PRIMARY RESEARCH PAPER

# Do faunal assemblages reflect the exchange intensity in groundwater zones?

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Abstract The exchange of water with groundwater is a key determinant of water quality and faunal assemblage. Water exchange not only occurs with running waters, but also through percolation, interception (soil, porous alluvium), and evaporation. The aim of this study was to identify how different types of exchange were related to the groundwater faunal assemblage of an alluvial aquifer. Hydrological exchange is largely governed by pore space and thus ultimately by geological formation. In the Marbling Brook catchment of Western Australia the different geological formations did not eventuate in hydrochemically distinct groundwater zones. The

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W. F. Humphreys Western Australian Museum, Locked Bag 49, Welshpool DC, WA 6986, Australia cluster analysis of faunal assemblages revealed five groups within the faunal samples which did not reflect spatial patterns such as geological, chemical or topographic features. Discriminant analysis showed that these five groups were best characterized by a range of abiotic features including dissolved oxygen, land-use, and temperature. These variables signal different types and intensities of exchange with the surface.

**Keywords** Groundwater fauna · Groundwater/ surface water interactions · Hydrological exchange · Catchment

## Introduction

Groundwater faunal assemblages are known to reflect the geological characteristics of the aquifer quality from large scale biogeographic studies (e.g. Ronneberger, 1975; Dole-Olivier et al., 2005; Hahn & Fuchs, 2005). Geological characteristics lead to different relative ion compositions in water bodies and the aquifer's exchange with the surface, contributes to shaping the hydrochemical groundwater characteristics and thus influences faunal assemblages (Strayer, 1994). The aim of this study was to compare relationships between different geological strata; here used to infer the type of hydrological exchange with the groundwater faunal assemblages. Our expectation was that differences in hydrological exchange will override the effects from geology.

Water exchange between surface environments and aquifers not only occurs with running waters, but also through percolation (rock crevices; e.g. karst, fractured rock; Bloomfield, 2005) and through interception (soil; porous alluvium). Groundwater/surface water exchange patterns along rivers and streams have been demonstrated and described for the riparian zone in numerous rivers and streams (e.g. review in Brunke & Gonser, 1997). Different types of exchange will alter the solute concentrations through e.g. evaporation, increased dissolution, and input of nutrients by land-use. Interception of rain water may lead to decreasing solute concentrations. Mixing of surface water such as from streams will often result in an altered relative ion composition (Harvey & Wagner, 2000). Hydrological exchange will thus be detectable in hydrogeochemical composition (Turner & Townley, 2006). Where aquifers are connected across different geological strata, the groundwater's hydrochemical composition will reflect the mixing of ions from those different strata. Zones of low exchange are expected to be low in nutrient and carbon concentrations and have low concentrations of oxygen. In the highexchange zones the groundwater is replenished regularly with nutrients and carbon, especially in areas influenced by land-use. Because of the high local-scale variability in dissolved oxygen, temperature, carbon, and redox state (Inwood et al., 2005), budgets of chemical compounds are difficult to establish. However, this input attracts invertebrates (e.g. Marmonier et al., 1992) which may reflect small-scale variability in chemical compounds and be good indicators for zones of increased nutrient and carbon input.

In the Marbling Brook catchment of Western Australia we tested two hypotheses:

- (1) The differences in hydrological exchange will override the effects from geological influence in shaping faunal assemblages. This will be discernible in the variability in electrical conductance versus relative ion composition.
- (2) The different zones of exchange between the aquifer and surface waters will be reflected

in the distribution of groundwater fauna assemblages.

As wells of different construction were used for sampling, we also tested whether the construction type resulted in different faunal characteristics.

# Materials and methods

## Study area

The study area, the 30 km<sup>2</sup> catchment of the Marbling Brook, is situated 60 km north east from Perth in Western Australia (easting: 0414602, northing: 6506513).

Groundwater wells were drilled in the catchment's valleys and near springs. Well locations are shown in Fig. 1 with abbreviations consisting of a letter for the site (S = Schmidt, L = Lambie,R = Read/confluence,C = Crees,J = Carr,M = Morley, T = Stringybark; N = Sandow Road) and a number to identify the different wells at each site. Twenty-three wells were drilled in March 2001 along the proposed direction of flow in the porous, shallow groundwater aquifer (Fig. 1). Groundwater wells were constructed of white PVC tubing (50 mm diameter) with horizontal slots of 1 mm width over the lowest 2 m. Some pre-existing wells were also used. The wells C1A, C1B, C2, C3, C4 (drilled between 1990 and 1999) on the Crees site were constructed of white PVC tubing (50 mm diameter) with horizontal slots of 1 mm width. The wells WO, J1 and J2 (drilled before 1990) were made of 100 mm diameter PVC with horizontal 1 mm wide slots over the whole length. The walls of the 1 m wide wells at Stringybark, Crees and Barrett sites (TW, CW, BW) were lined with bricks or concrete.

## Sampling

Groundwater levels, dissolved oxygen (DO), electrical conductance (EC), pH, and temperature were measured monthly with field probes (water level: electric dipper; DO, EC, pH, and temperature: WTW probes and instruments). Samples were pumped with a vacuum pump. DO was measured in a flow-through chamber. Since it was known that groundwater was of

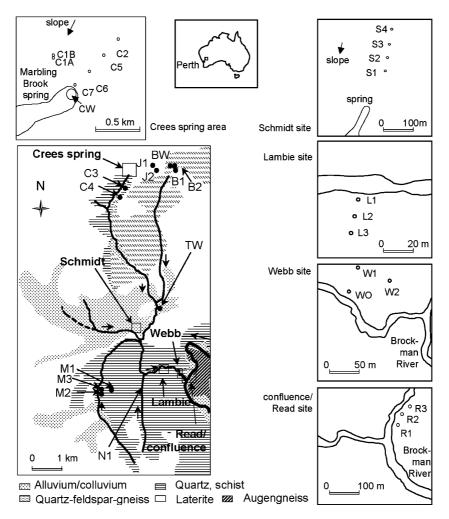


Fig. 1 Outline of the sampled wells in the Marbling Brook catchment, tributary to the Brockman River—sketch. Squares in the main map refer to insets to the top and right

different quality than stream water (Hahn, 2002), we used EC as the principal tracer for groundwater/surface water interactions and exchange (Freeze & Cherry, 1979, 140; Stanford et al., 1994; Turner & Townley, 2006).

