

## Research Article

# Phosphorus sources and demand during summer in a eutrophic lake

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**Abstract.** In pelagic systems, phytoplankton biomass may remain abundant or near equilibrium while concentrations of the limiting nutrient are below detection. In eutrophic lakes, it has been thought that episodic algal blooms are due to mixing events that break down this equilibrium by adding nutrients to the mixed layer. Alternatively, rapid rates of biotic recycling among primary producers and heterotrophic consumers could maintain high phytoplankton biomass, yet the recycling process has been difficult to observe in situ. Here we use free-water oxygen measurements and an associated metabolic model to infer rates of phosphorus (P) uptake and biotic mineralization in the epilimnion of a eutrophic lake. The rates of uptake and mineralization were compared to “external” sources of P such as loading and entrainment. Also, model results were assessed using

sensitivity analysis. We found that the majority of phytoplankton P demand during the period of low P availability could be accounted for by biotic mineralization, but that it was important to consider the effects of entrainment in order to account fully for P uptake. These general results were relatively insensitive to model parameterization, though the relative C:P ratio of material taken up versus mineralized was an important consideration. This study integrates modeling and measurement tools that monitor ecosystem processes at finer temporal resolution than has previously been possible, complementing other studies that use experimental incubation and elemental tracers. Extension of this approach could enhance models that aim to integrate biological and physical processes in assessment of water quality and prediction of phytoplankton biomass.

**Key words.** Ecosystem metabolism; entrainment; respiration; production; C:P ratio; phytoplankton bloom.

## Introduction

Phytoplankton in lake ecosystems may remain abundant under apparent nutrient limitation despite loss by sinking, grazing, natural mortality and bacterial- and viral-mediated mortality (Juday et al., 1927; De Pinto et al., 1986; Caraco et al., 1992). This paradox is

particularly puzzling in eutrophic lakes where strong summer stratification and nutrient limitation would seem to favor phytoplankton loss over production and growth, yet phytoplankton blooms may occur during the period when phosphorus concentrations are below detection (De Pinto et al., 1986). A number of mechanisms may explain the provisioning of phosphorus (P) to nutrient-limited primary producers including biotic recycling, loading from external

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sources and entrainment of P-rich metalimnetic waters.

Biotic recycling of organic P compounds by heterotrophic zooplankton and microbes is highly variable, but field and laboratory research suggests that much of the P needed for phytoplankton production may be supplied by heterotrophic mineralization (Barlow and Bishop, 1965; Cole et al., 1988; Sterner, 1989; Poister et al., 1994) and that mineralization may be the dominant supply of nutrients during bloom formation (De Pinto et al., 1986). Excretion of inorganic P by zooplankton alone may account for 4-239% of the P demand of primary producers, and this percentage varies among months (Gulati et al., 1995; Vanni, 2002). However, other analyses focused on rates of primary production that are corrected for loss of phytoplankton argue that biotic recycling is not sufficient to explain observed rates of production during the summer (Caraco et al., 1992). Thus, multiple lines of evidence suggest a variable and important role of heterotrophic consumers in P recycling, yet this process has been difficult to observe and quantify in lake ecosystems (Lehman, 1980).

External loading and entrainment are dominant sources of nutrients to lakes at an annual time scale, yet their importance during the period of summer stratification and low nutrient availability is not clear. External loading is often thought to be unimportant during summer months due to lower seasonal flows into lakes. Similarly, entrainment, or transport of P from deep water, has been noted to be low due to strong stratification (Lean et al., 1987). Recent work, however, suggests that the flux of nutrients across the thermocline may be an important driver of epilimnetic metabolism (MacIntyre et al., 2002; Staehr and Sand-Jensen, 2007). Another study shows that external P inputs fuel new production (Caraco et al., 1992). Notably, external loading and entrainment may be most important following storms (MacIntyre et al., 2006), but in these cases the event is difficult to monitor and the fate of nutrient influx can be difficult to trace. As such, the relationship between episodic storms, movement of P-rich waters, and blooms of phytoplankton is noisy and difficult to interpret (Soranno et al., 1997; MacIntyre et al., 2002).

The pathways of P supply are transient and occur at temporal and spatial scales that have been challenging to observe. This challenge has been met using light/dark bottle incubations with  $^{14}\text{C}$  as a proxy for metabolic P demand,  $^{32}\text{P}$  incubations as a measure of P uptake rates, and experimental isolation of heterotrophic organisms to estimate rates of mineralization (Ryther, 1956; Hargrave and Geen, 1968; Lean et al., 1983; Lean et al., 1987; Sterner, 1986; Vanni, 2002).

These approaches involve extended incubation times during which it is difficult to maintain representative environmental conditions. Additionally, there is unresolved debate about the relationship between rates of  $^{14}\text{C}$  fixation and gross versus net primary production (Peterson, 1980; Carignan et al., 2000). The challenges and limitations associated with these techniques are widely recognized (Bender et al., 1987; Smith and Prairie, 2004; Staehr and Sand-Jensen, 2007).

Here we explore a complementary free-water approach to estimate P uptake and mineralization. We derived estimates of in situ P uptake and heterotrophic mineralization using high-resolution oxygen data and an associated metabolic model. We then compared metabolically-inferred P uptake and mineralization rates to sources of P such as external loading and entrainment. Empirical estimates of uptake, mineralization, loading and entrainment were used to calibrate a model of P demand and supply and we used this model to explore the theoretical bounds of biotic and abiotic P transformation within the epilimnion of a eutrophic lake. Our goal was to examine the dynamic behavior of P demand and supply processes to elucidate the relative importance of biotic recycling and abiotic sources of P like external loading and entrainment, particularly during the period of low P availability.

## Materials and procedures

This study has four main components: empirical measurements of oxygen, carbon and P; integration of these empirical estimates into a whole-ecosystem metabolism model to estimate P uptake and mineralization rates; comparison of metabolically-derived uptake rates to potential sources of P such as in situ heterotrophic mineralization, external P loading and entrainment of P from the metalimnion of the lake; and sensitivity analysis of model results to changes in our assumptions. Estimated uptake rates served as a proxy for P demand, and throughout the text we use “uptake” and “demand” interchangeably.

All data were collected during the summer of 2007 on Lake Mendota, a eutrophic lake in Madison, Wisconsin (43°06' N, 89°25' W, 39.1 km<sup>2</sup> surface area, 12.3 m mean depth). Lake Mendota stratifies during the summer and soluble reactive P (SRP) typically falls below the limits of analytical detection in the epilimnion (North Temperate Lakes Long Term Ecological Research program (<http://lter.limnology.wisc.edu>)). Because SRP is often used as a proxy for the concentration of bioavailable P in the system, we targeted our assessment on this component of the P budget. We focused sampling efforts on the epilimn-

ion during the period of strong stratification and low concentrations of SRP (28 June 2007 until 8 September 2007) in order to explore how phytoplankton P demand during this period may be met.

### Field and laboratory methods

During the summer of 2007 an automated buoy, equipped with temperature and oxygen probes, was deployed at a central location in Lake Mendota. A TempLine (Apprise Technologies, Inc.) thermistor array recorded temperature every minute at 0.5–1.0 m intervals from 0–20 m depth. Dissolved oxygen concentrations were monitored using a D-Opto (Zebra-Tech LTD, New Zealand) optical dissolved oxygen probe that was suspended from the buoy at 2 m. The dissolved oxygen probe was compared weekly to manual measurements and corrected, assuming linear drift. The high-resolution temperature data were averaged over 24 hour periods and used to identify the maximum depth of the mixed layer for each day. Diel mixed layer depth was around 8 m for the season, though on a number of days microstratification was apparent within the first 2 or 3 meters of the surface. In order to avoid incorrectly identifying shifts in depth of the mixed layer due to microstratification, we defined the depth of the mixed layer as the final depth (starting from lake surface) at which the temperature was within 1.5 °C of the mean temperature of the first 5 m.

