Longitudinal analysis pipeline with longdat_disc()

Time (the proxy for treatment) as a discrete variable

Introduction

This is an example of running `longdat_disc()`. Note that the time variable (proxy of treatment) here should be discrete. If the time variable is continuous, please apply `longdat_cont()` instead.

```
# Load the packages
library(LongDat)
library(tidyverse)
library(kableExtra)
```

Explaining the input data frame format

The input data frame (called master table) should have the same format as the example data "LongDat_disc_master_table". If you have metadata and feature (eg. microbiome, immunome) data stored in separate tables, you can go to the section Preparing the input data frame with make_master_table() below. The function `make_master_table()` helps you to create master table from metadata and feature tables.

Now let's have a look at the required format for the input master table. The example below is a dummy longitudinal data set with 3 time points (1, 2, 3). Here we want to see if the treatment has a significant effect on gut microbial abundance or not.

```
# Read in the data frame. LongDat_disc_master_table is already lazily loaded.
master <- LongDat_disc_master_table
master %>%
    kableExtra::kbl() %>%
    kableExtra::kable_paper(bootstrap_options = "responsive", font_size = 12) %>%
    kableExtra::scroll_box(width = "700px", height = "200px")
```

Individual	Time_point	sex	age	DrugA	DrugB	BacteriumA	BacteriumB	BacteriumC
1	1	0	61	0.0	10	11	4	23
1	2	0	61	0.0	10	13	2	44

Individual	Time_point	sex	age	DrugA	DrugB	BacteriumA	BacteriumB	BacteriumC
1	3	0	61	0.0	20	7	13	48

As you can see, the "Individual" is at the first column, and the features (dependent variables), which are gut microbial abundances in this case, are at the end of the table. Any column apart from individual, test_var (e.g. Time_point) and dependent variables will be taken as potential confounders (confounding with the test_var). For example, here the potential confounders are sex, age, drug A and drug B. **Please avoid using characters that don't belong to ASCII printable characters for the column names in the input data frame.**

Preparing the input data frame with make_master_table()

If you have your input master table prepared already, you can skip this section and go to Run longdat_disc() directly. If your metadata and feature (eg. microbiome, immunome) data are stored in two tables, you can create a master table out of them easily with the function `make_master_table()`.

First, let's take a look at an example of the metadata table. Metadata table should be a data frame whose columns consist of sample identifiers (sample_ID, unique for each sample), individual, time point and other meta data. Each row corresponds to one sample_ID.

```
# Read in the data frame. LongDat_disc_metadata_table is already lazily loaded.
metadata <- LongDat_disc_metadata_table
metadata %>%
    kableExtra::kbl() %>%
    kableExtra::kable_paper(bootstrap_options = "responsive", font_size = 12) %>%
    kableExtra::scroll_box(width = "700px", height = "200px")
```

Sample_ID	Individual	Time_point	sex	age	DrugA	DrugB
1_1	1	1	0	61	0.0	10
1_2	1	2	0	61	0.0	10
1_3	1	3	0	61	0.0	20
2_1	2	1	0	66	0.0	640
2_2	2	2	0	66	0.0	320
2_3	2	3	0	66	0.0	640

This example is a dummy longitudinal meatadata with 3 time points for each individual. Besides sample_ID, individual, time point columns, there are also information of sex, age and drugs that individuals take. Here we want to see if the treatment has a significant effect on gut microbial abundance or not.

Then, let's see how a feature table looks like. Feature table should be a data frame whose columns only consist of sample identifiers (sample_ID) and features (dependent variables, e.g. microbiome). Each row corresponds to one sample_ID. Please do not include any columns other than sample_ID and features in the feature table.

```
# Read in the data frame. LongDat_disc_feature_table is already lazily loaded.
feature <- LongDat_disc_feature_table
feature %>%
    kableExtra::kbl() %>%
    kableExtra::kable_paper(bootstrap_options = "responsive", font_size = 12) %>%
    kableExtra::scroll_box(width = "700px", height = "200px")
```

Sample_ID	BacteriumA	BacteriumB	BacteriumC
1_1	11	4	23
1_2	13	2	44
1_3	7	13	48
2_1	344	0	48

This example is a dummy longitudinal feature data. It stores the gut microbial abundance of each sample.

