# Ecological dynamics of the vaginal microbiome in relation to health and disease



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The bacterial composition of the vaginal microbiome is thought to be related to health and disease states of women. This microbiome is particularly dynamic, with compositional changes related to pregnancy, menstruation, and disease states such as bacterial vaginosis. In order to understand these dynamics and their impact on health and disease, ecological theories have been introduced to study the complex interactions between the many taxa in the vaginal bacterial ecosystem. The goal of this review is to introduce the ecological principles that are used in the study of the vaginal microbiome and its dynamics, and to review the application of ecology to vaginal microbial communities with respect to health and disease. Although applications of vaginal microbiome analysis and modulation have not yet been introduced into the routine clinical setting, a deeper understanding of its dynamics has the potential to facilitate development of future practices, for example in the context of postmenopausal vaginal symptoms, stratifying risk for obstetric complications, and controlling sexually transmitted infections.

**Key words:** Bacterial vaginosis, community state types (CST), *Lactobacillus*, network, preterm birth, vaginal microbiome

The development of high-throughput DNA sequencing has dramatically increased the ability to study the microbiota inhabiting human bodies. It has revealed that microbiota compositions significantly vary between body sites,[1] and that these compositions are related to various health states.[2] The vaginal microbiome, unlike most other sites in the body, is very dynamic and is characterized by temporal perturbations that are influenced by sexual development, sexual intercourse, personal hygiene, menses, and hormone levels.[3,4] It is therefore not possible to define a universally "normal" microbial composition for all women, and even for a particular woman this composition may be unstable.[4] Understanding the relation between vaginal microbial composition in regard to health and disease states requires an approach that can capture complex interspecies dynamics. The field of ecology studies such complex dynamics under the concept of an "ecosystem," and thus the investigation of the vaginal microbiome could be pushed forward by studying it under that conceptual framework.[5,6]

An ecosystem is a broad term defined as a relatively closed group of organisms, along with the sum of the interactions between the organisms and between the organisms and their environment; the microbiome is a microbial ecosystem.[7] In some cases, it is possible to describe an ecosystem function that emerges out of such complex interactions, often a "desired" function. For example, an ecosystem function may be the preservation of a stable number of species in the system (biodiversity), or the maintenance of a certain environmental condition such as water quality. Under these definitions, an ecosystem is said to be "healthy" if it can perform its presumed function.

The ecosystem approach and its accompanying methodologies have been adopted in order to study the functions of human microbial communities, as evident from the study of the human intestinal tract[8] or the human skin.[9] In many cases, the function of an ecosystem cannot be understood by simply studying specific quantitative differences in the abundance of species, but rather by looking at the entire ecosystem, including all interactions between its elements.

In this review, we explore the idea of the vaginal microbiome as an ecosystem that changes over time, and focus upon the implications of such a perspective for understanding medical conditions and vaginal disease and health states.

## The "Normal" Vaginal Microbiome

The vagina is one of many sites in the human body where bacterial communities are normally present. The female neonate acquires vaginal microbiota shortly after delivery;[10] however, the time frame and stages of the vaginal microbiota acquisition process have not yet been adequately studied. During the course of a woman's life, the vagina is exposed to constant secretions, hormonal changes, and external influences such as douching and sexual activity.[4] Not only are the vast majority of bacteria that inhabit the vagina not harmful to their host, but the vaginal microbiome as a whole plays a crucial role in the maintenance of a healthy vaginal environment.[11] This mutuality is thought to have developed as a co-evolutionary process, which has yet to be fully elucidated.[5]

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The first step in understanding the forces and dynamics that shape the vaginal microbiota is identifying its composition. This may be challenging because of the wide variety of bacterial taxa comprising it. Major progress has been made with the development of culture-independent methods, mainly 16S ribosomal RNA amplicon sequencing, and with the accumulation of global data such as those arising from the human microbiome project.[12] Using these methods, first reports of vaginal microbiota of asymptomatic women at reproductive age were able to categorize all community types into 5−8 types.[13−15] Ravel et al termed 5 typical vaginal community state types (CST), each of which is characterized by a specific and typical composition and an abundance of taxa. However, typically, microbial communities in other sites of the body are not dominated by any single genus;[14] most vaginal communities, and the corresponding CSTs, are dominated by 1 or several species of the *Lactobacillus* genus.[13,14]

Lactobacilli are rod-shaped, Gram-positive facultative anaerobic bacteria,[16] and the main functionality attributed to the *Lactobacillus* genus is the ability to produce lactic acid that consequently reduces vaginal pH and thus facilitates an acidic environment in the vagina.[11] Lactobacilli are extremely common in the vagina, as exemplified by their detection in more than 98.8% of the cases.[13] In addition, Lactobacilli were identified from more than 50% of sequences obtained in studies, indicating a high bacterial load.[13,14] The high association of *Lactobacillus* species with vaginal bacterial communities of asymptomatic reproductive-aged women suggests a functional role as the acidifiers of the vagina, in the healthy state.

