Machine learning with R in the Life Sciences

Konstantin Pelz

2025-02-17

Predicting chickenpox from microbiome data

This notebook will show you the basics of using R to train a Random Forest Classifier. In theory, all kinds of features can be used for prediction, here we will use merged ASVs from a public dataset of the american gut project. It compares the gut microbial communities from 4,850 subjects. A total of 864 samples / subjects are represented in this dataset, and 507 genera. ID: PRJEB11419 / ERP012803

Preparations

First we have to load (and if necessary install) a few packages for later.

```r
if(!require(tidymodels)) install.packages('tidymodels')
```

```
## Loading required package: tidymodels
```

```
## -- Attaching packages -------------------------------------- tidymodels 1.3.0 --
```

```
## v broom        1.0.7     v recipes      1.1.1
## v dials        1.4.0     v rsample      1.2.1
## v dplyr        1.1.4     v tibble       3.2.1
## v ggplot2      3.5.1     v tidyr        1.3.1
## v infer        1.0.7     v tune         1.3.0
## v modeldata    1.4.0     v workflows    1.2.0
## v parsnip      1.3.0     v workflowsets 1.1.0
## v purrr        1.0.4     v yardstick    1.3.2
```

```
## -- Conflicts ----------------------------------------- tidymodels_conflicts() --
## x purrr::discard() masks scales::discard()
## x dplyr::filter()  masks stats::filter()
## x dplyr::lag()     masks stats::lag()
## x recipes::step()  masks stats::step()
```

```r
if(!require(tidyverse)) install.packages('tidyverse')
```

```
## Loading required package: tidyverse
## -- Attaching core tidyverse packages ------------------------ tidyverse 2.0.0 --
## v forcats   1.0.0     v readr     2.1.5
## v lubridate 1.9.4     v stringr   1.5.1
## -- Conflicts ------------------------------------------ tidyverse_conflicts() --
## x readr::col_factor() masks scales::col_factor()
## x purrr::discard()    masks scales::discard()
## x dplyr::filter()     masks stats::filter()
## x stringr::fixed()    masks recipes::fixed()
## x dplyr::lag()        masks stats::lag()
## x readr::spec()       masks yardstick::spec()
## i Use the conflicted package (<http://conflicted.r-lib.org/>) to force all conflicts to become errors
```

```
if(!require(vip)) install.packages('vip')
```

```
## Loading required package: vip
##
## Attaching package: 'vip'
##
## The following object is masked from 'package:utils':
##
##     vi
```

```
if(!require(ranger)) install.packages('ranger')
```

```
## Loading required package: ranger
```

```
library(tidyverse)
library(tidymodels)
library(vip)
library(ranger)
```

