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Full title:

**Exploring the role of hydraulic conductivity on the contribution of the hyporheic zone
to in-stream nitrogen uptake**

Short title: **The role of hydraulic conductivity on stream nitrogen uptake**

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Abstract

Nitrogen uptake (N-uptake) within the hyporheic zone provides key ecological services, such as nutrient removal, of stream ecosystems. We hypothesize that the hydraulic conductivity (Kf) of the hyporheic sediments governs nutrient uptake rates through effects on the: i) surface and subsurface flow (i.e. hyporheic flow); and ii) hyporheic N-uptake. Here, we worked at two hierarchical spatial scales (reach- and hyporheic-scale) to disentangle the role of Kf on N-uptake. At the reach scale, we performed co-injected N-NH₄⁺ and Cl⁻ additions in six reaches with contrasting reach Kf (10⁻¹ – 10⁻⁵ m/s) and simultaneously determined a) in-stream N-uptake (hyporheic+benthic N-uptake), and b) hyporheic flow, N-uptake, and c) microbial abundance at the hyporheic scale. Results suggest that Kf determines the contribution of the hyporheic zone to hydrological exchange, but that its role varies between scales to determine in-stream N-uptake. At the reach scale, Kf variability seems to determine the extent at which the hyporheic zone contribute to hyporheic flow; and thus, to N-uptake velocity. At the hyporheic scale, Kf seems to indirectly determine hyporheic N-uptake through the proportion of surface water that enters the hyporheic zone (i.e. relative connectivity) and the abundance of the microbial community. These results suggest an interplay between Kf at both scales and the spatial heterogeneity, which will ultimately drive in-stream N-uptake at reach-scale. In this sense, we found that Kf can be considered as a unifying variable for stream biogeochemical processes and as an important variable to derive the contribution of hyporheic zone to in-stream nutrient removal capacity.

Keywords: clogging, siltation, stream biogeochemistry, ammonia oxidizers, AOA, AOB

1. Introduction

Self-purification capacity of streams strongly relies on the uptake and immobilization of dissolved nutrients by in-stream biota (Newbold, 1996; von Schiller et al., 2017). Facing the increase in dissolved inorganic nitrogen (DIN; mostly available in the form of ammonium (NH_4^+) and nitrate (NO_3^-) (Ribot, von Schiller, & Martí, 2017)) inputs to streams caused by human activities in the drainage areas (Lammers & Bledsoe, 2017), self-purification capacity is an important ecosystem service reducing DIN loads during downstream transport (Loomis, Kent, Strange, Fausch, & Covich, 2000). Within the in-stream biota, microorganisms are responsible for most of DIN uptake (Saunders & Kalff, 2001). Microbial N-uptake is driven by three main biogeochemical pathways: (1) nitrification, the aerobic oxidation of NH_4^+ to NO_3^- to gain energy, (2) denitrification, the anaerobic reduction of NO_3^- to gaseous forms (N_2O and N_2) during oxidation of organic matter (OM), and (3) assimilation, the transitory incorporation of DIN into biomass (Sigee, 2005). While most microorganisms are able to assimilate DIN, which accounts for a large portion of DIN removal (Peterson et al., 2001; Ribot et al., 2017), nitrification and denitrification are restricted to two microbial functional groups active under distinct redox conditions: ammonia oxidizing bacteria and archaea under aerobic conditions, and denitrifying bacteria under anaerobic conditions (Sigee, 2005). In streams, the most biogeochemically dynamic compartment is the hyporheic zone, which is the saturated sediment area below and adjacent to stream channels where surface water and groundwater mix (White, 1993). The hyporheic zone hosts a great portion of the stream microbial community that can carry up to 97% of in-stream N-uptake (Battin, Kaplan, Newbold, & Hansen, 2003; Böhlke et al., 2009).

In-stream N-uptake at the reach scale results from the uptake in the benthic compartment (defined as the uppermost photic layer of the streambed) and in the hyporheic zone (Fig. 1A).

The contribution of the hyporheic zone to in-stream N-uptake at the reach scale is governed

by the balance between (i) hyporheic flow, the hydrologic interaction between surface and the hyporheic water (Boano et al., 2014), that determines the extent of the hyporheic zone that contributes to in-stream N-uptake (Boulton, Findlay, Marmonier, Stanley, & Valett, 1998), and (ii) the intensity of N-uptake in the hyporheic zone (Newcomer et al., 2018; Trimmer et al., 2012). Together with the hydraulic gradient, hyporheic flow is modulated by the hydraulic conductivity (Kf), defined as the ease with which a fluid is transported through a porous matrix (Bear, 1988; Tonina & Buffington, 2009). Kf is determined by the physical characteristics of the sediment (e.g. grain size, packing)(Bear, 1988). N-uptake in the hyporheic zone is determined by the abundance and activity of the microbial community which are controlled by: (i) the mass transfer of N and other solutes (e.g. oxygen, carbon), and (ii) the surface available for microbial colonisation (Hargrave, 1972; Mendoza-Lera et al., 2016; Wäsche, Horn, & Hempel, 2002). Mass transfer to the microbial community depends on the hyporheic flow and the interstitial velocity in the hyporheic zone that increases with Kf (Mendoza-Lera et al., 2016; Sawyer & Cardenas, 2009). Concomitantly, the surface available for microbial colonisation increases with decreasing particle size and Kf (Bear, 1988; Ren & Santamarina, 2017; Santmire & Leff, 2007). Based on these complex and antagonist influences, Mendoza-Lera and Datry (2017) predicted that at $Kf > 10^{-4}$ m/s, high hyporheic flow promotes mass transfer but the large sediment size limits microbial colonisable area, resulting in low hyporheic N-uptake and increasing the relative contribution of the benthic compartment. At intermediate Kf (10^{-4} – 10^{-6} m/s), hyporheic flow is relatively high and the intermediate colonisable area promotes high hyporheic N-uptake which maximizes the relative contribution of the hyporheic compartment. At $Kf < 10^{-6}$ m/s hyporheic flow is low or even null, thus the mass transfer limits the hyporheic N-uptake despite the high colonisable area and in-stream N-uptake is limited to the benthic compartment.