The existence of a "key" group of species in most healthy vaginal microbial communities suggests that these taxa play an important functional role in the ecosystem. Walker's "drivers and passengers" hypothesis[17] pertains to the role of such "keystone" species in ecosystems, and proposes that species can be divided into functional guilds, with each guild functioning in an ecologically similar way. Under Walker's hypothesis, only some of the guilds are the ones "driving" the function of the ecosystem (eg, maintaining diversity and stability), whereas the other guilds are "passengers" that inhabit the ecosystem but do not significantly alter the dynamics or contribute to its function (Figure 1). Given the ubiquitousness of lactobacilli in healthy microbial communities and their known effect on the vaginal environment, it has been suggested that the vaginal microbial ecosystem follows Walker's hypothesis, with Lactobacilli being the keystone group driving the function and composition of the ecosystem.[6,14]

However, the idea of *Lactobacillus* being crucial for a healthy vaginal environment, and the idea that a healthy vaginal environment must be acidic, are challenged by several observations. First, in some asymptomatic women, the vaginal microbial community is not dominated by Lactobacilli;[14] other dominating bacterial genera found include *Prevotella, Sneathia, Megasphaera, Streptococcus,* and *Gardnerella,* as well as CSTs that are characterized by the absence of any dominating bacteria.[13,14] Second, high abundances of Lactobacilli were also found in samples with elevated pH.[14] Third, different *Lactobacillus* species are associated with different levels of acidity, suggesting that some *Lactobacillus* species produce lower quantities of lactic acid or, alternatively, that they possess buffering capabilities as well.[14]

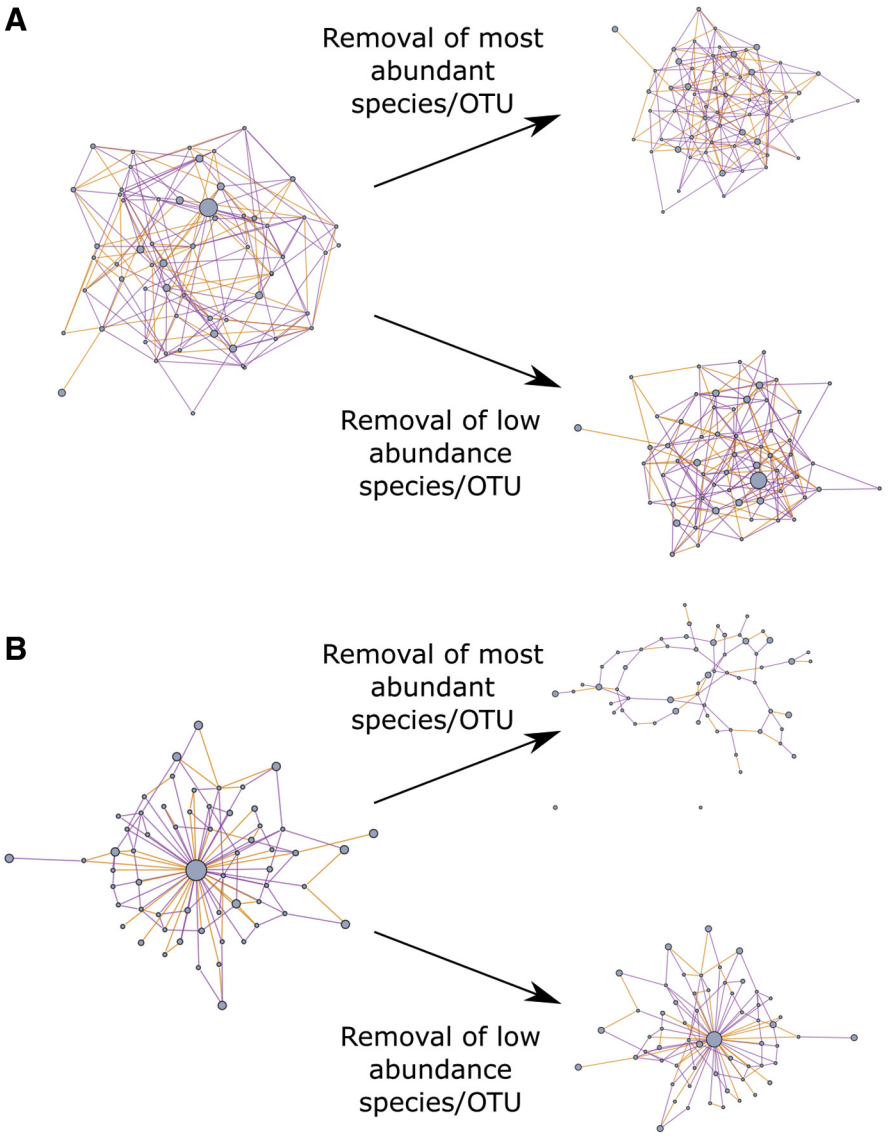
Apart from the dominating bacteria in each community, Drell et al[13] reported a mean of 26 other operational taxonomic units (OTU) in each sample. This is probably an underestimation, as some taxa may have been undetected because of their low abundance. However, such "rare" taxa could be significant in the dynamics of the community. In ecosystem dynamics, these OTU may function as a "seed bank," and their relative abundance may increase once environmental conditions change.[14] Thus, unlike more stable microbiomes, the presence or absence of rare species in the microbial community is important and cannot be overlooked when considering their role in the ecosystem, even if their abundance is very low at a given point in time.

The dynamics of such ecosystems, with many types of species and no clear "driver" guild, may also fall under a different ecological theory, Ehrlich's rivet hypothesis.[18,19] Here, species and taxon groups are considered to have overlapping roles, so that removing or adding species has little effect on the ecosystem function (Figure 1). Only when the changes in community structure cross a certain threshold does the system destabilize, and fundamental reorganization of the system results in alteration of the ecosystem function. If, in some cases, the vaginal microbiome function is determined according to this theory, studies focusing on specific bacteria taxa may provide only limited insights into the function of the vaginal microbiome. Instead, such a scenario may necessitate careful modeling of the full set of interactions between the species.

Therefore, there are currently 2 different types of vaginal microbiome function being considered: (1) a "drivers and passengers hypothesis"−type ecosystem, with *Lactobacillus* playing the role of the keystone species; and (2) a "rivet's hypothesis"−type ecosystem, in which many species contribute to the function and stability of the system. These 2 types of function only delineate 2 extremes; the dynamics in the vaginal microbiome may not necessarily follow either of them.

