# Microbial persistence in agricultural soil - code/output

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## Table of contents

## 1. Packages

Run this script from within the R project - the code now uses relative paths which may not work otherwise.

```
# Load packages
library(emmeans)
library(GGally)
library(ggpubr)
library(ggResidpanel)
library(here)
library(marginaleffects)
library(MASS)
library(multcomp)
library(multcompView)
library(nlme)
library(performance)
library(tidyverse)

# Register system fonts in R, if necessary
## library(extrafont)
## font_import()

# update.packages(ask = FALSE)
```

## 2. Reading persistence data and initial setup

```
readRDS(here('obj2', 'data', 'obj2_dat.RDS')) |>
  str()
```

```
'data.frame':	480 obs. of  8 variables:
 $ microbe  : Factor w/ 3 levels "ecoli","nv","tv": 1 1 1 1 1 1 1 1 1 1 ...
 $ matrix   : Factor w/ 2 levels "ps","s": 1 1 1 1 1 1 1 1 1 1 ...
 $ temp     : Factor w/ 1 level "12": 1 1 1 1 1 1 1 1 1 1 ...
 $ day      : num  0 0 0 1 1 1 3 3 3 7 ...
 $ replicate: num  1 2 3 1 2 3 1 2 3 1 ...
 $ assay    : Factor w/ 3 levels "pcr","plate",..: 2 2 2 2 2 2 2 2 2 2 ...
 $ survival : num  6.78 6.59 6.8 7.21 7.15 ...
 $ week     : num  0 0 0 0.143 0.143 ...
```

```r
# Limits of detection
LOD <- data.frame(
  microbe = as.factor(c('ecoli_plate', 'tv', 'tv_tcid50', 'nv')),
  lod = c(1.38, 3.78, 0.5, 2.15)
)

# Set random number generation state
SEED <- 5638

# Limit the number of significant digits to 3
options(digits=3)

# Turn in environments/packages/versions/etc
sessioninfo::session_info (pkgs = c("attached"))
```

```
─ Session info
───────────────────────────────────────────────────────────────

 setting  value
 version  R version 4.5.2 (2025-10-31)
 os       macOS Sequoia 15.7.2
 system   x86_64, darwin20
 ui       X11
 language (EN)
 collate  en_US.UTF-8
 ctype    en_US.UTF-8
 tz       America/New_York
 date     2025-12-08
 pandoc   3.6.3 @
/Applications/RStudio.app/Contents/Resources/app/quarto/bin/tools/x86_64/
(via rmarkdown)
 quarto   1.3.433 @ /usr/local/bin/quarto

─ Packages
───────────────────────────────────────────────────────────────────

 package         * version date (UTC) lib source
 dplyr           * 1.1.4   2023-11-17 [1] CRAN (R 4.5.0)
 emmeans         * 2.0.0   2025-10-29 [1] CRAN (R 4.5.1)
 forcats         * 1.0.1   2025-09-25 [1] CRAN (R 4.5.1)
 GGally          * 2.4.0   2025-08-23 [1] CRAN (R 4.5.1)
 ggplot2         * 4.0.0   2025-09-11 [1] CRAN (R 4.5.1)
 ggpubr          * 0.6.2   2025-10-17 [1] CRAN (R 4.5.1)
 ggResidpanel    * 0.3.0   2019-05-31 [1] CRAN (R 4.5.0)
 here            * 1.0.2   2025-09-15 [1] CRAN (R 4.5.1)
 lubridate       * 1.9.4   2024-12-08 [1] CRAN (R 4.5.0)
 marginaleffects * 0.30.0  2025-09-13 [1] CRAN (R 4.5.1)
 MASS            * 7.3-65  2025-02-28 [1] CRAN (R 4.5.2)
 multcomp        * 1.4-29  2025-10-20 [1] CRAN (R 4.5.1)
 multcompView    * 0.1-10  2024-03-08 [1] CRAN (R 4.5.0)
 mvtnorm         * 1.3-3   2025-01-10 [1] CRAN (R 4.5.0)
 nlme            * 3.1-168 2025-03-31 [1] CRAN (R 4.5.2)
 performance     * 0.15.2  2025-10-06 [1] CRAN (R 4.5.1)
 purrr           * 1.1.0   2025-07-10 [1] CRAN (R 4.5.1)
 readr           * 2.1.5   2024-01-10 [1] CRAN (R 4.5.0)
 stringr         * 1.5.2   2025-09-08 [1] CRAN (R 4.5.1)
 survival        * 3.8-3   2024-12-17 [1] CRAN (R 4.5.2)
 TH.data         * 1.1-4   2025-09-02 [1] CRAN (R 4.5.1)
 tibble          * 3.3.0   2025-06-08 [1] CRAN (R 4.5.0)
 tidyr           * 1.3.1   2024-01-24 [1] CRAN (R 4.5.0)
 tidyverse       * 2.0.0   2023-02-22 [1] CRAN (R 4.5.0)

 [1] /Library/Frameworks/R.framework/Versions/4.5-x86_64/Resources/library
 * ── Packages attached to the search path.
```

---

## 3. T1D

### 3.1 Model Functions

```
# Log linear and non-linear models

## Log-linear and reparameterized log-linear
ll <- function (x, log10n0, alpha) {
   log10n0 - (x*alpha)
}

llrepar <- function(x, log10n0, TD, D = 1){
  log10n0 - D*x/TD
}

## Nonlinear models
# Weibull and reparameterized Weibull
wb <- function (x, log10n0, alpha, beta) {
   log10n0 - (x/exp(alpha))^beta
 }

wbrepar <- function(x, log10n0, TD, beta, D = 1){
  log10n0 - D*(x/TD)^beta
```

```r
}

# Log-logistic and reparameterized log-logistic
lg = function(x, log10n0, kappa, sigma){
   log10n0 - log10(1+ exp((log(x)-kappa)/sigma^2))
 }

lgrepar = function(x, log10n0, TD, sigma, D = 1){
  log10n0 - log10(1+ exp((log(x)- (log(TD) - log(10^D -
1)*sigma^2))/sigma^2))
}

# To clean the environment
list_exc <- c(lsf.str(envir = .GlobalEnv), "LOD", "SEED", "list_exc",
"ec_m2")
```

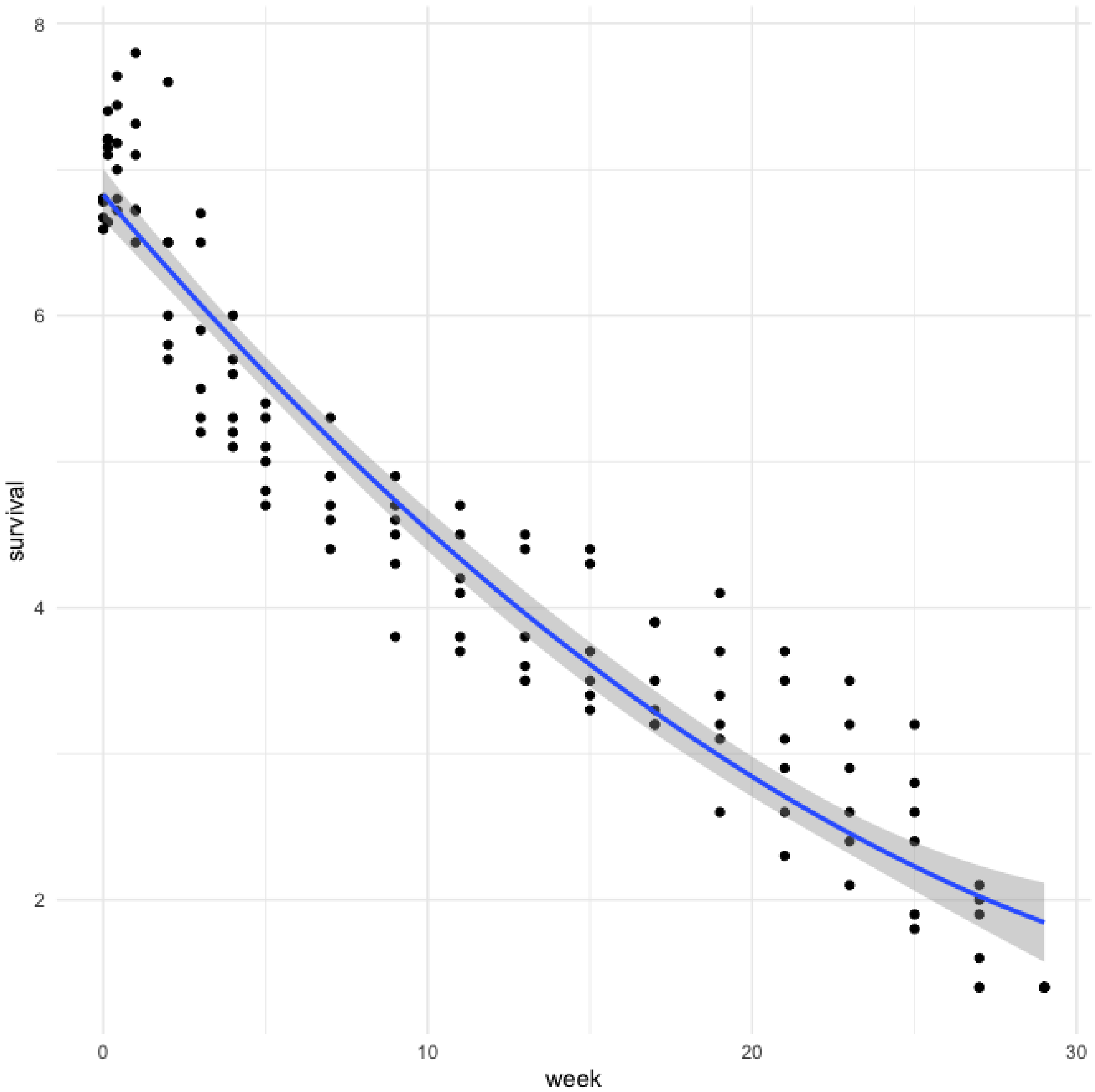
## 3.2 *E. coli*

### 3.2.1 Fitting models

```r
m2 <- readRDS(here('obj2', 'data', 'obj2_dat.RDS')) |>
  dplyr::filter(microbe == 'ecoli',
                assay == "plate") |>
  droplevels()

# Levels
contrasts(m2$matrix)
```

```
   s
ps 0
s  1
```

```r
# Initial visual inspection
ggplot(m2, aes(x = week, y = survival)) +
  geom_point() +
  stat_smooth(method = "lm", formula = y ~ poly(x, 2)) +
  theme_minimal()
```



```
m2_mod_null = nlme::gnls(survival ~ ll(x = week,
                                    log10n0 = log10n0,
                                    alpha = alpha),
                  data = m2,
                  params = log10n0+alpha ~ 1,
                  start = list(log10n0 = 5,
                               alpha = 1))

m2_mod_ll = nlme::gnls(survival ~ ll(x = week,
                                    log10n0 = log10n0,
                                    alpha = alpha),
                  data = m2,
                  params = log10n0+alpha ~ matrix,
                  start = list(log10n0 = c(5,0),
                               alpha = c(1,0)))
```

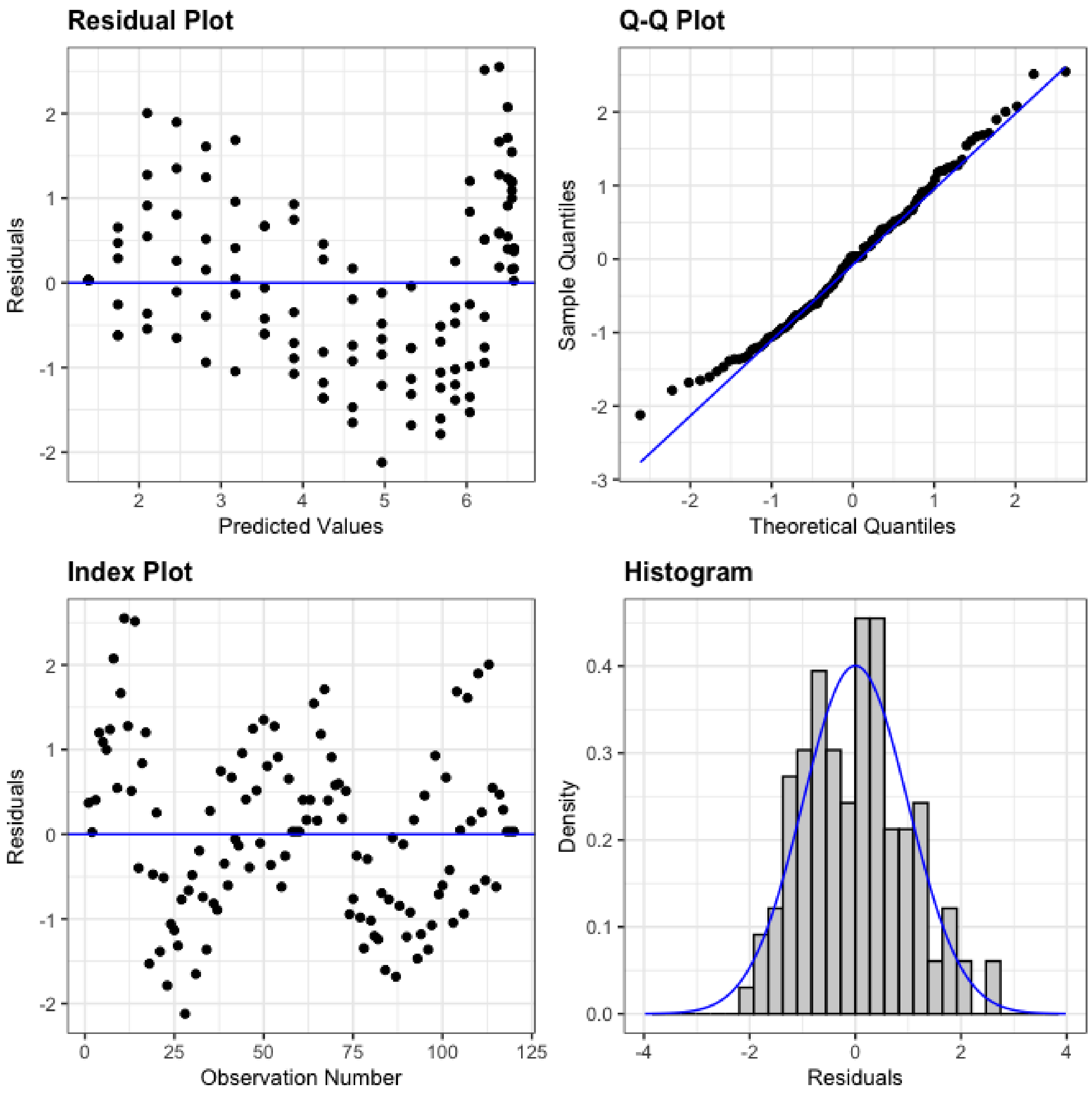
```
m2_mod_llrepar = nlme::gnls(survival ~ llrepar(x = week,
                                               log10n0 = log10n0,
                                               TD = TD),
                            data = m2,
                            params = log10n0+TD ~ matrix,
                            start = list(log10n0 = c(6, 0),
                                         TD = c(5, 0)))

m2_mod_wb = nlme::gnls(survival ~ wb(x = week,
                                     log10n0 = log10n0,
                                     alpha = alpha,
                                     beta = beta),
                       data = m2,
                       params = log10n0+alpha+beta ~ matrix,
                       start = list(log10n0 = c(5, 0),
                                    alpha = c(1, 0),
                                    beta = c(1, 0)))

m2_mod_wbrepar = nlme::gnls(survival ~ wbrepar(x = week,
                                               log10n0 = log10n0,
                                               TD = TD,
                                               beta = beta),
                            data = m2,
                            params = log10n0+TD+beta ~ matrix,
                            start = list(log10n0 = c(5, rep(0, 1)),
                                         TD = c(1, 0),
                                         beta = c(1, 0)))

m2_mod_lg = nlme::gnls(survival ~ lg(x = week,
                                     log10n0 = log10n0,
                                     kappa = kappa,
                                     sigma = sigma),
                       data = m2,
                       params = log10n0+kappa+sigma ~ matrix,
                       start = list(log10n0 = c(5, 0),
                                    kappa = c(2, 0),
                                    sigma  = c(1, 0)))

m2_mod_lgpar = nlme::gnls(survival ~ lgrepar(x = week,
                                             log10n0 = log10n0,
                                             sigma = sigma,
                                             TD = TD),
                          data = m2,
                          params = log10n0+sigma+TD ~ matrix,
                          start = list(log10n0 = c(4, 0),
                                       sigma = c(0.5, 0),
                                       TD  = c(4, 0)))
```

### 3.2.2 Model performance and inference

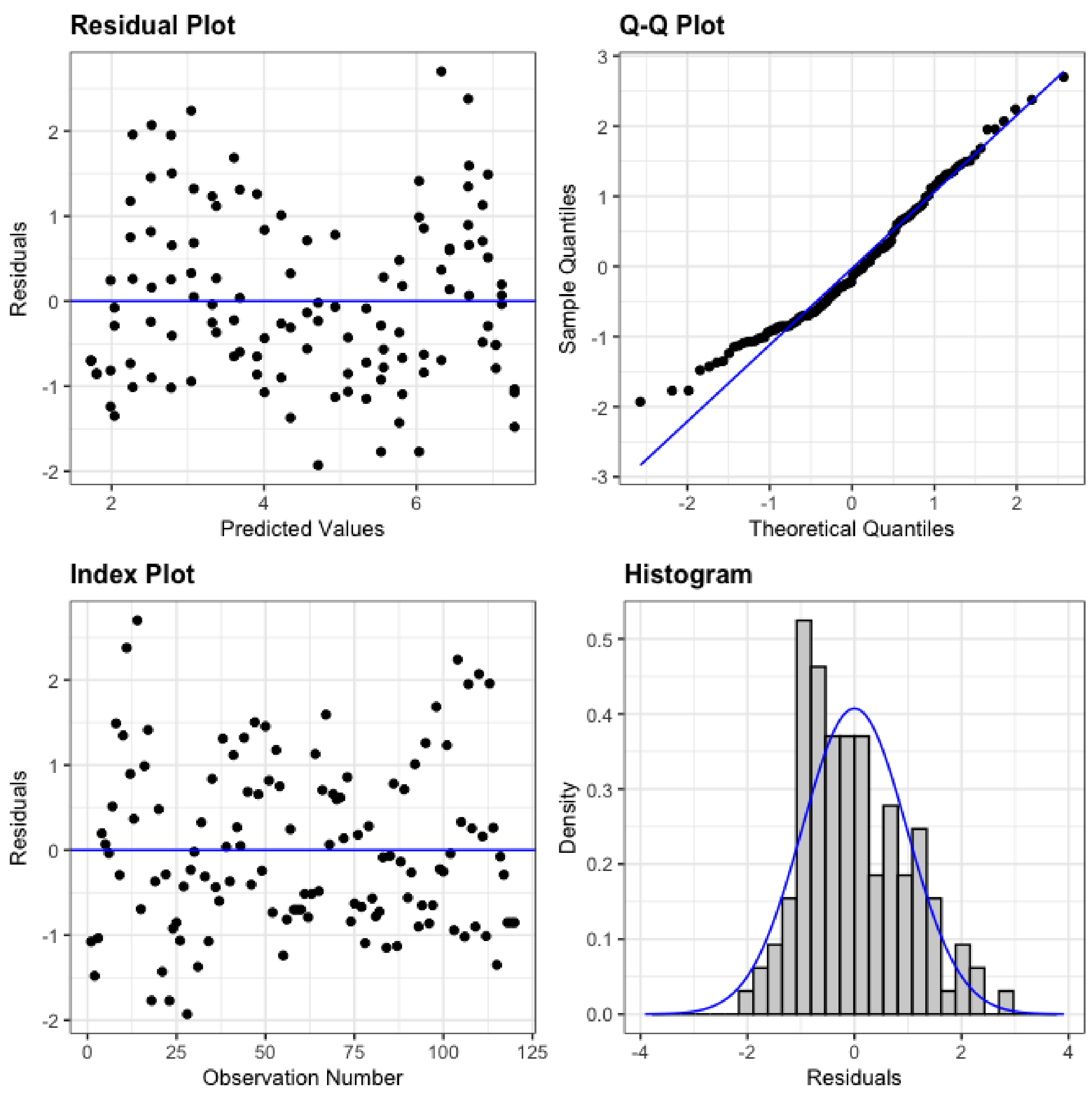
```
# Model comparisons
performance::compare_performance (
  m2_mod_null, m2_mod_ll, m2_mod_llrepar,
  m2_mod_lg, m2_mod_lgpar, m2_mod_wb, m2_mod_wbrepar,
  metrics = c('AICc'))
```

```
# Comparison of Model Performance Indices

Name            | Model | AIC (weights) | AICc (weights) | BIC (weights) |
R2
-----------------------------------------------------------------------------
--
m2_mod_null     |  gnls | 200.8 (<.001) |  201.0 (<.001) | 209.1 (<.001) |
0.908
m2_mod_ll       |  gnls | 202.2 (<.001) |  202.7 (<.001) | 216.1 (<.001) |
0.910
m2_mod_llrepar  |  gnls | 202.2 (<.001) |  202.7 (<.001) | 216.1 (<.001) |
0.910
m2_mod_lg       |  gnls | 186.9 (<.001) |  187.9 (<.001) | 206.4 (<.001) |
0.924
m2_mod_lgpar    |  gnls | 186.9 (<.001) |  187.9 (<.001) | 206.4 (<.001) |
0.924
m2_mod_wb       |  gnls | 167.9 (0.500) |  168.9 (0.500) | 187.4 (0.500) |
0.935
m2_mod_wbrepar  |  gnls | 167.9 (0.500) |  168.9 (0.500) | 187.4 (0.500) |
0.935

Name            |  RMSE | Sigma
------------------------------
m2_mod_null     | 0.545 | 0.549
m2_mod_ll       | 0.539 | 0.548
m2_mod_llrepar  | 0.539 | 0.548
m2_mod_lg       | 0.497 | 0.510
m2_mod_lgpar    | 0.497 | 0.510
m2_mod_wb       | 0.459 | 0.471
m2_mod_wbrepar  | 0.459 | 0.471
```

```
# Residual plots
m2_res_null = resid(m2_mod_null, type = 'normalized')
m2_fit_null = fitted(m2_mod_null)
resid_auxpanel(m2_res_null, m2_fit_null)
```



```
m2_res_wbrepar = resid(m2_mod_wbrepar, type = 'normalized')
m2_fit_wbrepar = fitted(m2_mod_wbrepar)
resid_auxpanel(m2_res_wbrepar, m2_fit_wbrepar)
```



```
pres_mod <- m2_mod_wbrepar

# gnls model tuning
m2_mod_exp = update(pres_mod, weights = varExp())
m2_mod_exp_wk = update(pres_mod, weights = varExp(form = ~week))
m2_mod_exp_sur = update(pres_mod, weights = varExp(form = ~survival))
m2_mod_idt_mtx = update(pres_mod, weights = varIdent(form = ~1 | matrix))
#m2_mod_idt_mic = update(pres_mod, weights = varIdent(form = ~1 | microbe))
#m2_mod_pow = update(pres_mod, weights = varPower())
m2_mod_pow_sur = update(pres_mod, weights = varPower(form = ~ survival))

#m2_mod_combined1 = update(pres_mod, weights = varComb(varIdent(form = ~1 |
microbe), varExp(form = ~ survival)))
#m2_mod_combined2 = update(pres_mod, weights = varComb(varIdent(form = ~1 |
microbe), varExp(form = ~ week)))
m2_mod_combined3 = update(pres_mod, weights = varComb(varIdent(form = ~1 |
matrix), varExp(form = ~ survival)))
m2_mod_combined4 = update(pres_mod, weights = varComb(varIdent(form = ~1 |
matrix), varExp(form = ~ week)))

compare_performance(
  pres_mod,
  m2_mod_exp,
  m2_mod_exp_wk,
  m2_mod_idt_mtx,
  #m2_mod_idt_mic,
  #m2_mod_pow,
  #m2_mod_combined1,
  #m2_mod_combined2,
  m2_mod_combined3,
  m2_mod_combined4,
  metrics = c('AICc'))

# Comparison of Model Performance Indices

Name              | Model | AIC (weights) | AICc (weights) | BIC (weights)
-------------------------------------------------------------------------
pres_mod          |  gnls | 167.9 (0.354) |  168.9 (0.400) | 187.4 (0.740)
m2_mod_exp        |  gnls | 169.8 (0.132) |  171.1 (0.129) | 192.1 (0.068)
m2_mod_exp_wk     |  gnls | 169.9 (0.131) |  171.2 (0.127) | 192.2 (0.068)
m2_mod_idt_mtx    |  gnls | 169.1 (0.191) |  170.4 (0.186) | 191.4 (0.099)
m2_mod_combined3 |  gnls | 170.0 (0.120) |  171.7 (0.099) | 195.1 (0.015)
m2_mod_combined4 |  gnls | 171.1 (0.072) |  172.7 (0.059) | 196.1 (0.009)

Name              |    R2 |  RMSE | Sigma
----------------------------------------
pres_mod          | 0.935 | 0.459 | 0.471
m2_mod_exp        | 0.935 | 0.459 | 0.459
m2_mod_exp_wk     | 0.935 | 0.459 | 0.473
m2_mod_idt_mtx    | 0.935 | 0.459 | 0.497
m2_mod_combined3 | 0.935 | 0.460 | 0.402
m2_mod_combined4 | 0.935 | 0.459 | 0.490

m2_mod <- pres_mod

summary (m2_mod)

Generalized nonlinear least squares fit
  Model: survival ~ wbrepar(x = week, log10n0 = log10n0, TD = TD, beta =
beta)
  Data: m2
  AIC BIC logLik
  168 187  -76.9

Coefficients:
                    Value Std.Error t-value p-value
```

```
log10n0.(Intercept)  7.29     0.181    40.2  0.0000
log10n0.matrixs     -0.24     0.259    -0.9  0.3460
TD.(Intercept)       2.13     0.567     3.8  0.0003
TD.matrixs           0.05     0.838     0.1  0.9511
beta.(Intercept)     0.66     0.062    10.6  0.0000
beta.matrixs        -0.02     0.089    -0.2  0.8521

 Correlation:
                 l100.( lg100. TD.(I) TD.mtr bt.(I)
log10n0.matrixs  -0.701
TD.(Intercept)   -0.876  0.615
TD.matrixs        0.593 -0.878 -0.676
beta.(Intercept) -0.792  0.556  0.981 -0.664
beta.matrixs      0.549 -0.794 -0.680  0.980 -0.694

Standardized residuals:
   Min     Q1    Med     Q3    Max
-1.930 -0.745 -0.180  0.690  2.700

Residual standard error: 0.471
Degrees of freedom: 120 total; 114 residual
```

```
saveRDS(m2_mod, here('obj2', 'results', 'ecoli_mod_sum.RDS'))

# Marginal means (predictions)
m2_mod <- readRDS(here('obj2', 'results', 'ecoli_mod_sum.RDS'))

expand.grid(
  matrix = levels(m2$matrix),
  microbe = levels(m2$microbe),
  assay = levels(m2$assay),
  week = seq(0, max(m2$week), 0.5)) |>
  marginaleffects::avg_predictions(
    model=m2_mod,
    conf_level = 0.95,
    by = c('matrix', 'week')) |>
  saveRDS(here('obj2', 'results', 'ecoli_mod_preds.RDS'))

# Extract parameters (coefficients) and their 95% CI
m2_mod_coef = coef(m2_mod)
m2_mod_confint = confint(m2_mod)

(mod_params <- as.data.frame (cbind(
  Param = names (m2_mod_coef),
  Est = round (m2_mod_coef, 3),
  lwr = round (m2_mod_confint[,1], 3),
  upr = round (m2_mod_confint[,2], 3)
  )))
```

```
                                  Param    Est    lwr   upr
log10n0.(Intercept) log10n0.(Intercept)  7.287  6.932 7.643
log10n0.matrixs         log10n0.matrixs -0.245 -0.752 0.262
TD.(Intercept)           TD.(Intercept)  2.127  1.017 3.238
TD.matrixs                   TD.matrixs  0.051 -1.591 1.694
beta.(Intercept)       beta.(Intercept)  0.657  0.536 0.778
beta.matrixs               beta.matrixs -0.017 -0.191 0.158
```

```
# Estimated marginal (group level) means
list(
  log10n0 = joint_tests (m2_mod, param = 'log10n0'),
  TD = joint_tests (m2_mod, param = 'TD'),
  beta = joint_tests (m2_mod, param = 'beta'))
```

```
$log10n0
 model term df1 df2 F.ratio p.value
 matrix       1 113   0.896  0.3460



$TD
 model term df1 df2 F.ratio p.value
 matrix       1 113   0.004  0.9510



$beta
 model term df1 df2 F.ratio p.value
 matrix       1 113   0.035  0.8520
```

```
# Estimated marginal (group level) means and contrasts
list(
  log10n0 = emmeans (m2_mod, pairwise ~ matrix, param = 'log10n0'),
  TD = emmeans (m2_mod, pairwise ~ matrix, param = 'TD'),
  beta = emmeans (m2_mod, pairwise ~ matrix, param = 'beta'))
```

```
$log10n0
$emmeans
 matrix emmean    SE  df lower.CL upper.CL
 ps       7.29 0.182 113     6.93     7.65
 s        7.04 0.184 113     6.68     7.41

Confidence level used: 0.95

$contrasts
 contrast estimate    SE  df t.ratio p.value
 ps - s      0.245 0.259 113   0.946  0.3460



$TD
$emmeans
```

```
 matrix emmean    SE  df lower.CL upper.CL
 ps       2.13 0.567 113    1.005     3.25
 s        2.18 0.617 113    0.956     3.40

Confidence level used: 0.95

$contrasts
 contrast estimate    SE  df t.ratio p.value
 ps - s    -0.0515 0.838 113  -0.061  0.9510



$beta
$emmeans
 matrix emmean     SE  df lower.CL upper.CL
 ps      0.657 0.0617 113    0.534    0.779
 s       0.640 0.0641 113    0.513    0.767

Confidence level used: 0.95

$contrasts
 contrast estimate    SE  df t.ratio p.value
 ps - s     0.0166 0.089 113   0.187  0.8520
```

```
# Overall marginal means
emmeans (m2_mod, ~ 1, param = 'log10n0')

 1       emmean    SE  df lower.CL upper.CL
 overall   7.16 0.129 113     6.91     7.42

Results are averaged over the levels of: matrix
Confidence level used: 0.95

emmeans (m2_mod, ~ 1, param = 'beta')

 1       emmean     SE  df lower.CL upper.CL
 overall  0.648 0.0445 113     0.56    0.736

Results are averaged over the levels of: matrix
Confidence level used: 0.95

(TD_values <- emmeans (m2_mod, ~ 1, param = 'TD'))

 1       emmean    SE  df lower.CL upper.CL
 overall   2.15 0.419 113     1.32     2.98

Results are averaged over the levels of: matrix
Confidence level used: 0.95

saveRDS (TD_values, here('obj2', 'results', "TD_values.RDS"))
```

