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Stress and stability: applying the Anna Karenina principle to animal microbiomes

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All animals studied to date are associated with symbiotic communities of microorganisms. These animal microbiotas often play important roles in normal physiological function and susceptibility to disease; predicting their responses to perturbation represents an essential challenge for microbiology. Most studies of microbiome dynamics test for patterns in which perturbation shifts animal microbiomes from a healthy to a dysbiotic stable state. Here, we consider a complementary alternative: that the microbiological changes induced by many perturbations are stochastic, and therefore lead to transitions from stable to unstable community states. The result is an 'Anna Karenina principle' for animal microbiomes, in which dysbiotic individuals vary more in microbial community composition than healthy individuals—paralleling Leo Tolstoy's dictum that "all happy families look alike; each unhappy family is unhappy in its own way". We argue that Anna Karenina effects are a common and important response of animal microbiomes to stressors that reduce the ability of the host or its microbiome to regulate community composition. Patterns consistent with Anna Karenina effects have been found in systems ranging from the surface of threatened corals exposed to above-average temperatures, to the lungs of patients suffering from HIV/AIDs. However, despite their apparent ubiquity, these patterns are easily missed or discarded by some common workflows, and therefore probably underreported. Now that a substantial body of research has established the existence of these patterns in diverse systems, rigorous testing, intensive time-series datasets and improved stochastic modelling will help to explore their importance for topics ranging from personalized medicine to theories of the evolution of host–microorganism symbioses.

Animals and plants evolved in the context of immense microbial diversity[1]. Multicellular life forms only a small and highly derived portion of universal trees of life[2,3], with the rest occupied by bacteria, archaea and microbial eukaryotes. In keeping with their phylogenetic diversity[4–7], these ancient microbial lineages have developed great genomic and metabolic diversity[8]. Exceeding even the immense diversity of cellular microorganisms is the vast array of phages, viruses and other transmissible genetic elements (conjugative transposons, integrative and conjugative elements, addiction plasmids, and so on) that infect them[9]. This wilderness of microorganisms, viruses and parasitic genetic elements was already long established before the emergence of animals and plants. Thus, the ecological context in which animals and plants emerged both necessitated defence against exploitation by opportunistic microorganisms, and provided many opportunities to benefit from microbial metabolic innovations through mutualistic symbioses[10].

Reflecting this heritage, symbiosis with microorganisms is ubiquitous in metazoans[1], influencing host health, development, disease susceptibility, and even behaviour[11,12]. As symbiotic microbial communities and their associated gene pools (microbiomes) influence many aspects of fitness, animals and plants regulate them. Animal mechanisms for microbiome regulation are diverse—ranging from the specialized light organs of the Hawaiian bobtail squid[13] to production of microbiome-targeted oligosaccharides in human breastmilk[14]. They have in common the aim of both preventing invasions by pathogens and managing symbiotic microorganisms to maximize their benefit to the host. These activities partially overlap, as mutualisms with beneficial microorganisms often provide animal hosts a measure of defence against pathogens[15] through mechanisms such as competition for resources, antibiotic production, metabolic inhibition and spatial occlusion[16], as well as through feedbacks with host immunity. Recent evidence suggests that even phages may have been recruited by hosts as defensive mutualists on animal mucosal surfaces—an especially important and flexible defence for animals lacking a host-derived adaptive immune system[17].

Predicting when and how normal regulation of microbiomes breaks down is at the heart of many important problems in microbial ecology. Under normal circumstances, the defensive activities of animal hosts, and their typical microbiota and phages, are thought to restrict microbiome membership, avoiding the many detrimental possibilities in favour of a smaller range of beneficial microbiome configurations. A corollary of this observation is that stressors need not, at least in principle, shift a microbiome to a specific dysbiotic configuration in order to reduce host fitness—they need only release normal regulation of microbiome membership.

Currently, most microbial ecology workflows attempt to identify a specific microbiome configuration associated with stress or disease. Overall community changes in host microbiomes are typically examined using high-throughput marker gene or shotgun metagenomic data. Commonly measured microbial community properties include richness (diversity of species within a sample), evenness (distribution of counts between one or many species), and β-diversity (turnover of species between samples).

