Bilayer infiltration system combines benefits from both coarse and fine sands promoting nutrient accumulation in sediments and increasing removal rates

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Abstract

Infiltration systems are treatment technologies based on water percolation through porous media where biogeochemical processes take place. Grain size distribution (GSD) acts as a driver of these processes and their rates, as well as it influences nutrient accumulation in sediments. Coarse sands inhibit anaerobic reactions such as denitrification and could constrain nutrient accumulation in sediments due to smaller specific surface area. On the other hand, fine sands provide higher nutrient accumulation but need a larger

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Combining both sand sizes in a bilayer system would allow infiltrating greater volume of water and the occurrence of aerobic/anaerobic processes. We studied the performance of a bilayer coarse-fine system compared to a monolayer fine one –by triplicate- in an outdoor infiltration experiment to close the C-N-P cycles simultaneously in terms of mass balances. Our results confirm that the bilayer coarse-fine GSD promotes nutrient removal by physical adsorption and biological assimilation in sediments, and further it enhances biogeochemical process rates (two-fold higher than the monolayer system). In overall, the bilayer coarse-fine system allows treating larger volume of water per surface unit achieving similar removal efficiencies as the fine system.

1. Introduction

High nutrient loads into freshwater ecosystems lead worldwide to eutrophication, associated harmful algal blooms, and “dead zones” due to hypoxia (1). In urban areas, the input of nutrients from wastewater treatment plants (WWTP) into freshwaters can contribute to eutrophication processes especially in areas characterized by flow intermittency (2). However, biogeochemical dynamics are affected by numerous factors which hinder the understanding of physical, chemical and biological dynamics related to the potential nutrient removal of water bodies (3). The implementation of infiltration systems in WWTPs before pouring water into streams is considered as a way to decrease nutrient load inputs to rivers, thus diminishing river eutrophication especially during low flow periods (4). Infiltration systems are water treatment technologies that rely on fluid percolation through a porous medium where a combination of biological, chemical, and physical processes help improving the quality of the influent water during the infiltration path (5).

The grain size distribution (GSD) of the porous medium is an important characteristic for infiltration systems (6-8). GSD, mostly linked to the pore size distribution and connectivity, modulates the distribution and transport of terminal electron acceptors, nutrients and organic matter in depth (7-8), and acts as a driver of the biogeochemical processes and their rates (9). Also, substrate grain size influences wastewater compounds’ adsorption (10). On one hand, coarse, well-sorted sediments imply large permeability values that result in high dissolved oxygen (DO) concentrations in depth, promoting aerobic processes such as nitrification, but inhibiting anaerobic reactions which are of great importance for the complete total dissolved nitrogen (TDN) removal (11). Furthermore, coarse sediments enhance high loads...
of nutrients and organic matter which are associated with high biogeochemical rates but with low
advection times (12). However, coarse sediments could constrain compounds’ adsorption due to smaller
specific surface area (13). On the other hand, in fine sediments, the low DO supply through infiltration
drives anoxic conditions, where bacteria catalyze full denitrification (14). It is also known that fine sand
has larger specific surface area and high adsorbing capacity (15-16) but lower permeability which can
constrain wastewater treatment (17) since the risk of substrate clogging may increase (13). Considering
all this, it is difficult to assess which substrate size is better to use since both grain sizes display benefits
and drawbacks. Accordingly, Perujo et al. (12) carried out an experiment with the aim of deepening in the
relationship between physicochemical and biological parameters in different GSDs and results suggested
that a bilayer coarse-fine system may integrate the positive aspects of coarse sands and those of fine
sands. Kauppinen et al. (18) studied the performance of multi-layered sand filters but focusing mainly on
pathogens removal and using combinations of 0-8 mm sand, 0-2 mm biotite and 2-4 mm which showed
no different performance between them in terms of nutrients removal. Latrach et al. (19) used a multi-
soil-layering system which consisted of a matrix of permeable material (mainly gravel) with soil mixture
“boxes” composed of coarse and fine sand, silt and clay, resulting in a high permeable system used to
treat raw wastewater, but without carrying out a comparison on amongst system designed with different
grain size distributions.

The study of biogeochemical processes in sand filters is relatively complex and several processes are
involved in the transformation and removal of carbon, nitrogen and phosphorous compounds (20). We are
not aware of any work aimed at studying the relevance of a bilayer coarse-fine grain size distribution
compared to a monolayer fine sand system to close the C-N-P cycles simultaneously, thus quantifying all
the biogeochemical pathways of C, N and P occurring both in the interstitial water and in the sediment
surfaces. The main objective of this research is to compare the performance of a bilayer coarse-fine sand
system with that of a monolayer fine sand–in triplicate- in an outdoor infiltration experiment and
specifically, to study (i) the distribution of biogeochemical processes in the depth profile, (ii) the
importance of nutrient accumulation in sediments in regards to adsorption and assimilation processes; and
(iii) the link of biogeochemical process rates to removal efficiencies at each system, in order to decipher
whether the bilayer system optimizes the performance of infiltration systems. To achieve these objectives,
we move away from qualitative process’ description, and rely on quantification based on measurements
(sampling of interstitial water and sediment and analysis of chemical species in depth from in-situ
replicated tanks during 104 days) and validated by means of mass balance evaluations in depth as well as
the study of C:N:P molar ratios in sediments to further understand nutrient accumulation processes in
sediments.

