

# Impact of mussel seed fishery on subtidal macrozoobenthos in the western Wadden Sea

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## Contents

Contents.....	3
Summary .....	4
1. Materials and Methods.....	6
a. Experimental design.....	6
b. Benthic Sampling.....	6
c. Data analyses .....	9
Univariate indices .....	9
Species composition.....	9
Power analysis .....	11
2. Results.....	12
a. Univariate indices .....	12
Short-term effects .....	12
Mid-term effects .....	16
b. Similarity.....	19
Short-terms effects.....	19
Mid-term effects .....	21
c. Principal Response Curves.....	23
A detailed example: Molenrak west .....	23
Overview .....	24
d. Power .....	25
3. Discussion .....	30
4. Conclusion .....	32
5. References.....	33
Quality Assurance .....	36
Justification.....	37
Appendix A. Rapport van de Audit commissie en reactie van de Probus auteurs.....	38

## Summary

Within the framework of PRODUS, the effect of mussel fishery on the macrofauna species sampled with a box-corer was investigated following a so-called split-plot design. Within areas where mussel seed fishery was allowed, 40 plots/locations were chosen within which one part was open for commercial fisheries and one part was closed of fisheries. Box-corers were taken in 21 of these locations, sampled one to several times.

The present study confirms the existence of small scale differences in macrofauna species composition: even within a subtidal mussel bed, within a few hundreds of meters. The present study confirms large temporal variation in species composition, independently of any human impact.

The study shows short-term effects of fishery activities: a change in total density and in species composition (e.g. associated species). The study also shows mid-term effects on species diversity (number of species and Shannon-Wiener index). The effect is different depending on the fishery season. Overall, any fishery effects seem to be less important in determining species composition than external factors controlling mortality and recruitment.

## Introduction

The research project on sustainable shellfish culture (PRODUS) is being implemented on behalf of the Ministry of Agriculture, Nature and Food Quality (LNV) and the shellfish sector. This assignment resulted from the new shellfish policy and the innovation agenda of the shellfish sector.

The 'PRODUS' research project is focussed on factors that determine the efficient exploitation of mussels in bottom culture, and on the impact of mussel culture, including mussel seed fishery, on the ecosystem. The standing stock dynamics and the yield of bottom culture of mussels is being studied in relation to environmental conditions, the role of predation, and the culture strategies of the farmers. A comparison is made of species composition and abundance of wild mussel seed beds, culture plots and other habitats.

It also investigates the effects of mussel seed fishing on mussel stocks and the nature values in sublittoral areas. For more information on the research questions and the research approach, we refer to Smaal et al (2013). In this report the effect of mussel seed fishery on the benthic fauna (as sampled by a box-corer) is analysed.

## **1. Materials and Methods**

### **a. Experimental design**

The experimental set up of the project is a so-called split-plot design (Ens et al., 2007). Within areas where mussel seed fishery is allowed (so-called whole plots), subplots of 400\*200m are chosen within which one part was open for commercial fisheries (further referred as open or Impact part) and one part was closed to fisheries further referred as closed or Control part). Plots are further referred to as locations.

At the start of the study, it was decided that 40 of these experimental plots were needed in order to have sufficient power (set at 80%, with a probability of 5% to reject the null hypothesis of no changes when in fact there are) to detect differences of 10% (of the range of observed values at the start of the experiment) (Ens et al., 2007). The locations were not defined a-priori to avoid the high risk of no spat fall during the study period (Ens et al., 2007). At the start of the study (2006), there was a very poor spat fall. It was therefore decided, to define ten locations on beds of year class 2005 in autumn 2006. Most of these had already been fished before the start of the study. Two of them were already abandoned in 2007 because of fisheries in the closed part of the location (Van Stralen et al., 2013). The other 32 locations could be defined in not disturbed mussel seed beds in the subsequent years (up to 2009). All fishing boats are equipped with a black box which permanently monitors the location of the boats. Based on these data the effective area fished and the fishery intensity was estimated (Van Stralen et al., 2013).

The macrobenthic infauna (as sampled with box-corer; see next chapter) was eventually studied in 21 locations (Figure 1). Some of them were sampled once, others several times (Table 1). Six of them were situated on mussel beds of year class 2005 (Afsluitdijk west, Breesem, Molenrak west, Stompe, Vlieter, ZuidWest). At some locations new spat fall events occurred in the years following the time the locations were marked out. In some, therefore, commercial fisheries took place more than once: three were fished twice (Breesem, Zuidwest, Stompe) and one was fished five times (Visjagersgaatje) (Van Stralen et al., 2013).

### **b. Benthic Sampling**

In 2006, 12 samples were taken within the closed (Control) and open (Impact) parts of each location, in 2007 20 cores and in later years again 12 samples, resulting in a total of 1896 samples. All samples were taken within the central 1 ha of both the open and closed parts of each location. The number of replicate samples per location was adapted after a power analysis in 2008 on the basis of the PRODUS data available so far (Meesters and Fey-Hofstede, 2009). They showed that sufficient power for the biodiversity parameters could be achieved by 12 replicates per plot.

Samples were taken with a box-corer. From each box-corer, two cylindrical subsamples were taken (total area: 0.1664 m<sup>2</sup>). Both subsamples were sieved together over a 1 mm sieve and samples were fixed in 10 % formalin. In the laboratory the organisms were sorted, identified to species level if possible and counted. The latter has been done by different laboratories. Therefore, identifications were checked for differences as different laboratories used different identification keys – moreover, some keys were updated during the study period - or have different opinions on the taxonomy of some species, and not everyone differentiated all species (some laboratories identified taxa as e.g. sea anemones to the species level while others did not). Therefore, some species were lumped to genus or a higher taxonomic level. The original list of 243 taxa was, thus, reduced to 185 taxa.

Table 1. Sampling locations and times of sampling. Only locations where samples were taken with box-corers are shown. Locations excluded from the analyses because of fishing disturbance are not shown.

location	T_0	T_1	T_2	T_3	T_4	T_5
Afsluitdijk West	25-sep-06	2-jul-07	19-jun-09	20-sep-10		
Breesem	25-sep-06	25-nov-06	14-jan-09	21-sep-09	24-nov-09	
Molenrak Oost	25-sep-06					
Molenrak West	25-sep-06	2-jul-07	19-jun-09	29-sep-10		
Omdraai	25-sep-06					
Txstroom-west	25-sep-06	25-nov-06				
Visjagersgaatje	25-sep-06	2-jul-07	19-jun-09	21-sep-09	24-nov-09	27-sep-10
Vlieter	25-sep-06	25-nov-06	14-jan-09	21-sep-10		
ZuidWest / Lutjewaard	25-sep-06	25-nov-06	14-jan-09	27-sep-10		
Stompe	17-apr-07	2-jul-07	19-jun-09	21-sep-10		
Gat van Stompe	27-aug-07	17-dec-07	24-nov-09	21-sep-10		
Stompe zuid	27-aug-07	17-dec-07	21-sep-09	24-nov-09	22-sep-10	
Afsluitdijk - AD10	14-apr-09	19-jun-09	20-sep-10			
Breezanddijk	14-apr-09	19-jun-09	22-sep-10			
Doovebalg DB23	14-apr-09	19-jun-09	23-sep-10			
Kornwerd / Boontjes	14-apr-09	19-jun-09	30-sep-10			
Zuidoostrak	14-apr-09	19-jun-09	30-sep-10			
Breesem W	21-sep-09	24-nov-09	24-sep-10			
Breesem Z	21-sep-09	24-nov-09	23-sep-10			
Griend / Blauwe Slenk noord	21-sep-09	24-nov-09	29-sep-10			
Inschot	21-sep-09	24-nov-09	30-sep-10			
Pollendam / Blauwe Slenk	21-sep-09	24-nov-09	29-sep-10			
Westkom	21-sep-09	24-nov-09	28-sep-10			
WestMeep	21-sep-09	24-nov-09	28-sep-10			

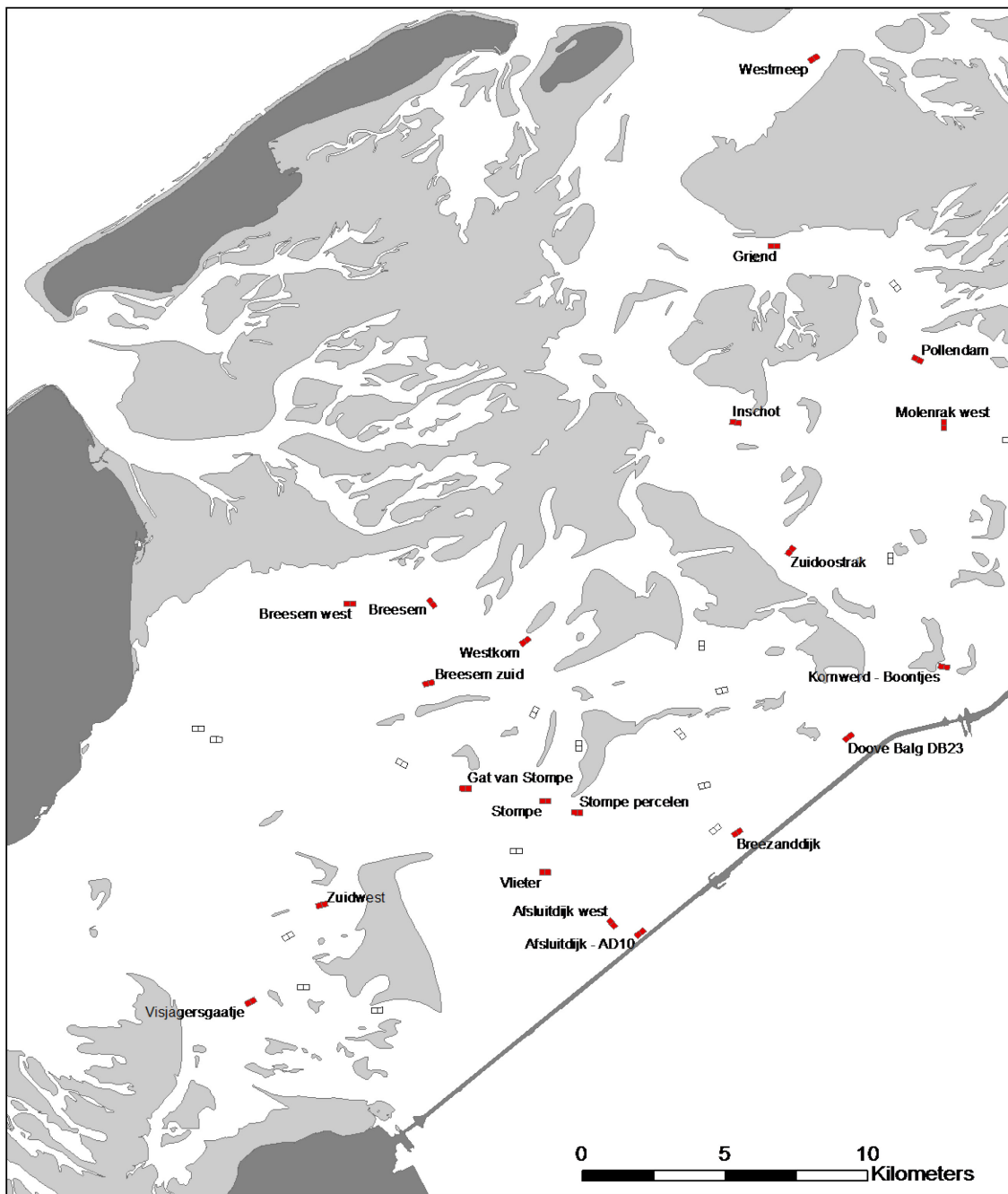


Figure 1. Map of the western Dutch Wadden Sea with locations used in the study of this project. The locations involved in this report (Table 1) are coloured red, and the location name is indicated. The other locations, not sampled by box-corer, are white coloured.



## c. Data analyses

### *Univariate indices*

In studies on temporal changes of the benthic community, a huge variety of indices is used (see e.g. Quintino et al., 2006). In this study, the following univariate indices were chosen: total density, number of species (S), Shannon-Wiener ( $H'$ ,  $\log_e$ ) and Pielou's evenness ( $J = H' / \log(S)$ ). Diversity indices S and  $H'$  were calculated using the library *vegan* (Oksanen et al., 2011) of R (R Development Core Team, 2011). Because mussels have been fished in the open site, an effect on the total density and on the relative abundance of all species will be seen, even if no other species than mussels are impacted by fishery activities. Therefore, all calculations were done excluding mussels. To analyse the effects of fisheries on these community indices, linear mixed effect models were used (Pinheiro, 2008). Box-plots of data for each location, histograms of the data and QQ-plots were used to check the normality assumption. If necessary, a data transformation was used that achieved the best normalization for the data analysed. If necessary different variance structures were used to achieve heterogeneity within groups.

The analyses on the univariate indices were done for short-term effects of fisheries, i.e. shortly after mussel seed fisheries (6 times spring fishery, 15 times autumn fishery), and mid-term effects, i.e. 1 to 1.5 years after fisheries (5 times spring fishery, 6 times autumn fishery). T0 – sampling was always in the same season as T1, except for Molenrak West, Afsluitdijk West and Visjagersgaatje (Table 1).

Analysis of long-term effects on these indices were not done because of the low number of locations sampled for a longer period.

The full data model included tree main effects, a three way interaction and three two way interactions. Main effects were time (before and after fishing activities), season (spring or autumn) and treatment (closed/Control or open/Impact). Since we were not so much interested in each specific location, but in the overall picture of fishing, sampling location was modelled as a random factor. Different variance structures were tested and applied if significantly improving the model fit. Nested models were compared using the Aikake Information Criteria (AIC) and tested using the F-test on the likelihood ratio which was calculated using restricted log likelihood (REML) for models with a different variance structure and maximum log likelihood (ML) for models with different fixed effects (see e.g. protocols in Zuur et al., 2009).

Model validation was done using validation graphs: residuals versus fitted values to check homogeneity, and box-plots of the residuals versus each explanatory variable to check independence.

All calculations were performed using R (R Development Core Team, 2011) and the package *nlme* (Pinheiro et al., 2011).

### *Species composition*

#### Similarity

The Bray-Curtis dissimilarity index was used to quantify the distance between the species composition of Control and Impact parts of each location. As for the univariate indices, calculations were done on species density averages per treatment per location. Density data were fourth root transformed. The effect was tested in two ways. First, a similars model was used as for the above mentioned univariate indices. However, because dissimilarity between open and closed areas is calculated, treatment could not be an explanatory variable and, thus the interaction term treatment\*time could not be tested. A model was set up with time and season as explanatory variables and the same procedure was followed for final model selection.

Secondly, a model was set up with only time as the main effect and location as random factor.

Differences in similarity between control and impact part of each location before and after fisheries were tested using simulation by informal Bayesian inference (Gelman and Hill, 2007). A linear mixed model

with similarity as response variable, time as explanatory variable and location as random factor was constructed and Monte Carlo Markov Chain simulation run (10000 simulations) to estimate model parameters and confidence intervals (McKechnie et al., 2009). The null model is: the difference in similarity between Control and Impact area is not different before and after fishing.

Changes in species composition were visualised using non-metric dimensional scaling (nMDS), an ordination method often used by ecologists. It may be preferred when the user wants to represent as much as possible of the distance relationships among objects in a few dimensions (Legendre and Birks, 2012). We used Bray-Curtis as dissimilarity index and set the number of dimensions to two.

All calculations were performed using R (R Development Core Team, 2011) and the packages *lme4* (Bates et al., 2011), *nlme* (Pinheiro et al., 2011), *arm* (Gelman et al., 2012) and *vegan* (Oksanen et al., 2011).

### Principal Response Curves

Principal response curves (PRC) were used to analyse the time and treatment-dependent multivariate response of the benthic infauna. PRC are a special case of RDA (partial Redundancy Analysis for multivariate responses in repeated observation design (a single factor for *treatment* and a single factor for *time* points in repeated observations). RDA is the canonical form of principal components analysis (PCA), and is based on a linear response model between species and explanatory variables (as e.g. expected in short segments of ecological gradients) (see e.g. Jongman et al., 1987; Legendre and Birks, 2012; ter Braak and Prentice, 1988). The advantage of PRC over other multivariate methods is that it is able to focus on the part of the variance explained by a treatment. Instead of presenting data in diagrams that are often too cluttered to allow easy interpretation of the changes in treatment effects over time, the principal components of the treatment effects are plotted against time, expressed as deviations from the control treatment. Thus, PRC diagrams are much easier to interpret and visualise much clearer than standard constrained ordination diagrams how treatment effects develop over a longer period. The vertical axis of a PRC diagram contrasts each treatment with the control, expressed as a canonical regression coefficient. Associated with each PRC is a set of species weights, shown on the right side of the PRC diagram. Species' weights denote the relative contributions to the PRC, i.e. the strength of the response of each species. Thus, PRC allows a direct interpretation down to the species level: species with high positive weights follow the same pattern as the PRC and are highly affected by the treatment, whereas taxa with negative values behave contrarily to the PRC. Statistical significance of each principal component can be assessed by Monte Carlo permutation testing (den Besten and van den Brink, 2005; Lepš and Šmilauer, 2003; van den Brink and Ter Braak, 1998; Van den Brink and Ter Braak, 1999).

The PRC method was specially designed for the analysis of time series obtained from microcosms and mesocosms experiments (Moser et al., 2007; Pernin et al., 2006; Van den Brink and Ter Braak, 1999) but proved to be very successful in demonstrating treatment effects in field experiments (Cébron et al., 2011; den Besten and van den Brink, 2005; Devotto et al., 2008; Dively, 2005; Marriott et al., 2009; Tschöpe et al., 2011). PRC can also be used for the study of data that were not obtained experimentally but are the result of a biomonitoring programme. In that case an internal reference can be defined to display changes in time, or different sampling sites can be compared (den Besten and van den Brink, 2005; van den Brink et al., 2009). Only a few studies have used PRC to analyse such monitoring data, where it proved valuable (Bollmohr and Schulz, 2009; Bollmohr et al., 2011; Leonard et al., 2000; Neher et al., 2005; Okullo and Moe, 2011).

PRC is most useful to highlight site differences in time if the initial differences between sites are not very large, as could be expected in our case where the Control and Impact part of each site are situated side by side. Van den Brink et al (2009) do not recommend PRC for large-scale studies with large initial differences among sites. We therefore analysed the data for each location separately. The gradient in terms of the main effect of time is not restricted by linearity and can be of any complexity. Resulting

species weights might identify species that may be tolerant or sensitive to disturbance (brought about by fisheries, in our case), thus could be interpreted as the affinity of the taxon with the PRCs. We checked for communality in the species involved and compared the percentage of the total variance that can be attributed to time with that attributable to the treatment regime.

The principal response curves method can be used to show trends over time with an internal reference or an external reference. Here we used the t0-situation as internal reference to show separately the temporal changes in the Impact and the Control part of each site. Secondly, again for each site separately, the data of the Control box were used as external reference for an analysis of the changes in the Impact box.

The analysis was carried out using the R package *vegan* (Oksanen et al 2011; R Development Core Team 2011). The species density data were square root transformed. The significance of the first canonical axis (first PRC diagram) was tested by a Monte Carlo permutation of the treatments. The significance of each time-point is indicated by the p values generated from unrestricted Monte Carlo permutations of the first axis of separate RDA analyses for each time point.

#### *Power analysis*

The design of the study was set up to be able to find 10% differences (= effect size) with a power of 80% (type II error  $\beta = 0.20$ ) and a significance level set to 5% (type I error  $\alpha = 0.05$ ). A priori estimates were done for 3 biodiversity indices: number of species, Simpson's index and the score on the first DCA axis (Ens et al., 2007).

We checked the realized effect size for total density, number of species, evenness and Shannon-Wiener index for the same type I and II errors ( $\alpha = 0.05$ ,  $\beta = 0.20$ ), given the realized standard deviations. The standard deviation of these community indices was calculated using the location averaged species abundances at T0. Separate calculations were done for short-term and mid-term effects as the number of locations differs (21 for short-term, 11 for mid-term; see further) .

## 2. Results

### a. Univariate indices

The boxplots in *Figure 2* show that there is a temporal variation in the chosen univariate community indices. In many cases temporal patterns were similar for Control and Impact areas. Total density, for instance, decreases in the first year after spat fall between spring and autumn, irrespective of the treatment (Closed/Open) or the season of fisheries (spring/autumn). There are differences as well. Evenness and Shannon-Wiener e.g., increases in the first year after spat fall between spring and autumn in the closed area after spring fisheries only. Differences at the end of the time series are likely due to the low number of locations.

#### *Short-term effects*

For the analysis of short-term effects, data collected before (T\_0) and after fishery (spring/01 or autumn/00) were selected. Data of 2006 (fishery on half grown mussels) were not excluded. Three-way interactions, indicating an effect of fishing, were never significant.

Total density is influenced by sampling time (Before/After) and treatment (Control/Impact) but the effect is not the same (*Table 2*). There is a significant interaction between both terms: the difference between control and impact sites is different before and after fishing, indicating an effect of fishing. The interaction plots indeed show that after spring fishery, densities have increased more in the closed than in the open parts of the locations. In autumn, densities decreased more in open than in closed areas (*Figure 3*). The interaction is stronger in spring (a disordinal interaction as lines cross) than in autumn (ordinal interaction as lines do not cross). There is also a significant interaction between season and time indicating that the densities before and after fishing depend on the season.

The evenness is only influenced by season and treatment (*Table 3*). No interaction terms are significant. Nevertheless, the interaction plots suggest that on average, the number of species increased less in open than in closed areas after spring fishery, and decreased more in open than in closed areas after autumn fisheries (*Figure 3*).

There is no significant interaction between time and treatment on the number of species (*Table 4*) and the Shannon-Wiener index (*Table 4, 5*). But the values before and after fishing depend on the season. The interaction plots (*Figure 3*) suggest that the decrease of both indices is the same in open and closed areas after autumn fishery but they develop different after spring fishery.

Model validation graphs are given in *Figure 4*. There was no sign for overdispersion of the models (dispersion values always around 1).

*Table 2. Short-term effects: model coefficients for total density. Final model:  
lme((tot\_density+1)^.15~season+time+treatment+season:time+time:treatment, random=~1|location, weights=varIdent(form=~1|time\*season)*

	Value	Std.Error	DF	t-value	p-value
(Intercept)	3.819819	0.17658359	1126	21.631791	0.0000
Season (autumn)	-0.217369	0.13452277	1126	-1.615852	0.1064
Time (After)	0.478977	0.13984980	1126	3.424940	0.0006
Treatment (Open)	0.026300	0.05308107	1126	0.495464	0.6204
Season (autumn):Time (After)	-0.632638	0.14113681	1126	-4.482446	0.0000
Time (After):Treatment (Open)	-0.200646	0.07443952	1126	-2.695421	0.0071

**Table 3. Short-term effects: model coefficients for evenness (J). Final model:  $lme(\exp(J)\sim season+treatment, random=\sim 1|location, weights=varIdent(form=\sim 1|time*season))$**

	Value	Std.Error	DF	t-value	p-value
(Intercept)	1.7124468	0.05934829	1092	28.854188	0.0000
Season (autumn)	0.3188247	0.05280578	1092	6.037687	0.0000
Treatment (Closed)	-0.0625565	0.02029236	1092	-3.082762	0.0021

**Table 4. Short-term effects: model coefficients for number of species (S). Final model:  $lme(\sqrt{S}\sim season+time+season:time, random=\sim 1|location)$**

	Value	Std.Error	DF	t-value	p-value
(Intercept)	2.4705754	0.1751029	1128	14.109278	0.0000
Season (autumn)	0.7886706	0.1334253	1128	5.910951	0.0000
Time (After)	0.3244655	0.1013994	1128	3.199876	0.0014
Season (autumn):Time (After)	-0.7449155	0.1151034	1128	-6.471707	0.0000

**Table 5. Short-term effects: model coefficients for Shannon-Wiener (H'). Final model:  $lme(H'\sim season+time+treatment+season:time, random=\sim 1|location, weights=varIdent(form=\sim 1|season))$**

	Value	Std.Error	DF	t-value	p-value
(Intercept)	1.1141786	0.13988814	1127	7.964783	0.0000
Season (autumn)	1.0497926	0.11712161	1127	8.963270	0.0000
Time (After)	0.2143926	0.08448724	1127	2.537573	0.0113
Treatment (Open)	0.0855698	0.04043957	1127	2.115991	0.0346
Season (autumn):Time (After)	-0.5766195	0.09872180	1127	-5.840853	0.0000

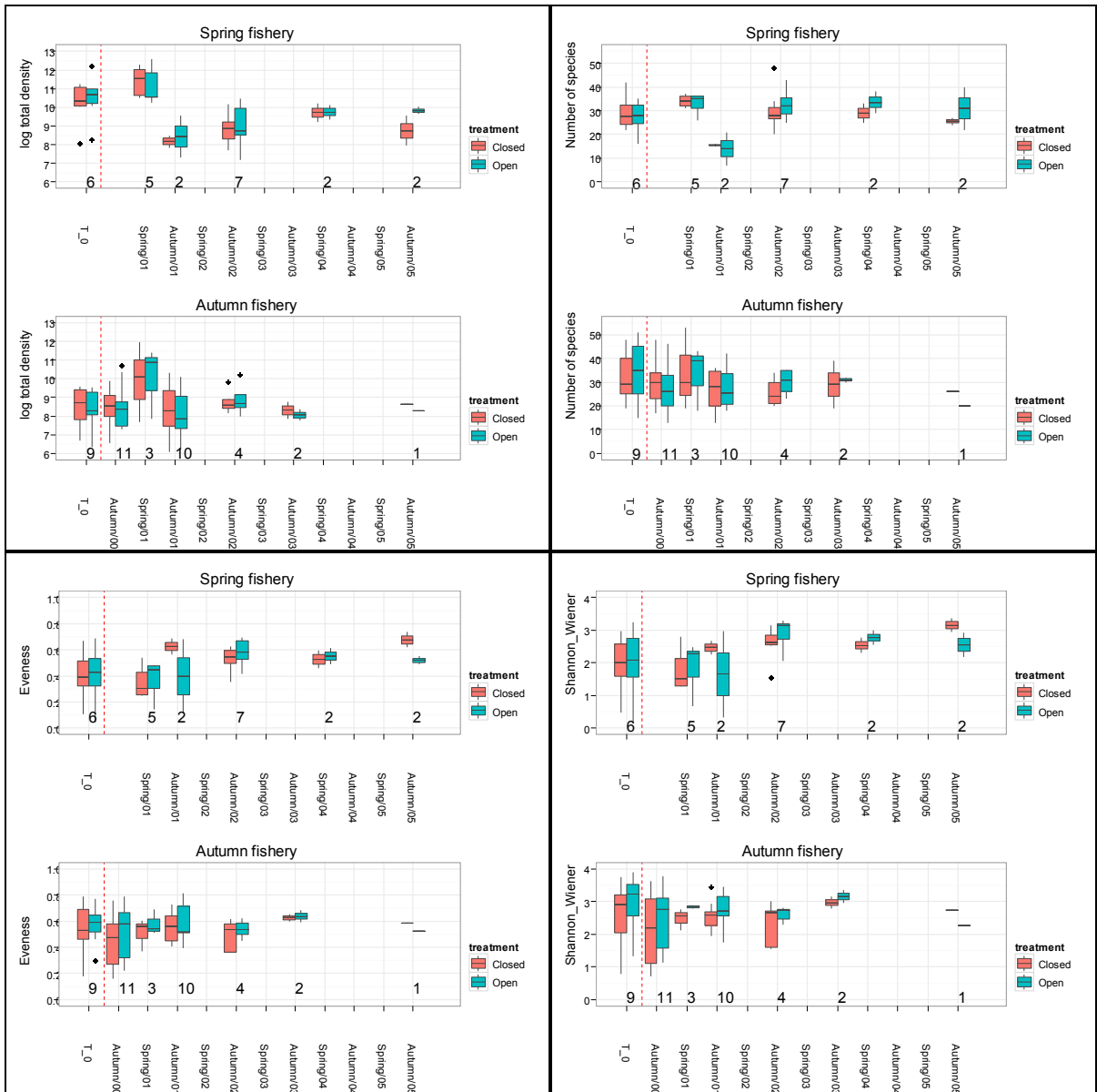


Figure 2. Boxplots of (location average) total density, number of species, evenness and Shannon-Wiener conditional on time, for locations where mussel seed fisheries took place the first time in spring or autumn, in areas open and closed for fisheries. T\_0 is the situation prior to the fishing activities (excluding samples taken in 2006 and 2007 on beds settled in 2005; see text for further explanation). The time axis is rescaled to the year of spat fall prior to the sampling date (e.g. spring/02 is referring to data in spring of the second year after the spat fall). The numbers below the boxes denote the number of locations sampled.

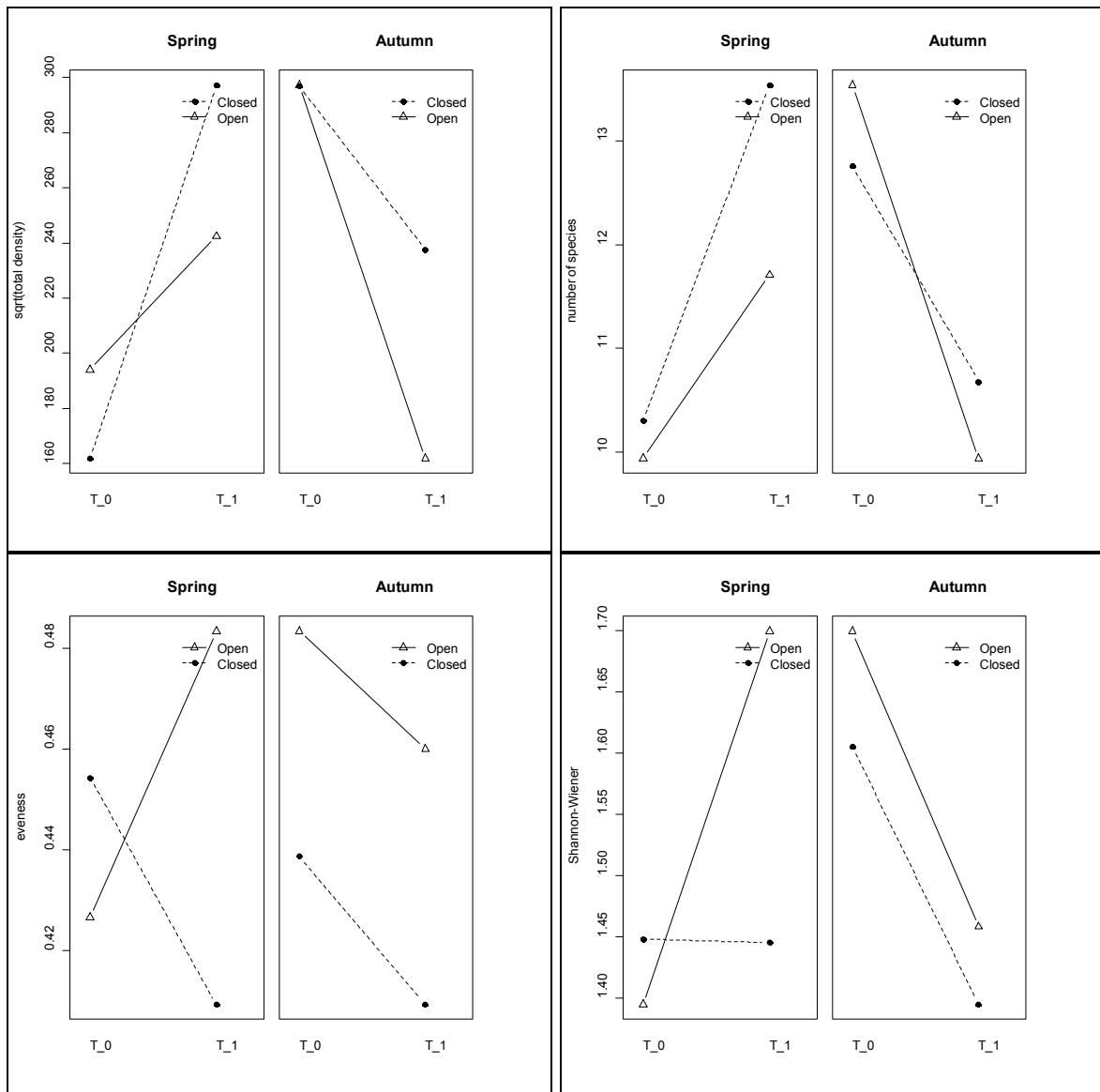


Figure 3. Interaction plot for short-term effects showing (on the y-axis) the mean of square root transformed total density (upper left), number of species (upper right), evenness (lower left) and Shannon-Wiener index (lower right) in spring and autumn. The x-axis gives the sampling time (t<sub>0</sub>, T<sub>1</sub>) and the lines the treatment type.

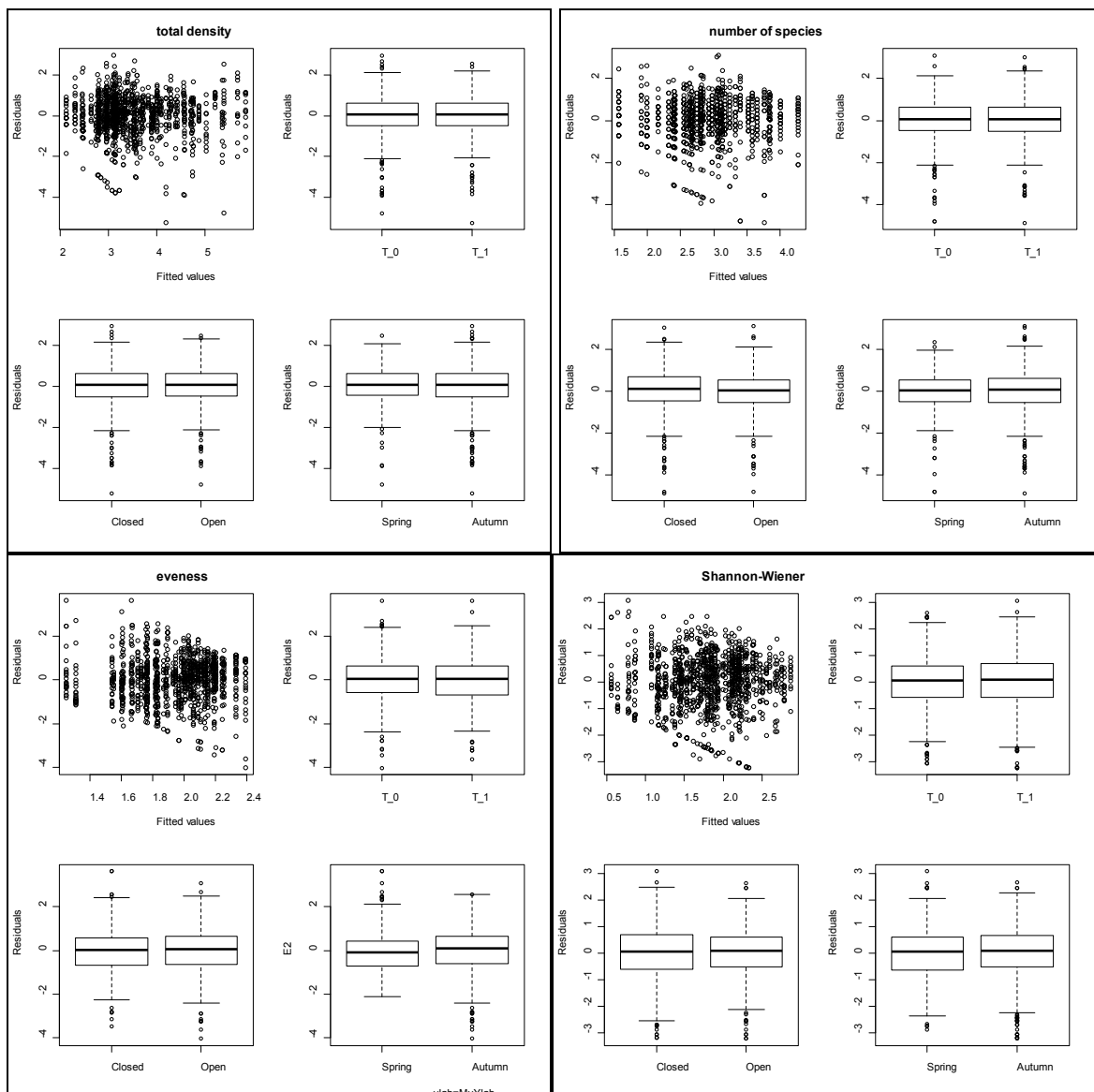


Figure 4. Model validation graphs for models on short-term effects in total density (upper left), number of species (upper right), evenness (lower left) and Shannon-Wiener index (lower right). Residuals are plotted versus the fitted values, sampling time, season, and treatment.

### Mid-term effects

At mid-term there is no significant interaction between time and treatment, thus no indication of fishery effects on total density (Table 6). The interaction plots indeed show the same trends in open and closed parts, both when fisheries took place in spring or autumn (Figure 5). For evenness too, there is no significant interaction between time and treatment (Table 7). The interaction plot suggest different trends after autumn (Figure 5). For the number of species and the Shannon-Wiener index, the three-way interaction (time: treatment:season) is significant (Table 8 and Table 9). Both in spring and autumn, the interaction between treatment and time (indicating possible fishery effects) are strong (disordinal) but depend on the season fishing took place. The interaction plots (Figure 5) show opposite trends in spring and autumn. The number of species, for instance, after spring fisheries increased in the open sites but decreased in the closed parts. After autumn fisheries, the number of species decreased both in the open



and closed parts, but more in the open parts. Shannon-Wiener index showed the same trends in open and closed parts, after spring (increase) and autumn (decrease) fisheries, but the changes were stronger in the open parts.

Model validation graphs are given in *Figure 6*. There was no sign for overdispersion of the models (dispersion values always around 1).

**Table 6. Mid-term effects: model coefficients for total density. Final model:  $\text{lme}(\text{tot\_density}^{0.25} \sim \text{time} + \text{treatment} + \text{season} + \text{time}:\text{season} + \text{treatment}:\text{season}, \text{random}=\sim 1|\text{location}, \text{weights}=\text{varIdent}(\text{form}=\sim 1|\text{season}*\text{Time}))$ .**

	Value	Std.Error	DF	t-value	p-value
(Intercept)	7.730013	0.8959457	536	8.627769	0.0000
Time (Before)	1.123072	0.2984812	536	3.762621	0.0002
Treatment (Open)	-0.383939	0.2746875	536	-1.397729	0.1628
Season (spring)	2.737416	1.3948135	10	1.962568	0.0781
Time (Before):Season (spring)	1.290995	0.6389239	536	2.020577	0.0438
Treatment (Open): Season (spring)	2.124881	0.5496475	536	3.865898	0.0001

**Table 7. Mid-term effects: model coefficients for evenness (J). Final model:  $\text{lme}(\exp(J) \sim \text{Time} + \text{season} + \text{time}:\text{season}, \text{random}=\sim 1|\text{location}, \text{weights}=\text{varIdent}(\text{form}=\sim 1|\text{time}))$ .**

	Value	Std.Error	DF	t-value	p-value
(Intercept)	1.5419500	0.08134407	523	18.955899	0.0000
Time (After)	0.2412979	0.04643726	523	5.196212	0.0000
Season (autumn)	0.3952492	0.10657521	10	3.708641	0.0041
Time (After): Season (autumn)	-0.1849276	0.06351386	523	-2.911610	0.0037

**Table 8. Mid-term effects: model coefficients for number of species (S). Final model:  $\text{lme}((S + 0.1)^{0.75} \sim \text{time} * \text{treatment} * \text{season}, \text{random}=\sim 1|\text{location}, \text{weights}=\text{varIdent}(\text{form}=\sim 1|\text{season}))$ .**

	Value	Std.Error	DF	t-value	p-value
(Intercept)	6.268876	0.7850769	534	7.985047	0.0000
Time (After)	-1.208268	0.3201871	534	-3.773631	0.0002
Treatment (Open)	-0.256489	0.3201871	534	-0.801060	0.4235
Season (autumn)	-0.105912	1.0349662	10	-0.102333	0.9205
Time (After):Treatment (Open)	1.452890	0.4528130	534	3.208588	0.0014
Time (After):Season (autumn)	0.579458	0.4662753	534	1.242738	0.2145
Treatment (Open):Season (autumn)	0.443509	0.4526288	534	0.979851	0.3276
Time (After):treatment(Open):Season (autumn)	-1.946924	0.6533027	534	-2.980125	0.0030

**Table 9. Mid-term effects: model coefficients for Shannon-Wiener (H'). Final model:  $\text{lme}(H' \sim \text{time} * \text{treatment} * \text{season}, \text{random}=\sim 1|\text{location}, \text{weights}=\text{varIdent}(\text{form}=\sim 1|\text{time}))$ .**

	Value	Std.Error	DF	t-value	p-value
(Intercept)	1.3872354	0.2808303	534	4.939763	0.0000
Time (After)	0.5017701	0.1311295	534	3.826522	0.0001
Treatment (Closed)	0.1161598	0.1205374	534	0.963683	0.3356
Season (autumn)	0.8099831	0.3676932	10	2.202878	0.0522
Time (After):Treatment (Closed)	-0.4155712	0.1854452	534	-2.240938	0.0254
Time (After):Season (autumn)	-0.8084444	0.1762691	534	-4.586422	0.0000
Treatment (Closed):Season (autumn)	-0.2648875	0.1578205	534	-1.678411	0.0939
Time (After):Treatment(Closed):Season (autumn)	0.7337067	0.2476257	534	2.962967	0.0032

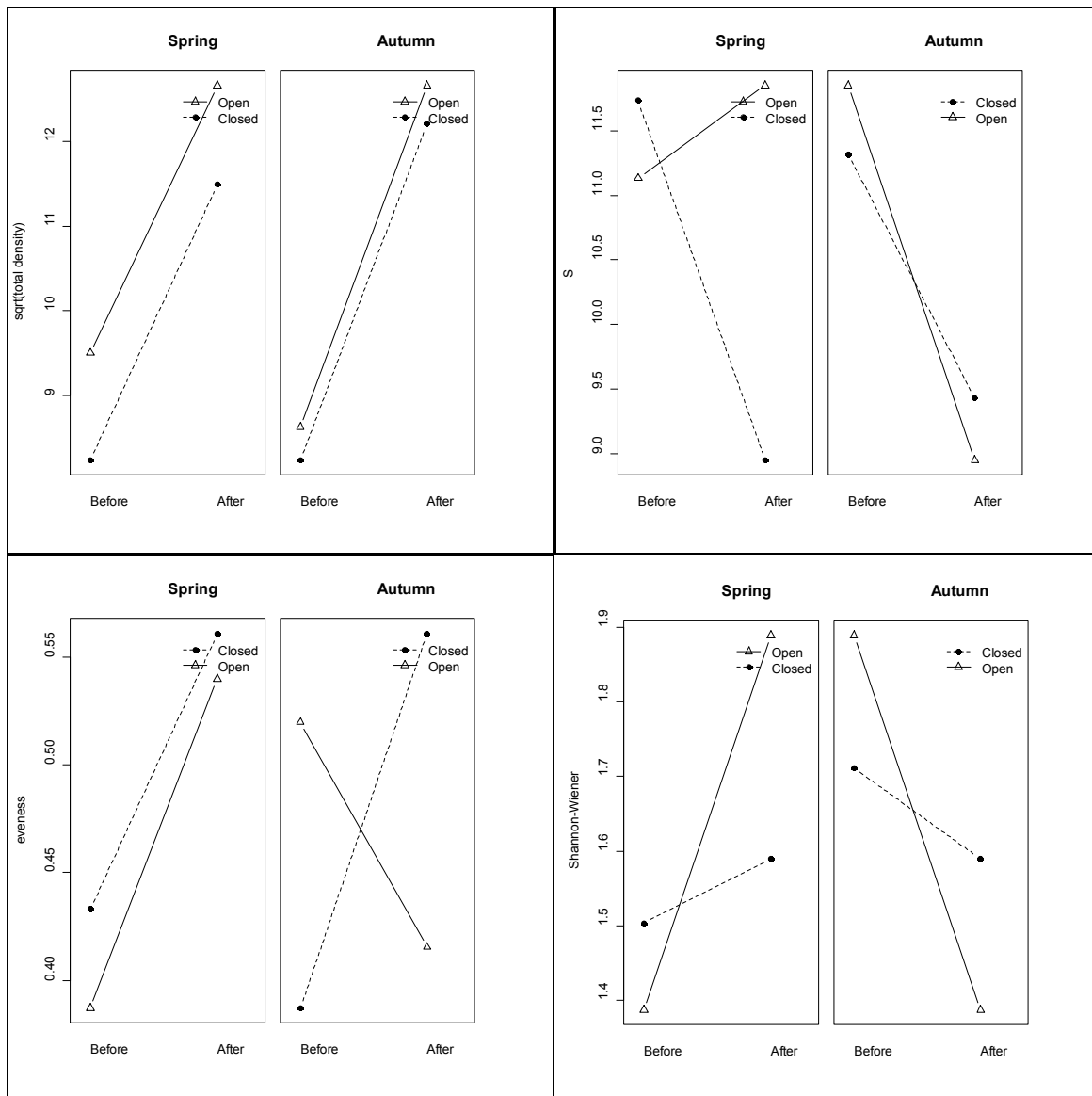


Figure 5. Interaction plot for mid-term effects showing (on the y-axis) the mean of square root transformed total density (upper left), number of species ( $S$ ; upper right), evenness (lower left) and Shannon-Wiener index (lower right) in spring and autumn. The x-axis gives the sampling time (before and 1-1.5 year after fishing activities) and the lines the treatment type.