Analysis of β-diversity has been particularly useful in microbial ecology. While β-diversity was originally defined as species turnover along a habitat gradient[18], microbial ecologists have applied measures of β-diversity to examine differences in communities separated by health status (for example, ref. 19), the passage of time (for example, ref. 20), or divergence in the evolutionary history of their host (for example, ref. 21). These differences are visualized by

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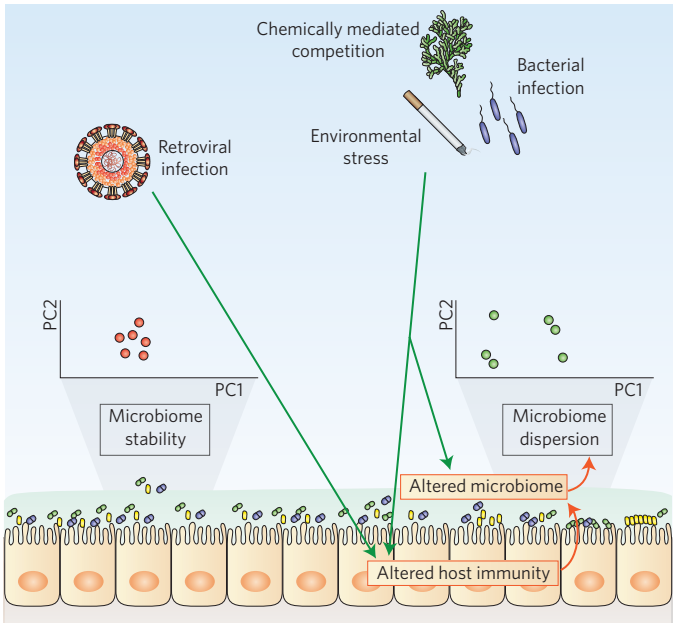

Figure 1 | Anna Karenina principle of perturbations inducing microbiome destabilization. Typically, healthy hosts possess relatively stable microbiomes that form tight clusters in ordination space (left plot). In contrast to movement of these clusters to a new place in ordination space (for example, due to a transition to an alternative stable state; see Fig. 2), a variety of external stressors have been shown to disrupt this stability, resulting in more dispersed microbiomes (right plot). More dispersed microbiomes have been associated with a variety of negative outcomes for the host, including increased invasibility[57], altered clinical parameters (for example, endotoxaemia in alcoholics[29]) and increased sensitivity to seasonal temperature changes[34]. In principle, these disruptions may act indirectly by affecting host immunity (as in HIV and SIVcpz), indirectly by altering the microbiome (for example, by displacing protective mutualists like antibiotic producers), or through a combination of both mechanisms. In practice, perturbations that directly destabilize the microbiome are challenging to distinguish from those that indirectly destabilize the microbiome by affecting host immunity (Box 1), and careful experimentation is needed to distinguish the mechanisms that produce microbiome dispersion, and to distinguish the health consequences of the deterministic versus stochastic portion of microbiome changes (Box 3).

computing a matrix of β-diversity distances between samples, then using ordination methods such as non-metric multidimensional scaling (NMDS) or principal coordinates analysis (PCoA) to represent this multidimensional matrix in two or three dimensions. In many cases, the microbial communities associated with diseased hosts will form distinct clusters in such two- or three-dimensional ordination spaces, indicating a correlation between microbiome change and degraded host health (for example, chronic periodontitis[22], corals challenged by turf algal competition[23], and so on). Additional experiments, such as microbiome transplants, test the direction of causation underlying these correlations. In many cases, important host phenotypes, ranging from increased energy harvest (and weight gain) to the capability to digest otherwise poisonous foods[24], are conferred by distinct microbial communities that are reflected by clusters in ordination space. However, despite their well-understood importance, these patterns of microbial community change may not reflect the full range of dynamics needed to understand the contribution of microorganisms to host health and disease.

In this Perspective, we examine findings in diverse systems—from threatened coral reefs to simian immunodeficiency virus (SIV)-infected chimpanzees—where host stress or disease produces stochastic rather than deterministic changes in the microbiome. These stochastic changes often induce dispersion, rather than location, effects on microbial community composition. That is, rather than shift the microbiome to a new discrete configuration, producing clusters, these stressors allow the microbiomes of stressed individuals to take on a wider range of possible configurations than healthy controls, producing a constrained 'core' of control microbiomes surrounded by a large 'halo' or 'smear' of stressed or diseased microbiomes (Fig. 1).

