

## Research Article

# Substrate-specific macroinvertebrate diversity patterns following stream restoration

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**Abstract.** We examined the effects of stream restoration efforts that re-established multiple-channel sections in otherwise single-channel streams on aquatic habitat diversity and macroinvertebrate assemblages. At seven pairs of sites (single- vs. multiple-channel) we analysed the diversity of aquatic habitat parameters at various spatial scales (e.g. shore length, channel features, substrate diversity, flow variability). We also sampled macroinvertebrates in all available substrates individually and compared alpha- and beta-diversity and nestedness patterns on substrates between single- and multiple-channel sections. Multiple-channel sections showed a considerably more diverse hydromorphology. Taxa number, abundance, and evenness of macroinvertebrate assemblages did not differ significantly. Ten Coleoptera and seven Trichoptera taxa were present exclusively in multiple-channel sections on loam, sand, living parts of terrestrial plants (LPTP), coarse particulate organic matter (CPOM) or large

wood. Non-metric Multidimensional Scaling showed that macroinvertebrate assemblages were substrate-specific rather than section-specific. Nestedness did not differ for samples from single- and multiple-channel sections, nor for individual substrates from different sections. We did not observe differences in alpha-diversity from substrates at single- and multiple-channel sections. However, different substrates host different assemblages and the increased substrate diversity in multiple-channel sections might result in higher beta-diversity in these sections. Our results indicate that stream restoration projects aimed at re-developing near-natural macroinvertebrate diversity should focus on generating several long multiple-channel stretches with large areas of high quality habitats (e.g. large wood), creating stepping stone habitats for re-colonisation, and should allow sufficient time for new assemblages to establish.

**Key words.** Hydromorphology; aquatic habitat; multiple-channel streams; mountain streams; alpha-diversity; beta-diversity; nestedness.

## Introduction

Both large and small scale parameters influence macroinvertebrate assemblages. Large scale influences stem from hydrological, physical, geomorphological and chemical processes. Small scale influences can originate from the texture of single stones, substratum complexity, the spatial distribution of sand and leaf

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patches, and the mosaic heterogeneity around a habitat (Beisel et al., 2000; Palmer et al., 2000; Lepori et al., 2005). Invertebrate species traits, e.g. those related to oviposition sites, migration, and drift, also play a role in shaping macroinvertebrate assemblages (Harper and Everard, 1998). There are data showing that physical complexity promotes biological richness at all spatial scales (Brosse et al., 2003; Townsend et al., 2003). Today, most streams in Central Europe exhibit only remnants of their former hydromorphological and aquatic habitat diversity (EC, 2000). Due mainly to poor hydromorphology, 86% of the water bodies assessed in Germany were found to be at risk of failing the Water Framework Directive (WFD) objectives (BMU, 2005), which include achieving a "good" ecological status of all surface water bodies by 2015. The situation is similar for the large, international Rhine and Danube basins (ICPDR, 2005; ICPR, 2005).

To achieve the goals set by the WFD, a variety of restoration measures has been conducted in Germany. Recently, sections of larger streams (100–1000 km<sup>2</sup> catchment area) have been restored, with the goal of recreating their stream type-specific reference conditions. Large parts of the central European highlands exhibit catchment geology, slope and discharge characteristics that naturally support multiple-channel streams (LUA NRW, 2001a; LUA NRW, 2001b; Sommerhäuser and Pottgiesser, 2005), which are characterised by a network of active and abandoned channels within the floodplain. Today, most streams have single-channel beds, with bank fixation and/or flow regulation that prevent or restrict lateral migration to a narrow stretch of the former floodplain. For these streams, restoration should aim at re-establishing multiple channels to increase habitat heterogeneity (Muotka et al., 2002; Moerke et al., 2004). Such measures could influence the macroinvertebrate assemblages in two ways: (1) Equal substrates are colonised differently in single- vs. multiple-channel sections, resulting in differences in alpha-diversity; (2) Substrates vary between single- and multiple-channel sections, increasing beta-diversity through substrate specific assemblages. Besides alpha- and beta-diversity, the degree of nestedness (Atmar and Patterson, 1993) is a valuable indicator of substrate-related macroinvertebrate assemblage heterogeneity. Nestedness analyses can thus help identify which substrates might be valuable for conservation.

While physical changes in the investigated sections have been achieved (Jähnig et al., 2008a), their biological implications, especially in terms of benthic macroinvertebrates used for stream assessment, remain unclear (Jähnig et al., 2008b). This study compares diversity patterns following restorations

that successfully increased habitat diversification. Specifically, we aim to:

- Quantify differences of alpha-diversity (within-substrate diversity) of the benthic macroinvertebrate assemblage on specific substrates at single- and multiple-channel sections.
- Quantify differences of beta-diversity (between-substrate diversity) of macroinvertebrate assemblages at single- and multiple-channel sections.
- Quantify differences in the degree of nestedness of macroinvertebrate assemblages on different substrates between single-channel and multiple-channel sections.

Understanding the relationships of substrate-related differences in macroinvertebrate assemblages in single- and multiple-channel stream sections, will enable more effective planning of restoration measures, as the relative importance of certain habitats for the macroinvertebrate assemblage can be estimated. We will also be able to determine whether past efforts in restoring in-stream habitat were sufficient to cause shifts in the macroinvertebrate assemblage.