Further samples were collected five times at the end of summer and following rain events, and analysed for nitrate, calcium, hydrogen carbonate, dissolved and total iron concentrations. In addition, magnesium concentration was measured seasonally. Hydrogen carbonate concentrations were measured by titration within 8 h of sampling in unfiltered samples (autotitrator TTT 85, Copenhagen). Subsamples were filtered (0.45  $\mu$ m) subsequently so that nitrate and dissolved iron (Fe(II)) concentration could be analysed. Nitrate concentration was analysed colourimetrically (Lachat Quikchem Automated Flow Injection Analyzer). Calcium, magnesium, sodium, potassium and total iron (Fe(II) + Fe(III))concentration were determined in an unfiltered, acidified subsample. All ion analyses except for nitrate were performed with the Varian 600 flame atomic absorption spectrophotometer. In April 2002, silica, sulphate, sodium and potassium concentrations were analysed by flame atomic absorption spectrophotometry. Previous analysis of ionic data indicated that the wells sampled aquifers which were uniform in terms of relative hydrochemical properties, but highly variable in terms of salinity.

Fauna were sampled with a 45 mm diameter plankton net with 125 µm mesh, whenever field measurements were taken, i.e. twice a month in stream sites and monthly in groundwater. After water had been pumped for chemical and physical analyses, the narrow plankton net was lowered down the well. The net was drawn up and lowered as described in Bou (1974). Dumas & Fontanini (2001) demonstrated the efficiency of nets to be similar to that of pumps. In some groundwater wells the net became clogged after two draws, therefore for consistency, all groundwater wells were sampled by lowering and pulling the net up twice. This type of sampling, although not entirely quantitative, allowed a regular and effective monitoring of the wells which would not have been possible by time-consuming and sediment-disrupting pumping. Fauna were sorted alive under 40× magnification and identified microscopically at a magnification of 400 x. Taxa were identified variously to order, but Crustacea and Acari were identified to species/morphotype where possible.

## Data preparation

Abiotic data were averaged for half year time spans (see later). All abiotic variables except for pH were log(x + 1) transformed to improve normality and to stabilize the variance (Sokal & Rohlf, 1995), and were standardized to the same scale for multivariate analysis (Quinn & Keough, 2002).

Seasonal variability was expected to be much higher in physical and chemical variables than in faunal characteristics, and this information was reduced using the averaging process. To include seasonal variability in the statistical analyses of the half-yearly data, we calculated physical and chemical indices. Maximum value, minimum value, (first) month of maximum value, (first) month of minimum value, and range were calculated from the data for EC, temperature, and water levels. These variables indicate the degree of connection between the aquifer and surface water input. In addition to these variables, measurement averages were used in the stepwise discriminant analysis to assess how much of the faunal variability can be explained by the variability in these variables and measurement averages (see below). Land-use was classified for each

well according to the dominant practice and was ranked from most to least intense (Table 1).

To increase sample size, faunal abundances were pooled for each site over summer and winter (1 =summer: December to May; 2 = winter: June to November) to give a half yearly aggregated sample. December was chosen as the start of the summer season because average groundwater temperature increased most between November and December (average water temperature November:  $22 \pm 1.8^{\circ}$ C; December:  $24.66 \pm 3.1^{\circ}$ C). Likewise, between May and June the highest decrease in water temperature was observed (average water temperature May:  $20.9 \pm 1.3^{\circ}$ C; June:  $19.4 \pm 1.8^{\circ}$ C). Therefore, the summer half year was set to end with the month of May. With the exclusion of samples lacking fauna, this aggregation left 31 half year faunal data sets per well.

Three types of well were sampled (50 mm, 100 mm and 1 m diameters). It was tested whether well size had an effect on sampled abundances or taxa numbers, by Kruskal–Wallis tests.

Statistical analyses

None of the values (abiotic or biotic) were normally distributed, and the variances were not

Table 1 Land-uses in Marbling Brook catchment

Category	Land-use	Wells
1	Viticulture	TW
2	Sheep and car mechanic's workshop, partly on unsealed ground	S1, S2, S3, S4
3	Low numbers of sheep and cattle	C1A, C1B, C2, C5, C6, C7, CW, M1, M2, M3, R1, R2, R3, N1
4	Cereal, extensively cultivated: one crop per year; buffer strips	C3, C4
5	Old citrus plantation, untreated	J1, J2
6	Paddock; no cattle but some gardening and spraying, or few horses	L1, L2, L3, W1, W2, WO
7	Near-pristine bush land	B1, B2, BW

homogeneous, even after transformation, so only nonparametric tests were performed. Kruskal– Wallis tests were used to test the hypothesis that there were no differences in abiotic values among geological units (R Development Core Team, 2004). Behrens–Fisher test with the Satterthwaite t-approximation were employed for nonparametric multiple comparisons within geological units (Munzel & Hothorn, 2001; R Development Core Team, 2004).

## Ordination of faunal data

Since the sampling was semi-quantitative, calculations were not performed on the abundances of taxa, but on taxonomic richness. Using the taxonomic richness instead of the abundances overcomes the issue of needing large numbers of replicates to estimate the average abundances representatively.

The aggregated samples were classified using a biologically based cluster algorithm (König, 2005). This hierarchical method uses a biologically based variance measure and merges the elements (in this case samples) to maximize the evenness of the assemblage composition, E.

$$E = \frac{\sum_{i=1}^{S} k_i \cdot k_i}{\sum_{i=1}^{S} k_i}, \text{ with } 0 < E \le 1$$

 $k_i$  is the frequency (also referred to as presence, constancy) of finding a species in the samples;  $0 < k_i \le 1$ .

Homogeneous compositions have an E value of 1 and indicate that all samples have an identical taxa composition. The fusion axis (unit E) is directly interpretable since the final groups are distinct at E > 0.5 or 50%.