Observations of microbiome variability associated with disease have led several workers to propose an Anna Karenina principle (AKP) for animal microbiomes. The principle derives from the opening line of Tolstoy's *Anna Karenina*: "all happy families are all alike; each unhappy family is unhappy in its own way". It was popularized by Diamond[25] in reference to the many reasons why animals might prove undomesticable. Translated into a hypothesis for host-associated microbiomes, the AKP predicts that certain stressors have stochastic rather than deterministic effects on community composition. In these cases, 'all healthy microbiomes are similar; each dysbiotic microbiome is dysbiotic in its own way'. Giongo and colleagues proposed that microbial changes during autoimmune misregulation in type 1 diabetes followed the AKP[26]. Independently, in applying a new method (Dirichlet multinomial mixtures) for clustering microbial samples into enterotypes, Holmes and colleagues noted that obese gut microbiomes may be associated with a broader range of configurations than lean[27]. On this basis, they suggested that there may be an AKP for human microbiomes, in that samples from obese patients are explained by a mixture of more enterotypes than samples from healthy patients. Later, Dey *et al.* made similar observations in investigating the microbiome of Crohn's disease[28]. Despite this remarkable convergence in findings, interpretation, and even metaphor across diverse specialties, AKP effects are little discussed in the microbiological literature. This situation makes it challenging for workers to compare notes. Indeed, even the above publications describing AKP effects in specific systems[26–28] do not reference the other works, and these effects are not well known outside of specialized publications on particular diseases.

We argue that AKP effects in animal microbiomes are common, important, and often linked to declining host health. We synthesize observations of microbiome dispersion in diverse systems into testable predictions, and show how the predicted patterns, although often quite clear, are easily missed—or dismissed as statistical artifacts—by the most commonly applied workflows. We describe different types of community dynamics that could produce apparent AKP effects (Box 1) and present methods that can help distinguish them. We also discuss scenarios that appear to decrease inter-individual variation relative to healthy controls (see 'Anti-AKP effects', Box 1). We then address new empirical and theoretical avenues for exploring the microbiome opened by these emerging perspectives, and discuss their broader importance for personalized medicine.

External stressors can induce microbial AKP effects

Recent research shows that under stress, many animal-associated microbiomes exhibit increased dispersion in microbial community composition. These findings have been reported in the early development of the microbiome; and in adult animals subject to environmental stress, immune dysregulation or pathogen infection. Although many questions remain, the commonalities among these findings suggest an area ripe for additional tools, theory and experimentation.

Microbiological studies of the effects of alcoholism and cigarette smoking have both reported increased dispersion of microbial communities. Alcoholic patients showed a much greater spread of microbial community composition than controls[29]. This was interpreted as evidence of dysbiosis. When analysed separately, patients with microbial samples that were dispersed away from the typical

Box 1 | Distinguishing biological patterns that can produce AKP effects.

Here we consider some possible hypotheses for how AKP effects might be produced, and the characteristics that distinguish these scenarios.

Sampling biases. In some cases, particular disorders may be associated with biases in the amount of technical variance produced during sampling, producing an artefactual AKP effect. Similarly, significant differences in sequencing depth across samples could produce artefactual AKP effects, and should be controlled by random resampling to even depth (rarefaction)[73].

Antibiotics. In humans or model organisms, antibiotic treatment can produce individualized responses[74], complicating the interpretation of increased β-diversity due to disease. Direct microbiome disruption by disease, and microbiome disruption by antibiotics applied to treat disease, are each a distinct biological mechanism for microbiome change and should be distinguished when possible.

Categories combining distinct biological conditions. If multiple true treatments, each with a simple deterministic effect on the microbiome, are lumped together as a single category, microbiome variance may increase. Past work has assessed this by testing any sub-categories of the treatment for AKP effects separately.

Hidden gradients within categorical factors. Many microorganisms may respond to a host parameter that is itself more variable in subjects of a particular class (for example, a broader range of body mass index scores in obese patients). In this case, increased variation within a given class should be arrayed in a particular direction in ordination space (Fig. 2b) rather than in all directions (Fig. 2c).

Sampling during the transition between stable states. Experimental treatments may create simple locational effects after a temporarily unstable period of transition. Such a shift could look like an AKP effect if timepoints are densely sampled and sampling is concentrated during the period of transition between states (resembling Fig. 2b).

Increased stochastic temporal change within individuals. If a condition reduces the ability of the host or the normal microbiome and virome[17] to regulate microbiome composition, then stochastic temporal changes in environmental conditions may have greater influence on the microbiome. This can lead to increases in the rate of random change in individuals' microbiomes over time, and multidirectional dispersion will increase across timepoints within individuals (Fig. 2c).

Increased range of stable states. Reduction in immune function may also lead to alternate stable states dominated by the effects of microbial migration and competition rather than habitat filtering by the host. To the extent that migration or intraspecific competition are stochastic, this may lead to very different outcomes in different individuals. Stochasticity in migration could be due to underlying variation in diet[68], patterns of physical contact[75] or hospitalization[76,77], while stochasticity in intra-specific competition often arises from priority effects. For example, if two or more species (or consortia of allied species) are mutually inhibiting, the first to establish will tend to exclude others (that is, bistability[78] or multistability[79]). If the outcome of this initial establishment is partially stochastic, then different species may stably dominate different individuals (the same argument can be made for stochasticity in competitive outcomes). Stochastic bistability could produce directional changes across timepoints within individuals (Fig. 2b), but would increase inter-individual variation (Fig. 2c).

