DNA metabarcoding diet analysis in reindeer is quantitative and integrates feeding over several weeks

Stefaniya Kamenova[a,b,c,*], Pernille Meyer[a], Anne Krag Brysting[a], Leo Rescia[d], Lars P. Folkow[d], Galina Gusarova[a,d,e,f], Eric Coissac[g]

[a] *Centre for Ecological and Evolutionary Synthesis, University of Oslo, Oslo, Norway,*

[b] *Departments of Ecology and Natural Resource Management, Norwegian University of Life Sciences, Ås,*

[c] *National Museum of Natural History, Bulgarian Academy of Sciences, Sofia,*

[d] *Department of Arctic and Marine Biology, The Arctic University of Norway, Tromsø,*

[e] *The Arctic University Museum of Norway, The Arctic University of Norway, Tromsø,*

[f] *Department of Botany, St Petersburg State University, St Petersburg,*

[g] *Laboratoire d'Ecologie Alpine, CNRS, Université Grenoble Alpes, Grenoble,*

Abstract

Filtering of the EUKA02 DNA metabarcoding raw data.

1. Setting up the R environment

1.1. Install missing packages

```
packages <- c(
  "tidyverse", "devtools", "vegan",
  "ggpubr", "colorspace", "R.utils", "ggthemes",
  "ggforce"
)

install.packages(
  setdiff(
    packages,
    rownames(installed.packages())
  ),
  dependencies = TRUE
)
```

1.2. Loads the used R packages

- `ROBITools` package is used to read result files produced by OBITools.
- `ROBITaxonomy` package provides function allowing to query OBITools formated taxonomy.

*Corresponding author

Email addresses: `stefaniya.kamenova@gmail.com` (Stefaniya Kamenova), `galina.gusarova@uit.no` (Galina Gusarova), `eric.coissac@metabarcoding.org` (Eric Coissac)

```
if (!"ROBITools" %in% rownames(installed.packages())) {
  # ROBITools are not available on CRAN and have to be installed
  # from http://git.metabarcoding.org using devtools

  metabarcoding_git <- "https://git.metabarcoding.org/obitools"

  devtools::install_git(paste(metabarcoding_git,
    "ROBIUtils.git",
    sep = "/"
  ))

  devtools::install_git(paste(metabarcoding_git,
    "ROBITaxonomy.git",
    sep = "/"
  ))
  devtools::install_git(paste(metabarcoding_git,
    "ROBITools.git",
    sep = "/"
  ))
}

library(ROBITools)
library(ROBITaxonomy)
```

- `tidyverse`[1] provides various methods for efficient data manipulation and plotting via `ggplot2`[2]

```
library(tidyverse)
```

- `vegan` is loaded for its `decostand` function[3]

```
library(vegan)
```

- `ggthemes` is loaded for its `theme_tufte` function

```
library(ggthemes)
```

- `ggpubr` is loaded for its `ggarrange` function[4]

```
library(ggpubr)
```

```
library(colorspace)
```

```
library(R.utils)
```

```
library(magrittr)
```

1.3. Initialising some global data

The blind color compliant color pallet for plant families.

```
family_color <- c(
  "#991919", "#fcff5d",
  "#0ec434", "#228c68", "#8ad8e8", "#235b54", "#29bdab",
  "#3998f5", "#37294f", "#277da7", "#3750db", "#f22020",
  "#ffc413", "#f47a22", "#2f2aa0", "#b732cc", "#772b9d",
  "#5d4c86"
)

# The palette with grey:
cbPalette <- c("#999999", "#E69F00", "#56B4E9",
               "#009E73", "#F0E442", "#0072B2",
               "#D55E00", "#CC79A7")

# The palette with black:
cbbPalette <- c("#000000", "#E69F00", "#56B4E9",
                "#009E73", "#F0E442", "#0072B2",
                "#D55E00", "#CC79A7")
```

2. Reading the data

2.1. Reading of the NCBI taxonomy

```
taxo = read.taxonomy("Data/ncbi20210212")
```

2.2. Reading of the metabarcoding data

2.3. For the EUKA02 data set

- The Read contingency table

```
reads <- read_csv("Data/Faeces/FE.Eukaryota.samples.reads.csv",
  show_col_types = FALSE
) %>%
  column_to_rownames("id") %>%
  as.matrix() %>%
  decostand(method = "total")
```

- The sample description table

```
samples <- read_csv("Data/Faeces/FE.Eukaryota.samples.samples.csv",
  show_col_types = FALSE
) %>%
  mutate(.id = sample_id) %>%
  column_to_rownames(".id") %>%
  mutate(
    Animal_id = factor(Animal_id,
      levels = c("9/10", "10/10", "12/10")
    ),
    Fed_biomass = factor(Fed_biomass,
      levels = c("20", "500", "2000")
    )
```

```
  )
```

- The MOTU description table

```
motus <- read_csv("Data/Faeces/FE.Eukaryota.samples.motus.csv",
  show_col_types = FALSE
) %>%
  mutate(.id = id) %>%
  column_to_rownames(".id")
```

- Create a `metabarcoding.data` object, where you merge the three tables

```
Euka02 <- metabarcoding.data(
  reads = reads,
  samples = samples,
  motus = motus
)
```

And sorts the table from the most to the less abundante MOTU.

```
motus.hist <- colMeans(reads(Euka02))
Euka02@motus$mean_ref_freq <- motus.hist
Euka02 <- Euka02[, order(motus.hist, decreasing = TRUE)]
```

2.4. For the SPER01 data set

- The Read contingency table

```
reads <- read_csv("Data/Faeces/FE.Spermatophyta.samples.reads.csv",
  show_col_types = FALSE
) %>%
  column_to_rownames("id") %>%
  as.matrix() %>%
  decostand(method = "total")
```

- The sample description table

```
samples <- read_csv("Data/Faeces/FE.Spermatophyta.samples.samples.csv",
  show_col_types = FALSE
) %>%
  mutate(.id = sample_id) %>%
  column_to_rownames(".id") %>%
  mutate(
    Animal_id = factor(Animal_id,
      levels = c("9/10", "10/10", "12/10")
    ),
    Fed_biomass = factor(Fed_biomass,
      levels = c("20", "500", "2000")
    )
  )
```

- The MOTU description table

```
motus <- read_csv("Data/Faeces/FE.Spermatophyta.samples.motus.csv",
  show_col_types = FALSE
) %>%
  mutate(.id = id) %>%
  column_to_rownames(".id")
```

- Create a `metabarcoding.data` object, where you merge the three tables

```
Sper01 <- metabarcoding.data(
  reads = reads,
  samples = samples,
  motus = motus
)
```

And sorts the table from the most to the less abundante MOTU.

```
motus.hist <- colMeans(reads(Sper01))
Sper01@motus$mean_ref_freq <- motus.hist
Sper01 <- Sper01[, order(motus.hist, decreasing = TRUE)]
```