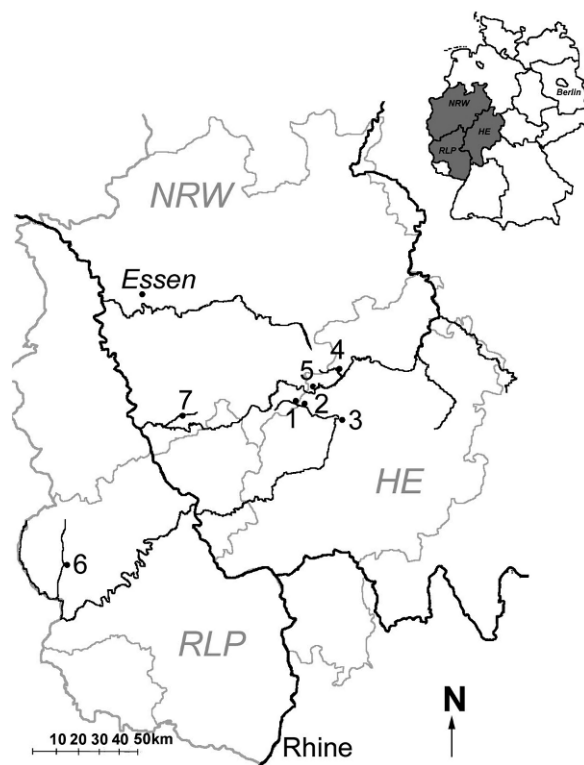
## Material and methods

### Study sites

The study area is located in the central German Highlands (Fig. 1). The sites are located in the upper reaches of the Lahn, Eder, and tributaries of the Sieg and Mosel. The catchment size at sampling sites ranges from 180 to 650 km<sup>2</sup> (Table 1). The catchment geology mostly comprises acid rock (schist). Land use in the study catchments consists of forest (60%), pasture and agriculture (30%), and around 10% urban areas (Corine land cover data Germany, 2000). The research is designed as a paired-site study. In the spring and summer of 2004 and 2005, seven multiple-channel sections were compared to nearby straightened single-channel sections in the same streams to examine differences in hydromorphology and stream macroinvertebrate assemblages. The multiple-channels either resulted from restoration measures (Lahn-W, Lahn-LH, Lahn-C) or developed autonomously in sections where maintenance ceased or was abandoned in recent years (Orke, Eder, Nims and Bröl). All multiple-channel sections are nested within a largely uniform single-channel environment. Hydromorphological details on the sites are provided in Jähnig et al. (2008a).

**Table 1.** Site characteristics. Information is valid for both single- and multiple-channel sections, as they are located some 100 meters distant.

Site name	Lahn-W	Lahn-LH	Lahn-C	Orke	Eder	Nims	Bröl
Stream	Lahn	Lahn	Lahn	Orke	Eder	Nims	Bröl
Location	Wallau	Ludwigshütte	Coelbe	Niederorke	Dodenau	Birtlingen	Waldbröl
Latitude (N)	50°55'37"	50°55'29"	50°51'47"	51°9'8"	51°1'38"	49°56'48"	50°49'36"
Longitude (E)	8°29'20"	8°29'59"	8°47'25"	8°50'37"	8°34'21"	6°29'3"	7°22'58"
Catchment size (km <sup>2</sup> )	278	288	650	289	480	222	181
Section area (ha) (single- vs. multiple-channel)	0.29	0.31	0.48	0.43	0.73	0.35	0.45
Altitude (m asl)	0.62	1.14	1.24	0.92	0.74	0.55	1.04
Catchment geology	300	300	190	300	300	240	104
Restoration year	100% acid rock (schist) 2001	100% acid rock (schist) 2002	100% acid rock (schist) 2000	100% acid rock (schist) n.a. (individually started ~ 1998)	100% acid rock (schist) n.a. (individually started ~ 2000)	40:60% acid : carbonate rock n.a. (individually started ~ 1996)	100% acid rock (schist) n.a. (individually started ~ 1995)
Mean discharge (m <sup>3</sup> /s)	5.1	5.2	8.3	6.3	10.5	2.8	3.4
Bankfull discharge (m <sup>3</sup> /s)	45	45	90	65	131	49	46
Local channel slope (m/km)	0.21	0.40	0.20	0.45	0.20	0.48	0.60

**Figure 1.** Location of sampling sites; light grey = borders of German Federal States: NRW = North Rhine-Westphalia; HE = Hesse; RLP = Rhineland Palatinate; site names: 1 = Lahn-W, 2 = Lahn-LH, 3 = Lahn-C, 4 = Orke, 5 = Eder, 6 = Nims, 7 = Bröl.

### Hydromorphological measurements and analyses

A stretch of approximately 200 m was investigated at each stream section. Along 20 equidistant transects, channel features (e.g. floodplain, bars, islands, main channel, sidearms) were measured (details in Jähnig et al., 2008a). In the aquatic (i.e. submerged) sections along each transect (main channel, secondary channel, connected or disconnected sidearm, permanent or temporary standing water body), current velocity, water depth and substrate types were examined at 20 points, resulting in a total of 400 data points for these three parameters per stream section. At the site Lahn-W, investigations were limited to 16 transects due to limited access to the riverbed. Depth was measured with a 2-m-long rule, fixed to a surveying pole; measuring accuracy was  $\pm 1$  cm. Water depths greater than 140 cm values were standardised to 145 cm. Current velocity was measured at 0.6 of the water depth from the water surface using a Schiltknecht MiniAir2 device with a MiniWater20 Mini water sensor, which automatically calculates a 6-second mean from 0.5-second values. With these data, 12 metrics were calculated to compare the stream sections. Metrics included the number of different channel features and substrates for each section, as

**Table 2.** Hydromorphological parameters at single- (\_1) and multiple-channel (\_2) sections. cv = coefficient of variation; SWI = Shannon-Wiener-Index; SDI = Spatial-Diversity-Index; higher values between paired sections are marked bold. Abbreviations and grainsize of substrates in brackets, according to multi-habitat sampling protocol (Hering et al., 2003): Boulders (>200 mm); cobbles (>60–200 mm); C-gravel = coarse gravel (>20–60 mm); F-gravel = fine gravel (>2–20 mm); sand (>0.006–2 mm); loam (<0.006 mm); LPTP = living parts of terrestrial plants; CPOM = coarse particulate organic matter; mud = organic mud.

