Biogeochemistry (2005) 72: 67–86
DOI 10.1007/s10533-004-0375-8

Estimation of denitrification potential in a karst aquifer using the ^{15}N and ^{18}O isotopes of NO_3^-

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Received: 1 December 2003; accepted in revised form 12 March 2004

Key words: Chemical tracers, Denitrification, Isotopes karst

Abstract. A confined aquifer in the Malm Karst of the Franconian Alb, South Germany was investigated in order to understand the role of the vadose zone in denitrifiaction processes. The concentrations of chemical tracers Sr^{2+} and Cl^- and concentrations of stable isotope ^{18}O were measured in spring water and precipitation during storm events. Based on these measurements a conceptual model for runoff was constructed. The results indicate that pre-event water, already stored in the system at the beginning of the event, flows downslope on vertical and lateral preferential flow paths. Chemical tracers used in a mixing model for hydrograph separation have shown that the pre-event water contribution is up to 30%. Applying this information to a conceptual runoff generation model, the values of $\delta^{15}N$ and $\delta^{18}O$ in nitrate could be calculated. Field observations showed the occurence of significant microbial denitrification processes above the soil/bedrock interface before nitrate percolates through to the deeper horizon of the vadose zone. The source of nitrate could be determined and denitrification processes were calculated. Assuming that the nitrate reduction follows a Rayleigh process one could approximate a nitrate input concentration of about 170 mg/l and a residual nitrate concentration of only about 15%. The results of the chemical and isotopic tracers postulate fertilizers as nitrate source with some influence of atmospheric nitrate. The combined application of hydrograph separation and determination of isotope values in $\delta^{15}N$ and $\delta^{18}O$ of nitrate lead to an improved understanding of microbial processes (nitrification, denitrification) in dynamic systems.

Introduction

The European community has recently adopted a new Water Framework Directive (WFD) that extends to protect all groundwater types and sets a legally binding objective of good status for those waters (6th Environment Action Programme). Europe has only begun to address this issue, recognizing the importance of groundwater protection. An increased and more efficient agricultural production derived from chemical use can have adverse effects of the quality of water resources, because of the accumulative effect of adding more nutrients and persistent organic pollutants. The continuing pollution causes problems not only on the availability of high quality freshwater resources but generates also economic and social pressures due to increasing conflict potentials between land use and the demands for protection of the water resources.

During the past several decades a number of field studies have been published showing the critical issue of nitrate in groundwater, e.g. Williard et al. (2001), Panno et al. (2001), Hill et al. (1999), Kendall et al. (1996), Wassenaar (1995), Aber et al. (1989). Intensive agricultural land use in the Franconian Alb (FRA), South Germany (Figure 1), results in higher NO_3^- concentrations in most of the springs and wells. The values are distinctly above German drinking water limits (> 50 mg/l). As a consequence a high influx of nitrate and pesticides into the rivers Altmühl and Anlauter (s. Figure 1) via springs or directly into the deep karst aquifer is observed downstream. However in some springs the nitrate concentrations do not correlate to catchment specific rates of N and to groundwater recharge in the Franconian Alb (Glaser 1998). Wendland et al. (1993) postulated that significantly higher nitrate concentrations in groundwater would be observed in the area of the Franconian Alb if the denitrification processes could be excluded. However, the concentration of infiltrating pollutants can be also altered due to chemical reaction and biodegradation, which strongly depends on the pathway length, the permeability of the flow system and the presence of bacteria and fungi (Meckenstock 1999; Biegert et al. 1996).

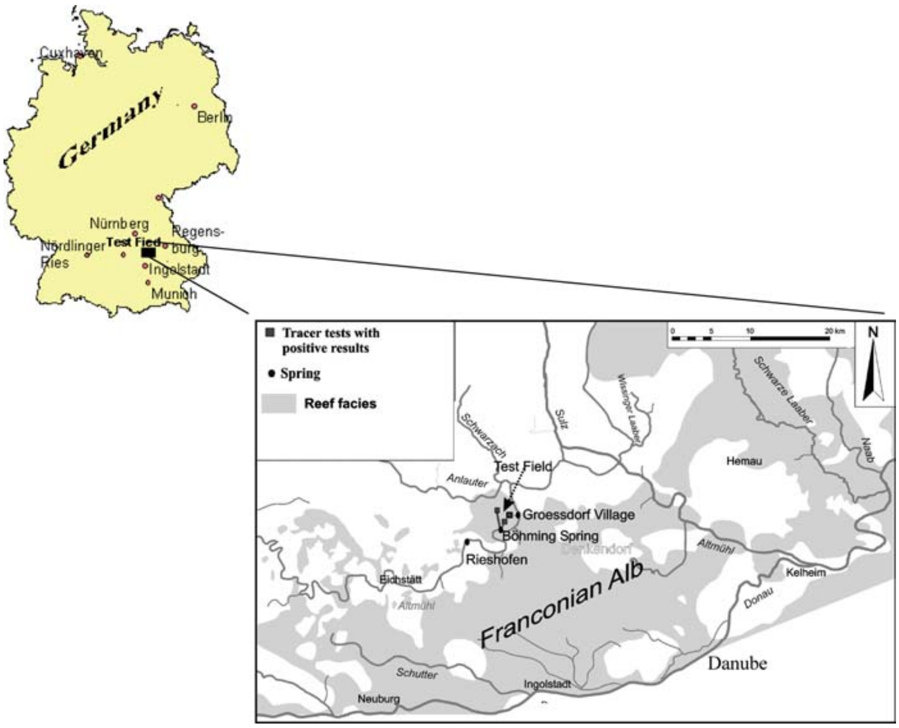

Figure 1. Site map of the Franconian Alb karstic chatchment area and the test fiel.

Some studies like: Mariotti et al. (1988), Böttcher et al. (1990), Aravena and Robertson (1998) or Voerkelius (1990) document that bacterial denitrification can take place in porous aquifers. In comparison to porous aquifers karst systems have low dispersivities in fissures (Seiler et al. 1989) and possess low purification capacity (Hötzl 1999). In the karst system of the FRA, groundwater velocities in the range of km/day can be observed (Seiler et al. 1989). Therefore pollutants can flow over considerable distances without retention. Information about the microbial influence on biodegradation rates (e.g. denitrification) in the vadose zone, before pollutants reach the groundwater table, is of central importance.

The goal of this study is to characterize the influence of the vadose zone on microbial denitrification processes in strong heterogeneous karst aquifers and to quantify the denitrification potential using the isotope ratios of $\delta^{15}N$ and $\delta^{18}O$ in nitrate. In addition, environmental chemical tracers were evaluated for their suitability for partitioning stream discharge into different runoff components. The results of the runoff separation based on a mixing model were interpreted: (i) to delineate the flow system, (ii) to understand better runoff processes in karst systems and (iii) to characterize the region (soils zone, epikarst, unsaturated zone) in the karst system of the catchment area, where microbiological degradation processes of NO_3^- are significant.

Materials and methods

Experimental site

The Southern Franconian Alb is situated in Southern Germany and stretches north of the Danube River from the Nördlinger Ries in the west to Regensburg in the east (Figure 1). The karst aquifer (Oxfordian to Tithonian age) represents an important source of water for industrial (e.g. brewery, car industry), municipal and domestic use. The upland of the springs and wells in the Franconian Alb is mainly used for agricultural purposes. Forests cover about 40% of the area (Glaser 1998). The aquifer of the Böhming spring in the Franconian Alb is a confined aquifer between the River Altmühl and Anlauter and consists of carbonates, which belongs to two consecutive cycles of sedimentation starting both with marls and ending with carbonates. In the upper cycle limestone was replaced mostly by reefs, which were diagenetically transformed into dolomites (reef facies). Bedded limestones have a porosity less than 2 vol%. To the contrary, the reef facies has an effective porosity of 7 vol%, determined in independent laboratory experiments (Michel 1999). The high matrix porosity of the reef facies demanded a double porosity concept for the tracer transport (Maloszewski and Zuber 1985) and was already used for the interpretation of artificial and environmental tracer experiments in the Franconian Alb by Seiler et al. (1989) and Rau (1999), respectively. The porous carbonate aquifer contains mobile water in fissures and immobile water in the

