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Quantifying in-stream retention of nitrate at catchment scales using a practical mass balance approach

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Abstract As field data on in-stream nitrate retention is scarce at catchment scales, this study aimed at quantifying net retention of nitrate within the entire river network of a fourth-order stream. For this purpose, a practical mass balance approach combined with a Lagrangian sampling scheme was applied and seasonally repeated to estimate daily in-stream net retention of nitrate for a 17.4 km long, agriculturally influenced, segment of the Steinlach River in southwestern Germany. This river segment represents approximately 70 % of the length of the main stem and about 32 % of the streambed area of the entire river network. Sampling days in spring and summer were biogeochemically more active than in autumn and winter. Results obtained for the main stem of Steinlach River were subsequently extrapolated to the stream network in the catchment. It was demonstrated that, for baseflow conditions in spring and summer, in-stream nitrate retention could sum up to a relevant term of the catchment's nitrogen balance if the entire stream network was considered.

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Introduction

River water quality mainly depends on the location and strength of contaminant inputs, the catchment's water yield diluting those contaminants, and attenuation processes within the river network, which have the potential to retard and degrade harmful compounds. In developed countries such as Germany, nitrate (NO_3^-), one of the world's most widespread contaminants, is introduced into aquatic systems mainly by agricultural activity (European Environment Agency 1999; Rothwell et al. 2010; van Grinsven et al. 2012). Elevated NO_3^- levels can lead to eutrophication and excess primary production (Petzoldt and Uhlmann 2006; Delong and Brusven 1991) in surface waters and may even be toxic to organisms (Camargo et al. 2005). Various known turnover pathways may lead to an attenuation of NO_3^- within aquatic systems. The most important pathways are (i) assimilation by plants and microorganisms, and the subsequent incorporation of NO_3^--N into newly formed biomass and (ii) the dissimilatory, microbially induced reduction to gaseous products such as N_2O and N_2, i.e. denitrification. This process is the only one which

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permanently eliminates NO_3^- from the water cycle. Assimilated nitrogen (N) may be re-released and oxidized to NO_3^- after decomposition of biomass (Saunders and Kalff 2001; Smith et al. 2009).

In headwater rivers, cycling of N species is mediated mainly by organisms attached to the surface of the riverbed substrate (Peterson et al. 2001); and these processes have been locally studied in detail (e.g. Triska and Oremland 1981; Claret et al. 1997; Deforet et al. 2008). It is desirable to extrapolate this point information of local transformation rates to an entire river network. Subsequently, this cumulative turnover in the river system may be related to the total loading of N supplied from the catchment. This relationship would indicate to which extent a stream network is capable of ‘digesting’ the input of nutrients and which proportion thereof is—at least temporally—prevented from reaching downstream ecosystems. These questions were sometimes tackled through modelling studies (e.g. Alexander et al. 2000; Wagenschein and Rode 2008) but they may be problematic because sufficient data for model inputs and parameters is typically unavailable for domains exceeding the reach scale (Marzadri et al. 2011). To obtain estimates of in-stream nitrate retention at catchment scales, field measurements of discharges and concentrations with relatively low costs and time requirements may be a useful and practical alternative. To this end, Burns (1998) adopted a mass balance approach to compare nitrate retention between two reaches of a stream in a forested catchment in the state of New York. A similar approach was presented by Battaglin et al. (2001) along the lower Mississippi River. Using Lagrangian sampling, the authors could show the loss of a small proportion of nitrogen within the river channel during spring and summer. A detailed mass balance coupled to a Lagrangian sampling scheme was recently applied to a short urban segment of the Steinlach River in southwestern Germany to study the reactivity of primarily organic micropollutants but also of nitrate in river water (Schwientek et al. 2016). However, all previously mentioned studies only investigated relatively short river reaches. In this present paper, the mass balance approach combined with Lagrangian type of sampling was repeatedly applied during different seasons to a segment of the Steinlach River which includes the most part of the main stem from the headwater region to the lower course of the river. The segment was investigated to quantify in-stream net retention of NO_3^- including its seasonal variability in a stream network draining an agriculturally influenced catchment. Experimental results obtained from the main river together with a field survey of the stream network were used to estimate the importance of net in-stream retention for regulating the export of nitrate from the Steinlach catchment.

