Using `asremlPlus`, in conjunction with `asreml`, to do a linear mixed model analysis of a wheat experiment using hypothesis tests

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This vignette shows how to use `asremlPlus` (Brien, 2024), in conjunction with `asreml` (Butler et al., 2020), to employ hypothesis tests to select the terms to be included in a mixed model for an experiment that involves spatial variation. It also illustrates diagnostic checking and prediction production and presentation for this experiment. Here, `asremlPlus` and `asreml` are packages for the `R` Statistical Computing environment (R Core Team, 2024).

It is divided into the following main sections:

1. Set up the maximal model for this experiment
2. Perform a series of hypothesis tests to select a linear mixed model for the data
3. Diagnostic checking using residual plots and variofaces
4. Prediction production and presentation

1. Set up the maximal model for this experiment

```
library(knitr)
opts_chunk$set("tidy" = FALSE, comment = NA)
suppressMessages(library(asreml, quietly=TRUE))
```

```
## Offline License checked out Wed Jun 19 15:36:56 2024
```

```
packageVersion("asreml")
```

```
## [1] '4.2.0.332'
```

```
suppressMessages(library(asremlPlus))
packageVersion("asremlPlus")
```

```
## [1] '4.4.34'
```

```
suppressMessages(library(qqplotr, quietly=TRUE))
options(width = 100)
```

Get data available in asremlPlus

The data are from a 1976 spring wheat experiment and are taken from Gilmour et al. (1995). An analysis is presented in the `asreml` manual by Butler et al. (2020, Section 7.6), although they suggest that it is a barley experiment.

```
data(Wheat.dat)
```

Fit the maximal model

In the following a model is fitted that has the terms that would be included for a balanced lattice. In addition, a term WithinColPairs has been included to allow for extraneous variation arising between pairs of adjacent lanes. Also, separable ar1 residual autocorrelation has been included. This model represents the maximal anticipated model,

```
current.asr <- asreml(yield ~ WithinColPairs + Variety,
                      random = ~ Rep/(Row + Column) + units,
                      residual = ~ ar1(Row):ar1(Column),
                      maxit = 30, data=Wheat.dat)
```

```
ASReml Version 4.2 19/06/2024 15:37:00
          LogLik        Sigma2     DF     wall
 1     -724.1213      23034.14    124   15:37:00
 2     -717.4149      9206.931    124   15:37:00  (  2 restrained)
 3     -694.8752      26492.99    124   15:37:00  (  2 restrained)
 4     -694.1600      33101.80    124   15:37:00  (  1 restrained)
 5     -692.0020      36912.26    124   15:37:00  (  1 restrained)
 6     -691.7892      46701.51    124   15:37:00  (  2 restrained)
 7     -691.8336      46208.51    124   15:37:00  (  1 restrained)
 8     -691.7749      47698.26    124   15:37:00
 9     -691.7711      47041.85    124   15:37:00
```

```
Warning in asreml(yield ~ WithinColPairs + Variety, random = ~Rep/(Row + : Some components changed
by more than 1% on the last iteration
```

The warning from `asreml` is probably due to a bound term.

Initialize a testing sequence by loading the current fit into an asrtests object

A label and the information criteria based on the full likelihood (Verbyla, 2019) are included in the `test.summary` stored in the `asrtests` object.

```
current.asrt <- as.asrtests(current.asr, NULL, NULL,
                            label = "Maximal model", IClikelihood = "full")
```

```
Warning in infoCriteria.asreml(asreml.obj, IClikelihood = ic.lik, bound.exclusions = bound.exclusions):
 Rep
```

```
Warning in asreml(fixed = yield ~ WithinColPairs + Variety, random = ~Rep/(Row + : Log-likelihood
not converged
```

Check for and remove any boundary terms

```
current.asrt <- rmboundary(current.asrt, IClikelihood = "full")
```

```
Warning in infoCriteria.asreml(asreml.obj, IClikelihood = ic.lik): The following bound terms were discou
 Rep

ASReml Version 4.2 19/06/2024 15:37:01
          LogLik        Sigma2     DF     wall
 1     -691.7710      47071.42    124   15:37:01

Warning in asreml(fixed = yield ~ WithinColPairs + Variety, random = ~Rep/(Row + : Log-likelihood
not converged
```

```
summary(current.asrt$asreml.obj)$varcomp
```

```
                          component    std.error    z.ratio bound %ch
Rep:Row                4.293282e+03 3.199458e+03  1.3418779     P 0.0
Rep:Column             1.575689e+02 1.480357e+03  0.1064398     P 0.7
units                  5.742689e+03 1.652457e+03  3.4752438     P 0.0
Row:Column!R           4.706787e+04 2.515832e+04  1.8708669     P 0.0
Row:Column!Row!cor     7.920301e-01 1.014691e-01  7.8056280     U 0.0
Row:Column!Column!cor 8.799559e-01 7.370402e-02 11.9390486     U 0.0
```

```
print(current.asrt, which = "testsummary")
```

```
####  Sequence of model investigations

(If a row has NA for p but not denDF, DF and denDF relate to fixed and variance parameter numbers)

          terms DF denDF  p      AIC     BIC         action
1 Maximal model 26     6 NA 1646.129 1742.47 Starting model
2           Rep  1    NA NA 1646.129 1742.47       Boundary
```

Rep has been removed because it has been constrained to zero. Following the recommendation of Littel et al. (2006, p. 150), the bound on all variance components is set to unconstrained (U) using `setvariances.asreml` so as to avoid bias in the estimate of the residual variance. Alternatively, one could move Rep to the fixed model.

