Resource landscape shapes the composition and stability of the human vaginal microbiota

Tsukushi Kamiya[1,2,*], Mircea T. Sofonea[2, 2bis], Michael France[3], Nicolas Tessandier[1,4], Ignacio G Bravo[4], Carmen Lia Murall[4,5], Jacques Ravel[3], and Samuel Alizon[1]

[1]Center for Interdisciplinary Research in Biology (CIRB), Collège de France, CNRS, INSERM, Université PSL, Paris, France
[2]PCCEI, Univ. Montpellier, Inserm, EFS, Montpellier, France
[2bis]CHU Nîmes, Nîmes, France
[3]Institute for Genome Sciences, Department of Microbiology and Immunology, University of Maryland School of Medicine, Baltimore, MD, USA
[4]MIVEGEC, CNRS, IRD, Université de Montpellier, France
[5]National Microbiology Laboratory, Public Health Agency of Canada, Montréal, Canada
[*]Corresponding author: samuel.alizon@college-de-france.fr; tsukushi.kamiya@college-de-france.fr

Abstract

The vaginal microbiota is associated with the health of women and newborns alike. Despite its comparatively simple composition relative to other human microbiota systems, the mechanisms underpinning the dynamics and stability of vaginal microbial communities remain elusive. A crucial, yet so far underexplored, aspect of vaginal microbiota ecology is the role played by nutritional resources. Glycogen and its derivatives, produced by vaginal epithelia, are accessible to all bacterial constituents of the microbiota. Concurrently, free sialic acid and fucose offer supplementary nutritional resources to bacterial strains capable of cleaving them from glycans, which are structurally integral to mucus. Notably, bacteria adept at sialic acid exploitation are often correlated with adverse clinical outcomes and are frequently implicated in bacterial vaginosis (BV). In this study, we introduce a novel mathematical model tailored to human vaginal microbiota dynamics to explore the interactions between bacteria and their respective nutritional landscape. Our resource-based model examines the impact of the relative availability of glycogen derivatives (accessible to all bacterial species) and sialic acid (exclusive to some BV-associated bacteria) on the composition of the vaginal microbiota. Our findings elucidate that the success of BV-associated bacteria is intricately linked to their exclusive access to specific nutritional resources. This private access fortifies communities dominated by BV-associated bacteria, rendering them resilient to compositional transitions. We empirically corroborate our model prediction with longitudinal clinical data on microbiota composition and previously unpublished metabolomic profiles obtained from a North American cohort. The insights gleaned from this study shed light on potential pathways for BV prevention and treatment.

Keywords: Vaginal microbiota; *Lactobacillus*; *Gardnerella*; women's health; community ecology; metabolomics; mathematical modelling

Significance statement

The vaginal microbiota has a notable impact on women's health at various stages of life, namely puberty, infection protection, sexual health, fertility, pregnancy, and menopausal changes. At present, most non-anti-microbial products developed to mitigate adverse vaginal symptoms emphasise competitive interactions through acids (boric or lactic acid) or probiotics as a means to "rebalance" microbiota communities. Despite recent advances in profiling the composition of vaginal microbiota communities, there remains a major gap in our mechanistic understanding of how to maintain or reinstate a resilient *Lactobacillus*-dominated microbiota that improves vaginal health and outcomes. This study explores the role of nutritional resources in the vaginal microbiota by introducing a mathematical model that analyses how access to specific nutrients like glycogen derivatives and sialic acid affects the balance of bacterial vaginosis (BV) and non-BV-associated bacteria. Our findings, supported by original cohort-derived microbiological and metabolomics data, demonstrate that exclusive access to these nutrients is linked to the dominance and resilience of BV-associated bacteria, providing new insights for BV prevention and treatment.

Background

The integration of community ecology theory in human microbiota studies remains a central goal, as ecological processes that shape microbial community composition hold the key to understanding the role of microbiota in health and disease (Costello et al., 2012). Gut microbiota garners most of the attention among human microbiota systems. Nevertheless, the application of traditional ecological theories to the intestinal milieu continues to be generic in scope. This is in part due to the vast diversity of these communities, which display between-individual variability, temporal composition fluctuations, as well as within-host spatial diversity in different regions of the digestive tract.

While detailed mechanistic understanding of the human gut microbiota ecology remains elusive, ecological theory has been increasingly applied to the dynamics in chemostat bioreactors, in which defined bacterial communities are cultured under controlled settings (Bucci et al., 2012; Kaul et al., 2016; Vet et al., 2020; Khazaei et al., 2020). Under such in-vitro, simplified conditions, mathematical models can describe interactions between bacteria and their environment to uncover factors shaping the composition of these synthetic microbial communities. Nonetheless, the extent to which these findings from in-vitro settings scale up to in-vivo microbial communities and, more generally, how ecological theory can improve our understanding of human microbiota dynamics remains an open question. In this work, we focus on human vaginal microbiota communities, whose relative simplicity makes them well-suited for applying ecological theory to a real-world setting and elucidate mechanisms underlying health and dysbiosis, understood here as imbalances or deviations from eubiotic and optimal states (Hooks and O'Malley, 2017).

Defining dysbiosis is essential for establishing a shared understanding of what microbial community compositions are considered beneficial or harmful. Yet this remains particularly

challenging in the gut microbiota since the classification — and even the existence itself — of distinct microbiota community types in the gut (known as 'enterotypes') continues to be contentious (Costea et al., 2018). By contrast, the existence of vaginal community state types, or CSTs, is widely recognised and has been well-documented in association with health and dysbiosis (Ravel et al., 2011; France et al., 2020). Briefly, the human vaginal microenvironment is most often dominated by one of four *Lactobacillus* species (Hill et al., 1984; Ravel et al., 2011): CST I, dominated by *L. crispatus*; CST II, dominated by *L. gasseri*; CST III, dominated by *L. iners*; and CST V, dominated by *L. jensenii*. CSTs I, II, and V are often regarded as 'optimal', offering protection against disease and adverse health effects, whereas CST III plays a more ambiguous or mixed role (Holm et al., 2023). In contrast, in CST IV, lactobacilli are not the most prevalent bacteria, and the microbiota is constituted of a diverse array of strict and facultative anaerobes. This microbial composition has been consistently linked to adverse gynaecological, obstetric, and mental health outcomes, an increased risk of sexually transmitted infections (van de Wijgert, 2017) as well as in-vitro fertilisation failure (Haahr et al., 2019). Consequently, CST IV is often regarded as a 'non-optimal' or dysbiotic vaginal microbiota state. Bacterial vaginosis (BV), a condition characterised by symptoms such as vaginal odour and discharge and associated with severe discomfort (McKinnon et al., 2019), is experienced by approximately 23 to 29% of women of reproductive age, depending on the region (Peebles et al., 2019). Microbiologically, the vaginal microbiota in women suffering from BV systematically corresponds to CST IV, although the reverse is not necessarily true as many women with CST IV do not exhibit any symptoms (Haahr et al., 2019). Despite the relative tractability and well-established health implications of the vaginal microbiota, the application of ecological theory to understanding this dynamic system remains limited.

Here, we adopt a resource ecology perspective, focusing on the role of shared and private nutritional resources in shaping the microbiota composition. Briefly, the nutritional resource

landscape of the vaginal environment is described as follows. The human vagina is lined with stratified layers of epithelial cells, which are renewed and shed from the basal and outer layers, respectively. Epithelial cells store carbohydrates in the form of glycogen, which may be released to the extracellular milieu by shedding outer epithelial cells and through the destruction of immature cells (i.e., cytolysis) by *G. vaginalis* and other CST IV species (France et al., 2022). The degree to which each mechanism contributes to the availability of extracellular glycogen is unclear. However, the strong negative association observed between pH and cell-free glycogen suggests that glycogen release is driven predominantly by a CST IV independent mechanism (Mirmonsef et al., 2014): as *G. vaginalis* dominance is associated with elevated pH, one would expect a positive association if cytolysis were a dominant driver. Once released by shedding outer epithelial cells, glycogen and its breakdown derivatives (e.g., glucose, maltose, maltotriose, maltopentaose, maltodextrins) constitute the primary fuel for vaginal bacterial growth, and they are utilised by both CST I and IV bacteria (Spear et al., 2014; Tester and Al-Ghazzewi, 2018). In addition to glycogen, CST IV bacteria are capable of metabolising additional resources. Specifically, *G. vaginalis* can cleave off sialic acid and fucose molecules from large sugar molecules that comprise the vaginal mucus layer (i.e., mucus sialoglycan) (Lewis et al., 2013; Agarwal and Lewis, 2021). As the CST I does not appear to metabolise the end products of mucus degradation (Agarwal and Lewis, 2021), we can consider sialic acid and fucose private to CST IV.

