



A comparative study on the bioturbation ability of seven freshwater snail species

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Abstract An assessment of the bioturbation potential of seven freshwater snail species was made in a laboratory microcosm study. Two non-operculate species, *Indoplanorbis exustus*, and *Racolina luteola* and five operculate species, namely *Pila globosa*, *Filopaludina bengalensis*, *Gabbia orcula*, *Melanoides tuberculata*, and *Brotia costula* were considered for the experiment extending for a period of 28-days. Grazing, burrowing and bulldozing activities of snails on sediments bed induced changes in physicochemical properties of substratum and modulated the organic mineralization process. All seven snail species increased $\text{NH}_4^+\text{-N}$ and $\text{PO}_4^{3-}\text{-P}$ flux from sediment to overlying water in comparison with the control. Moreover, in snail presence modulated NO_x^- ($\text{NO}_2^-\text{-N} + \text{NO}_3^-\text{-N}$) concentrations and other parameters (DO, pH, TDS, conductivity) to the column water. Snail activity induced changes in physicochemical properties (TN, AP, OC, porosity and WHC) of sediment and modulated the organic

mineralization process based on the length of the snails. The sediment texture was changed, more prominently in presence of the operculate snails than the non-operculate snails. Apparently, the operculate benthic snail *F. bengalensis* induced greater changes in the sediment in comparison to other snails. Nonetheless, all the snails appear to be efficient engineers in the management and habitat manipulation of the freshwater ecosystems.

Keywords Bioturbation · Operculate snails · Non-operculate snails · Wetlands · Nutrient cycling

Introduction

The freshwater benthic macroinvertebrates execute bioturbation in course of burrowing, feeding, secretion, excretion and transporting activities. As a result, the structure and properties of sediment are altered, featured by the translocation of nutrients and other solutes between sediment and water interface (SWI) (Mermillod-Blondin et al. 2003; Thibodeaux and Bierman 2003; Kristensen et al. 2012; Adámek and Maršálek 2013), accelerating the biogeochemical cycle (Mermillod-Blondin and Rosenberg 2006). Bioturbation mediated by the benthic macroinvertebrates can stimulate organic matter mineralization process by manipulating microbial growth (Mermillod-Blondin et al. 2004; Biswas et al. 2009). Increased oxygen penetration due to the reshuffling and reorientation

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of sediment strata by the active bioturbation can alter the redox potentials at SWI (Aller 1994; Gilbert et al. 2016). Such alteration thereby influence coupled nitrification–denitrification process (Gilbert et al. 2016; Saaltink et al. 2019; Mao et al. 2020) as well as alters phosphorus (P) release from the sediment (Cheng et al. 2014; Yang et al. 2020).

The functional attributes of the freshwater macroinvertebrates qualify them as ecosystem engineers (Gutiérrez et al. 2003; Biswas et al. 2009), bioindicators (Goswami et al. 2010) and sentinel species (Gnatyshyna et al. 2020). Many of these macroinvertebrates (oligochaete worms, dipteran and other insect larvae, isopods, and bivalve molluscs) adapted to benthic habitats are involved in bioturbation process play a significant role in consistent nutrient flux from the sediment to overlying water (Traunspurger et al. 1997; Zheng et al. 2009; Boeker et al. 2016; Gautreau et al. 2020). Besides, the active movement of benthic organisms on the superficial sediment interface also influences the biogeochemical processes through uncoupling compounds (Zheng et al. 2009; Zhong et al. 2015) and release of nutrients. Among the freshwater macroinvertebrates, the snails (Mollusca: Gastropoda) are abundant in several freshwater habitats (Basu et al. 2018; Meena et al. 2019), carrying out multiple functions that sustain the concerned ecosystem (Hall Jr et al. 2003; Zheng et al. 2009; Zhang et al. 2013; Meena et al. 2019). In course of grazing and filter-feeding on algal biomass (Fang et al. 2010; Mo et al. 2017), the freshwater snails regulate the producer community and thus influence the food web and species diversity (Turner et al. 2000; Aditya and Raut 2002 2005; Paul et al. 2020). Freshwater snails exhibit several feeding modes, from herbivory on submerged plants and periphyton (Cattaneo and Kalff 1986) to detritivory on fine sediments and filter-feeding on suspended particles (Brendelberger 1997). Therefore, snails exploit multiple trophic niches (Declerck 1995; Usseglio-Polatera et al. 2000) and in certain instances qualify as a keystone species in regulating community structure in freshwater systems (Loman 2001; Zhu et al. 2013). The secretions of freshwater snails act as coagulating substances to improve transparency of water (Pu et al. 1998; Das and Khangarot 2011). The shells of freshwater snails are often used as platform for the colonization and growth of algae and microbes (Abbott and Bergey 2007; Lukens et al. 2017). Having unique structural and chemical

properties (Parveen et al. 2020; Chakraborty et al. 2020), the shells of the snail regulate the alkalinity of water (House and Denison 2002), facilitate phosphate removal (Ewald et al. 2009; Pal et al. 2022) and biosorption of heavy metal (Hossain et al. 2015) from the contaminated water. The ability of the freshwater snails to withstand the contaminants establishes them as a sentinel species for the biological monitoring of the freshwater ecosystems (Gnatyshyna et al. 2020; Dhiman and Pant 2021). However, empirical assessment of the roles of the freshwater snails in bioturbation is yet to be deciphered at the species level, which prompted us to investigate the bioturbation ability of the freshwater snails observed in Kolkata, India.

A microcosm-based laboratory study was initiated using both non-operculate and operculate snails to highlight the bioturbation efficacy on a comparative scale. Among the model snails considered, two were non-operculate species, namely *Racessina luteola* (Lamarck, 1822) (Pulmonata: Lymnaeidae), *Indoplanorbis exustus* (Deshayes, 1834) (Pulmonata: Planorbidae) which are involved in the spread of several helminth diseases (Sangwan et al. 2017) and are commonly consumed by insect (Aditya and Raut 2002) and leech (Aditya and Raut 2005). The five operculate snails, namely *Melanoides tuberculata* (Müller, 1774) (Prosobranchia: Thiaridae), *Gabbia orcula* (Frauenfeld, 1862) (Prosobranchia: Bithyniidae), *Filopaludina bengalensis* (Lamarck, 1882) (Prosobranchia: Viviparidae), *Brotia costula* (Rafinesque, 1833) (Prosobranchia: Pachychilidae), and *Pila globosa* (Swainson, 1822) (Prosobranchia: Ampullariidae) were considered in the study as model snail species. All these seven snails are common in freshwater habitats of Kolkata and adjoining areas (Pal and Dey 2011), few prefer the benthic habitats and the others prefer the floating vegetation and the banks. As a consequence of the niche segregation of the freshwater snails, the prospective effects on the bioturbation ability are expected to vary accordingly. The adaptation of the snails to the different habitats may also contribute to the differential ability of bioturbation. A comparison of the bioturbation efficacy of the freshwater snails will enable to identify the relative importance of the different snails in the freshwater systems that would aid in the selection and application of the freshwater snails in the management and the manipulation of the concerned water bodies. Thus, the focus of

the study was to evaluate the species specific variations in the role as bioturbators and modulation of the freshwater biogeochemistry.