3. An overview of the diet

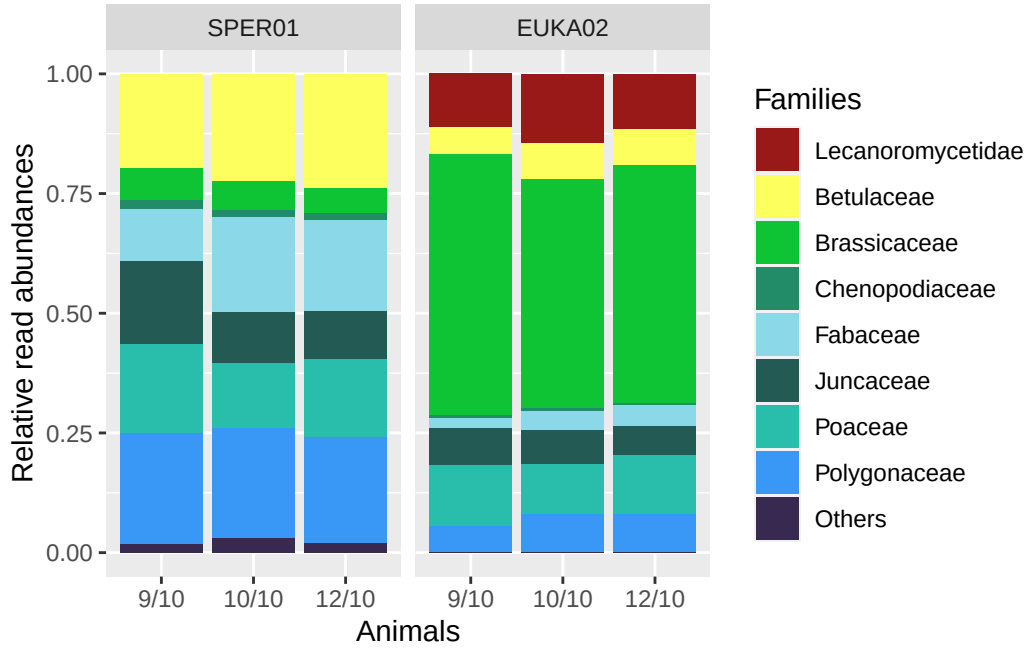
MOTUs are aggregated at family level.

```
Sper01_family <- aggregate(Sper01, by = list(family = Sper01@motus$family_name), MARGIN = 2, FUN =

Euka02@motus %<>%
  mutate(family_name = ifelse(category == "Lichen",
    "Lecanoromycetidae",
    family_name
  ))
Euka02_family <- aggregate(Euka02,
  by = list(family = Euka02@motus$family_name),
  MARGIN = 2,
  FUN = sum
)
```

```
Sper01_family@reads %>%
  as.data.frame() %>%
  rownames_to_column("sample_id") %>%
  left_join(Sper01_family@samples,
    by = join_by(sample_id)
  ) %>%
  group_by(Animal_id) %>%
  summarise(across(
    where(is.numeric),
    ~ mean(.x, na.rm = TRUE)
```

```
  )) %>%
  select(Animal_id, ends_with("aceae")) %>%
  pivot_longer(-Animal_id,
    names_to = "Family",
    values_to = "RRA"
  ) -> diet_sper01

Euka02_family@reads %>%
  decostand(method = "total") %>%
  as.data.frame() %>%
  rownames_to_column("sample_id") %>%
  left_join(Euka02_family@samples,
    by = join_by(sample_id)
  ) %>%
  group_by(Animal_id) %>%
  summarise(across(
    where(is.numeric),
    ~ mean(.x, na.rm = TRUE)
  )) %>%
  select(Animal_id, ends_with("ae")) %>%
  pivot_longer(-Animal_id,
    names_to = "Family",
    values_to = "RRA"
  ) -> diet_euka02

diet_sper01 %>%
  mutate(Marker = "SPER01") %>%
  bind_rows(diet_euka02 %>%
              mutate(Marker = "EUKA02")) %>%
  mutate(Marker = factor(Marker,
    levels = c("SPER01", "EUKA02")
  )) %>%
  group_by(Family) %>%
  mutate(
    merge = mean(RRA) < 0.01,
    Family = ifelse(merge, "Others", Family)
  ) %>%
  group_by(Family, Marker, Animal_id) %>%
  summarise(RRA = sum(RRA), .groups = "drop") -> diet_data

Families <- diet_data$Family %>%
  unique() %>%
  setdiff(c(
    "Lecanoromycetidae",
    "Betulaceae",
    "Others"
  )) %>%
  c(
    "Lecanoromycetidae",
    "Betulaceae",
```

```
    .,
    "Others"
  )

diet_data %>%
  mutate(Family = factor(Family, levels = Families)) %>%
  ggplot(aes(x = Animal_id, y = RRA, fill = Family)) +
  geom_col() +
  facet_wrap(. ~ Marker) +
  xlab("Animals") +
  ylab("Relative read abundances") +
  scale_fill_manual(
    name = "Families",
    values = family_color
  ) +
  theme(
    axis.title.x = ggtext::element_markdown(),
    axis.title.y = ggtext::element_markdown()
  ) -> comparative_diet_plot

ggsave("Figures/comparative_diet.pdf",
  comparative_diet_plot,
  dpi = 300,
  width = 20, height = 10, units = c("cm")
)
ggsave("Figures/comparative_diet.tiff",
  comparative_diet_plot,
  dpi = 300,
  width = 20, height = 10, units = c("cm")
)

comparative_diet_plot
```



Families representing less than one percent of the average diet with both markers are collapsed into the 'Others' category. *Lecanoromycetidae* is actually a sub-class and corresponds to the MOTUs representing the lichens in the `EUKA02` diet data.

```
diet_data %>%
  pivot_wider(names_from = c("Animal_id","Marker"),values_from = "RRA") %>%
  mutate(Family = factor(Family,levels = Families)) %>%
  arrange(Family) %>%
  mutate_if(is.numeric,~ round(.,3))
```