Anti-AKP effects. Of course, not all stressors increase β-diversity. Counterexamples that we predict will often significantly decrease β-diversity include polymicrobial infections stabilized by consortium interactions (for example, chronic periodontitis[80]), disorders associated with blooms of a specific microorganism (for example, *Staphylococcus aureus* in untreated atopic dermatitis flares[81]), and strong environmental filters on the microbiome (for example, iron insufficiency at weaning in mice[82]).

configuration for control patients varied in many clinical parameters, including higher endotoxin levels[29]. Similarly, both significant separation by mean location, and a large increase in microbiome dispersion were seen in the microbiome of patients with liver cirrhosis[30]. Patients with acute-on-chronic liver failure[31] also showed more microbiome dispersion than healthy controls.

Cigarette smoking has been associated with greater inter-individual variation in upper respiratory microbiomes. Sequencing of oropharynx and nasopharynx samples from 29 asymptomatic adult smokers and 33 healthy controls showed a significant increase in β-diversity (here, unweighted UniFrac scores within pairs of samples from smokers versus pairs of samples from non-smokers)[32]. These differences were significant for both the oropharynx and nasopharynx, and for both sides of the body. However, weighted UniFrac, which accounts for abundance, did not show clear differences. Similarly, a massive study of smoking in 1,204 individuals found a small but highly significant effect of smoking status on β-diversity dispersion in the oral microbiome[33]. β-Diversity dispersion was highest in current smokers, and lowest in those who had never smoked; former smokers had intermediate levels of β-diversity dispersion[33] (consistent with the scenario in Fig. 2c).

Surprisingly, similar effects of environmental stressors on the variability of microbial community composition extend beyond humans to several threatened marine invertebrates. Our own interest in AKP effects in animal microbiomes arose from the study of three-year time-series datasets tracking the cascading effects of overfishing and nutrient pollution on the microbiome of reef-building corals[34]. We had hypothesized that nutrient pollution and overfishing, which promote growth of macroalgal and turf-algal competitors of coral, would cause a shift between alternative stable states in the microorganisms on the coral surface—a sort of microbial analogue of the broader phase shifts from coral to macroalgal dominance seen on reefs. We found no evidence supporting this hypothesis. Instead, we found that contact with macroalgae such as *Dictyota* caused increased β-diversity in the surface microbiome of three genera of corals. The increased β-diversity corresponded to host tissue loss. Reanalysis of a previous study in which macroalgae were placed in contact with corals confirmed that macroalgal competition drove increased β-diversity[35] (reanalysed in ref. 34). Additionally, we found other similar reports in the literature. For example, corals that suffered high mortality after transplant into damselfish gardens[23], which have high turf- and macroalgal cover, showed both differences in mean location and a high degree of dispersion in PCoA plots relative to control corals.

The response of coral microbiomes to temperature told a similar story[34]. Here again, increased β-diversity was associated with coral mortality (most corals died in the hot months, which also showed increased microbiome β-diversity). Increased β-diversity

