Supplementary Text

Supplementary Text S1 – Supplementary Methods

Determination of moxifloxacin concentration in plasma and feces

Moxifloxacin concentrations were determined by reversed phase high performance liquid chromatography coupled with fluorescence detection for total plasma levels, and tandem mass spectrometry detection for free fecal concentrations as follows. Plasma samples containing moxifloxacin and spiked with ciprofloxacin as internal standard were extracted by solid phase extraction on a cation exchange sorbent (Strata™-X-C, Phenomenex) before being quantified by reversed phase high performance liquid chromatography coupled with fluorescence detection. The separation was performed on a Nucleoshell PFP column (50 x 4.6 mm; 2.7 µm, Macherey-Nagel) with binary gradient elution using 1% acetic acid and 0.3% triethylamine in 20 mM KH2PO4 buffer adjusted to pH 2.7 with H3PO4, and 50:50 (v/v) acetonitrile/methanol solution. Detection was performed with excitation wavelength at 295 nm and emission wavelength at 500 nm. The lower limit of quantification was 0.01 µg/mL.

For the assay of moxifloxacin in feces, the fraction containing free moxifloxacin was prepared by diluting 400 mg of feces 1:4 with 50 mM sodium phosphate buffer pH7 containing 80 mM NaCl, followed by homogenization, centrifugation and filtration of the supernatant on Nanosep MF GHP 0.45 µm. The samples were then purified by solid phase extraction on a molecularly imprinted polymer sorbent specific for fluoroquinolones (SupelMIP™ SPE cartridges, Supelco, Sigma-Aldrich France) before being quantified by reversed phase high performance liquid chromatography coupled to tandem mass spectrometry. The separation was performed on a Kinetex Phenylhexyl column (100 x 3.0 mm: 2.6 µm, Phenomenex) with binary gradient elution using 20 mM ammonium formate buffer pH 2.7 and 0.1% formic acid in acetonitrile. Detection was performed by using the multiple reaction monitoring mode and following the transitions, m/z 402→ m/z 384 for MXF and m/z 406→ m/z 388 for is the internal standard. The lower limit of quantification was 0.04 µg/g feces.

Statistical model

Nonlinear mixed effects models were used to analyse the different markers over time. A nonlinear mixed effects model for multiple responses is defined as follows. Let y_i denote the vector of observations for all responses and y_{ik} the vector of observations for the k^{th} response (e.g., $k = 1$ corresponds to plasma, $k = 2$ to feces, etc.) for the individual i. Let f denote the global structural model characacterizing all responses, based on a system of ordinary differential equations, similar for all individuals. Then one can define $y_{ik} = f_k(\theta_i, \xi_{ik}) + \varepsilon_{ik}$, where f_k is the component of the global model $f$describing the k^{th} repsonse, θ_i is the vector of individual parameters, ξ_{ik} is the vector of n_{ik} sampling times and ε_{ik} is the vector of residual errors for the k^{th} response in individual i. Each individual parameter θ_i can be decomposed as a fixed effect μ, which represents the mean value of the parameter in the population, and a random effect $b_i \sim \mathcal{N}(0, \Omega)$ where Ω accounts for the interindividual variability. Assuming an exponential random effect model, the individual parameters are modeled as: $\theta_i = \mu \times e^{b_i}$.

Lastly we assumed that $\varepsilon_{ik} \sim \mathcal{N}(0, \Sigma_{ik})$ where Σ_{ik} is a $n_{ik} \times n_{ik}$-diagonal matrix with k^{th} elements equal to $(\sigma_{inter,k} + \sigma_{slope,k} \times f_k(\theta_i, \xi_{ik}))^2$, with $\sigma_{inter,k}$ being the parameter for the additive part and $\sigma_{slope,k}$ the parameter for the proportional part of the variance error model. Constant ($\sigma_{inter,k} \neq 0$, $\sigma_{slope,k} = 0$), proportional ($\sigma_{inter,k} = 0$, $\sigma_{slope,k} \neq 0$) or combined ($\sigma_{inter,k} \neq 0$, $\sigma_{slope,k} \neq 0$) variance error models were tested for each response k.

Parameters estimation

Estimation of population parameters was performed using the stochastic approximation expectation maximisation algorithm (SAEM) (1) with 10 Markov chains, implemented in MONOLIX v4.3.2 (Lixoft, Orsay, France, www.lixoft.eu), a software devoted to parameter estimation by maximum likelihood in nonlinear mixed effect models. This algorithm handles data below the limit of quantification (2). It was for instance used to analyse viral load data under HCV treatment or joint modelling in oncology (3, 4).

First, we analysed total concentrations of moxifloxacin in plasma, then both total plasma and free fecal concentrations in moxifloxacin-treated subjects. Fecal weight was fixed to 200 g/day in all subjects. Then, individual pharmacokinetic parameters were estimated as the maximum of the *a posteriori* distribution, also called empirical Bayes estimates, and used to predict individual pharmacokinetic profiles. We used individual predicted fecal concentrations in the analysis of the diversity indices in moxifloxacin-treated subjects, and assumed the diversity indices were stable in the absence of moxifloxacin treatment. The two studied diversity indices were fitted simultaneously using data from moxifloxacin-treated subjects and controls, with a similar structural model for the effect of fecal concentration of free moxifloxacin on the 2 indices. As both indices were related to similar physical quantity, we also tested a simplified model assuming that they shared the same IC_{50} or EC_{50}.

For fixed effects, in case of high relative standard error (r.s.e.) (>60%), we computed the 95% confidence interval of the parameter by likelihood profiling (5). The profile likelihood function was obtained by fixing the parameter of interest at different values within a chosen range, and by maximizing the likelihood over the other parameters. The 95% confidence interval was deduced from the asymptotic χ^2 distribution with 1 degree-of-freedom. For random effects, in case of low estimated standard deviation (<0.1) and high relative standard error, parameter variability was set to 0.

Finally, in order to draw the evolution of plasma and fecal concentration of moxifloxacin, and of the two studied diversity indices, we performed Monte Carlo simulations using the final pharmacokinetic and pharmacodynamic models. We simulated 10'000 vectors of random effects using the estimated distribution of the parameters to obtain simulated individual parameters, and predicted the individual profiles of the plasma and fecal concentrations of moxifloxacin, and of the Shannon index and number of OTUs over time following a 5-day course of 400 mg/day of moxifloxacin.

