Vitamin B₁ in marine sediments: pore water concentration gradient drives benthic flux with potential biological implications

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Vitamin B₁, or thiamin, can limit primary productivity in marine environments, however the major marine environmental sources of this essential coenzyme remain largely unknown. Vitamin B₁ can only be produced by organisms that possess its complete synthesis pathway, while other organisms meet their cellular B₁ quota by scavenging the coenzyme from exogenous sources. Due to high bacterial cell density and diversity, marine sediments could represent some of the highest concentrations of putative B₁ producers, yet these environments have received little attention as a possible source of B₁ to the overlying water column. Here we report the first dissolved pore water profiles of B₁ measured in cores collected in two consecutive years from Santa Monica Basin, CA. Vitamin B₁ concentrations were fairly consistent between the two years ranging from 30 pM up to 770 pM. A consistent maximum at ∼5 cm sediment depth covaried with dissolved concentrations of iron. Pore water concentrations were higher than water column levels and represented some of the highest known environmental concentrations of B₁ measured to date, (over two times higher than maximum water column concentrations) suggesting increased rates of cellular production and release within the sediments. A one dimensional diffusion-transport model applied to the B₁ profile was used to estimate a diffusive benthic flux of ∼0.7 nmol m⁻² d⁻¹. This is an estimated flux across the sediment-water interface in a deep sea basin; if similar magnitude B-vitamin fluxes occur in shallow coastal waters, benthic input could prove to be a significant B₁-source to the water column and may play an important role in supplying this organic growth factor to auxotrophic primary producers.

Keywords: vitamin B₁, thiamin, coenzyme, sediment, flux, auxotroph

Introduction

Vitamin B₁ (thiamin) is a soluble, biotically synthesized, heterocyclic sulfur, and nitrogen-containing catalyst required in trace amounts by all organisms (Jurgenson et al., 2009). It is primarily used as a coenzyme in forming and breaking C-C bonds and is required in central metabolic processes including the pentose-phosphate pathway and tricarboxylic acid cycle as well as in acetolactate synthase utilized in the synthesis of branched-chain amino acids.
This vitamin was originally identified as the molecule in rice husks which cures the human disease beriberi, caused by vitamin B<sub>1</sub> deficiency, a discovery which was recognized with the 1929 Nobel Prize in Physiology and Medicine (Eijkman, 1990). In the 1950s and 60s it was discovered that some species of marine phytoplankton are unable to synthesize B<sub>1</sub> de novo (B<sub>1</sub> auxotrophs) and instead must acquire the coenzyme from an exogenous source (Droop, 1957; Provasoli, 1958; Carlucci and Silbernagel, 1969). This included many of the major marine primary producers (Croft et al., 2006; Bertrand and Allen, 2012; Sañudo-Wilhelmy et al., 2014) as well as some ubiquitous marine bacteria (Giovannoni et al., 2005) and picoeukaryotic algae (Paerl et al., 2015). However, not every marine microbe (including both bacterioplankton and phytoplankton) requires an exogenous source of B<sub>1</sub>; many microbes possess the full metabolic pathway needed to synthesize B<sub>1</sub> (Sañudo-Wilhelmy et al., 2014). Interestingly, B<sub>1</sub> auxotrophs do not appear to be related phylogenetically indicating that the loss of synthesis capability likely occurred multiple times (Helliwell et al., 2013). Additionally, it was discovered that B<sub>1</sub> synthetizers (or B<sub>1</sub> prototrophs) are able to self-regulate physiological concentrations within the cell via the use of a riboswitch (Croft et al., 2007). Recent work has revealed an additional layer of complexity regarding B<sub>1</sub> proto- and auxotroph dynamics, in that some species may only possess part of the synthesis pathway and can scavenge B<sub>1</sub> and/or its precursor moieties (4-amino-5-hydroxymethyl-2-methylpyrimidine or 4-methyl-5-β-hydroxyethylthiazole) in order to obtain the complete and active form of this vitamin (Jurgenson et al., 2009). Such organisms include climatologically relevant eukaryotic species such as Emiliania huxleyi (McRose et al., 2014) as well as environmentally abundant bacteria in the SAR11 clade (Carini et al., 2014). Field studies investigating dissolved B-vitamins in marine systems have shown that phytoplankton species succession and biomass production are influenced by the availability of vitamins B<sub>12</sub> (cobalamin) and B<sub>1</sub> (Sañudo-Wilhelmy et al., 2006; Panzeca et al., 2009; Koch et al., 2011; Bertrand et al., 2012). Additionally, it has been found that large regions of the ocean appear to be depleted in B<sub>1</sub> as well as other B-vitamins (Sañudo-Wilhelmy et al., 2012). Despite this, the sources of this organic growth factor have not been clearly identified and research in the area has mainly focused on B-vitamin production within the water column (e.g., Koch et al., 2012).