### 3.2.3 Assess covariance/collinearity

```
set.seed(SEED)

## Variance-covariance matrix
m2_mod <- readRDS(here('obj2', 'results', 'ecoli_mod_sum.RDS'))
m2_mod_coef <- coef (m2_mod)

(mod_vcov_joint <- vcov(m2_mod))
```

```
                    log10n0.(Intercept) log10n0.matrixs TD.(Intercept)
log10n0.(Intercept)             0.03293        -0.03293         -0.0901
log10n0.matrixs                -0.03293         0.06693          0.0901
TD.(Intercept)                 -0.09011         0.09011          0.3212
TD.matrixs                      0.09011        -0.19030         -0.3212
beta.(Intercept)               -0.00887         0.00887          0.0343
beta.matrixs                    0.00887        -0.01827         -0.0343
                    TD.matrixs beta.(Intercept) beta.matrixs
log10n0.(Intercept)     0.0901         -0.00887      0.00887
log10n0.matrixs        -0.1903          0.00887     -0.01827
TD.(Intercept)         -0.3212          0.03432     -0.03432
TD.matrixs              0.7021         -0.03432      0.07310
beta.(Intercept)       -0.0343          0.00381     -0.00381
beta.matrixs            0.0731         -0.00381      0.00792
```

```
mod_vcov_indep <- diag(diag(mod_vcov_joint))
dimnames(mod_vcov_indep) <- dimnames(mod_vcov_joint)

## Draw of coefficients
iter = 5000
draw_joint <- MASS::mvrnorm(n=iter, mu = m2_mod_coef, Sigma = mod_vcov_joint)
|>
  as.data.frame()

## Assuming no covariance
draw_indep <- MASS::mvrnorm(n=iter, mu = m2_mod_coef, Sigma = mod_vcov_indep)
|>
  as.data.frame()

## Extract TD values
TD_joint <- cbind (
  ps_joint = draw_joint[, "TD.(Intercept)"],
  s_joint = draw_joint[, "TD.(Intercept)"] + draw_joint[, "TD.matrixs"]
)

colMeans(TD_joint <= 0)
```

```
ps_joint  s_joint
   2e-04    0e+00
```

```
TD_indep <- cbind (
  ps_indep = draw_indep[, "TD.(Intercept)"],
  s_indep = draw_indep[, "TD.(Intercept)"] + draw_indep[, "TD.matrixs"]
)

colMeans(TD_indep <= 0)

ps_indep  s_indep
  0.0000   0.0154

## Calculate mean and 95% QIs
QIs <- function(x) c(
  mean=mean(x),
  lwr=quantile(x, 0.025),
  upr=quantile(x, 0.975))

round(t(apply(TD_joint, 2, QIs)),2)

         mean lwr.2.5% upr.97.5%
ps_joint 2.12     1.01      3.23
s_joint  2.18     0.95      3.37

round(t(apply(TD_indep, 2, QIs)),2)

         mean lwr.2.5% upr.97.5%
ps_indep 2.14     1.06      3.22
s_indep  2.19     0.25      4.14

## Again compare with emmeans
emmeans (m2_mod, pairwise ~ matrix, param = 'TD')$emmeans

 matrix emmean    SE  df lower.CL upper.CL
 ps       2.13 0.567 113    1.005     3.25
 s        2.18 0.617 113    0.956     3.40

Confidence level used: 0.95

saveRDS(mod_vcov_joint, here('obj2', 'results', 'ecoli_sim_mvrnorm.RDS'))

# Remove selected objects from the environment
rm(list = setdiff(ls(envir = .GlobalEnv, all.names = TRUE), list_exc),
   envir = .GlobalEnv)
```

### 3.2.4 Plot microbial inactivation

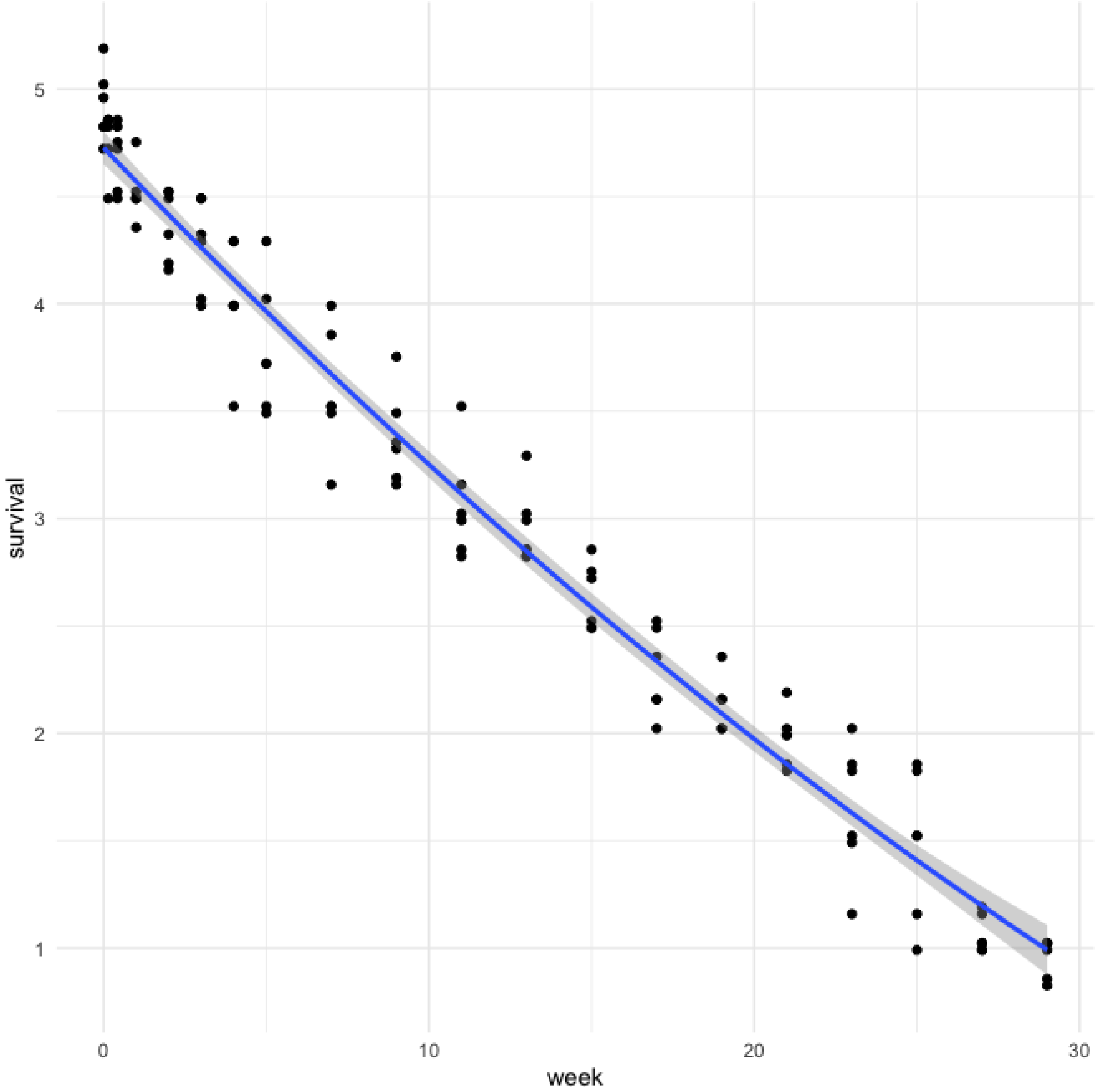
```
m2 <- readRDS(here('obj2', 'data','obj2_dat.RDS')) |>
  dplyr::filter(microbe == 'ecoli') |>
  dplyr::mutate(matrix = factor(matrix,
                         labels = c('Soil with plant', 'Soil'))) |>
  droplevels()

m2_preds <- readRDS(here('Obj2', 'results', 'ecoli_mod_preds.RDS'))
```

```
m2_preds$matrix <- factor(m2_preds$matrix, levels = c("ps", "s"),
                          labels = c("Soil with plant", "Soil"))

ec_m2 <- ggplot(m2_preds, aes(x = week, color = matrix, fill = matrix,
linetype = matrix)) +
  geom_hline(aes(yintercept = LOD$lod[LOD$microbe == "ecoli_plate"]),
             color = 'black', linetype = 3) +
  geom_ribbon(aes(ymin = conf.low, ymax = conf.high),
              alpha = 0.2,
              color = NA) +
  geom_line(aes(y = estimate), linewidth = 0.7) +
  geom_point(data = m2,
             aes(y = survival, shape = matrix),
             size = 1,
             position = position_jitter(width = 0.03)) +
  guides(
    color = guide_legend(title = 'Matrix:'),
    linetype = guide_legend(title = 'Matrix:'),
    shape = guide_legend(title = 'Matrix:')
  ) +
  labs(x = "",
       y = bquote(Log[10]~CFU~per~g)) +
  scale_fill_manual(values = c("#00529b", "#fb6502")) +
  scale_color_manual(values = c("#00529b", "#fb6502")) +
  scale_shape_manual(values = c(1, 2)) +
  scale_linetype_manual(values = c("longdash", "dotdash")) +
  scale_x_continuous(breaks = seq(0, 30, 3), labels = seq(0, 30, 3)) +
  scale_y_continuous(breaks = seq(0, 9, 1), labels = seq(0, 9, 1), limits =
c(0.5, 9)) +
  theme(text = element_text(family = "Arial"),
        legend.position="none",
        plot.title = element_text(color = "black", size = 7, face = "bold",
hjust = 0.5),
        axis.text.x = element_text(size = 7, color = "black", face = "bold",
hjust = 0.5, vjust = 0.5),
        axis.text.y = element_text(size = 7, color = "black", face = "bold"),
        axis.title.x = element_text(size = 7, vjust = -1),
        axis.title.y = element_text(size = 7, vjust = 2),
        panel.border = element_rect(color = "black", fill = NA, linewidth =
1),
        panel.grid.major = element_line(color = "gray", linewidth = 0.1,
linetype = "longdash"),
        panel.grid.minor = element_line(color = "lightgray", linewidth = 0.1,
linetype = "dotted"),
        panel.background = element_rect(fill = "transparent"),
        legend.background = element_rect(fill = "transparent", color =
"transparent"),
        plot.background = element_rect(fill = "transparent", color =
"transparent"))
```

## 3.3 *Tulane virus TCID50*

### 3.3.1 Fitting models

```
m2 <- readRDS(here('obj2', 'data','Obj2_dat.RDS')) |>
  dplyr::filter (microbe=='tv', assay=='tcid50') |>
  droplevels()

# Initial visual inspection
ggplot(m2, aes(x = week, y = survival)) +
  geom_point() +
  stat_smooth(method = "lm", formula = y ~ poly(x, 2)) +
  theme_minimal()
```



```
# Levels
contrasts(m2$matrix)
```

```
   s
ps 0
s  1
```

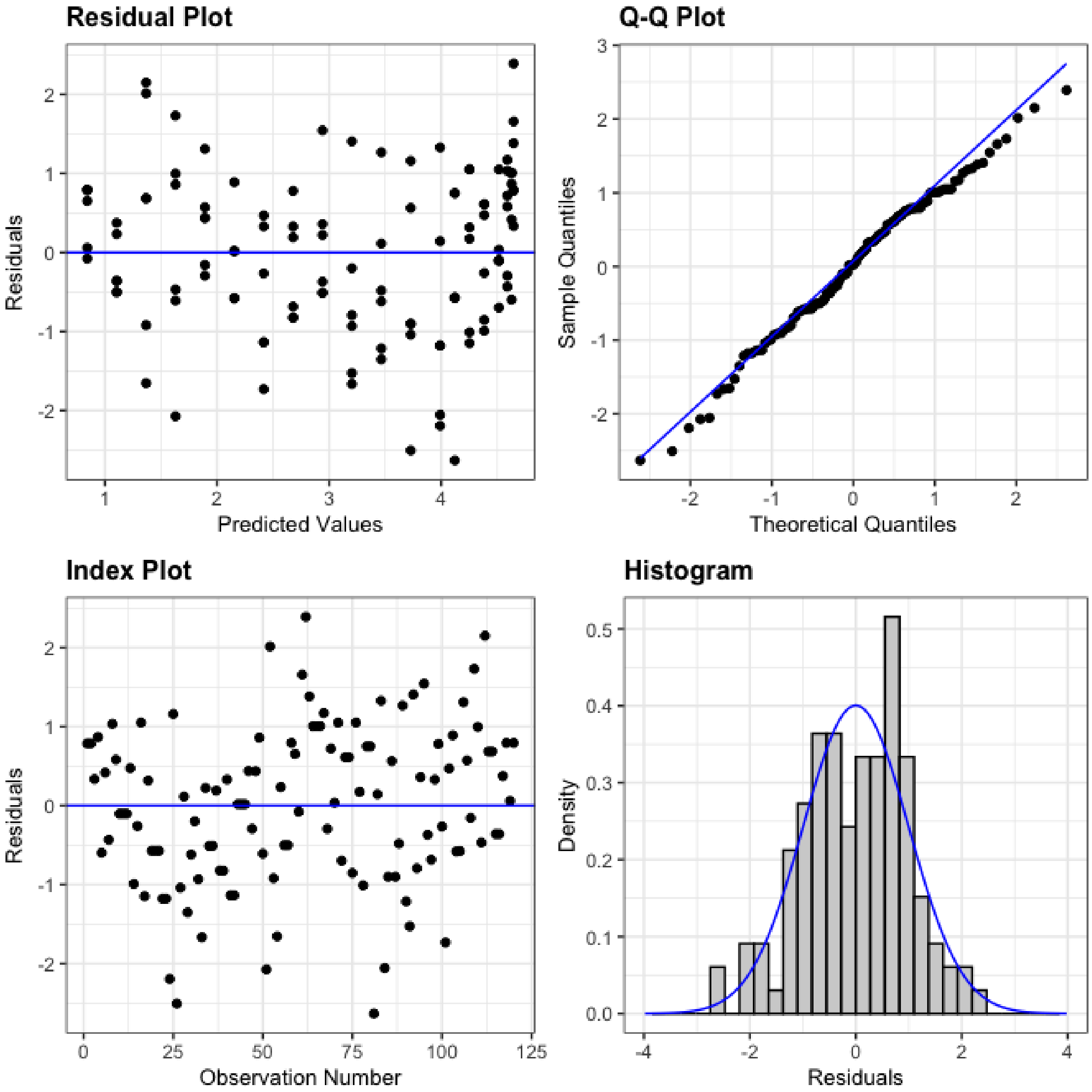
```
m2_mod_null = nlme::gnls(survival ~ ll(x = week,
                               log10n0 = log10n0,
                               alpha = alpha),
                 data = m2,
                 params = log10n0+alpha ~ 1,
                 start = list(log10n0 = 5,
                              alpha = 0.2))

m2_mod_ll = nlme::gnls(survival ~ ll(x = week,
                               log10n0 = log10n0,
                               alpha = alpha),
                 data = m2,
                 params = log10n0+alpha ~ matrix,
                 start = list(log10n0 = c(5, 0),
                              alpha = c(0.2, 0)))

m2_mod_llrepar = nlme::gnls(survival ~ llrepar(x = week,
                               log10n0 = log10n0,
                               TD = TD),
                 data = m2,
                 params = log10n0+TD ~ matrix,
                 start = list(log10n0 = c(5, 0),
                              TD = c(7, 0)))

m2_mod_wb = nlme::gnls(survival ~ wb(x = week,
                               log10n0 = log10n0,
                               alpha = alpha,
                               beta = beta),
                 data = m2,
                 params = log10n0+alpha+beta ~ matrix,
                 start = list(log10n0 = c(5, 0),
                              alpha = c(0.2, 0),
                              beta = c(0.2, 0)))

m2_mod_wbrepar = nlme::gnls(survival ~ wbrepar(x = week,
                               log10n0 = log10n0,
                               TD = TD,
                               beta = beta),
                 data = m2,
                 params = log10n0+TD+beta ~ matrix,
                 start = list(log10n0 = c(5, 0),
                              TD = c(5, 0),
                              beta = c(2, 0)))

m2_mod_lg = nlme::gnls(survival ~ lg(x = week,
                               log10n0 = log10n0,
                               kappa = kappa,
                               sigma = sigma),
                 data = m2,
                 params = log10n0+kappa+sigma ~ matrix,
                 start = list(log10n0 = c(5, 0),
                             kappa = c(1, 0),
                             sigma  = c(1, 0)))

m2_mod_lgpar = nlme::gnls(survival ~ lgrepar(x = week,
                               log10n0 = log10n0,
                               sigma = sigma,
                               TD = TD),
                 data = m2,
                 params = log10n0+sigma+TD ~ matrix,
                 start = list(log10n0 = c(5, 0),
                             sigma = c(1, 0),
                             TD   = c(6, 0)))
```

### 3.3.2 Model performance and inference

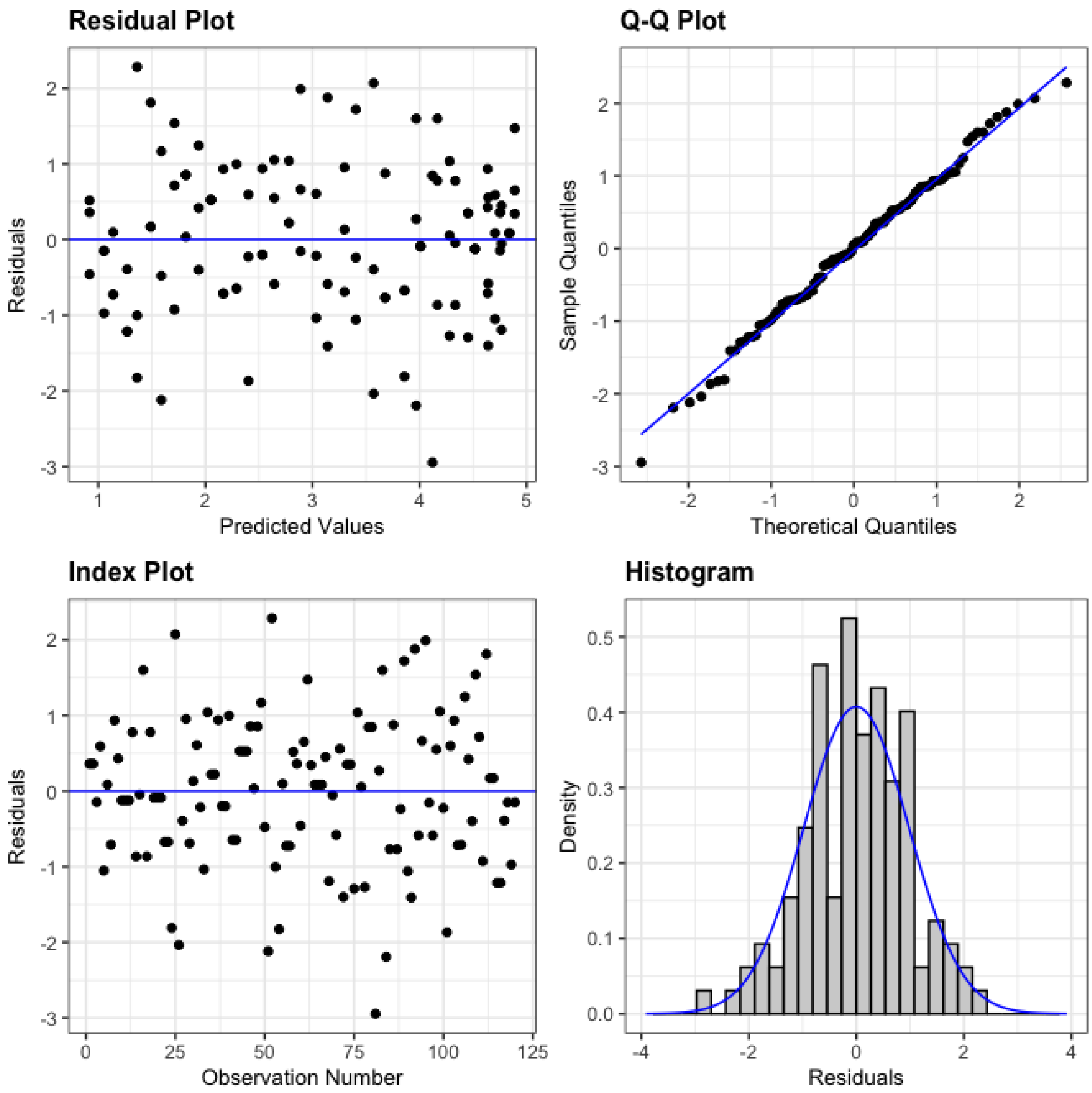
```
# Model comparisons
performance::compare_performance (
  m2_mod_null, m2_mod_ll, m2_mod_llrepar,
  m2_mod_lg, m2_mod_lgpar, m2_mod_wb, m2_mod_wbrepar,
  metrics = c('AICc'))
```

```
# Comparison of Model Performance Indices

Name           | Model | AIC (weights) | AICc (weights) | BIC (weights) |
R2
-----------------------------------------------------------------------------
--
m2_mod_null    |  gnls | -11.0 (<.001) |  -10.8 (<.001) |  -2.6 (0.001) |
0.969
m2_mod_ll      |  gnls | -15.6 (<.001) |  -15.1 (<.001) |  -1.7 (<.001) |
0.971
m2_mod_llrepar |  gnls | -15.6 (<.001) |  -15.1 (<.001) |  -1.7 (<.001) |
0.971
m2_mod_lg      |  gnls |  51.2 (<.001) |   52.2 (<.001) |  70.7 (<.001) |
0.951
m2_mod_lgpar   |  gnls |  51.2 (<.001) |   52.2 (<.001) |  70.7 (<.001) |
0.951
m2_mod_wb      |  gnls | -34.5 (0.500) |  -33.5 (0.500) | -15.0 (0.499) |
0.976
m2_mod_wbrepar |  gnls | -34.5 (0.500) |  -33.5 (0.500) | -15.0 (0.499) |
0.976

Name           |  RMSE | Sigma
------------------------------
m2_mod_null    | 0.225 | 0.227
m2_mod_ll      | 0.217 | 0.221
```

```
m2_mod_llrepar | 0.217 | 0.221
m2_mod_lg      | 0.283 | 0.290
m2_mod_lgpar   | 0.283 | 0.290
m2_mod_wb      | 0.198 | 0.203
m2_mod_wbrepar | 0.198 | 0.203

# Residual plots
m2_res_null = resid(m2_mod_null, type = 'normalized')
m2_fit_null = fitted(m2_mod_null)
resid_auxpanel(m2_res_null, m2_fit_null)
```



```
m2_res_wbrepar = resid(m2_mod_wbrepar, type = 'normalized')
m2_fit_wbrepar = fitted(m2_mod_wbrepar)
resid_auxpanel(m2_res_wbrepar, m2_fit_wbrepar)
```



