Differential incorporation of one-carbon substrates among microbial populations identified by stable isotope probing from the estuary to South China Sea

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Methanol (MOH) and monomethylamine (MMA) are two typical one-carbon (C$_1$) compounds found in natural environments. They play an important role in marine and atmospheric chemistry, cloud formation, and global climate. The main biological sink of MOH and MMA is rapid consumption by marine microbes. Here, field-based time-series incubations with supplemental $^{13}$C-labelled MOH and MMA and isotope ratio analyses were performed. A substantial difference in the MOH and MMA incorporation rates and bacterial taxa were observed between the South China Sea (SCS) and the Pearl River estuary. C$_1$ substrates were assimilated more quickly in the estuary than the SCS shelf where MOH and MMA had similar bio-availability. However, microbial responses to MMA may be faster than to MOH in the coastal and basin surface water of the SCS despite similar active bacterial populations. Three ecological types of bacteria, in terms of response to supplemented MOH and MMA, were identified: rapid incorporation (I, dominant C$_1$-incorporating group), slow incorporation (II, minor C$_1$-incorporating group), and no incorporation (III, C$_1$-non-incorporating group). Members of the families Methylophilaceae ($\beta$-Proteobacteria) and Piscirickettsiaceae ($\gamma$-Proteobacteria) belonged to type I and actively incorporated substrates in the estuary and SCS, respectively. Diverse MOH and MMA-incorporating type II bacteria were identified by stable isotope probing in the SCS, and could play a more important role in the transformation of C$_1$ compounds in marine environments than hitherto assumed.

One-carbon (C$_1$) compounds consist of a single carbon atom (lacking carbon-carbon bonds) such as methane, methanol (MOH), monomethylamine (MMA), halogenated methanes and methylated sulphur. C$_1$ compounds exert influence on marine and atmospheric chemistry, cloud formation, and global climate. For example, MOH is an oxygenated volatile organic compound that contributes to rainwater acidity through photochemistry; it positively influences the oxidising capacity and ozone-forming potential of the atmosphere, and is thus a climate-relevant gas. As one of the most abundant C$_1$ compounds on Earth, MOH occurs naturally in the environment since plants release it as they grow and decompose. A recent study found that the main source of MOH in the ocean is phytoplankton, which have a unique ability to produce MOH in quantities that rival what is produced on land. Experimental models have estimated that there are ~30 and ~100 million tons of MOH released from ocean and terrestrial plants, respectively, into the atmosphere per year, and globally ~600 million tons of methane released into the atmosphere per year. MOH is an “alcohol derivative” of methane. However, MOH has a higher solubility than methane and can be easily deposited in the ocean from the atmosphere. Thus, the ocean serves as a huge natural reservoir of MOH. Many previous studies showed that concentrations of MOH in seawater ranged from 27 to 429 nM and decreased with depth. MOH is a ubiquitous source of C$_1$ substrate, which aids the widespread distribution of methylotrophic organisms throughout the surface ocean.
Despite high volumes, MOH has a short residence time in seawater, suggesting that high production from phytoplankton is offset by rapid consumption. This, combined with its high solubility, suggests that consumption by microorganisms prevents MOH from escaping into the atmosphere in significant quantities.

MMA, another important C1 compound in atmospheric and marine environments, is a derivative of ammonia, with one hydrogen atom replaced by a methyl group. MMA is derived from the degradation of compatible solutes, choline, glycine betaine, and trimethylamine that is released by algae, invertebrates, and fish, as they adjust their osmotic pressure. Thus, MMA widely distributed in the ocean. The concentration of MMA generally ranges from 0 to 66 nM, with higher values in shoals, off shore, and high productivity areas, and lower values in oligotrophic areas, particularly the deep sea (<10 nM). As an ammonium analogue, MMA has an inhibiting effect for ammonium oxidation and chemooautotrophic growth of marine nitrifiers. MMA can act as a carbon or nitrogen source for marine microbes, and thus significantly participates in both the carbon and nitrogen cycles. Methylophaga use MMA as a carbon source, while non-methylotrophic microorganisms utilise MMA as a nitrogen source.

As the incorporation of MOH and MMA by microbes (mainly Methanotrophs) is the main biological sink, the microbes involved in this process have been intensively studied. Methylophaga are widespread in a diverse range of habitats, and most of them belong to the classes Alphaproteobacteria (α-Proteobacteria), Betaproteobacteria (β-Proteobacteria), and Gammaproteobacteria (γ-Proteobacteria), the phyla Actinobacteria, Firmicutes, and Verrucomicrobia, as well as a Candidatus phylum NC10. It has been reported that methanotrophs captured the majority (up to over 95%) of methane produced via methanogenesis or non-biogenically, whereas non-methanotrophic methylophaga constitute important barriers for accumulation of other environmentally important C1 compounds, such as MOH, methylated amines, and halogenated methanes. Previous studies on non-methanotrophic methylophaga have mainly focussed on the enrichment and isolation of methylophaga, but only a small number of microorganisms were available in pure culture. As a cultivation-independent analysis, DNA-based stable isotope probing (DNA-SIP) provides a powerful tool to characterise the metabolic activity of microorganisms under in-situ conditions by tracking the stable isotopes from isotopically labelled substrates into DNA. Compared with other SIP technologies, such as phospholipid fatty acid (PLFA)-SIP, RNA-SIP and protein-SIP, DNA-SIP can identify a broad spectrum of microorganisms involved in a particular process and with high phylogenetic resolution; thus, it can detect organisms that have previously been undetected using other methods. The identity of microorganisms responsible for the transformation of C1 compounds, which has not yet been determined, will fill major knowledge gaps regarding the role of these compounds in the marine carbon cycle. However, so far there is very few studies regarding the C1 compounds-incorporating microbes in marine systems. Neufeld et al. reported MOH and MMA utilization by Methylophaga and several clades of unclassified γ-Proteobacteria based on DNA-SIP and clone library analysis in the English Channel. Many open questions remain about the MOH and MMA-incorporating microbes, including diversity, incorporation rates, and difference among the typical marine systems.

