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**[Overview article]**

# Environmental change and human health: Can environmental proxies inform the biodiversity hypothesis for protective microbial-human contact?

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## Abstract

Microbiota from environmental sources overlap and interact with human microbiota, contribute to human microbial diversity and provide beneficial immunomodulatory stimuli. Meanwhile, reduced diversity in human microbiota and immune dysregulation have been associated with a range of diseases. Emerging evidence suggests landscape-scale drivers of microbial diversity may influence our health, but the area remains understudied because of its multidisciplinary nature. Here we attempt to widen the view on this subject by offering an environmental researcher's viewpoint, proposing a unifying conceptual framework to stimulate multidisciplinary interest. To focus research in this challenging area we propose greater emphasis on multi-scale ecological links, and that landscape-scale proxies for potential underlying microbial mechanisms be investigated to identify key environmental attribute and health relationships worthy of subsequent detailed examination. Wherever possible, ecological epidemiological studies should account for the temporal nature of environmental microbiota exposures, especially with respect to the early development of the human commensal microbiota.



Keywords: environmental microbiota, immunoregulation, biodiversity, dysbiosis, microbial old friends


## Introduction

People often express an intuitive sense that being amongst nature is good for their health. Further to well established risk-exposure scenarios in environmental health, modern scientific approaches are increasingly discovering there are a range of non-trivial mechanisms and co-

benefits linking natural surroundings, biodiversity and human health influence (table 1). Better understanding these relationships may have important implications for developing cost-effective and mutually beneficial outcomes to help address simultaneous challenges in public health and biodiversity conservation (von Hertzen et al. 2011, WHO and SCBD 2015).

**[--Insert table 1 near here--]**

The last mentioned mechanism in table 1 is among the least understood while also having wide potential to influence human health, due to the largely hidden but ubiquitous nature of microbes (or microorganisms). Microbes have dominated the evolution of life and comprise a dominant portion of the Earth's living biomass and its genetic diversity (Whitman et al. 1998). Microbes feature in every habitat where life is possible. The various human microbiotas, or communities of microbes (e.g. gut, skin, airway, oral cavity, genitourinary), exist in interdependent symbioses performing much of our metabolism (Wikoff et al. 2009). Their importance to human physiology is reflected in current knowledge that the combined human microbial genome (or microbiome) expresses over 100 times more genes than the human genome (Belizario and Napolitano 2015). Beneficial connections between microbiota and host health—influencing bodily development, mood and stress responses—have been observed in both humans and animal models (Round and Mazmanian 2009, Rook et al. 2013, Belizario and Napolitano 2015). The human microbiota is believed to play an important role in normal human development (of organs, gut, immune system, bone and brain) and actively participate in the homeostasis of the human body (McFall-Ngai et al. 2013). With important metabolic, immune and nutritional roles, the human intestinal microbiota has been described as a "super-organism" (Purchiaroni et al. 2013).

Dysbiosis of the human microbiota (i.e. reduced diversity or changes in composition, often with an increase in the ratio of pathogenic to commensal organisms) has been

48 associated with a range of immunological, gastrointestinal, metabolic, psychiatric and
49 behavioural disorders observed in humans and animal models, as reviewed elsewhere (Round
50 and Mazmanian 2009, Clemente et al. 2012, Parker and Ollerton 2013, Rook et al. 2013,
51 Belizario and Napolitano 2015). Ongoing research into particular microbiota–disease
52 associations is supporting increasing recognition of host microbiota-mediated mechanisms
53 across diverse disease outcomes, for example, in obesity (Ridaura et al. 2013), type 2 diabetes
54 (Forslund et al. 2015), rheumatoid arthritis (Zhang et al. 2015), stroke (Yin et al. 2015),
55 depression (Zheng et al. 2016) and some cancers (Sivan et al. 2015, Vétizou et al. 2015).

56 Multifactorial influences are known to drive the composition and diversity of the
57 human microbiota, including diet, genetics, antibiotic use, age, birth mode of delivery
58 (natural or caesarean), and geographic location (Clemente et al. 2012, Voreades et al. 2014,
59 Belizario and Napolitano 2015). However, at least a portion of the human microbiota is in
60 dynamic exchange with microbes from the surrounding environment and hence natural
61 microbial diversity is now appreciated as an important contributor to normal (healthy) human
62 immunological (and potentially other aspects of homeostatic) functioning (von Hertzen et al.
63 2011, WHO and SCBD 2015). Emerging experimental evidence also supports this line of
64 thinking. For example mice exposed to soil, house dust and decaying plants had enhanced gut
65 microbial diversity and innate immunity, when all other variables (diet, age, genetic
66 background, physiological status and original gut microbiota) were controlled for (Zhou et al.
67 2016). In a separate study, mice exposed to dog-associated house dust experienced changes in
68 gut microbiome that were associated with protective immune responses against airway
69 allergens and virus infection (Fujimura et al. 2014). Rook (2013, his figure 3) suggests
70 several potential pathways through which environmental microbiota might impact the human
71 microbiota and/or provide immunomodulatory stimuli. These pathways may include transient

72 contact or colonization; with either direct recognition by immune receptors or indirect
73 responses following interactions which alter the host microbiota.

74 Having emerged, in an evolutionary sense, from largely natural and biologically
75 diverse surroundings, a growing proportion of the global population are now surrounded by
76 relatively depauperate (low biodiversity) urban, industrialised or highly managed and largely
77 monocultural agro-ecological landscapes. As discussed later, these macroscale changes can
78 translate to microscale changes in biodiversity and ecosystem composition (Adams and Wall
79 2000, Bulgarelli et al. 2013, Turner et al. 2013). Meanwhile, the science of aerobiology (e.g.
80 Womack et al. 2010, Polymenakou 2012, Bowers et al. 2013) shows that human populations
81 have a real biological connection to their ambient surroundings (in addition to any direct
82 physical environmental contact). These lines of evidence suggest that different sources and
83 compositions of environmental microbiota—through interactions with the human microbiota
84 and other immunomodulatory pathways (von Hertzen et al. 2011, Rook 2013)—may
85 inadvertently provide protective or adverse background influences on human health.

