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Integrated Project to evaluate the Impacts of Global Change on European Freshwater Ecosystems

WP2: Climate-hydromorphology interactions

Task 2: Hydromorphological changes and aquatic and riparian biota

Subtask 2.3: Autecological and laboratory experiments

Deliverable No. 95

Testing the concept of habitat dynamics

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1 Testing the concept of habitat dynamics

In large parts of Europe hydromorphological alteration is the main stressor affecting rivers. Alterations include channel straightening, dam construction, disconnection of the river from its floodplain, and alteration of riparian vegetation. These changes also affect wetlands and lakes in the associated floodplain through, for example, a lowering of the groundwater level, increased siltation or changes in inundation regime (Hansen, 1998). Under predicted future climate conditions further stresses will be introduced including the combined effect of changes in precipitation and climate-induced changes in land-use patterns. These in turn may cause changes in catchment hydrology that will affect sediment transport and channel morphology, inundation frequency and extent, and impact aquatic ecosystems at both catchment and habitat scale.

In task 2 'Hydromorphological changes and aquatic and riparian biota' the question is addressed of how the distribution of taxa at the habitat scale is controlled by the environmental conditions at the catchment scale. Characteristic taxa to be used as response parameters to hydrological and morphological structures were therefore identified in task 1. These now called indicators of hydromorphology reflect key hydromorphological conditions at the catchment scale and were studied in task 2 in detail at the habitat scale. The habitats necessary for the occurrence of the indicator species was derived from information of preand post-restoration conditions. Hydromorphological restoration more often reduced habitat dynamics and offered the opportunity to extract the knowledge needed on the relation between habitat dynamics and species occurrences.

The objective was to determine the effects of hydromorphological measures on the structure and functioning of the instream communities. The key questions were: "What are the positive and negative effects of individual hydromorphological measures (=changes) on the instream functioning and indicators" and "What is the role of discharge and thus habitat dynamics upon these measures".

2 Study area

2.1 Catchment and stream description

Geographic position

The lowland stream Springendal is situated on the east side of a glacial hill-ridge in the eastern part of the Netherlands (figuur 1). The Springendal catchment comprises about 485 ha, most of this area (346 ha) is assigned nature reserve ("Het Springendal"). The total length of the stream is 5.5 km, with a slope of 40 m (TNO 1999). The Springendal stream consists of two major upper courses, a northern and a southern one (Figure 1), both fed by in total 7 helocrene springs. After about 600 m these two upper courses join into the middle course. Along the upper and middle courses several spring-fed ponds, and some additional helocrene springs and seepage zones occur. After about 2 km from the source the stream enters an agricultural area and becomes channalised. The stream discharges into the lower course of the stream "Hollander graven", which somewhat further downstream enters the river Dinkel.

The upper part of the catchment is covered with oak-birch, beech-oak and pine forest (large parts owned by 'Staatsbosbeheer', a nature management organization). In the upper and middle course the stream community is still well developed. Cold-stenothermic species occur as well as representatives of rheophilic inhabitatnts of gravel, sand and detritus habitats.

Figure 1. Geographic position of the Springendal stream.

Geology

The last two ice ages shaped the valley and surroundings of the Springendal stream. During the Saalien (the last but one ice age) the glacier ice pushed the depositions, from tertiary origin, up into hill ridges. These tertiary depositions are marked by a limited permeability for water. The tertiary hill ridges were then covered by a layer of fluvioglacial origin and by bottom moraine (Formation of Drente). The absence of plant cover during the Weichselien (last ice age), due to the cold climate, erosion further shaped the hill ridge landscape. Melt water transported sand and gravel and created a U-shaped glacial erosion valley. Later on, this valley was again partly filled with fluvioglacial depositions. Furthermore at the end of the Weichselien, wind transported also a lot of sand and the valley and hill ridges were covered by aeolic sand (Formation of Twente). During the warmer and wetter period of the Holocene, a precipitation surplus in combination with a limited bottom permeability, springs came into existence. These springs were situated close to the top of the hill ridge, there were the tertiary deposition reach the surface (TNO 1999).

Soil composition

The major soil type in the valley of the Springendal stream is podzol (a leached soil formed mainly in cool, humid climates). These soils are composed of fine with low to moderate loam content. In the sides of the hill ridge also coarse sandy soils with gravel occur. Loam/clay layers with gravel mainly occur in the south western part at $40 - 120$ cm depth. In the rest of the catchment these loam-gravel layers occur much deeper under the surface. Close to the stream and in the north western part stream ' beekeerdgronden' (a sandy soil with a humic upper layer) and peaty stream valley soils occur.

Hydrology

The Springendal stream originates from helocrene springs on the steep sides of the hill ridge, there were groundwater reaches the surface due to the presence of impermeable loam layers (TNO 1999). The helocrene springs mainly feed two upper courses, a northern and a southern one. Both upper courses join after about 600 m. The northern course is fed by a near natural helocrene spring and a near natural forested area of the catchment. The southern upper course is fed partly by some helocrene springs and partly through a drainage system from an agricultural enclave. The infiltration area is situated on the top of the hill ridge. The northern upper course is fed from an area of about 63 ha, the southern from about 48 ha.

The sandy top-layer of the hill ridge functions as a rain water reservoir. This top-layer is situated above the impermeable loam/clay layers. In the south western part of the catchment these layers are situated quite close to the surface and are scattered, in the north western part these layers are situated somewhat more regular and deeper. This causes the discharge in the northern springs to be more constant throughout the year in comparison the southern one.

Land-use

The catchment of the Springendal stream is partly used as forest and partly as agricultural area. Until 1850–1900 the area was mainly covered with heather. Around the year 1900 large parts of the north western part were forested and about 50 years later also the wetter south western part was forested or turned into fields and grasslands (Jalink 1997). Nowadays the north western part still is forested and is designated as nature reserve. The infiltration area of the south western part still is used for agricultural purposes. The agricultural areas are heavily fertilized and drained. In 1997 one of these agricultural enclaves was turned into nature area.

Disturbance

The last decades the Springendal stream was threatened by increasing discharge fluctuations, drought, and nutrient enrichment. In the stream valley also acidification occurred. The causes are related. The major cause of these disturbances is due to the agricultural use of the

upper part of the catchment, especially the southern upper course and the Nutterveld branch. Due to the drainage system rain water is directly transported towards the main course of the stream. This results in extreme discharge events, and in periods without rain duet o a less well filled groundwater reservoir, to low discharges or even drought events. Downstream canalization, widening and deepening of the profile caused the stream to incise upstream. These incisions lower the stream bottom and increase the streams draining capacity. This is an extra cause for an increase in discharge dynamics and drought events. Intensive fertilization of the agricultural land enriched the groundwater and surface water with nutrients. All these disturbances caused specific spring and rheophilic stream species to decrease or even to disappear (van Gerven et al. 1997).

The stream valley became dryer and the nutrient poor upper sandy soil acidified. The more organic and peaty soils mineralized and the inundation with nutrient rich water caused eutrophication, locally. Vulnerable stream valley vegetation types disappeared, especially those characteristic for wet and/or oligotrophic conditions

2.2 Major stream sections

Southern upper course

The total length of the southern upper course is about 720 m. This course mainly is forested, except for the most downstream 250 m where it passes a hayfield. Since 1998?, this field is not mowed anymore. Here, re-growth of *Alnus glutinosa* occurs. The uppermost helocrene spring is situated at about 65 m above sea level. Furthermore, the course is fed by a retention pond, the Onland branch and several adjacent springs and seepages areas. Before the construction of the retention pond, the hydrology of the southern upper course was disturbed with high discharge peaks and periods of very low flow. This hydrological condition caused the course to locally incise itself.

