Deciphering microbial robustness at the community level through synthetic ecology and molecular systems synecology

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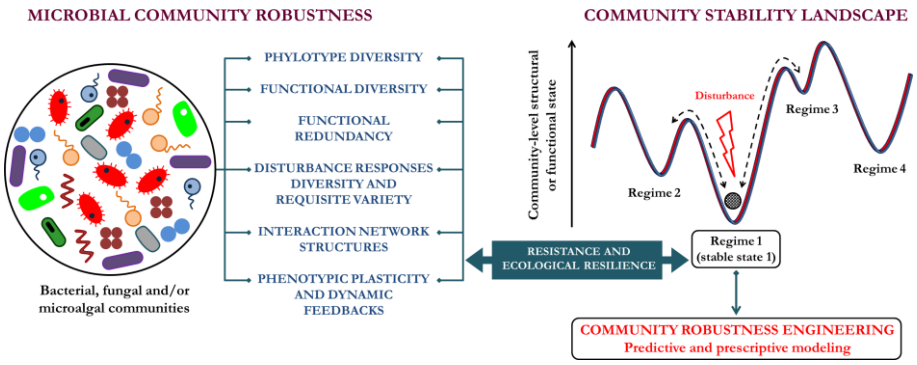
Abstract

Microbial ecosystems exhibit specific robustness attributes arising from the assembly and interaction networks of diverse, heterogeneous communities challenged by fluctuating environmental conditions. Synthetic ecology provides new insights into key biodiversity-stability relationships and robustness determinants of host-associated or environmental microbiomes. Driven by the advances of meta-omics technologies and bioinformatics, community-centered holistic approaches (defined as molecular systems synecology) combined with the development of dynamic and mechanistic mathematical models make it possible to decipher and predict the outcomes of microbial ecosystems under disturbances. Beyond discriminating the normal operating range and natural, intrinsic dynamics of microbial processes from systems-level responses to environmental forcing, predictive modeling is poised to be integrated within prescriptive analytical frameworks and thus provide guidance in decision-making and proactive microbial resource management.

Graphical abstract



Introduction

From the enzymatic to the global scale, biological robustness is a key attribute of biosystems that guarantees the maintenance of structural organization and/or function performance in the face of disturbances and uncertainty. A brief survey of the Earth's biogeochemical history after the first stirrings of the Industrial Revolution shows a broad range of anthropogenic and natural perturbations that have been triggering specific biological responses and ecosystem state transitions, up to the ultimate point of sudden collapse [1]. The intrinsic principles and mechanisms of microbial robustness have drawn special attention because microbial communities can provide host protection against diseases [2,3] and lie at the heart of biological processes that fulfill the premise of sustainable development. With the translation of the microbial resource management concept into practical applications [4•], microbial communities also play crucial roles in the cradle-to-cradle approach by supporting the valorization and recycling of natural resources, man-made products and their wastes.

Microbial robustness has mainly been addressed from an autecological perspective by identifying the key features and biomarkers of clonal microbial populations that promote robust industrial and biotechnological processes, e.g., modularity of metabolic networks, fault tolerance (enzyme redundancy and promiscuity), self-repair and protection systems (DNA repair and antioxidant activity) [5]. However, the metabolic capabilities, growth characteristics and robustness attributes of individual microbial populations differ significantly when they are living within dynamic consortia characterized by intricate networks of inter-population interactions. Because microbial community members are faced with a composite set of extracellular metabolic cues, display dissimilar or redundant functional traits and exchange genetic material and nutrients, the activities of a microbial community cannot be generally attributed to any of its individual phylotype components. Many essential microbial partnerships occur in nature, such as the cooperative biodegradation of trichloroethene (TCE) through syntrophic associations and cofactor exchanges [6•]. Because microbial consortia have the potential to achieve more diverse and robust functions than single phylotypes grown in isolation, there is a compelling impetus to move from individual-based analysis to synecological (i.e., community-centered) approaches to study microbial

resources within the context of natural environments and engineered systems. Cryoconservation strategies have recently been optimized for the stable preservation of ecologically- and industrially-relevant microbial consortia to complement the vast collection of pure culture strains [7]. Supported by the experimental evidences of the link between microbial diversity, productivity and robustness, the production of next generation, polymicrobial probiotics (i.e., microbial ecosystem therapeutics [3]) to substitute for single-strain probiotic formulations is an elegant example of the quest for more robust and optimized microbial-driven services.