The South China Sea (SCS) is one of the largest marginal seas, with a deep basin in the tropical to subtropical western North Pacific. Here, we carried out field-based time-series incubation experiments with supplemental 13C-labelled MOH or MMA in different water masses of the SCS (Table S1), covering the Pearl River estuary (site P1, 113.57°E, 22.90°N), coastal water (site C3, 109.48°E, 18.22°N), continental shelf water (site S2, 113.93°E, 21.34°N), and the oligotrophic central basin (site B4, 117.50°E, 18°N), during two cruises in July–October 2013 (Fig. 1). Subsequently, 13C and 12C-DNA separated through ultracentrifugation were analysed by terminal-restriction fragment length polymorphism (T-RFLP) and high-throughput sequencing. In addition, carbon isotopic compositions of particulate organic carbon (POC) in incubated seawater were measured...
were clearly distinguished from incubations with 12C-labelled MOH after one day. However, peaks were clearly
distinguishable between 13C and 12C-DNA after four days of incubation of samples at the coastal site C3 (5 m
depth), shelf site S2 (5 and 69 m depth), and 200 m of the central basin site B4 and two days at 5 m depth of site

| Station | Depth (m) | Substrate | Assimilation (13C) (μg L⁻¹ d⁻¹) | 13C% |
|---------|-----------|-----------|-------------------------------|------|
|         |           |           | 1 d | 4 d | 1 d | 4 d |
| P1      | 5         | MOH       | 165.6 | 77.4 | 27.9 | 17.9 |
| S2      | 5         | MOH       | 14.8 | 44.0 | 10.9 | 46.5 |
| 69      | MOH       | 0.4       | 26.7 | 0.5 | 48.2 |
|         | MMA       | 1.4       | 41.1 | 3.0 | 51.7 |
|         | MMA       | 11.4      | 39.8 | 20.0 | 58.4 |

Table 1. Microbial assimilation rates of 13C-labeled substrates and percent of 13C in total particulate organic carbon during incubation experiments. MOH, methanol; MMA, monomethylamine.

to estimate 13C-labelled substrate assimilation rates. The objectives of this study were to identify the key microbial players involved in the transformation of the two typical C₁ substrates (MOH and MMA), compare results among the typical water masses of the SCS, and evaluate microbially driven C₁ cycling in the ocean.

Results
13C-labelled substrate assimilation. The assimilation rate of 13C-labelled MOH was 165.6 μg L⁻¹ d⁻¹ after the seawater from 5 m depth of the estuary site P1 was incubated for one day, and decreased to 77.4 μg L⁻¹ d⁻¹ after four days of incubation. In contrast, samples at the shelf site S2 had a 13C-MOH assimilation rate of 14.8 μg L⁻¹ d⁻¹ (5 m water depth) and 1.4 μg L⁻¹ d⁻¹ (69 m water depth) after one day of incubation, which increased to 44.0 μg L⁻¹ d⁻¹ (5 m water depth) and 41.1 μg L⁻¹ d⁻¹ (69 m water depth) after four days of incubation. Consistent with the MOH assimilation rates of samples at site S2 from one to four days of incubation, the assimilation rate of 13C-labelled MMA was distinctly higher after four days than one. The assimilation rates of 13C-MMA were higher in samples at 69 m depth than 5 m (Table 1). Overall, there was no significant difference between the assimilation rates of 13C-MOH and 13C-MMA at site S2. The same trends were observed in the 13C percent in total POC (Table 1). It decreased with increasing incubation time of samples at site P1, indicating that some microbially assimilated 13C-MOH was degraded to carbon dioxide. The substrate assimilation rates were not measured at sites C3 and B4 as all culture volumes were collected for ultracentrifugation and molecular analysis.

DNA distribution in CsCl density gradient fractions. Relative abundances of DNA in the CsCl density gradient fractions were analysed to locate 13C and 12C-DNA (Fig. 2a; Figs S1–S4). CsCl density gradually decreased from the 1st to 12th fraction, ranging from 1.687 to 1.770 g mL⁻¹. Overall, DNA was distributed throughout all fractions, with one to two peak values in heavy and/or light density fractions. The DNA concentration peaks from incubations with supplemental 13C-labelled MOH at the estuary site P1 samples (5 m depth) were clearly distinguished from incubations with 12C-labelled MOH after one day. However, peaks were clearly distinguishable between 13C and 12C-DNA after four days of incubation of samples at the coastal site C3 (5 m depth), shelf site S2 (5 and 69 m depth), and 200 m of the central basin site B4 and two days at 5 m depth of site B4. For incubations with supplemental 13C and 12C-labelled MMA, 13C-DNA can be clearly distinguished after one day of incubation at 5 m depth of both sites C3 and B4 and after four days for sites S2 (5 and 69 m depth) and 200 m of site B4 (Figs S1–S4).

Bacterial communities in density gradient fractions based on T-RFLP analyses. Bacterial community composition in the density gradient fractions, produced from ultracentrifugation of DNA retrieved from incubation with supplemental 13C-labelled substrates, was analysed with T-RFLP. The T-RFLP results (Fig. 2b) showed that there was a significant difference in T-RFs between heavy (H) and light (L) fractions for each sample. Figure 2 shows the sample from the 13C-MOH supplemented seawater incubation experiment at site C3 (5 m depth) as an example. Nonmetric multidimensional scaling (NMDS) ordination based on the Bray-Curtis similarities between T-RFLP fingerprinting of bacterial communities further revealed that community was separated into two major clusters: group I contained fractions 1–3 and 5 (heavy group, Bray-Curtis similarity = 73%), and group II contained fractions 8–12 (light group, 32%) (Fig. 2c). Meanwhile, fractions 5 and 10 were located at the 13C and 12C-DNA concentration peaks, respectively (Fig. 2a). Based on the analysis, fractions 5 and 10 obtained from the incubation with supplemental 13C-labelled substrate at site C3 (5 m depth) were selected as the representative H and L fraction, respectively, and used for further sequencing. By this means the representative H and L fraction of other samples were also selected. The fractions selected for each sample are shown in Table S2.