	cv		no. Substrates	SWI		SDI		Substrate composition (%) (median rate of increase)							
	Current velocity	Depth				Boulders (0.3)	Cobbles (1.2)	C-gravel (1.4)	F-gravel (8)	Sand (2)	Loam (5.3)	LPTP (1)	Wood (1)	CPOM (3)	Mud (1.6)
Lahn-W_1	0.72	0.47	5	0.78	0.67	<b>20.63</b>	<b>73.44</b>	1.25	0.00	0.00	0.94	0.94	0.63	0.31	<b>1.88</b>
Lahn-W_2	<b>1.25</b>	<b>0.84</b>	<b>10</b>	<b>1.44</b>	<b>5.97</b>	10.63	50.00	<b>21.88</b>	<b>5.31</b>	<b>5.94</b>	<b>1.25</b>	<b>3.13</b>	0.63	<b>0.94</b>	0.31
Lahn-LH_1	0.79	0.57	3	0.47	1.05	22.06	73.68	1.00	0.50	2.01	0.00	0.25	0.00	0.50	0.00
Lahn-LH_2	<b>1.56</b>	<b>0.87</b>	<b>11</b>	<b>1.44</b>	<b>5.34</b>	5.25	45.00	<b>22.25</b>	<b>5.25</b>	<b>3.75</b>	<b>4.00</b>	<b>2.00</b>	<b>0.50</b>	<b>2.75</b>	<b>5.75</b>
Lahn-C_1	0.67	0.59	8	1.24	4.14	<b>8.25</b>	<b>28.50</b>	54.00	0.50	0.00	1.75	<b>1.25</b>	2.50	0.25	3.00
Lahn-C_2	<b>1.02</b>	<b>0.97</b>	<b>10</b>	<b>1.47</b>	<b>5.98</b>	2.26	16.29	<b>55.14</b>	<b>4.01</b>	<b>4.76</b>	<b>12.03</b>	0.75	<b>1.50</b>	0.25	3.01
Orke_1	0.61	0.35	<b>11</b>	<b>1.70</b>	<b>6.38</b>	6.75	41.75	<b>13.75</b>	<b>22.00</b>	<b>4.25</b>	0.75	<b>3.75</b>	<b>3.50</b>	0.25	<b>3.00</b>
Orke_2	<b>0.77</b>	<b>0.52</b>	10	1.14	5.28	<b>9.25</b>	<b>71.00</b>	6.75	1.00	1.50	<b>4.00</b>	1.25	0.75	<b>3.00</b>	1.50
Eder_1	0.91	0.61	<b>10</b>	<b>1.15</b>	2.14	4.50	64.00	<b>7.00</b>	0.00	0.25	0.25	0.25	0.25	0.00	2.00
Eder_2	<b>0.99</b>	<b>0.77</b>	8	0.79	<b>2.66</b>	<b>9.00</b>	<b>75.50</b>	5.50	<b>0.50</b>	<b>0.75</b>	0.25	0.25	0.25	0.00	<b>3.25</b>
Nims_1	0.86	<b>0.77</b>	10	<b>1.44</b>	2.41	<b>30.25</b>	56.50	3.25	<b>1.25</b>	0.25	0.75	1.25	1.25	0.25	5.00
Nims_2	<b>1.19</b>	0.66	<b>11</b>	1.43	<b>4.50</b>	9.25	<b>68.00</b>	<b>4.50</b>	0.25	<b>0.50</b>	<b>3.75</b>	1.25	<b>1.50</b>	0.25	<b>10.75</b>
Bröl_1	0.63	0.43	8	1.01	2.52	<b>49.50</b>	45.25	0.25	1.25	<b>1.25</b>	0.00	<b>0.75</b>	0.25	0.00	1.50
Bröl_2	<b>1.31</b>	<b>0.92</b>	<b>9</b>	<b>1.33</b>	<b>4.12</b>	5.00	<b>68.50</b>	<b>9.00</b>	<b>2.75</b>	0.75	<b>2.50</b>	0.25	<b>2.25</b>	<b>1.00</b>	<b>8.00</b>

well as Shannon-Wiener-Index (Shannon and Weaver, 1949) calculated for channel feature data and substrate data. The Spatial-Diversity-Index (Fortin et al., 1999) was calculated with the substrate data. This index links the number of patches of present substrates to the area occupied by them. Thus the spatial occupancy and sequence of substrates along transects is considered, reflecting spatial structure and patchiness of habitats. The coefficient of variation was calculated for depth and current velocity data. Substrate area in the submerged part of each transect was estimated from transect length and substrate point recordings. The area covered by each substrate type was calculated using the distance between measuring points and taking the increased width at the multiple-channel section into consideration.

### Macroinvertebrate sampling

Macroinvertebrate samples were collected during two sampling campaigns in early summer using a shovel sampler (500  $\mu$ m mesh size, 0.0625 m<sup>2</sup> sampling area; specifications of substrates in Table 2). Each available substrate was sampled independent of its frequency and relative cover. This procedure considers important but area-limited

substrates, which are usually missed when a strict area-weighted multi-habitat sampling protocol is applied (Rabeni, 2000). Two levels of detail were used in macroinvertebrate sampling. In 2004, a more elaborate sampling design was used at sites Lahn-C and Orke for detailed investigation of substrate-specific assemblages. The sampling involved the following procedure: the dominant substrate was sampled eight times, both in the single- and the multiple-channel section; every other substrate was sampled twice per stream section (two exceptions due to availability). The other sites were sampled in 2005. As the data collected in 2004 did not show variability of substrate-specific assemblages between single- and multiple-channel sections, the sampling design was simplified and each occurring substrate was sampled only once in the single-channel sections and once in the multiple-channel sections. Several substrates occurred only in the multiple-channel sections and were sampled twice there. No substrate occurred solely in the single-channel section.

