Defining the biosecurity risk posed by transported soil: Effects of storage time and environmental exposure on survival of soil biota

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# 1 Design

#### 1.1 Physical Setup

The design of the experiment was as follows. Two sites were selected; an organic orchard and a forest ( $\omega_i$ , i = 1, 2). From each site, three soil pits were excavated and samples taken. The three soil pits were at least 10m apart. The six soil samples ( $\delta_i$ , j = 1, ... 6) were then split into sixteen

- portions that were stored in the following locations: four in separate cupboards, and twelve stored on (i) top of, (ii) inside, and (iii) underneath each of four shipping containers. Measures of soil chemistry were taken from each of the six replicates, and measures of temperature were made for most of the physical locations of the experiment, for most of the time.
- 10 This design has split-plot elements (site is replicated only at the highest level), nested random effects (soil pit within site), crossed random effects (soil pit nested within site is crossed with treatment location), longitudinal measures (four repeated measurements in time).

Measures of various response variables were taken from some of the soil samples at duration t = 0, and thereafter from most of the soil samples at 3 months, 6 months, and 12 months. The response variables include: (i) scaled number of bacteria, (ii) scaled number of pseudomonas, (iii) scaled number of fungi, (iv) number of nematodes, (v) proportion of plant-eating nematodes

out of all nematodes, (vi) count of plants, and (vii) count of plant species.

The motivating null hypothesis was that there was no difference between the treatment levels upon the effect of time upon the response variables. If the hypothesis was rejected for the response variables, then we explored possible relationships between temperature, treatment,

#### 1.2 Model

and the response variable.

The statistical model fitted used the following template.

$$f_L(y_{ijkmt}) = \beta_0 + \omega_i + f_T(t, \tau_m) + \gamma_i + \delta_j + \psi_k + \epsilon_{ijkmt}$$
(1)

where

- $y_{ijkmt}$  is the response variable at duration t from soil pit  $\delta$  within origin i stored in site k under treatment m;
  - $f_L(\ldots)$  is a selected transformation of the response variable, usually a natural logarithm;
  - *t* refers to the duration of aging;
  - $f_T(t, \tau_m)$  is some function of the duration and the treatment (m = 1, ..., 4) that is constrained so that all treatments are identical at t = 0;
  - $\omega_i$  is a fixed effect representing origin (Forest / Orchard);
  - $\gamma_i \stackrel{d}{=} N(0, \sigma_{\gamma}^2), \ i = 1, 2$  is the site random effect, which may be confounded with  $\omega_i$ ;
  - $\delta_j \stackrel{d}{=} N(0, \sigma_{\delta}^2), \ j = 1, \dots, 6$  is the soil pit random effect, with 3 from each site;
  - $\psi_k \stackrel{d}{=} N(0, \sigma_{\psi}^2)$ ,  $k = 1, \dots, 8$  is the treatment location, where 1 4 are sea containers (corresponding to treatments 1 3 only), and 5 8 are cupboards (corresponding to treatment 4); and
  - $\epsilon_{ijkmt} \stackrel{d}{=} N(0, \sigma^2)$  is a random error.

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#### 1.3 Analysis Policies

The fixed effects were tested using likelihood ratio tests, with cutoff for statistical significanceset at 0.01 in order to loosely compensate for the multiplicity of tests. The random intercepts were not tested; they were included in order that the model faithfully represent the experimental design.

We initially fit a full model that included treatment, origin, and duration as a linear effect, and checked diagnostic graphics. The outcome of the check was generally some form of trans-

- 45 formation, e.g. natural log or square root. We next applied backwards elimination to assess whether origin should be retained. We then applied a backwards elimination approach to see how duration should enter the model, including: not at all, as a linear term, as a quadratic term, and as a constrained two-knot spline, which is equivalent in degrees of freedom to a cubic function but performed better visually. Invariably the most complex model was selected. The most
- 50 appropriate model was assessed using graphical diagnostics, and adjustments made if needed. The fitted models were then overlaid upon scatterplots of the data that were augmented with smooth means, as a visual aid to the testing and modeling process. These graphs are produced in the body of this report; all diagnostic graphs are in the appendix.
- For some of the models (namely, count of plants and count of plant species), it was necessary to use the volume of soil in the sample as an offset in the model. We initially tried to use a poisson distribution for the conditional response variable, but the resulting generalized linear mixed-effects models failed to converge. Accordingly for count of plants we divided the count by soil volume, to allow for different amounts tested. For count of plant species we analyzed the response both with and without the correction by soil volume.
- 60 Although it is commonly true in the documented analyses that the model assumptions are ratified by graphical diagnostics, sometimes the distribution of the residuals was a little more skewed than symmetric. In these instances we were reasonably confident that the Central Limit Theorem would hold, and that the test assumptions would be robust to the departure from the nominal assumptions. However, it would be wise to interpret the test results with caution.
- 65 In any case, a visual assessment of the graphs that superimpose the raw data, the smoothed mean, and the predictions from the final model, provide an indication of the importance of the contribution of the tested terms.

We used the following reasoning for assessing the importance of interactions between random effects. There are no reasons to assume that the random effects will interact with duration

- 70 above and beyond the effects of the interaction of treatment with duration, so the random interactions were not formally tested, however, graphs were constructed to act as diagnostics for this assumption. If there was an important interaction between the random effect and duration then we assume that it would be visible in these graphics. In cases where this occurred we formally tested the extended term using a whole-model likelihood ratio test, and if it was
- 75 significant, repeated the fixed effects test suite with the new random effects structure. In no case did the test of the fixed effects alter from the orignal result in any important way, hence we do not pursue these results further. We did not test further interactions between fixed and random effects.

If the soil pit random effect was non-zero then we informally assessed the effects of soil chemistry upon the response variable by examining scatterplots of the estimated soil pit random effects against the soil chemistry variables. More formal analysis did not seem appropriate owing to the large number of potential soil variable predictors.

If the interaction between treatment and duration was statistically significant, then further analysis was undertaken to assess the effect of the temperatures. The maximum, minimum, and average temperatures had been recorded at each location for each timespan (Figure 1). The analysis was performed by taking the temporal differences of the response variable, or transformations of the response variable where approriate (e.g. natural log for count data and

empirical logit for proportion data) and assessing the effect of the interaction of duration with

both treatment and temperature upon the change from measure time to measure time.

- 90 It would have been preferable to simply fit the same model as previously, with temperature data added in as possible. However, we were working with the first differences as the response variable, in order to capture the effect of temperature at specific times of the experiment. Therefore the structure of the model would have to be recreated in its differential form. The extra effort did not seem justified given that the intent of this section of the analysis was an ad-hoc assessment of the comparative effects of treatment and temperature upon the response
- variable, rather than a formal modeling undertaking.

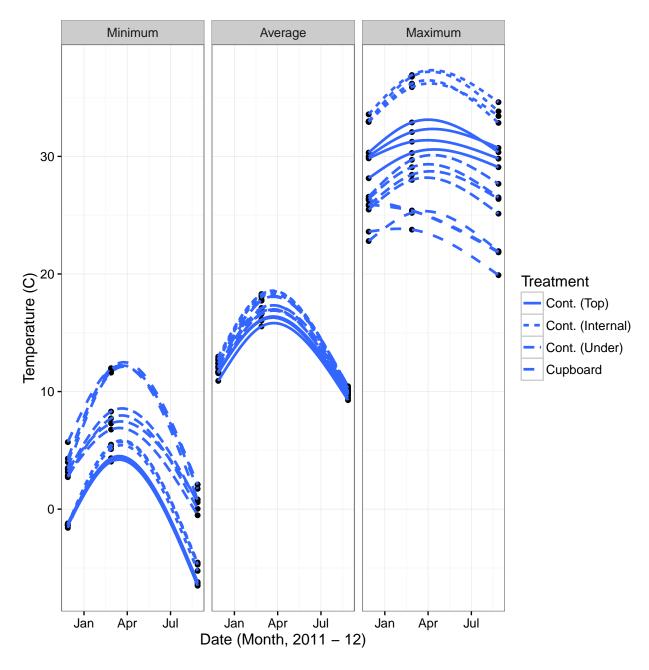


Figure 1: Plot of minimum, average, and maximum temperatures against month. Each treatment is represented using a different color. Each line represents a different replicate.

# 2 Results

The results of the analyses are represented as follows. Each response variable is provided with a two-part graphical summary that plot (i) duration and (ii) date against the raw data with smoothed means, constructed using loess. The first plot is augmented by dashed lines that represent the best-fitting statistical model. Further, two tables are provided for all variables, one that provides point and interval estimates of the random effects, and one that summarizes the tests used to assess the main hypothesis. We also provide a graphics that reports the interplay between temperature and treatment. For those response variables for which the soil pit effects were not zero, we also provide the scatterplot of the soil pit random effects with the

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5 pit effects were not zero, we also provide the scatterplot of the soil pit random effects with the soil chemistry variables. Graphical model diagnostics for the selected models are provided in the Appendix.

## 2.1 Bacteria

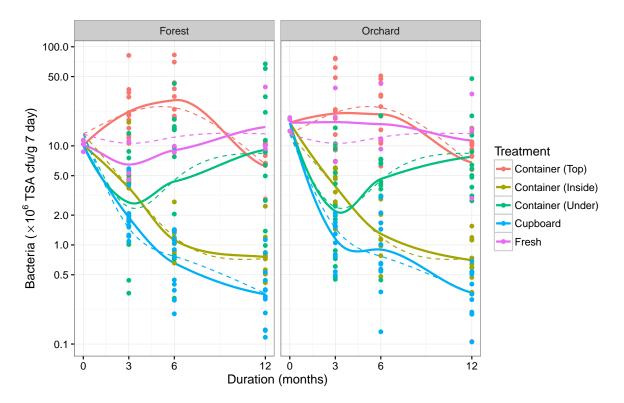


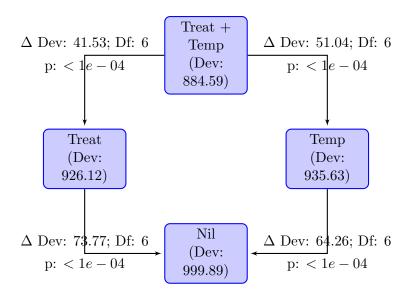
Figure 2: Scatterplot of bacteria response variable against time since start of experiment, augmented with smooth mean (solid lines) and predictions from preferred model (dashed lines).

**Table 1:** ANOVA for bacteria: comparison of duration models. In the row labels, Dur refers to duration, Dur.2 is the square of duration, Treat is Treatment, ns refers to a natural cubic spline, and the colon : signals an interaction term. In the columns, logLik reports the log likelihood of the model evaluated at its maximum, Deviance is negative twice the log likelihood, Chisq is the difference between the deviance of the current line and the line above it.

Fixed Effects	Df	logLik	deviance	Chisq	Chi Df	$\Pr(>Chisq)$
1	5	-530.34	1060.68			
Dur	6	-521.62	1043.24	17.44	1	$2.96\times10^{-05}$
Treat:Dur	10	-445.30	890.60	152.64	4	$5.53\times10^{-32}$
Treat:(Dur + Dur.2)	15	-389.25	778.49	112.11	5	$1.47\times 10^{-22}$
Treat:ns(Dur, knots = $c(0.25, 0.5)$ )	20	-379.13	758.26	20.23	5	$1.13\times 10^{-03}$

**Table 2:** Random effects for bacteria: standard deviations with 95% confidence intervals. These values are in the same units as the response variable for the model; namely  $\log_e$  of the count.

