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# Evidence for suboxic nitrification in recent marine sediments

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**ABSTRACT:** The classical scheme of biogeochemical zones (BGZ) is known to be an oversimplification of the microbial processes that occur in organic-rich marine sediments. Results from a coupled deployment of pore-water gel probes in Loch Duich, Scotland, provide direct evidence for rapid recycling within the iron reduction (FeR) and sulphate reduction (SR) zones. High resolution pore-water profiles obtained using diffusive equilibrium in thin films (DET) gel probes found a nitrate peak at the boundary between the FeR and SR zones. This non-steady state feature is consistent with recycling of reduced N occurring throughout the FeR zone. Both conventional pore-water iron profiles and results from diffusive gradient in thin films (DGT) probes indicate that iron is solubilised and precipitated in rapid Fe/S recycling reactions throughout the SR zone. The presence of such complex recycling reactions confirms the oversimplification of the classical BGZ scheme.

**KEY WORDS:** Biogeochemical recycling · Suboxic nitrification · DET · DGT

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## INTRODUCTION

Biogeochemical zones (BGZ) form the framework for describing the early diagenesis of aquatic sediments. They represent degradation of organic matter by bacteria using successively less energy-efficient terminal electron accepting processes (TEAPs). Evidence for the existence of these sequential processes has been known for more than half a century (e.g. Mortimer et al. 1941), but a formalised depth-related scheme was not developed until the 1970s (e.g. Claypool & Kaplan 1974, Froelich et al. 1979). During the last 20 yr, BGZ have been used to describe a wide variety of aquatic environments where the supply of labile organic matter exceeds diffusion of oxygen into the sediment, from hypertrophic systems (e.g. Barica & Mur 1980) to the oligotrophic deep ocean (e.g. Froelich et al. 1979). The depth range of each zone is determined from a charac-

teristic sequence of chemical changes in the sediment pore-water (Jørgensen 1983).

During the last decade, research has begun to indicate that sequential BGZ are an oversimplification of the biogeochemical processes occurring in sediments (e.g. Canfield et al. 1993, Coleman et al. 1993, Postma & Jackobsen 1996). Concomitantly, new advances in sampling pore-water geochemistry (e.g. Davison et al. 1991, 1994, Krom et al. 1994, Brendel & Luther 1995, Zhang et al. 1995, Mortimer et al. 1998, Kjaer et al. 1999) have begun to reveal new biogeochemical interactions (e.g. Luther et al. 1997).

In this paper, we present results from the first simultaneous field deployment of 2 of these new technologies, diffusive equilibrium in thin films (DET) gel probes (Davison et al. 1994, Krom et al. 1994, Mortimer et al. 1998) and diffusive gradient in thin films (DGT) probes (Zhang et al. 1995). These results show new pore-water features, and direct evidence for complex recycling processes within the iron reduction (FeR) and sulphate reduction (SR) zones.

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## MATERIALS AND METHODS

An undisturbed box core was collected from a depth of 120 m in the centre of Loch Duich, a marine fjord on the west coast of Scotland, in April 1997. Three DET gel probes and 1 DGT gel probe were inserted and left for 24 h. DET probes consist of a perspex spear containing a thin (0.5 to 1 mm) strip of polyacrylamide gel which equilibrates with the pore-water chemistry and can then be analysed at mm resolution. The DGT technique uses a perspex probe containing a strip of chelex resin with a diffusive gel layer in front (Zhang et al. 1995).

Metal ions diffuse through the gel layer and bind to the chelex, allowing a depth profile of metal flux to be obtained (Zhang et al. 1995). The probes were sampled and analysed using standard procedures (Zhang et al. 1995, Mortimer et al. 1998) to obtain high-resolution pore-water chemistry. A subcore was then taken from the central undisturbed portion of the box core to obtain conventional pore-water samples for comparison. This core was sliced inside a  $N_2$ -filled glovebox, centrifuged and the supernatant filtered through a  $0.45 \mu\text{m}$  filter. The samples were analysed for  $NH_4^+$  using a flow injection analyser (Hall & Aller 1992); for  $Cl^-$ ,  $NO_3^-$  and

