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EUTROPHICATION IN LAKES

Nitrogen dynamics and microbial food web structure during a summer cyanobacterial bloom in a subtropical, shallow, well-mixed, eutrophic lake (Lake Taihu, China)

Mark J. McCarthy · Peter J. Lavrentyev · Longyuan Yang · Lu Zhang · Yuwei Chen · Boqiang Qin · Wayne S. Gardner

Abstract Nitrogen dynamics and microbial food web structure were characterized in subtropical, eutrophic, large (2,338 km^2), shallow (1.9 m mean depth), and polymictic Lake Taihu (China) in Sept–Oct 2002 during a cyanobacterial bloom. Population growth and industrialization are factors in trophic status deterioration in Lake Taihu. Sites for investigation were selected along a transect from the Liangxihe River discharge into Meiliang Bay to the main lake. Water column nitrogen and microbial food web measurements were combined with sediment–water interface incubations to characterize and identify important processes related to system nitrogen dynamics. Results indicate a gradient from strong phosphorus limitation at the river discharge to nitrogen limitation or co-limitation in the main lake. Denitrification in Meiliang Bay may drive main lake nitrogen limitation by removing excess nitrogen before physical transport to the main lake. Five times higher nutrient mineralization rates in the water column versus sediments indicate that sediment nutrient transformations were not as important as water column processes for fueling primary production. However, sediments provide a site for denitrification, which, along with nitrogen fixation and other processes, can determine available nutrient ratios. Dissimilatory nitrate reduction to ammonium (DNRA) was important, relative to denitrification, only at the river discharge site, and nitrogen fixation was observed only in the main lake. Reflecting nitrogen cycling patterns, microbial food web structure shifted from autotrophic (phytoplankton dominated) at the river discharge to heterotrophic (bacteria dominated) in and near the main lake.

Keywords Lake Taihu · Eutrophication · Nitrogen · Cyanobacterial blooms · Denitrification

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Eutrophication of shallow lakes with special reference to Lake Taihu, China

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Introduction

Nitrogen (N) and phosphorus (P) often limit primary productivity in aquatic systems. Unlike P,

dissolved inorganic N (DIN) occurs in different forms and oxidation states. DIN is assimilated and converted to organic N by phytoplankton, other plants, or bacteria. Organic N can be regenerated as ammonium (NH_4^+) or dissolved organic N (DON) compounds, reassimilated by plants or heterotrophs, or oxidized by bacteria.

DIN composition and ratios can affect phytoplankton community structure. For example, NH_4^+ is conducive to cyanobacteria (Blomqvist et al., 1994; Hyenstrand et al., 1998; Dokulil & Teubner, 2000; Jacoby et al., 2000). Unlike diatoms, cyanobacteria are poor competitors for nitrate (NO_3^-; Hyenstrand et al., 1998). Some cyanobacteria can fix atmospheric dinitrogen (N_2), which can be regenerated as bioavailable DIN compounds (Shapiro, 1990; Downing et al., 2001). Cyanobacteria dominance often is regarded as an indicator of eutrophication (Dokulil & Teubner, 2000).

Nitrification coupled with denitrification converts biologically available N forms (NH_4^+ and NO_3^-, respectively) to N_2 gas and may reduce effects of excessive N inputs and eutrophication (Seitzinger, 1988). Nitrate for coupled denitrification derives from organic matter mineralization to NH_4^+ followed by nitrification (Seitzinger, 1988). Dissimilatory NO_3^- reduction to NH_4^+ (DNRA) is an alternative pathway for NO_3^- in sediments (Tobias et al., 2001; An & Gardner, 2002). The relative partitioning between NO_3^- reduction pathways (denitrification versus DNRA) is important since denitrification removes fixed N from the system while DNRA returns it as bioavailable NH_4^+ (Tobias et al., 2001).

The microbial food web (MFW) includes heterotrophic, autotrophic, and mixotrophic prokaryotes and eukaryotes. Multiple cascading trophic interactions within the MFW can affect the biogeochemical N cycle and have feedback effects on trophic conditions in coastal waters (Lavrentyev et al., 1998). MFW structure also is an important factor regulating algal community dynamics in eutrophic, cyanobacteria-dominated systems (Elser, 1999). MFW processes should be included in nutrient-food web models to understand complex processes in aquatic environments (Edwards et al., 2000).

Evaluating internal nutrient cycling and transformations, and conditions enhancing them, in lakes and other aquatic systems help managers address effects of excessive nutrient inputs and eutrophication, such as cyanobacteria dominance. Eutrophication studies in freshwater lakes often focus on P, since it is the most common limiting nutrient. This situation describes the current status of research on Lake Taihu, a large (2,338 km^2), subtropical, shallow (1.9 m mean depth), well-mixed, and eutrophic lake in China (Pu & Yan, 1998). Studies in this lake have been limited to monitoring nutrient sources and dominant compounds (i.e. nutrient concentrations).

Water column NH_4^+ regeneration and uptake, benthic nutrient fluxes and N sinks (i.e. denitrification), water column versus benthic N cycling, and MFW structure are important factors related to trophic status and water quality. These issues have not been addressed in Lake Taihu. The size, depth, trophic status, latitude, and basin land use for Lake Taihu are similar to Lake Okeechobee (Florida, USA; Havens et al., 2001). General information about Lake Taihu geography is presented elsewhere in this issue (Qin et al., 2007).