Commenté [S1]: En anglais "evidence" n'a pas de pluriel: on dit "evidence" et "many lines of evidence" mais pas « evidences » ;même « lines of evidence » est assez mal-fichu – donc, je propose de dire tout simplement "evidence" en singulier (evidence peut très bien inclure plusieurs points)

With the development of new molecular technology platforms and bioinformatic pipelines and the recent advances in microbial ecological theory [8•,9••,10•], new hypotheses can be formulated and tested to develop predictive models of microbial community robustness in controlled, artificial communities and ultimately more complex natural ecosystems. Synthetic ecology, i.e., the rational design and theory-driven manipulation of artificial microbial ecosystems [11], is highly relevant to decipher robustness attributes thanks to the analysis of replicate systems of lower complexity and higher controllability and reproducibility in the presence of specific perturbations. Similar to molecular (eco)systems- or community systems biology [12,13], molecular systems synecology seeks to achieve a systems-level understanding of community structure and function emerging from dynamic interactions and networks under different environmental scenarios. This holistic approach focuses on systems biology at the community level by integrating meta-omics techniques, computational systems bioinformatics and synecology (i.e., analysis of the activities of an entire natural or synthetic community as a whole and forming a unified functional entity). Engineered maintenance and perturbation studies in multiplexed continuous culture systems are particularly relevant to molecular systems synecology in order to single out and compare the effect of specific perturbations on microbial communities maintained under controlled, reproducible and environmentally-relevant conditions. The predictive models of robustness developed at the lab scale for synthetic and more complex communities can be further translated into natural microbiomes with the combination of multi-scale modeling of hydrogeochemical processes [11].

In this review, we highlight the need for quantitative community-level benchmarks of multiple microbial ecosystems in confined and open systems to discriminate natural dynamics (baseline) from community changes under the impact of environmental forcing factors, either natural or anthropogenic. We also emphasize the multi-faceted dimension of microbial community robustness with the description of its epistemic framework and give an overview of the conceptual and mechanistic principles governing community robustness. Finally, the prediction of systems-level robustness of microbial ecosystems is discussed in the light of molecular systems synecology using network inference and dynamic modeling of omics data sets.

Benchmarking in microbial ecology: Microbial community dynamics under normal (undisturbed) conditions

Before investigating the robustness of a microbial community towards a specific disturbance, its temporal stability (i.e., constancy in structure (see Box 1) and function over time) must be deciphered to define structural and functional pre-disturbance benchmarks (reference states) against which community robustness will be assessed (Figure 1). Perturbation-independent stability requires the description of the system (reference state of biological variables (descriptors) and environmental parameters) and the specification of its natural dynamics in terms of structure and function [14]. Similar to the normal operating range (NOR) concept of soil functioning [15], the normal limits of variation of microbial community processes under reference conditions are a prerequisite to properly assess the disturbance-specific responses of microbial ecosystems. A functional reference state with permissible limits of variation (i.e., engineering tolerance) can be obtained from (i) the measurement of quantitative end-points of functionally redundant and non-redundant processes (e.g., nitrification and denitrification rates [16,17]) or (ii) the definition of a functional core microbiome with quantifiable critical traits using community-level functional diversity metrics [2,18]. A structural reference state defined on the basis of a core set of phylotypes identified for a target microbial habitat (i.e., a core microbiome in terms of specific microbial phylotypes) is more difficult to achieve because of the intrinsic dynamic nature of microbial communities (temporal and spatial structural variation). A set of shared metabolic traits across microbial

habitats is frequently detected (e.g., human gut [2], activated sludge), notwithstanding high levels of variability in phylotype composition. As stated by Verstraete et al. [19], even under stable conditions, microbial ecosystems display a cooperative community continuum with continuous fluctuations of community composition and population abundance. A comprehensive survey of the temporal variability of different microbial habitats using targeted sequencing of 16S rRNA genes (illumina-based 16S-tags (*iTags*) or 16S pyrosequencing (16S *pyrotags*)) demonstrated species-time relationships (STRs) with consistent temporal scaling exponents across similar environments (*w* ranging from 0.24 to 0.61, see Box 1) [20]. Comparable *w* values generated from pyrotags analysis were obtained for a full-scale activated sludge system (ranging from 0.38 to 0.45 as a function of the targeted 16S region) highlighting significant phylotype turnover rates [21]. Microbial communities in controlled bioreactors also present intrinsic temporal variability. In a functionally-stable denitrifying continuous culture maintained in a chemostat for 50 days, Kraft et al. [22] observed that microbial communities can be highly dynamic in composition but achieve stable functional outputs thanks to the maintenance of a core set of functionally-redundant genes. In long-term replicate anaerobic reactors (362 days), Vanwonterghem et al. [23] have observed reproducible and predictable transient states and population dynamics that were mainly governed by deterministic (niche-based) processes. After the start-up phase characterized by an inevitable variation in functionality and phylotype composition due to operating conditions and early stages of community assembly, succession trajectories of hydrolytic, fermentative, acetogenic and methanogenic communities were synchronized among replicates to reach stable methane production rates and a more even distribution of functional guilds [23]. On the contrary, Zhou et al. [24] observed divergent community trajectories in electrolysis cell reactors inoculated with the same consortium under identical environmental conditions because of initial stochastic colonization followed by deterministic processes maintaining community variations between replicates. Consequently, an original microbial community can exhibit alternative differentiation pathways that lead to dissimilar structural and/or functional endpoints [24]. Chaotic dynamics and sensitivity to initial conditions have also been identified in microbial systems. The dynamics of nitrifying microbial communities have been studied in aerobic chemostats using the Lyapunov stability function (see Box 1) [25]. In the absence of an external