Outlet branch retention pond

In 1995 a retention pond was constructed west of the southern upper course to prevent nutrient rich drainage water from the upper most situated agricultural land to enter the stream. This drainage water was one of the major causes of a very instable hydrological condition. The outlet of the retention pond is to the southern upper course. The total length is about 90 m. In 1998 the drainage system from one of the agricultural enclaves was removed. Furthermore, the nutrient rich top soil layer was excavated from part of the enclave and shaped as a gully. There after, this wide gully started to carry a small network of temporary streams and spring or seepage areas.

Onland branch

The upper most part of the northern Onland branch is temporary, only transporting rain water during short wet periods. Though this branch originally emerged from a former helocrene spring area. Halfway it crosses a small man made pond. The temporary southern Onland branch emerges in two small erosion valleys of which only the northern one still provides water. Also these two branches originally emerged more upstream in a former pool and seepage area. Both are dry now. These two branches join in a more down stream situated seepage area. This area adds extra seepage water to the southern branch. The seepage area ends in a waterfall with a height of about 1 m. The southern Onland branch is about 225 m in length. The

whole system of the Onland branches is shaded, except the uppermost, now dry, parts. Both northern and southern branch join some tents of meters before joining the southern upper course of the Springendal stream.

Northern upper course

The total length of the northern upper course is about 560 m. This courses mainly is forested, except for the most downstream 180 m where it passes a hayfield. Since, 1998? this field is not mowed anymore. When entering this hayfield a waterfall was present caused by a big tree root and some large stones. To prevent this waterfall the break whereby the stream would incise, a cascade was constructed in 1998. The uppermost helocrene spring is situated at about 52 m above sea level. The northern branch lacks side branches, but receives water from adjacent helocrene springs and seepage areas. At about 150 m before joining the southern upper course, a dry erosion valley in very wet periods can add extra water. In 1998 a culvert situated just before the joining with the southern upper course was replaced by a square culvert and a small cascade made from stones. This construction caused part (the last 50 m about) of the bottom of northern upper course to rise because of sand sedimentation.

Middle course

Where northern and southern course join the middle course starts. The middle course first crosses small haylands and forested areas. Until the border of the nature reserve its length is about 1600 m. Over the last 210 m it crosses a wooded bank and a fertilized agricultural grassland. Some helocrene springs, seepage areas and three major spring ponds, all man made by damming former helocrene springs, feed the middle course. Furthermore, two major side branches are present, the temporary Nutterveld branch and the small Meerbekke branch. The two major upstream situated spring ponds supply the largest amount of water to the middle course. The third, more swampy, spring pond only adds little to no water anymore. The temporary Nutterveld branch is flashy and causes the middle course to become more instable. Together with the instable southern upper course, before the construction of the retention pond, both branch caused the middle course locally to incise deep into the landscape.

Nutterveld branch

The Nutterveld branch emerges in the Nutterveld area, and was drained and channalized in 19..? Lateron, the channalized part was culverted. Nowadays, the water reaches the surface when entering the nature area. Because of these parctises the water runoff became temporary and flashy. To buffer the flashy floods, in 2004 the branch was diverted through a hayfield towards the swampy spring pond. This pond collects the water and releases it slowly again back to the middle course.

Meerbekke branch

The Meerbekke branch emerges as a helocrene spring near the former farmhouse Meerbekke. It transports only little amount of water. The whole branch is more or less ditched and situated in wet hayfield. When joining the middle course it is fed by a second helocrene spring.

Lower course

The lower course starts at the road crossing Uelserdijk and runs down to the junction with the stream Hollander Graven. The lower course is regulated and receives, just after the road crossing Uelserdijk waste water from a laundry. Several reservoir 9acting as sand collector) and weirs interrupt the course of the stream.

2.3 Restoration measures

Four major restoration measures were undertaken:

- 1. *Stabilizing the discharge regime and nutrient load;* by the construction of a reservoir upstream of the southern upper course and the change of land use in a part of the agricultural enclaves in 1998. The reservoir should buffer surface and subsurface runoff and reduce nutrient run off. Therefore, the drainage system of the agricultural enclave in the south western part of the catchment was connected with the reservoir. The reservoir itself consists of two parts, a collection reservoir and a retention reservoir. The first will be overgrown with helophytes to further reduce the nutrient load, the second functions as discharge buffer. The transformation of a small enclave of agricultural land (this former intense fertilized corn field became natural land in 1996) into natural land in the south western part of the catchment should add to both discharge peak buffering as well as nutrient load reduction. To optimize nature development in this area the drainage was removed and part of the upper soil (nutrient enriched) was extracted and transformed into a gully. Part of the area will be covered by natural forest and part is mowed yearly to further reduce nutrient loads and to develop a natural hayfield (Gerven et al. 1999). Shortly after the implementation of these measures in 1998, a few temporary springs and a temporary stream emerged in the newly developed upstream natural area. A second major measure in this category was buffering the Nutterveld branch discharge peaks by diverting the down stream part of this stream towards a shallow pond which discharges more down stream into the main course of the Springendal stream. The pond will function like a helophyte filter and extract nutrients as well as a buffer to reduce discharge dynamics.
- 2. *Rising the incised stream bottom;* by adding clay (in 1997) a section of the southern upper course and rising the stream bottom with about 0.8-1 m. In another deeply incised section of the southern upper course, in 1997 tree stems were installed (no data available) and in 1999 submerged gravel dams were constructed to induce a slow but steady bottom rise by instream within dam sedimentation.
- 3. *Shading;* by stopping the yearly mowing regime (in 1998) in the grasslands along both the northern and southern upper course, so mainly elder (*Alnus glutinosa*) development can take its course. Shortly after, the southern upper course was invaded by young elder plants. Along the northern upper course the elder development is very slow.
- 4. *Remeandering* of a section of the middle course. This measure is foreseen in 2006.

2.4 Research hypotheses

I. A dynamic discharge pattern will result in a more dynamic substrate and/or stream bottom incision. Stream bottom incision results in an eroded bottom

substrate which is either hard or instable. Both situations will result in an impoverishment of the stream macroinvertebrate community.

Test: the differences between more and less hydrological dynamic stream sections in the upper courses can be analyzed by comparing:

- $\frac{1}{x}$ the southern upper course until 1996, the pre-restoration phase
- $\frac{\sqrt{3}}{2}$ the southern upper course after 1995, the post-restoration phase the northern upper course, the reference sites
- the northern upper course, the reference sites

Test: the differences between more and less hydrological dynamic stream sections in the middle course can be analyzed by comparing:

- √ the middle course after the junction of the Nutterveld branch before 2004, the pre-restoration phase
- √ the middle course after the junction of the Nutterveld branch after 2004, the post-restoration phase
- √ the middle course upstream of the Nutterveld branch junction, the references

II. Stream bottom rise will results in a more balanced process of erosion and sedimentation, a more stable stream substrate and a more diverse habitat mosaic that sustains a more diverse macroinvertebrate community.

