ORIGINAL RESEARCH

Epilithic algae from an urban river preferentially use ammonium over nitrate

Eduardo Cejudo ⬤ . William D. Taylor . Sherry L. Schiff

Abstract Epilithon is a taxonomically diverse assemblage of aquatic organisms which grow on rocks; this biological compartment is involved in several reactions that contribute to the dynamics of dissolved organic nitrogen in water. Using ceramic tiles colonized in an urban river, this experimental study assessed the relative importance of ammonium uptake by inhibiting nitrification blocking ammonium oxidation with acetylene, as well it tested the hypothesis that epilithon preferentially assimilated ammonium over nitrate. In our experiments, ammonium uptake by epilithon accounted for 46% – 100 % of the ammonium decrease in the water column, whereas nitrate uptake accounted for 0% – 11% of the nitrate decrease. Ammonium uptake rates ranged from 197 to 519 μmol N-NH$_4^+$ m^{-2} h^{-1}, while nitrate uptake rates were from 47 to 85 μmol N-NO$_3^-$ m^{-2} h^{-1}. The rate of preferential assimilation (RPI) was between 1.15 and 1.26, indicating preference for ammonium over nitrate. The results of this research provide valuable information regarding the relative contribution of algal uptake relative to nitrification in epilithon from an urban river.

Keywords Epilithon . Uptake . Ammonium . Nitrate

Introduction

Epilithon comprises organisms that grow on the surface of rocks in aquatic ecosystems. Epilithic biofilms in rivers play diverse roles in tightly coupled biogeochemical processes; for instance, photosynthesis and heterotrophic growth are often synergistic (Paerl and Pinckney 1996; Kuehn et al. 2014). Nutrient enrichment in rivers is commonly associated with an increase in biomass due to the response of the algal component (Stevenson et al. 2006; Elser et al. 2007; Kane et al. 2014); thus, the recovery of rivers impacted by nutrient point sources is largely dependent on the in-stream nutrient processing (Murdock et al. 2004; Ribot et al. 2012).

Epilithon assimilates ammonium (NH$_4^+$) and nitrate (NO$_3^-$) into biomass, whereas the mineralization of organic matter can regenerate NH$_4^+$ (Wetzel 1964; Galloway 2003). NH$_4^+$ is an energetically favorable nitrogen species because it requires less energy to be incorporated into organic nitrogen due to its reduced state (Taiz and Zeiger 2010). The expression NH$_4^+$ preference is used to stress that, in spite of the availability of dissolved inorganic nitrogen, ammonium is taken up and incorporated into biomass at higher rates than nitrate. Preference for one species over the other is not simply inhibition or competition, it is rather a combination of processes affected by concentration and environmental conditions (Dortch 1990). Changes in environmental nitrogen concentrations do not necessarily become variations in uptake rates (Dortch 1990; Kemp and Dodds 2002; Ribot et al. 2013). N fluxes due to mineralization, NH$_4^+$ oxidation or denitrification have been previously reported in epilithon (Duff et al. 1984; Binnerup et al. 1992; Kemp and Doods 2001; O'Brien et al. 2012). However, few papers have addressed autotrophic assimilation or the relative

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contribution of bacterial versus algal uptake. For instance, NH_4^+ assimilation by epilithon accounted for 9% – 23% of the total NH_4^+ loss in streams (Hamilton et al. 2001; Peipoch et al. 2016). One way to disentangle the multiple nitrogen transformations that occur in epilithon is using chemical inhibitors. Several substances inhibit ammonium oxidation by biocidal activity or by causing toxicity to specific microbes that oxidize ammonium (Huber et al. 1977). The activity of the enzyme ammonia monooxygenase (AMO) is reduced or inhibited by direct binding with alternative substrates such as acetylene (C_2H_2). Direct binding with an alternative substrate results into the irreversible inactivation of the enzyme and its recovery involves *de novo* protein synthesis (McCarty 1999). Acetylene has been previously used for simultaneously inhibiting both nitrification and denitrification (Knowles 1990; Teissier and Torre 2002). This type of chemical inhibition was resorted to in this research to distinguish autotrophic NH_4^+ uptake from NH_4^+ oxidation in epilithon; however, it does not account for heterotrophic uptake (Kirchman 1994). The objective of this research is to provide evidence regarding the importance of uptake and nitrification as pathways for NH_4^+ loss in aquatic ecosystems and the preferential uptake of NH_4^+ over NO_3^- by the autotrophic component in epilithon growing in the Grand River (southwestern Ontario, Canada) as an example of an urban river. Urban rivers receive large loads of N from wastewater treatment plants and information about nutrient processing is required (Gibson and Meyer 2007; Zhang et al. 2015). The Grand River (southwestern Ontario) is an example of an urban river impacted by economic activities. The land in the middle section of the watershed (where the epilithon for this research was grown) is used for intensive agriculture and urban development; thereby, high concentrations of nitrate and phosphorus are commonly measured owing to the extensive tile drainage and treated wastewater discharged from wastewater treatment plants (Grand River Conservation Authority 2006). Thus, understanding the preferential assimilation of one nitrogen species over the other will assist in assessing the impact of treated effluent into watercourses.