perturbation event, the mutualistic interactions between the ammonia-oxidizing bacteria (AOB) and nitrite-oxidizing bacteria (NOB) were prone to chaotic instability. Other non-linear dynamics with low predictability are commonly observed in microbial communities which do not stabilize to a climax condition but produce complex attractor structures [26].

Therefore, the definition of pre-disturbance structural benchmarks from snapshot analysis could be pointless due to the intrinsic temporal variability of the system. This normal variability in terms of community structure under undisturbed conditions can be obtained from time series analysis of omics data sets in order to predict natural community structure dynamics and microbial succession trajectories within multiple stable or transient states [9••,20]. In addition, pre-disturbance functional benchmarks can be defined in terms of (i) macroscopic, non-discriminating functional endpoints, such as, NOR of aerobic respiration and (ii) a functional core microbiome consisting of a set of specific functional guilds and metabolic pathways derived from systems-level, high-resolution functional diversity metrics. This understanding of pre-disturbance states and dynamics will enhance the proper assessment of microbial community robustness.

Epistemic framework for microbial community robustness

Microbial community robustness is defined as the resistance and resilience of the community towards any external or internal system perturbations relative to pre-disturbance dynamics of structure (structural robustness) and function (functional robustness). Robustness assessment is dependent on the type of disturbances (intensity and frequency, duration (press or pulse), etc.) and targeted system endpoints (structural stability (community-level and phylotype-specific responses), maintenance of specific or systems-level function). Resistance is the magnitude of a community metric change following a disturbance ([27], see Box 1). Engineering resilience (from the Latin *resilire*, to bounce back) is the rate at which a system returns to its unperturbed structural baseline or NOR of functioning after a perturbation ([27], see Box 1). Engineering resilience assumes that the system has built-in stabilizers to maintain the *status quo ante* (i.e., single stable state or fixed point attractor). The basin of attraction (attractor) of this single equilibrium can be defined by a Lyapunov stability function governing the return rate to the pre-

disturbance stable state [25]. The identification of at least one positive Lyapunov exponent in time series involves instability and chaotic dynamics [25]. Although the calculation of engineering resilience index is straightforward for a specific output and single performance metric from pre- and post-disturbance analysis, a single equilibrium reference state (e.g., climax community) seldom occurs in complex microbial ecosystems due to non-equilibrium or non-linear dynamics. Succession (transient state dynamics [28]) and ecological resilience, i.e., the intensity and frequency of disturbance a community can absorb before moving to an alternative stable state [29], are more ecologically-relevant concepts. Regime shift, i.e., a transition observed at a critical threshold (tipping point) between alternative stable states characterized by different structures, functions or processes, has been identified in microbial ecosystems [1,30]. The different attractors can be visualized through the three-dimensional stability landscape [31] or two-dimensional ball-in-cup diagrams that can be approximated using frequency distribution of states [1,32]. Alternative stable states were identified in the human gut microbiome using multimodal abundance distributions of specific phylotypes obtained from phylogenetic HITChip microarray analysis [33].