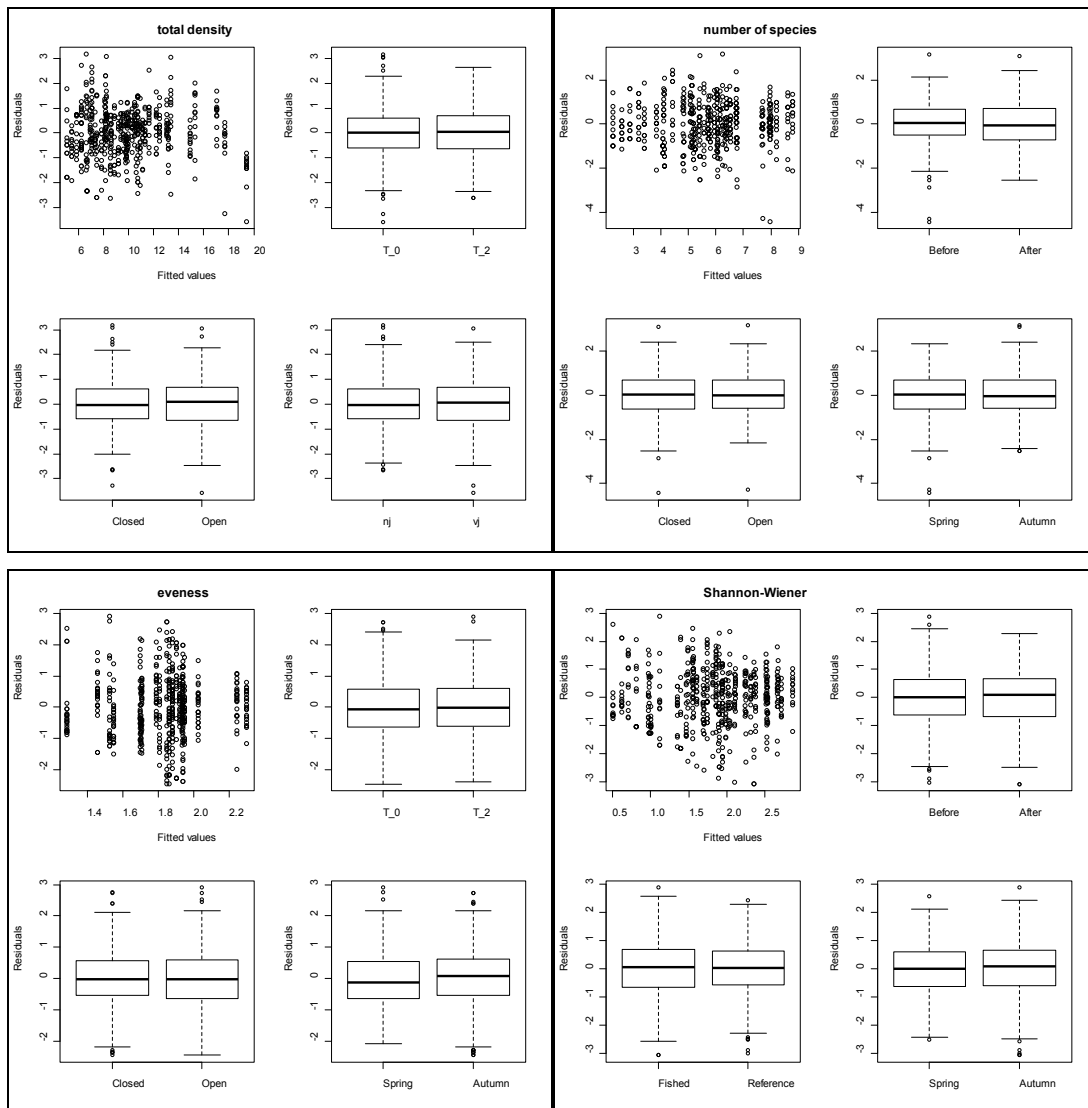


Figure 6. Model validation graphs for models on mid-term effects in total density (upper left), number of species (upper right), evenness (lower left) and Shannon-Wiener index (lower right). Residuals are plotted versus the fitted values, sampling time, season, and treatment.

## b. Similarity

### Short-terms effects

Model validation of a linear regression model did not point to heterogeneity. The final model did only include time as an explanatory variable (

Table 10) and indicated significantly less similarity (increased dissimilarity) between open and closed part of the locations after mussel seed fisheries. Hierarchical modelling with locations as random factor reveals the same results. The 95% confidence limits do not contain zero (Table 11) and the actual p-value is estimated as 0.0001. In the MDS plot the centroids of the samples taken in open and closed parts are, overall, further apart at T1 than at T0 (Figure 7), although that seems not to be the case for all locations (Figure 8).

Table 10. Short-term effects: model coefficients for linear model for dissimilarity. Final model:  $lm(\sqrt{\text{dissimilarity}} \sim \text{time})$ .

	Estimate	Std. Error	t value	Pr(> t )
(Intercept)	0.52178	0.01556	33.523	<2e-16 ***
Time (T1)	0.04681	0.02201	2.127	0.0397 *

Table 11. Short-term effects on dissimilarity. Model coefficients for hierarchical model using locations as random factor:  $lmer(\sqrt{\text{dissimilarity}} \sim \text{time} + (1|\text{location}))$ . T1 is given relative to T0. Confidence limits are based on 10000 simulations.

	Estimate	Std. Error	value	95%CI
T0	0.52178	0.01556	33.52	0.49 0.55
T1	0.04681	0.01310	3.57	0.02 0.07

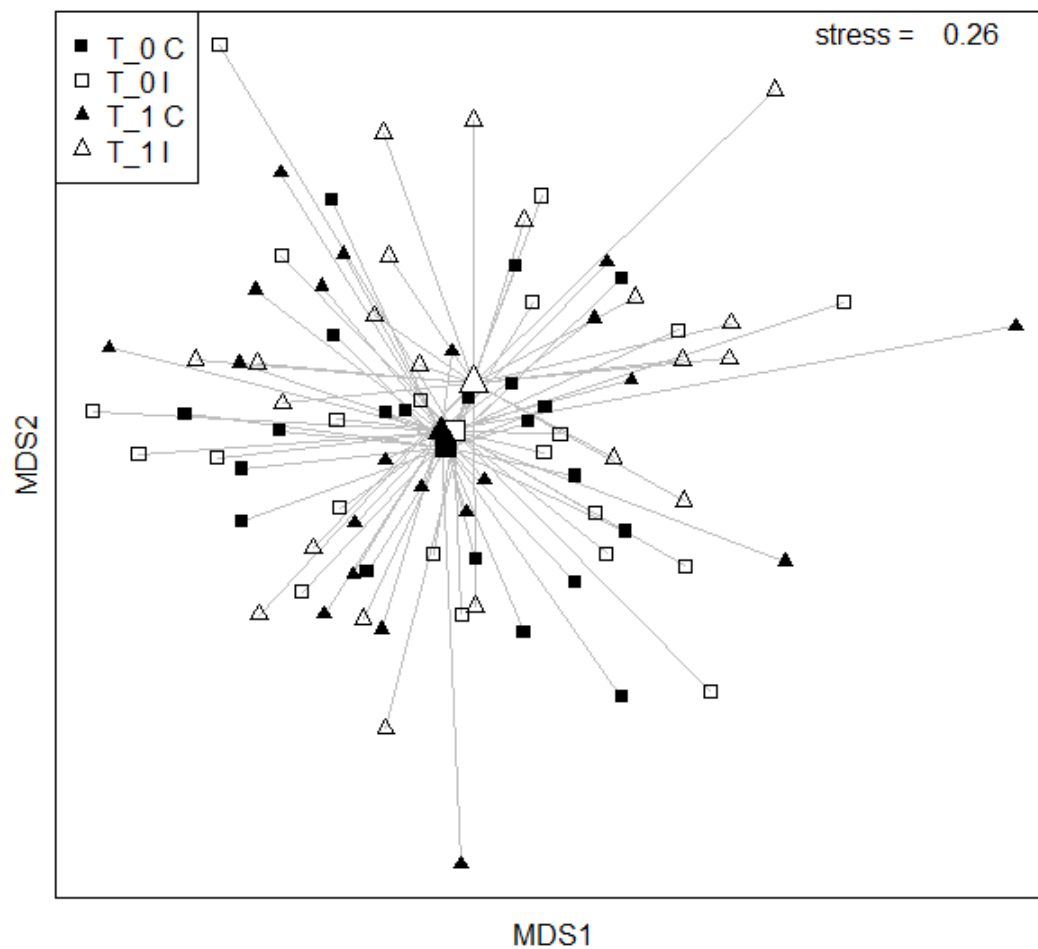


Figure 7. MDS graph of plots showing the ordination of the locations in relation to treatment (C = closed/Control, I = Impact/open) and time (T0, T1). Centroids for the four groups are shown and each location is connected to its group centroid by a grey line.

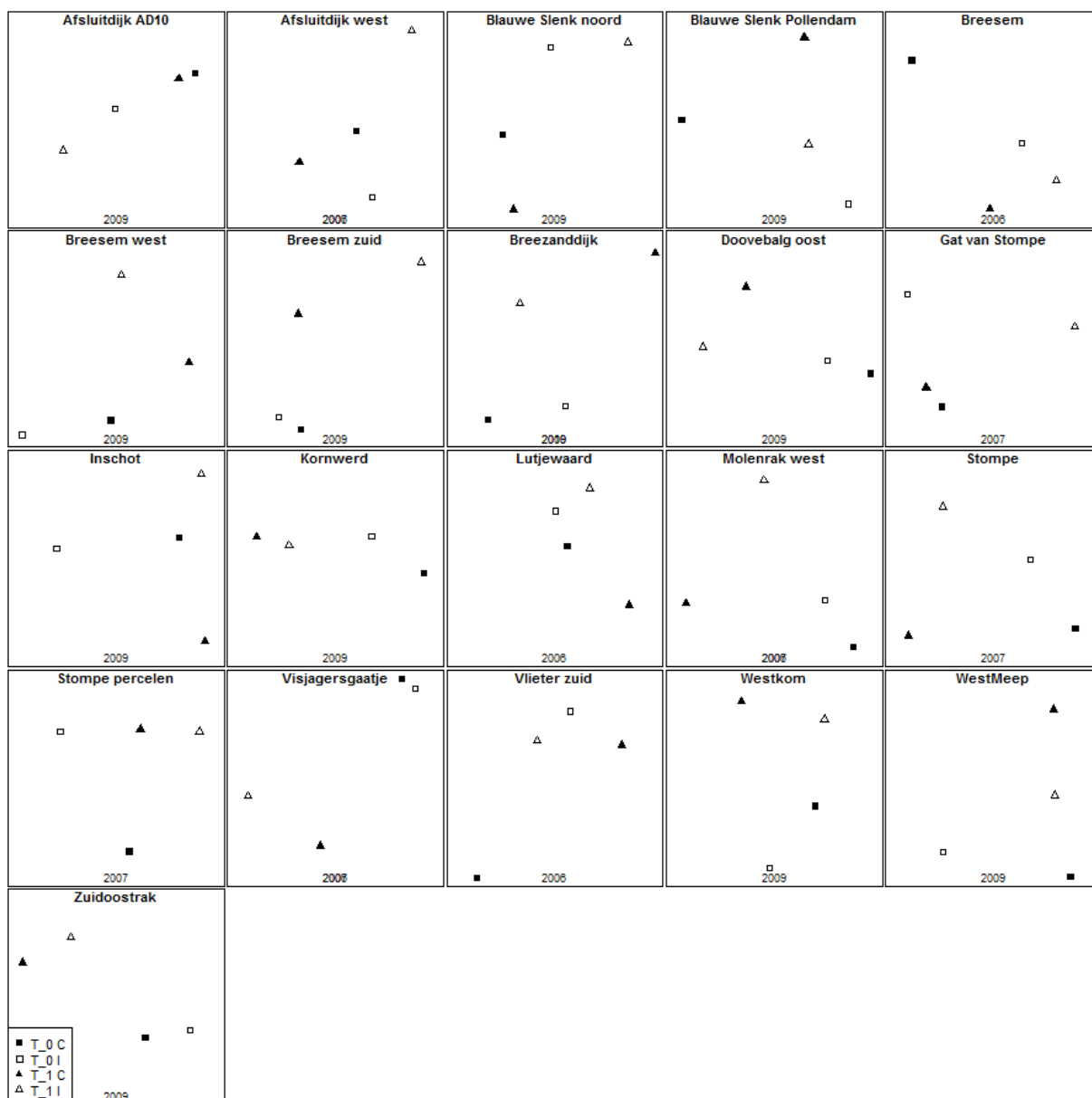


Figure 8. MDS graphs for each location separately.

#### Mid-term effects

There is no significant difference in similarity between the open and closed part of the locations 1-1.5 years after mussel seed fisheries. Neither time nor season or their interaction were significant in the linear regression model. Hierarchical modelling with locations as random factor revealed the same results. The 95% confidence limits contain zero (Table 12) and the actual p-value is estimated as 0.3111. In the MDS plot the centroids of the samples taken in open and closed parts are, overall, almost equally close to each other in T0 and Tmid (Figure 9), although for some locations the distance is larger (Figure 10).

Table 12. Mid-term effects on dissimilarity: model coefficients for hierarchical model using locations as random factor. Tmid is given relative to T0. Confidence limits are based on 10000 simulations.

	Estimate	Std. Error	value	95%CL	
T-0	0.26594	0.03121	8.521	0.21	0.33
T_mid	0.01996	0.04035	0.495	-0.06	0.10

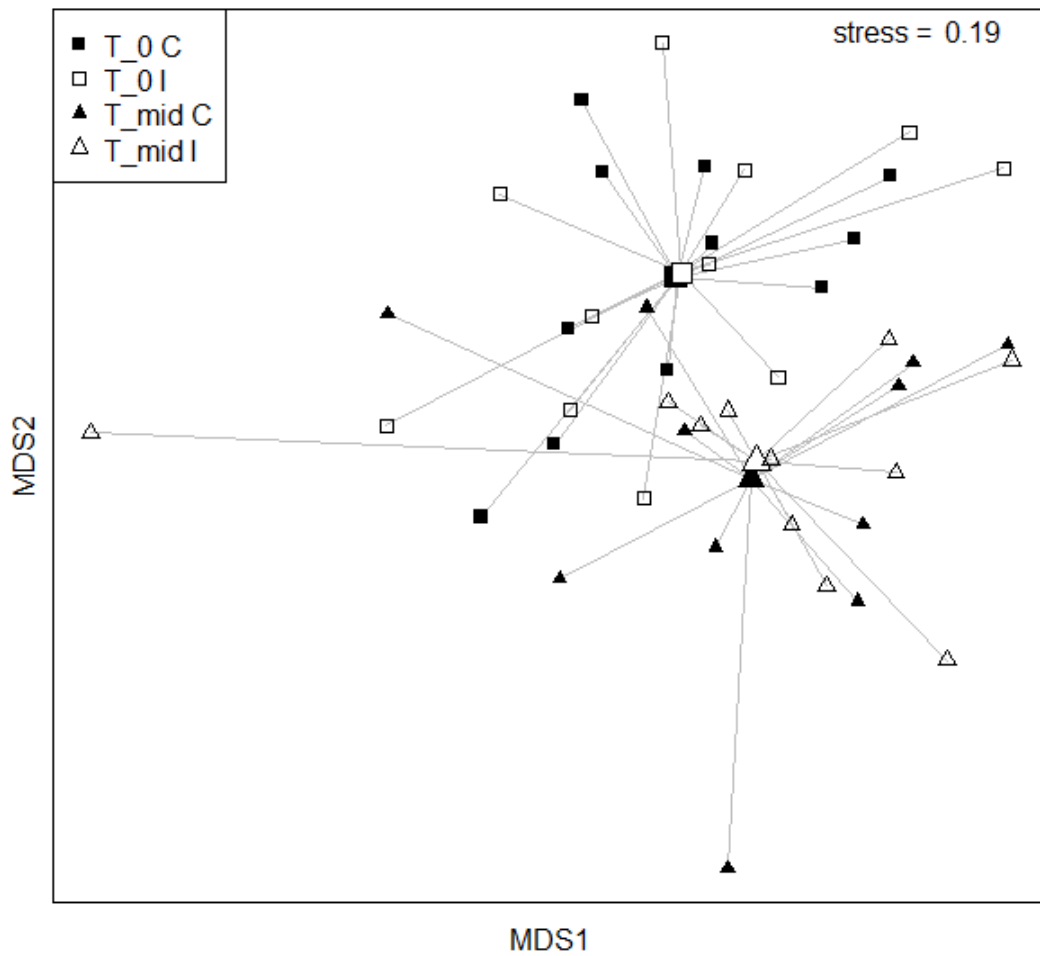


Figure 9. MDS graph of samples showing the ordination of the locations in relation to treatment (C = open/closed, I = closed/Impact/open) and time (T0, Tmid). Centroids for the four groups are shown.

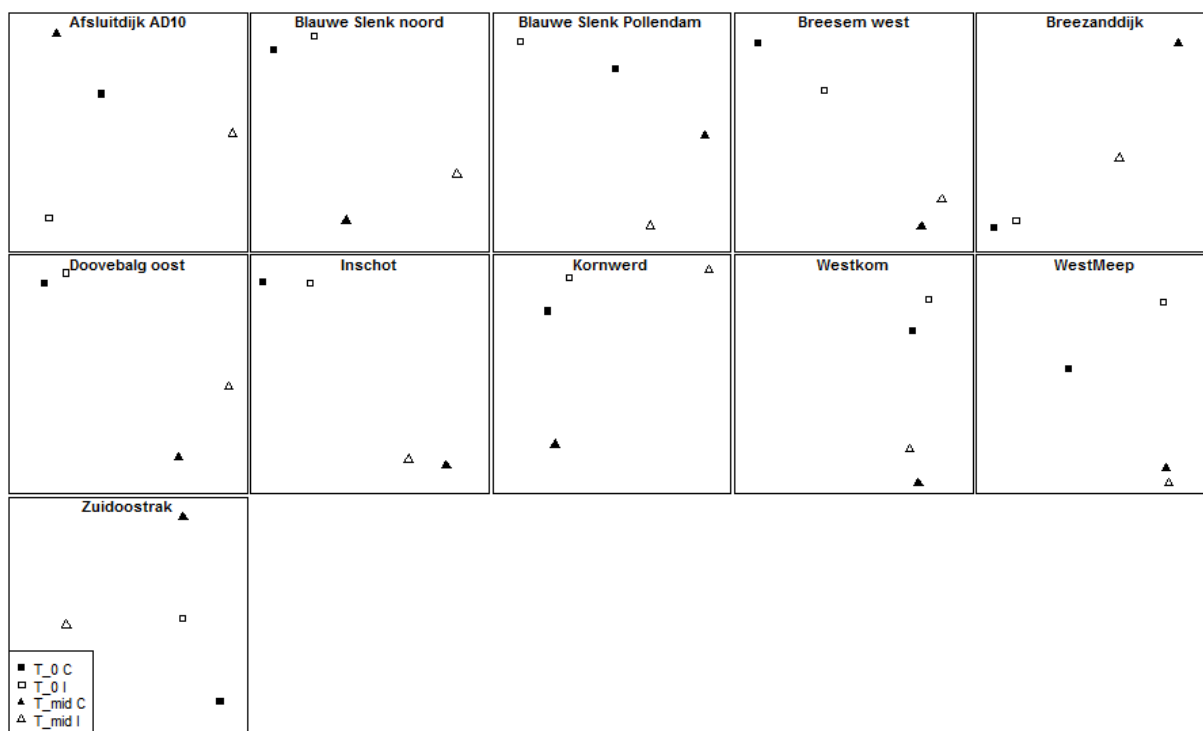


Figure 10. MDS graphs for each location separately.

### c. Principal Response Curves

A detailed example: Molenrak west

In order to better understand the results of the PRC method, we compared the results of one location (Molenrak west, MR-w) with that of an RDA analysis. The RDA analysis (Figure 11) clearly shows the changes in species composition in time, both in the open and closed parts. In the RDA diagram samples with similar species composition lie close together, samples with very different species composition lie far apart. The relative abundance of species in samples can be derived by projecting the sample points onto an imaginary line drawn through a species point and the origin of the plot.

Not surprisingly, mussels are one of the species abundant in samples taken at T0. At T2, the community is characterized by a larger abundance of species such as *Marenzelleria* sp., *Scoloplos armiger*, *Streblospio benedicti* and *Pygospio elegans*, both in the open and closed area. At the last sampling point (T3) the community composition has changed again. The species composition in the open part, however, has become more different from that in the closed part: the samples taken in each part (and their centroid) are lying far apart in the ordination diagram. The species composition in the open part seems to be very similar to that at the start of the study (T0).

The interpretation in terms of how the changes in species composition in open and closed parts differ, is difficult to derive from this diagram. The PRC method represents the time trajectory as a horizontal line, enabling an easier interpretation.

Figure 12 shows the first component of the PRC diagram using the species composition at T0 as reference point for the samples taken at later dates, separately for the parts of this location open and closed to mussel fishery. The diagram displays differences in species composition between the time series and the reference point. The reference points get a zero value at all other dates. At both parts the changes are significant ( $p=0.005$ ). The differences are largest at T2. At all sampling dates, differences with T0 are significant, except for T1 in the closed area, i.e. short after fisheries. In the RDA diagram T1

is almost overlapping with T0 (and, therefore, not visible in the diagram). The species axes on the right shows the affinity of the species with the response shown in the diagram. We choose for two axes, to make a distinction between the most numerically dominant species and the species with the largest weight. It is clear that both in the open as closed area, the polychaete *Pygospio elegans* e.g. has increased in time. This is also clear from the RDA diagram. At T2 (June 2009), both the control as the open part have the highest score on the second RDA axis, as is *P. elegans*. Thus, major changes in species composition are not restricted to the fished part of the location. In both parts the species composition tend to return to its original state: in the PRC diagram the canonical coefficients at T3 has decreased compared to the T2 situation.

The treatment effects, i.e. the deviations of treatment from the control and the species involved, are more clear in the PRC diagram using the development in the closed part as reference time series (Figure 13) than in the RDA diagram (Figure 11). Species such as *Alitta succinea*, *Oligochaeta*, *Polydora cornuta*, *Lanice conchilega* and *Heteromastus filiformis* are, in general, more abundant in the open part, *Hydrobia ulvae* and *Cerastoderma edule* in the closed part. On the basis of the Monte Carlo permutation tests per sampling date, the species composition in the control and open part differ significantly at all sampling dates except T2, i.e. when the population of *P. elegans* is at top, the species composition in the control and open part differ. At that time, there is no significant difference in species composition between the open and the closed part. The difference is largest at the end of the study period. This is also evident from the RDA diagram: the largest distance between control and open is at T3.

At Molenrak west 24% of the total variance can be attributed to time, whereas only 14% can be attributed to the treatment (including interaction with time). Sixty-two percent of the latter is explained by the first constrained axis, shown in the first PRC diagram. Thus, the changes in species composition do differ in the open and the closed parts of this location, which might be due to fisheries. Apparently, differences are largest at the end of the study period. However, open and closed part already differ at the start of the study, and common changes are much more important than explained by the treatment regime.

#### Overview

The PRC curves for the analyses of all locations are given in Appendix B, along with RDA diagrams per sampling date and some boxplots for selected species.

At almost all locations the benthic community composition changes significantly over time, irrespective of treatment (Table 14). At following 3 locations only, there was no overall significance change in one part of the location: Afsluitdijk-AD10 (closed part), Pollendam (open part) and Zuidoostrak (open part).

At 7 of the 21 locations, changes in species composition in the open part were not significantly different from those in the closed part of the location (see p-values in Table 15). This does not mean that the species composition was not different at all. Indeed, it was at most of the sampling dates (see Appendix A). At the other sites, the species composition was significantly different in the open and the closed part, indicating a possible effect of fisheries.

At all but 3 stations, the percentage variance explained by time is, however, larger than that by the treatment regime (Table 15). At Vlieter-zuid and WestMeep the percentage is about the same. At Pollendam, the treatment regime could explain much more of the variation than time: 17% versus 10%. At this location, *Mytilus edulis* and *Nereis virens* are more abundant in the open part, *Oligochaeta* and *Nereis succinea*, among others, more in the closed part. The density of mussels based on the box-corer samples was at T1, i.e. after fisheries, higher in the open part than in the closed part (see Appendix A). The data collected with the dredge show the same distribution van Stralen et al, 2013; Glorius et al, 2013).

The percentage variance explained by time and treatment regime (almost always much smaller than that by time) is not related to the moment of fisheries, or the length of the time series.



#### d. Power

The realized detectable effect size for the short-term analyses with a power of 80% is a bit larger than anticipated: 12 – 16% (Table 13). For the mid-term analyses (11 locations), the effect sizes are larger: 17-23%.

Table 13. Effect size that can be detected ( $\alpha = 0.05$ ,  $\beta = 0.20$ )

	Effect size T0-T1	Effect size T0-Tmid
Total density	11.76	16.98
Number of species	15.84	22.82
Evenness	15.63	21.83
Shannon-Wiener	15.89	22.42

Table 14. Significance of the first Principal Response Curve of differences in species composition of the benthic fauna in open and closed parts of a location, using T0 as internal reference point. Significant p-values point to significant changes in species composition over time.

location	Closed	Open
Afsluitdijk - AD10	0.220	0.017
Afsluitdijk West	0.005	0.005
Breesem	0.005	0.005
Breesem W	0.005	0.005
Breesem Z	0.005	0.010
Breezanddijk	0.005	0.005
Doovebalg DB23	0.005	0.005
Gat van Stompe	0.005	0.005
Griend	0.005	0.005
Inschot	0.005	0.005
Kornwerd (Boontjes)	0.005	0.005
Molenrak West	0.005	0.005
Pollendam	0.005	0.130
Stompe	0.005	0.005
Stompe percelen	0.005	0.005
Visjagersgaatje	0.010	0.015
Vlieter (zuid)	0.017	0.005
Westkom	0.005	0.005
WestMeep	0.041	0.010
Zuidostrak	0.005	0.060
ZuidWest (Lutjewaard)	0.005	0.005

Table 15. Percentages of the total variance that can be attributed to time and treatment regime (including interaction with time) of analyses using the closed part of the locations as reference time series, and p-values of the first PRC of these analyses, indicating different temporal changes in species composition in the open and the closed part of the location.

location	% variance accounted for by		p-value
	Time	Treatment regime	First PRC
Afsluitdijk - AD10	14.5	11.0	0.010
Afsluitdijk West	27.2	6.0	0.140
Breesem	26.2	7.8	0.005
Breesem W	37.3	7.7	0.005
Breesem Z	14.3	23.6	0.005
Breezanddijk	32.2	18.6	0.005
Doovebalg DB23	23.7	6.9	0.059
Gat van Stompe	22.5	7.4	0.010
Griend	21.8	4.3	0.180
Inschot	25.7	4.0	0.140
Kornwerd (Boontjes)	34.4	14.1	0.005
Molenrak West	27.4	15.9	0.005
Pollendam	9.8	17.0	0.005
Stompe	43.5	11.6	0.005
Stompe percelen	16.8	4.9	0.005
Visjagersgaatje	49.4	6.0	0.010
Vlieter (zuid)	20.1	17.1	0.005
Westkom	17.2	4.0	0.200
WestMeep	10.1	11.0	0.005
Zuidoostrak	26.3	7.7	0.086
ZuidWest (Lutjewaard)	27.6	4.4	0.051

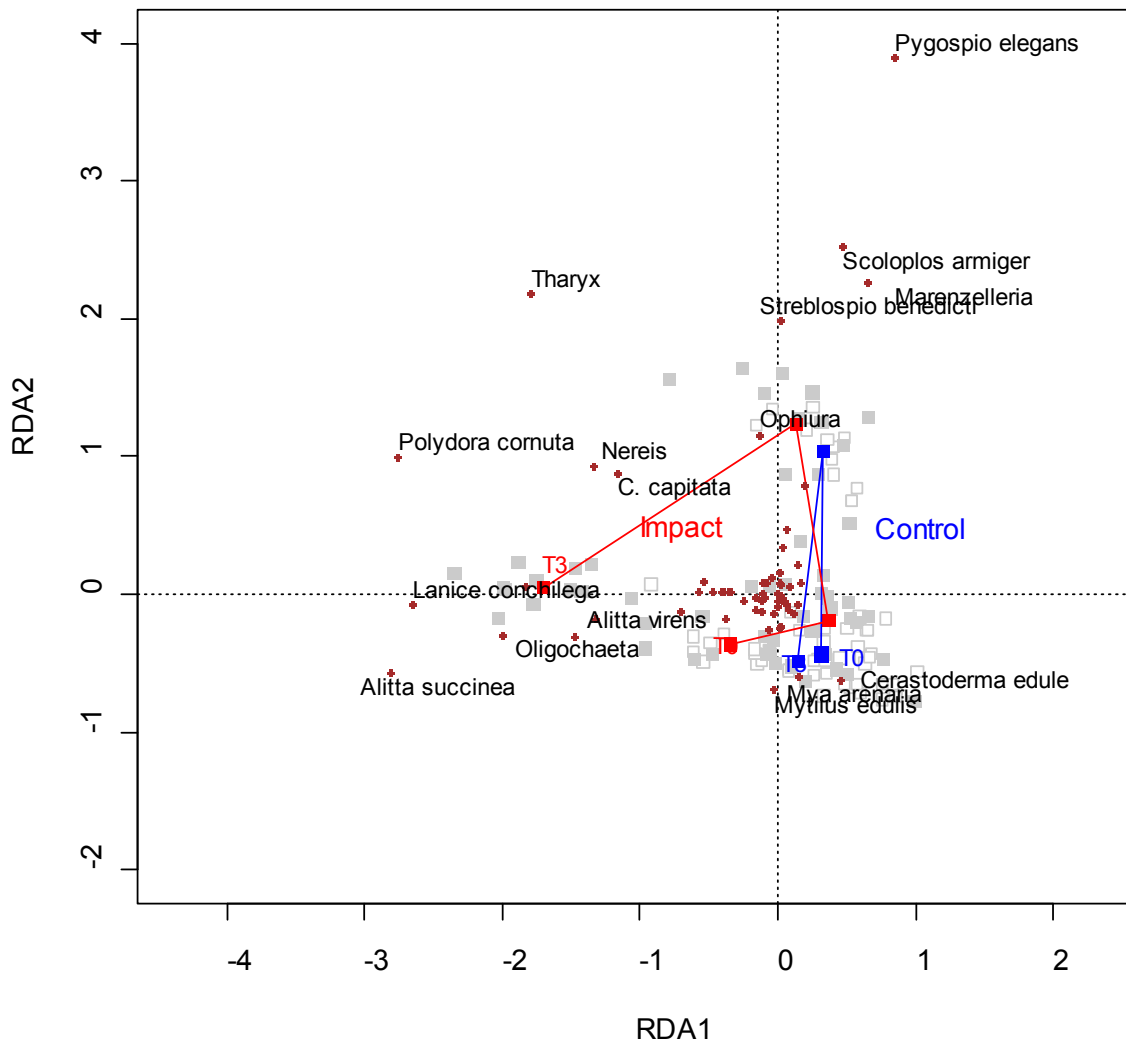


Figure 11. Ordination diagram (RDA) of square root transformed data at Molenrak west. Number of years after T0 and treatment level (Control, Impact), as well as their interactions, were taken as explanatory variables. Individual samples are given in grey (open/Impact part) or white (closed/Control part). The centroids of each sampling data (T0, ...T3) are given in red (open/Impact) and bleu (closed/Control). The lines represent the course of the (centroid of the) treatment levels in the ordination diagram. Of all variance, 43% can be attributed to the explanatory variables, Of this explained variance, 35% is explained in the diagram. Only those taxa are shown that are most discriminating in the diagram.

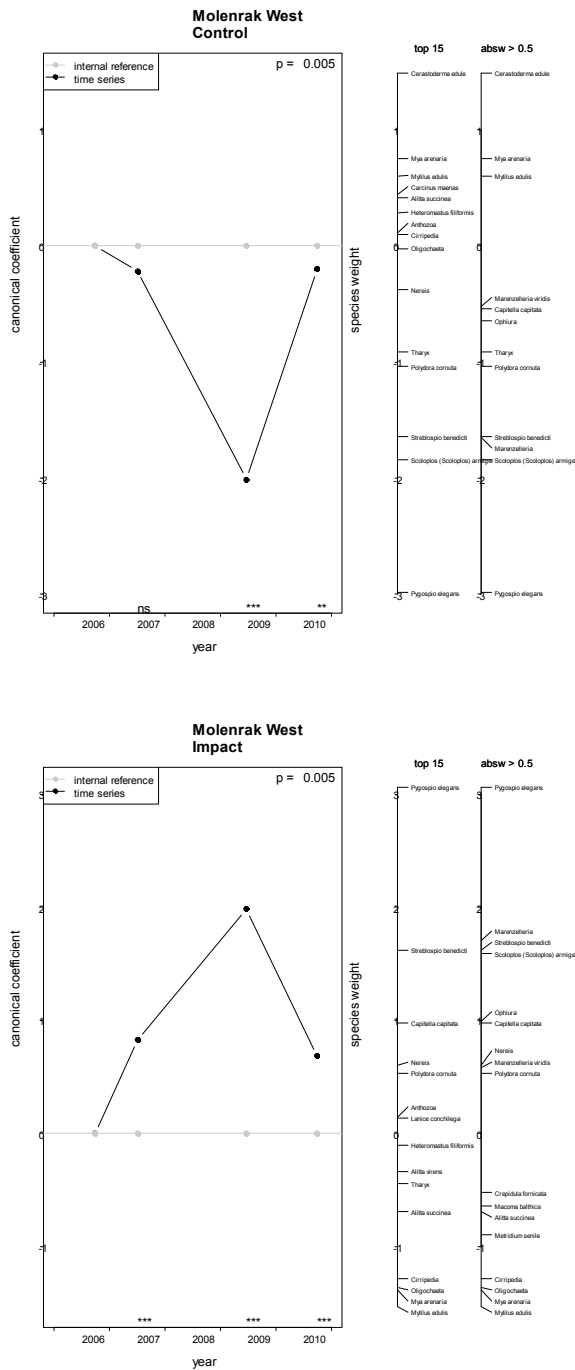


Figure 12. Diagrams for the first component of the PRC of differences in species composition resulting from separate analysis of the benthic fauna in the open and closed part of location Molenrak West, using T0 as an internal reference. The species weights in the right part of the diagrams represent the affinity of species with the response shown in the diagram. The 15 numerically dominant species (top 15), and species with a species weight larger than 0.5 or smaller than -0.5 ( $absw > 0.5$ ) are shown. The significance of differences between Control and Impact parts at each time point is indicated by the asterisks generated from unrestricted Monte Carlo permutation (\*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$ )

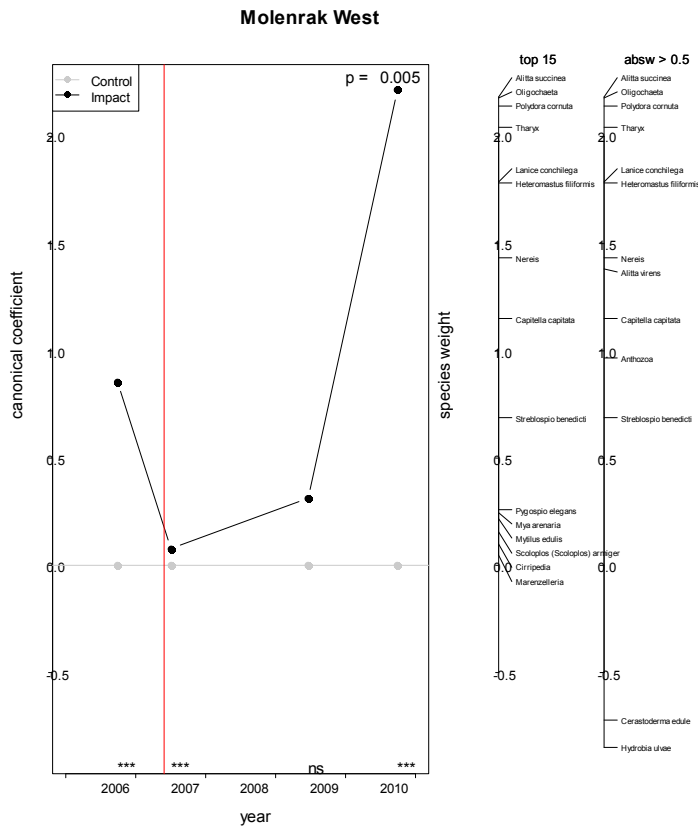


Figure 13. Diagram for the first component of the PRC of differences in species composition of the benthic fauna between the open (Impact) and closed (Control) part of location Molenrak West, using the closed part as reference. The species weights in the right part of the diagrams represent the affinity of species with the response shown in the diagram. The 15 numerically dominant species (top 15), and species with a species weight larger than 0.5 or smaller then -0.5 ( $absw > 0.5$ ) are shown. The red line indicates the moment of mussel seed fisheries. The significance of differences at each time point is indicated by the asterisks generated from unrestricted Monte Carlo permutation (\*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$ ). The differences between open and closed part explained 14% of all variation, of which 62% is displayed on the y-axis of the first PRC. Therefore, not all differences are well represented in the first response diagram, as e.g. the differences at T1.

### 3. Discussion

Benthic species composition is well-known to show spatial differences. At larger scales this is generated by differences in physical processes, at smaller scales biologically generated patterns are important (Herman et al., 1996; Legendre et al., 1997; McArdle et al., 1997; Thrush et al., 1997). Mussel beds too show spatial patterns, at different scales including small-scale variation (Lawrie and McQuaid, 2001; van de Koppel et al., 2005). There is also a spatial variation within locations in the silt content, and differences between open and closed parts of the locations (van Bemmelen et al., 2013). Such patterns will have consequences for patterns in community attributes such as species richness and species composition. This is confirmed by the present study. At many locations, the species composition of the open and closed part was significantly different already at T0.

Macrobenthic communities are also characterized by large temporal fluctuations, resulting from differences in recruitment success, recruitment patterns and mortality rates (see e.g. Kröncke et al., 2011; Van Hoey et al., 2007). And opportunistic species, with short reproductive and recruitment cycles, are periodically very abundant. Almost all study areas of this study indeed show significant changes in species composition, irrespective of fishing activities (principal response curves with T0 as internal reference, fig 12). Some of these changes were large-scale and could be seen at many locations. High densities of *Pygospio elegans*, for instance, are recorded in 2009 and contribute to the significant temporal changes. *P. elegans* appears to have high species weights in PRC analyses comparing the open and closed parts at 11 of the locations studied.

Mussel beds offer, because they form a three dimensional hard substrate structure, offers habitat for hard substrate epibenthic species, and shelter for mobile epibenthic species. Mussels change the sediment characteristics, favouring endobenthic species with a high tolerance for organic enriched sediments. Thus, mussel bed do influence biodiversity by facilitation and inhibition of species, but the result is a higher species richness than the surrounding soft sediment habitat (Drent et al., 2013a; Dolmer et al., 2001; Ens et al., 2007; Koivisto and Westerbom, 2012; Koivisto and Westerbom, 2010; Markert et al., 2010; Norling and Kautsky, 2008; Saier et al., 2002; Ysebaert et al., 2009). It is, therefore, reasonable to expect at least a temporarily effect of mussel seed fisheries. For instance, a decrease in densities of associated species, a decrease of the total density, or a decrease in species richness. And, as the species composition is changed due to the mussels, we also expect the species composition in fished areas to be more identical to that on sand banks than in mussel beds.

The present study showed a significant impact of fishing on the total density (excluding mussels) in the short term: after fishing densities were higher in the closed plots than in the open plots. This is in accordance with Dolmer et al (2001) who found 40 days after dredging lower density in the trawled area, particularly of polychaetes.

The differences in species composition in fished areas compared to closed areas were larger after mussel seed fisheries. In the diagrams of the RDA analysis at T1 (see appendix A) differences between open en closed parts were, irrespective of the fishery season, often due to higher densities in the closed parts of mussels - of course -, associated hard substrate species (barnacles) and species such as *Alitta virens*, *Lanice conchilega*, *Harmothoe imbricata*, *Polydora cornuta* and *Capitella capitata*. These species were associated with sublittoral mussels (Drent et al, 2013b)(Dolmer et al., 2001; Markert et al., 2010; Ysebaert et al., 2009).

After 1 to 1.5 years, the difference in the similarity in species composition in open and closed parts compared to differences before fishing activities is no longer significant. The interaction between time and treatment (open vs. closed) for total density was not significant, indicating no lasting effect of fisheries. The analyses point, however, to significant different changes over this period in the number of species and the Shannon-Wiener index. The response differs according to the period of fishing activities, in spring or in autumn. The number of species, for instance, increased after spring fishery in the open part and declined in the closed part, while after autumn fishery it decreased in both parts, but faster in the open part. The interaction terms at short term were not significant, although the interaction plots suggest different trends. Effects on number of species, thus, seem to last for at least 1-1.5 years. Dolmer et al (2001) studied the infauna in dredged and control areas up to 40 days after the dredged sites were dredged, and reported lower number of species in the dredged area at the end of the study period.

Long-term effects (3-4 years) on the univariate and multivariate indices could not be analysed. From the PRC's there does no common pattern in changes in species composition show up. This might be partly due to different developments of the mussel population at the different locations. In some of these locations more than one spat fall and fishery event occurred. At Breesem, e.g., the last sampling date is short after the last fishery event, and the closed area is then still characterized by mussels and associated species as *Lanice conchilega* and *Carcinus maenas*, thus only reflecting short-term effects. But also at other locations that were not fished for a long period, as e.g. Afsluitdijk west, species such as *Allita succinea* and *Polydora cornuta* were more abundant in the closed than in the open part at the end of the study period. This could be related to the higher densities of mussels in the open part of this location than in the closed part, at least as sampled by the box-corer. Even small patches of mussels have a large impact on the associated fauna, and the number of species present (Norling and Kautsky, 2008) Differences in species composition between open and closed parts are, however, at almost all locations much smaller than common temporal changes. And, thus, any fishery effects seem to be less important in determining species composition than external factors controlling mortality and recruitment. This is in agreement with Hoffmann & Dolmer (2000). In the Limfjord (Denmark) the closed area appeared to have no significant influence on the epibenthic fauna, suggesting that other factors than mussel dredging determine the observed spatial and temporal variability of the ecosystem.