2. Material and methods

2.1 Experimental setup

An outdoor infiltration experiment was performed with secondary treated municipal wastewater.
Physicochemical parameters of the infiltrated water and nutrient loadings are described in Table S1. Two
flow-through sand tank systems (three replicates per system) were created using different GSDs: (1) a
bilayer Coarse-Fine (CF) system, consisting of a 20 cm layer of coarse sand (0.9 – 1.2 mm) placed on top
of a 20 cm layer of fine sand (0.075 – 0.250 mm); and (2) a monolayer Fine (F) system, with 40 cm of
fine sand (0.075 – 0.250 mm) (Fig. S1). Sands were bought from a company dealing (and selling)
aggregates (Adicat Tribar, S.L.). They consisted of siliceous sand, rounded shape, free from clays and
organic matter and with low metal contents (Al₂O₃ < 0.5 % of the sand dry weight, Fe₂O < 0.04 %, CaO <
0.05 %, and K₂O < 0.5 %). Both systems consisted of tanks of 0.21 m³ capacity and 0.46 m² of infiltration
surface area; in the upper part of each one, a valve was used to ensure a constant water level creating a
constant surface water layer and a continuous infiltration in each system. At the bottom of each tank a
gravel layer was placed to facilitate drainage of water towards the outlet to resemble vertical infiltration
conditions. There was no water recirculation in the experiment. Three water ports were installed in the
wall of the tanks; at depths 4, 18 and 38 cm. The experiment was performed during 104 days from April
to July 2016. Outdoor temperature was 21.6 °C on average (21) and 234 mm of accumulated rainfall (22)
during the time the experiment ran. Sunlight conditions were allowed only in the surface of the tanks, to
mimic real infiltration basins. Since the accumulated rainfall only represented 0.12 % and 0.26 % of the
daily infiltrated water in our systems (CF and F, respectively) we consider that the effect of rainfall was
negligible. Hydraulic parameters of each system as well as organic and nutrient loadings are detailed in
Table S1.

Water and sediments (note that we use the term “sediment” to refer to the porous media (sand) which is
being colonized by biofilm) were sampled weekly at the start and biweekly at the end of the experiment,
for a total of 9 sampling campaigns. Samples were collected at about the same time (11 – 12 am), and
with consistent weather conditions (sunny, with no registered precipitations two days previous to
sampling) to minimize variability due to environmental factors. Water samples were collected from the inlet water, the surface pond, and the three water ports installed at each tank. Dissolved nutrient and organic carbon concentrations were analyzed for all samples.

Sediment sampling was performed using a sediment core sampler (Eijkelkamp 04.23.SA) to a depth of 40 cm and each core was subdivided in three specific depth layers (0-4 cm as “surface sediment”, 18-22 cm as “20 cm depth sediment” and 36-40 cm as “40 cm depth sediment”). Each sample was homogenized, and then subsamples of 1 cm$^3$ of sediment were collected using an uncapped syringe, and kept frozen (-20 ºC) until analysis to determine nutrient and carbon content within the sediments. After taking each sediment core, one methacrylate empty column was placed at the same place to avoid the collapse of the surrounding sediment in the tanks, in an attempt to minimize the disruption of the flow field.

2.2 Water analysis

Samples for dissolved nutrients and dissolved organic carbon (DOC) were filtered in pre-combusted (4h, 450 ºC) filters (GF/F, 0.7 µm, Whatmann). After filtering, dissolved nutrients were analyzed in fresh, while DOC samples were acidified and kept at 4 ºC until analysis. DOC concentrations were determined using a total organic carbon analyzer (TOC-V CSH Shimadzu). For inorganic nutrient determination, samples were filtered again (Nylon filters, 0.2 µm, Whatmann) and analyzed as follows: $\text{NO}_x$ - as the sum of $\text{NO}_2^-$ and $\text{NO}_3^-$ - by ionic chromatography (761 Compact IC 1.1 Metrôhm), and $\text{NH}_4$ by the spectrophotometric sodium salicylate protocol (23). Total dissolved nitrogen (TDN) and total dissolved phosphorous (TDP) samples were digested before analysis. The digestion protocol (adapted from Koroleff (24)) consisted in an oxidation where the reagent prepared in NaOH solution (0.375 M) containing $\text{K}_2\text{S}_2\text{O}_8$ (0.18 M) and $\text{H}_3\text{BO}_3$ (0.48 M) was added (2 ml) to filtered samples (20 ml) in tightly capped Pyrex glass tubes. Tubes were closed, shaken and autoclaved (90 min, 115 ºC). Absorbance was measured at 275 and 220 nm for TDN determination. TDP determination followed the protocol from Murphy and Riley (25). Dissolved organic nitrogen (DON) was calculated as the difference between TDN and inorganic nitrogen (DON = TDN – ($\text{NH}_4 + \text{NO}_x$)).

