INTERACTION BETWEEN GRAZING BY STARFISH (*Parvulastra exigua*) AND NUTRIENT ENRICHMENT ON INTERTIDAL SOFT-SEDIMENT COMMUNITIES

BY

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PLAGIARISM DECLARATION

I hereby declare that this is my own work and effort and that it has not been previously submitted at any institution for the award of the master's degree qualification. To the best of my knowledge, this thesis contains no material that has previously been published or written by any other person. Where other sources of information have been used, they have been acknowledged.

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Abstract

Top-down and bottom-up forces are important determinants of community structure, having significant control over diversity metrics. Bottom-up control is frequently represented by nutrient level, which determines ecosystem productivity. Top-down control includes consumer (e.g. grazers and predators) effects on lower trophic levels. Consumers determine community structure through 2 main interacting mechanisms viz. by creating physical disturbances to the habitat and by removing other species through consumption. Both of these mechanisms rely predominantly on the effectiveness of the consumer in terms of exerting dominance on members of lower trophic levels or in terms of generating physical disturbances to the ecosystem as well as the type of community being affected. Today, the pervasive overharvesting of important consumers and increased nutrient loading in coastal natural habitats are globally recognised as factors requiring urgent attention. Such activities can significantly modify the interaction between top-down and bottom-up forces and the consequent effects on communities.

This dissertation presents results of a field experiment investigating the interactive effects of top-down (grazing) and bottom-up (nutrients) effects on intertidal soft-sediment communities on the West coast of South Africa. The herbivorous starfish *(Parvulastra exigua*) was the grazer used in the study, and manipulated to generate five levels of increasing starfish densities. Slow-release fertilizer (Plantacote N: P: K) was utilized to generate three levels of increasing nutrient levels effects. The experiment was undertaken based upon three central aims, the first of which was to examine if nutrient enhancement can interact with increasing starfish densities to structure communities of producers (microalgae) and consumers (macrofauna). The second aim was to examine if the interactive effects of nutrients and starfish densities can influence the microalgal biomass (Chl a) and extracellular polymeric substances (EPS) associated with the sediment. The third aim was to investigate if the interactive effects of nutrients and starfish densities can affect functional group composition of macrofauna.

The findings did not show interactive effects of nutrients and starfish on microalgal and macrofaunal community assemblages. There was, however, a strong influence of nutrient enhancement on both communities. The responses of microalgal morphotypes and macrofaunal species to nutrient enhancement were limited. Seventeen of the 59 microalgal morphotypes which dominated community structure were affected by nutrient enhancement whereas two of the six dominant macrofaunal species were affected by nutrient enhancement. These results indicate that the effects of nutrients on both communities operate on a few taxa. Microalgal morphotypes showed varying responses to increasing nutrient levels including unimodal hump and U-shapes, and linear increases and decreases. The interactive effect of nutrient enrichment and starfish densities was only apparent for few microalgal morphotypes, macrofaunal species and EPS concentration. For diversity indices, only macrofaunal species diversity was influenced by nutrients but the response pattern was not graphically obvious. At the functional group level, increasing nutrient enhancement resulted in an increase in abundance of suspension feeders.

Starfish density had a significant effect on EPS concentration, with a hump-shaped response to increasing starfish densities at the first level of nutrient enrichment. Microalgal species richness and diversity were also affected by starfish densities and responded in a U-shape manner against increasing starfish densities at the first and second levels of nutrient enrichment. Surface and burrowing predators/scavengers and suspension feeders were affected by starfish densities. Suspension feeders showed a hump-shaped response against increasing starfish densities at all nutrient levels.

In conclusion, the top-down force exerted by *P. exigua* on producers and consumers was very limited in this study and the bottom-up effect of nutrients was much more important as a structuring agent of producer and consumer assemblages. The findings of the study are evaluated against the predictions of the grazer-reversal hypothesis, which predicts a promotive effect of grazing on diversity at high nutrient levels but a negative effect on diversity at low nutrient levels.

CHAPTER 1: GENERAL INTRODUCTION

1.1 Disturbance and productivity as regulators of community structure

Top-down and bottom-up forces are important determinants of community structure (Burkepile & Hay 2006, Posey *et al*., 2002, Mork *et al*., 2009), having important effects on diversity measures (e.g. species richness and evenness, Posey *et al.,* 1995). Generally, bottom-up forces include nutrient and sunlight availability. Both factors enhance ecosystem productivity by promoting primary producer abundance or biomass. Top-down forces are generally represented by consumers such as grazers and predators (Posey *et al.,* 1995, Uthicke 2001), which are both significant sources of disturbance. Disturbance can be defined as a destruction of biomass that may lead to an opening of space and resources to be used by new species in a habitat (van der Maarel 1993, Roxburgh *et al*., 2004, Wootton *et al*., 2009). It can be caused by either biotic or abiotic factors. Regarding biotic factors, consumers can cause disturbances by consumption and through the physical alteration of the habitat (Wootton *et al*., 2009).

Disturbances caused by consumption and/or the physical alteration of the habitat can play a crucial role in determining diversity and ecosystem structure and functioning (Pillay *et al.,* 2009). In terrestrial environments, the trampling effects of cattle on soil invertebrates have been reported to reduce the abundance, diversity, and richness of microarthropods (Cole *et al.,* 2008). Similarly, the effects of cultivation and soil mixing caused by humans have been previously reported to influence groups of microathropods (Crossley *et al.,* 1992, Berch *et al.,* 2007). After a disturbance, communities may undergo several successional changes via species recolonization, which includes species recruitment, immigration and growth. The response of the above variables depends substantially on the type of ecosystem involved and biological traits of the taxa present in the ecosystem (Plante *et al.,* 2010).

Grazers are prime examples of organisms that generate disturbance primarily through consumption and are known to structure communities in all major ecosystems. Grazing species may have positive and negative effects on the community with both direct and indirect influences (Morrisey 1988, Anderson 1999) depending on the density of grazers. Community responses to grazers also differ significantly depending on the type of grazer species and their densities and feeding mechanisms (Underwood 1978, Branch & Branch 1980). For example, grazers can have specialized feeding apparatus and exert significant control on assemblages, while others lack sophisticated feeding organs and have less of an impact on communities. Dominant grazers usually exhibit strong influences on a community, especially when occurring in dense aggregates. For instance, high densities of grazing limpets have been reported to reduce algal abundance (Guerry 2006) while also influencing sessile invertebrates (Dayton 1971) through bulldozing.

Limpets feed by lightly crawling over the rocks while efficiently scraping the rock surface for microalgae with a radula (Geury 2006). Sea urchins are also amongst the most effective benthic grazers that are capable of inducing changes in local community, and feed using a highly specialised feeding organ viz. Aristotle's Lantern. Some grazing species, however, such as herbivorous fish, lack specialised feeding organs, and exert less of an influence on community structure.

Ecosystem productivity is usually dependant on sunlight availability, nutrient input or primary producer abundance or biomass. Primary producers supply energy to upper trophic levels and at optimum nutrient levels, primary producer biomass can became enhanced (Worm *et al.,* 2000). However, if nutrient enrichment becomes excessive, especially over large spatio-temporal scale, algal primary productivity can increase rapidly (Guerry 2006, Baggett *et al.,* 2010) and lead to cascading effects in ecosystem (Armitage & Fong 2004).

Today, nutrient impacts on ecosystems have received significant attention (Sundback & Snoeijs 1991) following the high rate of nutrient input in coastal ecosystems (Mork *et al*., 2009, Shin & Cheung 2010). One basic way in which humans increase nutrients in ecosystems is through the generous application of fertilizers, mainly nitrogen and phosphorus, for agricultural purposes (Vitousek *et al.,* 1997b, Posey *et al.,* 1999, Mork *et al*., 2009). At the appropriate temperature, oxygen concentration and adequate sunlight, increased nutrient loading can result in eutrophication (Miller *et al.,* 1999, Mork *et* al., 2009, Shin & Cheung 2010) with general effects that include hypoxia, noxious algal blooms (Hagy *et al.,* 2005) and poor water quality (Rabalais *et al.,* 2007, Spivak *et al.,* 2009, Baggett *et al.,* 2010). Sediment hypoxia, which occurs as a result of oxygen depletion due to decomposition of algal blooms, may cause loss of species (Rabalais *et al.,* 2007). These factors can alter the trophic structure and the functioning of aquatic ecosystems (Baden *et al.,* 1990, Rosenburg *et al.*, 1992, Suding *et al.,* 2005). In lakes, eutrophication has been reported to create a shift in the dominance of taxonomic groups of phytoplankton, causing alterations in biomass and composition of various trophic levels (Sundback & Snoeijs 1991). Similarly, in other fresh water ecosystems, a shift in dominance of the main benthic algal groups has been reported (Carrick *et al.* 1988).

Many authors have examined the effects of nutrient enrichment on primary production (Sundback & Snoeijs 1991, Bucolo *et al.,* 2008, Mork *et al*., 2009, Guerry *et al.,* 2009, Werner & Matthiessen 2013), herbivores (Burkepile & Hay 2006) and predators (Posey *et al.,* 2002). In some instances, these factors have been examined together to investigate the interactive impacts on communities (Posey *et al.,* 2002). Nutrient enrichment has been shown to increase microalgal biomass in fresh water lakes (McQueen *et al.,* 1989, Power 1992, Posey *et al*., 2002), rivers (Deegan *et al.,* 1997) and in marine coastal ecosystems (Beukema 1991, Pitta *et al.,* 1998). Benthic microalgal diversity has been reported to be susceptible to higher nutrient levels in salt marshes (Sullivan 1976), fresh water (Carrick *et al.* 1988) and estuarine ecosystems (Admiraal *et al.,* 1989. Sundback & Snoeijs 1991). In coral reefs, increased algal biomass due to higher nutrient concentration has been reported to outcompete the corals for space (Mork *et al*., 2009). Increased microalgal biomass can cause cascading effects, which, in turn, increases food availability but only favours certain species possibly at higher trophic levels (Posey *et al.,* 1995, Sarda *et al.,* 1996). Elevated nutrients can also modify the species composition of primary producer assemblages by favouring fast growing, opportunistic algal species (Valiela *et al.,* 1997). Nutrient enrichment has been shown to modify the structure of aquatic fungal and bacterial communities (Beukema 1991, Artigas *et al*., 2007), elevate productivity and producer and consumer biomass (Beukema, 1991 Posey *et al*., 2006), while reducing overall diversity (Snelgrove, 1998).

In terms of soft-sediment invertebrates, positive responses of benthic fauna to nutrient enhancement have been documented in deep waters (Josefson & Rasmussen 1999). Josefson & Rosenberg (1988), however, reported no positive effects on benthic macrofauna in response to nutrient enrichment in shallow water ecosystems. Several authors reported an increase in growth rate and abundances of certain macrofaunal species due to nutrient increase (Tsutsumi 1990, Posey *et al.,* 1999, Wolfrath 1992). Others have reported a reduction in macrofaunal diversity, loss of certain deep burrowing taxa (Simboura *et al.,* 1995) and increased food source availability for other benthic grazers (Posey *et al.,* 1995, Posey *et al.,* 1999).

1.2 Relationship between Productivity and Disturbance

The interaction between disturbance and productivity on species diversity or richness has been explored using different models. The first model that predicted the effects of disturbance and productivity on diversity is the dynamic equilibrium model proposed by Huston (1979). This model has been supported by Proulx & Mazumder (1998) after examining the interactive effect of grazing and nutrient on plant species richness and developed the grazer-reversal hypothesis (Fig. 1.1). This model predicts that (1) under high-nutrient levels, an increase in grazing pressure may increase species richness by reducing competition, (2) at low-nutrient levels, grazing may decrease richness by adding to stress, and (3) at intermediate nutrient levels richness may increase in response to intermediate grazing pressure (Fig. 1.1). Kondoh (2001) also presented results supportive of this model using rocky shore communities and demonstrated a positive relationship between disturbance and diversity when productivity is high, a negative relationship when productivity is low and a unidomal correlation when productivity is at intermediate level.

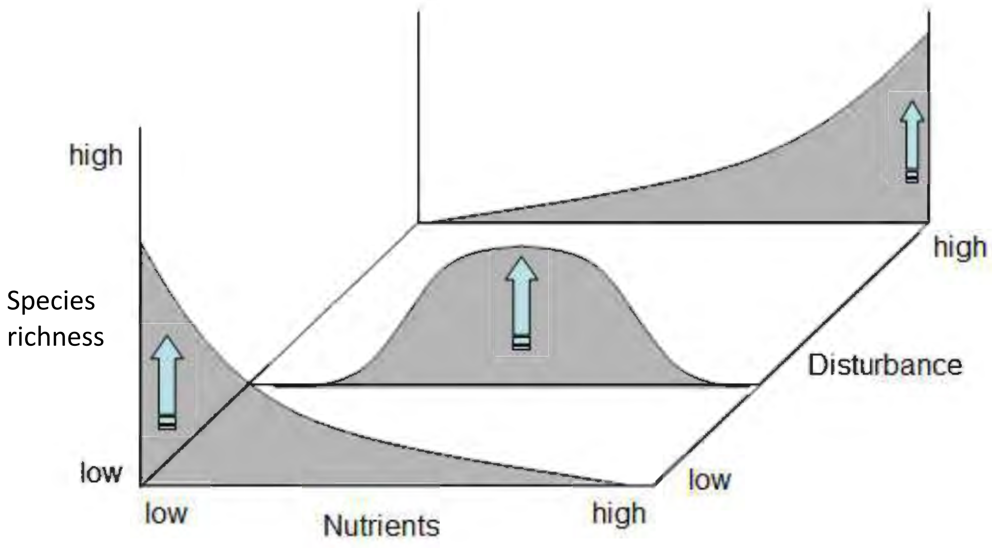


Fig. 1.1: Conceptual model showing the interacting effects of nutrients (z-axis) and disturbance (x axis) on richness (y axis) Huston 1979.

The grazer reversal model, however, does not necessarily apply to all communities or maintain consistency under different ecological scenarios, and different communities may respond differently to varying levels of nutrients and disturbance (Cole *et al.,* 2008). Community measures can also respond differently. For instance, Hillebrand (2003) showed that species evenness, but not species richness followed the predictions of the grazer reversal hypothesis. Local factors such as light availability, or some other unknown environmental trait, may be responsible for modifying grazer-nutrient interactions (Liess *et al.,* 2009). Some authors suggest that competitive abilities of different species may be the primary mechanism that is likely to cause different responses of diversity (Abrahams 1988).

Several studies have investigated interactive effects of productivity and disturbance particularly grazers (which act as agents of disturbance) and nutrients (which affect productivity) on diversity (Gough & Grace 1998, Wilson & Tilman 2002, Worm *et al.,* 2002). In some instances, the results oppose the predictions of the grazer-reversal hypothesis, while some cases are in agreement with predictions. Non-interactive effects between grazers and nutrients have also been documented (e.g. Nielsen 2003, Hillebrand 2003). In aquatic microcosms, Warren & Spencer (1996) found no existing interaction between productivity and disturbance on insect communities. Worm *et al.,* (2002) demonstrated a shift in the algal diversity peak from low to high productivity due to increasing grazing level. In lake ecosystems, Hillebrand (2003) found that nutrients and grazers can act individually and produce contrasting effects on diversity of periphyton. Guerry (2006) pointed out that a number of factors can contribute to the lack of agreement between studies. Experiments are usually conducted over different spatial scales, manipulate different levels of productivity but usually fail to attain a desirable disturbance level. Different diversity metrics are also used in analysing community responses. These and other factors are likely responsible for the lack of consensus in studies related to productivity and disturbance effects on communities.

Given the uncertainty regarding the applicability and generality of the grazer-reversal hypothesis, it is of interest to apply this hypothesis to soft-sediment communities. A point worth raising is that competition, which has been argued to be an important determinant of the outcome of interactions between grazing and nutrients, is considered of less significance in soft-sediments due to the natural structure of the habitat, being arranged in a three dimensional form, which potentially allows organisms to burrow into the sediment, thereby preventing competition for space (Wilson 1991, Dawson & Pillay 2011). This thesis therefore focuses on the interactive effects of grazing by starfish (*Parvulastra exigua)* as a form of disturbance, and nutrient enrichment in structuring intertidal soft-sediment communities, particularly producers (microalgae) and consumers (macrofauna), to determine if responses of producers and consumers follow the preditions of the grazer reversal hypothesis.

CHAPTER 2: EXPERIMENTAL EVALUATION OF GRAZING AND NUTRIENT ENRICHMENT IN INTERTIDAL SOFT-SEDIMENT

2.1 Introduction

Today, increasing rates of coastal development and human population growth have raised key questions regarding the modification of natural coastal habitats, species co-existence and maintenance of biodiversity (Mork *et al.,* 2009). Apart from the high rates of nutrient discharge (Burkepile & Hay 2006, Baggett *et al.,* 2010), these ecosystems also face a substantial threat due to human removal of consumers. Important consumers such as herbivorous fish, sea urchins and other grazing invertebrates are intensively harvested from shallow water coastal ecosystems (Duffy 2003, Mork *et al.,* 2009). These activities can exert significant stresses on different trophic levels which may result in trophic cascading, including changes in abundance of consumed organisms through impacts on higher consumers.

Harvesting of important consumers may cause population explosions of organisms at lower trophic levels. For instance, elimination of predatory fish has been reported to cause population explosions of jelly fish through the release of predation pressure (Lynam *et al.,* 2005, Xian *et al.,* 2005). In the absence of macro-and meso-grazers, fast growing species of ephemeral green algae have been reported to proliferate (Mork *et al.,* 2009). The removal of grazers favours some algal species, especially when nutrient levels are higher (McClanahan *et al.,* 2002). In terrestrial studies, the spraying of insecticides has been reported to increase the abundance of grass (Allan & Crawley 2011). Given the role of human activities in modifying consumer abundance and nutrient levels in coastal systems, it is important to understand the influence of grazing and nutrients on communities, since the magnitude of the impact of individual factors or their interaction may differ with regional biogeography (Mork *et al.,* 2009).

Grazers are often regarded as important determinants of community structure. They play a significant role in structuring the food web by linking primary producers and higher trophic levels, regulating plant biomass and altering the community composition (Spivak *et al.,* 2009) and altering competitive interaction between species (Allan & Crawley 2011). Grazers are also known to be important regulators of primary producer diversity. There are two major ways by which this can occur: grazers can promote diversity by consuming dominant plants or decrease diversity by feeding on infrequent species (Pacala & Crawley 1992). The effects of grazing have been well examined in marine benthic (Flint & Goldman 1975, Nicotri 1977, Jackson *et al.,* 2009, Pillay *et al.,* 2010), lake (Lowe & Hunter 1988) and terrestrial ecosystems (Gan *et al.,* 2012). Grazing can influence a wide range of biological processes including resource availability, habitat heterogeneity (Jackson *et al*., 2009) rates of succession and recruitment (Levinton & Stewart 1982) community biomass and diversity (Keleher *et al.,* 2003) and competitive interactions (Branch & Branch 1980).

This thesis focuses on the herbivorous asterinid starfish *Parvulastra exigua*, which is a small cushion starfish that can attain a maximum size of approximately 5 cm and frequently occurs in intertidal rocky shores in South Africa and Australia, but rarely inhabits intertidal soft-sediment (Pillay *et al.,* 2010). Langebaan Lagoon and Knysna Lagoon on the west and southern coasts of South Africa are the only known soft-sediment ecosystem where *P. exigua* occurs (Pillay *et al.,* 2010, Barnes & Ellwood 2011). In Langebaan Lagoon, *P. exigua* occurs predominately in eelgrass beds, which unfortunately, have been deteriorating over the years, which may be linked with human activities (Pillay *et al.,* 2010). Further declines in eelgrass may thus lead to significant reductions in populations of *P. exigua* in Langebaan Lagoon. A recent study by Pillay *et al.,* (2010) showed unstable historical population trends of *P. exigua* abundance in Langebaan Lagoon. *Parvulastra exigua* was first recorded in the area in the 1950s. During the start of monitoring the species was very rare, occurred at average densities of 1.05 ± 0.26 ind. m^{-2} in 1972 and 2.9 ± 0.59 ind. m^{-2} in 1973. An increase in densities was recorded in 1980, peaking at 172.4 ± 48.5 ind. m^{-2}. However, between 1990 and 2007 the densities then declined and stabilised (10.4 ± 0.2 and 2 ± 0.8 ind. m^{-2}).

