

The Changing State of N: P: Si Stoichiometry in Ganga River: Possible Implications for Production and Fate of Phytoplankton Biomass

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Abstract

Nutrient loading to surface waters has increased over recent decades while silicon loading has remained relatively constant or declined. A shift in N: P: Si stoichiometric ratios due to anthropogenic influences may become a feature of changing biogeochemistry in surface waters. We studied the changing state of NO_3^- , NH_4^+ , PO_4^{3-} , Si^{4+} , dissolved organic carbon (DOC), chlorophyll *a* (Chl *a*) biomass and gross primary productivity (GPP) along a 35 km long stretch of Ganga River at Varanasi (India). Concentrations of NO_3^- , NH_4^+ , PO_4^{3-} increased downstream characterized by anthropogenic influences, while silica showed an opposite trend. On a seasonal scale, unlike other nutrients, Si concentration was lowest in winter. The N: P stoichiometry showed a declining trend downstream indicating N limiting condition. The Si: N and Si: P ratios also showed deviation from classical Redfield ratios and indicated Si limitation as influenced by excessive N and P inputs. Chl *a* biomass and GPP followed a trend similar to nutrients. This relationship was also evident in DOC. Statistically significant differences in Chl *a*

biomass and GPP as well as in N: P: Si and C: Chl *a* ratios indicated the changing state of stoichiometric coupling of essential elements with possible implication on ecological status of Ganga River.

Introduction

In recent years an unprecedented increase in human population coupled with economic growth has dramatically altered the stoichiometric coupling of essential elements and in turn, the ecosystem functioning and climate change drivers (Gruber and Gallo-way, 2008; Pandey *et al.*, 2014). A specific stoichiometric ratio of N, P and Si, often called the Redfield ratio (N: P: Si: 16: 1: 16), is important for balanced growth of phytoplankton (Turner *et al.*, 2003). A deviation from this ratio associated with the deviation in the level of particular nutrients influence phytoplankton growth, community composition and quality of food for consumers (Turner, 2002) and consequently, has potential effect on food web structure (Elser *et al.*, 2000a; Turner, 2002; Dodds *et al.*, 2004).

The concentration of nutrients in rivers de-

pend on their loading from the catchment as influenced by catchment characteristics, such as human activities, climate change, biogeochemistry and the river's own assimilation potential. Human activities have potentially influenced the biogeochemical cycles of many elements, specifically carbon (C), nitrogen (N) and phosphorus (P) (Carcaco, 1993; Turner *et al.*, 2003; Howarth, 2008). In aquatic ecosystems, the turnover of N and P is relatively higher (Downing and McCauley, 1992; Downing, 1997), while the cycle of silica is relatively slower and stable (Sommer, 1988). The concentration of dissolved silica is largely controlled by runoff volume. The theory of resource competition among algal communities, consumer driven nutrient recycling and food chain dynamics based on the stoichiometric ratios of N: P: Si suggests a long term shift in trophic cascades and ecosystem resilience when such stoichiometric ratios are skewed in favour of one element relative to other (Teubner and Dokulil, 2002; Elser *et al.*, 2009). For instance, in phytoplankton assemblages, diazotrophic cyanobacterial dominance declines when nutrient supply leads to higher N: P ratio (Guildford and Robert, 2000; Elser *et al.*, 2000b). Similarly, a Si: N ratio below 1: 1 leads to reduced proportion of diatoms in phytoplankton communities and, by implication, a shift in higher trophic level structure. Although diatoms support herbivorous zooplankton–coupled fish production, they reduce the hatching success of copepod eggs (Kristiansen and Hoell, 2002). Changes in the Chl *a*: N: P ratio reflect a shift in phytoplankton production as a function of N and P. The turnover of pelagic phytoplankton regulates the dissolved organic carbon (DOC) and, consequently, the microbial loop in aquatic habitats. The overall DOC in receiving waters depends on the coupled effect of allochthonous input and autochthonous C-pool. The proportion of these two sources of C however, regulates the nutritional status of detritivores (Elser *et*

al., 2000b). Most of the studies so far on N: P: Si stoichiometry have focused mainly on oceans and lake ecosystems (Granelil *et al.*, 1999; Turner *et al.*, 1998; Kristiansen and Hoell, 2002). Data on the changing state of such stoichiometric relationships especially in major rivers of India are very scarce (Pandey *et al.*, 2013; 2014).