Random Effects	Estimate	L.025	U.975
Container	0.079	0.000	0.1528
Soil	0.000	0.000	0.1265
Origin	0.000	0.000	0.0794
Sigma	0.822	0.731	0.8626



**Figure 3:** Deviance diagram for bacteria. Each node represents a model. The response variable is the change in count between each measured time period.

## 2.2 Pseudomonas

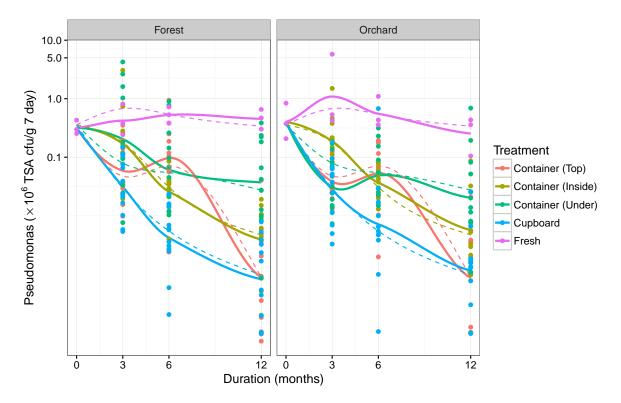


Figure 4: Scatterplot of pseudomonas response variable against time since start of experiment, augmented with smooth mean (solid lines) and predictions from preferred model (dashed lines).

**Table 3:** ANOVA for pseudomonas: comparison of duration models. In the row labels, Dur refers to duration, Dur.2 is the square of duration, Treat is Treatment, ns refers to a natural cubic spline, and the colon : signals an interaction term. In the columns, logLik reports the log likelihood of the model evaluated at its maximum, Deviance is negative twice the log likelihood, Chisq is the difference between the deviance of the current line and the line above it.

Fixed Effects		logLik	deviance	Chisq	Chi Df	$\Pr(>Chisq)$
1	5	-664.13	1328.3			
Dur	6	-597.31	1194.6	133.63	1	$6.57\times10^{-31}$
Treat:Dur	10	-558.59	1117.2	77.45	4	$6.04\times10^{-16}$
Treat:(Dur + Dur.2)	15	-544.69	1089.4	27.80	5	$3.98\times10^{-05}$
Treat:ns(Dur, knots = $c(0.25, 0.5)$ )	20	-533.64	1067.3	22.09	5	$5.02\times10^{-04}$

Table 4: Random effects for pseudomonas: standard deviations with 95% confidence intervals. These values are in the same units as the response variable for the model; namely  $\log_e$  of the count.

Random Effects	Estimate	L.025	U.975
Container	3.80e - 01	0.00	0.538
Soil	2.58e - 01	0.00	0.436
Origin	1.17e - 07	0.00	0.273
Sigma	1.32e + 00	1.18	1.400

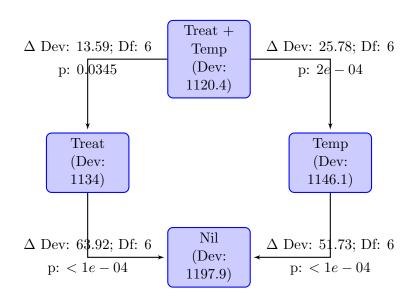


Figure 5: Deviance diagram for pseudomonas. Each node represents a model. The response variable is the change in count between each measured time period.

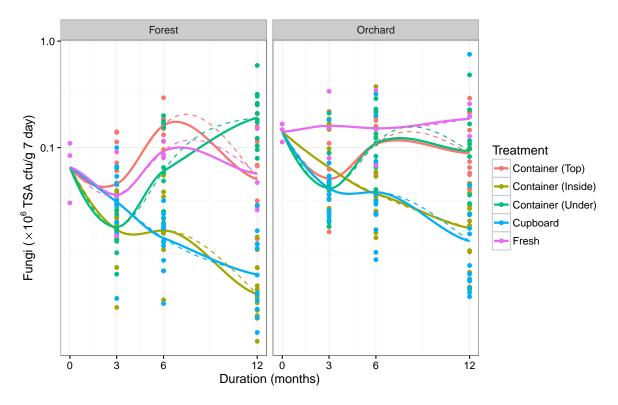


Figure 6: Scatterplot of fungi response variable against time since start of experiment, augmented with smooth mean (solid lines) and predictions from preferred model (dashed lines).

**Table 5:** ANOVA for fungi: comparison of duration models. In the row labels, *Dur* refers to duration, *Dur.2* is the square of duration, *Treat* is Treatment, *ns* refers to a natural cubic spline, and the colon : signals an interaction term. In the columns, logLik reports the log likelihood of the model evalauted at its maximum, *Deviance* is negative twice the log likelihood, *Chisq* is the difference between the deviance of the current line and the line above it.

Fixed Effects	Df	logLik	deviance	Chisq	Chi Df	$\Pr(>Chisq)$
origin	6	-482.96	965.92			
origin + Dur * origin	8	-481.36	962.72	3.204	2	$2.02\times10^{-01}$
origin + (origin * Treat): Dur	16	-392.81	785.62	177.097	8	$4.19\times10^{-34}$
origin + (origin * Treat): (Dur	26	-373.70	747.41	38.212	10	$3.49\times10^{-05}$
+ Dur.2)						
origin + (origin $*$ Treat):	36	-356.79	713.58	33.833	10	$1.97\times10^{-04}$
(ns(Dur, knots = c(0.25, 0.5)))						

Random Effects	Estimate	L.025	U.975
Container	0.170	0.00	$2.51 \times 10^{-01}$
Soil	0.129	0.00	$2.13\times10^{-01}$
Origin	0.000	0.00	$3.29\times10^{-08}$
Sigma	0.748	0.65	$7.70\times10^{-01}$

**Table 6:** Random effects for fungi: standard deviations with 95% confidence intervals. These values are in the same units as the response variable for the model; namely  $\log_e$  of the count.

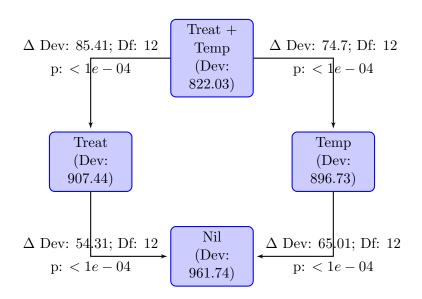


Figure 7: Deviance diagram for fungi. Each node represents a model. The response variable is the change in count between each measured time period.

### 2.4 Nematodes

The response variable was the square root of the nematode count, standardized by the volume of soil tested.

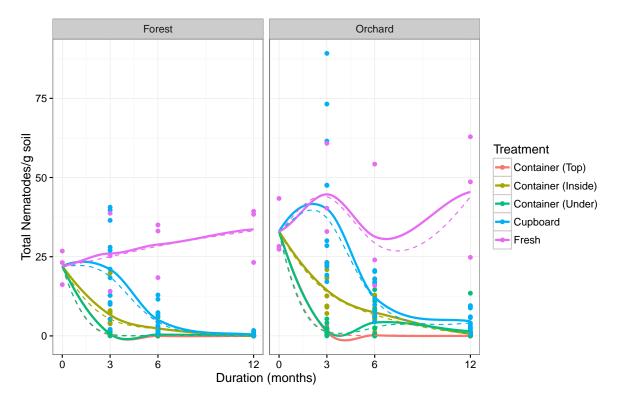


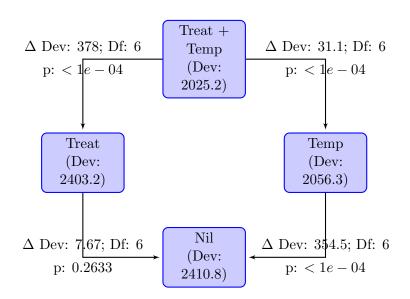
Figure 8: Scatterplot of nematodes response variable against time since start of experiment, augmented with smooth mean (solid lines) and predictions from preferred model (dashed lines).

**Table 7:** ANOVA for nematodes: comparison of duration models. In the row labels, Dur refers to duration, Dur.2 is the square of duration, Treat is Treatment, ns refers to a natural cubic spline, and the colon : signals an interaction term. In the columns, logLik reports the log likelihood of the model evaluated at its maximum, Deviance is negative twice the log likelihood, Chisq is the difference between the deviance of the current line and the line above it.

Fixed Effects	Df	$\log$ Lik	deviance	Chisq	Chi Df	$\Pr(>Chisq)$
origin	6	-552.05	1104.10			
origin + Dur * origin	8	-505.68	1011.35	92.75	2	$7.24\times10^{-21}$
origin + (origin * Treat):Dur	16	-449.41	898.83	112.53	8	$1.15\times10^{-20}$
origin + (origin * Treat):(Dur +	26	-393.03	786.06	112.76	10	$1.48\times10^{-19}$
Dur.2)						
origin + (origin $*$	36	-354.79	709.59	76.48	10	$2.45\times10^{-12}$
Treat):(ns(Dur, knots = $c(0.25,$						
0.5)))						

Random Effects	Estimate	L.025	U.975
Container	0.3620	$1.21 \times 10^{-01}$	0.613
Soil	0.1842	0.00e + 00	0.362
Origin	0.0944	$3.98\times10^{-07}$	0.398
Sigma	0.7706	$7.01\times10^{-01}$	0.834

**Table 8:** Random effects for nematodes: standard deviations with 95% confidence intervals. These values are in the same units as the response variable for the model; namely  $\log_e$  of the count.



**Figure 9:** Deviance diagram for nematodes. Each node represents a model. The response variable is the change in count between each measured time period.

#### 2.5 Nematodes (Plant-eating)

- 115 The count of plant-eating nematodes per unit soil is small and continuous, and has many zeros. The large count of zeros means that satisfying the previous modeling assumptions problematic. We tried to solve the problem by using a generalized linear mixed-effects model with conditional Poisson response and log link with the known (or estimated, where missing) soil sample volume as an offset, then by using a zero-inflated generalized linear mixed-effects model with condi-
- 120 tional Poisson response and log link with the known (or estimated, where missing) soil sample volume as an offset, and then by using a zero-inflated generalized linear mixed-effects model with conditional negative binomial response and log link with the known (or estimated, where missing) soil sample volume as an offset. All these attempts failed to gain traction. In the end we conditioned on the presence of nematodes, thus deleting all the observations with no ne-
- 125 matodes, and used a generalized linear mixed-effects model with conditional binomial response and logit link, where the response was the number of plant-eating nematodes conditional on the total number of nematodes. Interpretations of the graphics and summary tables are identical to previous analyses.

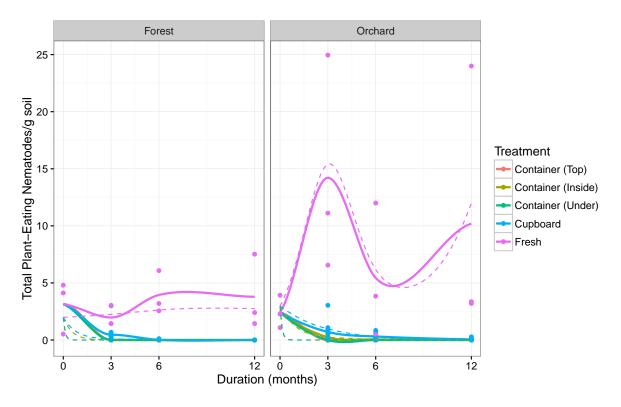


Figure 10: Scatterplot of plant-eating nematodes response variable against time since start of experiment, augmented with smooth mean (solid lines) and predictions from preferred model (dashed lines).