Marine sediments pose a potentially significant source for B<sub>1</sub> since sediments contain some of the highest cellular densities and diversity of any environment on Earth (where cellular abundance can reach as high as 10⁸ cells/cm²; Kallmeyer et al., 2012). Pioneering work in the 1950s and 1960s revealed that marine sediments may serve as a source of some B-vitamins including vitamin B<sub>1</sub> (Burkholder and Burkholder, 1958; Burkholder and Lewis, 1968). Based on our survey of required synthesis genes in whole genome sequenced sediment isolates (see Supplementary Material Table S1), marine sediments include many potential B<sub>1</sub> prototrophs. However, the majority of sediment microbes have remained uncultured (Eilers et al., 2000), and the dynamics of B<sub>1</sub> production and extracellular release remain largely unexplored. Thus, measuring dissolved B<sub>1</sub> in sediment pore waters is essential to determine if the marine sediment community as a whole serves as a source of this critically required vitamin. In comparison to the many decades of study on trace metal and inorganic nutrient requirements (e.g., Fe and NO<sub>3</sub>⁻), vitamins have received substantially less attention as a limiting agent to productivity. This is due in part to difficulties encountered measuring a labile molecule found in trace amounts (femto to pico molar concentrations) via the classic bioassay techniques or with the more recently developed liquid chromatography-mass spectrometry (LC/MS) techniques which can provide compound-specific information (Carlucci and Silbernagel, 1966; Okbamichael and Sañudo-Wilhelmy, 2005). As a result, the existing published environmental measurements of dissolved B<sub>1</sub> are almost entirely focused on the water column with little attention given to marine sediments and their pore waters. As such, we pose the following targeted research questions: (1) What are the sediment pore water B<sub>1</sub> concentrations? (2) Is there a flux of B<sub>1</sub> from sediments to the overlying water? (3) How relevant are these concentrations and fluxes to biological communities in the water column?

To address these questions, this study presents the first dissolved B<sub>1</sub> concentration profiles in marine pore waters, collected from the California Borderlands in Santa Monica Basin (SMB), CA from two sampling years (2011 and 2012). Pore water concentrations were compared to water column concentrations collected at the same station (Sañudo-Wilhelmy et al., 2012). Finally a simple diffusion-transport model was applied to the B<sub>1</sub> pore water concentrations in order to establish the first diffusive benthic flux estimates of B<sub>1</sub> from marine sediments.

