

# Manganese oxidation and bacterial diversity on different filter media coatings during the start-up of drinking water biofilters

I. L. Breda, L. Ramsay and P. Roslev

## ABSTRACT

Manganese removal is a typical concern in drinking water production. Biofiltration may be used when treating groundwater sources but the onset of manganese removal in virgin biofilters can vary considerably. The aim of this study was to investigate the effect of different filter media on manganese oxidation and bacterial diversity in biofilters during the start-up. The onset of manganese oxidation in four virgin granular filter media (quartz, calcium carbonate, polystyrene, and manganese oxide) and one matured medium (quartz) was followed during the start-up. Immediate manganese removal was achieved by manganese oxide, while 48, 57 and 72 days were required by virgin quartz, calcium carbonate and polystyrene, respectively. The bacterial community was investigated using DAPI staining, quantitative polymerase chain reaction (qPCR), 16S rRNA gene pyrosequencing, and bacterial enrichments. Bacterial abundance was greatest on polystyrene and matured quartz. Molecular community analysis and bacterial enrichments suggested the presence of manganese oxidizing bacteria on all media coatings after the start-up period. Virgin quartz and calcium carbonate showed similar bacterial communities whereas manganese oxide and polystyrene were distinct. This investigation suggests that when inoculating different filter media with an identical water source, the bacterial diversity and onset of manganese oxidation during start-up is strongly influenced by the filter media type.

**Key words** | bacterial diversity, bench-scale, drinking water, filter media, manganese oxidizing bacteria, start-up

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## INTRODUCTION

Manganese is a typical concern in drinking water production (Tekerlekopoulou *et al.* 2013) and should be limited for health and aesthetic reasons (WHO 2011). In northern Europe, drinking water treatment is commonly based on aeration and biofiltration (Mouchet 1992). Biofilters are defined as granular filters coated with both inorganics and biofilm capable of treating water to national drinking water criteria (Tekerlekopoulou *et al.* 2013).

The period during which virgin filter media matures into a fully functional biofilter is designated as the start-up period. Start-up of drinking water biofilters hinges on a set of interconnected physical, chemical and biological

processes (Mouchet 1992). When manganese is present, the duration of a start-up varies from weeks to more than a year (Tekerlekopoulou *et al.* 2013). During this time, produced water cannot be distributed to the consumers. Costs of labor, water and energy during the start-up are a major concern for water utilities. Further, frequent manganese breakthrough after some years of operation may require filter media replacement and consequently a new start-up period (Buamah *et al.* 2009). Knowledge regarding solutions to shorten the start-up period is of interest for water utilities.

Abiotic manganese oxidation is described by homogeneous and heterogeneous processes (Katsoyiannis &

Zouboulis 2004). Homogeneous oxidation of manganese by oxygen is slow at pH below 9 (Stumm & Morgan 1996). Thus, homogeneous oxidation of manganese in drinking water treatment using a groundwater source, where typically pH ranges between 6 and 8, is mostly mediated biologically (Tekerekopoulou et al. 2013). Heterogeneous oxidation of manganese is based on the autocatalytic activity of solid manganese oxides present in the coating of the filter medium grains (Katsoyiannis & Zouboulis 2004). Sahabi et al. (2009) conclude that manganese removal in matured biofilters is mainly based on autocatalytic action, whereas investigations by Bruins (2016) suggest that manganese removal in a non-coated virgin medium is initiated biologically, evolving to a predominantly physicochemical removal process over time. Thus, biological oxidation of manganese may be of particular importance during the start-up period.

Recent advances of analytical and bioinformatic methods have enabled characterization and quantification of the prokaryotic communities present in biofilters. Previous studies have found several genera related to manganese oxidation on matured biofilter media. These include *Leptothrix*, *Crenothrix*, *Hyphomicrobium*, *Metallogenium*, *Siderocapsa*, *Siderocystis*, *Pedomicrobium*, *Hydrogenophaga*, and some species of *Pseudomonas* and *Acinetobacter* (Mouchet 1992; Ehrlich 1996; Larsen et al. 1999; Katsoyiannis & Zouboulis 2004; Pacini et al. 2005; Tebo et al. 2005; Das et al. 2011; Abu Hasan et al. 2012; Beukes & Schmidt 2012; Marcus et al. 2017; Su et al. 2016). This suggests that multiple genera may participate in the biological oxidation of manganese. However, the bacterial diversity in drinking water biofilters during the initial stages of manganese removal is generally underexplored.