**Table 9:** ANOVA for plant-eating nematodes: comparison of duration models. In the row labels, Dur refers to duration, Dur.2 is the square of duration, Treat is Treatment, ns refers to a natural cubic spline, and the colon : signals an interaction term. In the columns, logLik reports the log likelihood of the model evalauted at its maximum, Deviance is negative twice the log likelihood, Chisq is the difference between the deviance of the current line and the line above it.

Fixed Effects	Df	logLik	deviance	Chisq	Chi Df	$\Pr(>Chisq)$
1	4	-1903.9	3807.7			
Dur	5	-1788.2	3576.3	231.4	1	$3.01\times 10^{-52}$
Treat*Dur	13	-1735.4	3470.8	105.5	8	$3.19\times10^{-19}$
$Treat^*(Dur + Dur.2)$	17	-1624.4	3248.7	222.1	4	$6.62\times10^{-47}$
Treat:ns(Dur, knots = $c(0.25,$	18	-1493.1	2986.2	262.5	1	$4.84\times10^{-59}$
(0.5))						

Table 10: Random effects for nematodes: standard deviations with 95% confidence intervals. These values are in the same units as the response variable for the model; namely  $\log_e$  of the count.

Random Effects	Estimate	L.025	U.975
Container	0.3620	$1.21 \times 10^{-01}$	0.613
Soil	0.1842	0.00e + 00	0.362
Origin	0.0944	$3.98 \times 10^{-07}$	0.398
Sigma	0.7706	$7.01 \times 10^{-01}$	0.834

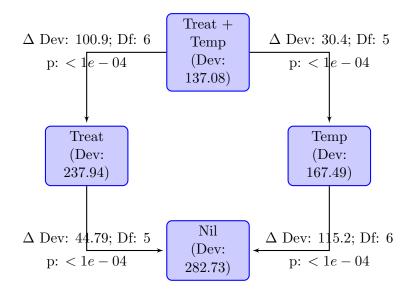


Figure 11: Deviance diagram for plant-eating nematodes. Each node represents a model. The response variable is the change in count between each measured time period.

### 2.6 Plant Count

130 Plant count is an integer-valued response. We tried to formulate the models as generalized linear mixed-effects models, for which the response variable was conditionally Poisson with log link function, and the (estimated) soil volume used as an offset in the model. These models failed to converge, so we instead used a mixed-effects model on the square root of the plant count, standardized by the sample soil volume.

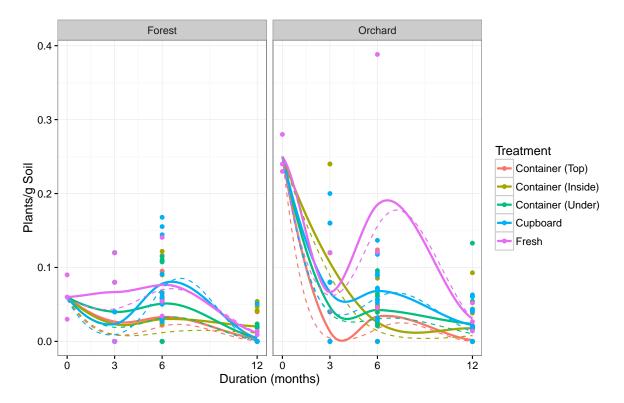


Figure 12: Scatterplot of plant count response variable against time since start of experiment, augmented with smooth mean (solid lines) and predictions from preferred model (dashed lines).

**Table 11:** ANOVA for plant count: comparison of duration models. In the row labels, Dur refers to duration, Dur.2 is the square of duration, Treat is Treatment, ns refers to a natural cubic spline, and the colon : signals an interaction term. In the columns, logLik reports the log likelihood of the model evaluated at its maximum, Deviance is negative twice the log likelihood, Chisq is the difference between the deviance of the current line and the line above it.

Fixed Effects	Df	logLik	deviance	Chisq	Chi Df	$\Pr(>Chisq)$
Dur	6	206.48	-412.96			
Treat:Dur	10	210.39	-420.78	7.817	4	$9.85\times10^{-02}$
Treat:(Dur + Dur.2)	15	217.72	-435.45	14.671	5	$1.19\times10^{-02}$
Treat:(ns(Dur, knots = $c(0.25,$	20	236.47	-472.94	37.492	5	$4.77 \times 10^{-07}$
(0.5)))						
Origin (All Interactions)	36	256.32	-512.64	39.700	16	$8.61\times10^{-04}$

**Table 12:** Random effects for plant count: standard deviations with 95% confidence intervals. These values are in the same units as the response variable for the model; namely  $\log_e$  of the count.

Random Effects	Estimate	L.025	U.975
Container	0.0358	0.00e + 00	0.0629
Soil	0.0192	0.00e + 00	0.0396
Origin	0.0204	$6.82\times10^{-08}$	0.0544

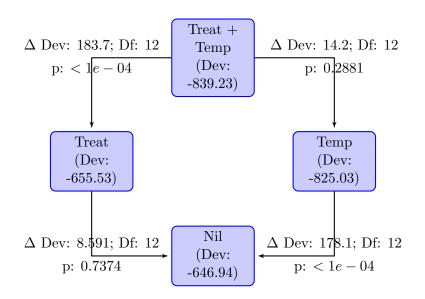


Figure 13: Deviance diagram for plant count. Each node represents a model. The response variable is the change in count between each measured time period.

#### 135 2.7 Plant Species Count

140

Species count is an integer-valued response. We tried to formulate the models as generalized linear mixed-effects models, for which the response variable was conditionally Poisson with log link function, and the (estimated) soil volume used as an offset in the model. These models failed to converge, so we instead used a mixed-effects model on the square root of the plant count, standardized by the sample soil volume.

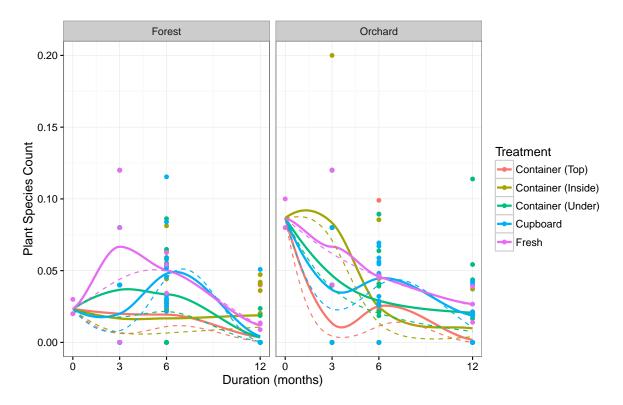


Figure 14: Scatterplot of plant species count response variable against time since start of experiment, augmented with smooth mean (solid lines) and predictions from preferred model (dashed lines).

**Table 13:** ANOVA for plant species count: comparison of duration models. In the row labels, Dur refers to duration, Dur.2 is the square of duration, Treat is Treatment, ns refers to a natural cubic spline, and the colon : signals an interaction term. In the columns, logLik reports the log likelihood of the model evalauted at its maximum, Deviance is negative twice the log likelihood, Chisq is the difference between the deviance of the current line and the line above it.

Fixed Effects	$\mathrm{Df}$	logLik	deviance	Chisq	Chi Df	$\Pr(>Chisq)$
origin	6	245.61	-491.21			
origin + Dur * origin	8	265.51	-531.02	39.80	2	$2.28\times10^{-09}$
origin + (origin * Treat):Dur	16	272.69	-545.39	14.37	8	$7.26\times10^{-02}$
origin + (origin * Treat):(Dur +	26	288.12	-576.24	30.85	10	$6.21\times10^{-04}$
Dur.2)						
origin + (origin $*$	36	304.69	-609.39	33.15	10	$2.57\times10^{-04}$
Treat):(ns(Dur, knots = $c(0.25,$						
(0.5)))						
$\operatorname{origin}^{*}$ + (origin * Treat):(ns(Dur, knots = c(0.25,	36	304.69	-609.39	33.15	10	$2.57 \times 10$

Table 14: Random effects for plant species count: standard deviations with 95% confidence intervals. These values are in the same units as the response variable for the model; namely  $\log_e$  of the count.

Random Effects	Estimate	L.025	U.975
Container	0.0305	$5.73 \times 10^{-09}$	0.0538
Soil	0.0104	0.00e + 00	0.0261
Origin	0.0191	$3.49\times10^{-06}$	0.0465

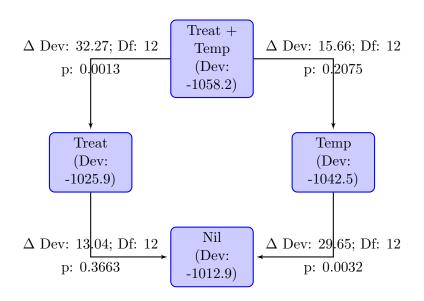


Figure 15: Deviance diagram for plant species count. Each node represents a model. The response variable is the change in count between each measured time period.

#### 2.8 Plant Species Count — no Offset

145

Plant species count is an integer-valued response. We tried to formulate the models as generalized linear mixed-effects models, for which the response variable was conditionally Poisson with log link function. These models failed to converge, so we instead used a mixed-effects model on the square root of the plant count.

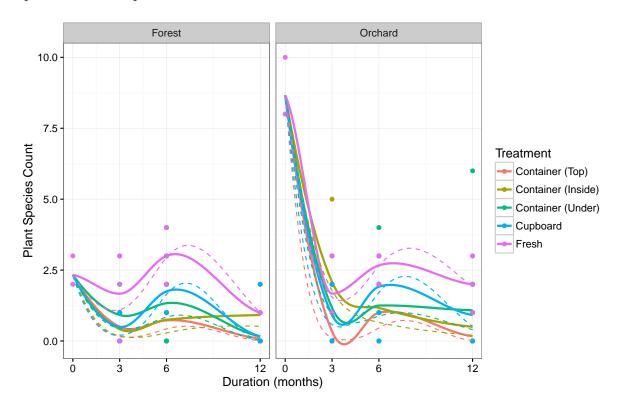


Figure 16: Scatterplot of plant species count (no offset) response variable against time since start of experiment, augmented with smooth mean (solid lines) and predictions from preferred model (dashed lines).

**Table 15:** ANOVA for plant species count (no offset): comparison of duration models. In the row labels, Dur refers to duration, Dur.2 is the square of duration, Treat is Treatment, ns refers to a natural cubic spline, and the colon : signals an interaction term. In the columns, logLik reports the log likelihood of the model evaluated at its maximum, Deviance is negative twice the log likelihood, Chisq is the difference between the deviance of the current line and the line above it.

