



# In situ soft sediment nutrient enrichment: A unified approach to eutrophication field experiments



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## ABSTRACT

Adding fertiliser to sediments is an established way of studying the effects of eutrophication but a lack of consistent methodology, reporting on enrichment levels, or guidance on application rates precludes rigorous synthesis and meta-analysis. We developed a simple enrichment technique then applied it to 28 sites across an intertidal sandflat. Fertiliser application rates of 150 and 600 g N m<sup>-2</sup> resulted in pore water ammonium concentrations respectively 1–110 and 4–580 × ambient, with greater elevations observed in deeper (5–7 cm) than surface (0–2 cm) sediments. These enrichment levels were similar to eutrophic estuaries and were maintained for at least seven weeks. The high between-site variability could be partially explained by the sedimentary environment and macrofaunal community (42%), but only at the high application rate. We suggest future enrichment studies should be conducted in situ across large environmental gradients to incorporate real world complexity and increase generality of conclusions.

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## 1. Introduction

Nutrient processing is deemed one of the most valuable ecosystem services globally and the majority of this occurs in coastal soft sediments (Costanza et al., 1997). This ecosystem service influences the supply and flux of nutrients within and between marine habitats and through denitrification in particular, can alleviate problems such as the loss of ecosystem functionality and biodiversity associated with excess nutrients. Indeed, excessive nutrient loading and eutrophication are stressing coastal marine environments throughout the world (Levin et al., 2015). The overabundance of nitrogen in particular the (nutrient usually limiting production (Herbert, 1999; Howarth and Marino, 2006)) causes changes in biomass, structure, and functioning of coastal communities and food webs (Abreu et al., 2006; Howarth et al., 2011; Rabalais et al., 2014). Yet, despite being of paramount importance to global environmental wellbeing, nutrient processing in soft sediments is still poorly understood and response to perturbations are rarely tested experimentally in situ. Reliable techniques are needed to empirically test the effects of excess nutrients, and its interactions with other stressors in real world settings that embrace ecological complexity,

and thereby allow broad scale inferences regarding response to change (Snelgrove et al., 2014).

Fertilisers have commonly been used to test the effects of increased nutrient loading on marine soft sediment habitats, but methodological development has been haphazard making cross-study comparisons near impossible. We extended the review of Worm et al. (2000) to include the recent literature, and found 47 enrichment studies conducted in intertidal and subtidal habitats (Appendix 1). Approximately half of the studies tested nutrient limitation and growth in macrophytes (mainly seagrasses), and half examined nutrient enrichment effects on benthic communities and food webs. Slow release fertilisers, such as Osmocote®, were used in 33 of 47 (70%) studies, but these fertilisers varied considerably in their elemental makeup. Similarly, studies had a very wide range of application rates (between 3 and 750 g N m<sup>-2</sup> (Fig. 1)); while some were based on previously published experiments or site-specific pilot studies (25 of 47), in >50% of studies application rates were not justified (27 of 47). Applications of fertiliser to surficial sediments were common; in 53% of studies additions were <5 cm deep, and in many studies (36%) only the top 1 cm of sediment received fertiliser. Moreover, in only 20 of 47 studies were enrichment levels (i.e. realised treatment effect) on sediment nutrient pore water concentrations reported. Relative increases in pore water nitrogen concentrations in these 20 studies ranged from 7 to 352 times ambient levels (Fig. 1) but enrichment level comparisons are difficult to make because the depth of sampling (0–20 cm) was not standardised. These inconsistencies and methodological limitations indicate a need for a more

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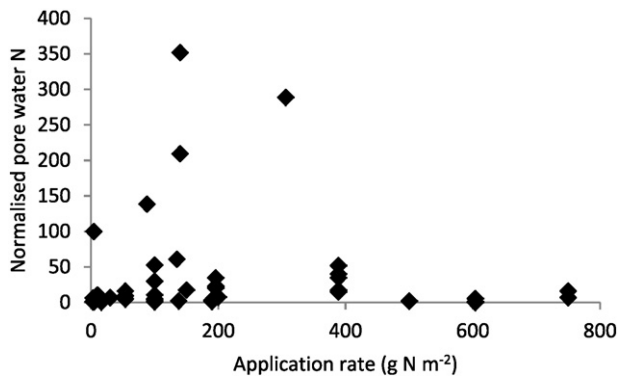


Fig. 1. Normalised (relative to ambient) pore water nitrogen concentration as a function of fertiliser application rate in the 20 studies for which such data were reported (Appendix 1).

informed approach to enrichment experiments that justifies fertiliser application rates, and improves understanding of the factors that may influence the resulting pore water nutrient concentrations.

Firstly, when planning manipulative field or mesocosm experiments it is useful to consider potential enrichment levels for a given application rate to avoid unrealistically high or undetectable pore water nutrient concentrations. Secondly, Worm et al. (2000) showed that enrichment level (i.e. pore water nutrient increase) could not be predicted by the initial fertiliser application rate, time since application and application depth using multiple linear regression analysis of literature studies (overall  $r^2 = 0.07$ ,  $p = 0.53$ ,  $n = 34$ ). We repeated this analysis on the larger set of literature and revealed a similar result ( $r^2 = 0.01$ ,  $p = 0.92$ ,  $n = 48$ ). The implication is that local environmental variables and variability in methods may strongly affect the enrichment level. We also note that previous studies have frequently overlooked co-variables or failed to assess their influence on the nutrient treatment.

