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Review

Surveying Gut Microbiome Research in Africans: Toward Improved Diversity and Representation

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Descriptive and translational investigations into the human gut microbiome (GM) are rapidly expanding; however, studies are largely restricted to industrialized populations in the USA and Europe. Little is known about microbial variability and its implications for health and disease in other parts of the world. Populations in Africa are particularly underrepresented. What limited research has been performed has focused on a few subject domains, including the impact of long-term lifestyle and dietary factors on GM ecology, its maturation during infancy, and the interrelationships between the microbiome, infectious disease, and undernutrition. Recently, international consortia have laid the groundwork for large-scale genomics and microbiome studies on the continent, with a particular interest in the epidemiologic transition to noncommunicable disease. Here, we survey the current landscape of GM scholarship in Africa and propose actionable recommendations to improve research capacity and output.

Background about Worldwide Gut Microbiome Research

With thousands of manuscripts published yearly on the topic, investigations into the human **gut microbiome** (**GM**) (see Glossary) have reached an inflection point. Research is beginning to leverage an expanding foundational knowledge base to inform novel interventions for disease treatment and prevention [1]. As the field transitions to translational applications, it is important to evaluate the generalizability of these findings. To date, most microbiome and genomics research has focused on human populations in Europe and the USA (often termed '**western**'); more recently, studies are emerging from a few additional countries with similar profiles of lifestyle and industrialization, including Israel, Japan, and China [2–4]. In these studies, lifestyle, diet, and environmental exposures have been shown to strongly affect GM composition. Little is known about the GM in nonwestern and other groups historically understudied in institutional research. Populations in South America, Africa, and regions in Asia, such as India, have been scarcely studied despite genetic, ethnic, sociocultural, lifestyle, and dietary diversity. As a result, we are left with significant biases and an incomplete understanding of microbiome variability around the world.

Over the past century, Africa has undergone a rapid period of industrialization, globalization, and economic expansion. Concomitantly, disease epidemiology, urban settlement, and population demographics have changed profoundly [5–7]. The adoption of western lifestyle and dietary practices has precipitated a rising burden of noncommunicable diseases (NCDs), such as obesity, cancer, cardiovascular disease, and type 2 diabetes. That said, the pace of transition has been heterogeneous, and most populations lie on a continuum of westernization, varying by country and along the urban–rural divide [5,6]. Undernutrition, poor sanitation, food insecurity, and communicable diseases remain formidable causes of morbidity and mortality.

Highlights

Most human GM research has concentrated on westernized societies. Whether these findings can be generalized to African populations is poorly understood.

Comparative studies between western individuals and hunter-gatherer and agriculturalist communities in Africa have revealed distinct taxonomic signatures based on lifestyle and dietary factors.

Microbiome alterations are associated with and may underlie malnutrition-associated pathologies. Contemporary nutrient replacement strategies have failed to durably correct these changes, limiting their therapeutic efficacy.

Infectious diseases have been linked to disruptions in microbial ecology. Colonization with parasites may be an important determinant of microbiome structure.

Representing diverse populations must be prioritized in future microbiome studies to capture the full ensemble of microbial diversity worldwide.

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Several genomic and bioinformatic initiatives have formed to better understand Africa's unique and multiform evolutionary and epidemiologic trajectories, beginning with the HapMap Project in 2002 [8]. Today, Human Heredity and Health and Africa (H3Africa) and other consortia are harnessing genomic technologies toward improving health outcomes [9]. Building on the success of these organizations, the GM is now emerging as a subject of substantial scientific inquiry in Africa. Here, we review the landscape of GM scholarship in Africa to date, emphasizing contributions to the field and outstanding knowledge gaps. Additionally, we propose actionable recommendations to overcome limitations that have slowed advances in GM research.

Insights from Agriculturalists and Hunter-Gatherers: The Effects of Western Lifestyles and Diet

Today, several remote African **agriculturalist** and **hunter-gatherer** societies practice subsistence strategies that resemble those dating back to before the Neolithic era, more than 10 000 years ago. Juxtaposing these traditional African groups with western populations has informed how changes in dietary and environmental factors accompanying industrialization can profoundly impact the GM (Figure 1).