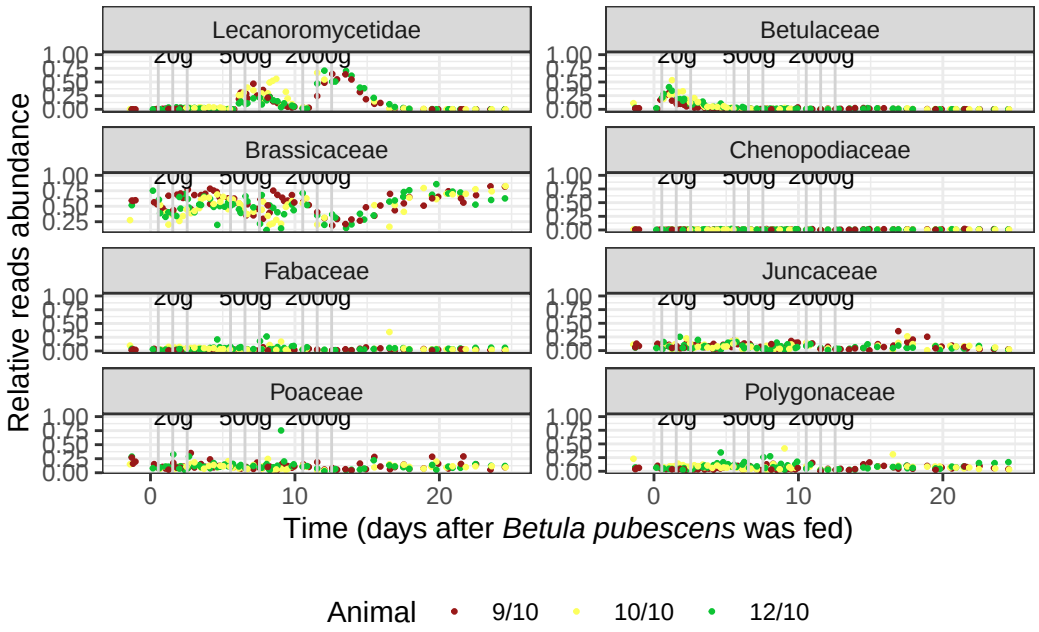
```
# A tibble: 9 x 7
  Family            `9/10_SPER01` `10/10_SPER01` `12/10_SPER01` `9/10_EUKA02`
  <fct>                     <dbl>          <dbl>          <dbl>         <dbl>
1 Lecanoromycetidae        NA             NA             NA             0.111
2 Betulaceae                0.196          0.223          0.239         0.057
3 Brassicaceae              0.069          0.062          0.052         0.545
4 Chenopodiaceae            0.017          0.014          0.014         0.006
5 Fabaceae                  0.11           0.199          0.191         0.021
6 Juncaceae                 0.172          0.107          0.101         0.077
7 Poaceae                   0.188          0.135          0.161         0.128
8 Polygonaceae              0.231          0.229          0.22          0.053
9 Others                    0.018          0.031          0.021         0.001
# i 2 more variables: `10/10_EUKA02` <dbl>, `12/10_EUKA02` <dbl>
```

4. Analysis of the diet

4.1. Evolution of the Food items accross time

4.1.1. For the Euka02 marker

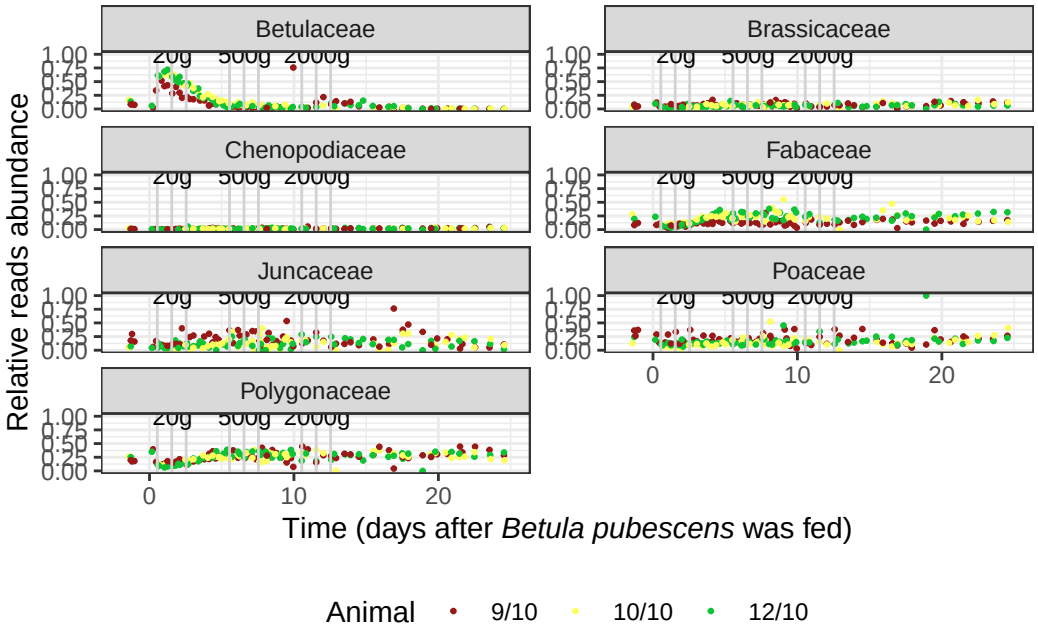
```
Euka02_family@reads %>%
  as.data.frame() %>%
  rownames_to_column("sample_id") %>%
  pivot_longer(cols = - "sample_id", names_to = "Family",values_to = "RRA") %>%
  mutate(Family = factor(Family,levels=Families)) %>%
  left_join(Euka02@samples, by = "sample_id")  %>%
  mutate(times_from_birch = times_from_birch/24) %>%
  filter(! is.na(Family)) %>%
  filter(! is.na(RRA)) %>%
  filter(times_from_birch >=-2 & times_from_birch <= 25 ) %>%
  ggplot(aes(x=times_from_birch,y=RRA, col = Animal_id)) +
  geom_point(size=0.5) +
  xlim(-2,25) +
  facet_wrap(. ~ Family, ncol=2,scales="free_y") +
  geom_vline (xintercept = 0.54, colour = "lightgrey") +
  geom_vline (xintercept = 1.54, colour = "lightgrey") +
  geom_vline (xintercept = 2.54, colour = "lightgrey") +
  geom_vline (xintercept = 5.54, colour = "lightgrey") +
  geom_vline (xintercept = 6.54, colour = "lightgrey") +
  geom_vline (xintercept = 7.54, colour = "lightgrey") +
  geom_vline (xintercept = 10.54, colour = "lightgrey") +
  geom_vline (xintercept = 11.54, colour = "lightgrey") +
  geom_vline (xintercept = 12.54, colour = "lightgrey") +
  annotate("text", x = 1.60, y = 1, label = "20g",size = 3) +
  annotate("text", x = 6.60, y = 1, label = "500g",size = 3) +
  annotate("text", x = 11.60, y = 1, label = "2000g",size = 3) +
  ylab("Relative reads abundance") +
  xlab("Time (days after *Betula pubescens* was fed)") +
  theme_bw() +
  theme(axis.title.x = ggtext::element_markdown(),
        legend.position="bottom") +
  scale_color_manual(name="Animal",values = family_color) -> euka02_family_plot

ggsave("Figures/Euka02_family_plot.pdf",
       euka02_family_plot,
       dpi = 300,
      width = 32, height = 35, units = c("cm"))

ggsave("Figures/Euka02_family_plot.tiff",
       euka02_family_plot,
       dpi = 300,
      width = 16, height = 17, units = c("cm"))

euka02_family_plot
```



