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Trace gas oxidizers are widespread and active members of soil microbial communities

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1. Extended Data

Figure #	Figure title One sentence only	Filename This should be the name the file is saved as when it is uploaded to our system. Please include the file extension. i.e.: <i>Smith_ED Fig1.jpg</i>	Figure Legend If you are citing a reference for the first time in these legends, please include all new references in the Online Methods References section, and carry on the numbering from the main References section of the paper.
Extended Data Fig. 1	Composition of the bacterial and archaeal communities sequenced in each soil metagenome.	Extended-Data-Figure-1.tif	Stacked barcharts depicting the relative abundance of different phyla in (a) Australian soils and (b) global soils based on reads for the single-copy ribosomal protein gene <i>rplP</i> . Alpha diversity is shown as observed and estimated richness (Chao1) in (c) Australian soils and (d) global soils. Boxplots show median, lower and upper quartile, and minimum and maximum values. This is based on four biological replicates for the four Australian soils (n = 16) at depths of 0-5, 5-10, 15-20, and 25-30 cm, and biological triplicates for the eight global soils (n = 24). For beta diversity, abundance-based distance matrix Bray-Curtis diversity is visualized on a multidimensional PcoA plot for both Australian and global soils. Testing for significant differences in community structure between depths and ecosystems was performed using a one-way PERMANOVA with 999 permutations.
Extended Data Fig. 2	Metatranscriptomic analysis comparing metabolic marker gene expression across three	Extended-Data-Figure-2.tif	(a) Relative abundance of quality-filtered metatranscriptomic short reads expressed as the total reads per kilobase per million (RPKM). (b) Relative abundance of genes in each metatranscriptome mapped to the corresponding assemblies

	global soils with paired metagenome and metatranscriptome datasets.		from each soil expressed as total transcripts per million (TPM).
Extended Data Fig. 3	Analysis of aerobic methane oxidation enzymes and pathways in sampled soils.	Extended-Data-Figure-3.tif	<p>(a) Maximum-likelihood tree of amino acid sequences of particulate methane monooxygenase A subunit (PmoA), a marker for aerobic methane oxidation. The tree shows 2 sequences from soil metagenome-assembled genomes (blue) and 12 sequences from unbinned contigs (red) alongside 93 representative sequences from NCBI reference genomes (black). The tree shows the affiliation of the PmoA from <i>Candidatus Methylotheobacterium kingii</i> with those of amplicons of the tropical upland soil cluster (TUSC). Also shown are reference sequences of other members of the copper membrane monooxygenase superfamily, namely ammonia monooxygenase (AmoA), hydrocarbon monooxygenase (HmoA), and groups of unknown function (including PxmA). The tree was constructed using the JTT matrix-based model, used all sites, and was bootstrapped with 50 replicates and midpoint-rooted. Source data is provided in Newick (nwk) format. (b) Metabolic reconstruction of the putative novel methanotroph <i>Candidatus Methylotheobacterium kingii</i>. The core pathways associated with energy conservation and carbon acquisition are shown, with genes detected shown in italics. The bacterium is predicted to use methane, methanol, and acetate as energy and carbon sources. In addition, it can use molecular hydrogen as an electron donor via a group 1f [NiFe]-hydrogenase. The bacterium is predicted to use the electron acceptors oxygen <i>via</i> a cytochrome <i>c</i> oxidase and nitrous oxide <i>via</i> a nitrous oxide reductase. Its particulate methane monooxygenase forms a distinct phylogenetic lineage with amplicons from the Tropical Upland Soil Cluster (TUSC), whereas its methanol dehydrogenase is closely related to those in previously sequenced Gemmatimonadota MAGs inferred to be methylotheobacterial. The genome encodes key</p>

			<p>enzymes for the serine cycle for assimilation of one-carbon sources. Abbreviations: H₄F = tetrahydrofolate; Hyd = group 1f [NiFe]-hydrogenase; pMMO = particulate methane monooxygenase; MDH = methanol dehydrogenase; PQQ = pyrroloquinoline quinone; I = NADH dehydrogenase (complex I); II = succinate dehydrogenase (complex II); IV = cytochrome <i>aa</i>₃ oxidase (complex IV). Dashed black lines indicate diffusion. Dashed gray lines indicate unknown process or undetected genes. (c) Molecular model of the putative particulate methane monooxygenase (Pmo) from <i>Candidatus</i> Methylophilum kingii and comparison with putative catalytic sites of other experimentally validated Pmos. (i) Molecular model of the functional homotrimer of the putative Pmo complex from <i>Ca. M. kingii</i>, shown as a cartoon representation. PmoA, PmoB, and PmoC subunits from one Pmo complex are colored in light blue, dark blue, and sky blue respectively. The other two Pmo complexes in the trimer are colored in transparent yellow. Cu ions bound in the putative active site of the color-coded Pmo complex are shown as ochre spheres. TM = transmembrane. (ii) Zoomed view of the Pmo complex from panel A, with the three Pmo subunits labelled and the putative active sites Cu_B and Cu_C highlighted. (iii) Stick representation of residues in the Cu_B and Cu_C active sites of Pmo molecular models from <i>Ca. M. kingii</i> (Gemmatimonadota) and <i>Methylophilum inferorum</i> (Verrucomicrobiota), and from the crystal structure (PDB ID: 3RGB) of Pmo from the <i>Methylococcus capsulatus</i> (Proteobacteria), showing that both sites are conserved between Pmo from <i>Ca. M. kingii</i> and <i>M. capsulatus</i>. Pmo from <i>M. inferorum</i> lacks the Cu_B site, suggesting this site is not responsible for methane oxidation. (iv) Sequence alignment of metal binding motifs from the Cu_B (left) and Cu_C (right) sites from Pmo from <i>Ca. M. kingii</i> (<i>cMk.</i>), <i>M. capsulatus</i> (<i>Mc.</i>), <i>Candidatus</i> Methylophilum limnetica (<i>cMl.</i>; Methylophilum limnetica), and <i>M. inferorum</i> (<i>Mi.</i>). Amino acids involved in Cu coordination are highlighted.</p>
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<p>Extended Data Fig. 4</p>	<p>Biogeochemical measurements of <i>in situ</i> soil gas concentrations and validation of static chamber setup.</p>	<p>Extended-Data-Figure-4.tif</p>	<p>(a) Mean soil-gas profiles normalized to the respective ambient air concentration (dashed line) of four biological replicates collected at depths of 0, 2, 4, 6, 8, 10, and 16 cm across four Australian ecosystems (n = 112). Note that the different gases were sampled at identical depths, but points are plotted slightly offset on the y-axis for better visibility of error bars. (b) Laboratory static chamber incubations (n = 3) to control for abiotic release of trace gases from chamber's plastic components. Chamber headspace mixing ratios of hydrogen (H₂), carbon monoxide (CO), and methane (CH₄) were measured at eight time points during ~25 minutes of incubation time of an inert stainless steel surface. Measurement setup, sampling procedure, and sampling frequency followed those applied during the <i>in situ</i> chamber incubations performed to measured soil-atmosphere flux of trace gases. Circles indicate mixing ratio averaged from three independent incubations, with the vertical lines denoting one standard deviation. Dashed lines indicate the mixing ratio of trace gas averaged across three air samples.</p>
<p>Extended Data Fig. 5</p>	<p>Maximum-likelihood tree of amino acid sequences of group 3 [NiFe]-hydrogenase large subunits, a marker for hydrogen production during fermentation processes.</p>	<p>Extended-Data-Figure-5.tif</p>	<p>The tree shows 129 sequences from soil metagenome-assembled genomes (blue) alongside 172 representative sequences from NCBI reference genomes (black). The subgroup of each reference sequence is denoted according to the HydDB classification scheme⁴¹. The tree was constructed using the JTT matrix-based model, used all sites, and was bootstrapped with 50 replicates and midpoint-rooted. All sequences shorter than 350 amino acids were omitted. Source data is provided in Newick (nwk) format.</p>
<p>Extended Data Fig. 6</p>	<p>Gas chromatography studies of one <i>ex situ</i> trace gas consumption experiment.</p>	<p>Extended-Data-Figure-6.tif</p>	<p>Shown are four biological replicates collected at depths of 0-5, 5-10, 15-20, and 25-30 cm across four Australian ecosystems (n = 64). Depicted are the oxidation rates recorded over time of (a) atmospheric H₂, (b) atmospheric CO, and (c) atmospheric CH₄ by soils at each depth compared to heat-</p>

			<p>killed control soils. Oxidation of the three gases was also measured in the dryland soils following hydration, i.e. Dryland (wet) samples. Points show average values and vertical lines the error bars representing one standard deviations of four biological replicates. Significance testing of differences in <i>ex situ</i> oxidation rates between ecosystem type and soil depth was carried out using a one-sided Kruskal Wallis H test followed by a pairwise Wilcoxon tests with adjusted <i>p</i>-values using a Benjamini Hochberg correction for multiple testing. Boxplots show median, lower and upper quartile, and minimum and maximum values comparing the <i>ex situ</i> rates of H₂, CO, and CH₄ oxidation between depths and ecosystems for (d) cell-specific rates and (e) bulk reaction rates per gram of dry soil.</p>
Extended Data Fig. 7	Copy number of the 16S rRNA gene per gram of soils of four biological replicates.	Extended-Data-Figure-7.tif	<p>Soil samples were collected at depths of 0-5, 5-10, 15-20, and 25-30 cm across four Australian ecosystems, with four biological replicates for each depth (n = 64). Boxplots show median, lower and upper quartile, and minimum and maximum values.</p>
Extended Data Fig. 8	Comparison of the theoretical and measured population of trace gas oxidizers in soils,	Extended-Data-Figure-8.tif	<p>Shown are four biological replicates collected at depths of 0-5, 5-10, 15-20, and 25-30 cm across four Australian ecosystems (n = 64). The theoretical populations, in line with the methods of Conrad⁶⁹, were estimated from thermodynamic modeling and measured <i>ex situ</i> oxidation rates assuming a single maintenance energy for all cells. Two different values were used: $8.9 \times 10^{-15} \text{ W cell}^{-1}$ as assumed by Conrad⁶⁹ and $1.9 \times 10^{-15} \text{ W cell}^{-1}$ as the median of metabolic rates reported by DeLong et al⁶¹. The measured populations of trace gas oxidizers were determined from 16S rRNA gene copy numbers and the relative abundance of the respective functional gene as determined from metagenomics. The ratio of theoretical vs. measured population is equivalent to dividing our calculated power per cell by the respective maintenance energy above. Boxplots show median, lower and upper quartile, and</p>

			minimum and maximum values.
Extended Data Fig. 9	Maximum-likelihood tree of amino acid sequences of ribulose 1,5-bisphosphate carboxylase / oxygenase (RbcL), a marker for carbon fixation through the Calvin-Benson cycle.	Extended-Data-Figure-9.tif	The tree shows 68 sequences from soil metagenome-assembled genomes (blue) alongside 126 representative sequences from NCBI reference genomes (black). The subtype of each reference sequence is denoted. The tree was constructed using the JTT matrix-based model, used all sites, and was bootstrapped with 50 replicates and midpoint-rooted. Source data is provided in Newick (nwk) format.
Extended Data Fig. 10	Summary of the processes and mediators of trace gas cycling at the soil-atmosphere interface.	Extended-Data-Figure-10.tif	The soil bacterial phyla capable of consuming the trace gases H ₂ (<i>via</i> group 1 and 2 [NiFe]-hydrogenases), CO (<i>via</i> form I carbon monoxide dehydrogenases), and CH ₄ (<i>via</i> particulate methane monooxygenases) are shown. They are listed in order of the number of metagenome-assembled genomes recovered (black for highly abundant and grey for less abundant trace gas oxidizers). The downward arrows show the net consumption of atmospheric H ₂ , CO, and CH ₄ by soil bacteria. The upward arrows show processes that result in endogenous production and internal cycling of trace gases. The green boxes indicate biotic processes, whereas the brown boxes indicate abiotic processes.

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7 **2. Supplementary Information:**

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9 **A. Flat Files**

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Item	Present?	Filename This should be the name	A brief, numerical description of file contents. i.e.: <i>Supplementary Figures 1-4, Supplementary Discussion, and</i>
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		the file is saved as when it is uploaded to our system, and should include the file extension. The extension must be .pdf	<i>Supplementary Tables 1-4.</i>
Supplementary Information	Yes	Supplementary information.pdf	Supplementary Tables 1 to 12 Supplementary Figures 1 to 10
Reporting Summary	Yes	AB Rep Summary.pdf	
Peer Review Information	Yes	Greening_TPR File.pdf	

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B. Additional Supplementary Files

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Type	Number If there are multiple files of the same type this should be the numerical indicator. i.e. "1" for Video 1, "2" for Video 2, etc.	Filename This should be the name the file is saved as when it is uploaded to our system, and should include the file extension. i.e.: <i>Smith_Supplementary Video 1.mov</i>	Legend or Descriptive Caption Describe the contents of the file
Supplementary Table		Supplementary Tables 1-12.xlsx	Supplementary Tables 1 to 12 in XLSX format.
Supplementary Data		Phylogenetic tree newick files.zip	Raw phylogenetic trees in NWK format for Figures 2a, 2b, ED Figure 3, ED Figure 5, ED Figure 9, and Supplementary Figures 1 to 10.

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17 **3. Source Data**

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Figure	Filename This should be the name the file is saved as when it is uploaded to our system, and should include the file extension. i.e.: <i>Smith_Source Data Fig1.xls</i> , or <i>Smith_Unmodified Gels_Fig1.pdf</i>	Data description i.e.: Unprocessed Western Blots and/or gels, Statistical Source Data, etc.
Source Data Fig. 1	Source Data Fig 1.xlsx	Numerical source data
Source Data Fig. 3	Source Data Fig 3.xlsx	Numerical source data
Source Data Extended Data Fig. 1	Source Data Extended Data Fig 1.xlsx	Numerical source data
Source Data Extended Data Fig. 2	Source Data Extended Data Fig 2.xlsx	Numerical source data
Source Data Extended Data Fig. 4	Source Data Extended Data Fig 4.xlsx	Numerical source data
Source Data Extended Data Fig. 6	Source Data Extended Data Fig 6.xlsx	Numerical source data
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Trace gas oxidizers are widespread and active members of soil microbial communities

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52 **Abstract**

53 Soil microorganisms globally are thought to be sustained primarily by organic carbon
54 sources. Certain bacteria also consume inorganic energy sources such as trace gases, but they are
55 presumed to be rare community members, except within some oligotrophic soils. Here we
56 combined metagenomic, biogeochemical, and modelling approaches to determine how soil
57 microbial communities meet energy and carbon needs. Analysis of 40 metagenomes and 757
58 derived genomes indicated over 70% of soil bacterial taxa encode enzymes to consume inorganic
59 energy sources. Bacteria from 19 phyla encoded enzymes to use the trace gases hydrogen and
60 carbon monoxide as supplemental electron donors for aerobic respiration. In addition, we
61 identified a fourth phylum (Gemmatimonadota) potentially capable of aerobic methanotrophy.
62 Consistent with the metagenomic profiling, communities within soil profiles from diverse
63 habitats rapidly oxidized hydrogen, carbon monoxide, and to a lesser extent methane below
64 atmospheric concentrations. Thermodynamic modelling indicated that the power generated by
65 oxidation of these three gases is sufficient to meet the maintenance needs of the bacterial cells
66 capable of consuming them. Diverse bacteria also encode enzymes to use trace gases as electron
67 donors to support carbon fixation. Altogether, these findings suggest that trace gas oxidation
68 confers a major selective advantage in soil ecosystems, where availability of preferred organic
69 substrates limits microbial growth. The observation that inorganic energy sources may sustain
70 most soil bacteria also has broad implications for understanding atmospheric chemistry and
71 microbial biodiversity in a changing world.