2.3 Sediment analysis

For carbon and nitrogen determination in sediments, distilled Milli-Q water (1.5 ml) was added to each sediment sample, then sonicated for 1 minute and shook. Aliquots of the extract (100 µl) were pipetted into pre-weight tin cups and placed in the oven at 60 ºC until drying. The remaining extract was kept at 4
C. Pipetting was repeated five times to obtain a final dry weight of ca. 1 - 3 mg. Tin cups were weighted and sediment carbon and nitrogen content were analyzed in a CN-analyzer (Carlo Erba).

The protocol used for P determination in sediments is adapted from Aspila et al. (26). The samples were dried at 50 °C, crushed and accurately weighted. Samples for total phosphorous (P_{tot}) determination were transferred into porcelain crucibles, ignited in a muffle furnace (550 °C for 1h, following Andersen (27)), and then let to cool for one hour. Samples for total phosphorous and inorganic phosphorous (P_{inorg}) determinations were transferred to 100 ml Erlenmeyer flasks with HCl acid (1.0 M, 25 ml). Mixtures were boiled for 15 minutes on a hot plate, filtered (Whatmann, 2.5 µm) to separate the liquid phase from the sediment, and ten-fold diluted with Milli-Q water. P_{tot} and P_{inorg} were determined by the Molybdate-blue method (25). P_{org} was determined by colorimetry, as the difference between P_{tot} and P_{inorg}. The ratio between P_{inorg}/P_{org} was calculated as an indicator of the proportion of inorganic phosphorous accumulated in sediments versus the organic phosphorous in sediments, where values >1 indicate dominance of inorganic P and values < 1 indicate dominance organic P. Sediment molar C:N:P ratios were also calculated.

2.4 Removal rates, removal efficiencies and mass balances

2.4.1 Removal rates

Biogeochemical transformation rates were calculated for different chemical species both in water and sediment. Data were transformed to homogeneous units to allow the comparison between the rates measured in water and in sediment. Total water nutrient loads were calculated at each sampling depth (surface water layer, 4 cm, 18 cm and 38 cm) for all the duration of the experiment, and balances between each two consecutive sampling points were calculated. The results were divided by the total duration of the experiment (104 days) and by the spatial volume of each layer. Data from the sediments were calculated separately for each depth (surface, 20 cm, and 40 cm), multiplied by the conversion factor (1.45 g DW-Dry Weight·per cm³) and divided by 104 days. All values are reported in µg·cm⁻³·day⁻¹. Negative rates indicate a removal process.

2.4.2 Removal efficiencies

Removal efficiencies were calculated from the nutrient loads following equation 1. Two calculations were performed: 1) from the inlet to the outlet, and 2) from the surface of the sediment to the outlet, this one to
exclude processes occurring in the layer of surface water and to focus on the processes occurring in the sediment. 

Removal efficiency (\%) = 100 \cdot \frac{\text{(inlet-outlet)}}{\text{inlet}}, \quad (1)

where, inlet – outlet are the nutrient loads (expressed in mg·day\(^{-1}\)).

2.4.3 Mass balances

Mass balances were calculated from the removal rates and results are reported for different depth infiltration layers (see Figure S1): top layer (includes water mass balance from surface water to 4 cm depth and the C-N-P contents measured in the surface sediment); mid layer (includes water mass balance from 4 cm to 18 cm depth and the sediment C-N-P contents measured at 20 cm depth); and finally, the bottom layer (includes water mass balance from 18 to 38 cm depth and the sediment C-N-P contents measured at 40 cm depth). As the potential processes occurring in the layer of surface water include complex biological (photosynthesis, planktonic uptake of nutrients, organic matter transformation) and chemical (photochemical) reactions that were not monitored, mass balances from inlet water to surface water were not assessed.