We will start with loading the data and inspecting it. As the dataset is really big, we need to load it in chunks

```
file_location <- "/home/konstantin/Downloads/13733642/"  #"INSERT_PATH_TO_DATA"
project_id <- "PRJEB11419"

# Loading all relevant files
f <- function(x, pos) subset(x, str_starts(sample, project_id))
taxonomic_table <- read_csv_chunked(paste0(file_location, "taxonomic_table.csv.gz"), DataFrameCallback$
```

```
## Warning: Missing column names filled in: 'X1' [1]
```

```
##
## -- Column specification --------------------------------------------------------
## cols(
##   .default = col_double(),
##   sample = col_character()
## )
## i Use `spec()` for the full column specifications.
```

```r
tags <- read_tsv(paste0(file_location, "tags.tsv.gz")) |> filter(project == project_id)
```

```
## Rows: 3489745 Columns: 5

## -- Column specification --------------------------------------------------------
## Delimiter: "\t"
## chr (5): project, srr, srs, tag, value
##
## i Use ‘spec()‘ to retrieve the full column specification for this data.
## i Specify the column types or set ‘show_col_types = FALSE‘ to quiet this message.
```

```r
sample_metadata <- read_tsv(paste0(file_location, "sample_metadata.tsv")) |> filter(project == project_
```

```
## Rows: 168464 Columns: 11
## -- Column specification --------------------------------------------------------
## Delimiter: "\t"
## chr  (9): srs, project, srr, library_strategy, library_source, instrument, g...
## dbl  (1): total_bases
## dttm (1): pubdate
##
## i Use ‘spec()‘ to retrieve the full column specification for this data.
## i Specify the column types or set ‘show_col_types = FALSE‘ to quiet this message.
```

```r
projects <- read_csv(paste0(file_location, "projects.csv")) |> filter(project == project_id)
```

```
## Rows: 482 Columns: 10
## -- Column specification --------------------------------------------------------
## Delimiter: ","
## chr (8): project, link, amplicon, kit, sample_type, condition, subjects, notes
## dbl (1): avg_length
## lgl (1): bead_beating
##
## i Use ‘spec()‘ to retrieve the full column specification for this data.
## i Specify the column types or set ‘show_col_types = FALSE‘ to quiet this message.
```

```r
# Remove columns which were not measured and thus contain only zeros
taxonomic_table_only_measured <- taxonomic_table |>
  select(-sample)
taxonomic_table_only_measured <- taxonomic_table_only_measured[,colSums(abs(taxonomic_table_only_measur
# Alternative
# taxonomic_table_only_measured <- taxonomic_table |> select_if(~ !all(. == 0))

# Split sample id into project and srr
taxonomic_table_only_measured <- taxonomic_table_only_measured |>
  mutate(project = strsplit(taxonomic_table$sample,"_", fixed = TRUE) |> map_chr(1)) |>
  mutate(srr = strsplit(taxonomic_table$sample,"_", fixed = TRUE) |> map_chr(2))

# Important: No balancing or normalization before we split the data!

# finally, we will inspect the metadata:
print('Number of samples per diagnosis:')
```

```
## [1] "Number of samples per diagnosis:"
```

```
tags |>
  filter(tag=="chickenpox") |>
  count(value) |>
  print()
```

```
## # A tibble: 6 x 2
##   value              n
##   <chr>          <int>
## 1 no               711
## 2 not collected      3
## 3 not provided      17
## 4 not sure         350
## 5 unspecified      110
## 6 yes             3651
```

```
print('Number of samples per sequencing technology:')
```

```
## [1] "Number of samples per sequencing technology:"
```

```
sample_metadata |>
  count(instrument) |>
  print()
```

```
## # A tibble: 2 x 2
##   instrument              n
##   <chr>               <int>
## 1 Illumina HiSeq 4000   419
## 2 Illumina MiSeq       4431
```

Random Forest

From here we will start with the actual setup of the model. We will set it up to predict whether a sample is classified as having chickenpox using the measured genera. Question: Which output variables do we want to predict? Answer: Only yes and no.

For better convenience, we will combine the ASV table with the one column from the metadata which holds the information about chickenpox (`chickenpox` values).

```
model_data <- taxonomic_table_only_measured |>
  left_join(filter(tags,tag=="chickenpox")) |>
  select(-X1) |>
  mutate(chickenpox = value) |>
  select(-project,-srr,-value,-tag,-srs)
```