It has been well established that long-term diet influences the taxonomic and functional signature of the microbiome. A consistent pattern is the inverse relationship between two members of the Bacteroidetes phylum, *Prevotella* and *Bacteroides*. Whereas *Prevotella* associates with a diet rich in plant-based foods found predominately in nonindustrialized populations, a higher intake of animal fats and proteins, characteristic of industrialized populations, correlates with increased relative abundance of *Bacteroides* [10–14]. Hunter-gatherer communities, such as the Hadza of Tanzania and the BaAka of the Central African Republic, consume diverse simple and complex

Subsistence strategy	Hunter-gatherer		Agriculturalist		Industrialized	
Lifestyle/Dietary characteristics	Settlement, Tubers, Wild berries, Wild animals		Settlement, Antibiotics, Vegetables, Legumes, Cereals, Fruits		Cities, Antibiotics, Sugary drinks, Animal fat and protein, Prepackaged foods	
Enriched taxa	*Prevotella* *Succinivibrio* *Treponema* *Eubacterium*	Proteobacteria Spirochaetes Clostridiaceae	*Prevotella* *Succinivibrio* *Treponema* *Eubacterium*	*Ruminococcus* Firmicutes Clostridiaceae	*Bacteroides* *Bifidobacterium* *Blautia*	*Ruminococcus* Firmicutes
Functional features	• Complex carbohydrate metabolism • Propionate SCFA production • Aromatic amino acid biosynthesis • Vitamin and co-factor synthesis		• Complex carbohydrate metabolism • Propionate SCFA production • Xenobiotic metabolism		• Simple sugar metabolism • Lipid and fat metabolism • Xenobiotic metabolism • Branched chain amino acid metabolism	
Relative alpha diversity	High		High		Low	

Trends in Microbiology

Figure 1. Gut Microbiome Features across Host Subsistence Strategies. Lifestyle and dietary factors exert a profound effect on gut microbiome configuration. Hunter-gatherer populations, who rely on foraging and have little exposure to therapeutic agents, are highly enriched in *Prevotella* and other fiber-degrading taxa. Conversely, the dietary profile and sanitation infrastructure found in westernized societies is associated with *Bacteroides* dominance and a concomitant decrease in microbial diversity. Traditional agriculturalists occupy a putative intermediate state, sharing features of both western and hunter-gatherer communities. Abbreviation: SCFA, short-chain fatty acid.

carbohydrates in the form of wild tubers, roots, and berries. For the Hadza and BaAka, a foraging lifestyle is associated with an enrichment in *Prevotella,* as well as *Treponema*, and *Succinivibrio*, all of which have fiber-degrading activity and may be involved in starch, hemicellulose, and xylan degradation [11,12,15–17]. Metagenomic analyses in the Hadza further reveal a high representation of genes related to broad-spectrum carbohydrate and amino acid utilization [15,18]. Notably, increased abundance of fibrinolytic lineages may be characteristic of an ancestral GM state, as observed by previous studies of coprolite samples from ancient civilizations [19]. Taxonomic and dietary parallels make it tempting to use extant hunter-gatherer populations as a direct proxy for the gut ecosystem of our ancestors. However, it should be noted that we are limited in our ability to confidently assess the appropriateness of this proxy without direct characterization of past GM composition. Furthermore, modern traditional societies have likely been exposed to classically western microbes and xenobiotics through contact with researchers.

Agriculturalist societies, marked by subsistence farming and animal domestication, share microbial features with hunter-gatherer communities. Populations from rural Burkina Faso and the Bantu community in the Central African Republic follow a vegetarian diet comprised of grains, vegetables, and legumes. Accordingly, taxa capable of metabolizing plant polysaccharides and fibers, including *Prevotella* and *Treponema*, dominate the gut, though at lower abundances than those in hunter-gatherers [10,12]. While the GM profiles of agriculturalist individuals more closely resemble the GM of hunter-gatherers, some features are shared with western populations – such as bacterial taxa and gene pathways associated with digestible sugars [12,16]. Robust evidence links the typical western diet – high in simple sugars and animal fats and proteins – to shifts in GM ecology. *Bacteroides* spp. emerge as the most prominent bacteria in western populations, with lower levels of fibrinolytic species, such as *Prevotella* and *Treponema* [10,12,13,17] (Box 1). This is consistent with increased access to refined sugars and animal fats and proteins. Additionally, an enrichment in genes involved in amino acid and bile salt metabolism has been described in individuals living in the USA who consume a typical western diet [20].

