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BRIEF REPORT

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A rapid method for measuring ATP + ADP + AMP in marine sediment

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Abstract

In this report, I describe a method for rapid measurement of total adenylate (ATP + ADP + AMP) in marine sediment samples for estimating microbial biomass. A simple 'boil and dilute' method is described here, whereby adding boiled MilliQ water to sediments increases the detection limit for ATP + ADP + AMP up to 100-fold. The lowered detection limit of this method enabled the detection ATP + ADP + AMP in relatively low-biomass sub-seafloor sediment cores with 10^4 16S rRNA gene copies per gram. Concentrations of ATP + ADP + AMP correlated with 16S rRNA gene concentrations from bacteria and archaea across six different sites that range in water depth from 1 to 6000 m indicating that the ATP + ADP + AMP method can be used as an additional biomass proxy. In deep sea microbial communities, the ratio of ATP + ADP + AMP concentrations to 16S rRNA genes >1 m below seafloor was significantly lower compared to communities in the upper 30 cm of sediment, which may be due to reduced cell sizes and or lower ATP + ADP + AMP concentrations per cell in the deep sea sub-seafloor biosphere. The boil and dilute method for ATP + ADP + AMP is demonstrated here to have a detection limit sufficient for measuring low biomass communities from deep sea sub-seafloor cores. The method can be applied to frozen samples, enabling measurements of ATP + ADP + AMP from frozen sediment cores stored in core repositories from past and future international drilling campaigns.

INTRODUCTION

Adenosine triphosphate (ATP) plays a critical role in role in intracellular energy transfer reactions because it provides energy that drives biochemical processes in cells. As an example, over the course of a day the amount of ATP turned over by a human is equivalent to body weight (Tornroth-Horsefield & Neutze, 2008). The use of ATP as a microbial biomass indicator for aquatic microbes has been applied since the 1960's (Holm-Hansen & Booth, 1966) (Holm-Hansen, 1973) (Karl, 1993). In a recent publication, the use of ATP as a metric of biomass for aquatic systems was extensively described and proven to be an effective quantitative measure for biomass in aquatic systems (Bochdansky et al., 2021). ATP has been measured previously in sediments for a proxy of microbial biomass in seafloor samples (Egeberg, 2000) (Kimura et al., 2003) (Karl & Larock, 1975) and lacustrine sediments (Vuillemin et al., 2015; Vuillemin et al., 2018), and also in terrestrial deep subsurface fluids (Eydal & Pedersen, 2007).

Adenosine triphosphate is produced via the phosphorylation of adenosine diphosphate [ADP] and adenosine monophosphate (AMP) and in the course of metabolism ATP is repeatedly dephosphorylated (to ADP and AMP) and rephosphorylated (from ADP and AMP). The commercially available 'A3 method' Test Kit (Kikkomann Biochemifa) works via a firefly luciferase assay that produces luminescence

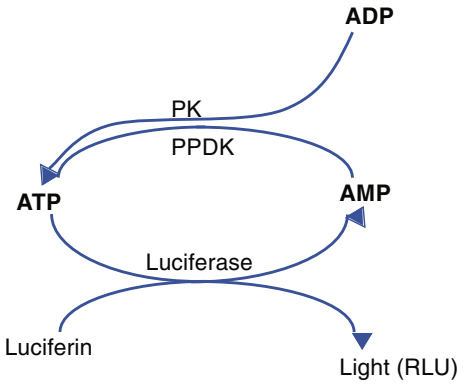

FIGURE 1 A simplified diagram showing the enzymatic steps used to quantify ATP, ADP and AMP in the LuciPac A3 Aqua water tester kit (Kikkomann Biochemifa). The light emitted from all the three molecules (AMP, ADP and ATP) are registered as a single relative fluorescent unit (RLU) value by the lumitometer. PK, pyruvate kinase; PPDK, pyruvate orthophosphate dikinase

detectable with a lumitometer whose intensity correlates with the amount of ATP + ADP + AMP present in the sample (Figure 1). This 'A3 method' is used to detect microbial contamination in the food industry on the basis of ATP + ADP + AMP values (Bakke & Suzuki, 2018) (Saito et al., 2020) (Bakke, 2022). The A3 assay provides qualitative values of ATP + ADP + AMP concentrations from a given sample based on relative fluorescent units (RLUs) (Saito et al., 2020) (Bakke & Suzuki, 2018) (Bakke, 2022). Therefore, applying the A3 method for simultaneous quantification of ATP + ADP + AMP could be useful as an additional proxy for microbial biomass in deep sub-seafloor sediment samples that often have ultra-low concentrations of cells that are living close to the energetic limit to life (Kallmeyer et al., 2012) (Inagaki et al., 2015) (Lever et al., 2015) (Roy et al., 2012) (Braun et al., 2017) (Schippers et al., 2005). The hand-held lumitometer also has the advantage of being small and portable, and therefore is easily brought into the field to make ATP + ADP + AMP measurements in real-time as samples are acquired to get a rapid assessment of microbial biomass during sampling campaigns.

Here, I describe a simple 'boil and dilute' method for rapid quantification of ATP + ADP + AMP in marine sediment samples and show that the concentrations generally correlate with 16S rRNA gene concentrations determined via quantitative PCR (qPCR). Sediment samples tested derived from six different locations with widely varying water depths and sediment depths (Table 1). Across all of the different sedimentary settings, the concentration of ATP + ADP + AMP correlates with16S rRNA gene concentrations indicating that the method can be used as an additional proxy for biomass in marine sediment samples, including deep biosphere sediment samples with concentrations of microbial biomass as low as 10^3–10^4 cells per gram sediment.

