Ecology meets human health: studies on human gut microbiota in health and disease

Pinto, S.

Chapter 6

Ecological dynamics of donor and host microbial species following FMT

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Ecological dynamics of donor and host microbial species in the treatment of ulcerative colitis with fecal microbiota transplantation

Abstract

Fecal microbiota transplantation (FMT) has emerged as a promising treatment for the chronic immune-mediated disease ulcerative colitis (UC). However, the ecological dynamics underlying clinical remission remain poorly understood. To investigate these dynamics, we analysed data from 24 UC patients treated with four rounds of FMT donated by two healthy donors. Microbiota samples from patients were collected at nine standardized time points before, during, and after treatment, covering a period of 14 weeks. Additionally, 27 donor samples were analysed. Species detected in the recipients' gut microbiota were categorized into ecological categories based on their origin and temporal dynamics: species already present in the host pre-FMT, species derived from the donor, or novel species, i.e., absent before FMT in both host and donor samples but detected later. Overdispersed Poisson regression models with random effects were employed to model the number of species within each category over time. Furthermore, we investigated the change in relative abundance for species present in the host pre-FMT. The results revealed that host species with higher relative abundances prior to FMT were more likely to persist following FMT. Notably, patients who achieved combined clinical and endoscopic remission at week 14 retained a significantly higher number of host species compared to non-responders. In contrast, non-responders initially exhibited a higher colonization of donor species than responders, but colonization rate decreased significantly over time in non-responders. These findings suggest that clinical remission following FMT is associated with a resilient patient gut community, capable of controlled incorporation of donor species, without replacing resident species.

Introduction

Fecal microbiota transplantation (FMT) is the transfer of fecal matter, including gut microorganisms, from the intestine of a healthy donor to a diseased recipient with the goal of modulating the recipient's disturbed microbiota.[189, 190, 381] FMT has been demonstrated to be effective in recurrent *Clostridioides difficile* infection,[189, 381] but the success rate is lower for more complex diseases, such as inflammatory bowel disease (IBD).[193, 413] A possible cause for the lower success rate of FMT in complex diseases is the tendency of the recipient's microbiota to revert to its original pre-FMT adverse state.[23] Transition to a healthier state is likely helped by the successful colonization (engraftment) of donor-derived microorganisms. Therefore, it has been suggested that the success of FMT depends on the donor's gut microbial diversity and composition.[210, 399] The extent to which shifts in the patient's microbiota towards the donor's microbiota are beneficial for resolving gut disturbances remains unclear.[23, 195, 200, 414] This donor-centric view has been challenged, and the importance of the recipient and procedural factors to determine FMT outcomes has been highlighted.[199, 415-417]

In previous analyses of the FMT trial for ulcerative colitis (UC) we examined the engraftment of specific microbial species following FMT, and their associations with clinical remission (see also Chapter 5).[384, 414] For this, we analysed the data from a randomized controlled trial (RCT) involving 24 UC patients treated with four rounds of FMT donated by two healthy donors. Interestingly, we observed that the rate of microbial engraftment did not correlate with successful clinical remission,[384] a paradox also noted in a meta-analysis conducted by Schmidt et al. (2022) involving 316 FMT procedures.[199] In their study, clinical success was not correlated with donor strain colonization or replacement of recipient species. Instead, recipient factors seemed to play a more important role in determining FMT outcomes than donor-related factors.[199] The seemingly limited role of engraftment in predicting clinical outcome of FMT defies the super-donor hypothesis and necessitates deeper investigation into the ecological changes underlying clinical remission.

In this study, the role of donor and host microbial species in determining clinical outcome of FMT is investigated further by applying the conceptual framework introduced by Schmidt et al. (2022)[199] to a longitudinal setting. We capitalize on a randomized controlled trial[384] with dense repeated sampling to map the succession dynamics in the recipient's gut microbiota of UC patients following FMT treatment in relation to clinical remission. Our analysis focuses on ecological dynamics on a species level, categorizing all taxa based on their origin and temporal presence: already present in the host before FMT, derived from the donor, or detected during or after the FMT therapies while absent in both the pre-FMT host and the donor.

Methods

The study population

A total of 24 adult patients experiencing mild to moderate exacerbations of UC were included in a double-blind randomized controlled trial conducted at LUMC.[384] Written informed consent was obtained from all study participants prior to their participation. Demographic variables and subject characteristics are provided in Appendix Table 6.1, with further details on the study population and clinical characteristics provided by van Lingen et al. (2024) and in Box 6.1.[384]

Following pretreatment with either budesonide (n = 12) or placebo (n = 12), patients received four fecal transplants at weekly intervals. Donors (D07 and D08) were randomly assigned. FMTs were infused in the duodenum via a nasoduodenal tube or gastroscope.[385] Stool samples were obtained before and after the pretreatment phase, before every FMT (four times), and 1 week, 4 weeks, and 8 weeks after treatment. At the end of the study, at week 14, a sigmoidoscopy was performed to assess the endoscopic Mayo score. Remission (i.e., response) was defined at week 14 as no symptoms (partial Mayo score of 2 with no individual sub score of > 2) and an endoscopic Mayo score 0–1. Partial remission was defined as a decrease of at least 3 points at the partial Mayo score and at least 1 point at the endoscopic Mayo score. A total of nine patients achieved remission, and one patient achieved partial remission. Of the 14 non-responders, 10 patients left the study early (in total 2 patients did not finish all four FMT treatments) because their symptoms worsened.[384, 414]

For this study, we defined a responder as a patient in remission after FMT (n = 9). Non-responders were defined as having activity despite FMT (non-responders and partial responders, n = 15).

Box 6.1 - Patient inclusion criteria, treatment protocols, and study design. The patients were included in the study if they had a full Mayo score of 4–9 and a colonoscopy with a Mayo endoscopic sub score of 1–2 within four weeks before study entry. Patients were excluded from this study if they had used antibiotics (< 6 weeks), used oral corticosteroids (< 8 weeks), surgical treatment (< 12 weeks), treatment with any investigational drug in another trial (< 12 weeks), significant signs of active infectious gastro-enteritis or enterocolitis, or any other significant medical illnesses. During the study, the medication and diet of the patients was not changed. Patients randomly received daily treatment for three weeks with either 9 mg budesonide or a placebo drug (Appendix Table 6.1). One day before the first FMT a bowel lavage with two liters of Kleanprep (macrogol solution) was performed to cleanse the intestine. Before every fecal transplantation the patients did not eat for at least six hours. The fecal donor suspensions were provided by the Netherlands Donor Feces Bank (NDFB). Collected fecal samples were stored and prepared at the LUMC following standard protocols.[385] Further details on the study population and clinical characteristics are provided by van Lingen et al. (2024).[384]

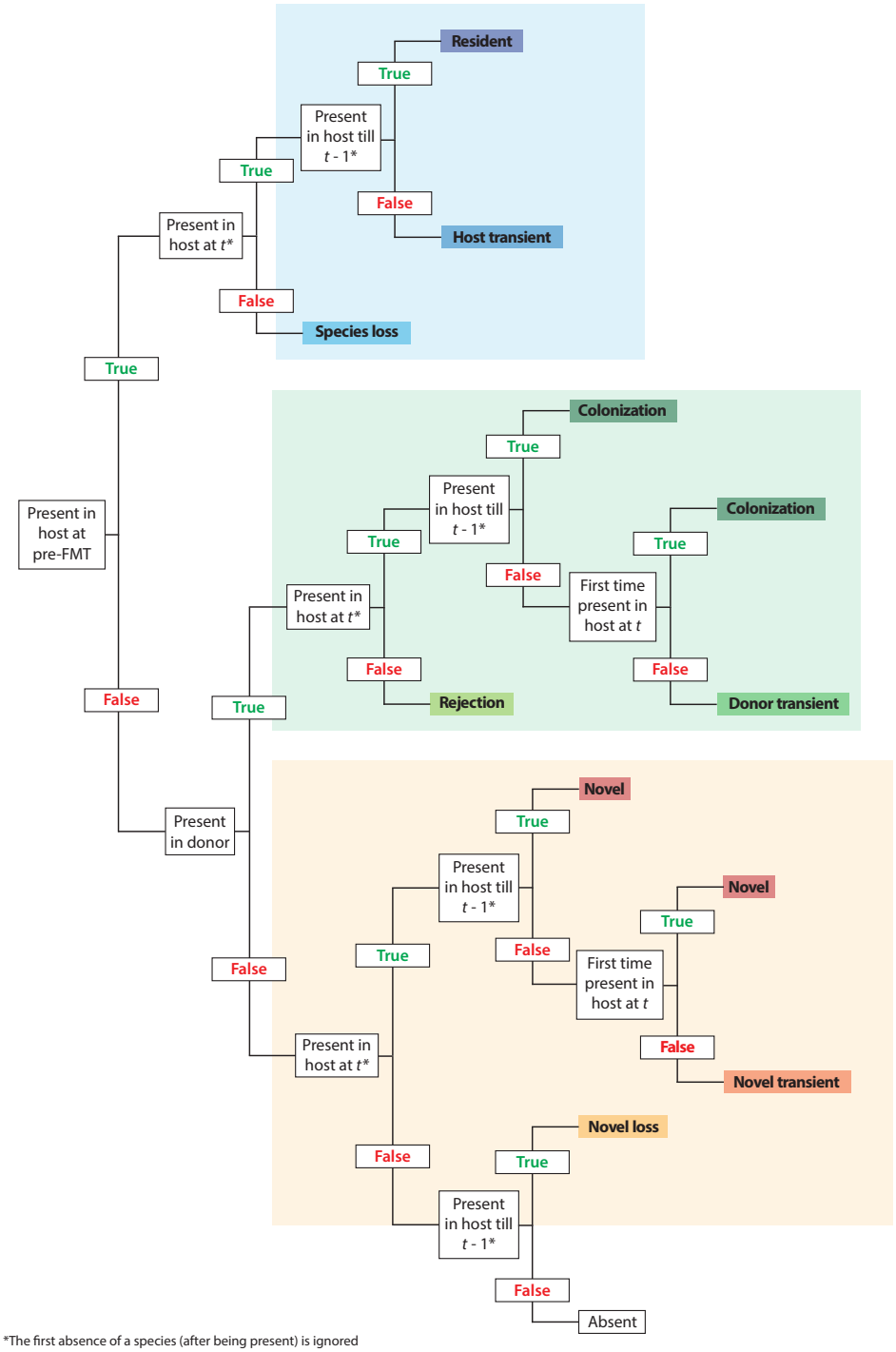
Microbiota data

DNA was extracted from the donor and recipient stool samples and shotgun sequenced with 100 bp single-end reads to a median depth of 2.9 million reads by Diversigen (New Brighton, Minneapolis, USA) using the Illumina NovaSeq platform. Raw reads mapping to the human genome were removed using bowtie2 (version 2.4.2)[386] and the GRCh37 reference genome and reads were quality-trimmed using fastp (version 0.20.1),[387] both part of an in-house workflow (git.lumc.nl/snooij/metagenomics-preprocessing). The mOTUs3 workflow (version 3.0.1) was used to generate taxonomic profiles.[388, 389] Unassigned, human-derived, archaeal, and low-quality reads were removed from the data, which resulted in 1552 unique mOTUs. For the sake of simplicity, we use the term 'species' to refer to unique mOTUs throughout. The results table was then imported into R (version 4.2.2) for analysing the data, visualizing the results and performing the statistical tests. R code is available on the GitHub repository (susannepinto/FECBUD_microbiome).