- *Test*: the differences between sections before (incised) versus after measures to rise the stream bottom were taken; clay test 1) and dam construction (test 2), respectively:
	- 1. the southern upper course clay section before (until 1998) versus after filling (from 1998 on)
	- 2. the southern upper course gravel dam section before (until 2000) versus after dam construction (from 2000 on)

III. Tree development along the stream ensures leaf and woody debris input, provides shade and reduces temperature fluctuations of the water, all processes contributing to a more diverse macroinvertebrate community.

Test: the differences between more and less shaded sections can be analyzed by comparing:

- $\frac{1}{2}$ the southern upper course in hayfield before 2000; the pre-shading phase
- $\frac{1}{x}$ the southern upper course in hayfield after 2000 (maybe even later as development of wooded bank goes on)
- $\sqrt{\ }$ the southern upper course in the forest

IV. Remeandering will result in a shallow stream with an asymmetric profile, reduced discharge dynamics and a more diverse macroinvertebrate community.

- *Test*: the differences between the current versus the future remeandering section of the middle course:
	- √ the middle course before and after 2006 (? if the restoration is finished) (not sampled yet)

3 Material and methods

3.1 Introduction

The study design followed a Before-After-Control-Impact (BACI) design. For both impact and control site series of samples from before and after the moment of the restoration were available. In some cases a space for time substitution was necessary to compose a complete series. The hydromorphological processes of erosion and sedimentation, partly resulting in incision of lowland streams, are reflected in the morphological structures in the stream by the parameters monitored were described in Deliverable 99.

3.2 Macroinvertebrate sampling

Macroinvertebrates were sampled with a micromacrofaunashovel (10 cm width, 10 cm high, 15 cm length, 0.5 mm mesh size) according to Tolkamp (1980). The shovel is made of stainless steel, on the top and the rear there are openings covered with nylon gauze. On the sides, adjustable wings are screwed, which decide the depth of the sample. This depth is fixed at 2 cm. The shovel is pushed into the substrate at an angle of 30-45o and brought in a horizontal position when it reaches its 2 centimeters depth. At the same moment the shovel is pushed through the substrate over a distance of 15 cm, tilted backwards and lifted above the water surface. The sample is transferred into a bucket. Thus, an area of 15 cm2 is sampled. All substrate-type/habitat samples were kept separate.

A multihabitat approach by sampling the dominant (occurrence $>$ 5%) habitat types in proportion to their frequency of occurrence in the stream reach was also used. At most of the sites fine gravel, sand, fine detritus, coarse detritus and leaves were combined and sampled in such way. Taxa-poor substrates/habitats were sampled 2-3 times (area of 30-45 cm2).

All samples were processed in the laboratory by rinsing the sample over two sieves (mesh size 1 and 0.25 mm), placing the residue in a white tray, and sorting the animals alive.

3.3 Taxonomic adjustment

A common problem in macroinvertebrate community samples is that many inconsistencies occur in the data after identification of the taxa. Many but not all specimens are identified to species level, others to higher taxonomic levels. Such inconsistencies can ultimately lead to pseudo replication and need to be resolved prior to analyses. Inconsistencies were resolved by removing the data from higher taxonomic groups when occurrence of higher groups was sparse, or by clustering species data to higher taxonomic groups when needed. More specifically, the methods described by Nijboer & Verdonschot (2000) were used. The original and new taxa lists are presented in Appendix 2.

3.4 Multivariate methods

The ordination techniques DCA and DCCA (CANOCO 4.5 for Windows) were used. All options used in the runs are listed in Table 1.

Analysis	Objective	Choice of method					
General	Transformation	$Log2$ (abundance+1)					
	Environmental variables	Nominal variables, unless specified otherwise					
	Rare species	Downweighting of rare species					
DCA	Detrending	By segments or $2nd$ order polynomials					
DCCA	Detrending	$2nd$ order polynomials					
	Scaling focus	Inter-sample distances					
	Scaling type	Hill's scaling $(L^{\wedge}a) / (1-L)$					
PCA & RDA	Scaling focus	Inter-sample distances					
	Species scores	Do not post transform					
	Sample centering	By samples					
	Species centering	By species					
Significance testing	Monte Carlo permutation test	-499 Permutations -Unrestricted permutations					

Table 1. Options used in the DCA and DCCA analyses.

4 Results: Analyses of macroinvertebrate communities in pre- and post restoration phases

4.1 Decreased hydrological dynamics in the southern upper course.

The macroinvertebrate communities were sampled in southern upper course in pre- and post- retention pond restoration phases and in the unchanged and relatively stable northern upper course. This sampling scheme allows a Before-After-Control-Impact comparison. A total of 61 samples were obtained between 1979 and 2006, of which 10 were from the prerestoration phase (1980-1995), 28 from the post-restoration phase (1996-2006) and 23 from the reference site (northern upper course, 1979-2006). A total of 248 taxa occurred in the samples. After taxonomic adjustment 142 taxa and taxa-groups remained for analyses. The taxonomic lists used for analyses after taxonomic adjustment are included in Appendix 1.

The ordination techniques DCA and DCCA (CANOCO 4.5 for windows, ter Braak & Smilauer 2002) were used successively to investigate the impact of the restoration measure. Additionally, the variation among sample years, sample seasons and sample locations was included in the analysis. Table 2 (a) shows the results of the first DCA run, which uses the detrending method 'by segments' and allows for determination of the gradient lengths. Gradient lengths of the first two ordination axes both approached two, indicating an intermediate homogeneity in species composition between samples (Verdonschot & ter Braak 1994). This suggests that, possibly, both linear and unimodel detrending techniques could be justified for subsequent analyses. However, the use of unimodel assumptions on species distributions should be preferred over linear assumptions in most biological systems (ter Braak & Verdonschot 1995). Therefore, in subsequent analyses, a unimodel detrending technique was used. The eigenvalues of the ordinal axes in the second DCA run, that uses a 'second order polynomial' detrending technique, were not lower compared to the previous analysis, suggesting justified use of unimodel detrending technique.

I able 2. Kesults of DCA with detrending methods (d) by segments and (b) by $\mathbb Z$					oraer polynomials.
(a) Axes	1	2	3	4	Total inertia
Eigenvalues	0.183	0.143	0.088	0.068	1.782
Lengths of gradient	2.195	1.965	1.529	2.387	
Cumulative percentage variance					
of species data	10.3	18.3	23.2	27.1	
Sum of all eigenvalues					1.782
(b) Axes	1	2	3	$\overline{4}$	Total inertia
Eigenvalues	0.183	0.147	0.120	0.072	1.782
Cumulative percentage variance					
of species data	10.3	18.6	25.3	29.3	
Sum of all eigenvalues					1.782

Table 2. Results of DCA with detrending methods (a) by segments and (b) by 2nd order polynomials.

In order to investigate the macroinvertebrate community differences during the pre- or postrestoration phases, a DCCA was carried out. The eigenvalues of the first two ordinal axes were substantially higher than axis 3 and 4, which indicates that a substantial amount of the total variation explained by the environmental variables is comprised in the first two ordinal axes (Table 3). Together the first two axes explained 15.7 % of all species variation among samples.

Table 3. DCCA results. Included were the restoration phase, year, sample location and season variable groups.

Axes		2°	3	$\overline{4}$	Total inertia
Eigenvalues ٠ Species-environment correlations :	0.159 0.945	0.122 0.927	0.085 0.901	0.056 0.917	1.782
Cumulative percentage variance					
of species data of species-environment relation :	8.9 15.0	15.7 26.5	20.5 34.5	23.7 39.8	
Sum of all eigenvalues Sum of all canonical eigenvalues					1.782 1.060

Figure 2. DCCA ordination diagram with the restoration phase (pre, post, and ref), year, sample location and season variable groups included. The triangles represent the relative effects of the environmental variables and the open circles represent the samples. For clarifying purposes the year variable group is illustratively suppressed in this diagram. Samples are labeled by year and location.

