Package: microbiomeDASim (via r-universe)

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Title Microbiome Differential Abundance Simulation

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Description A toolkit for simulating differential microbiome data designed for longitudinal analyses. Several functional forms may be specified for the mean trend. Observations are drawn from a multivariate normal model. The objective of this package is to be able to simulate data in order to accurately compare different longitudinal methods for differential abundance.

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`form_beta_check` *Beta Specification Check*

Description

Function for checking that the appopriate beta parameters are specified for each of the mean trend specifications

Usage

```
form_beta_check(form, beta, IP, timepoints)
```

Arguments

`form`	character value specifying the type of time trend. Options include 'linear', 'quadratic', 'cubic', 'M', 'W', 'L_up', and 'L_down'.
`beta`	vector specifying the appropriate parameters for functional trend. See details of `mean_trend` for explanation for each form
`IP`	vector specifying the inflection points. See details of `mean_trend` for explanation for each form
`timepoints`	numeric vector specifying the points to fit the functional trend.
	@keywords internal

Value

Nothing returned unless an error is returned.

gen_norm_microbiome *Generate Longitduinal Differential Abundance from Multivariate Normal*

Description

Generate Longitduinal Differential Abundance from Multivariate Normal

Usage

```
gen_norm_microbiome(
  features = 10,
  diff_abun_features = 5,
  n_control,
  n_treat,
  control_mean,
  sigma,
  num_timepoints,
  t_interval,
  rho,
  corr_str = c("ar1", "compound", "ind"),
  func_form = c("linear", "quadratic", "cubic", "M", "W", "L_up", "L_down"),
  beta,
  IP = NULL,
  missing_pct,
  missing_per_subject,
  miss_val = NA,
  dis_plot = FALSE,
  plot_trend = FALSE,
  zero_trunc = TRUE,
  asynch_time = FALSE
)
```

Arguments

features	numeric value specifying the number of features/microbes to simulate. Default is 10.
diff_abun_features	numeric value specifying the number of differentially abundant features. Default is 5.
n_control	integer value specifying the number of control individuals
n_treat	integer value specifying the number of treated individuals
control_mean	numeric value specifying the mean value for control subjects. all control subjects are assummed to have the same population mean value.
sigma	numeric value specifying the global population standard deviation for both control and treated individuals.

num_timepoints	integer value specifying the number of timepoints per subject.
t_interval	numeric vector of length two specifying the interval of time from which to draw observatoins [t_1, t_q]. Assumed to be equally spaced over the interval unless asynch_time is set to TRUE.
rho	value for the correlation parameter. must be between [0, 1]. see mvrnorm_corr_gen for details.
corr_str	correlation structure selected. see mvrnorm_corr_gen for details.
func_form	character value specifying the functional form for the longitduinal mean trend. see mean_trend for details.
beta	vector value specifying the parameters for the differential abundance function. see mean_trend for details.
IP	vector specifying any inflection points. depends on the type of functional form specified. see mean_trend for details. by default this is set to NULL.
missing_pct	numeric value that must be between [0, \1] that specifies what percentage of the individuals will have missing values.
missing_per_subject	integer value specifying how many observations per subject should be dropped. note that we assume that all individuals must have baseline value, meaning that the maximum number of missing_per_subject is equal to num_timepoints - 1.
miss_val	value used to induce missingness from the simulated data. by default missing values are assummed to be NA but other common choices include 0.
dis_plot	logical argument on whether to plot the simulated data or not. by default plotting is turned off.
plot_trend	specifies whether to plot the true mean trend. see mean_trend for details.
zero_trunc	logical indicator designating whether simulated outcomes should be zero truncated. default is set to TRUE
asynch_time	logical indicator designed to randomly sample timepoints over a specified interval if set to TRUE. default is FALSE.

