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Multivariate analysis of stress in experimental ecosystems by Principal Response Curves and similarity analysis

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Abstract

Experiments in microcosms and mesocosms, which can be carried out in an advanced tier of risk assessment, usually result in large data sets on the dynamics of biological communities of treated and control cosms. Multivariate techniques are an accepted tool to evaluate the community treatment effects resulting from these complex experiments. In this paper two methods of multivariate analysis are discussed on their merits: 1) the canonical ordination technique Principal Response Curves (PRC) and 2) the similarity indices of Bray-Curtis and Stander. For this, the data sets of a microcosm experiment were used to simultaneously study the impact of nutrient loading and insecticide application.

Both similarity indices display, in a single graph, the total effect size against time and do not allow a direct interpretation down to the taxon level. In the PRC method, the principal components of the treatment effects are plotted against time. Since the species of the example data sets, react in qualitatively different ways to the treatments, more than one PRC is needed for a proper description of the treatment effects. The first PRC of one of the data sets describes the effects due to the chlorpyrifos addition, the second one the effects as a result of the nutrient loading. The resulting principal response curves jointly summarize the essential features of the response curves of the individual taxa. This paper goes beyond the first PRC to visualize the effects of chemicals at the community level. In both multivariate analysis methods the statistical significance of the effects can be assessed by Monte Carlo permutation testing.

Introduction

Microcosm and mesocosm experiments may be carried out at higher tiers in the risk assessment procedure of pesticides. Normally, these experiments result in large data sets comprising information on temporal changes in the structure and functioning of control and treated experimental ecosystems. Of these large data sets, however, only the information on a limited number of taxa (usually the abundant ones) can be properly analyzed with standard univariate statistical methods. Describing the effects of chemical stress at the community level requires techniques that also take the information of less abundant taxa into account.

The first aim of this paper is to compare the results of two different multivariate techniques. For this purpose, we re-analyze the data from an experiment the published results of which were based solely on univariate analyses (Van Donk et al., 1995; Brock et al., 1995; Cuppen et al., 1995). The first multivariate technique used is a relatively simple method, based on an appropriately chosen similarity index, whereas the second, relatively complex method, called the Principal Response Curves (PRC) method, is based on ordination. The PRC method was specially designed by Van den Brink & Ter Braak (in press) for the analysis of data from mesocosms experiments. The multivariate methods are compared and their advantages and disadvantages discussed. The second aim

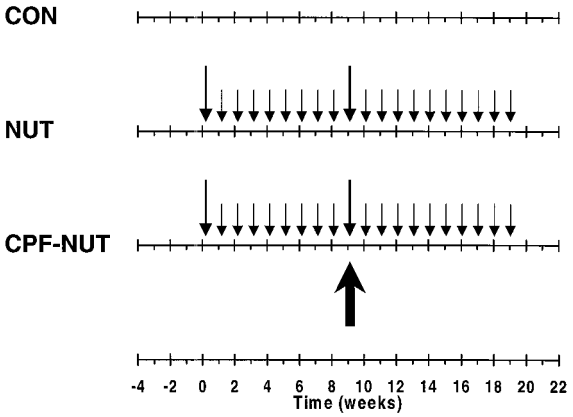

Figure 1. Set-up nutrient-chlorpyrifos experiment. Apart from 4 control microcosms (CON), there were 4 microcosms that received the multiple dosages of nutrients, as indicated (NUT), and 4 microcosms that received both the multiple nutrients dosages and the single chlorpyrifos addition at week 9 (CPF-NUT). Small and very small arrows refer to large and small nutrient dosages, the large arrow to the chlorpyrifos addition.

is to show the merits of an additional multivariate analysis over an exclusively univariate one.

Example data set

Experimental set-up

The example data sets resulted from an experiment in microcosms, simulating the community of drainage ditches, using the insecticide chlorpyrifos and nutrients as stressors. The experiment is outlined in Figure 1. Four microcosms were chosen as controls (CON) and 4 microcosms as NUT, which were treated with two high dosages of nutrients (0.15 mg P l^{-1} and 0.9 mg N l^{-1} in week 0 and 9) and several lower ones (0.025 mg P l^{-1} and 0.15 mg N l^{-1}). The remaining 4 microcosms received the same nutrient treatment but also a relatively high dose (35 μg/l^{-1}) of the insecticide chlorpyrifos in week 9 (CPF-NUT). Hence, before week 9 the NUT treatment consisted of 8 replicates (Figure 1). After the chlorpyrifos addition in week 9, both the NUT and CPF-NUT treatments consisted of four replicates.

A microcosm consisted of a glass aquarium (L: W: H; 1.1: 1.1: 1 m) with a natural sediment layer of 10 cm and a water layer of 60 cm. During the preparatory phase of the microcosm experiment, the macrophyte *Elodea nuttallii*, and indigenous invertebrate and algal species were introduced. Zooplankton, macro-invertebrates and phytoplankton were sampled biweekly, identified and counted, and recalculated to numbers per substratum or litre, in the period from 4 weeks before the first nutrient addition to 22 weeks hereafter. The zooplankton and macro-invertebrate data sets were combined into one invertebrate data set (for details see Van den Brink et al., 1996). A total of 120 invertebrate and 26 phytoplankton taxa were identified. Before using PRC analysis and the permutation tests using the F-type criterion, the invertebrate and phytoplankton data sets were Ln(x+1) and Ln(2x+1) transformed, respectively (for rationale see Van den Brink et al., 1995). The experiment and the example data sets are described and discussed in detail in Van Donk et al. (1995), Cuppen et al. (1995) and Brock et al. (1995).

Summary of effects found by univariate analysis

The columns labelled ‘references’ in Table 1 summarize the effects as recorded in Cuppen et al. (1995). Briefly, as a direct effect of the nutrient additions an increase in the biomass of the macrophytes and in the chlorophyll-a of the planktonic and periphytic algae was found. This increase in food availability resulted in an increase in the abundance of herbivores (Cladocera and Copepoda) and detritivores (Isopoda) and finally in an increase in abundance of the carnivores (*Plea minutissima*). As a direct effect of chlorpyrifos, the crustaceans (Amphipoda, Isopoda, Cladocera and Copepoda) and Insecta, decreased in abundance. The decrease in grazing pressure led to an increase in the chlorophyll-a content of planktonic and periphytic algae. This increase in food levels finally brought on an increase in numbers among the herbivores (Oligochaeta, Gastropoda and Rotifera).

Multivariate analyses

Similarity analyses using the Bray-Curtis and Stander’s Index

Similarity indices are in widespread use to express in a single number the similarity between biological communities (Washington, 1984). Following examples from marine ecology (Clarke, 1993; Clarke & Warwick, 1994), they have also been also used in ecotoxicology to quantify the effects of stressors at the community level. Clarke (1993) used the Bray-Curtis index for this purpose. The Bray-Curtis index, also known as the Czekanowski coefficient or the percentage of similarity, is defined by:

Table 1. Summary of effects of nutrients and chlorpyrifos. N.A. = comparison not applicable. A '+' indicates an increase compared to the control, a '-' a decrease. The more the plusses or minusses, the larger the increase or decrease

Endpoint	Effect nutrients		Effect chlorpyrifos	
	References	PRC	References	PRC
Primary producers				
Planktonic algae				
chlorophyll-a	+	N.A.	+	N.A.
Abundance *Volvox*	+	+	++	++
Abundance other species	0	+/−−	0	+/−−
Periphytic algae				
Chlorophyll-a	+	N.A.	++	N.A.
Macrophytes				
Biomass	+++	N.A.	(−)	N.A.
Invertebrates				
Cladocera	++	++	−−−	−−−
Copepoda	++	++	−	−
Rotifera	0	+/−	+	+
Insecta (*P. minutissima*)	+++	++	−−−	−−
Isopoda	+	+	−−	−−
Amphipoda	0	−−	−−−	−−
Gastropoda	0	+	++	++
Oligochaeta	0	+/−	+/−	++/−

$$BC_{jk} = 100 * \left(1 - \frac{\Sigma|y_{jj} - y_{ik}|}{\Sigma(y_{ij} + y_{ik}}\right), \qquad (1)$$

where BC_{jk} = Bray–Curtis index;
y_{ij}, y_{ik} = abundance of species k in samples i and j, respectively.