Another fundamental question that has been gaining much attention in the research of the human microbiome deals with the "core microbiome"—a group of taxa that are present in a specific anatomic site in almost all asymptomatic individuals. Core microbiomes have been identified in other body sites such as the oral cavity[20]; however, in the case of the dynamic vaginal microbiome, the limited evidence from multi-ethnic studies currently available suggests that a core vaginal microbiome may not exist.[14] This would be in agreement with the theoretical ecological consideration that a more dynamic ecosystem is not

**FIGURE 1**
**Schematic network representation of communities of different types and their stability**




The nodes of the networks represent species or OTUs, where the size of each node represents the abundance of the species/OTU in the community, and the edges (links) represent mutualistic (orange) and antagonistic (purple) interactions. A, A community with interactions not correlated with species abundances. B, A community with an abundant keystone species that is involved in most interactions. The community composition (number and sizes of nodes) and the number and types of interactions in A and B are similar, but the structure of the community is different. On the right, the consequences for the removal of the most abundant species/OTUs or a low abundance species/OTUs are shown. In (A), the removal of either type of species/OTU does not much affect the structure of the community, and it is likely that the function of the community will be preserved (under the "rivet hypothesis," only removal of many species/OTUs will result in functional change). In (B), the removal of a low-abundance species/OTU does not alter the structure significantly, but the removal of the keystone species/OTU results in major structural changes, which are likely to be associated with loss of community function and major reorganization of the community ("drivers and passengers hypothesis").

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likely to be characterized by a specific group of taxa but, rather, by the function of the ecosystem at different phases of the dynamics.

Different ethnic groups are associated with different compositions of vaginal communities.[14,21] This observation may be attributed to host, environmental, or genetic differences (eg, differences in epithelial ligands, innate and adaptive immune system response).[14] This variability in vaginal microbiota composition among women from different ethnic groups was observed in asymptomatic women, making the definition of a core vaginal microbiome that is associated with health state in the vagina questionable. The difficulty of defining a vaginal core microbiome in healthy women, and similar challenges in relation to other body sites,[22] have led to the conceptualization of a "functional microbiome."[23]

One way in which functionality of microorganisms in a community can be studied is metabolic profiling (metabolomics), mainly by measuring small molecules such as carbohydrates and amino acids. As a proof of concept, a recent study analyzing vaginal fluid samples of 130 pregnant and nonpregnant women was able to identify 1 molecule that was highly associated with increased community diversity and with vaginal symptoms.[24] New techniques that enable metabolite analysis by ionization mass spectrometry directly off a vaginal swab have the potential to lead towards clinical implementations.[25] The ability to sample and analyze metabolites in a clinical setting, and the identification of metabolites indicative of a microbiome function related to a disease state, provide a promising direction for development of diagnostic and prognostic tools.[26]

In ecosystems, taxa are considered "functionally redundant" when they generate and participate in similar biological processes in the ecosystem, and can therefore be interchanged without affecting the ecosystem function. In the microbiome, such redundancy can occur when microbiota share the ability to produce a certain biological compound that is important for the microbiome function. The common ability of all *Lactobacillus* species to produce lactic acid suggests that they could be related to a "vaginal core functionality." This explains both *Lactobacillus* variability among healthy women[14] and the abundance of the *Lactobacillus* genus; however, the existence of a healthy non−*Lactobacillus*-dominated CST suggests that this view is incomplete.

## Ecological Dynamics in the Vaginal Microbiome

The vaginal microbial ecosystem may be influenced by many physiological changes such as the menstrual cycle, pregnancy, menopause, and other hormonal changes.[3,4] Several longitudinal studies have monitored changes in the vaginal community composition during and after such events. In nature, ecosystems often change their composition in response to environmental changes, sometimes in a cyclical predictable manner (eg, seasonal cycles) or in response to extreme perturbations (eg, severe draughts, fires, or other catastrophes). Following severe perturbations, some ecosystems follow a series of compositional changes, known as ecological succession.[27] In ecological succession, pioneering and fast-growing species usually form the first community, which is followed by more stable and complex communities until the stable climax community is reached. Therefore, when studying changes in ecosystems dynamics through time, in response to environmental changes, the parameters of interest are most often the change in the community diversity (as a proxy to complexity) and community stability, over time (Figure 2).

Stability of the vaginal microbiome is usually not expressed in terms of changes in taxa composition, but rather in terms of consistency of the CSTs.[4] Four possible models were suggested in order to describe the dynamics of intrapersonal changes of the vaginal microbiome: (1) a single stable CST (no dynamics); (2) transition among all possible CSTs; (3) transition between a small number of CSTs; (4) a single basic CST, with short transitions to other CSTs in response to disturbances.[4]