72

73 **Introduction**

74 Bacteria mediate key supporting and regulatory services in soil ecosystems worldwide ^{1,2}.
75 Culture-independent surveys have shown that soils harbor abundant and diverse bacterial
76 communities ^{3,4}, with most cells thought to be in dormant states due to pressures such as resource
77 limitation ^{5,6}. Most soil bacteria use organic carbon derived from vegetation and other inputs as
78 energy and carbon sources ^{1,7}; reflecting this, isolates from all nine of the most abundant phyla in
79 soils are aerobic organotrophs ^{8,9}. However, various bacteria have been characterized that use
80 inorganic energy sources, including molecular hydrogen (H₂), carbon monoxide (CO), sulfide,
81 thiosulfate, ammonia, and nitrite ^{10,11}. While these lithotrophs have key ecological and
82 biogeochemical roles ^{1,10}, they are generally thought to be minor community members compared
83 to organotrophs ^{1,12}.

84 Emerging evidence suggests the trace gases H₂, CO, and to a lesser extent methane (CH₄)
85 are particularly important energy sources for bacterial growth and survival. These three gases are
86 ubiquitously available in the atmosphere at average global concentrations of 0.53, 0.10, and 1.85
87 parts per million (ppmv) respectively, with local CO levels varying greatly due to natural and
88 anthropogenic processes ¹³⁻¹⁶. These compounds are also produced within soils: H₂ through
89 biological nitrogen fixation and fermentation ^{13,17,18}, CO primarily through abiotic thermal and
90 photochemical processes ^{15,19}, and CH₄ primarily through methanogenesis ¹⁶. Biogeochemically,
91 soil bacteria are major sinks of reduced trace gases, accounting for the net loss of approximately
92 75% atmospheric H₂ (70 teragrams per year) ^{13,17}, 10% atmospheric CO (250 teragrams per year)
93 ^{15,20}, and 5% CH₄ when excluding wetlands (30 teragrams per year) ^{16,21}. Microorganisms have
94 evolved specialized metalloenzymes, namely uptake [NiFe]-hydrogenases, [MoCu]-carbon
95 monoxide dehydrogenases, and methane monooxygenases, to oxidize these gases, including
96 below atmospheric concentrations ²¹⁻²⁴. Some bacteria sustain autotrophic or mixotrophic growth
97 by using electrons derived from H₂ and CO to drive aerobic respiration and carbon fixation
98 through various pathways ²⁵⁻²⁸. In addition, bacterial cultures from four phyla (Actinobacteriota,
99 Proteobacteria, Acidobacteriota, Chloroflexota) have been shown to switch from growth on
100 organic carbon to persistence on these trace gases in response to carbon starvation ^{22-24,29,30}.
101 Genetic studies have shown that such metabolic flexibility provides a selective advantage under
102 conditions of low resource availability or stability ^{18,24,31-33}.

103 Despite these advances, we lack a holistic understanding of the relative contributions of
104 trace gases and other compounds in sustaining the energy and carbon needs of soil communities
105 ³⁴. Some have hypothesized that trace gas oxidizers may be widespread community members
106 ^{20,24,26}. However, previous soil surveys reported that atmospheric H₂ and CO oxidizers are

107 members of the rare biosphere, each comprising just 1% of the total bacterial community,
108 similarly to methane-oxidizing bacteria (methanotrophs)^{12,35,36}. Notable exceptions include some
109 extreme environments, such as Antarctic deserts, where trace gas oxidizers are major primary
110 producers in soils with otherwise low photosynthetic input³⁷⁻³⁹. Here, we combined
111 metagenomic, biogeochemical, and thermodynamic modelling approaches to profile metabolic
112 strategies of soil bacteria. We reveal facultative trace gas oxidizers are highly abundant and
113 phylogenetically diverse members of soil microbiomes. Moreover, consumption of trace gases
114 by these bacteria would be sufficient to sustain their basal energy needs.

115 **Results and Discussion**

116 **Most soil bacteria harbor the flexibility to oxidize organic and inorganic energy sources**

117 First, we sequenced metagenomes from depth profiles of four Australian soil ecosystems
118 (wetland, grassland, forest, dryland) (**Supplementary Tables 1 & 2**) and analyzed metagenomes
119 from eight further global sites (**Supplementary Table 2**). Assembly and binning yielded 757
120 high- or medium-quality metagenome-assembled genomes (MAGs) spanning 26 bacterial and
121 five archaeal phyla (**Supplementary Table 3**). Consistent with the community composition of
122 the sites (**Extended Data Fig. 1; Supplementary Table 4**), half of the genomes affiliate with
123 globally dominant soil phyla^{3,8} Actinobacteriota, Proteobacteria, and Acidobacteriota.

124 We used comprehensive reference databases (**Supplementary Table 5**) to search key
125 metabolic genes in metagenomic short reads and derived genomes (**Fig. 1; Supplementary**
126 **Tables 6 & 7**). As anticipated¹, most bacteria have the capability to conserve energy through
127 aerobic respiration of organic compounds. More surprisingly, many of these bacteria also
128 encoded the key enzymes (group 1 and 2 [NiFe]-hydrogenases and form I CO dehydrogenases)
129 to consume trace gases H₂ and CO: an average of 39% and 56% of the bacterial community
130 based on short reads, and 31% and 26% of assembled genomes. Also widespread was the
131 capacity for sulfide (28% community / 22% genomes) and thiosulfate (6.2% / 11%) oxidation,
132 whereas nitrite (4.6% / 3.7%), ammonium (1.5% / 2.4%), and methane (1.1% / 0.37%) oxidation
133 and photosynthesis (1.3% / 1.5%) were more restricted traits (**Fig. 1**). Altogether, on average
134 72% of bacterial genomes encoded enzymes to acquire energy *via* inorganic substrates. For
135 electron acceptor utilization, most bacteria encoded terminal oxidases for aerobic respiration and
136 many were predicted to conserve energy by nitrate reduction (25% / 14%), nitrite reduction (21%
137 / 21%), and hydrogenogenic fermentation (29% / 21%) (**Fig. 1**). Consistent with these results, for
138 three global sites with accompanying metatranscriptome data (**Supplementary Table 2**), read
139 mapping confirmed the most expressed respiratory genes encoded terminal oxidases (av. 594
140 transcripts per million, TPM) and enzymes for oxidation of NADH (198 TPM), H₂ (126 TPM),
141 CO (75 TPM), and ammonium (223 TPM) (**Extended Data Fig. 2; Supplementary Table 8**).
142 Altogether, these observations suggest that a large proportion of soil bacteria are metabolically
143 flexible organoheterotrophs, which encode and express enzymes to use alternative energy
144 sources such as trace gases to supplement energy needs.

145 **Diverse bacterial phyla are capable of oxidizing H₂, CO, and CH₄ in different soil**
146 **ecosystems**

147 To gain insight into phylogenetic diversity of trace gas oxidizers, we generated
148 phylogenetic trees of the uptake hydrogenases and CO dehydrogenases from binned and
149 unbinned assembled sequences (**Supplementary Table 9**). 19 phyla encoded one or both
150 enzymes, including all nine dominant soil phyla (Acidobacteriota, Actinobacteriota,
151 Bacteroidota, Chloroflexota, Firmicutes, Gemmatimonadota, Planctomycetota, Proteobacteria,
152 Verrucomicrobiota)^{3,8} and six candidate phyla (Binatota, Dormibacterota, Eremiobacterota,
153 Methyloirabilota, Tectomicrobia, UBP7). Among the uptake hydrogenases, 49% of the 1522
154 hits affiliated with group 1h [NiFe]-hydrogenases. This relatively recently discovered high-
155 affinity enzyme supports persistence of organotrophic bacteria by oxidizing atmospheric H₂
156 ^{22,23,26,30,40,41}. The phylogenetic tree revealed an unprecedented sequence diversity and broad
157 taxonomic distribution of this subgroup, with sequences affiliated with 13 phyla (**Fig. 2a;**
158 **Supplementary Fig. 1**). Also widespread were various radiations within the group 1d (8.9%), 1f
159 (19%), and 2a (3.6%) [NiFe]-hydrogenases known to support aerobic hydrogen oxidation
160 ^{23,26,28,42,43}, as well as a subclade of the group 1c hydrogenases (13%) encoded by
161 Acidobacteriota, Planctomycetota, and Gemmatimonadota (**Fig. 2a; Supplementary Figs. 2 &**
162 **3**). For CO dehydrogenases, we restricted our searches to the canonical form I enzyme using
163 recently described reference databases²⁴, given there is no strong evidence that CO is the
164 physiological substrate of the related form II enzyme^{20,24,29,44}. The form I CO dehydrogenase
165 was similarly prevalent across the MAGs; the tree shows large actinobacterial and
166 proteobacterial clades flanking a central mixed clade containing sequences from 11 different
167 phyla (**Fig. 2b; Supplementary Fig. 4**). In line with previous inferences^{24,26}, both trees suggest
168 enzymes for trace gas oxidation were horizontally disseminated between bacteria on multiple
169 occasions.

170 Phylogenetic analysis also validated the presence and affiliations of sulfide, thiosulfate,
171 ammonia, nitrite, and methane oxidizers (**Supplementary Figs. 5 to 10; Extended Data Fig. 3**).
172 These included genomes of soil comammox bacteria^{45,46} and a potential nitrite-oxidizing
173 bacterium within recently reported candidate phylum Eremiobacterota³⁷. A major finding was
174 the recovery of a high-quality Gemmatimonadota bin predicted to use methane, hydrogen, and
175 acetate as energy sources. Its genome encodes a putative particulate methane monooxygenase
176 that forms a sister lineage to those of the recently discovered *Candidatus* Methyloirabilota
177 methanotrophs (sharing ~60% sequence identity) and corresponds to amplicon sequences
178 previously identified for the ubiquitous uncultivated putative methanotroph lineage TUSC

179 (Tropical Upland Soil Cluster)^{47,48}. Protein modelling suggests that this enzyme shares a
180 common tertiary structure and copper-binding residues with canonical particulate methane
181 monooxygenases (**Extended Data Fig. 3**). Altogether, these results suggest that
182 Gemmatimonadota is a fourth phylum^{49–51} capable of aerobic methanotrophy, though cultivation
183 and biogeochemical studies are required to confirm its activity. We propose this bacterium is
184 named *Candidatus Methylootropicum kingii* (**Etymological Information**).

185

186 **Trace gas oxidizers are highly active across different soil habitats and depths**

187 Reflecting their abundance and diversity, we confirmed that trace gas oxidizers are active
188 in soil communities of all investigated Australian soils. *In situ* concentrations and soil-
189 atmosphere fluxes of H₂, CO, and CH₄ were measured across the four sites in biological
190 quadruplicate using a sensitive gas chromatograph (**Supplementary Table 10**). All three gases
191 were present within typical mixing ratios^{13,15,16} at the soil-atmosphere interface and top 20 cm of
192 each soil (**Fig. 3a**), confirming these substrates are available to bacteria. Gas concentrations
193 decreased with depth at some sites (**Extended Data Fig. 4**), potentially indicating microbial
194 consumption. There was also potential evidence of endogenous gas production within some soil
195 profiles: the elevated H₂ concentrations in deeper wetland soils may result from activity of the
196 numerous hydrogenogenic fermenters (33% community / 32% genomes; **Extended Data Fig. 5**)
197 under hypoxic conditions¹⁸, whereas the elevated gas levels in the dryland soils are consistent
198 with photochemical and thermal production processes⁵². In turn, endogenous production would
199 result in some cryptic gas cycling within the soil profile, though this remains to be
200 experimentally verified. In line with the abundance of H₂ oxidizers, net *in situ* H₂ uptake was
201 observed by soil communities at all sites and consumption was particularly rapid at the
202 grassland, forest, and wetland sites (**Fig. 3b**); ambient H₂ in the flux chambers was oxidized by
203 underlying soil down to a threshold of ~0.1 ppmv within a few minutes, resulting in typical
204 fluxes of -10 to -40 nmol m⁻² s⁻¹. In contrast, no significant fluxes of CO were observed; the high
205 rates of photochemical CO production known to occur during daylight potentially obscured
206 underlying microbial consumption^{19,53}, though this remains to be experimentally confirmed.
207 Reflecting global patterns⁵⁴, with the exception of forest sites, CH₄ uptake fluxes were low in
208 comparison with H₂. Control experiments confirmed the flux chambers were gas-tight and did
209 not mediate significant gas production or consumption during the timecourse of the flux
210 measurements (**Extended Data Fig. 4**). Overall, the trace gas fluxes observed are much lower
211 than typically reported for heterotrophic respiration⁷, although endogenous soil production
212 potentially obscures much consumption.

213 We subsequently measured *ex situ* oxidation rates by incubating samples from soil depth
214 profiles in serum vials containing ambient air headspaces (**Supplementary Table 10**). Under
215 these conditions, biological gas consumption could be accurately quantified given no significant
216 abiotic production of these gases was detected (**Extended Data Fig. 6**). Atmospheric H₂ and CO
217 were both rapidly oxidized by forest, grassland, and wetland soils at rates within fivefold of
218 previous literature^{10,55-58}. Bulk rates were significantly higher for topsoils (0-5 and 5-10 cm)
219 compared to deeper soils (15-20 and 25-30 cm) (**Supplementary Table 10; Extended Data Fig.**
220 **6**), though cell-specific rates (normalized to estimated number of respective trace gas oxidizing
221 cells; **Extended Data Fig. 7**) showed few significant variations with depth (**Fig. 3c;**
222 **Supplementary Table 11**). Across these sites, atmospheric CH₄ oxidation occurred at 76-fold
223 and 35-fold lower rates than H₂ and CO on average, with the most rapid consumption again
224 occurring at the forest site. Such observations are consistent with the much higher levels of
225 uptake hydrogenases and CO dehydrogenases compared to methane monooxygenases in the
226 metagenomes and derived genomes (**Fig. 1**). In line with the *in situ* observations, trace gas
227 oxidation was slowest for dryland soils. This possibly reflects that, as observed in previous
228 laboratory studies, low water content inhibits trace gas uptake^{44,45}. Contrary to the pattern
229 observed in other habitats, deeper dryland soils consumed trace gases more rapidly, perhaps
230 reflecting their higher measured water content (**Supplementary Table 1**). Experimentally
231 wetting dryland soils, in order to simulate rainfall events, enhanced rates by an average of 60-
232 fold (**Fig. 3c; Extended Data Fig. 6**). Together, these activity-based measurements complement
233 the metagenomic analysis by confirming soil communities contain highly active H₂, CO, and
234 sometimes CH₄ oxidizers. While net *in situ* gas fluxes were often low or variable, especially for
235 CO, the rapid *ex situ* rates and previous reports of endogenous gas production^{18,19,52,53,59} suggest
236 high levels of internal gas cycling likely occurs.