Using the Bray–Curtis index, the mean within-treatment similarity (Wdata) and the mean between-treatment similarity (Bdata) are calculated. For an evaluation of the size of the treatment effects, the Bdata/Wdata quotient is plotted against the sampling date (see the example in Figure 2A, where the quotient is referred to as the Bray–Curtis index quotient). When the quotient is more below 1 than can be expected by chance, the treatment is demonstrated to have an effect on the community in the cosms. But if the quotient is greater than or equal to 1, there is no evidence that the treatment has an effect. For the period before week 9, one quotient was calculated for each sampling date, to compare the microcosms that received nutrient addition with the controls. After week 9, two quotients were calculated, one to compare the NUT cosms with the controls and another to compare the CPF-NUT cosms with the controls. The untransformed data were used for the analyses, which were performed with the Community Analysis software package version 3.5 (CA; Hommen et al., 1994). CA is a computer program used to evaluate data from freshwater field tests and can, among other things, calculate a variety of similarity and dissimilarity indices. Note that other definitions of effect-size are possible. For example, Clarke (1993) used the difference between the between- and within-similarities.

Recently, Heimbach & Ratte (1997) proposed the use of the Stander's similarity index for the analysis of data from mesocosm experiments. The Stander's Index, also known as the Cosine or Ochiai coefficient (Jongman et al., 1995), is defined by:

$$S_{jk} = \frac{\Sigma(p_{ij} * p_{ik})}{\sqrt{(\Sigma p_{ij}^2 * \Sigma p_{ik}^2)}} \qquad (2)$$

where S_{jk} = Stander's index; p_{ij}, p_{ik} = proportion of species k in samples i and j, respectively.

Stander's index quotients were calculated analogously to the Bray-Curtis index quotients.

Ordination using Principal Response Curves (PRC)

The PRC method (Van den Brink & Ter Braak, in press; 1997) is based on the ordination technique

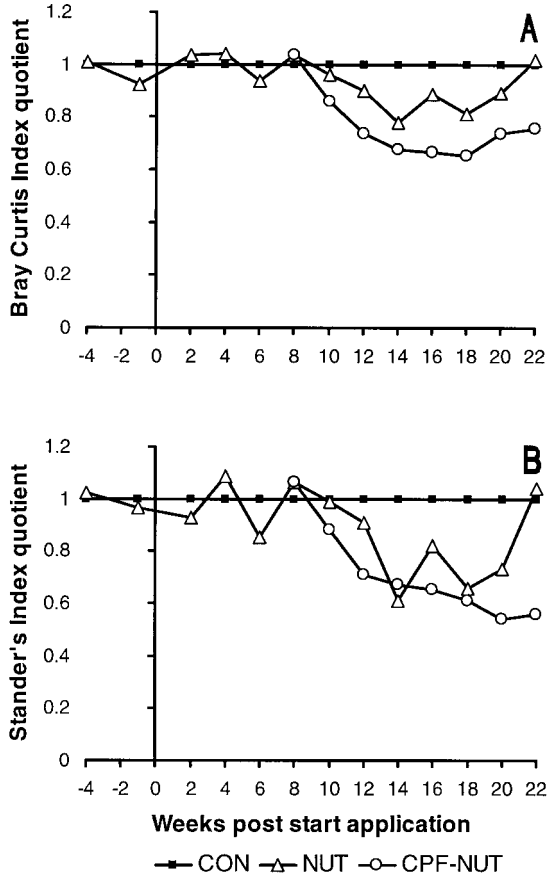

Figure 2. Size of the effects of nutrient additions (NUT) and the extra chlorpyrifos addition (CPF-NUT) in the invertebrate data set as defined by the Bray-Curtis (A) and Stander's (B) Index quotient. The quotient was defined as the between treatment similarity divided by the within treatment similarity. Values that are much smaller than the reference value of 1, indicate strong effects.

called partial redundancy analysis, which is a constrained form of principal component analysis (Jongman et al., 1995). Because people are more familiar with principal components than with redundancy analysis, we introduce PRC heuristically via principal components. Like many other multivariate techniques, the PRC technique uses dimension reduction to summarize all information on the investigated populations simultaneously, so as to elucidate effects of contaminants at the community level. Whereas similarity analysis plots the effect size against time, the PRC method plots the first principal component of the treatment effects against time (Figure 3A), expressing the treatment effects as deviations from the control treatment (reference coding). As a result, the vertical axis of a PRC diagram contrasts each treatment with the control. Associated with each PRC is a set of species weights shown on the right side (Figure 3). The species weights can be interpreted as the affinity of each species with the diagram. For instance, in Figure 3A, *Asellus aquaticus* has a higher positive weight than *Dero digitata* and thus markedly decreased after the chlorpyrifos treatment in week 9 (see results section). *Stylaria lacustris* has a negative weight, indicating an increase in the CPF-NUT treatment. More quantitatively, the model fitted is:

$$y_{d(j)tk} = \overline{y}_{0tk} + b_k c_{dt} + \epsilon_{d(j)tk}, \quad (3)$$

where $y_{d(j)tk}$ is the log-abundance of species k in replicate mesocosm j of treatment d at time t, $\overline{y}_{0tk}$ is the mean log-abundance of species k in week t in the control ($d = 0$), c_{dt} is the score of the dth treatment at time t, b_k is the weight of the kth species, and $\epsilon_{d(j)tk}$ is an error term with mean zero and variance σ_k^2. Note that by definition $c_{0t} = 0$ for every t.

The product $b_k{}^* c_{dt}$ gives the fitted change in log-abundance ($-$ or $+$) of the species in the treated cosms relative to the controls (Van den Brink & Ter Braak, in press). In terms of the original abundance counts, the fitted abundance in the treatment cosms is $\exp(b_k{}^* c_{dt})$ times the geometric mean of the abundance in the controls (see the result section for an example). So, PRC emphasises the percentage change in abundance count of a species in the treatments relative to the control, independent from its absolute abundance. For the theoretical background, computational details and a discussion of the PRC analysis, see Van den Brink & Ter Braak (in press; 1997).

Although the first principal component extracts the maximum amount of information from the multivariate treatment effects, it does not necessarily describe the effects of all treatments on all taxa in sufficient detail. Further components can be extracted from the residual variation. PRC diagrams based on the second, third and higher components display treatment effects that are not captured in earlier components. The model for extracting two components becomes:

$$y_{d(j)tk} = \overline{y}_{0tk} + b_{k1} c_{dt1} + b_{k2} c_{dt2} + \epsilon_{d(j)tk}, \quad (4)$$

where c_{dt1} is the first principal response curve (PRC) for treatment d, i.e. course of treatment d in time relative to the controls, c_{dt2} is the second principal response curves for treatment d, b_{k1} is the weight of species k on the first PRC, and b_{k2} is the weight of species k on the second PRC.