Mapping ecological categories

Respectively 13 and 14 samples were available for donor D07 and donor D08. Note that every recipient received FMT material from only one of the donors. We could not match every recipient sample to a specific donor sample used for the FMT, because not every donor sample used for FMT was sequenced. Therefore, we created a dataset with the core microbiota for each donor. The core donor microbiota was defined as having its relative abundance higher than 0.1% in at least one sample. The core donor microbiota yielded 120 and 84 unique species for donors D07 and D08, respectively.

Subsequently, we created a presence or absence dataset of all species per recipient and per time point, and every species was assigned to an ecological category per recipient and per time point based on its origin and presence over time, according to the decision tree presented in Figure 6.1 (detailed explanation Box 6.2).



*The first absence of a species (after being present) is ignored

Figure 6.1 - Decision tree used to assign species to ecological categories. The categories are based on the origin and presence of a species over time. First, the species was compared to the pre-FMT host samples, then to the core donor microbiota. Next, the presence or absence at all previous time points was considered to assign the species to an ecological category. Note that we ignored the first absence of a species when categorizing species as lost or as transient upon re-detection. In Sensitivity 1 we evaluated whether this choice had an impact on the results (Box 6.2).

Per recipient, for every species ever present at any time point in the recipient, or present in the microbiota of the associated donor, a comparison was made with the recipient's pre-FMT sample and with the microbiota of the corresponding donor. All species present in the recipient's pre-FMT sample were placed into a host category (Resident, Host transient, or Species loss), depending on the pattern of presence over time. If species were unique for the donor relative to the recipient's pre-FMT samples, species were placed into a donor category (Colonization, Donor transient, or Rejection). If species were not present in the host pre-FMT or in the microbiota of the donor, they were classified as a novel species (Novel, Novel transient, or Novel loss). Within these broad categories, a species was further categorized as a stable (Resident, Colonization, or Novel), intermittent (Host transient, Donor transient, or Novel transient), or previous occupant (Species loss, Rejection, or Novel loss) in the microbiota, depending on the presence at that moment and at the previous time points. Because absence in microbiota data can also mean that the abundance was under the detection limit, in the base case we allowed, for each species, the occurrence of one single absence without direct consequences for categorization in the rest of the time series. Due to the way the categories are defined, some categories cannot occur at the first time points. For example, a donor-derived species first had to colonize the gut (colonization), then be absent for at least two time points (absence ignored (NA) and Rejection), and then be detected again to be categorized as a Donor transient species (Box 6.2).

In sensitivity analyses, we tested some variations to the base case criteria regarding the temporal information used for categorizing the species. In Sensitivity 1 we did not allow the occurrence of any absence when categorizing species into either of the host, donor, or novel categories (Figure 6.1). In Sensitivity 2 we only considered the presence or absence at the previous time point instead of all the previous time points (Appendix Figure 6.3). In contrast, in Sensitivity 3 the presence of species at all time points is considered in the categorization of species at a particular time point (Appendix Figure 6.4). Sensitivity 4 is the same as Sensitivity 3 but with the added criterion of not allowing the occurrence of any absence (Appendix Figure 6.4). In Box 6.2 examples on categorization of species and the differences between the sensitivity analyses are illustrated.

Box 6.2 - Examples illustrating the categorization of the species in the base case and in the four sensitivity analyses.

Sensitivity analyses

In Sensitivity 1 we did not allow the occurrence of any single absence when categorizing species as lost or as transient upon re-detection (in either the host, donor, or novel categories). Secondly, in Sensitivity 2 we only considered the previous time point instead of all previous time points (Appendix Figure 6.3). Therefore, the species can switch more frequently between ecological categories. In Sensitivity 3 and in Sensitivity 4, we considered the full time series (also future points) before assigning them to a category with and without considering a single absence, respectively (Appendix Figure 6.4).

Species present in the host pre-FMT

In the base case scenario, a host species was present in one of the pre-FMT samples of the host (Example 6.1). The resident species has been present up to a specific time point, however, we have ignored a single absence of the species. If the species was absent for two or more time points up to the current one, the species was categorized as a host transient species. The third possible category for a host species is based on the absence of the species at a specific time point and is called 'Species loss'.

Example 6.1 - A species present in the host pre-FMT can be categorized as Resident (Res), Host transient (HT), or Species loss (SL).

			Time point						
	Donor	Host pre-FMT	1	2	3	4	8	10	14
	Absent	Present	Present	Present	Absent	Present	Present	Present	Present
A - Base case			Res	Res	NA	Res	Res	Res	Res
B - Sensitivity 1			Res	Res	SL	HT	HT	HT	HT
C - Sensitivity 2			Res	Res	SL	HT	Res	Res	Res
D - Sensitivity 3			Res	Res	NA	Res	Res	Res	Res
E - Sensitivity 4			HT	HT	SL	HT	HT	HT	HT

Species identified in both the host pre-FMT and the donor are categorized as host species into the groups: Resident (Res), Host transient (HT), and Species loss (SL) (Example 6.2).

Example 6.2 - A species both present in the host pre-FMT and in the donor will be categorized as a host species into: Resident (Res), Host transient (HT), or Species loss (SL).

			Time point						
	Donor	Host pre-FMT	1	2	3	4	8	10	14
	Present	Present	Absent	Absent	Present	Absent	Absent	Present	Present
A - Base case			NA	SL	HT	SL	SL	HT	HT
B - Sensitivity 1			SL	SL	HT	SL	SL	HT	HT
C - Sensitivity 2			SL	SL	HT	SL	SL	HT	Res
D - Sensitivity 3			NA	SL	HT	SL	SL	HT	HT
E - Sensitivity 4			SL	SL	HT	SL	SL	HT	HT

Donor-derived species

A donor species is a species that was not detected in the host pre-FMT, and that was present in the core donor microbiota (Example 6.3). Again, there are three possible categories: Colonization, Donor transient, and Rejection. Species are categorized according to rules similar to how the host species are categorized (Colonization similar to Resident, Donor transient similar to Host transient, and Rejection similar to Species loss). However, a species can still be placed in the Colonization category after being absent for some time points, as it is possible that a species does not colonize directly after the first FMT, but that it needs time to establish in the gut. Note that also in this category a species is allowed and ignored if it is absent once, but only after being present.

Example 6.3 - A species not present in the host pre-FMT, but present in the donor can be categorized as Colonization (C), Donor transient (DT), or Rejection (Rej).

			Time point						
	Donor	Host pre-FMT	1	2	3	4	8	10	14
	Present	Absent	Present	Present	Absent	Present	Present	Absent	Present
A - Base case			C	C	NA	C	C	Rej	DT
B - Sensitivity 1			C	C	Rej	DT	DT	Rej	DT
C - Sensitivity 2			C	C	Rej	DT	C	Rej	DT
D - Sensitivity 3			DT	DT	NA	DT	DT	Rej	DT
E - Sensitivity 4			DT	DT	Rej	DT	DT	Rej	DT

Novel species

A novel species has not been present or was under the detection limit in the pre-FMT host samples, as well as in the core donor microbiota (Example 6.4). Similar to colonizing species, novel species can also enter the microbiota of the host later. However, where a donor species is in that case categorized as 'Rejected', the novel species is not categorized as 'Novel loss', but as 'Absent' and not taken into account in the analyses, until the species has been present once.

Example 6.4 - A species not present in either the host pre-FMT or the donor can be categorized as Novel (N), Novel transient (NT), or Novel lost (NL), from the moment the species appeared in the patient samples.

			Time point						
	Donor	Host pre-FMT	1	2	3	4	8	10	14
	Absent	Present	Present	Present	Absent	Present	Present	Present	Present
A - Base case			-	N	N	N	NA	N	NL
B - Sensitivity 1			-	N	N	N	NL	NT	NL
C - Sensitivity 2			-	N	N	N	NL	NT	NL
D - Sensitivity 3			-	NT	NT	NT	NA	NT	NL
E - Sensitivity 4			-	NT	NT	NT	NL	NT	NL

Modeling the number of species across ecological categories

We modelled the number of species across ecological categories by means of overdispersed Poisson regression models with random effects to accommodate correlation between repeated measurements per recipient. For this, we employed a generalized linear mixed-effects model (GLMM) with a negative binomial family and a log-link using the 'glmer.nb' function from the 'lme4' R package.[395] The temporal evolution of the expected log-number of species in each category was modelled with a spline transformation of the original time variable (in weeks since start of FMT treatment). Estimates from the spline model were compared to those from a linear model in a sensitivity analysis, by modeling the expected log-number of species as a simple linear function of time. Possible differences in succession dynamics between responders and non-responders were investigated by adding the treatment response variable as a covariate to the model, and through specification of interaction terms with time and ecological category. Patient-specific variables, namely, donor (donor D07 vs. D08), pretreatment (budesonide vs. placebo), age, and sex (female vs. male), were included based upon their role as possible confounders.

Change in population abundances of host-derived species

To explore the dynamics of host-derived species in response to FMT in more detail, we investigated the relative abundance over time for the species that were already present

in the host pre-FMT. Results reveal the distribution of abundance differences at particular time points across subjects per ecological category for the species that were already present pre-FMT. In addition, we compared the baseline distributions among species that were later categorized as resident, host transient, and species lost among both responders and non-responders. Finally, we also calculated the differences in microbial abundance before and after FMT for all species that were present in the recipients' pre-FMT samples. Because several non-responder patients quitted early during the study, we only included patients who completed all four rounds of FMT and had at least one post-FMT sample (n = 18 patients, of whom 9 were defined as responders) and used the last available post-FMT measurement when calculating the difference in relative abundance before and after FMT. Because the abundance distributions were right-skewed, we used a natural-log transformation of the abundances. Consequently, the abundance differences on the log scale can be interpreted as proportional differences on the original scale (in percentage differences). To assess the significance of these differences between responders and non-responders, linear mixed-effects models (LMM) were applied, accounting for the correlation of repeated observations within each patient (using the 'lmer' function from the 'lme4' R package).[395]

Results

Succession of host-derived, donor-derived, and novel species following FMT

To study the succession dynamics of species during and after FMT in our UC cohort, we modelled the number of species across ecological categories and investigated differences between responders and non-responders (Figure 6.2). In these models, donor and sex were included as covariates, while pretreatment and age were not relevant as confounders. Appendix Figure 6.1 shows the specific parameter estimates of the model depicted in Figure 6.2.

At the start of the study, we observed a significantly higher number of host species in the resident categories (species that were present in the patient's gut pre-FMT) among responders compared to non-responders, and this difference persisted over time (Figure 6.2A). Although the number of resident species declined over time in both responders and non-responders this decrease was not statistically significant. In contrast, the number of host transient species increased significantly over time in both patient groups (Figure 6.2B). Of note, this increase may be partly attributable to the definition of host-derived species being transient upon re-detection after temporary absence. Non-responder patients exhibited a significantly greater loss of host species over time compared to responders, in whom the number of host species lost decreased significantly over time (Figure 6.2C).

Conversely, non-responders were initially colonized by a significantly higher number of donor species compared to responders. However, the number of colonizing species in non-responders significantly declined over time, whereas it remained constant in responders (Figure 6.2D). The number of donor transient species was similar between the two patient groups at the start of the study and showed a significant increase over time, especially in non-responders. However, this category remained relatively small and differences according to treatment response were not significant (Figure 6.2E). The number of rejected donor species was higher at baseline and over time for non-responders compared to responders, however this difference also did not reach statistical significance (Figure 6.2F).