The present study was an effort to quantify the changing state of the N: P: Si stoichiometric ratio to explore possible implications on the ecology of Ganga River, the second largest river in the world, as far as the amount of water discharge is concerned. The study addresses emerging trends in phytoplankton nutrient limitation and associated shift in Chl *a* biomass and productivity in the study river stretch. The major goal was to test the hypothesis that the changing state of the N: P: Si stoichiometry of the Ganga River in response to anthropogenic activities would lead to a shift in phytoplankton production in the river.

Materials and Methods

Study Area

The present study was conducted from March 2013 to February 2014 at 3 selected stations along a 35 km long stretch of the Ganga River at Varanasi (25°18' N lat and 83°1' E long). Site selection was based on sub-catchment characteristics, land use pattern, and anthropogenic disturbances and nutrient input. The climate of the region is tropical with distinct seasonality. The year can be divided into a hot and dry summer (March-June), a humid rainy season (July-October) and a cold winter season (Nov-February). The mean annual rainfall varied between 870-1130 mm, relative humidity between 27 and 83% (summer) and 58 and 99% (rainy season). More than 90% of the annual rainfall occurs during rainy season. During summer day temperature varied between 29 to 46°C and during winter night temperature at rare occasions drops below

4°C. The soil is alluvial fluvisol. The Ganga River is about 2,525 km long and its basin area in India is about 8, 61,404 km². The Ganga basin is surrounded by Himalaya from north and Vindhyas from south. The river system covers cool upland streams and warm water stretches together with deltaic habitats. Due to availability of water, fertile soil and suitable landscape, the Ganges basin is the most heavily populated river basin in the world. Water of this sacred river in India is used for drinking, clothes washing, bathing of domestic animals and irrigation.

The river receives pollutants from sewage, cremation of dead bodies, agricultural and urban runoff, atmospheric deposition and land use change. Station 1 was relatively natural and Station 3 was the most human disturbed. Among the selected stations, three sites (urban side, mid stream and offsite) were selected at each station. The samples were taken in replicate (n=3), in presterilized acid rinsed plastic containers, directly below the surface (15-30 cm depth) at monthly interval. The distance between replication sampling was about 50 m.

Measurements

The experimental design consisted of two tiers of study that include water chemistry and phytoplankton biomass. A total of 108 analyses were carried out (12 variables in 9 samples). The chemicals used for the analysis were Merck analytical grade.

Water Chemistry

Nitrate and ammonia together comprises dissolved inorganic nitrogen (DIN). Nitrate-N was quantified using brucine sulphanilic acid method (Voghe, 1971) and ammonia-N using Nessler's reagent (Maiti, 2001). Dissolved reactive phosphorous was quantified as orthophosphate using ammonium molybdate method (Mackereth, 1963). Dissolved reactive silica was measured following Dienert and Wandenbulke's (1923) method. Dissolved organic carbon (DOC)

was estimated using KMnO₄ digestion procedure (Michel, 1984). For this purpose, a known volume of filtered water was mixed with acidified N/80 potassium paramagnet and incubated at 37°C. Organic carbon was estimated by titrating to quantify oxygen after 4 hours of incubation (APHA, 1998).

Phytoplankton Production

Phytoplankton biomass measured in terms of chlorophyll *a* (chlorophyll *a* comprises about 1.5% of algal dry organic matter (APHA, 1998). For this purpose, water samples collected in acid rinsed plastic containers from each replication point, from directly below the surface (15-30 cm depth) were filtered by Whatman No. 42. From the filter paper the sample was extracted using acetone extraction procedure (Maiti 2001) and measured spectrophotometrically. The primary productivity was measured by light and dark bottle method (APHA, 1998).

Statistical Analysis

Significant effect of site and time was tested using analysis of variance (ANOVA). The study considered a well defined protocol with balanced experimental/sample collection designs which validate the use of ANOVA for the analysis. To give an indication of the uncertainty of the mean, arithmetic means are accompanied by $\pm 1SE$. The Mann-Kendall test and Sen's slope estimator (XLSTAT 2014) were used for detecting the trend direction and magnitude of change in seasonal data sets. Principal components analysis (PCA) was used to evaluate the overall spatial and temporal profile of nutrients, OC, productivity for the study period. SPSS package (version 16) was used for statistical analysis.