Our objective is to test the above predictions and determine the role of Kf in the contribution of the hyporheic zone to in-stream N-uptake. This is of great relevance for understanding the effects of Kf reduction by fine sediment infiltration and restoration practices aiming at increasing Kf on N-uptake and other ecosystem services (Hester & Gooseff, 2010; Mendoza-Lera & Datry, 2017; Nogaro, Datry, Mermillod-Blondin, Descoux, & Montuelle, 2010). We examined the role of Kf in both governing mechanisms (hyporheic flow and hyporheic N-uptake) that are implicitly interrelated at two hierarchical spatial scales (Boulton et al., 1998). At the reach scale (10^1 – 10^2 m), hyporheic flow represents the extent of the hyporheic zone that contributes to in-stream N-uptake. Hyporheic flow consists of a maze of flowpaths of different lengths, directions, and velocities (Boulton et al., 1998). At the habitat scale (10^{-1} m; hereafter named as hyporheic scale), each of these flowpaths will interact with Kf to determine its interstitial velocity and with the abundance of the microbial community to determine hyporheic N-uptake. We worked at these two spatial scales (reach and hyporheic) in six gaining reaches differing in reach-scale Kf (range: 10^{-1} – 10^{-5} m/s; high to intermediate Kf) and determined in-stream N-uptake at the reach scale (benthic+hyporheic compartment) and N-uptake in the hyporheic compartment at the hyporheic scale (Fig. 1A). Based on the predictions of Mendoza-Lera and Datry (2017), we propose that at the reach scale and given a comparable benthic N-uptake among reaches, the contribution of the hyporheic compartment will increase as Kf decreases (Fig. 1B). This will result from the interplay of hyporheic flow and abundance of the microbial community in the hyporheic compartment. That is, as Kf decreases at the hyporheic scale, the increase in microbial community abundance can compensate the effect of the reduction of the hyporheic flow.

2. Methods

2.1 Study reaches. We selected six morphologically homogeneous, gaining 100-m stream reaches from different streams within the same catchment (Isere) located in the Vercors Range, in the Alps Piedmonds, south-east France (Table S1). The reaches included at least 19 times the wetted width that is considered to comprise all the available geomorphic units (Table S1, Table 1) (Leopold, Wolman, & Miller, 1964). All reaches had canopy cover > 75% and drained limestone, forest/shrub dominated watersheds with zero to low anthropogenic impacts (Table S1). At the reach scale, the variation of hyporheic flow and N-uptake was determined in all the six reaches, and the variation at the hyporheic scale was determined in four of the six reaches (Table 1).

2.2 Kf determination. Along each of the six reaches, reach-scale Kf (rsKf) was measured following Datry et al. (2014). Briefly, 25 transects spaced every four meters along the reach were selected and, in each transect, one to three measurements of Kf were performed (for a total up to 33 points; Fig. S5). The position of each measurement along each transect, within the wetted width, was randomly selected *a priori* in the laboratory. At each point, Kf was determined at 25 cm depth with the falling head slug test using minipiezometers (see Datry, Lamouroux, Thivin, Descloux, & Baudoin (2014) for further details). rsKf was calculated averaging the 33 Kf measurements and ranged from 0.24 m/s to $7.5 \cdot 10^{-5}$ m/s (Table 1) (Datry et al., 2014). In the four reaches where hyporheic scale was studied, we selected 25 out of the 33 Kf measurements (one per transect, 25 per reach) and each one of the measurements corresponded to the hyporheic-scale Kf (hzKf; Fig. S5). At each selected Kf measurement, at least four days prior to the addition, we inserted an unplasticized polyvinyl chloride wells (1.6 cm internal diameter screened across the last 4 cm, 0.4-cm mesh screens) to a depth of 25 cm. An schematic representation of the parameters measured at each scale can be found in Figure S5.

2.3 Solute additions. Between 20th of June and 11th of July 2016, we conducted short-term, constant rate solute additions of a conservative tracer (Cl^- as NaCl) and ammonium (N-NH_4^+ as NH_4Cl) to estimate simultaneously hyporheic flow and N-uptake at the reach- and hyporheic-scale. The solute addition point was located 20 meters upstream of the first sampling site. This distance was set based on previous NaCl-additions and was the minimal distance ensuring a complete mixing of the released solution and the stream water. Additions started approximately at 12:00 and lasted until electric conductivity (EC; recorded every 10 seconds; 6920 V2-2 Multiparameter, YSI, Yellow Springs, OH, USA) reached a plateau at the bottom of the reach (2-4 h). The multiparametric probes were left at the end of the reach for 24h after the addition to capture the whole breakthrough curves. Prior to start the addition and at plateau conditions we measured EC and collected surface water samples at eight sampling sites (0, 10, 20, 30, 45, 65, 85 and 100 m from the uppermost sampling site) and hyporheic water samples at all wells. Surface and hyporheic water samples were collected using 50-mL acid clean syringes. At each surface water sampling site, two 50-mL analytical replicates were collected for N-NH_4^+ and N-NO_x^- ($\text{N-NO}_3^- + \text{N-NO}_2^-$) for background concentrations and two analytical replicates for N-NH_4^+ for plateau concentrations. At each well, a single 15-mL sample of hyporheic water was collected for N-NH_4^+ and N-NO_x^- background concentrations and one for N-NH_4^+ plateau concentrations. Additionally, three surface and hyporheic water samples were collected at the up-, middle- and downstream part of the reach for PO_4^{2-} characterization. At each sampling point we measured mean water depth. All water samplings were performed from down- to upstream. Water samples were immediately filtered in the field (Rotilabo CA syringe filters, 0.2 μm pore diameter) and frozen until analysis (Text S1).

