Tools for mining biomarkers from -omics data in case-control clinical studies

Host-Microbiome Factors in Cardiovascular Disease, PI Sofia Kirke Forslund[1,2,3,4]

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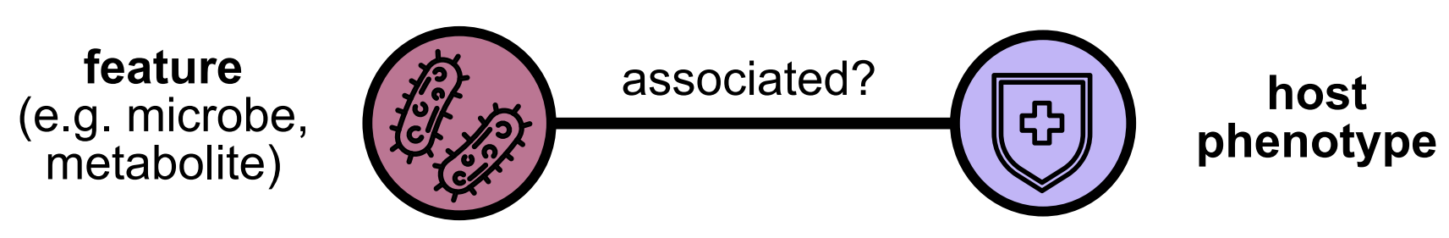
Univariate association tests are sensitive, but susceptible to confounding

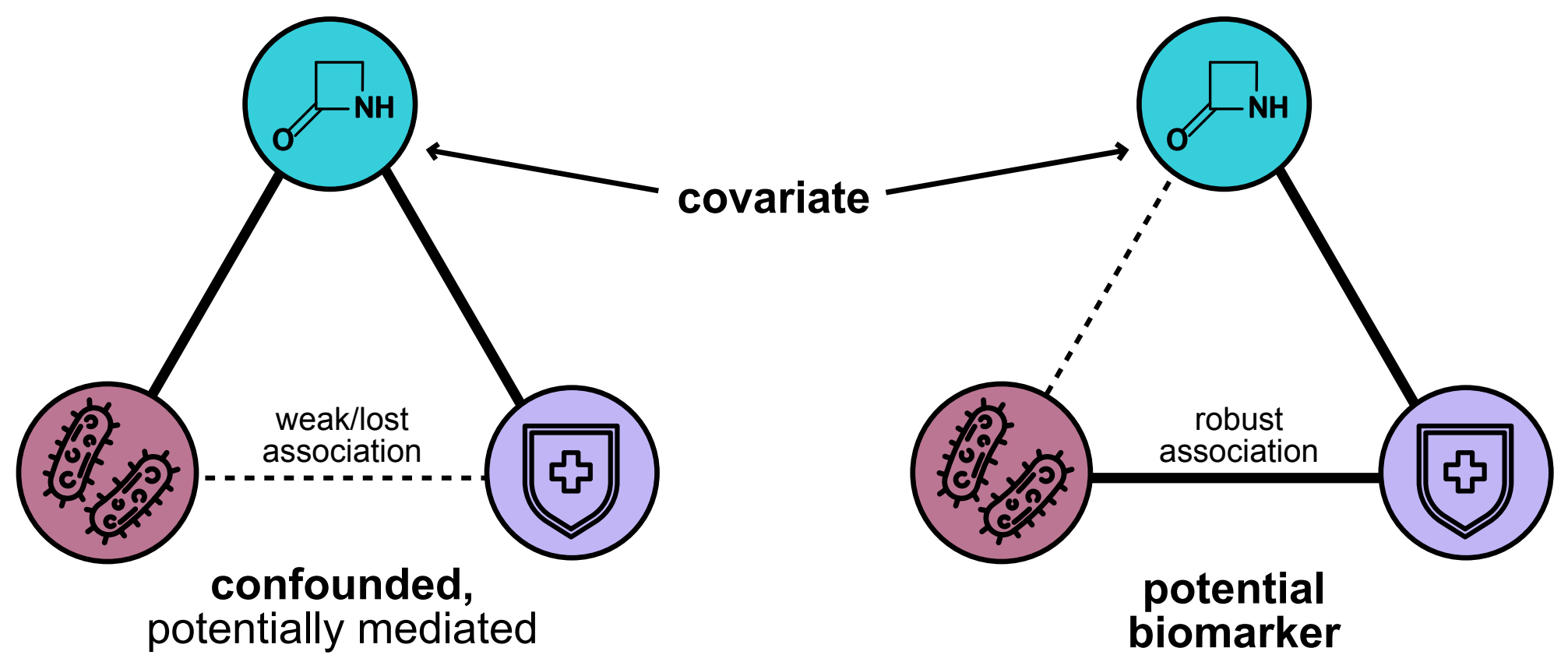
▶ Clinical microbiome research seeks to characterize **health and disease states** using **high-throughput molecular data** types, typically by comparing **case-control groups**

