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Aquifer recharge by stormwater infiltration basins: hydrological and vadose zone characteristics control the impacts of basins on groundwater chemistry and microbiology

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Abstract.

Stormwater infiltration systems (SIS) are designed to collect and infiltrate urban stormwater runoff into the ground for flood risk mitigation and artificial aquifer recharge. Many studies have demonstrated that infiltration practices can impact groundwater chemistry and microbiology. However, quantitative assessments of the hydrogeological factors responsible of these changes remain scarce. Thus, the present study aimed to quantitatively test whether changes of groundwater chemistry and microbiology induced by SIS were linked to two factors associated with vadose zone properties (vadose zone thickness, water transit time from surface to groundwater) and one factor associated with groundwater recharge rate (assessed by groundwater table elevation during rain events). To evaluate changes in chemistry (NO_3^- , PO_4^{3-} and dissolved organic carbon concentrations), groundwater samples were collected in wells located in SIS-impacted and non-SIS-impacted zones during experimental periods of 10 days. During the same periods, clay beads were incubated in the same wells to measure changes of groundwater microbial biofilms (microbial biomass, dehydrogenase and hydrolytic activities) induced by SIS. Results showed that changes in PO_4^{3-} supplied to groundwater during stormwater infiltration was negatively correlated with vadose zone thickness. A short water transit time from surface to groundwater increased dissolved organic carbon concentrations in the aquifer which, in turn, increased biofilm biomasses in groundwater. The groundwater recharge rate during rain events (assessed by groundwater table elevation) diluted NO_3^- concentrations in the aquifer but also influenced the changes of biofilm activities induced by SIS. Groundwater recharge rate during rain events probably increased the fluxes of water and dissolved organic carbon in groundwater, stimulating the activity of microbial biofilms. Overall, the present study is the first to quantify conjointly several factors and processes (water transfer, dilution, solute fluxes) that could explain the impact of stormwater infiltration on chemistry and/or microbiology in groundwater.

Keywords: infiltration basin; aquifer recharge; water transit time from surface to groundwater; groundwater table elevation; biofilm biomass and activity; stormwater runoff

1. Introduction

Urban areas host 55% of the world's population, a proportion that is expected to increase to 66% by 2050 (United Nations, 2019). One of the major landscape modifications associated with urbanization is an increase in the impervious surface coverage (Arnold and Gibbons, 1996). The sealing of urban surfaces alters the natural water cycle by reducing groundwater recharge and by increasing stormwater runoff during rainfall events that are responsible of urban flooding (Mays 2013; Naik et al., 2008; Tam and Nga, 2018). Since the last 30 years, stormwater management practices have been developed worldwide to limit stormwater runoff by infiltration to compensate for reduced groundwater recharge (Marsalek and Chocat, 2002; Dechesne et al., 2005; Hunt et al., 2010; Natarajan and Davis, 2015; Masetti et al., 2016; Beganskas and Fisher, 2017). The practices, based on stormwater infiltration systems (SIS) like infiltration basins, were mainly hydrologically-designed with the main aim to ensure the rapid infiltration of water drained from a given catchment area during stormwater rainfalls (Grebel et al., 2013). However, infiltrated urban stormwater can be highly contaminated with heavy metals, hydrocarbons, pathogens, organic matter and nutrients (Pitt et al., 1999; Grebel et al., 2013; Gasperi et al., 2014; Mermillod-Blondin et al. 2015; Colin et al., 2020) which can constitute a risk of pollution for receiving groundwater ecosystems. Therefore, assessing the environmental performance of stormwater infiltration systems is crucial for groundwater resource protection.

The environmental performances of aquifer recharge practices such as riverbank filtration (Kuehn and Mueller, 2000) or soil aquifer treatment (Amy and Drewes, 2007) have been assessed for nutrients (Dillon et al., 2010; Vanderzalm et al., 2013), organic chemicals (Rauch-Williams et al., 2010) or microorganisms (Levantesi et al., 2010; Barba et al., 2019; Fillinger et al., 2021) retention. It has been shown that these performances were mainly dependent on the

ability of the soil and the sedimentary environment to retain and metabolize contaminants during their transfer in/to the aquifer (Pabich et al., 2001; Holden and Fierer, 2005; Bekele et al., 2011; Tedoldi et al., 2016; Avishai et al., 2017; Alam et al., 2021). In “soil aquifer treatment” systems like stormwater infiltration systems (SIS, see Fig. S1 in Supplement), many authors (e.g., Datry et al., 2004; Foulquier et al., 2010a) reported the crucial role of the vadose zone (zone between the land surface and the groundwater table) in the protection of the aquifer against contaminants associated with infiltrated urban stormwater. Biotic (microbial processes, Marschner and Kalbitz 2003; Shen et al. 2015) and abiotic (sorption on clay particles, soil organic matter, or/and Fe and Al oxides/hydroxides, McKnight et al. 1992; Kalbitz et al. 2000; Saïdy et al. 2013) soil components are likely involved in the retention efficiency of the vadose zone. Consequently, the thicker the vadose zone, the more efficient a SIS should be to limit groundwater contamination with organic and inorganic compounds. Therefore, it has been demonstrated that SIS can enrich groundwater with dissolved organic carbon (DOC) and nutrients like orthophosphate when the vadose zone was not thick enough to retain the organic matter contained in stormwater runoff (Pabich et al. 2001; Datry et al. 2005; Voisin et al., 2020). Due to the carbon limitation of most groundwater ecosystems (Malard and Hervant, 1999; Hofmann and Griebler, 2018), these increased DOC concentrations in the aquifer associated with SIS may affect groundwater ecology by stimulating the microbial compartment (Starr and Gillham, 1993; Siemens et al., 2003; Mermillod-Blondin et al., 2013; Voisin et al., 2020). Thus, vadose zone thickness is recognized as a main factor mediating the impact of SIS on the chemistry and microbiology of groundwater ecosystems.

Nevertheless, the physical heterogeneity of the vadose zone that could lead to preferential flow paths can call into question the main influence of vadose zone thickness on SIS environmental performance (Goutaland et al., 2013; Slimene et al., 2015). By influencing the transit time of the infiltrating stormwater from the basin surface to the groundwater table, the physical

heterogeneity of the vadose zone may play a key role on groundwater quality (Vías et al., 2010; Andres and Sims, 2013). For example, Voisin et al. (2018) reported that the transit time from surface to groundwater might have more influence on groundwater microbiology than the vadose zone thickness because preferential flow paths could promote the transfer of nutrients to the aquifer and limit the efficiency of trapping and metabolizing mechanisms occurring in the vadose zone. Consequently, the transit time of water from surface to groundwater could be a more functional parameter to evaluate the retention processes occurring in the vadose zone than its thickness. Thus, the environmental performance of the SIS would be positively correlated with the transit time of stormwater from surface to the aquifer through infiltration basins.

In addition to these two main characteristics of SIS (vadose zone thickness and water transit time through the vadose zone), the response of groundwater ecosystems to aquifer recharge was also expected to differ depending on the groundwater recharge rate during rain events (Owor et al., 2009; Foulquier et al., 2010b; Wang et al., 2015; Lebon et al., 2021). Thus, evaluating the environmental performance of SIS on microorganisms needs to consider the recharge rate of stormwater effectively entering into the aquifer during rain events. Indeed, groundwater recharge rate has a direct influence on dissolved nutrient and DOC fluxes (Dobson et al., 2007; Jost et al., 2015; Legout et al., 2009; Rühle et al., 2015) and can also remobilize these compounds previously accumulated in the vadose zone (Gao et al., 2006; Zhuang et al., 2009). Both mechanisms are expected to stimulate the activities of groundwater microbial communities (Lebon et al., 2021).