Materials and methods

Study catchment

The Steinlach River, a fourth-order stream, is a tributary of the Neckar River, one of the principal tributaries of the Rhine in southwestern Germany (Fig. 1). It has a total length of 25 km whereas the length of its stream network is about 190 km. The Steinlach drains a total catchment area of 140 km^2 with a mean discharge of 1.7 m^3/s. Elevation ranges between 320 and 880 m asl. From a geological viewpoint, the catchment consists of a sequence of Mesozoic formations, starting with a shallow ridge of Triassic sandstones in the northwest. The middle part of the catchment is characterized by a gently sloped topography formed by organic-rich Lower Jurassic claystones. In the southeastern part of the catchment, Middle Jurassic clay- and mudstones constitute some ridges with steep slopes. These represent the transition to the prominent Upper Jurassic escarpment along the southeastern rim of the catchment. The Upper Jurassic limestone is the only karstic formation within the Steinlach catchment. Consequently, with the exception of a few headwater reaches, the whole stream network is developed in non-karstic formations with relatively low hydraulic conductivity. In its middle and lower reaches, the Steinlach runs through shallow alluvial deposits which are locally subject to river bank erosion and, thus, are an important source of bedload material (Osenbrück et al. 2013). The stream bed substrate is dominated by gravel substrata. Along the 17.4 km long river segment investigated in this study (Fig. 1), the slope declines from 2.5 to 1 %. Hydrology is characterized by relatively little baseflow (due to the limited storage capacity of the local geology) and flashy stream flow peaks that often occur during summer as a result of both convective precipitation events and the generation of fast runoff components along the steep hill slopes of the Middle Jurassic formation. Mean air temperature is approximately 8 °C (city of Tübingen) and areal precipitation is 900 mm/year (1980–2009) with a slight maximum during the summer months. Landuse (Fig. 1) is dominated by rain-fed agriculture (49 %). The largest proportion thereof is made up of pastures and mixed orchards while arable land is restricted to the lower parts of the main valleys. Forest represents 39 % of the catchment area and comprises mostly broadleaf trees. The population density is relatively high (approximately 340 inhabitants per km^2). Urban areas constitute 12 % of the catchment and include one city (Mössingen, 20,000 inhabitants) and a number of smaller towns. Wastewater is collected and conveyed to a central wastewater treatment plant near the catchment outlet. The investigated stream segment is unaffected by wastewater with the exception of intense rainfall events when the mixed sewer system may overflow into the stream network. Nitrate inputs to the catchment mostly originate from atmospheric deposition and agriculture, resulting in concentrations in streamwater that ranged between 0.6 and 2.5 mg l^{-1} NO_3^--N during the four sampling campaigns conducted during this study. Nitrate can be assumed to be the dominant dissolved N species in the Steinlach River.

Methods

Overview

Possible methodologies to gain experimental data on processes removing nitrate at stream reach or even network scale are listed by Birgand et al. (2007). The mass balance approach presented here provides an integral measure of the total net retention of nitrate. Furthermore, this approach is feasible at comparably low costs with respect to time, equipment and laboratory analyses. The core of the method is seasonally repeated mass flux balances of NO_3^--N for a defined stream section. In a first step, for all discrete and diffuse inflows and the outflow of this stream section, the NO_3^--N mass fluxes were quantified using the product of the volumetric flow rate of water (L^3/T) and the associated NO_3^--N concentrations (M/L^3). From a balance of the influxes and the outflux, the net retention of NO_3^--N was calculated (M/T). For extrapolation, normalized retention rates per reactive surface area (M/L^2/T) were computed. For this normalization, the reactive surface area of the balanced stream segment was estimated. Since many studies present results on nitrate retention as uptake velocity, for better comparability, we additionally report our results in this form. To this end, the area-normalized retention rates (M/L^2/T) were divided by the ambient concentration of NO_3^--N (M/L^3), resulting in the dimension of a velocity (L/T). In a second step, nitrate retention at catchment scales (M/T) was obtained by extrapolating the experimentally determined normalized retention rates (M/L^2/T) to the total surface area of the entire stream network (L^2). This total surface area was approximated by a field survey of wetted stream widths and a GIS analysis of stream lengths for the various stream orders. Finally, estimates of in-stream retention for the catchment (M/T) were related to the total estimated nitrate loading supplied from the catchment to the river network (M/T), i.e. the observed nitrate exported from the catchment (M/T) plus the calculated in-stream retention (M/T).

Mass balance approach

For our mass balance approach, we assumed that a stream segment of arbitrary length can be balanced using discrete and diffuse inflows, one outflow at the downstream end of the segment and potential internal turnover processes. Further, it is assumed that under baseflow conditions, diffuse inflows are restricted to groundwater inflows along the river bed. Losses to groundwater were unlikely for the Steinlach River since effluent conditions are typically observed all year round in this bedrock environment with humid climate.

For NO_3^--N, the turnover mass flux (m_{turnover}) comprises all processes that remove nitrate at least temporarily from the water column, which can be calculated as

$$m_{\mathrm{turnover}} = \sum_{i=1}^{n} m_{\mathrm{in},i}^{\mathrm{disc}} + \sum_{j=1}^{m} m_{\mathrm{in},j}^{\mathrm{diff}} - m_{\mathrm{out}}, \tag{1}$$

where $m_{\mathrm{in}}^{\mathrm{disc}}$ are n discrete influxes (M/T) into the river segment, $m_{\mathrm{in}}^{\mathrm{diff}}$ represent n diffuse influxes from groundwater (M/T) and m_{out} is the outflux at the downstream end of the river segment (M/T). Note that, if the stream segment is a gaining one and does not lose water to the aquifer system, the turnover mass flow m_{turnover} will be equal to zero for conservative constituents. Potentially existing internal sources, e.g. internal production of NO_3^- by oxidation of NH_4^+, NO_2^- or particulate organic N, are also comprised in m_{turnover} and thus this term may also become negative. Neither NH_4^+ nor NO_2^- were detected (detection limits 0.15 and 0.06 mg N/l, respectively) in streamwater of the Steinlach River during all sampling campaigns. The role of particulate forms of N