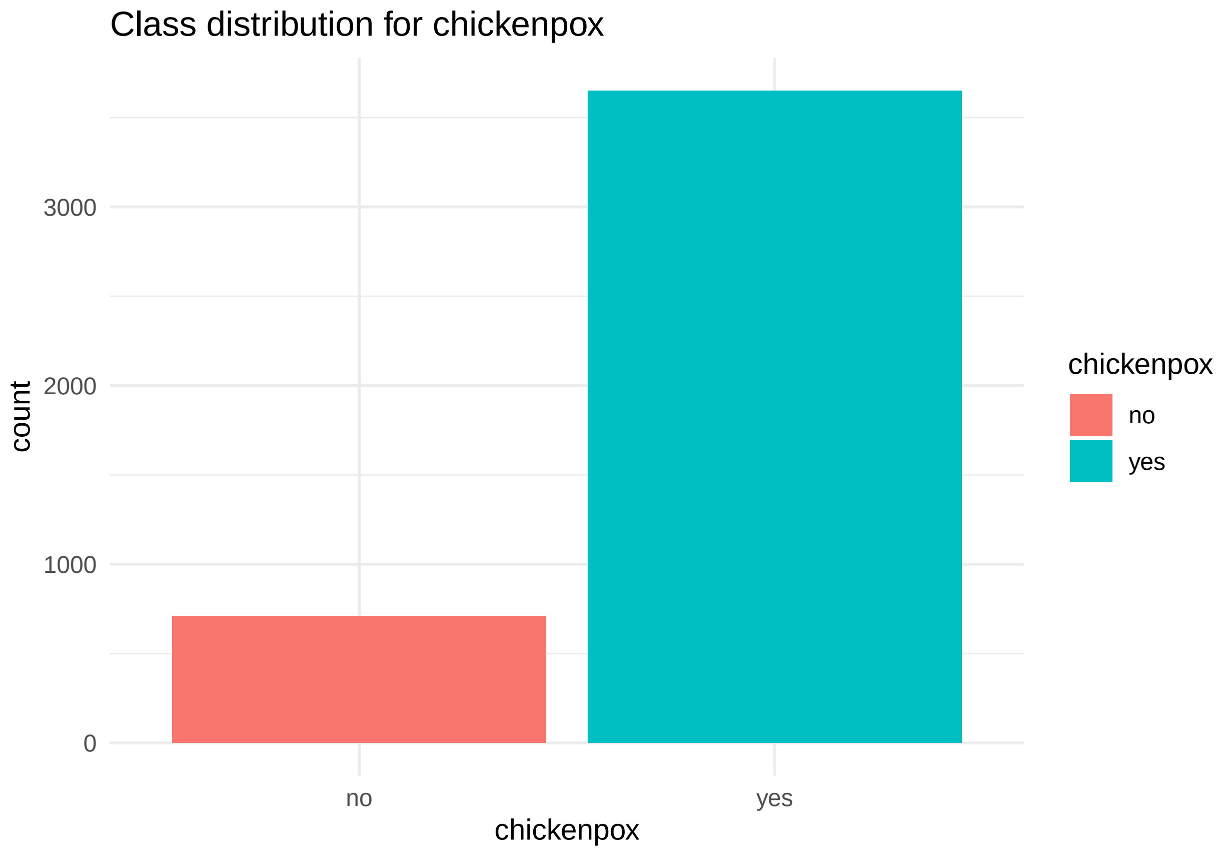
```
## Joining with ‘by = join_by(project, srr)‘
```

```r
non_information_values <- c('not collected', 'not provided', 'not sure', 'unspecified')

samples_without_information <- model_data |>
  filter(is.na(chickenpox) | chickenpox %in% non_information_values) |>
  mutate(chickenpox = as.factor(chickenpox))

model_data <- model_data |>
  filter(!is.na(chickenpox) & !(chickenpox %in% non_information_values)) |>
  mutate(chickenpox = as.factor(chickenpox))

ggplot(model_data, aes(x=chickenpox, fill=chickenpox))+
  geom_bar()+
  theme_minimal()+
  ggtitle('Class distribution for chickenpox')
```



## Prepare data splits

We want to split the data into training and testing. During data splitting, we have to take care that the proportion of chickenpox stays equal between both data splits.

```r
set.seed(123)
split <- initial_split(data = model_data,
                       prop = c(0.75),
                       strata = chickenpox)

split
```

```
## <Training/Testing/Total>
## <3271/1091/4362>
```

```
train_data <- training(split)
test_data <- testing(split)
```

