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ENVIRONMENTAL BIOTECHNOLOGY

Microbial biogeography across a full-scale wastewater treatment plant transect: evidence for immigration between coupled processes

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Abstract Wastewater treatment plants use a variety of bioreactor types and configurations to remove organic matter and nutrients. Little is known regarding the effects of different configurations and within-plant immigration on microbial community dynamics. Previously, we found that the structure of ammonia-oxidizing bacterial (AOB) communities in a full-scale dispersed growth activated sludge bioreactor correlated strongly with levels of NO_2^- entering the reactor from an upstream trickling filter. Here, to further examine this puzzling association, we profile within-plant microbial biogeography (spatial variation) and test the hypothesis that substantial microbial immigration occurs along a transect (raw influent, trickling filter biofilm, trickling filter effluent, and activated sludge) at the same full-scale wastewater treatment plant. AOB *amoA* gene abundance increased >30-fold between influent and trickling filter effluent concomitant with NO_2^- production, indicating unexpected growth and activity of AOB within the trickling filter. *Nitrosomonas europaea* was the dominant AOB phylotype in trickling filter biofilm and effluent, while a distinct "*Nitrosomonas*-like" lineage dominated in activated sludge. Prior time series indicated that this "*Nitrosomonas*-like" lineage was dominant when NO_2^- levels in the trickling filter effluent (i.e., activated sludge influent) were low, while *N. europaea* became dominant in the activated sludge when NO_2^- levels were high. This is consistent with the hypothesis that NO_2^- production may cooccur with biofilm sloughing, releasing *N. europaea* from the trickling filter into the activated sludge bioreactor. Phylogenetic microarray (PhyloChip) analyses revealed significant spatial variation in taxonomic diversity, including a large excess of methanogens in the trickling filter relative to activated sludge and attenuation of *Enterobacteriaceae* across the transect, and demonstrated transport of a highly diverse microbial community via

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the trickling filter effluent to the activated sludge bioreactor. Our results provide compelling evidence that substantial immigration between coupled process units occurs and may exert significant influence over microbial community dynamics within staged bioreactors.

Keywords Activated sludge · Ammonia-oxidizing bacteria · Immigration · PhyloChip · Sloughing · Trickling filter

Introduction

Wastewater treatment plants (WWTPs) are vitally important to the protection of human health and aquatic ecosystems. Central to the function of most WWTPs are secondary treatment bioreactors—controlled environments for the growth and maintenance of self-assembled microbial consortia that remove oxygen-depleting organics and nutrients from water. WWTP bioreactors can largely be divided into two broad categories: biofilm-based reactors, of which the trickling filter is a common example, and suspended growth systems, the archetype of which is the activated sludge process. Application of molecular tools in recent years has greatly expanded our knowledge of microbial community structure in both bioreactor types. Nonetheless, our understanding of factors influencing microbial community composition in these engineered systems, and temporal and spatial variation therein, remains limited. Increased knowledge of the ecology of the critical microbial biocatalysts in these systems is needed for rational development of operational and design strategies that can improve process efficiency and stability (Curtis et al. 2003).

WWTP bioreactors also serve as valuable testing facilities for fundamental questions in microbial ecology. One outstanding question is the importance of immigration in structuring microbial communities (Sloan et al. 2006). Classically, deterministic factors associated with niche differentiation have been invoked to explain variations in microbial community structure. Neutral dispersal processes, of which immigration is an example, have only recently begun to be explored in microbial ecosystems (Sloan et al. 2006). Indeed, few studies have experimentally investigated the taxonomic composition of microbial immigrants or the potential influence of immigration to the community assembly process. Lindstrom et al. (2004; 2006) reported taxonomic identity and immigration rate of microbial constituents to lakes and associated the influence of inlet bacteria on bulk lake microbial community composition with hydraulic residence time. More recently, Jones et al. (2008; 2009) investigated the influence of atmospheric deposition on freshwater bacterial communities and concluded that this route may not significantly affect community structure. The taxonomic identity of microbial immigrants and the potential role of immigration in the community assembly process in WWTP bioreactors remain largely unexamined.

In biofilm-based reactors, detachment is the primary process that balances microbial growth and thus sets the steady-state accumulation of biofilm (Stewart 1993). While mechanistic explanations can be provided for some detachment phenomena, factors influencing detachment are not well understood (Morgenroth 2003; Rittmann and Laspidou 2003). Nonetheless, detachment mechanisms can be divided into four categories: (1) sloughing, (2) erosion, (3) abrasion, and (4) predatory grazing (Stewart 1993). The dominant detachment mechanism in highly loaded trickling filters is expected to be sloughing—detachment of relatively large portions of biofilm whose characteristic size is comparable to or greater than the thickness of the biofilm itself (Morgenroth and Wilderer 2000). Researchers have begun to explore how detachment processes influence within-biofilm population dynamics (Elenter et al. 2007), and process engineers have long recognized that influent biomass can impact activated sludge process performance by changing solids retention time (Rittmann and McCarty 2001). However, little is known about the potential role of sloughing and other detachment mechanisms from biofilm-based reactors as sources of microbial immigration to downstream process units.

A key step in nitrogen removal in WWTP bioreactors is nitrification, the microbial oxidation of ammonia (NH_3) to nitrate (NO_3^-) via nitrite (NO_2^-). The rate-limiting step in nitrification, oxidation of NH_3 to NO_2^-, is catalyzed by two distinct groups of chemolithoautotrophic microorganisms—ammonia-oxidizing bacteria (AOB) and recently discovered ammonia-oxidizing archaea (AOA). Unfortunately, nitrification failures in WWTPs are common, thereby leading to excess discharge of reactive nitrogen to natural environments, and the reasons for these failures are often obscure. Failures have been linked in some cases to shifts in microbial community structure (Wittebolle et al. 2005), but the key factors influencing nitrifier community structure and dynamics in activated sludge, including a potential role for immigration, are not well defined.

We previously documented a strong correlation between influent NO_2^- to the activated sludge bioreactor and AOB population dynamics during a 1-year time series of weekly samples from a full-scale WWTP (Wells et al. 2009). Activated sludge bioreactor influent NO_2^- concentrations (e.g., NO_2^- in the effluent from an upstream trickling filter) were strongly positively correlated with the relative abundance of AOB from the *Nitrosomonas europaea* lineage and strongly negatively correlated with a novel *Nitrosomonas*-like lineage. Here, we compile independent converging lines of retrospective evidence suggesting that this influent NO_2^- may be a signature of microbial immigration via sloughing from an upstream trickling filter (detailed in "Results" section). To further examine this association, we subjected biological replicate biomass samples from a transect along the plant treatment train to a variety of molecular analyses. The goals of this study were twofold: (1) to prospectively test the

hypothesis that significant within-plant microbial immigration occurs and may in turn influence dynamics in microbial community structure in downstream processes and (2) to profile biogeography (spatial variation) in microbial community structure along a transect through a full-scale WWTP.

Materials and methods

Site description and sampling procedure

Samples described in this study were obtained at the Palo Alto Regional Water Quality Control Plant (PARWQCP) in Palo Alto, CA (37.2631° N, 122.0835° W). The PARWQCP treats 100,000–170,000 m^3/day of predominantly municipal wastewater, and treated effluent is discharged to the South San Francisco Bay. Raw sewage proceeds through grit screen and primary sedimentation prior to secondary treatment, which consists of two independent unit processes operated in series in a roughing reactor/activated sludge process configuration. Primary effluent is first equally distributed between two forced draft trickling filters (29 m diameter), normally operated without recirculation. Corrugated PVC plastic media (6.6 m depth) provide support for attached biological growth, thereby promoting ~65 % of total COD removal with minimal NH_4^+ removal. Second, trickling filter effluent proceeds to a highly aerated activated sludge bioreactor operated for nitrification and remaining COD removal. No settling tank or other solids separation unit separates these two processes, ensuring that all biomass sloughed from the trickling filter enters the activated sludge bioreactor. The activated sludge bioreactor consists of four parallel 6,880 m^3 aeration basins, previously demonstrated to be well mixed with respect to community structure (Wells et al. 2009). Activated sludge bioreactor solids and hydraulic retention times average 21 days and 6.2 h, respectively.

