Review

Ordination of Responses to Toxic Stress in Experimental Ecosystems

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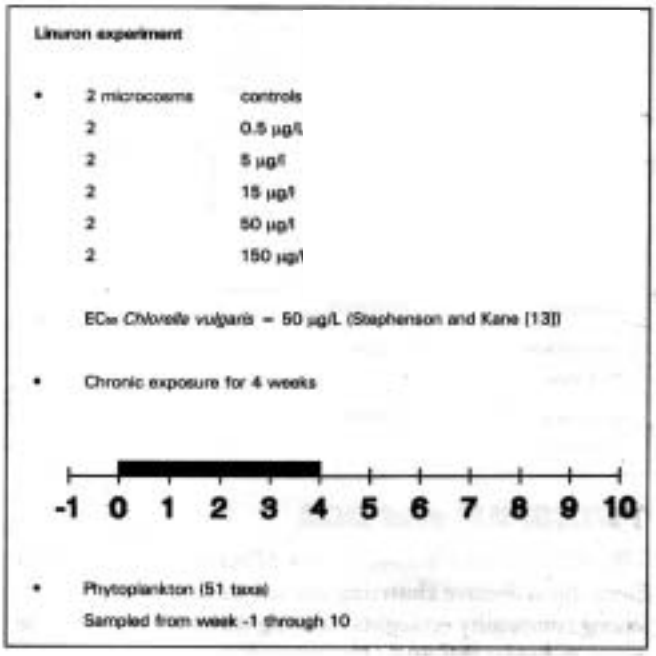
Pesticides used for crop protection may enter adjacent freshwater ecosystems by spray drift, leaching, run-off or accidental spills. For a hazard assessment of these pesticides, microcosm and mesocosm experiments can be carried out. Although smaller and less complex than real-world freshwater ecosystems, microcosms provide the opportunity to perform ecosystem-level research in replicable test systems under conditions that are manageable in terms of costs and logistics [1]. These experiments, however, involve great effort in sampling, identification of the biological communities and measurements of parameters. Data of only the most common taxa appear suitable for univariate statistical analysis (e.g. ANOVA). By contrast, multivariate statistical analysis analyses all available data and describes the effects of chemical stress at the community level. In this paper we will discuss four different multivariate techniques.