Prepare model workflow for parameter selection

Now we can prepare the workflow for our RF in order to find the best hyperparameters. We will use 5-fold cross-validation with 2 repeats on our training data to test the different parameter values.

```
train_recipe <- recipe(chickenpox ~ ., data = train_data) #|>
  #step_downsample(chickenpox) # Possible but we want to create a screening model, so it is best to hav

tuning_specs <- parsnip::rand_forest(
  mtry = tune(),
  trees = tune(),
  min_n = tune()
) |>
  set_mode('classification') |>
  set_engine('ranger', importance = 'impurity')

tuning_workflow <- workflow() |>
  add_recipe(train_recipe) |>
  add_model(tuning_specs)

training_folds <- rsample::vfold_cv(data = train_data, v = 5, repeats = 2)

tuning_workflow
```

```
## == Workflow ===================================================================
## Preprocessor: Recipe
## Model: rand_forest()
##
## -- Preprocessor ---------------------------------------------------------------
## 0 Recipe Steps
##
## -- Model ----------------------------------------------------------------------
## Random Forest Model Specification (classification)
##
## Main Arguments:
##   mtry = tune()
##   trees = tune()
##   min_n = tune()
##
## Engine-Specific Arguments:
##   importance = impurity
##
## Computational engine: ranger
```

Now we can create a grid of values for each hyperparameter that will be tested. *Advanced:* You can also try out random grid search instead of regular grid search, details are here: https://www.tmwr.org/grid-search#irregular-grids

```
tuning_grid <- grid_regular(
  trees(range = c(1,2000)),
  min_n(range = c(2,40)),
  mtry(range = c(2,20)),
  levels=5
)

tuning_grid
```

```
## # A tibble: 125 x 3
##    trees min_n  mtry
##    <int> <int> <int>
##  1     1     2     2
##  2   500     2     2
##  3  1000     2     2
##  4  1500     2     2
##  5  2000     2     2
##  6     1    11     2
##  7   500    11     2
##  8  1000    11     2
##  9  1500    11     2
## 10  2000    11     2
## # i 115 more rows
```

Train the RF model using cross-validation

With our set of 125 combinations for hyperparameter values, we are ready to tune! We will fit a model for all combinations and explore the results. *Advanced:* implement parallelization to speed up the training process, details are here: https://tune.tidymodels.org/articles/extras/optimizations.html#parallel-processing

```
# this can take some minutes, it has to create a RF for each of the 125 combinations ...
tuning_results <- tune_grid(
  tuning_workflow,
  resamples = training_folds,
  grid=tuning_grid,
  metrics = metric_set(f_meas, accuracy),
  control = control_grid(save_pred = TRUE, verbose = TRUE)
)

tuning_metrics <- tuning_results |>
  collect_metrics()

show_best(tuning_results, metric = 'f_meas', n = 3)
```

Check performance on the test dataset

In order to check if our model with the best hyperparameters is able to generalize, we will now apply it to the unseen test set.

```r
best_model <- tuning_results |>
  select_best(metric = 'f_meas')

final_workflow <- tuning_workflow |>
  finalize_workflow(best_model)

final_workflow
```