Sampling along a transect (Fig. S1) within the PARWQCP occurred on 12 November 2008. Upon temporary shutdown of the rotating distributor arm in one of the trickling filters, a surface PVC media module (0.9 m deep) was removed from the reactor, and 1–3 g biofilm samples were obtained with a sterile spatula, placed in sterile 1.5-ml tubes, weighed, and stored at −20 °C. Concurrently, samples of influent, trickling filter effluent, and activated sludge were obtained. Activated sludge samples represented a 24-h composite from the combined outlet of all four aeration basins using a Sigma 900 refrigerated automatic sampler (Hach, Loveland, CO). Eight 1.5 ml activated sludge samples were centrifuged onsite for 5 min at 5,000 g, decanted, and stored at −20 °C. Raw influent and trickling filter effluent grab samples were transported on ice to the laboratory, vacuum filtered in 10 ml aliquots onto 0.22 μm Hydrophilic Durapore PVDF filters (25 mm diameter, Millipore, Billerica, MA), and archived at −20 °C.

DNA extraction

Activated sludge and trickling filter biofilm samples were washed using 1-ml Tris-EDTA buffer (1 mM, pH=7.0) and genomic DNA (gDNA) was extracted in triplicate using the FastDNA Spin Kit for Soil (MP Biomedicals, Solon, OH) following the manufacturer's protocol, except for the initial bead-beating step. A Vortex Adapter (MO BIO laboratories, Inc., Carlsbad, CA) with the Vortex Genie 2 T (Scientific Industries, Inc., Bohemia, NY) was used to physically disrupt cells in lysing matrix at maximum speed for 15 min. gDNA extraction from raw influent and trickling filter effluent proceeded via incubation of filters in lysis solution (800 μl water, 100 μl 10 % SDS (Invitrogen, Carlsbad, CA) and 100 μl 10 mg/ml Proteinase K (Invitrogen, Carlsbad, CA) at 55 °C for 2.5 h). Filtrate from the lysis incubation was purified with the FastDNA Spin Kit for Soil (MP Biomedicals, Solon, OH) following the manufacturer's protocol but without the initial bead-beating step.

PCR, T-RFLP, qPCR, and clone library analyses

A 491 bp region of the betaproteobacterial ammonia monooxygenase subunit A (*amoA*) gene was PCR amplified in triplicate from gDNA using primers amoA-1F and amoA-2R, as previously described (Wells et al. 2009). Each 25 μl PCR mixture consisted of 0.4 μM of each primer, 1X Fail-Safe PCR buffer F (Epicentre, Madison, WI), 1 U AmpliTaq LD Taq polymerase (Applied Biosystems, Inc., Foster City, CA), and 20–50 ng of gDNA. The PCR temperature profile was as follows: 94 °C for 3 min, followed by manual addition of Taq polymerase; then 40 cycles consisting of 94 °C for 60 s, 55 °C for 90 s, and 72 °C for 60 s, with a 10 min final extension at 72 °C. AOB *amoA*-based dual-labeled T-RFLP and qPCR analyses followed previously described protocols (Wells et al. 2009) (detailed in supplementary methods.) archaeal 16S rRNA genes (~900 bp) were amplified with primers 21F (5′-TTCCGGTTGATCCYGCCGGA-3′) and 958R (5′-YCCGGCGTTGAMTCCAATT-3′). Each 50 μl PCR mixture consisted of 0.2 μM of each primer, 1X Fail-Safe PCR buffer F, 1.25 U AmpliTaq LD Taq polymerase, and 20–50 ng gDNA. The PCR temperature profile was as follows: 94 °C for 5 min, then 30 cycles consisting of 94 °C for 90 s, 55 °C for 90 s, and 72 °C for 90 s, with a 7 min final extension at 72 °C. Cloning, sequencing, and phylogenetic analyses followed Wells et al. (2009). Gene sequences have been deposited in GenBank under accession numbers HQ450810–HQ450847, JF305759–JF305767, and HQ592631–HQ592642.

PhyloChip analyses

We assessed overall microbial diversity in biological triplicate samples from the trickling filter biofilm, trickling filter

effluent, and activated sludge and biological duplicate samples from the plant influent (due to limited biomass available) via the PhyloChip™ assay (Second Genome, Inc., San Bruno, CA). The PhyloChip is a high-density oligonucleotide 16S rRNA gene microarray targeting 8,741 microbial taxa (OTUs, ~3 % sequence divergence) representing all 121 demarcated prokaryotic orders (Brodie et al. 2007). PCR amplification of 16S rRNA genes for microarray hybridizations employed the bacterial-specific primer set 27F.1 (5′-AGRGTTTGATCMTGGCTCAG-3′) and 1492R (5′-GGTTACCTTGTTACGACTT-3′) and the archaeal specific primer set 4Fa (5′-TCCGGTTGATCCTGCCRG-3′) and 1492R. Processing, scanning, probe set scoring, and data normalization followed previously defined methods (Brodie et al. 2007), as described in detail in the electronic supplementary materials (ESM) 1. PhyloChip data has been deposited in the NCBI Gene Expression Omnibus Web site (https://www.ncbi.nlm.nih.gov/geo/) under accession number GSE52079.

2.5 Statistical analyses

Spearman rank correlation analyses, ANOVA, and Student *t* tests were performed in SPSS v18 (SPSS Inc., Chicago, IL). Nonmetric multidimensional scaling (NMDS) analyses, multiresponse permutation procedures (MRPP), and analyses of similarity (ANOSIM) were performed in R v2.11.1 using the package "vegan." Nearest shrunken centroid, heatmap, and hierarchical clustering analyses were performed in R using packages "pamr" and "made4," respectively. Detailed statistical methods are provided in the electronic supplementary materials (ESM) 1.

Results

A puzzling previous association: influent NO_2^- as a putative signature of microbial immigration

The motivation for the current study stems from a previously documented strong association between activated sludge bioreactor influent NO_2^- and AOB population dynamics at the PARWQCP (Wells et al. 2009). This association was puzzling for two reasons: (1) the NO_2^- levels (0.05–1.18 mg N/L) entering the reactor were significantly below levels expected to influence AOB community structure (Anthonisen et al. 1976) and (2) the well-mixed activated sludge community is actually exposed to NO_2^- that is diluted and degraded to even lower levels (0.01–0.1 mg N/L).

At municipal wastewater treatment plants, NO_2^- in both the raw influent and the primary effluent rarely exceeds 0.1 mg NO_2^- N/L (Tchobanoglous et al. 2002). This is also true of the PARWQCP, but at this plant, we observed a small but consistent increase in NO_2^- concentration when primary clarifier effluent passed through a trickling filter designed for BOD removal (Fig. S2). Partial nitrification—as opposed to partial denitrification—was the most probable explanation: NH_4^+ levels (23.2±0.8 mg N/L) consistently dwarfed NO_3^- levels (0.5±0.34 mg N/L) in the trickling filter influent, and the highly variable change in NO_3^- across the trickling filter (0.34±0.37 mg N/L) was insufficient to explain the observed NO_2^- accumulation.

Based on the data above, we reasoned that activated sludge bioreactor influent NO_2^- originates at least in part from small levels of partial nitrification (NO_2^- accumulation) in the upstream trickling filter biofilm, despite filter operation strictly for BOD removal. We hypothesize that small but highly variable levels of partial nitrification are modulated by exposure of dormant, cloistered AOB populations to increased levels of oxygen after biofilm sloughing. Such control of biofilm nitrification and NO_2^- accumulation by sloughing of heterotrophic bacteria that have overgrown the biofilm surface has been predicted previously by mathematical modeling (Elenter et al. 2007; Morgenroth and Wilderer 2000; Wanner and Gujer 1986). We further hypothesize that sloughing in turn provides a significant microbial "seed" to an activated sludge unit located downstream from the trickling filter, thereby explaining observed correlations between bioreactor influent NO_2^- and AOB population dynamics. Influent NO_2^- may thus be a surrogate measure of immigration via sloughing from the upstream trickling filter.