During the start-up period, filter media provide a surface for the attachment of microorganisms. The use of alternative filter media in drinking water filters has been investigated in several studies (Qiu et al. 2010; Grace et al. 2015; Schöntag et al. 2015). However, the effect that these alternative filter media may have on the bacterial community structure has received little attention. More knowledge could potentially lead to the use of alternative filter media as biostimulators to shorten the start-up period, as well as possible management of the functional roles of the bacterial community present in biofilters. Hence, the aim of this study is to investigate the effect of different filter media on the start-up

period of manganese oxidation and on the diversity of bacterial communities present in the biofilters at the end of the start-up period.

## MATERIALS AND METHODS

### Setup configuration

Fredensborg waterworks (Skanderborg, Denmark) treats anaerobic groundwater by aeration and two stage biofiltration. The matured quartz filter medium located in the second stage biofilter had been in operation for 47 years without being replaced. The experiment was conducted in a bench-scale setup using virgin and matured filter media. An illustration of the experimental setup is available in the supplementary materials (S1), available with the online version of this paper.

Source water for the experiment was prepared by mixing non-chlorinated treated water from the storage tank of the waterworks with a concentrated solution of  $\text{MnCl}_2$ . Treated water from the waterworks has the advantage of ensuring concentrations of all treatment parameters below national drinking water criteria while maintaining stable physical and biological conditions. Further, direct interference from waterworks processes are also avoided (e.g. backwash and changes in production rates). The concentrated manganese solution was prepared using distilled water and  $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$  (Emsure ACS), which was then pumped to a mixing vase using a diaphragm pump (Digital DDC, Grundfos) and mixed with the treated water using a magnetic mixer (MR 1000, Heidolph), resulting in a manganese concentration of  $0.281 \pm 0.018 \text{ mg L}^{-1}$  before distribution to the filter columns (Table 1).

The source water was distributed by gravity to 15 polyethylene filter columns, each with a diameter of 5 cm and a 10 cm layer of granular medium. The columns were operated in downflow mode with a filtration rate of 3.5 m/h and an empty bed contact time of 1.8 min. No backwash was used during the experiment.

Five granular filter media were selected for the experiment and placed in triplicate filter columns. The media were: virgin quartz, calcium carbonate, polystyrene, manganese oxide, and matured quartz collected from the top layer

**Table 1** | Quality of the source water

Parameter	Unit	Average	Std. dev.
Manganese	mg/L	0.281	0.018
Iron	mg/L	0.027	0.01
Ammonium	mg/L	<0.02	–
Nitrite	mg/L	<0.001	–
pH <sup>a</sup>	–	7.7	0.1
Oxygen <sup>a</sup>	mg/L	11.1	0.1
Redox <sup>a,b</sup>	mV	298	58
Temperature <sup>a</sup>	°C	10.7	0.2
Turbidity	NTU	<0.1	–
Hydrogen carbonate	mg/L	278	15
NVOC	mg/L	1.0	0.1

<sup>a</sup>Field measurements.<sup>b</sup>Redox measurements increased over time.

of the second stage filter of the waterworks (Table 2). The latter served as a positive control since this filter medium successfully removes manganese in the full-scale waterworks.

Before the experiment commenced, all filter media were submitted to pretreatment to remove fines and disinfect the media. A sample of approximately 1.5 kg of each filter medium was dry-sieved (630 µm), then washed ten times in a 2-L blue cap bottle with distilled water to remove fines. Each medium was then subdivided using a rotary sample divider (DR100 and PT100, Retsch GmbH, Germany), washed with 65 °C distilled water and incubated for a period of 12 h at 67 °C. A disinfection procedure of heat treatment at 75 °C for 12 h was also applied to all tubing, filter columns and sampling valves.

