MICROBIAL ENDPOINTS

Endpoint	Species, community or other	Description of assay and measured endpoint(s)	Endpoint type (apical, biological, biochemical, functional, biomarker, other)	Additional information to help understand ecological relevance of endpoint	Acute/chronic based on current ANZG Guidance (Warne et al., 2025)	Currently accepted to derive ANZG DGVs?	Strengths as line of evidence	Weakness as line of evidence	Evidence of use in GVs?	Standardised/ Research/ Aspirational, with examples of published standard methods. NOTE: standardised assays are not crucial for ANZG, if QA/QC meet ANZG quality assessment criteria.	Functional Group	Potential Adverse outcome/ecological endpoint	Ecologically relevant for GV derivation, under 'new' definition (Score 5-10)	Percentage of participants who supported their use in deriving DGVs?	Methods - defined, availability, considerations - challenges/gaps?	Notes to consider
CELL-BASED (SINGLE AND MULTIPLE SPECIES) ENDPOINTS																
Bacterial luminescence inhibition	Species	Measures decrease in luminescence of a bacterial species following exposure (typically 5 minutes to 24 hrs) to a toxicant e.g. Microtox® test kit using marine bacterium *Aliivibrio fischeri* and more recently, an Australian tropical isolate.	Biological, biochemical	Bioluminescence inhibition is a non-specific indicator of cellular stress in a model bacterium (e.g., symbiont of squid) and does not directly represent microbial community structure, function, or ecosystem processes.	acute	No	Standardised, rapid, and highly reproducible assay; Suitable for screening complex matrices (water, sediment porewater leachates)	Marine bacterium not representative of target ecosystems; Non-specific response mechanism (general cytotoxicity); Affected by confounding factors (pH, salinity, DOC); Limited link to higher-tier ecological endpoints or population effects	Not used	Standards related - e.g. Environment Canada (1992).	Heterotrophic aerobic bacteria (general metabolic activity).	None, although potential impacts to asymbiont.	2.5	Unsure (%? No (%19 Yes(%7 Yes, with improvements(%7	Standardised commercially. Rapid and easy to use. Less ecologically relevant as based on one species and primarily indicates acute toxicity. Run at salinities of 15-35 ppt, so applicable to estuarine to marine ecosystems, standard method is 15ppt, but modifications enable up to 35 ppt. Relatively insensitive to metals.	Used in environmental management and risk assessment for mixtures.
Microalgae growth inhibition	Species	Growth inhibition (typically over 48 - 96h) test in defined media measuring growth rate (yield by cell counts, fluorescence, chlorophyll-a or optical density; optional subsets of live/dead using viability stains and/or fluorescence (e.g., PAM efficiency). Typically single species assays, but growth data for single species can also be extrapolated from community exposures, e.g., Stone et al., 2020.	Apical (growth), Biochemical (functional (photosynthesis)	Microalgae are primarily assessed as primary producers as they drive carbon fixation and oxygen production and underpin aquatic food webs; photosynthesis endpoints are biochemically linked to primary production. Assays have been developed using low nutrient media, but not all available data are derived from such assays.	chronic, if exposure is longer than 48 h; acute-to-subchronic for fluorescence endpoints depending on design.	Yes	High ecological relevance for primary production; widely used in aquatic ecotoxicology; produces robust concentration-response data suitable for SSD inputs; standardised and reproducible.	Single-species lab cultures may not represent natural community sensitivity; depends strongly on nutrient/light regime and species choice; growth inhibition does not necessarily translate to population decline; photosynthesis inhibition may not translate directly to long-term productivity under field conditions. Issues occur when growth curves exhibit hormesis, which effectively are not currently considered acceptable endpoints when deriving DGVs.	Yes, most DGVs include a microalgae growth endpoint.	Standards related - examples of standardised algae growth inhibition assays are OECD 201 and ISO 8692 (freshwater) and ISO 10253 (marine), and are routinely used in aquatic guideline derivation	Primary producers (photosynthesis) driving photosynthesis and primary production.	Reduced primary productivity and energy transfer to higher trophic levels, potentially altering food web structure and oxygen dynamics.	3	Unsure (%? No(%19 Yes, with improvements(%7	Routine laboratory method with established protocols and common instrumentation ; needs appropriate test species selection (freshwater vs marine), nutrient-relevant media, and light/temperature control; interpretation strengthened when paired with community/phytoplankton metrics in site-specific contexts.	