Value

This function returns a list with the following objects

Y The full simulated feature sample matrix where each row represent a feature and each column a sample. Note that the differential and non-differential bugs are marked by row.names

Examples

```
gen_norm_microbiome(features = 5, diff_abun_features = 2,
               n_control = 10, n_treat = 10, control_mean = 8, sigma = 1,
               num_timepoints = 5, t_interval=c(0, 4), rho = 0.8,
               corr_str = "compound", func_form = "linear", beta =  c(0, 1),
               missing_pct = 0.3, missing_per_subject = 2)
```

gen_norm_microbiome_obs *Generate Longitduinal Differential Abundance from Multivariate Normal with Observed Data*

Description

Generate Longitduinal Differential Abundance from Multivariate Normal with Observed Data

Usage

```
gen_norm_microbiome_obs(
  features = 10,
  diff_abun_features = 5,
  id,
  time,
  group,
  ref,
  control_mean,
  sigma,
  rho,
  corr_str = c("ar1", "compound", "ind"),
  func_form = c("linear", "quadratic", "cubic", "M", "W", "L_up", "L_down"),
  beta,
  IP = NULL,
  dis_plot = FALSE,
  plot_trend = FALSE,
  zero_trunc = TRUE
)
```

Arguments

features	numeric value specifying the number of features/microbes to simulate. Default is 10.
diff_abun_features	numeric value specifying the number of differentially abundant features. Default is 5.
id	vector of length N that identifies repeated measurements for each unit
time	vector of length N that determines when values will be sampled for each unit
group	factor vector with two levels indicating the group assignment for each respective id
ref	character value identifying which group value to treat as control and which value to treat as treatment
control_mean	numeric value specifying the mean value for control subjects. all control subjects are assummed to have the same population mean value.

sigma	numeric value specifying the global population standard deviation for both control and treated individuals.
rho	value for the correlation parameter. must be between [0, 1]. see mvrnorm_corr_gen for details.
corr_str	correlation structure selected. see mvrnorm_corr_gen for details.
func_form	character value specifying the functional form for the longitduinal mean trend. see mean_trend for details.
beta	vector value specifying the parameters for the differential abundance function. see mean_trend for details.
IP	vector specifying any inflection points. depends on the type of functional form specified. see mean_trend for details. by default this is set to NULL.
dis_plot	logical argument on whether to plot the simulated data or not. by default plotting is turned off.
plot_trend	specifies whether to plot the true mean trend. see mean_trend for details.
zero_trunc	logical indicator designating whether simulated outcomes should be zero truncated. default is set to TRUE

Value

This function returns a list with the following objects

Y The full simulated feature sample matrix where each row represent a feature and each column a sample. Note that the differential and non-differential bugs are marked by row.names

Examples

```
set.seed(011520)
id_list <- lapply(seq_len(60), function(i){
obs <- sample(5:10, size=1)
id_rep <- rep(i, obs)
})

time_interval <- c(0, 10)
time_list <- lapply(id_list, function(x){
time_len <- length(x)
times <- runif(time_len, min=time_interval[1], max=time_interval[2])
times <- times[order(times)]
})

group_list <- lapply(id_list, function(x){
group_len <- length(x)
tx_ind <- sample(seq_len(2), 1)
tx_group <- ifelse(tx_ind==1, "Control", "Treatment")
groups <- rep(tx_group, group_len)
})
id <- unlist(id_list)
group <- factor(unlist(group_list), levels = c("Control", "Treatment"))
time <- unlist(time_list)
```

```
# control times
ct <- unlist(lapply(unique(id[group=="Control"]), function(x){
length(id[id==x])
}))

tt <- unlist(lapply(unique(id[group=="Treatment"]), function(x){
length(id[id==x])
}))
mean(ct)
mean(tt)

gen_norm_microbiome_obs(features=4, diff_abun_features=2,
id=id, time=time, group=group, ref="Control", control_mean=2,
               sigma=1, rho=0.7, corr_str="compound", func_form="L_up",
               beta=1, IP=5, zero_trunc=TRUE)
```

`ggplot_spaghetti` *Spaghetti Plots using* `ggplot2`

Description

This function allows the user to create spaghetti plots for individuals with time varying covariates. You can also break this down into subgroups to analyze different trentds.

Usage

```
ggplot_spaghetti(
  y,
  id,
  time,
  alpha = 0.2,
  method = "loess",
  jit = 0,
  group = NULL
)
```

Arguments

`y`	This is the y-axis parameter to specify. Generally it is a continuous variable.
`id`	This is the id parameter that identifies the unique individuals or units.
`time`	This is the time vector and must be numeric.
`alpha`	Scalar value between [0,1] that specifies the transparencey of the lineplots.
`method`	Character value that specifies which type of method to use for fitting. Optional methods come from `geom_smooth` function.
`jit`	Scalar value that specifies how much you want to jitter each individual observation. Useful if many of the values share the same y values at a time point.
`group`	Specifies a grouping variable to be used, and will plot it by color on one single plot.

