



Benchmarking gut microbiome indices within and across populations

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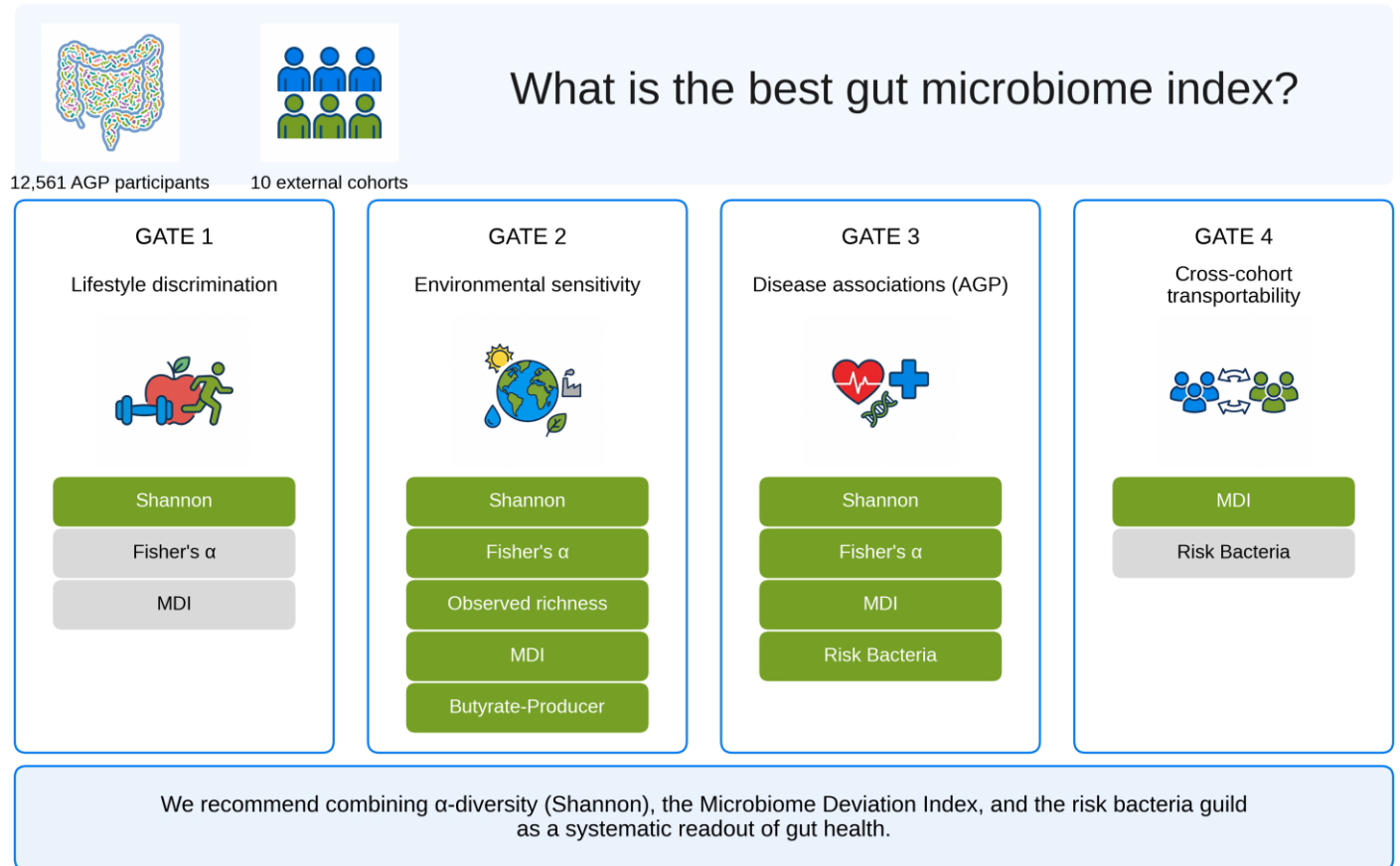


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Introduction

- Summary indices (alpha diversity, F/B ratio, functional guilds, MDI) are widely used to read gut health
- They are seldom benchmarked head-to-head, and rarely tested beyond a single cohort
- This study: 12,561 AGP participants x 10 indices x 3 within-cohort tasks + 10 external cohorts
- We propose a three-index panel and a reusable benchmarking framework