The number of novel species detected post-FMT was similar for both responders and non-responders and remained constant in time (Figure 6.2G). The number of novel transient species increased significantly over time; this increase was more or less similar for both the responders and non-responders (Figure 6.2H). Initially, the responders lost significantly more novel species than the non-responders, but over time the latter group lost significantly more novel species than the responders (Figure 6.2, panel I).

We also found significant differences between responders and non-responders in the host transient and novel transient categories when applying a linear model instead of splines for the temporal evolution of the number of species in each category (Appendix Figure 6.2). It should be noted that these categories contained relatively few species, and the lack of statistical significance when using splines is likely explained by a reduced statistical power. Importantly, all differences between responders and non-responders identified by the spline model were retained in the linear model for category size (Appendix Figure 6.2).

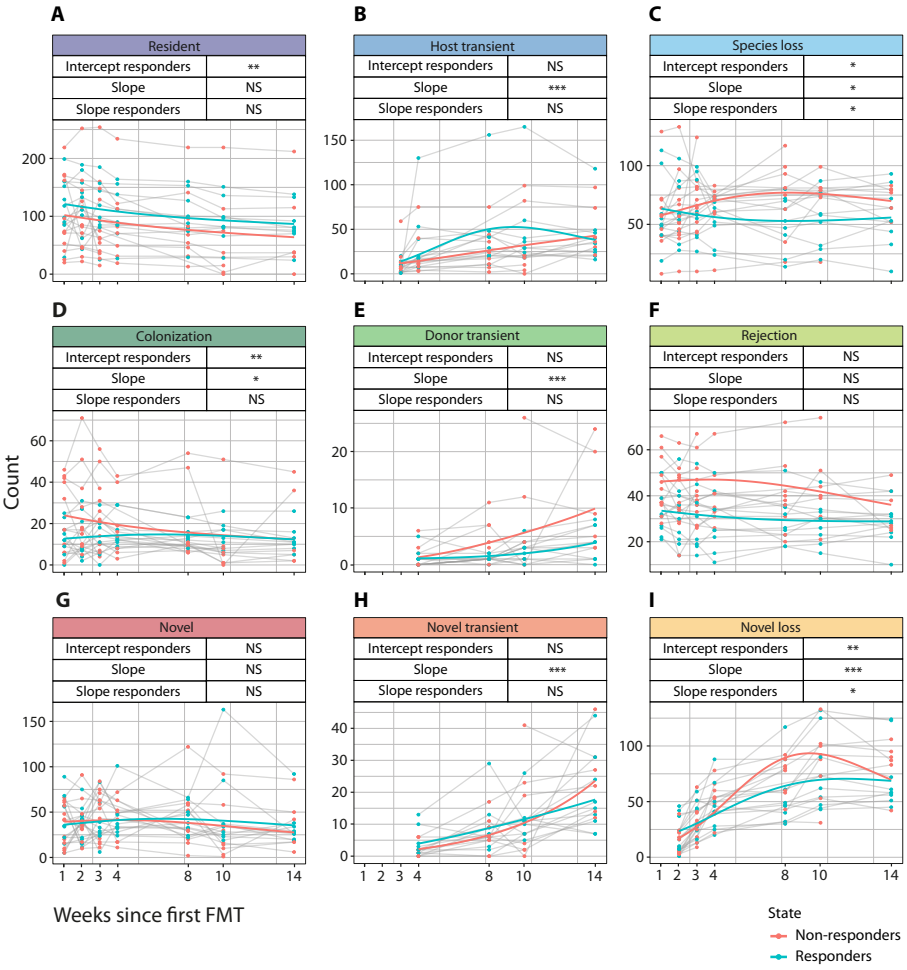


Figure 6.2 – Temporal changes in the number of species per ecological category. Average trajectories among responders to the treatment are indicated with blue lines, average trajectories among non-responders with red lines. Individual patient trajectories are shown with grey lines. Note the different scaling of the *y*-axis per category. The model contained a random intercept per patient to account for repeated measurements.

Time was modelled with a spline. The levels of significance are reported above each plot and are indicated by asterisks (*** = p-value < 0.001; ** = p-value < 0.01; * = p-value < 0.05; NS = not significant).

Sensitivity analyses

We conducted four different sensitivity analyses concerning the categorization of the species. To illustrate the effect of categorization on the rates of change over time, we generated a plot of the average slope estimates according to each sensitivity analysis (Appendix Figures 6.5 to 6.10). Sensitivity analysis 1 resulted in a slightly stronger decline in the number of species for the resident, colonization, and novel categories (Appendix Figures 6.5, 6.9, and 6.10). This outcome is a logical consequence of the criterion that a species can no longer be absent for a single time point. Consequently, the likelihood of a species moving to a different category (transient or loss) increased, since it was by definition not possible to return to the categories denoting stable presence over time. This resulted in transient categories having higher intercepts, but the average slopes remained unchanged for all other categories (Appendix Figures 6.5, 6.9, and 6.10). Similarly, for Sensitivity analysis 2, no substantial differences from the base case were found (Appendix Figures 6.6, 6.9, and 6.10). The most profound differences were noted in the slopes of the resident and transient categories. The slopes of the transient categories were smaller, especially for the host-derived species among non-responders (Appendix Figures 6.6, 6.9, and 6.10). Sensitivity analyses 3 and 4 led to more stable patterns over time, especially for the resident category, as compared to both the base case scenario and the other sensitivity analyses (Appendix Figures 6.7 to 6.10). This stability can be attributed to the modifications in the category assignment criteria in Sensitivity analyses 3 and 4, where stable presence is defined at all time points. Consequently, fewer species were assigned to the resident, colonization, and novel categories and more to the transient categories (Box 6.2).

Relative abundances of host resident species pre- and post-FMT

We further assessed changes in the relative abundance of species present in the gut prior to treatment to investigate whether the relative abundance pre-FMT is indicative of the category that a species will reach post-FMT. Host transient species displayed significantly lower relative abundances at all time points compared to resident species (Figure 6.3A and Appendix Table 6.2). In both responders and non-responders, recipient species with higher pre-FMT relative abundances were more likely to remain in the recipient's gut and become resident species, compared to recipient species that were transient or lost (Figure 6.3B, Appendix Figure 6.11, and Appendix Table 6.2). Therefore, our findings show that initial microbiota composition is associated with post-FMT composition. The differences in relative abundance of host resident species between the pre-FMT measurement and the last available post-FMT measurement were centered around zero (Figure 6.3C). A positive difference indicates an increase in the relative abundance of resident species following FMT, while a negative difference denotes a decrease. Thus, approximately equal numbers of resident species showed either a positive or negative response to FMT. No significant differences were found between responders and non-responders regarding relative abundances of resident species in response to FMT (Figure 6.3C, Appendix Figure 6.12, and Appendix Table 6.2).

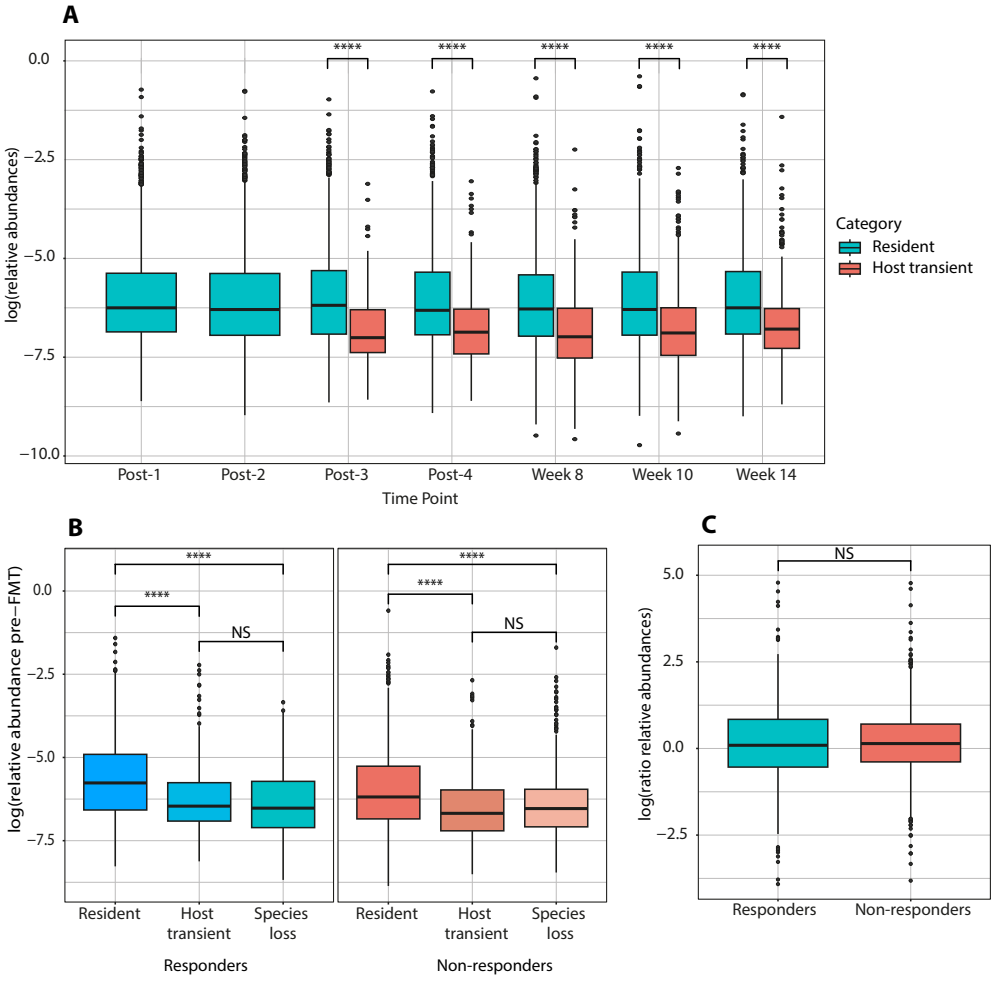


Figure 6.3 - Comparison of relative abundances of species in different categories.
A) Relative abundances of Resident (blue) and Host transient (red) species over time. Here, no distinction has been made between responders and non-responders. B) Relative abundance of host species at pre-FMT measurement. The relative abundances in species categorized as Resident, Host transient, and Species loss species between responders (blue) and non-responders (red) are not significant (Appendix Table 6.2). C) Difference in relative abundance in resident species between pre-FMT and last available post-FMT measurement for responders (blue) and non-responders (red). Significance was tested with linear mixed-models and shown in the plots (**** = p-value < 0.0001, *** = p-value < 0.001; ** = p-value < 0.01; * = p-value < 0.05; NS = not significant).

Discussion

The success of FMT for UC is ultimately determined by whether the patient achieves clinical and endoscopic remission after treatment. It has been suggested that treatment success is related to the extent to which the recipient's microbiota composition shifts towards that of the donor.[399, 418] However, we found no evidence supporting this link, in line with several other studies.[199, 200, 384, 414, 415]

We used an ecological framework of succession to investigate microbiota dynamics associated with clinical success of FMT. Microbial species were categorized as pre-existing in the host before FMT, donor-derived, or newly detected. We found that responders retained a higher number of host species compared to non-responders. Although non-responders initially exhibited colonization by more donor species than responders, this colonization in non-responders declined over time and eventually became equal to the levels observed in responders. These findings suggest that a successful clinical response to FMT may be facilitated by a microbiota receptive to colonization without compromising the resident microbiota. Additionally, non-responders lost substantially more novel species over time compared to responders, indicating that newly detected species failed to establish stably within the non-responder gut microbiota. This finding suggests less robust alterations in gut microbiota composition among non-responders. A successful FMT may induce a shift in which the recipient's microbiota integrates donor and novel species, achieving a balanced coexistence to restore the gut microbial ecosystem. This observation aligns with earlier research.[188, 199] Our study expands upon previous analyses using longitudinal analysis of UC patients, thereby providing a fine-grained view of the ecological dynamics over time of donor and host species following FMT.