Materials and methods

The middle section of the Grand River's watershed (southwestern Ontario) was used as an example of an urban river impacted by agriculture and urban development. The epilithon was grown on unglazed ceramic tiles, 25.8 cm^2 each. Sheets of tiles were incubated in uncovered plastic containers in the middle Grand River at a location 520 m downstream from Kitchener wastewater treatment plant's effluent (43.398° N, -80.4155° W) for 35 days (June 11th - July 17th 2013). Flow velocity and water depth varied over the growing period owing to fluctuating river discharges (40.8 ± 27.2 m^3/s, HYDAT station 02GA048), result of summer rainfall and water released from two flow control structures in the upper Grand River (Conestogo and Shand Dams). Biomass was quantified from tiles that were not used in the experiments. The biomass from 16 tiles was brushed and scraped and the slurry obtained was dried for 24 h at 60ºC, weighed, baked for 2 h at 550ºC and then reweighed to ascertain ash-free dry weight (AFDW). Similar incubation experiments in the Grand River reported tile colonization by *Cladophora* spp, diatoms and unidentified green algae; however, macroscopic filamentous material was not observed in our samples (Barlow-Busch et al. 2006). Chlorophyll was measured from four tiles not used in the experiments. The slurry brushed off from the tiles was taken to a final volume of 500 mL with ultrapure water and kept in cold and dark conditions until analysis. The samples were filtered (0.5-0.7 bar) using pre-baked Whatman GF/F filters (0.7 μm). After filtration, the filters were folded and placed into 20-ml glass vials with 10 mL of 90% acetone, shaken gently and stored in darkness overnight (4ºC). Before the analysis, samples were filtered to remove glass fibers from the liquid (0.45-μm Millipore syringe filter). The absorbance of both acetone blanks and extracts was measured at 750, 664, 647, and 630 nm and chlorophyll concentrations were calculated following Jeffrey and Humphrey (1975). Finally, chlorophyll *a* and ash-free dry mass (AFDM) were referred to as indicators of the community structure using the autotrophic index (AI = [AFDM/Chl *a*]).

The tiles colonized with epilithon and used for "uptake" treatments were pre-incubated in a solution of 1% C_2H_2 (v/v) overnight to inhibit nitrifying bacteria (following Herrmann et al. 2007). The chemical inhibition performed in this research assumes that nitrification was inhibited; hence, NH_4^+ loss in such treatments was considered to represent autotrophic uptake, whereas treatments without C_2H_2 accounted for "*uptake + nitrification*". All treatments (in duplicate) had 300 mL of Grand-River water filtered through a 0.45-μm filter (Millipore filter membrane) in 400-mL HDPE containers (Starplex®). The treatments

Table 1 Experimental design showing the nitrogen species present in each treatment (n=2) and the initial mass (C0 in µmol N) for assessing uptake rates in epilithon. Inhibition refers to incubation with acetylene (C2H2, 1% v/v) prior to the experiment.

N specie(s)	C_0 [µmol N]	Inhibition
	23	C_2H_2
	23	
NH_4^+	67	C_2H_2
	67	
	101	C_2H_2
	101	
	29	
NO_3^-	72	
	123	
	25 + 24	C_2H_2
	25 + 24	
$NH_4^+ + NO_3^-$	53 + 70	C_2H_2
	53 + 70	
	85 + 107	C_2H_2
	85 + 107	