#### **4. Conclusion**

- The present study confirms the existence of small scale differences in macrofauna species composition: even within a subtidal mussel bed, within a few hundreds of meters. The present study confirms large temporal variation in species composition, independently of any human impact
- The study shows short-term effects: a change in total density and in species composition (e.g. associated species)
- The study shows mid-term effects on species diversity (number of species and Shannon-Wiener index). The effect is different depending on the fishery season.



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## **Quality Assurance**

IMARES utilises an ISO 9001:2008 certified quality management system (certificate number: 124296-2012-AQ-NLD-RvA). This certificate is valid until 15 December 2015. The organisation has been certified since 27 February 2001. The certification was issued by DNV Certification B.V. Furthermore, the chemical laboratory of the Fish Division has NEN-EN-ISO/IEC 17025:2005 accreditation for test laboratories with number L097. This accreditation is valid until 27 March 2013 and was first issued on 27 March 1997. Accreditation was granted by the Council for Accreditation.

## Justification

Report number : C003/13  
Project Number : 4308501015

The scientific quality of this report has been peer reviewed by the a colleague scientist and the head of the department of IMARES.

Approved: Dr. I. Tulp



Signature:

Datum: 7 January 2013

Approved: Dr. B.D. Dauwe  
Head of department Delta



Signature:

Date: 7 January 2013

## Appendix A. Rapport van de Audit commissie en reactie van de Producers auteurs

Audit van het Project Onderzoek DUurzame Schelpdiercultuur (PRODUS)

### 4. Specifieke commentaren

#### 4.2. PR7: Impact of mussel seed fishery on subtidal macrozoobenthos in the western Wadden Sea

Het rapport is over het algemeen duidelijk, al laat de typografische kwaliteit van de grafieken veel te wensen over (te kleine letters, vreemde lijnen, onduidelijke symbolen). Het rapport heeft geen inleiding waarin de vragen worden gespecificeerd en de hypothesen uitgewerkt die zullen worden getoetst. Er is een groot scala aan responsvariabelen gemeten, de statistiek is goed uitgewerkt en de conclusies zijn helder. De belangrijkste conclusie is dat er significante korte en middellange termijn effecten van mosselvisserij op de geassocieerde gemeenschap zijn, zoals bemonsterd met de boxcorer. Op de lange termijn zijn er geen effecten aantoonbaar.

*In de inleiding wordt nu verwezen naar Smaal et al (2013; Samenvattend eindrapport) voor nadere informatie over vraagstelling en opzet van het onderzoek.*

De samenhang met rapport PR6 is beperkt. Dit rapport is geschreven in het Engels, maar het is niet duidelijk waarom. Er is meer geïnvesteerd in de analyse en interpretatie van de data, maar minder in de afwerking van de tekst. Er zijn veel univariate en multivariate variabelen gebruikt en geanalyseerd met mixed models om de effecten van visserij te onderzoeken.

*In feite is maar een beperkt aantal univariate variabelen gebruikt: totale dichtheid, aantal soorten, Shannon-Wiener index, evenness and Bray-Curtis similariteit. Multivariaat is eindelijk maar een type univariate analyse uitgevoerd: PRC, ondersteund door RDA per tijdstip. Verder is in discussie is nu wel aandacht besteed aan gepubliceerd onderzoek.*

Figuur 2 geeft een globale vergelijking van univariate variabelen tussen bevestigd en onbevestigd. Gezien de grote variatie tussen locaties en het gepaarde karakter van de proef geeft dit slechts een deel van de resultaten weer. Verdere details worden gegeven in zeer technische en gedetailleerde interactieplotjes, en in multivariate ordinatieplots. Het zou informatief kunnen zijn om als tussenvorm een plot te maken zoals figuur 2, maar met het gemiddelde relatieve verschil tussen bevestigd en onbevestigd. Ook hier zouden deze relatieve verschillen tussen paren gerelateerd kunnen worden aan omgevingsfactoren om te onderzoeken in welke mate het visserijeffect afhankelijk is van de lokale omstandigheden.

*Ook de audit-cie geeft aan dat de resultaten weergegeven (moeten) worden met behulp van interactieplots. Analyses met linear mixed effects model komen praktisch op hetzelfde neer als de klassieke standaardanalyse (variantieanalyse) en kunnen zelfs als een verbetering gezien worden. In de modellen zouden ook omgevingsvariabelen meegenomen kunnen worden. In eerste instantie*

*valt te deken aan sedimentkarakteristieken. Omdat vaak geen sedimentanalyses uitgevoerd zijn, is dit niet verder meegenomen bij de analyses.*

Op basis van informatie die elders wordt gegeven (bijvoorbeeld in het overzichtsrapport PR1) kan men aannemen dat de fauna zeer verschillend is in boxcores met en zonder mossels. Men vraagt zich af of dat hier ook het geval is. Indien het zo is, dan zou correctie voor het al dan niet voorkomen van mosselen in de boxcores een groot gedeelte van de variatie in de fauna kunnen verklaren. Dit zou toelaten te onderzoeken of visserij alleen effect heeft via het verwijderen van mosselen en hun geassocieerde fauna, of ook indirect via bijvoorbeeld bodemverstoring of beschadiging van grote organismen.

*In het rapport was al kort aangegeven dat verschillen zeker voor een deel aan geassocieerde fauna te wijten waren. Dat is nu iets explicieter opgenomen, met referenties naar rapport van Drent (2013).*

De commissie ondersteunt de eerste drie conclusies van het rapport (grote lokale verschillen, significante korte en middellange termijn effecten), maar niet de laatste. Hier wordt gesteld dat: “Fishery effects on the long-term, if any, seem to be less important in determining macrofauna species composition than external factors controlling mortality and recruitment”. Gezien de beperkingen in de proefopzet kan dit niet geconcludeerd worden. Vooraf was, op basis van power analyses gesteld dat er 40 proeflocaties nodig waren om een visserijeffect van 10% te kunnen aantonen. Analyse achteraf laat zien dat de ‘statistische slagkracht’ zelfs op de korte termijn al iets kleiner was dan vooraf voorzien. Door het verlies van locaties over de tijd, nam de mogelijkheid om verschillen aan te tonen verder af (tabel 13 in het rapport). Ondanks een lagere analysekracht, worden er toch significante verschillen aangetoond voor de korte en middellange termijn. Op de lange termijn zijn effecten niet meer aantoonbaar, maar het valt moeilijk uit te sluiten dat dit te wijten is aan de verder verkleinde power van de testen.

*De conclusies mbt de lange termijn, zijn enkel gebaseerd op de PRC-analyses. Daaruit blijkt dat andere factoren dan visserij een belangrijkere rol lijken te spelen bij de veranderingen in soortensamenstelling. Dat is in overeenstemming met bevindingen in Denemarken (Hoffmann & Dolmer 2000). Omdat de resultaten niet even hard zijn als deze op de korte en middenlange termijn, is een conclusie over lange-termijneffecten geschrapt.*

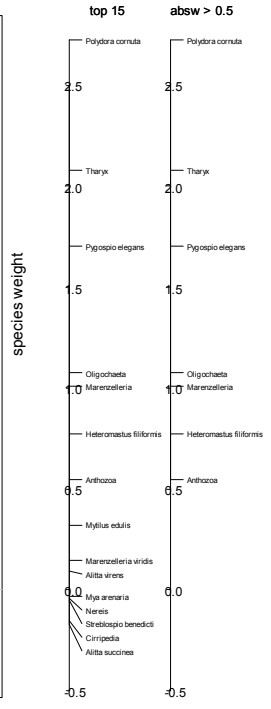
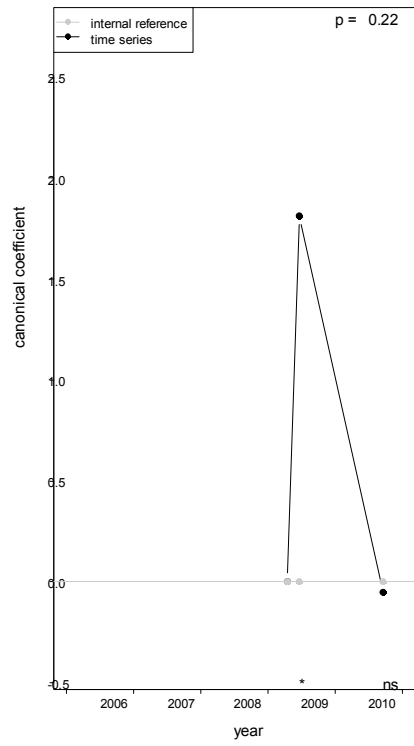
## Appendix B

Figures and diagrams as result from the PRC analyses per location:

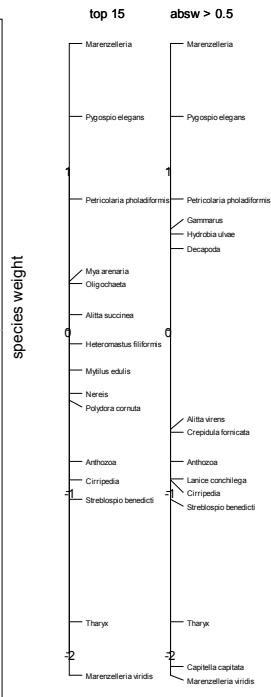
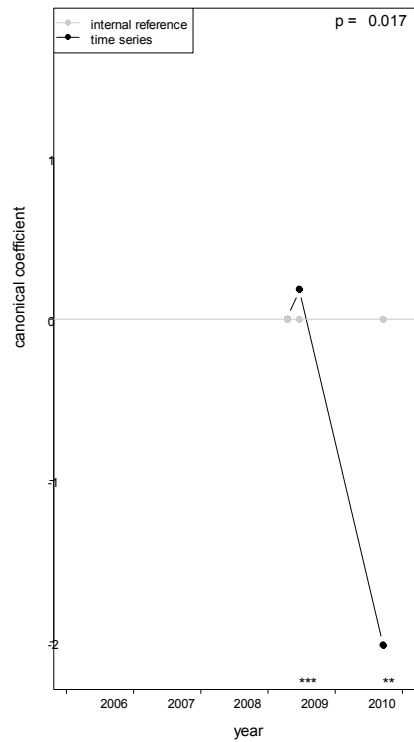
- diagrams first PRC of separate analyses of open and closed parts, using T0 as reference point
- diagrams for first PRC, using closed area as reference point
- RDA diagrams per sampling data (constrained axis = y-axis), with boxplots of scores along the constrained axis and weight of species
- Boxplots for selected species



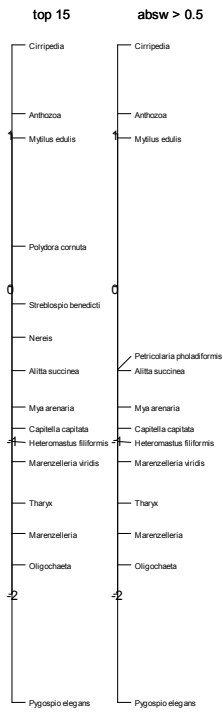
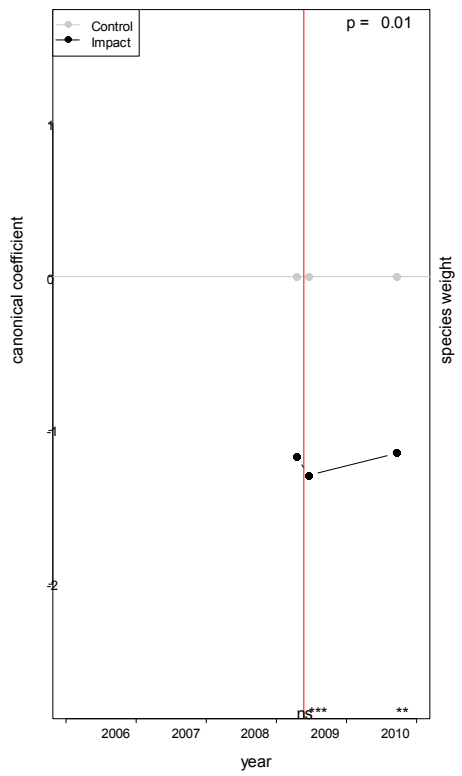
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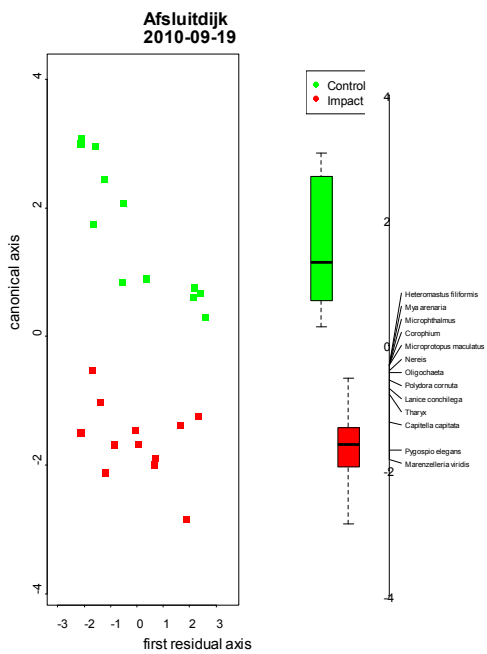
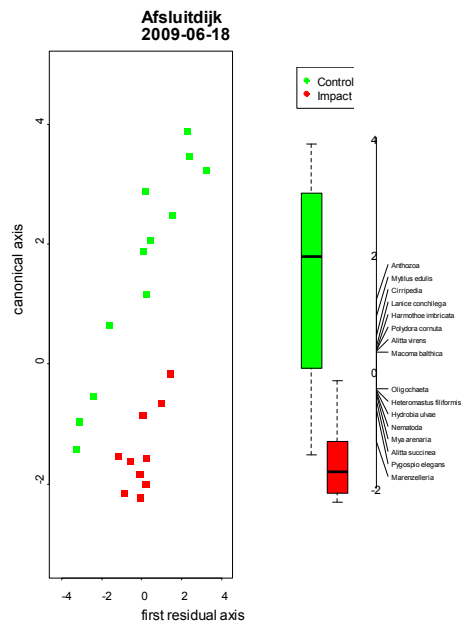
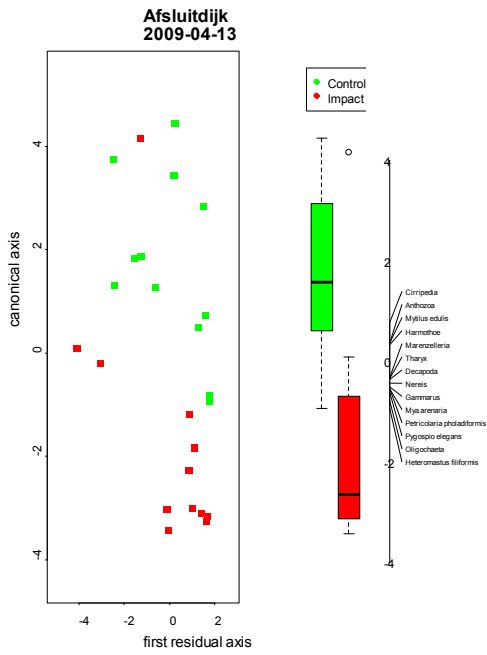


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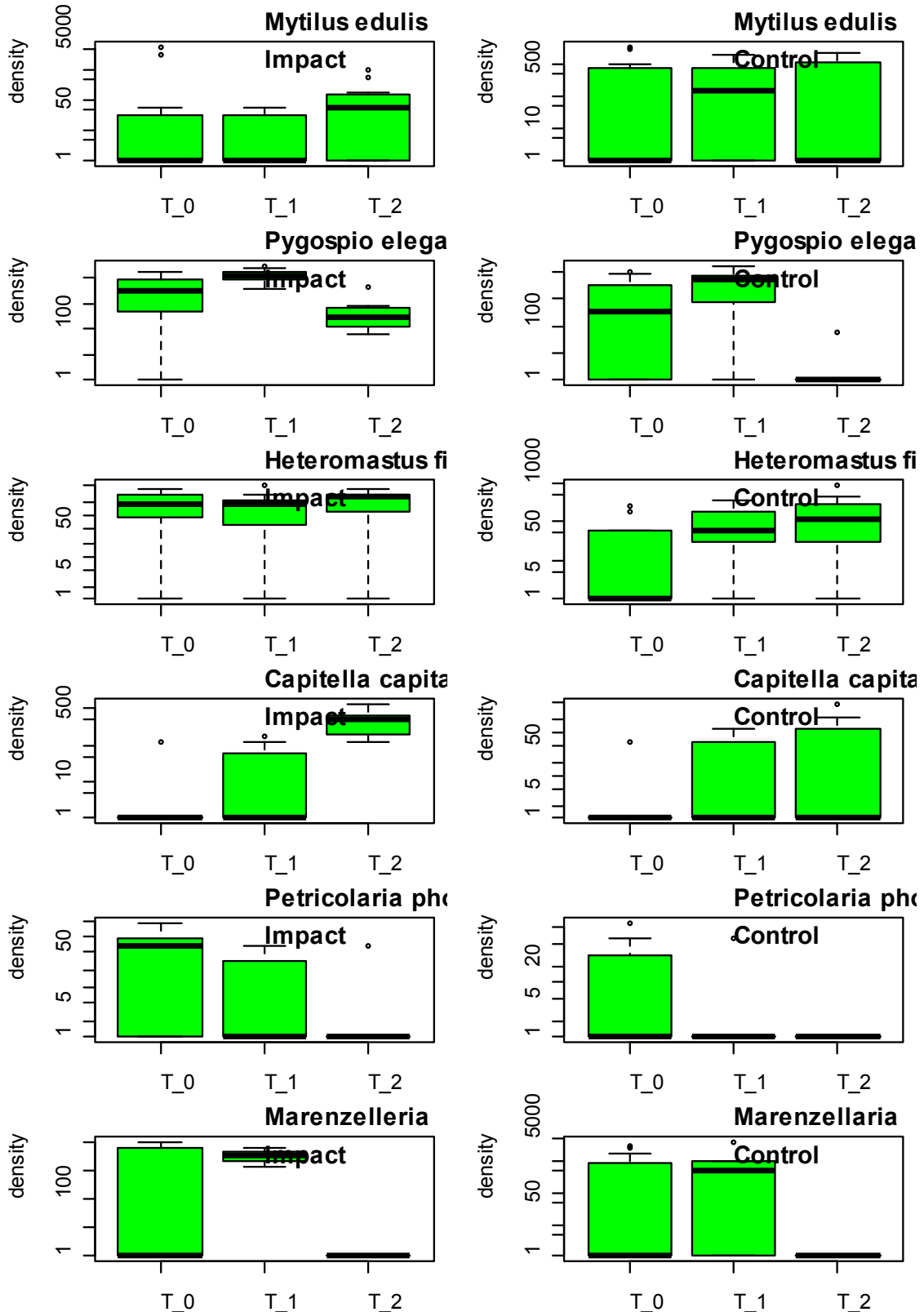


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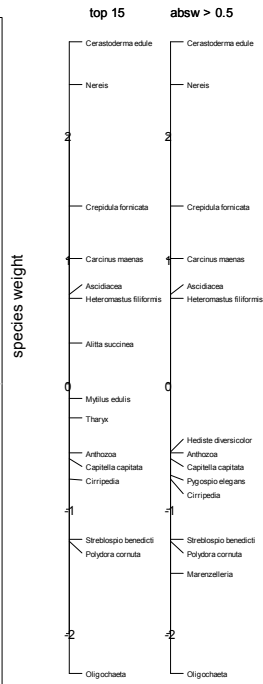
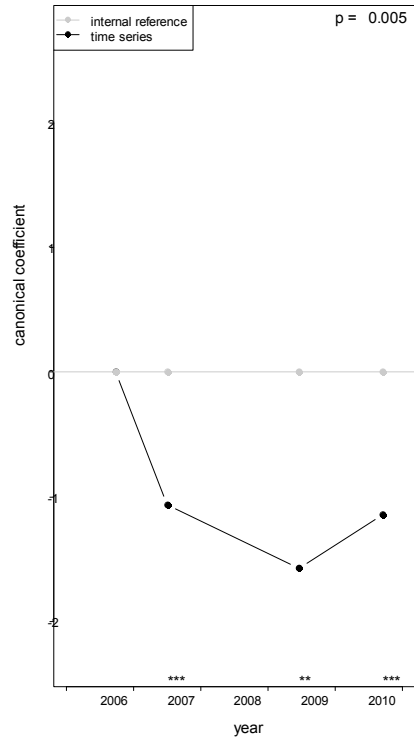




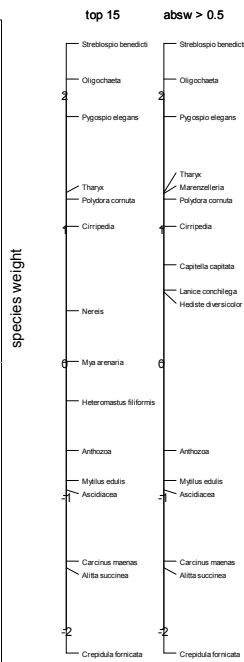
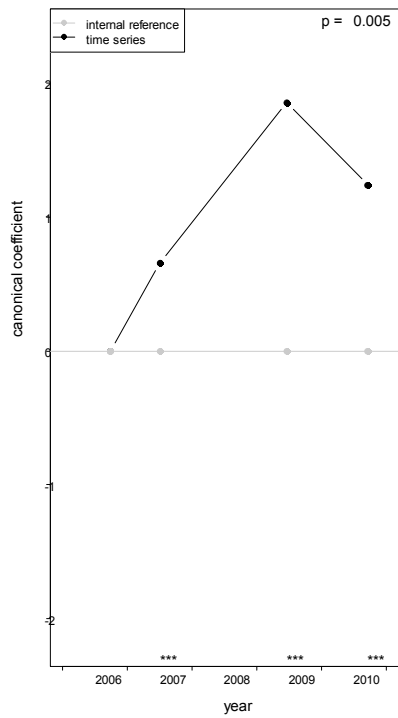
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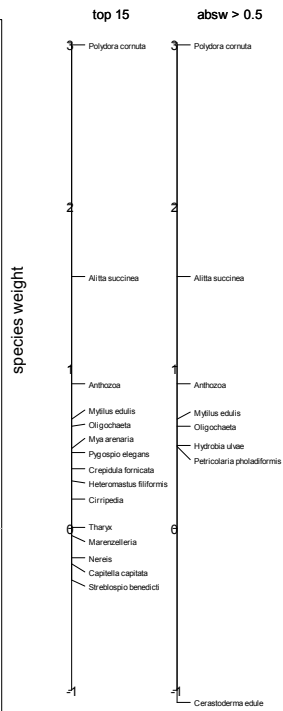
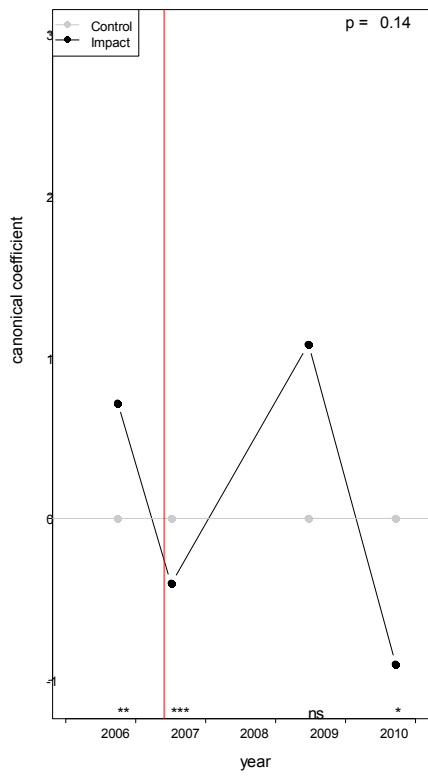
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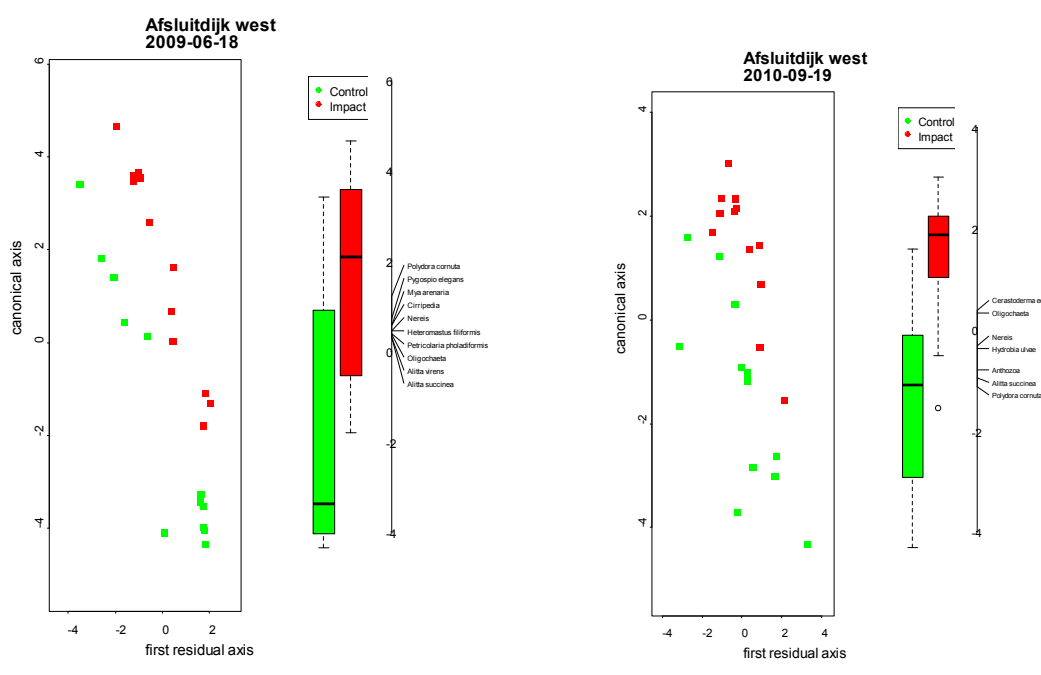
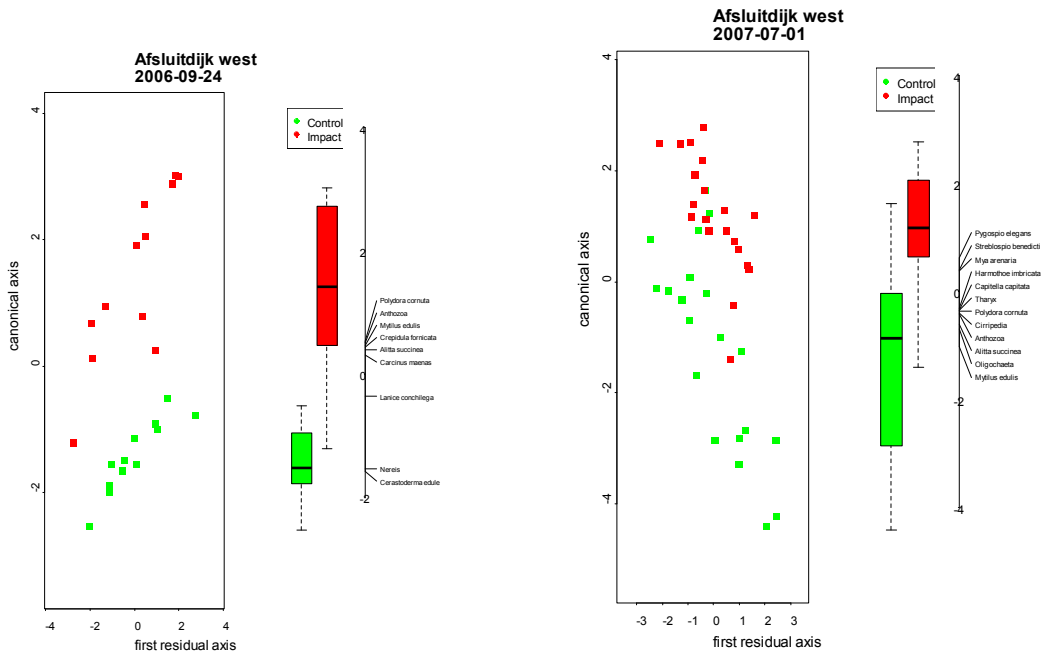


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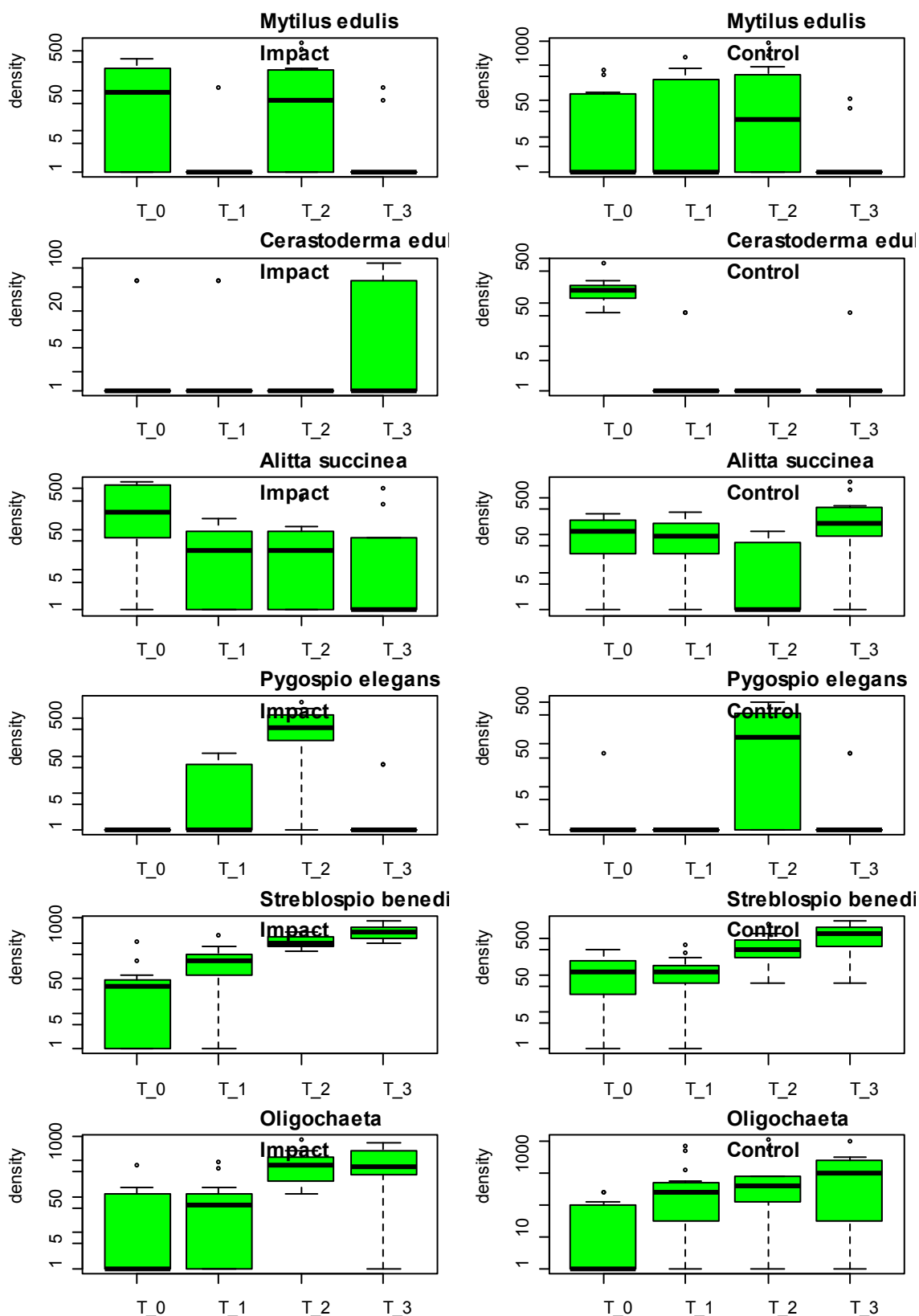


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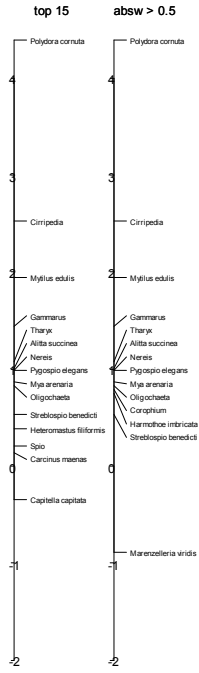
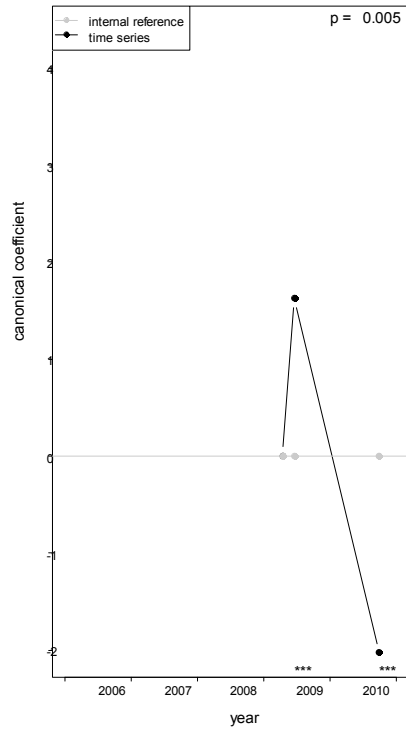


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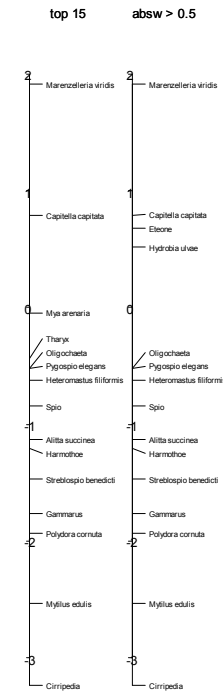
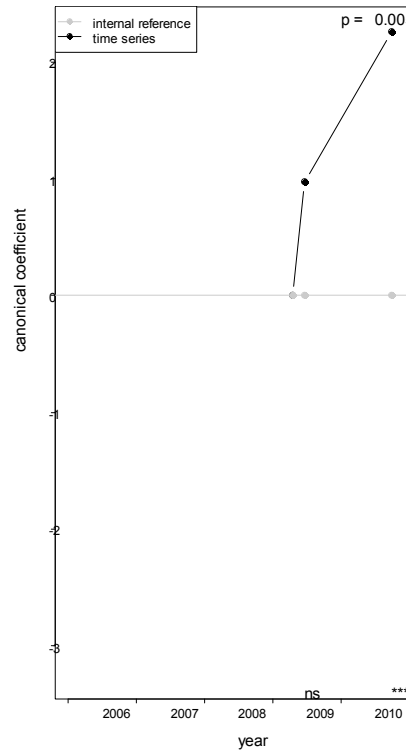




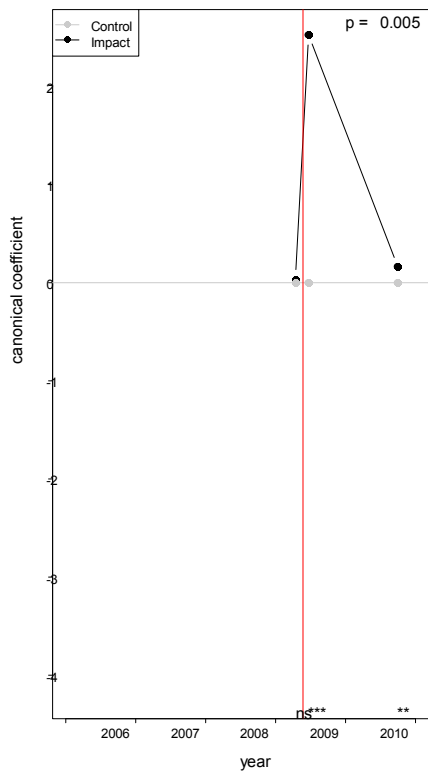
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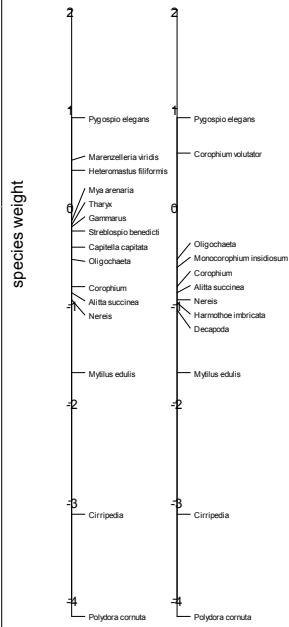
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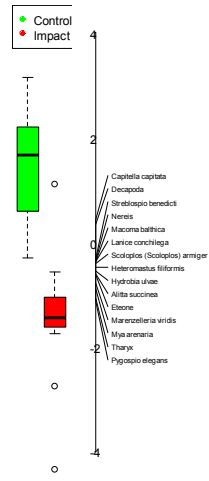
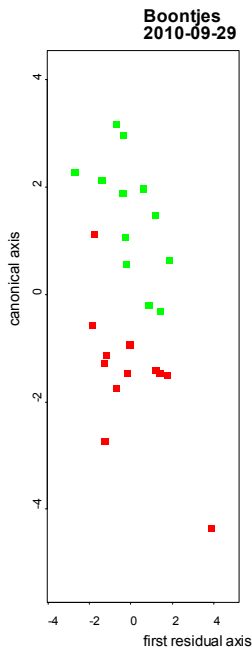
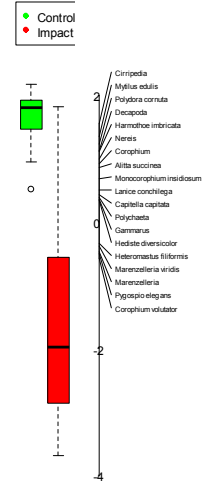
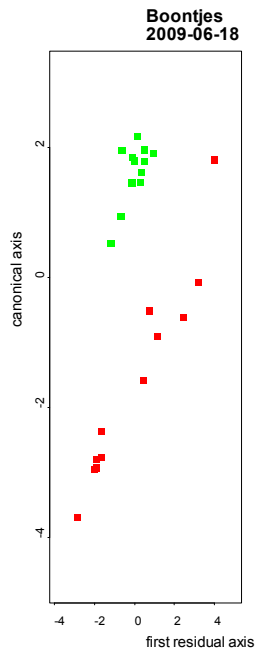
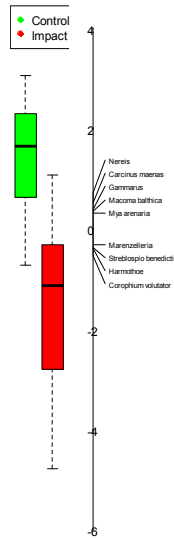
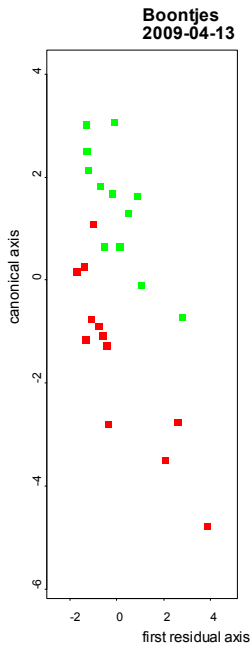


### Boontjes

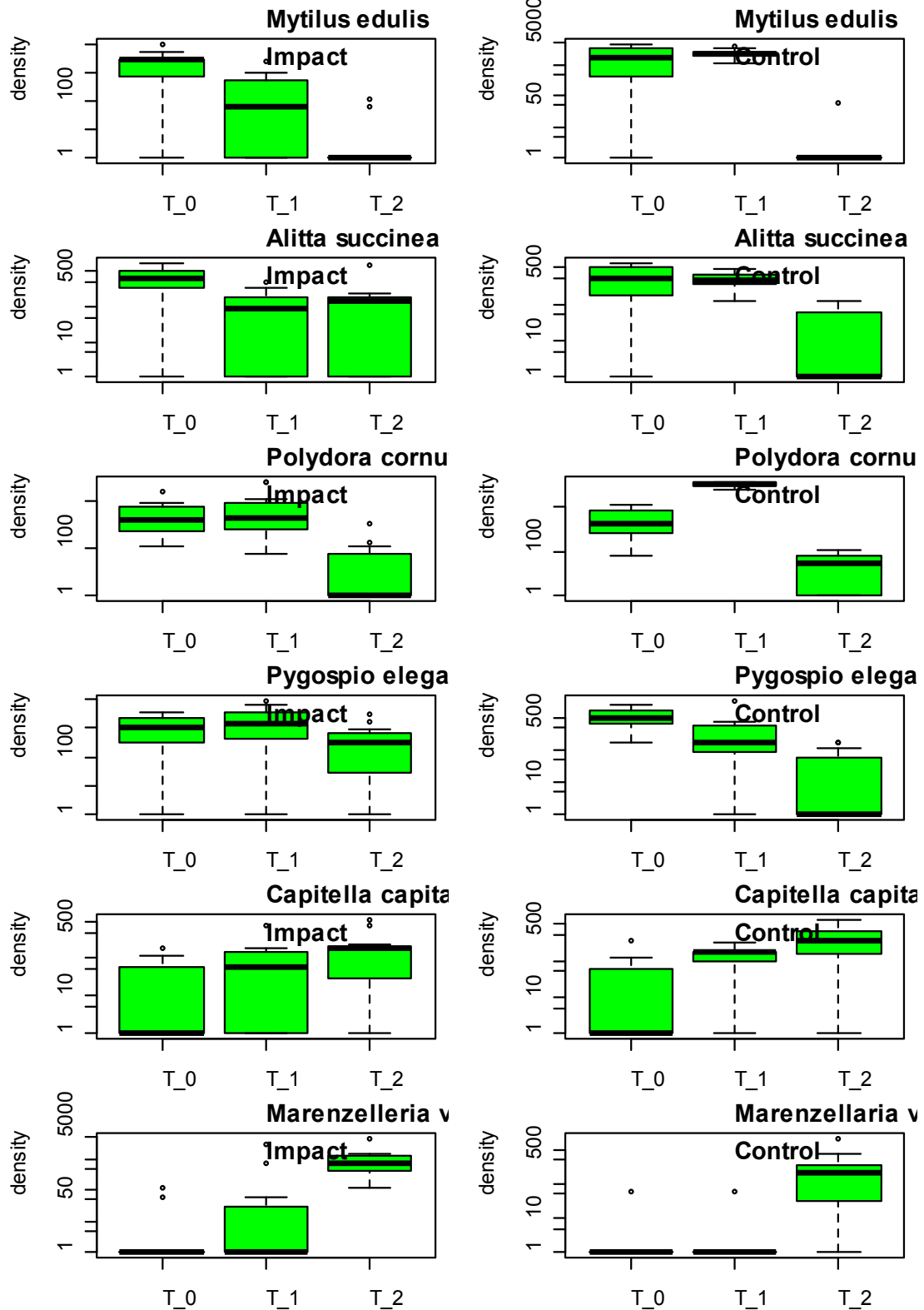


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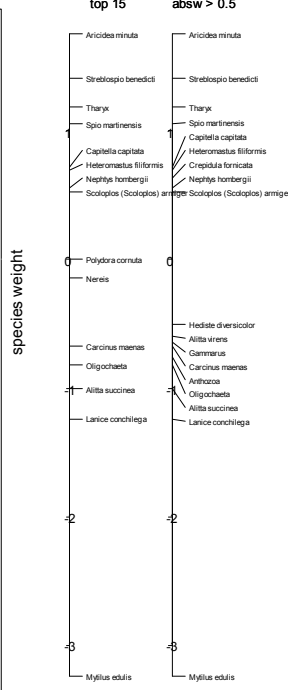
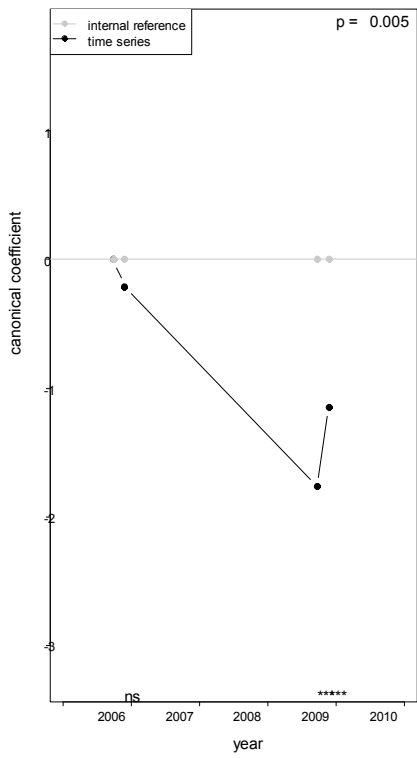




