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# Nitrogen and phosphorus removal rates using small algal turfs grown with dairy manure

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## Abstract

Conservation and reuse of nitrogen (N) and phosphorus (P) from animal manure is increasingly important as producers try to minimize transport of these nutrients from farms. An alternative to land spreading is to grow crops of algae on the N and P present in the manure. The general goal of our research is to assess nutrient recovery from animal manure using attached algae. The specific objective of this study was to evaluate the use of small subsections of algal turfs for determining N and P removal rates by attached algae under different loading rates of dairy manure. Algae were grown in a laboratory–scale algal turf scrubber (ATS) operated by recycling wastewater and adding manure effluent daily. Replicate subsections (0.032 $m^2$) of algal turf screens were removed and treated with five different loadings of anaerobically digested dairy manure containing 5 to 80 mg $L^{-1}$ $NH_4$-N and 1 to 20 mg $L^{-1}$ $PO_4$-P over a 2-h incubation period. $NH_4$-N removal rates were biphasic with a fast initial rate followed by a slower rate. Biphasic rates were more pronounced for the lowest loading rates but less so for the higher ones. $PO_4$-P removal rates were linear throughout the incubation period for all loading rates. N and P removal rates increased with increasing loading rate and biomass. In incubations using 1% dairy manure $NH_4$-N and $PO_4$-P removal rates averaged 0.72 and 0.33 g $m^{-2}$ $d^{-1}$, respectively. These rates were approximately 5 to 8-fold lower than rates measured on laboratory-scale ATS units using undisturbed turfs.

## Introduction

Agricultural non-point source pollution has become a sensitive issue as regulations become stricter to limit the contamination of groundwater and surrounding ecosystems (Baker 1992). Increasing use of intensive animal production has resulted in an excess of animal waste (Baumgarten et al. 1999) and has led to the imposition of these regulations on dairy farms (Wilkie and Mulbry 2002). Dairy manure is commonly applied to adjacent agricultural or forested lands. However, land application of animal waste is limited in its effectiveness because of the short time period it can be applied each year, the large areas required to spread the manure, and the loss of N during storage and application. These limitations increase possible transport of nutrients into the environment. A potential alternative to land spreading of manure is to grow crops of algae on the N and P present in the manure and convert these nutrients into algal biomass. Most efforts in using algae for wastewater treatment have been focused on tertiary treatment of municipal waste effluents using suspended microalgae (Oswald and Gotaas 1957; Soeder 1980). However, there has been increased interest in the use of attached algae (periphyton), primarily because of the ease of harvesting algal biomass (Hoffman 1998). One technology using periphyton, termed algal turf scrubbers (ATS) (Adey and Hackney 1989; Adey and Loveland 1998), is relatively simple in design and yields an algal biomass that can be easily harvested on adapted farm-scale equipment. An ATS is essentially an artificial stream designed to promote biological wastewater treatment using periphyton (Craggs et al. 1996). The essential elements of the ATS system are a solid support for the growth and harvest of periphyton, wave surge and optimal light (Adey and Loveland 1998).

The general goal of our research is to assess nutrient recovery from animal manure using attached algae. Previous work in this laboratory has demonstrated the use of ATS periphyton to remove N and P from three different dairy manure effluents (Mulbry and Wilkie 2001; Wilkie and Mulbry 2002). However, because of subtle differences in flow pattern, temperature, or light between laboratory ATS units, there was sometimes considerable heterogeneity in the productivity between units. The specific objective of this study was to test the effect of different loading rates of anaerobically digested dairy manure on N and P removal rates over short time periods using small ($0.032\ m^2$) subsections of a laboratory-grown ATS turf. Although the manure contained significant levels of organic nitrogen and organic phosphorus, we focused on removal of the most available forms of N and P in manure, namely ammonium and orthophosphate. The use of subsections from a single ATS unit was intended to minimize heterogeneity between turf samples and to increase the speed with which we could test different treatments using proper replicates.