Lake Taihu was oligotrophic as recently as the 1950s, but increased nutrient inputs related to population and economic growth have led to eutrophication (Cai et al., 1997; Chen et al., 2003). Most pollutants come from rivers discharging into Meiliang Bay and other parts of the lake (Huang, 2000). Meiliang and Wuli Bays (Fig. 1) suffer from severe eutrophication (Huang, 2000). Nutrient concentrations decrease with distance

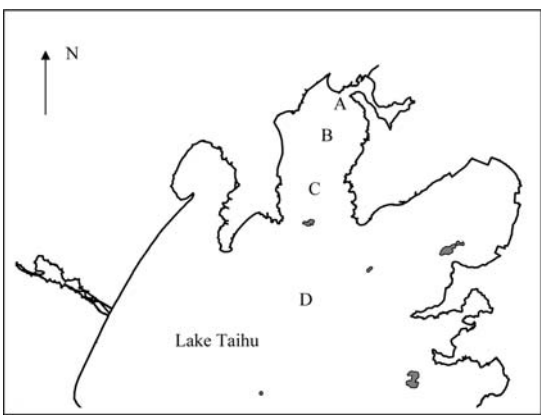

Fig. 1 Map of northern Lake Taihu showing locations of sampling stations in Meiliang Bay. A is the ''river'' site, B is ''inner bay'', C is ''outer bay'', and D is ''main lake''

from river outflows (Cai et al., 1997), but whole-lake nutrient concentrations have increased by an order of magnitude (Huang, 2000).

Wind-driven mixing and shallow depth prevent stratification in the lake, and bottom waters remain oxic (Dickman et al., 1998). Phytoplankton diversity has decreased since 1981, but cyanobacteria populations (*Microcystis* and *Anabaena*) have increased (Pu & Yan, 1998) and can comprise 85% of summer phytoplankton biomass (Chen et al., 2003). Annual blooms have clogged intakes at municipal waterworks, interrupted domestic and industrial water supply, disrupted tourism and capture fisheries, and caused cultured fish stock losses (Pu & Yan, 1998). Wind-driven sediment resuspension inhibits photosynthesis below 0.5–1 m depth. However, *Microcystis* use gas vesicles to maintain buoyancy and have a competitive advantage over other phytoplankton by avoiding light limitation (Shapiro, 1990; Chen et al., 2003).

The primary goal of this study was to characterize, for the first time, N dynamics relative to MFW structure in this understudied, eutrophic system. Specific objectives were to: (1) measure water column NH_4^+ regeneration and potential uptake rates; (2) measure sediment N fluxes and transformation rates; (3) compare sediment N transformation rates with water column N cycling rates; and (4) relate microbial plankton composition and distribution to N cycling rates.

Materials and methods

Site description

Four stations were selected along the Liangxihe River discharge gradient from Meiliang Bay to the main lake (Fig. 1). Stations were located at the Liangxihe River discharge into Meiliang Bay (river; ''A'' on Fig. 1), inner Meiliang Bay (inner bay; ''B'' on Fig. 1), outer Meiliang Bay (outer bay; ''C'' on Fig. 1), and the main lake (''D'' on Fig. 1). These stations correspond to monitoring stations (0, 1, 3, and 7, respectively) along a larger transect detailed in previous work (Cai et al., 1997; Chen et al., 2003). Sampling occurred on 26 Sept 2002 (outer bay and main lake) and 2 Oct 2002 (river and inner bay). Data from triplicate incubation chambers were averaged for each measured parameter or flux. Standard errors were calculated to determine replicate variation and are reported with means as plus/minus (±) one standard error (SE).

Water column characteristics

Water depth was estimated using a wooden pole and tape measure. Turbidity was estimated using a ~30 cm Secchi disc, and surface water temperature was measured with a mercury thermometer. Dissolved oxygen concentration (DO) was measured using Winkler titration, and chlorophyll *a* concentration (chl) was measured using hot ethanol extraction followed by spectrophotometry (Chen et al., 2003).

Water column nutrient {NO_3^- + nitrite (NO_2^-), NO_2^-, and ortho-phosphate (o-PO_4^{3-})} samples were filtered with 0.2 μm syringe filters (Osmonics) and frozen in 14 ml snap-cap tubes (Falcon). NH_4^+ samples were filtered (0.2 μm syringe filter) and frozen in 8 ml glass vials (Wheaton). Frozen samples were carried to the University of Texas Marine Science Institute (UTMSI) for flow-injection analysis of $NO_3^- + NO_2^-$, NO_2^-, and PO_4^{3-} (Lachat QuikChem 8000) and high performance liquid chromatographic (HPLC) NH_4^+ analysis (Gardner et al., 1995a). Total N (TN) and total P (TP) were determined by persulfate oxidation and spectrophotometry (Jin & Tu, 1990). Total P was oxidized to PO_4^{3-} at 120°C. TN and TP recovery efficiencies were 98.4% and 99.7%, respectively.

Water column NH_4^+ regeneration and uptake

Water from each site was collected in 2 l plastic bottles, returned to the Taihu Laboratory for Lake Ecosystem Research (TLLER), enriched with 99.8% $^{15}NH_4Cl$ (Isotec; 16 μmol l^{-1} final isotope concentration), and partitioned into triplicate light and dark 70 ml tissue culture bottles (Corning). Dark bottles were wrapped with aluminum foil. Initial samples were collected immediately after enrichment and mixing, filtered (0.2 μm syringe filter), and frozen in 8 ml glass vials (Wheaton). Bottles were incubated in a

mesh bag suspended from the TLLER pier into surface water. Intermediate samples were collected after 4 (river and inner bay) or 11 h (outer bay and main lake), filtered, and frozen. Intermediate samples were collected to coincide with day/night transition. Final samples were collected after 20 h, filtered, and frozen. All frozen samples were hand-carried to UTMSI for analysis of total NH_4^+ concentration and atom % ^{15}N using HPLC (Gardner et al., 1995a). Ammonium regeneration and potential uptake rates were calculated from these data using the Blackburn/Caperon isotope dilution model (Blackburn, 1979; Caperon et al., 1979). NH_4^+ uptake rates in this study are qualified as ''potential'', and no instances of complete isotope substrate depletion were observed.