In addition to diet, changes in sanitation and hygiene practices have placed new selective pressures on the gut microbiota of industrialized populations. Microbiomes of western and even some agriculturalist communities are enriched in xenobiotic-processing genes, potentially due to therapeutic medication and food additive exposure [12,16]. The disruptive effects of antibiotics on the GM are well-established [24,25]. In studies of children in Burkina Faso and Nigeria, oral administration of azithromycin significantly decreased GM **alpha-diversity** and altered taxonomic composition [26,27]. Antibiotic exposure via food and medicine may increase the reservoir of antibiotic-resistance genes (ARGs) in the GM, collectively known as the resistome. However, ARGs exist not only in the metagenomes of industrialized populations, but also in hunter-gatherer and other communities with little exposure to pharmacological agents [15]. Their resistome contains genes resembling those that naturally occur in soil, suggesting an alternative, environmentally-based model of ARG acquisition. Microbial transmission secondary to interactions with

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Box 1. The Influence of Short-chain Fatty Acids on Chronic Inflammatory Diseases

The higher intake of plant-derived carbohydrates by traditional populations compared with industrialized populations correlates positively with the synthesis of short-chain fatty acids (SCFAs) [20]. SCFAs are metabolites derived from the fermentation of soluble fibers and serve as an energy source for gut epithelial cells [21]. Low SCFA levels from insufficient dietary fiber consumption may predispose individuals to chronic inflammatory diseases. In a diet-switch study, African-Americans who consumed a low-fat, high-fiber South African-style diet exhibited increased GM saccharolytic fermentation and SCFA production as well as reduced markers of inflammation [22]. SCFAs are thought to protect against colorectal cancer and may partially explain the lower incidence of the disease in South Africa compared with the USA, although it should be noted that colorectal cancer screening strategies differ substantially in these two countries and diagnosis bias may contribute to the observed increased incidence of colorectal cancer in the USA [23].

Glossary

Agriculturalist: the primary subsistence strategy of the Neolithic age (~10 000 years ago). In contrast to hunter-gatherers, agriculturalists have stable settlements, and practice food-crop and animal domestication.

Alpha-diversity: a measure of the number of different species, or richness, within a microbial community.

Beta-diversity: a measure of the difference in microbial composition between samples.

Dysbiosis: a breakdown in the balance between putative species of 'protective' vs 'harmful' intestinal microorganisms.

Gut microbiome (GM): the genomes (genetic material [DNA or RNA]) of the ensemble of microorganisms – including eukaryotes, archaea, bacteria, and viruses, residing in the intestinal tract.

Hunter-gatherer: a person living in a society whose primary subsistence strategy involves. Hunting and gathering was dominant amongst early modern humans prior to the advent of agriculture (>10 000 years ago).

Metagenomics: the study of a collection of genetic material (genomes) from a mixed ecological community of organisms.

Ready-to-use therapeutic food (RUTF): a nutrient- and energy-dense peanut-based food indicated as a therapeutic food for severe malnutrition.

Severe acute malnutrition (SAM): is defined by a weight-for-height below three standard deviations from the World Health Organization (WHO) Standards median, visible severe wasting, or the presence of bilateral pitting edema.

Stunting: refers to a child with a length-for-age below two standard deviations from the WHO Standards median.

Westernization: the process by which a society adopts the customs and practices typical of Western European and North American culture. 'Western' populations are generally characterized by a more sedentary lifestyle, decreased exposure to microbes and pathogens, and a diet rich in energy-dense foods and animal fat and protein.

16S rRNA sequencing: 16S ribosomal RNA/DNA of prokaryotes is amplified via PCR and sequenced to identify the taxonomic classification of bacteria and archaea.

> **Box 2. Lifestyle and Dietary Factors Override Host Genetics in Determining GM Composition**
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> Mounting evidence indicates that lifestyle exerts a much stronger effect on microbiome composition than genetics and ethnicity [2]. This was initially reported by Yatsunenko et al. who analyzed hunter-gatherer, agriculturalist, and industrial populations from rural Venezuela, Malawi, and the USA [20]. Principal coordinate analysis showed a clear segregation of the populations based on lifestyle with little relationship to genetic identity. Reciprocally, individuals from a similar genetic background can diverge in GM composition if subjected to different environmental exposures. In a study of 2- to 8-year-old children in Burkina Faso of the same ethnicity, but living in rural, suburban, or urban settings, the microbial communities of urban subjects differed in accordance with the metabolic and dietary needs associated with industrialization [13].

individuals who have been exposed to antibiotics may also contribute to the resistome of hunter-gatherers.