## Materials and methods

### *Cultivation of algal turfs*

Laboratory-scale algal turf scrubber (ATS) units containing $1\ m^2$ of growing area were operated by continuously recycling 208 L effluent. In this system, ATS effluent was pumped into a wave surge bucket from a plastic drum below. The bucket filled up, tipped over, and the effluent washed over the turf screen before draining back into the drum to be recycled. Water depth over the screen was 1–2 cm. The units were illuminated for 23 hours per day using two 400-W metal halide lights (300–470 $\mu$mol $m^{-2}\ s^{-1}$). ATS units were originally seeded with algal consortia from a nearby stream. The most abundant genera of attached algae in the ATS units were *Ulothrix, Oedogonium* and *Rhizoclonium*. The algae were maintained by daily adding 0.4 L of anaerobically digested manure (from the USDA dairy unit in Beltsville, Maryland) and harvesting them weekly as described previously (Mulbry and Wilkie 2001). The manure contained 2010 mg $L^{-1}$ $NH_4$-N, 750 mg $L^{-1}$ organic-N, 2760 mg $L^{-1}$ TN, 370 mg $L^{-1}$ $PO_4$-P and 410 mg $L^{-1}$ TP. For short-term experiments, an ATS screen containing an established algal turf was cut into 16 × 20 cm subsections ($0.032\ m^2$ each) and 16 of these subsections were attached to a new $1\ m^2$ screen using plastic ties. The screen containing the attached subsections was cultivated in a laboratory ATS unit as described above.

### *Nitrogen and phosphorus removal experiments*

N and P removal experiments were conducted using digested dairy manure with concentrations of 0.25%, 0.5%, 1%, 2%, and 4%, corresponding to initial concentrations of approximately 5, 10, 20, 40, and 80 mg $L^{-1}$ $NH_4$-N and 1, 2, 4, 8, and 16 mg $L^{-1}$ $PO_4$-P, respectively (Table 1). Immediately prior to each experiment, the manure was diluted to the desired concentration with de-ionized water and adjusted to pH 7. For each treatment, four ATS subsections were randomly selected after 2–5 days of growth, removed from the larger screen and placed into 16 × 25 cm flat plastic containers, and 400 mL diluted manure was added to each container. The four containers were placed on a rocking shaker that simulated the water motion within an ATS and illuminated using two 400 W halide lights (340–475 $\mu$mol $m^{-2}\ s^{-1}$) for two hours at 23–25 °C. To minimize ammonia volatilization the pH was maintained between 7.0–7.5 by adding 0.1 N HCl. The number of replicates for each treatment is shown in Table 1.

Effluent samples (1 mL) were taken at 5-minute intervals for the first 40 minutes and at intervals of 10 and 15 minutes thereafter. Effluent samples were stored on ice and analyzed within 24 h for $NH_4$-N, $PO_4$-P, nitrite ($NO_2$-N) and nitrate ($NO_3$-N) (Mulbry and Wilkie 2001). At the end of the incubation period, algal biomass on subsection screens was harvested and dried at 70°C for dry weight determination. The four harvested subsection screens were returned to the ATS system for re-growth.

## Results

There were significant reductions in $NH_4$-N and $PO_4$-P levels at all five loading rates and the kinetics of their removal varied with the loading rate (Table 1). The results of three representative loading rate treatments (0.25%, 1%, 4% manure) using similar

*Table 1.* Removal rates of $NH_4$-N and $PO_4$-P using algal turfs and different loadings of manure. Values represent mean and standard deviation; number of replicates in parenthesis.

| % Manure | Initial Concentration | | | | Removal Rate ($\mu$mol min$^{-1}$ g$^{-1}$ DW) | | |
|---|---|---|---|---|---|---|---|
| | $NH_4$-N | | $PO_4$-P | | $NH_4$-N | | $PO_4$-P |
| | mM | mg L$^{-1}$ | mM | mg L$^{-1}$ | 15–40 min | 40–120 min | |
| 0.25 (4) | 0.35 ± 0.03 | 4.85 ± 0.44 | 0.03 ± 0.001 | 0.93 ± 0.03 | 0.97 ± 0.37 | 0.16 ± 0.07 | 0.07 ± 0.03 |
| 0.5 (4) | 0.65 ± 0.02 | 9.07 ± 0.26 | 0.05 ± 0.007 | 1.61 ± 0.22 | 1.78 ± 0.52 | 0.25 ± 0.12 | 0.13 ± 0.04 |
| 1 (8) | 1.41 ± 0.07 | 19.70 ± 0.97 | 0.11 ± 0.02 | 3.42 ± 0.75 | 1.36 ± 0.46 | 0.41 ± 0.15 | 0.12 ± 0.03 |
| 2 (4) | 2.70 ± 0.19 | 37.80 ± 2.62 | 0.24 ± 0.02 | 7.56 ± 0.76 | 3.64 ± 0.75 | 1.60 ± 0.58 | 0.61 ± 0.10 |
| 4 (4) | 5.34 ± 0.32 | 74.80 ± 4.53 | 0.50 ± 0.04 | 15.40 ± 1.39 | 3.68 ± 2.55 | 3.10 ± 1.41 | 0.40 ± 0.08 |