▶ **Univariate testing** compares groups **feature-by-feature** to identify disease- or phenotype-associated signals



▶ To ensure **robust** findings, and **generate more precise mechanistic hypotheses**, association testing frameworks must incorporate information about known **clinical covariates**

▶ If inclusion of a covariate (**adjustment**) weakens or displaces the feature-phenotype association, it is not robust!

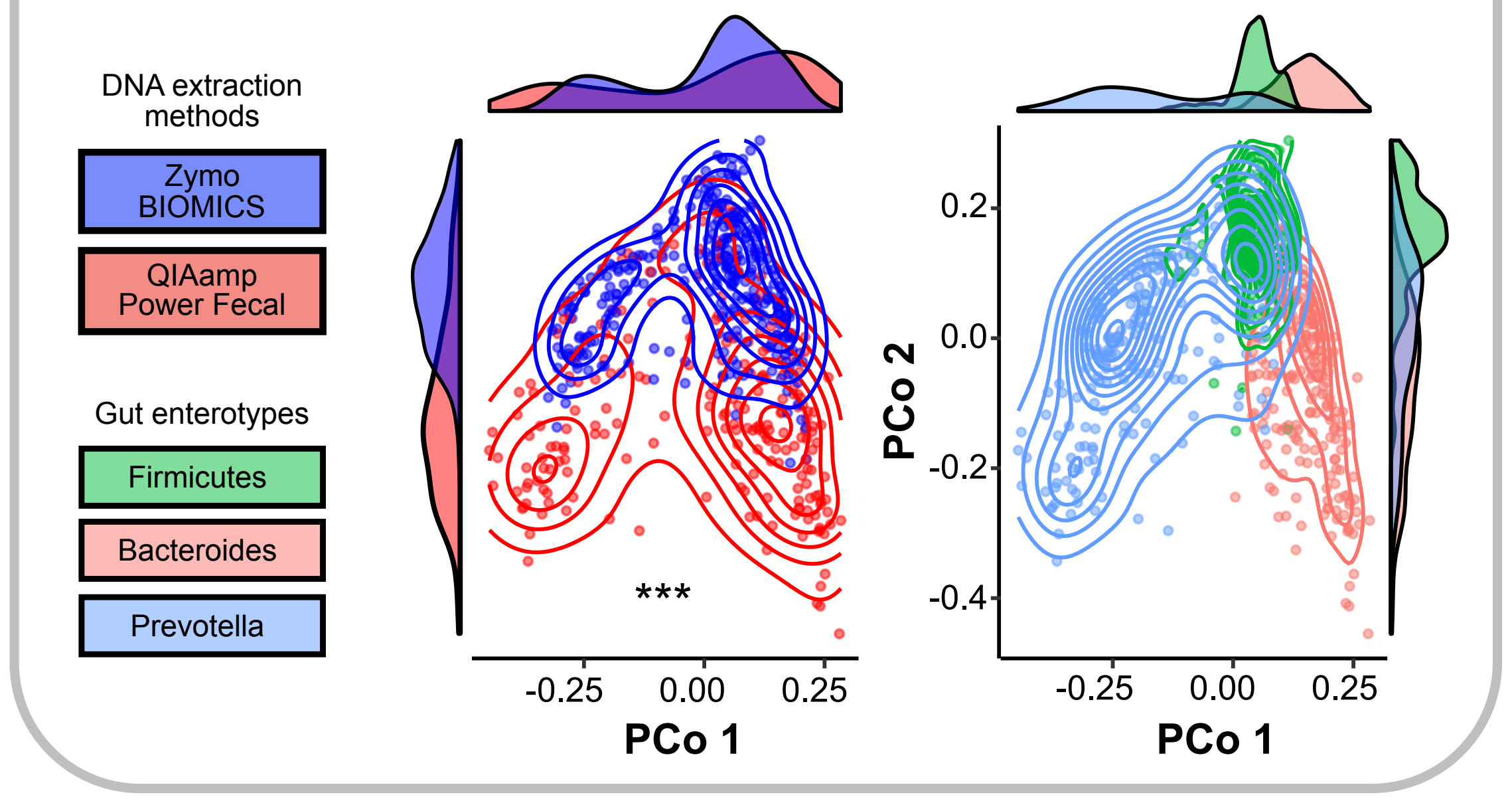


COLLECT Study¹

▶ Non-standardized **experimental protocols** can generate **technical effects** which can outweigh biological effects and obfuscate **downstream analysis**, if not accounted for