Unbind Rep, Row and Column components and reload into an asrtests object

```
current.asr <- setvarianceterms(current.asr$call,
                                terms = c("Rep", "Rep:Row", "Rep:Column"),
                                bounds = "U")
```

```
ASReml Version 4.2 19/06/2024 15:37:02
          LogLik        Sigma2     DF     wall
 1     -724.1213      23034.14    124   15:37:02
 2     -717.4149      9206.931    124   15:37:02  (  2 restrained)
 3     -694.8752      26492.99    124   15:37:02  (  2 restrained)
 4     -693.9744      33129.65    124   15:37:02  (  1 restrained)
 5     -692.8856      39662.12    124   15:37:02
 6     -691.4276      53103.83    124   15:37:02
 7     -691.2387      48092.17    124   15:37:02
 8     -691.1808      47278.94    124   15:37:02
 9     -691.1710      46850.98    124   15:37:02
10     -691.1700      46690.46    124   15:37:02

Warning in asreml(fixed = yield ~ WithinColPairs + Variety, random = ~Rep/(Row + : Some components
changed by more than 1% on the last iteration
```

```
current.asrt <- as.asrtests(current.asr, wald.tab = NULL, test.summary = current.asrt$test.summary,
                            IClikelihood = "full", label = "Max model & Unbound components")
current.asrt <- rmboundary(current.asrt)
summary(current.asrt$asreml.obj)$varcomp
```

```
                         component    std.error    z.ratio bound %ch
Rep                   -2458.3485841 1.197491e+03 -2.0529167     U 0.0
Rep:Row                5008.7151486 3.401335e+03  1.4725732     U 0.0
Rep:Column              916.4641198 1.699576e+03  0.5392309     U 0.2
units                  5959.0220817 1.609649e+03  3.7020634     P 0.0
Row:Column!R          46637.6303429 2.724392e+04  1.7118545     P 0.0
Row:Column!Row!cor        0.8150590 1.000281e-01  8.1483012     U 0.0
Row:Column!Column!cor     0.8856824 7.492514e-02 11.8208968     U 0.0
```

```
print(current.asrt, which = "testsummary")
```

```
####  Sequence of model investigations

(If a row has NA for p but not denDF, DF and denDF relate to fixed and variance parameter numbers)

                          terms DF denDF  p      AIC      BIC         action
1                 Maximal model 26     6 NA 1646.129 1742.470 Starting model
2                           Rep  1    NA NA 1646.129 1742.470       Boundary
3 Max model & Unbound components 26     7 NA 1647.193 1746.544 Starting model
```

```
print(current.asrt, which = "pseudoanova")
```

```
####  Pseudo-anova table for fixed terms


Wald tests for fixed effects.
```

```
Response: yield

               Df denDF   F.inc     Pr
(Intercept)     1   1.7 153.500 0.0115
WithinColPairs  1  15.6   2.545 0.1307
Variety        24  76.1  10.110 0.0000
```

Now the Rep component estimate is negative.

The `test.summary` output has been extended, by supplying the previous `test.summary` to `as.asrtests`, to show that there is a new starting model. The pseudo-anova table shows that Varieties are highly significant ($p < 0.001$)

2. Perform a series of hypothesis tests to select a linear mixed model for the data

The hypothesis tests in this section are Wald tests for fixed terms, with denominator degrees of freedom calculated using the Kenward-Rogers adjustment (Kenward and Rogers (1997), and Restricted Maximum Likelihood Ratio Tests (REMLRT) for random terms.

Check the term for within Column pairs (a post hoc factor)

The information criteria based on the full likelihood (Verbyla, 2019) is also included in the `test.summary` stored in the `asrtests` object.

```
current.asrt <- testranfix(current.asrt, term = "WithinColPairs",
                           drop.fix.ns=TRUE, IClikelihood = "full")
```

```
Warning in asreml(fixed = yield ~ Variety, random = ~Rep/(Row + Column) + : Some components changed
by more than 1% on the last iteration
Warning in asreml(fixed = yield ~ Variety, random = ~Rep/(Row + Column) + : Some components changed
by more than 1% on the last iteration
```

```
print(current.asrt)
```

```
####  Summary of the fitted variance parameters

                         component    std.error    z.ratio bound %ch
Rep                  -2391.9489939 1.194581e+03 -2.0023338     U 0.4
Rep:Row               5035.5311054 3.406006e+03  1.4784269     U 0.3
Rep:Column             761.9535622 1.612103e+03  0.4726458     U 1.2
units                 5933.2133794 1.610805e+03  3.6833848     P 0.1
Row:Column!R         45970.8383027 2.635124e+04  1.7445415     P 0.0
Row:Column!Row!cor       0.8101615 9.995498e-02  8.1052641     U 0.1
Row:Column!Column!cor    0.8846970 7.503039e-02 11.7911827     U 0.0


####  Pseudo-anova table for fixed terms
```

```
Wald tests for fixed effects.
Response: yield

            Df denDF  F.inc     Pr
(Intercept)  1   1.7 158.90 0.0112
Variety     24  76.8  10.27 0.0000


####  Sequence of model investigations

(If a row has NA for p but not denDF, DF and denDF relate to fixed and variance parameter numbers)

                           terms DF denDF      p      AIC      BIC         action
1                  Maximal model 26   6.0     NA 1646.129 1742.470 Starting model
2                            Rep  1    NA     NA 1646.129 1742.470       Boundary
3 Max model & Unbound components 26   7.0     NA 1647.193 1746.544 Starting model
4                 WithinColPairs  1  15.6 0.1307 1645.325 1741.666        Dropped
```