Our mathematical model demonstrates that the relative frequency of BV-associated bacteria hinges upon the relative supply of resources private to them (sialic acid and fucose from the mucus), and that the access to private resources makes communities dominated by BV-associated bacteria resistant to transition. By leveraging vaginal microbiota compositional data and original metabolomic data longitudinally collected from a cohort in North America, we corroborate the role of nutritional resources in shaping vaginal microbiota communities. These

findings have implications for BV prevention and provide indications for targeted interventions to improve women's sexual and reproductive health.

Modelling vaginal microbiota ecology

Mathematical models are instrumental in community ecology to study the consequences of non-linear interplay between organisms and their environment. A large body of theoretical exploration of microbiota systems has relied on classic competitive Lotka-Volterra models, often focusing on species interactions, while overlooking resource availability and consumption as a key driver of bacterial growth and population size (Bucci et al., 2016; Gonze et al., 2018; Lee et al., 2020). In contrast, the work described here builds upon classic models of consumer-resource theory (Tilman, 1980, 1982), which assumes resources to be the primary drivers of community composition and assemblage.

We adopt a parsimonious approach in modelling the composition of the vaginal microbiota and focus on two communities associated with distinct health outcomes (Fig. 1): on the one hand, CST I vaginal microbiota, dominated by *L. crispatus*, that maintains an acidic vaginal micro-environment and in which BV-associated bacteria tend to perform poorly; on the other hand, CST IV vaginal microbiota consisting of an assemblage of strict and facultative anaerobic bacteria, typically including species within *Gardnerella*, *Prevotella*, and *Fannyhessea* genera. Both CST II and CST V (dominated by *L. gasseri* and *L. jensenii*, respectively) are less prevalent and generally considered functionally analogous to CST I (France et al., 2022). As aforementioned, the association of CST III (dominated by *L. iners*) with a clinical presentation remains an open question and does not feature in our model as a distinct entity. Herein, we use the CST identity to refer to its typical microbial profile and implication on health outcomes: i.e., CST I is characterised by a high prevalence of lactobacilli and is compatible with optimal vaginal physiology, while CST IV is characterised by the presence of strict and facultative anaerobes

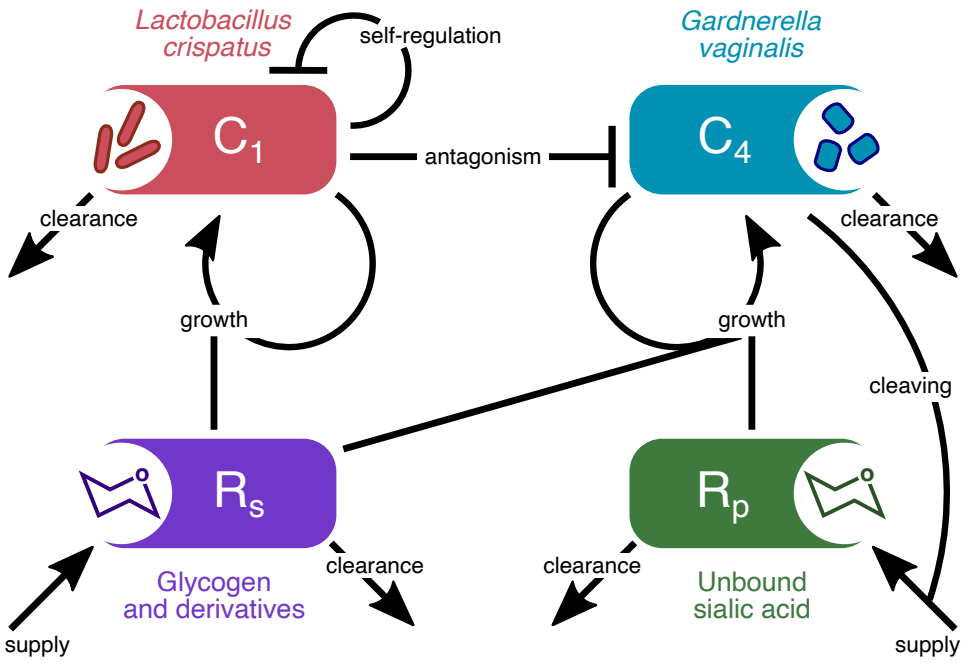

Figure 1: **Diagram of the consumer-resource vaginal microbiota model.** We track the dynamics of two vaginal microbiota types (CST I and CST IV associated bacteria; C_1 and C_4) and the shared (R_s) and private resource (R_p) utilised by the two communities. The arrows among compartments represent density-dependent interactions including resource-mediated bacterial growth; lactic-acid-mediated density regulation (self-regulation and antagonism); resource supply; sialic acid cleaving; and background clearance of microbes and resources. The representative species and, or molecules for each model component are listed. The corresponding mathematical model based on ordinary differential equations is detailed in the Methods section.

and is most often associated with adverse clinical presentations (France et al., 2022).

In our work, we track the availability of two types of nutritional resources (Fig. 1): first, glycogen and its derivatives, which constitute a universal energy source for all vaginal microbiota bacterial components (Tester and Al-Ghazzewi, 2018), and, second, sialic acid and fucose molecules, which are available as sialogycans attached to mucins that form mucus. Some common anaerobes can access these resources through the production of sialidases and fucosidases respectively, while *L. crispatus* is unable to leverage these resources for growth (Agarwal and

Lewis, 2021) as the majority of *L. crispatus* strains do not metabolise sialic acid. Furthermore, the strata formed by the sialic acid-cleaving bacteria (with potential biofilms) likely allow them to secure private access to this resource, as well as to the associated glycans.

Finally, we modelled the inhibitory interactions mediated by lactic acid produced by lactobacilli (Fig. 1). Acidity contributes to the ecology in the vaginal environment by differentially affecting bacterial activity. Bacteria commonly found in CST IV vaginal microbiota often require a vaginal pH > 4.5 to grow and are growth restricted at a lower pH (Aroutcheva et al., 2001; Ravel et al., 2011) while lactobacilli have a higher tolerance to acidic conditions but show growth inhibition at lower pH (< 4.0; i.e., self-inhibition) (Aroutcheva et al., 2001; Hammes and Hertel, 2015). We note that CST I antagonism towards CST IV in our model represents the net effect of all antagonism mechanisms, including lactic acid and other molecules produced by lactobacilli, such as bacteriocins, as well as the counter-adaptation processes that CST IV bacteria use (e.g., the production of biogenic amines) (France et al., 2022; Borgogna et al., 2021).

Changes in the composition and activity of vaginal microbiota can occur in rapid succession in the vaginal environment, including daily fluctuations (Ravel et al., 2013). Therefore, the model assumes that the resources and bacterial activity reach equilibrium rapidly, and our analysis focuses on steady-state conditions. Our model is based on a system of ordinary differential equations (ODEs): the mathematical formulation and analyses are described in Methods.

Insights from mathematical modelling

We find that the resource landscape of the vaginal microenvironment can explain the composition and structure of the vaginal microbiota. Broadly, our numerical analysis demonstrates that an initially CST I-dominated vaginal microbiota undergoes a transition to CST IV as the proportion of resource supply that is private to CST IV bacteria, χ, increases (Fig. 2). When

only a small fraction of resources is accessible privately to CST IV, CST I dominates the system since compounds like lactic acid directly inhibit bacteria associated with CST IV. When more private resources are available to CST IV, it gains dominance, as the benefits from the additional resources eventually outweigh the disadvantages caused by direct suppression from lactobacilli. We also observe an intermediate window of resource condition that allows for bistability (where either state, i.e., of single species-dominance, is stable, depending on the initial state of the system) and the stable coexistence of multiple species (Fig. 2; grey shading).