To avoid interference of N-NH_4^+ additions on DOC concentrations (Oviedo-Vargas, Royer, & Johnson, 2013), we sampled water for DOC 24h after the addition following the same

procedure as for background N-NO_x⁻ concentrations. Water samples were immediately filtered in the field (Rotilabo CA syringe filters, 0.45µm pore diameter) and frozen until analysis (Text S1).

After all water samples were collected, we dug around each well and collected ~500 g of sediment at the same depth as hyporheic water was sampled (21-25 cm). To avoid contamination, we dug up to 21 cm with a hoe, and collected the sediment at 21-25 cm with a shovel that was cleaned with stream water between samplings. Sediment was placed in zip-lock bags and frozen for later sediment characterization and determination of total abundance of Bacteria and Archaea, and total abundance of ammonia oxidizing archaea (AOA) and bacteria (AOB).

2.4 Sediment characteristics. About 200g of sediment was wet-sieved (20, 15, 8, 2, 1, 0.5, 0.2 and 0.05 mm sieve). The fraction <0.05mm (i.e. clay and silt content) was lost in the wet-sieving process, and its mass was estimated by subtraction. From the cumulative sediment grain size distribution we obtained d_{10} , the particle diameter representing the 10% cumulative percentile value; d_{10} is the effective diameter that controls porosity and together with sediment specific area (SSA) determines Kf (Bear, 1988; Ren & Santamarina, 2017). SSA (cm²/ g DM), was approximated following (Hillel, 2003):

$$SSA = \frac{6}{\rho_s} \sum \frac{mass_{dx}}{dx} \quad (\text{eq. 1})$$

where $mass_{dx}$ is the proportion of the fraction dx in the sample, ρ_s is the particle density (2.65 g/cm³) and dx is the mean diameter of each fraction except for fractions > 20 mm and < 0.05 mm which corresponded to the sieve pore size.

OM was determined from duplicate samples of 5-g sediment as loss of ignition and reported as ash-free dry mass (AFDM). Samples were dried to a constant mass (105°C) for dry mass calculation (DM) and then burned (4 h, 500°C) for AFDM calculation.

2.5 DNA extraction, quantification of microbial community and ammonia oxidizers.

After DNA extraction (Text S2), the abundance of the total bacterial and archaeal communities was assessed by qPCR quantifying the copy numbers of 16S rDNA gene. The abundance of ammonia-oxidizing archaea (AOA) and bacteria (AOB) was based on copy numbers of the enzyme ammonia monooxygenase (*amoA*) that catalyzes the first and rate limiting step of nitrification, the oxidation of NH_4^+ to NO_2^- (Text S3, and Table S2).

2.6 Data calculation-reach scale. Discharge (L/s) and average surface water velocity (v , m/s) were estimated using the EC breakthrough curve at the bottom of the reach (Gordon, McMahon, Finlayson, Gippel, & Nathan, 2004). v was calculated by dividing the reach length by the time needed to increase EC to one half of the plateau (i.e. nominal travel time).

We used moment methods (Luo, Cirpka, & Kitanidis, 2006; McGuire & McDonnell, 2006) to calculate the mass recovery of Cl^- in the stream as the zero moment and the mean arrival time (*MAT*) of Cl^- to the bottom of the reach (100 m) as the first moment:

$$\text{MAT} = \int_{t=t_1}^{t=t_{99}} tc(t)dt, \quad (\text{eq. 2})$$

We divided the length of the stream reach by the *MAT* to calculate a *MAT*-integrated velocity (v_{MAT} , m/s) that convolves the solute flow through both the free-flowing and water transient storage zones (Drummond, Bernal, von Schiller, & Martí, 2016). We calculated the ratio v/v_{MAT} as proxy of the relative flow in the free-flowing water compared to that in the water transient storage zone (Drummond et al., 2016). The higher the ratio v/v_{MAT} , the more skewed the breakthrough curve is, indicating a greater influence of the water transient storage zone

on solute transport within the reach. Therefore the ratio v/v_{MAT} can be used as in-stream proxy of reach-scale hyporheic flow.

We estimated reach-scale $N-NH_4^+$ uptake as $N-NH_4^+$ uptake velocity (V_f , mm/min; hereafter named as N-uptake velocity) (Stream Solute Workshop, 1990). V_f describes the velocity by which a nutrient molecule is removed from the water column, and it is considered an indicator of nutrient retention efficiency (von Schiller et al., 2008). The following equation describes uptake velocity:

$$V_f = \frac{v \times d}{S_w} \quad (\text{eq. 5})$$

being v surface water velocity (m/min), d mean water depth (m) and S_w (uptake length, m) calculated as the negative inverse of the slope of the regression of the ln-transformed and background corrected concentration:conductivity ratio versus distance downstream from the addition point. V_f standardizes S_w for stream-specific discharge (i.e., discharge/width) and provides a more appropriate variable for comparing N-uptake velocity across reaches (von Schiller et al., 2008).