**Table 2** | Characterization of filter media

Parameter	Matured quartz	Virgin quartz	Calcium carbonate	Polystyrene beads	Manganese oxide
Main composition day 0	–	100% SiO <sub>2</sub>	96.8% CaCO <sub>3</sub>	100%	80% MnO <sub>2</sub>
Grain size (mm, 10–90%)	1.03–1.86	1.10–1.76	1.64–5.08	0.97–1.22	1.75–3.44
Sphericity ( $4\pi\text{-area}/\text{perimeter}^2$ )	0.90	0.87	0.85	0.97	0.82
Particle density (kg/L)	2.50	2.60	2.56	1.00	3.56
Porosity (%)	42.60	40.63	46.93	39.84	51.66
Supplier	Fredensborg waterworks	Dansk Kvarst Ind. Denmark	Faxe Kalk, Denmark	Bewi Styrochem, Finland	Unitex, Poland
Cost (euros/L)	–	0.34	0.55	0.06	5.7

## Sampling and chemical analysis

Filter column inlet and outlet samples were collected two to three times a week during the experiment. Outlet samples were collected from each filter column after a contact time of 20 minutes by pausing the flow. Both inlet and outlet samples were collected manually, filtered immediately (0.45 µm) and analyzed within two hours for manganese according to manufacturer's instructions (kit LCK304 and DR3900 spectrophotometer, Hach, Denmark).

## Sampling for microbiological analysis

Samples of source water (4 L) were collected in the middle and end of the experiment and filtered using sterile 0.20 µm membrane filters (Advantec). The filters were stored at –21 °C for subsequent molecular analysis. Filter media from the 15 columns were collected at the termination of the experiment to characterize the bacterial community. Media samples for DAPI staining were fixed by submerging in a 3% formaldehyde solution and stored at 4 °C in the dark. Media samples for molecular analysis were stored at –21 °C until extraction.

## DAPI staining and cell counting

Formaldehyde-fixed filter media samples (5 g) were sonicated, vortexed, and aliquots of the supernatant were transferred to sterile test tubes. Homogenization was obtained by pulling/pushing several times through a syringe needle. The supernatant was then transferred to a sterile test

tube and diluted with filtered sterilized tap water. Cells were filtered onto 0.2  $\mu\text{m}$  black polycarbonate filters, and stained for 15 min with 4',6-diamidino-2'-phenylindole dihydrochloride (DAPI) at a concentration of 20 mg/L. DAPI stained cells were counted using an epi-fluorescence microscope at 630X or 1,000X magnification.

### DNA extraction, qPCR amplification, 16S rRNA gene amplicon sequencing and library preparation

DNA was extracted from water and filter media samples using the PowerWater DNA Isolation Kit (MOBIO) and the FastDNA spin kit for soil (MP Biomedicals), respectively; 4 L of water and 1.5 mL of filter medium samples were used for DNA extraction. Full description of the methodology used for quantitative polymerase chain reaction (qPCR) amplification, 16rRNA amplicon sequencing and library preparation can be found in the supplementary materials (S2), available online. Rarefaction curves for all individual samples were determined to ensure exhaustive sequencing of the diversity in the sample.

### Manganese oxidizing bacteria enrichment

Filter media homogenates (0.1 mL) were serially diluted and manganese oxidizing bacteria (MnOB) were enriched in MnOB broth and on MnOB agar containing: peptone from casein (0.5 g/L), peptone from soybean (0.5 g/L), meat extract (0.5 g/L), yeast extract (0.5 g/L), glucose (0.1 g/L), soluble starch (0.1 g/L), HEPES (10 mM),  $\text{MnCl}_2$  (10 mM), and 2.0% agar (agar plates only). Initial enrichment was carried out for 2 weeks at 20 °C. Manganese oxidation was subsequently compared in liquid cultures with and without heat treatment to inactivate MnOB (121 °C for 20 min). Manganese oxidation was detected after 24 hours incubation of 0.1 mL culture in 10 mL MnOB broth. Oxidized manganese was detected in 0.5 mL subsamples by adding 0.1 mL 0.04% Leucoberbelin blue I in 45 mM acetic acid. Leucoberbelin blue I reacts with oxidized Mn but not Mn (II) to form a blue color (Krumbein & Altmann 1973). Production of oxidized manganese was quantified as an increase in absorbance measured at 620 nm (Beukes & Schmidt 2012) using a Multiskan FC Microplate Photometer (Thermo Fisher Scientific).