Materials and Methods

Study Site

SMB lies ~10 miles offshore from Los Angeles within the California Continental Borderlands region. The basin is steep-walled with a flat-bottom covering an area of ~1800 km<sup>2</sup> with a basin floor at ~910 m and a sill at ~740 m isolating sub-sill waters from mixing with nearby basins; flushing events are estimated to occur every 1–8 years (Hammond et al., 1990; Berelson, 1991; Hickey, 1991; Berelson and Stott, 2003). Bottom waters and surface sediments are nearly but never completely anoxic (<10 µM oxygen) yet oxygen is undetectable within the first few millimeters of the sediment column (Shaw et al., 1990; Berelson et al., 2005). As a result of the low oxygen concentrations in bottom waters, the sediments are laminated with no evidence of infauna and minimal bioturbation indicating little to no advective mixing of pore waters (Jahneke, 1990; Christensen et al., 1994; Berelson et al., 2005; Tems et al., 2015). Previous studies indicate a ~5 cm deep ferruginous/manganese zone defined by maximum concentrations of dissolved iron, and manganese (Jahneke, 1990; McManus et al., 1998; Prokopenko et al., 2011). Beneath this lies a zone with decreasing dissolved iron and manganese concentrations (Jahneke, 1990; McManus et al., 1998; Burdige and Komada, 2011). Flushing events, minor bioturbation, and changes in surface primary productivity may cause seasonal changes in shallow sediment (~0–5 cm) geochemical zonation by introducing increased
concentrations of oxygen, nitrate, and/or particulate organic carbon (Berelson, 1991). Multiple studies have investigated SMB sediment accumulation rates and found that roughly 9–11% of surface water primary productivity is exported to the basin floor resulting in consistent hemipelagic-sourced sediments accumulating at ∼16.0 ± 3 mg cm⁻² y⁻¹ (Huh et al., 1990; Christensen et al., 1994; Berelson and Stott, 2003). In nearby San Pedro Basin particle flux was found to be seasonal and SMB likely experiences similar seasonality in sediment input (Collins et al., 2011). Of the organic carbon that reaches the sediment floor, ∼40% is buried and preserved while the rest is remineralized and escapes to the water column (Jahnke, 1990). SMB’s sediments are characterized as a silty-clay with ∼10% calcium carbonate content and ∼4–6% organic carbon (Craven and Jahnke, 1992; Gorsline, 1992). Sediments follow a typical porosity profile starting around a porosity of 0.98 which exponentially decreases with depth to values of ∼0.85 at 8 cm depth (Berelson et al., 2005; Komada et al., 2013).

Core Collection
Sediment cores were collected from SMB (33°48.76’ N, 118°46.60’ W; Figure 1) far enough away from basin walls and on a small regional high to avoid turbidite sampling. Cruises occurred in January 2011 and March 2012, just prior during the expected maximum particle flux but before any spring flushing. Cores of 25–45 cm length were collected with an Ocean Instruments (MC 400) multicorer (Barnett et al., 1984) containing 9.5 cm diameter core liners. Upon retrieval, cores were inspected for a well-preserved sediment-water interface, minimal overlying water turbidity, and a lack of bubbles in the sediment in order to minimize collection artifacts. All cores were stored on board ship in an ice bath protected from light until transport to the laboratory cold room for sampling ∼9 h after retrieval. Cores were sampled at depths of 1, 3, 5, 7, 9, 11, 15, 20, 25, and 35 cm in 2011 and 1.5, 3.5, 5.5, 7.5, 11.5, 15.5, 19.5, 25.5, 31.5, and 39.5 cm in 2012 using Rhizon soil samplers (Rhizosphere Research Products) fitted with 0.2 µm pore size filters. Rhizons were inserted into pre-drilled holes in the core liner and pore water was collected on cm-scale resolution using plastic syringes (Norm-ject) which had been acid-cleaned and methanol-rinsed. Sample volume ranged from 5 to 30 mL.

Figure 1: Santa Monica Basin station location (33°48.76’ N, 118°46.60’ W). This figure was generated using Ocean Data View (Schlitzer, R. Ocean Data View, http://odv.awi.de, 2015).

Analytical Methods
Vitamin B₁ was measured according to the technique described previously (Sanudo-Wilhelmy et al., 2012). The technique involves a solid-phase extraction onto a C₁₈ resin at pH 6.5 and 2.0 followed by elution with methanol, drying, and quantification using high-performance liquid chromatography/tandem quadrupole mass spectrometer (LC/MS) with an electrospray ionization interface. Reagent grade thiamin hydrochloride (≥99%) was obtained from Sigma-Aldrich and used as an external standard. Samples were triple injected into the LC/MS to confirm instrument stability. Because sample volume is so low replicate sample splits were not performed. Method analytical blanks were measured with Milli-Q water subjected to the same preconcentration and quantification steps resulting in 0 pM in 2011 and 0.53 pM in 2012 defined as three times the standard deviation of the procedural blank for 2012 and three times the standard deviation of the y-intercept of the calibration curve divided by the slope following the method outlined by Snyder et al. (2010) since the procedural blank was equal to zero in 2011. The improvement in detection limits between years resulted from an optimization of the method by increasing the sample injection volume from 50 to 100 µL. Dissolved iron concentrations were quantified by ICP-MS using external calibration curves and an internal indium standard.