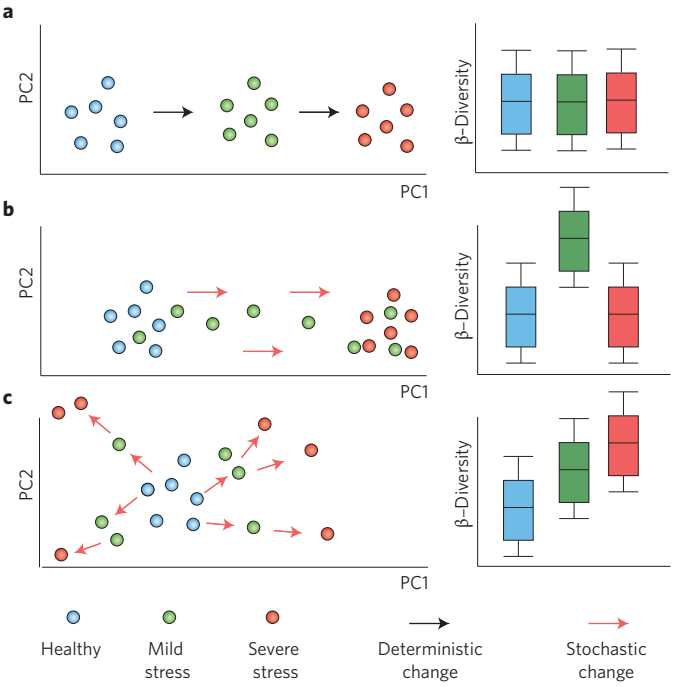

Figure 2 | Stochasticity produces contrasting effects of mild and severe perturbations under different models of microbiome dynamics. **a**, Hypothetical ordination results for a perturbation that alters microbiomes by driving them towards a new deterministic configuration (black arrows). For example, an environmental variable might linearly increase the relative abundance of certain taxa. Such deterministic changes produce new clusters of 'stressed' samples (green and red spheres) with similar dispersion as healthy controls. Under this scenario, the within-category β-diversity of healthy, mildly stressed or severely stressed hosts is identical (as shown by the box plot). **b**, Results for a scenario where the way in which a perturbation alters the microbiome is deterministic (all arrows point right), but the extent of alteration is stochastic based on the severity of the stressor. This produces clusters of healthy (blue spheres) and severely stressed (red spheres) samples. Under mild levels of stress, however, only a subset of samples is shifted to the stressed cluster. This produces elevated β-diversity in samples from moderately stressed hosts, but low β-diversity in both healthy and severely stressed hosts. Superficially, this effect on between-individual β-diversity follows a similar curve to observations of peak microbiome richness under intermediate levels of disturbance[86]. However, unlike species richness, β-diversity dispersion is not readily explained by tradeoffs between competition and colonization ability among microorganisms without additional assumptions. **c**, Results for a stressor that alters the microbiome in unpredictable ways. For example, a stressor that suppresses host immunity may allow invasion by myriad opportunists, with the specific outcome determined in part by chance effects such as exposure. Under these circumstances, increasing levels of the stressor produce greater dispersion around the healthy centroid. Thus, severely stressed hosts have greater microbiome β-diversity than healthy hosts, while mildly stressed hosts have microbiomes with an intermediate level of dispersion.

at above-average temperatures coincided with displacement of the normal surface microbiota by stochastic blooms of several different fast-growing opportunists[34].

These effects may extend to other threatened marine animals. Globally, sponge microbiomes are typically less variable than surrounding sediments or seawater[21]. However, evidence from mesocosms suggests that ocean acidification (but not temperature) increases variability in sponge microbiomes[36]. The similarities of microbiome response to certain environmental stressors in humans and marine invertebrates such as sponges and corals present intriguing parallels. One potential interpretation is that many environmental stressors can disrupt normal mechanisms for regulating the microbiome. These might include both innate and adaptive host immunity (for hosts with an adaptive immune system), and the action of beneficial members of the microbiome.

Compromised host immunity can induce AKP effects

Studies of microbiome development across populations demonstrate high microbiome variability during the early development of adult immunity. Children from three populations showed greater variation in microbial metabolic capabilities than did adults from the same population[37]. This suggests that immunity may play an important role in microbiome stability. If so, we might expect that disruption of normal immune function due to genetic defects, retroviral infection or immunosuppressive drugs might destabilize the microbiome.

In the absence of antiretroviral drugs, HIV/AIDs kills patients by decreasing $CD4^+$ T-cell counts, increasing susceptibility to a wide range of bacterial, viral and fungal opportunistic pathogens (reviewed in ref. 38). Only more recently have the consequences of HIV/AIDs on the broader host microbiome been considered (reviewed in ref. 39). In these studies, enrichment of specific microbial lineages and changes in α-diversity are more commonly tested than changes in β-diversity dispersion[39]. However, at least two studies have reported increased β-diversity dispersion as a consequence of HIV infection. While less severe HIV infection induced small differences in the microbiome[40], advanced HIV increased β-diversity between patients[41]. Other recent studies of the effects of HIV/AIDs on the gut microbiome, including of American[42] and Chinese[43] patient cohorts, did not report statistical tests for β-diversity dispersion. However, circumstantial evidence suggests its presence: PCoA plots in Sun *et al.*[43] show much tighter clustering of non-diarrhoeal control patients than HIV/AIDs patients (though this may be confounded with presence of diarrhoea), while Dinh *et al.*[42] report much higher interquartile ranges for the abundance of microbial phyla in HIV+ cases versus healthy controls. Additionally, these studies both show a subtle symptom of a particular subtype of AKP effect (discussed in section 'Destabilization of animal microbiomes influences statistical tests of microbiome dynamics'): many more significantly depleted taxa in cases versus controls, even in relative abundance data in which microbial proportions are constrained to sum to 100%.