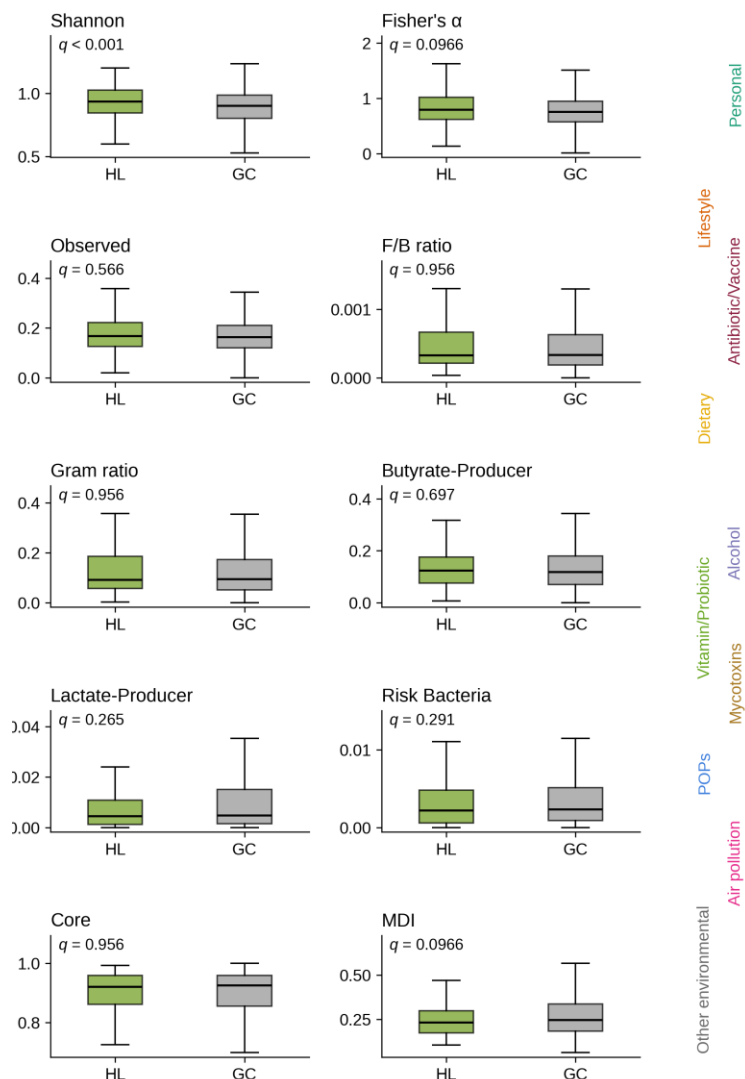
Highlights

- ① Ten widely used gut-microbiome indices benchmarked head-to-head in 12,561 AGP participants and tested in 10 external cohorts (5 T2D, 5 IBD)
- ② Shannon diversity is the only robust discriminator of a stringent healthy-lifestyle reference, but does not generalize across cohorts
- ③ The Microbiome Deviation Index (MDI) shows the strongest environmental sensitivity and disease associations, replicating externally for IBD (OR 1.85, $p = 0.017$)
- ④ No single index wins all tasks - we recommend a three-index panel: alpha diversity (Shannon) + MDI + risk bacteria guild



Results 1

(A) Discrimination of healthy-lifestyle individuals (HL n = 177 vs GC n = 12,384)



(B)



