

## Week 10: Experimental design

ANTH 674: Research Design & Analysis in  
Anthropology

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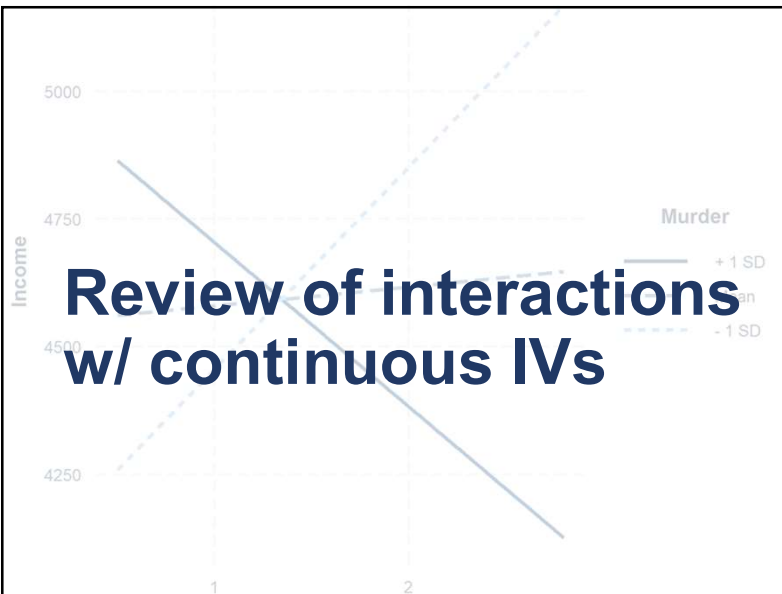
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## Lecture outline

1. Review of interactions w/ continuous IVs
2. What is experimental design?
  - Broadly used to encompass sampling design
3. How do experiments account for uncontrolled variation in DV?
  1. Replication
  2. Adequate spacing between replicates
  3. Randomization
  4. Blocking
4. Two-factor experiments
5. Moving from ANOVA to regression

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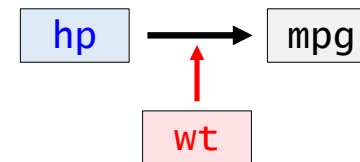
## Review of interactions w/ continuous IVs



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## Interaction w/ continuous IVs

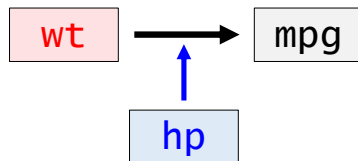
- $Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_1 X_2 + \varepsilon$
- Changing intercepts **AND** slopes, just like in ANCOVA
- E.g.,  $\text{mpg} \sim \text{hp} * \text{wt}$
- Intercept and slope of  $\text{mpg} \sim \text{hp}$  will change as  $\text{wt}$  increases



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## Interaction w/ continuous IVs

- $Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_1 X_2 + \varepsilon$
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## Interaction w/ continuous IVs

- $\text{mpg} = 49.81 - 0.12\text{hp} - 8.22\text{wt} + 0.03\text{hp}*\text{wt}$
- If  $\text{hp} = 0$ ,  $\text{mpg} = 49.81 - 8.22\text{wt}$
- If  $\text{hp} = 1$ ,  $\text{mpg} = 49.81 - 0.12 - 8.22\text{wt} + 0.03\text{wt}$
- If  $\text{hp} = 1$ ,  $\text{mpg} = 49.69 - 8.19\text{wt}$

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## Interaction w/ continuous IVs

- $\text{mpg} = 49.81 - 0.12\text{hp} - 8.22\text{wt} + 0.03\text{hp}*\text{wt}$
- If  $\text{wt} = 0$ ,  $\text{mpg} = 49.81 - 0.12\text{hp}$
- If  $\text{wt} = 1$ ,  $\text{mpg} = 49.81 - 8.22 - 0.12\text{hp} + 0.03\text{hp}$
- If  $\text{wt} = 1$ ,  $\text{mpg} = 41.59 - 0.09\text{hp}$