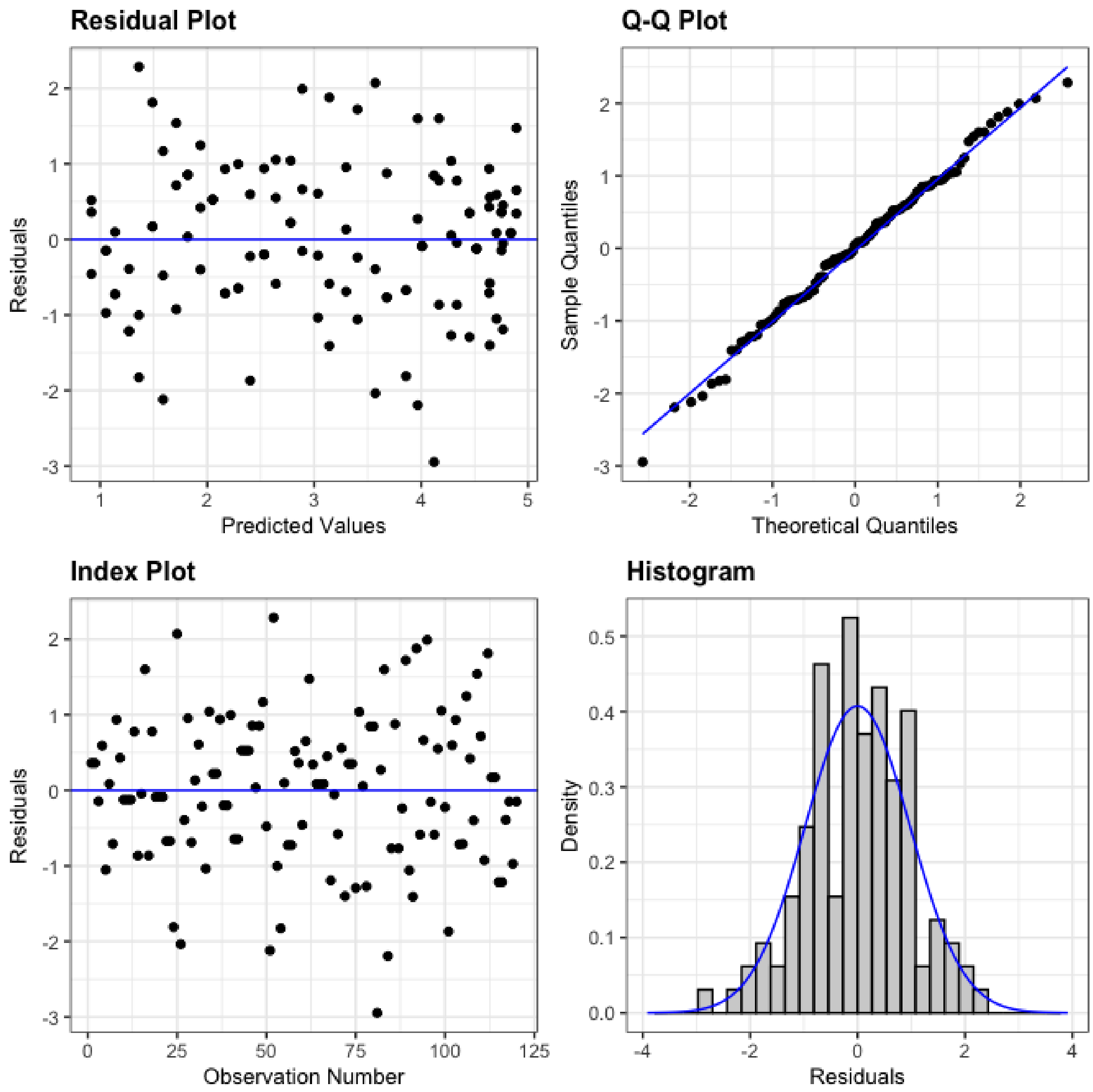
```
pres_mod <- m2_mod_wbrepar

# gnls model tuning
m2_mod_exp = update(pres_mod, weights = varExp())
m2_mod_exp_wk = update(pres_mod, weights = varExp(form = ~week))
m2_mod_idt_mtx = update(pres_mod, weights = varIdent(form = ~1 | matrix))
#m2_mod_idt_mic = update(pres_mod, weights = varIdent(form = ~1 | microbe))
m2_mod_pow = update(pres_mod, weights = varPower())

#m2_mod_combined1 = update(pres_mod, weights = varComb(varIdent(form = ~1 |
microbe), varExp()))
#m2_mod_combined2 = update(pres_mod, weights = varComb(varIdent(form = ~1 |
microbe), varExp(form = ~ week)))
m2_mod_combined3 = update(pres_mod, weights = varComb(varIdent(form = ~1 |
matrix), varExp()))
m2_mod_combined4 = update(pres_mod, weights = varComb(varIdent(form = ~1 |
matrix), varExp(form = ~ week)))

compare_performance(
  pres_mod,
  m2_mod_exp,
  m2_mod_exp_wk,
  m2_mod_idt_mtx,
  #m2_mod_idt_mic,
  m2_mod_pow,
  #m2_mod_combined1,
  #m2_mod_combined2,
  m2_mod_combined3,
  m2_mod_combined4,
  metrics = c('AICc'))

# Comparison of Model Performance Indices

Name              | Model | AIC (weights) | AICc (weights) | BIC (weights)
--------------------------------------------------------------------------
pres_mod          |  gnls | -34.5 (0.264) |  -33.5 (0.304) | -15.0 (0.647)
m2_mod_exp        |  gnls | -32.8 (0.113) |  -31.5 (0.112) | -10.5 (0.069)
m2_mod_exp_wk     |  gnls | -32.6 (0.107) |  -31.4 (0.106) | -10.3 (0.065)
m2_mod_idt_mtx    |  gnls | -34.0 (0.211) |  -32.7 (0.209) | -11.7 (0.128)
m2_mod_pow        |  gnls | -32.5 (0.100) |  -31.2 (0.099) | -10.2 (0.061)
m2_mod_combined3 |  gnls | -32.7 (0.109) |  -31.1 (0.091) |  -7.6 (0.016)
m2_mod_combined4 |  gnls | -32.4 (0.095) |  -30.8 (0.080) |  -7.3 (0.014)

Name              |    R2 |  RMSE | Sigma
-----------------------------------------
pres_mod          | 0.976 | 0.198 | 0.203
m2_mod_exp        | 0.976 | 0.198 | 0.224
m2_mod_exp_wk     | 0.976 | 0.198 | 0.195
m2_mod_idt_mtx    | 0.976 | 0.198 | 0.186
m2_mod_pow        | 0.976 | 0.198 | 0.211
m2_mod_combined3 | 0.976 | 0.198 | 0.214
m2_mod_combined4 | 0.976 | 0.198 | 0.174

(m2_mod <- pres_mod)

Generalized nonlinear least squares fit
  Model: survival ~ wbrepar(x = week, log10n0 = log10n0, TD = TD, beta =
beta)
  Data: m2
  Log-likelihood: 24.2

Coefficients:
log10n0.(Intercept)     log10n0.matrixs      TD.(Intercept)
TD.matrixs
             4.7517              0.1390              5.7305             -
0.2105
```

```
   beta.(Intercept)        beta.matrixs 
             0.8288             -0.0182 

Degrees of freedom: 120 total; 114 residual
Residual standard error: 0.203 
```

```
summary (m2_mod)
```

```
Generalized nonlinear least squares fit
  Model: survival ~ wbrepar(x = week, log10n0 = log10n0, TD = TD, beta = 
beta) 
  Data: m2 
    AIC BIC logLik
  -34.5 -15   24.2

Coefficients:
                    Value Std.Error t-value p-value
log10n0.(Intercept)  4.75     0.067    70.5   0.000
log10n0.matrixs      0.14     0.096     1.4   0.150
TD.(Intercept)       5.73     0.560    10.2   0.000
TD.matrixs          -0.21     0.788    -0.3   0.790
beta.(Intercept)     0.83     0.046    17.8   0.000
beta.matrixs        -0.02     0.065    -0.3   0.781

 Correlation: 
                 l100.( lg100. TD.(I) TD.mtr bt.(I)
log10n0.matrixs  -0.702                            
TD.(Intercept)   -0.869  0.610                     
TD.matrixs        0.617 -0.870 -0.710              
beta.(Intercept) -0.761  0.534  0.966 -0.686       
beta.matrixs      0.543 -0.763 -0.690  0.967 -0.714

Standardized residuals:
    Min      Q1     Med      Q3     Max 
-2.9461 -0.6764  0.0461  0.6183  2.2847 

Residual standard error: 0.203 
Degrees of freedom: 120 total; 114 residual
```

```
# Residual plots
m2_res_m2_mod = resid(m2_mod, type = 'normalized')
m2_fit_m2_mod = fitted(m2_mod)
resid_auxpanel(m2_res_m2_mod, m2_fit_m2_mod)
```



```
saveRDS(m2_mod, here('obj2', 'results', 'tv_tcid50_mod_sum.RDS'))

# Marginal means (prediction)
m2_mod <- readRDS(here('obj2', 'results', 'tv_tcid50_mod_sum.RDS'))

expand.grid(
  matrix = levels(m2$matrix),
  microbe = levels(m2$microbe),
  assay = levels(m2$assay),
  week = seq(0, max(m2$week), 0.5)) |>
  marginaleffects::avg_predictions(
  model=m2_mod,
  conf_level = 0.95,
  by = c('matrix', 'week')) |>
  saveRDS(here('obj2', 'results', 'tv_tcid50_mod_preds.RDS'))
```

```
# Extract parameters (coefficients) and their 95% CI
m2_mod_coef = coef(m2_mod)
m2_mod_confint = confint(m2_mod)

as.data.frame (cbind(
  Param = names (m2_mod_coef),
  Est = round (m2_mod_coef, 3),
  lwr = round (m2_mod_confint[,1], 3),
  upr = round (m2_mod_confint[,2], 3)
  ))
```

```
                                  Param    Est    lwr   upr
log10n0.(Intercept) log10n0.(Intercept)  4.752   4.62 4.884
log10n0.matrixs         log10n0.matrixs  0.139 -0.049 0.327
TD.(Intercept)           TD.(Intercept)  5.731  4.633 6.828
TD.matrixs                   TD.matrixs  -0.21 -1.756 1.335
beta.(Intercept)       beta.(Intercept)  0.829  0.738  0.92
beta.matrixs               beta.matrixs -0.018 -0.146 0.109
```

```
# Joint tests
list(
  log10n0 = joint_tests (m2_mod, param = 'log10n0'),
  TD = joint_tests (m2_mod, param = 'TD'),
  beta = joint_tests (m2_mod, param = 'beta'))
```

```
$log10n0
 model term df1 df2 F.ratio p.value
 matrix        1 113   2.096  0.1504



$TD
 model term df1 df2 F.ratio p.value
 matrix        1 113   0.071  0.7900



$beta
 model term df1 df2 F.ratio p.value
 matrix        1 113   0.078  0.7810
```

```
# Estimated marginal (group level) means and contrasts
list(
  log10n0 = emmeans (m2_mod, pairwise ~ matrix, param = 'log10n0'),
  TD = emmeans (m2_mod, pairwise ~ matrix, param = 'TD'),
  beta = emmeans (m2_mod, pairwise ~ matrix, param = 'beta'))
```

```
$log10n0
$emmeans
 matrix emmean     SE  df lower.CL upper.CL
 ps       4.75 0.0674 113     4.62     4.89
 s        4.89 0.0684 113     4.76     5.03
```

```
Confidence level used: 0.95

$contrasts
 contrast estimate    SE  df t.ratio p.value
 ps - s     -0.139 0.096 113  -1.448  0.1504



$TD
$emmeans
 matrix emmean    SE  df lower.CL upper.CL
 ps       5.73 0.560 113     4.62     6.84
 s        5.52 0.555 113     4.42     6.62

Confidence level used: 0.95

$contrasts
 contrast estimate    SE  df t.ratio p.value
 ps - s       0.21 0.788 113   0.267  0.7900



$beta
$emmeans
 matrix emmean     SE  df lower.CL upper.CL
 ps      0.829 0.0465 113    0.737    0.921
 s       0.811 0.0456 113    0.720    0.901

Confidence level used: 0.95

$contrasts
 contrast estimate     SE  df t.ratio p.value
 ps - s     0.0182 0.0651 113   0.279  0.7810
```

```
# Overall marginal means
emmeans (m2_mod, ~ 1, param = 'log10n0')
```

```
 1       emmean    SE  df lower.CL upper.CL
 overall   4.82 0.048 113     4.73     4.92

Results are averaged over the levels of: matrix
Confidence level used: 0.95
```

```
emmeans (m2_mod, ~ 1, param = 'TD')
```

```
 1       emmean    SE  df lower.CL upper.CL
 overall   5.63 0.394 113     4.84     6.41
```

```
Results are averaged over the levels of: matrix 
Confidence level used: 0.95 

emmeans (m2_mod, ~ 1, param = 'beta')

 1       emmean     SE  df lower.CL upper.CL
 overall   0.82 0.0326 113    0.755    0.884

Results are averaged over the levels of: matrix 
Confidence level used: 0.95 

TD_values <- list (
  ecoli_plate_1D = readRDS (here('obj2', 'results', 'TD_values.RDS')),
  tv_tcid50_1D = emmeans (m2_mod, ~ 1, param = 'TD')
)

saveRDS (TD_values, here('obj2', 'results', "TD_values.RDS"))
```

### 3.3.3 Assess covariance/collinearity

```
set.seed(SEED)

## Variance-covariance matrix
m2_mod <- readRDS (here('obj2', 'results', 'tv_tcid50_mod_sum.RDS'))
m2_mod_coef <- coef (m2_mod)

(mod_vcov_joint <- vcov(m2_mod))

                    log10n0.(Intercept) log10n0.matrixs TD.(Intercept)
log10n0.(Intercept)             0.00455        -0.00455        -0.0328
log10n0.matrixs                -0.00455         0.00922         0.0328
TD.(Intercept)                 -0.03280         0.03280         0.3133
TD.matrixs                      0.03280        -0.06589        -0.3133
beta.(Intercept)               -0.00239         0.00239         0.0251
beta.matrixs                    0.00239        -0.00477        -0.0251
                    TD.matrixs beta.(Intercept) beta.matrixs
log10n0.(Intercept)     0.0328         -0.00239      0.00239
log10n0.matrixs        -0.0659          0.00239     -0.00477
TD.(Intercept)         -0.3133          0.02515     -0.02515
TD.matrixs              0.6217         -0.02515      0.04964
beta.(Intercept)       -0.0251          0.00216     -0.00216
beta.matrixs            0.0496         -0.00216      0.00424

mod_vcov_indep <- diag(diag(mod_vcov_joint))
dimnames(mod_vcov_indep) <- dimnames(mod_vcov_joint)

## Draw of coefficients
iter = 5000
draw_joint <- MASS::mvrnorm(n=iter, mu = m2_mod_coef, Sigma = mod_vcov_joint)
|>
  as.data.frame()
```

```
## Assuming no covariance
draw_indep <- MASS::mvrnorm(n=iter, mu = m2_mod_coef, Sigma = mod_vcov_indep)
|>
  as.data.frame()

## Extract TD values
TD_joint <- cbind (
  ps_joint = draw_joint[, "TD.(Intercept)"],
  s_joint = draw_joint[, "TD.(Intercept)"] + draw_joint[, "TD.matrixs"]
)

colMeans(TD_joint <= 0)
```

```
ps_joint  s_joint 
       0        0 
```

```
TD_indep <- cbind (
  ps_indep = draw_indep[, "TD.(Intercept)"],
  s_indep = draw_indep[, "TD.(Intercept)"] + draw_indep[, "TD.matrixs"]
)

colMeans(TD_indep <= 0)
```

```
ps_indep  s_indep 
       0        0 
```

```
## Calculate mean and 95% QIs
QIs <- function(x) c(
  mean=mean(x),
  lwr=quantile(x, 0.025),
  upr=quantile(x, 0.975))

round(t(apply(TD_joint, 2, QIs)),2)
```

```
         mean lwr.2.5% upr.97.5%
ps_joint 5.72     4.62      6.82
s_joint  5.52     4.41      6.58
```

```
round(t(apply(TD_indep, 2, QIs)),2)
```

```
         mean lwr.2.5% upr.97.5%
ps_indep 5.74     4.68      6.81
s_indep  5.53     3.67      7.39
```

```
saveRDS(mod_vcov_joint, here('obj2', 'results', 'tv_tcid50_sim_mvrnorm.RDS'))

## Again compare with emmeans
emmeans (m2_mod, pairwise ~ matrix, param = 'TD')$emmeans
```

```
 matrix emmean    SE  df lower.CL upper.CL
 ps       5.73 0.560 113     4.62     6.84
 s        5.52 0.555 113     4.42     6.62

Confidence level used: 0.95
```

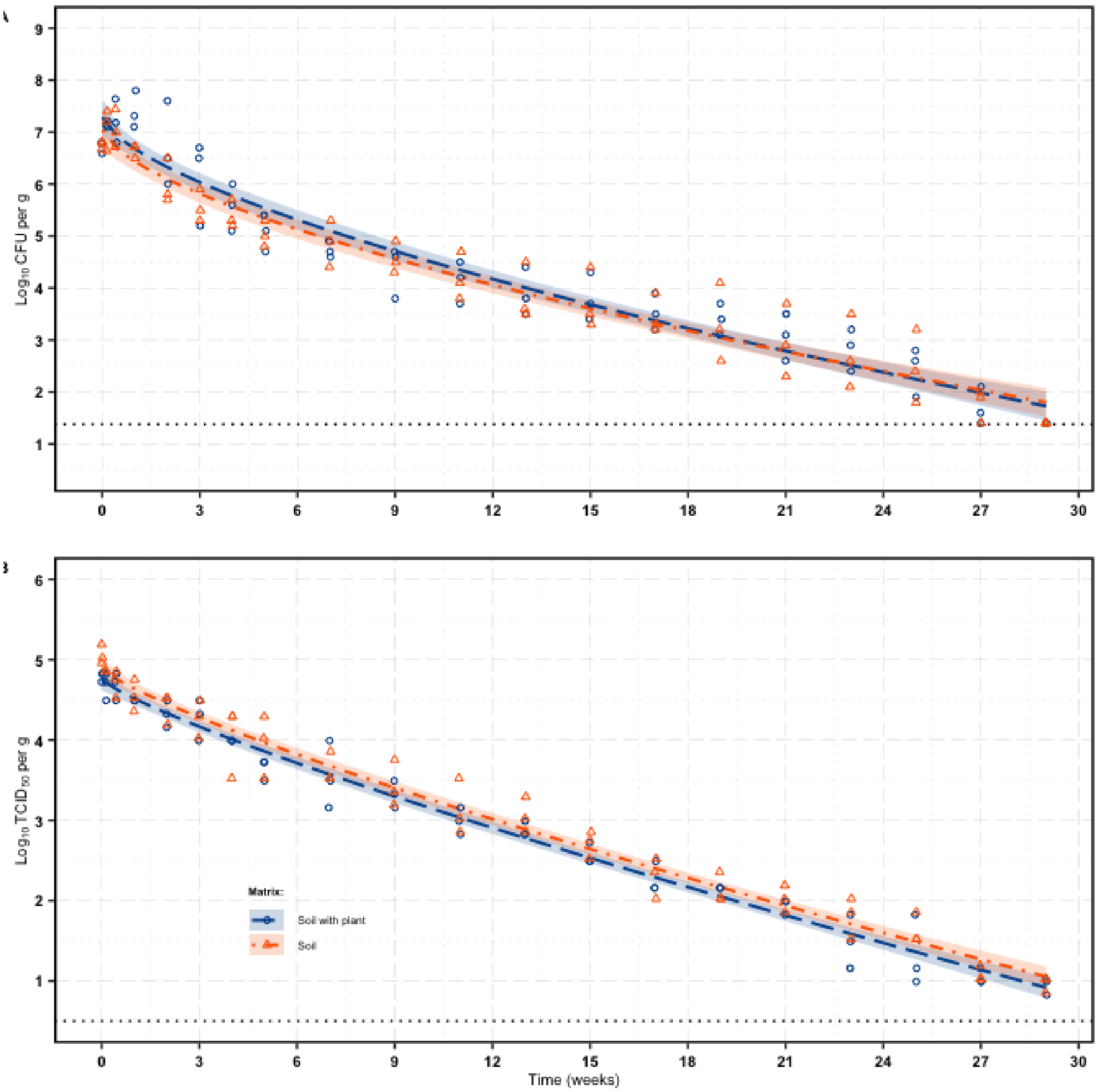
```
rm(list = setdiff(ls(envir = .GlobalEnv, all.names = TRUE), list_exc),
   envir = .GlobalEnv)
```

### 3.3.4 Plot microbial inactivation

```
m2 <- readRDS(here('obj2', 'data', 'Obj2_dat.RDS')) |>
  dplyr::filter (microbe=='tv', assay=='tcid50') |>
  dplyr::mutate(matrix = factor(matrix,
                                labels = c('Soil with plant', 'Soil'))) |>
  droplevels()

m2_preds = readRDS(here('obj2', 'results', 'tv_tcid50_mod_preds.RDS'))

m2_preds$matrix <- factor(m2_preds$matrix, levels = c("ps", "s"),
                          labels = c("Soil with plant", "Soil"))

microb_labs = c(tv = 'Tulane virus')

tv_m2 <- ggplot(m2_preds, aes(x = week, color = matrix, fill = matrix,
linetype = matrix)) +
  geom_hline(aes(yintercept = LOD$lod[LOD$microbe == "tv_tcid50"]),
             color = 'black', linetype = 3) +
  geom_ribbon(aes(ymin = conf.low, ymax = conf.high,
                  group = matrix),
              alpha = 0.2,
              color = NA) +
  geom_line(aes(y = estimate), linewidth = 0.7) +
  geom_point(data = m2,
             aes(y = survival, shape = matrix),
             size = 1,
             position = position_jitter(width = 0.03)) +
  guides(
    fill = guide_legend(title = 'Matrix:'),
    color = guide_legend(title = 'Matrix:'),
    linetype = guide_legend(title = 'Matrix:'),
    shape = guide_legend(title = 'Matrix:')) +
  labs(x = "Time (weeks)",
       y = bquote(Log[10]~TCID[50]~per~g)) +
  scale_fill_manual(values = c("#00529b", "#fb6502")) +
  scale_color_manual(values = c("#00529b", "#fb6502")) +
  scale_shape_manual(values = c(1, 2)) +
  scale_linetype_manual(values = c("longdash", "dotdash")) +
  scale_x_continuous(breaks = seq(0, 30, 3), labels = seq(0, 30, 3)) +
  scale_y_continuous(breaks = seq(0, 6, 1), labels = seq(0, 6, 1), limits =
c(0.5, 6)) +
  theme(text = element_text(family = "Arial"),
        legend.position = 'inside', legend.position.inside = c(0.25, 0.25),
        legend.direction = "vertical",
        legend.text = element_text(size = 5),
        legend.title = element_text(size = 5, face = "bold"),
        legend.key.height = unit(0.4, "cm"),
        plot.title = element_text(color = "black", size = 7, face = "bold",
hjust = 0.5),
        axis.text.x = element_text(size = 7, color = "black", face = "bold",
hjust = 0.5, vjust = 0.5),
        panel.border = element_rect(color = "black", fill = NA, linewidth =
1),
        axis.text.y = element_text(size = 7, color = "black", face = "bold"),
        axis.title.x = element_text(size = 7, vjust = -1),
        axis.title.y = element_text(size = 7, vjust = 2),
        panel.grid.major = element_line(color = "gray", linewidth = 0.1,
linetype = "longdash"),
        panel.grid.minor = element_line(color = "lightgray", linewidth = 0.1,
linetype = "dotted"),
        panel.background = element_rect(fill = "transparent"),
        legend.background = element_rect(fill = "transparent", color =
"transparent"),
        plot.background = element_rect(fill = "transparent", color =
"transparent"))
```

### 3.4 Combined *E. coli* and Tulane virus plot

```
combined_plots <- ggpubr::ggarrange (ec_m2,  tv_m2,
                                     labels = c("A", "B"),
                                     font.label = list(size = 7, face =
"bold"),
                                     label.x = -0.01,
                                     label.y = 0.98,
                                     nrow=2, ncol=1)

combined_plots
```

