<u>Citation</u>: Blakemore, R.J. (2022). **New Global Species Biodiversity: Soil soars, Ocean flounders**. *Veop.* 5: 1–9. Online: <u>https://veop.wordpress.com/2022/09/10/volume-5/</u>. Date: 10<sup>th</sup> Sept., 2022. Preprint DOI: <u>10.32942/osf.io/dgptw</u>. Addendum & updated file: Online: <u>here</u>. Date: 16<sup>th</sup> Sept., 2022.

#### New Global Species Biodiversity: Soil soars, Ocean flounders

Robert J. Blakemore PhD

VermEcology, 101 Suidomichi, Nogeyama, Kanagawa-ken 231-0064, Japan. C/- Kanagawa Prefectural Museum of Natural History, Odawara, Kanagawa-ken 247-0007. Email: <u>rob.blakemore@gmail.com</u>

# ABSTRACT

Based on topographic field data, an argument is advanced that Soil houses ~2.1 x  $10^{24}$  taxa and supports >99.9% of global species biodiversity, mostly Bacteria or other microbes. Contradictory claims that Soil is home to only a quarter of biota while Ocean harbours 80-99% of Life on Earth are both dismissed. Earlier guesstimates of ~8.8 million taxa (~2.2 million or 25% marine), of 1-6 billion, or up to over a trillion species worldwide are likely underestimations. Recent studies show >10<sup>12</sup> microbial OTUs (just 10<sup>10</sup> or ~1% in Ocean) soon raised to  $10^{12}$ - $10^{14}$  and speculated as high as  $10^{22}$ - $10^{23}$  species. Scaling of simple topsoil samples herein ups the total taxa to  $10^{24}$ . Biomass at 2 x  $10^{-13}$  g/cell of 2.1 x  $10^{30}$  soil cells = 4 x  $10^{17}$  g or 400 Gt (200 Gt carbon, 48 Gt N).

An Addendum estimates microbes in the gut of two key soil taxa: megadrile earthworms  $(10^{15}-10^{24} \text{ cells})$  and termites  $(10^{22}-10^{23} \text{ cells})$  that strictly are added to Soil's totals. Although values are large, neither makes major contribution to global Soil microbe counts of ~2.1 x  $10^{30}$  cells.

#### INTRODUCTION

An honest inventory of our global biotic stock is vitally important in order to estimate biodiversity and track extinctions. False claims that soil has "*more than 25% of our planet's biodiversity*" (<u>Ref</u>.) and simultaneously ocean has 80% (<u>Ref</u>.) or "97 percent of life in the world, maybe in the universe" (<u>Ref</u>.) or "99% of the habitable space on this planet" (<u>Ref</u>.) are clearly misguided.

Species	Earth			Otean		
	Catalogued	Fredicted	#5E	Catalogued	Predicted	± 56
Eukarpotes	1000 C 1000 C		21010205	A CONTRACT	a second second	1000000
Animula	953,494	2,770,000	958,000	171,042	2,1543,6840	145,068
Chipmiste	13,035	27,598	30.509	4.859	7,498	\$1,640
Fairings	43,271	811,000	2907,000	1,097	6,835	11,100
Plantae	215.644	299,000	8.200	8,690	16,009	9,130
Protocola	A.118	35,430	6,690	8,118	36,498	0.090
Tenur	6,233,000	8,740,000	1,300,000	181,756	2,310,008	182,000
Prokaryskes						
Archaee	343	455	166	.W.1	0 C	00
lacteria .	10,318	RARE	8,470	812	1,320	436
Forusi	10.890	10.100	3.630	451	1.330	436
Grand Total	1,344,300	8,756,009	1,300.000	104.409	3,210,060	182,000

Figure 1. Mora et al. (2011: tab. 2) with Earth's ~8.8 million taxa (~2.2 million or 25% Oceanic).

In a highly biased survey range of 3–100 million taxa  $(10^6-10^8)$ , Mora *et al.* (2011: tab. 2) chose a mere ~8.8 million (~2.2 million or 25% marine). Whereas Larsen *et al.* (2017) proposed a new Pie of Life projected for >1–6 billion ( $10^9$ ) species on Earth dominated by Bacteria (~70–90% of total) which they mainly considered for insect hosts. Mundanely, they also found on average six cryptic species per morphologically described arthropod taxon (their <u>tab. S1</u>) quite counterbalancing approximately 20% of published eukaryote names that are synonyms (<u>Ref</u>.).

Bahram et al. (2018) concluded soils are Earth's most diverse biomes, but fail to give figures.

Subsequently, Louca *et al.* (2019) claimed only "*about 2.2–4.3 million full-length OTUs* [unique taxa] *worldwide*" ( $10^6$ ) refuting predictions that billions or trillions of prokaryotic OTUs exist. Wiens (2021) explained how Louca *et al.* (2019) had made entirely avoidable underestimation errors whilst also revising Larsen *et al.*'s (2017) projected 1–6 billion estimate downwards to 0.183 to 4.2 billion ( $10^8$ – $10^9$ ) species with 58–88% Bacteria, again most of these in insect hosts.