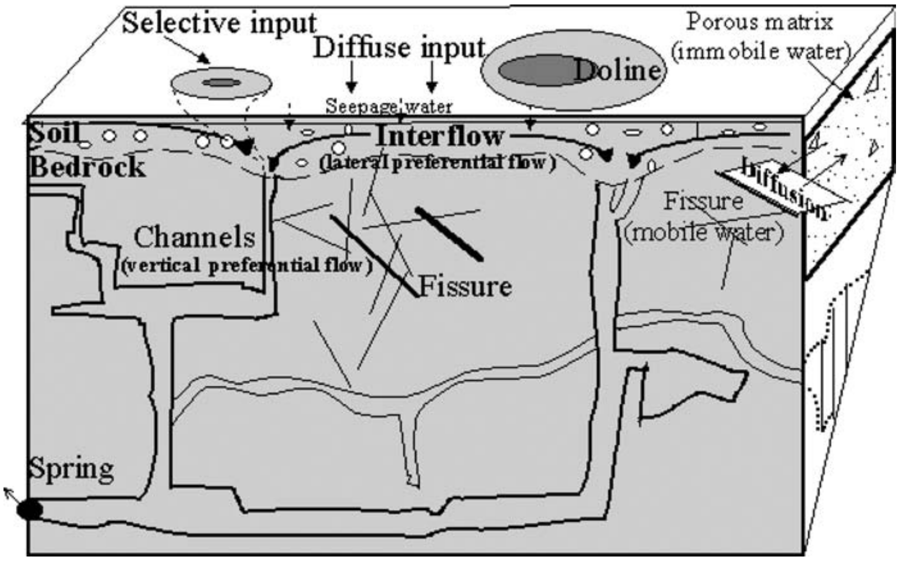

Figure 2. Conceptual model of runoff generation.

porous rock matrix (Figure 2). Therefore diffusion processes within the immobile water of the matrix control the contaminant transport during groundwater flow in the reef facies. The porous rock matrix has a significant storage capacity of immobile water about 98.5% of the total water volume while only 1.5% of the water volume is stored in the fissures (Pfaff 1987).

The Böhming spring with well known hydrogeology was selected as a representative landscape section of the Franconian Alb. This spring is located between the Rivers Altmühl and Anlauter and has a catchment area of about 7 km^2 (Einsiedl et al. 2002). The mean discharge of 29 l/s corresponds to a groundwater recharge of 0.13 m/a. This is in good agreement with calculations given for this region by the Bavarian Water Management Agency (1996). The vadose zone is up to 120 m thick and is composed of the reef and bedded facies. Twenty percentage of the catchment area is used agriculturally, while forests covered 80%. Only the agricultural land is covered by postjurassic overlay of up to 1.5 m in thickness (loess loam from the Pleistocene and clayey tertiary structures from the Miocene) (Schmidt-Kaler 1989). Groundwater is a Ca–HCO$_3$ type, pH and E_h are in the range of 7.5 and 220 mV, respectively. The DOC concentration during base-flow varies between 0.7 and 1.1 mg/l.

Two component-mixing model

Hydrograph separation with natural tracers and stabile isotopes has become a popular method to get information about runoff processes, e.g. Uhlenbrock et al. (2002), Maloszewski et al. (2002). Lee and Krothe (2001) described a simple mass balance approach to distinguish the runoff components from different reservoirs of the karst system. Buttle (1994) and Brown et al. (1999) discussed the theoretical basics for a two component separation model (Eq. 1). Various studies (e.g. Buttle and McDonald (2002), Rice and Hornberger (1998) or Turner et al. (1987)) have established the usefulness of chemical indicators for monitoring water movement and their application to distinguish between different runoff components.

The specific objective of this paper is the application of strontium as a tool to distinguish between the base-flow and surface near flow components. Strontium is predominating in the bedrocks (Ca sources are the bedded limestone and the reef facies). The contact time of water with the bedrock regulates the strontium concentration in the base-flow water. Assuming that strontium concentrations in event water (atmospheric deposition) and soil zone water (pre-event water) are equal to zero, one can estimate the (pre-) event water contribution by applying Eq. (1). The suitability of Sr^{2+} as natural tracer to separate event water and base-flow water was conducted in a catchment area next to the test field Böhming. This study has demonstrated (Einsiedl et al. 2002) that the amount of event water found using Sr^{2+} is consistent with values calculated with the conservative tracers O^{18} and Cl^- (Figure 3). As it is shown further, the calculation of event water content to total runoff using two-component mixing model (Eq. (1)) yields for Sr^{2+} the value of $10 \pm 5\%$. Applying Cl^- and ^{18}O data to the mixing equation a contribution of event water to total runoff of 10 and 13% were calculated, respectively. In contrary to Sr^{2+} no significant effect of solution processes of strontium bearing minerals in the carbonate bedrock were observed. Due to the fact, that Sr^{2+} concentration in precipitation is below detection limit and no significant variability of strontium concentration is observed in the base-flow during the investigation period, the proportion of event and pre-event water could be estimated using Eq. (2) (Hinton et al. 1994).

$$Q_{\mathrm{dir}}/Q_{\mathrm{T}} = (C_{\mathrm{M}} - C_{\mathrm{B}})/(C_{\mathrm{NS}} - C_{\mathrm{B}}), \tag{1}$$

$$Q_{\mathrm{indir}}/Q_{\mathrm{T}} = (C_{\mathrm{B}} - C_{\mathrm{M}})/(C_{\mathrm{B}}). \tag{2}$$

Q_{dir} and Q_{indir} are the event or pre-event flow rate component, respectively, Q_{T} is the total runoff, C_{M} is the concentration of tracer in the outflow during storm runoff, C_{B} is the concentration of tracer in base-flow and C_{NS} is the concentration of tracer in precipitation.

To calculate the ratio of pre-event water Q_{ind} and base-flow water Q_{B} to total runoff (Q_{T}), Eq. (2) was used. A Sr^{2+} concentration of 0.034 mg/l during base-flow condition (C_{B}) was measured. The subsurface concentration C_{M} during storm runoff generation and Q_{T} were integrated over the time period of the event $Q_{\mathrm{T}}(t_i)$, $i = 1,\ldots,N$).