were amended with three concentration levels of ammonium ($(NH_4)_2SO_4$, Fisher Scientific, CAS 7783-20-2) or nitrate (KNO_3, Fisher Scientific, CAS 7757-79-1) or both (Table 1). The initial concentration used in the experiments was similar to the annual average concentrations observed in the Grand River from 2010 to 2013 (0.1 to 0.8 mg N-NH_4^+ L^{-1}; 3.0 to 4.0 mg N-NO_3^- L^{-1}); except for the heavy NH_4^+ concentration treatment (4.7 mgN-NH_4^+ L^{-1}) which was higher than any value ever observed in the river. All treatments started at the same time under the following conditions: 25º C, 24-hour light regime (630 lumens, incandescent bulbs), continuous stirring. Containers were lid-closed and each tile was elevated 2 cm from the bottom by means of a plastic support. Water subsamples from each experimental container were collected at 0, 0.5, 1, 2, 3, 6, 12, 24 and 48 h, filtered through a 0.45-µm filter (0.45-µm Millipore filter membrane) and stored in 5-mL HDPE vials for their later analyses. NH_4^+ and NO_2^- were analyzed using colorimetric methods (blue indophenol and sulphanilamide + azo dye, respectively) with a Smartchem 200 Autoanalyzer (Westco, Brookfield CT; precision 5%, detection limit 0.05 mg N L^{-1}). NO_3^- was analyzed with an Ion Chromatograph (Dionex ICS-90 Thermo Scientific, Sunnyvale CA; precision 0.07 mg N L^{-1}, detection limit 0.05 mg N L^{-1}). Table 1 shows the conditions that express initial N mass per experimental unit (µmol N). Dissolved inorganic nitrogen (DIN) was calculated as the addition of $NO_2^- + NO_3^- + NH_4^+$. Since sampling continuously removed volume from all experimental units, mass removal due to volume withdrawal was accounted over time for accurate N mass per experimental unit.

NH_3 volatilization was estimated from the initial pH of the river's water amended with $(NH_4)_2SO_4$ at pH = 7.6; this way, free ammonia was ascertained by means of equation 1

$$\left[NH_{3\,free}\right] = \frac{\left[NH_4^+ \times pH^{10}\right]}{(ka/kw) + 10^{pH}} \qquad Eq\ 1$$

The estimated free NH_3 was less than 2.3% of the total ammonium nitrogen (TAN) and was not considered in the DIN balance.

NH_4^+ and NO_3^- uptake rates (k_{uptake} in µmol h^{-1}) were estimated as the slope of the linear regression of the N mass (µmol N) by time (h) over the 48-hour duration of the experiment. Areal uptake rates were obtained for the colonized tiles (A = 25.8 cm^2) extrapolating to µmol N m^{-2} h^{-1}. Finally, biomass-specific uptake rates (µmol N g biomass^{-1} h^{-1}) were ascertained by extrapolating the areal uptake rates measured in the tiles to the biomass that grew on them. Changes in biomass over the experiments (48 h) were not considered.

In order to quantify epilithon preference for NH_4^+, the rate of preferential incorporation (RPI, equation 2) was utilized following Dortch 1990, and Takahashi and Saijo 1981.

$$RPI_{NH_4^+} = \frac{P_{NH_4^+} / \Sigma\rho_N}{[NH_4^+] / [\Sigma N]} \qquad\qquad Eq\ 2$$

Where $P_{NH_4^+}$ is the ammonium uptake rate; $\Sigma\rho_n$ is the sum of rates of all inorganic nitrogen species; $[NH_4^+]$ ammonium concentration; and, $[\Sigma N]$ is the total concentration of inorganic nitrogen. RPI NH_4^+ values greater than 1.0 indicate preference for ammonium.

Statistical analyses

Statistical analyses were run on JMP 5.1 (SAS Institute). Graphs were made in SPSS 13 (SPSS Inc.). Effect tests (F) and Least Squares Mean plots were produced to assess significant effects of the variables and their interactions. Post-hoc tests (Tukey Kramer HSD, α=0.5) were performed within treatments by N species to evaluate differences over time.

Results

Epilithic biomass measurements

The epilithon which grew on the tiles in the middle Grand River had 73.6 g biomass AFDW m^{-2} (±37.2 g m^{-2}). Chlorophyll measurements were 42.4 ± 9.6 mg Chl a m^{-2}, 8.7 ± 3.9 mg Chl b m^{-2} and 3.6 ± 1.7 mg Chl c m^{-2}. The *Chl c: biomass* ratio was ≈ 0.0006 mg Chl a (mg C AFDW)$^{-1}$ and the autotrophic index AI ≈ 1700.