Water samples were collected at least weekly in acid-washed polyethylene bottles at 1–4 m intervals throughout the water column using a peristaltic pump for determination of total P (TP) and soluble reactive P (SRP) concentrations. In the field, SRP samples were filtered through a 0.45- $\mu\text{m}$  polycarbonate etched filter using in-line filtration, and stored on ice. TP samples were preserved using Optima HCl, while SRP samples were refrigerated and analyzed within 24 h. SRP samples were analyzed colorimetrically using the ascorbic acid method (American Public Health Association (APHA), 1995). TP samples were analyzed using a Technicon Auto Analyzer following persulfate digestion (APHA, 1995). All statistical analyses and model iterations were run using the mean P concentration of samples from the epilimnion.

Particulate carbon and P samples were collected from triplicate integrated epilimnetic samples. These samples were collected at least weekly from mid-June to mid-October at depths determined by concurrent temperature profiles. All samples were filtered onto glass fiber filters and stored for carbon or P analysis. Because there is no evidence that the C:P ratio of phytoplankton should differ markedly from that of the whole water seston (Healey and Hendzel, 1980; Sterner and Elser, 2002), particularly during the

summer when phytoplankton dominate seston composition (Hecky et al., 1993; Elser et al., 1995), the C:P ratios for uptake and mineralization are derived from the C:P ratio of the entire seston. Carbon values used in the C:P ratio were based on determination of ash-free dry mass (AFDM). Three replicates of each sample were filtered onto pre-weighed glass fiber filters (Proweigh GF/F, 47 mm, 1.5  $\mu\text{m}$  pore size). The filters with sample residue were placed in a drying oven for at least 48 h, transferred to a desiccator for at least 48 h, and weighed. The filters and samples were then combusted at 550 °C for 4 h, returned to the drying oven and desiccator, and weighed again. Combusted mass was subtracted from dry mass to determine AFDM. Seston carbon concentration was inferred as 48% of the AFDM (Round, 1965; Fogg, 1975). Whole seston particulate P concentrations used as the denominator in the C:P ratio were determined using the method described by Lampman et al. (2001). Integrated epilimnetic samples were filtered onto pre-combusted glass fiber filters and frozen until analysis. Samples, standards, and blanks were placed in 25 ml acid-washed serum vials and digested by adding 20.0 ml of 1% low N potassium persulfate (Fisher Scientific ID #P282-500). The vials were sealed with aluminum rings crimped over butyl rubber septa and autoclaved for 2.5 h at 120 °C. The concentration of P in the liquid portion of each sample was analyzed colorimetrically using the ascorbic acid method and all samples were corrected for P content of the filter and of the digestion reagent (APHA, 1995).

Water samples for the determination of chlorophyll *a* concentration were collected twice per week at 2 m using a peristaltic pump or Van Dorn sampler. Water was collected in dark 3.5 L bottles and stored on ice in the field. Within three hours of collection, samples were mixed well, filtered under low light conditions onto glass fiber filters (Whatman GF/F, 47 mm, 0.7  $\mu\text{m}$  pore size), and frozen until analysis. Chlorophyll *a* samples were extracted with methanol and analyzed fluorometrically on a Turner TD-700 fluorometer. The final concentrations were corrected for phaeopigments (Holm-Hansen and Riemann, 1978; Arar et al., 1997).

### Metabolic inference of phosphorus uptake and mineralization

Estimates of gross primary production (GPP) were derived as follows from free-water oxygen measurements collected during buoy deployment. For each 1-minute measurement interval,  $t$ , we calculated net ecosystem production ( $\text{NEP}_t$ ) from the measured change in oxygen concentration,  $\Delta\text{O}_2$  ( $\text{mmol O}_2 \text{ m}^{-3} \text{ min}^{-1}$ ), and atmospheric exchange,  $D_t$  ( $\text{mmol O}_2 \text{ m}^{-3} \text{ min}^{-1}$ ). To express metabolism in areal units ( $\text{mmol}$

$\text{m}^{-2} \text{min}^{-1}$ ), we multiplied by the depth of the mixed layer,  $z$  (m).

$$\text{NEP}_t = \text{GPP}_t - \text{R}_t = (\Delta\text{O}_2 - \text{D}_t) * z \quad (1)$$

Atmospheric exchange can be positive or negative. We use positive values to indicate addition of  $\text{O}_2$  to the lake and negative values for removal. Atmospheric exchange was estimated as

$$D_t = \frac{k([\text{O}_2]_{\text{SAT},t} - [\text{O}_2]_t)}{z} \quad (2)$$

$[\text{O}_2]_{\text{SAT},t}$  ( $\text{mmol m}^{-3}$ ) is the aqueous concentration of oxygen if the lake were in equilibrium with the atmosphere and was calculated from water temperature using the empirical equation of Weiss (1970).  $[\text{O}_2]_t$  ( $\text{mmol m}^{-3}$ ) is the measured concentration of dissolved oxygen in measurement interval  $t$ . We calculated the gas piston velocity,  $k$  ( $\text{m min}^{-1}$ ), using estimates of  $k_{600}$  ( $\text{m min}^{-1}$ ) as a function of wind speed (Cole and Caraco, 1998; Cole et al., 2000) and the water temperature dependent Schmidt number,  $Sc$ , (Wanninkhof, 1992).

$$k = k_{600} \left( \frac{Sc}{600} \right)^{-\frac{1}{2}} \quad (3)$$

We measured wind speed every minute at 2 m above the water surface and converted these measurements to values at 10 m height assuming a neutrally stable boundary layer and the empirical equation of Smith (1985) to calculate values of  $k_{600}$  ( $\text{m min}^{-1}$ ). In order to express atmospheric exchange in volumetric units, we divided by the depth of the mixed layer,  $z$  (m).

We used the values of NEP for each measurement interval ( $\text{NEP}_t$ ) to obtain daily estimates of NEP, GPP and ecosystem respiration ( $\text{R}$ ) (Eq. 1). During darkness, the  $\text{NEP}_t$  values are attributable solely to respiration. Therefore, we summed the nighttime  $\text{NEP}_t$  values and divided by the period of darkness to get the rate of ecosystem  $\text{R}$  at night. We followed the convention of assuming daytime  $\text{R}$  is equal to nighttime  $\text{R}$  and averaged the  $\text{R}$  rates obtained for the night preceding and night after each daylight period to estimate  $\text{R}$  for each day. Daily values of GPP were calculated by summing the interval measurement of  $\text{NEP}_t$  for the daylight hours and adding daytime  $\text{R}_t$ . We aggregated daily estimates of GPP and  $\text{R}$  to a weekly scale following the convention established by Cole et al. (2000) and justified by Staehr and Sand-Jensen (2007). Also, the weekly scale was most fitting for inference because P samples were collected at a weekly time step.

We used estimates of GPP and ecosystem respiration ( $\text{R}_{\text{tot}}$ ) to estimate P demand and mineralization (assuming photosynthetic and respiratory quotients of 1.0). The assimilation of P into algal cells should occur at a rate proportional to the net primary production (NPP) observed in the system. NPP is equivalent to GPP ( $\text{mg O}_2 \text{m}^{-2} \text{d}^{-1}$ ) corrected for the amount of oxygen used in autotrophic respiration ( $\text{R}_a$ ) ( $\text{mg O}_2 \text{m}^{-2} \text{d}^{-1}$ ). Thus, to determine the rate of NPP and convert the oxygen-based estimate to carbon we used the following equation:

$$\text{NPP} = 0.375 * (\text{GPP} - \text{R}_a) \quad (4)$$

Where:

NPP ( $\text{mmol C m}^{-2} \text{d}^{-1}$ ) is the rate of net primary production,  $\text{R}_a$  ( $\text{mg O}_2 \text{m}^{-2} \text{d}^{-1}$ ) is the rate of autotrophic respiration, and 0.375 is the mass ratio of C to  $\text{O}_2$ .

Autotrophic respiration is difficult to determine, but has been quantified in a number of studies with values generally ranging from 35–60% of total community respiration (del Giorgio and Peters, 1993; Duarte and Cebrian, 1996, Dodds and Cole, 2007). As a nominal value we assumed that  $\text{R}_a$  would be equal to 50% of total community respiration.