RESULTS

The upper and lower limits of RLU detectable by the lumitometer were determined to be 10^6 and 4 (+/−2) RLU, respectively (Figure 2). The lower limit of detection was determined by measuring MilliQ water, and was dependent on the cleanliness of the MilliQ water. The lowest possible RLU values from MilliQ were consistently between 2 and 5, which could be obtained if 1 L of MilliQ water was dispensed prior to making the measurement. If MilliQ was taken immediately from the machine without this rinsing step, then RLU values from the blanks were detected at a wide range of values ranging from 5 even up to 3500 RLU. This is likely due to the growth of bacteria on the outer plastic surfaces of the MilliQ dispenser after the water passes through the sterilization regions of the instrument. Rinsing with 1 L MilliQ prior to sampling was found to eliminate this problem and result in constant blank measurements of <5 RLU. It is therefore paramount to rinse the outer (post-sterilization) MilliQ surfaces well with at least 1 L, and confirm by measuring blanks, particularly when measuring low biomass samples. Because the post-rinsed MilliQ water blanks consistently gave an RLU value of <5, an RLU value of 5 was used as the detection limit for the method (contamination could not be ruled out from RLU values <5). Boiling the MilliQ water did not have an effect on the RLU detected in the MilliQ blanks.

The enzymatic steps used to quantify ATP, ADP and AMP in the LuciPac A3 Aqua water tester kit (Kikkomann Biochemifa) involve three different enzymes: pyruvate orthophosphate dikinase (PPDK), pyruvate kinase (PK) and luciferase (Figure 1). The light emitted from all the three molecules (AMP, ADP and ATP) from the firefly luciferase enzyme are registered as a single RLU value by the lumitometer. Standard curves of ATP, ADP and AMP serial dilutions showed that the relationship between concentration and corresponding RLU values followed a power-law function, and were strongly correlated (R^2 >0.98) (Figure 2). This is consistent with prior studies (Bakke & Suzuki, 2018). After completing the standard curve measurements, marine sediment samples were then processed for ATP + ADP + AMP measurements using the A3 kit. Following the observations of Bochdansky et al. (2021) that many extraction reagents interfere with the luciferin-luciferase reaction, a minimal sample processing protocol was employed that involved diluting the sample with boiling-hot ultrapure (MilliQ) water. This followed general guidelines discovered in prior studies that found incubating the sample with boiled ultrapure water increases the ATP yield (Bochdansky et al., 2021) (Yang et al., 2002). ATP starts to degrade into ADP and AMP at high temperatures (Fang et al., 2015) and therefore boiling a sample could result in an underestimate of ATP concentrations.

TABLE 1 Tested sediment samples across various locations.

Sample	Water depth (m)	Sediment depth below seafloor (m)	Location	Source
River sediment, hyporheic zone	0.6	0.02–0.2	Munich, Germany	Michaelis et al., 2022
Svalbard beach, sandy sediment	0.1	Surface sediment, tidal zone	Kongsfjorden, Ny-Ålesund, Svalbard	This study
Namibian shelf sediments	125	0.02–0.3	Benguela Upwelling Zone	Orsi, Morard, et al., 2020
Puerto Rico upper slope sediment	493	0.1–10	Puerto Rico trench	This study
Deep sea sediment (anoxic)	5500	0.1–20	North Atlantic Gyre	Vuillemin, Vargas, et al., 2020
Deep sea red clay (oxic)	6000	0.1–15	North Atlantic Gyre	Vuillemin et al., 2019

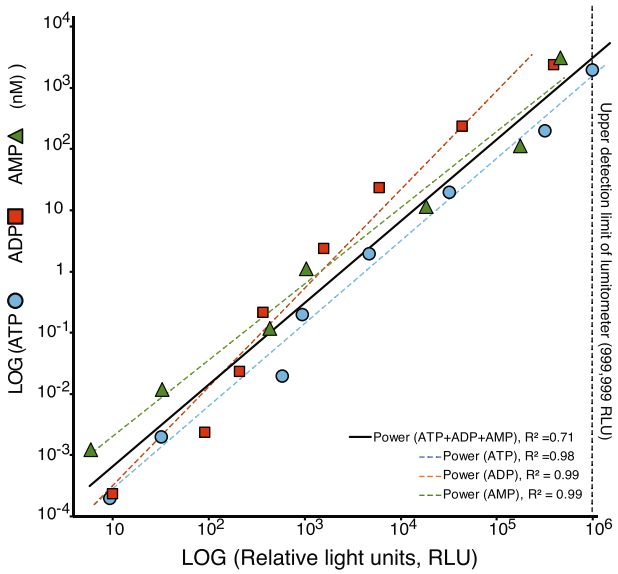

FIGURE 2 Standard curves of ATP, ADP and AMP serial dilutions using the LuciPac A3 Aqua water tester kit.

However, the A3 method measures the combined total of ATP + ADP + AMP and therefore is suitable for measuring total adenylate even in samples that have been extracted at high temperatures (Bakke & Suzuki, 2018).