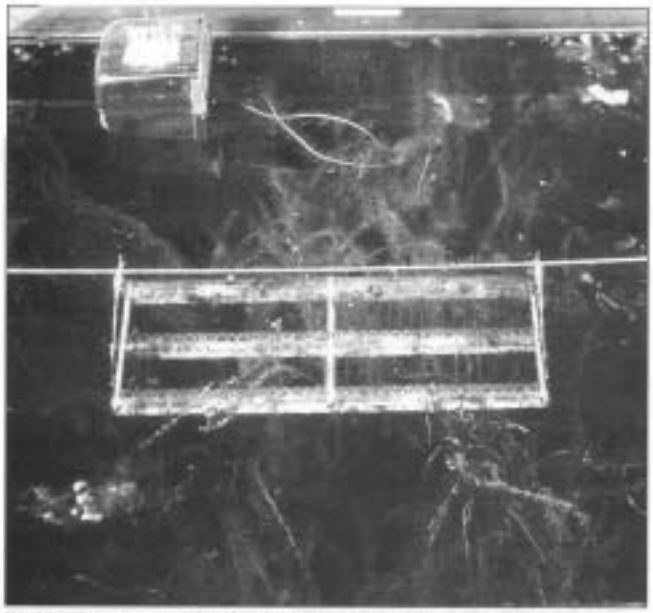
Example data set

The example data set resulted from an experiment in microcosms, simulating the community of drainage ditches, and with the herbicide linuron as a stressor. The outline of the experiment is summarised in Textbox 1. The microcosms consisted of a glass aquarium (volume approximately 1 m^3) with a natural sediment layer of 10 cm and a water layer of 60 cm (photographs 1 and 2). During the preparatory phase of the microcosm experiment the macrophyte *Elodea nuttallii* and indigenous invertebrate and algal species were introduced. Zooplankton, macro-invertebrates, phytoplankton and periphyton were sampled and identified (bi)weekly from 1 week before the start of the experiment until 10 weeks after. During this period several physico-chemical parameters were also measured. The experiment and the phytoplankton data set, which is used in this paper as an example, are described and discussed in detail in Van den Brink *et al.* [2] and Cuppen *et al.* [3]. In the application of the first two methods (TWINSPAN and DCA), abundance values were averaged over replicates. The later methods used the original samples.

Textbox 1



Photograph 1. *Overview of the climate room and the microcosms.*



Photograph 2. *Detailed view of macrophyte dominated microcosm.*

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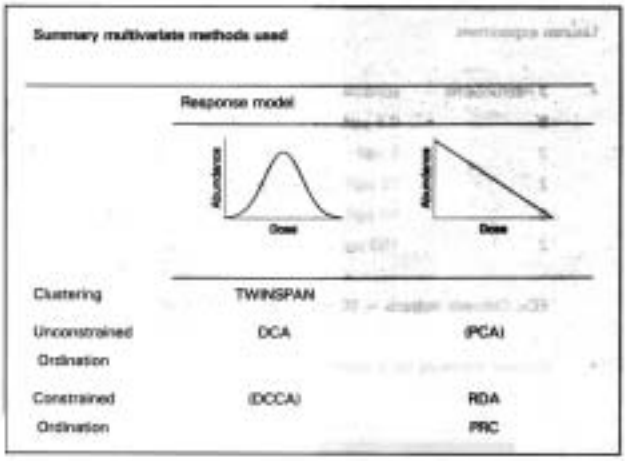
Textbox 2



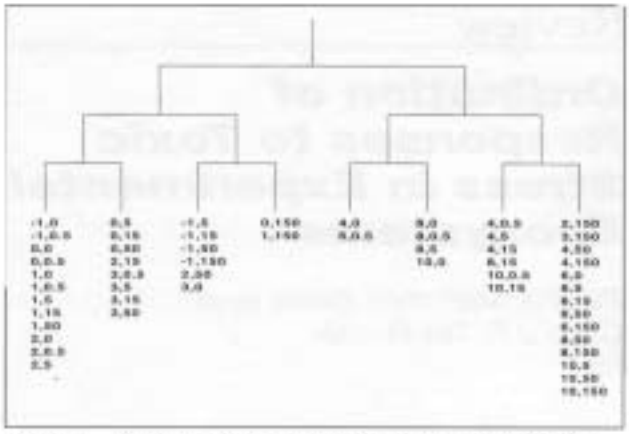


Figure 1. Dendrogram of the example data set using TWINSPAN. The sample codes, e.g. –1,0, are week number followed by dose.

TWINSPAN and DCA

TWINSPAN (Two-Way-INdicator-SPecies-ANalysis) [4] is a hierarchical divisive clustering method that is very popular among community ecologists. Starting with all samples in one group, it divides this group into two sub-groups. These sub-groups are then divided into sub-sub-groups, etc. Each division is based on Correspondence Analysis [5]. Correspondence Analysis has the desirable ability to detect clusters and to sequence sample data that arise from bell-shaped response curves (Textbox 2). Applied to the example data set, TWINSPAN yielded the dendrogram of Figure 1. The first division separates most early samples (weeks –1 to 3) from the later samples. Later divisions are more difficult to interpret, except for the final division on the right-hand side, which sets apart all samples from the 150 µg/L treatment taken in and after week 2 and all samples from the 50 µg/L treatment taken in and after week 4. This cluster contains an odd control sample. In its finer detail, the dendrogram thus suggests a treatment effect. It does not provide information on the magnitude and nature of the effects.