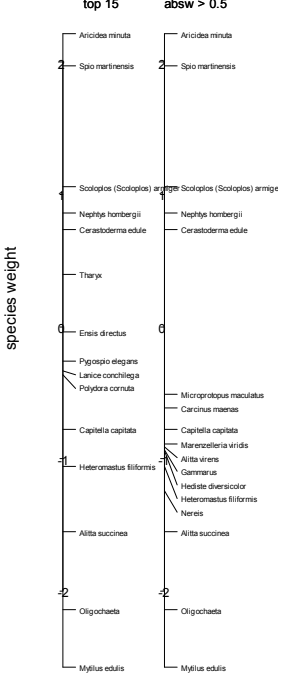
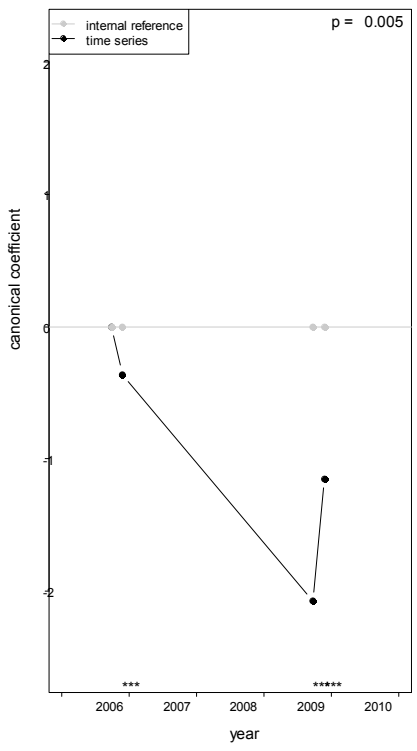
# Boontjes



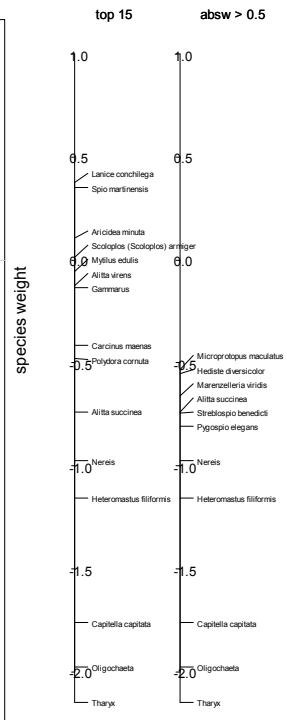
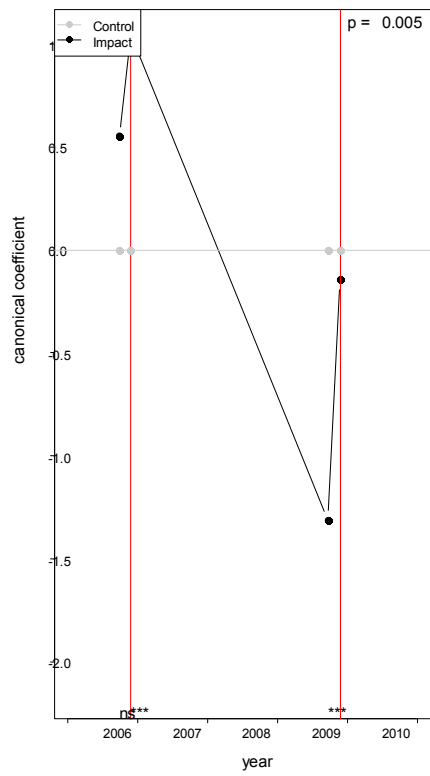
### Breesem Control

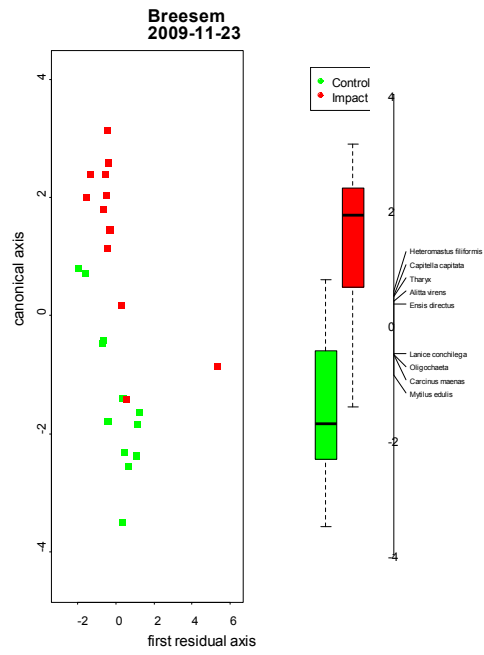
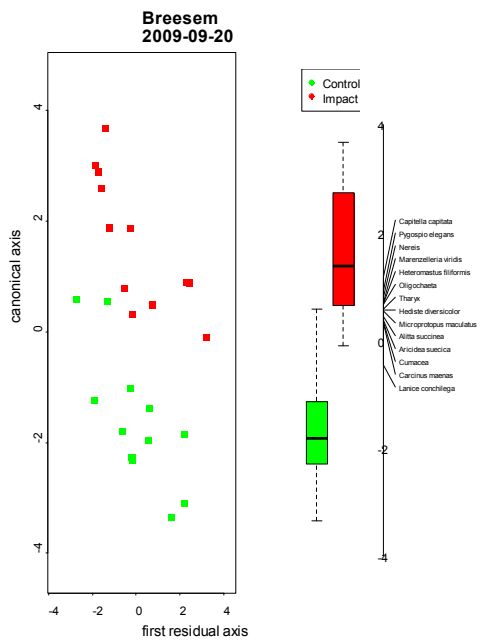
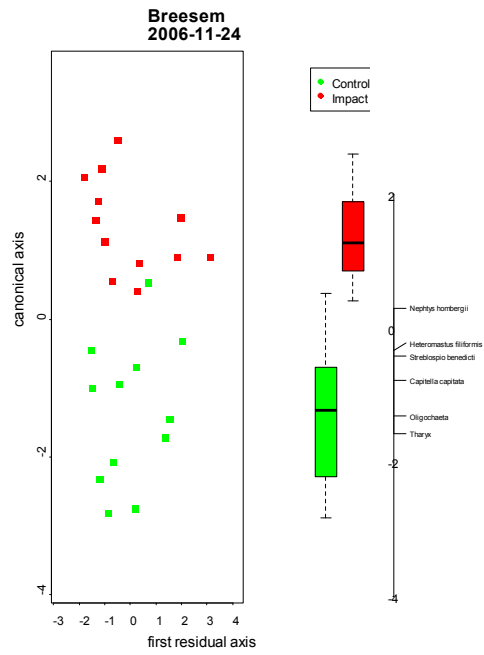
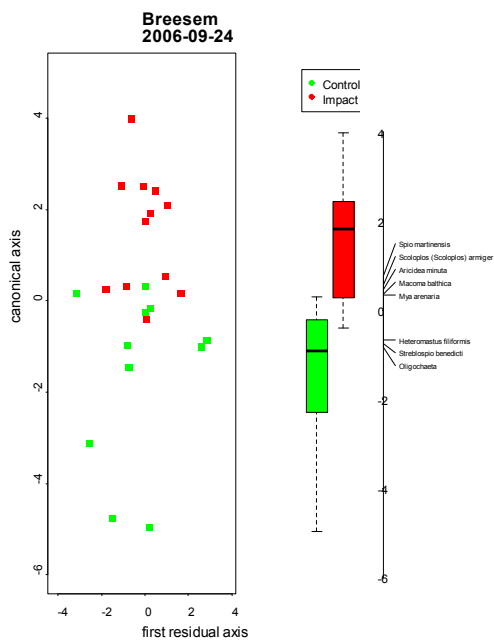


### Breesem Impact

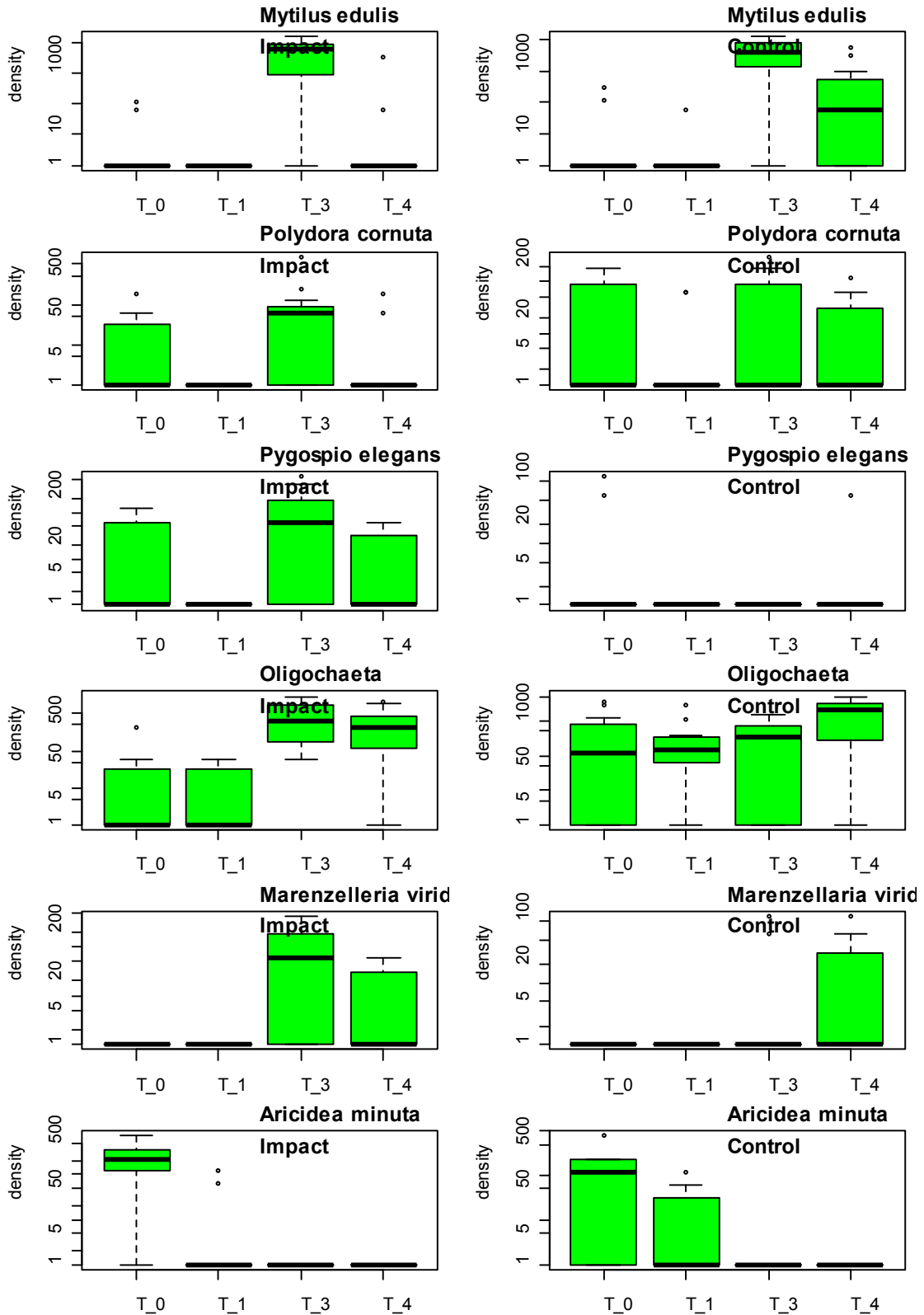


### Breesem



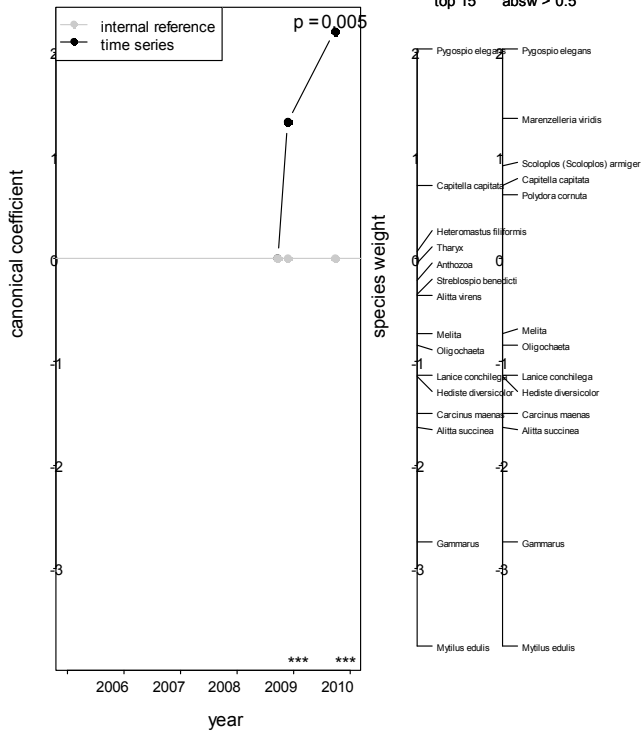


# Breeseem

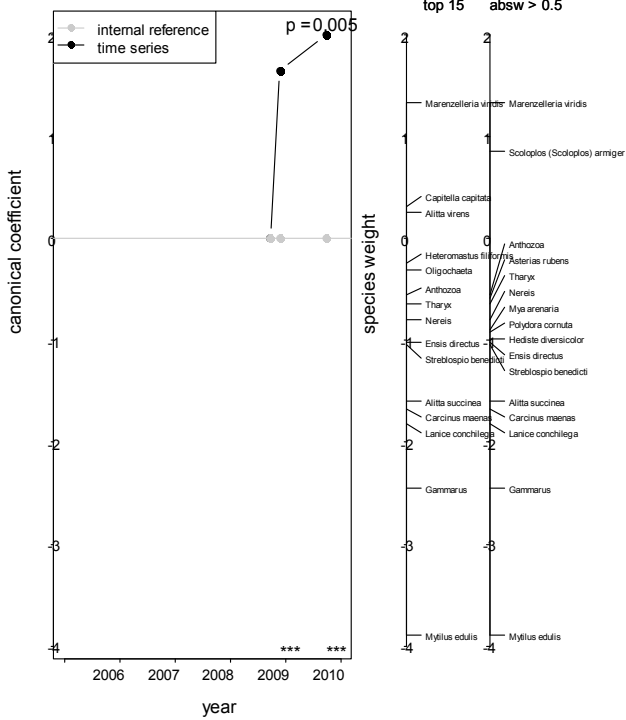




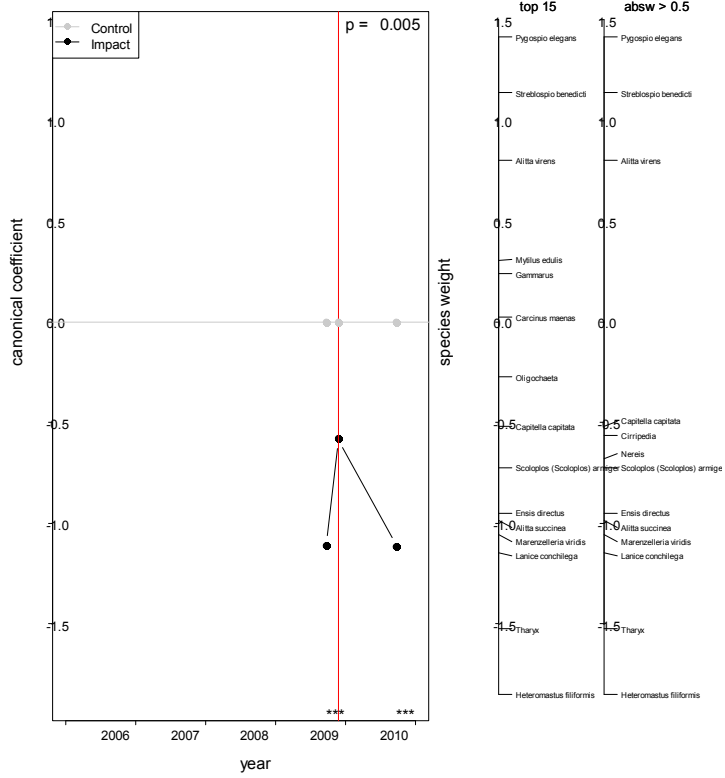
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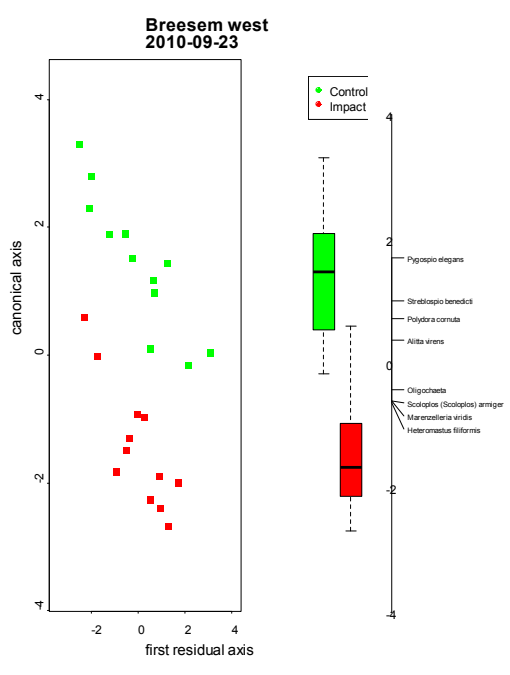
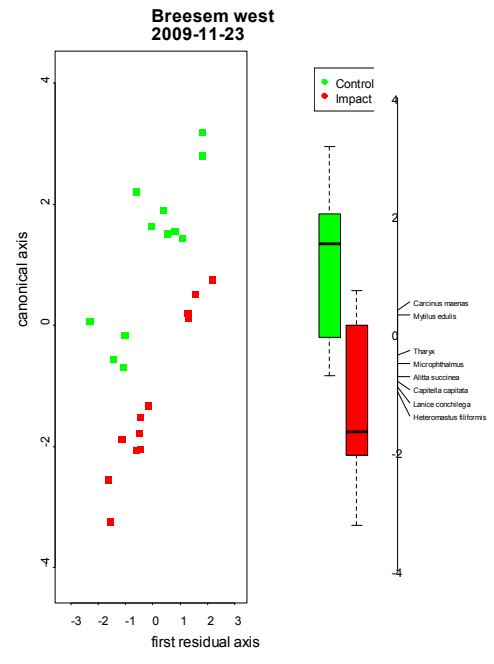
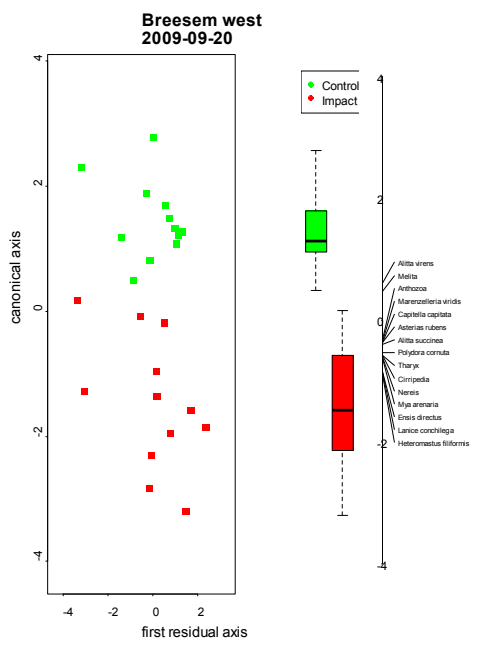


### Breesem west Impact

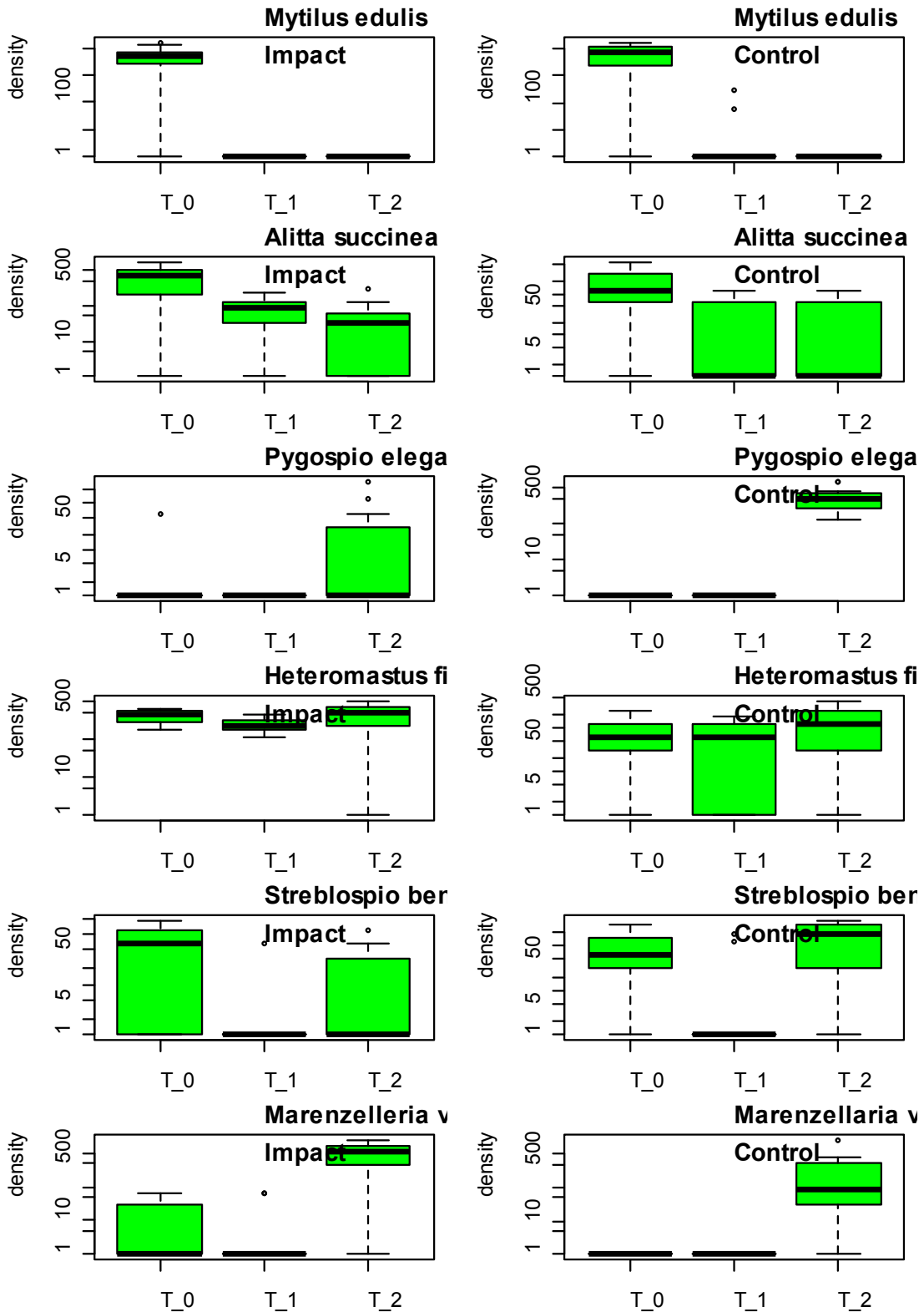


### Breesem west

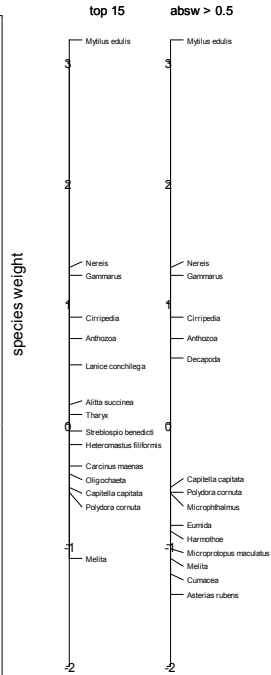
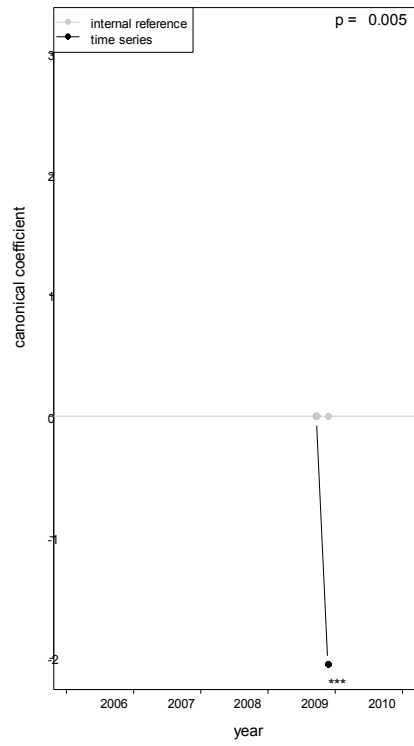




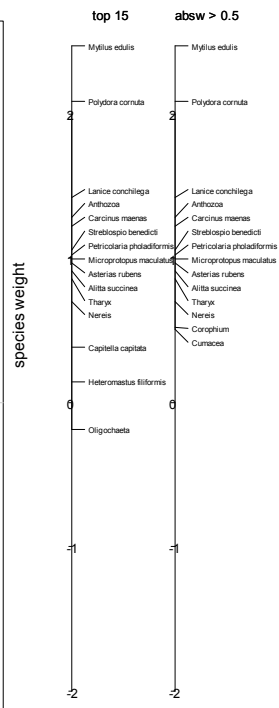
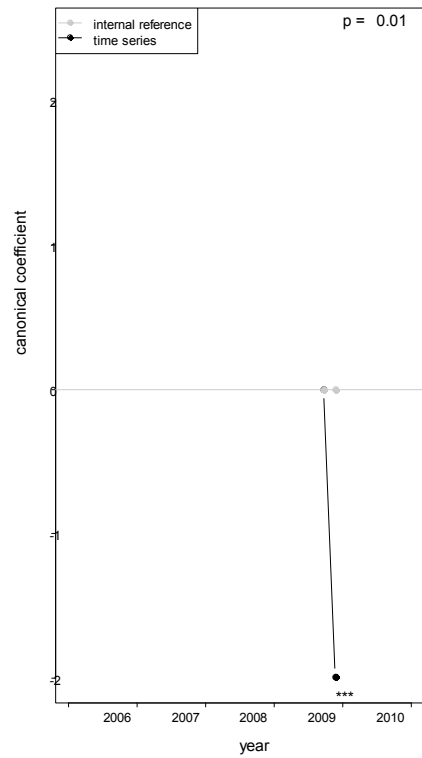
# Breesem west



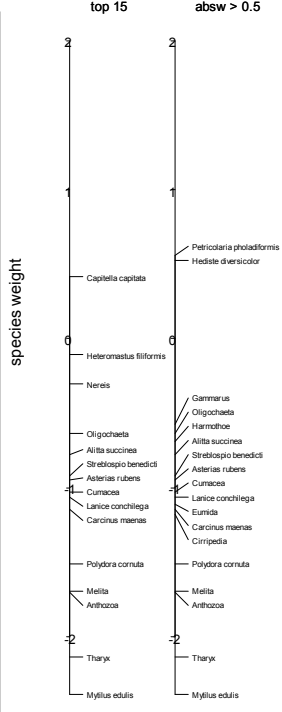
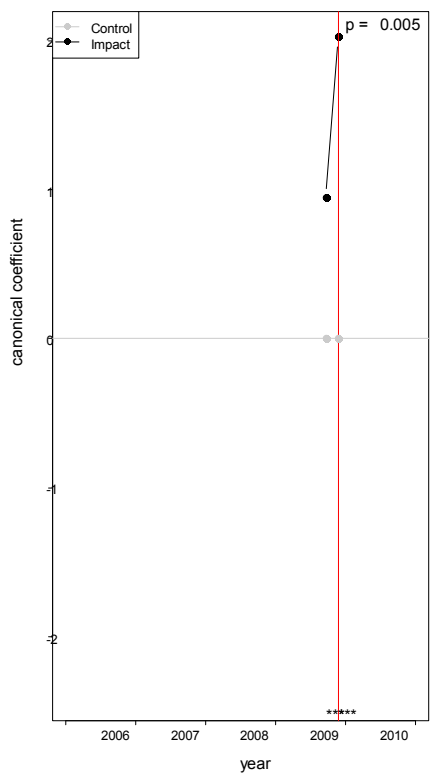
**Breesem zuid  
Control**



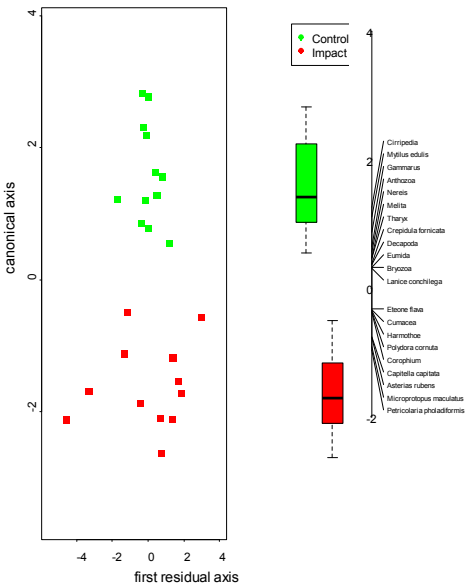
**Breesem zuid  
Impact**



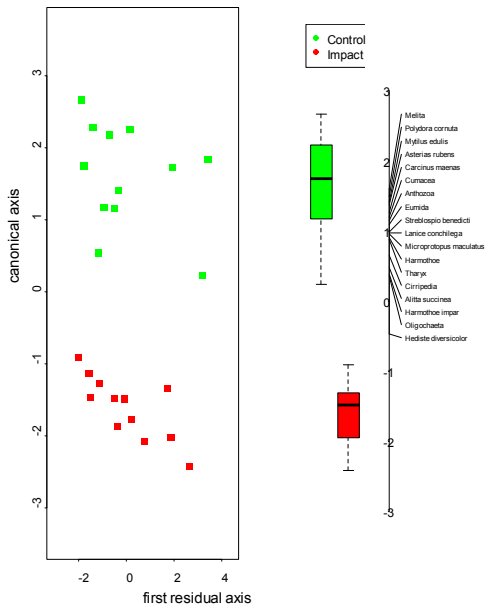
**Breesem zuid**



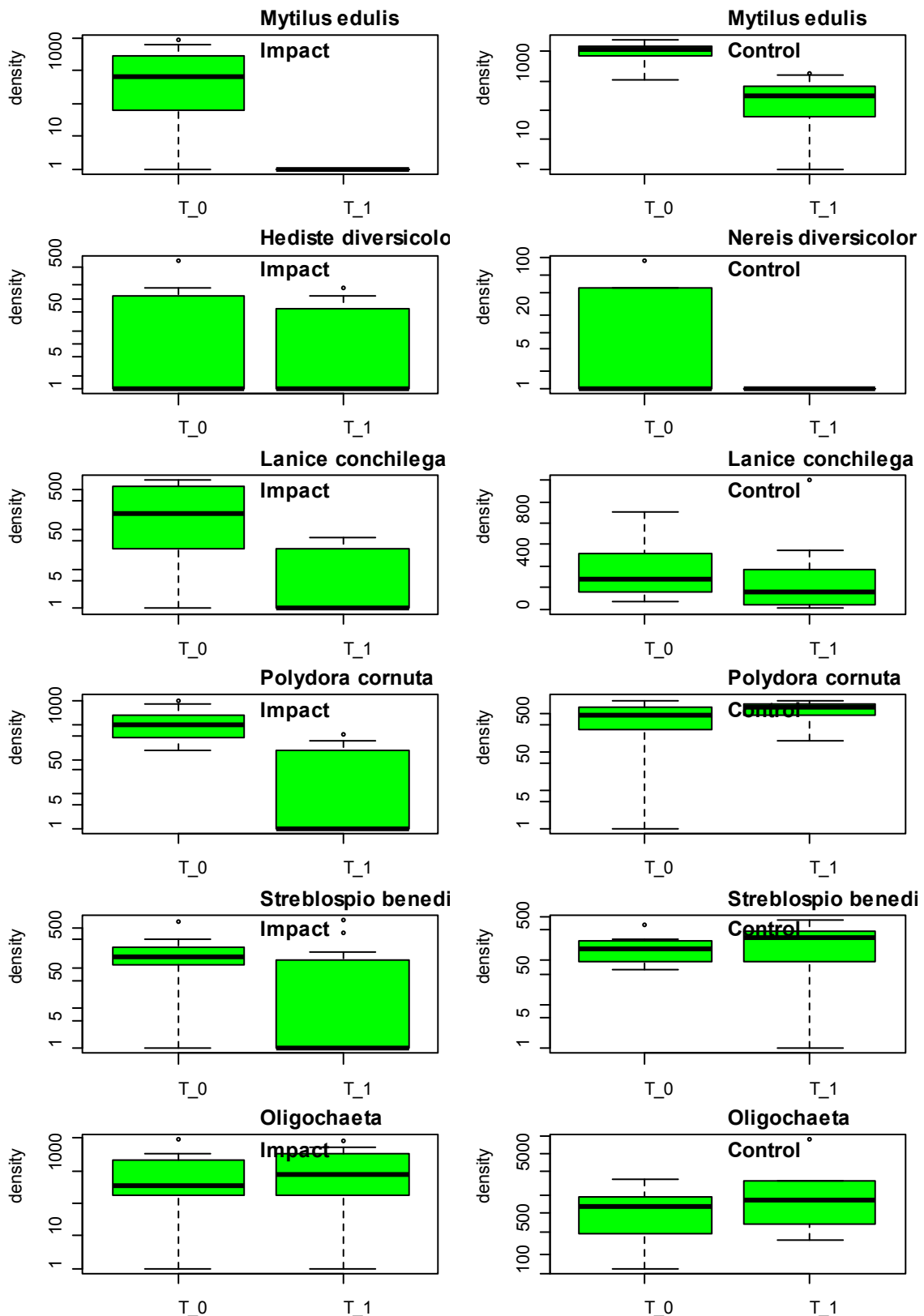
**Breesem zuid 2009-09-20**



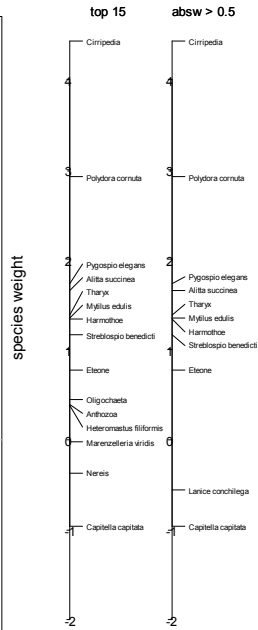
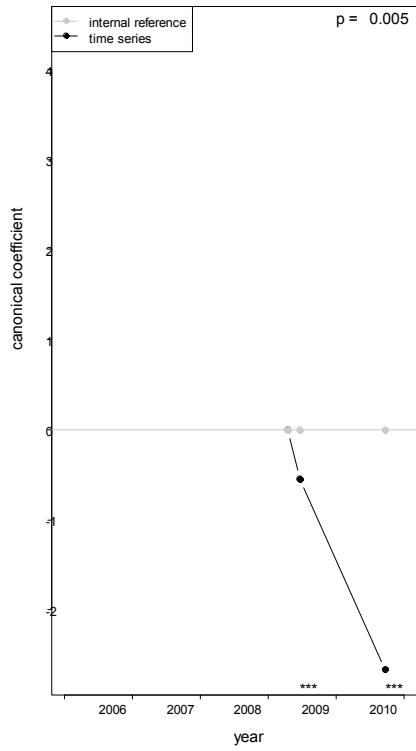
**Breesem zuid 2009-11-23**



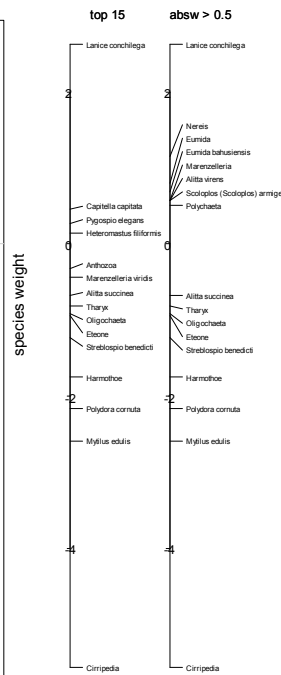
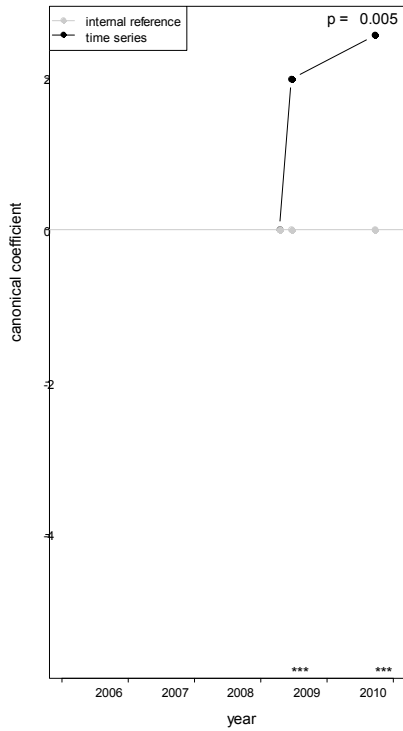
## Breesem zuid



### Breezanddijk Control

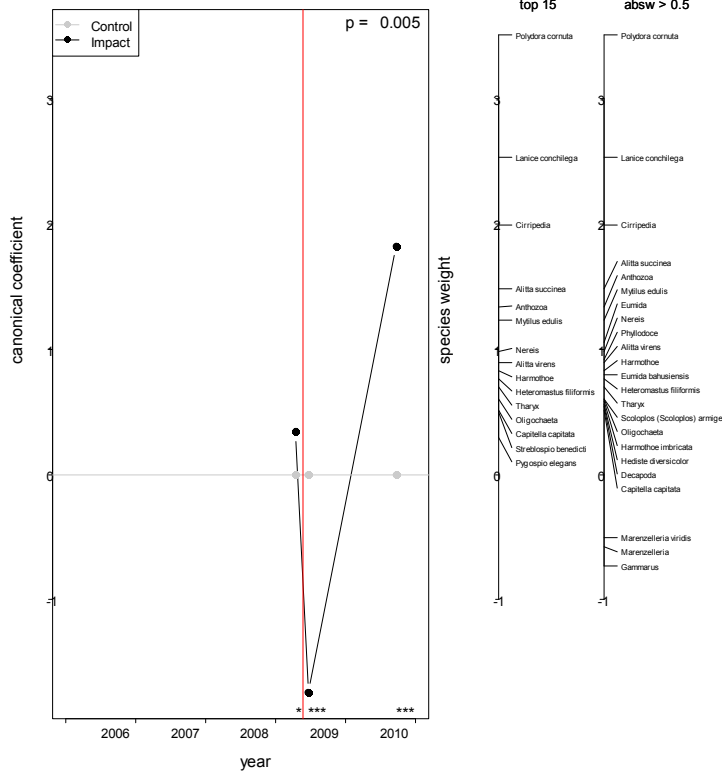


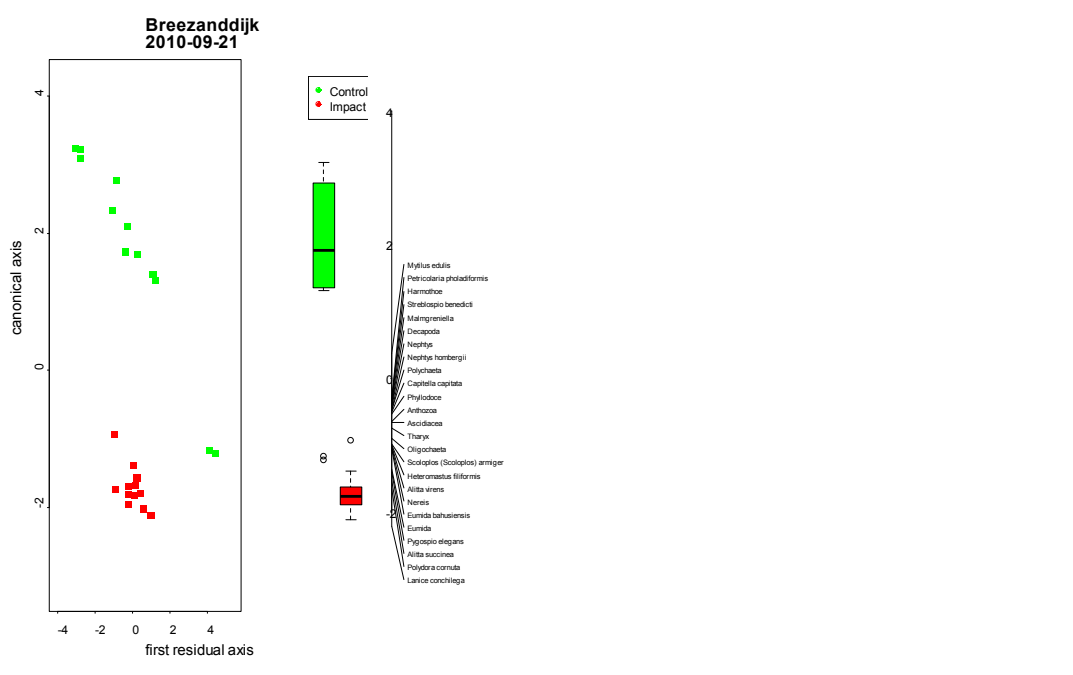
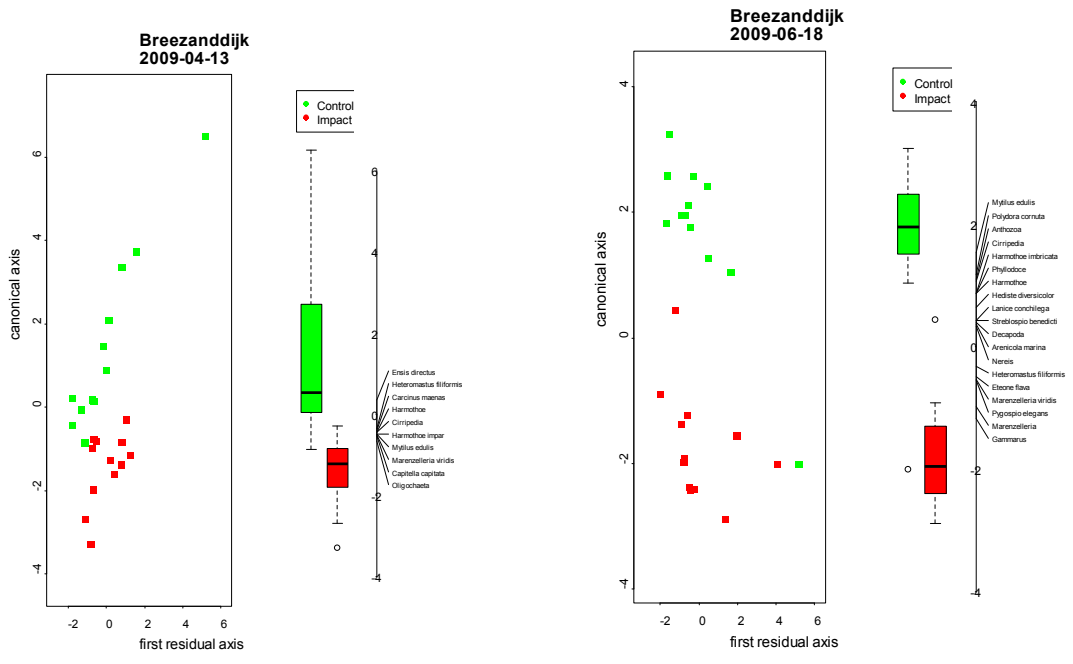
### Breezanddijk Impact