Fixed Effects	Df	logLik	deviance	Chisq	Chi Df	$\Pr(>Chisq)$
origin	6	-309.11	618.21			
origin + Dur * origin	8	-298.02	596.03	22.18	2	$1.53\times10^{-05}$
origin + (origin * Treat):Dur	16	-290.49	580.97	15.06	8	$5.79\times10^{-02}$
origin + (origin * Treat):(Dur + Dur.2)	26	-271.55	543.10	37.87	10	$3.99 \times 10^{-05}$
$\begin{array}{l} \text{origin} & + & (\text{origin} & * \\ \text{Treat}):(\text{ns}(\text{Dur}, \text{ knots} = c(0.25, \\ 0.5))) \end{array}$	36	-247.36	494.71	48.39	10	$5.28 \times 10^{-07}$

Table 16: Random effects for plant species count (no offset): standard deviations with 95% confidence intervals. These values are in the same units as the response variable for the model; namely  $\log_e$  of the count.

Random Effects	Estimate	L.025	U.975
Container	0.1903	0.02635	0.344
Soil	0.0618	0.00000	0.148
Origin	0.1550	0.00218	0.318

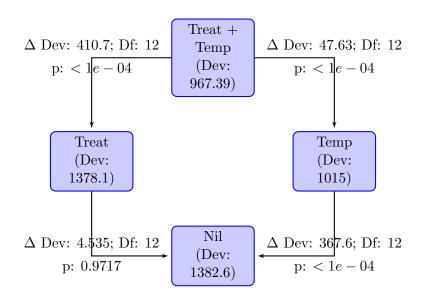
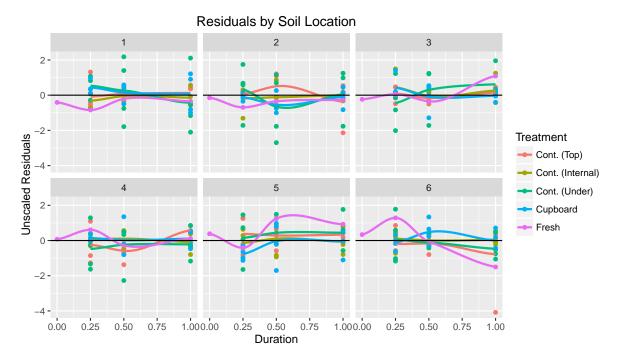


Figure 17: Deviance diagram for plant species count, no offset. Each node represents a model. The response variable is the change in count between each measured time period.

# A Appendix: Diagnostic Graphs

### A.1 Bacteria



**Figure 18:** Scatterplot of  $\log_e$  bacteria count residuals against duration by treatment, empanelled by soil location, with smooth means. Ideally, no patterns within location.

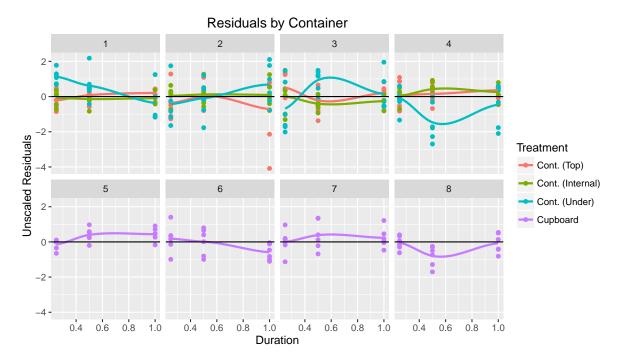
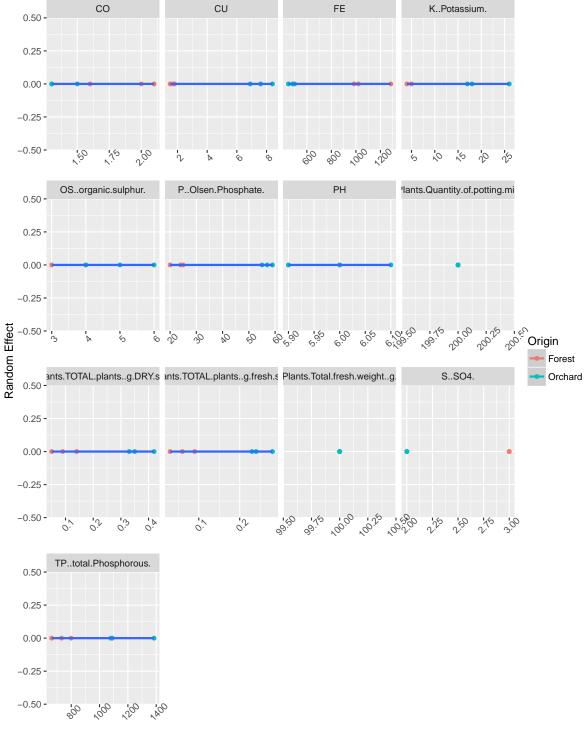
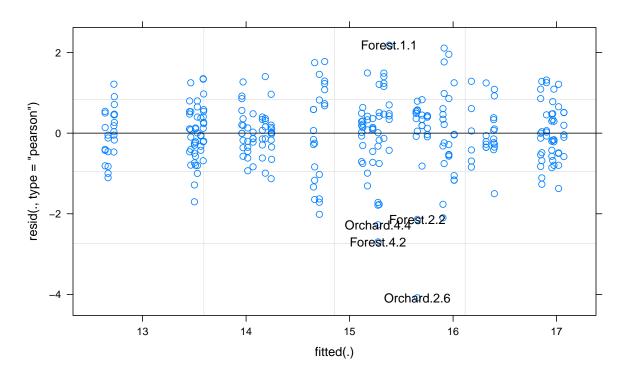


Figure 19: Scatterplot of  $\log_e$  bacteria count residuals against duration by treatment, empanelled by container, with smooth means. Ideally, no patterns within container.

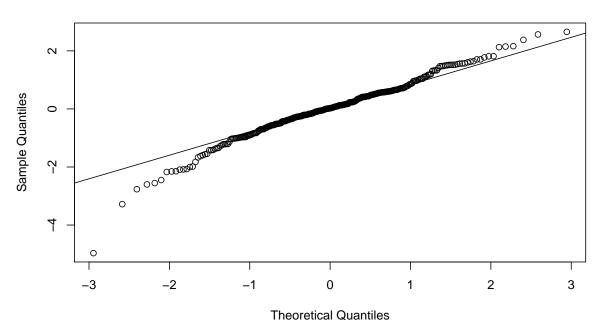


Soil Variable

**Figure 20:** Scatterplots of  $\log_e$  bacteria count soil random effects against soil random effect, coloured by origin (Forest / Orchard). Beware over-interpretation.

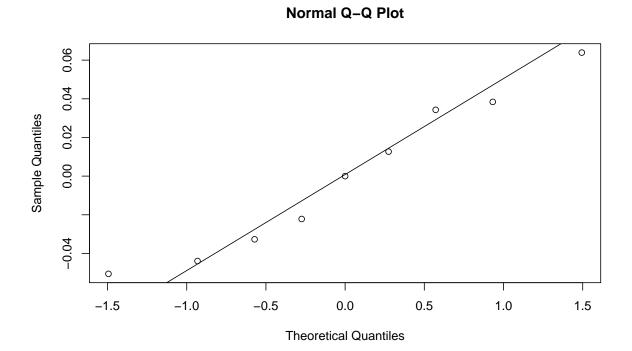


**Figure 21:** Diagnostic plot 1 for  $\log_e$  bacteria count: variance-corrected ("Pearson") residuals against fitted values. Ideally, no visible pattern.

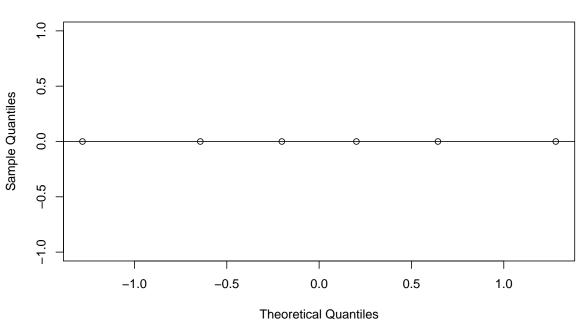


Normal Q-Q Plot

**Figure 22:** Diagnostic plot 2 for  $\log_e$  bacteria count: variance-corrected ("Pearson") residuals against equivalent normal quantiles. Ideally, a straight line.



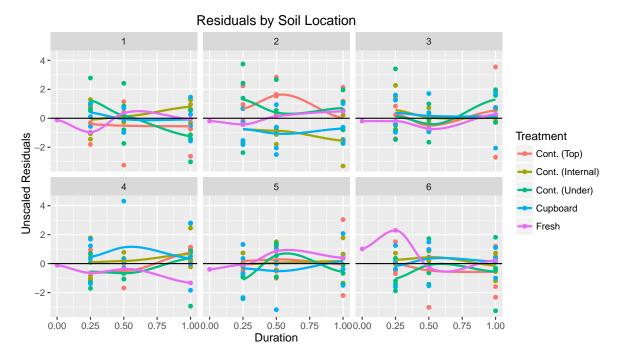
**Figure 23:** Diagnostic plot 3 for  $\log_e$  bacteria count: container-level random effects estimates against equivalent normal quantiles. Ideally, a straight line.



Normal Q-Q Plot

**Figure 24:** Diagnostic plot 3 for  $\log_e$  bacteria count: soil pit-level random effects estimates against equivalent normal quantiles. Ideally, a straight line.

# A.2 Pseudomonas



**Figure 25:** Scatterplot of  $\log_e$  pseudomonas count residuals against duration by treatment, empanelled by soil location, with smooth means. Ideally, no patterns within location.

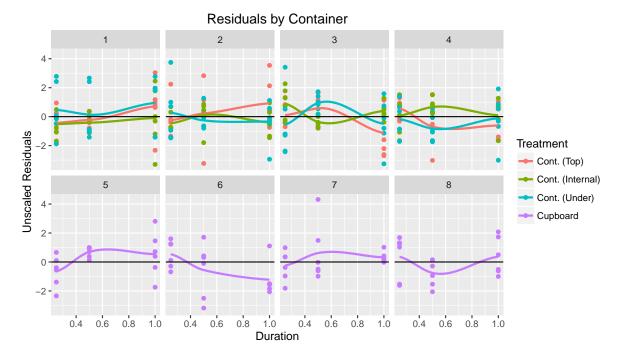
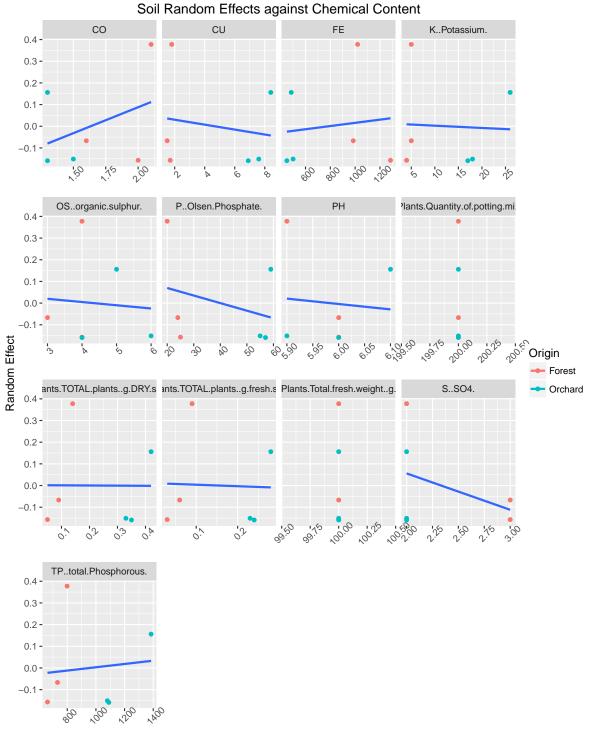
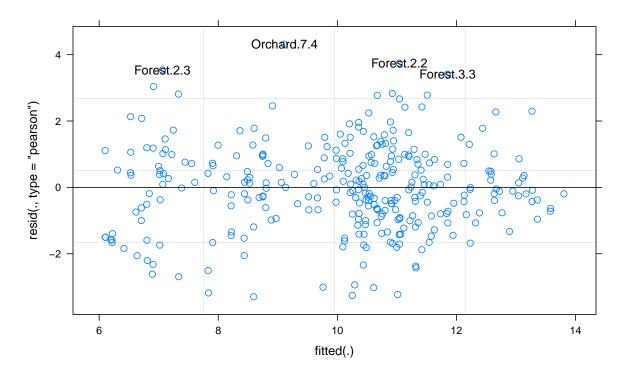


Figure 26: Scatterplot of  $\log_e$  pseudomonas count residuals against duration by treatment, empanelled by container, with smooth means. Ideally, no patterns within container.