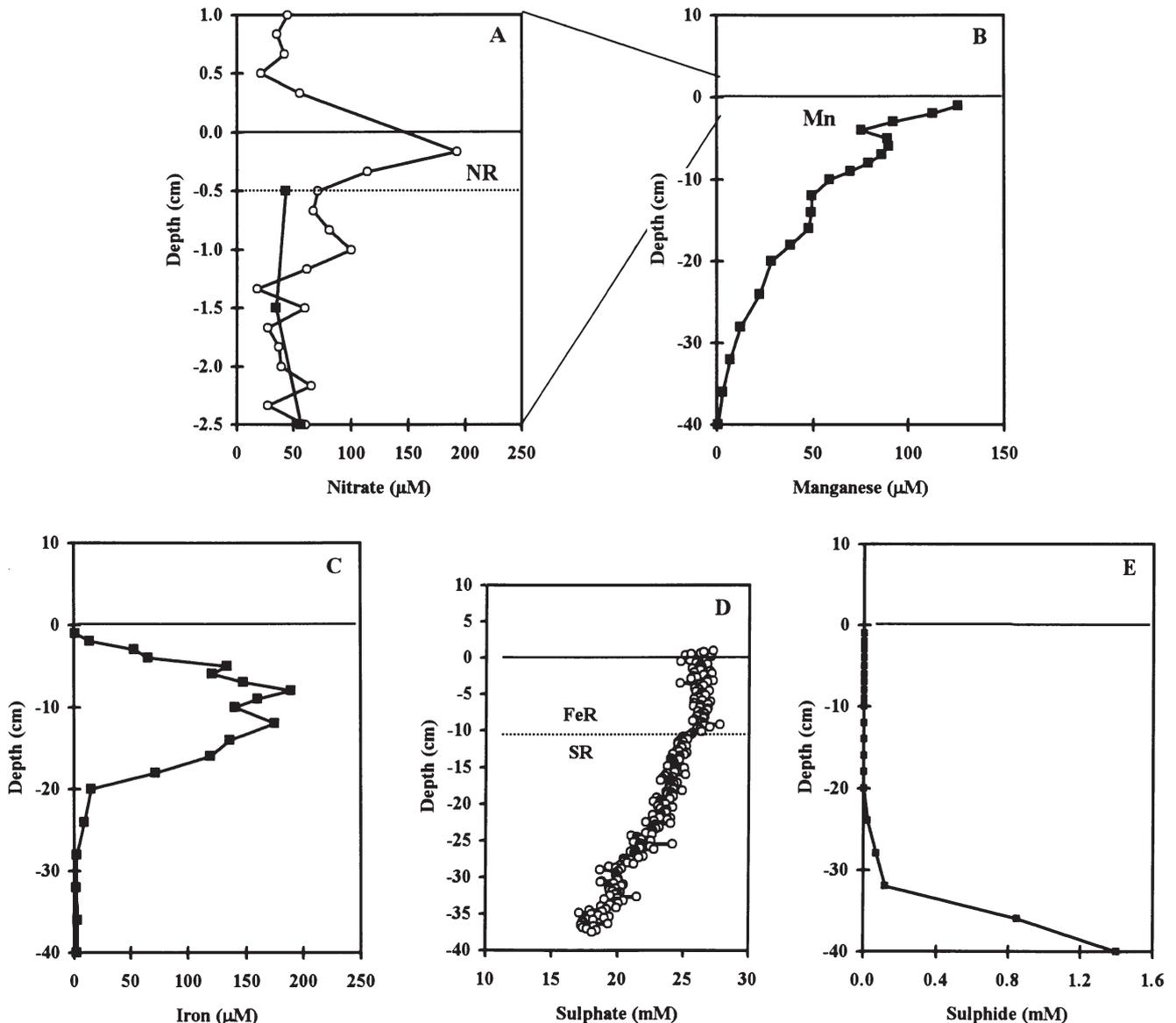


Fig. 1. Pore-water profiles of nitrate (A), manganese (B), iron (C), sulphate (D) and sulphide (E) in Loch Duich. Labels indicate position of biogeochemical zones (NR, MnR, FeR, SR = nitrate, manganese, iron and sulphate reduction, respectively). Note the different depth scale for nitrate (only upper 2.5 cm shown). (O) DET gel profiles and (■) conventional pore-water profiles obtained by core slicing are indicated