In order to infer the P uptake rate ( $\text{P}_{\text{uptake}}$ ), we assumed that over the short period of the study the *in situ* C:P ratio of phytoplankton serves as an adequate estimate of the C:P ratio at which inorganic materials are assimilated during photosynthesis. Thus, from estimates of NPP ( $\text{mmol C m}^{-2} \text{d}^{-1}$ ) and seston molar C:P, we calculated rates of P uptake based on the following:

$$\text{P}_{\text{uptake}} = \text{NPP} * (1/(\text{C} : \text{P}_{\text{up}})) \quad (5)$$

Mineralization of P ( $\text{P}_{\text{min}}$ ) was assumed to occur under conditions of heterotrophic metabolic equilibrium (i.e. growth  $\sim$  loss). Bacterial biomass is relatively constant during this period of the summer in Lake Mendota (epilimnetic bacterial biomass ranged from 130.3–177.8  $\text{mg C m}^{-2}$  in 1979 and 84.7–167.0  $\text{mg C m}^{-2}$  in 1980; Pedros-Alio and Brock, 1982) and it is likely that overall heterotrophic growth rates are approximately balanced by loss. Under a scenario of zero net heterotrophic growth, we infer that the C:P of mineralization is equivalent to the C:P ratio of the substrate. Therefore, the C:P ratio of mineralization was assumed to be equal to the C:P ratio of whole epilimnetic seston. This value was multiplied by the proportion of total respiration due to heterotrophs ( $\text{R}_h$ ) ( $\text{mmol C m}^{-2} \text{d}^{-1}$ ) according to the following equation:

$$P_{\min} = R_h * (1 / (C : P_{\min})) \quad (6)$$

Where:

$$R_h = R_{\text{tot}} - R_a$$

### Estimating non-metabolic sources of phosphorus

In addition to quantifying the metabolic processing of P, we also assessed alternate SRP sources to the lake during the period when SRP was below detection. Estimates of external load were based on an approach used previously (Lathrop et al., 1998; Carpenter and Lathrop, 2008). There are four streams and two storm water inlets that enter Lake Mendota. Two streams (Pheasant Branch and Yahara River) and one storm water inflow (Spring Harbor) are continuously monitored for hydrologic and chemical inputs into the lake by the United States Geological Survey (USGS). Loading was determined for these three inlets and these data were used to infer loading from other inlets based on previous estimates of the relative load entering the lake from each source (Lathrop et al., 1998). Baseline P loads were measured as input of total P, but data were also available to determine the approximate proportion of P entering the lake in the soluble form. For most inflows (Six Mile Creek, Pheasant Branch, Spring Creek, Spring Harbor and Willow Creek), the proportion of P entering as SRP was 24–52% of TP and did not depend on discharge. However, for the largest inflow, the Yahara River, the proportion entering the lake as SRP differed systematically based on flow rates (SRP = 0.045 ( $\pm 0.024$ )  $\times$  TP when discharge  $< 5.7 \text{ m}^3 \text{ s}^{-1}$ ; SRP = 0.56 ( $\pm 0.028$ )  $\times$  TP when discharge  $> 5.7 \text{ m}^3 \text{ s}^{-1}$ ) due to a wide river estuary immediately upstream of the lake. Therefore, when discharge  $> 5.7 \text{ m}^3 \text{ s}^{-1}$ , the “high flow” proportion (0.523  $\times$  TP) of SRP was attributed to the input, while during “low flow” SRP input was equal to 0.186  $\times$  TP, based on flow-weighted mean SRP:TP ratio of all inflows. This rate was used to convert estimates of TP loading from all inflows to areal estimates of SRP loading. Similarly, we were interested in accounting for any significant loss of SRP to downstream systems through the outlet of the lake. The hydrologic outflow was multiplied by the mean SRP concentration of the epilimnion during the periods when SRP was detectable, however the total mass of SRP lost through this pathway was negligible (mean outflow  $< 0.0001 \text{ mg SRP m}^{-2} \text{ d}^{-1}$ ).

Entrainment of SRP into the epilimnion from lower strata of the lake was also considered. Entrainment events were identified using the high-resolution temperature profiles, and entrainment was defined as an increase in the maximum depth of the mixed layer by 0.5 m or more. When an additional mass of water

was incorporated into the epilimnion we quantified the associated SRP flux based on the volume of water incorporated and the mean SRP concentration of that water mass. This flux includes SRP derived from hypolimnetic and benthic remineralization.

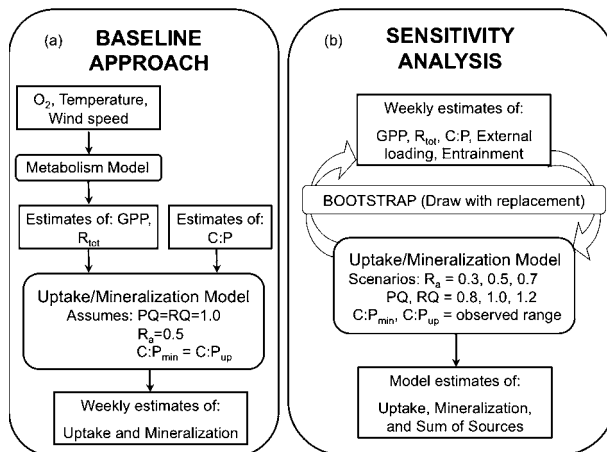
We also considered the input of P from rain water using total weekly precipitation, as measured at a weather station associated with the Dane County Truax airport, multiplied by a TP concentration of  $0.032 \text{ mg P L}^{-1}$ , an average used in previous budget calculations for the lake (Lathrop, 1979; Soranno et al., 1997). The mean estimate we derived ( $0.19 \text{ mg P m}^{-2} \text{ d}^{-1} \pm 0.26$ ) is likely an overestimate of the input of SRP because SRP makes up only a portion of total P input through precipitation.

Release of SRP from epilimnetic sediments in contact with the mixed layer was also considered as a source. The mean weekly depth of the mixed layer was used to calculate the area of the sediment surface that was in contact with the epilimnion, and this value was multiplied by an average SRP release rate for Lake Mendota of  $2.4 \text{ mg P m}^{-2} \text{ d}^{-1}$  (Stauffer, 1987; Soranno et al., 1997). These estimates amounted to a mean release rate of  $0.72 \text{ mg P m}^{-2} \text{ d}^{-1}$  ( $\pm 0.04$ ) for the sediments in contact with the epilimnion, as the sediment area in contact with the mixed layer is approximately 30% of total lake sediments. Average inputs from sediment release and from precipitation were consistently low, hence we focused on external loading and entrainment, which can be episodic and important during summer months.

### Sensitivity analysis

In order to assess all variables on a common temporal scale, the mean weekly values of each input variable (GPP,  $R_{\text{tot}}$ , C:P) were cast into the uptake/mineralization model to compare the relative magnitude of weekly SRP uptake and mineralization rates, and assess their magnitude in comparison to other sources of P (external load and entrainment) (Fig. 1a). Rather than rely solely on mean values to summarize the trends in P demand and supply, however, we explored a feasible range of values as input to the model through sensitivity analysis (Fig. 1b). The full suite of mean weekly values observed for each variable was incorporated into the sensitivity analysis through a bootstrapping procedure (Fig. 1b). The model was run for 2000 iterations by drawing values randomly with replacement from the observed dataset. Because GPP and  $R_{\text{tot}}$  were positively correlated (Pearson's correlation analysis,  $r = 0.83$ ,  $p = 0.002$ ), these two variables were drawn concurrently from the dataset, meanwhile C:P values were not correlated with GPP and  $R_{\text{tot}}$ , therefore the C:P ratio for uptake ( $C:P_{\text{up}}$ ) and mineralization ( $C:P_{\text{min}}$ ) were drawn independently

and randomly from the pool of observed weekly mean seston C:P ratios.