Time-series analysis of substrate-incorporating bacterial communities. The representative H and L fractions from all samples were analysed with high-throughput sequencing to identify the active substrate-incorporating bacterial populations and populations that did not incorporate substrate. NMDS was built from the Bray-Curtis dissimilarities, which were calculated based on the relative abundance of OTUs, to discriminate bacterial community differences between the H and L fractions over the incubation time. For site P1 (Fig. 3a), the dissimilarity of bacterial communities at 5 m depth between H and L were 76.36%, 68.21%, and 53.49% after one, three, and four days of incubation, respectively. Moreover, bacterial community structure after one day of incubation was significantly different from three and four-day incubation periods (R = 0.607, P < 0.05). Combining the community structure analysis and 13C and 12C-DNA concentration distributions in the density gradient fractions (Fig. S1), the sample at estuary site P1 after one day of incubation more accurately reflected the substrate-incorporating activity of the in-situ community. It also escaped the "bottle effect" and
cross-feeding effect. In this context, the “bottle effect” alludes to an apparent reaction of bacteria to batchwise incubation in a confined environment (see Discussion)39. Cross-feeding is the phenomenon that one species lives off the products of another species, which would cause the detection of microorganisms that utilized a labelled intermediate produced by the primary incorporator of the substrate or dead biomass of primary incorporator in long periods of incubation33. Here, gradual homogenisation between H and L communities after three and four days of incubation could reflect this effect. In contrast, samples at shelf site S2 (both 5 and 69 m water depth) and coastal site C3 (5 m depth) (Fig. 3b–d) showed dissimilarity in bacterial communities between H and L fractions after four days of incubation with both MOH and MMA higher than after one day. Statistically significant differences were observed between bacterial community structure in the H fractions after four days of incubation and the L fractions and after one-day incubation at site S2 (Fig. 3b,c; 5 m depth: R = 0.646, P < 0.05; 69 m depth: R = 0.875, P < 0.05). Combining this community structure analysis and 13C and 12C-DNA concentration distributions in the density gradient fractions (Figs S2 and S3), the samples at sites S2 and C3 after four days of incubation more appropriately reflected the active substrate-incorporating populations from the in-situ community. They also escaped the indistinguishable state between 13C and 12C-DNA that resulted from the short one-day incubation, but the influences of the “bottle effect” and the cross-feeding effect cannot be assessed. The dissimilarity of the bacterial community between H and L fractions from samples of the basin site B4 (5 m depth) after two-day

Figure 2. Bacterial community composition at 5 m water depth at site C3 based on T-RFLP analyses in density gradient fractions produced from ultracentrifugation of DNA after four days of incubation with supplemental 13C-labelled MOH. (a) Relative abundance of DNA in CsCl density gradients; (b) Relative abundance of T-RFLP peaks (colour bars) in each DNA fraction; (c) Nonmetric multidimensional scaling (NMDS) ordination based on the Bray-Curtis similarities between T-RFLP fingerprinting of bacterial communities in density gradient fractions. F = T-RFLP experiment failed.
incubation with both MOH and MMA was higher than one and four days (Fig. 3e), and meanwhile the bacte-
rial community structure after four days of incubation was significantly different from after one and two days
(R = 0.404, P < 0.05). Taken together, dissimilarity of the bacterial community between H and L fractions and
13C- and 12C-DNA concentration distributions in the density gradient fractions (Fig. S4a–d) indicated that the
sample after two days of incubation more appropriately reflected the active substrate-incorporating populations
from the in-situ community than one or four days of incubation. The sample escaped the indistinguishable state
between 13C- and 12C-DNA that resulted from the short one-day incubation as well as the influences of the "bottle
effect" and cross-feeding effect that resulted from the long four-day incubation. The sample at 200 m water depth
of site B4 was only collected after four days of incubation (Fig. 3f). In almost all of the samples, the Bray-Curtis
similarity of the bacterial community between the L fractions and their corresponding in-situ sample was higher
than between the H fraction and in-situ sample (Table S3).

Comparison among active substrate-incorporating bacterial communities. H fractions of sam-
ple from appropriate incubation times were selected (Table S4) to analyse the phylogenetic composition of the
active MOH and MMA-incorporating bacterial communities; the corresponding L fractions were also sequenced.
Shannon diversity index values showed that 13C-DNA bacterial community from the incubations with MOH
(0.93–2.39, average = 1.61 ± 0.55) and MMA (1.38–2.33, average = 1.75 ± 0.46) were significantly less diverse
than the 16C-DNA and in-situ bacterial community (MOH: 1.86–2.98, average = 2.57 ± 0.45; MMA: 1.8–2.98,
The SCS sites. The 12C-DNA bacterial communities were more similar to Methylophilaceae (Fig. 5). Overall, the active MOH and MMA-incorporating (13C-DNA) bacterial populations were mainly from ples, were phylogenetically analysed and their relative abundances in each library were depicted with heat maps. >1% in either H or L fraction, or 91 representative sequences of OTUs with relative abundances were distinctly abundant in the 12C-DNA communities as influence final cell concentrations41, viability/activity parameters42, cultivability43, and population composition44. Here, the difference between in-situ and 12C-DNA communities could reflect this effect. For instance, members from Alteromonadaceae were distinctly abundant in the 12C-DNA communities as compared to the in-situ communities. Members of Alteromonadaceae were the most frequently found lineages after incubation due to their capability of higher growth rate, faster response to nutrients, and better assimilation of carbon sources45,46.

**Phylogenetic analysis of the active substrate-incorporating bacterial populations.** A total of 91 representative sequences of OTUs with relative abundances >1% in either H or I fraction, or in-situ samples, were phylogenetically analysed and their relative abundances in each library were depicted with heat maps (Fig. 5). Overall, the active MOH and MMA-incorporating (13C-DNA) bacterial populations were mainly from the families Methylphilaceae (β-Proteobacteria) at estuary site P1 and Piscirickettsiaceae (γ-Proteobacteria) at the SCS sites. The 13C-DNA bacterial communities were more similar to in-situ communities, with major populations from β-Proteobacteria and Actinobacteria at site P1 and the SAR11 group of α-Proteobacteria at the SCS sites. In addition, members from Alteromonadaceae were abundant in the 12C-DNA communities.

Based on the framework of Nelson and Carlson40, we divided the OTUs into nine clades based on their relative abundance differences among H and I fractions, and in-situ samples (Fig. 5). Clade II (Flavobacteria), Clade III (α-Proteobacteria), Clade VI (γ-Proteobacteria), Clade VIII (β-Proteobacteria) and Clade IX (Actinobacteria) did not incorporate 13C-labelled MOH and MMA, as the relative abundance of OTUs in these five clades were lowest in H fractions compared with I fractions and in-situ samples. Clade V (γ-Proteobacteria) was the dominant MOH and MMA-incorporating group in the SCS sites (S2, C3, and B4), whilst Clade VII (β-Proteobacteria) was the dominant MOH-incorporating group in the estuary site (P1). Clade I (Flavobacteria and Bacteroidetes) and Clade IV (α-Proteobacteria) consist of diverse OTUs that incorporated 13C-labelled MOH and/or MMA; these OTUs were the minor MOH and MMA-incorporating groups with the similar relative abundance of OTUs in H and I fractions. Thus, the nine clades were classified as dominant, minor MOH and MMA-incorporating groups, and MOH and MMA-non-incorporating group. The representative phylogenetic taxa in the nine clades are shown in Fig. 6.