Although the impacts of SIS on groundwater chemistry and microbiology are expected to depend on SIS characteristics (thickness of the vadose zone, transit time from surface to groundwater) and the groundwater recharge rate during rain events, no study has yet tried to

evaluate quantitatively and conjointly the significance of these three factors on SIS impacts. The present study aims at filling these gaps by considering simultaneously the vadose zone thickness, the transit time of water from infiltration basins to groundwater and the groundwater recharge rate during rain events (assessed by groundwater table elevation during rain events, Healy and Cook, 2002; Labrecque et al., 2020) as most probable factors driving the impacts of SIS on groundwater chemistry and microbiology. To disentangle the roles of these factors in chemical and microbial changes associated with SIS practices under realistic conditions, field experiments were performed in five SIS over six experimental periods of 10 days characterized by contrasted groundwater recharge rates due to rain events. For each SIS and experimental period, the impacts of SIS were assessed on groundwater chemistry (dissolved nutrients NO_3^- , PO_4^{3-} and DOC) and microbiology (biomass, dehydrogenase and hydrolytic activities in biofilms developed on artificial substrates).

2 Materials et methods

2.1 Study sites

Studied stormwater infiltration systems (SIS) were located in highly permeable glaciofluvial sediments (hydraulic conductivity: 10^{-3} – 10^{-2} m.s⁻¹ – Foulquier et al., 2009) of the eastern aquifer of Lyon, France (Fig. S1 in Supplement). In this aquifer, groundwater was characterized by low concentrations in dissolved organic carbon (mean value \pm standard deviation: 0.87 ± 0.33 mg L⁻¹) and P- PO_4^{3-} (18 ± 9 $\mu\text{g L}^{-1}$) in comparison with stormwater runoff supplied to infiltration basins (5.51 ± 1.96 mg L⁻¹ for DOC concentrations and 165 ± 125 $\mu\text{g L}^{-1}$ for P- PO_4^{3-} concentrations) (data reported from Voisin et al., 2018 and Lebon et al., 2021). In contrast, N- NO_3^- concentrations were more than 10-fold higher in groundwater (10.2 ± 5.6 mg L⁻¹) than in stormwater runoff (0.65 ± 0.49 mg L⁻¹) due to maize agriculture in the East of Lyon. Due to

these differences, the impact of infiltrated stormwater in groundwater tended to increase DOC and orthophosphate concentrations in the aquifer (Voisin et al., 2018) and to decrease nitrate concentrations in the aquifer (Datry et al., 2004). The five selected SIS covered a wide range of vadose zone thickness (from 1.9 to 20.8 m, Table 1). To cover a wide range of groundwater recharge rates (and associated groundwater table elevations) among SIS, we selected basins with surfaces varying from 0.09 to 0.87 ha and collecting stormwater runoff from catchment areas varying from 2.5 to 315 ha (Table 1). Each SIS was equipped with an upstream well and a downstream well along the groundwater flow (Fig. S1 in Supplement). The downstream well was located in the SIS-impacted zone of the aquifer because it intersects the plume of infiltrated runoff water entering the aquifer during rain events (Bahar et al., 2021). The upstream well was located outside of the plume of infiltrated runoff water (non-SIS-impacted zone of the aquifer). Based on this dedicated equipment, it was possible to evaluate the impact of each SIS on chemical and microbiological groundwater variables by comparing data obtained from SIS-impacted and non-SIS-impacted (reference) zones of the aquifer.

Table 1: Main characteristics of the five studied stormwater infiltration systems (SIS). The mean vadose zone thickness presented in this table corresponds to the mean +/- SD measured on each SIS (see 2.3 and 2.4) for the six experimental periods.

SIS	Mean vadose zone thickness over the six experimental periods (m)	Infiltration surface (ha)	Catchment area (ha)
Grange Blanche (GB)	1.94±0.60	0.32	300
Minerve (Min)	3.68±0.20	0.42	165
IUT (IUT)	3.60±0.18	0.09	2.50
Django Reinhardt (DjR)	19.9±0.48	0.87	185
Chemin de Feyzin (Fey)	20.8±0.49	0.25	315

2.2 Experimental periods

Six experiments of 10 days were performed to evaluate the impact of groundwater recharge rate on groundwater chemistry and microbiology (Table 2). These six 10-day periods were characterized by widely contrasted cumulative rainfalls and maximum rain intensities (Table 2, Fig. S2 in Supplement). The experimental period of September 2015 was characterized by the highest cumulative rainfall and rain intensity whereas the experimental period of December 2017 presented more than 3-fold lower cumulative rainfall and intensity (Table 2). The three other rainy experimental periods (November 2018, April 2019, November 2019) were characterized by intermediate rain intensities and cumulative rainfalls (Table 2). In addition, a dry period in August 2016 has been also considered as a control period without groundwater recharge. This selection of widely contrasted rain event characteristics (associated with contrasted SIS characteristics) was particularly important to identify the influence of stormwater infiltration on chemistry and groundwater biofilms.

Table 2: For each 10-day period considered in this study, cumulative rainfall (mm) and maximum rain intensity (mm/h) as mean +/- SD measured on the five SIS.

Period	Cumulative rainfall (mm) during the 10-day experiment	Maximum hourly rain intensity (mm/h) during the experiment
September 2015	94.4±3.80	15.0±1.51
August 2016	0	0
December 2017	38.55±0.55	4.00±0.49
November 2018	61.0±3.28	4.80±0.28
April 2019	53.5±3.56	8.80±1.26
November 2019	66.3±1.22	6.53±0.46

2.3 Monitoring and sampling of groundwater and microbial biofilms

To evaluate factors associated with SIS characteristics (vadose zone thickness and transit time of water from surface to groundwater) and groundwater recharge rate (groundwater table elevations), electric conductivity (EC) and groundwater table level were monitored on a 1 h step basis with field probes (LTC Levelogger® Junior, Solinst, Canada) in non-SIS-impacted and SIS-impacted wells of all SIS from January 2015 to December 2019. During each experimental period of 10 days, groundwater was sampled 50 cm below the groundwater table at three occasions in non-SIS-impacted and SIS-impacted zones of each SIS to evaluate the influence of SIS on nitrate (NO_3^-), orthophosphate (PO_4^{3-}) and DOC concentrations. While stormwater runoff is relatively low in NO_3^- which is the predominant source of nitrogen in groundwater at the study sites (Voisin et al., 2018), PO_4^{3-} and DOC are mainly supplied by stormwater runoff and can contaminate groundwater due to SIS practices (Datry et al., 2005; Mermillod-Blondin et al., 2015). Groundwater samplings were performed with the Bou-Rouch method (Bou and Rouch, 1967) for SIS with thin vadose zone (< 5 m) and with an immersed electric pump for SIS with a thick (> 5 m) vadose zone (see Voisin et al., 2020 for details). Groundwater samples (1 L) were stored at 4 °C during travel back to the laboratory and analyzed within 24 h for inorganic nutrients (NO_3^- and P-PO_4^{3-}). For assessing the biomass and the activities of microbial biofilms, expanded clay beads (clay beads with diameters between 7.1 and 9 mm and colonizable areas varying between 155 and 255 mm^2) sterilized at 550 °C were used as artificial substrates for sampling groundwater microorganisms (Voisin et al., 2016, 2020; Mermillod-Blondin et al., 2019). Three replicated artificial substrates (i.e. three nylon mesh bags containing thirty expanded clay beads each) were incubated in each well (non-SIS-impacted/SIS-impacted) of each SIS for 10-days experimental period (starting just before the rain event). The rationale for a ten-day incubation period was that (1) it was long enough to develop microbial communities which are representative of biofilms developing in the aquifer (see Voisin et al., 2016 and Mermillod-Blondin et al., 2019) and (2) it corresponded to the

duration of the impact induced by stormwater runoff infiltration in the aquifer (duration of electric conductivity decrease in the aquifer, Fig. 1a). At the end of the artificial substrate incubation, the clay beads from each bag were collected and randomly distributed in groups of 5 beads to perform the three microbial analyses (i.e. biomass; hydrolytic activity; dehydrogenase activity). For each analysis, five clay beads were used per bag and the three replicated bags enabled triplicate analyses in non-SIS-impacted and in SIS-impacted zones of each SIS at each period.