**Fig. 1.** Flow chart depicting the steps taken in the baseline model run and in the sensitivity analysis.

While we lack evidence that the C:P ratio of natural phytoplankton populations differs significantly from that of overall seston, one might think of scenarios of high detrital content or low phytoplankton biomass in which such a difference could occur. Also, while difference in the C:P ratio among components of the seston may be imperceptible based on current seston separation techniques, it is certainly possible that different trophic groups (bacteria versus zooplankton) are differentially using seston components in metabolic processes. Thus, there is likely to be an imbalance between the C:P ratio taken up by phytoplankton and the C:P ratio of substrate that is metabolized and subsequently mineralized during respiration. We explored the sensitivity of the model results to the influence of an imbalance between the C:P ratio of uptake and mineralization. Such exploration was based on the observed range in weekly C:P ratios (180.1–483.8), which was comparable to sestonic C:P ratios that had been previously documented for temperate lakes (range = 122–441) (Hecky et al., 1993; Dobberfuhl and Elser, 2000)

Sensitivity analyses were used to explore the full range of values that may feasibly be observed for the photosynthetic quotient (PQ), respiratory quotient (RQ) and  $R_a$ . PQ and RQ vary depending on the biochemical composition of the molecules produced or broken down during the metabolic process. Many researchers assume a baseline value of 1.0 for both PQ and RQ in aquatic ecosystems (del Giorgio and Peters, 1993; Hanson et al. 2003), and empirical estimates support this assumption (Bender et al., 1987), yet reported values may range from 0.8–1.2 (del Giorgio

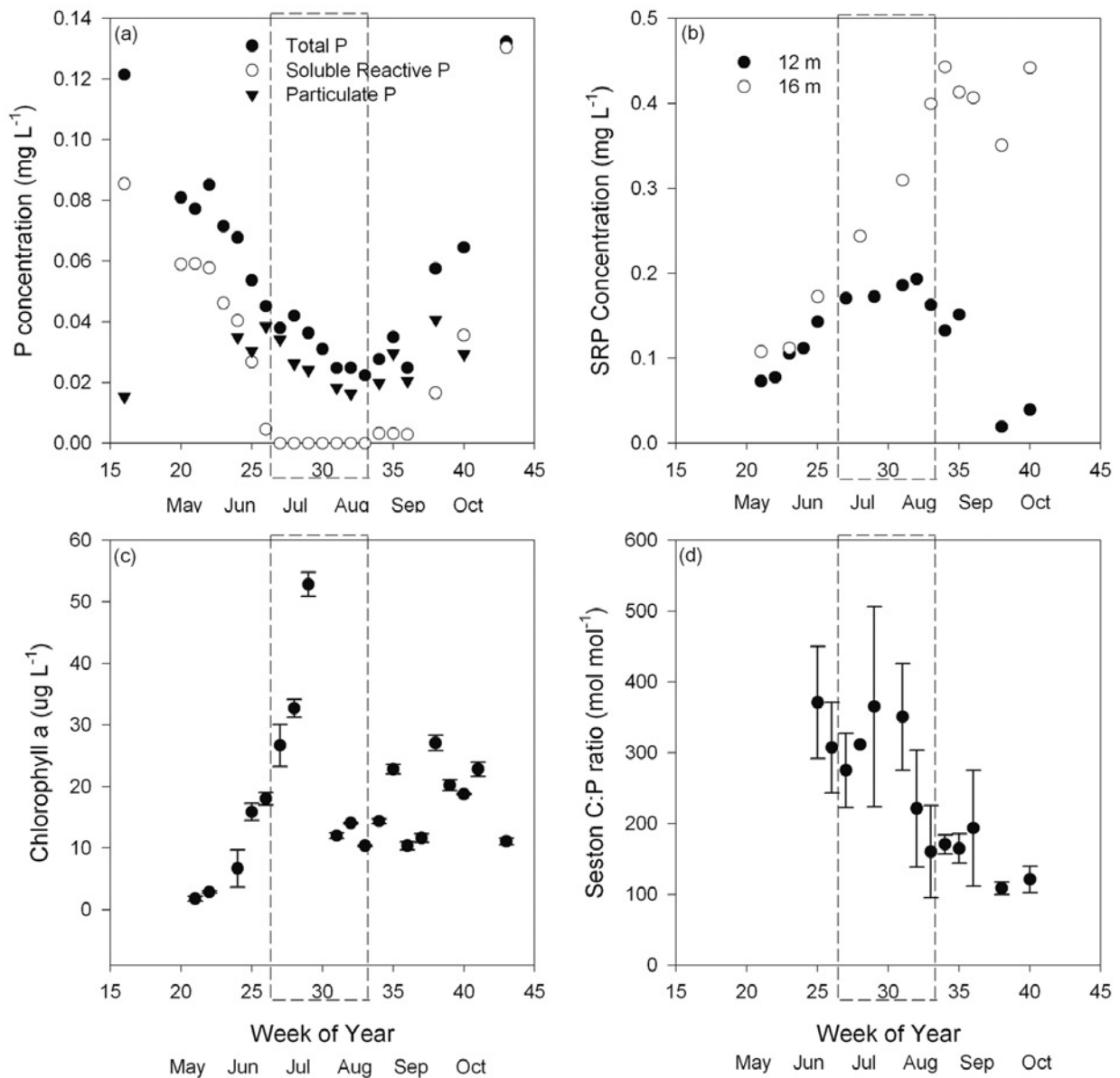
and Peters, 1993). We explored uncertainty in the quotients by running the model using a range of PQ and RQ values from 0.8–1.2.

Another variable with potentially high uncertainty was the percent of total community respiration ( $R_{tot}$ ) that could be attributed to autotrophs ( $R_a$ ) and heterotrophs ( $R_h$ ). Total respiration tends to increase with lake trophicity, and respiration by autotrophs becomes an increasing part of total respiration as lakes become more eutrophic (del Giorgio and Peters, 1993; Biddanda et al., 2001; Roberts and Howarth, 2006). A literature review by del Giorgio and Peters (1993) suggests that autotrophic respiration may contribute 35% in oligotrophic systems and over 60% in eutrophic systems. As a conservative estimate of the range expected in natural lakes, we used  $R_a$  values that ranged from 30–70% of total community respiration in the sensitivity analysis.

## Results

P concentrations followed a seasonal trend typical for Lake Mendota (Stauffer, 1987; North Temperate Lakes Long Term Ecological Research program (<http://lter.limnology.wisc.edu>)). Mean epilimnetic SRP concentrations declined through the spring and reached the limit of analytical detection by week 27 (1 July 2007–7 July 2007) (Fig. 2a). During spring and early summer (week 16–25, 15 April 2007–23 June 2007), SRP and TP concentrations were correlated ( $r = 0.97$ ,  $p < 0.001$ ). In contrast, TP was closely related to the particulate P concentration after week 25 (24 June 2007–8 September 2007) ( $r = 0.75$ ,  $p = 0.008$ ), while SRP fell below detection. Following week 25 (24 June 2007), the majority of P mass in the epilimnion was bound within the particulate pool ( $TP = 0.032 \pm 0.002 \text{ mg P L}^{-1}$ , Particulate P =  $0.025 \pm 0.002 \text{ mg P L}^{-1}$ ). At the same time, the SRP concentration in the bottom waters of the lake increased (Fig. 2b). Our analysis was focused on the period of the summer between weeks 26 and 34 (24 June 2007–25 August 2007) when the lake was strongly stratified and SRP concentrations were below the limit of detection ( $< 0.003 \text{ mg L}^{-1}$ ).