Studies examining the effects of grazing by starfish are limited, especially in soft-sediments (Pillay *et al.,* 2010), compared to what is known about the role of starfish as predators (Jackson *et al.,* 2009). Pillay *et al.,* (2010) identified two main mechanisms by which *P. exigua* structures soft-sediment communities other than through consumption viz: physical disturbance of sediment and alteration of sediment biogeochemistry. These types of starfish-sediment interactions have also been discussed by Dawson & Pillay (2011) who identified three major non-consumptive pathways by which *P. exigua* may structure communities: (1) pelletisation, in which starfish increase microbial colonisation rates through excretion, (2) bioturbation or physical disturbance of sediment which enhances its resuspension, and increases smothering and emigration of other species, and (3) production of extracellular polymeric substance (EPS). Generally, EPS can be defined as polysaccharides that hydrate on contact with water and link to create a mucus-like substance (Wotton 2004). It can be produced by microalgae, bacteria or larger invertebrates including the gastropods and polychaeta, in the form of mucus (Wotton 2004). EPS plays a significant ecological role, including (1) protection of organisms from desiccation, abrasion, predation, re-suspension, (2) stabilization of sediments (Paterson & Hagerthey 2001), (3) promotion of laminar flow of overlying water and (4) recruitment of benthic invertebrates (van Loosdrecht *et al.,* 1990, Lam *et al.,* 2005, Pillay *et al.,* 2007).

The aim of this thesis was to examine the interactive effects of grazing by the starfish *P. exigua* and nutrient enrichment on intertidal sandflat communities. More specifically, the investigation aimed to determine if producer (microalgae) and consumer (macrofauna) assemblages responded similarly to the interactive effects of grazing and nutrient enrichment and whether responses agree with the predictions of the grazer reversal hypothesis presented in Fig 1.1

It is hypothesized that diversity of both producers and consumers will:

  1.1 decrease with increasing *P. exigua* densities at background (unmanipulated) nutrient levels.
  1.2 follow a unimodal hump-shaped pattern against the increasing *P. exigua* densities at intermediate nutrient levels.
  1.3 increase with increasing *P. exigua* densities at higher nutrient levels.

It is also hypothesized that:

1. Microalgal biomass (Chl a) and Extracellular Polymeric Substances (EPS) will be influenced at each nutrient treatment as above.

2. At the functional level, suspension feeders, surface scavengers/predators and surface deposit feeders will:
   2.1 decrease with increasing *P. exigua* densities at background (unmanipulated) nutrient levels,
   2.2 follow a unimodal hump-shaped pattern against the increasing *P. exigua* densities at intermediate nutrient levels and,
   2.3 increase with increasing *P. exigua* densities at higher nutrient levels
3. Burrowing predators and burrowing deposit feeders will not be influence by nutrients and starfish as these factors are likely to be most influential at the sediment surface but not deeper.

2.2 Materials and methods

2.2.1 Study site

To test the interactive effects of starfish grazing and nutrients on microalgal and macrofaunal communities, a field experiment was conducted in the mid-shore zone of intertidal sand-flats at Bottelary in Langebaan Lagoon (Fig 2.1), located on the west coast of South Africa. Unlike most lagoons created from river flow into the sea, Langebaan Lagoon was formed as a result of fluctuations in the sea level over millions of years (Shaefer & Shaefer 1993). It is a 16km lagoon system divided into three management zones viz: a multipurpose recreational area (mostly water sports and controlled exploitation of marine resources), a limited recreational area (power boats and prohibited exploitation of marine resources) and a wilderness or sanctuary area (closed to the public). This study was conducted in the sanctuary area. Langebaan Lagoon is highly productive because of the fringing salt marshes and seagrass beds in the system (Shaefer & Shaefer 1993). Additional nutrients enter into the system during each tide as a result of the west coast upwelling.

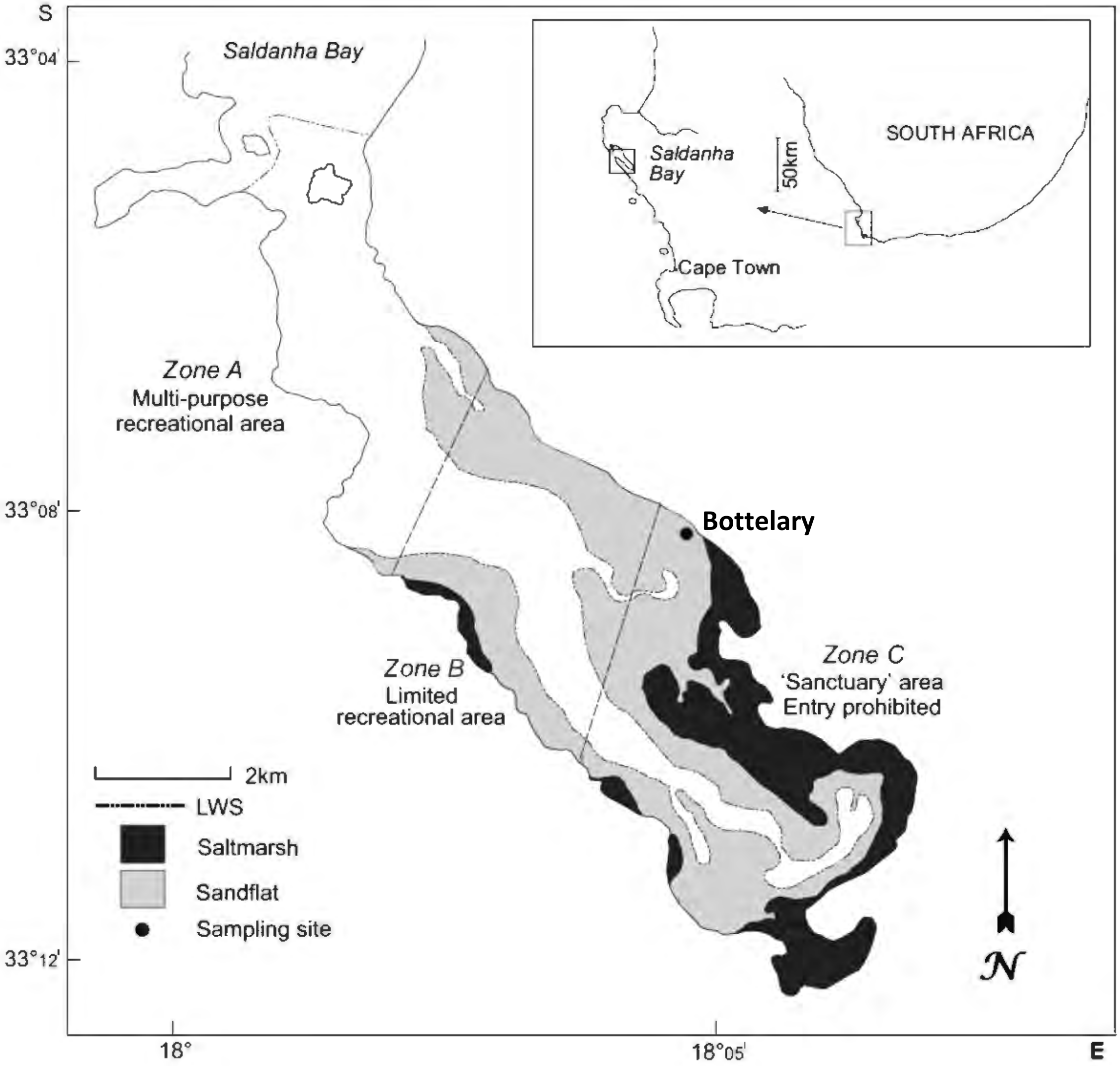


Fig. 2.1: Map of Langebaan Lagoon showing the position of Bottelary in which the experiment was undertaken.

2.2.2 Experimental design

The experiment was designed to manipulate (1) nutrient levels using controlled slow-release fertilizer (Plantacote-Plus™ 8M Aglukon) and (2) grazing disturbance by starfish (*Parvulastra exigua*). Nutrients enrichment was generated by using increasing quantities of fertilizer capsules composed of nitrogen, phosphorus, and potassium (N: P: K, 14: 9: 15). Black plastic crates (size 45 x 58 x 43 cm) were utilized to construct cages for the experiment. The bottom and sides of the crates were cut out and replaced by fly meshing (mesh diameter 1 mm). The cages were then randomly pushed to a depth of 10-15 cm into the sediment. Once installed into the sediment, the meshed top and sides of cages protrudes roughly 10-15cm above the sediment surface. A distance of 5 m was demarcated between the cages to avoid interference between the cages of different treatments.

Three levels of nutrients were used in the experiment (Fig 2.2) viz. (1) background or natural nutrient level, in which no fertilizer was added, (2) an intermediate level of enrichment, in which bags of fertilizer (250g/bag), were attached to the sides of each cage (1 bag per side), and (3) a high nutrient enrichment treatment, in which bags of fertilizer (500g/bag), were attached to the sides of each cage (1 bag per side). These nutrient levels were used based on data from previous field nutrient-enrichment trials, which showed that the above fertiliser quantities produced levels of enrichment in proportion to treatment designations (Cooper 2011).

The cages were left undisturbed for one week after installation in the field before starfish were introduced. Five levels of starfish densities were used in the experiment: control cages which were left free of starfish, and inclusion cages in which starfish were added to cages to achieve final densities of 10, 20, 30 and 40 starfish/m^2 following Dawson & Pillay (2011). Three replicate cages for each treatment were used in the experiment (Fig 2.2). The experiment was undertaken for the duration of 6 months (from November 2011 to April 2012), after which samples were collected to assess responses to treatments. All cages were randomly interspersed to avoid interference of unknown spatial variables with the main variables of interest (viz. nutrients and grazing).

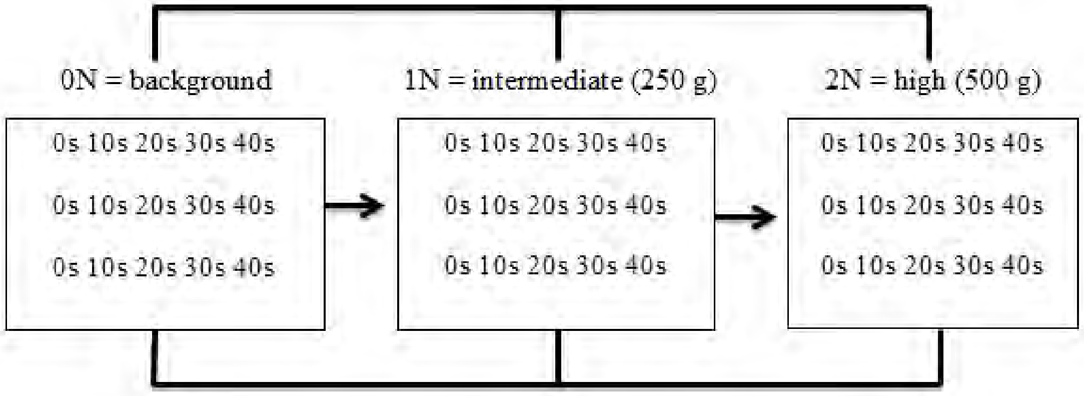


Fig. 2.2: Simplified illustration of the factorial experimental design with three replicates of five starfish density levels (s) within each nutrient treatment level (N). NB: all cages in the field were randomly interspersed and not blocked as illustrated above.

2.2.3 Data collection and analysis

Samples for extracellular polymeric substances (EPS), microalgal biomass (Chl a), and microalgal and macrofaunal assemblages were collected at the termination of the experiment. For EPS data, 3 cores (diameter = 2 cm, length = 1 cm) were taken from each cage. The samples were stored unpreserved in 50 ml vials and kept frozen at -20 °C for 24 h before they were analysed. To determine the EPS concentration, the phenol-sulfuric acid assay (Underwood & Paterson 1995) was employed, which entailed the addition of 2 ml of distilled water, 1 ml of 5 % aqueous phenol (wt/vol) and 5 ml of concentrated sulfuric acid (H_2SO_4) to a 2 g homogenised sub-sample of each sediment core. The mixture was then diluted 10 fold and absorbance was measured spectrophotometrically (Merck Spectroquant Pharo 100) against a reagent blank. The calibration was performed using a standard curve of absorbance against glucose concentration at 485 nm. EPS levels were expressed as mg g^{-1} sediment (wet weight).

Microalgal biomass (Chl a) was measured from sediment cores collected from cages (n = 3, diameter = 2 cm, length = 1 cm) and stored in 50 ml vials filled with 30 ml of 90 % acetone. Samples were then refrigerated for 48 h to extract Chl a before the fluorescence was measured using a Turner Designs Trilogy fluorometer (Welschmeyer 1994, Pillay *et al.,* 2010; Dawson and Pillay 2011).

Microalgal community structure data were obtained from cores (n = 3, diameter = 2 cm, length = 1 cm) which were preserved in Lugol's solution (1 % final solution, Taylor *et al.,* 2007). Each sample was homogenised for 10 s using a vortex mixer, and allowed to settle for 30 s before extracting a 5 ml sub-sample. The sub-sample was then centrifuged for 10 min at 3000 rpm, after which the supernatant was pipetted off and mixed with 0.3 ml of distilled water. The prepared sample was re-suspended using a vortex mixture. Using a Leica DM 750 compound microscope fitted with a Leica ICC50 camera, a 30 µl of the liquid sub-sample was viewed from the slide at 40 times magnification. Microalgal cells were counted from five fields of view per core. Data from the five fields of view for each of the three cores sampled from each cage were pooled into one sample per cage. Microalgae were classified to morphotype due to complications in identifying microalgal species.

Samples of macrofauna were obtained from sediment cores (diameter 10 cm, depth 10 cm). Two cores were collected within each of the 45 cages. Using a mesh of 500 µm, the samples were sieved through and preserved in a 70 % solution of ethanol with Rose Bengal to stain the organisms. Macrofauna were then identified to the lower possible taxonomic level. Where the identification of polychaetes was unfeasible due to their smaller size, those polychaetes were classified as juvenile polychaetes. Once identified, macrofauna were also classified into functional groups.

2.2.4 Statistical analyses

The EPS data were pooled to generate 3 replicates and then tested using a two-way ANOVA on IBM SPSS statistics 20, with nutrient treatment and starfish densities as the fixed factors. Where significant results were detected, a Tukey post-hoc test was employed to determine where the differences lay. Chl a data was treated and tested as above.

To test for the effects of grazing and nutrients on macrofaunal and microalgal community structure, the data were pooled to generate three replicates and analysed in PRIMER 6.1.11 with PERMANOVA+ 1.0.1. Prior to the test, the data were square root transformed, to reduce the effects of disproportionately abundant taxa. Using PERMANOVA (permutational multivariate analysis of variance) analysis with both nutrient and starfish fixed, the main and interactive effects of nutrients and starfish on both communities were examined. Where significant differences were found, a PAIR-WISE test was employed to reveal differences within treatments. Multidimensional scaling (MDS) ordinations were constructed based on resemblance matrices produced from Bray-Curtis similarities to illustrate spatial variation either due to nutrients or starfish effects. The DIVERSE function was used to calculate various community descriptors such as species richness (S), species evenness (J'), abundance (N) and Shannon-Wiener diversity (H') based on untransformed and unstandardized abundance data. Species richness in this experiment was effectively a measure of the number of microalgal morphotypes recorded per sample. Community measures were tested using a two-way ANOVA to determine the effects of grazing and nutrients with post-hoc tests to identify significant differences within treatments. The dominant taxa in microalgal and macrofaunal communities were selected using the SIMPER routine based on 90 % cut-off limit. Two-way ANOVA was applied to each of these taxa to test the influence of nutrients and starfish. The response of macrofaunal functional groups to treatments were also tested using a two-way ANOVA.

2.3 RESULTS

2.3.1 Extra cellular polymeric substances (EPS)

Total sediment EPS levels (Fig. 2.3) were significantly influenced by nutrients (ANOVA, $F_{2,45}$ = 3.997, p = 0.029), especially between treatments 0N and 2N (Tuky post hoc, p = 0.033). Mean EPS levels in the 2N treatment (0.378 ± 0.023 SE) were marginally lower than the 0N treatment (0.432 ± SE 0.026). Starfish densities also significantly affected the EPS levels (ANOVA, $F_{4,45}$ = 10.807, p < 0.005), with a clear hump-shaped pattern of EPS in response to increasing starfish densities at the 1N treatment (Fig. 2.3). There was no clear pattern in response of EPS levels against increasing starfish densities at 0N or 2N treatments. However, the 0N treatment showed lowest EPS level at densities of 30 starfish.m^{-2} whereas, highest EPS occurred at starfish densities of 20 ind.m^{-2} in 2N treatment. Nutrients also interacted with starfish densities to influence the EPS levels (ANOVA, $F_{8,45}$ = 6.032, p < 0.005).

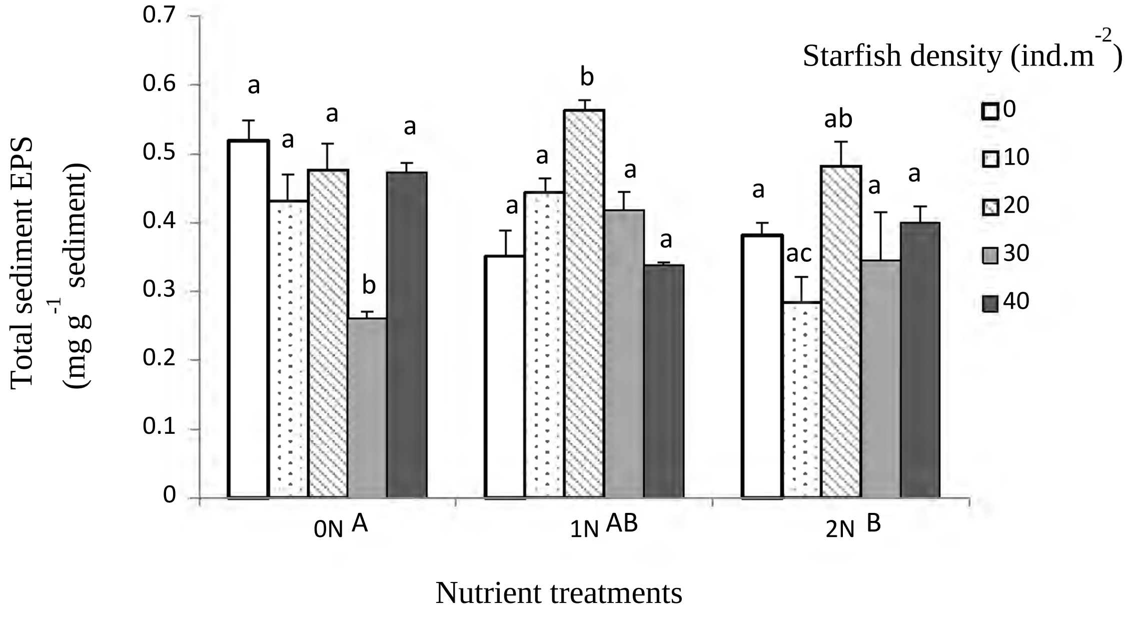


Fig 2.3: Sediment extracellular polymeric substance (EPS means ± 1SE) levels with increasing densities of *Parvulastra exigua* at each nutrient treatment. Different letters denote significant differences of total sediment EPS between nutrient treatments (in upper case) and among starfish densities within nutrient treatments (lower case).