In principle, part of the increased inter-individual microbiome variability seen in HIV+ patients could be due to the effects of antivirals or antibiotics (Box 1). However, similar microbiome disruption arises following retroviral immune suppression in unmedicated non-human primates (for example, SIV in chimpanzees (SIVcpz)). Wild chimpanzees with SIVcpz did not show a single distinct community configuration relative to healthy animals. Instead, they showed increased β-diversity across a range of different microbial community dissimilarity measures[44]. This increased dispersion of community composition was linked to more rapid microbiome change over time in SIV+ chimpanzees, and increased abundance of multiple potential pathogens or opportunists[44]. This SIV-induced microbiome destabilization appears to be absent for much of the course of infection, but becomes pronounced around five months prior to death[45]. Based on these findings, the presence or absence of virally induced gut microbiome dysbiosis is now proposed as a diagnostic of pathogenic versus non-pathogenic SIV infections in primates[46].

It remains an open question whether other related retroviruses, such as feline immunodeficiency viruses (FIVs), destabilize the microbiome. Although not formally characterized for differences in community dispersion, a recent study of the oral and conjunctival microbiome of domestic cats with FIVs showed significantly

Box 2 | Relationship of the AKP to personalized medicine.

The human microbiome is increasingly understood to influence the efficacy of many drugs. As one example, strains of the human gut actinobacterium *Eggerthella lenta* can convert the heart drug digoxin into the ineffective form dihydrodigoxin[64]. Remarkably, this problematic interaction could be greatly reduced by a high-amino-acid diet[64]. Even extremely common and seemingly well-understood drugs may be affected by variation in bacterial metabolism across individuals. For example, individuals with greater bacterially generated *p*-Cresol in their urine show altered metabolism of acetaminophen[83]. Observations such as these have led to much interest in incorporating microbiome information into personalized medicine in general[84], and into personalized prediction of drug pharmacokinetics and pharmacodynamics in particular[85].

The AKP for host microbiomes may help to guide these efforts. If, as currently appears probable, many disorders destabilize microbiomes rather than shifting them to a particular predictable configuration, then patients with these disorders will have variable secondary changes in their microbiomes. This implies that the same underlying disorder could have widely varying impacts on drug metabolism, depending on the outcome of stochastic ecological processes in each patient's microbiome. Understanding which disorders tend to destabilize microbiomes, versus driving them to new stable states, may help to shape screening regimes for microbiome-based personalized medicine. For example, patients with disorders that increase microbiome change over time might require more frequent screenings for microorganisms that interact with medication. Conversely, patients with disorders that have predictable and consistent effects on the microbiome across individuals may gain little benefit from personalized microbiome screenings.

reduced evenness and increased dispersion in PCoA plots of the conjunctival microbiome[47].

Immunosuppressive drugs also often induce AKP effects. In lung transplant recipients, post-operative upper and lower respiratory tract microbiomes showed much larger differences than did the same sites in healthy controls due to stochastic outgrowth of varied lung microorganisms[48]. Post-operative liver transplantation patients, who received both antibiotics and immune suppression by tacrolimus, showed greater variation in follow-up samples relative to asymptomatic hepatitis B positive controls[49], although these differences were not tested for significance.

In addition to immune suppression by retroviral infection or immunosuppressive drugs, physical trauma with immune repercussions has also been reported to show these patterns. For example, severely burned human patients showed both strong location effects driven by an increase in Enterobacteriaceae, and greater microbiome dispersion in NMDS plots[50]. When burns were experimentally inflicted on mice, similar patterns were observed[50]. Ultimately, these ideas are best tested by experiments that manipulate immunity directly, some of which have already been conducted. For example, *Rag1*$^{-/-}$ (recombination-activating gene 1) mice, which lack adaptive immunity, showed significantly increased β-diversity relative to healthy controls[51]. Emerging methods such as IgA-seq[52] will help fuel future work dissecting the relationship between different immune pathways and AKP effects in the microbiome.

AKP effects in allergenic and autoimmune disorders

Given that immunosuppression increases microbiome variability, one might expect that autoimmune disorders would decrease microbiome variability. Contrary to this expectation, allergenic and autoimmune disorders are often associated with strong AKP effects.