Differences in the faunal assemblages between different geological strata were tested with the ANOSIM (analysis of similarities) procedure (Clarke & Green, 1988; Primer v5, Plymouth Laboratories, Plymouth, UK). ANOSIM is a nonparametric and multivariate procedure similar to ANOVA, and tests for differences among groups (Clarke, 1993), in this case among groups of faunal assemblages. Frequencies were fourth root-transformed prior to analysis and Bray Curtis dissimilarity data were used in the analysis.

Reciprocal averaging analysis (RA: Hill, 1973; algorithm implemented by B. König, BfG, Koblenz; unpublished) was performed of the frequencies of the taxa within the groups. This analysis, which is an ordination or gradient analysis method, leads to a visual display of a simultaneous sorting of taxa and sampling sites. Only those individuals identified to the lowest level possible (class; species/morphotype for Crustacea and Acari) were included.

Relationships between faunal assemblages and physical and chemical factors

Patterns of chemical features in the catchment were investigated with the multivariate Principle Component Analysis (PCA; R Development Core Team, 2004). Prior to this analysis, correlations between variables were tested, and consequently, the following factors were not included in the PCA:  $HCO_3^-$  concentration, maximum electrical conductance, minimum standing water level, and maximum temperature.

Relationships between faunal assemblages and physical and chemical factors were tested with the BIO-ENV routine (Primer v5; Plymouth Laboratories), a multivariate correlation test. This procedure searches stepwise to see which combinations of environmental variables provide a good match (in this case using the normalized Euclidean distance) between the biotic and the abiotic configuration (Clarke & Ainsworth, 1993). For this procedure and for the linear discriminant analysis (LDA), abiotic values were log(x + 1)transformed, except for pH, and standardized. Faunal data were fourth root transformed (Clarke & Warwick, 1994).

A stepwise linear discriminant function analysis (LDA; SPSS 12.2) was conducted to determine which environmental (abiotic) variables discriminated best among the groups characterized by the structure of the faunal data. The variables were added to the regression if they contributed a significant amount of information to the discriminant model (Brosius, 2002). Prior probability for a sample to fall into any group was set to the

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same for all groups. Silica, sulphate, sodium, and potassium concentrations were omitted from the analysis because data were available only for the summer samples. The remaining variables are listed in Table 2. Among these, only those entered the final model which added significant amount of explanation to the model, based on the Wilks  $\lambda$ criterion (Brosius, 2002). This criterion was set not to exceed 1.2 for the partial F of a new variable, and was set not to exceed 1.1 for the partial F of a variable already in the model, after insertion of a new variable. Iteration was set to stop when the variable insertion did not satisfy the selection criterion (Brosius, 2002). The approximate F statistic was calculated according to Rao (Rao, 1952). The predicted group membership was tested by cross validation. In this validation, models were calculated. For each model one sample was omitted sequentially and used as a test sample to calculate the functions (Brosius, 2002). The remaining samples, or cases, were then tested using these model functions. This procedure is an improvement over the overly optimistic jackknife procedure (Engelman, 1998).

## Results

The multivariate analysis with PCA showed that the variation of chemical features across the catchment were best described by average stand-

**Table 2** List of independent variables which were used inthe stepwise linear discriminant analysis

•	•
Nitrate	Minimum SWL
Land-use	Maximum SWL
Distance to brook	Range of SWL
Standing water level (SWL)	Month of minimum SWL
pH	Month of maximum SWL
Dissolved oxygen	Minimum EC
Electrical conductance	Maximum EC
(EC $\mu$ S cm <sup>-1</sup> )	
Temperature (°C)	Range of EC
Hydrogen carbonate	Month of minimum EC
Iron in the filtered samples	Month of maximum EC
Total iron	Minimum temperature
Calcium	Maximum temperature
Magnesium	Month of minimum temperature
	Month of maximum temperature

ing water level, maximum standing water level, and temperature range on the first axis, and average electrical conductance, maximum electrical conductance, and range of electrical conductance on the second axis (Fig. 2). The first two axes accounted for 42% of the variation. Wells from the same area and thus the same geological background were not necessarily chemically similar. For example, the well L3 was chemically similar to the wells C1B and R2, while the wells L2 and L1, at 30 and 100 m from L3, grouped close together with the well S4. However, the wells C5, C6, C7, C3, and C4, were situated along a downward gradient in the same sequence as in the PCA diagrams (refer to Fig. 1 for the respective position of wells).

Physical and chemical characteristics differed among the geological units for standing water levels, dissolved oxygen, and nitrate concentration (Kolmogoroff-Smirnoff test; significance level P = 0.05; Fig. 3). However, fauna occurred in all geological strata. Standing water levels were significantly different (Behrens Fisher test; Satterthwaite approximation; P < 0.05) between some pairs of geological strata (Fig. 3a). Dissolved oxygen concentrations ranged from 0 to 7.3 mg  $l^{-1}$  and varied throughout the year without an obvious pattern (Schmidt, unpubl. data). Dissolved oxygen concentrations were significantly different (Behrens Fisher test; Satterthwaite approximation; P < 0.05) between alluvium and laterite (Fig. 3b). Nitrate concentrations were different between quartz/gneiss and quartz/schist as well as between quartz/gneiss and alluvium, with lowest values in quartz/gneiss (Fig. 3c).

## Methodology

The highest abundances and taxa numbers were sampled in the medium diameter wells. This was mainly due to the well WO, which showed significantly higher abundances ( $66.3 \pm 34.3$  versus  $0.4 \pm 1.3$  individuals per sample; Behrens Fisher test, Satterthwaite approximation, significance level P = 0.05; Table 3) and taxa numbers ( $4.7 \pm 2$  taxa on average in WO, versus  $0.03 \pm 0.9$ taxa for the rest) than the other 100 mm wells and the smaller and larger diameter wells. Regularly, highest numbers of individuals and taxa were