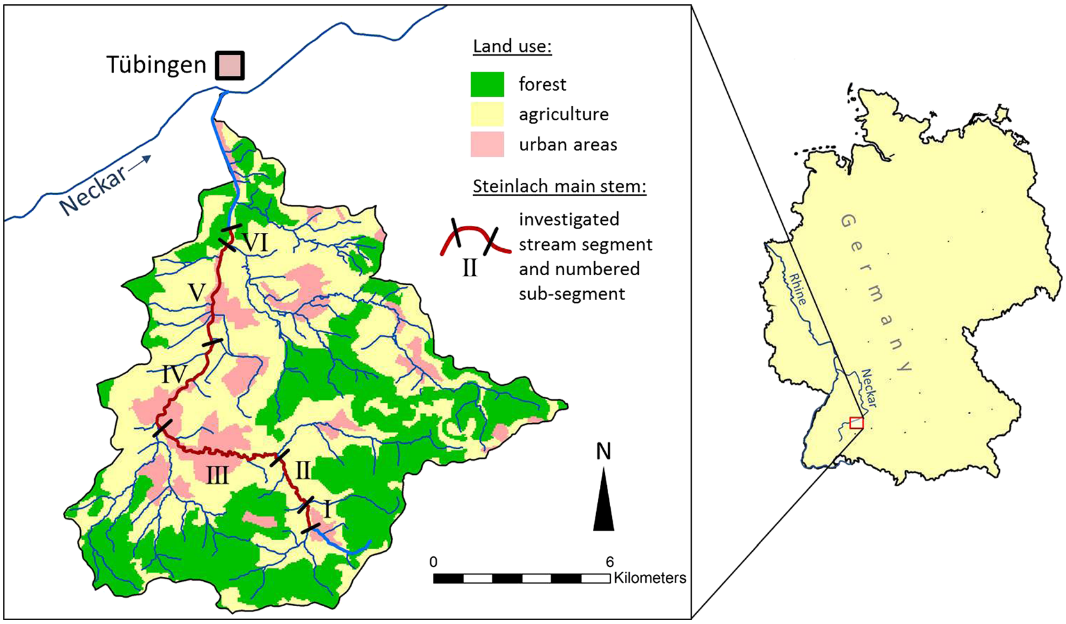

Fig. 1 The Steinlach catchment in Southwest Germany with landuse and the studied section of the main stem. The six different sub-sections are indicated

was not assessed in this study. Dissolved organic carbon concentrations ranged between 1.5 and 2.5 mg/l along the Steinlach main stem. The well-known Redfield ratio (e.g. Tamelander et al. 2013) may be used to estimate concentrations of dissolved organic N (DON) which, accordingly, could be up to 0.44 mg N/l. Brookshire et al. (2005) demonstrated that DON may play an active role in N cycling in forest streams. However, compared to forest streams, dissolved organic matter in the Steinlach River is low and likely dominated by refractory compounds (Aitkenhead et al. 1999), so net transformations of dissolved organic N may be negligible.

Delineation of stream sub-segments

The 17.4 km long study segment of the Steinlach River was divided into sub-segments such that each sub-segment started with the confluence of a major tributary and ended immediately upstream of a subsequent major confluence. The investigated stream segment was subdivided to obtain a better quantification of diffuse inflows for each of the sub-segments and also to continuously check the consistency of our discharge measurements. A total of six sub-segments were delineated (Figs. 1 and 2). The delineation was based on longitudinal profiles of discharge (Q), water temperature (T), and electrical conductivity (EC) measured on 2 March 2011. The corresponding discontinuities and steps for the EC, T and Q profiles (Fig. 2) clearly show where the relevant inflows were. Moreover, it became apparent that at least 90 % of the total stream flow of the Steinlach River was supplied by a relatively small number of discrete inflows or tributaries.

An exception is evident in sub-segment III with a relatively large quasi-discrete inflow of water and solute within the sub-segment. Around river kilometre 10, a sequence of springs on the left bank discharges into the river, the largest one being located at kilometre 10.2. The springs emerge from the outcropping Lower Jurassic formation and resemble each other in terms of their hydrochemistry. Therefore, they were treated as a homogeneous tributary.

Sampling and measuring campaigns

Four campaigns were conducted to capture the seasonal conditions in spring (16 March 2011), summer (24 August 2011), autumn (10 November 2011), and winter (28 February 2012). The sampling days may be characterized hydrologically by the probabilities of flow exceedance during the entire sampling period (16 March 2011–28 February 2012). Those probabilities were 72 % (March), 49 % (August), 97 % (November), and 30 % (February). An overview of the whole period is

