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MASTERARBEIT

Titel der Masterarbeit

„Monitoring bacterial community shifts in a non-carbonated bottled mineral water after induction of volatile, methylated sulfur and selenium compound formation“

Verfasser

Claus Pelikan BSc

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Betreuer:	Ass.-Prof. Dipl.-Biol. Dr. Alexander Loy

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1. Introduction

1.1. A complex microbial community in still natural mineral waters

Mineral water is derived from protected groundwater sources, oligotrophic environments where the planktonic microbial cells are dead, dormant or severely starved (Egli, 2010). When the water is drawn from a well, filled into a bottle and stored at ambient temperature, microbiota start proliferating (Buttiaux and Boudier, 1960). Whereas freshly bottled mineral water contains up to 10^3-10^4 cells per mL of which only a minor fraction is metabolically active, total cell counts (TCC) are increasing to approximately 10^5 cells per mL upon storage for one week (Loy et al., 2005; Vachee et al., 1997).

Even though microbial growth in bottled mineral water is a well described phenomenon, the reasons for growth are still under debate. However, a variety of responsible factors was discussed by Leclerc and Moreau (2002). They argued, that the amount of available nutrients in the water bottle might be higher, due to an increase in temperature and dissolved oxygen concentration during and after the bottling process. Furthermore, it was hypothesized that nutrients are concentrated and readily degraded at the inner bottle surface. If bacteria are able to adhere to this surface, depending on other factors i.e. surface roughness, proliferation can occur. In addition, bacterial growth was found to be positively correlated with storage temperature and light exposure. The latter factor causes photodegradation of recalcitrant organic substances what can further increase nutrient availability.

The microbial communities that are developing in bottled mineral waters appear relatively complex. So far, most of our knowledge about the composition of these communities was obtained by employing culture dependent techniques (Bischofberger et al., 1990; Casanovas-Massana and Blanch, 2012; Falcone-Dias et al., 2012; Manaia et al., 1990; Morais and da Costa, 1990; Vaz-Moreira et al., 2011; Vachee et al., 1997). *Pseudomonas spp.* (*Gammaproteobacteria*) was isolated most frequently from different waters and therefore, it was assumed that pseudomonad species, common in the groundwater habitat and apparently preserved through the bottling system (Vachee et al., 1997), are important members of the bottled mineral water flora (Leclerc and Moreau, 2002). However, this concept has been challenged by Loy et al. (2005), who revealed, by the use of the culture independent full-cycle 16S rRNA approach (Amann et al., 1995), that the actively growing bacterial

community in a bottled mineral water was dominated by *Betaproteobacteria* while *Gammaproteobacteria* constituted only a minor fraction of the community. Although the observed differences are likely explained by the well-known biases of culture dependent techniques (Wagner et al., 1993), the dominance of *Betaproteobacteria* in other bottled mineral waters remains to be proven.

Usually, bacterial proliferation in bottled mineral water remains unnoticed by the consumer, however, in rare cases the bacteria tend to produce the methylated sulfur (S) and selenium (Se) compounds dimethyl sulfide (DMS), dimethyl selenide (DMSe), and their derivates dimethyl disulfide (DMDS), dimethyl trisulfide (DMTS) and dimethyl diselenide (DMDSe). These volatile organic S/Se compounds (VOSCs) are known flavor compounds of various foods like vegetables, truffles, cheese and honey and of drinks like beer, wine, juices and coffee (Schäfer et al., 2010). Furthermore DMS gives the sea its typical smell (Curson et al., 2011). Evidently, VOSC production in bottled mineral water is problematic, as it is associated with the development of unpleasant odours.

1.2. VOSC production processes in the environment: focus on freshwater systems

On a global scale the oceans are by far the most important source of DMS, as they account for 80 % of the total DMS flux from the sea to the air (Watts, 2000). In the marine environment bacteria degrade the algal osmoprotectant dimethyl-sulfoniopropionate (DMSP) and form DMS (Curson et al., 2011; Dickson et al., 1980; Yoch, 2002). This process is important for the global S cycle (Lovelock et al., 1972) and bacterial degradation of DMSP followed by a release of DMS was also reported from a freshwater ecosystem (Ginzburg et al., 1998). Nonetheless, the formation of DMS from DMSP might play a minor role in bottled mineral water, where heterotrophic bacteria are the dominating microorganisms and in general no algae are found (Leclerc and Moreau, 2002). Notwithstanding, major routes of VOSC formation in freshwater are the degradation of S/Se-containing amino acids and the methylation of inorganic S/Se compounds (Franzmann et al., 2001; Ginzburg et al., 1999; Ranjard et al., 2004). Any of these processes are possibly involved in VOSC production in bottled mineral water and are therefore discussed in the following.

The supplementation of L-methionine to drinking water stimulated the production of significant amounts of DMDS and DMTS (Franzmann et al., 2001). DMDS formation from L-methionine is a two-step process, where this compound is first degraded to methanethiol (MT), ammonium and

2-oxobutanic acid (Figure 1; reaction 1), a compound that is used for energy generation (Sato and Nozaki, 2009) and two MT molecules are subsequently oxidized to form DMDS (Figure 1; reaction 2). Alternatively, it was hypothesized that MT can be methylated to form DMS (Figure 1; reaction 3) by a reaction that requires a methylating agent (i.e. S-adenosylmethionine). However, according to the results from incubation experiments with *Acinetobacter lwoffii* cultures, DMS production from L-methionine is favored by lower oxygen concentrations (Ginzburg et al., 1999). DMTS formation in drinking water incubations with L-methionine, was explained by Franzmann et al. (2001) by the potential of this amino acid to methylate naturally occurring polysulfides (H_2Sn), according to Figure 2, reaction 4. These H_2Sn can be also formed directly through bacterial degradation of L-methionine (Ginzburg et al., 1999).

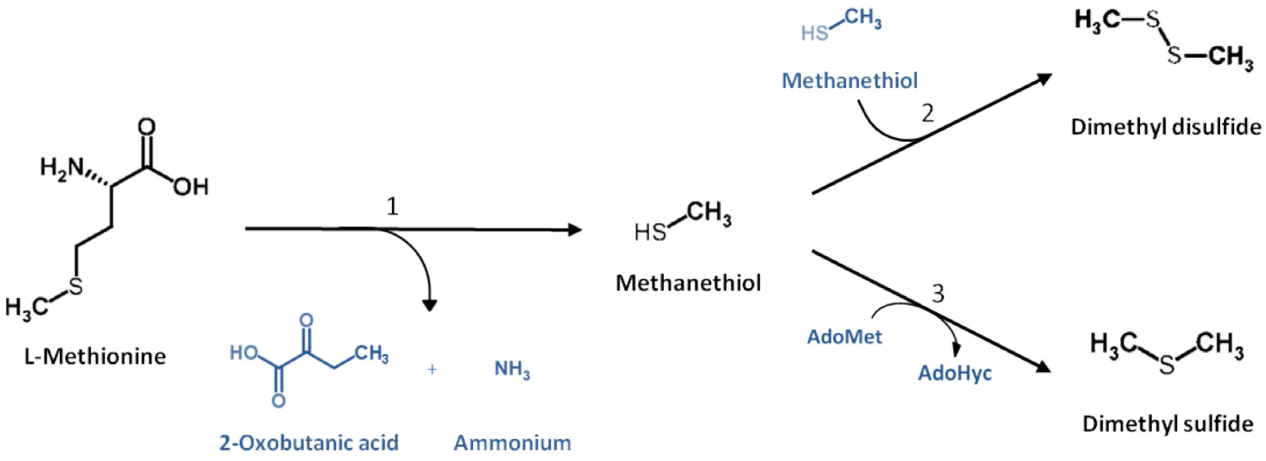

Figure 1. Reactions involved in degradation and volatilization of L-methionine. 1 Degradation of L-methionine to methanethiol, 2-oxobutanic acid and ammonium (Sato an Nozaki, 2009). **2** Oxidation of two methanethiol molecules to dimethyl disulfide (Segal and Starkey, 1969). **3** Methylation of methanethiol to dimethyl sulfide and demethylation of S-adenosylmethionine (AdoMet) to S-adenosylhomocysteine (Adohyc) (Drotar et al., 1987a).

Seleno-L-methionine, the Se-containing analogue of L-methionine, induced the production of DMDSe when added to drinking water (Franzmann et al., 2001). This can be explained by the fact that seleno-L-methionine is metabolized in a similar way to its S-containig analogue L-methionine (Doran and Alexander, 1977). However, instead of MT (Figure 1, reaction 1), methaneselenol is produced during seleno-L-methionine degradation (Esaki et al., 1979) and subsequent oxidation of two methaneselenol molecules results, according to Figure 1, reaction 2, in the formation of DMDSe (Doran and Alexander, 1977).

Drinking water incubations with L-cysteine produced DMDS and DMTS, but the yields were low (Franzmann et al., 2001). It was reviewed that the degradation of L-methionine and L-cysteine can happen in a similar way, with L-cysteine being degraded to hydrogen sulfide (H_2S), ammonium and pyruvate (Figure 2, reaction 1), which is, analogous to 2-oxobutanic acid, used for energy generation (Sato and Nozaki, 2009). Subsequent formation of DMDS from H_2S might happen through methylation of H_2S to form MT (Figure 2; reaction 2), which is followed by the oxidation of two MT molecules to form DMDS (Figure 1, reaction 2). However, H_2S is unstable under oxygen rich conditions (Ginzburg et al., 1999). Therefore VOSC production from L-cysteine might rather be the result of its degradation to H_2Sn (Figure 2, reaction 3), as shown for the aerobic freshwater bacterium *Acinetobacter lwoffii* (Ginzburg et al., 1999), followed by the methylation of these H_2Sn to form the dimethyl-polysulfides DMDS and DMTS (Figure 2, reaction 4).

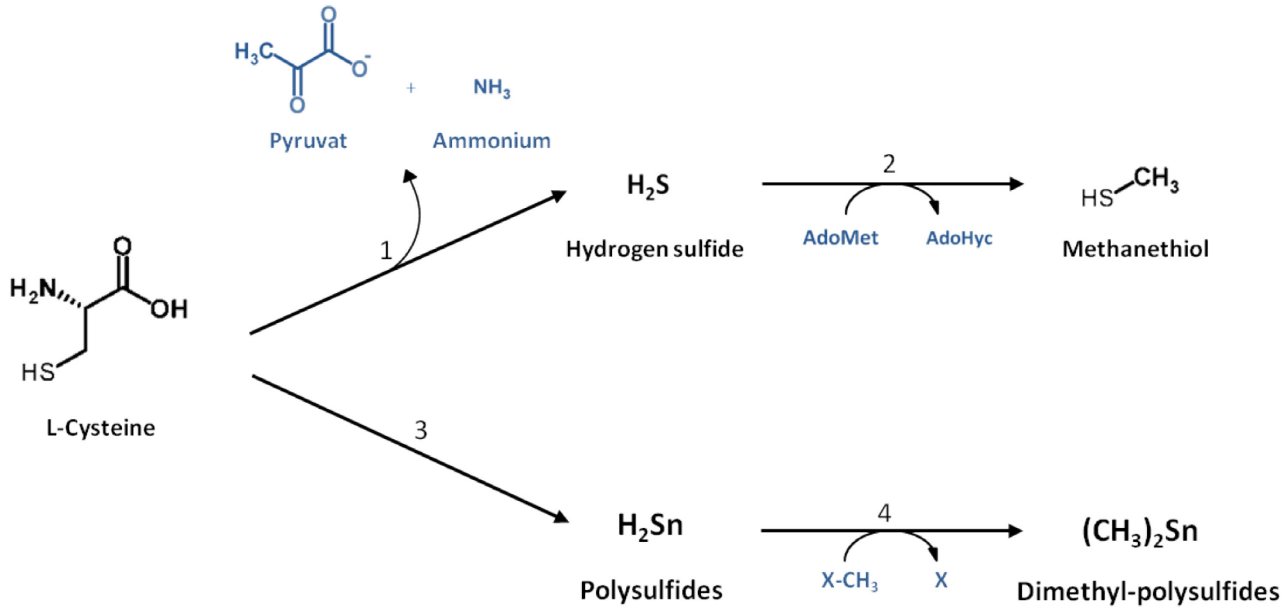

Figure 2. Reactions involved in degradation and volatilization of L-cysteine. 1 Degradation of L-cysteine to hydrogen sulfide, pyruvat and ammonium (Sato and Nozaki, 2009). **2** Methylation of hydrogen sulfide to dimethyl sulfide and demethylation of S-adenosylmethionine (AdoMet) to S-adenosylhomocysteine (Adohyc) (Drotar et al., 1987a). **3** Degradation of L-cysteine to polysulfides (Ginzburg et al., 1999). **4** Methylation of polysulfides to dimethyl-polysulfides by the aid of a methylating agent (i.e. methyl iodide) (Franzmann et al., 2001).

The amendment of methyl-selenocysteine, a compound consisting of a "CH_3Se - R" group attached to a L-cysteine molecule, to freshwater, stimulated the production of DMSe and DMDSe (Ranjard et al., 2004). This was explained by a reduction of methyl-selenocysteine into elemental Se or

methaneselenol followed by methylation of either one of these derivates to form DMSe and DMDSe. Nonetheless, the underlying processes were not discussed in detail.

Doran and Alexander (1977) found that the aerobic soil isolate *Corynebacterium sp.* was able to produce DMSe from the precursor seleno-L-cystine. They proposed that this compound is first reduced to seleno-L-cysteine, which is then metabolized, in a similar way like its S-containing analouge L-cysteine (Figure 2, reaction 1), to form hydrogen selenide, followed by the methylation of hydrogen selenide to form volatile DMSe.

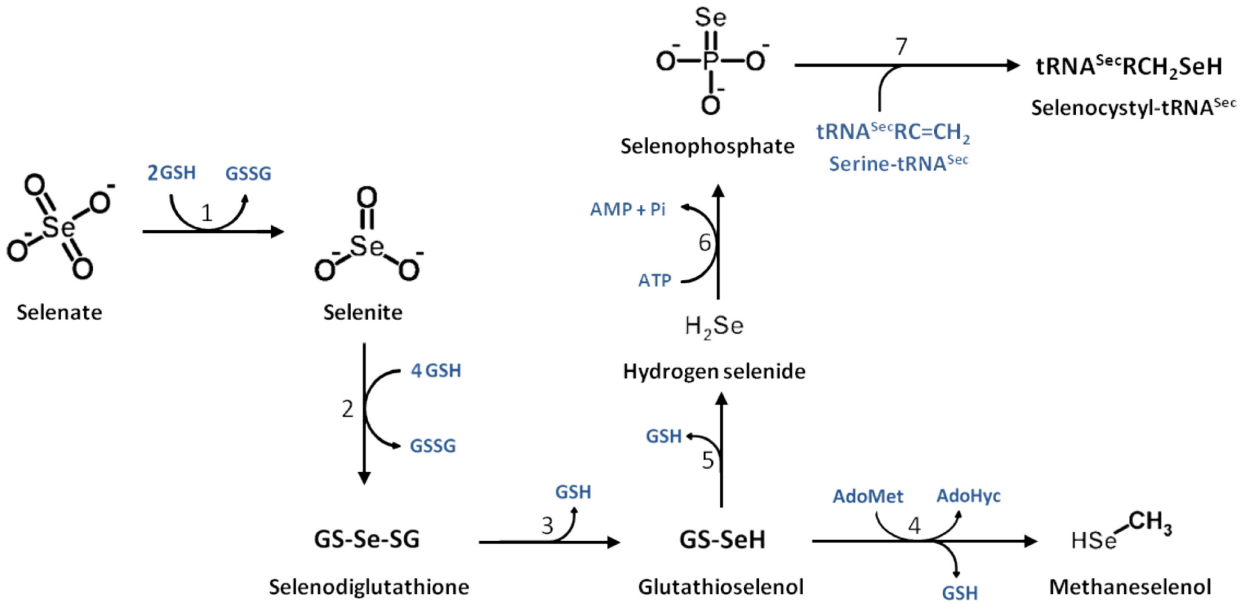

Figure 3. Reactions involved in volatilization of selenate and selenite (Figure modified from Turner et al., 1998). 1 Reduction of selenate to selenite. **2** Reduction of selenite to selenodiglutathione. **3** Reduction of selenodiglutathione to glutathioselenol. **4** Methylation of glutathioselenol to methaneselenol and demethylation of S-adenosylmethionine (S-adomet) to S-adenosylhomocysteine (S-adohyc). **5** Reduction of glutathioselenol to hydrogen selenide. **6** Production of selenophosphate. **7** Generation of selenocystyl-tRNA^{Sec} from serine-tRNA^{Sec}. GSH - reduced glutathione; GSSG - oxidized glutathione.

The inorganic Se compounds selenate (SeO_4^{2-}) and selenite (SeO_3^{2-}) are potentially toxic to microorganisms, as highly reactive oxygen species (H_2O_2 and O_2^-) are formed during their cellular metabolism, which can damage cell membranes and DNA (Bébien et al., 2002). Some bacteria are able to avoid damage by SeO_4^{2-} and SeO_3^{2-} through methylation, resulting in the production of volatile DMSe and DMDSe (Ranjard et al., 2003). The biochemical steps that are likely employed by bacteria (described for *Escherichia coli*) to metabolize these inorganic Se compounds, including the production of VOSCs, were previously described (Turner et al., 1998). In a first step SeO_4^{2-} and SeO_3^{2-} are

reduced and glutathioselenol is formed (Figure 3; reaction 1-3). Glutathioselenol is then either reduced to form hydrogen selenide (Figure 3; reaction 5) or methylated to form methaneselenol (Figure 3; reaction 4). Methaneselenol is subsequently oxidized to form DMDSe (Doran and Alexander, 1977) or methylated again to form DMSe (Drotar et al., 1987a). Reduced glutathione is found in several intermediate reactions of the described pathway (Figure 3), therefore Iodoacetate can inhibit the volatilization of SeO_4^{2-} and SeO_3^{2-}, as it reacts with reduced glutathione and forms inactive thioethers (Dickens, 1933). Alternatively, hydrogen selenide that is produced by reduction of glutathioselenol (Figure 3, reaction 5) could be activated by ATP to form selenophosphate (Figure 3, reaction 6). This compound is used to charge serine-$tRNA^{Sec}$ with Se to generate selenocystyl-$tRNA^{Sec}$ (Figure 3, reaction 7), which is subsequently incorporated into the amino acid seleno-L-cysteine (Böck et al., 1991). Seleno-L-cysteine or its derivates (i.e. seleno-L-methionine) might serve as precursors for VOSC production as described above. In order to test this hypothesis, Na-borohydride can be used, as it inhibits the activity of selenocysteine synthase, the enzyme that catalyzes the production of selenocystyl-RNA^{Sec} from serine-$tRNA^{Sec}$ (Forchhammer et al., 1991).

1.3. Aims of the study

The aims of the study were to (i) determine whether the production of VOSCs could be induced in bottled mineral water with the addition of selected organic and inorganic S/Se compounds and/or other substrates at different concentrations, (ii) reveal processes that could be involved in VOSC production through the use of inhibitors for specific metabolic pathways, (iii) investigate shifts in bacterial community composition that are induced under the different incubation conditions, and (iv) identify selectively enriched organisms that could be involved in the production of VOSCs.

2. Materials & Methods

2.1. Equipment, Software and Consumables

Table 1. Technical equipment used and corresponding software.

Machine	Software (Version)	Company
Milli Q water purification system	-	Millipore
3- Branch manifold 16828 (vacuum filtration unit)	-	Sartorius
Reusable funnel (500 mL stainless steel funnel)	-	Sartorius
Infinite® M200 (microplate reader)	i-controlTM (1.6)	Tecan
Axioplan 2 imaging (fluorescence microscope)	AxioVision (4.7)	Zeiss

Most of the equipment that was used in this study belongs to the Department of Microbial Ecology (University of Vienna). The Infinite® M200 - microplate reader that was used for fluorescence measurements belongs to the Department of Terrestrial Ecosystem Research (University of Vienna).

Table 2. Software used for data analysis.

Software (Version)	Reference
ARB (5.1)	(Ludwig et al., 2004)
DAIME (2.0)	(Daims et al., 2006)
Mothur (1.30.2)	(Schloss et al., 2009)
QIIME (1.7.0)	(Caporaso et al., 2010)
R (2.15.2)	(R Development Core Team, 2011)

Table 3. Consumables.

Consumable	Company
PCR soft tubes, 0.2 mL, colourless	Biozym
Omnifix® syringes, 50 mL	Braun
1.5 mL reaction tubes	Greiner Bio One
2 mL reaction tubes	Greiner Bio One
15 mL reaction tubes	Greiner Bio One
Microplate, 96 wells, black	Greiner Bio-One
Polycarbonate filter, diameter of 47 mm, pore size of 0.2 µM	Millipore
Lysis A matrix tubes	MP Biomedicals
Microscopic slides, standard	ROTH
Precision cover slip	ROTH
Cellulose nitrate membrane filter, diameter of 50 mm, pore size of 0.45 µM	Sartorius
Standard petri dish, diameter of 90 mm	Sterilin
Syringe filters, pore size of 0.2 µM	Thermo Scientific

2.2. Kits, Primers, Chemicals and Enzymes

Table 4. Molecular biology kits.

Kit	Company
Agencourt AMPure XP - PCR purification	Beckman Coulter
Quant-iT™PicoGreen®dsDNA reagent and kit	Invitrogen

Table 5. Primers used for first step PCR and barcoding PCR.

Name	Sequence	Reference
909F	5'-ACTCAAAKGAATWGACGG-3'	(Berry et al., 2011)
1492R	5'-NTACCTTGTACGACT-3'	(Berry et al., 2011)
Titanium B	5'-CTATGCGCCTTGCCAGCCCGCTCAGCCACTCAAAKGAATWGACGG-3'	(Berry et al., 2011)
Barcoding primers	5'-CGTATCGCCTCCCTCGCGCCATCAG--------TANTACCTTGTTACGACT-3'	(Berry et al., 2011)

The barcoded primers contain an 8 mer barcode, this is indicated by hyphens. A detailed list of the barcodes used in this study is given in Table S1.

Table 6. Chemicals and reagents.

Chemical Product	Company
Ethanol PRIMA 96 %	Australco
Agarose	Biozym
Citifluor AF1 antifadent solution	Citifluor Ltd
GeneRuler™ 100 bp plus DNA ladder	Fermentas
25 mM $MgCl_2$	Fermentas
10x Taq buffer with KCl and 15 mM $MgCl_2$	Fermentas
Aqua bidest. Fresenius (PCR water)	FRESENIUS KABI
4',6-Diamidino-2-phenylindole (DAPI)	Lactan
Ethanol ROTISOLV® HPLC gradient grade, min 99,9 %	Lactan
Formaldehyde solution 37 % (PFA)	ROTH
Roti® phenol/chloroform/isoamylalcohol, 25:24:1	ROTH
Tris-(hydroxymethyl)-aminomethan (Tris)	ROTH
Ethylendiamine tetraacetic acid disodiumsalt dihydrate (EDTA)	ROTH
Sodium dodecyl sulfate (SDS)	ROTH
Sodium chloride (NaCl)	ROTH
Hydrochloric acid 37 % (HCl)	ROTH
Sodium dihydrogen phosphate monohydrate ($NaH_2PO_4 \cdot H_2O$)	ROTH
Disodium hydrogen phosphate dihydrate ($Na_2HPO_4 \cdot 2H_2O$)	ROTH
Sodium acetate trihydrate	ROTH
Sodium hydroxide, ≥99 %, pearls (NaOH)	ROTH
Glycogen, RNA grade, 20 mg mL^{-1}	Thermo Scientific
dNTP Mix, 2 mM each	Thermo Scientific

Table 7. Enzymes.

Enzyme	Company
Taq DNA polymerase recombinant, 5 u μL^{-1}	Fermentas
Bovine serum albumin (BSA)	Invitrogen
Proteinase K, recombinant, PCR grade	Roche

Table 8. Chemicals for incubations.

Compound	Company	Catalogue Number
Sodium sulfate (Na-sulfate)	Roth	8560.3
Sodium sulfite (Na-sulfite)	Sigma-Aldrich	S4672
Sodium selenate (Na-selenate)	Sigma-Aldrich	71948
Sodium selenite (Na-selenite)	Sigma-Aldrich	S5261
L-cysteine	Roth	3467.2
Seleno-L-cystine	Sigma-Aldrich	545996
Se-(Methyl)selenocysteine hydrochloride (Methyl-selenocysteine)	Sigma-Aldrich	M6680
L-methionine	Roth	9359.1
S-(5'-adenosyl)-L-methionine iodide (S-adenosylmethionine)	Sigma-Aldrich	A4377
Seleno-L-methionine	Sigma-Aldrich	S3132
Sodium acetate (Na-acetate)	Sigma-Aldrich	S8750
Glucose	Roth	X997.2
Sodium iodoacetate (Na-iodoacetate)	Sigma-Aldrich	57858
Sodium borohydride (Na-borohydride)	Sigma-Aldrich	21346

2.3. Solutions and buffers

The solutions and buffers were prepared with water derived from a Milli-Q water purification system (MQ water). After preparation, all solutions and buffers used for DNA extraction and purification of barcoded 16S rRNA amplicons were autoclaved, with the exception of 10 % SDS, which was sterilized by filtration through a syringe in combination with a 0.2 µM pore-size syringe filter. The solutions used for mineral water incubations were sterilized by filtration as described above. Ethanol ROTISOLV® HPLC gradient grade was used pure (100 % EtOH) and in a 70 % dilution with autoclaved MQ water (70 % EtOH).

Table 9. Solutions used for cell fixation, total cell counts, DNA extraction and purification of barcoded 16S rRNA amplicons.

Solution	Chemical Substance	Final Concentration	Amount
3 % PFA	37 % PFA	3 % (w/v)	21.6 mL
	H_2O		add 200 mL
200 mM NaH_2PO_4 solution	$NaH_2PO_4 \cdot H_2O$	0.2 mol L^{-1}	13.8 g
	H_2O		add 500 mL
200 mM Na_2HPO_4 solution	$Na_2HPO_4 \cdot 2H_2O$	0.2 mol L^{-1}	17.8 g
	H_2O		add 500 mL
PBS stock solution pH of the NaH_2PO_4 solution was adjusted to 7.2-7.4 using the Na_2HPO_4 solution	200 mM NaH_2PO_4 solution	-	400 mL
	200 mM Na_2HPO_4 solution	-	add 500 mL
3M Sodium acetate pH was adjusted to 5.2 with HCl	Sodium acetate trihydrate	3 mol L^{-1}	102.1 g
	H_2O		add 250 mL
1 M Tris HCl pH was adjusted to 8.0 with HCl	Tris	1 mol L^{-1}	30.275 g
	H_2O		add 250 mL
0. 5 M EDTA pH was adjusted to 8.0 with NaOH	EDTA	0.5 mol L^{-1}	46.53 g
	H_2O		add 250 mL
5 M NaCl	NaCl	5 mol L^{-1}	73.05 g
	H_2O		add 250 mL
10 % SDS	SDS	10 % (w/v)	25 g
	H_2O		add 250 mL

Table 11. Buffers used for cell fixation, DNA extraction and purification of barcoded 16S rRNA amplicons.

Buffer	Chemical Substance	Final Concentration	Amount
1 x Phosphate buffered saline (PBS)	5 M NaCl	130 mmol L^{-1}	7.6 g
	PBS stock solution	-	50 mL
	H_2O		add 1 L
Lysis buffer	1 M Tris HCl	10 mmol L^{-1}	0.5 mL
	0. 5 M EDTA	1 mmol L^{-1}	0.1 mL
	5 M NaCl	100 mmol L^{-1}	1 mL
	10 % SDS	0.5 % (w/v)	2.5 mL
	H_2O		add 50 mL
TE buffer	1 M Tris HCl	10 mmol L^{-1}	0.5 mL
	0.5 M EDTA	1 mmol L^{-1}	1 mL
	H_2O		add 50 mL
Tris buffer	1 M Tris HCl	10 mmol L^{-1}	0.5 mL
	H_2O		add 50 mL

Table 10. Solutions used for incubations.

Compound	Final Concentration (mmol L^{-1})	Volume Prepared (ml)
Na-sulfate	1000	150.00
Na-sulfite	1000	250.00
Na-selenate	10	7.00
Na-selenite	10	25.00
L-cysteine	1000	13.00
Seleno-L-cystine	100	7.48
Methyl-selenocysteine	100	4.58
L-methionine	310	40.41
S-adenosylmethionine	10	0.95
Seleno-L-methionine	100	5.10
Na-acetate	1000	15.00
Glucose	1000	40.00
Na-iodoacetate	1000	24.00
Na-borohydride	1000	115.00

2.4. Amendment of compound combinations and storage

The non-carbonated mineral water that was used for the present study was filled into 0.5 L polyethylene terephthalate (PET) bottles. It had a pH of 7.4 and contained 83 mgL^{-1} of Ca^{2+}, 57 mgL^{-1} Cl^{-}, 414 mgL^{-1} HCO_3^{-}, 24 mgL^{-1} Mg^{2+}, 1 mgL^{-1} NO_3^{-}, 7 mgL^{-1} K^{+}, 114 mgL^{-1} Na^{+} and 136 mgL^{-1} SO_4^{2-}. Freshly bottled water was sent to us by our industrial cooperation partner on the 8th of March 2012. After the water arrived in Vienna, on the 9th of March 2012 (day 1 after bottling), a part of it was amended with 16 different compound combinations listed in Table 12. The amendment of compound combinations was performed by Claus Pelikan. The different S/Se compounds that were added to the water, were selected according to their potential to stimulate VOSC formation (see Introduction). These compounds included the S/Se-containing amino acids L-cysteine, seleno-L-cystine, methyl-selenocysteine, L-methionine and seleno-L-methionine, the methylating agent S-adenosylmethionine and the potentially toxic inorganic S/Se compounds Na-sulfite, Na-selenate and Na-selenite which might induce volatilization reactions (no evidence was found in the literature for Na-sulfite volatilization). The organic compounds glucose and Na-acetate as well as the inorganic compound Na-sulfate were added in order to stimulate the metabolic activity of the bottled water microbiota and to subsequently induce the conversion of naturally occurring VOSC precursors like L-methionine (Franzmann et al., 2001). Glucose was added together with Na-sulfite and Na-selenite as to increase methylation efficiency (Favre-Bonté et al., 2005). In order to test for pathways that are potentially

employed by bacteria for methylation of Na-selenite, the two inhibitors Na-iodoacetate and Na-borohydride were used (see Introduction). S-adenosylmethionine was added at one concentration, due to cost reasons, whereas the other compound combinations were added at two different concentrations. These concentrations were selected, according to concentrations that were used in previous supplementation experiments with freshwater and bacterial cultures isolated from freshwater (Favre-Bonté et al., 2005; Franzmann et al., 2001; Ranjard et al., 2003, 2003, 2004). In total 22 bottles (11 L) were amended with every compound combination in all designated concentrations. These bottles included duplicate samples (each sample consisted of 1 to 4 bottles) for TCC, 454 pyrosequencing analysis as well as for chemical and sensory analysis. The amount of water (L) that contained one compound at one concentration per respective analysis and sampling time point (day 1, day 3 or day 28) is indicated in Table S3. A part of the water was left untreated for TCC and as a negative control for 454 pyrosequencing analysis as well as for chemical and sensory analysis. The amount of untreated water that was used per respective analysis and sampling time point (day 1, day 3, day 6, day 8, day 14, day 28 and day 56) is indicated in Table S2. Bottles with and without amendment were stored at 20 °C in the dark.

Table 12. Compound combinations amended to bottled mineral water.

Compound Combination	Low Concentration	High Concentration
Na-sulfate	1.5 mM	10 mM
Na-sulfite	0.1 mM	10 mM
Na-selenate	0.1 µM	5 µM
Na-selenite	0.1 µM	5 µM
L-cysteine	10 µM	1 mM
Seleno-L-cystine	1 µM	50 µM
Methyl-selenocysteine	1 µM	35 µM
L-methionine	10 µM	1 mM
S-adenosylmethionine	0.5 µM	-
Seleno-L-methionine	1 µM	35 µM
Na-acetate	100 µM	1 mM
Glucose	100 µM	1 mM
Na-sulfite + Glucose	0.1 mM + 100 µM	10 mM + 1 mM
Na-selenite + Glucose	0.1 µM + 100 µM	5 µM + 1 mM
Na-selenite + Glucose + Na-iodoacetate	0.1 µM + 100 µM + 1 mM	5 µM + 1 mM + 1 mM
Na-selenite + Glucose + Na-borohydride	0.1 µM + 100 µM + 5 mM	5 µM + 1 mM + 5 mM

Values represent the final concentration in the 0.5 L PET bottles.

2.5. Sampling of bottled mineral water and planktonic microbiota

Water bottles with and without amendment were sampled according to Table S2 and Table S3. Sampling was performed by Celine Lesaulnier and Claus Pelikan.