Details

Note that the data must be in long format.

Value

Plots a time series data by each individual/unit with group trends overlayed.

Examples

```
library(ggplot2)
num_subjects_per_group <- 15
sim_obj <- mvrnorm_sim(n_control=num_subjects_per_group,
                       n_treat=num_subjects_per_group,
                       control_mean=5, sigma=1, num_timepoints=5,
                       t_interval = c(0, 4),
                       rho=0.95, corr_str='ar1', func_form='linear',
                       beta=c(0, 0.25),
                       missing_pct=0.6, missing_per_subject=2)

with(sim_obj$df, suppressWarnings(ggplot_spaghetti(y=Y_obs, id=ID, time=time,
                                                   jit=0.1, group=group)))+
  labs(title="Simulated Microbiome Data from Multivariate Normal",
       y="Normalized Reads", x="Time") +
  scale_linetype_manual(values=c("solid","dashed"), name="Group") +
  scale_color_manual(values=c("#F8766D", "#00BFC4"), name="Group")
```

`mean_trend` *Function for Generating Various Longitudinal Mean Trends*

Description

In order to investigate different functional forms of longitudinal differential abundance we allow the mean time trend to take a variety of forms. These functional forms include linear, quadratic, cubic, M, W, L_up, or L_down. For each form the direction/concavity/fold change can be specified using the beta parameter.

Usage

```
mean_trend(
  timepoints,
  form = c("linear", "quadratic", "cubic", "M", "W", "L_up", "L_down"),
  beta,
  IP = NULL,
  plot_trend = FALSE
)
```

Arguments

`timepoints`	numeric vector specifying the points to fit the functional trend.
`form`	character value specifying the type of time trend. Options include 'linear', 'quadratic', 'cubic', 'M', 'W', 'L_up', and 'L_down'.
`beta`	vector specifying the appropriate parameters for the equation. In the case of 'linear', beta should be a two-dimensional vector specifying the intercept and slope. See details for the further explanation of the beta value for each form.
`IP`	vector specifying the inflection points where changes occur for functional forms M, W, and L trends.
`plot_trend`	logical value indicating whether a plot should be produced for the time trend. By default this is set to TRUE.

Details

Linear Form Notes:

$$f(x) = \beta_0 + \beta_1 x + \beta_2 x^2$$

- Sign of β_1 determines whether the trend is increasing (+) or decreasing (-)

Quadratic Form Notes:

$$f(x) = \beta_0 + \beta_1 x + \beta_2 x^2$$

- Critical point for quadratic function occurs at the point $\frac{-\beta_1}{2\beta_2}$
- β_2 determines whether the quadratic is concave up (+) or concave down (-)

Cubic Form Notes:

$$f(x) = \beta_0 + \beta_1 x + \beta_2 x^2 + \beta_3 x^3$$

- Point of Inflection for cubic function occurs $\frac{-\beta_2}{(3\beta_3)}$
- Critical points for cubic function occur at $\frac{-\beta_2 \pm \sqrt{\beta_2^2 - 3\beta_1\beta_3}}{3\beta_3}$
- Can generate piecewise linear trends, i.e. 'V' form, by placing either one of the IP points outside of the timepoints specified

M/W Form Notes:

- Must specify beta as (β_0, β_1) and IP as (IP_1, IP_2, IP_3)
- This form should be specified with an initial intercept, β_0, and slope, β_1, that will connect to the first point of change (IP) specified.
- Subsequent slopes are constructed such that the mean value at the second IP value and final timepoint are 0
- The mean value at the third IP is set to be equal to the calculcated mean value at the first IP based on the specified intercept and slope.
- β_0=intercept, i.e. timepoint when y=0
- β_1=slope between β_0 and IP_1

L_up Form Notes:

The structure of this form assumes that there is no trend from t_1 to IP_1. Then at the point of change specified, IP_1, there occurs a linearly increasing trend with slope equal to β_{slope} up to the last specified timepoint t_q.

- Must specify beta as (β_{slope}), and must be positive
- Specify a single point of change (IP) variable where positive trend will start
- IP must be between [t_1, t_q]

L_down Form Notes:

Similarily, the L_down form assumes that there are two region within the range of timepoints. The first region is a decreasing trend and the second region has no trend. The decreasing trend must start with a Y intercept greater than zero, and the slope must be specified as negative. There is one point of change (IP), but this is calculated automatically based on the values of the Y intercept and slope provided, IP=$-\beta_{yintercept}/\beta_{slope}$.