Model selection and evaluation

At each step, the best model was chosen using the Bayesian information criteria (BIC), derived for each model from the likelihood computed by importance sampling with 2.10^5 iterations (1). Model evaluation was conducted by investigating several goodness-of-fit plots: individual fits, plots of predictions versus

observations, distribution of the individual weighted residuals (IWRES) and normalized prediction distribution errors (NPDE) versus time and versus model predictions, as well as the visual predictive check (VPC). NPDE and VPC were generated using 500 Monte Carlo simulations.

Measures of antibiotic impact on the microbiome

Derived parameters were computed for each subject i treated by moxifloxacin using the individual parameters.

Using the pharmacokinetic model, we computed the mean residence time of moxifloxacin in the compartment k ($k = \{1,2\}$, with 1 is the plasma and 2 is the feces) at steady state as: $MRT_{ik} = \frac{AUMC_{ik}}{AUC_{ik}}$. Let $C_{ik}(t)$ be the function depicting the evolution of the concentration of moxifloxacin over time in the compartment k, then $AUMC_{ik} = \int_{t=0}^{\infty} C_{ik}(t)\ t\ dt$ is the area under the first moment curve of the concentration of moxifloxacin in the compartment k over time at steady state and $AUC_{ik} = \int_{t=0}^{\infty} C_{ik}(t)\ dt$ is the area under the curve of the concentration of moxifloxacin in the compartment l over time at steady state. The elimination half-life from compartment k was computed as: $t_{1/2,ik} = MRT_{ik} \times \log(2)$. The mean transit time between the plasma compartment and the lower intestinal tract ($MTT_{pf,i}$) was computed as $MTT_{pf,i} = \sum_{l=1}^{L} \frac{1}{k_{t,il}}$, where $k_{t,il}$ is the first-order elimination rate for the L compartments identified between the plasma compartment and the lower intestinal tract.

We determined the maximal loss of each bacterial diversity index in the intestinal microbiome after the beginning of treatment (nadir) and the time for which this maximal loss was achieved (time to nadir). We then determined the time at which each diversity index returned to 95% of its baseline value.

We finally quantified the impact of moxifloxacin on diversity by computing the area under the curve for each diversity index up to 42 days after the beginning of treatment ($AUC_{D,i}$). This metric takes into account both the extent of the loss of diversity following moxifloxacin administration, and the duration of the dysbiosis. This period was chosen because the risk of developing severe antibiotic-associated diarrhea such as *Clostridium difficile* colitis has been reported to be maximal in the first few weeks following antibiotic administration (6). It was computed as:

$$AUC_{D,i} = \int_{t=0}^{42} (D_i(t) - D_{i0})\ dt$$

where $D_i(t)$ is the function depicting the evolution of the diversity index over time and D_{i0} is the value of the diversity index at baseline for the subject i and t is in days.

References of the Supplementary methods

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2. Samson A, Lavielle M, Mentré F. 2006. Extension of the SAEM algorithm to left-censored data in nonlinear mixed-effects model: Application to HIV dynamics model. Computational Statistics & Data Analysis 51:1562-74.

3. Laouenan C, Guedj J, Mentre F. 2013. Clinical trial simulation to evaluate power to compare the antiviral effectiveness of two hepatitis C protease inhibitors using nonlinear mixed effect models: a viral kinetic approach. BMC Med Res Methodol 13:60.
4. Desmee S, Mentre F, Veyrat-Follet C, Sebastien B, Guedj J. 2017. Nonlinear joint models for individual dynamic prediction of risk of death using Hamiltonian Monte Carlo: application to metastatic prostate cancer. BMC Med Res Methodol 17:105.
5. Cole SR, Chu H, Greenland S. 2014. Maximum likelihood, profile likelihood, and penalized likelihood: a primer. Am J Epidemiol 179:252-60.
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Supplementary Text 2 – Supplementary results

Pharmacokinetic model

The evolution of the amount of moxifloxacin in the various compartments was described using the following system of ordinary differential equations:

$$\frac{dA_a}{dt} = Dose \times F \times k_{tr} \times \frac{(k_{tr} \times t)^n \times e^{-k_{tr} \times t}}{n!} - k_a \times A_a$$

$$\frac{dA_c}{dt} = k_a \times A_a + k_{fc} \times A_f + k_{21} \times A_p - (k_e + k_{ct1} + k_{12}) \times A_c$$

$$\frac{dA_p}{dt} = k_{12} \times A_c - k_{21} \times A_p$$

$$\frac{dA_{t1}}{dt} = k_{ct1} \times A_c - k_t \times A_{t1}$$

$$\frac{dA_{t2}}{dt} = k_t \times A_{t1} - k_t \times A_{t2}$$

$$\frac{dA_f}{dt} = k_t \times A_{t2} - (k_{fc} + k_f) \times A_f$$

where A_a is the amount of moxifloxacin in the absorption compartment; A_c is the amount of moxifloxacin in the central compartment; A_p is the amount of moxifloxacin in the peripheral compartment; A_{t1} is the amount of moxifloxacin in the first transit compartment between the central compartment and the lower intestinal tract; A_{t2} is the amount of moxifloxacin in the second transit compartment between the central compartment and the lower intestinal tract; A_f is the amount of moxifloxacin in the lower intestinal tract.

We denote F the bioavailability of moxifloxacin, n the number of compartments between the depot compartment and the absorption compartment; Mtt the mean transit time from the depot compartment to the absorption compartment; k_{tr} the transfer rate between each compartment for the absorption delay; k_a the absorption rate to the central compartment; k_e the extraintestinal elimination rate from the central compartment; k_{12} and k_{21} the transfer rates between the central compartment and the peripheral compartment; k_{ct1} the elimination rate from the central compartment to the intestinal tract; k_{fc} the transfer rate between the lower intestinal tract and the central compartment; k_t the transfer rate between the intestinal transit compartments; and k_f the elimination rate from the lower intestinal tract;

The relationship between n, Mtt and k_{tr} is: $n = (Mtt \times k_{tr}) - 1$.

Initial conditions of these equations are: $A_{a,0} = 0$; $A_{c,0} = 0$; $A_{p,0} = 0$; $A_{t1,0} = 0$; $A_{t2,0} = 0$ and $A_{f,0} = 0$.

The concentration of total moxifloxacin in the central compartment C_c is computed as: $C_c = \frac{A_c}{V}$, where V is the volume of distribution.