2.5.1. Filtration

Filtration was done with a vacuum filtration unit consisting of three 500 mL stainless steel funnels in combination with an electronic evacuation pump. Before usage, the funnels and the filtration unit were sterilized with 70 % EtOH and flamed, and were allowed to cool down afterwards. A supportive cellulose nitrate membrane filter was dipped into sterile MQ water and placed between the funnel and the filtration unit. A sterile polycarbonate filter was dipped into sterile MQ water as well and placed on top of the supportive membrane filter. After filtration of the water the polycarbonate filter was removed from the system and placed in a standard petri dish for drying. Filters used for TCC were treated as described below and dry filters used for DNA extraction were cut in two halves, put into 15 mL reaction tubes and stored at -80 °C.

2.5.2. Cell fixation

Filters used for TCC were covered with 4 mL of 3 % PFA and incubated for 25 minutes. After removal of the solution the filters were washed twice with 4 mL 1× PBS for 3 minutes. Fixed filters were air-dried, cut in halves and stored in 15 mL reaction tubes at -20 °C.

2.6. Total cell counts

TCC were performed by Celine Lesaulnier and Claus Pelikan and were determined for the untreated water samples from day 1, day 3, day 6, day 8, day 14, day 28 and day 56 (Table S3). Small sections of PFA-fixed filters were cut on ice to avoid freeze/thaw cycles and put on microscopic slides afterwards. Each slide was covered with 200 µl DAPI working solution (concentration of 0.1 µg mL^{-1} dissolved in 1 x PBS) and stained for three minutes in the dark. DAPI was removed then and filter sections were washed twice with 200 µL 1 x PBS for three minutes in the dark. Afterwards, filters were air-dried, mounted with a drop of Citifluor AF1 antifadent solution and covered with a precision cover slip. The slides were either analyzed immediately or stored for short periods at 4 °C. Pictures were taken with a fluorescence microscope and the corresponding imaging software. After images were taken, filter sections were either removed or washed with ice cold MQ, air-dried and stored at -20 °C. Image analysis was performed with the software DAIME (Daims et al., 2006).

2.7. Chemical and sensory analysis

The chemical and sensory analysis was performed by our industrial cooperation partner at day 28 after bottling (27 days of incubation). Included in the analyisis were all incubations, except the incubations with Na-selenite & glucose & Na-borohydride, and three bottles of untreated water (Table S2 and Table S3). In order to quantify VOSC formation in the water, all samples were analyzed chemically by purge-and-trap gas chromatography-mass spectrometry (GC-MS). The sensory analysis was performed by specially trained personnel (5 to 7 independent assessors) who screened every sample for off-odour production and determined the intensity of the defect. According to the average off-odour intensity, every sample was categorized into one of the six possible levels of off-odour formation (no off-odour < low off-odour < medium off-odour < clear off-odour < strong off-odour < very strong off-odour). For all samples the concentrations of SeO_4^{2-}, SeO_3^{2-}, seleno-L-methionine and seleno-L-cystine were determined. Therefore, it was possible to quantify the uptake of these Se-containing compounds by bottled mineral water bacteria and their conversion into Se-containing VOSCs during the 27 days of incubation. The calculations were based on the concentrations (in µM) of residual, amended compounds and the concentrations (in µM) of produced DMSe/DMDSe in the liquid phase of each incubation. The concentration of VOSCs in the bottle headspace was not included in these calculations as it was assumed that the volume of the gas phase in each bottle is negligibly small compared to the volume of the liquid phase.

2.8. DNA isolation and bacterial 16S rRNA gene amplicon pyrosequencing

The bacterial community composition was analyzed according to a recently established 2-step 454 pyrosequencing protocol (Berry et al., 2011). DNA extraction was performed by Claus Pelikan. First step PCR was performed by Celine Lesaulnier. Barcoding PCR as well as purification, quantification and pooling of amplicons were performed by Claus Pelikan. Bioinformatic and statistical analysis was performed by David Berry. Included in the analysis were the samples taken from the incubations after 4 hours and after 27 days as well as the untreated water samples from day 1 and day 28 after bottling (Table S2 and Table S3).

2.8.1. DNA extraction

Filters with enriched planktonic microbiota were cut in smaller pieces with sterile scissors and transferred into 2 mL Lysis A matrix tubes without ¼ inch ceramic sphere. 400 µL of Lysis Buffer and

20 µg Proteinase K were added to each Lysis A matrix tube and the reactions were incubated at room temperature for 15 minutes. After that, 500 µL of phenol/chloroform/isoamylalcohol was added to each tube, followed by vortexing for 2 minutes at room temperature and subsequent centrifugation at approximately 18.000 g for 2 minutes at 4 °C. The aqueous phase of each sample was recovered and transferred into a sterile 2 mL reaction tube for a second round of phenol/chloroform/isoamylalcohol purification. The supernatant aqueous phase, recovered from the second round of purification was then precipitated with 2.5 x volume 100 % EtOH, 1 µL sodium acetate (3 M) and 1 µL glycogen RNA grade (20 mg mL^{-1}), followed by incubation for 2 hours at -20 °C. This was followed by centrifugation at approximately 18.000 g for 10 minutes at 4 °C for pelleting of the sample DNA. The DNA pellets were washed with ice cold 70 % EtOH and air-dried. Dry pellets were resuspended in 50 µL TE buffer and stored at -20 °C.

2.8.2. First step PCR

Table 13. Components in first step PCR.

Reagent	Amount (µL)
10x Taq buffer	2.5
dNTP mix	2.5
$MgCl^2$	1.5
Primer 909F, 50 pmol $µL^{-1}$	0.5
Primer 1492R, 50 pmol $µL^{-1}$	0.5
Taq DNA polymerase	0.2
BSA	0.1
Template DNA	1-2
PCR water	15.2-16.2
Total reaction volume	25

Table 14. First step PCR thermal cycling program.

Repeats	Temperature	Time (min)
1	94°C	03:00
25	94°C	00:40
	51°C	00:40
	70°C	01:00
1	70°C	07:00
1	4°C	∞

Bacterial 16S rRNA genes were amplified from the DNA extracts with PCR. The components in the PCR reaction and the thermal cycling program used, are described in Table 13 and Table 14, respectively. The primers 909F and 1492R, which were used for first step PCR, are shown in Table 5. BSA was added to the PCR reaction in certain cases in order to increase the PCR yields. If no BSA was used, the same volume of PCR water was added to the reaction instead. A DNA extract from an *E.coli* pure culture was amplified in parallel to the samples as a positive control. In addition PCR water was added to one of the PCR reactions instead of a DNA extract, to screen for possible contaminations. PCR products were analyzed by the use of agarose gel electrophoresis (1.5 % agarose gel, 125 V, 65 min). To every agarose gel a Generuler™ 100 bp plus DNA ladder was applied for size control of the amplicons. First step PCR products were stored at 4 °C.

2.8.3. Barcoding PCR

Table 15. Components in the bcPCR.

Reagent	Amount (µL)
10x Taq buffer	5
dNTP mix	4
$MgCl^2$	4
Titanium B, 100 pmol μL^{-1}	0.6
Barcoding primer, 50 pmol μL^{-1}	1
Taq DNA polymerase	0.2
BSA	0.1
Template DNA	2-6
PCR water	28.8 - 32.8
Total reaction volume	50

Table 16. bcPCR thermal cycling program.

Repeats	Temperature	Time (min)
1	94°C	03:00
5	94°C	00:40
	51°C	00:40
	70°C	01:00
1	70°C	07:00
1	4°C	∞

First step PCR products were used as a template for a barcoding PCR (bcPCR). The components in the bcPCR reaction and the thermal cycling program used, are described in Table 15 and Table 16,

respectively. For bcPCR the primer Titanium B and a set of barcoding primers was used (Table 5). The 8 mer barcode sequences of the barcoding primers are listed in Table S1. Depending on the concentration of the first step PCR amplicon, estimated via agarose gel electrophoresis, an appropriate amount was used for the bcPCR. One first step PCR product was amplified in duplicates with the same barcoding primer. For a negative control, PCR water was amplified instead of first step PCR product for a third reaction with the same barcoding primer. After amplification, all duplicate barcoded amplicons were analyzed by the use of agarose gel electrophoresis, as described above. Products of the barcoding PCR were stored at 4 °C.

2.8.4. Purification of amplicons

The barcoded amplicons were purified with the Agencourt AMPure XP PCR purification system. Purification was performed according to manufacturer's instructions, with the following modifications. The amplicons were purified in 0.2 mL PCR soft tubes and were allowed to air-dry for 15-20 minutes after washing with 70 % EtOH. After drying, the amplicons were resuspended in 20-30 µL Tris buffer instead of TE buffer, in order to not disturb downstream 454 pyrosequencing reactions. Duplicate amplicons were pooled in sterile 1.5 mL reaction tubes and stored at -20 °C.

2.8.5. Quantification of amplicons

The purified amplicons were quantified by the application of the Quant-iT™PicoGreen® dsDNA reagent and kit. Quantification was performed according to the manufacturer's instructions, with the following modifications. The assay was done in black 96 well microplates. Standards were applied in triplicate on the plate and had final concentrations of 1000, 800, 500, 200, 160, 80, 40, 20, 0 $ngmL^{-1}$. Samples were diluted 1:10 with 1 x TE buffer and applied once to the plate. In the end, each well was filled with 50 µL of diluted sample or standard. Another 50 µL of PicoGreen®dsDNA reagent was added on top of every well to a final volume of 100 µL. The fluorescence was measured with a microplate reader.

2.8.6. Pooling of amplicons

40 ng of every amplicon was combined to a final pool in a sterile 1.5 mL reaction tube (Table S4). One amplicon pool contained the amplicons from all mineral water incubations and had a final concentration of 6.15 $ng\mu L^{-1}$ in a volume of 780.5 µL. The amplicons derived from untreated water formed part of a second amplicon pool, which had a final concentration of 9.85 $ng\mu L^{-1}$ in a volume of

483 µL. 1000-1200 ng of each amplicon pool was sent to Eurofins MWG Operon (Ebersberg, Germany, http://www.eurofinsgenomics.eu/) for 454 pyrosequencing.

2.8.7. Data analysis

454 pyrosequencing data processing and quality control was done as described previously (Berry et al., 2011). Sequences were demultiplexed and clustered into phylotypes at 97 % sequence similarity using QIIME (Caporaso et al., 2010). Taxonomic affiliation of the sequences was done with the Ribosomal Database Project naïve Bayesian classifier (Wang et al., 2007). For identification of abundant phylotypes (greater than 1 % relative abundance in at least one sample), sequence similarity with full length 16S rRNA sequences of related type strains (Yarza et al., 2008) was calculated using the neighbor joining function of the ARB software (Ludwig et al., 2004). In order to find close relatives of non-abundant phylotypes that were identified during the indicator species analysis, described below, the Basic Local Alignment Tool (BLAST) was used (http://www.ncbi.nlm.nih.gov/BLAST/). Alpha- and beta-diversity metrics were calculated applying re-sampling (bootstrapping and jackknifing) at 100/1000 and 350 sequences, respectively, using QIIME (Caporaso et al., 2010). Statistical analysis was performed in the R software environment (R Development Core Team, 2011). Statistical significance of factors (e.g. days after bottling, production of VOSCs) was calculated using the nonparametric permutational multivariate ANOVA (permANOVA) with 1000 permutations (Anderson, 2001). An indicator species analysis was performed (Cáceres and Legendre, 2009), determining the strength of the association between phylotypes found in the samples and the production of VOSCs. Heatmaps were produced showing the relative abundance of abundant phylotypes. Correlations between the relative abundance of phylotypes and the concentrations of the five measured VOSCs were calculated, employing the rank abundance based spearman coefficient.

3. Results

3.1. Microbial growth in bottled mineral water

In order to determine the microbial growth profile, the total amount of cells in the mineral water was quantified at days 1, 3, 6, 8, 14, 28 and 56 after bottling. Growth occurred in the water after bottling and storage at ambient temperature (Figure 4). At day 1, 45 cells per mL were detected on average and no considerable increase in cell number was observed in the first three days. Then TCC dramatically increased from an approximate mean of 700 cells per mL at day 3 to about 10^5 cells per mL at day 6 and remained rather constant during the stationary growth phase.

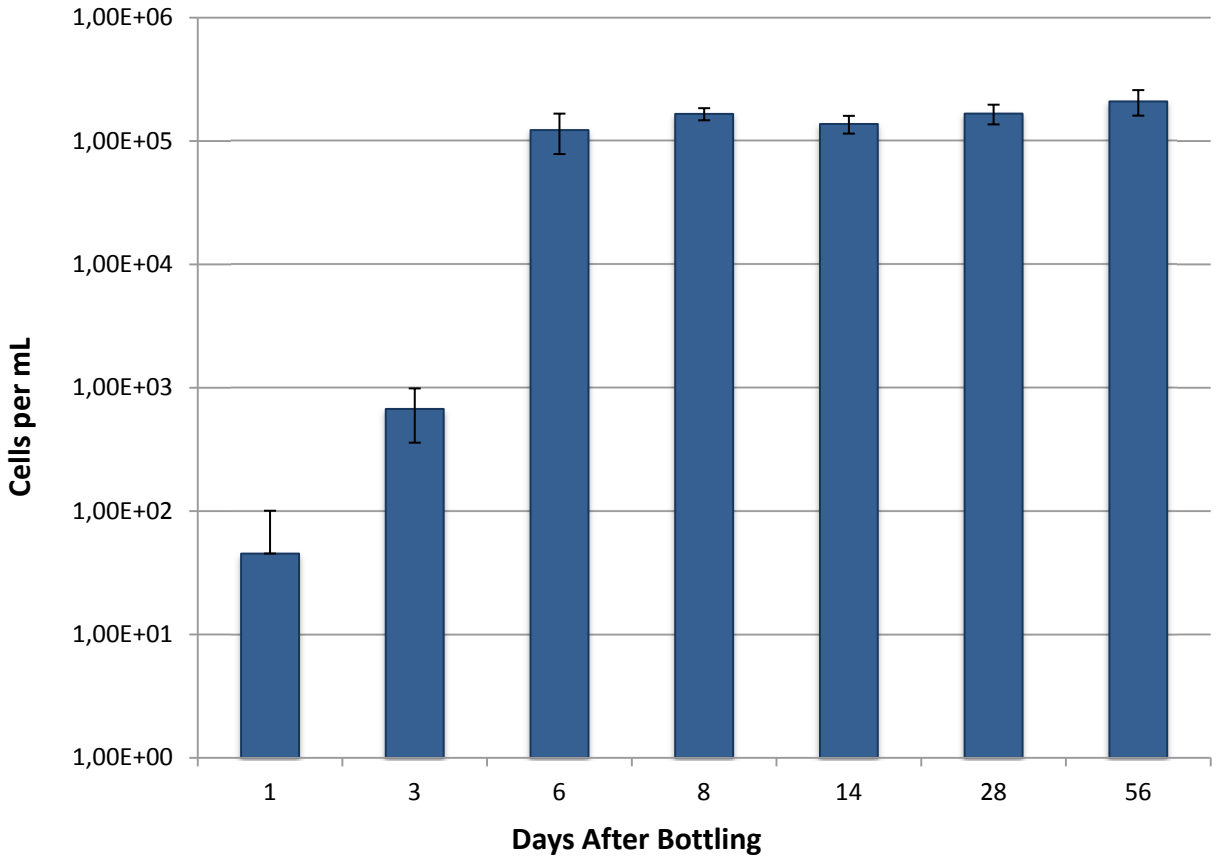

Figure 4. Growth of the planktonic microbial community of mineral water filled into 0.5 L PET bottles and stored at ambient temperature over 56 days post bottling. Mean values of total cell counts, quantified on replicate images from the same filter with planktonic biomass, are shown. Error bars show the standard deviation.

3.2. Production of volatile organic S/Se compounds and of off-odours in bottled mineral water incubated with different S/Se compounds and/or other substrates

Formation of VOSCs in the incubations and the untreated water was quantified at day 28 after bottling (27 days of incubation) via GC-MS. VOSC production was stimulated by all supplemented organic S/Se compounds, either at low, high or both concentrations, but only in few incubations with inorganic S/Se compounds (Figure 5). No VOSC formation was observed in the untreated water (Table S5).

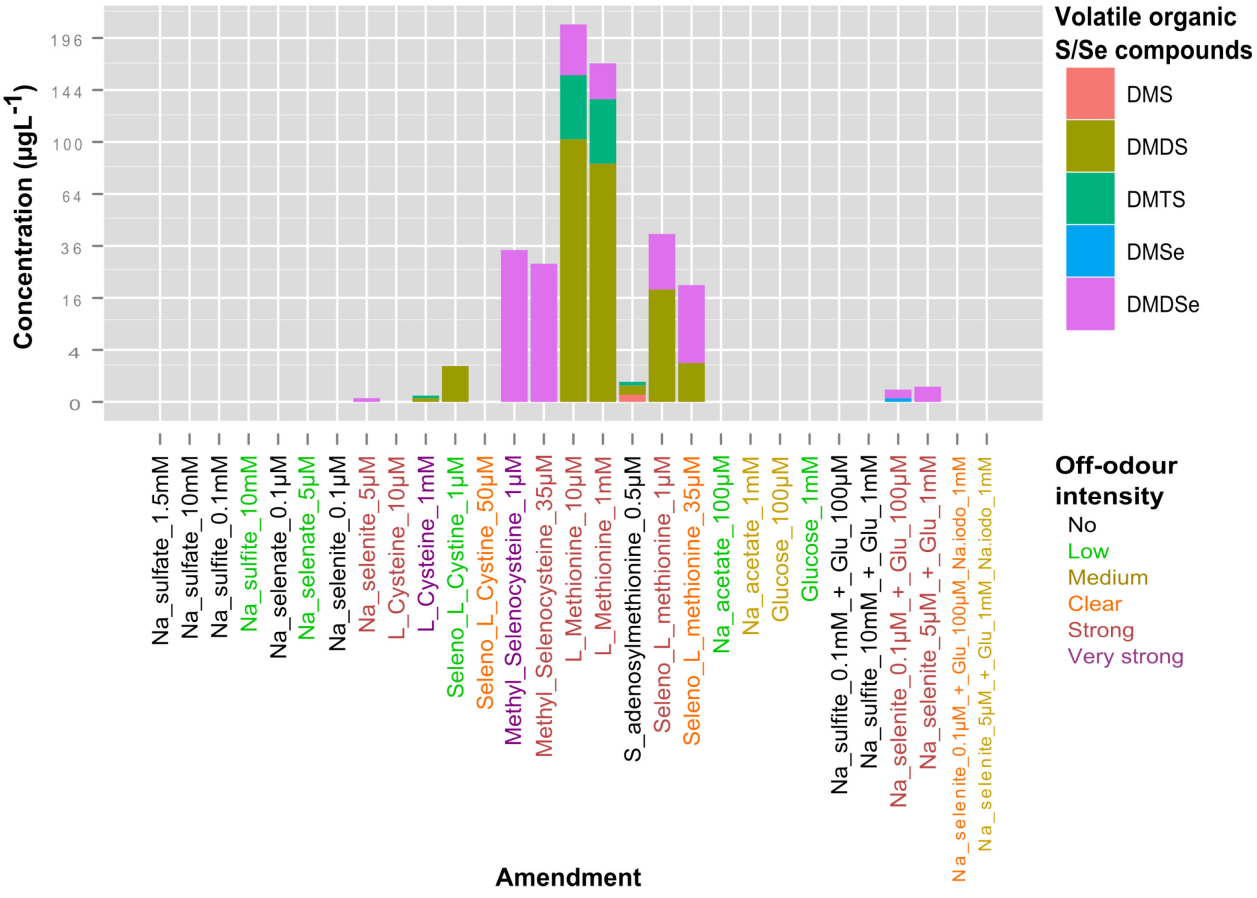

Figure 5. Concentrations of volatile organic S/Se compounds in bottled mineral water incubated for 27 days with different S/Se compounds and/or other substrates and development of off-odours. The means of volatile organic S/Se compound concentrations in duplicate water incubations are shown. Highest possible off-odour intensity detected in duplicate incubations with the same compound combination is indicated by the color of the x-axis lable.

In the incubations, supplemented with L-methionine relatively high amounts of DMDS and low amounts of DMTS and the Se-containing compound DMDSe were detected (Figure 5 and Table S5).

The concentration of DMDS ranged from 98.3 to 105.8 μgL^{-1} in the 10 µM incubations and was slightly lower, with 77.2 to 90.8 μgL^{-1}, in the 1 mM incubations. DMTS concentrations were similar in the two different incubations; yields ranged from 5.6 to 6.6 μgL^{-1} in the 10 µM incubations and from 5.8 to 6.5 μgL^{-1} in the 1 mM incubations. 2.8 to 4.8 μgL^{-1} DMDSe was detected in the 10 µM incubations with L-methionine, but only 1.9 μgL^{-1} DMDSe was detected in 1 mM incubations. Addition of seleno-L-methionine induced production of DMDSe and its S-containing analogue DMDS. In 1 µM incubations more DMDS than DMDSe was detected and concentrations ranged from 5.4 to 32 μgL^{-1} and 4 to 5.1 μgL^{-1}, respectively. In the 35 µM incubations DMDS concentrations were lower and ranged from 2 to 2.5 μgL^{-1}, whereas DMDSe concentrations were higher and ranged from 4 to 13.9 μgL^{-1}. The amendment of 0.5 µM S-adenosylmethionine stimulated the formation of 0.07 to 0.08 μgL^{-1} DMS, 0.09 to 0.15 μgL^{-1} DMDS and 0.01 and 0.02 μgL^{-1} DMTS.

In one of the duplicate incubations with 1 mM L-cysteine production of 0.2 μgL^{-1} DMDS and 0.1 μgL^{-1} DMTS was induced (Figure 5 and Table S5). No VOSC formation was observed in the incubations with 10 µM L-cysteine. In the methyl-selenocysteine incubations, DMDSe was detected. Concentrations ranged from 33.6 to 34.6 μgL^{-1} in the 1 µM incubations and from 26.4 to 30.1 ugL^{-1} in the 35 µM incubations. The amendment of seleno-L-cystine to the water resulted in the production of DMDS (1.9 $\mu gL^{-}1$), but only in one of the duplicate 1 µM incubations (Table S5).

0.02 $\mu gL^{-}1$ DMDSe was detected in one of the duplicate incubations with 5 µM Na-selenite, whereas the amendment with 0.1 µM Na-selenite had no effect on VOSC production (Figure 5 and Table S5). The addition of 100 µM glucose to the incubations with 0.1 µM Na-selenite stimulated the production of DMSe and DMDSe, at concentrations ranging from 0.01 to 0.03 μgL^{-1} and 0.9 to 0.12 μgL^{-1}, respectively. In the incubations with 5 µM Na-selenite & 1 mM glucose, the concentrations of DMDSe were increased, compared to the 5 µM incubations with Na-selenite, and ranged from 0.29 to 0.38 $\mu gL^{-}1$. The addition of 1 mM of the inhibitor Na-iodoacetate to the Na-selenite & glucose incubations suppressed the production of DMSe and DMDSe. No VOSC production was stimulated by the addition of Na-sulfate, Na-sulfite and Na-selenate as well as by the addition of the easily biodegradable compounds, glucose and Na-acetate.

Besides specific VOSC analyses, levels of off-odour formation were also evaluated at day 28 (27 days of incubation) by sensory analysis through trained personnel. Most of the compounds stimulated the production of off-odours, at least when they were added at high concentrations (Figure 5 and Table S6). However, the detected off-odours were not always linked to DMS, DMDS, DMTS, DMSe or

DMDSe production (e.g. L-cysteine). On the contrary, VOSCs were detected with the addition of S-adenosylmethionine though no off-odours were detected in the sensory analyses. Post 4 and 48 hours of incubation, off-odours were detected in incubations with L-methionine and seleno-L-methionine. As VOSC quantification was only performed after 27 days of incubation, there is no data available for these early time points.

Table 17. Total amounts of amended Se compounds taken up and amounts of volatile organic Se compounds produced by the microbiota of bottled mineral water during the 27 days of incubation.

Compound Combination	Amended (μML^{-1})	Measured (μML^{-1})	Uptake (%)	DMSe (μML^{-1})	DMSe (%)	DMDSe (μML^{-1})	DMDSe (%)
Na-Selenate	0.10	0.04	60.0	0.00	0.0	0.00	0.0
Na-Selenate	0.10	0.04	60.0	0.00	0.0	0.00	0.0
Na-Selenate	5.00	1.34	73.2	0.00	0.0	0.00	0.0
Na-Selenate	5.00	1.35	73.0	0.00	0.0	0.00	0.0
Na-Selenite	0.10	0.05	50.0	0.00	0.0	0.00	0.0
Na-Selenite	0.10	0.05	50.0	0.00	0.0	0.00	0.0
Na-Selenite	5.00	3.77	24.6	0.00	0.0	0.00	0.0
Na-Selenite	5.00	3.53	29.4	0.00	0.0	<0.01	<0.1
Seleno-Cystine	1.00	0.04	96.0	0.00	0.0	0.00	0.0
Seleno-Cystine	1.00	0.03	97.0	0.00	0.0	0.00	0.0
Seleno-Cystine	50.00	5.39	89.2	0.00	0.0	0.00	0.0
Seleno-Cystine	50.00	6.59	86.8	0.00	0.0	0.00	0.0
Seleno-Methionine	1.00	0.60	40.0	0.00	0.0	0.01	1.4
Seleno-Methionine	1.00	0.61	39.0	0.00	0.0	0.01	1.1
Seleno-Methionine	35.00	19.84	43.3	0.00	0.0	0.04	0.1
Seleno-Methionine	35.00	20.65	41.0	0.00	0.0	0.01	<0.1
Na-Selenite + Glucose	0.10	0.04	60.0	<0.01	0.1	<0.01	0.2
Na-Selenite + Glucose	0.10	0.04	60.0	<0.01	0.2	<0.01	0.3
Na-Selenite + Glucose	5.00	2.55	49.0	0.00	0.0	<0.01	<0.1
Na-Selenite + Glucose	5.00	2.22	55.6	0.00	0.0	<0.01	<0.1
Na-Selenite + Glucose + Na-Iodoacetate	0.10	0.05	50.0	0.00	0.0	0.00	0.0
Na-Selenite + Glucose + Na-Iodoacetate	0.10	0.04	60.0	0.00	0.0	0.00	0.0
Na-Selenite + Glucose + Na-Iodoacetate	5.00	2.56	48.8	0.00	0.0	0.00	0.0
Na-Selenite + Glucose + Na-Iodoacetate	5.00	2.63	47.4	0.00	0.0	0.00	0.0

The concentrations of amended compounds were not measured, but represent the concentrations that were theoretically amended to the water. The uptake of Se compounds (%) was calculated from the concentrations in which the compounds were amended to the water and the concentrations of these compounds in the water after 27 days of incubation. Total amounts of produced DMSe and DMDSe (%) were calculated from the concentrations in which the compounds were amended to the water and the concentrations of the respecitve volatile Se compounds in the water after 27 days of incubation.

The concentrations of SeO_4^{2-}, SeO_3^{2-}, seleno-L-cystine and seleno-L-methionine were determined for all 27-days incubations (Table S7). Based on these values, the uptake of Se-containing compounds from the water was calculated (Table 17). Significant amounts (25 to 97 %) of Se compounds were taken up by bacteria in all incubations with Na-selenate, Na-selenite, seleno-L-cystine and seleno-L-methionine. Nonetheless, no VOSCs were detected in most of these incubations or the fraction of Se

compound that was volatilized was very low (DMSe 0.1 to 0.2 %; DMDSe <0.1 % to 1.4 %). In the incubations with seleno–L-cystine, uptake was highest with 87 to 97 %, but no VOSCs were detected.

3.3. Bacterial community shifts in bottled mineral water induced by the amendment with different S/Se compounds and/or other substrates

The bacterial community composition of the untreated water and all incubations was analyzed at day 1 after bottling (4 hours of incubation) and at day 28 after bottling (27 days of incubation) with a recently established 2-step 454 pyrosequencing approach targeting the bacterial 16S rRNA gene (Berry et al., 2011). DNA was extracted from duplicate filters with planktonic biomass from incubated or untreated water samples. 16S rRNA genes were amplified from every DNA extract and amplicons were barcoded from the reverse end. After sequencing and quality control, 415,810 16S rRNA gene sequences were obtained with an average of 3,300 sequences per sample (Table S8 and Table S9). No sequences were obtained from one of the duplicate 0.1 µM Na-selenite & 100 µM glucose incubations. High quality sequences from the entire dataset were clustered on a 97 % identity level into 1,118 phylotypes of which 63 were abundant (higher than 1 % relative abundance in at least one sample). Abundant phylotypes that could be related to type strains (≥ 97 % 16S rRNA gene sequence similarity) are shown in Table 18. During storage of the water with and without amendment, the number of observed and estimated phylotypes (resampling at 100/1000 sequences) was reduced from approximately 20/79 observed and 48/142 estimated phylotypes at day 1 to an approximate mean of 8/26 observed and 14/45 estimated phylotypes at day 28 (Table S8 and Table S9). Accordingly, the alpha-diversity measures and the equitability (resampling at 100/1000 sequences) of the samples were reduced between day 1 and day 28. The Shannon index was reduced from approximately 2.8/3.3 to 1.5/1.7, the Simpson index was reduced from an approximate mean of 0.76/0.78 to 0.52/0.52 and the equitability was reduced from about 0.66/0.52 to 0.51/0.35. Good's coverage slightly increased from approximately 0.876/0.960 at day 1 to an approximate mean of 0.960/0.988 at day 28.

The beta-diversity plot shows that planktonic bacterial communities, found in the incubations and in the untreated waters strongly clustered by storage time i.e. days after bottling (Figure 6). This was supported by the permANOVA, which revealed that 56 % of the variance in phylotype diversity could be explained by time after bottling (Table S10). Most of the day 1 samples (4-hours incubations and untreated water) clustered together (Figure 6). However, three day 1 samples, obtained from untreated water in the year 2011 and shown here for comparison, were closely related to samples

from day 28. Furthermore one of the 4-hours incubation with 1 mM Na-acetate did not cluster together with the other samples from 2012 at day 1.

Table 18. Taxonomy, next relatives and mean relative abundance of depicted abundant phylotypes (relative abundance above 1 % in at least one sample) detected in bottled mineral water incubations with different S/Se compounds and/or other substrates or in bottled mineral water without amendment.

Phylotype	Phylum/Class	Species	Accesion number	Similarity	Mean abundance (%)
p1494	*Alphaproteobacteria*	Magnetospirillum gryphiswaldense	AM085146	100.0%	2,9
p8915		Caulobacter segnis	CP002008	100.0%	2
p6572		Methylobacterium populi	CP001029	99.5%	0,2
p2977		Novosphingobium taihuense	AY500142	99.5%	0,1
p4795		Hyphomicrobium zavarzinii	Y14305	97.8%	0,1
p4048		Caulobacter sp. strain FWC38	AJ227774	98.7%	<0,1
p3209		Brevundimonas vesicularis	EU024137	100.0%	<0,1
p7475		Paracoccus seriniphilus	AJ428275	100.0%	<0,1
p7607		Phenylobacterium koreense	AB166881	97.2%	<0,1
p7802		Agrobacterium tumefaciens	AJ389904	100%	<0,1
p388		Hyphomicrobium zavarzinii	Y14305	98.90%	<0,1
p2289		Caulobacter sp. strain FWC38	AJ227774	98.0%	<0,1
p8635		Alfipia birgiae	AF288304	98.7%	<0,1
p6525	*Betaproteobacteria*	Curvibacter fontana	AB120963	100.0%	18,4
p8483		Aquabacterium parvum	AF035052	100.0%	14,8
p2380		Acidovorax facilis	AF078765	100.0%	3,2
p1685		Undibacterium parvum	AM397629	98.7%	1,9
p2483		Undibacterium pigrum	AM397630	99.0%	1,4
p4629		Methyloversatilis universalis	DQ442273	99.7%	0,3
p7869		Curvibacter fontana	AB120963	100.0%	0,2
p3840		Curvibacter fontana	AB120963	100.0%	0,1
p2964		Limnohabitans australis	FM178226	98.8%	<0,1
		Variovorax paradoxus	AB008000	98.8%	
		Variovorax boronicumulans	AB300597	98.8%	
p4757		Limnohabitans australis	FM178226	98.8%	<0,1
		Variovorax paradoxus	AB008000	98.8%	
		Variovorax boronicumulans	AB300597	98.8%	
p7126		Albidiferax ferrireducens	AF435948	99.4%	<0,1
		Rhodoferax ferrireducens	CP000267	99.4%	
p6328	*Gammaproteobacteria*	Pseudomonas borbori	AM114527	98.5%	18,4
		Pseudomonas stutzeri	AJ308315	98.5%	
p8404		Serratia quinivorans	AF286867	99.8%	16,9
p4236		Leclercia adecarboxylata	JN175338	99.7%	3,6
p4589		Acinetobacter bouvetii	HQ180181	100.0%	2
p4680		Aeromonas caviae	AB626132	99.5%	1,7
		Aeromonas hydrophila subsp. anaerogenes	FR870443	99.5%	
		Aeromonas aquariorum	EU085557	99.5%	
p3868		Pseudomonas borbori	AM114527	98.5%	0,4
		Pseudomonas stutzeri	AJ308315	98.5%	
p7078		Enterobacter amnigenus	AB004749	99.2%	0,2
p1872		Leclercia adecarboxylata	JN175338	99.7%	0,1
p7058		Yersinia frederiksenii	AALE02000076	99.4%	0,1
p8159		Acinetobacter towneri	HQ180191	98.9%	0,1
p3198		Enhydrobacter aerosaccus	AJ550856	100.0%	0,1
p8934		Xanthomonas oryzae	X95921	99.5%	<0,1
p394		Pseudomonas borbori	AM114527	98.5%	<0,1
		Pseudomonas stutzeri	AJ308315	98.5%	
p8154	Bacilli	Streptococcus gallinaceus	AJ307888	99.5%	<0,1

Identification was based on 16S rRNA gene sequence similarity with related type strains (Yarza et al., 2008) using the software ARB (Ludwig et al., 2004). Only these phylotypes are shown that had a sequence similarity of ≥97 %.