The ordination diagram (Figure 2) shows that the different sample location variables mainly follow the restoration phase separation in the samples (i.e. mainly represent the difference between pre and post vs. reference sites), and are relatively homogenously distributed within restoration phases. This may indicate a relatively minor contribution of the sample location variable group to explaining the inter-sample variation. To test the explanatory importance of the restoration phase variable group versus the variable group sample location, the analysis was repeated without the sample location variable group.

Table 4 represents the results of the DCCA, without the sample location variable group. Compared to the previous analysis, for the first two ordinal axes combined, 1.1 % of the explained variance was lost by excluding sample location. However, the variance in speciesenvironment relationship is now better explained by the ordinal axes (Table 4). The ordination diagram for this analysis (Figure 3.31) shows a relatively strong separation of the pre-restoration samples, but also relatively strong effects of the year and season variable groups.

Figure 3. DCCA ordination diagram with the restoration phase (pre, post, ref), year and season variable groups included. The triangles represent the relative effects of the environmental variables and the open circles represent the samples. Samples are labeled by year and location.

Axes			2 3	$\overline{4}$	Total inertia
Eigenvalues Species-environment correlations : 0.922 0.912 0.871 0.820 Cumulative percentage variance		0.146 0.115 0.078		0.043	1.782
of species data of species-environment relation : 18.3 32.7 42.4			\therefore 8.2 14.6 19.0	21.4 47.8	
Sum of all eigenvalues Sum of all canonical eigenvalues					1.782 0.799

Table 4. DCCA results. Included were the restoration phase, year and season variable groups.

Unfortunately not all restoration phases were sampled equally over the seasons (Figure 4). The two environmental variable groups, restoration phase and season, may thus explain in part the same variance in the species data and their separate effects are difficult to tease apart. To explore which of the two factors is most important, two separate DCCAs, one with the season variable group excluded and one with the restoration phase variable group excluded, were carried out (Table 5). Both analyses yielded similar results; eigenvalues of the first two ordinal axes and the cumulative percentage of explained variation in the species data by the first two ordinal axes were relatively equal (Table 5). This suggests that, by using these data, it is not possible to distinguish between the relative effects of season versus restoration phase.

Table 5. DCCA results. (a) Without the season variable group and (b) without the restoration phase variable group. The variable group year was included in both analyses.

(a) Axes		1	2	3	4	Total inertia
Eigenvalues		0.142	0.110	0.078	0.038	1.782
Species-environment correlations Cumulative percentage variance		0.911	0.908	0.872	0.853	
of species data		7.9	14.1	18.5	20.6	
of species-environment relation		19.9	35.3	46.2	51.6	
Sum of all eigenvalues						1.782
Sum of all canonical eigenvalues						0.713
(b) Axes		1	2	3	$\overline{4}$	Total inertia
Eigenvalues	0.145	0.102	0.068	0.043		1.782
Species-environment correlations	$\ddot{\cdot}$	0.917	0.899	0.816	0.798	
Cumulative percentage variance		8.1	13.9	17.7	20.1	
of species data of species-environment relation		21.0	35.8	45.7	51.9	
Sum of all eigenvalues						1.782

The main problem is that the post-restoration phase was more than the other phases characterized by autumn samples and the pre-restoration phase has a disproportionably high amount of winter samples (Figure 4). Furthermore, the species abundance data, combined for all post-restoration samples and the data combined for all autumn samples were strongly correlated (non-parametric correlation: $r_s = 0.87$, n = 142, p < 0.001, Figure. 4.33a), which was similar for the species abundance data of the pre-restoration phase and the data for the winter samples ($r_s = 0.54$, $n = 142$, $p < 0.001$, Figure 5b). This also indicates the difficulty in distinguishing between the relative effects of seasonality and restoration phase on the species abundance data. To isolate the effect of the restoration measure, the spring and autumn samples were analyzed separately. Spring and autumn samples were both relatively frequently represented in all three restoration phases (Figure.4.32).

Figure 4. Histogram representing the relative frequencies of the seasons within the samples taken in the preand post- restoration phases and at the reference sites.

Figure 5. (a) Correlation between the species abundance data from the post-restoration samples combined and the species abundance data from the autumn samples combined. (b) Correlation between the species abundance data from the pre-restoration samples combined and the species abundance data from the winter samples combined.

Within autumn sample analysis

A DCCA with the restoration phase variable group and the year variable group was carried out, on a total of 25 autumn samples (Table 6). Eigenvalues for the first two ordinal axes were higher than for the other axes and the first two axes together explained 21.7 % of the species data. All ordinal axes were significant (Monte Carlo permutation tests, first axis: $f =$ 2.26, $p = 0.002$; all axes: $f = 1.82$, $p = 0.002$).

Axes			2	3	$\overline{4}$	Total inertia
Eigenvalues 1.502		0.197	0.129 0.084 0.097			
Species-environment correlations Cumulative percentage variance	\sim 1.	0.981	0.942 0.908 0.000			
of species data		13.1	21.7	27.3	33.7	
of species-environment relation	$\ddot{}$		26.6 44.1 55.4 0.0			
Sum of all eigenvalues 1.502						

Table 6. Autumn samples DCCA results. The included variable groups were restoration phase and year.

The ordination diagram shows strong effects of the year variables (Figure 6). Especially the year 2000 strongly influenced the first ordinal axis. However, also the restoration phase variables seemed to have considerable effect, and are mainly separated along the second axis. The pre- restoration samples are mostly separated, in the bottom-left quadrant of the diagram, but this should be interpreted with caution. There were only two pre- restoration phase samples, both from the year 1992, and the reference site samples from that year are closely situated in the diagram. This suggests that another factor has specific effect on the year 1992. The sampling in 1992 was carried out by an external sampler, the 'waterschap Regge & Dinkel'. An explanation may be that their sampling methods deviate somewhat from the methods used for this study. However, it is also likely that there is strong year effect for other, unknown reasons, as other years have strong effects too.

Figure 6. DCCA ordination diagram for the autumn samples, with the restoration phase variable group (pre, post, and ref) and the year group nominal variables. The triangles represent the relative effects of the environmental variables; the open circles represent sample sites. Sample sites are labelled by sample year and location.

Within spring sample analysis

A DCCA with the restoration phase variable group and the year variable group was carried out (Table 7), on a total of 23 spring samples. Eigenvalues of the first two ordinal axes higher than the other axes and together explain 20.0 % of the species data. The ordinal axes significantly fit the species data (Monte Carlo permutation tests, first axis: $f = 1.746$, $p =$ 0.002; all axes: $f = 1.89$, $p = 0.002$)

Table 7. Spring sample DCCA results. Included were the restoration phase and year variable groups.

Axes			\mathcal{L}	3	4	Total inertia
Eigenvalues	$\ddot{\cdot}$	0.179	0.123	0.109	0.116	1.510
Species-environment correlations	$\ddot{}$	0.962	0.936	0.929	0.000	
Cumulative percentage variance of species data	٠	11.8	20.0	27.2	34.9	
of species-environment relation	\therefore	20.9	35.3	48.1	0.0	
Sum of all eigenvalues Sum of all canonical eigenvalues						1.510 0.855

Figure 7. DCCA ordination diagram for the spring samples with the restoration phase variable group (pre, post, and ref) and the year group as variables. The triangles represent the relative effects of the environmental variables; the open circles represent the sample sites. Sample sites are labelled by sample year and location.