To enable the joining process of metadata and feature tables, please pay attention to the following rules.

1. The row numbers of metadata and feature tables should be the same.
2. Sample_IDs are unique for each sample (i.e. no repeated sample_ID)
3. Metadata and feature tables have the same sample_IDs. If sample_IDs don't match between the two tables, the joining process will fail.
4. As mentioned above, feature table should include only the columns of sample_ID and features.
5. Avoid using characters that don't belong to ASCII printable characters for the column names.

Now let's create a master table and take a look at the result!

```
master_created <- make_master_table(metadata_table = LongDat_disc_metadata_table,
    feature_table = LongDat_disc_feature_table, sample_ID = "Sample_ID", individual =
        "Individual")
#> [1] "Finished creating master table successfully!"

master_created %>%
    kableExtra::kbl() %>%
    kableExtra::kable_paper(bootstrap_options = "responsive", font_size = 12) %>%
    kableExtra::scroll_box(width = "700px", height = "200px")
```

Individual	Time_point	sex	age	DrugA	DrugB	BacteriumA	BacteriumB	BacteriumC
1	1	0	61	0.0	10	11	4	23
1	2	0	61	0.0	10	13	2	44
1	3	0	61	0.0	20	7	13	48
2	1	0	66	0.0	640	344	0	48
2	2	0	66	0.0	320	3	0	80

The table "master_created" is just the same as the table "master" or "LongDat_disc_master_table" in the previous section, with the "Individual" as the first column, and the features (dependent variables), which are gut microbial abundances in this case, are at the end of the table. Any column apart from individual, test_var (e.g. Time_point) and dependent variables will be taken as potential confounders (confounding with the test_var). For the details of the arguments, please read the help page of this function by using `? make_master_table`.

OK, now we're ready to run `longdat_disc()`!

Run longdat_disc()

The input is the example data frame LongDat_disc_master_table (same as "master" or "master_created" in the previous sections), and the data_type is "count" since the dependent variables (features, in this case they're gut microbial abundance) are count data. The "test_var" is the independent variable you're testing, and here we're testing "Time_point" (time as the proxy for treatment). The variable_col is 7 because the dependent variables start at column 7. And the fac_var mark the columns that aren't numerical. For the details of the arguments, please read the help page of this function by using `?longdat_disc`.

The run below takes less than a minute to complete. When data_type equals to "count", please remember to set seed (as shown below) so that you'll get reproducible randomized control test.

```
# Run longdat_disc() on LongDat_disc_master_table
set.seed(100)
test_disc <- longdat_disc(input = LongDat_disc_master_table, data_type = "count",
    test_var = "Time_point", variable_col = 7, fac_var = c(1:3))
#> [1] "Start data preprocessing."
#> [1] "Finish data preprocessing."
#> [1] "Start selecting potential confounders."
#> [1] 1
#> [1] 2
#> [1] 3
#> [1] 1
#> [1] 2
#> [1] 3
#> [1] "Finished selecting potential confounders."
#> [1] "Start null model test and post-hoc test."
#> [1] 1
#> [1] 2
#> [1] 3
#> [1] "Finish null model test and post-hoc test."
#> [1] "Start confounding model test."
#> [1] 1
#> [1] 2
#> [1] 3
#> [1] "Finish confounding model test."
#> [1] "Start unlisting tables from confounding model result."
#> [1] "Finish unlisting tables from confounding model result."
#> [1] "Start calculating effect size."
#> [1] 1
#> [1] 2
#> [1] 3
#> [1] "Finish calculating effect size."
#> [1] "Start randomized negative control model test."
#> [1] 1
#> [1] 2
#> [1] 3
#> [1] 1
#> [1] 2
#> [1] 3
#> [1] "Finish randomized negative control model test."
#> [1] "Start Wilcoxon post-hoc test."
#> [1] "Finish Wilcoxon post-hoc test."
#> [1] "Start removing the dependent variables to be exlcuded."
#> [1] "Finish removing the dependent variables to be exlcuded."
#> [1] "Start multiple test correction on null model test p values."
#> [1] "Finish multiple test correction on null model test p values."
#> [1] "Start generating result tables."
#> [1] "Strictly_deconfounded"
#> [1] "Finished successfully!"
```

If you have completed running the function successfully, you'll see the message "Finished successfully!" at the end. The results are stored in list format.