2.3.2 Microalgal biomass (Chl *a*)

Chl *a* levels were not significantly affected by nutrients ((Fig 2.4, ANOVA, $F_{2,45}$ = 1.140, p = 0.333) or starfish densities (ANOVA, $F_{4,45}$ = 1.147, *p* = 0.344). There was also no interactive effects of nutrients and *P. exigua* densities on microalgal biomass (ANOVA, $F_{8,45}$ = 0.566, *p* = 0.797).

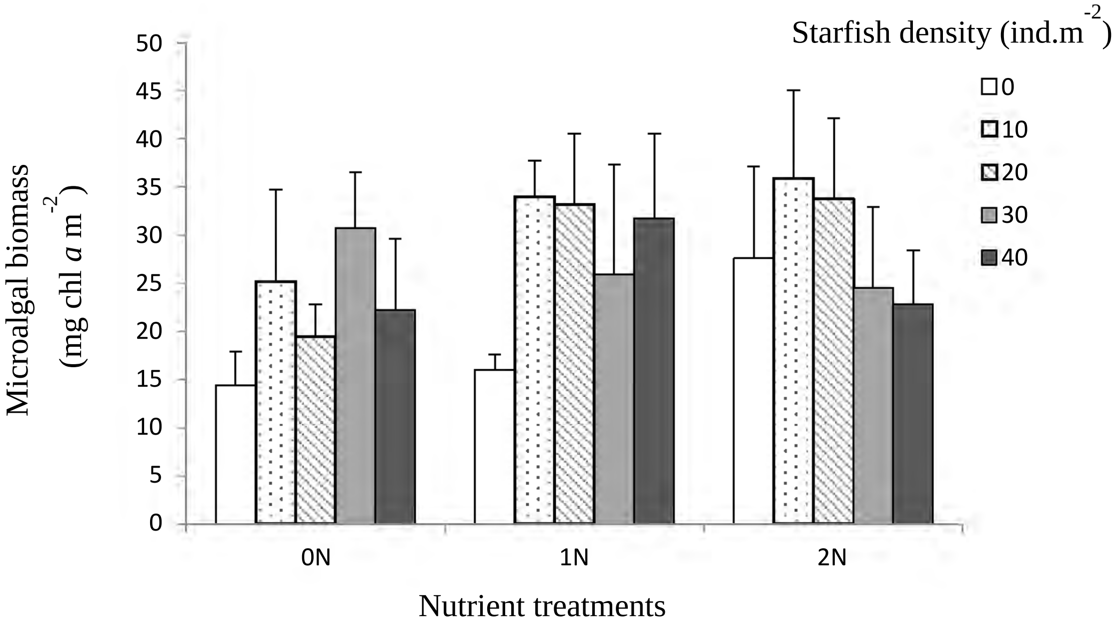


Fig. 2.4: Microalgal biomass (Chl *a*, means ± 1SE), with increasing densities of *Parvulastra exigua* at the three different nutrient treatments.

2.4 Microalgal community structure

2.4.1 Overall composition

One hundred and forty-two different microalgal morphotypes were identified and used for community analyses. Nutrient treatments had a significant influence on microalgal community structure (PERMANOVA, Pseudo-$F_{2,44}$ = 3.475, *p* = 0.001). Pair-wise tests further showed that the background nutrient level (0N) differed from both enriched nutrient treatments 1N (*t* = 1.765, *p* = 0.001) and 2N (*p* = 0.001, *t* =1.975), but the 1N treatment also differed from 2N (*t* = 1.838, *p* = 0.001). Neither densities of *P. exigua* (PERMANOVA, Pseudo-$F_{4,44}$ = 1.072, p = 0.309) nor the interaction between nutrients and *P. exigua* densities (PERMANOVA, Pseudo-$F_{8,44}$ = 0.853, p = 0.95) was found to influence microalgal community structure. Spatial variation in microalgal community structure in response to nutrients and starfish treatments is given in Fig. 2.5.

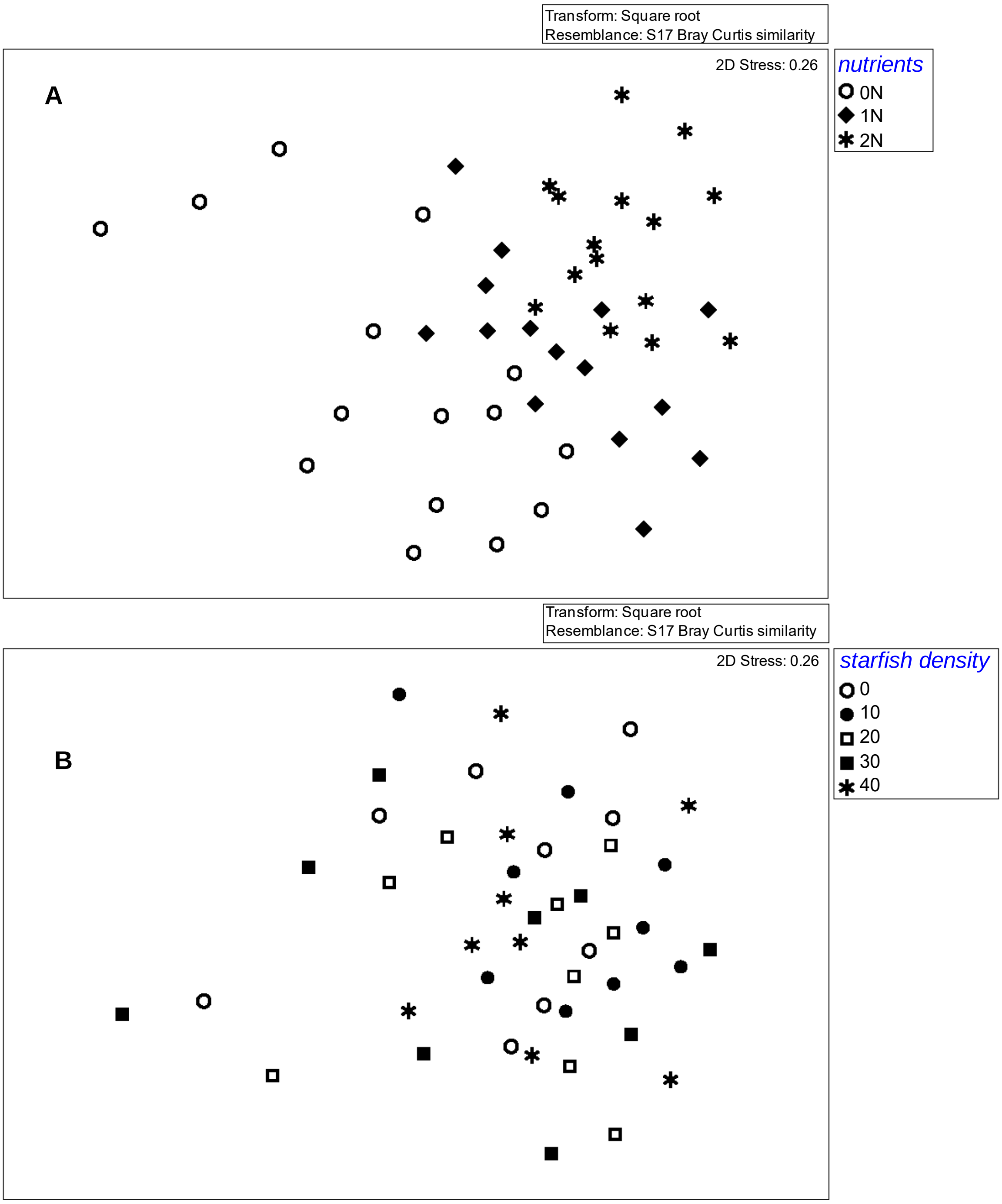


Fig. 2.5: Multidimensional scaling (MDS) ordination illustrating spatial variability in microalgal communities in response to increasing nutrient levels (**A**) and starfish densities (**B**).

2.4.2 Dominant microalgal morphotypes

Fifty-nine of the 142 microalgal morphotypes that were identified by SIMPER to contribute 90% to community structure were selected for the analysis. Seventeen of these morphotypes were significantly influenced by nutrient treatments (ANOVA, Table 2.1), with most of them differing significantly between 0N and 1N and 0N and 2N treatments. There were very few morphotypes that significantly differed between 1N and 2N (Fig. 2.6). The major pattern that emerged was that the abundances of several morphotypes were reduced with increasing nutrient enhancement (Fig. 2.6: Type 13, 34, 59, 70, 72, 78, 82, 102 and 118). The opposite pattern was observed for some morphotypes showing an increase in abundances with increasing nutrient enhancement (Fig. 2.6: Type 3, 28, 33 and 142). A humped-shape pattern in abundances related to increasing nutrients appeared for six morphotypes (Fig. 2.6: Type 21, 23, 61, 64, 79 and 120) in which abundances were mostly enhanced at intermediate nutrient levels (1N) compared to 0N and 2N treatments. There was no significant influence of starfish densities on any of the microalgal morphotype but starfish and nutrient interacted to influence three of the morphotypes (Table 2.1, Fig. 2.6: Type 61, 64 and 82). Type 61 showed increased abundances in the 0N treatment from 0 to 30 starfish.m^{-2}, whereas, no clear pattern was observed at 1N and 2N treatments except that at the 1N treatment abundances were higher. Abundances of type 64 declined with increasing starfish densities at the 0N treatment and increased with starfish densities at 1N, whereas, a humped-shaped pattern was formed against increasing starfish densities at the 2N treatment. Type 82 also showed no clear pattern but at the 0N treatment abundances were higher at 10 and 40 starfish.m^{-2} and at 0 starfish.m^{-2} in 1N. Type 82 was only recorded at 40 starfish.m^{-2} in the 2N treatment.

Table 2.1: ANOVA summary statistics showing significant responses of specific microalgal taxa to nutrient treatments (N) and the interaction between starfish densities and nutrients (N&S). Df = degrees of freedom, *F* = test statistic, *p* = significance level.

ANOVA summary statistics				
Morphotype	**Source**	***F***	**Df**	***P***
Type 13	N	4.094	2	0.027
Type 34	N	4.722	2	0.016
Type 102	N	3.680	2	0.037
Type 3	N	6.428	2	0.005
Type 120	N	5.883	2	0.007
Type 70	N	8.896	2	0.001
Type 72	N	8.855	2	0.001
Type 78	N	3.338	2	0.049
Type 118	N	3.335	2	0.049
Type 59	N	5.571	2	0.009
Type 33	N	3.765	2	0.035
Type 61	N & S	3.731	8	0.004
Type 82	N	5.864	2	0.007
	N & S	3.022	8	0.013
Type 28	N	4038	2	0.028
Type 21	N	4.844	2	0.015
Type 79	N	4.282	2	0.023
Type 23	N	6.612	2	0.004
Type 64	N & S	2.612	8	0.027
Type 142	N	5.471	4	0.009

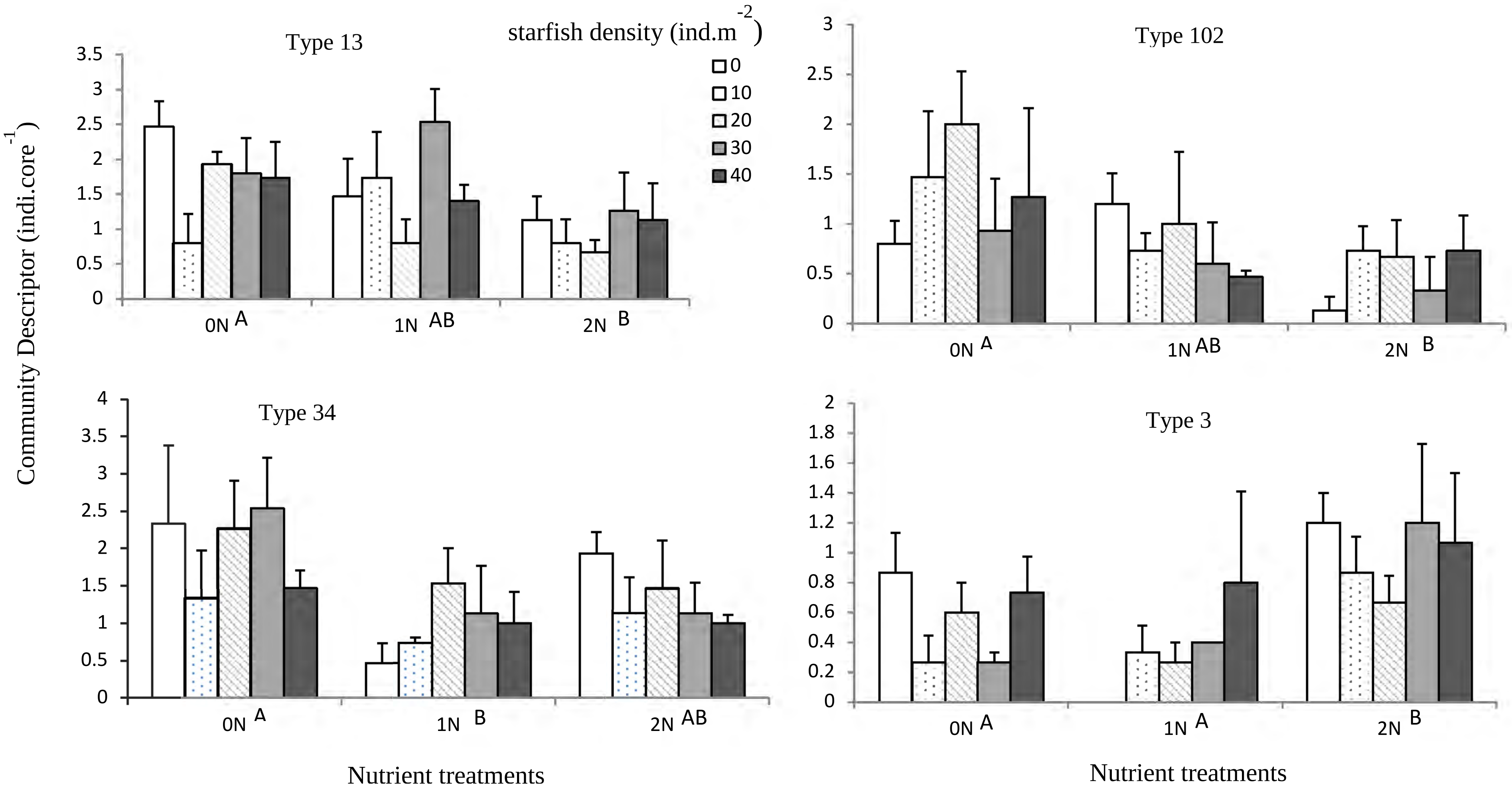


Fig. 2.6: Abundances (means ± 1SE) of dominant microalgal morphotypes in response to increasing starfish and nutrient levels. Different letters denote significant differences in mean abundance of microalgal morphotypes between nutrient treatments (in upper case).

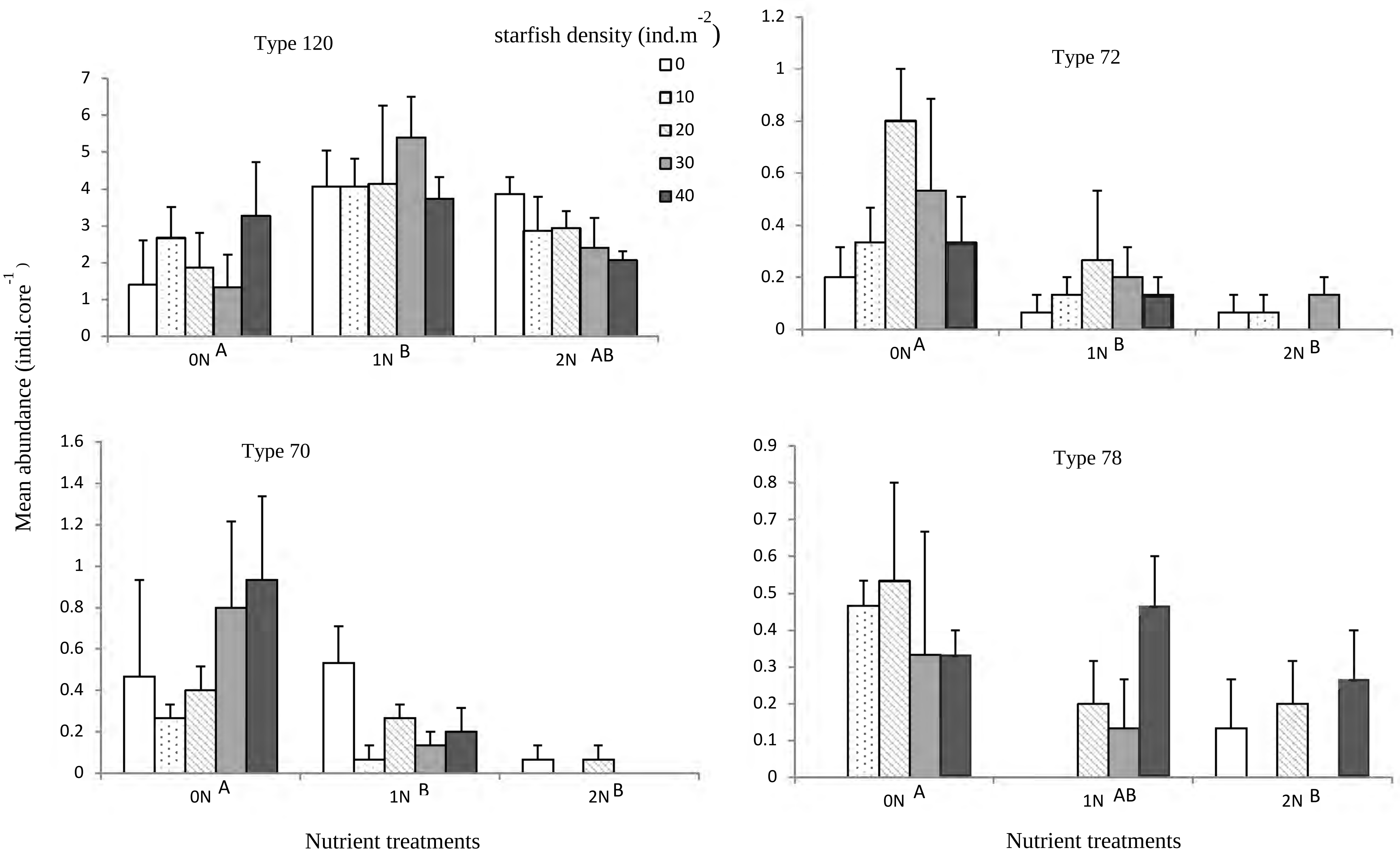
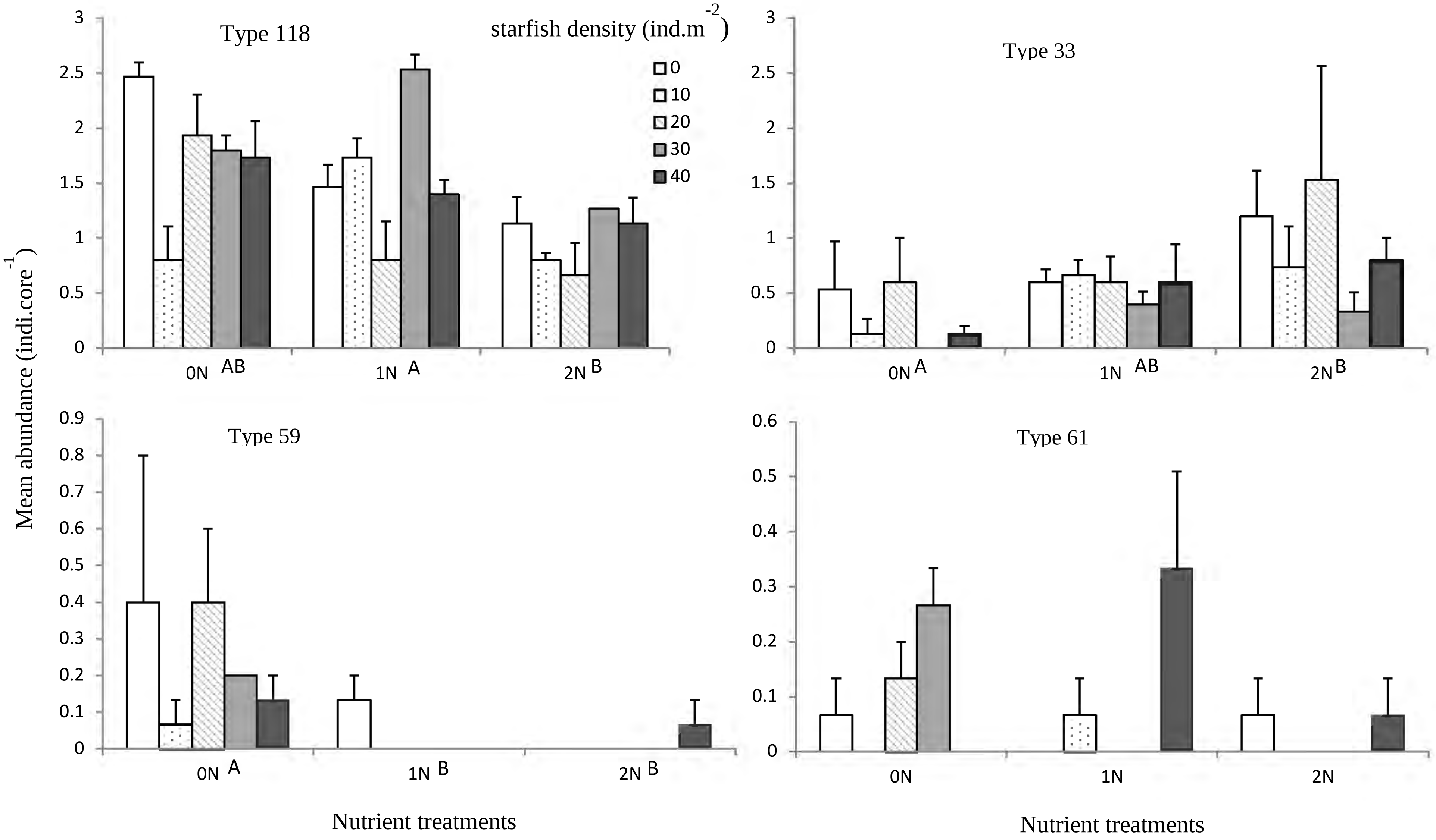