At the community level, microbial diversity differs between populations with distinct lifestyle practices. Hunter-gatherer and agriculturalist cohorts have consistently higher GM alpha-diversity than western populations, contributing to the hypothesis that GM diversity declines with industrialization [10,12–14,16,20]. This trend is attributed to differences in dietary habits and sanitation standards. Colonization with parasites, which have been largely eradicated in western countries, may also promote GM diversity [28]. Interestingly, alpha-diversity tends to be inversely related to **beta-diversity** [10–13,16,20]. Traditional societies may have greater contact with their natural environment as well as with other village- and family-members and thus may have comparatively fewer barriers to microbial transmission and dispersal. Consequently, population-level GM composition tends to converge while local host diversity increases [14,29]. Collectively, descriptions of traditional African groups have revealed shifts in GM structure and function, driven primarily by diet and lifestyle, along the axis of westernization, (Box 2).

Early Microbial Colonization and Maturation

Differences in GM composition can be observed well before adult life between traditional and industrialized populations. In the immediate postparturition time period, the microbial consortium undergoes a complex succession of growth and maturation [30,31]. It is influenced by exposures, including mode of delivery, the maternal gut and skin microbiome, and feeding practices. The process of GM maturation may carry important health implications. Aberrant or inadequate colonization has been associated with an increased susceptibility to immune- and inflammatory-related diseases as well as adverse metabolic outcomes that disproportionately burden industrialized countries [32]. Moreover, differences in GM composition between western and 'non-western' infants may be partially responsible for divergences in vaccine responsiveness (Box 3). Toward this end, investigations into the temporal and microbial features of the GM in African infants may help to elucidate risk factors related to these outcomes.

Delivery mode and setting are among the earliest external factors that shape the nascent GM [38–40]. Brazier et al. noted significant differences in taxonomic abundance in African neonates based on birth mode. Bacteroides and Collinsella were enriched with vaginal birth, while Sarcina and Klebsiella were associated with Cesarean section [41]. Sanitary conditions in the delivery

> **Box 3. Clinically-Relevant Phenotypes May Be Shaped by GM Ecology**
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> There is evidence that differences in infant GM composition have important clinical implications. For example, differences in the early microbiota can impact the efficacy of early and important clinical interventions, such as vaccines. Children in low-income countries in Africa generally have poor responsiveness to the oral rotavirus vaccines (RVV), with rates as low as 48–64% [33,34]. RVV response in Europe and other industrialized populations reaches as high as 98% [35,36]. Microbiome composition has been hypothesized to play a role in this discrepancy: in a study of 6-week old infants from Ghana and the Netherlands, overall taxonomic composition significantly differed between RVV responders and nonresponders [37]. Taken together, these data suggest that prospective clinical trials will be necessary to determine whether the GM can explain or drive variability in vaccine efficacy.

environment can also influence the infant GM. A hospital-based study in Nigeria found that greater than 90% of newborns had GM colonization with principally facultative anaerobes on the first day of life [42]. European neonates appear to have a lower rate of first-day GM colonization , perhaps due to differences in hospital hygiene practices [43,44].

In the weeks and months after parturition, feeding practices largely mediate GM dynamics in developing infants [40,45]. The gut becomes increasingly diversified after birth and, after a period of dominance by facultative anaerobic or aerobic lineages, transitions to predominately strictly anaerobic bacteria in the first 6 to 9 months of life. Among the most prevalent genera is *Bifidobacterium*, which can be transferred in breast milk [46]. Bifidobacteria modulate education of the immune system and govern early gut community structure [47]. The relative proportions of high-abundance taxa differ between African and western guts. Malawian infants had higher levels of *Bifidobacterium*, *Clostridium histolyticum*, and the *Bacteroides–Prevotella* group than did Finnish infants at 6 months of age [48]. Additionally, *Lactobacillus* species, which are also transmitted from mother to infant, were more abundant and diverse in the Malawian cohort [49]. Other studies have identified functional signatures in Malawian and nonindustrialized neonates, namely an enrichment in host glycan metabolism and urease genes [20]. This is primarily explained by differences in breast milk oligosaccharide composition and the earlier introduction to complementary feeding (solid foods) in traditional groups [13]. Interestingly, extensive environmental contact nullifies the classical progression of GM maturation in hunter-gatherer infants [14]. In a study of Bassa infants, *Bifidobacterium* was largely absent, and GM stability and diversity profiles resembled those of adults. The authors speculated that GM assembly can be altered or shortened through dispersal of microbes amongst community members.

