Assessing the composition of microbial communities in textile wastewater treatment plants in comparison with municipal wastewater treatment plants

Ken Meerbergen1 | Maarten Van Geel2 | Michael Waud1 | Kris A. Willems1 | Raf Dewil3 | Jan Van Impe4 | Lise Appels3 | Bart Lievens1

1Laboratory for Process Microbial Ecology and Bioinspirational Management (PME&BIM), Department of Microbial and Molecular Systems (M2S), Technology Campus De Nayer, KU Leuven, Sint-Katelijne-Waver, Belgium
2Plant Conservation and Population Biology, Department of Biology, KU Leuven, Leuven, Belgium
3Process and Environmental Technology Lab (PETLab), Department of Chemical Engineering, Technology Campus De Nayer, KU Leuven, Sint-Katelijne-Waver, Belgium
4Chemical and Biochemical Process Technology and Control (BioTeC), Department of Chemical Engineering, Technology Campus Gent, KU Leuven, Gent, Belgium

Correspondence
Bart Lievens
Laboratory for Process Microbial Ecology and Bioinspirational Management (PME&BIM), Technology Campus De Nayer, KU Leuven, Sint-Katelijne-Waver, Belgium. Email: bart.lievens@kuleuven.be.

Funding information
Industrial Research Council of KU Leuven, Grant/Award Number: KP/10/006; Research Council of KU Leuven, Grant/Award Number: OT/13/063

Abstract
It is assumed that microbial communities involved in the biological treatment of different wastewaters having a different chemical composition harbor different microbial populations which are specifically adapted to the environmental stresses encountered in these systems. Yet, little is known about the composition of these microbial communities. Therefore, the aim of this study was to assess the microbial community composition over two seasons (winter and summer) in activated sludge from well-operating textile wastewater treatment plants (WWTPs) in comparison with municipal WWTPs, and to explain observed differences by environmental variables. 454-pyrosequencing generated 160 archaeal and 1645 bacterial species-level Operational Taxonomic Units (OTUs), with lower observed richness in activated sludge from textile WWTPs compared to municipal WWTPs. The bacterial phyla Planctomycetes, Chloroflexi, Chlorobi, and Acidobacteria were more abundant in activated sludge samples from textile WWTPs, together with archaeal members of Thaumarchaeota. Nonmetric multidimensional scaling analysis of the microbial communities showed that microbial communities from textile and municipal WWTPs were significantly different, with a seasonal effect on archaea. Nitrifying and denitrifying bacteria as well as phosphate-accumulation bacteria were more abundant in municipal WWTPs, while sulfate-reducing bacteria were almost only detected in textile WWTPs. Additionally, microbial communities from textile WWTPs were more dissimilar than those of municipal WWTPs, possibly due to a wider diversity in environmental stresses to which microbial communities in textile WWTPs are subjected to. High salinity, high organic loads, and a higher water temperature were important potential variables driving the microbial community composition in textile WWTPs. This study provides a general view on the composition of microbial communities in activated sludge of textile WWTPs, and may provide novel insights for identifying key players performing important functions in the purification of textile wastewaters.

KEYWORDS
454-pyrosequencing, activated sludge, environmental fit, microbial community, quantitative real-time PCR, wastewater
Wastewater treatment using activated sludge processes has been commonly practiced to purify municipal and industrial wastewater, mainly because of the high treatment efficiency and low operating cost. Activated sludge processes are based on the ability of microorganisms to utilize organic material as a source of energy and/or as a source of carbon and other minerals for growth (Carneiro, Umbuzeiro, Oliveira, & Zanoni, 2010), thereby playing important roles in the biodegradation of organic materials, transformation of toxic compounds into harmless products, and removal of nutrients such as ammonia, nitrate, sulfate, and phosphate (Gentile, Jessup, Nyman, & Criddle, 2007; Wang et al., 2011). The stable operation of biological wastewater treatment plants (WWTPs) relies upon the occurrence, relative abundance and activity of several microbial populations in the activated sludge performing these processes (Gentile et al., 2007; Wagner & Loy, 2002; Yang et al., 2014). As variations in microbial community composition are often associated with changes in the functional capabilities of the communities, microbial community and functional stability are generally recognized as important factors for efficiently treat wastewater (Wang, Xia, Wen, Yang, & Zhou, 2014).

Microbial communities of activated sludge in WWTPs have been intensively studied over the last decade, especially for WWTPs dealing with municipal wastewater (e.g., López-Vázquez, Hoöljmans, Brdjanovic, Gijzen, & van Loosdrecht, 2008; Miura et al., 2007; Sanapareddy et al., 2009; Wang et al., 2010, 2011). In general, trends are observed with members of the phylum Proteobacteria, frequently being the most abundant in municipal WWTPs (accounting for 30%–60% of the total number of sequences), followed by Actinobacteria and Bacteroidetes (Hu, Wang, Wen, & Xia, 2012; Ju, Guo, Ye, Xia, & Zhang, 2014; Saunders, Albertsen, Vollertsen, & Nielsen, 2016; Wang, Hu, Xia, Wen, & Ding, 2012; Wei et al., 2015; Ye & Zhang, 2013; Zhang, Shao, & Ye, 2012; Zhao et al., 2014). Moreover, in a recent Illumina MiSeq-based study of 13 municipal WWTPs across Denmark, it was shown that the plants contained a core community of 63 abundant genus-level operational taxonomic units (OTUs), indicating that microbial communities in activated sludge in municipal WWTPs are quite similar across multiple plants (Saunders et al., 2016). However, it is reasonable to assume that communities involved in the biological treatment of more hazardous wastewaters, such as those originating from the textile industry, harbor different microbial populations that are specifically adapted to the environmental stresses encountered in these systems. Textile industry effluents typically contain high concentrations of dyes, dyeing additives, and diverse chemicals, some of which are nonbiodegradable, toxic, mutagenic, or carcinogenic, which pose a major threat to health and environment. Additionally, textile wastewater generally has a low biological oxygen demand/chemical oxygen demand (BOD/COD) ratio (around 20%), a wide range of pH (4–12), and may contain several inhibitor compounds (hampering effective biological wastewater treatment), active substances, adsorbable organic halogens (e.g., chlorine compounds) (AOX) and high salt concentrations, altogether making textile wastewater difficult to treat (Sandhya & Swaminathan, 2006; Selcuk, 2005; Verma, Dash, & Bhunia, 2012; Wu, Wang, Kong, Liu, & Xia, 2007). Therefore, it can be hypothesized that microbial communities in textile WWTPs are different from those observed in municipal WWTPs. However, so far only little is known about the microbial community composition and their functioning in activated sludge from textile wastewater treatment systems (but see Yang et al., 2014).