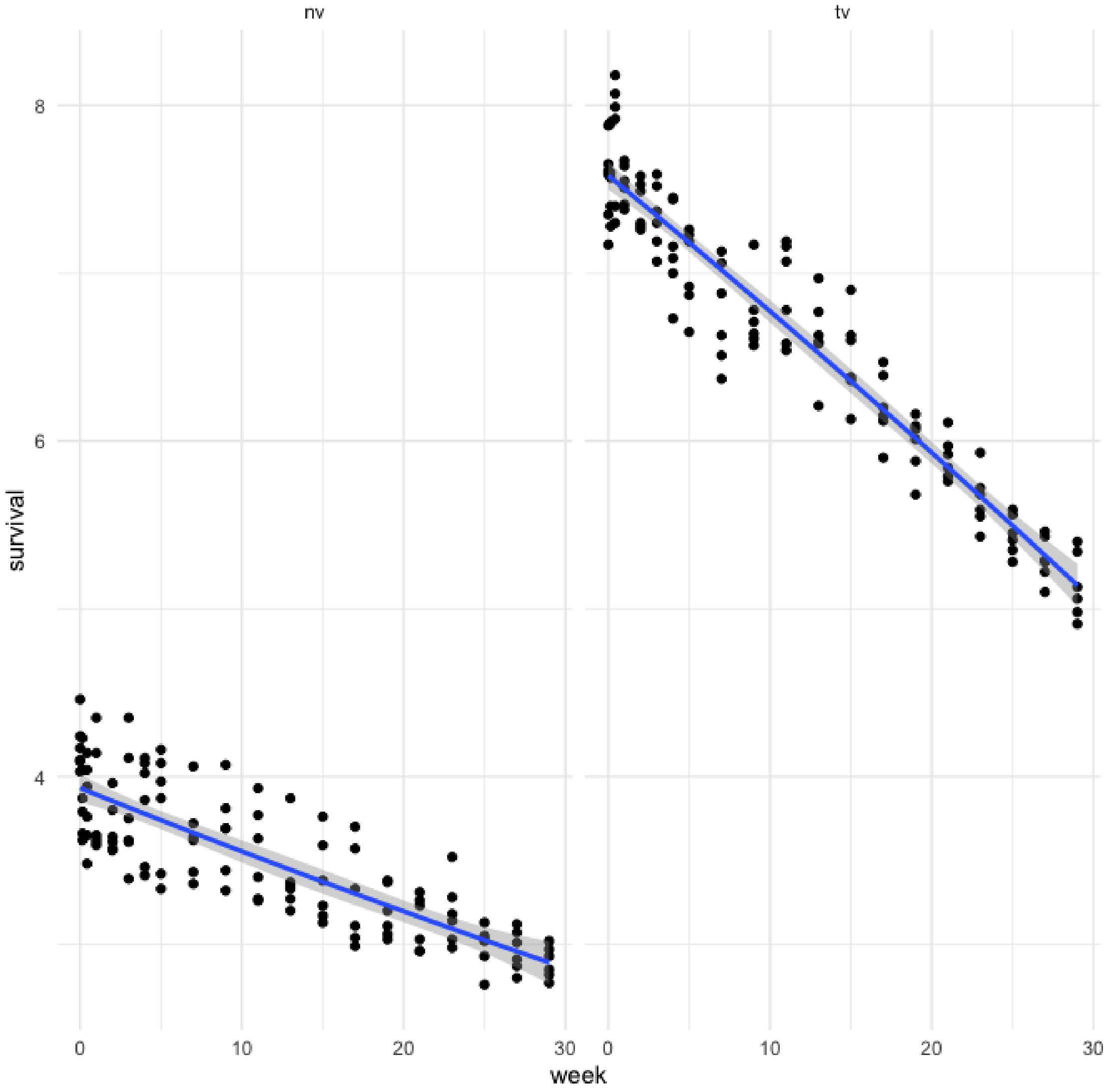


```
ggsave(
  filename = here('obj2', 'results', 'Fig 1 ec_tv_plots.tiff'),
  plot = combined_plots,
  width = 6,
  height = 9,
  units = "cm",
  dpi = 600
)

# Remove selected objects from the environment
rm(list = setdiff(ls(envir = .GlobalEnv, all.names = TRUE), list_exc),
   envir = .GlobalEnv)
```

## 3.5 TuV and HuNoV - *RNase RT-qPCR*

### 3.5.1 Fitting models

```
m2 <- readRDS(here('obj2', 'data', 'Obj2_dat.RDS')) |>
  dplyr::filter (assay=='pcr') |>
  droplevels()

# Initial visual inspection
ggplot(m2, aes(x = week, y = survival)) +
  facet_wrap(~ microbe) +
  geom_point() +
    stat_smooth(method = "lm", formula = y ~ poly(x, 2)) +
    theme_minimal()
```



```
# Levels and combinations
contrasts(m2$matrix)
```

```
   s
ps 0
s  1
```

```
contrasts(m2$microbe)
```

```
   tv
nv  0
tv  1
```

```
levels(interaction(m2$matrix, m2$microbe))
```

```
[1] "ps.nv" "s.nv"  "ps.tv" "s.tv"
```

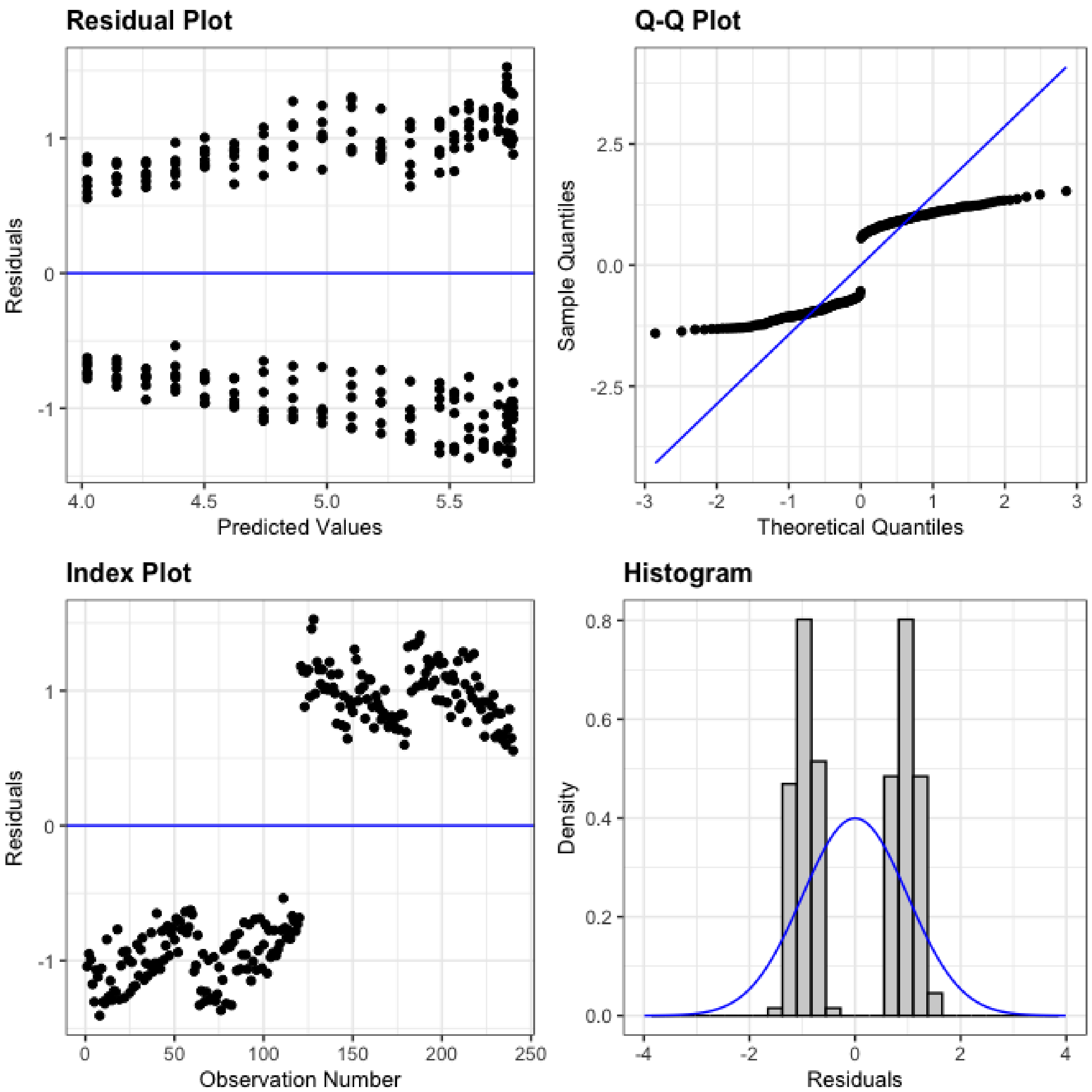
```
m2_mod_null = nlme::gnls(survival ~ ll(x = week,
                                       log10n0 = log10n0,
                                       alpha = alpha),
                         data = m2,
                         params = log10n0+alpha ~ 1,
                         start = list(log10n0 = 4,
                                      alpha = 0.1))


m2_mod_ll = nlme::gnls(survival ~ ll(x = week,
                               log10n0 = log10n0,
                               alpha = alpha),
                 data = m2,
                 params = log10n0+alpha ~ matrix*microbe,
                 start = list(log10n0 = c(4, rep(0,3)),
                              alpha = c(0.1, rep(0,3))))


m2_mod_llrepar = nlme::gnls(survival ~ llrepar(x = week,
                                               log10n0 = log10n0,
                                               TD = TD),
                            data = m2,
                            params = log10n0+TD ~ matrix*microbe,
                 start = list(log10n0 = c(4, rep(0,3)),
                              TD = c(25, rep(0,3))))


m2_mod_wb = nlme::gnls(survival ~ wb(x = week,
                               log10n0 = log10n0,
                               alpha = alpha,
                               beta = beta),
                 data = m2,
                 params = log10n0+alpha+beta ~ matrix*microbe,
                 start = list(log10n0 = c(4, rep(0,3)),
                              alpha = c(4, rep(0,3)),
                              beta = c(1, rep(0,3))))


m2_mod_wbrepar = nlme::gnls(survival ~ wbrepar(x = week,
                               log10n0 = log10n0,
                               TD = TD,
                               beta = beta),
                 data = m2,
                 params = log10n0+TD+beta ~ matrix*microbe,
                 start = list(log10n0 = c(4, rep(0,3)),
                              TD = c(1, rep(0,3)),
                              beta = c(1, rep(0,3))))

m2_mod_lg = nlme::gnls(survival ~ lg(x = week,
                               log10n0 = log10n0,
                               kappa = kappa,
                               sigma = sigma),
                 data = m2,
                 params = log10n0+kappa+sigma ~ matrix*microbe,
                 start = list(log10n0 = c(4,rep(0,3)),
                             kappa = c(2, rep(0,3)),
                             sigma  = c(1, rep(0,3))))

m2_mod_lgpar = nlme::gnls(survival ~ lgrepar(x = week,
                                log10n0 = log10n0,
                                sigma = sigma,
                                TD = TD),
             data = m2,
             params = log10n0+sigma+TD ~ matrix*microbe,
             start = list(log10n0 = c(4, rep(0,3)),
                          sigma =   c(1, rep(0,3)),
                          TD =      c(10, rep(0,3))))
```

### 3.5.2 Model performance and inferences

```
# Model comparisons
performance::compare_performance (
  m2_mod_null, m2_mod_ll, m2_mod_llrepar,
  m2_mod_lg, m2_mod_lgpar, m2_mod_wb, m2_mod_wbrepar,
  metrics = c('AICc'))

# Comparison of Model Performance Indices

Name           | Model | AIC (weights) | AICc (weights) | BIC (weights) |
R2
-----------------------------------------------------------------------------
--
m2_mod_null    |  gnls | 911.1 (<.001) |  911.2 (<.001) | 921.6 (<.001) |
0.115
m2_mod_ll      |  gnls |  -0.3 (0.461) |    0.4 (0.474) |  31.0 (0.500) |
0.981
m2_mod_llrepar |  gnls |  -0.3 (0.461) |    0.4 (0.474) |  31.0 (0.500) |
0.981
m2_mod_lg      |  gnls |  46.3 (<.001) |   47.9 (<.001) |  91.5 (<.001) |
0.978
m2_mod_lgpar   |  gnls |  46.3 (<.001) |   47.9 (<.001) |  91.5 (<.001) |
0.978
```

```
m2_mod_wb       |  gnls |   4.6 (0.039) |    6.2 (0.026) |  49.9 (<.001) |
0.981
m2_mod_wbrepar |  gnls |   4.6 (0.039) |    6.2 (0.026) |  49.9 (<.001) |
0.981

Name           |  RMSE | Sigma
------------------------------
m2_mod_null    | 1.595 | 1.601
m2_mod_ll      | 0.233 | 0.237
m2_mod_llrepar | 0.233 | 0.237
m2_mod_lg      | 0.252 | 0.259
m2_mod_lgpar   | 0.252 | 0.259
m2_mod_wb      | 0.231 | 0.237
m2_mod_wbrepar | 0.231 | 0.237
```

```
# Residual plots
m2_res_null = resid(m2_mod_null, type = 'normalized')
m2_fit_null = fitted(m2_mod_null)
resid_auxpanel(m2_res_null, m2_fit_null)
```



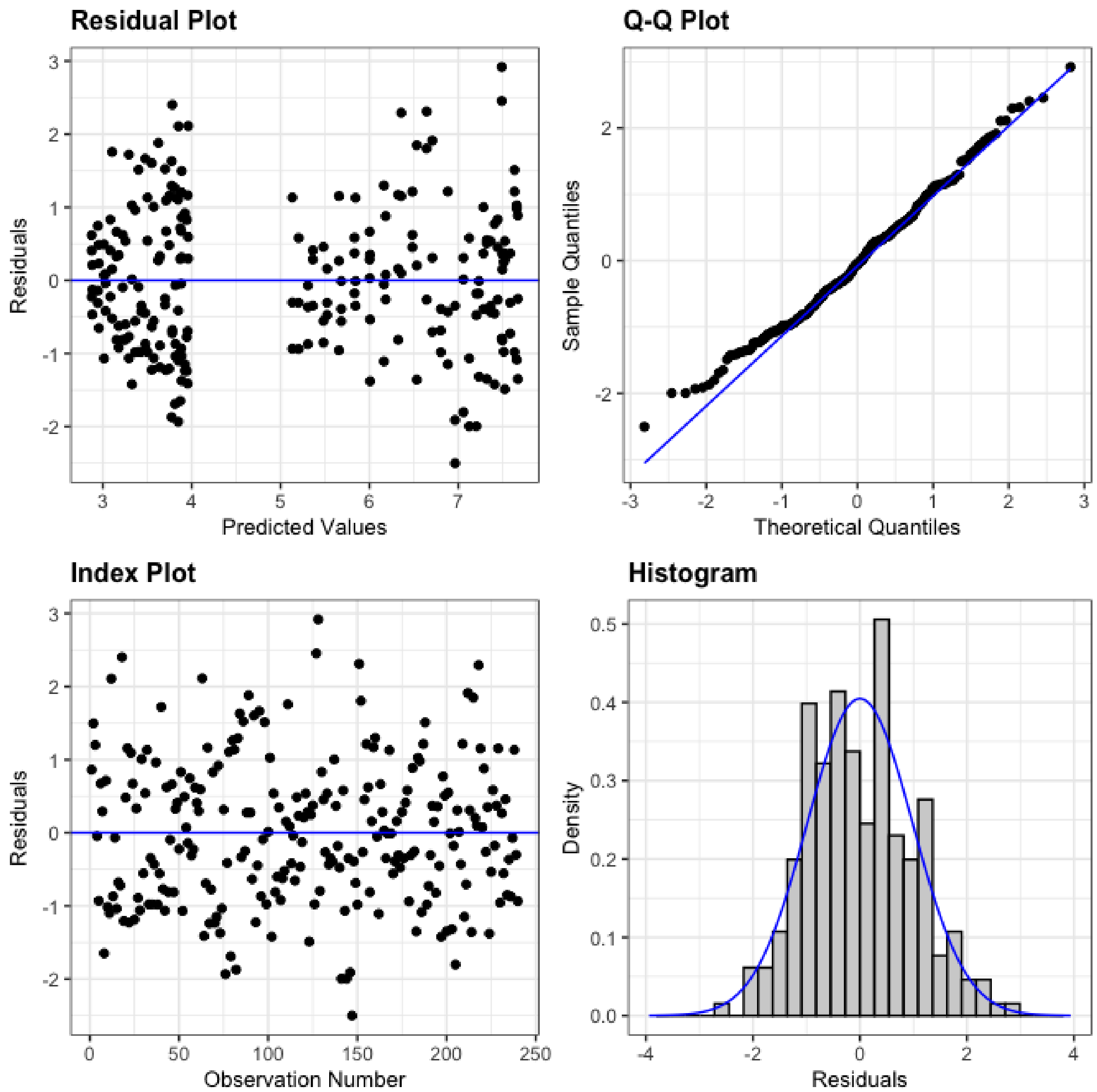
Residual Plot

Q-Q Plot

Index Plot

Histogram

```
m2_res_llrepar = resid(m2_mod_llrepar, type = 'normalized')
m2_fit_llrepar = fitted(m2_mod_llrepar)
resid_auxpanel(m2_res_llrepar, m2_fit_llrepar)
```



```
(pres_mod <- m2_mod_llrepar)

Generalized nonlinear least squares fit
  Model: survival ~ llrepar(x = week, log10n0 = log10n0, TD = TD)
  Data: m2
  Log-likelihood: 9.17

Coefficients:
      log10n0.(Intercept)           log10n0.matrixs         log10n0.microbetv
                   3.8856                    0.0740                    3.6372
log10n0.matrixs:microbetv            TD.(Intercept)                TD.matrixs
                   0.0731                   28.6548                   -1.7829
             TD.microbetv      TD.matrixs:microbetv
                 -16.1565                    0.7094

Degrees of freedom: 240 total; 232 residual
Residual standard error: 0.237
```

```
# gnls model tuning
m2_mod_exp = update(pres_mod, weights = varExp())
m2_mod_exp_wk = update(pres_mod, weights = varExp(form = ~week))
m2_mod_idt_mtx = update(pres_mod, weights = varIdent(form = ~1 | matrix))
m2_mod_idt_mic = update(pres_mod, weights = varIdent(form = ~1 | microbe))
m2_mod_pow = update(pres_mod, weights = varPower())

m2_mod_combined1 = update(pres_mod, weights = varComb(varIdent(form = ~1 |
microbe), varExp()))
m2_mod_combined2 = update(pres_mod, weights = varComb(varIdent(form = ~1 |
microbe), varExp(form = ~ week)))
m2_mod_combined3 = update(pres_mod, weights = varComb(varIdent(form = ~1 |
matrix), varExp()))
m2_mod_combined4 = update(pres_mod, weights = varComb(varIdent(form = ~1 |
matrix), varExp(form = ~ week)))

compare_performance(
  pres_mod,
  m2_mod_exp,
  m2_mod_exp_wk,
  m2_mod_idt_mtx,
  m2_mod_idt_mic,
  m2_mod_pow,
  m2_mod_combined1,
  m2_mod_combined2,
  m2_mod_combined3,
  m2_mod_combined4,
  metrics = c('AICc'))
```

```
# Comparison of Model Performance Indices

Name             | Model |   AIC (weights) | AICc (weights) | BIC (weights)
---------------------------------------------------------------------------
pres_mod         |  gnls |     -0.3 (<.001) |    0.4 (<.001) |  31.0 (<.001)
m2_mod_exp       |  gnls |      0.3 (<.001) |    1.2 (<.001) |  35.1 (<.001)
m2_mod_exp_wk    |  gnls |    -20.8 (0.545) |  -19.8 (0.569) |  14.0 (0.872)
m2_mod_idt_mtx   |  gnls |      1.7 (<.001) |    2.6 (<.001) |  36.5 (<.001)
m2_mod_idt_mic   |  gnls |      1.7 (<.001) |    2.6 (<.001) |  36.5 (<.001)
m2_mod_pow       |  gnls | 5.6e-03 (<.001) |    1.0 (<.001) |  34.8 (<.001)
m2_mod_combined1 |  gnls |    -12.7 (0.010) |  -11.5 (0.009) |  25.6 (0.003)
m2_mod_combined2 |  gnls |    -18.8 (0.206) |  -17.7 (0.195) |  19.5 (0.058)
m2_mod_combined3 |  gnls |      2.2 (<.001) |    3.4 (<.001) |  40.5 (<.001)
m2_mod_combined4 |  gnls |    -19.1 (0.240) |  -18.0 (0.227) |  19.2 (0.067)

Name             |    R2 |  RMSE | Sigma
----------------------------------------
pres_mod         | 0.981 | 0.233 | 0.237
m2_mod_exp       | 0.981 | 0.233 | 0.200
m2_mod_exp_wk    | 0.981 | 0.233 | 0.305
m2_mod_idt_mtx   | 0.981 | 0.233 | 0.236
```

```
m2_mod_idt_mic    | 0.981 | 0.233 | 0.238
m2_mod_pow        | 0.981 | 0.233 | 0.178
m2_mod_combined1 | 0.981 | 0.233 | 0.070
m2_mod_combined2 | 0.981 | 0.233 | 0.302
m2_mod_combined3 | 0.981 | 0.233 | 0.197
m2_mod_combined4 | 0.981 | 0.233 | 0.298
```

```
(m2_mod <- m2_mod_exp_wk)
```