In sediment from the Namibian continental shelf (Table 1), the method was applied to frozen samples using three different preparation techniques (1) 1:10 dilution of sediment with MilliQ water, (2) 1:10 dilution of sediment with boiled MilliQ water, (3) 1:2 dilution of sediment with boiled MilliQ water (see Methods). The technical variability (standard deviation) associated with the measurement was <10% of the average RLU value for all samples (Figure 3) indicating a high reproducibility and relatively low amount of technical variability. Adding boiled MilliQ increased the RLU from the samples by ca. 10-fold, and the highest recorded RLU measurements (>80,000 RLU) could be obtained when diluting the sample 1:2 with boiled MilliQ (Figure 3). ATP + ADP + AMP concentration obtained from a samples diluted 1:2 with boiled MilliQ water provided RLU values up to 100 fold higher compared to no boiling, and mirrors most closely (compared to all three treatments tested) the vertical profile of 16S rRNA gene concentrations from the same sediment core (Figure 3). With the boil and dilute method, the concentration of ATP + ADP + AMP from the Namibian shelf sediment was in the nM range (Figure 3).

In contrast, deep sea anoxic sediment (Table 1) (Vuillemin, Vargas, et al., 2020) had ATP + ADP + AMP concentrations in the lower pM range (Figure 4). In the deep sea sediment samples, no ATP + ADP + AMP was detectable without boiling the MilliQ water (Figure 4). However, when boiled MilliQ water was used, ATP + ADP + AMP was detectable above the detection limit (RLU = 5), and there is a clear trend of ATP + ADP + AMP decreasing exponentially with increasing sediment depth below the seafloor (Figure 4). Similar to the Namibian sediments, diluting 1:2 with boiled MilliQ water results in roughly an order of magnitude higher RLU measurement for most samples compared to a 1:10 dilution (Figure 4). As with the Namibian site, the technical variability (standard deviation) associated with the measurement was <10% of the differences between treatments (Figure 4) indicating that the observed differences are real and not a result of technical variation. As opposed to the Namibian site, the upper 2 m interval of the ATP + ADP + AMP concentrations shows an opposite trend compared to the concentrations of 16S rRNA genes. However, in the deeper section of the core below 2 m there is consistency between the ATP + ADP + AMP and 16S gene concentration profiles (Figure 4).

The 1:2 boil and dilute method for ATP + ADP + AMP was then applied to additional samples from the deep sea, Puerto Rico slope, Svalbard tidal zone (Kongsfjorden, Ny-Ålesund), and river sediments (Table 1) and a correlation analysis was performed

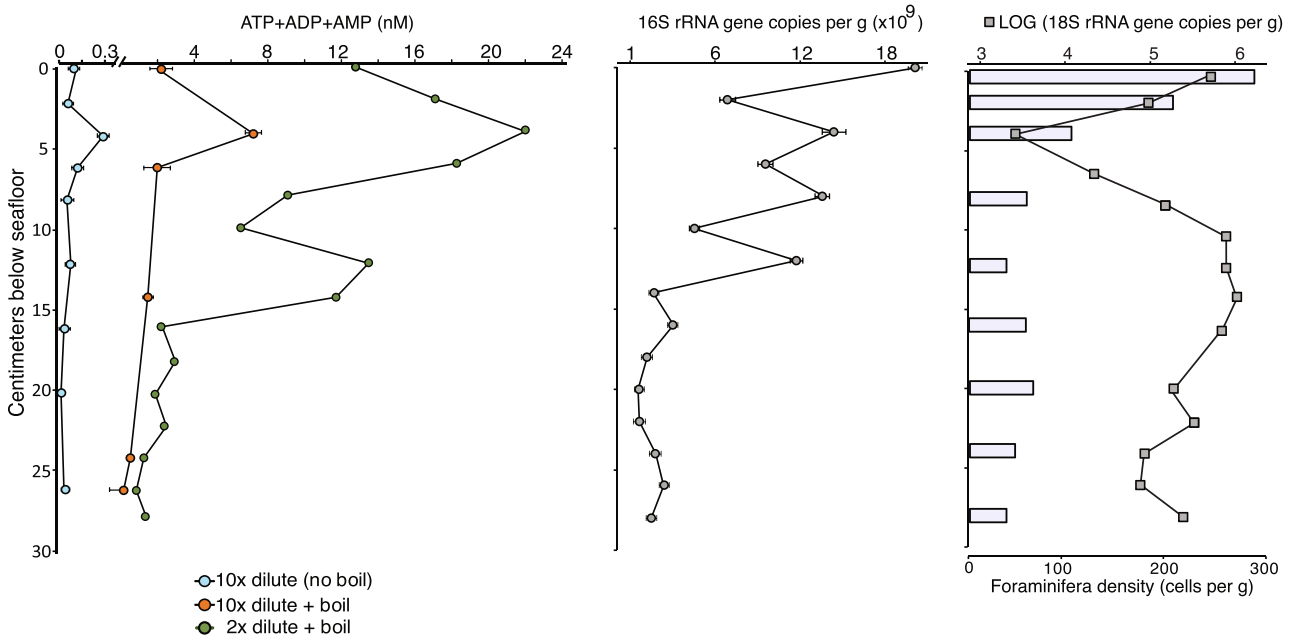

FIGURE 3 Left panel: vertical profiles of ATP + ADP + AMP concentrations from the Namibian shelf core using three different preparation techniques (1) 10× dilution with MilliQ water: blue, (2) 10× dilution with boiled MilliQ water: orange, (3) 2× dilution with boiled MilliQ water: green. Middle panel: vertical profile of 16S rRNA gene concentrations from the same sediment core as reported previously (Orsi, Vuillemin, et al., 2020). Right panel: vertical profile of 18S rRNA genes from fungi and cell concentrations of Foraminifera as reported in previous studies (Orsi et al., 2022; Orsi, Morard, et al., 2020). Error bars represent standard deviations across three technical replicates.