Fig. 2.6 continued



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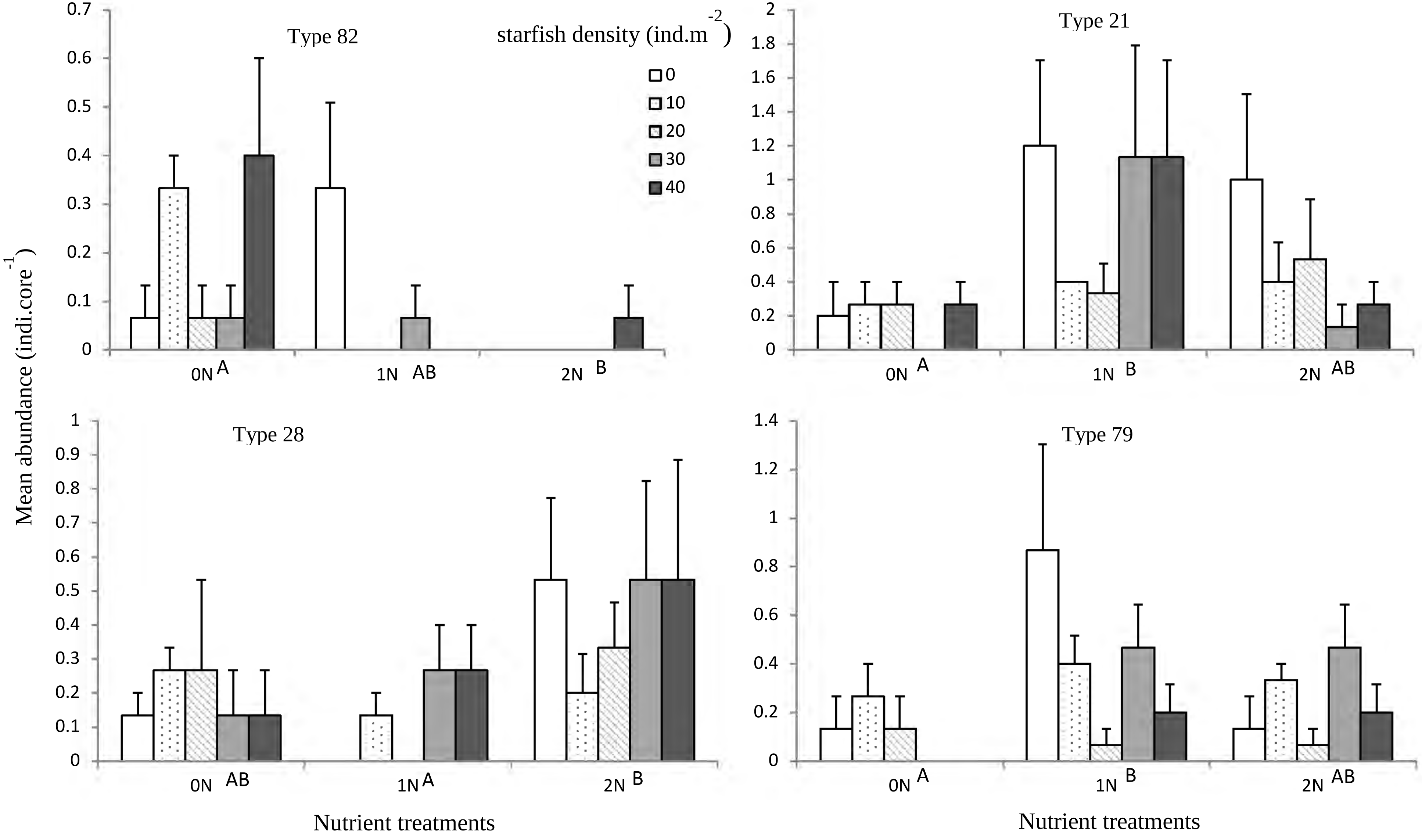

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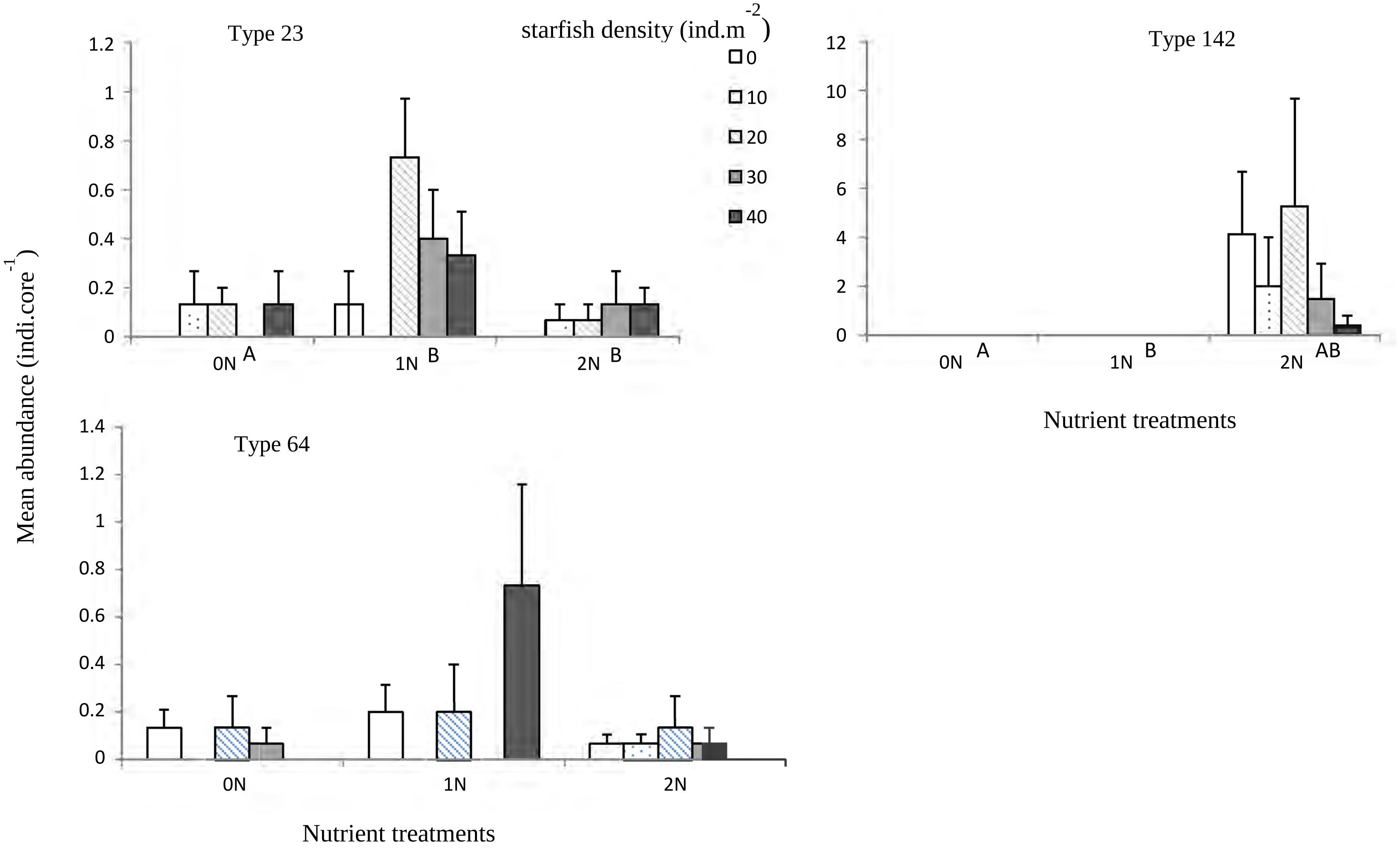

Fig. 2.6 continued

2.4.3 Microalgal diversity indices

Nutrient levels did not affect any of the microalgal diversity measures. They also did not interact with starfish densities to influence the microalgal indices (Table 2.2, Fig. 2.7). However, there were marginally non-significant effects of nutrients on species evenness (J') and Shannon-Wiener diversity (H'). ANOVA (Table 2.2) revealed significant starfish effects on species richness (S) and Shannon-Wiener diversity (H′), but post hoc tests could not identify any differences among starfish densities within each nutrient treatment (*P* > 0.05 for all starfish densities). Both indices that were affected by starfish displayed a U-shape pattern against increasing starfish densities at 1N and 2N nutrient levels. Enrichment appeared to increase the magnitude of difference between intermediate and minimum and maximum starfish densities.

Table 2.2: ANOVA summary statistics showing significant responses of microalgal community indices to nutrient treatments (N), starfish (S) and the interaction between starfish densities and nutrients (N&S). Df = degrees of freedom, *F* = test statistic, *p* = significance level. * denotes if *p* value is significant.

Diversity indices	**Source**	***F***	**Df**	***p***
Species richness (S)	N	1.174	2	0.323
	S	2.925	4	0.037 *
	N & S	1.776	8	1.22
Species evenness (J')	N	2.551	2	0.095
	S	1.443	4	0.244
	N & S	0.235	8	0.981
Abundance (N)	N	0.328	2	0.732
	S	0.446	4	0.775
	N & S	0.864	8	0.556
Shannon-Wiener diversity (H')	N	3.006	2	0.065
	S	2.747	4	0.047 *
	N & S	0.350	8	0.938

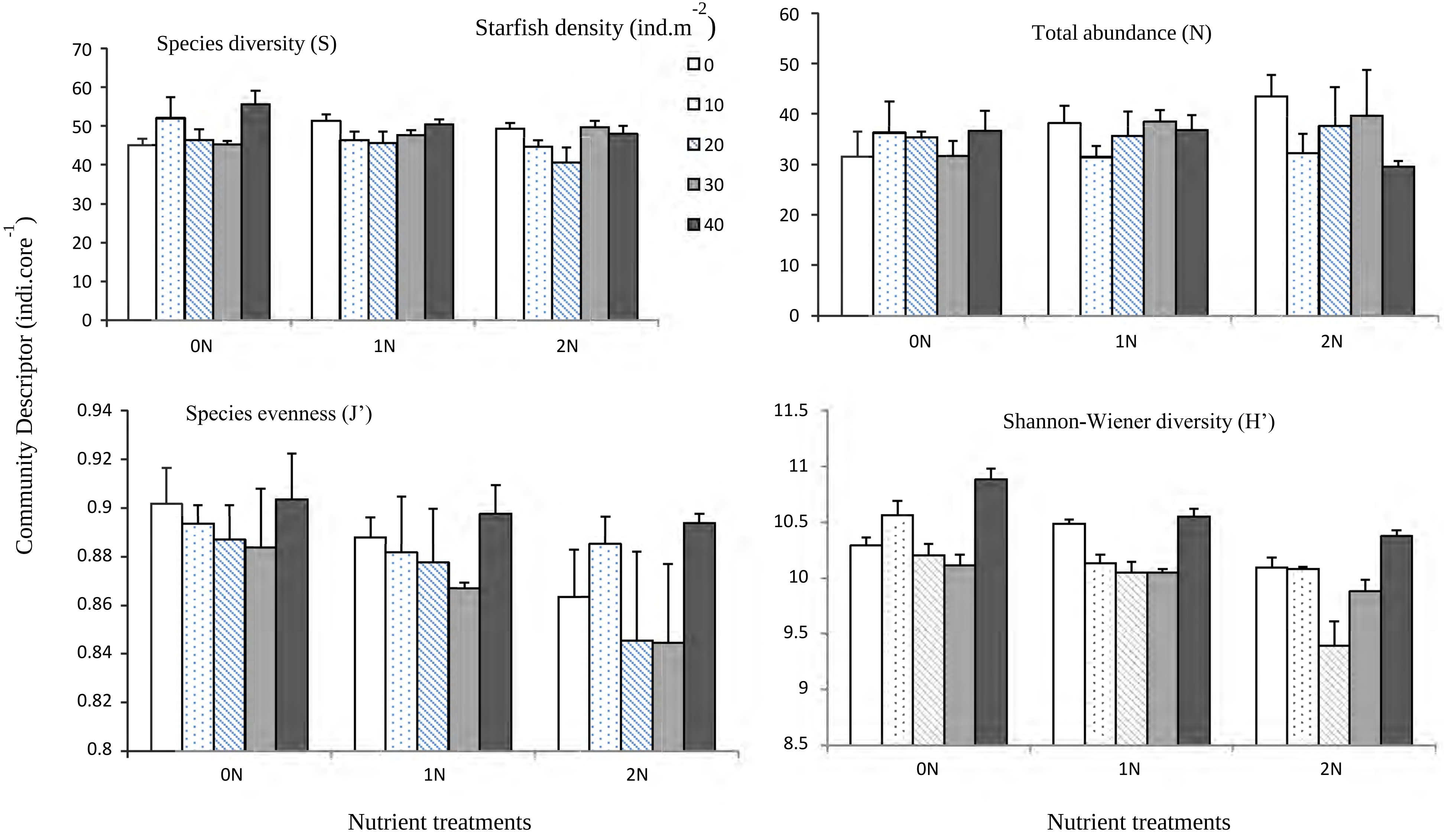

Fig. 2.7: Microagal community indices (Means ± 1SE) with increasing starfish densities at each nutrient treatment.

2.5 Macrofaunal community structure

2.5.1 Overall composition

Macrofaunal community structure was significantly influenced by nutrient treatments (PERMANOVA, Pseudo-$F_{2,44}$ = 2.257, p = 0.006). This was not obvious on the MDS ordination (Fig. 2.8A), as no clear segregation of macrofauna samples in response to nutrient treatments was observed. Pair wise tests showed that the background nutrient treatment did not differ from the 1N treatment (t = 1.188, p < 0.188), but differed from the 2N treatment (t = 1.611, p < 0.015) whereas, the 1N treatment differed from 2N (t = 1.695, p < 0.024). There was no significant starfish effects (PERMANOVA, Pseudo-$F_{4,44}$ = 1.025, p = 0.417) or interactive effect between starfish and nutrients (PERMANOVA, Pseudo-$F_{8,44}$ = 0.828, p = 0.807) on macrofuanal community structure. This was visually supported in the MDS ordination (Fig 2.8B) indicating no clear separation of samples according to starfish densities.

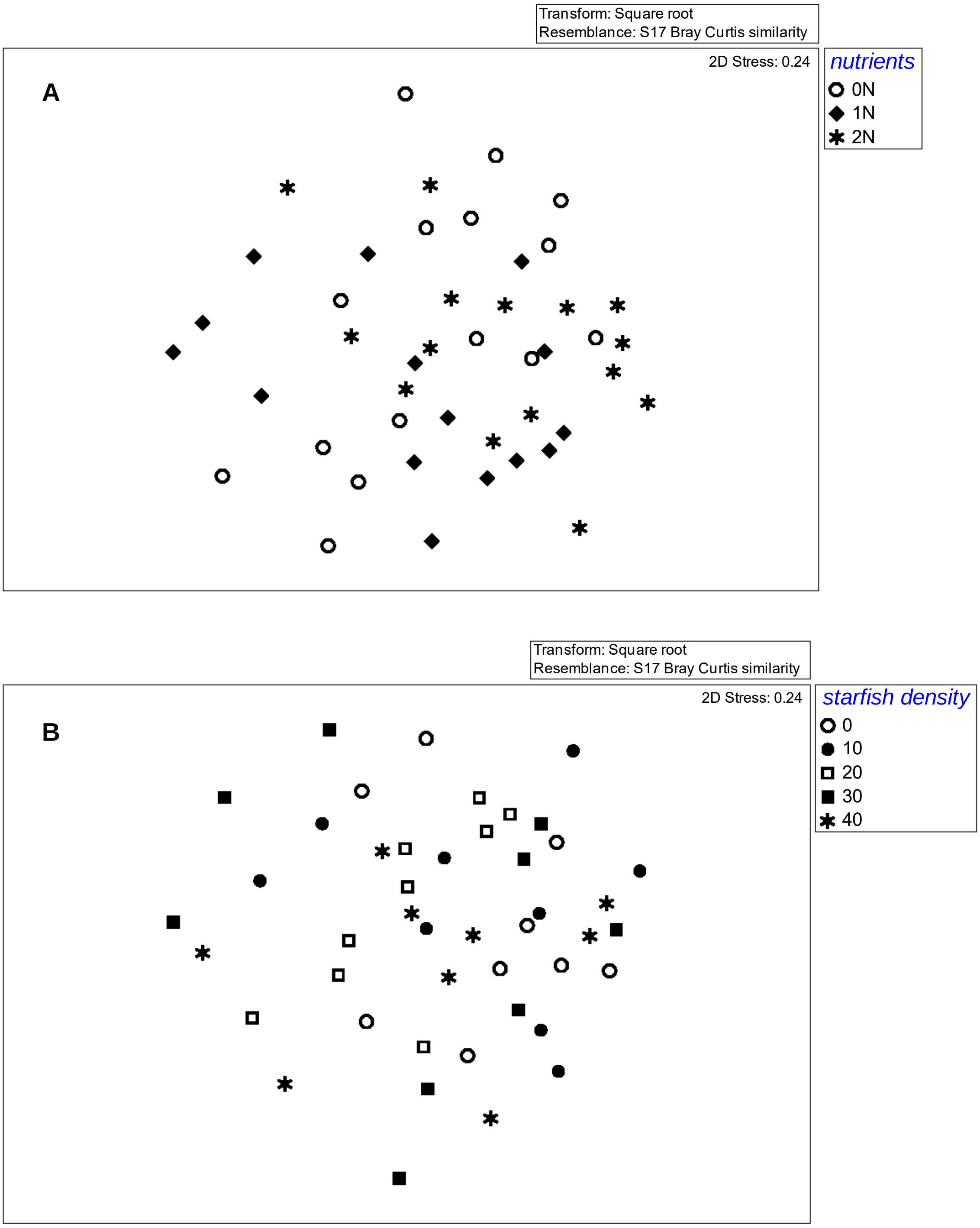

Fig. 2.8: Multidimensional scaling (MDS) ordination illustrating spatial variability in macrofaunal communities in response to increasing nutrient levels (**A**) and starfish densities (**B**).