The aim of this study was to assess the microbial community composition in activated sludge from textile WWTPs in comparison with municipal WWTPs in Flanders (Belgium), and to explain observed differences by environmental factors. The first objective of this study was to assess differences in the microbial (both bacterial and archaeal) communities using 454 amplicon pyrosequencing and real-time quantitative PCR (qPCR). Secondly, we aimed at determining which environmental factors drive species richness, diversity, and community composition in activated sludge from different WWTPs dealing with different wastewaters.

## MATERIALS AND METHODS

### 2.1 Study samples

Activated sludge samples (0.5 L) were collected in triplicate from five textile WWTPs and five municipal WWTPs. Additionally, samples were taken from one plant dealing with both textile and municipal wastewater. All WWTPs were located in Flanders (Belgium) and were characterized by a stable operating system, discharging wastewater effluents within legal standards. Sampling was performed in two different seasons, including winter (February 2015) and summer (July 2015). Following sampling, samples were immediately centrifuged at 3500 g using a portable Hettich EBA 20 centrifuge (Hettich Lab Technology, Tutlingen, Germany) to precipitate the sludge. Approximately 1 g of precipitated sludge was resuspended in 20 ml RNAlater (Life Technologies, Carlsbad, CA, USA) to preserve the nucleic acids present in the samples. At the same time, influent samples (1 L) were collected from each wastewater for chemical analysis. Samples were transported in an ice-cooled container to the laboratory and stored overnight at 4°C prior to further analysis.

### 2.2 DNA extraction, PCR amplification, and 454 amplicon pyrosequencing

Following centrifugation of the samples (10 ml), genomic DNA was extracted from 0.15 g precipitated material using the Power Soil DNA isolation kit (MoBio Laboratories Inc., Solana Beach, CA, USA) according to the manufacturer’s instructions. Subsequently, DNA extracts from the three samples taken per studied WWTP were pooled and stored at −80°C until further processing.

Amplicon libraries were created using two PCR primer sets, targeting part of the bacterial and archaeal 16S ribosomal RNA (rRNA) genes, including the primer pairs S-D-Bact-0341-b-S-17 / S-D-Bact-0785-a-A-21 (covering the V3-V4 region; amplicon size of approximately 464 bp; Klindworth et al., 2013) and S-D-Arch-0519-a-S-15 /
sequences obtained from the 454-pyrosequencing run were assigned to the appropriate sample based on their barcodes and primer sequences, allowing zero discrepancies, and were subsequently trimmed using a custom Python script implemented within the USEARCH v.7 analysis pipeline (Edgar, 2013). Sequences obtained from both PCR replicates per sample were combined and further trimmed based on a minimum Phred score of 30 (base call accuracy of 99.9%) averaged over a 50 bp moving window. Sequence length was determined for each primer pair assessed, with the average being 250 bp. Sequences with ambiguous base calls or homopolymers with a length of more than eight sequences were rejected, as were chimeric sequences detected by UCHIME 4.2 chimera detection (de novo algorithm) (Edgar, Haas, Clemente, Quince, & Knight, 2011). Remaining sequences were aligned and grouped into species-level OTUs based on a 3% sequence dissimilarity cut-off using the UPARSE algorithm implemented in USEARCH (Edgar, 2013). To minimize the risk of retaining sequences from sequencing errors, “global” singletons (i.e., OTUs representing only a single unique sequence in the entire dataset) were removed after UPARSE clustering (Brown et al., 2015; Waud, Busschaert, Ruyters, Jacquemyn, & Lievens, 2014). Due to uneven sequencing depth and correlation between number of sequence reads and number of OTUs per sample (data not shown), the number of sequences was rarefied to 1,000 sequences per sample for both bacteria and archaea. OTU representative sequences were assigned taxonomic identities using the “classify.seqs” command in Mothur (v. 1.36.1) (Schloss et al., 2009) against the Silva taxonomy database, v. Jul 2014 (Quast et al., 2013), manually curated to include organisms previously observed in activated sludge (Midas database; McLroy et al., 2015).

Sequence data for all samples have been deposited in the Sequence Read Archive under the BioProject Accession PRJNA317527. OTU representative sequences were also submitted to GenBank under the Accession Numbers KX029477 to KX031991. A mock bacterial community DNA sample obtained from BEI Resources (HM-276D; even, high-concentration v5.1H) was included as a positive control for 454-pyrosequencing using the bacterial primers, undergoing the same processing steps as all other samples. The results of this mock community were according to the expectations, illustrating the robustness of our results.

2.3 Real-time quantitative PCR

To confirm and further assess the occurrence and distribution of two bacterial OTUs that could be specifically attributed to textile or municipal WWTPs (based on the 454 data), a qPCR analysis was performed. To this end, specific primers were designed for OTU217 (Planctomyces sp.) and OTU23 (Rhodoferax sp.), respectively (Table S2). Specificity of the primers was evaluated using the BLAST algorithm against GenBank, and further evaluated against the 454 datasets obtained in this study. Furthermore, qPCR analyses were performed for two bacterial genes involved in nitrogen removal, including the amoA and nirK gene, encoding a functional nitrifying (ammonium monooxygenase alpha subunit) and denitrifying enzyme (copper-containing nitrite reductase) (for primers see Table S2) (Geets et al., 2007). Analyses were
performed on an ABI StepOnePlus real-time PCR system. Each reaction contained 1.0 μl 10× diluted genomic DNA, 0.5 μl of each primer (20 μM stock), 10.0 μl 2× iTaq universal SYBR Green supermix, and 8.0 μl nuclease-free water. The qPCR run consisted of the same thermal profile as described above except for the annealing temperature which was 64°C (for OTU23 and OTU217) or 59°C (for the amoA and nirK genes). At the end of each qPCR run, a melting curve analysis was performed as described above. Quantification was performed using a standard curve based on known concentrations of DNA standard dilutions from 10^2 copies μl^-1 down to 10^5 copies μl^-1. All qPCR analyses were conducted in duplicate.

2.4 | Chemical analyses

In order to determine the environmental conditions to which the different microbial communities have been exposed, a number of chemical analyses were performed on the influent samples. Analyses were performed using Nanocolor test tubes and a Nanocolor 500D photometer (Macherey-Nagel, Düren, Germany) according to manufacturer’s instructions, and included measurement of ammonium (NH_4^+), nitrite (NO_2^-), nitrate (NO_3^-), COD, BOD, AOX, total phosphorus (TP), and total nitrogen (TN) concentrations. Conductivity (salinity) and pH were measured using the Inolab conductivity and pH level 2 benchtop meters, respectively (WTW, Weilheim, Germany) (for each test one measurement was performed). Additionally, dissolved oxygen (DO) and temperature were measured at the sampling site using a HI9146 DO and temperature meter, respectively (Hanna Instruments, Temse, Belgium).