FMT can be seen as a perturbation experiment on the gut microbiota, creating a dynamic interplay between donor and recipient communities, which may open ecological niches for other microorganisms.[95, 199] The balance between the engraftment of beneficial microorganisms and competition with deleterious microorganisms in the recipient gut, combined with systemic host processes, such as the modulation of immune responses and the interaction with (external) environmental factors and genetic characteristics, could initiate clinical remission.[413] The process of microbial invasion involves various challenges that incoming microorganisms need to overcome to establish colonization and influence the existing microbial community. It is important for the invading species to achieve sufficient metabolic activity in the gut to interact with the resident community. This interaction may also be achieved by transient species, indicating that permanent colonization is not always necessary.[95] Analogous to nurturing an ecosystem such as a crop field through biological control, FMT necessitates the introduction of donor species with healthy functional properties to modify the recipient's system rather than inducing wholesale changes that might lead to the extinction and replacement of existing microbial inhabitants. Therefore, the recipient microbiota must exhibit a degree of resilience, allowing it to integrate donor species without completely altering its composition. FMTs may also strengthen recipient species by introducing beneficial spores or metabolites, thereby enhancing the stability and functionality of the recipient's own microbiota.[103] The stability of the microbiota is maintained through controlled species loss, ensuring that introduced organisms integrate harmoniously with the pre-existing ecosystem.

The outcome of FMT is influenced by a range of ecological processes, spanning from neutral or stochastic factors (e.g., donor propagule pressure) to adaptive or selective factors (e.g., niche competition and differentiation).[199, 419] This indicates a complex mechanism of action of FMT in patients with UC, necessitating the establishment of a novel homeostasis between the donor and recipient microbiota. This complexity may also explain why prolonged FMT treatment with multiple donor infusions appears necessary in UC, as repeated exposure may be required to achieve an optimal balance between recipient and donor microbiota. This approach contrasts with the FMT treatment of recurrent *Clostridioides difficile* infections (rCDI), which is characterized by a depleted microbiota that can be effectively restored with a single infusion, with a cure rate of about 80%.[190]

The success of FMT may not be reliant on resembling the donor's microbiota, but rather on establishing a complementary relationship, emphasizing the importance of selecting donors whose microbiota optimally aligns with the recipient's specific needs.[399] Unlike the developmental stages of a child's microbiota, the gut microbiota of a UC patient is already an established, independent microbial community. This pre-existing microbiota makes the introduction of new species and the induction of change considerably more challenging.[67, 68] Tailoring the selection of FMT donors to those enriched in taxa capable of restoring disturbed metabolic pathways in the recipient might enhance the effectiveness of FMT, particularly in metabolic dysfunction associated diseases.[23, 200, 399] For example, incoming species that are metabolically complementary to the recipient's community, by introducing novel functions or by occupying previously unfilled niches, may be more likely to colonize the resident community.[87, 420] In addition, a high gut microbial diversity in the donor and low diversity in the recipient may further influence the success of colonization.[1, 200]

From an ecological perspective, our findings suggest that donor and recipient species can coexist. We might hypothesize that they occupy distinct metabolic niches. Moreover, we observed that species with a higher abundance prior to FMT (the main 'founders') are more likely to persist during the FMT than species with a lower abundance. This implies that the competitive strength of the resident species is related to their abundance, indicating that within each metabolic niche, communities are built by random winners, driven by stochastic colonization.[84] This is in line with ecological studies showing that functional differences create opportunities for coexistence (niche theory). However, within each niche functionally similar species can coexist, and communities are structured to random stochastic rules (neutral theory).[421] Within the gut microbiota, species often have overlapping functions, allowing them to replace each other and take over specific functional traits if one species is perturbed or removed.[22]

This study has several limitations. The first concerns the classification of patients into responders and non-responders. Patients who dropped out early due to worsening symptoms were classified as non-responders. Microbiota data were not collected for these patients, which potentially introduces bias into the results for the non-responder group. Moreover, the study concerns only 24 UC patients and the time series up to 14 weeks represents only a snapshot of the dynamic process of microbial succession. This sample size is too small to draw definite conclusions and further investigation into longer-term outcomes is necessary to gain a more comprehensive understanding.[422] A third limitation is the sequencing depth (2.9 million 100 bp single-end Illumina reads), which does not allow for definitive determination of whether an absent species was actually absent in the host or donor, or simply undetected.[87]

Also, the low sequencing resolution makes it impossible to determine whether the same strain present in the donor sample successfully colonized the recipient's gut microbiota or whether the donor and host strains coexisted or were replaced following FMT. Lastly, we did not have data to directly link the unique donor sample used for FMT to the corresponding recipient samples. Therefore, we used the combined microbiota data, which may have led to the misclassification of some low-abundance colonizing species from the donor as novel species.

By applying an ecological perspective to FMT, our study sheds new light on the importance of ecological principles, such as succession of microorganisms and the resilience of the recipient's system, in shaping therapeutic outcomes. Our study reveals the ecological dynamics of the gut microbiota during and after FMT in patients with UC, with a particular focus on the dynamics of recipient, donor, and novel species. Contrary to some previous studies, the overall engraftment of the donor microbiota did not emerge as the most important factor for FMT success in this study.[399, 415] The key factor influencing the response may not be the overall engraftment of donor species, but rather the recipient's ability to retain resident species while simultaneously enriching with novel and donor species. Thus, successful FMT hinges on fostering a microbiota shift that complements rather than compromises the existing ecosystem. This ecological interpretation aids in understanding the mechanism through which FMT may induce clinical remission and also underscores the nuanced interplay between donor and recipient microbiota essential for therapeutic efficacy.

Appendices of Chapter 6

Appendix Table 6.1 - Clinical and demographic information of responders and non-responders.		
	Responders[a]	**Non-responders**[b]
	Number (Percentage)	**Number (Percentage)**
Patients	9 (38%)	15 (63%)
Samples	81 (45%)	99 (55%)
Missing	0	36
Sex		
% Female[c]	6 (67%)	6 (40%)
Pretreatment		
% Budesonide[c]	5 (56%)	8 (53%)
Donor		
% D07[c]	2 (22%)	10 (67%)
	Mean (SD)	**Mean (SD)**
Age	45 (17)	48 (16)

[a] Remission (i.e., response) was defined at week 14 as no symptoms (partial Mayo score of 2 with no individual sub score of > 2) and an endoscopic Mayo score 0–1.

[b] All other patients, including those with a partial response (a decrease of at least 3 points in the partial Mayo score and at least 1 point at the endoscopic Mayo score) at week 14 and patients who left the study early, were classified as non-responders.

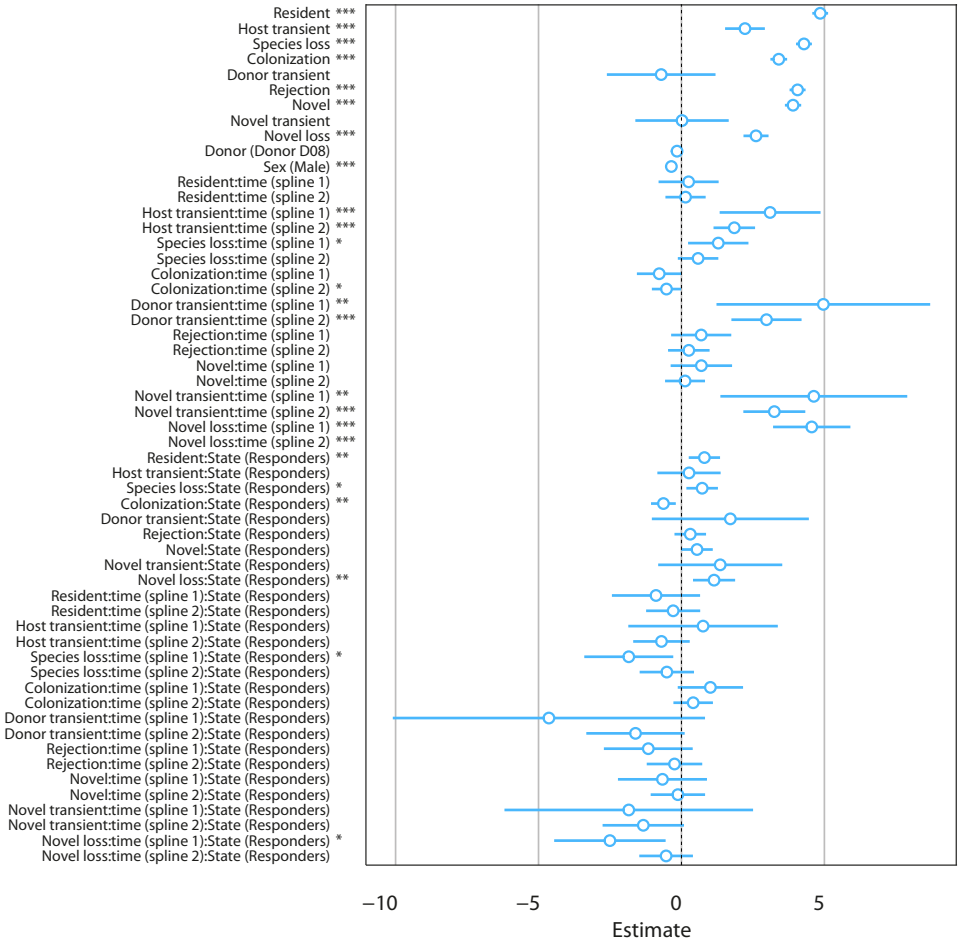
[c] Percentages calculated separately for responders and non-responders.

Appendix Table 6.2 - Model estimates and *p*-values for the differences in relative abundances.

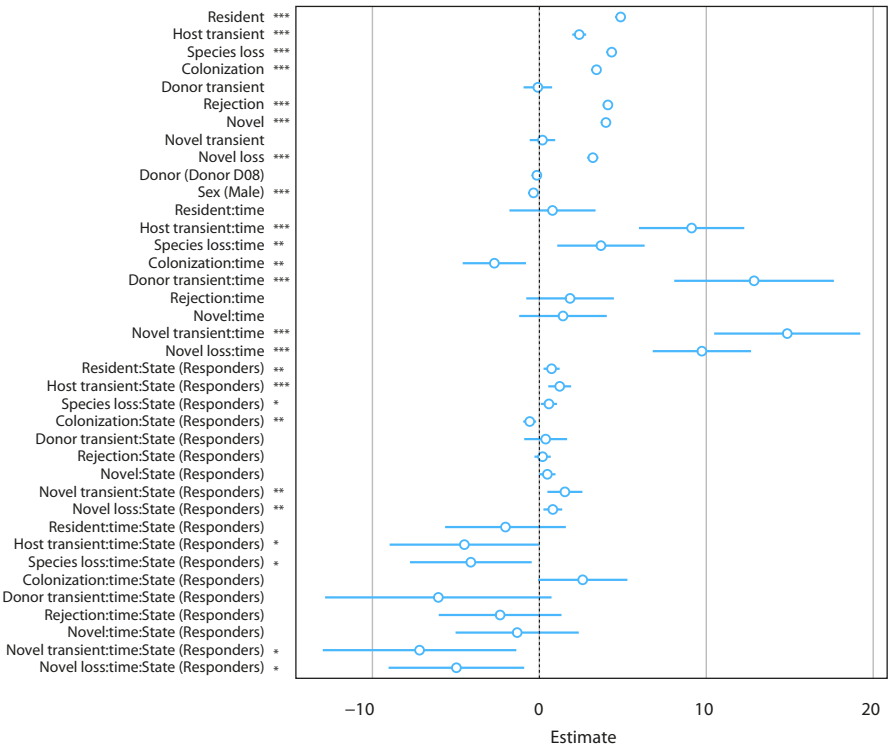
Results are visualized in Figure 6.3. Multiple models were used to test the differences. Significant results are highlighted in bold and blue.