$\text{SO}_4^{2-}$  using a Dionex DX100 Ion Chromatograph with an AS14 column; for  $\text{S}^{2-}$  using the methylene blue method; and for  $\Sigma\text{Fe}$  using a Perkin-Elmer Zeeman 4100 ZL atomic absorption spectrometer.

## RESULTS AND DISCUSSION

### Biogeochemical zones

The sediments of Loch Duich show the classical sequence of biogeochemical zones (Fig. 1). The conven-

tional core profile for nitrate did not resolve any structure, but in the high resolution DET gel profile there was a decrease from a maximum immediately below the sediment surface due to coupled nitrification-denitrification (Fig. 1A). There was a peak of Mn immediately below the sediment water interface which decreased due to precipitation of  $\text{Mn}^{2+}$  at depth (Fig. 1B; Krom & Sholkovitz 1978). The Fe concentration increased down-core to a peak of approximately  $190 \mu\text{M}$  at a depth of 10 cm, which delineates the base of the iron reduction (FeR) zone (Fig. 1C). The sulphate concentration was constant throughout the FeR zone (down to 10 cm), but then decreased below 10 cm (top of the SR zone) (Fig. 1D). As has been observed previously (e.g. Goldhaber et al. 1974), there was no measurable sulphide in the upper part of the SR zone (10 to 20 cm), presumably due to removal by uptake on labile Fe oxides and precipitation with  $\text{Fe}^{2+}$  (Canfield et al. 1993). When labile Fe oxides were depleted ( $>25$  cm), free dissolved sulphide accumulated in the porewaters (Fig. 1E).

### Nitrate peak within the suboxic zone

The complete depth profile of nitrate shows a striking non steady-state peak at a depth of 10 cm, the boundary between the FeR and SR zones (Fig. 2B). This peak was up to  $900 \mu\text{M}$  and defined by 6 gel samples spread over a depth of 0.5 cm and a single point in the 0.5 cm spaced conventional measurements.

The presence of this nitrate peak at the FeR-SR boundary within Loch Duich sediments was confirmed by a subsequent re-deployment of a nitrate DET probe at the same site in May 2000. This time, analysis was performed using the standard colorimetric method (Strickland & Parsons 1972), modified for small samples (Harris et al. in press), resulting in a lower detection limit ( $0.25 \mu\text{M}$ ) and increased precision (relative SD  $< 4\%$ ). A large nitrate peak was again found exactly at the