2.6 Data calculation-hyporheic scale. At each well we measured the magnitude of hyporheic flow at the hyporheic scale as relative connectivity by comparing the increase in EC in the wells between background and when the plateau was reached in the surface water with increases in EC at the adjacent surface-sampling point (Argerich, Martí, Sabater, & Ribot, 2011; Wondzell, 2006):

$$\text{Relative connectivity} = \left(\frac{EC_{plat}^h - EC_{bckg}^h}{EC_{plat}^s - EC_{bckg}^s} \right) \quad (\text{eq. 3})$$

Where EC is electric conductivity ($\mu\text{S}/\text{cm}$), subscript *plat* and *bckg* indicate concentrations at plateau and background respectively, and superscripts *h* and *s* indicate hyporheic water (in the well) and water at the adjacent surface-sampling point, respectively.

To assess the direction and magnitude of ground- and surface water discharge (i.e. up- or downwelling) we determined Darcy flux as

$$Q_f = -K_f \times (\Delta h / \Delta l) \quad (\text{eq. 4})$$

where Q_f is Darcy flux (m/s), Δh is the difference measured between the hydraulic head inside and outside of each well (m) and Δl is the length of porous media crossed by water, equivalent to the depth at which the well was placed (25 cm). Positive values indicate downwelling and negative ones indicate upwelling.

N-NH₄⁺ uptake in the hyporheic zone was determined as the variation in apparent N-NH₄⁺ loss or gain in the wells (hereafter named as hzN-uptake or hzN-production, respectively) based on data from N-NH₄⁺ and Cl⁻ additions following Argerich et al. (2011). We calculated hzN-uptake or production for each well by comparing observed nutrient concentrations in wells when surface plateau was achieved to those predicted based on relative connectivity.

$$C_{pred,plat}^{Hz} = (C_{obs,plat}^S - C_{obs,bckg}^S) \times \text{relativeconnectivity} + C_{obs,bckg}^{Hz} \quad (\text{eq. 6})$$

Where C is N-NH₄⁺ concentration; superscripts Hz and S refer to water in the well and at the adjacent surface-sampling point, respectively; subscripts $pred$ and obs refer to predicted and observed values, respectively; subscript $plat$ refers to surface plateau conditions; subscript $bckg$, refers to background conditions; and relative connectivity is based on EC (eq. 3). hzN-uptake or production was calculated as

$$\text{hzN-uptake} \vee \text{production} = \left(1 - \frac{C_{obs,plat}^{Hz}}{C_{pred,plat}^{Hz}} \right) \quad (\text{eq. 7})$$

Values > 1 indicate hzN-production and values < 1 indicate hzN-uptake.

To gain information about the biogeochemistry of the hyporheic zone and its influence on hzN-uptake or production, we further examined the variation of the ratio between background

hyporheic and surface nutrient concentration for each well (N-NH_4^+ , N-NO_x^- and DOC).

Values of a ratio <1 indicated that surface water was nutrient-enriched compared to hyporheic water, whereas values >1 indicated that hyporheic water was nutrient-enriched compared to the surface water.

2.7 Statistical analysis. We compared the studied reaches for chemical and physical parameters characterizing each stream reach using permutation analysis of variance (permANOVA; R-package *lmPerm* 1.1-2, function *aovp*; 5000 permutations (Anderson, 2001)) using stream reach as factor; when significant differences were found, we ran pairwise comparisons (R-package *stats* 3.0.3; function *TukeyHSD*).

At the reach-scale, we described the relationships between rsKf , rsKf variance, N-uptake velocity and hyporheic flow (v/v_{MAT}) using linear exponential models (R-package *stats* 3.3, function *lm* and *nls*).

At the hyporheic-scale, original data did not meet normality requirements, so all explanatory and response variables were log-transformed, except hzN-uptake and production and relative connectivity that were square-root transformed ($\sqrt{x}$). We described the relationships between hzKf , relative connectivity, hyporheic zone biogeochemistry, Q_f and hzN-uptake and -production using general linear mixed-effects models accounting for the random effects of the sampled reaches (R-package *nlme* 3.1-127, function *lme*). For each linear mixed-effect model we calculated the marginal coefficient of determination (r^2_m), to determine the proportion of variance predictable by the fixed factors. To identify the factors determining hzN-uptake , the abundance of the total bacterial and archaeal microbial community, AOA and AOB, we performed selection of the best models by minimizing Akaike information criterion (AIC) values after the following routine (R-package *nlme* 3.1-127, function *lme*

followed by package MASS 7.3-29, function *stepAIC*). Due to the limited number of wells the best model selection could not be applied to estimate the factors determining hzN-production. Explanatory variables were arranged in three groups: hydrological (hzKf, Q_f and relative connectivity), physicochemical (N-NH_4^+ , N-NO_x^- , DOC) and sediment (SSA, d_{10} , OM). An additional group was included for hzN-uptake: microbial community (16SBacteria, 16SArchea, AOA, AOB). Clay and silt content was not considered within sediment group because of its strong link to SSA. We used general linear mixed-effects models accounting for the random effects of the sampled reaches to identify the best explanatory variable(s) for each group, and then the best explanatory variables from each group were tested all together. We considered tests significant at $p < 0.05$. All statistical tests were done using R 3.1.0 (R Development Core Team, 2014), except for permANOVA which was done using R 2.15.2 (R Development Core Team, 2012).

3. Results

3.1 Reach-scale

rsKf was the highest in Dououlouche and the lowest in Correncon (Table 1). The variance of rsKf increased with mean rsKf ($r^2 = 0.99$, $p < 0.0001$). We observed lower proportion of null-points (Kf measurements where no infiltration was observed after 2 minutes (Datry et al., 2014)) at reaches with higher rsKf (Table 1 and Fig. S6).