2.4.3.1 Carbon mass balances

It is assumed that the main DOC removal pathways are depth dependent. In the upper infiltration layer, the main C transformation pathways are C respiration (\(C_{\text{resp}}\)) and C accumulation in sediments (\(C_{\text{sed}}\)), and the mass balance can be expressed as:

\[ C_{\text{DOC inlet}} - C_{\text{sed}} - C_{\text{resp}} = C_{\text{DOC outlet}} \quad (2) \]

In depth, for the mid and bottom layers, transformation pathways are related to C used in denitrification (\(C_{\text{denitr}}\)), aside from respiration and accumulation in sediments (28). Nonetheless, as the two terms \(C_{\text{resp}}\) and \(C_{\text{denitr}}\) were not directly measured in the deeper layers, they were considered as a single term, denoted as \(C_{\text{resp/denitr}}\), so that the mass balance equation can be written as:

\[ C_{\text{DOC inlet}} - C_{\text{sed}} - C_{\text{resp/denitr}} = C_{\text{DOC outlet}} \quad (3) \]

2.4.3.2 Nitrogen mass balances

It is assumed that the main nitrogen transformation pathways are depth dependent: (i) TDN removal pathways in the top infiltration layer are driven by \(\text{NH}_4\) volatilization (29) and N accumulation in sediments; (ii) in depth TDN removal pathways consist of denitrification to N gas (30) and N
accumulation in sediments (31). Furthermore, (iii) DON can be mineralized to NH₄ (32); (iv) in depth NO₃ can be transformed to DON (33). Also, (v) under oxic conditions NH₄ is nitrified to NO₃ (32); (vi) in depth NO₃ can be denitrified to N₂ (14) or reduced to NH₄ (34).

According to these assumptions, N mass balances are described. TDN mass balances are given by equation 4 for the top infiltration layer and 5 for the mid and bottom ones. The main N removal paths are NH₄ volatilization (denoted in the equations as TDN$_{NH4\ volat}$), N being accumulated in sediments (TDN$_{N\ sed}$), and NO₃ denitrification (TDN$_{NO3\ denitr}$).

\[
TDN_{inlet} - TDN_{NH4\ volat} - TDN_{N\ sed} = TDN_{outlet}
\] (4)

\[
TDN_{inlet} - TDN_{N\ sed} - TDN_{NO3\ denitr} = TDN_{outlet}
\] (5)

Ammonium mass balances are given by equation 6 for the top layer and 7 for the mid and bottom ones. Positive contributions on NH₄ mass balances are the DON being mineralized to NH₄ (denoted by NH₄$_{DON\ mineral}$), and the NO₃ being reduced to NH₄ (NH₄$_{NO3\ reduction}$). Negative contributions on ammonium mass balance are the NH₄ that is being transformed to DON (NH₄$_{DON\ prod}$), the one that left the system via volatilization (NH₄$_{volat}$), the one being nitrified to NO₃ (NH₄$_{nitrif}$), and that accumulated in the sediment (NH₄$_{N\ sed}$).

\[
NH₄_{inlet} + NH₄_{DON\ mineral} - NH₄_{volat} - NH₄_{nitrif} - NH₄_{N\ sed} = NH₄_{outlet}
\] (6)

\[
NH₄_{inlet} + NH₄_{NO3\ reduction} - NH₄_{DON\ prod} - NH₄_{N\ sed} = NH₄_{outlet}
\] (7)

In parallel, NO₃ mass balances are given by equation 8 for the top infiltration layer (where there is a net production) and equation 9 for the mid and bottom ones (with a net reduction). Positive contribution on NO₃ mass balances include NH₄ being nitrified to NO₃ (NO₃$_{prod}$). The negative terms in the mass balance equations include the NO₃ leaving the system via denitrification (NO₃$_{denitr}$), that transformed to NH₄ via reduction (NO₃$_{reduction}$ -in the text called DNRA-), and the NO₃ being transformed to DON (NO₃$_{DON\ prod}$).

\[
NO₃_{inlet} + NO₃_{prod} = NO₃_{outlet}
\] (8)

\[
NO₃_{inlet} - NO₃_{denitr} - NO₃_{reduction} - NO₃_{DON\ prod} = NO₃_{outlet}
\] (9)

2.4.3.3 Phosphorous mass balances

For the phosphorus mass balances, the assumption is that the TDP removal pathways at any depth are driven by phosphorous content in sediments, TDP$_{P\ sed}$ (6). Thus, the TDP mass balance equation is written as follows:
\[ TDP_{inlet} - TDP_{P\,sed} = TDP_{outlet} \]  
\[ \text{(10)} \]

Where \( TDP_{P\,sed} \) can be also expressed as the sum of phosphorous adsorption in sediments, \( TDP_{P\,inorg} \) and phosphorous assimilation, \( TDP_{P\,org} \), resulting in the following mass balance equation:

\[ TDP_{inlet} - TDP_{P\,inorg} - TDP_{P\,org} = TDP_{outlet} \]  
\[ \text{(11)} \]