Despite a decline in biologically available P, phytoplankton biomass remained relatively high. Chlorophyll *a* concentration reached a peak in week 29 (15 July 2007–21 July 2007) ( $52.9 \pm 1.9 \text{ ug L}^{-1}$ ), and thereafter centered around a mean of  $16.8 \pm 5.8 \text{ ug L}^{-1}$  for the remainder of the summer (Fig. 2c). The molar C:P ratio of seston in the epilimnion was generally high early in the summer (275.1–370.9) (20 June 2008–4 August 2008) and was lower during the later part of the summer (108.7–221.2) (5 August 2008–6

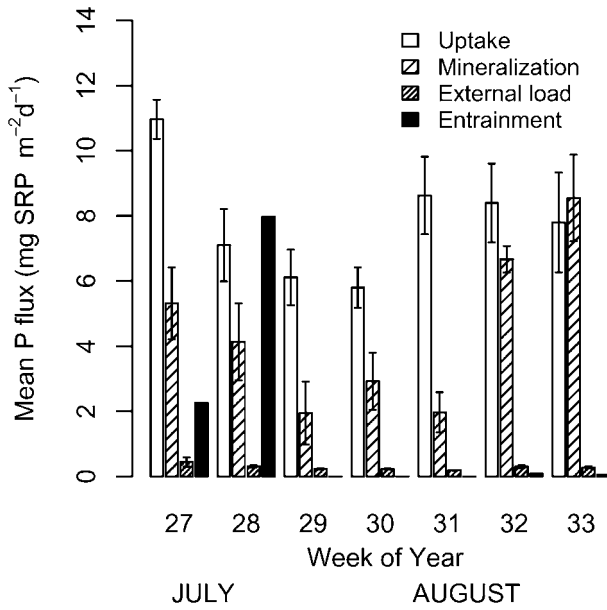


**Fig. 2.** Summer trends in epilimnetic phosphorus concentration (a), selected hypolimnetic soluble reactive phosphorus (SRP) concentration (b), phytoplankton biomass (as measured by chlorophyll *a* concentration) (c) and seston C:P ratio (d). Values are mean estimates for each week, error bars represent one standard deviation. The dotted box delineates the focal period for this study.

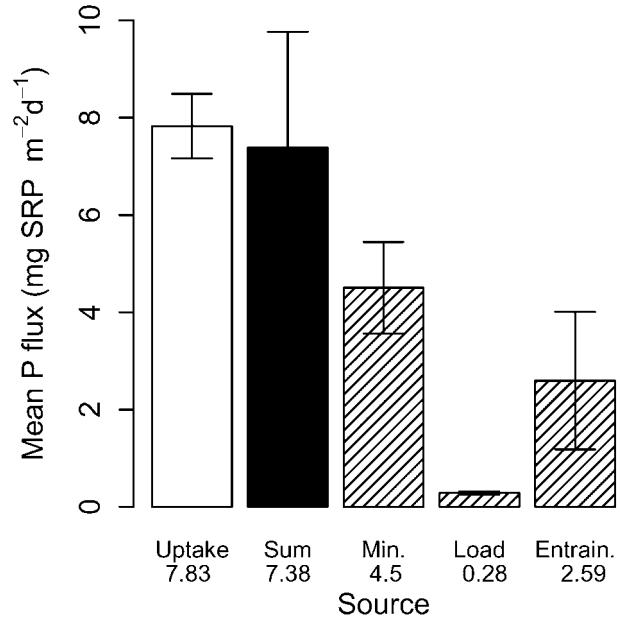
October 2008) (Fig. 2d). Gross primary production (GPP) ranged from 0.86–2.30, with an average of 1.52  $\text{g C m}^{-2} \text{d}^{-1}$ . Total community respiration ( $R_{\text{tot}}$ ) ranged from 0.32–1.56, with an average of 1.08  $\text{g C m}^{-2} \text{d}^{-1}$ .

TN:TP ratios in the epilimnion ranging from 25.2 to 84.7 (on molar basis) suggested that phosphorus was limiting. Similarly, DIN:SRP ratios in the metalimnion (8–10 m) during the period of interest ranged from 15.4–30.7, suggesting that water entering the epilimnion through entrainment would be relatively rich in bioavailable N (based on Redfield's N:P ratio of 16:1). Thus, P was considered the limiting nutrient during our study.

During the period that P was below the detection limit (24 June 2007–25 August 2007), weekly rates of SRP uptake were relatively constant (Fig. 3). While uptake rates were relatively low in weeks 28–30, rates were not significantly different among weeks 28–33. In most weeks, uptake exceeded the amount of P supplied by any single source, with the exception of weeks 28 and 33 (8 July 2007–14 July 2007 and 12 August 2007–18 August 2007). Rates of P supply via mineralization were more variable than rates of uptake during the period of interest. Mineralization ranged from 23–109% of uptake, thus meeting an average of 57% of documented phytoplankton P



**Fig. 3.** Mean weekly estimate of rates of soluble reactive phosphorus (SRP) uptake, mineralization, external loading and entrainment for the period when SRP was below the limit of detection during summer 2007. Error bars represent one standard error around the mean and account for the daily variation in estimates of GPP and  $R_{tot}$ .



**Fig. 4.** Overall mean rate of phosphorus uptake compared to estimated magnitudes of possible sources of phosphorus. Sum Sources represents the sum of all documented sources of SRP input during the P-deficient stratified period. The components of Sum Sources (mineralization, external load, and entrainment) are shown to the right. Error bars represent one standard error around the mean ( $n = 7$  weeks). Input of SRP from precipitation and epilimnetic sediment release were less than  $1 \text{ mg SRP m}^{-2} \text{ d}^{-1}$  and are not shown here.

demand. Mineralization represented a roughly consistent source of SRP to phytoplankton throughout the summer (mean =  $4.5 \pm 0.94 \text{ mg SRP m}^{-2} \text{ d}^{-1}$ ), while entrainment (mean =  $2.59 \pm 1.41 \text{ mg SRP m}^{-2} \text{ d}^{-1}$ ) occurred during irregular pulses. During such events, the SRP flux via entrainment was comparable to observed rates of mineralization. P loading from external sources was consistently low during this mid-summer period and did not satisfy a significant portion of phytoplankton P demand ( $0.28 \pm 0.03 \text{ mg SRP m}^{-2} \text{ d}^{-1}$ , 3.5% of demand).

These trends taken together showed that on average the mean daily uptake rate ( $7.83 \pm 0.66 \text{ mg SRP m}^{-2} \text{ d}^{-1}$ ) was met by the sum of documented sources ( $7.38 \pm 2.38 \text{ mg SRP m}^{-2} \text{ d}^{-1}$ ) (t-test,  $p = 0.39$ ); note that these two groups are independent over this period so a t-test was appropriate (Fig. 4). However, no single source of P was sufficient to explain the variation in P uptake. The mean rate of mineralization was not significantly different than that of entrainment (t-test,  $p = 0.41$ ).

**Sensitivity analysis**

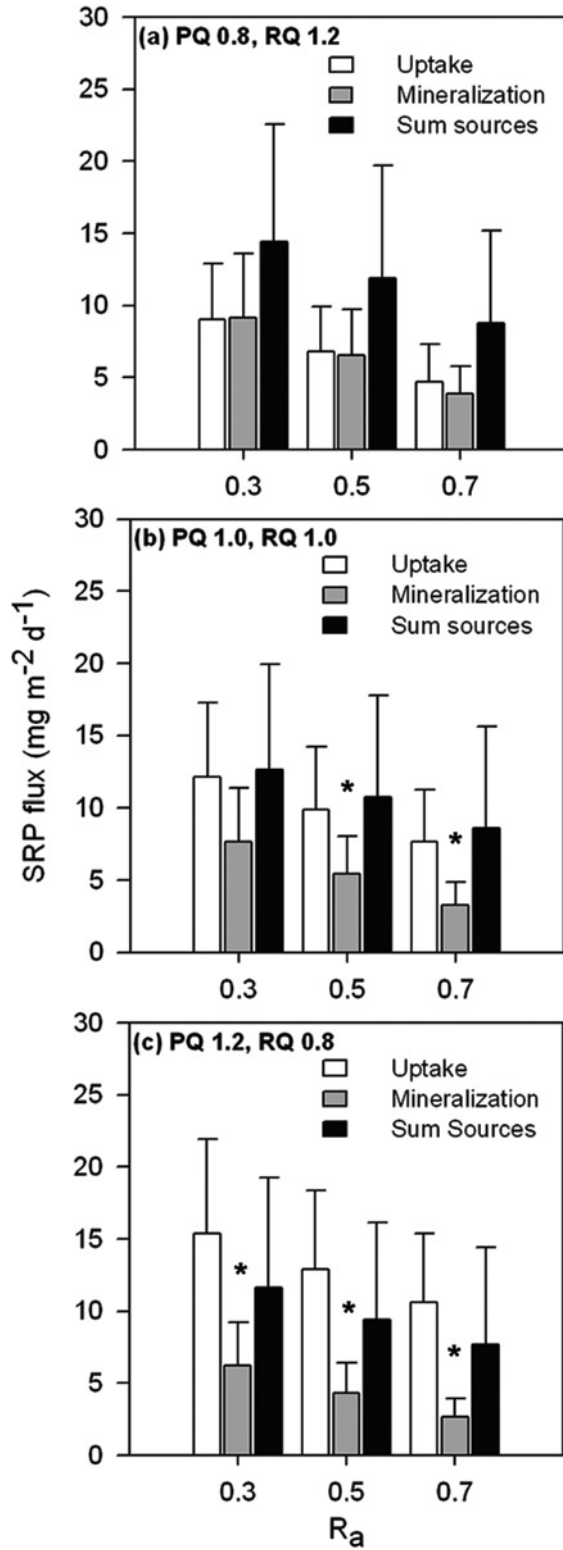
Model iterations using combinations of low, nominal and high values for PQ, RQ and  $R_a$  (Fig. 1b) demonstrated that the rate of uptake and the sum of all sources of P were not significantly different (by t-test) in any of the model scenarios (Fig. 5). The large error bars around the sum of sources in figure 5,