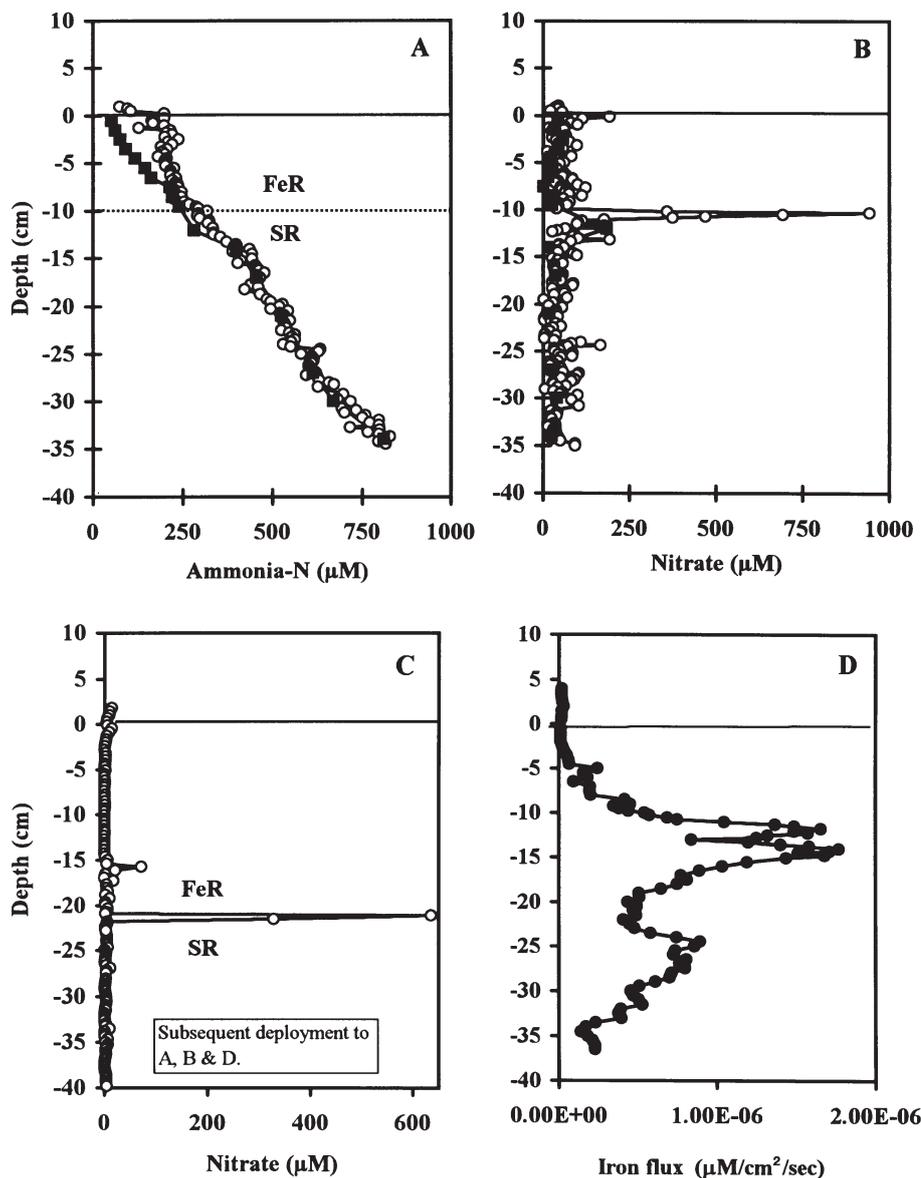
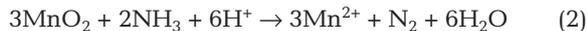
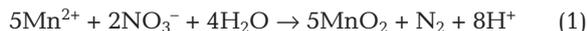


Fig. 2. Pore-water profiles of ammonia-N (A), nitrate (B,C) and DGT iron (D) in Loch Duich. Labels indicate position of iron and sulphate reduction zones (FeR and SR, respectively) as determined from relevant pore-water profiles

FeR-SR boundary (Fig. 2C). We have measured nitrate profiles in 11 cores from Loch Duich over 3 sampling trips and have found large suboxic nitrate peaks in 2 and smaller peaks in a further 4.

A possible explanation for this nitrate peak is the presence of macrofaunal burrows (cf. Mortimer et al. 1999). This is considered implausible here because the nitrate peak was present in both the gel sample and conventional sample at approximately the same depth, yet these samples were horizontally separated by several cm in the box core. It is considered extremely unlikely that a burrow would have intersected these samples at the same depth and at no other. Furthermore, if the sediment contained macrofauna capable of introducing enough oxygen to trigger conventional nitrification at a depth of 10 cm, it would be expected to cause a minimum in the ammonia-N profile (Luther et al. 1998, Mortimer et al. 1999) which was not observed (Fig. 2A).