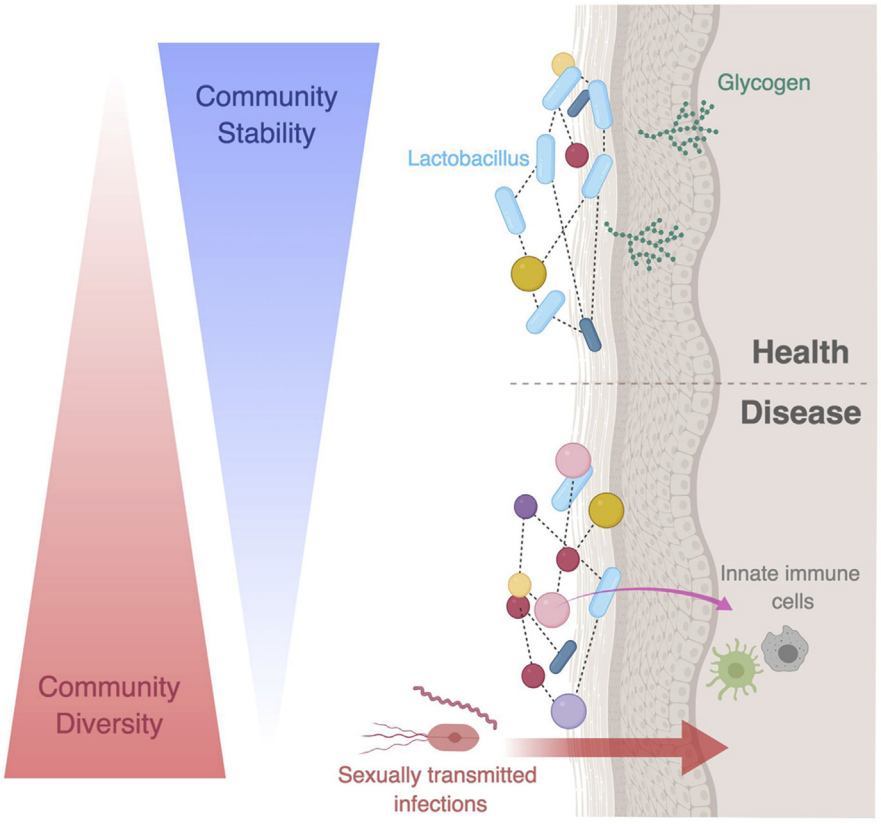
## The Vaginal Microbiome During Pregnancy

Several studies have looked into the community dynamics related with pregnancy, and were able to use the same CST definitions that were previously used to describe nonpregnant women (Table 1). In pregnant women, transition between different CSTs does not seem to occur randomly with specific transition patterns more likely to be associated with specific CSTs.[3] Even when transition between the different CSTs is taken into account, the overall stability is increased (ie, in variance of composition shifts) during pregnancy.[28,29] The observed increased stability during pregnancy suggests that it may play an important functional role, by lowering susceptibility to an ascending infection that might result in an intrauterine infection and subsequent preterm labor. The increased vaginal ecosystem stability during pregnancy may therefore be an evolutionary adaptation aimed at increasing the host's fecundity and hence its fitness.[28] This hypothesis would suggest that the host actively shapes the vaginal microbiome during pregnancy or, in a way, instigates a compositional change that would be favorable to the pregnancy.

Another important trait used to describe the ability of an ecosystem to respond to perturbation[3] is the diversity of the community.[30] In many ecological systems, increased diversity is associated with increased resilience. This is explained by response diversity, the available array of possible responses to perturbations in the system, which enables the ecosystem to react to environmental changes and to reorganize (eg, under the rivet hypothesis).[31] In the gut microbiome, for example, loss of diversity has been shown to be associated with inflammatory bowel diseases.[32]

Surprisingly, in the vaginal microbiome, this rule does not seem to apply. The vaginal microbiome is one of the least diverse microbiomes in the human body, and even more so during pregnancy.[28−30] In black American women, for whom a diverse CST is relatively

**FIGURE 2**
**An illustration of the relation between the vaginal microbiome and health and disease states**




In the vaginal microbiome, health states, unlike the gut microbiome and other human microbiomes, are associated with low community diversity. Many, but not all, community state types (CSTs) of healthy reproductive-aged women are dominated by Lactobacillus species, and remain fairly stable, but not permanent (eg, the community composition changes during menstruation). Glycogen deposits in the vaginal epithelium are being used by Lactobacillus species in anaerobic glycolysis, which results in lactic acid production. In disease states, for example in bacterial vaginosis (BV), the communities observed are more diverse and less stable. Vaginosis-associated bacteria can negatively modify host innate immune response, and are associated with predisposition for sexually transmitted infections (STIs).

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common, this community state is rare in pregnancy.[14,28] In white American women, non−*Lactobacillus*-dominated vaginal community state type is not common; nevertheless it is even rarer in pregnancy.[3,29] Therefore, it would seem that during pregnancy the vaginal microbial community shifts toward a less diverse, more *Lactobacillus*-dominated, state (Figure 2).

Notably, 2 studies found that the specific dominant *Lactobacillus* species in pregnant women are different from those found in nonpregnant women.[28,33] From the perspective of the "drivers and passengers hypothesis," the findings of increased Lactobacillus abundance during pregnancy may be interpreted as an increase in the abundance of a dominant keystone species, which induces stability on the ecological dynamics, and supports a community core functionality of lactic acid production. On the other hand, the shift of lactobacilli species during pregnancy does not sit well with this hypothesis, and suggests more complex ecological mechanisms at play. The low diversity during pregnancy, characterized by the dominance of the *Lactobacillus* genus, is possibly related to increased levels of estrogen that may confer a relative advantage to *Lactobacillus* species. The positive correlation between hormonal levels and proliferation of Lactobacilli is attributed to the effect of estrogen on the maturation of the vaginal epithelium, which results in accumulation of glycogen, a metabolite used by Lactobacilli in lactic acid production.[28,29] However, due to technical difficulties in measuring local estrogen concentrations in the vagina, further research is required in order to assess the association between hormonal change and diversity of the vaginal microbiota.[29]

## Vaginal Microbiota and Preterm Birth

An important question arises regarding causation between vaginal microbial dysbiosis and preterm birth (defined as delivery before 37 weeks' gestation). DiGiulio et al[3] identified an association between a low abundance of *Lactobacillus* and a high abundance of *Gardnerella* in the vaginal community and preterm birth in a predominantly white cohort. A follow-up study was able to reproduce these findings in a similar cohort with a low risk of preterm birth, but not in a black American population with a high risk of preterm birth.[34] In a recent prospective study of women at high risk for preterm birth, preterm delivery cases were associated with high community diversity, and community instability in the first trimester.[35] Despite robust methodology, these 3 studies did not distinguish between spontaneous and induced preterm labor, thereby possibly obscuring the true relationship between vaginal dysbiosis and preterm birth.