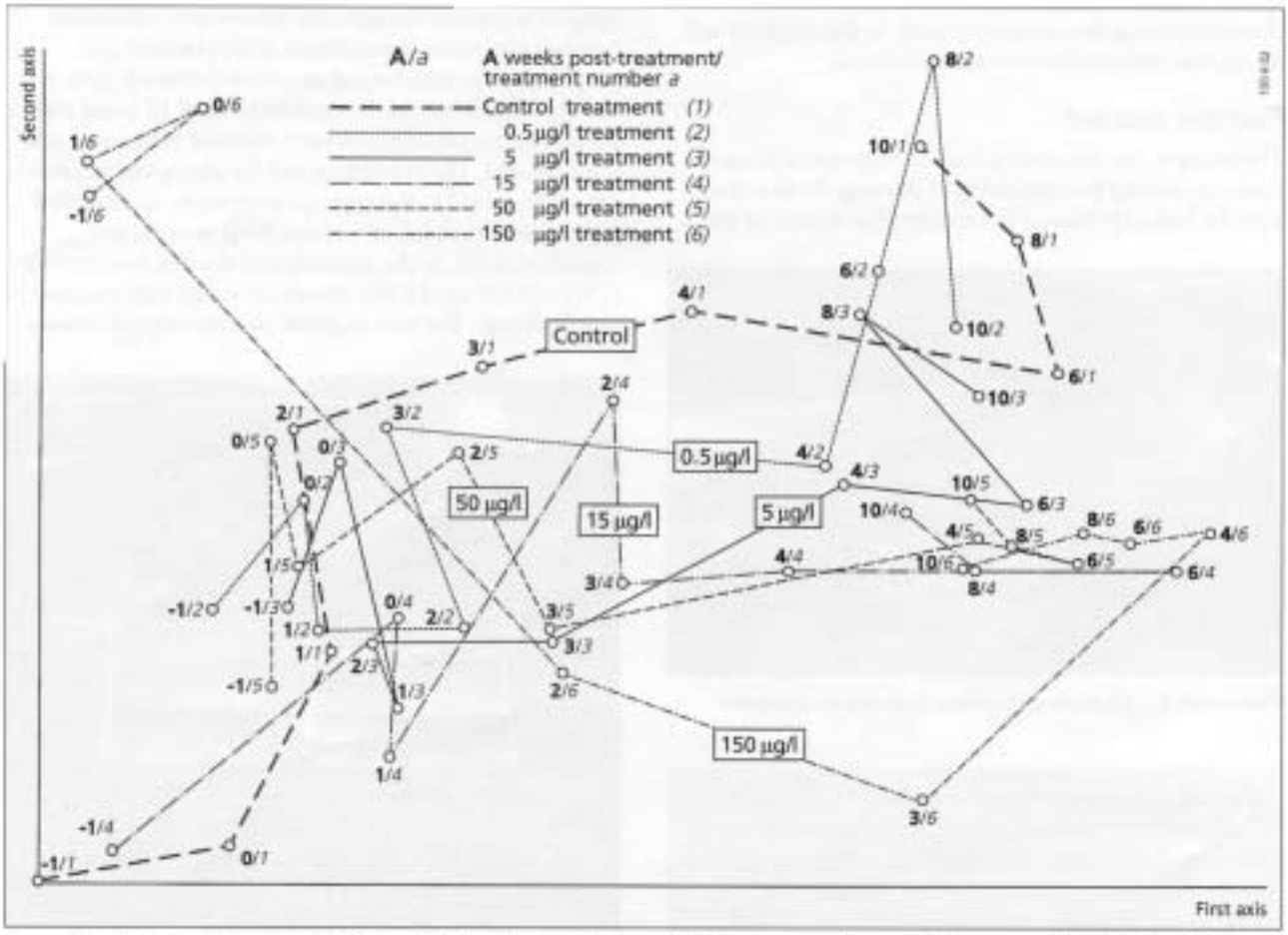


Figure 2. Ordination diagram resulting from a DCA on the example data set. The lines represent the course of the treatment levels in time. Of all variance, 18% is displayed on the horizontal axis and 7% on the vertical axis.