Type 1 diabetes (T1D) is an autoimmune disorder in which T-cells attack insulin-producing pancreatic β-cells. Giongo *et al.* show how patients with T1D exhibit reduced similarity between pairs of individuals, and more rapid microbiome changes over time, than healthy patients[26]. Several other allergenic and autoimmune disorders thought to involve some combination of microbial dysbiosis and changes in host immunity also show strong AKP effects. In a study of patients with Crohn's disease, Dey and colleagues found that Crohn's patients showed significantly greater β-diversity than healthy controls[28]. Additionally, patients that would go on to have a recurrence of Crohn's disease following surgery had greater β-diversity distances from healthy or non-inflammatory bowel disorder surgery controls[28]. Further tests will be required to determine if this increased distance from healthy patients is due to AKP effects or deterministic effects. Pérez-Brocal and colleagues reached a similar conclusion based on visual inspection of 16S rRNA data from 19 patients[53]. Ulcerative colitis patients in remission again showed much lower temporal stability than did controls[54], as measured by steady reductions in community similarity to the initial timepoint. In a mouse model of colitis, in which inflammation is induced by exposure to dextran sodium sulfate (DSS), repeated rounds of DSS exposure increasingly destabilized the gut microbiome, eventually shifting it to a distinct colitic configuration[55]. Finally, a recent time-series analysis of 137 individuals with multiple inflammatory bowel disorders elaborated on these findings by demonstrating that the microbiomes of patients with inflammatory bowel disease changed more rapidly over time, and showed greater stochastic deviations from a 'healthy plane' defined by microbiomes of healthy individuals[56].

Thus, AKP effects are present in several forms of abnormal positive or negative immunomodulation. These observations, while perhaps counterintuitive, could be explained by the hypothesis[26] that animal regulation of immunity evolved to both screen out pathogens and carefully regulate commensal and mutualistic microorganisms. If so, it may be more apparent why microbiome instability is observed in multiple types of immune dysregulation.

Pathogen infection can induce AKP effects

If, as we argue above, both autoimmunity and immunosuppression can induce AKP effects in animal microbiomes, then we should expect that pathogens, particularly those that are adept at manipulating host immunity for their own benefit, may also induce these effects.

Mouse models of infection by bacteria[57] and parasites[58,59] have shown AKP effects that illustrate the feedbacks among immunity, commensal microbiome stability and pathogen invasion. For example, in a mouse model of *Salmonella* infection, artificially reduced intestinal expression of the mouse glycosyltransferase *B4galnt2* (β-1,4-N-acetylgalactosaminyltransferase 2) caused both greater microbiome variability and increased susceptibility to *Salmonella typhimurium* infection[57]. Critically, this increased susceptibility to infection was dependent on the presence of the microbiome, and did not arise from direct mouse–*Salmonella* interactions. In turn, *Salmonella* infection greatly exacerbated microbiome variability, suggesting that AKP effects can be both a cause and a consequence of successful pathogen infection.

Similar evidence arises from fungal infections of amphibians. The chytrid fungus *Batrachochytrium dendrobatidis* (hereafter *Bd*) has been implicated in amphibian declines worldwide. The mechanism by which it harms its host is still being explored, but may involve secretion of harmful water-soluble compounds, as water incubated with *Bd* also causes pathological effects[60]. Microbiome variability between frogs at a single time point was not different between lakes with or without *Bd* infection. However, a *Bd* outbreak was accompanied by significantly increased temporal change in community β-diversity relative to lakes without outbreaks[20]. Experimental infection of bullfrogs showed both that differences

Box 3 | An experimental test disentangling AKP effects.

Understanding the biological consequences of AKP effects will require experiments that separate the effects of predictable shifts in the microbiome versus dispersion. Pooling experiments provide one way to disentangle the physiological consequences of these dynamics for hosts. If a perturbation causes pure dispersion effects (Fig. 2c), then a simple but surprising property should hold: the average microbiome of diseased individuals will be similar in composition to that of a healthy individual. In contrast, perturbations that predictably shift the microbiome (Fig. 2a) or in which the microbiome shifts in varying degrees to a dysbiotic state (Fig. 2b) will not exhibit this property. This difference could allow for separation of the relative contribution of dispersion and location effects to a particular health outcome. For example, a mouse model of a disease with AKP effects on the microbiome could compare the effects of microbiome transplants into gnotobiotic animals formed by single donors versus pooled donors. In the latter scenario, pure dispersion effects will be averaged out. Differences in outcome between the two transplant pools will therefore separate AKP effects from deterministic differences. This approach should be especially useful in cases that present a mixture of deterministic and stochastic alterations to the microbiome.

in microbial community structure (for example, weighted UniFrac distances) influenced *Bd* infectivity, and that *Bd* infection induced microbiome dispersion in a controlled experiment[61].