▶ We **benchmarked** several sample **storage, isolation, and extraction methods** and found the latter to explain about **6% of overall microbiome variability**

▶ Below, the extraction method dramatically impacted **enterotype determination**, with Zymo and Qiagen enriched for Firmicutes and Bacteroidetes, respectively

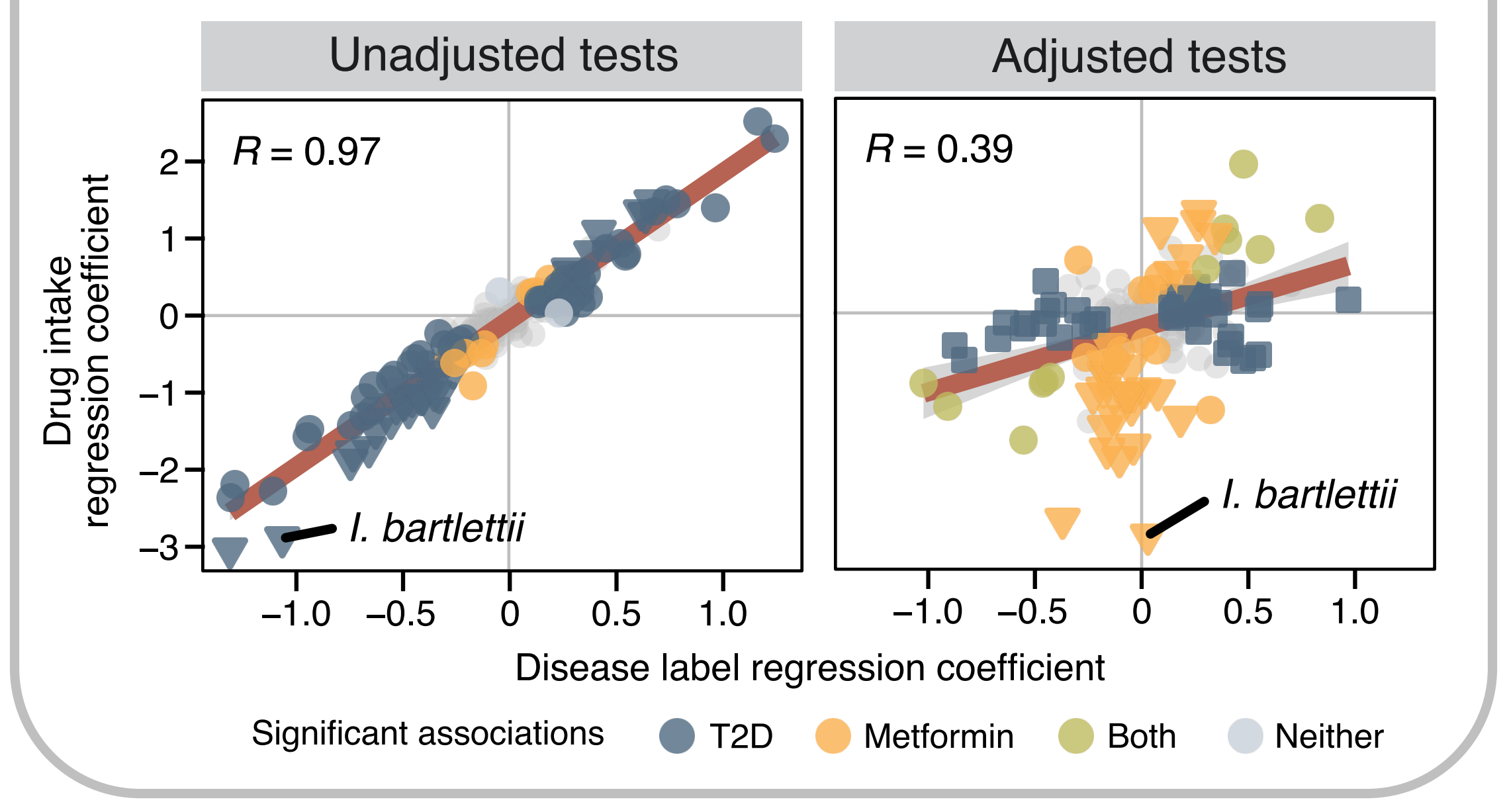


MetaCardis² and MetaDrugs

▶ **Multiple and combination drug therapies** are common in cardiometabolic disease groups, producing myriad effects on host systems and **potentially confounding** simple comparisons with **non-medicated healthy controls**

▶ ***Post hoc* stratification** and adjusted tests are able to diagnose confounding and **disentangle disease signals**

▶ Below, metformin intake is strongly correlated with T2D (ϕ=0.72), and many metformin-sensitive taxa appear **naively** T2D-associated in **unadjusted** tests