The concentration of free moxifloxacin in the lower intestinal tract C_f is computed as: $C_f = \frac{A_f}{P_f}$, where P_f is the fecal weight.

Pharmacodynamic model

The evolution of the diversity indices in the lower intestinal tract is described using the following system of ordinary differential equations:

$$\frac{dS}{dt} = R_{in,S} - \left(1 + \frac{E_{max,S} \times C_f}{EC_{50} + C_f}\right) \times k_{out,S} \times S$$

$$\frac{dOTU}{dt} = R_{in,OTU} - \left(1 + \frac{E_{max,OTU} \times C_f}{EC_{50} + C_f}\right) \times k_{out,OTU} \times OTU$$

where S is the value of the Shannon index in the lower intestinal tract; OTU is the number of OTUs in the lower intestinal tract; $R_{in,S}$ is the zero-order constant for production of the Shannon index; $k_{out,S}$ is the first-order elimination rate of the Shannon index from the fecal compartment; $R_{in,OTU}$ is the zero-order constant for production of the number of OTUs; $k_{out,OTU}$ is the first-order elimination rate of the number of OTUs from the fecal compartment; $E_{max,S}$ is the maximal effect of moxifloxacin on the elimination rate of the Shannon index; $E_{max,OTU}$ is the maximal effect of moxifloxacin on the elimination rate of the number of OTUs, and EC_{50} is the concentration of moxifloxacin leading to 50% of the maximal effect. S_0 is the value of the Shannon index at baseline; OTU_0 is the number of OTUs at baseline.

Initial conditions of these equations are: $S_0 = \frac{R_{in,S}}{k_{out,S}}$ and $OTU_0 = \frac{R_{in,OTU}}{k_{out,OTU}}$.

Supplementary Tables

Supplementary Table S1

Estimated population parameters and their relative standard errors (r.s.e) for the final pharmacokinetic model of moxifloxacin in the plasma and feces.

The model was fitted on the data from the 14 subjects treated by moxifloxacin. F is the bioavailability of moxifloxacin; Mtt is the mean transit time between the first transit compartment to the absorption compartment; k_{tr} is the transfer rate between each compartment for the absorption delay; k_a is the absorption rate to the central compartment; V is the volume of distribution; k_e is the extraintestinal elimination rate from the central compartment; k_{12} and k_{21} are the transfer rates between the central compartment and the peripheral compartment; k_{ct1} is the elimination rate from the central compartment to the intestinal tract; k_{fc} is the transfer rate between the lower intestinal tract and the central compartment; k_t is the transfer rate between the intestinal transit compartments; k_f is the elimination rate from the lower intestinal tract; $\sigma_{\text{slope,c}}$ is the proportional component of the residual error for the plasma pharmacokinetic model; $\sigma_{\text{inter,f}}$ and $\sigma_{\text{slope,f}}$ are the additive and proportional components of the residual error for the fecal pharmacokinetic model, respectively.

		Fixed effects		Standard deviation of the exponential random effects (%)	
Model	**Parameter**	**Estimate**	**r.s.e. (%)**	**Estimate**	**r.s.e. (%)**
Plasma	Mtt (day)	0.014	30	92.3	26
	k_{tr} (day^{-1})	151	52	166.0	24
	k_a (day^{-1})	101	47	160.0	22
	V/F (L)	99.6	6	17.2	27
	k_e (day^{-1})	1.63	4	10.8	26
	k_{12} (day^{-1})	0.125	11	0 (fixed)	-
	k_{21} (day^{-1})	1.06	<1	0 (fixed)	-
	k_{ct1} (day^{-1})	0.205	10	31.2	27
Lower gastrointestinal tract	k_{fc} (day^{-1})	0.407	18	52.4	27
	k_t (day^{-1})	3.46	15	48.5	23
	k_f (day^{-1})	1.23	16	57.2	20
Residual error	$\sigma_{\text{slope,c}}$	0.216	5		
	$\sigma_{\text{inter,f}}$ (µg/g)	0.0557	36		
	$\sigma_{\text{slope,f}}$	0.481	8		

Supplementary Table S2

Estimated population parameters and their relative standard errors for the final pharmacodynamic model of moxifloxacin on the bacterial diversity in the intestinal microbiome.

The model was fitted on the data from all subjects. S_0 is the value of the Shannon index at steady state; OTU_0 is the number of OTUs at steady state; $k_{out,S}$ the first-order elimination rate of the Shannon index from the lower intestinal tract; $k_{out,OTU}$ is the first-order elimination rate of the number of OTUs from the lower intestinal tract; $E_{max,S}$ is the maximal effect of moxifloxacin on the elimination rate of the Shannon index; $E_{max,OTU}$ is the maximal effect of moxifloxacin on the elimination rate of the number of OTUs, EC_{50} is the concentration of moxifloxacin leading to 50% of the maximal effect, $\sigma_{inter,S}$ is the additive component of the residual error for the pharmacodynamic model of the Shannon index; $\sigma_{inter,OTU}$ is the additive component of the residual error for the pharmacodynamic model of the number of OTUs.

Parameter	**Fixed effects**		**Standard deviation of the exponential random effects**	
	Estimate	**r.s.e. (%)**	**Estimate**	**r.s.e. (%)**
S_0 (Shannon unit)	4.75	2	0.0729	22
OTU_0 (OTU)	163	5	0.203	17
$k_{out,S}$ (day^{-1})	0.585	27	0 (fixed)	-
$k_{out,OTU}$ (day^{-1})	0.275	26	0 (fixed)	-
$E_{max,S}$	0.384	14	0 (fixed)	-
$E_{max,OTU}$	0.938	4	0 (fixed)	-
EC_{50} (µg/g)	0.128	137	4.74	21
$\sigma_{inter,S}$ (Shannon unit)	0.506	7		
$\sigma_{inter,OTU}$ (OTU)	23.2	7		

