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Distribution and Dynamics of Nitrogen and Microbial Plankton in Southern Lake Michigan During Spring Transition 1999-2000

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[1] Ammonium and amino acid fluxes were examined as indicators of N and microbial food web dynamics in southern Lake Michigan during spring. Either $^{15}NH_4^+$ or a mixture of ^{15}N-labelled amino acids (both at 4 μM N final concentration) was added to Lake Michigan water. Net fluxes were measured over 24 h under natural light and dark conditions using deck-top incubators and compared to microbial food web characteristics. Isotope dilution experiments showed similar light and dark NH_4^+ regeneration rates at lake (6 versus 5 nM N h^{-1}) and river-influenced (20 versus 24 nM N h^{-1}) sites. Ammonium uptake rates were similar to regeneration rates in dark bottles. Dark uptake (attributed mainly to bacteria) accounted for ~70% of total uptake (bacteria plus phytoplankton) in the light at most lake sites but only ~30% of total uptake at river-influenced sites in or near the St. Joseph River mouth (SJRM). Cluster analysis grouped stations having zero, average, or higher than average N-cycling rates. Discriminant analysis indicated that chlorophyll concentration, oligotrich ciliate biomass, and total P concentration could explain 66% of N-cycling rate variation on average. Heterotrophic bacterial N demand was about one third of the NH_4^+ regeneration rate. Results suggest that, with the exception of SJRM stations, bacterial uptake and protist grazing mediated much of the N dynamics during spring transition. Since NH_4^+ is more available to bacteria than NO_3^-, regenerated NH_4^+ may have a strong influence on spring, lake biochemical energetics by enhancing N-poor organic matter degradation in this NO_3^- -replete ecosystem. *INDEX TERMS:* 1845 Hydrology: Limnology; 4805 Oceanography: Biological and Chemical: Biogeochemical cycles (1615); 4845 Oceanography: Biological and Chemical: Nutrients and nutrient cycling; *KEYWORDS:* nitrogen, microbial food web, Lake Michigan

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1. Introduction

[2] Aquatic ecosystem ecology relates to cycling patterns of essential nutrients, such as C, N, and P. For example, N can serve as a metabolic currency to assess microbial processes in aquatic ecosystems [*Legendre and Rassoulzadegan*, 1995]. Ammonium (NH_4^+), the dominant N form produced by mineralization of organic N [*Campbell*, 1973], is assimilated by phytoplankton or heterotrophic bacteria or converted to nitrate (NO_3^-) via nitrification (Figure 1). Phytoplankton use light-derived energy to assimilate NO_3^- and NH_4^+, but bacteria prefer reduced forms of N, such as NH_4^+ or organic N, because the energetic cost for assimilating these forms is lower than for NO_3^- [*Kirchman*, 2001]. Heterotrophic bacteria can assimilate NH_4^+ at about one fifth of the energy cost needed for NO_3^- assimilation [*Vallino et al.*, 1996].

[3] Community NH_4^+ regeneration reflects heterotrophic (bacteria, microbial grazers, or other secondary producers) processes, whereas NH_4^+ uptake reflects consumption by phytoplankton or bacteria, depending on light, nutrient, and organic-substrate availability (Figure 1). Light/dark differences in NH_4^+ uptake or release rates provide insights about the importance of heterotrophic versus autotrophic processes in N cycling [e.g., *Gardner et al.*, 1987, 1989, 1996]. Dissolved N uptake by autotrophs is enhanced by light energy, whereas assimilation by bacteria depends more on the availability of organic substrates or other limiting nutrients. Organic substrates range from fresh materials produced by phytoplankton or other organisms to degraded organic residues (e.g., riverine or resuspended organic materials) with high C:N ratios. If available organic substrates are low in N or P, heterotrophic bacterial growth may

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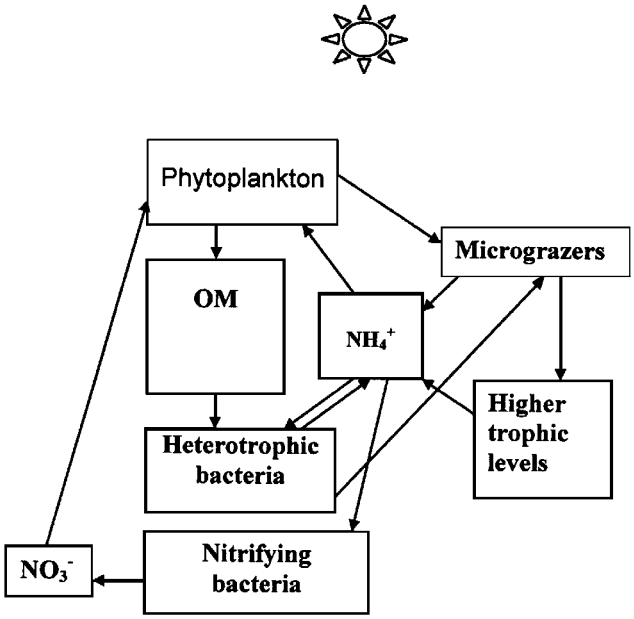

Figure 1. Schematic diagram depicting interactions of ammonium and organic matter with pelagic organisms in the lake.

depend on inorganic forms of these nutrients [*Kirchman*, 2001].

[4] Amino acids are a labile, N-rich, organic C source and can be produced by phytoplankton exudation [*Bronk and Glibert*, 1993; *Bronk et al.*, 1994] or as a byproduct of grazing [*Nagata and Kirchman*, 1991], then incorporated back into the food web by bacterial uptake [*Kirchman et al.*, 1989; *Fuhrman*, 1990]. If amino acids are produced by phytoplankton but taken up by bacteria, most amino acid production may occur in the light, or soon after light exposure, but uptake rates would be similar in the light and dark. In some cases, extra-cellular enzymes released by N-starved phytoplankton may de-aminate the amino acids to provide a NH_4^+-N source [*Palenik and Morel*, 1990]. The degree of $^{15}NH_4^+$ regeneration from added ^{15}N-labeled amino acids provides insights into whether bacteria are limited more by C or N [*Gardner et al.*, 1996].

[5] Lake Michigan is a large, temperate lake with pronounced seasonal changes in physical dynamics driven by temperature. The lake stratifies thermally in summer but is isothermal in winter and spring with enhanced internal mixing. These physical changes affect particle and nutrient distributions, which, along with seasonal light and temperature variations, affect food web dynamics [*Eadie et al.*, 2002]. Studies during stratification development and occurrence have clarified our understanding of food web dynamics during this period (see conceptual model of *Scavia and Fahnenstiel* [1987]). Strong temporal and spatial disequilibrium of bacterial production, with respect to autotrophic fixation, have been reported for Lake Michigan. Phytoplankton exudation does not always provide sufficient C to support bacterioplankton growth in Lake Michigan [*Laird et al.*, 1986]. Autotrophic production was not sufficient to account for measured seasonal bacterioplankton production in Lake Michigan, which suggests temporal decoupling of the two processes [*Scavia and Laird*, 1987; *Biddanda and Cotner*, 2002].

[6] Abundant protists (ciliates and flagellates) occur in the pelagic zone of the Great Lakes [*Taylor and Heynen*, 1987; *Carrick and Fahnenstiel*, 1990; *Fahnenstiel et al.*, 1998]. These eukaryotic microorganisms have high growth rates [*Carrick et al.*, 1992; *Lavrentyev et al.*, 1995], can exert considerable grazing pressure on bacteria [*Gardner et al.*, 1986; *Hwang and Heath*, 1997] and picophytoplankton [*Fahnenstiel et al.*, 1991], form direct trophic links to planktonic crustaceans [*Carrick et al.*, 1991; *LeBlanc et al.*, 1997], and recycle P [*Taylor*, 1984] and trace elements [*Twiss et al.*, 1996].

[7] Little information is available about biological processes during the isothermal winter-spring period when lake physics are unstable. Observations in Lake Constance, Germany [*Müller et al.*, 1991; *Gaedke and Straile*, 1994] and small, temperate lakes [*Lavrentyev and Maslevtsov*, 1988; *Sommaruga and Psenner*, 1995] indicated sizable microbial populations, including protists, during winter and early spring. These observations suggest that microbial food web (MFW) processes can be active in temperate lakes during the winter-spring transition (e.g., March–June) despite low water temperatures and sub-optimal light regimes during the early part of this transition.

[8] Coastal turbidity plumes, from resuspension of nearshore sediments during storms, may affect nutrient and food web dynamics [*Eadie et al.*, 2002]. For example, elevated concentrations of bacteria and loricated ciliates occurred in a turbidity plume along the eastern shore of Lake Michigan after a severe storm in early April 1996 [*Eadie et al.*, 2002]. During a major resuspension event in March 1998, bacterial production was high (64% of summertime rates [*Cotner et al.*, 2000]). The Episodic Events Great Lakes Experiment (EEGLE) was funded by The National Science Foundation and National Oceanic and Atmospheric Administration to provide information on effects of recurring plumes on lake dynamics during winter/spring in southern Lake Michigan.

[9] Our research effort was directed at estimating N-cycling rates relative to MFW characteristics in southern Lake Michigan during spring transition when particle resuspension is prevalent. Although Lake Michigan is P-limited [*Scavia and Fahnenstiel*, 1987], studying N dynamics can provide insights about plankton dynamics at the community and population levels [*Haga et al.*, 1995; *Miller et al.*, 1995]. In addition to diatoms, we hypothesized that nondiatom autotrophic and heterotrophic organisms were significant contributors to MFW dynamics in spring.