Many studies correlate microbial robustness to community composition (richness, i.e., number of phylotypes, and evenness, i.e., relative abundance of phylotypes) [16] and community structure (structural organization emerging from network of inter-phylotype interactions) [34]. Since the late 19th century, plant and animal ecology has been providing significant theoretical advances on the diversity-complexity debate and the biodiversity-ecosystem function (BEF) coupling in fluctuating environments. These fundamental ecological principles are now testable in microbial ecology by investigating natural or artificial microbial ecosystems in the presence of pulse or press disturbances. Natural and in vivo microbial communities are commonly analyzed through a *top-down* approach to examine the interactions between the system components and the overall behavior of the system in response to various perturbations at the community level [9••,35]. Naturally occurring microbial communities can also be manipulated using dilution-to-extinction experiments to assess their robustness as a function of phylotype richness gradient at constant community evenness [e.g., 36]. Alternatively, combinatorial biodiversity

experiments can provide new insights into the relative contribution of individual community members using system deconstruction into pure cultures and artificially-assembled mixed cultures of different composition and structure. Artificial microbial communities can be assembled with specific interacting microbial strains [37] or with different diversity indices on the basis of traditional phylogenetic marker genes (e.g., 16S rRNA) or an emergent functional trait-based approach [18,38•]. This *bottom-up* approach includes different experimental designs, such as additive *vs.* substitutive (replacement series) [39•] and factorial *vs.* D-optimal [40]. Synthetic ecology is suitable to reduce practical constraints on knowledge acquisition in the mechanisms of microbial community robustness which can be further extended to more complex ecosystems. Although perturbation studies of synthetic microbial communities are commonly performed under batch conditions to investigate the diversity-resistance coupling [16], community culture techniques under continuous and controlled conditions are best suited for studying physiological responses, causal relationships and ecological resilience towards different environmental stressors challenging microbial community structure, spatial architecture and function [41]. The engineering of multiplexed continuous culture systems can provide several advantages over batch systems, such as environmentally-relevant conditions (e.g., oligotrophic environments), highly reproducible platforms for dynamic perturbation studies and relevance to systems biology approaches that require data obtained under rigorously defined, regulated and reproducible conditions. Multiplexing of chemostats, auxostats (e.g., turbidostat, pH-auxostat and morbidostat [42]) and continuous flow biofilm systems is relevant to compare disturbance responses of planktonic and sessile cultures using sustained selective pressure (press disturbance), pulse perturbation events, or both. Bioprocess and genetic engineering strategies and model-based community designs have recently been developed to circumvent inherent issues related to (i) the co-existence of selected community members and (ii) the stability and long-term maintenance of synthetic consortia [e.g., 43].

Mechanisms of microbial community robustness

Dynamic control (feedback loops), modularity, heterogeneity, redundancy, disturbance-specific response diversity and system response variety to compensate different perturbations (Ashby's law of requisite

183 variety) are important attributes of community robustness [1,5]. Modularity, diversity and redundancy
184 trade-offs are pervasive in natural microbial communities with different Pareto optimal solutions as a
185 function of environmental gradients and physiological status. Nitrogen removal processes are catalyzed
186 by diverse sets of dynamic and interacting modules (e.g., denitrifiers, NOB and AOB, anaerobic
187 ammonium-oxidizing bacteria (AnAOB)) which fluctuate in relative abundance in the face of
188 disturbances. Despite low functional redundancy of phylogenetically-restricted nitrifying populations,
189 Gruber-Dorninger et al. [44] observed from pyrosequencing of the functional marker gene *nxrB* an
190 unexpected high diversity of *Nitrospira* with different ecophysiological features which can stabilize
191 nitrite oxidation in activated sludge (beneficial diversity-stability coupling). Under different dissolved
192 oxygen conditions, a shift in NOB community is observed between *Nitrospira*-like NOB and
193 *Nitrobacter*-like NOB with no net changes in nitrification activity [45]. Lücker et al. [46] also identified
194 *Nitrotoga* as novel functionally important nitrite oxidizers in wastewater treatment plants (WWTP), a
195 genus which complements the diversity of WWTP-related NOB communities (i.e., *Nitrolancetus*,
196 *Nitrospira* and *Nitrobacter*). Within the BEF framework, the functionality and robustness of microbial
197 ecosystems decrease concomitantly with a decline of phylotype richness [47]. The net positive
198 biodiversity effect on ecosystem functioning (e.g., biomass productivity) can be measured by additive
199 partitioning of the complementarity effect (positive species interactions (facilitation) and complementary
200 resource use (niche differentiation)) and the selection effect (higher probability to contain particularly
201 productive populations with key functional traits in phylotype-rich communities) [48]. The selection can
202 be further partitioned in trait-dependent complementarity and dominance effects [38•,39•]. The
203 complementarity effect is enhanced within a microbial community composed of specialists displaying
204 greater niche differentiation than generalists [49]. A positive complementarity effect of phylotype
205 richness has been observed during the microbial degradation of crude oil composed of structurally
206 diverse chemicals which promote high niche dimensionality and complementary resource uses [50]. A
207 negative complementarity effect (relative to root colonization and plant protection against fungal
208 pathogens) has also been detected in artificial rhizospheric communities of dissimilar *Pseudomonas*
209 *fluorescens* strains due to resource competition and antagonistic interactions [34]. Under environmental