2.5 Data Analysis

Dissolved nutrient concentrations and DO were analyzed by repeated measures ANOVA (factors: depth and system, \( p < 0.05 \)). Data for each system were further analyzed separately through Tukey post-hoc analysis to detect significant different groups in depth. The parameters measured in sediments (C, N and P) were analyzed by covariance ANCOVA (factors: depth and system, \( p < 0.05 \)) and further analyzed using the Bonferroni post-hoc test to detect significant different groups in depth and significant differences between systems at each depth. \( P_{inorg}/P_{org} \) ratios were also analyzed (ANOVA, factor: depth and system, \( p < 0.05 \)) and the Tukey post-hoc analysis was then applied to all systems separately (factor: depth). Removal rates and removal efficiencies were subject to an ANOVA analysis to detect differences between systems at a given depth and for the overall systems (factor: system, \( p < 0.05 \)). All statistical analyses have been performed using R software (R version 3.1.1, Stats Package).

3. Results

3.1 Distribution of chemical species in depth

Concentrations of dissolved chemical species measured at each depth are shown in Figures S2 - S5. Both systems showed an increase in DOC, DON and DO concentrations in the surface water layer compared to those in the inlet water. In the surface water layer, there was also a slight decrease in the concentrations of \( \text{NH}_4, \text{NO}_x \) and TDP for the F system. These slight changes from the inlet water to the surface water layer (although not being statistically significant) resulted in higher \( \text{NO}_x \) and TDP concentrations in the surface water in the CF system; and higher DO concentration in the F one. A decrease in DOC and DON concentrations was observed in the top sediment layer (surface to 5 cm depth). TDN concentrations gradually decreased in depth, but higher values were measured in CF at 20 cm, as compared to those in F. \( \text{NH}_4 \) and \( \text{NO}_x \) concentration profiles in depth were similar in both systems; \( \text{NH}_4 \) concentration decreased at 5 cm depth but then increased. Oppositely, \( \text{NO}_x \) concentration increased at 5 cm depth but then decreased. The F system displayed higher \( \text{NH}_4 \) and lower \( \text{NO}_x \) concentrations at 20 cm depth, as
compared to CF. Both systems displayed a similar pattern for the TDP concentration profile, with a
decrease from the surface to 5 cm depth and then stabilization. Higher TDP concentrations were reported
in the CF system in the surface and at 20 and 40 cm depth than in the F system. DO concentrations
decreased with depth, with lowest values found in the F system at 20 and 40 cm depth.
C, N and P concentrations in sediments (Figure 1) were higher in the surface than in the deep layers. The
CF system showed higher inorganic P concentration in the surface sediment than F, while for total
phosphorous no significant differences between systems were detected. At depths 20 and 40 cm, C, N,
inorganic P and total P concentrations in sediments were significantly higher in the CF system than in F.
The ratio P_{inorg}/P_{org} (Table S2) decreased significantly in depth indicating higher accumulation of organic
P than inorganic P in deeper sediments in both systems. In the surface, the CF system showed
significantly higher P_{inorg}/P_{org} ratio than the F one. Sediment molar ratios are described as follows,
in the top layer both systems showed similar C:N ratio (≈ 7.8 - 7.6), N:P_{org} ratio (≈ 14.7 – 17.7), and C:P_{org} ratio
(≈ 115 – 146). In the mid layer, C:N ratio increased slightly in both systems (≈ 8.3 – 9), and N:P_{org} ratio
decreased (≈ 4.2 – 3.1) as well as C:P_{org} ratio (≈ 35.8 – 22.7). Molar ratios in the bottom layer are similar
than in the mid layer, C:N ratio (≈ 10.2 – 9.0), N:P_{org} ratio (≈ 2.7 – 4.3) and C:P_{org} ratio (≈ 23.8 – 32.5 in
CF and F, respectively).

3.2 Carbon, nitrogen and phosphorus mass balances

3.2.1 Carbon mass balances
Carbon mass balances per unit volume of sediment at each sediment layer are described in Figure 2.
Removal rates decreased in depth in both systems. In the top layer, DOC removal and C respiration rates
were largest in CF while C accumulation rates in sediments were similar between GSDs. This resulted in
C accumulation in sediments accounting for 25.1 % and 50.3 % of the DOC removal in CF and F,
respectively. Thus, respiration resulted in the main C removal path in the CF system (74.9 %), while in
the F one an equal contribution of C accumulation and C respiration to the global DOC balance was
found (50.3 % and 49.7 %, respectively). In the middle infiltration layer, both systems were estimated to
receive unaccounted C inputs as DOC balances were positive; for this reason, we could not estimate the
relative contribution of each carbon removal pathway in that layer, however greatest C accumulation rate
in sediments was found in CF. In the bottom layer (20 to 40 cm depth), greatest C accumulation rate was
found in CF but similar C respiration rates were described in both systems. In this layer, C_{sed} accounted
for 19.1 % and 17.3 %, while C. resplendens accounted for 80.9 % and 82.7 % of the total DOC removed in the CF and F systems, respectively.