86 Indeed, many medical researchers including the World Allergy Organization now
87 suggest that microbiota-mediated mechanisms—and disruption to these, arising from
88 environmental change—at least partly explain the pandemic of allergic, auto-immune and
89 chronic inflammatory diseases (AACIDs, discussed later) occurring across developed nations
90 in recent decades (Haahtela et al. 2013). Described variously as the microbial Old Friends
91 (MOF) mechanism (Rook et al. 2013), high microbial turnover hypothesis (Matricardi and
92 Bonini 2000), biodiversity hypothesis (von Hertzen et al. 2011), or the evolutionary
93 mismatch of 'biome depletion' (Parker and Ollerton 2013)—it is suggested that a lack of
94 microbial diversity, or reduced contact with the right type of microbes (or MOF as discussed
95 later), in our modern surroundings is an important contributor to the rising incidence of
96 immune dysregulation underlying AACIDs, and possibly a range of other diseases including

some cancers (Rook and Dalgleish 2011). Highlighting concerns (shared by von Hertzen et al. 2011) for the impacts of biodiversity loss leading to reduced immunoregulation from natural environments, Rook (2013) proposed that environmental microbiota (as supplements to MOF) may provide an unappreciated ecosystem service that is essential to our well-being, and that "this insight will allow green spaces to be designed to optimize health benefits and will provide impetus from health systems for the preservation of ecosystem biodiversity".

However, large gaps remain in our knowledge. "Hardly anything is known about the interactions between environmental and indigenous [host commensal] microbiotas" (Haahtela et al. 2013). There are still many unknowns concerning the protective roles and membership of MOF, their possible modes of action, and broader relationships with biodiversity and the surrounding environment (Stanwell-Smith et al. 2012, WHO and SCBD 2015). Important research questions in the context of potential environmental microbiota-mediated influences on human health include: (a) can we characterize environments through their microbiota? (b) what are the effects of macro- to landscape-scale environmental change and biodiversity loss on environmental microbiota? (c) is landscape-scale biodiversity associated with human health outcomes? (d) are different types, or condition (quality), of environment potentially more beneficial than others? (e) how might protective environmental influences compare to recognised drivers of human health such as socioeconomic status, diet and lifestyle risk factors? (f) can we identify and prioritise particular environment (or environmental change) and health associations to target subsequent detailed research? (g) under what circumstances might environmental microbiota (or other microscale bioactive agents) be associated with health benefits? Answers to these questions may help to build insight and hypotheses, and prioritise research opportunities, before tackling more detailed investigations of possible environmental microbiota-mediated mechanisms.

To-date the MOF mechanism has principally been investigated from a medical research focus, with limited emphasis placed on the potential role, and analysis, of broad-scale ecology or environmental change. This is despite a call to "bridge the chasm between ecology and medicine/immunology" (Rook 2013). Here we further the argument for greater integration of ecological insight and environmental analyses into studying potential protective environmental microbiota-mediated mechanisms. In particular, we suggest that a comprehensive examination of broad-scale spatially variable environmental attributes, in the context of spatially distributed public health data, may progress knowledge in this area. If we adopt the view that microbial diversity in the environment should be viewed as an inherent ecosystem service that is essential to our wellbeing (Rook 2013) and this can be related to environmental biodiversity (von Hertzen et al. 2011), then we suggest that protective health effects should be observable and associable with recognisable environmental attributes (e.g. land use, vegetation, soil types, and their diversity)—acting as proxies for as-yet unknown microbial agents and mechanisms.

## Immunomodulation, 'old friends' and the biodiversity hypothesis

Microbes play a key role in educating and regulating the immune system (Purchiaroni et al. 2013, Belizario and Napolitano 2015, WHO and SCBD 2015). Having co-evolved with a diverse range of microbes (and their metabolic and decay products) in the surrounding environment, the human immune system has needed to develop defence mechanisms against harmful pathogens as well as tolerance mechanisms to other commonly encountered, and mostly harmless, microbial agents. As developed societies around the world have improved standards of sanitation we have witnessed a decline in infectious diseases. However, in recent

144 decades this has been paralleled by a corresponding increase in AACIDs (Haahtela et al.
145 2013).

146 Initial attempts to explain this trend gained most attention via the hygiene hypothesis
147 (Strachan 1989). However this has since been revised and expanded, and is perhaps most
148 notably described in terms of the MOF mechanism (Rook et al. 2013). Alternatively, WHO
149 and SCBD (2015) use the terminology 'supplements to the human symbiotic microbiota from
150 the natural environment'. The MOF mechanism suggests that, following prolonged microbial
151 exposure over evolutionary timescales, a dependence evolved between the immune system of
152 mammals and some microorganisms. Possibly, this involved ancestral humans losing the
153 need for gene expression associated with essential functions that could be readily performed
154 by these partner microorganisms. In particular, this concerns a key function of the immune
155 system in recognizing when *not* to activate, to avoid unwarranted and potentially self-
156 harming inflammatory responses to the body's own cells and normally harmless microbes
157 from the surrounding environment.

158 Exposure to a broad diversity of microorganisms following birth (e.g. from vaginal
159 delivery, diet, human contact and the environment) provides important training inputs to the
160 human immune system (O'Hara and Shanahan 2006, Wopereis et al. 2014). Microbes are
161 sampled by immune cells associated with mucosal barrier tissues prompting the
162 establishment of complex immunoregulatory circuits that balance inflammatory responses (to
163 suppress dangerous pathogens) with tolerance mechanisms that induce, for example, anti-
164 inflammatory cytokines (signalling proteins) and regulatory T cells ($T_{reg}$), in order to avoid
165 undue responses to common antigens (O'Hara and Shanahan 2006, von Hertzen et al. 2011,
166 Purchiaroni et al. 2013).

In contrast, AACIDs have been associated with immune dysfunction, dysbiosis, and inappropriate inflammatory responses to: (a) our own tissues, manifesting as autoimmune diseases such as type 1 diabetes, multiple sclerosis, rheumatoid arthritis; (b) normally harmless allergens and foods, manifesting as allergic disorders, eczema, asthma, hay fever; and (c) gut contents including commensals, manifesting as inflammatory bowel diseases such as ulcerative colitis and Crohn's disease. These associations are reviewed in detail elsewhere (e.g. Round and Mazmanian 2009, Clemente et al. 2012, Parker and Ollerton 2013, Purchiaroni et al. 2013, Belizario and Napolitano 2015). Risk of AACIDs may be further enhanced by lack of physical activity and sunlight, poor diet, pollution and other factors, which may act in synergy with dysbiosis of the gut flora (Stanwell-Smith et al. 2012, Haahtela et al. 2013). Haahtela et al. (2013) also review and speculate on possible connections between dysbiosis and AACIDs. They suggest it is possible that some common members of the normal (healthy) commensal microbiota may play an active role in the development of Treg cells, responsible for mediating suppression of T-cell mediated inflammatory responses. They speculate that altered environmental microbiota may play a role in the development of dysbiosis, for example, through reduced signalling of pattern recognition receptors (used by the innate immune system to identify particular microbes and thereby amplify or suppress responses). Reduced immune signalling may then lead to immune dysfunction which enhances the colonization and growth of a biased microbiota, thus reinforcing the host-microbe interaction towards an unhealthy state (Haahtela et al. 2013).