2.5 | Data analysis

Rarefaction curves were generated for each sample using the Vegan package (v. 2.2-1) for R (Oksanen et al., 2013; R Development Core Team, 2013) to visualize the overall coverage of the studied microbial communities. Additionally, OTU richness, Chao1 and ACE richness estimators, and Shannon diversity were calculated using Mothur (v. 1.36.1) (Schloss et al., 2009). To detect OTUs that are specific for the textile or municipal activated sludge, we performed an indicator species analysis (ISA) in PC-ORD 6 (Dufrêne & Legendre, 1997; McCune & Mefford, 2006). This analysis calculates an indicator value based on fidelity and relative abundance of an OTU in relation to the different sludge groups (textile or municipal). By definition, an indicator value of 100 (perfect indicator) implies that the presence of a given OTU identifies a treatment without error. The obtained indicator values were tested for significance using a Monte Carlo randomization test with 1000 permutations.

For all chemical parameters, the mean and standard error were calculated for every sample origin (textile or municipal) and sampling time (February and July). Further on, chemical parameters were linked to origin and time using general linear models (univariate analysis) conducted in SPSS 22.0 for Windows (SPSS Inc., Chicago, IL). Nonmetric multidimensional scaling (NMDS) was performed on the sample * OTU matrix, using Bray-Curtis distances to visualize differences in the microbial communities (Vegan package v. 2.2-1). Subsequently, the measured chemical parameters were fitted onto the ordination and tested for significance based on a permutation test with 1000 iterations, using the function “envfit”. Finally, the fitted chemical parameters were compared to the results in the univariate analysis.

3 | RESULTS AND DISCUSSION

3.1 | Archaeal and bacterial community composition

Bacterial and archaeal communities were profiled for a total of 22 activated sludge samples (11 samples from February and 11 from July) from five textile WWTPs, five municipal WWTPs and one plant dealing with both textile and municipal wastewater. Strikingly, low amounts of sequences were obtained for the archaeal communities in sludge of the textile WWTPs sampled in February (varying between five and 1,350 sequences; only one sample yielded more than 1,000 archaeal sequences (sample T2F). The reason for this is not clear, but can most likely be attributed to the 454 process as no differences in band intensities were observed across all samples studied when amplicons were loaded on an agarose gel (suggesting that the variability in the number of sequences cannot be attributed to the PCR step).

As a result, only one out of the five samples taken in February from textile WWTPs was retained for further analysis of the archaeal community (sample T2F). For archaea, rarefaction curves generally tended to approach saturation; for bacteria, rarefaction curves did not reach clear saturation, indicating that further sequencing would be necessary to fully cover the bacterial diversity (Fig. S1). Based on Chao1, the sample coverage ranged between 56.9% and 100.0% for the archaeal communities and between 34.1 (outlier; in general >50%) and 92.8% for the bacterial communities (Table 1), suggesting that the most dominant community members were covered in our study. In total, 160 archaeal OTUs and 1645 bacterial OTUs were detected in the samples studied, global singletons excluded. Per sample, observed archaeal and bacterial richness varied between 18 and 60 OTUs (average of 39 ± 3.16 (SE)), and between 119 and 353 OTUs (average of 248 ± 14.5 (SE)), respectively (Table 1). Both archaeal (p = .01) and bacterial (p = 5.03E-05) richness were significantly higher for samples from municipal WWTPs compared to those from textile WWTPs, indicating the presence of a specialized microbial community in activated sludge of textile WWTPs. More particularly, on an average, 29 (±2.94 (SE)) archaeal and 191 (±15.7 (SE)) bacterial OTUs were recovered from a textile WWTP sample, whereas on an average, 45 (±3.63 (SE)) and 298 (±12.3 (SE)) OTUs were recovered from a municipal WWTP sample, respectively. No significant differences were found between archaeal (p = .371) and bacterial (p = .622) richness for both seasons studied.

Taxonomic assignment of the OTUs revealed the presence of two archaeal and 35 bacterial phyla in the activated sludge samples investigated in this study (Table S3). With regard to the archaea, Euryarchaeota represented 94.6% of the total number of sequences, while Thaumarchaeota represented 5.4%, respectively. This is in line with previous studies where Euryarchaeota were found to dominate...
The archaeal community in activated sludge from municipal WWTPs (Ju et al., 2014; Zhang et al., 2014). Further, as also noticed in previous studies on microbial communities in activated sludge (Hu et al., 2012; Saunders et al., 2016; Wang et al., 2012; Wei et al., 2015; Yang et al., 2014; Zhang et al., 2012; Zhao et al., 2014), Proteobacteria was the most abundant bacterial phylum detected (44.3% of the total number of sequences), followed by Bacteroidetes (24.8%). When comparing samples from municipal WWTPs with those from textile WWTPs, while Planctomycetes, Chloroflexi, Acidobacteria, and Candidatus Parcubacteria was higher in February, whereas Planctomycetes, Actinobacteria, Chloroflexi, Chlorobi, and Acidobacteria were more abundant in July (Fig. 1). Thaumarchaeota represents a ubiquitous, relatively recently described archaeal phylum (Brochier-Armanet, Boussau, Gribaldo, & Forterre, 2008), constituting the chemolithoautotrophic ammonia-oxidizers that play important roles in biogeochemical cycles, such as the nitrogen cycle and carbon cycle (Offre, Spang, & Schleper, 2013; Tourna et al., 2011), and can therefore be considered as an important player in activated sludge processes (Park, Wells, Bae, Criddle, & Francis, 2006). Planctomycetes, Chloroflexi, Chlorobi, and Acidobacteria are known to contain halotolerant species that are found in extreme, heavily polluted habitats such as coastal salt marshes and contaminated soils (Canfora et al., 2014; Kutovaya, Lebedeva, Tkakakhova, Ivanova, & Andronov, 2015; Strous et al., 1999). They fulfill important roles in global carbon, nitrogen and/or sulfur cycles, degrading carbohydrates, hydrocarbons, and heavy pollutants (Baker, Lazar, Teske, &

### TABLE 1  Microbial community diversity indices for activated sludge from textile and municipal wastewater treatment plants (WWTPs)