Materials and methods

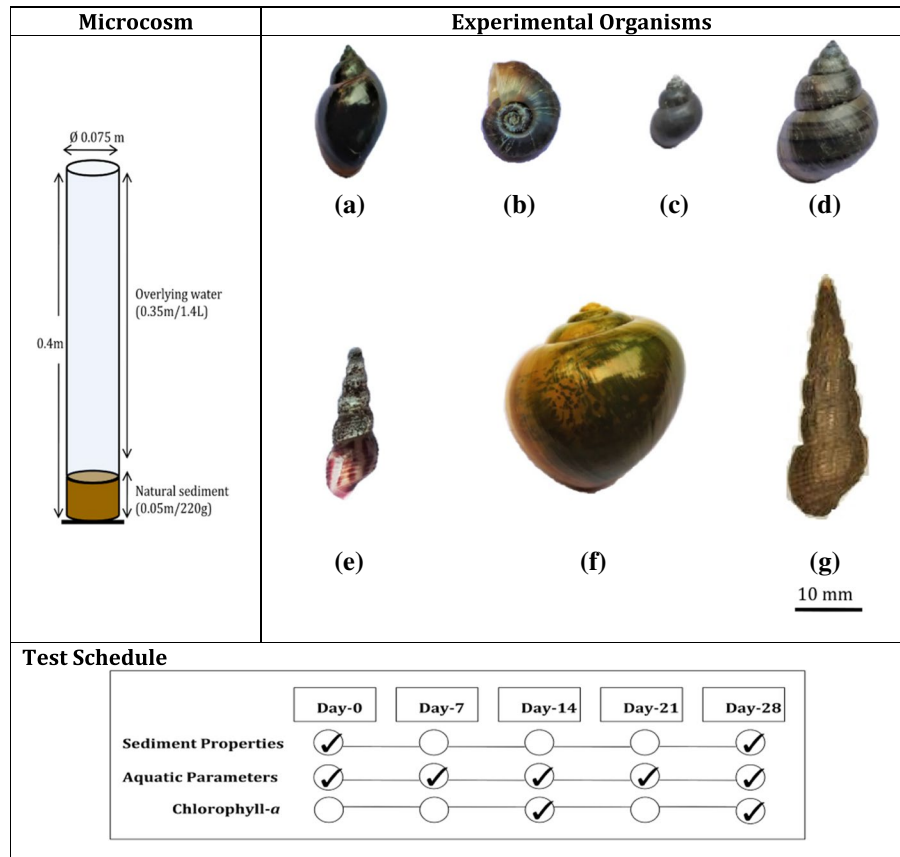
Collection of molluscs and sediment preparation

The sediment and freshwater snails were collected from randomly selected ponds and wetlands in and around Kolkata metropolitan area (22° 32' 27.96" N, 88° 20' 16.08" E), West Bengal, India and from an urban wetland, Santragachi *Jheel* (22° 34' 51.72" N, 88° 16' 58.92" E), located near Santragachi Railway Station, Howrah, West Bengal, India. Using a grab sampler, the sediment was procured from the upper surface layer (upto the depth of 10 cm) and sun dried for 5 days, subsequently sieved through 500 µm mesh to eliminate undesired particles (Lacoste et al. 2018). The sediment sample was homogenized before introducing to the experiment and to get identical properties in initial condition. Individuals of the snails *R. luteola*, *I. exustus*, *M. tuberculata*, *G. orcula*, *F. bengalensis*, *B. costula*, and *P. globosa* (abbreviation used as RL, IE, MT, GO, FB, BC, and PG, respectively, to denote treatments) were collected by dragging nylon hand net (frame size 0.3×0.3 m; mesh size 500 µm) along bottom region of littoral zone or simply hand-picked from the bottom sediment (Dalu et al. 2012). All the organisms were brought to laboratory and acclimated for 7 days under controlled environment to the sedimentary habitats in aquarium (0.37×0.3×0.35 m) containing procured unpolluted sediment and overlying tap water with some free floating hydrophytes and detritus in the form of dead leaves and twigs. Intact individuals of *R. luteola* (17.99±0.26 mm in length), *I. exustus* (15.28±0.1 mm in diameter), *M. tuberculata* (26.06±0.37 mm in length), *G. orcula* (9.8±0.17 mm in length), *F. bengalensis* (21.26±0.34 mm in length), *B. costula* (40.77±0.76 mm in length) and *P. globosa* (27.93±0.18 mm in length) with normal activities were chosen for the study. Before introducing the snails to the microcosms they were washed properly to remove the attached biota and debris from their shell surfaces. No external food particles were added to the microcosms during the experiment.

Experimental organisms

All the seven experimental snail species have an affinity for organic-rich sediments (Sarkar 1992; Zheng et al. 2009; Tripathy and Mukhopadhyay 2015), and are common throughout the year reaching their maximum densities (up to 3000–4300 ind/m²) during the monsoon season (Mukherjee and Nandi 2004). The chosen species (Fig. 1) are particularly common and may attain local dominance in India (SubbaRao 1989; Tripathy Mukhopadhyay 2015; Chandra et al. 2017; Ghosh and Panigrahi 2018; Meena et al. 2019). The snails *R. luteola* and *I. exustus* are intermediate hosts of disease causing helminth worms (Sangwan et al. 2016; 2017), known to thrive well in the water bodies of India having a broad range of environmental variability (SubbaRao 1989; Aditya and Raut 2002). Though the herbivory is common in these snails (Raut et al. 1992; Madsen 1992), they also prefer to aggregate over the organic content of sediment and other substratum materials. These snails tend to have the bacterial fraction of food (Callow 1975; Dillon 2000) from aquatic ecosystem. *M. tuberculata*, is a globally invasive thiarid, native across Middle East to Southeast Asia and East Africa (Facon et al. 2003). They occur in large number (ranging from 170 to 2040 ind/m²) to the various freshwater habitats (Subba Rao 1989) having fine sediment substratum (Giovanelli et al. 2005; Ramakrishna and Dey 2007). *M. tuberculata* are known to feed by scraping on sedimentary organic matter, detritus and graze on periphyton (Raw et al. 2016). *F. bengalensis* is most widely dispersed freshwater snail in India (Goswami et al. 2010; Baag et al. 2020), known to serve as nutritious food item for human (Datta et al. 2016). They are facultative suspension-feeder, found to graze and move on sediment in course of foraging and to scrap on solid detritus by using radula (Zheng et al. 2009; Olden et al. 2013). *B. costula* and operculate snail *G. orcula* are also very common benthic epifauna (Roy et al. 2014; Ghosh and Panigrahi 2018), having same ecological traits like viviparids (Usseglio-Polatera et al. 2000; Aditya and Raut 2005; Pyron and Brown 2015). *P. globosa* is another widely distributed snail in India with its prominent ecological significance as an indicator species to study the effects of contaminations in freshwater ecosystem (Bhattacharya et al. 2016; Parveen et al. 2020). This snail has clinical importance (Prasad et al. 2019) and is also considered

Fig. 1 The microcosm and the snails [a *Racemina luteola*, b *Indoplanorbis exustus*, c *Gabbia orcula*, d *Filopaludina bengalensis*, e *Melanoides tuberculata*, f *Pila globosa*, g *Broteria costula*] used in the present study along with the test schedule followed during the experiment to measure the changes in physicochemical properties of sediment and water and to observe the change in Chlorophyll-*a* measures



for its potential utilization in aquaculture industry as a food (Panda et al. 2021). However, the Ampullarids are mostly amphibious, they usually inhabit aquatic life during active period when the resource is available to their habitat (Aditya and Raut 2001; Panda et al. 2021). Like the other members of Viviparaceae they also feed on detritus and utilize bacteria and other suspended particles trapped over the mucus layer which they stretched over the substratum and then ingest it (Dudgeon 1999).

Experiment design

The microcosm was prepared by adding sediments. The sieved and homogenized sediment was added to cylindrical glass columns (0.4 m long with internal diameter of 0.075 m) to make a 0.05 m thick sediment layer (220 g of dry weight), overlain by 0.35 m (volume: 1.4 L) of deionized water (Represented in Fig. 1). The mixing of the sediment with the deionized water added the ions to the experimental water.

