Aquatic Ecosystems

23.02.-03.03.2016

‚Brownification‘
- Lecture 3 -

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Overview of this teaching unit

- **Lecture 1**
  - Nitrogen (Importance, sources, transformations and recent trends)
  - Environmental Quality Criteria

- **Exercise 1**
  - Application of the Environmental Quality Criteria and mapping using R

- **Lecture 2** on Thursday – 10.15 – 12.00
  - Feedbacks, interactions, stable states
  - Eutrophication Management (Directives, examples)

Overview of this teaching unit

- **Lecture 2** on Thursday – 13.15 – 15.00
- ‚Brownification' in inland waters
- Time series analysis using linear mixed effects models

- **Exercises 2 to 5** – Fr 26/02 to Tue 01/03
- Case study biogeochemistry in aquatic systems in Sweden over the past decades
- Time series analyses, mixed effects modeling, R

- **Final seminar** – Thursday 03/03 – 10.15 – 12.00
- Presentation of case study results and discussion

Learning goals

1. Drivers of DOC and CDOM concentrations in aquatic systems
2. pH dependent DOC solubility in soils
3. Linear mixed effects models, pseudoreplication, correction for multiple comparisons

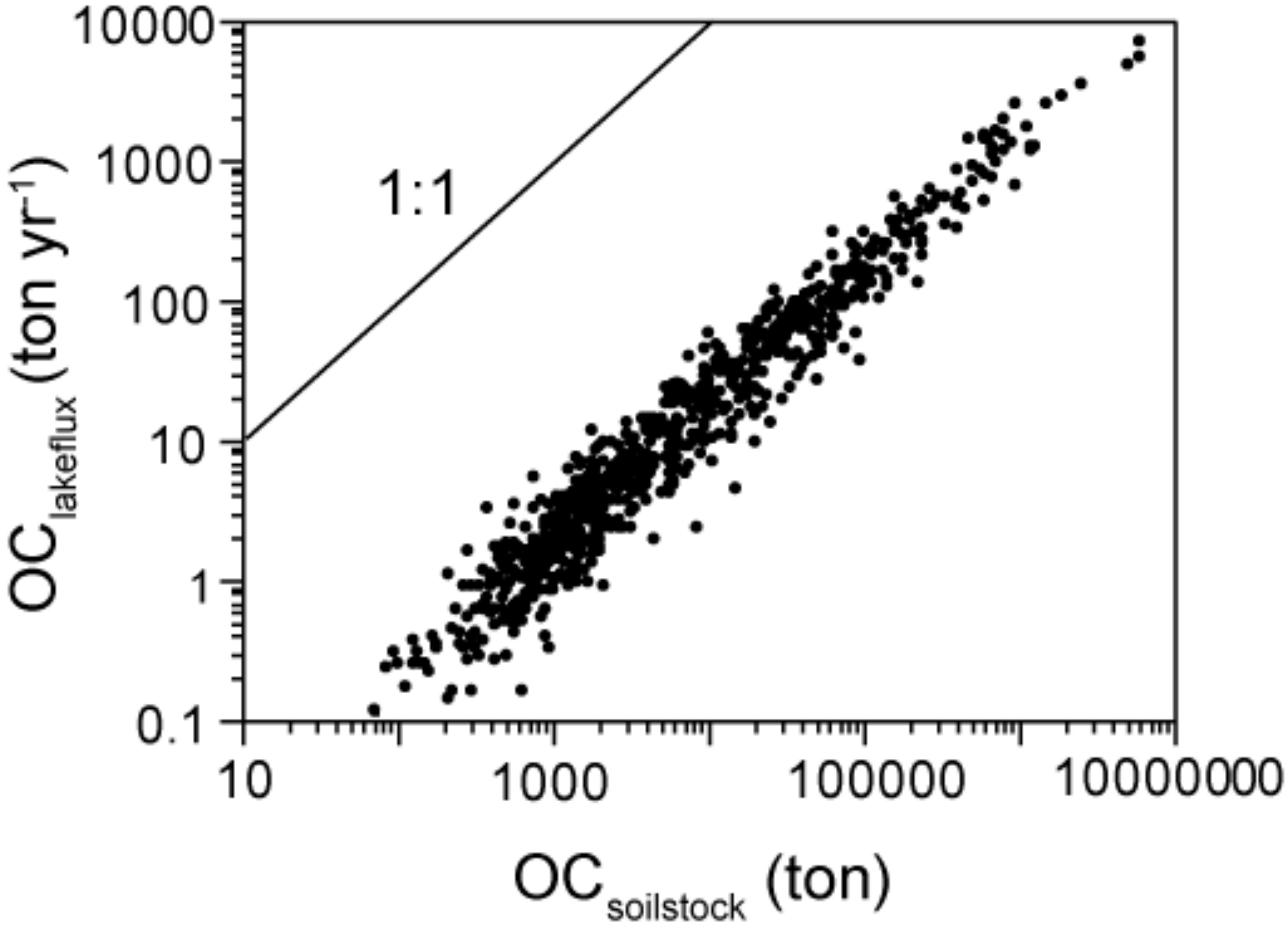
Which factors influence DOC concentrations in aquatic systems?

Questions welcome at all times!!