| Wastewater WWTP | Sampling time | Sample | Archaea | Bacteria |
|-----------------|---------------|--------|---------|----------|
|                 |               |        | Sobs%   | Chao1 | Coverage [%]b | Acec | Shannond | Sobs% | Chao1 | Coverage [%]b | Acec | Shannond |
| Municipal 1     | February      | R1_F   | 48      | 51.93  | 92.43   | 56.05 | 1.98     | 233   | 336.21| 69.30   | 420.79| 4.71    |
| Municipal 1     | July          | R1_J   | 60      | 85.50  | 70.18   | 75.22 | 2.66     | 353   | 672.05| 52.53   | 1025.07| 5.26    |
| Municipal 2     | February      | R2_F   | 40      | 62.67  | 63.83   | 101.23| 1.74     | 324   | 702.07| 46.15   | 962.89| 5.08    |
| Municipal 2     | July          | R2_J   | 46      | 81.00  | 56.79   | 57.67 | 2.00     | 324   | 493.32| 65.68   | 518.40| 5.24    |
| Municipal 3     | February      | R3_F   | 44      | 49.00  | 89.80   | 52.57 | 2.18     | 308   | 543.56| 56.66   | 741.99| 5.08    |
| Municipal 3     | July          | R3_J   | 57      | 76.13  | 74.88   | 74.63 | 2.73     | 331   | 970.29| 34.11   | 1267.17| 5.05    |
| Municipal 4     | February      | R4_F   | 40      | 49.17  | 81.36   | 49.77 | 1.50     | 274   | 438.41| 62.50   | 446.92| 4.87    |
| Municipal 4     | July          | R4_J   | 54      | 70.25  | 62.63   | 71.10 | 2.18     | 320   | 359.92| 72.24   | 393.20| 4.86    |
| Municipal 5     | February      | R5_F   | 18      | 23.00  | 78.26   | 23.23 | 1.07     | 314   | 555.38| 56.54   | 957.48| 4.93    |
| Municipal 5     | July          | R5_J   | 50      | 68.20  | 73.31   | 61.99 | 2.72     | 258   | 340.73| 75.72   | 372.89| 4.88    |
| Textile 1       | February      | T1_F   | 30      | 36.00  | 83.33   | 44.17 | 1.90     | 228   | 414.58| 55.00   | 416.50| 4.79    |
| Textile 1       | July          | T1_J   | N.D.    | N.D.   | N.D.    | N.D.  | N.D.     | 233   | 322.02| 72.36   | 392.87| 4.89    |
| Textile 2       | February      | T2_F   | 21      | 24.75  | 84.85   | 26.37 | 0.96     | 181   | 270.52| 66.91   | 264.25| 4.01    |
| Textile 2       | July          | T2_J   | 42      | 55.00  | 76.36   | 72.99 | 2.42     | 119   | 173.08| 68.75   | 153.68| 3.85    |
| Textile 3       | February      | T3_F   | 64      | 34.00  | 88.38   | 28.06 | 0.77     | 126   | 183.95| 68.50   | 223.01| 3.15    |
| Textile 3       | July          | T3_J   | 29      | 44.00  | 65.91   | 32.24 | 1.96     | 141   | 151.90| 72.82   | 192.31| 4.10    |
| Textile 4       | February      | T4_F   | 64      | N.D.   | N.D.    | N.D.  | N.D.     | 174   | 245.03| 71.01   | 259.15| 4.21    |
| Textile 4       | July          | T4_J   | 24      | 25.43  | 94.38   | 28.06 | 0.77     | 126   | 183.95| 68.50   | 223.01| 3.15    |
| Textile 5       | February      | T5_F   | 64      | N.D.   | N.D.    | N.D.  | N.D.     | 261   | 433.94| 60.15   | 592.25| 4.84    |
| Textile 5       | July          | T5_J   | 30      | 39.00  | 76.92   | 56.58 | 1.96     | 222   | 291.45| 76.17   | 305.62| 4.79    |
| Combinedd 1     | February      | TR1_F  | 20      | 20.00  | 100.00  | 20.00 | 1.19     | 256   | 404.22| 63.33   | 493.67| 4.82    |
| Combinedd 1     | July          | TR1_J  | 58      | 79.38  | 73.07   | 77.13 | 2.27     | 306   | 441.71| 69.28   | 510.93| 4.98    |

N.D., not determined due to too few sequences.
*a*Observed richness.
*b*Abundance-based coverage estimator.
*d*Shannon-Wiener diversity index.

dSample from a WWTP dealing with textile and municipal wastewater.
In total, recovered species-level OTUs could be classified in 21 archaeal and 259 bacterial genera (Table S4, approximately 40% of all OTUs), among which 76 genera were uniquely found in municipal WWTPs, 68 were uniquely found in textile WWTPs, and 136 were shared by both. A number of these genera have been functionally characterized and some of these are important constituents of activated sludge processes.

In total, recovered species-level OTUs could be classified in 21 archaeal and 259 bacterial genera (Table S4, approximately 40% of all OTUs), among which 76 genera were uniquely found in municipal WWTPs, 68 were uniquely found in textile WWTPs, and 136 were shared by both. A number of these genera have been functionally characterized and some of these are important constituents of activated sludge processes.

FIGURE 1 Relative abundance of bacterial phyla in activated sludge samples from textile and municipal wastewater treatment plants (WWTPs) (data combined for February and July; 22 samples) (a) sampled in February and July (data combined for textile and municipal WWTP samples; 22 samples) (b). Phyla representing less than 1% of the total amount of sequences are referred to as “Other”.

Dick, 2015; García-Fraile, Benada, Cajthaml, Baldrian, & Lladó, 2016; Glöckner et al., 2003; Hiras, Wu, Eichorst, Simmons, & Singer, 2016; Hug et al., 2013), making them important constituents of activated sludge processes.

In total, recovered species-level OTUs could be classified in 21 archaeal and 259 bacterial genera (Table S4, approximately 40% of all OTUs), among which 76 genera were uniquely found in municipal WWTPs, 68 were uniquely found in textile WWTPs, and 136 were shared by both. A number of these genera have been functionally characterized and some of these are important constituents of activated sludge processes.

These filamentous bacteria were present (albeit at low densities) in both municipal (eight OTUs representing these four genera) and textile (four OTUs belonging to the genera Anaerolinea, Microthrix, and Thiothrix) WWTPs, but were generally more abundantly present in municipal WWTP samples (data not shown). Furthermore, nitrifying and denitrifying bacteria as well as phosphate-accumulating bacteria showed a higher relative read abundance in municipal WWTPs (Fig. 2). This was confirmed by a qPCR analysis targeting the bacterial amoA and nirK genes, the first being involved in nitrification, the second in denitrification: samples from municipal WWTPs were significantly higher in amoA abundance during winter (p = 6.75E-03) and summer (p = 8.34E-05) as opposed to textile WWTP samples. Also, nirK abundance was higher in municipal samples, albeit not significantly (p = .518). These findings suggest that removal of ammonium, nitrate, and phosphate is likely more efficient in municipal WWTPs. Indeed, effluent measurements of the different municipal wastewaters showed an enhanced removal of phosphate and ammonium for the investigated municipal WWTPs in comparison with the investigated textile WWTPs (data not shown).

In contrast, sulfate-reducing bacteria were almost solely found in textile WWTPs (Fig. 2). Notably, a great number of OTUs (40) belonging to the genus Planctomyces (Planctomycetes) were specifically found in textile WWTPs, suggesting that these bacteria can thrive in salt-rich environments (Zhang et al., 2014). Additionally, members of the genera Leucobacter (Actinobacteria) and Hydrogenophaga (Proteobacteria)
were predominantly found in the textile-activated sludge samples. For both genera, species have been described isolated from dye wastewater (Kim & Lee, 2011; Yoon, Kang, Ryu, Jeon, & Oh, 2008). Additionally, some Leucobacter species have already been used in microbial consortia to degrade disperse and reactive dyes (Franciscon et al., 2010, 2015).