Before filling water, few plastic tapes (0.07 × 0.025 m) were fixed to the inner surface of columns which could act as substratum for colonization of algae (Wood and Richardson 2009). The estimation of chlorophyll-*a* (Chl-*a*) content of periphyton developed on the surface of substrates could inform about the effects of nutrient availability and grazing pressure on algal biomass (Wood and Richardson 2009; Mo et al. 2017). The prepared microcosms were stabilized for 2 weeks (pre-incubation period) before the introduction of organisms to the systems (Lacoste et al. 2018). The day of introduction of snails to the microcosms was considered as day-0 of the experiment. The total experiment was set aside in outdoor environment in the availability of air and sunlight for another 28 days after the introduction of snails (i.e., on July 25, 2017). The study was conducted under normal daylight condition (daily period of light at 13:23-h). The average temperature during the study was 28 ± 1.5 °C. To compare the effects of different organisms, the biomass of organism was considered as fixed parameter

(Michaud et al. 2006). Therefore, in this experiment the number of individuals of each snail species introduced was considered to obtain the initial biomass (wet weight) comparable ($F_{6, 32}=1.87$; $p=0.12$) among snail treatments. The wet (living) biomass of the individual snails was determined by placing the living snails on absorbent paper for a few minutes (Gosselin 1993), after that the weights were measured in a Pan balance (ADAM®, ADA 71/L, Adam Equipment, UK). The complete setup comprised 8 treatments, one control treatment without fauna and seven snail treatments (see Table 1). Three replicates for *P. globosa* treatment and five replicates for the other treatments were considered for the study. The relative densities of the snails (represented in Table 1) were more or less closed to their natural densities reported in earlier observations (Mukherji and Nandi 2004; Pal and Nandi 2006; Sharma et al. 2013; Janagal and Khatri 2016) and substantiated during the field study carried out in nearby ponds and lakes around Kolkata metropolitan area, West Bengal, India, during the period of May 2016 to July 2016. Every microcosm was examined daily during the experiment and dead snails if found, were removed and replaced with individuals of identical size.

Sampling and measurements

To study the impacts of the selected snail on the physicochemical properties of sediment and overlying water as well as on the algal growth, scheduled sampling and measurements were performed during the experiment. The sediment properties (total-N, available-P, organic-C and porosity and water holding

capacity), aquatic parameters ($\text{NH}_4^+\text{-N}$, $\text{NO}_2^-\text{-N}$, $\text{NO}_3^-\text{-N}$, $\text{PO}_4^{3-}\text{-P}$, DO, pH, TDS, COND) and periphytic chl-*a* were estimated and recorded. To estimate the changes in nutrients, water samples were collected at a depth of 10 cm from each column by using glass syringe and inorganic nutrients ($\text{NH}_4^+\text{-N}$, $\text{NO}_2^-\text{-N}$, $\text{NO}_3^-\text{-N}$, $\text{PO}_4^{3-}\text{-P}$) concentrations were measured 5 times: on Day-0, just before the introduction of fauna and on days- 7, 14, 21 and 28. The volume of water (50 ml) taken from each column in course of estimations of nutrients was renewed immediately with same amount of deionized water. Since the weekly collected samples corresponded to less than 4% of the total water volume in each experimental column, such sampling did not affect concentrations appreciably. The water sample was filtered and stored in 4 °C before analysis. All nutrients were measured by spectrophotometric method using Labman LMSP-UV1000B UV-VIS Spectrophotometer. $\text{NH}_4^+\text{-N}$ in water was measured at 640 nm by phenate method (APHA 2005). $\text{NO}_2^-\text{-N}$ concentration was measured at 543 nm by sensitive diazotization method (APHA 2005). $\text{NO}_3^-\text{-N}$ concentration was estimated by Brucine method (following EPA test method, 1971). $\text{PO}_4^{3-}\text{-P}$ of water was estimated by following ascorbic acid method (APHA 2005). Dissolved oxygen was measured manually by a dissolve oxygen meter (Lutron PDO-519). Other physico-chemical parameters like pH, TDS, COND were estimated following the same schedule, on days 0, 7, 14, 21 and 28, by using multi-parameter tester (Model PCSTestr35).

To assess the treatment effects on physical and chemical properties of sediment, the samples were collected 2 times (before fauna introduction and at the

Table 1 Density (individuals/m²), number and biomass (wet weight) of seven freshwater snail species added to the glass columns

Treatment Name	Treatment description				
	Added snail species	Added snails (column ⁻¹)	Individual biomass (mg/ind)	Total biomass (g/column)	Density (ind/m ²)
C	Control without snail				
RL	<i>R. luteola</i>	5	493.6 ± 11.5	2.7 ± 0.5	1136
IE	<i>I. exustus</i>	5	519.7 ± 11.5	2.8 ± 0.7	1136
MT	<i>M. tuberculata</i>	5	691.7 ± 23.7	3.4 ± 0.1	1136
PG	<i>P. globosa</i>	1	3806.6 ± 512.4	3.7 ± 0.4	227
FB	<i>F. bengalensis</i>	3	1109.7 ± 59.5	3.4 ± 0.1	681
BC	<i>B. costula</i>	3	1255.9 ± 105.3	3.3 ± 0.4	681
GO	<i>G. orcula</i>	25	119.9 ± 3.9	2.8 ± 0.1	5680

Values are presented as mean ± standard error(SE)

end of experiment) from three experimental columns of each treatment (viz. C, RL, IE, MT, FB, GO, BC and PG). The sediment sample was sliced from the upper 2 cm surface of sediment bed. Organic-C content of sediment samples were quantified by Walkley–Black's rapid titration method by using 0.5 (N) Mohr's salt solution in presence of diphenyl amine indicator (Nelson and Sommer 1982). Total kjeldahl nitrogen (TN) was estimated with the standard method (Jackson 1958). The available-P (AP) of the sediment was determined following suitable method (Olsen et al. 1954). The porosity and water holding capacity (WHC) of the sediment were estimated following the suitable methods (Ragab et al. 1982 and Cassel et al. 1986). The Chl-*a* content was estimated on day 14 and 28 by removing single plastic tape from each experimental microcosm. Deposited algal film on the plastic tape was scraped off by using brush and razor (Zhang et al. 2013; Mo et al. 2017). The resultant sample was taken and Chl-*a* estimated by 90% acetone extraction procedure followed by Sadasivam and Manickam (1992).

Statistical analysis

Before the snails were introduced into the microcosms, on day-0, the concentrations of inorganic nutrients in all treatment cores were compared using one-way ANOVA. After the introduction of each snail species to their respective treatment cores, their effects on physicochemical and Chl-*a* variables were compared by performing one-way RM-ANOVAs, considering fauna treatment (control without snail, *R. luteola*, *I. exustus*, *M. tuberculata*, *G. orcula*, *F. bengalensis*, *B. costula* and *P. globosa* treatments) as main factor and time (days 7, 14, 21 and 28 for aquatic parameters and days 14, 28 for Chl-*a*) as repeated factor. If any significant difference (i.e. $p < 0.05$) was observed among the treatments, a post hoc Tukey test was carried out to determine which treatments differed after 28 days of microcosm stabilization. To determine the difference among treatments affecting the sediment properties (total-N, available-P, organic-C, porosity and water holding capacity) one-way ANOVA was performed using treatments as main factor. Within-column data were calculated to evaluate the change between times (start to end) for each treatment. Data transformations (log or square-root

transformed) were made to meet the assumptions of homoscedasticity. For all tests when $p < 0.05$, the differences were considered as statistically significance. All statistical analyses were performed following Zar (1999), and using XLSTAT software (Addinsoft 2010).