In addition to reducing the supply of private resources to CST IV, our model predicts that the transition from CST IV to CST I always requires the (re-)introduction or external seeding of CST I-associated bacteria, which was approximated in the model with a non-zero initial density of CST I ($q - 1 = 10^{-10}$ to 0.1; Fig. 2). Importation of external microbes to the vaginal milieu may take place through sexual intercourse, contamination from other body parts, and lactobacilli-based probiotics (Morison et al., 2005; Khan et al., 2018; Liu et al., 2023). In our model, if the external seeding of CST I bacteria is insufficient, the CST IV dominance is irreversible (Fig. 2a). This finding is consistent with our assumption of a closed system, wherein any increase in the bacterial populations is assumed to be generated solely by bacterial growth, precluding CST I from increasing in frequency if they are initially absent. However, a small initial seeding of CST I (e.g., at least 0.1% of the total initial frequency is made up of CST I despite CST IV dominance) is enough to cross over an unstable equilibrium (i.e., separatrix) and escape the basin of attraction associated with CST IV dominance as long as the resource landscape allows for CST I existence (i.e., sufficiently low χ; Fig. 2 b–d).

A larger external seeding of CST I-associated bacteria allows for a stable CST I community to be achieved across a broader range of resource conditions (Fig. 2), supporting that external seeding of CST I (i.e., lactobacilli-based probiotics) may represent a promising strategy to restore the lactobacilli-rich community. Conversely, the more the resource landscape is biased

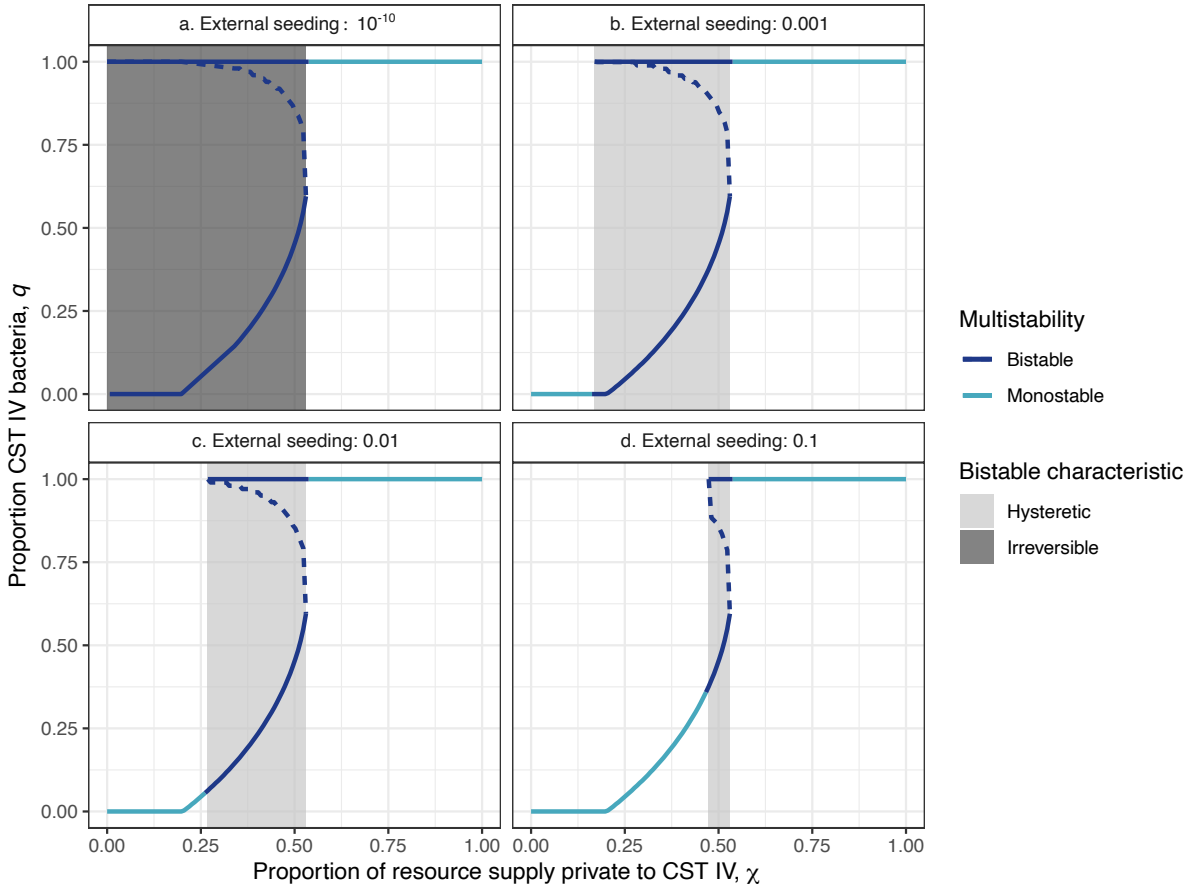

Figure 2: **Vaginal microbiota communities undergo a hysteretic or even irreversible transition to CST IV dominance as the proportion of resource supply private to CST IV increases.** Solid lines indicate stable steady states with light blue and dark blue indicating monostable and bistable states, respectively. The dashed lines indicate the separatrix, which separates two basins of attraction, each with a stable state. The derivation of χ is found in the Extended Methods S1.

toward the needs of CST I (smaller χ; Fig. 2), the smaller the perturbation required to establish stability involving CST I bacteria, i.e., away from a stable state dominated by CST IV bacteria ($q = 1$).

We observe that transitions to CST IV can be hysteretic, meaning that recovery from CST IV requires a larger reduction in the CST IV private resources (e.g., sialic acid and fucose) than the initial increase that triggered a transition from CST I to IV. While it is challenging to demon-

strate hysteresis in natural settings, hysteretic shifts along a resource gradient have been recently demonstrated in chemostats of intestinal microbes (Khazaei et al., 2020).

Corroborating theory with clinical data

We leverage human vaginal microbiota and metabolomics data to corroborate our theoretical prediction that the relative supply of resources private to CST IV is a driver of the vaginal microbiota composition. A key challenge in interrogating mechanistic model predictions with empirical data is that ecological processes of interest are often difficult or impossible to measure directly. For instance, it is not possible to directly observe the quantity of incoming resources (i.e., rates of glycogen and sialic acid supply). Fortunately, however, the measured quantity of resource-associated metabolites, such as sialic acid and glycogen derivatives (e.g., glucose, maltose, and maltotriose) can yield valuable insights when interpreted through the lens of a mechanistic ecological model. On their own, these metabolites may be misleading as proxies for resource supply because they are influenced by distinct ecological processes, including the resource supply rate, resource processing (e.g., sialic acid and fucose cleavage by CST IV bacteria), and consumption. For instance, a high concentration of unbound sialic acid might reflect either increased input of the bound form or elevated density of CST IV bacteria that release it through enzymatic cleavage. Our model addresses this issue by explicitly delineating the processes of resource consumption and sialic acid cleavage from resource supply, allowing us to derive an expression for resource supply that is independent of other processes that influence the concentration of unbound sialic acid (eq. 8 in Extended Methods S1): in brief, the key derived quantities, independent of bacterial densities, are the proportion of resource supply private to CST IV (χ) and the overall resource productivity ($\overline{\tau}$). By incorporating these expressions with in-vivo data on resource-associated metabolites and microbiota composition, as well as the relative growth rate of *Gardnerella vaginalis* to *Lactobacillus crispatus* (Figure