Cyanobacterial growth inhibition	Species	Growth inhibition (typically over 48-96 h) assay, yield, growth rate, measured based on cell counts or surrogate such as optical density/fluorescence	apical	Similar to microalgae, cyanobacteria are primarily assessed as primary producers as they drive carbon fixation and oxygen production and underpin aquatic food webs. Overgrowth (biomass) can be problematic, as they can trigger eutrophication, hypoxia and toxin release. Assays have been developed using low nutrient media, but not all available data are derived from such assays.	chronic, if exposure is longer than 48 h	Yes	Standardised and reproducible assay; Represents primary producers and photosynthesis functions; Mechanistically interpretable (photosynthesis inhibition); Directly applicable to water quality guidelines. Note these assays do not measure photosynthesis inhibition - that's a different assay	Sensitivity varies by strain and culture conditions; Some test species not regionally native; Single-species assays miss community-level interactions; Issues occur when growth curves exhibit hormesis. Hormesis effects are not currently considered as acceptable endpoints when deriving DGVs.	Yes, used in many DGVs, including ... (to look up)	Standards related - e.g., OECD 201 (2011) ... Freshwater Algae and Cyanobacteria, Growth Inhibition Test; Organisation for Economic Co-operation and Development, Paris; ISO 8692:2012 *Water quality — Freshwater algal growth inhibition test with unicellular green algae*. International Organization for Standardization, Geneva.	Photoautotrophic cyanobacteria (primary producers, nitrogen-fixing taxa).	Reduced primary productivity and nitrogen fixation; altered oxygen dynamics and food web basis.	3.5	Unsure (%21 No(%27 Yes(%50 Yes, with improvements(%7	Difficulties counting long chains of cells e.g. Anabaena. However methods exist to add bias.	Low nutrient test medium essential. Should use low initial cell densities
Microbial growth inhibition (bacteria, protozoa)	Species	Growth inhibition (typically over 24-48 h), measured using spectrophotometric method or radiological detection e.g. TdC - Including Minimum Inhibitory Concentration (MIC) assay, 16-24 h growth inhibition. Typically single species assays, but multiple single species exposures can be assayed in a single microwell plate, e.g. The Microbial Assay for Risk Assessment (MARA) is a system based on a array of 11 different microbial species frozen-dried in a 96-well micro-litre plate format.	apical	Microbial productivity is the foundation for nutrient cycling, energy flow, and the maintenance of ecosystem health. However, microbes exist on a complex continuum.	chronic, if exposure is longer than 24h	Yes	Standardised and rapid assay for general toxicity; High reproducibility and low cost; Useful for screening and comparative assessments; Mechanistically simple and easy to interpret.	Often uses non-native or laboratory strains; Non-specific response mechanism (general cytotoxicity); May not reflect chronic or community-level effects; Influenced by confounding factors such as pH, salinity, and nutrient status	Yes (Copper GV - bacteria; BPA GV - protozoa)	Published standard methods available (e.g., Stauber and Davies, 2000; ISO 20776-1 (2019), ISO 20776-2 (2021), Wu Bin, 2013)	Various groups	Reduced organic matter turnover and nutrient regeneration, altering soil and aquatic productivity	4.5	Unsure (%14 No(%21 Yes(%50 Yes, with improvements(%14	Simple culture based assays available. Limited to culturable taxa. Doesn't reflect whole community effects. MIC assays used internationally for AMR PNECs.	Low nutrient test medium essential
Fungal growth inhibition	Species	Growth inhibition assay measuring radial mycelial growth, colony area, or biomass on solid media following exposure (typically over 3-14 days) to a contaminant.	Apical (growth)	Growth rate reflects fungal physiological stress but is a simplified laboratory surrogate that does not directly represent fungal-mediated ecosystem processes such as decomposition	Not specifically defined, but for general microbes standard is minimum 24 h.	Yes	Simple and sensitive assay for detecting direct toxicity to fungal organisms; useful for mechanistic screening and comparative sensitivity assessment	Single-species laboratory assay with limited ecological representativeness; growth inhibition does not always translate to population-level resistance dynamics; High ecological and public health relevance — links contaminant exposure to selection pressure for tolerance or resistance; Reproducible and quantitative through growth-rate-based selection coefficients; Reflects functional adaptation over short-term (16–48 h) exposures using environmentally relevant inocula such as sewage microbiomes.	Yes (limited use; bacterial growth as a fungal growth data included in single-species datapoints in the copper GV but not routinely applied across GVs)	Research - assay reported in the literature and used in a copper GV; but not standardised under OECD/ISO and not routinely applied in guideline derivation	Fungal taxa (saprotrophic or yeast species); does not directly represent functional guild activity in natural systems	Indication of potential fungal stress but weakly linked to ecosystem-level outcomes such as organic matter decomposition or nutrient cycling	4.4	Unsure (%21 No(%14 Yes(%29 Yes, with improvements(%29	Widely used in research laboratories using agar-based media; results depend on growth medium composition, temperature, and species choice; lack of standardisation and regulatory benchmarks limits applicability for guideline derivation	Included in copper DGV derivation as a individual microbial data point; represents opportunistic use of available data rather than a established part of a general fungal toxicity test framework