Broader significance of AKP effects

The AKP not only provides insight into host microbiome dynamics, but also has broader implications for microbial data analysis and for our interpretation of the biologically meaningful effects of different biotic and abiotic forcing that in the past have generally been considered noise or negative results. As the dominant paradigm in our field has emphasized shifts between alternative stable states, which produce distinct clusters in ordination space (that is, location effects), stressors that increase variability (dispersion effects) are often discarded as statistical artifacts. Empirical data connecting increased variability to health outcomes suggests that we should analyse rather than throw away these datasets.

Destabilization of animal microbiomes influences statistical tests of microbiome dynamics. Biological interpretation of dispersion effects, although not commonplace, has roots in the literature. Detection of both dispersion and location effects was originally presented as an advantage of ANOSIM (analysis of similarities) tests[62]. Later, PERMANOVA (permutational multivariate analysis of variance) and PERMDISP (permutational analysis of multivariate dispersions) allowed for separate detection and quantification of location and dispersion effects[63]. However, in practice, the detection of location effects has been seen as a primary goal, while dispersion effects have been regarded as nuisances. This can probably be traced to the lack of a meaningful biological interpretation for increased β-diversity dispersion in animal microbiomes, which is what we propose in this Perspective.

AKP effects shed light on paradoxical enrichment patterns. The AKP helps to resolve apparently paradoxical changes in species enrichment. A stressor that affects the microbiome by increasing its rate of stochastic change over time will tend to increase inter-individual variation as the microbiome of individuals increasingly drift apart. Imagine regressing the abundances of microorganisms across individuals against an index of stress or disease severity. If stressed individuals have greater variability, such an analysis will often detect significant loss of 'normal' microorganisms, paired with no corresponding statistically significant gains in pathogens. This situation appears paradoxical, because marker gene surveys of microbiomes produce compositional data: proportional losses of one microorganism must be compensated with proportional gains somewhere else in the community. However, the systematic differences in variance between stressed and unstressed conditions means that even though compensatory shifts are happening, they may not rise to the level of significance because they vary in each stressed individual. This is especially true at more detailed taxonomic levels (strain or species), where statistical power is most limited by the need to correct for multiple comparisons.

Conclusions

Recent research has demonstrated that AKP effects are common to many disturbed animal microbiomes. Important pathogens, environmental stressors and immune dysfunctions can lead to increased stochasticity in the microbiome. Our goal in discussing the prevalence of AKP effects is not to minimize the importance of deterministic changes in community composition, which are well appreciated. Instead, it is to suggest an alternative framework for the interpretation of data that do not easily fit into these paradigms.

AKP effects probably matter a great deal in the clinic, as microbiome status can affect the efficacy of drugs ranging from cardiac glycosides[64] to chemotherapeutics[65]. Understanding which underlying conditions produce more variable microbiome may help to target microbiome-aware personalized medicine screens (Box 2).

The associations between microbiome instability and many stressors and diseases suggest that microbiome resistance and resilience are a hallmark of healthy physiology, consistent with the evolution of animals in a sea of microorganisms and viruses[66]. However, microbiome instability due to AKP effects can only be seen in contrast to normal variation[67]. Time-series studies are increasingly critical for documenting background microbiome stability across normal development[37]; with dietary changes[68]; and also across generations[69] and between species[70], so that this normal variation can be contrasted with pathological changes.

Many widely used software packages provide tools that can test for AKP effects in microbiome data. For example, the PERMDISP procedure is available in the *vegan* R package. Both this procedure and a separate permutational test for pairwise distances between samples are available through the QIIME package for microbial community analysis (see the compare_categories.py and make_distance_boxplots.py scripts in QIIME 1.9)[71]. We recommend the widespread reporting of these tests. Additional methods for analysis of temporal variation are reviewed in ref. 72.

However, current tools are less well-suited to distinguishing biological mechanisms that might produce AKP effects. Therefore, new theory and applied computational software are needed to routinely distinguish these hypotheses. Observation of increased microbiome variability under multiple host stressors suggests the opportunity to elaborate on existing ecological models of interactions within the microbiome. Increased attention is also needed to develop computational methods for inferring stochastic and deterministic effects of a stressor on animal microbiomes in a unified statistical framework. These computational approaches should be paired with experimental protocols in model systems designed to explicitly test the effects of stochastic versus deterministic microbiome change. Such designs include pooled versus unpooled microbiome transplants from diseased individuals into healthy gnotobiotic recipients (Box 3). This new toolkit, along with broader recognition and reporting of AKP effects, will help clarify and contextualize the connections between host stress and microbiome stability in diverse systems.

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Author contributions

J.R.Z. wrote the manuscript. All authors conducted research, contributed intellectually, and edited the manuscript.

Additional information

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Competing interests

The authors declare no competing financial interests.