4.1.2. For the Sper01 marker

```
Sper01_family@reads %>%
  as.data.frame() %>%
  rownames_to_column("sample_id") %>%
  pivot_longer(cols = - "sample_id", names_to = "Family",values_to = "RRA") %>%
  mutate(Family = factor(Family,levels=Families)) %>%
  left_join(Sper01@samples, by = "sample_id")  %>%
  mutate(times_from_birch = times_from_birch/24) %>%
  filter(! is.na(Family)) %>%
  filter(! is.na(RRA)) %>%
  filter(times_from_birch >=-2 & times_from_birch <= 25 ) %>%
  ggplot(aes(x=times_from_birch,y=RRA, col = Animal_id)) +
  geom_point(size=0.5) +
  xlim(-2,25) +
  facet_wrap(. ~ Family, ncol=2,scales="free_y") +
  geom_vline (xintercept = 0.54, colour = "lightgrey") +
  geom_vline (xintercept = 1.54, colour = "lightgrey") +
  geom_vline (xintercept = 2.54, colour = "lightgrey") +
  geom_vline (xintercept = 5.54, colour = "lightgrey") +
  geom_vline (xintercept = 6.54, colour = "lightgrey") +
  geom_vline (xintercept = 7.54, colour = "lightgrey") +
  geom_vline (xintercept = 10.54, colour = "lightgrey") +
  geom_vline (xintercept = 11.54, colour = "lightgrey") +
  geom_vline (xintercept = 12.54, colour = "lightgrey") +
  annotate("text", x = 1.60, y = 1, label = "20g",size = 3) +
  annotate("text", x = 6.60, y = 1, label = "500g",size = 3) +
  annotate("text", x = 11.60, y = 1, label = "2000g",size = 3) +
```

```
  ylab("Relative reads abundance") +
  xlab("Time (days after *Betula pubescens* was fed)") +
  theme_bw() +
  theme(axis.title.x = ggtext::element_markdown(),
        legend.position="bottom") +
  scale_color_manual(name="Animal",values = family_color)  -> sper01_family_plot

ggsave("Figures/Sper01_family_plot.pdf",
       sper01_family_plot,
       dpi = 300,
      width = 32, height = 35, units = c("cm"))

ggsave("Figures/Sper01_family_plot.tiff",
       sper01_family_plot,
       dpi = 300,
      width = 16, height = 17, units = c("cm"))

sper01_family_plot
```



4.2. Normalisation of the Diet by a constant item

In the relative read frequency approach, the sum of all elements is, by definition, equal to one. This means that one degree of freedom is lost. Thus, if one item increases (birch or lichen in our experience), other items are forced to decrease because of the lost degree of freedom. Throughout the experiment, pellets were provided in a constant amount and therefore must be constantly retrieved in the feces. To recover the degree of freedom, the relative frequencies of the food items are divided by the pellet components. The new amount of food is therefore expressed in an arbitrary unit of DNA, and the amounts don't add up to one in every sample.

4.2.1. Normalizing the *Euka02* data set

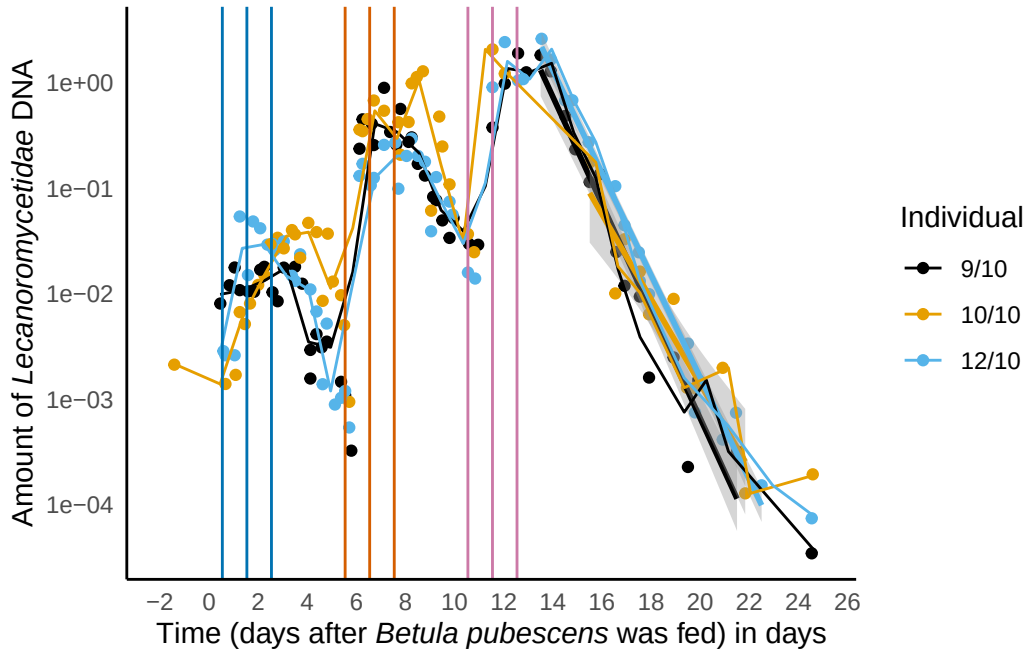
```
Euka02_family@motus %<>%
  mutate(food = ifelse(family_name =="Betulaceae","Birch",
                       ifelse(family_name =="Lecanoromycetidae","Lichen","Pellet")))

Euka02_food <- aggregate(Euka02_family,MARGIN = "motus",
                         by=list(Food=Euka02_family@motus$food),
                         FUN = sum)


Euka02_food$dna_amount <- sweep(Euka02_food@reads,
                                  MARGIN = 1,
                                  STATS = Euka02_food@reads[,"Pellet"],
                                  FUN = "/"
                                 )
```