Detrended Correspondence Analysis (DCA), is an improved form of Correspondence Analysis. Incorporated in the computer programmes DECORANA [6] and CANOCO [7] it is among the most popular ordination methods used in ecology. It results in a diagram that represents the major pattern of between-sample variation (Figure 2). In the diagram, samples with nearly identical species composition lie close together, while samples with very different species composition lie far apart (for more explanation see Ter Braak, [8]). So, the samples taken at weeks –1, 0 and 1 in the highest treatment have a similar species composition, which differs from the species composition of all other samples. In an attempt to highlight the structure of the experimental design, time-trajectories are added to Figure 2. The trajectories for all treatments can hardly be separated from each other, so the treatment effects are difficult to discern from Figure 2. Usually the species are also presented in the diagram but in our study the species were placed too far outside the range of the samples to be shown.

RDA and PRC

Outside community ecology, Principal Component Analysis (PCA) is the most frequently used multivariate technique. In contrast to DCA, PCA is based on a linear response model. Redundancy Analysis (RDA) is the constrained form of PCA (Textbox 2). RDA constrains the ordination to that part of the total variance of a data set that is explained by a given set of explanatory variables. We used treatment, time and their interaction as explanatory variables. In this particular example, the same ordination could have been obtained by applying a PCA on abundance values averaged over replicates, as was done in DCA. Van Wijngaarden *et al.* [9] compare DCA, PCA and RDA and their usage in mesocosm