In general the day 28 samples (27-day incubations and untreated water) were more dispersed over the beta-diversity plot. This was consistent with the permANOVA, which revealed that 47 % of the

observed variance in phylotype diversity at day 28 could be explained by the type of amendment (Table S11). Still, the majority of day 28 samples clustered together and was closely related to various real DMS/DMSe cases, which are colored differently from day 1 and day 28 samples (Figure 6).

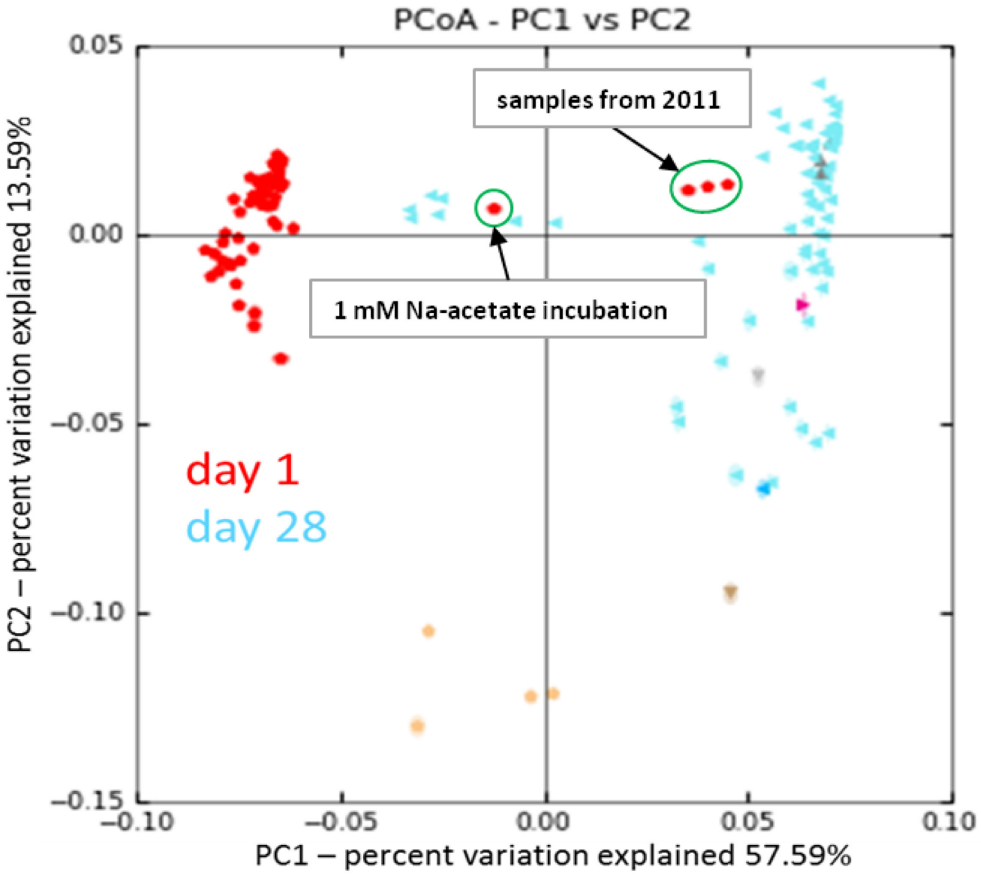

Figure 6. Similarity between planctonic bacterial communities detected in bottled mineral water incubations with different S/Se compounds and/or other substrates and in bottled mineral water without amendment. Sampling was performed after 4 hours (day 1 after bottling) and after 27 days (day 28 after bottling) of incubation. Weighted UniFrac values were calculated using bootstrapping at 350 sequences per sample. The communities are colored according to days after bottling. Samples with a different bottling time than day 1 or day 28 derive from bottles with water classified as "DMS/DMSe case". Those and samples of the year 2011 are shown for comparison. Each dot represents the bacterial community of an individual sample.

Shifts in bacterial community composition induced by incubation of the water with different S/Se compounds and/or other substrates were analyzed by bacterial community profiling of all incubations after 4 hours (day 1) and after 27 days (day 28) and comparison of these profiles to that of the untreated water at day 1 and at day 28 after bottling. At day 1 the untreated water was predominated by *Gammaproteobacteria*, with relative abundances ranging from 78.7 to 83 % (Figure 7 and Table S12). Representatives of the bacterial phyla/classes *Betaproteobacteria* (2.2 to 7.4%),

Alphaproteobacteria (2.6 to 2.9 %), *Nitrospirae* (1.4 to 2.3 %), *Actinobacteria* (1 to 1.3 %) and *Cyanobacteria* (0.5 to 1.3 %) were also found in the water and 4.5 to 4.7 % of all phylotypes could not be affiliated to any known bacterial phyla/class. *Gammaproteobacteria* were predominantly represented by phylotypes related to *Pseudomonas sp.* (p6328), *Serratia quinivorans* (p8404), *Leclercia adecarboxylata* (p4236), *Acinetobacter bouvetii* (p4589) and *Aeromonas sp.* (p4680), at respective relative abundances of 27.3 to 33.5 %, 31.1 to 34.9 %, 7.7 to 8.4 %, 2.12 to 2.24 % and 2.7 to 3.5 % (Figure 8 and Table S13). The only *Betaproteobacteria* affiliated phylotype that was abundant in both replicates of the untreated water was closely related to the species *Undibacterium pigrum* (p2483), and comprised 1.3 to 1.7 %, of the bacterial community. The even smaller fraction of *Alphaproteobacteria* was primarily represented by the *Methylobacterium populi* related phylotype p6572 that was found at a relative abundance of 1.6 to 2.1 %.

After 4 hours of incubation no major shifts in bacterial community composition were observed in the majority of incubations and like in the untreated control, representatives of the class *Gammaproteobacteria* comprised the largest fraction of the community (47.4 to 94.6 %) (Figure 7 and Table S12). Accordingly the phylotypes related to *Pseudomonas sp.* (p6328), *Serratia qinivorans* (p8404), *Leclercia adecarboxylata* (p4236) and *Aeromonas sp.* (p4680) contributed substantially to a gammaproteobacterial predominance in the 4-hours incubations, with relative abundances ranging from 19.3 to 46.5 %, 16 to 44.5 %, 2.7 to 20.2 % and 1.8 to 5.2 %, respectively (Figure 8 and Table S13). In contrast to the untreated water, the relative abundance of *Acinetobacter bouvetii* phylotype p4589 was below 1 % in most of the incubations, but was highest in one of the duplicate 0.1 mM Na-sulfite incubations (9.7 %). Only 1.6 to 4.7 % of all phylotypes found in the 4-hours incubations were affiliated to the class *Betaproteobacteria*, with the exception of an individual 1 mM Na-acetate incubation, where representatives of this class comprised 41.4 % (Table S12). *Betaproteobacteria* were predominantly represented by the *Undibacterium pigrum* related phylotype p2483 that was found, according to the untreated control in all incubations at a relative abundance of 1.1 to 3.8 % (Table S13). However, in the 1 mM Na-acetate incubation with higher relative abundance of *Betaproteobacteria*, the *Curvibacter fontana* related phylotype p6525 and *Aquabacterium parvum* related phylotype p8483 comprised 18 % and 11.8 % of the bacterial community, respectively.

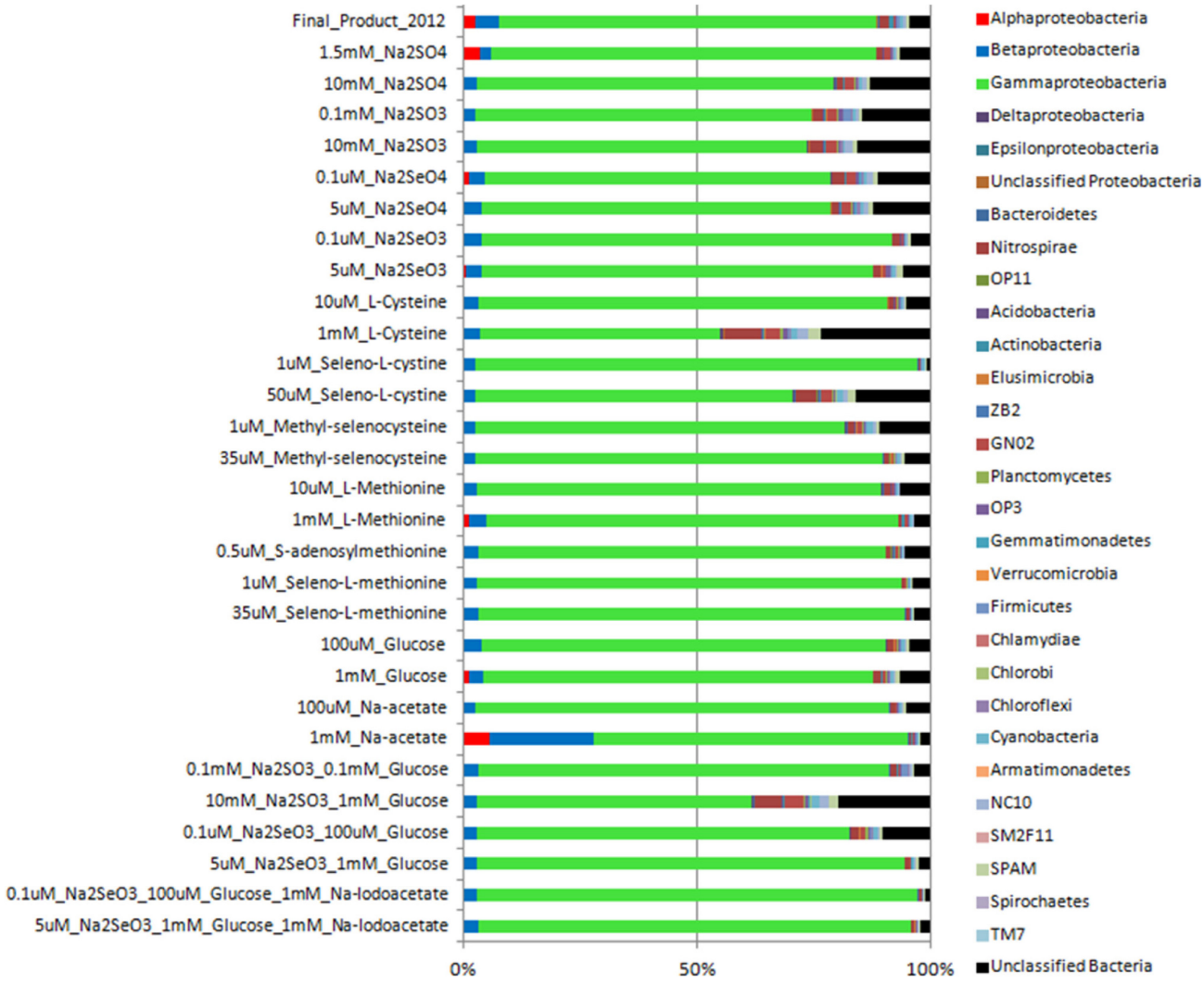

Figure 7. Relative abundance of major bacterial phyla/classes in bottled mineral water incubated for 4 hours (day 1 after bottling) with different S/Se compounds and/or other substrates and in bottled mineral water without amendment (Final_Product_2012) at the first day after bottling. Mean values of replicates are shown.

In contrast to the untreated control, *Alphaproteobacteria* were not abundant in most of the incubations (Figure 7), but representatives of this class accounted for 7.2 %, 2.5 %, 1.3 %, 2.5 % and 2.3 % in individual incubations with 1.5 mM Na-sulfate, 0.1 µM Na-selenate, 5 µM Na-selenite, 1 mM L-methionine and 1 mM glucose, respectively and comprised 5.2 to 5.8 % of the bacterial community in the individual 1 mM Na-acetate incubation (Table S12). This observation corresponded to a relative abundance of 4.6 % of *Magnetospirillum gryphiswaldense* phylotype, p1494 in one of the duplicate 1 mM Na-acetate incubations and to relative abundances of 2.4 %, 1.3 %, 2.5 %, 5.3 % and 2.2 % of the *Methylobacterium populi* phylotype, p6572 in the respective individual incubations with 0.1 µM Na-selenate, 5 µM Na-selenite, 1 mM L-methionine, 1 mM Na-acetate and 1 mM glucose (Table S13). An

increase in unclassified bacteria was observed in a variety of incubations and was most pronounced in 1 mM L-cysteine and 10 mM Na-sulfite & 1 mM glucose incubations, with respective relative abundances ranging from 21.4 to 24.8 % and 18.3 to 21.1 % (Table S12).

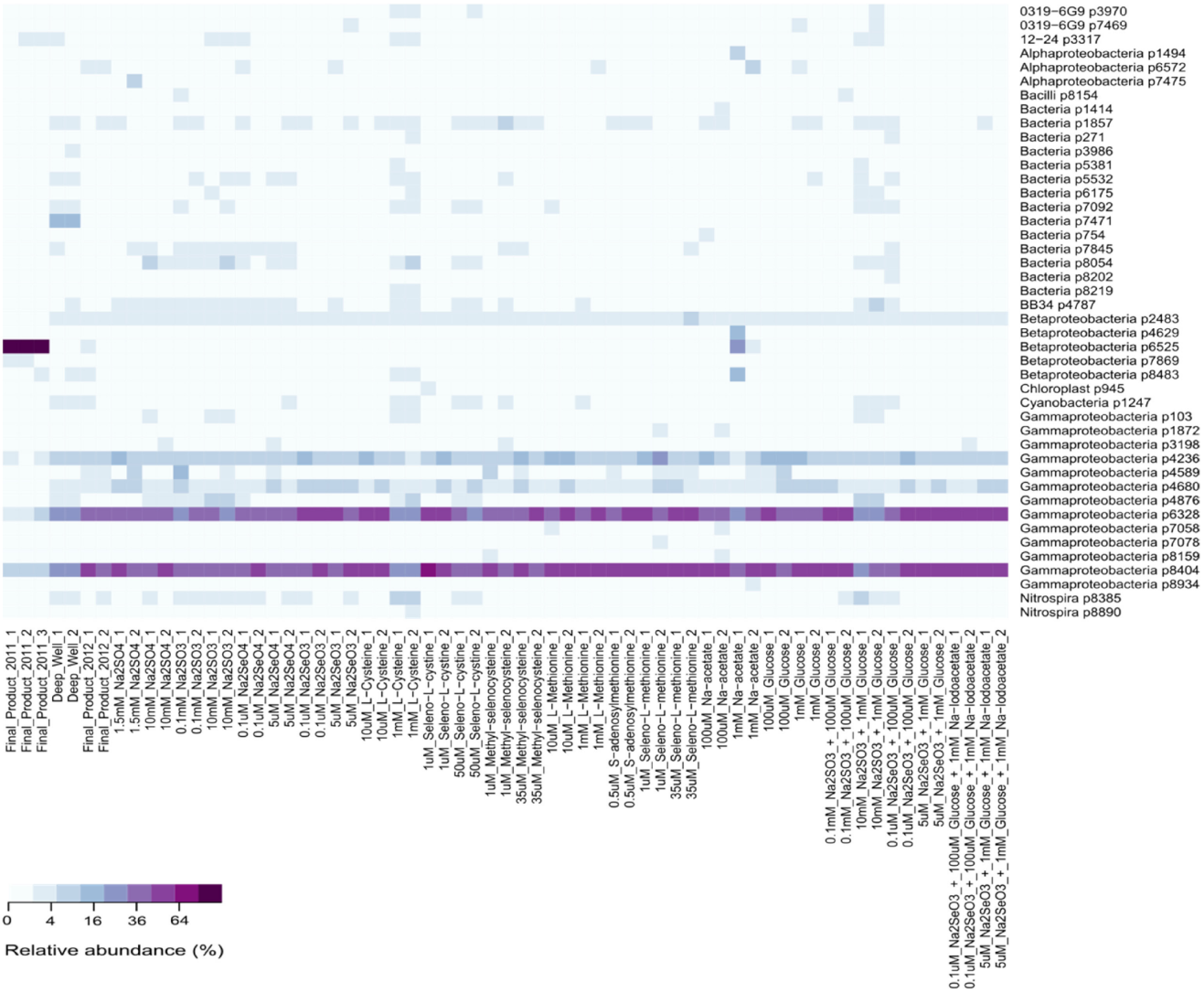

Figure 8. Heatmap of abundant phylotypes (>1 % relative abundance in at least one sample) in bottled mineral water incubated for 4 hours (day 1 after bottling) with different S/Se compounds and/or other substrates and in bottled mineral water without amendment at day 1 after bottling.

After 28 days of storage at ambient temperature the bacterial community of the untreated water was completely shifted towards a dominance of *Betaproteobacteria* (Figure 9). The fraction of *Gammaproteobacteria* was small, with 0.2 to 0.8 %, and *Betaproteobacteria* comprised 88.7 to 96.8 % of the bacterial community (Table S14). The betaproteobacterial predominance was explained by high relative abundances of *Curvibacter fontana* related phylotype p6525 and *Aquabacterium parvum*

related phylotype p8483 (Figure 10). They accounted for 34.3 to 36.3 % and 48.6 to 59.1 % of the bacterial community, respectively (Table S15). A minor fraction of 2.4 to 10.9 % was constituted of *Alphaproteobacteria*, which was mostly due to *Magnetospirillum gryphiswaldense* phylotype p1494 with relative abundances of 1.3 to 9.6 %. The dominance of *Betaproteobacteria* in the untreated water at day 28 after bottling, was confirmed by the use of fluorescence in situ hybridization (Table S16).

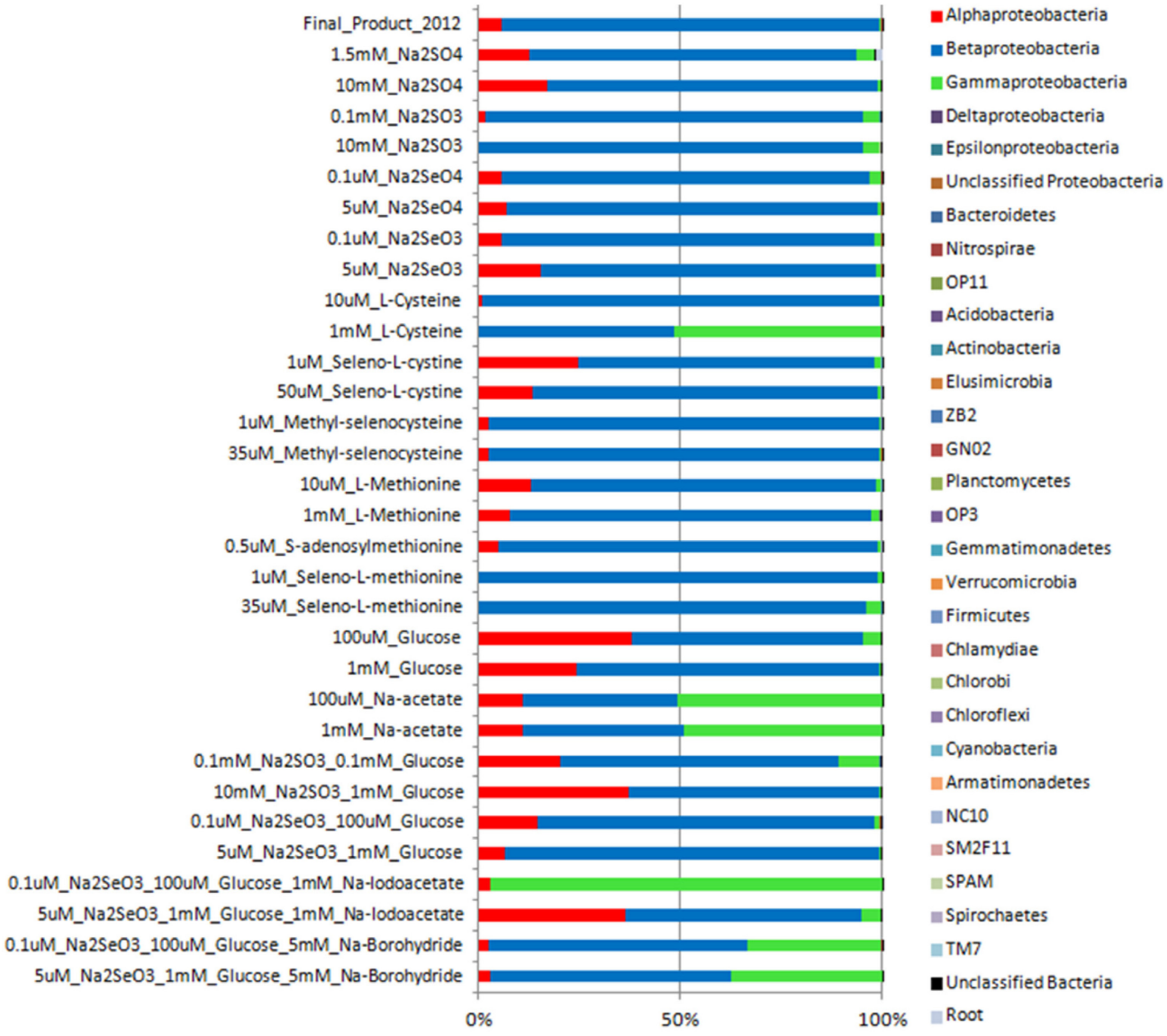

Figure 9. Relative abundance of major bacterial phyla/classes in bottled mineral water incubated for 27 days (day 28 after bottling) with different S/Se compounds and/or other substrates and in bottled mineral water without amendment (Final_Product_2012) at day 28 after bottling. Mean values of replicates are shown.

Analogues to the untreated control, the bacterial communities of most 27-days incubations were shifted from gammaproteobacterial predominance towards betaproteobacterial predominance (Figure 9). Nevertheless, in one of the duplicate incubations with 100 µM Na-acetate and 1 mM Na-acetate and in all incubations with 0.1 µM Na-selenite & 100 µM glucose & 1 mM Na-iodoacetate, *Gammaproteobacteria* were still dominating after 27 days of incubation (99.6 %, 97.1 % and 96.5 to 97.4 %), and *Betaproteobacteria* had no higher relative abundance than 1 % (Table S14). In individual incubations with 1 mM L-cysteine, 0.1 µM Na-selenite & 100 µM glucose & 5 mM Na-borohydride and 5 µM Na-selenite & 1 mM glucose & 5 mM Na-borohydride the fraction of *Betaproteobacteria* was lower (9.8 %, 34 % and 24.4 %) than the fraction of *Gammaproteobacteria* (90.1 %, 63.7 % and 74.1 %). In all other incubations, including the 100 µM Na-acetate and 1 mM Na-acetate incubations without gammaproteobacterial predominance, *Betaproteobacteria* accounted for 50.3 to 99.1 % of the bacterial community. In these incubations *Betaproteobacteria* were represented by phylotypes closely related to *Curvibacter fontana* (p6525), *Aquabacterium parvum* (p8483), *Undibacterium parvum* (p1685) and *Acidovorax facilis* (p2380) in varying quantities (Figure 10). p6525 was found, like in the untreated control, in most of the incubations, except the *Gammaproteobacteria* dominated incubations with 1 mM L-cysteine, 100 µM Na-acetate, 1 mM Na-acetate and 0.1 µM Na-selenite & 100 µM glucose & 1 mM Na-iodoacetate. Furthermore, in an individual incubation with 0.1 mM Na-sulfite & 100 µM glucose the relative abundance of p6525 was below 1 %. However, it was present in all other incubations, with relative abundances of 1.4 to 87.8 % (Table S15). p8483, also found in the untreated mineral water, was present in most of the incubations without glucose, and comprised, if abundant, 3.7 to 94.8 % of the bacterial community. Nonetheless, this phylotype was not found in the non-glucose-containing incubations with 10 mM Na-sulfite and its abundance was below 1 % in the *Gammaproteobacteria* dominated individual incubations with 100 µM Na-acetate and 1 mM Na-acetate. In glucose-containing incubations p8483 was either not found or its relative abundance was very low (0.2 to 4.8 %). The two phylotypes p6525 and p8483 were observed in all incubations with VOSC production, except in one of the duplicate incubations with 1 mM L-cysteine (Figure 10). Coherence was observed between the relative abundances of these two phylotypes and the presence of certain organic S/Se compounds in the water. The amendment of 50 µM seleno-L-cystine, methyl-selenocysteine and seleno-L-methionine enriched p8483 (49 to 94.8 %) in relation to p6525 (2 to 26.9 %), whereas the amendment of 10 µM L-cysteine, L-methionine and S-adenosylmethionine enriched p6525 (30.9 to 75.2 %) in relation to p8483 (3.7 to 27.7 %) (Table S15). In the 50 µM incubations with seleno-L-cystine the described abundance pattern was reversed, with p6525 (51.3 to 62.6 %) being more abundant than p8483 (15.9 to 16.8 %), and in one of the duplicate 1 mM L-cysteine incubations

p6525 was not found at all. The two phylotypes p1685 and p2380 were not abundant in the untreated control, but contributed substantially to the betaproteobacterial predominance in some of the incubations (Figure 10). p1685 was strongly enriched by 1 mM glucose incubations and 5 µM Na-selenite & 1 mM glucose incubations, with relative abundances ranging from 54.4 to 57 % and 56.4 to 61.7 %, respectively (Table S15). p2380 had higher than 1 % relative abundance in incubations with 10 mM Na-sulfate (6.6 to 8.7 %), Na-sulfite (3.5 to 4.7 %), 1 µM methyl-selenocysteine (12.2 to 16.2 %) and L-methionine (11.1 to 23.6 %) as well as in individual incubations with 50 µM seleno-L-cystine (14.1 %) and 0.5 µM S-adenosylmethionine (1.8 %). Furthermore this phylotype was abundant in a variety of glucose-containing incubations like glucose (4.6 to 35.8 %), Na-sulfite & glucose (1.1 to 64.4 %) and Na-selenite & glucose (1.9 to 4.6 %) as well as in individual incubations with 5 µM Na-selenite & 1 mM glucose & 1 mM Na-iodoacetate (54.8 %), 0.1 µM Na-selenite & 100 µM glucose & 5 mM Na-borohydride (4.1 %) and 5 µM Na-selenite & 1 mM glucose & 5 mM Na-borohydride (2.8 %). The *Pseudomonas* sp. related phylotype p6328 was responsible for the gammaproteobacterial predominance in incubations with 1 mM L-cysteine (11 to 78.3 %) and 0.1 µM Na-selenite & 100 µM glucose & 1 mM Na-iodoacetate (79.1 to 85.8 %) and in individual incubations with 0.1 µM Na-selenite & 100 µM glucose & 5 mM Na-borohydride (55.3 %) and 5 µM Na-selenite & 1 mM glucose & 5 mM Na-borohydride (64 %). The *Acinetobacter bouvetii* related phylotype p4589 dominated in individual incubations with 100 µM Na-acetate and 1 mM Na-acetate, with relative abundances of 99.4 % and 96.8 %, respectively. Like in the water without amendment, *Alphaproteobacteria* were present in most of the incubations (Figure 9). However, they were missing or found at low relative abundances in incubations with Na-sulfite (0 to 2.8 %), L-cysteine (0 to 1.2 %), seleno-L-methionine (0 to 0.2 %) and in one of the duplicate incubations with 100 µM Na-acetate (0.2 %) (Table S14). In all other incubations *Alphaprotoebacteria* comprised 1.4 to 42.6 % of the bacterial community. *Alphaproteobacteria* were primarily represented by the *Magnetospirillum gryphiswaldense* related phylotype p1494 and by the *Caulobacter segnis* related phylotype p8915 (Figure 10). p1494 a member of the untreated water accounted, if abundant, for 1.6 to 28.6 % of the bacterial community and was enriched compared to the untreated control, in incubations with Na-sulfate (10 to 13.9 %) and seleno-L-cystine (10.8 to 28.9 %) (Table S15). However, this phylotype was not found in incubations with Na-sulfite, L-cysteine, seleno-L-methionine, 10 mM Na-sulfite & 1 mM glucose, Na-selenite & glucose & Na-iodoacetate and 5 µM Na-selenite & 1 mM glucose & 5 mM Na-borohydride as well as in one of the duplicate incubations with 0.1 µM Na-selenite & 100 µM glucose & 5 mM Na-borohydride. Moreover p1494 was found at relative abundances below 1 % in individual incubations with 100 µM Na acetate (0.2 %), 1 mM Na acetate (0.1 %), 0.1 mM Na-sulfite & 100 µM glucose (0.1 %) and 0.1

µM Na-selenite & 100 µM glucose & 5 mM Na-borohydride (0.3 %). If no glucose was present in the water, p8915 was either found at very low relative abundances (0.1 to 1.6 %), like it was observed in the untreated control, or it was not found at all. In several glucose-containing incubations this phylotype constituted 1.4 to 39.3 % of the bacterial community. However, it was not abundant in incubations with 0.1 µM Na-selenite & 100 µM glucose (0.4 %) and 0.1 µM Na-selenite & 100 µM glucose & Na-iodoacetate (0.1 to 0.5 %).

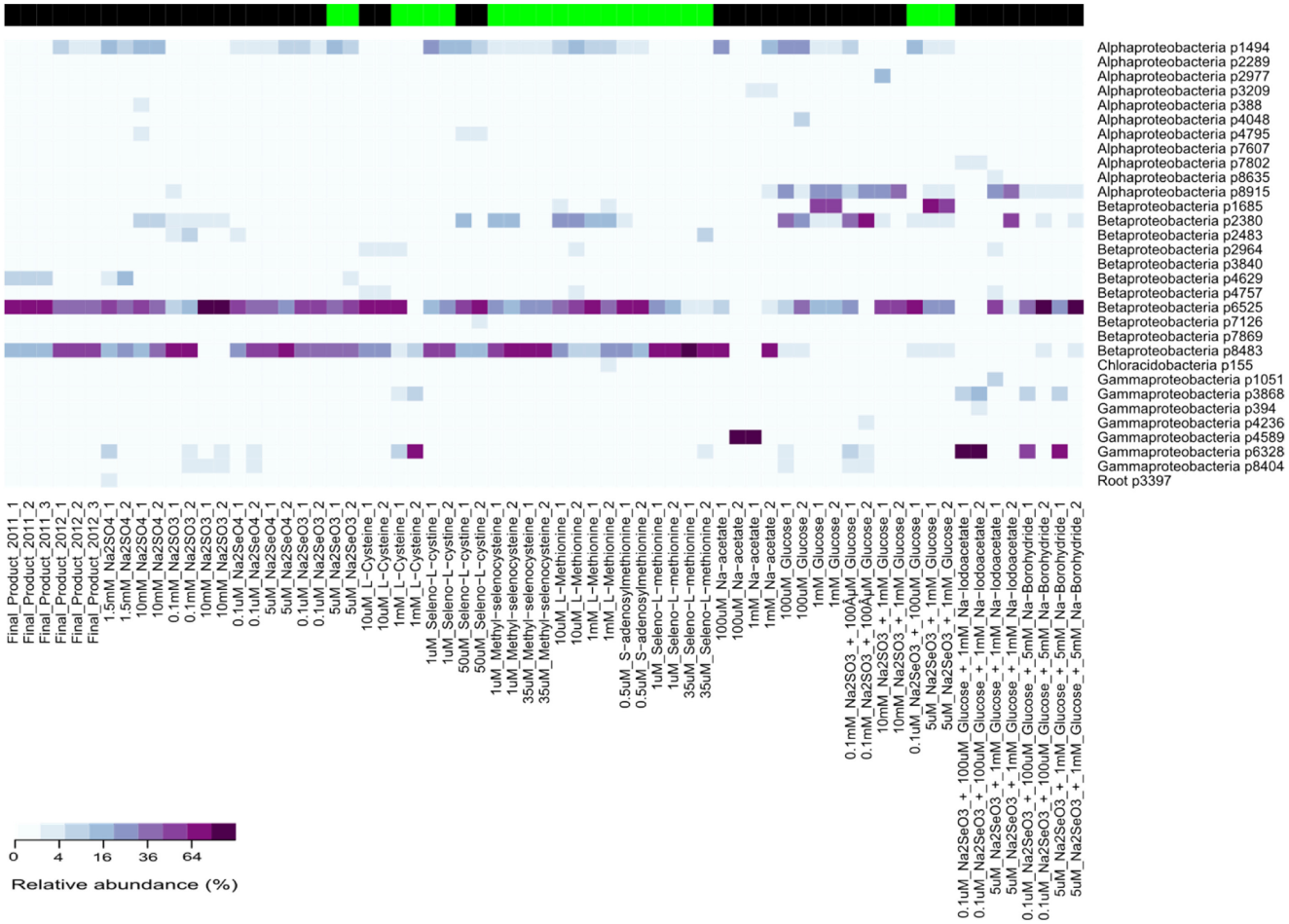

Figure 10. Heatmap of abundant phylotypes (>1 % relative abundance in at least one sample) in bottled mineral water incubated for 27 days (day 28 after bottling) with different S/Se compounds and/or other substrates and in bottled mineral water without amendment at day 28 after bottling. Production of volatile organic S/Se compounds (VOSC) in incubations is indicated by green colored bar above the heatmap.