Benthic Flux Model
We applied a one-dimensional diffusion-transport model following Fick’s First Law to the B₁ pore water concentration profile. The data (from 0 to 9 cm) was fit with a polynomial function:

\[ C = C_0 + m_1x + m_2x^2 \]

Where \( x \) is depth (cm), \( m_1 \) and \( m_2 \) are fitting parameters, and \( C_0 \) is the concentration at the sediment water interface (SWI). The diffusive flux of B₁ across the SWI was determined using Fick’s First Law, applied to the derivative of the polynomial function fit evaluated at \( x = 0 \):

\[ J = -\phi^3D_0 \left( \frac{dc}{dx} \right) \]
Where $\varphi$ represents sediment porosity at the SWI and $D_0$ is the molecular diffusion coefficient, and $\frac{dx}{ds}$ is the slope at the SWI. The $D_0$ of citrate ($3.22 \times 10^{-6}$ cm$^2$ s$^{-1}$) was used due to similarities in composition and molecular weight to $B_1$. The model ignores advection as is standard in similar sedimentation rate environments lacking bioturbation (e.g., Hammond et al., 1996).

**Results**

**Pore Water Profiles**

Vitamin $B_1$ concentrations in sediment pore water showed a consistent depth-profile shape in both sampling years (Figure 2). Concentrations were higher than water column values in most sampling depths of 2012 and all depths of 2011. Pore water concentrations ranged from 330 to 770 pM in 2011 and 30–480 pM in 2012 as compared to water column concentrations of 30–280 pM previously reported by Sanudo-Wilhelmy et al. (2012) for the same station location (Supplementary Material Table S3). Additionally, the water column concentrations consistently increased with depth such that the deepest water column sample (890 m) had the highest $B_1$ concentration, ~280 pM. In both pore water profiles, vitamin $B_1$ exhibited consistent maximum concentrations at ~5 cm sediment depth and subsequently decreased with depth in both sampling years. The maximum concentrations of $B_1$ at ~5 cm sediment depth coincided with a maximum of dissolved iron (Figure 2; Supplementary Material Table S4).

**Modeled Flux**

The well-defined convex-upward $B_1$ profile in 2011 allowed a one dimensional diffusion-transport model, based on Fick’s First Law, to be applied to the pore water concentrations (see Supplementary Figure S1). A simple quadratic curve fit evaluated the inflection point to be at ~9 cm. This model was then applied to the five pore water data points within the top 9 cm of sediment and the bottom water concentration was fixed at 280 pM based on the deepest water column value. The model produced a statistically significant model fit with a high chi-squared value ($\text{chisq} = 160$). The concentration gradient was evaluated at the SWI and a potential $B_1$ flux of 0.7 nmol m$^{-2}$ d$^{-1}$ was calculated out of the sediment. The less smooth shape of the 2012 profile (Figure 2) especially just below the sediment water interface, due to possible disturbance during transport and sampling or simply spatial variability, did not allow a good model fit for this sampling year. This is not uncommon for pore water sampling in deep marine sediments, for example of 8 total cores collected for DOC analysis in the same basin only 4 showed profiles consistent enough to allow a model fit (Komada et al., 2013).

**Potential Algal Growth Yield**

The calculated vitamin $B_1$ flux described above is for the exchange across the SWI at depths of 900 m in a sedimentary basin, yet this represents the first flux estimate for any marine setting. Assuming our calculated flux is representative of similar fluxes occurring in shallow water environments, and that $B_1$ degradation is minimal, a mass balance was applied to estimate the hypothetical growth response of such a sediment flux on a $B_1$-limited surface ocean phytoplankton community. Using the estimated sediment flux (0.7 nmol m$^{-2}$ d$^{-1}$), a series of experimental phytoplankton cell growth yields ranging from to $2.2 \times 10^{-8}$ to $3.6 \times 10^{-8}$ pmol $B_1$ cell$^{-1}$ (Paerl et al., 2015), and a photic zone of ~20 m (Small et al., 1989), we estimated that this magnitude sediment flux could support an algal growth yield ranging from $9.8 \times 10^3$ to $1.6 \times 10^6$ cells L$^{-1}$ d$^{-1}$ (Supplementary Material Table S2).