The DCCA ordination diagram for the spring samples shows, like for the autumn samples, a strong effect of year that mostly determines the first ordinal axis. The year 1992 is again strongly separated. In this analysis, the pre-restoration samples were clearly separated in the top right quadrant of the diagram, and contrasting to the autumn analyses, this is not matched by the 1992 reference site samples. The reference site samples were in large part concentrated in the bottom right quadrant. This analysis suggests there is separation between all three restoration phases, with the post-restoration samples and the reference site samples showing most similarity.

4.2 Decreased hydrological dynamics in the middle course

The macroinvertebrate communities were sampled in the middle course downstream of the junction of the Nutterveld branch in pre- and post restoration phases. The samples were then compared to reference site samples taken upstream of the Nutterveld branch junction. This sampling scheme allows a Before-After-Control-Impact comparison. A total of 41 samples were obtained between 1981 and 2006, of which 27 were from the pre-restoration phase (1981-2004), 7 from the post-restoration phase (2005-2006) and 7 from the reference site (2002-2006). A total of 269 taxa occurred in the samples. After taxonomic adjustment 135 taxa and taxa-groups remained for further analyses. The taxonomic lists, resulting after taxonomic adjustment, are included in Appendix 2.

The ordination techniques DCA and RDA (RDA is the linear alternative for DCCA) were used successively to investigate the impact of the restoration measure, and the effects of different sample years and sample seasons. Table 8 (a) shows the results of the first DCA, which uses the detrending method 'by segments' and allows for a determination of the gradient lengths. Gradient lengths of the first two ordinal axes are considerably low, especially for the first axis which was \leq 2. This suggests that analyses with a linear model would best suit these data (Verdonschot & ter Braak 1994). Table 8 (b) shows the results of the initial PCA, which is the alternative for DCA, based on linear assumptions on species distributions. The eigenvalues have not decreased compared to the DCA analysis, which suggest it is appropriate to proceed with linear methods of analysis.

	1	$\mathfrak{D}_{\mathfrak{p}}$	3	$\overline{4}$	Total inertia
$\ddot{\cdot}$	0.224	0.113	0.081	0.054	1.730
	1.534	2.100	1.841	1.407	
	13.0	19.5	24.2	27.3	
					1.730
	1	2	3	$\overline{4}$	Total variance
	0.220	0.135	0.071	0.061	1.000
	22.0	35.5	42.7	48.8	
	Cumulative percentage variance Cumulative percentage variance				

Table 8. (a) Initial DCA (by segments) results and (b) PCA results.

A RDA is carried out to investigate the relationships between the macroinvertebrate communities and three environmental variables including the restoration phase variable group (Table 9). The eigenvalues of the first two ordinal axes are substantially higher than the axis 3 and 4, which indicates that a substantial proportion of the total variance explained by the environmental variables is comprised in the first two ordinal axes. Together the first two axes explain 32.4 % of all species variation among samples, which is close to the amount of variance explained by the first two ordinal axes in the PCA (i.e. without any environmental variables specified).

Axes			2	3	4	Total variance
Eigenvalues Species-environment correlations		0.202	0.122 0.059	0.962 0.956 0.952	0.045 0.880	1.000
Cumulative percentage variance of species data of species-environment relation	٠ $\ddot{}$ \therefore	20.2 30.2	32.4 48.4	38.3 57.1	42.8 63.9	
Sum of all eigenvalues Sum of all canonical eigenvalues						1.000 0.670

Table 9. RDA results. Included were the restoration phase, year, sample location and season variable groups.

Figure 8. RDA ordination diagram for the Nutterveld branch restoration measure. Included variables: restoration phase (pre, post, and ref), year, sample location and season variable groups. The triangles represent the relative effects of the environmental variables and the open circles represent the sample locations. Samples are labeled by year and location.

The ordination diagram shows a relatively strong effect of year, particularly 2004, but also indicates a separation in the samples by the restoration phase variable group, with the prerestoration samples mostly separated from the others in the top right half of the diagram (Figure 8). Sample location seemed of negligible importance compared to the other environmental variables, and to test this, a second RDA without the sample location variable group is carried out Table (10).

Axes variance			\mathcal{L}	3		Total	
Eigenvalues Species-environment correlations	$\ddot{}$	0.201 0.959	0.112 $0.922 \quad 0.937$	0.056	0.042 0.855		1.000
Cumulative percentage variance of species data of species-environment relation	\sim :	20.1 34.3	31.2 53.3	36.9 62.9	41.0 70.1		
Sum of all eigenvalues Sum of all canonical eigenvalues							1.000 0.585

Table 10. RDA results. Included were the restoration phase, year and season variable groups.

Compared to the first RDA only 1.2 % of the explained variance by the first two ordinal axes was lost (Table 10), which is relatively minor. This suggests that the sample location variable group indeed had little effect on the species community in the samples. The ordinal axes in this analysis fit the data significantly (Monte Carlo permutation test; first axis: $f =$ 6.27, $p = 0.002$; all ordinal axes: $f = 2.35$, $p = 0.002$).

Year had a relatively strong effect (Figure 9), particularly 2004, which may be explained by the fact that 2004 was the year in which the restoration measure was carried out, the samples were taken on November, short after the restoration measure. The samples are also clearly separated by the restoration phase variable group (Figure 9). The pre-restoration samples are all grouped in the top-right half of the diagram, with post- (m4-8) and ref-samples (m1-2) all being grouped in the bottom-left half of the diagram. There seems some but relatively minor separation between post- and ref-samples, indicating that the species community has shifted from the pre-restoration composition to a species composition that more resembles that of the reference samples. The seasonal effect was relatively minor.

Figure 9. Second RDA ordination diagram for the Nutterveld branch restoration measure. Included variables: restoration phase (pre, post, ref), season and year variable groups. The triangles represent the relative effects of the three environmental variables; the open circles represent each sample site. Sample sites are labelled by sample year and location. The variable 'year' is not represented in this graph for the clarifying purposes; the year- information can in large part be obtained from the sample labels. .

4.3 Stream bottom rise in the southern upper course

Clay filling

The macroinvertebrate communities were sampled in the clay section of the southern upper course in pre- and post-filling restoration phases. A total of 7 samples were obtained between 1980 and 2005, of which 4 were from the pre-restoration phase (1980-1997), 3 from the post-restoration phase (2004-2005). A total of 107 taxa occurred in our samples. After taxonomic adjustment 75 taxa and taxa-groups remained for analysis (Appendix 3).

The ordination techniques DCA and DCCA were used successively to investigate the impact of the restoration measure. All samples were taken from the same location, hence sample locations were not used as variables in this analysis. Table 11 shows the results of the first DCA, which uses the detrending method 'by segments' and allows for a determination of the gradient lengths. Gradient length of especially the first axis is considerably higher than two which suggests that unimodel species distributions can be assumed (Verdonschot & ter Braak 1994) and therefore unimodel techniques are used in subsequent analyses (DCCA).