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## Interaction w/ continuous IVs

- $\text{mpg} = 49.81 - 0.12\text{hp} - 8.22\text{wt} + 0.03\text{hp}*\text{wt}$
- 49.81 is intercept when both IVs equal zero
- Focusing on  $\text{mpg} \sim \text{wt}$ 
  - -0.12 is change in intercept as  $\text{hp}$  increases by one
  - -8.22 is slope when  $\text{hp}$  is zero
  - 0.03 is change in slope as  $\text{hp}$  increases by one

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## Interaction w/ continuous IVs

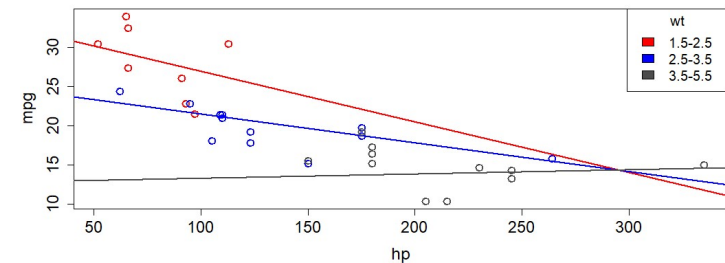
- $\text{mpg} = 49.81 - 0.12\text{hp} - 8.22\text{wt} + 0.03\text{hp}*\text{wt}$
- 49.81 is intercept when both IVs equal zero
- Focusing on  $\text{mpg} \sim \text{hp}$ 
  - -8.22 is change in intercept as **wt** increases by one
  - -0.12 is slope when **wt** is zero
  - 0.03 is change in slope as **wt** increases by one

Interaction term is symmetrical!  
Affects both slopes in the same way

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## Interaction w/ continuous IVs

- Focusing on  $\text{mpg} \sim \text{hp}$ 
  - -8.22 is change in intercept as **wt** increases by one
  - 0.03 is change in slope as **wt** increases by one



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## Questions?



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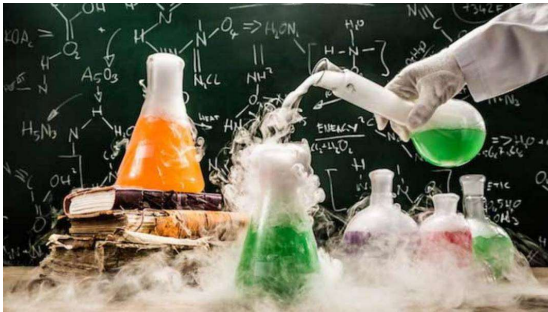
## What is experimental design?



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## What is experimental design?

- What do you think of when I say “experiment”?



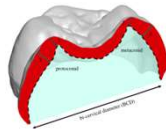
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## What is experimental design?

- Planning the logical structure of your experiment to anticipate statistical issues
- Good ED recognizes sources of unmeasured variation & plans statistics around them
  - Increases statistical power
  - Analysis of data goes hand-in-hand w/ ED
- If there are serious problems w/ ED, very difficult to correct afterwards w/ statistics
- Important to think through ED **before** data collection (clear research question helps **a lot**)!

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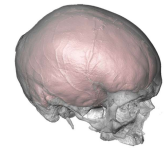
## Not only experiments!



- Sampling design
- E.g., Interested in primate enamel thickness as it relates to diet
  - How many primate species to sample in museum?
  - How many individuals per species?
  - How many species or individuals per diet?
  - From what regions/years should I sample?
- Also applies to collecting data from literature if there's a lot of data to sample from

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## Not only experiments!



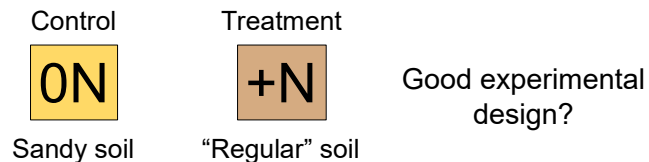
- If not much data, have no choice but to use all of it, so sampling design not as applicable
- E.g., how hominin brain size evolution was affected by climate change
- Must use statistics to account for a lot of the things we'll be talking about

Everything that follows applies to experimental **AND** sampling design!