Failing immunoregulatory mechanisms can also lead to continuous background inflammation, even without a specific chronic inflammatory disorder. Persistent raised levels of inflammatory mediators have been associated with increased susceptibility to a range of diseases including insulin resistance, metabolic syndrome, type 2 diabetes, obesity,

192 cardiovascular disease, reduced stress resilience and psychiatric disorders such as depression
193 (Parker and Ollerton 2013, Rook et al. 2013, Belizario and Napolitano 2015). Several forms
194 of cancer are also associated with increases in AACIDs which may be explained via chronic
195 inflammation providing growth factors and mediators that stimulate the vascularisation and
196 metastasis of tumours (Rook and Dalgleish 2011).

197 Temporal dimensions of human and environmental microbiota interactions also
198 require consideration. Early stimulation is viewed as particularly crucial for supporting the
199 maturation of immunoregulatory mechanisms (Wopereis et al. 2014) and dysbiosis during
200 early developmental periods may have lasting adverse health impacts (Cox et al. 2014).
201 However, immunoregulatory effects associated with dysbiosis (Parker and Ollerton 2013,
202 Belizario and Napolitano 2015) and helminth infections (Versini et al. 2015) are also
203 observed in later childhood and in adults, while immune-boosting effects of mycobacteria (a
204 suggested Old Friend) are known to be transient (Matthews and Jenks 2013); suggesting that
205 ongoing diverse exposures are also important (Matricardi and Bonini 2000). Temporal effects
206 are also discussed later in relation to variability of environmental microbiota exposures and
207 addressing confounders in more detailed work.

208 Drawing on multiple lines of evidence, von Hertzen et al. (2011) extended the notion
209 of a MOF mechanism to suggest that: “declining biodiversity might actually increase the risk
210 to humanity from chronic diseases”. This idea arises because transient beneficial members of
211 the human microbiota overlap with environmental microbiota, suggesting a dynamic
212 interaction with the environment. As reviewed elsewhere (von Hertzen et al. 2011, Stanwell-
213 Smith et al. 2012, Haahtela et al. 2013, Rook 2013, WHO and SCBD 2015, and references
214 therein), grounds for the notion of a wider association between dysbiosis, AACIDs and a lack
215 of biodiverse microbial stimuli from the surrounding environment comes from: (a)
216 metagenomic studies of the microbiota in the gut and other sites; (b) epidemiological studies

on immigrants moving to more affluent but more depauperate countries; (c) urban-rural AACID comparative studies; and (d) studies of immunomodulatory effects due to epigenetic mechanisms, farm and livestock exposures, proximity to agricultural land, and exposure to pets. Reduced exposure to biodiverse environments and urban green space is also suggested to partly explain the higher incidence of AACIDs associated with lower socioeconomic status (Rook et al. 2014).

Emerging evidence lends support to von Hertzen et al. (2011)'s biodiversity hypothesis. Hanski et al. (2012) found associations between atopic sensitization (allergic disposition), skin microbiota and surrounding land use types in a random sample of 118 adolescents living in a heterogeneous 100 × 150 km region of Finland. Atopic individuals had reduced generic diversity of gammaproteobacteria on the skin compared with healthy individuals. In contrast, healthy individuals showed a significant correlation between the relative abundance of the gammaproteobacterial genus *Acinetobacter* and expression of interleukin (IL)-10, a key anti-inflammatory cytokine in immune tolerance. Atopic sensitization was significantly explained by land use, decreasing with the amount of forested and agricultural land within 3 km of the study subjects' homes. In cohort studies from Finland and Estonia, Ruokolainen et al. (2015) found that land use patterns explained 20% of the variation in the relative abundance of proteobacteria on the skin of healthy individuals, and the amount of green environment (forest and agricultural land had similar effects) was inversely associated with the risk of atopic sensitization in children. They concluded that "the environmental effect may be mediated via the effect of environmental microbiota on the commensal microbiota influencing immunotolerance". There are, however, limited studies of this type, and more research to test the biodiversity hypothesis in different environments is needed.

## Why focus on environmental proxies?

Sources of microbial diversity in the natural environment include soil, vegetation, animals, and aquatic and marine environments. The environment is highly multifaceted and here we discuss landscape-scale attributes as potential drivers of environmental microbiota diversity, and therefore health. We might expect macroscale environments to be linked to microscale environments through provision of characteristic feedstocks and micro-habitats. Changes to above-ground macroscale features can impact on microscale ecosystem dynamics of terrestrial, freshwater and marine systems through: (a) changes in resource supply, (b) physical and structural habitat heterogeneity, (c) biotic (ecological) interactions, and (d) cross-surface migration of above- and below-ground organisms (Adams and Wall 2000). Broad-scale geographic variation may also contribute to variation in human microbiota, for example Suzuki and Worobey (2014) suggest that higher latitude colder climates are associated with changing proportions of dominant bacterial phyla linked to increased body mass. Key environmental themes linked to sources of microbiota are highlighted below.

***Vegetation / land use.*** Different plant species are associated with different microbiota of the phyllosphere and rhizosphere (i.e. microbial habitats of aerial vegetation and below-ground roots respectively) (Bulgarelli et al. 2013, Turner et al. 2013). The composition of, and similarities between, plant microbiota are driven by factors including: (a) biochemically-induced mutualism between particular plant and microbial species, (b) genetic relatedness between plants, (c) climate, (d) anthropogenic influences (e.g. pesticide use) and (e) spatial proximity (Bulgarelli et al. 2013, Bringel and Couée 2015). The connection between above-ground (macroscale) and below-ground (microscale) components within terrestrial ecosystems typically results from powerful mutual feedback mechanisms. For example, plant characteristics will dictate organic matter inputs to soil microbiota while soil

266 microbiota will dictate the breakdown and re-supply of nutrients to plants. These feedbacks
267 will vary depending on the natural fertility and productivity of an ecosystem (Wardle et al.
268 2004) and also with anthropogenic changes in land use and management (Coleman et al.
269 2004).

