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Leaves stimulate aquatic phosphorus uptake by dark-grown but not by light-grown microbial communities in sediments: A laboratory study

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Abstract

1. It is a widely discussed topic in restoration ecology to use organic carbon (OC) management to increase nutrient uptake in chronically loaded agricultural streams. However, although nitrate uptake has been shown to correlated closely with the availability of dissolved OC, this relationship is less clear for phosphate. Likewise, the role of particulate OC (POC) on stimulating nutrient uptake has to be clarified. We aimed at investigating the effects of leaf additions on the net uptake of soluble reactive phosphorus and nitrate-nitrogen from the water column by benthic biofilms in combined laboratory flume and batch experiments.
2. Fine sediments were colonised under nutrient-enriched conditions in the dark (dark-grown heterotrophic biofilms) and under a 12/12-hr dark/light cycle (light-grown photoautotrophic biofilms) in flumes. In each light treatment, half of the biofilms were grown under strong OC-limitation, the other half under near-optimal C:N:P ratios. For the uptake experiments, the colonised sediments were incubated in nutrient-enriched water to which (1) fresh alder leaves, (2) pre-leached leaves, or (3) no leaves were added. Net nutrient uptake (or release) rates were determined via the change in nutrient concentrations in the water column over time.
3. Net phosphorus uptake by dark-grown biofilms was significantly increased by the addition of leaves. Despite their high initial nutrient leaching, fresh leaves showed stronger stimulating effects on the net P uptake than leached leaves over the entire experiment. Furthermore, biofilms grown under near-optimum C:N:P ratios responded stronger to the POC supply than biofilms grown under C-limited conditions. In contrast to dark-grown biofilms, leaf additions had no significant effects on the net P uptake by light-grown biofilms. We also found no effects of leaf additions on net nitrate uptake.
4. Our study shows that an increased POC supply in nutrient-impacted streams may boost heterotrophic phosphate uptake. However, the stimulating effect of the POC addition depends on a variety of POC-dependent (e.g. respiration,

heterotrophic assimilation, denitrification) and independent (e.g. adsorption, photoautotrophic assimilation) processes, making nutrient control via POC management challenging. We suggest application of management approaches that focus on restoration of riparian areas and wetlands to increase the amount of natural, complex POC with a moderate degradability, thus keeping the heterotrophic metabolism low but potentially stimulating in-stream N and P retention.

KEYWORDS
batch experiments, microbes, nutrient uptake, organic carbon, stoichiometry

1 | INTRODUCTION

Aquatic microbial biofilms play an important role in the nutrient uptake and self-purification capacity of streams (Hanrahan et al., 2018; Stutter, Graeber, & Weigelhofer, 2020; Stutter, Wyness, et al., 2020). One key factor controlling microbial nutrient uptake is stoichiometry, i.e. the ratio of organic carbon (OC) to reactive N and P in the food source compared to that of the consumers' demand (Cross et al., 2005; Dodds et al., 2004; Stutter et al., 2018; Wymore et al., 2016). Studies have found that optimal molar ratios of OC:N:P are around 68:14:1 for efficient heterotrophic nutrient uptake (Godwin & Cotner, 2018; Graeber et al., 2021). However, in intensively used agricultural landscapes, streams are exposed to high P and N loads from adjacent fields (Dupas et al., 2015; Ezzati et al., 2020; Weigelhofer et al., 2018; Withers et al., 2014) while agricultural soils are OC depleted and riparian forests are missing. As a consequence, a high proportion of agricultural streams are OC-limited, showing OC:N:P ratios far below the microbial demand (Stanley et al., 2012; Stutter et al., 2018), and heterotrophic nutrient uptake is stoichiometrically constrained (Gibson & O'Reilly, 2012; O'Brien et al., 2017). A shift towards an increased supply with natural OC via, e.g., the rehabilitation of riparian forests, could restore or at least improve the nutrient uptake capacity of agricultural streams and mitigate nutrient downstream transport (Goeller et al., 2019; Johnson et al., 2012; Robbins et al., 2017; Wymore et al., 2016).

Both empirical and experimental studies have shown positive correlations between the concentrations of dissolved OC (DOC) and nitrate uptake (Ghosh & Leff, 2013; Johnson et al., 2009, 2012; Rodríguez-Cardona et al., 2016; Wymore et al., 2016). However, the relation between OC supply and in-stream phosphorus (P) uptake is far less clear (Bechtold et al., 2012; Coble et al., 2016; Gibson & O'Reilly, 2012; Oviedo-Vargas et al., 2013). Oviedo-Vargas et al. (2013), for example, found no stimulation of P uptake through acetate additions in streams, probably due to autotrophic competition for the P and/or the microbial use of P and OC already stored in the sediments. Besides, P adsorption may dominate P retention in streams (Griffiths & Johnson, 2018; Robbins et al., 2023; Stutter, Graeber, & Weigelhofer, 2020) but this process is mostly independent of the OC availability (Li et al., 2020).

So far, most studies on the coupling between OC supply and nutrient uptake have focused on the role of DOC while the effects of particulate OC (POC) on in-stream nutrient uptake have been largely neglected. Leaves amount to *c*. 41%–98% of total terrestrial POC inputs to streams and deliver OC of high complexity to the stream biota (Zhang et al., 2019). Leaves provide habitat for the aquatic microbiome (Marks, 2019; O'Brien et al., 2017; Zhang et al., 2019) and can also act as adsorption sites for P (Mehring et al., 2015; Robbins et al., 2023). Numerous studies have shown a strong coupling between nutrient availability and leaf degradation (Bastias et al., 2018; Danger et al., 2021; Marks, 2019) but the reverse effects of POC availability on the microbial nutrient uptake have rarely been investigated so far (but see, e.g., O'Brien et al., 2017; Gibson & O'Reilly, 2012). Leaves do not only provide OC for nutrient uptake but they also deliver nutrients to the water column via leaching and mineralisation, thus potentially diminishing the positive effects of the OC supply on nutrient uptake. Furthermore, OC availability may promote heterotrophic uptake while P uptake by algae as well as P adsorption to the sediments are (mostly) independent of the OC supply (Stutter, Wyness, et al., 2020). Thus, a higher availability of natural POC can but may not necessarily stimulate P uptake in agricultural streams.

Our study aimed at analysing the effects of POC in the form of leaves on the net uptake of reactive inorganic P and N by benthic microbial communities using experimental flumes and nutrient addition batch experiments. Specifically, we investigated the potential coupling between POC supply and net nutrient uptake by biofilms grown under dark/light conditions and strong/moderate OC-limitation in the laboratory. For the POC, we used both fresh and leached leaves as they occur naturally in streams but may have different effects on the net nutrient uptake due to higher amounts of leached nutrients from fresh leaves. Based on the stoichiometric control of nutrient uptake outlined above, we hypothesised that leaves will generally increase the microbial uptake of both soluble reactive phosphorus (SRP) and nitrate by benthic biofilms, with leached leaves leading to higher net uptake rates than fresh leaves. Furthermore, we hypothesised that nutrient uptake by mainly heterotrophic (dark-grown) biofilms will be stimulated stronger by the POC supply, based on their OC demand for growth, than that of mainly photoautotrophic (light-grown) biofilms. We also expected biofilms grown under strong OC limitation to respond stronger to the stimulation by POC than biofilms grown under almost optimal C:N:P ratios due to their higher need for OC.

