>pilG</i>	1	Urban Square	Urban
<i>pilH</i>	1	Urban Square	Urban
<i>algR</i>	1	Urban Square	Urban
<i>fliA</i>	1	Urban Square	Urban
<b>Total</b>	<b>32</b>		

**Table S8.** Summary of the identified VFs by VFDB in sequenced soil metagenomes on farming land use system.

<b>VF</b>	<b>Count</b>	<b>Soil</b>	<b>Land Use</b>
<i>phoP</i>	1	Hen House	Farming
<i>acpXL</i>	10	Hen House	Farming
<i>cheW</i>	1	Hen House	Farming
<i>esxH</i>	1	Hen House	Farming
<i>hsiB1/vipA</i>	2	Hen House	Farming
<i>mbtH</i>	2	Hen House	Farming
<i>ideR</i>	1	Hen House	Farming
<i>pilG</i>	2	Hen House	Farming
<i>icl</i>	1	Hen House	Farming
<b>Total</b>	<b>21</b>		
<i>phoP</i>	2	Orchard	Farming
<i>mbtH</i>	2	Orchard	Farming
<i>pilT</i>	1	Orchard	Farming
<i>hsiB1/vipA</i>	4	Orchard	Farming
<i>esxM</i>	4	Orchard	Farming
<i>acpXL</i>	10	Orchard	Farming
<i>esxN</i>	4	Orchard	Farming
<i>ideR</i>	1	Orchard	Farming
<i>pilG</i>	1	Orchard	Farming
<b>TOTAL</b>	<b>29</b>		
<i>acpXL</i>	9	Pasture	Farming
<i>mbtH</i>	1	Pasture	Farming
<i>hsiB1/vipA</i>	3	Pasture	Farming
<b>TOTAL</b>	<b>13</b>		
<i>pilG</i>	1	Cattle Site	Farming
<i>acpXL</i>	8	Cattle Site	Farming
<b>TOTAL</b>	<b>9</b>		
<i>acpXL</i>	5	Agriculture	Farming
<i>pilG</i>	2	Agriculture	Farming
<i>esxM</i>	1	Agriculture	Farming
<i>icl</i>	1	Agriculture	Farming
<i>pilT</i>	2	Agriculture	Farming
<i>fliA</i>	1	Agriculture	Farming
<i>esxG</i>	1	Agriculture	Farming
<i>hsiB1/vipA</i>	1	Agriculture	Farming
<i>phoP</i>	1	Agriculture	Farming
<b>Total</b>	<b>15</b>		
<b>Total Farming</b>	<b>143</b>		