### 3.2.2 Nitrogen mass balances

Nitrogen mass balances per unit volume of sediment are described in Figure 3. In general, process rates decreased in depth in both systems. In the top infiltration layer, ammonium volatilization was estimated as the main TDN removal path (92.4 % in CF and 90.7 % in F) while N accumulation in the surface sediments accounted for 7.6 % (CF) and 9.3 % (F) of the TDN removal. Notice that only small amounts of N were unaccounted for (3.0 and 0.7 µg N·cm⁻³·day⁻¹ for CF and F, respectively). In the mid and bottom layers, denitrification was estimated to be the main TDN removal pathway in both systems (90 – 95 %) while N accumulation in sediments accounted for only 2 – 10 %. Other N transformation pathways were estimated in the infiltration systems: DON mineralization to NH₄ and NH₄ nitrification to NOₓ in the top infiltration layer; DNRA and NOₓ transformation to DON in the middle one, and DNRA in the bottom one.

The contributions of these processes in the general balance of intermediate species of N (NH₄ and NOₓ) vary between GSDs. Thus, in CF we find that with regard to NH₄ transformation in the upper layer, dominates nitrification (65%). Regarding NOₓ transformation in this system, in the middle layer dominates the transformation to DON (43%) and denitrification (55%) and in the lower layer dominates DNRA (72%). However, in the F system, in the top layer dominates both nitrification and ammonium volatilization (∼ 50% each), in the middle layer dominates DNRA (43%) and denitrification (60%), and in the bottom layer dominates denitrification (80 %). Mass balance closure discrepancies in the mid and bottom layers were very low (0.17 - 0.25 and 0.41 – 0.44 µg N·cm⁻³·day⁻¹ for CF and F, respectively).

### 3.2.3 Phosphorous mass balances

Mass balances of the P species per unit volume of sediment are described in Figure 4. We assumed that all TDP removed was accumulated in the sediment through adsorption or assimilation. However, in some of the mass balance evaluations, TDP removal was lower than P accumulated in sediments, potentially indicating an unaccounted P input. From Total P accumulated in sediments, proportions between organic and inorganic P were calculated; in surface sediments, results suggested similar accumulation of inorganic P (49 %) and organic P (51 %) in CF, while accumulation of organic P (60 %) contributed more than inorganic P (40 %) in F. At 40 cm depth, organic P accumulation accounted for higher proportion (56 – 63 %) compared to inorganic P (37 – 44 %) in both systems. Higher TDP and P accumulation rates
were measured in the top infiltration layer. Comparing systems, TDP removal rate was higher in CF in
top and bottom layers and P accumulation rate was higher in the CF system in the mid infiltration layer.

3.3 Removal efficiencies

We report in Table 1 the removal efficiencies of each infiltration system. Considering only the layers that
constitute the sediments, both systems showed similar DOC (7 - 11 %), TDN (18 – 23 %) and TDP (14 –
16 %) removal efficiencies, but higher TDP and TDN removal rates were reported in CF as compared to
F. Including the processes in the full system (three sediment layers plus the surface water layer), the F
system resulted in higher TDP removal efficiency (23 %), compared to the CF (12 %).

4. Discussion

Our data support that biogeochemical processes of C-N-P species are depth and GSD dependent, both in
qualitative (most relevant processes) and in quantitative terms. As expected, highest rates occurred in the
top layer which might be controlled by high nutrient, and DO and electron acceptors availability as well
as higher biomass developed in surface sediments compared to deeper ones (35). Regarding our results,
nutrient accumulation in the top layer sediments played a key role on nutrient removal efficiencies,
especially for DOC and TDP removals (representing up to 50% of their respective removal, while N
accumulation in sediment represents 10% of its removal). This removal is suggested to be mainly due to
biological processes (assimilation) since the C:N:P elemental molar ratios (calculated from total C and N
and organic P in sediments) are close to the known Redfield ratio described for autotrophic biomass
(106:16:1, Redfield (36)). Interestingly, even though we expected higher nutrient accumulation rates in
the fine sediment due to higher surface area available in fine compared to coarse sediments (37), both
systems showed similar rates. Furthermore, in the case of P, where we distinguished the inorganic to the
organic fraction, inorganic P showed greater accumulation in the bilayer coarse-fine system. This
contradiction could be due to the fact that difference in sediment texture has mostly been associated with
the presence of silts and clays (< 2 µm) (38) and in our study the presence of silts and clays was excluded.
Furthermore, higher inorganic P accumulation measured in the bilayer system could be related to higher
TDP concentrations in the surface water layer (39) which could enhance physical adsorption of inorganic
P. It is also worth noting that we are not comparing coarse and fine sands, but GSDs where the bilayer
coarse-fine system does not act like coarse homogeneous sediment since physical characteristics such as
K and flow velocity are lower than a coarse sediment (12) and therefore both GSDs the bilayer coarse-
fine and the monolayer fine systems promote the removal of nutrients through the accumulation of these
nutrients in the sediments via adsorption and assimilation.