2.5.2 Dominant macrofaunal species

Six species were identified by SIMPER (based on 90 % cut-off) that dominated macrofaunal community structure (Fig. 2.9). Two of these species viz. *Notomastus latericeus* (Polychaeta) and *Carditella rugosa* (Bivalvia) were significantly influenced by nutrient levels and starfish densities (ANOVA, Table 2.3). Post hoc tests however did not identify any significant starfish effects on *N. latericeus* abundance within each nutrient treatment ($p >$ 0.05 for starfish densities). Abundance of *N. latericeus* was significantly greater in the 1N nutrient enrichment than the 2N treatment ($p < 0.012$) whereas for *C. rugosa*, densities were lowest in the 0N treatment relative to the 1N ($p = 0.050$) and 2N treatments ($p = 0.003$). The abundance of *C. rugosa* generally increased with nutrient enrichments (Fig. 2.9), but for *N. latericeus,* a U-shaped pattern was observed, in which abundance was enhanced at 0N and 2N treatments with 1N treatment showing lower abundance. Increasing starfish density did not show any positive increase or negative decrease in abundances of both species within nutrient treatments, instead abundances were mostly enhanced at particular starfish densities forming U-and hump-shaped patterns (Fig. 2.9).

Table 2.3: ANOVA summary statistics showing significant responses of dominant macrofaunal species to nutrient treatment, starfish (S) and the interaction between starfish densities and nutrients (N&S). Df = degrees of freedom, *F* = test statistic, *p* = significance level, * denotes if *p* value is significant.

Taxonomic group	**Species**	**Source**	***F***	**Df**	***p***
Amphipoda	*Urothoe grimaldii*	N	0.660	2	0.524
		S	1.336	4	0.280
		N&S	1.107	8	0.386
Bivalvia	*Carditella rugosa*	N	6.728	2	0.004 *
		S	3.120	4	0.029 *
		N&S	0.755	8	0.644
Polychaeta	*Notomastus latericeus*	N	5.080	2	0.013 *
		S	2.706	4	0.049 *
		N&S	1.399	8	0.237
Polychaeta	*Marphysa elitueni*	N	0.718	2	0.496
		S	0.201	4	0.936
		N&S	1.196	8	0.334
Anomura	*Callianassa kraussi*	N	0.265	2	0.769
		S	0.576	4	0.682
		N&S	1.613	8	0.163
Polychaeta	Juvenile polychaeta	N	1.903	2	0.167
		S	0.553	4	0.698
		N&S	0.888	8	0.538

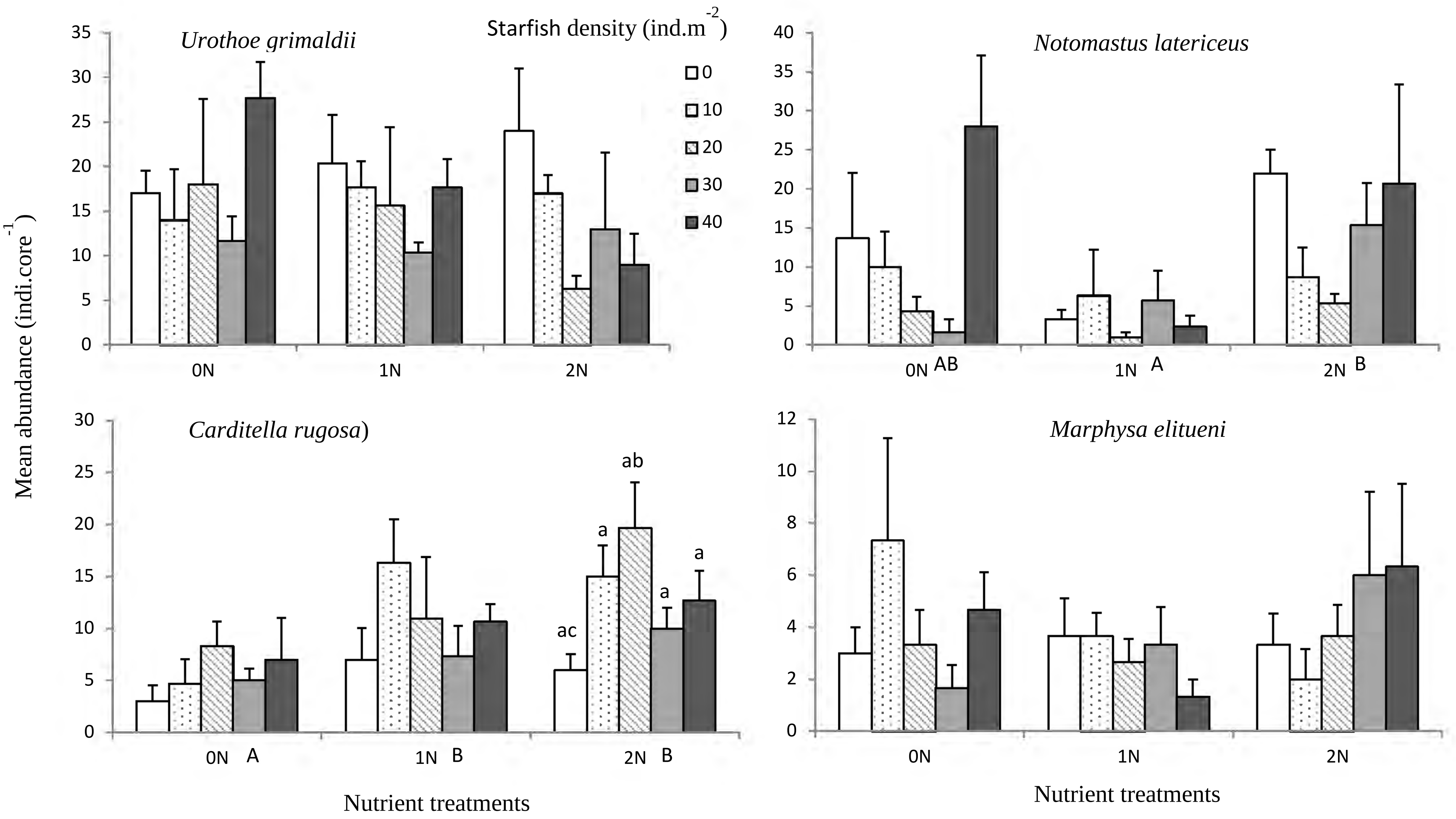

Fig. 2.9: Abundances (means ± 1SE) of dominant macrofaunal species in response to increasing starfish and nutrient levels. Different letters denote significant differences in species abundance between nutrient treatments (in upper case) and among starfish densities within nutrient treatments (lower case).

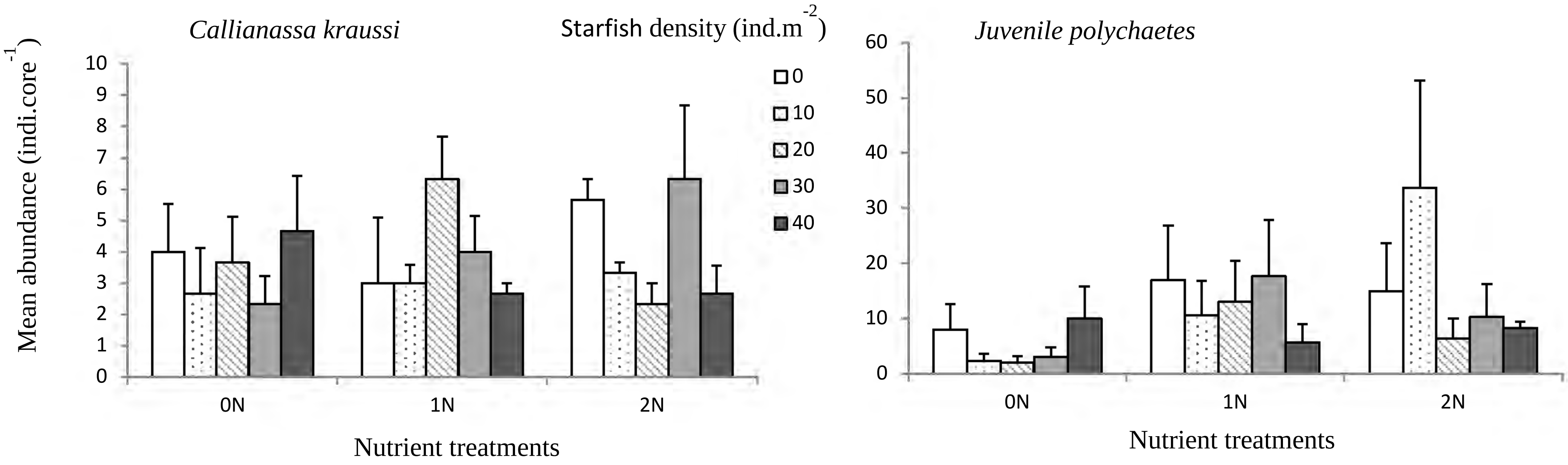

Fig. 2.9 continued.

2.5.3 Macrofaunal diversity indices

Nutrients significantly affected Shannon-Wiener diversity (H′), with marginally non-significant effects on Pielou's species evenness (J′) and total abundance (N). Starfish densities and its interaction with nutrient levels did not influence any of the macrofaunal community measures (Table 2.4). For Shannon-Wiener diversity, Post hoc tests indicated that the 1N level of nutrient enrichment differed significantly from the 2N level (*p* = 0.027) but this was not clearly evident graphically (Fig.2.10)

Table 2.4: ANOVA summary statistics showing significant responses of macrofaunal indices to nutrient treatments (N), starfish (S) and the interaction between starfish densities and nutrients (N&S). Df = degrees of freedom, *F* = test statistic, *p* = significance level. * denotes if *p* value is significant.

Diversity indices	**Source**	***F***	**Df**	***p***
Species richness (S)	N	1.651	2	0.209
	S	0.726	4	0.581
	N & S	0.887	8	0.539
Species evenness (J')	N	2.897	2	0.071
	S	0.852	4	0.504
	N & S	1.024	8	0.440
Abundance (N)	N	2.599	2	0.091
	S	0.932	4	0.459
	N & S	1.174	8	0.347
Shannon-Wiener diversity (H')	N	3.803	2	0.034*
	S	1.071	4	0.388
	N & S	0.527	8	0.826

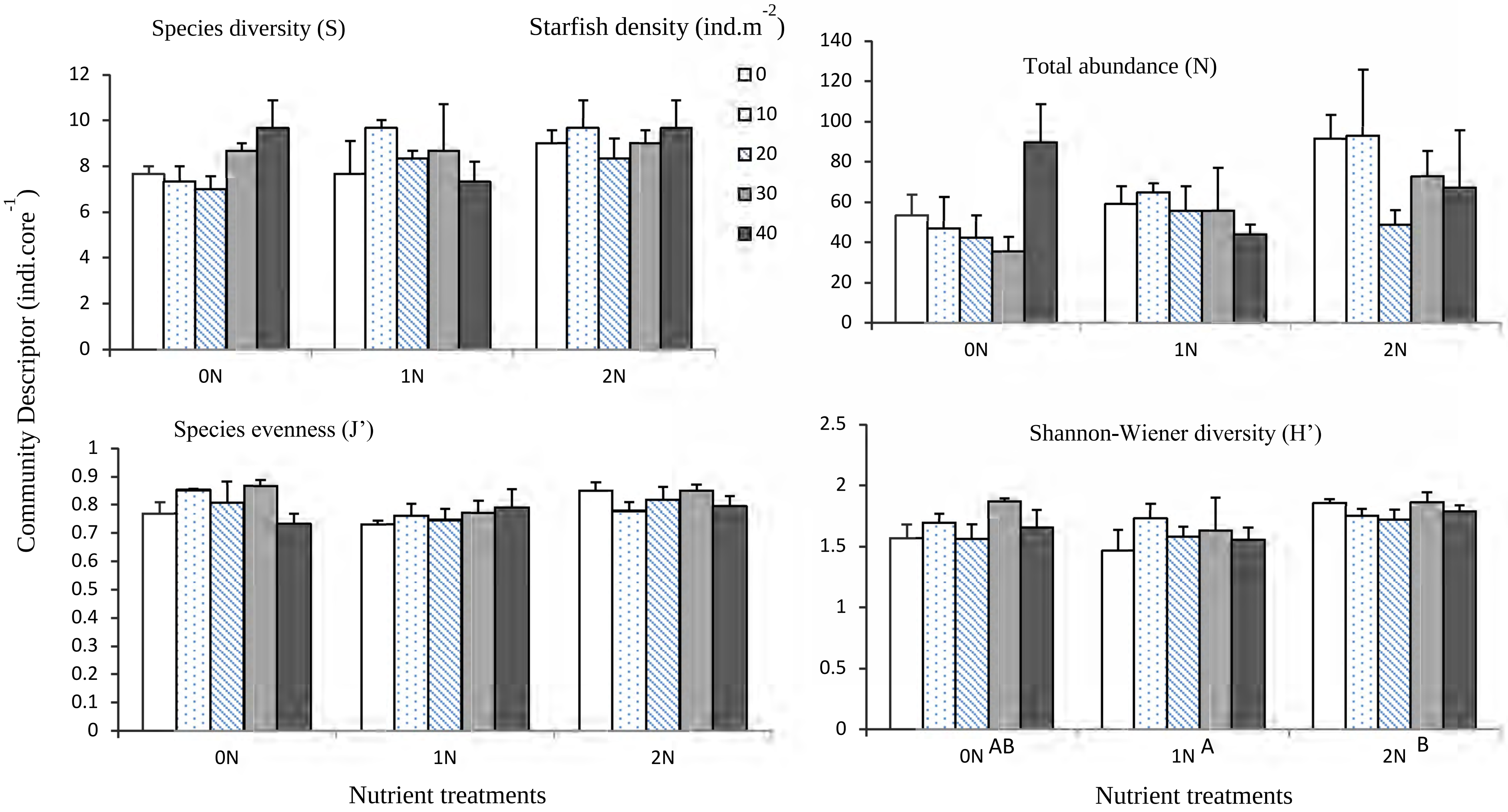

Fig. 2.10: Macrofaunal community indices (Means ± 1SE), with increasing starfish densities at each nutrient treatment. Different letters denote significant differences in community descriptors between nutrient treatments (in upper case).

2.6 Functional groups

2.6.1 Surface and burrowing scavengers/predators

At the functional level, abundance of surface predators that may also scavenge (scavengers/predators, Fig. 2.11 A) were not influenced by nutrient levels (ANOVA, $F_{2,45}$ = 0.514, p = 0.604) or the interactive effects of nutrients and starfish densities (ANOVA, $F_{8,45}$ = 1.414, p = 0.231) but were, however, influenced by starfish densities (ANOVA, $F_{4,45}$ = 2.914, p = 0.038). In general, surface scavengers/predators increased in abundance with increasing starfish densities at the 0N and 1N nutrient treatments. At the 2N treatments, there was no obvious pattern, but certain starfish densities appeared to enhance the abundance of surface scavengers (Fig.2.11).

Nutrient enrichment did not affect the abundance of burrowing scavengers/predators ($F_{2,45}$ = 1.259, p = 0.298). Similarly, the densities of starfish (ANOVA, $F_{4,45}$ = 0.801, p = 0.534) and interaction between starfish densities and nutrients (ANOVA, $F_{8,45}$ = 0.994, p = 0.461) did not influence this functional group (Fig. 3.4).

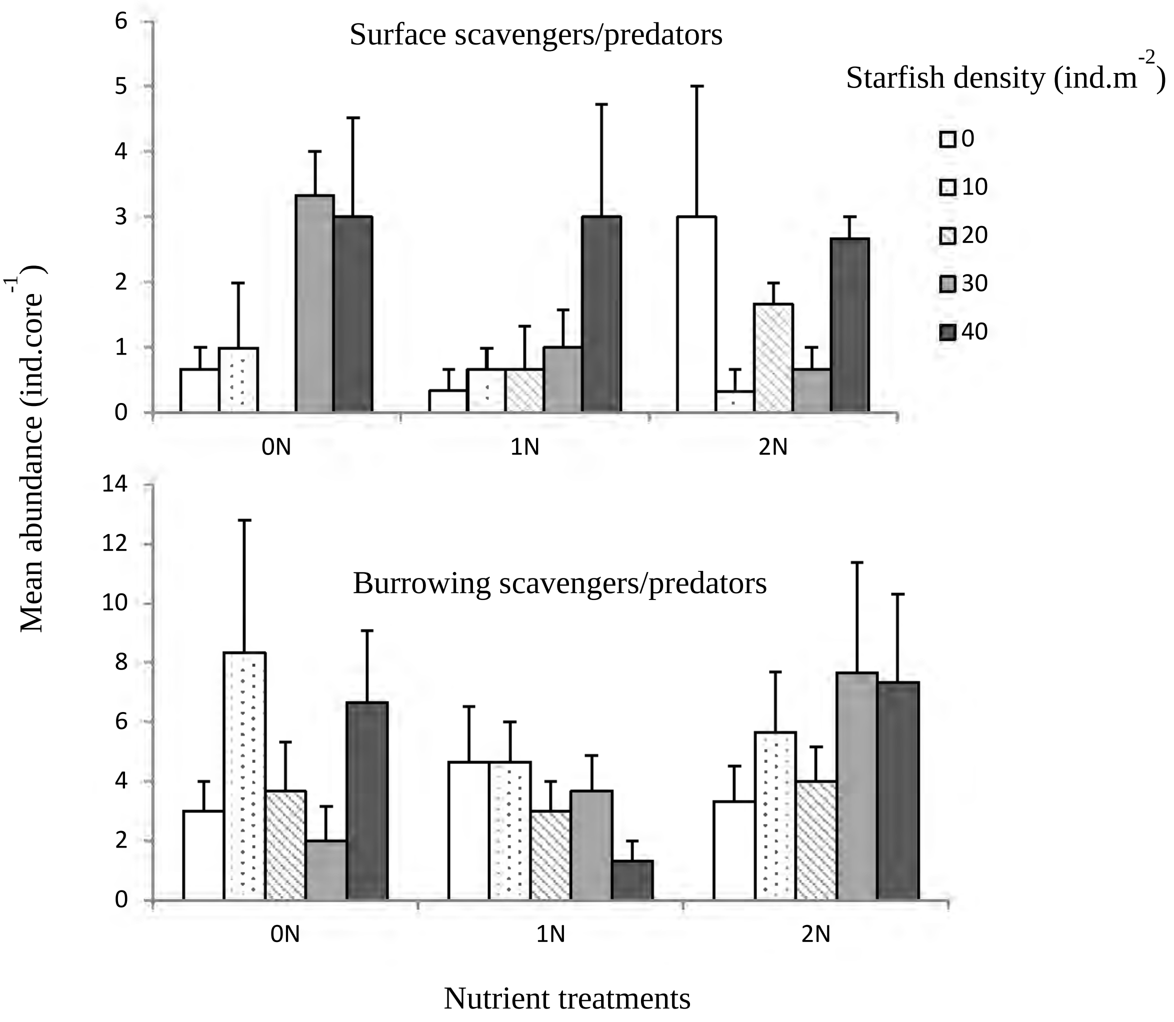

Fig. 2.11: Abundances of surface and burrowing scavengers/predators (Means ± 1SE), with increasing starfish densities at each nutrient level.