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## Main goal of experiments

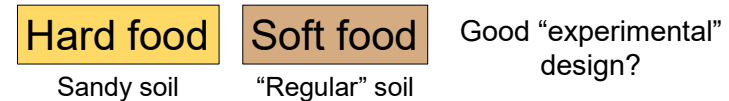
- To see if and how one or more IVs affect a DV
- Controls for other factors affecting DV, thereby isolating IV's effect on DV
- E.g., how does nitrogen affect plant growth rate?



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## Main goal of experiments

- To see if and how one or more IVs affect a DV
- Controls for other factors affecting DV, thereby isolating IV's effect on DV
- E.g., How does diet affect primate enamel thickness?



Uncontrolled factor confounded w/ IV—not good!

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## Good ED accounts for:

1. Confounding factors
  - Randomization, blocking
2. Experimenter bias
  - Randomization
3. Noise and variation in data
  - Replication
4. Non-independence in data
  - Adequate spacing between replicates, randomization

Basically, **clearly** outline your research question & think about all the factors that can screw up your analyses and inference!

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## Two main types of experiments

### Manipulative

- Experimenter varies IV & measures DV
- E.g., manipulating N levels → plant growth
- Expensive and time-consuming
- Good for controlling confounding factors
- Too "artificial"?

### Natural

- Uses natural variation in IV & measures DV
- E.g., measure areas w/ different N levels
- Cheaper and faster
- More difficult to control confounders
- More "realistic"?

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## Questions?



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## How to account for uncontrolled variation in ED

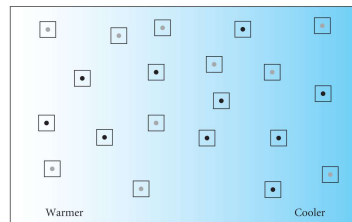
Warmer

Cooler

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## ED strategies

1. Replication
2. Adequate spacing between replicates
3. Randomization
4. Blocking



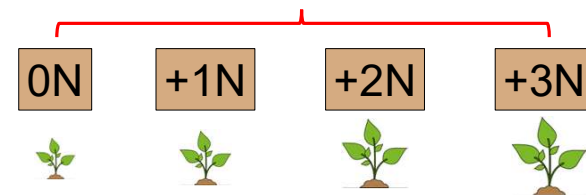
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## Basic experimental design

- One or more factors
- One or more levels w/in each factor

Interested in plant growth rate ~ N level

One factor: nitrogen; 4 levels: 0, +1, +2, +3



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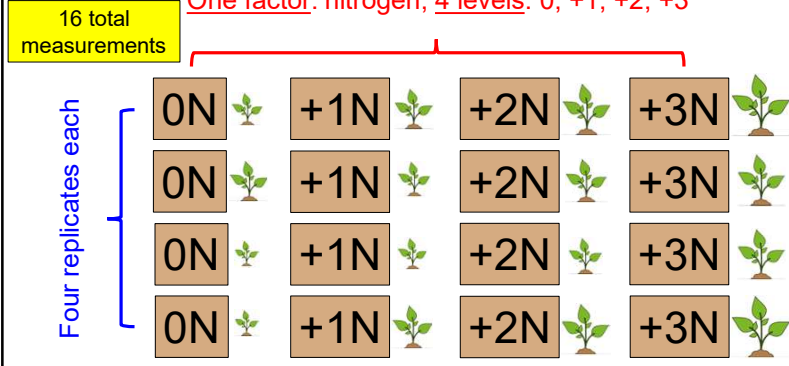
# 1. Replication