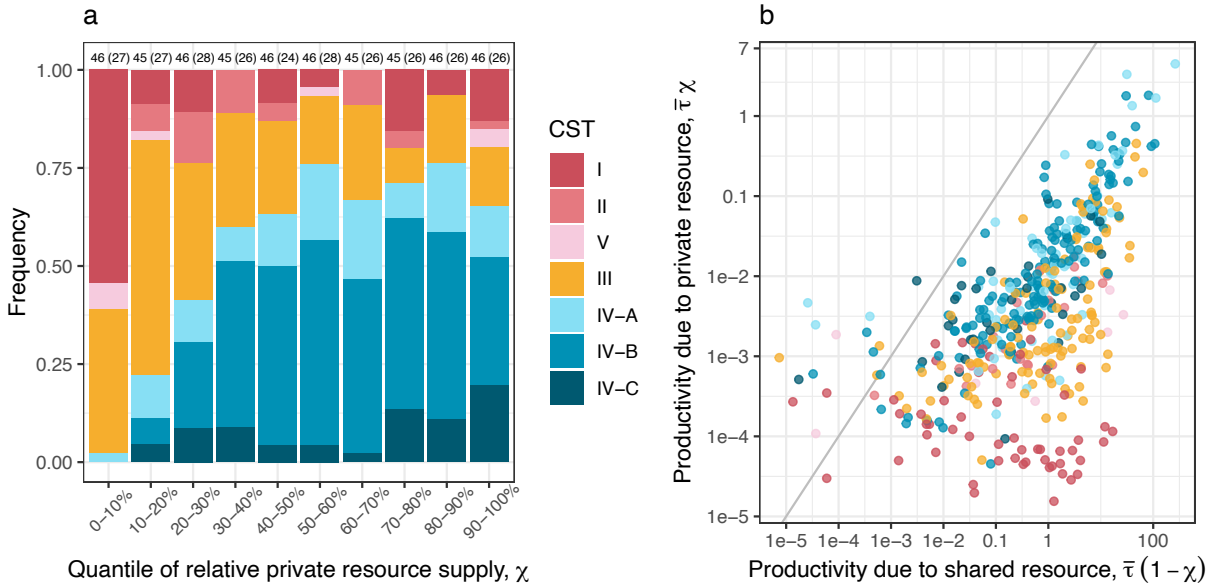

Figure 3: **Microbiota and metabolomics data support our model prediction that resource availability shapes human vaginal microbiota structure.** a) Higher supplies of private resources more frequently support CST IV-dominated communities. Shown are the frequencies of CSTs as a function of quantiles of empirically estimated χ, the proportion of supplied resource private to CST IV (i.e., informed by metabolomics and microbiological data). The numbers on top of each bar indicate the number of samples (and women in parentheses), respectively. b) Extremes in private resource availability lead to the exclusion of distinct community types—CSI I excluded under high supply and CST IV under low supply. CST classifications are shown as a function of productivity due to shared (x-axis; $\overline{\tau}(1-\chi)$) and private resources (y-axis; $\overline{\tau}\chi$), where $\overline{\tau}$ denotes the overall productivity. The grey diagonal line indicates an equal supply of the two resource types. The derivations of χ and $\overline{\tau}$ are found in the Extended Methods S1. Sensitivity analyses of these results to different model assumptions are provided in Supplementary Text S3.

S1), we estimate the extent of shared (i.e., glucose, maltose, and maltotriose) and private (i.e., sialic acid) resource supply for each clinical sample.

Analysis of data from this cohort corroborates that the composition of human vaginal microbiota is associated with variations in resource supply: an increased relative supply of private resources (χ) is associated with increased colonization by CST IV bacteria (Fig. 3a). In particular, at the lowest 10% quantile of the relative private resource supply, *L. crispatus*-dominated

CST I was the most common type (25 out of 46 samples) while the occurrence of CST IV was very rare (1 out of 46 samples). Beyond the 30% quantile, the CST IV frequency consistently exceeded 50% (Fig. 3a). Our findings were qualitatively identical across a wide range of model assumptions, with the exception of a scenario in which private resources are virtually inaccessible due to sialidase inactivity (Supplementary Text S3).

Visualising the CSTs over the productivity owing to the distinct resource types corroborates that the relative supply of shared versus private resources has strong explanatory power for a large window of the resource landscape — i.e., at intermediate levels of sialic acid supply — ($10^{-3} \lesssim \overline{\tau}\,\chi \lesssim 0.1$; Fig. 3b). In this regime, either increasing the supply of shared resources or decreasing the supply of private resources is associated with a reduction in CST IV, most notably in favour of *L. iners*-dominated CST III, which has been hypothesised to act as a functional gateway between CST I and CST IV (France et al., 2022). While resource-ratio theory—centred on relative resource supply—captures much of the observed variation in CST composition, deviations emerge at the extremes of the resource landscape. In particular, CST I and its functional analogues II and V are rarely found at high sialic acid supply (beyond $\overline{\tau}\,\chi = 10^{-2}$) while CST IV is rarely found at low sialic acid supply (below $\overline{\tau}\,\chi = 10^{-4}$) (Fig. 3b). These edge cases suggest that, at the extremes of sialic acid supply, community composition may be driven primarily by sialic acid availability alone.

Discussion

Community ecology has long been proposed as a useful framework for understanding human microbiota dynamics. Our theoretical and empirical findings provide compelling evidence that resource ecology is a key factor shaping the composition and structure of the vaginal microbiota. While previous studies have demonstrated that elevated glycogen levels favour lactobacilli (Mirmonsef et al., 2014) and that sialidase activity affords *G. vaginalis* access to resources not acces-

sible to lactobacilli (Lewis et al., 2013), our findings bring into focus the ecological mechanisms linking the resource landscape to the microbiota composition. Specifically, we demonstrate that CST IV communities depend on relatively high levels of private resources to gain a growth advantage over CST I, which otherwise creates a hostile environment for CST IV — for example, through the production of lactic acid. As a consequence, across much of the resource landscape, either increasing the supply of private resources or decreasing the shared resources would shift the balance in favour of CST IV.

Supplementing private resources to help beneficial microbiota has been proposed in the context of improving gut microbiota health (Boza et al., 2019). To date, it remains an open question if there are private resources for lactobacilli in the vaginal milieu. A recent study shows that these bacteria can hydrolyse innate immune peptides into free amino acids and metabolise them for their growth (Lebeau et al., 2022). However, the relative importance of this additional source and the extent to which CST IV-associated bacteria engage in similar metabolic feats remain an open question. A search for lactobacilli-private resources warrants further inquiry as our model demonstrates that private resource availability is a major force shaping the vaginal microbiota.

Mechanistic ecological modelling of vaginal microbiota is in its infancy (Lee et al., 2020, 2023). Thus, there are ample opportunities for the future development of system-specific details. For example, one could explicitly model *L. iners* (CST III), whose unique features (i.e., it has a small genome size and carries a cytotoxin, inerolysin) and role as a transitional, or gateway taxon between CST I and IV has been increasingly recognised in recent years (Holm et al., 2023). Even within CST I and IV, there are known differences between lineages. For example, some *L. crispatus* strains can metabolise sialic acid, when most cannot. Therefore, incorporating within-CST diversity into our model could yield equally interesting results as increasing the number of CSTs.

Another notable simplifying assumption in the present work is the omission of the immune system, despite its increasingly recognised role in shaping the vaginal microbiota (Ntuli et al., 2022). Recent studies have shed light on the complex interplay between immune responses and microbial composition in the vaginal environment (Delgado-Diaz et al., 2020; López-Filloy et al., 2022; Decout et al., 2024). The direction of causality, however, remains unclear—does the microbiota shape the immune profile, or does immune signalling drive microbial changes? In all likelihood, both processes occur, but the relative magnitude and context-dependence of their effects remain insufficiently understood (Smith and Ravel, 2017; France et al., 2022). Characterising these signalling pathways and summarising the net immune response to various constituents of the vaginal milieu will be a key challenge in the future.