Biofilm sloughing events are little understood and apparently sporadic (Morgenroth and Wilderer 2000) and are therefore difficult to predict or control, particularly in full-scale systems. Directly testing our hypothesis that sloughing of trickling filter biofilm is linked to both NO_2^- accumulation (via partial nitrification) and downstream activated sludge population dynamics (via microbial immigration) is thus extremely challenging. However, this hypothesis suggests a number of readily testable corollaries that are the focus of this paper:

Corollary (1) AOB are present in the upstream trickling filter, despite operation solely for BOD removal, and are transported via immigration in the trickling filter effluent to the activated sludge bioreactor.

Corollary (2) Distinct AOB communities coexist under normal operating conditions in the trickling filter and activated sludge bioreactor. Specifically, we hypothesize that the trickling filter AOB community is dominated by the *N. europaea* lineage, while the activated sludge community is dominated by the "*Nitrosomonas*-like" lineage. This hypothesis is consistent with the strong positive correlation previously observed between the *N. europaea* lineage and influent NO_2^-.

Corollary (3) Distinct heterotrophic communities also coexist in the trickling filter and activated sludge bioreactor. Specifically, we hypothesize that the trickling filter harbors a diverse heterotrophic microbial assemblage, including anaerobes (e.g., methanogens) due to anaerobic microenvironments within the biofilm, and provides a seed of microbial diversity via the trickling filter effluent to downstream activated sludge.

We employed retrospective and prospective lines of evidence to test these corollaries. Our intent was to test the hypothesis that significant within-plant microbial immigration occurs and to profile within-plant biogeography (spatial variation) in microbial community structure.

Retrospective evidence of within-plant microbial immigration

Corollary 3 implies that if influent NO_2^- is indeed a surrogate measure of sloughing and associated microbial immigration, a significant association between influent NO_2^- and activated sludge heterotrophic community structure (including anaerobes) should be evident. Three independent and converging lines of retrospective evidence support this corollary, as summarized in Table 1. We have noted these connections to influent NO_2^- previously in passing in a Ph.D. thesis (Wells 2011) and (for 16S rRNA-based T-RFLP results) in a publication (Wells et al. 2011), but these associations have not been analyzed in detail, nor have these lines of evidence been presented together.

First, over the same time period in which we detailed associations between AOB population dynamics and bioreactor influent NO_2^-, we observed a significant correlation (p=0.014) between influent NO_2^- and overall bacterial community dynamics, based on redundancy analysis of 16S rRNA-based T-RFLP assays (Wells et al. 2011).

Second, bioreactor influent NO_2^- was significantly positively correlated to activated sludge taxonomic richness (ρ=0.8, p=0.001 [one-way test of significance]), based on PhyloChip analyses of 12 monthly samples from the same time series (Wells 2011). PhyloChip analyses also indicated the routine presence of a diverse community of putative anaerobes, particularly methanogens, and the relative abundance of *Methanomicrobia* was significantly positively correlated to influent NO_2^- (ρ=0.49, p=0.05 [one-way test of significance]). An archaeal 16S rRNA PCR clone library confirmed the presence of methanogens (Fig. S3).

Third, influent NO_2^- to the activated sludge bioreactor was also significantly positively correlated to functional gene richness (ρ=0.52, p=0.05 [one-way test of significance]), based on functional gene microarray (GeoChip 3.0) analyses of 12 monthly activated sludge samples over the same time period (Wells 2011). This correlation is consistent with the hypothesis that elevated influent NO_2^- levels cooccur with immigration of a functionally diverse microbial consortium to the activated sludge bioreactor, thus leading to elevated functional gene richness in this location.

Taken together, these three lines of retrospective evidence provide support for corollary 3 and suggest that immigration between coupled process units (putatively associated with influent NO_2^-) may be substantial and may play an important role in structuring the PARWQCP activated sludge microbial community. To prospectively test the hypothesis that substantial immigration occurs between process units and to profile within-plant microbial biogeography, we subjected replicate biomass samples from a transect through the PARWQCP (Fig. S1) to a battery of independent but complementary molecular analyses: qPCR to quantify bacterial *amoA* gene abundance, bacterial *amoA*-based T-RFLP corroborated by clone libraries to assess spatial variation in AOB community structure, and phylogenetic microarray (PhyloChip) analyses to characterize spatial variation in overall microbial diversity.

Table 1 Summary of statistical associations between bioreactor influent NO_2^- and activated sludge microbial community structure at the PARWQCP

	Statistical analysis method	Strength of association	Significance (p value)	Reference
N. europaea lineage relative abundance (*amoA*-based T-RFLP)[a]	Spearman's rank correlation analysis	ρ=0.37	0.007	Wells et al 2009
Nitrosomonas-like lineage relative abundance (*amoA*-based T-RFLP)[a]	Spearman's rank correlation analysis	ρ=−0.36	0.006	Wells et al 2009
Overall bacterial community dynamics (16S rRNA-based T-RFLP)[a]	Redundancy analysis	F=1.54	0.014	Wells et al 2011
Taxonomic richness (PhyloChip)[b]	Spearman's rank correlation analysis	ρ=0.80	0.001[c]	Wells 2011
Unique functional gene richness (GeoChip)[b]	Spearman's rank correlation Analysis	ρ=0.52	0.05[c]	Wells 2011

[a] Applied to weekly activated sludge samples for 1 year

[b] Applied to monthly activated sludge samples for 1 year

[c] One-way test of significance

Bacterial *amoA* gene abundance across a WWTP transect

Betaproteobacterial AOB *amoA* gene abundances at transect sampling points are documented in Fig. S4. AOB *amoA* was not detectable in the plant influent but was readily quantifiable in both the trickling filter biofilm and effluent. This result supports our hypothesis that despite operation solely for BOD removal, AOB are present in significant quantities in the trickling filter (corollary 1). Moreover, successful AOB *amoA* quantification in trickling filter effluent and not in plant influent demonstrates a net accumulation, and thus growth, of AOB in the trickling filter itself, and provides strong prospective evidence of transport of AOB from this reactor to the downstream activated sludge bioreactor (corollary 1), i.e., immigration into the activated sludge bioreactor. Trickling filter effluent AOB *amoA* gene abundance averaged 2.6×10^4 copies/ml (1.2×10^5 copies/(μg DNA)), while biofilm samples from the top of the trickling filter averaged 1.1×10^6 copies/g biofilm, wet weight (1.2×10^4 copies/(μg DNA)). Activated sludge averaged 5.1×10^6 copies/ml (2.0×10^6 copies/(μg DNA)), more than two orders of magnitude greater abundance per volume than the trickling filter effluent. AOA *amoA* was not detected via PCR screening of all samples.

Spatial variation in AOB community structure

To characterize variation in AOB community structure along this WWTP transect, all sampling locations except the plant influent were successfully subjected to dual-labeled AOB *amoA*-based T-RFLP analysis, and clone libraries were generated in parallel. In silico analyses of sequences allowed association of T-RFs with specific AOB lineages (Fig. S5). Dual-labeled T-RFLP yields two electropherograms per digested sample—one each corresponding to forward and reverse T-RFs. Forward T-RFs identified in this study were 48, 219, 354, and 491 bp, while 135, 206, 270, and 491 bp reverse T-RFs were measured. In this study and previous studies (Wells et al. 2009), the 270 bp reverse T-RF associated exclusively with the *N. europaea* lineage. Similarly, the 354 bp and 48 bp forward T-RFs and 135 bp reverse T-RF associated exclusively with the *Nitrosomonas oligotropha* lineage in this WWTP, and the 491 bp T-RFs (forward and reverse) associated with *N. oligotropha* or *N. communis* lineages. We previously documented a unique *Nitrosomonas*-like cluster as the dominant AOB lineage in >70 % of 52 weekly activated sludge samples from this bioreactor (Wells et al. 2009). As confirmed here, this *Nitrosomonas*-like cluster is distinct from previously reported AOB lineages and has a unique 219 bp forward and 206 bp reverse T-RF pair. The 219 bp forward T-RF is shared with the *N. europaea* lineage. Subtraction of the normalized 219 bp T-RF peak height in a given electropherogram from the normalized 270 bp T-RF peak height (diagnostic for *N. europaea*) thus yields the relative abundance of this unique *Nitrosomonas*-like cluster.