### Bioinformatics and statistical analysis

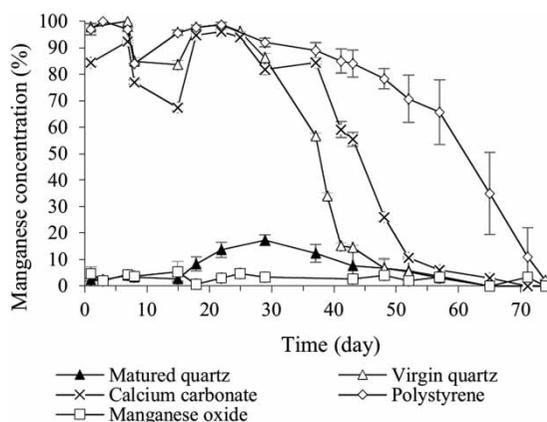
Results from 16S rRNA gene amplicon sequencing were analyzed in R (R Core Team 2017) through Rstudio IDE using the ampvis package v.2.0.0 (Albertsen et al. 2015). To compare the bacterial communities in the different filter media samples, multivariate statistics based on principal component analysis (PCA) was carried out using amp\_ordi function with Hellinger transformed OTU counts. Distribution of principal components axes in percentage of total inertia are available in the supplementary materials (S4), available online.

## RESULTS AND DISCUSSION

### Manganese removal by different filter media

Manganese removal in the 15 filter columns was monitored for a period of 75 days (Figure 1). The start-up period was considered finished when the manganese concentration of each medium's outlet reached 10% of the manganese concentration in the source water.

On day 75, all columns removed manganese to concentrations below the method detection limit (0.005 mg/L). Virgin quartz, calcium carbonate and polystyrene share a common onset of manganese removal after approximately 28 days (Figure 1). However, the length of the maturation



**Figure 1** | Manganese concentration in the column outlets over time as a percentage of manganese concentration in the source water (0.281 mg/L). Each datum point represents the average of three filter columns and the error bars represent standard deviations.

process was somewhat different. The start-up period ended at day 48 for virgin quartz, day 57 for calcium carbonate and day 72 for polystyrene. A similar pattern of manganese start-up by virgin quartz has been reported by Bruins (2016).

Investigations on the potential use of polystyrene as filter media for treatment of surface water conclude that energy savings can be achieved during backwash (Schöntag et al. 2015). In our study using polystyrene as a biofilter, this medium required a longer start-up period which will likely result in higher water and energy consumption during the start of a full-scale waterworks.

Matured quartz showed efficient removal of manganese (>90%) at the beginning of the experiment (day 1 to day 21). The manganese concentration in the outlet increased slightly during the following 8 days, returning to efficient manganese removal from thereon (Figure 1). Minor fluctuations in manganese removal have also been observed for filter media in other studies (Tekerekopoulou et al. 2013; Bruins 2016). The exact reason for these fluctuations is not known but may be related to varying roles of abiotic and biotic removal mechanisms. These results emphasize the complex nature of manganese oxidation processes (Ehrlich 1996; Tebo et al. 2005; Das et al. 2011).

Virgin manganese oxide media showed efficient manganese removal from day 1, which is consistent with its abiotic autocatalytic capacity to remove manganese. Collectively, the results suggest that the start-up length and stability of manganese removal is dependent not only on the initial filter media properties but also on the maturing process of the biofilter.