NMDS ordination of the community composition, inferred from the archaeal \( (p = .033; R^2 = .280) \) and bacterial \( (p = .001; R^2 = .663) \) OTU relative abundance, revealed that there was a significant difference (Goodness-of-Fit) between activated sludge samples from textile and municipal WWTPs (Fig. 3). Furthermore, samples from municipal WWTPs were much more similar than those from textile WWTPs (Fig. 3). Interestingly, NMDS ordination plotted the samples from the plant dealing with both municipal and textile wastewater (TR1) in between samples from municipal WWTPs on one hand and samples from textile WWTPs on the other hand (Fig. 3). Significant differences were found in community composition of archaeal communities sampled in February and July \( (p = .034; R^2 = .179) \), but not for bacteria \( (p = .694; R^2 = .014) \).

Strikingly, only one archaeal and one bacterial OTU was shared by all samples investigated. These included an OTU corresponding to Methanosaeta sp. (Euryarchaeota) and an OTU corresponding to an unidentified member of Proteobacteria, respectively. The first one covered approximately 23% of all archaeal sequences, while the second covered about 3% of all bacterial sequences. A core microbial community, consisting of five archaeal and 30 bacterial OTUs could be identified for municipal WWTPs that made up 33.0% and 19.3% of the total archaeal and bacterial sequences, respectively. Two of these bacterial genera, Dechloromonas and candidatus Epiflobacter, were also identified as one of the 23 to the genus-level identified core genera in a recent study about activated sludge microbial communities in 13 Danish WWTPs (in total, 63 abundant genus-level

**FIGURE 2** Read abundance of bacterial genera performing essential functions in activated sludge processes (nonexhaustive list), including nitrification (a), denitrification (b), sulfate reduction (c), and phosphate accumulation (d), in samples from textile and municipal wastewater treatment plants (WWTPs) (data combined for February and July; 22 samples). The number of Operational Taxonomic Units (OTUs) belonging to the genus is reported between brackets.
OTUs were identified based on a 6% 16S rRNA gene sequence dissimilarity cut-off (Saunders et al., 2016), suggesting a ubiquitous occurrence of these genera in municipal WWTPs. In contrast, only one archaeal and one bacterial OTU was shared by all textile WWTPs, suggesting that microbial communities in textile WWTPs are driven by diverse factors. ISA, allowing to identify one more given species/OTUs to serve as an indicator of a particular ecosystem, revealed the presence of two and six archaeal OTUs that could be attributed to textile and municipal WWTPs, respectively. For the bacteria, ISA revealed 10 and 34 indicator OTUs, respectively (Table S5). In order to confirm and generalize these results, all 22 samples investigated as well as six additional sludge samples from three textile WWTPs (sampled in February and July) and 10 additional samples from five municipal WWTPs (sampled in February and July) were subjected to qPCR analysis targeting two randomly selected indicator bacteria. These included OTU23, representing a member of the genus *Rhodoferax* (Proteobacteria), which was found as an indicator for municipal activated sludge, and OTU217, member of the genus *Planctomyces* (Planctomycetes), which was found as an indicator for activated sludge from textile WWTPs. OTU23 was found in all municipal WWTP samples analyzed, while it was absent in the textile WWTP samples. Additionally, OTU23 was found in activated sludge from the plant purifying both municipal and textile wastewater. OTU217 was found at five textile WWTPs, both in February and July (10 positive samples on a total of 16), while it was not detected in any sample from the municipal WWTPs (Table S6).

### 3.2 Environmental factors explaining differences in microbial communities

In order to determine environmental factors potentially explaining the differences in microbial communities in activated sludge from textile WWTPs and municipal WWTPs, several environmental variables were measured on the influent wastewater (Table 2). Samples from textile WWTPs had significantly higher salt levels and were higher in temperature (Table 2 and 3). Further, textile wastewater was found to contain a significantly higher organic load, as shown by the high COD and BOD values as opposed to municipal wastewater, whereas the DO level was significantly lower (Table 2 and 3), supporting previous findings (Verma et al., 2012). Also AOX values were slightly, but not significantly higher for textile wastewater. Little or no differences were found for NH$_4^+$, NO$_2^-$, NO$_3^-$, TP, TN, and pH (Table 2 and 3). Wastewater from the plant treating both municipal and textile wastewater was characterized by values situated between textile and municipal wastewater (Table 2). Notably, for some plants, differences were observed for particular influent characteristics (e.g., NH$_4^+$, TP, and COD) between the two sampling periods (Table 2), suggesting that these companies treat wastewaters with a variable composition.

Archaeal community composition significantly ($p < .05$) varied with temperature, COD, BOD, conductivity, pH, and TN (Table 4), whereas the bacterial communities varied with temperature, conductivity, pH, DO, COD, BOD, and NH$_4^+$ (Table 4). When fitting the environmental variables on the NMDS ordination plot of the microbial communities (Fig. 3), temperature, conductivity, COD, BOD, and DO differentiated both archaeal and bacterial communities from textile and municipal wastewater (Table 2). Notably, for some plants, differences were observed for particular influent characteristics (e.g., NH$_4^+$, TP, and COD) between the two sampling periods (Table 2), suggesting that these companies treat wastewaters with a variable composition.

Archaeal community composition significantly ($p < .05$) varied with temperature, COD, BOD, conductivity, pH, and TN (Table 4), whereas the bacterial communities varied with temperature, conductivity, pH, DO, COD, BOD, and NH$_4^+$ (Table 4). When fitting the environmental variables on the NMDS ordination plot of the microbial communities (Fig. 3), temperature, conductivity, COD, BOD, and DO differentiated both archaeal and bacterial communities from textile and municipal WWTPs, with an increasing gradient toward samples from textile WWTPs except for DO, which shows an increasing gradient toward municipal activated sludge samples. For archaea, also TN and pH significantly divided samples from both groups. Fitting
### TABLE 2  Influent wastewater characteristics