270 It is also possible that a range of (non-microbe) microscale bioactive agents may
271 provide immunomodulatory influences. For example, Li et al. (2006) found immune-boosting
272 effects from phytoncides (wood essential oils), and Stanwell-Smith et al. (2012) suggest that
273 protective agents may extend beyond the living MOF themselves, to include their cellular
274 components (e.g. endotoxin), decay products and metabolites. In view of this potential wider
275 context for health influences from the environment, plants are also known to emit pollens,
276 aerosols, and a wide variety of volatile organic compounds (VOCs) (Bulgarelli et al. 2013).
277 VOCs can promote or inhibit (and thus shape) adjacent microbial communities; while
278 phyllosphere microbiota are also active in the production, interception and alteration of
279 various plant-related VOC emissions (Bringel and Couée 2015).

280 ***Animals.*** Through interactions with their surrounding environment, animals may
281 inadvertently sample and collect a wide variety of environmental microbiota. Characteristic
282 microbial sources most relevant to human interactions will likely include fur, or hides, and
283 fecal matter. At the landscape-scale, different vegetation and land use types are often
284 associated with different animals (e.g. livestock grazing or feedlots on agricultural land,
285 native species in conservation areas, pest species in poorly managed areas). Human exposure
286 to animal microbiota will be influenced by proximity, and the amount and volatility of source
287 material, as well as prevailing winds for airborne microbiota. Bowers et al. (2013) measured
288 airborne bacterial signatures of cow fecal microbiota in a rural city surrounded by agricultural
289 land containing cattle feedlots. Exposure to pet dogs in early infancy has been shown to
290 reduce the risk of childhood allergic disease development, and dog-associated house dust has

been found to be associated with beneficial immunomodulatory effects (Fujimura et al. 2014). Microbiota associated with animals and farm exposures are further reviewed elsewhere (Stanwell-Smith et al. 2012, Rook 2013).

***Soils.*** Soils are the most complicated biomaterial on the planet (Young and Crawford 2004). They support an immense diversity of microbes which remain largely unexplored; with drivers of variability in soil microbiota including variation in soil types and microhabitats (arising from environmental conditions, anthropogenic and organic inputs, and soil texture or clay content) (Torsvik and Øvreås 2002). Microbes from soils have produced many of the most important medicinal drugs, including the majority of antibiotics and many anti-cancer compounds (Charlop-Powers et al. 2015). Soil eating (geophagy) is widespread in vertebrates and many human cultures, typically targeting clay-rich soils and suggested to provide protective health benefits (Young et al. 2011); this is consistent with the mechanisms discussed here. Particular soil constituents may have biological effects and seasonal mobilisation patterns, for example as shown in studies of coccidioidomycosis (valley fever) caused by a soil-dwelling fungus (Kolivras et al. 2001). Loss of contact with soil (and associated microbiota) has been suggested as a possible contributor to the rise in AACIDs arising from broad-scale sealing of soils in urban developments (von Hertzen and Haahtela 2006).

Aside from soil itself, biological soil crusts can comprise up to 70% of the living groundcover across many diverse natural environments (Belnap and Lange 2001). These crusts form an aggregation of soil particles and cyanobacteria, algae, microfungi, lichens, and bryophytes which live in or upon the top few millimetres of soil, and may also be important contributors to beneficial human-environmental microbiota contact.

***Coastal / marine environments.*** The marine microbiome is also diverse and largely unexplored, has biomass (cell densities) concentrated in near surface layers, and shares over 70% of microbial gene functionality with the human gut microbiome (Sunagawa et al. 2015). Mobilisation, via aerosols, of bioactive substances associated with marine microorganisms—thus influencing the health of near-coastal human populations—is demonstrated through the adverse example of harmful algal blooms or red tides (Weinstein 2013).

***Air.*** Aerobiology demonstrates there is a real biological connection between humans and ambient environmental microbiota. The air is alive with microbial diversity, including bacteria, viruses, fungi, pollen and algae; and acts as a source of both pathogenic and beneficial microbes to humans (Womack et al. 2010, Polymenakou 2012). Spatial and temporal variability may be expected in the composition of airborne microbiota. From sampling the near-surface atmosphere across three distinct land use types (agricultural fields, suburban areas and forests), Bowers et al. (2011) found the composition of airborne microbiota was significantly related to land use type, and that differences were likely driven by shifts in the sources of bacteria rather than local meteorological conditions. Also, Bowers et al. (2013) observed seasonal fluctuations in the composition and sources of near-surface airborne microbes, with soils and leaves representing important microbial sources across both urban and rural sites, and cow fecal bacteria (associated with neighbouring feedlots) also featuring in the rural location on a seasonal basis. They observed that microbial sources varied in prominence under seasonal conditions, potentially explained by climatic conditions, deciduous plant growth and senescence, and seasonal soil disturbance from surrounding agricultural land use practices. Continental-scale patterns in the distribution of dust-associated bacteria and fungi have also been observed (Barberán et al. 2015), where geographic patterns were associated with climatic and soil variables. That work also found

that urban areas were exposed to more homogenized airborne microbiota compared to the geographic variability found across rural areas.

Given the preceding evidence of relationships connecting various ecosystems (or ecosystem components), environmental microbiota and potential human health effects, it may be possible to improve human health outcomes through environmental management specifically targeting microbiota-mediated linkage mechanisms. We envisage theoretical links between landscape-scale environmental change, environmental microbiota and human health as shown in figure 1. However, in order to prioritise where more detailed study of underlying mechanisms and/or public health interventions might be most cost-effectively targeted, it is first necessary to quantify the strength of associations between environmental exposures and health outcomes. In the absence of temporal change data, we might examine these relationships using spatial analogues (environmental mapping) for differences in the landscape. We suggest that the links depicted in figure 1, while not comprehensive, may provide a useful conceptual framework for multidisciplinary research. In the next section we briefly discuss methodological approaches for establishing priorities, addressing confounders, and outline subsequent more detailed approaches required to progress knowledge in this emerging field of study.