**Discussion**

To clearly separate and obtain sufficient 13C-DNA under low substrate conditions, we supplemented the 13C-labelled substrates with a final concentration of 100 μM, which is higher than in-situ content. High substrate concentration has no influence on assessing whether a population incorporates it or not, although it might result in a “bottle effect” which could influence final cell concentrations41, viability/activity parameters42, cultivability43, and population composition44. Here, the difference between in-situ and 12C-DNA communities could reflect this effect. For instance, members from Alteromonadaceae were distinctly abundant in the 12C-DNA communities as compared to the in-situ communities. Members of Alteromonadaceae were the most frequently found lineages after incubation due to their capability of higher growth rate, faster response to nutrients, and better assimilation of carbon sources45,46.

**Figure 4.** Dendrogram of the group average model based on Bray-Curtis similarities between the representative 13C-DNA communities. Colour bars indicate relative abundances of phylogenetic taxa in each library. MOH = methanol; MMA = monomethylamine; d = day.
The trade-offs between obtaining enough $^{13}$C-DNA and cross-feeding is a balancing act in SIP experiments. Thus, in this study, time-series incubations were performed to assess the best incubation time for accurate identification of substrate-incorporating populations. One day of incubation was enough to label sufficient DNA from samples of estuary site P1, four days were needed for SCS sites S2 and C3, and two days for site B4. This is broadly consistent with a previous study of samples from the English Channel that indicated MOH and MMA can be well incorporated after three or four days of incubation\(^6\). However, it is clear that the optimal incubation time varies in different environments, which is shorter in eutrophic estuarine waters than in oligotrophic open waters. Isotope ratio analysis also indicated that the substrate uptake rate in estuary site P1 samples were at least one order of magnitude higher than shelf site S2 after one day of incubation. These results suggest that C\(_1\) substrates could be more quickly assimilated by microbes in fresh water than in the shelf water of the SCS, and thus estuaries could be important MOH and MMA sinks. Overall, MOH and MMA had similar bio-availabilities in the SCS shelf, as indicated by time-series incorporation rates and active community composition. However, in the coastal and open ocean basin surface water, DNA distribution in CsCl density gradient fractions (Figs S3 and S4) indicated that the microbial response to MMA may be faster than to MOH despite similar active bacterial populations. A possible explanation is that MMA can act as a nitrogen source for marine microbes besides carbon source, particularly in the nutrient-limiting oligotrophic environments (Table S1). MOH is mainly used as an energy source (degraded to CO\(_2\)), and only a small part of its uptake is attributed to cellular carbon source (incorporation into cell material)\(^5,47\). MMA can be used as both an energy and carbon source\(^48\). $^{13}$C-DNA indicates incorporation of

Figure 5. Phylo-genetic tree of OTU sequences with relative abundance >1% of total sequences in either the representative heavy (H) or light (L) fractions, or in-situ samples. Relative abundances of OTUs are shown as heat maps to the right of the phylogenetic tree. Figure was produced from the Interactive Tree Of Life (iTOL, http://itol.embl.de/). E, estuary; SCS, South China Sea.
the substrate, whilst the $^{12}$C-DNA community might include the groups utilising MOH or MMA only as a source of energy, such as SAR1169, as well as the groups that did not incorporate MOH or MMA.

There are three ecological types of bacterial taxa, characterised according to their response to supplemented MOH and MMA: rapid incorporation (I, dominant $C_1$-incorporating group), slow incorporation (II, minor $C_1$-incorporating group), and no incorporation (III, $C_1$-non-incorporating group). Most of the taxa were ecological type III, which lack the ability to assimilate MOH and MMA, but might have the capacity of degrading them to CO$_2$50. Most ecological type I taxa were fairly rare in the SCS sites, but quickly responded to supplemented MOH and MMA. Ecological type II had more diverse bacterial taxa, which could incorporate either MOH, MMA, or native organic matter as indicated by their relative abundance in L fraction and in-situ communities; however, it is possible these $^{13}$C-DNA taxa were derived from $^{12}$C-DNA due to cross-feeding.

Members of Methylophilaceae ($\beta$-Proteobacteria), belonging to ecological type I, are major MOH-incorporating populations in the estuary. They are widespread in a diverse range of fresh water habitats, such as mud flats, rivers, lakes, and ponds, and capable of assimilating MOH or methylamine51,52. In the SCS, MOH and MMA-incorporating populations included more diverse bacterial taxa belonging to $\gamma$-Proteobacteria (ecological type I), $\alpha$-Proteobacteria (type II), Flavobacteriia (type II), and Sphingobacteria (type II), of which Methylophaga ($\gamma$-Proteobacteria, Piscirickettsiaceae) was the most dominant group. These are widespread in a diverse range of salt water habitats and are involved in $C_1$ compounds assimilation6,53. Members of Methylophaga use the ribulose monophosphate pathway to assimilate $C_1$ compounds6 and have been defined as strictly aerobic methylotrophs. The second most dominant group was Thalassobius (ecological type II), a newly defined genus54 of the Rhodobacteraceae that has not been tested the capability to assimilate MOH and MMA under natural or culture conditions to-date. Other identified ecological type II MOH and MMA-incorporating groups belong to Shimia, Erythrobacter, and Tenacibaculum, but these have not been reported to incorporate MOH and MMA to-date. Many $^{13}$C-DNA OTUs belonged to unclassified Piscirickettsiaceae (2.5% to 78.9% of total reads, ecological type I), Rhodobacteraceae (type II), Flavobacteriaceae (type II), and Saprospiraceae (type II), and had quite high relative abundances in some samples. These results indicate that there are more diverse MOH and MMA-incorporating bacterial taxa in marine environments than assumed previously55. To gain insight on the phylogeny and physiology of these methylotrophic bacteria, further investigations combining DNA-SIP with metagenomics or metatranscriptomics should be conducted50,56.