Sediment–water interface incubations

Triplicate sediment cores (depth 15–20 cm) were collected from each site using a coring device allowing acrylic tube (30.5 cm length, 7.6 cm inside diameter, 0.32 cm wall thickness) insertion into the sediment with minimal disturbance and overlying water retention. Sediment cores with overlying water were sealed immediately at both ends with butyl caps and electrical tape. Near-bottom water from each site was collected into two ~20 l carboys using a submersible pump.

Cyanobacteria formed a ''scum'' covering the water surface at all sampling stations. The water column also was turbid from wind-driven sediment resuspension. Thus, the sediment–water interface was expected to receive little or no light, and intact sediment cores with overlying water were wrapped with aluminum foil to prevent light effects. Wind-driven turbulence and a shallow water column also maintain bottom water normoxia. Therefore, carboys with bottom water were aerated using an aquarium air pump to maintain oxic conditions in overlying water.

Cores were installed into a flow-through incubation system (Lavrentyev et al., 2000; McCarthy & Gardner, 2003) consisting of aerated bottom water, a multi-channel proportioning pump (Technicon), transmission tubing (Teflon), and an acetol plunger with Viton o-ring. The plunger was positioned ~5 cm above the sediment surface to give ~230 ml of overlying water volume (An & Gardner, 2002). This system maintained a gas-tight setting with inflow (flow rate $\cong 0.072$ l h^{-1}) from the bottom water carboy and outflow from positive displacement of overlying water. The cores were placed in a water bath maintained at in situ temperature and allowed to reestablish steady-state overnight.

After the pre-incubation period, discreet inflow (from bottom water carboy) and outflow samples were collected daily for 2 days for nutrient analyses as described above. Nutrient fluxes (in μmol m^{-2} h^{-1}) were calculated by: $(C_o - C_i)\ x\ f/a$, where C_o is the outflow concentration in μmol l^{-1}, C_i is the inflow concentration, f is the flow rate (0.072 l h^{-1}), and a is the sediment surface area (0.0045 m^2).

Dissolved gas samples were collected from core outflow by overflowing 15 ml ground-glass stopper test tubes (Chemglass; 19.8 cm length and 1 cm ID), injecting 200 μl 50% $ZnCl_2$, and inserting the ground-glass stopper quickly while twisting to prevent air bubble entrapment. Parafilm was wrapped around the top of the tubes, which were stored under water in 4 l bottles (Nalgene) to prevent large temperature changes. Samples were hand-carried to UTMSI for dissolved gas (N_2, O_2, and argon) analysis via membrane inlet mass spectrometry (MIMS; Kana et al., 1994; An et al., 2001; An & Gardner, 2002; McCarthy & Gardner, 2003). Net N_2 flux and sediment O_2 demand (SOD; O_2 flux) were calculated from the flux formula above. In the absence of N fixation, net N_2 flux provides a reasonable estimate for ''actual'' denitrification rates.

Bottom water was enriched with 98% $Na^{15}NO_3^-$ (~100 μmol l^{-1} final isotope concentration) after the second sampling day. Samples were collected for two more days for $^{28}N_2$, $^{29}N_2$, and $^{30}N_2$ analysis via MIMS (An et al., 2001; An & Gardner, 2002). These data allowed isotope pairing (Nielsen, 1992) analysis of potential denitrification (sum of $^{28}N_2$, $^{29}N_2$, and $^{30}N_2$ fluxes and N fixation) driven by ^{14}N and ^{15}N pools and simultaneous evaluation of N fixation. Potential denitrification, ^{14}N and ^{15}N denitrification, and N fixation calculations are detailed in An et al. (2001). Ammonium isotope ratios obtained by HPLC (Gardner et al., 1995a) allowed calculation of $^{15}NH_4^+$ concentrations and, using the above flux

formula, $^{15}NH_4^+$ production (potential DNRA; An & Gardner, 2002).

MIMS uses a quadrupole mass spectrometer (QMS) to detect dissolved gases in water. The QMS ion source ionizes gases and produces O^+ ions, which react with N_2 to form nitric oxide (NO; Eyre et al., 2002). This scavenging results in a lower N_2 signal at high O_2 concentrations, and vice versa, and may lead to over- or underestimation of denitrification rates. The error, however, is machine dependant (Eyre et al., 2002), and the extent of this effect was small (0.13%) on the QMS used in this study (McCarthy & Gardner, 2003). Also, sulfide can inhibit the final step in denitrification (nitrous oxide (N_2O) $\rightarrow$ N_2) and lead to N_2O release (Nedwell & Dong, 2002). Since MIMS uses N_2:Ar to determine N_2 and a liquid N_2 trap to remove interferences (Kana et al., 1994), denitrification leading to N_2O release is not detected and may cause rate underestimation.

Water column MFW structure

Heterotrophic bacteria and phototrophic picoplankton were quantified from 1% formalin-fixed samples using epifluorescence microscopy (EFM; Olympus BX-40). Heterotrophic bacteria were stained with DAPI (Porter & Feig, 1980) and sonicated to count attached bacteria (Velji & Albright, 1993). Attached bacterial abundance was the calculated difference between total cell number (treated samples) and total free-suspended cells (untreated samples). Bacterial cells were measured with a SPOT-2 digital camera and Image Pro 4.5 software. Bacterial and picocyanobacterial biovolumes were converted to carbon following Loferer-Krossbacher et al. (1998) and Menden-Deuer & Lessard (2000), respectively.