237

238 **Trace gases enable persistence of most soil bacteria and may support mixotrophic growth**

239 Trace gas oxidation is likely to primarily support the persistence of organotrophic
240 bacteria. Most sequenced soil bacteria encoded uptake hydrogenases and CO dehydrogenases
241 together with the genes for organotrophy. Based on observations from pure culture studies,
242 bacteria consume trace gases to conserve energy for cellular maintenance when organic carbon
243 supplies are limiting for growth^{3-5,30}. We used thermodynamic modelling to predict the amount
244 of power (i.e. energy per time in J s⁻¹ (W)) per cell (**Supplementary Table 12**) that could be
245 generated based on the *ex situ* oxidation rates (**Extended Data Fig. 6**) and the total number of
246 putative trace gas oxidizers detected per gram of dry soil (**Fig. 1 & Extended Data Fig. 7**).

247 Oxidation rates were sufficient to generate an average power of 2.2×10^{-15} W per H₂-oxidizing
248 cell, 2.0×10^{-15} W per CO-oxidizing cell, and 1.1×10^{-13} W per CH₄-oxidizing cell, and trends
249 were similar across habitats and depths with the exception of the dryland soils (**Fig. 3d &**
250 **Supplementary Table 12**). The calculated cell-specific power values are within the broad range
251 of the maintenance and endogenous energy requirements previously reported for various
252 laboratory-grown cultures of aerobic heterotrophs (average 1.9×10^{-15} W per cell⁶⁰; range 10^{-12}
253 to 10^{-17} W per cell⁶⁰⁻⁶³) and greatly exceed the *in situ* maintenance energies of aerobic
254 heterotrophs in highly energy-limited ecosystems (10^{-17} to 10^{-19} W per cell⁶⁴⁻⁶⁶). Previous
255 studies estimated the theoretical maximum population of trace gas oxidizers based on observed
256 oxidation rates and the assumption of a single static maintenance energy⁶⁷⁻⁶⁹. Although our
257 experimentally observed population is in reasonable agreement with such estimates, results are
258 sensitive to the assumed maintenance energy (**Extended Data Fig. 8**). Literature data and our
259 cell-specific power calculations based on empirical parameters suggest that metabolic rates are
260 highly variable between microbial populations, metabolic strategies, and soil habitat. Therefore,
261 the use of a single maintenance energy to estimate the fraction of the population that can be
262 supported by trace gas oxidation may be too simplistic. It should also be noted that our estimates
263 did not account for relic DNA⁷⁰, inactive cells, and cryptic gas cycling. Thus, gross oxidation
264 rates and available power per active cell may be considerably higher *in situ*. Altogether, this
265 suggests that trace gases alone can theoretically sustain the persistence of the entire gas-
266 consuming community.

267 Trace gases may also support mixotrophic growth of a significant proportion of bacteria
268 across these soil habitats. Our analysis indicates that most community members, including trace
269 gas oxidizers, acquire carbon heterotrophically. However, some bacteria (13% community / 12%
270 genomes) encoded the capacity to assimilate CO₂ through the Calvin-Benson cycle (**Fig. 1**).
271 Genome-resolved analysis indicated most of these autotrophs were capable of oxidizing H₂
272 (79%) and/or CO (63%), with some predicted to mediate sulfide oxidation (32%), thiosulfate
273 oxidation (17%), or photosynthesis (11%) (**Supplementary Table 7**). Putative autotrophic H₂
274 and CO oxidizers were patchily distributed among seven dominant soil phyla and candidate
275 phylum Dormibacterota, suggesting autotrophy has been acquired multiple times (**Fig. 1**).
276 Consistently, phylogenetic analysis showed that the 93% of the binned ribulose 1,5-bisphosphate
277 carboxylase / oxygenase (RuBisCO / RbcL) hits were from clades known to support
278 chemosynthesis rather than photosynthesis (**Extended Data Fig. 9**). Reads for other CO₂ fixation
279 pathways were also detected, but with exception of the peatland sample, were encoded by less
280 than 1% of the total community (**Fig. 1; Supplementary Table 6**). Individual atmospheric trace
281 gases are thought to be insufficiently concentrated to sustain growth as the sole energy source

282 ^{10,67}, though there is increasing evidence that they can sustain mixotrophic growth ^{27,28,71}.
283 Moreover, autotrophic growth may be favorable in environments where there are significant
284 rates of edaphic gas production, for example due to hydrogenogenic fermentation (**Extended**
285 **Data Fig. 5**) during hypoxia ²⁸. Remarkably, this suggests that the process of atmospheric
286 chemosynthesis recently discovered in Antarctic deserts ⁹ extends to other habitats.

287

288 **Conclusions and Outlook**

289 Overall, bacteria with the metabolic flexibility to use both organic and inorganic energy
290 sources are likely to have a selective advantage in soil environments. In most soils, organic
291 carbon is the main factor limiting microbial growth ^{13,49}; this reflects the inherent spatiotemporal
292 variability in organic carbon availability of soils, together with the recalcitrance of many organic
293 polymers and the intense competition for more degradable compounds ¹. Thus, the ability to
294 consume alternative energy sources is likely to be critical for adaptation and resilience of many
295 taxa. H₂ and CO are ideal compounds in this regard given they are readily available from both
296 atmospheric and edaphic sources; likewise, their high energy content, low activation energy, and
297 diffusibility into microbial cells make these gases dependable for persistence ^{31,50}.

298 Our findings in turn suggest that oxidation of these trace gases is a generalist process,
299 rather than a specialist one as previously suggested ⁶. We provide multiple lines of evidence that
300 H₂ and CO oxidizers are abundant, diverse, and active across different soil habitats and depths.
301 These findings strikingly contrast with some previous reports that trace gas oxidizers comprise
302 just 1% of the community ^{12,35}. Such discrepancies might reflect that previous work on
303 atmospheric H₂ oxidizers relied on non-degenerate quantitative PCR primers that only capture a
304 small proportion of the total diversity of soil uptake hydrogenases; indeed, we observed high
305 proportions of high-affinity hydrogenases (30% community / 40% genomes) in the Canadian
306 cropland metagenome where H₂ oxidizers were previously inferred to be minor community
307 members ^{6,7}. Moreover, while previous genome surveys indicate Actinobacteriota predominantly
308 mediate atmospheric H₂ oxidation ^{33,37}, our analysis indicates all major bacterial soil phyla can
309 mediate this process. However, an important caveat is that our findings are based primarily on
310 genomic inferences and bulk rates, meaning it is unclear if all putative trace gas oxidizers were
311 active during the experiments. Such metabolically flexible generalists co-exist with more
312 specialist taxa that are predicted to use more niche substrates, including methane, ammonia, and
313 nitrite. The finding that some Gemmatimonadota encode genes for aerobic methane oxidation
314 further indicates the diversity of trace gas oxidizers in soils. Our results also reveal an

315 unexpected diversity and abundance of chemoautotrophs in soils, while hinting at roles for
316 sulfur-based compounds as further supplementary energy sources.

317 The thermodynamic calculations suggest that trace gas oxidation is sufficient to sustain
318 the persistence of all trace gas oxidizers and thus most bacteria in soils. Power per cell values are
319 well within the range of maintenance or endogenous energies typically reported for pure cultures,
320 and exceed the range for energy-limited ecosystems⁶⁰⁻⁶⁴. Nevertheless, there is likely to be
321 considerable heterogeneity in the rates that individual cells oxidize trace gases and the way they
322 allocate energy derived from this process. For example, it is plausible that whereas some cells
323 encoding uptake hydrogenases minimally use H₂, many others will depend on this gas for
324 survival and yet others may use it to support growth. It is also critical to note that most bacteria
325 harboring genes for trace gas oxidation are predicted to be metabolically flexible. Many of them
326 are likely to simultaneously consume organic and inorganic energy sources, or indeed several
327 trace gases together as recently inferred for a methanotroph predicted to grow on atmospheric
328 energy sources alone²⁷, in order to grow or persist mixotrophically. Innovative studies are
329 required to gain a detailed perspective on how trace gases are differentially used within and
330 between bacterial populations and cells.

331 More broadly, the extensive soil-atmosphere interaction described here appears to be a
332 key regulator of soil biodiversity and atmospheric chemistry. As summarized in **Extended Data**
333 **Fig. 10**, soil bacteria consume trace gases of atmospheric, biological, and geochemical origin.
334 Given trace gases are major energy sources sustaining the dormant soil majority, their oxidation
335 will in turn influence wider community ecology; indeed, it is well-established that dormancy
336 contributes to the maintenance of microbial biodiversity and the resilience of soil communities
337¹⁷. Moreover, the observation that trace gas oxidizers are more numerous and diverse than
338 previously thought suggests the global atmospheric sinks of H₂ and CO are relatively resilient.
339 This may explain why, despite high anthropogenic emissions of H₂, global mixing ratios have
340 remained quite stable²⁶. Nevertheless, various human activities could undermine this soil-
341 atmosphere interaction, for example through changing soil properties (e.g. through agricultural
342 practices and desertification) and atmospheric composition (e.g. through urban pollution or a
343 hydrogen economy⁵¹), with potential ecological and biogeochemical ramifications. Further
344 studies are required to understand what physicochemical factors influence the abundance and
345 activities of trace gas oxidizers, and how they respond to local and global change. More work is
346 also needed to understand the role of chemosynthetic CO₂ fixation by soil trace gas oxidizers,
347 which appear to include the most abundant bacterial autotrophs in aerated soils, and in turn
348 determine whether this process serves as a significant CO₂ sink.

350 **Methods**

351

352 **Site description and sampling.** Sampling was conducted in four sites within Australia: (i)
353 wetland (-37.908647°, 145.139591°; Jock Marshall Reserve, Clayton, VIC; JMR), (ii) forest (-
354 37.446379°, 144.470078°; Wombat State Forest, VIC; WSF), (iii) grassland (-37.926514°,
355 145.313136°; Don Bosco Grassland, Lysterfield, VIC; DBG), and (iv) dryland (-23.977765°,
356 133.712524°; 25.5 km south of Alice Springs, NT; ASD). The sites were sampled on December
357 19 2018, January 5 2019, January 21 2019, and January 29 2019 respectively. At each site (~100
358 m²), four sampling plots (~1 m²) were selected. At each plot, *in situ* depth-resolved gas
359 concentrations profiles and soil-atmosphere fluxes were measured. In addition, a core was used to
360 collect soil samples at four depths (0-5, 5-10, 15-20, and 25-30 cm) for *ex situ* oxidation
361 measurements, physicochemical analysis, and DNA extractions to perform quantitative
362 polymerase chain reactions (qPCR) and metagenomic sequencing. All sampling occurred during
363 daylight hours and dry weather conditions, gas samples were processed within 48 h of collection,
364 and soil samples were incubated for *ex situ* oxidation measurements within 24 h of collection.

365

366 **Sampling and measurement of soil gas profiles.** Depth-resolved *in situ* gas concentrations of
367 H₂, CO, and CH₄ were measured using stainless steel capillaries of 1 mm internal diameter fitted
368 with a Luer Lock and Discifix three-way stopcock. Prior to sampling, each capillary was inserted
369 into the soil at depth intervals of 2, 4, 6, 8, 10, and 16 cm. All capillaries were installed
370 simultaneously, arranged in a hexagonal grid (diameter ~20 cm), and left to equilibrate for ~30
371 min. Samples of 15 mL were withdrawn using a 20 mL gas-tight Terumo syringe. A gas sample
372 was also collected at the soil-atmosphere interface (0 cm). All gas samples were stored in
373 evacuated 12 mL glass exetainers sealed with rubber septum lids and analyzed using gas
374 chromatography. Gas concentrations in samples were measured by gas chromatography using a
375 pulsed discharge helium ionization detector (model TGA-6791-W-4U-2, Valco Instruments
376 Company Inc.) as previously described⁷². Samples were calibrated against H₂, CO and CH₄
377 standards that were prepared using ultra-pure concentrations of each gas (1% in N₂ gas cylinder,
378 99.999% pure, Air Liquide Australia) down to the limit of quantification (H₂ 20 ppbv, CO 90
379 ppbv, CH₄ 500 ppbv. Pressurized air (Air Liquide Australia) with known trace gas concentrations
380 was used as an internal reference standard.

381

382 **Measurement of soil-atmosphere gas fluxes.** *In situ* soil-atmosphere fluxes of H₂, CO, and CH₄
383 were measured using static flux chambers. The chamber consisted of a polyvinylchloride (PVC)
384 pipe of 20 cm height and 15 cm diameter with a threaded access cap. The cap was fitted with a

385 gastight O-ring, two butyl rubber septa (one for air sampling and one for a thermometer), and an
386 axial fan on the inside to promote internal mixing. At each plot, a PVC base collar of 10 cm
387 height and 14.8 cm diameter was inserted ~5 cm into the soil and left to equilibrate for ~30
388 minutes prior to sampling to reduce lateral gas fluxes. Once the chamber was fitted over the
389 collar, the cap was closed and the axial fan was started. Three consecutive gas measurements
390 were taken at approximately one-minute intervals, followed by either four or five measurements
391 at approximately five-minute intervals. For each measurement, 15 mL of gas was collected using
392 a gas-tight 20 mL Terumo syringe fitted with a Luer Lock and Discifix three-way stopcock and
393 measured by gas chromatography as described above. Control gas measurements of ambient air
394 were taken directly before, during, and after sampling. The temperature of the chamber, ambient
395 air, and soil were monitored throughout. Concentrations were then converted to nmol m^{-3} at
396 ambient pressure and temperature using the ideal gas law. Atmospheric flux (J_{atm}) was calculated
397 from the concentration (C_0) and rate change (dC/dt) at chamber deployment using a linear
398 regression (eq. 1) and an exponential model fit (eq. 2 and eq. 3) ⁵⁹ for each chamber
399 measurement:

400

$$401 \quad J_{\text{atm}} = h \frac{dC}{dt} \quad (\text{EQ. 1})$$

402

$$403 \quad C_{(t)} = \frac{P}{k} + \left(C_0 - \frac{P}{k} \right) e^{(-kt)} \quad (\text{EQ. 2})$$

404

$$405 \quad J_{\text{atm}} = h(P - kC_0) \quad (\text{EQ. 3})$$

406

407 For the exponential model, parameters P and k were fitted via non-linear regression using the
408 `nls()` function in R. h denotes the effective chamber height in m. The best model was chosen
409 according to the lower Akaike information criterion (AIC). Conservative flux detection limits
410 incorporating errors of sample handling and storage were calculated using mean and standard
411 deviation of air samples ⁷³. Three independent incubations were conducted to control for trace
412 gases potentially leaked from the chamber or released in the chamber headspace by plastic
413 components due to abiotic processes. Chambers were placed on an inert stainless steel surface
414 and sealed on the surface with silicone grease. Headspace samples from eight time points and
415 three air samples were collected following measurement setup, incubation time, and sampling
416 procedures described above for the *in situ* sampling of actual samples. No variation in trace gas
417 concentration was observed during the incubation time (**Extended Data Fig. 8**).