NH_4^+ and NO_3^- uptake

The change in NH_4^+ ($NH_{4\ initial}^+$ - $NH_{4\ final}^+$) was different between treatments with and without inhibitor per concentration level (Tukey Kramer HSD, q = 2.47, p = 0.01, df = 10). In the treatments without inhibitor
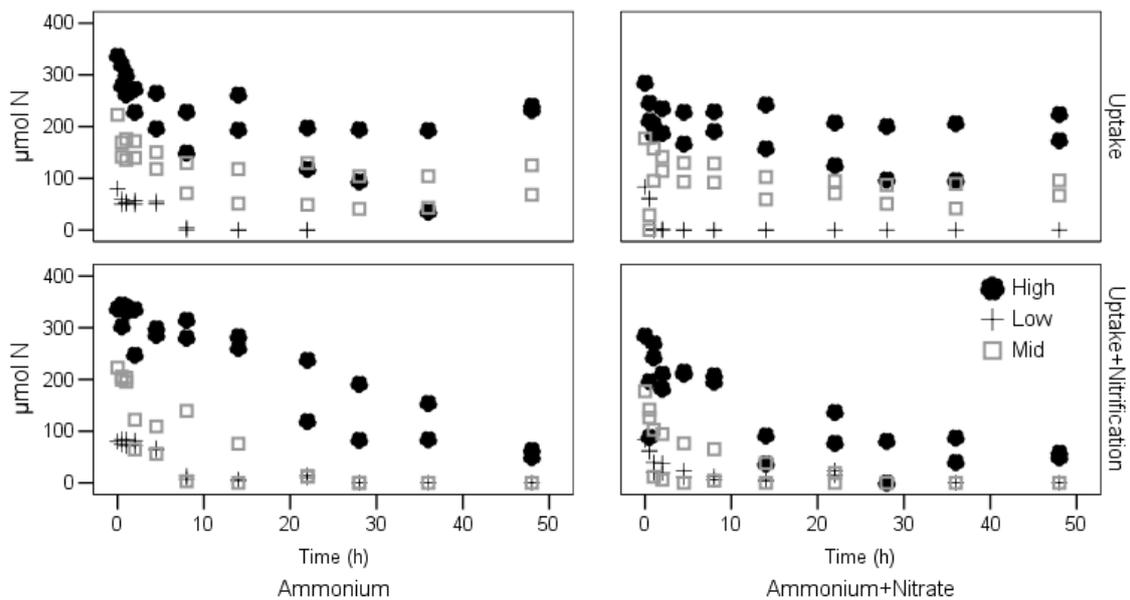
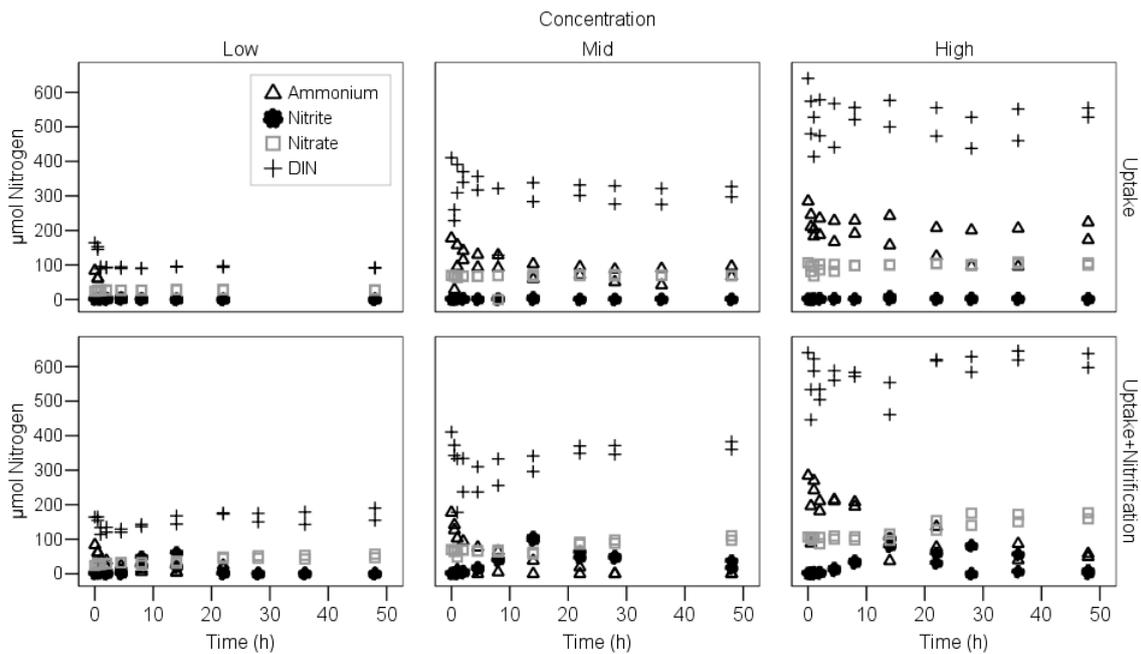**Fig. 1** Ammonium decrease (μmol N-NH$_4^+$) over time in treatments amended with ammonium only (*left*) and ammonium + nitrate (*right*). Uptake represents experimental units with tiles previously incubated with inhibitor (acetylene 1% v/v). Nitrogen concentrations (high, mid, low) as described in Table 1.
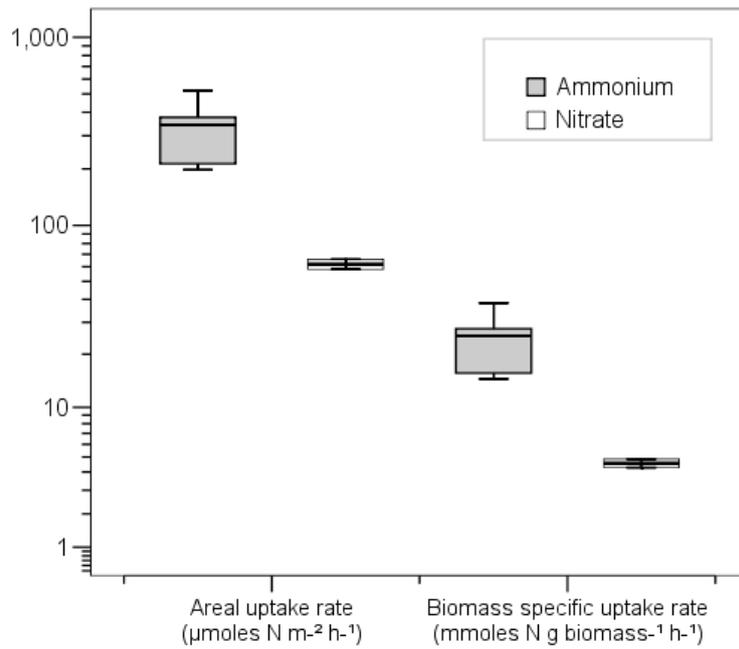
Fig. 2 Estimated ammonium and nitrate areal uptake rates (µmol N m^{-2} h^{-1}) and biomass specific uptake rates (mmol N per g biomass^{-1} h^{-1}). NB: Y axis is in log scale.