Variations in AOB community structure across sampling locations are documented in Fig. 1. The *N. europaea* lineage predominated in both the trickling filter biofilm (76±2 %) and trickling filter effluent (89±2 %). The *Nitrosomonas*-like and *N. oligotropha* lineages constituted minority members in these locations. In contrast, the *Nitrosomonas*-like lineage maintained a large majority (72±2 %) of the AOB community in the activated sludge bioreactor. The *N. europaea* lineage constituted <3 % of the activated sludge AOB population. T-RFLP and clone library results thus provide strong support for our hypothesis of spatial variation in AOB community structure across this WWTP transect (corollary 2) and demonstrate immigration via trickling filter effluent of the *N. europaea* lineage from an area of high relative abundance (the trickling filter) to an area of low relative abundance (the activated sludge bioreactor).

Spatial variation in overall microbial diversity

In addition to dramatic shifts in AOB abundance and community structure, PhyloChip analyses revealed significant spatial variation in overall microbial taxonomic diversity across this WWTP. In general, microbial species richness increased along the transect (Fig. S6). Indeed, one-way ANOVA indicated that richness differed significantly between the four sampling locations ($p=0.014$). Richness varied at the OTU level (~97 % identity (Brodie et al. 2007)) from minimums of 1,110±76 and 1,098±162 in the plant influent and trickling filter biofilm, respectively, to a maximum of 1,527±155 in activated sludge. Richness was significantly higher ($p=0.05$, Tukey's HSD test) in activated sludge than in plant influent or trickling filter biofilm. Trickling filter effluent displayed an intermediate level of richness (1,341±41 OTUs), in agreement with its status as a combination of trickling filter biofilm and plant influent. Trickling filter effluent richness was significantly higher ($p=0.02$, Student's t test) than the plant influent, although this result was not confirmed by Tukey's HSD test in conjunction with one-way ANOVA.

We used NMDS to visually represent patterns of taxa relative abundance between sampling locations in a reduced set of ordinates (Fig. 2). Based on NMDS analysis, global microbial community composition overlapped substantially in biological replicates. In contrast, microbial communities diverged substantially between sampling locations, based on ANOSIM ($R=1$, $p=0.0004$) and MRPP ($A=0.6297$, $p=0.001$) tests. Interestingly, raw influent, activated sludge, and trickling filter biofilm microbial community structures define the boundaries of the ordination, while trickling filter effluent community composition was intermediate between these three locations. This is consistent with our view of the trickling filter effluent as a composite of the trickling filter

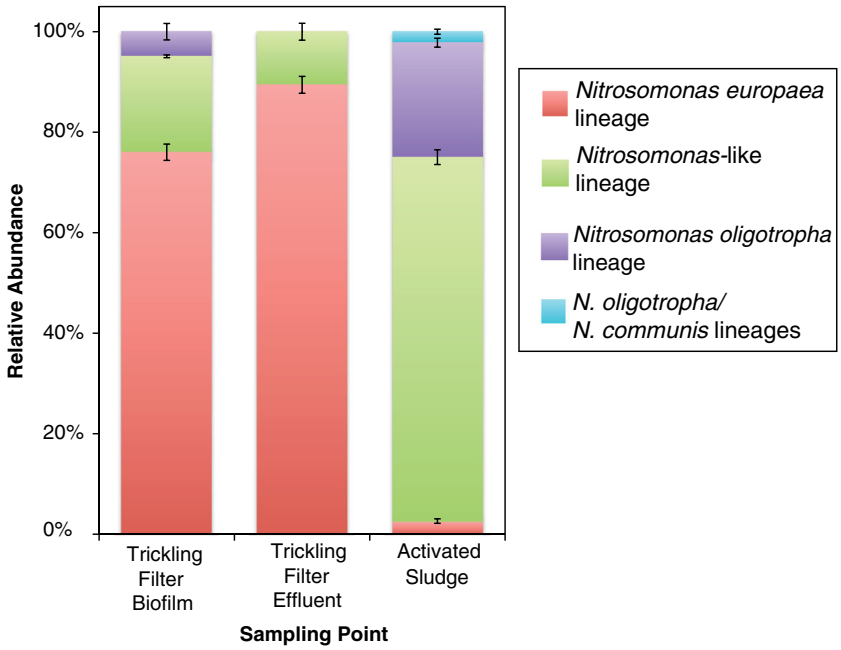

Fig. 1 Spatial variation within AOB lineages detected along a transect at the PARWQCP. Relative abundance (%) of each AOB lineage was determined by the corresponding normalized T-RFLP electropherograms. Standard deviations in T-RFLP normalized peak heights ($n>3$, minimum of triplicate sample extractions) are indicated by *error bars*

biofilm and plant influent, as well as a source of diversity for the downstream activated sludge bioreactor (corollary 3).

We plotted mean differences in PhyloChip hybridization intensities to explore spatial variations in relative abundance across broad taxonomic affiliations between the trickling filter biofilm and activated sludge (Fig. 3). Of 430 detected subfamilies, 179 displayed statistically significant hybridization intensity differences ($p=0.05$; indicated by columns extending beyond the gray region in the plot) in pairwise comparisons between these locations. In some cases, entire phyla showed consistent differences. All ten euryarchaeal subfamilies displaying statistically meaningful signal intensity differences were more abundant in trickling filter biofilm than in activated sludge. Six of these euryarchaeal subfamilies belong to the *Methanomicrobia*, in line with our hypothesis that the trickling filter provides anaerobic habitats for methanogens (corollary 3). Similarly, all five cyanobacterial subfamilies with significantly different mean signal intensities were more abundant in the trickling filter than in activated sludge. Biofilm samples were collected by necessity from the upper 3 ft of the trickling filter, where phototrophic (i.e., green) biomass was present. In contrast, all subfamilies with statistically significant mean signal intensity differences associated with the *Nitrospira* (two subfamilies), TM7 (two subfamilies), *Planctomycetes* (six subfamilies), and *Verrucomicrobia* (nine subfamilies) were more abundant in activated sludge than in the trickling filter.

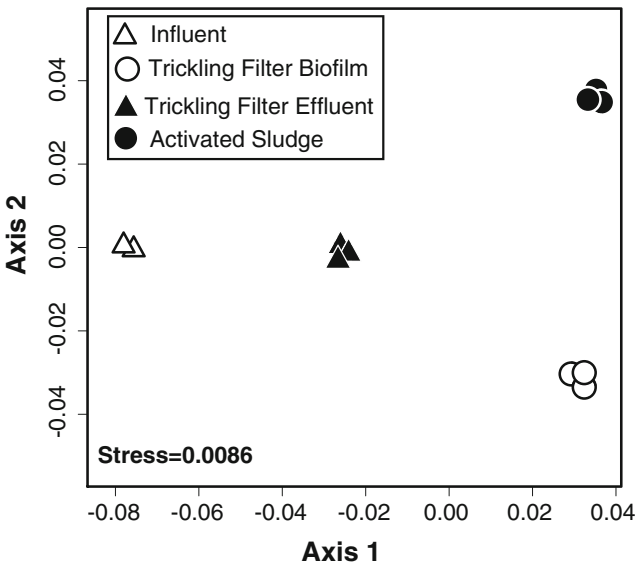

Fig. 2 Ordination of NMDS analysis of PhyloChip OTU signal intensities across the PARWQCP transect. Wisconsin double standardization of the square root of hybscores was performed, as per the default recommendations of the vegan R package documentation. Transformed hybscores were then used to construct a Bray–Curtis dissimilarity matrix, which was the basis for the NMDS analysis. The significance of the ordination is represented as a stress value. Based on the stress value (0.0086), Bray–Curtis dissimilarities between samples in the original data are well-preserved in this ordination (Fig. S7)