Results and Discussion

The concentration of nutrients increased downstream, and there was an over 1.4 fold increase in NO₃⁻, a 1.2 fold increase in NH₄⁺ and an over 3.4 fold increase in PO₄³⁻ at Site

3 (Rjht) compared to Site 1 (Adpr) (Figure 1). Dissolved organic carbon (DOC) also followed a similar trend. In contrast, the concentration of silica decreased downstream from Adpr to the Rjht site. Gross primary productivity and Chl *a* biomass increased downstream by about 1.6 and 1.9 fold re-

spectively. Productivity variables showed distinct synchrony with nutrients (Figure 1). Seasonally, the concentrations of DOC and silica were highest in monsoon season and lowest in summer. Unlike DOC however, concentration of silica was recorded higher in summer in comparison to winter season.

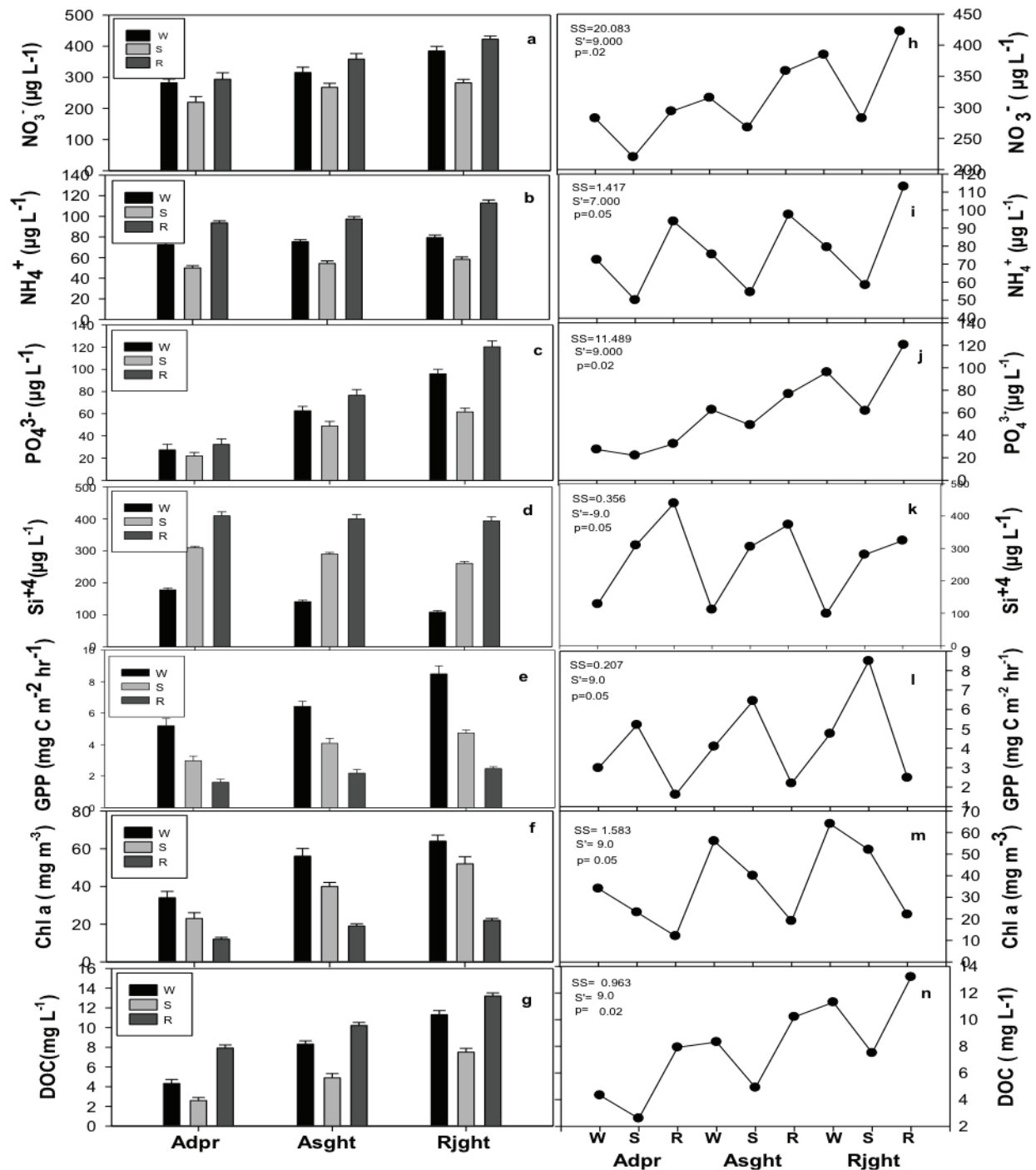


Figure 1: Seasonal trends and Mann-Kendall test with Sen's slope statistics for NO_3^- (a,h), NH_4^+ (b,i), PO_4^{3-} (c,j), Si^{4+} (d,k), GPP (e,l), Chl *a* (f,m) and DOC (g,n) at three study sites. S', Sen's estimate; SS, Sen's slope; GPP: gross primary productivity; Chl *a*: chlorophyll *a*

Chl *a* biomass and productivity was highest in winter season and lowest in monsoon. On a spatial scale, the concentration of river nutrients was highest at Rjht and lowest at the Adpr site (Figure 1). Seasonal ($p < 0.01$) as well as spatial ($p < 0.05$) variations in these variables were significant (ANOVA). The Mann-Kandall test with Sen's slope statistics revealed a positive rising trend across site for NO_3^- , NH_4^+ , PO_4^{3-} , DOC and produc-

tivity. In case of silica however, a decreasing trend was noticed (Figure 1).

The N: P: Si stoichiometric ratio declined downstream from Adpr to the Rjht site (Figure 2). The N: P, Si: N and Si: P ratios were highest at Adpr and lowest at the Rjht site. Seasonally, N: P: Si stoichiometry was highest in summer and lowest in winter season. Chl *a*: DOC ratio also showed

