Mixed Effect ANOVAs

Mixed-effects models handle repeated-measures designs to avoid pseudoreplication. They also handle spatial units that are considered random effects. Those spatial units might also be considered fixed-effects blocks (a "philosophical" choice that can be evaluated statistically). Here we work with data from a large, repeated-measures experiment using whole wetlands as units in a 3-way factorial design, where treatments were:

- cattle excluded with fence (or not), and
- prescribed fire applied or not, in
- either intensive land use or seminatural pastures (Boughton et al. 2015).

2006 was a pasture-only year. Fences were installed in winter 2006-2007, and then fire was applied in winter 2007-2008 and periodically since. So we expect changes through time and use 2006 as a baseline.

The goal here is to build toward a model that evaluates the interactive effects of treatments on plant diversity through the years. We start with a simpler model for the last year (2015) and then add layers of complexity to represent random effects in space and time – just to illustrate what mixed effect models can do. We analyze simple, total species richness here (i.e., the number of species in a wetland (or "pond" in the data). The data set lists species richness per wetland from 2006 (when only pasture treatments had been applied) through 2015.

<u>Getting Organized</u>:

1. Load these packages to use (if not already installed, first do that):

```
library(tidyverse) # dplyr, readr, ggplot, etc. etc. in one line
library(MASS) # for stepAIC
library(glmmTMB) # to run glm and glmm
library(MuMIn) # for R2 estimates
library(bbmle) # for AICs
library(performance) # for assumptions and R2 values
```

2. Import and attach the **`plantSR0615.txt`** file. Below I assume you called it "data." Note that this is all the raw data but we want Species Richness. So next we count species in each row and make factors of year, block, fence, and burn.

```
data$SR <- rowSums(data[,7:203]!=0) # counts nonzero entries per row to make
Species Richness (SR) - Should use neg binom or poisson...
data$fyear <- factor(data$year)
data$fblock <- factor(data$block)
data$ffence <- factor(data$fence)
data$fburn <- factor(data$burn)
```

3. We need to use initial conditions (year 2006) as a covariate in analyses because wetlands already varied (i.e., this "levels" the pre-existing conditions):

```
yr2006 <- filter(data, fyear == "2006")
SR06 <- yr2006$SR # this matches length of any one year
```

5. Finally, let's set apart year 2015 to analyze for single-year spatial effects only, as a point of comparison for mixed effects models to follow.

```
yr2015 <- filter(data, fyear == "2015")
```

6. Now let's run a relatively simple model for 2015 only (despite effects of intervening years on 2015 plant communities. We use year 2006 values as a covariate and blocks as *fixed effects*. We also evaluate residual assumptions.

```
nullmodel <- glmmTMB(SR ~ 1, data=yr2015, family=Gamma(link="log")) # fixed
effects only, using gamma with log link (a flexible version of a poisson/neg
binomial)
model1 <- glmmTMB(SR ~ scale(SR06) + pasture*ffence*fburn + fblock,
data=yr2015, family=Gamma(link="log")) # fixed effects only, but otherwise as
for null
check_model(model1)
r.squaredLR(model1,nullmodel) # in MuMIn: compares fit to a null to estimate a
pseudo-R^2 value
summary(model1)
```

Feel free to try different families to see if another works better.

7. What do you see in results for year 2015?

8. Now we handle blocks as a ***random effect***, rather than a fixed effect, because *blocks did not represent actual spatial patterns on the ranch* – instead they were units of sampling convenience.

```
mmodel2 <- glmmTMB(SR ~ scale(SR06) + pasture*ffence*fburn + (1|fblock),
data=yr2015, family=Gamma(link="log"))
check_model(mmodel2)
r2_nakagawa(mmodel2) # from performance
summary(mmodel2)
```

9. Did treating blocks as random effects alter the "story"? How about assumptions?

10. Now let's analyze results **across all the years.** For that repeated measures analysis, time can be treated as a random effect. So now we replicate SR06 for all the years (to make the data match up):

```
SR06rep <- rep(SR06, 9) # to make a SR06 for all-years analyses (9 years)
```

And run the model again but using random effects for space (blocks) and time (years).

```
mmodel3 <- glmmTMB(SR ~ scale(SR06rep) + pasture*ffence*fburn + (1|fblock) +
(1|fyear), data=data, family=Gamma(link="log")) # fixed effects only, but
otherwise as for null
check_model(mmodel3)
r2_nakagawa(mmodel3) # from performance
summary(mmodel3)
```

11. What are the overall experimental results for simple SR?
12. How important are the random effects? Compare Random Effect Std. Dev. to coefficients as a way to compare them.
13. And how much variation did the model “explain”?
14. And how well do analyses meet assumptions about residuals?

15. Can the relatively complex mmodel3 be simplified using stepAIC?

16. Now let's consider what would happen if we mistakenly considered each year as an independent replicate (i.e., temporal pseudoreplication), blindly used a plain ol' lm (family="gaussian"), AND didn't think of spatial variation (blocks), AND didn’t think about initial SR in 2006 as already setting up some variation:

```
wrongmodel <- lm(SR ~ pasture*ffence*fburn, data=data)
check_model(wrongmodel)
summary.aov(wrongmodel)
summary(wrongmodel)
```

17. Compare coefficients (effect sizes) and their std. errors among the “correct” and wrong models. And how do R^2 values compare? Would we have written about a different story?

<u>Bottom Lines</u>: Mixed effect models can represent a lot of variation in space and time that fixed effect models cannot, and so better tease out the story, even if it may be counter to our “need” to find effects. ***Expect to use mixed effect models in your research!***

<u>References</u>

- Boughton EH, et al. 2015. Interactive effects of pasture management intensity, release from grazing, and prescribed fire on forty subtropical wetland plant assemblages. J. Applied Ecology 53:159-170.
- Jost, L. 2006. Entropy and diversity. Oikos 113:363-375.