Ecological details of the vaginal milieu are often tentatively known and variability among women and vaginal microbiota is rarely characterised quantitatively. Yet, our local sensitivity analyses (Supplementary Text S2) demonstrate that details of within-host ecology — including resource supply and acquisition, relative growth rates, and competition as well as external microbial input — play a key role in shaping the structure of the human vaginal microbiota. Specifically, the factors identified to promote CST IV communities included: low productivity of the vaginal microbiota, sialidase activity efficiency, high CST IV-associated bacteria growth rate, low lactic acid production (slow acidification affords increased competition for resources) and low antagonism from CST I (Supplementary Text S2). Fully parameterised with relevant data, mechanistic models would be able to generate quantitative predictions of the vaginal microbiota dynamics and compositions. Additionally, these models could facilitate *in silico* experiments that explore the consequences of interventions and counterfactual scenarios. To gain a systematic understanding of the ecological diversity of the vaginal microenvironment, the recent development of biomimetic *in vitro* models of vaginal epithelium offers a promising avenue (Mahajan et al., 2022). Biomimetic *in vitro* models enable the cultivation and manipulation of

reconstructed vaginal microbiota sourced from biological samples, providing a high-throughput approach for cataloguing the diversity of vaginal microbiota across different contexts.

While our work emphasises the pivotal role of the resource landscape in shaping the vaginal microbiota communities, it is worth noting other potential drivers. Factors such as the menstrual cycle, pregnancy, obesity, alcohol and tobacco use, antibiotics, ethnicity, stress, and the use of certain vaginal hygiene products have been implicated (Gajer et al., 2012; Mayer et al., 2015; Si et al., 2017; Amabebe and Anumba, 2018a; France et al., 2022; Kamiya et al., 2025). These factors may alter the physico-chemical conditions of the vaginal milieu (e.g., the formation of the vaginal mucus plug during pregnancy), while others (e.g., drug use) may also influence sexual behaviour, which in turn affects the exposure to BV-associated bacteria and sexually transmitted infections. To advance our mechanistic understanding of community transitions in real-world settings, mathematical models can be leveraged to interrogate how the influence of certain factors — for example, antibiotics that target certain bacteria — translates to the dynamics of vaginal microbiota communities.

A key advantage of the development of a mathematical model is to shed light on knowledge gaps in the literature, which future empirical studies may address. For example, the two resource types in our model are assumed perfectly substitutable, i.e., offer equal value to growth. Given that glycogen — a shared universal resource — is freely available in the extracellular milieu while sialic acid molecules — private resource only accessible to CST IV-associated bacteria — require activity by sialidases that cleave sialic acid from sialoglycans associated with mucins with potential handling time by the bacteria, it is conceivable that glycogen-fueled growth may be more energy efficient for CST IV-associated bacteria. Here again, in-vitro experiments could study the growth rate of CST IV-associated bacteria under varied nutritional combinations and identify optimal nutritional requirements.

In addition, our mechanistic model integrates both resource production and consumption,

enabling the role of the private resource to be inferred indirectly through modelling. Future studies employing direct sialidase activity assays, short-interval longitudinal sampling, or in-vitro perturbations would enable a more direct empirical assessment of the causal mechanisms governing sialic acid dynamics. Such positive feedback between mathematical model development and targeted experiments will facilitate the development of model-assisted prognostic tools and evidence-based treatment options.

Materials and methods

Mechanistic ecological model of vaginal microbiota

At the fundamental level, our model can be interpreted as a consumer-resource model (MacArthur, 1970) in which n communities of consumers (C_i, here bacteria) and their resource (R) are described by a set of ordinary differential equations:

$$\frac{\mathrm{d}C_i}{\mathrm{d}t} = \quad -\gamma_C\, C_i + \lambda_i \frac{R}{\kappa + R} C_i - \alpha_{j,i}\, C_j\, C_i \tag{1a}$$

$$\frac{\mathrm{d}R}{\mathrm{d}t} = \eta_R - \gamma_R\, R - \sum_{i=1}^{n} \lambda_i \frac{R}{\epsilon(\kappa + R)}\, C_i \tag{1b}$$

where the growth of consumer type i is determined by the maximum intrinsic growth rate (λ_i), conditioned upon the availability of the resource (R) following a Monod (or Holling type II) function ($R \mapsto \frac{R}{\kappa+R}$ where κ represents the half-saturation constant, i.e., resource needed to achieve half the maximum growth). The resource is supplied to the system at a constant rate (η_R) and the resource and consumers exit the system at a background rate of γ_R and γ_C, respectively. The resource is consumed by C_i at a rate $\lambda_i \frac{R}{\epsilon(\kappa+R)}$ where ϵ corresponds to the yield (i.e., units of resource consumed per bacterial growth). Unlike classic consumer-resource models (e.g., (Tilman, 1982)), in which the dynamics of a species only depend on its own density and that of the resources, we consider inhibitory interactions between two species through an $\alpha_{j,i}$ term capturing the impact of type j on i. Note that more generic versions of similar formulation,

but without Holling type II functions, have been developed to study microbial interactions (Sofonea, 2019), in which the resources correspond to interaction molecules such as bacteriocins or siderophores. Our work develops this generic model (eq. 1) focusing on two aspects of vaginal microbiota that functionally distinguish CST I from CST IV, namely differential resource use and lactic acid production with its inhibitory effect on bacterial populations.

a) *Differential resource use.* We model the availability of unbound sialic acid (i.e., private resource to CST IV) as a function of bacteria-independent resource supply η_{Rp} and the density of CST IV bacteria that cleave off sialic acid from mucus sialoglycan. Thus, we express the realised input of CST IV private resource as $\eta_{Rp}\frac{C_4}{\theta+C_4}$ where θ represents the half-saturation constant of sialic acid cleaving. There is considerable variability in CST IV bacteria to cleave off and utilise sialic acid from bound sources (Lewis et al., 2013). In our model, small θ represents strong sialidase activity, where the majority of bound sialic acid can be made available by a few CST IV bacteria. We carry out sensitivity analyses to explore the impact of θ (Supplementary Text S3).

b) *Inhibitory interactions mediated by lactic acid and other molecules.* Acidity is a key abiotic factor regulating microbial ecology in the vaginal environment (O'Hanlon et al., 2013). The acidity is primarily maintained by lactobacilli through the production of lactic acid during the fermentation of glycogen and its derivatives that fuel the majority of their growth (Amabebe and Anumba, 2018b). When dominated by lactobacilli, the vaginal milieu remains acidic with the pH ranging between 3.5 and 4.5. Acidity differentially affects bacterial communities. CST IV bacteria are commonly associated with vaginal pH > 4.5 as their growth is inhibited at a lower pH (Aroutcheva et al., 2001; Ravel et al., 2011). In contrast, lactobacilli have a higher tolerance to acidic conditions (with an inhibition pH below 4.0 (Aroutcheva et al., 2001; Hammes and Hertel, 2015)). In our model,

the lactic acid-mediated inhibitory effect on CST I and CST IV are denoted by $\alpha_{1,1}$ and $\alpha_{1,4}$, respectively, where $\alpha_{1,4} > \alpha_{1,1}$ because lactobacilli are more tolerant to acidic condition (France et al., 2022). We note that the inhibition rate $\alpha_{1,4}$ also encompasses other mechanisms through which CST I inhibits CST IV bacteria including hydrogen peroxide (H_2O_2) and bacteriocins (France et al., 2022). We ignore the reciprocal impact of CST IV to CST I ($\alpha_{4,1}$) as co-culture experiments have shown that *G. vaginalis* (CST IV) has negligible effects on *L. jensenii* (CST V, which is functionally akin to CST I) (Jackman et al., 2019). Several CST IV bacteria have evolved defence mechanisms including biogenic amines synthesis, which elevates the extracellular pH and alleviates the acidic stress for themselves as well as other bacteria (Borgogna et al., 2021; Shah and Swiatlo, 2008). Thus, $\alpha_{1,4}$ includes the net effect of inhibition by CST I and counteracting defence mechanisms of CST IV.