The *Hyphomicrobium zavarzinii* related phylotype p4795 was only found at a relative abundance higher than 1 % in one of the duplicate incubations with 10 mM Na-sulfite (2.8 %) and in the 50 µM

incubations with seleno-L-cystine (1.9 to 2.6 %) but was low abundant in a variety of other incubations (i.e. S-adenosylmethionine at a relative abundance of 0.2 to 0.5 %) as well as in the untreated control.

Although community shifts were observed in a number of incubations, they did not correlate with the detection of VOSCs (Figure 10). This was supported by the permANOVA, which revealed that the detection of VOSCs in the incubations could only explain 6.7 % of the variation in phylotype diversity (Table S11). A correlation between the relative abundance of phylotypes and the concentrations of all five individual VOSCs showed that the *Hyphomicrobium zavarzinii* related phylotype p388 was correlating significantly (58 %) with DMS (Figure 11). However, p388 was only found in individual incubations with 10 mM Na-sulfate and 0.5 µM S-adenosylmethionine, at relative abundances of 3.1 % and 0.1 %, respectively (Figure 10 and Table S15).

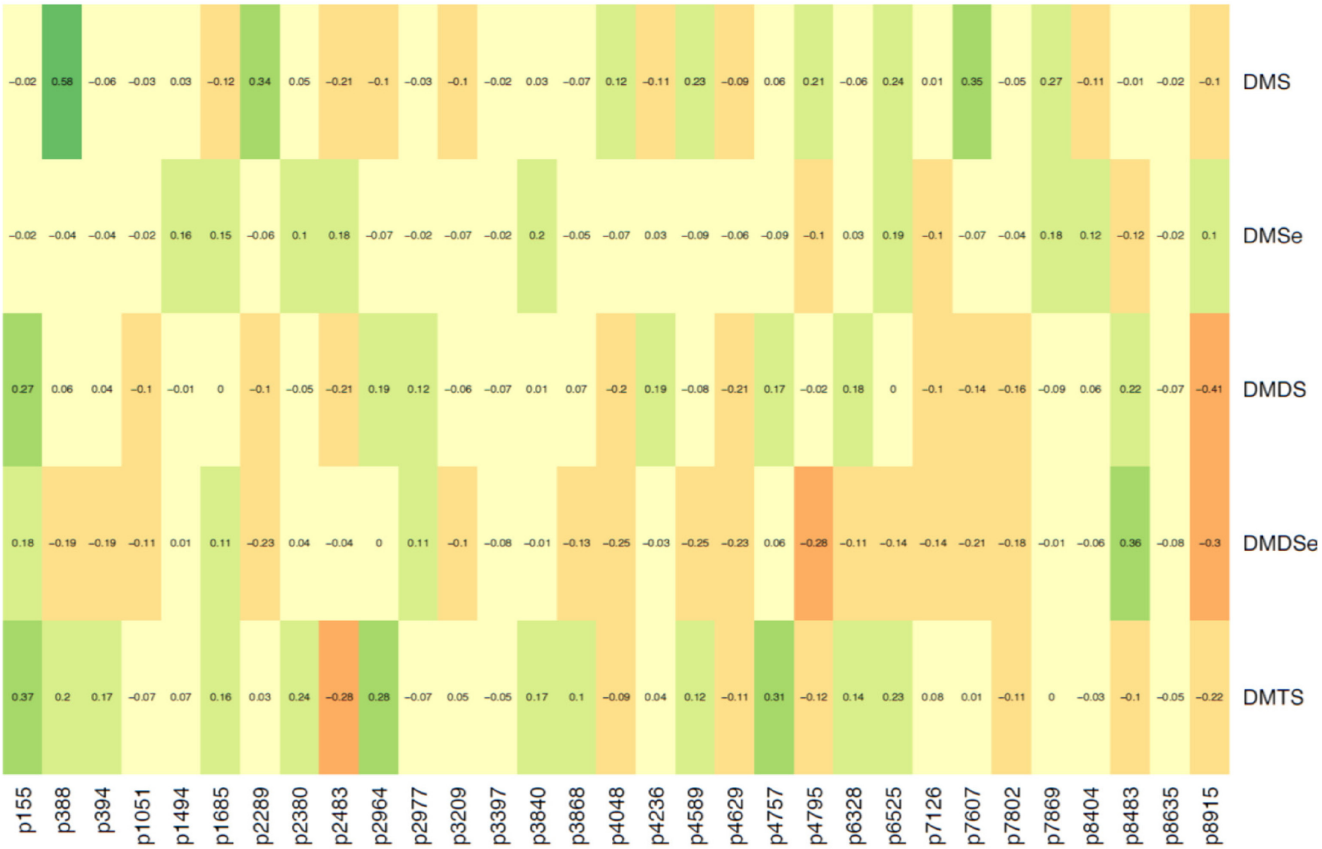

Figure 11. Correlations between relative abundances of phylotypes and production of the volatile organic S/Se compounds dimethyl sulfide (DMS), dimethyl selenide (DMSe) and their derivates dimethyl disulfide (DMDS), dimethyl trisulfide (DMTS) and dimethyl diselenide (DMSe) in bottled mineral water incubated for 27 days with different S/Se compounds and/or other substrates. Color shows the strenght of the positive (green) and negative (orange) correlation. Calculations were performed by the use of the rank abundance based spearman coefficient.

In order to find potential indicators for “VOSC production” or “no VOSC production” an indicator species analysis was performed (Table 19). This analysis was based on presence/absence information. It was revealed that the phylotype p7506, was an indicator for “VOSC production”, with a high significance. By the use of BLAST, *Ideonella sp.*, *Rubrivivax sp.*, *Methylibium petroleiphilum* and *Aquabacterium sp.* were identified as the closest relatives of p7506, with a sequence identity of 100 % to all of them. However the relative abundance of p7506 never exceeded 0.2 % (Table S15). The presence of *Caulobacter segnis* related phylotype p8915 in the incubations was found to be an indicator for “no VOSC production” (Table 19). The phylotypes p3515, which was found to be closely related to *Caulobacter sp.* (maximum identity of 99 %), and p4876, which was found to be related to *Solemya sp.* (maximum identity of 92 %), weakly indicated “no VOSC production”, though, their relative abundances were always below 1 % (Table S15).

Table 19. Indicator species for VOSC production and no VOC production found in the planktonic bacterial communities derived from bottled mineral water incubated for 27 days (day 28 after bottling) with different S/Se compounds and/or other substrates.

VOSC Production	Stat	P.value	Significance
p7506	0.724	0.001	***

No VOSC Production	Stat	P.value	Significance
p8915	0.882	0.001	***
p3515	0.613	0.021	*
p4876	0.573	0.023	*

stat – statistical strength (varying between 0 and 1); p.value - probability

4. Discussion

4.1. Delayed growth in mineral water started from a very low abundant microbial community

The microbial growth profile revealed in this study was similar to a previously reported growth profile for bottled water microbiota (Loy et al., 2005). In an analysis performed in 2011 with the same water, 10^3 cells per mL were observed at day 1 after bottling (Lesaulnier unpublished) and values from the literature were ranging from about 10^3 to 10^4 cells per mL (Loy et al., 2005; Vachee et al., 1997). Therefore, TCC of approximately 45 cells per mL at day 1 after bottling, as reported in this study, was unexpectedly low. However, it is possible that TCC are generally lower in the investigated water and the observed variability in TCC between the years 2011 and 2012 is rather explained by differences during the bottling process. In the year 2011, the bacteria were in the exponential growth phase at day 3 after bottling (Lesaulnier unpublished). As no considerable growth was observed in this study between day 1 and day 3 after bottling, samples taken after 48 hours of incubation (3 days after bottling) were not included in the 454 pyrosequencing analysis.

4.2. Bottled water microbiota can produce VOSC through degradation of S/Se-containing amino acids and methylation of Na-selenite

The formation of VOSCs in bottled mineral water was induced by the addition of a variety of S/Se compounds. It was observed that the addition of organic compounds was far more stimulating than the addition of inorganic compounds. Therefore, VOSC production in bottled mineral water might be more closely tight to amino acid metabolism, than to cycling of inorganic S/Se compounds. As indicated by our data, bacterial metabolism of inorganic Se for VOSC formation under natural conditions should, however, not be underestimated.

The amino acid L-methionine predominantly induced the production of DMDS. This suggests that L-methionine was degraded, according to Figure 1, to MT, by a process that was found in a variety of aerobic and anaerobic bacterial systems (Sato and Nozaki, 2009), followed by oxidation of two MT molecules to form DMDS and H_2O (Figure 1, reaction 2) as described for aerobic bacterial soil isolates (Segal and Starkey, 1969). It was reported from incubations with aerobic *Acinetobacter lwoffii* cultures, that oxygen concentration is positively correlated with DMDS production from L-methionine (Ginzburg

et al., 1999). Previous analysis revealed that the oxygen concentration of the water stayed close to atmospheric conditions (7.6 mg L^{-1} at 20°C) throughout the whole growth process (Lesaulnier unpublished). The aerobic conditions in the water further supports the auto-oxidation of MT to DMDS (Chin and Lindsay, 1994). Drotar et al. (1987a) speculated that bacteria could alternatively methylate MT under aerobic conditions to form DMS (Figure 1, reaction 3), though here there is no evidence for methylation of MT as no DMS was found in the L-methionine incubations. However, any produced DMS might have been converted aerobically to DMSO or MT and formaldehyde (Schäfer et al., 2010), or consumed by methylotrophs during the 27 days of incubation. Even though the detection of DMS in other incubations (0.5 µM S-adenosylmethionine) contradicts this hypothesis, consumption of DMS might be dependent on concentration and it is possible that the concentration of DMS in the incubations with 0.5 µM S-adenosylmethionine (0.07-0.08 μgL^{-1}) was too low to trigger its consumption. Furthermore, Lu et. al (2013) reported that freshwater incubations with L-methionine produced DMS at a relatively low concentration compared to the concentrations of dimethyl polysulfides. This low concentration was reportedly due to the a high volatility of DMS and potentially different production mechanisms for DMS and other dimethyl polysulfides (Lu et al., 2013). Therefore, it can be speculated that DMS was produced in our L-methionine incubations but its concentration in the water was below detection limit. To unambiguously prove if DMS can be produced from L-methionine as a precursor, future experiments on VOSC production have to be monitored in more detail. This would include hourly or daily measurements at early time points, incubations with methanemonooxygenase inhibitors (i.e. allylthiourea) to prevent the consumption of DMS by methylotrophes, and include the concentration of DMS in the bottled headspace. DMTS formation in L-methionine incubations was previously observed, though it is not well understood how DMTS is formed (Franzmann et al., 2001). It was speculated that molecules containing a so called "methanethiol" (CH_3-S-R) group, like L-methionine, can methylate naturally occurring H_2Sn (Franzmann et al., 2001). In addition, H_2Sn was produced by the aerobic bacterium *Acinetobacter lwoffii* directly from L-methionine (Ginzburg et al., 1999). Consequently, DMTS (and DMDS) production in bottled mineral water might be the result of a conversion of L-methionine into H_2Sn, followed by its subsequent methylation. Alternatively, Chin and Lindsay (1994) reported that MT can be readily oxidized through non-enzymatic auto-oxidation to DMTS (and other dimethyl polysulfides). As MT is reportedly produced during bacterial degradation of L-methionine, it is possible that DMTS production in the water occurred through a similar process. In order to obtain a better understanding about the DMTS production mechanisms, it is necessary to prove in future experiments whether H_2Sn is present in the water or the cells. In addition to polysulfides, DMDSe was detected in the incubations

with L-methionine. This was unexpected, as L-methionine was added at a high purity of ≥99 %. However, selenate is naturally occurring in the water (1.2 μgL^{-1}) and bacteria (described for *Escherichia coli*) can produce seleno-L-cysteine and seleno-L-methionine from SeO_4^- (Turner et al., 1998). Furthermore, Doran and Alexander (1977) proposed that the metabolism of S- and Se-containing amino acids appear to be similar. Therefore, it is likely that through the addition of L-methionine to the water we have triggered the conversion of naturally occurring seleno-L-methionine or seleno-L-cysteine to DMDSe (Doran and Alexander, 1977). Compared to the 1 µM incubations with L-methionine, less DMDS and DMDSe were detected when the compound was added at the higher concentration of 1 mM. It was reported from freshwater incubations with L-methionine, that besides MT, DMS, DMDS and DMTS, dimethyl tetrasulfide (DMTeS) was formed (Lu et al., 2013). These authors further noted that the addition of a supplementary carbon source (glucose) resulted in a shift in the production of larger volatile polysulfides (DMTS & DMTeS). Similarly, we might have shifted the production of volatiles from L-methionine (and seleno-L-methionine) towards the production of higher molecular weight compounds like DMTeS (and its Se-containig analogue), by amending the water with L-methionine at a higher concentration. Furthermore, it was found that dimethyl selenyl sulfides (CH_3-S-Se-CH_3) can be formed from gas mixtures of DMDS and DMDSe in the presence of selenium resistant bacteria (Chasteen, 1993). Though not included in our analyses, similar processes might have occurred in the mineral water and could therefore explain the lower DMDS and DMDSe concentrations in the 1 mM incubations with L-methionine. We also cannot dismiss the possibility that bacteria in the mineral water may have degraded DMDS (and DMDSe), as previously reported from aerobically growing soil isolates (Smet et al., 1996). A complementary control was carried out whereby L-methionine was added at a concentration of 1 mM to two seperate bottles containing 500 mL of sterilized Carola water from day 1. No VOSCs were detected after 28 days of incubation, indicating that the formation of VOSCs is not and autooxidation process but is dependent on the metabolic activity of the indigenous microorganisms.

The formation of DMDSe from seleno-L-methionine incubations supports the hypothesis that bacteria degrade this compound, according to Figure 1 (Doran and Alexander, 1977). Notwithstanding, seleno-L-methionine was found to be toxic to cultures of the Cyanobacterium *Anabaena flos-aquae* when added at concentrations of 0.1 mgL^{-1} (Kiffney and Knight, 1990). This was explained by a loss in enzyme function resulting from the synthesis of polypeptides in which S-containing amino acids have been replaced by their Se-containing analogues. In the present study, seleno-L-methionine was added to mineral water at concentrations of approximately 0.2 and 7 mgL^{-1}, thus the observed DMDSe

formation might be the result of a detoxification process. The additional formation of DMDS in seleno-L-methionine incubations, was consistent with the results of oxic drinking water incubations with this compound (Franzmann et al., 2001). Through the addition of seleno-L-methionine we have apparently triggered the conversion of naturally occurring L-methionine (for references see above). However, Franzmann et al. (2001) reported that the concentration of DMDSe in seleno-L-methionine incubations was approximately 50 times higher than the concentration of DMDS. Surprisingly, in the present study the amount of DMDSe in seleno-L-methionine incubations was only two times higher than the amount of DMDS (35 µM incubations) or was even lower than the amount of DMDS (1 µM incubations). As seleno-L-methionine was purchased at a purity of ≥98 %, this is unlikely a consequence of contamination with L-methionine. Interestingly, it has previously been reported that an *Escherichia coli* K-12 SO_4^{2-} transporter also bound to SeO_4^{2-} and SeO_3^{2-} though with a 5 and 37 times respective lower affinity (Lindblow-Kull et al., 1985). Therefore, the high concentrations of DMDS, might be explained by the fact that the transporters involved in amino acid metabolism are not Se specific in the bottled mineral water microbiota and have a higher affinity to S containing ligands than to their Se-containing analogues. Interestingly, the ratio between the amount of DMDSe and DMDS, was shifted from DMDS>DMDSe to DMDS<DMDSe, when seleno-L-methionine was added at the higher concentration of 35 µM. The amendment of this compound at the higher concentration seemingly forced the bacteria to focus more on the volatilization of potentially toxic seleno-L-methionine. It is surprising that, unlike L-methionine incubations, no DMTS was found in the incubations with seleno-L-methionine. This possibly indicates that the production of DMDS in the seleno-L-methionine incubations cannot be uniquely explained by degradation of naturally occurring L-methionine and other processes may be involved in DMDS production.

Franzmann et al. (2001) reported that oxic drinking water produced DMDS and DMTS when amended with methylating agents like methyl iodide. This was explained by the methylation of naturally occurring H_2Sn in drinking water (Figure 2, reaction 4), that are likely a legacy of sulfate-reducing bacteria that occur throughout the drinking water distribution system (Franzmann et al., 2001). These H_2Sn are intermediate products during the oxidation of H_2S (Luther, 2001), which is the end product of sulfate-reduction (Muyzer and Stams, 2008). Consequently, it could be speculated that the detection of DMS, DMDS and DMTS in incubations with the methylating agent S-adenosylmethionine indicates the presence of H_2S and H_2Sn in the investigated mineral water. The formation of DMS might be the result of an aerobic, S-adenosylmethionine dependent, two step methylation of H_2S. Where in a first step MT is formed (Figure 2, reaction 2), followed by a (speculative) second methylation of MT to form

DMS (Figure 1, reaction 3). However, as field experiments in a lake showed that H_2S is rather unstable under oxygen rich conditions (Ginzburg et al., 1999), other processes for DMS production must be involved. It is possible that S-adenosylmethionine was metabolized back into L-methionine, or other compounds involved in amino acid cycling, by a central process of all living systems (Bottiglieri, 2002). The produced L-methionine could have been subsequently degraded to MT, followed by the S-adenosylmethionine dependent methylation of MT to DMS, as discussed above. By using methylating agents that do not contain S (i.e. methyl iodide) in future experiments, this hypothesis could be tested. H_2Sn are more stable in oxygen rich freshwaters (Gun et al., 2000), hence, the observed production of DMDS and DMTS is potentially indicative of the presence of H_2Sn in the mineral water. However, taking into account that S-adenosylmethionine might be derivatized into L-methionine, DMDS and DMTS could also be the product of degradation of this amino acid, as described above. Therefore, the presence of sulfides must be proven. Compared to the 10 µM L-methionine incubations, yields of VOSCs triggered by 0.5 µM S-adenosylmethionine were 25 times lower in respect to the concentration of the amended compounds. Nonetheless, this difference fell within a similar range as observed previously and were explained by limited concentration or availability of (potentially present) H_2Sn (Franzmann et al., 2001). The fact that negligible amounts of off-odours were detected in the 0.5 µM S-adenosylmethionine incubations during the sensory analysis (Table S6) is not surprising, as the detected DMTS concentrations (0.01 to 0.02 μgL^{-1}) corresponded to the proposed odour threshold concentration of 0.01 μgL^{-1} (Wajon et al., 1985).

Amendment of L-cysteine to the water could stimulate production of very low amounts of DMDS and DMTS, but only when amended at a concentration of 1 mM. The observation that low yields of VOSC were found in L-cysteine incubations, was consistent with the findings of a previous supplementation experiment with drinking water and can be explained by the terminal position of the S in L-cysteine, which is different compared to L-methionine, where the S is part of the previously mentioned CH_3-S-R group (Franzmann et al., 2001). Our results indicated that L-cysteine in the water was predominantly degraded to H_2Sn (Figure 2, reaction 3), like it was reported from aerobic *Acinetobacter lwoffii* cultures (Ginzburg et al., 1998), followed by the methylation of these H_2Sn to DMDS and DMTS as described above. However, it cannot be ruled out that L-cysteine was first degraded to H_2S (Figure 2, reaction 1), by a widespread bacterial mechanism (Sato and Nozaki, 2009), as H_2Sn are intermediate products during the oxidation of H_2S (Luther, 2001). DMDS and DMTS production was only observed in one of the duplicate 1 mM L-cysteine incubations, however the concentrations of DMDS and DMTS in the other 1 mM L-cysteine incubation were close to the threshold value of 0.01 μgL^{-1}, hence it is likely that

DMDS and DMTS were produced in the other 1 mM L-cysteine incubation, but at concentrations that were below the detection limit. In order to definitely prove the potential of L-cysteine as a precursor for VOSC production, further experiments are needed. It is evident that other processes for L-cysteine degradation are possible and might have competed with derivatization of L-cysteine to H_2Sn and subsequently to VOSCs. One such process is the oxidation of L-cysteine to SO_4^{2-}, like it was observed in a mixed population of soil microorganisms (Freney, 1958). This would be a less energy consuming way of L-cysteine degradation under oxic conditions.

DMDSe (and DMSe) formation from methyl-selenocysteine was previously reported from supplementation experiments with freshwater (Ranjard et al., 2004). However, Ranjard et al. (2004) argued that methyl-selenocysteine is first degraded to methaneselenol or elemental Se, which are subsequently methylated to form DMDSe and DMSe. In the present study no DMSe was detected in the incubations with methyl-selenocysteine. Therefore, it can be speculated that methyl-selenocysteine is indeed degraded to methaneselenol but is then, according to Figure 1, reaction 2, rather oxidized to DMDSe. Notwithstanding, the lack of DMSe in the methyl-selenocysteine incubations might be explained by its consumption, as described for DMS (Schäfer et al., 2010) or by higher volatility of DMSe compared to DMDSe, as described for DMS and DMDS (Lu et al., 2013). It was reported from an incubation experiment with (oxygen deprived) *Rhodobacter sphaeroides* cultures that the concentration of DMSe, produced from selenite and selenate, was reduced after 7 days, whereas DMDSe concentration stayed constant over the 10 days of observation (Fleet-Stalder et al., 1997). This suggests that DMSe might have been produced from the precursor methyl-selenocysteine, but was less stable than DMDSe and was consequently not found after 27 days of incubation. In order to unambiguously prove if DMSe was produced, more detailed future experiments are needed, as described above for L-methionine and DMS.

The Se-containing compound seleno-L-cystine stimulated production of DMDS in one of the 1 µM incubations but did not in all the other incubations. Doran and Alexander (1977) reported, based on their experiments with the aerobic soil isolate *Corynebacterium sp.* and various bacterial isolates from sewage and clay, that seleno-L-cystine can be degraded, according to Figure 2, reaction 1, to hydrogen selenide. Through methylation of hydrogen selenide, which is a highly toxic compound (Tarze et al., 2007), DMSe (and DMDSe) can be formed, as reported from cell extracts of the freshwater protozoan *Tetrahymena thermophila* (Drotar et al., 1987b). However, even though 87 to 97 % of the seleno-L-cystine was taken up by the bacteria during incubation for 27 days, we could not detect any Se-containing VOSCs. It should be mentioned that seleno-L-cystine did not dissolve

completely during amendment and this might partly explain the high calculated uptake of this compound. Nonetheless, a variety of processes for seleno-L-cystine consumption/assimilation, other than VOSC production, could have been employed by the bottled water bacteria. One such process is the oxidation of seleno-L-cystine to SeO_4^{2-} as it was shown for L-cystine and SO_4^{2-} in soil (Freney, 1958). However, no SeO_3^{2-} (a possible intermediate) was detected in the water and the amount of SeO_4^{2-} did not increase in seleno-L-cystine incubations in comparison to the water without amendment. Another possibility is the degradation of seleno-L-cystine to elemental Se, as it was reported from individual bacterial isolates from sewage and clay (Doran and Alexander, 1977). Similar processes in the bottled mineral water cannot be verified in this study as the concentration of elemental Se was not determined. In addition it could be speculated that seleno-L-cystine was aerobically reduced, analogues to L-cystine (Thomas, 1984), to seleno-L-cysteine, which was subsequently included into proteins (Böck et al., 1991). The occurrence of DMDS in the seleno-L-cystine incubations and the fact that it was found in just one of the duplicate incubations with this compound is hard to explain. Contamination of the individual incubation with a S-containing amino acid (i.e. L-methionine) cannot be ruled out. However, the amendment of different compound combinations was performed successively with the same equipment, thus this possibility seems unlikely.

The addition of 5 µM Na-selenite to the water could stimulate DMDSe production. This is likely due to a an accepted detoxification mechanism for volatilization of potentially toxic SeO_3^{2-} (Favre-Bonté et al., 2005; Ranjard et al., 2003, 2004). However, in contrast to previous studies, it could be shown the first time that bacterial volatilization of SeO_3^{2-} is possible without the addition of a complementary carbon source. This is possibly explained by the fact that compound supplementation occurred prior to any considerable growth in the water. The positive effect of glucose on biological volatilization of SeO_3^{2-} that was observed (10 fold increase in 5 µM Na-selenite incubations and detection of VOSCs in incubations with 0.1 µM Na-selenite) was consistent with the results of previous analysis (Favre-Bonté et al., 2005). Apparently, the additional energy source stimulated the metabolic activity of the bacteria and allowed them to methylate the added Na-selenite more efficiently. No DMSe was detected in the incubations with 5 µM Na-selenite & 1 mM glucose. It was reported, based on the results from experiments with anaerobic peat soil, that the addition of glucose correlated negatively with the natural formation of DMS (Kiene and Hines, 1995). This was explained by a not further specified inhibition of the thiol (R-SH) methylation pathway, that could have prevented DMSe from being produced in the incubations with 5 µM Na-selenite & 1 mM glucose. Furthermore, Lu et al. (2013) reported from his

supplementation experiments with L-methionine and freshwater, that glucose supplementation may extend disulfide bonds of S-containing VOSCs (DMDS and DMTS) to form DMTeS. Consequently, it is possible that DMSe was converted into DMDSe when the additional glucose was ameded to the Na-selenite incubations at the higher concentration of 1 mM. The production of VOSC was clearly inhibited by the addition of Na-iodoacetate, likely through a reaction of this inhibitor with reduced glutathione under the formation of inactive disulfides (Dickens, 1933). This indicates that bottled water bacteria volatilize potentially toxic SeO_3^{2-} according to Figure 3. It could not be proven if the hydrogen selenide, that was potentially produced through reduction of glutathioselenol (Figure 3, reaction 6), was included into seleno-L-cysteine (Böck et al., 1991), as the incubations with Na-selenite & glucose & Na-borohydride were not analyzed chemically. However, 29.4 to 60 % of the amended Na-selenite were taken up by bacteria during the 27 days of incubations and only a negligible part of the uptake was related to volatilization. Consequently, it is possible that other processes were acting on SeO_3^{2-}, like the inclusion into seleno-amino acids.

In contrast to the incubations with Na-selenite, no VOSC production was observed in the incubations with Na-selenate. This is not consistent with a previous study, that reported DMDSe production from freshwater spiked with SeO_3^{2-} and SeO_4^{2-} (Ranjard et al., 2004). However, this could indicate that Na-selenate is not toxic to the bottled mineral water bacteria possibly explained by the fact that SeO_4^{2-} is naturally occurring in the water (1.2 μgL^{-1}). Alternatively, Na-selenate could have been predominantly "detoxified" through reduction into elemental Se, as shown for aerobic *Pseudomonas stutzeri* cultures (Lortie et al., 1992). This might be reflected by the fact that the uptake of Na-selenate (60-73.2%) was considerably higher than the uptake of Na-selenite (24.6-50%). Contrary to the study of Lindblow-Kull et al. (1984), various studies found that bacteria have different transporters for the uptake of SeO_3^{2-} and SeO_4^{2-} (Brown and Shrift, 1982; Bryant and Laishley, 1988; Hudman and Glenn, 1984). Therefore, it can be speculated that the uptake of Na-selenite and Na-selenate by bottled water bacteria was performed by different transporters. Furthermore, the concentration of DMDSe in the 5 µM incubation with Na-selenite was close to the detection limit (0.02 μgL^{-1}) and was only observed in one of the duplicate incubations, thus the differences between the metabolism of Na-selenate and Na-selenite might not be notable. In none of the incubations with the inorganic S compound SO_3^{2-} were VOSCs detected. This highly reactive and therefore toxic compound is apparently rather oxidized to SO_4^{2-} (Kappler and Dahl, 2001), than volatilized through energy consuming methylation, though we cannot prove this hypothesis in our study.

None of the compounds that were added in order to stimulate the metabolism of the bottled water microbiota (glucose, Na-acetate and Na-sulfate) had an effect on VOSC production. This is not consistent with the results from Franzmann et al. (2001), who reported VOSC formation from drinking water, amended with easily biodegradable compounds (i.e. glucose) and explained that by an increase in metabolic activity, which lead to a conversion of naturally occurring precursors, like L-methionine, to DMTS. However, the drinking water incubations described above were performed in bioreactors with extensive growth of biofilms (Franzmann et al., 2001). In the bottled mineral water that was tested in the present study, we did not observe visible biofilm formation, thus the concentration of naturally occurring precursors for VOSC production might have been much lower. Even though, the addition of Na-sulfate to the water had no effect on VOSC production, SO_4^{2-} might still be important for off-odour formation under natural conditions. Compared to the median concentration of 24.4 mgL^{-1}, as determined for 571 European bottled mineral waters (Bertoldi et al., 2011), SO_4^{2-} is found in the investigated mineral water in high concentrations of 136 mgL^{-1}. Franzman et al. (2001) suggested that H_2Sn, which are a legacy of sulfate-reducing bacteria from biofilms growing in drinking water distribution systems, can act as precursors for VOSC formation (Franzmann et al., 2001). It is possible that the reduction of SO_4^{2-} into H_2S and subsequent formation of H_2Sn, as described above, is happening in the bottling system of the investigated mineral water. Through methylation of these H_2Sn, VOSC and off-odours could be formed. According to the results obtained from the S-adenosylmehionine incubations, it is possible that H_2Sn are present in the water.

From all incubations with inorganic and organic Se-containing compounds, a considerable amount of the amended substance was taken up by the bottled water bacteria. Nevertheless, only a very small part of the uptake happened through production of volatile DMSe and DMDSe and in most of the incubations no volatilization of Se compounds was observed at all. This is consistent with the findings of a supplementation experiment with cultures of the purple nonsulfur bacterium *Rhodobacter sphaeroides* were volatilization was statistically insignificant compared to total uptake (Van Fleet-Stalder et al., 2000). Se is an important cell nutrient (Pinsent, 1954)., and the bacteria, growing in the water, apparently incorporate the added Se (i.e. into proteins) rather than volatilizing it. Another possible explanation for these observations, could be the degradation of Se-containing VOSC by certain bacteria.

Previous investigations of real “DMS/DMSe cases” linked the formation of DMS, DMSe, DMDS and DMDSe to the occurrence of unpleasant off-odours in bottled mineral water. As the mechanisms

involved in the degradation of the two analogues amino acids L-methionine and seleno-L-methionine did not discriminate between the formation of volatile S- and Se-containing compounds, they might play a crucial role in VOSC production under natural conditions. However, other processes must be involved as well, as no DMS and DMSe were detected in the incubations with these two amino acids. DMSe could be produced through volatilization of SeO_3^{2-}. Even though we found DMS in the S-adenosylmethionine incubations, the underlying mechanisms for DMS formation remain unexplained. In conclusion, VOSC production in bottled mineral water is likely explained by a variety of processes, rather than exclusively by a single process. In addition, the results of the sensory analysis suggest that the chemical composition of off-odours is more diverse and is not only linked to the occurrence of DMS, DMSe and/or their derivates. According to the literature, L-cysteine might get degraded to H_2S (Sato and Nozaki, 2009), a compound that is olfactory active (Robert J. McGorrin, 2011). This would explain the strong to very strong off-odours that were detected in these incubations. However, the fact that H_2S is not stable under oxygen rich conditions contradicts this hypothesis. Consequently, it remains to be proven if other compounds than the VOSCs that were analyzed in the present study cause unpleasant off-odours in the mineral water.