**[--Insert figure 1 near here--]**

There are a number of factors supporting the use of environmental proxies to investigate the MOF mechanism and related biodiversity hypothesis. Knowledge of the membership of MOF is incomplete and inconsistent (Stanwell-Smith et al. 2012); it is not known whether biodiversity, total biomass or the particular source or species of environmental microbe(s) is important (Rook 2013); and there are still considerable computational challenges and base knowledge limitations in trying to characterize and

362 understand the genetic makeup and biological function of complex natural environments such
363 as soil (Howe et al. 2014). Knowledge is still building on the microbiota of different
364 environments, for example through the Earth Microbiome Project (Gilbert et al. 2014). A
365 range of previously unappreciated (non-microbial) microscale bioactive agents from the
366 environment may also be contributing to human health. There is a growing consensus that
367 living in close proximity to the natural environment can provide a broad range of health
368 benefits (WHO and SCBD 2015), but what type of natural environment? And are some
369 environments better than others? Using a spatial analogue approach may help answer this
370 question.

371 A focus on identifying particular environmental microbiota-mediated mechanisms
372 impacting human health may also be hampered by redundancy that is likely to be found both
373 in the microbial agents providing immunomodulatory stimuli and human immune system
374 pathways (Stanwell-Smith et al. 2012). Microbiota can drive epigenetic responses (Shenderov
375 2012), bringing further potential complexity and requisite expertise to the examination of
376 underlying mechanisms. Required doses are unknown, and it may be that subclinical
377 (asymptomatic) exposures are all that is required to deliver protective health benefits
378 (Stanwell-Smith et al. 2012). If so, this poses a challenge as subclinical exposure is much
379 harder to detect in epidemiological studies. This means for subsequent detailed study into
380 underlying mechanisms, immunological markers will be important, not just disease
381 outcomes.

382 When it comes to analysing environmental exposures, the question of required dose is
383 worth exploring further. This is because emerging knowledge of the predominantly beneficial
384 role of microbes (as discussed here) is at odds with the traditional focus of microbiology,
385 concerned with the negative role of microbes in driving infectious disease. Here we raise the
386 possibility that hormetic, U- or J-shaped dose-response relationships (i.e. characterised by

low dose stimulation and high dose inhibition, Calabrese et al. 2007) may provide a bridging paradigm between protective MOF and the traditional toxicological view of common pathogenic microbes, by spanning divergent health outcomes inferable from varying microbial dosage rates. For example, known pathogens including *Escherichia coli*, *Helicobacter pylori*, species of *Salmonella* and *Staphylococcus*, enteroviruses and parasitic helminth worms are among those microorganisms suggested to have protective roles (Stanwell-Smith et al. 2012). Calabrese et al. (2007) suggest that hormetic responses are generalizable and commonly encountered across a range of biological systems.

In figure 2 we conceptualise an idealized dose-response curve for a generic MOF. When otherwise expected, missing or very low doses of a MOF are associated with greater risk of AACIDs and related adverse health outcomes (zone 1). Nominally low to moderate doses are associated with protective benefits (zone 2; due to appropriate stimulation of immunoregulatory circuits). Increasingly elevated microbial doses are expected to be associated with disease (zone 3). Figure 2 mirrors Calabrese et al. (2007)'s biphasic response curve except—instead of setting the reference response level at 100% of control (zero dose)—by setting the reference response at some low-moderate dose range (perhaps corresponding to an evolutionary norm), three response zones instead of two are depicted. In this context, an evolutionary norm would correspond to long-term exposures to diverse environmental microbiota and natural allergens consistent with expected normal immunomodulation via the MOF mechanism. Such a curve also parallels the triphasic deficiency-adequacy-toxicity concept familiar in plant nutrition (Smith and Loneragan 1997), further supporting Calabrese et al. (2007)'s claim that such hormetic curves are generalizable across many biological systems. Selecting spatial environmental attributes (proxies) that might mimic varying amplitudes of microbial exposure (e.g. soil erodibility, soil microbial

activity indices), and using natural experiments, may provide a means to test (or at least build support for) this hypothesis of a hormetic relationship.

**[--Insert figure 2 near here--]**

Ecological interactions (e.g. competition, predation, mutualism, commensalism) operate at the microbial scale (Coleman et al. 2004), so applying general principles we might speculate on the ecological context corresponding to the three zones in figure 2. In zone 2, where some low-moderate concentration of the MOF is present, this would correspond to an evolutionary norm or long-term steady-state microbiota—consistent with the establishment of immunoregulatory norms. Such a long-term, well-established microbiota is also suggestive of a balanced composition with maximal biodiversity (and hence buffering to change) compared to the other zones. In zone 1, we might envisage that environmental conditions or microbial ecosystem dynamics have reduced the populations of the particular MOF. Such a shift in environmental conditions (e.g. feedstocks, temperature, air, moisture) will likely favour the proliferation of another microbial species to fill the vacant, or newly emergent, ecological niche. The loss of the MOF with a rise in some other remaining species would correspond to an overall reduction in biodiversity of the microbiota. Alternatively, the original environmental microbiota could have been largely substituted, for example, where people have moved from rural to urban areas. In zone 3, we might speculate that it is actually the particular MOF that has been favoured by a shift in environmental conditions. This would be at the expense of reduced numbers or loss of other species, also corresponding to a loss of biodiversity. In this hypothetical scenario it is interesting to note that appropriate, protective doses (and exposures) to a particular MOF might be entirely consistent with exposure to a high diversity environmental microbiota. However a chain of evidence would be required to test this hypothesis in detail, for example, as outlined in figure 1.

435 Other known microbial ecology mechanisms also underlie the importance of
436 microbiota composition and diversity. Greater diversity suggests greater redundancy in gene
437 functionality, and genetic adaptability (including horizontal gene transfer). Quorum sensing
438 will also play a role, referring to intra- and inter-species signalling used to synchronise gene
439 expression among bacterial groups to control production of, for example, anti-bacterial
440 substances, disease-causing virulence factors, and immune system suppressors (Belizario and
441 Napolitano 2015). Alcock et al. (2014) suggest that, through mechanisms such as quorum
442 sensing, more abundant microbial species can coordinate their secretions to influence host
443 mood and behaviour, and even manipulate host eating habits to increase their survival.