fluctuations, biodiversity stabilizes aggregate system attributes (i.e., insurance hypothesis [48]) because of (i) compensatory processes from asynchronous phylotype responses to disturbance and temporal niche complementarity (buffering and storage effect) and (ii) the performance-enhancing effect from asynchronicity, selection and functional over-compensation of best-performing, disturbance-tolerant phylotypes. The insurance hypothesis is directly linked to ecological strategies (*r*-/*K*-strategists [51], niche width [49]), disturbance responses diversity of community members coupled to degeneracy and multi- and unifunctional redundancy (i.e., back-up reservoir of multiple phylotypes per functional guild due to redundancy of gene function and non-orthologous gene displacement). The rare biosphere (low-abundance phylotypes active or in dormancy) can also play a role in microbial community robustness by maintaining significant levels of biodiversity in temporarily disturbed environments. For instance, time series metagenomic analysis of a temperate lake microbial community subject to a whole-ecosystem disturbance experiment (forced mixing) show that specific conditionally rare taxa (CRT) significantly increase in relatively high abundance after the mechanical pulse perturbation and temporary modifications of environmental conditions [52].

The performance-enhancing effect of biodiversity has been identified as a main stabilization driver in initially even microbial communities containing five glucose-assimilating phylotypes challenged by high temperature fluctuation treatments [53]. Synthetic ecological experiments also proved that built-in flexibility of a microbial community characterized by a high richness is minimized by uneven phylotype abundances. Wittebolle et al. [16] demonstrated that high initial evenness of functionally-redundant denitrifying communities guarantees rapid adjustment and stable microbial community functioning in the face of selective saline stress. High phylotype evenness [40] and richness [36] are also key features enhancing the microbial community robustness against invasion because of efficient niche partitioning. By manipulating different *Pseudomonas fluorescens* genotypes (functional dissimilarity and niche differentiation) and carbon source formulations (niche dimensionality), Eisenhauer et al. [10•] reported that functionally-dissimilar microbial communities are more resistant to invasion at high niche dimensionality due to the complementarity effect. On the contrary, at low niche dimensionality, the

selection effect of highly productive phylotypes decreases the invasibility of the microbial community [10•]. Last but not least, Saleem et al. [39•] integrate different trophic levels and food webs in synthetic bacterial communities disturbed by predation (three different bacterivorous protists). In predation-free synthetic communities, a positive selection effect was observed combined with a negative complementarity effect likely due to antimicrobial metabolite production by the dominant phylotype [39•]. Bacterial cell yield in the presence of multiple predation increased concomitantly with bacterial richness due to the increased complementarity effect and predation-enhanced bacterial community evenness [39•]. Because of the direct link between system stability and microbial biodiversity (phylotype richness and evenness, functional diversity and redundancy), factors governing microbial community assembly and ecological succession are of primary importance for robustness prediction [8,24,54,55••]. Microbial community assembly processes are governed by deterministic (niche-based), stochastic (drift) and deterministic and/or stochastic (diversification, dispersal) processes, with a trade-off between determinism (environmental selection) and the priority effect (historical contingency and species arrival order) [56]. Using time series GeoChip functional microarray analysis, time-dependent deterministic and stochastic-neutral processes were found to govern microbial community responses after emulsified vegetable oil (EVO) biostimulation of uranium-contaminated groundwater [55••]. The two processes are not mutually exclusive but stochasticity was predominant after the EVO amendment as supported by a null model analysis [55••]. In soil bacterial communities after a wildfire and in deglaciated soil communities after a reciprocal transplant experiment [54,57], the community responses to disturbances also involve time-dependent stochastic and niche-based processes. The future states of the system challenged by specific disturbance(s) can be predictable if the microbial community responses to the disturbance(s) are deterministic and if biological variables and disturbance-associated environmental changes are known. On the other hand, neutral responses can be predominant at the onset of a disturbance event and lead to unpredictable trajectories in the post-disturbance landscape (Figure 1).

Ecological resilience: Bouncing back or bouncing forward?