Heterotrophic nanoflagellates (HNF) were preserved with 1% formaldehyde, concentrated onto black 0.8 μm polycarbonate membrane filters, and counted using EFM following dual-staining with FITC/DAPI (Sherr et al., 1993). Lugol's iodine preserved microplankton were settled overnight in 20–50 ml chambers and counted with an Olympus IX-70 inverted differential interference contrast (DIC) microscope. Linear dimensions of 30–90 individuals (fewer for less abundant taxa) were measured at 400–600× and converted to volumes using appropriate geometric shapes. Tintinnid volume was determined using the same approach as for aloricate protists, since they were clearly visible under DIC. Phytoplankton and HNF volumes were converted to carbon following Menden-Deuer & Lessard (2000). Ciliate and rotifer volumes were converted following Putt & Stoecker (1989) and Fahnenstiel et al. (1998), respectively.

Results

Water column characteristics

Site data for sampling stations are provided in Table 1. All sites except 'river' were deeper than the mean lake depth. Mean DO (8.4 ± 0.2 mg O_2 l^{-1}) was near saturation (8.6; Colt, 1984) for the mean temperature (22.7 ± 0.4°C). Nutrient concentrations decreased along the transect except PO_4^{3-} concentrations were low at all sites. TN:TP was about eight-fold higher at 'river' versus the other sites.

Water column NH_4^+ regeneration and uptake

Water column NH_4^+ regeneration and potential uptake rates (hereafter ''regeneration'' and ''uptake'') were an order of magnitude higher at 'river' than the other sites (Fig. 2). Light/dark uptake differences were observed at all sites. Light/dark differences in regeneration were observed at 'river' and 'outer bay'. Light uptake was higher than light regeneration at all sites.

Sediment–water interface nutrient fluxes

Benthic nutrient fluxes were higher at 'river' than the other sites (Table 2). Mean sediment DIN flux for all sites (129 ± 34 μmol N m^{-2} h^{-1}) was almost two orders of magnitude higher than PO_4^{3-} flux (1.4 ± 0.6 μmol P m^{-2} h^{-1}). Mean NO_2^- (-7.2 ± 3.3 μmol N m^{-2} h^{-1}) and NO_3^- (-13.1 ± 9.6 μmol N m^{-2} h^{-1}) fluxes for all sites were negative due to high fluxes into the sediment at 'river'. Lake Taihu sediments were a source of NH_4^+ (149 ± 47 μmol N m^{-2} h^{-1}).

Table 1 Site characteristics for Lake Taihu sampling stations

Lat (N) = north latitude in degrees, minutes, seconds (d, m, s). Long (E) = east longitude. Temp = temperature. NH_4^+ = ammonium. NO_3^- = nitrate. NO_2^- = nitrite. NO_x = NO_3^- + NO_2^-. PO_4^{3-} = ortho-phosphate. TN = total N. TP = total P. Chl *a* = chlorophyll *a*. DO = dissolved oxygen

Parameter	Units	River	Inner Bay	Outer Bay	Main Lake
Lat (N)	d, m, s	31, 32, 17	31, 29, 37	31, 26, 16	31, 21, 0
Long (E)	d, m, s	120, 13, 17	120, 11, 51	120, 11, 12	120, 10, 58
Depth	m	1.4	2.6	2.9	2.8
Temp	°C	23.8	22.4	22.4	22.0
pH	su	8.65	9.04	9.08	8.55
Secchi	m	0.35	0.35	0.50	0.40
NH_4^+	μmol l^{-1}	185	3.4	2.9	0.07
NO_3^-	μmol l^{-1}	73.3	6.4	0.14	0.35
NO_2^-	μmol l^{-1}	13.8	0.6	0.02	0.06
NH_4^+:NO_x		2.1	0.5	17.9	0.2
PO_4^{3-}	μmol l^{-1}	0.18	0.25	0.22	0.04
TN	μmol l^{-1}	135	20.7	20.6	27.4
TP	μmol l^{-1}	1.5	1.7	2.5	2.0
TN:TP		89	12	8	13
Chl *a*	μg l^{-1}	85.4	4.5	3.9	14.0
DO	Mg l^{-1}	8.3	9.0	8.3	8.0

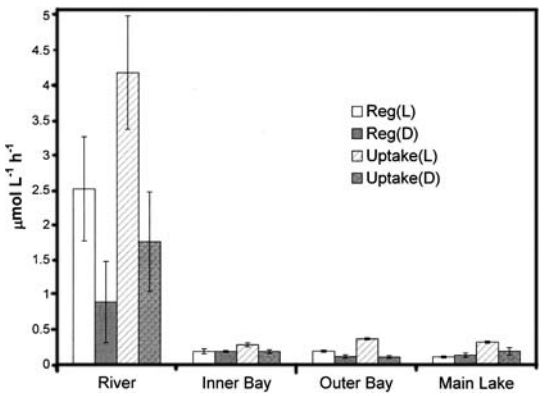

Fig. 2 Light (L) and dark (D) water column ammonium (NH_4^+) regeneration (Reg) and potential uptake rates plus/minus one standard error

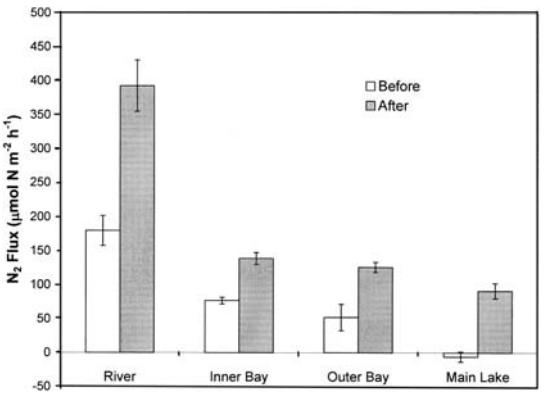

Fig. 3 Sediment–water interface net dinitrogen (N_2) flux before and after isotope ($^{15}NO_3^-$) addition plus/minus one standard error