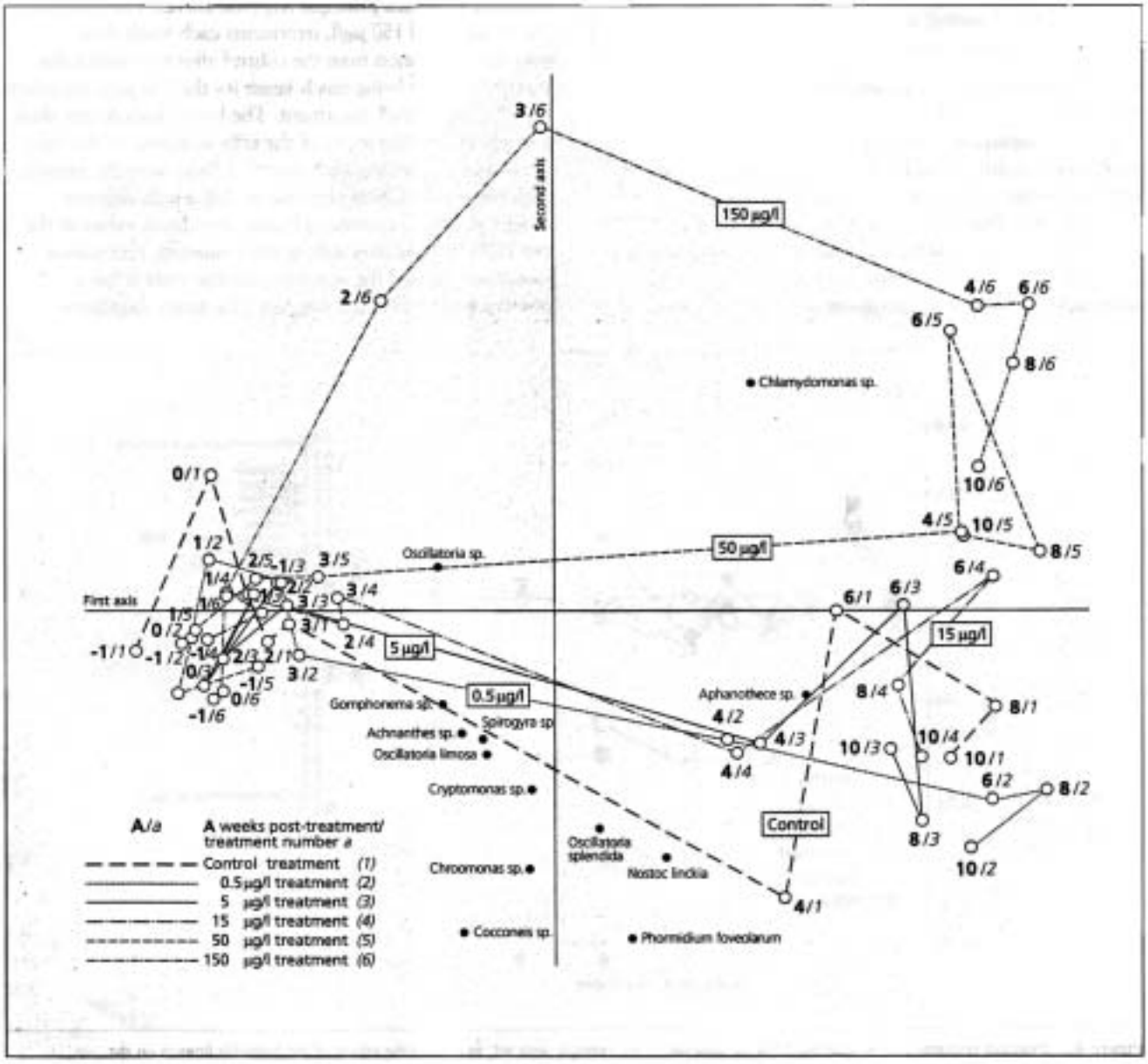


Figure 3. Ordination diagram (RDA) indicating effects of the herbicide linuron on the phytoplankton. Sampling week and treatment level, as well as their interactions, were taken as explanatory variables. The lines represent the course of the treatment levels in time. Of all variance, 78% could be attributed to the explanatory variables. Of this explained variance, 61% is explained in the diagram.

Textbox 3.

Principle Response Curves

- based on RDA
- Zooms in at differences in measurement endpoints between treatments and the control as reference
- Model used:

  $y_{tkjb} = y_{tk0} + b_k h_{tb} + e_{tkjb}$

  with

  y_{tkjb} : abundance counts of taxon k at time t in replicate j of treament b.

  y_{tk0} : mean abundance in controls (0) for each sampling date

  h_{tb} : principal response of treatment b at time t (PRC)

  b_k : weight of species k with respect to $\{h_{tb}\}$

  e_{tkjb} : error term
- In diagram horizontal axis : time
  vertical axis : h_{tb} and b_k

research in more detail. Van den Brink *et al.* [10] provide more examples of RDA.

Figure 3 displays the ordination with added time-trajectories resulting from RDA applied to the example data set. The horizontal axis shows mainly the changes in species composition in time. The vertical axis shows a difference compared to the control for the 150 µg/L treatment and less pronounced also for the 50 µg/L treatment. The samples taken at the start of the experiment are all placed close together. The placement of the species indicates higher abundance values in the highest treatments for *Chlamydomonas* sp. and lower abundance values for *Cocconeis* sp., *Chroomonas* sp. *Phormidium foveolarum*, etc.

Diagrams such as Figure 3 may become very cluttered (see for instance Kersting and Van den Brink, [11]). Even from Figure 3, it is hard to discern how the treatment effects change in time. To overcome these problems we recently developed a variant of RDA, called Principal Response Curves analysis (PRC [12]). PRC is designed to show optimally the major changes in treatment effects over time. The new method zooms in at differences in species-composition between the treatments and control at each particular timepoint. The model of PRC is outlined in Textbox 3. The result is a PRC diagram such as Figure 4. The horizontal axis denotes the week relative to the start of the treatment and the vertical axis the treatment effect, expressed as deviation from the control. The curves so obtained are called principal response curves. The curves for the 50 µg/L and 150 µg/L treatments each reach their maximum deviation from the control after four weeks, the maximum effect being much larger for the 150 µg/L treatment than for the 50 µg/L treatment. The lower doses do not show a strong effect. The scores of the taxa as shown on the right are weights expressing each taxon's affinity with the principal response curves. *Chlamydomonas* sp. has a high negative weight and thus occurred in higher abundance values at the two highest doses after start of the treatment. *Phormidium foveolarum* showed the opposite response since it has a positive weight with the diagram. The mean abundance