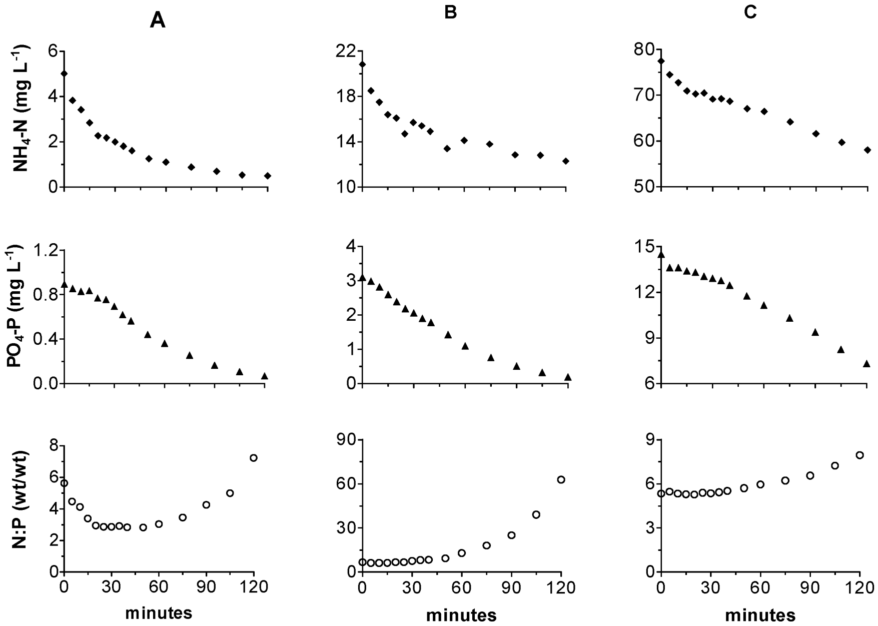


*Figure 1.* $NH_4$-N and $PO_4$-P concentrations and N:P ratios by weight over time from three representative experiments using algal turfs in (A) 0.25%, (B) 1% and (C) 4% manure effluent. The levels of algal biomass in the experiments were roughly comparable with dry weight values of 1.82 g, 1.35 g and 1.69 g, respectively.

amounts of algal biomass are shown in Figure 1. $PO_4$-P removal rates were roughly linear throughout the 2-h incubation period for all five treatments. However, at the three lower loading levels (0.25, 0.5%, 1% manure) $NH_4$-N removal was rapid during the first 40 minutes of incubation followed by a slower removal rate for the remaining 80 minutes. This phenomenon was most evident at the two lowest loading rates (0.25 and 0.5% manure). At the two highest loading rates (2% and 4% manure) $NH_4$-N removal was roughly linear throughout the incubation period. Effluent N:P ratios by weight for all treatments decreased or were unchanged for the first 30 minutes of incubation and increased for the remaining period (Figure 1).