4.2.2. Normalizing the *Sper01* data set

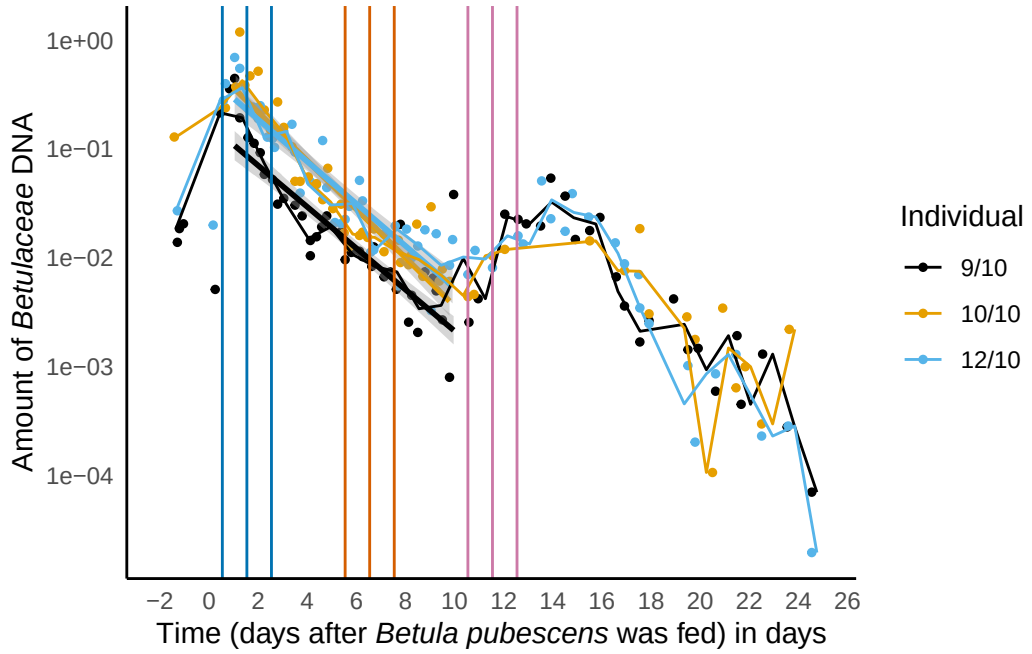
```
Sper01_family@motus %<>%
  mutate(food = ifelse(family_name =="Betulaceae","Birch",
                       ifelse(family_name =="Lecanoromycetidae","Lichen","Pellet")))

Sper01_food <- aggregate(Sper01_family,MARGIN = "motus",
                         by=list(Food=Sper01_family@motus$food),
                         FUN = sum)

Sper01_food$dna_amount <- sweep(Sper01_food@reads,
                                  MARGIN = 1,
                                  STATS = Sper01_food@reads[,"Pellet"],
                                  FUN = "/"
                                 )
```

```
Euka02_food$dna_amount %>%
  as.data.frame() %>%
  rownames_to_column("sample_id") %>%
  pivot_longer(cols = - "sample_id", names_to = "Food",values_to = "amount") %>%
  left_join(Euka02_food@samples, by = "sample_id") %>%
  mutate(times_from_birch = times_from_birch/24,
         time_group = floor(times_from_birch)) %>%
  filter(Food=="Lichen") %>%
  filter(amount > 0) %>%
  filter(times_from_birch <= 26) -> lichen_data_Euka02

lichen_start_time=13
lichen_end_time=24

ggplot(data = lichen_data_Euka02,
                       aes(x = times_from_birch,
                           y =  amount,
```

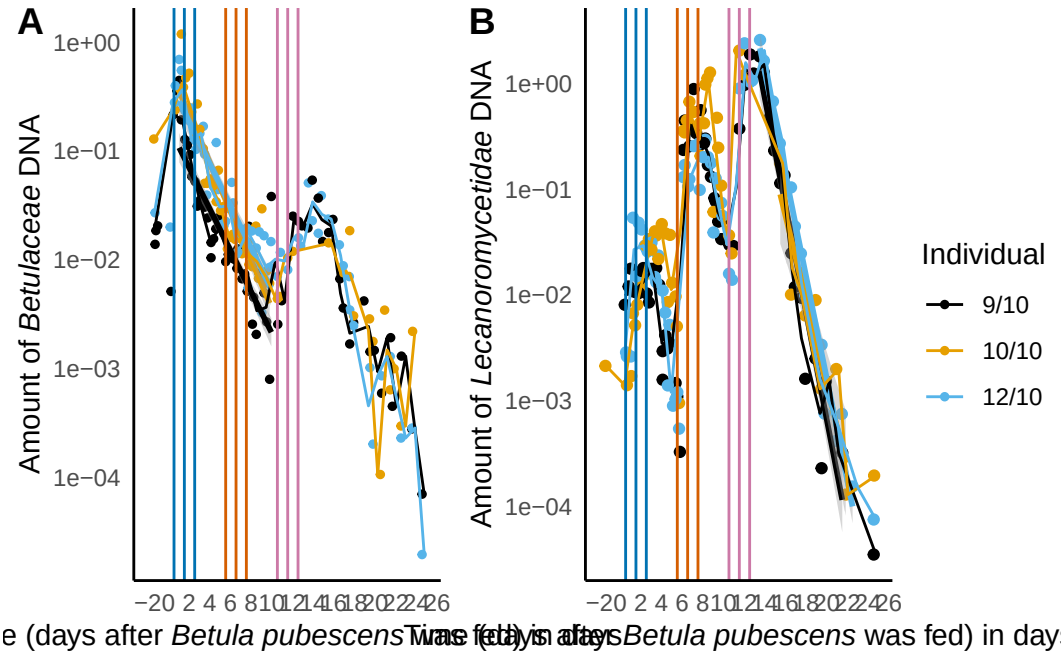
```
                          color = Animal_id)) +
  geom_point() +
  geom_smooth(data = lichen_data_Euka02 %>%
                filter(times_from_birch >= lichen_start_time &
                       times_from_birch <= lichen_end_time),
              method = MASS::rlm,show.legend = FALSE,
              formula = y~x) +
  scale_color_manual(values=cbbPalette) +
  stat_summary_bin(fun = median, geom = "line") +
  scale_y_log10() +
  geom_vline (xintercept = c(0.54,1.54,2.54), colour = cbbPalette[6]) +
  geom_vline (xintercept = c(5.54,6.54,7.54), colour = cbbPalette[7]) +
  geom_vline (xintercept = c(10.54,11.54,12.54), colour = cbbPalette[8]) +
  scale_x_continuous(breaks = scales::pretty_breaks(n = 15),limits = c(-2,25)) +
  guides(color=guide_legend(title="Individual")) +
  theme_minimal() +
  theme(panel.grid.major = element_blank(),
        panel.grid.minor = element_blank(),
        panel.background = element_blank(),
        axis.title.x = ggtext::element_markdown(),
        axis.title.y = ggtext::element_markdown(),
        axis.line = element_line(colour = "black")) +
  ylab("Amount of *Lecanoromycetidae* DNA") +
  xlab('Time (days after *Betula pubescens* was fed) in days') -> decay_leuca_euka02

decay_leuca_euka02
```



```r
Euka02_food$dna_amount %>%
  as.data.frame() %>%
  rownames_to_column("sample_id") %>%
  pivot_longer(cols = - "sample_id", names_to = "Food",values_to = "amount") %>%
  left_join(Euka02_food@samples, by = "sample_id") %>%
  mutate(times_from_birch = times_from_birch/24,
         time_group = floor(times_from_birch)) %>%
  filter(Food=="Birch") %>%
  filter(amount > 0) %>%
  filter(times_from_birch <= 25) -> birch_data_Euka02

birch_start_time=1
birch_end_time=10

ggplot(data = birch_data_Euka02,
                      aes(x = times_from_birch,
                          y =  amount,
                          color = Animal_id)) +
  geom_point(size=1) +
  geom_smooth(data = birch_data_Euka02 %>%
                filter(times_from_birch >= birch_start_time &
                         times_from_birch <= birch_end_time),
              method = MASS::rlm,
              show.legend = FALSE,
              formula = y~x) +
  scale_color_manual(values=cbbPalette) +
  stat_summary_bin(fun = median, geom = "line") +
  scale_y_log10() +
  geom_vline (xintercept = c(0.54,1.54,2.54), colour = cbbPalette[6]) +
  geom_vline (xintercept = c(5.54,6.54,7.54), colour = cbbPalette[7]) +
  geom_vline (xintercept = c(10.54,11.54,12.54), colour = cbbPalette[8]) +
  scale_x_continuous(breaks = scales::pretty_breaks(n = 15),
                     limits = c(-2,25)) +
  guides(color=guide_legend(title="Individual")) +
  theme_minimal() +
  theme(panel.grid.major = element_blank(),
        panel.grid.minor = element_blank(),
        panel.background = element_blank(),
        axis.title.x = ggtext::element_markdown(),
        axis.title.y = ggtext::element_markdown(),
        axis.line = element_line(colour = "black")) +
  ylab("Amount of *Betulaceae* DNA") +
  xlab('Time (days after *Betula pubescens* was fed) in days') -> decay_betula_euka02

decay_betula_euka02
```




```
ggarrange(decay_betula_euka02,
          decay_leuca_euka02,
          common.legend = TRUE,
          legend="right",labels = c("A","B")) -> decay_euka02_plot
ggsave("Figures/decay_euka02.pdf",
       decay_euka02_plot,
       dpi=300,
       width=12,height=5)
ggsave("Figures/decay_euka02.tiff",
       decay_euka02_plot,
       dpi=300,
       width=12,height=5)
decay_euka02_plot
```