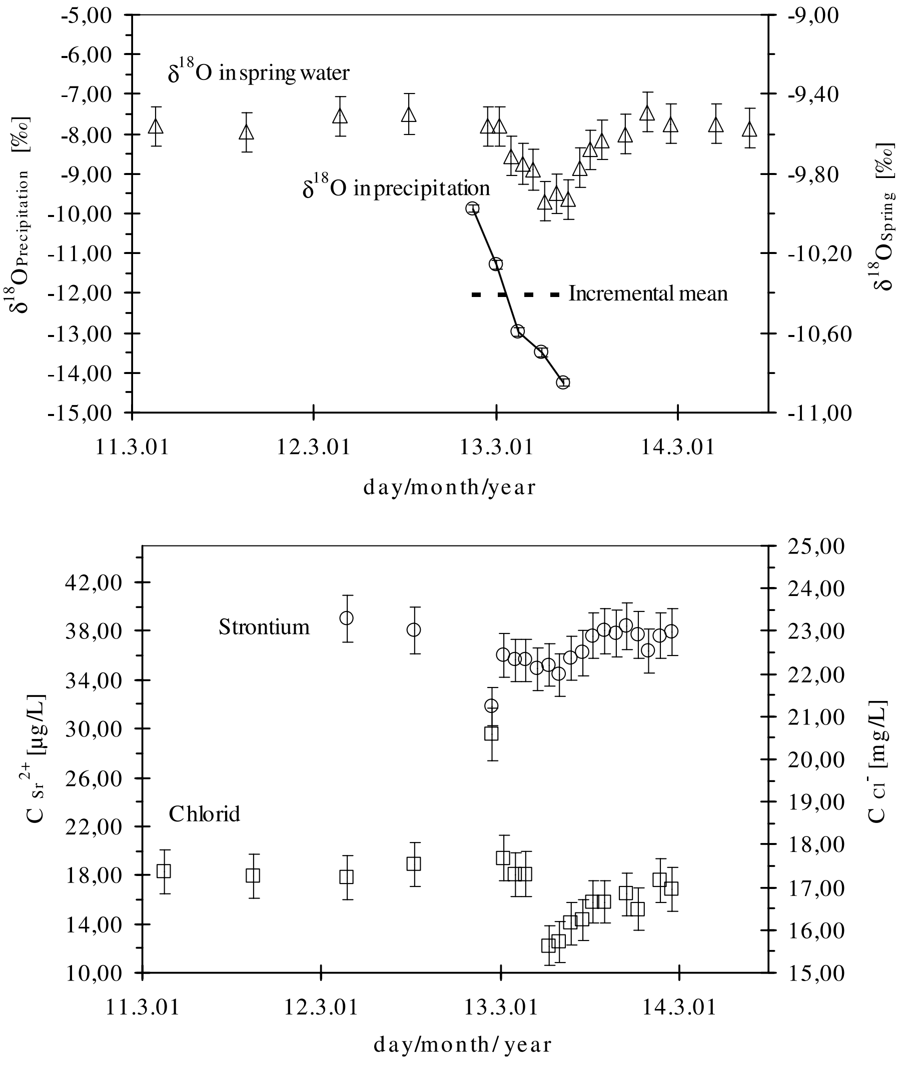

Figure 3. Temporal variability of ^{18}O, Sr^{2+} and Cl$^-$ during rainfall runoff event.

δ^{15}N und δ^{18}O values of NO_3^- in the hydrologic cycle

Measurements of stable isotope values of δ^{15}N and δ^{18} O in NO_3^- can characterize the sink and source terms of N in an ecosystem and help to determine the influence of microbiological activity of the NO_3^- concentration (see Figure 4). The values of δ^{18}O and δ^{15}N in nitrate are strongly different dependent on the nitrate source. The δ^{15}N shows a wide range of nitrate deposition, which lies for bedding/forests between −8 and 0‰ (Nattelhofer and

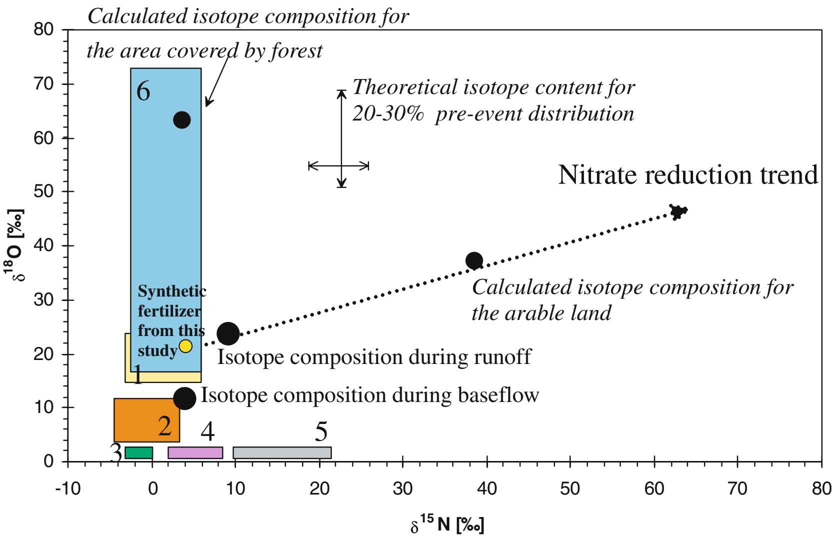

Figure 4. Natural isotope values of various sources of nitrate (synthetic fertilizer (1), nitrification process (2), bedding/forest (3), soil organic matter (4), manure (5), precipitation (6)) modified after Clark and Fritz (1997) and calculated isotope ratios of precipitation nitrate and microbiological influenced fertilizer nitrate of Böhming karst aquifer.

Fry 1988) and can reach up to $+22$‰ for manure (Heaton 1986; Wassenaar 1995). Studies have been conducted to characterize the origin of nitrate in karst water with the δ^{18}O signature of the nitrate. Kendall (1998) found a wide range of δO^{18} values (14 and 75‰) of nitrate in precipitation. Williard et al. (2001) postulated a seasonal variation of δ^{18}O–NO_3^- in atmospheric nitrate deposition. Durka et al. (1994) and Voerkelius (1990) have characterized atmospheric nitrate with values between 73.4 and 52.5‰ of δ^{18}O and values between 6 and -3‰ of δ^{15}N in nitrate. Voerkelius (1990), Böttcher et al. (1990), Amberger and Schmidt (1996) observed a significant concentration of δ^{18}O and δ^{15}N in NO_3^- if denitrification processes occurred in the hydrogeological system. Field studies and the evidence from soil incubation experiments suggest that microbial denitrification leads to an increase of δ^{15}N and δ^{18}O in the NO_3^- at a constant ratio of 2.1:1. ε (enrichment factor) values of -8 and -15.9‰ for oxygen and nitrogen were used, respectively (Voerkelius 1990) to calculate initial theoretical nitrate concentrations.

Clark and Fritz (1997) have demonstrated that the synthetic fertilizer nitrate has a remarkably constant δ^{18}O value of 22 ± 3‰ as well as low δ^{15}N values (-3 to $+5$‰). Therefore denitrification processes increase to clearly distinguishable ratios of δ^{18}O and δ^{15} (Figure 4) in nitrate. The dashed line in Figure 4 illustrates the denitrification process. The concentrations of δ^{18}O and δ^{15}N of different nitrate sources taken from the literature are shown in Figure 4. The sampled and calculated isotope ratios of nitrate show the effect of isotope enrichment in $\delta^{18}O$ and $\delta^{15}N$ from the biologically newly formed nitrate.

Field and laboratory methods

Water samples from the Böhming spring (s. Figure 1) were collected between November 2001 and March 2002. The spring water was monitored with automatic samplers two times a day to determine the tracer concentrations of the stream base-flow. During the runoff event the temporal resolution of the rain samplers and the samplers for the spring water was set to sample every 2 h.

The water samples for major cations (Na^+, K^+, Ca^{2+}, Mg^{2+}) and anions (Cl^-, $SO_4{}^{2-}$, $NO_3{}^-$) were 0.45 μm field filtered, collected in 0.5 l plastic bottles and stored at 5 °C. Standard ion chromatography (Dionex DX 100) was used for the measurements. The analytical error of the measurement was less than $\pm 3\%$. Water samples for strontium filtered, acidified with HNO_3 and measured according to standard ICP technique (Varian 200) with an analytical error of approximately $\pm 5\%$. Samples for the $\delta^{18}O$, $\delta^{15}N$ and DOC were collected in 0.01–5 l glass bottles. The samples were analyzed for $\delta^{18}O$ by CO_2 equilibrium method using a mass spectrometer. The contents were expressed relatively to the Vienna standard mean ocean water (V-SMOW). The analytical Gaussian standard error was approximately $\pm 0.1‰$ for $\delta^{18}O$ in water. The analyses for $\delta^{15}N$ and $\delta^{18}O$ in $NO_3{}^-$ were performed at the Environmental Isotope Laboratory 'Hydroisotop' in Schweitenkirchen, Germany. After removal of $HCO_3{}^-$, $SO_4{}^{2-}$ and DOC the cations were collected using a cation exchange column. Dried KCl and KNO_3 were mixed with graphite and converted to CO and N_2 and measured using isotope ratio mass spectrometry. The analytical Gaussian standard error was approximately ± 0.3 and 0.5‰ for $\delta^{15}N$ and $\delta^{18}O$ in nitrate, respectively.