2.3.1 Chemical analyses

Groundwater samples were filtered (0.7 μm pore size Whatman™ GF/F filters, GE Healthcare, Boston, MA, USA) to remove particles. Nitrate (N-NO_3^-) and orthophosphate (P-PO_4^{3-}) concentrations were quantified with an automatic analyzer based on colorimetric methods (Smartchem 200, AMS Alliance, Frépillon, France). DOC analyses were performed on water samples previously treated with hydrochloric acid (350 μL , HCl 2 N) to remove dissolved inorganic C. After this step, DOC concentration was measured by combustion of water samples at 900 °C and infrared detection of CO_2 stripped under O_2 flow with a total carbon analyzer (multi-N/C 3100, Analytik Jena, Jena, Germany).

2.3.2 Microbial biomass

Total protein content was used as a proxy of microbial biomass developed on clay beads during the experimental period of 10 days. The analysis was performed according to the protocol of Peterson (1977) described in Voisin et al. (2016). Briefly, collected clay beads ($n=5$) were treated with 2 mL of Lowry's reagent diluted with 2 mL of ultrapure water (Sigma Protein Assay Kit P-5656, Sigma-Aldrich, St Louis, MO, USA) for 20 min under an orbital shaker to improve the contact between bead surfaces and the reagent. Then, 0.5 mL of Folin reagent was added and a colored reaction developed for 30 min in the dark at 15 °C. The final solution was filtered

with a Whatman™ GF/F filter (0.7 µm pore size, GE Healthcare, Boston, MA, US) to remove particles before its optical density measurement at 750 nm with a spectrophotometer (Aquamate, ThermoSpectronic, Cambridge, UK). In parallel, a calibration curve was performed with BSA standards (bovine serum albumin lyophilized powder ≥96%, Sigma-Aldrich, St Louis, MO, US) to convert optical densities into total protein content. Finally, total protein content was related to bead surface (see Lebon et al., 2021 for details) and corrected by results obtained from controls (sterile clay beads) to express microbial biomass in µg of protein per cm².

2.3.3 Microbial activities

The impact of SIS on microbial activities was assessed by measuring hydrolytic and respiratory activities of biofilms developed on clay beads.

Hydrolytic activity of the biofilm was quantified by measuring the hydrolysis of fluorescein diacetate (FDA, 3,6-diacetyl-fluorescein), a compound which can be hydrolyzed by a wide spectrum of non-specific extracellular and membrane-bound enzymes such as esterases, proteases, and lipases (Fontvieille et al., 1992). Thus, FDA hydrolysis is considered as a good proxy of hydrolytic activity involved in hydrolysis of high-molecular-weight organic compounds into smaller molecules which are available for microbial uptake (Fontvieille et al., 1992). For each incubated bag, five clay beads were incubated with 3 ml of a phosphate buffer solution (pH = 7.6) and 0.1 mL of FDA solution (10 mg of FDA dissolved in 6 mL of acetone and 4 mL of ultrapure water) at 15 °C in the dark until a green coloration occurred (fluorescein production) (Voisin et al., 2018). Three mL of acetone were added to stop the incubation. Then, the supernatant was filtered to remove particles (0.7 µm pore size Whatman™ GF/F filters, GE Healthcare, Boston, MA, USA) and the optical density of the filtrate was measured at 490 nm with a spectrophotometer (Aquamate, ThermoSpectronic, Cambridge, UK). Optical density

was then converted into hydrolytic activity expressed in quantity of FDA hydrolyzed per hour and cm^2 of clay bead surface ($\mu\text{mol FDA}\cdot\text{h}^{-1}\cdot\text{cm}^{-2}$) following the equation reported in Lebon et al. (2021). As microbial biomass analyses, hydrolytic activity measurements performed on samples were corrected by subtracting results of the control samples (sterile clay beads).

Respiratory activity was assessed by measuring the activity of intracellular enzymes (dehydrogenase) involved in electron transfer processes, as a proxy of biofilms metabolic activities (e.g., Mermillod-Blondin et al., 2005). Measurements were performed using the reduction rate of 2-(4-iodophenyl)-3-(4-nitrophenyl)-5-phenyl tetrazolium chloride (INT) into INT-formazan (Hourri-Davignon et al., 1989). For each sample, five clay beads were incubated in the dark at 15 °C with 0.1 mL of INT solution (20.2 mg of INT in 1 mL of methanol) diluted in 4 mL of filtered groundwater (0.2 μm pore size Whatman™ nuclepore polycarbonate membrane, GE Healthcare, Boston, MA, USA) collected from the well where clay beads were incubated. Following the same incubation times as those performed for hydrolytic activity, the dehydrogenase activity was stopped with the addition of 0.5 mL of formaldehyde at 37%. Then, an extraction of accumulated INT-formazan (light red coloration) in cells was performed. For each incubated sample, supernatants were filtered with 0.2 μm pore size membrane (Whatman™ nuclepore polycarbonate membrane, GE Healthcare, Boston, MA, USA) to retain free microbial cells. Filters were then put back in glass flasks containing the clay beads and the extraction of INT-formazan on microorganisms started by adding 4 mL of methanol. To increase this extraction step, 2 minutes of sonication were applied before starting an incubation of 12 hours in the dark. After this incubation, supernatant was filtered to remove particles (0.7 μm pore size Whatman™ GF/F filters, GE Healthcare, Boston, MA, USA) and the optical density of the filtrate was measured at 480 nm with a spectrophotometer (Aquamate, ThermoSpectronic, Cambridge, GB). Measured optical density was converted into dehydrogenase activity expressed in quantity of INT hydrolyzed per hour and cm^2 of clay bead

surface ($\mu\text{mol INT.h}^{-1}.\text{cm}^{-2}$) following the equation described in Lebon et al. (2021). Obtained result of dehydrogenase activity was then corrected by subtracting results obtained from control samples (sterile clay beads).

2.3.4 Assessment of SIS impacts on groundwater chemistry and microbiology

The impact of each SIS on groundwater chemistry and microbial biofilms was assessed by measuring the differences between the SIS-impacted zone and the non-SIS-impacted zone of the aquifer for each experimental period. The chemical and microbial biofilm changes due to SIS were approximated by the ratios in orthophosphate, nitrate, dissolved organic carbon, total protein content (proxy of microbial biomass), hydrolytic activity and dehydrogenase activity between SIS-impacted (SI) and non-SIS-impacted (NSI) zones for each SIS and each experimental period. Ratios greater than 1 indicated that SIS increased the concentrations of the analyzed nutrient in groundwater or stimulated the biomass or the activities of groundwater biofilms. Inversely, ratios lower than 1 indicated that SIS diluted the concentration of nutrients in groundwater or inhibited the biomass and activities of groundwater biofilms.

2.4 Estimations of SIS characteristics and rain events characteristics during the 6 experimental periods

The thickness of the vadose zone was measured as the depth (in meters) between the soil and the groundwater table for each SIS and experimental period before rain events. Data were obtained from long-term continuous measurements of groundwater table level (see section 2.3.). The second SIS characteristics (the characteristic time of stormwater transfer from surface to groundwater table, water transit time) was estimated as the delay between the rain peak and the peak of infiltrated stormwater reaching the aquifer in hours. As stormwater runoff had a low electric conductivity ($< 100 \mu\text{S}$) in comparison with groundwater ($> 500 \mu\text{S}$), electric conductivity in groundwater monitored with field probes in SIS was used to track the arrival of

low-mineralised stormwater into the aquifer (Fig. 1a). The water transit time was thus calculated as the time between the rain peak and the minimum electric conductivity measured in the SIS-impacted well of the studied SIS (Fig. 1a). As changes in electric conductivity in the aquifer could be only detected from rain events having intensities > 3 mm/h, we calculated an average water transit time for each SIS and each experimental period by considering rain events that occurred close to the experimental periods (within 3 months before and after the dates) and that were significant enough to be processed. Moreover, rain events selected to calculate average water transit times were also characterized by a unique significant rainfall to clearly associate a decrease of electric conductivity in the aquifer to a given rainfall event. Due to this procedure, calculated water transit times corresponded to a main characteristic of each SIS for each experimental period that described the dynamics of stormwater transfer from surface to groundwater through the vadose zone. In most of the SIS, this water transit time exhibited a small dependence to the cumulative rainfall with relative standard deviation lower than 13%, except in the Fey SIS where a large variability (relative standard deviation of 42%) and an increasing trend with cumulative rainfall were observed (Table S1 in Supplement).