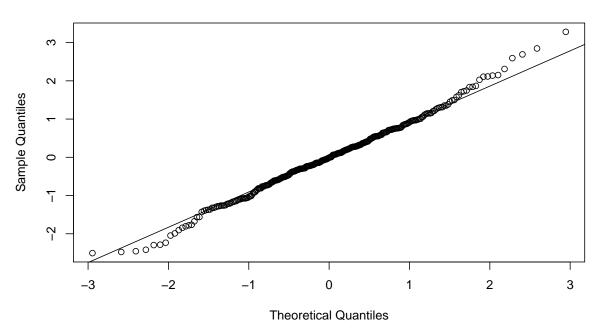


Soil Variable

**Figure 27:** Scatterplots of  $\log_e$  pseudomonas count soil random effects against soil random effect, coloured by origin (Forest / Orchard). Beware over-interpretation.

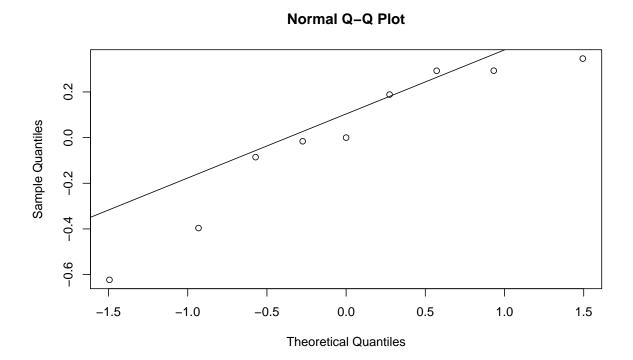


**Figure 28:** Diagnostic plot 1 for  $\log_e$  pseudomonas count: variance-corrected ("Pearson") residuals against fitted values. Ideally, no visible pattern.

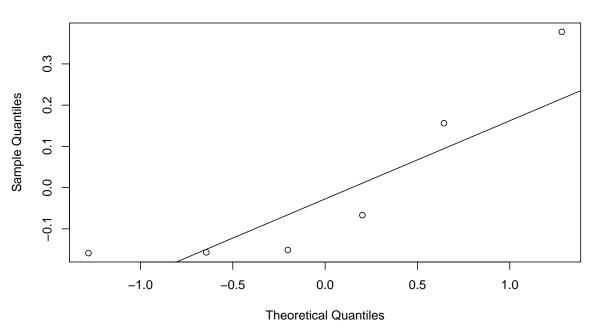


Normal Q-Q Plot

**Figure 29:** Diagnostic plot 2 for  $\log_e$  pseudomonas count: variance-corrected ("Pearson") residuals against equivalent normal quantiles. Ideally, a straight line.

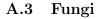


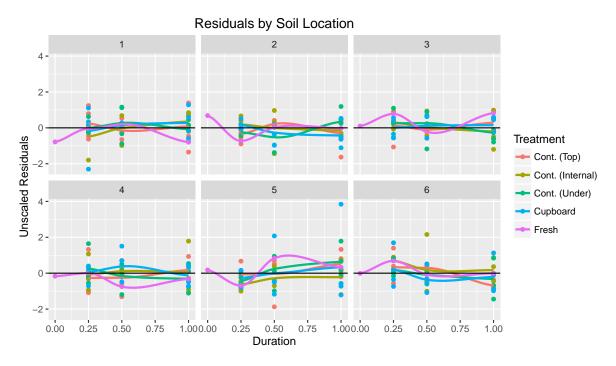
**Figure 30:** Diagnostic plot 3 for  $\log_e$  pseudomonas count: container-level random effects estimates against equivalent normal quantiles. Ideally, a straight line.



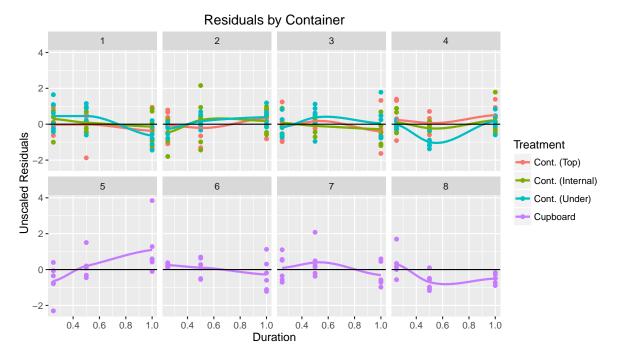
Normal Q-Q Plot

**Figure 31:** Diagnostic plot 3 for  $\log_e$  pseudomonas count: soil pit-level random effects estimates against equivalent normal quantiles. Ideally, a straight line.

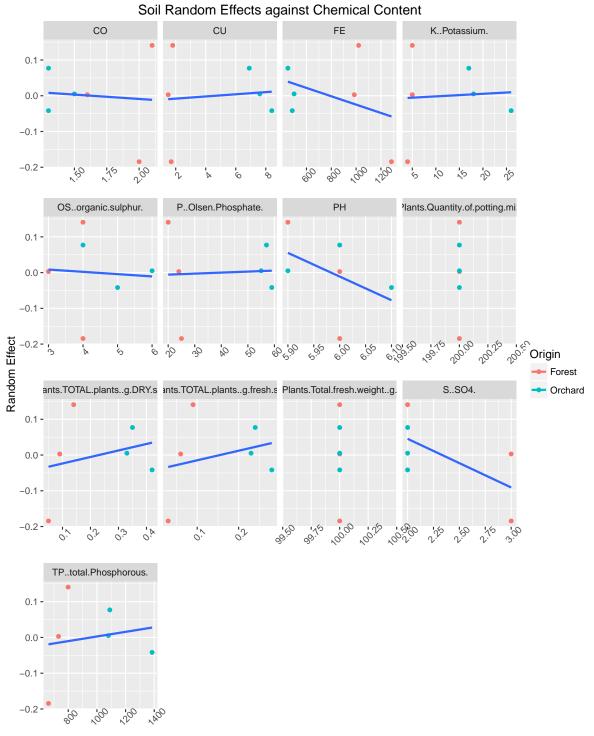




**Figure 32:** Scatterplot of  $\log_e$  fungi count residuals against duration by treatment, empanelled by soil location, with smooth means. Ideally, no patterns within location.

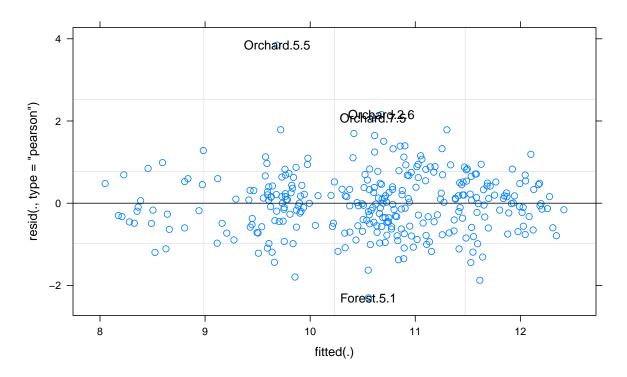


**Figure 33:** Scatterplot of  $\log_e$  fungi count residuals against duration by treatment, empanelled by container, with smooth means. Ideally, no patterns within container.

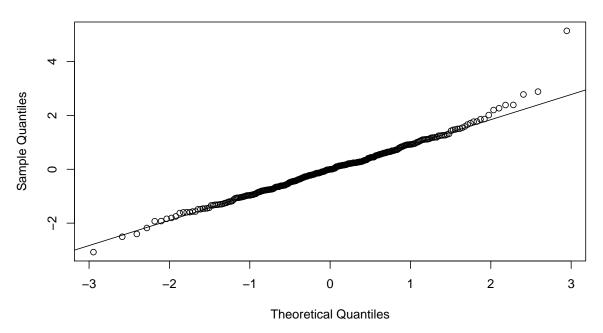


Soil Variable

**Figure 34:** Scatterplots of  $\log_e$  fungi count soil random effects against soil random effect, coloured by origin (Forest / Orchard). Beware over-interpretation.

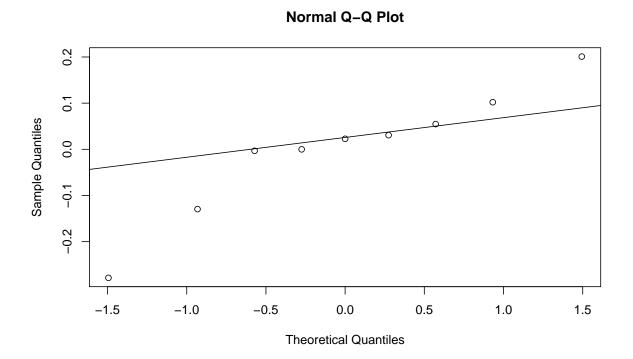


**Figure 35:** Diagnostic plot 1 for  $\log_e$  fungi count: variance-corrected ("Pearson") residuals against fitted values. Ideally, no visible pattern.

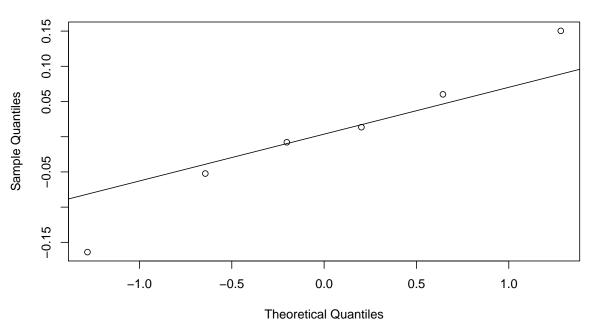


Normal Q-Q Plot

**Figure 36:** Diagnostic plot 2 for  $\log_e$  fungi count: variance-corrected ("Pearson") residuals against equivalent normal quantiles. Ideally, a straight line.



**Figure 37:** Diagnostic plot 3 for  $\log_e$  fungi count: container-level random effects estimates against equivalent normal quantiles. Ideally, a straight line.



Normal Q-Q Plot

**Figure 38:** Diagnostic plot 3 for  $\log_e$  fungi count: soil pit-level random effects estimates against equivalent normal quantiles. Ideally, a straight line.