This study combined DNA-SIP time-series incubation with isotope ratio analyses to investigate the bio-availabilities of typical $C_1$ compounds, MOH and MMA, and the microbes responsible for the assimilation of these $C_1$ compounds in different water masses of the Pearl River estuary, coast, shelf, and central basin of the SCS. Our findings demonstrate a substantial difference in the rates of MOH and MMA incorporation and bacterial taxa, particularly between the estuary and SCS. The estuary microbial community can more quickly
incorporate MOH than the SCS shelf community, and *Methylophilaceae* (β-Proteobacteria) are major microbial sinks of MOH. MOH and MMA generally had similar bio-availabilities in the SCS shelf, based on comparable incorporation rates and similar active bacterial populations. However, microbial response to MMA may be faster than to MOH in the coastal and basin surface water of the SCS despite similar active bacterial populations. *Piscirickettsiaceae* (γ-Proteobacteria) are major MOH and MMA-incorporating bacteria in the SCS. *Thalassobius* (α-Proteobacteria) were predominant at 200 m water depth in the central basin. Moreover, unexpectedly diverse MOH and MMA-incorporating bacterial groups were identified, which suggests a more important role in the transformation of C, compounds in marine environments than hitherto assumed.

**Methods**

**Sampling.** Seawater was collected from 5 m water depth (about 100 L total) at sites P1 and C3, 5 and 69 m (near the bottom) water depth at site S2 (200 L collected from each depth), and 5 and 200 m water depth at site B4 (100 L from each depth) by a SeaBird CTD (SBE 9/11 plus) equipped with 12 L Niskin bottles during two cruises in July–October 2013. Two litres of seawater for *in-situ* community DNA extraction were collected on a 0.2 μm filter (47 mm diameter, Millipore Sterivex filters, EMD Millipore Corp., Merck KGaA, Germany) at each of the sites. The filters were flash-frozen in liquid nitrogen for 10 min and subsequently stored at −80 °C until analysis. Seawater (500 mL) for *in-situ* microbial ^13^C content measurements was collected from sites P1 and S2 on 0.3 μm glass fibre filters (25 mm diameter, Advantec, Toyo Roshi Kaisha, Japan), which were pre-combusted for 4 h at 525 °C. All of the glass fibre filters were frozen at −20 °C until analysis.

**Biogeochemical parameters.** Temperature, salinity, and depth data were obtained from the CTD system. Water samples for inorganic nutrients were filtered through 0.45 μm cellulose acetate membranes and then analyzed onboard. Ammonium was analyzed by the indophenol blue spectrophotometric method. Nitrite, nitrate, and silicate concentrations were measured with a four-channel continuous flow Technicon AA3 Auto-Analyzer (Bran-Lube GmbH, Germany). The basic physical and chemical parameters were showed in Table S1.

**Field-based incubation experiments.** Seawater collected for incubation was immediately filtered through a 3 μm polycarbonate filter (254 mm diameter, Pall Life Science, USA) to remove macrophytoplankton and grazers. Twenty litres of filtered seawater were incubated in 20 L polycarbonate bottles, previously washed with a 10% HCl solution and filtered seawater. 99% ^13^C-labelled or ^12^C (light), respectively, present in DNA from each sample were selected for high-throughput DNA extraction, CsCl density gradient ultracentrifugation, and gradient fractionation. DNA was extracted using the phenol-chloroform-isooamyl alcohol method. Purified DNA was checked with a NanoDrop device (ND2000, Thermo Fisher Scientific, Inc., Waltham, MA, USA), and fluorometrically quantified using a Qubit dsDNA Assay Kit (Invitrogen, Thermo Fisher Scientific, USA) and Qubit 2.0 Fluorometer (Invitrogen, Life Technologies, Singapore). Ultracentrifugation was performed according to previous protocols with minor modifications. Briefly, about 3 μg of DNA from each sample was mixed with a gradient buffer containing 0.1 M Tris, 0.1 M KCl, and 1 mM EDTA, after which the mixture was added to a CsCl solution (1.89 g mL−1) to a final density of 1.725 g mL−1. The final solutions were poured into 5.1 mL ultracentrifuge tubes and centrifuged at 140,000 × g (~37,700 rpm) in a vertical rotor (VT6 56.2, Beckman Coulter, Inc., Brea, CA, USA) at 20 °C for 69 h under vacuum. After centrifugation, the solution was divided into 12 equal fractions as soon as possible and the densities of all fractions were determined as described by Zhang et al. DNA in each fraction was retrieved by adding two volumes of PEG solution (30% polyethylene glycol 6000, w/v, 1.6 M NaCl, and 20–40 μg of glycogen), resuspended in 35 μL of TE (10 mM Tris-HCl, 1 mM EDTA, pH 8.0), and then fluorometrically quantified.

**T-RFLP analyses.** Fractions were analysed by T-RFLP according to previous procedures. Bacterial 16S rRNA genes were PCR-amplified from each DNA fraction with primers 27F-FAM (5′-AGAGTTTGATCMTGGGCTCAC-3′, 5′ end-labelled with the dye carboxy fluorescein) and 927R (5′-ACGCGGTGTGCGGGCC-3′). PCR products were purified with the Agarose Gel DNA Extraction kit (Tiangen Biotech Co., Ltd., China). Purified PCR products were digested with FastDigest Mspl and Rsal (Thermo Fisher Scientific, Inc.) at 55 °C for 4–12 h. Digested products were purified by precipitation with ethyl alcohol and then resuspended in 20 μL of sterile deionised water. Purified products were then mixed with 0.5 μL of an internal standard and detected on an Applied Biosystems Automated 3730 DNA analyser (Applied Biosystems, USA). T-RFLP data were analysed with MegaBACE software (Amersham Biosciences Corp., USA). The fragment profiles of the 12 density gradient fractions from each incubation experiment, as well as community similarity between fractions, were analysed using Primer 5 software.

**High-throughput sequencing and statistical analysis.** Two fractions, corresponding to the ^13^C (heavy) and ^12^C (light), respectively, present in DNA from each sample were selected for high-throughput
sequencing. The V3–V4 hypervariable region of bacterial 16S rRNA genes was amplified with the universal primers 341F (CCTACGGGNGGCWGCAG) and 805R (GACTACHVGGGTATCTAATCC). Sequencing was carried out using an Illumina MiSeq platform at the Chinese National Human Genome Center (Shanghai, China).