Yeast growth inhibition	Species (unicellular yeasts)	Growth inhibition assay measuring yeast cell density, optical density, or colony counts in liquid or solid media following exposure (typically over 24-48 h) to a contaminant	Apical (growth)	Yeasts represent a limited subset of microbial eukaryotes; growth inhibition reflects cellular stress but is a simplified laboratory surrogate with weak linkage to ecosystem-level microbial functions	chronic, if exposure is longer than 24h	No	Simple, rapid, and sensitive assay for detecting direct toxic effects on eukaryotic microbial cells; useful for screening and comparative toxicity assessment	Yeasts are not dominant ecosystem drivers; Indication of ecosystem-level relevance is indirect; Not a standard test for regulatory guideline derivation	No	Research - no OECD, ISO, or ASTM standards for yeast growth inhibition in environmental quality guideline derivation	Unicellular fungal taxa (yeasts); not representative of dominant microbial functional guilds in most aquatic ecosystems	Indication of cellular level toxicity only; weakly linked to adverse ecosystem outcomes or microbial process impairment	3	Unsure (%50 No(%50	Widely available laboratory method using standard culture media and spectrophotometric or plate-count measurements; results highly dependent on media composition, strain selection, and growth conditions; lack of ecological context and regulatory benchmarks limits applicability for guideline derivation	Best treated as a screening or mechanistic assay; provides little additional ecological insight beyond existing bacterial or algal growth inhibition endpoints
Change in viable microbial abundance	Species	Quantitative Microbiome Profiling (QMP) via PMA and Flow Cytometry. Combines propidium monoazide (PMA) treatment with flow cytometric cell counts to normalise amplicon read data to absolute microbial abundances for molecular species or taxonomic groups (cells ml^{-1}). Excludes DNA from membrane-compromised or dead cells, converting relative sequence counts into ecologically meaningful cell-based abundance estimates.	Structural	Provides a direct measure of change in abundance for individual species or taxa, when exposed to a whole community, linking lower specific abundance changes to potential biomass shifts in the environment. Improves detection and accurate modelling of growth responses that are masked in relative datasets.	chronic, if exposure is longer than 24h for "microbes" and longer than 48 h for microalgae or cyanobacteria. No guidance for complex communities with multiple taxa types.	Yes, if data are extracted for individual species.	Improves metabarcoding analyses. Quantifies viable microbial cells and supports detection of no-significant effect concentrations (NSECs) in toxicity tests; Comparable to eukaryotic species sensitivity distributions used in guideline setting;	Flow cytometric counts may vary with staining efficiency and gating strategy; PMA effectiveness depends on membrane integrity and temperature/time; Requires specialised instrumentation and calibration; Still limited to DNA-level inference rather than functional activity.	No	Research	Measures all groups and ring acts on functional groups might be affected	Potentially multiple adverse outcomes might be inferred	7.6	Unsure (%14 No(%14 Yes(%36 Yes, with improvements(%36)	Measured using flow cytometry fluorescence-activated cell sorting (FCM) or qPCR based absolute gene copies; methods are standard but for soils and widely applied to freshwater and marine sediments, while aquatic water column applications are less established; sediment-specific factors (organic content, redox conditions, mineral binding) require careful interpretation, and biomass reflects standing stock rather than realised function	
COMMUNITY FUNCTION ENDPOINTS																
Decrease in microbial biomass	Community	Can be measured in different ways, dry/wet weight, Chloroform fumigation-extraction, qPCR, PLFA analysis.	Biological, apical (infers growth)	Biomass is a measure of growth, so has similar ecological relevance as microbial growth endpoints, i.e. Microbial productivity is the foundation for nutrient cycling, energy flow, and the maintenance of ecosystem health.	chronic, if exposure is longer than 24h for "microbes" and longer than 48 h for microalgae or cyanobacteria. No guidance for complex communities with multiple taxa types.	No. None, biomass is an accepted endpoint. However, community based biomass endpoints have not been considered. Likely, would be acceptable as an additional line of evidence.	Integrates effects across the whole microbial community; Provides direct measure of long-microbial biomass; Established and widely used methods (CFE, qPCR, qRCR, PLFA with good reproducibility; Can indicate reduced soil or sediment function and nutrient cycling potential	Does not identify which taxa are affected or disrupt the ecology; Biomass can fluctuate naturally with season, moisture, or organic inputs; Requires careful calibration and consistent methodology; Cannot distinguish between toxic suppression and natural variability; Increases in biomass (e.g., bloooms) may also represent adverse ecological imbalance rather than recovery.	No but can be used within OECD 216 (S7) soil function tests as a supporting endpoint	↑ - could be targeted, Total living microbial biomass, including heterotrophs, chemoautotrophs, and decomposers.	Heterotrophic aerobic bacteria (general metabolic activity).	Reduction in microbial standing biomass, indicating loss of microbial capacity to support ecosystem processes, and considered an adverse ecological outcome even where downstream functional consequences are not fully resolved	4.8	Unsure (%14 No(%14 Yes(%14 Yes, with improvements(%56)	Measured using chloroform fumigation-extraction, PLFA analysis, or qPCR based absolute gene copies; methods are standard but for soils and widely applied to freshwater and marine sediments, while aquatic water column applications are less established; sediment-specific factors (organic content, redox conditions, mineral binding) require careful interpretation, and biomass reflects standing stock rather than realised function	