It is clear in the call to `testranfix` that the model is being changed by dropping the `withinColPairs` term, which could also be achieved using `update.asreml`. However, an `asremlPlus` model-changing function operates on an `asrtests` object, that includes an `asreml` object, and, except for `changeTerms.asrtests`, results in an `asrtests` object that may contain the changed model or the supplied model depending on the results of hypothesis tests or comparisons of information criteria. In addition, the result of the test or comparison will be added to a `test.summary` data.frame stored in the new `asrtests` object and, if the model was changed, the `wald.tab` in the new `asrtests` object will have been updated for the new model.

In this case, as can be seen from the summary of `current.asrt` after the call, the p-value for the `withinColPairs` was greater than 0.05 and so now the model stored in `current.asrt` does not include `withinColPAirs`. The `wald.tab` has been updated for the new model.

Test the nugget term

The nugget term represents non-spatial variance, such as random plot and measurement error. It is fitted using the `asreml` reserved word `units`.

```
current.asrt <- testranfix(current.asrt, "units", positive=TRUE, IClikelihood = "full")
```

```
Warning in asreml(fixed = yield ~ Variety, random = ~Rep + Rep:Row + Rep:Column, : Some components
changed by more than 1% on the last iteration
Warning in asreml(fixed = yield ~ Variety, random = ~Rep + Rep:Row + Rep:Column, : Some components
changed by more than 1% on the last iteration
```

Test Row autocorrelation

We begin testing the autocorrelation by dropping the Row autocorrelation. Because of messages about the instability of the fit, `iterate.asrtests` is used to execute extra iterations of the fitting process.

```
current.asrt <- testresidual(current.asrt, "~ Row:ar1(Column)",
                             label="Row autocorrelation",
                             simpler=TRUE, IClikelihood = "full")
```

```
Warning in asreml(fixed = yield ~ Variety, random = ~Rep/(Row + Column) + : Some components changed
by more than 1% on the last iteration
Warning in asreml(fixed = yield ~ Variety, random = ~Rep/(Row + Column) + : Some components changed
by more than 1% on the last iteration
```

```
current.asrt <- iterate(current.asrt)
```

Test Column autocorrelation (depends on whether Row autocorrelation retained)

The function `getTestPvalue` is used to get the p-value for the Row autocorrelation test. If it is significant then the Column autocorrelation is tested by by dropping the Column autocorrelation, while retaining the Row autocorrelation. Otherwise the model with just Row autocorrelation, whose fit is returned via `current.asrt` after the test, is compared to one with no autocorrelation.

```
(p <- getTestPvalue(current.asrt, label = "Row autocorrelation"))
```

```
[1] 4.676754e-06
```

```
{ if (p <= 0.05)
  current.asrt <- testresidual(current.asrt, "~ ar1(Row):Column",
                               label="Col autocorrelation",
                               simpler=TRUE, IClikelihood = "full")
  else
    current.asrt <- testresidual(current.asrt, "~ Row:Column",
                                 label="Col autocorrelation",
                                 simpler=TRUE, IClikelihood = "full")
}
```

```
Warning in DFdiff(bound.h1, bound.h0, DF = DF, bound.exclusions = bound.exclusions): There were a total
  The following bound terms occur in only one of the models compared and so were discounted:
   Row:Column!Row!cor
```

Output the results

```
print(current.asrt)
```

```
####  Summary of the fitted variance parameters

                         component    std.error    z.ratio bound %ch
Rep                   -2385.8697551 1.211207e+03 -1.9698276     U 0.0
Rep:Row                5027.7123253 3.415391e+03  1.4720753     U 0.0
Rep:Column              753.5913536 1.609865e+03  0.4681086     U 0.6
units                  5920.3547038 1.611274e+03  3.6743304     P 0.0
Row:Column!R          45870.0971595 2.623601e+04  1.7483638     P 0.0
Row:Column!Row!cor        0.8098786 1.001805e-01  8.0841906     U 0.0
Row:Column!Column!cor     0.8845768 7.510598e-02 11.7777144     U 0.0
```

```
####  Pseudo-anova table for fixed terms


Wald tests for fixed effects.
Response: yield

            Df denDF  F.inc     Pr
(Intercept)  1   1.7 159.20 0.0111
Variety     24  76.8  10.27 0.0000


####  Sequence of model investigations

(If a row has NA for p but not denDF, DF and denDF relate to fixed and variance parameter numbers)

                           terms DF denDF      p      AIC      BIC         action
1                  Maximal model 26   6.0     NA 1646.129 1742.470 Starting model
2                            Rep  1    NA     NA 1646.129 1742.470       Boundary
3 Max model & Unbound components 26   7.0     NA 1647.193 1746.544 Starting model
4                  WithinColPairs  1  15.6 0.1307 1645.325 1741.666        Dropped
5                           units  1    NA 0.0006 1645.325 1741.666       Retained
6             Row autocorrelation  1    NA 0.0000 1645.325 1741.666      Unswapped
7             Col autocorrelation  2    NA 0.0000 1645.318 1741.658      Unswapped
```

```
printFormulae(current.asrt$asreml.obj)
```

```
#### Formulae from asreml object

fixed: yield ~ Variety
random: ~ Rep/(Row + Column) + units
residual: ~ ar1(Row):ar1(Column)
```

```
print(R2adj(current.asrt$asreml.obj, include.which.random = ~ .))
```

```
ASReml Version 4.2 19/06/2024 15:37:08
          LogLik        Sigma2     DF     wall
 1     -694.6149      45855.31    125   15:37:08
 2     -694.6149      45854.06    125   15:37:08
[1] 44.62413
attr(,"fixed")
~.
<environment: 0x000001f1ef829858>
attr(,"random")
~.
```