Quality controlled sequences were analysed with the standard operating procedure using mothur software (www.mothur.org/wiki/ MiSeq_SOP). Briefly, the sequences were simplified to unique sequences with the unique.seqs command, and then aligned to the SILVA bacterial database using the align.seqs command. Then, screen.seqs and filter.seqs commands were applied to remove the sequences that had lengths outside the desired range and columns with only gaps. The sequences within 2 bp of difference to a more abundant sequence were merged with the pre.cluster command to reduce sequencing error. Chimeras were identified and removed using the chimera.uchime and remove.seqs commands, respectively. Finally, classify.seqs and remove.lineage commands were run to remove reads that were classified as “Cyanobacteria_Chloroplast”, “Mitochondria” or could not be classified at the kingdom level. The confidence cut-off was set to 80%. Sequences were further clustered into Operational Taxonomic Units (OTUs) with a cut-off value of 0.03. The taxonomy for each of the OTUs was detected by classify.otu. For further normalisation, sequences in all samples were rarefied and subsampled to an equal number using the sub.sample command. Bray-Curtis similarities were calculated based on OTU relative abundance matrices and the community similarity between fractions was analysed by nonmetric multidimensional scaling (NMDS) and dendrograms using Primer 5. One-way analysis of similarity (ANOSIM) with 999 permutations was performed to test the null hypothesis of no significant difference between clusters in the NMDS charts.

**Phylogenetic analysis.** Representative sequences of each OTU were selected out through the bin.seqs and get.otuREP commands for further phylogenetic analysis. Sequences were aligned and compiled using the MEGA7 program and the maximum likelihood phylogenetic tree was constructed.

**Microbial substrate assimilation.** The amount of organic carbon retained from each glass fibre filter was measured according to the following methods. Briefly, glass fibre filter samples were freeze-dried for at least 24 h, and then steamed in concentrated HCl for 48 h. Samples were placed in 5 × 9 mm tin cups and measured for particulate organic carbon (POC) in 1 L of incubated seawater (mPOC). Carbon isotopic compositions (δ13C) were measured with an elemental analyser coupled to a stable isotope ratio mass spectrometer (Flash EA 1112 HT-Delta V Advantage, Thermo). Two reference materials (Actanilide#1: δ13C = −29.53‰; Urea#2: δ13C = −8.02‰) were used to calibrate the δ13CPOC of the samples. The analytical precision for δ13CPOC was better than ±0.2‰. The mass of 13C in microbial biomass was calculated using the equations:

\[ R_{\text{sample}} = \left( \frac{\delta^{13}C_{\text{POC}}}{1000} + 1 \right) \times R_{\text{ref}} \]

\[ m^{(13)C} = \frac{13 \times m_{\text{POC}} \times R_{\text{sample}}}{13 \times R_{\text{sample}} + 12} \]

where \( m^{(13)C} \) is the mass of microbially assimilated 13C in 1 L of incubated seawater, \( R_{\text{sample}} \) is the atomic percent of 13C in the total organic carbon of the incubated sample, and \( R_{\text{ref}} \) is the atomic percent of 13C in the international reference material Vienna Pee Dee Belemnite (VPDB), and \( R_{\text{ref}} = 0.0112372 \).

In this study, there were two sources of 13C in the microorganisms, from the natural environment and supplemented by the 13C-substrate. The microbial assimilation rate of 13C from the supplementation into cells was calculated using the equation:

\[ \text{Assimilation rate (13C)} = \frac{m_{\text{sample}}^{(13)C} - m_{\text{blank}}^{(13)C}}{t} \]

where \( m_{\text{sample}}^{(13)C} \) and \( m_{\text{blank}}^{(13)C} \) is the microbially assimilated 13C mass in 1 L of incubated seawater from the 13C and 12C-substrate supplemented incubations, respectively, and \( t \) is the incubation time. The percentage of 13C in the POC pool was calculated using the equation:

\[ \text{Percentage of 13C in POC pool} = \frac{m_{\text{sample}}^{(13)C} - m_{\text{blank}}^{(13)C}}{m_{\text{POC}}} \]

where \( m_{\text{POC}} \) is the total mass of POC.

**Data Availability**

The sequencing data were deposited in NCBI Sequence Read Archive (SRA) with Accession Number SRP142566. The representative sequences used in the phylogenetic tree are under GenBank Accession Numbers MH121322 to MH121412.

**References**

1. Hines, M. E., Duddleston, K. N. & Kiene, R. P. Carbon flow to acetate and C1 compounds in northern wetlands. *Geophysical Research Letters* **28**, 4251–4254 (2001).
2. Jacob, D. J. Chemistry of OH in remote clouds and its role in the formation of formic acid and peroxymonosulfate. *Journal of Geophysical Research: Atmospheres* **91**, 9807–9826 (1986).
3. Jacob, D. J. Heterogeneous chemistry and tropospheric ozone. *Atmospheric Environment* **34**, 2131–2159 (2000).
4. Prather, M. J. & Jacob, D. J. A persistent imbalance in HOx and NOx photochemistry of the upper troposphere driven by deep tropical convection. *Geophysical Research Letters* **24**, 3189–3192 (2013).

5. Sargeant, S. L., Murrell, J. C., Nightingale, P. D. & Dixon, J. L. Seasonal variability in microbial methanol utilisation in coastal waters of the western English Channel. *Marine Ecology Progress Series* **550**, (2016).

6. Neufeld, J. D. et al. Stable-isotope probing implicates Methylophaga spp and novel Gammaproteobacteria in marine methanol and methylene metabolism. *The ISME Journal* **1**, 480–491 (2007).

7. Mincer, T. J. & Aicher, A. C. Methanol production by a broad phylogenetic array of marine phytoplankton. *PloS one* **11**, e0150820 (2016).

8. Heikes, B. G. et al. Atmospheric methanol budget and ocean implication. *Global Biogeochemical Cycles* **16** (2002).

9. Galbally, I. & Kirstüne, W. The production of methanol by flowering plants and the global cycle of methanol. *Journal of Atmospheric Chemistry* **43**, 195–229 (2002).

10. Dlugokencky, E. J., Nisbet, E. G., Fisher, R. & Lowry, D. Global atmospheric methane: budget, changes and dangers. *Philosophical Transactions of the Royal Society of London A: Mathematical, Physical and Engineering Sciences* **369**, 2058–2072 (2011).

11. Carpenter, L. J. et al. Uptake of methanol to the North Atlantic Ocean surface. *Global Biogeochemical Cycles* **18** (2004).

12. Williams, J. et al. Measurements of organic species in air and seawater from the tropical Atlantic. *Geophysical research letters* **31** (2004).