High-throughput genomic sequencing and bioinformatics studies allow scaling values based on Locey & Lennon (2016: fig. 3 below) showing Earth with ~10<sup>12</sup> microbial OTU taxa (just 10<sup>10</sup> or ~1% in global Ocean). These totals were later raised to  $10^{12}$ – $10^{14}$  microbial taxa by Lennon & Locey (2020) and then by Fishman & Lennon (2022) who had "a soft upper constraint of  $10^{22}$ – $10^{23}$  due to neutral drift". Most of these taxa at any time are likely dormant (Ref.) and/or unculturable as fewer than 1% of soil species are culturable (Schloss & Handelsman, 2006), or possibly as low as only 0.001% to 0.1% (Ref.) of all bacterial species! Such counts are minima.

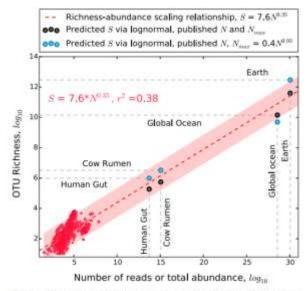


Fig. 3. The microbial richness-abundance scaling relationship (dashed red

Figure 2. From Locey & Lennon (2016: fig. 3) with Earth's 10<sup>12</sup> taxa (just 10<sup>10</sup> or 1% Oceanic).

However, to date, none of the estimates of terrestrial biodiversity have considered terrain that may easily double soil surface area (Blakemore, <u>2018</u>b). All estimates based upon planimetrically flat land areas are manifestly deficient since land is hilly and soil bumpy. Thus this study aims to estimate soil microbial totals taking terrain into consideration for the first time.

As Blakemore (2018b) stated: "A single gramme (~1 cm<sup>3</sup>) of fertile topsoil may have three billion microbes (Bacteria, Actinomycetes, Archaea, Fungi, Protozoa, etc.), up to 60 km of fungal hyphae, with 10,000 to 50,000 microbial species having 1,598 km of DNA some dating to the beginning of life four billion years ago." Average soil carbon is ~1–2% and global soil organic carbon (SOC) alone amounts to >10,000 Gt, thus if one gram of soil has >10,000 spp, its total biodiversity must be truly astronomical and lower values or figures above are likely most modest.

#### **METHODS**

Two main steps to determine the status of soil biota are to review sample surveys to obtain a consensus per gram then to determine the soil matrix extent to give an overall estimate of totals.

#### How many species per gram or tonne of soil?

Curtis *et al.* (2002) stated: "*the entire bacterial diversity of the sea may be unlikely to exceed 2 x*  $10^6$ , while a ton of soil could contain  $4 \times 10^6$  different taxa" (or 4 million taxa) which seems a substantial underestimation error as "*species of bacteria per gram of soil vary between 2,000 and 8.3 million*" (Gans *et al.*, 2005; Roesch *et al.* 2007) (=  $10^4$ – $10^6$  spp/g or  $10^{10}$ – $10^{12}$  spp/t that, if all unique taxa, is equivalent to twenty billion up to a trillion spp per ton of topsoil). [Discrepancies in Gans *et al.* are samples of 10 g soil so strictly 0.83 x  $10^6$  spp/g, yet their fig. 4 shows total species number computed as up to  $10^7$  thus a million or so spp per g seems correct].

Raynard & Nunan (2014) had: "densities commonly found in bulk soil ( $10^8$  cells g<sup>-1</sup> soil)" or "a single gram of soil can harbour up to  $10^{10}$  bacterial cells and an estimated species diversity of between 4 x  $10^3$  [1] to 5 x  $10^4$  species [2]" (=  $10^{14}$ – $10^{16}$  cells/t and 4 x  $10^9$ –5 x  $10^{10}$  spp/t).

Bickel & Orr (2020) found: "bacterial phylotypes ranges between  $10^2$  and  $10^6$  per gram of soil<sup>1,2,4</sup>, with high values similar to the diversity in all of earths environments<sup>3</sup>" (=  $10^8 - 10^{12}$  spp/t).

Madison James *et al.* (2022) summarize: "Soil microorganisms are the largest biodiversity pool on earth, with more than  $10^{30}$  microbial cells [total surely!?],  $10^4 - 10^6$  species, and nearly 1,000 Gbp of microbial genome per gram of soil (Vogel et al., 2009; Mendes and Tsai, 2018)".

Soils naturally include root-zone Rhizosphere: "the most diverse microbiomes on Earth, containing up to 10<sup>11</sup> microbial cells and ~30,000 bacterial species per gram of root [1]. The rhizosphere microbiome exists through an interwoven tapestry of bacteria, viruses, archaea,

protists, fungi, nematodes, and small arthropods interacting directly with plant roots and each other" (White et al., 2021). McNear (2013) has " $10^{10}$ - $10^{12}$  cells per gram rhizosphere soil".

# Thus soil has up to $10^8$ – $10^{12}$ cells/g or $10^{14}$ – $10^{18}$ cells/t, there being $10^6$ grams in a tonne.

# Soil biodiversity ranges $10^2 - 10^6$ spp/g or $10^8 - 10^{12}$ spp/t, there being $10^6$ grams in a tonne.

How many tonnes of topsoil on Earth?

Estimates by Whitman *et al.* (<u>1998</u>: tab. 2 below) cites 2.6 x  $10^{29}$  prokaryotic cells in 1.2 x  $10^{14}$  m<sup>2</sup> soil. They footnote 1 m of topsoil ranges from  $10^7$  to  $10^9$  cells per gram (median  $10^8$  /g) with 1.3 t per cubic metre to give a global total of (1.2 x 1.3 =) **~1.6 x 10^{14} t of topsoil to 1 m depth**.