The `test.summary` shows is that the model with Row and without Column autocorrelation failed to converge. The `asreml.obj` in `current.asrt` contains the model selected by the selection process, which has been printed using `printFormulae.asrtests`. It is clear that no changes were made to the variance terms. The adjusted R^2 value shows that the fixed and random terms in the fitted model account for 45% of the total variation in the yield.

3. Diagnosing checking using residual plots and variofaces

Get current fitted asreml object and update to include standardized residuals

```
current.asr <- current.asrt$asreml.obj
current.asr <- update(current.asr, aom=TRUE)
```

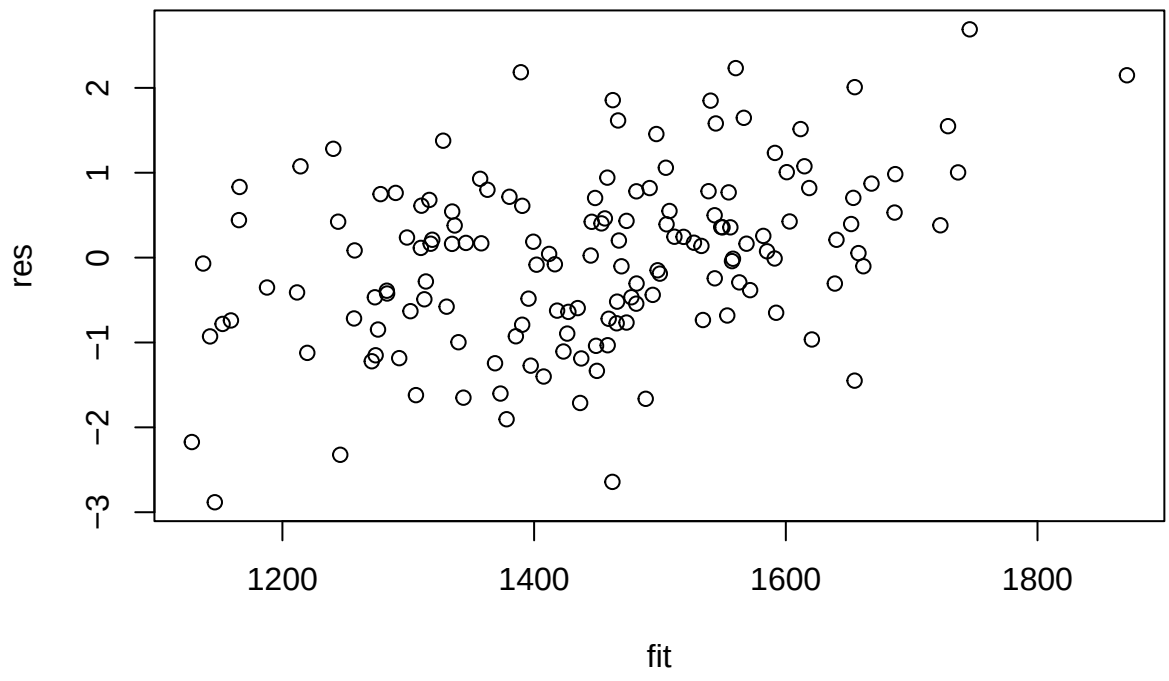
```
ASReml Version 4.2 19/06/2024 15:37:08
          LogLik        Sigma2     DF     wall
 1     -694.6149      45855.31    125   15:37:08
 2     -694.6149      45854.06    125   15:37:08
 3     -694.6149      45851.09    125   15:37:08
```

```
Wheat.dat$res <- residuals(current.asr, type = "stdCond")
Wheat.dat$fit <- fitted(current.asr)
```