Results

The major output from `longdat_disc()` include a result table and a confounder table. If you have count data (data_type equals to "count"), then there are chances that you get a third table "randomized control table". For more details about the "randomized control table", please read the help page of this function by using `?longdat_disc`.

Result table

Let's have a look at the result table first.

```
# The first dataframe in the list is the result table
result_table <- test_disc[[1]]
result_table %>%
    kableExtra::kbl() %>%
    kableExtra::kable_paper(bootstrap_options = "responsive", font_size = 12, position =
        "center") %>%
    kableExtra::scroll_box(width = "700px")
```

Feature	Prevalence_percentage	Mean_abundance	Signal	Effect_1_2	Effect_1_3	Effect_2_3	EffectSize_1_2	EffectSize_1_3	EffectSize
BacteriumA	93.333	59.567	OK_sd	Decreased	NS	Enriched	-0.6	0.2	
BacteriumB	46.667	29.500	NS	NS	NS	NS	-0.1	-0.2	
BacteriumC	90.000	69.533	OK_nc	NS	Enriched	NS	0.3	1.0	

The second and third columns show the prevalence and mean abundance of each feature According to the "Signal" column, treatment is a significant predictor for BacteriumA as it shows "OK_sd" (which represents "OK and strictly deconfounded"), meaning that there are potential confounders, however there is an effect of time (proxy of treatment) and it is independent of those of confounders. To find out what the confounders are, you'll need to see the confounder table, but we'll get to that later. On the other hand, the signal for BacteriumC is "OK_nc" (which represents OK and no confounder), meaning that the abundance of BacteriumC alters significantly throughout time (proxy of treatment), and that there is no potential confounder. As for BacteriumB, time (proxy of treatment) has no effect on its abundance.

The following columns "Effect_1_2", "Effect_1_3" and "Effect_2_3" describe how features (dependent variables) change from time point a to b. Here we can tell that BacteriumA decreases from time point 1 to 2, and then enriched from time point 2 to 3. From the columns "EffectSize_1_2" and "EffectSize_2_3", we know that the effect size are -0.6 and 0.8. The most relevant information for users ends here, which are listed from the first column to "EffectSize" columns.

Then the following columns contain the details of model test p values ("Null_time_model_q"), the post-hoc test p values (Post.hoc_q_1_2, Post.hoc_q_1_3 and Post.hoc_q_2_3). For more detailed information of the columns in the result table, please refer to the help page by using `?longdat_disc`.

The explanation of each type of "Signal" is listed below.

Signal	Meaning	Explanation
NS	Non-significant	There's no effect of time.
OK_nc	OK and no confounder	There's an effect of time and there's no potential confounder.

Signal	Meaning	Explanation
OK_d	OK but doubtful	There's an effect of time and there's no potential confounder, however the confidence interval of the test_var estimate in the model test includes zero, and thus it is doubtful. Please check the raw data (e.g. plot feature against time) to confirm if there is real effect of time.
OK_sd	OK and strictly deconfounded	There are potential confounders, however there's an effect of time and it is independent of those of confounders.
AD	Ambiguously deconfounded	There are potential confounders, and it isn't possible to conclude whether the effect is resulted from time or confounders.
C	Confounded	There's an effect of time, but it can be reduced to the confounding effects.

Confounder table

Next, let's take a look at the confounder table.

```r
# The second dataframe in the list is the confounder table
confound_table <- test_disc[[2]]
confound_table %>%
    kableExtra::kbl() %>%
    kableExtra::kable_paper(bootstrap_options = "responsive", font_size = 12, position =
        "center") %>%
    kableExtra::scroll_box(width = "700px")
```

Feature	Confounder1	Confounding_type1	Effect_size1	Confounder2	Confounding_type2	Effect_size2
BacteriumA	DrugB	Strictly_deconfounded	0.4943542	NA	NA	NA

The columns of this confounder table are grouped every three columns. "Confounder1" is the name of the confounder, while "Confounding_type1" is the confounding type of confounder1 with time (or the test_var), and "Effect_size1" is the effect size of the dependent variable values between different levels of confounder1. Here, time is strictly deconfounded with confounder1, so we don't need to worry about the confounding effect of confounder1 on bacteriumA. If there are more than one confounders, they will be listed along the rows of each dependent variable.