DOC concentration / water color influences…

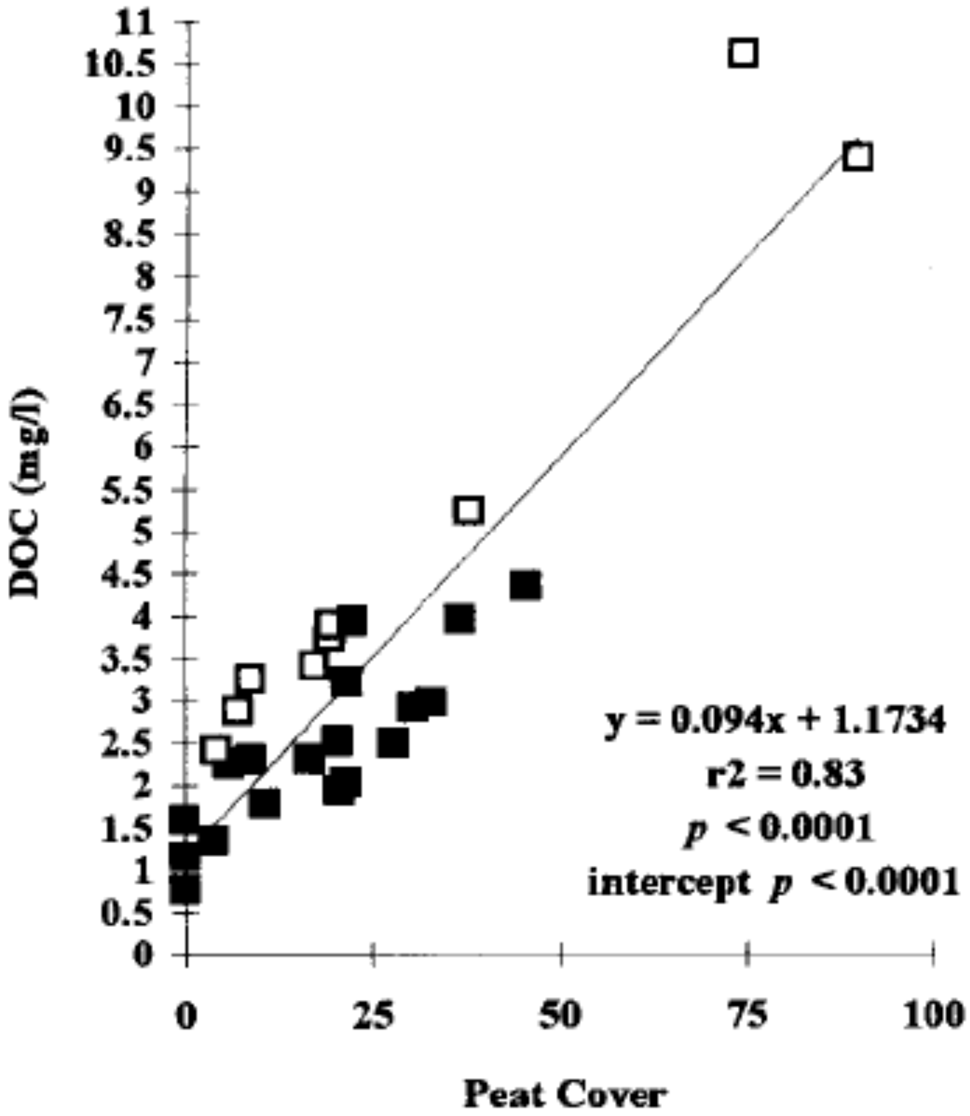
- Light and thermal regime
- Water chemistry
- Nutrient availability
- Bioavailability of toxic substances
- Drinking water quality/treatment
- Carcinogenic chlorination by-products
- Lake metabolism/greenhouse gas fluxes