Groundwater table elevation was measured using continuous monitoring of groundwater level with field probes in all SIS (see section 2.3.). These elevations corresponded to the maximum increase in groundwater level observed during each experimental period in SIS-impacted wells of each SIS (Fig. 1b – black arrow). This elevation is considered as a proxy of groundwater recharge rate during rain events since it is mainly controlled by the injected flow rate in the SIS. The absolute value of elevation is strongly controlled by the aquifer transmissivity since a high transmissivity promotes a quick lateral flow in the aquifer which limits the elevation. In this study, all the studied SIS are set on a similar fluvio-glacial geological formation (Foulquier et al., 2009) which tends to promote similar behaviours. The elevation is also impacted by vadose

zone properties since longer residence times could correspond to a limitation of the percolation flow rate and thus a time spreading of the groundwater table elevation curve.

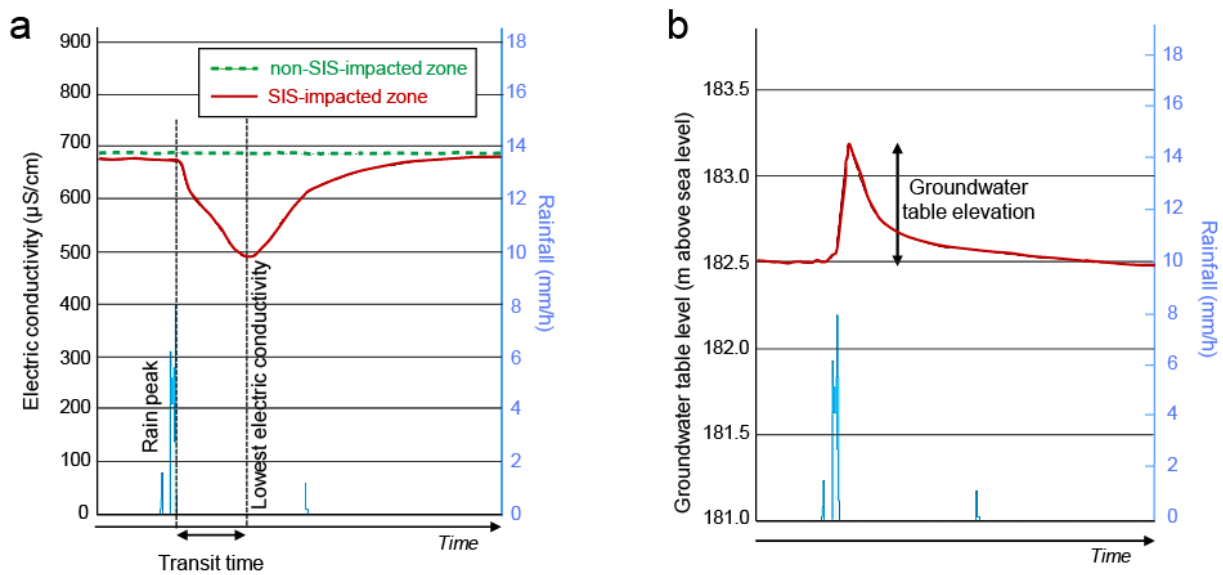


Figure 1: Examples of (a) electric conductivity monitoring to estimate stormwater transit time from surface to groundwater; and of (b) groundwater table level monitoring to measure groundwater table elevation (in meters above sea level) obtained in Django-Reinhardt (DjR) site.

2.5 Statistical analyses

Over the six experimental periods and for the five studied SIS, the obtained dataset comprised 21 points (ratios between measures made in SIS-impacted wells and measures made in non-SIS-impacted zone for each SIS) for which it was possible to measure the impacts of SIS on chemical and microbial groundwater variables and to assess the influence of the three factors associated to SIS characteristics (vadose zone thickness and transit time of water from surface to groundwater) and groundwater table elevations on these impacts. In a first step, vadose zone thicknesses, water transit times and groundwater table elevations were compared among SIS using non parametric Kruskal-Wallis tests. Then, generalized linear models were used to evaluate the influence of vadose zone thickness, transit time of water from surface to groundwater and groundwater table elevation on chemical and microbiological changes

associated with SIS impacts. As Shapiro-Wilk normality tests showed that a majority of variables did not present normal distributions, all variables were $\ln(x+1)$ transformed to perform generalized linear models. We checked for multi-collinearity among the three factors (vadose zone thickness, transit time of water from surface to groundwater, and groundwater table elevation) using variance inflation factors (VIF, Zuur et al., 2010) and found them to be acceptable (all VIF < 3; Hair et al., 2010). As the three factors could influence nutrients and microbial variables, a procedure of model selection was performed for each nutrient and microbial variable based on information theory (Burnham and Anderson 2002). The different models were ranked according to Akaike's Information Criterion adapted for small sample size (AICc, "car" R package) by starting from the most complete model (including all factors in addition). For all tested models, a comparable estimation of the amount of explained variance among models with variable numbers of parameters was performed by calculating the adjusted McFadden's pseudo- R^2 statistics (McFadden 1974). Statistical analyses (Kruskal-Wallis tests, generalized linear models) as well as graphics were performed with R 3.4 software (R Development Core Team, 2018).

3 Results

3.1 Variability in vadose zone thickness, water transit time and groundwater table elevation among SIS

Vadose zone thickness and water transit time through the vadose zone significantly differed among the studied SIS based on measurements performed in SIS-impacted wells (Fig. 2, Kruskal-Wallis tests, $\chi^2=17.576$, $df = 4$, $p\text{-value} = 0.001493$ for vadose zone thickness, $\chi^2 = 17.955$, $df = 4$, $p\text{-value} = 0.00126$ for water transit time). Although water transit time was expected to increase with the vadose zone thickness, these two variables were not significantly

correlated (Pearson’s correlation, $R^2 = 0.089$, $p > 0.19$). It was mainly due to GB site which had a thin vadose zone like Min and IUT sites but was characterized by a water transit time comparable to thick vadose zone sites like Fey and DjR (Fig. 2). In contrast with these two first variables, groundwater table elevations were not significantly different among the studied SIS (Fig. 2, Kruskal-Wallis tests, $\chi^2=6.17$, $df = 4$, $p\text{-value} > 0.18$) because they were significantly correlated with the “experimental period-specific” cumulative rainfalls reported on Table 2 (Pearson’s correlation, $R^2 = 0.30$, $p < 0.01$).