Our vaginal microbiota model (Fig. 1) is described by the following set of equations:

$$\frac{\mathrm{d}C_1}{\mathrm{d}t} = -\gamma_C\, C_1 + \frac{\lambda_1\, R_s\, C_1}{\kappa + R_s} - \alpha_{1,1}\, C_1^2 \tag{2a}$$

$$\frac{\mathrm{d}C_4}{\mathrm{d}t} = -\gamma_C\, C_4 + \frac{\lambda_4\, (R_p + R_s)\, C_4}{\kappa + R_p + R_s} - \alpha_{1,4}\, C_1\, C_4 \tag{2b}$$

$$\frac{\mathrm{d}R_p}{\mathrm{d}t} = \eta_{Rp}\frac{C_4}{\theta + C_4} - \gamma_R\, R_p - \frac{\lambda_4\, R_p\, C_4}{\epsilon(\kappa + R_p + R_s)} \tag{2c}$$

$$\frac{\mathrm{d}R_s}{\mathrm{d}t} = \eta_{R_s} - \gamma_R\, R_s - \frac{\lambda_1\, R_s\, C_1}{\epsilon(\kappa + R_s)} - \frac{\lambda_4\, R_s\, C_4}{\epsilon(\kappa + R_p + R_s)} \tag{2d}$$

The subscripts 1 and 4 refer to CST I and CST IV bacteria and the subscripts p and s refer to private and shared resources, respectively.

Model restructuring

To facilitate model exploration, we first restructure the above model (eq. 2) into equations tracking the dynamics of the total bacterial ($C = C_1 + C_4$) and resource ($R = R_p + R_s$)

densities and proportions that make up the CST IV bacteria ($q = \frac{C_4}{C}$) and their private resource ($p = \frac{R_p}{R}$). We focus our analysis on the relative quantities, p and q, in order to reduce the dimensionality of the original system — which involves two consumers and two resources — to a more tractable, one-dimensional framework. By working with relative rather than absolute quantities, we simplify the analysis without losing key ecological insights, allowing for a clearer and more tractable exploration than would be possible with the full two-by-two system. We then introduce χ, the proportion of newly supplied resource that is private to C_4 (such that $\eta_{R_p} = \chi\, \eta_R$ and $\eta_{R_s} = (1-\chi)\, \eta_R$ where η_R is the overall supply). We also define ψ as the ratio of CST IV inhibition and self-inhibition (i.e., $\frac{\alpha_{1,4}}{\alpha_{1,1}}$).

We then set the time scale of the system relative to the CST I growth rate (λ_1) such that selected parameters become relative to it and variables are dimensionless (i.e., non-dimensionalisation). Next, we assume a separation of timescales between consumers and resources and solve (owing to the Tikhonov-Fenichel theorem) for quasi-equilibrium conditions for resources such that the resource availability is held constant for a given density of consumers. In doing so, we now only explicitly track the consumer dynamics. In resource–consumer models, separating timescales is biologically justified when the dynamics of one variable — such as resource availability — occur on a much faster timescale than others, like consumer population changes. This reflects real biological systems, where resource concentrations can fluctuate rapidly due to environmental inputs or microbial uptake, while their assimilation into biomass (i.e., bacterial growth) typically proceeds more slowly, constrained by intracellular metabolic processing and regulation. By leveraging this disparity, time-scale separation simplifies model analysis and captures essential system behaviour without sacrificing biological realism.

Details about non-dimensionalisation are found in the Extended Methods.

Clinical data

We took advantage of the previously published human vaginal microbiota data from a longitudinal clinical study (Ravel et al., 2013) and previously unpublished metabolomics data from the same cohort to parameterise the mechanistic ecological model and evaluate model predictions.

In particular, we could measure proxies for four of our variables. For the total amount of bacteria (C), the proxy was the ratio between the total bacterial DNA quantity in a sample and the median total bacterial DNA quantity in each of the samples using the qPCR data. For the proportion of CST IV bacteria (q), we used the proportion of CST IV-associated bacteria (here, the ones listed by the CST classification algorithm, VALENCIA (France et al., 2020)) in the 16S metabarcoding data. For the total amount of resources (R), we used the sum of the abundance of four nutrients (glucose, maltose, maltotriose, and sialic acid) in a sample normalised by the median of these sums in all the samples using the metabolomics data. Finally, for the proportion of sialic acid among the resources (p), we used the ratio between the abundance of sialic acid and that of the four nutrients in the metabolomics data.

Details about the interfacing between the analytical expressions of the model and this data to parameterise the model can be found in the Extended Methods. The clinical study protocol was approved by the Institutional Review Board of the Johns Hopkins University School of Medicine and the University of Maryland School of Medicine. Written informed consent was appropriately obtained from all participants, which included permission to use the samples obtained in future studies.

Participants and sample collection

Briefly, the study — originally published in Ravel et al. (2013)— focused on investigating bacterial vaginosis and included 135 women from Birmingham, Alabama, USA. At the beginning of the study, participants underwent an assessment that included an extensive questionnaire on

their medical, dental, obstetric, hygiene, sexual, and behavioural histories. They were also asked to self-collect three mid-vaginal swabs daily for ten weeks for use in genomic sequencing and metabolomics analysis. In addition, participants recorded their daily hygienic practices and sexual activities in a diary using a standardised form. Pelvic examinations were conducted at the time of enrolment and at weeks 5 and 10 or at interim times if the participant reported vaginal symptoms. Any symptomatic BV was diagnosed by the clinician and treated using a standard of BV care practices (Workowski and Bolan, 2015). Integration with our mechanistic model requires a sample to be processed for both genomic sequencing (16S metabarcoding and qPCR DNA quantity) and resource-related metabolomics profiling. In total, 540 samples from 82 women met our criteria.

Microbiota data: DNA was extracted from vaginal swabs and then the V3-V4 region of the bacterial 16S rRNA gene was amplified and sequenced on an Illumina HiSeq 2500 as described previously (Holm et al., 2019). The paired end sequences were then processed using DADA2 (Callahan et al., 2016) generating amplicon sequence variants (ASVs). The ASVs were then assigned taxonomy using a combination of the RDP Naive Bayesian Classifier (Wang et al., 2007) trained on the SILVA 16S rRNA gene database (Quast et al., 2012) and speciateIT software (Holm et al., 2024). Finally, community state types (CSTs) were assigned using VALENCIA software (France et al., 2020). The total bacterial DNA was quantified using qPCR. We normalised the bacterial DNA quantity with the median total qPCR quantity of all samples such that the default value ($C = 1$) corresponds to the study median as explained above. These data have been published in Ravel et al. (2013).

Metabolomics data: As indicated above, we also provide original data on metabolites from the same vaginal swabs. These were processed by Metabolon for global liquid chromatography-mass spectrometry (LC-MS) metabolomic profiling, which provides information roughly proportional to the true concentration of the metabolites in a sample (Lawton et al., 2008). Metabolomics

readings were batch-standardised by Metabolon. An important limitation of global metabolomic profiling is that it provides an imperfect reflection of the metabolite quantity in a sample. Specifically, the Pearson's correlation coefficient between the raw global metabolomics output and concentrations measured by the targeted metabolomics approach (which offers the most accurate quantitative information) is generally around 0.7 (Wulff et al., 2018). As metabolites are small molecules less than 1.5 kDa in size (Thevis et al., 2017), whole glycogen molecules are not detected by metabolomics profiling. Thus, we focused on three glycogen breakdown products (namely, glucose, maltose, and maltotriose) as a proxy for the shared resource. We refer to the sum of these three metabolites as glycogen derivatives. As a proxy for the private resource used by CST IV, we used N-acetylneuraminate, the anionic form of the acetylneuraminic acid (Neu5Ac), which is the form of sialic acid found in the vaginal environment (Lewis et al., 2013). Fucose, another private resource for CST IV, was not found in the metabolomics panel. With the aforementioned caveat that global metabolomic profiling provides an imperfect reflection of metabolite quantity, we use resource-related metabolomics data in two ways: total resource-related metabolite quantity (i.e., the sum of glucose, maltose, maltotriose and sialic acid) and proportion of CST IV private resource (i.e., sialic acid relative to the total resource quantity). We normalised raw resource metabolite values by the total resource-related metabolites across all samples such that the default total resource value ($R = 1$) corresponds to the study median.