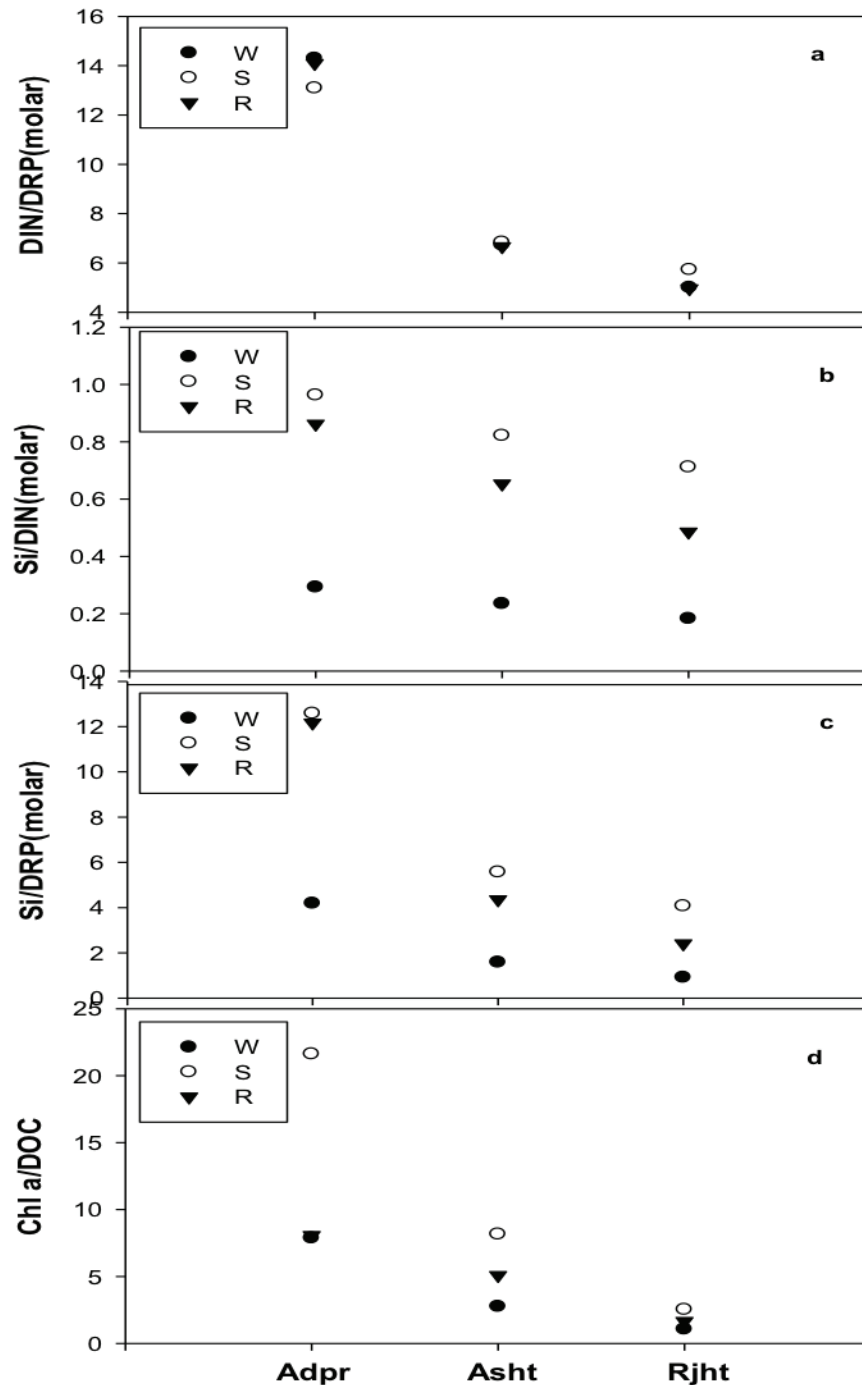


Figure 2: The seasonal trend in DIN: DRP (a), Si:DIN (b), Si: DRP (c) and Chl *a*: DOC ratios at three study sites

a similar trend from Adpr to the Rjht site and was highest in summer season (Figure 2). Principal components analysis (PCA) separated Adpr, the least anthropogenically influenced site, from rest of the sites

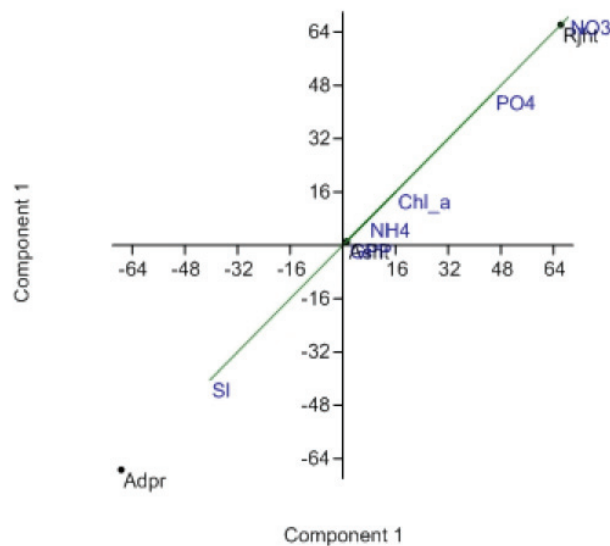


Figure 3: Principal Component Analysis (PCA) showing the position of three sampling sites based on water quality characteristics. At the center of the axis, variable GPP overlaps with site Asht.

demarcating water quality characteristics including nutrients and productivity variables (Figure 3).

Enhanced nutrient loading is seen as the key factor in enhancing excessive algal growth and other problems associated with eutrophication and the poor ecological status of fresh waters. The results of this study indicate that the concentration of nutrients in river increased in response to changes in site characteristics and season. Increases in nutrient concentration could be a combined effect of natural input from weathering and erosion and anthropogenic activities such as land use changes, sewage input and other sources. In addition, air-borne materials also add nutrients to waterways either through direct deposition on water surfaces or indirectly through catchment deposition-coupled surface runoff (Pandey *et al.*, 2014). In this study, Site 1 (Adpr) is a comparatively natural site whereas Site 3

(Rjht) represents downstream urban core with strong influence of industrial and sewage outlets and atmospheric deposition. Thus, the downstream rising trend in concentrations of nutrients and DOC indicates strong influence of urban core. This effect was clearly evidenced in PCA results wherein Adpr (Site 1), with low level of nutrients and DOC, is grouped away from rest of the sites experiencing local controls (Figure 3).

Results showed marked seasonality in concentrations of N, P and Si wherein storm water runoff appeared to be the major determinant for high concentrations in the rainy season. In the aquatic systems, especially in rivers, a major part of silica comes from weathering and erosion of lithogenic materials which is accelerated by rainfall. The influence of surface runoff has been shown to be one of the most important causations regulating seasonality in nutrient concentration in Ganga River (Pandey *et al.*, 2014).