Supplementary Table S3

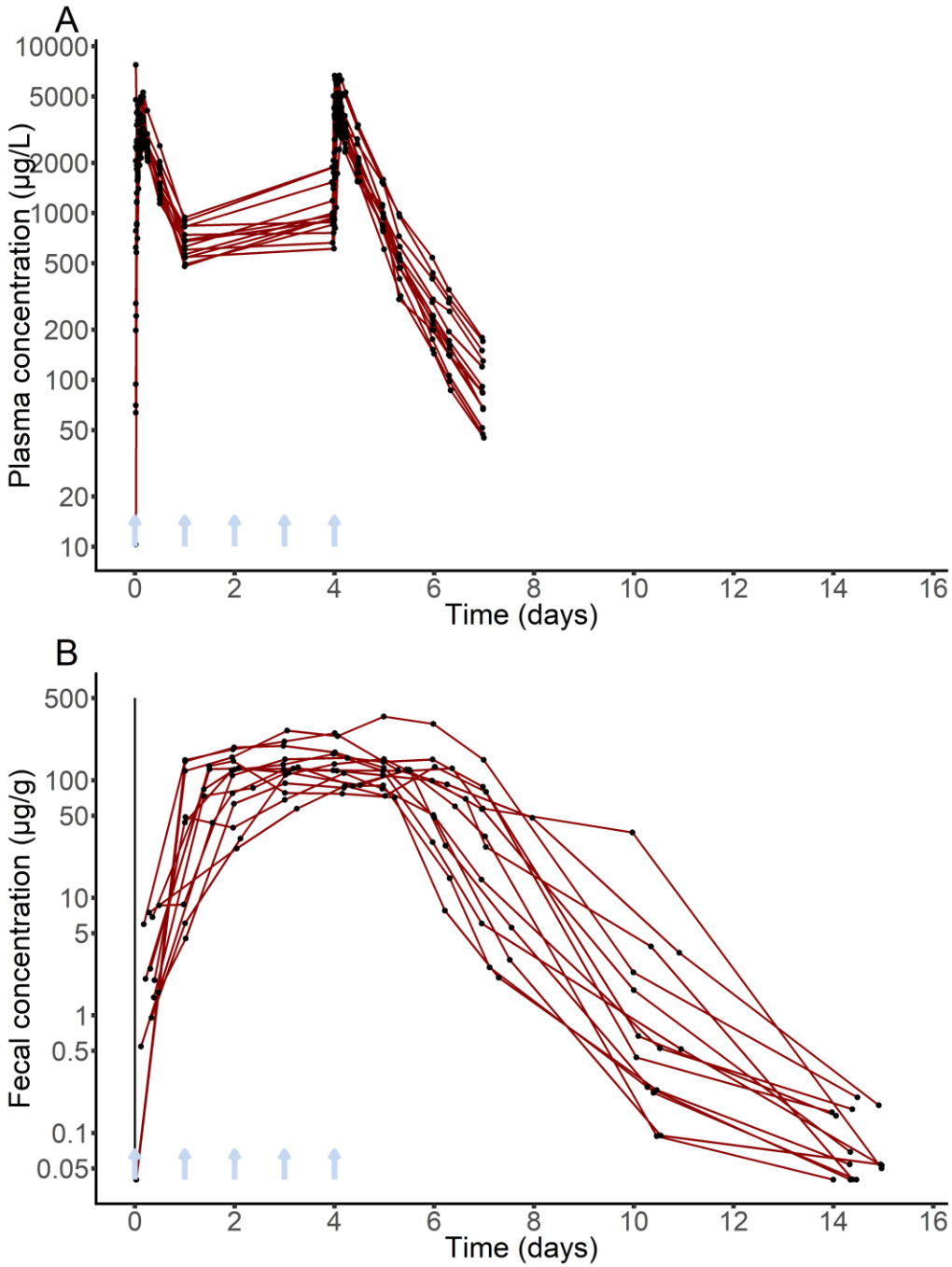
Measures of the impact of moxifloxacin on the gut microbiome diversity according to moxifloxacin daily dose and to treatment duration. Measures were derived from the estimated individual pharmacodynamic parameters in the 14 treated individuals. Data are presented as median (min; max).

	5-day treatment	**10-day treatment**	
Pharmacodynamic indices	**Moxifloxacin 800 mg**	**Moxifloxacin 400 mg**	**Moxifloxacin 800 mg**
Shannon index			
Time to maximal loss (days)	7.4 (5.1; 11.8)	10.5 (9.9; 14.0)	10.6 (9.9; 14.4)
Maximal loss (unit)	1.3 (1.0; 1.4)	1.3 (0.9; 1.4)	1.3 (1.0; 1.4)
Maximal loss (% of baseline value)	27.6 (21.3; 27.7)	27.7 (18.4; 27.8)	27.7 (22.1; 27.8)
Time to return to 95% of baseline value (days)	17.1 (9.4; 26.2)	21.3 (13.8; 30.4)	22.1 (14.4; 31.2)
AUC between day 0 and day 42 of the change of diversity indices from day 0 (unit.day)	-17.8 (-29.8; -6.7)	-23.3 (-35.4; -9.5)	-24.4 (-36.5; -11.9)
Number of OTUs			
Time to maximal loss (days)	8.9 (5.5; 14.3)	11.8 (10.0; 16.9)	12.1 (10.0; 17.4)
Maximal loss (number of OTUs)	77 (56; 100)	77 (54; 100)	77 (58; 100)
Maximal loss (% of baseline value)	47.6 (36.5; 48.3)	48.2 (34.8; 48.4)	48.2 (40.4; 48.4)
Time to return to 95% of baseline value (days)	22.1 (14.2; 31.2)	26.3 (18.6; 35.4)	27.2 (19.4; 36.2)
AUC between day 0 and day 42 of the change of diversity indices from day 0 (number of OTUs.day)	-1147 (-2405; -475)	-1440 (-2821; -657)	-1515 (-796; -2902)