A reduced microbial set diagnostic for spatial variation across the WWTP

To explore the dominant microbial constituents differentiating the trickling filter biofilm and activated sludge biomass, we employed the method of nearest shrunken centroids (Tibshirani et al. 2002), with the goal of deriving a reduced set of OTUs that can both accurately classify samples and is of manageable size. Our analysis yielded a core set of 50 OTUs that enabled 100 % accuracy in classifying samples from the

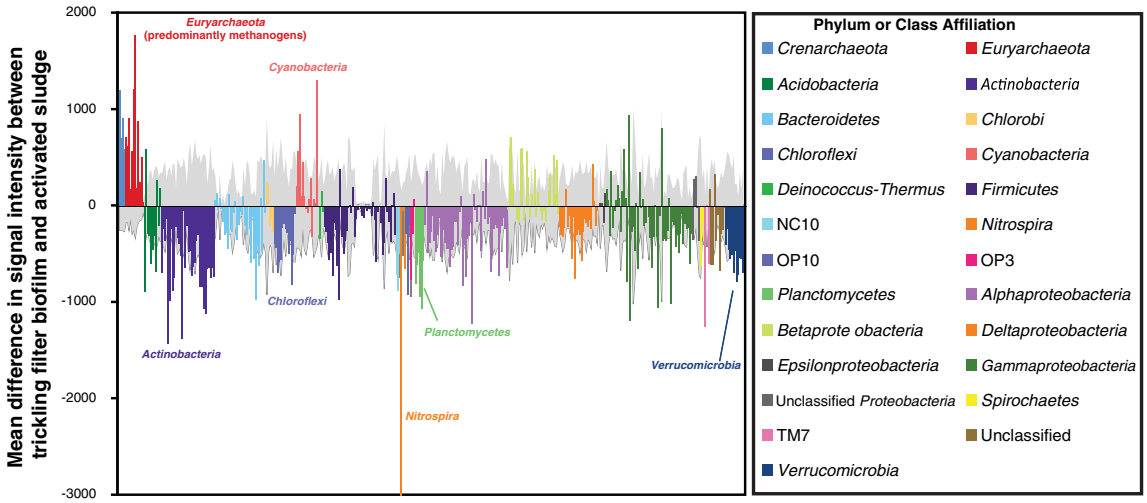

Fig. 3 Difference plot in PhyloChip hybridization intensities at the subfamily level between the trickling filter biofilm and activated sludge, averaged over three biological replicates in each sampling location. Mean differences between trickling filter and activated sludge hybridization intensities are color coded and ordered according to phylum or class affiliation. Subfamily rather than OTU affiliations were employed in this analysis in order to simplify the figure. A *positive column* indicates higher mean signal intensity in the trickling filter biofilm than in activated sludge, and vice versa for a *negative column*. Mean signal intensity differences associated with columns extending beyond the *gray region* in the plot are statistically significantly different at the $p=0.05$ level. Phyla demonstrating consistent mean signal intensity differences between locations are highlighted in the difference plot

trickling filter biofilm and the activated sludge bioreactor. Hierarchical cluster analysis of these 50 OTUs is shown in Fig. 4. This minimal OTU set mirrored broad variations identified via difference plot analysis. Approximately 40 % of the OTUs were more abundant in the trickling filter biofilm than in activated sludge, including eight methanomicrobial OTUs (putative methanogens), four cyanobacterial OTUs, and four gammaproteobacterial OTUs associated with the *Chromatiales* (putative purple sulfur bacteria capable of anoxygenic photosynthesis). Of 31 diagnostic OTUs more abundant in activated sludge, five *Nitrospira*-associated OTUs (putative nitrite oxidizing bacteria (NOB)) were observed, as were three OTUs each associated with the *Verrucomicrobia* and *Planctomycetes*, five actinobacterial OTUs, and a variety of alpha-, beta-, and gammaproteobacterial OTUs.

Both difference plot and nearest shrunken centroid analyses indicated a substantial reservoir of putative methanogens in the trickling filter biofilm relative to activated sludge. Heatmap analysis of all detected methanomicrobial OTUs suggests that the trickling filter biofilm is a reservoir specifically for methanogens associated with the family *Methanosarcinaceae*, while the plant influent is abundant in *Methanosaetaceae* (Fig. 5a). Indeed, all four observed *Methanosaetaceae* OTUs displayed significantly higher relative abundances in raw influent than trickling filter biofilm (one-way *t* test, $p<0.005$). Similarly, five of six observed *Methanosarcinaceae* OTUs displayed significantly higher relative abundances in the trickling filter biofilm than in the influent (one-way *t* test, $p<0.005$). Both families were also detected in trickling filter effluent and activated sludge, though at reduced levels relative to the influent and trickling filter biofilm. Phylogenetic inferences of archaeal 16S rRNA sequences previously recovered from PARWQCP activated sludge confirmed the presence of both methanomicrobial families (Fig. S3).

Attenuation of enteric bacteria

Of the minimal 100 PhyloChip OTUs adequate to properly classify each of our four sampling locations with 100 % accuracy via nearest shrunken centroid analysis, 35 associated with the *Enterobacteriaceae* family (data not shown). Nearly all *Enterobacteriaceae* OTUs included in this set were most abundant in the plant influent. We used hierarchical cluster analysis to further explore spatial variation in *Enterobacteriaceae* (Fig. 5b). Of 70 total *Enterobacteriaceae* detected, 68 displayed higher mean abundance in the plant influent than the trickling filter biofilm or activated sludge. Sixty-four of these OTUs were more abundant in the trickling filter effluent than in either the trickling filter biofilm or in activated sludge. The average change in *Enterobacteriaceae* OTU hybscore between plant influent and activated sludge was >1,000 FU, indicating approximately an order of magnitude change in abundance between these locations. Our results demonstrate attenuation of *Enterobacteriaceae* along this transect, with moderate survival across the trickling filter followed by a strong decrease in abundance in the activated sludge bioreactor.