Results

Visual observations

After the introduction of the seven snail species to their respective treatment cores, the individuals were found to glide actively over the sediment surface or to attach to the wall of glass columns. The activities of snails usually restricted to the top few centimeters of sediment substrate. The gliding, burrowing and grazing activities of the snails altered the natural texture of the surface sediment. The seven freshwater snail species introduced in this experiment modified the sediment surface texture diversely corresponding to activities on the sediment bed (Fig. 2). Observations on the activities of *F. bengalensis*, *B. costula* and *M. tuberculata* demonstrated that in course of their crawling and grazing activities the concave trails were formed on the top few millimetres of the sediment surface. Periodic pattern of movement in and out of burrow, bulldozing and nudging the sediment surface caused extensive mixing and reshuffling of the upper sediment layer up to the depth of 1–2 cm by the snail *F. bengalensis* and few millimetres by the activities of *B. costula* and *M. tuberculata*. Initially *G. orcula* was also found very active to crawl over the sediment, leaving trails on the surface layer. At the later stage of the experiment a few individuals of *F. bengalensis*, *G. orcula* and *M. tuberculata* became less active and buried themselves for several days within the sediment. In addition to the gliding on sediment and scrapping over the surface materials *R. luteola*, *I. exustus* and *P. globosa* produced and aggregated granular pellets. The pelletization during the ingestion and egestion process modified fine surface texture of sediment with larger granules having greater inter-particle voids. Mucus coated pellets deposited on the sediment surfaces in the presence of *R. luteola*, *I. exustus* and *P. globosa* contained mainly the mucus and egested sediment.

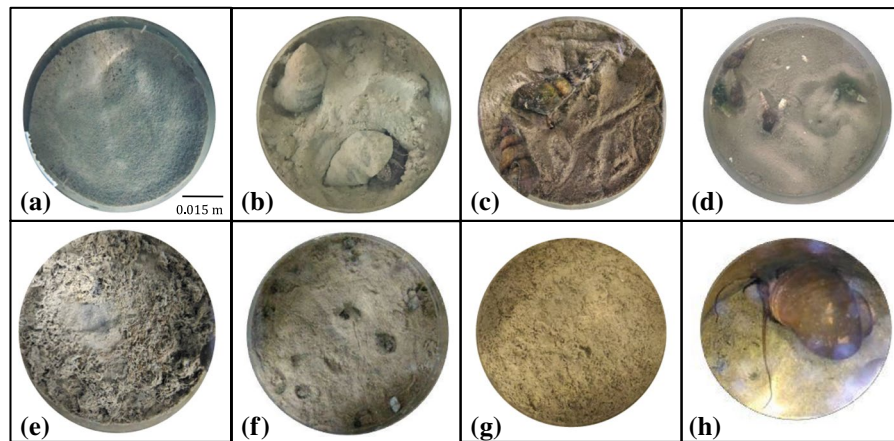


Fig. 2 Photographic view of the microcosms: **a** control treatment without snail showing undisturbed sediment bed, **b** activity of *F. bengalensis* on sediment produced crawling traces and shallow grooves, **c** *B. costula* crawling traces to the surface sediment to form shallow groove and distorting the normal structure of sediment bed, **d** *M. tuberculata* nudging their anterior part of body to the surface sediment to form shallow

groove, **e** *I. exustus* modified fine surface texture of sediment with larger granules and greater inter-particle voids having mucus coated pellets deposited on the sediment surfaces, **f** small burrows and gliding trails formed due to the activities of *G. orcula* on sediment bed, **g** *R. luteola* and **h** *P. globosa* modified texture of sediment with larger granules having mucus coated pellets deposited on the sediment surfaces

Table 2 The results of one-way ANOVA on the initial values of water parameters

Parameters	$F_{(7, 64)}$	p -values
NO_3^- -N	0.454	0.863
NO_2^- -N	0.377	0.912
NH_4^+ -N	3.514	0.003
PO_4^{3-} -P	1.134	0.355
TDS	1.506	0.184
COND	1.078	0.389

Water quality dynamics induced by snail bioturbation

On day-0, prior to the introduction of the snails in microcosms, except for NH_4^+ -N, the concentrations of NO_2^- -N, NO_3^- -N, PO_4^{3-} -P, COND and TDS in the overlying water were comparable (represented in Table 2) among the eight treatment groups (Control, *R. luteola*, *I. exustus*, *M. tuberculata*, *G. orcula*, *F. bengalensis*, *B. costula*, *P. globosa*) and did not exhibit significant differences revealed through ANOVA.

After introducing the fauna to the microcosms, the NH_4^+ -N concentrations in the overlying water of all the treatment columns showed distinct treatment and time dependent variations (Fig. 3a; Table 3;

RM-ANOVA, $p < 0.0001$). Except for the control columns, all the other treatments showed maximum rise in NH_4^+ -N concentrations during the earlier 7 to 14 days of the experiment and then began to decrease. The changes were significant ($p < 0.05$) for all the treatment groups with snails except treatment *R. luteola* and *P. globosa*. Throughout the experiment the control columns showed steady decline in NH_4^+ -N measures over time. The microcosms in presence of snail *F. bengalensis* and *P. globosa* showed maximum and minimum NH_4^+ -N flux from sediment to water column, respectively. Except day-7 for treatment *B. costula* and *R. luteola* and Day-28 for *M. tuberculata* in each sampling events all of the six treatment groups (with the snails *R. luteola*, *I. exustus*, *M. tuberculata*, *G. orcula*, *F. bengalensis*, *B. costula*) showed significantly ($p < 0.05$; Tukey's HSD) higher NH_4^+ -N effluxes compared to the control and *P. globosa*. After 28 days of experiment stabilization, NH_4^+ -N concentrations were 9.1, 9.3, 4.1, 11.6, 14.2, 9.8 and 1.9-fold higher in treatments *R. luteola*, *I. exustus*, *M. tuberculata*, *G. orcula*, *F. bengalensis*, *B. costula* and *P. globosa*, respectively, in comparison with the control group. Incidentally, the ability to increase the NH_4^+ -N flux to the water column seemed to be species-specific rather than related to the individual body size (average shell length) of snail (Fig. 4a).