| Wastewater | WWTP | Sampling time | Sample | Conductivity (mS/cm) | DO (ppm) | pH | Temperature (°C) | NH$_4^+$ (mg/L) | NO$_2^-$ (mg/L) | NO$_3^-$ (mg/L) | COD (mg O2/L) | BOD (mg O2/L) | TP (PO$_4^-$ P) (mg/L) | TN (mg/L) | AOX (mg/L) |
|------------|------|---------------|--------|----------------------|----------|----|-----------------|----------------|----------------|----------------|----------------|----------------|-----------------------------|-----------|----------|
| Municipal  | 1    | February      | R1_F   | 0.959                | 6.27     | 6.97| 10.4            | 29.1           | 0.02           | 1.3            | 167            | 6              | 2.22                                      | 26        | 0.03     |
| Municipal  | 1    | July          | R1_J   | 1.000                | 1.81     | 7.52| 19.9            | 43.7           | 0.02           | 3.1            | 199            | 25             | 6.66                                      | 38        | 0.03     |
| Municipal  | 2    | February      | R2_F   | 0.580                | 10.96    | 7.01| 8.3             | 8.8            | 0.02           | 1.3            | 21             | 2              | 0.73                                      | 9         | 0.04     |
| Municipal  | 2    | July          | R2_J   | 1.000                | 0.04     | 7.64| 20.6            | 53.0           | 0.06           | 2.1            | 240            | 22             | 7.30                                      | 53        | 0.03     |
| Municipal  | 3    | February      | R3_F   | 0.652                | 10.27    | 6.88| 9.4             | 0.2            | 0.02           | 24.4           | 52             | 2              | 1.65                                      | 10        | 0.04     |
| Municipal  | 3    | July          | R3_J   | 1.000                | 1.05     | 7.59| 9.6             | 80.0           | 0.02           | 3.2            | 380            | 34             | 8.68                                      | 75        | 0.09     |
| Municipal  | 4    | February      | R4_F   | 1.080                | 9.02     | 6.77| 11.0            | 17.8           | 0.04           | 5.2            | 519            | 116            | 6.35                                      | 33        | 0.04     |
| Municipal  | 4    | July          | R4_J   | 2.000                | 0.53     | 7.76| 10.3            | 41.2           | 0.03           | 1.3            | 92             | 13             | 14.78                                     | 33        | 0.04     |
| Municipal  | 5    | February      | R5_F   | 1.541                | 8.52     | 7.08| 14.2            | 41.0           | 0.02           | 1.6            | 307            | 29             | 2.97                                      | 43        | 0.04     |
| Municipal  | 5    | July          | R5_J   | 2.000                | 3.09     | 7.62| 21.1            | 61.0           | 0.02           | 2.4            | 261            | 34             | 5.33                                      | 52        | 0.05     |
| Textile    | 1    | February      | T1_F   | 3.960                | 0.67     | 7.23| 15.4            | 4.0            | 0.06           | 6.6            | 1476           | 90             | 1.75                                      | 24        | 0.06     |
| Textile    | 1    | July          | T1_J   | 4.010                | 0.30     | 7.40| 34.8            | 12.9           | 0.11           | 3.7            | 1724           | 300            | 9.84                                      | 24        | 0.06     |
| Textile    | 2    | February      | T2_F   | 3.900                | 0.91     | 7.67| 18.2            | 76.0           | 0.02           | 6.3            | 1153           | 125            | 4.19                                      | 96        | 0.14     |
| Textile    | 2    | July          | T2_J   | 3.000                | 0.20     | 7.84| 34.5            | 80.0           | 0.05           | 4.0            | 1124           | 320            | 9.34                                      | 91        | 0.14     |
| Textile    | 3    | February      | T3_F   | 1.356                | 1.46     | 5.80| 22.3            | 1.0            | 0.09           | 14.3           | 2818           | 28             | 2.66                                      | 51        | 0.08     |
| Textile    | 3    | July          | T3_J   | 1.000                | 0.30     | 6.62| 27.5            | 1.6            | 0.04           | 13.2           | 2993           | 480            | 5.61                                      | 42        | 0.04     |
| Textile    | 4    | February      | T4_F   | 8.140                | 2.28     | 8.11| 15.6            | 6.5            | 0.02           | 9.1            | 1426           | 134            | 14.20                                     | 29        | 0.02     |
| Textile    | 4    | July          | T4_J   | 9.000                | 0.90     | 7.98| 26.2            | 2.2            | 0.03           | 4.8            | 2771           | 130            | 7.42                                      | 7         | 0.44     |
| Textile    | 5    | February      | T5_F   | 3.280                | 0.88     | 7.55| 16.0            | 76.0           | 0.03           | 2.6            | 693            | 35             | 8.10                                      | 81        | 0.03     |
| Textile    | 5    | July          | T5_J   | 2.000                | 0.42     | 7.53| 25.2            | 37.1           | 0.06           | 8.8            | 786            | 62             | 7.79                                      | 54        | 0.02     |
| Combineda | 1    | February      | TR1_F  | 0.812                | 3.56     | 6.77| 3.3             | 11.0           | 0.07           | 2.5            | 580            | 2              | 1.52                                      | 17        | 0.06     |
| Combineda | 1    | July          | TR1_J  | 1.000                | 0.99     | 7.67| 10.3            | 47.5           | 0.09           | 5.4            | 720            | 43             | 9.79                                      | 53        | 0.06     |

AOX, Adsorbable organic halogens; BOD, biological oxygen demand; COD, chemical oxygen demand; DO, dissolved oxygen; TN, total nitrogen; TP, total phosphorus.  

aSample from a WWTP dealing with textile and municipal wastewater.
the environmental variables on the NMDS ordination of the bacterial communities also revealed that \( \text{NH}_4^+ \) and pH significantly discriminated samples from textile WWTPs, with an increasing gradient toward samples of two textile WWTPs (T2 and T5; irrespective of sampling time).

Altogether, our study shows that activated sludge from textile WWTPs harbors a highly specialized microbial community which is different from those from municipal WWTPs. High salinity, high organic loads, and a higher water temperature were important factors driving the microbial community composition in activated sludge from textile WWTPs. Earlier research confirms the importance of these factors in establishing microbial community structures (Griffiths, Ritz, Ebblewhite, & Dobson, 1998; Liu, Yang, Gong, & Su, 2008; Pietikäinen, Pettersson, & Bååth, 2005; Rietz & Haynes, 2003; Siggins, Enright, & O’Flaherty, 2011; Wakelin et al., 2012; Wang et al., 2012). In addition to these general parameters, other variables specifically linked to the textile dyeing industry (e.g., the dyes used, chemical additives etc.) are also likely to be involved in the

### TABLE 3
Univariate analysis of the environmental variables corresponding to the analyzed activated sludge samples. Further, each environmental parameter is investigated through multiple comparisons in the origin subgroups (i.e., textile, municipal, and combined)

| Environmental variable | F-value | p-value | Textile/municipal | Textile/combined | Combined/ municipal |
|-------------------------|---------|---------|-------------------|-----------------|-------------------|
| Conductivity           | 6.309   | .008    | 0.009             | 0.112           | 0.980             |
| DO                     | 5.150   | .016    | 0.013             | 0.814           | 0.451             |
| pH                     | 0.097   | .908    | 0.933             | 0.934           | 0.988             |
| Temperature            | 9.641   | .001    | 0.005             | 0.007           | 0.374             |
| \( \text{NH}_4^+ \)    | 0.196   | .824    | 0.826             | 1.000           | 0.930             |
| \( \text{NO}_2^- \)    | 5.397   | .014    | 0.071             | 0.311           | 0.025             |
| \( \text{NO}_3^- \)    | 0.720   | .500    | 0.527             | 0.719           | 0.988             |
| COD                    | 15.177  | .000    | 0.000             | 0.089           | 0.639             |
| BOD                    | 5.110   | .017    | 0.018             | 0.189           | 0.977             |
| AOX                    | 1.694   | .210    | 0.191             | 0.707           | 0.967             |
| TP                     | 0.336   | .719    | 0.781             | 0.892           | 1.000             |
| TN                     | 0.601   | .558    | 0.580             | 0.765           | 0.992             |

AOX, adsorbable organic halogens; BOD, biological oxygen demand; COD, chemical oxygen demand; DO, dissolved oxygen; TN, total nitrogen; TP, total phosphorus.