Do diagnostic checking

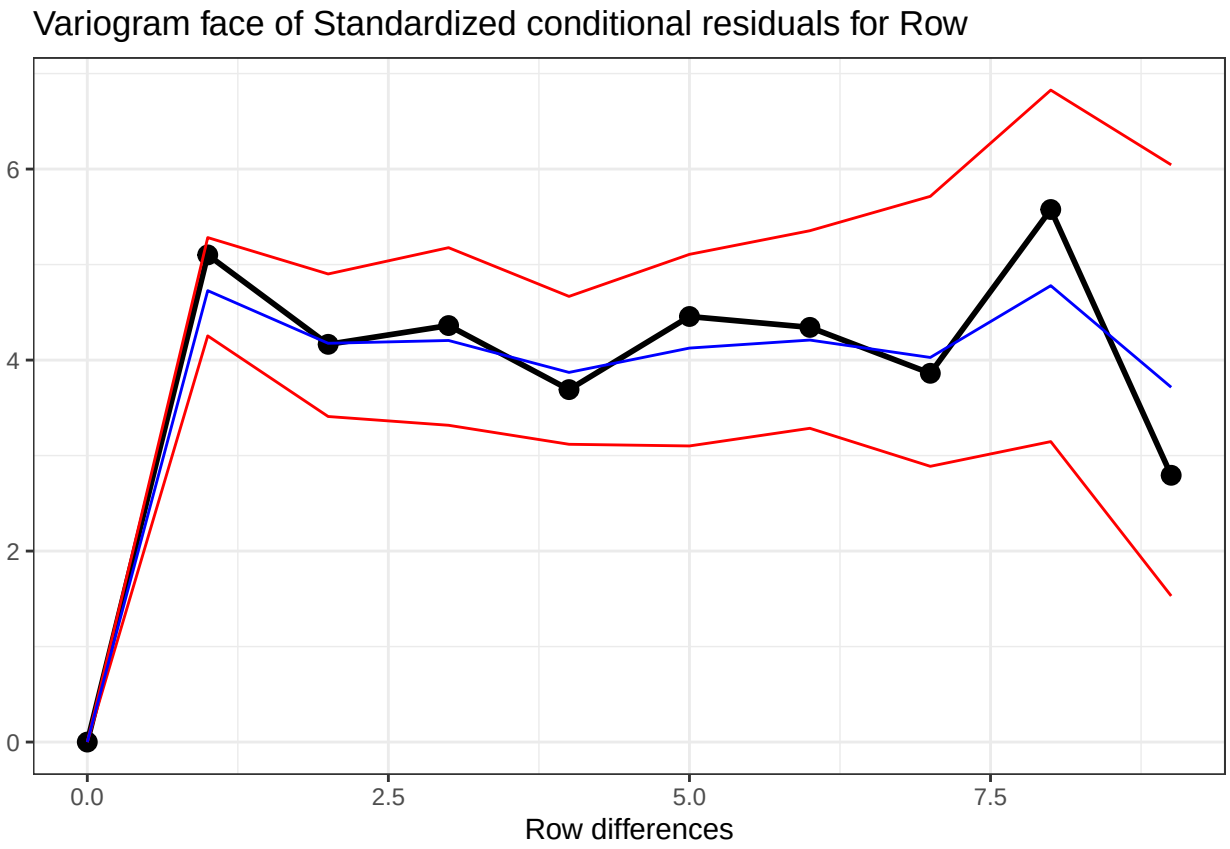
Do residuals-versus-fitted values plot

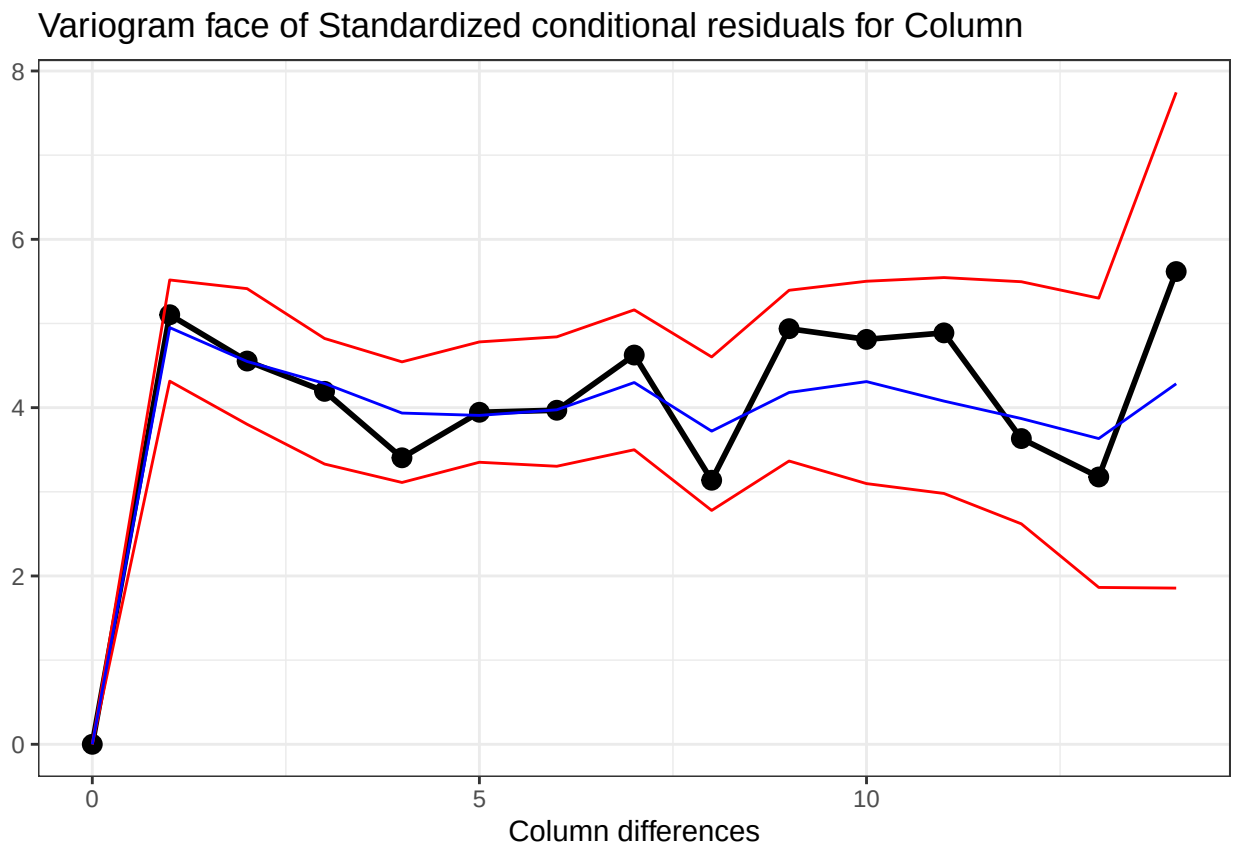
```
with(Wheat.dat, plot(fit, res))
```



Plot variofaces

```
variofaces(current.asr, V=NULL, units="addtores",