2.6.2 Surface and burrowing deposit feeders

There was no significant effects of nutrients on surface (Fig. 2.12 A) (ANOVA, $F_{2,45}$ = 0.206, p = 0.815) and burrowing deposit feeders (Fig. 2.12 B) (ANOVA, $F_{2,45}$ = 2.376, p = 0.110). Starfish densities also showed no effects on surface deposit feeders (ANOVA, $F_{4,45}$ = 0.471, p = 0.757) but did show a marginally non-significant effect on burrowing deposit feeders (ANOVA, $F_{4,45}$ = 2.251, p = 0.087). Neither of these two functional groups appeared to be influenced by the interaction between nutrients and starfish densities (surface deposit feeders: ANOVA, $F_{8,45}$ = 1.088, p = 0.398), burrowing deposit feeders: ANOVA, $F_{8,45}$ = 2.106, p = 0.067), although it must be noted that for burrowing deposit feeders the interaction was marginally non-significant.

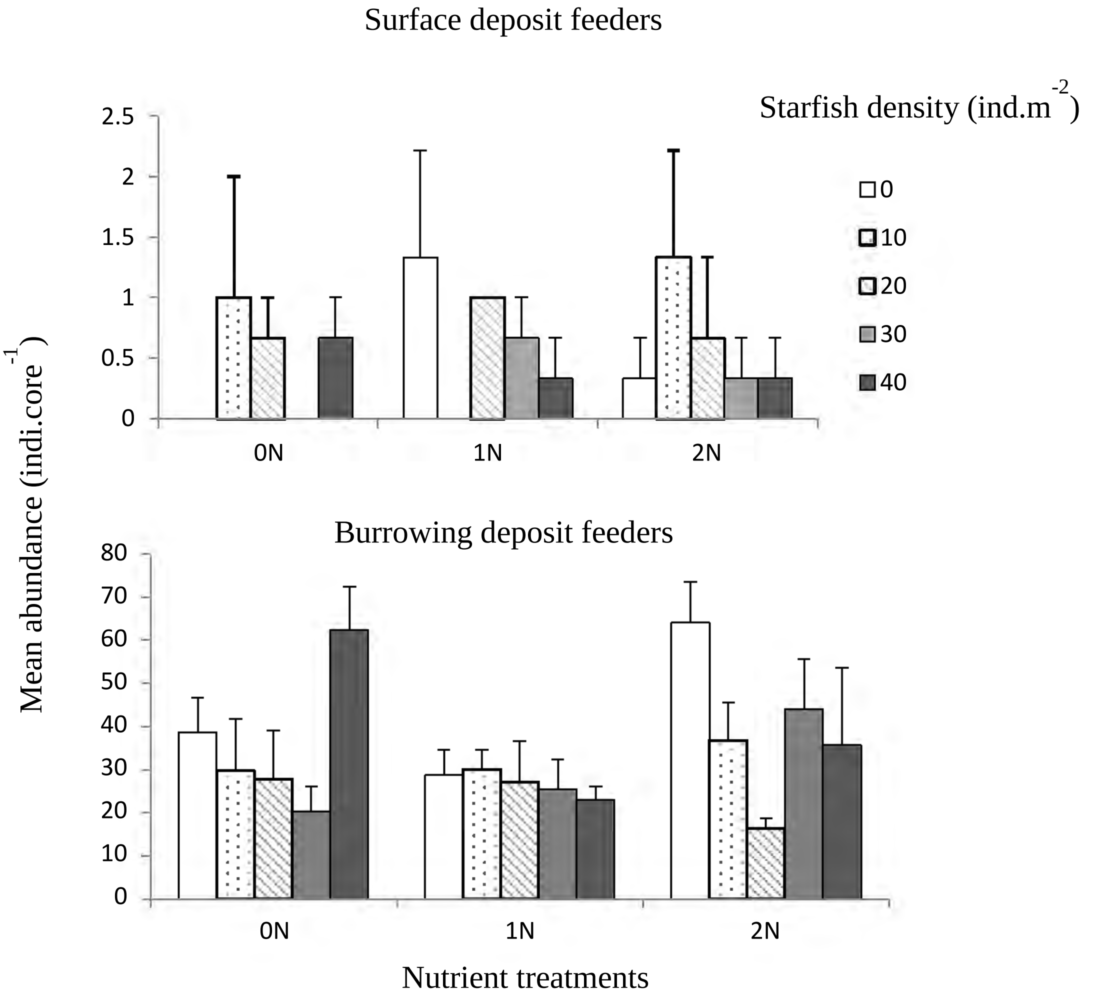

Fig. 2.12: Abundances of surface and burrowing deposit feeders (Means ± 1SE), with increasing starfish densities at each nutrient level.

2.6.3: Suspension feeders

Suspension feeders were influenced by nutrients (ANOVA, $F_{2,45}$ = 6.728, p = 0.004, mainly between the 0N and 1N nutrient enrichment levels (p = 0.050), and between 0N and 2N (p = 0.003), with a gradual increase in abundance across the three nutrient treatments (Fig. 2.13). Starfish densities also had a significant effect on suspension feeders (ANOVA, $F_{4,45}$ = 3.120, p = 0.029) but Post hoc tests could not identify significant starfish effects within nutrient levels (p > 0.05). Suspension feeder abundance was generally enhanced from low to intermediate starfish densities within each enrichment level (Fig. 2.13). The interactive effects of nutrients and starfish densities did not influence suspension feeder abundance (ANOVA, $F_{8,45}$ = 0.755, p = 0.644).

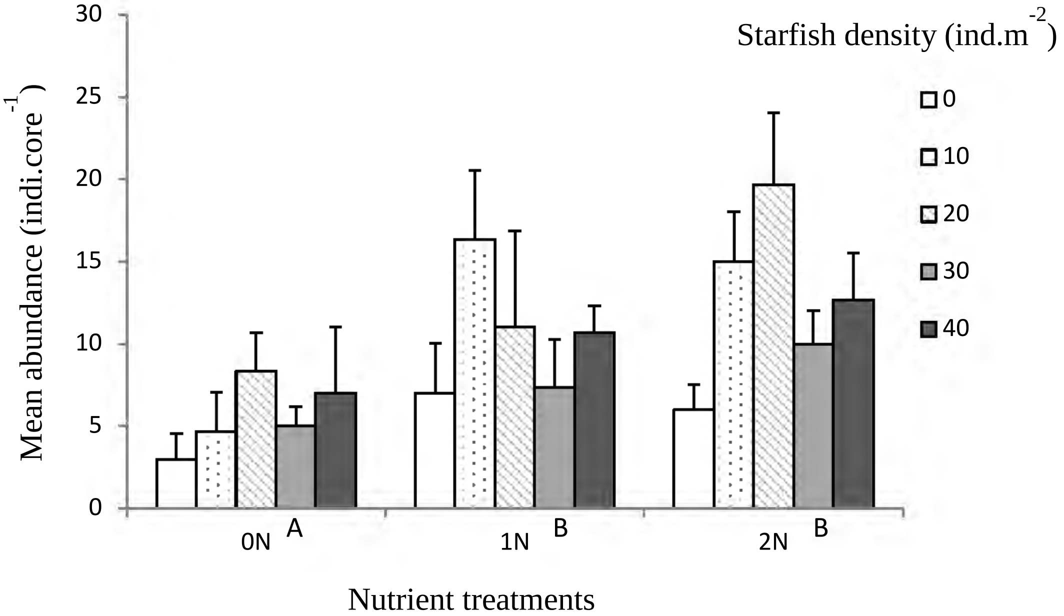

Fig. 2.13: Abundances of suspension feeders (Means ± 1SE), with increasing starfish densities at each nutrient level. Different letters denote significant differences in abundance of suspension feeders between nutrient treatments (in upper case).

2.7 Discussion

This study provides insight into the interactive effects of nutrient enhancement and starfish grazing on producers and consumers in intertidal soft-sediment communities. Producer (microalgal) and consumer (macrofaunal) assemblages responded differently to increasing starfish densities and nutrient levels. These results were generally not overwhelmingly supportive of the predictions of the grazer reversal hypothesis, and re-enforce the variable nature of responses of ecological systems to ecological theories. However, the results obtained nevertheless shed light on the relative importance of nutrients and starfish grazing on intertidal soft-sediment ecosystem. Interestingly, bottom-up control by nutrients in this study emerged to be more important than top-down grazer regulation of community structure, which is in agreement with previous studies conducted in marine soft-sediment and other ecosystems (Werner & Matthiessen 2013, Rober *et al.,* 2011, Mork *et al.,* 2009).

2.7.1 EPS and Chl *a*

One of the most significant outcomes of the experiment was the main and interactive effects of nutrients and starfish on sediment EPS levels. In terms of nutrient effects, EPS levels were marginally lower in the 2N level of nutrient enrichment compared to 1N level. In terms of starfish grazing, at the 2N level of enrichment, low to intermediate starfish density elevated the EPS level whereas in the absence of nutrient enrichment, lowest concentration of EPS occurred at intermediate starfish. A clear hump-shaped pattern of EPS level against increasing starfish densities was observed at the 1N nutrient level. The increase in EPS levels at low to intermediate starfish densities of the hump-shaped pattern are likely due to different mechanisms. Firstly, the secretion of a mucus web by *P. exigua* during feeding events could potentially enhance sediment EPS levels. Mucus secreted by various invertebrates and algae is known to be enriched in carbohydrates and contribute to substrate associated EPS (Underwood & Paterson 1995, Hirst *et al.,* 2003, Wotton 2011). Secondly, sediment-associated bacteria are important producers of EPS in marine ecosystems (Underwood & Paterson 1995, Hirst *et al.,* 2003). It is possible that, as *P. exigua* feeds, it indirectly stimulates surficial (surface) bacterial densities by removing older cells thereby promoting growth of new cells and the quantity of EPS exuded (Pillay *et al.*, 2010). Although *P. exigua* cannot effectively select particular cells to be consumed, older cells could be eliminated by *P. exigua* based on their larger sizes. The addition of bacteria in *P. exigua* gut content or directly on its faeces on sediment can stimulate the bacterial growth (Pillay *et al.,* 2010). Thirdly, sediment EPS levels could be increased through exudation by microalgal morphotypes that increased in abundance at the 1N nutrient enrichment level with increasing starfish density (eg. Fig 2.6, Type 3, 78, 64, 21 and 28).

The reduction of EPS level following a peak at intermediate starfish density could be attributed to starfish consuming EPS on the sediment surface. Under high starfish densities, consumption of EPS may outweigh EPS production. Alternatively, a reduction in sediment EPS could be related to consumption pressure induced by surface dwelling macrofauna. The increase in abundances of macrofaunal suspension feeders (Fig. 2.13) such as the bivalve *Carditella rugosa* (Fig. 2.9) at intermediate nutrient levels could exert significant top-down control of EPS through consumption. Suspension feeders usually feed on suspended particles in the water column (Reise 2001), however it may be possible that they switch to a deposit-feeding mode when food availability increases on surface sediments, as is known for certain suspension feeding species (Reise 2001). Irregular patterns of EPS level at 0N and 2N treatments could be caused by either high scale variability between the cages or complex biological interactions between EPS production by starfish, exudation by bacteria and microalgae or consumption by starfish or other Infaunal grazers.

Microalgal biomass (Chl *a*) was unresponsive to grazing by starfish. The high variability levels displayed by microalgal biomass with increasing starfish densities at each nutrient treatment resulted in an irregular or unclear pattern. Perhaps greater sample size would have reduced such variability and revealed more clearly the underlying ecological patterns and processes.

The lack of a grazing effect of starfish on Chl *a* in this study is consistent with previous results documented by Dawson & Pillay (2011) working on the same starfish species (*Parvulastra exigua*) on soft sediment, but differs from other studies which reported a reduction in microalgal biomass in response to grazing by *P. exigua* in rocky shore (Jackson *et al.,* 2009) and soft sediment (Pillay *et al.,* 2010). The lack of *P. exigua* effect on Chl *a* could be due to the uneven or mobile nature of soft-sediments which hindered feeding by *P. exigua*. Jackson *et al.* (2009) indicated that starfish may be less effective on rough surfaces than on smooth ones.

Reduction in microalgal biomass has also been reported for other grazers such as juvenile blue crabs (Spivak *et al.,* 2009) and snails (Kehde & Jerry 1972, Lamberti *et al.,* 1987). Inconsistent reports between the various studies may be due to life-history differences in grazer species used, their densities, feeding mechanisms and the nature of the local environments. Subtle differences between experimental designs and sample sizes could also explain the inconsistency in results between studies.

Given the fact that the microalgal community structure was influenced by nutrient enrichment, it is very surprising that microalgal biomass was not affected by increasing nutrient levels, as it would be expected that microalgal biomass would increase in the presence of increasing nutrients. The lack of a response from microalgal biomass, however, should be interpreted cautiously, considering the variability in data. Shading of the sediment from the cages used in the experiment could also have affected light penetration into the cages.

Increases in microalgal biomass in response to nutrient enrichment have been reported for fresh water lakes (Power 1992, Posey *et al*., 2002), rivers (Deegan *et al.,* 1997) and marine shallow water ecosystems (Beukema 1991, Pitta *et al.,* 1998). Posey *et al*., (2002) found that nutrients significantly enhanced benthic Chl a, with greater Chl a levels in nutrient addition plots versus controls. Bucolo *et al*., (2008) also showed greater Chl *a* in the experimental plots relative to controls in a study that examined effects of nutrient enhancement on primary production and biomass using sediment microalgae. Sullivan (1981) however reported no effect of nutrient enrichment on microalgal biomass in salt marshes.

2.7.2: Microlagal community: Overall composition, dominant morphotypes and diversity indices

Multivariate analysis indicated a strong response of microalgal communities to nutrient enrichment, but not to starfish grazing. The sensitivity of microalgae to nutrient enhancement in this study is consistent with other studies (Cahoon 1999, Posey *et al.,* 2002, Hillerband & Kahlert 2001). In terms of responses to nutrients, there was a limited response of individual morphotypes, with 17 of the 59 morphotypes identified by SIMPER being statistically linked with enrichment. Three of the 19 morphotypes were affected by the interaction between nutrients and starfish densities, further re-enforcing the limited response observed. The restricted responses of microalgae may indicate a high level of insensitivity of dominant microalgal taxa to the level of nutrient enrichment used in this study.

The patterns of microalgal responses to nutrient enrichment documented here varied considerably between morphotypes. Some morphotypes increased in abundance with nutrient enrichment while others became less abundant. U- and hump-shaped patterns were also observed for some of the dominant morphotypes. Linear increases in abundance of morphotypes with increasing nutrient levels could indicate that the nutrient levels used in this study favour these taxa, allowing them to grow and proliferate with increasing nutrient enrichment. The latter could also indicate that these microalgal taxa may be nutrient limited under natural conditions. In contrast, the reduction in abundance of certain microalgal morphotypes with increasing nutrient enrichment could be related to the degree of sensitivity of individual morphotype to the level of nutrient enrichment. Those taxa that were completely eliminated at higher nutrient levels (e.g. Fig. 2.6.Type 59 and 82) are likely the most sensitive of microalgal taxa to enrichment. These taxa likely to survive best under non-nutrient enriched conditions. There is some evidence in the scientific literature that microalgal abundances are often enhanced by lower nutrient levels (Roll *et al.,* 2005).

Hump-shaped responses of microalgal taxa to nutrient enrichment reflect an initial increases in abundance of these morphotypes between low and intermediate nutrient levels, after which further nutrient enrichment reduces abundance, either because these taxa prefer low nutrient conditions or are outcompeted by microalgae that proliferate under high nutrient levels. U-shaped patterns in responses of microalgal taxa to increasing nutrient levels may be due to initial decreases in abundance of theses morphotypes between low and intermediate nutrients levels, after which further nutrient enrichment elevates abundances because these morphotypes prefer high nutrient conditions.

It has been previously reported that only a few benthic microalgal species respond significantly to nutrient enrichment in marine shallow-water (Hillebrand & Sommer, 1997) and freshwater ecosystems (Carrick *et al.,* 1988). In some cases nutrient enrichment has been shown to favour opportunistic and fast growing algal species (Rosenberg *et al.,* 1990). Sundback & Snoeijs (1991) showed that high water column nutrients can selectively stimulate the growth rate of benthic microalgae, by specifically favouring filamentous algae, cyanobacteria, diatoms, and flagellates. These results, along with the findings of the present experiment, point to an important but limited role of bottom-up nutrient effects on producers and that nutrient effects override starfish effects in regulating producer dynamics and community structure. The latter are not fully consistent with other findings like those of Hillebrand & Kahlert (2001) who document strong effects of both nutrient enrichment and grazers on algal species composition. Hillebrand & Kahlert (2001) showed that algal biomass and taxonomic composition can be strongly influenced by grazing pressure but also to be enhanced by increasing nutrient levels.

There have been no previous studies investigating the combined effects of grazing by *P. exigua* and increasing nutrients on microalgal communities, although grazing by *P.*

exigua has been reported to influence microalgal communities on soft-sediment (Dawson & Pillay 2011) and in rocky shore communities (Jackson *et al.,* 2009). Of these studies, Jackson *et al.,* (2009) showed that *P. exigua* can extrude it stomach on the rock surface and remove up to 60% of the superficial microalgae in a single feeding event. The stomach eversion feeding mode of *P. exigua* may not be very effective when feeding on soft-sediments compared with rocky substrata. Jackson *et al.,* (2009) and Dawson & Pillay (2011) pointed out that grazing by *P. exigua* cannot be compared to other dominant grazers that can exert strong top-down control on producers. The reason for this is that *P. exigua* lacks a sophisticated feeding organ comparable to those of other dominant grazers such as limpets (Branch & Branch 1980). *Parvulastra exigua* simply digests and absorbs all the organic matter beneath its extruded stomach, potentially making it less efficient as a feeder and in exerting strong influences over communities.

Theoretically, increasing grazing pressure of *P. exigua* at lower nutrient levels was proposed to decrease microalgal diversity. Higher nutrient levels were hypothesized to interact with increasing starfish densities and cause an increase in microalgal diversity, whereas, intermediate nutrient levels were proposed to produce a hump-shaped pattern of diversity against the increasing grazing pressure of *P. exigua*. In terms of effects on community descriptors, nutrient enrichment had marginally non-significant effects on species evenness (J’) and Shannon-Wiener diversity (H’). Grazing by *P. exigua* was more influential and affected both microalgal species richness (S) and Shannon-Wiener (H’) diversity, with both showing U-shaped responses to increasing densities of *P. exigua* at 1N and 2N nutrient enrichment treatments. The initial decrease in these indices at low to intermediate starfish densities could be caused by a combination of direct and indirect effects of *P. exigua*. Direct effects could include the consumption of microalgae whereas, indirect effects could occur due to the negative effects of physical disturbance caused by the movement of *P. exigua* over sediments. The promotive role of starfish on microalgal indices emerged at highest *P. exigua* densities and could be linked with the increased production of EPS at highest starfish densities, which could prevent resuspension of microalgae at the surface sediment into the water column (Wotton 2004). Alternatively, consumption of dominant microalgal species by *P. exigua* could indirectly favour the establishment of rare species that could not previously co-exist with dominants. Feeding by *P. exigua* involves eversion of its stomach over the sediment and adhesion of organic matter onto the stomach. In this mechanism, starfish are unlikely to be selective in preferentially consuming particular taxa. However, they could reduce the abundance of dominant taxa simply on the basis that dominant taxa are likely to be disproportionately more abundant on sediments.