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## Replication

Multiple observations w/in each level of a factor

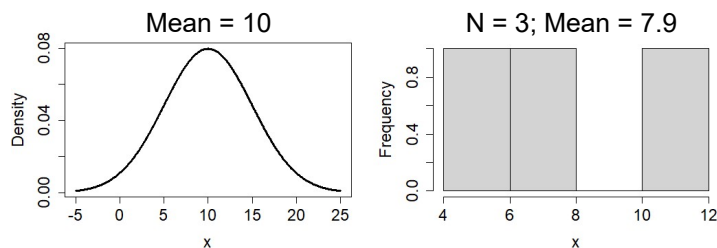
One factor: nitrogen; 4 levels: 0, +1, +2, +3



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## Why replicate?

- To average out individual variation among observations w/in a treatment level
- Same reason why a small sample may not be representative of the population



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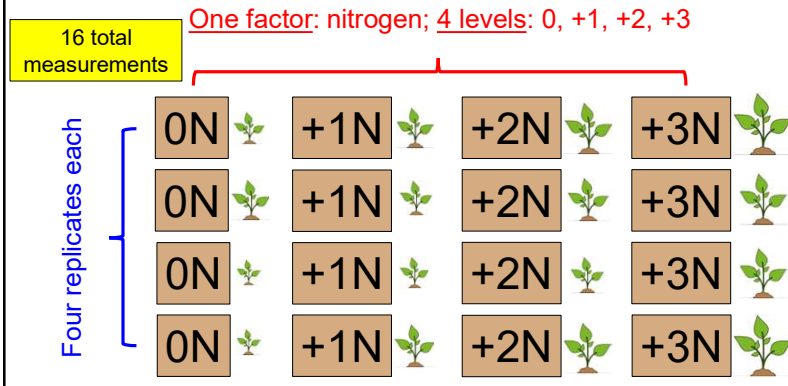
## How many replicates?

- Ultimately determined by money, effort, and time, so usually there's a max. # measurements
- A trade-off between number of factors, levels, and replicates

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## Replication

- If I had two levels, I could have eight replicates



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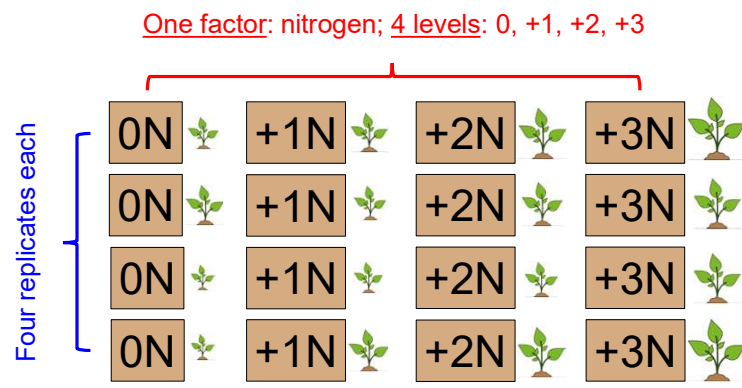
## How many replicates?

- Ultimately determined by money, effort, and time, so usually there's a max # measurements
- Is a trade-off between number of factors, levels, and replicates
- Depends on your question!
  - Usually 1–3 factors is most that is manageable
  - Usually 2–5 levels is adequate
- Gotelli & Ellison's "Rule of 10": absent other information, need 10 replicates
- If data are variable and effect sizes (i.e., mean differences) are small, need more replicates

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## Which statistical test?

- This is a one-way ANOVA:  $\text{aov}(\text{plant} \sim \text{N})$



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## Questions?