418

419 **Soil sampling and physicochemical analysis.** Four soil cores of 30 cm depth were collected
420 from each site (16 total). Cores were carefully extracted and immediately segmented at 5 cm
421 depth intervals before being transferred into 50 mL Falcon tubes. At four different depth intervals
422 (0-5, 5-10, 15-20, 25-30 cm), a subset of 10 g of soil was frozen at -20°C for community DNA
423 extraction and a subset of 5 g was used within 24 hours for gas chromatography studies.
424 Additional surface soil was collected using a 10 × 5 cm bulk density ring to estimate bulk density
425 and soil water content, which was measured gravimetrically using a drying oven at 140°C. For
426 soil chemistry analysis, surface soil samples (0-5 cm) from each plot were pooled to form one
427 representative composite sample per site and sent to the Environmental Analysis Laboratory
428 (EAL), Southern Cross University. In total, 21 separate soil chemical parameters were selected
429 for analysis, based on commonly reported drivers of soil microbial composition globally. These
430 included: soil acidity (pH), electrical conductivity (EC), effective cation exchange capacity
431 (ECEC), total organic carbon, total nitrogen, sodium (Na), sulfur (S), phosphate (P), potassium
432 (K), nitrate (NO₃⁻), and ammonium (NH₄⁺), as well as bioavailable manganese (Mn), copper
433 (Cu), zinc (Zn), boron (B), aluminium (Al), iron (Fe), and silicon (Si). In addition, particle size
434 analysis by hydrometry was performed to estimate the percentage of gravel, sand, silt, loam, and
435 clay (**Supplementary Table 1**).

436

437 ***Ex situ* oxidation rates.** To determine the capacity of the soils to oxidise trace gases at
438 atmospheric concentrations, 5 g soil from the four depth intervals (0-5, 5-10, 15-20, 25-30 cm)
439 were placed in 120 mL serum vials. The headspace was repeatedly flushed with air from a
440 pressurized cylinder (Air Liquide, Australia) to achieve headspace mixing ratios reflecting
441 atmospheric levels (~0.5 ppmv H₂, ~0.6 ppmv CO, ~1.8 ppmv CH₄). Sampling commenced
442 immediately after sealing the vial and headspace samples of 2 mL were taken at 10 min intervals
443 for 40 minutes. While this timecourse sufficiently captured oxidation rates for most samples,
444 additional gas samples were collected every 24 hrs for up to nine days to capture oxidation rates
445 of soils that mediated slow gas consumption. Gas concentrations were measured by gas
446 chromatography as described above. Heat killed soils (two 30-minute autoclave cycles at 121°C)
447 and blank measurements (empty serum vials) were used as controls, confirming trace gas
448 oxidation from samples occurred due to biotic processes. Given the low capacity of dryland soils
449 to oxidize atmospheric trace gases, we simulated soil wetting conditions due to a rainfall event to
450 determine whether soil hydration enhances activity. To do this, we used a custom Perspex collar
451 fitted with a water-draining stainless steel woven mesh (0.17 mm) and a water-catching tray.
452 Collars were sterilised using ethanol and soils (5 g) were placed in the centre of the mesh surface.
453 Soils were watered until fully saturated by repeated addition of MilliQ water. Once fully

454 saturated, each collar was sealed at the top using clingfilm to avoid evaporation and left to drain
455 for 24 hrs in the dark. Once drained, soil samples were transferred using sterile techniques into a
456 120 mL serum vial and gas oxidation was measured as described in the previous section. Similar
457 to *in situ* fluxes above, reaction rates were calculated from the first five data points using first-
458 and zero-order reaction models, with the best rate chosen according to AIC. The data was tested
459 for normality using a Shapiro-Wilk test. To test for significant difference in oxidation rates
460 between ecosystems and depths, a non-parametric one-way Kruskal-Wallis test was used. This
461 was followed by a pairwise Wilcoxon Rank Sum test to test significant relationships between pairs.
462 *p* values were adjusted using a Benjamini Hochberg correction.

463

464 **Community DNA extraction.** At each soil depth sampled per site, soils from the four plots were
465 pooled together. For each of the 16 resultant samples, total community DNA was extracted using
466 0.25 g soil. Extractions were performed using the MoBio PowerSoil Isolation kit according to the
467 manufacturer's instructions. Samples were eluted in DNase- and RNase-free UltraPure Water
468 (ThermoFisher). A sample-free negative control was also run. Nucleic acid purity and yield were
469 measured using a NanoDrop ND-1000 spectrophotometer and a Qubit Fluorometer 2.0.

470

471 **Quantitative PCR.** Quantitative polymerase chain reactions (qPCR) were used to estimate total
472 bacterial and archaeal biomass. The 16S rRNA gene was amplified using the degenerate primer
473 pairs 515F (GTGYCAGCMGCCGCGGTAA) and 806R (GGACTACNVTGGGTWTCTAAT)⁷⁴.
474 A synthetic *E. coli* 16S rRNA gene sequence in a pUC-like cloning vector (pMA plasmid;
475 GeneArt, ThermoFisher Scientific) was used as a standard. PCR reactions were set up in each
476 well of a 96-well plate using a LightCycler 480 SYBR Green I Master Mix. Each sample was run
477 in triplicate and standards in duplicate on a LightCycler 480 Instrument II (Roche). The qPCR
478 conditions were as follows: pre-incubation at 95°C for 3 min and 45 cycles of denaturation 95°C
479 for 30 s, annealing at 54°C for 30 s, and extension at 72°C for 24 s. 16S rRNA gene copy
480 numbers were calculated based on a standard curve constructed by plotting average Cp values of
481 a serial dilution of the plasmid-borne standard against their copy numbers.

482

483 **Sequencing, assembly, and binning of Australian metagenomes.** Metagenomic shotgun
484 libraries were prepared for the 16 samples using the Nextera XT DNA Sample Preparation Kit
485 (Illumina Inc., San Diego, CA, USA). Sequencing was performed on an Illumina NextSeq500
486 platform with a 2 × 150 bp High Output run. Raw reads derived from the 16 metagenome
487 libraries were quality-controlled by clipping off primers and adapters then filtering out artifacts
488 and low-quality reads using Read_QC module within the metaWRAP pipeline⁷⁵. For each

489 ecosystem, the four quality-controlled metagenomes were co-assembled using MEGAHIT v1.1.3
490 ⁷⁶ (default parameters) and individually assembled using SPAdes v3.13.0 ⁷⁷ (metaSPAdes mode,
491 default parameters), producing five assemblies for each ecosystem. Short contigs (<1000 bp)
492 were removed. Each assembly was binned using the binning module within the metaWRAP ⁷⁵
493 pipeline (MetaBAT ⁷⁸, MetaBAT2 ⁷⁹, and MaxBin2 ⁸⁰). For each assembly, the three bin sets
494 were then consolidated into a final bin set with the bin_refinement module of metaWRAP ⁷⁵. For
495 each ecosystem, the final bin sets were aggregated and de-replicated using dRep ⁸¹ (-comp 50 -
496 con 10 options). Completeness, contamination, and heterogeneity of each bin were estimated
497 using CheckM ⁸², with medium- and high-quality bins (completeness >50%, contamination
498 <10%) ⁸³ retained for further analysis. After dereplication, a total of 93 metagenome-assembled
499 genomes (MAGs) were obtained for the four ecosystems. Each bin was taxonomically assigned
500 according to the Genome Taxonomy Database (GTDB; release 04-RS89) ⁸⁴ using GTDB-tk ⁸⁵.

501

502 **Assembly and binning of global public soil metagenomes.** A total of 24 previously sequenced
503 metagenomes from eight different soil ecosystems (three metagenomes for each ecosystem)
504 (**Supplementary Table 2**) were downloaded from the Integrated Microbial Genomes database ⁸⁶
505 and the NCBI Sequence Read Archive (SRA) ⁸⁷. These comprised: Barrow Environmental
506 Observatory site, Barrow, Alaska, USA (Arctic Tundra, BEO) ⁸⁸; St. Claude, Quebec, Canada
507 (Agricultural Land – Crop Rotation, SCQ) ⁸⁹; Kellogg Biological Station, Michigan, USA
508 (Agricultural Land – Switchgrass, KBS) ⁹⁰; Algoma, Ontario, Canada (Coniferous Forest, ALO)
509 ⁹¹; Anza Borrego Desert, California, USA (Hot Desert, ABD) ⁹²; Department of Meta, Colombia
510 (Tropical Peatland, DMC) ⁹³; National Park of Serra do Cipo, Brazil (Rupestrian Grassland, NPS)
511 ⁹⁴; and Luquillo Experimental Forest, Rio Grande, Puerto Rico (Tropical Rainforest, LEF) ⁹⁵.
512 Raw reads derived from the 24 metagenome libraries were quality-controlled by clipping off
513 primers and adapters and filtering out artefacts and low-quality reads using Read_QC module in
514 the metaWRAP pipeline ⁷⁵. For each ecosystem, the three quality-controlled metagenomes were
515 both co-assembled and individually assembled using MEGAHIT v1.1.3 ⁷⁶, producing four
516 assemblies for each ecosystem. For the assembly process, all of them used default parameters
517 except co-assembly of metagenomes for Kellogg Biological Station (--k-min 27). Short contigs
518 (<1000 bp) were removed. Each assembly was binned using the binning module within the
519 metaWRAP ⁷⁵ options (MetaBAT ⁷⁸, MetaBAT2 ⁷⁹ and MaxBin2 ⁸⁰) except assemblies derived
520 from Kellogg Biological Station and Luquillo Experimental Forest where only MetaBAT2 ⁷⁹ was
521 used. Dereplication and quality-control of produced bins were performed as above. After
522 dereplication, a total of 664 high- or medium-quality MAGs were obtained for the eight
523 ecosystems. Each bin was taxonomically assigned as above.

524

525 **Functional annotation of binned and unbinned contigs.** The sequences of 43 marker genes
526 representing energy conservation and carbon acquisition processes were retrieved from binned
527 and unbinned contigs. Open reading frames (ORFs) were first predicted using Prodigal v.2.6.3⁹⁶
528 and genes were annotated using a combination of homology-based searches and hidden Markov
529 model (HMM) searches. For homology-based searches, predicted ORFs were searched using
530 DIAMOND *blastp*⁹⁷ against 32 custom protein databases described below. These encompassed
531 the genes encoding ATP-citrate lyase (AclB), acetyl-CoA synthase (AcsB), ammonia
532 monooxygenase (AmoA), anaerobic sulfite reductase (AsrA), anaerobic carbon monoxide
533 dehydrogenase (CooS), form I aerobic carbon monoxide dehydrogenase (CoxL), dissimilatory
534 sulfite reductase (DsrA), flavocytochrome *c* sulfide dehydrogenase (FCC), 4-hydroxybutyryl-
535 CoA synthase of Crenarchaeota (HbsC), 4-hydroxybutyryl-CoA synthase of Thaumarchaeota
536 (HbsT), hydrazine synthase (HzsA), malonyl-CoA reductase of Chloroflexota (Mcr),
537 methyl/alkyl-CoM reductase (McrA), soluble methane monooxygenase (MmoX), periplasmic
538 nitrate reductase (NapA), dissimilatory nitrate reductase (NarG), nitrogenase (NifH), copper-
539 containing nitrite reductase (NirK), cytochrome *cd*₁ nitrite reductase (NirS), nitrous oxide
540 reductase (NosZ), ammonia-forming nitrite reductase (NrfA), nitrite oxidoreductase (NxrA),
541 particulate methane monooxygenase (PmoA), ribulose 1,5-bisphosphate carboxylase/oxygenase
542 (RbcL), succinate dehydrogenase / fumarate reductase (SdhA / FrdA), sulfur
543 oxygenase/reductase (Sor), thiosulfohydrolase (SoxB), sulfide-quinone oxidoreductase (Sqr), and
544 three hydrogenase classes (NiFe-hydrogenase large subunit, FeFe-hydrogenase catalytic domain,
545 Fe-hydrogenase). DIAMOND mapping was performed with a query coverage threshold of 80%
546 for all databases, and a percentage identity threshold of 60% (AmoA, PmoA, MmoX, CoxL,
547 HbsT, NxrA, RbcL) or 50% (all other databases) and e-value thresholds of 10⁻²⁰. HMM searches
548 were performed against Pfam and Tigrfam databases using the *annotate* function of EnrichM
549 v.0.5.0 (<https://github.com/geronimp/enrichM>), with domain noise-cut-off scores as previously
550 described⁹⁸. Eleven genes encoding subunits of ATP synthase (AtpA), two NADH
551 dehydrogenases (NuoF, NqrF), four terminal oxidases (CcoN, CoxA, CydA, CyoA), two
552 photosystems (PsaA, PsbA), formate dehydrogenase (FdhA), and reductive dehalogenase
553 (RdhA) were searched.

554

555 **Metabolic annotation of metagenomic short reads.** For the functional annotation of short
556 reads, paired-end reads in each sample were stripped of adapter and barcode sequences, then
557 contaminating PhiX and low quality sequences were removed (minimum quality score 20) using
558 the BBDuk function of BBTools v. 36.92 (<https://sourceforge.net/projects/bbmap/>). Resultant

559 quality-filtered forward reads with lengths of at least 100 bp were searched for the presence of the
560 43 metabolic marker genes described above using DIAMOND *blastx* algorithm ⁹⁹. Specifically,
561 reads were searched against the 32 custom-made reference databases and hits from the 11 HMM
562 searches, using a query coverage of 80% and an identity threshold of either 60% (AmoA, PmoA,
563 MmoX, CoxL, HbsT, NxrA, RbcL) or 50% (all other databases) and a maximum e-value
564 threshold of 10⁻¹⁰. Read counts were normalized to reads per kilobase per million (RPKM) and
565 further normalized against a mean RPKM value estimated from 14 single copy ribosomal marker
566 genes to infer the percentage of the community encoding the gene.

567

568 **Metabolic annotation of metatranscriptomic reads.** Three previously sequenced soil
569 metatranscriptomes collected from sampling locations matching three metagenomes (Extended
570 Data Table S2) were downloaded from the Integrated Microbial Genomes database ¹⁶ and the
571 NCBI Sequence Read Archive (SRA) ¹⁷. These comprised: Kellogg Biological Station,
572 Michigan, USA (Agricultural Land – Switchgrass, KBS) ⁹⁰; Anza Borrego Desert, California,
573 USA (Hot Desert, ABD) ⁹²; Department of Meta, Colombia (Tropical Peatland, DMC) ⁹³. Raw
574 metatranscriptomes were quality filtered as described above and SortMeRNA (version 4.2.0) ¹⁰⁰
575 was used to remove ribosomal RNA (rRNA) reads. To determine transcript abundances of 43
576 metabolic marker genes described above, filtered reads with lengths of at least 100 bp were
577 searched for their presence using DIAMOND *blastx* and read counts were normalized to reads
578 per kilobase per million (RPKM). To calculate transcript abundances of genes annotated from
579 metagenomic assemblies and MAGs, Salmon v1.3.0 ¹⁰¹ was used in mapping-based mode
580 (salmon quant, --validate Mappings) and read counts were normalised to transcripts per million
581 (TPM). For genes predicted from metagenomic assemblies, they were firstly clustered at 95%
582 sequence identity to produce a non-redundant gene catalog as a ‘reference transcriptome’ using
583 CD-HIT v4.8.1 ¹⁰² before Salmon quantifications.