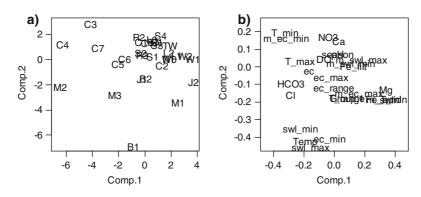


Fig. 2 Principal Component Analysis (PCA) of the chemical features in the wells

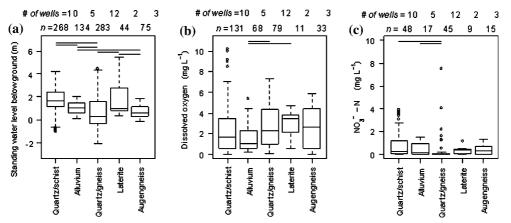


Fig. 3 Boxplots of (a) standing water level below ground (m), (b) dissolved oxygen concentration, and (c) nitrate concentration in the different geological units. Geological

sampled in the 100 mm diameter well WO, while at the same site numbers were very low in the narrow diameter wells W1 and W2 (WO:  $66.3 \pm 34.3$  individuals on average; W1:  $2,8 \pm 3.9$ ; W2:  $0.5 \pm 0.9$ ; taxa numbers in WO:  $4.7 \pm 2$ ; W1:  $1.5 \pm 1.7$ ; W2:  $0.7 \pm 0.9$ ).

No fauna was found in the three 1,000 mm diameter wells, except for one dipteran. However, at the respective sites, Crees, Barrett, and Stringybark, fauna was not found in any of the narrower wells (if existing) either (except for the well C1B).

#### Faunal assemblage patterns

The cluster analysis of faunal assemblages revealed five discrete groups within faunal samples. In Fig. 4 the fusion nodes are numbered consecutively starting at the number of aggregated

units that are connected with a line (on the top of the sub plots) are significantly different (P = 0.05; Behrens–Fisher test with the Satterthwaite t-approximation)

samples (31) on which the agglomerative clustering was performed, plus one. This means that the first fusion node of aggregated samples starts at 32. The consecutive four nodes, e.g., all resulted in assemblages with an evenness of 100%. The five groups resulting at an  $E \ge 50\%$  were made up of those branches (largest groups) which would not result in a homogeneous assemblage if clustered with the next, most similar branch. The five cluster groups only partly grouped samples together that were spatially and temporally related. Groups 1 and 2 only comprised samples from the winter half year (prefix 2 in the label to the left in Fig. 4).

The faunal assemblages in the cluster groups were significantly and distinctly different (ANO-SIM Global R = 0.71, P = 0.001). A strong effect is indicated by a Global R > 0.75. Differences between pairs of cluster groups were strong and

Well size (mm)	Abundances per sample	Number of taxa per sample
50 100 100: WO 100: rest 1000	$\begin{array}{c} 0.92 \pm 3.25 \\ 28.38 \pm 39.65 \\ 66.29 \pm 34.25 \\ 0.35 \pm 1.27 \\ 0.03 \pm 0.17 \end{array}$	$\begin{array}{c} 0.42 \pm 0.97 \\ 2.18 \pm 2.62 \\ 4.71 \pm 1.96 \\ 0.30 \pm 0.88 \\ 0.03 \pm 0.17 \end{array}$

 
 Table 3
 Abundances and number of taxa per sample and standard deviation in the three different well sizes

The well size 100 mm is presented separately for WO and the rest

significant at the P = 0.05 level. Only the pair of cluster groups 1 and 2 was strongly, but not significantly, different (ANOSIM R = 0.75; P = 0.067). The differences in faunal assemblages among geological strata were weak though (ANOSIM Global R = 0.22, P = 0.01).

Almost half of the taxa occurred only in group 4. This group comprised wells within 100 m from the Marbling Brook at different seasons (Table 4). The closer the similarity values in Table 4 were, the more similar were the groups and taxa. Groups 3 and 1, grouping wells from different areas that had all been found to be unconnected with the brook (see below), shared no taxa at all. Three of the species occurring in group 5 did not occur anywhere else. Groups 5 and 3 and groups 2 and 4 were considerably more similar to each other than to the group 1 which harboured only few crustaceans.

The cluster groups did not reflect the patterns in geological or topographic features (Fig. 5). Some wells were grouped into different cluster groups in the different seasons. Therefore, a

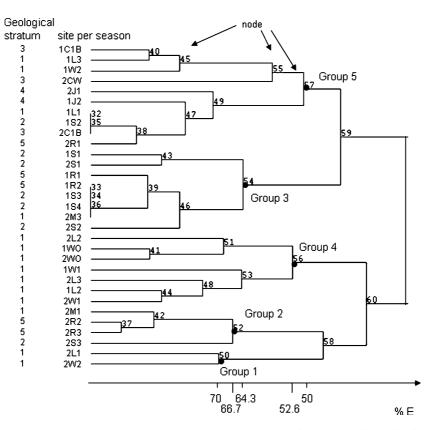


Fig. 4 Dendrogram of the cluster analysis of the faunal assemblages at the wells. Labelling of the aggregated samples indicates the half year (first number: 1 = summer; 2 = winter) and the well (letter/number combination; refer to Fig. 1 for relative position in the catchment). Numbering of geological stratum is as follows:

1 = quartz/schist, 2 = alluvium/colluvium, 3 = quartz/feldspar/gneiss, 4 = laterite, 5 = Augengneiss. Dots indicate where the five groups are fusioned which are used for subsequent analysis. The numbers at the nodes indicate the consecutive clustering of samples to groups

 
 Table 4
 Frequencies of
 Group 5 Group 3 Group 1 Group 2 Group 4 the taxa in the aggregated (n = 9)(n = 8)(n = 2)(n = 4)(n = 7)samples, sorted according 40.4 47.7 0 28.6 100 Species to reciprocal averaging score analysis according to Hill Site score (1973). n = number of aggregated samples in the Bathynella sp. 1 0 100 group 0 100 Chironomidae 0 100 Metacyclops arnaudi (Sars 1908) Australocamptus similis 28.6 100 Karanovic 2004 Metacyclops sp. 1 32.0 25.9 70.7 3.4 Nematoda 37.6 56.1 8.8 35.1 Oligochaeta 45.9 12.8 16.0 25.2 46.0 Malaconothridae sp. 2 49.6 25.3 67.5 7.2 32.7 20.5 Hurleva sp. 1 52.6 46.8 Protocrangonyx fontinalis 90.6 15.8 84.2 Nicholls 1926 Atopobathynella sp. 1 93.1 3.7 6.2 90.1 Diacyclops sp. 1 0.8 4.0 95.3 96.9 100 100 Canthocamptus sp. 1 100 Canthocamptus sp. 2 100 100 Canthocamptus sp. 3 100 Collembola indet. 100 100 Candonidae sp. 5 100 100 Insecta 100 100 Malaconothridae sp. 3 100 100 Malaconothridae sp. 4 100 100 Malaconothridae sp. 5 100 100 Parapseudoleptomesochra 100 100 sp. 1 Turbellaria 100 100

separate map with the cluster groups found in the wells is given for each season.