between ATP + ADP + AMP concentrations and the corresponding 16S rRNA gene concentrations. The relationship between RLU and 16S rRNA gene concentrations can be described using a power law function (Figure 5) ($R^2 = 0.45$). The lowest ATP + ADP + AMP values obtained were from oxic deep sea red clay (Table 1) where the detection limit for the method was reached (Figure 5). The oxic red clay sub-seafloor sediment derive from a core reaching down to 15 m below seafloor with an approximate age of 15 million years (Vuillemin et al., 2019). Across all sites (Table 1), sediment microbial communities that have higher concentrations of 16S rRNA genes (and therefore bacteria and archaea) have correspondingly higher RLU values (Figure 5) indicating that the 'boil and dilute' ATP + ADP + AMP method described here can be used as an additional proxy for assessing microbial biomass in marine sediments. The considerations and suggestions for interpretations of this method are discussed below.

DISCUSSION

The results show that ATP + ADP + AMP can be measured rapidly from frozen marine sediments samples by simply diluting the sediment sample 1:2 with boiled MilliQ water, which can lower the detection limit for ATP + ADP + AMP up to 100-fold. Since the measurement is made with a handheld lumitometer, it is ideal for field based measurements of microbial biomass. The addition of the boiling step likely increases the amount of detectable ATP + ADP + AMP by thermal lysis of the cells, which then releases their intracellular ATP + ADP + AMP pools that can be measured in the supernatant. This is consistent with prior studies that found incubating the sample with boiling ultrapure water increases the ATP yield (Bochdansky et al., 2021) (Yang et al., 2002). Previous studies showed that after 2 min in water at 150°C, that ATP readily degraded to ADP and AMP (Fang et al., 2015). Because the A3 method used here simultaneously quantifies ATP + ADP + AMP by constant enzymatic conversion of ADP and AMP back to ATP (Figure 1), it is not biased by ATP degradation during sample heating (Bakke & Suzuki, 2018). The ATP + ADP + AMP method described here can serve as an additional proxy to DNA based assessments of microbial biomass for an additional assessment of the living fraction of cells in cases where extracellular DNA ('dead DNA') may be preserved (Vuillemin et al., 2015).

The organic matter in sub-seafloor sediments such as humic acids have the potential to inhibit enzymatic reactions. For example, DNA extracted from sediment samples often needs to be diluted between 1:10 and 1:1000 to remove inhibitory effects of the humic acids on the Taq polymerase in order to obtain a successful PCR reaction (Vuillemin et al., 2018) (Vuillemin, Kerrigan, et al., 2020) (Vuillemin, Vargas, et al., 2020). Therefore, humic acids could also influence the enzymatic reactions in the ATP + ADP + AMP (A3) method