Driver soil OC content



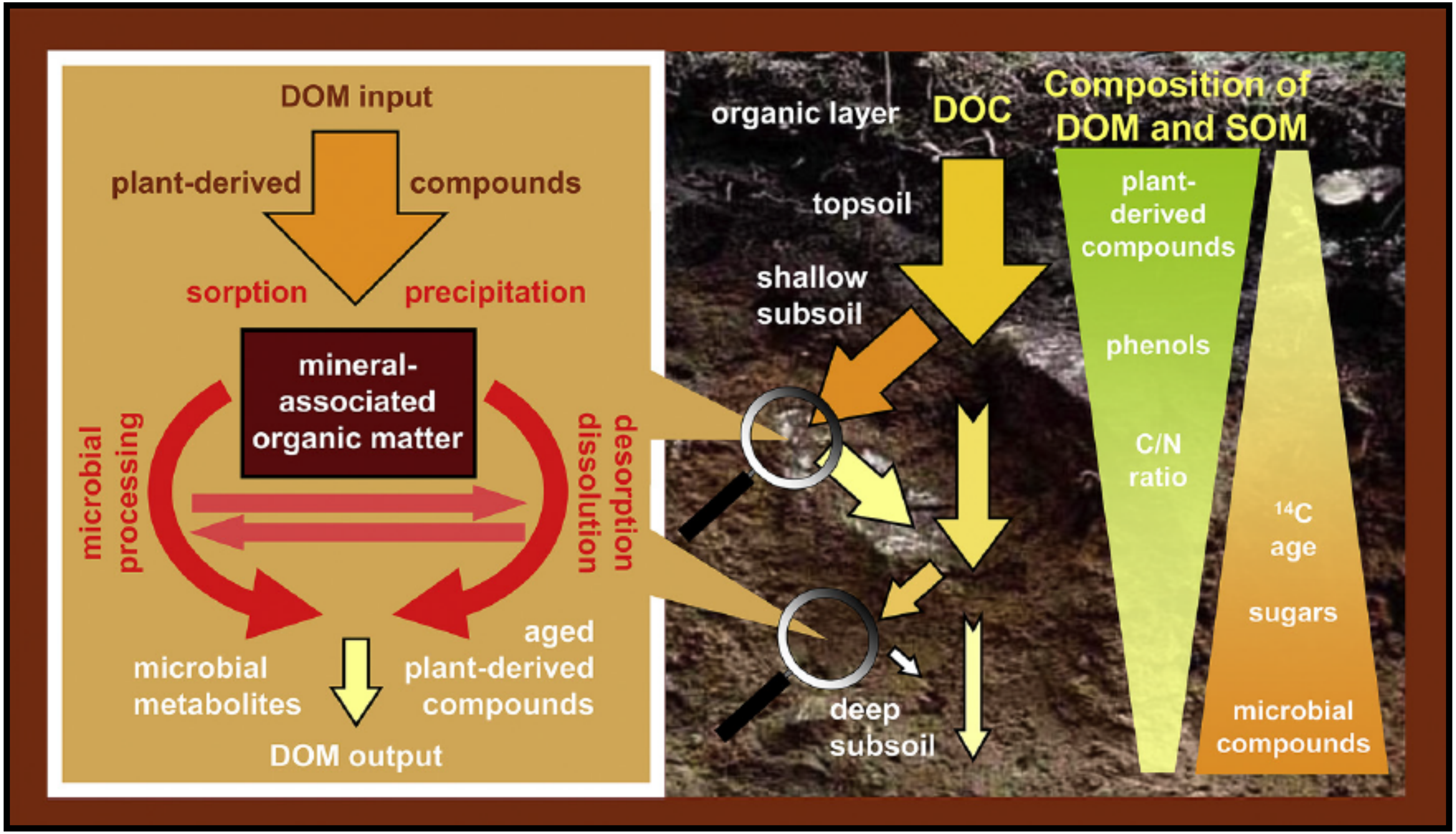
Weyhenmeyer et al. 2012

Importance of soil type



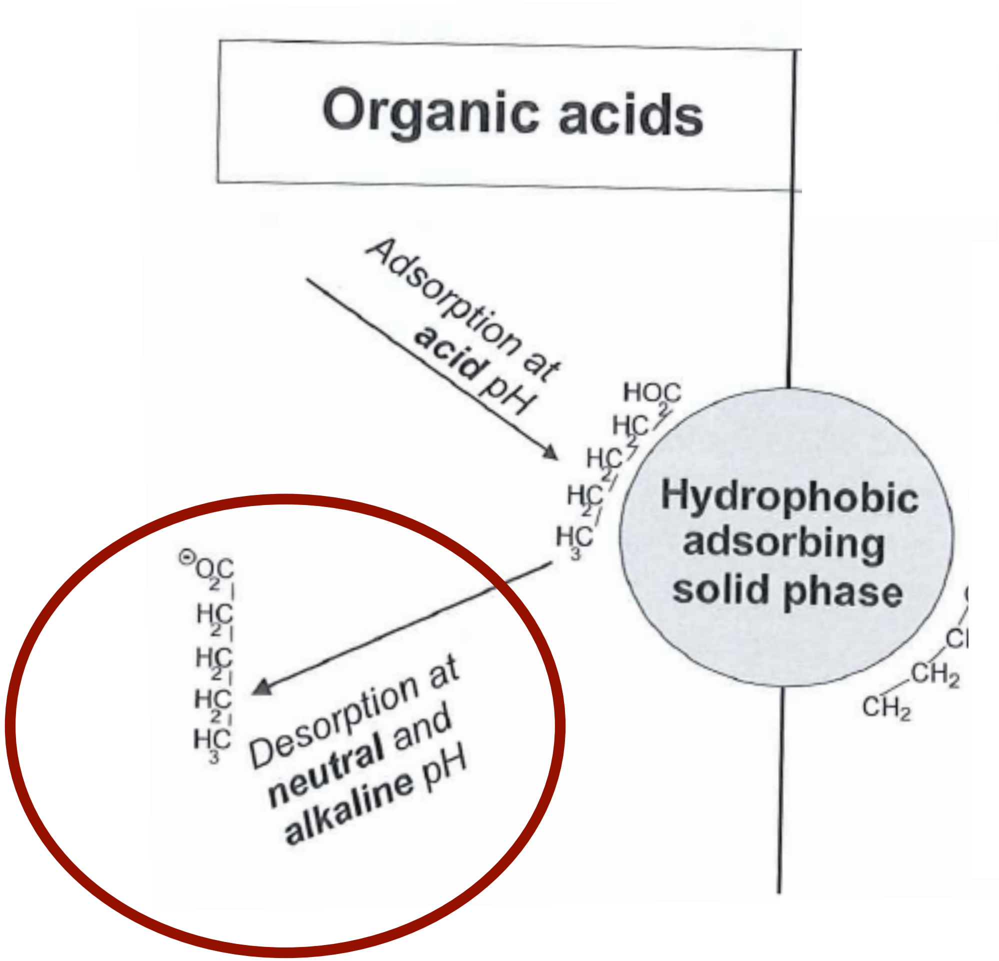
Aitkenhead et al. 1999

Hydrology influences C export



Kaiser and Kalbitz, 2012

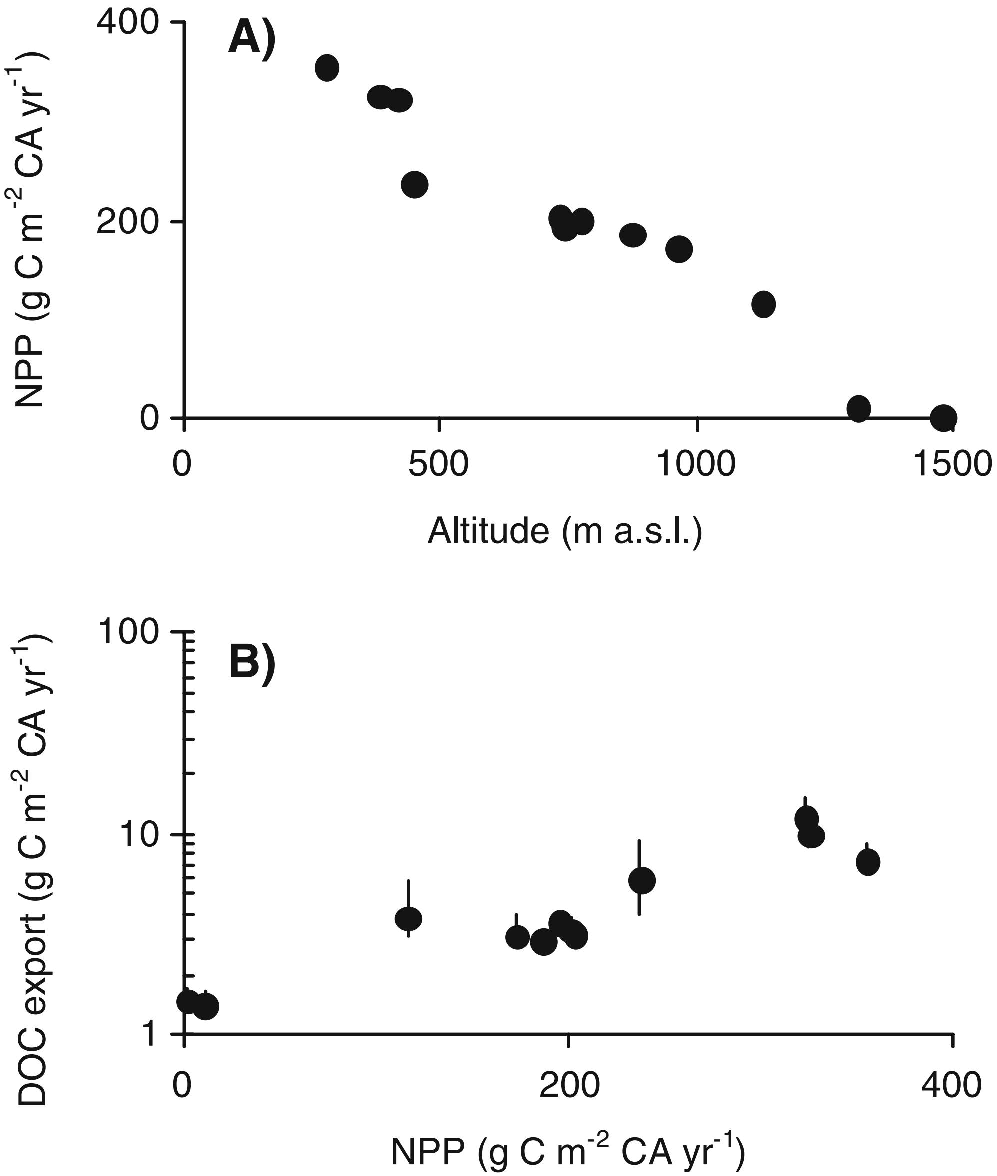
pH dependent DOC leakage from soils



Organic acids are negatively charged. With decreasing pH (more H^+) ionic strength increases, hence DOC solubility decreases.

