

C:N Ratios

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Summary Tables

C:N ratios

- ar = Annual Rye
- cr = Cereal Rye
- pc = Wild Type Pennycress
- gm = Gene Edited AOP2 Pennycress

Table 1. Carbon to nitrogen ratio, percent carbon, and percent nitrogen by species.

spp	avg_pct_n	avg_pct_c	c_n_ratio
ar	1.741304	35.23478	20.77427
cr	1.471064	37.33830	26.15907
gm	1.500851	41.41702	27.92590
pc	1.847447	41.87021	23.27899

C:N ratios by plot

Table 2. Carbon to nitrogen ratio, percent carbon, and percent nitrogen by species with plot. Plots were determined by placing samples in the two most prevalent soil types in central Illinois.

spp	plot	avg_pct_n	avg_pct_c	c_n_ratio
ar	1	1.657727	33.89545	20.80510
ar	2	1.817917	36.46250	20.74602
cr	1	1.433043	36.73478	26.18316
cr	2	1.507500	37.91667	26.13600
gm	1	1.453809	41.26190	28.80456
gm	2	1.538846	41.54231	27.21621
pc	1	1.792174	41.24348	23.57482
pc	2	1.900417	42.47083	22.99549

Summary Figure

Figure 1. Mean and standard error of carbon to nitrogen ratio by species with color as plot.