N-uptake velocity followed an exponential relationship with rsKf (Fig. 2B): below rsKf = 0.03 m/s, N-uptake velocity decreased very slowly with rsKf, and above rsKf = 0.05 m/s, N-uptake velocity increased. Hyporheic flow (v/v_{MAT}) weakly increased with rsKf (Fig. 2A). N-

uptake velocity increased with hyporheic flow (v/v_{MAT}) when excluding Correncon from the relationship (Fig. 2C).

3.2 Hyporheic scale

Not all the wells were hydrologically connected with the surface water, regardless of their Kf in some cases the wells had very low volume of water (< 5mL) and in others no change in EC was detected (relative connectivity = 0). Irrespective of the direction and magnitude of the Darcy flux, the proportion of non-hydrologically connected wells was 100% in Correncon, 61% in Vernaison, 59% in Dououlouche and 8% in La Fauge. These values indicated that in general, hyporheic zone was dominated by upwelling fluxes (negative Darcy flux Q_f , Table 2), except for one downwelling well in La Fauge and one in Vernaison.

On average, relative connectivity was lower than 0.5 and was the highest in La Fauge, and lower and comparable in Dououlouche, Vernaison and Correncon (Table 2). The median of the relative connectivity was lower than the mean (La Fauge: 0.39; Dououlouche: 0.0; Vernaison: 0.0; Correncon: 0.0), indicating positively skewed data distribution which indicates that there are more wells with low to zero relative connectivity (Fig. S6B). Neither the magnitude of the upwelling Darcy flux nor $hzKf$ significantly influenced relative connectivity.

The hyporheic biogeochemistry varied among reaches (Table 2, Table S1). DOC was more concentrated in the hyporheic zone than in the surface water. Concentration of $N-NH_4^+$ was also higher in the hyporheic zone, except for Vernaison, where concentration was slightly higher in surface water (Table 2, Table S1). Concentration of $N-NO_x^-$ was higher in the hyporheic zone than in the surface water in Dououlouche and La Fauge (Table 2, Table S1), and lower in the surface water in Correncon and Vernaison (Table 2, Table S1). Except for $N-NH_4^+$, which surface water concentration increased with relative connectivity ($N-NH_4^+ Hz/S$)

($r^2_m = 0.092$, $p = 0.009$), hyporheic biogeochemistry was not significantly influenced by $hzKf$, magnitude and direction of Darcy flux or relative connectivity.

Descriptors of sediment characteristics varied differently among reaches. The reaches did not differ in d_{10} (Table 2). The higher clay and silt content of Vernaison and Correncon compared to La Fauge and Doulouche, which determines a big portion of the SSA (eq. 1), resulted in higher SSA in the former and in the latter two (Table 2). OM was the lowest in Doulouche and La Fauge and the highest in Correncon (Table 2).

$N-NH_4^+$ uptake in the hyporheic zone (hzN -uptake) in Correncon was zero and lower than in the other reaches which showed higher and similar levels of hzN -uptake (0.4 on average; Table 2). The median hzN -uptake was 0.38 for Doulouche, 0.18 for Vernaison, 0.57 for La Fauge and 0.00 for Correncon, indicating that the data were positively skewed in Vernaison and Doulouche and slightly negatively skewed in La Fauge (Fig. S6C). Production of hzN was observed at relative connectivity below 44% and was restricted to few wells (Doulouche = 5, Vernaison = 4 and La Fauge = 1; Fig. 3B, Fig. S6D). Neither $hzKf$, Darcy flux nor hyporheic zone biogeochemistry influenced significantly hzN -uptake or -production.

However, hzN -uptake increased with relative connectivity (Figure 2A).

As single linear mixed model comparisons, none of the sediment characteristics, biogeochemistry or microbial community parameters showed a relationship with hzN -uptake. Overall, the selection of the best model revealed that less variance ($r^2_m = 19\%$) of hzN -uptake was explained by the inclusion of sediment characteristics, physicochemical and microbial community parameters (Table S3) than by relative connectivity as single fixed factor ($r^2_m = 23\%$, Fig. 3A). Due to the limited number of wells that showed hzN -production, the selection of the best model could not be applied, as well single comparisons with

sediment parameters, biogeochemistry or microbial community descriptors did not show any relationship.

The microbial community was overall dominated by bacteria, since its total abundance was two orders of magnitude higher than that of archaea (Table 2). The total abundance of bacteria was similar among reaches, while the total abundance of archaea was the highest in Vernaïsson and the lowest in Doulouche (Table 2). The best model selection indicated that SSA was the best descriptor of total bacterial and archaeal abundances (explaining 43-44% of their variability; Table S4). The abundance of the AO genes was comparable in all four reaches (Table 2). The best model selection indicated that OM and N-NH_4^+ explained 23% of AOA variability, while SSA explained 47% of the AOB variability (Table S4).

4. Discussion

4.1. Kf represents the extent of the hyporheic compartment contributing to in-stream N-uptake at the reach scale

Following the conceptual model by Mendoza-Lera and Datry (2017) we predicted that within the Kf range of the study reaches, the contribution of the hyporheic compartment to in-stream N-uptake would increase as rsKf decreases due to a higher abundance of the microbial community and a lower hyporheic flow (Fig. 1B). Our results, which are to our best knowledge the first effort to explore in the field the role of rsKf on in-stream N-uptake, indicated the inverse pattern. We found that N-uptake velocity increased exponentially with rsKf. This suggests that the contribution of hyporheic compartment relative to that of the benthic zone on N-uptake increased with rsKf. N-uptake velocity increased exponentially with hyporheic flow (v/v_{MAT}) and with rsKf, suggesting a secondary role of the abundance of the microbial community. However, we did not observe the predicted relationship between

rsKf and hyporheic flow (v/v_{MAT}). We attribute this outcome to two reasons. First, the method estimating hyporheic flow can be influenced by transient storage in surface areas (i.e., benthic compartment) with low biogeochemical activity resulting in high hyporheic flow and low N-uptake velocity (Kurz et al., 2017; Li, Aubeneau, Bolster, Tank, & Packman, 2017).