Regime shifts of microbial communities can be detrimental changes or opportunities for beneficial changes reflecting the intrinsic dynamic nature of microbial ecosystems. A system may shift to an alternative state of higher functionality (new community-level fitness) after a press disturbance through beneficial responses to changes and overcompensation under the new environmental conditions (i.e., antifragility [58]). Disturbance-driven structural shifts in microbial communities are frequently observed with different responses from complete recovery of the pre-disturbance state to bouncing forward to another state through positive feedback loops [35,59,60]. Freshwater microbial communities from a temperate lake were shown to return to the pre-manipulation structural state after a short-term artificial mixing of epi- and hypolimnion [35]. Time series shotgun metagenomic analysis revealed community-level compositional and functional shifts of soil communities in temperate grassland after an artificial 2 °C infrared heating for 10 years, resulting in increases of cellulose degradation, CO_2 production, denitrification and genome G+C content [60]. The recovery of community structure after a disturbance event can be a requirement for maintaining particularly specialized functions that are driven by the selection effect of specific bacterial strains or functional guilds of low redundancy (e.g., anaerobic ammonium oxidation (anammox) and *Dehalococcoides mccartyi*-catalyzed reductive dechlorination of trichloroethene to ethene [6•]). Long-term heavy metal stress of ryegrass grasslands show low resilience of key specialized microbial functions that cannot be compensated by functional redundancy to the same extent as general functions, such as soil respiration [61]. The anammox process is sensitive to various stresses (nitrite accumulation, high oxygen levels, competition with denitrifiers, etc.) and relies on the activity of AnAOB for which strategies to manage the robustness have recently been reviewed [4•]. By monitoring a one-stage nitritation/anammox process over a 1.5-year period, Bürgmann et al. [30] observed a regime community shift between AnAOB and denitrifier populations in sequencing batch reactors treating urine under fluctuating operator-controlled parameters. The system shifted to a new stable state marked by reduced annamox activity that could not be compensated to maintain stable nitrogen removal performance. In this case, intervention of the operator will be required for the community to bounce back and for the stable state to be restored ensuring optimal function performance. The reductive dechlorination of TCE to the non-toxic ethene is catalyzed by highly specialized

288 *Dehalococcoides mccartyi* strains that are characterized by very restrictive ecological niches and an
289 absolute requirement for community-level networks of metabolic interdependencies (Figure 2, [6•]).
290 Following treatment with sodium persulfate in a combined in situ chemical oxidation and bioremediation,
291 Sutton et al. [62] showed very low resistance and resilience of *D. mccartyi* populations compared to other
292 phylotypes. In order to maximize the robustness of in situ bioremediation of TCE-contaminated
293 groundwater, the complementarity and selection effects are both essential and provide guidance to
294 strategies for manipulating microbial communities in the field and ensuring functional diversity,
295 functional redundancy and selection for particular functional traits (Figure 2).

296 Microbial ecosystems characterized by low ecological resilience in community structure and composition
297 can also exhibit high ecological resilience in functioning due to adaptive capacity and built-in flexibility
298 of community members. By decreasing the solid retention times (SRTs) in a full-scale activated sludge,
299 Vuono et al. [63] observed a community composition shift concomitant with loss of nitrification,
300 denitrification and biological phosphorus removal function (i.e., low structural and functional resistance).
301 Complete functional recovery was observed upon the return to the initial SRT whereas a different post-
302 disturbance microbial structure was reported with a significant biodiversity loss of the rare biosphere
303 [63]. This study confirms the high functional redundancy in WWTP for carbon, nitrogen and phosphorus
304 removal and the presence of multiple structural stable states with equivalent functionality [63]. Regime
305 shift induced by ammonia pulse has also been observed in anaerobic digesters from aceticlastic
306 methanogenesis to hydrogenotrophic methanogenesis and syntrophic acetate oxidation which is catalyzed
307 by very diverse phylotypes [64]. This compositional shift enabled the maintenance of high rates of
308 acetate removal and thus the stabilization of the bioreactor performance. Following high ammonia stress,
309 microbial community evenness strongly decreased to finally return to pre-disturbance community
310 evenness when the bioreactor recovered steady-state optimal performance [64]. During and after the
311 *Deepwater Horizon* (BP) oil spill in the Gulf of Mexico, the microbial community in the oil plume
312 shifted to a community enriched in hydrocarbon-degrading microorganisms (alternative transient state)
313 [2]. Regime shift after the BP oil spill was also observed in sediment AOA communities that stabilized to

a new alternative state with a higher AOA community diversity which persists for at least one year due to long-term oil impact or oil persistence in sediments [65].