The change in log-abundance as calculated per principal component must be summed across com-

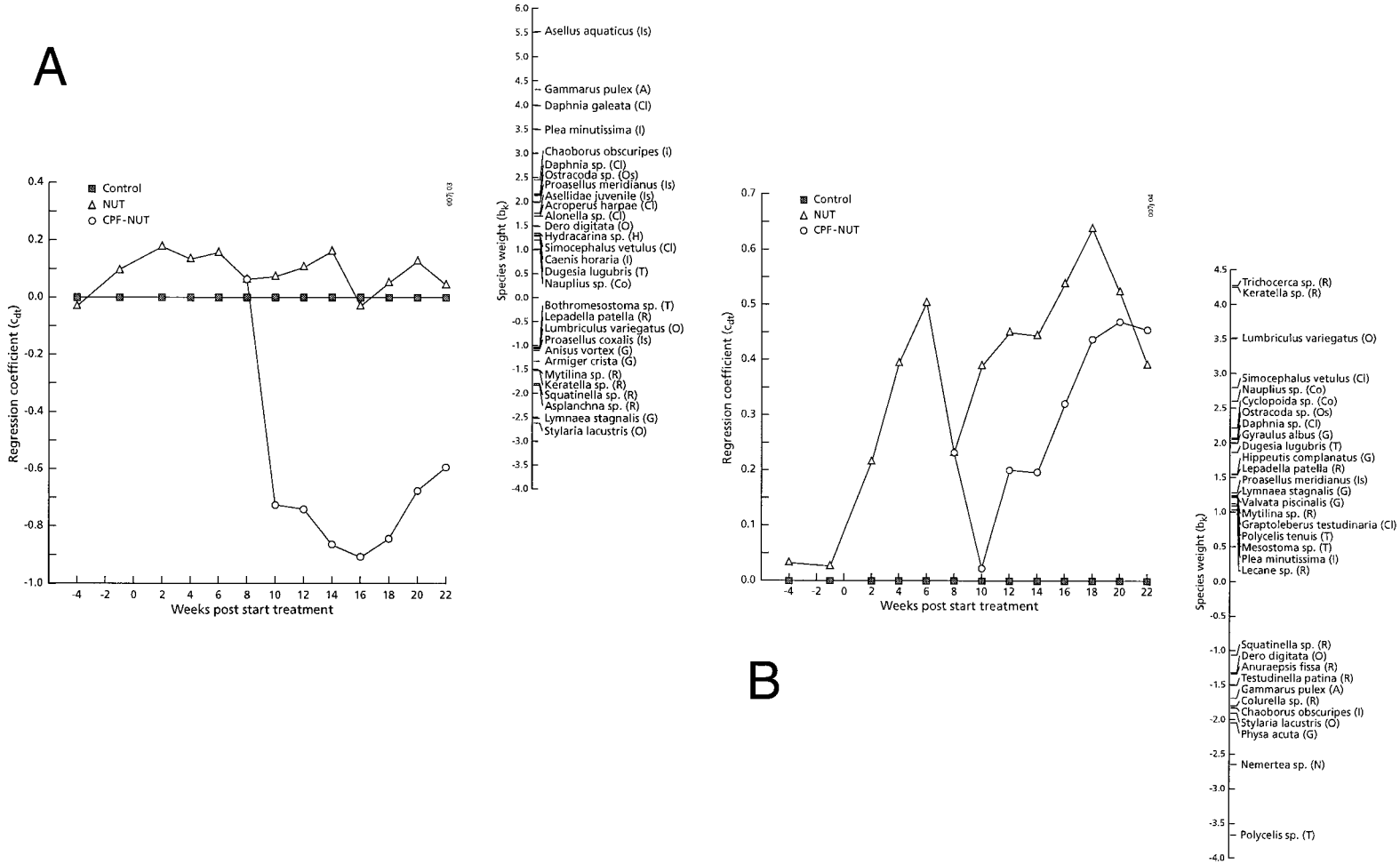

Figure 3. Principal Response Curves for the invertebrate data set, indicating the effects of nutrient additions (NUT) and the extra chlorpyrifos addition (CPF-NUT). The first PRC is given in A; the second PRC in B. See Table 2, for explained and displayed variance. Values deviating from the reference value of 0 indicate treatment effects. The species weight (b_k) can be interpreted as the affinity of the taxon with the principal response curves. Only the species with a weight of 1 or higher or -1 or lower with the diagrams are displayed. Explanation of the codes: A = Amphipoda; Cl = Cladocera; Co = Copepoda; G = Gastropoda; H = Hirudinea; I = Insecta; Is = Isopoda; N = Nemertea; O = Oligochaeta; Os = Ostracoda; R = Rotatoria; T = Turbellaria.

Table 2. Percentages of the total variance which can be attributed to time and treatment regime for the analyzed data sets. The treatment component includes the interaction between treatment and time. The remaining fraction of the variance is residual variance. The Table also indicates which fraction of the variance explained by the treatment regime is captured by the first and second Principal Response Curves (PRC)

Data set	% of variance accounted for by		% of variance explained by treatment regime captured by (p-value of PRC between brackets)	
	Time	Treatment regime	First PRC	Second PRC
Invertebrates	31	20	43 ($p \leq 0.01$)	15 ($p \leq 0.01$)
Phytoplankton	41	13	29 ($p \leq 0.01$)	26 ($p \leq 0.01$)

ponents to yield the joint fit of the change. The fitted change can be plotted against time to yield fitted response curves for individual species or species groups.

The significance of each principal component was tested by Monte Carlo permutation of the microcosms, i.e., by permuting whole time series in the partial redundancy analysis from which PRC is obtained, using an F-type test statistic based on the eigenvalue of the component (Van den Brink & Ter Braak, in press). In these tests, components that had already been extracted were added to the covariables. The PRC analyses and associated permutation tests were performed using the CANOCO software package, version 3.14 (Ter Braak, 1988, 1990).

We close this section with a technical remark. As described above, the PRC is the principal component of the treatment-by-species matrix of treatment effects. In Van den Brink & Ter Braak (in press), the PRC consists of the canonical coefficients of a partial redundancy analysis, in which the input data sets are the sample-by-species matrix of log-abundance values, the sample-by-week matrix of covariables, and the sample-by-(treatment in week) matrix of explanatory variables. It is known (Ter Braak & Looman, 1994) that the canonical coefficients of the explanatory variables in the redundancy analysis are the generalized principal components of the matrix of the regression coefficients, which in our context are the treatment effects. The heuristic introduction given above thus coincides with the PRC analysis by Van den Brink & Ter Braak (in press) in that they both apply principal component analysis with a particular weighting scheme. The weighting scheme becomes particularly important if treatments are not equi-replicated. In conclusion, the treatment scores $\{c_{dt}\}$ are both principal component scores and canonical coefficients of the treatments.

Tests of significance of treatment effects

To test on which sampling dates the treatments had significant effects on the biological communities, permutation tests were performed for each sampling date. Before week 9, the 8 treated cosms were tested against the 4 controls. After week 9, each of the two treatments was tested against the control and against the other treatment. The tests were carried out with the CA (Hommen et al., 1994) and CANOCO (Ter Braak, 1988) software packages. The permutation procedure in CA tests whether the similarity quotient is more below 1 than can be expected by chance, i.e. whether the quotient is significantly below 1. These tests were performed using the Bray-Curtis and Stander's index. The non-parametric method in CANOCO uses Monte Carlo permutation and the F-type test statistic of redundancy analysis (RDA). The reported P-values are based on 999 Monte Carlo permutations under the null model. Specific details for the application of Monte Carlo permutation in model ecosystem experiments have been described by Verdonschot and Ter Braak (1994), Van den Brink et al. (1996) and Van Wijngaarden et al. (1995).

Results

Invertebrate data set

Both the Bray-Curtis index and Stander's index quotients showed an effect of the treatments on the invertebrate communities after the second high nutrient addition and chlorpyrifos treatment in week 9 (Figure 2), with a higher effect size for the CPF-NUT