[10] In this paper, we report light and dark regeneration and potential uptake rates for NH_4^+ and net amino acid flux in southern Lake Michigan during March 1999 and 2000, June 1999, and May 2000. These results are compared to MFW structure and other information to gain insights about autotrophic and heterotrophic factors controlling N and MFW dynamics during spring transition. An intriguing question considered in this study was:

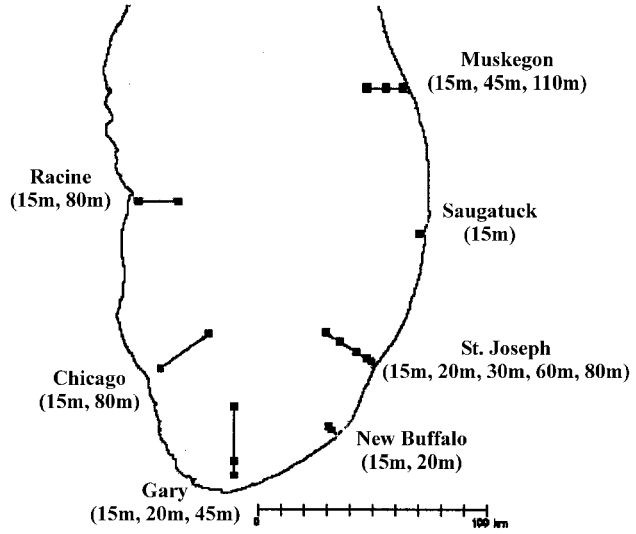

Figure 2. Map of sampling locations in southern Lake Michigan. Specific samples are indicated in the text by sequential listing of the transect, station depth, and sample depth.

"What nutrients or other factors limit microbial activity during spring transition?"

2. Methods

2.1. Sampling Strategy and Water Column Parameters

[11] Samples were collected during cruises on the R/V Lake Guardian from March 8–15 and June 3–7, 1999, and March 10–15 and May 14–20, 2000 (Figure 2). Most sampling sites were located on nearshore-offshore transects, but a station in the St. Joseph River mouth (SJRM) provided information about N cycling and MFW characteristics at the mouth of an important tributary delivering materials into southern Lake Michigan. Temperature and turbidity were measured at each station with a SeaBird STE-911 multisensor unit. Total suspended materials (TSM) concentrations were determined gravimetrically using a Mettler AT250 balance. Two-L aliquots of lake water were filtered through prerinsed, dried, combusted and preweighed 45mm GF/F filters (unless the filter became clogged sooner). Concentrations were determined to the nearest 0.1 mg l^{-1} based on the volume filtered. Nutrient and chloride concentrations were measured using standard colorimetric procedures on an Auto Analyzer II as detailed in *Davis and Simmons* [1979]. Dissolved nutrient samples were filtered through a 0.2 μm nylon filter after collection and frozen until analyses. Total P and total dissolved P samples were stored in acid-cleaned Pyrex test-tubes in the refrigerator, digested in an autoclave after addition of potassium persulfate (5% final concentration [*Menzel and Corwin*, 1965]), and analyzed for soluble reactive P (SRP) with an auto analyzer.

2.2. Ammonium and Amino Acid Flux Experiments

[12] Experiments with and without P additions were conducted to measure N-cycling rates and attempt to isolate factors limiting community microbial activity. Unfiltered lake water was amended with 0.40 μM PO_4^{3-}, except in March 1999. ^{15}Nitrogen-labeled NH_4^+ or an amino acid mixture (Algal Amino Acid mixture-U-^{15}N, 96-99/NLM-2161, Cambridge Isotope Laboratories, Inc.) was added to untreated or P-amended lake water (4 μM final concentration). Seventy-ml aliquots from each treatment were dispensed into each of 6 tissue culture bottles (Corning 25 cm^2 Polystyrene Tissue Culture Bottles) giving a total of 24 incubation bottles per experiment. Initial (T-0) samples were collected, filtered through a rinsed 0.2 μm syringe filter, and analyzed onboard for NH_4^+ and primary amines [*Gardner and St. John*, 1991]. Remaining filtrates from $^{15}NH_4^+$ samples were frozen in 8-ml Wheaton vials for direct analysis of atom % $^{15}NH_4^+$ by high performance liquid chromatography (HPLC [*Gardner et al.*, 1995]). Three replicate bottles from each treatment were wrapped with aluminum foil (dark treatments). Treatment bottles were placed in a deck-top incubator maintained at *in situ* temperature with a Neslab controller and incubated for 24 h under natural light [*Lohrenz et al.*, 1988]. Twenty-four hour incubations were needed for measurable concentration changes to occur and make light-dark comparisons consistent among samples collected at different times of the day. At the end of the incubations, samples were collected and filtered, and the filtrates were analyzed immediately for NH_4^+ and primary amines. Remaining filtrates were frozen for atom % $^{15}NH_4^+$ analysis.

2.3. Low-Level $^{15}NH_4$-Addition Uptake Experiments

[13] Duplicate bottle experiments were conducted in daylight and dark (duct tape covered), except samples collected in the evening or night, which were incubated only in the dark. Two-liter portions of lake water were placed in light and dark polycarbonate bottles and spiked with $^{15}NH_4^+$ (0.5 μM final concentration). Water from an initial T-0 bottle was filtered through a 47 mm combusted (4 hours at 450 °C) glass fiber filter. Experimental bottles were incubated for at least 6 h at ambient temperature and light in the deck-top incubator, and experimental water was filtered to collect particles for T-1 measurements. One liter was filtered for experiments conducted in 1999, and two liters were filtered in 2000. One-N HCl was applied to all filters to remove inorganic material. The filters were stored frozen in a small Petri dish until analysis of ^{15}N:^{14}N [*Dugdale and Goering*, 1967; *Glibert and Capone*, 1993] using a VG PRISM stable isotope mass spectrometer.

2.4. Microbial Plankton Measurements

[14] Microzooplankton (ciliates, heterotrophic dinoflagellates, and rotifers), heterotrophic nanoflagellates (HNF), and chlorophyll *a* (Chl) were sampled using 10-l Niskin bottles mounted on a SeaBird carousel water sampler. Heterotrophic nanoflagellates, preserved with 1% formaldehyde (final concentration), were stained with DAPI on 0.8 μm black polycarbonate filters and counted under an Olympus IX-70 microscope at 1,000× [*Sherr et al.*, 1993]. Microzooplankton, preserved in 1% (final concentration) acid Lugol's iodine, were counted after settling 25 to 100-ml aliquots in a chamber for at least 18 hours. The entire chamber was scanned at 200–400×. Two to 3 liters of lake water were filtered through a 25 μm Nitex mesh using gravity reverse flow to quantify rotifers, which often were

less abundant than protists. The resulting 100-ml sample was preserved, stored, and processed as described above. Linear dimensions of 30 to 90 individual protists and rotifers (fewer for less abundant taxa) were measured at 400–600× and converted to volumes using appropriate geometric shapes to estimate plankton biomass. Ciliate and rotifer volumes were converted to C following *Putt and Stoecker* [1989] and *Fahnenstiel et al.* [1998], respectively. Dinoflagellate and HNF volumes were corrected for shrinkage using a factor of 1.3 [*Montagnes et al.*, 1994] and converted to C [*Menden-Deuer and Lessard*, 2000]. Bacterial secondary productivity (BSP) was measured with tritiated leucine incorporation (20-nM final concentration) and a theoretical conversion factor of 3.1 kg C per mol leucine incorporated [*Kirchman*, 1993]. Bacterial abundance and size were measured with epifluorescence microscopy [*Hobbie et al.*, 1977]. A SPOT digital camera was used to photograph 500–1000 cells at each sampling site. Image analysis was performed with Image Pro Plus software. Cell C was estimated from cell volume assuming an ellipsoidal shape and applying a conversion of 0.25×10^{-12} g C μm^{-3} [*Psenner*, 1993; *Cotner et al.*, 2000].

[15] Chl was collected by filtering 100-ml of lake water through a 45-mm Whatman GF/F filter under low vacuum pressure and freezing the filters in polypropylene tubes until extraction with N, N-dimethylformamide (DMF [*Speziale et al.*, 1984]. DMF was added to the tubes and heated in a water bath at 65°C for 15 min. After agitation and centrifugation, the supernatant was analyzed on a fluorometer using the acid correction method [*Strickland and Parsons*, 1972; *Speziale et al.*, 1984]. The reported values are means from duplicate analyses. The established coefficient of variation for this procedure is less than 4%.

2.5. Statistical Comparisons

[16] Statistical analyses, using Systat 8.0 (SPSS 1998) and JMP 4.0 (SAS 2000) software, were performed to compare N-cycling data to MFW characteristics. Descriptive statistics showed that N-cycling rate data were not distributed normally (skewness coefficient values ranged from 1.6 to 3.5 for N dark regeneration and N light uptake, respectively), which is typical for small data sets. Owing to some negative values resulting from experimental measurement variations, these data could not be log transformed in an unbiased manner.

[17] The data were compared with heuristic statistics, which do not rely on the normal distribution assumption in contrast to linear techniques. A hierarchical cluster tree was derived for uptake and regeneration rates using the Ward linkage algorithm. Stepwise interactive discriminant analysis was performed using the clusters as a grouping variable and other chemical and biological parameters as explanatory variables. The jackknifed classification matrix was calculated to cross-validate disciminant analysis results. Group-means of variables included in the model were analyzed by the two-group t-test.

3. Results

3.1. Site Description and Characteristics

[18] Temperature, TSM, and nutrients varied among stations and sampling times (Table 1). Mean water temperatures (°C) for the different cruises were 1.5 ± 0.2 (March 1999), 3.5 ± 0.3 (March 2000), 10.2 ± 1.6 (June 1999), and 11.0 ± 1.1 (May 2000). Nutrient and particle concentrations were higher in or near SJRM than at most open lake stations. TSM ranged from 0.7 to 21 mg l^{-1} among sites. Particulate organic materials expressed as C (POC) ranged from 13 to 67 μM C (with the highest value being offshore from Chicago) and had C:N ratios ranging from 5 to 13. Lake Michigan has high NO_3^- levels during the winter-spring period (e.g., 17–30 μM for stations not influenced directly by the river; Table 1). By contrast, ambient NH_4^+ levels ranged from 0.1 to about 1.0 μM, except at SJRM where concentrations reached 4.3 μM. Most SRP concentrations were less than 0.05 μM, but higher values were observed at stations near SJRM. Chloride ion, indicative of coastal or riverine influence, ranged from 11 to 13 mg l^{-1} at most stations but was elevated at SJRM and, occasionally, at nearshore stations along the St. Joseph transect.