Ecosystem type*	Area, × $10^{12}$ m <sup>2</sup>	No. of cells, $^{\dagger} \times 10^{27}$
Tropical rain forest	17.0	1.0
Tropical seasonal forest	7.5	0.5
Temperate evergreen forest	5.0	0.3
Temperate deciduous forest	7.0	0.4
Boreal forest	12.0	0.6
Woodland and shrubland	8.0	28.1
Savanna	15.0	52.7
Temperate grassland	9.0	31.6
Desert scrub	18.0	63.2
Cultivated land	14.0	49.1
Tundra and alpine	8.0	20.8
Swamps and marsh	2.0	7.3

Table 2. Number of prokarvotes in soil

\*From ref. 73.

<sup>†</sup>For forest soils, the number of prokaryotes in the top 1 m was  $4 \times 10^7$  cells per gram of soil, and in 1–8 m, it was  $10^6$  cells per gram of soil (16). For other soils, the number of prokaryotes in the top 1 m was  $2 \times 10^9$  cells per gram of soil, and in 1–8 m, it was  $10^8$  cells per gram of soil (18). The boreal forest and tundra and alpine soils were only 1 m deep. A cubic meter of soil was taken as  $1.3 \times 10^6$  g.

123.0

255.6

Figure 3. Land from Whitman et al. (1998: tab. 2) excluding Antarctica/Greenland (Ref.).

Total

Conversely, Blakemore (2018b: fig. 4; tab. 5) has "*habitable land*" of  $104 \times 10^{6}$  km<sup>2</sup> presumably with rich humic topsoil, say ~1 m deep (and ~1 t per m<sup>3</sup>), there being  $10^{6}$  m<sup>2</sup> in a km<sup>2</sup>, thus 1.04 x  $10^{14}$  t or 104,000 Gt. Doubled for terrain is about 208,000 Gt or ~2.1 x  $10^{14}$  t global topsoil.

# RESULTS

If  $10^{14}-10^{18}$  cells/t soil x 2.1 x  $10^{14}$  t the range is 2.1 x  $10^{28}-10^{32}$  cells (median ~2.1 x  $10^{30}$ ).

A range of  $10^8 - 10^{12}$  spp/t x 2.1 x  $10^{14}$  t gives 2.1 x  $10^{22} - 10^{26}$  spp total (median ~2.1 x  $10^{24}$ ).

Biomass carbon Whitman et al. (<u>1998</u>) took as half average soil prokaryotic dry mass (C:N = 1:0.24) thus 2 x  $10^{-13}$  g/cell of all 2.1 x  $10^{30}$  cells = 4 x  $10^{17}$  g or 400 Gt (200 Gt carbon, 48 Gt N).

#### DISCUSSION

Properly allowing for terrain, Earth's surface as exposed to Sun, air and rain of Land vs. Ocean from Blakemore (2018b) is 64:36%; so Soil has by far the largest area and supports most biota.

Whitman *et al.* (<u>1998</u>) initially estimated microbial prokaryotes cell numbers in Soil vs. Ocean as 2.6 x  $10^{29}$  vs. 1.2 x  $10^{29}$ , respectively (68% vs. 32%); biomass soil carbon was 26 vs. 2.2 Gt C (92% vs. 8%). Soil was to 1 or 8 m depth, Ocean included open water and 10 cm of sediment. My present value of ~2.1 x  $10^{30}$  soil cells to just 1 m increases their Soil tally by about ten times.

Remote subsurface biota are not my major concern, but Whitman *et al.*'s (<u>1998</u>: tab. 5) 3.6 x  $10^{30}$  & 2.5 x  $10^{30}$  cells in Oceanic & Terrestrial subsurfaces as revised by Kallmayer *et al.* (<u>2012</u>) and Magnabosco *et al.* (<u>2018</u>) to just 3–5 x  $10^{29}$  & 2–6 x  $10^{29}$ , respectively; a total range of 0.5– 1.1 x  $10^{30}$  cells and subsurface biomass of 4 & 23–31 Gt C. New soil cell total herein of ~2.1 x  $10^{30}$  is two to four times this and soil microbe biomass of ~200 Gt C is over six times as large.

For global biodiversity, a reasonable status summary by Fishman & Lennon (2022) had: "*the present number of bacterial and archaeal taxa*  $S_{present}$  *is between* 10<sup>6</sup> *and* 10<sup>23</sup>." My present ~2.1 x 10<sup>24</sup> soil species increases their upper value by a factor of about twenty times but a lower estimate of ~2.1 x 10<sup>20</sup> soil species—as calculated below—is within their range. Adding weight to the argument, the methods to achieve these similar conclusions were calculated independently.

Whereas terrain doubles area at metre scale, at cm–mm sampling scale it is likely quadrupled. Blakemore (2018b: tabs. 5–6) had x1, x2 and x4 soil as 162,000 Gt (agreeing with Whitman *et al.*, <u>1998</u>: tab. 2), 324,000 and 648,000 Gt, respectively; or 1.6, 3.2 and 6.5 x  $10^{14}$  t soil. My median scaling estimate of around ~2.1 x  $10^{24}$  soil microbial taxa is therefore likely a low value that may be readily doubled (see - <u>https://vermecology.wordpress.com/2022/08/04/different-f3/</u>). Accounting for neglected terrain & topography at cm–mm scale, if soil microbial tallies double again would total ~4.2 x  $10^{30}$  cells, ~4.2 x  $10^{24}$  spp and biomass total of ~800 Gt (= ~400 Gt C).