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## 2. Adequate spacing

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## Adequate spacing

- Virtually everything is temporally, spatially, and phylogenetically autocorrelated
- Tobler's first law of geography: "everything is related to everything else, but near things are more related than distant things"
- This non-independence artificially inflates sample size, making P-values too small (increases Type I error)

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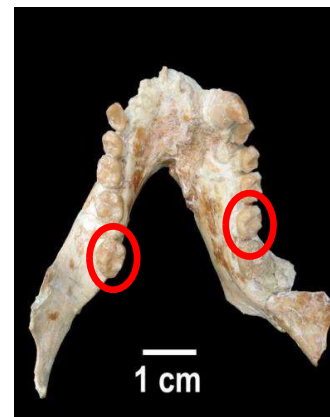
## A spatial example

Seedlings in shade are smaller (size is spatially autocorrelated)



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## An extreme example



- Independent replicates?
- **NO!** Products of the same genetics, habitat, diet, & whatever processes affected this individual
- Replicates are not truly independent (i.e., "**pseudoreplication**")

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## Adequate spacing

- Adequate spacing in time and/or space ensures replicates are truly independent
- How much time or space? Depends on process of interest
  - E.g., predation of mantis requires less spacing than lion predation
- Only worry about this if you care about P-values

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## Questions?



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## 3. Randomization

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## Randomization



- **Randomly assign replicates to treatment levels**
- Must be truly random!
  - Use random number generator, e.g., `sample()`
  - Coin flips, roll a die
- Counteracts experimenter bias, confounding factors, and non-independence
- Replication and randomization only effective when used together

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## Randomization



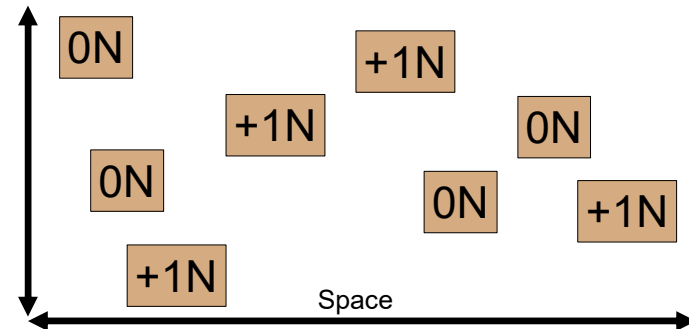
- Take individual plants and randomly assign them to treatment levels

|    |     |     |     |
|----|-----|-----|-----|
| 0N | +1N | +2N | +3N |
| 0N | +1N | +2N | +3N |
| 0N | +1N | +2N | +3N |
| 0N | +1N | +2N | +3N |

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## Battling non-independence

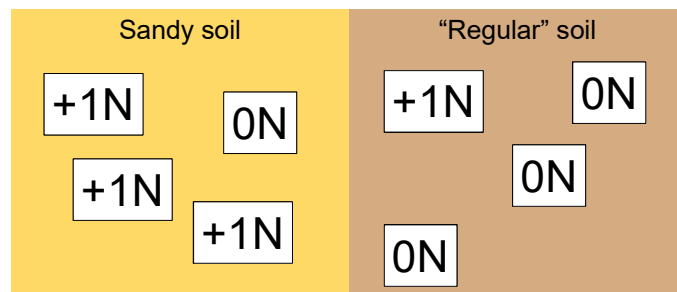
- Random distribution ensures levels that are close are canceled by levels that are far



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## Battling confounding factors

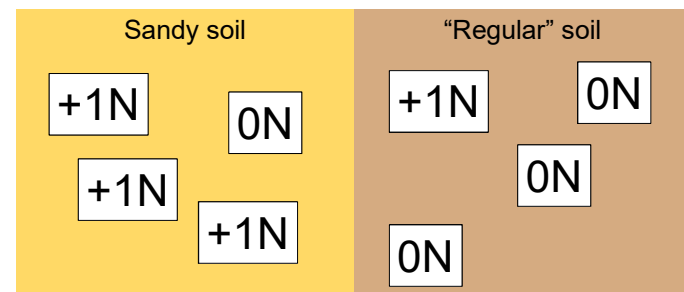
- Plots are randomly distributed
- Assigning treatments to plots is random
- Assigning individuals to treatments is random



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## Battling confounding factors