Marine soft sediment ecosystems vary greatly in their physical and biological makeup, and consequently their biogeochemical processes (Braeckman et al., 2014). For example, sediment properties are important to consider in studies of benthic nutrient cycling since these influence diffusion and solute transport (e.g. Blackburn and Henriksen, 1983; Glud, 2008; Hohaia et al., 2013; Huettel et al., 2003), as well as macrofauna behaviour and ecosystem functioning (e.g. Lohrer et al., 2004; Pratt et al., 2013; Woodin et al., 2012). Benthic macrofauna are known to influence nitrogen cycling (Aller, 1988; Kristensen et al., 1991; Laverock et al., 2011), and the presence of macrophytes and microphytobenthos is also expected to influence pore water nutrient concentrations and the level of experimental enrichment. The majority of enrichment experiments have been conducted in vegetated sediments (28 of 47) and only 10 of the 19 studies conducted in un-vegetated sediments reported significant increases in pore water concentrations (Appendix 1). Our literature review shows that there is insufficient information for researchers designing enrichment experiments in un-vegetated sediments, and that there is a need to experimentally assess the role of habitat and biological processes in ameliorating pore water nutrient concentrations.

Our study develops protocols that are simple and cost-effective for in situ nitrogen enrichment experiments. The method was developed based on the published literature and a recent intertidal sandflat experiment that encompassed a wide range of sediment types, macrophyte coverage, and variations in benthic macrofauna community composition (Table 1). Our study design allowed us to document the degree to which surface and sub-surface sediment pore water nitrogen concentrations were elevated as a function of fertiliser application rate and time since application, in relation to environmental variables to serve as a guide for future studies.

Table 1

Sediment properties and macrofauna variables as a function of fertiliser application rate. Values are medians with minimum and maximum in parentheses ( $n = 28$ ).

Variable	Control (0 g N m <sup>-2</sup> )	Medium (150 g N m <sup>-2</sup> )	High (600 g N m <sup>-2</sup> )
<b>Sediment properties</b>			
Seagrass (% cover)	16 (0–84)	20 (0–97)	21 (0–75)
OC (%)	0.9 (0.6–2.0)	0.9 (0.6–2.0)	1.0 (0.6–1.8)
Mud (% <63 μm)	1.78 (0–15)	0.62 (0–14)	0.42 (0–12)
GSM (μm)	215 (177–241)	220 (182–242)	219 (190–250)
Chl- <i>a</i> (μg g <sup>-1</sup> sediment)	9.3 (3–23)	10.0 (5–32)	9.5 (5–28)
<b>Macrofauna</b>			
S (taxa core <sup>-1</sup> )	26 (11–38)	23 (7–40)	26 (11–45)
N (n core <sup>-1</sup> )	107 (19–419)	58 (8–345)	62 (22–574)
H'	2.4 (1.1–3.1)	2.4 (1.6–3.0)	2.4 (1.1–3.0)

OC = sediment organic content, Mud = sediment mud content, GSM = grain size median, Chl-*a* = chlorophyll *a* content, S = number of species, N = number of individuals, H' = Shannon diversity.

## 2. Methods

### 2.1. Experiment setup

A large scale nitrogen enrichment experiment was set up on a 300,000 m<sup>2</sup> area of intertidal sand flat on the Tapora Bank in the Kaipara Harbour, northern New Zealand (36° 39' S, 174° 29' E). The study area is composed mostly of permeable sediments of varying mud (particle size < 63 μm) content (Table 1), and is subject to tidal flushing, wind waves, and run off from a mostly agricultural catchment. Treatment plots (1 m × 1 m) consisting of control (no addition), medium (150 g N m<sup>-2</sup>) and high (600 g N m<sup>-2</sup>) nitrogen enrichment were established at 28 sites (each in a 5 × 5 m area) across the study area. These application rates were based on the median and upper quartile values from the literature review (Appendix 1). We used Nutricote® N (70 d, 40-0-0 N:P:K), a controlled release coated urea fertiliser containing no phosphorus, potassium or trace elements. A nitrogen-only fertiliser was used since it is typically the limiting nutrient in these systems, and urea quickly hydrolyses to ammonium (NH<sub>4</sub><sup>+</sup>) (Lomstein et al., 1989), the most common form of nitrogen in New Zealand estuaries (Tay et al., 2013).

Fertiliser was applied to each plot in a series of 20 evenly spaced 3 cm diameter 15 cm deep holes made in the sediment using a hand held corer. Each hole received an equal volume of fertiliser and the intact sediment core plugs were replaced immediately to minimise disturbance to the sediment. For less cohesive sediments, an outer core sleeve was used to prevent holes from infilling while fertiliser was added. Control plots were similarly cored and received an equal volume (as the high treatment) of pea gravel of similar diameter to the fertiliser pellets. With this method we were able to establish 84 1 m<sup>2</sup> experimental plots across a 300,000 m<sup>2</sup> study site in one low tide (4–5 h) with a team of six people. In a preliminary study, this technique provided even elevation of pore water NH<sub>4</sub><sup>+</sup> throughout a 1 m<sup>2</sup> plot (1.3–2.0 fold variation in concentration between the plot centre, edge and half-way in between) when sampled four weeks after application, with enrichment effects undetectable 0.5 m beyond the plot boundary.

### 2.2. Sampling

Samples were collected four weeks (pore water and sediment properties) and seven weeks after the fertiliser addition (pore water, sediment properties, macrofauna). Sampling times were chosen to allow enough time for the system to respond (based on our literature review and pilot study), and were within the 70 d release period of the fertiliser. Replicate, randomly placed sediment cores (2.6 cm dia.) from each plot were pooled and homogenised for analysis of sediment properties ( $n = 5$ , 0–2 cm depth) and pore water nutrients ( $n = 4$ , 0–2 cm and 5–7 cm depths, separated). Sediment samples were kept in the dark and

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