[19] Chlorophyll concentration and bacterial biomass and production rates were higher at or near SJRM than other stations (Table 2). Chlorophyll concentrations ranged from 0.7 to 18 μg Chl l^{-1} (0.7 to 2.8 μg Chl l^{-1} excluding SJRM). Bacterial biomass ranged from 10 to 104 μg C l^{-1} (10 to 40 μg C l^{-1} excluding SJRM). Mean bacterial biomass for the same samples ranged from 23 ± 4 μg C l^{-1} in March to 28 ± 4 μg C l^{-1} in June 1999 and were not significantly different among the 4 cruises. Bacterial production rates were less than 6 μg C l^{-1} d^{-1} at most stations but were higher at some stations in the St. Joseph transect. Other heterotrophs, considered for biomass comparison with N-cycling rates, were HNF, oligotrichs, and other microbial grazers including nonoligotrichous ciliates, heterotrophic dinoflagellates, and rotifers (Table 2). Total grazer biomass ranged from 0.4 μg C l^{-1} off Chicago in March to 54 μg C l^{-1} at SJRM in May 2000. Excluding SJRM and Chicago stations, total grazer biomass ranged from 2 to 24 μg C l^{-1} (Table 2).

3.2. Ammonium Uptake and Regeneration Rates

[20] Light NH_4^+ uptake rates for high-level NH_4^+ additions (−7 to 25 nM h^{-1}) were the same order of magnitude as rates from low-level additions (1.5 to 3 nM h^{-1}) except for stations near SJRM (right three points on graph; Figure 3). In dark bottles, a linear relationship was observed between the two methods with progressively lower rates from the low-level addition experiments as rates increased for the high-level additions, again with differences emphasized at SJRM.

[21] Potential uptake rates in March of both years ranged from about zero to 30 nM h^{-1} except for light SJRM samples, which had uptake rates ~60 nM h^{-1} (Figures 4a and 4b). Light uptake rates were higher than dark at SJRM but not other stations. Ammonium regeneration rates were low and comparable in March with standard error (SE) bars often overlapping zero except at SJRM, where rates were higher in March 2000.

[22] In May and June, NH_4^+ uptake rates ranged from zero to 30 nM h^{-1} at all sites except SJRM, where mean light uptake rates were about 160 nM h^{-1} in May 2000 and 110 nM h^{-1} in June 1999 (Figures 5a and 5b). Ammonium regeneration rates for the same samples ranged from near zero to 29 nM h^{-1} (Figures 5a and 5b). Most

Table 1. Concentrations of Total Suspended Materials (TSM), Total P (TP), Total Dissolved P (TDP), Particulate P (PP), Soluble Reactive P (SRP); NO_3^-, NH_4^+, Particulate Organic N (PON), PON; POC Ratio, Temperature and Cl^- in March 1999, June 1999, March 2000, May 2000

Cruise	Station	TSM, mg L^{-1}	TP, μM	TDP, μM	PP, μM	SRP, μM	NO_3^-, μM	NH_4^+, μM	PON, μM	POC, μM	C:N, Molar	Temp, °C	Cl^-, mg L^{-1}
Mar-99	M15-5	1.0	0.2	0.03	0.12	0.003	20.4	0.1	1.1	15.3	13.3	1.3	12.4
	M45-5	0.8	0.2	0.03	0.05	0.003	19.8	0.1	1.8	13.5	7.6	2.4	11.5
	S15-5	1.2	0.2	0.04	0.08	0.006	28.8	0.2	3.5	29.6	8.5	ND	ND
	G20-5	6.8	0.3	0.04	0.15	0.006	23.4	0.7	1.9	17.5	9.4	1.1	12.4
	J15-5	3.0	0.4	0.05	0.18	0.003	54.5	0.3	4.3	32.6	7.6	1.0	17.6
	J20-5	2.1	0.3	0.06	0.15	0.006	48.9	0.2	3.9	31.3	8.0	0.9	16.4
	J60-5	1.8	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	J80-5	0.8	0.1	0.03	0.06	0.003	22.4	0.2	2.3	18.5	8.1	2.4	11.7
	C15-5	21.1	0.5	0.06	0.36	0.010	20.4	0.8	2.7	29.7	10.9	0.6	12.0
	C80-5	1.0	0.2	0.05	0.06	0.003	19.0	0.1	1.8	15.6	8.7	1.9	11.5
	R15-5	8.2	0.3	0.04	0.21	0.006	18.8	0.2	3.9	37.2	9.5	1.4	12.0
	R80-5	0.9	0.2	0.05	0.07	0.003	18.5	0.1	2.1	18.9	9.1	2.4	11.4
Jun-99	M15-5	1.0	0.2	0.05	0.20	0.010	25.9	0.6	2.8	27.8	10.0	11.3	13.3
	M110-5	0.9	0.2	0.04	0.19	0.010	22.3	0.3	2.8	25.6	9.2	10.2	11.8
	M110-25	0.9	0.2	0.04	0.18	0.010	22.1	0.7	2.4	21.7	8.9	7.0	11.4
	J15-5	2.4	0.4	0.08	0.36	0.013	37.9	0.3	5.9	47.9	8.2	16.6	14.6
	J80-5	0.7	0.1	0.04	0.16	0.003	23.9	0.2	2.1	19.8	9.2	14.0	12.1
	J80-24	0.9	0.1	0.04	0.18	0.003	20.0	0.3	2.6	27.3	10.6	6.0	11.6
	C80-25	1.1	0.2	0.04	0.23	0.010	21.3	0.4	2.9	31.7	11.1	6.1	11.6
Mar-00	SJRM-3	9.3	0.7	0.14	0.67	0.042	55.4	1.3	8.0	61.7	7.7	5.5	18.7
	J15-5	4.5	0.2	0.06	0.19	0.029	24.3	0.2	2.4	23.6	10.0	3.2	11.4
	J30-5	4.3	0.2	0.07	0.18	0.042	24.3	0.4	2.2	26.6	12.0	3	11.2
	NB20-10	6.3	0.3	0.05	0.24	0.010	26.7	0.2	2.8	25.3	9.1	3.7	12.7
	G15-5	4.8	0.2	0.04	0.18	0.006	23.5	0.3	1.6	16.1	9.8	4.2	11.2
	G45-5	0.8	0.1	0.05	0.12	0.010	24.3	0.3	2.2	17.7	8.0	2.5	11.1
	C15-5	1.1	0.1	0.07	0.12	0.023	25.1	0.7	1.1	12.7	11.1	2.8	11.9
	C80-10	0.8	0.2	0.05	0.11	0.023	17.4	0.3	3.6	67.5	18.5	3	10.9
May-00	M45-5	1.3	0.2	0.03	0.15	0.045	26.5	0.1	ND	ND	ND	9.3	13
	M45-15	1.2	0.2	0.04	0.15	0.042	25.2	0.2	1.9	15.4	8.0	8	12.7
	SJRM-4	19.4	2.8	0.89	1.31	0.313	120.5	4.3	ND	ND	ND	17.5	35.5
	J15-5	2.6	0.2	0.04	0.14	0.042	25.9	0.3	2.0	12.0	6.0	12.2	14
	J30-5	2.2	0.2	0.03	0.15	ND	24.6	0.4	1.7	10.8	6.3	9.7	12.3
	NB15-5	2.4	0.1	0.03	0.12	0.026	24.6	0.7	1.1	6.3	5.5	11.5	12.7
	G15-5	1.3	0.1	0.04	0.09	0.026	24.2	0.9	1.7	12.4	7.2	11.5	13
	G45-5	0.8	0.1	0.04	0.09	0.013	21.9	0.2	1.9	18.1	9.4	8	11.3

sites had positive mean regeneration rates but some SE overlapped with zero. Sites near SJRM had higher NH_4^+ regeneration rates, but differences among sites were not as pronounced as for uptake. The effects of darkness, P additions, and interactions between the two processes were not uniform for either uptake or regeneration rates. Both light and P-addition effects on NH_4^+ uptake rates were most pronounced at stations near SJRM, where regeneration rates also were elevated. In contrast to uptake results, NH_4^+ regeneration rates were not much different for light versus dark and P addition experiments near SJRM.

3.3. Ammonium Cycling Rates and Their Relationship to Chemical/Biological Sampling-Site Characteristics

[23] Data from all experiments and treatments were combined to observe relationships between NH_4^+ uptake and regeneration rates in light and dark bottles. The relationship was not useful for comparisons among stations because multiple treatments were done on water from the same stations, but it provided a useful tool to examine light effects and explain outlying data. In dark bottles, NH_4^+ uptake and regeneration rates were related ($R^2 = 0.836$, $y = 1.015 + 0.0030$; $p < 0.001$) and near equal on average. Light-bottle comparisons of uptake and regeneration rates were similar except uptake was higher than regeneration at SJRM and J15 in June 1999. These outliers were separated from other lake data to form general conclusions about mean N cycling rates in "lake" and "river-influenced" regions but were included in the statistical comparisons of N-cycling data to MFW characteristics.

[24] The mean dark uptake rate at the "lake" sites (i.e., without outlier sites) was 7 ± 1 nM N h^{-1} compared to a mean light uptake rate of 10 ± 1 nM N h^{-1} ($n = 52$). The mean dark NH_4^+ regeneration rate (5 ± 1 nM N h^{-1}) did not differ significantly from the mean rate under natural light (6 ± 1 nM N h^{-1}) at "lake" sites. The mean NH_4^+ regeneration rate at "river-influenced" sites was higher than "lake" sites but also did not differ significantly between light (20 ± 2 nM N h^{-1}) and dark (24 ± 5 nM N h^{-1}) bottles.