A) Abundance differences per time point				
Post-3				
Intercept	–5.72735	0.15259	–37.535	**< 2e-16**
Host transient	–0.68310	0.08039	–8.497	**< 2e-16**
Post-4				
Intercept	–5.91299	0.10204	–57.95	**< 2e-16**
Host transient	–0.83215	0.06461	–12.88	**< 2e-16**
Week 8				
Intercept	–5.95367	0.10251	–58.08	**< 2e-16**
Host transient	–0.83115	0.05676	–14.64	**< 2e-16**
Week 10				
Intercept	–5.75390	0.18769	–30.66	**3.73e-10**
Host transient	–0.84484	0.06146	–13.75	**< 2e-16**
Week 14				
Intercept	–5.90331	0.09321	–63.33	**< 2e-16**
Host transient	–0.78173	0.05636	–13.87	**< 2e-16**
B) Relative abundance pre-FMT				
Categories within responders				
Intercept	–5.48590	0.19461	–28.189	**2.17e-09**
Host transient[a]	–0.63418	0.07692	–8.245	**3.91e-16**
Species loss[a]	–0.71556	0.06999	–10.224	**< 2e-16**
Categories within non-responders				
Intercept	–5.60069	0.18367	–30.49	**5.3e-10**
Host transient[a]	–0.72155	0.07792	–9.26	**< 2e-16**
Species loss[a]	–0.80577	0.06514	–12.37	**< 2e-16**
Differences in Resident species between responders and non-responders				
Intercept	–5.6414	0.1732	–32.57	**2.69e-16**
State (Responders)	0.1314	0.2432	0.54	0.597
Differences in Host transient species between responders and non-responders				
Intercept	–6.3584	0.1989	–31.973	**5.92e-16**
State (Responders)	0.2003	0.2797	0.716	0.484
Differences in Species loss species between responders and non-responders				
Intercept	–6.3788	0.2133	–29.902	**1.34e-14**
State (Responders)	0.1883	0.3053	0.617	0.546
C) Ratio relative host species abundances between responders and non-responders				
Intercept	0.184309	0.152030	1.212	0.246
State (Responders)	0.006944	0.213633	0.033	0.975

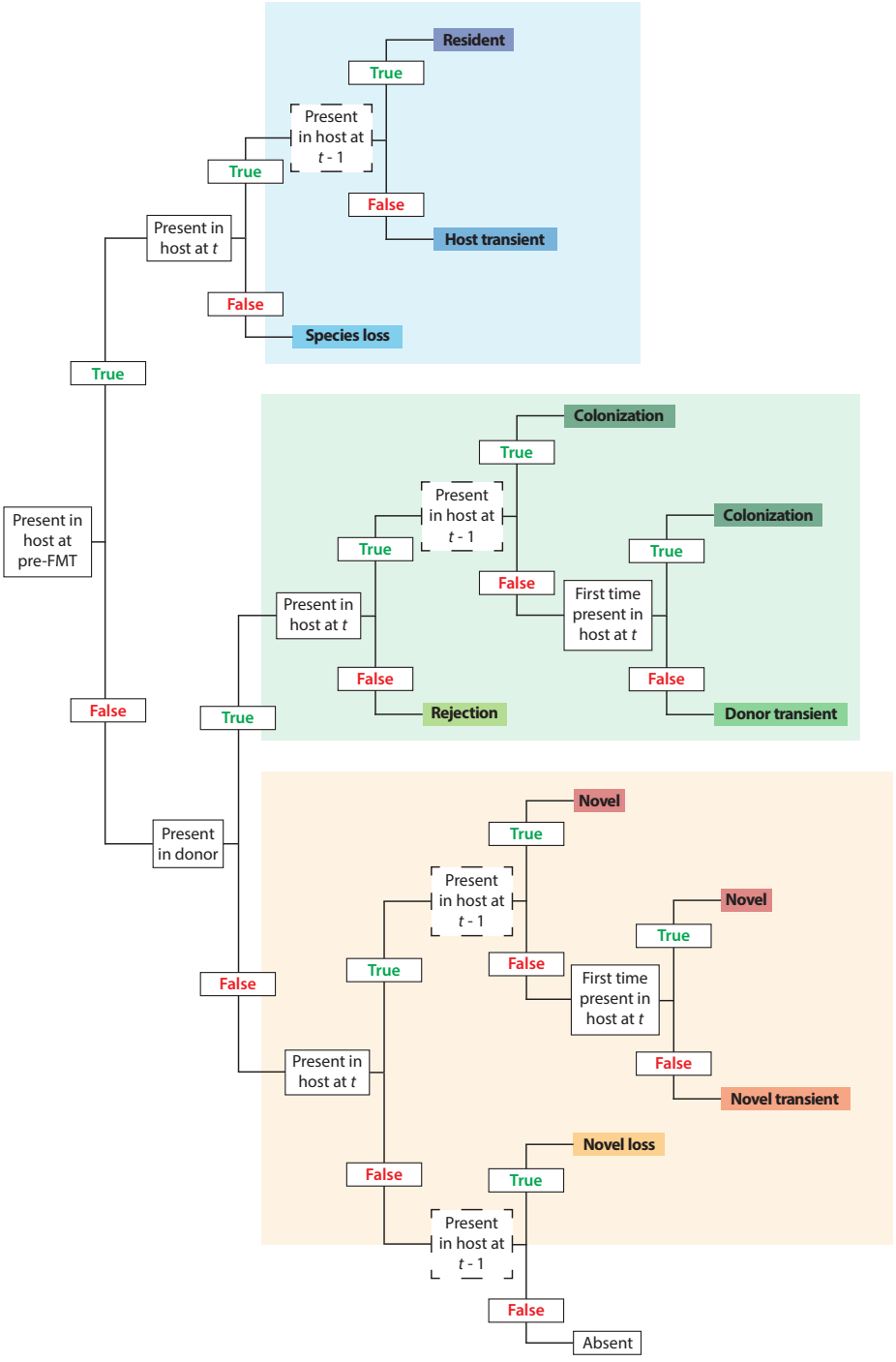
[a] The difference between the host transient and species loss categories for responders and non-responders was tested in a separate model and was not significant (*p*-values were 0.303 and 0.343 for responders and non-responders, respectively).



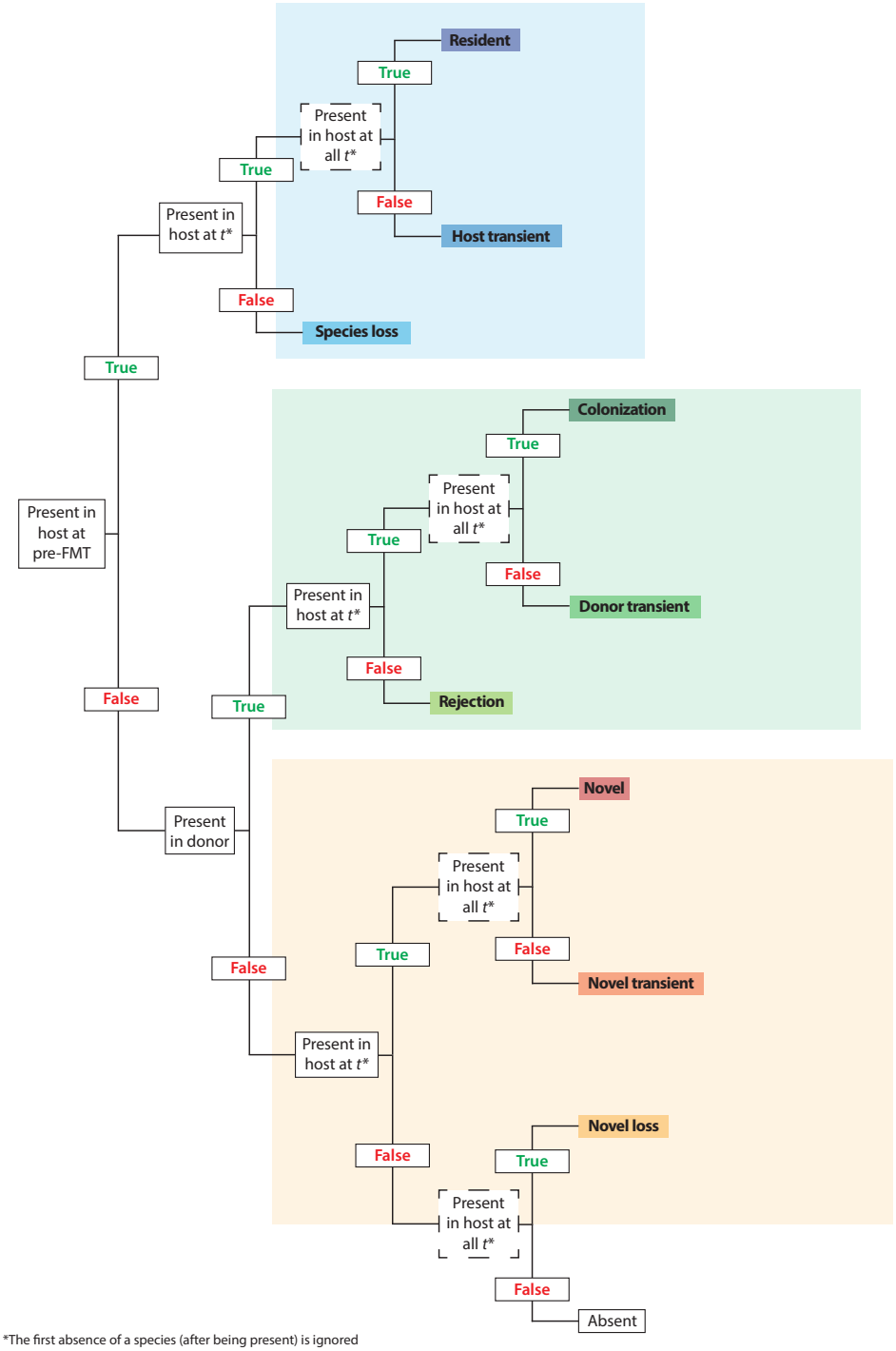
Appendix Figure 6.1. Results of modeling (with a spline) the number of species per ecological category in the base case. The point estimates, 95% confidence intervals, and a reference line at 0 are shown. When the horizontal lines do not cross the vertical reference line, this means that the coefficients are significantly different from 0. The original time variable was modelled with a spline rescaled to denote time in weeks since the start of FMT. The model contained a random intercept per patient to account for repeated measurements.