### qPCR and DAPI

DAPI counts and qPCR measurements at day 75 with broad range primers showed that the abundance of bacteria was greatest on polystyrene and matured quartz media compared with virgin quartz, calcium carbonate and manganese oxide (Table 3). The apparent differences between media with low bacterial abundance (e.g. virgin quartz) and material with high bacterial abundance (e.g. matured quartz) was >10-fold. Larger relative differences were generally observed with qPCR compared with DAPI staining. Variations in abundance determined with the two methods may be explained by differences in extraction

**Table 3** | qPCR and DAPI numbers for filter media at day 75

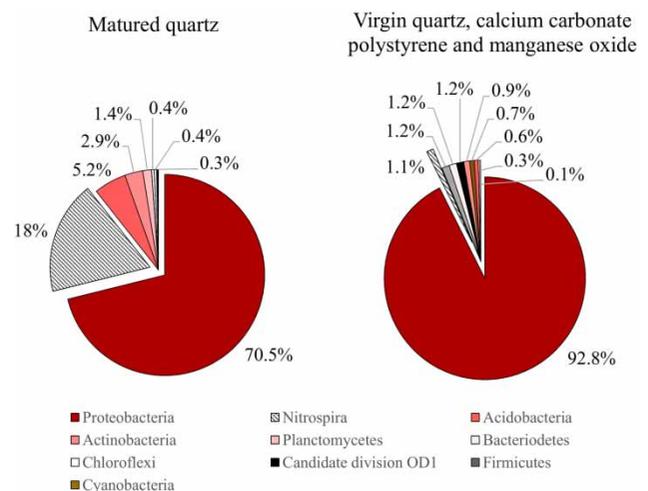
Filter medium	qPCR (copies/g)	DAPI (cells/g)
Matured quartz	$1.9 \times 10^8$	$1.8 \times 10^7$
Virgin quartz	$2.0 \times 10^6$	$1.1 \times 10^6$
Calcium carbonate	$4.0 \times 10^4$	$1.6 \times 10^6$
Polystyrene	$3.4 \times 10^8$	$3.6 \times 10^7$
Manganese oxide	$3.9 \times 10^6$	$8.2 \times 10^5$

Each number represents the average of three filter columns.

procedures and detection principles (DNA vs. intact cells). In general, the abundance of bacteria determined with the two cultivation independent methods in the present study is in the same range as related studies of microorganisms in drinking water biofilters from groundwater sources (Nitzsche et al. 2015; Gülay et al. 2016).

### 16S rRNA amplicon sequencing analysis

The bacterial diversity of the source water (day 44 and day 75) and the filter media coating (day 75) was investigated using 16S rRNA gene amplicon sequencing analysis. The microbial diversity present on the coating of the filter medium at the phylum level indicated a clear dominance of *Proteobacteria* phylum but significant differences in *Nitrospira* phylum abundance between matured quartz and initially virgin media (Figure 2).



**Figure 2** | Read abundance (%) at the phylum level on the coating of matured quartz and virgin quartz, calcium carbonate, polystyrene and manganese oxide at day 75. Data per medium available in the supplementary materials (S3), available with the online version of this paper.