Changes in surface water quality are most often reflected in primary production and, if persist for long time, in the trophic cascades. Productivity variables such as GPP and Chl *a* biomass increased consistently downstream and showed synchrony with the concentration of nutrients. The variation in the N: P: Si ratio in the river is the result of net change in the concentration of these nutrients caused mainly by anthropogenic factors. A classical study by Redfield (1958) has revealed the importance of N: P: Si (16: 1: 16) ratios for phytoplankton growth. In the present study, the N: P ratio ranged from 4.96 to 14.29. Since, the N: P stoichiometry below 16:1 shows N limiting condition, the trend observed in N: P ratios downstream indicate N limitation as we move from Adpr to the Rjht site. Lower N compared to P downstream could be due to excess P addition from anthropogenic sources, or loss of N to the atmosphere through denitrification. Similar to N: P, we

found a clear deviation in the Si: N and Si: P ratios. The former varied between 0.18 and 0.96 and the latter between 0.91 and 12.58. Lower Si: N ratio than the classical Redfield ratio of 1: 1 merits attention especially for growth of diatoms (Ittekkot *et al.*, 2000; Gilpin *et al.*, 2004). Low DSi: DIN ratios cause potential Si limitation, especially during diatom blooms in human impacted waters (Viaroli *et al.*, 2013). Further, lower Si: P stoichiometry than 16: 1, resulting possibly from high P input through sewage etc. has concern for phytoplankton growth, especially for diatoms.

Competition for resources (N: P: Si) in fresh water is often used to explain the structure of phytoplankton communities (Tilman *et al.*, 1982; Elser *et al.* 2000b). Variation in N: P: Si ratio observed in present study could influence phytoplankton growth and community composition especially with silicious algal groups. For instance, silica, a major component of the cell wall of diatoms, is required for the balanced growth of diatoms.

A consistent and long-term increase of N and P at low Si may cause dramatic shift in the phytoplankton community with dominance of green or blue-green algae (Tilman *et al.*, 1982; Teubner and Dokulil, 2002). Experimental evidence suggest the dominance of diatoms at high Si: N ratios and that of non-silicious flagellates at low Si: N ratio in marine ecosystems (Sommer, 1994). Further, low N: P ratio favours the dominance of diazotrophic cyanobacteria (Havens *et al.*, 2002; Smith and Schindler, 2009). Thus, along a N-limiting gradient, as may be the case of present study, the contribution of cyanobacterial population, especially N-fixing species to overall phytoplankton biomass would be higher. Similarly, the Si- limiting condition, as observed spatially toward downstream, could slowly replace diatoms (Sommer *et al.*, 1986).

Additionally, during eutrophication, bio-

geochemical feedbacks further increase the supply of P, but decrease the availability of silica favouring the formation and persistence of harmful algal blooms (Howarth *et al.*, 2011). In fresh waters, cyanobacteria commonly bloom and fix N when the availability of N relative to P is low (Howarth *et al.*, 2011).

The increase in Chl *a* biomass and GPP downstream indicate enhanced growth of phytoplankton associated with nutrient enrichment. The concentration of DOC also indicate an increasing trend due to the coupled effect of autochthonous C- pool and allochthonous C input. The Chl *a*/ DOC ratio is the measurement of autochthonous C in the river. In this study, the Chl *a*/ DOC ratios indicated major contribution of phytoplankton in winter season. Despite enhanced phytoplankton development however, Chl *a*/ DOC ratio declined at downstream sites indicating the loading of organic carbon in the river at a rate faster than the rate of phytoplankton turnover.

This indicates the influence of the loading of organic input from terrestrial sources (Pandey *et al.*, 2014). This has relevance for the long term shift in trophic cascades since the nature of C available in surface waters regulates the nutritional status of consumers.

Conclusions

Anthropogenic loading of nutrients in surface waters has substantially increased over recent decades. This may lead to a shift in the N: P: Si stoichiometric elemental coupling and, by implication, the production and fate of phytoplankton in surface waters. Our study, conducted along a 35 km stretch of the Ganga River, showed a shift in N: P: Si stoichiometric ratios in the river at Varanasi. Such changes are expected to be the result of anthropogenic release coupled with biogeochemical feedbacks, including denitrification and sediment efflux regulat-

ing N and P levels in the river water. Since the N: P: Si ratio for phytoplankton growth is specific, a long-term shift in N: P: Si stoichiometry may induce a dramatic change in the ecological dominance of the phytoplankton community in major rivers experiencing anthropogenic controls.

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