444 At the landscape-scale, the highly faceted nature of the environment is reflected in the
445 growing availability of diverse large-area environmental mapping datasets. Geographic
446 information systems (GIS) are being increasingly used in epidemiology studies, including the
447 use of spatial association (e.g. proximity analysis) to design surrogate exposure metrics to
448 better understand environmental influences on disease (Nuckols et al. 2004). Environmental
449 proxies to investigate possible relationships between environmental microbiota (and other
450 microscale bioactive agents) and human health could include spatial measures: (a) of relative
451 exposure to particular environmental features or attributes (e.g. via GIS focal statistics
452 calculations of proportions of different classes of vegetation, land cover, land use, or other
453 themes within a predetermined neighbourhood) that might subsequently be related to changes
454 in airborne microbiota; (b) of biodiversity where we might expect to find positive correlations
455 with human health outcomes; and (c) that might mimic ambient exposure to particular MOF
456 (e.g. soil erodibility, soil microbial activity indices) where we may find non-linear (e.g.
457 hormetic or U-shaped) relationships with health outcomes.

458 Spatial environmental mapping data vary from expert-assessed polygon-based
459 thematic mapping, to raster-based remote sensing data (with varying levels of processing and

460 interpretability), and statistically-based spatial predictive modelling/mapping for all manner
461 of environmental attributes. Following the approach of McBratney et al. (2003), predictive
462 mapping of soil microbiota (for example) may be developed using a wide array of
463 environmental variables as potential predictors. Predictors can be chosen to span various soil-
464 influencing themes such as previously measured soil attributes, climate, organisms (including
465 vegetation and land use), topography and terrain attributes, lithology, age, and spatial or
466 geographic position. By extension, we might also consider a wide array of environmental
467 variables as potential predictors, or proxies, for as-yet-undefined potential protective
468 environmental microbiota and non-microbial influences, in a broad-scale environmental
469 correlation analysis with spatially-defined public health outcomes (or ecological
470 epidemiological study). Such correlative studies could potentially involve tens to hundreds of
471 environmental variables—where often many of these variables are correlated. Traditional
472 multivariate approaches such as principal components analysis can deal with correlation in
473 predictor variables, however this may be at the expense of ease of interpretation—for
474 example, when attempting to compare the relative importance of environmental variables
475 (which may have lower effect size) amongst other known public health predictors (e.g.
476 socioeconomic status, lifestyle risk factors, etc.).

477 Contemporary machine learning methods such as the LASSO (least absolute
478 shrinkage and selection operator) penalised regression (Tibshirani 1996) are designed to
479 tackle high dimensional problems with large numbers of (including often correlated) potential
480 explanatory variables, and yield interpretable results. Using LASSO penalised regression
481 modelling in the environmental correlation analysis of Liddicoat et al. (2015) enabled direct
482 interpretation of the relative effect and direction of important environmental predictors from
483 the size and sign of standardised regression coefficients. Using alternative methods such as
484 the LASSO in ecological epidemiological studies may complement traditional multivariate

approaches to highlight key environmental attributes to assist in hypothesis building and establishing priorities for subsequent work. Therefore, the availability of diverse environmental spatial mapping datasets, coupled with natural experiments that influence human health, can provide a wealth of data from which to draw key associations, and thus point the way for subsequent studies to investigate causal links.

## Limitations, confounders, and more detailed work

A complex interplay of factors can influence human health, including known confounders (e.g. socioeconomic status, diet, lifestyle risk factors, exercise, health support services, genetics, age, sex) and environmental influences (table 1). A broad human health-environmental correlation analysis will obviously not restrict findings to environmental microbiota mechanisms. Follow-up work will be needed—in those environments of interest—to test connections (see figure 1) through characterising: environmental features and their related environmental microbiota, human exposures, interactions with human microbiota, immunomodulatory responses, and consequent human health responses. Natural experiments, whereby particular population groups can be found that provide inherent controls for other important confounding factors (e.g. diet, lifestyle, antibiotic use) will assist this subsequent detailed work. Focussing analysis on lower socioeconomic groups, reflecting their stronger association with AACIDs (Rook et al. 2014), or children due to the important role of early immune stimulation (Wopereis et al. 2014), may also assist in the identification of environmental microbiota-mediated health mechanisms.

Individual responses to environmental microbiota are expected to vary due to differences in host commensal microbiota. Studies in mice (Seedorf et al. 2014) investigating the colonization of host microbiota show that established indigenous host microbiotas are

509 resilient to perturbation and resist colonization by foreign microbiota. However they also
510 found in the case of germfree or gnotobiotic (with no or limited known microbiota) mice,
511 with a limited suite of environmental microbiota sources, there are reproducible selective
512 processes that can drive initially disparate host microbiota compositions of separate co-
513 housed animals to converge to similar phylogenetic structures. This included colonization of
514 host gut microbiota by foreign microbes from highly divergent environmental habitats (e.g.
515 soil microbiota). From this we might speculate that the immunomodulatory influence of
516 environmental microbiota could be greatest on individuals with immature or compromised
517 (dysbiotic) commensal microbiota. Voreades et al. (2014) found that short-term diet
518 interventions may transiently alter the gut microbiota composition but long-term diet changes
519 are required to shift to a new steady-state. If we were to extrapolate these results more
520 broadly this could suggest that lasting protective influences of environmental microbiota may
521 depend on long-term exposures. Important temporal factors such as: (a) the timing and
522 duration of exposure to potential beneficial environmental microbiota, (b) seasonal variations
523 in environmental microbiota sources, (c) short-term fluctuations, succession and maturation
524 in host commensal microbiota (Clemente et al. 2012, Wopereis et al. 2014) —would need to
525 be accounted for in any subsequent detailed work.

526 Recognised health drivers may also be correlated with underlying environmental
527 variables. For example, biodiversity and landscape productivity can be drivers of local
528 economic activity which in-turn can drive higher socioeconomic status of communities.
529 Investigating a large number of (including often correlated) environmental variables presents
530 obvious challenges in attempting to identify links between health outcomes and microbiota-
531 associated environmental proxies. Despite this challenge, medical researchers are calling for
532 new approaches that invest ecological knowledge (Rook 2013), and investigate multiple

533 interacting environmental influences, that may potentially act across multiple health
534 outcomes (Myers et al. 2013).