Substrate-specific samples were preserved individually in 70% ethanol and sorted in the laboratory, following the RIVPACS sorting scheme (Murray-Bligh et al., 1997). The organisms were identified to

species level where possible, except Oligochaeta, which were recorded as such or identified to the family level, and Chironomidae, identified mostly to the family or tribe level. Prior to data analysis, all taxa lists were corrected to the same (but group-specific) taxonomic identification level. Taxa represented by a single individual were omitted from further analyses; this applied to 19 taxa.

### Macroinvertebrate data analyses

Sample data from all streams were pooled for substrate types, but kept separately in single- and multiple-channel sections. Following this, single- and multiple-channel substrates were analysed for taxa number, abundance, and evenness. Samples of Lahn-C and Orke were analysed separately and ranked using Non-metric Multidimensional Scaling (NMS) with Bray-Curtis dissimilarity as a distance measure (PC-ORD, McCune and Mefford, 1999). Dissimilarity was calculated with log-transformed abundance data. Based on NMS results, substrates were merged into five substrate groups to increase sample size for analyses of within- and between-substrate group dissimilarity. Differences within substrate samples and between samples from single- and multiple-channel sections were calculated using the Multi-Response Permutation Procedures (MRPP) of PC-ORD. The resulting groups were tested using the MEANSIM-calculator of Van Sickle (1998). The calculated dissimilarity was used to estimate the degree of differences between and within substrates (independent of channel type), as well as differences of substrate specific assemblages depending on channel type.

Nestedness was calculated for samples of sections and substrates using the "nestedness calculator" (Atmar and Patterson, 1993). The nestedness calculator calculates the "temperature"  $T$ , whereby  $T = 0$  indicates a perfectly nested data set, i.e. taxa of species-poor samples all occur in species-rich samples and  $T = 100$  is a maximally disordered data set. Generally, a higher  $T$  means less predictability. The nestedness calculator uses presence-absence data of taxa to analyse the probability of nestedness by a Monte Carlo permutation (500 runs per test),  $T_{calc}$ . If  $T < T_{calc}$  the assemblage is considered nested. We hypothesised that nestedness is higher in the single-channel sections, as the species pool is smaller and various substrates are colonised in a similar fashion. Correspondingly, we hypothesised that nestedness is higher in the various substrates of multiple-channel sections because a multiple-channel section is thought to support a greater variety of species. Therefore substrate-specific assemblages should vary more when compared to each other.

## Results

### Hydromorphological diversity

Detailed results for all measured parameters of different scales are published in Jähnig et al. (2008a), so results here are limited to an overview. The multiple-channel sections showed a considerable diversity of hydromorphological structures (Table 2). The mean overall width (between embankments) was increased by a factor of 2.1 in multiple-channel sections compared to the single-channel sections. Shore length increased by a factor of 2.4. The coefficient of variation of depth and current velocity was highest in the multiple-channel sections. The multiple-channel sections usually had more substrates and a more complex arrangement of substrates, which was reflected by higher values for the Spatial-Diversity-Index.

### Overview of macroinvertebrate assemblages

In total, 163 taxa were found at the 14 stream sections in 192 samples. A total of 48,947 individuals in 66 families were identified. The mean density was 6631 individuals/m<sup>2</sup> (ranging from 113 to 100,720). The most diverse groups were Trichoptera (40 taxa), Coleoptera (36 taxa), Diptera (21 taxa) and Ephemeroptera (22 taxa). The average number of taxa per sample was  $23.48 \pm 8.96$  (ranging from 2 to 47).

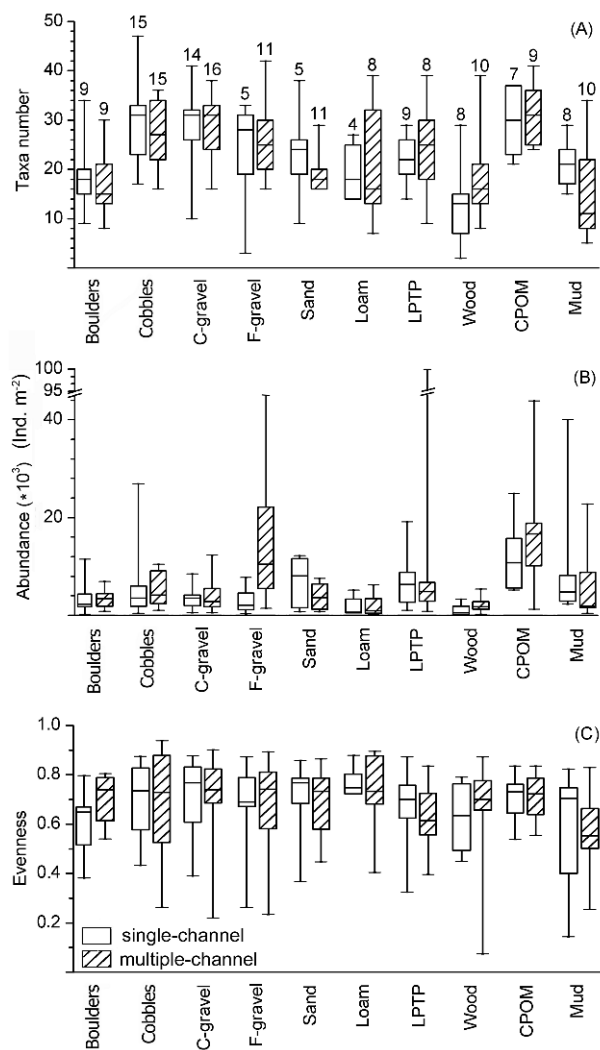
### Substrate-specific assemblages

The lowest mean number of taxa were found on wood and boulders (Fig. 2A). High numbers of taxa were found in mineral substrates such as cobbles, coarse and fine gravel, together with CPOM. Abundances in the samples showed a stochastic distribution (Fig. 2B). Highest abundances were found in fine gravel and CPOM of the multiple-channel sections. Despite the fact that the relative proportion of fine mineral and organic substrates increased in the multiple-channel sections (Table 2), abundances did not differ for substrates sampled in different channel forms. Average evenness of all samples was 0.68 (Fig. 2C). Multiple-channel substrates had highest mean evenness in boulders, coarse gravel, loam and CPOM. The Mann-Whitney-U-test between substrates from single- and multiple-channel sections was not significant for any metric.