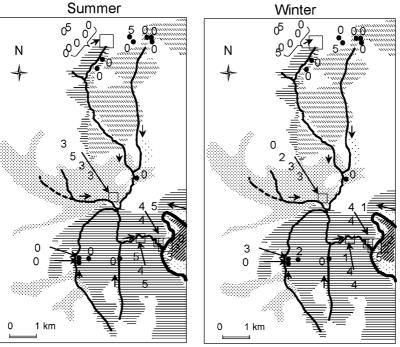
Relating faunal assemblages with physical and chemical variables

No correlations were found between taxonomic richness and any single physical or chemical factor. Neither was the species composition well-related to any combination of factors (BIO-ENV results; highest Spearman rank correlation coefficient = 0.35 for the combination of land-use, maximum standing water level, range of electrical conductance, and month of maximum electrical conductance).

Ninety seven percent of the five faunal sample groups that were the result of the cluster analysis were correctly classified by the LDA procedure (upper half of Table 5; 56.7% by cross validation, lower part of Table 5). The LDA models were developed for the five groups of wells per half year based on faunal data. A selected set of variables was used for this (for the stepwise derivation of the data set see below). This percentage of correct classification is high compared to a maximum arbitrary probability of 29% (maximum arbitrary probability: the largest number of cases per cluster group divided by the total number of cases  $\times 100$ , i.e.  $9/31 \times 100$ ). The main discrepancies between the full model and the cross-validated one were with groups 1 and 5—new samples had a low chance to be grouped into these two groups (Table 5).

Of the available variables (Table 2), eight were found by the stepwise procedure to add a significant amount of information to the discriminant model (Table 6). They contributed to the correct classification as outlined in Table 5.

With five groups among which to discriminate, four functions needed to be derived. These func-



🖾 Alluvium/colluvium 🗎 Quartz, schist 🖾 Quartz-feldspar 🜌 Augengneiss 🗆 Laterite

**Fig. 5** Catchment outline for summer (left) and winter (right) faunal assemblages. The cluster groups for each well is indicated schematically by numbers. Wells denoted

tions were constructed using the eight variables selected (Table 6). The first two functions explained a considerable proportion of the betweengroup variance versus the within-group variance (64.2 and 23%; Table 7), i.e. the eigenvalue was greater than 1 for these first two axes. The third axis did not add much further information (10.6%).

The canonical correlation coefficient measures the strength of the relationships between the function values of the discriminant functions and the groups of the dependent variables. With the maximum value for the correlation being 1, the values for the first two axes are high (0.9 and 0.8, respectively; Table 7). This goodness of fit test was corroborated by the Wilks' test: the amount of information which was contributed to the overall model was high and significant for the first two functions (Wilks'  $\lambda < 0.2$ ; chi<sup>2</sup> values 81 and 41, respectively; P < 0.01).

The first function was best correlated with dissolved oxygen and land-use (largest absolute value of the coefficient, Table 8). The second function was best correlated with the month,

with 0 indicate wells where aggregated samples did not yield fauna within the half year

when the highest temperature was measured, as well as with the average temperature and dissolved oxygen again. The canonical discriminant function coefficients may be impacted by correlation within the variables (although such correlation was not likely given that correlated variables had been omitted from analysis and that the stepwise procedure was employed; see above). To corroborate the results from the canonical coefficients, additionally the structure matrix (common correlation within one group between the discriminant variables and the standardized canonical discriminant functions) was calculated. The first discriminant function correlated best with land-use. The second function correlated best with temperature.

An overview of the means and ranges of the variables that described faunal groups is presented in Fig. 6. The group means were tested for differences, and Wilk's  $\lambda$ , the *F* statistic and the probability value are given for each of the variables singled out by LDA. Additionally, the values for electrical conductance and water level

<b>Table 5</b> Classificationresults: percentages of			Cluster series	Predicted group					Total
successful prediction and cross-validated				1	2	3	4	5	
percentages of successful	Original	Value	1	2	0	0	0	0	2
prediction			2	0	4	0	0	0	4
			3	0	0	8	0	0	8
			4	0	0	0	7	0	7
			5	0	0	2	0	7	9
	Cross-validated	%	1	100	0	0	0	0	100
			2	0	100	0	0	0	100
			3	0	0	100	0	0	100
			4	0	0	0	100	0	100
			5	0	0	22	0	78	100
		Value	1	0	1	0	1	0	2
			2	1	2	1	0	0	4
			3	0	1	6	0	1	8
			4	0	0	0	7	0	7
			5	2	2	2	1	2	9
		%	1	0	50	0	50	0	100
			2	25	50	25	0	0	100
			3	0	13	75	0	13	100
			4	0	0	0	100	0	100
			5	22	22	22	11	22	100

 Table 6
 Discriminant analysis: stepwise insertion of variables into the model

Iteration step	Variable inserted	Wilks' lambda								
		Statistic	df1	Exact F	Exact F		Approximate F			
_				Statistic	df1	Significance	Statistic	df1	df2	Significance
1	Land-use	0.28	1	15.9	4	< 0.001				
2	Month <sub>max. temp.</sub>	0.22	2	6.9	8	< 0.001				
3	$NO_{3}^{-}N (mg l^{-1})$	0.16	3				5.1	12	61.1	< 0.001
4	Temperature (°C)	0.10	4				4.8	16	67.8	< 0.001
5	Month <sub>min. SWL</sub>	0.08	5				4.1	20	70.6	< 0.001
6	Dissolved oxygen (mg $l^{-1}$ )	0.06	6				3.8	24	71	< 0.001
7	Minimum temp. (°C)	0.04	7				3.6	28	69.9	< 0.001
8	Month <sub>max. ec.</sub>	0.03	8				3.5	32	68	< 0.001

max. = maximum value of; min. = minimum value of; temp. = temperature; SWL = standing water level (m); ec. = electrical conductance

During each step the variable is inserted which minimizes overall Wilks' lambda 52 iterations

variation are presented in Fig. 6, since they had explained much of the variation among wells in PCA. The intensity of land-use was significantly different among the groups. The values for the earliest month when electrical conductance was highest, differed among groups at a P = 0.06level. For comparison, the values for the wells without fauna are plotted to the left of each subset (Fig. 6). Nitrate concentrations were significantly lower in the wells where no fauna was found (group 0 in Fig. 6; Mann Whitney W = 158, P < 0.001).