With increasing pH (less H^+) DOC solubility increases.

Climate, here: temperature



Jansson et al. 2008

CDOM at Galten (Mälaren) in March

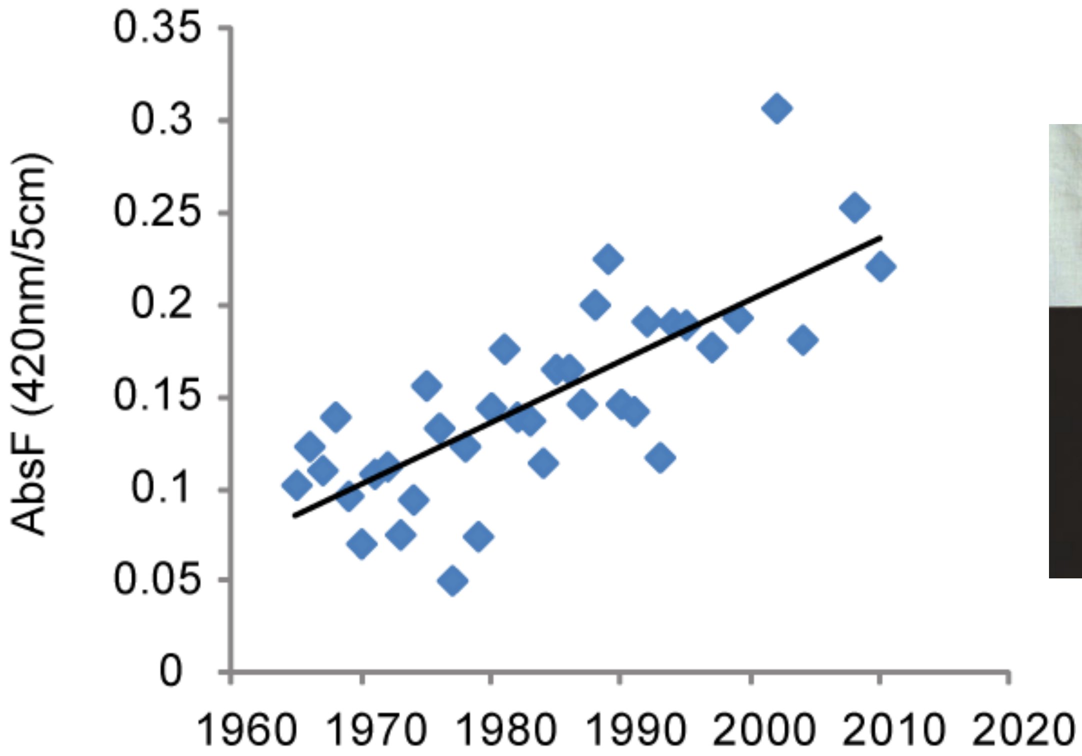
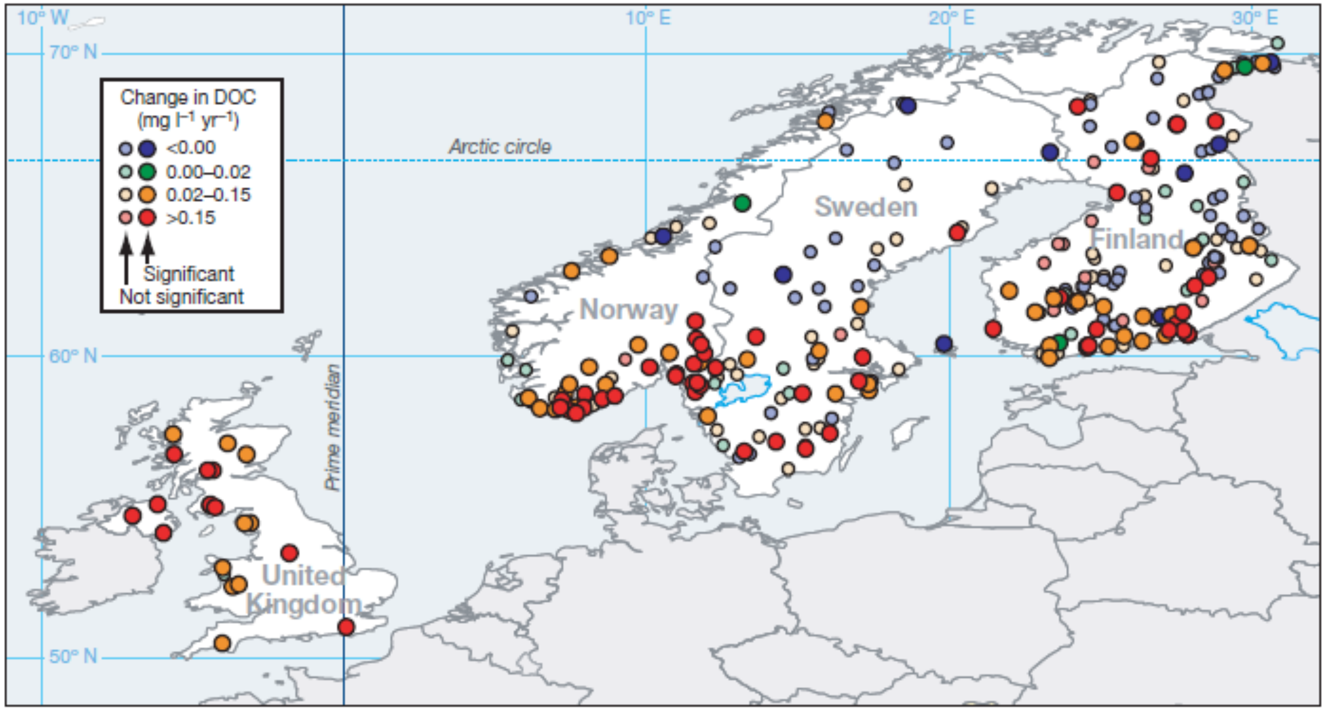
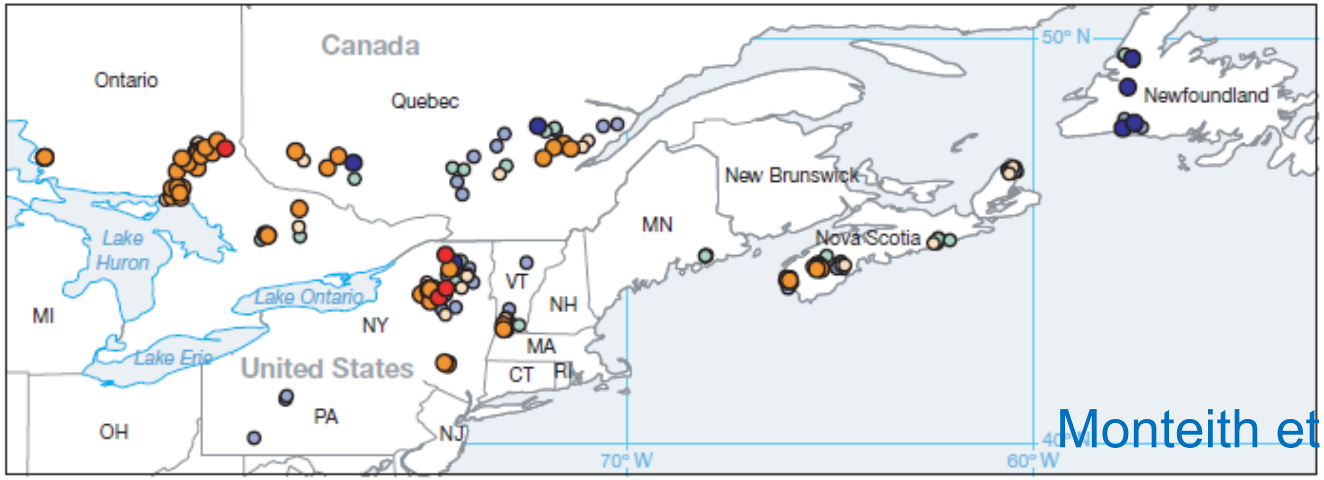