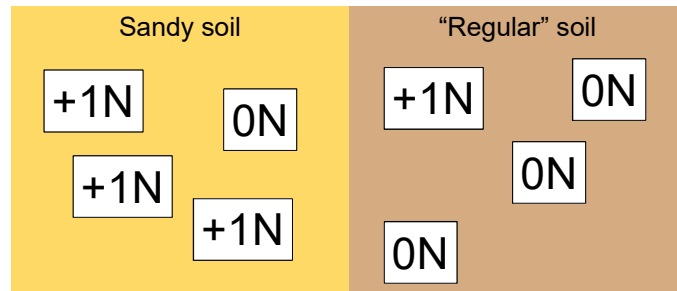
- Treatments found in both soil types now
- Treatment is now independent of confounder
- Works for "known unknown" confounders too!



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## Which statistical test?

- One-way ANOVA: `aov(plant ~ N)`
- Can include soil type as covariate to increase power (move DV variation from error term to covar.)
- Two-way ANOVA: `aov(plant ~ soil + N)`



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## Questions?



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## 4. Blocking

### Blocking

- In soil example, three "+1N" and only one "0N" in sandy soil (uneven sample sizes)
- Blocking solves this issue (**randomized block design**)
- **Block**: delineated area or time period w/in which environment is homogeneous
- Environmental variation between blocks must be greater than w/in blocks

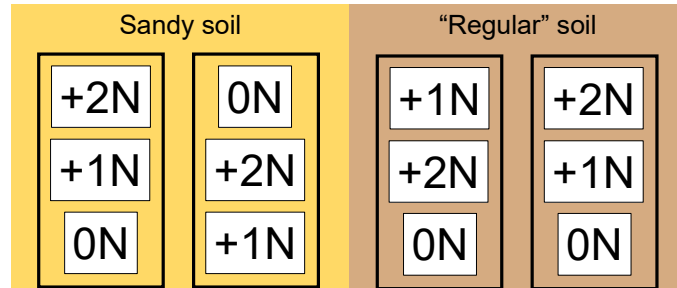
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## Blocking



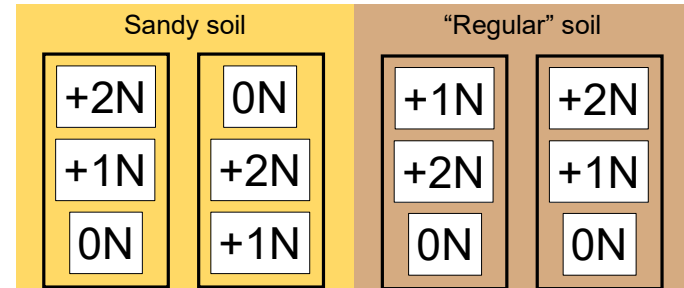
- Each treatment level assigned to a block but randomized within
- Replicates randomly assigned to levels



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## Blocking

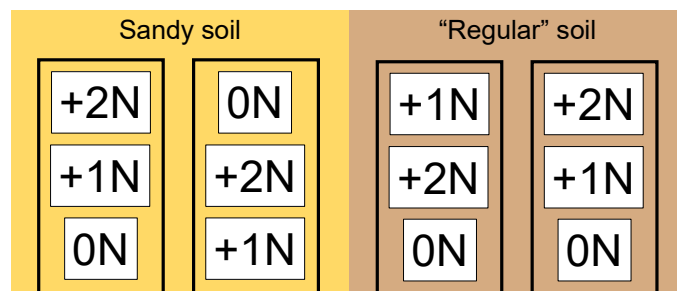
- Treatment now independent of known and unknown (spatially autocorrelated) confounders



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## Which statistical test?

- Can include block as covariate to increase power
- Two-way ANOVA: `aov(plant ~ block + N)`



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## Caveats for blocking

- Assumes that there is no interaction between blocks & treatments  
(e.g., effects of +2N > +1N > 0N for all blocks)
- What if this is not the case? Need two-factor experiment

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## Questions?