Results

Precipitation, spring water ^{18}O measurements and conceptual model

The influence of event-water to runoff processes was obtained by comparing $\delta^{18}O$ contents in precipitation and in spring water. The application of this method requires heavy rainfalls with distinctly different $\delta^{18}O$ values in precipitation and base-flow. Four events ($E_1,\ldots,E_4$) with different intensities and isotope contents were investigated during November and December 2001. According to McDonnel (1991), a temporal variable incremental mean method was used for the calculation of the mean $\delta^{18}O$ value in precipitation yielding: −11.2, −10.8, −9.2, and −14.5‰ for the first, second, third and fourth event, respectively (see Figure 5). The $\delta^{18}O$ values in spring water (Figure 6) were: −9.9, −9.7, −9.9, and −9.8‰ for these events, respectively. The isotope composition for the first event could not be detected completely. Therefore the interpretation was done using events E_2–E_4. The $\delta^{18}O$ value of the base-flow, before storm runoff, was determined to be $-9.9‰$. In general low ^{18}O concentrations in precipitation cause lower ^{18}O concentrations in the outflow after mixing of event water with base-flow water. During four rain events the $\delta^{18}O$ values in precipitation were decreased (lighter water) while at the spring an increase of $\delta^{18}O$ values (heavier water) was observed (compare Figures 5 and 6). This effect can only be explained if pre-event water in the system was mobilized by the rain event and observed at the spring. Lee and Krothe (2001,

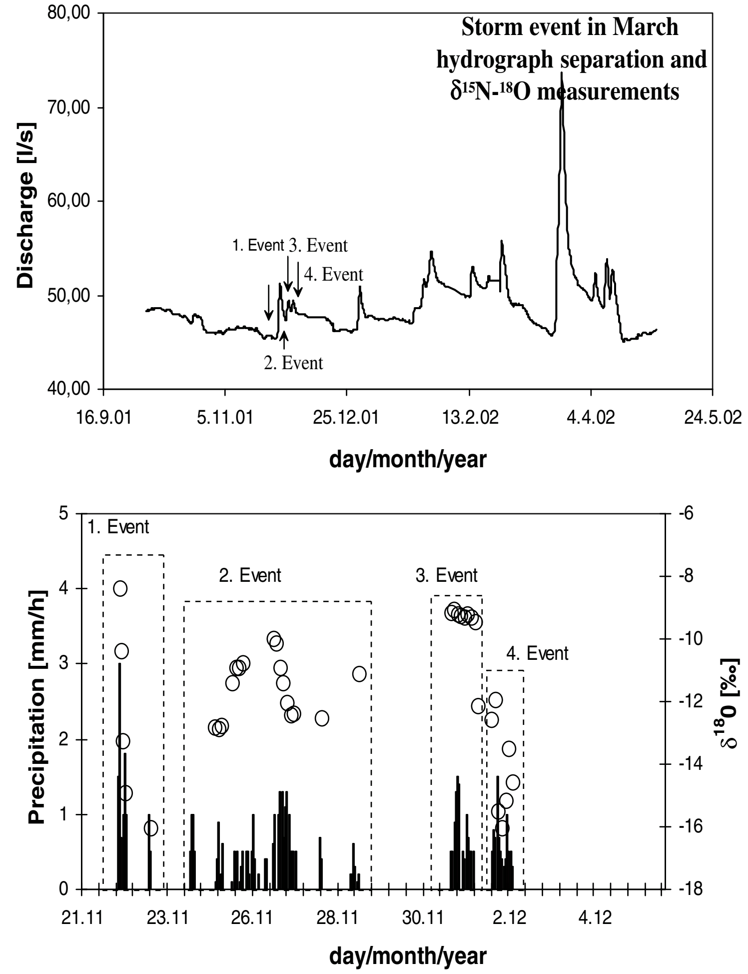

Figure 5. $\delta^{18}O$ value of precipitation and rainfall (black lines) during four events (lower part), long term discharge (upper part) and discharge peaks for interpretation observed in Böhming spring.

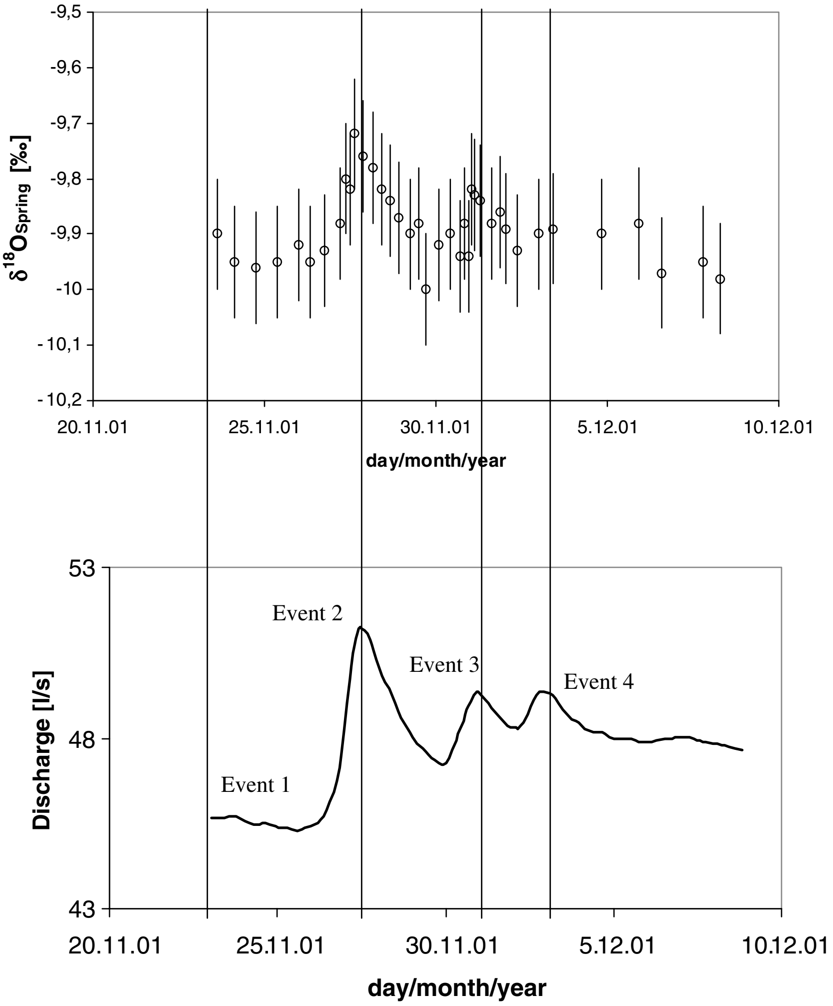

Figure 6. δ^{18}O value in spring water (upper) and discharge of Böhming spring (lower) for four observed events.

2003), Swistock et al. (1989), McDonnell et al. (1991), Bazemore et al. (1994) have already shown in their studies that the mobilized soil water (pre-event water) is an important contributor to the storm-flow during dry periods. During the research period at Böhming spring all observed runoff results have shown the absence of event water at this spring. Probably pre-event water has controlled the storm event in March 2002, although the δ^{18}O values of the precipitation would not clearly show the mixing between pre-event and base-flow water (Figure 7). The δ^{18}O value in precipitation was −9.7, −8.6, −10.5, −9.6‰, which show higher or identical values compared to those of soil or