Photo: Stefan Löfgren

G. Weyhenmeyer, unpublished data

Trends in DOC: 1990 to 2004





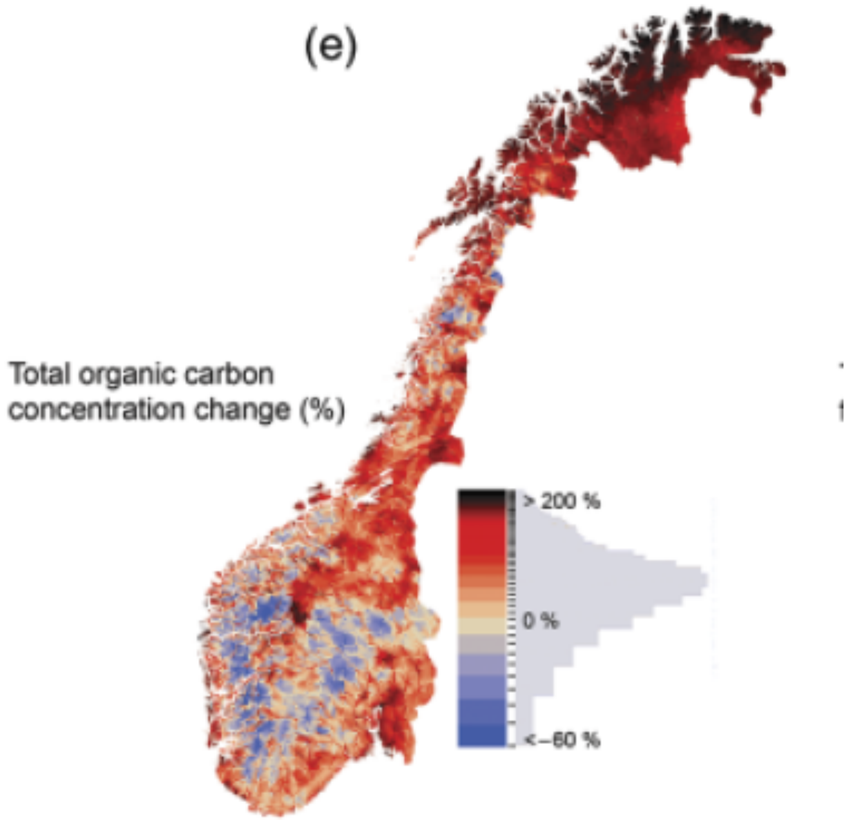
Monteith et al., 2007

Increasing DOC concentrations in recent years…driving factors?

- Higher temperatures (Freeman et al. 2001)
- Hydrological factors, e.g. dominant flowpaths, water retention times (Evans et al. 2005)
- Increasing CO_2 concentrations (Freeman et al. 2004)
- Nitrogen deposition (Findlay 2005)
- Decreased sulfate deposition, recovery from acid deposition (Evans et al. 2006)

➢ Results in very different future projections!

TOC concentrations & predicted changes in Norway (100-200 yr perspective)