Supplementary Figures

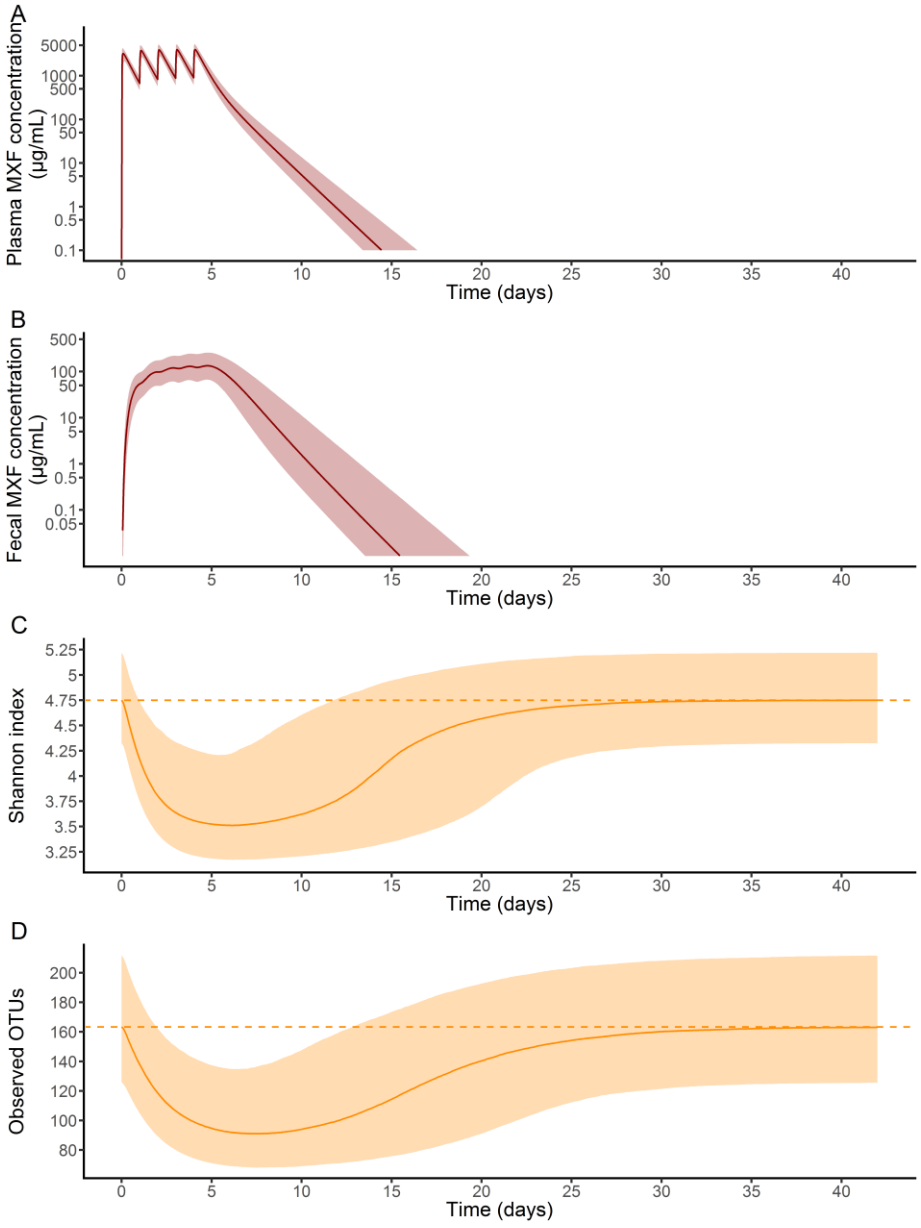
Supplementary Figure S1

Spaghetti plot of the observed concentrations of free moxifloxacin in plasma (panel A) and feces (panel B) for the 14 subjects treated with moxifloxacin. Blue arrows represent moxifloxacin administration.