535 In spatial epidemiology there are typically trade-offs between the availability and
536 spatial resolution of health and key contextual data. Often some level of spatial aggregation
537 may occur for privacy or data reliability purposes, and hence environmental parameters will
538 also need to be summarised to match the available area-based health data. In these situations,
539 due to the scale and availability of data, there can be difficulty in separating influences due to
540 spatial variability versus differences in ecological processes. Also, Ruokolainen et al. (2015)
541 found that the spatial scale of land use description affects the ability to detect a significant
542 relationship between land use gradients and allergic disorder; statistically significant
543 relationships were observed at intermediate scales from 2 to 5km. The potential for ecological
544 bias and ecological fallacy (Elliot et al. 2000) also needs to be recognised. When examining
545 possible environmental proxies for MOF via spatial mapping, these will at best represent
546 potential exposure (not dose). Mapping data for environmental variables is often extrapolated
547 from limited field-truthed sites to provide exhaustive spatial coverages. Such data often carry
548 uncertainty that is unquantified, but may represent the best available knowledge. These
549 limitations need to be borne in mind, but should not be seen as roadblocks for the purpose of
550 hypothesis building and pointing to areas where more detailed research is required.

551 A sequence of progressively detailed studies is envisaged (in the context of potential
552 links in figure 1). As outlined here, we recommend that broad-scale environmental
553 correlation analyses be examined to firstly identify particular environments and health
554 outcome scenarios of interest. Where possible, this may take advantage of existing
555 environmental and public health datasets. In areas of interest, prospective epidemiological
556 cohort studies are then recommended when possible, to further establish possible associations
557 between environmental exposures and possible protective health outcomes. Studies will need

to account for recognised confounders (e.g. demographics, diet, social indicators, lifestyle risk factors such as smoking status, environmental pollution), temporal factors including the timing, duration and seasonality of environmental exposures; and incorporate immune biomarkers to track asymptomatic (or subclinical) exposures. As discussed earlier, a focus on children (reflecting the importance of early immune system development) and/or lower socioeconomic groups (reflecting a stronger association with AACIDs) may also assist in the identification of environmental microbiota-health mechanisms.

More detailed ecological epidemiological studies based on environmental proxies may lead to hypotheses that can subsequently be tested with experimental studies on animal models. This has been demonstrated elsewhere, for example Hanski et al. (2012) reported a special role for the gammaprotebacterial genus *Acinetobacter* in enhancing immunotolerance and in increasing the expression of anti-inflammatory cytokine IL-10. Subsequently Fyhrquist et al. (2014) reported strong support for this hypothesis with a mouse model. Further research to understand relationships between potentially beneficial environmental microbiota and corresponding recognizable environmental features (e.g. plant species, soil types, etc) will also benefit subsequent implementation of public health policy. For example, in translating new knowledge of protective environmental microbiota-mediated mechanisms into new urban green space design.

## Conclusion

Microbes and other microscale bioactive agents provide a real biological connection to our surrounding environment and represent an understudied influence on human health. Emerging evidence suggests that microbial Old Friends and/or diverse environmental microbiota supplement human microbiota and may provide protective background

immunomodulatory stimuli; while their absence may play a role in dysbiosis, immune dysregulation and disease. To progress understanding we advocate the use of environmental proxies as a pragmatic investigation tool. In this, we suggest that soils have been underrepresented in studies to-date, as a source of environmental microbial diversity with potential for a protective role in microbiota-mediated human health. Similarly, the influence of different types of vegetation, land cover and land use (among other themes) also remain largely untested. We suggest that comprehensive environmental correlation analyses examining recognisable environmental attributes and allergic, auto-immune and chronic inflammatory diseases (as well as other dysbiosis-associated diseases) could help build understanding, and provide greater focus for subsequent detailed studies of potential underlying microbiota-mediated mechanisms. In this way we can progress the 'eating of the elephant'—we provide a first step. The timing and duration of environmental microbiota exposures also require consideration, with respect to the establishment, maturation and long-term stability of the human commensal microbiota. Knowledge gaps regarding potential sources of microbial Old Friends and their relationship with recognisable features in the environment need to be addressed, for example, to prescribe new urban design (green space) health treatments. In short, it remains to be demonstrated convincingly that landscape-scale environmental influences can affect our human microbiota and health. However, the public health implications of such a connection, warrant further research into this area. Such work will ultimately inform concurrent improvements in environmental stewardship, biodiversity conservation and human health.

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608 ***Table 1. Broad mechanisms of environmental, and environmental-change, impacts on***

609 ***human health (WHO and SCBD 2015, and references therein).***

| |
|---|
| (1) Increased exposure to anthropogenic hazards and environmental pollution |
| (2) Increased exposure to natural hazards, including:<br>▪ Harmful biotic agents: emerging infectious disease arising from land use change, encroachment, biodiversity loss and altered human-animal-environment dynamics<br>▪ Physical hazards: due to reduced buffering from extreme weather and other natural disasters |
| (3) Declining food security and nutritional deficiency |
| (4) Lifestyle: health benefits from exercise and sunlight influenced by surrounding natural environments |
| (5) Mental health, social and cultural wellbeing: linked to natural surroundings and sense of place |
| (6) Global change: including climate change, globalisation and conflicts over depleting natural resources |
| (7) Biomedicines: loss of biodiversity-related potential new pharmaceuticals and traditional biomedicines |
| (8) Reduced contact with protective environmental microbial diversity |