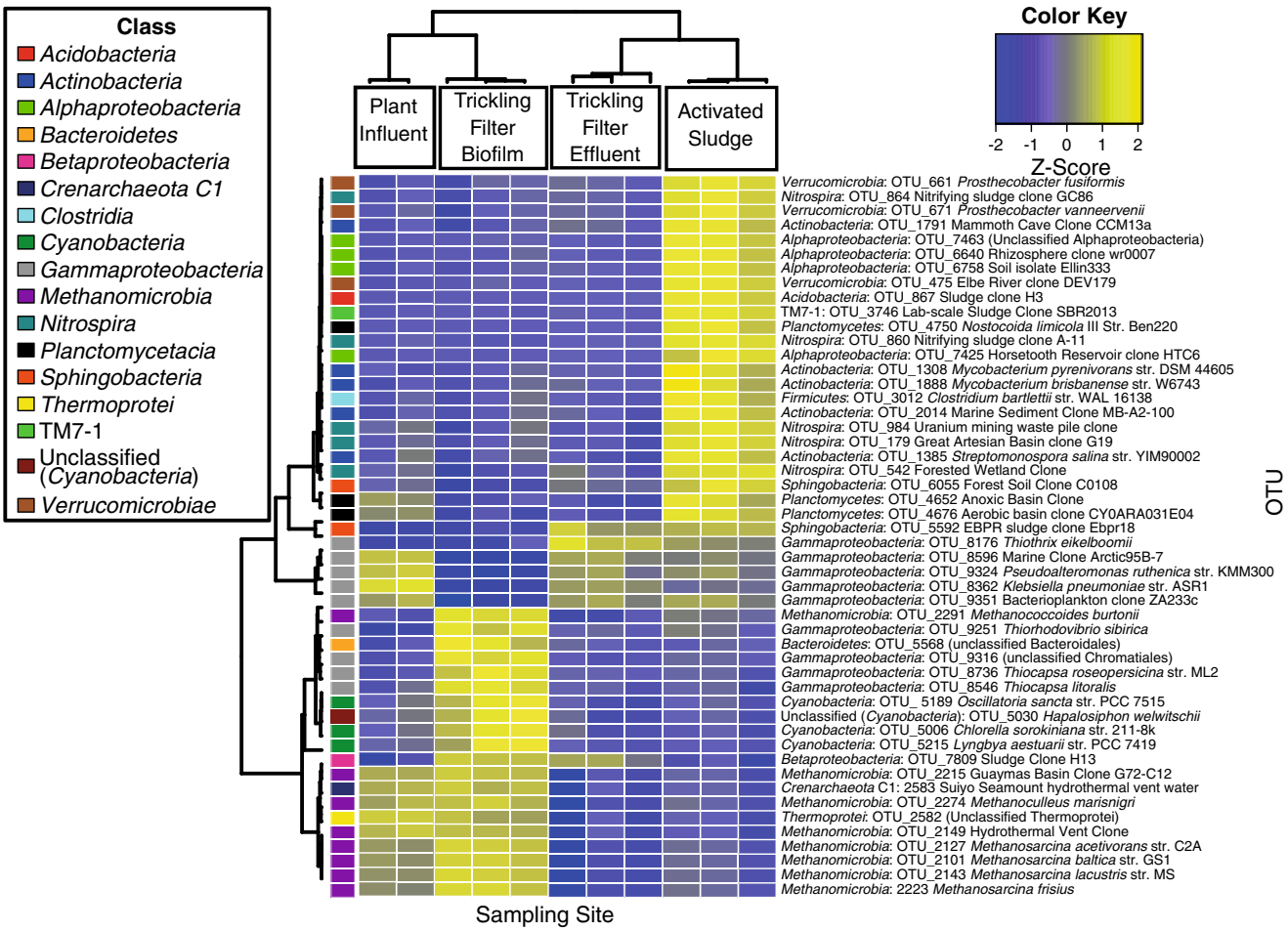

Fig. 4 Heatmap and hierarchical clustering analysis of the 50 microbial OTUs diagnostic for differences between activated sludge and trickling filter biofilm, based on Nearest Shrunken Centroid analysis of PhyloChip data with a shrinkage parameter of Δ=2.814. The shrinkage parameter was chosen based on visual inspection of the cross-validation error. Sampling locations are clustered on the *x axis*, and the reduced microbial set is clustered on the *y axis*. Class-level associations of OTUs are color-coded to the left of the heatmap. *Blue* indicates low relative abundance across an OTU (row); *yellow* indicates high relative abundance. The *color key* indicates correspondence between blue–yellow coloring and standard deviations from the mean signal intensity (e.g., *z* score) within an OTU. A representative sequence type associated with each OTU number is given on the far right of the heatmap. These sequence types were identified from the greengenes G2_Chip phylogeny (prokMSA; greengenes.lbl.gov)

Discussion

As hypothesized in corollary 1, we demonstrated a population of AOB in the trickling filter biofilm, despite operation solely for BOD removal, and substantial transport (immigration) of AOB between coupled unit processes—namely, between an upstream trickling filter and a downstream activated sludge unit. We also observed significant differences in AOB community structure between the trickling filter biofilm and the downstream activated sludge bioreactor, with the upstream biofilm dominated by the *N. europaea* lineage and the downstream activated sludge dominated by a "*Nitrosomonas*-like" lineage, precisely as predicted in corollary 2. Finally, as postulated in corollary 3, PhyloChip analyses revealed highly distinct heterotrophic microbial communities and immigration of a diverse microbial consortium between these staged bioprocesses. The observed (and predicted) differences between trickling filter and activated sludge microbial community structures may be due in part to species sorting via spatial niche partitioning. Within the AOB community, the *Nitrosomonas*-like lineage that dominates PARWQCP activated sludge is only distantly related to known AOB isolates, with 80–81 % *amoA* sequence identity to the closest cultivated isolate, *N. communis* NM33 (AF272399). Speculation regarding the ecophysiology of this unique lineage is thus premature. However, the *N. europaea* lineage that dominates the trickling filter biofilm and effluent is well studied. This lineage is often found in eutrophic environments embedded in flocs or biofilms (Koops and Pommerening-Roser 2001). Moreover, selected *N. europaea* strains have exceedingly high affinities for oxygen (Park and Noguera 2007). AOB populations in the trickling filter biofilm are exposed to high NH_4^+ and low O_2, relative to the activated sludge bioreactor. It is tempting to speculate that this combination of conditions promotes selective

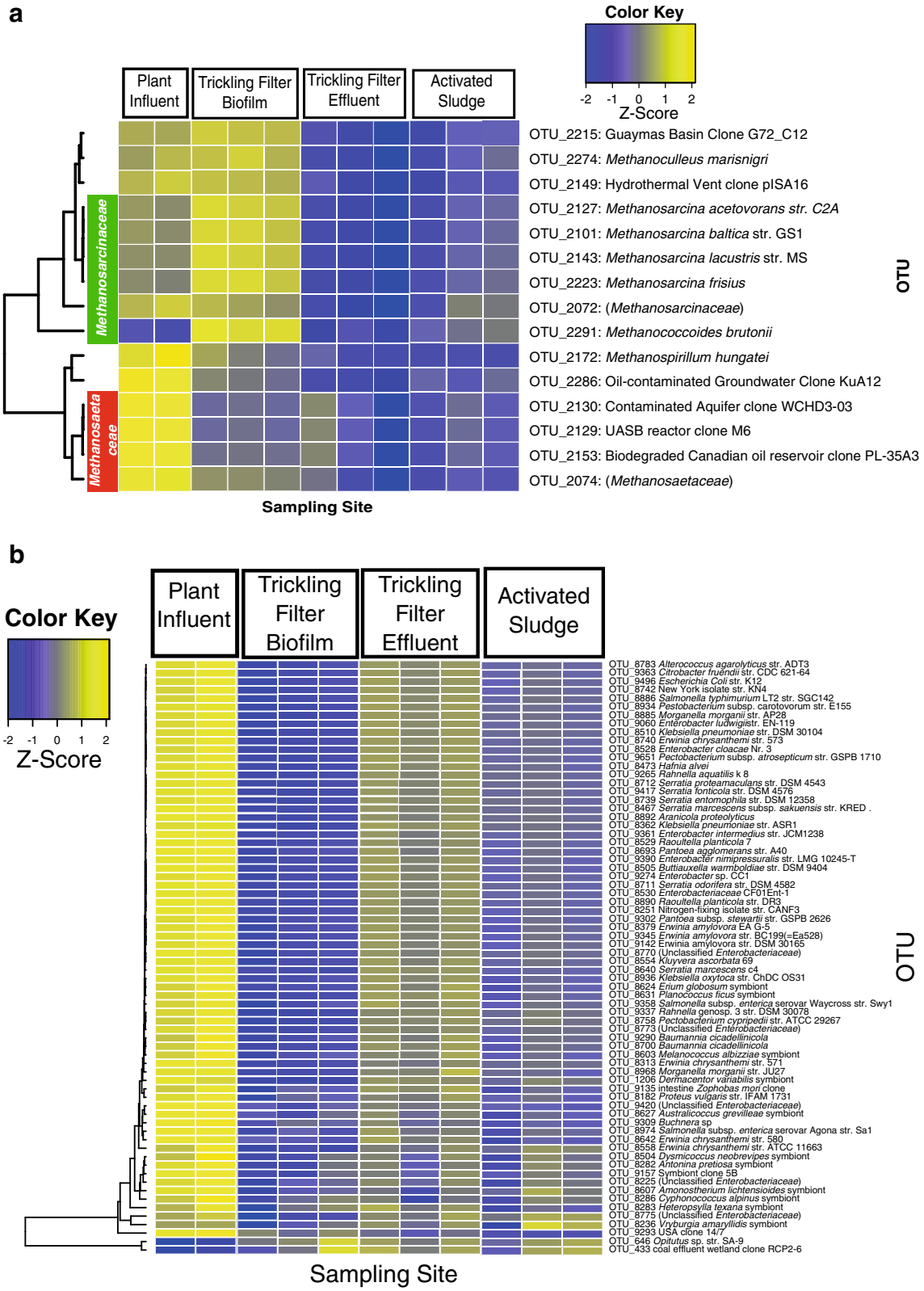

Fig. 5 Heatmap and hierarchical clustering analysis of *Methanomicrobia* (**a**) and *Enterobacteriaceae* (**b**) OTUs detected by the PhyloChip. Sampling locations are clustered on the *x axis*, and OTUs on the *y axis*. *Blue* indicates low relative abundance across an OTU (row); *yellow* indicates high relative abundance. The *color key* indicates correspondence between blue–yellow coloring and standard deviations from the mean signal intensity (e.g., *z* score) within an OTU. Representative sequence types for OTUs are displayed on the far right of both heatmaps, and Methanomicrobial OTUs affiliated with the families *Methanosaetaceae* and *Methanosarcinaceae* are highlighted on the far left of the heatmap in (**a**)

maintenance of the *N. europaea* lineage over other AOB populations in the trickling filter and that inverse conditions in the activated sludge bioreactor may explain the low relative abundance of the *N. europaea* lineage in this process unit.