3.4. Statistical Comparison of Microbial Food Web Characteristics and Ammonium Cycling Rates

[25] Hierarchical cluster analysis for all stations and treatments without P additions resulted in a cluster tree for N cycling rates consisting of three major groups (A, B, C) and one outlying station, SJRM-May 2000 (D; Figure 6). These clusters grouped similar observations of N cycling rates. The first cluster (A) combined stations where rates

Table 2. Chlorophyll Concentrations, Biomass for Bacteria, Heterotrophic Nanoflagellates (HNF), Oligotrichs (OLIG), Other Ciliates, and Total Microbial Grazers, and Bacterial Secondary Production (BSP) in Samples From Southern Lake Michigan and the SJRM During March 1999, March 2000, June 1999, and May 2000

Cruise	Station	Chl A, μg Chl L^{-1}	Bacteria, μg C L^{-1}	HNF, μg C L^{-1}	Oligotrichs, μg C L^{-1}	Other Ciliates, μg C L^{-1}	Total Grazers, μg C L^{-1}	BSP, μg C L^{-1} d^{-1}
Mar-99	M15-5	1.50	24.1	0.86	1.1	7.77	8.87	1.832
	M45-5	1.39	25.0	1.53	0.9	3.41	5.66	1.031
	S15-5	2.36	ND	ND	ND	ND	ND	1.132
	J15-5	3.41	43.9	4.33	9.2	38.45	53.59	9.479
	J20-5	3.24	33.2	2.22	7.8	35.81	45.24	7.223
	J60-5	ND	ND	ND	ND	ND	ND	ND
	J80-5	2.34	25.0	0.73	10.3	15.34	17.03	1.295
	G20-5	0.92	ND	1.20	4.3	ND	11.30	1.633
	C15-5	0.79	13.8	0.35	0.0	0.00	0.35	3.841
	C80-5	2.36	10.1	0.82	1.3	3.64	4.94	ND
	R15-5	2.13	14.2	0.85	0.2	1.26	2.11	3.484
	R80-5	2.42	40.5	1.35	2.5	6.56	9.11	1.039
Mar-00	SJRM-5	7.43	35.7	4.01	5.4	8.15	18.89	0.416
	J15-5	1.73	17.3	2.18	3.9	11.06	15.40	0.139
	J30-5	1.14	22.0	1.34	1.5	7.53	11.03	0.091
	NB20-5	1.85	15.8	2.00	1.0	5.05	7.53	0.088
	G15-5	0.91	28.8	1.50	2.1	3.50	6.20	1.794
	G45-5	1.52	ND	0.30	1.3	ND	4.10	0.043
	C15-5	0.47	35.2	0.34	0.3	0.14	0.96	1.418
	C80-5	1.48	18.6	0.93	3.7	8.95	13.49	1.193
Jun-99	M15-5	1.58	27.6	6.98	7.9	7.08	23.55	5.737
	M110-5	1.29	26.8	1.38	4.3	10.05	12.69	1.556
	M110-DCL	2.12	20.5	2.04	8.9	12.56	18.37	3.610
	J15-5	4.99	71.3	7.63	7.4	5.72	44.44	10.377
	J80-5	0.76	17.7	1.42	0.4	0.66	20.18	0.896
	J80-DCL	2.49	38.4	3.63	7.0	5.92	18.68	1.612
	C80-DCL	2.78	38.0	3.17	5.9	8.20	17.12	1.730
May-00	M45-5	2.10	51.8	2.07	2.0	7.50	16.78	3.526
	M45-DCL	1.93	ND	2.30	1.8	ND	17.00	3.221
	SJRM-5	18.36	103.5	10.43	4.7	19.78	46.62	33.730
	J15-5	0.99	48.9	3.65	7.8	19.41	27.62	4.552
	J30-5	0.72	25.3	2.08	10.4	19.13	23.29	4.488
	NB15-5	0.80	19.3	2.26	1.8	5.06	8.20	2.564
	G15-5	1.17	18.1	2.16	3.9	6.07	9.35	2.520
	G45-5	1.37	16.3	2.07	15.8	19.00	23.45	3.646

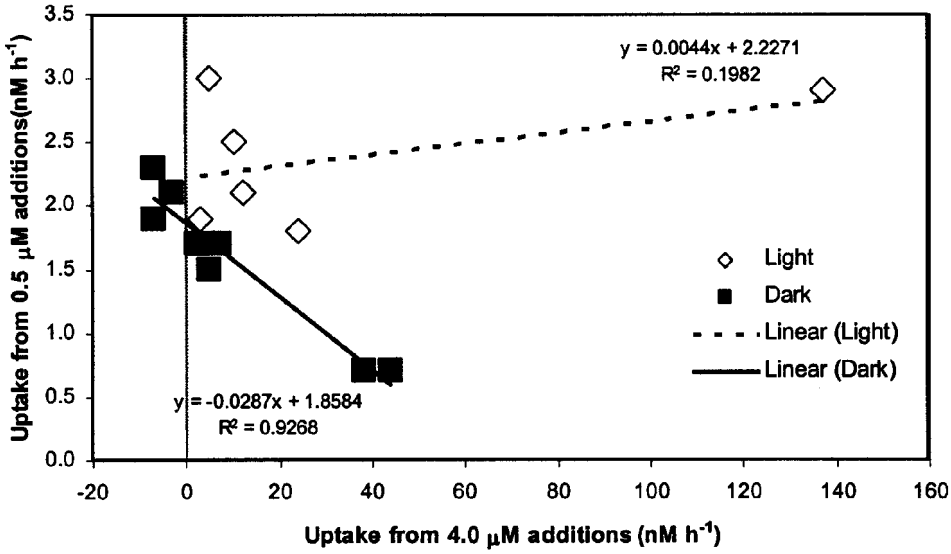

Figure 3. Comparison of ammonium removal rates in small bottles with high-level (4 μM) ammonium substrate additions to those collected on particles from the large bottles with low-level (0.5 μM) additions.

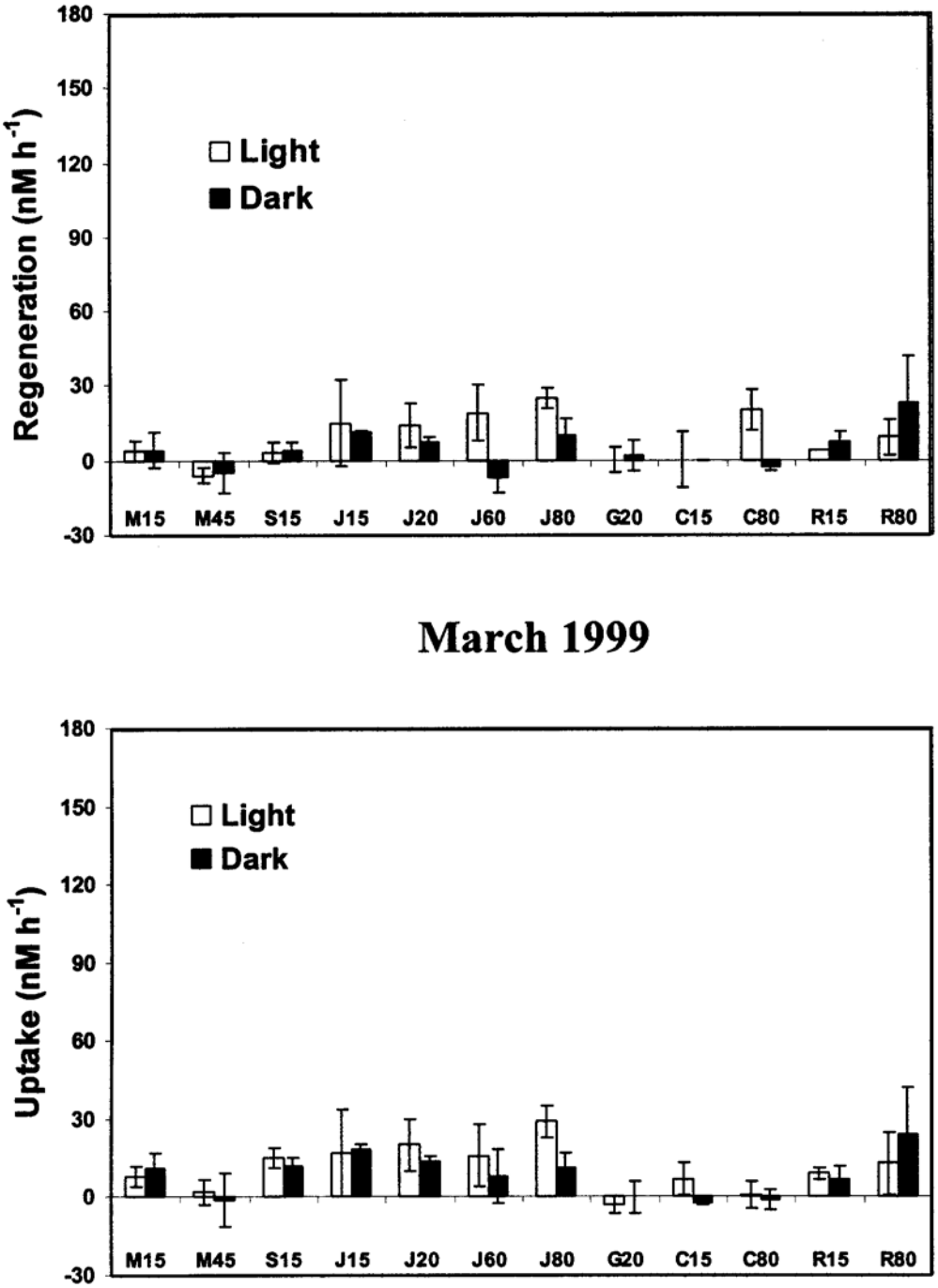

Figure 4a. Potential uptake and regeneration rates (±SE) for ammonium at southern Lake Michigan sites sampled in March 1999.

were not significantly ($p > 0.05$) different from zero. The second cluster (B) included sites with "average" rates (i.e., significantly above zero but not different from average values). The third cluster (C) combined sites with above average rates, and the fourth cluster (D) consisted of the SJRM station with much higher rates (Figure 7).