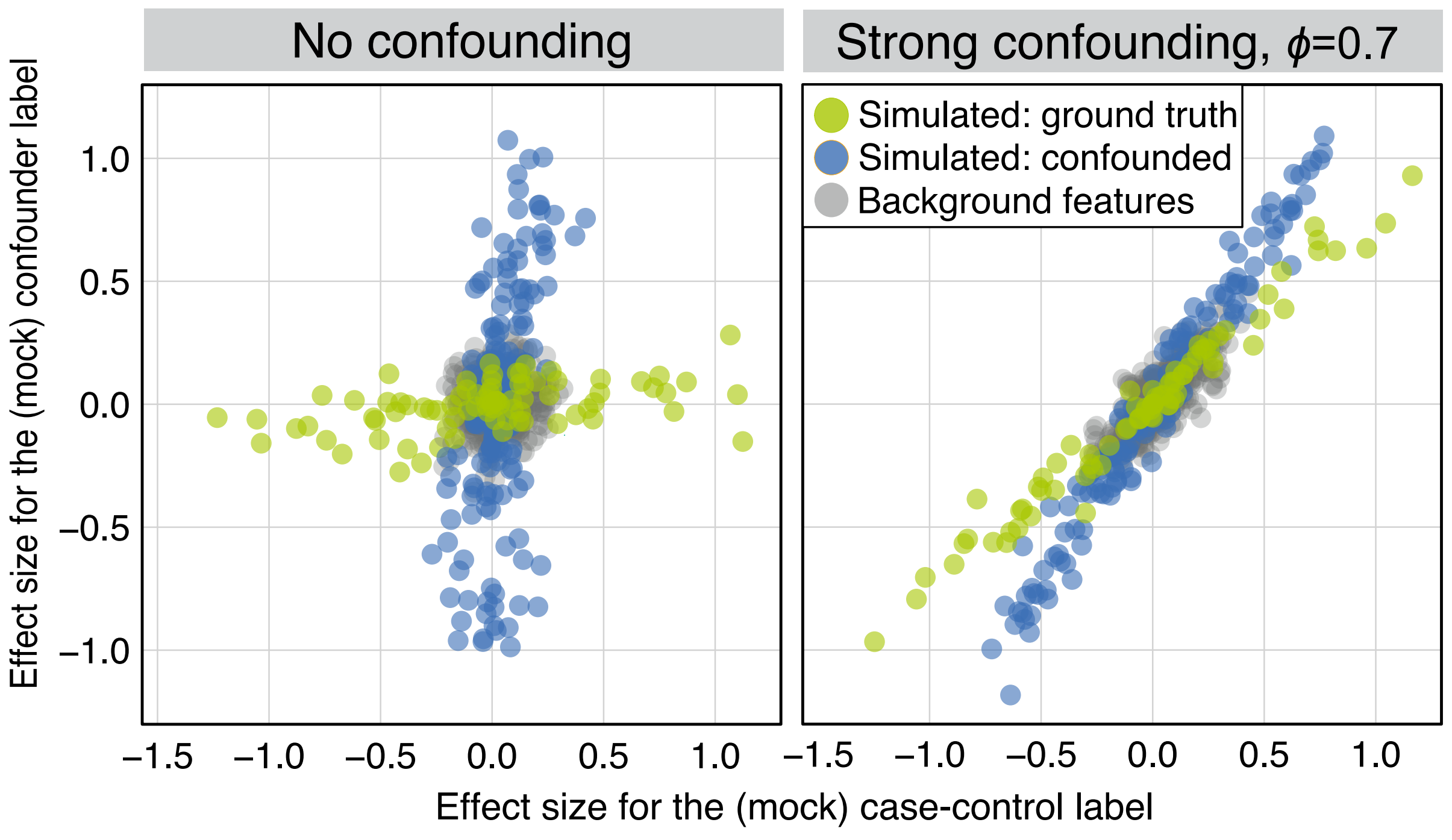


Adjusting for known confounders can disentangle true disease signals

▶ Previous differential abundance benchmarks relied on overly synthetic simulations and **oversimplified evaluations,** which generated recommendations that **do not generalize**

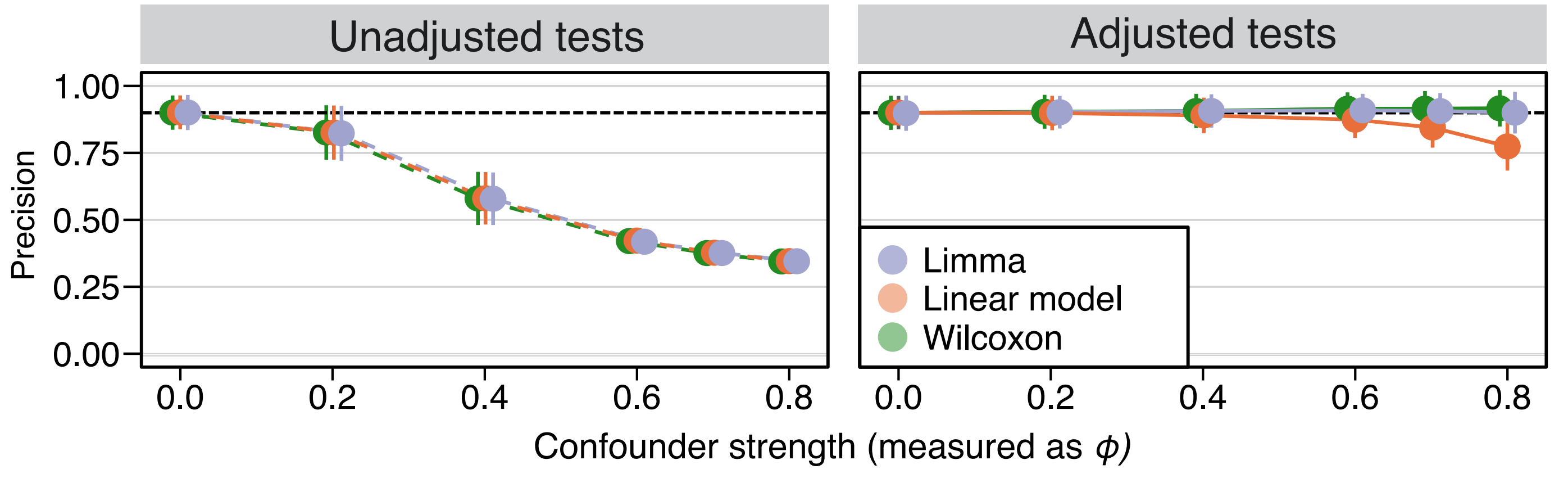
▶ We built and validated an open source **simulation framework in R** to implant calibrated signals into **real metagenomic data,** including **known confounding patterns**

▶ We used these simulations to perform a **comprehensive method benchmark** under the most **realistic conditions** possible



▶ **Unadjusted tests failed** to distinguish ground truth features and **suffered from low precision** under moderate to strong confounding, but adjusting for the (mock) confounder variable **restored good performance**

▶ **Linear models** are considerably more **flexible** than other methods tested



metadeconfoundR: a fast and flexible R package for robust association testing

▶ In a first step, metadeconfoundR calculates **standardized, non-parametric effect sizes** (Cliff's delta or Spearman correlations) paired with appropriate **statistical tests** to identify naively disease-associated features

▶ In a second step, **nested models** including covariates achieving significance in the first step are used in **likelihood ratio tests**, checking for **two conditions** needed to **classify the robustness** of feature associations

▶ **Rank-based methods** are robust to non-normal distributions; **mixed-effect models** are robust to pseudoreplication and repeated measures designs; **iterative, integrated status determination** robust to inclusion of an arbitrarily large number of covariates