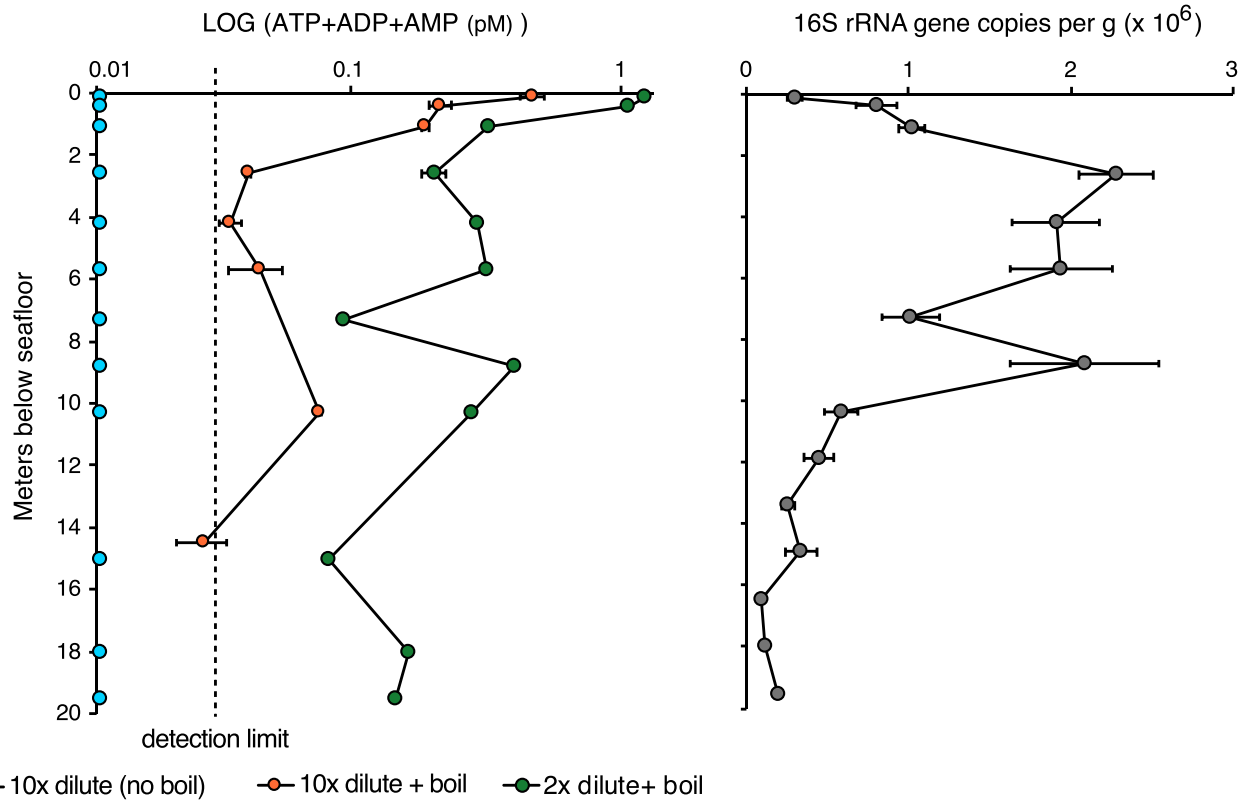

FIGURE 4 Left panel: vertical profiles ATP + ADP + AMP concentrations from a deep sea anoxic sediment core (5500 m water depth, Table 1) using three different preparation techniques (1) 10× dilution with MilliQ water: blue, (2) 10× dilution with boiled MilliQ water: orange, (3) 2× dilution with boiled MilliQ water: green. Right panel: vertical profile of 16S rRNA gene concentrations from the same sediment core as reported previously (Vuillemin, Kerrigan, et al., 2020). Note that no ATP + ADP + AMP was detectable without boiling MilliQ, whereas dilution 2× with boiled MilliQ water increased the amount of detectable ATP + ADP + AMP by up to 100 fold. Error bars represent standard deviations across three technical replicates.

(Figure 1). However, the results suggest that inhibitory organic substances did not play a major role inhibiting the enzymatic reactions used in this kit. As an example, the 1:10 dilution gave consistently lower RLU values compared to the 1:2 RLU. If organic compounds (such as humic acids) were inhibiting the enzymatic reactions, inhibitory effects would be increased at higher inhibitor concentrations. However, the 1:2 dilution gives consistently higher RLU compared to the 1:10 dilution (Figures 3 and 4). Therefore, potential inhibitors do not appear to play a major role in inhibiting the enzymatic reactions in the method.

Some considerations for interpretation of the 'boil and dilute' ATP + ADP + AMP method are provided by comparing the vertical profiles from two of the coring locations. First, there are two subsurface peaks in the vertical profile of ATP + ADP + AMP in Namibian shelf sediments (Table 1) that are not apparent in the 16S rRNA gene qPCR profile (Figure 3). Nevertheless, the vertical trend of ATP + ADP + AMP generally follows that of the qPCR whereby there are two zones: (1) a sharp decline in concentration over the top 12 cm, and (2) a less rapid decline over the bottom half of the core (Figure 3). This shows that the ATP + ADP + AMP quantified concentrations from the 1:2 dilute and boil method are generally consistent with the vertical trend in microbial biomass obtained with the conventional qPCR technique.

The amount of ATP per cell is a function of cell size (Bochdansky et al., 2021). Because many eukaryotes (Caron et al., 2009) can have larger cell sizes compared to bacteria and archaea, the concentration of larger eukaryote cells in a sample could potentially influence the ATP + ADP + AMP concentration. In the Namibia shelf sediment (Table 1) the ATP + ADP + AMP concentrations mirrors more closely the 16S rRNA gene concentrations from bacteria and archaea, compared to the concentration of eukaryotic Foraminifera cells (Orsi, Morard, et al., 2020) and fungi 18S rRNA genes (Orsi et al., 2022) (Figure 3). This is explained by several orders of magnitude higher concentration of 16S rRNA genes from bacteria and archaea, compared to the fungi 18S rRNA gene and Foraminifera cell concentrations (Figure 3). However, there is a second deeper peak in ATP + ADP + AMP at 10 cm that coincides with a 1000 fold increase in fungal 18S rRNA gene concentrations (Figure 3). At this depth, no peak of 16S rRNA genes (from bacteria and