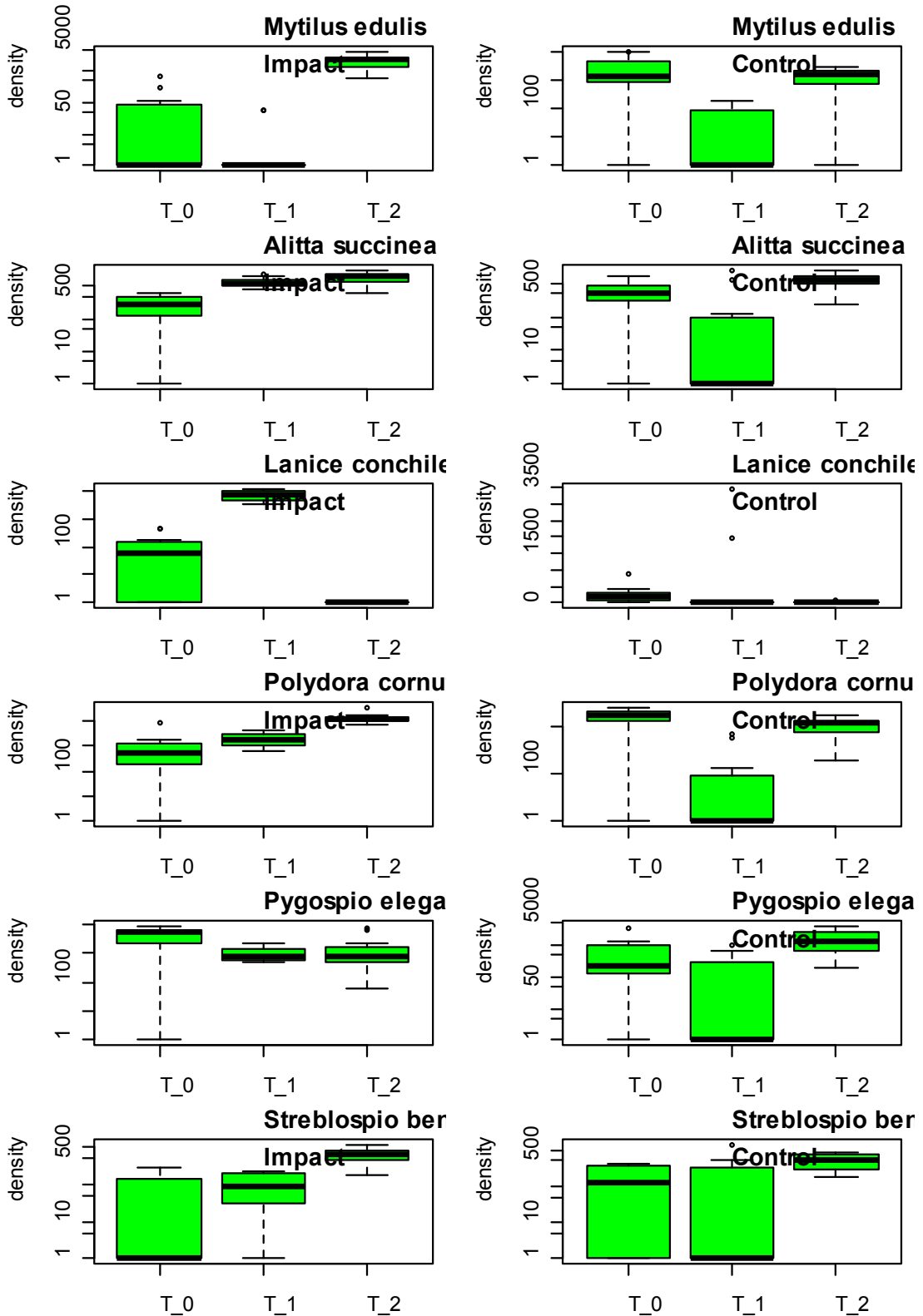


### Breezanddijk

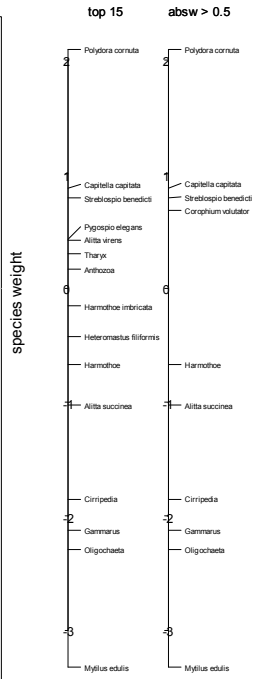
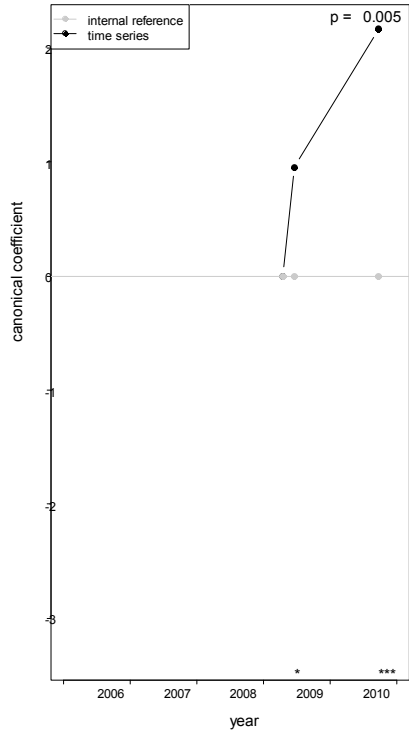




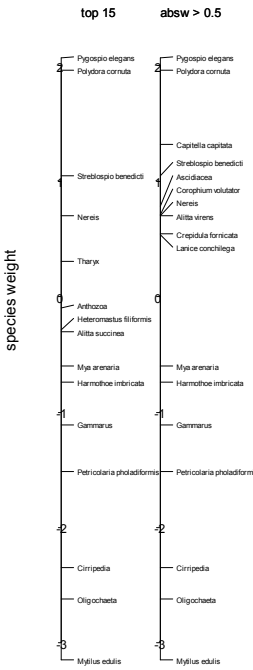
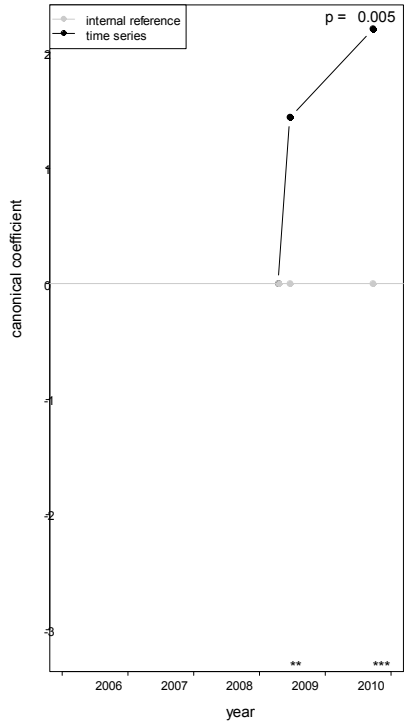
# Breezanddijk



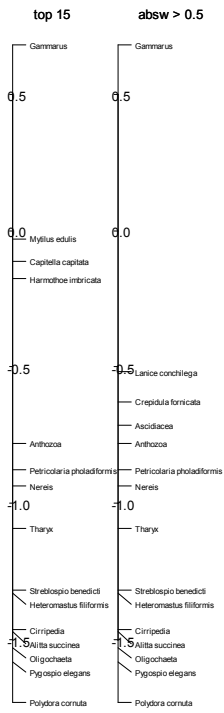
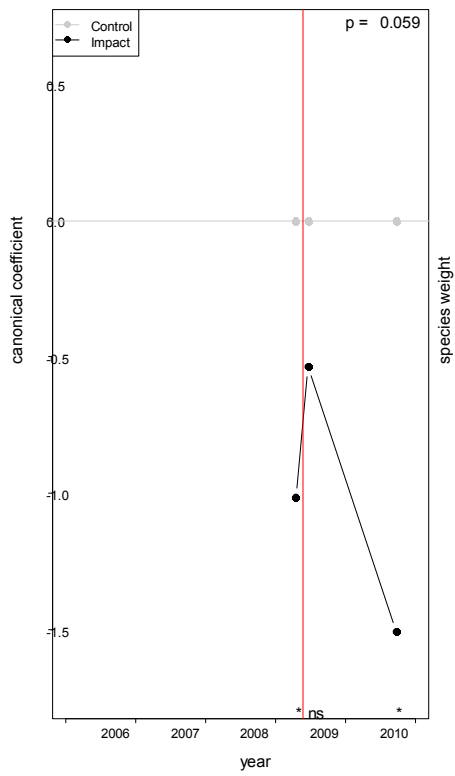
### Doovebalg Control

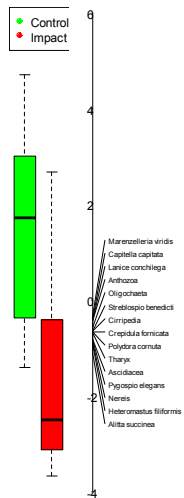
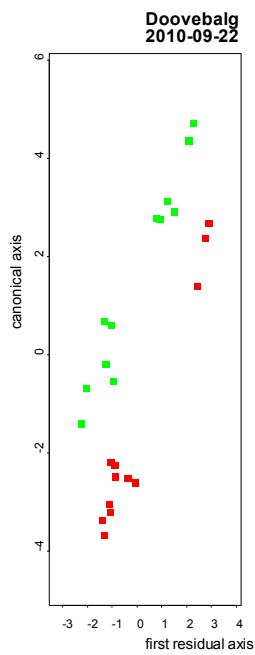
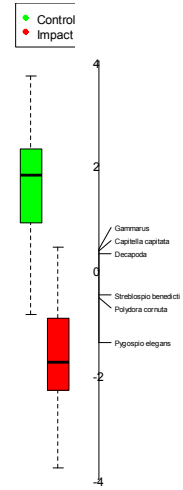
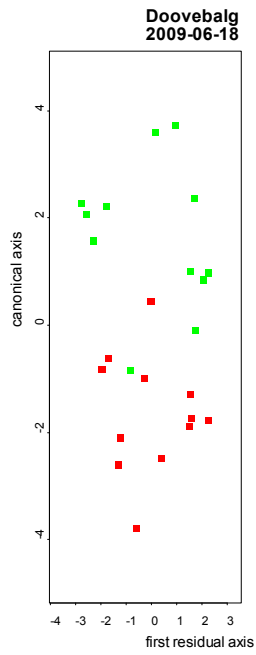
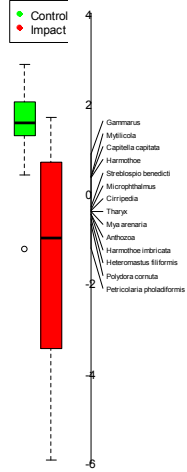
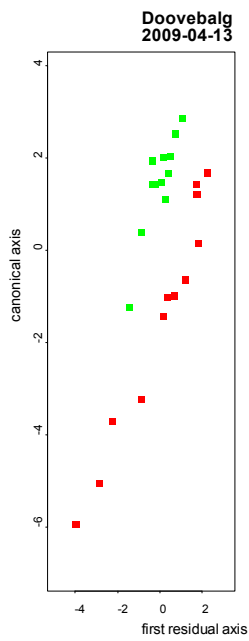


### Doovebalg Impact

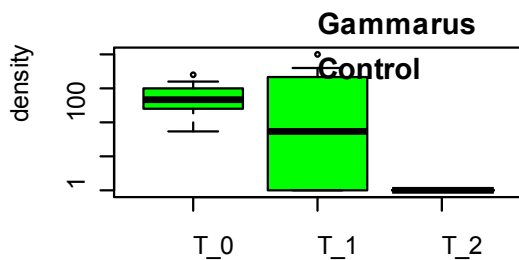
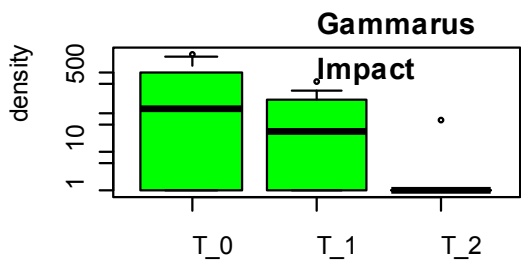
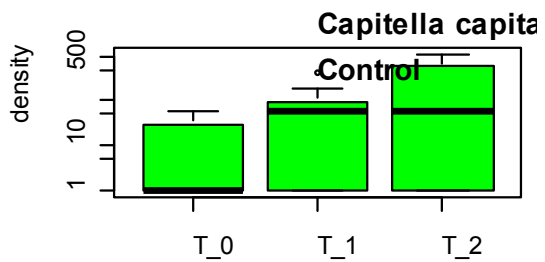
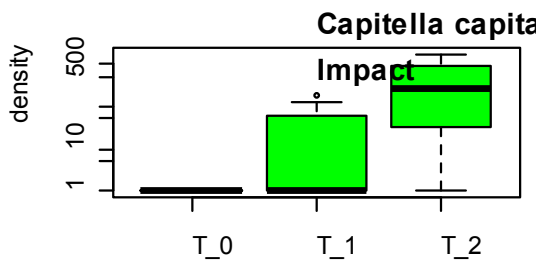
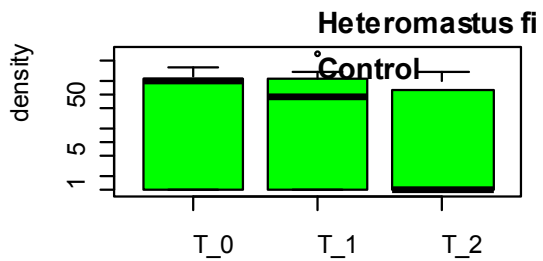
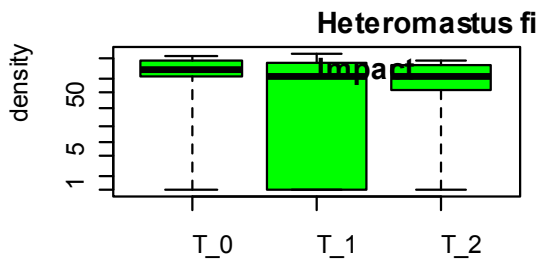
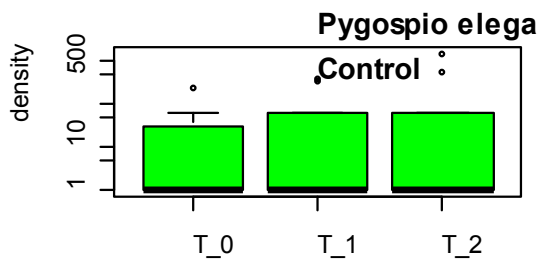
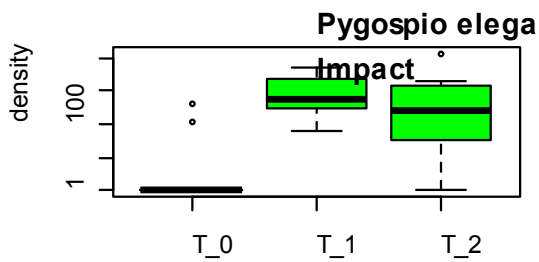
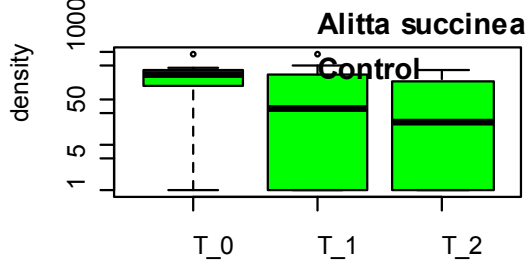
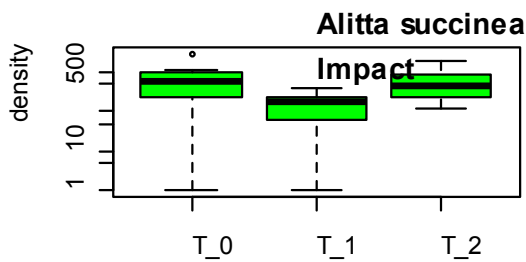
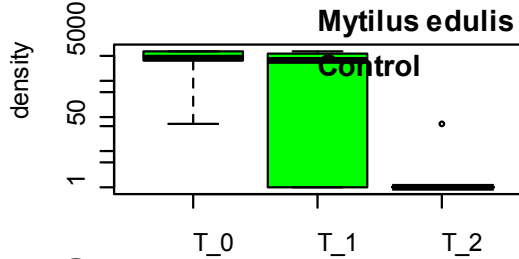
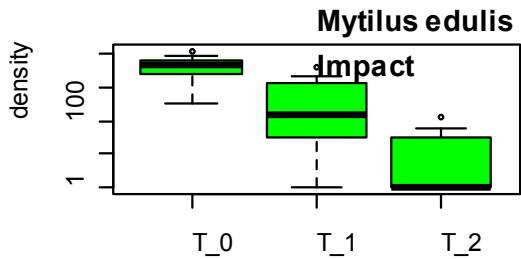


### Doovebalg

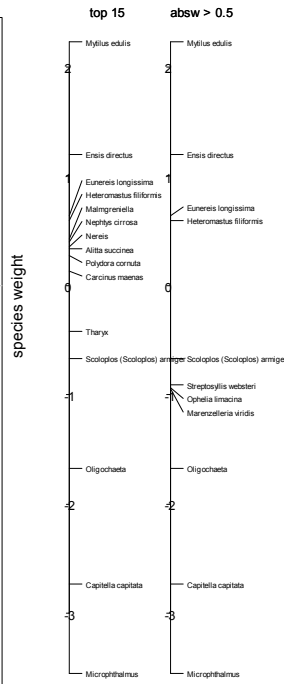
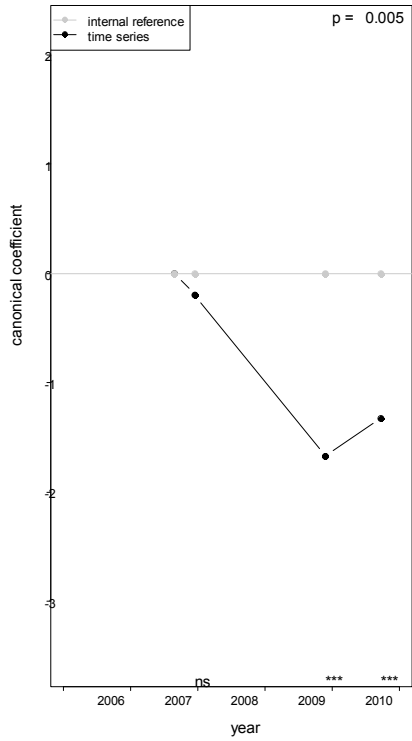




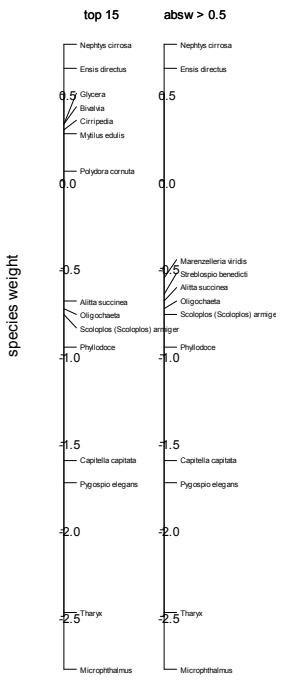
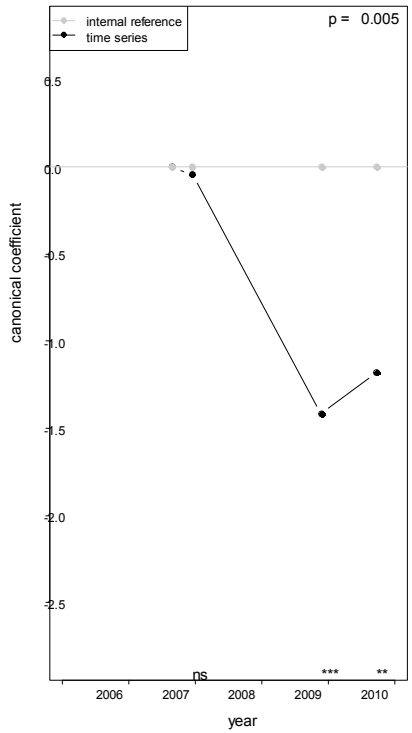
# Doovebalg



### Gat van Stompe Control

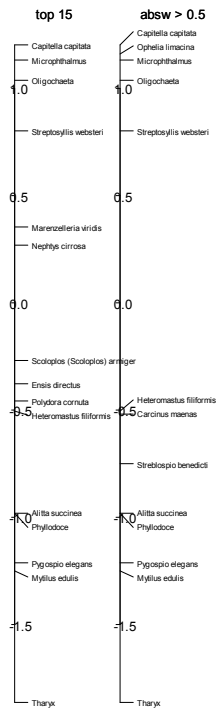
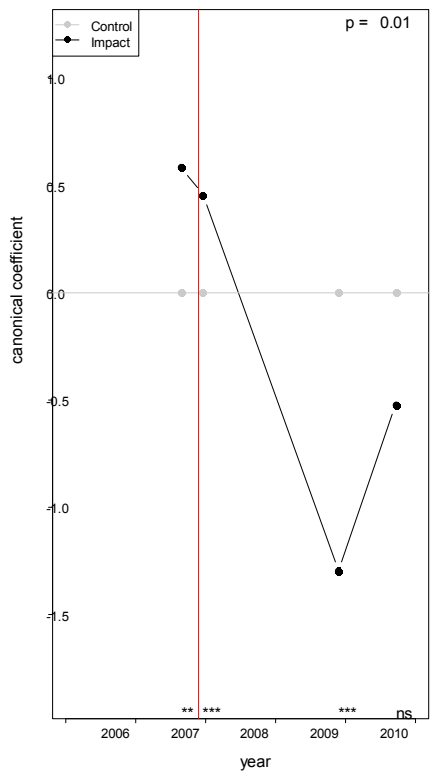


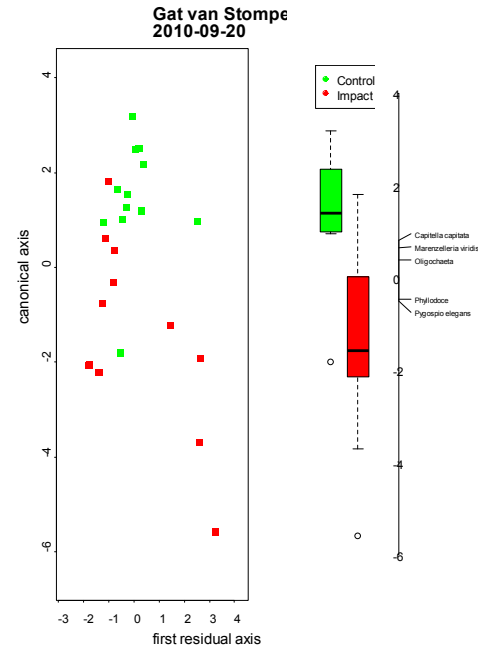
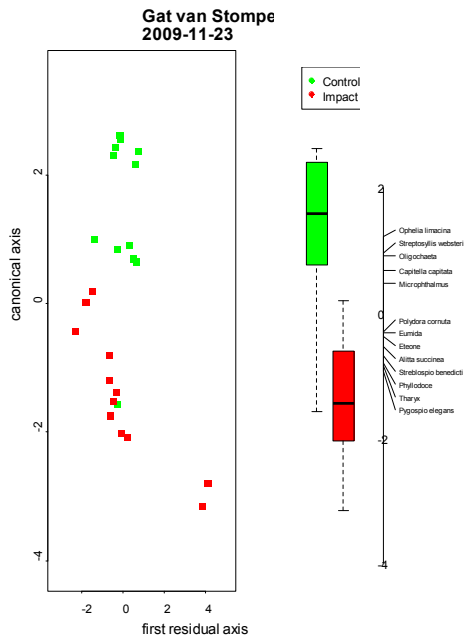
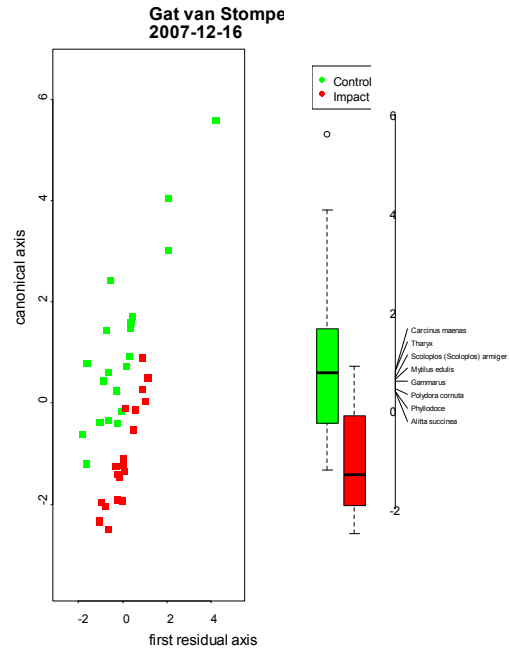
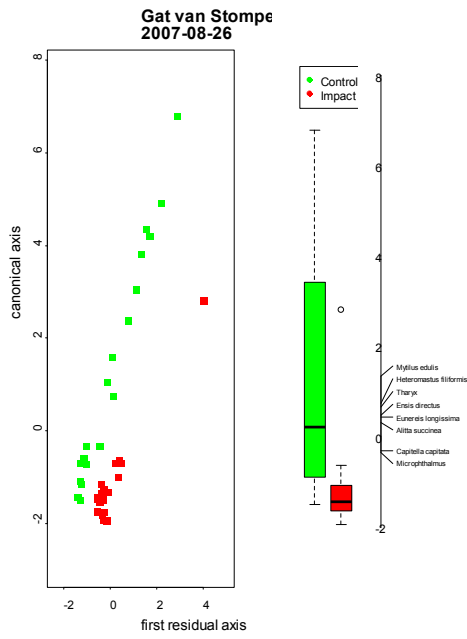
### Gat van Stompe Impact



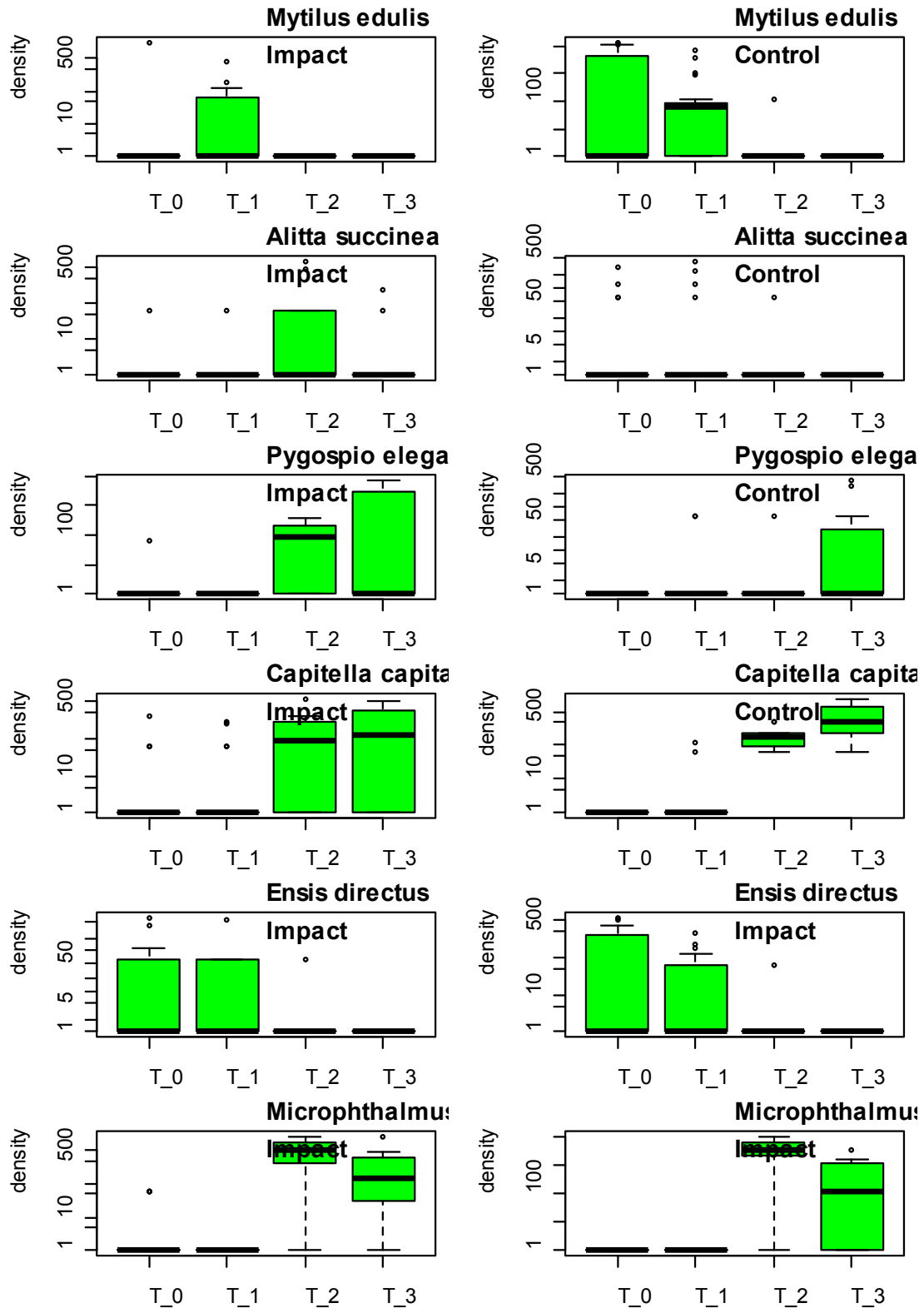


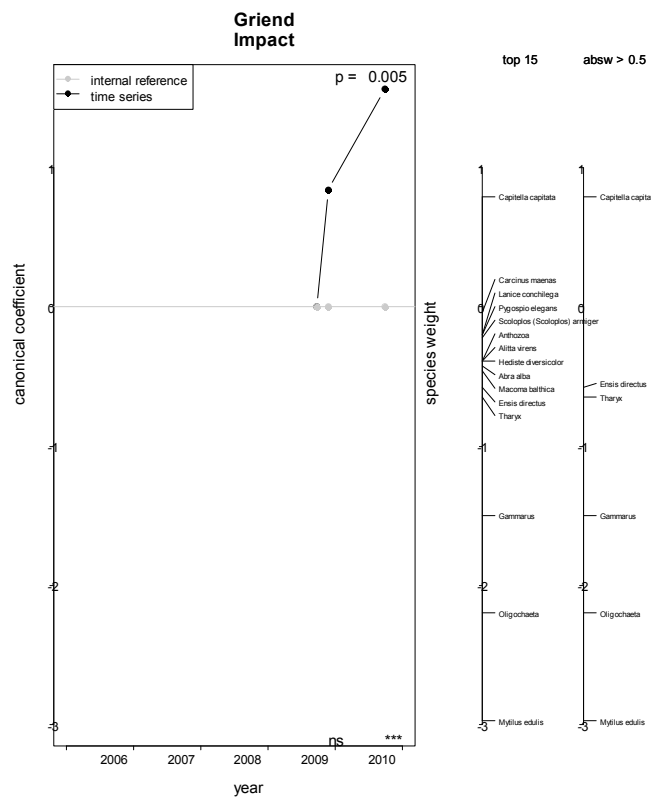
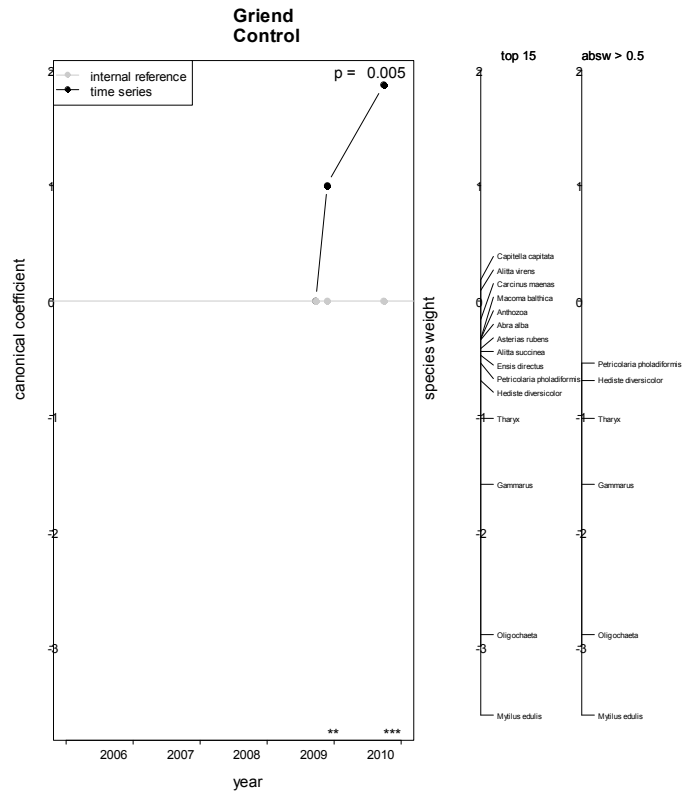
### Gat van Stompe



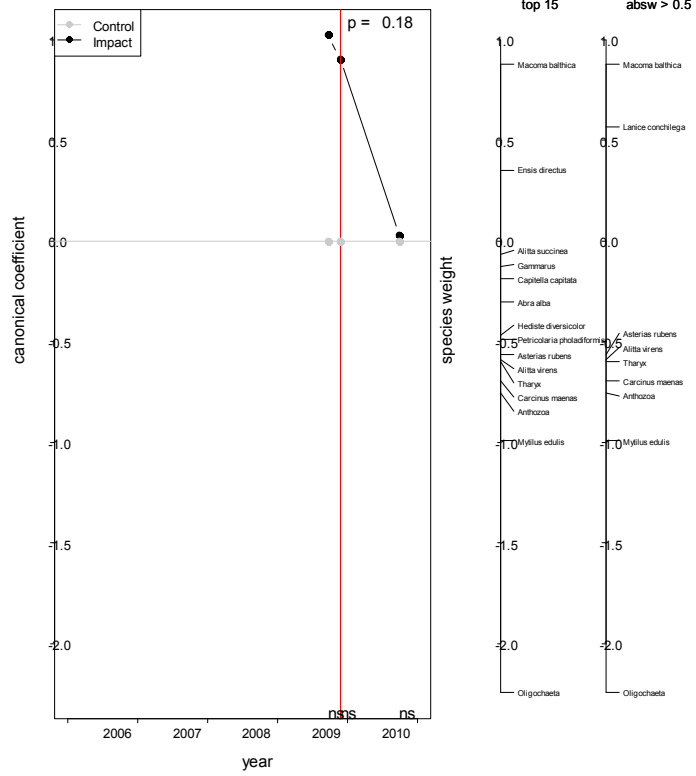


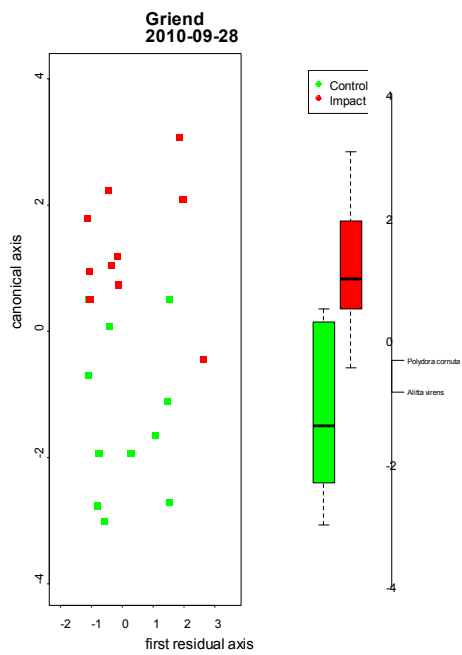
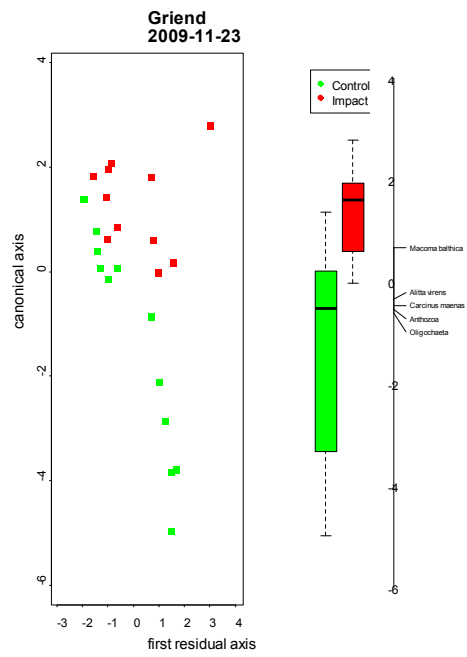
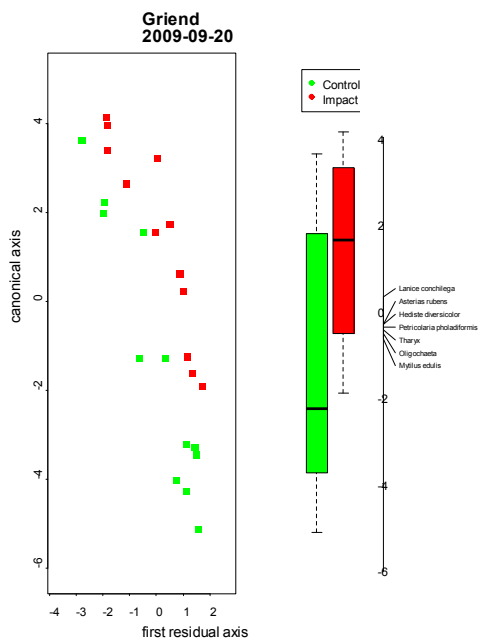
# Gat van Stompe



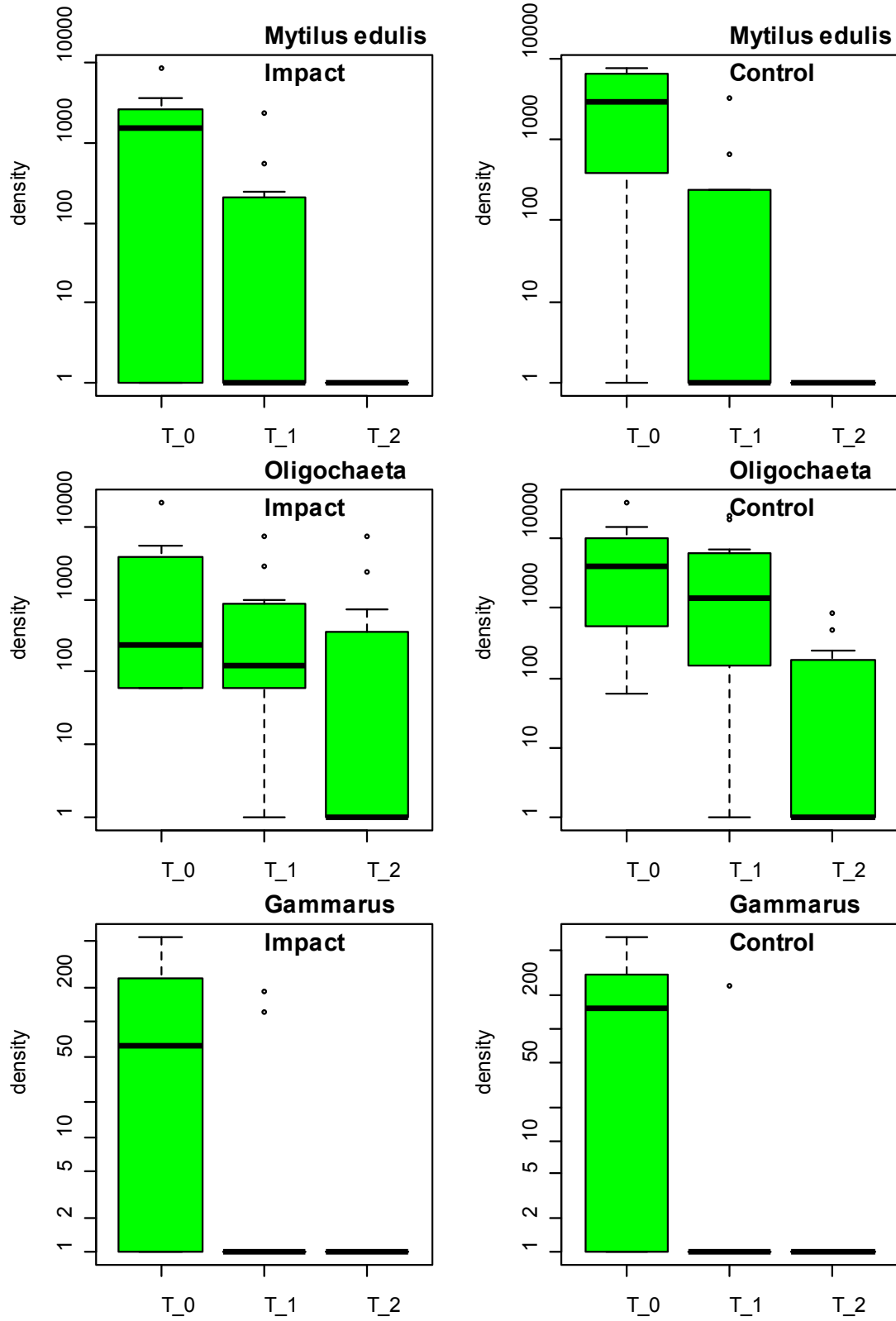


### Griend

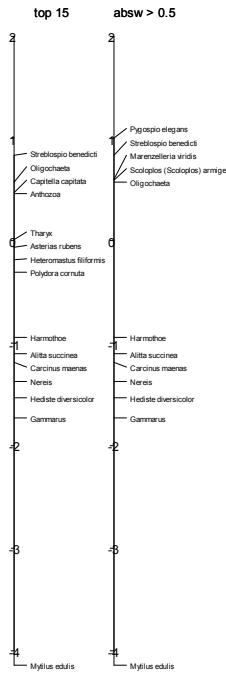
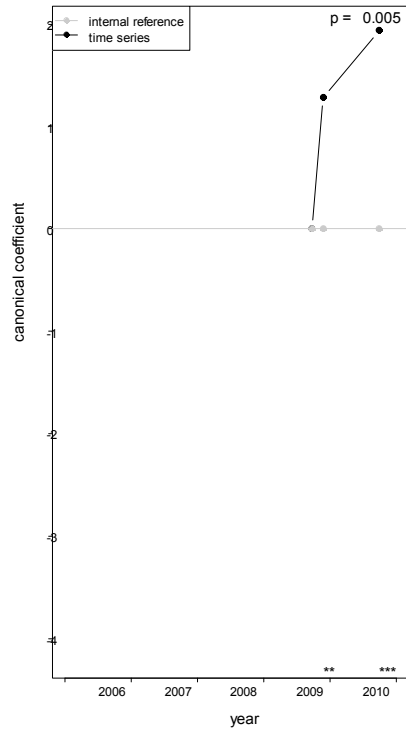