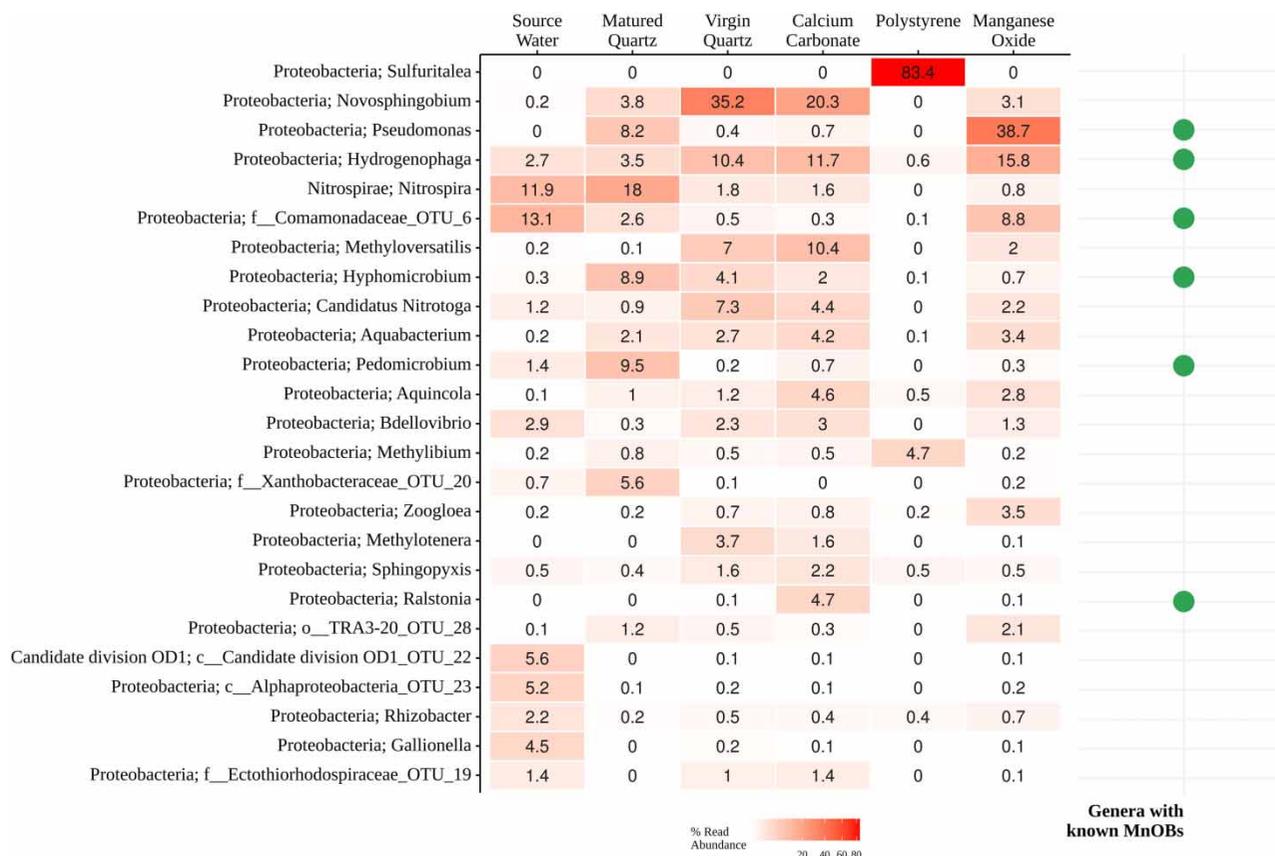
At the genus level (Figure 3), the bacterial community of the source water included families and genera previously reported in matured drinking water biofilters, such as: *Comamonadaceae*, *Gallionellaceae*, *Hydrogenophaga*, *Sulfuricurvum*, *Nitrospira* and *Gallionella* (Zhu et al. 2010; Lührig et al. 2015; Bruins 2016). The most dominant genus present on the coating of each media was *Nitrospira* (matured quartz), *Novosphingobium* (virgin quartz and calcium carbonate), *Sulfuritalea* (polystyrene) and *Pseudomonas* (manganese oxide).

Matured quartz maintained its original bacterial community fingerprint (dominance of *Nitrospira*) even after 75 days of contact with the nitrite and ammonium-free source water used in this experiment (Table 1). A recent study on rapid gravity filter microbial communities suggested a novel physiology of *Nitrospira* spp., which included a potential capability to oxidize manganese (Palomo et al. 2016).

Abundant bacteria found in the media coating include genera with known MnOB (Figure 3), such as

*Pedomicrobium*, *Hyphomicrobium*, *Ralstonia*, *Pseudomonas*, *Hydrogenophaga* and *Leptothrix* as part of the *Comamonadaceae* family (Mouchet 1992; Ehrlich 1996; Larsen et al. 1999; Katsoyiannis & Zouboulis 2004; Pacini et al. 2005; Tebo et al. 2005; Das et al. 2011; Abu Hasan et al. 2012; Marcus et al. 2017). These genera were part of the bacterial community of all five filter media, with exception of *Pseudomonas* and *Pedomicrobium* which were not detected on polystyrene, and *Ralstonia* which was not detected on polystyrene and matured quartz. The low abundance of *Hyphomicrobium* and *Comamonadaceae* and absence of *Ralstonia*, *Pseudomonas* and *Pedomicrobium* in the polystyrene samples coincide with a long start-up of this medium.