2 | MATERIALS AND METHODS

2.1 | Experimental set-up and colonisation

We used laboratory flumes for the biofilm colonisation combined with batch experiments to determine nutrient uptake. Due to time and space restrictions, the experiment was divided into two parts: First, biofilms were grown under dark conditions (experiment 1) for 5–6 weeks, followed by the uptake experiments (October–November 2020). Then, the entire equipment was sterilised overnight with sodium hypochlorite, rinsed and soaked in tap water overnight, and then rinsed twice with distilled water. The experiment was repeated under light conditions (experiment 2) with the same colonisation period from February to March 2021. For the colonisation, 12 flumes made of acrylic glass (55 cm length, 9 cm width, 10.5 cm depth) were used in circulation flow mode using aquarium pumps (EDEN, 126, 800 L/hr, Cartigliano [VI] Italy; flow velocity in the flumes approximately 6 cm/s; for details, see [Weigelhofer et al., 2020]). In both experiments, commercially available pre-cleaned 0.7–1.2-mm quartz sand was filled in petri dishes, which were placed into the flumes for colonisation at 20°C in a climate chamber. Initial nutrient and DOC concentrations as well as microbial abundances in the sand were below detection limit. The dishes facilitated the undisturbed removal of the sediments from the flumes for the batch experiments. In experiment 1, all work was done in the dark with red headlamps and all flumes were covered with fine, black nylon mesh to ensure light deprivation for algal growth. In experiment 2, all flumes followed a 12/12 h light–dark cycle using light-emitting diodes (TRU Light LED-Board Grow Light 12 V, 18 cm, White, Blue; 21.5 $\mu mol\, m^{-2} s^{-1}$ photosynthetically active radiation TRU COMPONENTS, Grünwald, Germany). Light intensity used in this study is similar to forested catchments and previous studies have shown that this relatively low intensity is sufficient to grow phototrophic biofilms (Bengtsson et al., 2018; Hill et al., 2009).

In each experiment, the flumes were divided into two sets (six replicates per set) with a separated water reservoir of 35 L capacity each. In the first set, the sand was colonised under strongly C-limited conditions (LIM), receiving nutrient-poor filtered well-water enriched to end concentrations of 6 mg C/L (added as glucose), 6 mg N/L (added as $NaNO_3$), and 0.5 mg P/L (added as NaH_2PO_4), typical for streams in intensively cultured agricultural regions in Austria (molar C:N:P ratios 4.6:5.4:1) (Weigelhofer et al., 2018). In the second set (OPT), the sand received enriched filtered well-water with concentrations of 7 mg C/L, 1 mg N/L, and 0.06 mg P/L, resulting in C:N:P ratios of 45.7:7.6:1 approximating those of optimum conditions (Godwin & Cotner, 2018). Thus, we had the following four combinations: dark*LIM and dark*OPT in experiment 1, and light*LIM and light*OPT in experiment 2 (Figure 1). At the start of the colonisation phase, all flumes were inoculated with a homogenised suspension of periphyton collected from both shaded and sun-exposed sections of a nearby forested headwater stream which was added to each water reservoir once at the start of the colonisation period. Once per week, the water in the reservoir was substituted by fresh water and the CNP solution was replenished.

2.2 | Uptake experiments and analyses

After the colonisation, the batch experiments for assessing the microbial nutrient uptake were conducted over 48 hr with the addition of leaves. Fresh alder leaves (*Alnus glutinosa*) in senescent state were collected from trees growing adjacent to the forested headwater stream directly before each experiment in November 2020 (dark colonisation) and in March 2021 (light colonisation). To ensure better comparability, the leaves collected in March were old leaves from the preceding year that had remained on the trees. Half of the leaves were leached in nutrient-poor filtered well-water over 48 hr before the start of the incubations (leached leaves). Petri dishes were randomly taken out of the flumes and carefully submerged into 400 mL beakers containing 200 mL filtered well-water with a natural DOC concentration of 1 mg C/L amended with 5 mg N/L (as $NaNO_3$) and 0.2 mg P/L (as NaH_2PO_4).

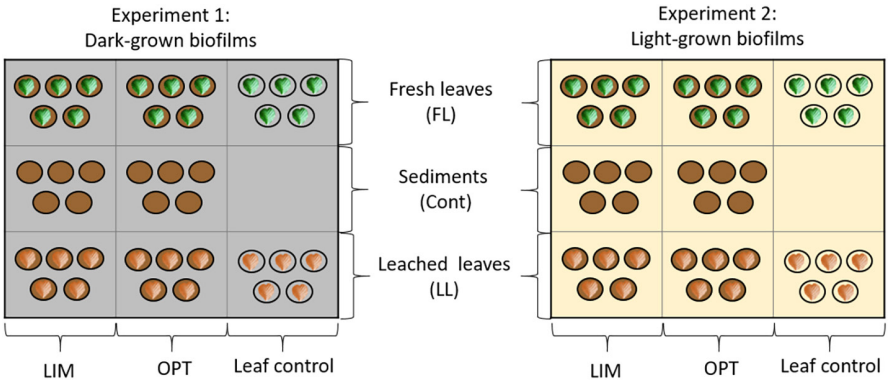

FIGURE 1 Design of experiment 1, using biofilms colonised under dark conditions (dark-grown biofilms) and experiment 2, using biofilms colonised under light conditions (light-grown biofilms). Cont, sediment control without leaf addition; FL, addition of fresh leaves; Leaf control, Leaves without sediments; LIM, biofilms grown under strongly C-limited conditions; LL, addition of leached leaves; OPT, biofilms grown under near-optimum stoichiometric ratios.

As nutrient uptake is a function of concentration (Weigelhofer et al., 2018), we used the same nutrient concentrations for both LIM and OPT biofilms to ensure comparability between treatments. All combinations mentioned above (dark*LIM, dark*OPT in experiment 1, and light*LIM and light*OPT in experiment 2) were exposed to three different treatments: addition of fresh leaves (FL), addition of leached leaves (LL), and no leaf addition (sediment control; Cont) (Figure 1; 5 replicates each). For this purpose, the leaves were cut uniformly into 1-cm^2 large square discs and three leaf discs were added to each beaker, respectively, amounting to 28 mg dry weight of leaves to 15 g dry weight of sediment Additionally, in each experiment, we incubated the two leaf types (FL and LL) in the solute without sediments to observe the effects of nutrient uptake or release by the leaves only (leaf control; 5 replicates each).

Water samples (20 mL) were collected 10 mins after the beginning of the treatments (start point *t*0) and 1 (*t*1), 3 (*t*3), 24 (*t*24), and 48 (*t*48) h after the start via consumptive sampling (i.e. from the same beaker without replacing the sampled water). The samples were filtered immediately with pre-combusted Whatman GF/F Filters (0.75 µm) and stored in pre-combusted glass vials at 4°C in the dark. Nutrient concentrations (SRP, NO_3-N, NH_4-N, and NO_2-N) were determined with a continuous flow analyser (CFA, Alliance Instruments GmbH, Salzburg, Austria) and DOC concentrations were analysed with a Sievers*900 portable TOC-Analyser (GE Analytical Instruments, Boulder, USA) within 24 hr after sampling. Dissolved organic phosphorus (DOP) and dissolved organic nitrogen (DON) were determined at the end of the uptake experiment. First, total dissolved P and N were determined based on potassium persulfate digestion in an autoclave (APHA, 2005). After this, DOP and DON were calculated from the differences between SRP and total dissolved P, and NO_3-N and total dissolved N, respectively.

Biofilm properties were analysed immediately after the uptake experiments and the removal of the leaves. Respiration rates were measured as a surrogate for the microbial community activity. For this purpose, the petri dishes with the sediments were placed in 200-mL air-tight glass beakers equipped with oxygen-sensitive optical sensor spots (Fibox 3 optode system, PreSens GmbH, Regensburg, Germany). Each beaker was filled carefully with groundwater, closed without air-bubbles, and incubated in the dark on a laboratory shaker at 20°C. The decrease in dissolved oxygen concentrations was measured at the beginning and after 4, 8, 12, 24, and 30 hr. The respiration rates were calculated from the decrease in oxygen over time corrected for the water volume and sediment weight.

For bacterial abundances, 1.3 g sediments were suspended in 3 mL GF/F filtered autoclaved groundwater and fixed with 0.75 mL 37% formaldehyde. Bacterial abundances were determined after extraction with Tween 80 (10%) and staining with Sybr Green II on a flow cytometer (CytoFLEX, Beckman Coulter GmbH, Krefeld, Germany; Weigelhofer et al., 2020). Chlorophyll-a concentrations were determined after extraction in 90% cold acetone with a Hitachi Fluorescence Spectrophotometer F-7000 (Hitachi High-Tech Science Corporation, Tokyo, Japan; APHA, 2005). The alkaline phosphatase activity was determined via the fluorescence enzyme assay method by incubating the sediments in 2 mM 4-methylumbelliferyl phosphate and determining the fluorescence at 365 nm excitation and 450 nm emission using a 96-well plate reader (VARIOSCAN FLASH, Thermo Fisher Scientific, Waltham, Massachusetts, USA; [Bell et al., 2013; Marx et al., 2001]). The potentially bioavailable P in the sediments was extracted sequentially via Milli-Q water and then 1 M NaOH, with subsequent peroxydisulfate digestion for the dissolved organic P (Lukkari et al., 2007). Bioavailable inorganic N in the sediments was determined as NO_3-N, NO_2-N, and NH_4-N after Milli-Q water extraction (APHA, 2005). Sediment samples were dried at 75°C and combusted at 450°C for 4 hr to determine the organic matter (OM) content.