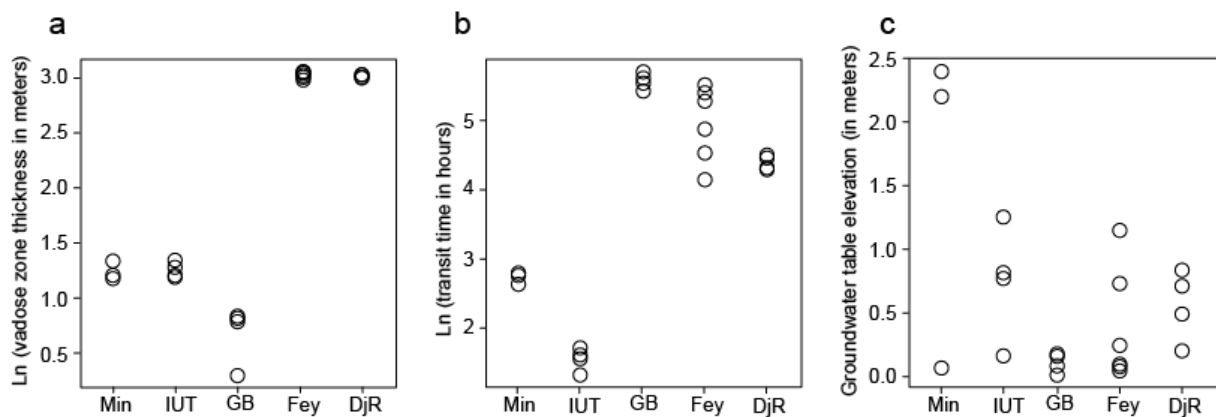


Figure 2: Distribution of (a) vadose zone thicknesses, (b) water transit times from surface to groundwater and (c) groundwater tables elevations measured during six rain events for the five stormwater infiltration systems ($n = 21$). Ln (vadose zone thickness) and Ln (water transit time) were displayed on the graphs. Codes for SIS: Min = Minerve, IUT = IUT, GB = Grange Blanche, Fey= Feyzin, DjR = Django Reinhardt.

3.2 Impact of SIS on groundwater chemistry

Overall, SIS reduced the concentrations of NO_3^- in groundwater (i.e. NO_3^- concentration measured in SIS-impacted well was lower than the concentration measured in the corresponding non-SIS-impacted well leading to ratios < 1) at the exception of two experimental periods for GB site (i.e. ratios higher than 1, Fig. 3a). The model selection procedure starting from the most complete model including vadose zone thickness, transit time of water from surface to groundwater and groundwater table elevation resulted in the selection of a model with

groundwater table elevation as unique predictor of NO_3^- changes due to SIS in groundwater (generalized linear model, $F_{(1,19)} = 7.24$, $p < 0.05$, Table 3a). Higher was the groundwater table elevation, higher was the reduction of NO_3^- concentration induced by SIS (Fig. 3b).

PO_4^{3-} concentration was not consistently impacted by SIS as most points reported on Fig. 3c were close to a ratio of 1. Nevertheless, we observed high enrichments of PO_4^{3-} concentrations in groundwater below SIS at three experimental periods for IUT site and at two periods for GB site. Overall, PO_4^{3-} changes due to SIS were negatively linked with vadose zone thickness (Fig. 3d, generalized linear model, $F_{(1,19)} = 9.86$, $p < 0.01$) and vadose zone thickness was the unique factor selected in the model (Table 3b).

The impact of SIS on DOC concentrations in groundwater varied strongly in each site among experimental periods (Fig. 3e). Overall, SIS increased the concentrations of DOC in groundwater as observed in 76% of occasions (points > 1 on Fig. 3e). The model selection showed that DOC changes due to SIS were uniquely influenced by the water transit time from surface to groundwater (generalized linear model, $F_{(1,19)} = 4.27$, $p < 0.05$, Table 3c). Highest groundwater enrichments in DOC due to SIS were observed in SIS characterized by the shortest water transit time from surface to the aquifer (Fig. 3f).

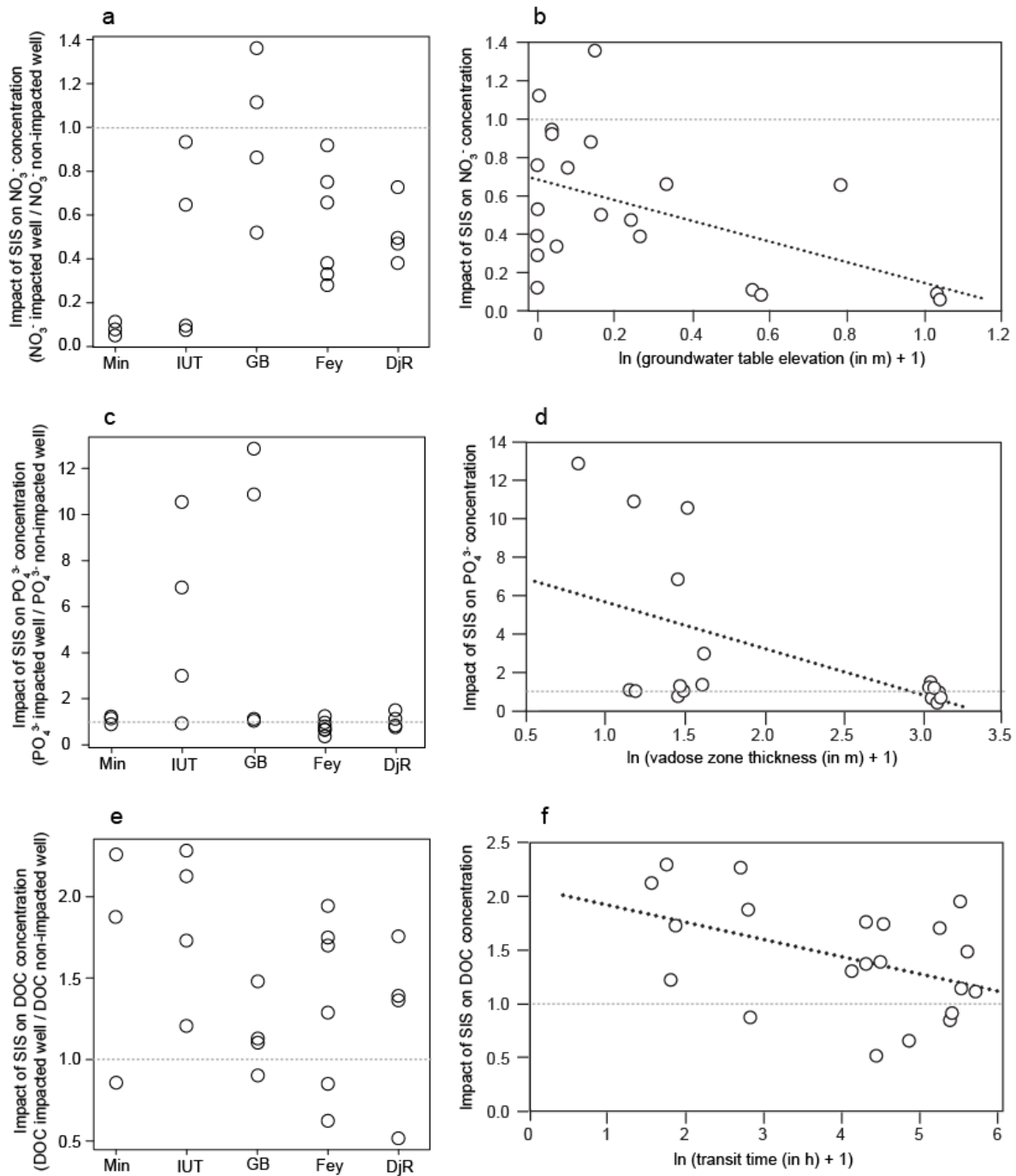


Figure 3: Left panels: Influence of SIS on (a) NO_3^- , (c) PO_4^{3-} and (e) DOC concentrations measured during rain events for the five stormwater infiltration systems ($n = 21$). Points below the dotted lines (at 1 on the Y-axis) indicate that SIS reduced nutrient concentrations in groundwater whereas points above the dotted lines indicate that SIS produced an enrichment of nutrients in groundwater. Codes for SIS: Min = Minerve, IUT = IUT, GB = Grange Blanche, Fey= Feyzin, DjR = Django Reinhardt. Right panels: Relationships between the significant explanatory variables (vadose zone thickness, water transit time from surface to groundwater and groundwater table elevation) and changes of (b) NO_3^- , (d) PO_4^{3-} and (f) DOC concentrations associated with SIS.