- Must specify beta as ($\beta_{yintercept}$, β_{slope}) where $\beta_{yintercept}$>0 and β_{slope}<0
- IP variable should be specified as NULL, if value is provided it will be ignored.

Value

This function returns a list of the following

`form` - character value repeating the form selected

`trend` - data.frame with the variables `mu` representing the estimated mean value at `timepoints` used for fitting the trend

`beta` - returning the numeric vector used to fit the functional form

Examples

```
#Quadratic Form
mean_trend(timepoints=seq(0, 6, length.out=20),
               form='quadratic', beta=1/4 * c(-1, 3, -0.5), plot_trend=TRUE)
#M Form
mean_trend(timepoints=seq(0, 10,length.out=100), form='M',
               beta=c(0, 5), IP=10 * c(1/4, 2/4, 3/4), plot_trend=TRUE)
#in this case the IP points are selected so that peaks are evenly
#distributed but this does not have to be true in general

#L_up Form
mean_trend(timepoints=seq(0, 10, length.out=100), form='L_up',
           beta=1, IP=5, plot_trend=TRUE)

#L_down Form
mean_trend(timepoints=seq(0, 10,length.out=100), form='L_down',
           beta=c(4, -0.5), IP=NULL, plot_trend=TRUE)
```

`mvrnorm_corr_gen` *Generate Multivariate Random Normal Longitudinal Data*

Description

For this methodology we assume that we draw a set of `n` independent each with q_i observations.

Usage

```
mvrnorm_corr_gen(
  n,
  obs,
  t,
  mu,
  sigma,
  rho,
  corr_str = c("ar1", "compound", "ind"),
  zero_trunc = TRUE
)
```

Arguments

`n`	integer scalar representing the total number of individuals
`obs`	vector of length `n` specifying the number of observations per indivdiual.
`t`	vector corresponding to the timepoints for each individual.
`mu`	vector specifying the mean value for individuals.
`sigma`	scalar specifying the standard deviation for all observations.
`rho`	numeric scalar value between [0, 1] specifying the amount of correlation between. assumes that the correlation is consistent for all subjects.
`corr_str`	character value specifying the correlation structure. Currently available methods are \'ar1\', \'compound\', and \'ind\' which correspond to first-order autoregressive, compound or equicorrelation, and independence respecitvely.
`zero_trunc`	logical value to specifying whether the generating distribution should come from a multivariate zero truncated normal or an untruncated multivariate normal. by default we assume that zero truncation occurs since this is assummed in our microbiome setting.

Value

This function returns a list with the following objects:

`df` - data.frame object with complete outcome `Y`, subject ID, time, group, and outcome with missing data

`Y` - vector of complete outcome

`Mu` - vector of complete mean specifications used during simulation

Sigma - block diagonal symmetric matrix of complete data used during simulation

N - total number of observations

Examples

```
size <- 15
reps <- 4
N <- size*reps
mvrnorm_corr_gen(n=size, obs=rep(reps, size), t=rep(seq_len(4), size),
mu=rep(1, N), sigma=2, rho=0.9, corr_str="ar1")
```

---

mvrnorm_sim *Simulate Microbiome Longitudinal Data from Multivariate Random Normal*

Description

This function is used in the gen_norm_microbiome call when the user specified the method as mvrnorm.

Usage

```
mvrnorm_sim(
  n_control,
  n_treat,
  control_mean,
  sigma,
  num_timepoints,
  t_interval,
  rho,
  corr_str = c("ar1", "compound", "ind"),
  func_form = c("linear", "quadratic", "cubic", "M", "W", "L_up", "L_down"),
  beta,
  IP = NULL,
  missing_pct,
  missing_per_subject,
  miss_val = NA,
  dis_plot = FALSE,
  plot_trend = FALSE,
  zero_trunc = TRUE,
  asynch_time = FALSE
)
```