4.3. The shifts in composition of the actively growing bottled mineral water community were not correlated to VOSC formation

The phylotypes, that are discussed below, had either a higher than 1% relative abundance in all replicates of the untreated water at day 1/day 28 after bottling, or were enriched by certain amendments with different S/Se compounds and/or other substrates. At day 1, the only enrichment that is discussed below was found in an individual 1 mM Na-acetate incubation. This incubation did not cluster together with the other 4-hours incubations in the beta-diversity plot. Among the day 28-incubations those (enriched) phylotypes were discussed, that were not abundant in the untreated control, but had higher than 10% relative abundance in at least two incubations. Only exception is the *Hyphomicrobium zavarzinii* related phylotype p4795, which is discussed because p388, another phylotype that was related to the same species, was identified as an indicator for VOSC production.

In general it must be stated that the DNA that was extracted from the water at day 1 after bottling might be also derived from dead, inactive or lysed bacteria. Consequently, all phylotypes that were abundant at day 1 do not necessarily represent actively growing bacterial population. One such phylotype is p6328, most closely related to representatives of the genus *Pseudomonas*. It was

previously reviewed that pseudomonad species are important members of the mineral water flora (Leclerc and Moreau, 2002). *Pseudomonas spp.* are perfectly adapted to oligotrophic environments as they do not depend on the availability of specific vitamins or amino acids and can grow on a variety of carbon sources (Leclerc and Moreau, 2002). However, as p6328 was not found in the untreated water at day 28, this hypothesis cannot be supported by our data. This adaptation might be the reason for the good susceptibility of *Pseudomonas spp.* to isolation and could therefore explain the overestimated relevance of this species as a member of the bottled mineral water flora. Phylotype p8404 is closely related to the soil isolate *Serratia qinivorans* and was found at a high relative abundance in the untreated water at day 1. The leaching of soil bacteria into groundwater has previously been shown (Brennan et al., 2010) and is, therefore, a reasonable explanation for the occurrence of this phylotype. Two other phlyotypes, closely related to *Leclercia adecarboxylata* (p4236) and Aeromonas sp. (p4680), were found in the water with representatives of the later having previously been isolated from drinking water (Kuhn et al., 1997). However, both p4236 and p4680 did not proliferate beyond day 1. The phylotype p4589, closely related to an isolate from activated sludge named *Acinetobacter bouvetii* (Carr et al., 2003), was found in the untreated water. Representatives of the genus *Acinetobacter* were isolated from bottled mineral water before (Bischofberger et al., 1990; Manaia et al., 1990) and they were considered as common members of the growing mineral water flora (Leclerc and Moreau, 2002). As we did not find p4589 in the untreated water at day 28 after bottling, this hypothesis cannot be supported by our data. The occurrence of *Undibacterium pigrum* related phylotype p2483 in bottled mineral water is not surprising, as this species was isolated from a german drinking water (Kämpfer et al., 2007). Finally, the detection of the *Methylobacterium populi* related phylotype p6572, raised the general question if such methylotrophic bacteria are degrading VOSCs in the water. However, this phylotype was not found in the untreated water at day 28 after bottling and is therefore, not likely involved in VOSC degradation.

After 4 hours of incubation the bacterial communities appeared to be almost identical to that of the untreated water. This was also reflected by the beta-diversity measures, as most samples from 2012 at day 1 (all 4-hours incubations and the untreated water) clustered together. Only exception was an individual incubation with 1 mM Na acetate, where three phylotypes related to the species *Magnetospirillum gryphiswaldense* (p1494), *Curvibacter fontana* (p6525) and *Aquabacterium parvum* (p8483) constituted 34.4 % of the bacterial community. The fact that these “day-28” phylotypes proliferated in the water, even though nutrient availability was increased, might indicate that this factor does not affect the composition of the bottled mineral water community. To the contrary, considerable

community shifts were observed in some of the incubations with Na-acetate and in a variety of glucose-containing incubations after 27 days. Hence, it remains unexplained which factors are shaping the community composition of the bottled mineral water. In general, a high variability in relative abundance of phylotypes (i.e. p4589), phyla/classes (i.e. *Alphaproteobacteria*) and unclassified bacteria was observed between biological replicates. The reproducibility of 16S rRNA gene amplicon pyrosequencing was recently proven for aquifer microbes (Pilloni et al., 2012) and the 2-step barcoding approach, that was used in the present study, is known to further improve this reproducibility (Berry et al., 2011). Therefore, the observed variability might be rather explained by biological variation (bottle-to-bottle variability), than by low technical reproducibility. The fraction of unclassified bacteria was increased, compared to the untreated control, in both replicates of incubations with 1 mM L-cysteine and 10 mM Na-sulfite & 1 mM glucose. This was related to the highest observed alpha-diversity within the 4-hours incubations, with Shannon diversity indices of 4.8 to 5.2 and 4.7, respectively. However, the reasons for this diversification are unclear. In contrast to the untreated control *Methylobacterium populi* related phylotype p6572 was only found in a few individual incubations (0.1 µM Na-selenate, 5 µM Na-selenite, 1 mM L-methionine, 1 mM Na- acetate and 1 mM glucose). This gives further support to the hypothesis that this phylotype is not relevant for VOSC degradation in the water. During sampling of L-methionine and seleno-L-methionine incubations after 4 hours, we noticed considerable production of off-odours. This is surprising, as bacteria that were able to produce VOSCs in the water must have been metabolically active at day 1. Phylotypes closely related to the species *Pseudomonas sp.* (p6328) and *Aeromonas sp.* (p4680) were found in all incubations at day 1. *Pseudomonas fluorescens* was able to produce VOSCs from L-methionine (Segal and Starkey, 1969) and the production of DMDS, methaneselenol, DMSe, dimethyl selenyl sulfide and DMDSe was reported from an isolate from seleniferous agricultural drainage water named *Aeromonas vinies* (Rael and Frankenberger Jr, 1996). Consequently, p6328 and p4680 might have played a role in VOSC production at this early time point, despite the fact that they were apparently not proliferating in the bottle. Alternatively, VOSC formation at day 1 might have been due to a few actively growing cells that started to proliferate and produce VOSC despite very low relative abundances. Unfortunately, the incubations were not analyzed chemically at these early time points, hence, there is no data to confirm that VOSCs were produced at day 1.

After proliferation, at day 28, the bacterial community of the untreated water was completely shifted towards a dominance of *Betaproteobacteria.* Accordingly, storage time was identified as the factor that shaped the bacterial communities of the water strongest. The alpha-diversity decreased during

storage, what is reflected by a dominance of two betaproteobacterial phylotypes that were closely related to the species *Curvibacter fontana* (p6525) and *Aquabacterium parvum* (p8483). p6525 and p8483 constituted 85 to 95 % of the actively growing bacterial community. The finding, that *Betaproteobacteria* were the dominant members of the actively growing community (88.7 to 96.8 %), is consistent with previous findings (Loy et al., 2005) and gives further support to the hypothesis that *Betaproteobacteria* are generally the dominant members of the bottled mineral water flora. Furthermore, betaproteobacterial predominance in the investigated water was also shown by fluorescence in situ hybridization (Table S16), hence, adequate recovery of relative species abundances by the use of 16S rRNA gene amplicon pyrosequencing seems likely. As *Curvibacter fontana* was isolated from a well (Ding and Yokota, 2010) and *Aquabacterium parvum* is an isolate from a biofilm of a drinking water distribution system (Kalmbach et al., 1999) the occurrence of p6525 and p8483 in the mineral water is not suprising. The third abundant phylotype in the untreated mineral water at day 28 after bottling, was closely related to *Magnetospirillum gryphiswaldense (Alphaproteobacteria*), which was first isolated from a lake sediment (Geelhoed et al., 2010). The phylotypes p6525 and p8483 dominated the actively growing community in the present study and were the only phylotypes found in the water at day 28 after bottling in the year 2011 (Lesaulnier unpublished). Therefore, they can be defined as the most important members of the actively growing flora in the investigated mineral water, whereas p1494 is obviously not a stable member of the community.

The community composition of most 27-days incubations was shifted, according to the untreated water, towards a dominance of *Gammaproteobacteria*. Nonetheless, the beta-diversity measures and the permANOVA revealed that the amendment with different S/Se compounds and/or other substrates clearly affected the composition of some of the incubation. The three “day-28” phylotypes that were related to the species *Curvibacter fontana* (p6525), *Aquabacterium parvum* (p8483) and *Magnetospirillum gryphiswaldense* (p1494) were affected by the addition of S/Se compounds and/or other substrates in different ways. p6525 was found in almost all incubations and was therefore the most stable member of the bacterial community. In contrast to that, p8483 is apparently adapted to lower nutrient concentrations as it was overgrown by other phylotypes when glucose was added to the water. p8483 and p6525 were found in all incubations with VOSC production, with the exception of an individual incubation with 1 mM L-cysteine. Therefore, they might be relevant for off-odour development under natural conditions. Unfortunately, there is not much known about the metabolism of *Curvibacter fontana* (p6525) and *Aquabacterium parvum* (p8483), hence, this hypothesis remains to

be proven. Furthermore, p6525 and p8483 were selectively enriched in relation to each other by the addition of S- and Se-containing amino acids. 1 µM Seleno-L-cystine, methyl-selenocysteine and seleno-L-methionine enriched p8483 in relation to p6525, whereas the amendment of L-cysteine, L-methionine and S-adenosylmethionine enriched p6525 in relation to p8483. This observation might be explained by different food preferences or by differences in toxicity of these compounds to the respective species. p1494 was enriched, compared to the untreated control by Na-sulfate and seleno-L-cystine, but was inhibited by Na-sulfite, L-cysteine, seleno-L-methionine and by the two inhibitors Na-iodoacetate and Na-borohydride. It was recently shown that *M. gryphiswaldense* is able to oxidize H_2S to SO_4^{2-} under microaerobic conditions (6 -10 % of atmospheric oxygen concentration) (Geelhoed et al., 2010). Hence, a positive effect of SO_4^{2-} might be related to the use of this compound by p1494 as a source of S. In the experiments from Geelhoed et al. (2012), *M. gryphiswaldense* strains did not respire SO_3^{2-}, but no toxic effects of this compound were reported. Therefore, an inhibitory effect of SO_3^{2-} to p1494 is surprising. Notwithstanding, the absence of this phylotype from L-cysteine incubations might then be explained as follows. L-cysteine could have been oxidized by some bacteria to SO_4^{2-}, like it was described for a mixed population of soil microorganisms (Freney, 1960), and the proposed intermediate SO_3^{2-} might have inhibited the proliferation of p1494 in the L-cysteine incubations. The enrichment of p1494 by seleno-L-cystine possibly indicates that this organism is not only involved in the metabolism of S in the water, but also in the metabolism of Se. This hypothesis is further supported by a fact that p1494 was absent from incubations with Na-iodoacetate and Na-borohydride, as these compounds inhibit certain reactions of the bacterial metabolism of Se (see section 1.2.). However, it is thus far unexplained, why p1494 was not found in the seleno-L-methionine incubations. Strong shifts in community composition were observed in a variety of glucose-containing incubations. In these incubations we found the *Caulobacter segnis* related phylotype p8915 (in incubations with glucose, Na-sulfite & glucose and 5 µM Na-selenite & 1 mM glucose & 1 mM Na-iodoacetate), the *Acidovorax facilis* related phylotype p2380 (in incubations with 100 µM glucose, 0.1 mM Na-sulfite & 100 µM glucose and in one of the duplicate incubations with 5 µM Na-selenite & 1 mM glucose & 1 mM Na-iodoacetate) and the *Undibacterium parvum* related phylotype p1685 (in incubations with 1 mM glucose and 5 µM Na-selenite & 1 mM glucose). The fact that glucose addition selectively enriched p8915 in certain incubations, potentially explains why representatives of the genus *Caulobacter* were isolated from bottled mineral water in the past (González et al., 1987). An *A. facilis* isolate was able to use glucose as a source of carbon and one of the tested strains of *A. facilis* was also able to use L-methionine (Willems et al., 1990). This might explain the selective enrichment of p2380 in incubations with glucose and L-methionine. On the contrary, *U. parvum*, an isolate from

drinking water, weakly utilized glucose as a substrate (Eder et al., 2011). Therefore, the enrichment of p1685 by this compound in certain incubations is surprising. However, when grown in liquid media, this species was mostly attached to the glass surface (Eder et al., 2011). This feature might be helpful for proliferation in the bottle habitat, as it was hypothesized that the nutrients are accumulating on the bottle surface (Leclerc and Moreau, 2002). Two phylotypes were selectively enriched by certain incubations that were found in the untreated water at day 1 but were not found in the untreated water at day 28 after bottling. The *Pseudomonas sp.* (*Pseudomonas borbori* and *Pseudomonas stutzeri*) related phylotype p6328 (an individual incubation with 1 mM L-cysteine, incubations with 0.1 µM Na-selenite & 100 µM glucose & 1 mM Na-iodoacetate one of the duplicate incubations with 0.1 µM Na-selenite & 100 µM glucose & 5 mM Na-borohydride and with 5 µM Na-selenite & 1 mM glucose & 5 mM Na-borohydride) and the *Acinetobacter bouvetii* related phylotype p4589 (individual incubations with 100 µM Na-acetate and 1 mM Na-acetate). A *Pseudomonas stutzeri* isolate could reduce SeO_3^{2-} and SeO_4^{2-} into Se under aerobic conditions (Lortie et al., 1992). Therefore, the enrichment of p6328 in certain incubations with Na-selenite & glucose (& Na iodoacetate or Na-borohydride) might be explained by reduction of SeO_3^{2-} for the purpose of energy generation. No enrichment of p6328 in any of the Na-selenite & glucose incubations without Na-iodoacetate or Na-borohydride, is possibly explained by the fact that bacteria that were able to volatilize Na-selenite in these incubations were more competitive. The enrichment of p6328 in incubations with 1 mM L-cysteine remains unclear. p4589 was enriched by the addition of Na-acetate. However, no requirements of the p4589 related species *A. bouvetii* for Na-acetate are known (Carr et al., 2003).

The fact that none of the observed community shifts corresponded with the detection of VOSCs, indicates that VOSC production is an 'inherent' trait of bacteria found in the water under natural conditions. This is also reflected by the fact that almost no significant correlations were found, between the occurrence of VOSCs and the relative abundance of certain phylotypes. Only exception is a correlation between *Hyphomicrobium zavarzinii* and DMS. Close relatives of the methylotroph *H. zavazrinii* (Goenrich et al., 2002), were shown to degrade DMS and MT (Bont et al., 1981). Therefore, the presence of *H. zavarzinii* (p388) in one of the DMS producing 0.5 µM S-adenosylmethionine incubations (0.1 %) might be explained by DMS consumption. However, p388 was not found in the other duplicate 0.5 µM S-adenosylmethionine incubation. Additionally, most samples from day 28 (27-days incubations and untreated water) clustered together with a number of real "DMS/DMSe cases". This indicates that the community composition of these problematic waters was also not different from those found in the mineral water incubations or the untreated waters.

The phylotype p4795 was also affiliated to the species *H. zavarzinii*. As this phylotype was present in both 0.5 µM S-adenosylmethionine incubations, there is the potential that different strains of *H. zavarzinii* consume DMS in bottled mineral water. The phylotype p7506, that apparently formed part of a *Leptothrix*, *Ideonella*, *Rubrivivax* sub-cluster within the family *Burkholderiales incertia sedis* (Loy et al., 2005), was identified as an indicator for VOSC production, but its relative abundance was never above 1 %. This raises the general question if a low abundant (<1 %) community member might be involved in the production of VOSC? *Caulobacter segnis* was found to be an indicator for "no VOSC production" but this is most likely a coincidence, as *C. segnis* was enriched by the addition of glucose while VOSCs were detected only in a few glucose-containing incubations. In addition, no significant negative correlation was found between the relative abundance of *C. segnis* and VOSC production in the 27-days incubations. The other indicator species that were found by the indicator species analysis had a relative abundance below 1 % in all samples, but analogues to p7506 their role might have been underestimated by our analysis.

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5. Conclusions

Bacterial growth occurred in a mineral water after bottling and storage at ambient temperature and the growing bacterial community was dominated by members of the class *Betaproteobacteria*. It was possible to induce the production of volatile organic S/Se compounds in the water through the addition of several organic and inorganic S/Se substrates. Furthermore, two processes/pathways, possibly involved in VOSC and off-odour production, were identified, like the degradation of S/Se-containing amino acids (i.e. L-methionine and seleno-L-methionine) and the methylation of SeO_3^{2-}. Our data indicates that the composition of off-odours in this bottled mineral water might be more diverse, as L-cysteine incubations showed strong off-odours, but only weak or no production of volatile organic compounds. Some of the added compounds were able to shift the community composition of the water, however, no clear correlations were found between the enrichment of certain phylotypes and the occurrence of VOSCs. In conclusion, off-odour production seems to be an inherent trait of the 'natural' bottled mineral water community and is due to shifts in metabolic activity, induced by the availability of certain substances, rather than to unambiguous shifts in microbial community composition.

6. Summary

Bottled mineral water originates from protected groundwater sources, which are generally cold and nutrient-poor ecosystems where the majority of planktonic bacteria are dead, dormant or severely starved. The number of bacteria in non-carbonated mineral water increases rapidly, to 10^5 cells per ml, within a few days after bottling and storage at ambient temperature, but the identity and temporal community dynamics of the bottled water microbiota are largely unknown. In rare cases the microbiota tends to produce an unpleasant smell that is often related to the occurrence of volatile organic sulfur/selenium compounds such as dimethyl sulfide, dimethyl selenide and their derivates dimethyl disulfide, dimethyl trisulfide and dimethyl diselenide. Aim of this study was to gain a better understanding of the mechanisms involved in volatile sulfur/selenium compound formation and to identify responsible bacteria through parallel incubations of bottled water supplemented with different organic and inorganic sulfur- and selenium-containing compounds and/or other substrates. Formation of methylated sulfur/selenium compounds was followed by chemical analysis using GC-MS, bacterial communities were profiled by 16S rRNA gene amplicon pyrosequencing, and total cell counts were determined by fluorescence microscopy. Bacterial proliferation was observed a few days after bottling and the growing bacterial community, dominated by *Betaproteobacteria*, was able to produce volatile organic sulfur/selenium compounds in a variety of incubations. This revealed possible processes/pathways involved in methylated sulfur/selenium compound formation, including the degradation of sulfur- and selenium-containing amino acids (i.e. L-methionine and seleno-L-methionine) and the methylation of selenite. However, although the community composition of the water was influenced by some of the added compounds, no clear correlations were found between the production of volatile organic sulfur/selenium compounds and the enrichment of certain bacteria. This indicates that the potential to produce unpleasant smell is an inherent trait of the indigenous, oligotrophic microbiota growing in bottled water and (ii) volatile sulfur/selenium compound formation is due to shifts in metabolic activity, induced by the availability of certain substances, rather than to significant shifts in community composition.

7. Zusammenfassung

Natürliches Mineralwasser entstammt geschützten Grundwasserquellen. In diesen kalten nährstoffarmen Ökosystemen ist der Großteil des bakteriellen Planktons tot, dormant oder ernsthaft ausgehungert. Wenn Grundwasser jedoch in Flaschen gefüllt und bei Raumtemperatur gelagert wird, vermehren sich die planktonischen Bakterien innerhalb von wenigen Tagen und erreichen Zellzahlen von bis zu 10^5 Zellen pro mL. In seltenen Fällen kann es zu unangenehmer Geruchsentwicklung in Mineralwasser kommen, die auf die bakterielle Produktion der volatilen organischen Schwefel/Selenium Verbindungen Dimethylsulfid, Dimethylselenid und deren Derivate Dimethyldisulfid, Dimethyltrisulfid und Dimethyldiselenid zurückgeführt wird. Ziel dieser Studie war es, ein besseres Verständnis über die Prozesse zu bekommen die zur Entstehung dieser volatilen organischen Schwefel/Selenium Verbindungen in natürlichem Mineralwasser führen und die dafür verantwortlichen Bakterien zu identifizieren. Zu diesem Zweck wurde frisch abgefülltes Wasser mit verschiedenen organischen und anorganischen schwefel- und seleniumhaltigen Substraten und/oder anderen Substanzen inkubiert. In weiterer Folge wurde die Produktion von volatilen organischen Schwefel/Selenium Verbindungen in den Inkubationen gemessen, die Bakteriengemeinschaften mittels 16S rRNA Gen Amplikon-Pyrosequenzierung charakterisiert und das Zellwachstum in unbehandeltem Mineralwasser mittels Fluoreszenzmikroskopie bestimmt. Nach der Abfüllung und Lagerung bei Raumtemperatur, wurde Zellwachstum im Mineralwasser beobachtet und die entwickelte Bakteriengemeinschaft, dominiert von *Betaproteobakterien*, war in der Lage verschiedene Substrate in volatile organische Schwefel/Selenium Verbindungen umzuwandeln. Dadurch konnten Prozesse/Stoffwechselwege identifiziert werden die für die Entstehung von volatilen organischen Schwefel/Selenium Verbindungen verantwortlich sind, wie der Abbau von schwefel- und seleniumhaltigen Aminosäuren (z.B. L-Methionin und Seleno-L-Methionin) sowie die Methylierung von Selenit. Obwohl in einigen Inkubationen Veränderungen in der Zusammensetzung der Bakteriengemeinschaft induziert werden konnten, wurden keine Korrelationen zwischen dem Auftreten von volatilen organischen Schwefel/Selenium Verbindungen und der Anreicherung von bestimmten Bakterien gefunden. Daraus folgt, dass die unangenehmen Gerüche von den selben Bakterien produziert werden, die auch dann im Mineralwasser vorkommen, wenn keine Geruchsentwicklung erfolgt und dass (ii) die Entstehung von volatilen organischen Schwefel/Selenium Verbindungen auf Veränderungen der Stoffwechselaktivität, induziert durch die Verfügbarkeit von bestimmten

Substraten, zurückgeführt werden kann und nicht auf Veränderungen in der Zusammensetzung der Bakteriengemeinschaft.

8. Appendix

8.1. Supplementary Materials and Methods

Table S1. 8 mer barcodes used for identification of 16S rRNA amplicons.

Barcode ID	Barcode (5' -3')	Barcode ID	Barcode (5' -3')	Barcode ID	Barcode (5' -3')
8	AACCGGTT	213	AGAGCTCT	676	CTAGTGGT
23	AACGTACC	225	AGCAAGCA	677	CTCAACAG
26	AACGTTGC	228	AGCACATC	679	CTCAAGAC
28	AAGCAAGC	232	AGCAGATG	684	CTCACTGT
36	AAGCGGTA	234	AGCAGTTC	701	CTCTGACT
40	AAGCTTGC	246	AGCTCTTC	709	CTGAACAC
64	ACACACTG	247	AGCTGAAG	755	CTTCTGCA
73	ACACGTCA	254	AGCTTGGT	756	CTTCTGGT
74	ACACGTGT	288	AGTCACTG	759	CTTGAGCA
77	ACACTGAC	290	AGTCAGTC	761	CTTGCAAG
86	ACAGCTGT	293	AGTCCTCT	763	CTTGCTAC
88	ACAGGAGT	301	AGTCTGAC	805	GACAACAG
92	ACAGTCTG	305	AGTGAGAC	806	GACAACTC
102	ACCACTTC	310	AGTGCTGT	812	GACACTGT
104	ACCAGATC	316	AGTGTCTG	816	GACAGTGA
115	ACCTCAAG	317	AGTGTGAG	838	GAGAACTG
116	ACCTCATC	318	AGTGTGTC	866	GAGTTCTG
119	ACCTGAAC	332	ATCCATGG	869	GATCACCA
132	ACGACATC	336	ATCCGGTA	879	GATCGTAC
141	ACGATGCT	353	ATCGTTCC	884	GATCTGGT
147	ACGTCAAC	360	ATGCCGTA	887	GATGAGCA
148	ACGTCATG	368	ATGCTTGG	1022	GGTTATGC
149	ACGTCTAG	403	CAAGACCT	1023	GGTTCCAA
150	ACGTCTTC	407	CAAGCAAG	1024	GGTTCCTT
151	ACGTGAAG	409	CAAGCTAC	1137	GTTCGTAG
155	ACGTTCCT	410	CAAGCTTG	1172	TACCGGTA
156	ACGTTCGA	418	CAAGTGGA	1189	TACGTTCC
157	ACGTTGCA	419	CACAACAC	1196	TAGCCGTA
166	ACTCCTGT	442	CACTCTGT	1204	TAGCTTGG
168	ACTCGAGT	448	CACTTCTG	1237	TCACGTCT
172	ACTCTCTG	451	CAGAACAG	1238	TCACGTGA
173	ACTCTGAG	458	CAGACTGT	1239	TCACTCAC
176	ACTGACTG	487	CATCCAAG	1240	TCACTCTG
198	AGACCTGT	499	CATGACCA	1241	TCACTGAG
201	AGACGTCT	514	CATGTGGT	1243	TCAGACAC
204	AGACTCTG	534	CCATCGTT	1265	TCCACTAC
205	AGACTGAG	556	CCTACGTT	1267	TCCAGAAC
206	AGACTGTC	572	CCTTCGTA	1268	TCCAGATG
208	AGAGACTG	647	CTACAGCA	1279	TCCTCAAC
210	AGAGAGTC	649	CTACCAAG	1285	TCCTGTAC

Table S2. Sampling scheme for total cell counts (TCC), 454 pyrosequencing and chemical and sensory analysis of bottled mineral water without amendment.

		TCC		454 pyrosequencing		Chemical and senory analysis
Sampling date	Days after bottling	Filters	H_2O per filter [L]	Filters	H_2O per filter [L]	H_2O [L]
09.03.2012	1	1	3	1	3	-
11.03.2012	3	1	2.5	-	-	-
16.03.2012	8	1	0.5	-	-	-
22.03.2012	14	1	0.5	-	-	-
05.04.2012	28	1	0.5	1	3	1.5
03.05.2012	56	1	0.5	-	-	-

Table S3. Sampling scheme for total cell counts (TCC) 454 pyrosequencing and chemical and sensory analysis of bottled mineral water incubated with different S/Se compounds and/or other substrates.

			TCC		454 pyrosequencing		Chemical and senory analysis
Amendment	Conc.	Days after bottling	Filters	H_2O per filter [L]	Filters	H_2O per filter [L]	H_2O [L]
Na-sulfate	low	1	2	0.5	2	2	-
Na-sulfate	high	1	2	0.5	2	2	-
Na-sulfite	low	1	2	0.5	2	2	-
Na-sulfite	high	1	2	0.5	2	2	-
Na-selenate	low	1	2	0.5	2	2	-
Na-selenate	high	1	2	0.5	2	2	-
Na-selenite	low	1	2	0.5	2	2	-
Na-selenite	high	1	2	0.5	2	2	-
L-cysteine	low	1	2	0.5	2	2	-
L-cysteine	high	1	2	0.5	2	2	-
Seleno-L-cystine	low	1	2	0.5	2	2	-
Seleno-L-cystine	high	1	2	0.5	2	2	-
Methyl-selenocysteine	low	1	2	0.5	2	2	-
Methyl-selenocysteine	high	1	2	0.5	2	2	-
L-methionine	low	1	2	0.5	2	2	-
L-methionine	high	1	2	0.5	2	2	-
S-adenosylmethionine	-	1	2	0.5	2	2	-
Seleno-L-methionine	low	1	2	0.5	2	2	-
Seleno-L-methionine	high	1	2	0.5	2	2	-
Na-acetate	low	1	2	0.5	2	2	-
Na-acetate	high	1	2	0.5	2	2	-
Glucose	low	1	2	0.5	2	2	-
Glucose	high	1	2	0.5	2	2	-
Na-sulfite + Glucose	low	1	2	0.5	2	2	-
Na-sulfite + Glucose	high	1	2	0.5	2	2	-
Na-selenite + Glucose	low	1	2	0.5	2	2	-
Na-selenite + Glucose	high	1	2	0.5	2	2	-
Na-selenite + Glucose + Na-iodoacetate	low	1	2	0.5	2	2	-
Na-selenite + Glucose + Na-iodoacetate	high	1	2	0.5	2	2	-
Na-sulfate	low	3	2	0.5	2	0.5	-
Na-sulfate	high	3	2	0.5	2	0.5	-
Na-sulfite	low	3	2	0.5	2	0.5	-
Na-sulfite	high	3	2	0.5	2	0.5	-
Na-selenate	low	3	2	0.5	2	0.5	-
Na-selenate	high	3	2	0.5	2	0.5	-
Na-selenite	low	3	2	0.5	2	0.5	-
Na-selenite	high	3	2	0.5	2	0.5	-
L-cysteine	low	3	2	0.5	2	0.5	-
L-cysteine	high	3	2	0.5	2	0.5	-

Seleno-L-cystine	low	3	2	0.5	2	0.5	-
Seleno-L-cystine	high	3	2	0.5	2	0.5	-
Methyl-selenocysteine	low	3	2	0.5	2	0.5	-
Methyl-selenocysteine	high	3	2	0.5	2	0.5	-
L-methionine	low	3	2	0.5	2	0.5	-
L-methionine	high	3	2	0.5	2	0.5	-
S-adenosylmethionine	-	3	2	0.5	2	0.5	-
Seleno-L-methionine	low	3	2	0.5	2	0.5	-
Seleno-L-methionine	high	3	2	0.5	2	0.5	-
Na-acetate	low	3	2	0.5	2	0.5	-
Na-acetate	high	3	2	0.5	2	0.5	-
Glucose	low	3	2	0.5	2	0.5	-
Glucose	high	3	2	0.5	2	0.5	-
Na-sulfite + Glucose	low	3	2	0.5	2	0.5	-
Na-sulfite + Glucose	high	3	2	0.5	2	0.5	-
Na-selenite + Glucose	low	3	2	0.5	2	0.5	-
Na-selenite + Glucose	high	3	2	0.5	2	0.5	-
Na-selenite + Glucose + Na-iodoacetate	low	3	2	0.5	2	0.5	-
Na-selenite + Glucose + Na-iodoacetate	high	3	2	0.5	2	0.5	-
Na-sulfate	low	28	2	0.5	2	1	1
Na-sulfate	high	28	2	0.5	2	1	1
Na-sulfite	low	28	2	0.5	2	1	1
Na-sulfite	high	28	2	0.5	2	1	1
Na-selenate	low	28	2	0.5	2	1	1
Na-selenate	high	28	2	0.5	2	1	1
Na-selenite	low	28	2	0.5	2	1	1
Na-selenite	high	28	2	0.5	2	1	1
L-cysteine	low	28	2	0.5	2	1	1
L-cysteine	high	28	2	0.5	2	1	1
Seleno-L-cystine	low	28	2	0.5	2	1	1
Seleno-L-cystine	high	28	2	0.5	2	1	1
Methyl-selenocysteine	low	28	2	0.5	2	1	1
Methyl-selenocysteine	high	28	2	0.5	2	1	1
L-methionine	low	28	2	0.5	2	1	1
L-methionine	high	28	2	0.5	2	1	1
S-adenosylmethionine	-	28	2	0.5	2	1	1
Seleno-L-methionine	low	28	2	0.5	2	1	1
Seleno-L-methionine	high	28	2	0.5	2	1	1
Na-acetate	low	28	2	0.5	2	1	1
Na-acetate	high	28	2	0.5	2	1	1
Glucose	low	28	2	0.5	2	1	1
Glucose	high	28	2	0.5	2	1	1
Na-sulfite + Glucose	low	28	2	0.5	2	1	1
Na-sulfite + Glucose	high	28	2	0.5	2	1	1
Na-selenite + Glucose	low	28	2	0.5	2	1	1
Na-selenite + Glucose	high	28	2	0.5	2	1	1
Na-selenite + Glucose + Na-iodoacetate	low	28	2	0.5	2	1	1
Na-selenite + Glucose + Na-iodoacetate	high	28	2	0.5	2	1	1
Na-selenite + Glucose + Na-borohydride	low	28	2	0.5	2	1	-
Na-selenite + Glucose + Na-borohydride	high	28	2	0.5	2	1	-

Table S4. Concentration of purified barcoded 16S rRNA amplicons and respective volume added to the final amplicon pool, which corresponds to 40 ng, for 454 pyrosequencing. Samples without amendment were pooled and sequenced in a separate run.