## 150 A.4 Nematodes

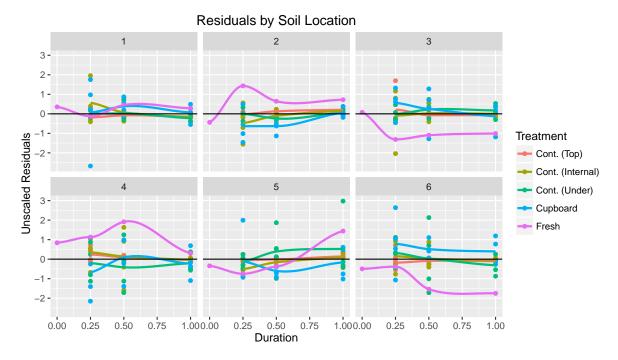


Figure 39: Scatterplot of square-root nematodes count residuals against duration by treatment, empanelled by soil location, with smooth means. Ideally, no patterns within location.

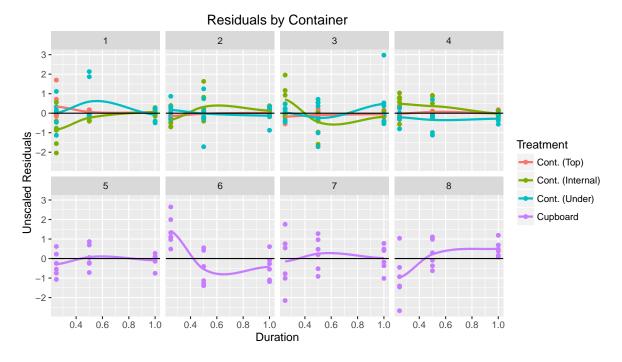
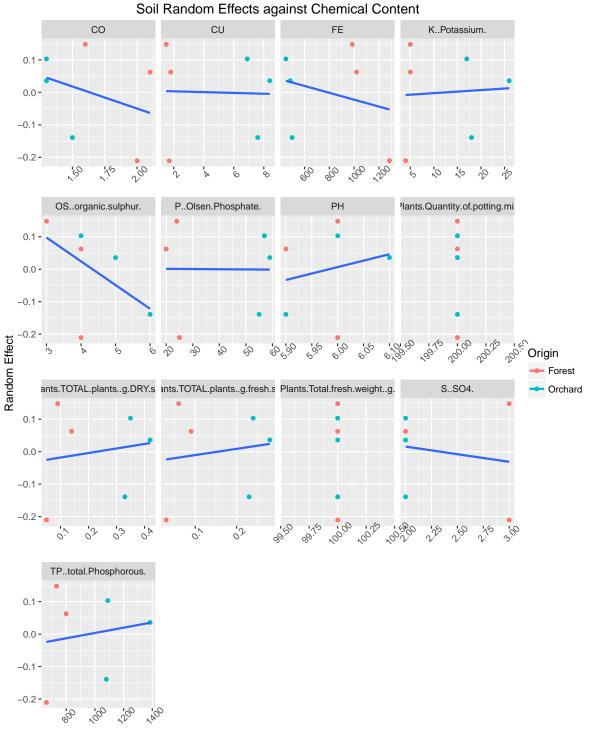


Figure 40: Scatterplot of square-root nematodes count residuals against duration by treatment, empanelled by container, with smooth means. Ideally, no patterns within container.



**Figure 41:** Scatterplots of square-root nematodes count soil random effects against soil random effect, coloured by origin (Forest / Orchard). Beware over-interpretation.

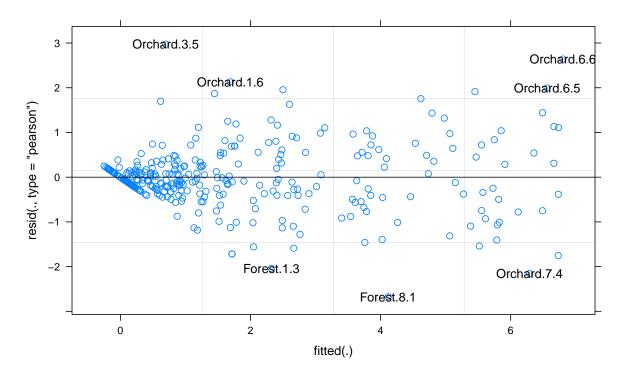
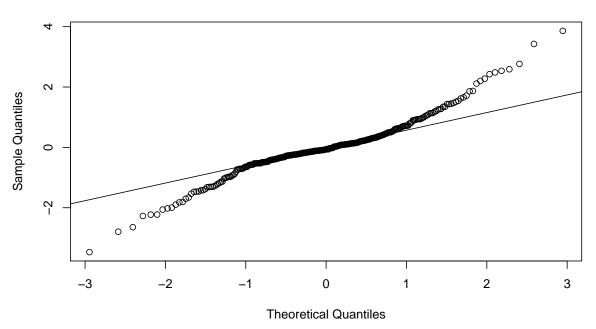


Figure 42: Diagnostic plot 1 for square-root nematodes count: variance-corrected ("Pearson") residuals against fitted values. Ideally, no visible pattern.



Normal Q-Q Plot

Figure 43: Diagnostic plot 2 for square-root nematodes count: variance-corrected ("Pearson") residuals against equivalent normal quantiles. Ideally, a straight line.

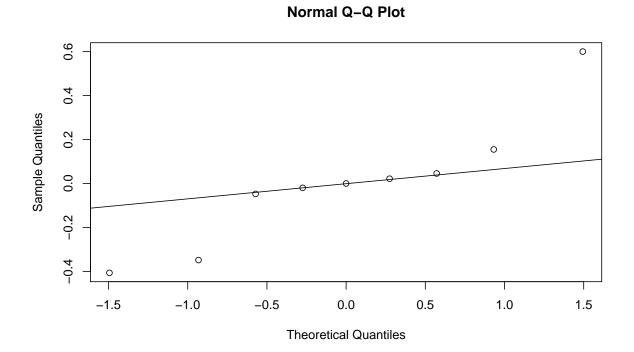
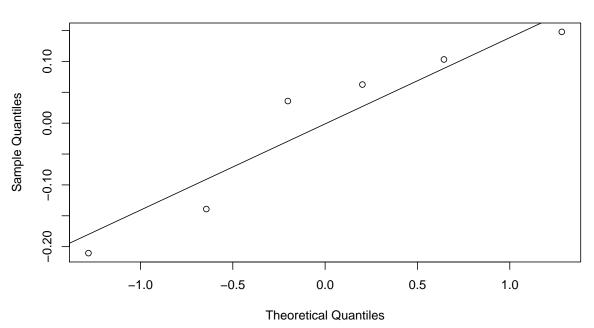


Figure 44: Diagnostic plot 3 for square-root nematodes count: container-level random effects estimates against equivalent normal quantiles. Ideally, a straight line.



Normal Q-Q Plot

Figure 45: Diagnostic plot 3 for square-root nematodes count: soil pit-level random effects estimates against equivalent normal quantiles. Ideally, a straight line.

## A.5 Plant-Eating Nematodes

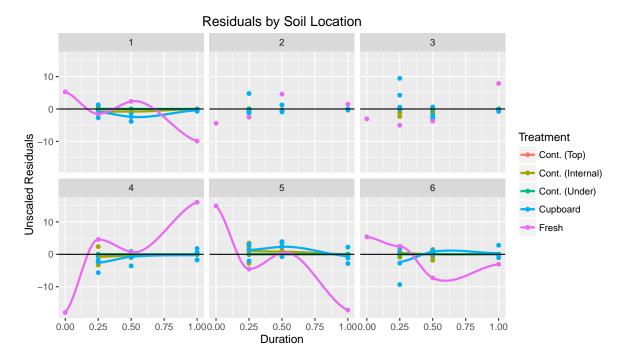


Figure 46: Scatterplot of logit plant-eating nematodes ratio residuals against duration by treatment, empanelled by soil location, with smooth means. Ideally, no patterns within location.

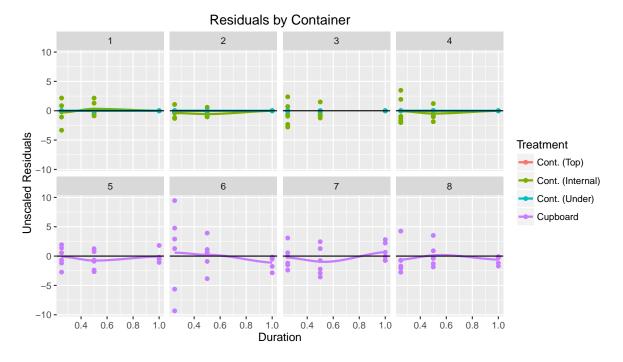


Figure 47: Scatterplot of logit plant-eating nematodes ratio residuals against duration by treatment, empanelled by container, with smooth means. Ideally, no patterns within container.

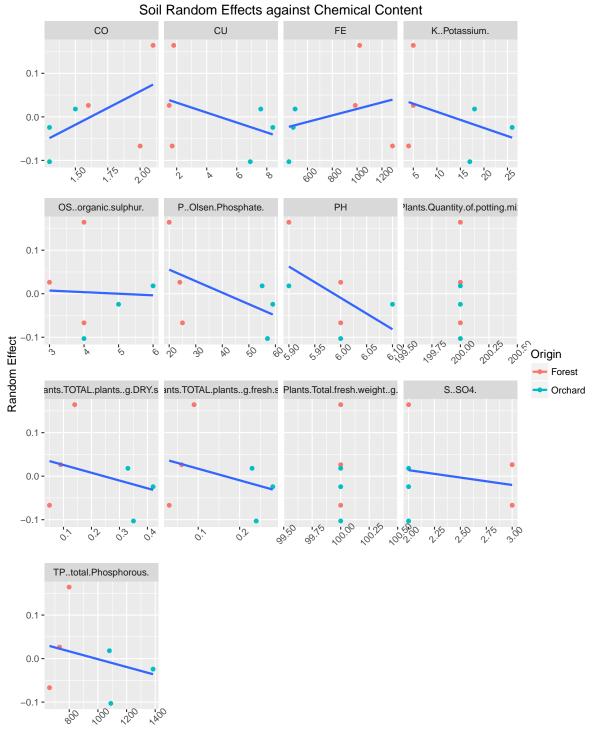


Figure 48: Scatterplots of logit plant-eating nematodes ratio soil random effects against soil random effect, coloured by origin (Forest / Orchard). Beware over-interpretation.

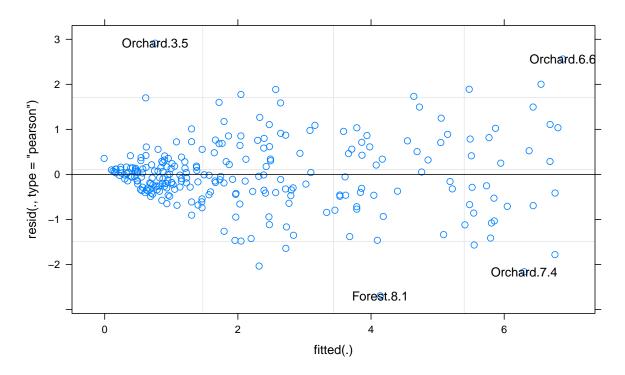
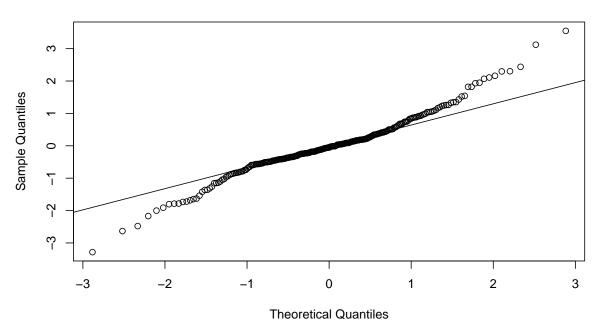


Figure 49: Diagnostic plot 1 for logit plant-eating nematodes ratio: variance-corrected ("Pearson") residuals against fitted values. Ideally, no visible pattern.