[26] Results of multivariate discriminant analysis, which used cluster order as a grouping variable, are presented in Table 3. On average, the combination of Chl, oligotrich ciliate biomass, and total P (TP) correctly predicted 81% (66% after the jackknifed cross-validation) of the observed cycling rates. The other variables (temperature, Cl^-, NH_4^+ and NO_3^- concentrations, C:N ratios, bacterial biomass and production) did not show significant relationships to NH_4^+ cycling rates. Both Chl concentration and oligotrich biomass followed patterns similar to N-cycling rates among the clusters (Figure 8). Comparison of group means suggested that Chl helped distinguish between the "zero" and "average" clusters (A versus B, $p = 0.02$), and oligotrich biomass distinguished more between the "average" and "high" categories (B versus C, $p = 0.014$). Although TP was not significantly different among the groups, it helped

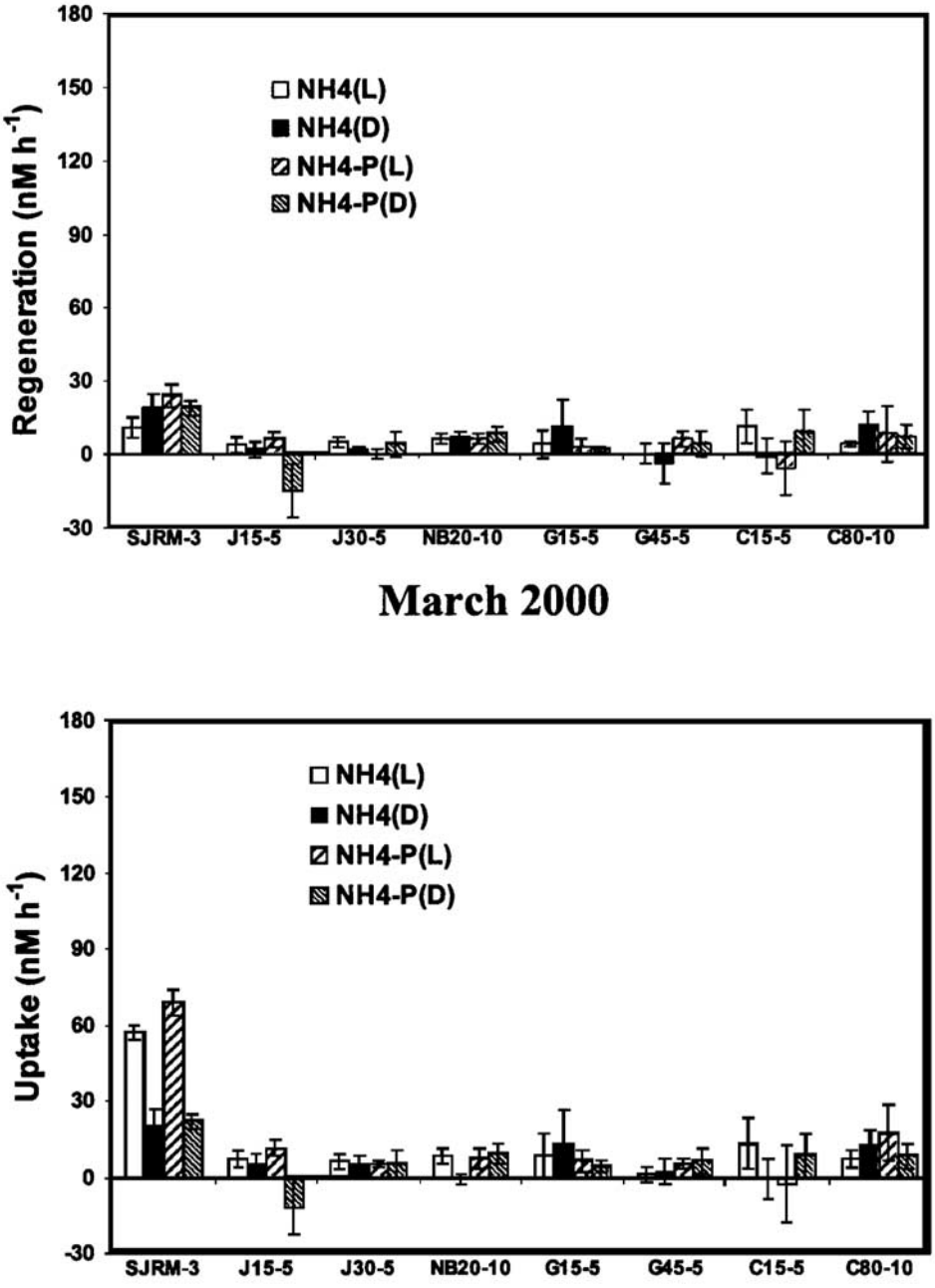

Figure 4b. Potential uptake and regeneration rates (±SE) for ammonium at southern Lake Michigan sites sampled in March 2000.

improve the model by ca. 10% (based on the jackknife; Table 3).

3.5. Net Amino Acid Fluxes

[27] In March 1999, net amino acid flux ranged from uptake of 20 nM N h^{-1} to production of 20 nM N h^{-1} (Table 4). In March 2000, net amino acid flux overlapped with zero for one or more treatments at all sites except SJRM and J15, which had uptake rates of 5 and 30 nM N h^{-1}, respectively, and C15, which had production rates of 20 nM N h^{-1} (Table 4). Except for J15 with uptake rates of 15–20 nM N h^{-1}, amino acid flux was <10 nM N h^{-1} in either direction in June 1999 (Table 5). Significant uptake was observed at one site in May 2000 (30 nM N h^{-1} at SJRM; Table 5). Significant production (12 to 60 nM N h^{-1}) occurred at three sites in May, but rates at the other four sites were low and often overlapped with zero. Increases in NH_4^+ regeneration in response to amino acid additions were not observed at any sites (data not shown).

4. Discussion

4.1. Methodological Considerations

[28] Although NO_3^- is the dominant inorganic N form in Lake Michigan, we focused on NH_4^+ and amino acid dynamics because they are active N forms mediated by

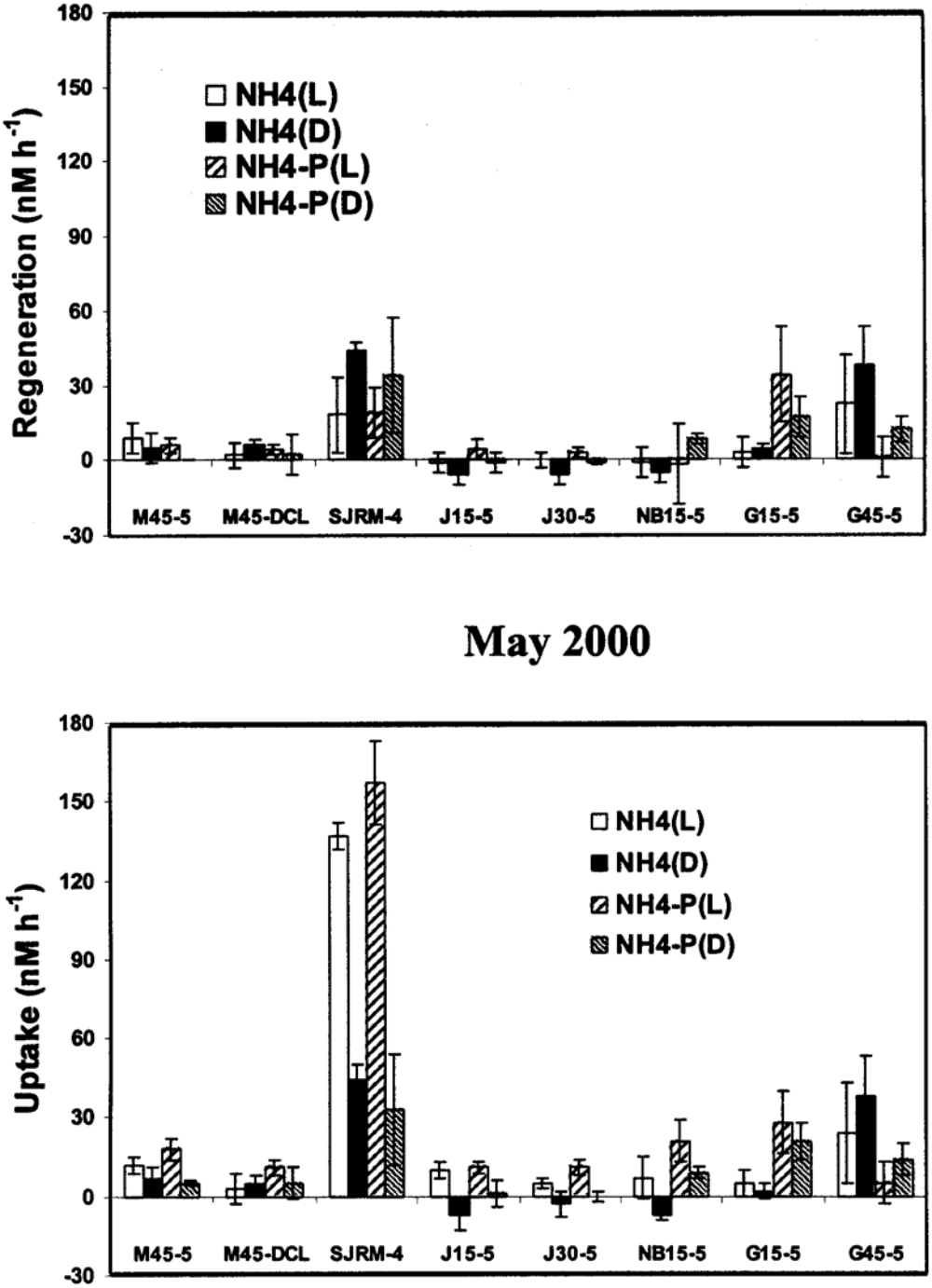

Figure 5a. Potential uptake and regeneration rates (±SE) for ammonium at southern Lake Michigan sites sampled in May 2000.