SOD, denitrification, and N_2 fixation

Before isotope addition, SOD (in μmol O_2 m^{-2} h^{-1}) ranged from 750 ± 0.2 at 'outer bay' to 1350 ± 83 at 'river' and was similar at 'inner bay' (950 ± 32) and 'main lake' (970 ± 23). Net N_2 flux (in μmol N m^{-2} h^{-1}) decreased along the transect before and after $^{15}NO_3^-$ addition (Fig. 3). N fixation (after $^{15}NO_3^-$ addition) was observed only at 'main lake' (14 ± 10 μmol N m^{-2} h^{-1}). Thus, net N_2 fluxes before isotope addition likely reflect ''actual'' denitrification rates at the other sites.

Potential denitrification was equivalent to net N_2 flux after $^{15}NO_3^-$ addition (Fig. 4) except at 'main lake', where N fixation was observed. Denitrification fueled by $^{15}NO_3^-$ and the ^{14}N pool

Table 2 Lake Taihu sediment–water interface nutrient fluxes (μmol N or P m^{-2} h^{-1}) plus/minus one standard error

See Table 1 legend for nutrient abbreviations

Nutrient	River	Inner Bay	Outer Bay	Main Lake
NH_4^+	490 ± 31	40 ± 19	20 ± 0.5	45 ± 1.4
NO_3^-	–83 ± 6.8	5.8 ± 11	12 ± 0.1	13 ± 1.2
NO_2^-	–31 ± 3.8	–2.0 ± 0.4	0.08 ± 0.05	4.2 ± 0.5
DIN	380 ± 27	44 ± 7.2	33 ± 0.6	63 ± 3.1
PO_4^{3-}	5.2 ± 0.2	1.2 ± 0.5	0.8 ± 1.0	–1.5 ± 0.9

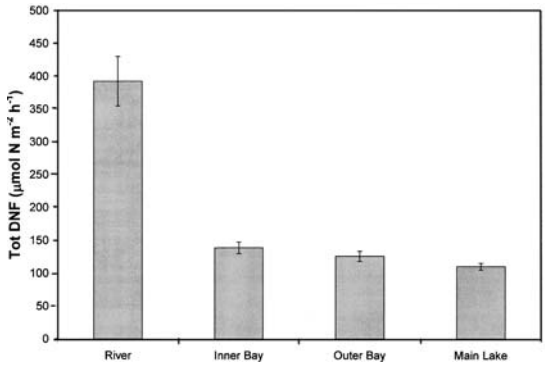

Fig. 4 Sediment–water interface potential (Tot) denitrification (DNF) after isotope ($^{15}NO_3^-$) addition plus/minus one standard error

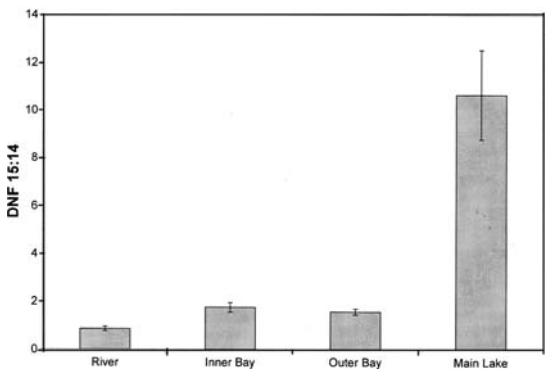

Fig. 5 Ratio of denitrification (DNF) fueled by ^{15}N versus ^{14}N nitrate (15:14) pools plus/minus one standard error

were similar at 'river', but the ^{15}N pool accounted for more potential denitrification than the ^{14}N pool at the other sites (Fig. 5).

Potential DNRA

Potential DNRA (in μmol N m^{-2} h^{-1}) was observed at all sites in Lake Taihu (data not shown). Potential DNRA rates ranged from 0.4 ± 0.1 at 'outer bay' to 250 ± 80 at 'river' and were 9.5 ± 2.4 at 'inner bay' and 3.0 ± 1.9 at 'main lake.

Water column MFW structure

Cyanobacteria were abundant at all sites during sampling (Table 3). Maximum phytoplankton biomass was observed at 'river' and dominated by large (>10,000 μm^3) euglenophytes (~80%; Fig. 6). The ''edible'' (to microbial grazers) fraction (i.e. < 200 μm^3) was mostly cryptophytes. Bacteria also reached maximum abundance (5×10^7 cells ml^{-1}) at 'river', but >67% were aggregated or attached to particles, and the median cell volume was minimal (0.033 μm^3). Rotifers (mostly *Polyarthra remata*) accounted for >50% of micrograzer biomass (HNF + ciliates + rotifers). The choreotrich ciliate, *Strobilidium* spp., and colorless nano-chysophytes dominated heterotrophic protists.

Meiliang Bay sites (inner bay and outer bay) had lower phytoplankton biomass consisting of green algae, cyanobacteria, and diatoms (Table 3; Fig. 6). Heterotrophic biomass in Meiliang Bay also was lower versus 'river', but to a lesser extent than for bacterioplankton alone, which were represented by large cells (median vol. = 0.085 μm^3). The colonial peritrich ciliate *Epystilis rotans*, chrysophytes, and rotifers *Asplanchna priodonta* and *Brachionus* spp. dominated the microbial grazers.

The main lake site had higher phytoplankton biomass than Meiliang Bay sites and was dominated by *Anabaena flos-aquae* and *Synechococcus*-like picocyanobacteria (>50%; Fig. 6). Bacterial biomass was lowest (cell volume 0.05 μm^3), but micrograzer biomass was high, primarily due to the tintinnid ciliate *Codonella cratera* and a diverse HNF assemblage, including chysophytes, kinetoplastids, and choanoflagellates.