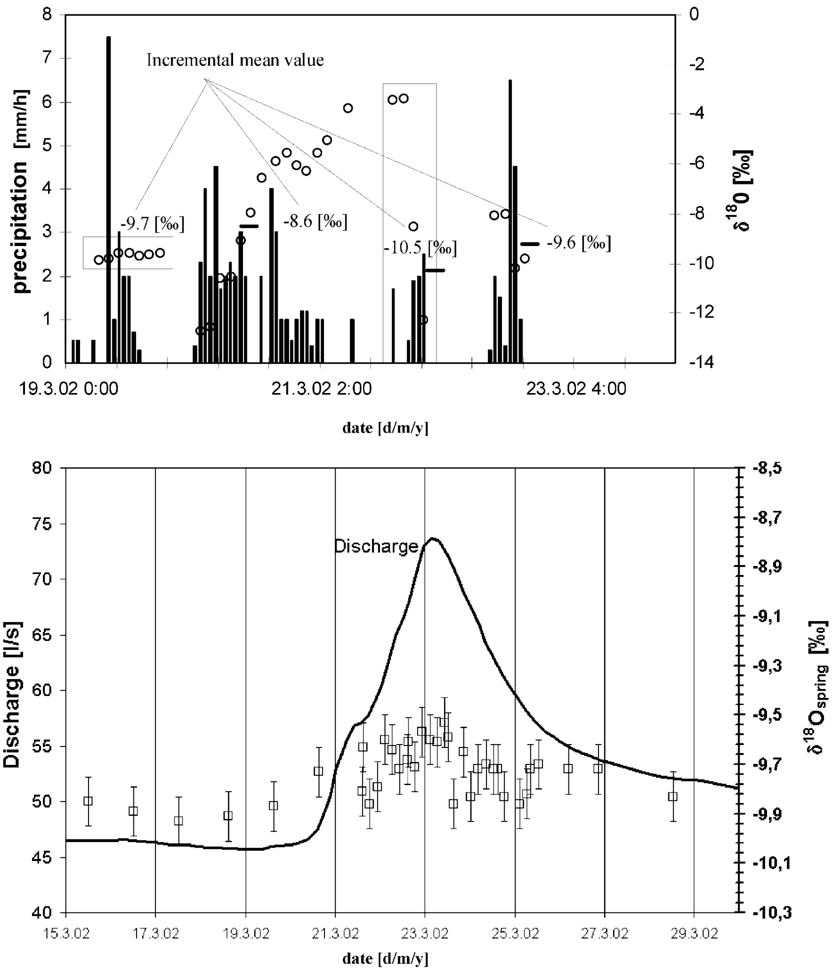

Figure 7. δ^{18}O values of precipitation in March at Böhming spring including incremental mean values obtained by a weighted average (upper) and temporal variation of δ^{18}O in spring water (lower).

base-flow water (−9.9‰). Thus, it was not possible to distinguish between soil zone and event water. However the measurements of sodium and potassium observed during the runoff event support previous findings (Figure 8). The concentrations of sodium, potassium and sulphate in precipitation were very low ($Na^+ < 2$ mg/l, $K^+ < 2$ mg/l, $SO_4^{2-} < 7$ mg/l). Generally, after event water had infiltrated, a significant dilution process of Na^+ and SO_4^{2-} and a higher concentration of K^+ in the base-flow could be observed if the contribution of event water to total flow was up to 30% March 21 2002. Based on water chemistry the water of the storm runoff event in March was stored in the soil

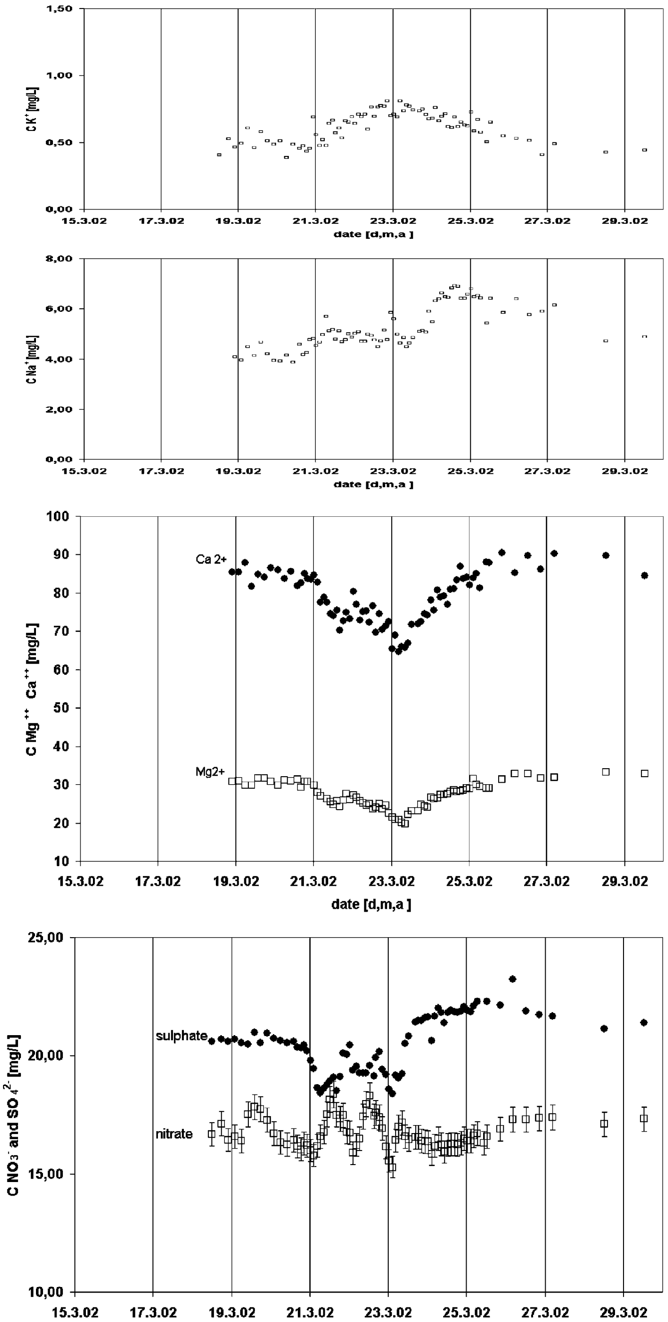

Figure 8. Temporal variability of major ions during the runoff event in March.

zone in spite of the fact that the isotope values did not permit the identification of pre-event water.

A conceptual runoff model was constructed (Figure 2) to interpret the runoff generation processes in the karst system. Runoff in forested slopes with thin soil cover occurred often via rapid flow along the soil bedrock interface (Buttle and Turcotte 1999). Vertical preferential flow paths, characterized by solution channels associated with sinkholes are probably connected with lateral preferential flow paths (interflow) in a thin saturated layer above the bedrock surface. The existence of the lateral surface-near preferential flow paths (interflow) can be documented in this case by the DOC measurement. Generally, colloids like DOC can be easily transported through macropores (preferential flow) (Langdsmand et al. 1999; Sun et al. 2001). During the runoff event high DOC concentrations up to 7 mg/l were observed, which probably resulted from physical heterogeneities (bypass flow) in the system. This is in agreement with earlier findings by Kaiser et al. (2002) who have found that preferential flow increases the loss of DOC from soils at rainstorm events. The rapid inflow of injected fluorescence tracers in two sinkholes of the catchment area and the registration of the tracer curves at the outflow of the spring after some hours (Einsiedl et al. 2002) support the assumption of the existence of vertical preferential flow paths down to the groundwater table in the area under investigation.

Water chemistry, solute tracer results and variations in $\delta^{15}N$ and δ^{18} of NO_3^-

Spring-water nitrate concentrations varied around 17 mg/l. Values for δ^{15}N, sampled during base-flow observed in November and March varied between 3.8 and 4.5‰. The δ^{18}O–NO_3^- values in the spring-water were on average equal to 12.5‰. In March 2003 the runoff systematic differed in chemistry (Figures 8 and 9). Stream discharges (*Q*), strontium and stable isotope δ^{18}O values as well as major concentrations of cations and anions measured were used for determining the pre-event water contribution to total runoff from the area covered by forests and the arable land. Strontium and some anions (Cl^-, Ca^{2+}, Mg^{2+}) have shown a significant dilution during runoff. Although Cl^- is a conservative tracer, the dilution effect was less distinct in comparison to strontium (Figure 9). Highest values in Cl^- concentrations were observed at the beginning and the end of the runoff. These findings suggest leaching of water from agricultural fields which is enriched in Cl^-. Simultaneously dilutions of Cl^- from forest floor take place, which has significantly lower Cl^- concentrations (Glaser 1998). Concentration of Na^+ rose significantly during peak discharge while in comparison only low increase of K^+ is observed. This effect is probably caused by the contribution of K^+ percolated from soils rich in organic content (Uhlenbrook et al. 2002). During the second flood peak DOC concentrations rose to values of about 7 mg/l. Results from soils rich in organic content indicate that it is assumed that 30% of the total runoff

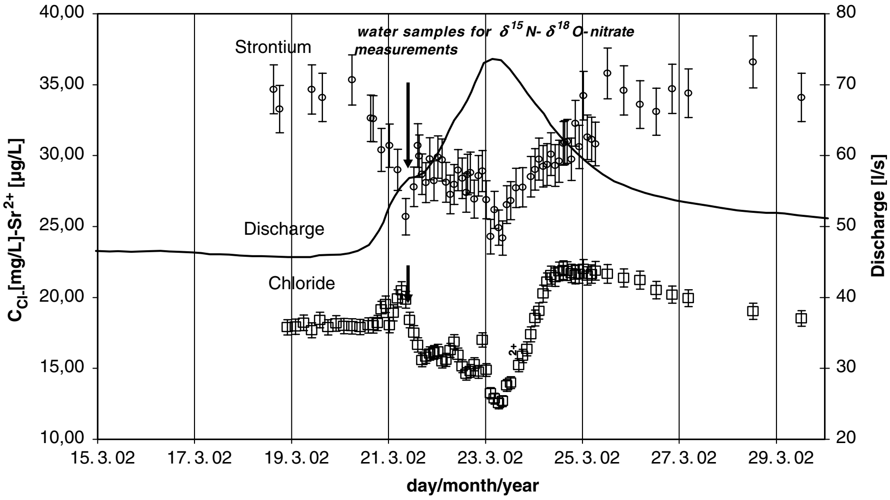

Figure 9. Temporal variability of the discharge, dissolved Sr^{2+} and Cl^- during the rainfall-runoff event.

originates from the pre-event fraction then the DOC concentrations leaching from the soil zone can be estimated to be about 20 mg/l.