(a) Axes		1	2	3	$\overline{4}$	Total inertia	
Eigenvalues	$\ddot{\cdot}$	0.419	0.209	0.020	0.000		1.517
Lengths of gradient		2.486	1.600	2.146	2.369		
Cumulative percentage variance							
of species data	$\ddot{\cdot}$	27.6	41.4	42.7	42.8		
Sum of all eigenvalues							1.517
(b) Axes			$\mathbf{1}$	2	3	$\overline{4}$	Total inertia
Eigenvalues Cumulative percentage variance	٠		0.419	0.271	0.191	0.000	1.517
of species data	$\ddot{\cdot}$		27.6	45.5	58.1	0.0	
Sum of all eigenvalues							1.517

Table 1.1 DCA result detrended by (a) segments and (b) second order polynomials.

A DCCA was carried out to investigate the relationships between the macro invertebrate communities and the environmental variable groups (Table 12). The eigenvalues of the first two ordinal axes are substantially higher than the axis 3 and 4, which indicates that a substantial amount of the total variance was explained by the environmental variables. Together the first two axes explain 44.9 % of all species variation among samples which is nearly as high as that of the initial DCA (i.e. without environmental variables specified, Table 11 b). In the DCA ordination diagram (Figure 10) it can be seen that this analysis is based on relatively few samples, even with more environmental variables included, and these results may therefore be unreliable. Furthermore the diagram indicates that several years only represent one sample and thus coincide exactly in the diagram. This indicates that year is not an appropriate variable group and therefore a second DCCA, with only the restoration phase and season variable groups as environmental variables is carried out (Table 13).

Axes	$\mathbf{1}$	$\overline{2}$	\mathfrak{Z}	$\overline{4}$	Total inertia
Eigenvalues Species-environment correlations Cumulative percentage variance	0.413 0.997	0.268 0.999	0.232 0.000	$0.000\,$ 0.000	1.517
of species data of species-environment relation $\ddot{\cdot}$	27.2 32.2	44.9 53.0	60.2 $0.0\,$	$0.0\,$ 0.0	
Sum of all eigenvalues Sum of all canonical eigenvalues					1.517 1.286
2.0					
				92.May 1992	
spring					
1997. 97.Nov \bigcirc 05. Nov 200 05. March 04. Nov 2004	pre				
ယ္ $\overline{\tau}$			winter	1980 80.Feb.a 80.Feb.b	
-1.0				2.0	

Table 12. DCCA results. Included were the restoration phase, year and season variable groups.

Figure 10. DCCA ordination diagram for the clay-filling restoration measure with the restoration phase (pre, post), season and year variable groups included. The triangles represent the relative effects of the three environmental variables; the open circles represent each sample site. Sample sites are labelled by sample year and season.

Axes			2	3	4	Total inertia
Eigenvalues		0.348	0.219	0.291	0.000	1.517
Species-environment correlations	÷	0.969	0.989	0.000	0.000	
Cumulative percentage variance						
of species data		22.9	37.4	56.5	0.0	
of species-environment relation		43.2	70.4	0.0	0.0	
Sum of all eigenvalues						1.517
Sum of all canonical eigenvalues						0.805

Table 13. DCCA results. Included were the restoration phase and season variable groups.

A total of 7.5% explained variance was lost by removing the year variable group from the analysis (Table 13). In the DCCA ordination diagram, the samples sites from pre- and postrestoration phases are separated, with the post- restoration samples on the left and the prerestoration samples on the right of the diagram (Figure 11). The seasonal effect seemed stronger than the effect of the restoration measure and it should be noted that the two 1980 samples are the only winter samples and hence the winter variable overlaps automatically with those two samples (Figure 11). The two ordinal axes are not significant according to a Monte Carlo permutation test (first axis $f = 0.89$, $p = 0.23$. all ordinal axes: $f = 1.13$, $p =$ 0.08), which is probably caused by the low number of samples (7) in analysis. The results of this ordination analysis should therefore be treated with caution.

Figure 11. Second DCCA ordination diagram for the clay-filling restoration measure with the restoration phase and season variable groups included. The triangles represent the relative effects of the environmental variables; the open circles represent each sample site. Sample sites are labelled by sample year and season.

In spite of the low sample size and the unreliability of this analysis, the DCCA at least suggests a possible effect of the restoration by clay-filling. However the absence of reference-site samples makes it hard to determine if any effect acts positively or otherwise on the macroinvertebrate community. Therefore, additionally, the mean frequency and abundance of species in the pre- and post-restoration samples was compared. The mean abundance (individuals per m², averaged over all species, \log_2 transformed) was mean \pm 1SD $= 1.04 \pm 1.24$ for the pre-restoration samples and 1.64 \pm 2.17 for the post-restoration samples. Mean species abundance is significantly higher in post-restoration samples (non parametric test for two related samples; Wilcoxon signed rank test: $z = -2.50$, $n = 75$, $p =$ 0.012, SPSS 12.0.1 for Windows). It thus seemed that the overall species abundance increased after the clay-filing restoration measure. To provide more insight in the effect on macroinvertebrate species diversity, the mean number of different species per sample for the pre- and post-restoration samples is presented: mean \pm 1SD = 22.75 \pm 6.18 and 28.00 \pm 1.73 respectively. The post-restoration samples presumably contained a higher diversity of species although the low number of samples (4 and 3) do not allow for accurate significance testing.

Dam construction

Samples were taken from the gravel dam section of the southern upper course in pre- and post- dam construction restoration phases and at a reference site in the southern upper course. This sampling scheme allows for a Before-After-Control-Impact comparison. A total of 16 samples were obtained between 1999 and 2006, of which only one was from the prerestoration phase (1999), 7 from the post-restoration phase (2001-2005) and 8 from the reference site (1999-2006). A total of 133 taxa occurred in the samples. After taxonomic adjustment 84 taxa and taxa-groups remained for analyses. The taxonomic lists that were used after adjustment are included in Appendix 4.

The ordination techniques DCA and RDA were used successively to investigate the impact of the restoration measure. Table 14 (a) shows the results of the first DCA, which uses the detrending method 'by segments' and allows for a determination of the gradient lengths. Gradient lengths of the first two ordinal axes were both \leq 2, indicating a homogenous species composition between samples. This suggests that linear model based analyses would best suit these data (Verdonschot & ter Braak 1994).

(a) Axes		1	\mathcal{L}	3	$\overline{4}$	Total inertia	
Eigenvalues	$\ddot{\cdot}$	0.217	0.170	0.081	0.032		1.343
Lengths of gradient	$\ddot{\cdot}$	1.837	1.483	1.385	1.212		
Cumulative percentage variance							
of species data	$\ddot{\cdot}$	16.1	28.8	34.8	37.2		
Sum of all eigenvalues (b) Axes variance		1	\mathcal{L}	3	4	Total	1.343
Eigenvalues		0.212	0.173	0.113	0.104		1.000
Cumulative percentage variance							
of species data	٠	21.2	38.5	49.8	60.2		
\sim $\overline{11}$ 1 \cap							1.000

Table 14. (a) DCA results, (detrended by segments) and (b) PCA results.

Before proceeding with the first RDA, two problems in these data should be noted. 1) There is only one pre-restoration sample, and any interpretations concerning the pre-restoration phase should therefore be treated with caution. 2) Partly because there is only one prerestoration sample, the variable 'sample location' represented mostly the same information as the difference between post-restoration phase and reference site. Therefore sample location was not used as an environmental variable group in this analysis.