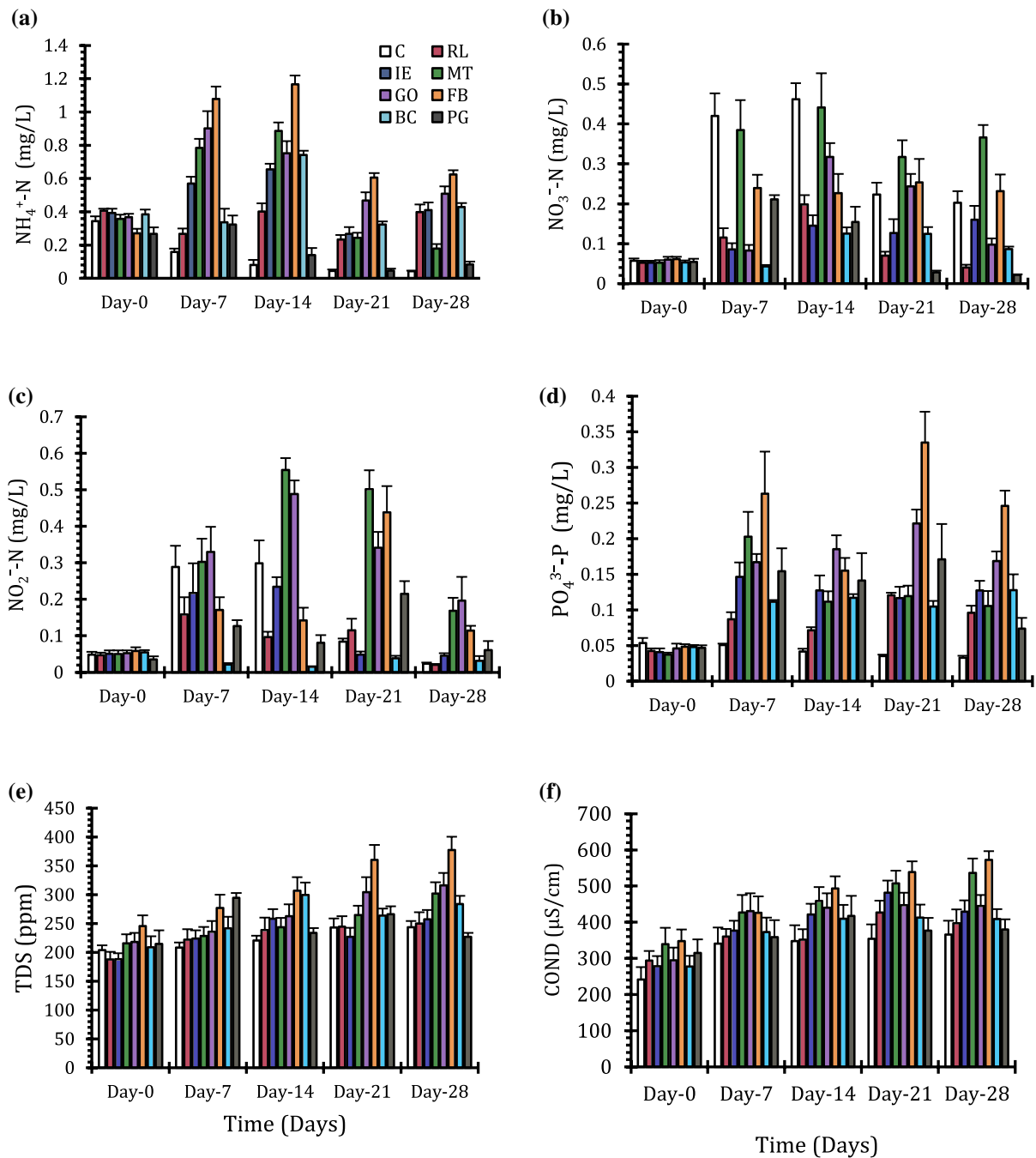


Fig. 3 Changes in concentrations of **a** $\text{NH}_4^+\text{-N}$; **b** $\text{NO}_3^-\text{-N}$; **c** $\text{NO}_2^-\text{-N}$, **d** $\text{PO}_4^{3-}\text{-P}$ **e** TDS and **f** COND in the water columns of microcosms during the incubation period of eight treatment groups [C=Control, RL=*R. luteola* treatment; IE=*I. exilis*

tuberculosis treatment MT=*M. tuberculosis* treatment, GO=*G. orcula* treatment FB=*F. bengalensis* treatment, BC=*B. costula* treatment; PG=*P. globosa* treatment]. Values are presented as mean \pm SE

Table 3 The results of one-way RM-ANOVAs, considering fauna treatment (control without snail, *R. luteola*, *I. exustus*, *M. tuberculata*, *G. orcula*, *F. bengalensis*, *B. costula* and *P. globosa* treatments) as main factor and time (days 7, 14, 21 and 28 for aquatic parameters and days 14, 28 for Chl-*a*) as the repeated factor

Parameter	Source	df	F	p	Parameter	Source	df	F	p
NH ₄ ⁺ -N	Treatment	7	110.6	<0.001*	TDS	Treatment	7	12.22	0.002*
	Time	3	97.32	<0.001*		Time	3	5.94	0.001*
	Interaction	21	9.74	<0.001*		Interaction	21	1.27	0.19
	Error	174				Error	174		
NO ₂ ⁻ -N	Treatment	7	34.56	<0.001*	COND	Treatment	7	8.13	0.046*
	Time	3	19.51	<0.001*		Time	3	3.46	0.004*
	Interaction	21	5.53	<0.001*		Interaction	21	0.46	0.97
	Error	174				Error	174		
NO ₃ ⁻ -N	Treatment	7	32.81	<0.001*	Chl- <i>a</i>	Treatment	7	5.01	<0.001*
	Time	3	10.3	<0.001*		Time	1	8.04	0.007*
	Interaction	21	3.04	0.001*		Interaction	7	1.22	0.31
	Error	174				Error	51		
PO ₄ ³⁻ -P	Treatment	7	37.71	<0.001*					
	Time	3	4.76	0.003*					
	Interaction	21	2.64	0.043*					
	Error	174							

[*indicates significant results (*p* < 0.05)]

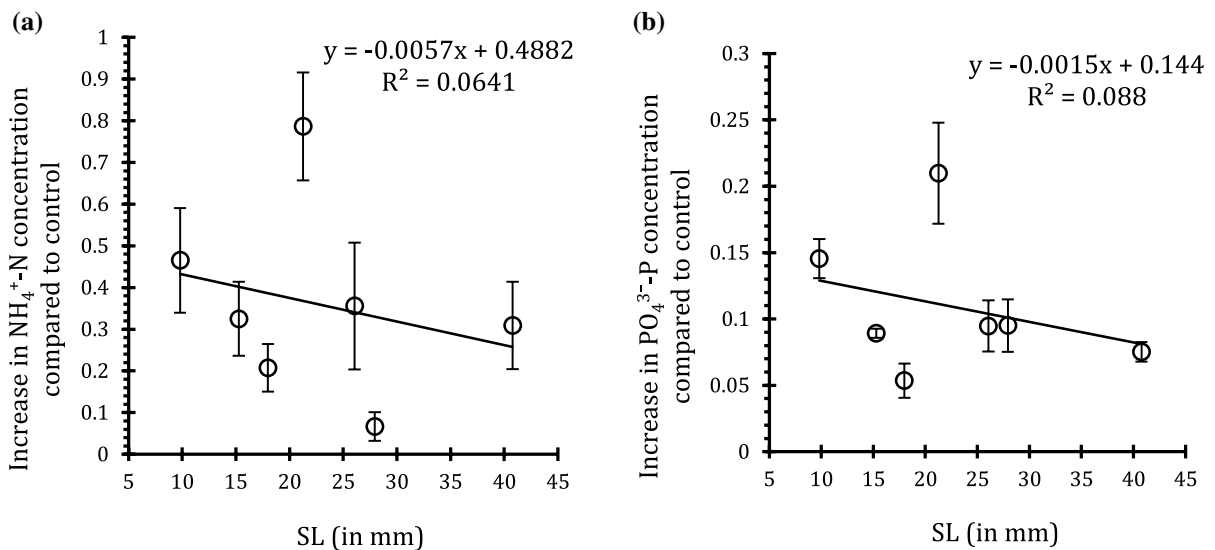


Fig. 4 Relationship between average shell length (SL, in mm) of snails and increasing concentrations of **a** NH₄⁺-N, **b** PO₄³⁻-P to the overlying water. Values are presented as mean ± SE

Both the NO₃⁻-N and NO₂⁻-N concentrations measured in the overlying water varied significantly with the time (Fig. 3b and c; Table 3; RM-ANOVA, time effect, *p* < 0.001) and also among treatments (Fig. 3b and c; Table 3; RM-ANOVA, treatment effect, *p* < 0.001). The NO₃⁻-N effluxes in the water columns of all treatment groups were noticed in between 7th day and 21st day of the experiment. The

increased concentrations were significant (*p* < 0.05; Tukey’s HSD) in *B. costula* and *M. tuberculata*. The NO₃⁻-N concentration measures to the water columns steadily decreased in treatments *R. luteola*, *B. costula*, *P. globosa* from day 14 to the rest of the experiment keeping the lowest NO₃⁻-N flux in treatment cores with snail PG. Treatment cores in the presence of *F. bengalensis* maintained steady rate of

NO_3^- -N flux during the experiment. Between day-7 and day-14, NO_3^- -N efflux was significantly higher ($p < 0.05$; Tukey's HSD) in control and *M. tuberculata* compared to the other six treatment groups. At the end of the experiment only the treatment *M. tuberculata* and *F. bengalensis* showed 1.8 fold and 1.1 fold higher NO_3^- -N compared to the control. *P. globosa* showed 9.3 times lower NO_3^- -N concentrations compared to the control at the end of 28 days of experiment.