Appendix Figure 6.2 - Results of modeling (without a spline) the number of species per ecological category in the base case. The point estimates, 95% confidence intervals, and a reference line at 0 are shown. When the horizontal lines do not cross the vertical reference line, this means that the coefficients are significantly different from 0. Contrary to the base case, the original time variable was not modelled with a spline. Time was rescaled to denote time in weeks since the start of FMT. The model contained a random intercept per patient to account for repeated measurements.

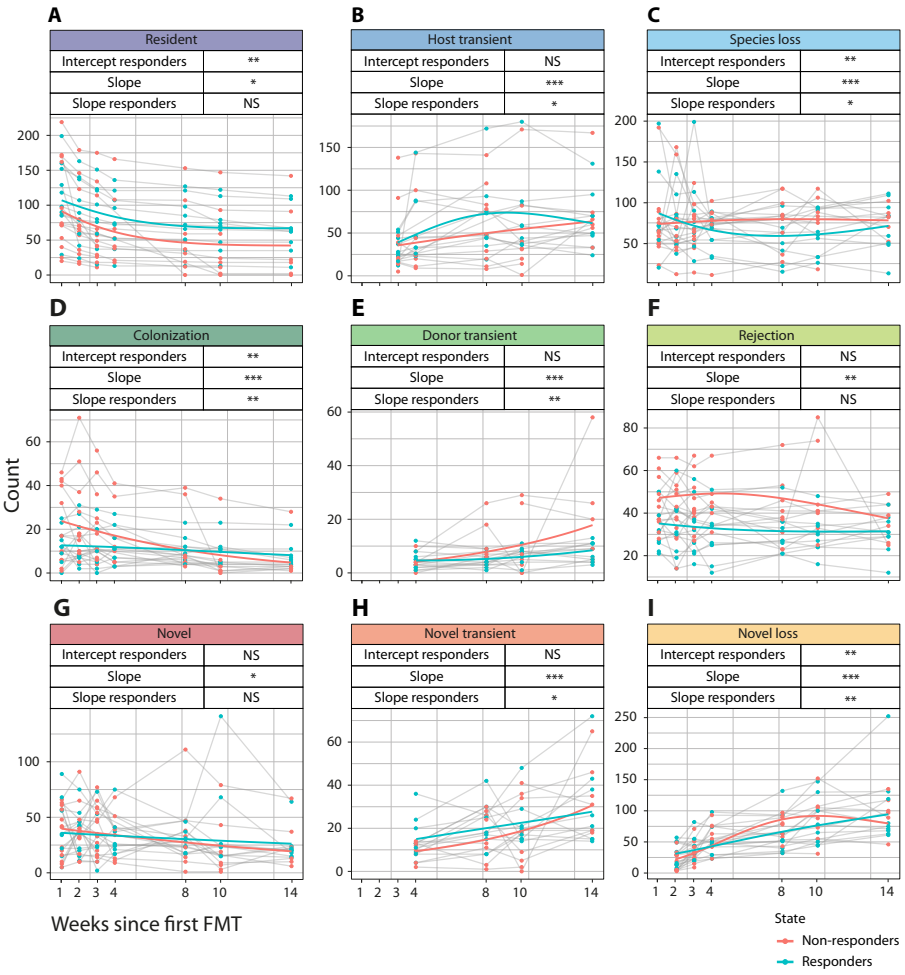


Appendix Figure 6.3 - Decision tree for Sensitivity 2 analysis to assign species to ecological categories according to different inclusion criteria as in the base case analysis. The categories are based on the origin and presence of a species over time. First, the species was compared to the pre-FMT host samples, then to the core donor microbiota. Next, the presence or absence at only the previous time point was considered to assign the species to an ecological category. Differences with the base case scenario, where all previous time points were considered, are indicated with a dotted line around the box (see also Box 6.2).

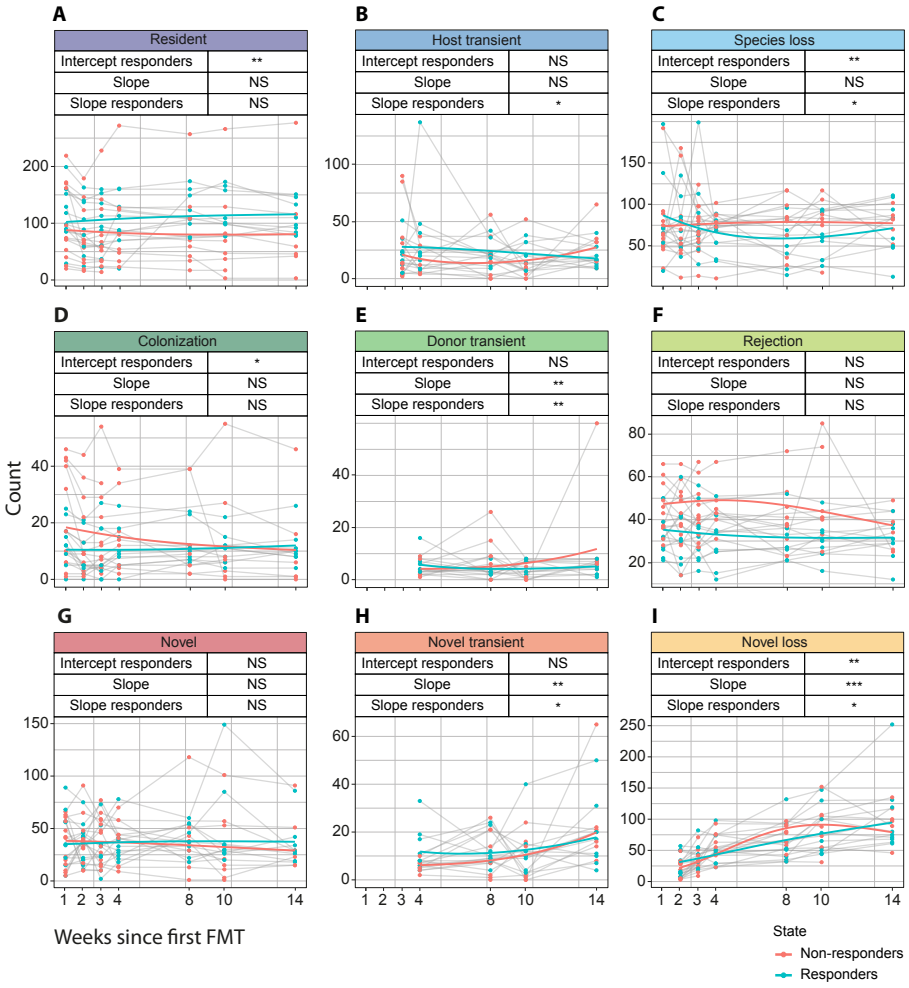


*The first absence of a species (after being present) is ignored

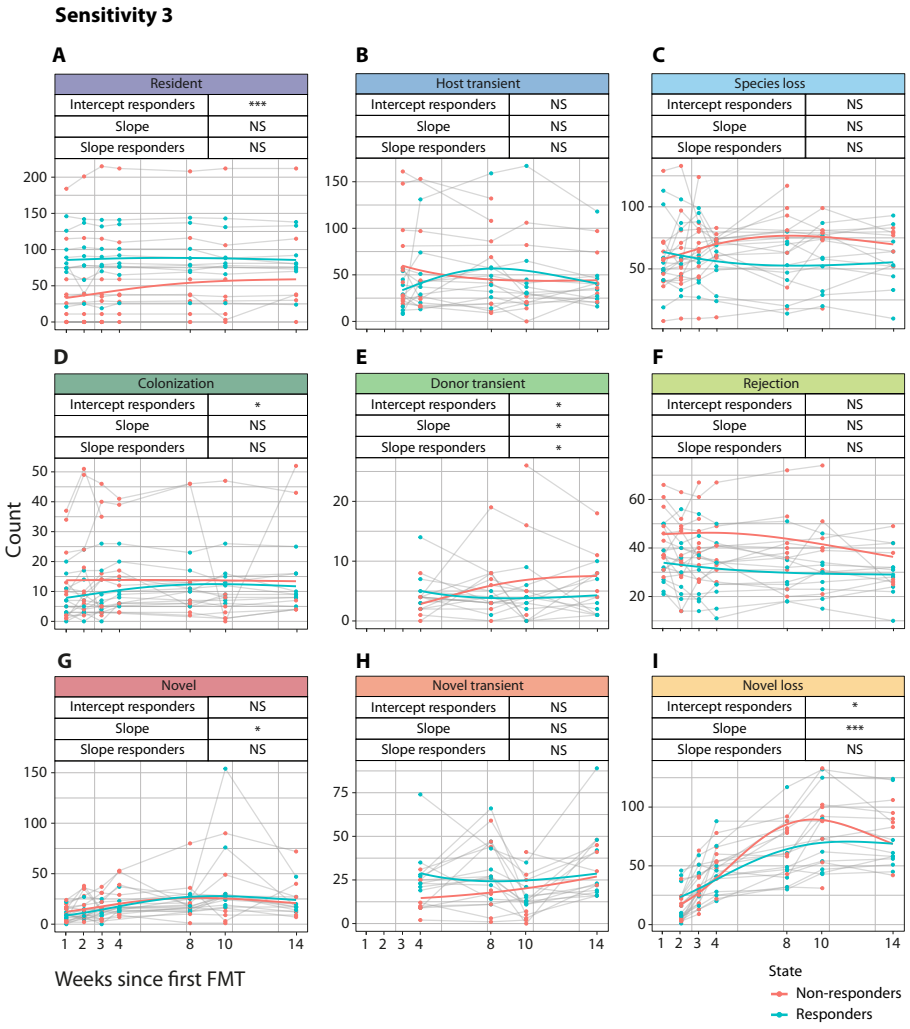
Appendix Figure 6.4 - Decision tree for Sensitivity 3 and 4 analyses to assign species to ecological categories according to different criteria as in the base case analysis. The categories are based on the origin and presence of a species over time. First, the species was compared to the pre-FMT host samples, then to the core donor microbiota. Next, the presence or absence at all time points was considered to assign the species to an ecological category. Differences with the base case scenario, where only previous time points were considered, are indicated with a dotted line around the box (see also Box 6.2).