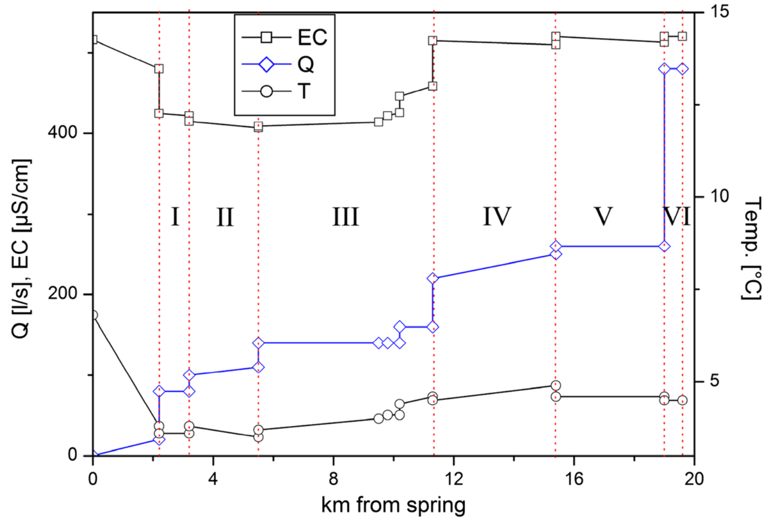

Fig. 2 Profiles of electrical conductivity (*EC*), discharge (*Q*), and water temperature (*T*) along the Steinlach River, measured on 2 March 2011, and delineation of six sub-segments

given in Fig. 3, further information on the hydrological conditions during sampling throughout the catchment are given below in section "Discharges and nitrate concentrations" .

During sampling, no fast runoff generation processes were active and presumably all discharge was supplied by slow components, i.e. the hydrological system could be assumed to be in steady-state in terms of both river discharge and nitrate concentrations. This was observed over the whole day near to the catchment outlet by water level data from the gauging station in Tübingen and an automated sampler (Maxx TP4 C) which took water samples every 2 h. Sampling and measurements were performed in a Lagrangian manner, starting at the upstream end of the uppermost sub-segment and then proceeding downstream, following the flowing water. Each campaign took between 10 and 14 h which is similar to the travel time of a parcel of water moving downstream along the sampled stream segment as could be roughly estimated from numerous measurements of flow velocity and cross-sectional areas, which were measured to infer the discharges along the river profile. A precise determination of travel velocities, e.g. by tracer injections as demonstrated by Brown et al. (2009) or Writer et al. (2013), was not deemed useful, as it is impractical at various locations along an extended river segment. The basic assumption of the presented method is that flow rates and concentrations do not change abruptly during the sampling day. The problem of relatively small, continuous changes can be addressed by the Langrangian sampling scheme. This procedure minimized possible transient effects as natural systems are rarely in a real steady state. Water samples were taken from the main stem upstream of a confluence, from the tributary, and from the main stem downstream of a confluence at a distance which allowed sufficient

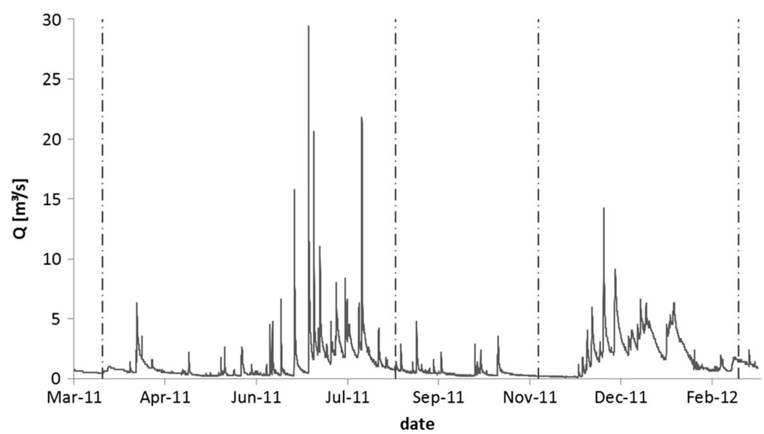

Fig. 3 Hydrograph during the sampling period measured at the gauging station in Tübingen at the outlet of the Steinlach catchment. *Dashed vertical lines* mark the sampling campaigns

mixing of the two flows. At each of these sampling points, T and EC were measured using a Cond 340i handheld probe (WTW, Weilheim, Germany) and discharge was carefully determined for at least two of the three sampling points using a flow metre (Ott C2, Kempten, Germany). The discharge measurements were checked using mixing calculations based on concentrations of relatively conservative ions. Standard deviations for the estimated Q from the different methods were mostly <3 %. The mass fluxes added by each of the recorded tributaries correspond to the term m_{in}^{disc} in Eq. (1). Additional water samples were taken at springs and perennial streams that contributed water to the respective sub-segment but were too small for a reliable quantification of discharge. Discharges of all unmeasured inflows along each sub-segment, corresponding to the term m_{in}^{diff} in Eq. (1), were estimated by balancing all measured discharges. In order to assign a nitrate concentration to these inflows, the average NO_3^- concentration of the small streams and springs sampled along each sub-segment were considered as its best possible representation as those are fed by diffuse inflows and groundwater and integrate over their entire catchments. During the sampling in November and February, not all sub-segments were measured as the length of daylight was too short. In November, the measurements covered sub-segments II, III, and VI. The input into sub-segments IV and V were estimated by balancing the measured flow rates at the input of sub-segment IV and the outlet of sub-segment V. As the approximate nitrate concentration in the inflowing water, the flow-weighted mean concentration of the respective sub-segments determined during the previous August campaign was used. In February, sub-segments II, III, the combined IV-V, and VI were measured.