2.3 | Data analysis

For simplification, we use the term *nutrient exchange* hereinafter if both uptake and release processes are meant. As most of the added P and N was gone after 24 hr, only the first three time steps (*t*0–*t*1, *t*1–*t*3, and *t*3–*t*24) were used to calculate net nutrient exchange rates. The net nutrient mass exchange R_{netM} (in µg/hr) between two time intervals t_n and t_{n+1} was calculated for each time interval separately:

$$R_{\text{netM}}\left(t_{n,n+1}\right) = \frac{\Delta\left(V\,C\right)}{\Delta\,t} = \frac{V_{t_{n+1}}\,C_{t_{n+1}} - V_{t_n}\,C_{t_n}}{t_{n+1} - t_n} \quad (1)$$

Here, V_{t_n} and C_{t_n} are the volume of the water and the concentration of the compound at one time step (e.g. 1 h after beginning of treatment), respectively. Similarly, $V_{t_{n+1}}$ and $C_{t_{n+1}}$ are at the following time step (e.g. 3 h after beginning of treatment). t_n and t_{n+1} represent the previous and following time steps during the treatment, respectively. This calculation was needed because both the concentration and volume underwent changes due to exchange with the sediments and consumptive sampling, respectively. To obtain net nutrient exchange rates R_{net}, R_{netM} was divided per gram dry weight of sediments (µg g DW^{-1} hr^{-1}) as well as per gram ash-free dry weight (µg g $AFDW^{-1}$ hr^{-1}) in order to compare with the controls and to standardise against OM content respectively biofilm biomass of the sediments.

The effects of different leaf conditions (FL, LL, no leaves) on the net nutrient exchange by different biofilms (OPT and LIM for both dark- and light-grown biofilms) were analysed by two-way ANOVA followed by Tukey's Honestly Significant Difference post hoc tests after checking that equal sample size, the assumptions of normal distribution (Shapiro–Wilk test) and/or homogeneity of variance (Bartlett's test) were met. Changes in biofilm properties (bacterial respiration, bacterial abundances, chlorophyll-a, phosphatase activity, OM, bioavailable P, bioavailable N) at the end of the uptake experiments as well as among experiment 1 (dark) and experiment 2 (light) were checked by two-way ANOVA. Results at $p < 0.05$ were considered significant. All statistical analyses were performed using statistical software R (v4.0.3, R Core Team, 2020).

3 | RESULTS

3.1 | Properties of dark-grown and light-grown biofilms

Generally, biofilms from leaf treatments did not differ significantly from the respective sediment controls, indicating that the leaf additions had no or only small effects on biofilm properties over the 48-hr incubations (Table 1). The largest differences in biofilm properties were observed between light- and dark-grown biofilms. On average, light-grown biofilms showed significantly higher respiration rates (1.04 ± 0.12 µg O_2 g $DW^{-1} hr^{-1}$; ANOVA, $p < 0.001$, $n = 60$), bacterial abundances (402.45 ± 68.38 cells 10^6 g DW^{-1}; $p < 0.001$), chlorophyll-a concentrations (4.32 ± 1.24 µg g^{-1} DW; $p < 0.001$) and phosphatase activities (819.17 ± 300.12 nmol $g^{-1} hr^{-1}$; $p < 0.001$) than dark-grown biofilms. The respective mean values for dark-grown biofilms were 0.21 ± 0.1 µg O_2 g $DW^{-1} hr^{-1}$, $146.95 \pm 38.72 \times 10^6$ cells/g DW, 0.22 ± 0.33 µg/g DW and 192.31 ± 33.25 nmol $g^{-1} hr^{-1}$ on average (Table 1, Table S2). In contrast, mean bioavailable inorganic N was significantly higher in dark-grown biofilms (4.44 ± 2.19 µg/g DW) than in light-grown biofilms (0.04 ± 0.02 µg/g DW; $p < 0.001$; Table S2).

Interestingly, OPT and LIM biofilms only differed in the FL treatments. In experiment 1 (dark), LIM biofilms had significantly higher mean respiration rates (0.35 ± 0.11 µg O_2 g $DW^{-1} hr^{-1}$; ANOVA, $p < 0.001$, $n = 30$) but lower mean bacterial abundances ($144.86 \pm 38 \times 10^6$ cells/g DW; $p < 0.05$), and phosphatase activities (149.46 ± 19 nmol $g^{-1} hr^{-1}$; $p < 0.01$) than OPT biofilms (Table 1). The respective mean values for OPT biofilms amounted to 0.24 ± 0.08 µg O_2 g DW^{-1} hr^{-1}, $220.92 \pm 35 \times 10^6$ cells/g DW, and 236.78 ± 57 nmol $g^{-1} hr^{-1}$, respectively. In experiment 2 (light), LIM biofilms in the FL treatments showed lower mean phosphatase activities (814.96 ± 157 nmol $g^{-1} hr^{-1}$) than OPT biofilms (1325.1 ± 428 nmol $g^{-1} hr^{-1}$; $p > 0.05$; Table 1).

3.2 | Effects of nutrient leaching from leaves and sediments on C:N:P ratios

The nutrient solution used in the batch experiments had initial molar C:N:P ratios of approximately 2:11:1 and was, thus, strongly C-limited. When the solution was added to the sediments/biofilms in experiment 1, the DOC concentrations increased to approximately 4–5 mg C/L, changing the molar C:N:P ratios to 8–10:11:1 (data from

TABLE 1 Biofilm properties in the treatments (LIM = biofilms grown under C-limiting conditions; OPT = biofilms grown under near-optimum stoichiometric ratios; FL = addition of fresh leaves; LL = addition of leached leaves) and the sediment controls (Cont; no leaves added) in experiment 1 with dark-grown biofilms and experiment 2 with light-grown biofilms at the end of the uptake experiments.

	Bacterial respiration	Bacterial abundances	Chl-a	Phosphatase activity	OM	Bioavailable P	Bioavailable N
	µg O_2 g $DW^{-1} hr^{-1}$	10^6 cells/g DW	µg/g DW	nmol $g^{-1} hr^{-1}$	%	µg/g DW	µg/g DW
Experiment 1 (Dark)							
LIM							
Cont	0.17^b (0.03)	124.75^a (45)	0.89^a (1.82)	177.85^{ab} (25)	0.03^a (0.01)	0.33^a (0.03)	7.40^b (0.72)
FL	0.35^a (0.11)	144.86^a (38)	0.05^a (0.04)	149.46^b (19)	0.16^a (0.05)	0.27^a (0.04)	2.57^a (0.44)
LL	0.23^b (0.08)	150.5^{ab} (17)	0.07^a (0.01)	227.65^{ab} (31)	0.03^a (0.02)	0.29^a (0.07)	3.32^a (0.11)
OPT							
Cont	0.10^b (0.03)	127.34^a (34)	0.05^a (0.02)	181.92^{ab} (60)	0.24^a (0.18)	0.30^a (0.01)	7.09^b (0.36)
FL	0.24^b (0.08)	220.92^b (35)	0.12^a (0.27)	236.78^a (57)	0.14^a (0.06)	0.31^a (0.02)	3.01^a (1.02)
LL	0.16^b (0.04)	113.34^a (30)	0.04^a (0.02)	180.22^{ab} (41)	0.11^a (0.05)	0.32^a (0.12)	3.24^a (0.16)
Experiment 2 (Light)							
LIM							
Cont	1.04^a (0.42)	474.43^a (81)	4.68^a (1.82)	548.03^a (110)	0.24^a (0.08)	1.50^a (0.73)	0.05^a (0.02)
FL	1.23^a (0.14)	355.44^a (78)	5.79^a (2.82)	814.96^a (157)	0.23^a (0.05)	0.82^a (0.19)	0.02^a (0.02)
LL	1.08^a (0.38)	388.57^a (211)	5.51^a (1.36)	578.28^a (200)	0.96^a (1.09)	1.45^a (0.27)	0.05^a (0.04)
OPT							
Cont	0.97^a (0.19)	350.77^a (53)	3.70^a (1.66)	647.15^a (213)	0.57^a (0.76)	1.08^a (0.27)	0.05^a (0.03)
FL	0.88^a (0.16)	501.17^a (159)	2.49^a (0.55)	1325.1^b (429)	0.27^a (0.08)	0.99^a (0.58)	0.03^a (0.05)
LL	1.02^a (0.22)	344.34^a (97)	3.74^a (2.43)	1001.48^{ab} (218)	0.17^a (0.12)	0.89^a (0.24)	0.06^a (0.05)

Note: Values are means with standard deviations in brackets ($n = 5$ per treatment). Small letters denote significantly different groups within dark-grown biofilms and within light-grown biofilms (ANOVA, $n = 30$).