Coleoptera and Trichoptera contributed most to differences observed for taxa numbers between single- and multiple-channel sections (Fig. 3). Coleoptera showed highest dissimilarity, with one to 10 additional taxa in the multiple-channel sections. Trichoptera had up to seven more taxa in fine gravel, but otherwise more or fewer taxa occurred equally often. For Ephemeroptera, up to seven additional taxa

**Table 3.** Mean assemblages metrics (taxa number, abundance, evenness, dissimilarity) in dominant substrates at single- (\_1) and multiple-channel (\_2) sections at Lahn-C and Orke. n=8; t-test not significant.

	Taxa number	p	Abundance (Ind./m <sup>2</sup> )	p	Evenness	p	Between-group dissimilarity (Bray-Curtis-Index)
Lahn-C_1 (c-gravel)	28.3		209.8		0.81		
Lahn-C_2 (c-gravel)	26.1	n.s.	209.3	n.s.	0.78	n.s.	47.63
Orke_1 (cobble)	31.5		381.8		0.71		
Orke_2 (cobble)	24.8	n.s.	318.8	n.s.	0.68	n.s.	49.11 (p<0.1)

**Figure 2.** Box-and-Whisker Plots (Median; Box: 25%-75%; Whisker: Min-Max) of assemblage metrics (taxa number, abundance, evenness) for substrates in single- and multiple-channel sections. Sample n for substrates indicated in (A) only and valid for (A), (B), (C). Mann-Whitney-U-test between single- and multiple-channel substrates is not significant for any metric.

were found, with a mean of 1.6 additional taxa. However, fewer Ephemeroptera taxa were recorded in sand and CPOM. Plecoptera, the order with the overall fewest taxa, had only one or two additional taxa in the multiple-channel sections.

### Substrate-specific assemblages at single- and multiple-channel sections

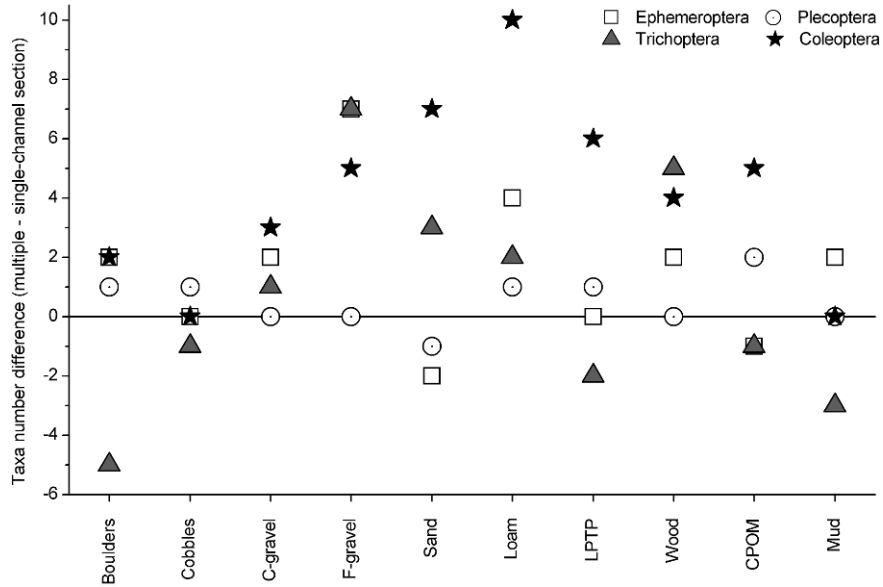
The macroinvertebrate assemblages in the dominant substrate at sites Lahn-C and Orke – coarse gravel and cobbles, respectively – did not differ between the single- and multiple-channel sections (Table 3). The mean dissimilarity between samples from single- and multiple-channel sections was < 50 % for both. When within- and between-stream section dissimilarities were compared, the Orke had a 3 % higher mean between-group dissimilarity than within-group dissimilarity. This implies a small difference of the macroinvertebrate assemblages in different stream sections. Lahn-C showed no differences in that respect.

The results of the NMS-analyses of Lahn-C samples (Fig. 4) suggest similar assemblages in substrates of single- and multiple-channel sections. Three groups were identified: The topmost part of figure 4 shows assemblages in the substrates of living parts of terrestrial plants and large wood (Group A). In the lower right corner, assemblages of finer and lighter substrates such as organic mud and CPOM are displayed (Group B), and an intermediate part summarises various mineral substrates (Group C). Similar results were obtained for the Orke (not shown). For both streams, substrates taken in the single- or multiple-channel section cannot be differentiated from one another. Analyses of within- and between-group dissimilarity for both streams distinguished between substrate groups with dissimilarities of 61 and 66 %, respectively.