MFW organisms, and occur at low concentrations since they are often taken up as rapidly as they are produced. Ammonium is the dominant N form produced by heterotrophic mineralization of organic N and is preferred by phytoplankton and bacteria [*Valiela*, 1995; *Kirchman*, 2001]. We assumed that phytoplankton amino acid production would occur in the light, or soon after light exposure, but bacterial uptake rates would be similar in the light and dark.

[29] The addition of $^{15}NH_4^+$ allowed measurement of NH_4^+ concentration and isotope ratio changes over time [*Gardner et al.*, 1995] and calculation of potential NH_4^+ uptake and regeneration rates [*Blackburn*, 1979; *Caperon et al.*, 1979]. Since concentrations of added NH_4^+ were higher than ambient concentrations, uptake rates must be considered "potential" rather than "actual" rates. However, regeneration rates should not be affected directly by NH_4^+ additions. Differences between potential and actual uptake rates should have been minimized for low-level (0.5 μM) additions where added concentrations were similar to natural levels (0.1–0.7 μM N). Our small-bottle experiments were

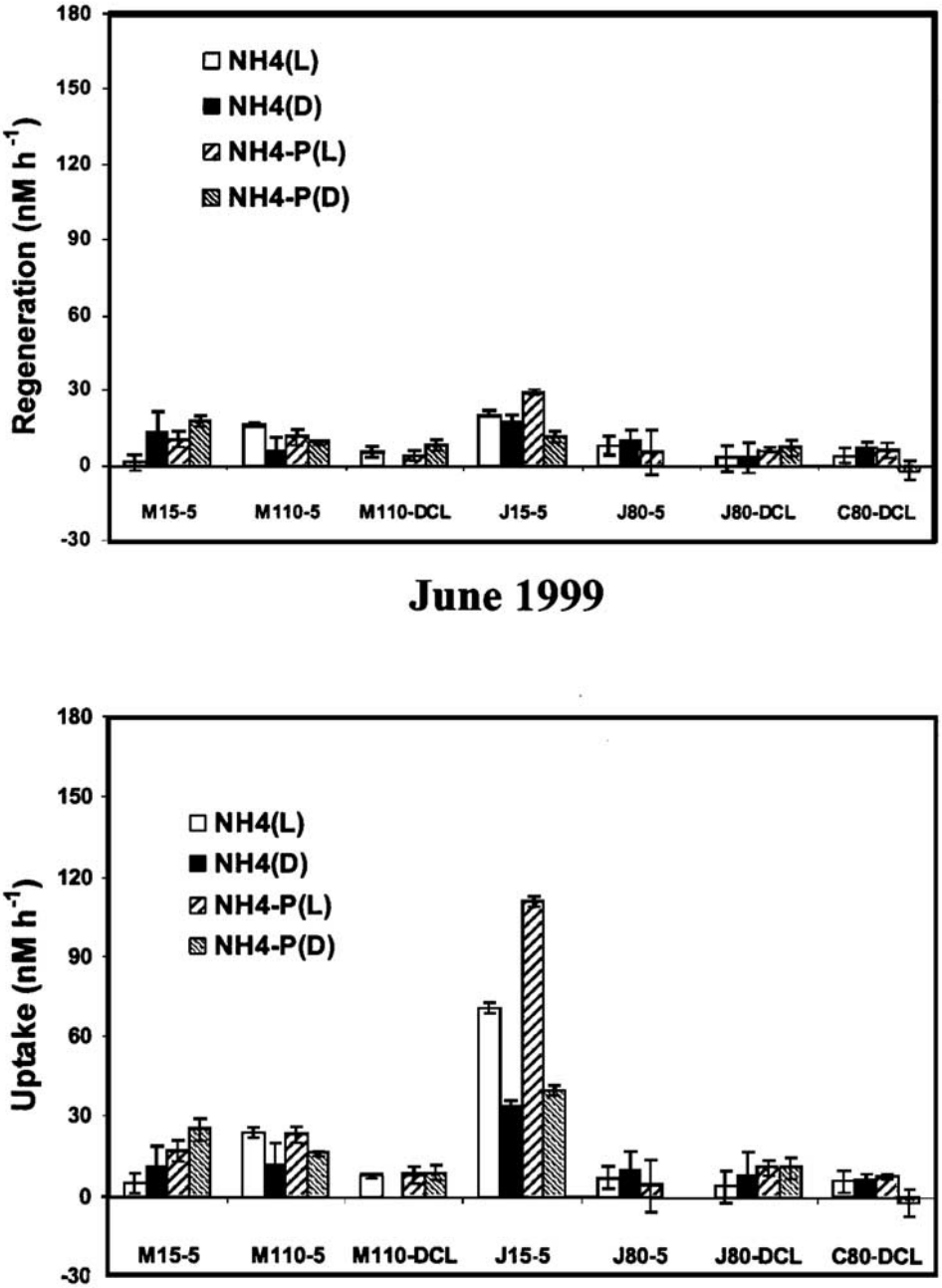

Figure 5b. Potential uptake and regeneration rates (±SE) for ammonium at southern Lake Michigan sites sampled in June 1999.

conducted over 24-h incubation intervals in order to obtain measurable concentration changes over a light-dark cycle. Large-bottle experiments with tracer additions were run only for about 6 h to keep the tracer from being removed quantitatively. However, even the 6-h period was too long for tracer additions in May at SJRM because significant quantities of the elevated NH_4^+ were removed before the first time point.

[30] A feasible explanation for these exceptions at SJRM is that high biomass (Table 2) caused $^{15}NH_4^+$ to be removed before sample collection at the first time point. Rapid substrate removal causes underestimation of calculated uptake rate, which is based on sampling interval length. High-level addition experiments gave reasonable estimates of potential NH_4^+ uptake and were more robust for long incubation times since $^{15}NH_4^+$ was not depleted from solution between time points. The small magnitude of the observed rates, the similarities between results from high and tracer level additions at lake stations, and the agreement between dark regeneration and uptake suggest that measured potential uptake rates may have approached actual uptake rates in our experiments. The incubation period of 24 h was needed to obtain light-dark comparisons among stations sampled at different times of the day. We believe

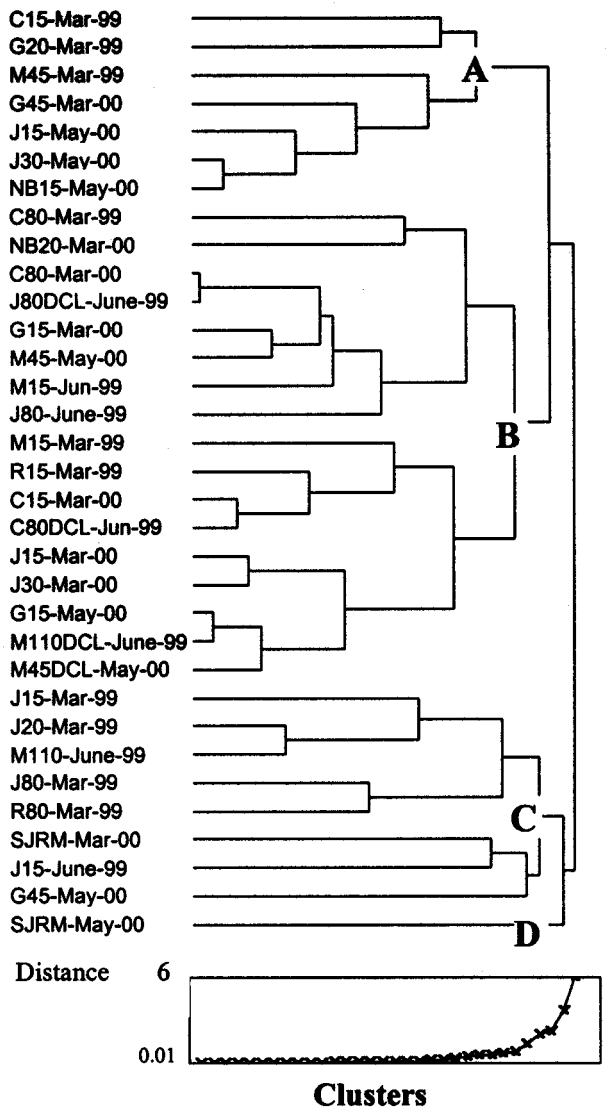

Figure 6. The results of hierarchical cluster analysis of N cycling rates. The plot beneath the dendrogram has a point for each cluster join. The ordinate is the distance that was bridged to join the clusters at each step. The distance between each two clusters is the ANOVA sum of squares between the two clusters added up over all the variables.

that bottle effects over the 24 h incubation period were minimal considering the low temperatures and high substrate additions.