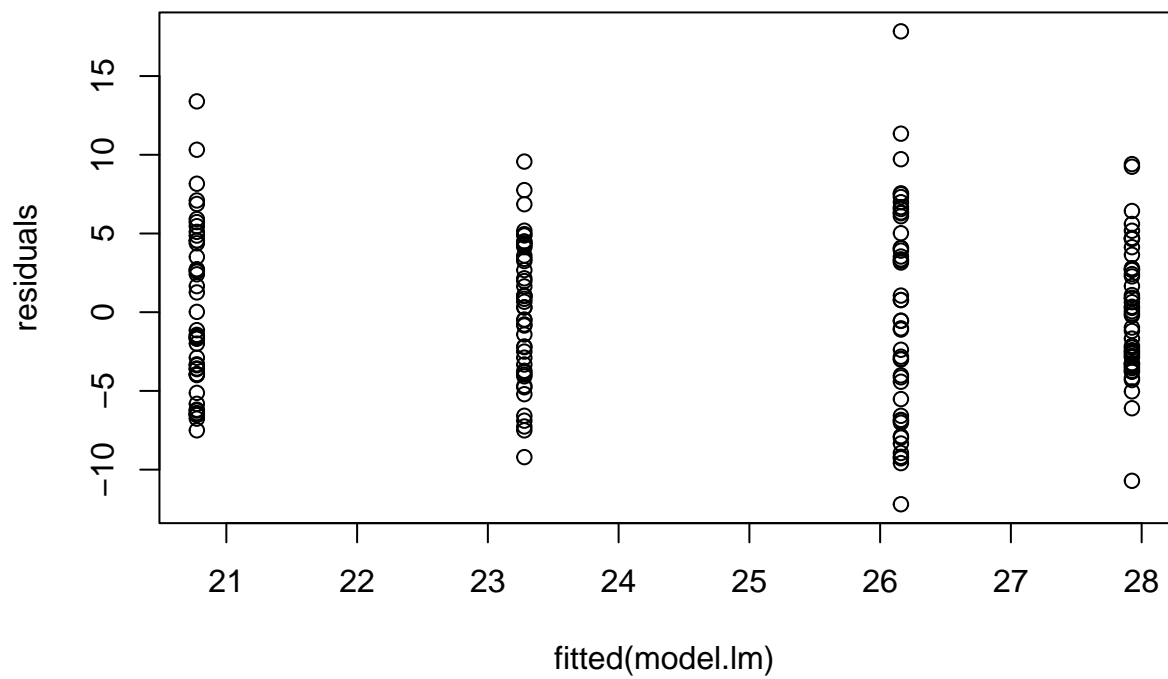
Statistics

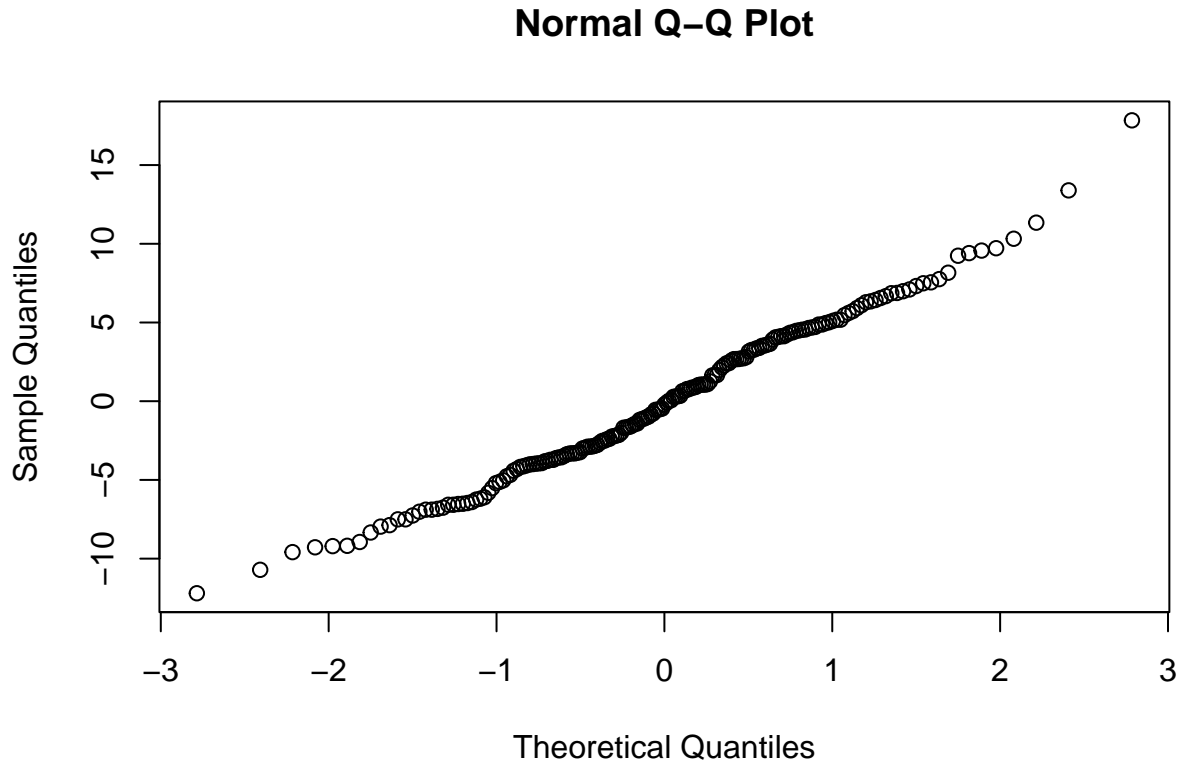
Do species significantly differ in their C:N ratio, does soil type have a significant effect?

Model:

- DV: C:N ratio
- Fixed = Species
- Random = Soil type (plot) and Row

Using AIC the model with plot and subplot fit the best so those terms are included as random effects, but they do not have a significant effect on the model using the -2 Log Likelihood method.





Figures 2 and 3. Graphed residuals for visual analysis of homogeneity of variance and normality.

Table 3. Data used for model selection.

	npar	logLik	AIC	LRT	Df	Pr(>Chisq)
	7	-568.9479	1151.896	NA	NA	NA
(1 plot)	6	-568.9479	1149.896	0	1	1
(1 subplot)	6	-568.9479	1149.896	0	1	1