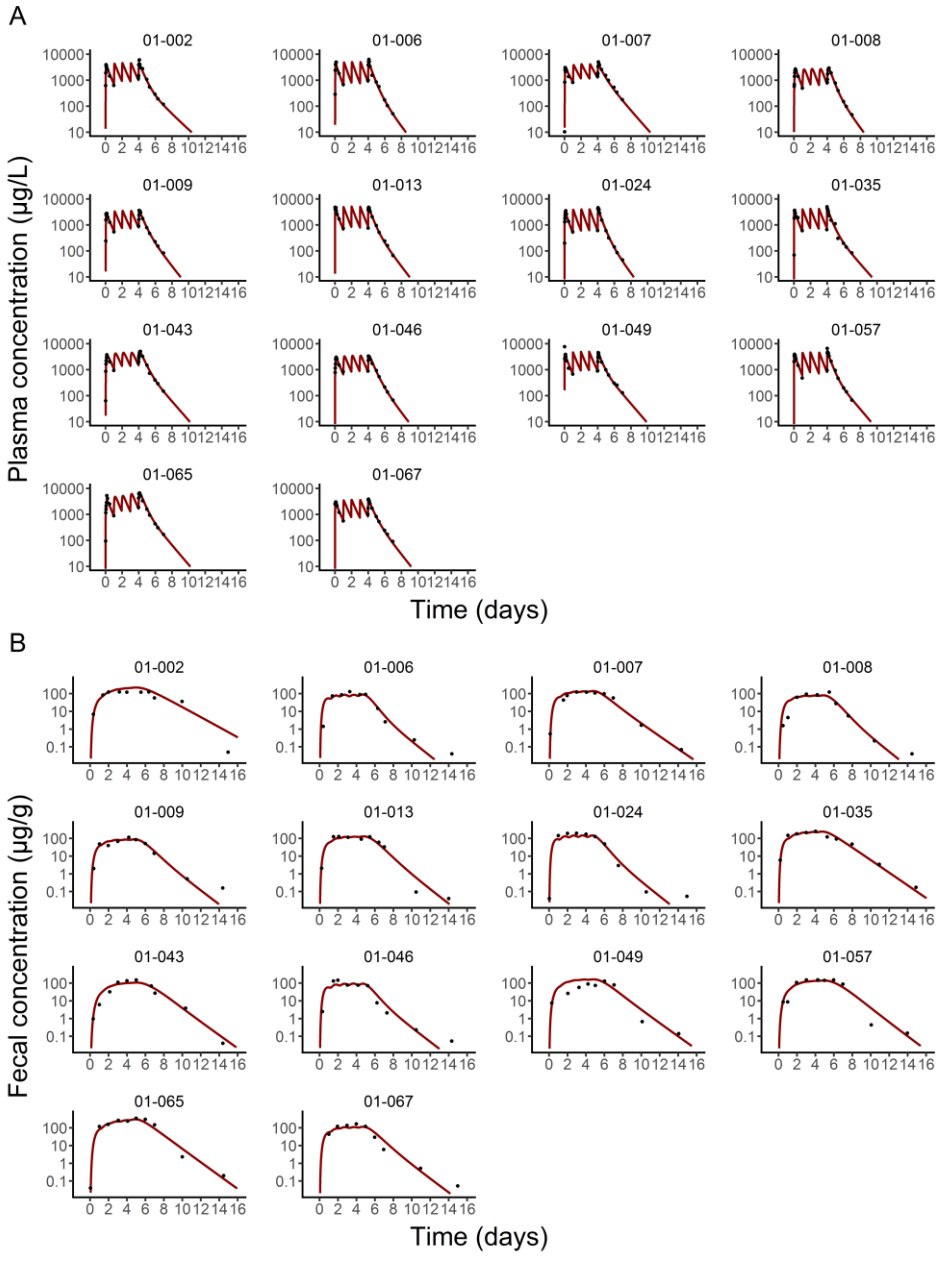
Supplementary Figure S2

Simulated profiles of the concentration of total moxifloxacin in plasma (panel A), of free moxifoxacin in feces (panel B), of the Shannon index (panel C) and of the number of OTUs (panel D) following a course of 400 mg/day moxifloxacin for 5 days. We simulated 10,000 vectors of random effects using the estimated distribution of the parameters and predicted the individual profiles of the Shannon index and number of OTUs over time for a 5-day course of 400 mg/day of moxifloxacin. Plain lines represent the median simulations, whereas ribbons represent the 90% prediction intervals. In panels C and D, orange dashed lines represent the median baseline value of diversity indices without moxifloxacin treatment.



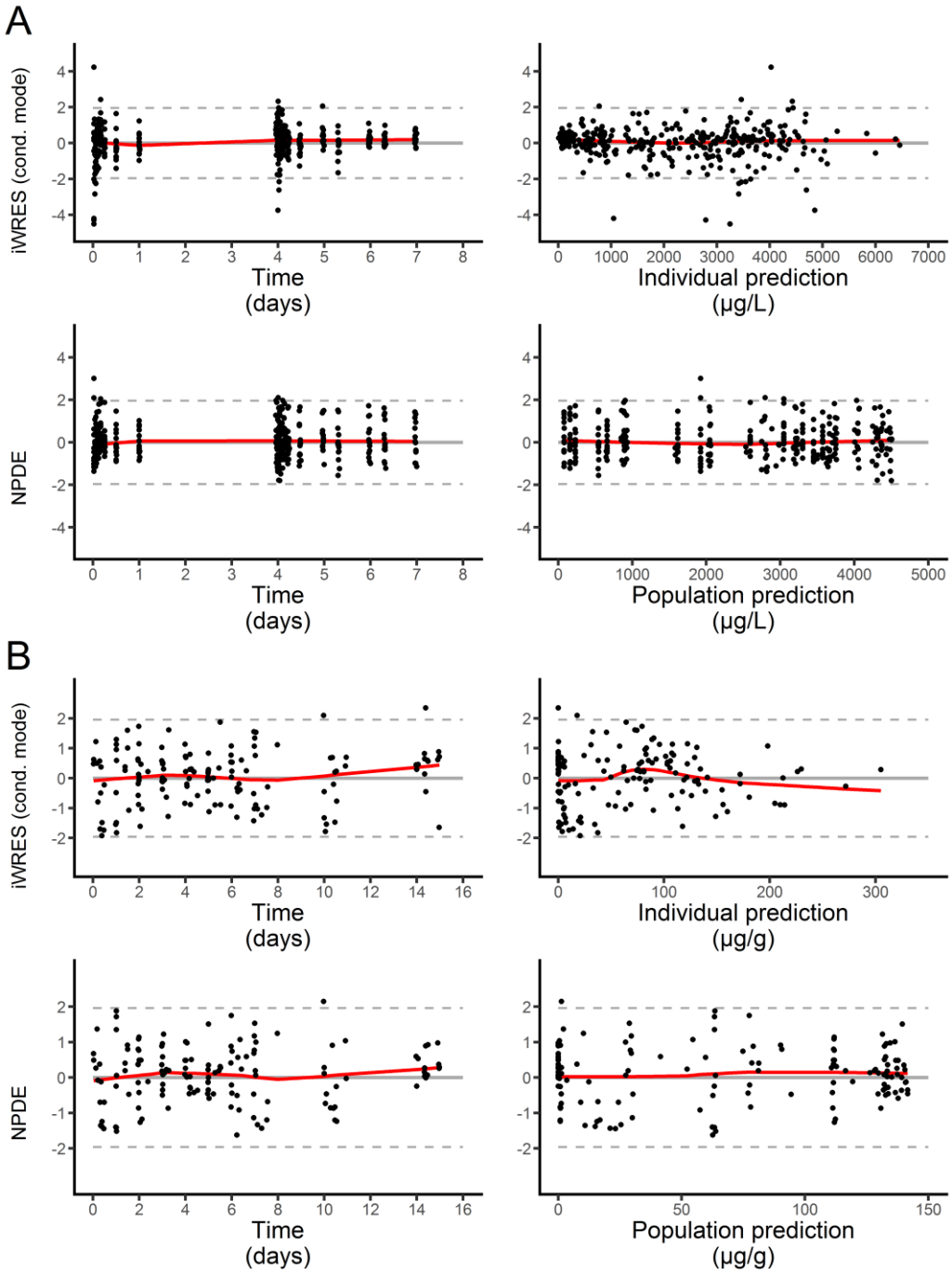
Supplementary Figure S3

Individual fits for the plasma total (panel A) and fecal free (panel B) moxifloxacin concentrations for the final pharmacokinetic model in the 14 subjects treated by moxifloxacin. Black dots represent observed moxifloxacin concentrations. Red curves represent the individual pharmacokinetic profiles predicted by the model using estimated individual parameters.