Characterisation of the faunal groups

Wells in group 1 (only two wells) and group 4 were situated only on quartz schist. Wells in the other three groups were situated on more than

Function	0		Cumulative %	Canonical correlation
1	5.1	64.2	64.2	0.9
2	1.8	23	87.1	0.8
3	0.8	10.6	97.8	0.7
4	0.2	2.2	100	0.4

 Table 7 Goodness of fit: Eigenvalues and canonical correlation of the discriminant functions

 Table 8
 Standardized
 canonical
 discriminant
 function

 coefficients

	Discriminant function					
	1	2	3	4		
$NO_{3}^{-}-N \ (mg \ l^{-1})$	-0.35	0.95	0.77	-0.09		
Temperature (°C)	-0.19	1.14	0.33	-0.10		
Land-use	-1.20	0.12	0.13	-0.17		
Dissolved oxygen $(mg l^{-1})$	1.44	-1.09	0.75	0.17		
Month <sub>min. SWL</sub>	-0.54	0.99	-0.60	0.68		
Month <sub>max. ec.</sub>	0.27	-0.83	0.35	0.46		
Minimum temp. (°C)	0.80	-0.06	0.47	0.92		
Month <sub>max. temp.</sub>	-0.15	-1.16	-0.16	0.26		

max. = maximum value of; min. = minimum value of; temp. = temperature; SWL = standing water level (m); ec. = electrical conductance

one type of geological strata. Fauna in group 1 comprised two crustacean species, which occurred in the groups 4 and 5 (Table 4). The wells L1 and W2 at the Lambie and Webb sites were situated on quartz/schist at sites that were not impacted heavily by land-use. Maximum EC and minimum water level occurred late in the year. Minimum temperature was high. Average EC was very low and the range of water level very high (Fig. 6).

Fauna in group 2 was sparse and shared all taxa with group 4 (Table 4). Only winter samples were included. Land-use was intense. At the same time, nitrate concentrations were not elevated. Minimum water level occurred late in the year, and temperature was high. EC was high and water level varied by one meter within the investigation period (Fig. 6).

Few taxa were found in the wells of group 3. This group included the only well where *Austra-locamptus similis* Karanovic 2004 occurred, a species usually found in surface water (Table 4). The group included samples from both winter and summer. Electrical conductance was high. Water level variation was close to the surface throughout the year. Temperature was variable and maximum temperature occurred in either July or August (Fig. 6).

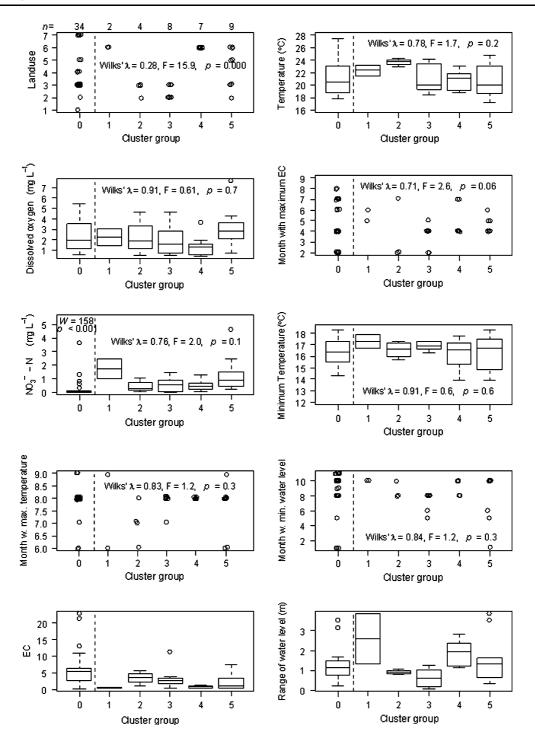
Group 4 contained the wells with the most diverse assemblages. Four crustacean species as well as some mite morphotypes and other taxa occurred exclusively in this group. *Protocrangonyx fontinalis* Nicholls 1926, *Atopobathynella* sp. 1, and *Diacyclops* sp. 1 had their highest frequencies in this group (Table 4). Samples from both winter and summer, but exclusively from the Lambie and Webb sites, were included. Land-use in this group was not intense, DO and EC were low, and the range of water level was high (Fig. 6).

Group 5 included wells from several sites and seasons in the catchment and was the most heterogeneous of all groups. Two crustacean species occurred exclusively in this group. The low frequencies of *Diacyclops* sp. 1 and *Atopobathynella* sp. 1 distinguished it further from the other groups, especially from group 4 (Table 4). Physical and chemical results were variable for this group (Fig. 6).

## Discussion

The effect of geology in shaping the relative hydrochemical and physical characteristics of groundwater at Marbling Brook was smaller than that of hydrological exchange. Faunal assemblages reflected hydrological exchange rather than geological stratification or intensive landuse per se.

PCA explained less than half of the amongwell variation. The PCA results demonstrated that variation among wells was characterized best by features that are related to hydrological exchange, such as electrical conductance, temperature, and the variability of both over time. The characteristics that differed among geological strata are some that depend on hydrological exchange, which in turn is determined by geological structure. Therefore, these differences were indirect effects from geological structure and did not reflect background rock dissolution.