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## Two-factor experiments

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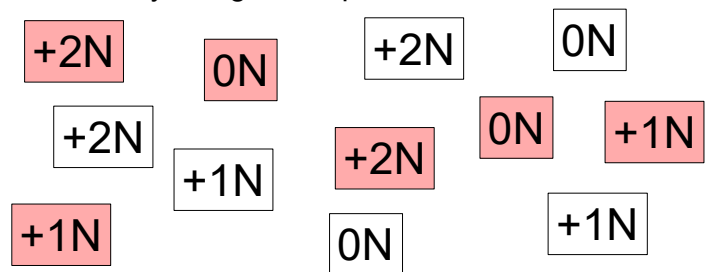
## Two-factor experiments

- Interested in how DV responds to manipulating **TWO** factors simultaneously
- Every level of factor 1 *must* be combined w/ every level of factor 2 (**fully crossed** or **orthogonal** design)
- Can look at interaction between factors 1 & 2
- With blocking, this becomes a **split-plot design**

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## Two-factor experiments

- E.g., Plant growth rate ~ nitrogen level **AND** presence of herbivores
- Plots randomly distributed, treatments randomly assigned to plots, replicates randomly assigned to plots



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## Which statistical test?

- This is a two-way ANOVA w/ an interaction between the IVs (often interested in how effect of IV1 on DV is affected by IV2)
- If you have unequal # replicates among levels for the two factors, ANOVA can be problematic ([http://onlinestatbook.com/2/analysis\\_of\\_variance/unequal.html](http://onlinestatbook.com/2/analysis_of_variance/unequal.html)) (<https://mcfromnz.wordpress.com/2011/03/02/anova-type-iii-ss-explained/>)

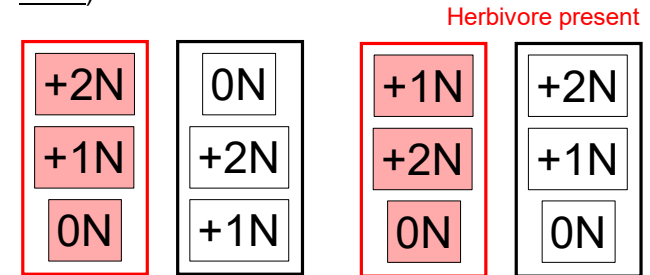
Confounding factors all over again!

|               | 0N | +1N |
|---------------|----|-----|
| Herb. absent  | 1  | 10  |
| Herb. present | 10 | 1   |

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## Split-plot design

- All N treatment levels (i.e., subplot factor) are represented in each block, which is itself a different herbivore treatment (i.e., whole-plot factor)



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## Questions?



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## Getting away from ANOVAs



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## Getting away from ANOVAs

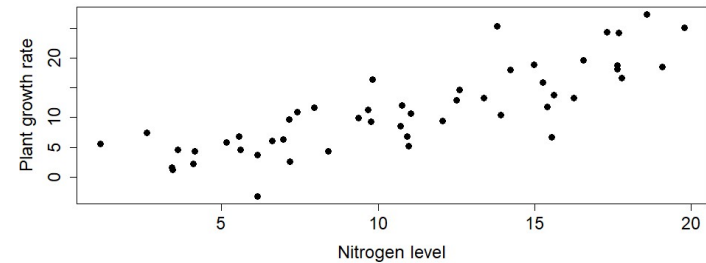
- Thus far, I have presented IVs as categorical factors w/ different levels
- Indeed, ANOVA was invented by R.A. Fisher in the context of agricultural experiments
- But many times, different levels w/in a factor can be converted to continuous data

|    |     |     |     |     |      |     |
|----|-----|-----|-----|-----|------|-----|
| 0N | +1N | +2N | +3N | ... | +20N |     |
| 0H | 1H  | 2H  | 3H  | 4H  | ...  | 20H |