Evaluating model predictions in light of clinical data

To corroborate the role of resources in shaping real vaginal microbiota communities, we graphically explored the relationship between the empirically informed proportion of CST IV private resources (χ; see eq. 8b in the Extended Methods S1) and CST classification. We also visualised CST classification as a function of shared productivity (i.e., yield-adjusted glycogen derivative supply, i.e., $\overline{\tau}(1 - \chi)$) and private productivity (i.e., yield-adjusted sialic acid supply, $\overline{\tau}\ \chi$) to

explore the influence of the absolute resource supply in shaping bacterial community composition. As the half-saturation constants (i.e., κ and θ) are not precisely informed by data, we do not rely on the absolute value of $\overline{\tau}$ and χ. Nonetheless, our sensitivity analyses demonstrate that the rank order of $\overline{\tau}$ and χ estimates are largely consistent across a range of θ and κ such that our findings are qualitatively unaffected except in the case of very large θ such that private resources are virtually inaccessible. (Supplementary Text S3).

Acknowledgements

The authors acknowledge support from the Fondation pour la Recherche Medicale (SPF202005011951 to TK), the Centre National de la Recherche Scientifique, the Agence Nationale de la Recherche contre le Sida (ANRS—MIE, to NT), the US National Institute of Health (NR015495 to JR).

Data and software programmes used in this study are provided as Auxiliary files. Amplicon sequence data used in this study are available in the NCBI Short Read Archive (SRA) deposited under the BioProject PRJNA208535. Vaginal shotgun metagenomics data used to estimate *in vivo* growth rates can be found in the NCBI SRA under the BioProject PRJNA797778.

Supporting Information

The supporting information PDF file contains the Extended Methods and Supplementary Results. All the data and scripts used to generate the figures can be accessed at `https://doi.org/10.57745/3GJF2Z`.

References

Agarwal K, Lewis AL. **2021**. Vaginal sialoglycan foraging by *Gardnerella vaginalis*: mucus barriers as a meal for unwelcome guests? *Glycobiology* **31**:667–680.

Amabebe E, Anumba DOC. **2018**a. Psychosocial stress, cortisol levels, and maintenance of vaginal health. *Frontiers in Endocrinology* **9**:568. doi:10.3389/fendo.2018.00568.

Amabebe E, Anumba DOC. **2018**b. The vaginal microenvironment: The physiologic role of *Lactobacilli*. *Frontiers in Medicine* **5**:1–11. doi:10.3389/fmed.2018.00181.

Aroutcheva A, Gariti D, Simon M, Shott S, Faro J, Simoes JA, et al. 2001. Defense factors of vaginal lactobacilli. *American Journal of Obstetrics and Gynecology* **185**:375–379.

Borgogna JLC, Shardell MD, Grace SG, Santori EK, Americus B, Li Z, et al. 2021. Biogenic amines increase the odds of bacterial vaginosis and affect the growth of and lactic acid production by vaginal *Lactobacillus* spp. *Applied and Environmental Microbiology* **87**:e03068–20. doi:10.1128/AEM.03068-20.

Boza G, Worsley SF, Yu DW, Scheuring I. **2019**. Efficient assembly and long-term stability of defensive microbiomes via private resources and community bistability. *PLOS Computational Biology* **15**:e1007109.

Bucci V, Bradde S, Biroli G, Xavier JB. **2012**. Social interaction, noise and antibiotic-mediated switches in the intestinal microbiota. *PLOS Computational Biology* **8**:e1002497.

Bucci V, Tzen B, Li N, Simmons M, Tanoue T, Bogart E, et al. 2016. Mdsine: Microbial dynamical systems inference engine for microbiome time-series analyses. *Genome Biology* **17**:1–17.

Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJA, Holmes SP. **2016**. Dada2: High-resolution sample inference from illumina amplicon data. *Nature methods* **13**:581–583.

Costea PI, Hildebrand F, Arumugam M, Bäckhed F, Blaser MJ, Bushman FD, et al. 2018.

Enterotypes in the landscape of gut microbial community composition. *Nature Microbiology* **3**:8–16.

Costello EK, Stagaman K, Dethlefsen L, Bohannan BJ, Relman DA. **2012**. The application of ecological theory toward an understanding of the human microbiome. *Science* **336**:1255–1262.

Decout A, Krasias I, Roberts L, Gimeno Molina B, Charenton C, Brown Romero D, et al. 2024. Lactobacillus crispatus s-layer proteins modulate innate immune response and inflammation in the lower female reproductive tract. *Nature Communications* **15**:10879.

Delgado-Diaz DJ, Tyssen D, Hayward JA, Gugasyan R, Hearps AC, Tachedjian G. **2020**. Distinct immune responses elicited from cervicovaginal epithelial cells by lactic acid and short chain fatty acids associated with optimal and non-optimal vaginal microbiota. *Frontiers in Cellular and Infection Microbiology* **9**:446.

France M, Alizadeh M, Brown S, Ma B, Ravel J. **2022**. Towards a deeper understanding of the vaginal microbiota. *Nature Microbiology* **7**:367–378.

France MT, Ma B, Gajer P, Brown S, Humphrys MS, Holm JB, et al. 2020. Valencia: a nearest centroid classification method for vaginal microbial communities based on composition. *Microbiome* **8**:1–15.

Gajer P, Brotman RM, Bai G, Sakamoto J, Schütte UM, Zhong X, et al. 2012. Temporal dynamics of the human vaginal microbiota. *Science Translational Medicine* **4**:132ra52–132ra52.

Gonze D, Coyte KZ, Lahti L, Faust K. **2018**. Microbial communities as dynamical systems. *Current opinion in microbiology* **44**:41–49.

Haahr T, Zacho J, Bräuner M, Shathmigha K, Skov Jensen J, Humaidan P. **2019**. Reproductive outcome of patients undergoing in vitro fertilisation treatment and diagnosed with bacterial vaginosis or abnormal vaginal microbiota: a systematic PRISMA review and meta-analysis. *BJOG* **126**:200–207. doi:10.1111/1471-0528.15178.

Hammes WP, Hertel C. **2015**. *Lactobacillus*, John Wiley & Sons, Ltd, 1–76. doi:https://doi.org/10.1002/9781118960608.gbm00604. URL `https://onlinelibrary.wiley.com/doi/abs/10.1002/9781118960608.gbm00604`.

Hill G, Eschenbach D, Holmes K. **1984**. Bacteriology of the vagina. *Scandinavian Journal of Urology and Nephrology. Supplementum* **86**:23–39.

Holm JB, Carter KA, Ravel J, Brotman RM. **2023**. Lactobacillus iners and genital health: molecular clues to an enigmatic vaginal species. *Current Infectious Disease Reports* **25**:67–75.

Holm JB, Gajer P, Ravel J. **2024**. SpeciateIT and vSpeciateDB: novel, fast, and accurate per sequence 16S rRNA gene taxonomic classification of vaginal microbiota. *BMC Bioinformatics* **25**:313. doi:10.1186/s12859-024-05930-3.

Holm JB, Humphrys MS, Robinson CK, Settles ML, Ott S, Fu L, et al. 2019. Ultrahigh-throughput multiplexing and sequencing of >500-base-pair amplicon regions on the illumina hiseq 2500 platform. *mSystems* **4**:10.1128/msystems.00029–19. doi:10.1128/msystems.00029-19.

Hooks KB, O'Malley MA. **2017**. Dysbiosis and its discontents. *MBio* **8**:e01492–17.

Jackman CM, Deans KW, Forney LJ, Lin XN. **2019**. Microdroplet co-cultivation and interaction characterization of human vaginal bacteria. *Integrative Biology* **11**:69–78.

Kamiya T, Tessandier N, Elie B, Bernat C, Boué V, Grasset S, et al. 2025. Factors shaping vaginal microbiota long-term community dynamics in young adult women. *Peer Community Journal* **5**:pcjournal.527. doi:10.24072/pcjournal.527.

Kaul RB, Kramer AM, Dobbs FC, Drake JM. **2016**. Experimental demonstration of an allee effect in microbial populations. *Biology Letters* **12**:20160070.