13. Gibb, S. W. & Hatton, A. D. The occurrence and distribution of trimethylamine-N-oxide in Antarctic coastal waters. *Marine Chemistry* **91**, 65–75 (2004).

14. Chistoserdova, L. et al. Genome of Methylobacillus flagellatus, molecular basis for obligate methylother, and polyphyletic origin of methylothery. *Journal of bacteriology* **189**, 4020–4027 (2007).

15. King, G. M. Ecophysiological characteristics of obligate methanotrophic bacteria and methane oxidation in situ. NASA (1993).

16. Naqvi, S. et al. Biogeochemical ocean-atmosphere transfers in the Arabian Sea. *Progress in Oceanography* **65**, 116–144 (2005).

17. Glover, H. E. Methylamine, an inhibitor of ammonium oxidation and chemooautotrophic growth in the marine nitrifying bacterium Nitrosococcus oceanus. *Archives of Microbiology* **132**, 37–40 (1982).

18. Taubert, M. et al. Methylamine as a nitrogen source for microorganisms from a coastal marine environment. *Environmental Microbiology* **19**, 2246–2257 (2017).

19. Flöret, A. M. Characterisation of novel methylophytes and the role of oxS in coastal marine environments, University of East Anglia (2017).

20. McDonald, I. R., Radajewski, S. & Murrell, J. C. Stable isotope probing of nucleic acids in methanotrophs and methylotrophs: a review. *Organic Geochemistry* **36**, 779–787 (2005).

21. Söhngen, N. Über bakterien, welche methan als kohlenstoffnahrung und energiequelle gebrauchen. *Zentralbl Bakteriol Parasitenk Infektionskr* **15**, 513–517 (1906).

22. Anthony, C. *Biochemistry of methylotrophs*. (Academic Press, 1982).

23. Chistoserdova, L. Kalyuzhnaya, M. G. & Lidstrom, M. E. The expanding world of methylorophic metabolism. *Annual review of microbiology* **63**, 477–499 (2009).

24. Chistoserdova, L. & Lidstrom, M. E. In *The prokaryotes*. Springer (2013).

25. Le Mer, J. & Roger, P. Production, oxidation, emission and consumption of methane by soils: a review. *European Journal of Soil Biology* **37**, 25–50 (2001).

26. Kuivila, K., Murray, J., Devol, A., Lidstrom, M. & Reimers, C. E. Methane cycling in the sediments of Lake Washington. *Limnology and Oceanography* **33**, 571–581 (1988).

27. Chistoserdova, L. Methylotrophs in natural habitats: current insights through metagenomics. *Applied Microbiology & Biotechnology* **99**, 5763–5779 (2015).

28. Sieburth, J. N. et al. The first methane-oxidizing bacterium from the upper mixing layer of the deep ocean: Methylomonas pelagica sp. nov. *Current Microbiology* **14**, 285–293 (1987).

29. Doronina, N., Krauzova, V. & Trossten, Y. A. Methylphaga limanica sp. nov.: a new species of moderately halophilic, aerobic, methylotrophic bacteria. *Microbiology* **66**, 434–439 (1997).

30. Schäfer, H., McDonald, I. R., Nightingale, P. D. & Murrell, J. C. Evidence for the presence of a CmuA methyltransferase pathway in novel marine methyl halide-oxidizing bacteria. *Environmental Microbiology* **7**, 839–852 (2005).

31. Radajewski, S., Ineson, P., Parekh, N. R. & Murrell, J. C. Stable-isotope probing as a tool in microbial ecology. *Nature* **403**, 646–649 (2000).

32. Dumont, M. G. & Murrell, J. C. Stable isotope probing—linking microbial identity to function. *Nature Reviews Microbiology* **3**, 499–504 (2005).

33. Ulhik, O., Jecná, K., Leigh, M. B., Macková, M. & Nisbet, E. G. Differential incorporation of carbon substrates among microbial populations identified by field-based, DNA stable-isotope probing in South China Sea. *PloS one* **11**, e0157178 (2016).

34. Buckley, D. H., Huangyuttitham, Y., Hsu, S.-F. & Nelson, T. A. Stable isotope probing with 15N achieved by disentangling the effects of genome G + C content and isotope enrichment on DNA density. *Applied and environmental microbiology* **73**, 3189–3195 (2007).

35. Neufeld, J. D., Chen, Y., Dumont, M. G. & Murrell, J. C. Marine methylotrophs revealed by stable-isotope probing, multiple displacement amplification and metagenomics. *Environmental microbiology* **10**, 1526–1535 (2008).

36. Morton, B. & Blackmore, G. South China Sea. *Marine Pollution Bulletin* **62**, 1226–1263 (2001).

37. McBeth, R. Ocean Data View, http://odv.awi.de (2017).

38. Hammes, F., Vital, M. & Egli, T. Critical evaluation of the volumetric “bottle effect” on microbial batch growth. *Appl Environ Microbiol* **76**, 1278–1281 (2010).

39. Nelson, C. E. & Carlson, C. A. Tracking differential incorporation of dissolved organic carbon types among diverse lineages of Sargasso Sea bacterioplankton. *Environmental Microbiology* **14**, 1500–1516 (2012).

40. Bischotterger, T., Cha, S. K., Schmitt, R., König, B. & Schmidt-Loerzen. W. The bacterial flora of non-carbonated, natural mineral water from the springs to reservoir and glass and plastic bottles. *International Journal of Food Microbiology* **73**, 65–75 (2004).

41. Bischofberger, T., Cha, S. K., Schmitt, R., König, B. & Schmidt-Loerzen, W. The bacterial flora of non-carbonated, natural mineral water from the springs to reservoir and glass and plastic bottles. *International Journal of Food Microbiology* **73**, 65–75 (2004).

42. Jürgens, K., Gasol, J. M. & Vaqué, D. Bacteria–flagellate coupling in microcosm experiments in the Central Atlantic Ocean. *Journal of Experimental Marine Biology & Ecology* **245**, 127–147 (2000).

43. Ferguson, R. L., Buckley, E. N. & Palumbo, A. V. Response of marine bacterioplankton to differential filtration and confinement. *Applied and Environmental Microbiology* **47**, 49–55 (1984).

44. Agis, M., Granada, A. & Dolan, J. R. A cautionary note: Examples of possible microbial community dynamics in dilution grazing experiments. *Journal of Experimental Marine Biology & Ecology* **341**, 176–183 (2007).