Post Hoc Analysis

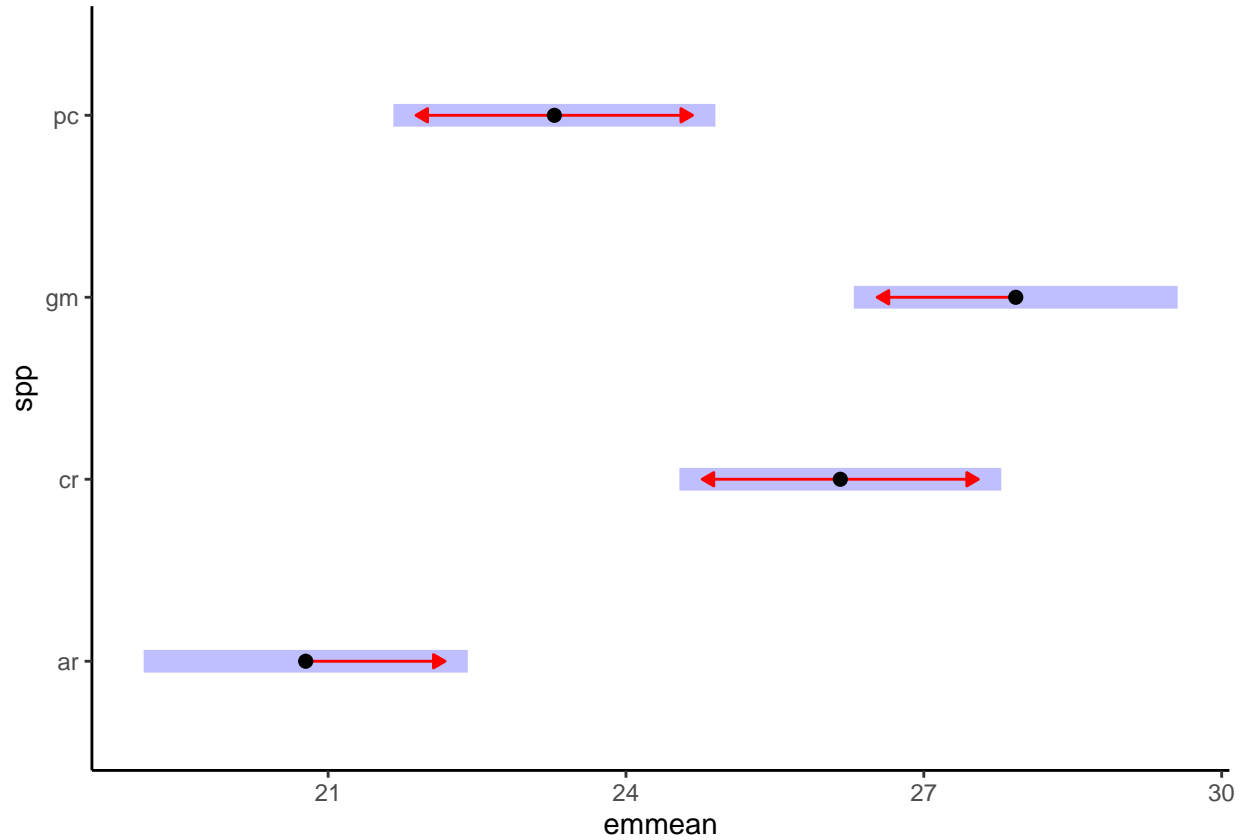


Figure 4. Estimated marginal mean by species with 95% confidence intervals, red arrows demonstrate overlap in confidence intervals.

Table 4. Estimated Marginal Means and Results.

contrast	estimate	SE	df	t.ratio	p.value
ar - cr	-5.384801	1.078188	174.6924	-4.994306	0.0000085
ar - gm	-7.151629	1.079122	174.6969	-6.627264	0.0000000
ar - pc	-2.504717	1.078845	175.4337	-2.321665	0.1284006
cr - gm	-1.766828	1.074375	174.9262	-1.644516	0.6111931
cr - pc	2.880084	1.073031	175.3090	2.684065	0.0478235
gm - pc	4.646912	1.075062	175.6710	4.322460	0.0001547

Conclusion

To determine if statistical differences were present in the carbon to nitrogen ratios by species a general linear mixed model was used to analyze the data with species as a fixed effect and plot and row (subplot) as random effects. Assumptions were analyzed visually with the null hypotheses that data are not normal and homogeneous. Visual analysis rejects these null hypotheses, these data meet the assumptions for this test. The significance of the random effects in the model were analyzed using AIC and the -2 log likelihood method. While the -2 log likelihood method indicates that the random effects do not have a significant

impact on the model the model with the lowest AIC included the random effects, so they were left in the model.

To account for the effects of the random effects and determine differences between all pairwise comparisons estimated marginal means were calculated and compared using the bonferroni method with an alpha of 0.05. Results indicate significant differences in the C:N ratio between annual rye and cereal rye ($p < 0.05$), annual rye and gene edited pennycress ($p < 0.05$), cereal rye and wild type pennycress ($p = 0.48$), and gene edited and wild type pennycress ($p < 0.05$). Annual rye has a significantly lower C:N ratio than cereal rye and gene edited pennycress. Cereal rye has a higher C:N ratio than wild type pennycress. Most importantly gene edited pennycress has a significantly higher C:N ratio than wild type pennycress.