Arguments

n_control	integer value specifying the number of control individuals
n_treat	integer value specifying the number of treated individuals
control_mean	numeric value specifying the mean value for control subjects. all control subjects are assummed to have the same population mean value.
sigma	numeric value specifying the global population standard deviation for both control and treated individuals.
num_timepoints	either an integer value specifying the number of timepoints per subject or a vector of timepoints for each subject. If supplying a vector the lenght of the vector must equal the total number of subjects.
t_interval	numeric vector of length two specifying the interval of time from which to draw observatoins [t_1, t_q]. Assumed to be equally spaced over the interval unless asynch_time is set to TRUE.
rho	value for the correlation parameter. must be between [0, 1]. see mvrnorm_corr_gen for details.
corr_str	correlation structure selected. see mvrnorm_corr_gen for details.
func_form	character value specifying the functional form for the longitduinal mean trend. see mean_trend for details.
beta	vector value specifying the parameters for the differential abundance function. see mean_trend for details.
IP	vector specifying any inflection points. depends on the type of functional form specified. see mean_trend for details. by default this is set to NULL.
missing_pct	numeric value that must be between [0, \1] that specifies what percentage of the individuals will have missing values.
missing_per_subject	integer value specifying how many observations per subject should be dropped. note that we assume that all individuals must have baseline value, meaning that the maximum number of missing_per_subject is equal to num_timepoints - 1.
miss_val	value used to induce missingness from the simulated data. by default missing values are assummed to be NA but other common choices include 0.
dis_plot	logical argument on whether to plot the simulated data or not. by default plotting is turned off.
plot_trend	specifies whether to plot the true mean trend. see mean_trend for details.
zero_trunc	logical indicator designating whether simulated outcomes should be zero truncated. default is set to TRUE
asynch_time	logical indicator designed to randomly sample timepoints over a specified interval if set to TRUE. default is FALSE.

Value

This function returns a list with the following objects:

`df` - data.frame object with complete outcome `Y`, subject ID, time, group, and outcome with missing data

`Y` - vector of complete outcome

`Mu` - vector of complete mean specifications used during simulation

`Sigma` - block diagonal symmetric matrix of complete data used during simulation

`N` - total number of observations

`miss_data` - data.frame object that lists which ID's and timepoints were randomly selected to induce missingness

`Y_obs` - vector of outcome with induced missingness

Examples

```
num_subjects_per_group <- 20
sim_obj <- mvrnorm_sim(n_control=num_subjects_per_group,
                       n_treat=num_subjects_per_group,
                       control_mean=5, sigma=1, num_timepoints=5,
                       t_interval=c(0, 4), rho=0.95, corr_str='ar1',
                       func_form='linear', beta=c(0, 0.25),
                       missing_pct=0.6, missing_per_subject=2)
#checking the output
head(sim_obj$df)

#total number of observations is 2(num_subjects_per_group)(num_timeponts)
sim_obj$N

#there should be approximately 60% of the IDs with missing observations
length(unique(sim_obj$miss_data$miss_id))/length(unique(sim_obj$df$ID))

#checking the subject covariance structure
sim_obj$Sigma[seq_len(5), seq_len(5)]
```

`mvrnorm_sim_obs` *Simulate Microbiome Longitudinal Data from Multivariate Random Normal with Observed Data*

Description

This function is used in the `gen_norm_microbiome_obs` call.

Usage

```
mvrnorm_sim_obs(
  id,
  time,
  group,
  ref,
```

```
  control_mean,
  sigma,
  rho,
  corr_str = c("ar1", "compound", "ind"),
  func_form = c("linear", "quadratic", "cubic", "M", "W", "L_up", "L_down"),
  beta,
  IP = NULL,
  dis_plot = FALSE,
  plot_trend = FALSE,
  zero_trunc = TRUE
)
```

Arguments

id	vector of length N that identifies repeated measurements for each unit
time	vector of length N that determines when values will be sampled for each unit
group	factor vector with two levels indicating the group assignment for each respective id
ref	character value identifying which group value to treat as control and which value to treat as treatment
control_mean	numeric value specifying the mean value for control subjects. all control subjects are assummed to have the same population mean value.
sigma	numeric value specifying the global population standard deviation for both control and treated individuals.
rho	value for the correlation parameter. must be between [0, 1]. see mvrnorm_corr_gen for details.
corr_str	correlation structure selected. see mvrnorm_corr_gen for details.
func_form	character value specifying the functional form for the longitduinal mean trend. see mean_trend for details.
beta	vector value specifying the parameters for the differential abundance function. see mean_trend for details.
IP	vector specifying any inflection points. depends on the type of functional form specified. see mean_trend for details. by default this is set to NULL.
dis_plot	logical argument on whether to plot the simulated data or not. by default plotting is turned off.
plot_trend	specifies whether to plot the true mean trend. see mean_trend for details.
zero_trunc	logical indicator designating whether simulated outcomes should be zero truncated. default is set to TRUE