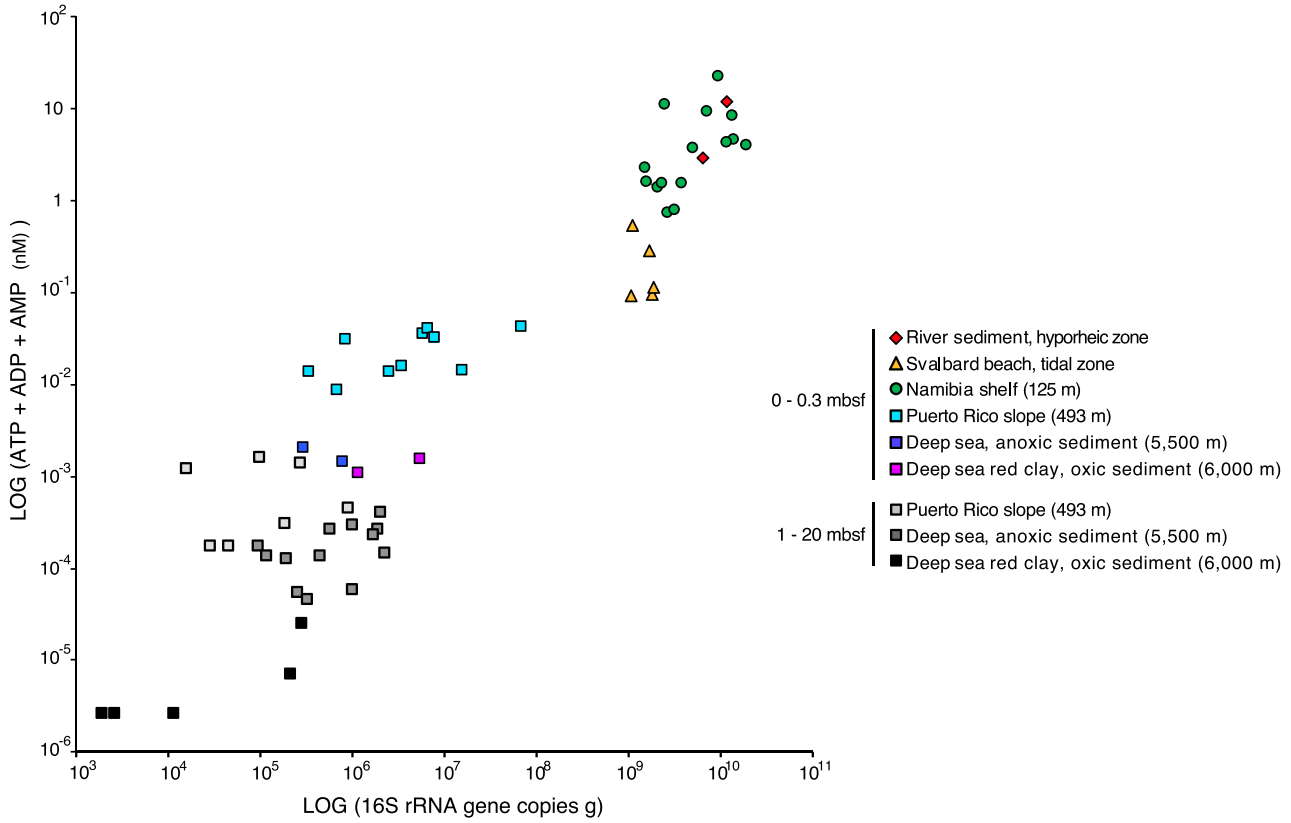

FIGURE 5 A log–log plot showing the power-law relationship between the concentration of ATP + ADP + AMP (*y*-axis), and 16S rRNA gene concentrations from the same samples. Water depth of the coring locations (Table 1) are given in meters in the legend, as are the ranges of sediment depths below the seafloor.

archaea) or Foraminifera cells was observed indicating that this second sub-seafloor peak in ATP + ADP + AMP is due to increased fungal biomass. Therefore, the presence and abundance of benthic eukaryotes should be considered when interpreting the ATP + ADP + AMP measurements from marine sediments.

Generally, the concentration of ATP + ADP + AMP correlates positively with 16S rRNA gene concentrations across all sites (Figure 5). However, an exception to this trend was found in deep sea anoxic sediment (Table 1) where the vertical profiles of ATP + ADP + AMP and 16S rRNA gene concentrations over the top two meters of the core are inverted (Figure 4). The inverted trends could be explained by decreasing cell sizes with increasing depth below the seafloor, since cell volume decreases over an order of magnitude in the sub-seafloor (Braun et al., 2016). Reduced cell size below the seafloor would reduce the amount of biomass per cell and thereby the concentration of ATP + ADP + AMP per gram. The inverted profiles of ATP + ADP + AMP and 16S rRNA gene concentrations in the deep sea anoxic sediment core (Figure 4) could therefore be explained by a proliferation of cells >1 mbsf (as indicated by the 16S rRNA gene concentrations), that have smaller volumes compared to the cells living in the upper 20 cm of sediment (registered by the ATP + ADP + AMP concentrations). A reduction of cell size and volume under laboratory conditions is a common feature of energy limitation in bacteria (Amy et al., 1993; Amy & Morita, 1983) (Kieft et al., 1997) (Novitsky & Morita, 1976), and has been observed for deep biosphere cells persisting under energy limitation (Braun et al., 2016). Because energy limitation is a general feature of the sub-seafloor biosphere, a reduced cell size due to energy limitation could be a contributing factor behind the reduced adenylate concentrations in the sub-seafloor communities.

The detection limit for the ATP + ADP + AMP method was reached in the oxic deep sea red clay subseafloor sediment (Table 1, Figure 5), that are millions of years old and are dominated by ammonia-oxidizing archaea (Vuillemin et al., 2019). The relatively small size of ammonia-oxidizing (Konneke et al., 2005) archaea could furthermore contribute to the low ratio of ATP + ADP + AMP versus 16S rRNA gene concentrations in the deep sea sub-seafloor samples (Figure 6). Considering this, the ratio of archaea to bacteria could also influence the ATP + ADP + AMP measurement in marine sediments.

In the deep sub-seafloor samples >1 mbsf, increased water depth correlates with a lower concentration of ATP + ADP + AMP, relative to 16S rRNA