*Sample from a WWTP dealing with textile and municipal wastewater.

### TABLE 4
Results of the permutation test of the nonmetric multidimensional scaling coordinates (NMDS 1 and NMDS 2) testing for significant relationships between activated sludge samples from textile and municipal wastewater treatment plants (WWTPs) and influent chemical variables

| Environmental variable | \( R^2 \) | p-value | Environmental variable | \( R^2 \) | p-value |
|-------------------------|-----------|---------|-------------------------|-----------|---------|
| AOX                     | 0.3795    | .068    | AOX                     | 0.2964    | .053    |
| BOD                     | 0.3892    | .041*   | BOD                     | 0.4630    | .007*   |
| COD                     | 0.4960    | .014*   | COD                     | 0.8605    | .004*   |
| Conductivity            | 0.5433    | .017*   | Conductivity            | 0.5997    | .001*   |
| DO                      | 0.2908    | .088    | DO                      | 0.2868    | .047*   |
| \( \text{NH}_4^+ \)     | 0.2985    | .061    | \( \text{NH}_4^+ \)     | 0.4298    | .006*   |
| \( \text{NO}_2^- \)     | 0.0759    | .558    | \( \text{NO}_2^- \)     | 0.1211    | .269    |
| \( \text{NO}_3^- \)     | 0.0201    | .811    | \( \text{NO}_3^- \)     | 0.1149    | .321    |
| pH                      | 0.3122    | .046*   | pH                      | 0.3608    | .012*   |
| Temperature             | 0.5423    | .005*   | Temperature             | 0.4674    | .004*   |
| TN                      | 0.3504    | .034*   | TN                      | 0.2468    | .066    |
| TP                      | 0.2796    | .093    | TP                      | 0.1001    | .343    |

AOX, adsorbable organic halogens; BOD, biological oxygen demand; COD, chemical oxygen demand; DO, dissolved oxygen; TN, total nitrogen; TP, total phosphorus.

The results are based on 999 permutations.

\( R^2 \) and p-values are shown for the different environmental variables, where significant p-values are indicated with *. 
mechanisms behind the assembly of these microbial communities. Further research is needed to unravel their importance in driving the assembly of textile WWTP communities. Future research building on our results could also aim at the identification of key players in the community that may be exploited for enhanced purification of textile wastewaters. In this regard, the phyla Planctomycetes (Bacteria) and Thaumarchaeota (Archaea), both abundantly present in activated sludge from textile WWTPs and possibly performing important functions in the purification of textile wastewaters, may provide promising candidates.

ACKNOWLEDGMENTS
The authors thank the Industrial Research Council of KU Leuven (KP/10/006) and the Research Council of KU Leuven (OT/13/063) for financial support. Special thanks goes out to Stefan Ruyters for his help with designing the experiments. The DNA mock community was obtained through BEI Resources, NIAID, NIH as part of the Human Microbiome Project: Genomic DNA from Microbial Mock Community B (Even, High Concentration), v5.1H, for Whole Genome Shotgun Sequencing, HM-276D.

CONFLICT OF INTEREST
No conflict of interest declared.

REFERENCES
Baker, B. J., Lazar, C. S., Teske, A. P., & Dick, G. J. (2015). Genomic resolution of linkages in carbon, nitrogen, and sulfur cycling among widespread estuary sediment bacteria. Microbiome, 3, 14.

Brochier-Armanet, C., Boussau, B., Gribaldo, S., & Forterre, P. (2008). Mesophlic Crenarchaeota: Proposal for a third archaeal phylum, the Thaumarchaeota. Nature Reviews Microbiology, 6, 245–252.

Brown, S. P., Veach, A. M., Rigdon-Huss, A. R., Grond, K., Lickteig, S. K., Brochier-Armanet, C., Boussau, B., Gribaldo, S., & Forterre, P. (2008). Potential of a bacterial consortium to degrade azo dye Disperse Red 1 in a pilot scale anaerobic-aerobic reactor. Process Biochemistry, 48, 816–825.

Franciscon, E., Piubelli, F., Fantinatti-Garbogni, F., de Menezes, C. R., Silva, I. S., Cavaco-Paulo, A., ... Durrant, L. R. (2010). Polymerization study of the aromatic amines generated by the biodegradation of azo dyes using the laccase enzyme. Enzyme and Microbial Technology, 46, 360–365.

García-Fraile, P., Benada, O., Cajthaml, T., Baldrían, P., ... Lladó, S. (2016). Terracidiophilus gabretensis gen. nov., sp. nov., an Abundant and Active Forest Soil Acidobacterium Important in Organic Matter Transformation. Applied and Environmental Microbiology, 82, 560–569.

Geets, J., De Cooman, M., Wittebolle, L., Heylen, K., Vanparys, B., ... De Vos, P., ... Boon, N. (2007). Real-time PCR assay for the simultaneous quantification of nitrifying and denitrifying bacteria in activated sludge. Applied Microbiology and Biotechnology, 75, 211–221.

Gentile, M. E., Jessup, C. M., Nyman, J. L., & Criddle, C. S. (2007). Correlation of functional instability and community dynamics in denitrifying dispersed-growth reactors. Applied and Environmental Microbiology, 73, 680–690.

Glöckner, F. O., Kube, M., Bauer, M., Teeling, H., Lombardot, T., Ludwig, W., ... Reinhart, R. (2003). Complete genome sequence of the marine planctomycete Pirellula sp. strain 1. Proceedings of the National Academy of Sciences USA, 100, 8298–8303.

Griffiths, B. S., Ritz, K., Ebbewehte, N., & Dobson, G. (1998). Soil microbial community structure: Effects of substrate loading rates. Soil Biology & Biochemistry, 31, 145–153.

Hiras, J., Wu, Y. W., Eichorst, S. A., Simmons, B. A., & Singer, S. W. (2016). Refining the phylum Chlorobi by resolving the phylogeny and metabolic potential of the representative of a deeply branching, uncultivated lineage. ISME Journal, 10, 833–845.

Hu, M., Wang, X., Wen, X., & Xia, Y. (2012). Microbial community structures in different wastewater treatment plants as revealed by 454-pyrosequencing analysis. Bioresource Technology, 117, 72–79.