Fig. 3 Changes in ammonium, nitrite, nitrate and dissolved inorganic nitrogen (DIN) in treatments amended with ammonium + nitrate. Uptake represents experimental units with tiles previously incubated with inhibitor (acetylene 1% v/v). Nitrogen concentrations (high, mid, low) as described in Table 1

(*Uptake + Nitrification*) between 26% and 100% of the initial NH$_4^+$ was removed from the water. The treatment with the highest concentration (> 101 µmol N-NH$_4^+$) was the only one to display NH$_4^+$ above the detection limit after 48 h. The treatments with inhibitor (*Uptake*) had a less steep decrease in NH$_4^+$ (Fig. 1). NH$_4^+$ decrease patterns were similar when both NH$_4^+$ and NO$_3^-$ were present in the same experimental unit. Only in the low concentration treatment was NH$_4^+$ removed from the water column below detection limit after 48 h. Considering the change in NH$_4^+$ from experimental units inhibited with C$_2$H$_2$, it was deemed that autotrophic uptake accounted for 46% and 56% decrease in the initial NH$_4^+$ mass in the mid and high
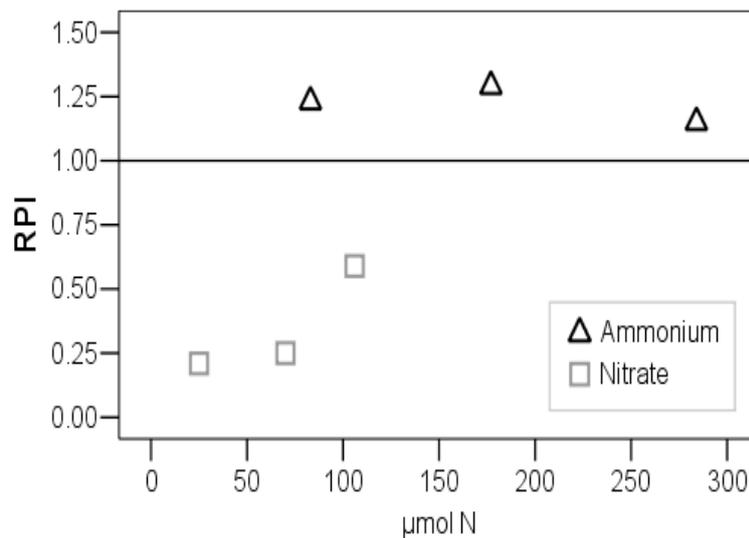
Fig. 4 Rate of preferential incorporation (RPI) of ammonium and nitrate in treatments amended with both nitrogen species at different initial N mass. Values above 1.0 represent NH_4^+ preference.