All water samples were immediately transferred to the lab, 0.45 μm-filtered, and stored at 4 °C. For all water samples, major ion concentrations were measured. More specifically, measurements of NO_3^--N were accomplished by ion chromatography (Dionex D 120, equipped with a Dionex AS23 anion column) with a detection limit of 0.025 mg N/l.

Morphological features of stream channels

To facilitate comparison of turnover rates for different streams and reaches of various length and width, normalization by the reactive surface is a common practice (Kaushik and Robinson 1976; Hill 1979; Smith et al. 2009). The reactive surface is basically represented by the stream bed substrate and the plants on the stream bottom (Vincent and Downes 1980; Triska and Oremland 1981). A direct measurement of this reactive surface is difficult in the field. As a proxy, the wetted surface area was used as the projection of the reactive surface. A survey of the wetted widths of the main stem was conducted on 13 October 2011. Measurements were performed at 28 randomly selected locations along the 17.4 km study segment of the Steinlach River. The reactive surface area was estimated for each of the sub-segments as the product of the median measured wetted widths and the length of the sub-segment (obtained by GIS analysis). An additional survey was performed on 6 October 2011 throughout the catchment covering streams of several stream orders. During both surveys, discharge was relatively low (about 0.4 m^3/s at the outlet), which was similar to the conditions during the spring, summer and autumn sampling campaigns. In total, wetted widths at 51 locations were measured in order to derive a relation between stream order and the median wetted width. The measurements were used to estimate the total wetted surface area of the catchment's stream network and, subsequently, to extrapolate the net retention measured in the main stem to the entire stream network. We would like to mention that the number of measurements taken here, were a compromise between effort and benefit, and the authors are confident that the measurements represent the average stream width and its variability sufficiently well.

Results and discussion

Discharges and nitrate concentrations

During the four sampling campaigns, different discharge conditions were encountered with respect to quantity and hydrochemistry. Discharge was highest in winter (1.315 m^3/s at the outlet of sub-segment VI) followed by summer (0.533 m^3/s), spring (0.380 m^3/s) and autumn (0.160 m^3/s). The measured longitudinal profiles of discharge for the various sampling campaigns are shown in Fig. 4.

Different hydrological situations resulted in different NO_3^--N concentrations along the Steinlach River. Concentrations were positively correlated with Q and were highest throughout the profile in winter, followed by summer, spring and autumn (Fig. 5). An

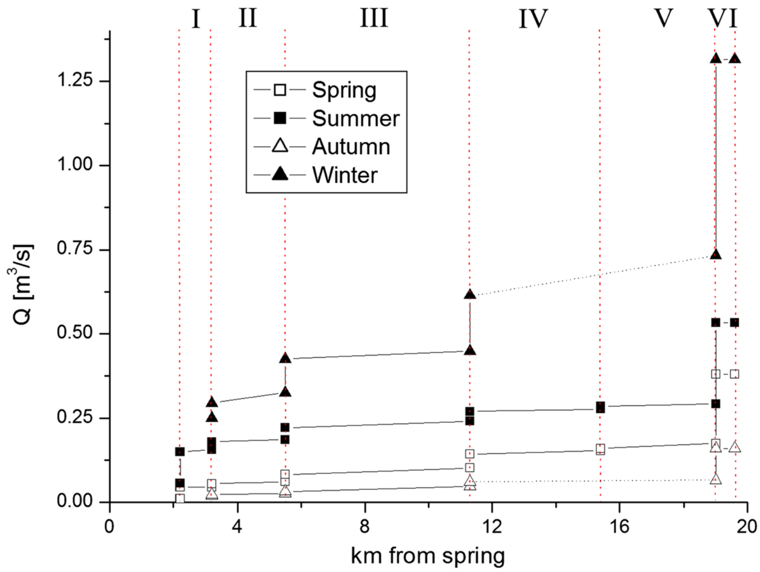

Fig. 4 Profiles of discharge (*Q*) along the Steinlach River for all four sampling campaigns

increase of NO_3^- concentrations with increasing discharges may be due to higher groundwater levels and, thus, mobilization of N from zones closer to the surface. During low stream flow, typically deeper and older groundwater is discharged which is potentially depleted in NO_3^-(Pauwels et al. 2000).

Net retention of nitrate for the investigated stream segment

Table 1 summarizes the total input, the output, and the corresponding net retention of NO_3^--N for the investigated river segment for each sampling campaign. The total input comprised all quantified discrete and diffuse inputs from the catchment that were determined as described above (section "Sampling and measuring campaigns"). The output is the measured NO_3^--N mass flux at the catchment outlet. The total input should match the output in the case of no net retention of NO_3^-.