The U-shaped pattern of microalgal richness and diversity document here is opposite to the findings of Dawson & Pillay (2011), who indicated a unimodal hump-shaped pattern of microalgal species richness, diversity and abundance in response to increasing grazing pressure by *P. exigua*. Hillebrand *et al.,* (2000) showed that grazers reduce species richness in marine microbenthic communities in an interactive experimental study that manipulated grazers and nutrients. They also illustrated that grazers can interact with nutrients to reduce the species diversity and evenness of marine microbenthic community at low nutrient levels but increase both indices at high nutrient supply. However, in their study the level of grazers was not specified and also included more than one grazing species. The impacts as a result of grazing by more than one species may be higher since the species likely differ in terms of grazing mode (Underwood & Jernakoff 1981).

2.7.3 Macrofaunal community: Overall composition, dominant species and diversity indices

Macrofaunal community structure was significantly influenced by nutrient enrichment but not by starfish grazing pressure. Even though ordination techniques showed ambiguous support for nutrient effects (Fig. 2.8 A), the latter was evident in pair-wise tests that showed differences in macrofaunal assemblages between the 0N and 2N and 1N and 2N treatments. Nutrient treatments were however, not very influential at the level of individual species, as only two of the six dominant macrofaunal species selected by SIMPER were affected viz. the bivalve *Carditella rugosa* and the burrowing polychaete *Notomastus latericeus*. The abundance of *C. rugosa* showed a linear increase against increasing nutrient levels whereas *N. latericeus* revealed a U-shaped response to increasing nutrient levels, with lower abundance at intermediate nutrient levels (1N). Both of these species were also influenced by grazing effects of *P. exigua*, even though for the *N. latericeus* post hoc tests failed to identify significant differences between starfish densities.

A lack of significant nutrient effects on other macrofaunal species could be due to these species being insensitive to nutrient increases. It is possible that "insensitive" species consume food sources other than microalgae which do not respond to nutrient enrichment. In estuaries and lagoons, detritus has often been viewed as an important food source of consumers (Dunn *et al.,* 2007). If there is a high dependence on detritus and detritus is highly abundant, then consumers may not necessarily respond to increases in nutrient levels through microalgal enrichment.

The linear increase in abundance of the bivalve *C. rugosa* with increasing nutrient levels may be due to indirect enhancement of food quantities available to this species. *C. rugosa* is a suspension surface feeder that mainly consumes microalgae and other organic particles suspended in the water column (Josefsona & Rasmussen 1999). As primary producer abundance is strongly linked with nutrient availability, it is possible that *C. rugosa* abundance was indirectly enhanced by nutrient enrichment. Elevated nutrient levels may have caused localised increases in phytoplankton biomass at the sediment-water interface within the immediate vicinity of nutrient bags.

Grazing pressure generated by *P. exigua* influenced the abundance of *C. rugosa* in a hump-shaped manner, which was most obvious in the 2N nutrient enrichment treatment. This pattern could be generated due to the balance between EPS production and physical disturbance caused by *P. exigua*, with EPS exudation playing a promotive role in elevating abundances of *C. rugosa* at low to intermediate starfish densities. Elevated EPS levels in turn, could favour a shift from suspension to the deposit feeding, as is known for other suspension feeding species (Reise 2001). At greater starfish densities, sediment disturbance and interference of the siphons by *P. exigua* may have caused a reduction in *C. rugosa* density.

The results of this experiment, which showed a limit impact of nutrients on macrofaunal species, are partially consistent with those of Shin & Cheung (2010), who concluded that nutrient enrichment increases the abundance of certain species over a long time period and that responses are not quick. Posey *et al.,* (1995) and Beukema (1991) also noted an increase in growth and abundance of certain species with nutrient enhancement. Some studies, however, have documented the opposite effects of nutrient enhancement on macrofaunal communities including a decrease in abundance, diversity, evenness and richness (Simboura *et al.,* 1995, Shin & Cheung 2010), or undetectable responses (Wiltse *et al.*, 1984). In some cases, sensitive species have been reported to be completely removed from a habitat and be replaced by tolerant ones (Pearson & Rosenberg 1978, Diaz & Rosenberg 1995, Shin & Cheung 2010) or fast growing opportunistic ones (Fitch & Crowe 2012) due to high nutrient levels.

The effects of nutrient enrichment on macrofaunal indices were only apparent for Shannon-Wiener diversity (H’). Even though post hoc tests located significant differences between the 1N and 2N nutrient enrichment treatments for Shannon-Wiener diversity (H’) index, graphically (Fig 2.10), this was not overwhelmingly supported. Nutrient effects were marginally non-significant for species evenness (J’) and abundance (N). The unresponsiveness of individual macrofaunal species to nutrient enrichment may have contributed to negligible nutrient effects on macrofaunal indices. These results, therefore, indicate subtle effects of bottom-up control on macrofaunal indices.

2.7.4 Functional groups

This study documented interesting responses of surface suspension feeders to nutrient enrichment and grazing by starfish. A gradual increase in suspension feeder abundance was recorded across all nutrient enhancements, with a hump-shaped response within each nutrient treatment in relation to increasing starfish densities. These results indicate a strong non-interactive control of top-down and bottom-up mechanisms on the abundance of suspension feeders. These results are similar to those reported by Posey *et al.,* (2002), who documented positive influences of nutrients on grazers and near-surface taxa. One possible reason for the positive effects of nutrients on suspension feeders in the current study is that suspension feeders are reported to be limited by food availability (Heip *et al.,* 1995), predominantly phytoplankton concentrations. In this study, nutrient enrichment may have enhanced phytoplankton production and, as a result, the abundance of suspension feeding species.

The influence of *P exigua* on suspension feeders may indicate that this functional group does not entirely depend on suspended particles or phytoplankton for food. It is likely that the suspension feeders, which were dominated by the bivalve *Carditella rugosa*, also consume food particles from surface sediments under certain conditions. Hump-shaped responses of suspension feeders due to increasing *P. exigua* densities at each nutrient level may be indicate an ability of low to intermediate starfish densities to facilitate abundances of suspension feeders, by enhancing EPS levels

The effect of nutrient enrichment or grazing by starfish failed to influence burrowing deposit feeders and scavengers/predators. Burrowing species are unlikely to be affected by nutrient effects. Prominent nutrient effects could be expected on species inhabiting the surface sediment where sunlight availability is often not limiting. Similarly, sediment disturbance by *P. exigua* may also be negligible for infaunal species that live below the sediment surface.

Surface scavengers/predators were also one of the functional groups affected by the grazing effects of *P. exigua* but not by nutrient enhancement. This functional group showed a steady increase in abundance against increasing grazing pressure at 0N and 1N. At the highest nutrient enhancement, the pattern was not very obvious but can be described as a U-shape response for simplicity. At low nutrient levels, increasing densities of *P. exigua* could elevate densities of surface scavengers/predators through promotive effects on other species that are potential prey for predators. Increasing EPS production by increasing densities of *P. exigua* could also positively influence surface scavengers/predators by providing an alternate food source for members of this functional group.

2.8 Methodological considerations

Caging experiments, which are frequently used in the ecological field experiments in intertidal shores (Miller & Gaylord 2007), were the main research tool used in this investigation to test the interactive effects of grazing and nutrient levels on intertidal soft-sediment community structure. The rationale behind using cages is that cages enable controlled manipulation of densities of organisms of interest (such as grazers and predators) to test various hypothesis in ecology. However, the major criticism encountered in the literature regarding caging experiments is the development of caging artifacts which can influence the outcome of ecological investigations. In some cases, cage artifacts can be mitigated by the use of a cage control, which is usually a partial cage (Miller & Gaylord 2007). The inevitable problem facing most ecologists when dealing with caging experiments is that a perfect control cage is unlikely to be achieved (Pillay 2006), as one can never create a cage control that has all the physical features of the experimental cages but not be a cage.

For the purpose of this experiment, the approach was to use multiple starfish levels and not just inclusions and exclusions, with the rationale being that whatever artefact was present in the experiment would have been held consistently across all cages and that the patterns observed would be due to starfish grazing or nutrients (Dawson & Pillay 2011).

Another important consideration in this study and in ecology generally, is the challenge of determining adequate sample sizes. A high level of variability was observed in some data (eg. microalgal biomass) between the cages in this experiment. However, other variables showed less variability, making it difficult to determine optimal sample size at the termination of the experiment. In hindsight, additional samples could have been collected for highly variable data, and may be necessary in future studies, to increase statistical power and reveal ecological patterns and processes more clearly.

CHAPTER 3: FINAL SUMMATION

3.1 General conclusion and the lessons learned

In a world where human impacts on ecosystems are diverse, a wide range of ecological interactions need to be examined in order to increase our understanding of community ecology and ecosystem functioning. We know a lot about the fundamental effects of increased nutrients in ecosystems (Burkepile & Hay 2006, Mork *et al*., 2009), and we also know that primary consumers can temper most effects of high resource on the autotrophs (Poore *et al.,* 2012). However, we don't know much about the different herbivores that can interact with productivity to temper these effects. Identifying the relative role of nutrients and primary consumers has become more significant as their contributions in structuring communities can differ with the type of ecosystem and primary producers involved (Burkepile & Hay 2006). Unfortunately, field experiments limit us through various experimental artifacts (Miller & Gaylord 2007) and the spatial and temporal scale over which experiments can be conducted (Cummings *et al.,*2001) which make it more difficult to extrapolate on the role of particular species in communities impacted by anthropogenic effects.

Here, I experimentally ascertained if localised productivity (nutrients) enhancement could interact with grazing by starfish to structure intertidal soft-sediment microalgal and macrofaunal communities. The EPS concentration secreted by the starfish and microalgal biomass (Chl a) were the additional variables measured in the study, as they could potentially influence community patterns.

Microalgal community structure responded to nutrient enhancement but not to the interactive effects of nutrients and starfish grazing. Nutrient levels and its interaction with starfish densities only affected a few of the microalgal morphotypes from those that were identified as dominating community structure (Table 2.1). It must be noted that, although the microalgal community structure was influenced by the nutrient enhancement, very few morphotypes were affected. For diversity indices, none of the microalgal indices were influenced by nutrient enhancement but starfish densities did influence microalgal species diversity and richness. Nutrients and starfish also interacted to influence EPS concentrations (Figure 2.3). The macrofaunal community responded to nutrients but not to starfish densities or the interaction between nutrient levels and starfish densities. The effects of nutrient and grazing treatments were, however, very limited for macrofaunal community discriptors. Species diversity was the only macrofaunal index influenced by nutrient levels, while species evenness and abundance showed marginally non-significant responses to nutrient levels. In terms of impacts at the species level, only two of the six dominant macrofaunal species were influenced by nutrient treatments and starfish densities (Table 3.1). For the bivalve *Carditella rugosa,* increasing nutrients increased abundance of this species, whereas, increasing starfish densities created a hump-shaped pattern within each nutrient level. The polychaete *Notomastus latericeus* showed a U-shaped response at the 0N and 2N nutrient levels with increasing starfish densities.

At the functional level, suspension feeders were affected by nutrient enhancement and starfish densities, while surface scavengers/predators were affected by starfish densities. Increasing nutrient enhancement led to increases in the abundance of suspension feeders, whereas increasing grazing pressure resulted in a hump-shaped response of suspension feeders across all nutrient treatments. Increasing starfish grazing at the 0N and 1N nutrient levels caused an increase in abundance of surface scavengers/predators.

Although nutrient availability and grazing pressure have often been hypothesized to interact and shape natural ecosystem (Power 1992, Proulx & Mazumder 1998), results of this investigation emphasise the importance of nutrient levels in structuring benthic communities. Subtle effects of *P. exigua* on producers and consumers indicate that this species may not always affect communities as has been reported previously (Jackson et al., 2009, Dawson & Pillay 2011). However, caution is necessary when drawing conclusions about the strength of the top-down force exerted by *P. exigua* on both communities. Factors such as high scale variability between the cages or complex biological interaction between the EPS production by the starfish and microalgae or consumption could have been related to the minor grazing effects of *P.exigua*.

Based on the hypothesis of this study that grazing by *P. exigua* will influence microalgal and macrofaunal communities according to the grazer-reversal hypothesis (Proulx & Mazumder 1998), the findings were not consistent with the prediction, although, variables like EPS, suspension feeders and particular macrofaunal species (*C. rugosa*) appeared to follow a prediction of hump-shaped pattern with increasing grazing pressure at intermediate nutrient levels. There was no agreement with the grazer reversal hypothesis, however, with increasing nutrient enrichment. According to Proulx & Mazumder (1998) not all communities produce consistent patterns predicted by the grazer-reversal hypothesis. These results may also shed light on the importance of intermediate *P. exigua* densities at intermediate nutrient levels because of promotive effects in soft-sediments. In this study, the lack of consistent or prominent effects of *P. exigua* on both communities could be related to non-specialized feeding behaviour of this species. Previous studies highlighted that the subtle grazing effect of *P. exigua* on microalgal and macrofaunal communities is likely to be caused by a lack of specialised feeding organs by this species. Based on the fact that *P. exigua* extrudes its stomach on the rock surface and digests all organic matter underlying the stomach (Jackson *et al.,* 2009), its effect can be very subtle. Feeding by *P. exigua* in soft-sediment may be more challenging than on rocky surfaces due to the unconsolidated nature of sediment, that make extracting microalgae and other organic material less efficient.

REFERENCES

ABRAHAMS, P.A. 1988. Resource productivity-consumer species diversity: simple models of competition in spatially heterogeneous environment. *Ecol. Socie. Americ* **69**: 1428-1433.

ADMIRAAL, W.E., DE RUYTER VAN STEVENINCK, E.D. & DE KRUIJF, H.A.M. 1989. Environmental stress in five ecosystems in the floodplain of the River Rhine. *Sci.Total Environ* **78***:* 59-75.

ALLAN, E. & CRAWLEY, M.J. 2011. Contrasting effects of insect and molluscan herbivores on plant diversity in a long-term field experiment. *Ecol. Lett* **14**: 1246-1253

ANDERSON, M.J. 1999. Distinguishing direct from indirect effects of grazers in estuarine intertidal assemblages. *J. Exp. Mar Biol. Ecol* **234**:199-218.

ARTIGAS, J., ROMANI, A.M. & SABATER, S., 2007. Effects of nutrients on the sporulation Mediterranean and diversity of aquatic hyphomycetes on submerged substrata in a stream. *Aquat. Bot*. **88**: 32-38.

ARMITAGE, A.R. & FONG, P. 2004. Upward cascading effects of nutrients: shifts in a benthic microalgal community and a negative herbivore response. *Oecologia* **139**: 560-567.

BADEN, S.P., LOO, L.O., PIHL, L. & ROSENBERG, R.1990. Effects of eutrophication on benthic community fish-Swedish West coast. *Ambio* **19**:**113**-122.

BAGGETT, L., HECKT JR, K.L., FRANKOVICH, T.A, ARMITAGE, A.R. & FOURQUREAN, J.W. 2010. Nutrient enrichment, grazer identity, and their effects on epiphytic algal assemblages: field experiments in subtropical turtle grass *Thalassia testudinum* meadows. *Mar. Ecol. Prog. Ser* **406**: 33-45.

BARNES, R.S.K. & ELLWOOD, M.D.F. 2011. The significance of shore height in intertidal macrobenthic seagrass ecology and conservation. Aquatic Conservation: Marine and Freshwater Ecosystems .Volume 21, pages 614-624.

BEUKEMA, J. 1991. Changes in composition of bottom-fauna of a tidal flat area during a period of eutrophication. *Mar. Bio* **111**:292-301.

BERCH, S.M, BATTIGELLI, J.P. & HOPE, G.D., 2007. Responses of soil mesofauna communities and oribatid mite species to site preparation treatment in high-elevated culblocks in southern British Columbia. *Pedobiologia* **51**: 23-32.

BUCOLO, P., SULLIVAN, M.J. & ZIMBA, P.V. 2008. Effects of nutrient enrichment on primary production and biomass of sediment microalgae in a subtropical sea grass bed. *J. Phycol*. **44**: 874-881.

BRANCH, G.M., GRIFFITHS, C.L., BRANCH, M.L. & BRECKLEY, L.E. 2010. Two Oceans. A guide to the marine life of Southern Africa. Struik Nature, Cape Town, South African.

BRANCH, G.M. & BRANCH, M.L. 1980. Competition between *Callana tramoserica* (Sowerby) (Gastropoda) and *Patiriella exigua* (Lamarck) (Asteroidea), and their influence on algal standing stocks. *J. Exp. Mar.Bio. Ecol* **330**: 245-260.

BURKEPILE, D.E. & HAY, M.E. 2006. Herbivore vs. nutrient control of marine primary producers: context-dependent effects. *Ecology* **87**: 3128–3139.

CAHOON, L. B. 1999. The role of benthic microalgae in neritic ecosystems. *Oceanogr. Mar. Bio* **37**: 47-86.

CARRICK JR H, J. & LOWE, R.L. 1988. Response of Lake Michigan benthic algae to in situ enrichment with Si, N, and P. *Can. J. Fish. Aquat. Sci* **45***:* 271-279.

COLE, L, BUCKLAND, S.M. & BARDGETT, R. D. 2008. Influence of disturbance and nitrogen addition on Plant and soil animal diversity in grassland. *Soil. Bio. Biochem* **40**: 505-514.

COOPER, R. 2011. The interactive effects of sand prawn (*Callichirus kraussi*) stebbing bioturbation and nutrients on macrofaunal communities. MSc thesis. University of Cape Town. South Africa. Cape Town.

CROSSLEY, D.A., MUERLLER, R.D. & PERDUE, J.C. 1992. Biodiversity of microathropods in agricultural soils: relations to processes. *Agric. Ecosys. Environ* **40**: 37-46.

CUMMINGS, V.J., THRUSH, J.E. & FUNNELL, G.A. 2001. Variable effect of a large suspension-feeding bivalve on infauna: experimenting in a complex system. *Mar. Ecol .Prog. Ser* **209**: 159-175.

DAWSON, J. & PILLAY, D. 2011. Influence of starfish grazing on lagoonal microalgal communities: non-competitive mechanisms for unimodal effects on diversity. *Mar. Ecol. Prog. Ser* **435**: 75-82.

DAYTON, P.K. 1971. Competition, Disturbance and Community organization: The provision and subsequent utilization of space in a rocky intertidal. PhD thesis. University of Washington, Seattle, Washington.

DEEGAN, L. A., PETERSON, B. H., GOLDER, H., MCIVER, C. C. & MILLER. M. C. 1997. Effects of fish density and river fertilization on algal standing stock, invertebrate communities, and fish production in an Arctic river. *Can. J. Fish. Aquat. Sci* **54**: 269-283.

DIAZ, R. J. & ROSENBERG, R. 1995. Marine Benthic hypoxia: a review of its ecological effects and the behavioural responses of benthic macrofauna. *Oceanogr. Mar.Bio* **33**: 245.

DUFFY, J.E. 2003. Biodiversity loss, trophic skew, and ecosystem functioning. *Ecol. Lett* **6**: 680-687.

DUNN, R.J.K., ALI, A., LERMCKERT, C.J., TEASDALE, P.R. & WELSH, D.T. 2007. Short-term Variability of Physico-chemical Parameters and the Estimated Transport of Filterable Nutrients and Chlorophyll-*a* in the Urbanised Coombabah Lake and Coombabah Creek System, Southern Moreton Bay, Australia. *J. coast. Res* **50**: 1062-1068.

FITCH, J.E. & CROWE, T.P. 2012. Combined effects of inorganic nutrients and organic enrichment on intertidal benthic macrofauna: an experimental approach. *Mar. Ecol Prog. Ser* **461**: 59-70.

FLINT, R.W. & GOLDMAN, C.R. 1975. The effects of a benthic grazer on the primary productivity of the littoral zone of Lake Tahoe. *Limnol. Oceanogr* **20**: 935-944.