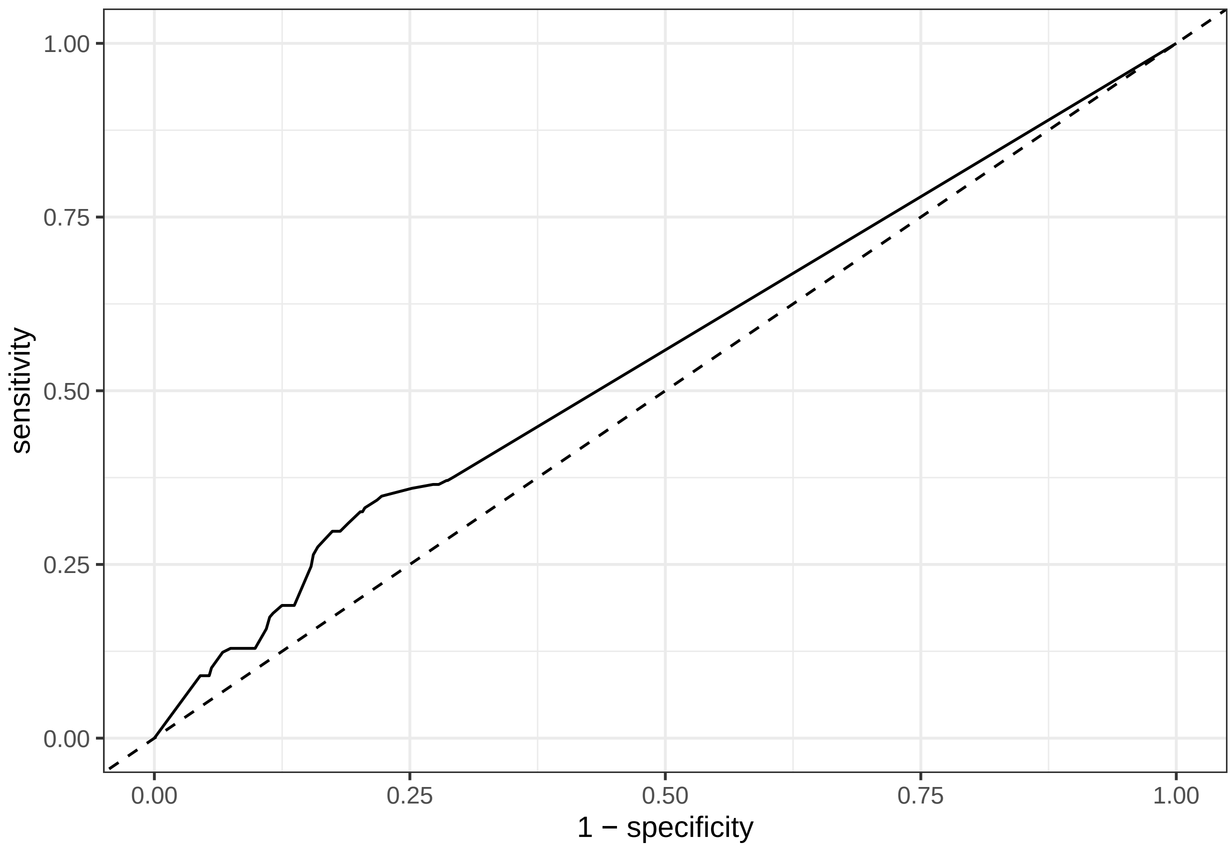
```
## == Workflow ====================================================================
## Preprocessor: Recipe
## Model: rand_forest()
##
## -- Preprocessor ----------------------------------------------------------------
## 0 Recipe Steps
##
## -- Model -----------------------------------------------------------------------
## Random Forest Model Specification (classification)
##
## Main Arguments:
##   mtry = 20
##   trees = 1
##   min_n = 11
##
## Engine-Specific Arguments:
##   importance = impurity
##
## Computational engine: ranger
```

First, we fit the best model to the whole training data again (remember, before we only fit it to the cross-validation sets one at a time). This command will also automatically apply the model to the test set after re-fitting to the training data.

```r
final_fit <- final_workflow |>
  last_fit(split, metrics = metric_set(accuracy, f_meas, roc_auc))

roc_df <- final_fit |>
    collect_predictions() |>
    dplyr::rename('prediction' = .pred_class) |>
    dplyr::rename('no' = .pred_yes) |>
    roc_curve(truth = chickenpox, starts_with('.pred_'))

 ggplot(roc_df, aes(x=1-specificity, y=sensitivity))+
   geom_path()+
   geom_abline(linetype = 'dashed')+
   theme_bw()
```



We can also take a look at the F1 measure (`f_meas`) and Accuracy of our model on the test set: Advanced: Also look at other performance metrics, details are here: https://www.tmwr.org/performance#multiclass-classification-metrics and https://yardstick.tidymodels.org/articles/metric-types.html#metrics