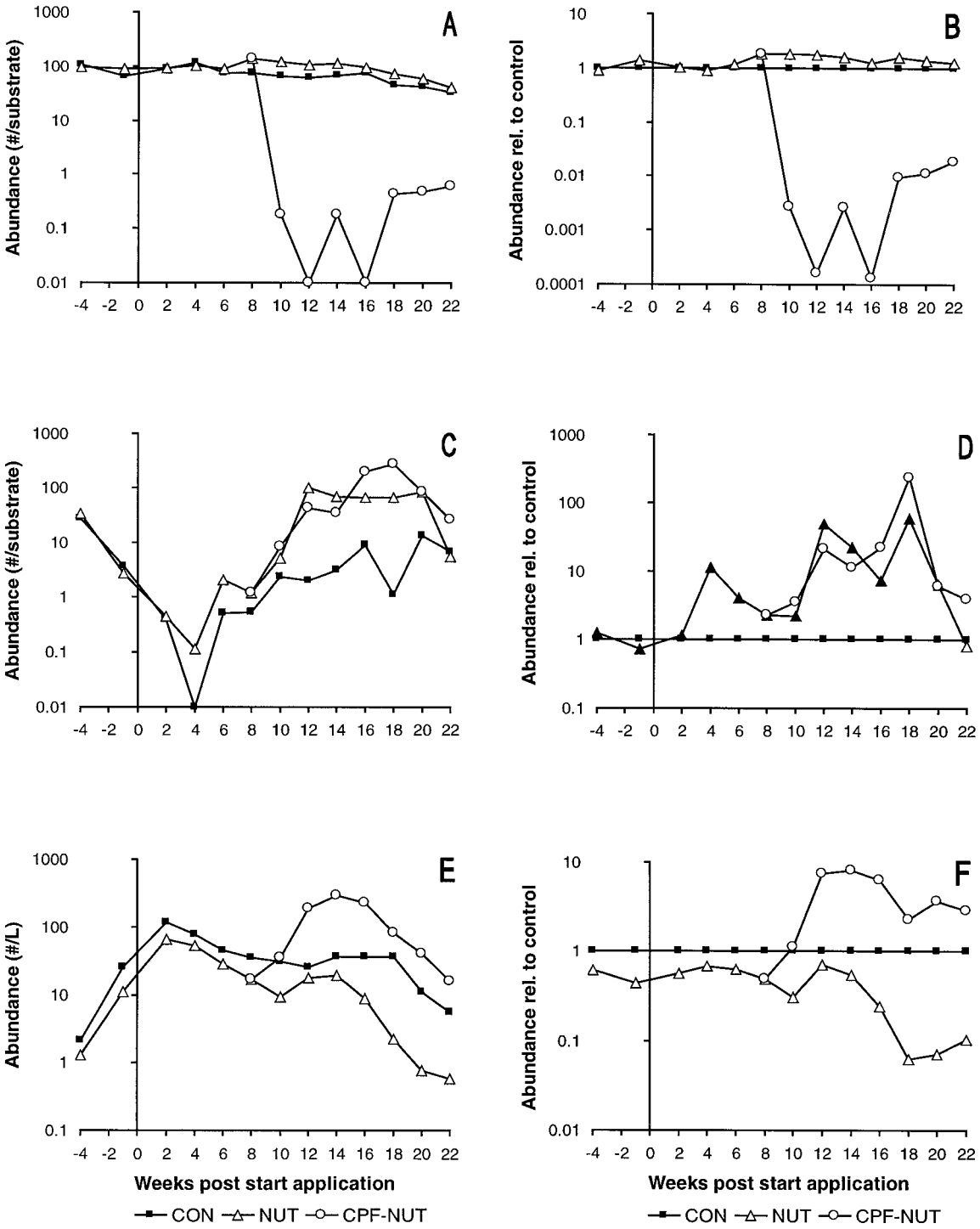

Figure 4. Dynamics in numbers of three invertebrate taxa. Figures 4A, 4C and 4E show the geometric means of the counted numbers per treatment, of *Asellus aquaticus*, *Stylaria lacustris* and *Keratella* sp. respectively. B, D and F shows their abundance relative to the control treatment, respectively.

treatment. The Bray–Curtis index showed a more stable pattern of effect sizes in time than Stander's index (Figure 2).

The PRC analysis indicated for the invertebrate data set that the overall variation among sampling dates was higher than that among treatments (Table 2). The first and second PRC components were statistically significant (Table 2), whereas the third component was not ($P>0.05$). The first PRC diagram (Figure 3A) shows that the curve for the CPF-NUT treatment drops abruptly after the chlorpyrifos addition (week 9), whereas the curve for the NUT treatment stays close to the zero line for the control during the whole sample period. In the second PRC (Figure 3B) the curve for the NUT treatment starts to deviate from the control after the first high nutrient dose, rises to a peak in week 6, drops and, after the second high nutrient dose in week 9, rises to a new peak in week 18. The curve for CPF-NUT in the period after week 9, starts at the zero level (week 10) and then roughly follows the trend in the NUT curve. The broad pattern that emerges is that the first PRC shows the dominant effect of the chlorpyrifos addition, whereas as the second PRC shows the subdominant nutrient effects.

Figure 4 shows the observed response patterns of three distinctive species. The first column of figures shows their (geometric) mean counts on a logarithmic scale, whereas the second column was obtained from the first one by plotting differences with respect to the controls. The decreases or increases shown by the species in the treatments compared with the level in the control, as displayed in the second column, constitute the treatment effects that the PRC method attempts to summarise. We now compare the response curves of the individual species with the response curves fitted by the first two PRCs.

Asellus aquaticus is the taxon with the highest weight with the first PRC, but a near zero weight with the second. Its observed response (Figure 4B) is, indeed, very similar in shape to the first PRC (Figure 3A). Quantitatively, the fitted reduction due to chlorpyrifos is also of the right order of magnitude, as we will now show. In week 10 the CPF-NUT score was about -0.8 (Figure 3A). The weight of *A. aquaticus* was 5.52, so that the fitted reduction was $\exp(-0.8 * 5.52) = 0.012$. The PRC analysis thus indicates that *A. aquaticus* is reduced to ca. 1% of its abundance in the control.

Keratella sp. has relatively higher weights with both PRCs. The fitted response pattern of *Keratella* sp.

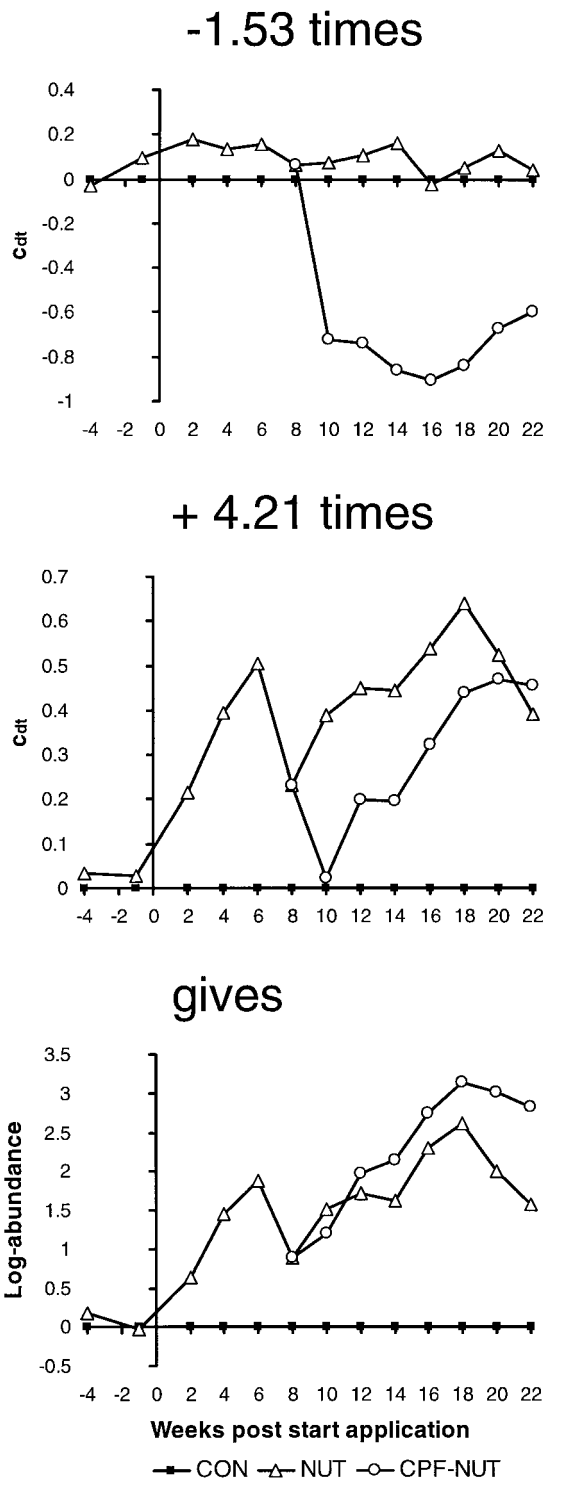

Figure 5. Outline of how the two PRC diagrams of Figure 3 must be combined so as to obtain the fitted response curves of *Keratella* sp., which has weights -1.53 and 4.21 on the first and second PRC, respectively.

can be obtained by multiplying these weights with the treatment scores in the corresponding PRCs and then summing the two products (Figure 5). The resulting fitted curves are similar to the observed response as given in Figure 4D, except for its response at the end of the experimental period (week 20 and 22). Quantitatively speaking, week 16, for example, shows a fitted change on a log scale of ca. 2.5 for both treatments (Figure 5). In terms of abundance, *Keratella* sp. was present with $\exp(2.5) = 12$ times more individuals in the treatments than in the control, which roughly corresponds to the observed response.