A first RDA was carried out to investigate the relationships between the macroinvertebrate communities and the environmental variables (Table 15). The eigenvalues for the first two ordinal axes were substantially higher than for axis 3 and 4, which indicates that a substantial amount of the total variation explained by the environmental variables was comprised in the first two ordinal axes. Together the first two axes explained 37.7% of variation among samples, which is comparable to that in the initial PCA. This indicates that little information was lost by excluding sample location from the analysis.

I WORK TO CHILD I T FORWARD. INVERSION WORK WAS TODDED WHOM PROWDER WHEN SOUND HE CONTROLLY CHIPPER							
Axes			\mathcal{L}	3	4	Total	
variance							
Eigenvalues		0.207	0.169	0.101	0.094		1.000
Species-environment correlations	$\ddot{\cdot}$	0.992	0.992 0.961		0.975		
Cumulative percentage variance							
of species data		20.7	37.7	47.8	57.2		
of species-environment relation		26.4	48.0	60.9	72.9		
Sum of all eigenvalues							1.000

Table 15. RDA results. Included were the restoration phase, year and season variable groups.

Figure 12. RDA ordination diagram for the dam construction restoration measure with the restoration phase (pre, post, ref), year and season variable groups included. The triangles represent the relative effect of the variables; the open circles represent each sample site. Sample sites are labelled by sample year and season and location.

In the RDA ordination diagram (Figure 12) the samples are mostly separated by the year and season variable groups and there is relatively little separation between post- and ref-samples. It should thus be concluded at this stage that season and especially sample year explained more variation than the restoration phase variable group.

However, more insight in the specific effect of the restoration could be obtained by investigating relationships between the macro invertebrate communities and environmental variables while first controlling for the strong effect of year. I.e. investigating the effect of the restoration measure after the variance explained by the year group is removed. Therefore a partial RDA, with year as a covariable group, is carried out.

Axes			2 $\frac{3}{2}$		Total variance	
Eigenvalues \therefore 0.169 0.075 0.038 0.026 Species-environment correlations : 0.995 0.968 0.951 0.898 Cumulative percentage variance					1.000	lable
of species data of species-environment relation : 54.7 79.2 91.5	32.1	46.4	53.6	58.6 100.0		lonte
Sum of all eigenvalues Sum of all canonical eigenvalues					0.526 0.308	

Table 16. Partial RDA results. Included were the restoration phase and season variable group, covariable = year group.

Carlo permutation test; first axis: $f = 2.83$, $p = 0.018$; all axes $f = 2.13$, $p = 0.006$). The ordination diagram for this analysis is presented in Figure 13.

Figure 13. Partial RDA ordination diagram for the dam construction restoration measure with the restoration phase (pre, post, and ref) and season variable groups included. The year variable group is included as a covariable. The triangles represent the relative effect of the variables; the open circles represent each sample site. Sample sites are labelled by sample year, season and location.

The effect of winter was strongest and mainly determines the first ordinal axis. However, there now is a relatively clear separation of the pre-, post- and reference samples along the second ordinal axis (Figure 13). Despite that there was only one pre-restoration sample, these results indicate that the composition of macroinvertebrate community has shifted somewhat toward the reference situation after the dam-construction, suggesting a positive effect of the restoration measure. However, it should be noted that seasonal- and year effects had substantially more influence on the community than the restoration measure.

4.4 Shading by tree development in the southern upper course

Samples were taken from the hayfield-section of the southern upper course in pre- and postrestoration (i.e. forestation) phases and at a reference site in the natural forest section of southern upper course. Because the tree development in the newly forested area (postrestoration) is gradual process, with the shading intensity increasing over years, a Before-After-Control-Impact comparison is not appropriate for this analysis. Instead, the pre-, postand reference sample information was combined with temporal continuity of tree development to create a continuous environmental variable. For this 'tree development' variable, the pre-restoration phase samples all received score 1 (i.e. no shading by trees). The years from the post-restoration phase got scores 2-9 for years 1998-2006 respectively (i.e.

increasing intensity of shading by developing trees). All the reference samples received score 9 (i.e. maximum shading intensity). A total of 23 samples were obtained between 1980 and 2006, of which 8 were from the pre-restoration phase (1980-1997), 4 from the postrestoration phase (2001-2005) and 11 from the reference site (1997-2006). A total of 190 taxa occurred in the samples, after taxonomic adjustment 109 taxa and taxa-groups remained for analyses (Appendix 5).

The ordination techniques DCA and RDA were used successively to investigate the impact of the restoration and subsequent tree development. Table 17 (a) shows the results of the first DCA, which used the detrending method 'by segments' and allows for a determination of the gradient lengths. Gradient length of the first ordination axis was \leq 2. This suggests that analyses by a linear model would best suit our data (Verdonschot & ter Braak 1994). Table 17 (b) shows the PCA results, with eigenvalues of the first two axes being similar to those in the DCA.

(a) Axes		1	2	3	4	Total inertia
Eigenvalues	٠	0.226	0.147	0.074	0.047	1.603
Lengths of gradient	\bullet	1.910	2.298	2.024	1.337	
Cumulative percentage variance						
of species data	$\ddot{\cdot}$	14.1	23.3	27.9	30.8	
Sum of all eigenvalues						1.603
(b) Axes variance		1	2	3	4	Total
Eigenvalues	$\ddot{\cdot}$	0.245	0.148	0.103	0.084	1.000
Cumulative percentage variance of species data	$\ddot{\cdot}$	24.5	39.3	49.6	58.0	

Table 17. (a) DCA (by segments) and (b) PCA results.

Preliminary analysis with and without the location variable group indicated that only a minor, negligible amount of explained variation was lost by removing the sample location variables (0.3%). Therefore the sample location variable group was not included in this analysis, but samples are labeled by location in the subsequent diagrams.

Axes			\mathcal{L}	3		Total variance
Eigenvalues		0.232	0.137	0.099	0.078	1.000
Species-environment correlations	$\ddot{}$	0.977	0.968	0.985 0.971		
Cumulative percentage variance						
of species data	٠	23.2	36.9	46.8	54.6	
of species-environment relation	$\ddot{\cdot}$	28.8	45.8	58.1	67.7	
Sum of all eigenvalues						1.000
Sum of all canonical eigenvalues						0.806

Table 18. RDA results. Included were the nominal season and year variable groups and the continuous tree development variable.

Figure 14. RDA ordination diagram for the sahding restoration measure with the nominal year and season variable groups and the continuous tree development variable included. The triangles represent the relative effect of the nominal variables; the open circles represent each sample site. The arrow represents the relative effect of the continuous tree development variable. Sample sites are labelled by sample year and restoration phase (pre, post, ref).

The RDA ordination diagram (Figure 14) shows that year most strongly effects the separation of the samples. In the years 2001-2005 the samples are highly clustered by year regardless of their classification as reference or post restoration samples. This could indicate that either the year effect was particularly strong in those years, or that the species community in post restoration samples was very similar to the reference samples overall. It is difficult to interpret which of these two scenarios is most likely, because there were no samples from the pre restoration phase and the reference site with overlapping years. The tree development variable seemed to have strong effect too, but this was mainly caused by the large difference between scores the for pre restoration samples versus the scores of the reference samples. There seems to be a gradual change in species community in the post restoration samples, coinciding with subsequent years (2001-2005), however, notably, this gradual change is directed more toward the pre restoration samples and not toward the reference samples. Given that the reference samples from those years follow the post restoration samples closely; this change in species community was most likely caused by a year effect other than the tree development, possibly due to between-year climatological variation.

5 Conclusions

Decreased hydrological dynamics in the southern upper course.