           maxiter=50, update = FALSE,
           ncores = parallel::detectCores())
```



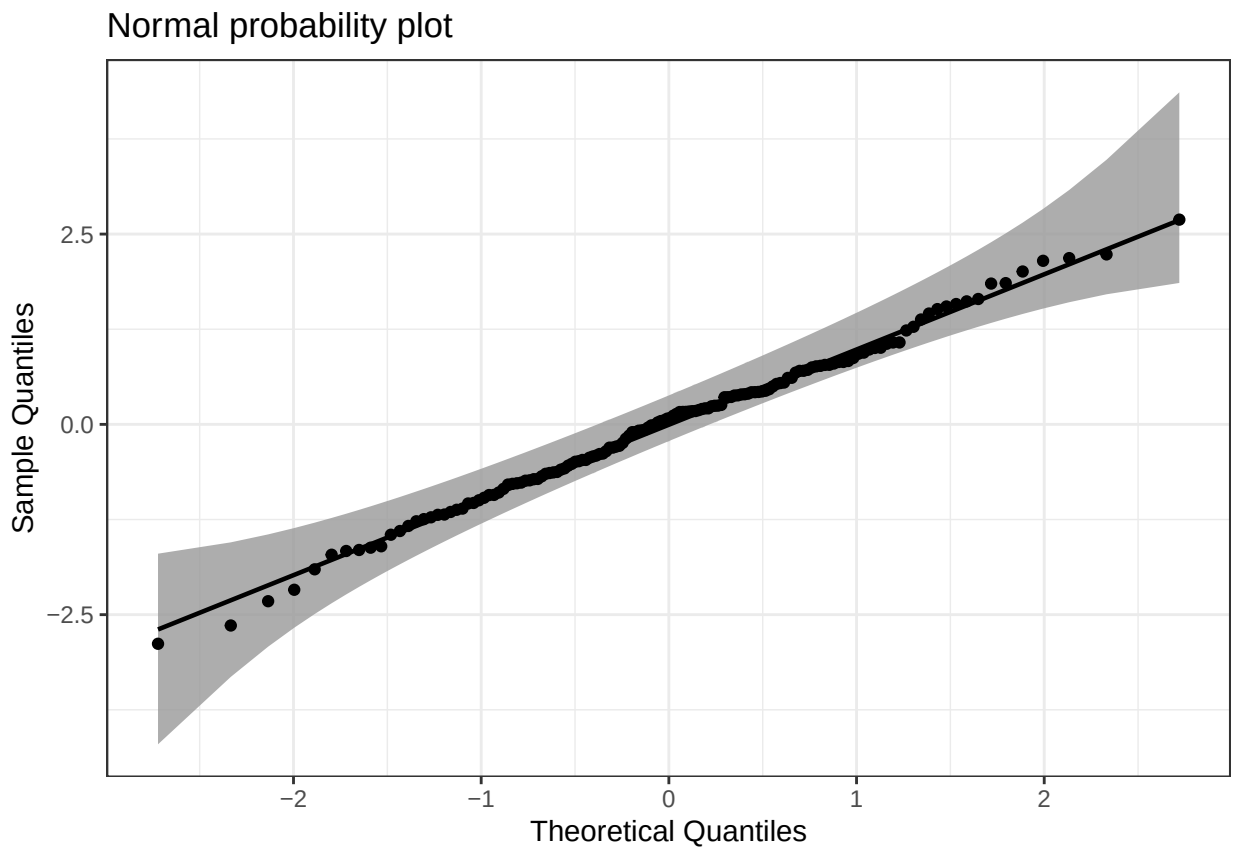


The variofaces are the lag 1 plots of the sample semivariogram with simulated confidence envelopes (Stefanova et al., 2009).