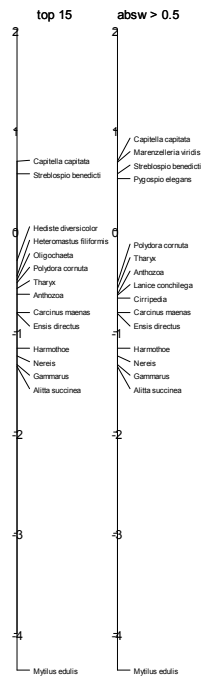
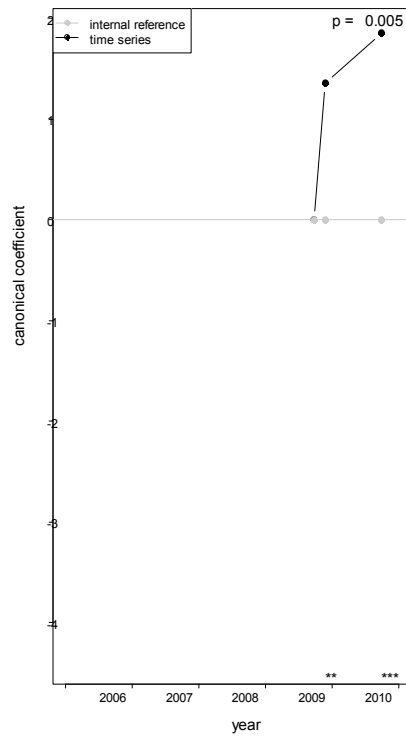
# Griend



### Inschot Control

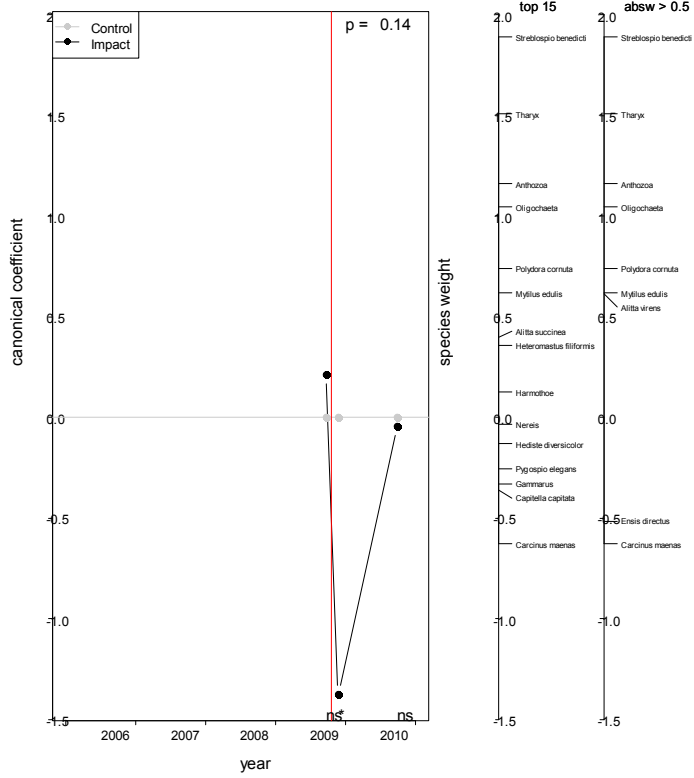


### Inschot Impact

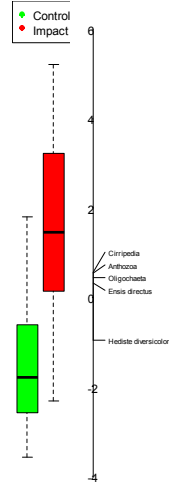
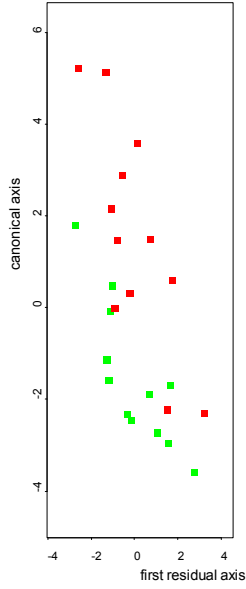




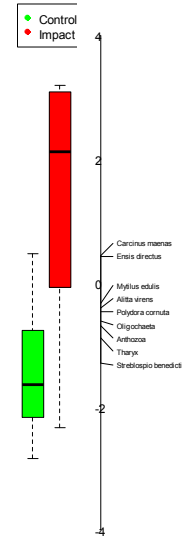
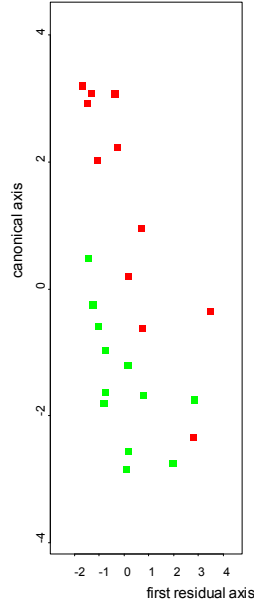
### Inschot



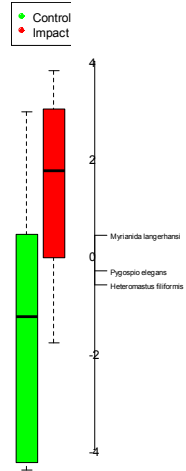
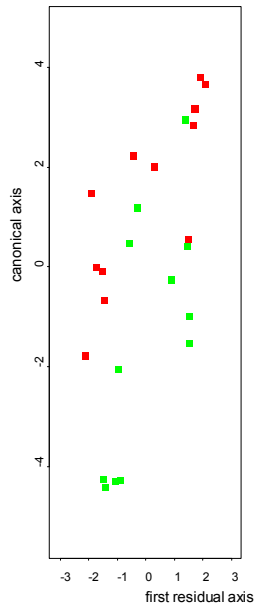
**Inshot  
2009-09-20**



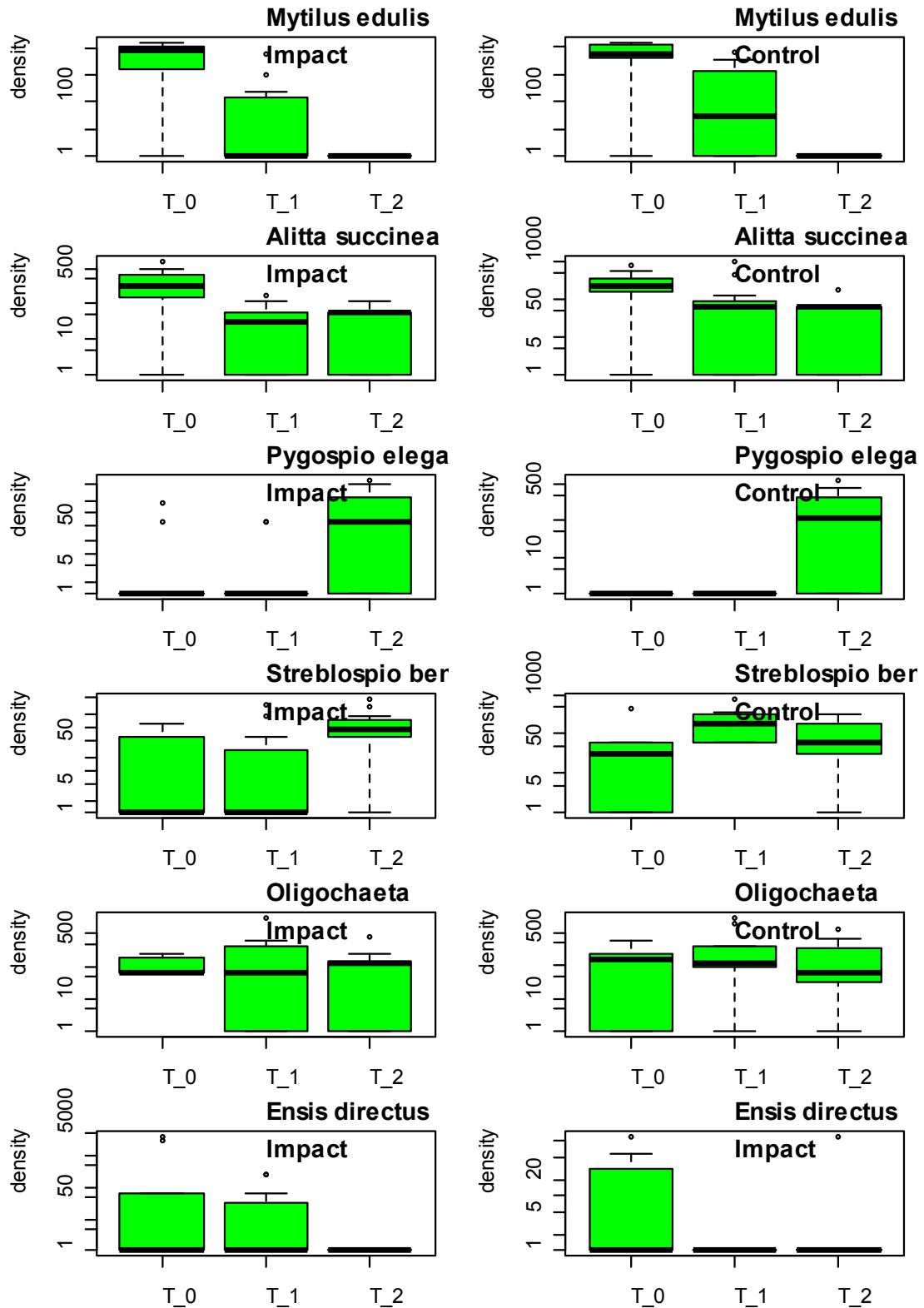
**Inshot  
2009-11-23**



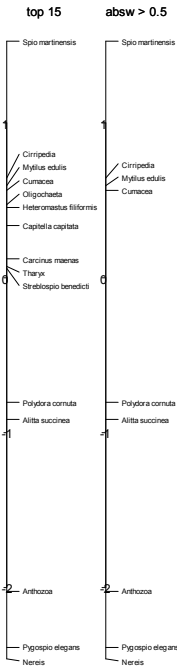
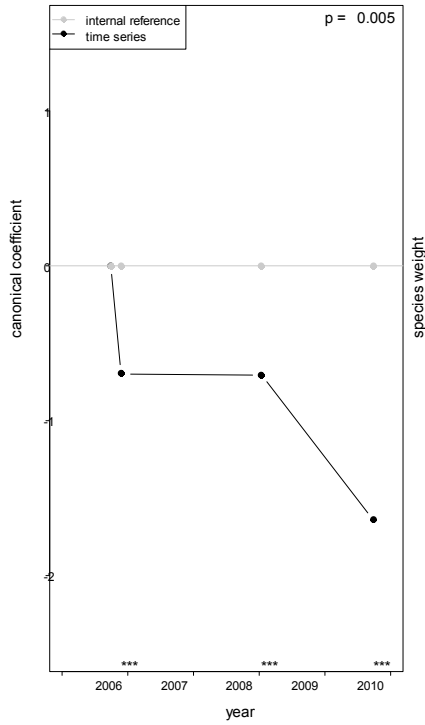
**Inshot  
2010-09-29**



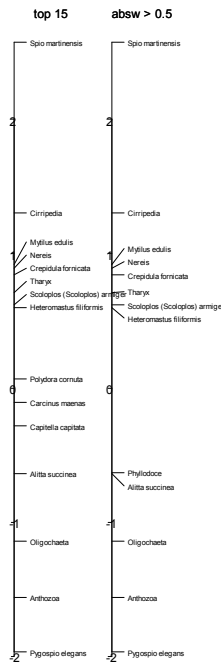
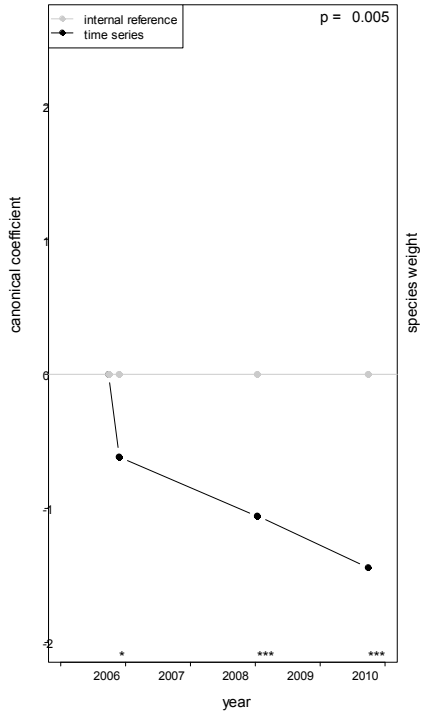
# Inschot



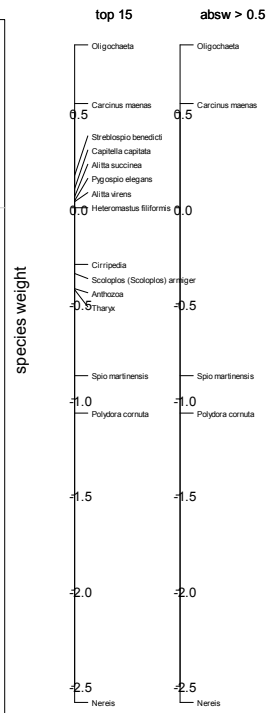
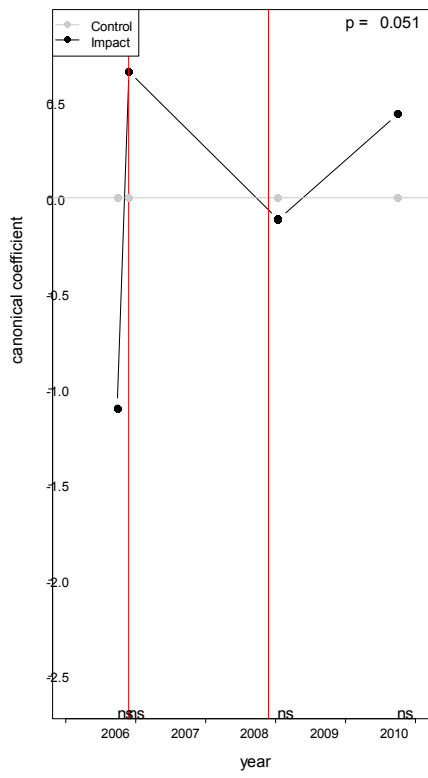
### Lutjewaard Control

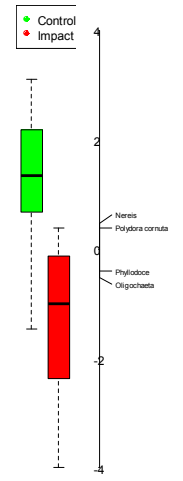
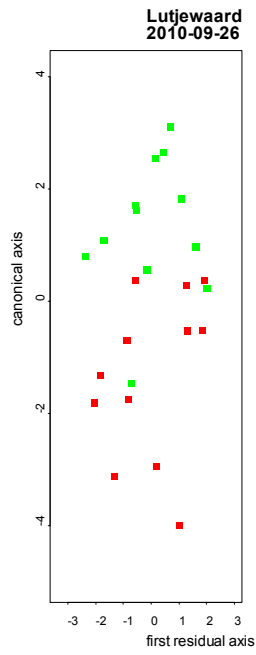
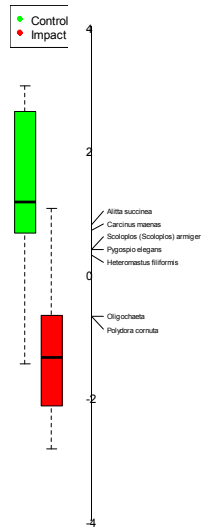
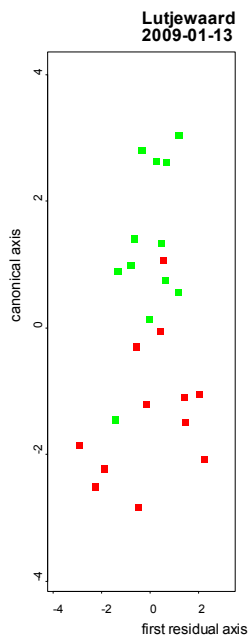
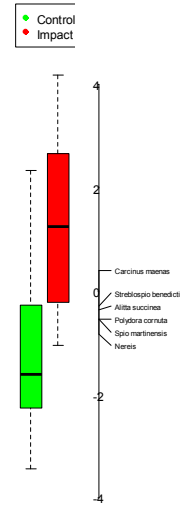
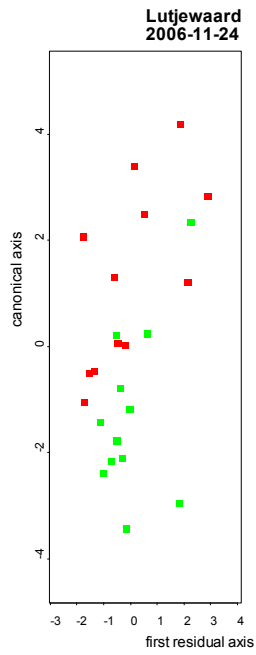
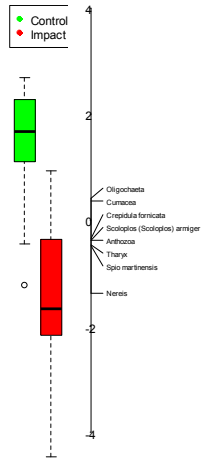
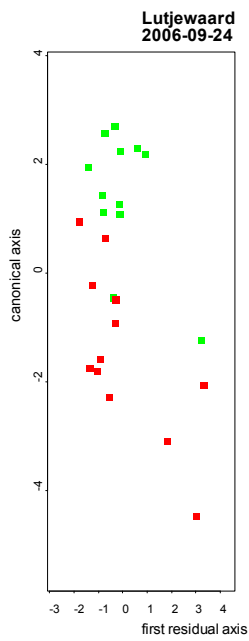


### Lutjewaard Impact

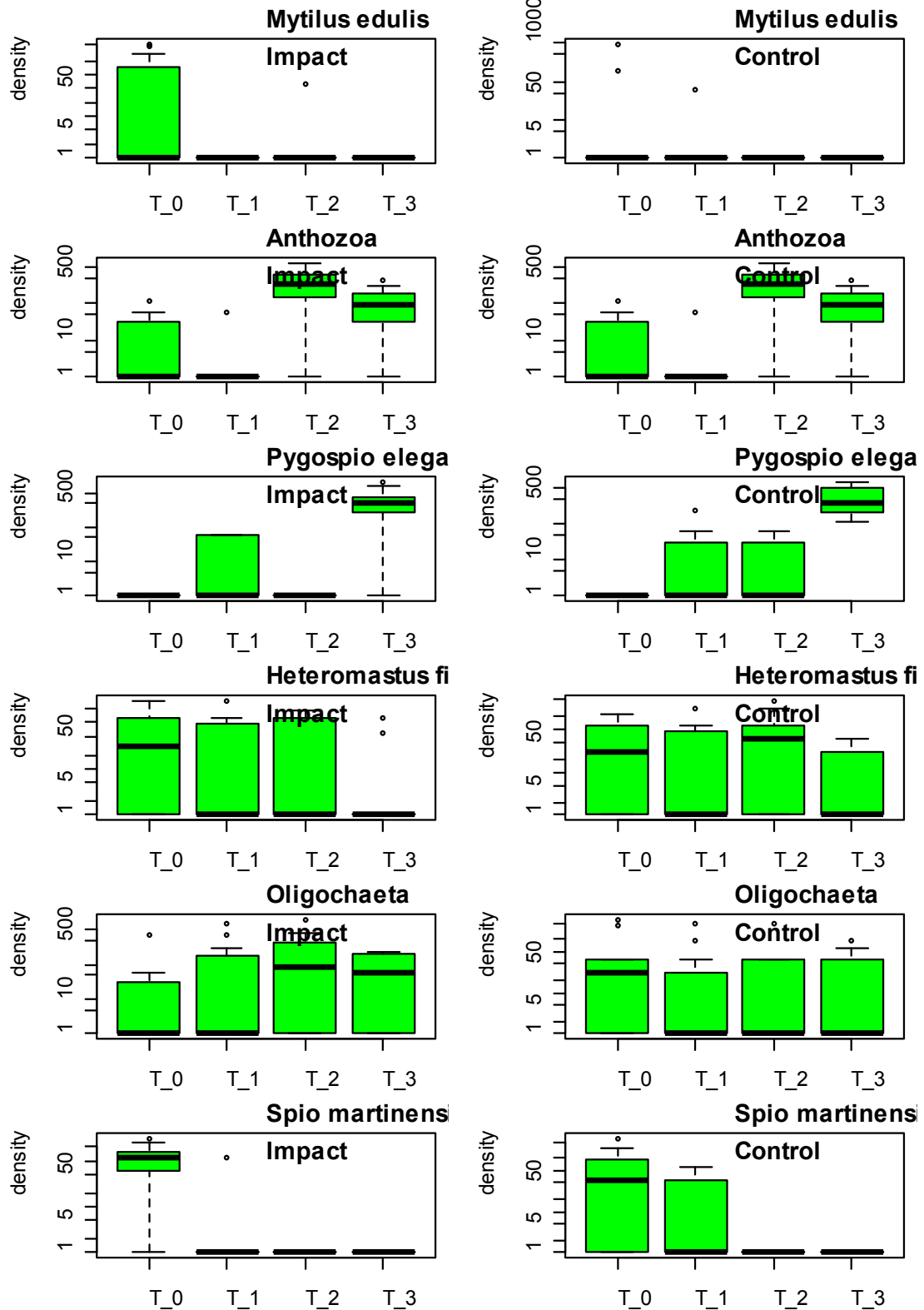


### Lutjewoord

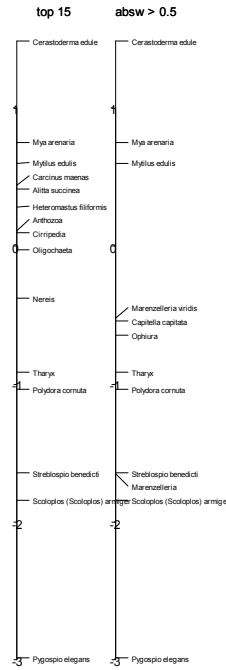
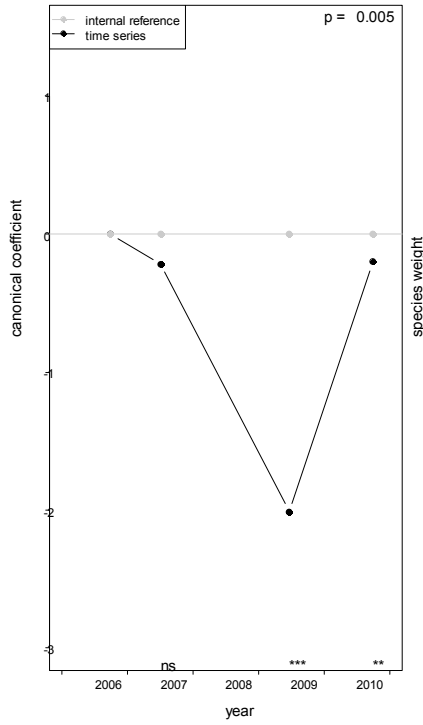




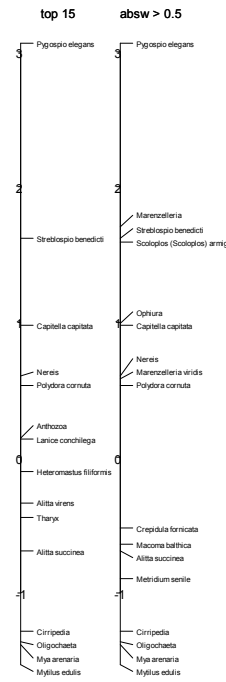
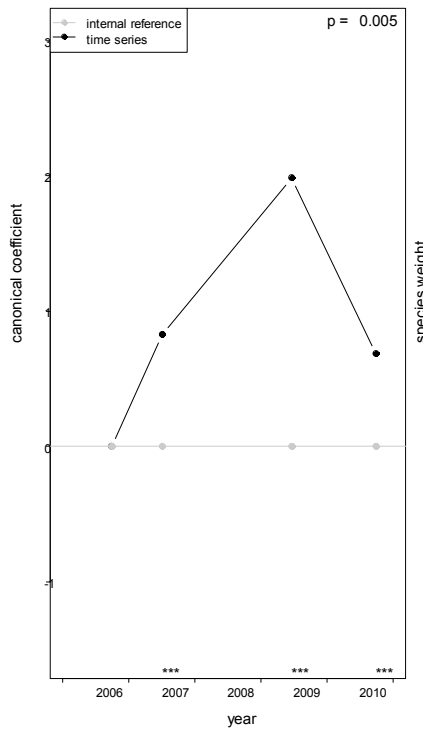
# Lutjewoord



### Molenrak West Control

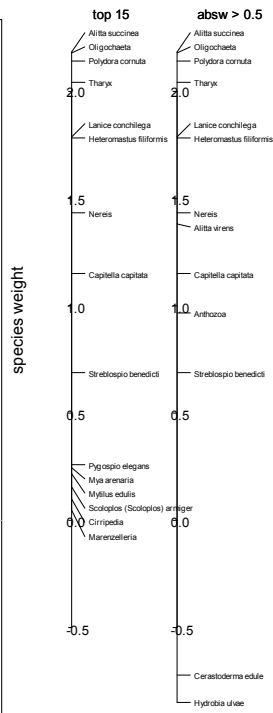
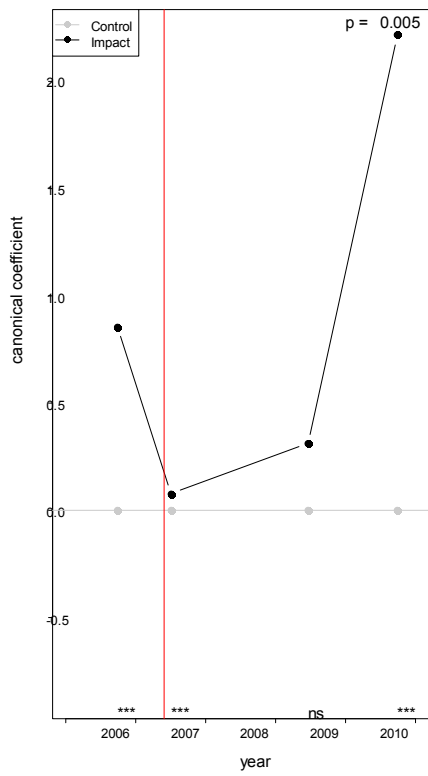


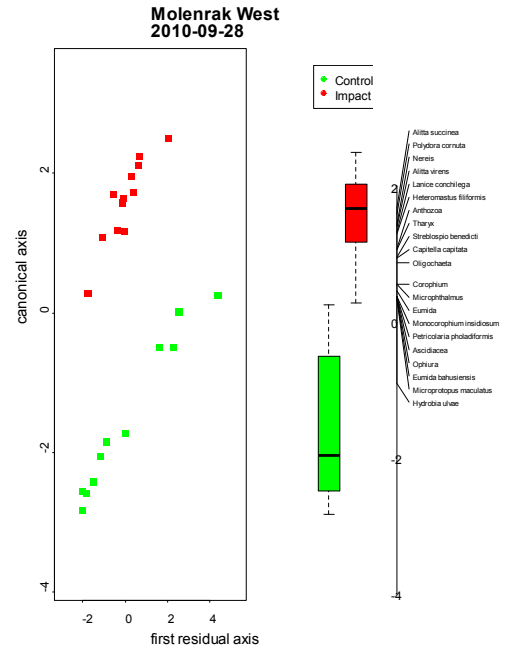
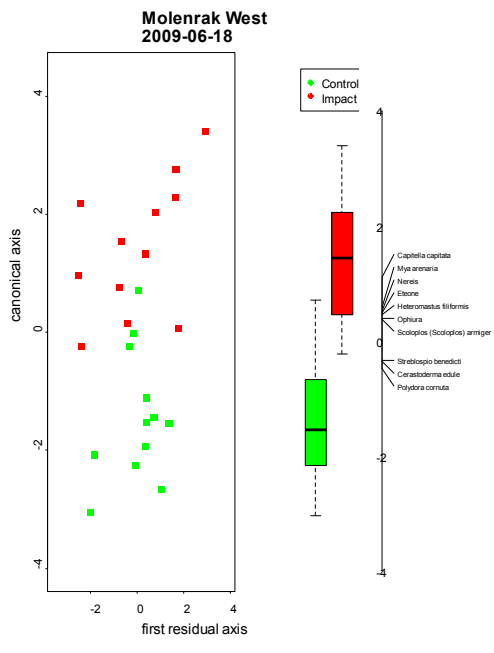
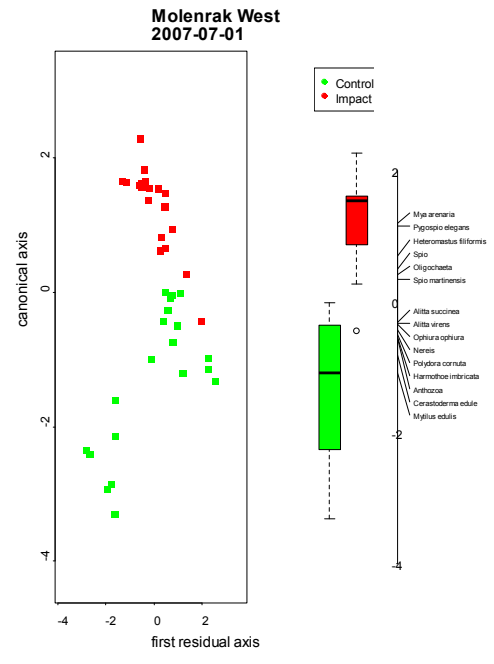
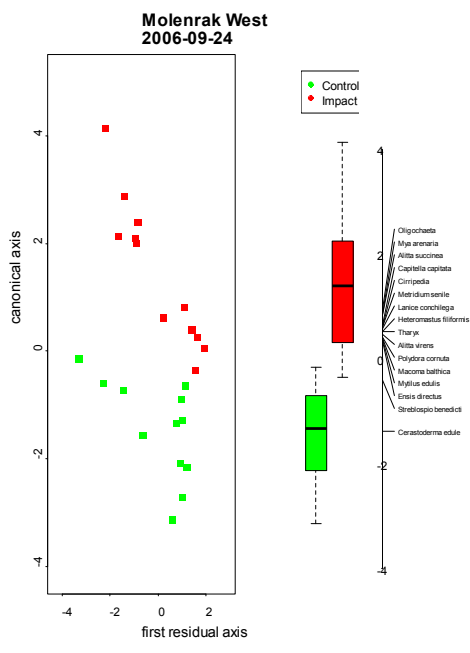
### Molenrak West Impact



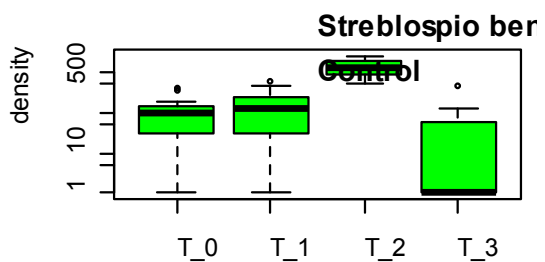
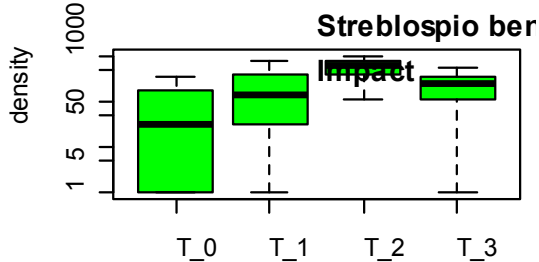
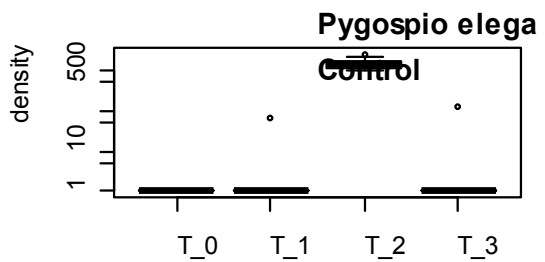
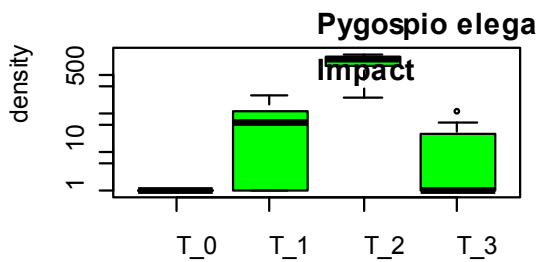
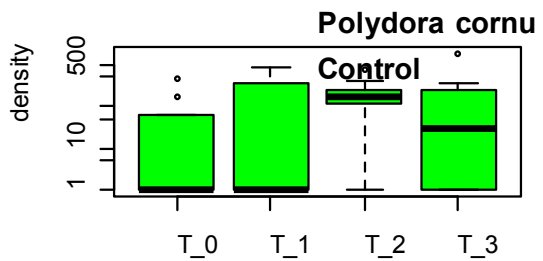
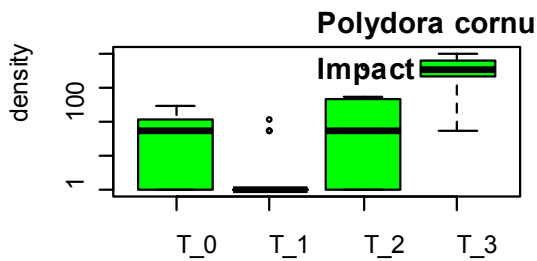
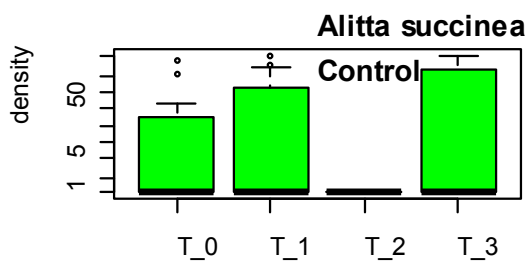
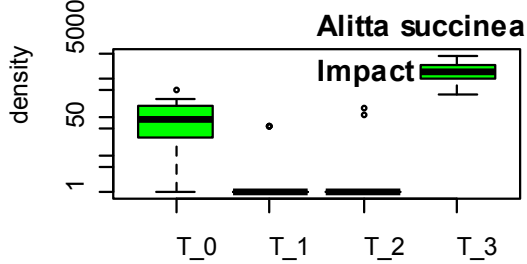
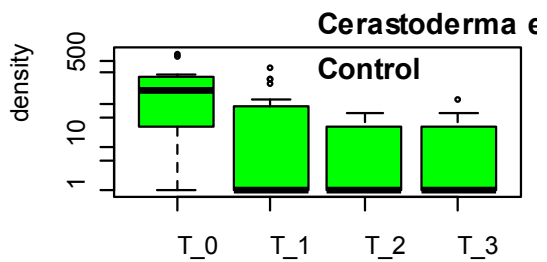
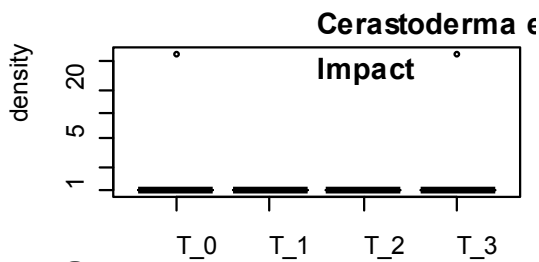
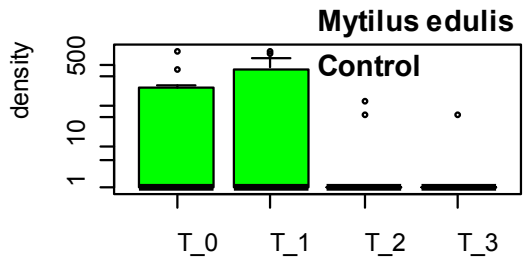
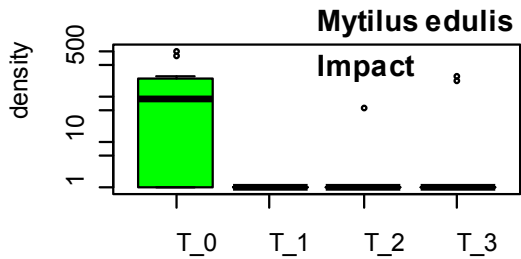


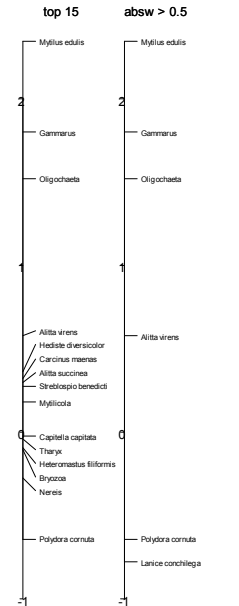
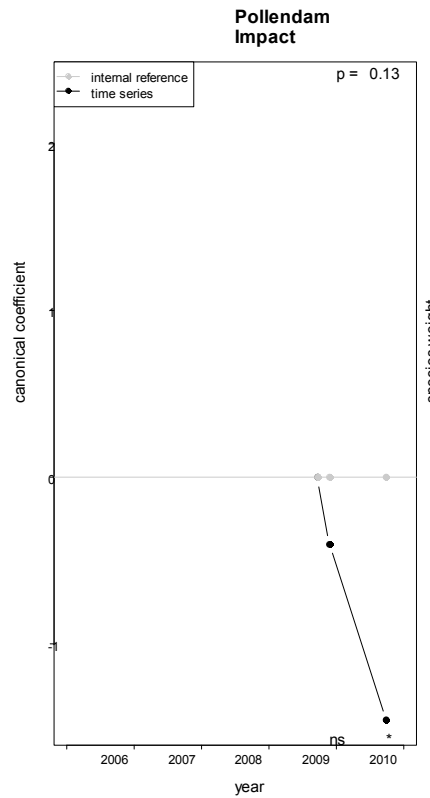
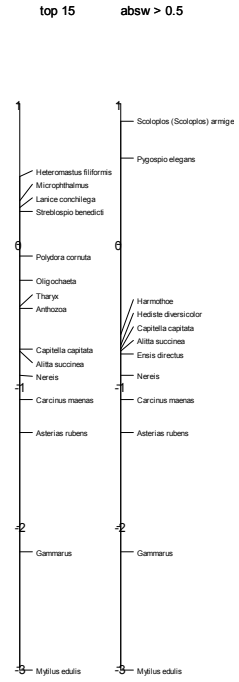
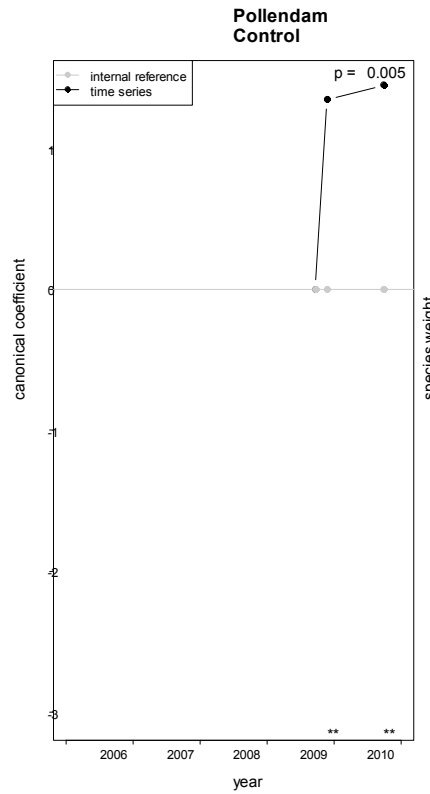
### Molenrak West



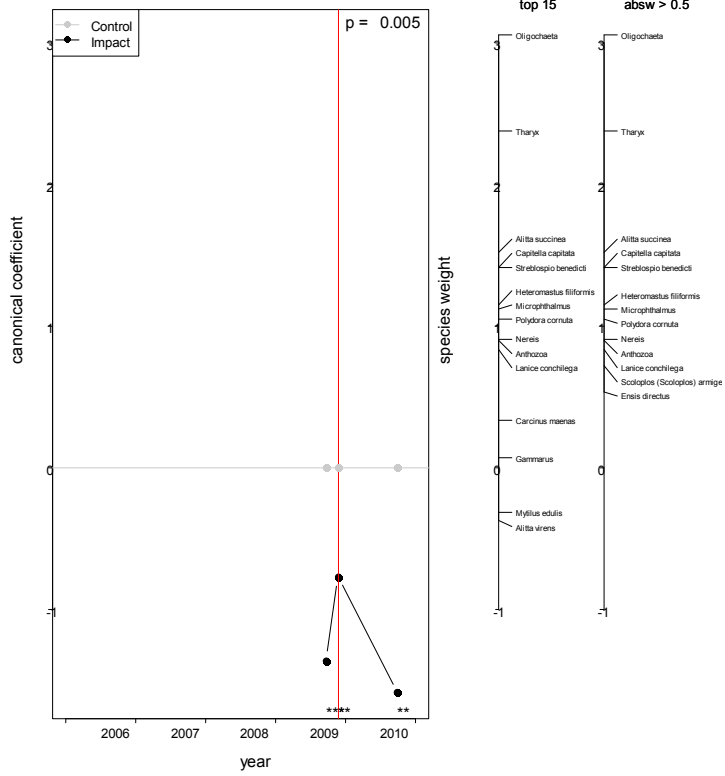


# Molenrak West

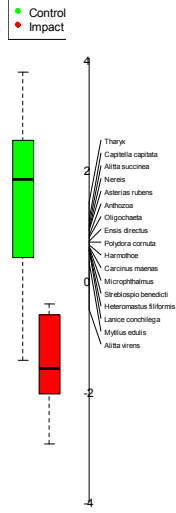
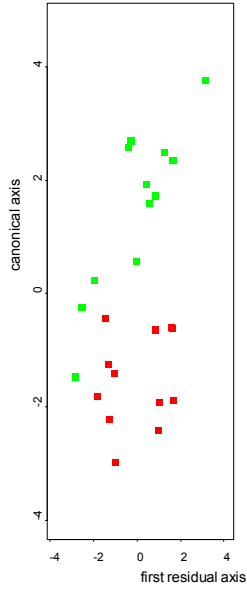