Normal Q-Q Plot

**Figure 50:** Diagnostic plot 2 for logit plant-eating nematodes ratio: variance-corrected ("Pearson") residuals against equivalent normal quantiles. Ideally, a straight line.

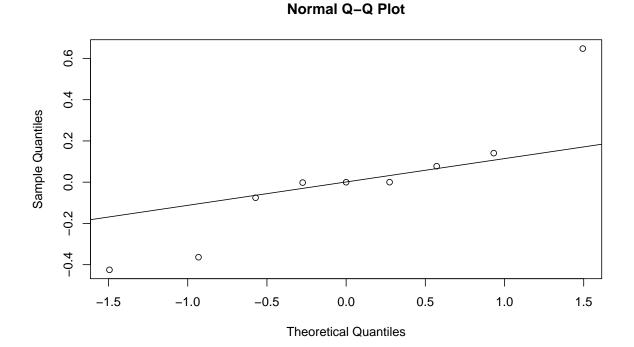


Figure 51: Diagnostic plot 3 for logit plant-eating nematodes ratio: container-level random effects estimates against equivalent normal quantiles. Ideally, a straight line.

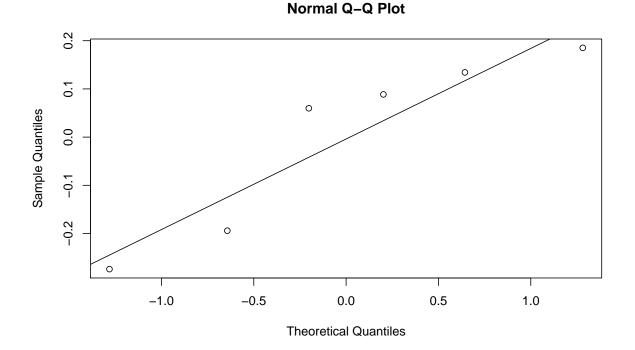


Figure 52: Diagnostic plot 3 for logit plant-eating nematodes ratio: soil pit-level random effects estimates against equivalent normal quantiles. Ideally, a straight line.

## A.6 Plant Count

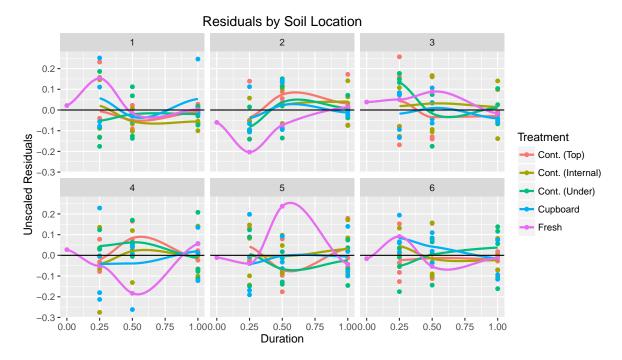
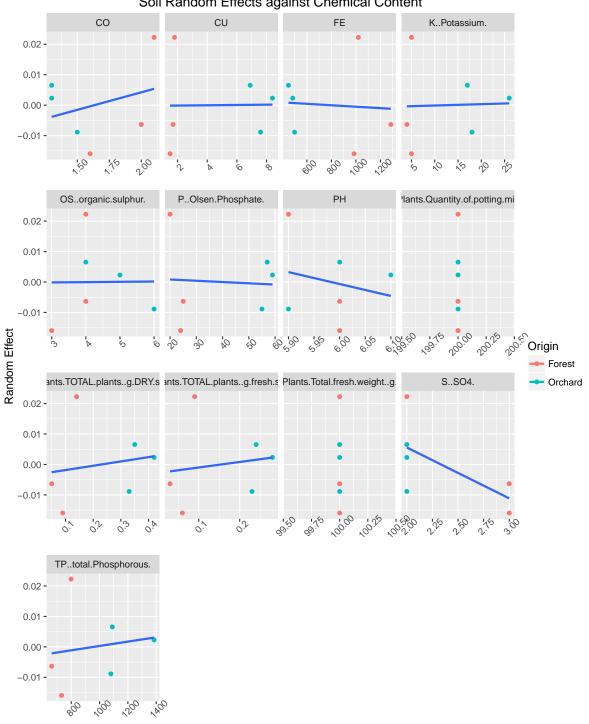


Figure 53: Scatterplot of square-root plant count residuals against duration by treatment, empanelled by soil location, with smooth means. Ideally, no patterns within location.



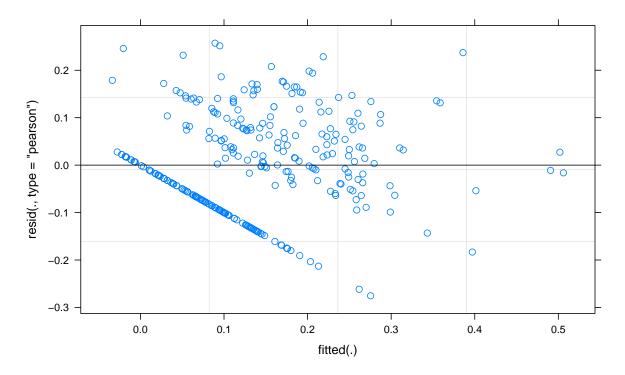
Figure 54: Scatterplot of square-root plant count residuals against duration by treatment, empanelled by container, with smooth means. Ideally, no patterns within container.



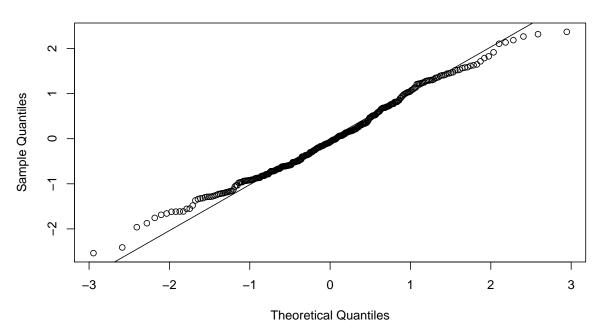
Soil Random Effects against Chemical Content

Soil Variable

Figure 55: Scatterplots of square-root plant count soil random effects against soil random effect, coloured by origin (Forest / Orchard). Beware over-interpretation.



**Figure 56:** Diagnostic plot 1 for square-root plant count: variance-corrected ("Pearson") residuals against fitted values. Ideally, no visible pattern.



Normal Q-Q Plot

**Figure 57:** Diagnostic plot 2 for square-root plant count: variance-corrected ("Pearson") residuals against equivalent normal quantiles. Ideally, a straight line.



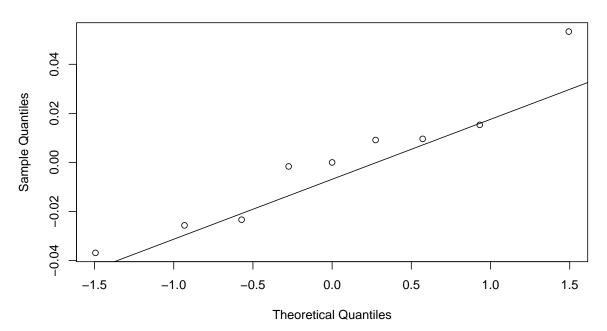
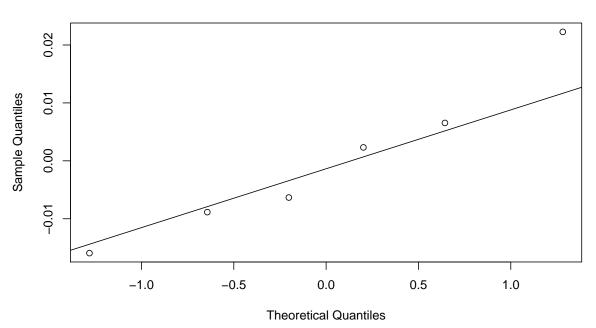


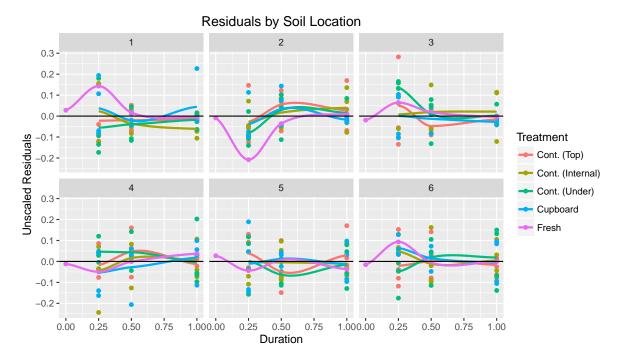
Figure 58: Diagnostic plot 3 for square-root plant count: container-level random effects estimates against equivalent normal quantiles. Ideally, a straight line.



Normal Q-Q Plot

Figure 59: Diagnostic plot 3 for square-root plant count: soil pit-level random effects estimates against equivalent normal quantiles. Ideally, a straight line.

## A.7 Plant Species Count



**Figure 60:** Scatterplot of square-root plant species count residuals against duration by treatment, empanelled by soil location, with smooth means. Ideally, no patterns within location.