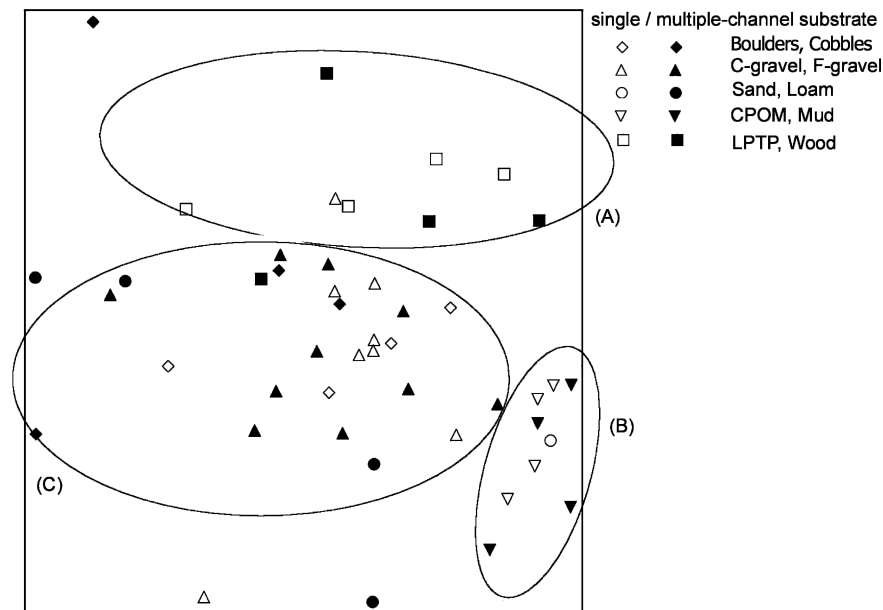
Analogous results were obtained from dissimilarity analyses of section-pooled substrate samples (Fig. 5). For the substrate groups, differences are larger on average between the groups than within the groups (ratio of 1.1,  $p < 0.05$ ). This was not true for single- and multiple-channel differences, where this ratio is close to 1.

### Nestedness

The multiple-channel sections of Orke, Nims and Lahn-W had a higher T (i.e. less predictability) compared to their single-channel control stream sections (Table 4). While this is in accordance with



**Figure 3.** Taxa number difference of four insect orders. Each dot is calculated as "number of taxa of an order in multiple-channel section samples" minus "number of taxa of the same order in single-channel section samples".



**Figure 4.** NMS graph of macroinvertebrate assemblages at site Lahn-C. Comparison of substrate groups in single- and multiple-channel section. Stress: 13.3; MRPP mean between-group dissimilarity: 61%. Similar results were obtained for NMS analyses at the site Orke: MRPP mean between-group dissimilarity: 66%.

the stated hypothesis, it is not a regular pattern in this study and single-channel and multiple-channel sections were similar in their mean T (Mann-Whitney-U-test not significant).

The substrates mud, loam, boulders, and living parts of terrestrial plants had a lower T when found in the multiple-channel sections; three of their respective single-channel samples were not nested (Table 5). Again, this is in accordance with the stated hypothesis,

but mean T is yet again equal for single- and multiple-channel sections (Mann-Whitney-U-test not significant).

**Table 4.** Nestedness analysis for single- (\_1) and multiple-channel (\_2) sections. Rows sorted according to ascending System T. If  $T < T_{\text{calc}}$  and  $p < 0.1$  the assemblage can be stated nested. Bold section names are in accordance with hypothesis  $T_{\text{single-section}} < T_{\text{multiple-section}}$  of a site. Fill (%) = presence (%) in the section taxon matrix; T = system temperature;  $T_{\text{calc}}$  = system temperature generated by Monte Carlo randomisation (500 iterations).

	No. samples	No. taxa	Fill (%)	T	$T_{\text{calc}}$ (mean $\pm$ SD)	p
<b>Orke_1</b>	23	113	22.0	22.7	61.2 $\pm$ 2.6	<0.05
Orke_2	28	115	21.0	24.2	60.7 $\pm$ 2.6	<0.05
Lahn-C_2	28	96	24.2	26.3	64.4 $\pm$ 2.8	<0.05
<b>Nims_1</b>	10	78	30.0	27.0	55.6 $\pm$ 4.4	<0.05
<b>Lahn-W_1</b>	11	79	33.2	27.4	59.2 $\pm$ 4.0	<0.05
Lahn-LH_2	13	66	27.3	29.2	57.5 $\pm$ 4.4	<0.05
Lahn-C_1	21	94	27.4	29.9	64.9 $\pm$ 3.1	<0.05
Bröl_2	13	74	31.8	33.8	60.3 $\pm$ 4.2	<0.05
Lahn-W_2	8	62	29.8	34.6	51.0 $\pm$ 5.8	<0.05
Eder_2	11	76	25.0	34.8	52.7 $\pm$ 4.7	<0.05
Bröl_1	7	49	41.1	39.5	51.5 $\pm$ 5.7	<0.05
Lahn-LH_1	7	56	41.5	44.7	52.7 $\pm$ 5.5	<0.1
Nims_2	10	74	32.0	45.7	57.2 $\pm$ 4.4	<0.05
Eder_1	7	74	29.3	49.4	49.2 $\pm$ 5.7	n.s.

**Table 5.** Nestedness analysis for single- (\_1) and multiple-channel (\_2) substrates. Rows sorted according to ascending System T. If  $T < T_{\text{calc}}$  and  $p < 0.1$  the assemblage can be stated nested. Bold substrate names are in accordance with hypothesis  $T_{\text{multiple-section}} < T_{\text{single-section}}$  of a site. Fill (%) = presence (%) in the substrate taxon matrix; T = system temperature,  $T_{\text{calc}}$  = system temperature generated by Monte Carlo randomisation (500 iterations).