**Discussion**

**Vitamin $B_1$ Pore Water Profiles**

The pore water depth profiles of $B_1$ from two sampling years showed high maximum concentrations compared to previous...
field measurement as well as a consistent profile shape, especially considering the vitamin is present in such trace concentrations. This is in contrast to upper water column concentrations of B1 which can vary widely on fairly short time scales (months to days; e.g., Gobler et al., 2007; Koch et al., 2012) and do not necessarily show consistent profile shape (Sañudo-Wilhelmy et al., 2012). This is likely due to the stratified nature of deep marine sediments which result in predictable geochemical zones that are not as susceptible to mixing or large diurnal shifts in bacterio-phytodplankton activity which likely affect vitamin production and uptake in the surface ocean (Sañudo-Wilhelmy et al., 2014). The consistent B1 pore water concentration profile shape points to the existence of a stable mechanism for B1 release to the dissolved phase. Furthermore, the maximum dissolved pore water concentrations for B1 (770 pM) in 2011 were among the highest concentrations of any previously published values (see Table 1) almost 2 times higher than maximum water column concentrations. In fact the only other measurement that is within the same range was a single pore water value measured via HPLC (Okbamichael and Sañudo-Wilhelmy, 2005), supporting the hypothesis that sediments may represent universally elevated concentrations. Of the other water column measurements (Table 1), we note that some of the highest measurements are either found in shallow embayments likely affected by high benthic fluxes (Okbamichael and Sañudo-Wilhelmy, 2005) or anoxic marine basins such as SMB (Sañudo-Wilhelmy et al., 2012). These high concentrations suggest that vitamin production within the pore waters could be an important vitamin source for both the sediments and the water column. Additionally, of the 56 sediment bacteria and archaea surveyed in our genomic review, 74% of them were B1 prototrophs with all of the genes necessary to synthesize the vitamin de novo (see Supplementary Material Table S1).

The shape of the B1 pore water profile showed similarity to our dissolved iron profile as both showed peaks at ∼5 cm. Manganese, which has been measured in the same basin in other studies, also shows a coincident peak at ∼5 cm depth (Jahne, 1990). This implies that the largest B1 production was occurring within a geochemical zone of iron and manganese reduction as defined by the classic redox cascade of terminal electron acceptors (Figure 2; Froelich et al., 1979). We are unaware of any biological mechanism linking B1 to metal reduction, however many iron and manganese reducers are B1 prototrophs (see Supplementary Material Table S1). Future culture experiments on sediment isolates from this sediment zone may help to explain why the elevated pore water concentrations occurred at this depth.

Previous measurements of dissolved organic carbon (DOC) in this same basin also showed elevated concentrations starting around ∼5 cm (Komada et al., 2013). As an organic molecule, B1 is part of the DOC pool, albeit a very small proportion (pM versus mM concentrations). Thus, processes driving changes in pore water DOC may also contribute to the profile shape of B1, namely organic carbon remineralization. B1 is a required cofactor for many important C-C breaking decarboxylase enzymes (Sañudo-Wilhelmy et al., 2014) which may serve as a possible mechanism linking the dissolved concentrations of DOC to B1. Future environmental sampling and culture experiments targeted at carbon remineralization and B1 production will be needed to confirm the validity of this proposed connection.

### Vitamin B1 Benthic Flux

The diffusive flux of 0.7 nmol m⁻² d⁻¹ out of the sediment represents the first such estimate ever made and therefore we lack a good comparison in order to judge the magnitude or significance of this flux. However, the algal growth yield calculation resulted in rates of cellular production (see Supplementary Material Table S2) some of which fall within the range for a phytoplankton bloom (Anderson et al., 2002). Certain caveats go along with these calculations, the most important being that we are explicitly not implying that the flux measured in SMB is reaching the surface waters. Instead, we assume that the calculated sediment flux may be representative of similar fluxes in more shallow environments as has been hypothesized in other studies (Okbamichael and Sañudo-Wilhelmy, 2005). Such a shallow water environment where a B1 sediment flux would be particularly relevant would include shallow embayments, marshes, lagoons, or other environments that experience significant mixing and/or deep seasonal upwelling in order to allow transport of B1-rich bottom water to surface waters.