Although a variety of factors influence N and P uptake by algal turfs, two factors that can be manipulated in large-scale manure treatment operations are the manure loading rate and biomass density. With increasing manure loading rate, $NH_4$-N and $PO_4$-P removal rates increased (Table 1, Figure 2). Due to the nonlinear nature of $NH_4$-N removal observed, two $NH_4$-N removal rates were calculated for all treatments, an initial ″fast″ rate (from T = 15–40 min) and a final ″slow″ rate (from T = 40–120 min). Both the fast and slow $NH_4$-N removal rates increased linearly with increasing $NH_4$-N concentration up to approximately 40 mg L$^{-1}$ (2% manure). However, the differences between initial and final $NH_4$-N removal rates at each treatment decreased as the manure loading in-

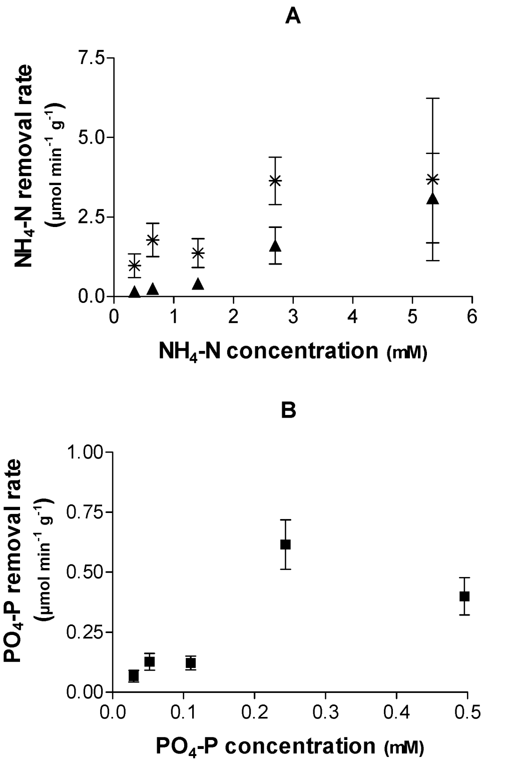


*Figure 2.* (A) $NH_4$-N and (B) $PO_4$-P removal rates by algal turfs as a function of manure $NH_4$-N and $PO_4$-P concentrations. Two $NH_4$-N removal rates are shown: (*) ″fast″ rate (from T = 15–40 min); (▲) ″slow″ rate (from T = 40–120 min). Points represent averages of 4–8 replicates; bars indicate standard deviation.

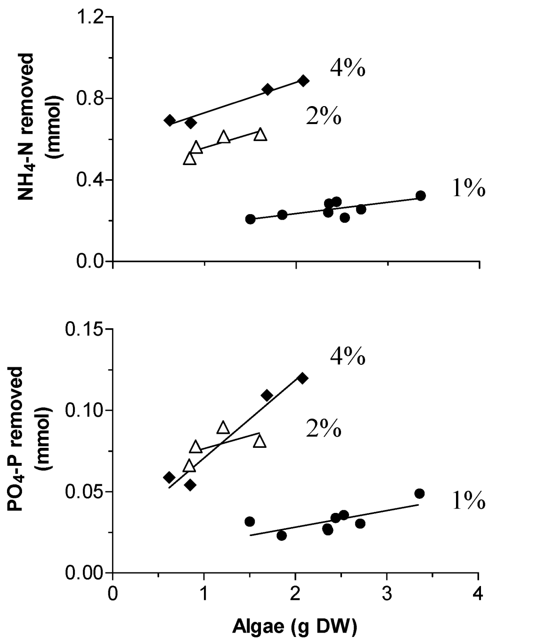


*Figure 3.* Amounts of (A) $NH_4$-N and (B) $PO_4$-P removed by algal turfs as a function of algal biomass. Points represent values from individual experiments.

creased. The fast rate was 6–7 fold higher than the slow rate at 0.25 and 0.5% manure loading rates but only 1.2–3 fold higher at 1, 2 and 4% manure loading rates (Table 1). For $PO_4$-P, a single removal rate was calculated since the rate was relatively linear throughout the incubation periods. The $PO_4$-P removal rate increased with increasing manure loading up to 8 mg $L^{-1}$ (2% manure) but decreased in incubations using 4% manure (16 mg $L^{-1}$).