The weighted summation of PRC curves for each individual taxon can be avoided, as is shown in Figure 6, where species have been positioned by their weights on the first and second PRCs (Figure 3). In this system of coordinates, species that lie on the same line through the origin have proportional response curves. Their fitted response curves thus have the same shape. Figure 6 shows the shape of the response curves for species situated on lines with 45° intervals and they give an impression of the type of shapes that can be obtained by combining PRCs. The four diagrams in the corners apply to species that have equal weights on the two PRCs (except for the sign). The PRC diagrams corresponding with the axes are precisely the PRCs of Figure 3. For example, the species positioned on the right side along the horizontal axis in Figure 6 (e.g., *Asellus aquaticus*) decreased due to chlorpyrifos, while those at the top of the vertical axis (e.g., *Trichocerca* sp.) increased due to the nutrient additions. Those at 45° (e.g., *Ostracoda*) decreased due to chlorpyrifos and increased due to nutrients, whereas those at the other end of the ray, i.e., at 225°, (e.g. *Stylaria lacustris*) show the opposite pattern. In this way, taxa can be grouped on the basis of similarity of response pattern.

The statistical significance of the effects of chlorpyrifos on the invertebrate community is demonstrated by the permutation tests per sampling date (comparison CPF-NUT versus NUT; Table 3). Using the F-type criterion and the Bray-Curtis index quotient, significant effects were found for the whole period after week 9, whereas Stander's index quotient indicated effects for only a few sampling dates (Table 3). The P-values obtained with the Bray–Curtis index quotient were, however, not always robust (weeks 10 and 12; Table 3).

The effects of the nutrient additions were significant from week 14 to week 20, as assessed on the basis of the similarity indices (NUT versus CON; Table 3). Using the F-type criterion, a few sampling dates showed significant effects (weeks 6, 16 and 20).

All tests indicate significant effects of the CPF-NUT treatment compared with the control (Table 3). Only for week 10 did the tests produce different results: the effect was judged to be significant using the F-type criterion only.

Phytoplankton data set

Both the Bray–Curtis and Stander's index showed minor effects of the first large nutrient addition on the phytoplankton community. After the second large nutrient addition, the effects were larger (weeks 10–14; Figure 7). There were no consistent differences between NUT and CPF-NUT.

As for the invertebrate data set, the overall variation among sampling dates was higher than among treatments (Table 2). Whereas the first and second PRC components were statistically significant, the third was not ($P > 0.05$). The percentage of variance explained by treatment was smaller in the phytoplankton data set than in the invertebrate data set. In the phytoplankton data set, the second PRC was about equally important as the first, as assessed on the basis of the percentage of variance accounted for. This means that the joint interpretation of the PRCs (Figures 8A and B) is even more important in the phytoplankton data set than it was in the invertebrate data set. Together, the two PRCs encompass 55% of the treatment variance, which is only slightly less than was the case with the invertebrate data set.

The first PRC indicates a deviation of the NUT treatment from the control for week 4. After the second high nutrient treatment and the chlorpyrifos application in week 9. Both treatments differed more consistently from the control, with a larger deviation for the CPF-NUT compared to the NUT treatment (Figure 8A). The second PRC shows opposite responses to both treatments after week 9. The differences between NUT and CPF-NUT shown by the first two PRCs are reduced when the second PRC is added to the first, and are enhanced when the second PRC is subtracted from the first, as shown in Figure 9 in the right upper and lower quadrants at 45° and 135°, respectively.

Figure 10 shows the observed dynamics of the three species that were in extreme positions in terms of their weight with the first PRC, namely *Volvox* sp., *Chroomonas* sp. and Algae sp. The lay-out of Figure 10 is the same as that of Figure 4. We now compare the response curves of these species with the

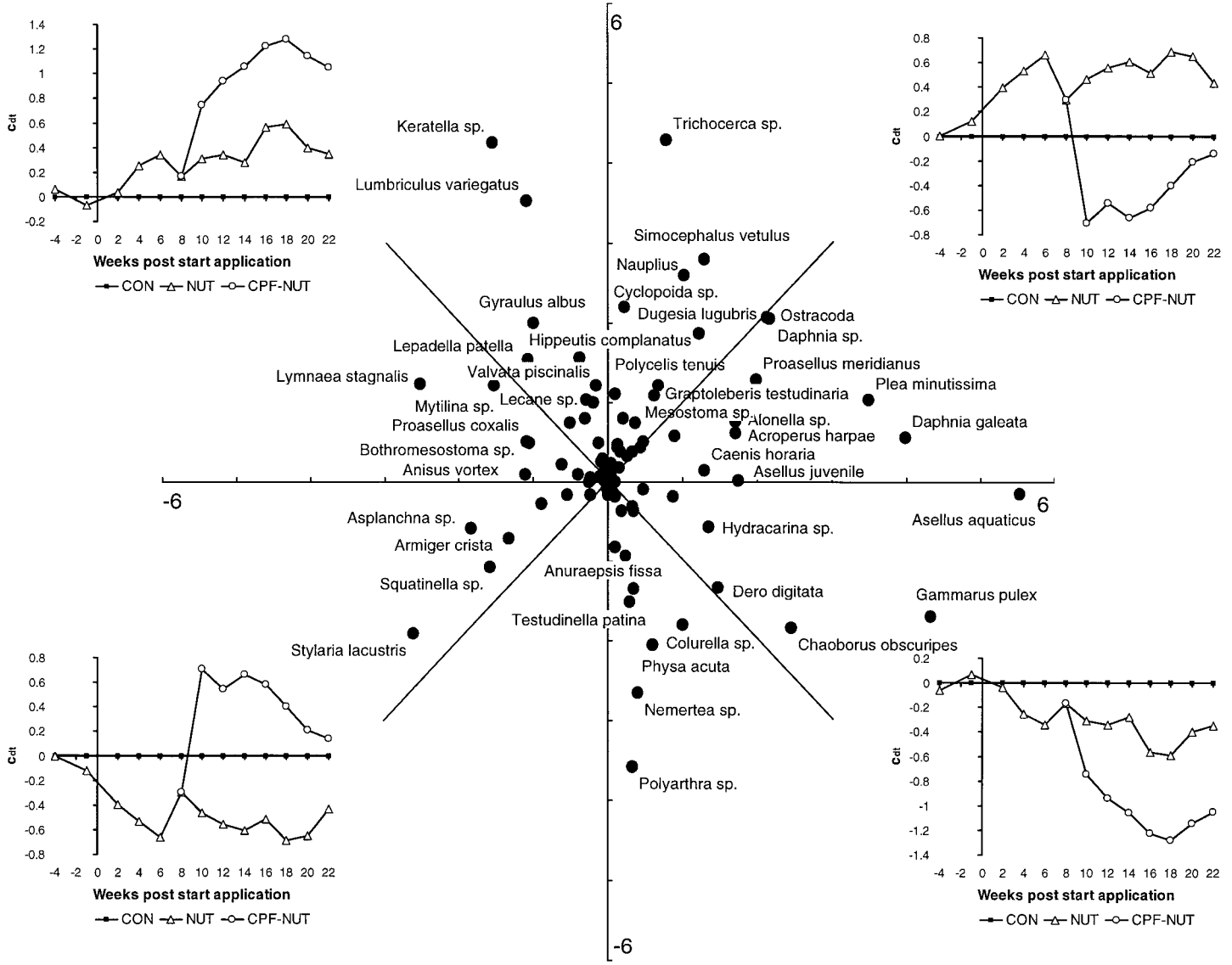

Figure 6. Two-dimensional plot of the weights of the invertebrate species on the first and second PRC, as given in Figure 3. The four diagrams in the corners apply to species that have equal weights on the two PRC's (except for the sign).