Accepting ~2.1 x  $10^{30}$  soil microbial cells with ~2.1 x  $10^{24}$  taxa implies one unique taxon per million microbial cells. Albeit if as many as 99% of soil microbes were eventually proven ubiquitous "species", ~2.1 x  $10^{22}$  different genetic phylotypes would remain. Soils are often deeper than 1 m, but if such high species richness occurred in only the top 10 cm of 10% of Earth's topsoils, it would yet total in the order of ~2.1 x  $10^{20}$  species. Higher values seem reasonable as random samples separated by up to 9,000 km had only <1.5% bacterial taxa in common to all, the majority of 88% OTUs unique to just one soil (Fulthorp *et al*, 2008). A similar figure of 80% wholly endemic taxa per soil sample was found by Schloss & Handelsman (2006). Moreover, Operational Taxonomic Unit (OTU) often applies to Genera comprising many species.

Such findings attest to and support a massive Soil microbial biodiversity, orders of magnitude above that a cold, dark, densely saline, nutrient- and oxygen-depleted and ever-mixing Ocean's.

For the Ocean, Ferrer *et al.* (2019) categorically state: "*It is estimated that the ocean.*. *hosts the largest population of microbes on Earth. More than 2 million eukaryotic and prokaryotic species are thought to thrive both in the ocean and on its surface.*" Hoshino *et al.* (2020) claim: "Global marine sedimentary taxonomic richness predicted by species–area relationship models is 7.85  $\times$  10<sup>3</sup> to 6.10  $\times$  10<sup>5</sup> for Archaea and 3.28  $\times$  10<sup>4</sup> to 2.46  $\times$  10<sup>6</sup> for Bacteria as amplicon sequence variants, which is comparable to the richness in seawater and that in topsoil [!]" also "the global diversity of marine prokaryotes (Archaea and Bacteria) in the near-surface ocean (0 to 1,000 m below sea level [mbsl]) was estimated (3.75  $\times$  10<sup>4</sup> operational taxonomic units)".

# Remarkably, a couple of grams of topsoil has an equivalent to all Ocean's total 10<sup>4</sup>–10<sup>6</sup> taxa!

Furthermore, Whitman *et al.* (<u>1998</u>) had 5 x 10<sup>19</sup> cfu (colony forming units) in the atmosphere, and, since only 0.001-1% of Bacteria are culturable, this likely equates to >>5 x 10<sup>21</sup> cells. That is at least 500 times the estimate of Curtis *et al.* (<u>2002</u>) who said: "*The atmosphere is thought to have an NT* [total "individuals" (sic for microbes with binary fission!) of aerobiotic cells] *value of*  $10^{19}$ , *which is sufficient to accommodate*  $4 \times 10^6$  *taxa*" or 4 million species (most taxa shared with soils?). This is also twice the Ocean's best tally whilst also exceeding it as the Earth's largest biome. Aquatic taxa totals are proportionately infinitesimal. Prior to latest microbial totals, mainly in soil on land was already shown to support 99.7% of biomass and 98.0% of biodiversity (Blakemore, <u>2020</u>: fig. 2; - <u>https://vermecology.wordpress.com/2020/05/27/realms-of-the-soil/</u>).

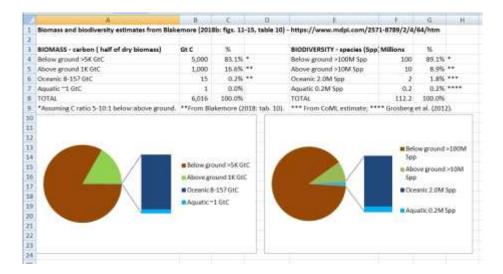


Figure 4. Blakemore (2020: fig. 2) Global soil biomass & biodiversity (~90% now upped >99.9%).

How much soil biota is currently known and why is it so important?

Soil provides 99.7% human food (Pimental & Burgess, 2013), all our fibres and building materials, many of our medicines (such as Penicillin, Streptomycin or Ivermectin), filters and stores most of our freshwater supplies, and supports >99.9% of biodiversity albeit we know <0.0001% of soil fauna, fungi or flora (my estimate is 310,000 soil species currently described, mostly microbes or invertebrates such as earthworms that enhance microbes, etc.). Moreover, an argument may be to include plant seeds or roots as valid soil flora (~4-500,000 spp - Ref.).