Inhibition of Microbial respiration/fermentation	Community function	Microcosm test measuring aerobic respiration during, or following exposure to toxicant.	Biochemical	Indicator of microbial activity and organic matter breakdown. Affects carbon cycling.	chronic, if exposure is longer than 24h for "microbes" and longer than 48 h for microalgae or cyanobacteria. No guidance for complex communities with multiple taxa types.	No	Indirectly measures microbial metabolic activity and overall ecosystem function; Provides integrative assessment of microbial community health in realistic environmental matrices (soil, sediment, or water microcosms); Rapid and cost-effective using oxygen consumption or CO_2 evolution assays; Useful for detecting substantial stress before changes in community composition or biomass occur.	Respiration rates can vary naturally with organic matter content, temperature, and oxygen availability; Does not distinguish between non-toxic and toxic causes of reduced activity; Can be influenced by non-biological oxygen demand or chemical oxidation; Requires careful calibration and controls to account effects from natural variability; Interpretation of "inhibition" versus "adaptation" depends on exposure duration and environmental context.	no	Pe mutly standardised — OECD 209 (activated sludge), ISO 17184 & ISO 14672 (soil CO_2 evolution), ASTM E2172 (sediment microcosms); strong for soil and wastewater applications, moderate for sediment; highly relevant when following standard protocols. Algae extracellular activity tests are used for water and sediments, but these are acute (24 h exposures), e.g. Stauber et al., 2006; Adams et al., 2006)	Aerobic heterotrophic community (carbon and energy metabolism)	Suppressed soil/sediment respiration, indicating ecosystem-level microbial dysfunction and lower decomposition rates	4.7	Unsure (%50 No(%7 Yes(%7 Yes, with improvements(%29	Measured using microcosm or laboratory incubations quantifying microbial respiration or fermentation (e.g. CO_2 production, O_2 consumption, electron acceptor depletion); ancillary enzyme extracellular hydrolytic enzyme activity (e.g. esterases) following contaminant exposure; methods are well established and widely available, with some standardisation in soils and sediments but limited formal guidance for aquatic applications; respiration rates are highly sensitive to environmental conditions (temperature, to ensure internal conditions in (sub-)state availability, redox, temperature), reflect potential or short-term activity rather than realised ecosystem process rates, and lack universally defined functional thresholds for guideline derivation	Biochemical endpoint, generally not used in water and sediment quality SV derivations. For soils, functional thresholds are typically defined as acceptable deviations from reference conditions or controls (e.g. <30% change in soil processes under OECD guidelines), with aquatic applications requiring site-specific interpretation rather than fixed universal limits.
Carbon digestion inhibition	community	Biologic Respirometers measure the ability of communities to metabolise a carbon substrate	Biochemical	Important function but ecological relevance unclear	chronic, if exposure is longer than 24h for "microbes" and longer than 48 h for microalgae or cyanobacteria. No guidance for complex communities with multiple taxa types.	no	Assesses microbial functional and metabolic potential; Provides detection of stress-induced inhibition of carbon substrate utilisation; Rapid and reproducible with standardised kinetic methods; Useful for screening bioactive contaminants in microbial activity; Links directly to ecosystem processes like carbon cycling and energy flow	Limited ecological realism — relies on culturable heterotrophic bacteria; Not all environmental microbes utilise the provided substrates; Influenced by nutrient availability, temperature, and substrate availability; Data interpretation can be complex and semi-quantitative; Functional inhibition may not always translate to ecosystem-level effects.	Yes (acids)	OECD 217 2000 Soil Microorganisms: Carbon Transformation Test; ISO 17155 2012 Soil quality — Determination of the effects of pollutants on soil microflora — Carbon dioxide evolution test; ISO 14239 2012 Soil quality — Determination of effects on Collembola (Sminthurus viridis) — Part 1: Substrate-induced respiration method; ISO 14372 2012 Soil quality — Laboratory methods for determination of microbial soil respiration; OECD 209 2010 Activated Sludge, Respiration Inhibition Test	Heterotrophic carbon-utilising bacteria - and fungi	Reduced carbon mineralisation and CO_2 release, leading to slower organic matter decomposition and energy flow	5.3	Unsure (%33 No(%7 Yes(%7 Yes, with improvements(%14	Measured using static lab or microcosm assays assessing microbial utilisation of organic carbon substrates, including substrate-induced respiration, CO_2 evolution, or community-level metabolic profiles (e.g. Biolog EcoPlates); methods are well established and widely used in aquatic and sediment contaminant exposure; methods are well established and partly standardised for soils and sediments, but aquatic applications require adaption; results reflect potential carbon utilisation capacity rather than in situ decomposition rates, are sensitive to substrate selection, nutrient status, and redox conditions, and interpretation is constrained by functional redundancy and the absence of defined regulatory thresholds for guideline derivation	Biochemical endpoint, generally not used in water and sediment quality SV derivations. For soils, functional thresholds are typically defined as acceptable deviations from reference conditions or controls (e.g. <30% change in soil processes under OECD guidelines), with aquatic applications requiring site-specific interpretation rather than fixed universal limits.