Other significant biogeochemical processes occurring in the top layer of infiltration systems are mostly
related to nitrogen (ammonium volatilization and the coupled processes of nitrification and DON
mineralization) but also include carbon respiration. As expected, C respiration, nitrification and DON
mineralization rates were approximately double in the bilayer system compared to the monolayer system
which would indicate that those rates could be proportional to input loads. Greater attention should be
focused on ammonium volatilization as it is the main TDN removal pathway in the aerobic top layer of
infiltration systems and it seems to be independent of input loads. Ammonium volatilization is a
physicochemical process driven by temperatures higher than 20 °C and pH ranging 6.6-8.0 (40) for this
reason showed similar rates in both systems.

In depth, accumulation of nutrients in sediments was less relevant as a removal path to that observed for
the top layer. The slight increase in C:N sediment molar ratios as well as the decrease in C:P_{org} and N:P_{org}
ratios compared to the top sediment layer could be linked to the fact that the dominant living biomass in
depth is microbial biomass which showed a different elemental molar ratio than autotrophic biomass.
Cleveland and Liptzin (41) stated C:N:P = 60:7:1 as the elemental molar ratio for microbial biomass.
According to this, sediment molar ratios at 20 and 40 cm depth following the elemental molar ratio
described for microbial biomass could be indicative of nutrient assimilation as a main pathway of nutrient
accumulation in sediments in depth, but also they could be linked to physical adsorption of microbial
biomass that is being transported from upper to lower sediment layers. Specifically, higher accumulation
rates in the interface of the bilayer system could be linked to physical entrapment of microbial biomass
when reaching the transition from the coarse to the fine sediment layers, as well as retention of carbon,
nitrogen and phosphorous species which are then potential to be biologically assimilated or physically
adsorbed. At the same time, the imbalance of the molar ratios to a greater accumulation of organic P (low
C:P_{org} and N:P_{org} ratios) suggests the deposition of dead organic matter and the accumulation and
adsorption of P in lower layers where the mineralization processes of this organic matter could possibly
be limited by reduced microbial activity and lower DO. We further contend that unaccounted C inputs
reported in the experiment at intermediate depths were probably due to the generation of microbial
soluble products (42), or to desorption of organic carbon retained in top sediments (43). Similarly,
unaccounted P inputs in mass balances at intermediate depths could be related to the release of
phosphates previously assimilated in upper layers by bacteria and algae following their death and subsequent degradation (44).

In depth, TDN concentration is the only parameter decreasing in the mid and bottom layers mainly due to anaerobic processes such as denitrification that could take place even in DO concentrations of around 4 mg O$_2$·L$^{-1}$ (45). Larger loading inputs could explain the larger denitrification rates reported in the bilayer system which resulted in higher TDN removal rates especially in the mid layer as compared to the monolayer fine one. However, sediment GSD in depth modulated N transformation pathways. Specifically, the larger C accumulation in the interface between the coarse and fine layer in CF could favor the conversion of NO$_x$ to DON, most relevant in organic horizons (33). NO$_x$ transformation to DON has been mainly attributed to abiotic processes (46) and specific DON reactions remain unknown. Interestingly, at 40 cm depth, DNRA dominated over denitrification in the CF system, while the opposite happened in the F system. Higher hydraulic conductivity in the former system could have favored interstitial DO transport in depth (11), driving the high DO concentration measured at 20 and 40 cm depth, and being the reason for DNRA being the dominant pathway in depth compared to denitrification (under oxic conditions the denitrification/DNRA ratio is low, Roberts et al. (47)). In this study, different contributions of N transformation pathways between the bilayer and the monolayer systems did not affect the overall TDN removal efficiencies, possibly due to the fact that main differences were achieved in the mid and bottom layers where N concentrations and transformation rates were far smaller than in the top ones.

To complete the picture, some comments can be made regarding biogeochemical processes occurring in the surface water layer. Although the observed changes were small, these may have implications in the global nutrient balance in infiltration systems. The most significant changes were the increase in DOC and DON concentrations, related to the potential release of soluble microbial products and algal exudates (48-49), and combined with NH$_4$, NO$_x$ and TDP concentrations decrease, these associated with possible nutrient uptake by planktonic microorganisms and algae (50-51). Photosynthetic activity in the surface water layer might be responsible for DO concentration increase (52) which, in turn, favours nitrification in the top sediment layer due to high DO concentration in infiltrated water (nitrification has high DO requirements: 4.57 g of O$_2$ per gram of NH$_4$, Tchobanoglous (53)).
Biogeochemical processes occurring in the surface water layer seem to be directly related to the hydraulic retention time (HRT). This should be interpreted carefully since, as large HRT could favor TDN and TDP removal efficiencies in monolayer fine systems, it could also decrease DOC removal efficiency.