Soil-Invertebrate Group:	Individuals as a (approx.):	Biomass g- m <sup>-1</sup> 0	Known- specieso	%
Bacteria and Archaeat	10110	20-500*s	3,2000	<<19670
Fungia	(500+km)=	20-500*c	80,0000	0.5%
Protozoa=	1050	6-300	1,5000	8360
Rotifera (Bdelloid soil rotifers)	10 <sup>5</sup> n	20	3000	20
Nematodan	10*0	1-300	25.000G	1.3%"0
Lobopodia (Onychophora, Tardigrada)o	0	0	-1,2000	<<50990
Lobopodia (Onschophora, 10	20	20.	<2000	50%0.
Lobopodia (Tardigrada)=	p	0	-1,0000	90
Arachnida Opiliones:	0	0	6.3000	70
Arachnida, Pseudoscorpionidati	0	8	3.3000	70
Acari (mites):	1040	0.2-40	45,2000	4%60
Hexapoda (totals):	10 <sup>4</sup> g	0.2-40	~9,0000	17960
Hexapoda (Collembolai)	Up to 100.0000	0	6.5000	0
Hexapada (Dipluraj⊂	0	0	8000	12
Hexapada (Protrura coneheads)0	0	0	7310	0.
Soil Insects and their larvae	50-5000	4.50	55.000+70	20%/20
Myriapoda (centi-, millipedes)o	100-11000	1.5-22.50	18,0000	20%80
Myriapoda (Simpiniai=	2	8	160=	
Pauropoda (Miriapodarelative)=	D	a	5000	8
laopoda (slaters, woodlice, etc.) II	Up to 18000	<41	5,0000	70
lsoptera (Termites)::	Colonies	2.5	2,6000	60%/7a
Blattodea (Cockroaches)	90	20	4,5000	20
Ants (Hymenoptera : Formicidae)	Colonies□	20	13,0000	50%60
Molluses (Soil Gastropode)	?p	20	24,0000	40%22
Terrestrial Turbellaria (Planarians)	70	70	830+0	70
Terrestrial Polychaeta:	20	70	70	.70
Oligochaeta (Mega-, Microdriles)	50-2,0000	20-5000	10,0000	20%670
Microdriles (Enchytrastidae)=	1,000-300,000=	1-33=	~700=	20
Microdriles (excluding enclytracid)	20	20	1-2,300??=	20
Megadriles (true earthworms)=	50-2.0200	20-305*0	7,0000	<20%20
Total species (approx.)p	0	0	310,000::	70

 Total species (approx.)
 a
 a
 310,000
 ?a

 \* Sub-surface biomass (even excluding plant roots and tabers) exceed those above-ground - Highest earthworm values are from Lee (1985: tab.?) in NZ partners (mean 2.020 m² with 305 gm² from McColl & Lautour, 1978). Encloyeasid maxima are from Springett (1967: fig. 24) Gragg (1963: tab.?) from Moor House, UK (mis)quoted by Spain & Lavelle (2001) §



Blakemore (2016: tab. 3) compiled list of 310,000 known of many millions soil species is greater than a \$1 billion, 10 yr Census of Marine Life of 230,000 spp (Ref.). After this CoML survey completed, Mora et al. (2011: tab. 2) totalled just 194,409 catalogued Ocean spp; both predicted only ~2 million total taxa. The current study substantially increases Soil taxa totals.

As biodiversity estimates grow so too they decline as soil erosion and species extinctions take their toll (Blakemore, 2018a; Ref., Ref.). Despite vital importance and massive biodiversity inventory, not a single SOIL ECOLOGY INSTITUTE yet exists anywhere on Earth (except perhaps on my humble office desk – see: https://vermecology.wordpress.com/2015/12/10/iseiinternational-soil-ecology-institute-and-soil-ecology-exchange-yokohama-seexy-open-day-5thdecember-2015-for-unfao-international-soil-day/). In all honestly, for our rational and reasonable survival, this major Soil research deficiency requires redress with urgent redirection of resources.

Table 3. Abundance and biodiversity estimates for common soil Invertebrates (from Brunand et a).

#### REFERENCES

- Bahram, M., Hildebrand, F., Forslund, S.K. *et al.* (2018). Structure and function of the global topsoil microbiome. *Nature*, **560**, 233–237. <u>https://doi.org/10.1038/s41586-018-0386-6</u>.
- Bickel, S. & Or, D. (2020). Soil bacterial diversity mediated by microscale aqueous-phase processes across biomes. *Nat Commun*, **11**, 116. doi.org/10.1038/s41467-019-13966-w.
- Blakemore, R. J. (2016). *Cosmopolitan earthworms*. (6<sup>th</sup> Edn.). VermEcology, Yokohama, Japan, 1,250 pp. + 150 figs.
- Blakemore, R.J. (2018a). Critical Decline of Earthworms from Organic Origins under Intensive, Humic SOM-Depleting Agriculture. Soil Syst, **2**, 33. https://doi.org/10.3390/soilsystems2020033.
- Blakemore, R.J. (2018b). Non-Flat Earth Recalibrated for Terrain and Topsoil. *Soil Syst*, **2**, 64. <u>https://doi.org/10.3390/soilsystems2040064</u>.
- Blakemore, R.J. (2020). *Realms of the Soil (vs. Solar Lunacy)*. Online: <u>https://vermecology.wordpress.com/2020/05/27/realms-of-the-soil/</u>.
- Curtis, T.P., Sloan, W.T. & Scannell, J.W. (2002). Estimating prokaryotic Diversity and Its Limits. *Proc Natl Acad Sci USA*, **99**, 10494-10499. <u>http://dx.doi.org/10.1073/pnas.142680199</u>.
- Ferrer M., Méndez-García C., Bargiela R., Chow J., Alonso S., García-Moyano A., Bjerga G.E.K., Steen I.H., Schwabe T., Blom C., Vester J., Weckbecker A., Shahgaldian P., de Carvalho CCCR, Meskys R, Zanaroli G, Glöckner FO, Fernández-Guerra A, Thambisetty S, de la Calle F, Golyshina OV, Yakimov MM, Jaeger KE, Yakunin AF, Streit WR, McMeel O, Calewaert JB, Tonné N, Golyshin PN; INMARE Consortium. (2019). Decoding the ocean's microbiological secrets for marine enzyme biodiscovery. *FEMS Microbiol Lett*, **366(1)**, fny285. doi: 10.1093/femsle/fny285.
- Fishman, F.J. & Lennon, J.T. (2022). Macroevolutionary constraints on global microbial diversity. *bioRxiv*, <u>https://doi.org/10.1101/2022.06.04.494835</u>. <u>https://www.biorxiv.org/content/10.1101/2022.06.04.494835v1.full</u>.
- Fulthorpe, R., Roesch, L., Riva, A. et al. (2008). Distantly sampled soils carry few species in common. ISME J, 2, 901–910. <u>https://doi.org/10.1038/ismej.2008.55</u>.
- Gans, J., Woilinsky, M. & Dunbar, J. (2005). Computational improvements reveal great bacterial diversity and high metal toxicity in soil. Science, **309**, 1387–1390. <u>https://www.science.org/doi/10.1126/science.1112665</u>.
- Hoshino T, Doi H, Uramoto GI, Wörmer L, Adhikari RR, Xiao N, Morono Y, D'Hondt S, Hinrichs KU, Inagaki F. (2020). Global diversity of microbial communities in marine sediment. *Proc Natl Acad Sci USA*, **117(44)**, 27587-27597. doi: 10.1073/pnas.1919139117.
- James, Madison T., Farrisi, S.T., Shah, S. & Shah, V. (2022). Identification of Major Organisms Involved in Nutritional Ecosystem in the Acidic Soil From Pennsylvania, USA. *Front Env Sci*, **10**, doi:10.3389/fenvs.2022.766302.
- Kallmeyer, J., Pockalny, R., Adhikari, R.R., Smith, D.C. & D'Hondt, S. (2012). Global distribution of microbial abundance and biomass in subseafloor sediment. *Proc. Natl Acad Sci USA*, **109**, 16213–16216, doi:pnas.1203849109. <u>pnas.org/doi/pdf/10.1073/pnas.1203849109</u>.
- Larsen, B.B., Miller. E.C., Rhodes, M.K. & Wiens, J.J. (2017). Inordinate fondness multiplied and redistributed: the number of species on Earth and the new pie of life. *Q Rev Biol*, **92(3)**, 229–65. doi: 10.1086/693564.