Table 3: Model selection for estimating the impacts of stormwater infiltration systems on nutrient concentrations in groundwater. Models are ranked by decreasing Akaike's information criterion corrected for small sample size (AICc). A difference of AICc between two models is considered as a significant improvement in favor of the model with the lowest AICc. The 'adjusted R²' (modified R² that has been adjusted for the number of factors included in the model) is indicated here as an estimation of the proportion of variance explained by the corresponding model. GTE = Groundwater Table Elevation, TT = Transit Time of water from surface to the aquifer, VZT = Vadose Zone Thickness

a) Impact on NO₃⁻ concentrations

General Linear models	AICc	adjusted R²
ln (impact on NO ₃ ⁻ +1) ~ ln (GTE+1)	-0.760	0.238
ln (impact on NO ₃ ⁻ +1) ~ ln (TT+1)	-0.218	0.218
ln (impact on NO ₃ ⁻ +1) ~ ln (TT+1) + ln (GTE+1)	0.820	0.251
ln (impact on NO ₃ ⁻ +1) ~ ln (VZT+1) + ln (GTE+1)	1.407	0.230
ln (impact on NO ₃ ⁻ +1) ~ ln (TT+1) + ln (VZT+1)	1.962	0.210
ln (impact on NO ₃ ⁻ +1) ~ ln (TT+1) + ln (VZT+1) + ln (GTE+1)	2.789	0.263
ln (impact on NO ₃ ⁻ +1) ~ ln (VZT+1)	6.023	-0.053

b) Impact on PO₄³⁻ concentrations

General Linear models	AICc	adjusted R²
ln (impact on PO ₄ ³⁻ +1) ~ ln (VZT+1)	43.33	0.307
ln (impact on PO ₄ ³⁻ +1) ~ ln (VZT+1) + ln (GTE+1)	45.52	0.299
ln (impact on PO ₄ ³⁻ +1) ~ ln (TT+1) + ln (VZT+1)	46.41	0.269
ln (impact on PO ₄ ³⁻ +1) ~ ln (TT+1) + ln (VZT+1) + ln (GTE+1)	48.65	0.271
ln (impact on PO ₄ ³⁻ +1) ~ ln (TT+1)	51.46	-0.020
ln (impact on PO ₄ ³⁻ +1) ~ ln (GTE+1)	52.10	-0.052
ln (impact on PO ₄ ³⁻ +1) ~ ln (TT+1) + ln (GTE+1)	54.27	-0.063

c) Impact on dissolved organic carbon (DOC) concentrations

General Linear models	AICc	adjusted R²
ln (impact on DOC +1) ~ ln (TT+1)	-1.233	0.140
ln (impact on DOC +1) ~ ln (TT+1) + ln (GTE+1)	0.708	0.141
ln (impact on DOC +1) ~ ln (TT+1) + ln (VZT+1)	1.828	0.094
ln (impact on DOC +1) ~ ln (VZT+1)	2.417	-0.023
ln (impact on DOC +1) ~ ln (GTE+1)	2.758	-0.040
ln (impact on DOC +1) ~ ln (TT+1) + ln (VZT+1) + ln (GTE+1)	4.100	0.095
ln (impact on DOC +1) ~ ln (VZT) + ln (GTE)	5.421	-0.075

3.3 Impact of SIS on groundwater microbiology

The impact of SIS on biofilm biomass varied strongly among experimental periods and among SIS (Fig. 4a). The biofilm biomass changes between non SIS-impacted and SIS impacted wells showed that only around 50% of points indicated a stimulation of biofilm biomass by SIS (11 points out of 21 had values > 1 , Fig. 4a). Generalized linear model selections showed that biofilm biomass was only negatively linked with water transit time from surface to groundwater (Fig. 4b, generalized linear model, $F_{(1,19)} = 8.27$, $p < 0.01$, Table 4a). Interestingly, DOC and biofilm biomass changes due to SIS, both influenced by water transit time, were also correlated to each other (Pearson's correlation, $R^2 = 0.198$, $p < 0.05$): highest groundwater enrichments in DOC due to SIS were associated with the highest stimulations in biofilm biomass associated with SIS.

The stimulations of biofilm activities by SIS represented only 33% of points for dehydrogenase activity (7 points from 21 had values > 1 , Fig. 4c) and around 50% of points for hydrolytic activity (11 points from 21, Fig. 4e). Generalized linear models showed that the impacts of SIS on the two microbial activities (dehydrogenase and hydrolytic) were positively correlated with groundwater table elevation (Fig. 4df, generalized linear models, $F_{(1,19)} = 10.1$, $p < 0.01$ and Table 4b for dehydrogenase activity, and $F_{(1,18)} = 15.86$, $p < 0.001$ and Table 4c for hydrolytic activity). Thus, groundwater table elevation was clearly the main factor influencing both the impacts of SIS on dehydrogenase and hydrolytic activities of microorganisms in groundwater. More precisely, the strongest stimulations of dehydrogenase and hydrolytic biofilm activities due to SIS were measured where the strongest groundwater table elevations were observed (Fig. 4df).