Amendment	Conc.	Days after bottling	Barcode ID	ng/µl	µL in final pool
no amendment	nd	1	26	6.86	5.83
no amendment	nd	1	28	9.08	4.40
Na-sulfate	low	1	8	4.33	9.23
Na-sulfate	low	1	23	4.06	9.85
Na-sulfate	high	1	26	4.76	8.41
Na-sulfate	high	1	28	4.98	8.03
Na-sulfite	low	1	36	4.49	8.91
Na-sulfite	low	1	40	4.01	9.99
Na-sulfite	high	1	64	6.73	5.95
Na-sulfite	high	1	73	4.92	8.13
Na-selenate	low	1	74	4.83	8.29
Na-selenate	low	1	77	7.13	5.61
Na-selenate	high	1	86	4.14	9.65
Na-selenate	high	1	1285	5.30	7.55
Na-selenite	low	1	88	7.59	5.27
Na-selenite	low	1	92	9.33	4.29
Na-selenite	high	1	102	4.92	8.13
Na-selenite	high	1	104	6.50	6.15
L-cysteine	low	1	115	5.26	7.60
L-cysteine	low	1	116	9.29	4.31
L-cysteine	high	1	119	9.31	4.29
L-cysteine	high	1	132	7.02	5.70
Seleno-L-cystine	low	1	141	4.75	8.42
Seleno-L-cystine	low	1	147	8.57	4.67
Seleno-L-cystine	high	1	148	5.42	7.37
Seleno-L-cystine	high	1	149	7.76	5.16
Methyl-selenocysteine	low	1	150	5.11	7.83
Methyl-selenocysteine	low	1	151	5.39	7.42
Methyl-selenocysteine	high	1	155	6.89	5.80
Methyl-selenocysteine	high	1	156	5.66	7.07
L-methionine	low	1	157	4.24	9.43
L-methionine	low	1	166	4.77	8.39
L-methionine	high	1	168	7.24	5.53
L-methionine	high	1	172	6.48	6.18
S-adenosylmethionine	-	1	173	4.14	9.65
S-adenosylmethionine	-	1	176	4.32	9.26
Seleno-L-methionine	low	1	198	5.02	7.98
Seleno-L-methionine	low	1	201	6.33	6.32
Seleno-L-methionine	high	1	204	6.74	5.94
Seleno-L-methionine	high	1	205	5.13	7.79
Na-acetate	low	1	206	5.77	6.93
Na-acetate	low	1	208	6.14	6.52
Na-acetate	high	1	210	11.57	3.46
Na-acetate	high	1	213	7.02	5.70
Glucose	low	1	225	8.61	4.65
Glucose	low	1	228	4.29	9.32
Glucose	high	1	232	6.37	6.28
Glucose	high	1	234	4.70	8.52
Na-sulfite + Glucose	low	1	246	4.46	8.97
Na-sulfite + Glucose	low	1	247	6.82	5.87
Na-sulfite + Glucose	high	1	254	4.15	9.64
Na-sulfite + Glucose	high	1	288	4.22	9.47
Na-selenite + Glucose	low	1	290	4.23	9.45
Na-selenite + Glucose	low	1	293	4.97	8.05
Na-selenite + Glucose	high	1	301	4.01	9.97
Na-selenite + Glucose	high	1	305	5.96	6.71
Na-selenite + Glucose + Na-iodoacetate	low	1	310	7.41	5.40
Na-selenite + Glucose + Na-iodoacetate	low	1	316	8.28	4.83
Na-selenite + Glucose + Na-iodoacetate	high	1	317	7.67	5.21
Na-selenite + Glucose + Na-iodoacetate	high	1	318	6.62	6.04
no amendment	nd	28	102	17.04	2.35
no amendment	nd	28	104	18.09	2.21

no amendment	nd	28	115	16.14	2.48
Na-sulfate	low	28	332	7.25	5.51
Na-sulfate	low	28	336	4.60	8.69
Na-sulfate	high	28	353	7.42	5.39
Na-sulfate	high	28	360	5.76	6.95
Na-sulfite	low	28	368	6.41	6.24
Na-sulfite	low	28	403	8.07	4.95
Na-sulfite	high	28	407	4.50	8.88
Na-sulfite	high	28	409	4.63	8.63
Na-selenate	low	28	410	5.31	7.53
Na-selenate	low	28	418	4.03	9.93
Na-selenate	high	28	419	12.23	3.27
Na-selenate	high	28	442	10.53	3.80
Na-selenite	low	28	448	9.32	4.29
Na-selenite	low	28	451	4.09	9.79
Na-selenite	high	28	458	5.85	6.84
Na-selenite	high	28	487	7.62	5.25
L-cysteine	low	28	499	5.83	6.86
L-cysteine	low	28	514	12.95	3.09
L-cysteine	high	28	534	9.66	4.14
L-cysteine	high	28	556	6.35	6.30
Seleno-L-cystine	low	28	572	9.00	4.44
Seleno-L-cystine	low	28	647	8.63	4.64
Seleno-L-cystine	high	28	649	5.17	7.74
Seleno-L-cystine	high	28	676	14.36	2.79
Methyl-selenocysteine	low	28	677	4.29	9.32
Methyl-selenocysteine	low	28	679	5.17	7.74
Methyl-selenocysteine	high	28	684	6.00	6.66
Methyl-selenocysteine	high	28	701	6.41	6.24
L-methionine	low	28	709	8.97	4.46
L-methionine	low	28	755	5.43	7.36
L-methionine	high	28	756	5.31	7.53
L-methionine	high	28	759	8.51	4.70
S-adenosylmethionine	-	28	761	6.53	6.12
S-adenosylmethionine	-	28	763	4.22	9.48
Seleno-L-methionine	low	28	805	7.27	5.50
Seleno-L-methionine	low	28	806	5.13	7.79
Seleno-L-methionine	high	28	812	4.10	9.75
Seleno-L-methionine	high	28	816	9.40	4.26
Na-acetate	low	28	838	6.40	6.25
Na-acetate	low	28	866	12.15	3.29
Na-acetate	high	28	869	12.65	3.16
Na-acetate	high	28	879	11.68	3.42
Glucose	low	28	884	5.28	7.58
Glucose	low	28	887	4.39	9.11
Glucose	high	28	1022	7.45	5.37
Glucose	high	28	1023	8.57	4.67
Na-sulfite + Glucose	low	28	1024	5.20	7.69
Na-sulfite + Glucose	low	28	1137	6.91	5.78
Na-sulfite + Glucose	high	28	1172	10.24	3.91
Na-sulfite + Glucose	high	28	1189	6.93	5.77
Na-selenite + Glucose	low	28	1196	6.22	6.43
Na-selenite + Glucose	low	28	1204	6.42	6.23
Na-selenite + Glucose	high	28	1237	17.27	2.32
Na-selenite + Glucose	high	28	1238	12.49	3.20
Na-selenite + Glucose + Na-iodoacetate	low	28	1239	12.33	3.25
Na-selenite + Glucose + Na-iodoacetate	low	28	1240	7.84	5.10
Na-selenite + Glucose + Na-iodoacetate	high	28	1241	6.25	6.40
Na-selenite + Glucose + Na-iodoacetate	high	28	1243	5.20	7.69
Na-selenite + Glucose + Na-borohydride	low	28	1265	11.60	3.45
Na-selenite + Glucose + Na-borohydride	low	28	1267	8.76	4.57
Na-selenite + Glucose + Na-borohydride	high	28	1268	12.82	3.12
Na-selenite + Glucose + Na-borohydride	high	28	1279	11.72	3.41

8.2. Supplementary Results

Table S5. Concentration of volatile organic S/Se compounds in mineral water incubated with different organic and inorganic S/Se compounds and/or other substrates for 27 days and in bottled mineral water without amendment at day 28 after bottling.

Amendment	Conc.	DMS (µg L^{-1})	DMSe (µg L^{-1})	DMDS (µg L^{-1})	DMDSe (µg L^{-1})	DMTS (µg L^{-1})
no amendment	nd	0.00	0.00	0.00	0.00	0.00
no amendment	nd	0.00	0.00	0.00	0.00	0.00
no amendment	nd	0.00	0.00	0.00	0.00	0.00
Na-sulfate	low	0.00	0.00	0.00	0.00	0.00
Na-sulfate	low	0.00	0.00	0.00	0.00	0.00
Na-sulfate	high	0.00	0.00	0.00	0.00	0.00
Na-sulfate	high	0.00	0.00	0.00	0.00	0.00
Na-sulfite	low	0.00	0.00	0.00	0.00	0.00
Na-sulfite	low	0.00	0.00	0.00	0.00	0.00
Na-sulfite	high	0.00	0.00	0.00	0.00	0.00
Na-sulfite	high	0.00	0.00	0.00	0.00	0.00
Na-selenate	low	0.00	0.00	0.00	0.00	0.00
Na-selenate	low	0.00	0.00	0.00	0.00	0.00
Na-selenate	high	0.00	0.00	0.00	0.00	0.00
Na-selenate	high	0.00	0.00	0.00	0.00	0.00
Na-selenite	low	0.00	0.00	0.00	0.00	0.00
Na-selenite	low	0.00	0.00	0.00	0.00	0.00
Na-selenite	high	0.00	0.00	0.00	0.00	0.00
Na-selenite	high	0.00	0.00	0.00	0.02	0.00
L-cysteine	low	0.00	0.00	0.00	0.00	0.00
L-cysteine	low	0.00	0.00	0.00	0.00	0.00
L-cysteine	high	0.00	0.00	0.02	0.00	0.01
L-cysteine	high	0.00	0.00	0.00	0.00	0.00
Seleno-L-cystine	low	0.00	0.00	0.00	0.00	0.00
Seleno-L-cystine	low	0.00	0.00	1.90	0.00	0.00
Seleno-L-cystine	high	0.00	0.00	0.00	0.00	0.00
Seleno-L-cystine	high	0.00	0.00	0.00	0.00	0.00
Methyl-selenocysteine	low	0.00	0.00	0.00	33.60	0.00
Methyl-selenocysteine	low	0.00	0.00	0.00	34.60	0.00
Methyl-selenocysteine	high	0.00	0.00	0.00	26.40	0.00
Methyl-selenocysteine	high	0.00	0.00	0.00	30.10	0.00
L-methionine	low	0.00	0.00	105.80	4.80	6.60
L-methionine	low	0.00	0.00	98.30	2.80	5.60
L-methionine	high	0.00	0.00	90.80	1.90	6.50
L-methionine	high	0.00	0.00	77.20	1.90	5.80
S-adenosylmethionine	-	0.08	0.00	0.15	0.00	0.02
S-adenosylmethionine	-	0.07	0.00	0.09	0.00	0.01
Seleno-L-methionine	low	0.00	0.00	5.40	5.10	0.00
Seleno-L-methionine	low	0.00	0.00	32.00	4.00	0.00
Seleno-L-methionine	high	0.00	0.00	2.50	13.90	0.00
Seleno-L-methionine	high	0.00	0.00	2.00	4.00	0.00
Na-acetate	low	0.00	0.00	0.00	0.00	0.00
Na-acetate	low	0.00	0.00	0.00	0.00	0.00
Na-acetate	high	0.00	0.00	0.00	0.00	0.00
Na-acetate	high	0.00	0.00	0.00	0.00	0.00
Glucose	low	0.00	0.00	0.00	0.00	0.00
Glucose	low	0.00	0.00	0.00	0.00	0.00
Glucose	high	0.00	0.00	0.00	0.00	0.00
Glucose	high	0.00	0.00	0.00	0.00	0.00
Na-sulfite + Glucose	low	0.00	0.00	0.00	0.00	0.00
Na-sulfite + Glucose	low	0.00	0.00	0.00	0.00	0.00
Na-sulfite + Glucose	high	0.00	0.00	0.00	0.00	0.00
Na-sulfite + Glucose	high	0.00	0.00	0.00	0.00	0.00
Na-selenite + Glucose	low	0.00	0.01	0.00	0.09	0.00
Na-selenite + Glucose	low	0.00	0.03	0.00	0.12	0.00
Na-selenite + Glucose	high	0.00	0.00	0.00	0.29	0.00
Na-selenite + Glucose	high	0.00	0.00	0.00	0.38	0.00
Na-selenite + Glucose + Na-iodoacetate	low	0.00	0.00	0.00	0.00	0.00

Na-selenite + Glucose + Na-iodoacetate	low	0.00	0.00	0.00	0.00	0.00
Na-selenite + Glucose + Na-iodoacetate	high	0.00	0.00	0.00	0.00	0.00
Na-selenite + Glucose + Na-iodoacetate	high	0.00	0.00	0.00	0.00	0.00

Table S6. Intensity of off-odours in bottled mineral water incubated with different organic and inorganic S/Se compounds and/or other substrates for 27 days and in bottled mineral water without amendment at day 28 after bottling.

Amendment	Conc.	Assessors giving a defect	Sample average intensity	Conclusion
no amendment	nd	0/7	0.0	no off-odour
no amendment	nd	0/7	0.0	no off-odour
no amendment	nd	1/7	1.4	no off-odour
Na sulfate	low	0/5	0.0	no off-odour
Na sulfate	low	0/5	0.0	no off-odour
Na sulfate	high	0/5	0.0	no off-odour
Na sulfate	high	0/5	0.0	no off-odour
Na sulfite	low	1/5	0.8	no off-odour
Na sulfite	low	0/5	0.0	no off-odour
Na sulfite	high	2/5	2.8	low off-odour
Na sulfite	high	0/5	0.0	no off-odour
Na selenate	low	0/5	0.0	no off-odour
Na selenate	low	0/5	0.0	no off-odour
Na selenate	high	3/5	6.0	low off-odour
Na selenate	high	2/5	3.2	low off-odour
Na selenite	low	0/5	0.0	no off-odour
Na selenite	low	1/5	2.0	no off-odour
Na selenite	high	5/5	15.2	strong off-odour
Na selenite	high	5/5	16.0	strong off-odour
L-cysteine	low	5/5	15.6	strong off-odour
L-cysteine	low	5/5	18.0	strong off-odour
L-cysteine	high	5/5	18.0	strong off-odour
L-cysteine	high	5/5	18.8	very strong off-odour
Seleno-L-cystine	low	4/7	4.3	low off-odour
Seleno-L-cystine	low	2/7	2.3	low off-odour
Seleno-L-cystine	high	6/7	11.0	clear off-odour
Seleno-L-cystine	high	7/7	12.0	clear off-odour
Methyl-selenocysteine	low	6/7	19.0	very strong off-odour
Methyl-selenocysteine	low	7/7	16.0	strong off-odour
Methyl-selenocysteine	high	7/7	16.3	strong off-odour
Methyl-selenocysteine	high	7/7	15.7	strong off-odour
L-methionine	low	7/7	16.3	strong off-odour
L-methionine	low	7/7	16.6	strong off-odour
L-methionine	high	7/7	16.3	strong off-odour
L-methionine	high	7/7	16.3	strong off-odour
S-adenosylmethionine	-	0/7	0.0	no off-odour
S-adenosylmethionine	-	3/7	1.1	no off-odour
Seleno-L-methionine	low	7/7	14.3	strong off-odour
Seleno-L-methionine	low	7/7	12.0	clear off-odour
Seleno-L-methionine	high	7/7	12.0	clear off-odour
Seleno-L-methionine	high	7/7	10.9	clear off-odour
Na-acetate	low	2/6	1.3	no off-odour
Na-acetate	low	3/6	3.3	low off-odour
Na-acetate	high	6/6	9.3	medium off-odour
Na-acetate	high	6/6	8.7	medium off-odour
Glucose	low	6/6	6.7	medium off-odour
Glucose	low	5/6	6.3	medium off-odour
Glucose	high	3/6	2.7	low off-odour
Glucose	high	2/6	1.7	no off-odour
Na-sulfite + Glucose	low	1/6	1.0	no off-odour
Na-sulfite + Glucose	low	0/6	0.0	no off-odour
Na-sulfite + Glucose	high	0/6	0.0	no off-odour
Na-sulfite + Glucose	high	0/6	0.0	no off-odour
Na-selenite + Glucose	low	6/6	14.7	strong off-odour
Na-selenite + Glucose	low	6/6	16.3	strong off-odour
Na-selenite + Glucose	high	6/6	15.3	strong off-odour
Na-selenite + Glucose	high	6/6	15.0	strong off-odour

Na-selenite + Glucose + Na-iodoacetate	low	5/6	10.3	clear off-odour
Na-selenite + Glucose + Na-iodoacetate	low	5/6	10.7	clear off-odour
Na-selenite + Glucose + Na-iodoacetate	high	5/6	10.0	medium off-odour
Na-selenite + Glucose + Na-iodoacetate	high	5/6	10.0	medium off-odour

Table S7. Chemical parameters determined in bottled mineral water incubated with different organic and inorganic S/Se compounds and/or other substrates for 27 days and in bottled mineral water without amendment at day 28 after bottling.

Sample	Conc.	pH	Na^+ (mg L^{-1})	K^+ (mg L^{-1})	Seleno-L-methionine (µg L^{-1})	Seleno-L-cystine (µg L^{-1})	Selenite (µg L^{-1})	Selenate (µg L^{-1})
no amendment	nd	7.19	129.86	7.42	<0.25	<0.10	<0.10	1.19
no amendment	nd	7.28	131.86	7.35	<0.25	<0.10	<0.10	1.20
no amendment	nd	7.30	131.90	7.47	<0.25	<0.10	<0.10	1.19
Na-sulfate	low	7.23	202.40	7.46	<0.25	<0.10	<0.10	1.17
Na-sulfate	low	7.29	201.15	7.53	<0.25	<0.10	<0.10	1.16
Na-sulfate	high	7.30	568.00	7.70	<1.25	<0.50	<0.50	1.30
Na-sulfate	high	7.31	572.20	7.74	<1.25	<0.50	<0.50	1.15
Na-sulfite	low	7.32	137.88	7.85	<0.25	<0.10	<0.10	1.27
Na-sulfite	low	7.35	137.96	7.56	<0.25	<0.10	<0.10	1.25
Na-sulfite	high	7.90	568.20	7.99	<1.25	<0.50	<0.50	1.45
Na-sulfite	high	7.90	567.90	7.70	<1.25	<0.50	<0.50	1.55
Na-selenate	low	7.39	141.50	7.95	<1.25	<0.50	<0.50	8.45
Na-selenate	low	7.43	136.88	8.03	<1.25	<0.50	<0.50	8.15
Na-selenate	high	7.33	134.46	7.66	<0.25	<0.10	<0.10	253.30
Na-selenate	high	7.34	131.08	7.79	<0.25	<0.10	<0.10	254.30
Na-selenite	low	7.33	130.50	7.75	<1.25	<0.50	8.50	1.40
Na-selenite	low	7.35	129.80	7.46	<1.25	<0.50	8.55	1.40
Na-selenite	high	7.38	127.70	7.61	<0.25	<.0.10	652.00	1.24
Na-selenite	high	7.34	138.64	7.63	<0.25	<0.10	610.00	1.22
L-cysteine	low	7.36	136.24	7.81	<0.25	<0.10	<0.10	1.45
L-cysteine	low	7.38	130.94	7.65	<0.25	<0.10	<0.10	1.45
L-cysteine	high	7.36	133.46	7.79	<0.25	<0.10	<0.10	1.58
L-cysteine	high	7.35	131.62	7.68	<0.25	<0.10	<0.10	1.57
Seleno-L-cystine	low	7.33	129.68	7.67	<2.50	11.80	<1.00	1.10
Seleno-L-cystine	low	7.33	145.52	7.56	<2.50	9.90	<1.00	1.10
Seleno-L-cystine	high	7.32	140.84	7.70	<10.00	1800.00	<4.00	<4.00
Seleno-L-cystine	high	7.35	136.30	7.74	<10.00	2200.00	<4.00	<4.00
Methyl-selenocysteine	low	7.37	135.94	7.89	<0.25	<0.10	<0.10	1.15
Methyl-selenocysteine	low	7.38	136.06	7.80	<0.25	<0.10	<0.10	1.15
Methyl-selenocysteine	high	7.37	136.94	7.80	<0.25	<0.10	<0.10	1.20
Methyl-selenocysteine	high	7.38	132.36	7.70	<0.25	<0.10	<0.10	1.24
L-methionine	low	7.40	136.04	7.72	<0.25	<0.10	<0.10	1.20
L-methionine	low	7.42	131.40	7.65	<0.25	<0.10	<0.10	1.19
L-methionine	high	7.42	133.24	7.69	<0.25	<0.10	<0.10	1.21
L-methionine	high	7.45	131.48	7.65	<0.25	<0.10	<0.10	1.14
S-adenosylmethionine	-	7.44	133.66	7.79	<0.25	<0.10	<0.10	1.15
S-adenosylmethionine	-	7.42	144.80	7.68	<0.25	<0.10	<0.10	1.19
Seleno-L-methionine	low	7.43	141.12	7.76	116.80	<1.00	<1.00	1.40
Seleno-L-methionine	low	7.47	132.52	7.76	120.30	<1.00	<1.00	1.40
Seleno-L-methionine	high	7.45	137.06	7.90	3890.00	<4.00	<4.00	<4.00
Seleno-L-methionine	high	7.45	136.70	7.90	4050.00	<4.00	<4.00	<4.00
Na-acetate	low	7.18	139.38	7.83	<0.25	<0.10	<0.10	0.96
Na-acetate	low	7.24	138.00	7.87	<0.25	<0.10	<0.10	1.14
Na-acetate	high	7.32	154.58	7.80	<0.25	<0.10	<0.10	1.17
Na-acetate	high	7.33	150.32	7.94	<0.25	<0.10	<0.10	1.10
Glucose	low	7.33	130.66	7.92	<0.25	<0.10	<0.10	1.17
Glucose	low	7.34	129.68	7.78	<0.25	<0.10	<0.10	1.07
Glucose	high	7.35	137.98	7.99	<0.25	<0.10	<0.10	1.14
Glucose	high	7.35	133.82	7.46	<0.25	<0.10	<0.10	1.11
Na-sulfite + Glucose	low	7.37	136.36	7.65	<0.25	<0.10	<0.10	1.17
Na-sulfite + Glucose	low	7.39	136.22	7.65	<0.25	<0.10	<0.10	1.14
Na-sulfite + Glucose	high	7.93	552.10	7.65	<1.25	<0.50	<0.50	1.00
Na-sulfite + Glucose	high	7.93	557.10	7.67	<1.25	<0.50	<0.50	1.05
Na-selenite + Glucose	low	7.40	130.74	7.57	<1.25	<0.50	7.40	1.25
Na-selenite + Glucose	low	7.43	130.70	7.80	<1.25	<0.50	7.55	1.00

Na-selenite + Glucose	high	7.45	128.60	7.69	<0.25	<0.10	441.00	1.19
Na-selenite + Glucose	high	7.43	128.14	7.77	<0.25	<0.10	384.00	1.16
Na-selenite + Glucose + Na-iodoacetate	low	7.44	131.60	7.83	<1.25	<0.50	8.25	1.20
Na-selenite + Glucose + Na-iodoacetate	low	7.43	131.12	7.85	<1.25	<0.50	7.45	1.16
Na-selenite + Glucose + Na-iodoacetate	high	7.44	128.26	7.78	<0.25	<0.10	442.00	1.21
Na-selenite + Glucose + Na-iodoacetate	high	7.46	129.98	7.90	<0.25	<0.10	454.00	1.20

Table S8. Number of observed and estimated phylotypes (Chao1), Good's coverage and alpha-diversity indices at the species level (97% 16S rRNA gene identity) applying re-sampling (bootstrapping and jackknifing) at 100 and 1000 sequences of bottled mineral water incubated with different organic and inorganic S/Se compounds and/or other substrates for 4 hours and of bottled mineral water without amended at day 1 after bottling.

			Observed phylotypess		Singles		Good's Coverage		Chao1		Equitibility		Shannon		Simpson	
Description	**Conc.**	**Reads**	**100**	**1000**	**100**	**1000**	**100**	**1000**	**100**	**1000**	**100**	**1000**	**100**	**1000**	**100**	**1000**
no amendment	nd	2402	23	92	15	48	0.850	0.952	58	165	0.68	0.54	3.1	3.5	0.79	0.79
no amendment	nd	1879	22	81	14	39	0.860	0.961	56	138	0.66	0.52	2.9	3.3	0.77	0.78
Na-sulfate	low	1413	19	72	12	32	0.880	0.968	48	105	0.64	0.49	2.7	3.0	0.75	0.76
Na-sulfate	low	5633	20	78	12	42	0.880	0.958	52	149	0.70	0.53	3.0	3.4	0.80	0.80
Na-sulfate	high	3647	28	104	18	49	0.819	0.951	69	170	0.74	0.61	3.6	4.1	0.84	0.85
Na-sulfate	high	2583	22	80	14	36	0.856	0.964	55	127	0.65	0.52	2.9	3.3	0.76	0.77
Na-sulfite	low	3055	28	102	17	52	0.832	0.948	63	186	0.76	0.61	3.6	4.1	0.86	0.86
Na-sulfite	low	3123	28	103	18	47	0.816	0.953	68	162	0.71	0.59	3.4	3.9	0.82	0.83
Na-sulfite	high	2267	26	99	16	51	0.835	0.949	62	178	0.71	0.57	3.3	3.8	0.81	0.82
Na-sulfite	high	3442	30	111	18	56	0.815	0.944	69	199	0.76	0.63	3.7	4.3	0.86	0.87
Na-selenate	low	3587	29	103	18	49	0.818	0.951	65	172	0.73	0.60	3.5	4.0	0.83	0.84
Na-selenate	low	4042	23	93	16	47	0.844	0.953	65	160	0.66	0.52	3.0	3.4	0.77	0.78
Na-selenate	high	2744	26	98	16	51	0.837	0.949	61	181	0.70	0.56	3.3	3.7	0.81	0.81
Na-selenate	high	2976	27	101	18	49	0.824	0.951	67	172	0.70	0.57	3.3	3.8	0.81	0.82
Na-selenite	low	1800	19	73	12	36	0.880	0.964	51	123	0.65	0.50	2.8	3.1	0.76	0.77
Na-selenite	low	4416	13	51	7	28	0.928	0.972	29	96	0.61	0.43	2.2	2.4	0.69	0.70
Na-selenite	high	2153	19	76	13	39	0.872	0.961	52	136	0.63	0.49	2.7	3.1	0.74	0.75
Na-selenite	high	2175	20	78	13	40	0.870	0.960	53	142	0.63	0.50	2.7	3.1	0.75	0.75
L-cysteine	low	1692	16	61	10	30	0.904	0.970	39	97	0.62	0.45	2.4	2.7	0.72	0.72
L-cysteine	low	4252	18	76	12	40	0.881	0.960	50	137	0.62	0.47	2.6	2.9	0.73	0.74
L-cysteine	high	2927	36	120	22	54	0.781	0.946	73	193	0.81	0.70	4.2	4.8	0.90	0.91
L-cysteine	high	4132	40	136	24	66	0.758	0.934	86	241	0.85	0.73	4.5	5.2	0.93	0.93
Seleno-L-cystine	low	1129	10	35	5	19	0.951	0.981	18	64	0.60	0.41	2.0	2.1	0.65	0.66
Seleno-L-cystine	low	2782	10	36	5	20	0.951	0.980	18	71	0.63	0.43	2.1	2.2	0.69	0.69
Seleno-L-cystine	high	1302	29	106	18	55	0.817	0.945	66	199	0.72	0.59	3.5	4.0	0.83	0.84
Seleno-L-cystine	high	1963	32	120	21	60	0.792	0.940	77	216	0.77	0.64	3.8	4.4	0.87	0.87
Methyl-selenocysteine	low	2443	17	67	10	36	0.897	0.964	42	132	0.65	0.49	2.7	3.0	0.75	0.76
Methyl-selenocysteine	low	1639	29	91	18	37	0.818	0.963	63	141	0.73	0.62	3.6	4.0	0.84	0.84
Methyl-selenocysteine	high	1925	20	76	13	39	0.874	0.961	53	133	0.66	0.50	2.8	3.2	0.77	0.77
Methyl-selenocysteine	high	1710	15	59	10	28	0.902	0.972	40	93	0.59	0.44	2.3	2.6	0.69	0.70
L-methionine	low	710	18	na	11	na	0.886	na	45	na	0.63	na	2.6	na	0.74	na
L-methionine	low	957	18	na	12	na	0.882	na	51	na	0.62	na	2.6	na	0.74	na
L-methionine	high	648	17	na	11	na	0.890	na	45	na	0.61	na	2.5	na	0.72	na
L-methionine	high	1144	14	54	8	30	0.924	0.970	29	94	0.61	0.44	2.3	2.5	0.70	0.71
S-adenosylmethionine	nd	819	18	na	11	na	0.888	na	41	na	0.63	na	2.7	na	0.74	na
S-adenosylmethionine	nd	759	17	na	10	na	0.897	na	41	na	0.61	na	2.5	na	0.71	na
Seleno-L-methionine	low	1349	15	61	9	36	0.912	0.964	34	130	0.62	0.45	2.4	2.6	0.72	0.72
Seleno-L-methionine	low	772	14	na	8	na	0.923	na	29	na	0.66	na	2.5	na	0.76	na
Seleno-L-methionine	high	829	13	na	7	na	0.928	na	28	na	0.60	na	2.2	na	0.69	na
Seleno-L-methionine	high	1084	15	48	8	25	0.923	0.975	28	104	0.63	0.48	2.5	2.7	0.72	0.73
Na-acetate	low	471	17	na	10	na	0.899	na	37	na	0.63	na	2.5	na	0.73	na
Na-acetate	low	406	19	na	12	na	0.879	na	45	na	0.62	na	2.7	na	0.73	na
Na-acetate	high	2432	15	56	7	32	0.931	0.968	29	109	0.78	0.56	3.0	3.3	0.84	0.85
Na-acetate	high	1223	19	66	11	30	0.890	0.970	41	100	0.64	0.50	2.7	3.0	0.74	0.74
Glucose	low	3144	17	73	11	42	0.890	0.958	47	150	0.63	0.47	2.6	2.9	0.73	0.74
Glucose	low	1868	19	75	12	39	0.881	0.961	52	145	0.68	0.52	2.9	3.2	0.79	0.79
Glucose	high	2029	17	65	10	33	0.896	0.967	40	126	0.64	0.49	2.6	2.9	0.75	0.75
Glucose	high	3313	20	81	13	43	0.870	0.957	56	148	0.64	0.49	2.7	3.1	0.75	0.75
Na-sulfite + Glucose	low	3850	12	47	6	27	0.935	0.973	25	93	0.60	0.42	2.1	2.3	0.68	0.68
Na-sulfite + Glucose	low	3366	18	72	11	39	0.889	0.961	44	134	0.63	0.48	2.6	2.9	0.73	0.74
Na-sulfite + Glucose	high	3196	35	129	22	67	0.785	0.933	77	247	0.80	0.67	4.1	4.7	0.89	0.90
Na-sulfite + Glucose	high	3269	35	128	22	65	0.781	0.935	81	232	0.80	0.67	4.1	4.7	0.89	0.89
Na-selenite + Glucose	low	3648	30	108	19	54	0.813	0.946	68	195	0.74	0.61	3.6	4.1	0.84	0.85
Na-selenite + Glucose	low	2728	12	46	6	28	0.936	0.972	25	105	0.62	0.43	2.2	2.4	0.70	0.70

Na-selenite + Glucose	high	3285	14	56	9	29	0.913	0.971	35	98	0.60	0.44	2.3	2.6	0.70	0.71
Na-selenite + Glucose	high	393	12	na	7	na	0.928	na	29	na	0.59	na	2.1	na	0.68	na
Na-selenite + Glucose + Na-iodoacetate	low	2386	11	48	6	29	0.938	0.971	24	98	0.58	0.39	2.0	2.2	0.65	0.66
Na-selenite + Glucose + Na-iodoacetate	low	1890	11	39	6	21	0.944	0.979	20	68	0.59	0.41	2.0	2.2	0.66	0.66
Na-selenite + Glucose + Na-iodoacetate	high	1425	13	49	7	28	0.928	0.972	28	105	0.60	0.43	2.2	2.4	0.68	0.69
Na-selenite + Glucose + Na-iodoacetate	high	2895	13	55	8	33	0.923	0.967	31	117	0.59	0.42	2.2	2.4	0.68	0.69
Average	-	2292	20	79	12	40	0.876	0.960	48	142	0.66	0.52	2.8	3.3	0.76	0.78

Table S9. Number of observed and estimated phylotypes (Chao1), Good's coverage and alpha-diversity indices at the species level (97% 16S rRNA gene identity) applying re-sampling (bootstrapping and jackknifing) at 100 and 1000 sequences of bottled mineral water incubated with different organic and inorganic S/Se compounds and/or other substrates for 27 days and of bottled mineral water without amended at day 28 after bottling.