Two studies have demonstrated an association between preterm labor and carriage of specific bacteria, such as Bacterial Vaginosis Associated Bacterium 1 (BVAB)−1, in populations at high risk for preterm birth.[36,37] However, these studies were targeting a

**TABLE 1**
**Selected studies of vaginal microbiome analysis, by chronological order**

| Study population | Age of study group (y) | Sample size | Pregnancy status | Method used for DNA processing | Comments | Reference |
|---|---|---|---|---|---|---|
| Japanese women | 19–44 | 132 | Pregnant | Selected polymerase chain reaction amplification of specific bacteria (14 *Lactobacilli* species and 4 vaginosis associated bacteria), followed by electrophoresis | Analysis by subgroups according to Nugent score | Tamrakar et al, 2007 |
| Asymptomatic North American women (different ethnicities) | 12–45 | 396 | Non-pregnant | Amplification of bacterial 16S rRNA (V1–V2 hypervariable region) and sequencing using 454 pyrosequencing | This study defined 5 vaginal community structure types (CSTs) that are commonly referred to in vaginal microbiome studies; this study included analysis by ethnic group | Ravel et al, 2011 |
| Mexican women | 13–43 | 64 | Pregnant | Amplification of bacterial 16S rRNA (V3 hypervariable region) using multiple phases of PCR amplification, followed by Sanger sequencing | One sampling event at different trimesters; only women with normal Nugent scores were included | Hernandez-Rodriguez et al, 2011 |
| Asymptomatic reproductive-aged Estonian women | 15–44 | 494 | Non-pregnant | Amplification of bacterial 16S rRNA (V1–V2 hypervariable region) and sequencing using 454 pyrosequencing technology | In addition to vaginal microbiome analysis, this study analyzed the vaginal mycobiome; large-scale study | Drell et al, 2013 |
| American women, mostly of African American race | 20-28 | 90 | Pregnant | Amplification of bacterial 16S rRNA (V1-V3 hypervariable region) and DNA sequencing using 454 pyrosequencing technology | Longitudinal sampling every 2–4 wk; analysis included only spontaneous preterm birth; the definition of preterm delivery was <34 wk gestation | Romero et al, 2014 |
| Mostly white non-Hispanic | 19–45 | 49 | Pregnant | Amplification of bacterial 16S rRNA (V3–V5 hypervariable region), followed by sequencing using methods independently: 454 pyrosequencing and Illumina HiSeq | Weekly sampling until delivery and monthly after delivery. In addition to vagina, stool, oral saliva sampling; analysis for preterm birth but half of the preterm group are not spontaneous | DiGiulio et al, 2015 |
| British women (different ethnicities) | >18 | 42 | Pregnant | Amplification of bacterial 16S rRNA (V1–V2 hypervariable region) and DNA sequencing by Illumina MiSeq; | Four sampling points during pregnancy and 1 postpartum | MacIntyre et al, 2015 |
| American women, African American and white | 17-42 | 135 | Pregnant | Amplification of bacterial 16S rRNA (V4 hypervariable region and DNA sequencing by Illumina HiSeq) | Longitudinal sampling during gestation. Suggests defining vaginal bacterial communities by frequencies of *G. vaginalis, L. crispatus, and L. iners* instead of discrete CSTs. | Callahan et al, 2017 |

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limited number of bacteria, which may oversimplify the complexity of the community structure and bias findings toward certain species. A different obstetric outcome, preterm premature rupture of membranes (PPROM), was recently investigated, and was found to be associated with a *Lactobacillus*-depleted community.[38] Because PPROM is the presenting manifestation in only a subgroup of spontaneous preterm deliveries, this finding may not be directly generalized to all preterm deliveries. In the scenario of cervical cerclage, the use of a monofilament suture was associated with a more subtle inflammatory reaction (compared to a braided thread), and was associated with a lower rate of vaginal dysbiosis and preterm birth.[39]

However, a prospective study that looked at longitudinal sampling of women without known risk of preterm birth did not identify any differences in community compositions.[40] Of note, preterm delivery in this study was defined as <34 weeks' gestation, and women in this group were mainly of black American ethnicity. Discrepancies among the different studies may be related to background characteristics of participants, small sample size in most studies, and differences in definition of preterm delivery. Overall, despite the increasing interest in vaginal dysbiosis as a possible cause of preterm birth, evidence does not currently exist to support screening and treatment of bacterial vaginosis in pregnant women at low risk or high risk for preterm labor, and therefore is not recommended.[41−43]

## Vaginal Microbiota in the Postpartum Period

Although vaginal dysbiosis as a cause for preterm delivery is still under debate, evidence strongly supports that drastic vaginal bacterial community alterations occur after pregnancy.[3,29] The postpartum vaginal microbiome includes more vaginosis-associated bacteria (VAB), less *Lactobacillus*,[29] and higher resemblance to gut communities.[3] Furthermore, these changes persist for up to 1 year, and do not correlate with similar changes in microbial communities in other body sites.[3] The underlying mechanisms that may explain these observations are yet unknown; however, it seems that they cannot be explained merely by translocation of stool bacteria to the vagina during vaginal delivery, as similar observations were made in women after cesarean delivery.[3] One explanation is that the alkalinity of the lochial discharge has an inhibitory effect on Lactobacilli.[29] Alternatively, an abrupt drop in estrogen levels after delivery may aggravate environmental conditions that are deleterious for *Lactobacillus*.[29] This observation may have clinical importance in the setting of a future recommendation regarding the minimal interval between pregnancies, in order to enable the microbial community to retain its stable pre-pregnancy state. However, as current data regarding vaginal community disruption and obstetric complications are controversial, it is still too early to draw conclusions regarding an ideal interpregnancy interval.