Result interpretation

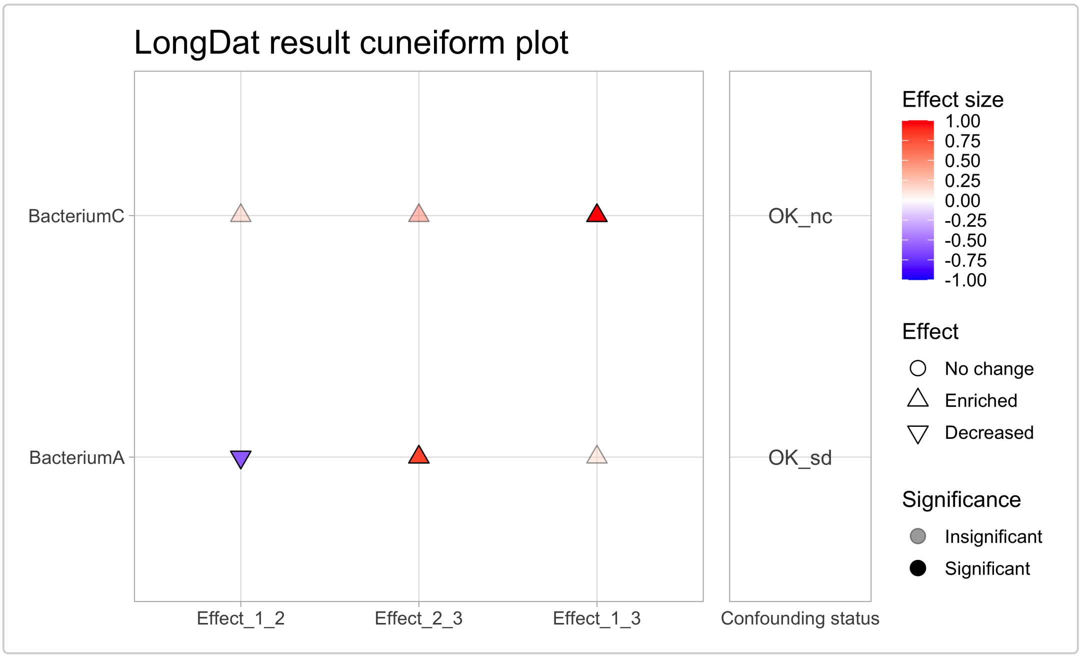
From the result above, we see that the potential confounder for BacteriumA is DrugB, but we don't need to worry about the effect of time (proxy for the treatment) confounded by DrugB since it's confounding type is "strictly deconfounded", meaning that the effect of time is strictly deconfounded from the effect of DrugB. With this information, we confirm that the treatment can solely explain the changes in BacteriumA abundance. All together, the treatment induces significant changes on the abundance of BacteriumA and BacteriumC, while causing no alteration in that of BacteriumB.

Plotting the result

Finally, we can plot the result with the function `cuneiform_plot()`. The required input is a result table from `longdat_disc()` (or any table with the same format as a result table does).

```r
test_plot <- cuneiform_plot(result_table = test_disc[[1]], x_axis_order = c("Effect_1_2",
    "Effect_2_3", "Effect_1_3"), title_size = 15)
#> [1] "Finished plotting successfully!"
```

```r
test_plot
```



Here we can see the result clearly from the cuneiform plot. It shows the features whose signals are not “NS”. The left panel displays the effects in each time interval. Red represents positive effect size while blue describes negative one (colors can be customized by users). Signficant signals are indicated by solid shapes, whereas insignificant signals are denoted by transparent ones. The right panel displays the confounding status of each feature, and users can remove it by specifying `confound_panel = FALSE`. For more details of the arguments, please read the help page of this function by using `?cuneiform_plot`.

Wrap-up

This tutorial ends here! If you have any further questions and can’t find the answers in the vignettes or help pages, please contact the author (Chia-Yu.Chen@mdc-berlin.de).