Khan KN, Fujishita A, Hiraki K, Kitajima M, Nakashima M, Fushiki S, et al. 2018. Bacterial contamination hypothesis: a new concept in endometriosis. *Reproductive Medicine and Biology* **17**:125–133.

Khazaei T, Williams RL, Bogatyrev SR, Doyle JC, Henry CS, Ismagilov RF. **2020**. Metabolic multistability and hysteresis in a model aerobe-anaerobe microbiome community. *Science Advances* **6**:eaba0353.

Lawton KA, Berger A, Mitchell M, Milgram KE, Evans AM, Guo L, et al. 2008. Analysis of the adult human plasma metabolome. *Pharmacogenomics* **9**:383–397. doi:10.2217/14622416.9.4.383.

Lebeau A, Bruyere D, Roncarati P, Peixoto P, Hervouet E, Cobraiville G, et al. 2022. Hpv infection alters vaginal microbiome through down-regulating host mucosal innate peptides used by lactobacilli as amino acid sources. *Nature Communications* **13**:1076.

Lee CY, Cheu RK, Lemke MM, Gustin AT, France MT, Hampel B, et al. 2020. Quantitative modeling predicts mechanistic links between pre-treatment microbiome composition and metronidazole efficacy in bacterial vaginosis. *Nature Communications* **11**:1–12.

Lee CY, Diegel J, France MT, Ravel J, Arnold KB. **2023**. Evaluation of vaginal microbiome equilibrium states identifies microbial parameters linked to resilience after menses and antibiotic therapy. *PLOS Computational Biology* **19**:e1011295.

Lewis WG, Robinson LS, Gilbert NM, Perry JC, Lewis AL. **2013**. Degradation, foraging, and depletion of mucus sialoglycans by the vagina-adapted actinobacterium *Gardnerella vaginalis*. *Journal of Biological Chemistry* **288**:12067–12079.

Liu P, Lu Y, Li R, Chen X. **2023**. Use of probiotic lactobacilli in the treatment of vaginal infections: In vitro and in vivo investigations. *Frontiers in Cellular and Infection Microbiology* **13**:391.

López-Filloy M, Cortez FJ, Gheit T, Cruz y Cruz O, Cruz-Talonia F, Chávez-Torres M, et al. 2022. Altered vaginal microbiota composition correlates with human papillomavirus and mucosal immune responses in women with symptomatic cervical ectopy. *Frontiers in Cellular and Infection Microbiology* **12**:884272.

MacArthur R. **1970**. Species packing and competitive equilibrium for many species. *Theoretical Population Biology* **1**:1–11. doi:10.1016/0040-5809(70)90039-0.

Mahajan G, Doherty E, To T, Sutherland A, Grant J, Junaid A, et al. 2022. Vaginal microbiome-host interactions modeled in a human vagina-on-a-chip. *Microbiome* **10**:201.

Mayer BT, Srinivasan S, Fiedler TL, Marrazzo JM, Fredricks DN, Schiffer JT. **2015**. Rapid and profound shifts in the vaginal microbiota following antibiotic treatment for bacterial vaginosis. *The Journal of Infectious Diseases* **212**:793–802. doi:10.1093/infdis/jiv079.

McKinnon LR, Achilles SL, Bradshaw CS, Burgener A, Crucitti T, Fredricks DN, et al. 2019. The Evolving Facets of Bacterial Vaginosis: Implications for HIV Transmission. *AIDS Research and Human Retroviruses* **35**:219–228. doi:10.1089/aid.2018.0304.

Mirmonsef P, Hotton AL, Gilbert D, Burgad D, Landay A, Weber KM, et al. 2014. Free glycogen in vaginal fluids is associated with lactobacillus colonization and low vaginal ph. *PLOS One* **9**:e102467.

Morison L, Ekpo G, West B, Demba E, Mayaud P, Coleman R, et al. 2005. Bacterial vaginosis in relation to menstrual cycle, menstrual protection method, and sexual intercourse in rural gambian women. *Sexually Transmitted Infections* **81**:242–247.

Ntuli L, Mtshali A, Mzobe G, Liebenberg LJ, Ngcapu S. **2022**. Role of immunity and vaginal microbiome in clearance and persistence of human papillomavirus infection. *Frontiers in Cellular and Infection Microbiology* **12**:927131.

O'Hanlon DE, Moench TR, Cone RA. **2013**. Vaginal ph and microbicidal lactic acid when lactobacilli dominate the microbiota. *PLOS One* **8**:e80074.

Peebles K, Velloza J, Balkus JE, McClelland RS, Barnabas RV. **2019**. High global burden and costs of bacterial vaginosis: a systematic review and meta-analysis. *Sexually transmitted diseases* **46**:304–311.

Quast C, Pruesse E, Yilmaz P, Gerken J, Schweer T, Yarza P, et al. 2012. The silva ribosomal rna gene database project: improved data processing and web-based tools. *Nucleic acids research* **41**:D590–D596.

Ravel J, Brotman RM, Gajer P, Ma B, Nandy M, Fadrosh DW, et al. 2013. Daily temporal dynamics of vaginal microbiota before, during and after episodes of bacterial vaginosis. *Microbiome* **1**:1–6.

Ravel J, Gajer P, Abdo Z, Schneider GM, Koenig SS, McCulle SL, et al. 2011. Vaginal microbiome of reproductive-age women. *Proceedings of the National Academy of Sciences* **108**:4680–4687.

Shah P, Swiatlo E. **2008**. A multifaceted role for polyamines in bacterial pathogens. *Molecular Microbiology* **68**:4–16.

Si J, You HJ, Yu J, Sung J, Ko G. **2017**. Prevotella as a hub for vaginal microbiota under the influence of host genetics and their association with obesity. *Cell Host & Microbe* **21**:97–105. doi:10.1016/j.chom.2016.11.010.

Smith SB, Ravel J. **2017**. The vaginal microbiota, host defence and reproductive physiology. *Journal of Physiology* **595**:451–463.

Sofonea MT. **2019**. A global asymptotic stability condition for a Lotka–Volterra model with indirect interactions. *Applicable Analysis* **98**:1636–1645. doi:10.1080/00036811.2018.1437417.

Spear GT, French AL, Gilbert D, Zariffard MR, Mirmonsef P, Sullivan TH, et al. 2014. Human α-amylase present in lower-genital-tract mucosal fluid processes glycogen to support vaginal colonization by *Lactobacillus*. *Journal of Infectious Diseases* **210**:1019–1028.

Tester R, Al-Ghazzewi FH. **2018**. Intrinsic and extrinsic carbohydrates in the vagina: a short review on vaginal glycogen. *International Journal of Biological Macromolecules* **112**:203–206.

Thevis M, Krug O, Geyer H, Schänzer W. **2017**. Expanding analytical options in sports drug testing: Mass spectrometric detection of prohibited substances in exhaled breath. *Rapid Communications in Mass Spectrometry* **31**:1290–1296.

Tilman D. **1980**. Resources: a graphical-mechanistic approach to competition and predation. *American Naturalist* **116**:362–393.

Tilman D. **1982**. *Resource Competition and Community Structure*. Monographs in Population Biology. Princeton University Press.

van de Wijgert JH. **2017**. The vaginal microbiome and sexually transmitted infections are interlinked: consequences for treatment and prevention. *PLOS Medicine* **14**:e1002478.

Vet S, Gelens L, Gonze D. **2020**. Mutualistic cross-feeding in microbial systems generates bistability via an allee effect. *Scientific Reports* **10**:7763.

Wang Q, Garrity GM, Tiedje JM, Cole JR. **2007**. Naive bayesian classifier for rapid assignment of rrna sequences into the new bacterial taxonomy. *Applied and environmental microbiology* **73**:5261–5267.

Workowski KA, Bolan GA. **2015**. Sexually transmitted diseases treatment guidelines, 2015. *Morbidity and Mortality Weekly Report: Recommendations and reports* **64**:1–137.

Wulff JE, Mitchell MW, et al. 2018. A comparison of various normalization methods for lc/ms metabolomics data. *Advances in Bioscience and Biotechnology* **9**:339.