Abbreviations: Chl-a, chlorophyll-*a*; DW, dry weight; OM, organic matter.

sediment controls). In contrast, no DOC leaching was observed from the sediments/biofilms in the second experiment.

Nutrient and DOC leaching from the leaves differed between fresh and pre-leached leaves but also between the two experiments due to the different leaf age. In experiment 1, fresh leaves leached about 20 mg DOC/L, 0.5 mg P/L, and 1 mg NO_3-N/L in the leaf controls (i.e. without sediments) during the first hour of incubation. This amounts to initial leaching rates of 4.4 and 87.5 $\mu g\,L^{-1}\,hr^{-1}$ for P and N, respectively (Table 2). The addition of fresh leaves to the dark-grown biofilms increased the initial DOC concentrations in the solution to approximately 20 mg C/L in the OPT treatment but only 10 mg C/L in the LIM treatment (Table 3). Thus, fresh leaf additions resulted in initial molar DOC:N:P ratios of about 40:11:1 for OPT and 20:11:1 for LIM biofilms, respectively. Leached leaves showed an initial leaching of only about 10 mg DOC/L in the first experiment and no nutrient release. When leached leaves were added to the biofilms, initial DOC concentrations changed to 5–9 mg/L (Table 3). Thus, leached leaf additions resulted in initial molar DOC:N:P ratios of about 6–10:11:1 for both OPT and LIM biofilms in experiment 1.

In experiment 2, the addition of both fresh and leached leaves increased the DOC concentrations to approximately 30–40 mg/L, which equalled the DOC leached from the leaves. The amounts of nutrient leached from the leaves were similar as in experiment 1. Initial molar DOC:N:P ratios were approximately 60–80:11:1 for both LIM and OPT biofilms in experiment 2.

3.3 | Effects of leaf additions on nutrient uptake by dark-grown biofilms

Sediment controls (i.e. without leaves) showed slight net SRP uptake throughout the entire incubation period. After 24 hr, between 76% (OPT biofilms) and 88% (LIM biofilms) of the initial SRP concentrations remained in the solute. Mean net uptake rates for the last time step (*t*3–*t*24) amounted to 0.004 ± 0.003 and 0.011 ± 0.005 μg P g $DW^{-1}\,hr^{-1}$ for LIM and OPT biofilms, respectively. Based on the OM content of the sediments, mean net uptake rates were 4.2 ± 3.4 (LIM) and 1.2 ± 0.5 μg P g $AFDW^{-1}\,hr^{-1}$ (OPT).

When leaves were added, net SRP release was observed at the beginning, which was especially high in the FL treatment, followed by net SRP uptake until the end of the incubation period (Figure 2). The first release pulse in the FL treatment was equally high for OPT biofilms as in the leaf controls but considerably smaller for LIM biofilms. After 24 hr, the net SRP uptake of OPT biofilms in the FL treatment was significantly higher by 2–3 times than in the sediment control (ANOVA, $p < 0.001$; Figure 2), with about 3% of the initial SRP concentrations remaining after 24 hr. For LIM biofilms, the net uptake was also significantly, albeit only slightly higher than in the control, with an average of 71% of the SRP still remaining in the FL treatments. Mean net uptake rates for the last time step (*t*3–*t*24) amounted to 0.017 ± 0.003 and 0.04 ± 0.004 μg P g $DW^{-1}\,hr^{-1}$ for LIM and OPT biofilms, respectively (3.4 ± 0.6 μg P g $AFDW^{-1}\,hr^{-1}$ for LIM and 8 ± 0.7 μg P g $AFDW^{-1}\,hr^{-1}$ for OPT). In contrast to the FL treatment, we did not observe any initial SRP release after the addition of leached leaves to the biofilms (Figure 2), corresponding to the leaf controls (Table 2). In LIM biofilms, SRP uptake in the LL treatment was similar to that of the control, with approximately 77% of the initial SRP remaining. In OPT biofilms, uptake was significantly higher than in the control after 24 hr (approximately 52% SRP remaining; ANOVA, $p < 0.001$; Figure 2). Mean net uptake rates between *t*3 and *t*24 amounted to 0.008 ± 0.002 and 0.03 ± 0.003 μg P g $DW^{-1}\,hr^{-1}$ or 8 ± 2.3 and 7.4 ± 0.7 μg P g $AFDW^{-1}\,hr^{-1}$ for LIM and OPT biofilms, respectively.

Net NO_3-N exchange showed a more variable pattern than SRP (Figure 3). In the sediment controls, we observed a slight NO_3 uptake during the first 3 hr followed by a slight release after 24 hr for both LIM and OPT biofilms. Mean net release rates for the last time step (*t*3–*t*24) amounted to 0.306 ± 0.09 (LIM) and 0.186 ± 0.21 μg N g^{-1} DW hr^{-1} (OPT) or 305.8 ± 92.3 (LIM) and 20.7 ± 23.8 μg N g^{-1} AFDW hr^{-1} (OPT) for the sediment controls. In the FL treatments, a significantly increased net NO_3-N release for OPT biofilms was observed during the first hour, which turned into net uptake until the end of the incubation (ANOVA, $p < 0.001$; Figure 3). In contrast, LIM biofilms showed an initial net NO_3-N uptake, followed by a net release which had turned again into uptake after 24 hr. Remaining NO_3-N concentrations were around 77% for OPT and 94% for LIM biofilms on average, with mean net uptake rates of 0.173 ± 0.02 and 0.27 ± 0.07 μg N g^{-1} DW hr^{-1} or 34.5 ± 4.1

TABLE 2 Exchange rates of dissolved inorganic phosphorus (SRP), nitrate (NO_3), and dissolved organic carbon (DOC) from fresh leaves (FL) and leached leaves (LL) in the leaf controls of experiment 1 (dark) and experiment 2 (light; $n = 5$ per treatment).

	SRP ($\mu g\,L^{-1}\,hr^{-1}$)			NO_3 ($\mu g\,L^{-1}\,hr^{-1}$)			DOC ($mg\,L^{-1}\,hr^{-1}$)		
	1	3	24	1	3	24	1	3	24
Experiment 1 (Dark)									
FL	4.42 (2.61)	−0.16 (0.29)	−0.05 (0.03)	87.50 (24.84)	−21.42 (7.31)	−0.62 (0.44)	0.19 (0.08)	0.08 (0.03)	0.04 (0.01)
LL	−0.73 (0.11)	−0.63 (0.10)	−0.09 (0.03)	7.38 (3.17)	6.38 (2.74)	−2.10 (0.6)	1.64 (0.49)	0.14 (0.21)	0 (0.01)
Experiment 2 (light)									
FL	5.31 (1.85)	0.25 (0.60)	−0.08 (0.11)	−27.07 (26.01)	2.74 (14.51)	−1.93 (1.97)	0.11 (0.1)	0.06 (0.03)	0.01 (0)
LL	−1.09 (0.83)	0.93 (0.24)	−0.18 (0.04)	23.19 (39.76)	−26.38 (9.49)	0.27 (0.98)	0.03 (0.03)	0 (0.01)	0.03 (0)

Note: Positive and negative values show net release and uptake rates, respectively. Shown are mean and standard deviations in brackets.

TABLE 3 Concentrations of nitrite (NO_2), ammonium (NH_4) and dissolved organic carbon (DOC) at the beginning (0 hr) and end (24 hr) of experiment 1 (dark-grown biofilms) and experiment 2 (light-grown biofilms). Shown are means and standard deviations in brackets ($n = 5$ per treatment).