The pre-event water can be directly sampled from the soil zone due to the existence of interflow (vertical preferential flow) (see Figure 2). Therefore during the runoff a time slot was selected to monitor the $\delta^{18}O–NO_3^-$ and $\delta^{15}N–NO_3^-$ of the pre-event water at the spring. The results of the calculations using the mixing model with two-components, pre-event- and base-flow waters, allowed the estimation of the theoretical isotope values of the $\delta^{15}N$ and $\delta^{18}O$ for the soil zone.

The contribution of the pre-event water was estimated based on Sr^{2+} data (Figure 9) to be 25% of total runoff during the flood peak (21 March 2003). The Sr^{2+} concentration was measured with an accuracy of ± 5%. Unfortunately, the Sr^{2+} dilution did not correspond to decrease in Cl^-, being also considered as a conservative tracer e.g. Turner et al. (1987). However, it probably results from the leaching of water enriched in Cl^- from agricultural soil into the karst system.

At the beginning and the end of the March-event a strong rise in the Cl^- concentration was observed. These observations strongly suggest that Cl^- rich water from the agricultural ecosystem was detected at the outflow of the spring. The values of 5–6 mg/l which were observed in the neighboring forested catchment areas Größdorf Village and Rieshofen (Figure 1) were taken to estimate the portion of pre-event water stored in the soil covered by forests. The Cl^- concentration originating from the agricultural ecosystem (21.9 mg/l) was sampled as representative concentration during runoff (Figure 9). At that time only the interflow from the agricultural land could be measured, thus no significant dilution effect from the forested area was observed. Additional measurements of soil water samples taken within the agricultural land (ceramic suction cups at 0.6 and 0.95 m under the soil surface) show Cl^- concentrations of 18–22 mg/l, which agrees well with concentrations of 21.9 mg/l estimated before. Using estimated end-member concentrations of Cl^- for the mixing equation, the portion of pre-event water from the agricultural area was calculated to be 15%. Assuming, that the portion of pre-event water is equal to 20–30%, in consequence, the portion of pre-event water from the forested area is between 5 and 15%.

During storm runoff the $\delta^{15}N$ and $\delta^{18}O$ values of NO_3^- contents rose from about 4–8‰ and 12–24‰, respectively. Taking into account that the portion of pre-event water in the runoff is in the range between 30 and 20%, the $\delta^{15}N$ value in pre-event water can be estimated to be between 19 and 26‰, respectively (Figure 4). The isotope value of $\delta^{18}O$ in NO_3^- was between 52 and 70‰. This could be estimated that the portion of pre-event water was equal to 30 and 20%, for both components (forested and agricultural areas), respectively. Limited data indicate that $\delta^{15}N$ values of nitrate in precipitation are near 0‰ or slightly higher (Voerkelius 1990; Coplen et al. 2002). The $\delta^{15}N$ value derived from the forested area was not measured directly, but obtained from calculations that indicated that $\delta^{15}N$ is equal to 1.2 and 1.4‰. These values were found during the base-flow of neighboring forested catchments of Rieshofen and Größdorf. Using this information for the Böhming spring, the $\delta^{15}N$ value equal to 38‰ and more than 100‰ (25% portion of the arable land and calculated $\delta^{15}N$ value in pre-event water of 26‰) resulting from agricultural area could be estimated, assuming 50 and 25% of the arable land surface in the whole catchment, respectively.

Assuming that the portion of the agricultural area is 25%, one can estimate an initial nitrate concentration of some g/l using the fractionation factors ε of $\delta^{15}N$ from Voerkelius (1990) and the $\delta^{15}N = 100‰$ found above. Such high $\delta^{15}N$ value (100‰) is astonishing and was rarely found (Schroeder et al. 1991). Normally, the highest value of $\delta^{15}N$ in soil extracts was reported to be equal to 46‰ (Kreitler 1975). However, the residual nitrate concentration of about 20 mg/l would have to appear during the runoff event. Such a concentration would suggest an unrealistically high denitrification potential of the soil zone (1:100). In addition, based on mass balances of nitrate concentration from springs with fertilizer application, nitrate concentration should be in the range of 150 mg/l if nitrate leaches through the soil without microbial transformation. As a consequence the portion of the agricultural area equal to 25% of whole catchment can be excluded.

Finally, the portion of the agricultural area equal to 50% of the whole catchment area and resulting $\delta^{15}N$ equal to 38‰ were taken into further consideration. Using this $\delta^{15}N$ value for the arable land, the enrichment factors $\varepsilon = -8‰$ of $\delta^{18}O$ in NO_3 and $\delta^{15}N$ in nitrate is equal to $-15.9‰$ (Voerkelius 1990) an initial nitrate concentrations in the soil horizon equal to 170 mg/l can be calculated. Such a value is also in agreement with findings of Hellmeier

(2001), who has calculated similar concentrations of nitrate in similarly used agricultural catchments in Tertiary hills south of the Franconian Alb. A $\delta^{18}O$ in NO_3^- value equal to 64‰ was calculated for the forested area of the catchment, further suggesting that the runoff component in the forested area was mostly stored in the summer months but flushed into the groundwater during winter and fall months. Between August and November the $\delta^{18}O$ in NO_3^- in atmospheric nitrate was equal to 61 and 52.5‰, respectively with the highest concentration of summer precipitation (Voerkelius 1990).

The $\delta^{15}N$ and $\delta^{18}O$ values found above document that the fertilizer and a small part of atmospheric NO_3^- are the primary source of NO_3^-. In this study, the isotope ratios of $\delta^{15}N$ and $\delta^{18}O$ in NO_3^- observed during runoff could not be interpreted in a conventional way considering manure and/or sewage as NO_3^- source. Nitrate originating from manure is characterized by high $\delta^{15}N$ concentration (> 8‰) and significantly lower $\delta^{18}O$ values as compared to nitrate from fertilizers (Figure 4). Nitrate derived from manure or produced from the soil source is characterized by $\delta^{18}O$ values around 0‰ (Voerkelius 1990). It can be easily shown using mixing equations, that nitrate sources other than manure cannot explain the $\delta^{18}O$ content in nitrate. $\delta^{18}O$ values of more than 75‰ in the atmospheric nitrate are needed to explain the $\delta^{18}O$ values observed in the spring during runoff. However, the highest $\delta^{18}O$ values in atmospheric nitrate were found to be about 75‰ (Voerkelius 1990; Durka et al. 1994; Kendall 1998). Figure 4 shows the isotope ratios of nitrate oxygen and nitrate nitrogen modified from Clark and Fritz (1997) and further the calculated results of $\delta^{18}O$–NO_3^- and $\delta^{15}N$–NO_3^- ratios for the area studied.

To determine the residence time of the pre-event water in the flow system, the $\delta^{18}O$ values measured in water can be used. If the mean transit time through the soil zone is larger than 3.5 years, the temporal variations of $\delta^{18}O$ in precipitation are usually smoothed out and a constant mean content is observed in the outflow (Stichler and Herrmann 1983). Based on experiments performed by Pfaff (1987) in the agricultural land in the Franconian Alb a transit time of about 3 years can be estimated, taking into account maximal thickness of the soil zone of 1.5 m, average saturated porosity of 25% and mean groundwater recharge rate of 0.13 m/a.