4.3. Estimate of the Half-time detection

```
lichen_data_Euka02 %>%
  filter(times_from_birch >= lichen_start_time &
    times_from_birch <= lichen_end_time) %>%
  MASS::rlm(times_from_birch ~ log(amount):Animal_id + Animal_id,
    data = .
  ) %>%
  summary() %>%
  .[["coefficients"]] %>%
  as.data.frame() %>%
  rownames_to_column("Effect") %>%
  filter(str_starts(Effect, "log")) %>%
  mutate(Animal = str_replace(Effect, "^.*Animal_id", "")) %>%
  bind_rows(
    lichen_data_Euka02 %>%
      filter(times_from_birch >= lichen_start_time &
        times_from_birch <= lichen_end_time) %>%
      MASS::rlm(times_from_birch ~ log(amount) + Animal_id, data = .) %>%
      summary() %>% .[["coefficients"]] %>%
      as.data.frame() %>%
      rownames_to_column("Effect") %>%
      filter(Effect == "log(amount)") %>%
      mutate(Animal = "All")
  ) %>%
  mutate(
    HalfTime = -Value * log(2) * 24,
    HalfTime_sd = `Std. Error` * log(2) * 24,
```

```
    HalfTime_ci_low = qnorm(0.025, mean = HalfTime, sd = HalfTime_sd),
    HalfTime_ci_high = qnorm(0.975, mean = HalfTime, sd = HalfTime_sd)
  ) %>%
  select(Animal, HalfTime, HalfTime_ci_low, HalfTime_ci_high, HalfTime_sd)
```

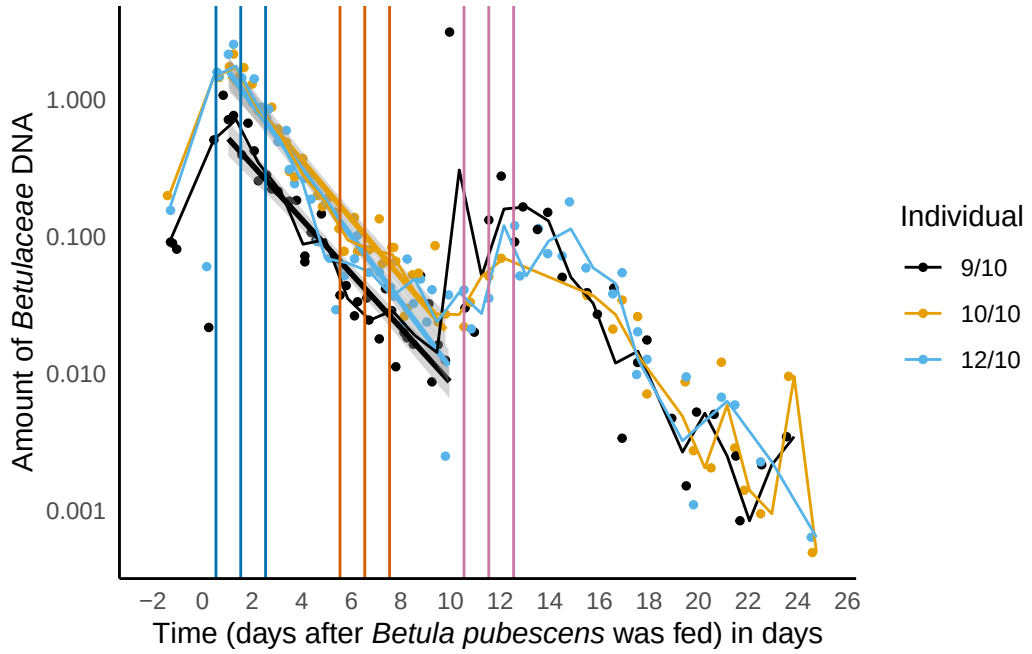
```
  Animal HalfTime HalfTime_ci_low HalfTime_ci_high HalfTime_sd
1   9/10 12.65668        11.03263         14.28073   0.8286128
2  10/10 14.65908        11.76153         17.55662   1.4783678
3  12/10 14.53293        13.06507         16.00078   0.7489193
4    All 14.09341        13.13104         15.05579   0.4910176
```

```
birch_data_Euka02 %>%
  filter(times_from_birch >= birch_start_time &
    times_from_birch <= birch_end_time) %>%
  MASS::rlm(times_from_birch ~ log(amount):Animal_id + Animal_id,
    data = .
  ) %>%
  summary() %>%
  .[["coefficients"]] %>%
  as.data.frame() %>%
  rownames_to_column("Effect") %>%
  filter(str_starts(Effect, "log")) %>%
  mutate(Animal = str_replace(Effect, "^.*Animal_id", "")) %>%
  bind_rows(
    birch_data_Euka02 %>%
      filter(times_from_birch >= birch_start_time &
        times_from_birch <= birch_end_time) %>%
      MASS::rlm(times_from_birch ~ log(amount) + Animal_id, data = .) %>%
      summary() %>% .[["coefficients"]] %>%
      as.data.frame() %>%
      rownames_to_column("Effect") %>%
      filter(Effect == "log(amount)") %>%
      mutate(Animal = "All")
  ) %>%
  mutate(
    HalfTime = -Value * log(2) * 24,
    HalfTime_sd = `Std. Error` * log(2) * 24,
    HalfTime_ci_low = qnorm(0.025, mean = HalfTime, sd = HalfTime_sd),
    HalfTime_ci_high = qnorm(0.975, mean = HalfTime, sd = HalfTime_sd)
  ) %>%
  select(Animal, HalfTime, HalfTime_ci_low, HalfTime_ci_high, HalfTime_sd)
```