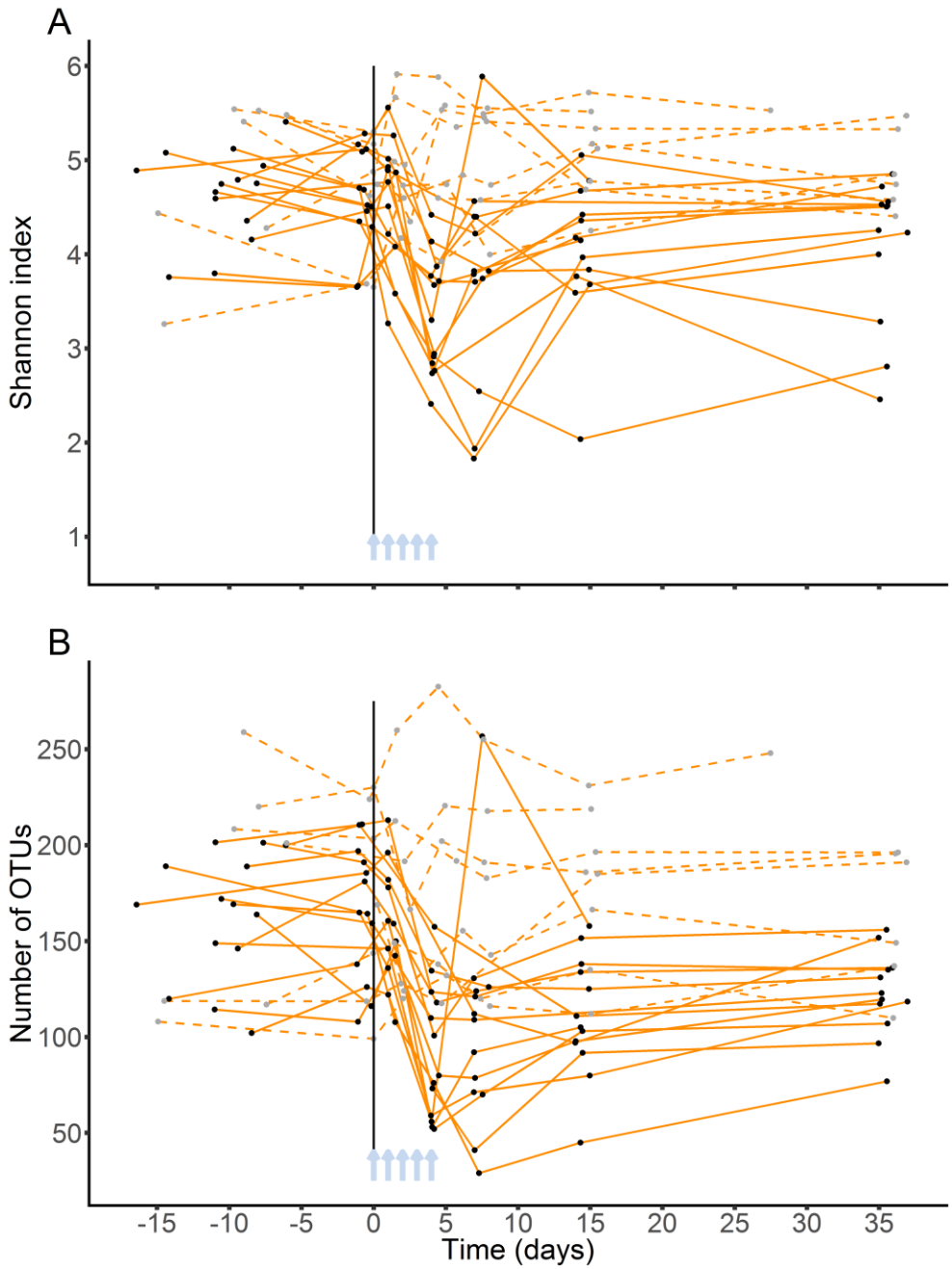
Supplementary Figure S4

Goodness of fit plots for the pharmacokinetic model. Plots of the individual weighted residuals (iWRES) and normalized prediction distribution errors (NPDE) over time (left) or model predictions (right) for the plasma (panel A) or fecal (panel B) concentrations. The iWRES and NPDE are shown as black points, and spline lines are also added as red curves.