Discussion and conclusion

Intensive agricultural land use in the Franconian Alb causes extensive nitrate contamination of groundwater. Nitrate concentrations above drinking water standards were observed in the springs indicating a mean transit time of only some years in base-flow and simultaneously the lack of postjurassic soil cover. Isotope analyses indicate that the attenuation of nitrate does not result from simple mixing of different water components (seepage water from the agricultural and forested land and the water from the porous matrix of the unsaturated zone). Based on the calculated $\delta^{15}N$ and $\delta^{18}O$ in NO_3^- in the water of the Böhming spring it was shown that significant remediation by microbial denitrification has to take place (see Figure 4). The existence of a residual nitrate of 20 mg/l (15% of initial 170 mg/l) would suggest a high denitrification potential of the postjurassic soil layer and/or the soil/bedrock interface. Possible variations in the isotope contents of different nitrate sources have documented that fertilizer has to be assumed as main nitrate source in area under investigation. It was shown that $\delta^{18}O$ values NO_3^- of larger than 74‰ in atmospheric NO_{3atm}^- are needed to explain the spring observations. However such a value is outside of possible range observed in terrestrial ecosystems (Voerkelius 1990; Durka et al. 1994; Kendall 1998) using nitrate of manure or nitrate produced from the soil zone as main nitrate source.

One could expect that the uncertainties in the calculations with the mixing model, have yielded an underestimation of the pre-event water contribution and an overestimation of the $\delta^{15}N$ value for the arable land. On one hand, the uncertainties in the calculation of the contribution of pre-event water could be undoubtedly large by interpreting storms with smaller peak discharge and relatively dry antecedent conditions as it was shown by Rice and Hornberger (1998). On the other hand, the hydrograph separations yield the plausible results for large storm conditions and wet conditions (Rice and Hornberger 1998). Such conditions existed only during the storm runoff in March 2003 (87 mm/m^2).

The fertilizers were found as a main nitrate source in the study area. Calculated $\delta^{15}N$ and $\delta^{18}O$ values of the potential NO_3^- sources agreed with the isotope signature ($\delta^{18}O = 22.6‰$ and $\delta^{15}N = 3.1‰$) of the fertilizer used in the catchment area of Böhming spring. These contents are typical for synthetic fertilizers as reported by Clark and Fritz (1997).

The study from Mengis et al. (2001) provided clear evidence that microbial processes (e.g. nitrification) could mask the original isotope ratios of fertilizer nitrate in groundwater because nitrate from both NH_4^+ and NO_3^- in N-fertilizers have a $\delta^{18}O$ in NO_3^- of about 10‰. In comparison $\delta^{18}O$ in NO_3^- originating only from nitrate in NH_4NO_3 has a significantly higher content ($>18‰$). The isotope results found in Böhming spring for the base-flow conditions $\delta^{15}N$ and $\delta^{18}O$ in NO_3 of about 4 and 12‰, respectively, support the hypothesis that N-fertilizer is the key source in this region. In addition it could also been shown that other processes such as denitrification are of relevance in karst systems. The denitrification processes take place above the soil/bedrock interface, probably nitrification in the unsaturated zone of the karst system (Figure 2), which masked the isotope value in $\delta^{18}O$ and $\delta^{15}N$ of the denitrification process.

The tracer methods were used to identify the hydrodynamic processes of the Böhming karst aquifer and to develop a conceptual model of water flow. Both vertical and lateral preferential flow paths in the karst system were found. The extent of pre-event soil water along these bypasses was examined using DOC and fluorescent tracer. The combination of hydrogeological data and stable isotope contents suggest that the denitrification is predominantly above the bedrock and synthetic fertilizer is the primary source of nitrate contamination. The findings of this study further suggest that the interface between bedrock and soil zone (interflow zone) in the vadose zone can play a dominant role for biogeochemical and transport processes. Observed DOC concentration of more than 6 mg/l during runoff generation would support the hypothesis that microbial denitrification could exist in the interflow.

The future of the methodology proposed in the study might be promising if the method is applied in dynamic systems where aerobic and anoxic microbial processes can be postulated for different flowpaths (soil zone, epikarst, unsaturated zone, saturated zone, rock matrix). Concluding, by combining the results of hydrograph separation with measurements of $\delta^{15}N$ and $\delta^{18}O$ values in nitrate for heavy storm runoff event it is possible to demonstrate the significance and the area of biodegradation in the karst aquifer. In that way a first step to quality assurance of drinking water reservoirs in the area under investigation can be attained.

References

Aber J.D., Nadelhoffer K.J., Steudler P. and Melillo J.M. 1989. Nitrogen saturation in northern forest ecosystems. Bioscience 39: 378–386.

Amberger A. and Schmidt H.-L. 1996. Natürliche Isotopengehalte für Nitrat als Indikatoren für dessen Herkunft. Geochim. Cosmochim. Acta 51: 1699–2705.

Aravena R. and Robertson W.D. 1998. Use of multiple isotope tracers to evaluate denitrification in ground water: study of nitrate from a large-flux septic system plume. Ground Water 36: 975–982.

Buyarian water Management Agency 1996. Groundwater Recharge in Bavaria. Heft 5/96, (in German).

Bazemore D.E., Eshleman K.N. and Hollenbeck K.J. 1994. The role of soil water in storm flow generation in a forested headwater catchment: synthesis of natural tracers and hydrometric evidence. J. Hydrol. 162: 47–75.

Biegert T., Fuchs G. and Heider J. 1996. Evidence that anaerobic oxidation of toluene in the denitrifying bacterium Thauera aromatica is initiated by formation of benzylsuccinate from toluene and fumarate. Eur. J. Biochem. 238: 661–668.

Böttcher J., Strebel O., Voerkelius S. and Schmidt H.L. 1990. Using isotope fractionation of nitrate nitrogen and nitrate oxygen for evaluation of denitrification in a sandy aquifer. J. Hydrol. 114: 413–424.

Brown A., McDonnell J.J., Burns D.A. and Kendall C. 1999. The role of event water, a rapid shallow flow component, and catchment size in summer stormflow. J. Hydrol. 217(3–4): 171–190.

Buttle J.M. 1994. Isotope hydrograph separations and rapid delivery of preevent water from drainage basins. Prog. Phys. Geogr. 18: 16–41.

Buttle J.M. and McDonald D.J. 2002. Coupled vertical and lateral preferential flow on a forested slope. Water Resour. Res. 38(5): 10.1029/2001 WR000773.

Buttle J.M. and Turcotte D.S. 1999. Runoff processes on a forested slope on the Canadian Shield. Nordic Hydrol. 30: 1–20.

Clark I. and Fritz P. 1997. Environmental Isotopes in Hydrogeology. Lewis Publishers, Boca Raton, USA, p.151

Coplen T.B., Hopple J.A., Böhlke J.k., Peiser H.S., Rieder S.E., Krouse H.R., Rosman K.J.R., Ding T., Vocke R.D., Revez K.M., Lamberty A., Tayler P. and De Bievre P. 2002. Compilation of Minimum and Maximum Isotope Ratios of Selected Elements in Naturally Occurring Terrestrial materials and Reagents. Water-Resources Investigations Report 01-4222. US Geological Survey, Reston, Virginia.

Durka W., Schulze E.D., Gebauer B. and Voerkelius S. 1994. Effects of forest decline on uptake and leaching of deposited nitrate determined from ^{15}N and ^{18}O measurements. Nature 372: 765–768.

Einsiedl F., Stichler W. and Maloszewski P. 2002. Untersuchung hydrodynamischer Prozesse eines Karstsystems unter Verwendung natürlicher und künstlicher Tracer. In: Herbert D., Merkel B. and Wolkersdorf C.H. (eds), Workshop "Isotope und Tracer in der Wasserforschung". Freiberg, pp. 95–102 (in German).

Glaser St. 1998. Der Grundwasserhaushalt in verschiedenen Faziesbereichen des Malms der Südlichen und Mittleren Frankenalb. GSF-Bericht 2/98, München (in German).