Systems-level prediction of microbial community robustness: From cross-sectional analysis to mechanistic predictive modeling of time series data sets

As discussed in the previous sections, major breakthroughs have been made in understanding the mechanisms of microbial community robustness and the responses of microbial ecosystems challenged by a perturbation. The ultimate goal aims at exploiting integrated omics data sets to model microbial ecosystems for the assessment of community robustness using predictive ecological models which must include causal relationships. Up to now, the development of systems-level predictive models of complex microbial communities is still in its infancy [66] and requires mathematical modeling of ecological time series. Cross-sectional analysis of high-resolution molecular data provides a static snapshot of multiple microbial ecosystems at a single time point and enables transversal comparisons using standard multivariate statistical tools which assume data independence (e.g., cluster analysis and ordination in reduced space [67•]). This approach was used to visualize community-level variation across similar microbial habitats and to identify alternative stable states in the human intestine [33]. In addition, network inference from cross-sectional omics data enables the reconstruction of microbial relationships and interdependencies using phylotype interaction coefficients from correlations in phylotype abundance or absence/presence data [68••,69]. Co-occurrence network reconstruction tools, such as the CoNet pipeline [70], accommodate transversal independent samples but cannot deal with the non-independence of longitudinal (auto-correlated) data sets. Topological network features associated with keystone phylotypes and gatekeepers (i.e., critical nodes controlling network flow) have been identified from co-occurrence networks [71,72], which can inform on the fragility of microbial ecosystems due to potential network fragmentation and disproportionately high negative effects on system behavior after disturbances. Several network inference tools are tailored to analyze time series data, such as extended Local Similarity Analysis (eLSA) [73] and regression-based approaches [32]. However, inferred networks of microbial interactions rely on static topology models and causal relationships between

ecological descriptors and system predictions cannot be derived from correlation matrix. In spite of violation of independence in ecological time series, phenomenological analysis of microbial ecosystems in terms of structure and function before and after a perturbation event is commonly achieved through multivariate data matrices and descriptive multidimensional statistical methods (e.g., principal response curves (PRC) analysis designed for time-dependent multivariate community-level responses [74••]). Using paired-difference testing (Wilcoxon signed-rank test), the recovery of human gut microbial communities recurrently infected by *Clostridium difficile* was observed after fecal transplantation [74••]. The same approach was used to demonstrate the functional recovery of activated sludge microbial communities disturbed by intentional decrease of SRTs [63]. Time series modeling of subarctic invertebrate community dynamics has been carried out using canonical analysis (redundancy analysis (RDA) and Principal Coordinates of Neighbor Matrices (PCNM)) from Hellinger-transformed abundance data matrices. Thanks to this RDA-PCNM modeling approach, relative ecosystem resilience in the face of global change was inferred that was supported by functional diversity, redundancy and response diversity within and across temporal scales [75]. However, emerging approaches based on biological interaction network inference and dynamic system modeling are poised to fully exploit large data sets from time-series analyses that are required to forecast the future community behavior under relatively constant variables and parameters (correlative modeling) and to predict the future system states by analyzing mechanistic processes and causal relationships among ecological variables (predictive modeling) (Figure 3) [68••,76]. Dynamic linear modelling (DLM) is a Bayesian technique for the analysis of auto-correlated time series data sets, such as Box-Jenkins models used to forecast future system behaviors (auto-regressive (integrated) moving average, ARMA and ARIMA). Autocorrelation of first-order autoregressive process (AR (1)) [63], temporal eigenfunction analysis of multivariate data sets [77] or multivariate recurrence plots and recurrence quantification analysis [78] are suitable approaches to characterize information-rich ecological time series (e.g., autocorrelation, periodic variability, discontinuities, succession, future trends). The generalized additive model (GAM) is a multivariate regression method that can be used for time series modeling and temporal dynamic analysis of microbial communities [76]. Computational longitudinal analysis tools can also be included within a mechanistic

