



Making SPF mice great again: Symbiotic Microbiota Restoration in SPF Mice through Dirty Facility Exposure

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1. Background

The specific pathogen-free (SPF) house mouse is the most widely used animal model in biological research. SPF environment profoundly alters the symbiotic microbial communities (compared to those of wild house mice), resulting in the loss of long-standing co-evolved symbionts.

These microbiota shifts can significantly affect the phenotype of SPF mice, potentially complicating the interpretation of experimental results. Considerable effort has therefore been dedicated to restoring natural microbiota in SPF models (a process often referred to as ‘rewilding’)

In addition to SPF facilities, house mice are also maintained in conventional facilities, where no deliberate interventions are made to alter their microbiota. As a result, the microbiota in these mice more closely resembles that of wild populations.

Here, we investigated whether long-term exposure of SPF mice to the environment in dirty facilities results in convergence of their microbiota and immune phenotype toward that typically observed in wild populations

2. Experimental design

Three experimental groups:

1. **Barrier** - SPF mice 5 inbred strains, 3 facilities (134 individuals in total)

2. **Conv** - ex-SPF mice from group 1 raised for more than 2 generations in 2 conventional (“dirty”) breeding facilities, (100 individuals)

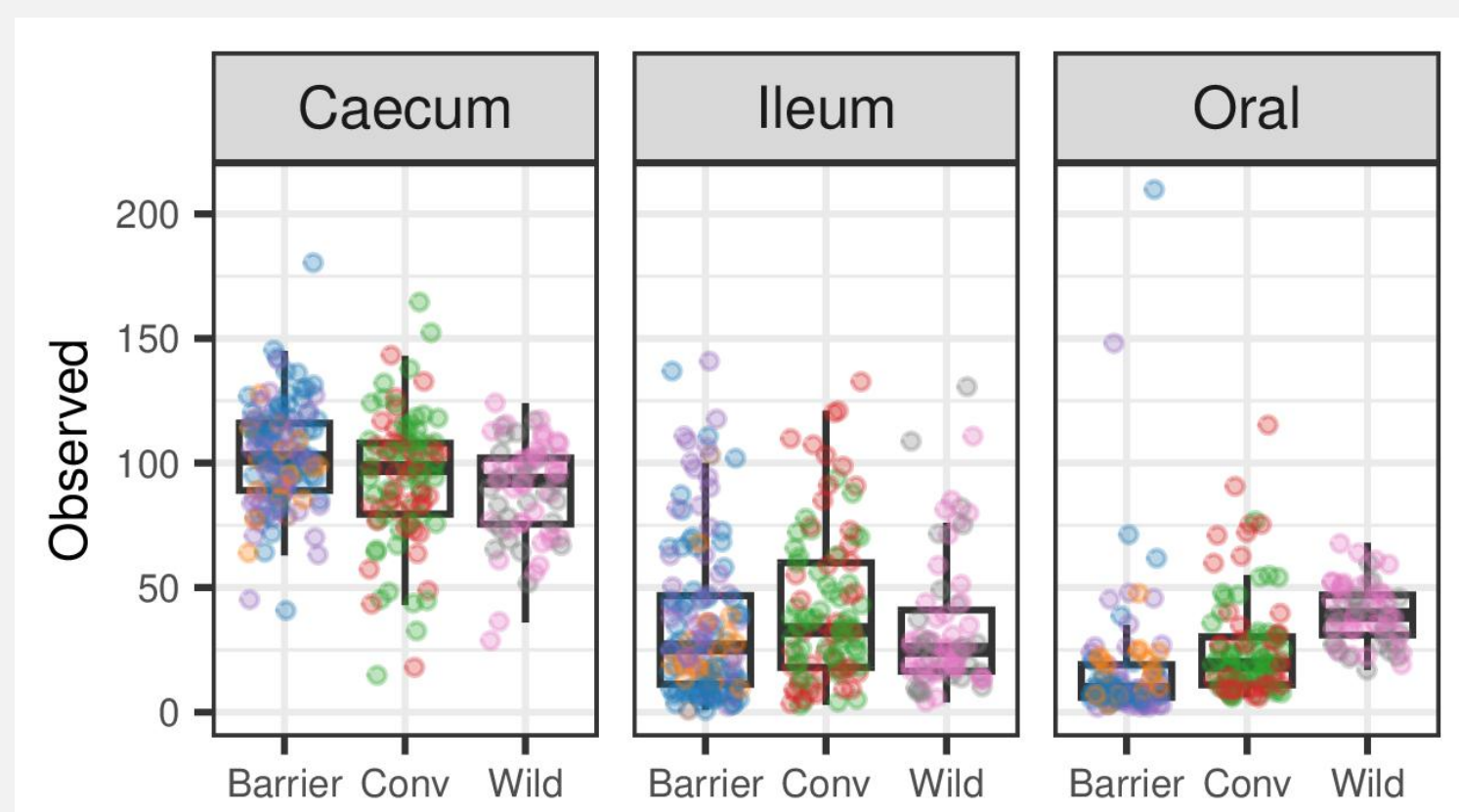
3. **Wild mice** - samples collected in free living populations, Two subspecies (*Mus musculus musculus* and *M. m. domesticus*), 67 individuals