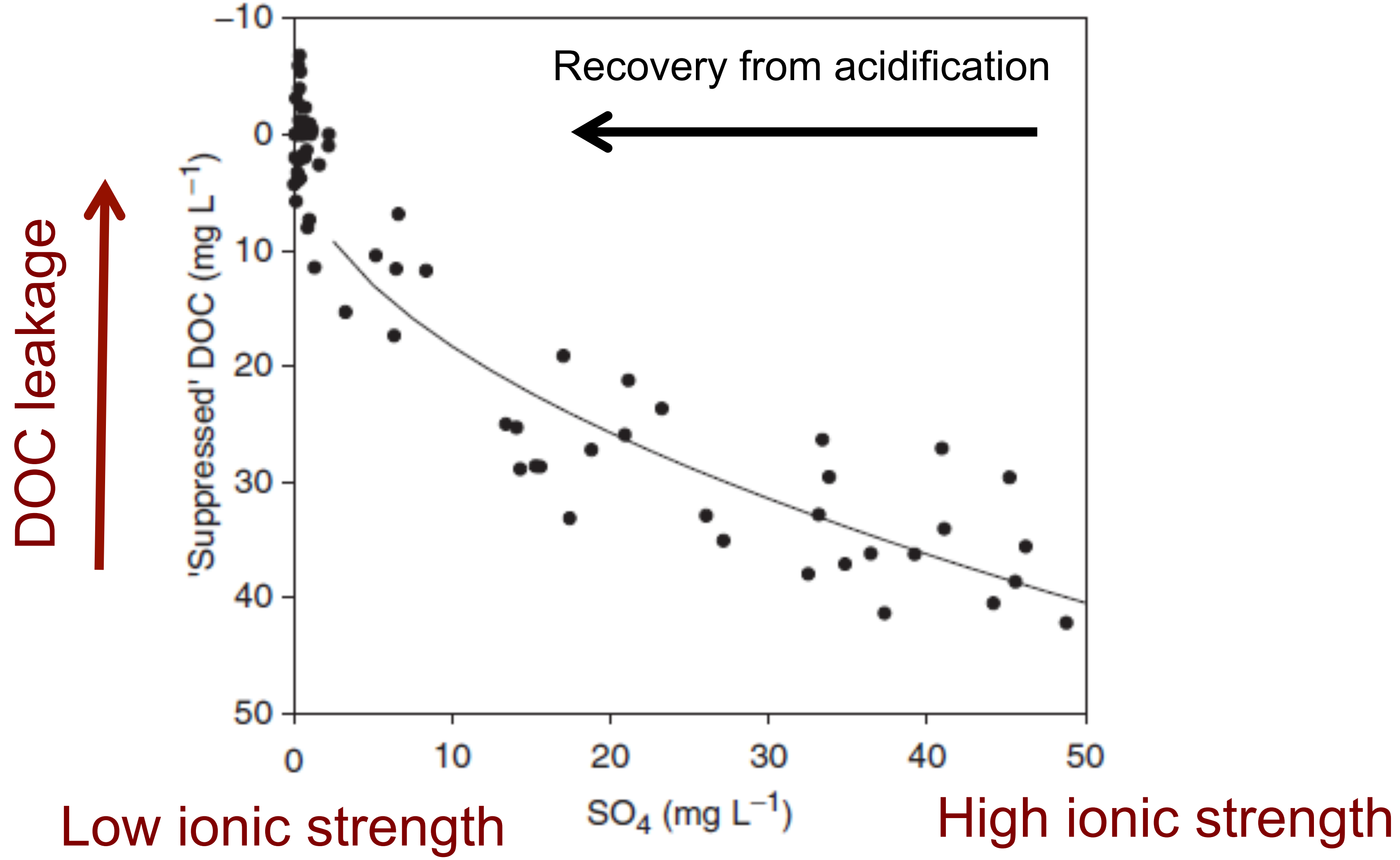
- Primarily mediated by increased terrestrial vegetation cover in response to climate change.

Larsen et al. 2010

With increasing soil acidification (decreasing pH)...

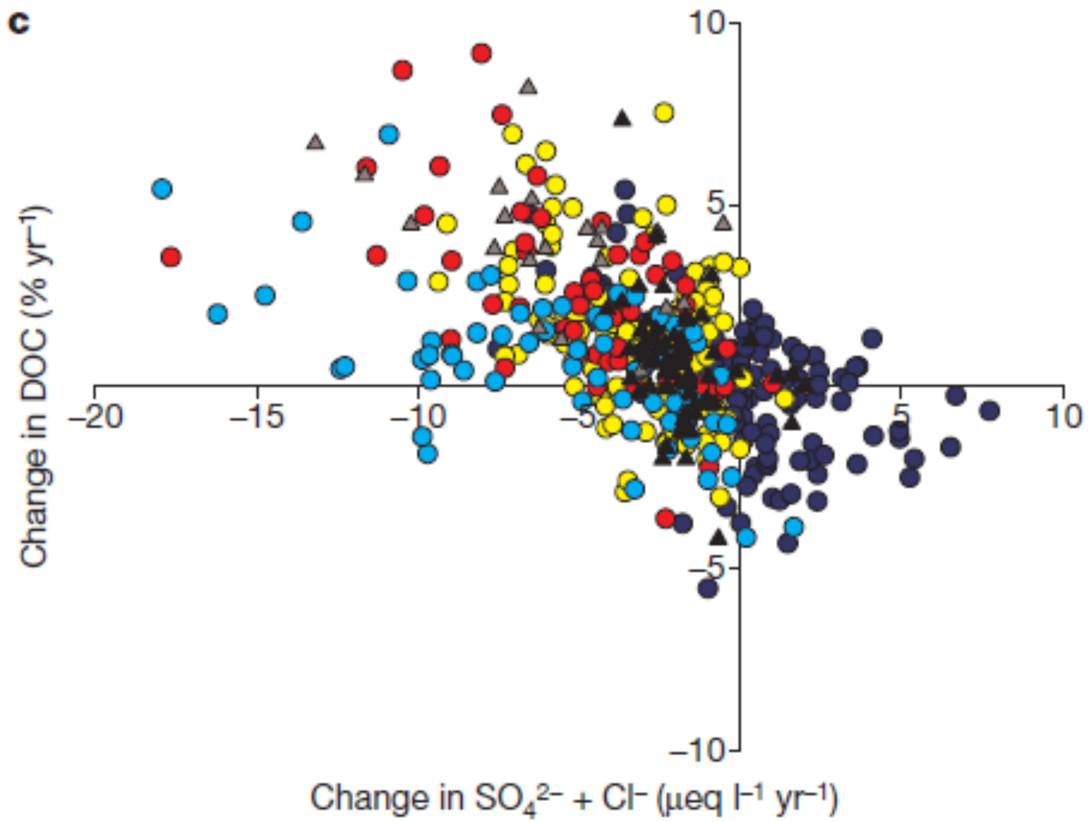
1. Ionic strength INcreases and DOC solubility/export DEcreases
2. Ionic strength DEcreases and DOC solubility/export INcreases
3. DOC export from soils is unaffected

Recovery from acidification



Evans et al. 2006

Decreasing sulfate and chloride deposition



Mechanism: Soil water acidity and ionic strength are negatively related to DOC solubility