## Vaginal Microbiota and Menstruation

Several longitudinal studies have monitored dynamics in the vaginal microbiome in relation to the menstrual cycle. In reproductive-aged women, stability of the microbial community decreases during menses and correlates with estrogen levels.[4] With respect to bacterial composition, *Gardnerella vaginalis* abundance increases during menses with a concurrent decrease in abundance of *Lactobacillus* species, excluding *Lactobacillus iners*.[44] This may be explained by the lysis of vaginal blood during menses and increased iron levels, which support the accelerated growth of both *G. vaginalis* and *L. iners*.[44]

Data regarding composition and dynamics of the vaginal microbiome using culture-independent methods in young girls are sparse, and are entirely missing in female neonates, possibly because of the difficulties involved in recruitment of healthy patients. A longitudinal study in adolescents prior to menarche found that the composition of the vaginal microbiome resembles that of reproductive-aged women.[45] In contrast, studies in postmenopausal women are available, and have somewhat revised what was previously thought about the postmenopausal vaginal microbiome. Postmenopausal vaginal communities were considered poor in *Lactobacillus* but rich in anaerobic taxa (eg, *Bacteroides*, *Mobiluncus*) and VAB such as *G. vaginalis*.[46] Surprisingly, it was observed that in more than 50% of postmenopausal women the dominating bacteria is *Lactobacillus*, irrespective of climacteric symptoms such as vaginal dryness or vulvovaginal atrophy.[47,48] Nevertheless, in both studies,[47,48] a low abundance of *Lactobacilus* was indeed associated with vaginal symptoms.

New technologies, such as microablative fractional $CO_2$ laser, are increasingly being used for treatment of genitourinary syndrome of menopause in postmenopausal women. Although this technology has not been approved by the U.S. Food and Drug Administration (FDA) for treatment of genitourinary syndrome of menopause,[49] studies report amelioration of vaginal symptoms, and re-population of the vagina with *Lactobacillus*.[50,51] Positive clinical response is proposed to be the result of the tissue-restorative effect of this laser treatment. This restoration is demonstrated by thickening of the epithelium and increased abundance of glycogen-rich shedding cells, which promote proliferation of glycogen-dependent bacteria such as *Lactobacillus*.[52]

Overall, the vaginal microbiome is characterized by low community diversity and high community stability in fertile healthy women (although this stability is temporary). Although resilience of ecosystems is often correlated with high diversity, in the vagina high diversity is associated with disease states. This puzzling observation could possibly be explained by the involvement of an external force, such as estrogen: Estrogen grants an advantage for the *Lactobacillus* genus,[28,29] and may supports a low-diversity/high-resilience equilibrium state, which again becomes unstable as estrogen levels are depleted. However, this explanation fails to account for the high frequency of the

*Lactobacillus*-dominant microbiome in postmenopausal women.[47,48]

## Vaginal Microbiome and Bacterial Vaginosis

Bacterial vaginosis (BV) is the most common vaginal infection in reproductive-aged women, and therefore is a commonly used example for a disease state in the study of the vaginal microbiome.[11] Around half of the women with BV report an increased malodorous vaginal discharge, which inflicts discomfort and has a major effect on quality of life.[53] BV correlates closely with high vaginal pH,[11] which is attributed to a decreased production of lactic acid by vaginal bacteria, mainly of the *Lactobacillus* species (Figure 2). However, data collected so far on BV are not supportive of *Lactobacillus* species as the single causative pathogen for this condition, and the relation between microbiota and BV has been suggested to be polymicrobial.[5]

Until recently, studies have focused mainly on identifying simple quantitative changes in the vaginal bacterial composition, for example, the proliferation of certain pathogens with simultaneous depletion of others. However, this approach fails to explain the presence of anaerobic bacteria in healthy asymptomatic women,[5,54] and it could be postulated that anaerobic bacteria are most likely not the cause of BV but are rather opportunistic bacteria that flourish in a disease state.[5] This, again, emphasizes the problematic assignment of a single keystone species, *Lactobacillus*, as an indicator of a well-functioning vaginal microbiome. In the absence of basic understanding of the etiology and natural history of this common condition, advancement toward the development of an effective treatment for BV will be difficult. Therefore, considering the healthy state as a well-balanced ecosystem and disease as a disrupted state may be beneficial in attempting to develop new treatment strategies.[5]

## Bacterial Vaginosis and Sexually Transmitted Infections

BV not only affects personal well-being, but also has public health and epidemiological implications, as it is associated with a higher acquisition rate of various sexually transmitted infections (STIs) such as *Neisseria gonorrhoeae*,[55–57] *Chlamydia trachomatis*,[56,57] *Trichomonas vaginalis*,[57] herpes simplex virus,[58] and human immunodeficiency virus (HIV)[59–61] (Figure 2).

In order to delineate the effect of BV on the acquisition rate of HIV, longitudinal studies have focused on high-risk populations such as commercial sex workers in Africa,[62] and have demonstrated that diagnosis of BV correlates with higher HIV acquisition.[63,64] Moreover, a higher viral load[60] and severity of immunodeficiency status[65] are also correlated with BV. These findings have attracted considerable attention to treating BV as a promising way of mitigating the HIV pandemic.[66] However, apart from 1 study,[67] studies of empiric antimicrobial treatment for BV did not decrease the HIV seroconversion rate.[68–71] Moreover, most studies noted that BV prevalence did not decrease as a result of intervention.[69,71] In 1 study, decreased STI (eg, syphilis and trichomoniasis) incidence following intervention had no effect on the incidence of HIV-1.[68] A possible explanation for the persistence of a high HIV seroconversion rate could be poor compliance with treatment or high BV recurrence rates.[68] The fact that BV rates did not change in the course of treatment undermines the premise of these studies, and may give an alternative explanation as to why these interventional studies did not yield positive results.[68]

The association of BV with increased STI risk is unclear. In vaginitis, where STI risk has also been shown to be increased, tissue inflammation and ulceration result in loss of cell-to-cell adhesion and decreased epithelial integrity, which facilitates pathogen penetration.[72] However, in BV, the tissue is not inflamed. Therefore, an alternative explanation is required in order to explain the association of BV with STIs.