Nitrogen fixing and nitrification/ denitrification inhibition	community	Acetylene reduction assay, labelled nitrogen incorporation measures conversion of N_2 gas to ammonia by nitrogen-fixing microbes. Measurement of nitrate/nitrite/ammonium over time 15N tracers	Biochemical	Important function but ecological relevant is unclear	chronic, if exposure is longer than 24h for "microbes" and longer than 48 h for microalgae or cyanobacteria. No guidance for complex communities with multiple taxa types.	no	Directly measures functional processes fundamental to nitrogen cycling; Provides mechanistic insight into contaminant effects on fixation and nitrification-denitrification pathways; Links closely to ecosystem-level nutrient transformations and productivity; Can distinguish between nitrogen-fixing and denitrifying microbial activity using established methods (acetylene reduction, $15^N/^{14}N$ tracers).	Technically complex and resource intensive; Sensitive to incubation conditions and substrate availability; Requires careful interpretation to separate microbial inhibition from abiotic effects; Spatial and temporal variability in processes can obscure contaminant effects; Isotopic analyses demand specialised equipment and expertise; The distinction between healthy and dysfunctional nitrogen cycling is context dependent — rates can vary naturally with season, hydrology, and redox conditions, so reference site data and environmental context are essential for interpretation	No	OECD 216 2000 Soil Microorganisms: Nitrogen Transformation Test ; ISO 14238 2012 Soil quality — Biological methods — Determination of nitrogen mineralisation and nitrification in soils and the influence of chemicals ; ISO 15685: 2012 Soil quality — Determination of potential nitrification and inhibition of nitrification — Rapid test by ammonium oxidation ; ISO 21772-1 : 2024 Soil quality — Determination of dehydrogenase activity — Part 1: Method using triphenylmethazolium chloride (TTC) (optional functional indicator for microbial oxidation).	Nitrogen-transforming microbes: phototrophs, ammonia-oxidisers, nitrite/nitrate-reducers, denitrifiers	Impaired nitrogen cycling (ammonia oxidation, nitrate formation N_2 release), causing nutrient imbalances and reduced plant growth	6.8	Unsure (%21 No(%21 Yes(%29 Yes, with improvements(%29	Acetylene reduction widely used but an indirect proxy; calibration with 15N2 required for accuracy. Taxa/condition dependent. Gaps include field in lab comparability.	Biochemical endpoint, generally not used in water and sediment quality SV derivations. For soils, functional thresholds are typically defined as acceptable deviations from reference conditions or controls (e.g. <30% change in soil processes under OECD guidelines), with aquatic applications requiring site-specific interpretation rather than fixed universal limits.
Sulfate reduction capacity inhibition	community	Measured with labeled sulfate tracer and HGS production	Biochemical	Less important function but ecological relevance is unclear	chronic, if exposure is longer than 24h for "microbes" and longer than 48 h for microalgae or cyanobacteria. No guidance for complex communities with multiple taxa types.	no	Directly measures microbial sulfate reduction, a key anaerobic process in carbon and sulfur cycling; Provides clear functional link between contaminant exposure and redox-sensitive microbial activity; Sensitive to metals that disrupt electron transport and enzyme systems; Indicates broader sediment or soil biogeochemical health and oxygen dynamics; Can be quantified using well established tracer or sulfide production methods.	Technically demanding and requires controlled anaerobic conditions; Sulfide production can be affected by abiotic reactions and sediment chemistry; High natural variability in sulfate pools, sediments, organic matter, and temperature; Requires specialised analytical methods and methods; Interpretation can be complicated by "sulfide paradoxes" versus "sulfide chemical" activity; depends strongly on environmental context — both suppression and stimulation of sulfate reduction can indicate ecological imbalance.	No	Research - Established in research	Anaerobic sulfate-reducing bacteria (SRB) — key in sulfur cycling and metal precipitation.	Reduced sulfur cycling and metal sulfide formation; accumulation of sulfides and altered redox balance in sediments	6.8	Unsure (%33 No(%29 Yes(%14 Yes, with improvements(%14	Methods are well established in sediment biogeochemistry and research laboratories but are not standardised for regulatory toxicity testing; results are highly sensitive to redox conditions, organic carbon availability, and sediment characteristics, and interpretation is limited by lack of defined functional thresholds and by challenges in extrapolating laboratory inhibition to ecosystem-scale sulfur cycling	Biochemical endpoint, generally not used in water and sediment quality SV derivations. For soils, functional thresholds are typically defined as acceptable deviations from reference conditions or controls (e.g. <30% change in soil processes under OECD guidelines), with aquatic applications requiring site-specific interpretation rather than fixed universal limits.