Hug, L. A., Castelle, C. J., Wrighton, K. C., Thomas, B. C., Sharon, I., Frischkorn, K. R., ... Banfield, J. F. (2013). Community genomic analyses constrain the distribution of metabolic traits across the Chloroflexi phylum and indicate roles in sediment carbon cycling. Microbiome, 1, 22.

Ju, F., Guo, F., Ye, L., Xia, Y., & Zhang, T. (2014). Metagenomic analysis on seasonal microbial variations of activated sludge from a full-scale wastewater treatment plant over 4 years. Environmental Microbiology Reports, 6, 80–89.

Kim, H. J., & Lee, S. S. (2011). Leucobacter kyeonggiensis sp. nov., a new species isolated from dye waste water. The Journal of Microbiology, 49, 1044–1049.

Klintworth, A., Prcerus, E., Schweer, T., Peplis, J., Quast, C., Horn, M., & Glöckner, F. O. (2013). Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-generation sequencing-based diversity studies. Nucleic Acids Research, 41, e1.

Kutovaya, O. V., Lebedeva, M. P., Tikhakakhova, A. K., Ivanova, E. A. & Andronov, E. E. (2015). Metagenomic characterization of biodiversity in the extremely arid desert soils of Kazakhstan. Eurasian Soil Science, 48, 493–500.

Liu, S., Yang, F., Gong, Z., & Su, Z. (2008). Assessment of the positive effect of salinity on the nitrogen removal performance and microbial composition during the start-up of CANON process. Applied Microbiology and Biotechnology, 80, 339–348.

López-Vázquez, C. M., Hooijmans, C. M., Brdjanovic, D., Gijzen, H. J., & van Loosdrecht, M. (2008). Factors affecting the microbial populations at full-scale enhanced biological phosphorus removal (EBPR) wastewater treatment plants in The Netherlands. Water Research, 42, 2349–2360.
McCune, B., & Mefford, M. J. (2006). PC-ORD, Multivariate Analysis of Ecological Data. MJM Software, Glenden Beach, OR, home.centurytel.net/~mj/mj/pdf/pcord.htm.

McIlroy, S. J., Saunders, A. M., Albertsen, M., Nierchilo, M., McIlroy, B., Hansen, A. A., ..., Nielsen, P. H. (2015). MIDAS: The field guide to the microbes of activated sludge. Database, 2015, bav062.

Miura, Y., Hiraiwa, M. N., Ito, T., Itonaga, T., Watanabe, Y., & Okabe, S. (2007). Bacterial community structures in MBRs treating municipal wastewater: Relationship between community stability and reactor performance. Water Research, 41, 627–637.

Nielsen, P. H., Kragelund, C., Seviour, R. J., & Nielsen, J. L. (2009). Identity and ecophysiology of filamentous bacteria in activated sludge. FEMS Microbiology Reviews, 33, 969–998.

Offre, P., Spang, A., & Schleper, C. (2013). Archaea in biogeochemical cycles. Annual Review of Microbiology, 67, 437–457.

Oksanen, J., Blanchet, F. G., Kindt, R., Legendre, P., Minchin, P. R., O’Hara, R.,.Abate, P., … Schleper, C. (2013). Archaea in biogeochemical cycles. Annual Review of Microbiology, 67, 437–457.

Park, H. D., Wells, G. F., Bae, H., Criddle, C. S., & Francis, C. A. (2006). Occurrence of ammonia-oxidizing archaea in wastewater treatment plant bioreactors. Applied and Environment Microbiology, 72, 5643–5647.

Pietikäinen, J., Pettersson, M., & Bäåth, E. (2005). Comparison of temperature effects on soil respiration and bacterial and fungal growth rates. FEMS Microbiology Ecology, 52, 49–58.

Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., … Richert, I., Dinasquet, J., Logares, R., Riemann, L., Yager, P. L., & Wendeberg, H. (2015). Bacterial community composition and function in sewage treatment systems. Current Opinion in Biotechnology, 13, 218–227.

Rossetti, S., Tomei, M. C., Nielsen, P. H., & Tandoli, V. (2005). "Microthrix parvicella", a filamentous bacterium causing bulking and foaming in activated sludge systems: A review of current knowledge. FEMS Microbiology Ecology, 29, 49–64.

Sanapareddy, N., Hamp, T. J., Gonzalez, L. C., Hilger, H. A., Fodor, A. A., & Clinton, S. M. (2009). Molecular diversity of a North Carolina wastewater treatment plant as revealed by pyrosequencing. Applied and Environment Microbiology, 75, 1688–1696.

Sandhya, S., & Swaminathan, K. (2006). Kinetic analysis of treatment of textile wastewater in hybrid column upflow anaerobic fixed bed reactor. Chemical Engineering Journal, 122, 87–92.

Savio, D., Sinclair, L., Ijaz, U. Z., Parajka, J., Reischer, G. H., Stadler, P., … Eiler, A. (2015). Bacterial diversity along a 2600 km river continuum. Environmental Microbiology, 17, 4994–5007.

Selcuk, H. (2005). Decolorization and detoxification of textile wastewater by ozonation and coagulation processes. Dyes Pigments, 64, 217–222.

Shan, D., Wei, G., Li, M., Wang, W., Li, X., Gao, Z., & Shao, Z. (2015). Distribution and diversity of bacterioplankton communities in subtropical seawater around Xiamen Island, China. Microbiological Research, 175, 16–23.

Siggins, A., Enright, A. M., & O’Flaherty, V. (2011). Temperature dependent (37–15 C) anaerobic digestion of a trichloroethylene-contaminated wastewater. Bioresource Technology, 102, 7645–7656.

Strous, M., Fuerst, J. A., Kramer, E. H., Logemann, S., Muyzer, G., van de Pas-Schoonen, K. T., … Jetten, M. S. M. (1999). Missing lithotroph identified as new planctomycete. Nature, 400, 446–449.

Tourna, M., Stieglmeier, M., Spang, A., Könneke, M., Schintlmeister, A., Urih, T., … Schleper, C. (2011). Nitrososphaera viennensis, an ammonia oxidizing archaeon from soil. Proceedings of the National Academy of Sciences USA, 108, 8420–8425.

Verma, A. K., Dash, R. R., & Bhunia, P. (2012). A review on chemical coagulation/floculation technologies for removal of colour from textile wastewaters. Journal of Environmental Management, 93, 154–168.

Weaver, M., & Loy, A. (2002). Bacterial community composition and function in sewage treatment systems. Current Opinion in Biotechnology, 13, 56–64.
Zhao, D., Huang, R., Zeng, J., Yu, Z., Liu, P., Cheng, S., & Wu, Q. L. (2014). Pyrosequencing analysis of bacterial community and assembly in activated sludge samples from different geographic regions in China. *Applied Microbiology and Biotechnology, 98*, 9119–9128.

**SUPPORTING INFORMATION**

Additional Supporting Information may be found online in the supporting information tab for this article.