Plot normal quantile plot

The plot is obtained using the `ggplot` function with extensions available from the `qqplotr` package (Almeida, A., Loy, A. and Hofmann, H., 2023).

```
suppressWarnings(
  ggplot(data = Wheat.dat, mapping = aes(sample = res)) +
    stat_qq_band(bandType = "ts") + stat_qq_line() + stat_qq_point() +
    labs(x = "Theoretical Quantiles", y = "Sample Quantiles",
         title = "Normal probability plot") +
    theme(plot.title = element_text(size = 12, face = "bold")) + theme_bw())
```



4. Prediction production and presentation

Get Variety predictions and all pairwise prediction differences and p-values

```
Var.diffs <- predictPlus(classify = "Variety",
                         asreml.obj=current.asr,
                         error.intervals="halfLeast",
                         wald.tab=current.asrt$wald.tab,
                         sortFactor = "Variety",
                         tables = "predictions")
```

```
#### Predictions for yield from Variety


Notes:
- The predictions are obtained by averaging across the hypertable
  calculated from model terms constructed solely from factors in
  the averaging and classify sets.
- Use 'average' to move ignored factors into the averaging set.
- The ignored set: Rep,Row,Column,units
```

```
- Variety is included in this prediction
- (Intercept) is included in this prediction
- units is ignored in this prediction
```

	Variety	predicted.value	standard.error	upper.halfLeastSignificant.limit	lower.halfLeastSignificant.limit	est.status
1	10	1168.989	120.4768	1228.315	1109.663	Estimable
2	1	1242.750	119.8104	1302.076	1183.424	Estimable
3	9	1257.137	119.9708	1316.463	1197.811	Estimable
4	16	1285.718	119.9400	1345.045	1226.392	Estimable
5	14	1293.526	119.9227	1352.853	1234.200	Estimable
6	23	1313.653	120.2929	1372.979	1254.327	Estimable
7	11	1322.159	120.1964	1381.485	1262.832	Estimable
8	7	1374.447	120.2407	1433.773	1315.120	Estimable
9	3	1394.070	120.4032	1453.396	1334.743	Estimable
10	4	1410.980	120.1055	1470.306	1351.653	Estimable
11	12	1444.557	120.6034	1503.883	1385.231	Estimable
12	8	1453.396	120.5940	1512.723	1394.070	Estimable
13	15	1458.383	120.4346	1517.709	1399.057	Estimable
14	5	1473.782	120.4455	1533.108	1414.456	Estimable
15	17	1487.828	120.2896	1547.154	1428.501	Estimable
16	6	1498.294	120.1189	1557.620	1438.968	Estimable
17	21	1517.121	120.2262	1576.447	1457.795	Estimable
18	2	1520.466	119.6322	1579.792	1461.140	Estimable
19	24	1533.769	120.2995	1593.095	1474.443	Estimable
20	18	1541.148	120.3664	1600.474	1481.821	Estimable
21	25	1575.795	120.5142	1635.121	1516.468	Estimable
22	22	1610.482	120.3281	1669.808	1551.156	Estimable
23	13	1610.762	120.4575	1670.088	1551.436	Estimable
24	20	1627.971	120.2328	1687.297		
25	19	1652.992	120.3435	1712.318		

```
24                          1568.645  Estimable
25                          1593.666  Estimable


LSD values

minimum LSD =  114.0128

mean LSD =  118.6523

maximum LSD =  123.3578

(sed range / mean sed =  0.0788 )
```

We have set `error.intervals` to `halfLeast` so that the limits for so that the limits for each prediction $\pm$ (0.5 LSD) are calculated. When these are plotted overlapping error bars indicate predictions that are not significant, while those that do not overlap are significantly different (Snee, 1981).

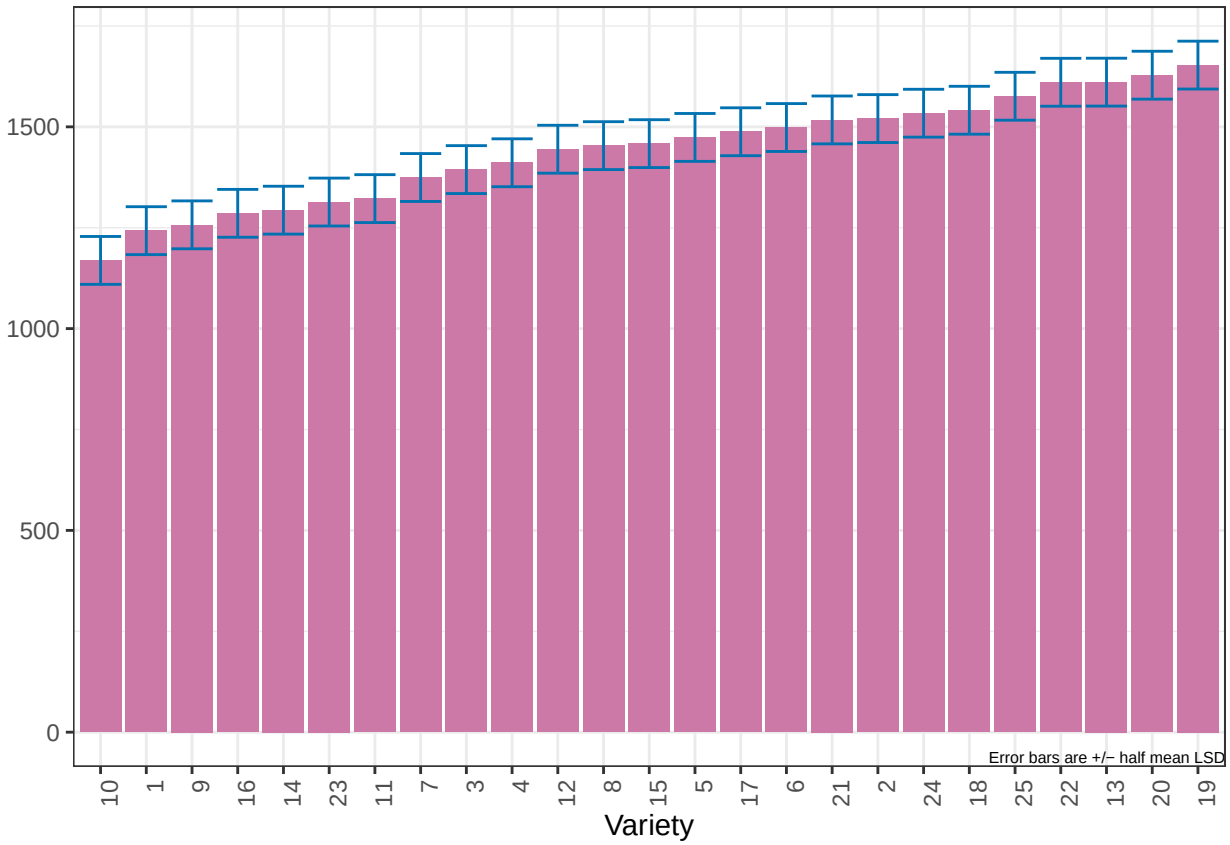
Also set was `sortFactor`, so that the results would be ordered for the values of the predictions for Variety.