From day-7, a significant ($p < 0.05$) rise in NO_2^- -N concentration was observed in control columns and in presence of the snail *M. tuberculata* and *G. orcula*. With an initial increase in NO_2^- -N efflux between day 7 and day 14, in control columns, a constant decrease was observed in the subsequent sampling event. During the experiment no such considerable changes in NO_2^- -N measures observed in presence of *R. luteola*, *I. exustus*, *B. costula* and *P. globosa*. At the end of 28 days of experiment stabilization, compared to the control, the treatment *I. exustus*, *M. tuberculata*, *G. orcula* and *P. globosa* showed 2.2 fold, 3.7 fold, 1.2 fold and 1.9 fold much higher NO_2^- -N concentrations, respectively. The increased concentrations were significant ($p < 0.05$; Tukey's HSD) only for the treatments with *M. tuberculata* and *G. orcula*.

For all the snail species, the release of PO_4^{3-} -P from the sediment to the overlying water varied significantly during the period study (Fig. 3d; Table 3; RM-ANOVA, time effect, $p < 0.01$). The extent of the PO_4^{3-} -P release varied with the concerned snail species (Fig. 3d; Table 3; RM-ANOVA, treatment effect, $p < 0.01$). PO_4^{3-} -P concentration in the snail treatments began to increase significantly from day 7 that either sustained or further increased for rest of the experiment. At the end of the experiment maximum efflux of PO_4^{3-} -P was observed in presence of *F. bengalensis*. From day 7 all the snail treatments always maintained higher PO_4^{3-} -P concentrations compared to the control. At the end of the experiment (on day 28) PO_4^{3-} -P concentrations were 2.9 fold, 3.9 fold, 3.2 fold, 5.2 fold, 7.5 fold, 3.9 fold and 2.3-fold much higher in treatments *R. luteola*, *I. exustus*, *M. tuberculata*, *G. orcula*, *F. bengalensis*, *B. costula* and *P. globosa* respectively in comparison with the control group. It was also observed that the ability to increase the PO_4^{3-} -P flux to the water column in presence of snail seemed to be species-specific rather than related

to the individual body size (average shell length) of snail (Fig. 4b).

However, before introduction of the snails (Day-0) to the microcosms, there was no significant difference ($p > 0.05$; Tukey's HSD) in dissolved inorganic nitrogen (NH_4^+ -N + NO_3^- -N + NO_2^- -N) to PO_4^{3-} -P ratio (N:P) among the treatments. Following addition of snail species to the respective treatment cores, a sharp decrease in N:P were noticed in all sampling occasions for all snail treatment columns compared to the control columns (Fig. 5).

Except on day 21 for *I. exustus* and on day 28 for *P. globosa*, in each sampling event, all the snail treatments showed higher TDS measures compared to the control (Fig. 3e), and differed significantly ($p < 0.05$; Tukey's HSD) in treatment *F. bengalensis*. Water columns in presence of *F. bengalensis* also showed significant ($p < 0.05$; Tukey's HSD) increase in TDS measures compared to the other six snail treatments from the sampling day 21.

In each sampling event all the snail treatments showed higher COND measures compared to the control (Fig. 3f). Increase in COND measures were significant ($p < 0.05$; Tukey's HSD) from the day 21 to the end of the experiment for the treatment *F. bengalensis* and *M. tuberculata*. Water columns in presence of *F. bengalensis* showed significant ($p < 0.05$; Tukey's HSD) increase in COND measures compared to the treatments *R. luteola* and *B. costula* at the end of the experiment. It is importance to note that the effects of time on TDS and COND in the water column were not dependent on snail treatments (Table 3; RM-ANOVA, treatment* time interaction effect, $p > 0.05$).

Effects on developing periphyton

The Chl-*a* levels measured from the substrate surface showed distinct variability with reference to the snail species concerned (Fig. 6; Table 3; RM-ANOVA, treatment effect, $p < 0.01$). While no significant differences in the Chl-*a* content was observed for the mesocosms with different snails, but, at the end of the experiment (on day 28), compared to control, the Chl-*a* measures were lower in most of the treatments except *R. luteola* and *B. costula* (Fig. 6). In addition, compared to the control columns the significant reductions ($p < 0.05$; ANOVA, Tukey's test) in Chl-*a* contents were noticed in treatment groups

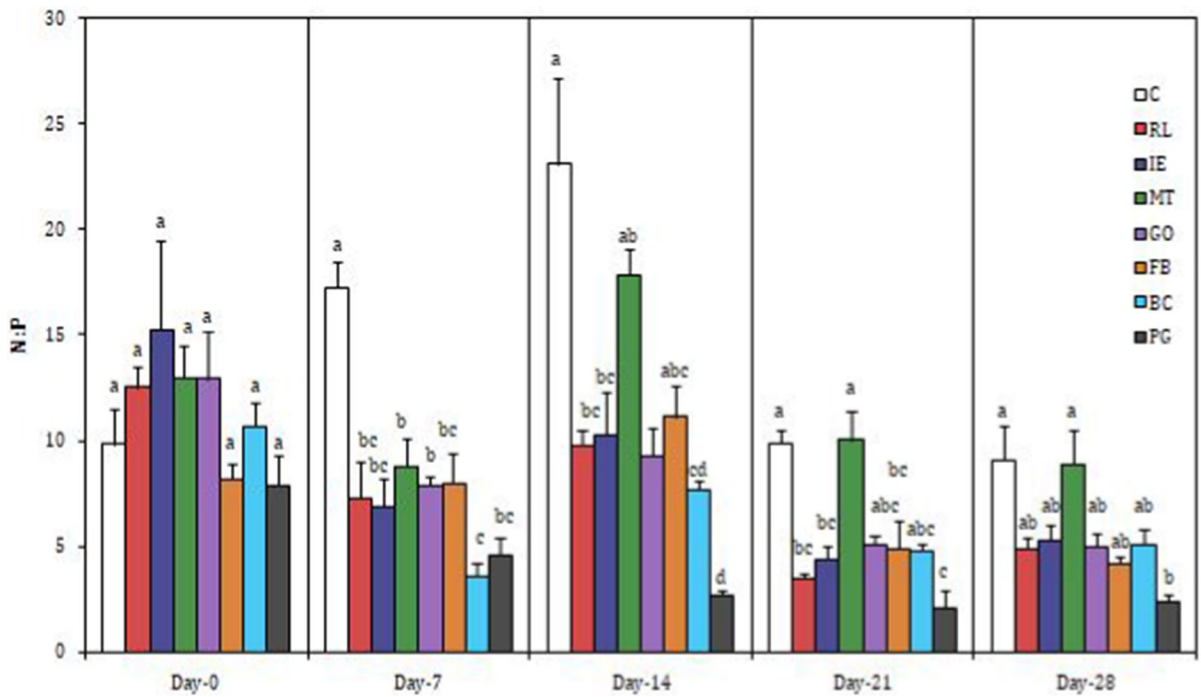
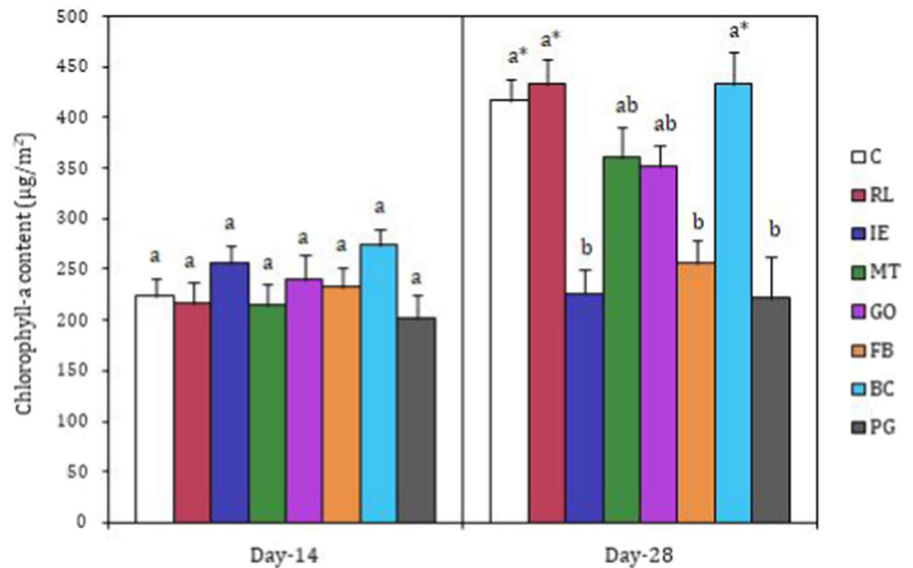