GAN, L., PENG, X. & HORN, R. 2012. Effects of grazing intensity on soil thermal properties and heat flux under Leymus Chinensis and Stipa grandis vegetation in Inner Mongolia, China. *Soil & Tillage Res* 1**18**: 147-158.

GOUGH, L. & GRACE, J.B. 1998. Herbivore effects on plant species density at varying productivity levels. *Ecology* **79**: 1586-1594.

GUERRY, A.N. 2006. Grazing, Nutrients, and Marine Benthic Algae: Insights into the Drivers and Protection of Diversity. PhD Thesis. Oregon State University.

GUERRY, A.D, MENG, B.A & DUNMORE, A.R. 2009. Effects of consumers and enrichment on abundance and diversity of benthic algae in a rocky intertidal community. *J.Exp. Mar. Bio and Ecol* **369**: 155–164.

HAGY, J.D, BOYNTONA, W.R. & JASINSKI, D.A. 2005. Modelling phytoplankton deposition to Chesapeake Bay sediments during winter–spring: interannual variability in relation to river flow. *Estuar. Coast. and Shelf* Scie **62**: 25-40.

HEIP, C. H. R., GOOSEN, N. K., HERMAN, P. M. J., KROMKAMP, J., MIDDELBURG, J. J. & SOETAERT, K. 1995. Production and consumption of biological particles in temperate tidal estuaries. *Oceanogr. Mar. Biol. Annu. Rev* **33:**1-149.

HILLEBRAND, H, WORM, B. & LOTZE, H.K. 2000. Marine microbenthic community structure regulated by nitrogen loading and grazing pressure. *Mar. Ecol. Prog. Ser* 204: 27-38.

HILLEBRAND, H. & SOMMER, U. 1997. Response of epilithic microphytobenthos of the Western Baltic Sea to in *situ* experiments with nutrient enrichment. *Mar. Ecol. Prog. Ser* **160**:35-46.

HILLEBRAND, H. 2003. Opposing effects of grazing and nutrients on diversity. *Oikos* **100**: 592-600.

HILLEBRAND, H. & KAHLERT, M. 2001. Effect of grazing and nutrient supply on periphyton biomass and nutrient stoichiometry in habitats of different productivity. *Limnol. Oceanogr* **46**(8):1881-1898.

HIRST, C.N, CYR, H & JORDON, I.A. 2003. Distribution of Exopolymeric Substances in the Littoral Sediments of an Oligotrophic Lake. *Microb. Ecol* **64**: 22-32.

HUSTON, M.A. 1979. A general hypothesis on diversity. *Am. Nat.* **113**: 81-101.

JACKSON, A.C., MURPHY, R.J. & UNDERWOOD, A.J. 2009. *Patiriella exigua*: grazing by a starfish in an overgrazed intertidal system. *Mar. Ecol. Prog. Ser* **376**:153-163.

JOSEFSON, A. B. & ROSENBER, R. 1988. Long-term soft-bottom faunal changes in three shallow fjords, West Sweden. *Neth. J. Sea Res* **22**:149-159.

JOSEFSON, A.B. & RASMUSSEN, B. 1999. Nutrient Retention by Benthic Macrofaunal Biomass of Danish Estuaries: Importance of Nutrient Load and Residence Time. *Estuar.Coast. Shelf. Sci* **50**: 205-216.

KEHDE, P.M. & JERRY.L.W. 1972. The Effects of Grazing by Snails on Community Structure of Periphyton in Laboratory Streams. *Americ. Mid. Nat* **87**: 8-24.

KELAHER, B.P., LEVINTON, J.S. & HOCH, J.M. 2003. Foraging by the mud snail *Ilyanassa obsoleta* (Say) modulates spatial variation in benthic community structure. *J. Exp. Mar. Biol. Ecol* **292**: 139-157.

KONDOH, M. 2001. Unifying the relationships of species richness to productivity and disturbance. *Royal Society* **268**: 269-271.

LAM, C., HADER, T. & QIAN, P. 2005. Induction of larval settlement in the polychaete *Hydroides elegans* by extracellular polymers of benthic diatoms. *Mar. Ecol .Prog. Ser* **286**:145-154.

LAMBERTI, G.A., ASHKENAS, R.L. & GREGORY. A.V. 1987. Effects of three herbivores on periphyton communities in laboratory streams: J*. N. Am. Benthol. Soc* **6**(2): 92-10.

LEVINTON, J.S. & STEWART, S. 1982. Marine succession: the effect of two deposit-feeding gastropod species on the population growth of *Paranais litoralis* (Oligochaeta). *J.Exp. Mar Bio. Ecol* **59**: 231-241.

LIESS, A., LANGE, K., SCHULZ, F., PIGGOTT, J.J., MATTHAEI, C.D. & TONWNSEND, C.R. 2009. Light, nutrients and grazing interact to determine diatom species richness via changes to productivity, nutrient state and grazer activity. *J. Ecol* **97**: 326-336.

LYNAM, C.P., HAY, S.J. & BRIERLEY, A.S. 2005. Jellyfish abundance and climatic variation: contrasting responses in oceanographically distinct regions of the North Sea, and possible implications for fisheries. *J. Mar. Biol. Ass* **85**: 435-450.

LOWE, R. L. & HUNTER, R.D. 1988. Effect of grazing by *Physa integra* on periphyton community structure. J. N. Am. *Benthol. Soc.* **7:** 29-36.

MCCLANAHAN, T.R., COKOS, B.A. & SALA,E. 2002. Algal growth and species composition under experimental control of herbivory, phosphorus and coral abundance in Glovers Reef, Belize. *Mar. Poll. Bull* **44**: 441-451.

MCQUEEN, D. J., JOHANNES, M. R. S., POST, J. R., STEWART, T. J. & LEAN, D. R. S. 1989. Bottom-up and top-down impacts on freshwater pelagic community structure. *Ecol. Mon* **59**: 289-309.

MILLER, M.W., HAY, M.E., MILLER, S.L., MALONE, D., SOTKA, E.E. & SAMANT, A.M. 1999. Effects of nutrients versus herbivores on reef algae: a new method for manipulating nutrients on coral reefs. *Limn. Ocean* **44**: 1847-1861.

MILLER, L.P. & Gaylord. B. 2007. Barriers to flow: The effects of experimental cage structures on water velocities in high-energy subtidal and intertidal environments. J. *Exp. Mar. Bio. Ecol* **344**: 215-228.

MORK, E., SJOO, G.L., KARUTSKY, N. & MACLANAHAN, T.R. 2009. Top–down and bottom-up regulation of macroalgal community structure on a Kenyan reef. *Estuar. Coast. Shelf. Sci* **84**: 331-336.

MORRISEY, D.Y.1988. Differences in effects of grazing by deposit-feeders *Hydrobia ulvae* (Pennant) (Gastropoda: Prosobranchia) and *Corophium arenarium* (Crawford) (Amphipoda) on sediment microalgal populations. II. Quantitative effects. *J. Exp. Mar Biol. Ecol* **118**: 43-53.

NICOTRI, M. E. 1977. Grazing effects of four marine intertidal herbivores on the microflora. *Ecology* **58**: 1020-1032.

NIELSEN, K. J. 2003. Nutrient loading and consumers: Agents of change in open-coast macrophyte assemblages. *Proceedings of the National Academy of Sciences*, USA **100**: 7660-7665.

PACALA, S.W. & CRAWLEY, M.J. 1992. Herbivores and plant diversity. *Am. Nat* **140**, 243-260.

PARTSON, D.M. & HAGERTHEY, S.E. 2001. Microphytobethos in contrasting coastal ecosystems: *biology and dynamics*. In: Reise K (ed) Ecological comparisons of sedimentary shores. Ecological studies, volume 151: Pp: 276-293. Springer-Verlag, Berlin, Heidelberg.

PEARSON, T.H. & ROSENBERG, R. 1978. Macrobenthic succession in relation to organic enrichment and pollution of the marine environment. *Oceanogr Mar. Bio. Annu Rev* **16**: 229-311.

PILLAY, D. 2006. The influence of bioturbation by *Callianassa kraussi* Stebbing on the macrobenthic assemblages of Little Lagoon. PhD Thesis, University of Kwa-Zulu-Natal, Durban, South Africa.

PILLAY, D., BRANCH, G.M. & FORBES, A.T. 2007. Effects of *Callianassa kraussi* on microbial biofilms and recruitment of macrofauna: a novel hypothesis for adult–juvenile interactions. *Mar. Ecol. Prog .Ser* **347**: 1-14.

PILLAY, D. BRANCH, G. M. & STEYN.A. 2009. Complex effects of the gastropod Assiminea globulus on benthic community structure in a marine-dominated lagoon. *J. Exp. Mar. Bio. Ecol.* **380**:47-52.

PILLAY, D. BRANCH. G. M, & STEYN.A. 2010. Unexpected effects of starfish grazing on sand-flat communities following an outbreak. *Mar. Ecol. Prog. Ser* **398**: 173-182.

PITTA, P., GIANNAKOUROU, A., DIVANAUH, P. & KENTOURI. M. 1998. Planktonic food in marine mesocosms in the eastern Mediterranean: Bottom-up or top-down regulation? *Hydrobiologia* **363**: 97-105.

PLANTE, C.J, FEIPEL, S. & WILKIE, J.L. 2010. Disturbance effects of deposit feeding on microalgal community structure and mechanisms of recolonization. *J. Phycol* **46**: 907-916.

POSEY, M.H., DUMBAULD, B.R. & ARMSTRONG, D.A. 1991. Effects of a burrowing mud shrimp, *Upogebia pugettensis* (Dana), on the abundances of macro-infauna. *J. Exp. Mar. Biol. Ecol* **148**, 283-294.

POSEY, M., POWELL.C, CAHOON, L. & LINDQUIST.D. 1995. Top down vs. bottom up-control of benthic community composition on an intertidal tide flat. *J. Exp. Mar. Bio. Ecol* **185:** 19-31.

POSEY, M. H., ALPHIN, T., DCAHOON, L., LINDQUIST, D. & BECKER. M.E. 1999. Interactive effects of nutrient additions and predation on infaunal communities. *Estuaries* **22**: 785-792.

POSEY, M.H., ALPHIN, T., CAHOON, L.B., LINDQUIS, D.G., MALLIN, M.A. & NEVERS, M.B. 2002. Top-down versus bottom-up limitation in benthic infaunal communities: direct and indirect effects. *Estuaries* **25**: 999-1014.

POSEY, M.H., ALPHIN, T. & CAHOON, L. 2006. Benthic community responses to nutrient enrichment and predator exclusion: influence of background nutrient concentrations and interactive effects. *J. Exp. Mar. Biol. Ecol* **330**: 105-118

POORE, A.G., CAMPBELL, A.H., COLEMAN, R.A., EDGAR, G.J., JORMALANEN, V., REYNOLDS, P.L., SOTKA, E.E., STACHOWICZ, J.J., TAYLOR, R.B., VANDERKLIFT, M.A. & DUFFY, J.E. Global patterns in the impact of marine herbivores on benthic primary producers. *Ecol. Lett* **15**(8): 912-922.

POWER, M. E. 1992. Top-down and bottom-up forces in food webs: Do plants have primacy? *Ecology* **73**: 733-746.

PROXY, M. & MAZUMDER A. 1998. Reversal of grazing impact on plant species richness in nutrient-poor vs. nutrient-rich ecosystems. *Ecologia* **79**: 2581-2592.

RABALAIS, N.N, TURNER, R.E, BARUN, K. GUPTA, S, PLATON, E. & PARSONS, M.L. 2007. Sediment tells the history of the eutrophication and sediment hypoxia in the Northern Gulf of Mexico. *Ecol. Appl***17**(5): 129–143.

REISE, K. 2001. In: Reise K (ed). Ecological comparisons of sedimentary shores. Springer Verlag Berlin Heidelberg New York.

ROBEN, A. R., WYATT, K.H. & STEVENSON, R.J. 2011. Regulation of algal structure and function by nutrients and grazing in a boreal wetland. *J. N. Am. Benthol. Soc* **30**(3): 787–796.ROLL, S. K., DIELH, S. & COOPER, S. D. 2005. Effects of grazer immigration and nutrient enrichment on an open algae-grazer system. *Oikos* **108**: 386-400.

ROSENBERG, R., ELMGREN, R., FLEISCHER, S., JONSSON, P., PERSSON, G. & DAHLIN, H. 1990. Marine eutrophication. Case studies in Sweden. *Ambio* **19***:* 102-108.

ROSENBERG, R., LOO, L.O. & MOELLER, P. 1992. Hypoxia salinity and temperature as structuring factors for marine benthic community in eutrophication area. *N. J. sea res* **30**: 121.129.

ROSENBERG, R.1985. Eutrophication-the future marine coastal nuisance? *Mar. Pollut. Bull* **16**: 227-231.

ROXBURGH, S.H., SHEA, K. & WILSON., J.B. 2004. The intermediate disturbance hypothesis: Patch dynamics and mechanisms of species coexistence. *Ecol. Socie. Amer* **85**: 359-371.

SARDA, R., VALIELA, I. & FOREMAN, K. 1996. Decadal shifts in a salt marsh macroinfaunal community in response to sustained long-term experimental nutrient enrichment. *J. Exp. Mar Bio. Ecol* **205**: 63-81.

SHAEFER, A. & SHAEFER, P. 1993. Lagoon: A companion to the West Coast National Park. Yoshi Publishing.Pp 89. Cape Town.

SHIN, R.T.S. & CHEUNG, S.G. 2010. Effects of Nutrient Enrichment on Intertidal Macrobenthic Community in Subtropics: A field manipulation Experiment. In:

Eutrophication, Ecological Effects, Sources, Prevention. Carolann D. Webber (ed). Chapter 1. Nova science publisher Inc. City University of Hong Kong.

SIMBOURA, N., ZONOTOS, A., PANAYOTIDES, P. & MAKRA, A. 1995. Changes in benthic community structure along an environmental pollution gradient. *Mar. Poll. Bull* **30**:470-74.

SNELGROVE, P.V. R. 1998. The biodiversity of macrofaunal organisms in marine sediments. *Biodiv.Cons* **7**:1123-1132.

SPIVAK, A.C., CANUEL, E.A., DUFFY, J.M. & RICHARDSON, J.P. 2009. Nutrient Enrichment and Food Web Composition Affect Ecosystem Metabolism in an Experimental Sea grass Habitat. PloS ONE **4**: 10- e7473.

SUNDBACK, K. & SNOEIJS, P. 1991. Effects of Nutrient Enrichment on Microalgal Community Composition in a Coastal Shallow-water Sediment System: An Experimental Study. *Bot.Mar* **34**: 341-358.

SUDING, K. N., COLLINS, S.L., GOUGH, L., CLARK, C., CLELAND, E.E., GROSS, K.L, MILCHUNAS, D.G. & PENNINGS, S. 2005. Functional and abundance-based mechanisms explain diversity loss due to N fertilization. Proceedings of the National Academy of Sciences, USA **102**: 4387-4392.

SULLIVAN, M. J. 1976. Long-term effects of manipulating light intensity and nutrient enrichment on the structure of a salt marsh diatom community. *J. Phycol.* **12***:* 205-2010.

SULLIVAN, M. J. 1981. Effects of canopy removal and nitrogen enrichment on a *Distichlis spicata-edaphic* diatom complex. *Estuar. Coast. Shelf Sci* **13***:*119-129.

TAYLOR, J.C., HARDING, W.R. & ARCHIBARD, C.G.M. 2007. A methods manual for the collection, preparation and analysis of diatom samples. Water Research Commission Report TT **281/07**:1-49.

TSUTSUMI, H., FUKUNAGA, S., FUJITA, N. & SUMIDA. M. 1990. Relationship between growth of *Capitella* sp. and organic enrichment of the sediment. *Mar. Ecol. Prog. Ser* **63:** 157-162.

UNDERWOOD, A.J. 1978. An experimental evaluation of competition between three species of intertidal Prosobranch Gastropods. *Oecologia* **33** (2): 185-202.

UNDERWOOD, A.J. & JERNAKOFF, P. 1981. Effects of interactions between algae & grazing gastropods on structure of low-shore intertidal algal community. *Oecologia* **48**(2): 221-233.

UNDERWOOD, G.J.C. & PATERSON, D.M. 1995. The measurement of microbial carbohydrate exopolymers from intertidal sediments. *Limn. Oceanogr* **40**: 1243-1253.

UTHICKE, S. 2001. Interactions between sediment-feeders and microalgae on coral reefs: losses versus production enhancement. *Mar. Ecol. Prog. Ser* **210**: 125-138.

VALIELA, I., MCCLELLAND, J., HAUXEWELL, J., BEHR, P.J., HERSH, D. & FOREMAN, K. 1997. Macroalgal blooms in shallow estuaries: Controls and ecophysiological and ecosystem Consequences. *Limn.Oceanogr* **42**: 1105-1118.

VAN LOOSDRECHT, M.C.M., NORDE, W., LYKLEMA, J. & ZEHNDER, A.J.B. 1990. Influences of interfaces on microbial activity. *Microbio. Review* **54**: 75-87.

VAN DER MAAREL, E. 1993. Some remarks on disturbance and its relationship to diversity and stability. *J. Veget. Sci* **4**: 733-736.

VITOUSEK, P. M., MOONEY, H. A., LUBCHENCO, J. & MELILLO, J.M. 1997b. Human Domination of Earth's Ecosystems. *Science* **277**:494-499.

WELSCHMEYER, N.A. 1994. Fluorometric analysis of chlorophyll-a in the presence of chlorophyll-b and phaeopigments. *Limnol Oceanogr* **39**: 1985-1992.

WERNER, F.J & MATTHIESSEN, B. 2013. Temperature indirectly affects benthic microalgal diversity by altering effects of top – down but not bottom – up control.*Oikos* **122**: 52–63.

WARREN, P.H. & SPENCER, M. 1996. The effects of habitat size and productivity on food web structure in small aquatic microcosm. *Oikos* **75**: 419-430.

WILSON, W.H .1991. Competition and predation in marine soft-sediment communities. *Annu. Rev.Ecol. systemat* **21**: 221-241.

WILSON, S. D. & TILMAN, D. 2002. Quadratic variation in old-field species richness along gradients of disturbance and nitrogen. *Ecology* **83**: 492-504.

WILTSE, W.I., FOREMAN, K.H., TEAL, J.M. & VALIELA, I., 1984. Effects of predators and food resources on the macrobenthos of salt marsh creeks. *J. Mar. Res* **42:** 923-942.

WOLFRATH, B. 1992. Field experiments on feeding of European fiddler crab *Uca tangeri. Mar. Ecol. Prog. Ser* **90**: 39-43.

WOTTON, R.S. 2011. EPS (Extracellular Polymeric Substances), silk, and chitin: vitally important exudates in aquatic ecosystems. *J.N. Am. Benthol. Soc* **30** (3): 762-769.

WOOTTON, J.T., CUSSON, M., NAVARRETE, S. & PETRAITIS, P.S. 2009. Disruption, Succession and Stochasticity. Marine Hard Bottom Communities, *Ecologic. Stud* **206** (3): 201-212.

WOTTON, R.S. 2004. The ubiquity and many roles of exopolymers (EPS) in aquatic systems. *Sci. Mar* **68**: 12-21.

WORM, B., REUSCH, T.B.H. & LOTZE, H.K., 2000. *In situ* nutrient enrichment: methods for marine benthic ecology. *Internat. Rev. Hydrobiologia* **85**: 359-375.

WORM, B., LOTZE, H.K., HILLEBRAND, H. & SOMMER. U. 2002. Consumer versus resource control of species diversity and ecosystem functioning. *Nature* **417**: 848-851.

XIAN, W., KANG, B. & LIU, R. 2005. Jellyfish blooms in the Yangtze Estuary. *Science, New York* **307** 41.