- Lennon, J.T. & Locey, K.J. (2020). More support for Earth's massive microbiome. *Biol Direct*, **15**, 5. www.ncbi.nlm.nih.gov/pmc/articles/PMC7055056/. doi: 10.1186/s13062-020-00261-8.
- Locey, K.J. & Lennon, J.T. (2016). Scaling laws predict global microbial diversity. *Proc Natl Acad Sci USA*, **113**, 5970–5975. https://www.pnas.org/doi/pdf/10.1073/pnas.1521291113.
- Louca, S., Mazel, F., Doebeli, M. & Parfrey, L.W. (2019). A census-based estimate of earth's bacterial and archaeal diversity. *PLoS Biol*, **17**, e3000106.
- Magnabosco, C., Lin, L.-H., Dong, H., Bomberg, M., Ghiorse, W., Stan-Lotter, H., ... Onstott, T. C. (2018). The biomass and biodiversity of the continental subsurface. *Nat. Geosc*, *11*, 707-717, doi:10.1038/s41561-018-0221-6.
- McNear, D.H., Jr. (2013) The Rhizosphere Roots, Soil and Everything In Between. Nature Education Knowledge, **4(3)**, 1.
- Mora, C., Tittensor, D.P., Adl, S., Simpson, A.G.B. & Worm, B. (2011). How many species are there on earth and in the ocean? *PLoS Biol*, **9**, e1001127.
- Pimentel, D. & Burgess, M. (2013). Soil Erosion Threatens Food Production. *Agriculture*, **3**, 443-463. <u>https://doi.org/10.3390/agriculture3030443</u>.
- Raynaud, X. & Nunan, N. (2014). Spatial ecology of bacteria at the microscale in soil. *PLoS ONE*, **9**, e87217. DOI: 10.1371/journal.pone.0087217.
- Roesch, L., Fulthorpe, R., Riva, A. et al. (2007). Pyrosequencing enumerates and contrasts soil microbial diversity. ISME J, 1, 283–290. <u>https://doi.org/10.1038/ismej.2007.53</u>.
- Schloss P.D. & Handelsman J. (2006). Toward a census of bacteria in soil. *PLoS Comput Biol*, **2(7)**, e92. doi: 10.1371/journal.pcbi.0020092.
- White, R.A., III, Rosnow, J., Piehowski, P.D., Brislawn, C.J. & Moran, J.J. (2021). In Situ Non-Destructive Temporal Measurements of the Rhizosphere Microbiome 'Hot-Spots' Using Metaproteomics. Agronomy, 11, 2248. <u>https://doi.org/10.3390/agronomy1112248</u>.
- Whitman, W.B., Coleman, D.C. & Wiebe, W.J. (1998). Prokaryotes: The unseen majority. Proc Natl Acad Sci USA, 95, 6578–6583. <u>https://www.pnas.org/doi/pdf/10.1073/pnas.95.12.6578</u>.
- Wiens, J.J. (2021). Vast (but avoidable) underestimation of global biodiversity. *PLoS Biol*, **19**, e3001192. <u>https://doi.org/10.1371/journal.pbio.3001192</u>.

### ADDENDUM

# Global Microbiome Counts in Intestines of Earthworms and Termites Robert J. Blakemore PhD

VermEcology, 101 Suidomichi, Nogeyama, Kanagawa-ken 231-0064, Japan.