Homogenized by plot the C:N ratios of the crops are as follows

- ar = 20.8:1
- cr = 26.2:1
- gm = 28:1
- pc = 23.3:1

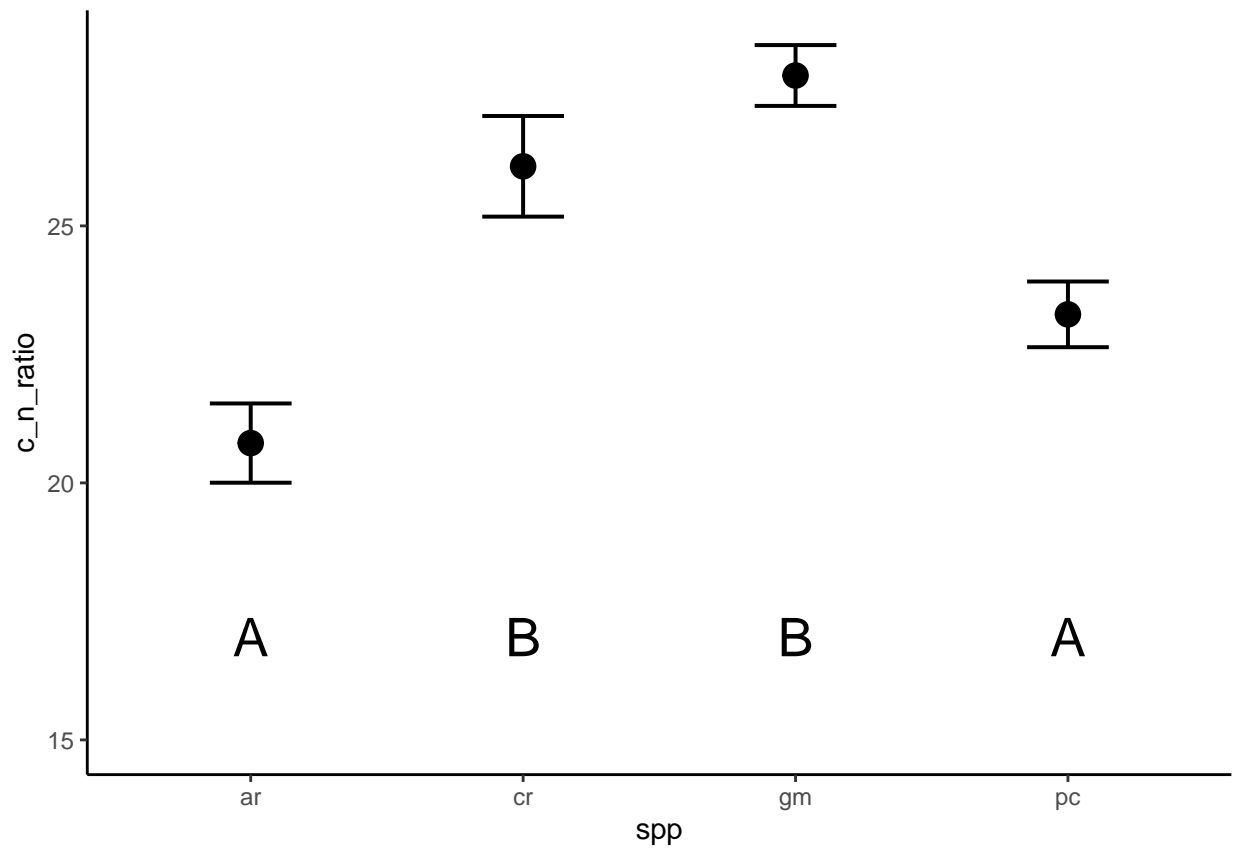


Figure 5. Mean and standard error of carbon to nitrogen ratios by species with letters indicating significant differences between groups.

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```
n.plot <- decomp.df %>%
  rename(t = y_m_d) %>%
  ggplot(aes(x = t, y = percent_n, color = spp)) +
    stat_summary(
      fun = mean, na.rm = TRUE, geom = "point", size = 4) +
    stat_summary(
      fun.data = mean_se, na.rm = TRUE, geom = "errorbar", width = .3, size = .7) +
    theme_classic()

c.plot <- decomp.df %>%
  rename(t = y_m_d) %>%
  ggplot(aes(x = t, y = percent_c, color = spp)) +
    stat_summary(
      fun = mean, na.rm = TRUE, geom = "point", size = 4) +
    stat_summary(
      fun.data = mean_se, na.rm = TRUE, geom = "errorbar", width = .3, size = .7) +
    theme_classic()

n.plot + c.plot + c_n.plot + plot_layout(guides = "collect", ncol = 3)
```