Value

This function returns a list with the following objects:

df - data.frame object with complete outcome Y, subject ID, time, group, and outcome with missing data

`Y` - vector of complete outcome

`Mu` - vector of complete mean specifications used during simulation

`Sigma` - block diagonal symmetric matrix of complete data used during simulation

`N` - total number of observations

Examples

```
set.seed(011520)
id_list <- lapply(seq_len(30), function(i){
obs <- sample(seq_len(10), size=1)
id_rep <- rep(i, obs)
})

time_interval <- c(0, 10)
time_list <- lapply(id_list, function(x){
time_len <- length(x)
times <- runif(time_len, min=time_interval[1], max=time_interval[2])
times <- times[order(times)]
})

group_list <- lapply(id_list, function(x){
group_len <- length(x)
tx_ind <- sample(seq_len(2), 1)
tx_group <- ifelse(tx_ind==1, "Control", "Treatment")
groups <- rep(tx_group, group_len)
})
id <- unlist(id_list)
group <- factor(unlist(group_list), levels = c("Control", "Treatment"))
time <- unlist(time_list)

# N=173 total repeated measurements
length(id)

# 15 control and 15 treated subjects
table(group[unique(id)])

# control times
ct <- unlist(lapply(unique(id[group=="Control"]), function(x){
length(id[id==x])
}))

#treatment times
tt <- unlist(lapply(unique(id[group=="Treatment"]), function(x){
length(id[id==x])
}))

# on average the treatment group has one more observation than control
mean(ct)
mean(tt)

mvrnorm_sim_obs(id=id, time=time, group=group, ref="Control", control_mean=2,
               sigma=1, rho=0.7, corr_str="compound", func_form="L_up",
```

```
beta=1, IP=5, plot_trend=TRUE, dis_plot=TRUE, zero_trunc=TRUE)
```

`simulate2MRexperiment` *Convert simulated output to MRexperiment object*

Description

In order to allow investigators to more easily incorporate simulated data, this package converts the raw output into an MRexperiment object used in the `metagenomeSeq` package.

Usage

```
simulate2MRexperiment(obj, missing = FALSE)
```

Arguments

`obj`	output from either `gen_norm_microbiome` or `mvrnorm_sim`
`missing`	logical indicator for objects from `mvrnorm_sim`. If missing = TRUE then create MRexperiment object with `Y_obs` else use `Y`.

Value

An MRexperiment object

Examples

```
bug_gen <- gen_norm_microbiome(features=6, diff_abun_features=3,
                               n_control=30, n_treat=20, control_mean=2,
                               sigma=2, num_timepoints=4, t_interval=c(0, 3),
                               rho=0.9, corr_str="compound", func_form="M",
                               beta=c(4, 3), IP=c(2, 3.3, 6),
                               missing_pct=0.2, missing_per_subject=2,
                               miss_val=0, asynch_time=TRUE)
bug_gen_MR <- simulate2MRexperiment(bug_gen)
class(bug_gen_MR)
```

`simulate2phyloseq` *Convert simulated output to phyloseq object*

Description

This function will convert simulated data into a `phyloseq` object.

Usage

```
simulate2phyloseq(obj, missing = FALSE)
```

Arguments

`obj`	output from either `gen_norm_microbiome` or `mvrnorm_sim`
`missing`	logical indicator for objects from `mvrnorm_sim`. If missing = TRUE then create MRexperiment object with `Y_obs` else use `Y`.

Value

A phyloseq object

Examples

```
bug_gen <- gen_norm_microbiome(features=6, diff_abun_features=3,
                               n_control=30, n_treat=20, control_mean=2,
                               sigma=2, num_timepoints=4, t_interval=c(0, 3),
                               rho=0.9, corr_str="compound", func_form="M",
                               beta=c(4, 3), IP=c(2, 3.3, 6),
                               missing_pct=0.2, missing_per_subject=2,
                               miss_val=0, asynch_time=TRUE)
bug_gen_phyloseq <- simulate2MRexperiment(bug_gen)
class(bug_gen_phyloseq)
```

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