	NO_2 (µg/L)		NH_4 (µg/L)		DOC (mg/L)	
	0 hr	24 hr	0 hr	24 hr	0 hr	24 hr
Experiment 1 (Dark)						
LIM						
Cont	21.0^{b}(3.8)	13.8^{b}(4.5)	55.0^{b}(35.4)	18.4^{b}(8.5)	5.1^{c}(0.3)	5.4^{b}(0.2)
FL	6^{a}(4.3)	52.8^{ab} (30.1)	–	13.5^{ab}(6.4)	10.5^{a}(0.4)	20.5^{a}(1.1)
LL	19.8^{b}(6.1)	75.4^{a} (34.9)	17.8^{b}(1.3)	3.8a^{c}(3.0)	9.1^{b}(0.8)	8.4^{b}(0.6)
OPT						
Cont	17.8^{b}(4.7)	12.4^{b}(2.3)	161.3^{b} (62.3)	12.2^{ab}(4.1)	4.1^{c}(0.4)	5.4^{b}(0.2)
FL	5.3^{a}(2.4)	90.2^{a} (27.5)	15.4^{c}(1.9)	<2.0^{c}(0.95)	19.5^{b}(0.5)	24.2^{c}(4.4)
LL	29.2^{c}(2.2)	59.8^{a} (16.1)	113.2^{b} (43.3)	6.4^{ac}(5.14)	5.3^{c}(0.7)	6.6^{b}(0.4)
Experiment 2 (Light)						
LIM						
Cont	9.4^{b}(2.3)	29.8^{ab}(23.1)	6.8^{b}(3.7)	21.0^{a}(6.8)	0.6^{c}(0.1)	0.9^{b}(0.7)
FL	1.5^{a}(1.4)	71.4^{a} (19.9)	43.3^{a}(15.9)	30.5^{a}(26.5)	25.5^{a}(11.5)	29.5^{a}(1.3)
LL	9.6^{b}(1.7)	65.4^{a} (46.1)	6.9^{b}(4.6)	31.8^{a}(22.6)	30.5^{ab}(0.9)	29.5^{a}(0.7)
OPT						
Cont	9.6^{b}(3.2)	8.2^{b}(6.8)	6.3 ^{b}(3.3)	74^{a}(70.2)	0.5^{c}(0.2)	1.3^{b}(0.5)
FL	0.5^{a}(0.4)	57.8^{ab} (27.7)	70.6^{c}(18.8)	15.5^{a}(17.3)	29.4^{ab}(0.9)	30.4^{a}(1.0)
LL	5.6^{c}(0.9)	11.4^{b}(9.7)	4.7^{b}(1.4)	65.4^{a}(61)	38.8^{b}(1.1)	40.3^{c}(8.7)

Note: Small letters denote significantly different groups within dark-grown biofilms and within light-grown biofilms (ANOVA, $n = 30$).

Abbreviations: Cont, sediment control without leaf addition; FL, addition of fresh leaves; LIM, biofilms grown under strongly C-limited conditions; LL, addition of leached leaves; OPT, biofilms grown under near-optimum stoichiometric ratios.

and 54 ± 13.6 µg N g^{-1} AFDW hr^{-1} for LIM and OPT biofilms for the last time step (*t*3–*t*24), respectively. In the LL treatment, no initial NO_3-N release was observed in any of the biofilms. The NO_3-N uptake rates were generally low and did not differ significantly from that of the controls, with 80%–100% initial NO_3-N concentrations remaining after 24 hr.

The initial NH_4-N concentrations were high in the sediment controls and in the OPT-LL treatment (55–161 µg/L) while all other treatments showed concentrations below 16 µg/L (Table 3). After r, average NH_4-N concentrations decreased in all treatments, with values ranging between <2 and 18.4 µg N/L. The initial mean NO_2-N concentrations ranged between 5.3 and 29.2 µg/L, with lower concentrations in the FL treatments, respectively. After 24 hr, mean NO_2-N concentrations had increased in all treatments to values between 12.4 and 90.2 µg/L. Here, the controls showed the lowest concentrations. Both DOP and DON concentrations were below detection limit, probably due to the high SRP and nitrate concentrations. DOC concentrations remained stable in the sediments controls, but increased in the FL treatments by about 5 mg C/L (OPT) to 10 mg C/L (LIM) until 24 hr (Table 3). Due to these changes, the average DOC:N:P ratios were 50:12:1 for LIM-FL and 290:50:1 for OPT-FL after 24 hr. DOC concentrations remained more or less the same in the LL treatments.

3.4 | Effects of leaf additions on nutrient uptake by light-grown biofilms

In the second experiment using light-grown biofilms, the sediment controls of both OPT and LIM treatments showed slight net SRP release during the first 1–3 hr, followed by net uptake (Figure 4). After 24 hr, only about 29% (OPT biofilms) and 65% (LIM biofilms) of the initial SRP concentrations remained, which was markedly lower than in experiment 1 with dark-grown biofilms. Mean uptake rates for the last time step (*t*3–*t*24) amounted to 0.029 ± 0.002 and 0.035 ± 0.004 µg P g DW^{-1} hr^{-1} or 2.9 ± 0.2 and 1.4 ± 0.2 µg P g AFDW^{-1} hr^{-1} for LIM and OPT biofilms, respectively.

A trend of initial SRP release followed by SRP uptake was also observed in the FL and LL treatment. After 24 hr, the OPT biofilms showed lower remaining SRP concentrations with fresh (56%) and leached leaves (31%) than the LIM biofilms (88% and 74% for FL and LL, respectively). Net uptake rates of the leaf treatments were lower than those of the control (ANOVA, $p < 0.05$). In the FL treatment, mean net uptake rates for the last time step (*t*3–*t*24) amounted to 0.009 ± 0.006 and 0.024 ± 0.002 µg P g DW^{-1} hr^{-1} or 0.3–1.0 ± 0.7 and −2.2 ± 0.2 µg P g AFDW^{-1} hr^{-1} for LIM and OPT biofilms, respectively. In the LL treatment, mean net uptake rates for the last time step (*t*3–*t*24) amounted to 0.022 ± 0.004 (LIM) and 0.022 ± 0.005 µg

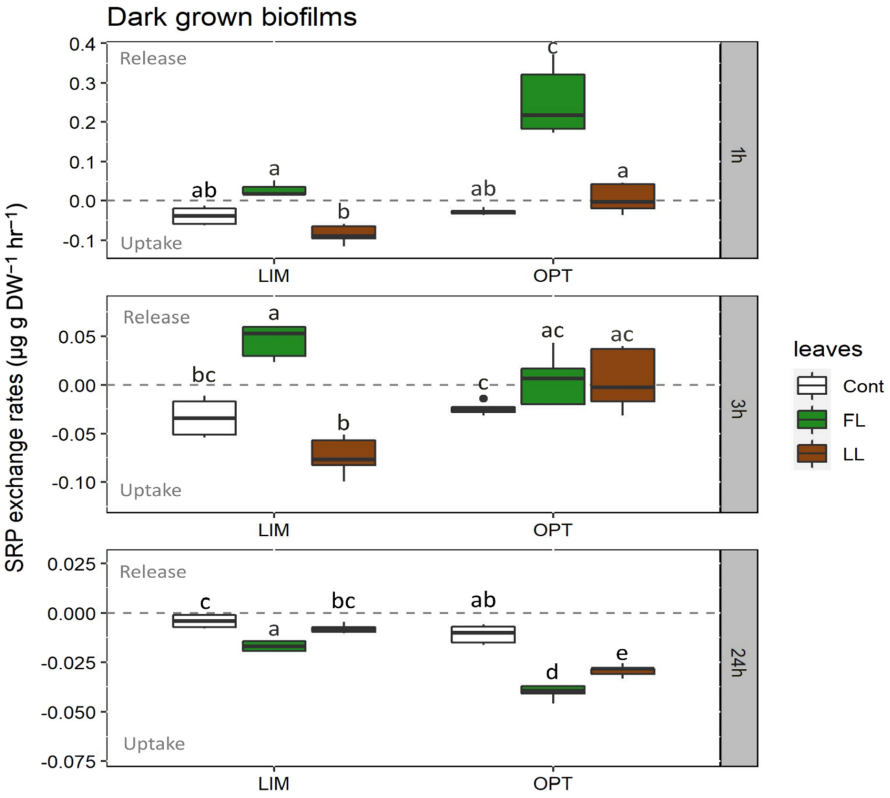

FIGURE 2 Soluble reactive phosphorus (SRP) exchange rates of dark-grown biofilms (experiment 1) between *t*0–*t*1 (1 hr), *t*1–*t*3 (3 hr), and *t*3–*t*24 (24 hr) after the SRP addition in four treatments: fresh leaves (FL) and leached leaves (LL) added to biofilms grown under limited (LIM) and optimum (OPT) C:N:P ratios. Cont, Control without leaf additions; small letters denote statistically different groups (ANOVA, $p < 0.001$, $n = 30$). Positive and negative values show release and uptake rates, respectively. Boxplots show 10%, 25%, 50%, 75%, and 90% percentiles and outliers.