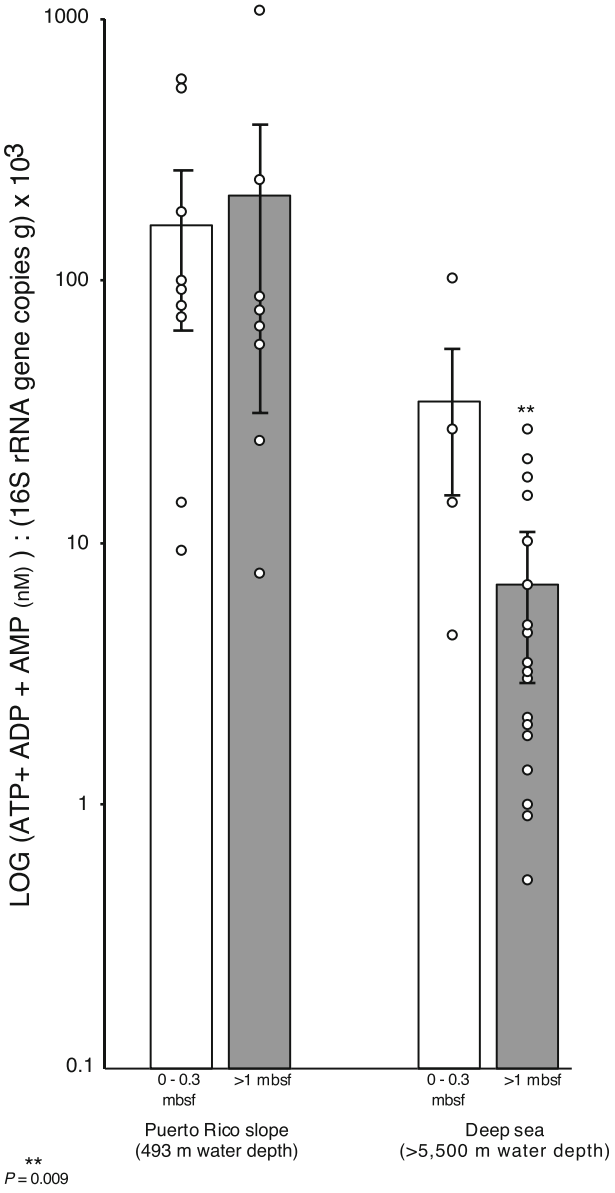

FIGURE 6 Ratio of ATP + ADP + AMP and 16S rRNA gene concentrations in sediment from the Puerto Rico slope (493 m water depth) and deep sea (>5500 m water depth). Samples are grouped according to depth below seafloor: white: 0–0.3 mbsf, grey: >1 mbsf. Histograms represent average values per group, the error bars represent standard deviations, and the individual data points are displayed. Note that there was significantly lower average ATP + ADP + AMP: 16S rRNA gene concentration ratio between the shallow and deep sub-seafloor samples from the deep sea (two sided *T*-test: $P = 0.009$), but not from the shallower water Puerto Rico slope (two sided *T*-test: $P > 0.1$).

gene concentration. Namely, deep sub-seafloor microbial communities (>1 mbsf) at deep sea (> 5500 m water depth) locations have significantly lower ATP + ADP + AMP: 16S rRNA gene concentration ratios (two sided *T*-test: $P = 0.009$), compared to the sediment communities residing in the top 30 centimetres of sediment (Figure 6). However, at the shallower water (493 m water depth) Puerto Rico upper slope site no significant difference in ATP + ADP + AMP: 16S rRNA gene concentration ratios was observed between the top 30 centimetres, and deep (>1 mbsf) sub-seafloor communities (Figure 6). These results indicate that the biomass of sub-seafloor cells in the deep sea is reduced compared to sub-seafloor cells subsisting underneath shallower waters. This could be due to the effect of high hydrostatic pressure in the deep sea, that can result in a reduction of bacterial cell size (Grossart & Gust, 2009). Nutrient availability in the surface waters and associated primary productivity can also control cell concentrations at the seafloor (Kallmeyer et al., 2012), and can explain the higher ATP + ADP + AMP concentrations in the shallower waters of the Namibian shelf compared to the lower ATP + ADP + AMP concentrations in the deep sea sediment that underly oligotrophic waters (Figure 5).

The clear positive correlation between ATP + ADP + AMP concentrations measured with the 'boil and dilute' method and 16S rRNA gene concentrations across multiple sediment sites with varying age, biomass, and lithology (Figure 5), shows that the new method provides a measure of microbial biomass in the sediments. In the future, the method should be cross checked by comparing ATP + ADP + AMP concentrations with direct cell counts, as has been done in the past to calibrate qPCR based assessments of microbial biomass in sediments (Lloyd et al., 2013). The detection limit for ATP + ADP + AMP concentrations in sediments demonstrated here could also be potentially lowered, to increase sensitivity allowing for quantification of ultra-low biomass samples.

METHODS

Standard curves for quantification of ATP, ADP and AMP

Relative fluorescent units corresponding to known concentrations across serial dilutions of ATP, ADP and AMP (Sigma-Aldrich) were prepared in MilliQ water and measured using a lumitometer (Lumitester PD-30; Kikkomann Biochemifa) using the LuciPac A3 Aqua water tester kit (Kikkomann Biochemifa) following the manufacturer's instructions. This test kit system exploits a cyclic method based on a combination of firefly luciferase, PPDK and a PK to detect ATP, ADP and AMP in a sample (Figure 1). The method produces a defined amount of luminescence proportional to the amount of ATP + ADP + AMP that is registered in RLU (Bakke & Suzuki, 2018) (Saito et al., 2020) (Bakke, 2022). Standard curves of ATP, ADP and AMP concentrations were created individually and plotted as a function of RLU (Figure 2). The resulting power law function was used to calculate molarity of total adenylate from the RLU obtained from sediment samples.