4.2. Microbial Food Web Composition

[31] Results indicate that planktonic protists, particularly ciliates, are abundant in spring in southern Lake Michigan and account for 35.4 ± 3.31 (SE) and 15.0 ± 1.87 percent of heterotrophic and total plankton biomass, respectively (Table 2; based on C:chl of 30, crustacean zooplankton biomass excluded). Although microzooplankton biomass can reach 25 μg C l^{-1} in the epilimnion or deep chlorophyll layer in offshore Lake Michigan during late stratification [*Carrick and Fahnenstiel*, 1990], depth-weighted averages were comparable between winter-spring (this study) and summer stratification [*Fahnenstiel et al.*, 1998]. Ciliate biomass in Lake Michigan is high in early spring when cladocera are absent and copepod numbers are low [*Barbiero et al.*, 2001]. Combined with their high specific growth rates at low temperature [*Carrick et al.*, 1992; *Kovalcik*, 2001], these data are consistent with the enhanced role of microzooplankton during the winter-spring transition. Heterotrophic nanoplankton are at their seasonal minimum in offshore Lake Michigan during winter-early spring [*Carrick et al.*, 1991]. Nanoflagellates peaked near SJRM in late spring-early summer, but remained low (<2 μg C l^{-1}) at mid-transect and offshore waters in this study. These results are consistent with high predation pressure from microzooplankton-sized ciliates, the primary factor controlling nanoflagellate populations in early spring [*Weisse*, 1991].

[32] In offshore Lake Michigan, microbial plankton had an almost uniform distribution during the winter-spring transition. Along the lake margins, spatial variability was profound reflecting the complexity of physical processes in the southern basin. Similar trends have been described in Lake Ontario [*Taylor and Heynen*, 1987]. The greatest and most variable concentrations of microzooplankton, nanoflagellates, and bacteria occurred nearshore at St. Joseph. Because these stations are immediately offshore the St. Joseph River confluence, this variability may reflect the co-influence of tributary inflows and sediment resuspension on coastal assemblages.

[33] Heterotrophic or autolithotrophic, rather than autotrophic, organisms may dominate in winter/early spring

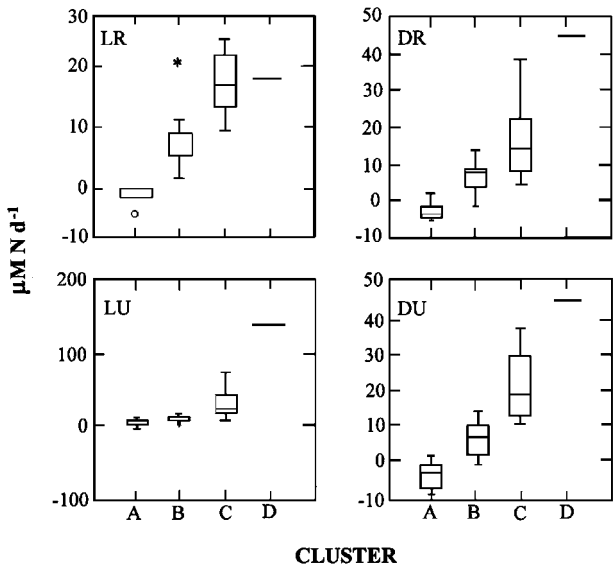

Figure 7. Box plots of nitrogen cycling rates arranged in clusters. LR - light regenration, DR, dark regeneration; LU, light uptake; DU, dark uptake. The center vertical line marks the median of the sample. The length of each box shows the range within which the central 50% of the values fall, with the box edges at the first and third quartiles. Fences define outside and far outside values. The whiskers show the range of observed values that fall within the inner fences. Values between the inner and outer fences are plotted with asterisks.

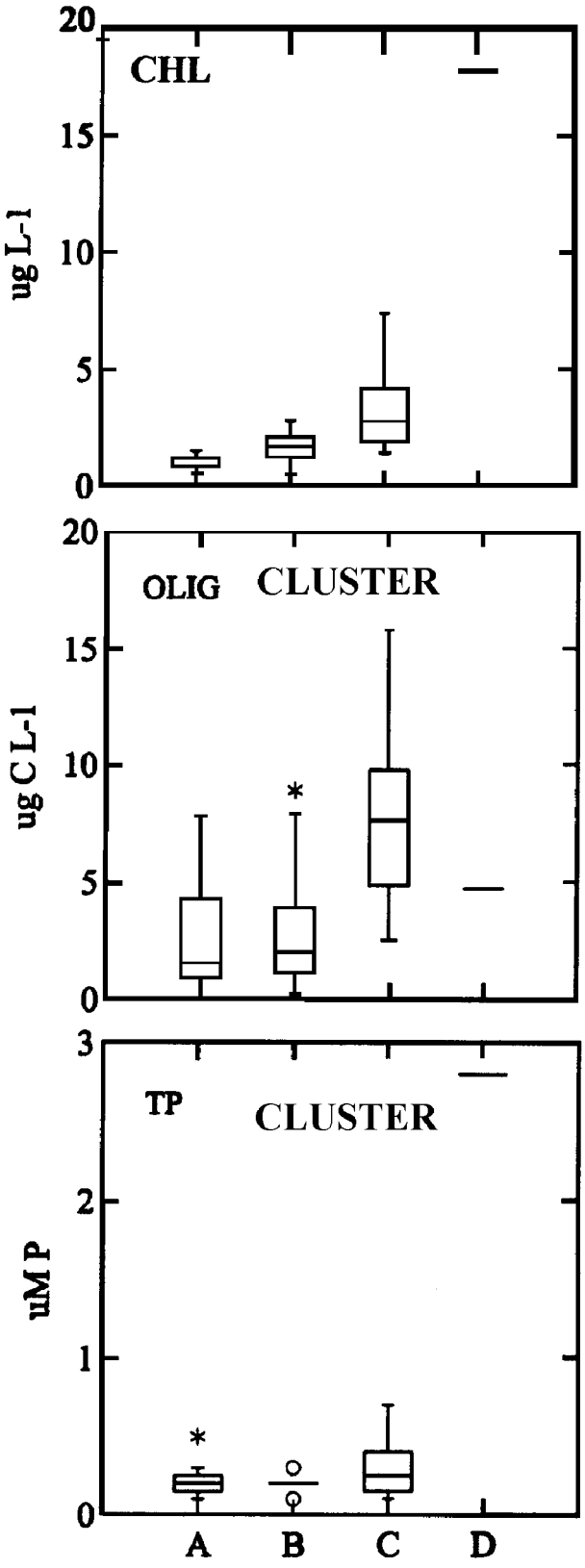

Figure 8. Box plots of explanatory variables from the discriminant analysis (Table 3). The center vertical line marks the median of the sample. See Figure 6 for explanation of symbols.

because "nephloid layer" organic materials are distributed throughout the water column [*Cotner et al.*, 2000]. Riverine nutrient and organic matter inputs also are amplified in the spring when river flow is high. These plumes of resuspended and riverine particles reduce available light for phytoplankton [*Fahnenstiel et al.*, 2000] but can provide labile organic substrates and other nutrients to heterotrophic bacteria during spring [*Eadie et al.*, 1984; *Cotner et al.*, 2000].

4.3. Nitrogen Fluxes and Microbial Food Web Characteristics

[34] Not including the outlying SJRM sites, NH_4^+ uptake (mean = 10 nM N h^{-1}) and regeneration rates (6 nM N h^{-1}) under natural light were at the low end of rates observed in coastal waters (10–7,600 nM N h^{-1} [e.g., *Lipschultz et al.*, 1986; *Bode and Dortch*, 1996; *Paasche*, 1988; *Selmer*, 1988; *Hanson et al.*, 1990; *Cotner and Gardner*, 1993; *Gardner et al.*, 1993, 1997; *Gardner et al.*, unpublished data, 1996–2002). This result is not surprising because low rates are expected for cold waters with low primary production rates. The SJRM sites had higher rates and exhibited greater response to light or added P than lake sites, which is an indication of high microbial activity and phytoplankton importance versus other lake stations.

[35] A temporal pattern in N cycling rates was not observed despite mean temperatures ranging from 1.5 to 11.0°C during March, May, and June cruises. Spring diatoms in May and June [*Fahnenstiel and Scavia*, 1987] did not have an obvious effect on NH_4^+ cycling rates, perhaps because diatoms use NO_3^- rather than NH_4^+ as their primary N source. In addition, epilimnetic diatom production accounted for <20% of total primary production in Lake Michigan, even when diatoms dominated [*Laird et al.*, 1988].

[36] Discriminant analysis identified three variables (Chl, oligotrichs, and TP) that helped explain differences among clusters of stations with different N cycling rates. The inclusion of Chl concentration helped differentiate between sites characterized by low and average N cycling rates, and oligotrichs and TP corresponded to the difference between average and high N cycling rate sites. The results are consistent with the idea that observed regeneration was driven by protists, such as oligotrichs, which may release NH_4^+ while grazing. Microzooplankton-sized oligotrich ciliates are major consumers of primary production in offshore southern Lake Michigan during winter-spring [*Kovalcik*, 2001]. Small oligotrichs, like *Rimostrombidium brachykinetum,* often are abundant at locations characterized by elevated populations of bacteria and picophytoplankton. Higher biomass (>15 μg C l^{-1} compared to background abundance <5 μg C l^{-1}at other stations) of

Table 3. Result of Discriminant Analysis Using Clusters (Figure 6) as a Grouping Variable[a]

Cluster	% Correct	% Correct Jack-Knifed	Between Group F	A	B	C
A	57	57	A	0.0		
B	82	65	B	2.5	0.0	
C	78	75	C	8.2	7.6	0.0

[a]Multivariate statistics: Wilk's lambda = 0.391; F = 5.39, p = 0.0002.