The weaning process marks the next major milestone in GM development. Around 1–2 years of age, the presence of Bacteroidetes and Firmicutes increases as the microbiome further diversifies [48,49]. The transition to a varied solid-food diet coincides with the divergence in microbial ecology based on subsistence strategy. De Filipo *et al*. described similar overall community structure and Bifidobacterium profiles in breast-fed children from Burkina Faso and Italy [10]. A distinct separation into geographical groupings occurred after weaning, underscoring the dominance of dietary specialization in shaping the young GM. Dietary influences persist through 18–36 months, when the GM stabilizes and approaches the adult state with respect to taxonomic composition and diversity [50,51]. Ultimately, patterns of GM maturation in African relative to non-African infants may be an important source of variation that drives differences in host phenotypes later in life.

The Gut Microbiome and Nutritional Status

Clearly, lifestyle and diet contribute substantially to GM development and composition. One of the major health issues faced in low- and middle-income settings is undernutrition. Chronic deficiencies in carbohydrate, fat, protein, and/or micronutrient intake are a leading cause of under-five mortality, primarily affecting low- and middle-income countries [52]. The World Health Organization (WHO) estimates that sub-Saharan Africa accounts for nearly 40% of cases globally[j].

Severe acute malnutrition (SAM) is the most extreme form of undernutrition. Just as SAM can drive significant alterations in GM, the GM can contribute to, or exacerbate, SAM by impeding the ability to extract dietary nutrients [53]. Furthermore, numerous reports link the GM as a causative agent of SAM. This was first revealed in a seminal transplantation study, wherein gnotobiotic mice receiving fecal samples from Malawian children with kwashiorkor, a SAM subtype, reproduced the weight-loss phenotype [54]. This supported the hypothesis that the GM is sufficient to induce substantial weight loss even when controlling for oral dietary intake. Microbial communities from malnourished hosts have lowered alpha-diversity levels and are taxonomically immature

compared with healthy controls, reflecting a disruption in normal GM assembly. Malnutrition also impacts the viral component of the GM, otherwise known as the virome. Studying the same Malawian cohort, Reyes *et al*. showed that SAM significantly perturbs the normal program of virome development [55].

SAM is also associated with a dramatic loss of obligate anaerobes and oxygen-sensitive prokaryotes, such as *Methanobacter*, in conjunction with overgrowth of aerobic taxa and increased redox potential [56,57]. The enriched aerobic fraction includes numerous enteropathogenic organisms, most notably Enterobacteriaceae and *Enterococcus*. Overall, it is unclear whether this enrichment for Enterobacteriaceae and *Enterococcus* is sufficient to induce adverse clinical phenotypes. On the one hand, transplantation experiments implicate this pathogenic consortium in causing increased diarrhea, enteric inflammation, sepsis, and gut mucosal barrier permeability [58]. However, on the other hand, the presence of intestinal pathogens did not correlate with either enteropathy or death in a study of hospitalized malnourished Malawian children [59]. The leading hypothesis suggests that the loss of protective factors conferred by a predominantly anaerobic GM rather than pathogen overgrowth underlies SAM pathophysiology.

Stunting, the most common manifestation of undernutrition worldwide, also correlates with GM **dysbiosis**. In an investigation of stunted infants in the Central African Republic and Madagascar, the GM harbored an overrepresentation of taxa that normally reside in the oropharyngeal cavity [60]. The authors propose that bacterial translocation to lower GI regions – or 'decompartmentalization' – could contribute to linear growth deficits and enteropathy.

Given the complex interplay with the microbiome, effectively addressing malnutrition demands an intervention that resolves both nutritional deficiencies and alterations in GM composition. **Ready-to-use therapeutic food** (**RUTF**), a calorie-dense peanut mixture, is the current international standard for SAM therapy[ii]. While short-term RUTF administration has dramatically reduced malnutrition-associated morality, high rates of relapse and comorbidities, from stunting to immune dysfunction, have limited its efficacy [61]. Disappointing findings have been partially attributed to interactions with the GM. In Malawian co-twins discordant for kwashiorkor, RUTF yielded temporary improvements in microbial composition, namely enrichments in Bifidobacteria and *Lactobacillus*, and amino acid and carbohydrate processing, but the effect rapidly diminishes after treatment cessation [54]. Additionally, it fails to repair aberrations in the proper assembly of the gut virome [55].