584

585 **Phylogenetic analysis.** Phylogenetic trees were constructed to understand the distribution and
586 diversity of bacteria and archaea consuming inorganic energy sources. Trees were constructed
587 for the group 1 and 2 [NiFe]-hydrogenase large subunits, group 3 [NiFe]-hydrogenase large
588 subunits, CoxL, PmoA, RbcL, AmoA, NxrA, Sqr, SoxB, and DsrA. In all cases, protein
589 sequences retrieved from the MAGs reads by homology-based searches were aligned against a
590 subset of reference sequences from the custom protein databases using ClustalW in MEGA7 ¹⁰³.
591 Evolutionary relationships were visualized by constructing a maximum-likelihood phylogenetic
592 tree; specifically, initial trees for the heuristic search were obtained automatically by applying
593 Neighbour-Join and BioNJ algorithms to a matrix of pairwise distances estimated using a JTT

594 model, and then selecting the topology with superior log likelihood value. All residues were used
595 and trees were bootstrapped with 50 replicates. To further visualize the diversity of trace gas
596 oxidizers, neighbor-joining trees were constructed using the binned and unbinned sequences for
597 group 1 and 2 [NiFe]-hydrogenase large subunits and CoxL; these trees were constructed using
598 the Poisson model with gaps treated with pairwise deletion and bootstrapped with 50 replicates.

599

600 **Protein homology modelling.** Structural homology models for particulate methane
601 monooxygenase subunits PmoA, PmoB, and PmoC from *Candidatus Methylophilum kingii*,
602 *Methylacidiphilum infernorum* (PmoA: WP_012463843, PmoB: WP_012463845, PmoC:
603 WP_012463844) and *Candidatus Methylophilum limnetica* (PmoA: WP_107560874, PmoB:
604 WP_107560875, PmoC: WP_107560873) were generated using the Phyre2 webserver¹⁰⁴. Direct
605 threading of Pmo subunit amino acid sequences was performed using the closest structurally
606 characterized particulate methane monooxygenase homologue to each strain, namely those of
607 *Methylocystis* sp. strain M (PDB ID: 3RFR)¹⁰⁵ for *Ca. M. kingii* and *Methylococcus capsulatus*
608 (PDB ID: 3RGB)¹⁰⁵ for *M. infernorum* and *Ca. M. limnetica*. Direct threading was used to avoid
609 modelling using earlier erroneously modelled Pmo structures (PDB IDs: 1YEW and 3CHX)
610 present in the PDB.

611

612 **Analysis of community composition and diversity.** Bacterial and archaeal community
613 composition was determined from the pre-processed metagenomic reads with SingleM v0.12.1
614 (<https://github.com/wwood/singlem>). In total, 28 HMM searches were performed against 14
615 single-copy ribosomal marker genes. The gene for single-copy ribosomal protein L16/L10E
616 (*rplP*) was selected for downstream analysis and sequences were clustered *de novo* into
617 operational taxonomic units at a sequence identity threshold of 97%. Taxonomic assignment was
618 carried out using the Genome Taxonomy Database⁸⁴. Community richness and beta diversity
619 were calculated using the phyloseq¹⁰⁶ and R package VEGAN¹⁰⁷. To account for differences in
620 richness between samples, all sequences were rarefied to within 90% of the minimum sequence
621 count. Observed richness and estimated richness (Chao1) were calculated. First, beta diversity
622 (Bray-Curtis) was calculated and visualised using a multidimensional scaling ordination (MDS),
623 then significance testing was carried out using a permutational analysis of variance
624 (PERMANOVA) to test for significant differences in community structure between ecosystems
625 and between depth profiles. A beta dispersion test (PERMDISP) was used to ascertain if
626 significant differences in community structure were due to data dispersion.

627

628 **Thermodynamic modelling.** Cell-specific power (i.e. Gibbs energy per unit time per microbial
629 cell), P (W), was calculated for each reaction using measured *ex situ* oxidation rates according to:
630

$$631 \quad P = \frac{r \cdot \Delta G_r}{B} \quad (\text{EQ. 4})$$

632
633 where r denotes the rate of reaction ($\text{mol s}^{-1} \text{g}_{\text{dry soil}}^{-1}$), ΔG_r represents the Gibbs energy of the
634 reaction (J mol^{-1}), and B ($\text{cells g}_{\text{dry soil}}^{-1}$) is the number of microbial cells carrying out each of the
635 following the reactions: $\text{H}_2 + 0.5 \text{O}_2 \rightarrow \text{H}_2\text{O}$ (dihydrogen oxidation); $\text{CO} + 0.5 \text{O}_2 \rightarrow \text{CO}_2$
636 (carbon monoxide oxidation); and $\text{CH}_4 + 2 \text{O}_2 \rightarrow \text{CO}_2 + 2 \text{H}_2\text{O}$ (methane oxidation). Values of
637 ΔG_r are calculated using:

$$638 \quad \Delta G_r = \Delta G_r^0 + RT \ln Q_r \quad (\text{EQ. 5})$$

639
640
641 where ΔG_r^0 and Q_r refer to the standard Gibbs energy and the reaction quotient of the indicated
642 reaction, respectively, R represents the gas constant, and T denotes temperature in Kelvin. Values
643 of ΔG_r^0 were calculated using the revised-HKF equations of state^{108–110}, the SUPCRT92
644 software package¹¹¹, and thermodynamic data taken from references^{109,112–115}.

645
646 Values of Q_r are calculated for each reaction using:

$$647 \quad Q_r = \prod a_g^{v_i} \quad (\text{EQ. 6})$$

648
649
650 where a_i stands for the activity of the i^{th} species and v_i corresponds to the stoichiometric
651 coefficient of the i^{th} species in the reaction of interest. Activities of gas-phase compounds, a_g ,
652 were calculated using:

$$653 \quad a_g = \frac{f_g}{f_g^0} \quad (\text{EQ. 7})$$

654
655 where f_g and f_g^0 designate the fugacity and standard state fugacity of the respective gas. Due to
656 the low temperatures and pressures of soil ecosystems, fugacity coefficients for all gases are one
657 (see¹¹⁶). Therefore, partial pressures are equivalent to fugacity since f_g^0 was taken to be 1 bar.
658 Gibbs energy calculations were carried out at 20°C and 1 bar. The concentrations of reactants in
659 the catabolic reactions and reaction rates were measured by gas chromatography as described
660 above.

661

662 Estimates of the number of microbial cells carrying out each reaction (B) were obtained by
663 calculating the proportion of cells performing a specific catabolic reaction in our 16S rRNA gene
664 copy number dataset, assuming all cells to be active. The proportion of a specific functional
665 community was retrieved from the relative abundance of the individual biomarker genes
666 (encoding the large subunits of group 1c, 1d, 1f, 1h, and 2a [NiFe]-hydrogenase, carbon
667 monoxide dehydrogenase (CoxL), particulate methane monooxygenase (PmoA), and soluble
668 methane monooxygenase (MmoX)) in our metagenomics dataset. An average of 4.2 16S rRNA
669 copies per cell was assumed for cell number estimation¹¹⁷.

670

671 To compare our approach to previous literature, we repeated an earlier attempt to estimate the
672 theoretical maximum population of trace gas oxidizing bacteria from modelled ΔG_r and measured
673 reaction rates⁶⁸:

674

$$675 \quad N_t = \frac{N_c}{m_E} \Delta G_r r \quad (\text{EQ. 8})$$

676

677 Here, m_E is a mean maintenance energy at 293K in [kJ (C-mol biomass)⁻¹ h⁻¹], estimated from
678 pure-culture data of different metabolisms⁶³; N_c is the number of cells per C-mol of biomass (1.4
679 $\times 10^{14}$ cells C-mol⁻¹⁶⁸, equivalent to 8.6×10^{-14} g C dry cell⁻¹); and r is the trace-gas reaction rate
680 as defined above. The ratio N_t / B (the fraction of the estimated trace gas oxidizing population that
681 can be supported given a specific maintenance energy) is equivalent to dividing cell-specific
682 power by the given maintenance energy, after conversion to W cell⁻¹ via N_c :

683

$$684 \quad \frac{N_t}{B} = \frac{\frac{N_c \Delta G_r r}{m_E}}{B} = \frac{P}{\frac{m_E}{N_c}} \quad (\text{EQ. 9})$$

685

686

687 **Reporting summary.** Further information on research design is available in the Nature Research
688 Reporting Summary linked to this article.

689 **Data Availability**

690 All data supporting the findings of the present study are available. All numerical data used to
691 make figures is provided in **Source Data 1** (XLSX format) and all raw phylogenetic trees are
692 provided in Newick (NWK) format in **Source Data 2** (ZIP format). All metagenomes sequenced
693 for this project can be accessed at the Sequence Read Archive with accession number
694 PRJNA656125 (<https://www.ncbi.nlm.nih.gov/bioproject/PRJNA656125>) and all metagenome-
695 assembled genomes are available at FigShare
696 (https://figshare.com/articles/dataset/Metagenome_Assembled_Genomes/12782543). All
697 previously sequenced metagenomes and metatranscriptomes analysed in this study are available
698 at NCBI BioProject with the accession numbers listed in **Supplementary Table 2**.

699

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982

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991

992 **Author information**

993

994 **Contributions**

995 C.G. conceived and supervised this study. S.K.B., C.G., E.C., and P.A.N. designed experiments
996 and analyzed data. Different authors were responsible for performing fieldwork (S.K.B., E.C.,
997 P.M.L., C.G.), laboratory work (S.K.B., T.J., E.C.), metagenome assembly (X.D.), metagenome
998 analysis (S.K.B., C.G., X.D., E.C.), phylogenetic analysis (C.G.), protein modelling (R.G.), and
999 thermodynamic modelling (J.A.B., P.A.N., D.L., E.C., S.K.B.). S.K.A. and P.L.M.C. provided
1000 logistical and theoretical support. C.G., S.K.B., and P.A.N. wrote the paper with input from all
1001 authors.

1002

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1007 **Ethics declarations**

1008

1009 **Competing interests**

1010 The authors declare no competing financial interests.

1011

1012 **Additional information**

1013

1014 **Etymological Information**

1015 *Candidatus* Methylophilum (Me.thy.lo.tro'pi.cum.) N.L. n. *methylum* (from
1016 French *méthyle* back-formation from French *méthylène*, coined from Gr. n. *methu*, wine and Gr.
1017 n. *hulê*, wood), the methyl group; N.L. pref. *methylo-*, pertaining to the methyl radical; L. masc.
1018 adj. *tropicus* pertaining to tropical zone of the Earth; N.L. neut. n. *Methylophilum* referring to a
1019 methyl-using bacterium from the tropics. *Candidatus* Methylophilum kingii (ki'ngi.i.) N.L.
1020 gen. n. *kingii* in honor of Prof. Gary King, who has pioneered research into the microbial
1021 oxidation of methane, CO, and H₂.
1022

1023 **Figure legends**

1024

1025 **Fig. 1.** Energy conservation and carbon acquisition strategies of global soil bacteria. Homology-
1026 based searches were used to detect marker genes for key metabolic processes in the metagenomic
1027 short reads and metagenome-assembled genomes. **(a)** Heatmaps showing the abundance of each
1028 gene in the metagenomic short reads of the four Australian depth profiles and eight global sites.
1029 The percentage of the total community predicted to encode at least one of each gene for a
1030 process is shown, based on normalization to single-copy marker genes. The genes detected are
1031 usually present in single copies in genomes and, where genes performing similar functions are
1032 collapsed together, the values are summed up to 100%. **(b)** Dot plot showing the metabolic
1033 potential of the 757 metagenome-assembled genomes (MAGs). The size of each point represents
1034 the number of genomes in each phylum that encode the gene of interest and the shading
1035 represents the average genome completeness. Taxonomy assignment is based on the Genome
1036 Taxonomy Database (GTDB)⁵².