One proposed explanation of this association focuses on VAB presence in the vaginal microbiome and their adverse effects on the host innate immune system.[73,74] The innate immune system is the first line of defense following breach of the physical barrier. Innate immune cells, such as resident macrophages, can rapidly recognize and attack invading bacteria, and can also be triggered by epithelial cells via cytokine inter-talk. Genital secretions of women with BV have been repeatedly associated with alteration in cytokines and antimicrobial peptides, notably increased IL1-$\beta$,[61] which in turn can initiate innate immune response and promote inflammation. A recent in vitro study of vaginal epithelial cell aggregates showed increased expression of cytokines, including IL1-$\beta$ and chemokine ligand 20, when co-cultured with *Atopobium vaginae*.[75] As part of their pro-inflammatory effect, these cytokines stimulate lymphocyte migration to the site, which has been proposed to modulate risk of HIV acquisition through facilitating an abundance of HIV target cells in the mucosa.[61,76] Such findings may help elucidate the role of the vaginal microbiome in transmission of STIs.

## Future Research

Understanding the relation of the vaginal microbiome to health and disease states lies crucially in elucidating the ecological dynamics in this system. Much attention has been given to the role of *Lactobacillus* in the vaginal microbiome, given its high abundance in many cases, promoting a "drivers and passengers hypothesis" perspective of this community, with a dominant role conveyed on a single species. However, many findings indicate that this community cannot be so simplistically understood, and that complex interaction between species and their effect on the ecosystem function must be incorporated in analysis of this system.

Future research should focus on the interrelations and interactions between species within the vaginal microbiome. Some observations of this kind have already been made, for example *Lactobacillus crispatus* appear to have a strong negative influence on *G. vaginalis*, whereas *L. iners* does not.[34] Another example is the positive influence of *Prevotella sp.* on the growth of *Peptostreptococcus anaerobius* and *Gardnerella*

*vaginalis* due to the production of nutrients (ammonia and amino acids) that are consumed by both taxa.[14] Interrelationships between different taxa may play a vital role in the functionality of bacterial communities, and hence have a crucial impact on "health" and "disease" states. A better understanding of interspecies interactions may also be crucial to understand how composition is translated into function in the ecosystem, and how succession dynamics play out in the vaginal environment.

A promising and increasingly used approach to study ecosystem function is network analysis.[77,78] A network is a mathematical construct composed of "nodes," the elements of a system, and "edges," the interactions between those elements.[79,80] The field of network theory is concerned with analyzing and understanding complex systems and emerging phenomena, which includes understanding functions such as stability and resilience of ecosystems. Ecosystems can be naturally described as networks, where the nodes are the different species and the edges represent positive or negative interactions between them. The medical sciences have seen many useful applications of network theory, such as in the study of epidemics,[79] in cancer research,[81] and in neuroscience.[82] Microbiome ecosystems have also been analyzed using the networks.[83–87] Specifically, network methods applied in human microbiome study have proved very successful,[88] for example in analyzing infant[89] and adult gut microbiomes.[90]

The vaginal bacterial microbiome as a network is composed of the different bacteria as nodes and the co-occurrence of bacteria (ie, findings of both types of bacteria in the same microbial community) as the edges.[88] For example, considering 3 taxa found in the vaginal microbiome, *L. iners* and *G. vaginalis* have been found to co-occur, and would therefore be connected by an edge representing positive association, whereas *G. vaginalis* and *L. crispatus* do not occur together, and therefore would be connected by an edge representing negative association.[34] These networks can be constructed for microbial communities in healthy and in nonhealthy women. Network methods can help clarify the issue of the core functional microbiome by treating not only compositional changes but also functional structural properties of the ecosystem. Importantly, networks can also be analyzed by comparing the structure, resilience, and stability of the vaginal microbiome in health and disease states.

Understanding the network structure of the ecosystem can help predict the consequences of environmental perturbations, and whether the system is more likely to follow the "drivers and passengers hypothesis" or the "rivet hypothesis" (Figure 1). Dynamic network analysis may also elucidate the resilience and stability of communities in the face of cyclical changes such as menstrual cycles, or even in noncyclical but predictable changes such as pregnancy or menopause; such an approach is perhaps more appropriate for the study of the dynamic vaginal microbiome than is the classic static network approach. Dynamic networks incorporate information not only of a single static community but also of the changes that the ecosystem experiences over time.

In cases when specific disease states can be associated with a certain microbial community composition, the next step should be to investigate the underlying molecular pathways. Once a molecular mechanism can be hypothesized, therapeutic options can be tested. Given the network structure, solutions may be nontrivial. For example, if a paucity of bacteria A and B is recognized as related to the disease state, the solution may turn out to be weakening of bacteria C that is competing with bacteria A and B, rather than just supplementing them.