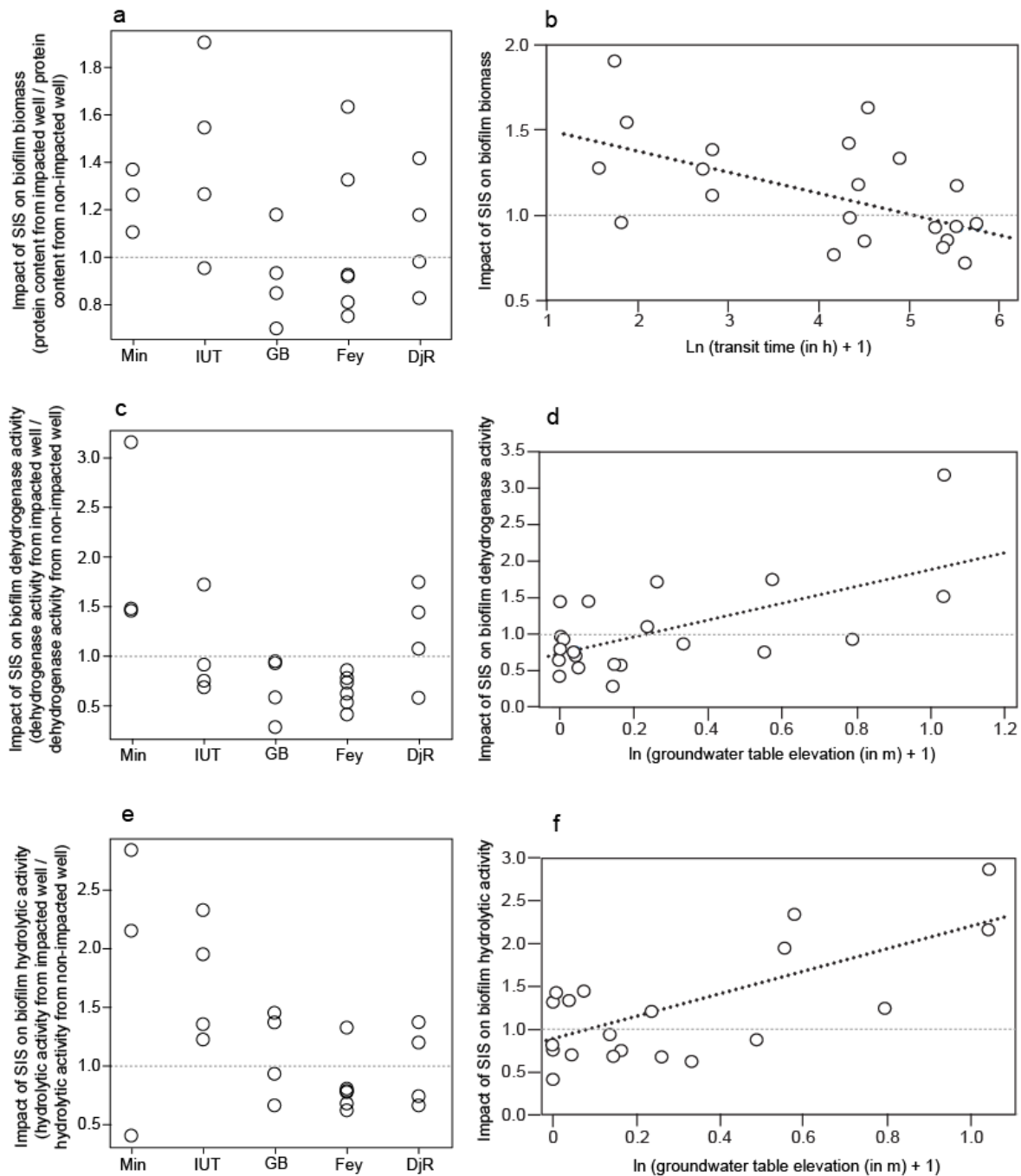


Figure 4: Left panels: Influence of SIS on (a) biofilm biomass, (c) dehydrogenase activity and (e) hydrolytic activity measured during rain events for the five stormwater infiltration systems ($n = 21$). Points below the dotted lines (at 1 on the Y-axis) indicate that SIS reduced microbial growth or activity in groundwater whereas points above the dotted lines indicate that SIS stimulated microbial biofilms in groundwater. Codes for SIS: Min = Minerve, IUT = IUT, GB = Grange Blanche, Fey= Feyzin, DjR = Django Reinhardt. Right panels: Relationships between the significant explanatory variables (vadose zone thickness, water transit time from surface to groundwater and groundwater table elevation) and changes of (b) biofilm biomass, (d) dehydrogenase activity and (f) hydrolytic activity associated with SIS.

Table 4: Model selection for estimating the impacts of stormwater infiltration systems on microbial variables in groundwater. Models are ranked by decreasing Akaike's information criterion corrected for small sample size (AICc). A difference of AICc between two models is considered as a significant improvement in favor of the model with the lowest AICc. The 'adjusted R²' (modified R² that has been adjusted for the number of factors included in the model) is indicated here as an estimation of the proportion of variance explained by the corresponding model. GTE = Groundwater Table Elevation, TT = Transit Time of water from surface to the aquifer, VZT = Vadose Zone Thickness

a) Impact on microbial biomass (MB)

General Linear models	AICc	adjusted R²
$\ln(\text{impact on MB}+1) \sim \ln(\text{TT}+1)$	-22.88	0.267
$\ln(\text{impact on MB}+1) \sim \ln(\text{TT}+1) + \ln(\text{GTE}+1)$	-21.72	0.294
$\ln(\text{impact on MB}+1) \sim \ln(\text{TT}+1) + \ln(\text{VZT}+1)$	-20.06	0.236
$\ln(\text{impact on MB}+1) \sim \ln(\text{TT}+1) + \ln(\text{VZT}+1) + \ln(\text{GTE}+1)$	-18.33	0.257
$\ln(\text{impact on MB}+1) \sim \ln(\text{VZT}+1)$	-15.45	-0.044
$\ln(\text{impact on MB}+1) \sim \ln(\text{GTE}+1)$	-15.83	-0.026
$\ln(\text{impact on MB}+1) \sim \ln(\text{VZT}+1) + \ln(\text{GTE}+1)$	-12.77	-0.081

b) Impact on dehydrogenase activity (DA)

General Linear models	AICc	adjusted R²
$\ln(\text{impact on DA}+1) \sim \ln(\text{GTE}+1)$	2.431	0.313
$\ln(\text{impact on DA}+1) \sim \ln(\text{TT}+1) + \ln(\text{GTE}+1)$	5.308	0.282
$\ln(\text{impact on DA}+1) \sim \ln(\text{VZT}+1) + \ln(\text{GTE}+1)$	5.448	0.277
$\ln(\text{impact on DA}+1) \sim \ln(\text{TT}+1)$	6.928	0.149
$\ln(\text{impact on DA}+1) \sim \ln(\text{TT}+1) + \ln(\text{VZT}+1) + \ln(\text{GTE}+1)$	8.684	0.244
$\ln(\text{impact on DA}+1) \sim \ln(\text{TT}+1) + \ln(\text{VZT}+1)$	10.02	0.101
$\ln(\text{impact on DA}+1) \sim \ln(\text{VZT}+1)$	10.98	-0.033

c) Impact on hydrolytic activity (HA)

General Linear models	AICc	adjusted R²
$\ln(\text{impact on HA}+1) \sim \ln(\text{GTE}+1)$	-1.979	0.426
$\ln(\text{impact on HA}+1) \sim \ln(\text{VZT}+1) + \ln(\text{GTE}+1)$	-0.631	0.443
$\ln(\text{impact on HA}+1) \sim \ln(\text{TT}+1) + \ln(\text{GTE}+1)$	0.827	0.402
$\ln(\text{impact on HA}+1) \sim \ln(\text{TT}+1) + \ln(\text{VZT}+1) + \ln(\text{GTE}+1)$	2.762	0.413
$\ln(\text{impact on HA}+1) \sim \ln(\text{TT}+1)$	4.866	0.205
$\ln(\text{impact on HA}+1) \sim \ln(\text{TT}+1) + \ln(\text{VZT}+1)$	5.956	0.237
$\ln(\text{impact on HA}+1) \sim \ln(\text{VZT}+1)$	6.954	0.122

4 Discussion

SIS characteristics (vadose zone thickness, water transit time) were found to be stronger determinants of SIS impacts on groundwater chemistry and microbial biomass than groundwater recharge rate (estimated by groundwater table elevations, Moon et al. 2004; Labrecque et al. 2020). More precisely, the impacts of SIS on PO_4^{3-} concentrations, DOC concentrations, and biofilm biomass were inversely correlated with the vadose zone thickness and/or the transit time of stormwater from surface to groundwater table.

The impact of SIS on PO_4^{3-} concentrations in groundwater decreased with vadose zone thickness. This relationship was probably associated with the influence of vadose zone thickness on aerobic-anaerobic processes occurring in infiltration porous media. As highlighted in experimental columns simulating water infiltration through SIS (Nogaro et al., 2007), a sharp depletion of dissolved oxygen concentrations occurred in the first cm of infiltration basins during recharge due to microbial activities. This decrease in dissolved oxygen concentrations observed in the top cm of SIS can be counterbalanced by a re-oxygenation of infiltrated water during its transfer in the vadose zone (Foulquier et al., 2010a; McMahon and Chapelle, 2008). Nevertheless, this re-oxygenation could not occur when the vadose zone is thin and rapidly saturated with water during stormwater runoff infiltration (Goutaland et al., 2008). In these conditions, anaerobic conditions could occur and lead to a facilitated transport of PO_4^{3-} from surface to groundwater by limiting the adsorption of P on sediments and/or inducing a release of P from previously adsorbed PO_4^{3-} (Nogaro et al., 2007). Such process could explain why SIS impacts on PO_4^{3-} enrichment in groundwater were the highest in SIS with a thin vadose zone. Nevertheless, these impacts were not reproducible from one rain event to another for each SIS. For example, strong enrichments in PO_4^{3-} (more than 10-fold increases) were only observed in GB SIS during two experimental periods (Fig. 3c). It means that processes leading to PO_4^{3-}

enrichment by SIS also depended on local conditions during experimental periods. We can suppose that the water saturation of the vadose zone preceding the experimental periods had a significant effect on the set up of aerobic-anaerobic conditions in the vadose zone during rain events.

The impact of SIS on DOC concentrations reaching the aquifer was negatively correlated with the water transit time from surface to groundwater. These results highlighted that the retention of DOC supplied with stormwater runoff was linked to the contact time between infiltrated stormwater and the vadose zone. Thus, rapid infiltration (short water transit time between surface to groundwater) led to a low retention of DOC in the vadose zone and a groundwater enrichment of DOC by SIS. Several studies (Datry et al., 2005; McMahon and Chapelle, 2008; Pabich et al., 2001, Voisin et al. 2020) showed a significant impact of vadose zone thickness on DOC retention through the vadose zone as the retention time of infiltrated water in vadose zone classically increases with its thickness. However, we showed that the water transit time from surface to groundwater determining the DOC retention in the vadose zone was not always linked with vadose zone thickness. Indeed, the long water transit time observed in GB site despite its thin vadose zone thickness clearly highlighted that a potential reduction of hydraulic conductivity in the bed of an infiltration basin limits the impact of this SIS on groundwater DOC enrichment. Thus, water transit time that takes into account the average time taken by stormwater to reach the aquifer appears as a better indicator of the role of the vadose zone on DOC retention than the vadose zone thickness.