Fig. 5 Variations in dissolved inorganic nitrogen (N) to orthophosphate (P) ratios in the water columns among eight treatment groups. Values are presented as mean ± SE. For

every panel, different letters represent significant difference between treatments ($p < 0.05$)

Fig. 6 Comparison of Chlorophyll-*a* measured on surface of substrates from the microcosms of eight treatment groups. For every panel, different superscripted letters represent significant difference between treatments ($p < 0.05$). *represents significant difference ($p < 0.05$) of a treatment between time interval. Values are presented as mean ± SE



with *I. exustus*, *F. bengalensis* and *P. globosa*. The treatment *M. tuberculata* and *G. orcula* also showed lower Chl-*a* content compared to the control, however their influences were not significant ($p > 0.05$;

Tukey’s HSD). The Chl-*a* measures also changed over time in all treatment columns with or without snails (Fig. 6; Table 3; RM-ANOVA, time effect, $p < 0.01$). Except *I. exustus*, in all seven treatments,

the Chl-*a* concentration measures at the end of the experiment (on day 28) were higher than their initial values (on day 14). Moreover, increase in concentrations between two sampling events were significant ($p < 0.05$; ANOVA, Tukey's test) for the control columns and in presence of snail *R. luteola* and *B. costula*. Thus, the differences in the outcome of the Chl-*a* content can be attributed to the differences in the species specific snail sizes, trophic status and the feeding activities.

Changes in sediment properties

A trend in the change of each of the sediment parameters to change over time, and the changes estimated in between the start to end of the experiment were significant (Student's t-test, $p < 0.05$) in some of the snail treatments (Table 4). However, at the end of the experiment there were no significant snail dependent treatment variations observed on the percentage OC contents of the sediment ($F_{7, 23} = 1.35$; $P = 0.29$), the significant treatments variability were prominent for AP contents ($F_{7, 23} = 7.49$; $P < 0.05$), TN contents ($F_{7, 23} = 6.82$; $P < 0.05$), porosity ($F_{7, 23} = 43.6$; $P < 0.05$) and WHC ($F_{7, 23} = 12.11$; $P < 0.05$) of the sediment samples that received various treatments. Variations in physicochemical properties of sediment among different treatment microcosms

are represented in Table 4. However, considering the %OC content of the sediment no such significant differences observed, the AP, TN, porosity and WHC measures were found to differ significantly among few treatment groups (Table 4) at the end of the 28 days of experiment. At the end of the study, in presence of *R. luteola*, *I. exustus*, *M. tuberculata* and *F. bengalensis* the AP contents increased significantly ($p < 0.05$; Tukey's HSD) compared to the control sediment samples. The TN contents in the sediment samples in presence of *R. luteola* and *F. bengalensis* also increased significantly ($p < 0.05$; Tukey's HSD) compared to the control columns. Nevertheless, the presence of any snail species could increase the porosity and WHC of the sediment. Indeed significant changes ($p < 0.05$; Tukey's HSD) were noticed in *M. tuberculata*, *G. orcula* and *F. bengalensis* for porosity and *I. exustus*, *M. tuberculata*, *F. bengalensis* and *B. costula* for WHC (Table 4).

Discussion

The freshwater operculate snails *F. bengalensis*, *G. orcula*, *M. tuberculata* and *B. costula* were observed to be more active on the sediment surface, altering the natural texture in the upper few millimetres of the sediment layer, as in *V. viviparus* (Estragnat et al.

Table 4 Sediment properties (%OC=percent of organic carbon, AP=available phosphorous, TN=total nitrogen, WHC=water holding capacity) at the beginning (day-0) and at the end (day-28) of experiment for eight treatments (C=Con-

trol, RL=*R. luteola*, IE=*I. exustus*, MT=*M. tuberculata*, GO=*G. orcula*, FB=*F. bengalensis*, BC=*B. costula*, PG=*P. globosa* treatment)

	% OC	AP (ppm)	TN (gm/kg)	Porosity	WHC
Day-0					
Fresh Sediment	1.01 ± 0.05 ^B	0.83 ± 0.02 ^B	1.11 ± 0.01 ^B	0.310 ± 0.000 ^B	0.279 ± 0.001 ^B
Day-28					
C	1.06 ± 0.16 ^{Ba}	0.94 ± 0.09 ^{Bd}	1.13 ± 0.07 ^{Bc}	0.318 ± 0.001 ^{Bc}	0.285 ± 0.001 ^{Bbd}
RL	1.22 ± 0.1 ^{Ba}	1.69 ± 0.06 ^{Aa}	1.77 ± 0.04 ^{Aa}	0.319 ± 0.001 ^{Bc}	0.301 ± 0.003 ^{Ac}
IE	0.86 ± 0.39 ^{Ba}	1.41 ± 0.02 ^{Aabc}	1.41 ± 0.02 ^{Aabc}	0.329 ± 0.005 ^{Bc}	0.314 ± .01 ^{Aac}
MT	1.33 ± .07 ^{Aa}	1.42 ± 0.02 ^{Aabc}	1.48 ± 0.02 ^{Aabc}	0.354 ± 0.003 ^{Ab}	0.337 ± 0.007 ^{Aa}
GO	1.19 ± 0.08 ^{Ba}	1.16 ± 0.23 ^{Abcd}	1.19 ± 0.23 ^{Bbc}	0.348 ± 0.001 ^{Ab}	0.307 ± 0.002 ^{Ac}
FB	1.48 ± 0.06 ^{Aa}	1.54 ± 0.01 ^{Aab}	1.59 ± 0.03 ^{Aab}	0.372 ± 0.002 ^{Aa}	0.335 ± 0.005 ^{Aa}
BC	1.08 ± 0.03 ^{Ba}	1.09 ± 0.02 ^{Ac}	1.14 ± 0.02 ^{Bc}	0.331 ± 0.005 ^{Ac}	0.295 ± 0.007 ^{Bc}
PG	1.17 ± 0.04 ^{Ba}	1.38 ± 0.01 ^{Aabcd}	1.42 ± 0.04 ^{Aabc}	0.321 ± 0.003 ^{Bc}	0.289 ± 0.003 ^{Bbc}

Values are presented as mean ± SE; $n = 3$. Different superscripted capital letters represent significant difference ($P < 0.05$) of within-column data between times (Day-0–Day-28) for each treatment. Different superscripted small letters represent significant difference ($P < 0.05$) between treatments compared for each variable at the end of the experiment