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## Regression

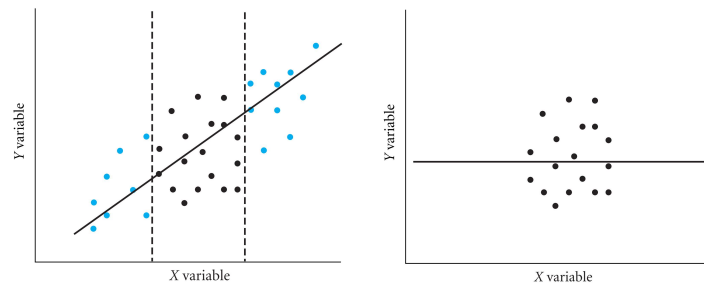
- Becomes regression instead of ANOVA
  - Can get change in DV ~ change in IV (slope)
  - Do predictions
  - Get goodness of fit ( $R^2$ )



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## Regression

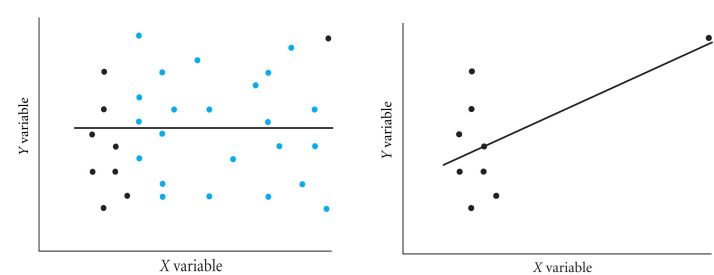
- Make sure IV is sampled over large enough range to get full range of response in DV



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## Regression

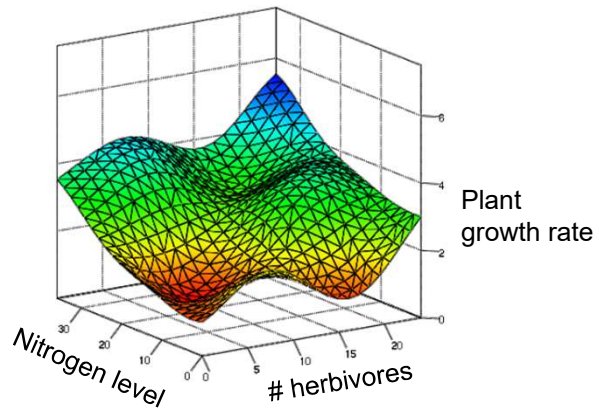
- Make sure IV is sampled uniformly within its range



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## Can get a response surface



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## Questions?



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## Summary

- Experiments account for sources of uncontrolled variation in DV → more powerful tests
- Accounts for confounding factors, non-independence, & noise in DV w/ replication, randomization, spacing btw plots, & blocking
- Try to make IV continuous rather than categorical
- In the end, it's all about thinking hard to account for confounding factors in your own research!

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## Statistics vignette

Brain cancer death rates in the US (example from Ellenberg, 2014)

### Top 5 States

- South Dakota
- Nebraska
- Alaska
- Delaware
- Maine

### Bottom 5 States

- Wyoming
- Vermont
- North Dakota
- Hawaii
- District of Columbia

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## Statistics vignette

Brain cancer death rates in the US (example from Ellenberg, 2014)

What's going on?  
What do these ten states have in common?

### Top 5 States

- South Dakota
- Nebraska
- Alaska
- Delaware
- Maine

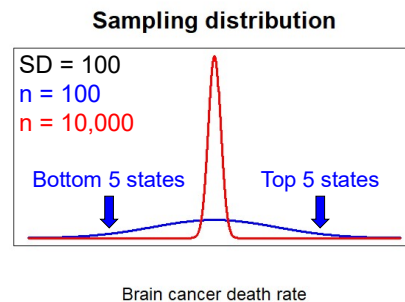
- Wyoming
- Vermont
- North Dakota
- Hawaii
- District of Columbia

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## Small population sizes!

- Leads to noisy death rate estimates (related to Law of Large Numbers & standard errors)

$$SE = \frac{SD}{\sqrt{n}}$$



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