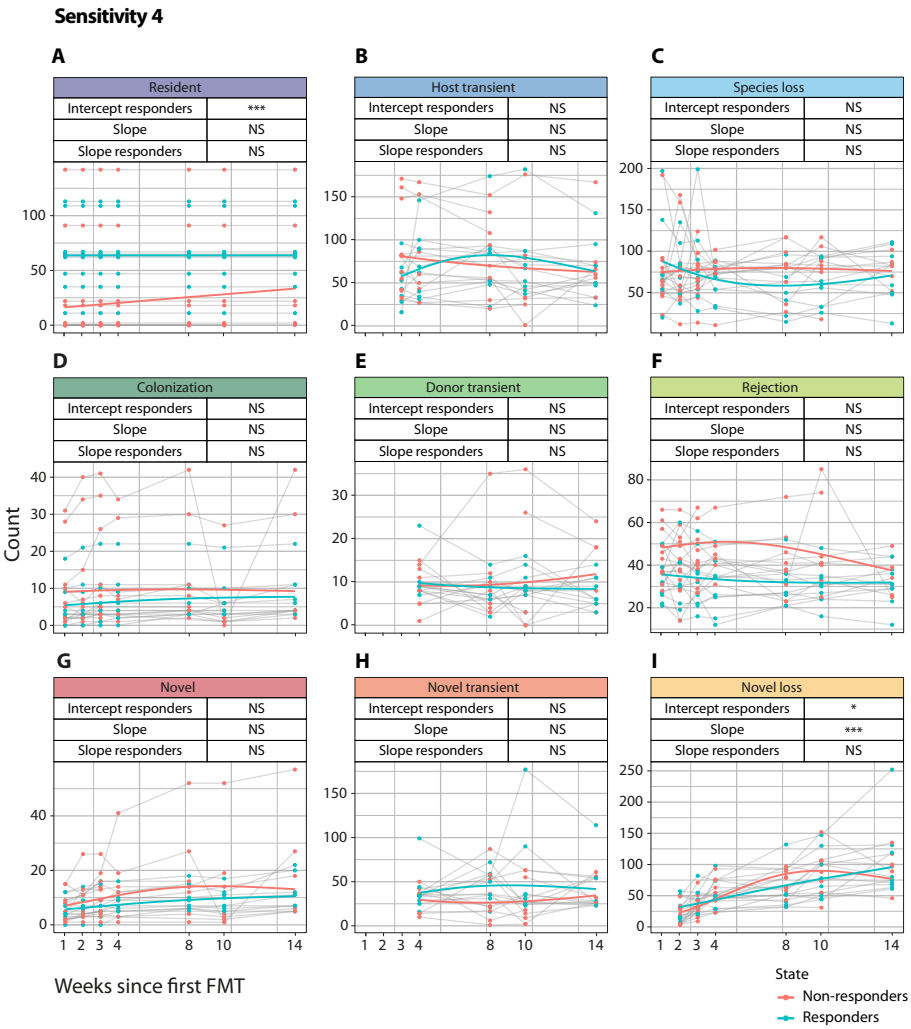
Appendix Figure 6.5 – Temporal changes in the number of species per ecological category for Sensitivity 1. Average trajectories among responders to the treatment are indicated with blue lines, average trajectories among non-responders with red lines. Individual patient trajectories are shown with grey lines. Note the different scaling of the *y*-axes. The model contained a random intercept per patient to account for repeated measurements. Time was modelled with a spline. The levels of significance are reported above each plot and are indicated by asterisks (*** = *p*-value < 0.001; ** = *p*-value < 0.01; * = *p*-value < 0.05; NS = not significant).



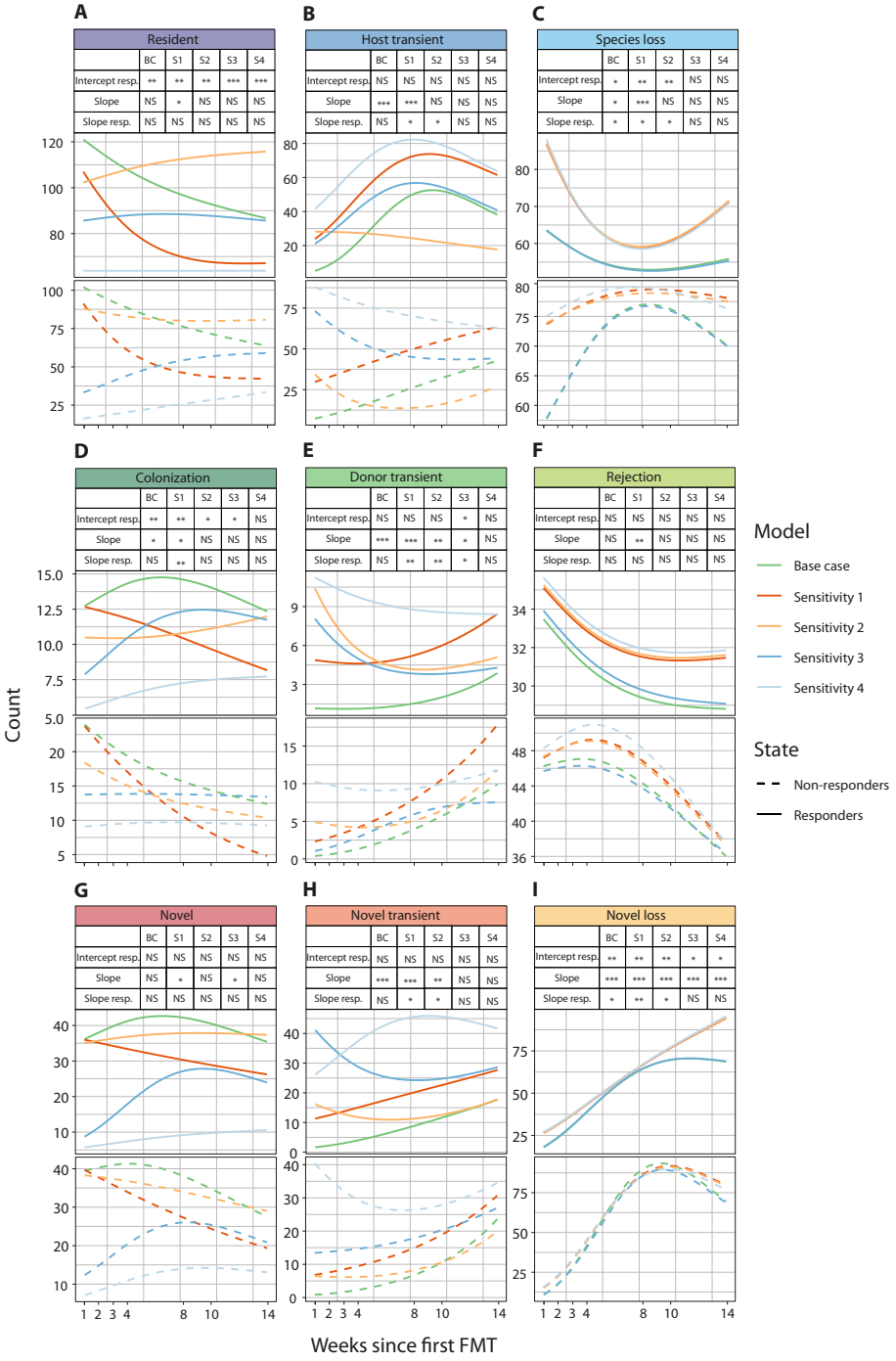
Appendix Figure 6.6 - Temporal changes in the number of species per ecological category for Sensitivity 2. Average trajectories among responders to the treatment are indicated with blue lines, average trajectories among non-responders with red lines. Individual patient trajectories are shown with grey lines. Note the different scaling of the *y*-axes. The model contained a random intercept per patient to account for repeated measurements. Time was modelled with a spline. The levels of significance are reported above each plot and are indicated by asterisks (*** = *p*-value < 0.001; ** = *p*-value < 0.01; * = *p*-value < 0.05; NS = not significant).



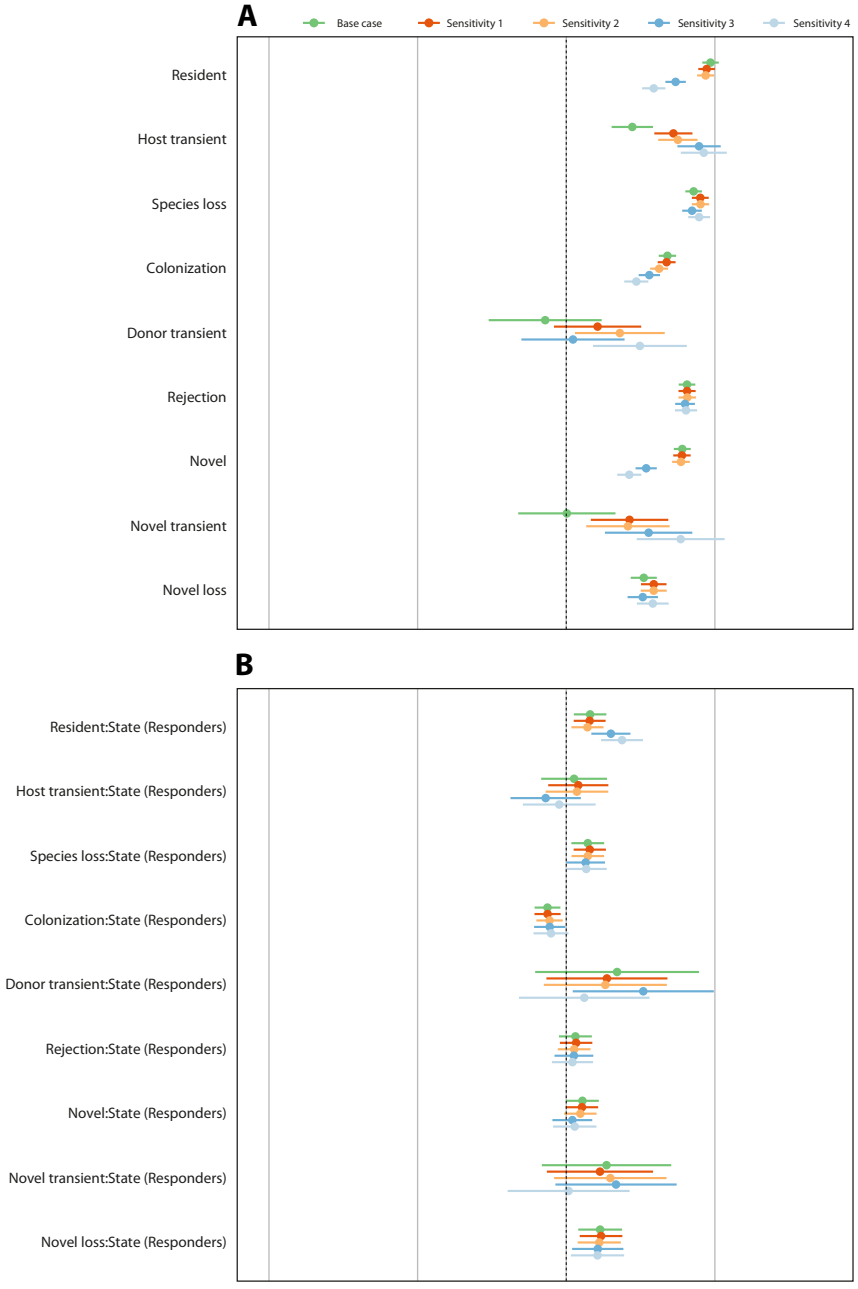
Appendix Figure 6.7 - Temporal changes in the number of species per ecological category for Sensitivity 3. Average trajectories among responders to the treatment are indicated with blue lines, average trajectories among non-responders with red lines. Individual patient trajectories are shown with grey lines. Note the different scaling of the *y*-axes. The model contained a random intercept per patient to account for repeated measurements. Time was modelled with a spline. The levels of significance are reported above each plot and are indicated by asterisks (*** = *p*-value < 0.001; ** = *p*-value < 0.01; * = *p*-value < 0.05; NS = not significant).



Appendix Figure 6.8 - Temporal changes in the number of species per ecological category for Sensitivity 4. Average trajectories among responders to the treatment are indicated with blue lines, average trajectories among non-responders with red lines. Individual patient trajectories are shown with grey lines. Note the different scaling of the *y*-axes. The model contained a random intercept per patient to account for repeated measurements. Time was modelled with a spline. The levels of significance are reported above each plot and are indicated by asterisks (*** = *p*-value < 0.001; ** = *p*-value < 0.01; * = *p*-value < 0.05; NS = not significant).