concentration treatments, respectively. The change in NO_3^- was much less noticeable than the changes in NH_4^+ in the mid and high concentration treatments, while it increased in the low concentration treatment despite the fact that NH_4^+ was not added (not showed). The NO_3^- loss was 0 and 11% of the initial mass supplied for the mid and high concentration treatments, respectively.

The NH_4^+ uptake rate (k_{uptake}) in the treatments inhibited with C_2H_2 was: 0.97 μmol $N-NH_4^+$ h^{-1} in the low concentration treatment ($R^2 = 0.78$, $s.e._{slope} = 0.16$); 0.89 μmol $N-NH_4^+$ h^{-1} in the mid concentration treatment ($R^2 = 0.68$, $s.e._{slope} = 0.16$); and, 1.34 μmol $N-NH_4^+$ h^{-1} in the high concentration treatment ($R^2 = 0.65$; $s.e._{slope} = 0.23$). The areal uptake rate in these experiments was between 197.7 and 519.4 μmol $N-NH_4^+$ m^{-2} h^{-1} (Fig. 2).

NO_3^- uptake rates ($NO_3^- k_{uptake}$) were: 0.15 μmol $N-NO_3^-$ h^{-1} in the mid concentration treatment ($R^2 = 0.55$, $s.e._{slope} = 0.05$); and, 0.17 μmol $N-NO_3^-$ h^{-1} in the high concentration treatment ($R^2 = 0.41$, $s.e._{slope} = 0.03$). NO_3^- areal uptake was calculated from 58 to 65 μmol $N-NO_3^-$ m^{-2} h^{-1}.

Biomass specific uptake rates were calculated extrapolating the measured areal uptake rates to the biomass growth on tiles (73.6 g biomass AFDW m^{-2}). Thus, the estimated biomass specific uptake was 24.4 $\pm$ 8.7 mmol of $N-NH_4^+$ g biomass^{-1} h^{-1} and 4.6 $\pm$ 0.3 mmol of $N-NO_3^-$ g biomass^{-1} h^{-1} (Fig. 2).

Interaction of NO_3^- and NH_4^+

The third treatment assessed the dynamic of NH_4^+ and NO_3^- together in the same experimental unit. NH_4^+ decrease and NO_3^- increase were different between the concentrations assessed (Tukey Kramer HSD, q = 2.35, p = 0.05, df= 14). The *Uptake + Nitrification* treatments showed the expected trend of NH_4^+ decrease and later NO_3^- increase due to assimilatory and dissimilatory ammonium oxidation. The NO_2^- measured with C_2H_2 inhibition was only 6% of the NO_2^- measured without C_2H_2 (Fig. 3), providing information that nitrification was greatly reduced. The final DIN was always lower than the initial DIN between treatments; however, there was a less steep decrease in DIN in the treatments with inhibitor, which suggests that little N disappeared from the water column. The NH_4^+ and NO_3^- uptake rates from the treatments, without inhibitor when the two species were present at the same time in our experimental units, were used to evaluate the rate of preferential incorporation (RPI), which showed that NH_4^+ was preferred over NO_3^- (RPI >1.0; Fig. 4).