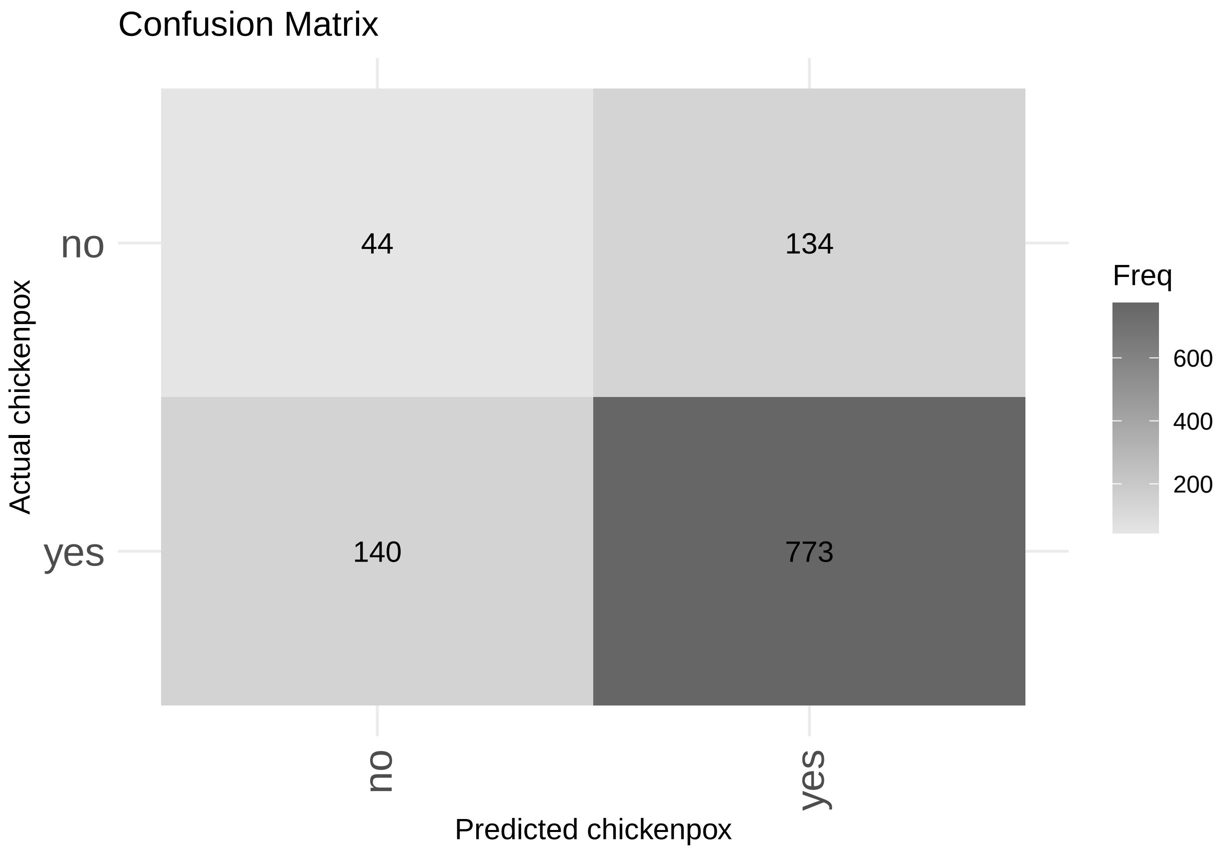
```
final_fit |>
    collect_metrics()
```

```
## # A tibble: 3 x 4
##   .metric  .estimator .estimate .config
##   <chr>    <chr>          <dbl> <chr>
## 1 accuracy binary         0.749 Preprocessor1_Model1
## 2 f_meas   binary         0.243 Preprocessor1_Model1
## 3 roc_auc  binary         0.552 Preprocessor1_Model1
```

Finally, here are the actual predictions of our model in form of a confusion matrix:

```
confusion_matrix <- final_fit |>
  collect_predictions() |>
  conf_mat(truth = `.pred_class`, estimate = chickenpox)

autoplot(confusion_matrix, type = "heatmap") +
    labs(
        title = "Confusion Matrix",
        x = "Predicted chickenpox",
        y = "Actual chickenpox"
    ) +
    theme_minimal()+
  theme(axis.text = element_text(size=15), axis.text.x = element_text(angle = 90, vjust = 0.5, hjust=1)
```