Biogeochemical rates - influenced by GSDs - have implications on removal efficiencies in infiltration systems. Main processes related to DOC, TDN and TDP removals are located in the top sediment layer and include C respiration and assimilation, NH\textsubscript{4} volatilization, as well as P assimilation and adsorption.

On one hand, the bilayer coarse-fine system allows to infiltrate greater volume of water, and so greater input nutrient loads, than the monolayer fine system. This results in greater rates of biogeochemical processes that are dependent on input loads such as C respiration in the bilayer system. Furthermore, in the bilayer coarse-fine system the convergence of characteristics such as grain size distribution and advection times allows to achieve higher TDN and TDP removal rates in the bilayer, as well as similar or even higher (if comparing values in the interface) nutrient accumulation rates in sediments through physical adsorption and biological assimilation, compared to the monolayer system. Overall, the bilayer coarse-fine system allows to treat a larger volume of water per surface unit achieving similar removal efficiencies as the monolayer fine one.

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Supporting Information. Scheme of the two systems used in the experiment; physicochemical parameters measured at the inlet water hydraulic parameters and input loads of each infiltration sand system used; boxplots of dissolved species measured at different depths at each system (DOC, TDN, NO\textsubscript{x}, NH\textsubscript{4}, DON, TDP and DO) and ratios between inorganic and organic P measured in the sediment.

References

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**Figures**

**Figure 1** Boxplots for carbon, nitrogen and phosphorous species (total P, inorganic P and organic P) concentrations measured in sediments as a function of depth (sup: superficial, 20: 20 cm depth, 40: 40 cm depth) and GSDs (CF: bilayer coarse-fine system; F: monolayer fine system) and Tukey’s post-hoc analysis after ANCOVA analysis for depth (p < 0.05). Asterisks determine differences between GSDs at each given depth (ANCOVA, p < 0.05).
Figure 2 Carbon mass balances for the main C transformation processes and removal paths in the sediment layers. Values in **bold** indicate mass balances calculated from experimental data while values in *italic* are parameters estimated through equations 2 and 3. Asterisk indicates significant difference between systems (ANOVA, factor: system, p < 0.05). Dotted oval circles refer to dissolved species that leave the system via gas; solid line oval circles refer to dissolved species that are being accumulated in the sediment. Results are expressed as µg C·cm⁻³·day⁻¹. Negative values indicate dissolved components removal.
Figure 3 Nitrogen mass balances for the main N transformation processes and removal paths in the sediment layers. Values in **bold** indicate mass balances calculated from experimental data while values in *italic* are parameters estimated through equations 4-9. Asterisk indicates significant difference between systems (ANOVA, factor: system, p < 0.05). Dotted oval circles refer to dissolved species that leave the system via gas; solid line oval circles refer to dissolved species that are being accumulated in the sediment. Results are expressed as µg N·cm⁻³·day⁻¹. Negative values indicate dissolved components removal. DON balances in the bottom layer resulted in low and highly uncertain values, and so, they were excluded from mass balance calculations.
Figure 4 Phosphorous mass balances for the main P transformation processes and removal paths in the sediment layers. Values in **bold** indicate mass balances calculated from experimental data while values in *italic* are parameters estimated through equations 10 and 11. Asterisk indicates significant difference between systems (ANOVA, factor: system, p < 0.05). Solid line oval circles refer to dissolved species that are being accumulated in the sediment. Results are expressed as µg P·cm$^{-3}$·day$^{-1}$. Negative values indicate dissolved components removal.
<table>
<thead>
<tr>
<th></th>
<th>Surface – 40 cm depth</th>
<th>Inlet – 40 cm depth</th>
<th></th>
<th></th>
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<tbody>
<tr>
<td></td>
<td>CF</td>
<td>F</td>
<td>CF</td>
<td>F</td>
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<tr>
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<tr>
<td>TDN</td>
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<tr>
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<td>Removal rate</td>
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<tr>
<td>TDP</td>
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<td>11.87 ± 2.6</td>
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<td>Removal rate</td>
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</table>

Removal rates are expressed as µg C·cm⁻³·day⁻¹ for DOC, µg N·cm⁻³·day⁻¹ for TDN and µg P·cm⁻³·day⁻¹ for TDP. Considering only the sediment part of the systems (three infiltration layers) correspond to the column “Surface – 40 cm depth”, while removal efficiencies for the whole system, including the surface water layer correspond to the column “Inlet – 40 cm depth”. Values in bold indicate the system with significant higher value of the specified parameter comparing systems (ANOVA, factor: system, p < 0.05).