In correlation with DOC dynamics through the vadose zone, the highest stimulations of biofilm biomass due to SIS were observed in SIS characterized by the shortest water transit times from surface to groundwater. As the studied aquifer was C-limited (e.g., Mermillod-Blondin et al. 2013), the ability of microorganisms to develop biomass as biofilms in groundwater was

inextricably linked with the availability of DOC. Several studies (Foulquier et al. 2011, Voisin et al. 2020, Lebon et al. 2021) performed on stormwater infiltration basins already highlighted that SIS-associated DOC enrichments in groundwater stimulated biofilm biomass. We also observed a positive correlation between the SIS impacts on DOC concentrations and their impacts on biofilm biomass. Based on this relationship, our results clearly indicate that water transit time from surface to groundwater was the main factor determining the impact of SIS on microbial biomass through its influence on DOC transfer from surface to the aquifer. Thus, the assessment of the water transit time through the vadose zone should be considered in the future to evaluate the risks of urban stormwater infiltration practices on microbial development in groundwater.

Contrasting with the main influences of SIS characteristics (vadose zone thickness or water transit time) on SIS impacts on PO_4^{3-} and DOC concentrations in groundwater, NO_3^- concentrations in groundwater were significantly influenced by groundwater recharge rates during rain events (estimated by groundwater table elevations). As observed for electric conductivity (Fig. 1), the recharge of groundwater with stormwater runoff produced a dilution of NO_3^- in the aquifer. Stormwater runoff had low concentrations of NO_3^- compared to the Eastern aquifer of Lyon (Datry et al. 2004, Voisin et al. 2018). In such conditions, NO_3^- in the aquifer was diluted by the flux of stormwater below infiltration basins, explaining the correlation between groundwater table elevation (groundwater recharge rate with stormwater) and changes of NO_3^- concentrations due to SIS.

Overall, the present study based on several SIS and rain events demonstrated that changes in nutrients induced by SIS depended on the main source of nutrient in the system. For nutrients supplied to groundwater by stormwater runoff (PO_4^{3-} and DOC), SIS characteristics (vadose zone thickness and transit time of water from surface to groundwater) which reflected

biogeochemical conditions and water transfer in the vadose zone mainly influenced the impact of SIS on nutrient contamination of the aquifer. For nutrients predominantly present in groundwater like NO_3^- , the flux of stormwater recharging the aquifer determined the impact of SIS as this recharge diluted nutrients in groundwater. Thus, predicting the impacts of SIS on groundwater chemistry needs to consider SIS characteristics or groundwater recharge rates depending on the origin of the chemical compounds (as stressed by Pinasseau et al. 2020 for pharmaceuticals and pesticides).

As suggested by Lebon et al. (2020), groundwater recharge rate during rain events (estimated by groundwater table elevation) was the main determinant of SIS impacts on microbial activity in groundwater. The highest stimulations of dehydrogenase and hydrolytic activities due to SIS were measured for the highest groundwater table elevations. This microbial stimulation might have been due to increased dissolved nutrient fluxes due to increased water flow velocities in the aquifer during recharge (Dobson et al. 2007; Rühle et al., 2014 and 2015) and/or a remobilization of nutrients accumulated in the vadose zone during groundwater table elevation (Gao et al., 2006; Zhuang et al., 2009). This second explanation based on a remobilization of nutrients and organic compounds trapped in the vadose zone during previous rain events could not be validated as the impact of SIS on groundwater DOC, which is the main limiting element for microorganisms in the studied aquifer (Mermillod-Blondin et al. 2013), did not increase with groundwater table elevation due to recharge. Thus, increased water flow velocities in the aquifer during recharge was the most probable mechanism explaining the stimulations of microbial activities by increasing the fluxes of DOC fueling the groundwater biofilms. Such positive influence of the fluxes of solutes and DOC on biofilm activity has been already observed in aquifer and streambed sediments (Battin, 2000; Foulquier et al. 2011; Battin et al. 2016) but, to our knowledge, the present study is the first to highlight a link between the

groundwater recharge rate and the SIS-associated changes of microbial biofilm activities on a short temporal scale (10 days).

Surprisingly, the present study performed on 6 experimental periods with contrasted meteorological conditions demonstrated that SIS could reduce the biomass and the activities of microbial biofilms in groundwater. This result contrasts with previous studies performed on a limited number of experimental periods (1 or 2) which showed a clear stimulation of biofilm biomass and activities in SIS-impacted zones compared with non-SIS-impacted zones (Voisin et al. 2020, Lebon et al. 2021). By increasing the number of experimental periods, we observed that more than 50% of measured ratios for microbial variables between SIS-impacted and non-SIS impacted zones were lower than 1, indicating cases when microbial biomass and/or activities were lower in SIS-impacted well than in non-SIS-impacted well of the studied SIS. These microbial inhibitions due to infiltration could have been associated with the presence of biocides in SIS-impacted zone of the aquifer because these kinds of compounds are often present in urban stormwater runoff (Masoner et al., 2019; Linke et al., 2021) and in SIS-impacted groundwater (Pinasseau et al., 2019). However, this microbial inhibition could be also due to the fact that stormwater reaching the aquifer could be less rich in biodegradable nutrients and elemental microelements (e.g., Fe, Mg and Mn) than the aquifer. In this case and for specific campaigns and basins, the stormwater infiltration could dilute the biodegradable substrates present in groundwater, reducing the availability of nutrients for microbial growth and activities (Brindha et al., 2019; Da Costa et al., 2020). Further experiments are clearly needed to finely decrypt the mechanisms (dilution of elemental compounds for microbial biofilms, impact of biocides etc.) explaining the inhibitory effect of SIS on groundwater microorganisms and their potential consequences on groundwater quality.

5 Conclusions

The present study clearly showed that vadose zone thickness, water transit time from surface to groundwater and groundwater recharge rate significantly influenced the impact of SIS on groundwater chemistry and/or microbiology. However, the significance of each investigated factor depended on the studied chemical and microbial variables. SIS characteristics associated with vadose zone (vadose zone thickness and water transit time from surface to groundwater) influenced the enrichment of groundwater with nutrients supplied to SIS by stormwater runoff (PO_4^{3-} and DOC) and the resulting effects on biofilm development (increase in biofilm biomass) in groundwater. In addition, stormwater fluxes reaching the aquifer could significantly explain the dilution of NO_3^- in groundwater below SIS but also the changes of microbial activities. Based on these conclusions, we can make recommendations about the implementation of infiltration basins in urban areas for stormwater management to limit the impact of SIS on groundwater ecosystems. Indeed, SIS with thick vadose zones and long water transit times from surface to groundwater should be favored to reduce the contamination of groundwater with nutrients supplied with stormwater runoff and their resulting effect on microbial growth in groundwater that could be associated with the presence of pathogenic bacteria (Colin et al. 2020). The installation of a homogeneous sediment layer of lower permeability than the surrounding soil at the bottom of the infiltration basin could be also an option to increase the retention time of infiltrated stormwater in the soil and the vadose zone, favoring water purification processes and then groundwater quality protection. Recommendations could be also made to control groundwater recharge rate during rain events: reduction of the ratio between the catchment area and the infiltration area, development of retention basins limiting the infiltrated water flow rate during rain event. However, the inhibitions of groundwater microbial biofilms due to SIS were commonly observed in the present study and yet the mechanisms explaining these microbial responses remain unclear. Further experiments in

environmental chemistry are clearly needed to evaluate whether these microbial inhibitions might be associated with an enrichment of the groundwater with biocides and a degradation of groundwater quality.

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