Table 3. Results of Monte Carlo permutation tests per sampling date; '−': calculation not applicable, F = $p < 0.05$ using the F-type criterion; $B = p < 0.05$ using the Bray–Curtis index quotient; S = $p < 0.05$ using Stander's index quotient; blanc = $P > 0.05$; * = when permutation procedure is repeated, sometimes a $p < 0.05$ is reported, sometimes $p > 0.05$. For abbreviations see Figure 1

Week	Invertebrates									Phytoplankton								
	NUT vs CON			CPF-NUT vs CON			CPF-NUT vs NUT			NUT vs CON			CPF-NUT vs CON			CPF-NUT vs NUT		
−4				–			–						–			–		
−1				–			–						–			–		
2				–			–						–			–		
4				–			–			F			–			–		
6	F		S	–			–						–			–		
8				–			–			F			–			–		
10				F			F	B*		F				B	S	F		
12				F	B	S*	F	B*		F	B	S	F	B	S*			
14		B	S	F	B	S	F	B	S	F	B	S		B	S*	F	B	S
16	F	B	S	F	B	S	F	B	S							F	B	S
18		B	S	F	B	S	F	B										
20	F	B	S	F	B	S	F	B										
22				F	B	S	F	B	S									

fitted curves that can be inferred from Figures 8 and 9. *Volvox* sp. increased in abundance in the NUT and CPF-NUT treatments, with a greater increase just after week 9 for the CPF-NUT treatment (Figures 10A and 10B). This response pattern is predicted well by Figure 9, where *Volvox* sp. had a high negative weight with the first PRC and a low weight with the second one. *Chroomonas* sp. had a high positive weight with the first PRC and near zero weight with the second (Figure 9), thus decreasing in abundance in both treatments, with a much larger decrease in the CPF-NUT treatment (Figures 10C and D). Algae sp. had high weights with both PRC diagrams. The predicted response curves of Algae sp. as indicated in Figure 9 at 45° are very much like the actual response curves (Figure 10F).

The effects of the prolonged nutrient additions and the single chlorpyrifos addition on the phytoplankton communities were both judged to be statistically significant during short sequences of sampling dates (Table 3). In this data set, the Bray–Curtis index and Stander's index quotients yielded similar test results, which sometimes differed from those based on the F-type criterion of RDA.

Discussion

Comparison of similarity analysis and PRC

Similarity analysis expresses the systematic differences among communities that are subjected to different treatments, in a single number. This number, defined as the quotient of the mean between-treatment and the mean within-treatment similarities, is a statistical measure of the total size of the treatment effects on the species. Similarity analysis results in a graph of the development of the size of the treatment effects with time, and is complemented by permutation tests that assess the statistical significance of the treatment effects. Although different in detail, the results obtained using the Bray–Curtis index and those obtained with Stander's index showed the same global pattern, namely, weak treatment effects before week 9 and strong treatment effects in the period after week 9 (Figures 2 and 7). This demonstrates the effect of the chlorpyrifos treatment and prolonged nutrient additions on both the invertebrate and the phytoplankton communities. The F-type statistic of RDA showed the same global pattern of effect sizes (Table 3).

In PRC, the principal components of the treatment effects on the species are each plotted against time. The first PRC diagram may sometimes resemble the graph from similarity analysis as, for example, in the phytoplankton data set. An important advantage is that PRC allows a direct interpretation down to the species

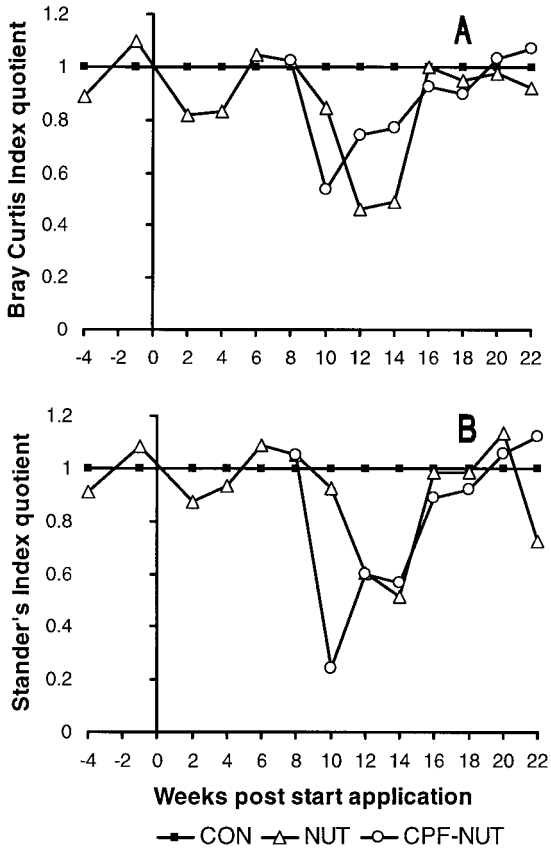

Figure 7. Size of the effects of nutrient additions (NUT) and extra the chlorpyrifos addition (CPF-NUT) in the phytoplankton data set as defined by the Bray–Curtis (A) and Stander's (B) Index quotient. For explanation see text and Figure 2.

level. In particular, PRC shows whether a particular species increased or decreased in the treated cosms relative to its abundance in the control cosms. In other words, PRC gives a signed difference (increase *vs.* decrease) whereas the similarity analysis provides an unsigned difference only, namely the total treatment size.

In both example data sets, PRC successfully reduced the treatment effects of the many species to two dimensions. The fact that more than one PRC was needed, demonstrates that the species reacted in qualitatively different ways to the treatments. When the PRCs are combined and displayed in a two-dimensional diagram of species weights, such as Figure 6, an overview is obtained of the main types of response curves that occur, and also of the species that react according to each particular type of response curve. For example, Figure 6 shows that all possible combinations of increase/decrease due to the nutrient additions (NUT) and increase/decrease due to both nutrient and chlorpyrifos additions (CPF-NUT) actually occurred. In addition, there are species that did not respond to nutrient additions but did decrease strongly when chlorpyrifos was added as well. These are the species that lie along the positive first axis. *Asellus aquaticus* shows this type of response most prominently.

In summary, both the PRC method and the similarity analysis provide endpoints for an evaluation of effects of toxicants at the community level. Both methods also allow the effects to be tested statistically. The main difference, however, is that the PRC method also allows the treatment effects to be evaluated directly down to the taxon level.

Comparison of effects as reported in Cuppen et al. (1995) versus PRC outcomes

The results reported by Cuppen et al. (1995) are summarized in Table 1. A qualitative interpretation of the PRC results of the invertebrates is also given, in the columns labelled 'PRC'. At first glance, no differences in interpretation between the reported effects on the invertebrates are apparent. This means that the PRC method succeeded in comprising the most important treatment effects of the invertebrate data set into two diagrams.

The increase in the chlorophyll-a content of the phytoplankton can of course not be compared with the abundance counts used in the example data set. For PRC, the entries in Table 1 are therefore empty as far as the chlorophyll-a content or biomass of the primary producers are concerned. In Van Donk et al. (1995), the only taxon reported to show a treatment effect was *Volvox* sp. (Van Donk et al., 1995). Although the chlorophyll-a content of the phytoplankton increased due to the nutrient additions and the chlorpyrifos application, PRC analyses indicated a decrease in abundance for most taxa that showed treatment-related effects (Figure 8A; e.g. *Chroomonas* sp.: Figure 10D). Only for *Volvox* sp. is an increase indicated (Table 1). Because of its size, this taxon evidently accounted for a considerable fraction of the chlorophyll-a content of the phytoplankton.

In conclusion, PRC not only revealed the same responses reported in Cuppen et al. (1995), but it also provided an overview of the effects at the community level.