The initial impetus for this study stemmed from the hypothesis that microbial immigration via trickling filter sloughing events—likely linked to production of small levels of NO_2^- due at least in part to partial nitrification in the filter itself—are associated with microbial population dynamics in the downstream activated sludge bioreactor. It should be emphasized that directly testing this hypothesis is not the goal of this paper, given the long time scales needed and the extreme difficulty in predicting and sampling over mass sloughing events in a full-scale reactor. Rather, our goals here are to test the related hypothesis that substantial within-plant immigration occurs and to profile biogeography in microbial community structure across this full-scale wastewater treatment plant transect. Nonetheless, a back-of-the-envelope examination of potential trickling filter biomass production and detachment by nitrifiers is informative to assess the theoretical impact of AOB immigration from the upstream trickling filter on the downstream activated sludge microbial community. Based on previously described methods (see electronic supplementary materials (ESM) 1) (Rittmann and McCarty 2001), we estimate that ~6 % of AOB biomass in the activated sludge is theoretically attributable to growth, detachment, and immigration from the upstream trickling filter. It should be emphasized that this is a rough estimate of average AOB biomass theoretically attributable to detachment and immigration. We previously noted significant variations in influent NO_2^- to the activated sludge bioreactor (Wells et al. 2009), suggesting that detachment (and thus AOB immigration rates) may vary strongly over time.

A similar estimation of heterotrophic detachment and immigration is complicated by the fact that both aerobic and anaerobic heterotrophs likely play important roles in the trickling filter biofilm. Growth yields differ substantially between these groups, and thus estimation of biomass production is difficult. However, roughly 65 % of COD removal occurs in the PARWQCP trickling filter, suggesting substantial biomass accumulation and intermittent detachment is necessary to avoid plugging. Based on daily measurements over a 2-week period, organic carbon removal in the trickling filter averaged 98 mg COD/L, while COD removal over 1 year in the activated sludge bioreactor averaged 49 mg COD/L (Wells et al. 2009). A conservative estimate based on typical anaerobic biomass yields (Rittmann and McCarty 2001) suggests that roughly 19 % of total activated sludge biomass is theoretically attributable to growth, detachment, and immigration from the upstream trickling filter. Critically, no solids separation (e.g., settling tank) separates the trickling filter from the downstream activated sludge bioreactor. Thus, detached biomass from the trickling filter proceeds directly to the activated sludge unit. It should be noted that TSS values in the effluent from the trickling filter would therefore likely provide a simple proxy for assessing the magnitude of filter sloughing events over time. While trickling filter effluent TSS was not routinely monitored here, the results of this study prompted initiation of a long-term monitoring program for this parameter. We anticipate that results from this monitoring program will further elucidate both the magnitude and frequency of sloughing events and the impacts of such events on downstream community structure and dynamics.

Coexistence of heterotrophs and autotrophic AOB in biofilms analogous to biomass from the trickling filter examined here has been documented both experimentally and via mathematical modeling. Kissel et al. (1984) modeled competition between autotrophs and heterotrophs and suggested that heterotrophs come to dominate biofilms at high organic carbon concentrations, independent of NH_4^+ concentrations. Subsequent studies confirmed that the majority of O_2 in systems with high organic substrate loadings, such as the PARWQCP trickling filter, is used by heterotrophs dominating the biofilm surface, and within-biofilm nitrification can thus be O_2-limited (Elenter et al. 2007; Okabe et al. 1995). Indeed, by employing the methods of Elenter and colleagues (Elenter et al. 2007) (see electronic supplementary materials (ESM) 1), we predict that under steady-state conditions in the PARWQCP trickling filter, aerobic heterotrophs are strongly O_2 limited and active nitrifiers are outcompeted.

However, modeling has also demonstrated that competition between autotrophs and heterotrophs is significantly influenced by the extent and frequency of detachment (Morgenroth and Wilderer 2000; Wanner and Gujer 1986). Kissel et al. (1984), whose model did not include detachment, acknowledged that periodic sloughing or continuous shearing can lead to reduced biofilm thickness and altered microbial community composition that can substantially effect biofilm behavior, notably by promoting loss of the fastest growing organisms (e.g., aerobic heterotrophs). Morgenroth and Wilderer (2000) arrived at a similar conclusion via simulations of competition in biofilms with different detachment patterns. Elenter et al. (2007) also suggested via 1D mathematical modeling that sloughing increases NH_4^+ oxidation by AOB in biofilms if surficial heterotrophic bacteria are removed, assuming a stratified biofilm structure with faster growing heterotrophs overlying autotrophs, as has been shown experimentally in a some systems (Okabe et al. 1995). When nitrification was observed in Elenter's model, NH_4^+ was oxidized solely to NO_2^- with no NO_3^- accumulation. The apparent lack of activity of NOB was attributed to competition for space and O_2 between NOB and AOB. We documented a similar increase in NO_2^- with minimal NO_3^- production across a full-scale trickling filter, with significant variations in NO_2^- accumulation that may be linked to biofilm sloughing and microbial immigration events.

We recognize that some of the production of NO_2^- in the trickling filter may be due to heterotrophic denitrification. However, given the low levels of NO_3^- relative to NH_4^+ in the trickling filter influent and the fact that change in NO_3^- across the trickling filter is insufficient to support heterotrophic denitrification as the sole source of NO_2^- accumulation, we reason that NO_2^- in the influent to the activated sludge bioreactor originates at least in part from partial nitrification in the upstream trickling filter biofilm. Results from our qPCR, T-RFLP, and clone library analyses fully support this reasoning, as do prior modeling studies of nitrifier/hererotroph competition in biofilms (Elenter et al. 2007; Kissel et al. 1984). Moreover, regardless of the fraction of NO_2^- production attributable to partial nitrification or heterotrophic denitrification in this system, our primary intent in this study was to provide evidence that significant within-plant immigration occurs between coupled process units. Retrospective and prospective lines of evidence support this contention by verifying corollaries 1, 2, and 3.

In addition to investigating immigration between coupled process units, the second goal of this study was to profile microbial biogeography across a transect in a full-scale WWTP. We documented strong spatial variation in microbial richness and community structure, including significant differences in community structure and abundance of *Methanomicrobia* at all sampling sites profiled. Given its highly aerated nature and the sensitivity of methanogens to even low levels of oxygen (Jarrell 1985), methanogens are likely allochthonous to the activated sludge bioreactor. Gray et al. (2002) also found low levels of methanogens in aerated activated sludge bioreactors and attributed their presence to both immigration from raw sewage and to putative active growth in the anoxic secondary settlers and return activated sludge line. Interestingly, of methanomicrobial OTUs detected along our transect, those associated with the family *Methanosaetaceae* tended to be more abundant in the plant influent, while those associated with the family *Methanosarcinaceae* were more abundant in the trickling filter biofilm. *Methanosaetaceae* are strictly acetoclastic methanogens, while *Methanosarcinaceae* can catabolize methyl compounds as well as display acetoclastic or hydrogenotrophic metabolisms (Kendall and Boone 2006). Dominance of *Methanosarcinaceae* in the trickling filter may thus reflect a greater diversity of catabolic substrates relative to the plant influent. Our results provide support for corollary 3 and suggest that observed methanogens in activated sludge are likely immigrants in part from anaerobic habitats in the trickling filter (*Methanosarcinaceae*) and raw influent (*Methanosaetaceae*).