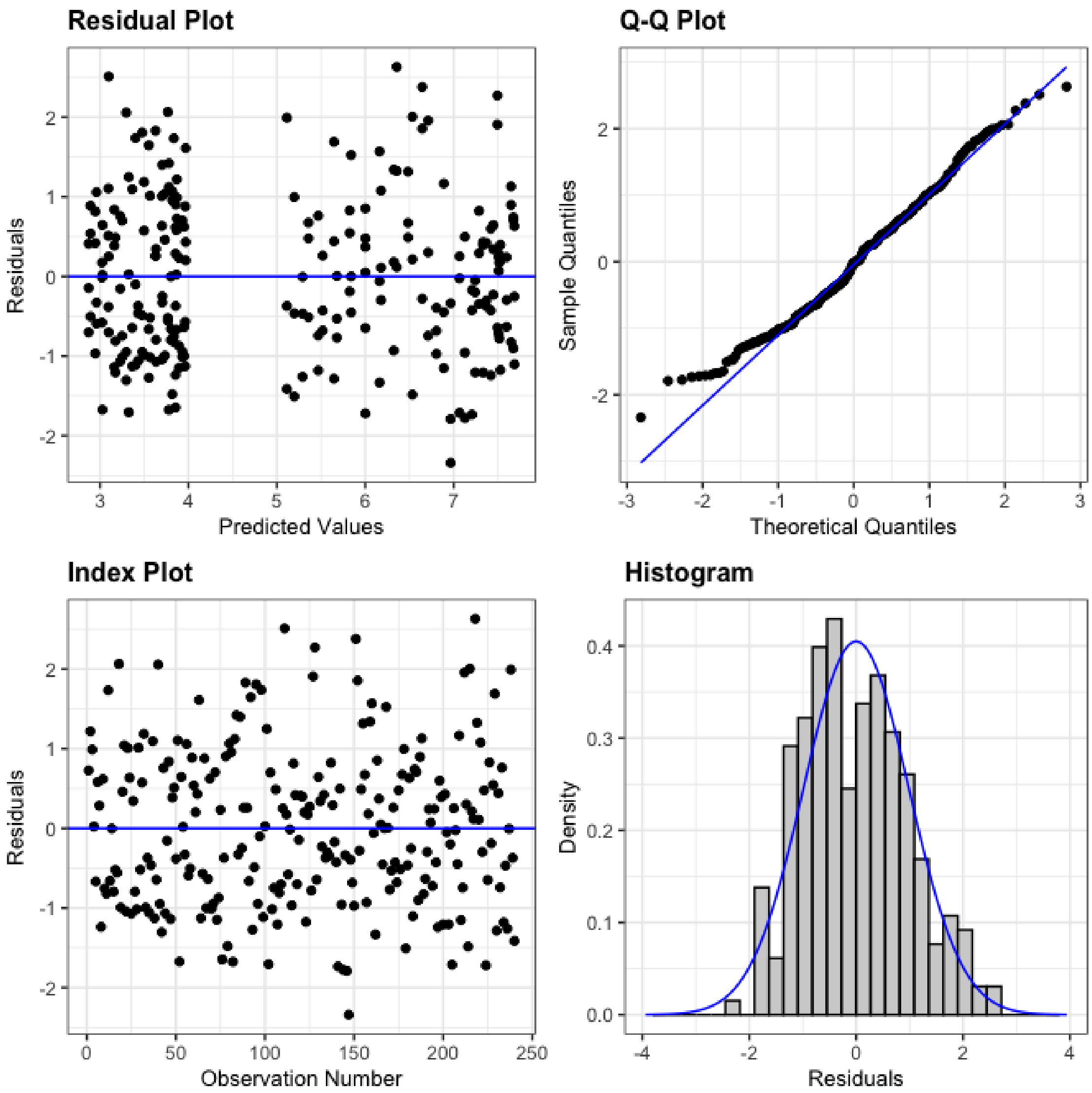
```
Generalized nonlinear least squares fit
  Model: survival ~ llrepar(x = week, log10n0 = log10n0, TD = TD)
  Data: m2
  Log-likelihood: 20.4

Coefficients:
      log10n0.(Intercept)           log10n0.matrixs         log10n0.microbetv
                    3.868                     0.100                     3.661
log10n0.matrixs:microbetv            TD.(Intercept)                TD.matrixs
                    0.058                    29.728                    -3.303
             TD.microbetv      TD.matrixs:microbetv
                  -17.292                     2.135

Variance function:
 Structure: Exponential of variance covariate
 Formula: ~week
 Parameter estimates:
  expon
-0.0259
Degrees of freedom: 240 total; 232 residual
Residual standard error: 0.305
```

```
summary (m2_mod)
```

```
Generalized nonlinear least squares fit
  Model: survival ~ llrepar(x = week, log10n0 = log10n0, TD = TD)
  Data: m2
    AIC BIC logLik
  -20.8  14   20.4

Variance function:
 Structure: Exponential of variance covariate
 Formula: ~week
 Parameter estimates:
  expon
-0.0259

Coefficients:
                           Value Std.Error t-value p-value
log10n0.(Intercept)         3.87      0.05    71.0   0.000
log10n0.matrixs             0.10      0.08     1.3   0.194
```

```
log10n0.microbetv           3.66      0.08    47.5   0.000
log10n0.matrixs:microbetv   0.06      0.11     0.5   0.595
TD.(Intercept)             29.73      2.53    11.8   0.000
TD.matrixs                 -3.30      3.22    -1.0   0.306
TD.microbetv              -17.29      2.56    -6.7   0.000
TD.matrixs:microbetv        2.14      3.27     0.7   0.514

 Correlation:
                          l100.( lg10n0.mt lg10n0.mc l100.: TD.(I) TD.mtr
log10n0.matrixs           -0.707                                         
log10n0.microbetv         -0.707  0.500                                  
log10n0.matrixs:microbetv  0.500 -0.707    -0.707                        
TD.(Intercept)            -0.865  0.611     0.611    -0.432              
TD.matrixs                 0.678 -0.859    -0.480     0.607 -0.785       
TD.microbetv               0.852 -0.602    -0.708     0.500 -0.985  0.773
TD.matrixs:microbetv      -0.668  0.845     0.555    -0.704  0.773 -0.985
                          TD.mcr
log10n0.matrixs                 
log10n0.microbetv               
log10n0.matrixs:microbetv       
TD.(Intercept)                  
TD.matrixs                      
TD.microbetv                    
TD.matrixs:microbetv      -0.784

Standardized residuals:
    Min      Q1     Med      Q3     Max 
-2.3405 -0.7486 -0.0182  0.6523  2.6306 

Residual standard error: 0.305 
Degrees of freedom: 240 total; 232 residual
```

```
# Residual plots
m2_res_m2_mod = resid(m2_mod, type = 'normalized')
m2_fit_m2_mod = fitted(m2_mod)
resid_auxpanel(m2_res_m2_mod, m2_fit_m2_mod)
```



```r
saveRDS(m2_mod, here('obj2', 'results', 'nv_tv_pcr_mod_sum.RDS'))

# Marginal means (prediction)
m2_mod <- readRDS(here('obj2', 'results', 'nv_tv_pcr_mod_sum.RDS'))

expand.grid(
  matrix = levels(m2$matrix),
  microbe = levels(m2$microbe),
  assay = levels(m2$assay),
  week = seq(0, max(m2$week), 0.5)) |>
  marginaleffects::avg_predictions(
    model=m2_mod,
    conf_level = 0.95,
    by = c('matrix', 'microbe', 'week')) |>
  saveRDS(here('obj2', 'results', 'nv_tv_pcr_mod_preds.RDS'))
```

```
# Extract parameters (coefficients) and their 95% CI
m2_mod_coef = coef(m2_mod)
m2_mod_confint = confint(m2_mod)

as.data.frame (cbind(
  Param = names (m2_mod_coef),
  Est = round (m2_mod_coef, 3),
  lwr = round (m2_mod_confint[,1], 3),
  upr = round (m2_mod_confint[,2], 3)
  ))

                                                 Param    Est     lwr     upr
log10n0.(Intercept)             log10n0.(Intercept)  3.868   3.761   3.975
log10n0.matrixs                     log10n0.matrixs    0.1  -0.051   0.251
log10n0.microbetv                 log10n0.microbetv  3.661    3.51   3.812
log10n0.matrixs:microbetv log10n0.matrixs:microbetv  0.058  -0.156   0.272
TD.(Intercept)                       TD.(Intercept) 29.728  24.777  34.678
TD.matrixs                               TD.matrixs -3.303  -9.612   3.006
TD.microbetv                           TD.microbetv -17.292 -22.317 -12.266
TD.matrixs:microbetv             TD.matrixs:microbetv  2.135  -4.273   8.543

# Estimated marginal (group level) means and contrasts
## Interaction model
list(
  log10n0 = emmeans (m2_mod, pairwise ~ matrix*microbe, param = 'log10n0'),
  TD = emmeans (m2_mod, pairwise ~ matrix*microbe, param = 'TD'))

$log10n0
$emmeans
 matrix microbe emmean     SE  df lower.CL upper.CL
 ps     nv        3.87 0.0545 230     3.76     3.98
 s      nv        3.97 0.0545 230     3.86     4.08
 ps     tv        7.53 0.0545 230     7.42     7.64
 s      tv        7.69 0.0545 230     7.58     7.79

Confidence level used: 0.95

$contrasts
 contrast      estimate     SE  df t.ratio p.value
 ps nv - s nv     -0.10 0.0771 230  -1.300  0.5630
 ps nv - ps tv    -3.66 0.0771 230 -47.500  <.0001
 ps nv - s tv     -3.82 0.0771 230 -49.600  <.0001
 s nv - ps tv     -3.56 0.0771 230 -46.200  <.0001
 s nv - s tv      -3.72 0.0771 230 -48.300  <.0001
 ps tv - s tv     -0.16 0.0771 230  -2.100  0.1720

P value adjustment: tukey method for comparing a family of 4 estimates


$TD
```

```
$emmeans
 matrix microbe emmean    SE  df lower.CL upper.CL
 ps     nv        29.7 2.530 230     24.8     34.7
 s      nv        26.4 2.000 230     22.5     30.4
 ps     tv        12.4 0.442 230     11.6     13.3
 s      tv        11.3 0.363 230     10.6     12.0

Confidence level used: 0.95

$contrasts
 contrast      estimate   SE  df t.ratio p.value
 ps nv - s nv      3.30 3.22 230   1.030  0.7340
 ps nv - ps tv    17.29 2.56 230   6.740  <.0001
 ps nv - s tv     18.46 2.55 230   7.230  <.0001
 s nv - ps tv     13.99 2.04 230   6.840  <.0001
 s nv - s tv      15.16 2.03 230   7.470  <.0001
 ps tv - s tv      1.17 0.57 230   2.040  0.1760

P value adjustment: tukey method for comparing a family of 4 estimates
```

```
## Interaction model (with matrices combined)
list(
  log10n0 = emmeans (m2_mod, pairwise ~ microbe, param = 'log10n0'),
  TD = emmeans (m2_mod, pairwise ~ microbe, param = 'TD'))
```

```
$log10n0
$emmeans
 microbe emmean     SE  df lower.CL upper.CL
 nv        3.92 0.0385 230     3.84     3.99
 tv        7.61 0.0385 230     7.53     7.68

Results are averaged over the levels of: matrix
Confidence level used: 0.95

$contrasts
 contrast estimate     SE  df t.ratio p.value
 nv - tv     -3.69 0.0545 230 -67.700  <.0001

Results are averaged over the levels of: matrix


$TD
$emmeans
 microbe emmean    SE  df lower.CL upper.CL
 nv        28.1 1.610 230     24.9     31.2
 tv        11.8 0.286 230     11.3     12.4

Results are averaged over the levels of: matrix
Confidence level used: 0.95
```

```
$contrasts
 contrast estimate   SE  df t.ratio p.value
 nv - tv      16.2 1.64 230   9.920  <.0001

Results are averaged over the levels of: matrix
```

```
# Joint tests
list(
  log10n0 = joint_tests (m2_mod, param = 'log10n0'),
  TD = joint_tests (m2_mod, param = 'TD'))
```

```
$log10n0
 model term     df1 df2  F.ratio p.value
 matrix           1 230    6.000  0.0180
 microbe          1 230 4585.000  <.0001
 matrix:microbe   1 230    0.000  0.5950



$TD
 model term     df1 df2 F.ratio p.value
 matrix           1 230   1.900  0.1730
 microbe          1 230  98.500  <.0001
 matrix:microbe   1 230   0.400  0.5140
```

```
# Overall marginal mean
emmeans (m2_mod, ~ microbe, param = 'log10n0')
```

```
 microbe emmean     SE  df lower.CL upper.CL
 nv        3.92 0.0385 230     3.84     3.99
 tv        7.61 0.0385 230     7.53     7.68

Results are averaged over the levels of: matrix
Confidence level used: 0.95
```

```
emmeans (m2_mod, ~ matrix, param = 'TD')
```

```
 matrix emmean   SE  df lower.CL upper.CL
 ps       21.1 1.28 230     18.6     23.6
 s        18.9 1.01 230     16.9     20.8

Results are averaged over the levels of: microbe
Confidence level used: 0.95
```

```
emmeans (m2_mod, ~ microbe, param = 'TD')
```

```
 microbe emmean    SE  df lower.CL upper.CL
 nv        28.1 1.610 230     24.9     31.2
 tv        11.8 0.286 230     11.3     12.4
```

```
Results are averaged over the levels of: matrix 
Confidence level used: 0.95 
```

```
TD_values <- readRDS (here('obj2', 'results', "TD_values.RDS"))
TD_values$nv_pcr_1D = emmeans (m2_mod, ~ microbe, param = 'TD')[1]
TD_values$tv_pcr_1D = emmeans (m2_mod, ~ microbe, param = 'TD')[2]
saveRDS (TD_values, here('obj2', 'results', "TD_values.RDS"))
```

### 3.5.3 Assess covariance/collinearity

```
set.seed(SEED)

## Variance-covariance matrix
m2_mod <- readRDS (here('obj2', 'results', 'nv_tv_pcr_mod_sum.RDS'))
m2_mod_coef <- coef (m2_mod)

(mod_vcov_joint <- vcov(m2_mod))
```

```
                          log10n0.(Intercept) log10n0.matrixs
log10n0.microbetv
log10n0.(Intercept)                   0.00297        -0.00297          -
0.00297
log10n0.matrixs                      -0.00297         0.00594
0.00297
log10n0.microbetv                    -0.00297         0.00297
0.00594
log10n0.matrixs:microbetv             0.00297        -0.00594          -
0.00594
TD.(Intercept)                       -0.11898         0.11898
0.11898
TD.matrixs                            0.11898        -0.21299          -
0.11898
TD.microbetv                          0.11898        -0.11898          -
0.13980
TD.matrixs:microbetv                 -0.11898         0.21299
0.13980
                          log10n0.matrixs:microbetv TD.(Intercept) TD.matrixs
log10n0.(Intercept)                         0.00297        -0.119      0.119
log10n0.matrixs                            -0.00594         0.119     -0.213
log10n0.microbetv                          -0.00594         0.119     -0.119
log10n0.matrixs:microbetv                   0.01188        -0.119      0.213
TD.(Intercept)                             -0.11898         6.379     -6.379
TD.matrixs                                  0.21299        -6.379     10.362
TD.microbetv                                0.13980        -6.379      6.379
TD.matrixs:microbetv                       -0.25091         6.379    -10.362
                          TD.microbetv TD.matrixs:microbetv
log10n0.(Intercept)              0.119               -0.119
log10n0.matrixs                 -0.119                0.213
log10n0.microbetv               -0.140                0.140
log10n0.matrixs:microbetv        0.140               -0.251
TD.(Intercept)                  -6.379                6.379
TD.matrixs                       6.379          -10.362
TD.microbetv                     6.575           -6.575
TD.matrixs:microbetv            -6.575           10.689

mod_vcov_indep <- diag(diag(mod_vcov_joint))
dimnames(mod_vcov_indep) <- dimnames(mod_vcov_joint)

## Draw of coefficients
iter = 5000
draw_joint <- MASS::mvrnorm(n=iter, mu = m2_mod_coef, Sigma = mod_vcov_joint)
|>
  as.data.frame()

## Assuming no covariance
draw_indep <- MASS::mvrnorm(n=iter, mu =m2_mod_coef, Sigma = mod_vcov_indep)
|>
  as.data.frame()

## Extract TD values
TD_joint <- cbind (ps_nv_joint =
                     draw_joint[["TD.(Intercept)"]],
                   s_nv_joint =
                     draw_joint[["TD.(Intercept)"]] +
                     draw_joint[["TD.matrixs"]],
                   ps_tv_joint =
                     draw_joint[["TD.(Intercept)"]] +
                     draw_joint[["TD.microbetv"]],
                   s_tv_joint =
                     draw_joint[["TD.(Intercept)"]] +
                     draw_joint[["TD.matrixs"]] +
                     draw_joint[["TD.microbetv"]] +
                     draw_joint[["TD.matrixs:microbetv"]]
                   )
colMeans(TD_joint <= 0)

ps_nv_joint  s_nv_joint ps_tv_joint  s_tv_joint
          0           0           0           0

TD_indep <- cbind (ps_nv_indep =
                     draw_indep[["TD.(Intercept)"]],
                   s_nv_indep =
                     draw_indep[["TD.(Intercept)"]] +
                     draw_indep[["TD.matrixs"]],
                   ps_tv_indep =
                     draw_indep[["TD.(Intercept)"]] +
                     draw_indep[["TD.microbetv"]],
                   s_tv_indep =
                     draw_indep[["TD.(Intercept)"]] +
                     draw_indep[["TD.matrixs"]] +
                     draw_indep[["TD.microbetv"]] +
                     draw_indep[["TD.matrixs:microbetv"]]
                   )

colMeans(TD_indep <= 0)
```

```
ps_nv_indep  s_nv_indep ps_tv_indep  s_tv_indep
     0.0000      0.0000      0.0000      0.0262
```

```
## Calculate mean and 95% QIs
QIs <- function(x) c(
  mean=mean(x),
  lwr=quantile(x, 0.025),
  upr=quantile(x, 0.975))

round(t(apply(TD_joint, 2, QIs)),2)
```

```
            mean lwr.2.5% upr.97.5%
ps_nv_joint 29.7     24.7      34.8
s_nv_joint  26.4     22.4      30.2
ps_tv_joint 12.4     11.6      13.3
s_tv_joint  11.3     10.6      12.0
```

```
round(t(apply(TD_indep, 2, QIs)),2)
```

```
            mean lwr.2.5% upr.97.5%
ps_nv_indep 29.7    24.76      34.7
s_nv_indep  26.4    18.53      34.3
ps_tv_indep 12.4     5.31      19.6
s_tv_indep  11.2    -0.09      22.8
```

```
## Again compare with emmeans
emmeans (m2_mod, pairwise ~ matrix:microbe, param = 'TD')$emmeans
```

```
 matrix microbe emmean    SE  df lower.CL upper.CL
 ps     nv        29.7 2.530 230     24.8     34.7
 s      nv        26.4 2.000 230     22.5     30.4
 ps     tv        12.4 0.442 230     11.6     13.3
 s      tv        11.3 0.363 230     10.6     12.0

Confidence level used: 0.95
```

```
## If considering filtering out negative simulated numbers
TF_pos_joint <- rowSums(TD_joint <= 0 | !is.finite(TD_joint)) == 0
TF_pos_indep <- rowSums(TD_indep <= 0 | !is.finite(TD_indep)) == 0

mean(TF_pos_joint)
```

```
[1] 1
```

```
mean(TF_pos_indep)
```

```
[1] 0.974
```

```
CI <- function(x) c(mean = mean(x),
                    lwr  = unname(quantile(x, 0.025)),
                    upr  = unname(quantile(x, 0.975)))

as.data.frame(t(apply(TD_joint[TF_pos_joint, , drop = FALSE], 2, QIs)))
```

```
            mean lwr.2.5% upr.97.5%
ps_nv_joint 29.7     24.7      34.8
s_nv_joint  26.4     22.4      30.2
ps_tv_joint 12.4     11.6      13.3
s_tv_joint  11.3     10.6      12.0
```

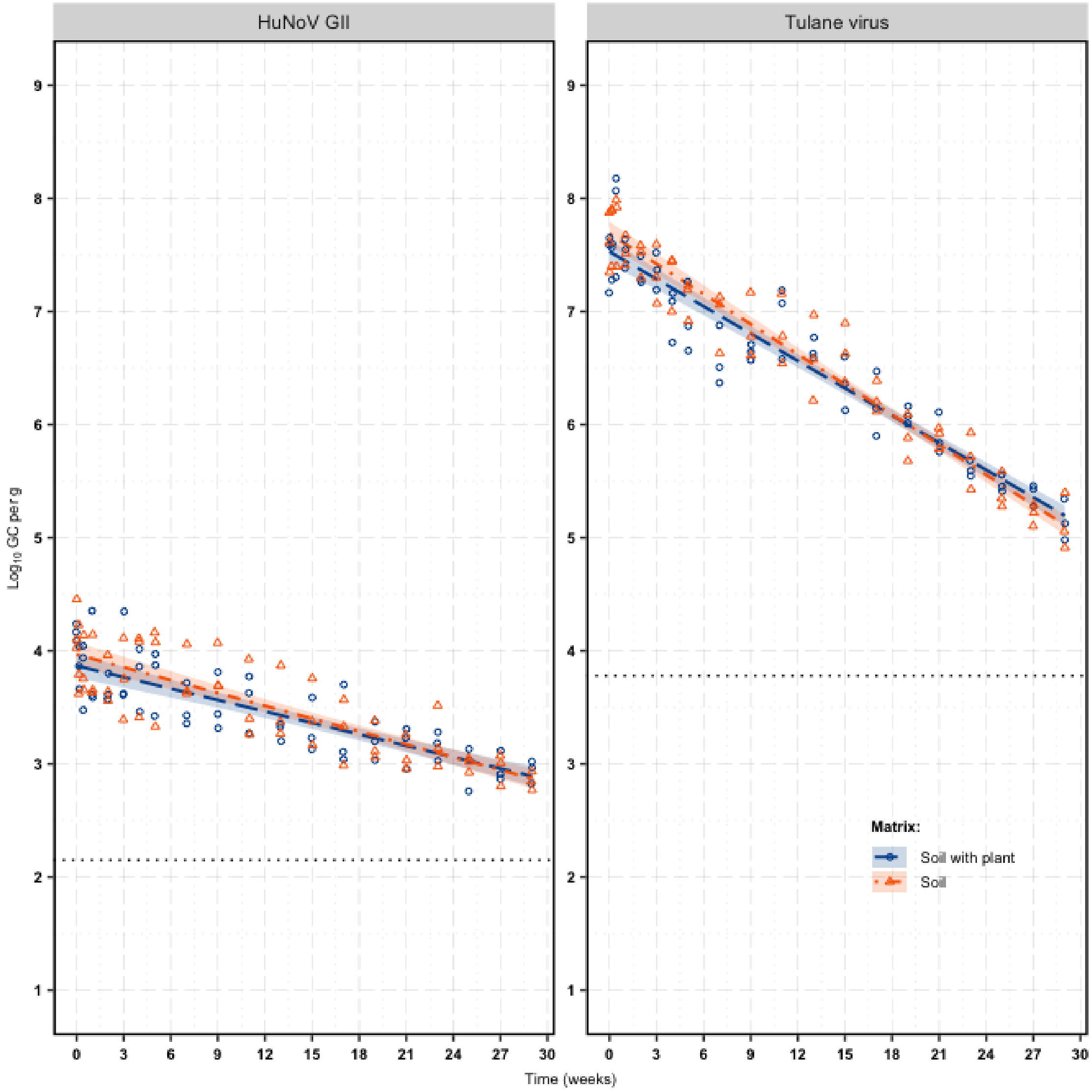
```
as.data.frame(t(apply(TD_indep[TF_pos_indep, , drop = FALSE], 2, QIs)))
```

```
            mean lwr.2.5% upr.97.5%
ps_nv_indep 29.7    24.87      34.7
s_nv_indep  26.6    19.06      34.3
ps_tv_indep 12.5     5.69      19.6
s_tv_indep  11.6     1.52      22.8
```

```
saveRDS(mod_vcov_joint, here('obj2', 'results', 'nv_tv_pcr_sim_mvrnorm.RDS'))

# Remove selected objects from the environment
rm(list = setdiff(ls(envir = .GlobalEnv, all.names = TRUE), list_exc),
   envir = .GlobalEnv)
```

### 3.5.4 Plot microbial inactivation

```
m2 <- readRDS(here('obj2', 'data', 'Obj2_dat.RDS')) |>
  dplyr::filter (assay=='pcr') |>
  dplyr::mutate(matrix = factor(matrix,
                                labels = c('Soil with plant', 'Soil'))) |>
  droplevels()

m2_preds =  readRDS(here('obj2', 'results', 'nv_tv_pcr_mod_preds.RDS'))
m2_preds$matrix <- factor(m2_preds$matrix, levels = c("ps", "s"),
                          labels = c("Soil with plant", "Soil"))

microb_labs = as_labeller(c(tv = 'Tulane virus', nv = "HuNoV GII"))

LOD1 <- LOD |>
  dplyr::filter (microbe=='nv' | microbe=='tv')

p_m2 = ggplot(m2_preds, aes(x = week, color = matrix, fill = matrix, linetype
= matrix)) +
  facet_wrap(~ microbe, labeller = microb_labs, scales = 'free_y') +
  geom_hline(data = LOD1, aes(yintercept = lod, group = microbe),
             color = 'black', linetype = 3) +
  geom_ribbon(aes(ymin = conf.low, ymax = conf.high,
                  group = matrix),
              alpha = 0.2,
              color = NA) +
  geom_line(aes(y = estimate), linewidth = 0.7) +
  geom_point(data = m2,
             aes(y = survival, shape = matrix),
             size = 1,
             position = position_jitter(width = 0.03)) +
  guides(
    fill = guide_legend(title = 'Matrix:'),
    color = guide_legend(title = 'Matrix:'),
    linetype = guide_legend(title = 'Matrix:'),
    shape = guide_legend(title = 'Matrix:')) +
  labs(x = "Time (weeks)",
       y = bquote(Log[10]~GC~per~g)) +
  scale_fill_manual(values = c("#00529b", "#fb6502")) +
  scale_color_manual(values = c("#00529b", "#fb6502")) +
  scale_shape_manual(values = c(1, 2)) +
  scale_linetype_manual(values = c("longdash", "dotdash")) +
  scale_x_continuous(
    breaks = seq(0, 30, 3),
    labels = seq(0, 30, 3)) +
  scale_y_continuous(
    breaks = seq(1, 9, 1),
    labels = seq(1, 9, 1),
    limits = c(1,9)) +
  theme(text = element_text(family = "Arial"),
        legend.position = 'inside', legend.position.inside = c(0.86, 0.18),
        legend.direction = "vertical",
        legend.text = element_text(size = 7),
        legend.title = element_text(size = 7, face = "bold"),
        legend.key.height = unit(0.4, "cm"),
        plot.title = element_text(color = "black", size = 7, face = "bold",
hjust = 0.5),
        axis.text.x = element_text(size = 7, color = "black", face = "bold",
hjust = 0.5, vjust = 0.5),
        axis.text.y = element_text(size = 7, color = "black", face = "bold"),
        axis.title.x = element_text(size = 7, vjust = -1),
        axis.title.y = element_text(size = 7, vjust = 2),
        panel.border = element_rect(color = "black", fill = NA, linewidth =
1),
        panel.grid.major = element_line(color = "gray", linewidth = 0.1,
linetype = "longdash"),
        panel.grid.minor = element_line(color = "lightgray", linewidth = 0.1,
linetype = "dotted"),
        panel.background = element_rect(fill = "transparent"),
        legend.background = element_rect(fill = "transparent", color =
"transparent"),
        plot.background = element_rect(fill = "transparent", color =
"transparent"))

p_m2
```



```
ggsave(file = here('obj2', 'results', 'Fig 2 tv_nv_pcr.tiff'),
       plot = p_m2,
       width = 12, height = 6, units = c("cm"), dpi=400)

# Remove selected objects from the environment
rm(list = setdiff(ls(envir = .GlobalEnv, all.names = TRUE), list_exc),
   envir = .GlobalEnv)
```

# 4. T2D

## 4.1 Model Functions

```
# Log linear and non-linear models

## Log-linear and reparameterized log-linear
llrepar <- function(x, log10n0, TD, D = 2){
  log10n0 - D*x/TD
}

## Nonlinear models
# Weibull and reparameterized Weibull
wbrepar <- function(x, log10n0, TD, beta, D = 2){
  log10n0 - D*(x/TD)^beta
}

# To clean the environment
list_exc <- c(lsf.str(envir = .GlobalEnv), "list_exc")
```

## 4.2 *E. coli*

```
m2 <- readRDS(here('obj2', 'data', 'obj2_dat.RDS')) |>
  dplyr::filter(microbe == 'ecoli',
                assay == "plate") |>
  droplevels()


m2_mod_wbrepar = nlme::gnls(survival ~ wbrepar(x = week,
                                               log10n0 = log10n0,
                                               TD = TD,
                                               beta = beta),
                             data = m2,
                             params = log10n0+TD+beta ~ matrix,
                             start = list(log10n0 = c(5, rep(0, 1)),
                                          TD = c(1, 0),
                                          beta = c(1, 0)))

(m2_mod <- m2_mod_wbrepar)
```

```
Generalized nonlinear least squares fit
  Model: survival ~ wbrepar(x = week, log10n0 = log10n0, TD = TD, beta =
beta)
  Data: m2
  Log-likelihood: -76.9

Coefficients:
log10n0.(Intercept)     log10n0.matrixs      TD.(Intercept)
TD.matrixs
             7.2874             -0.2448              6.1146
0.3220
   beta.(Intercept)        beta.matrixs
             0.6566             -0.0166

Degrees of freedom: 120 total; 114 residual
Residual standard error: 0.471
```

```
summary (m2_mod)
```

```
Generalized nonlinear least squares fit
  Model: survival ~ wbrepar(x = week, log10n0 = log10n0, TD = TD, beta = 
beta)
  Data: m2
  AIC BIC logLik
  168 187  -76.9

Coefficients:
                    Value Std.Error t-value p-value
log10n0.(Intercept)  7.29     0.181    40.2   0.000
log10n0.matrixs     -0.24     0.259    -0.9   0.346
TD.(Intercept)       6.11     1.040     5.9   0.000
TD.matrixs           0.32     1.549     0.2   0.836
beta.(Intercept)     0.66     0.062    10.6   0.000
beta.matrixs        -0.02     0.089    -0.2   0.852

 Correlation:
                 l100.( lg100. TD.(I) TD.mtr bt.(I)
log10n0.matrixs  -0.701                            
TD.(Intercept)   -0.910  0.638                     
TD.matrixs        0.611 -0.912 -0.672              
beta.(Intercept) -0.792  0.556  0.953 -0.640       
beta.matrixs      0.549 -0.794 -0.661  0.950 -0.694

Standardized residuals:
   Min     Q1    Med     Q3    Max 
-1.930 -0.745 -0.180  0.690  2.700 

Residual standard error: 0.471 
Degrees of freedom: 120 total; 114 residual
```

```
# Variance-covariance matrix
(mod_vcov <- vcov(m2_mod))
```

```
                    log10n0.(Intercept) log10n0.matrixs TD.(Intercept)
log10n0.(Intercept)             0.03293        -0.03293        -0.1717
log10n0.matrixs                -0.03293         0.06693         0.1717
TD.(Intercept)                 -0.17174         0.17174         1.0823
TD.matrixs                      0.17174        -0.36532        -1.0823
beta.(Intercept)               -0.00887         0.00887         0.0612
beta.matrixs                    0.00887        -0.01827        -0.0612
                    TD.matrixs beta.(Intercept) beta.matrixs
log10n0.(Intercept)     0.1717         -0.00887      0.00887
log10n0.matrixs        -0.3653          0.00887     -0.01827
TD.(Intercept)         -1.0823          0.06118     -0.06118
TD.matrixs              2.3980         -0.06118      0.13094
beta.(Intercept)       -0.0612          0.00381     -0.00381
beta.matrixs            0.1309         -0.00381      0.00792
```

```
# Overall marginal means
emmeans (m2_mod, ~ 1, param = 'log10n0')

 1       emmean    SE  df lower.CL upper.CL
 overall   7.16 0.129 113     6.91     7.42

Results are averaged over the levels of: matrix 
Confidence level used: 0.95 

emmeans (m2_mod, ~ 1, param = 'beta')

 1       emmean     SE  df lower.CL upper.CL
 overall  0.648 0.0445 113     0.56    0.736

Results are averaged over the levels of: matrix 
Confidence level used: 0.95 

emmeans (m2_mod, ~ 1, param = 'TD')

 1       emmean    SE  df lower.CL upper.CL
 overall   6.28 0.774 113     4.74     7.81

Results are averaged over the levels of: matrix 
Confidence level used: 0.95 

TD_values <- readRDS (here('obj2', 'results', "TD_values.RDS"))
TD_values$ecoli_plate_2D = emmeans (m2_mod, ~ 1, param = 'TD')
saveRDS (TD_values, here('obj2', 'results', "TD_values.RDS"))

# Remove selected objects from the environment
rm(list = setdiff(ls(envir = .GlobalEnv, all.names = TRUE), list_exc),
   envir = .GlobalEnv)
```