Discussion

Water column nutrients and phytoplankton

Nutrients in Lake Taihu followed the expected pattern of decreasing concentrations with distance from river outflow. Possible explanations include dilution via physical transport (i.e. wind-driven currents), higher nutrient uptake versus regeneration rates along the transect, and nutrient sinks, such as sediment burial and denitrification.

High TN:TP ratios at 'river'(Table 1) support P-limitation observations (Cai et al., 1997; Huang, 2000; Chen et al., 2003). However, TN:TP ratios below Redfield (~16) at the other sites

Table 3 Phytoplankton (Phyto) and microbial food web (MFW) characteristics during Lake Taihu sampling

			River	Inner Bay	Outer Bay	Main Lake
MFW	$\mu\mu$g C l^{-1}	Phytoplankton	6930	149	55.4	518
		Bacteria	577	292	263	157
		HNF	20.8	8.94	6.97	15.1
		Ciliates	35.3	13.5	10.3	52.9
		Rotifers	63.8	3.57	5.75	2.62
Phyto.	%phyto	Chlorophytes	4.90	41.8	27.4	33.5
		Cyanobacteria	1.90	28.6	37.2	59.7
		Diatoms	2.00	6.60	16.7	5.87
		Euglenophytes	78.9	0	0	0
		Varia (i.e. cryptophytes)	12.2	23.0	18.7	0.96
Cyanos	%phyto	*Anabaena*	0.14	14.5	6.01	38.9
		Synechococcus	0.2	6.8	29.9	11.7
		Aphanizomenon	0	0	0	3.68
		Total N-fixers	0.33	21.3	35.9	54.3
		Microcystis	0.80	7.33	1.27	0.14
Ratios	1	Bacteria:NH_4^+	0.22	6.13	6.48	160.2
	2	Chl:DIN	0.02	0.03	0.09	2.08
	3	Chl:NH_4^+	0.03	0.09	0.09	14.28
	4	Chl:NO_3^-	0.08	0.05	1.96	2.86
	5	Chl:PO_4^{3-}	15.3	0.58	0.56	11.3
	6	Diatom:NO_3^-	3.18	8.5	216	278
	7	Diatom:PO_4^{3-}	41.8	7.02	4.43	78.5
	8	Heterotrophs:Chl	0.10	2.13	5.16	0.44
	9	MZP:total phyto	0.01	0.11	0.29	0.11
	10	MZP:edible phyto	0.56	0.63	0.58	0.54
	11	Protists:bacteria	0.10	0.08	0.07	0.43
	12	Light uptake:Chl	49.0	63.0	95.3	23.1
	13	Light reg:hetero C	3.61	0.59	0.66	0.48
	14	Dark reg:hetero C	1.28	0.59	0.39	0.60

% phyto = proportion of phytoplankton groups to total phytoplankton; Cyanos = cyanobacteria; C = Carbon; HNF = heterotrophic nanoflagellates; Chl = chlorophyll; MZP = microzooplankton; Reg = regeneration; Hetero = heterotrophic. See Table 1 legend for nutrient abbreviations

indicate surplus P and conditions conducive to cyanobacteria (Jacoby et al., 2000). Nutrient concentrations in Meiliang Bay are lowest in summer (wet season) and highest in winter (dry season), when biological activity decreases and inverse water flow occurs (Cai et al., 1997). Thus, nutrient limitation status may change in different seasons.

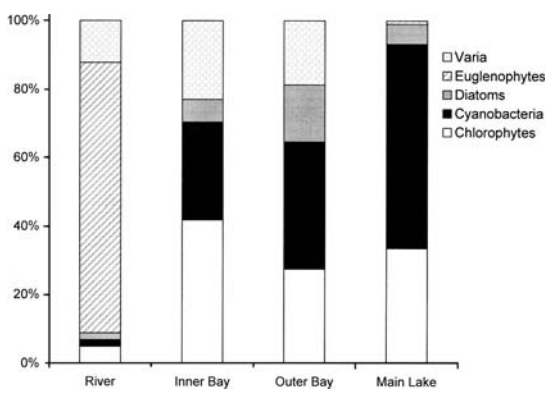

Fig. 6 Proportions of chlorophytes, cyanobacteria, diatoms, euglenophytes, and varia (mostly cryptophytes) in total phytoplankton biomass

Nutrient concentrations and algal standing stocks are important factors affecting cyanobacteria dominance (Downing et al., 2001). Transect NH_4^+:NO_x ratios were >1 at 'river' and 'outer bay' and <1 at 'inner bay' and 'main lake' (Table 1). Cyanobacteria percentage biomass, relative to total phytoplankton biomass, was highest at 'main lake' (Table 3), where the NO_3^- proportion peaked and NH_4^+ likely was depleted by cyanobacteria. Diatom percentage biomass peaked at 'outer bay', where highest NH_4^+:NO_x was observed, likely due to NO_3^- uptake by diatoms (Hyenstrand et al., 1998). Percentage cyanobacteria

biomass was greater than for diatoms at all sites except 'river'. Phytoplankton biomass at 'river' was dominated (79%) by *Euglena* spp., which are common in hypereutrophic systems (Hewson et al., 2001).