Evans et al. 2006; Monteith et al. 2007

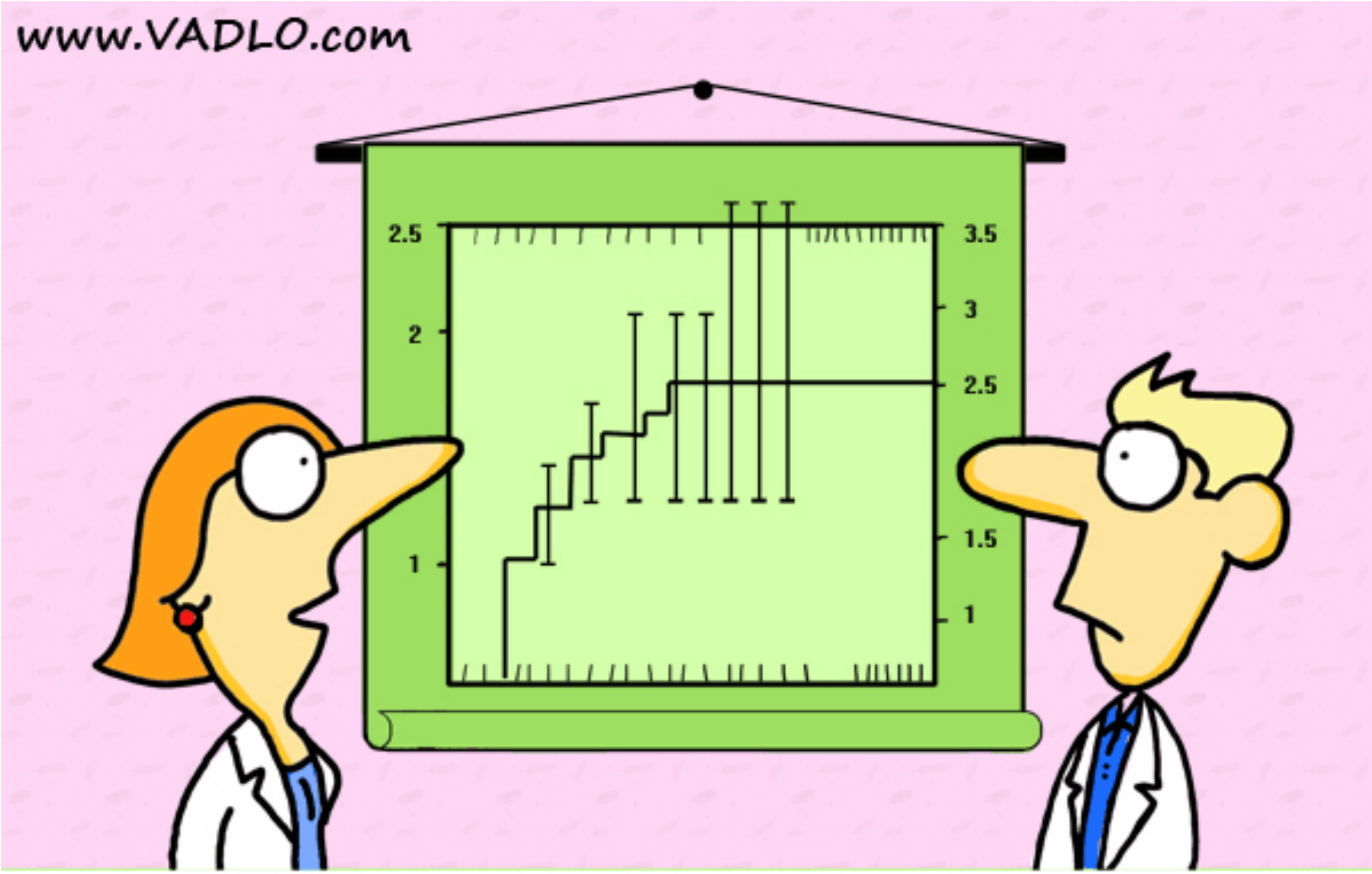
2 times 2 minutes 'speed dating' with your neighbour

a) What was unclear for you so far?
   2 minuter

b) What was the most interesting for you so far?
   2 minuter

Questions to the big group?

STATISTICS



"Did you really have to show the error bars?"

How do we analyse environmental changes over time?

- ➢ Repeated measurements / Time series
- ➢ Mixed-effects models!
- Are unaffected by randomly missing data
- Can properly account for correlation between repeated measurements
- Allow to specify the within-group variance of a stratification variable

Gueorguieva & Krystal, 2004; Pinheiro & Bates, 2004

Mixed-effects models

- Fixed effects
  - Influence the **mean** of the variable
  - Informative factor levels – we are interested in them!

- Random effects
  - Influence the **variance** of the variable
  - Uninformative factor levels – we are not interested in them!
  - Random samples from a much larger population

Crawley, The R Book, 2007

Examples of fixed and random effects

Fixed effects
Drug administered or not
Insecticide sprayed or not
Nutrient added or not
One country versus another
Male or female
Upland or lowland
Wet versus dry
Light versus shade
One age versus another

Crawley, The R Book, 2007

Examples of fixed and random effects

Fixed effects	Random effects
Drug administered or not	Genotype
Insecticide sprayed or not	Brood
Nutrient added or not	Block within a field
One country versus another	Split plot within a plot
Male or female	History of development
Upland or lowland	Household
Wet versus dry	Individuals with repeated measures
Light versus shade	Family
One age versus another	Parent

Crawley, The R Book, 2007

The trap of pseudoreplication

- In statistical testing the number of statistically INDEPENDENT samples needs to be correctly specified, and hence the degrees of freedom
- Often ‚pseudoreplicates‘ are erroneously assumed to be true replicates, inflates the degrees of freedom in testing, makes it more likely to declare a statistically significant effect!
- Most commonly from wrongly treating multiple samples from one experimental unit as multiple experimental units, or from using experimental units that are not statistically independent
- Temporal and spatial pseudoreplication

e.g. Hurlbert, 1984

The trap of pseudoreplication

- Meta-analysis Hurlbert 1984:

  For 101 manipulative field experiments statistics of 48% had pseudoreplication

- Revisited Heffner et al. 1996:

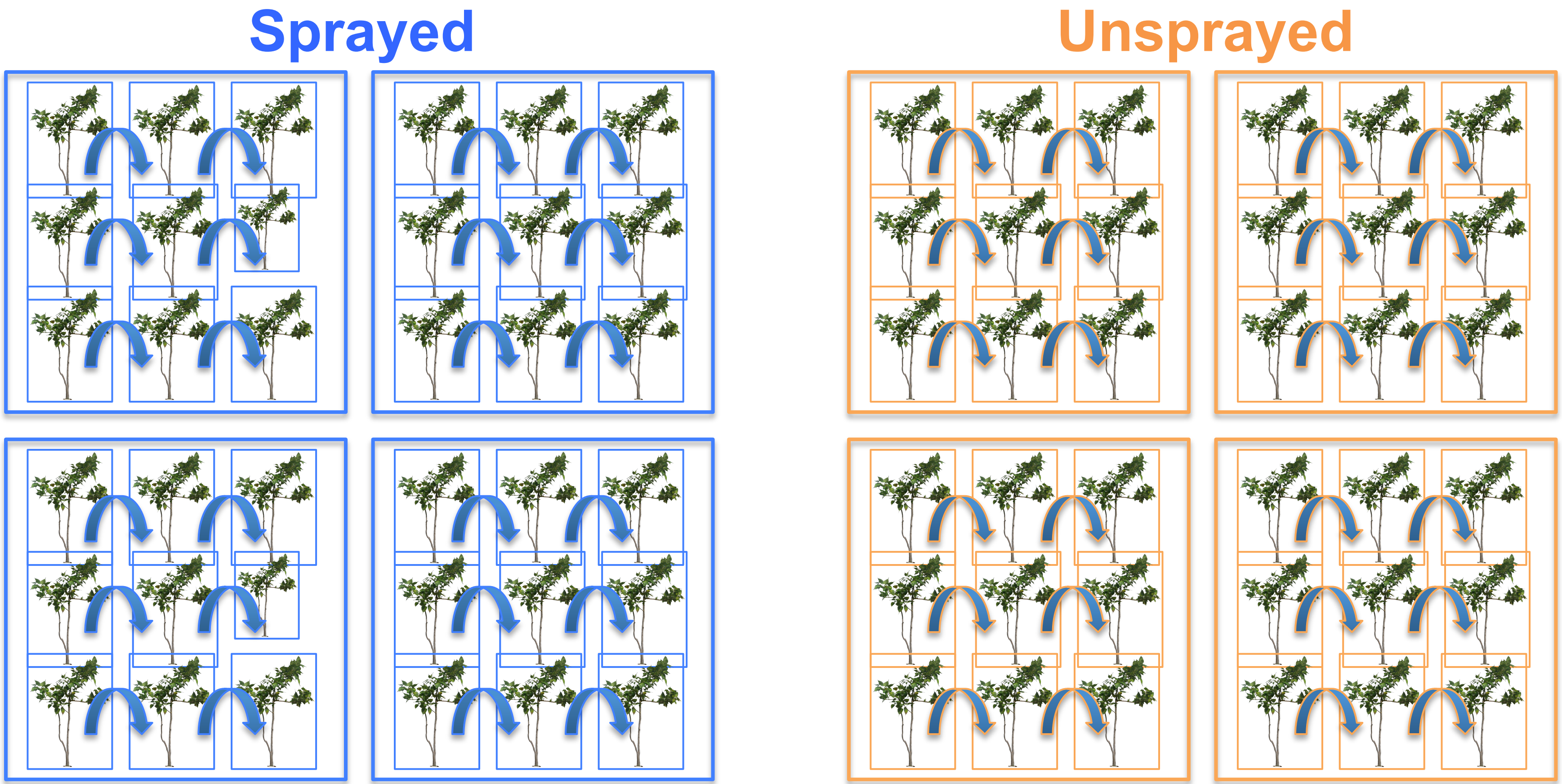
  119 manipulative field studies, pseudoreplication in 12% of these, i.e. 1 in 8 published studies

True replicates

- Independent!
- Not part of a time series
- Not be grouped together in one place
- Of an appropriate spatial scale
- Ideally, one replicate from each treatment ought to be grouped together into a block, and
- each treatment repeated in many different blocks

Crawley, The R Book, 2007

Which are the random and fixed effects?



- 8 plots (4 sprayed and 4 unsprayed)
- 3 trees in each plot
- Each tree measured three times for leaf damage

Define a linear mixed-effects model in R

- I use the function *lme* in the library *nlme* (library lme4 is another option)
- Syntax (here, y is the response variable, a-d are explanatory variables)
  - fixed = y~a (optional term)
  - random = ~ 1 | b/c/d (obligatory term)

Crawley, The R Book, 2007

Define a linear mixed-effects model in R

- For example:

- Nested design with different spatial scales:
- model<-lme(yield~irrigation*density*fertilizer,random=~1|block/irrigation/density)

- Repeated measurements
- model<-lme(root~fertilizer,random=~week|plant)

- summary(model)
- Diagnostic plots (qqplot, plot(model))

Crawley, The R Book, 2007

Correcting for multiple hypothesis testing

- Not doing it is unfortunately very common and as much a trap as mistaking pseudoreplicates and true replicates…
- Multiple comparisons influence the reliability of α, the probability to conduct a type I error
- I.e. ONE test at $\alpha=0.05$ the probability for a false positive is 5%
- At 40 comparisons we expect at least 2 of them to appear significant only by chance!
- Therefore, when doing multiple comparisons, make α smaller! But this is a trade-off with becoming ‘too conservative’ since type II errors become more likely

Correcting for multiple hypothesis testing

- Several methods, one of the more well-know traditional but very conservative ones is the Bonferroni correction (divide α by the number of tests)
- I use the function *glht* in the library *multcomp*
- Syntax
- model=lme(fixed=x~y,random=~time|plot)
- summary(model)
- K <- diag(1,4);glht.model <- glht(model, linfct = K)
- summary(glht.model)

  -> get multiplicity-adjusted *P*-values

Case study - Coming 4 exercises

- Work in three teams (2-3 people per team)
- Work with real life aquatic and environmental data (water chemistry and optics, climate, etc.)
- Analysis using mixed-effects modeling in R

  with multiple comparison corrections
- Prepare a presentation to present your results to the group during the final seminar of this teaching unit

  (Thu March 3):

➢ Statistical results

➢ Graphs

➢ Discuss in light of the context of this lecture and the literature

Final presentation on March 3

- 15-minute presentation by each team
- Questions to be answered:
  - What are the biogeochemical similarities and dissimilarities between system lake, stream and river mouth water?
  - Which variables have changed over time in these systems?
  - Which changes were influenced by temperature, precipitation and pH, and in which way?
  - Discuss related to this lecture and the literature (e.g. Zhang et al. 2010), discuss as well which other drivers you would like to test.

Mixed-effects model setup – think!

- You have a set of variables for three lakes, three streams and three river mouths in Sweden
- You want to make general conclusions about the aquatic systems based on these data
- You are interested in some response variables (e.g. TOC)
- You are interested if the response variables changed over time, differ between the systems, and if and how precipitation, temperature and pH are related to the response variables.
- **What are your fixed effects and what are your random effects?**

Some notes

- You need to have enough data to test your hypothesis, i.e. possible model complexity depends on the amount of data
- One reason why a lme model might not converge is that the structure is too complex for the amount of data