3. Biological samples

Microbiota profiles - for (A) caecum, (B) ileum, and (C) oral cavity samples (16S rRNA amplicon sequencing of the V3–V4 region).

Immune phenotype – FACS analyses of mesenteric nodes (5 markers for CD8⁺ and 4 markers for CD4⁺ cells).

4. Alpha diversity

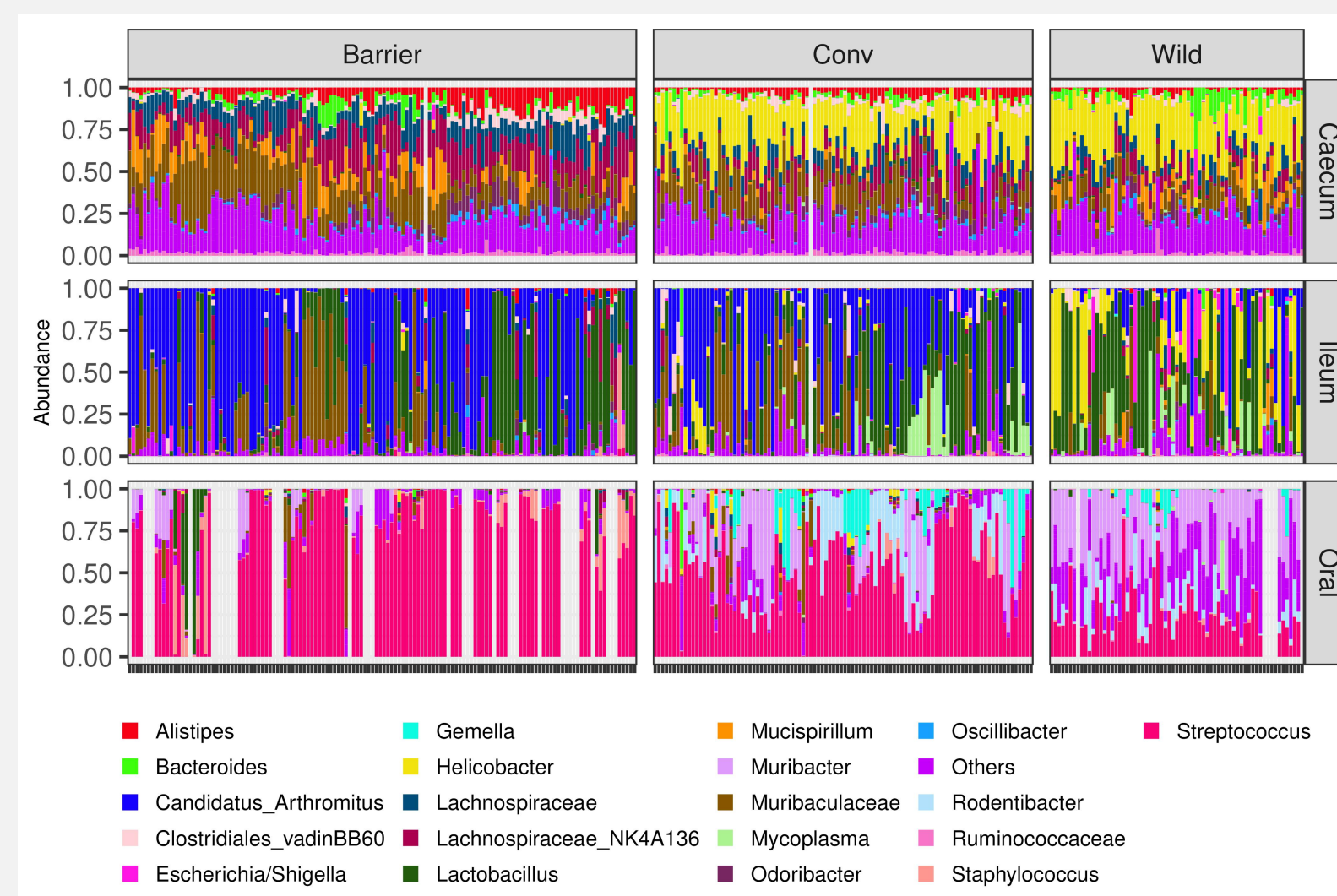


Caecum - Alpha diversity Increased in SPF compared with wild mice

Ileum - No differences between groups

Oral - Diversity increased in wild mice compared with SPF and ex-SPF

5. Taxonomic composition

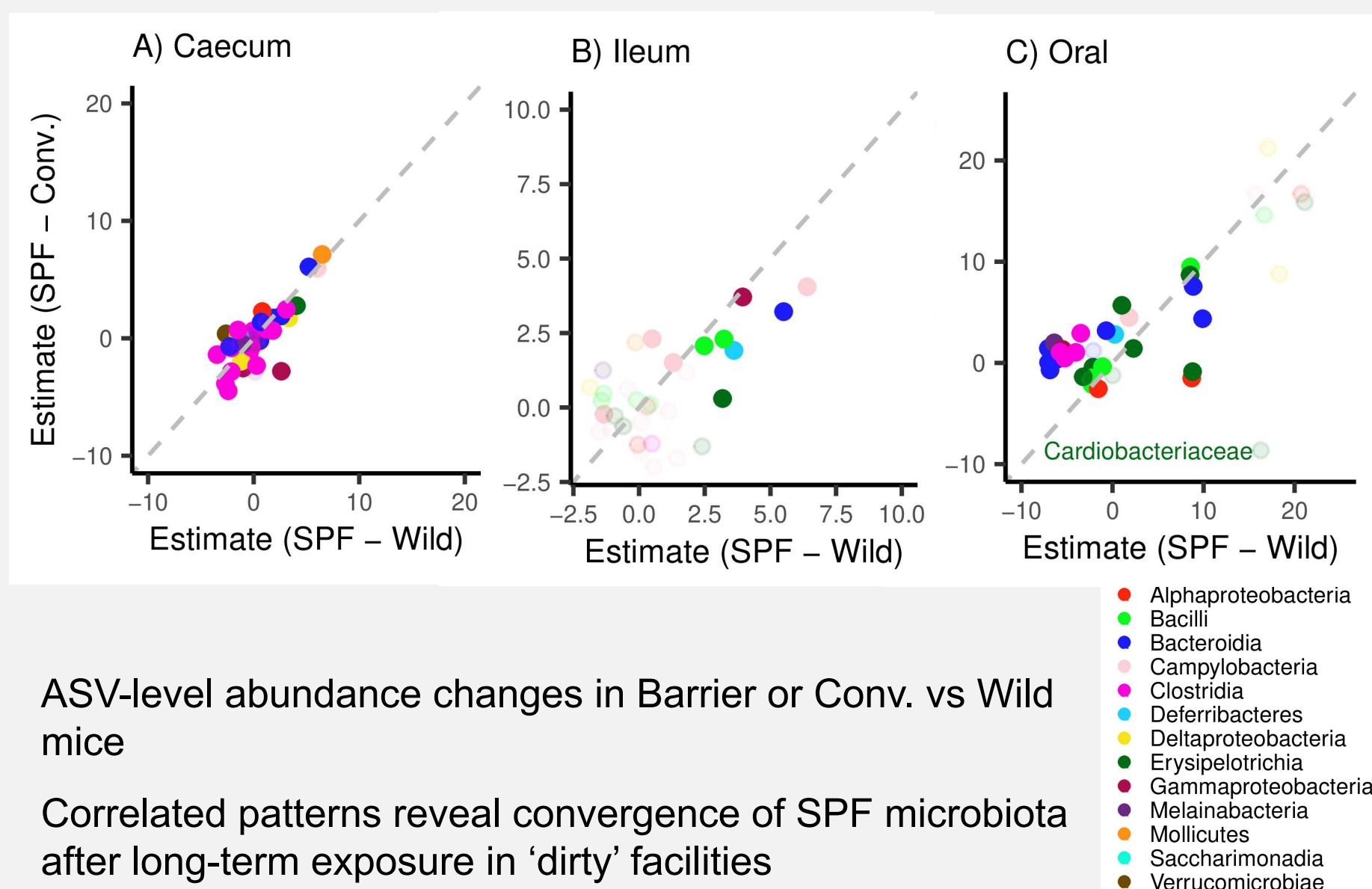