### TABLE 1 | Environmental marine measurements of vitamin B1.

| Study area | Concentration range (pM) | References |
|------------|--------------------------|------------|
| SMB pore water | 30–770 | This study |
| Marine pore water from Flax Pond, NY | 750 | Okbamichael and Sañudo-Wilhelmy, 2005 |
| Shallow Embayments in Peconic River and Stony Brook Harbor, NY | 230–310 | Okbamichael and Sañudo-Wilhelmy, 2005 |
| California-Baja Pacific Margin | <0.81–314 | Sañudo-Wilhelmy et al., 2012 |
| Western Tropical North Atlantic, Amazon River Plume | <0.81–230 | Barada et al., 2013 |
| Long Island Sound, NY | <10–220 | Vishniac and Riley, 1961 |
| Peconic River, NY | 12–190 | Gobler et al., 2007 |
| Quantuck Bay, NY | 7–169 | Koch et al., 2013 |
| Old Fort Pond, NY | 0.1–112 | Gobler et al., 2007; Koch et al., 2012 |
| Long Island Sound, NY | <0.10–99 | Koch et al., 2012 |
| Scripps Institute of Oceanography Pier | 20–40 | Carlucci and Silbernagel, 1966 |
waters. Furthermore, if the hypothesis that B$_1$ is linked to DOC proves correct, we would expect that shallow sediments, which are generally more organic rich and host higher bacterial abundances, likely produce significantly higher B$_1$ fluxes, and therefore the hypothetical growth yield can be considered a conservative estimate. Another assumption is that the surface algal community could be vitamin B$_1$-limited, which is possible given that 20% of genomic surveyed algae and 27% of cultured phytoplankton are B$_1$ auxotrophs (Croft et al., 2006; Sañudo-Wilhelmy et al., 2014), including many harmful algal bloom species (Tang et al., 2010). Of course additional variables would affect the initiation and response of the microbial community to the addition of B$_1$ including potential degradation prior to biologic uptake, competitive auxotrophic consumption of B$_1$, and additional release or transport during bloom die off (Sañudo-Wilhelmy et al., 2014). Despite these unknowns, our model suggests that benthic fluxes of B$_1$ occur at physiologically relevant rates and could impact surface primary production under a vitamin-limited regime, which appears to exist in many regions of today's oceans (Bertrand and Allen, 2012; Sañudo-Wilhelmy et al., 2012).

### Summary and Future Directions

Here we presented the first deep-sea sediment pore water profiles of the universally required vitamin B$_1$. Our data showed a stable profile, hinting at a yet-to-be-determined link to a fundamental metabolic sediment cycle. Additionally we showed that sediments might serve as a source of B$_1$ to the water column. Future studies are needed in order to constrain spatial and temporal variability of sediment B$_1$ fluxes. While we cannot provide an unequivocal link between B$_1$ and microbial producers or consumers, these pore water profiles serve as a starting point to formulate future hypotheses. Interesting avenues for future studies include whether B$_1$ can be limiting to sediment microbes in a similar way to their demonstrated limitation to surface water organisms despite the high pore water concentrations. Recent studies support the idea that B$_1$ has the potential to act as an ectocrine intermediate and perhaps a limiting nutrient based on a recent finding of auxotrophy in the widespread and highly abundant phylum chloroflexi (Rodionova et al., 2014), the members of which represent a significant abundance of bacteria in some shallow and deep sediments based on genomic studies (e.g., Blazek and Schippers, 2010; Jorgensen et al., 2012). Such hypotheses, when coupled with in situ prokaryotic diversity techniques, and physiological studies using bacterial isolates from the different biogeochemical zones, will elucidate the role of vitamin B$_1$ on community function and composition both within the sediment and as a source to the water column.

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### Supplementary Material

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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