Extracellular enzyme activity inhibition	community	Measures inhibition of extracellular enzyme activities (e.g. esterase, phosphatase, β-glucosidase) using colourimetric or fluorometric substrates to quantify enzyme activity following contaminant exposure.	Biochemical	Extracellular enzymes mediate acquisition of carbon (e.g. esterases) and nutrients (e.g. phosphatases) from organic matter and are critical to microbial-driven nutrient cycling	chronic, if exposure is longer than 24h for "microbes" and longer than 48 h for microalgae or cyanobacteria. No guidance for complex communities with multiple taxa types.	no	Directly measures enzymatic activity linked to nutrient acquisition; Potentially provides early indication of microbial or biochemical stress; Standardised colorimetric and fluorometric assays are rapid and cost-effective; Reflects both microbial and extracellular enzyme responses; Useful for linking contaminant exposure to impaired nutrient availability and ecosystem productivity	Activity can vary naturally with temperature, pH, and substrate type; Enzyme activity may persist extracellularly and not represent living microbial function; Inhibition may reflect enzyme denaturation rather than cellular stress; Interpretation can be confounded by soil or sediment properties affecting substrate availability; Does not distinguish between microbial community shifts and enzyme inhibition; Requires environmental context and reference baselines to determine whether decreased or increased activity indicates ecosystem dysfunction therefore difficult to define ecologically meaningful thresholds; responses may be non-linear or transient.	No	ISO 22939:2019 Soil quality — Measurement of enzyme activity patterns in soil samples using fluorogenic substrates in micro-well plates; ISO 20130:2018 Soil quality — Measurement of enzyme activity patterns using colorimetric substrates.	Heterotrophic microbial functional guilds involved in organic matter processing and substrate acquisition, inferred from composite extracellular hydrolytic enzyme activity, including carbon-processing enzymes (e.g. esterases) and phosphorus-mobilising enzymes (phosphatases), contributing to substrate availability for downstream microbial metabolism	Reduced microbial capacity for organic matter breakdown and nutrient mobilisation, leading to decreased substrate availability for microbial metabolism and potential impairment of ecosystem-level nutrient cycling.	EXTRAMACELLAR ACTIVITY: 4.6 EXTRACELLULAR ENZYME ACTIVITY: 5.0	EXTRAMACELLAR ACTIVITY: Unsure (%29 No(%43 Yes(%29 Yes, with improvements(%14 EXTRACELLULAR ENZYME ACTIVITY: Unsure (%29 No(%43 Yes(%29 Yes, with improvements(%29	Measured using colorimetric or fluorometric assays (e.g. FDA hydrolysis, phosphatase substrates) to quantify composite extracellular enzyme activity following contaminant exposure; methods are well established in research laboratories (e.g. ISO-based assays), but quality control, substrate specificity, and interpretation of functional capacity remain a challenge; results are sensitive to environmental conditions (sub-state availability, temperature, redox), and lack clearly defined regulatory thresholds for guideline derivation	Biochemical endpoint, generally not used in water and sediment quality SV derivations. For soils, functional thresholds are typically defined as acceptable deviations from reference conditions or controls (e.g. <30% change in soil processes under OECD guidelines), with aquatic applications requiring site-specific interpretation rather than fixed universal limits.