The function `predictPlus` returns an `alldiffs` object, a list consisting of the following components:

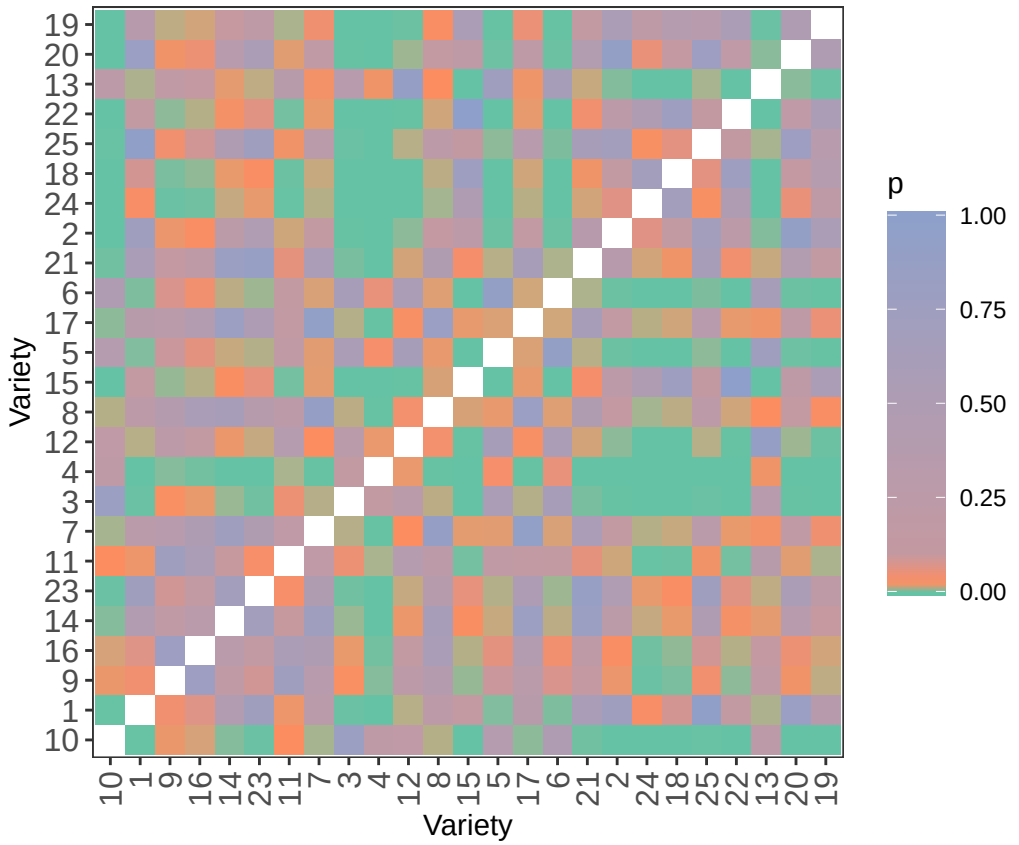
- `predictions`: the predictions, their standard errors and error intervals;
- `vcov`: the variance matrix of the predictions;
- `differences`: all pairwise differences between the predictions,
- `p.differences`: p-values for all pairwise differences between the predictions;
- `sed`: the standard errors of all pairwise differences between the predictions;
- `LSD`: the mean, minimum and maximum LSDs.

Plot the Variety predictions, with halfLSD intervals, and the p-values

```
plotPredictions(Var.diffs$predictions,
                classify = "Variety", y = "predicted.value",
                error.intervals = "half")
```




```
plotPvalues(Var.diffs)
```



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