Net retention was positive in spring, summer, and autumn and slightly negative in winter. Note that the net retentions for autumn and winter do not comprise sub-segment I. Furthermore, in autumn, the nitrate inputs for sub-segments IV and V were not measured, but estimated as described in section "Sampling and measuring campaigns". Since the concentrations determined in August were used for this estimate and concentrations were generally lower in November than in August, the net retention may be slightly overestimated. However, the flow entering along sub-sections IV and V was low, and therefore, this overestimation effect is expected to be small. The net retention flux was highest in spring and summer with a maximum in summer. A smaller yet significant retention was observed in autumn and even a net release of NO_3^--N was quantified in winter. It has to be noted, however, that the uncertainty in winter is large and hence the magnitude of retention cannot be reliably quantified. A seasonal pattern with positive net retention from spring through autumn has been reported previously in other studies (e.g. Kaushik and Robinson (1976)). The relative proportions of assimilative N-uptake by plants and microorganisms, as well as denitrification, cannot be quantified based on our measurements. However, an observed relationship between net retention and the annual cycles of both temperature and radiation appears reasonable. The latter promotes assimilation in spring when radiation is not yet blocked by the closed canopy of trees along the water courses (Mulholland 2004). The highest water temperatures occur in summer enhancing all biochemical activity; turnover processes are in turn hampered by the low temperatures in winter (Hill 1979). Still, a slight net release of NO_3^--N, potentially deriving from mineralization of organic matter, may be indicated by the data.

As detailed before, the net retention of NO_3^--N was normalized by the respective wetted surface area. The lengths and median wetted widths of each individual sub-segment are given in Table 2. The sum of the resulting wetted surface areas was used for the normalization of net retentions. To quantify uncertainty bounds

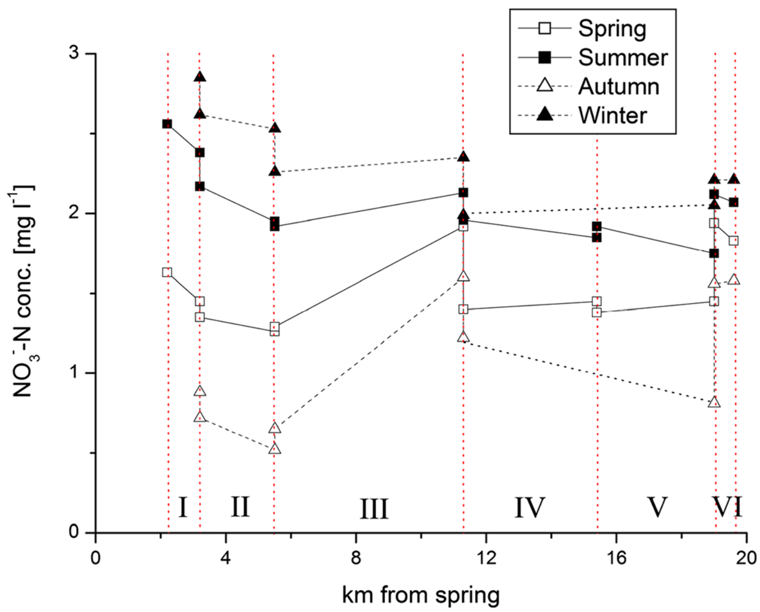

Fig. 5 Profiles of NO_3^--N concentrations along the Steinlach River for all four sampling campaigns

of normalized net retentions, we accounted for both the uncertainty in net retentions and the wetted widths (Table 2). In Table 1, the estimated rates are presented as normalized loadings per hour for better comparability with other published studies. The interquartile range of retention rates per area from reach scale measurements of more than 20 agricultural streams across the USA (generally performed during spring or summer) were 1 to 10 mg N/m^2/h (Mulholland et al. 2008); and our spring and summer estimates are within this range. When our estimated rates per area of streambed were again normalized by the average ambient nitrate concentrations during the various sampling campaigns, we obtained uptake velocities of 0.0018 ± 0.0011 m/h (spring), 0.0029 ± 0.0012 m/h (summer), 0.0016 ± 0.0006 m/h (autumn) and −0.0006 ± 0.0031 m/h (winter). The uptake velocity can be understood as an average downward velocity at which nitrate is removed from water column. The expected range from the literature was 0.003 to 0.01 m/h. This range is based on a review paper by Birgand et al. (2007) who compiled results more than 40 reach scale studies of agricultural streams worldwide. With our spring, summer, and autumn estimates, we are close enough to this published range.

Accuracy and precision of the calculated balances

The accuracy of the calculated results was determined by an uncertainty analysis using a Monte Carlo approach that was carried out by applying the following steps. For every measurement that was obtained either in the field or in the lab, an uncertainty was assigned. Subsequently, the calculations of total NO_3^--N input,

Table 1 Total input, output, net retention (total inputs minus output), and area-normalized net retention (for explanation see text) of NO_3^--N for the seasonal sampling campaigns. The given uncertainty ranges denote standard deviations determined as detailed in section 'Accuracy and precision of the calculated balances' . Note that the measurements for autumn and winter only comprise sub-sections II–VI