### 4.3 *Tulane virus TCID50*

```
m2 <- readRDS(here('obj2', 'data','Obj2_dat.RDS')) |>
  dplyr::filter (microbe=='tv', assay=='tcid50') |>
  droplevels()

m2_mod_wbrepar = nlme::gnls(survival ~ wbrepar(x = week,
                               log10n0 = log10n0,
                               TD = TD,
                               beta = beta),
                 data = m2,
                 params = log10n0+TD+beta ~ matrix,
                 start = list(log10n0 = c(5, 0),
                              TD = c(5, 0),
                              beta = c(2, 0)))

(m2_mod <- m2_mod_wbrepar)
```

```
Generalized nonlinear least squares fit
  Model: survival ~ wbrepar(x = week, log10n0 = log10n0, TD = TD, beta =
beta)
  Data: m2
  Log-likelihood: 24.2

Coefficients:
log10n0.(Intercept)     log10n0.matrixs      TD.(Intercept)
TD.matrixs
             4.7517              0.1390             13.2252             -
0.2451
   beta.(Intercept)        beta.matrixs
             0.8288             -0.0182

Degrees of freedom: 120 total; 114 residual
Residual standard error: 0.203
```

```
summary (m2_mod)
```

```
Generalized nonlinear least squares fit
  Model: survival ~ wbrepar(x = week, log10n0 = log10n0, TD = TD, beta =
beta)
  Data: m2
    AIC BIC logLik
  -34.5 -15   24.2

Coefficients:
                    Value Std.Error t-value p-value
log10n0.(Intercept)  4.75     0.067    70.5   0.000
log10n0.matrixs      0.14     0.096     1.4   0.150
TD.(Intercept)      13.23     0.710    18.6   0.000
TD.matrixs          -0.25     1.011    -0.2   0.809
beta.(Intercept)     0.83     0.046    17.8   0.000
beta.matrixs        -0.02     0.065    -0.3   0.781

 Correlation:
                 l100.( lg100. TD.(I) TD.mtr bt.(I)
log10n0.matrixs  -0.702
TD.(Intercept)   -0.916  0.643
TD.matrixs        0.643 -0.917 -0.702
beta.(Intercept) -0.761  0.534  0.884 -0.621
beta.matrixs      0.543 -0.763 -0.631  0.885 -0.714

Standardized residuals:
    Min      Q1     Med      Q3     Max
-2.9461 -0.6764  0.0461  0.6183  2.2847

Residual standard error: 0.203
Degrees of freedom: 120 total; 114 residual
```

```
# Variance-covariance matrix
(mod_vcov <- vcov(m2_mod))

                    log10n0.(Intercept) log10n0.matrixs TD.(Intercept)
log10n0.(Intercept)             0.00455        -0.00455        -0.0439
log10n0.matrixs                -0.00455         0.00922         0.0439
TD.(Intercept)                 -0.04386         0.04386         0.5047
TD.matrixs                      0.04386        -0.08902        -0.5047
beta.(Intercept)               -0.00239         0.00239         0.0292
beta.matrixs                    0.00239        -0.00477        -0.0292
                    TD.matrixs beta.(Intercept) beta.matrixs
log10n0.(Intercept)     0.0439         -0.00239      0.00239
log10n0.matrixs        -0.0890          0.00239     -0.00477
TD.(Intercept)         -0.5047          0.02920     -0.02920
TD.matrixs              1.0229         -0.02920      0.05831
beta.(Intercept)       -0.0292          0.00216     -0.00216
beta.matrixs            0.0583         -0.00216      0.00424

# Overall marginal means
emmeans (m2_mod, ~ 1, param = 'log10n0')

 1       emmean    SE  df lower.CL upper.CL
 overall   4.82 0.048 113     4.73     4.92

Results are averaged over the levels of: matrix
Confidence level used: 0.95

emmeans (m2_mod, ~ 1, param = 'TD')

 1       emmean    SE  df lower.CL upper.CL
 overall   13.1 0.506 113     12.1     14.1

Results are averaged over the levels of: matrix
Confidence level used: 0.95

emmeans (m2_mod, ~ 1, param = 'beta')

 1       emmean     SE  df lower.CL upper.CL
 overall   0.82 0.0326 113    0.755    0.884

Results are averaged over the levels of: matrix
Confidence level used: 0.95

TD_values <- readRDS (here('obj2', 'results', "TD_values.RDS"))
TD_values$tv_tcid50_2D = emmeans (m2_mod, ~ 1, param = 'TD')
saveRDS (TD_values, here('obj2', 'results', "TD_values.RDS"))

# Remove selected objects from the environment
rm(list = setdiff(ls(envir = .GlobalEnv, all.names = TRUE), list_exc),
   envir = .GlobalEnv)
```

### 4.4 TuV and HuNoV - *RNase RT-qPCR*

```
m2 <- readRDS(here('obj2', 'data', 'Obj2_dat.RDS')) |>
  dplyr::filter (assay=='pcr') |>
  droplevels()

m2_mod_llrepar = nlme::gnls(survival ~ llrepar(x = week,
                                               log10n0 = log10n0,
                                               TD = TD),
                            data = m2,
                            params = log10n0+TD ~ matrix*microbe,
                 start = list(log10n0 = c(4, rep(0,3)),
                              TD = c(25, rep(0,3))))


m2_mod_exp_wk = update(m2_mod_llrepar, weights = varExp(form = ~week))

(m2_mod <- m2_mod_exp_wk)
```

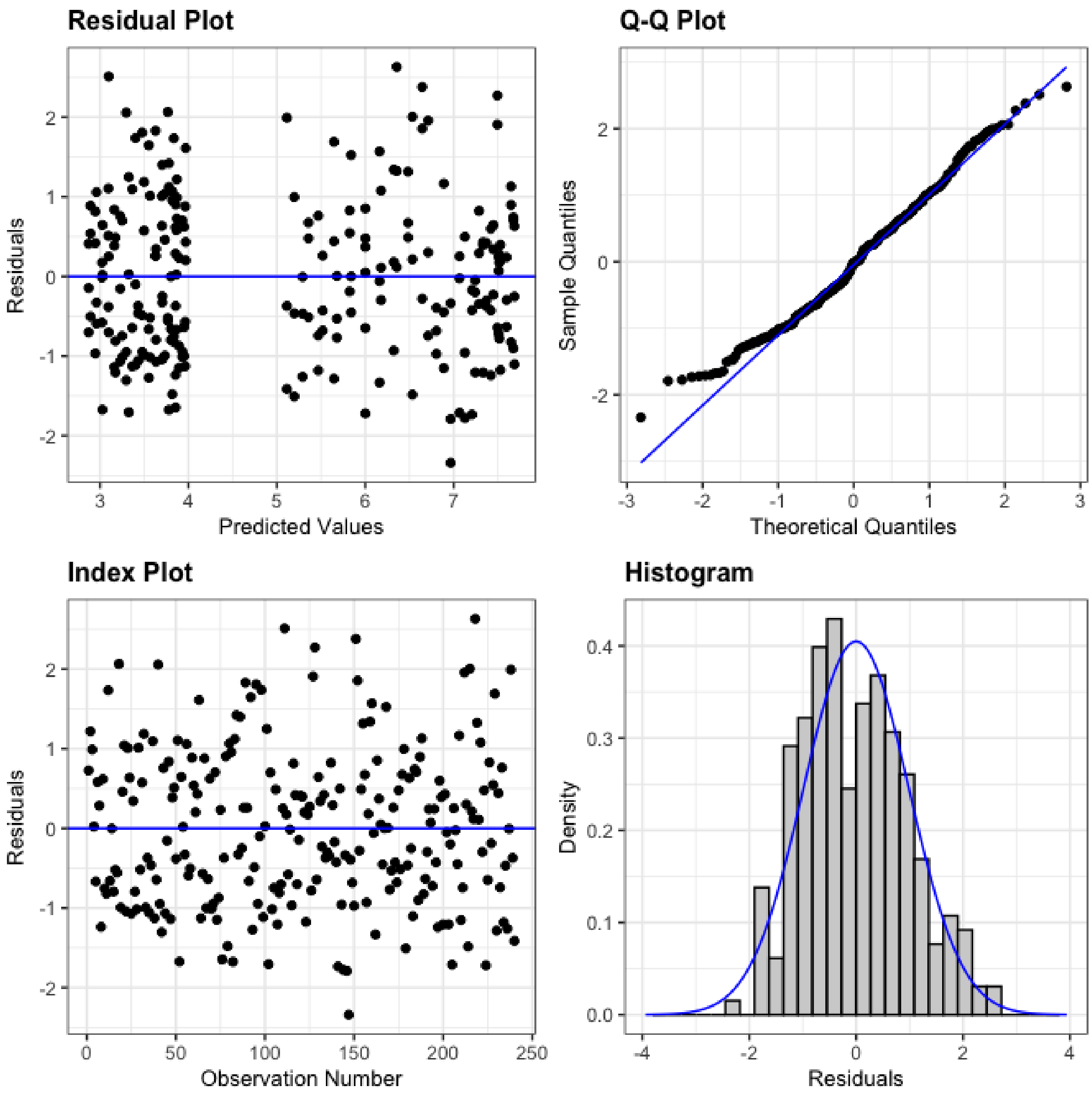
```
Generalized nonlinear least squares fit
  Model: survival ~ llrepar(x = week, log10n0 = log10n0, TD = TD)
  Data: m2
  Log-likelihood: 20.4

Coefficients:
      log10n0.(Intercept)            log10n0.matrixs         log10n0.microbetv
                    3.868                      0.100                     3.661
log10n0.matrixs:microbetv             TD.(Intercept)                TD.matrixs
                    0.058                     59.456                    -6.606
             TD.microbetv       TD.matrixs:microbetv
                  -34.584                      4.271

Variance function:
 Structure: Exponential of variance covariate
 Formula: ~week
 Parameter estimates:
  expon
-0.0259
Degrees of freedom: 240 total; 232 residual
Residual standard error: 0.305
```

```
summary (m2_mod)
```

```
Generalized nonlinear least squares fit
  Model: survival ~ llrepar(x = week, log10n0 = log10n0, TD = TD)
  Data: m2
    AIC BIC   logLik
  -20.8  14   20.4

Variance function:
 Structure: Exponential of variance covariate
 Formula: ~week 
 Parameter estimates:
  expon 
-0.0259 

Coefficients:
                          Value Std.Error t-value p-value
log10n0.(Intercept)         3.9      0.05    71.0   0.000
log10n0.matrixs             0.1      0.08     1.3   0.194
log10n0.microbetv           3.7      0.08    47.5   0.000
log10n0.matrixs:microbetv   0.1      0.11     0.5   0.595
TD.(Intercept)             59.5      5.05    11.8   0.000
TD.matrixs                 -6.6      6.44    -1.0   0.306
TD.microbetv              -34.6      5.13    -6.7   0.000
TD.matrixs:microbetv        4.3      6.54     0.7   0.514

 Correlation: 
                          l100.( lg10n0.mt lg10n0.mc l100.: TD.(I) TD.mtr
log10n0.matrixs           -0.707                                          
log10n0.microbetv         -0.707  0.500                                   
log10n0.matrixs:microbetv  0.500 -0.707    -0.707                         
TD.(Intercept)            -0.865  0.611     0.611    -0.432               
TD.matrixs                 0.678 -0.859    -0.480     0.607 -0.785        
TD.microbetv               0.852 -0.602    -0.708     0.500 -0.985  0.773 
TD.matrixs:microbetv      -0.668  0.845     0.555    -0.704  0.773 -0.985 
                          TD.mcr
log10n0.matrixs                 
log10n0.microbetv               
log10n0.matrixs:microbetv       
TD.(Intercept)                  
TD.matrixs                      
TD.microbetv                    
TD.matrixs:microbetv      -0.784

Standardized residuals:
    Min      Q1     Med      Q3     Max 
-2.3405 -0.7486 -0.0182  0.6523  2.6306 

Residual standard error: 0.305 
Degrees of freedom: 240 total; 232 residual
```

```
# Residual plots
m2_res_m2_mod = resid(m2_mod, type = 'normalized')
m2_fit_m2_mod = fitted(m2_mod)
resid_auxpanel(m2_res_m2_mod, m2_fit_m2_mod)
```

```
Warning: Removed 2 rows containing missing values or values outside the scale
range
(`geom_bar()`).
```



```
# Overall marginal mean
emmeans (m2_mod, ~ microbe, param = 'log10n0')

NOTE: Results may be misleading due to involvement in interactions

 microbe emmean     SE  df lower.CL upper.CL
 nv        3.92 0.0385 230     3.84     3.99
 tv        7.61 0.0385 230     7.53     7.68

Results are averaged over the levels of: matrix
Confidence level used: 0.95

emmeans (m2_mod, ~ matrix, param = 'TD')

NOTE: Results may be misleading due to involvement in interactions

 matrix emmean   SE  df lower.CL upper.CL
 ps       42.2 2.56 230     37.1     47.2
 s        37.7 2.03 230     33.7     41.7

Results are averaged over the levels of: microbe 
Confidence level used: 0.95 
```

```r
emmeans (m2_mod, ~ microbe, param = 'TD')
```

```
NOTE: Results may be misleading due to involvement in interactions

 microbe emmean   SE  df lower.CL upper.CL
 nv        56.2 3.22 230     49.8     62.5
 tv        23.7 0.57 230     22.6     24.8

Results are averaged over the levels of: matrix 
Confidence level used: 0.95 
```

```r
TD_values <- readRDS (here('obj2', 'results', "TD_values.RDS"))
TD_values$nv_pcr_2D = emmeans (m2_mod, ~ microbe, param = 'TD')[1]
```

```
NOTE: Results may be misleading due to involvement in interactions
```

```r
TD_values$tv_pcr_2D = emmeans (m2_mod, ~ microbe, param = 'TD')[2]
```

```
NOTE: Results may be misleading due to involvement in interactions
```

```r
saveRDS (TD_values, here('obj2', 'results', "TD_values.RDS"))

# Remove selected objects from the environment
rm(list = setdiff(ls(envir = .GlobalEnv, all.names = TRUE), list_exc),
   envir = .GlobalEnv)
```

# 5. T3D

## 5.1 Model Functions

```r
# Log linear and non-linear models

## Log-linear and reparameterized log-linear
llrepar <- function(x, log10n0, TD, D = 3){
  log10n0 - D*x/TD
}

## Nonlinear models
# Weibull and reparameterized Weibull
wbrepar <- function(x, log10n0, TD, beta, D = 3){
  log10n0 - D*(x/TD)^beta
}

# To clean the environment
list_exc <- c(lsf.str(envir = .GlobalEnv), "list_exc")
```

### 5.2 *E. coli*

```
m2 <- readRDS(here('obj2', 'data', 'obj2_dat.RDS')) |>
  dplyr::filter(microbe == 'ecoli',
                assay == "plate") |>
  droplevels()

m2_mod_wbrepar = nlme::gnls(survival ~ wbrepar(x = week,
                                               log10n0 = log10n0,
                                               TD = TD,
                                               beta = beta),
                            data = m2,
                            params = log10n0+TD+beta ~ matrix,
                            start = list(log10n0 = c(5, rep(0, 1)),
                                         TD = c(1, 0),
                                         beta = c(1, 0)))
```

```
(m2_mod <- m2_mod_wbrepar)
```

```
Generalized nonlinear least squares fit
  Model: survival ~ wbrepar(x = week, log10n0 = log10n0, TD = TD, beta =
beta)
  Data: m2
  Log-likelihood: -76.9

Coefficients:
log10n0.(Intercept)     log10n0.matrixs      TD.(Intercept)
TD.matrixs
             7.2874             -0.2448             11.3389
0.7902
   beta.(Intercept)        beta.matrixs
             0.6566             -0.0166

Degrees of freedom: 120 total; 114 residual
Residual standard error: 0.471
```

```
summary (m2_mod)
```

```
Generalized nonlinear least squares fit
  Model: survival ~ wbrepar(x = week, log10n0 = log10n0, TD = TD, beta =
beta)
  Data: m2
  AIC BIC logLik
  168 187  -76.9

Coefficients:
                    Value Std.Error t-value p-value
log10n0.(Intercept)  7.29     0.181    40.2   0.000
log10n0.matrixs     -0.24     0.259    -0.9   0.346
TD.(Intercept)      11.34     1.317     8.6   0.000
TD.matrixs           0.79     1.960     0.4   0.688
```

```
beta.(Intercept)     0.66     0.062    10.6   0.000
beta.matrixs        -0.02     0.089    -0.2   0.852

 Correlation:
                 l100.( lg100. TD.(I) TD.mtr bt.(I)
log10n0.matrixs  -0.701                            
TD.(Intercept)   -0.936  0.657                     
TD.matrixs        0.629 -0.938 -0.672              
beta.(Intercept) -0.792  0.556  0.895 -0.602       
beta.matrixs      0.549 -0.794 -0.621  0.888 -0.694

Standardized residuals:
   Min     Q1    Med     Q3    Max 
-1.930 -0.745 -0.180  0.690  2.700 

Residual standard error: 0.471
Degrees of freedom: 120 total; 114 residual
```

```
# Variance-covariance matrix
(mod_vcov <- vcov(m2_mod))
```

```
                    log10n0.(Intercept) log10n0.matrixs TD.(Intercept)
log10n0.(Intercept)             0.03293        -0.03293        -0.2238
log10n0.matrixs                -0.03293         0.06693         0.2238
TD.(Intercept)                 -0.22383         0.22383         1.7353
TD.matrixs                      0.22383        -0.47576        -1.7353
beta.(Intercept)               -0.00887         0.00887         0.0728
beta.matrixs                    0.00887        -0.01827        -0.0728
                    TD.matrixs beta.(Intercept) beta.matrixs
log10n0.(Intercept)     0.2238         -0.00887      0.00887
log10n0.matrixs        -0.4758          0.00887     -0.01827
TD.(Intercept)         -1.7353          0.07281     -0.07281
TD.matrixs              3.8430         -0.07281      0.15489
beta.(Intercept)       -0.0728          0.00381     -0.00381
beta.matrixs            0.1549         -0.00381      0.00792
```

```
# Overall marginal means
emmeans (m2_mod, ~ 1, param = 'log10n0')
```

```
 1       emmean    SE  df lower.CL upper.CL
 overall   7.16 0.129 113     6.91     7.42

Results are averaged over the levels of: matrix 
Confidence level used: 0.95 
```

```
emmeans (m2_mod, ~ 1, param = 'beta')
```

```
 1       emmean     SE  df lower.CL upper.CL
 overall  0.648 0.0445 113     0.56    0.736
```

```
Results are averaged over the levels of: matrix
Confidence level used: 0.95
```

```
emmeans (m2_mod, ~ 1, param = 'TD')
```

```
 1        emmean   SE  df lower.CL upper.CL
 overall   11.7 0.98 113     9.79     13.7

Results are averaged over the levels of: matrix
Confidence level used: 0.95
```

```
TD_values <- readRDS (here('obj2', 'results', "TD_values.RDS"))
TD_values$ecoli_plate_3D = emmeans (m2_mod, ~ 1, param = 'TD')
saveRDS (TD_values, here('obj2', 'results', "TD_values.RDS"))

# Remove selected objects from the environment
rm(list = setdiff(ls(envir = .GlobalEnv, all.names = TRUE), list_exc),
   envir = .GlobalEnv)
```

## 5.3 *Tulane virus TCID50*

```
m2 <- readRDS(here('obj2', 'data','Obj2_dat.RDS')) |>
  dplyr::filter (microbe=='tv', assay=='tcid50') |>
  droplevels()

m2_mod_wbrepar = nlme::gnls(survival ~ wbrepar(x = week,
                               log10n0 = log10n0,
                               TD = TD,
                               beta = beta),
                 data = m2,
                 params = log10n0+TD+beta ~ matrix,
                 start = list(log10n0 = c(5, 0),
                              TD = c(5, 0),
                              beta = c(2, 0)))

(m2_mod <- m2_mod_wbrepar)
```

```
Generalized nonlinear least squares fit
  Model: survival ~ wbrepar(x = week, log10n0 = log10n0, TD = TD, beta =
beta)
  Data: m2
  Log-likelihood: 24.2

Coefficients:
log10n0.(Intercept)     log10n0.matrixs      TD.(Intercept)
TD.matrixs
             4.7517              0.1390             21.5708             -
0.1663
   beta.(Intercept)        beta.matrixs
             0.8288             -0.0182
```

```
Degrees of freedom: 120 total; 114 residual
Residual standard error: 0.203

summary (m2_mod)

Generalized nonlinear least squares fit
  Model: survival ~ wbrepar(x = week, log10n0 = log10n0, TD = TD, beta =
beta)
  Data: m2
    AIC BIC logLik
  -34.5 -15   24.2

Coefficients:
                    Value Std.Error t-value p-value
log10n0.(Intercept)  4.75     0.067    70.5   0.000
log10n0.matrixs      0.14     0.096     1.4   0.150
TD.(Intercept)      21.57     0.693    31.1   0.000
TD.matrixs          -0.17     0.992    -0.2   0.867
beta.(Intercept)     0.83     0.046    17.8   0.000
beta.matrixs        -0.02     0.065    -0.3   0.781

 Correlation:
                 l100.( lg100. TD.(I) TD.mtr bt.(I)
log10n0.matrixs  -0.702
TD.(Intercept)   -0.881  0.619
TD.matrixs        0.615 -0.883 -0.698
beta.(Intercept) -0.761  0.534  0.624 -0.436
beta.matrixs      0.543 -0.763 -0.446  0.629 -0.714

Standardized residuals:
    Min      Q1     Med      Q3     Max
-2.9461 -0.6764  0.0461  0.6183  2.2847

Residual standard error: 0.203
Degrees of freedom: 120 total; 114 residual

# Variance-covariance matrix
(mod_vcov <- vcov(m2_mod))

                    log10n0.(Intercept) log10n0.matrixs TD.(Intercept)
log10n0.(Intercept)             0.00455        -0.00455        -0.0412
log10n0.matrixs                -0.00455         0.00922         0.0412
TD.(Intercept)                 -0.04116         0.04116         0.4801
TD.matrixs                      0.04116        -0.08414        -0.4801
beta.(Intercept)               -0.00239         0.00239         0.0201
beta.matrixs                    0.00239        -0.00477        -0.0201
                    TD.matrixs beta.(Intercept) beta.matrixs
log10n0.(Intercept)     0.0412         -0.00239      0.00239
log10n0.matrixs        -0.0841          0.00239     -0.00477
TD.(Intercept)         -0.4801          0.02011     -0.02011
TD.matrixs              0.9843         -0.02011      0.04064
beta.(Intercept)       -0.0201          0.00216     -0.00216
beta.matrixs            0.0406         -0.00216      0.00424

# Overall marginal means
emmeans (m2_mod, ~ 1, param = 'log10n0')

 1       emmean    SE  df lower.CL upper.CL
 overall   4.82 0.048 113     4.73     4.92

Results are averaged over the levels of: matrix
Confidence level used: 0.95

emmeans (m2_mod, ~ 1, param = 'TD')

 1       emmean    SE  df lower.CL upper.CL
 overall   21.5 0.496 113     20.5     22.5

Results are averaged over the levels of: matrix
Confidence level used: 0.95

emmeans (m2_mod, ~ 1, param = 'beta')

 1       emmean     SE  df lower.CL upper.CL
 overall   0.82 0.0326 113    0.755    0.884

Results are averaged over the levels of: matrix
Confidence level used: 0.95

TD_values <- readRDS (here('obj2', 'results', "TD_values.RDS"))
TD_values$tv_tcid50_3D = emmeans (m2_mod, ~ 1, param = 'TD')
saveRDS (TD_values, here('obj2', 'results', "TD_values.RDS"))

# Remove selected objects from the environment
rm(list = setdiff(ls(envir = .GlobalEnv, all.names = TRUE), list_exc),
   envir = .GlobalEnv)
```

### 5.4 TuV and HuNoV - *RNase RT-qPCR*

```
m2 <- readRDS(here('obj2', 'data', 'Obj2_dat.RDS')) |>
  dplyr::filter (assay=='pcr') |>
  droplevels()

m2_mod_llrepar = nlme::gnls(survival ~ llrepar(x = week,
                                               log10n0 = log10n0,
                                               TD = TD),
                            data = m2,
                            params = log10n0+TD ~ matrix*microbe,
                 start = list(log10n0 = c(4, rep(0,3)),
                              TD = c(25, rep(0,3))))

m2_mod_exp_wk = update(m2_mod_llrepar, weights = varExp(form = ~week))
```

```
(m2_mod <- m2_mod_exp_wk)
```

```
Generalized nonlinear least squares fit
  Model: survival ~ llrepar(x = week, log10n0 = log10n0, TD = TD)
  Data: m2
  Log-likelihood: 20.4

Coefficients:
      log10n0.(Intercept)           log10n0.matrixs         log10n0.microbetv
                    3.868                     0.100                     3.661
log10n0.matrixs:microbetv            TD.(Intercept)                TD.matrixs
                    0.058                    89.183                    -9.909
             TD.microbetv      TD.matrixs:microbetv
                  -51.876                     6.406

Variance function:
 Structure: Exponential of variance covariate
 Formula: ~week
 Parameter estimates:
  expon
-0.0259
Degrees of freedom: 240 total; 232 residual
Residual standard error: 0.305
```