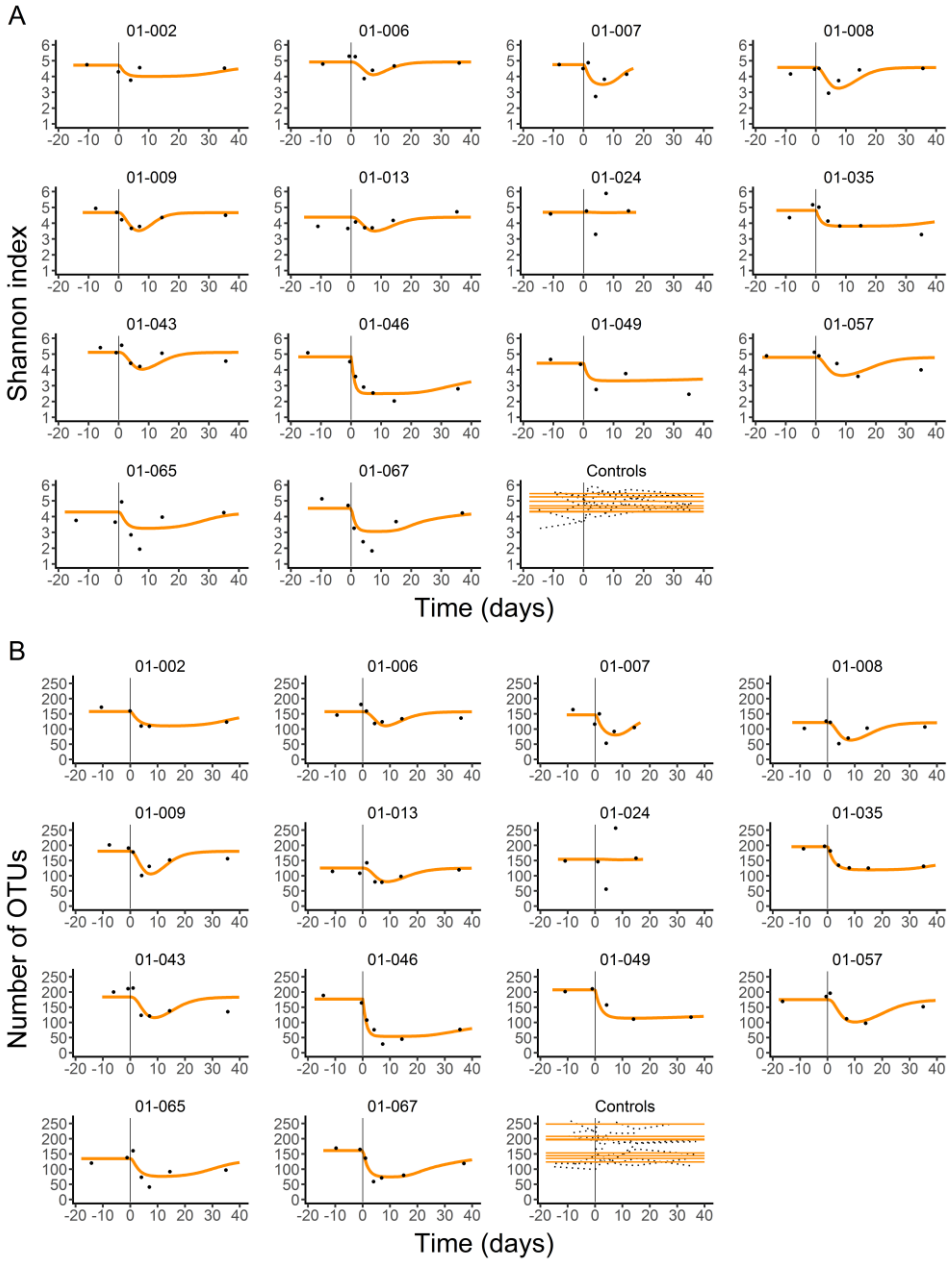
Supplementary Figure S5

Spaghetti plots of the bacterial diversity within the intestinal microbiome (Shannon index, panel A, and number of OTUs, panel B). Subjects treated with moxifloxacin (N=14) are represented with plain line and black points, and subjects from the negative control group (N=8) are represented with dashed lines and grey points. Blue arrows represent moxifloxacin administration.



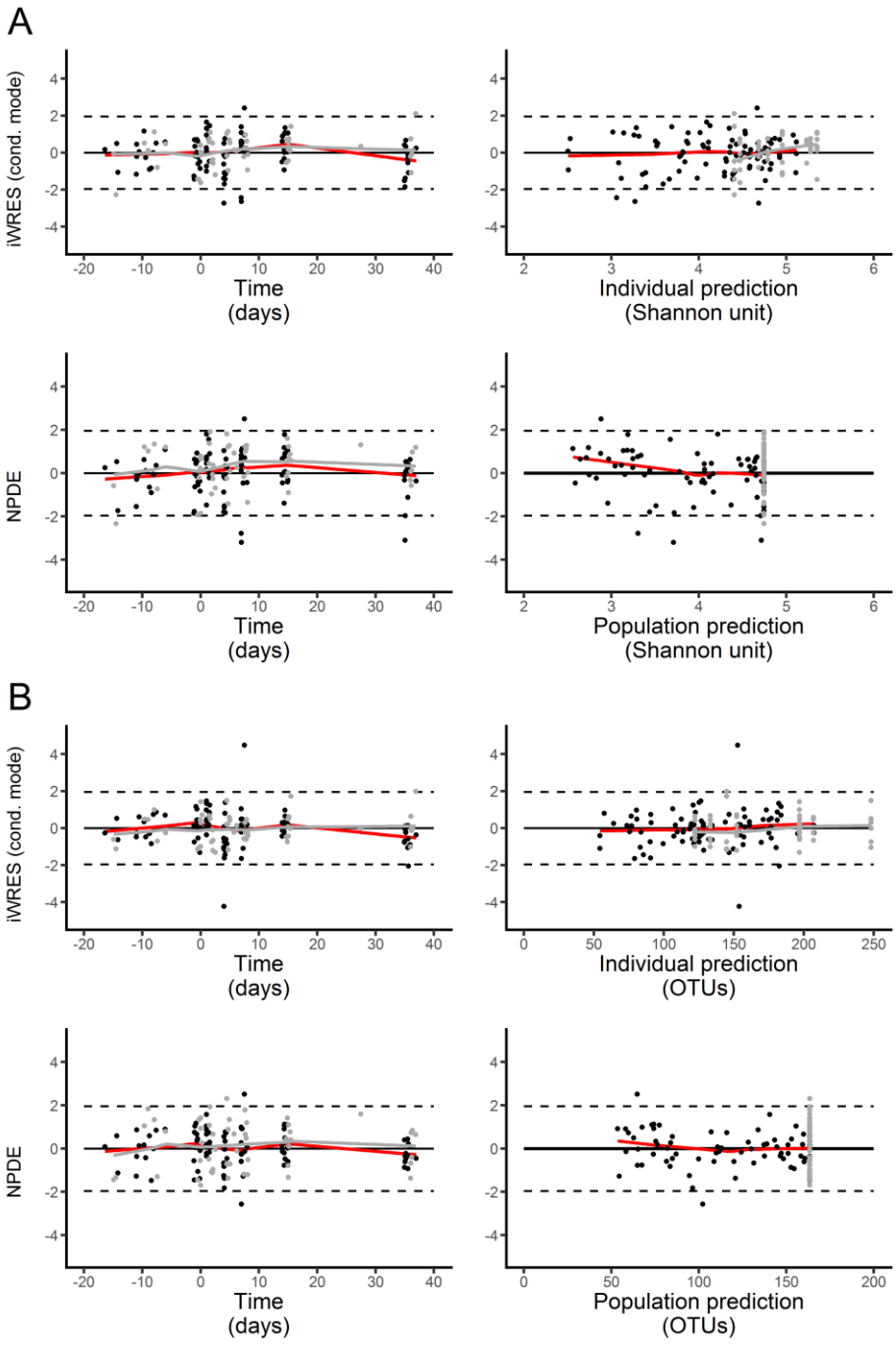
Supplementary Figure S6

Individual fits for the Shannon index (panel A) and number of OTUs (panel B) for the final pharmacodynamic model. Individual plots are represented for moxifloxain-treated subjects, whereas negative control subjects are all represented on the same plot. Black dots represent observed values of the diversity indices of moxifloxacin-treated subjects, and black dotted lines represent observed values of the diversity indices of negative control subjects. Orange curves represent the individual pharmacodynamic profiles predicted by the model using estimated individual parameters.



Supplementary Figure S7

Godness of fit plots for the pharmacodynamic model (Shannon index, panel A, and number of OTUs, panel B). Individual weighted residuals (iWRES) and normalized prediction distribution errors (NPDE) over time (left) or model predictions (right) for moxifloxacin-treated subjects are shown as black points and spline lines are added as red curves, whereas iWRES and NPDE are shown as grey points and spline lines are added as grey curves for negative controls.



Supplementary Figure S8

Profile of the log-likelihood according to the value of the EC_{50} parameter. The profile was obtained by fixing the EC_{50} parameter at different values ranging from 10^{-3} to 10, while maximizing the likelihood over the other parameters. Horizontal dashed line represent the value for which the variation of the log-likelihood from its maximum is $\frac{3.84}{2}$, where 3.84 is the 95th percentile of a 1-degree of freedom χ^2 variable. The blue shaded area represents the 95% confidence interval of the EC_{50} parameter computed using the Student's t-distribution.