representing the standard deviation around the mean, can be partly attributed to variation in estimates of entrainment and external loading. External loading and entrainment estimates were independent of the metabolic parameters of the model, thus the effect of changes in model parameters may be best explored by comparing the differences between P uptake and mineralization.

In comparing P uptake to mineralization alone, high PQ/low RQ scenarios showed that uptake was significantly greater than mineralization in all cases (Fig. 5c). Given nominal PQ/RQ values (PQ = RQ = 1.0), we found the relationship between uptake and mineralization was mediated by the value of  $R_a$ . When autotrophic respiration ( $R_a$ ) was low, uptake was more likely to be balanced by mineralization (Fig. 5b). When PQ was low (0.8) and RQ (1.0) was high, however, model results showed that mineralization could feasibly meet the demands of phytoplankton P uptake under any  $R_a$  scenario. Model results were most clearly affected by changes in the relative values of PQ and RQ, while model results were less sensitive to changes in the proportion of total community respiration attributed to autotrophs ( $R_a$ ).

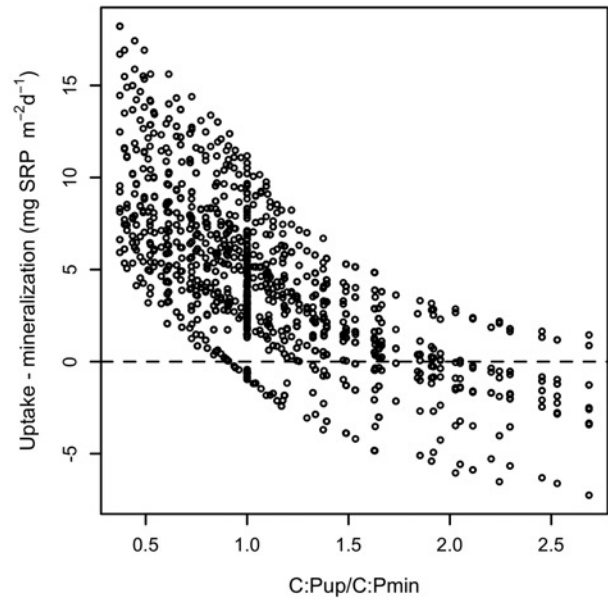
Given the difficulty in measuring C:P ratios of different sestonic components, we explored the sensi-





**Fig. 5.** Results of sensitivity analysis based on alternate values (0.8, 1.0, 1.2) for the photosynthetic quotient (PQ) and the respiratory quotient (RQ) as well as alternate values for the percent of total respiration that is autotrophic ( $R_a = (0.3, 0.5 \text{ or } 0.7) * R_{tot}$ ). Error bars represent the standard deviation around the mean. Asterisks (\*) indicate a significant difference between mean uptake and mineralization (t-test,  $\alpha = 0.05$ ). The difference between uptake and the sum of sources was not significant in any of the scenarios tested (t-test,  $\alpha = 0.05$ ).

tivity of model output to the C:P ratio used in calculating uptake and mineralization rates. We found the relative C:P ratio ( $C:P_{up}/C:P_{min}$ ) was a critical factor in determining the magnitude of the difference between uptake and mineralization rates inferred from the model (Fig. 6). Results of all model iterations (2000) are presented in figure 6 as the difference between uptake and mineralization. For this sensitivity analysis, GPP,  $R_{tot}$ , external loading and entrainment were integrated into the model as a dependent dataset, while the C:P ratios for uptake and mineralization were randomly and independently chosen from the entire pool of observed C:P ratios of seston. Thus, the results presented in figure 6 demonstrate the effect of our assumption that the C:P ratios of uptake and mineralization are equal. As long as  $C:P_{up}$  was smaller than  $C:P_{min}$  ( $C:P_{up}/C:P_{min} < 1.0$ ) the rate of P uptake exceeded that of P supplied through mineralization. We found that if  $C:P_{min}$  and  $C:P_{up}$  are significantly different in nature (which is possible, but hard to measure), then these differences could affect our estimates of the relative balance between  $P_{uptake}$  and  $P_{min}$ .



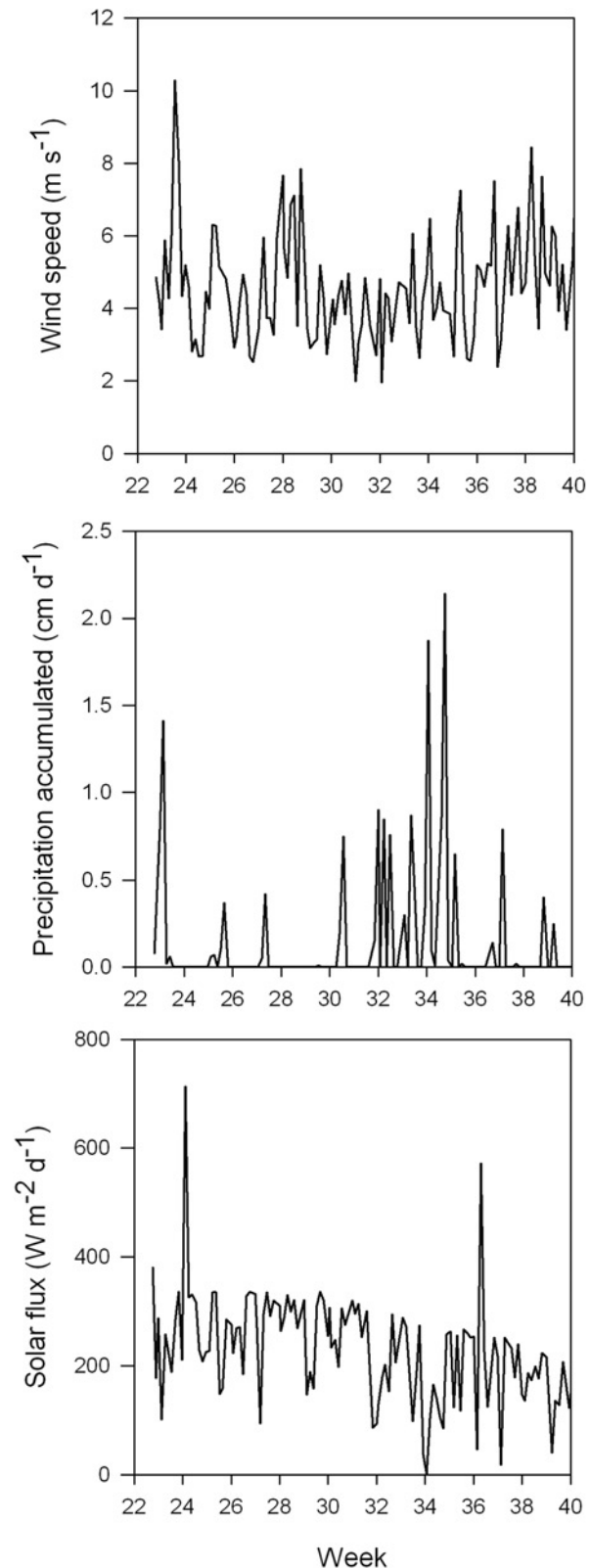
**Fig. 6.** The relationship between the relative difference between uptake and mineralization and the ratio of C:P for uptake relative to mineralization. When the relative C:P ratio is close to one, as was assumed in the baseline portion of our results, the difference between uptake and mineralization is slightly positive. When the C:P ratio of mineralization is less than C:P of uptake ( $C:P_{up}/C:P_{min} > 1.0$ ), then the difference between uptake and mineralization approaches a mean of zero. However, when C:P of mineralization is greater than uptake ( $C:P_{up}/C:P_{min} < 1.0$ ), the difference between uptake and mineralization is always positive.