367 modeling framework to predict the system fate under fluctuating conditions through inference of inter-
368 phylotype interaction parameters and ordinary differential equations (ODEs) (e.g., dynamic population-
369 based modeling using generalized Lotka-Volterra (gLV) equations, Figure 3) [9••,66,68••,69]. Stein et al.
370 [9••] used a mechanistically-based dynamic modeling approach to analyze mouse gut microbiome time
371 series data (pyrotags analysis and absolute phylotype abundances) under time-dependent external
372 perturbations (serial antibiotic clindamycin pulse treatment and *Clostridium difficile* invasion). Using
373 multiple linear (ridge) regression to infer the interaction matrix, the disturbance susceptibility matrix and
374 the growth rate vector of the ten most abundant genus-level phylotypes, prediction of community
375 dynamics under perturbations was obtained from gLV modeling at short and long-time scales. Low
376 ecological resilience (disturbance-induced structural regime shifts) was observed after clindamycin
377 treatment, *C. difficile* invasion, or both. This was confirmed by the identification of alternative stable
378 states on the basis of the most negative eigenvalue of the corresponding stability Jacobian matrix
379 (analogous to Lyapunov stability) [9••]. In a similar approach, Fisher et al. [69] developed the LIMITS
380 framework (Learning Interactions from MIcrobial Time Series) to predict the dynamics of human gut
381 microbiomes through sparse linear regression for network inference and dynamic ecological modeling
382 using discrete-time Lotka-Volterra (dLV) equations. Distinct keystone members of the gut microbiomes
383 were identified within the LIMITS framework which can have important implications for microbial
384 community robustness towards dissimilar perturbations [69]. Finally, cause-and-effect relationships
385 should be modeled in order to properly predict the future state of the system and the fate of ecological
386 series under disturbances. Sugihara et al. [79] have proposed an alternative method to the Granger
387 causality framework for detecting causality in ecological systems and inferring causal networks from
388 time series data. Convergent cross mapping (CCM) is able to distinguish causality from correlation using
389 state space reconstruction which could be of practical value for predicting microbial community
390 responses in the face of different disturbances [79].

391 **Conclusions and outlook**

Microbial community robustness hypotheses tested in replicate synthetic microbial systems under controlled conditions (e.g., batch reactors with defined initial conditions or continuous flow reactors with or without pulse, press or sustained disturbances) are poised to generate microbial ecological theories of practical significance for bioengineering practitioners who seek to predict process stability and optimize microbial resource management for environmental biotechnological purposes. Synthetic ecology has been providing new insights into key microbial features and determinants of community robustness and functionality using artificial microbial communities tailored for environmentally friendly processes (e.g., lipid productivity in microalgal communities or wastewater treatment by bacterial consortia) [11,38]. The emergence of holistic approaches to decipher complex ecological structures (unified in this review as molecular systems synecology) combined with the development of correlative or predictive mathematical modeling pipelines are progressively enabling the formulation of (i) ecological benchmarks for natural microbial community dynamics, (ii) functional core microbiomes critical for function maintenance and (iii) community-level rules of microbial resistance and resilience in fluctuating environments. Particularly, intensive meta-omics-based studies of the host-associated microbiomes have been developing statistical and mechanistic analysis tools of microbial community dynamics in the presence of a set of biotic and abiotic disturbances that can be extrapolated for earth microbiomes. Dynamic modeling and prediction of microbial ecosystems using time series data sets will be enhanced by the integration of shotgun metagenomics, metatranscriptomics, metaproteomics and meta-metabolomics within a comprehensive system-level and function-targeted approach. This integration will be facilitated in the future by the development of novel bioinformatic pipelines and technology platforms, such as the patented biomolecular extraction method, called BioMolXtract, designed for the isolation of DNA, RNA, proteins and metabolites from single, unique samples [80]. For example, recently Muller et al. [81••] used a comprehensive integrated omics approach to investigate oleaginous mixed microbial communities (OMMCs) containing lipid-rich filamentous bacteria (e.g., *Candidatus* Microthrix parvicella) that are responsible for bulking and foaming in sewage treatment systems, but also represent opportunities for resource recovery from WWTP through for example biodiesel synthesis from the lipid stores. *Candidatus* Microthrix spp. was identified as the most dominant population in winter and its ecological success was

related to fine-tuned gene expression allowing optimal resource usage in the fluctuating WWTP ~~wastewater treatment plant~~ environment [81••]. In another integrated approach, Lu et al. [82] combined *iTags* analysis and meta-metabolomics to identify structural community shifts and metabolic profile changes in the mouse gut microbiome disturbed by arsenic exposure.

In addition, predictive tools need to be developed by integrating structural and functional community dynamics with new multi-scale models of microbial processes [e.g., 83] and the combination of stoichiometric, thermodynamic and transport calculations. For example, constraint-based stoichiometric modeling is another strategy for the analysis of microbial communities using community-level metabolic networks. Flux balance analysis (FBA) has recently been applied to model inter-phylotype metabolite-mediated interactions in microbial consortia (community-level FBA (cFBA)), such as Computation of Microbial Ecosystems in Time and Space (COMETS) and multi-objective dynamic analysis of microbial communities (d-OptCom) [37•,84•]. Combined with gLV modeling and perturbation studies, these cFBA tools are relevant to achieve dynamic metabolic modeling of microbial communities under different environmental scenarios with the ultimate goal of optimizing the community-level fitness, an engineering objective for the whole community [84•].

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