Other nutrient replacement strategies have caused similar concerns. Oral iron supplementation or fortification through micronutrient powders (MNPs) are effective for iron-deficiency anemia, but early high-dose formulations led to increased hospitalizations, deaths, and diarrhea [62,63]. A putative mechanism lies in the GM, given the increased colonic iron secondary to poor fractional absorption in the duodenum. Many gut bacteria rely on iron as a key, growth-limiting factor [64]. Thus, high iron levels in the colon favor colonization by, and virulence of, enteric Gram-negative pathogens while exerting little effect on Bifidobacteria, *Lactobacillus*, and other beneficial commensal microbes. Unsurprisingly, iron fortification and supplementation has been found to produce adverse shifts in GM ecology [65,66]. The guts of Kenyan infants receiving iron-fortified maize porridge exhibited acute gut inflammation, an increased and decreased prevalence of Enterobacteria and *Bifidobacterium*, respectively, as well as an enrichment in enteropathogens [65]. Results have been mixed, however, raising the possibility that environmental factors play a role in observed outcomes. In a trial of high-dose iron supplementation, South African children, who have comparatively better sanitation and hygiene standards, were spared dysbiosis and gut inflammation [39]. Another complicating factor is antibiotic coadministration. Compared with those without fortification, oral broad-spectrum antibiotic use in conjunction with iron-

containing MNPs in Kenyan infants resulted in elevated pathogen carriage and decreased abundances of commensal *Bifidobacterium*, suggesting that iron modifies therapeutic efficacy [67]. As novel nutritional replacement strategies emerge (Box 4), potential solutions are compelled to navigate the complex interrelationship between malnutrition and the microbiome.

Microbial Dynamics in Infectious Disease

Despite enormous strides in public health infrastructure, communicable diseases – mainly malaria, tuberculosis, HIV/AIDS, and respiratory and diarrheal pathologies – remain the foremost cause of mortality in Africa[iii]. Disease risk is, in part, governed by the GM, which modulates the mucosal immune system and is thought to confer resistance to pathogen colonization [76]. The high infectious disease burden makes Africa an appropriate setting to better understand these dynamics.

HIV/AIDS, in particular, is a major public health concern, with Africa accounting for more than 70% of the global burden of infection [77]. The relationship of HIV infection to the GM, namely dysregulation of the host immune–epithelial interface, is a subject of active inquiry. The virus leads to disruption of key mucosal lymphoid compartments, including T helper (Th)17 and dendritic cells [78]. Disrupting the intestinal barrier can permit microbial translocation into the bloodstream and lead to chronic systemic inflammation and GM dysbiosis. Low CD4 counts in HIV$^+$ patients in Uganda correlated with an expansion in adenovirus populations in the GM [79]. Furthermore, bacterial communities in HIV$^+$ compared with HIV$^-$ individuals exhibited reduced alpha-diversity and increases in Enterobacteraceiae, which may contribute to the enteropathy, diarrhea, and related inflammatory comorbidities associated with disease progression. Many millions of HIV$^+$ patients receive long-term antiretroviral therapy (ART). Although effective, ART carries significant side-effects, and is known to have a potent *in vitro* antibacterial effect [80]. How these life-saving medications interact with the GM is poorly understood and is in need of urgent research [81].

Moderate-to-severe diarrhea (MSD) is another major public health problem, particularly amongst children under the age of five [82]. This enteritis has been linked to large-scale GM alterations, from loss of alpha-diversity to reduced microbial community stability [83,84]. Additionally, there is an inverse relationship in the proportions of obligate anaerobes and facultative anaerobes, many of which cause diarrhea. In a report on children from countries in West Africa, East Africa, and South Asia, Pop *et al*. identified an enrichment of not only known enteropathogens in MSD relative to controls, such as *Shigella* and *Campylobacter jejuni*, but also several novel pathogenic lineages, including *Streptococcus* and *Granulicatella* species [84].

Much like bacterial and viral components, parasites – including protozoa and helminths – have complex transkingdom interactions within the GM. On one hand, they exert pathogenic effects

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Box 4. 'Rehabilitating' Microbial Communities with GM-sensitive Nutritional Replacement Strategies