## ABSTRACT

Earthworms and termites are two of the wholly soil-dwelling invertebrate faunal groups. Both are known to support vibrant and enhanced microbial gut communities of symbionts and parasites. Estimates of gut microbial abundance for earthworms with  $1.1-2.3 \times 10^9$  tonnes dry biomass – including their soil ingesta – is an intestinal count of  $10^6-10^{13}$  cells/g thus  $10^{15}-10^{24}$  cells total. Termite gut microbiome range is around  $10^{22}-10^{23}$  cells. Although large, neither makes a major contribution to soil counts (~2.1 x  $10^{30}$ ), microbe biodiversity (~2.1 x  $10^{24}$  taxa), nor biomass. Total global soil biomass for ~2.1 x  $10^{30}$  cells is 4 x  $10^{17}$  g or 400 Gt (with 200 Gt C and 48 Gt N).

### INTRODUCTION

Earthworms (Annelida: Oligochaeta: Megadrilacea) are the major component of healthy soils. Earthworms and microorganisms are co-evolved and interdependent thus their interactions regulate Ecology of terrestrial soils. Megadrile earthworms comprise 20 families, approximately 1,000 genera and ~8,000 described species (Blakemore, 2016b). They represent up to 90% of invertebrate biomass present in soil and microbes are known to increase during digestion up to x 1,000 and after gut passage in their castings (Lee, 1985: 27, 206). Their total abundance is in the order of  $1.3 \times 10^{12}$  worms with a dry biomass around  $1.1-2.3 \times 10^{9}$  tonnes (1–2 Gt). (https://web.archive.org/web/20220828201550/https://en.wikipedia.org/wiki/Biomass\_(ecology)).

Soil terrain consideration (Blakemore, <u>2018</u>) increases evenly dispersed earthworm counts, but taxa such as ants or termites in discrete colonies or nests per unit area are not so enhanced.

Earthworms digestive systems have calciferous glands, typhlosoles and/or caeca thus, during their passage, ingested micro-organisms may increase 10 to 1,000 times (Ref. below quoting Parle, 1963). Whereas Parle (1963a) says the microbes only increase ten times in mid- to hindgut he showed x  $10^3$  in antilogs of his table 1. Later, Parle (1963b: tab. 1) reported soil to casts for Actinomycetes & Bacteria went from  $4 \times 10^7$  to  $6 \times 10^9$  & from  $8 \times 10^8$  to  $8 \times 10^{10}$ , respectively, or an increase about 100 times. Stockli (1928) had shown Actinomycetes & Bacteria increased 600 times & 1,000 times in intestines with hindgut counts raised to  $15 \times 10^9$  &  $4.4 \times 10^{11}$  cells, respectively. Of note is many such studies use culturable cfu plate counts that produce a small proportion of total soil microbes, perhaps just 1% (Ref.) or possibly as low as 0.001% to 0.1% (Ref.) of all bacterial species! Thus final results or current estimates of  $10^8$ - $10^{12}$  microbe cells/g in bulk topsoil ingesta as taken as initial base range, may both be far too low.

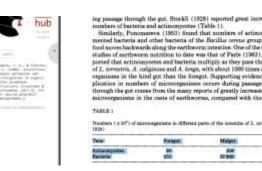


Figure 1. With report of 600–1,000 times increase in microbes in earthworms gut (data in blue).

Parle (<u>1963</u>a: tab. 1 below) for three reasonably widely distributed arable earthworm species had about the same number of Yeast or Fungi cells in surrounding soil compared to earthworms' gut, but Actinomycetes & Bacteria were substantially increased on average from  $2 \times 10^7$  to  $5 \times 10^8$  /g and from  $1 \times 10^8$  to  $1 \times 10^{11}$  /g, respectively. Thus antilog counts, rather than log numbers, were increased by one to three orders of magnitude or up ten to about 1,000 times. This supports claims that "*ingested soil may contain 10<sup>9</sup> or more microbial cells per gram dry weight*" (Ref.), the earthworm gut microbiome can have ~ $10^{10}$  cells per gram (Ref.), or passage through their digestive system increases microbes up to 1,000 times (Ref.; Ref.; Ref.; Lee, 1985: 27; cf. Ref. "*a Review*" that ignores any references prior to 1986!).

outents or soil			22331	
	Yoasta	Fungi × 10 <sup>4</sup>	Actino- asyortes = 10 <sup>9</sup>	Bacteria. × 10*
	La	indricas invest	TIA	
Gut content	3-09 ±0.110	$-0.25 \pm 0.122$	1-97 ±0154	4-01 ±0-100
Soft	4/70 ±0-178	-0.50 ±0.164	0-93 ±0-9598	2-07 ±0-244
	dila	biopliere calig	NAME	
Gat content	5-05 ±0-070	-0.08 $\pm 0.061$	8-12 ±-0-198	4-82 ±0-848
Soll	4/19 ±0-108	-0.89 ±0.119	1-54 ±0-290	2:04 ±0:500
		A. longs		
Gut content	5-46 ±0-074	0.52 ±0.988	0.95 ±0-302	5-00 ±0-070
Sell	3-00 ± 0-100	0-15 ± 0-099	1-00 ±0140	2:05 ±0:100

Figure 2. After Parle (<u>1963</u>a: tab. 1) with ~10–1,000 times microbe increase.

Earthworm-rich organic vs. conventional farm soils in Philippines had higher Bacteria & Fungi populations (+44% & +55%), counts up to  $1 \times 10^7$  &  $5 \times 10^4$  cfu/g (Blakemore, <u>2016</u>b: tab. 5). Vermicompost Bacteria & Fungi were  $1.2 \times 10^7$  &  $1.5 \times 10^5$  cfu/g (Blakemore, <u>2016</u>b: tab. 7).