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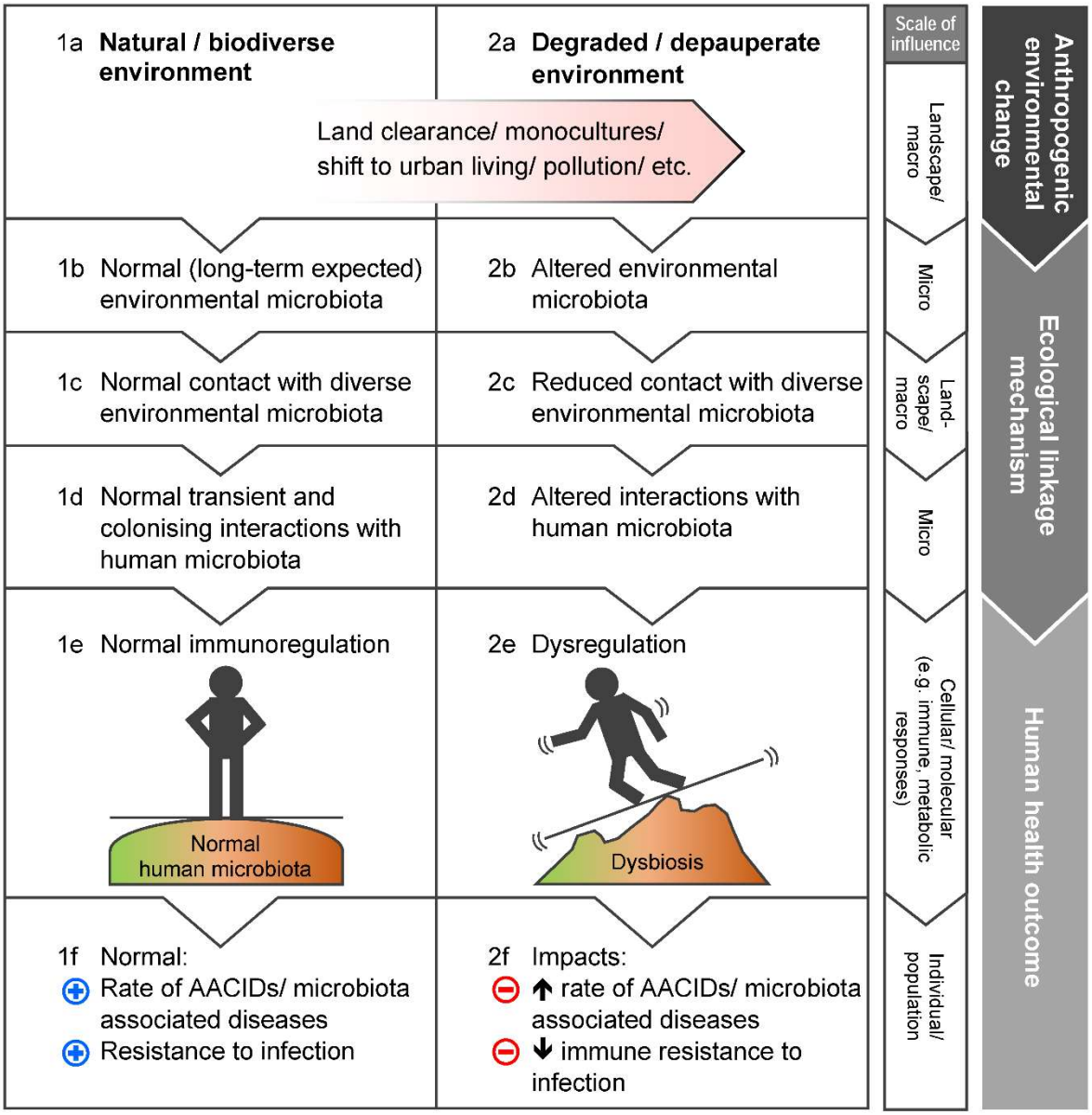


612

613 ***Figure 1. Theoretical multi-scale links between environmental change, protective***
614 ***environmental microbiota and human health. Environmental degradation (1a > 2a) alters***
615 ***feedstocks and microbial habitats (e.g. phyllosphere, rhizosphere, soil, animals), altering***
616 ***microbial ecosystem dynamics and hence the composition of environmental microbiota***
617 ***(2b). Consequently, exposure to environmental microbiota (2c) will differ from more***
618 ***natural and biodiverse surroundings (1c). Long-term expected (immunologically normal)***
619 ***interactions with human microbiota (1d) are therefore lacking in degraded environments***
620 ***(2d), and contribute to immune dysregulation (2e), autoimmune, allergic, chronic***

***inflammatory diseases (AACIDs) and other microbiota associated diseases (2f). Environmental supplements to human microbiota are indicated in green shading; normally these form part of a balanced human microbiota (1e), but are deficient or imbalanced in the case of dysbiosis (2e). Temporal effects (e.g. timing and duration of environmental exposures) and other potential drivers of human microbiota (e.g. diet, genetics, age) will also be important but for simplicity are not included in this diagram.***

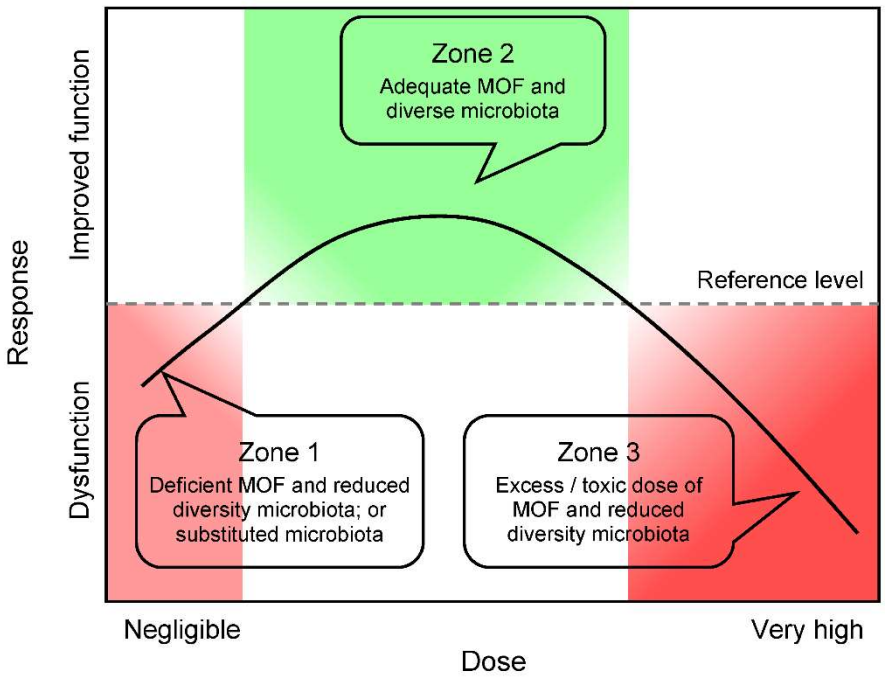


***Figure 2. Theoretical dose-response curve for a generic microbial Old Friend (MOF), with inferred ecological context (as discussed in main text). Immune dysregulation and disease is associated with absence of MOF (zone 1). At some low to moderate dose, appropriate immune stimulation is provided to establish and maintain immunoregulatory circuits (zone 2). Pathogenic effects would be expected at increasingly high doses (zone 3). This theoretical curve follows a generalizable hormetic response proposed by Calabrese et al. (2007), except for a shift in the reference level that creates three response zones instead of two.***

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