Inhibition of Biofilm formation	Other (functional trait)	Crystal violet assays/XPS quantification following exposure to toxicant	Functional	Measures capacity for surface-attachment etc colonisation, relevant for persistence	chronic, if exposure is longer than 24h for "microbes" and longer than 48 h for microalgae or cyanobacteria. No guidance for complex communities with multiple taxa types.	No	Provides direct measure of microbial capacity to form surface-attached biofilms; Sensitive to toxicants that disrupt cell adhesion, quorum sensing, or extracellular polymeric substances (EPS) production; Simple and cost-effective assay using standard laboratory equipment; suitable for rapid screening; Mechanistically interpretable — inhibition reflects impaired microbial communication and colonisation ability; Can serve as an early indicator of stress before changes in biomass or community composition occur.	Limited ecological realism — typically performed in single-species or simplified cultures; Biofilm formation can vary with nutrient status, surface type, and hydrodynamic conditions; Assay does not capture recovery or adaptive tolerance; Results are highly sensitive to assay conditions and can be affected by non-ecological material loss or cell density; Requires careful interpretation as both inhibition and stimulation of biofilm formation can indicate ecological imbalance depending on the contaminant and context. Recent work shows that this method produces sensitive results when looking at environmental biofilms. Biofilms formed in different environments vary and are not standard.	No	Research - common in research	Surface-attached microbial consortia (heterotrophs, quorum-sensing bacteria).	Impaired biofilm stability and colonisation; decreased habitat for microfauna and reduced nutrient retention on surfaces	5.8	Unsure (%21 No(%29 Yes(%29 Yes, with improvements(%21	Measured using static assay quantifying biofilm biomass or extracellular polymeric substances (e.g. crystal violet staining, EPS extraction); following contaminant exposure; methods are widely used in research and readily available, but are not standardised for regulatory use; results are sensitive to surface type, hydrodynamics, nutrient conditions, and species composition, and reflect simplified laboratory biofilms that may not represent natural multispecies biofilms or their ecosystem functions, limiting direct extrapolation to field-scale outcomes	
Protein synthesis	community	BONCAT (Bioorthogonal Non-Canonical Amino Acid Tagging),	Biochemical	Incorporation of non-canonical amino acids (e.g. HPG, Aha) into newly synthesised proteins of metabolically active microbes, detected via click-chemistry and fluorescence microscopy, flow cytometry, or sequencing (BONCAT-FACS / BONCAT-seq).	chronic, if exposure is longer than 24h for "microbes" and longer than 48 h for microalgae or cyanobacteria. No guidance for complex communities with multiple taxa types.	No	Direct measure of microbial activity rather than presence; discriminates active vs inactive taxa; can be combined with sequencing to link activity to taxonomy.	Requires specialised reagents and microscopy/flow cytometry; amino acid uptake varies among taxa; incubation conditions may influence activity; does not directly quantify ecosystem-level processes rates;	No	Research (emerging, not standardised)	All metabolically active microbial guilds (heterotrophs, autotrophs, nutrient cyclers)	Loss or suppression of active microbial populations leading to reduced rates of general microbial activity	4.5	Unsure (%29 No(%21 Yes(%50 Yes, with improvements(%29	Method is based on incorporation of non-canonical amino acids (e.g. HPG or AHA) into newly synthesised proteins of metabolically active cells, followed by click-chemistry labeling and detection via fluorescence microscopy, flow cytometry, or sequencing; methods are well established in research but require specialised research laboratories with access to flow cytometry or advanced microscopy. Challenges include scalable amino acid uptake across taxa, dependence on incubation conditions vs. potential artefacts from short incubations, limited standardisation, and difficulty scaling results to ecosystem-level process rates.	
Substrate-coupled microbial growth	Community	DNA-SIP, RNA-SIP	Functional	Links incorporation of isotopically labelled substrates (e.g. ^{13}C-, ^{15}N) into microbial DNA to identify taxa actively assimilating specific resources.	chronic, if exposure is longer than 24h for "microbes" and longer than 48 h for microalgae or cyanobacteria. No guidance for complex communities with multiple taxa types.	No	Provides direct causal linkage between microbial identity and biogeochemical function by identifying taxa that are actively growing while assimilating a defined substrate; avoids inferences inferred function from gene presence alone; offers high ecological relevance for nutrient cycling endpoints.	Technically complex and costly; biased toward fast-growing organisms and high substrate concentrations; Limited temporal resolution; long incubation times may alter natural community structure; low suitability for routine, high-throughput guideline assessments. Very much still a research tool and will be difficult to use as a methodology.	NO	Research (emerging, not standardised)	Specific substrate-utilising guilds (e.g. carbon assimilators, nitrogen fixers, sulfate reducers)	Impaired nutrient cycling and reduced ecosystem productivity due to loss of functionally active taxa	4.5	Unsure (%33 No (%14 Yes(%14 Yes, with improvements(%21	Requires isotope-labelled substrates, ultracentrifugation, and seq sencing; limited scalability and low suitability for routine guideline derivation	