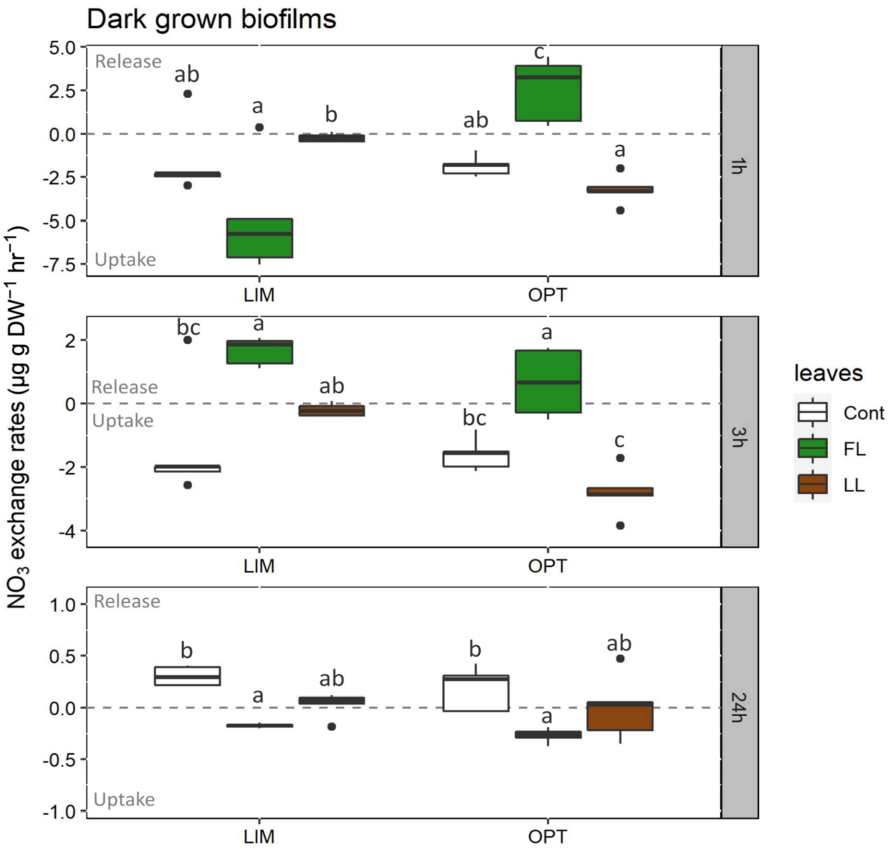

FIGURE 3 NO_3-N exchange rates of dark-grown biofilms (experiment 1) between *t*0–*t*1 (1 hr), *t*1–*t*3 (3 hr), and *t*3–*t*24 (24 hr) after the NO_3-N addition in four treatments: fresh leaves (FL) and leached leaves (LL) added to biofilms grown under limited (LIM) and optimum (OPT) C:N:P ratios. Cont, Control without leaf additions; small letters denote statistically different groups (ANOVA, $p < 0.001$, $n = 30$). Positive and negative values show release and uptake rates, respectively. Boxplots show 10%, 25%, 50%, 75%, and 90% percentiles and outliers.

P g DW^{-1} hr^{-1} (OPT) or 0.6 ± 0.1 (LIM) and 2.8 ± 0.6 µg P g AFDW $^{-1}$ hr^{-1} (OPT) Thus, net P uptake rates per gram OM were lower in the second experiment with the light-grown biofilms than in the first experiment with the dark-grown biofilms.

In all treatments, the NO_3-N concentrations remaining after 24 hr were high with values ranging between 94% and 99% of the initial concentrations (Figure 5). The NO_3-N exchange in the leaf treatments switched between uptake and release over the duration

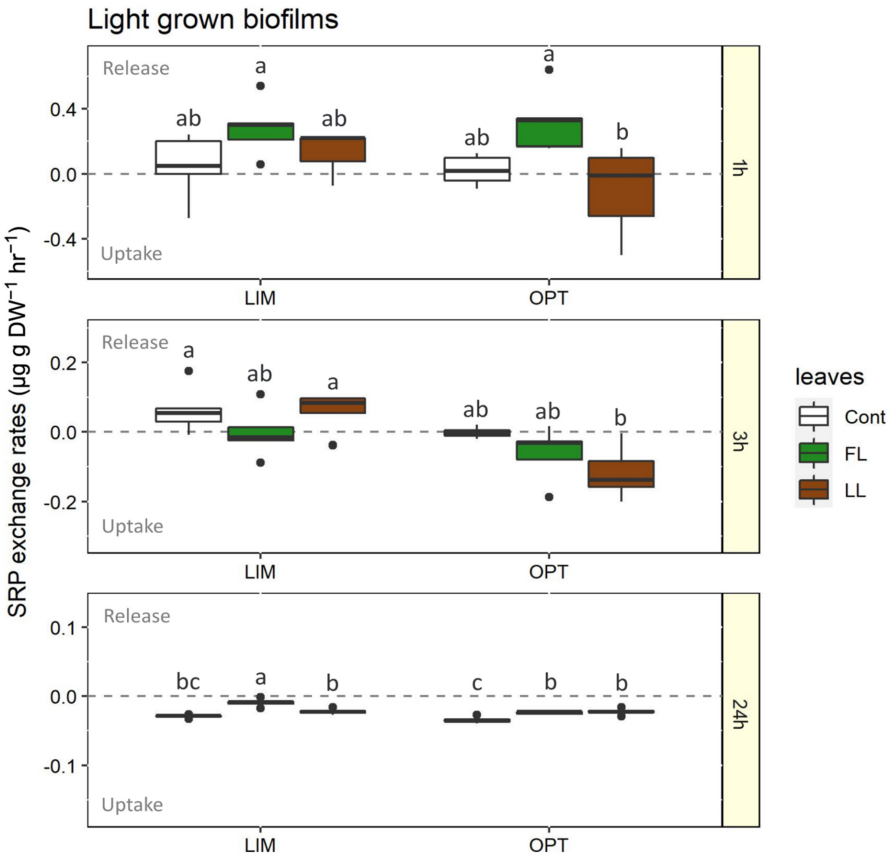

FIGURE 4 Soluble reactive phosphorus (SRP) exchange rates of light-grown biofilms (experiment 2) between *t*0–*t*1 (1 hr), *t*1–*t*3 (3 hr), and *t*3–*t*24 (24 hr) after the SRP addition in four treatments: fresh leaves (FL) and leached leaves (LL) added to biofilms grown under limited (LIM) and optimum (OPT) C:N:P ratios. Cont, Control without leaf additions; small letters denote statistically different groups (ANOVA, $p < 0.05$, $n = 30$). Positive and negative values show release and uptake rates, respectively. Boxplots show 10%, 25%, 50%, 75%, and 90% percentiles and outliers.