Water column NH_4^+ regeneration and uptake

Water column NH_4^+ cycling rates in Lake Taihu were within ranges observed in other eutrophic systems. High regeneration and uptake (Fig. 2) at 'river' are not surprising given high DIN concentration and phytoplankton biomass. Similar rates were observed in the Mississippi River plume (Bode & Dortch, 1996) and hypereutrophic Lake Maracaibo (Gardner et al., 2000). Uptake in Lake Okeechobee (0.67 μmol l^{-1} h^{-1}; Gu et al., 1997) was lower than the rate at 'river' but in the same range as those from Meiliang Bay and the main lake. Regeneration and uptake at sites other than 'river' were higher than oligotrophic Great Lakes (Gardner et al., 1995b) but lower than south Texas coastal waters (McCarthy et al., unpub. data).

Light/dark uptake differences were observed at all sites (Fig. 2). This result supports the idea that N is not limiting, since phytoplankton uptake depends less on light during N stress (Cochlan et al., 1991). However, light/dark uptake differences are less pronounced in N limited versus N replete systems (Cochlan et al., 1991). Light-to-dark uptake ratio was ~1.7 (less pronounced) at 'inner bay' and 'main lake' and 2.4 and 3.5 (more pronounced) at 'river' and 'outer bay', respectively. This observation suggests possible N limitation at 'inner bay' and 'main lake'. Lack of light/dark regeneration differences at these sites also is consistent with N limitation (Fig. 2; Gardner et al., 2000), since N-starved heterotrophs may retain N for biomass rather than release it. Light/dark uptake differences also may reflect autotrophic versus heterotrophic dominance, especially when those differences are more pronounced ('river' and 'outer bay').

Sediment–water interface nutrient fluxes

Cyanobacteria may acquire sufficient P to sustain all pelagic growth from sediments, thus avoiding competition with other phytoplankton (Blomqvist et al., 1994). Low water column PO_4^{3-} concentrations (Table 1) and PO_4^{3-} flux out of the sediments (Table 2) at 'river' and 'inner bay' indicate rapid P uptake and support P limitation observations at these sites. No sediment P flux at 'outer bay' and negative flux at 'main lake' (Table 2) are consistent with water column TN:TP observations below Redfield and suggest N limitation or co-limitation in outer Meiliang Bay and the main lake.

Positive NH_4^+ flux at all sites (Table 2) indicates high organic matter inputs and rapid remineralization (including DNRA). Cyanobacteria in Lake Taihu may be fueled in part by sediment NH_4^+ flux into the water column, especially at 'main lake' where DIN concentration was below 0.5 μmol l^{-1} (Table 1). Negative NO_3^- flux at 'river' may reflect denitrification and/or DNRA fueled by water column NO_3^-. Positive NO_3^- flux at 'outer bay' and 'main lake' can be explained by sediment nitrification. Small positive NO_2^- fluxes at these sites may result from incomplete nitrification and/or denitrification, since NO_2^- is an intermediate in both processes (Zumft, 1997).

N_2 fixation

Nitrogen fixation was not observed in Meiliang Bay (including 'river'). Before isotope addition, net N_2 flux at 'main lake' was negative, but standard error bars overlapped with zero, indicating that denitrification and N fixation were in balance. This site had the highest water column N-fixer biomass (Table 3). *Anabaena* comprised nearly 40% of total phytoplankton biomass, and 'main lake' is the only site where *Aphanizomenon* was observed. Phytobenthos were not evaluated, so it cannot be assumed that these N-fixers were responsible for observed sediment N fixation. However, high water column N-fixer abundance and sediment N fixation further support possible N limitation at this site (Dokulil & Teubner, 2000).

Denitrification

Sediments provide a site for denitrification, which can drive aquatic systems toward N limitation and

regulate nutrient supply ratios (Seitzinger, 1988). Denitrification in Meiliang Bay may be a factor in observed evidence for N limitation in the main lake. If excess N is denitrified before physical transport via wind-driven currents moves it to the main lake, then it follows that organisms in the main lake may have to rely on other N sources, such as fixation. Lack of N fixation at Meiliang Bay sites (including 'river') indicates that net N_2 fluxes before isotope addition (Fig. 3) may reflect ''actual'' denitrification rates, assuming no N_2O release from incomplete denitrification. Increases in net N_2 flux after $^{15}NO_3^-$ addition (Fig. 3) indicate that potential denitrification may be substrate limited, especially at 'outer bay' and 'main lake', where water column NO_3^- was low (Table 1). Another possible explanation for increased net N_2 flux after isotope addition is that the sediment core incubations eliminated wind-driven turbulence and resuspension, thus allowing settlement of suspended particles and development of a more defined redox gradient. After 2 days of incubation, redox conditions may have become inhibitory for nitrifiers and stimulative for denitrifiers, which could take advantage of increased substrate (as $^{15}NO_3^-$) to replace NO_3^- supply from decreased nitrification.

Decreases in potential denitrification along the transect may reflect diminishing organic matter availability (Fig. 4). Denitrification fueled by $^{15}NO_3^-$ versus the $^{14}NO_3^-$ pool (from water column or coupled to nitrification) shows substrate stimulation (Fig. 5), especially at sites further from river discharge. Decreasing water column NO_3^- concentrations along this gradient may result from lower nitrification and lead to reduced nitrification/denitrification coupling and increased dependence on water column NO_3^-.

DNRA

Since NH_4^+ is a primary factor in cyanobacterial dominance, it is important to characterize N transformations capable of maintaining NH_4^+ in the system. DNRA is associated with sulfidic estuarine or marine sediments (Tiedje, 1988; An & Gardner, 2002) but also can comprise up to 30% of NO_3^- reduction in lake sediments (Tiedje, 1988). Potential denitrification to DNRA partitioning ratios (DNF:DNRA) in Lake Taihu were 1.6 at 'river', 14.6 at 'inner bay', 315 at 'outer bay', and 35 at 'main lake' (data not shown). Since DNRA depends more on organic matter availability than NO_3^- concentration (Tiedje, 1988), higher DNF:DNRA may reflect organic matter limitation of DNRA away from river discharge. While potentially a meaningful process at 'river', DNRA is not as important as fixed N removal via denitrification at the other sites.