The macroinvertebrate communities were sampled in southern upper course in pre- and post- retention pond restoration phases and in the unchanged and relatively stable northern upper course as reference. The analyses showed that the pre- and post- retention pond restoration phases and the reference samples separated well, and that the within group sample variability was small. Furthermore, the pre-restoration samples were most different while the post-restoration samples and the reference site samples showed most similarity. As the samples were not taken using exactly the same method, this affected the deviation of the pre-restoration samples. Furthermore, the results were influenced by differences in season and years of sampling.

Decreased hydrological dynamics in the middle course

The macroinvertebrate communities were sampled in the middle course downstream of the junction of the Nutterveld branch in pre- and post restoration phases. The samples were then compared to reference site samples taken upstream of the Nutterveld branch junction. Again the pre-restoration samples were most separated from the others. The results indicated that the species community has shifted from the pre-restoration composition to a species composition that more resembles that of the reference samples. The seasonal effect was relatively minor.

Stream bottom rise in the southern upper course Clay filling

The macroinvertebrate communities were sampled in the clay section of the southern upper course in pre- and post-filling restoration phases. The seasonal effect was stronger than the effect of the restoration measure. As the analyses were based on relatively few samples the results may therefore be unreliable. Still, despite the low sample size and the unreliability of this analysis, a further analysis suggested a possible effect of the restoration by clay-filling. The average species abundance and the number of species was significantly higher in postrestoration samples.

Dam construction

Samples were taken from the gravel dam section of the southern upper course in pre- and post- dam construction restoration phases and at a reference site in the southern upper course. The results indicated that the composition of macroinvertebrate community shifted somewhat toward the reference situation after the dam-construction, suggesting a positive effect of the restoration measure. However, it should be noted that seasonal- and year effects had substantially more influence on the community than the restoration measure.

Shading by tree development in the southern upper course

Samples were taken from the hayfield-section of the southern upper course in pre- and postrestoration (i.e. forestation) phases and at a reference site in the natural forest section of southern upper course. Because the tree development in the newly forested area (postrestoration) is gradual process, with the shading intensity increasing over years, the development over time was tested. The results showed a gradual change in species

community in the post-restoration samples which coincided with the subsequent years 2001- 2005). However, this gradual change was directed more toward the pre-restoration samples and not towards the reference samples. Given that the reference samples from those years follow the post-restoration samples closely, this was possibly due to between-year climatological variation.

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Appendices: Adjusted taxonomic list with frequency and abundance.

The 'new taxon code' represents the species code as used in the analyses after adjusting the taxonomic list. If a new taxon code is absent, this taxon was excluded from analyses.

Appendix 1- Retention pond restoration measure.

taxon code	new taxon code	taxon name	taxon	frequency	total
			number		abundance
TRICLADI	TRICLADI	Tricladida	116	$\mathbf{1}$	41.14
DUGESISP	DUGESISP	Dugesia sp	120	$\overline{2}$	174.25
DUGEGONO	DUGEGONO	Dugesia gonocephala	122	36	10907.88
DUGELUPO	DUGELUPO	Dugesia lugubris/polychroa	126	$\mathbf{1}$	16.80
DUGEPOLY	DUGEPOLY	Dugesia polychroa	130	1	16.80
POLISSPE	POLISSPE	Polycelis sp	136	3	37.78
POLITENU	POLITENU	Polycelis tenuis	144	\overline{c}	35.56
NERITIAE	NERITIAE	Neritidae	184	$\mathbf{1}$	26.67
LYMNAEAE	LYMNAEAE	Lymnaeidae	323	$\mathbf{1}$	0.46
PHYSFONT	PHYSFONT	Physa fontinalis	378	$\mathbf{1}$	1.37
HIPPCOMP	HIPPCOMP	Hippeutis complanatus	449	$\mathbf{1}$	2.00
PLBACORN	PLBACORN	Planorbarius corneus	462	1	13.33
PISIDNAE	PISIDNAE	Pisidiinae	529	$\mathbf{1}$	13.33
PISIDISP	PISIDISP	Pisidium sp	531	18	1111.00
PISICASE	PISICASE	Pisidium casertanum	534	13	370.17
PISIHIBE	PISIHIBE	Pisidium hibernicum	545	$\mathbf{1}$	13.33
PISINITI	PISINITI	Pisidium nitidum	549	1	106.67
PISIOBOB	PISIOBOB	Pisidium obtusale obtusale	556	3	15.57
PISIPERS	PISIPERS	Pisidium personatum	557	14	355.36
SPUMSPEC	SPUMSPEC	Sphaerium sp	573	$\mathbf{1}$	133.33
GLSIPHAE	GLSIPHAE	Glossiphoniidae	708	$\mathbf{1}$	1.37
GLSICOMP	GLSICOMP	Glossiphonia complanata	716	1	11.11
HEBDSTAG	HEBDSTAG	Helobdella stagnalis	741	\overline{c}	1.60
ERPOBDAE	ERPOBDAE	Erpobdellidae	796	$\mathbf{1}$	13.33
ERPOOCTO	ERPOOCTO	Erpobdella octoculata	801	11	106.76
OLCHAETA	OLCHAETA	Oligochaeta	825	\overline{c}	27.12
NAIDIDAE	NAIDIDAE	Naididae	865	$\mathbf{1}$	0.46
CHTEDIAS	CHTEDIAS	Chaetogaster diastrophus	871	$\mathbf{1}$	6.86
NAISCOVA	NAISCOVA	Nais communis/variabilis	890	$\mathbf{1}$	13.33
NAISCOMM	NAISCOMM	Nais communis	891	$\overline{4}$	81.37
NAISVARI	NAISVARI	Nais variabilis	892	5	122.22
SLAVAPPE	SLAVAPPE	Slavina appendiculata	927	$\mathbf{2}$	40.00
PRNEAMPH	PRNEAMPH	Pristinella amphibiotica	973	\overline{c}	31.77
PRNEJENK	PRNEJENK	Pristinella jenkinae	975	3	51.11
TUFICIAE	TUFICIAE	Tubificidae	979	\overline{c}	26.67
TUFICJZH	TUFICJZH	Tubificidae juveniel zonder haarsetae	981	7	49.94
TUFICJMH	TUFICJMH	Tubificidae juveniel met haarsetae	983	27	744.98
TUFETUBI	TUFETUBI	Tubifex tubifex	994	18	392.03
LIDRHOFF	LIDRHOFF	Limnodrilus hoffmeisteri	1001	3	22.13
LIDRUDEK	LIDRUDEK	Limnodrilus udekemianus	1004	3	37.78
AUDRJAPO	AUDRJAPO	Aulodrilus japonicus	1046	1	19.05
AUDRPLUR	AUDRPLUR	Aulodrilus pluriseta (zie opmerking)	1049	2	66.67
RHDRCOCC	RHDRCOCC	Rhyacodrilus coccineus	1056	$\mathbf{2}$	18.00
ENCHYTAE	ENCHYTAE	Enchytraeidae	1099	17	490.12
LUCULIAE	LUCULIAE	Lumbriculidae	1142	26	883.67
STLOHERI	STLOHERI	Stylodrilus heringianus	1147	16	248.90
LUCUVARI	LUCUVARI	Lumbriculus variegatus	1151	6	14.81
LUMBRIAE	LUMBRIAE	Lumbricidae	1156	5	14.54

Appendix 2- Nutterveldbranch restoration measure.

Appendix 4- Dam-construction restoration measure.