**Fig. 6** Boxplots for the continuous variables and scatter plots for the discrete variables and factors in each group that were used for building the model. Average electrical conductance and range of water level (m) are plotted below as well. The group 0 contains all groundwater wells where fauna was never found and which were not classified

before. Points for superimposed data are offset. Group means of the groups 1–5 were tested by Wilks' lambda ( $\lambda$ ; df1 = 4, df2 = 25). Wilks' lambda, F statistic and probability level *P* are given. For the nitrate plot, additionally the differences in nitrate levels between group 0 and all other groups were tested by the Mann Whitney test

# Methods

The size of the fauna samples was small. The wells had been constructed and drilled in a way not to overly disturb the sediments around the wells. Therefore, no gravel filter was installed around the wells, as done in production wells. Besides, this would have introduced a new habitat for fauna and it would not have been possible to distinguish this gravel fauna from that representative of the surrounding aquifer. However, this led to well water having a suspension of the soil fines and clay which made it difficult to sample larger volumes with more net draws. Although net sampling offers many advantages (Schmidt et al., 2004), the issue of small volumes needs to be addressed in future investigations.

This small sampling effort explains the low number of individuals and taxa in each sample. It is possible that the distinction between faunal assemblages would have been clearer if the samples had been larger. However, the plankton net enabled us to sample the large number of wells regularly and we feel that in this type of exploratory study the advantage of the method outweighs the inherent compromise to the clarity of the statistical analyses.

The relative efficiency of the 45 mm net in these different wells will probably vary. One might expect that the narrower the well, the more effective the plankton net might be. If this was true, then the abundances would have been expected to be lowest in the 1 m diameter well, as indeed they were. However, faunal abundances and taxa numbers were highest in the medium size wells. This was solely due to abundances and taxa numbers in the well WO, which were significantly higher than those in other wells. In conclusion, rather than well size specific conditions around certain wells determine faunal abundances when sampled by the net sampler.

Faunal assemblages differed unexpectedly between seasons, considering that until a few years ago groundwater faunal assemblages were believed to be stable over time (Dole & Chessel, 1985). In this study, seasons were defined as the time span when temperature varied least from one month to the other. This delimitation of seasons might not correspond to faunal assemblage variation in time. Future studies have to focus on temporal variation of faunal assemblages.

## Factors influencing faunal assemblages

While geological strata were clearly distinguished from each other by differences in characteristics reflecting hydrological exchange (water level, DO, nitrate), the faunal assemblages did not differ among geological strata. In fact, the correlation between faunal distribution in the wells and physical or chemical factors in general was weak altogether (see the results from BIO-ENV). Fauna occurred in wells with dissolved oxygen concentrations close to anoxia and wells where fauna were absent were not significantly lower in dissolved oxygen than other wells. Therefore, dissolved oxygen was not a limiting factor to fauna in Marbling Brook catchment. The lack of a relationship between fauna and abiotic conditions in general is in agreement with other studies. For example, Boulton et al. (1992) found that fauna occurs in a wide range of DO, and Hakenkamp et al. (1994) did not find any correlations between faunal numbers and abiotic factors in their study in a sandy Atlantic Coastal Plain aquifer. Malard & Hervant (1999) demonstrated some of the effective adaptations of groundwater fauna to dissolved oxygen concentrations  $<0.3 \text{ mg L}^{-1}$ . However, Hahn (2002) had found that MDS dimensions calculated from Marbling Brook sediment fauna sampled in an earlier study were correlated with land-use and DO. The effect of these two characteristics on groundwater is compromised by hydrological exchange: the lower the exchange, the more DO and land-use-derived nutrients and carbon are stripped during the slow passage through the soil and the less DO, nutrients, and carbon actually reach the groundwater (Malard & Hervant, 1999). While in the present study the two factors per se did not correlate to faunal assemblage patterns, the hydrological exchange patterns which are reflected in e.g. standing water levels, electrical conductance, dissolved oxygen and nitrate concentrations did (see below).

# Fauna and hydrologic exchange

Discriminant analysis identified the variables land-use, DO, and temperature to be related to differences among faunal assemblage groups. Land-use was significantly different between the well groups differentiated by fauna. As shown above though, it was not the direct effect of the single variables that shaped faunal assemblage patterns. Rather, the combination of these variables indicated processes that were more important to groundwater fauna than the sole concentration of single variables. These processes can best be related to groundwater recharge.

Land-use may influence hydrological exchange (Grimm et al., 2005). Agricultural practices lead to an input of particles which clog the interstices and thus reduce hydrological exchange. Microbial growth plays an important role in these interactions (Brunke, 1999). If we were able to describe hydrological exchange with an index comparable to the land-use intensity index, we suggest that close concordance between hydrological exchange and land-use would be found. Such an index could be complementary to the GW Fauna Index (Hahn, 2006). Since detritus amounts, which contribute to the GW Fauna Index, were not recorded in Marbling Brook samples, the GW Fauna Index cannot be tested for these samples.

## Characterisation of the faunal groups

In the following the five well groups from faunal assemblages are characterized in terms of hydrological exchange patterns. Some of these characterizations may be based on weak evidence since data patterns partially lack statistical significance. The characterizations should therefore be seen as hypotheses which need to be tested in further studies.

Group 1 wells (L1, W2) were characterized by input from fresh precipitation. Maximum electrical conductance and minimum water level occurred late in the year in this group indicating that recharge had a lagged effect here compared to other wells. The timing of changes to dissolved oxygen, temperature, standing water level, and electrical conductance indicate how fast or slow exchange occurs. Minimum temperature was

high, indicating that these water bodies were not affected by the cold winter climate and thus by the surface to the same degree as wells L2, L3, and WO at the respective sites (see below). Wells L1 and W2 were thus characterized by slow exchange with other fresh water bodies. Since other wells at the Lambie and Webb sites were more saline (average electrical conductance and range of water level), this influence was most likely from fresh precipitation percolating through the covering soil. This interception led to considerable water level variations, and seasonally high nitrate concentrations (discussed below). Wells in this group provided a suitable habitat for some taxa, and the groundwater cyclopoid Metacyclops sp. 1 might in the future serve as an indicator for a groundwater habitat characterized by frequent recharge from precipitation. Although only two wells were included in this group, they combined a taxon diversity similar to that in the other, larger well groups. This is in concordance with Datry et al. (2005) who showed that total invertebrate as well as hypogean taxa densities and richness were higher in recharge than in reference sites.