Water column versus sediment NH_4^+ regeneration

Depth-averaged water column NH_4^+ regeneration (water column regeneration converted from μmol N l^{-1} h^{-1} to μmol N m^{-2} h^{-1} given depth) and sediment N recycling rates (sum of denitrification and net DIN flux, which includes DNRA; Table 4) indicates that Lake Taihu nutrient mineralization was four to six times higher in the water column versus sediments. Thus, primary production appears to be driven primarily by

Table 4 Comparison of depth averaged water column (WC) and sediment (Sed) nitrogen regeneration (reg) in Lake Taihu

	River	Inner Bay	Outer Bay	Main Lake
DNF	180	77	52	8
DIN flux	380	44	33	63
Sed reg	560	121	85	71
WC reg	3520	480	560	310
WC:sed	6.3	4.0	6.6	4.4
WC:DIN	9.3	10.9	17.0	4.9

Fluxes in μmol N m^{-2} h^{-1}. Sediment regeneration determined by adding denitrification (DNF) and net dissolved inorganic N (DIN) flux before $^{15}NO_3^-$ addition. DNF at 'main lake' determined by adding net N_2 flux before $^{15}NO_3^-$ addition to calculated nitrogen fixation rate

water column processes. This dependence is more pronounced if denitrification is excluded from the sediment calculation (WC Reg:DIN; Table 4), since denitrification results in N removal from the system.

MFW structure and N cycling

MFW structure shows a general shift from autotrophic to heterotrophic dominance along the river to lake transect. Nutrient concentration and cycling rate comparisons with MFW structure indicate increasing N limitation along the transect (ratio #'s 2, 3, 4, and 6; Table 3). Interestingly, the light uptake-to-chl ratio (#12) was lowest at 'main lake', but N fixers may have accumulated sufficient N and, thus, did not respond to ^{15}N addition. Diatom biomass-to-NO_3^- ratio (#6) indicates severe N limitation at 'outer bay' and 'main lake'. High regeneration-to-heterotrophic biomass ratios (#'s 13 and 14) at 'river' may indicate heterotrophic bacterial NH_4^+ recycling. Recycling at all other sites, particularly 'main lake', likely is driven by grazing, since bacteria may become N limited as indicated by a higher bacteria-to-NH_4^+ ratio (#1).

The microzooplankton (ciliates and rotifers)-to-''edible'' phytoplankton biomass ratio (#10) remained remarkably constant along the transect, indicating tight trophic coupling between these groups, despite sharp variation in the microzooplankton-to-total phytoplankton biomass ratio (#9). Strong grazing pressure on nanoplankton may help cyanobacteria by eliminating competitors for limiting nutrients. Grazers often are ineffective at preying on colonial cyanobacteria due to colony size, potential toxicity, and/or gelatinous envelopes (Dokulil & Teubner, 2000). However, grazers prey on competing phytoplankton (i.e. diatoms) and transform organic N to NH_4^+ via excretion (Shapiro, 1990). Although large colonial cyanobacteria and euglenophytes may not be involved in direct herbivorous trophic transfers within the MFW, the latter group are mixotrophs (i.e. can combine autotrophic and heterotrophic modes of nutrition). Therefore, nutrient limitation is less likely to affect these taxa, since they can obtain nutrients by grazing on bacteria and picocyanobacteria.

Low chl:PO_4^{3-} (#5) and diatom:PO_4^{3-} ratios (#7) at 'inner bay' may be due to top-down pressure rather than N limitation. Greater than 1,000 cladocerans l^{-1} were present at this site. Higher heterotrophic C-to-chl ratio (#8), predominance of mesoplankton-sized ciliate colonies (less vulnerable to cladoceran grazing), and lower protist-to-bacteria ratio (#11) also support this suggestion. The latter ratio also indicates strong bacterial grazing pressure at 'river' and 'main lake', particularly if only suspended cells are considered at 'river' (ratio increases to 0.29). Bacteria may dominate plankton biomass in Meiliang Bay due to sediment resuspension introducing benthic bacteria into the water column, indicated by unusually large bacterial cells and balanced dark NH_4^+ regeneration and uptake.

Conclusions

Nitrogen dynamics relative to MFW composition were characterized along the Liangxihe River discharge gradient in and around Meiliang Bay, Lake Taihu, during late summer/early fall cyanobacteria bloom conditions. The observations provide insights into ecological characteristics of a subtropical, shallow, well-mixed, eutrophic lake. The most interesting finding is that N limits or co-limits primary production in and near central Lake Taihu, contrary to the previous paradigm of exclusive P limitation. A gradient was observed from strong P limitation at the river discharge to N limitation or co-limitation in the main lake. Denitrification in Meiliang Bay may drive main lake N limitation by removing excess N before physical forces transport it to the main lake. This result also exemplifies the importance of characterizing N cycling in freshwater systems, where most studies have focused on P dynamics.

Another important finding is the importance of water column N recycling relative to sediment processes. Nutrient flux from Lake Taihu sediments do not appear to drive primary production during late summer/early fall when cyanobacteria bloom conditions are beginning to wane. However, sediments have an important role in nutrient and MFW dynamics by providing a site for denitrification, which, in conjunction with N

fixation and other processes, can regulate available nutrient ratios. Reflecting N cycling patterns, MFW structure shifted from autotrophic (phytoplankton dominated) at the river discharge to heterotrophic (bacteria dominated) in and near the main lake. More studies are needed to evaluate seasonal and spatial variability in nutrient limitation status, N transformation rates, and phytoplankton and MFW structure and function in Lake Taihu.

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