The lack of efficacy of contemporary nutrient supplementation strategies has made way for efforts to explore alternative, GM-sensitive methods to treat malnutrition and associated pathologies [68–70]. For example, lipid-based nutrient supplementation comprises essential macro- and micronutrients in edible fats. Although their impact on undernourishment and linear growth are not clear, evidence indicates that lipid-based supplementation does not negatively affect GM composition or development [68,71]. There is also emerging interest in microbiome-based therapies. A metagenomic and culturomic study of malnourished and healthy children from Senegal and Niger identified a distinct microbial signature of 12 bacterial species in healthy samples that included *Alistipes indistinctus*, *Anaerostipes caccae*, and *Bacillus licheniformis* [72]. Prebiotics or probiotics designed with selected taxa could restore normal GM configuration and prevent long-term complications related to enteropathogen colonization [73,74]. That said, early findings from a controlled interventional trial in Malawi have been inconclusive [75]. While further work is needed to validate their efficacy and safety, targeted microbiome interventions may help to overcome the shortcomings of current approaches.

that are partially mediated through the gut [85,86]. Colonization with *Giardia duodenalis*, for example, significantly alters microbial community structure and supports the expansion of facultative anaerobic and potentially pathogenic taxa [85]. Meanwhile, GM composition may influence malaria disease progression and outcome, such as the predisposition to infection with *Plasmodium falciparum* [87]. Specifically, enrichments in *Lactobacillus, Bifidobacterium,* and *Streptococcus* were associated with reduced *P. falciparum* infection in a Malian cohort. Only recently have resident eukaryotic microbes been appreciated for their potentially beneficial role in GM ecology. The presence of *Entamoeba* is associated with increased alpha-diversity and favorable shifts in taxonomic composition in studies of various rural African populations [28]. Nonwestern groups also exhibit a high prevalence of *Blastocystis,* but its clinical significance and relationship to the gut ecosystem are unresolved [88]. According to the 'old friends' hypothesis, parasites belonged to an ancient GM consortium, exerting important immunoregulatory functions [89]. The loss of persistent colonization connected with modern lifestyle and sanitation practices may have precipitated decreased bacterial diversity and the rising prevalence of allergic and other inflammatory disorders in industrialized populations.

Concluding Remarks

Research in African populations has deepened our understanding of GM ecology by leveraging the unique make-up of sociocultural, demographic, and epidemiologic features that cannot be captured in other geographies. Rural African hunter-gatherers and agriculturalists have provided a window into the relative influence of subsistence strategy and industrialization. These factors begin to shape GM composition as early as infancy, leading to differences in microbial development and maturation. Moreover, we have come to appreciate the complex interplay between the GM and infectious disease and malnutrition, both of which are pervasive public health issues on the continent.

Despite major progress, there are still gaps in our knowledge of GM characteristics in African populations (see Outstanding Questions). The current research landscape has not kept pace with the contemporary scientific and public health needs on the continent. Comparative studies have focused on the 'extremes' of lifestyle characteristics, most commonly rural traditional and industrialized subsistence modes in African and western countries, respectively. The relative dearth of studies invites the tendency to over-apply the findings from one subgroup, such as hunter-gatherers and agriculturalists, which actually constitute a small proportion of the population, to the entire African continent. This also comes at the expense of understanding populations that have transitioned or are transitioning to western lifestyles. Relatedly, little attention has been given to the epidemiological shift from communicable, maternal, neonatal, and nutritional pathologies to NCDs [5,6]. The WHO estimates that NCDs will become the leading causes of morbidity and mortality in Africa by 2050. Yet, we found little scholarship – and no large-scale studies – that examine the relationship between the GM and most prevalent NCDs, namely cardiovascular and respiratory disease, cancer, and type II diabetes [90].

Limitations in the current microbiome literature on Africans are compounded by constraints in sequencing methodology. To date, most investigations have used **16S ribosomal RNA gene sequencing.** While cost-effective and valuable for quantitative descriptions of microbial composition, this approach is constrained to bacterial and archaeal classification and is fraught with issues of amplification bias [91]. Shotgun metagenomic sequencing affords more comprehensive taxonomic characterization as well as enhanced prediction of gene and pathway content. However, the associated technical and computational costs have prevented its widespread adoption on a global scale. Consequently, our present understanding likely dramatically underestimates GM diversity in African populations, restricting our ability to understand and act on key scientific and medical issues.

We urgently need greater investment in GM research in Africa, but proposed solutions must be prepared to reckon with systemic and multifaceted barriers, not the least of which include: resource and financial constraints; challenges related to funding and government regulation; and limited participation of local scientists. These and other obstacles are the targets of several international organizations, most notably H3Africa, that seek to improve the ecosystem for modern health-related research [9]. Funded by the US National Institutes of Health, the Africa Academy of Sciences, and the Wellcome Trust, H3Africa supports large-scale genomic and epidemiological studies of specific relevance to Africa. Additionally, it advances capacity-building measures focused on developing local scientific infrastructure and educational programming. The strategies espoused by H3Africa and related efforts are being directly and indirectly harnessed to augment GM scholarship. In this setting, we offer the following recommendations on areas of high priority.