The top three most abundant genera detected on the manganese oxide coating included genera with known MnOB (*Pseudomonas*, *Hydrogenophaga* and *Leptothrix* as part of the *Comamonadaceae* family). Even though the main



**Figure 3** | Heatmap of the 25 most abundant genera present in filter media coatings at day 75 (average of three filter columns). The most abundant genera in the source water are shown for comparison (average of day 44 and day 75). Shadings are based on read abundance (%) and green dots indicate genera with known MnOB. Standard deviations available in the supplementary materials (S3), available with the online version of this paper.

manganese removal on this medium is likely based on an abiotic autocatalytic process, the presence of genera with known MnOB on this medium may suggest that MnOB are taking advantage of the morphology of the manganese oxide medium (80% MnO<sub>2</sub>) or of the autocatalytic oxidation process occurring at the surface of the medium grains. However, the underlying biochemical mechanisms behind bacterial oxidation of manganese are yet to be fully understood (Tebo et al. 2005). Further investigations on the potential use of manganese oxide medium to initiate the microbial growth in the virgin media biofilters might be of interest to the industry.

*Sphingomonadaceae* genera were detected as part of the 25 most abundant genera of filter media samples with  $0.57 \pm 0.05$ ,  $0.767 \pm 0.330$ ,  $0.333 \pm 0.249$ ,  $1.000 \pm 0.510$  and  $0.233 \pm 0.047$  percent for matured quartz, virgin quartz, calcium carbonate, polystyrene and manganese oxide media, respectively. *Sphingomonadaceae* has previously been reported in drinking water systems, and it has been suggested that *Sphingomonas* spp. plays a unique role assisting the initial formation and spatiotemporal development of microbial biofilms (Bereschenko et al. 2010; Lührig et al. 2015; Bruins 2016).

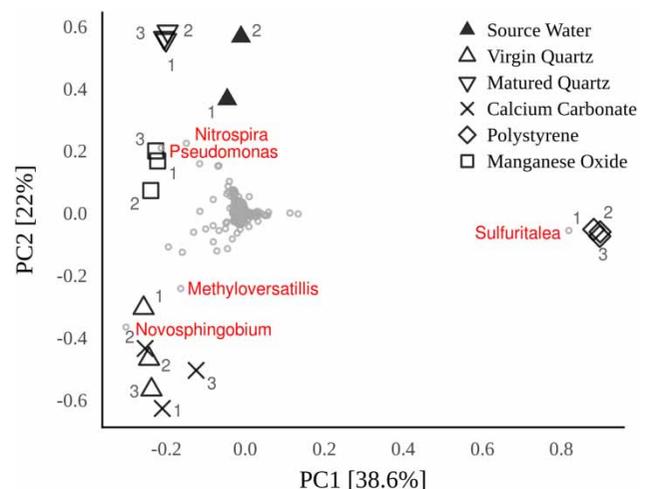
### MnOB enrichment

MnOB were enriched from all filter media samples to confirm the presence of culturable manganese oxidizers at the end of the experiment (day 75). The Leucobertelin blue assay for detection of oxidized manganese (Krumbein & Altmann 1973) was performed after enrichment of MnOB from filter media samples taken from all columns. Control samples and subsamples with autoclaved inoculum showed no color formation after 24 h in MnOB broth while color was displayed in subsamples containing live inoculum. Spectrophotometric measurements with a signal/background ratio >1.5 were considered positive (data not shown). Differences in absorbance between autoclaved enrichments and live enrichments confirmed that live bacteria capable of oxidizing manganese were present on filter coatings from all filter media at the end of the start-up periods. The concentration of culturable heterotrophic MnOB corresponded to  $\geq 10^1$  presumptive MnOB/g. This is likely a minimum estimate because a significant number of viable but not immediately culturable MnOB may also be present in the source material.