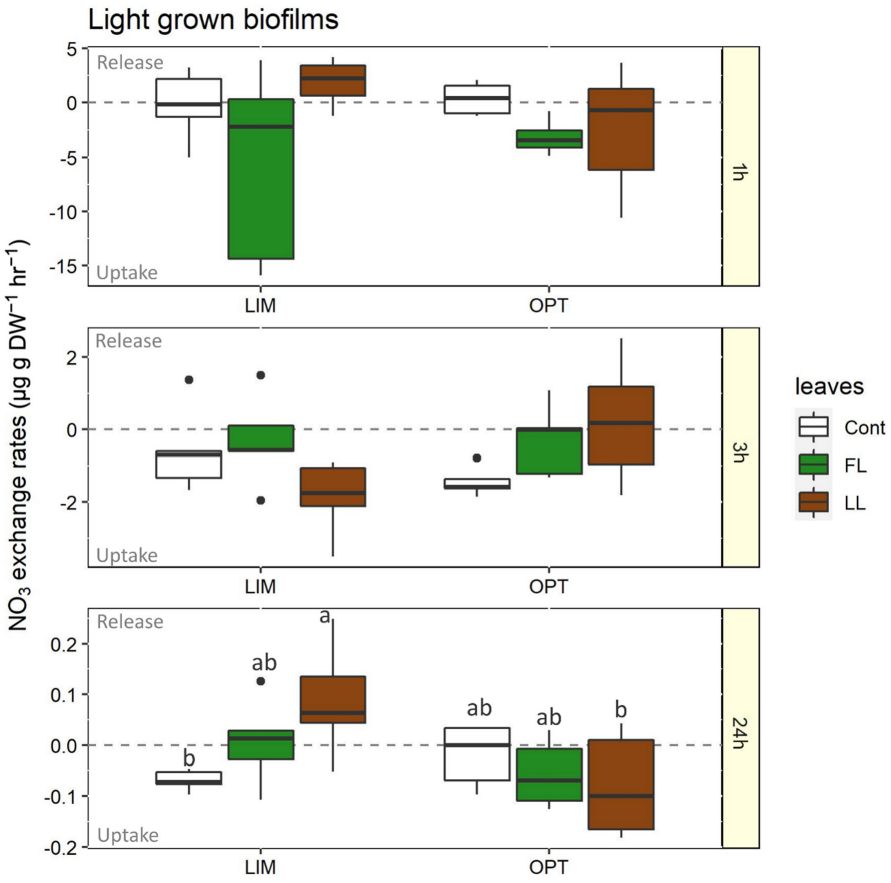

FIGURE 5 NO_3-N exchange rates of light-grown biofilms (experiment 2) between *t*0–*t*1 (1 h), *t*1–*t*3 (3 h), and *t*3–*t*24 (24 h) after the NO_3 addition in four treatments: fresh leaves (FL) and leached leaves (LL) added to biofilms grown under limited (LIM) and optimum (OPT) C:N:P ratios. Cont, Control without leaf additions; small letters denote statistically different groups (ANOVA, $p < 0.05$, $n = 30$) and no letters denote statistically no different group (ANOVA, $p > 0.05$, $n = 30$). Positive and negative values show release and uptake rates, respectively. Boxplots show 10%, 25%, 50%, 75%, and 90% percentiles and outliers.

of the incubation and did not differ significantly from that of the controls, except for the LL treatment of the LIM biofilms. There, we observed net NO_3-N release amounting to $0.088 \pm 0.1\,\mu g\,N\,g\,DW^{-1}\,hr^{-1}$ or $2.2 \pm 2.8\,\mu g\,P\,g\,AFDW^{-1}\,hr^{-1}$ for the last time step (*t*3–*t*24). The initial NH_4-N concentrations ranged between 5 and 7 μg/L in the control and the LL treatment and increased to an average of 25 μg/L (LIM) and 70 μg/L (OPT) after 24 hr (Table 3). In the FL treatment, the opposite trend was observed, with 40–70 μg/L at the beginning and 15–31 μg/L after 24 hr. The initial mean NO_2-N concentrations ranged between 0.5 and 9.6 μg/L, with lower concentrations in the FL treatments. After 24 hr, the mean NO_2-N concentrations had increased in all LIM treatments and in the OPT-FL treatment. As in the first experiment, DOP and DON concentrations were below detection limit. The initial DOC concentrations were very low in the sediment controls but ranged between 25 and 40 mg μg/L in the leaf treatments. The DOC concentrations remained relatively stable in all treatments throughout the experiment. In the second experiment, the average DOC:N:P ratios were 55:8:1 for LIM-FL, 70:11:1 for LIM-LL, 100:16:1 for OPT-FL, and 340:37:1 for OPT-LL after 24 hr.

4 | DISCUSSION

This study investigated the effects of leaf addition on nutrient uptake by heterotrophic and photoautotrophic benthic biofilms. We found that the presence of leaves had significant effects on the P uptake by dark-grown heterotrophic biofilms but not on the uptake by light-grown photoautotrophic microbes. In contrast, nitrate uptake rates did not respond to leaf additions in any of the experiments. Contrary to our expectations, heterotrophic biofilms grown under strongly C-limited conditions showed a stronger stimulation of P uptake by the additional POC supply than those grown under near optimal C:N:P ratios. Interestingly, over 24 hr, fresh leaves enhanced P uptake more than leached leaves despite their initially higher nutrient leaching at the beginning.

4.1 | Effects of leaf additions on the nutrient uptake by dark-grown biofilms

In agreement with our hypotheses, the addition of POC in the form of leaves improved the P uptake by dark-grown heterotrophic biofilms significantly over 24 hr. In the short term (maximum 3 hr), leaching and mineralisation of P from leaves dominated the nutrient exchange rates. Stimulating effects of bioavailable (D)OC on the heterotrophic microbial P uptake have been found also in other studies (Graeber et al., 2021; Stutter, Graeber, & Weigelhofer, 2020). In laboratory flow-through columns, the addition of glucose together with N and P increased the net P uptake by heterotrophic hyporheic biofilms by a factor of 1.5 compared to the NP treatment only (Stutter, Graeber, & Weigelhofer, 2020). There, the P retention was already high in the NP treatment without glucose addition which the authors ascribed to either P sorption processes or the microbial use of native OC in the sediments for P assimilation. Likewise, we observed P uptake already in our sediment controls concurrent with relatively high amounts of native DOC leached from the biofilms that probably stimulated microbial P assimilation. Graeber et al. (2021) also found a strong correlation between P uptake by heterotrophic planktonic stream microbes and molar DOC:P ratios between 100 and 400 in a laboratory study using leaf leachate as carbon source. They identified a zone of maximum (heterotrophic) P uptake at C:N:P ratios of >170:21:<1. According to this study, optimum C:N:P ratios in experiment 1 of our study occurred only in the OPT-FL treatment which also showed the highest P uptake after 24 hr.

The C:N:P ratios in our study refer only to the dissolved phase but do not consider OC that may be taken up by microbes directly from the decomposing leaves without reaching the overlying water column. Both the C:N:P ratios and the bioavailability of these macronutrients in the water column are the net result of various biological and physical uptake (e.g., adsorption, assimilation, respiration) and release processes (e.g., desorption, leaching, mineralisation) occurring in the benthic layer (Webster et al., 2009). Thus, they may not necessarily reflect the actual conditions in the immediate environment of the biofilm microbes (Scott et al., 2008). For example, Kamjunke et al. (2015) observed responses to the quantity and quality of dissolved OM only in planktonic stream microbes but not in benthic biofilms. This indicates that sediment-associated biofilms may be more controlled by internal nutrient and carbon cycling than by the external nutrient supply (Brailsford et al., 2019; Graeber et al., 2018; Wagner et al., 2017; Weigelhofer et al., 2020). In contrast, leaf- and detritus-associated biofilms have OC directly available in their immediate surroundings (Cheever et al., 2012) and may thus require additional nutrients from the water column for enhanced growth. Several studies have shown positive effects of nutrient additions on leaf decomposition (Cheever et al., 2012; Ferreira et al., 2015; Niyogi et al., 2003; Vivanco & Austin, 2011), indicating that leaf litter input to streams may reciprocally increase nutrient uptake (Dodds et al., 2004; Stutter et al., 2018; Wymore et al., 2016).