			Observed phylotypess		Singles		Good's Coverage		Chao1		Equitibility		Shannon		Simpson	
Description	**Conc.**	**Reads**	**100**	**1000**	**100**	**1000**	**100**	**1000**	**100**	**1000**	**100**	**1000**	**100**	**1000**	**100**	**1000**
no amendment	nd	5336	8	28	4	13	0.957	0.987	15	46	0.58	0.38	1.7	1.8	0.62	0.62
no amendment	nd	4198	7	25	4	13	0.962	0.987	12	44	0.50	0.32	1.4	1.5	0.53	0.53
no amendment	nd	4735	6	27	4	15	0.962	0.985	12	50	0.50	0.30	1.3	1.4	0.52	0.53
Na-sulfate	low	4643	12	39	6	19	0.940	0.981	22	69	0.64	0.47	2.3	2.5	0.70	0.70
Na-sulfate	low	3468	9	31	4	15	0.955	0.985	17	52	0.69	0.46	2.1	2.3	0.72	0.73
Na-sulfate	high	4373	12	42	6	22	0.942	0.978	23	77	0.65	0.46	2.3	2.5	0.70	0.71
Na-sulfate	high	4087	8	29	4	15	0.963	0.985	14	53	0.68	0.44	2.0	2.1	0.69	0.70
Na-sulfite	low	4170	10	29	5	14	0.952	0.986	16	51	0.42	0.31	1.4	1.5	0.39	0.39
Na-sulfite	low	4786	11	33	5	16	0.952	0.984	18	60	0.55	0.40	1.9	2.0	0.56	0.57
Na-sulfite	high	4627	9	31	5	15	0.949	0.985	16	52	0.29	0.21	0.9	1.0	0.23	0.24
Na-sulfite	high	4861	9	29	5	14	0.951	0.986	15	51	0.29	0.21	0.9	1.0	0.24	0.24
Na-selenate	low	3860	10	29	5	11	0.949	0.989	17	43	0.53	0.39	1.7	1.9	0.58	0.58
Na-selenate	low	4043	9	28	5	12	0.953	0.988	16	41	0.54	0.38	1.7	1.8	0.59	0.60
Na-selenate	high	5251	7	25	4	12	0.962	0.988	13	42	0.54	0.34	1.5	1.6	0.56	0.56
Na-selenate	high	4859	7	26	4	13	0.962	0.987	13	46	0.52	0.34	1.5	1.6	0.51	0.52
Na-selenite	low	4263	8	26	4	11	0.956	0.989	15	40	0.56	0.39	1.7	1.8	0.60	0.61
Na-selenite	low	3489	8	26	4	12	0.955	0.988	14	45	0.52	0.35	1.5	1.6	0.57	0.58
Na-selenite	high	3569	8	26	4	12	0.960	0.988	14	44	0.64	0.42	1.9	2.0	0.67	0.67
Na-selenite	high	2786	9	33	5	16	0.950	0.984	18	56	0.60	0.41	1.9	2.1	0.66	0.66
L-cysteine	low	3384	8	23	4	9	0.960	0.991	13	36	0.49	0.35	1.5	1.6	0.50	0.50
L-cysteine	low	2641	8	23	4	9	0.958	0.991	13	32	0.47	0.33	1.4	1.5	0.48	0.49
L-cysteine	high	4065	10	28	5	11	0.949	0.989	17	46	0.43	0.32	1.4	1.6	0.41	0.42
L-cysteine	high	5150	6	16	2	7	0.975	0.993	8	28	0.44	0.30	1.1	1.2	0.36	0.37
Seleno-L-cystine	low	2550	7	24	4	11	0.963	0.989	13	38	0.61	0.39	1.7	1.8	0.61	0.62
Seleno-L-cystine	low	2495	7	24	4	12	0.963	0.988	12	41	0.63	0.41	1.8	1.9	0.64	0.65
Seleno-L-cystine	high	3594	10	33	5	16	0.953	0.984	17	53	0.65	0.45	2.1	2.3	0.67	0.68
Seleno-L-cystine	high	1862	10	31	5	15	0.950	0.985	18	60	0.54	0.39	1.8	1.9	0.56	0.57
Methyl-selenocysteine	low	3342	7	23	3	11	0.966	0.989	12	37	0.59	0.39	1.7	1.8	0.59	0.59
Methyl-selenocysteine	low	2721	7	22	3	10	0.971	0.990	10	36	0.58	0.38	1.6	1.7	0.55	0.56
Methyl-selenocysteine	high	4317	6	22	3	11	0.967	0.989	10	38	0.47	0.29	1.2	1.3	0.45	0.45
Methyl-selenocysteine	high	3748	6	19	3	9	0.973	0.991	9	30	0.49	0.31	1.2	1.3	0.45	0.46
L-methionine	low	1322	9	30	4	16	0.959	0.984	15	56	0.72	0.49	2.3	2.4	0.75	0.76
L-methionine	low	2794	11	33	5	14	0.947	0.986	19	49	0.63	0.45	2.1	2.3	0.68	0.68
L-methionine	high	2610	11	32	5	13	0.946	0.987	20	49	0.51	0.38	1.7	1.9	0.52	0.52
L-methionine	high	1415	12	36	6	16	0.942	0.984	21	53	0.66	0.48	2.3	2.5	0.72	0.73
S-adenosylmethionine	nd	3595	9	30	5	14	0.953	0.986	15	52	0.49	0.34	1.5	1.6	0.52	0.52
S-adenosylmethionine	nd	2478	8	28	4	14	0.958	0.986	14	53	0.43	0.29	1.2	1.4	0.41	0.41
Seleno-L-methionine	low	2638	5	21	3	11	0.969	0.989	9	35	0.48	0.27	1.1	1.2	0.44	0.44
Seleno-L-methionine	low	2536	5	17	3	9	0.974	0.991	8	32	0.43	0.25	0.9	1.0	0.36	0.36
Seleno-L-methionine	high	3347	5	14	3	7	0.974	0.993	7	25	0.17	0.12	0.4	0.4	0.10	0.10
Seleno-L-methionine	high	1302	9	27	4	12	0.956	0.988	15	39	0.43	0.31	1.4	1.5	0.40	0.40
Na-acetate	low	4389	8	24	4	11	0.962	0.989	13	39	0.49	0.34	1.4	1.5	0.49	0.49
Na-acetate	low	8472	2	5	1	2	0.995	0.998	2	6	0.09	0.03	0.1	0.1	0.01	0.01
Na-acetate	high	14844	3	8	1	3	0.988	0.997	3	11	0.16	0.09	0.2	0.3	0.06	0.06
Na-acetate	high	8386	8	22	4	10	0.964	0.990	12	38	0.44	0.32	1.3	1.4	0.42	0.42
Glucose	low	3571	11	29	4	15	0.962	0.985	15	59	0.73	0.54	2.5	2.6	0.76	0.77
Glucose	low	3602	12	32	5	14	0.953	0.986	18	63	0.72	0.55	2.6	2.7	0.78	0.78
Glucose	high	3492	8	24	3	13	0.969	0.987	12	45	0.62	0.42	1.8	1.9	0.61	0.62
Glucose	high	4649	8	24	3	12	0.968	0.988	12	43	0.63	0.43	1.9	2.0	0.63	0.64
Na-sulfite + Glucose	low	5494	12	36	5	18	0.948	0.982	20	71	0.69	0.50	2.4	2.6	0.74	0.75
Na-sulfite + Glucose	low	1546	9	31	4	20	0.958	0.980	15	143	0.52	0.36	1.6	1.8	0.53	0.54
Na-sulfite + Glucose	high	3650	9	27	5	10	0.955	0.990	15	39	0.59	0.41	1.8	2.0	0.62	0.63
Na-sulfite + Glucose	high	4100	7	26	4	13	0.957	0.987	14	47	0.49	0.32	1.4	1.5	0.53	0.53
Na-selenite + Glucose	low	2831	9	29	5	13	0.949	0.987	17	46	0.44	0.32	1.4	1.6	0.44	0.44
Na-selenite + Glucose	low	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na
Na-selenite + Glucose	high	2625	9	27	4	12	0.956	0.988	15	43	0.51	0.37	1.6	1.7	0.54	0.55
Na-selenite + Glucose	high	7609	9	27	4	13	0.962	0.987	14	45	0.56	0.40	1.8	1.9	0.59	0.60
Na-selenite + Glucose + Na-iodoacetate	low	8398	5	13	2	4	0.978	0.996	7	17	0.34	0.23	0.8	0.8	0.25	0.26
Na-selenite + Glucose + Na-iodoacetate	low	4896	6	15	3	5	0.972	0.995	9	21	0.40	0.29	1.1	1.1	0.35	0.35

Na-selenite + Glucose + Na-iodoacetate	high	2901	11	26	5	10	0.954	0.990	16	45	0.59	0.46	2.0	2.1	0.64	0.65
Na-selenite + Glucose + Na-iodoacetate	high	2748	7	22	4	11	0.963	0.989	11	42	0.51	0.33	1.4	1.5	0.54	0.54
Na-selenite + Glucose + Na-borohydride	low	11201	8	22	4	8	0.961	0.992	13	35	0.56	0.40	1.7	1.8	0.59	0.59
Na-selenite + Glucose + Na-borohydride	low	5954	9	26	4	11	0.957	0.989	14	43	0.36	0.27	1.1	1.3	0.32	0.32
Na-selenite + Glucose + Na-borohydride	high	8478	7	20	3	7	0.966	0.993	12	28	0.54	0.37	1.5	1.6	0.53	0.53
Na-selenite + Glucose + Na-borohydride	high	8140	7	20	3	8	0.965	0.992	11	31	0.29	0.21	0.8	0.9	0.22	0.23
Average	-	4333	8	26	4	12	0.960	0.988	14	45	0.51	0.35	1.5	1.7	0.52	0.52

Table S10. Permutational multivariate ANOVA with the 16S rRNA sequence dataset derived from planktonic bacterial communities of bottled mineral water incubated with different S/Se compound and/or other substrates after 4 hours and 27 days of incubation and of bottled mineral water without amendment at day 1 and at day 28 after bottling.

	Df	SumsOfSqs	MeanSqs	F.Model	R2	Pr(>F)	Significance
Days_After_Bottling	0.01	20.18	20.18	188.66	0.56	0.00	***
Amendment_type	0.18	4.64	0.26	2.41	0.13	0.00	***
Residuals	1.04	11.13	0.10		0.31		
Total	1.23	35.95			1.00		

Df – degrees of freedom; SumsOfSqs – sums of squares; MeanSq – Mean of squares; F.Model – F ratio in the analysis; R2 – coefficient of determination; Pr(>F) – probability;

Table S11. Permutational multivariate ANOVA with the 16S rRNA sequence dataset derived from planktonic bacterial communities of bottled mineral water incubated with different S/Se compound and/or other substrates for 27 days and of bottled mineral water without amendment at day 28 after bottling.

	Df	SumsOfSqs	MeanSqs	F.Model	R2	Pr(>F)	Significance
VOSC	2	0.933	0.46651	3.7612	0.06732	0.000999	***
Sensory_analysis	4	1.3283	0.33208	2.6774	0.09585	0.003996	**
Amendment_type	16	6.5121	0.40701	3.2815	0.46989	0.000999	***
Residuals	41	5.0852	0.12403		0.36694		
Total	63	13.8587			1		

Df – degrees of freedom; SumsOfSqs – sums of squares; MeanSq – Mean of squares; F.Model – F ratio in the analysis; R2 – coefficient of determination; Pr(>F) – probability;

Table S 12. Relative abundance of depicted bacterial phyla/classes in bottled mineral water incubated for 4 hours with different S/Se compounds and/or other substrates and in bottled mineral water without amendment 1 day after bottling.

Amendment	Conc.	Alphaproteobacteria	Betaproteobacteria	Gammaproteobacteria	Nitrospirae	Actinobacteria	Cyanobacteria	Unclassified Bacteria
no amendment	nd	2.6	7.4	78.7	1.4	1.3	1.3	4.5
no amendment	nd	2.9	2.2	83.0	2.3	1.0	0.5	4.7
Na-sulfate	low	0.1	2.7	84.6	1.1	0.1	0.1	7.2
Na-sulfate	low	7.2	2.3	79.5	1.2	0.1	0.4	5.8
Na-sulfate	high	0.1	2.7	69.9	2.0	0.3	0.7	17.4
Na-sulfate	high	0.3	2.8	82.6	0.9	0.2	0.5	8.0
Na-sulfite	low	0.3	2.1	72.7	1.9	0.6	0.7	12.7
Na-sulfite	low	0.0	2.7	70.4	2.1	0.2	0.7	16.2
Na-sulfite	high	0.2	2.9	74.6	2.4	0.3	0.6	12.9
Na-sulfite	high	0.0	2.6	66.4	3.1	0.1	0.6	18.5
Na-selenate	low	2.5	2.7	69.0	3.3	0.3	0.8	13.4
Na-selenate	low	0.0	3.8	79.2	1.7	0.2	0.8	8.9
Na-selenate	high	0.0	3.4	76.1	1.1	0.2	0.9	12.2
Na-selenate	high	0.1	4.2	72.8	1.8	0.5	1.0	12.2
Na-selenite	low	0.1	4.7	83.9	2.1	0.2	0.3	5.4
Na-selenite	low	0.3	2.9	91.3	1.1	0.1	0.3	2.6

Na-selenite	high	1.3	2.5	84.7	1.4	0.1	0.8	5.4
Na-selenite	high	0.1	3.8	82.7	1.5	0.2	0.6	6.3
L-cysteine	low	0.0	3.4	89.4	0.8	0.1	0.1	4.1
L-cysteine	low	0.0	3.2	85.5	1.6	0.1	0.8	6.1
L-cysteine	high	0.1	3.4	55.1	5.8	0.3	1.1	21.4
L-cysteine	high	0.3	3.0	47.4	9.1	0.3	1.8	24.8
Seleno-L-cystine	low	0.1	2.4	94.6	0.0	0.0	1.2	0.8
Seleno-L-cystine	low	0.0	2.6	94.4	0.6	0.0	0.6	0.9
Seleno-L-cystine	high	0.2	2.8	71.2	3.1	0.2	1.2	14.6
Seleno-L-cystine	high	0.1	2.4	64.0	6.1	0.3	1.2	17.3
Methyl-selenocysteine	low	0.1	3.3	87.1	0.7	0.2	0.5	5.9
Methyl-selenocysteine	low	0.1	1.8	70.9	2.3	0.2	2.3	16.0
Methyl-selenocysteine	high	0.2	3.2	84.4	1.1	0.1	0.4	7.0
Methyl-selenocysteine	high	0.3	1.6	89.7	1.2	0.0	0.7	4.0
L-methionine	low	0.0	3.5	85.3	1.8	0.0	0.6	6.4
L-methionine	low	0.0	2.4	87.3	1.4	0.1	0.3	6.3
L-methionine	high	0.0	3.9	86.2	1.1	0.5	1.1	4.9
L-methionine	high	2.5	3.6	89.8	0.3	0.4	0.1	2.1
S-adenosylmethionine	-	0.4	3.2	86.1	1.5	0.0	0.0	5.9
S-adenosylmethionine	-	0.0	2.9	88.1	0.8	0.3	0.8	5.0
Seeno-L-methionine	low	0.0	2.8	89.1	0.9	0.1	0.7	4.8
Seleno-L-methionine	low	0.0	2.9	92.9	0.4	0.1	0.1	2.9
Seleno-L-methionine	high	0.0	2.5	92.6	0.4	0.0	0.2	3.0
Seleno-L-methionine	high	0.1	4.0	89.9	1.1	0.0	0.4	3.5
Na-acetate	low	0.0	3.0	88.1	0.6	0.0	0.9	5.7
Na-acetate	low	0.0	2.2	88.4	1.5	0.0	0.2	4.4
Na-acetate	high	5.2	41.4	50.7	0.2	0.0	0.1	1.2
Na-acetate	high	5.8	3.8	83.4	0.6	0.7	0.2	2.9
Glucose	low	0.2	4.6	86.8	0.9	0.2	0.4	4.0
Glucose	low	0.1	2.9	86.3	1.6	0.1	0.9	5.1
Glucose	high	2.3	2.7	84.3	1.9	0.6	0.5	4.6
Glucose	high	0.0	3.9	81.9	1.3	0.2	0.9	8.2
Na-sulfite + Glucose	low	0.2	3.3	92.0	0.3	0.1	0.3	2.8
Na-sulfite + Glucose	low	0.0	3.2	83.5	2.6	0.4	0.4	4.2
Na-sulfite + Glucose	high	0.1	2.9	57.0	7.3	0.1	1.5	21.1
Na-sulfite + Glucose	high	0.2	2.6	60.0	4.5	0.1	1.6	18.3
Na-selenite + Glucose	low	0.2	3.1	65.7	2.5	0.1	1.6	18.3
Na-selenite + Glucose	low	0.4	2.3	93.3	0.5	0.0	0.3	1.8
Na-selenite + Glucose	high	0.1	3.7	89.0	1.6	0.0	0.6	2.7
Na-selenite + Glucose	high	0.3	2.0	93.6	0.3	0.0	0.3	2.3
Na-selenite + Glucose + Na-iodoacetate	low	0.3	2.3	94.5	0.5	0.0	0.2	1.1
Na-selenite + Glucose + Na-iodoacetate	low	0.1	3.0	94.5	0.6	0.2	0.0	1.2
Na-selenite + Glucose + Na-iodoacetate	high	0.7	2.9	92.2	0.6	0.1	0.0	2.4
Na-selenite + Glucose + Na-iodoacetate	high	0.1	2.9	92.6	0.8	0.0	0.1	1.9

Values are given in %

Table S13. Relative abundance of depicted phylotypes in bottled mineral water incubated for 4 hours with different S/Se compounds and/or other substrates and in bottled mineral water without amendment 1 day after bottling.

Amendment	Conc.	p1494	p6572	p2483	p6525	p8483	p4236	p4589	p4680	p6328	p8404
no amendment	nd	0.5	1.6	1.7	2.3	2.2	7.7	2.12	2.7	27.3	34.9
no amendment	nd	0.0	2.1	1.3	0.2	0.1	8.4	2.24	3.5	33.5	31.1
Na-sulfate	low	0.0	0.0	2.3	0.0	0.1	8.6	0.2	3.9	32.4	35.6
Na-sulfate	low	0.0	0.0	1.6	0.1	0.1	7.6	4.2	3.9	30.0	30.0
Na-sulfate	high	0.0	0.0	1.8	0.1	0.2	8.3	0.2	3.4	26.4	25.5
Na-sulfate	high	0.0	0.0	1.9	0.0	0.1	6.7	0.5	2.8	30.2	36.6
Na-sulfite	low	0.0	0.0	1.7	0.1	0.2	6.7	9.7	3.9	21.2	26.6
Na-sulfite	low	0.0	0.0	2.0	0.0	0.4	6.1	0.4	3.4	27.1	29.8
Na-sulfite	high	0.0	0.0	2.3	0.1	0.2	4.0	0.2	3.1	27.9	30.7
Na-sulfite	high	0.0	0.0	1.6	0.1	0.7	5.3	1.0	1.8	21.5	27.0
Na-selenate	low	0.0	2.4	1.7	0.2	0.6	7.8	0.0	2.8	24.7	29.6

Na-selenate	low	0.0	0.0	3.0	0.0	0.2	7.0	0.0	3.5	29.4	35.4
Na-selenate	high	0.0	0.0	2.8	0.1	0.1	4.5	1.0	4.3	26.7	32.7
Na-selenate	high	0.0	0.0	3.4	0.2	0.3	8.0	0.2	2.9	25.3	32.5
Na-selenite	low	0.0	0.1	3.6	0.1	0.6	9.0	0.1	4.8	34.0	32.2
Na-selenite	low	0.0	0.2	2.5	0.0	0.2	7.6	0.2	4.9	37.9	38.7
Na-selenite	high	0.0	1.3	1.3	0.0	0.7	8.1	0.2	4.3	36.4	32.5
Na-selenite	high	0.0	0.0	3.3	0.0	0.3	5.4	0.7	3.8	32.6	36.5
L-cysteine	low	0.0	0.0	2.9	0.1	0.2	8.9	0.9	3.4	38.0	34.9
L-cysteine	low	0.0	0.0	2.7	0.0	0.2	6.7	0.2	4.0	35.1	36.0
L-cysteine	high	0.0	0.0	1.5	0.2	1.3	5.0	0.1	2.6	21.5	19.3
L-cysteine	high	0.0	0.0	1.3	0.3	1.1	2.7	0.0	2.1	16.0	16.1
Seleno-L-cystine	low	0.0	0.0	2.0	0.0	0.0	7.7	0.0	3.6	34.4	46.5
Seleno-L-cystine	low	0.0	0.0	2.4	0.1	0.1	10.8	0.4	4.5	39.5	37.2
Seleno-L-cystine	high	0.0	0.1	1.8	0.1	0.2	5.6	0.2	3.2	28.1	27.7
Seleno-L-cystine	high	0.0	0.0	1.9	0.1	0.0	7.4	0.3	4.4	22.4	25.1
Methyl-selenocysteine	low	0.0	0.0	2.7	0.0	0.3	8.3	4.3	3.4	33.0	34.7
Methyl-selenocysteine	low	0.0	0.0	1.1	0.0	0.5	6.8	0.1	3.3	29.1	24.6
Methyl-selenocysteine	high	0.0	0.0	2.7	0.0	0.2	9.9	0.9	5.0	30.1	35.0
Methyl-selenocysteine	high	0.0	0.3	1.2	0.0	0.1	7.8	0.6	3.6	44.5	30.6
L-methionine	low	0.0	0.0	3.3	0.0	0.3	9.2	0.0	3.1	31.5	37.5
L-methionine	low	0.0	0.0	1.8	0.4	0.0	8.9	0.1	5.0	34.8	35.3
L-methionine	high	0.0	0.0	3.6	0.2	0.0	5.6	0.0	3.1	31.7	41.4
L-methionine	high	0.0	2.5	2.7	0.8	0.0	7.3	0.2	3.1	36.7	38.7
S-adenosylmethionine	-	0.0	0.2	2.4	0.0	0.4	7.3	2.2	3.1	31.7	38.1
S-adenosylmethionine	-	0.0	0.0	2.6	0.0	0.1	6.1	0.0	3.7	36.8	37.3
Seleno-L-methionine	low	0.0	0.0	2.5	0.1	0.1	10.0	0.1	3.3	37.1	35.8
Seleno-L-methionine	low	0.0	0.0	2.7	0.1	0.0	20.2	1.6	4.2	28.1	33.7
Seleno-L-methionine	high	0.0	0.0	2.2	0.0	0.4	5.3	2.7	4.5	35.1	42.5
Seleno-L-methionine	high	0.0	0.0	3.8	0.1	0.0	6.4	2.9	3.7	36.3	36.2
Na-acetate	low	0.0	0.0	3.0	0.0	0.0	8.9	0.2	2.8	29.1	41.5
Na-acetate	low	0.0	0.0	2.0	0.0	0.0	4.9	0.2	2.7	29.6	41.4
Na-acetate	high	4.6	0.0	1.2	18.0	11.8	3.6	0.2	1.8	19.6	23.8
Na-acetate	high	0.0	5.3	2.3	1.2	0.0	5.6	1.3	2.0	26.7	42.2
Glucose	low	0.0	0.0	3.4	0.2	0.4	8.9	1.1	3.2	34.1	36.3
Glucose	low	0.0	0.1	2.3	0.0	0.3	9.5	7.9	3.8	29.9	31.6
Glucose	high	0.0	2.2	2.1	0.0	0.1	10.0	0.1	3.9	30.8	37.1
Glucose	high	0.0	0.0	3.2	0.0	0.4	5.8	0.0	4.6	30.1	38.5
Na-sulfite + Glucose	low	0.0	0.0	3.0	0.0	0.0	6.8	0.0	4.3	39.2	39.2
Na-sulfite + Glucose	low	0.0	0.0	2.3	0.3	0.3	5.8	0.0	3.6	35.4	35.5
Na-sulfite + Glucose	high	0.0	0.0	1.2	0.1	0.7	6.5	0.0	1.8	22.1	19.5
Na-sulfite + Glucose	high	0.0	0.1	1.5	0.2	0.6	4.9	0.2	3.0	19.7	23.3
Na-selenite + Glucose	low	0.0	0.0	2.1	0.0	0.7	5.3	0.2	3.2	25.6	28.2
Na-selenite + Glucose	low	0.0	0.4	2.0	0.0	0.1	10.7	0.0	5.1	35.4	39.5
Na-selenite + Glucose	high	0.0	0.0	3.0	0.1	0.3	7.1	0.4	3.5	38.1	37.7
Na-selenite + Glucose	high	0.0	0.0	1.3	0.0	0.5	8.2	0.0	4.1	38.5	40.3
Na-selenite + Glucose + Na-iodoacetate	low	0.0	0.2	2.0	0.0	0.1	6.8	0.0	3.4	37.1	44.3
Na-selenite + Glucose + Na-iodoacetate	low	0.0	0.0	2.3	0.0	0.0	6.6	0.0	3.3	38.2	42.8
Na-selenite + Glucose + Na-iodoacetate	high	0.0	0.6	2.4	0.3	0.1	5.9	0.0	5.2	33.6	44.0
Na-selenite + Glucose + Na-iodoacetate	high	0.0	0.0	2.0	0.0	0.1	7.8	0.6	3.7	36.5	40.9

Values are given in %

Table S 14. Relative abundance of depicted bacterial phyla/classes in bottled mineral water incubated for 27 days with different S/Se compounds and/or other substrates and in bottled mineral water without amendment 28 days after bottling.

Amendment	Conc.	Alphaproteobacteria	Betaproteobacteria	Gammaproteobacteria	Nitrospirae	Actinobacteria	Cyanobacteria	Unclassified Bacteria
no amendment	nd	10.9	88.7	0.2	0.0	0.0	0.0	0.0
no amendment	nd	3.5	95.7	0.8	0.0	0.0	0.0	0.0
no amendment	nd	2.4	96.8	0.6	0.0	0.0	0.0	0.1
Na-sulfate	low	14.1	73.7	8.6	0.0	0.0	0.0	0.1
Na-sulfate	low	10.8	88.3	0.7	0.0	0.0	0.0	0.1
Na-sulfate	high	21.2	77.3	0.8	0.0	0.0	0.0	0.2
Na-sulfate	high	12.8	86.1	0.9	0.0	0.0	0.0	0.1
Na-sulfite	low	2.4	94.5	2.6	0.0	0.0	0.0	0.2
Na-sulfite	low	0.8	93.2	5.3	0.0	0.1	0.0	0.1
Na-sulfite	high	0.0	95.6	3.3	0.2	0.0	0.0	0.6
Na-sulfite	high	0.0	95.2	4.2	0.0	0.0	0.0	0.3
Na-selenate	low	5.8	91.6	2.4	0.1	0.0	0.0	0.0
Na-selenate	low	5.1	91.3	3.5	0.0	0.0	0.0	0.0
Na-selenate	high	4.6	94.3	1.0	0.0	0.0	0.0	0.1
Na-selenate	high	9.3	89.2	1.3	0.0	0.0	0.0	0.0
Na-selenite	low	8.4	90.3	1.1	0.0	0.0	0.0	0.1
Na-selenite	low	3.1	94.6	2.3	0.0	0.0	0.0	0.0
Na-selenite	high	20.3	78.4	1.2	0.0	0.0	0.0	0.0
Na-selenite	high	10.1	87.9	1.7	0.0	0.0	0.0	0.1
L-cysteine	low	1.2	98.0	0.7	0.0	0.0	0.0	0.1
L-cysteine	low	0.4	98.9	0.7	0.0	0.0	0.0	0.0
L-cysteine	high	0.0	86.8	13.1	0.0	0.0	0.0	0.1
L-cysteine	high	0.0	9.8	90.1	0.1	0.0	0.0	0.0
Seleno-L-cystine	low	29.3	68.5	2.0	0.0	0.0	0.0	0.0
Seleno-L-cystine	low	19.9	78.4	1.5	0.0	0.0	0.2	0.1
Seleno-L-cystine	high	13.3	85.7	0.7	0.0	0.0	0.0	0.1
Seleno-L-cystine	high	13.7	85.2	0.9	0.0	0.1	0.0	0.0
Methyl-selenocysteine	low	1.8	97.6	0.5	0.1	0.0	0.0	0.1
Methyl-selenocysteine	low	3.3	96.0	0.6	0.0	0.0	0.0	0.1
Methyl-selenocysteine	high	1.6	97.8	0.5	0.0	0.0	0.0	0.1
Methyl-selenocysteine	high	3.7	95.8	0.5	0.0	0.0	0.0	0.0
L-methionine	low	11.5	86.7	1.6	0.0	0.0	0.0	0.2
L-methionine	low	14.2	84.6	0.9	0.0	0.0	0.0	0.0
L-methionine	high	7.8	90.5	1.5	0.0	0.0	0.0	0.2
L-methionine	high	7.2	88.9	2.4	0.0	0.0	0.0	0.1
S-adenosylmethionine	-	4.7	94.3	0.6	0.0	0.0	0.1	0.1
S-adenosylmethionine	-	5.2	93.8	0.8	0.0	0.0	0.0	0.0
Seleno-L-methionine	low	0.2	99.1	0.7	0.0	0.0	0.0	0.0
Seleno-L-methionine	low	0.0	98.6	1.3	0.0	0.0	0.0	0.0
Seleno-L-methionine	high	0.1	98.6	1.1	0.0	0.1	0.0	0.1
Seleno-L-methionine	high	0.0	93.4	6.5	0.0	0.0	0.0	0.1
Na-acetate	low	21.5	76.4	2.0	0.0	0.0	0.0	0.0
Na-acetate	low	0.2	0.2	99.6	0.0	0.0	0.0	0.0
Na-acetate	high	2.5	0.4	97.1	0.0	0.0	0.0	0.0
Na-acetate	high	19.4	79.5	0.9	0.0	0.0	0.0	0.1
Glucose	low	42.6	50.3	6.6	0.2	0.0	0.0	0.1
Glucose	low	33.6	63.8	2.4	0.0	0.0	0.0	0.1
Glucose	high	23.1	76.1	0.5	0.0	0.0	0.0	0.2
Glucose	high	25.2	74.0	0.6	0.0	0.0	0.0	0.1
Na-sulfite + Glucose	low	19.0	70.6	9.8	0.0	0.1	0.1	0.1
Na-sulfite + Glucose	low	21.3	67.1	10.8	0.0	0.0	0.1	0.5
Na-sulfite + Glucose	high	38.8	60.7	0.3	0.0	0.0	0.0	0.1
Na-sulfite + Glucose	high	35.8	63.3	0.4	0.0	0.0	0.0	0.3

Na-selenite + Glucose	low	14.7	83.3	1.5	0.0	0.0	0.0	0.2
Na-selenite + Glucose	high	5.0	94.4	0.3	0.0	0.0	0.0	0.1
Na-selenite + Glucose	high	8.1	91.2	0.5	0.0	0.0	0.0	0.1
Na-selenite + Glucose + Na-iodoacetate	low	2.4	0.2	97.4	0.0	0.0	0.0	0.0
Na-selenite + Glucose + Na-iodoacetate	low	3.3	0.1	96.5	0.0	0.0	0.0	0.0
Na-selenite + Glucose + Na-iodoacetate	high	32.8	59.3	7.5	0.0	0.0	0.0	0.1
Na-selenite + Glucose + Na-iodoacetate	high	40.1	57.6	1.7	0.1	0.0	0.0	0.4
Na-selenite + Glucose + Na-borohydride	low	2.2	34.0	63.7	0.0	0.0	0.0	0.1
Na-selenite + Glucose + Na-borohydride	low	3.0	93.8	3.0	0.1	0.0	0.0	0.1
Na-selenite + Glucose + Na-borohydride	high	1.4	24.4	74.1	0.0	0.0	0.0	0.0
Na-selenite + Glucose + Na-borohydride	high	4.5	94.6	0.8	0.0	0.0	0.0	0.0

Values are given in %

Table S15. Relative abundance of depicted phylotypes in bottled mineral water incubated for 27 days with different S/Se compounds and/or other substrates and in bottled mineral water without amendment 28 days after bottling.