Season	Total input [mg/s]	Output [mg/s]	Net retention [mg/s]	Normalized net retention [mg/m^2/h]
Spring	760 ± 24	695 ± 31	64 ± 39	2.8 ± 1.7
Summer	1245 ± 32	1106 ± 50	139 ± 60	6.0 ± 2.5
Autumn	295 ± 11	253 ± 11	42 ± 16	1.8 ± 0.7
Winter	2873 ± 102	2911 ± 129	−38 ± 162	−1.4 ± 7.1

Table 2 Sub-segments of the Steinlach River study segment with relevant morphometric features. Standard errors of average wetted widths per sub-segment were obtained by bootstrapping the measured data

Sub-segment	Length [m]	Median wetted width [m]	Watertable surface area [m^2]
I	977	2.5 ± 0.22	2443 ± 214
II	2341	2.4 ± 0.32	5618 ± 760
III	5844	4.2 ± 0.77	24,545 ± 4488
IV	4081	5.05 ± 0.83	20,609 ± 3382
V	3580	7.0 ± 0.67	25,060 ± 2403
VI	595	7.85 ± 0.56	4671 ± 336
Total	17,418		82,946 ± 6185

output, and retention were repeated with the measurements randomly varying within the uncertainty margins. For input and output fluxes, it was assumed that the probabilities within the uncertainty margins were uniformly distributed to allow for both random and systematic errors that may result from imprecise and inaccurate field or lab data. Uncertainty margins of ±5 % were set for every measurement of discharge and concentration in the Steinlach main stem and major tributaries. Regarding the unmeasured diffuse inflows, ±20 % uncertainty were assigned to the balanced flow rates and ±50 % for the concentrations determined as average of all small streams and springs along the respective sub-segments. ±20 % uncertainty were assigned to the concentrations of diffuse inflows along sub-sections IV and V and ±10 % to the flow rate and concentration of diffuse inflows into sub-segment III since the latter, mainly stemming from a series of springs, could be determined fairly well by means of a mixing calculation. For the calculation of the area-normalized retention, also the uncertainty of the wetted surface area, obtained from bootstrapping the measured wetted widths, was included in the Monte Carlo runs. The uncertainties of the uptake velocities were similarly quantified using a normally distributed error with a standard deviation of ±5 % for the mean NO_3^--N concentration along the whole studied river segment.

In addition to the Monte Carlo analysis, a bias check was conducted by calculating respective mass balances for chloride (Cl^-) and potassium (K^+).

Cl^- and K^+ can be assumed to behave conservatively within the studied system as they are not taken up by organisms in relevant amounts and show only minor interaction with rocks or organic matter (e.g. Burns 1998; Haggerty et al. 2009). The balance results for each sampling campaign are summarized in Table 3. For Cl^- and K^+, all balances were negative. The underestimation of these ions' inputs unlikely resulted from a release of water stored within the sub-segments due to transient discharge conditions. This is because all campaigns were conducted during flat parts of hydrograph recession curves (Fig. 3) and transient effects were further minimized by the Lagrangian sampling scheme. Therefore, it is believed that a more likely source of this biased uncertainty for the conducted mass balance approach are inflows that were not accounted for. Along sub-segment III, the Steinlach passes through the urban area of Mössingen with 20,000 inhabitants. Leakages of sewer systems are a well-known and regularly observed fact in urban areas and are capable of adding locally restricted inputs of seepage with elevated concentrations of organic and inorganic nitrogen species as well as Cl^- and K^+ into receiving waters. The apparent additional input of K^+ is maximum in summer. This may be related to low groundwater levels which allow for larger leakage fluxes, while during high groundwater levels, the flow may be reduced or even inverted and groundwater enters the sewage system. In contrast, additional inputs of Cl^- seem to peak during winter time. This may result from the use of chloride salts to roads which is common in winter in the study area. Potentially, sewer leakages can occur also in smaller towns along the Steinlach main stem. They will always be difficult to localize as, due to high concentrations, noticeable loads of solutes might be added to the river in spite of very small flow rates.

In summary, an underestimation of nitrate retention is suggested by the negative balances of Cl^- and K^+. However, one may argue that in catchments with relatively large proportions of both urban and agricultural areas typically a larger percentage of Cl^- and K^+ than nitrate is added to rivers via wastewater and, thus, the bias of the nitrate retention is likely smaller. In any case, this effect remains difficult to quantify exactly.

Table 3 Total input, output, and net retention (total input minus output) of (a) chloride (Cl^-) and (b) potassium (K^+) for the seasonal sampling campaigns. Note that the numbers for autumn and winter only comprise sub-sections II–VI

(a)			
Season	Total Cl^- input [mg/s]	Cl^- output [mg/s]	Net Cl^- retention [mg/s]
Spring	8981	9132	−151
Summer	11,381	12,161	−781
Autumn	4250	5011	−761
Winter	31,708	35,751	−4042
(b)			
Season	Total K^+ input [mg/s]	K^+ output [mg/s]	Net K^+ retention [mg/s]
Spring	673	706	−32
Summer	978	1227	−248
Autumn	419	526	−107
Winter	2074	2239	−165

Net retention of nitrate for the entire stream network

For an estimation of the nitrate net retention within the unmeasured part of the stream network, the total reactive area thereof was required. The reactive area could be estimated using the results of the stream order-dependent survey of wetted widths that are compiled in Table 4 and the wetted surface areas presented in Table 2. Accordingly, the wetted area of the investigated segment of the Steinlach main stem had a total area of 82,946 ± 6182 m^2 whereas the remaining area of the entire stream network was 172,681 ± 16,290 m^2. Note that Table 4 also gives the total lengths of the different stream orders as derived from the GIS analysis, as well as the approximate wetted areas for all stream orders, calculated as the product of median widths and total lengths. First-order streams contributed by far the most length to the stream network. The largest wetted area in the catchment, however, was provided by fourth-order streams.