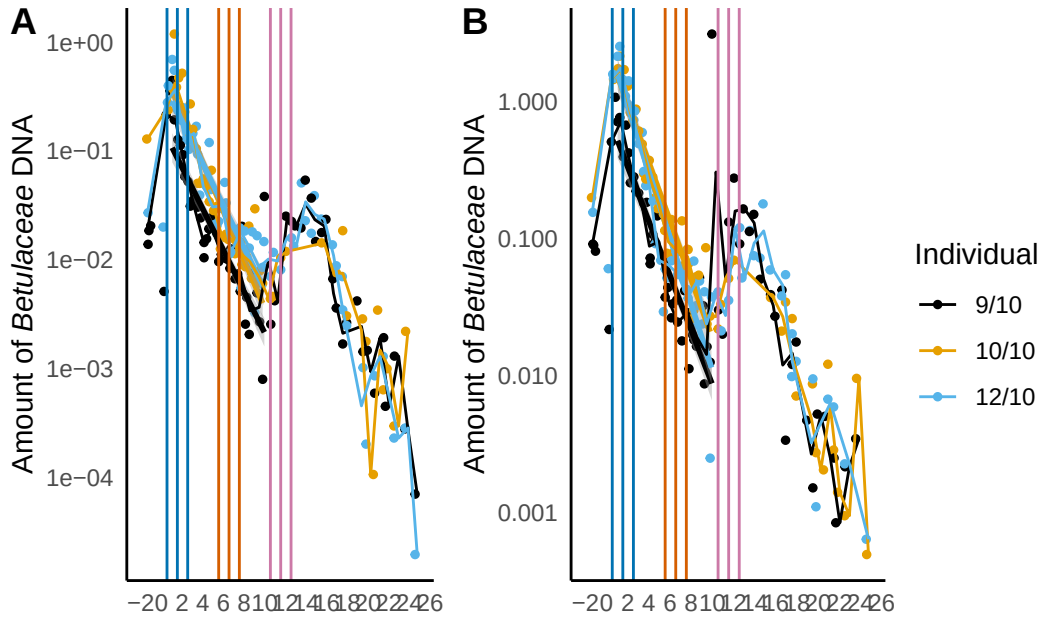
```
  Animal HalfTime HalfTime_ci_low HalfTime_ci_high HalfTime_sd
1   9/10 29.47525        24.86308         34.08742    2.353192
2  10/10 27.60222        23.36034         31.84409    2.164261
3  12/10 30.00489        25.33700         34.67278    2.381620
4    All 28.96248        26.49283         31.43212    1.260047
```

```
Sper01_food$dna_amount %>%
  as.data.frame() %>%
```

```
  rownames_to_column("sample_id") %>%
  pivot_longer(cols = - "sample_id", names_to = "Food",values_to = "amount") %>%
  left_join(Sper01_food@samples, by = "sample_id") %>%
  mutate(times_from_birch = times_from_birch/24,
         time_group = floor(times_from_birch)) %>%
  filter(Food=="Birch") %>%
  filter(amount > 0) %>%
  filter(times_from_birch <= 25) -> birch_data_Sper01

birch_start_time=1
birch_end_time=10

ggplot(data = birch_data_Sper01,
                      aes(x = times_from_birch,
                          y =  amount,
                          color = Animal_id)) +
  geom_point(size=1) +
  geom_smooth(data = birch_data_Sper01 %>%
                filter(times_from_birch >= birch_start_time &
                         times_from_birch <= birch_end_time),
              method = MASS::rlm,
              show.legend = FALSE,
              formula = y~x) +
  scale_color_manual(values=cbbPalette) +
  stat_summary_bin(fun = median, geom = "line") +
  scale_y_log10() +
  geom_vline (xintercept = c(0.54,1.54,2.54), colour = cbbPalette[6]) +
  geom_vline (xintercept = c(5.54,6.54,7.54), colour = cbbPalette[7]) +
  geom_vline (xintercept = c(10.54,11.54,12.54), colour = cbbPalette[8]) +
  scale_x_continuous(breaks = scales::pretty_breaks(n = 15),
                     limits = c(-2,25)) +
  guides(color=guide_legend(title="Individual")) +
  theme_minimal() +
  theme(panel.grid.major = element_blank(),
        panel.grid.minor = element_blank(),
        panel.background = element_blank(),
        axis.title.x = ggtext::element_markdown(),
        axis.title.y = ggtext::element_markdown(),
        axis.line = element_line(colour = "black")) +
  ylab("Amount of *Betulaceae* DNA") +
  xlab('Time (days after *Betula pubescens* was fed) in days') -> decay_betula_sper01

decay_betula_sper01
```




```
ggarrange(decay_betula_euka02,
          decay_betula_sper01,
          common.legend = TRUE,
          legend="right",labels = c("A","B")) -> decay_betula_plot
ggsave("Figures/decay_betula.pdf",
       decay_betula_plot,
       dpi=300,
       width=12,height=5)
ggsave("Figures/decay_betula.tiff",
       decay_betula_plot,
       dpi=300,
       width=12,height=5)
decay_betula_plot
```