Amendment	Conc.	p388	p1494	p3515	p4795	p8915	p1685	p2380	p6525	p7506	p8483	p4589	p4876	p6328
no amendment	nd	0.0	9.6	0.0	0.2	0.4	0.4	0.3	36.3	0.0	48.6	0.0	0.0	0.1
no amendment	nd	0.0	2.1	0.0	0.4	0.4	0.0	0.0	34.3	0.0	59.1	0.0	0.1	0.2
no amendment	nd	0.0	1.3	0.0	0.4	0.1	0.1	0.1	36.2	0.0	58.5	0.0	0.1	0.2
Na-sulfate	low	0.0	12.2	0.0	0.3	0.2	0.0	0.2	49.1	0.0	19.1	0.0	0.0	6.6
Na-sulfate	low	0.0	10.0	0.1	0.3	0.4	0.1	0.4	39.7	0.0	25.6	0.1	0.0	0.3
Na-sulfate	high	3.1	13.9	0.0	2.8	0.0	0.0	6.6	47.9	0.0	19.4	0.1	0.0	0.2
Na-sulfate	high	0.0	11.7	0.0	0.0	0.0	0.0	8.7	35.4	0.0	39.6	0.0	0.0	0.3
Na-sulfite	low	0.0	0.0	0.0	0.5	1.6	0.0	4.1	8.4	0.0	77.2	0.0	0.0	1.2
Na-sulfite	low	0.0	0.0	0.0	0.6	0.2	0.0	4.7	13.3	0.0	63.5	0.0	0.0	2.1
Na-sulfite	high	0.0	0.0	0.0	0.0	0.0	0.0	3.5	87.2	0.0	0.0	0.0	0.2	1.1
Na-sulfite	high	0.0	0.0	0.0	0.0	0.0	0.0	3.5	87.2	0.0	0.0	0.0	0.3	1.4
Na-selenate	low	0.0	4.5	0.0	0.3	0.1	0.0	0.3	58.0	0.0	28.0	0.0	0.0	0.7
Na-selenate	low	0.0	4.2	0.0	0.1	0.1	0.8	0.1	35.0	0.0	52.6	0.0	0.0	1.3
Na-selenate	high	0.0	4.5	0.0	0.0	0.0	0.1	0.1	35.4	0.0	55.6	0.0	0.0	0.2
Na-selenate	high	0.0	8.5	0.0	0.3	0.1	0.0	0.4	21.6	0.0	65.1	0.0	0.0	0.6
Na-selenite	low	0.0	7.5	0.0	0.3	0.2	0.0	0.8	51.4	0.0	34.7	0.0	0.0	0.5
Na-selenite	low	0.0	2.3	0.0	0.6	0.0	0.0	0.4	48.4	0.0	43.3	0.0	0.0	0.8
Na-selenite	high	0.0	18.6	0.0	0.3	0.0	0.0	0.1	33.7	0.1	42.0	0.0	0.0	0.4
Na-selenite	high	0.0	9.5	0.0	0.2	0.0	0.0	0.3	44.3	0.0	36.1	0.0	0.0	0.8
L-cysteine	low	0.0	0.0	0.0	0.0	1.1	0.0	0.4	66.7	0.0	22.6	0.0	0.0	0.1
L-cysteine	low	0.0	0.0	0.0	0.0	0.4	0.1	0.3	67.2	0.0	24.4	0.0	0.0	0.2
L-cysteine	high	0.0	0.0	0.0	0.0	0.0	0.0	0.1	75.2	0.0	3.7	0.0	0.0	11.0
L-cysteine	high	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	9.1	0.0	0.0	78.3
Seleno-L-cystine	low	0.0	28.6	0.0	0.4	0.0	0.0	0.1	13.6	0.0	52.9	0.0	0.0	0.6
Seleno-L-cystine	low	0.0	19.3	0.0	0.4	0.0	0.0	0.0	26.9	0.1	49.0	0.0	0.0	0.7
Seleno-L-cystine	high	0.0	11.3	0.0	1.9	0.0	0.0	14.1	51.3	0.0	15.9	0.1	0.0	0.3
Seleno-L-cystine	high	0.0	10.8	0.0	2.6	0.0	0.0	0.9	62.6	0.1	16.8	0.0	0.0	0.3
Methyl-selenocysteine	low	0.0	1.7	0.0	0.0	0.0	0.0	12.2	24.4	0.0	57.7	0.0	0.0	0.2
Methyl-selenocysteine	low	0.0	3.2	0.0	0.0	0.0	0.0	16.2	14.8	0.1	62.7	0.0	0.0	0.0
Methyl-selenocysteine	high	0.0	1.6	0.0	0.0	0.0	0.0	0.1	24.8	0.0	69.7	0.0	0.0	0.2
Methyl-selenocysteine	high	0.0	3.6	0.0	0.0	0.0	0.0	0.0	23.4	0.1	69.7	0.0	0.0	0.2
L-methionine	low	0.0	11.0	0.0	0.0	0.2	2.3	23.6	30.9	0.0	27.7	0.0	0.0	0.6
L-methionine	low	0.0	13.8	0.0	0.0	0.2	1.2	22.7	49.4	0.0	5.2	0.0	0.0	0.2
L-methionine	high	0.0	6.6	0.0	0.0	0.0	0.0	11.1	67.8	0.0	6.3	0.0	0.0	0.5
L-methionine	high	0.0	7.2	0.0	0.0	0.0	1.3	16.0	43.1	0.1	24.0	0.1	0.0	0.7
S-adenosylmethionine	-	0.1	3.1	0.0	0.5	0.1	0.0	1.8	64.5	0.0	24.9	0.1	0.0	0.3
S-adenosylmethionine	-	0.0	4.4	0.0	0.2	0.0	0.0	0.5	75.0	0.0	15.2	0.0	0.0	0.3
Seleno-L-methionine	low	0.0	0.0	0.0	0.2	0.0	0.0	0.0	26.6	0.1	69.7	0.0	0.0	0.2
Seleno-L-methionine	low	0.0	0.0	0.0	0.0	0.0	0.0	0.0	18.9	0.0	77.8	0.0	0.0	0.4
Seleno-L-methionine	high	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.0	0.1	94.8	0.0	0.0	0.6
Seleno-L-methionine	high	0.0	0.0	0.0	0.0	0.0	0.0	0.2	3.0	0.2	76.3	0.0	0.0	4.8
Na-acetate	low	0.0	20.3	0.0	0.0	0.9	0.0	0.1	5.4	0.1	68.2	1.2	0.0	0.2
Na-acetate	low	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	99.4	0.0	0.0
Na-acetate	high	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2	96.8	0.0	0.0
Na-acetate	high	0.0	15.1	0.0	0.0	1.3	0.0	0.1	2.8	0.0	74.4	0.4	0.0	0.1
Glucose	low	0.0	19.7	0.5	0.0	22.1	0.1	35.8	9.3	0.0	3.7	0.0	0.0	2.4

Glucose	low	0.0	21.7	0.1	0.0	4.6	0.4	21.1	34.3	0.0	4.4	0.0	0.0	1.1
Glucose	high	0.0	2.8	0.2	0.0	20.1	57.0	4.6	12.4	0.0	0.5	0.0	0.0	0.1
Glucose	high	0.0	2.4	0.3	0.0	22.3	54.4	4.8	12.3	0.0	0.3	0.0	0.0	0.2
Na-sulfite + Glucose	low	0.0	9.9	0.1	0.0	8.4	1.0	37.2	29.8	0.0	0.7	0.0	0.1	5.4
Na-sulfite + Glucose	low	0.0	0.1	0.2	0.0	21.0	0.6	64.4	0.3	0.0	0.8	0.0	0.0	4.0
Na-sulfite + Glucose	high	0.0	0.0	0.3	0.0	21.3	0.0	1.6	54.5	0.0	0.4	0.0	0.0	0.2
Na-sulfite + Glucose	high	0.0	0.0	0.4	0.0	35.4	0.0	1.1	58.3	0.0	0.0	0.0	0.0	0.0
Na-selenite + Glucose	low	0.0	13.8	0.0	0.0	0.4	0.1	4.6	73.4	0.0	1.3	0.0	0.0	0.5
Na-selenite + Glucose	high	0.0	3.2	0.0	0.0	1.7	61.7	1.9	26.1	0.0	1.5	0.0	0.0	0.2
Na-selenite + Glucose	high	0.0	3.9	0.1	0.0	3.9	56.4	2.6	28.0	0.0	1.6	0.0	0.0	0.2
Na-selenite + Glucose + Na-iodoacetate	low	0.0	0.0	0.0	0.0	0.1	0.0	0.1	0.0	0.0	0.0	0.2	0.0	85.8
Na-selenite + Glucose + Na-iodoacetate	low	0.0	0.0	0.0	0.0	0.5	0.0	0.1	0.0	0.0	0.0	0.3	0.0	79.1
Na-selenite + Glucose + Na-iodoacetate	high	0.0	0.0	0.8	0.0	28.6	0.0	0.6	51.6	0.0	0.0	0.0	0.0	0.3
Na-selenite + Glucose + Na-iodoacetate	high	0.0	0.0	0.8	0.0	39.3	0.0	54.8	1.4	0.0	0.0	0.1	0.0	0.4
Na-selenite + Glucose + Na-borohydride	low	0.0	0.0	0.0	0.0	2.2	0.0	0.5	30.9	0.0	0.8	0.0	0.0	55.3
Na-selenite + Glucose + Na-borohydride	low	0.0	0.3	0.0	0.0	2.5	0.0	4.1	82.2	0.0	4.8	0.0	0.0	1.1
Na-selenite + Glucose + Na-borohydride	high	0.0	0.0	0.0	0.0	1.4	0.1	0.2	22.6	0.0	0.2	0.0	0.0	64.0
Na-selenite + Glucose + Na-borohydride	high	0.0	0.0	0.0	0.0	4.5	0.0	2.8	87.8	0.0	1.3	0.0	0.0	0.3

Values are given in %

Table S16. Total cell counts and percentages of all bacteria and *Betaproteobacteria*, detected by the use of Fluresecence in situ hybridization, in mineral water at day 8 and day 28 after bottling into 0.5 L PET bottles stored at ambient temperature in two consecutive years.

		Total cell counts		All bacteria			Betaproteobacteria		
Year	**Days after bottling**	**Cells per mL**	**SD**	**Cells per mL**	**SD**	**%**	**Cells per mL**	**SD**	**%**
2011	8	**8,54E+04**	6,08E+03	**6,80E+04**	3,03E+03	79,64	**6,62E+04**	2,33E+03	77,51
2011	28	**6,83E+04**	4,72E+03	**6,54E+04**	3,03E+03	95,75	**5,97E+04**	6,87E+03	87,41
2012	8	**3,88E+04**	8,42E+03	**3,13E+04**	6,04E+03	80,76	**2,70E+04**	1,23E+04	69,60
2012	28	**6,02E+04**	1,12E+04	**5,53E+04**	7,03E+03	91,93	**4,64E+04**	8,39E+03	77,07

9. References

Aken, B.V., Peres, C.M., Doty, S.L., Yoon, J.M., and Schnoor, J.L. (2004). Methylobacterium populi sp. nov., a novel aerobic, pink-pigmented, facultatively methylotrophic, methane-utilizing bacterium isolated from poplar trees (Populus deltoides×nigra DN34). Int. J. Syst. Evol. Microbiol. *54*, 1191–1196.

Amann, R.I., Ludwig, W., and Schleifer, K.H. (1995). Phylogenetic identification and in situ detection of individual microbial cells without cultivation. Microbiol. Rev. *59*, 143–169.

Anderson, M.J. (2001). A new method for non-parametric multivariate analysis of variance. Austral Ecol. *26*, 32–46.

Ashelford, K.E., Fry, J.C., Bailey, M.J., and Day, M.J. (2002). Characterization of Serratia isolates from soil, ecological implications and transfer of Serratia proteamaculans subsp. quinovora Grimont et al. 1983 to Serratia quinivorans corrig., sp. nov. Int. J. Syst. Evol. Microbiol. *52*, 2281–2289.

Bébien, M., Lagniel, G., Garin, J., Touati, D., Verméglio, A., and Labarre, J. (2002). Involvement of Superoxide Dismutases in the Response of Escherichia coli to Selenium Oxides. J. Bacteriol. *184*, 1556–1564.

Berry, D., Mahfoudh, K.B., Wagner, M., and Loy, A. (2011). Barcoded Primers Used in Multiplex Amplicon Pyrosequencing Bias Amplification. Appl. Environ. Microbiol. *77*, 7846–7849.

Bertoldi, D., Bontempo, L., Larcher, R., Nicolini, G., Voerkelius, S., Lorenz, G.D., Ueckermann, H., Froeschl, H., Baxter, M.J., Hoogewerff, J., et al. (2011). Survey of the chemical composition of 571 European bottled mineral waters. J. Food Compos. Anal. *24*, 376–385.

Bischofberger, T., Cha, S.K., Schmitt, R., König, B., and Schmidt-Lorenz, W. (1990). The bacterial flora of non-carbonated, natural mineral water from the springs to reservoir and glass and plastic bottles. Int. J. Food Microbiol. *11*, 51–71.

Böck, A., Forchhammer, K., Heider, J., Leinfelder, W., Sawers, G., Veprek, B., and Zinoni, F. (1991). Selenocysteine: the 21st amino acid. Mol. Microbiol. *5*, 515–520.

Bont, J. a. M.D., Dijken, J.P.V., and Harder, W. (1981). Dimethyl Sulphoxide and Dimethyl Sulphide as a Carbon, Sulphur and Energy Source for Growth of Hyphomicrobium S. J. Gen. Microbiol. *127*, 315–323.

Bottiglieri, T. (2002). S-Adenosyl-l-methionine (SAMe): from the bench to the bedside—molecular basis of a pleiotrophic molecule. Am. J. Clin. Nutr. *76*, 1151S–1157S.

Brennan, F.P., O'Flaherty, V., Kramers, G., Grant, J., and Richards, K.G. (2010). Long-Term Persistence and Leaching of Escherichia coli in Temperate Maritime Soils. Appl. Environ. Microbiol. *76*, 1449–1455.

Brown, T.A., and Shrift, A. (1982). Selective assimilation of selenite by *Escherichia coli*. Can. J. Microbiol. *28*, 307–310.

Bryant, R.D., and Laishley, E.J. (1988). Evidence for two transporters of sulfur and selenium oxyanions in *Clostridium pasteurianum*. Can. J. Microbiol. *34*, 700–703.

Buttiaux, R., and Boudier, A. (1960). Comportement des bacte´ries autotro- phes dans les eaux mine´rales conserve´esenre´cipients herme´tiquement clos.

Cáceres, M.D., and Legendre, P. (2009). Associations between species and groups of sites: indices and statistical inference. Ecology *90*, 3566–3574.

Caporaso, J.G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F.D., Costello, E.K., Fierer, N., Peña, A.G., Goodrich, J.K., Gordon, J.I., et al. (2010). QIIME allows analysis of high-throughput community sequencing data. Nat. Methods *7*, 335–336.

Carr, E.L., Kämpfer, P., Patel, B.K.C., Gürtler, V., and Seviour, R.J. (2003). Seven novel species of Acinetobacter isolated from activated sludge. Int. J. Syst. Evol. Microbiol. *53*, 953–963.

Casanovas-Massana, A., and Blanch, A.R. (2012). Diversity of the heterotrophic microbial populations for distinguishing natural mineral waters. Int. J. Food Microbiol. *153*, 38–44.

Chasteen, T.G. (1993). Confusion between dimethyl selenenyl sulfide and dimethyl selenone released by bacteria. Appl. Organomet. Chem. *7*, 335–342.

Chin, H.-W., and Lindsay, R.C. (1994). Ascorbate and transition-metal mediation of methanethiol oxidation to dimethyl disulfide and dimethyl trisulfide. Food Chem. *49*, 387–392.

Curson, A.R.J., Todd, J.D., Sullivan, M.J., and Johnston, A.W.B. (2011). Catabolism of dimethylsulphoniopropionate: microorganisms, enzymes and genes. Nat. Rev. Microbiol. *9*, 849–859.

Daims, H., Lücker, S., and Wagner, M. (2006). daime, a novel image analysis program for microbial ecology and biofilm research. Environ. Microbiol. *8*, 200–213.

Dickens, F. (1933). Interaction of halogenacetates and SH compounds. Biochem. J. *27*, 1141–1151.

Dickson, D.M., Jones, R.G.W., and Davenport, J. (1980). Steady state osmotic adaptation inUlva lactuca. Planta *150*, 158–165.

Ding, L., and Yokota, A. (2010). <I>Curvibacter fontana </I>sp. nov., a microaerobic bacteria isolated from well water. J. Gen. Appl. Microbiol. *56*, 267–271.

Doran, J.W., and Alexander, M. (1977). Microbial Transformations of Selenium. Appl. Environ. Microbiol. *33*, 31–37.

Drotar, A., Burton, G.A., Tavernier, J.E., and Fall, R. (1987a). Widespread occurrence of bacterial thiol methyltransferases and the biogenic emission of methylated sulfur gases. Appl. Environ. Microbiol. *53*, 1626–1631.

Drotar, A., Fall, L.R., Mishalanie, E.A., Tavernier, J.E., and Fall, R. (1987b). Enzymatic methylation of sulfide, selenide, and organic thiols by Tetrahymena thermophila. Appl. Environ. Microbiol. *53*, 2111–2118.

Eder, W., Wanner, G., Ludwig, W., Busse, H.-J., Ziemke-Kägeler, F., and Lang, E. (2011). Description of Undibacterium oligocarboniphilum sp. nov., isolated from purified water, and Undibacterium pigrum strain CCUG 49012 as the type strain of Undibacterium parvum sp. nov., and emended descriptions of the genus Undibacterium and the species Undibacterium pigrum. Int. J. Syst. Evol. Microbiol. *61*, 384–391.

Egli, T. (2010). How to live at very low substrate concentration. Water Res. *44*, 4826–4837.

Esaki, N., Tanaka, H., Uemura, S., Suzuki, T., and Soda, K. (1979). Catalytic action of L-methionine .gamma.-lyase on selenomethionine and selenols. Biochemistry (Mosc.) *18*, 407–410.

Falcone-Dias, M.F., Vaz-Moreira, I., and Manaia, C.M. (2012). Bottled mineral water as a potential source of antibiotic resistant bacteria. Water Res. *46*, 3612–3622.

Favre-Bonté, S., Ranjard, L., Colinon, C., Prigent-Combaret, C., Nazaret, S., and Cournoyer, B. (2005). Freshwater selenium-methylating bacterial thiopurine methyltransferases: diversity and molecular phylogeny. Environ. Microbiol. *7*, 153–164.

Figueras, M.J., Guarro, J., and Martínez-Murcia, A. (2000). Clinically Relevant Aeromonas Species. Clin. Infect. Dis. *30*, 988–989.

Fleet-Stalder, V.V., Gürleyük, H., Bachofen, R., and Chasteen, T.G. (1997). Effects of growth conditions on production of methyl selenides in cultures of Rhodobacter sphaeroides. J. Ind. Microbiol. Biotechnol. *19*, 98–103.

Van Fleet-Stalder, V., Chasteen, T.G., Pickering, I.J., George, G.N., and Prince, R.C. (2000). Fate of Selenate and Selenite Metabolized by Rhodobacter sphaeroides. Appl. Environ. Microbiol. *66*, 4849–4853.

Forchhammer, K., Boesmiller, K., and Böck, A. (1991). The function of selenocysteine synthase and SELB in the synthesis and incorporation of selenocysteine. Biochimie *73*, 1481.

Franzmann, P.D., Heitz, A., Zappia, L.R., Wajon, J.E., and Xanthis, K. (2001). The formation of malodorous dimethyl oligosulphides in treated groundwater: the role of biofilms and potential precursors. Water Res. *35*, 1730–1738.

Freney, J.R. (1958). Aerobic Transformation of Cysteine to Sulphate in Soil. Nature *182*, 1318–1319.

Freney, J.R. (1960). The oxidation of cysteine to sulphate in soil. Aust. J. Biol. Sci. *13*, 387–392.

Geelhoed, J.S., Kleerebezem, R., Sorokin, D.Y., Stams, A.J.M., and Van Loosdrecht, M.C.M. (2010). Reduced inorganic sulfur oxidation supports autotrophic and mixotrophic growth of Magnetospirillum strain J10 and Magnetospirillum gryphiswaldense. Environ. Microbiol. *12*, 1031–1040.

Ginzburg, B., Chalifa, I., Gun, J., Dor, I., Hadas, O., and Lev, O. (1998). DMS Formation by Dimethylsulfoniopropionate Route in Freshwater. Environ. Sci. Technol. *32*, 2130–2136.

Ginzburg, B., Dor, I., Chalifa, I., Hadas, O., and Lev, O. (1999). Formation of Dimethyloligosulfides in Lake Kinneret: Biogenic Formation of Inorganic Oligosulfide Intermediates under Oxic Conditions. Environ. Sci. Technol. *33*, 571–579.

Goenrich, M., Bursy, J., Hübner, E., Linder, D., Schwartz, A., and Vorholt, J. (2002). Purification and characterization of the methylene tetrahydromethanopterin dehydrogenase MtdB and the methylene tetrahydrofolate dehydrogenase FolD from Hyphomicrobium zavarzinii ZV580. Arch. Microbiol. *177*, 299–303.

González, C., Gutiérrez, C., and Grande, T. (1987). Bacterial flora in bottled uncarbonated mineral drinking water. Can. J. Microbiol. *33*, 1120–1125.

Gun, J., Goifman, A., Shkrob, I., Kamyshny, A., Ginzburg, B., Hadas, O., Dor, I., Modestov, A.D., and Lev, O. (2000). Formation of Polysulfides in an Oxygen Rich Freshwater Lake and Their Role in the Production of Volatile Sulfur Compounds in Aquatic Systems. Environ. Sci. Technol. *34*, 4741–4746.

Hudman, J.F., and Glenn, A.R. (1984). Selenite uptake and incorporation by Selenomonas ruminatium. Arch. Microbiol. *140*, 252–256.

Kadota, H., and Ishida, Y. (1972). Production of Volatile Sulfur Compounds by Microorganisms. Annu. Rev. Microbiol. *26*, 127–138.

Kalmbach, S., Manz, W., Wecke, J., and Szewzyk, U. (1999). Aquabacterium gen. nov., with description of Aquabacterium citratiphilum sp. nov., Aquabacterium parvum sp. nov. and Aquabacterium commune sp. nov., three in situ dominant bacterial species from the Berlin drinking water system. Int. J. Syst. Bacteriol. *49*, 769–777.

Kämpfer, P., Rosselló-Mora, R., Hermansson, M., Persson, F., Huber, B., Falsen, E., and Busse, H.-J. (2007). Undibacterium pigrum gen. nov., sp. nov., isolated from drinking water. Int. J. Syst. Evol. Microbiol. *57*, 1510–1515.

Kappler, U., and Dahl, C. (2001). Enzymology and molecular biology of prokaryotic sulfite oxidation. Fems Microbiol. Lett. *203*, 1–9.

Kiene, R.P., and Hines, M.E. (1995). Microbial formation of dimethyl sulfide in anoxic sphagnum peat. Appl. Environ. Microbiol. *61*, 2720–2726.

Kiffney, P., and Knight, A. (1990). The toxicity and bioaccumulation of selenate, selenite and seleno-L-methionine in the cyanobacteriumAnabaena flos-aquae. Arch. Environ. Contam. Toxicol. *19*, 488–494.

Kristiana, I., Heitz, A., Joll, C., and Sathasivan, A. (2010). Analysis of polysulfides in drinking water distribution systems using headspace solid-phase microextraction and gas chromatography–mass spectrometry. J. Chromatogr. A *1217*, 5995–6001.

Kuhn, I., Allestam, G., Huys, G., Janssen, P., Kersters, K., Krovacek, K., and Stenstrom, T.A. (1997). Diversity, persistence, and virulence of Aeromonas strains isolated from drinking water distribution systems in Sweden. Appl. Environ. Microbiol. *63*, 2708–2715.

Leclerc, H., and Moreau, A. (2002). Microbiological safety of natural mineral water. Fems Microbiol. Rev. *26*, 207–222.

Lindblow-Kull, C., Kull, F.J., and Shrift, A. (1985). Single transporter for sulfate, selenate, and selenite in Escherichia coli K-12. J. Bacteriol. *163*, 1267–1269.

Lortie, L., Gould, W.D., Rajan, S., McCready, R.G.L., and Cheng, K.-J. (1992). Reduction of Selenate and Selenite to Elemental Selenium by a Pseudomonas stutzeri Isolate. Appl. Environ. Microbiol. *58*, 4042–4044.

Lovelock, J.E., Maggs, R.J., and Rasmussen, R.A. (1972). Atmospheric Dimethyl Sulphide and the Natural Sulphur Cycle. Publ. Online 23 June 1972 Doi101038237452a0 *237*, 452–453.

Loy, A., Beisker, W., and Meier, H. (2005). Diversity of Bacteria Growing in Natural Mineral Water after Bottling. Appl. Environ. Microbiol. *71*, 3624–3632.

Lu, X., Fan, C., He, W., Deng, J., and Yin, H. (2013). Sulfur-containing amino acid methionine as the precursor of volatile organic sulfur compounds in algea-induced black bloom. J. Environ. Sci. *25*, 33–43.

Ludwig, W., Strunk, O., Westram, R., Richter, L., Meier, H., Yadhukumar, Buchner, A., Lai, T., Steppi, S., Jobb, G., et al. (2004). ARB: a software environment for sequence data. Nucleic Acids Res. *32*, 1363–1371.

Luther (2001). Sulfur speciation monitored in situ with solid state gold amalgam voltammetric microelectrodes: Polysulfides as a special case in sediments, microbial mats and hydrothermal vent waters. J. Environ. Monit. *3*, 61–66.

Manaia, C.M., Nunes, O.C., Morais, P.V., and da Costa, M.S. (1990). Heterotrophic plate counts and the isolation of bacteria from mineral waters on selective and enrichment media. J. Appl. Bacteriol. *69*, 871–876.

Morais, P.V., and da Costa, M.S. (1990). Alterations in the major heterotrophic bacterial populations isolated from a still bottled mineral water. J. Appl. Bacteriol. *69*, 750–757.

Vaz-Moreira, I., Egas, C., Nunes, O.C., and Manaia, C.M. (2011). Culture-dependent and culture-independent diversity surveys target different bacteria: a case study in a freshwater sample. Antonie Van Leeuwenhoek *100*, 245–257.

Muyzer, G., and Stams, A.J.M. (2008). The ecology and biotechnology of sulphate-reducing bacteria. Nat. Rev. Microbiol. *6*, 441–454.

Pilloni, G., Granitsiotis, M.S., Engel, M., and Lueders, T. (2012). Testing the Limits of 454 Pyrotag Sequencing: Reproducibility, Quantitative Assessment and Comparison to T-RFLP Fingerprinting of Aquifer Microbes. Plos One *7*, e40467.

Pinsent, J. (1954). The need for selenite and molybdate in the formation of formic dehydrogenase by members of the Coli-aerogenes group of bacteria. Biochem. J. *57*, 10–16.

R Development Core Team (2011). R: A Language and Environment for Statistical Computing. *1*, 409.

Rael, R.M., and Frankenberger Jr, W.T. (1996). Influence of pH, salinity, and selenium on the growth of Aeromonas veronii in evaporation agricultural drainage water. Water Res. *30*, 422–430.

Ranjard, L., Nazaret, S., and Cournoyer, B. (2003). Freshwater Bacteria Can Methylate Selenium through the Thiopurine Methyltransferase Pathway. Appl. Environ. Microbiol. *69*, 3784–3790.

Ranjard, L., Prigent-Combaret, C., Favre-Bonté, S., Monnez, C., Nazaret, S., and Cournoyer, B. (2004). Characterization of a novel selenium methyltransferase from freshwater bacteria showing strong similarities with the calicheamicin methyltransferase. Biochim. Biophys. Acta Bba - Gene Struct. Expr. *1679*, 80–85.

Robert J. McGorrin (2011). The Significance of Volatile Sulfur Compounds in Food Flavors. In Volatile Sulfur Compounds in Food, (American Chemical Society), pp. 3–31.

Sato, D., and Nozaki, T. (2009). Methionine gamma-lyase: The unique reaction mechanism, physiological roles, and therapeutic applications against infectious diseases and cancers. Iubmb Life *61*, 1019–1028.

Schäfer, H., Myronova, N., and Boden, R. (2010). Microbial degradation of dimethylsulphide and related C1-sulphur compounds: organisms and pathways controlling fluxes of sulphur in the biosphere. J. Exp. Bot. *61*, 315–334.

Schloss, P.D., Westcott, S.L., Ryabin, T., Hall, J.R., Hartmann, M., Hollister, E.B., Lesniewski, R.A., Oakley, B.B., Parks, D.H., Robinson, C.J., et al. (2009). Introducing mothur: Open-Source, Platform-Independent, Community-Supported Software for Describing and Comparing Microbial Communities. Appl. Environ. Microbiol. *75*, 7537–7541.

Segal, W., and Starkey, R.L. (1969). Microbial Decomposition of Methionine and Identity of the Resulting Sulfur Products. J. Bacteriol. *98*, 908–913.

Smet, E., Chasaya, G., Langenhove, H.V., and Verstraete, W. (1996). The effect of inoculation and the type of carrier material used on the biofiltration of methyl sulphides. Appl. Microbiol. Biotechnol. *45*, 293–298.

Tarze, A., Dauplais, M., Grigoras, I., Lazard, M., Ha-Duong, N.-T., Barbier, F., Blanquet, S., and Plateau, P. (2007). Extracellular production of hydrogen selenide accounts for thiol-assisted toxicity of selenite against Saccharomyces cerevisiae. J. Biol. Chem. *282*, 8759–8767.

Temesgen, Z., Toal, D.R., and Cockerill, F.R. (1997). Leclercia adecarboxylata Infections: Case Report and Review. Clin. Infect. Dis. *25*, 79–81.

Thomas, E.L. (1984). Disulfide reduction and sulfhydryl uptake by Streptococcus mutans. J. Bacteriol. *157*, 240–246.

Turner, R.J., Weiner, J.H., and Taylor, D.E. (1998). Selenium metabolism in Escherichia coli. Biometals *11*, 223–227.

Vachee, A., Vincent, P., Struijk, C.B., Mossel, D.A.A., and Leclerc, H. (1997). A Study of the Fate of the Autochtonous Bacterial Flora of Still Mineral Waters by Analysis of Restriction Fragment Length Polymorphism of Genes Coding For rRNA. Syst. Appl. Microbiol. *20*, 492–503.

Wagner, M., Amann, R., Lemmer, H., and Schleifer, K.H. (1993). Probing activated sludge with oligonucleotides specific for proteobacteria: inadequacy of culture-dependent methods for describing microbial community structure. Appl. Environ. Microbiol. *59*, 1520–1525.

Wajon, J.E., Alexander, R., Kagi, R.I., and Kavanagh, B. (1985). Dimethyl trisulphide and objectionable odours in potable water. Chemosphere *14*, 85–89.

Wang, Q., Garrity, G.M., Tiedje, J.M., and Cole, J.R. (2007). Naïve Bayesian Classifier for Rapid Assignment of rRNA Sequences into the New Bacterial Taxonomy. Appl. Environ. Microbiol. *73*, 5261–5267.

Watts, S.F. (2000). The mass budgets of carbonyl sulfide, dimethyl sulfide, carbon disulfide and hydrogen sulfide. Atmos. Environ. *34*, 761–779.

Willems, A., Falsen, E., Pot, B., Jantzen, E., Hoste, B., Vandamme, P., Gillis, M., Kersters, K., and Ley, J.D. (1990). Acidovorax, a New Genus for Pseudomonas facilis, Pseudomonas delafieldii, E. Falsen (EF) Group 13, EF Group 16, and Several Clinical Isolates, with the Species Acidovorax facilis comb. nov., Acidovorax delafieldii comb. nov., and Acidovorax temperans sp. nov. Int. J. Syst. Bacteriol. *40*, 384–398.

Yarza, P., Richter, M., Peplies, J., Euzeby, J., Amann, R., Schleifer, K.-H., Ludwig, W., Glöckner, F.O., and Rosselló-Móra, R. (2008). The All-Species Living Tree project: a 16S rRNA-based phylogenetic tree of all sequenced type strains. Syst. Appl. Microbiol. *31*, 241–250.

Yoch, D.C. (2002). Dimethylsulfoniopropionate: Its Sources, Role in the Marine Food Web, and Biological Degradation to Dimethylsulfide. Appl. Environ. Microbiol. *68*, 5804–5815.

10. Authors' contributions

The work presented here was carried out by Celine Lesaulnier, David Berry, Alexander Loy and Claus Pelikan from the Department of Microbial Ecology at the University of Vienna and by our industrial cooperation partner. Celine Lesaulnier and Alexander Loy designed the experiments. Celine Lesaulnier and Claus Pelikan carried out most of the laboratory experiments. The chemical and sensory analysis was performed by the industrial cooperation partner. David Berry performed the bioinformatic and statistical analysis. Claus Pelikan interpreted the results and wrote the thesis. Celine Lesaulnier and Alexander Loy revised the thesis.

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12. Curriculum Vitae

Personal Data:

Name	Pelikan, Claus Wolfgang
Date of birth	September 17, 1987
Place of birth	Mödling, Austria
Nationality	Austria
Languagues	German, English, Spanish

Education:

1993-1997	Öffentliche Volksschule Harald Lowatschek, Mödling, Austria
1997-2005	Bundesgymnasium Mödling, Austria
2005-2006	Study of Biology and Environmental Studies – Subject of instruction (LA)
2006-2007	European volunteer service (EVS) in Torredembarra, Spain
2007-2010	Bachelor in Biology at the University of Vienna, branch of study: Ecology
2010-2012	Master in Ecology at the University of Vienna
Since 2012	Master thesis at the Department of Microbial ecology, University of Vienna

Scientific presentations:

2012	Contribution to the Poster: “Microbial Ecology Surrounding Methylated Sulfur and Selenium Compounds Formation in Freshwater; Celine Lesaulnier, Jutta Niggemann, Gabriel Singer, Cédric Gerard, Xavier LeCoz, **Claus Pelikan**, Thorsten Dittmar, Alexander Loy; presented at the 14th ISME conference