### Principal component analysis

Figure 4 illustrates a PCA plot where each point represents the bacterial community present in the coating of the filter medium of each column at the end of the experiment based on all taxa. The replicates of each medium share a clear clustering, which indicates comparable bacterial composition of the biofilm developed on the coating of the three replicates.

The PCA identifies four main clustering areas (Figure 4). The bacterial community present on the matured quartz was rather similar to the one found in the source water whereas virgin quartz and calcium carbonate shared strong similarities in their bacterial community composition. Interestingly, these two media also share a similar manganese removal pattern (Figure 1). Contrarily, manganese oxide and polystyrene have distinct clustering areas when compared with the other media. For example, the distinct clustering of polystyrene was strongly affected by the large relative abundance of *Sulfuritalea*, while manganese oxide and virgin quartz/calcium carbonate clustering areas were influenced by the relative abundance of *Pseudomonas* and *Novosphingobium*. These results suggest that when inoculating different filter media with an identical water source, the bacterial community formed in the filter media coatings during the start-up period is influenced by the media type.



**Figure 4** | PCA of bacterial communities present in the source water (day 44 and day 75) and on the coating of each filter medium (day 75). Numbers 1 to 3 represent medium replicates. Grey dots illustrate the taxa detected in all samples. Red labels identify selected key taxa. Please refer to the online version of this paper to see this figure in color: <http://dx.doi.org/10.2166/aqua.2017.084>.

Characterization and comparisons of the microbial diversity in biofilters treating groundwater has been reported in relatively few studies using 16S rRNA amplicon sequencing (e.g. quartz sand by Gülay et al. 2016; Palomo et al. 2016 and Nitzsche et al. 2015; and anthracite/sand by White et al. 2012). Gülay et al. (2016) found similarities among the core microbial communities at five different Danish drinking water treatment plants, all of them using quartz as filter medium to treat groundwater. This is in alignment with our study which indicates a cluster of microbial communities found in the quartz sand columns (Figure 2). Similar to Gülay et al. (2016), our matured quartz microbiome was dominated by different *Proteobacteria* and a high relative abundance of *Nitrospira*. White et al. (2012) investigated the microbial community of a drinking water biofilter with anthracite and quartz and also observed a significant presence of *Nitrospira*. However, the role of filter media for microbial community composition and metabolic activity is not well understood for many alternative media suggested for treating groundwater. Even though all our different filter media completely removed the manganese by day 75, the results of the present study indicate that the potential role of biotic manganese oxidation likely differs among the different filter media. Hence, biostimulation of MnOB by selection of proper media may be a possibility for the industry to enhance performance of drinking water biofilters for manganese removal during start-up.

Further, Figure 4 shows that during the maturation process, there is a tendency of different genera to develop in the different filter media. Biological inoculation using MnOB to accelerate the start-up of biofilters is of interest and is being investigated (Burger et al. 2008; McKee et al. 2016). Preselection of MnOB species to inoculate biofilters could potentially be based on the predisposition of the biofilter towards specific genera.

## CONCLUSIONS

Manganese removal and bacterial diversity of five different filter media were investigated during a 75-day start-up period. This investigation concludes the following:

- The start-up period for virgin quartz, calcium carbonate and polystyrene ended at day 48, day 57 and day 72,

respectively. In contrast, no start-up period was needed for efficient manganese removal by virgin manganese oxide media. All media completely removed manganese by day 75. Quartz and calcium carbonate showed comparable start-up length and bacterial communities. Polystyrene required a longer start-up period compared with virgin quartz and calcium carbonate, and fewer genera with known MnOB were detected in the bacterial community of this medium.

- Matured quartz maintained its original bacterial community fingerprint (dominance of *Nitrospira*) after 75 days of exposure to source water in which ammonia and nitrite were virtually absent.
- Culturable MnOB capable of oxidizing manganese were detected on all filter media at the end of the start-up period. Manganese oxide medium included genera with known MnOB even though the main oxidation process is likely based on abiotic processes.
- When inoculating different filter media with an identical water source, the bacterial communities formed during the start-up period are strongly influenced by the filter media type. Hence, management of bacterial communities may be possible by selecting specific filter media to enhance growth and activity of specific bacteria.

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