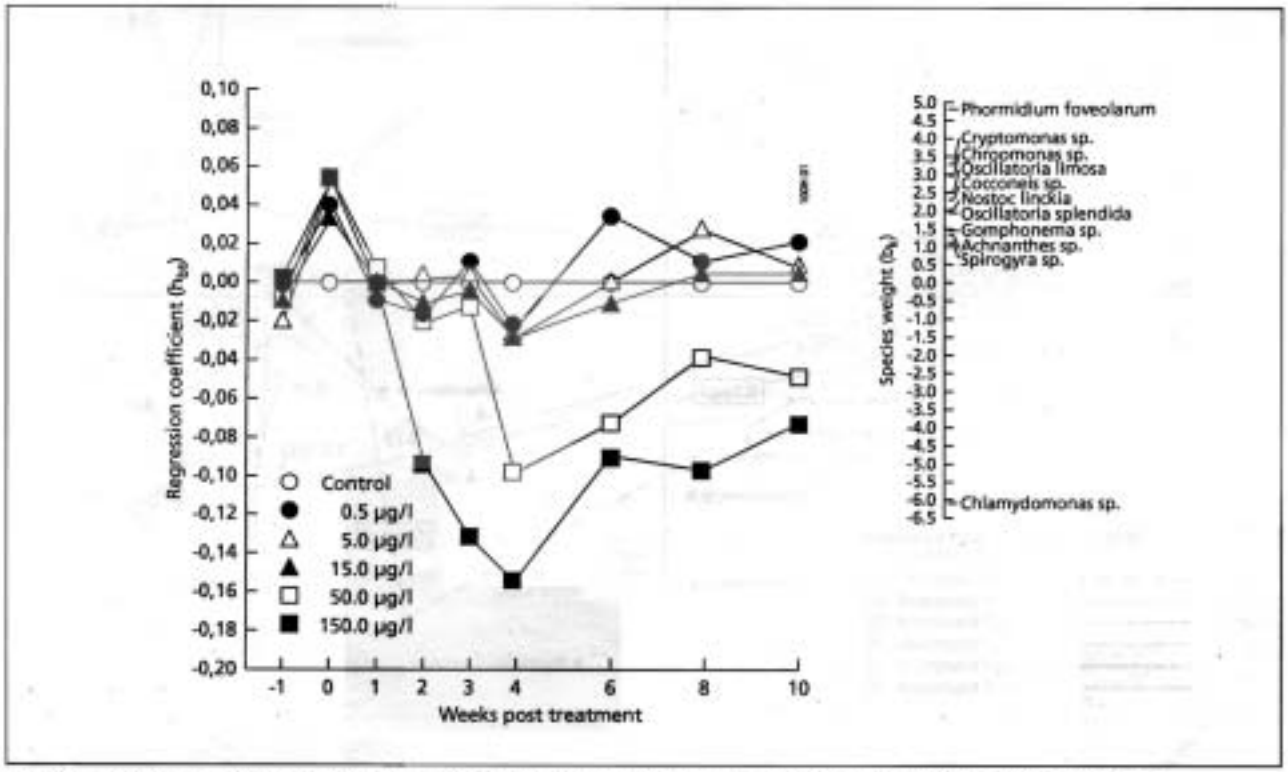


Figure 4. *Principal response curves resulting from the analysis of the example data set, indicating the effects of the herbicide linuron on the phytoplankton community. Of all variance, 47% could be attributed to sampling date, and is displayed on the horizontal axis. Of all variance, 30% could be attributed to treatment. Of the variance explained by treatment, 23% is displayed on the vertical axis. The lines represent the course of the treatment levels in time. The species weight (b_k) can be interpreted as the affinity of the taxon with the principal response curves.*