Table 4. Net Amino Acid Fluxes (±SE) in Amino Acid-Fortified Incubation Bottles for Waters Sampled in March 1999 and March 2000

		Net AA Uptake(L)		Net AA Uptake(D)	
Cruise	Station	nM N h^{-1}	Std. Err.	nM N h^{-1}	Std. Err.
Mar-99	M15-5	−2	7	−1	4
	M45-5	−11	0	−13	1
	S15-5	9	0	8	1
	J15-5	7	1	5	1
	J20-5	5	1	7	1
	J60-5	4	1	4	2
	J80-5	−19	1	−23	2
	G20-5	−13	1	−11	0
	C15-5	−11	2	−8	0
	C80-5	−1	1	−3	0
	R15-5	20	1	23	0
	R80-5	−8	2	−3	0
Mar-00	SJRM-3	5	1	5	1
	SJRM-3P	6	2	7	1
	J15-5	32	1	29	1
	J15-5P	25	2	30	1
	J30-5	−1	1	3	1
	J30-5P	0	1	4	2
	NB20-10	7	1	4	3
	NB20-10P	−2	5	2	1
	G15-5	−11	6	−7	0
	G15-5P	−4	10	3	9
	G45-5	−1	5	−7	3
	G45-5P	−7	2	−7	1
	C15-5	−19	2	−20	1
	C15-5P	−18	1	−17	2
	C80-10	−3	3	−5	1
	C80-10P	−13	8	−3	1

small (<3 μm) picophytoplankton (Synechococcus-like cyanobacteria) occurred at most sites where regeneration rates were enhanced [*Hersha*, 2002]. Although their contribution to total phytoplankton is not as high during spring transition as summer stratification [*Fahnenstiel et al.*, 2000], they grow faster than diatoms and cryptophytes and are consumed by ciliates at high rates [*Kovalcik*, 2001].

[37] The riverine zone near St. Joseph was characterized by enhanced bacterivory rates [*Hersha*, 2002]. In contrast, shallow sites away from the river often had low populations of cyanobacteria and ciliates and corresponding low N-cycling rates.

[38] Our light-dark NH_4^+ cycling data and comparisons with MFW characteristics support the conclusion that N uptake by bacteria and regeneration by ciliates are important mechanisms for NH_4^+ cycling during spring transition. Results suggest that heterotrophic and nitrifying bacteria (rates from dark bottles) may be responsible for about 70% of total NH_4^+ uptake (rates from light bottles) observed at "lake" sites. This observation agrees with the conclusion that bacterial activity drives MFW dynamics in regions influenced by resuspension [*Cotner et al.*, 2000]. However, our light-dark comparison may be biased by the possibility that nitrification is inhibited by light [*Ward*, 2000], and thereby cause uptake rates to be conservative during daylight hours in light, but not dark, bottles. Previous work in southern Lake Michigan indicated that nitrification rates were higher in dark than light bottles (Personal communication, Harvey Bootsma, University of Wisconsin at Milwaukee).

[39] Comparing uptake to regeneration rates for all sites and treatments revealed that potential uptake was higher than regeneration in some light bottles. However, in dark bottles, NH_4^+ uptake and regeneration rates were correlated ($p < 0.001$) and about equal in magnitude. This 1:1 relationship between dark uptake and regeneration suggests that bacterial uptake and community regeneration were coupled tightly and may indicate that the amount of NH_4^+ taken up was regulated by the production rate.

[40] SJRM and June 1999 J15 "river-influenced" samples, with high Chl values, had uptake rates three times higher in the light (100 ± 17 nM N h^{-1}, n = 6) than dark (32 ± 4 nM N h^{-1}, n = 6), except for May 2000 J15 site, which had low Chl levels and N-cycling rates similar to other lake samples. That site was the only one where HNF dominated the biomass. Light/dark comparisons revealed that photoautotrophic (light minus dark) uptake was small (ca. 30%) relative to heterotrophic or nitrifier uptake (dark) or regeneration rates in lake samples but important at two sites near SJRM (ca. 70%).

4.4. Possible Factors Limiting Microbial Activity During Spring Transition: A Case for the Importance of Ammonium as an N Substrate for Bacteria

[41] Bacterial growth rates could be limited by P, N, or organic-C depending on the composition of the organic substrate. Riverine or resuspended organic material in the early spring has a higher ratio of C to N or P than fresh organic material (e.g., Table 1), which could cause bacteria to be limited by inorganic nutrients. Phosphorus limits primary production in the lake during stratification but should be less important in spring, since P is associated with resuspended particles. Nitrogen is complex because of

Table 5. Net Amino Acid Fluxes (±SE) in Amino Acid-Fortified Incubation Bottles for Waters Sampled in May 2000 and June 1999

		Net AA Uptake(L)		Net AA Uptake(D)	
Cruise	Station	nM N h^{-1}	Std. Err.	nM N h^{-1}	Std. Err.
Jun-99	M15-5	1	1	3	3
	M15-5P	−2	2	−6	2
	M110-5	−3	1	−6	1
	M110-5P	4	1	−1	3
	M110-25	4	2	2	3
	M110-25P	5	2	3	1
	J15-5	15	2	17	1
	J15-5P	19	0	12	4
	J80-5	−6	5	2	1
	J80-5P	1	1	4	0
	J80-24	8	1	8	2
	J80-24P	5	1	5	1
	C80-25	−5	1	−6	2
	C80-25P	−9	2	−3	3
May-00	M45-5	−32	2	−37	1
	M45-5P	−34	1	−37	0
	M45-15	−49	6	−54	5
	M45-15P	−57	6	−63	1
	SJRM-4	31	2	32	0
	SJRM-4P	30	1	23	3
	J15-5	0	1	−6	3
	J15-5P	−2	1	0	0
	J30-5	−5	1	−6	0
	J30-5P	−8	1	−1	4
	NB15-5	1	1	1	1
	NB15-5P	4	1	2	2
	G15-5	−2	1	−4	1
	G15-5P	2	1	1	1
	G45-5	−12	0	−10	1
	G45-5P	−11	2	−13	0

its different oxidation states. The degree of NO_3^- as a N source for natural-water bacteria is not well understood, but, energetically, it is an inefficient N source for bacterial assimilation [*Kirchman*, 2001]. Although NH_4^+ and amino acids are reduced N forms, low concentrations or supply rates of these compounds may limit their availability to bacteria during spring transition.

[42] Added amino acids or P did not enhance community microbial processes, reflected by N cycling rates, at most sites in our study. Net amino acid fluxes were small at most stations indicating low demand for organic C or N supplied as amino acids. Native microbial populations must not have been adapted to high concentrations or inputs of amino acids. Even in summer, a lag of >24 h was observed before amino acids were taken up at significant rates in Lake Michigan [*Gardner et al.*, 1986, 1987, 1989]. In contrast, rapid amino acid uptake occurred within a few hours in mid-salinity regions of the Mississippi River plume [*Gardner et al.*, 1993, 1996]. Phosphorus additions did not cause NH_4^+ or amino acid uptake rates to increase except near SJRM, where the P effect was moderate. Soluble reactive P concentrations were low in most of the unspiked lake samples, but available P, associated with particles, is abundant in surface waters during the spring transition period [*Eadie et al.*, 1984].

[43] Nitrogen limitation initially seemed unlikely, because dissolved NO_3^- was abundant, but our experiments suggest that NH_4^+ could be an important factor. Ammonium may enhance microbial degradation of N-poor organic matter [e.g., *Goldman et al.*, 1987; *Gardner et al.*, 1996], because NH_4^+ requires five times less energy for assimilation than NO_3^- [*Vallino et al.*, 1996]. Available NH_4^+ regenerated by microbes in response to autotrophic production could provide reduced N for heterotrophic bacteria, which derive energy from partially-degraded organic materials with high C:N ratios. Reduced N forms, such as NH_4^+ or amino acids, may enhance degradation of organic residues with high C:N ratios [e.g., *Amon et al.*, 2001]. The C:N ratios of the particles in our samples (7.5 to 13.9, mean 9.3 ± 0.3) were higher than normal Redfield ratios for bacteria or phytoplankton (4.6 to 7 [*Kirchman*, 2001]).

[44] Our amino acid addition experiments argue against C limitation of bacterial growth but are consistent with the idea that bacteria and organic C interactions could be enhanced by NH_4^+ regeneration. Rapid amino acid uptake was not observed when labeled amino acids were added except at SJRM. Production of $^{15}NH_4^+$ from ^{15}N-labeled amino acids removed from solution would be expected if bacteria were limited by organic C [*Gardner et al.*, 1996]. The absence of $^{15}NH_4^+$ accumulation at SJRM (data not shown), where significant amounts of labeled amino acids were removed (positive uptake; Table 5), suggests that amino acid N was incorporated into biomass or any NH_4^+ produced was taken up before accumulating in the water.

[45] Ammonium regeneration and uptake rates were about equal in most experiments, indicating a close coupling between these two processes. To compare potential bacterial requirements with observed NH_4^+ regeneration rates, we estimated mean N demand from measured bacterial growth rates (Table 2) and compared it to dark NH_4^+ regeneration rates. Assuming that bacteria have a molar C:N ratio of five [*Kirchman*, 2001] and a mean growth rate of 3.7 (SE = 1.0, N = 33) μg C l^{-1} day^{-1} (average bacterial production rate from Table 2), the calculated N demand was 2.6 nM N h^{-1} compared to mean dark NH_4^+ regeneration of 9.4 nM N h^{-1} for the same stations. This calculation implies that NH_4^+ regeneration provides more than sufficient N for heterotrophic bacterial production with the remainder available for uptake by nitrifiers or autotrophs. Nitrifying bacteria, which obtain energy from NH_4^+ and fix inorganic C, contribute to the bacterial base of microbial populations [*Lavrentyev et al.*, 1997], but their biomass production is low since they convert most NH_4^+ to NO_3^- rather than into biomass [*Ward*, 2000].

[46] We conclude that microbial and N cycling processes are active and interactive during spring transition. Ammonium regenerated in the water column may provide reduced N needed to supplement resuspended or river-derived organic C for bacterial production, which drives much of the food web during spring transition. These results contrast to summer stratification when P and light, as well as top-down grazing, are major forces limiting autotrophic production and consequent food web processes [*Scavia and Fahnenstiel*, 1987]. More studies are needed to define the role of NH_4^+ versus NO_3^- (and organic N) in the degradation of organic residues with high C:N ratios.

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