	No. samples	No. taxa	Fill (%)	T	$T_{\text{calc}}$ (mean $\pm$ SD)	p
<b>Mud_2</b>	10	64	23.9	16.7	50.5 $\pm$ 5.3	<0.05
F-gravel_1	5	52	44.2	20.2	44.9 $\pm$ 5.8	<0.05
Wood_1	7	50	25.7	22.0	46.3 $\pm$ 6.4	<0.05
Sand_1	5	54	34.0	23.3	47.1 $\pm$ 7.8	<0.05
<b>Loam_2</b>	8	73	31.1	26.5	52.4 $\pm$ 4.9	<0.05
Wood_2	10	79	23.5	28.7	50.2 $\pm$ 4.8	<0.05
<b>Boulders_2</b>	9	60	28.5	31.0	52.1 $\pm$ 5.8	<0.05
<b>LPTP_2</b>	8	74	34.1	32.1	54.9 $\pm$ 5.1	<0.05
Boulders_1	9	63	26.6	33.3	50.2 $\pm$ 5.3	<0.05
C-gravel_1	14	89	33.0	36.2	63.2 $\pm$ 3.7	<0.05
C-gravel_2	16	89	31.8	36.4	64.6 $\pm$ 3.4	<0.05
Cobble_1	14	93	30.3	36.7	62.2 $\pm$ 3.5	<0.05
Cobble_2	15	84	32.7	37.8	63.7 $\pm$ 3.4	<0.05
F-gravel_2	11	79	31.7	44.2	58.3 $\pm$ 4.4	<0.05
Sand_2	11	72	25.3	46.5	53.5 $\pm$ 4.7	<0.1
Mud_1	8	60	30.6	47.2	52.6 $\pm$ 5.7	n.s.
LPTP_1	9	71	29.1	47.9	53.2 $\pm$ 5.1	n.s.
CPOM_1	7	83	34.2	50.7	52.3 $\pm$ 5.1	n.s.
Loam_1	4	56	36.1	51.0	41.9 $\pm$ 7.1	n.s.
CPOM_2	9	91	31.7	51.4	55.6 $\pm$ 4.4	n.s.

## Discussion

### Hydromorphological diversity

Morphological diversity increased at the multiple-channel sections at all scales. The paired sections differ in terms of overall and aquatic width. Additional channel features occur in the multiple-channel sections as a consequence of the removal or absence of bank fixation and more open space for the stream. Metrics summarising the substrate composition and spatial arrangement indicate a monotonous substrate distribution throughout the single-channel sections, while substrate arrangement in multiple-channel sections is more diverse. Although habitats show a greater resemblance to

reference conditions in the multiple-channel sections, many of the site- or catchment-scale controlling factors, such as land use in adjacent areas or the overall bank fixation situation along the river, have not been addressed in the restoration schemes. More natural patterns have developed in the multiple-channel sections, but these are presumably not yet in a near-natural state (Thomson et al., 2001; Molnar et al., 2002).

### Substrate-specific assemblages

The analysed samples follow the common principle of substrate specificity (Jenkins et al., 1984). There is similar taxa richness in mineral and vegetation substrates, but much lower abundance in mineral



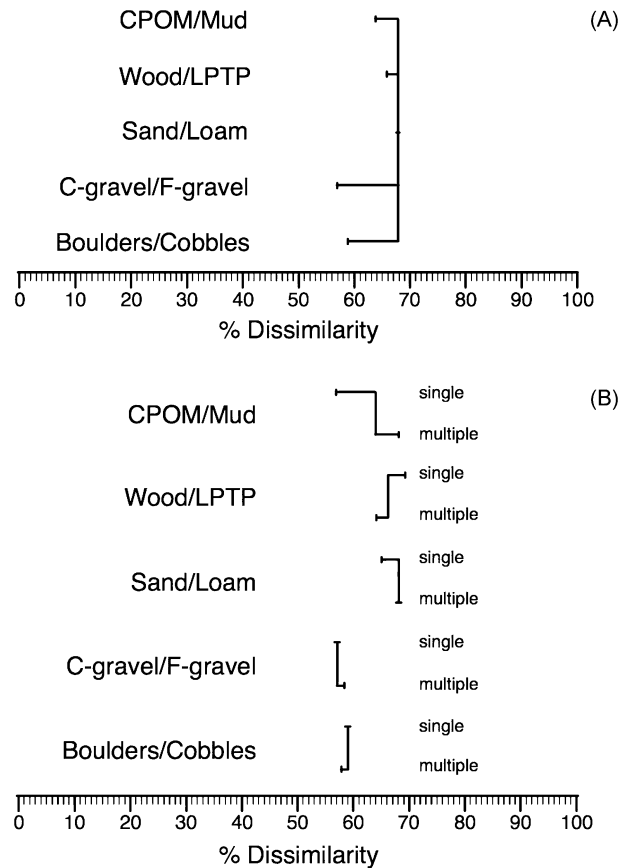
substrates. This result was also observed by Beauger et al. (2006). The highest taxa numbers and highest evenness scores are found for cobbles and coarse gravel substrates, a result congruent to those of Harrison et al. (2004). The special relevance of certain substrates in streams has been observed many times (Beisel et al., 2000; Grafahrend-Belau and Brunke, 2005). Organic substrates such as large wood or living parts of terrestrial plants act as colonisation substrates, which give stable ground in faster flowing areas, offer biofilm for grazers, and feed the detritus pool. Such habitats sustain predators as well. The rich structure and the highly complex surface of these substrates accounts for the higher abundances observed (Hoffmann and Hering, 2000). This is generally reflected in our data with high taxa numbers and high abundances for living parts of terrestrial plants and CPOM, although wood was among the poorest substrates.

**Substrate-specific assemblages at single- and multiple-channel sections**

This study aimed at answering the question whether macroinvertebrate assemblages differ in substrate samples taken in multiple-channel sections compared to substrate samples taken in single-channel sections. Our reasoning is that increased habitat diversity should result in a different colonisation of the same substrate. When the number of patches of the substrate mosaic increases, macroinvertebrate habitats are assumed to be more varied – the macroinvertebrate assemblage might then be more diverse because a higher number of taxa can find suitable ecological niches (Beisel et al., 1998).