Feature importance

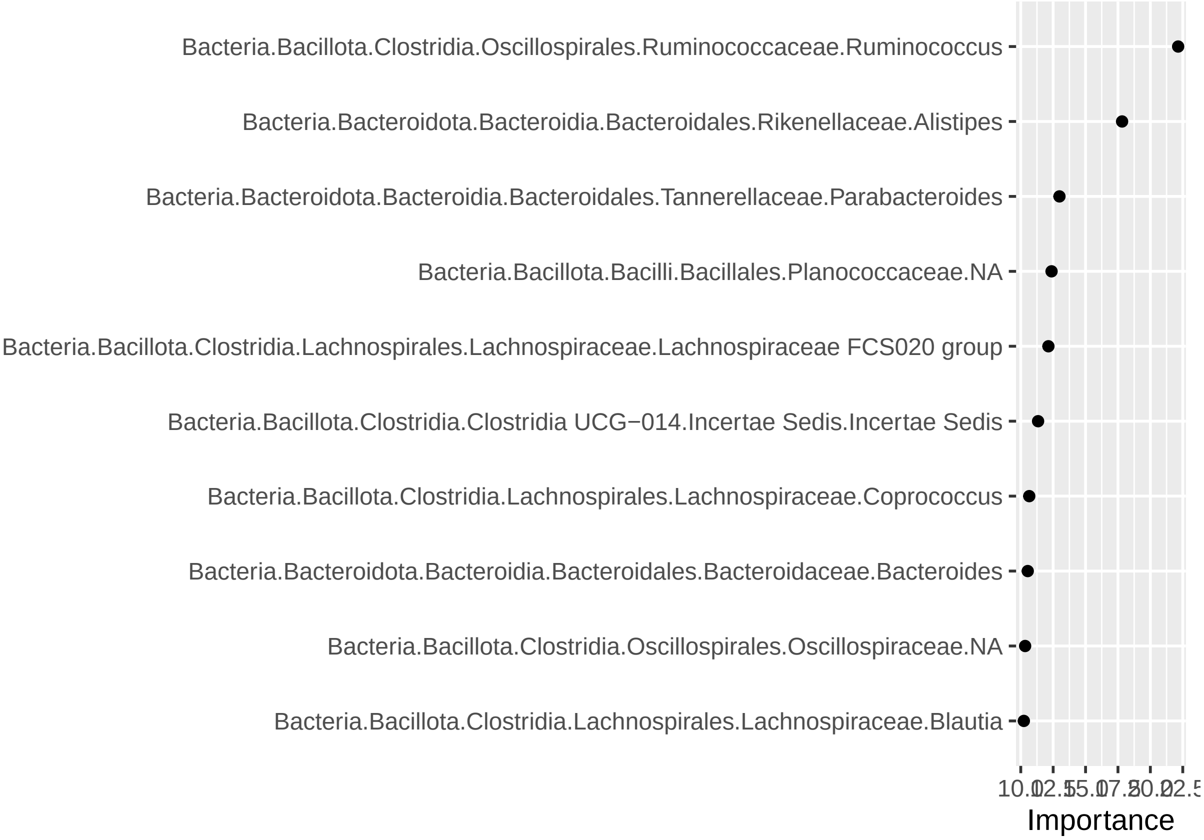
Now lets inspect our model in more detail. First we will extract it and check the OOB error value.

```
# extract the actual model from the final fit object
final_rf <- extract_workflow(final_fit)
final_rf
```

```
## == Workflow [trained] ==========================================================
## Preprocessor: Recipe
## Model: rand_forest()
##
## -- Preprocessor ----------------------------------------------------------------
## 0 Recipe Steps
##
## -- Model -----------------------------------------------------------------------
## Ranger result
##
## Call:
##  ranger::ranger(x = maybe_data_frame(x), y = y, mtry = min_cols(~20L,      x), num.trees = ~1L, min.
##
## Type:                            Probability estimation
## Number of trees:                 1
## Sample size:                     3271
## Number of independent variables: 1048
## Mtry:                            20
## Target node size:                11
## Variable importance mode:        impurity
## Splitrule:                       gini
## OOB prediction error (Brier s.): 0.2367154
```

Now we can also check, which ASV features were most important in this final model. *More Advanced:* compare the importance values you get using gini impurity and permutation accuracy, details are here: https://www.rdocumentation.org/packages/ranger/versions/0.16.0/topics/ranger

```
final_rf |>
  extract_fit_parsnip() |>
  vip(geom = 'point')
```