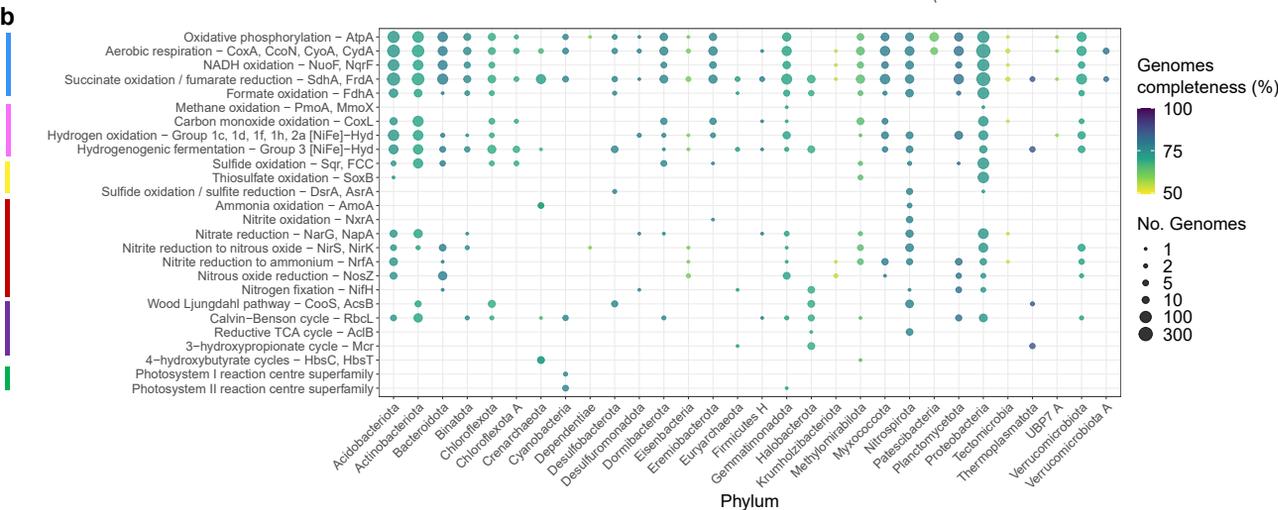
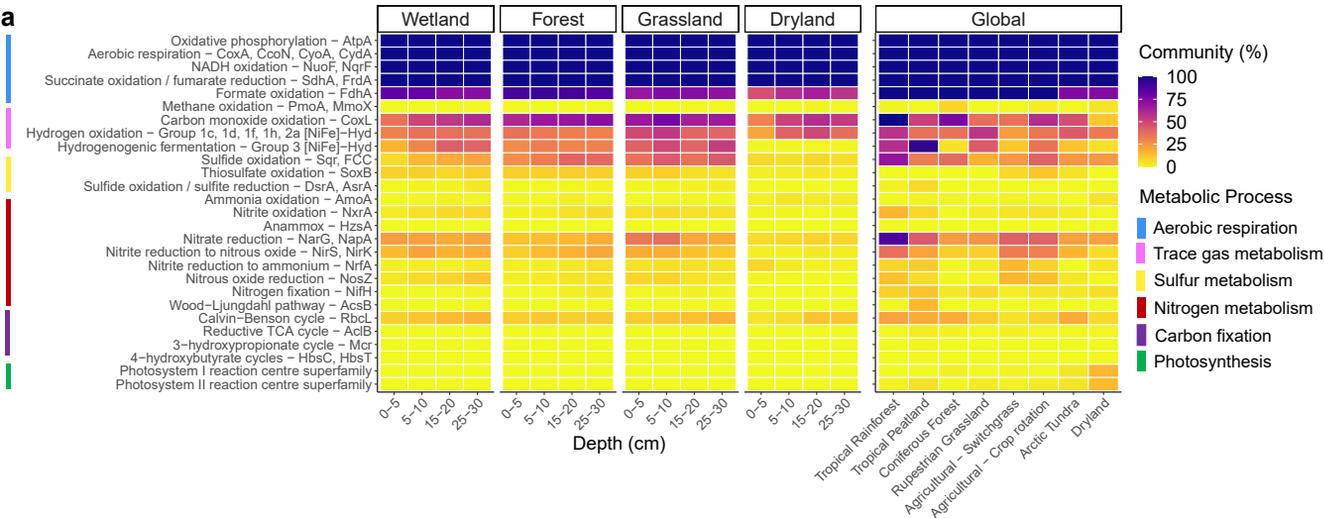
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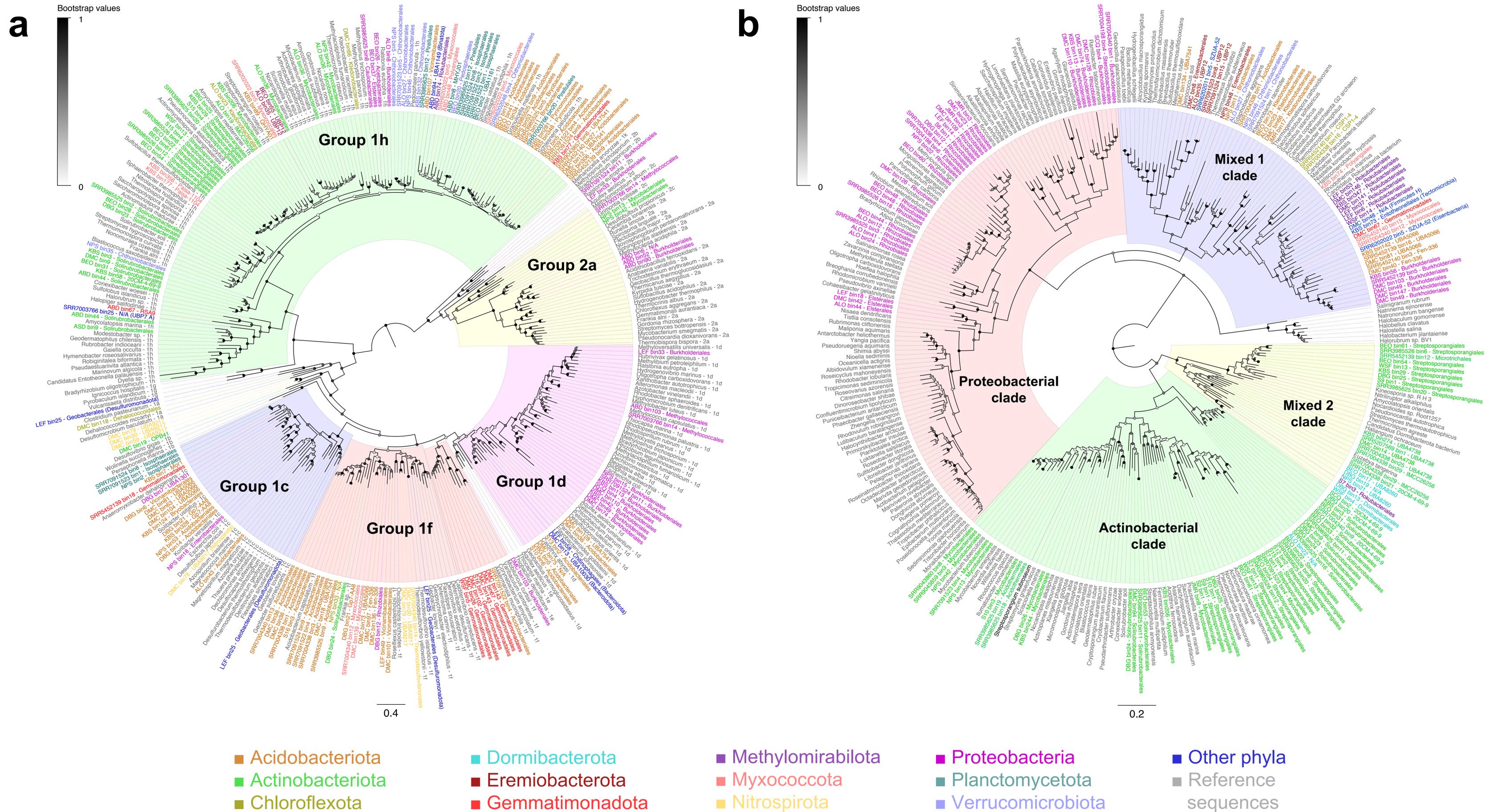
1038 **Fig. 2.** Radial maximum-likelihood phylogenetic trees showing the sequence diversity and
1039 taxonomic distribution of key enzymes associated with trace gas oxidation. The trees show the
1040 amino acid sequences of **(a)** group 1 and 2 [NiFe]-hydrogenase large subunits and **(b)** form I
1041 [MoCu]-carbon monoxide dehydrogenase large subunit (CoxL). The tree shows sequences from
1042 the soil metagenome-assembled genomes (taxa names colored by phylum; 167 hydrogenase
1043 sequences, 141 CO dehydrogenase sequences) alongside representative sequences from NCBI
1044 reference genomes (taxa names shown in grey; 168 hydrogenase sequences, 171 CO
1045 dehydrogenase sequences). The hydrogenase subgroups and carbon monoxide dehydrogenase
1046 clades predicted to support trace gas oxidation in the sampled soils are highlighted. Both trees
1047 were constructed using the JTT matrix-based model, used all sites, and were bootstrapped with
1048 50 replicates. Source data is provided in Newick (nwk) format.

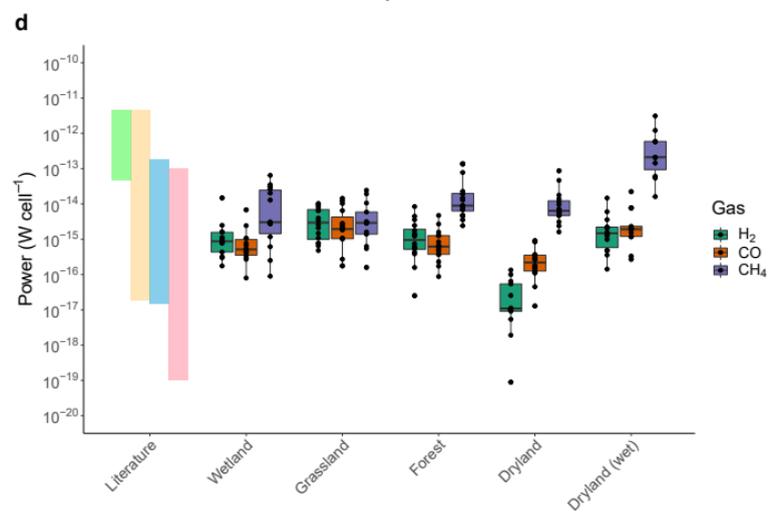
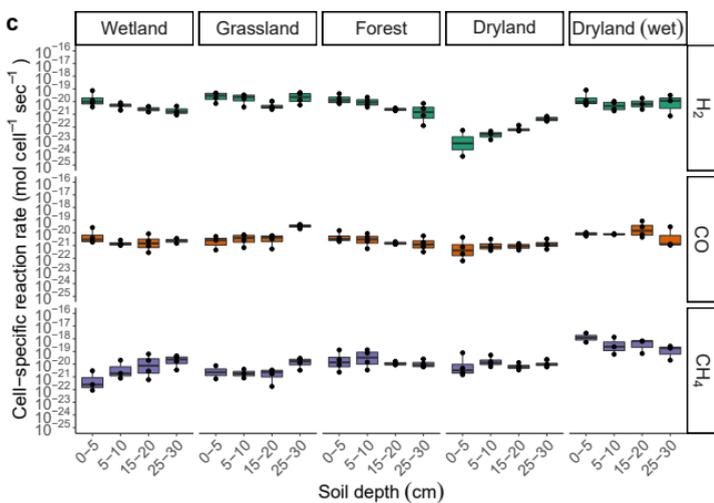
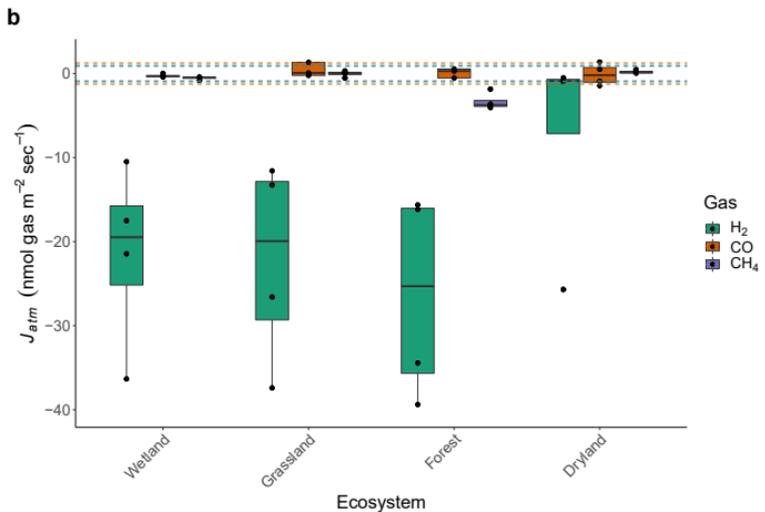
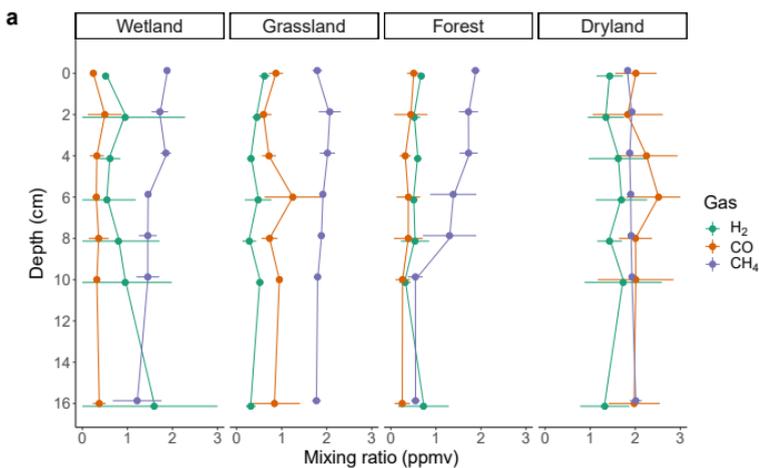
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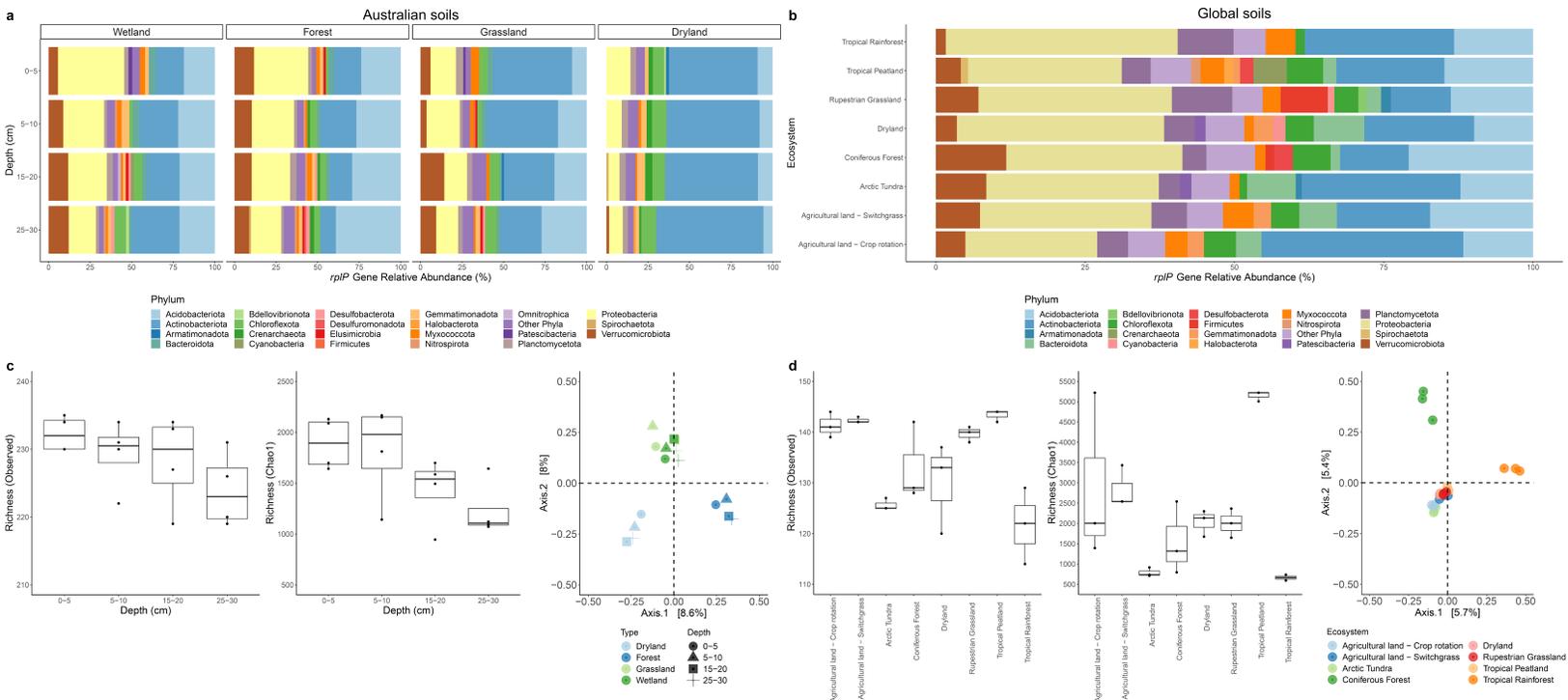
1050 **Fig. 3.** Measurement of oxidation of the trace gases H₂, CO, and CH₄ across four Australian soil
1051 ecosystems. **(a)** Depth-resolved *in situ* gas concentrations from 0 to 16 cm depth at each
1052 ecosystem (wetland, grassland, forest, dryland). **(b)** *In situ* soil-atmosphere gas fluxes (J_{atm} ;
1053 positive values indicate net gas production, negative values indicate net gas consumption).
1054 Dashed lines represent upper and lower simulated flux detection limits, with most CO and CH₄
1055 flux values falling within them. For **a** and **b**, measurements were performed in four separate soils
1056 per biome and error bars showing standard deviations. **(c)** Depth-resolved, cell-specific *ex situ*
1057 oxidation rates for each ecosystem of core samples from four different depths (0-5, 5-10, 15-20,