Caecum - Conv resemble wild mice. *Helicobacter*: 100% prevalence in Wild and Conv, but absent in Barrier

Ileum - Conv still resemble Barrier mice. Wild mice - increased abundance of *Candidatus Arthromitus* compared with Conv and Barrier.

Oral - Compared with Barrier, both Conv and Wild mice harbour higher abundance of *Muribacter*, *Rodentibacter*, and *Gemella*

6. ASV-level convergence

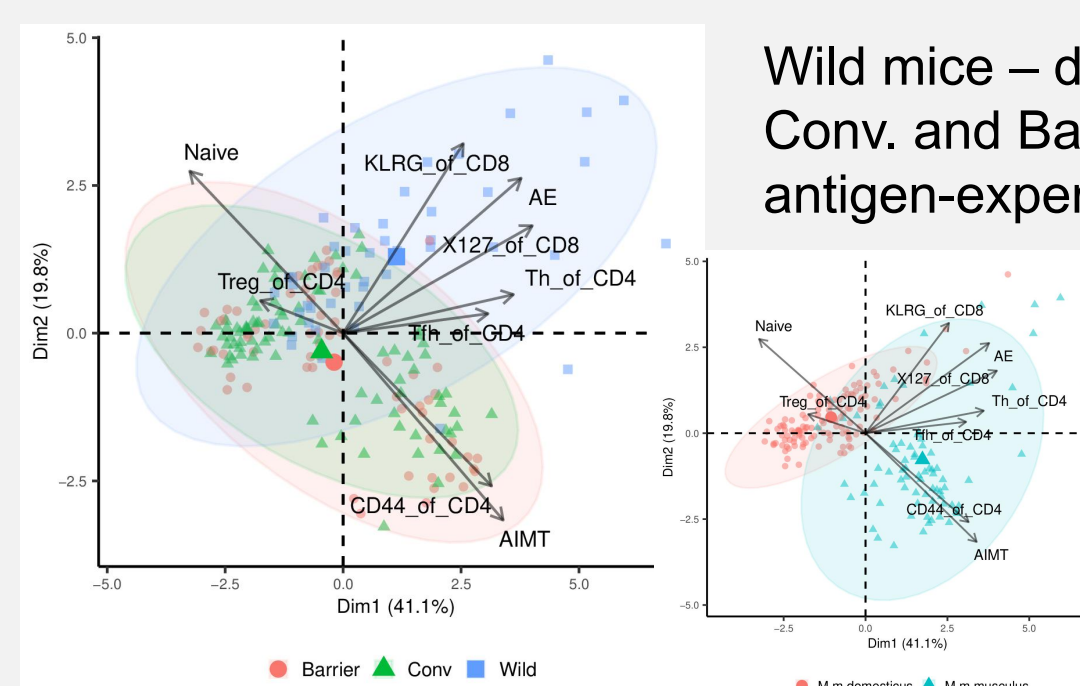


ASV-level abundance changes in Barrier or Conv. vs Wild mice

Correlated patterns reveal convergence of SPF microbiota after long-term exposure in ‘dirty’ facilities

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7. FACS analyses



Wild mice – distinct phenotype compared with both Conv. and Barrier (e.g., higher abundance of KLGR, antigen-experienced cells, etc.).

Conv. and Barrier – similar profiles.

Variation mostly driven by differences between mouse subspecies.

Conclusions

- Significant (but not fully complete) convergence of SPF microbiota after long-term exposure in ‘dirty’ facilities.
- A straightforward alternative to more complex microbiota ‘re-wilding’ methods, such as embryo transfer or direct microbiota transplantation.
- Limited convergence of immune phenotype.