▶ We have also developed a **sister R package** with similar logic geared toward **longitudinal biomarker analysis**: LongDat³

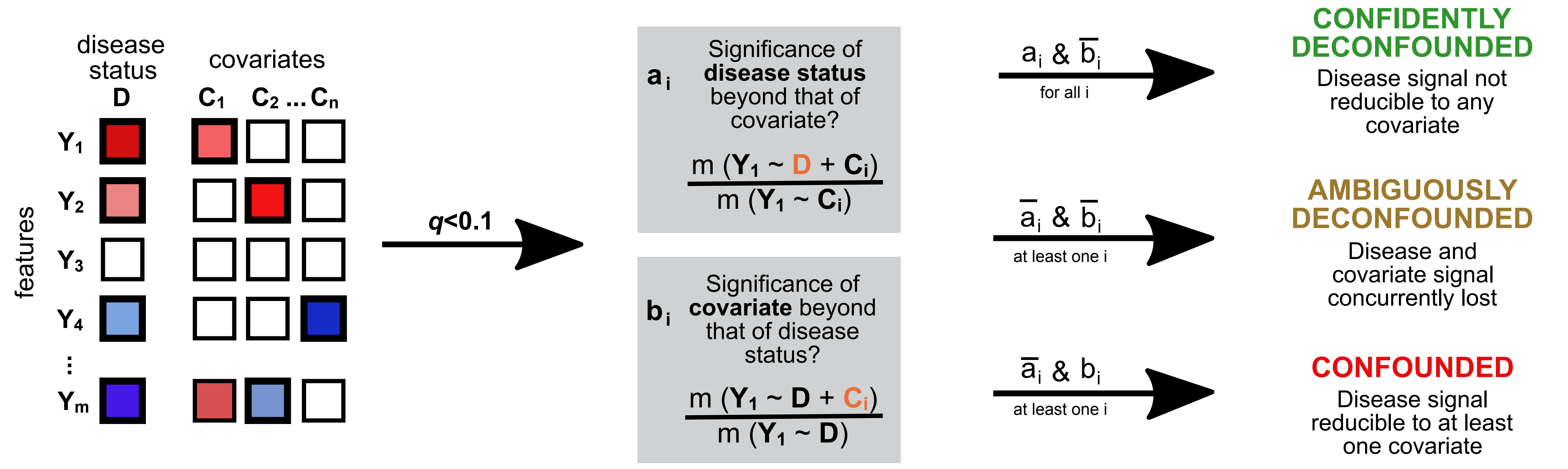
Naive association testing

between all feature-covariate pairs

Confounder modeling and *post hoc* testing

for all significant covariates, $\mathbf{C}_i$

Association status determination



disease status **D**

covariates $\mathbf{C_1}$ $\mathbf{C_2}$ ... $\mathbf{C_n}$

features $\mathbf{Y_1}$ $\mathbf{Y_2}$ $\mathbf{Y_3}$ $\mathbf{Y_4}$ ⋮ $\mathbf{Y_m}$

q<0.1

$\mathbf{a}_i$ Significance of **disease status** beyond that of covariate?

$$\frac{m\,(\mathbf{Y_1} \sim \mathbf{D} + \mathbf{C_i})}{m\,(\mathbf{Y_1} \sim \mathbf{C_i})}$$

$\mathbf{b}_i$ Significance of **covariate** beyond that of disease status?

$$\frac{m\,(\mathbf{Y_1} \sim \mathbf{D} + \mathbf{C_i})}{m\,(\mathbf{Y_1} \sim \mathbf{D})}$$

a_i & $\overline{b_i}$ for all i → **CONFIDENTLY DECONFOUNDED** Disease signal not reducible to any covariate

$\overline{a_i}$ & $\overline{b_i}$ at least one i → **AMBIGUOUSLY DECONFOUNDED** Disease and covariate signal concurrently lost

$\overline{a_i}$ & b_i at least one i → **CONFOUNDED** Disease signal reducible to at least one covariate

References

(1) Bartolomaeus TUP *et al.* Quantifying technical confounders in microbiome studies. *Cardiovascular Research* (2021); **(2) Forslund SK ... Birkner, Till** *et al.* Combinatorial, additive and dose-dependent drug–microbiome associations. *Nature* (2021); **(3) Chen CY, Löber U, and Forslund, SK**. LongDat: an R package for covariate-sensitive longitudinal analysis of high-dimensional data. *Bioinformatics Advances* (2023)

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