Appendix Figure 6.9 - Average temporal changes in the number of species per ecological category for the base case (BC) and all Sensitivity analyses (S1, S2, S3, and S4). Upper plots are for responders (solid lines) and lower plots for non-responders (dashed lines) to the treatment. The model contained a random intercept per patient to account for repeated measurements. Time was modelled with a spline. The levels of significance are reported above each plot and are indicated by asterisks (*** = *p*-value < 0.001; ** = *p*-value < 0.01; * = *p*-value < 0.05; NS = not significant).



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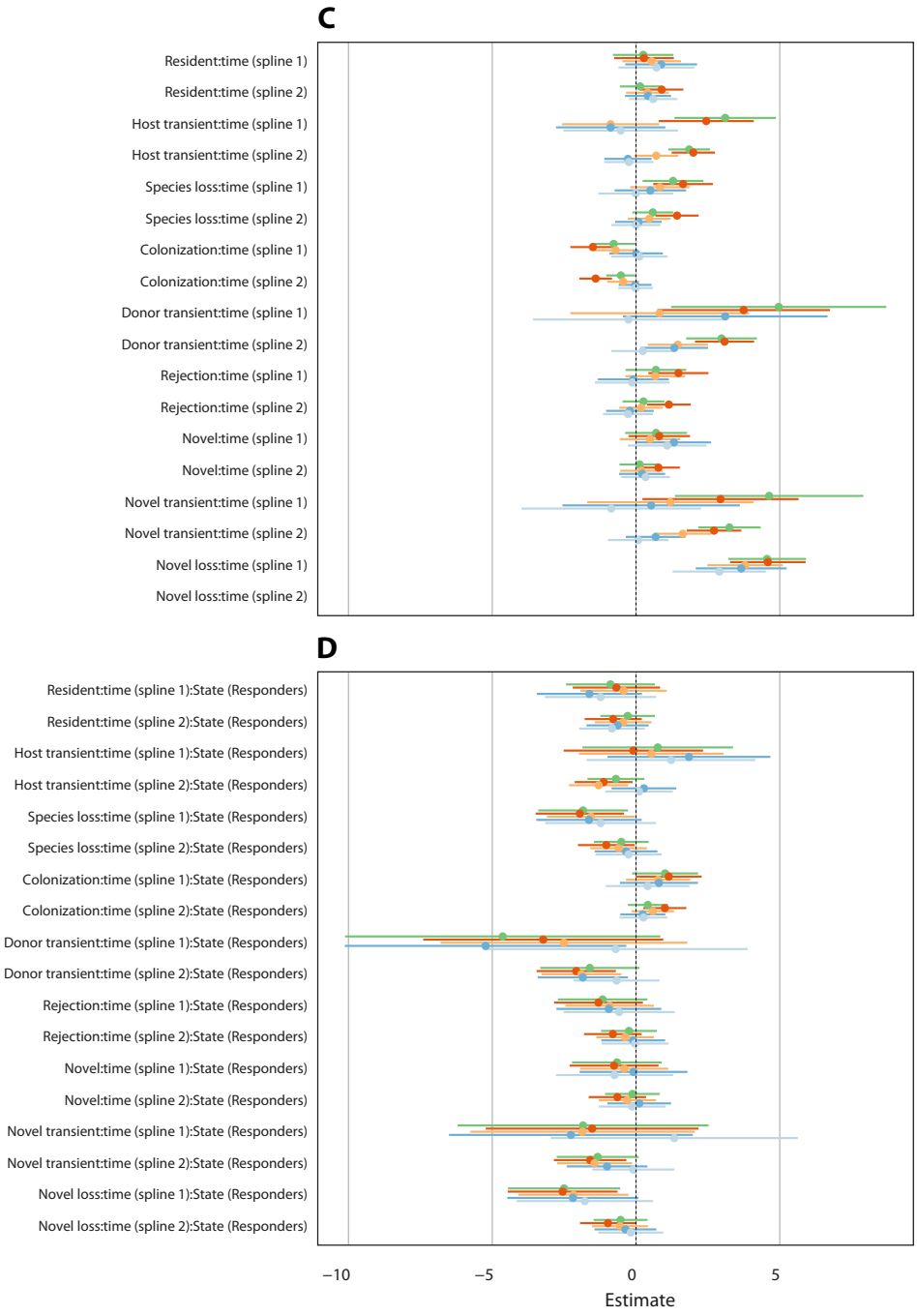
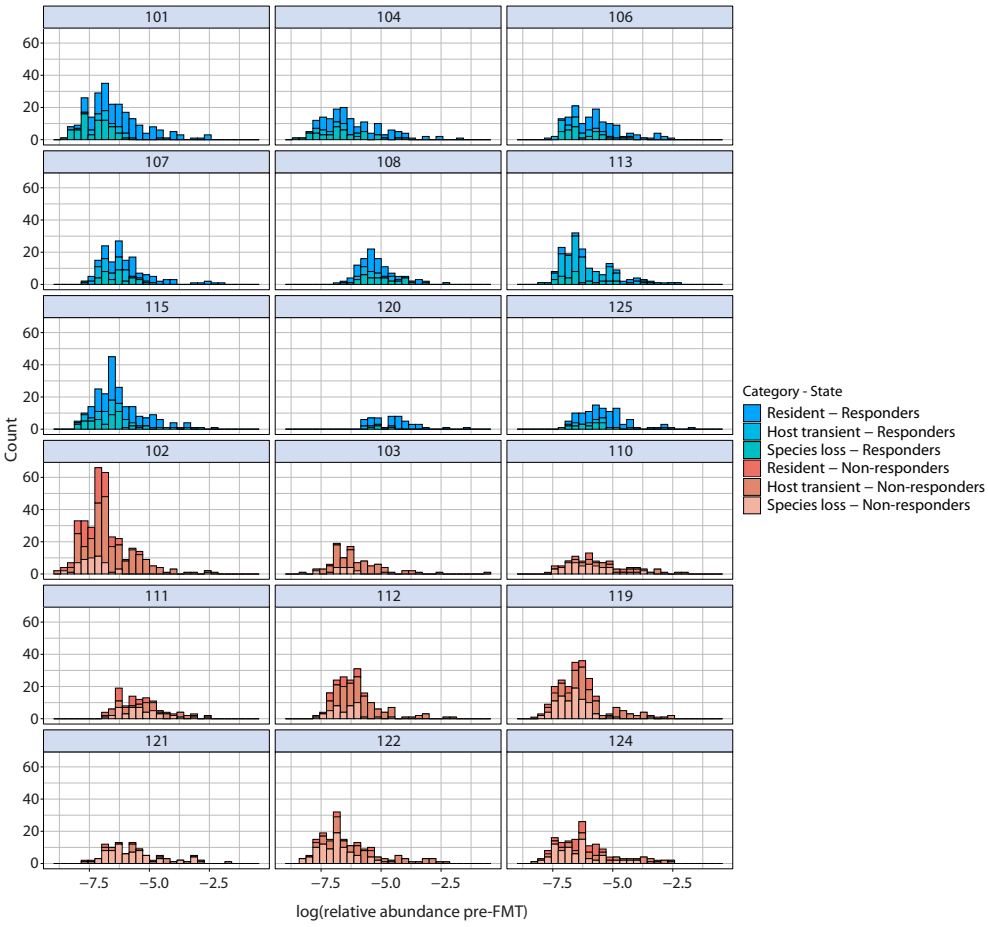
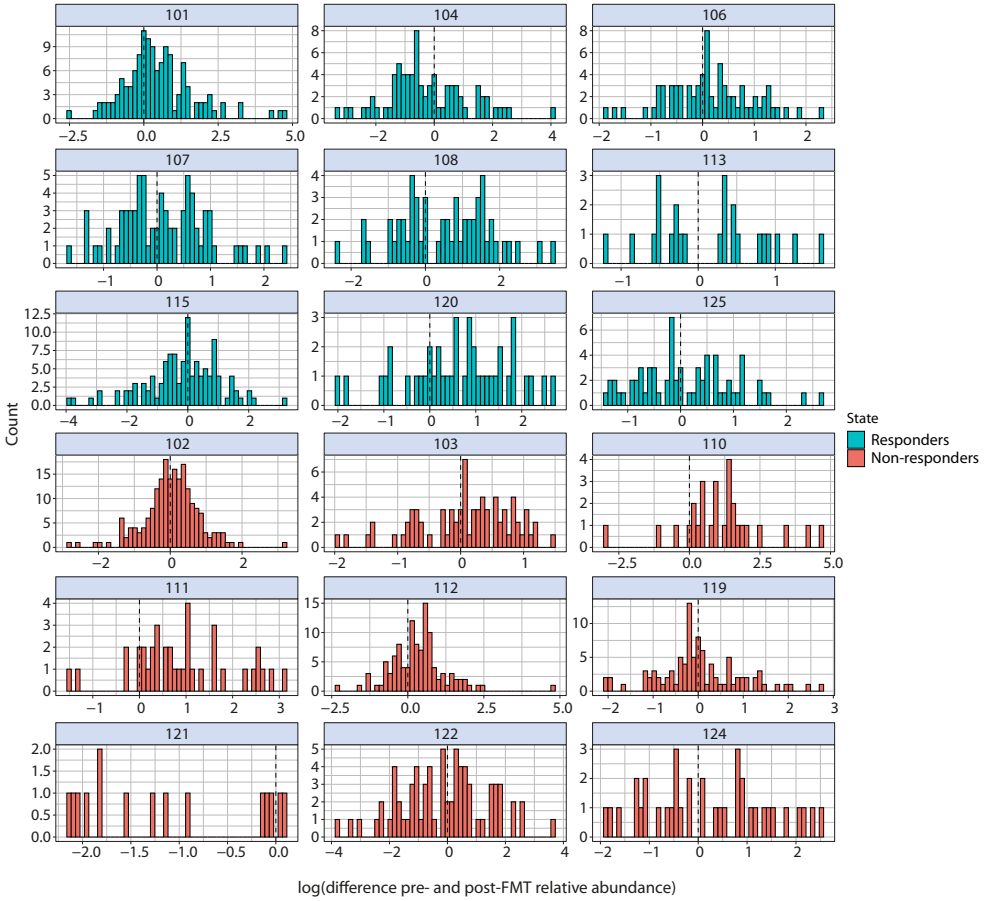


Appendix Figure 6.10 - Distribution of the number of species per ecological category for the base case and all sensitivity analyses, estimated by overdispersed Poisson regression models with random effects and splines.
The models contain random intercepts per patient to account for repeated measurements. The point estimates, 95% confidence intervals, and a reference line at 0 are shown. When the horizontal lines do not cross the vertical reference line, the coefficients are significantly different from 0. A - D) Model output is presented for variables grouped into four categories for clarity.



Appendix Figure 6.11 - Histograms showing the relative abundances of host species (Resident, Host transient, and Species loss) pre-FMT. Only patients that completed the treatment and had at least one post-FMT sample are included in this plot. Because the data had skewed distributions, we used a natural-log transformation of the abundances to normalize the data and homogenize the variance.



Appendix Figure 6.12 - Histograms showing the distribution of the differences in relative abundances (between pre- and post-FMT) of resident species.

Only patients that completed the treatment and had at least one post-FMT sample are included in this plot. The striped vertical line indicates no change in abundance between pre- and post-FMT. Because the data had skewed distributions, we used a natural-log transformation of the abundances to normalize the data and homogenize the variance.