45. Zhang, R., Weinbauer, M. G., Tam, Y. K. & Qian, P. Response of bacterioplankton to a glucose gradient in the absence of lysis and grazing. *FEMS Microbiology Ecology* **85**, 443–451 (2013).

46. Allers, E. et al. Response of Alteromonadaceae and Rhodobacteriaceae to glucose and phosphorus manipulation in marine mesocosms. *Environmental Microbiology* **9**, 2417–2429 (2007).

47. Dixon, J. L., Beale, R. & Nightingale, P. D. Methyl alcohol uptake in northeast Atlantic waters. *The ISME Journal* **5**, 704 (2011).

48. Harder, W., Dijkhuizen, L. & Postgate, J. Strategies of mixed substrate utilization in microorganisms. *Philosophical Transactions of the Royal Society of London B: Biological Sciences* **297**, 459–480 (1982).
49. Sun, J. et al. One carbon metabolism in SAR11 pelagic marine bacteria. *PLoS One* **6**, e23973 (2011).
50. Grob, C. et al. Combining metagenomics with metaproteomics and stable isotope probing reveals metabolic pathways used by a naturally occurring marine methylophag. *Environmental microbiology* **17**, 4007–4018 (2015).
51. Nercissian, O., Noyes, E., Kalyuzhnaya, M. G., Lidstrom, M. E. & Chistoserdova, L. Bacterial populations active in metabolism of C1 compounds in the sediment of Lake Washington, a freshwater lake. *Applied and environmental microbiology* **71**, 6885–6899 (2005).
52. Dornonina, N., Kaparulina, E. & Trotsenko, Y. *In The Prokaryotes* 869–880 (Springer, 2014).
53. Boden, R. *Emended description of the genus Methylophaga Janvier et al. 1985. International journal of systematic and evolutionary microbiology* **62**, 1644–1646 (2012).
54. Arahal, D., Macián, M., Garay, E. & Pujalte, M. Thalassobius mediterraneus gen. nov., sp. nov., and reclassification of Ruegeria gelatinovorans as Thalassobius gelatinovorus comb. nov. *International journal of systematic and evolutionary microbiology* **55**, 2371–2376 (2005).
55. Neufeld, J. D., Boden, R., Moussard, H., Schäfer, H. & Murrell, J. C. Substrate-specific clades of active marine methylophag associated with a phytoplankton bloom in a temperate coastal environment. *Applied and environmental microbiology* **74**, 7321–7328 (2008).
56. Duan, Y. et al. Complete genome sequence of citrus huanglongbing bacterium, *Candidatus Liberibacter asiaticus* obtained through metagenomics. *Molecular Plant-Microbe Interactions* **22**, 1011–1020 (2009).
57. Cherrier, J., Bauer, J. & Druffel, E. Utilization and turnover of labile dissolved organic matter by bacterial heterotrophs in eastern North Pacific surface waters. *Marine Ecology Progress Series* **139** (1996).
58. Pai, S. C., Tsau, Y. J. & Yang, T. I. pH and buffering capacity problems involved in the determination of ammonia in saline water using the indophenol blue spectrophotometric method. *Analytica chimica acta* **434**, 209–216 (2001).
59. Han, A. et al. Nutrient dynamics and biological consumption in a large continental shelf system under the influence of both a river plume and coastal upwelling. *Limnology & Oceanography* **57**, 486–502 (2012).
60. Ma, W. & Tian, J. Modeling the contribution of dissolved organic carbon to carbon sequestration during the last glacial maximum. *Geo-Marine Letters* **34**, 471–482 (2014).
61. Goodwin, K. D., Varner, R. K., Crill, P. M. & Oremland, R. S. Consumption of tropospheric levels of methyl bromide by C1 compounds-utilizing bacteria and comparison to saturation kinetics. *Applied and environmental microbiology* **67**, 5437–5443 (2001).
62. Neufeld, J. D. et al. DNA stable-isotope probing. *Nature protocols* **2**, 860–866 (2007).
63. Hutchens, E., Radajewski, S., Dumont, M. G., McDonald, I. R. & Murrell, J. C. Analysis of methanotrophic bacteria in Movie Cave by stable isotope probing. *Environmental Microbiology* **6**, 111–120 (2004).
64. Lee, S.-H. et al. Identification of airborne bacterial and fungal community structures in an urban area by T-RFLP analysis and quantitative real-time PCR. *Science of the total environment* **408**, 1349–1357 (2010).
65. Chen, L., Lu, Y., Liu, Z., Xiao, S. & Li, D. Nocturnal community analysis using high-throughput sequencing. *Biodegradation* **27**, 47–57 (2016).
66. Kozich, J. J., Westcott, S. L., Baxter, N. T., Highlander, S. K. & Schloss, P. D. Development of a dual-index sequencing strategy and curation pipeline for analyzing amplicon sequence data on the MiSeq Illumina sequencing platform. *Applied and environmental microbiology* **79**, 5112–5120 (2013).
67. Song, Z. Q. et al. Bacterial and archaeal diversities in Yunnan and Tibetan hot springs, China. *Environmental microbiology* **15**, 1160–1173 (2013).
68. Hama, T. et al. Measurement of photosynthetic production of a marine phytoplankton population using a stable 13C isotope. *Marine Biology* **73**, 31–36 (1983).
69. Wegener, G. et al. Assessing sub-seafloor microbial activity by combined stable isotope probing with deuterated water and 14C bicarbonate. *Environmental Microbiology* **14**, 1517–1527 (2012).
70. Schimmelmann, A. et al. Nicotine, acetanilide and urea multi-level 13H-13C and 14N-abundance reference materials for continuous-flow isotope ratio mass spectrometry. *Rapid Communications in Mass Spectrometry* **23**, 3513–3521 (2009).
71. Lin, Y. S., Lipp, J. S., Elvert, M., Holler, T. & Hinrichs, K. U. Assessing production of the ubiquitous archaeal diglycosyl tetraetheter lipids in marine subsurface sediment using intramolecular stable isotope probing. *Environmental microbiology* **15**, 1634–1646 (2013).

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**Author Contributions**
YZ. conceived and designed the experiments. L.P. and W.D. performed the experiments. W.D., L.P., and Y.Z. analysed the data. Y.Z., W.D. and L.P. wrote the paper. N.J. contributed to the interpretation of results and critical revision. All authors were involved in approval of the final version.

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