2020). On the other hand, *R. luteola*, *I. exustus* and *P. globosa* did not exhibit burrowing, bulldozing or nudging activities and enormous mucus coated pellets were deposited on the sediment surfaces in presence of these snails, consistent with the activities of some pelletizer gastropods like *Hydrobia ulvae* (Orvain et al. 2004). In comparison with the control, all the snail species (treatments) showed higher $\text{NH}_4^+\text{-N}$ and $\text{PO}_4^{3-}\text{-P}$ flux to the overlying water (Fig. 3a and d). Among the snail treatments, the maximum efflux was estimated in *F. bengalensis*, whereas, minimum in presence of *P. globosa* and *R. luteola* for $\text{NH}_4^+\text{-N}$ and $\text{PO}_4^{3-}\text{-P}$, respectively. An initial rise in $\text{NH}_4^+\text{-N}$ concentrations after the introduction of snails to the microcosms was possibly due to acceleration of decomposition of sediment organic matter (Bowen et al. 2014) and dispersion of solutes (Li et al. 2019; Bartl et al. 2019) stimulated by the biotic activities on sediment (Zheng et al. 2009; Nicholaus and Zheng 2014). As the heterotrophic mineralization on the snail mucus and faeces is a 'time-lagged' process (Li et al. 2019), initially it contributed to the changes in $\text{NH}_4^+\text{-N}$ and $\text{PO}_4^{3-}\text{-P}$ concentrations measured in the overlying water as compared to the bioturbation driven release of nutrients (Pieczyńska and Jachimowicz-Janaszek 1998; Wilhelm et al. 1999; Pinowska 2002). Surface level bioturbation activities by the freshwater snails enhance sediment oxygen consumption, which reduce the oxygen saturation (Zhang et al. 2013; Chen et al. 2016; Yang et al. 2020). Decreasing oxygen penetration enhances anoxic condition, thereby promoting reduction of Fe (III) and Al (III) forms of elements in the sediment into soluble and labile forms to increase the release of $\text{PO}_4^{3-}\text{-P}$ into water (Zheng et al. 2009; Yang et al. 2020). Evidently activities of all the snail species resulted in increased $\text{NH}_4^+\text{-N}$ and $\text{PO}_4^{3-}\text{-P}$ concentrations in the water column as observed in the present study. The quantitative differences in $\text{NH}_4^+\text{-N}$ and $\text{PO}_4^{3-}\text{-P}$ flux among the snail treatments declined with the shell length of the snails (Fig. 4). These results are consistent with the findings of some previous work on the influences of various freshwater gastropods on nutrient release (Underwood 1991; Pinowska 2002; Zhang et al. 2013; Yang et al. 2020).

Complex nitrification–denitrification process along with altered redox condition at the interface and microbial processes regulate the oxidized forms of nitrogen in aquatic ecosystem (Pelegri and Blackburn

1996). During the course of the experiment certain decline in $\text{NH}_4^+\text{-N}$ concentration with subsequent increase in oxidized forms of nitrogen compounds in presence of *M. tuberculata*, *G. orcula* and *F. bengalensis* represented higher rate of nitrification (Pignoneret et al. 2016; Gautreau et al. 2020). Increase rate of nitrification may be associated with higher mixing activities of organisms to superficial oxic zone of the sediment and subsequent raise in oxygen absorption by the sediment from oxygenated water attributed in course of organismic respiration (Zhang et al. 2013; Nicholaus and Zheng 2014). These results and interpretations are somehow consistent with the findings of earlier work on the freshwater snail *B. aeruginosa* (Zhu et al. 2013).

Some previous work already demonstrated the ability of suspension feeder to increase the conductivity of the overlying water (Zhu et al. 2013; Li et al. 2019). The ingestion and excretion by the snail increase the amount of biodeposits on the sediment. The mixing of sediment by the organisms increases the porosity of the substratum which alters the permeability of the substratum (Gingras et al. 2012) that may influence the diffusive removal of soluble substances to its habitat to increase the TDS of the medium. As the TDS shows positive correlation with the conductivity (Chidya et al. 2016), confirming that the existence of snails can increase the ionic content in the water. It was difficult to determine the main factors contributing to changes in ionic content of the water which required further investigations.

The littoral dwelling snails show specific affinity for organic rich sediment (Sarkar 1992; Zheng et al. 2009; Tripathy and Mukhopadhyay 2015) and their preferences on organic deposits may reduce the organic content of the sediment (Zheng et al. 2009). Conversely, the active ingestion and subsequent excretion of these snails can enhance the amount of biodeposits (Giles and Pilditch 2006; Zheng et al. 2009; Zhu et al. 2013). In the present study there was no such significant difference in sediment OC content observed in any microcosms with snail compared to the microcosms without snail (Table 4). This may be because of low bioturbation effect of snail, i.e. restricted only to the superficial sediment layer and short duration of the experiment (28 days). Except for *R. luteola* and *F. bengalensis*, none of the snail treatments showed significant changes in sediment TN content compared to the control. Higher rate of

total inorganic nitrogen release and PO_4^{3-} -P flux to the overlying water accompanied with minimal rise in TN and AP contents in the sediment samples of all snail treatments were observed. Such modifications may have been due to enhanced organic mineralisation (Kuntz and Tyler 2018) and nutrient transformation (Zheng et al. 2009). The impact of the impounding of organic matter and/or bacterial assimilation of dissolved nutrients present in the overlying water (Mermillod-Blondin et al. 2004) may also contribute to such modifications. Benthic macro-fauna may modify the physical characteristics of the sediments, as observed in *F. bengalensis*, *G. orcula* and *M. tuberculata*, where, the high sediment mixing rate may have contributed to the enhanced porosity of the superficial sediment layer. A corresponding increase in mineralization, diffusive flux of nutrients and solubilisation may increase the concentration of dissolved solid and ionic release to the water (Nicholaus and Zheng, 2014; Hannides and Aller 2016). Species specific ability in alteration in the physical characteristics of the sediment may vary in accordance with the biomass or the shell length of the snails.

Different empirical studies reveal that the presence of snails can stimulate algal growth (Hillebrand and Kahlert 2002; Zhang et al. 2013). Complying with this observation, in *R. luteola* and *B. costula* treatments, the amount of chl-*a* was increased compared to the control columns. However, in the presence of *I. exustus*, *M. tuberculata*, *G. orcula* and *F. bengalensis* and *P. globosa*, the amount of periphytic chl-*a* decreased compared to control (Fig. 6). During the experiment in all snail treatment cores, the snails were either found to crawl over sediment or graze over the algal deposition on the inner wall of glass column. Earlier studies suggest that snail bioturbation can enhance both planktonic or periphytic algal growth by increasing release of nutrients from the sediment (Rybak 2002; Zheng et al. 2009; Nicholaus and Zheng 2014; Mo et al., 2017; Kuntz and Tyler 2018). Simultaneous grazing activity of snail also reduces algal biomass (Li et al. 2019). Therefore, the variation in algal deposition among the treatment cores in presence or absence of snail was the consequences of these two opposite forces.

Considering the freshwater snails as common elements of the lake, pond, and allied wetlands in varied landscapes, the functional roles, particularly bioturbation property bears considerable significance.

In situations where the snails are the dominant members of the freshwater community, the bioturbation function may unleash the nutrients in the water column facilitating the uptake by the producer community. As a consequence, the growth and productivity of the system may increase providing further source for the sustenance of diverse taxa. While reports are available for the displacement of the sediments and thus nutrients by other macroinvertebrates, in Indian context as well as in similar regions with rich malacofauna, the role of the snails will prove important in restoration and sustenance of the freshwater bodies as observed in this study.

Conclusion

The results of the present study confirm that the common freshwater snails of India can influence the physicochemical features of the water and sediment through bioturbation. The sediment texture was changed, more prominently in presence of the operculate snails, namely, *F. bengalensis*, *M. tuberculata*, and *B. costula* than the non-operculate snails, namely, *R. luteola* and *I. exustus*. The maximum deposition of mucus coated pellets on the sediment surface was recorded in presence of non-operculate snails. While presence of the recorded snails increased the porosity and water holding capacity in sediment, but no significant variations in the OC, TN and AP were observed with reference to the control. Except in case of *R. luteola* and *B. costula*, the periphytic Chl-*a* content was reduced in all other snail treatments. A species-specific and significant increase in the NH_4^+ -N and PO_4^{3-} -P flux to the water column was observed. Thus, inclusion of the snails as biological components in the management of the freshwater ecosystem can be recommended to facilitate habitat manipulation in ecological processes.

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Availability of data and materials The data concerning experiments of the present study can be shared upon authentic and reasonable request.

Declarations

Conflict of interests As authors of this article we declare no competing interest.

Consent for publication Not applicable.

Consent to participate Not applicable.

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