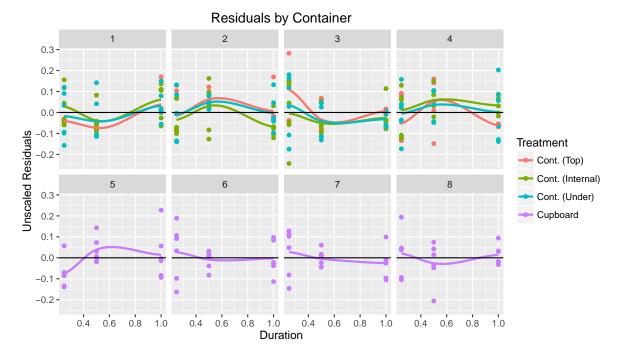
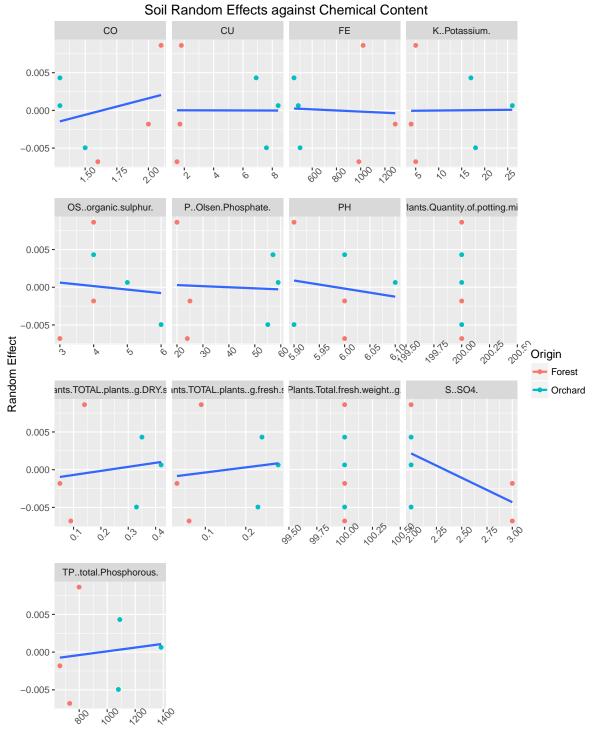
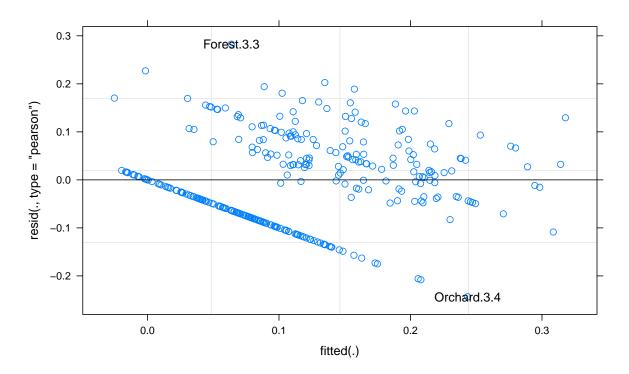


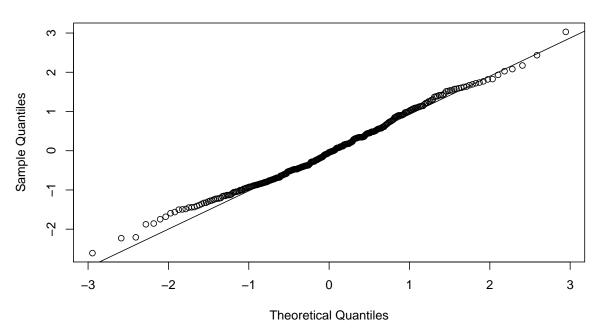
Figure 61: Scatterplot of square-root plant species count residuals against duration by treatment, empanelled by container, with smooth means. Ideally, no patterns within container.



**Figure 62:** Scatterplots of square-root plant species count soil random effects against soil random effect, coloured by origin (Forest / Orchard). Beware over-interpretation.



**Figure 63:** Diagnostic plot 1 for square-root plant species count: variance-corrected ("Pearson") residuals against fitted values. Ideally, no visible pattern.



Normal Q-Q Plot

**Figure 64:** Diagnostic plot 2 for square-root plant species count: variance-corrected ("Pearson") residuals against equivalent normal quantiles. Ideally, a straight line.



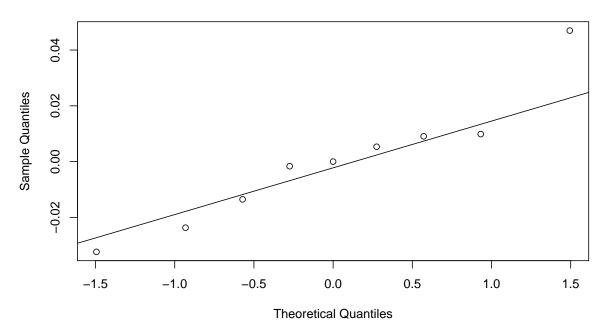
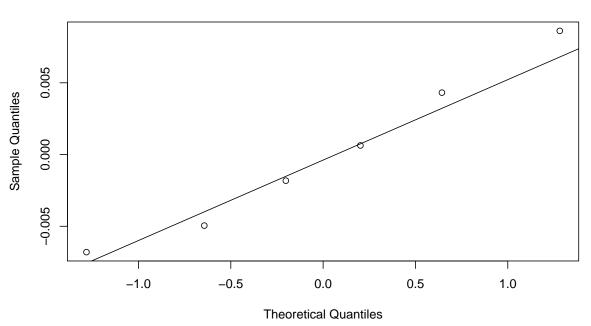


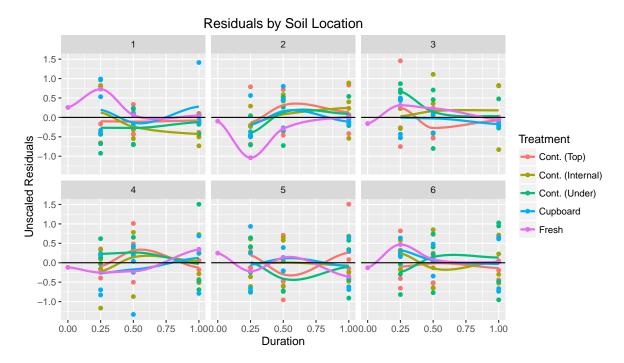
Figure 65: Diagnostic plot 3 for square-root plant species count: container-level random effects estimates against equivalent normal quantiles. Ideally, a straight line.



Normal Q-Q Plot

Figure 66: Diagnostic plot 3 for square-root plant species count: soil pit-level random effects estimates against equivalent normal quantiles. Ideally, a straight line.





**Figure 67:** Scatterplot of square-root plant species count (no offset) residuals against duration by treatment, empanelled by soil location, with smooth means. Ideally, no patterns within location.

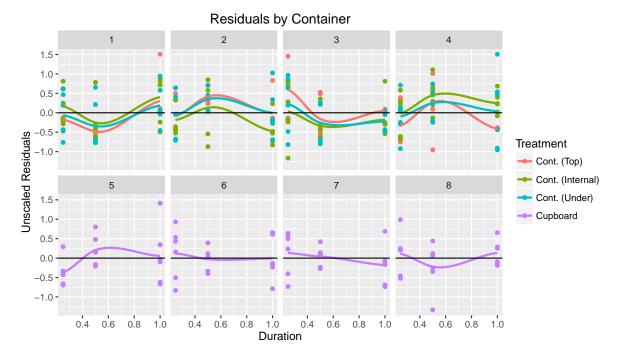
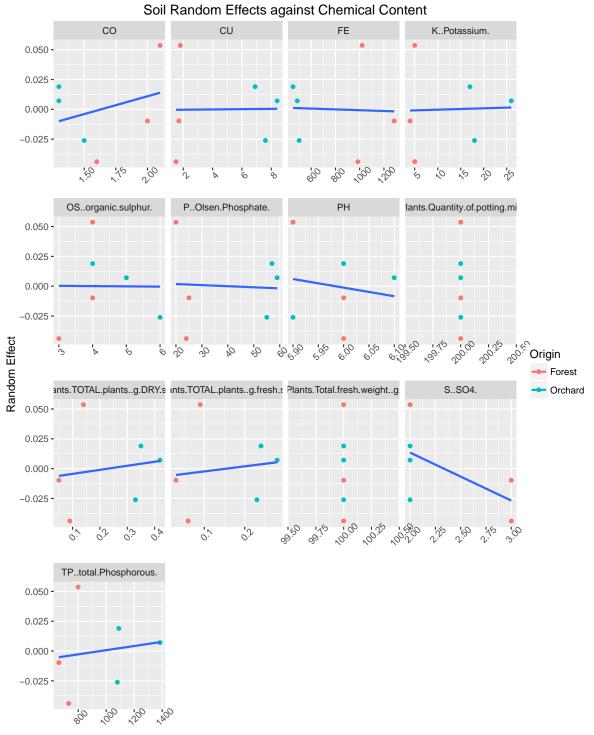
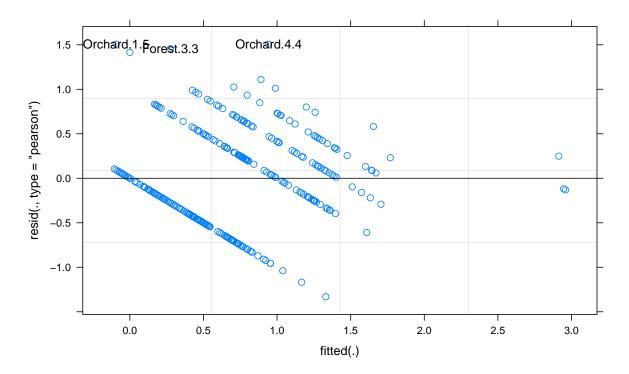


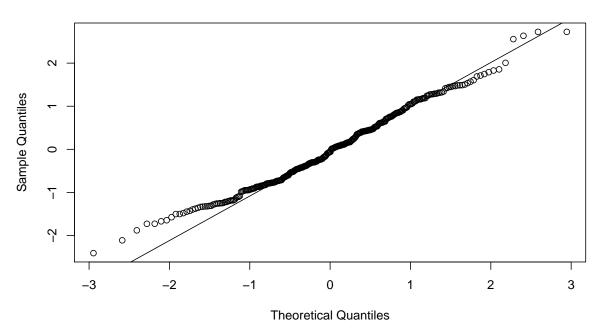
Figure 68: Scatterplot of square-root plant species count (no offset) residuals against duration by treatment, empanelled by container, with smooth means. Ideally, no patterns within container.



**Figure 69:** Scatterplots of square-root plant species count (no offset) soil random effects against soil random effect, coloured by origin (Forest / Orchard). Beware over-interpretation.



**Figure 70:** Diagnostic plot 1 for square-root plant species count (no offset): variance-corrected ("Pearson") residuals against fitted values. Ideally, no visible pattern.



Normal Q-Q Plot

**Figure 71:** Diagnostic plot 2 for square-root plant species count (no offset): variance-corrected ("Pearson") residuals against equivalent normal quantiles. Ideally, a straight line.

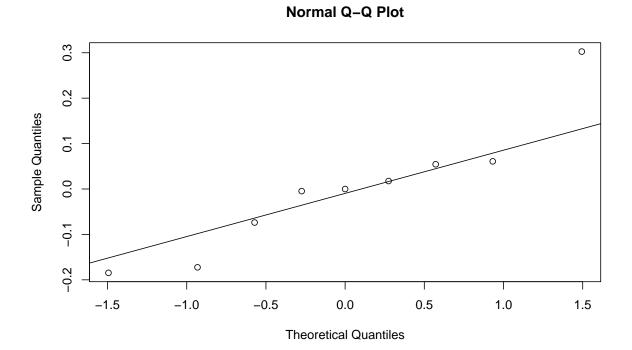
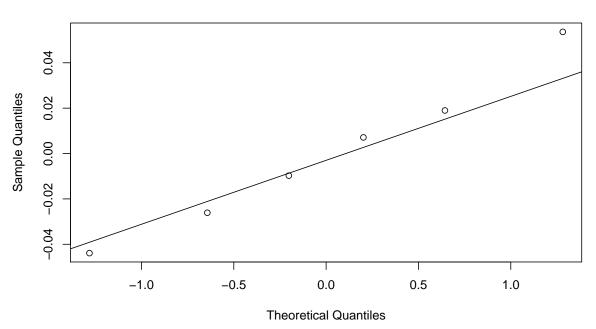


Figure 72: Diagnostic plot 3 for square-root plant species count (no offset): container-level random effects estimates against equivalent normal quantiles. Ideally, a straight line.



Normal Q-Q Plot

Figure 73: Diagnostic plot 3 for square-root plant species count (no offset): soil pit-level random effects estimates against equivalent normal quantiles. Ideally, a straight line.