Correncon had the lowest rsKf and the relative connectivity was zero, and hyporheic flow (v/v_{MAT}) was similar to Doulouche (16.9 and 13.9, respectively). Since all reaches were dominated by upwelling flow, we excluded the effect of groundwater on this pattern. Hence our results suggest that most of the N-uptake velocity in Correncon occurred in storage or very shallow zones in benthic compartments, so that when Correncon is excluded from the relationship, N-uptake velocity significantly increases with hyporheic flow. Second, hyporheic flow (v/v_{MAT}) and rsKf provide different information on the hydrology of the reach.

While the determination of the hyporheic flow as v/v_{MAT} or relative connectivity is limited to the “window of detection” of the tracer addition (minutes to tens of hours) (Argerich et al., 2011; Harvey & Wagner, 2000), rsKf encompasses the whole hyporheic zone including areas with low Kf and null points (i.e. $K_f < 10^{-7}$ m/s; Datry et al., 2014), such as the reach

Correncon, that might neither contribute to hyporheic flow nor to N-uptake velocity (Sawyer & Cardenas, 2009; Tonina & Buffington, 2009; Ward, Gooseff, & Johnson, 2011). The variance of rsKf increased with its mean and we observed more null points at low rsKf.

Therefore, our results suggest that rsKf likely represents the relative proportion of the hyporheic zone that contributes to in-stream N-uptake, highlighting the significance of heterogeneity in the hyporheic zone (Braun, Auerswald, & Geist, 2012; Ward et al., 2011).

4.2. The relative connectivity is the major determinant of hyporheic N-uptake at the hyporheic-scale

At the hyporheic scale, we hypothesized hzN-uptake to vary as function of the combined effect of two factors determined by hzKf, which are the relative connectivity and the abundance of the microbial community that, in turn, rely on the sediment specific area (SSA)(Mendoza-Lera et al., 2016). Uptake of hzN increased with relative connectivity, regardless of the Darcy flux (i.e. up- and downwelling pattern; (Argerich et al., 2011). We attribute most hzN-uptake to biotic assimilation as suggested by Ribot et al. (2017); despite a portion might had been potentially adsorbed to sediment particle surfaces (i.e., abiotic uptake) (Triska, Jackman, Duff, & Avanzino, 1994). The best descriptor of hzN-uptake was the relative connectivity regardless of local descriptors of the sediment or microbial community highlighting that the flowpath of water, since it enters the hyporheic zone until it reaches the well, prevails over local conditions at the sampling well. This was reflected by the increase of hzN-uptake with the relative connectivity while neither relative connectivity nor hzN-uptake were correlated with hzKf. Interestingly, a similar pattern was observed at background concentrations. As the relative connectivity increased the hyporheic water contained less N-NH_4^+ than surface water, which indicated that more N-NH_4^+ uptake occurred at higher connectivity while OM mineralization and ammonification promoted N-NH_4^+ accumulation at lower connectivity (Nogaro et al., 2010). Similarly, we observed hzN-production at low relative connectivity. However, the combined effect of the different hzKf patches likely determined the relative connectivity by defining the flowpath of the water (Sawyer & Cardenas, 2009; Ward et al., 2011). Therefore, hzKf likely determined the mass transfer by means of hyporheic flow and, in turn, hzN-uptake.

The abundances of bacteria and archaea were mainly driven by SSA regardless of nutrient concentration or relative connectivity. Therefore, the abundance of the microbial community

that can potentially assimilate N-NH_4^+ could be expected to increase as hzKf decreases (Ren & Santamarina, 2017). Since at low hzKf and relative connectivity anoxic conditions are likely promoted (Merbt, Auguet, Casamayor, & Marti, 2011; Nogaro et al., 2010), the potential for nitrification, as derived by AOA and AOB abundance, was expected to increase with hzKf and relative connectivity. Yet, this hypothesis was not supported by our data. Instead AOB abundance was driven by SSA, and AOA abundance was related to OM and N-NH_4^+ concentration. This pattern may be explained by contrasted sensitivities to N-NH_4^+ concentrations and metabolic differences between AO phyla as AOB are obligate chemoautotrophs using CO_2 as carbon source while AOA can have hetero- or mixotrophic metabolism (Mußmann et al., 2011; Verhamme, Prosser, & Nicol, 2011). The lack of influence of relative connectivity on the microbial community suggests that hyporheic N-uptake will depend first on the relative connectivity, and second on the abundance of the microbial community. Hence areas without hyporheic flow will not assimilate N regardless of SSA. In areas with hyporheic flow, the interplay between hyporheic flow and SSA will determine hyporheic N-uptake (Mendoza-Lera et al., 2016). Hence, contrary to the common assumption of spatially invariant microbial reaction rates (Li et al., 2017), our results show that N-uptake is heterogeneous in the hyporheic zone. Despite hzKf may not be a good predictor of hzN -uptake or relative connectivity, it may indirectly determine hyporheic N-uptake by means of driving hyporheic flow, interstitial velocity, and the abundance of the microbial community (noteworthy that microbial community dynamics can also influence hzKf e.g., bioclogging; Mendoza-Lera & Mutz, 2013). Thus improved predictions of the contribution of the hyporheic compartment to in-stream N-uptake require integration of the interplay between the relative hydrological connectivity and microbial community.