We were primarily interested in how P demand was met during periods of low P availability, yet we also had data to investigate patterns in P uptake and supply during the later period of the summer when SRP was available. An expanded time series showed the influence of a large storm with significant precipitation (and runoff, not shown) that occurred between week 33 and week 34 (Fig. 7). This storm event resulted in input of SRP from both external loading and entrainment (Fig. 8). Likely in response, mean uptake rates increased during week 35 (Fig. 8). During weeks 37 and 38, progressive deepening of the mixed layer at the end of the summer season resulted in significant entrainment and subsequent increases in the observed uptake and mineralization rates during weeks 38, 39 and 40.

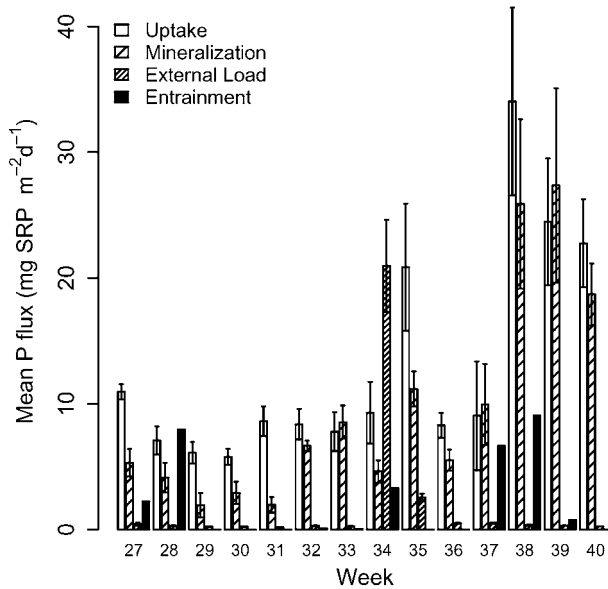
When these results were averaged over the extended 14-week period, mineralization and uptake became statistically indistinguishable (t-test,  $p = 0.29$ ) (Fig. 9). This was largely due to higher variation in uptake and mineralization represented in the larger data set (CV increased from 0.46 to 0.64 and from 0.55 to 0.91 for uptake and mineralization, respectively). The sum of sources, though not statistically different from uptake, indicated that P was supplied in excess of what was needed by primary producers. The availability of SRP at the end of this period was also apparent in the epilimnetic P trends presented in figure 2a.

## Discussion

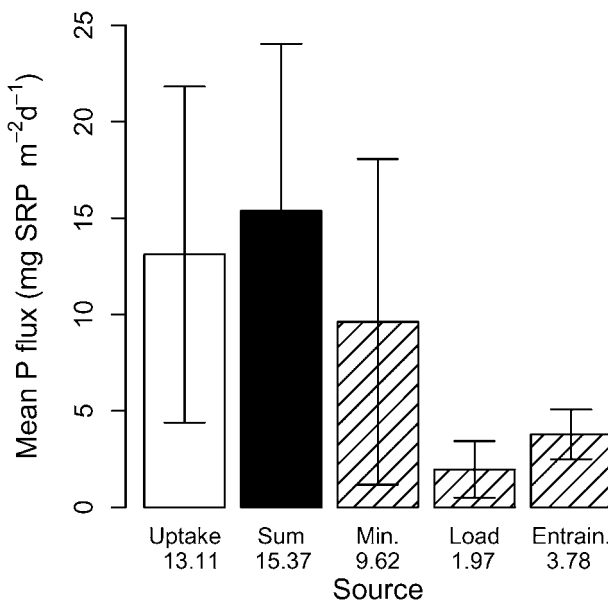
We explored the mechanics and magnitude of P demand and supply using a combination of empirical data and modeling. Results indicated that the amount of SRP used by primary producers during the period of low SRP availability was predominantly supplied via biotic mineralization. P demand, however, could not be met by mineralization alone and the relative balance among P sources (mineralization, external loading and entrainment) varied over the summer. Entrainment was also an important pathway of P transport, and the episodic input of P occurred during a period of higher than average wind speeds (week 28). While these results were generally insensitive to model conditions (i.e., independent of changes in  $R_a$ , PQ and RQ), the balance between P uptake and mineralization was mediated by the relative C:P ratios of uptake and mineralization. Thus, our results, which showed that patterns of P demand and supply were dictated by the relative C:P ratio of producers and consumers, support the importance of stoichiometric relationships in foodweb interactions and ecosystem



**Fig. 7.** Meteorological data for the summer of 2007. All data were monitored at the Atmospheric and Oceanic Sciences Building at University of Wisconsin – Madison, located 0.8 km south of Lake Mendota. Data available at: <http://rig.ssec.wisc.edu/>.



**Fig. 8.** Mean weekly estimate of rates of soluble reactive phosphorus (SRP) uptake, mineralization, external loading and entrainment for an extended period during summer 2007 ( $n=14$  weeks). Error bars represent one standard error around the mean and account for the daily variation in estimates of GPP and  $R_{\text{tot}}$ .



**Fig. 9.** Overall mean rate of phosphorus uptake compared to estimated magnitudes of possible sources of phosphorus. Sum Sources represents the sum of all documented sources of SRP. The components of Sum Sources (mineralization, external load, and entrainment) are shown to the right. Error bars represent one standard error around the mean. ( $n = 14$  weeks). Input of SRP from precipitation and epilimnetic sediment release were  $< 1$  mg SRP  $\text{m}^{-2} \text{d}^{-1}$  and are not shown here.

processes (Elser et al., 1988; Elser et al., 1995; Dobberfuhl and Elser, 2000).

Our finding that entrainment was necessary to account for observed uptake suggests that entrain-

ment can be important not only in annual budgets but also as a contributor to meeting P demand at a weekly scale during the stratified P-deficient season. Thus, part of the variation observed in patterns of gross primary production (GPP) and net ecosystem production (NEP) may be attributed to exchange of material across the thermocline (Staeher and Sand-Jensen, 2007). Also, entrainment occurred during a period of higher than average wind speeds ( $5.3 \text{ m s}^{-1}$  during week 27 and 28, compared to June-September average of  $4.5 \text{ m s}^{-1}$ ), but this entrainment event was independent of other storm indicators (precipitation, changes in solar flux). Such observations may help explain why the relationship between phytoplankton blooms and storm events are exceedingly noisy (Soranno, 1997).