### Pollendam

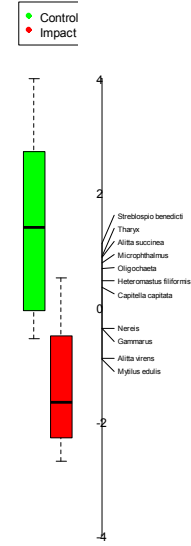
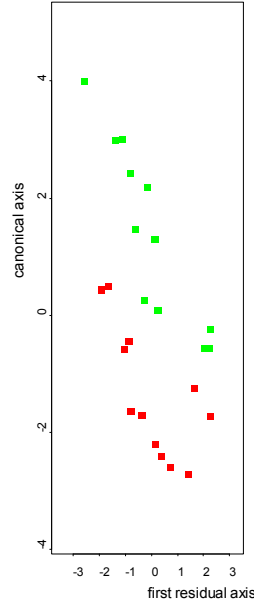


**Pollendam  
2009-09-20**



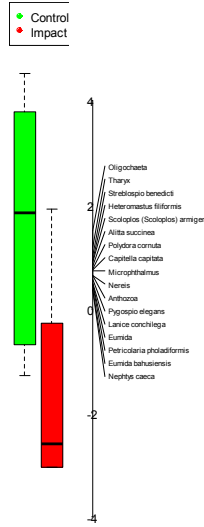
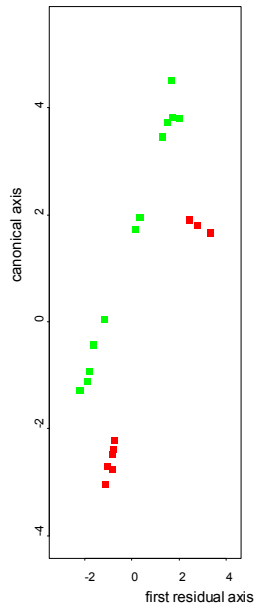
- Tharyx
- Capitella capitata
- Alitta succinea
- Nereis
- Ardalius rubrus
- Arthoeca
- Oligochaeta
- Ensis directus
- Polydora cornuta
- Hannadina
- Carcinus maenas
- Microphthalmus
- Streblospio benedicti
- Heteromastus filiformis
- Lanice conchilega
- Mytilus edulis
- Alitta virens

**Pollendam  
2009-11-23**



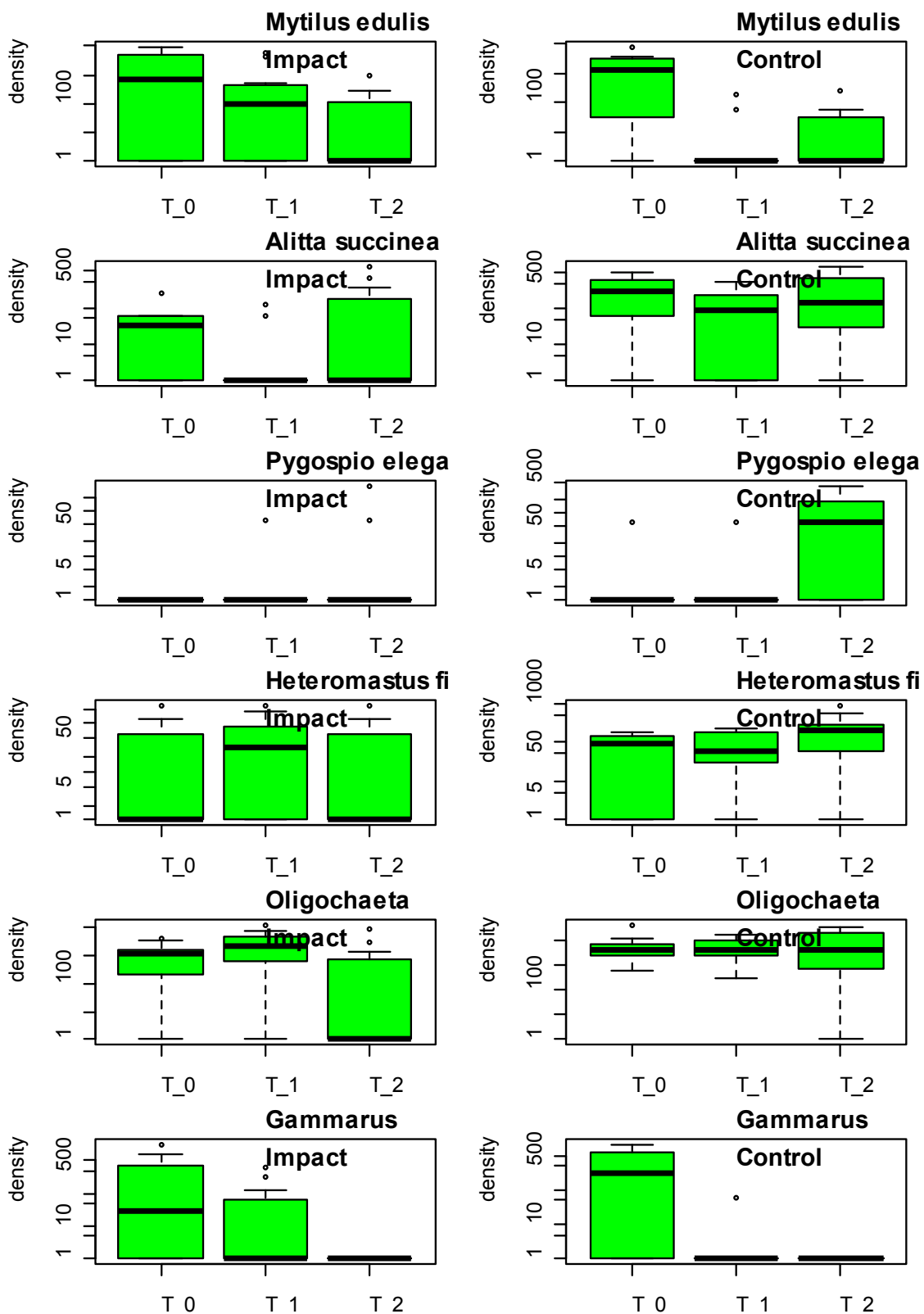
- Streblospio benedicti
- Tharyx
- Alitta succinea
- Microphthalmus
- Oligochaeta
- Heteromastus filiformis
- Capitella capitata
- Nereis
- Gammarus
- Alitta virens
- Mytilus edulis

**Pollendam  
2010-09-28**

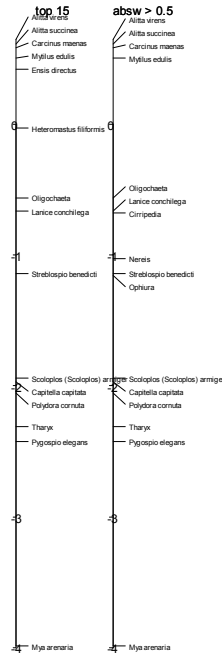
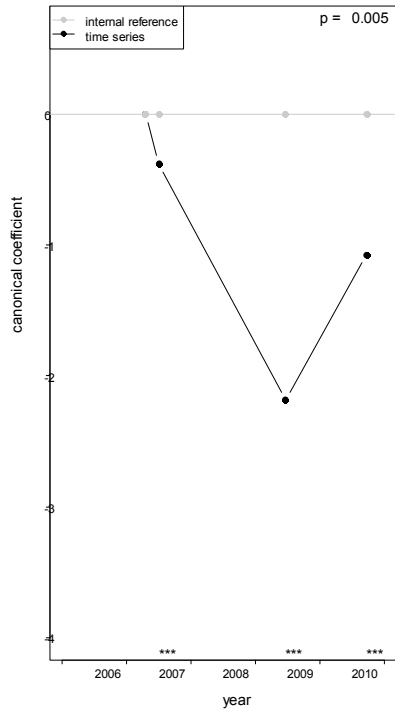


- Oligochaeta
- Tharyx
- Streblospio benedicti
- Heteromastus filiformis
- Scoloplos (Scoloplos) armiger
- Alitta succinea
- Polydora cornuta
- Capitella capitata
- Microphthalmus
- Nereis
- Arthoeca
- Pygospio elegans
- Lanice conchilega
- Eumida
- Pterocordia phthaliformis
- Eumida tuberosa
- Nephtys caeca

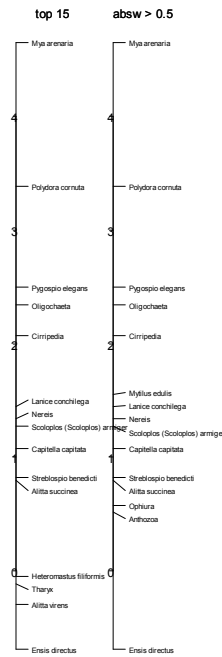
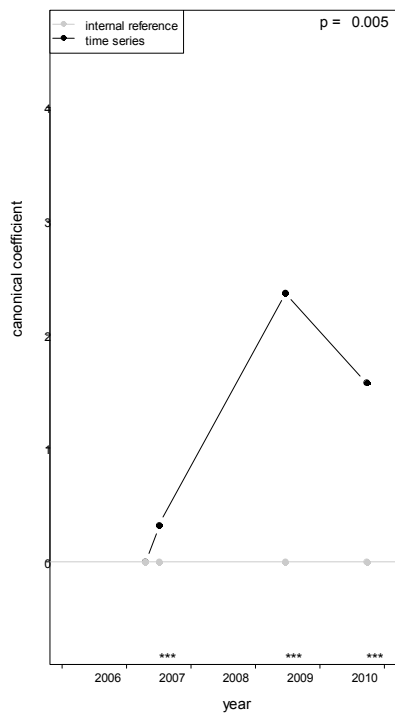
# Pollendam



### Stompe Control

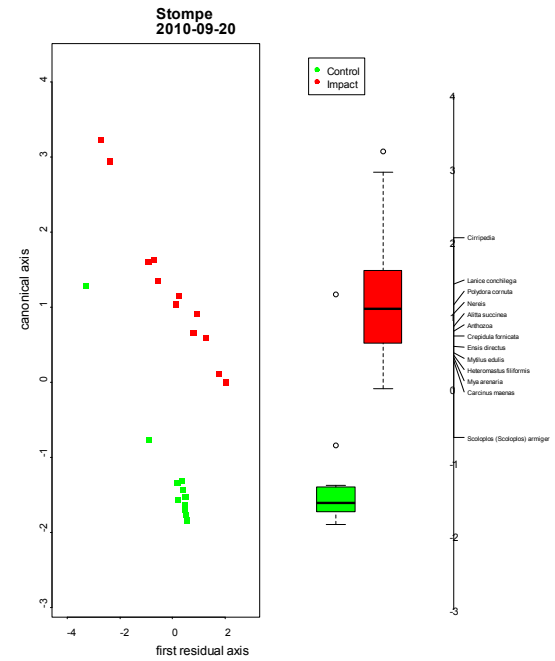
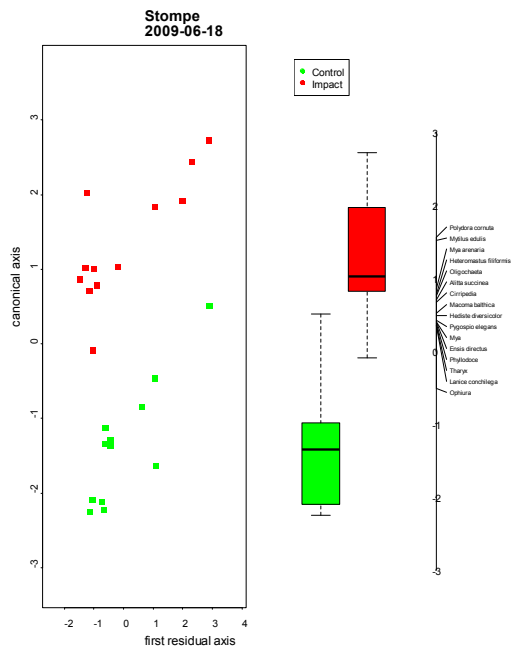
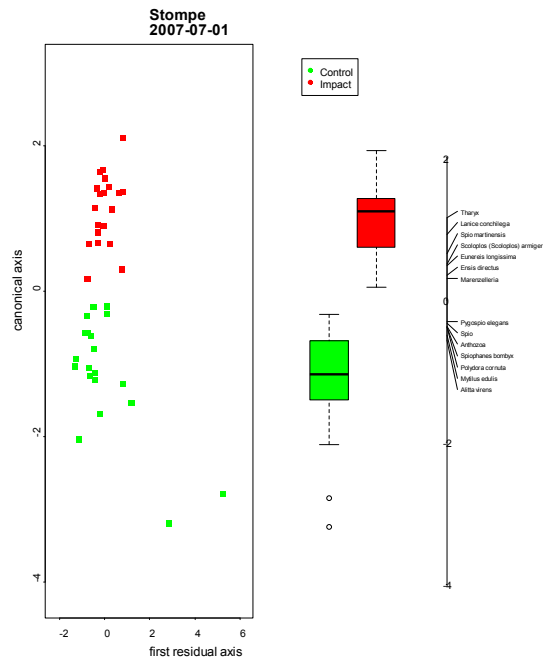
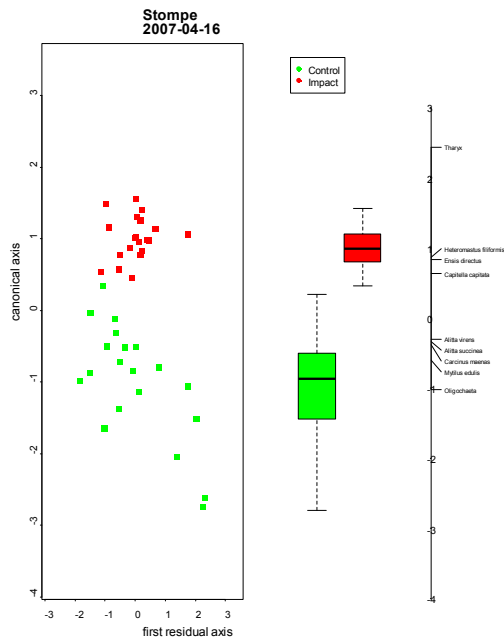


### Stompe Impact

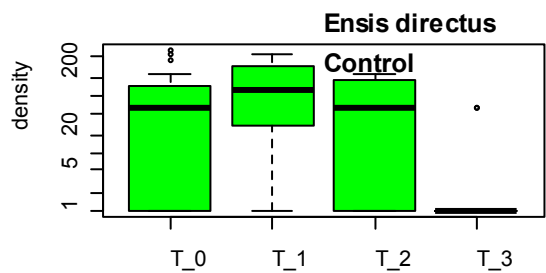
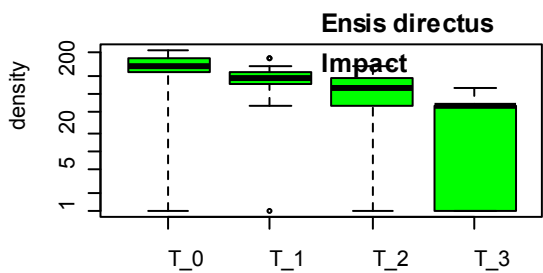
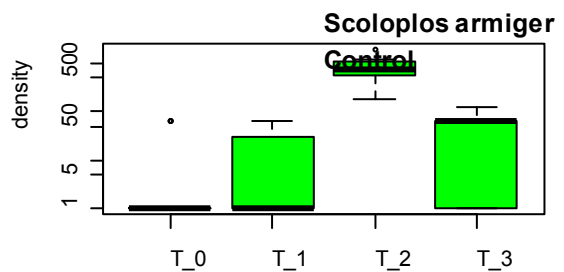
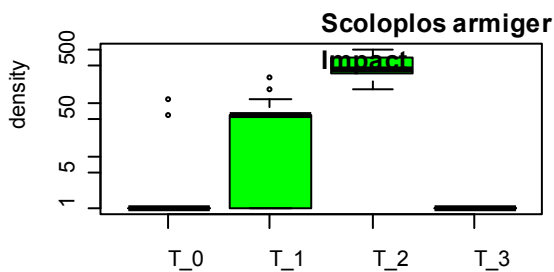
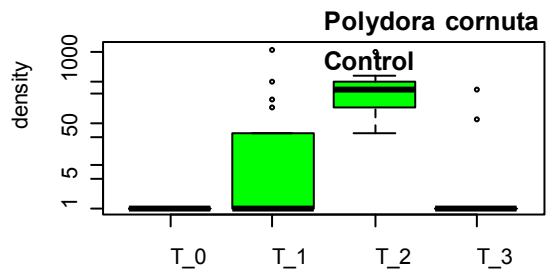
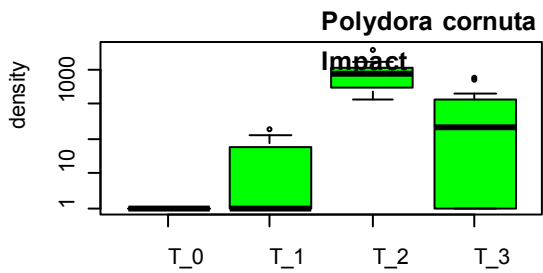
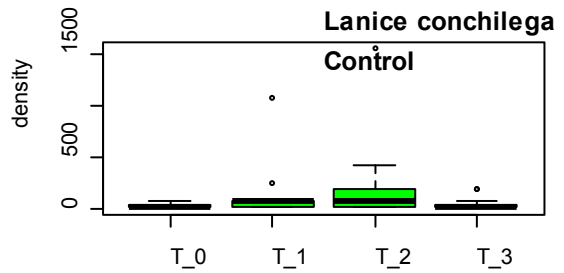
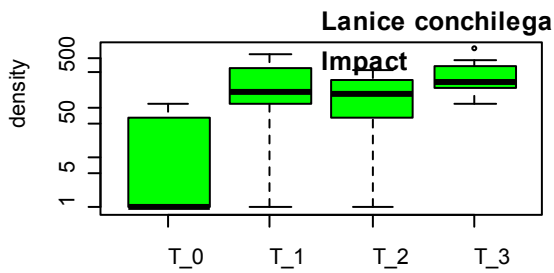
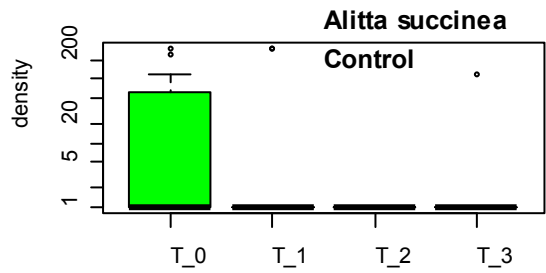
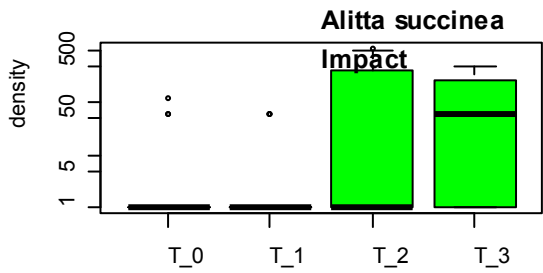
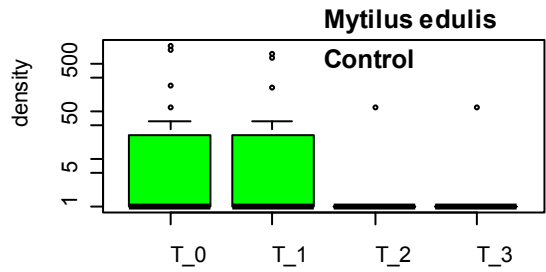
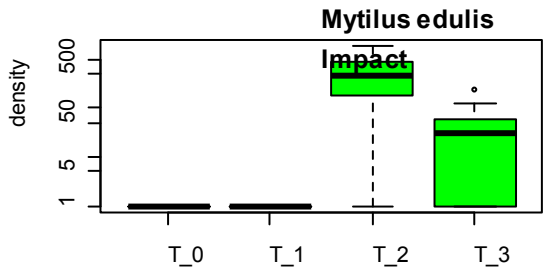




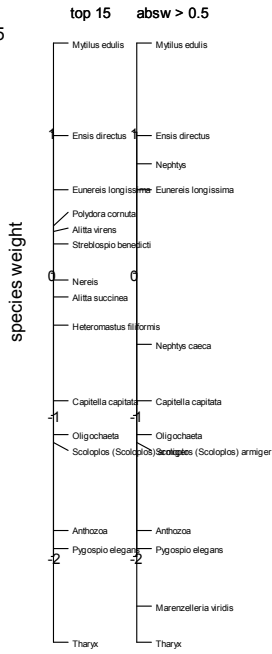
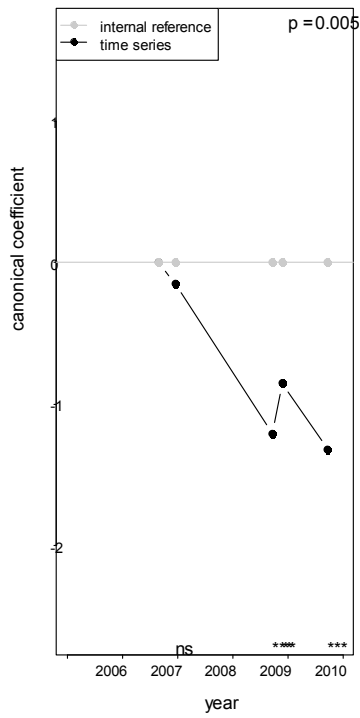




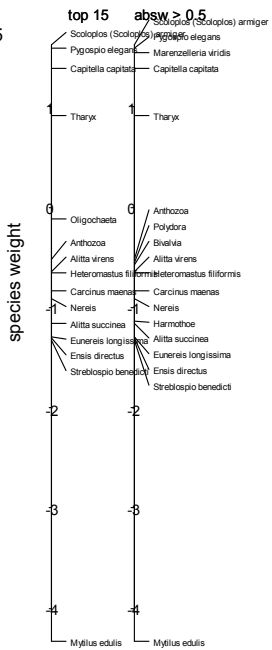
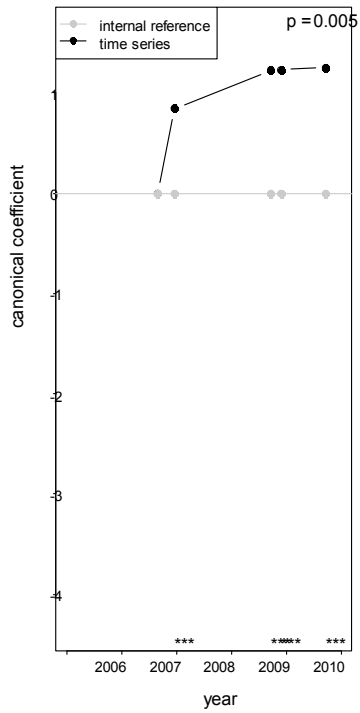
# Stompe



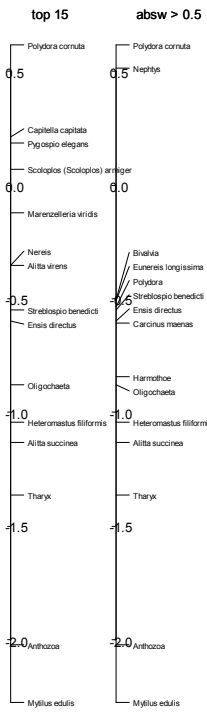
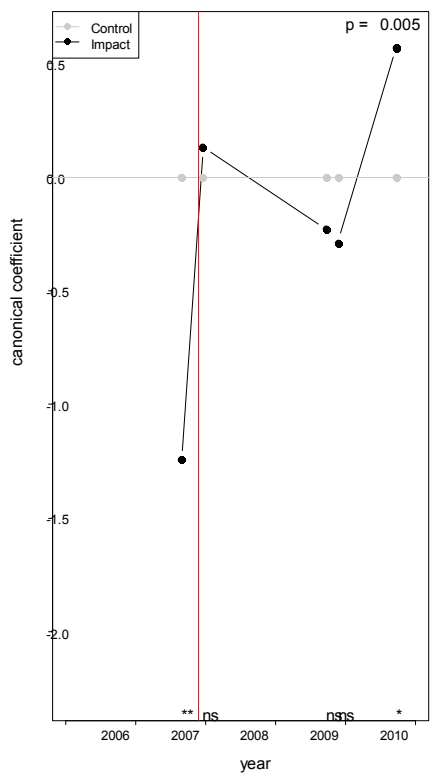
### Stompe percelen Control



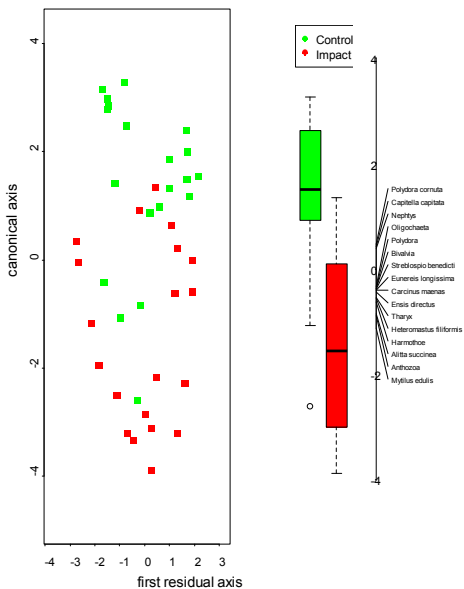
### Stompe percelen Impact



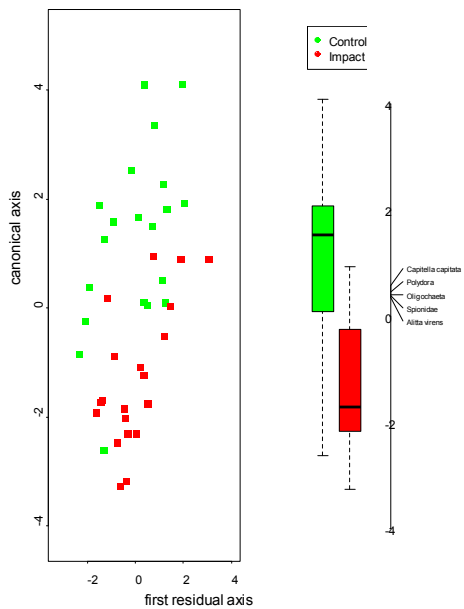
**Stompe percelen**

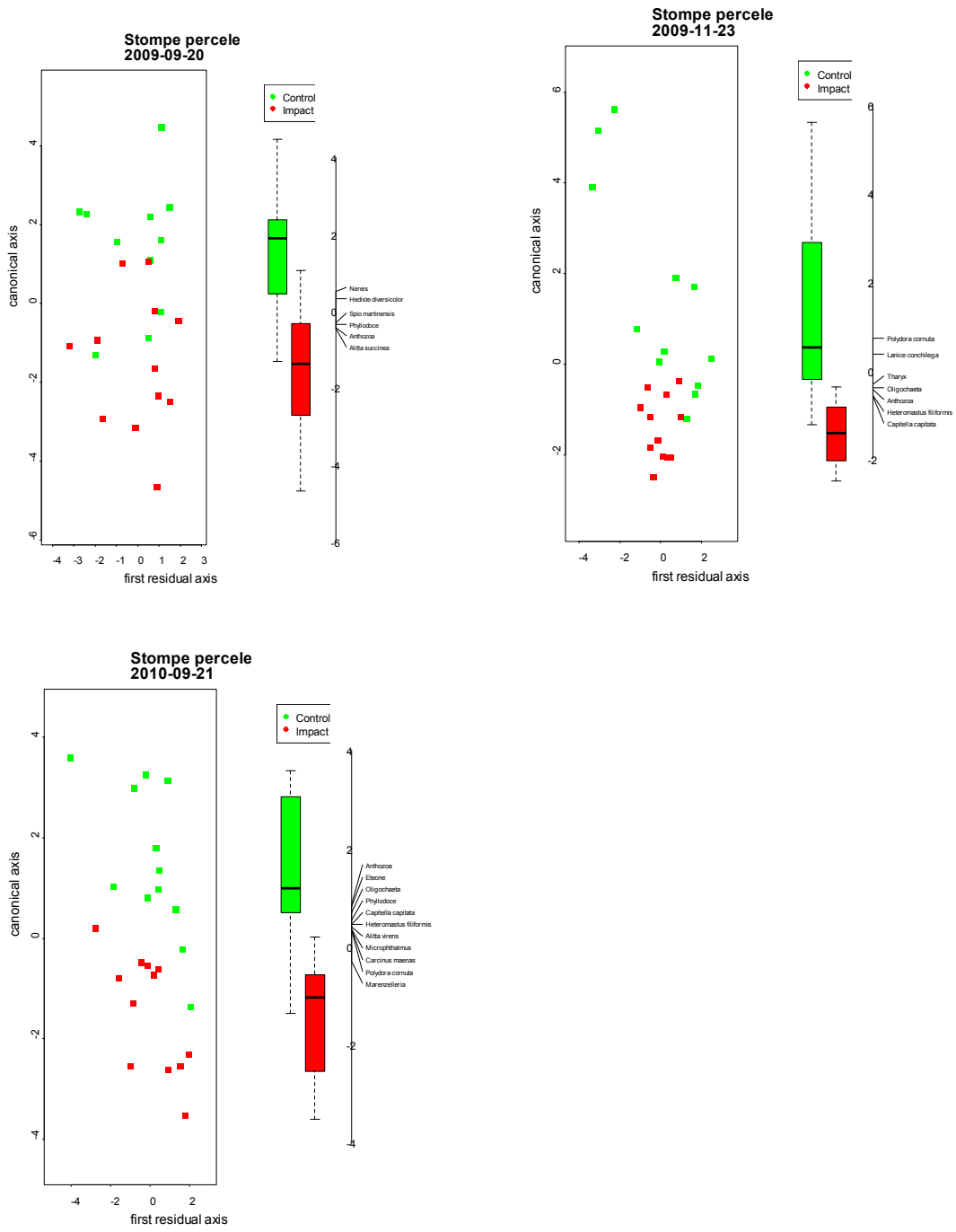


**Stompe percele 2007-08-26**

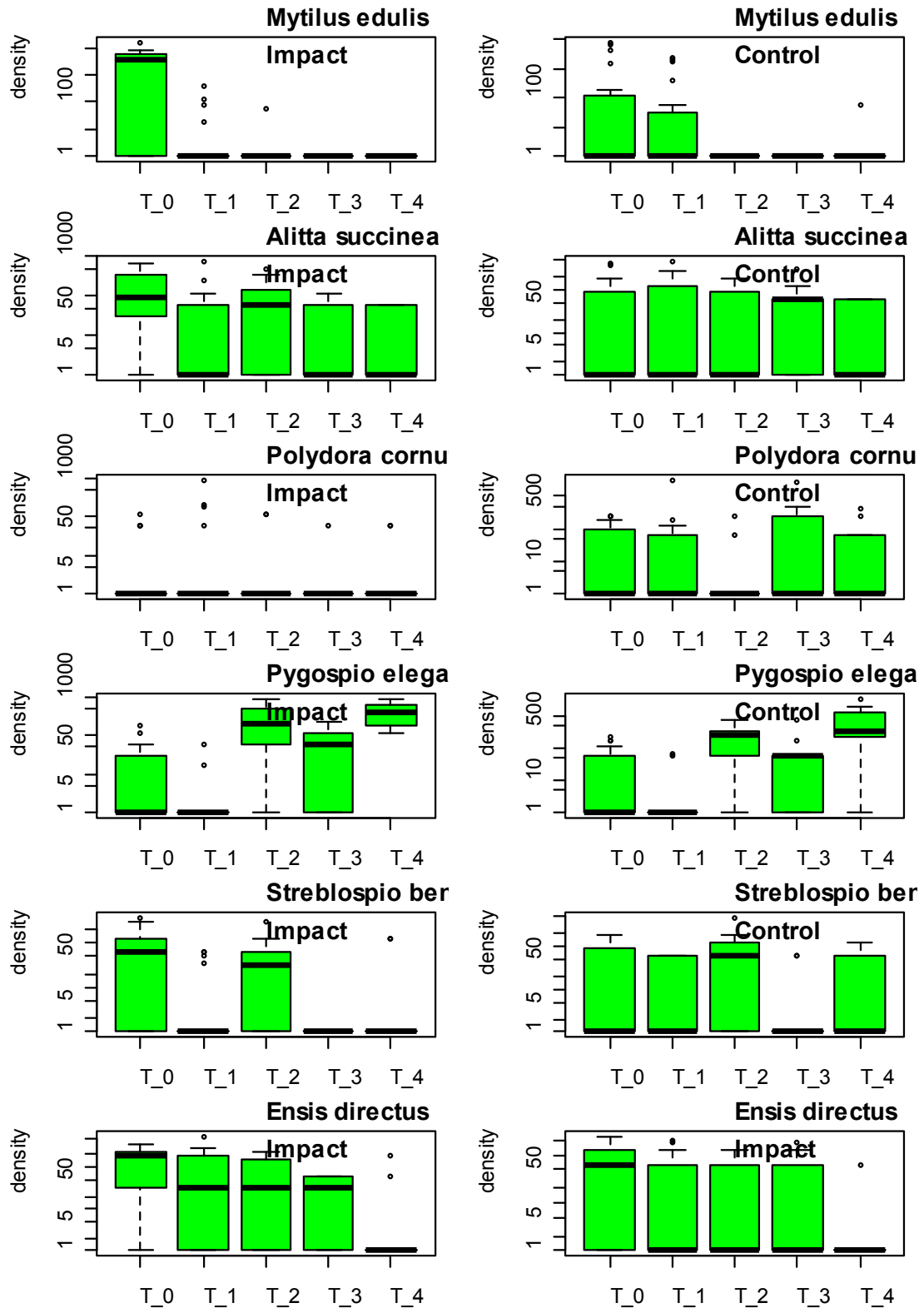


**Stompe percele 2007-12-16**

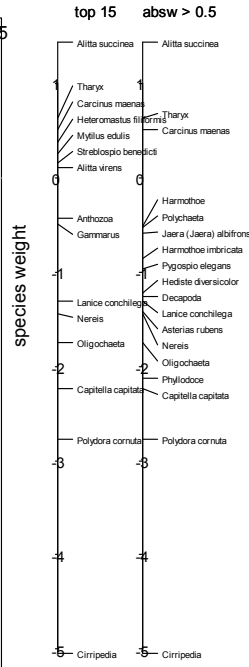
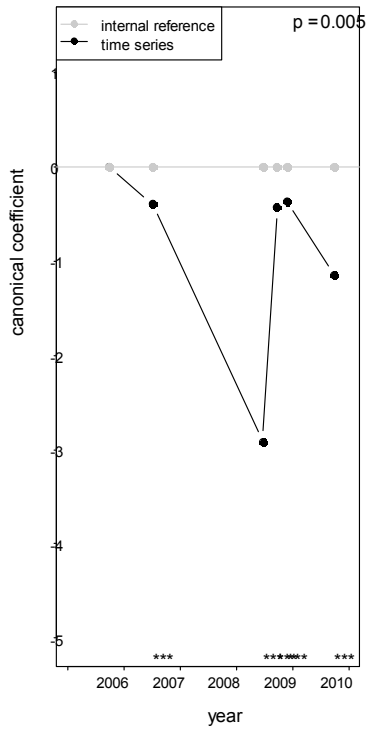




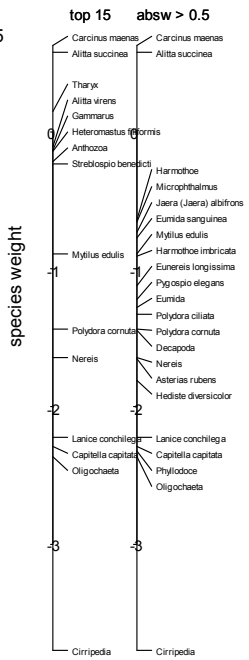
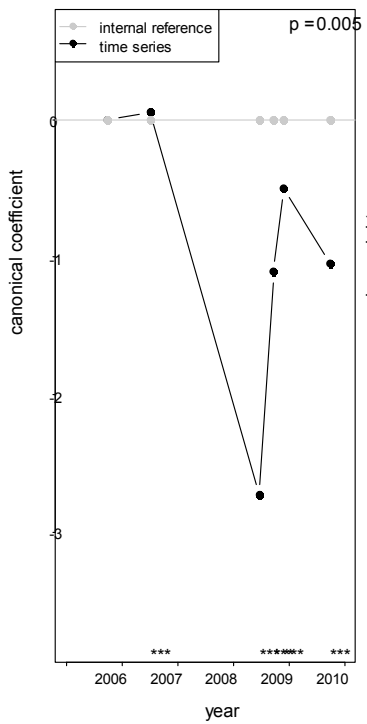
# Stompe percelen



### Visjagersgatje Control

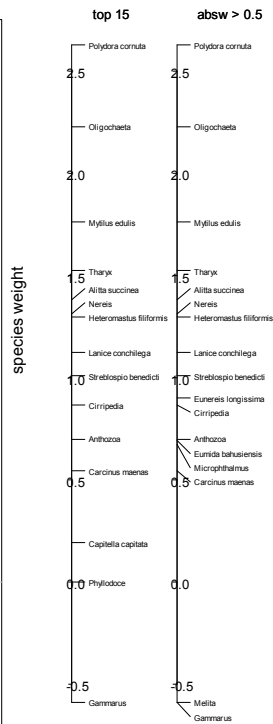
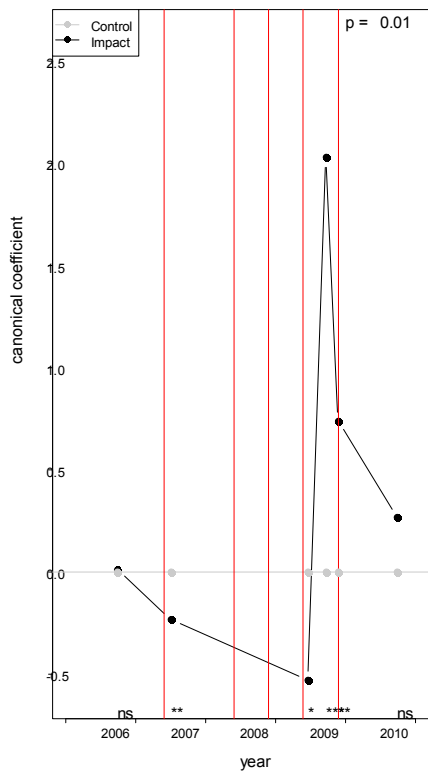


### Visjagersgatje Impact

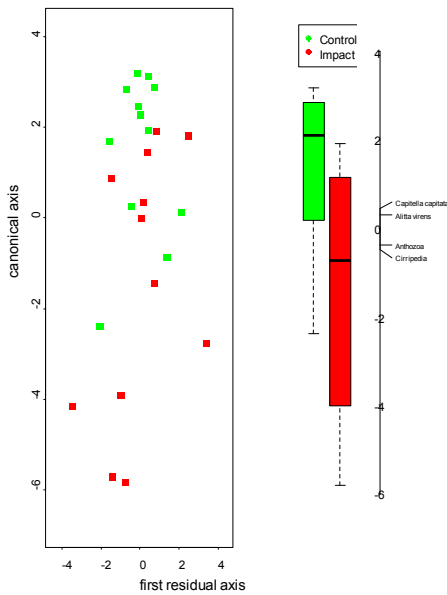




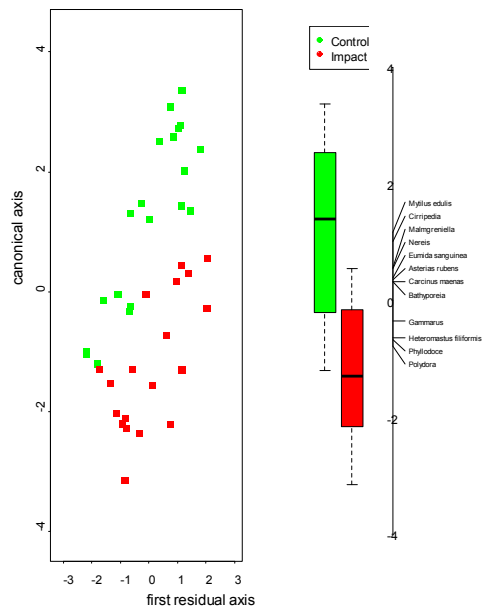
### Visjagersgatje

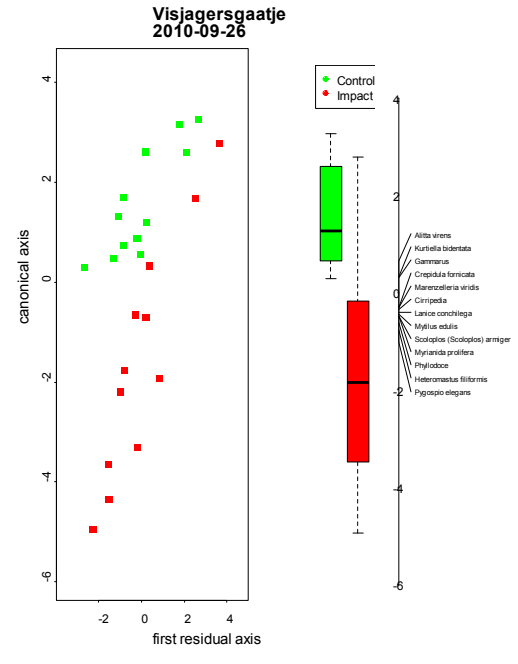
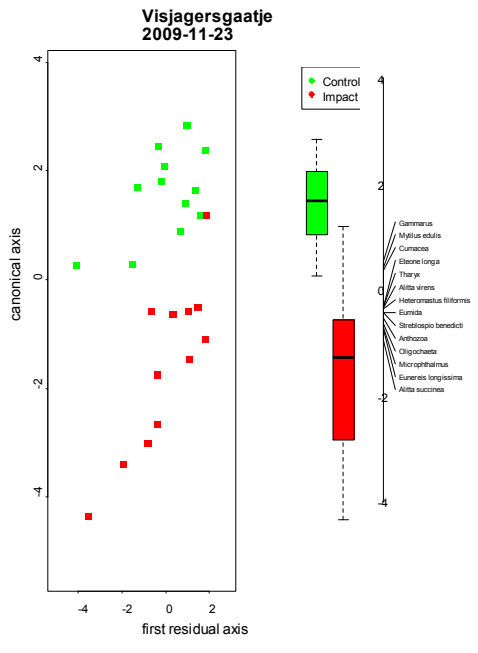
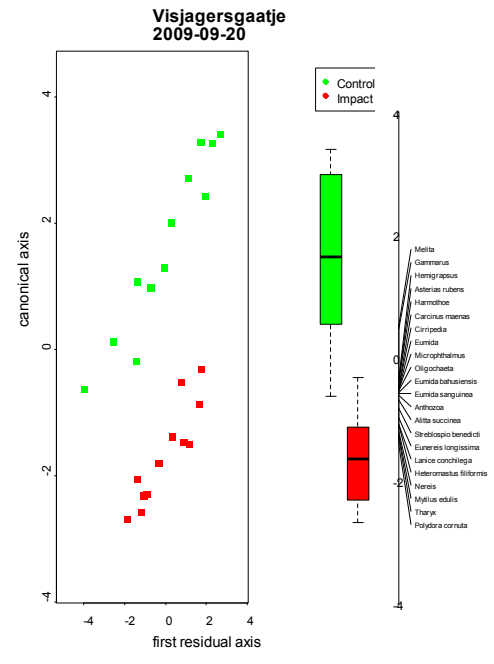
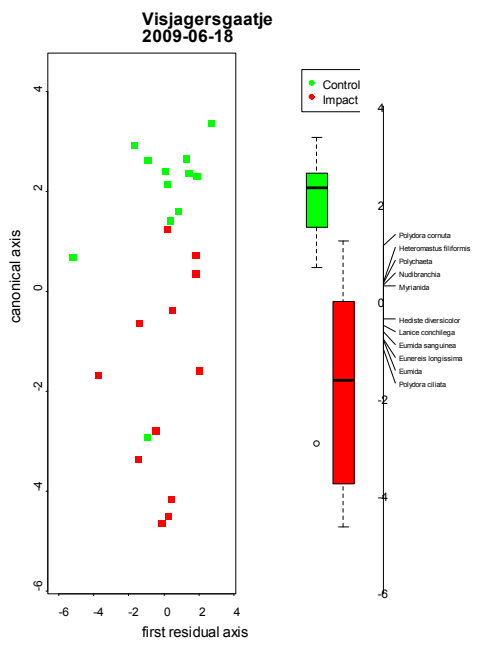