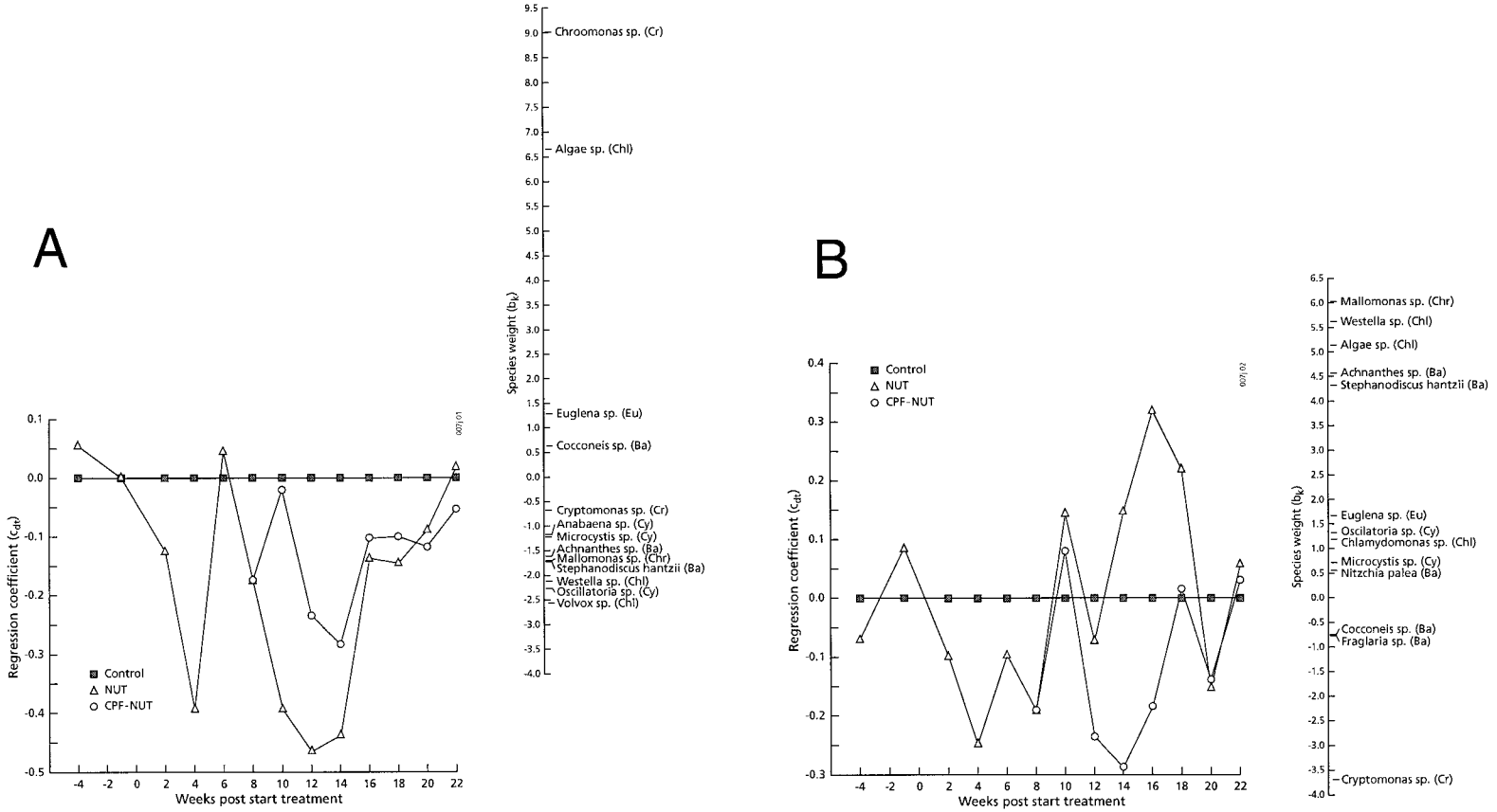

Figure 8. Principal Response Curves for the phytoplankton data set, indicating the effects of nutrient additions (NUT) and the extra chlorpyrifos addition (CPF-NUT). The first PRC is given in A; the second PRC in B. See Table 2, for explained and displayed variance, and Figure 3 for explanation. Only the species with a weight of 0.5 or higher or −0.5 or lower with the diagrams are displayed. Explanation of the codes: Ba = Bacillariophyceae; Chl = Chlorophyceae; Chr = Chrysophyceae; Cr = Cryptophyceae; Cy = Cyanophyta; Eu = Euglenophyta.

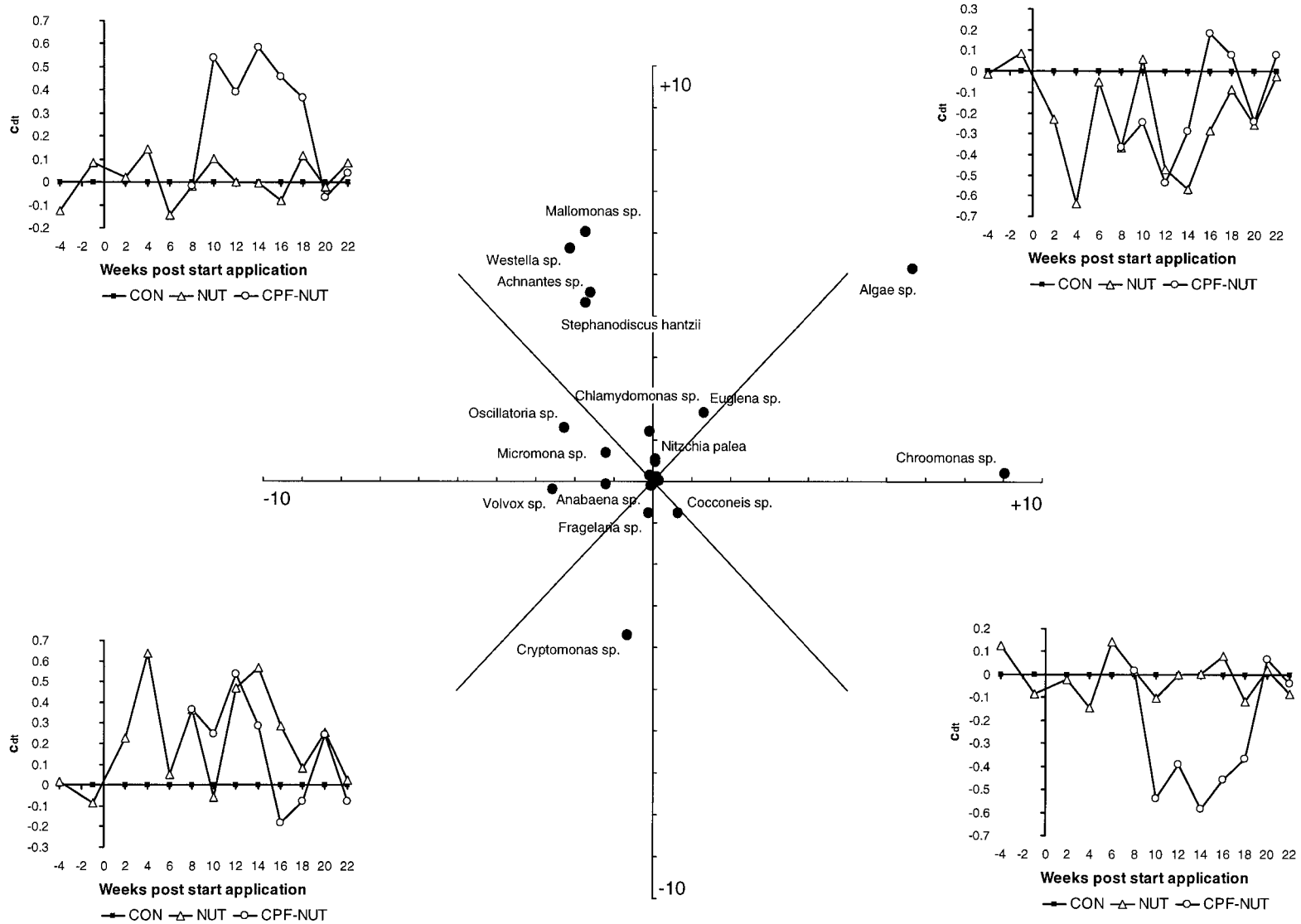

Figure 9. Two-dimensional plot of the weights of the phytoplankton species on the first and second PRC, as given in Figure 8. The four diagrams in the corners apply to species that have equal weights on the two PRC's (except for the sign).