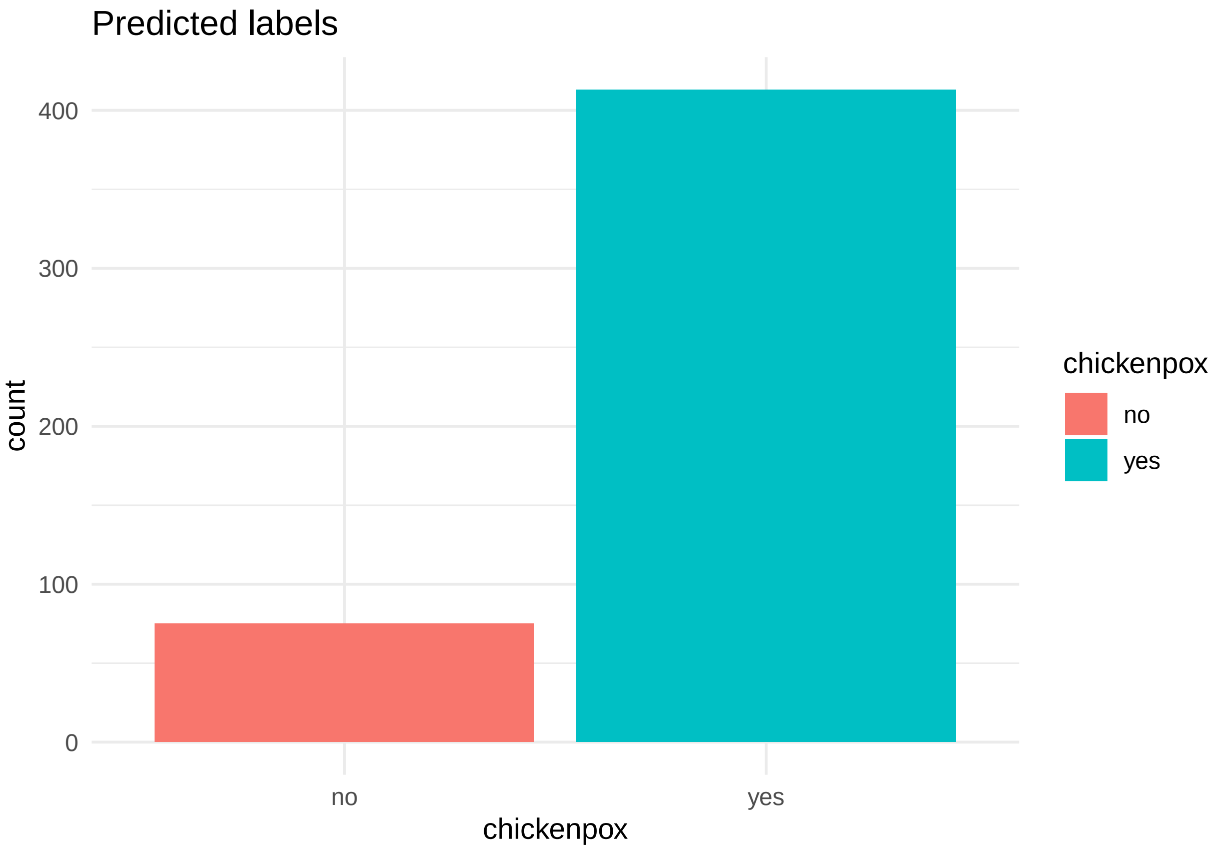


## Apply model to unkown samples

Remember the samples we removed earlier, because they had no definitive label regarding chickenpox? It looks like our model generalized well enough that we can apply it now to predict the disease label of these samples using their microbial composition!

```
predicted_labels <- predict(final_rf, new_data = samples_without_information)
samples_without_information$chickenpox <- predicted_labels$.pred_class

ggplot(samples_without_information, aes(x=chickenpox, fill=chickenpox))+
  geom_bar()+
  theme_minimal()+
  ggtitle('Predicted labels')
```


Predicted labels
count
no
yes
chickenpox
chickenpox
no
yes