4. 3. Implications for in-stream N-uptake

The proportion of water that reached the hyporheic zone (i.e. relative connectivity) was highly variable within the reach and a large number of wells had low to null surface water connection. On average less than the 25% of the hyporheic water was surface water. Since we do not know whether the hyporheic zone had reached plateau conditions during the solute addition when we sampled the wells, the observed patterns of relative connectivity could indicate either that indeed relatively low surface water reached the well (if plateau conditions were reached at the hyporheic zone) or that water travelled to the wells more slowly than the time considered as surface plateau when wells were sampled (Argerich et al., 2011). Despite of this, at the sampling time time about 40% of the nitrogen transported with surface water was assimilated in the hyporheic zone highlighting the disproportionate reactivity of the hyporheic zone considered the low connection with water surface.

Based on the relationship between the relative connectivity and hzN -uptake, it can be expected that, at the reach scale the contribution of the hyporheic zone to in-stream N-uptake will increase as function of the relative proportion of the hyporheic zone more connected to the surface water. Since hzK_f likely determined hyporheic N-uptake, the larger the relative proportion of high hzK_f areas at the reach scale (i.e. larger rsK_f), we would expect the larger the contribution to in-stream N-uptake of a given reach. This result highlights that not only K_f , but also its heterogeneity plays a significant role on in-stream N-uptake at reach scale. Previous research at the hyporheic scale (10^{-1} m) revealed that the impact of fine sediment infiltration on microbial processes, such respiration or nitrification, is a function of hzK_f heterogeneity. That is, homogeneous low- K_f layers have stronger negative influence on hydrologic flowpaths and microbial processes than heterogeneous layers (Nogaro et al., 2010).

Our results suggest that the mechanisms by which Kf drives the contribution of the hyporheic zone to in-stream N-uptake are linked to Kf across scales. Although rsKf was a better predictor of the patterns observed at the reach scale than hzKf at the hyporheic scale; and thus, further research is needed to clarify this relationship, our results suggest that taking into account the role of Kf across scales can provide further information about mechanisms driving the variability in N-uptake among streams.

4.4 Implications for stream management and restoration practices

Our study corroborates the recommendation that stream restoration practices aimed at overcoming clogging should focus on in-stream features (e.g. rsKf, geomorphology, flow; Auerswald & Geist, 2017; Pander, Mueller, & Geist, 2014). Although further research is needed to complement our findings, such as considering reaches covering a wider range of rsKf, being gaining and losing (e.g. Trauth, Schmidt, Vieweg, Oswald, & Fleckenstein, 2015), including the presence of bedforms (e.g. Harvey & Gooseff, 2015), a wider range of nutrient concentrations, and other biogeochemical processes such as denitrification or organic carbon dynamics, our results suggest that the non-linear relationship between rsKf and in-stream N-uptake has a potential to guide restoration efforts (Mendoza-Lera & Datry 2017). rsKf could be used as a functional indicator (ie. as a proxy) of self-purification capacity of low order stream reaches providing a potential tool for stream managers. Since rsKf can be determined using a neutral approach (Datry et al., 2014; Stewardson et al., 2016), once the its relationship with in-stream N-uptake is refined, we suggest that the rsKf based index could be of great applicability to assess the success of stream restoration to improve in-stream biogeochemical processing and associated ecosystem services (Hester & Gooseff, 2010; Lammers & Bledsoe, 2017; Pander et al., 2014). This would be particularly useful when efforts to restore and enhance hyporheic flow manipulate rsKf (Hester & Cranmer, 2014).

5. Conclusion

In this study we explored the role of Kf modulating the contribution of the hyporheic zone to whole-reach in-stream N-uptake considering two interrelated compartments (benthic and hyporheic) and its role at two hierarchical spatial scales (Boulton et al., 1998). Despite a relatively low number of study sites, our results suggest that Kf determines the contribution of the hyporheic compartment and that its role varies between scales. Our results point to that Kf modulates hyporheic N-uptake by means of hyporheic flow that becomes limiting at higher $rsKf$ than expected, and that the abundance of the microbial community in the hyporheic zone plays a secondary role. At the reach scale, Kf variably could determine the extent of the hyporheic zone contributing to hyporheic flow, and thus, N-uptake velocity.

However, at the hyporheic scale, Kf determines the hyporheic flowpaths (Sawyer & Cardenas, 2009; Ward et al., 2011); and thus, its influence on hyporheic N-uptake seems to be indirect through other interrelated factors such as the relative connectivity and the abundance of the microbial community at local scale. Finally, results suggest that the interplay of Kf between the two scales can drive in-stream N-uptake suggesting that Kf can be considered a unifying variable for stream biogeochemistry with implications for stream restoration strategies aiming at increasing self-purification capacity of streams. This is, to our best knowledge, the first study to explore via *in situ* co-located, simultaneous measurements the different mechanisms driving the in-stream N-uptake patterns across several scales (reach to habitat and microbial community scale).

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Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Supplementary material

Additional methods details (Text S1-S2), tables (Table S1-S2), results tables (Table S3-S4), methods figure (Fig. S5) and results figure (Fig. S6).

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Table 1

Characterization of the hydrology and Kf of the studied reaches. Where applicable, mean $\pm$ SD. S corresponds to surface water and Hz to hyporheic zone water.