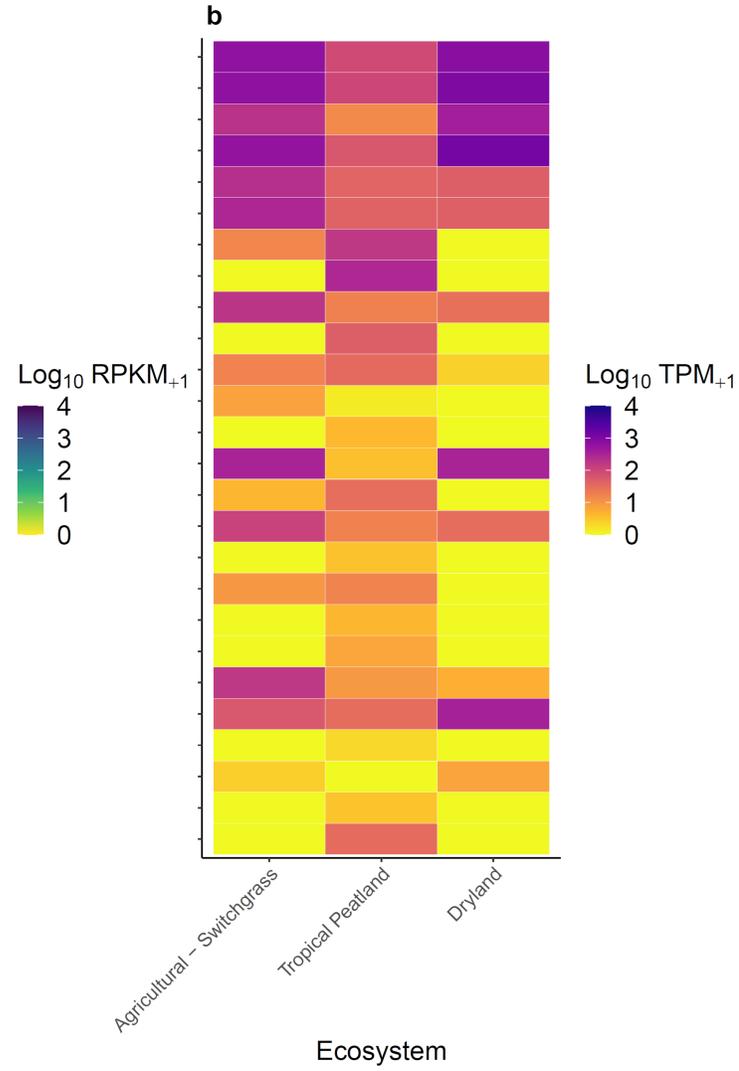
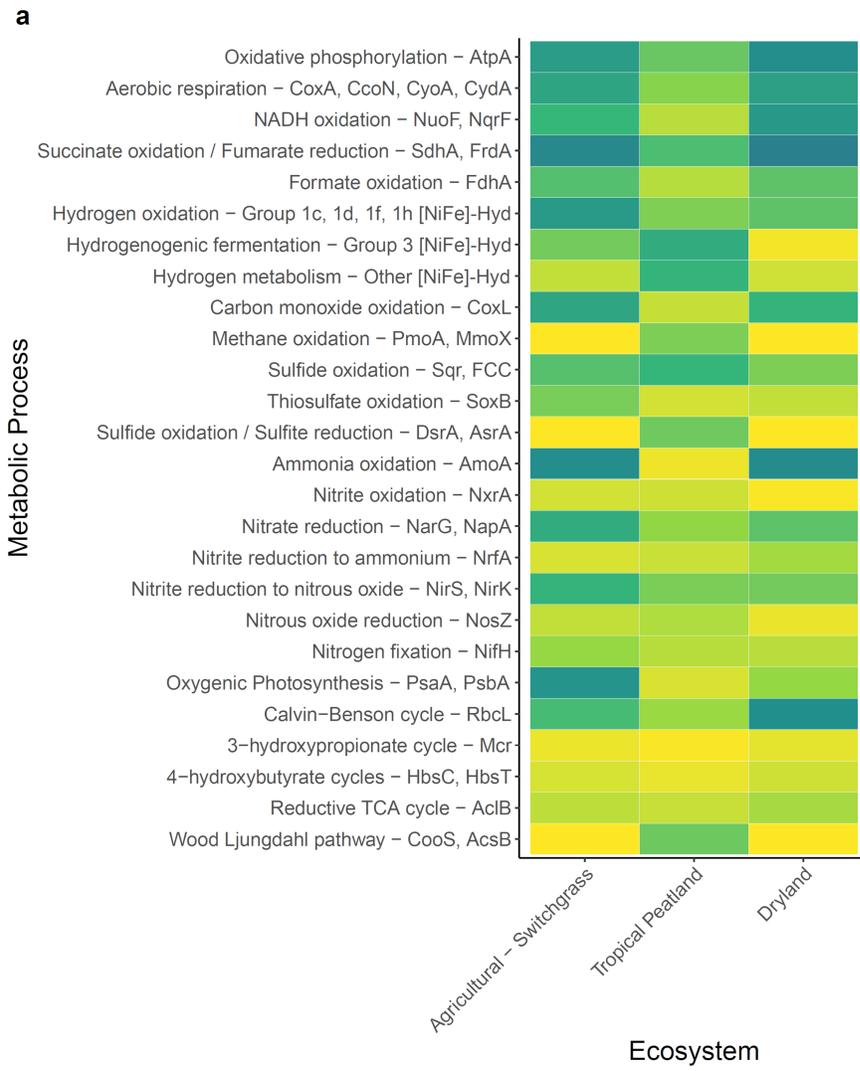
1058 25-30 cm). Measurements were performed with four biological replicates for each soil ecosystem
1059 and treatment (dryland wet). Oxidation rate values are normalized to total predicted cell number
1060 of trace gas oxidizers based on 16S rRNA gene qPCR and percentage of the community
1061 predicted to oxidize each gas. **(d)** Amount of power per cell derived from the oxidation of each
1062 trace gas. These were calculated using thermodynamic models based on *ex situ* rates measured
1063 across the four depths per habitat. The colored bars depict the range of literature values of
1064 maintenance energy requirements or endogenous metabolic rates of different pure cultures (green
1065 ⁶³, yellow ⁶⁴, blue ⁶⁰) and hydrogen oxidizers in deep marine sediments (pink ^{64,65}). For **c** and **d**,
1066 boxplots show means, lower and upper quartile, and minimum and maximum values.
1067

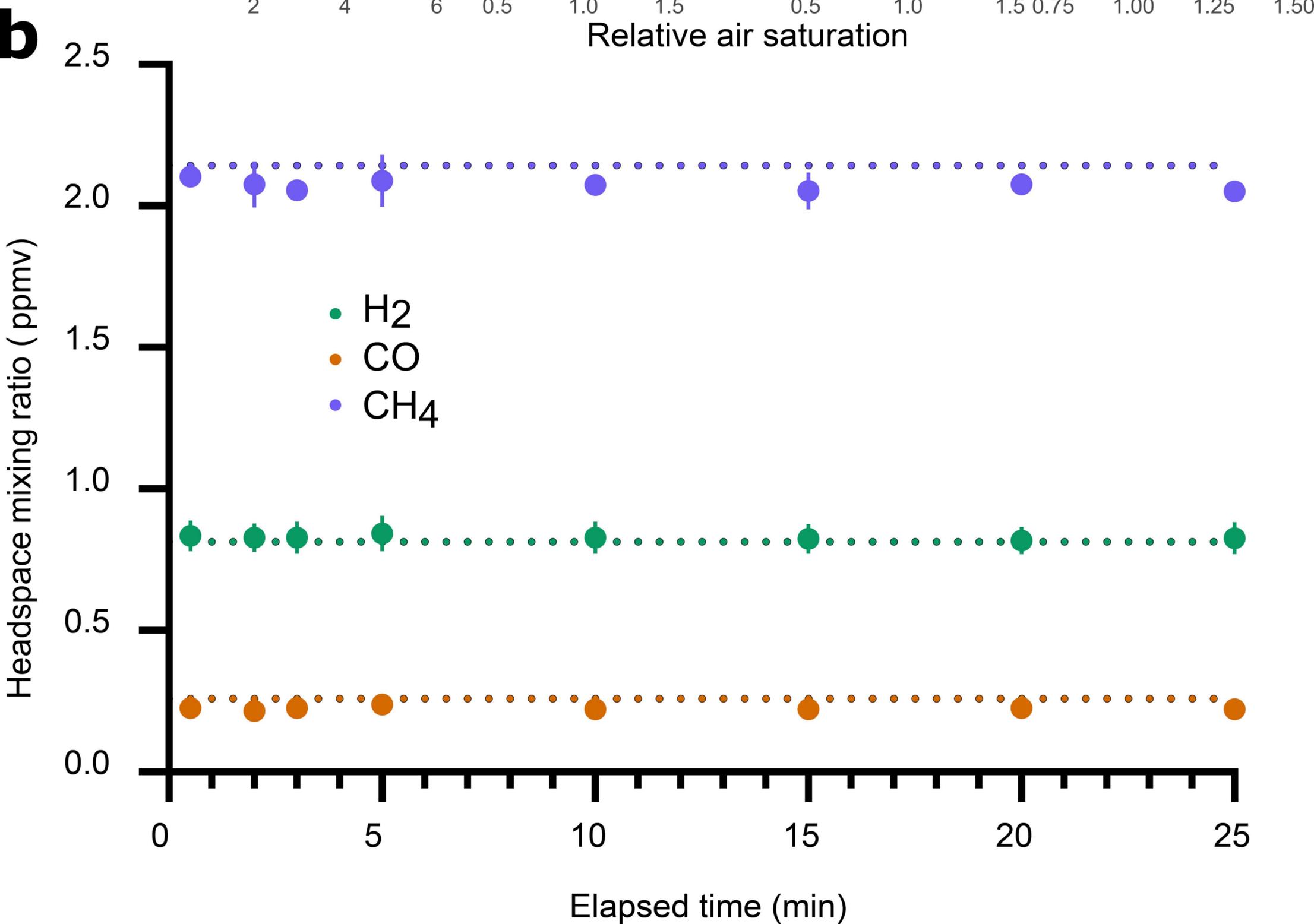
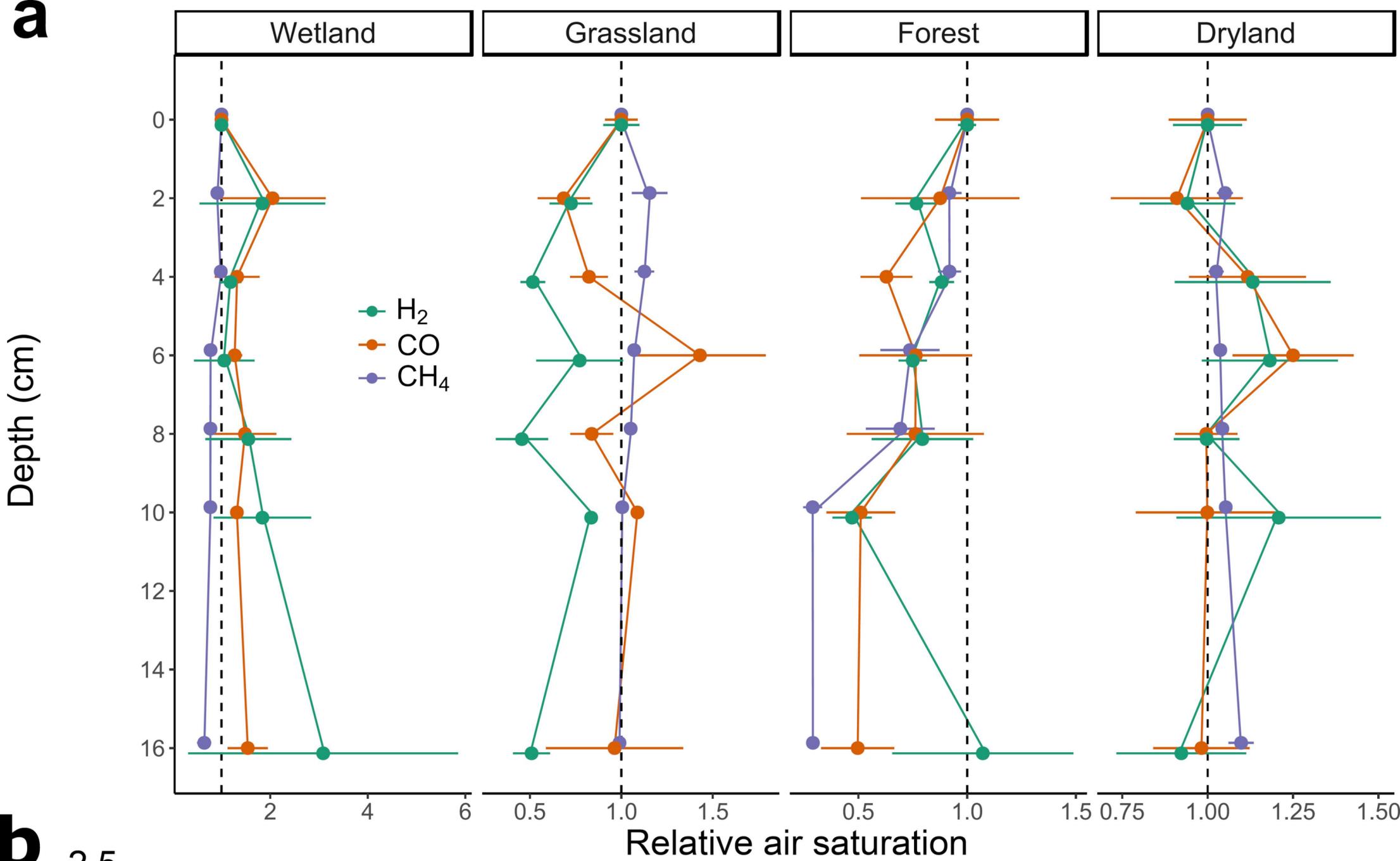