Sample processing and ATP + ADP + AMP quantification from sediments

Following the guidelines of Bochdansky et al. (2021) who found that extraction reagents can interfere with the luciferin-luciferase reaction for ATP measurements, a minimal sample processing protocol was employed that involved diluting the sample with boiling-hot MilliQ water. This followed prior studies that showed incubating samples with boiled ultrapure water increases the amount of detectable ATP (Bochdansky et al., 2021) (Yang et al., 2002). Between 0.2 and 0.5 g of wet sediment was transferred aseptically to a sterile 2 ml microfuge tube and diluted either 1:2 or 1:10 with ultra-pure MilliQ water that had been pre-heated to 100°C. The samples were then incubated on a heating block set to 95°C for 5 min, vortexed and returned to the heating block for another 5 min at 95°C. Samples were then allowed to cool to room temperature, and centrifuged at 13,000 rpm for 1 min to pellet the sediment particles. The supernatant containing dissolved ATP + ADP + AMP that had been released from the lysed cells was sampled using ATP sticks (LuciPac A3 assay; Kikkomann Biochemifa) according to the manufacturer's instructions. After injecting the sampling stick into the LuciPac reaction tube, the tube was inverted five times to ensure adequate mixing of the various reagents (Figure 1) and the liquid inside the tube was allowed to settle to the bottom prior to measuring on the lumitometer. The RLU was measured using a lumitometer (Lumitester PD-30; Kikkomann Biochemifa). During sampling of the supernatant, care was taken to avoid the pelleted sediment with the LuciPac stick since particles transferred to the luminescence reaction vials was found to result in anomalously lower RLU values, presumably due to their blocking of light from the luciferase reaction. Background values (extraction blanks) were performed using the exact same method but measured on MilliQ water only (with no added sediment). The extraction blanks ranged between 0 and 5 RLU. Thus, any samples with RLU values below 5 were not considered since contamination from the added milliQ water could not be ruled out.

Sampling

Marine sediment samples derived from five locations with widely varying water depth, sedimentation rate and lithology. The first site was a 30 cm long sediment core obtained from a water depth of 125 m on the Namibian continental shelf (18.0 S, 11.3 E), as described previously (Orsi, Vuillemin, et al., 2020). Sediments were sampled with a multi corer (diameter 10 cm), which yielded an intact sediment/water interface and the upper 30 cm of sediment. After retrieval, cores were moved immediately to a 4°C cold room and sectioned every 2 cm within 24 h. Sections were transferred immediately into sterile, DNA/RNA free 50 ml falcon tubes and then frozen immediately at −20°C until being processed for DNA extraction and ATP quantification. The second sampling site is a beach in Svalbard in front of the Midtre Lovénbreen glacier (78°.88′ N, 12°.07′ W), whereby sand from six different spots in the tidal zone were collected with sterile falcon tubes and frozen immediately until further processing (sampling took place during June of 2021). The third sampling site is shelf sediment ranging from 0.1 to 10 m below seafloor collected off the coast of Puerto Rico (site PR02: 18°.35′ N, 65°.34′ W) during an oceanographic expedition with the R/V Neil Armstrong (AR 64-02). Sediment cores were obtained via multi-coring, gravity coring, and piston coring sectioned on board immediately after retrieval and frozen at −80°C until further processing. The fourth and fifth sites are deep-sea clay sediment from 5500 and 6000 m water depth in the ultra-oligotrophic open ocean of the North Atlantic. These two coring sites (KN223-11, KN223-15) are characterized by a mean sedimentation rate of 1–3 m per million years, and was obtained using a 30 m long piston core (Vuillemin et al., 2019; Vuillemin, Vargas, et al., 2020). After retrieval, cores were moved immediately to a 4°C cold room and sectioned within 24 h. Sections were transferred immediately into sterile, cutoff syringes and then frozen immediately at −20°C until being processed for DNA extraction and ATP quantification. As a sixth site for comparison, surface sediments from the Moosach River hyporheic zone (Munich, Germany) were also included, and sampled as described previously (Michaelis et al., 2022).

DNA extraction and qPCR

The 16S rRNA gene data have been published previously (Orsi, Vuillemin, et al., 2020) (Vuillemin et al., 2019, Vuillemin, Kerrigan, et al., 2020) (Michaelis et al., 2022) and were used in this study to compare against the ATP + ADP + AMP measurements. In brief, DNA was sampled from sediment aseptically in a sterile laminar flow hood using a sterile spatula, and extractions were carried out using the Amicon concentration method as described previously (Coskun et al., 2021; Vuillemin, Kerrigan, et al., 2020). DNA extracts were purified using the PowerClean Pro DNA Clean-up Kit (MO BIO Laboratories) and quantified using the Qubit dsDNA HS Assay kit (Thermo Fisher Scientific) according to manufacturer's instructions. qPCR determination of the concentration of 16S rRNA genes was done using with primer pair 515F/806R using the qPCR protocol as described previously (Orsi, Morard, et al., 2020) (Vuillemin, Vargas, et al., 2020). Three technical replicates were prepared with the

epMotion 5070 robotic pipetting system (Eppendorf), with a technical variation of <5%. qPCR standards consisted of 10-fold dilution series of the genes of interest that were PCR amplified from the sample using the same primers. Reaction efficiencies in all qPCR assays were between 90% and 100%, with an r^2 of >0.9. Gene copies and ATP + ADP + AMP values were normalized to the wet weight of the sediment.

AUTHOR CONTRIBUTIONS

William Orsi: Conceptualization (equal); data curation (equal); formal analysis (equal); funding acquisition (equal); investigation (equal); methodology (equal); project administration (equal); resources (equal); software (equal); supervision (equal); validation (equal); visualization (equal); writing – original draft (equal); writing – review and editing (equal).

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

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