Interestingly, peaks in phytoplankton biomass occurred approximately one week after significant P fluxes from entrainment and external loading (weeks 29, 35 and 38). The initial chlorophyll *a* peak (week 29), however, occurred soon after depleted P conditions were apparent. It is possible that the bloom we witnessed during week 29 was due to growth of the phytoplankton population that began under SRP replete conditions, possibly sustained by luxury uptake. Given our observed average uptake rate of  $10 \text{ mg m}^{-2} \text{ d}^{-1}$  during week 26 and 27 and a mean mineralization of approximately  $5 \text{ mg m}^{-2} \text{ d}^{-1}$ , we can come to a net P demand of approximately  $5 \text{ mg m}^{-2} \text{ d}^{-1}$ , which translates to a daily uptake rate of  $196 \text{ kg d}^{-1}$ . Meanwhile, the observed epilimnetic SRP concentration in week 26 was  $0.0046 \text{ mg L}^{-1}$ , equaling an estimated 955 kg of SRP in the epilimnion. Given the conditions in week 26, we would expect the SRP available in the epilimnion to have been taken up within approximately 5 days. This uptake may also have included luxury uptake under nutrient replete conditions. Given that phytoplankton may sustain three or four cell-doublings without taking up additional phosphorus (Reynolds 2006), a growth rate of  $0.2 \text{ d}^{-1}$  (or a doubling time of  $\sim 3.5$  days) may allow phytoplankton to persist for an additional 14 days on luxury uptake. Based on the above assumptions, we can conclude that a bloom beginning in week 26 may have grown and persisted over a maximum of 19 days without a new source of phosphorus.

Later peaks in chlorophyll *a* concentration (weeks 35 and 38), though, demonstrate that entrainment and external loading can induce peaks in phytoplankton biomass that are accompanied by increases in the rates of uptake. It seems that mineralization generally provides sufficient P to sustain production during periods of low P availability, but that an external pulse is required to induce higher-than-average chlorophyll *a* concentrations. Our results corroborate other work

showing that physical processes, including entrainment, are linked to external drivers (meteorological variation, evaporative cooling, temperature of inflow) in complex ways and can play an important role in nutrient cycling and ecosystem processes (MacIntyre et al., 2002; Fragoso et al., 2008). Our approach based on free-water metabolism estimates can be used to assess the relative importance of biotic recycling, entrainment and external loading among systems, and to reduce uncertainties in the relationship between P supplies and phytoplankton biomass during summer months.

We explored uncertainties in the model using alternative sets of model conditions (i.e. a range in values for  $R_a$ , PQ and RQ); in all cases inferred P uptake was equal to the sum of sources to the epilimnion during the period of low P availability. Under scenarios of unbalanced photosynthetic and respiratory quotients (PQ = 0.8, RQ = 1.2, all  $R_a$ ) or low  $R_a$  (PQ = 1.0, RQ = 1.0,  $R_a$  = 0.3), mineralization alone was sufficient to account for total P demand, these model scenarios, however, are relatively unlikely to occur. Theoretical calculations and empirical observations suggest that PQ values are likely to be > 1.0 (Ryther, 1956; Williams and Robertson, 1991). The PQ was estimated to be 1.34 for a typical algal cell comprised of 40% protein, 40% carbohydrate, 15% lipid, and 5% nucleic acid (Williams and Robertson, 1991). Meanwhile, best estimates of RQ, while variable, tend to be < 1.0 (Hutchinson and Edmondson, 1957; Lampert and Bohrer, 1984). Thus the conditions represented by the PQ = 0.8 and RQ = 1.2 scenario are unlikely in aquatic ecosystems, while the conditions of our PQ = 1.2 and RQ = 0.8 (Fig. 5c) scenario are more plausible. The results of our sensitivity analysis demonstrate that different values of  $R_a$  may affect the magnitude of uptake and mineralization, yet the difference between uptake and mineralization is relatively insensitive to changes in the proportion of total community respiration attributed to autotrophs ( $R_a$ ) versus heterotrophs ( $R_h$ ). When PQ and RQ are unbalanced, the pattern of uptake and mineralization will be dictated by the PQ:RQ relationship rather than the balance between autotrophic and heterotrophic respiration.

While model output was generally insensitive to changes in PQ, RQ, and  $R_a$ , the relative balance between uptake and mineralization was sensitive to the stoichiometric relationship between primary producers and organisms contributing to heterotrophic mineralization. Transfer of carbon and nutrients through foodwebs is mediated by stoichiometric relationships among foodweb components and trophic patterns may be structured by the stoichiometry of primary producers at the base of the foodweb (Sternner and Elser, 2002).

There is variability in the C:N:P ratios of seston in lakes, and many organisms at the base of the foodweb have some plasticity in maintenance of tissue C:N:P ratios (Hecky et al., 1993; Sternner and Elser, 2002; Diehl et al., 2005). While we were not able to empirically distinguish among sestonic foodweb components (bacteria, phytoplankton, micrograzers), we were able to explore the influence of variation in C:P ratios within the modeling framework.

Our model results help constrain expectations of the patterns of P supply and demand likely in natural systems. Given the relationship presented in figure 6 and previously published stoichiometric relationships, we argue that P demand is likely to outweigh P supplied via mineralization alone. There is no conclusive evidence that the C:P ratio of seston, the component available as substrate for mineralization, should differ from the C:P ratio of phytoplankton, the component that represents the ratio of uptake (Healey and Hendzel, 1980; Sternner and Elser, 2002). Yet, there is evidence that mineralization of organic matter by zooplankton can exacerbate P limitation due to excretion at relatively high C:P ratios (Elser et al., 1988; Sternner, 1990). Also, measured C:P ratios of bacteria and zooplankton tend to be lower than those documented for whole-water seston (Goldman et al., 1987; Fagerbakke et al., 1996; Dobberfuhl and Elser, 2000; Hochstadter, 2000). As such, retention of P in bacterial and zooplankton pools likely results in C:P ratios of mineralization that are greater than the C:P ratios of primary producers (Elser et al., 1995), and thus the relative ratio of  $C:P_{up}:C:P_{min}$  is likely to be < 1.0. Under such conditions, our model results indicate that natural lake ecosystems are likely to cluster within the upper, left-hand portion of figure 6, and it may not be possible to explain the mid-summer rate of primary production by P supplied through mineralization alone.

Bacteria have been highlighted as dominant decomposers and mineralizers of organic material, yet they may also compete with phytoplankton for uptake of bioavailable P (Rigler, 1956; Currie and Kalff, 1984; Cotner and Wetzel, 1992). If bacteria are significant contributors to rates of heterotrophic respiration and these bacterial populations are net consumers of P, then our model would underestimate the true ecosystem demand for bioavailable P, and overestimate the rate of mineralization. While we do not have data to address this possibility directly, previous estimates of bacterial populations in Lake Mendota during late summer demonstrate relative consistency in bacterial biomass (Pedros-Alio and Brock, 1982). Given this consistency, it is likely that growth in the bacterial population is closely mirrored by loss and that the net population growth and rate of P uptake are close to zero. Also, Cotner and Wetzel (1992) suggest that P

taken up by bacteria does not represent net uptake because they generally lack the capacity for P storage, thus bacterial uptake is comparable to the amount lost from internal pools. In light of the small influence of heterotrophic  $R_h$  versus autotrophic respiration  $R_a$  on model results, we do not expect changes in our assumptions about the influence of bacteria on uptake and mineralization to have large effects on the results of the analysis.

Our approach and findings contribute to understanding of the relationship between primary production and nutrient cycling in aquatic ecosystems and complement other studies conducted using experimental incubation and elemental tracers. Using an approach that relies on free-water estimates, we can explore ecosystem processes in natural ecosystems, instead of under laboratory, light/dark bottle, or microcosm conditions. Our work integrates modeling approaches and monitoring data enhanced by tools for high-frequency data acquisition that can allow monitoring of processes at finer temporal resolution than previously possible. The assumptions of our approach are different from those inherent in previous analyses and therefore, this approach could be used to corroborate or question results from other modeling, experimental and observational approaches. Also, our work highlights an application for the emerging area of research related to whole-ecosystem metabolism (Hanson et al., 2003; Staehr and Sand-Jensen, 2007). As our estimates of whole-ecosystem metabolism improve, we stand to gain a better understanding of ecosystem processes like nutrient cycling. Further application and refinement of this approach could also enhance models that assess water quality and predict phytoplankton biomass through integrated representation of biological and physical processes (Hamilton and Schladow, 1997; Fragoso et al., 2008).

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