As leaf decomposition changes with time, the decomposition state of leaves controls their impact on the uptake of externally supplied nutrient (Cheever et al., 2012; Ferreira et al., 2015; Robbins et al., 2023; Webster et al., 2009). Robbins et al. (2023) modelled nutrient dynamics of decomposing litter from data of published studies. They found that P dynamics were strongly affected by the initial P content. Phosphorus-poor detritus acted as nutrient sink initially but shifted to net P mineralisation after losing *c*. 40% of its mass. In contrast, detritus always acted as a net source of N regardless of the initial N content. Likewise, Webster et al. (2009) developed a model showing a shift from net nutrient uptake at an early state of leaf decomposition to net release at a later stage. We also observed significant differences between the treatments with fresh and leached leaves, in which the stimulating effects of POC on the P uptake was higher when fresh leaves were added despite their initially high net P leaching. Fresh leaves from riparian trees initially leach mainly low-molecular nutrient-rich carbon compounds from their cell contents with a high bioavailability (Pisani et al., 2020). In our study, the DOC leached from fresh leaves could possibly compensate the additional nutrient supply and stimulate microbial P uptake stronger

than the leached leaves. The higher bioavailability of the DOM from fresh leaves is also reflected in increased respiration rates, as microbes tend to respire readily available low-molecular DOM compounds while more refractory molecules are assimilated (Berggren & Giorgio, 2015).

In addition, we observed significant differences in the net P uptake over time between heterotrophic biofilms grown under strongly C-limited conditions and those grown under near optimal C:N:P ratios. LIM biofilms reacted faster than OPT biofilms to the additional POC source and showed lower net DOC and nutrient leaching during the first hour, indicating immediate uptake of the leached macronutrients. However, after 24 hr, net P uptake was significantly higher in OPT than in LIM biofilms independent of the conditions of the leaves. Higher bacterial abundances and an increased P demand of the OPT biofilms (indicated by higher phosphatase activities) can only partly explain these differences, as they occurred only in the FL treatment. Instead we assume that the near-optimal C:N:P conditions during the colonisation phase stimulated the growth of a different microbial community that was better adapted to the additional OC supply and showed a higher efficiency in consuming OC, N, and P than in the LIM treatments. We made similar observations of a temporally dependent uptake efficiency in near-natural versus chronically loaded streams (Weigelhofer et al., 2018). There, biofilm communities not adapted to extended nutrient enrichments reacted faster to nutrient additions than biofilms in nutrient-loaded streams but could not sustain the initially high uptake efficiency over longer time periods. Future experiments should thus also look into the composition of the respective microbial biofilm communities to get a better insight into the mechanisms of coupled OC-nutrient uptake.

In contrast to P, the heterotrophic N uptake did not increase through the addition of leaves in our study although other laboratory studies have found stimulating effects of bioavailable DOC on the N uptake by heterotrophic biofilms (Graeber et al., 2021; Stutter, Graeber, & Weigelhofer, 2020). Stutter, Graeber, and Weigelhofer (2020), for example, observed four times larger effects of glucose on nitrate uptake than on P uptake in their study. The authors presumed that the bioavailable DOC stimulated both nitrogen assimilation and denitrification. Due to the well-oxygenated conditions, however, we assume that denitrification was mainly inhibited in our experiments. The laboratory study by Graeber et al. (2021) showed a strong correlation between N uptake and molar DOC:N ratios between 7 and 35 with a zone of maximum (heterotrophic) N uptake at C:N:P ratios of >114:9:<1. According to this study, C:N:P ratios in experiment 1 of our study occurred in the very low or no N uptake zone, which fits to the lack of significant N uptake.

4.2 | Effects of leaf additions on the nutrient uptake by light-grown biofilms

In contrast to heterotrophic biofilms, phosphorus uptake by light-grown photoautotrophic biofilms did not differ between treatments with and without leaves. On average, light-grown biofilms showed higher overall biomass, chlorophyll-a content, and bacterial abundances than dark-grown biofilms. While net phosphorus uptake per gram sediment was higher in the light-grown than in the dark-grown biofilms, the reverse pattern was observed when compared on OM and biomass base. Thus, the increased net P uptake by light-grown biofilms compared to dark-grown biofilms was rather due to the larger microbial biomass than to an enhanced microbial activity. Contrary to experiment 1, the sediment controls showed no initial DOC leaching in the second experiment. The lack of both, POC stimulation of nutrient uptake and initial DOC leaching, indicate even stronger internal nutrient and OM cycling in the light-grown than in the dark-grown biofilms.

Internal cycling within algae-dominated biofilms have also been reported in other studies (Francoeur et al., 2020; Rier & Stevenson, 2002; Romaní et al., 2014; Scott et al., 2008; Wyatt & Rober, 2019). While the growth of algae is independent of OC, algae provide protein-rich labile DOC in the form of algal exudates which can be directly used by biofilm heterotrophs (Wagner et al., 2017; Ylla et al., 2009). In contrast, heterotrophic microbes supply algae with nutrient from mineralisation. This internal nutrient and OC cycling is stronger when biofilms are thicker due to the decreasing solute exchange between the water column and the biofilms (Pereda et al., 2019). As a result, nutrient uptake by mainly photoautotrophic biofilms is largely controlled by the availability of light (Rier & Stevenson, 2002; Wagner et al., 2017) and the supply of dissolved (inorganic) N and/or P (Jarvie et al., 2018) but not (or hardly) by the amount and quality of the OM.

The coupling between photoautotrophic and heterotrophic microbes in stream biofilms and their dependence on external C, N, and P supply is highly complex and depends on the respective biological interactions (competition vs. mutualism; Scott et al., 2008; Wyatt & Rober, 2019; Ylla et al., 2009), physical constraints (solute transport, nutrient gradients; Scott et al., 2008; Pereda et al., 2019), and (mainly) growth-related environmental factors, such as external nutrient supply, light availability, stream velocity, and the substrate for colonisation (Rier & Stevenson, 2002; Stutter, Wyness, et al., 2020). The use of externally supplied vs. internally recycled nutrients and OM may also change in space and time. Wagner et al. (2017) found that high light availability shifted periphyton communities from using allochthonous DOC to autochthonous DOC. Thus, nutrient uptake from the water column may shift with daytime or shading conditions in mainly algae-dominated biofilms.

4.3 | Consequences for the stream management

Interactions between leaf decomposition and nutrient uptake are widely reported for stream ecosystems but are highly complex due to the variety of OC-dependent and independent processes involved (Bastias et al., 2018; Cheever et al., 2012; Gibson & O'Reilly, 2012). As the OC supply to streams varies seasonally, these processes may change throughout the year (Gibson & O'Reilly, 2012; Wagner et al., 2017), blurring the role of stoichiometry on in-stream nutrient

uptake further. Additionally, higher trophic levels may influence the relationship between POC supply and in-stream nutrient retention as macro-invertebrate feeding on biofilms may stimulate leaf breakdown and thus enhance nutrient uptake (Cheever et al., 2012). These complex abiotic and biotic interactions make nutrient control via OC management in nutrient loaded streams challenging. Given the still poorly understood effects of OC quantity and quality on nutrient uptake, management approaches are usually suggested that focus on the restoration of riparian areas and wetlands to increase the amount of natural, complex OC with a moderate degradability that keeps heterotrophic metabolism (and thus oxygen consumption) low but potentially stimulates in-stream N and P retention (e.g., Dodds et al., 2004; Robbins et al., 2017; Stanley et al., 2012; Stutter et al., 2018; Wymore et al., 2016). Such restoration approaches have various other positive effects on the stream ecosystem, such as temperature control, increased habitat availability for both the aquatic and riparian fauna, and mitigation of terrestrial N and P inputs among others, which will compensate any missing or potentially negative side effects of the increased DOC supply (e.g., water colouring, increased oxygen consumption) by far. Our study supports such management approaches insofar as leaves actually stimulated heterotrophic nutrient uptake. However, our study also shows that sun-exposed stream sections with well-developed autotrophic biofilms (or macrophytes) may exhibit a much higher nutrient uptake efficiency than shaded and mainly heterotrophic reaches (see also Fellows et al., 2006; Julian et al., 2011; Reisinger et al., 2019). Thus, further studies are needed on the coupling between POC and nutrient uptake regarding not only the nutrient uptake efficiency of different biofilms but also the duration of nutrient storage to develop nature-based and sustainable management approaches for nutrient loaded agricultural streams.

AUTHOR CONTRIBUTIONS

Conceptualisation: E.A., G.W. Conducting the research: E.A. Data analysis: E.A., D.B, G.W. Data interpretation: E.A., D.B., A.W., G.W. Preparation of figures and tables: E.A., D.B. Writing: E.A., D.B., A.W., G.W. All authors contributed to the revised version.

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CONFLICT OF INTEREST STATEMENT

The authors have no conflict of interest to declare.

DATA AVAILABILITY STATEMENT

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

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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