Our results suggest that there is no variation between assemblages on a particular substrate whether it occurs in single- or multiple-channel sections. Differences in taxa number, abundances, and evenness are not significant. The most dominant substrates cover the largest part of the stream bottom and are not greatly influenced by habitat diversification (Table 3). Ordination of macroinvertebrate samples that were pooled in substrate groups reflects these groups visually and statistically (Figs. 4, 5). Potential differences in macroinvertebrate assemblages in multiple-channel sections may result from higher beta-diversity due to a more varied substrate composition, but not because of increased alpha-diversity of the individual substrates. However, the overall differences appear small. Some reasons for this could be:

*Micro-scale insufficiency.* Macroinvertebrate assemblage structure is dependent on substrate diversity and spatial patch configuration (Beisel et al., 2000). Thus more substrate types with similar relative areas



**Figure 5.** Mean dissimilarity dendrograms of section-pooled groups of substrate samples. (A) Difference between substrate groups independent of channel form (groups significantly different,  $p < 0.05$ ; ratio of between- and within-group dissimilarity of substrates: 1.1). (B) Differences between substrate groups in single- and multiple-channel sections separately (upper and lower branches respectively; no significant differences; ratio of between- and within-group dissimilarity of substrates: 1.01). Vertical lines: mean between-group dissimilarity; horizontal branches: mean within-group dissimilarity; horizontal branches to the left represent a decrease in dissimilarity in that group.

promote higher taxa numbers as shown in several studies (Boyero, 2003; Brown, 2003; Beauger et al., 2006). In our system, additional substrates or shifts in substrate abundance in the multiple-channel sections might not (yet) differentiate these sections sufficiently from the habitat composition of prevailing single-channel sections in the streams. One indication for this was similar substrate numbers in most of the single- and multiple-channel sections (Table 2). Only a few invertebrates were added to the species pool, which were not present in other substrates (1–10 at the most, Fig. 3). However, taxa that were already common on other substrates became more abundant. Similar results were shown in a post restoration study by Friberg et al. (1998), who found different assemblages among substrate types but did not observe an effect attributable to restoration. A trend towards higher

macroinvertebrate abundances, as reported by Boyero (2003), is at least partially true for our samples (Fig. 2B). However, abundance is the biological descriptor least influenced by the surrounding bottom heterogeneity (Beisel et al., 1998), which in this study is reflected by highly variable abundances.

*Macro-/meso-scale prevalence.* There are many processes acting at larger scales that can influence the complex life cycles of macroinvertebrates, e.g. that different life stages use different parts of the aquatic and riparian environment (Bond and Lake, 2003). Such requirements might not yet be fulfilled in the investigated sections and streams, as they are usually outside the jurisdiction of authorities responsible for stream restoration (H. Diehl, *pers. comm*). Other studies also show that mere substrate placement does not imply that substrate-specific assemblages are re-created (Hughes, 2007; Clarke et al., 2003).

*Meta-population simplicity.* Local numbers of taxa or macroinvertebrate colonisation rates are directly proportional to prevailing local numbers of individuals and taxa (Marchant et al., 1991). Multiple-factor impairments and cumulative alterations of sites are common (Rabeni, 2000), and populations have been under pressure for a long time, so diverse source-populations might not be available any more. If restored stream channels are considered as islands to be colonised, then the main factors governing this process – ‘source distance’ and ‘stepping stone availability’ – also need to be addressed (Gore, 1985). The study sites are all located in mountainous regions of Western Germany, with much anthropogenic land use pressure (10–15% high density areas, up to 30% agricultural, ~60% forested areas; Kail et al., 2008) and restored stream sections are fairly short. Distances to possible re-colonisation sources might be great (Jähnig et al., 2008b). The size of the restored sections might be too small or stepping stones too scarce to sustain viable meta-populations, which provide sources for re-colonisation.

*Lag of time.* Although the investigated multiple-channel sections are characterised by higher hydro-morphological and habitat diversity, the development of some important “secondary substrates”, such as decaying wood and roots may take decades. It is likely that physical and biological recovery at restored stream sections is incomplete, as only a relatively short time elapsed between development of multiple-channel sections (end 1990s) and the investigation (2004–2005).

### **Nestedness**

Nestedness is a measure of predictability. If nestedness is high, the few species present in certain substrates will be those that are found everywhere, so only larger or more taxon-rich sites will support the more uncommon species (Patterson, 1987). We hypothesised that nestedness and predictability should be lower in the multiple-channel sections, as the species pool would be increased and various substrates might host different assemblages. This was observed for three sites, but overall the results are not significant. This result is partially explained by the algorithm. Every analysis maximally packs the taxasection matrix and zero-frequent taxa are excluded, so different taxa may be excluded for single- and multiple-channel sections.

As many stream taxa are remarkably mobile and rapidly colonise habitat after disturbance, an overall higher nestedness is characteristic (Atmar and Patterson, 1993; Malmqvist and Hoffsten, 2000). Much lower  $T$  ( $<20$ ) were observed for amphibians (M'Closkey and Hecnar, 1997; Tockner et al., 2006) or mammals and birds ( $<10$ , Atmar and Patterson 1993). Our results are in the range of those found in other studies of aquatic macroinvertebrates ( $T = 30–40$ , Schmera, 2004; Yoshimura et al., 2006).

We expected a higher nestedness in the various substrates of multiple-channel sections due to stronger differentiation. This pattern is confirmed for four substrates, three of these are organic substrates, suggesting that assemblages are currently becoming more differentiated in these habitats. These substrates play a major role in distinguishing multiple-channel section from single-channel section assemblages.

### **Conclusions and implications**

Assemblages on similar substrates in single- and multiple-channel sections showed only minor differences. Despite higher habitat diversity, alpha-diversity has not been changed. Influences from other scales seem to prevail and restoration might have partially failed as certain important habitats have not yet been sufficiently restored to their near-natural extent. Furthermore, hydrological and sedimentation processes have not been consciously addressed. A certain lag time for a biological response need to be allowed for, which might be additionally aggravated due to simplified source populations available for re-colonisation. Our results show that substrates have different assemblages, meaning that beta-diversity of a section can be influenced. For implementing or evaluating stream restoration projects, we need to focus on creating and facilitating access to high quality habitats such as large wood.

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