For termites, Whitman *et al.* (1998: tab. 4) have prokaryote cells per termite hindgut of  $2.7 \times 10^{6}$  cells and, for a world population of  $2.4 \times 10^{17}$  of these insects, they conclude as  $6.5 \times 10^{23}$  cells total. An estimate of global termite biomass is  $4.4 \times 10^{8}$  t wet (Ref.) or ~2.2 x 10<sup>8</sup> t dry weight.

### **METHODS**

Earthworm and termite total biomass estimates are multiplied by their known microbial residents.

### **RESULTS & DISCUSSION**

Earthworm global dry biomass of  $1.1-2.3 \times 10^9$  t with  $10^6-10^{11}$  cells/g of Acinomycetes, Bacteria & Archaea (unknown at time of Stockli's or Parle's studies) totals  $1.1-2.3 \times 10^{15}-10^{20}$  cells. Or, Kieft & Simmonds (2015: fig. 1) of  $10^8$  microbes per worm for  $1.3 \times 10^{12}$  worms =  $1.3 \times 10^{20}$  cells. Alternatively, if actual increase during passage is  $10^2-10^3$  with bulk soil range of  $10^8-10^{12}$  cells/g (from Blakemore, 2022) this goes to  $10^{10}-10^{15}$  cells/g and totals  $1.1-2.3 \times 10^{19}-10^{24}$  cells. Although large, it is a minor contribution to total soil microbes. Unique species are poorly known.

For termites,  $2.2 \times 10^{14}$  g for  $2.4 \times 10^{17}$  population =  $10^{-3}$  g/termite (dry). Hindgut count of 2.7 x  $10^{6}$  cells/termite is 2.7 x  $10^{9}$  cells/g; by 2.2 x  $10^{14}$  g = 6 x  $10^{23}$  cells. Or, Kieft & Simmonds (2015: fig. 1) of  $10^{6}$  cells/termite for  $10^{-2}$  g/termite (wet); by 4.4 x  $10^{14}$  g = 4.4 x  $10^{22}$  cells. Slightly smaller than Whitman *et al.*'s 6.5 x  $10^{23}$  cells, also a minor contribution to soil biomass.

## CONCLUSION

Although terrain consideration increases all soil biometrics, new estimates of the resident microbes of earthworm intestines or termite gut make minor contributions to total soil microbial abundances, biomass, nor biodiversity of  $2.1 \times 10^{30}$  cells at  $2 \times 10^{-13}$  g/cell = 400 Gt (200 Gt C, 48 Gt N) and  $10^{24}$  taxa (Blakemore, 2022). Therefore, any unique, host-specific parasitic or symbiotic microbial species would likely contribute relatively little to overall total soil biodiversity.

#### REFERENCES

- Blakemore, R. J. (2016a). *Cosmopolitan earthworms*. (6<sup>th</sup> Edn.). VermEcology, Yokohama, Japan. Pp. 1,250 + 150 figs.
- Blakemore, R.J. (2016b) Veni, Vidi, Vermi... II. EARTHWORMS IN ORGANIC FIELDS RESTORE SOM & H<sub>2</sub>O AND FIX CO<sub>2</sub>. *Veop*, **2** (2), 1-26. <u>orgprints.org/id/eprint/31189/</u>. Online: <u>https://veop.wordpress.com/volume-2-1-veni-vidi-vermi-part-ii/</u>.
- Blakemore, R.J. (2018b). Non-Flat Earth Recalibrated for Terrain and Topsoil. *Soil Syst*, **2**, 64. <u>https://doi.org/10.3390/soilsystems2040064</u>.
- Blakemore, R.J. (2022). New Global Species Biodiversity: Soil soars, Ocean flounders. *Veop.* **5**, 1–9. <u>https://veop.wordpress.com/2022/09/10/volume-5/; https://ecoevorxiv.org/dgptw/</u>.
- Kieft T.L., Simmons K.A. (2015). Allometry of animal-microbe interactions and global census of animal-associated microbes. *Proc Biol Sci*, 282. <u>https://doi.org/10.1098/rspb.2015.0702</u>.
- Lee, K.E. (1985). *Earthworms: their ecology and relationships with soils and land use.* Sydney: Academic Press. Pp. 411. ISBN 978-0-12-440860-9.
- Parle, J.N. (1963a). Micro-Organisms in the Intestines of Earthworms. *J. gen. Microbiol*, **31**, 1-11. Online: <u>https://repository.rothamsted.ac.uk/download/934559/mic-31-1-1.pdf</u>.
- Parle, J.N. (1963b). A Microbiological Study of Earthworm Casts. *J gen Microbiol*, **31**, 13-22. <u>https://citeseerx.ist.psu.edu/viewdoc/download?doi=10.1.1.631.3323&rep=rep1&type=pdf</u>.
- Stöckli, A. (1928). Studien über den Einfluss des Regenwurmes auf die Beschaffenheit des Bodens. Lanwirtschaft Jahrb Schweiz, 42, 1-121.
- Whitman, W.B., Coleman, D.C. & Wiebe, W.J. (1998). Prokaryotes: The unseen majority. *Proc Natl Acad Sci USA*, **95**, 6578–6583. <u>https://www.pnas.org/doi/pdf/10.1073/pnas.95.12.6578</u>.