To estimate a sensible range of NO_3^- retention rates across the entire catchment, the net retention rates for the investigated stream segment from the spring and summer campaigns (Table 1) were used. Normalized retention rates were 2.8 ± 1.7 and 6.0 ± 2.5 mg N/m^2/h in spring and summer, respectively. This yielded retention rates for the entire stream network—exclusive of the investigated stream segment—of 469 ± 293 g N/h (spring) and 1024 ± 446 g N/h (summer), whereas retentions for the investigated stream segment were 231 ± 140 g N/h (spring) and 503 ± 206 g N/h (summer). Nitrate exports measured at the catchment outlet were 2502 and 3982 g N/h in spring and summer, respectively. In spring, we calculated net retentions of 6.8 ± 3.7 and 20.4 ± 11.2 % of the total estimated nitrate inputs to the river system for the investigated stream segment and the entire stream network, respectively. For the sampling campaign in summer, we obtained a net retention of 8.8 ± 2.9 % (investigated stream segment) and 26.7 ± 8.8 % (entire stream network) of all nitrate inputs. It is therefore plausible that, under favourable conditions such as during summer 2011, 27 % of the catchment loading of nitrate (or more, taking into account the uncertainty) is at least temporarily retained within the stream network.

Table 4 Results of a survey of wetted widths for the various stream orders throughout the whole catchment. Standard errors of average wetted widths per sub-segment were obtained by bootstrapping the measured data. Total length denotes the cumulative length of all segments of the respective stream order for the entire catchment

Stream order	Length [m]	Median wetted width [m]	Watertable surface area [m^2]
1	107,789	0.40 ± 0.05	43,116 ± 5757
2	34,357	0.80 ± 0.16	27,486 ± 5336
3	32,300	2.80 ± 0.30	90,440 ± 9781
4	13,322	7.10 ± 0.63	94,586 ± 8404
Total	187,768		255,627 ± 15,120

Note that the total net nitrate retention increased by a factor of 3 if the whole stream network instead of only the main stem was considered. Our estimates may be too conservative as large parts of the stream network are formed by low-order reaches, which are probably best represented by retention rates of the upper sub-segments; and their retention rates could be higher than the estimated rates for the entire stream segment. In the upper sub-segments, hyporheic exchange is likely facilitated by relatively steep slopes and high hydraulic conductivities of the streambed. Based on their literature review, Birgand et al. (2007) estimated that in-stream processes may—at annual catchment scales—retain as much as 10 to 70 % of the total N load to the drainage network. With our instantaneous estimates, we are within this range but we infer that annual in-stream retentions for the Steinlach catchment are likely <27 % because our relative retentions were obtained for single days with low streamflow and during periods of high biotic activity. Nitrate exports from the catchment will disproportionately occur during the winter season when higher discharges and nitrate loads are common and nitrate retention could be relatively small (Table 1). However, receiving ecosystems are probably most sensitive to nitrate inputs in summer and hence nitrate retentions in winter may not be as relevant as in summer.

Concluding remarks

Our study provided a quantification of in-stream processes leading to a net retention of NO_3^- within the stream network of the Steinlach catchment in southwestern Germany. It was estimated that, under baseflow conditions and during the period of vegetation, net retentions of nitrate could be about 27 % of the total nitrate inputs to the stream network. Measured data for these processes are still rare, particularly at scales exceeding the reach scale. Our results highlight the importance of the spatial scale when assessing the magnitude and relevance of in-stream NO_3^- retention. Relatively small retentions observed at reach scales will become relevant if the entire stream network is considered. As a first estimate, the ratio between reach scale and catchment scale retention may be approximated by the ratio between the surface area of the investigated reach and the surface area of the entire stream network. The respective areas can be estimated using a GIS analysis.

The applied mass balance approach was useful to quantify the approximate magnitude of in-stream retention processes for the Steinlach catchment, including its general temporal pattern, although it only provided limited information on the specific processes involved. From a practical point of view, the methods appeared to be feasible for catchment scale studies due to relatively low cost and time requirements. If such type of analysis is conducted for different catchments, these measurements will help to identify key variables that govern the variability of retention processes across a multitude of catchments.

The uncertainties of the applied approach due to measurement errors were quantified using a Monte Carlo approach. However, also a potential bias was identified which could most likely be attributed to unaccounted inflows from urban areas, probably from leakages of the sewer system. Locations of such inputs could possibly be identified if longitudinal profiles of electrical conductivity are recorded at very high spatial resolution in order to detect any change in solute concentration. In this study, the resulting uncertainties likely led to an underestimation of the overall nitrate retention.

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