Heaton T.H.E. 1986. Isotope studies of nitrogen pollution in the hydrosphere and atmosphere: a review. Chem. Geol. 59: 87–102.

Hellmeier C. 2001. Stofftransport in der ungesättigten Zone der landwirtschaftlich genutzten Flächen in Scheyern/Oberbayern (Tertiärhügelland). Ph.D. thesis, LMU. GSF-Bericht 5/01, Munich (in German).

Hill A.R., Kemp W.A., Buttle J.M. and Goodyear D. 1999. Nitrogen chemistry of subsurface storm runoff on forested Canadian Shield hillslopes. Water Resour. Res. 35(3): 811–821.

Hinton M.J., Schiff S.L. and English M.C. 1994. Examining the contributions of glacial till water to storm runoff using two- and three component hydrograph seperations. Water Resour. Res. 30: 983–993.

Hötzl H 1999. Der Karst als Trinkwasserressource. In: Seiler K.-P., Grundwasserschutz im Karst der Südlichen Frankenalb, GSF-Bericht 4/99. pp. 99–114 (in German).

Kaiser K., Guggenberg G., Kaupenjohann M. and Zech W. 2002. Refractory organic substances in aggregated forest soils – retention versus translocation. In: Frimmel F.H. Abbt-Braun G. Heumann K.G. Hock B. Lüdemann H.D. and Spiteller M. (eds) Refractory Organic Substances in the Environment.

Kendall C. 1998. Tracing nitrogen sources and cycling in catchments. In: Kendall C. and McDonnell J.J. (eds), Isotope Tracers in Catchment Hydrology. Elsevier, Amsterdam.

Kendall C., Silva S.R., Chang C.C.Y., Burns D.A., Cambell D.H. and Shanley J.B. 1996. Use of the $\delta^{18}O$ and $\delta^{15}N$ of nitrate in earlier spring runoff in forested chatchments. Isotopes in Water Resources Management, International Atomic Energy Agency, Vol. 1. Vienna, pp. 167–176.

Kreitler C.W. 1975. Determining the source of nitrate in groundwater by nitrogen isotope studies. Bureau of Economic Geology, Report of Investigation No. 83. University of Texas, Austin, Texas, Austin, p. 57.

Langdsmand M., Villholth K.G., Ullum M. and Jensen K.H. 1999. Processes of colloid mobilization and transport in macropours soil monoliths. Geoderma 93: 33–59.

Lee E.S. and Krothe N.C. 2001. A four-component mixing model for water in a karst terrain in south-central Indiana, USA. Using solute concentration and stable isotopes as tracers. Chem. Geol. 179: 129–143.

Lee E.S. and Krothe N.C. 2003. Delineating the karstic flow system in the upper Lost River drainage basin, south central Indiana: using sulphate and δ^{34} $S_{S-34(SO_4)}$ as tracers. Appl. Geochem. 18: 145–153.

Maloszewski P., Stichler W., Zuber A. and Rank D. 2002. Identifying the flow systems in a karstic-porous aquifer, the Schneealpe, Austria, by modelling of environmental ^{18}O und ^{3}H isotopes. J. Hydrol. 256: 48–59.

Maloszewski P. and Zuber A. 1985. Mathematical models for interpreting tracer experimets in fissured rocks with a porous matrix. J. Hydrol. 79: 333–358.

Mariotti A., Landreau A. and Simon B. 1988. ^{15}N isotope biogeochemistry and natural denitrification process in groundwater: application to the chalk aquifer of northern France. Geochim. Cosmochim. Acta 52: 1869–1878.

McDonnel J.J., Owens I.F. and Stewart M.K. 1991. A case study of shallow flow paths in a steep zero-order basin. Water Resour. Bull. 27: 679–685.

Meckenstock R.U. 1999. Fermenative toluene degradation in anaerobic defined syntrophic cocultures. FEMS Microbiol. Lett. 177: 67–73.

Mengis M., Walther U., Berasconi S. and Wehrli B. 2001. Limitations of using δ^{18}O for the source identification of nitrate in Agricultural soils. Environ. Sci. Technol. 35: 1840–1844.

Michel U. 1999. Gesteinsphysikalische Eigenschaften und fazielle Ausbildung der oberjurassischen Massenfazies (Kimmeridge) der Südlichen Frankenalb. In: Seiler K.-P. (ed.), Trinkwasserschutz im Karst der Südlichen Frankenalb, GSF-Bericht 4/99, München, pp. 48–57 (in German).

Nattelhofer K.J. and Fry B. 1988. Controls on natural nitrogen-15 and carbon-13 abundances in forest soil organic matter. Soil Sci. Soc. Am. J. 52: 1633–1640.

Panno S.V., Hackley K.C., Hwang H.H. and Kell W.R. 2001. Determination of the sources of nitrate contamination in karst springs using isotopic and chemical data. Chem. Geol. 179: 113–128.

Pfaff T. 1987. Grundwasserumsatzräume im Karst der Frankenalb. Ph.D. thesis, LMU, München, GSF-Bericht 3/87 (in German).

Rau I. 1999. Untersuchungen zur langfristigen Speicherung von persistenten Schadstoffen in heterogenen porösen Medien mit Umweltracermethoden. Ph.D. thesis, LMU, Munich (in German).

Rice K.C. and Hornberger G.M. 1998. Comparison of hydrochemical tracers to estimate source contributions to peak flow in a small, forested headwater catchment. Water Resour. Res. 34: 1755–1766.

Schmidt-Kaler H. 1989. Geologische Karte 1.25000, Erläuterungen zum Blatt Nr. 7136 Neustadt a. d. Donau.-167 S., München, (Geol. Survey) (in German).

Schroeder R.A., Setmire J.G. and Densmore J.N. 1991. Use of stabile isotopes, tritium, soluble salts and redox-sensitive elements to distinguish groundwater from irrigation water in the Salton Sea basin. In: Ritter W.F. (ed.), Irrigation and Drainage. American Society of Civil Engineers, New York, pp. 524–530.

Seiler K.P., Maloszewski P. and Behrens H. 1989. Hydrodynamic dispersion in karstified limestones and dolomites in the Upper Jurassic of the Franconian Alb, F.R.G. J. Hydrol. 108: 235–247.

Stichler W. and Herrmann A. 1983. Application of environmental isotope techniques in water balance studies of small basins. New Approaches in Water Balance Computations IAHS Publication no. 148, pp. 93–112.

Sun N., Elimelech M., Sun Ne-Zheng. and Ryan J. 2001. A novel two-dimensional model for colloid transport in a physically and geochemically heterogeneous porous media. J. Contam. Hydrol. 49: 173–199.

Swistock B.R., DeWalle D.R. and Sharpe W.E. 1989. Sources of acidic storm flow in an Appalachian headwater stream. Water Resour. Res. 25(10): 2139–2147.

Turner J.V., MacPherson D.K. and Stokes R.A. 1987. The mechanisms of chatchment flow processes using natural variations in deuterium and oxygen-18, J. Hydrol. 94: 143–162.

Uhlenbrook S., Frey M., Leibundgut Ch and Maloszewski P. 2002. Hydrograph separations in a meso-scale mountainous basin at event and seasonal time scales. Water Resour. Res. 38(6): 31.1–31.14.

Voerkelius S. 1990 Isotopendiskriminierungen bei der Nitrifikation und Denitrifikation; Grundlagen und Anwendungen der Herkunfts-Zuordnung von Nitrat und Distickstoffmonoxid. Ph.D. thesis, Tech. Univ., Munich, Germany (in German).

Wassenaar L.I. 1995. Evaluation of the origin and fate of nitrate in the Abbotsford Aquifer using the isotopes of ^{15}N and ^{18}O in NO_3^-. Appl. Geochem. 10: 391–405.

Wendland F., Albert H., Bach M. and Schmidt R. 1993. Atlas zum Nitratstrom in der Bundesrepublik Deutschland. Springer, Berlin, Heidelberg (in German).

Williard K.W.J., DeWalle D.R., Edwards P.J. and Sharpe W.E. 2001. ^{18}O isotopic separation of stream nitrate sources in mid-Appalachian forested watersheds. J. Hydrol. 174–188.