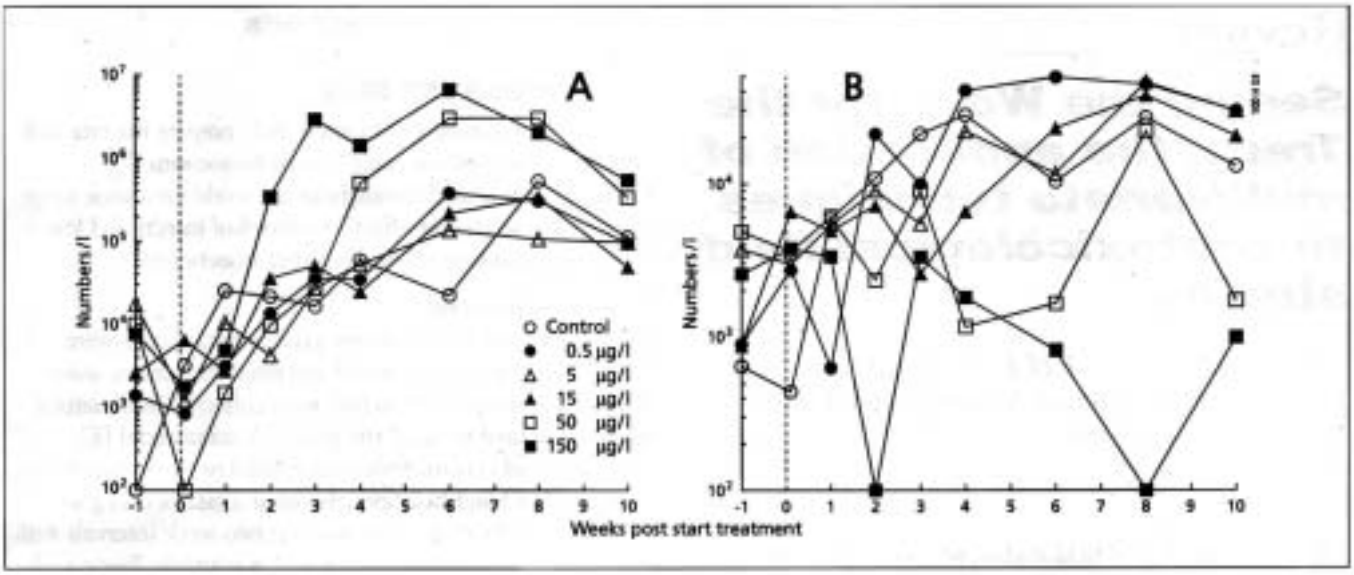


Figure 5. Dynamics in numbers of two phytoplankton taxa. Shown are the geometric means of the counted numbers per treatment level, of (A) Chlamydomonas *and (B)* Phormidium foveolarum.

patterns of both taxa are given in Figures 5A and 5B respectively. These patterns correspond very well with the treatment effects as indicated by the PRC diagram.

Conclusions

From this case study we conclude that RDA more clearly displayed the treatment effects than TWINSPAN and DCA. A possible explanation for this is that ecotoxicological dose–response relationships are usually logistic, which is better approximated by a linear model than by a unimodal model. In addition, Correspondence Analysis focuses on the relative abundances of taxa whereas RDA concentrates on the absolute abundances. An overall decrease in abundance may therefore go unnoticed in Correspondence Analysis.

The results of PRC were more easy to interpret than those of RDA. By filtering out the mean abundance pattern across time in the control, PRC focused on the deviation between treatment and associated control. The principal response curves displayed the major pattern in these deviations and were a good summary of response curves of individual taxa.

Noise is sometimes believed to be an overriding property of ecological data. Our most important conclusion is perhaps that proper statistical analysis revealed clear response patterns in noisy ecological data.

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