Group 2 was characterized by evaporation, while input from the surface was low. In addition, land-use was intense in group 2, temperature was high, oxygen concentration was variable, and the minimum water level occurred late in the year. Electrical conductance was high and water level varied considerably throughout the year, presumably from evaporation. Nitrate concentrations were not elevated (discussed below). Nutrients from land-use are likely to be stripped from the water before reaching this part of the groundwater body. Therefore, this groundwater body is believed to be fairly unconnected from any inputs from land-use and hydrological exchange other than evaporation. Thus, wells in this group were rather inhospitable to fauna.

Group 3 wells indicated influence from saline water bodies. Elevated electrical conductance, high variation in water level throughout the year, variable temperature and dissolved oxygen, and maximum temperature in either July or August indicate that groundwater in this group was connected to and exchanging regularly, with rather saline water bodies. There was little direct influence from the surface, as indicated by the high temperature in the coldest months in Marbling Brook catchment. Few taxa were found in these wells. This group included the only well where *Australocamptus similis* Karanovic 2004 occurred. This species is usually found in surface water and it might be indicative of exchange between groundwater and saline surface water bodies. Such upwelling water was indeed observed regularly at the Schmidt site, where most of the group 3 wells occurred.

Group 4 wells are likely to have been in temporal hydrological contact with the Marbling Brook. According to physical and chemical characteristics, wells in group 4 were similar to those in group 1, but nitrate and dissolved oxygen levels were lower. The slightly higher electrical conductance in these wells compared to those in other groups indicates concentration of salts through high evaporation, or exchange with water bodies more saline than precipitation, most likely exchange with the slightly saline Marbling Brook. Exchange with brook water will provide less nutrients and less oxygen than exchange with precipitation, since during stream sediment respiration nutrients and oxygen are stripped from the water. The exchange between brook and groundwater did not include fauna though (Schmidt, 2005), thus this diverse subterranean fauna (the taxa numbers were the highest among all well groups) was not challenged by the more competitive surface water taxa, but profited from the seasonal input of resources such as nutrients and carbon sources. Protocrangonyx fontinalis Nicholls 1926, Atopobathynella sp. 1, and Diacyclops sp. 1 had their highest frequencies in this group. These groundwater taxa might serve as indicators of inputs of surface water such as with the brook. Note that surface water taxa (except for Australocamptus similis; see above) have not been observed even in the wells suggested to exchange hydrologically with the Marbling Brook. Consequently, exchange seemed to have been restricted to water and solutes and did not include invertebrates.

Group 5 was heterogeneous. Land-use and physical and chemical results were variable for this group and indicated various sources of ions. The fauna in group 5 was diverse and shared many taxa with group 4. The low frequencies of *Diacyclops* sp. 1 and *Atopobathynella* sp. 1 distinguished it from the other groups, especially from group 4. Dissolved oxygen was high, although not significantly higher than in the other groups. However, this indicates frequent input from the surface. Group 5 shared elevated nitrate values with group 1 (although not significantly higher than in the other groups) further demonstrating influence from the surface. With its characterization by input from the surface this group is close to group 4, although wells in group 5 probably received—at least seasonally—more input from older groundwater.

## Nitrate, land-use, and hydrological exchange

Seasonally increased nitrate concentrations occurred where input from the surface was indicated by water level variation (see above). Seasonally elevated nitrate concentrations were observed to occur together with seasonal recharge from percolation/interflow (regional groundwater originating from percolating precipitation; Duff & Triska, 2000; Hinkle et al., 2001, Tesoriero et al., 2005). Especially in fine sediment soils combined with deeper water tables, nitrate is stripped from the soil water before reaching deeper layers (Malard et al., 2002). While fauna groups 1 and 4 were both characterized by recharge from the surface, in group 4, where hydrological exchange seemed to be more intense with the brook, nitrate levels were lower.

While elevated nitrate concentrations are believed to originate from intense land-use, a direct relationship between land-use and nitrate was not found. Intense land-use leads to a momentary input of nitrate concentrations, but these high concentrations are transformed quickly in the soil, depending on microbial respiration (Bärlocher & Murdoch, 1989) and hydrological exchange. The classification of land-use stayed the same for the investigation period, but event-based elevated nitrate concentrations are temporary. Direct relationships between land-use intensity and nitrate concentrations can only be expected when microbial respiration does not play an important role. Zones that lacked hydrologic exchange

Surface influence and input was probably lowest for the artesian wells (C3, C4, C5, C6, C7, M2). At such zones, aquifers are believed to offer inhospitable conditions for faunal assemblages, and in accordance with this, no fauna was observed in these wells. Hydraulic conductivity in these wells, as estimated from the grain size distribution according to Beyer (1964; except for the older C3 and C4), ranged from 25 to 86  $\mu$ m s<sup>-1</sup> (Schmidt, 2005). These values indicated very low transmissivity. Water upwelling in these wells is therefore believed to be older than other groundwater in the Marbling Brook catchment area, stripped from nutrients and consequently poor in food supply. However, even deep artesian aquifers may support diverse stygofauna in karst areas (Holsinger & Longley, 1980) with open conduit flow and adequate energy inputs. The lack of fauna in artesian wells in the shallow and porous Marbling Brook catchment aquifer seems to reflect the combined effect from small void space and the lack of exchange, as a consequence of the low transmissivity, and nutrient poor waters owing to long groundwater residence times.

## Conclusion

Our expectation was met that faunal assemblages would represent different types of exchange. Distribution patterns in groundwater fauna appear to be related to complex interaction patterns rather than single factors. The present work suggests that faunal assemblages reflect hydrological exchange not only at the interaction zone with the brook, but at zones throughout the aquifer showing varying interaction with the surface. Faunal assemblages differentiate between connection with surface water bodies such as the brook and surface influence from e.g. precipitation. In the future we might be able to make use of groundwater taxa such as Protocrangonyx fontinalis Nicholls 1926, Atopobathynella sp. 1, Diacyclops sp. 1, and Metacyclops sp. 1 to indicate recent and frequent surface connection.

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