The presence of high nitrate concentrations at the onset of the SR zone shows that microbial nitrification was occurring deep within the anaerobic layers. This confirms other recent work that has shown that nitrification can occur in suboxic sediments (e.g. Luther et al. 1997, 1998, Hulth et al. 1999). The most likely explanation is that there was recycling of reduced N occurring throughout the FeR zone. Since the nitrate peak is at the base of the FeR zone, it is tempting to postulate that sub-oxic nitrification occurred by oxidation of ammonia with Fe oxyhydroxides. However, Luther et al. (1997, 1998) calculated that nitrification of ammonia with Fe oxides is not thermodynamically possible, although their calculation used crystalline goethite rather than the labile Fe oxyhydroxides which are the reactive phase at this depth zone. Instead, they provided indirect evidence for coupling between the Mn and N cycles whereby  $Mn^{2+}$  was oxidised by nitrate (Eq. 1) and of oxidation of ammonia by Mn oxides (Eq. 2) (Luther et al. 1997, 1998).



In our sediments, nitrification by  $MnO_2$  (Eq. 3) could have occurred throughout the FeR zone, causing the observed conventional Mn (II) profile (Fig. 1B). Excess labile  $MnO_2$  has been determined within the FeR zone in this location (Burns 1996). Throughout most of the FeR zone, any nitrate produced was immediately denitrified to  $N_2$  which accounts for the low observed concentrations.

This coupled nitrification-denitrification mechanism would result in reduced accumulation of ammonia-N in the FeR zone compared to the SR zone and is there-

fore consistent with the observed change in gradient of the ammonia-N profile at 10 cm depth (Fig. 2A). By this mechanism, the nitrate peak is attributed to a selective inhibition of the denitrification part of the couple, possibly by local production of sulphide or diffusion up from the SR zone below. Denitrification is known to be more susceptible than nitrification to inhibition by sulphide (Jørgensen 1983, Sørensen 1987).

Since the nitrate peak is so sharp, the process must be transient and non-steady state. One mechanism is that it is caused by boundary effects between an upper region of Mn/N cycling and a lower region of Fe/S cycling (see below). An alternative mechanism is that a sediment slump has occurred, burying a thin horizon of Mn oxides to a depth of 10 cm. Whilst the pore-waters have had time to equilibrate, there is sufficient Mn oxide in the sediment to significantly increase suboxic nitrification within this narrow zone. This in turn drives higher rates of denitrification immediately above and below the peak. Both of these mechanisms are non-steady state effects and are caused by a perturbation of the suboxic nitrification-denitrification couple.

### Iron-sulphur recycling

The Fe profile obtained using the DGT probe shows that rapid recycling of Fe and S was also occurring in the SR zone (Fig. 2D) (Krom et al. unpubl.). The DGT Fe profile increases towards the base of the FeR zone. Assuming that the FeR rate decreases exponentially with depth, this increase may be due to decreasing rates of Fe removal. Alternatively, there may be a gradual increase in SR within the FeR zone, with the sulphide produced reacting with Fe minerals to produce more dissolved iron. At the FeR-SR boundary, there is a minimum in the DGT-Fe profile due to non-steady state iron removal. Below the boundary, the DGT Fe decreases through the SR zone. This may be caused by a decrease in SR rates, producing less sulphide to react with Fe minerals, or to a decrease in the availability of reactive Fe minerals to react with any sulphide produced.

### Significance

The existence of complex recycling reactions suggests that our understanding of sedimentary biogeochemical processes is far from complete. The original BGZ scheme relied on specific groups of bacteria using a single electron acceptor in a particular zone. However, it has been shown that bacteria are capable of switching between different electron acceptors (Sørensen 1982, 1987, Coleman et al. 1993, Dollhopf et

al. 2000). FeR and SR can also occur concomitantly (Jacobson 1994, Postma & Jakobsen 1996).

Luther et al. (1997, 1998) suggested that the cycles of Mn and N should not be viewed separately, but as inherently linked. Direct evidence for coupling between these 2 cycles was recently obtained in laboratory experiments by Hulth et al. (1999). They showed that Mn reduction could be coupled to anoxic nitrification (Eq. 3) and that the latter would not always be evident in pore-waters due to rapid coupled denitrification. Our results from Loch Duich provide direct field evidence for similar reactions occurring throughout the FeR zone, as well as for an additional complex coupling of the Fe and S cycles in the SR zone.

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