Considering the vaginal disease states (such as BV) as a disruption in the composition of the bacterial ecosystem, rather than a result of the acquisition of a specific pathogen, may support the idea of bacterial community transplantation as a therapeutic modality that is superior to antimicrobial treatment. Fecal microbiota transplantation (FMT) for gastrointestinal disease has yielded positive results, for example in treatment of recurrent infections with *Clostridioides difficile*,[91,92] and some negative results, for example in treatment of ulcerative colitis.[93,94] These mixed results emphasize the complexity of customizing treatment for a specific indication. In a similar manner, clinical trials have been done for urogenital infections (reviewed elsewhere[95]), including in pregnant women.[96] As Lactobacilli are abundant in dairy products, and are registered as dietary supplements, these studies did not transfer vaginal communities from a donor to the recipient (as was done in FMT studies), but rather supplemented study participants with *Lactobacillus*-rich products (eg, yogurt), either via oral administration or intravaginally. In a randomized, placebo-controlled, triple-blind study, 144 pregnant women received supplementation with oral probiotics, with no significant difference in duration of pregnancy, symptoms, or Nugent score.[97] To date, probiotics are not considered a standard treatment modality either for BV or for prevention of preterm birth.

## Conclusion

The goal of this review was to provide an overview of the vaginal microbiome from an ecological perspective, emphasizing its dynamic nature and its relation to health and disease states. The accumulating data point toward a diverse, complex, and dynamic vaginal microbial ecosystem, which is most commonly dominated by *Lactobacillus* species. Ecological perspectives have the potential to further increase our understanding of the composition and functionality of the vaginal microbiota. Future studies of the systems-level temporal dynamics of the vaginal ecosystem and its function may expand our understanding of genuine states of health and disease and the underlying mechanisms involved. Moreover, improved understanding of the microbial community networks in the vaginal ecosystem could facilitate the development of personalized modalities for treatment of vaginal diseases and improvement of obstetric outcomes. ■

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**A Glossary of Ecological Terms**

**Community** — A group of organisms typically occupying the same space at the same time. For example: a community of trees in a particular forest, a community of microbes in a particular type of soil.

**Community diversity** — The diversity of the species in the community, often measured simply as the number of species in the community (species richness).

**Community composition** — The composition of species in a given community, including the abundances of the different species.

**Community structure** — The composition of the community and the way different species interact with one another.

**Ecosystem** — A system that includes a community, the interactions among the organisms in the community, and the interactions between the organisms and the abiotic environment.

**Microbiome** — An ecosystem of microorganisms and their abiotic environment. Some researchers limit the term to refer only to the sum of genes of microorganisms in a microbial ecosystem.

**Community state type (CST)** — Different community compositions can be classified into typical types. These typical types are called CSTs, in the context of the microbiome.

**Dominating species** — A specific species, or closely related group of species (eg, genus), that are found in all communities of a certain type and typically at high abundances.

**Operational taxonomic unit (OTU)** — A pragmatic definition of taxonomic units, similar to the classic taxonomic definition of species, most often used in microbial biology where the regular definitions are often irrelevant. In practice, OTUs are clusters of genetically similar microbes that are presumed to interact with the ecosystem in a similar manner.

**Co-evolution** — An evolutionary process in which two or more species interact to affect the evolution their evolution. For example, the evolution of many flowering plants and their pollinators is considered a co-evolutionary process.

**Ecosystem function** — A high-level phenomenon or process that emerges from the sum of the interactions in the ecosystem. Ecosystem functions are often regulations of processes or quantities such as biodiversity, environmental quality (soil, water, etc), and habitat support for species. In the context of the human microbiome, an ecosystem function may be seen as related to the health state of the host, and a functioning microbial ecosystem may be seen as one that is associated with a healthy host.

**Community stability** — The tendency of a community to remain at a stable community composition. Stable communities display lower variance of abundances shifts.

**Community robustness** — The ability of a community to retains its typical functions in the face of perturbations, such as fluctuations in species abundances, addition or removal of species, or changes in the environment.

**Community resilience** — The ability of a community to rebound and return to its typical state and function after a major change has resulted in the ecosystem losing its function.

**Response diversity** — The range of responses to perturbations in a community contributed by the diversity of species in the community. It is hypothesized that diverse communities should be more robust and more resilient due to response diversity.

**Seed bank** — In plants, the total of seeds dormant in the soil. Although the seeds of some species may have no role in the typical state of a community, when large disturbances occur these seeds contribute to the response diversity of the community, and therefore their presence or absence from the seed bank is crucial to the resilience of the system. The term may also be used to refer to non-plant species (eg, microbes) that are typically found in low abundance in the community but have an important role in the maintenance of response diversity.

**Ecological succession** — The process of change of community compositions after a major catastrophic perturbation (eg, wildfire). Classic ecological theory predicts that the first community to populate would be composed of a few fast-growing, easily dispersing species (primary succession), and later communities would be more complex and stable. The stable community at the end of the succession process is called the climax community.

**Core microbiome** — The typical, nontransient, microbial community associated with a particular habitat (eg, body site).

**Community network** — A representation of community structure using networks, where nodes represent species or OTUs and edges (links) represent antagonistic or positive interactions between the nodes. Network theory methodologies can be used to study questions relating to stability, robustness, resilience, and function in ecosystems.

**Driver and passengers hypothesis** — The hypothesis that ecosystem function is driven by few driver species, whereas other species in the community (passengers) do not contribute to its function, and are therefore not essential for maintaining the function.

**Rivet hypothesis** — The hypothesis that species have overlapping functions in the community, and therefore removing or adding species would not result in a change of ecosystem function. Only when enough structure has been perturbed in an ecosystem ("rivets") is a critical threshold breached and the system reorganizes itself, most often in a way that the original function is lost.

**Invasive meltdown hypothesis** — The hypothesis that when species are invading a community, successful invasions alter the community in a way that facilitates further invasions. This means that once the first invasions take hold, the community may cross a "meltdown" threshold, after which invasions are frequent and the community rapidly loses its function.