To examine the effect of increasing algal biomass on nutrient removal, the total amounts of $NH_4$-N and $PO_4$-P removed throughout the two-hour experiment were calculated for manure loadings of 1%, 2% and 4% and turfs grown for 2–5 days. The amounts of $NH_4$-N and $PO_4$-P removed increased with algal biomass at a constant loading rate and increased with loading rate at a constant biomass (Figure 3). At each loading rate there was an increase of about 25% in the amounts of $NH_4$-N and $PO_4$-P removed for each two-fold increase in biomass, except at the 4% manure loading where there was an increase of 68% in the amount of $PO_4$-P removed.

## Discussion

Spatial heterogeneity within periphyton communities is well known (Trotter and Hendricks 1979) and such heterogeneity within the algal turf scrubber community is easily observed. As a consequence of this heterogeneity, it has not been possible to take representative samples of turf before and after incubation with manure in order to determine accurate removal rates. Instead, we have had to rely on nutrient disappearance from the ATS effluent as an approximate measure of nutrient uptake. In this regard, maintaining pH of 7–7.5 during incubation was important since increased pH (> 8.5) due to photosynthesis promotes volatilization of ammonia and precipitation of phosphorus. We believe that $NH_4$-N and $PO_4$-P disappearance during the incubation of algal turfs was due solely to algal removal, since incubations conducted under identical conditions but with very low algal biomass (< 0.1 g DW) at pH of 7 and 8.5 showed no detectable loss of $NH_4$-N or $PO_4$-P over a 2-h period (data not shown). In addition, there was no significant production of $NO_3$-N or $NO_2$-N in any of the experiments. However, nutrient removal by algal turfs is a combination of uptake by the turf's microbial flora and fauna as well as by nonspecific adsorption and physical trapping of organic particles. At present, we cannot differentiate between these processes.

The reason for the nonlinear $NH_4$-N removal rates at low loading rates using turf subsections is not clear and this is not a phenomenon that we have observed in the laboratory scale ATS units. Others have suggested that nutrient removal values during the first 10 minutes of incubations should be excluded in calculating removal rates because their inclusion may lead to substantial errors in uptake rate measurements (Goldman and Glibert 1982, 1983). However, even when the removal rate calculations excluded the first 15 minutes, a non-linearity was still present. In our experiments, one possible explanation for the nonlinear rates may be $PO_4$-P limitation. Although the $PO_4$-P concentrations used in these dilutions of manure are much higher (and the N: P ratio by weight much lower) than those normally associated with $PO_4$-P limitation, it is possible that these turfs (or some component of their flora) are acclimated to relatively high $PO_4$-P levels. Another possibility may be carbon limitation due to the relatively large amount of biomass in relation to the small volume of effluent used in these experiments.

The removal of $NH_4$-N and $PO_4$-P from manure effluent is clearly influenced by both manure concentration and turf biomass density. The increase in $NH_4$-N removal rates with increasing manure concentration reveals the potential of these algal turfs to function efficiently at relatively high concentrations of $NH_4$-N. At present, the basis of the decline of the $PO_4$-P removal rates using 4% manure is unknown. However, it is possible that either the maximal $PO_4$-P removal rate has been reached or that a component in the manure has reached an inhibitory level. The amounts of $NH_4$-N and $PO_4$-P removed increased approximately 25% for each doubling in algal biomass. At these relatively high biomass values, corresponding to 15–100 g DW $m^{-2}$ on laboratory ATS units, it is likely that light and nutrient exchange become limiting factors as turf density increases.

Use of these replicate subsections helps in overcoming the heterogeneity in algal turfs grown in different ATS units. However, the extrapolated removal rates using the subsections are significantly lower than those from laboratory scale ATS units. Recent experiments using laboratory ATS units with undisturbed turfs and 1% digested manure have yielded average $NH_4$-N and $PO_4$-P removal rates of 5.7 and 1.5 g $m^{-2}$ $d^{-1}$, respectively (E. Kebede-Westhead, unpublished results). Results from the removal experiments in this study using turf subsections and 1% manure correspond to overall removal values of about 0.72 g $NH_4$-N $m^{-2}$ $d^{-1}$ and 0.33 g $PO_4$-P $m^{-2}$ $d^{-1}$. In addition to the possibility of carbon or phosphorus limitation mentioned above, it is possible that disturbing the turf subunit community prior to the experiments and changes in water motion over the turf subsections may be factors in these lower rates.

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