Build Diverse Reference Databases
Accurate taxonomic and functional characterization of GM samples relies on comprehensive reference databases. Compared with traditional culture-based methods, culture-independent **metagenomic** approaches – such as the assembly of genomes from metagenomes – have dramatically expanded the catalog of identifiable organisms. Reference databases derived from these methods are biased toward well-profiled western populations. As a result, a large fraction of the microbial composition in understudied groups has been unexplored. Recently, Pasolli et al. reconstructed 154 723 microbial genomes and 4930 species-level genome bins from metagenomes of various ages, lifestyles, and geographies, including Madagascar and Ethiopia [92]. A comparable meta-analysis was performed by Nayfach et al. who recovered more than 60 000 genomes corresponding to 2058 novel species [93]. These newly described genomes particularly avail the taxonomic classification of non-western populations, dramatically improving the mapability of metagenomic reads. Continuing to incorporate diverse cohorts in large-scale metagenomic assembly efforts will enable greater insights into microbial variability around the world.

Invest in Bioinformatics and Biotechnology Capacity
Authors of GM studies from Europe and the USA vastly outnumber those from African institutions. While the cause of this disparity is unknown, we suspect that the underrepresentation of local scientists reflects the limited opportunities for specialized training [94]. GM researchers can tremendously benefit from H3Africa's commitment to education, which includes fostering competencies in bioinformatics, genomics, statistical analysis, and high-throughput technologies. Furthermore, organizations like H3ABioNet, a subsidiary of H3Africa, are poised to further research capacity by formalizing a pan-African bioinformatics network comprising conferences, workshops, and forums [95].

Forge International Partnerships
Given limitations in obtaining strong, ongoing domestic funding and/or technical resources, longitudinal collaborations with international research institutions can fill resource gaps in African countries. Indeed, H3Africa identifies links between African and non-African organizations as sustainable channels for financial support, equipment, and training. Importantly, relationships must be premised on the meaningful participation and contribution of both parties so as to minimize 'parachute research', that is, the practice of western institutions mining scientific data from low- and middle-income countries without consulting or involving academics from those institutions. A precedent set by H3Africa, this dynamic can be combated with agreed upon ethical guidelines for empowering local researchers, data and sample management, and community engagement.

Leverage Advances in Sequencing Technologies
Developments in next-generation sequencing approaches have been accompanied by innovative products that circumvent the requirement for extensive computational or laboratory

infrastructure. For example, the MinION, engineered by Oxford Nanopore Technologies, affords lower-cost, portable, real-time generation of high-quality GM sequencing data [96]. While such technologies have only recently been commercialized, they have and will continue to expand the penetration of modern genomics tools in low-resource settings.

Beyond Africa, we believe that these recommendations can be generalized to analogous microbiome efforts in other geographic contexts. It will be crucial to integrate ethical research principles and multilateral partnerships in the Global Microbiome Conservancy, for example, which aims to comprehensively catalog global gut microbial diversity with an emphasis on traditional and indigenous communities. [97] Additionally, our proposals can potentially inform emerging national-level associations, such as the Indian Human Microbiome Initiative, in providing a framework for large-scale microbiome studies in underrepresented settings[iv].

As our knowledge of GM ecology rapidly evolves, so too must the inclusion of diverse populations be prioritized. Africa contains some of the richest genetic diversity with unique socio-cultural, epidemiologic, and biological characteristics. Future studies promise to elucidate novel host–microbiome interactions that influence human health and disease. Scientific imperatives are coupled with critical matters of justice. For as long as GM scholarship stays concentrated in predominantly western, industrialized countries, disparities in characterizing and serving historically understudied populations will persist. Still in its relative infancy, the global GM community has the opportunity to codify practices that value the full ensemble of microbial variability around the world. We envision a future where the GM in Africa not only receives its due attention, but also where such knowledge is produced by, and for, those it affects most.

Resources

[i]https://data.unicef.org/topic/nutrition/malnutrition/

[ii]https://apps.who.int/iris/handle/10665/44295

[iii]www.afro.who.int/publications/who-african-region-communicable-diseases-cluster-annual-report-2016

[iv]www.bisep.karnataka.gov.in/images/pdf/news/22_25.01.2017.pdf

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