Growth-inferred resistance (SELECT assay)	Community	Growth response over 16-48 h of a sewage inoculum to a toxicant in dose-response assays. Resistance endpoints are measured in a growth assay using the selection coefficient.	Resistance inferred from growth	Measuring growth in a sewage microbiome to infer resistance potential in environmental microbiomes has limited relevance	chronic, if exposure is longer than 24h for "microbes" and longer than 48 h for microalgae or cyanobacteria. No guidance for complex communities with multiple taxa types (i.e. not TWI as currently used)	No	Quantifies contaminant-induced selection for resistant microbial subpopulations under realistic exposure; Provides direct measure of growth-based selection relevant to resistance dynamics; High ecological and public health relevance — links contaminant exposure to selection pressure for tolerance or resistance; Reproducible and quantitative through growth-rate-based selection coefficients; Reflects functional adaptation over short-term (16–48 h) exposures using environmentally relevant inocula such as sewage microbiomes.	Currently not standardised — promotion very emerging research groups; Requires careful experimental design, controls, and interpretation to distinguish genuine selective pressure from growth stimulation or inhibition; Interpretation of resistance versus tolerance can be context dependent; Limited ecological realism for complex biofilm or sediment systems; Data analysis requires fitting dose–response models to selection coefficients, making it more computationally intensive than routine growth assays.	No	Standards relevant but limited by inherent variability of sewage microbial consortia, and other community compositional effects; effective, but lack of standardised results. High ecological relevance but low reproducibility; currently viewed at grade and not yet suitable for inter-laboratory standardisation	Rapidly reproducing heterotrophic bacteria (potentially resistant subpopulations).	Selection for resistant microbial strains, leading to community shifts and a persistence of pollutant-relevant traits	4.4	Unsure (%27 No(%21 Yes(%29 Yes, with improvements(%21	Assessments using integrated multi-omics approaches combining genomics or transcriptomics, sidewide proteomic and metabolomic profiling, and functional activity measurements provide a mechanistic overview of microbial communities to contaminant exposure; method is available in advanced research settings but requires substantial analytical infrastructure, cross-platform data integration, and expert bioinformatics, and are not standardised for regulatory guideline derivation; while offering comprehensive insight into stress mechanisms and functional disruption, interpretation is challenged by high data complexity, temporal variability, and the absence of agreed threshold linking integrated molecular changes to adverse ecological outcomes or loss of ecosystem function	
AMPLICON-INFORMED COMMUNITY STRUCTURE ENDPOINTS																
Change in Microbial Community Richness	Community	Using metabarcoding (e.g. 16S/18SrRNA and ITS amplicons) from microcosms, mesocosms or field studies, quantifies the number of distinct taxa (ASVs, OTUs, or species) within a sample using richness or diversity indices (e.g., Chao1, Shannon, Simpson). Statistical comparisons typically use t-tests, ANOVA, or non-parametric tests (Kruskal-Wallis, Wilcoxon) to detect significant differences in richness between treatments or time points.	Structural	This is a structural endpoint, functional changes need to be inferred. Reflects potential loss of taxa but declines in richness can indicate reduced functional redundancy, lower ecosystem resilience, and diminished capacity to recover from disturbances.	chronic, if exposure is longer than 24h for "microbes" and longer than 48 h for microalgae or cyanobacteria. No guidance for complex communities with multiple taxa types.	No, but may be considered as an alternative line of evidence (?)	Provides an integrative measure of ecosystem biodiversity response; sensitive to cumulative and chronic stress; relatively easy to interpret across treatments; indicates potential loss of resilience and functional redundancy before higher-tier effects occur.	Sensitive to sampling effort and sequencing depth; does not distinguish between rare and functionally important taxa; richness changes may not directly translate to adverse ecosystem function; high natural variability can mask contaminant effects.	No	Research - Established in research but many methods/pipelines exist	Measures all groups and ring acts on functional groups might be inferred	Potentially multiple adverse outcomes might be inferred	4.6	Unsure (%29 No(%21 Yes(%29 Yes, with improvements(%36	Genomics are widely available however requires bioinformatics capacity. Gaps include linking diversity changes to functions and inferring ecosystem outcomes.	
Change in Microbial Abundance (via Sequence Reads)	Community	Using metabarcoding (e.g. 16S/18SrRNA and ITS amplicons) from microcosms, mesocosms or field studies, reads data differs taxa relative or absolute abundances of specific taxa or groups based on sequencing read counts. Analysed using differential abundance models such as DESeq2, edgeR, ANCOM-BC2, or ALDEx2, often with log-ratio or compositional transformations (CLR, TSS). Results indicate taxa that increase or decrease significantly due to exposure/treatment.	Structural	This is a structural endpoint, function changes need to be inferred. Indicates shifts in ecosystem density or dominance of key microbial groups. Increases or decreases in abundance can alter nutrient turnover rates, organic matter breakdown, and energy flow, influencing higher-trophic interactions and ecosystem productivity.	chronic, if exposure is longer than 24h for "microbes" and longer than 48 h for microalgae or cyanobacteria. No guidance for complex communities with multiple taxa types.	No	Offers taxa-specific, quantitative evidence of contaminant effects; sensitive to subtle and community-wide responses; allows linkage to functional shifts through taxa-function associations; scalable from microcosm to field.	Relative read counts do not necessarily reflect true biomass or cell abundance; compositional bias and library preparation artefacts can distort abundance estimates; requires careful normalisation and often lacks clear ecological thresholds.	No	Research - Established in research but many methods/pipelines exist	Measures all groups and ring acts on functional groups might be inferred	Potentially multiple adverse outcomes might be inferred	7.2	Unsure (%29 No(%14 Yes(%7 Yes, with improvements(%43	Genomics are widely available however requires bioinformatics capacity. Gaps include linking diversity changes to functions and inferring ecosystem outcomes.	
Change in Microbial Community Composition	Community	Using metabarcoding (e.g. 16S/18SrRNA and ITS amplicons) from microcosms, mesocosms or field studies, assesses shifts in the overall taxonomic or functional structure between samples using β-diversity metrics (Bray–Curtis, Jaccard, UniFrac). Multivariate statistics such as PERMANOVA (adonis), ANOSIM, or ordination