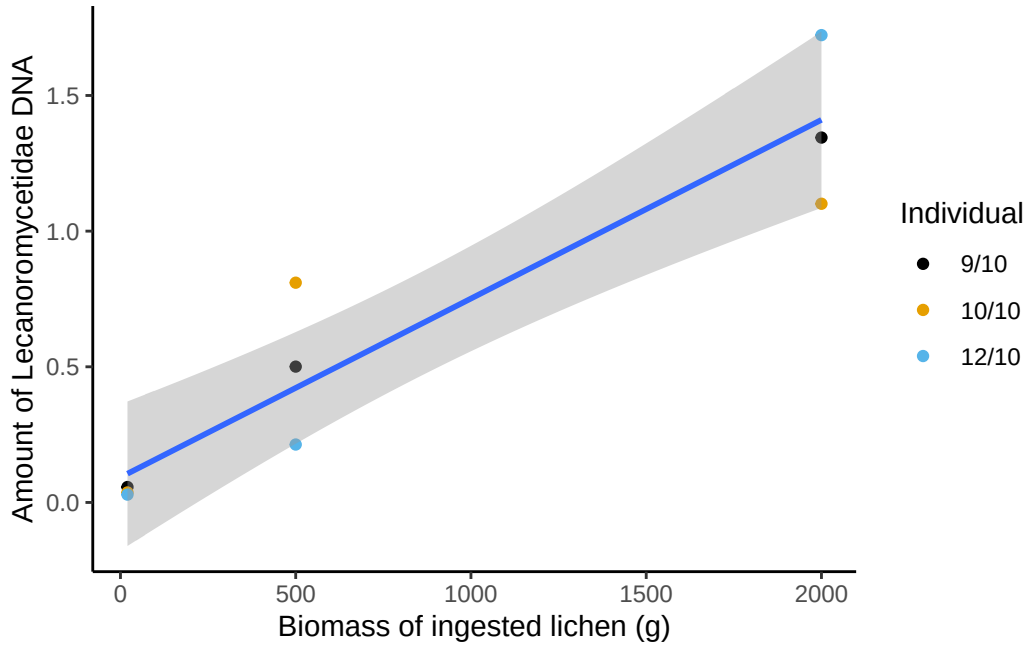
e (days after *Betula pubescens* was fed) in days Time (days after *Betula pubescens* was fed) in days

```
birch_data_Sper01 %>%
  filter(times_from_birch >= birch_start_time &
    times_from_birch <= birch_end_time) %>%
  MASS::rlm(times_from_birch ~ log(amount):Animal_id + Animal_id,
    data = .
  ) %>%
  summary() %>%
  .[["coefficients"]] %>%
  as.data.frame() %>%
  rownames_to_column("Effect") %>%
  filter(str_starts(Effect, "log")) %>%
  mutate(Animal = str_replace(Effect, "^.*Animal_id", "")) %>%
  bind_rows(
    birch_data_Sper01 %>%
      filter(times_from_birch >= birch_start_time &
        times_from_birch <= birch_end_time) %>%
      MASS::rlm(times_from_birch ~ log(amount) + Animal_id, data = .) %>%
      summary() %>% .[["coefficients"]] %>%
      as.data.frame() %>%
      rownames_to_column("Effect") %>%
      filter(Effect == "log(amount)") %>%
      mutate(Animal = "All")
  ) %>%
  mutate(
    HalfTime = -Value * log(2) * 24,
    HalfTime_sd = `Std. Error` * log(2) * 24,
    HalfTime_ci_low = qnorm(0.025, mean = HalfTime, sd = HalfTime_sd),
    HalfTime_ci_high = qnorm(0.975, mean = HalfTime, sd = HalfTime_sd)
```

```
  ) %>%
  select(Animal, HalfTime, HalfTime_ci_low, HalfTime_ci_high, HalfTime_sd)
```

```
  Animal HalfTime HalfTime_ci_low HalfTime_ci_high HalfTime_sd
1   9/10 30.06365        26.81057         33.31673   1.6597649
2  10/10 31.36567        27.81484         34.91650   1.8116813
3  12/10 25.96125        23.06194         28.86057   1.4792684
4    All 29.06789        27.19449         30.94130   0.9558374
```

```
Euka02_food$dna_amount %>%
  as.data.frame() %>%
  rownames_to_column("sample_id") %>%
  pivot_longer(cols = -sample_id,names_to = "Food", values_to = "amount") %>%
  left_join(Euka02_food@samples, by = "sample_id") %>%
  filter(!is.na(Fed_biomass)) %>%
  group_by(Animal_id,Fed_biomass,Food) %>%
  mutate(amount = slider::slide_mean(amount,after = 2,before = 2)) %>%
  summarise(amount = max(amount,na.rm = TRUE),.groups = "drop") %>%
  filter(Food == "Lichen") %>%
  mutate(Fed_biomass = as.integer(as.character(Fed_biomass))) %>%
  ggplot(aes(x=Fed_biomass,y=amount)) +
  geom_point(aes(col=Animal_id)) +
  stat_smooth(method = lm,formula = 'y ~ x')+
  theme_classic() +
  guides(color=guide_legend(title="Individual")) +
  scale_color_manual(values=cbbPalette) +
  labs(y="Amount of Lecanoromycetidae DNA") +
  labs(x=expression('Biomass of ingested lichen (g)'),
       fill="Var1")
```




```
Euka02_food$dna_amount %>%
  as.data.frame() %>%
  rownames_to_column("sample_id") %>%
  pivot_longer(cols = -sample_id,names_to = "Food", values_to = "amount") %>%
  left_join(Euka02_food@samples, by = "sample_id") %>%
  filter(!is.na(Fed_biomass)) %>%
  group_by(Animal_id,Fed_biomass,Food) %>%
  summarise(amount = max(amount,na.rm = TRUE),.groups = "drop") %>%
  filter(Food == "Lichen") %>%
  mutate(Fed_biomass = as.integer(as.character(Fed_biomass))) %>%
  lm(amount ~ Fed_biomass:Animal_id + 1, data=.) -> dna_rra_lm

  summary(dna_rra_lm)
```

```
Call:
lm(formula = amount ~ Fed_biomass:Animal_id + 1, data = .)

Residuals:
       1        2        3        4        5        6        7        8
 0.03048  0.26044 -0.06541 -0.16119  0.58525 -0.14470 -0.15696 -0.46597
       9
 0.11806

Coefficients:
                            Estimate Std. Error t value Pr(>|t|)
(Intercept)                0.1878469  0.1783062   1.054  0.34033
Fed_biomass:Animal_id9/10  0.0008862  0.0002121   4.179  0.00866 **
Fed_biomass:Animal_id10/10 0.0010101  0.0002121   4.763  0.00504 **
```

```
Fed_biomass:Animal_id12/10 0.0011459  0.0002121   5.403  0.00293 **
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 0.379 on 5 degrees of freedom
Multiple R-squared:  0.9035,    Adjusted R-squared:  0.8456
F-statistic: 15.61 on 3 and 5 DF,  p-value: 0.005683
```

```
shapiro.test(residuals(dna_rra_lm))
```

```
    Shapiro-Wilk normality test

data:  residuals(dna_rra_lm)
W = 0.95163, p-value = 0.708
```

References

[1] H. Wickham, M. Averick, J. Bryan, W. Chang, L. McGowan, R. François, G. Grolemund, A. Hayes, L. Henry, J. Hester, M. Kuhn, T. Pedersen, E. Miller, S. Bache, K. Müller, J. Ooms, D. Robinson, D. Seidel, V. Spinu, K. Takahashi, D. Vaughan, C. Wilke, K. Woo, H. Yutani, Welcome to the tidyverse, Journal of open source software 4 (43) (2019) 1686. doi:10.21105/joss.01686.
URL https://joss.theoj.org/papers/10.21105/joss.01686

[2] H. Wickham, ggplot2: Elegant Graphics for Data Analysis, Springer-Verlag New York, 2016.
URL https://ggplot2.tidyverse.org

[3] J. Oksanen, F. G. Blanchet, R. Kindt, P. Legendre, P. Minchin, R. O'Hara, G. Simpson, P. Solymos, M. Henry, M. Stevens, et al., Vegan: community ecology package. ordination methods, diversity analysis and other functions for community and vegetation ecologists, R package ver (2015) 2–3.

[4] A. Kassambara, M. A. Kassambara, Package 'ggpubr' (2020).