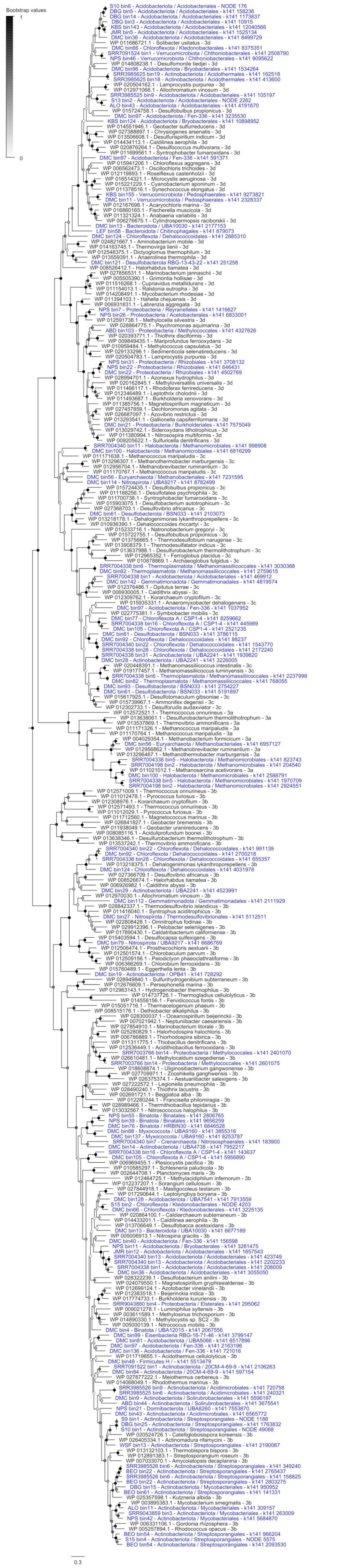


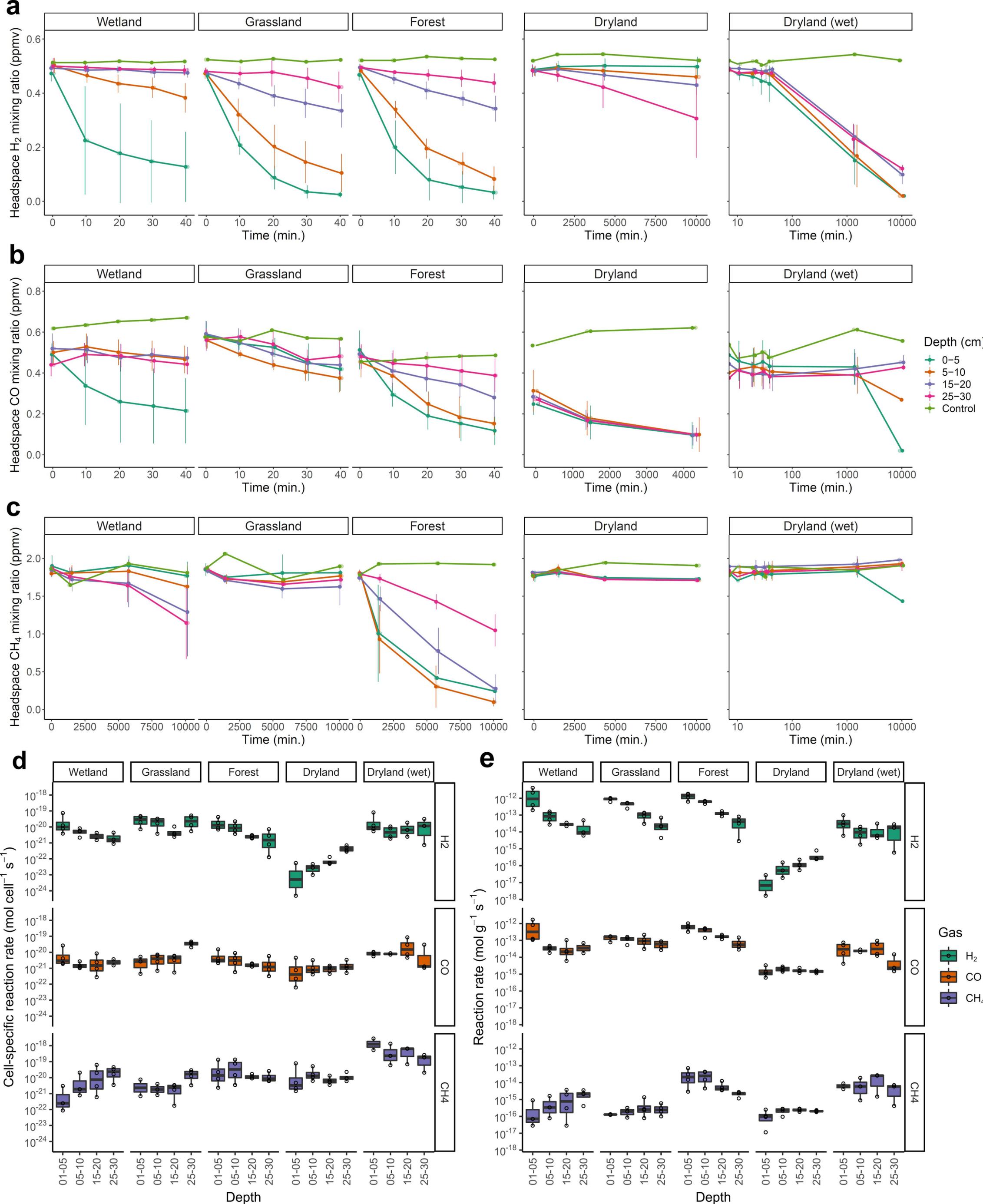


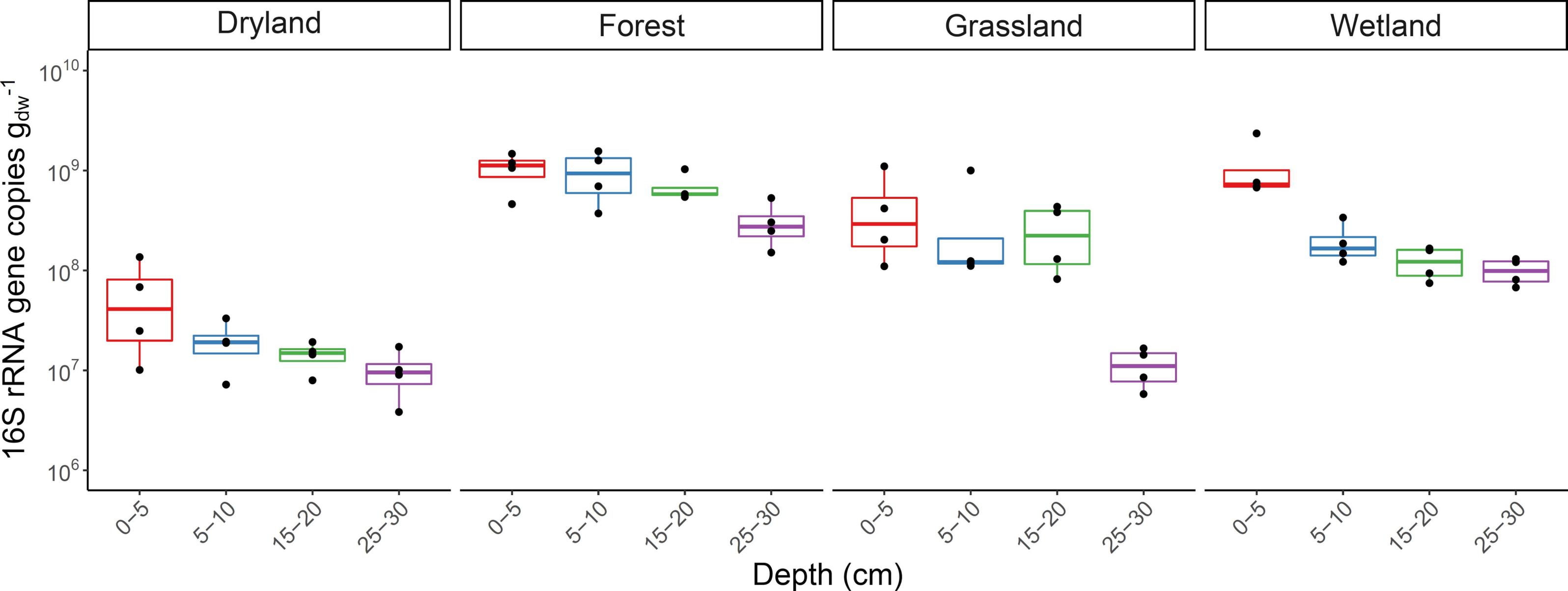


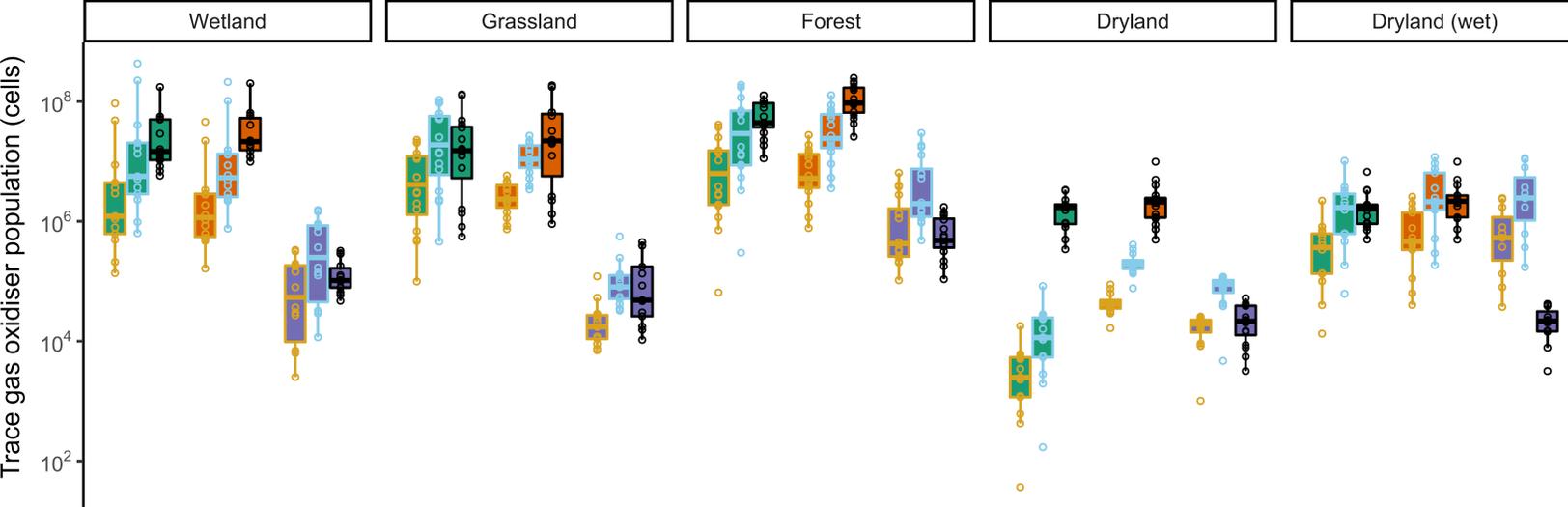


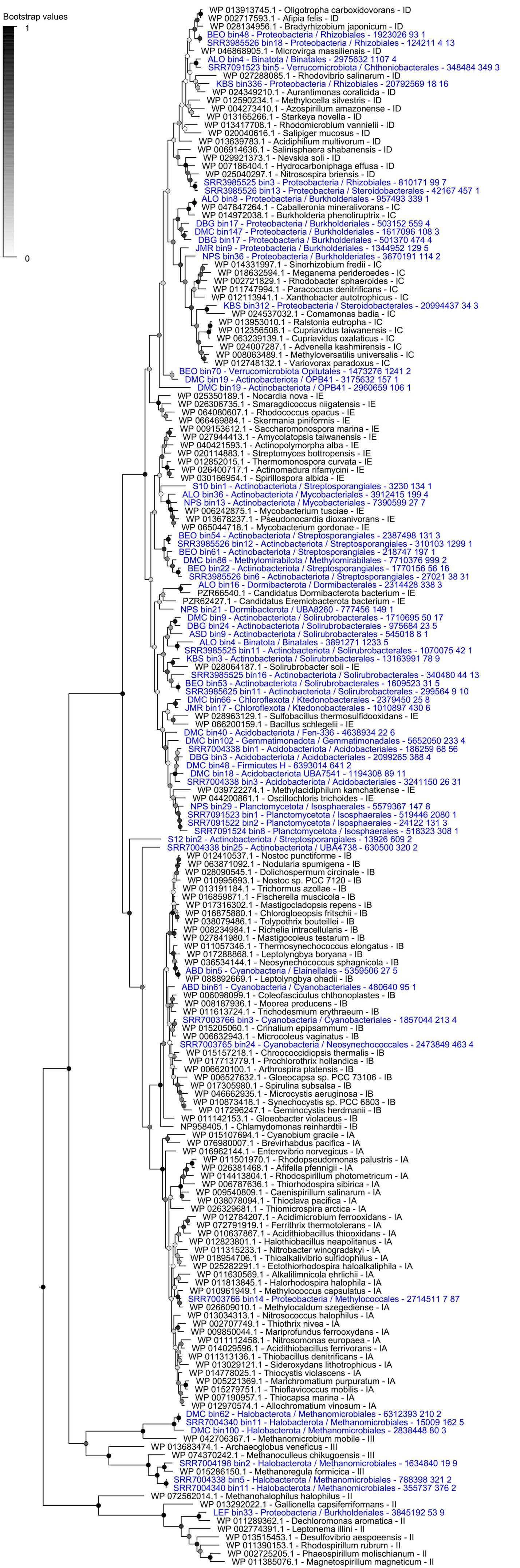




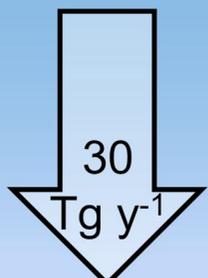
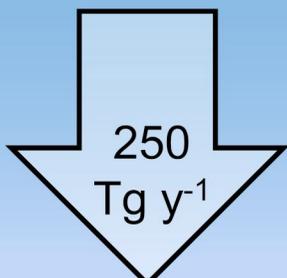
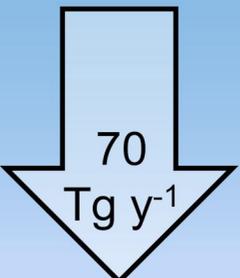
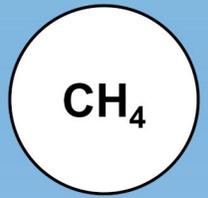
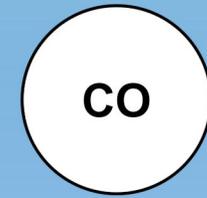
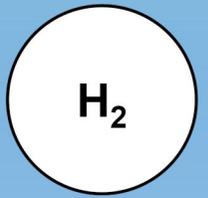








ATMOSPHERE



TRACE GAS OXIDIZERS

- Acidobacteriota
- Actinobacteriota
- Proteobacteria
- Gemmatimonadota
- Verrucomicrobiota
- Planctomycetota
- Myxococcota
- Nitrospirota
- Bacteroidota

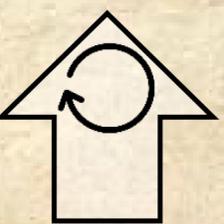
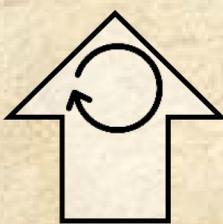
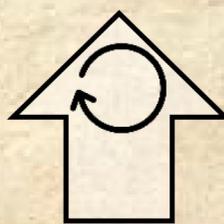
- Binatota
- Chloroflexota
- Desulfuromonadota
- Dormibacterota
- Eisenbacteria
- Eremiobacterota
- Methylomirabilota
- UBP7 A

- Actinobacteriota
- Proteobacteria
- Acidobacteriota
- Dormibacterota
- Eremiobacterota
- Methylomirabilota
- Myxococcota

- Chloroflexota
- Chloroflexota A
- Firmicutes H
- Gemmatimonadota
- Tectomicrobia
- Verrucomicrobiota

- Proteobacteria
- Gemmatimonadota (Methylomirabilota)
- (Verrucomicrobiota)

O₂



NITROGEN FIXATION

THERMAL/PHOTOCHEMICAL DEGRADATION

METHANOGENESIS

GEOLOGICAL SOURCE (e.g., serpentinization)

FERMENTATION

GEOLOGICAL SOURCE (e.g., natural gas seeps)

SOIL