	Doulouche	Bellecombe	Vernaison	Furon	La Fauge	Correncon
rsKf (m/s)	0.24 $\pm$ 0.44	0.04 $\pm$ 0.2	1.05 10 ⁻⁴ $\pm$ 10 ⁻⁴	2.20 10 ⁻⁴ $\pm$ 3.410 ⁻⁵	2.51 10 ⁻⁴ $\pm$ 2.8 10 ⁻⁴	7.5 10 ⁻⁵ $\pm$ 3.0 10 ⁻⁵
Variance reach-Kf	0.19	0.04	1.17 10 ⁻⁹	1.32 10 ⁻⁸	8.02 10 ⁻⁸	8.53 10 ⁻¹⁰
Proportion of null points*	0.33	0.24	0.70	0.82	0.37	0.82
Vertical hydraulic gradient (vhg)	0.57 $\pm$ 0.44	2.98 $\pm$ 1.11	0.55 $\pm$ 0.35	3.42 $\pm$ 0.38	2.9 $\pm$ 1.12	2.79 $\pm$ 0.24
Proportion of downwelling wells	0.06	0.03	0.09	0	0.09	0
Discharge (L/s)**	11.9	1.72	0.74	2.03	9.44	41.95
Mean water depth (m)	0.063	0.097	0.05	0.053	0.077	0.074
Surface water velocity (m/s)**	0.27	0.025	0.031	0.04	0.06	0.20

* Null points correspond to point Kf measurements in which no infiltration was observed after 2 minutes (Kf < 10⁻⁷ m/s). (Datry et al., 2014)

** On addition date

Table 2

Average and Standard Deviation and Differences among study reaches of Physical, Chemical, Hydrological and Microbial characteristics in the hyporheic zone. Bacteria, Archea, bacterial and archeal ammonia oxidizers (AOB and AOA) abundances. Differences among study reaches were determined with permANOVA, significant differences are indicated with a, b and c. Significant p-values are indicated in bold.

Parameter	P value	Doulouche	Vernaison	La Fauge	Correncon
hzKf (m/s)	0.046	2.38e-1 ± 2.01e-2 ab	1.05e-4 ± 3.42e-6 ab	2.51e-4 ± 1.29e-5 a	7.46e-5 ± 5.84e-6 b
Q_f (m/s) * 10 ⁻⁵	0.36	-6.42 ± 0.12 a	-2.33 ± 4.17 a	-10.44 ± 79.8 a	-0.83 ± 2.04 a
Relative connectivity	<0.0001	0.13 ± 0.006 a	0.088 ± 0.005 a	0.49 ± 0.014 b	0 ± 0 a
hzN-uptake	0.0002	0.45 ± 0.32 a	0.32 ± 0.28 a	0.54 ± 0.25 a	0 ± 0 b
hzN-production	0.5	0.88 ± 0.62 a	2.03 ± 2.11 a	0.34 ± 0 a	- -
No _x ⁻ Hz/S	0.018	1.74 ± 0.03 a	0.98 ± 0.07 ab	1.53 ± 0.03 ab	0.87 ± 0.02 b
DOC Hz/S	0.001	2.10 ± 0.05 b	4.15 ± 0.17 ab	5.63 ± 0.28 a	4.77 ± 0.20 ab
N-NH ₄ ⁺ Hz/S	0.01	3.28 ± 0.16 a	0.97 ± 0.0076 a	5.41 ± 0.22 a	4.77 ± 0.26 b
SSA (cm ² /g DM)	<0.0001	23.95 ± 3.30 a	122.94 ± 4.60 b	21.10 ± 0.50 a	103.17 ± 1.10 b
OM (g AFDM/gDM)	<0.0001	0.02 ± 0.00074 a	0.031 ± 0.00033 b	0.021 ± 0.00035 a	0.034 ± 0.00057 c
d_{10} (mm)	0.05	0.15 ± 0.10 a	0.091 ± 0.10 a	0.088 ± 0.075 a	0.079 ± 0.0081 a
Clay and silt content (%; <0.05mm)	<0.0001	4.66 ± 10.46 a	20.46 ± 22.21 b	0.94 ± 0.71 a	16.70 ± 19.94 b
Bacteria * 10e5 (#/g DM)	0.07	7.55 ± 0.61 a	87.8 ± 5.86 a	16.1 ± 1.07 a	13.2 ± 0.41 a
Archea * 10e3(#/g DM)	0.003	2.74 ± 0.22 a	33.8 ± 2.21 b	8.44 ± 0.59 c	9.2 ± 0.48 c
AOA * 10e3(#/g DM)	0.27	1.15 ± 0.075 a	13.4 ± 0.95 a	2.27 ± 0.14 a	14.6 ± 0.99 a
AOB * 10e2 (#/g DM)	0.07	2.05 ± 0.16 a	16.6 ± 1.2 a	7.2 ± 0.48 a	3.44 ± 0.19 a

A.

B.

Figure 1. A. Schematic representation of the two different scales and compartments studied. B. Schematic representation of the hypothesis, left diagram represents the N-uptake of each compartment and the in-stream N-uptake relative to K_f and the right diagram represents the changes in microbial community abundance and hyporheic flow in the hyporheic compartment relative to K_f .

Figure 2. Reach-scale relationships between hyporheic flow (v/v_{MAT}) and $rsKf$ (m/s) (A), N-uptake velocity (V_f , mm/min) and $rsKf$ (m/s) (B) and N-uptake velocity (V_f , mm/min) and hyporheic flow (v/v_{MAT}) (C). Note log-scale in x-axis in panels A and B. Regressions excluding Correncon are represented by dotted lines.

Figure 3. Hyporheic-scale relationships between relative connectivity and hzN-uptake (A), and between relative connectivity and hzN-production (B). Note both variables were squared root transformed. Note Correncon does not present regression lines because no relative connectivity nor hzN-uptake or -production was detected.