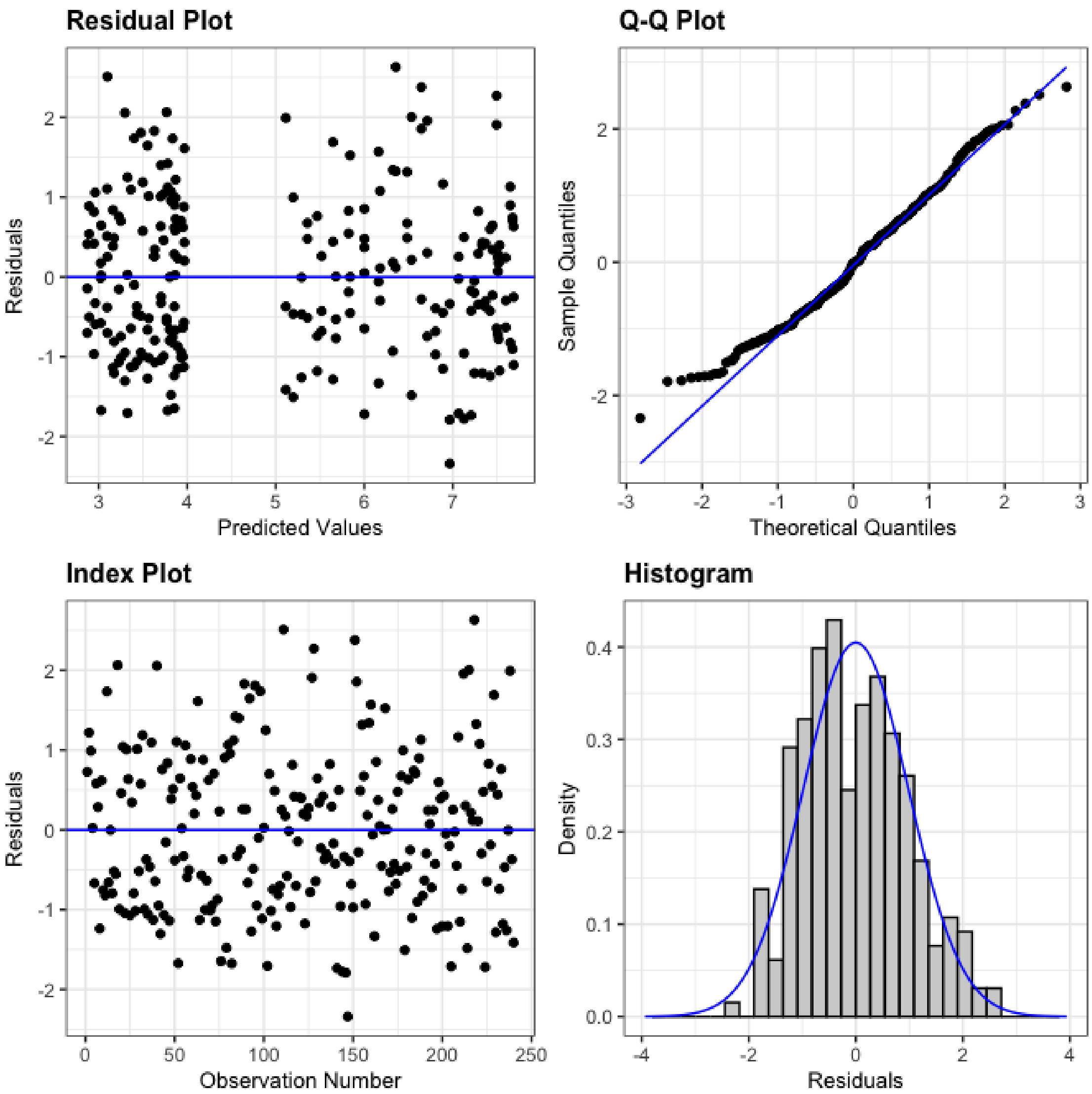
```
summary (m2_mod)
```

```
Generalized nonlinear least squares fit
  Model: survival ~ llrepar(x = week, log10n0 = log10n0, TD = TD)
  Data: m2
    AIC BIC logLik
  -20.8  14   20.4

Variance function:
 Structure: Exponential of variance covariate
 Formula: ~week
 Parameter estimates:
  expon
-0.0259

Coefficients:
                          Value Std.Error t-value p-value
log10n0.(Intercept)         3.9      0.05    71.0   0.000
log10n0.matrixs             0.1      0.08     1.3   0.194
log10n0.microbetv           3.7      0.08    47.5   0.000
log10n0.matrixs:microbetv   0.1      0.11     0.5   0.595
TD.(Intercept)             89.2      7.58    11.8   0.000
TD.matrixs                 -9.9      9.66    -1.0   0.306
TD.microbetv              -51.9      7.69    -6.7   0.000
TD.matrixs:microbetv        6.4      9.81     0.7   0.514
```

```
 Correlation:
                          l100.( lg10n0.mt lg10n0.mc l100.: TD.(I) TD.mtr
log10n0.matrixs           -0.707
log10n0.microbetv         -0.707  0.500
log10n0.matrixs:microbetv  0.500 -0.707    -0.707
TD.(Intercept)            -0.865  0.611     0.611    -0.432
TD.matrixs                 0.678 -0.859    -0.480     0.607 -0.785
TD.microbetv               0.852 -0.602    -0.708     0.500 -0.985  0.773
TD.matrixs:microbetv      -0.668  0.845     0.555    -0.704  0.773 -0.985
                          TD.mcr
log10n0.matrixs
log10n0.microbetv
log10n0.matrixs:microbetv
TD.(Intercept)
TD.matrixs
TD.microbetv
TD.matrixs:microbetv      -0.784

Standardized residuals:
    Min      Q1     Med      Q3     Max
-2.3405 -0.7486 -0.0182  0.6523  2.6306

Residual standard error: 0.305
Degrees of freedom: 240 total; 232 residual
```

```
# Residual plots
m2_res_m2_mod = resid(m2_mod, type = 'normalized')
m2_fit_m2_mod = fitted(m2_mod)
resid_auxpanel(m2_res_m2_mod, m2_fit_m2_mod)
```

```
Warning: Removed 2 rows containing missing values or values outside the scale
range
(`geom_bar()`).
```



```
# Overall marginal mean
emmeans (m2_mod, ~ microbe, param = 'log10n0')

NOTE: Results may be misleading due to involvement in interactions

 microbe emmean     SE  df lower.CL upper.CL
 nv        3.92 0.0385 230     3.84     3.99
 tv        7.61 0.0385 230     7.53     7.68

Results are averaged over the levels of: matrix
Confidence level used: 0.95

emmeans (m2_mod, ~ matrix, param = 'TD')

NOTE: Results may be misleading due to involvement in interactions

 matrix emmean   SE  df lower.CL upper.CL
 ps       63.2 3.85 230     55.7     70.8
 s         56.5 3.04 230     50.5     62.5

Results are averaged over the levels of: microbe 
Confidence level used: 0.95 
```

```r
emmeans (m2_mod, ~ microbe, param = 'TD')
```

```
NOTE: Results may be misleading due to involvement in interactions

 microbe emmean   SE  df lower.CL upper.CL
 nv        84.2 4.83 230     74.7     93.7
 tv        35.6 0.86 230     33.9     37.2

Results are averaged over the levels of: matrix 
Confidence level used: 0.95 
```

```r
TD_values <- readRDS (here('obj2', 'results', "TD_values.RDS"))
TD_values$nv_pcr_3D = emmeans (m2_mod, ~ microbe, param = 'TD')[1]
```

```
NOTE: Results may be misleading due to involvement in interactions
```

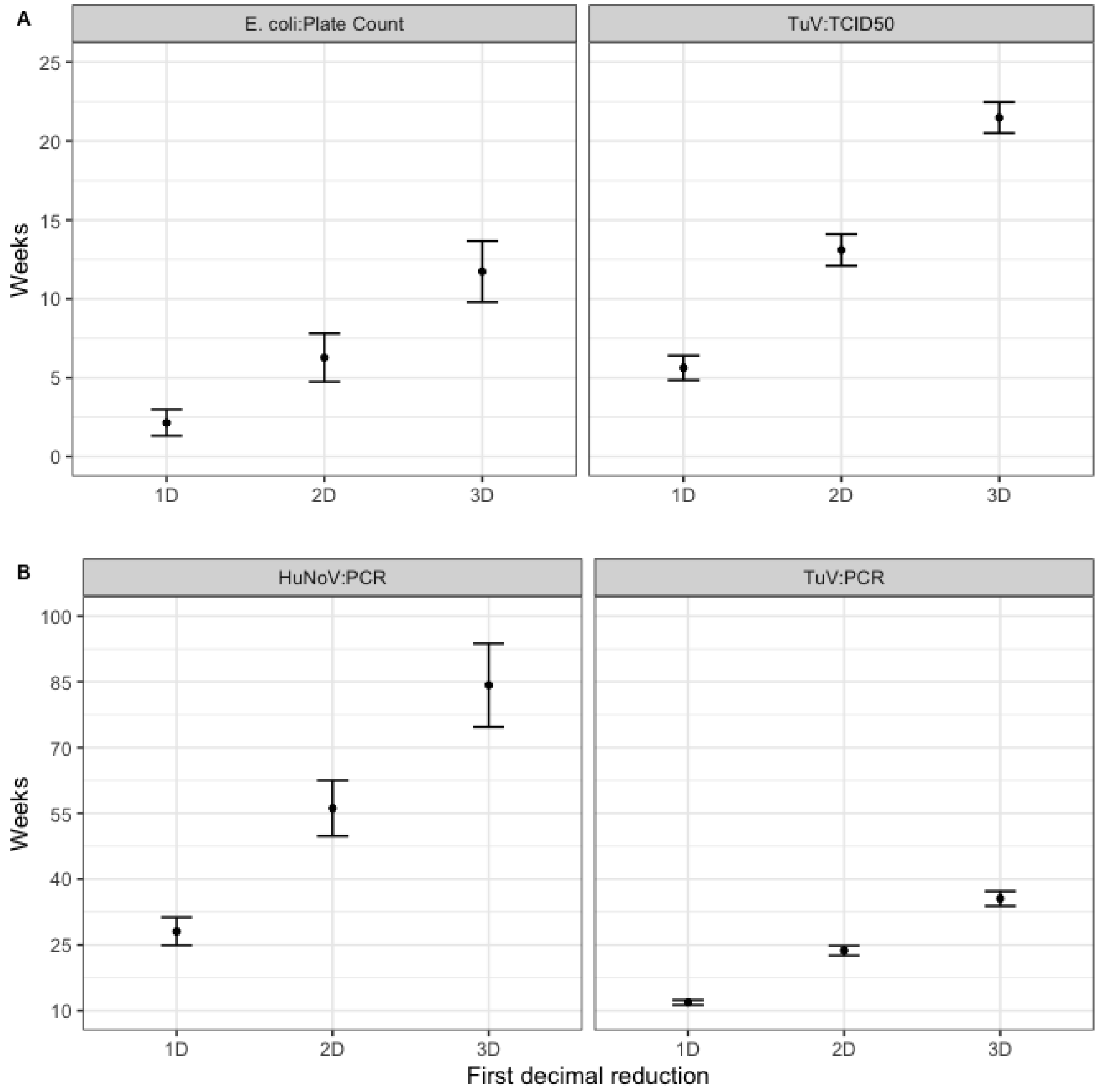
```r
TD_values$tv_pcr_3D = emmeans (m2_mod, ~ microbe, param = 'TD')[2]
```

```
NOTE: Results may be misleading due to involvement in interactions
```

```r
saveRDS (TD_values, here('obj2', 'results', "TD_values.RDS"))

# Remove selected objects from the environment
rm(list = setdiff(ls(envir = .GlobalEnv, all.names = TRUE), list_exc),
   envir = .GlobalEnv)
```

## 6. Combined TD values

```r
TD_values <- readRDS(here("obj2", "results", "TD_values.RDS"))

TD_df1 <- imap_dfr(TD_values, function(x, id) {
  TD_sum <- as.data.frame(summary(x))

  TD_sum <- TD_sum |>
    dplyr::mutate(ID = factor(id)) |>
    dplyr::select(ID, emmean, lower.CL, upper.CL)

    return(TD_sum)
})

TD_df2 <- TD_df1 |>
  separate(ID, into = c("microbe", "assay", "TnD"), sep = "_") %>%
  mutate(
    microbe = factor(microbe),
    TnD = factor(TnD, levels = c("1D", "2D", "3D")),
    assay = factor(assay)
  )

TD_df2 <- TD_df2 |>
  dplyr::mutate(
    microbe = recode(microbe,
                     "tv" = "TuV",
                     "nv" = "HuNoV",
                     "ecoli" = "E. coli"),
    assay = recode(assay,
                   "pcr" = "PCR",
                   "tcid50" = "TCID50",
                   "plate" = "Plate Count")
  )

nv_tv <- TD_df2 |>
  filter (assay == "PCR") |>
  ggplot(aes(x = TnD, y = emmean)) +
  geom_point(size = 1.1) +
  facet_wrap ( . ~ microbe:assay) +
  geom_errorbar(aes(ymin = lower.CL, ymax = upper.CL),
                width = 0.2) +
  scale_y_continuous(
    breaks = seq(10, 100, 15),
    labels = seq(10, 100, 15),
    limits = c(10, 100)) +
  labs(
    x = "First decimal reduction",
    y = "Weeks") +
  theme_bw()

ec_tv <- TD_df2 |>
  filter (assay != "PCR") |>
  ggplot(aes(x = TnD, y = emmean)) +
  geom_point(size = 1.1) +
  facet_wrap ( . ~ microbe:assay) +
  geom_errorbar(aes(ymin = lower.CL, ymax = upper.CL),
                width = 0.2) +
  scale_y_continuous(
    breaks = seq(0, 25, 5),
    labels = seq(0, 25, 5),
    limits = c(0, 25)) +
  labs(
    x = "",
    y = "Weeks") +
  theme_bw()

combined_plots <- ggpubr::ggarrange (ec_tv,  nv_tv,
                                     labels = c("A", "B"),
                                     font.label = list(size = 9, face =
"bold"),
                                         label.x = 0.01,
                                         label.y = 0.98,
                                         nrow=2, ncol=1)

combined_plots
```



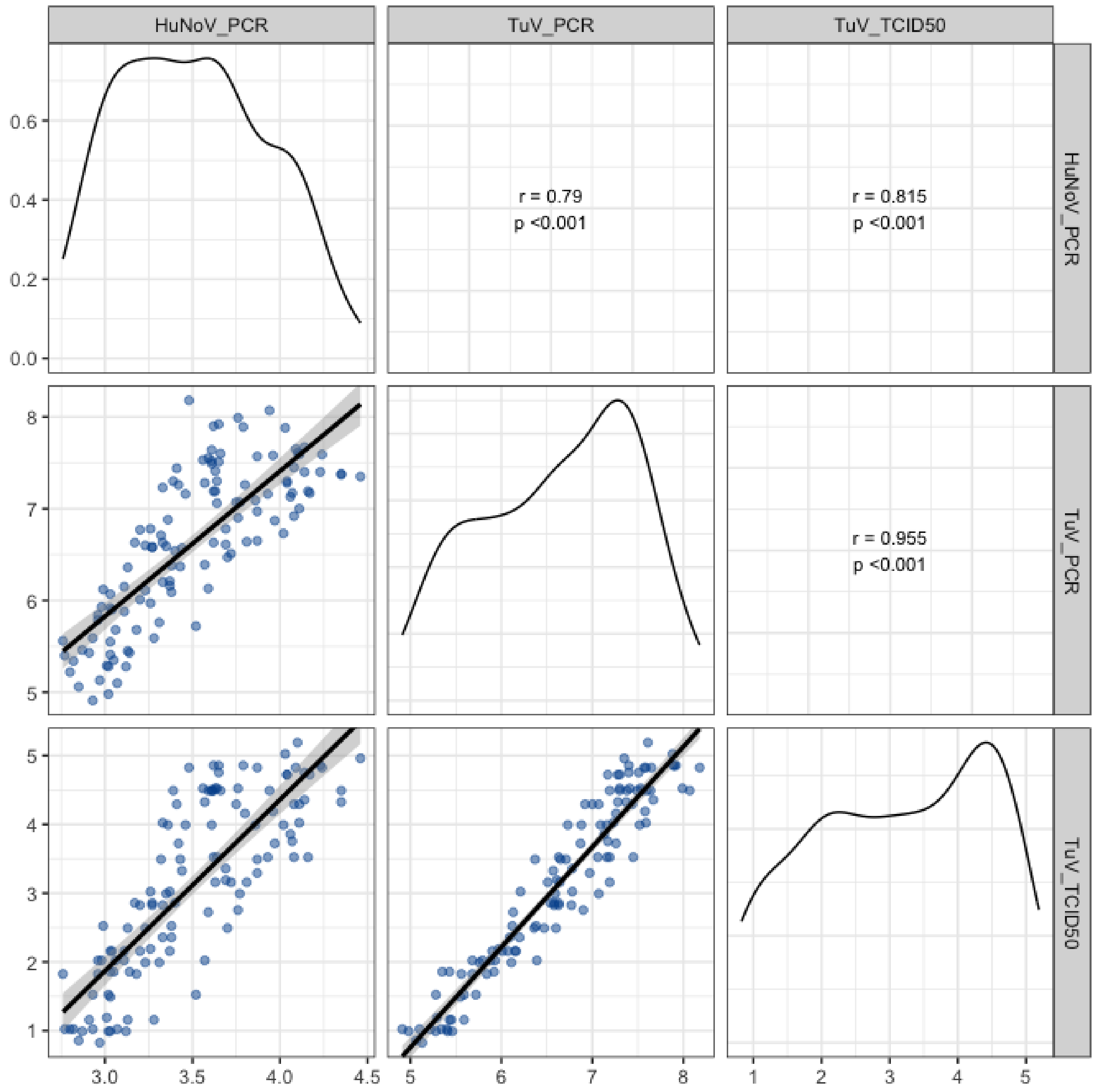
```
#ggsave(filename = here('obj2', 'results', 'Fig S1 TnD_plot.tiff'), plot =
combined_plots, width = 11, height = 10, units = "cm", dpi = 600)

TD_df1 |>
  mutate(
    Estimate_CI = sprintf("%.2f [%.2f, %.2f]", emmean, lower.CL, upper.CL)
  ) |>
  dplyr::select(ID, Estimate_CI)
```

```
               ID          Estimate_CI
1  ecoli_plate_1D    2.15 [1.32, 2.98]
2    tv_tcid50_1D    5.63 [4.84, 6.41]
3       nv_pcr_1D 28.08 [24.91, 31.25]
4       tv_pcr_1D 11.85 [11.29, 12.42]
5  ecoli_plate_2D    6.28 [4.74, 7.81]
6    tv_tcid50_2D 13.10 [12.10, 14.10]
7       nv_pcr_2D 56.15 [49.81, 62.50]
8       tv_pcr_2D 23.70 [22.58, 24.83]
9  ecoli_plate_3D  11.73 [9.79, 13.68]
10   tv_tcid50_3D 21.49 [20.50, 22.47]
11      nv_pcr_3D 84.23 [74.72, 93.74]
12      tv_pcr_3D 35.56 [33.87, 37.25]
```

```
# Remove selected objects from the environment
rm(list = setdiff(ls(envir = .GlobalEnv, all.names = TRUE), list_exc),
   envir = .GlobalEnv)
```

## 7. Correlation matrix

```
# Reshape data
m2 <- readRDS(here('obj2', 'data', 'Obj2_dat.RDS')) |>
  dplyr::filter (microbe=="nv" | microbe=="tv") |>
  dplyr::mutate(id = paste(microbe, assay, sep = "_")) |>
  dplyr::mutate(id = factor(id)) |>
  dplyr::select (survival, week, id) |>
  droplevels()

m2_wide <- m2 |>
  group_by(id, week) |>
  mutate(row = row_number()) |>
  pivot_wider(names_from = id, values_from = survival) |>
  as.data.frame() |>
  ungroup()

# rename columns
label_ids <- c(
  "HuNoV_PCR" = "nv_pcr",
  "TuV_PCR" = "tv_pcr",
  "TuV_TCID50" = "tv_tcid50")

m2_wide <- m2_wide |>
  dplyr::select(-week, -row) |>
  rename(!!!label_ids)

# Correlation matrix
# Custom function to show both r and p-values
cor_fun <- function(data, mapping, ...){
  x <- eval_data_col(data, mapping$x)
  y <- eval_data_col(data, mapping$y)
  cor_test <- cor.test(x, y)
  r <- round(cor_test$estimate, 3)
  p <- cor_test$p.value
  txt <- paste0("r = ", r, "\n",
                "p ", ifelse(p < 0.001, "<0.001", round(p, 3)))
  ggplot(data = data, mapping = mapping) +
    annotate("text", x = 0.5, y = 0.5, label = txt, size = 3, hjust = 0.5) +
    theme_void()
}

p_m2 <- GGally::ggpairs (m2_wide,
        lower = list(continuous = wrap("smooth",
                                        method = "lm",
                                        alpha=0.5,
                                        se = TRUE,
                                        color = "#00529b")),
        upper = list(continuous = cor_fun),
        diag = list(continuous = wrap("densityDiag",
                                       color = "#000"))
        ) + theme_bw()

p_m2
```



```
ggsave(file = here('obj2', 'results', 'Fig 3 cor_matrix.tiff'),
       plot = p_m2,
       width=12, height=12, units = c("cm"), dpi=400)

# Correlations with 95% CI
cor.test(m2_wide$TuV_PCR,
         m2_wide$HuNoV_PCR,
         use = "pairwise.complete.obs")


    Pearson's product-moment correlation

data:  m2_wide$TuV_PCR and m2_wide$HuNoV_PCR
t = 14, df = 118, p-value <2e-16
alternative hypothesis: true correlation is not equal to 0
95 percent confidence interval:
 0.712 0.849
sample estimates:
 cor
0.79

cor.test(m2_wide$TuV_PCR,
         m2_wide$TuV_TCID50,
         use = "pairwise.complete.obs")


    Pearson's product-moment correlation

data:  m2_wide$TuV_PCR and m2_wide$TuV_TCID50
t = 35, df = 118, p-value <2e-16
alternative hypothesis: true correlation is not equal to 0
95 percent confidence interval:
 0.936 0.969
sample estimates:
  cor
0.955

cor.test(m2_wide$HuNoV_PCR,
         m2_wide$TuV_TCID50,
         use = "pairwise.complete.obs")


    Pearson's product-moment correlation

data:  m2_wide$HuNoV_PCR and m2_wide$TuV_TCID50
t = 15, df = 118, p-value <2e-16
alternative hypothesis: true correlation is not equal to 0
95 percent confidence interval:
 0.745 0.868
sample estimates:
  cor
0.815

# Remove selected objects from the environment
rm(list = setdiff(ls(envir = .GlobalEnv, all.names = TRUE), list_exc),
   envir = .GlobalEnv)
```

## 8. PCR:Inf ratio for Tulane virus

```
readRDS(here('obj2', 'data', 'Obj2_dat.RDS')) |>
  dplyr::filter (microbe=='tv') |>
  droplevels() |>
  dplyr::select(matrix, week, replicate, assay, survival) |>
  tidyr::pivot_wider(names_from = assay, values_from = survival) |>
  dplyr::mutate(gc2inf = pcr-tcid50,
                week_fac = as.factor(week)) |>
  saveRDS(here('obj2', 'results', 'gc2inf.RDS'))

m2 <- readRDS(here('obj2', 'results', 'gc2inf.RDS'))

# Ratio across matrices
lm2 <- nlme::gls(gc2inf ~ matrix * week_fac,
            weights = varIdent(form = ~1 | week_fac),
            data = m2)

# Overall significant differences
joint_tests(lm2, by = NULL)

 model term      df1  df2 F.ratio p.value
 matrix            1 49.2   1.300  0.2630
 week_fac         19  4.4  37.300  0.0010
 matrix:week_fac  19  4.4   1.100  0.5180

joint_tests(lm2, by = "week_fac")

week_fac = 0:
 model term df1  df2 F.ratio p.value
 matrix       1 8.27   0.420  0.5350

week_fac = 0.142857142857143:
 model term df1  df2 F.ratio p.value
 matrix       1 3.92   0.090  0.7770

week_fac = 0.428571428571429:
 model term df1  df2 F.ratio p.value
 matrix       1 3.98   0.080  0.7870

week_fac = 1:
 model term df1  df2 F.ratio p.value
 matrix       1 2.47   0.060  0.8220

week_fac = 2:
 model term df1  df2 F.ratio p.value
 matrix       1 1.82   0.050  0.8510

week_fac = 3:
 model term df1  df2 F.ratio p.value
 matrix       1 4.00   0.010  0.9160

week_fac = 4:
 model term df1  df2 F.ratio p.value
 matrix       1 3.99   0.460  0.5330

week_fac = 5:
 model term df1  df2 F.ratio p.value
 matrix       1 3.99   0.120  0.7450
```

```
week_fac = 7:
 model term df1  df2 F.ratio p.value
 matrix       1 2.84   1.770  0.2800

week_fac = 9:
 model term df1  df2 F.ratio p.value
 matrix       1 4.00   1.570  0.2780

week_fac = 11:
 model term df1  df2 F.ratio p.value
 matrix       1 3.98   6.040  0.0700

week_fac = 13:
 model term df1  df2 F.ratio p.value
 matrix       1 2.85   1.620  0.2960

week_fac = 15:
 model term df1  df2 F.ratio p.value
 matrix       1 3.99   0.310  0.6070

week_fac = 17:
 model term df1  df2 F.ratio p.value
 matrix       1 3.91   0.020  0.9010

week_fac = 19:
 model term df1  df2 F.ratio p.value
 matrix       1 4.00   5.650  0.0760

week_fac = 21:
 model term df1  df2 F.ratio p.value
 matrix       1 3.94   0.460  0.5340

week_fac = 23:
 model term df1  df2 F.ratio p.value
 matrix       1 3.96   0.810  0.4190

week_fac = 25:
 model term df1  df2 F.ratio p.value
 matrix       1 3.99   1.980  0.2330

week_fac = 27:
 model term df1  df2 F.ratio p.value
 matrix       1 4.00   8.980  0.0400

week_fac = 29:
 model term df1  df2 F.ratio p.value
 matrix       1 3.61   0.070  0.8050
```

```
joint_tests(lm2, by = "matrix")

matrix = ps:
 model term df1  df2 F.ratio p.value
 week_fac     19 4.44  23.070  0.0024

matrix = s:
 model term df1  df2 F.ratio p.value
 week_fac     19 4.44  15.290  0.0057

# Pairwise comparisons between matrices at each week level
# emmeans(lm2, ~ matrix | week_fac, mode = "df.error") |>
#  contrast(method = "pairwise", adjust = "none", infer = TRUE)

# Ratio across matrices at each week
emmeans(lm2, ~ week_fac | matrix, mode = "df.error") |>
  multcomp::cld(decreasing = TRUE, Letters = letters) |>
  arrange(week_fac)

matrix = ps:
 week_fac          emmean     SE df lower.CL upper.CL .group
 0                   2.68 0.1350 61     2.41     2.95        g
 0.142857142857143   2.80 0.1630 61     2.48     3.13       fg
 0.428571428571429   3.17 0.2720 61     2.63     3.71   bcdefg
 1                   3.03 0.1310 61     2.77     3.29      efg
 2                   3.02 0.1300 61     2.76     3.28      efg
 3                   3.09 0.2500 61     2.59     3.59    cdefg
 4                   3.00 0.2690 61     2.46     3.54    cdefg
 5                   3.28 0.2310 61     2.82     3.74   bcdefg
 7                   3.04 0.1420 61     2.76     3.32      efg
 9                   3.32 0.0592 61     3.20     3.43     def
 11                  3.96 0.0755 61     3.80     4.11   bc
 13                  3.78 0.1410 61     3.50     4.06   bcd
 15                  3.80 0.1660 61     3.46     4.13  abcde
 17                  3.90 0.1700 61     3.56     4.25  abcd
 19                  3.92 0.0513 61     3.82     4.02   bc
 21                  3.97 0.1000 61     3.77     4.17  abc
 23                  4.12 0.1740 61     3.77     4.46  abc
 25                  4.15 0.1890 61     3.77     4.53  abc
 27                  4.34 0.0516 61     4.24     4.45  a
 29                  4.20 0.1270 61     3.95     4.46  ab

matrix = s:
 week_fac          emmean     SE df lower.CL upper.CL .group
 0                   2.56 0.1350 61     2.29     2.83       f
 0.142857142857143   2.87 0.1630 61     2.55     3.20      ef
 0.428571428571429   3.06 0.2720 61     2.51     3.60   bcdef
 1                   2.99 0.1310 61     2.72     3.25      ef
 2                   3.06 0.1300 61     2.80     3.32      ef
 3                   3.05 0.2500 61     2.55     3.55    cdef
 4                   3.26 0.2690 61     2.72     3.80  abcdef
 5                   3.17 0.2310 61     2.71     3.63    cdef
 7                   3.31 0.1420 61     3.02     3.59    cde
 9                   3.42 0.0592 61     3.30     3.54     de
 11                  3.69 0.0755 61     3.54     3.84   bcd
 13                  3.53 0.1410 61     3.25     3.81   bcde
 15                  3.93 0.1660 61     3.59     4.26  abcd
 17                  3.94 0.1700 61     3.60     4.28  abcd
 19                  3.75 0.0513 61     3.65     3.85   bc
 21                  3.87 0.1000 61     3.67     4.07  abc
 23                  3.89 0.1740 61     3.54     4.24  abcd
 25                  3.77 0.1890 61     3.39     4.15  abcde
 27                  4.12 0.0516 61     4.02     4.23  a
 29                  4.16 0.1270 61     3.90     4.41  ab

Degrees-of-freedom method: df.error 
Confidence level used: 0.95 
P value adjustment: tukey method for comparing a family of 20 estimates 
significance level used: alpha = 0.05 
NOTE: If two or more means share the same grouping symbol,
      then we cannot show them to be different.
      But we also did not show them to be the same.
```

```
# ratio at weeks 0 and 29
em2 <- emmeans(lm2, ~ week_fac, mode = "df.error")

summary(em2) |>
  dplyr::filter(week_fac == c('0', '29'))
```

```
 week_fac emmean     SE df lower.CL upper.CL
 0          2.62 0.0955 61     2.43     2.81
 29         4.18 0.0896 61     4.00     4.36

Results are averaged over the levels of: matrix 
Degrees-of-freedom method: df.error 
Confidence level used: 0.95 
```

```
# Overal average at each matrix level
(em2 <- emmeans(lm2, ~ matrix, mode = "df.error"))
```

```
 matrix emmean     SE df lower.CL upper.CL
 ps       3.53 0.0369 61     3.45     3.60
 s        3.47 0.0369 61     3.40     3.54

Results are averaged over the levels of: week_fac 
Degrees-of-freedom method: df.error 
Confidence level used: 0.95 
```

```
# contrast(em2, method = "pairwise", adjust = "none", infer = TRUE)
# Remove selected objects from the environment
rm(list = setdiff(ls(envir = .GlobalEnv, all.names = TRUE), list_exc),
   envir = .GlobalEnv)
```