Discussion

Preferential NH_4^+ assimilation has been reported in submerged macrophytes (Schuurkes et al. 1986; Hood et al. 2014; Volkmann et al. 2016). The epilithic community from the middle Grand River grown on ceramic

tiles preferentially took up NH_4^+ over NO_3^-, as indicated by the NH_4^+ uptake rate, greater than the NO_3^- uptake rate, and from the RPI calculated when both N species were present. The observed pattern of NH_4^+ decrease in the *Uptake + Nitrification* treatments was similar to the pattern observed for aerobic ammonia oxidizers in water (Sonthiphand et al. 2013) and sediments (Henriksen 1980). The NH_4^+ removal due to uptake and nitrification in our experiments was higher than in other studies, a NH_4^+ removal from 26% even up to 100% of the initial mass in each experimental unit was measured. Previous reports stated that the total contribution of epilithon to NH_4^+ removal oscillated from 18% in a river that received treated wastewater (Ogura et al. 2009) to as much as 66% of the total N removal in grasslands streams (Simon et al. 2007). The NH_4^+ and NO_3^- uptake rates measured in this research were higher than previously reported rates at lower dissolved inorganic nitrogen concentrations (Biggs 1990; Tank et al. 2000; Mulholland et al. 2004). We consider that the high concentration used in our experiments may have turned into higher experimental rates, as the biomass had a larger amount of dissolved nitrogen available per unit area, though it may also be due to the greater biomass that grew on the tiles, compared with other aquatic systems (Teissier et al. 2007).

Chlorella sp cultures showed a one-day delay in NO_3^- uptake when NO_3^- and NH_4^+ were added simultaneously (Malerba et al. 2015). NO_3^- uptake in our experiments may have also been delayed and longer incubation periods might have produced different outcomes. The uptake of NO_3^- by epilithon in temperate forested streams has been reported from undetectable (Hamilton et al. 2001) to as much as 850 µmol N-NO_3^- g biomass^{-1} h^{-1} (Triska et al. 1985). In our experiments, NO_3^- uptake by epilithon was small (< 25 µmol N-NO_3^- m^{-2} h^{-1}) or non-measurable, and it was only observed at experimental conditions higher than 72 µmol N (3.3 mg N-NO_3^- L^{-1}). Epilithon uptake rates can be low compared with uptake rates of macrophytes on an areal basis due to the large biomass of macrophytes and the associated periphyton (Sand-Jensen and Borum 1991). Nonetheless, estimated gross N uptake by macrophytes with attached periphyton in different sections of the Grand River was similar to our results, from 3 to 7.1 µmol N per g macrophyte^{-1} h^{-1} (Hood 2012).

The chemical inhibition used in these experiments allowed us to distinguish the dissolved inorganic nitrogen dynamics and uptake between treatments distinguishing autotrophic *uptake* from *Uptake + Nitrification* in epilithon growing in an urban river. In our experiments, the calculated RPI supports the hypothesis that NH_4^+ was preferred over NO_3^-. Preference for NH_4^+ might be common because the reduced state of this N species confers an energetic advantage, as it is readily available for organic N synthesis (Lam et al. 1996). Pulses of ^{15}N in the green algae *Enteromorpha intestinalis* showed also that more NH_4^+ was removed and assimilated into tissue than NO_3^- (Cohen and Fong 2004). The observed low NO_3^- uptake rates could have been the result of the experimental conditions or else, the epilithon that colonized the tiles was not very effective removing nitrate. Given that uptake rates were measured as NO_3^- removed from the water column, it is also possible that NO_3^- increased in the experimental chambers as a result of organic matter mineralization (Böckelmann et al. 2000; Araya et al. 2003; Teissier et al. 2007), which was neither expected nor controlled in this research. The fact that some experimental units of the same treatment showed different uptake patterns suggested that there were differences in N cycling at biofilm scale, regardless of the same experimental treatment to all tiles.

In these experiments, the NO_3^-/NH_4^+ mass ratio was similar across treatments (low=0.96; mid=1.3; and, high=1.25) and similar to the field conditions observed in the middle Grand River (NO_3^-/ NH_4^+ between 1 and 3 during the summers from 2010 to 2012). The biomass uptake rates reported in this research represent biofilm that grows in a river impacted by agricultural runoff and sewage discharges (Grand River Conservation Authority 2006).