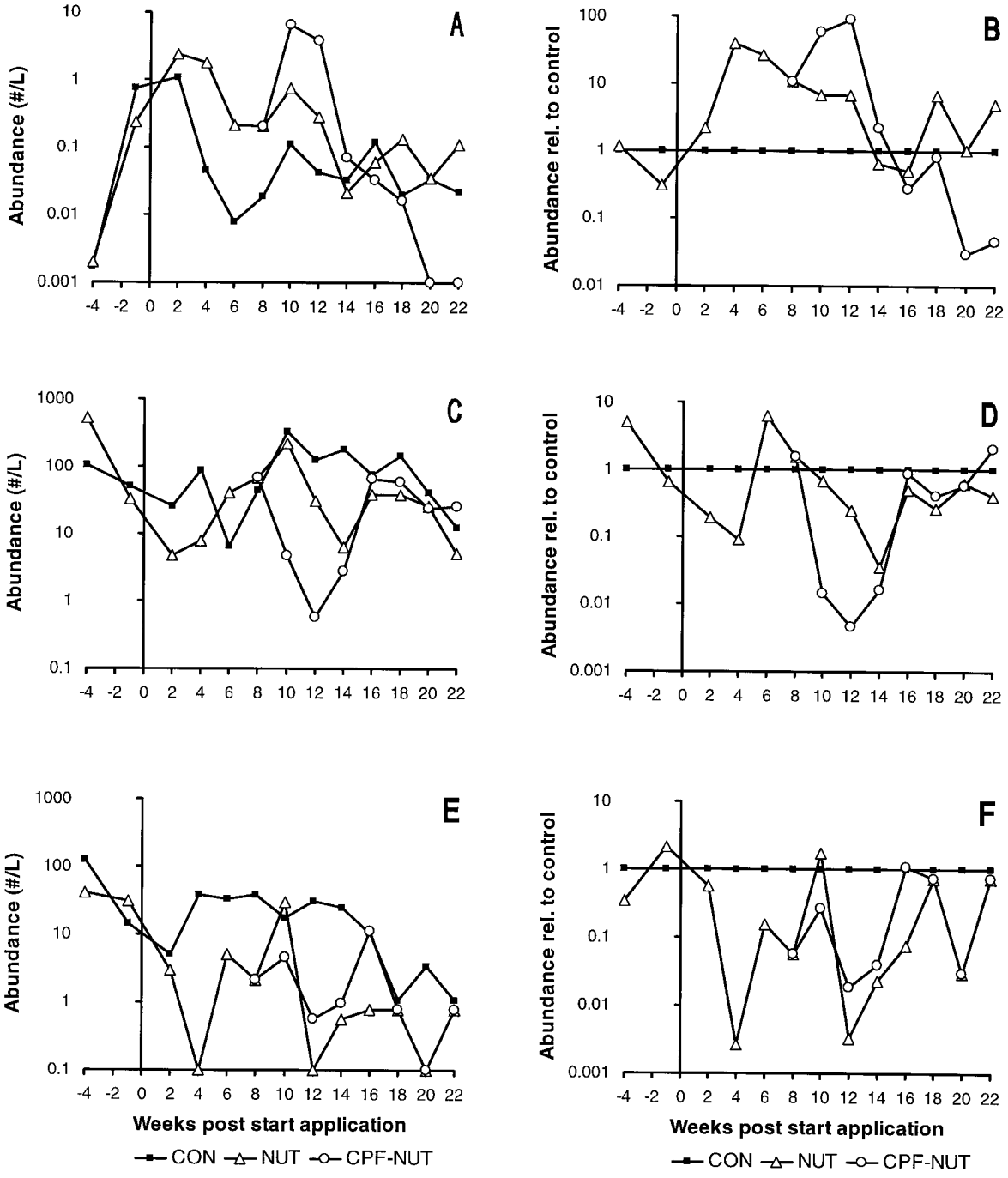

Figure 10. Dynamics in numbers of three phytoplankton taxa. A, C and E show the geometric means of the counted numbers per treatment, of *Volvox* sp., *Chroomonas* sp. and Algae sp. respectively. B, D and F shows their abundance relative to control, respectively.

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References

Brock TCM, Roijackers RMM, Rollon R, Bransen F and Van der Heyden L (1995) Effects of nutrient loading and insecticide application on the ecology of *Elodea*–dominated freshwater microcosms. II. Responses of macrophytes, periphyton and macroinvertebrate grazers. Arch Hydrobiol 134: 53–74

Clarke KR (1993) Non-parametric multivariate analyses of changes in community structure. Aust J Ecol 18: 117–143

Clarke KR and Warwick RM (1994) Similarity-based testing for community pattern: the 2-way lay-out with no replication. Mar Biol 118: 167–176

Cuppen JGM, Gylstra R, Van Beusenkom S, Budde BJ and Brock TCM (1995) Effects of nutrient loading and insecticide application on the ecology of *Elodea* – dominated freshwater microcosms. II. Responses of macroinvertebrate detritivores, breakdown of plant litter, and final conclusions. Arch Hydrobiol 134: 157–177

Heimbach, F and Ratte HT (1997) Use of a similarity index to compare the species composition of aquatic communities in order to investigate ecosystem recovery. Abstract Seventh Annual Meeting of SETAC-Europe, Amsterdam, the Netherlands.

Hommen U, Düllmer U, and Veith D (1994) A computer program to evaluate plankton data from freshwater field tests. In: Hill IA, Heimbach F, Leeuwangh P and Matthiesen P (eds), Freshwater field tests for hazard assessment of chemicals. Lewis Publishers. Boca Raton, FL, (pp. 503–513)

Jongman RGH, Ter Braak CJF and Van Tongeren OFR (1995) Data Analysis in Community and Landscape Ecology, Cambridge University Press, Cambridge, UK

Ter Braak CJF (1988) CANOCO – a FORTRAN program for canonical community ordination by [partial] [detrended] [canonical] correspondence analysis, principal component analysis and redundancy analysis (version 2.1). No. LWA-88-02. Technical Report. DLO-Agricultural Mathematics Group, Wageningen, The Netherlands

Ter Braak CJF (1990) Update Notes: CANOCO version 3.10. Technical Report. DLO-Agricultural Mathematics Group, Wageningen, The Netherlands.

Ter Braak CJF and Looman CWN (1994) Biplots in reduced-rank regression. Biom. J 36: 983–1003

Van den Brink PJ, Van Donk E, Gylstra R, Crum SJH, and Brock TCM (1995) Effects of chronic low concentrations of the pesticides chlorpyrifos and atrazine in indoor freshwater microcosms. Chemosphere 31: 3181–3200

Van den Brink PJ, Van Wijngaarden RPA, Lucassen WGH, Brock TCM and Leeuwangh P (1996) Effects of the insecticide Dursban® 4E (a.i. chlorpyrifos) in outdoor experimental ditches. II. Invertebrate community Responses. Environ Toxicol Chem 15: 1143–1153

Van den Brink PJ and Ter Braak CJF (1997) Ordination of responses to toxic stress in experimental ecosystems. Toxicol Ecotoxicol News 4: 174–178

Van den Brink PJ and Ter Braak CJF (in press) Principal Response Curves: Analysis of Time-dependent Multivariate Responses of a Biological Community to Stress. Acc Environ Toxicol Chem

Van Donk E, Prins H, Voogd HM, Crum SJH and Brock TCM (1995) Effects of nutrient loading and insecticide application on the ecology of *Elodea* – dominated freshwater microcosms. I. Responses of plankton and zooplanktivorous insects. Arch Hydrobiol 133: 417–439

Van Wijngaarden RPA, Van den Brink PJ, Oude Voshaar JH and Leeuwangh P (1995) Ordination techniques for analysing response of biological communities to toxic stress in experimental ecosystems. Ecotoxicology 4: 61–77

Verdonschot PFM and Ter Braak CJF (1994) An experimental manipulation of oligochaete communities in mesocosms treated with chlorpyrifos or nutrient additions: multivariate analyses with monte carlo permutation tests. Hydrobiologia 287: 251–266

Washington HG (1984) Diversity, biotic and similarity indices: a review with special relevance to aquatic ecosystems. Water Res 18: 653–694