### Visjagersgatje 2006-09-24

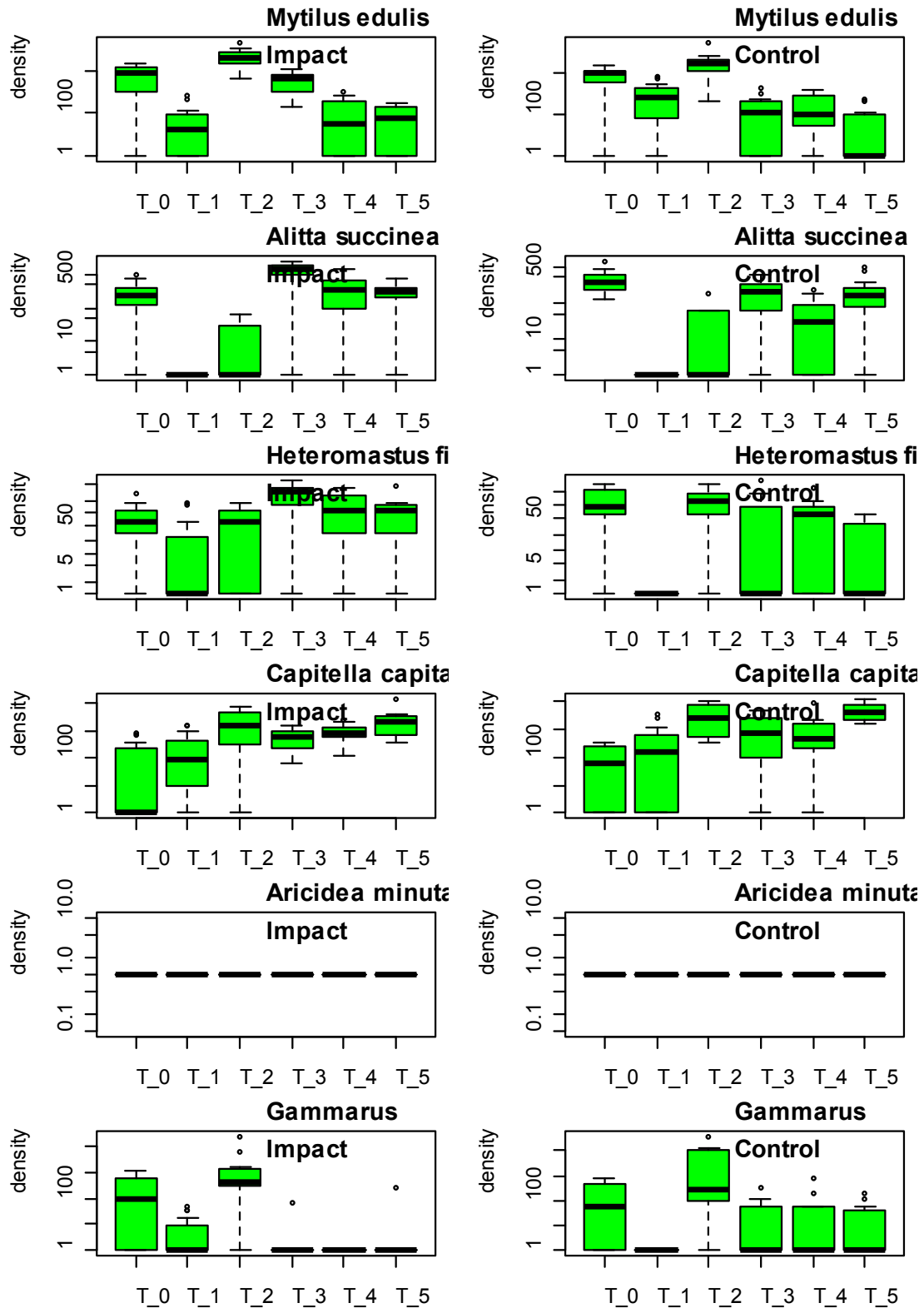


### Visjagersgatje 2007-07-01

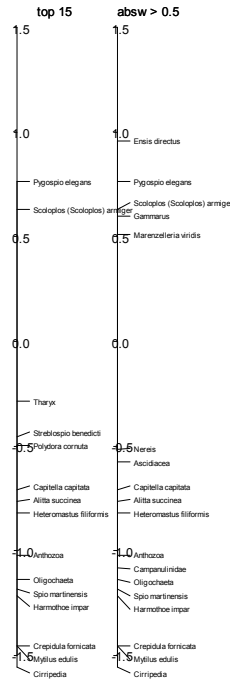
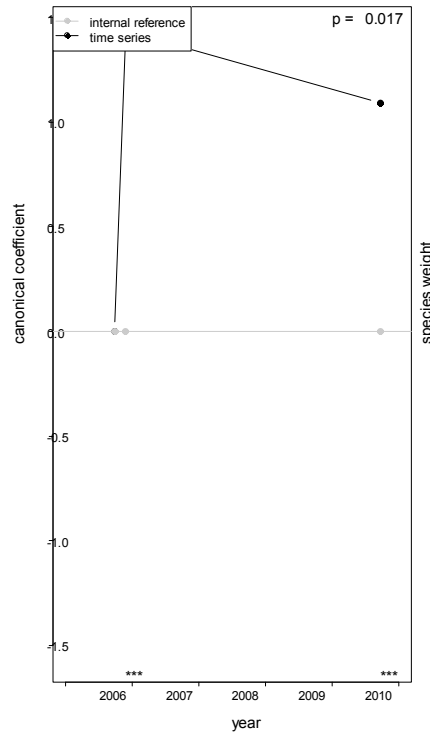




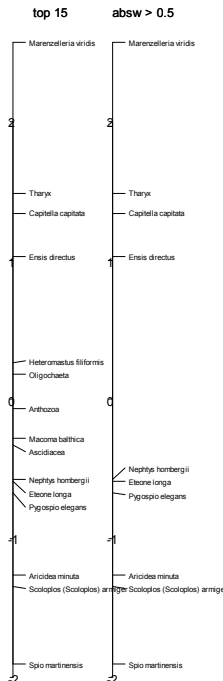
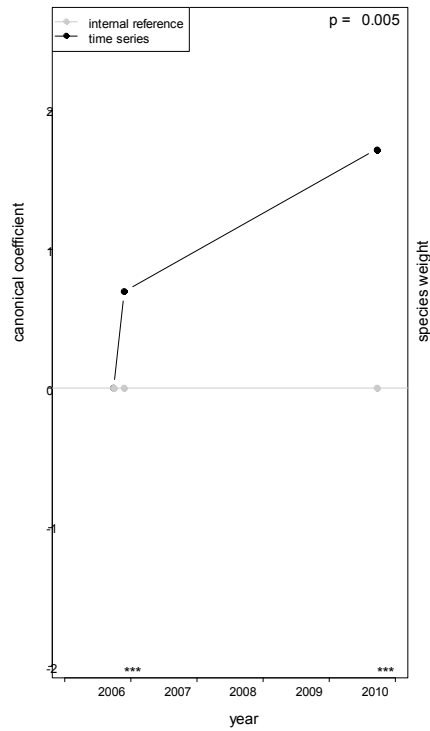
# Visjagersgaatje



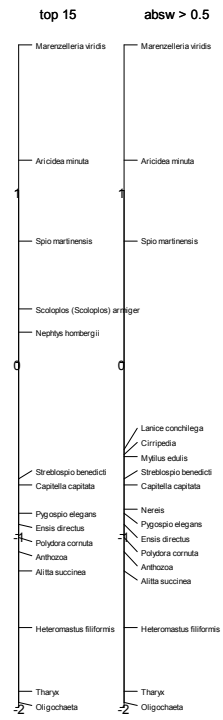
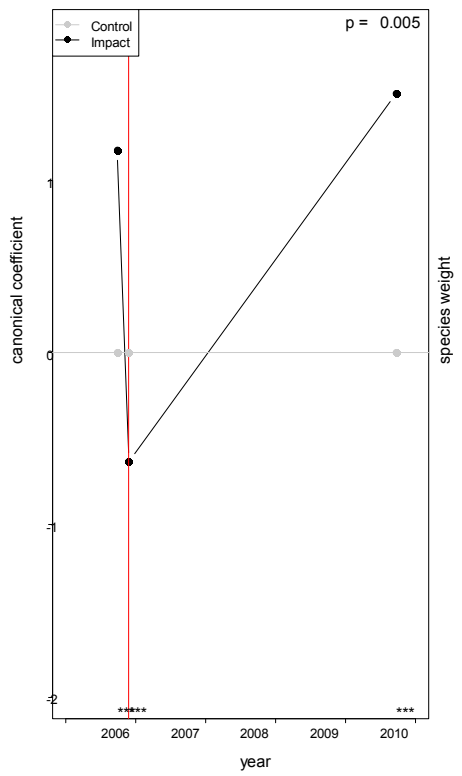
### Vlieter zuid Control



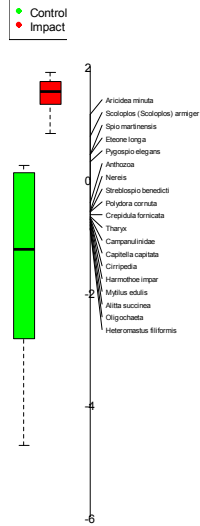
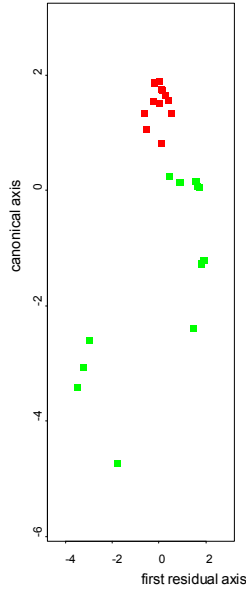
### Vlieter zuid Impact



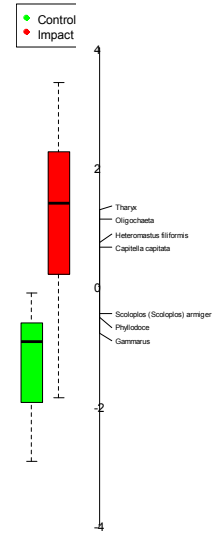
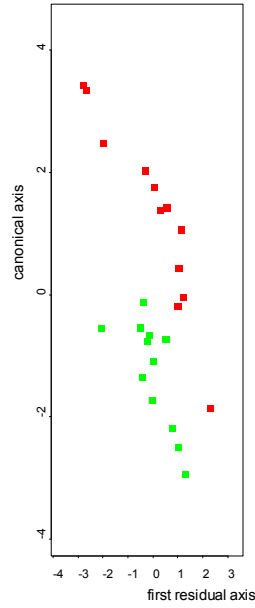
### Vlieter zuid



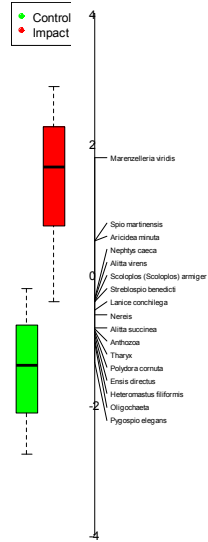
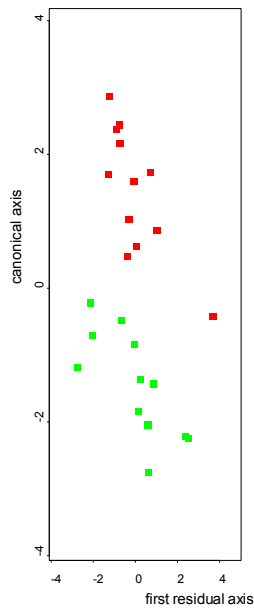
**Vlieter zuid  
2006-09-24**



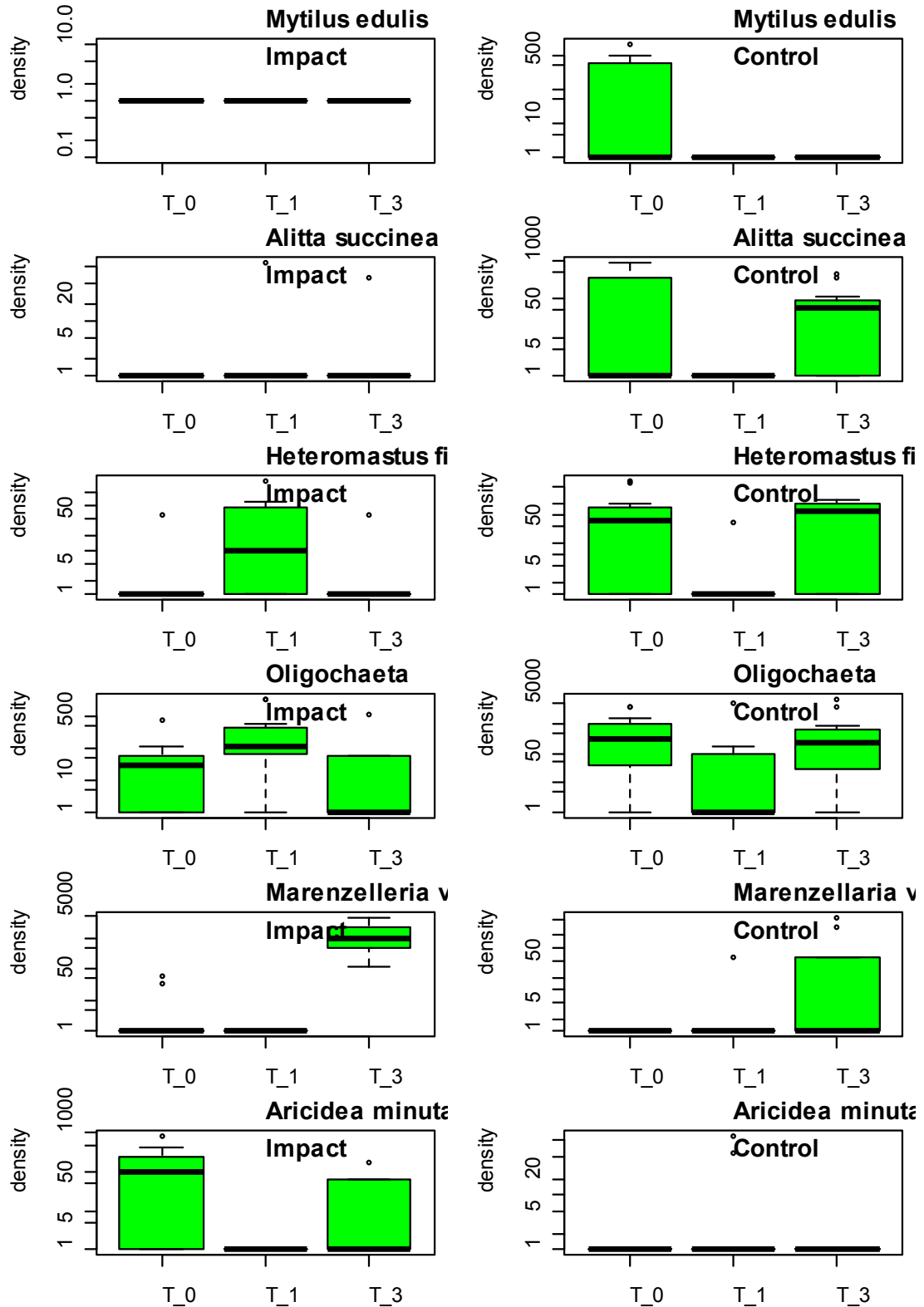
**Vlieter zuid  
2006-11-24**



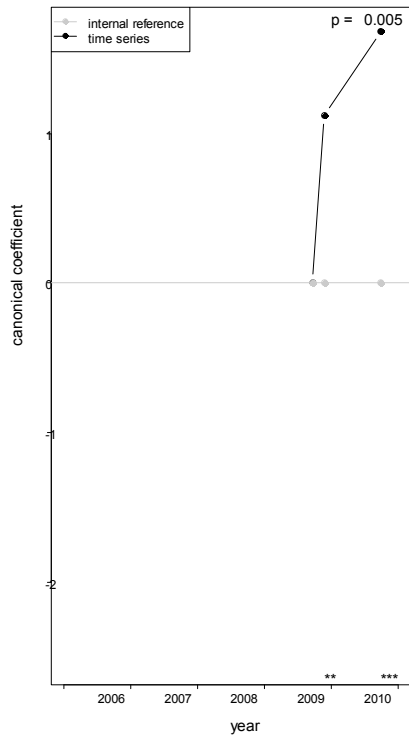
**Vlieter zuid  
2010-09-20**



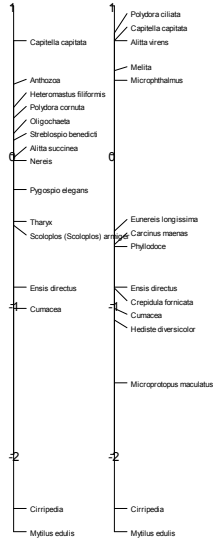
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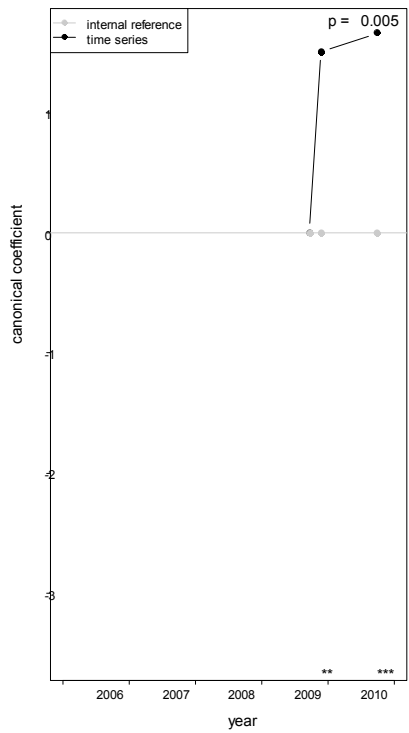
### Westkom Control



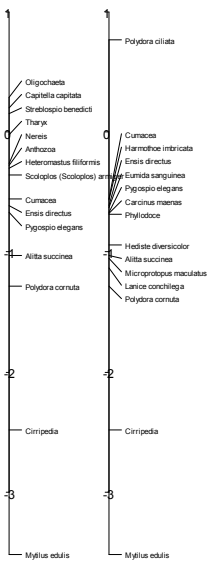
top 15 absw > 0.5



### Westkom Impact

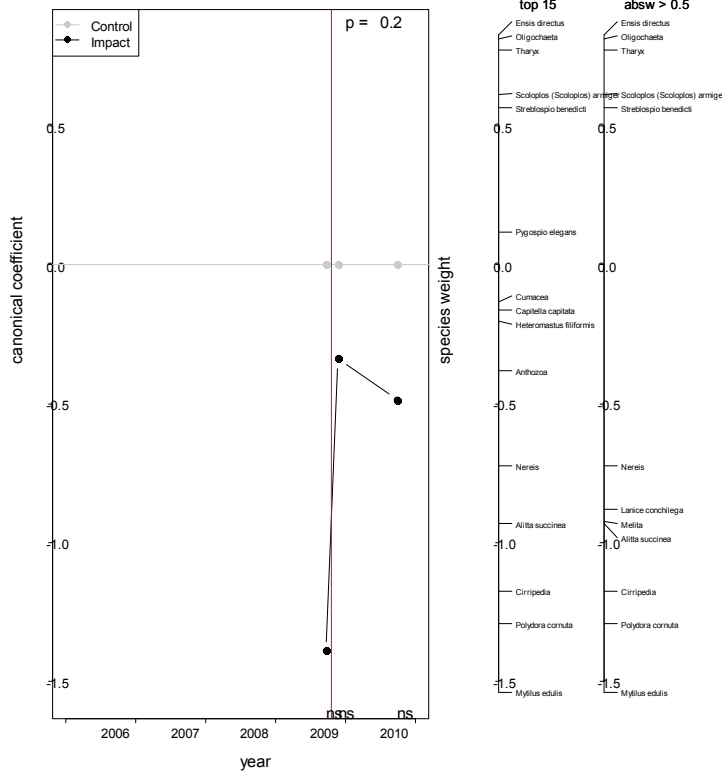


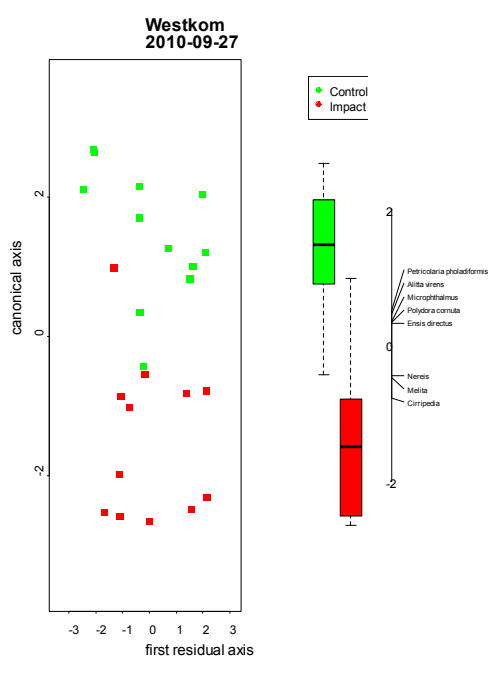
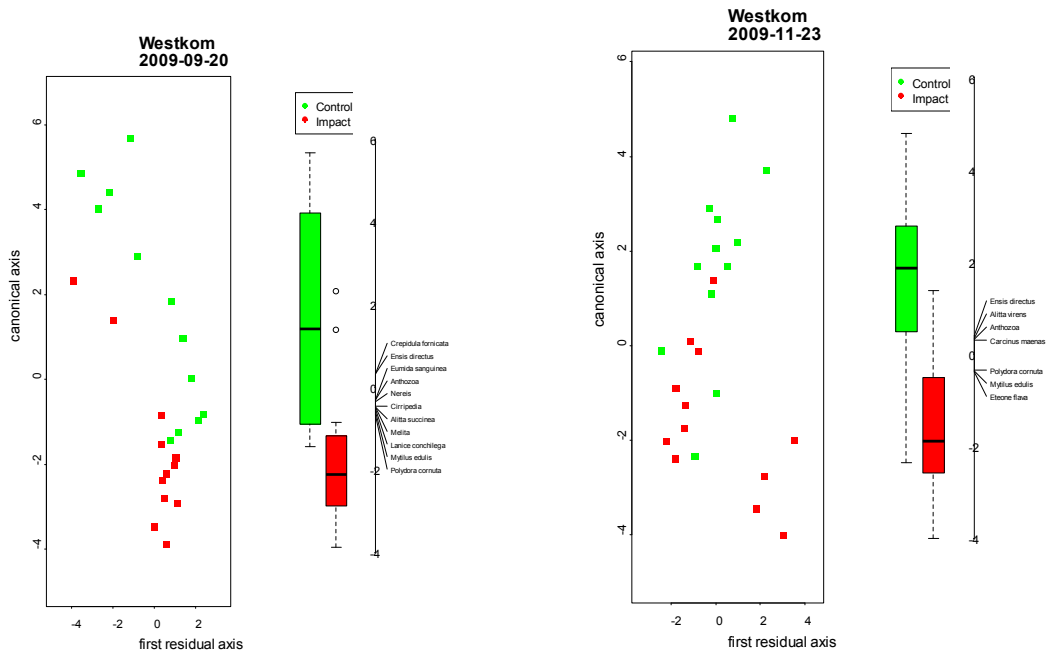
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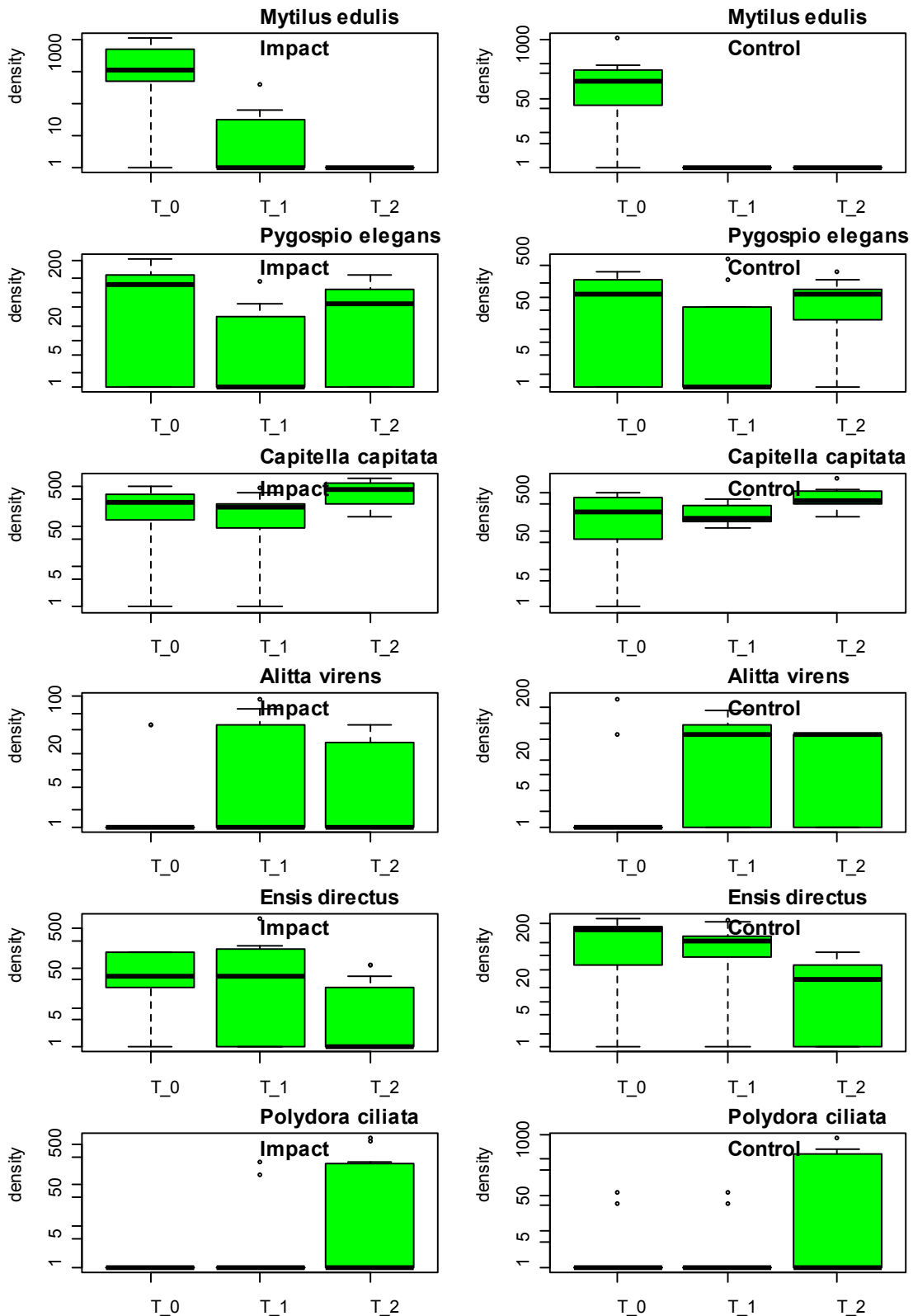


### Westkom



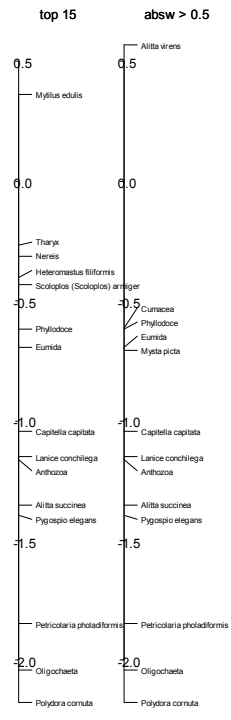
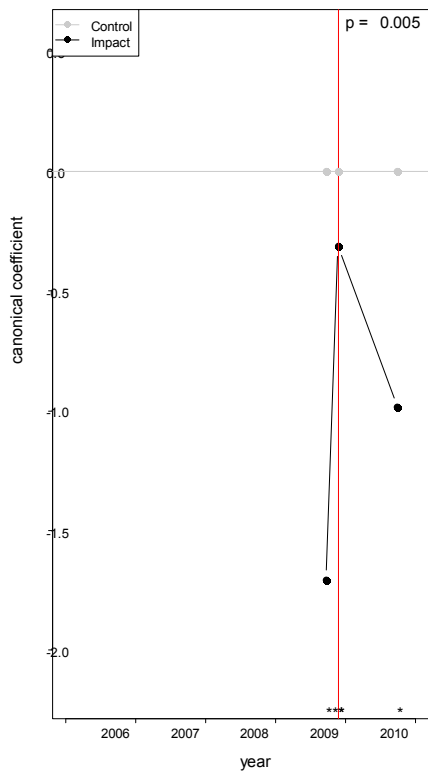


# Westkom

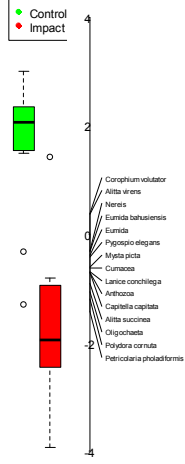
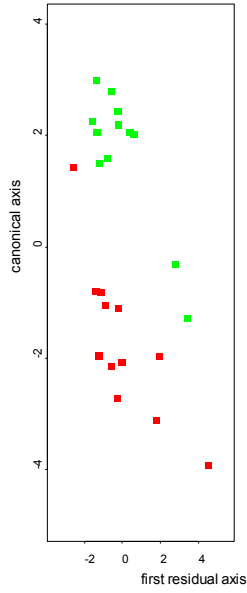




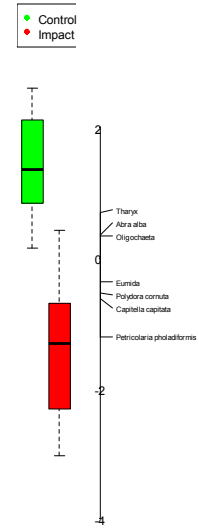
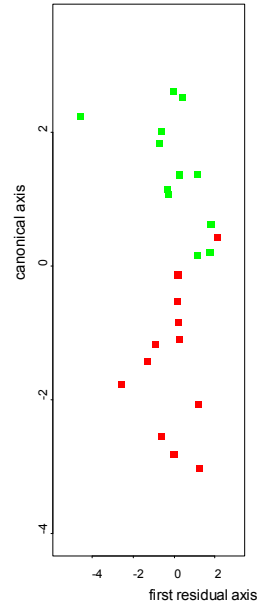
### Westmeep



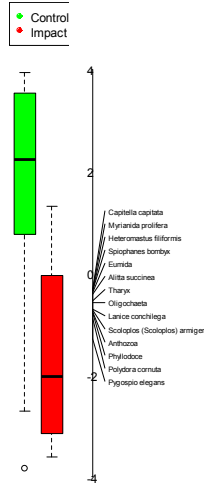
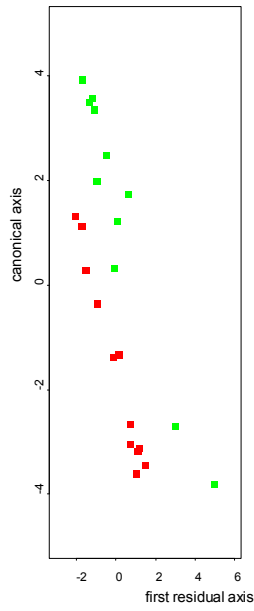
**Westmeep  
2009-09-20**



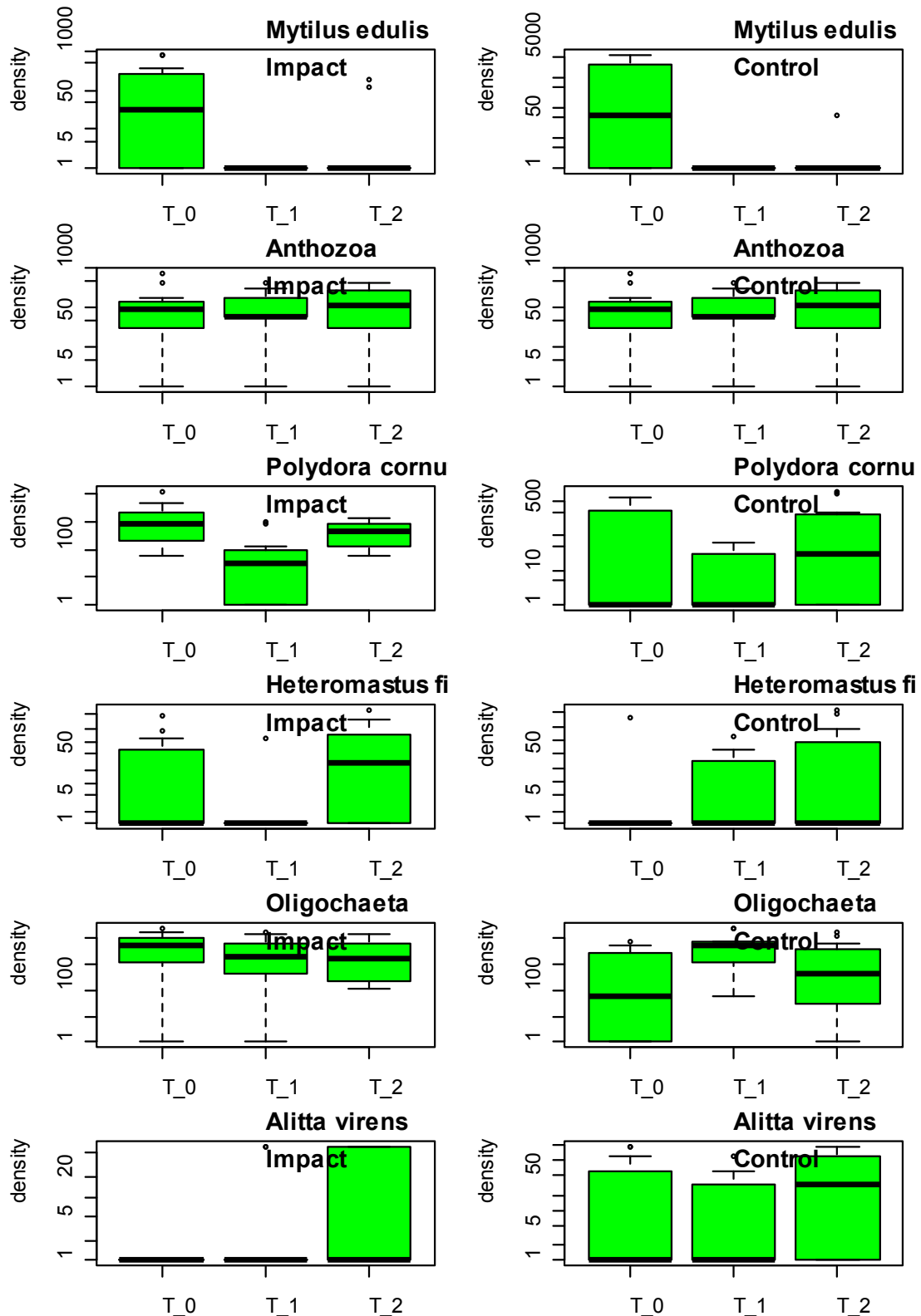
**Westmeep  
2009-11-23**



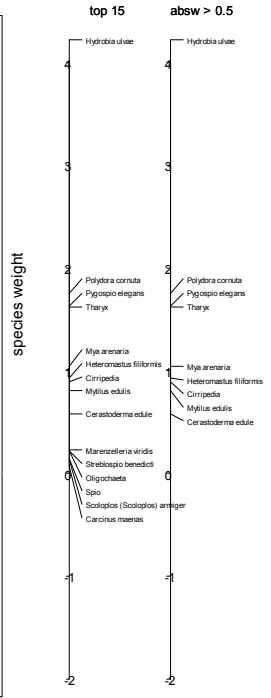
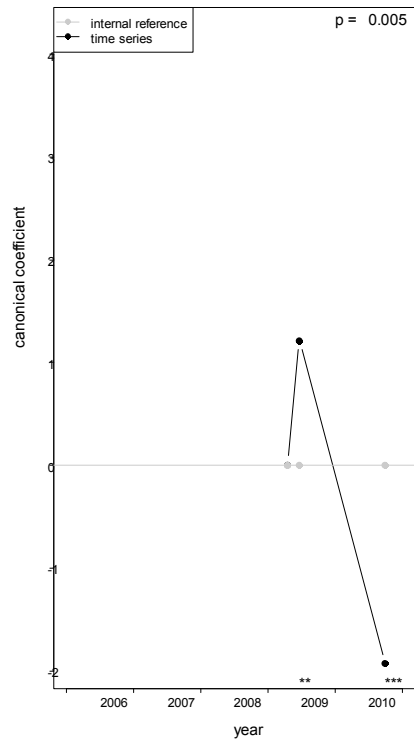
**Westmeep  
2010-09-27**



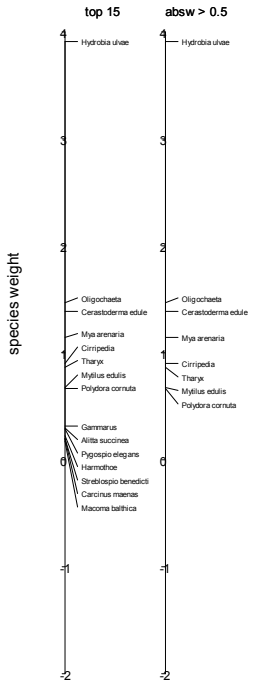
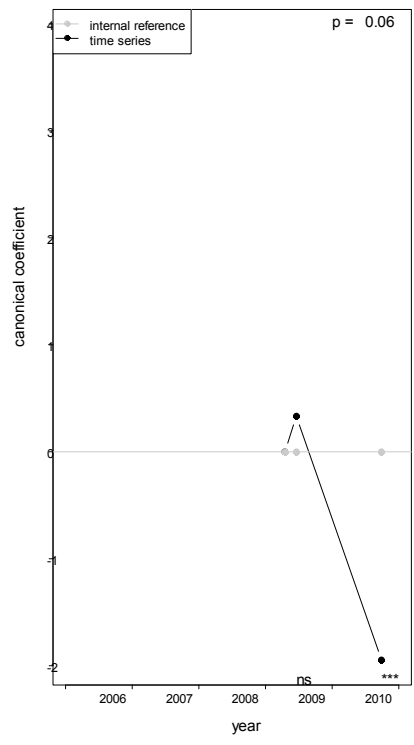
# Westmeep



### Zuidostrak Control

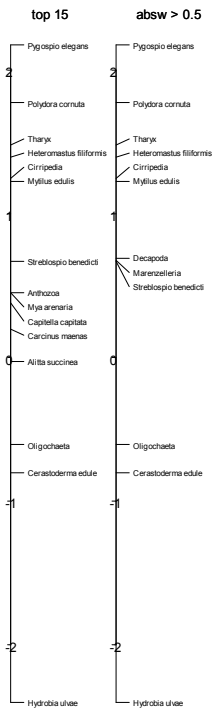
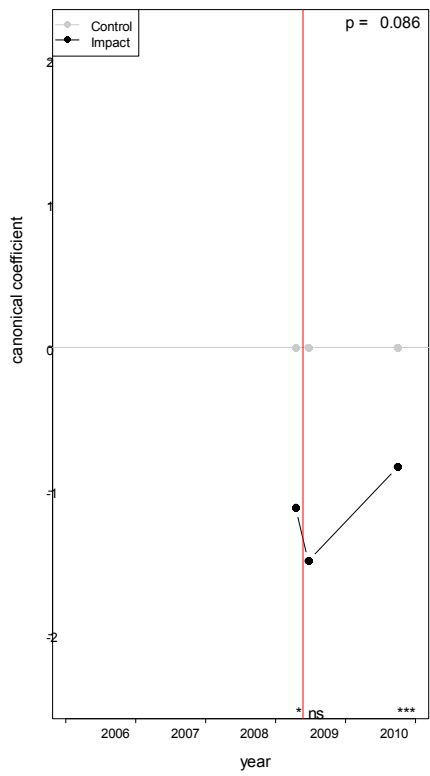


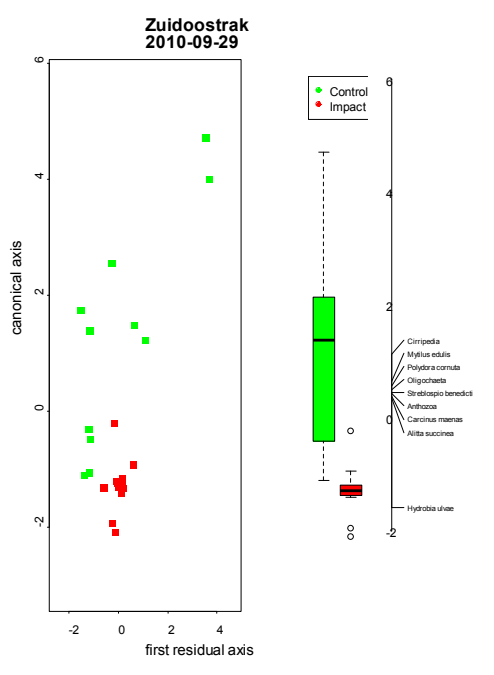
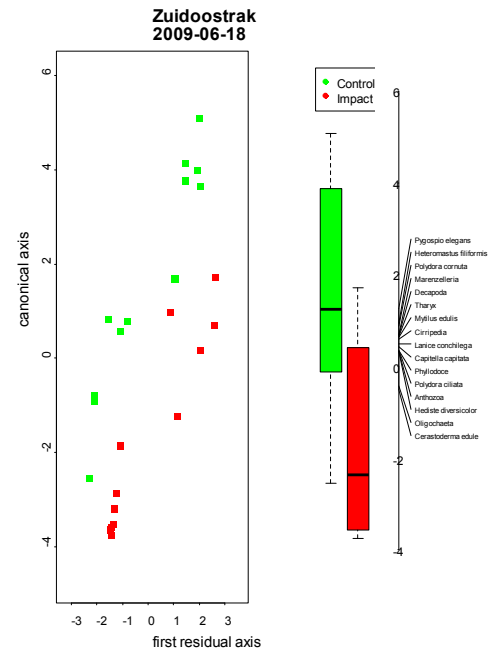
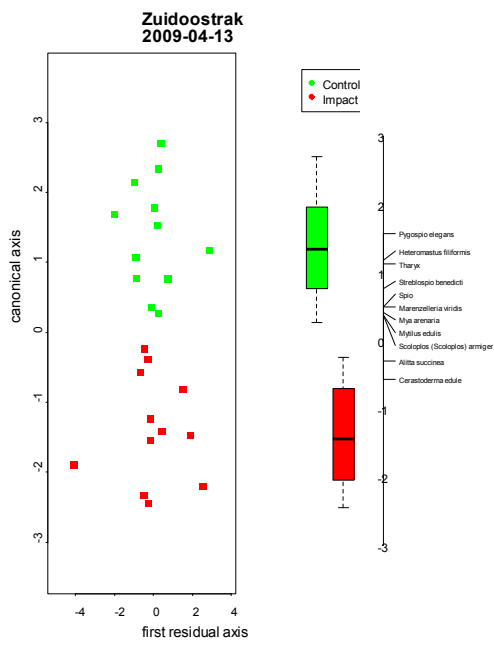
### Zuidostrak Impact





### Zuidostrak





# Zuidostrak