Additional factors such as grazing (Hillebrand and Kahlert 2001), sediment abrasion (Francoeur and Biggs 2006) and the availability of other nutrients can modify the outcomes hereby reported. For instance, the large surface area of the tiles relative to the small volume of the experimental units can be an important factor when assessing nutrient assimilation rates (Nielsen and Jensen 1990). Photoinhibition (Merbt et al. 2017) and competitive exclusion of ammonia oxidizing bacteria by microalgae (Risgaard-Petersen et al. 2004) have been previously reported in experimental conditions. Regarding nutrient availability, the dissolved organic nitrogen (DON) was not measured; however, previously collected water samples in the middle Grand River downstream from the Kitchener wastewater treatment plant's effluent had an average DON of 1.2 (±0.6) mg N L^{-1} (2011-2012, *unpublished data*), comprising between 12% and 25% of the total nitrogen measured in that reach of the river. It is possible that DON mineralization was an additional NH_4^+

source. Organic matter mineralization was not expected to be an important factor during the experiment due to the abundant nutrient supply; however, there was an increase in NO_3^- at the low concentration treatments with NO_3^- as the only nitrogen source, possibly a result of internal NH_4^+ regeneration. Finally, the biomass measured on the tiles was probably different from the biomass existent in the river throughout the year and such difference might have produced a different community composition. Previous studies measured 14 – 303 mg *chl a* m^{-2} (Teissier et al. 2007). Our results showed a more constrained range of chlorophyll (42.4 ± 9.6 mg *chl a* m^{-2}); probably the result of the duration of the incubation and the relatively homogeneous community that grew on the tiles.

Algal vs bacterial uptake

For the purposes of this section, uptake is understood as the introduction or translocation of a nutrient into cells, measured as the removal of the nutrient from the water column. Coupled assimilatory and dissimilatory NH_4^+ removal have been amply recognized as important processes that transform and remove N from aquatic ecosystems (Sprent 1987; Bitton 2011); nevertheless, there is little information available on the relative contribution of algal uptake and assimilation compared with microbial oxidation. In this research, we generate knowledge on autotrophic uptake rates measured in an experiment with chemical inhibition estimated as >200 µmol N-NH_4^+ m^{-2} h^{-1} and >40 µmol N-NO_3^- m^{-2} h^{-1} for the epilithic community of an urban river. We consider these results valuable information regarding N removal from the water column with an experimental approach to algal uptake relative to bacterial ammonia oxidation. Reported NH_4^+ uptake rates varied from 14.6 to 50 µmol N m^{-2} h^{-1} and NH_4^+ oxidation at a rate of 1.16 µmol N m^{-2} h^{-1} (Peipoch et al. 2016). In our experiments, inhibition of dissimilatory NH_4^+ oxidation highlighted the relevance of algal uptake, given that C_2H_2 has been previously used for simultaneously inhibiting nitrification and denitrification (Teissier and Torre 2002). In like manner, a part of the NO_3^- decrease measured in our experiments is assumed to be algal uptake, though denitrification was not measured and its magnitude is unknown. The small Chl *a*: biomass ratio and the elevated autotrophic index suggest that the epilithic community had a large heterotrophic component or abundant organic detritus and materials other than biomass (Weitzel 1979; Biggs 1988; Cloern et al. 1995). Thus, it cannot be ruled out that the inhibited treatments also estimated assimilatory uptake by heterotrophic organisms instead of algal uptake alone.

The conceptual model of epilithic growth and maturity presented by Teissier et al. (2007) implies a tipping point where the biomass drives the balance of N removal. Assuming that the epilithic biomass grew on the tiles for these experiments was young, thin and actively growing, we can expect that N is taken up from the water column at rates exceeding mineralization and transient storage. This way, there were high uptake rates, low mineralization and reduced denitrification given that the biomass is thin enough and there is no limited O_2 exchange within the biofilm and with the water column.

Conclusions

This study showed that algal uptake was measured by chemical inhibition of nitrification using C_2H_2, and that NH_4^+ was preferred over NO_3^- by the epilithic algae growth on ceramic tiles, as indicated by the RPI >1.0. Based on the rates obtained in these experiments, epilithon from the middle section of the Grand River assimilated NH_4^+ in controlled conditions at rates of 197.7 to 519.4 µmol N-NH_4^+ m^{-2} h^{-1}. NO_3^- assimilation rates were lower, from 58 to 65 N-NO_3^- m^{-2} h^{-1} and sometimes NO_3^- was produced instead of being consumed. The estimated biomass specific uptake rates were 24.4 ± 8.7 mmol of N-NH_4^+ g biomass^{-1} h^{-1} and 4.6 ± 0.3 mmol of N-NO_3^- g biomass^{-1} h^{-1}. The uptake rates measured in these experiments provide valuable information on the contribution of algal uptake compared with nitrification in epilithic biofilms from an urban river.

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