## 9. Convert TCID50 to PFU

```
# based on the median and CI calculated from simulated ratios from FEV
article (to be cited once the article is out). Median Log10 TCID50:PFU of
-0.65 [95%CI: -1.15, -0.16]. TCID50-PFU = log ratio => PFU = TCID50 - log
ratio

# Convert using raw data
readRDS(here('obj2', 'data', 'Obj2_dat.RDS')) |>
  dplyr::filter (microbe=='tv' & assay=='tcid50') |>
  droplevels() |>
  group_by(week) |>
  summarise(
    survival_ave = mean(survival, na.rm = TRUE)) |>
  dplyr::mutate(
    pfu_med = survival_ave + 0.65,
    pfu_lwr = survival_ave + 0.16,
    pfu_upr = survival_ave + 1.15) |>
  as.data.frame()

     week survival_ave pfu_med pfu_lwr pfu_upr
1   0.000        4.924    5.57    5.08    6.07
2   0.143        4.768    5.42    4.93    5.92
3   0.429        4.695    5.35    4.86    5.85
4   1.000        4.518    5.17    4.68    5.67
5   2.000        4.368    5.02    4.53    5.52
6   3.000        4.269    4.92    4.43    5.42
7   4.000        4.014    4.66    4.17    5.16
8   5.000        3.796    4.45    3.96    4.95
9   7.000        3.591    4.24    3.75    4.74
10  9.000        3.379    4.03    3.54    4.53
11 11.000        3.063    3.71    3.22    4.21
12 13.000        2.969    3.62    3.13    4.12
13 15.000        2.640    3.29    2.80    3.79
14 17.000        2.285    2.93    2.44    3.43
15 19.000        2.146    2.80    2.31    3.30
16 21.000        1.979    2.63    2.14    3.13
17 23.000        1.646    2.30    1.81    2.80
18 25.000        1.479    2.13    1.64    2.63
19 27.000        1.063    1.71    1.22    2.21
20 29.000        0.957    1.61    1.12    2.11

# Remove selected objects from the environment
rm(list = setdiff(ls(envir = .GlobalEnv, all.names = TRUE), list_exc),
   envir = .GlobalEnv)
```

# 10. Weather/soil conditions

## 10.1 Soil moisture changes

```
dat1 <- tidyr::tibble(
  week = factor(rep(c("0", "29"), each = 6)),
  matrix = factor(rep(c("s", "ps"), each = 3, times = 2)),
  moisture = as.numeric(c(
    4.319, 4.102, 5.086,
    5.932, 5.434, 5.330,
    4.096, 3.347, 4.096,
    3.717, 3.635, 4.589)
    ))

# Average moisture

dat1 |>
  group_by(matrix, week) |>
  do({
    data.frame(
      moisture_per = mean(.$moisture),
      sd = stats::sd(.$moisture))
  })
```

```
# A tibble: 4 × 4
# Groups:   matrix, week [4]
  matrix week  moisture_per    sd
  <fct>  <fct>        <dbl> <dbl>
1 ps     0             5.57 0.322
2 ps     29            3.98 0.529
3 s      0             4.50 0.517
4 s      29            3.85 0.432
```

```
dat1 |>
  group_by(week) |>
  do({
    data.frame(
      moisture_per = mean(.$moisture),
      sd = stats::sd(.$moisture))
  })
```

```
# A tibble: 2 × 3
# Groups:   week [2]
  week  moisture_per    sd
  <fct>        <dbl> <dbl>
1 0             5.03 0.698
2 29            3.91 0.438
```

```
fit1 <- aov(moisture ~ matrix * week, data=dat1)

joint_tests (fit1)
```

```
 model term   df1 df2 F.ratio p.value
 matrix         1   8   5.130  0.0532
 week           1   8  17.990  0.0028
 matrix:week    1   8   3.090  0.1167
```

```
emmeans(fit1, ~ matrix * week) |>
  pairs()
```

```
 contrast              estimate    SE df t.ratio p.value
 ps week0 - s week0       1.063 0.374  8   2.850  0.0830
 ps week0 - ps week29     1.585 0.374  8   4.240  0.0120
 ps week0 - s week29      1.719 0.374  8   4.600  0.0080
 s week0 - ps week29      0.522 0.374  8   1.400  0.5340
 s week0 - s week29       0.656 0.374  8   1.760  0.3580
 ps week29 - s week29     0.134 0.374  8   0.360  0.9830

P value adjustment: tukey method for comparing a family of 4 estimates
```

## 10.2 Monthly soil temperature

Data were collected from the UF/IFAS Florida Automated Weather Network (FAWN): https://fawn.ifas.ufl.edu (Live Oak, FL station)

```
dat1 <- read.csv(here('obj2', 'data', 'FAWN_report_2022_2025 daily.csv')) |>
  dplyr::mutate(
    Tweather.c = (`X10m.T.avg..F.`-32) * (5/9),
    Tsoil.c = (`Tsoil.avg.10cm...F.` -32) * (5/9),
    rain.cm = `X2m.Rain.tot..in.` * 2.54
    )
```

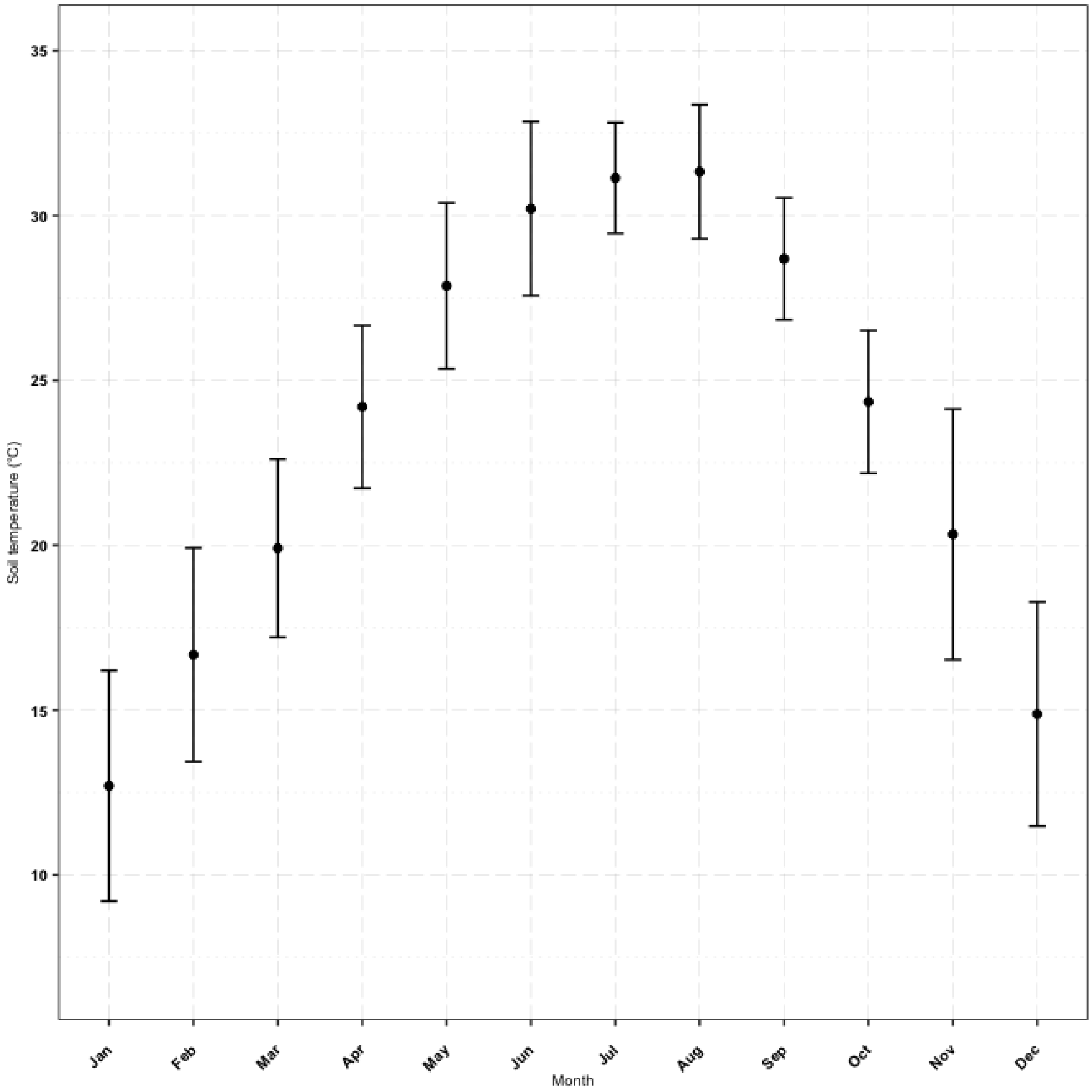
```
str (dat1)
```

```
'data.frame':   1096 obs. of  18 variables:
 $ FAWN.Station                : chr  "Live Oak" "Live Oak" "Live Oak" "Live
Oak" ...
 $ Period                      : chr  "1 Aug 2022" "2 Aug 2022" "3 Aug 2022"
"4 Aug 2022" ...
 $ X10m.T.avg..F.              : num  81.6 82.2 80.7 80.8 82.4 ...
 $ X10m.T.min..F.              : num  72.9 72.4 73.6 71.2 73.6 ...
 $ X10m.T.max..F.              : num  93.1 93.4 92.6 92.1 94.2 ...
 $ Tsoil.avg.10cm...F.         : num  93.7 92.9 94.3 91.6 93.2 ...
 $ Tsoil.min.avg..10cm...F.    : num  88.4 86.4 88.9 84.7 86.3 ...
 $ Tsoil.max.avg..10cm...F.    : num  99.5 100.4 101.1 99.7 100.8 ...
 $ X2m.DewPt.avg..F.           : num  75.4 74.3 74.3 74.8 74.9 ...
 $ RelHum.avg.2m...pct.        : int  82 77 81 81 78 87 81 80 83 84 ...
 $ X2m.Rain.tot..in.           : num  0.01 0 0.2 0 0 0 0 0 0.02 0 ...
 $ X2m.Rain.max.over.15min..in.: num  0.01 0 0.1 0 0 0 0 0 0.02 0 ...
 $ SolRad.avg2m...w.m.2.       : num  205 269 260 260 258 ...
 $ BP.avg..mb.                 : int  1016 1013 1012 1013 1014 1016 1016 1015
1015 1015 ...
 $ N....obs.                   : int  96 96 96 96 96 96 96 96 96 96 ...
 $ Tweather.c                   : num  27.6 27.9 27.1 27.1 28 ...
 $ Tsoil.c                      : num  34.3 33.9 34.6 33.1 34 ...
 $ rain.cm                      : num  0.0254 0 0.508 0 0 0 0 0 0.0508 0 ...
```

```
dat1_monthly <- dat1 |>
  mutate(
    Period_clean = str_squish(Period),
    date = dmy(Period_clean),
    month = month(date, label = TRUE, abbr = TRUE)   # Jan, Feb, Mar ...
  ) |>
  group_by(month) |>
  summarise(
    Tweather.ave.c = round(mean(Tweather.c, na.rm = TRUE), 2),
    Tweather.sd.c = round(sd(Tweather.c, na.rm = TRUE), 2),
    Tsoil.ave.c = round(mean(Tsoil.c, na.rm = TRUE), 2),
    Tsoil.sd.c = round(sd(Tsoil.c, na.rm = TRUE), 2),
    RH.ave = round(mean(RelHum.avg.2m...pct., na.rm = TRUE), 2),
    RH.sd = round(sd(RelHum.avg.2m...pct., na.rm = TRUE), 2),
    rain.ave = round(mean(rain.cm, na.rm = TRUE), 3),
    rain.sd = round(sd(rain.cm, na.rm = TRUE), 3),
    SolarRad.ave = round(mean(SolRad.avg2m...w.m.2., na.rm = TRUE), 2),
    SolarRad.sd = round(sd(SolRad.avg2m...w.m.2., na.rm = TRUE), 2),
    .groups = "drop"
  ) |>
  arrange(match(month, month.abb))

dat1_monthly
```

```
# A tibble: 12 × 11
   month Tweather.ave.c Tweather.sd.c Tsoil.ave.c Tsoil.sd.c RH.ave RH.sd
   <ord>          <dbl>         <dbl>       <dbl>      <dbl>  <dbl> <dbl>
 1 Jan             11.7          5.35        12.7       3.5    76.0 10.9
 2 Feb             15.7          4.02        16.7       3.24   77.5 10.2
 3 Mar             17.9          3.48        19.9       2.7    72.7 12.5
 4 Apr             20.9          2.84        24.2       2.47   72.0  9.59
 5 May             24.0          1.92        27.9       2.52   76.8  8.22
 6 Jun             26.3          1.79        30.2       2.64   79.2  7.5
 7 Jul             27.4          1.26        31.1       1.68   83.1  5.48
 8 Aug             27.1          1.4         31.3       2.03   82.7  5.45
 9 Sep             25.0          1.49        28.7       1.85   82.0  8.06
10 Oct             20.5          3.11        24.4       2.17   75.7  8.93
11 Nov             17.7          4.58        20.3       3.8    80.5 10.2
12 Dec             13.4          5.06        14.9       3.4    79.1 11.2
# ℹ 4 more variables: rain.ave <dbl>, rain.sd <dbl>, SolarRad.ave <dbl>,
#   SolarRad.sd <dbl>
```

```
# Warm and cool seasons
dat1 |>
  mutate(
    Period_clean = str_squish(Period),
    date = dmy(Period_clean),
    month_num = month(date),
    season = case_when(
      month_num %in% c(10, 11, 12, 1, 2, 3) ~ "Cool season (Oct-Mar)",
      month_num %in% c(4:9) ~ "Warm season (Apr-Sep)")) |>
  group_by(season) |>
  summarise(
    Tsoil.ave.c = mean(Tsoil.c, na.rm = TRUE),
    Tsoil.sd.c = sd(Tsoil.c, na.rm = TRUE),
    .groups = "drop")

# A tibble: 2 × 3
  season                Tsoil.ave.c Tsoil.sd.c
  <chr>                       <dbl>      <dbl>
1 Cool season (Oct-Mar)        18.2       5.01
2 Warm season (Apr-Sep)        28.9       3.29

# Plot monthly averages
p_m2 <- ggplot(dat1_monthly,
       aes(x = month, y = Tsoil.ave.c)) +
  geom_point(size = 1.5, color = "black") +
  geom_errorbar(aes(ymin = Tsoil.ave.c - Tsoil.sd.c,
                    ymax = Tsoil.ave.c + Tsoil.sd.c),
                width = 0.2, color = "black") +
  labs(
    x = "Month",
    y = "Soil temperature (°C)") +
  scale_y_continuous(
    breaks = seq(10, 35, 5),
    labels = seq(10, 35, 5),
    limits = c(7, 35)) +
  theme_bw() +
  theme(text = element_text(family = "Arial"),
        legend.position = 'inside', legend.position.inside = c(0.86, 0.18),
        legend.direction = "vertical",
        legend.text = element_text(size = 7),
        legend.title = element_text(size = 7, face = "bold"),
        legend.key.height = unit(0.4, "cm"),
        axis.text.x = element_text(angle = 45, size = 7, color = "black",
face = "bold", hjust = 1, vjust = 0.5),
        axis.text.y = element_text(size = 7, color = "black", face = "bold"),
        axis.title.x = element_text(size = 7, vjust = -1),
        axis.title.y = element_text(size = 7, vjust = 2),
        panel.border = element_rect(color = "black", fill = NA, linewidth =
0.5),
        panel.grid.major = element_line(color = "gray", linewidth = 0.1,
linetype = "longdash"),
        panel.grid.minor = element_line(color = "lightgray", linewidth = 0.1,
linetype = "dotted"),
        panel.background = element_rect(fill = "transparent"),
        plot.background = element_rect(fill = "transparent", color =
"transparent"))

p_m2
```



```
ggsave(file = here('obj2', 'results', 'Fig S1 ave_monthly_soil_temp.tiff'),
       plot = p_m2,
       width = 7, height = 5, units = c("cm"), dpi=400)

rm(list = setdiff(ls(envir = .GlobalEnv, all.names = TRUE), list_exc),
   envir = .GlobalEnv)
```

## 10.3 Seven days prior to sample collection

```
dat1 <- read.csv(here('obj2', 'data', 'FAWN_report_seven_day_hourly.csv')) |>
  dplyr::mutate(
    Tweather.c = (`X10m.T.avg..F.`-32) * (5/9),
    Tsoil.c = (`Tsoil.avg.10cm...F.` -32) * (5/9),
    rain.cm = `X2m.Rain.tot..in.` * 2.54
    )

str (dat1)
```

```
'data.frame':   192 obs. of  18 variables:
 $ FAWN.Station                : chr  "Live Oak" "Live Oak" "Live Oak" "Live
Oak" ...
 $ Period                      : chr  "22 Mar 2024  12:00 AM" "22 Mar 2024
1:00 AM" "22 Mar 2024  2:00 AM" "22 Mar 2024  3:00 AM" ...
 $ X10m.T.avg..F.              : num  64.8 65 64.9 62.4 61.3 ...
 $ X10m.T.min..F.              : num  64.7 64.8 64.3 61.1 61.2 ...
 $ X10m.T.max..F.              : num  65 65.2 65.2 63.8 61.6 ...
 $ Tsoil.avg.10cm...F.         : num  70.2 69.6 69.1 68.7 68.4 ...
 $ Tsoil.min.avg..10cm...F.    : num  69.9 69.4 68.9 68.6 68.2 ...
 $ Tsoil.max.avg..10cm...F.    : num  70.4 69.8 69.3 68.8 68.5 ...
 $ X2m.DewPt.avg..F.           : num  54 55.9 57 58.5 59.7 ...
 $ RelHum.avg.2m...pct.        : int  69 73 77 88 94 96 97 96 96 96 ...
 $ X2m.Rain.tot..in.           : num  0 0 0 0.02 0.05 0.25 0.37 0.03 0.04
0.08 ...
 $ X2m.Rain.max.over.15min..in.: num  0 0 0 0.01 0.03 0.15 0.19 0.02 0.02
0.03 ...
 $ SolRad.avg2m...w.m.2.       : num  0 0 0 0 0 ...
 $ BP.avg..mb.                 : int  1012 1013 1012 1011 1010 1010 1010 1010
1010 1010 ...
 $ N....obs.                   : int  4 4 4 4 4 4 4 4 4 4 ...
 $ Tweather.c                  : num  18.2 18.3 18.3 16.9 16.3 ...
 $ Tsoil.c                     : num  21.2 20.9 20.6 20.4 20.2 ...
 $ rain.cm                     : num  0 0 0 0.0508 0.127 ...
```

```
dat1 |>
  mutate(
    Period_clean = str_squish(Period),
    datetime = parse_date_time(Period_clean, orders = "d b Y I:M p"),
    date = as_date(datetime)
  ) |>
  group_by(date) |>
  summarise(
    Tweather.ave.c = round(mean(Tweather.c, na.rm = TRUE), 3),
    Tweather.sd.c = round(sd(Tweather.c, na.rm = TRUE), 3),
    Tsoil.ave.c = round(mean(Tsoil.c, na.rm = TRUE), 3),
    Tsoil.sd.c = round(sd(Tsoil.c, na.rm = TRUE), 3),
    RH.ave = round(mean(RelHum.avg.2m...pct., na.rm = TRUE), 3),
    RH.sd = round(sd(RelHum.avg.2m...pct., na.rm = TRUE), 3),
    rain.ave = round(mean(rain.cm, na.rm = TRUE), 3),
    rain.sd = round(sd(rain.cm, na.rm = TRUE), 3),
    SolarRad.ave = round(mean(SolRad.avg2m...w.m.2., na.rm = TRUE), 3),
    SolarRad.sd = round(sd(SolRad.avg2m...w.m.2., na.rm = TRUE), 3),
    .groups = "drop"
  )

# A tibble: 8 × 11
  date       Tweather.ave.c Tweather.sd.c Tsoil.ave.c Tsoil.sd.c RH.ave RH.sd
  <date>              <dbl>         <dbl>       <dbl>      <dbl>  <dbl> <dbl>
1 2024-03-22           18.2         0.965        19.6      0.622   91.3  7.50
2 2024-03-23           19.2         1.71         20.1      1.59    84.9 11.2
3 2024-03-24           15.8         2.76         19.0      1.60    75.2 11.3
4 2024-03-25           16.7         4.42         18.2      2.1     77.5 12.0
5 2024-03-26           21.3         4.04         20.0      1.98    74.3 12.1
6 2024-03-27           21.1         1.01         21.1      0.423   87.8  5.34
7 2024-03-28           18.6         2.68         20.4      1.35    76.0 20.7
8 2024-03-29           16.0         3.94         18.6      2.17    51.4 18.6
# ℹ 4 more variables: rain.ave <dbl>, rain.sd <dbl>, SolarRad.ave <dbl>,
#   SolarRad.sd <dbl>
```

```
# Average weekly weather conditions prior to sample collection
dat1 |>
  summarise(Tweather.ave.c = mean(Tweather.c, na.rm = TRUE),
            Tweather.sd.c = sd(Tweather.c, na.rm = TRUE),
            Tsoil.avg.c = mean(Tsoil.c, na.rm = TRUE),
            Tsoil.sd.c = sd(Tsoil.c, na.rm = TRUE),
            RH.avg = mean(`RelHum.avg.2m...pct.`, na.rm = TRUE),
            RH.sd = sd(`RelHum.avg.2m...pct.`, na.rm = TRUE),
            rain.avg.cm = mean((`X2m.Rain.tot..in.`*2.54), na.rm = TRUE),
            rain.sd.cm = sd((`X2m.Rain.tot..in.`*2.54), na.rm = TRUE),
            SolaRad.avg = mean(`SolRad.avg2m...w.m.2.`, na.rm = TRUE),
            SolaRad.sd = sd(`SolRad.avg2m...w.m.2.`, na.rm = TRUE),
            .groups = "drop") |>
  as.data.frame() |>
  mutate(across(where(is.numeric), ~ round(.x, 2)))

  Tweather.ave.c Tweather.sd.c Tsoil.avg.c Tsoil.sd.c RH.avg RH.sd
rain.avg.cm
1           18.3          3.54        19.6       1.82   77.3  17.4
0.05
  rain.sd.cm SolaRad.avg SolaRad.sd
1       0.21         168        250
```

```
rm(list = setdiff(ls(envir = .GlobalEnv, all.names = TRUE), list_exc),
   envir = .GlobalEnv)
```