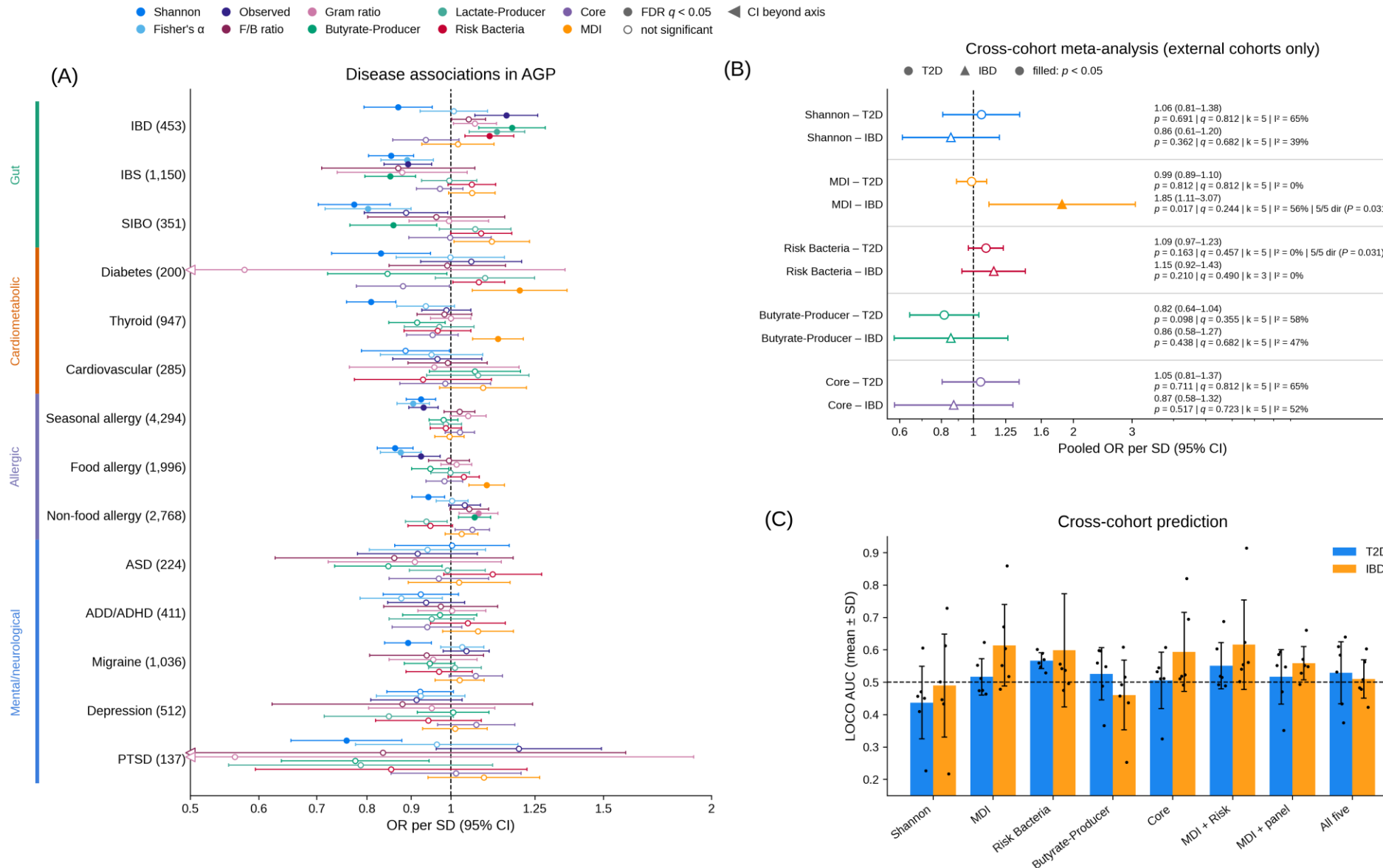
Key findings: Within-cohort benchmark - lifestyle discrimination and environmental sensitivity

- Only Shannon diversity survives demographically matched healthy-lifestyle discrimination
- Shannon, Fisher's alpha, observed richness, MDI and butyrate producers respond most to environment
- Taxonomic ratios (F/B, Gram ratio) are essentially inert

Figure 1. Healthy-lifestyle discrimination (A) and environmental sensitivity (B) of the 10 indices.



Results 2



Key findings: Disease associations, cross-cohort validation, and prediction

- Shannon protective for 10/14 chronic conditions
- MDI replicates externally for IBD: OR 1.85 (1.11-3.07), $p = 0.017$
- Risk bacteria direction-consistent in 5/5 T2D cohorts (sign test $p = 0.031$)
- Single-index prediction is modest (AUC 0.52-0.62)

Figure 2. Disease associations (A), cross-cohort pooled estimates (B), and prediction benchmark (C).



Conclusions: a three-index panel

- ❑ No single index is universally best - each captures a different facet of the ecosystem
- ❑ Alpha diversity (Shannon): best readout of within-cohort community state, but cohort-bound
- ❑ MDI: strongest environmental sensitivity and disease association; replicated for IBD
- ❑ Risk bacteria guild: most direction-consistent cross-cohort disease signal (5/5 T2D cohorts)
- ❑ Recommended: alpha diversity + MDI + risk bacteria as a systematic readout of gut health



Summary

- ❑ We benchmarked 10 widely used gut-microbiome indices in 12,561 AGP participants and tested their cross-cohort transportability in 10 independent cohorts.
- ❑ A generalizability hierarchy emerged: diversity metrics dominate within a cohort but are cohort-bound; MDI combines environmental sensitivity with disease-association strength and transfers partially; the risk bacteria guild shows the most consistent cross-cohort direction of effect.
- ❑ We recommend a three-index panel - alpha diversity (Shannon), MDI, and the risk bacteria guild - as a systematic readout for population-level microbiome assessment.

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