We also observed a striking attenuation of *Enterobacteriaceae* across this WWTP transect, with high abundance in 68 of 70 OTUs in the plant influent, moderate survival across the trickling filter (with low abundance in the trickling filter biofilm), and a strong decrease in abundance in activated sludge. *Enterobacteriaceae* are gammaproteobacterial facultative anaerobes and are often associated with human or animal gut microflora (Brenner and Farmer 2001). Indeed, the common fecal indicator bacteria (FIB) *Escherichia coli* is a member of this family, as are several common pathogens in the genera *Enterobacter*, *Shigella*, *Salmonella*, *Erwinia*, *Yersinia*, *Klebsiella*, and *Serratia* (Brenner and Farmer 2001). Few studies have employed molecular methods to monitor FIB or pathogen attenuation at multiple sampling points within a WWTP, and, to our knowledge, none have offered a high resolution picture of the relative abundance of *Enterobacteriaceae* across this environment. Wéry et al. (2008) monitored four pathogenic or indicator bacteria, including two *Enterobacteriaceae* members, at five points in a municipal WWTP, and concluded that survival rates were variable. Shannon (2007) monitored pathogens via 13 qPCR assays at five locations across a WWTP, and found three to four order of magnitude declines in abundance between raw wastewater and final effluent of *E. coli*, *K. pneumoniae*, *Clostridium perfringens*, and *Enterococcus faecilis*. Attenuation of these pathogens is somewhat higher than that suggested by our PhyloChip data for *Enterobacteriaceae* OTUs, although direct comparison is difficult due to the different platforms employed and to the fact that we did not monitor final effluent microbial community composition or abundance. Interestingly, our data suggests that biofilm-based reactors may be less efficacious at removing potentially harmful microbial invaders such as *Enterobacteriaceae* than dispersed growth reactors. Additional work is warranted to accurately assess attenuation of pathogens and FIB at multiple locations within biological WWTPs, particularly in attached and suspended growth systems designed for similar functions (e.g., for both C removal and nitrification) and to test the efficacy of different treatment plant configurations on pathogen removal.

Both AOB and heterotrophic microbial community dynamics in PARWQCP activated sludge were recently found to be consistent with a combined neutral and niche-based community assembly model (Ofiteru et al. 2010). Neutral community assembly processes specify random dispersal and chance as predominant drivers of community structure. The evidence we present here of significant immigration between coupled process units suggests that neutral dispersal processes may be an important contributor to the microbial community assembly process in this plant, but additional work is necessary to test this hypothesis.

Our data also suggest that the related process of mass effects (Leibold et al. 2004) may be important for at least some microbial guilds. Mass effects refer to the mechanism for spatial dynamics in which there is a net flow (dispersal) of individuals from local areas of high success (core areas) to unfavorable areas. Mass effects always function to increase

alpha diversity and have been shown in some cases to have strong influences on observed relationships between local conditions and community structure (Shmida and Wilson 1985). We observed a high relative abundance of putative methanogens in the trickling filter (presumably a favorable habitat) and low but significant associated populations in trickling filter effluent and in downstream highly aerated activated sludge (likely an unfavorable habitat). It is likely that the observed methanogen diversity in activated sludge is maintained by immigration from the more favorable upstream trickling filter—that is, by mass effects.

Microbial immigration between coupled process units has potentially profound implications for bioprocess engineering and control. Our data suggests that upstream biofilm reactors can serve as a continuous inoculum of functionally important organisms to dispersed growth reactors—essentially a form of unplanned or "blackbox" bioaugmentation. Analogous to the "rescue effect" in classical ecology (Leibold et al. 2004)—the prevention of extinction of a species in a local community by immigration—within-plant microbial immigration may serve to protect against process upsets by preventing washout of critical microbial populations. It may also maintain desirable catabolic capabilities.

This work demonstrates significant microbial immigration between staged unit bioprocesses coupled to strong biogeographical (spatial) variation in microbial community structure across a full-scale wastewater treatment plant. It also assembles converging lines of retrospective and prospective evidence supportive of the hypothesis that immigration impacts downstream bioreactor community structure and dynamics, with important implications for bioprocess design and operation. Additional work is needed to assess the quantitative impact of such immigration relative to environmental (local) parameters and the nature of the indigenous microflora on both microbial community structure and on process performance. A rigorous statistical framework, such as the variance partitioning approach of Cottenie (2005), would be of value for this purpose, especially in conjunction with a well-controlled lab-based system.

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References

Anthonisen AC, Loehr RC, Prakasam TBS, Srinath EG (1976) Inhibition of nitrification by ammonia and nitrous acid. J Water Pollut Con F 48(5):835–852

Brenner DJ, Farmer JJ III (2001) Family I. *Enterobacteriaceae*. In: Garrity GM, Brenner DJ, Krieg NR, Staley JT (eds) Bergey's manual of systematic bacteriology, vol 2, 2nd edn. Springer, New York

Brodie EL, DeSantis TZ, Parker JPM, Zubietta IX, Piceno YM, Andersen GL (2007) Urban aerosols harbor diverse and dynamic bacterial populations. Proc Natl Acad Sci U S A 104(1):299–304

Cottenie K (2005) Integrating environmental and spatial processes in ecological community dynamics. Ecol Lett 8(11):1175–1182

Curtis TP, Head IM, Graham DW (2003) Theoretical ecology for engineering biology. Environ Sci Technol 37(3):64A–70A

Elenter D, Milferstedt K, Zhang W, Hausner M, Morgenroth E (2007) Influence of detachment on substrate removal and microbial ecology in a heterotrophic/autotrophic biofilm. Water Res 41(20):4657–4671

Gray ND, Miskin IP, Kornilova O, Curtis TP, Head IM (2002) Occurrence and activity of archaea in aerated activated sludge wastewater treatment plants. Environ Microbiol 4(3):158–168

Jarrell KF (1985) Extreme oxygen sensitivity in methanogenic archaebacteria. Bioscience 35(5):298–302

Jones SE, McMahon KD (2009) Species-sorting may explain an apparent minimal effect of immigration on freshwater bacterial community dynamics. Environ Microbiol 11(4):905–913

Jones SE, Newton RJ, McMahon KD (2008) Potential for atmospheric deposition of bacteria to influence bacterioplankton communities. FEMS Microbiol Ecol 64(3):388–394

Kendall MM, Boone DR (2006) The order *Methanosarcinales*. In: Dworkin M, Falkow S, Rosenberg E, Schleifer KH, Stackebrandt E (eds) The prokaryotes: a handbook on the biology of bacteria, vol 3, 3rd edn. Springer Science + Business Media, LLC, pp 244–256

Kissel JC, McCarty PL, Street RL (1984) Numerical simulation of mixed-culture biofilm. J Environ Eng 110(2):393–411

Koops HP, Pommerening-Roser A (2001) Distribution and ecophysiology of the nitrifying bacteria emphasizing cultured species. FEMS Microbiol Ecol 37(1):1–9

Leibold MA, Holyoak M, Mouquet N, Amarasekare P, Chase JM, Hoopes MF, Holt RD, Shurin JB, Law R, Tilman D, Loreau M, Gonzalez A (2004) The metacommunity concept: a framework for multi-scale community ecology. Ecol Lett 7(7):601–613

Lindström ES, Bergström A-K (2004) Influence of inlet bacteria on bacterioplankton assemblage composition in lakes of different hydraulic retention time. Limnol Oceanogr 49(1):125–136

Lindström ES, Forslund M, Algesten G, Bergström A-K (2006) External control of bacterial community structure in lakes. Limnol Oceanogr 51(1):339–342

Morgenroth E (2003) Detachment: an often overlooked phenomenon in biofilm research and modeling. In: Wuertz S, Wilderer PA, Bishop PL (eds) Biofilms in Wastewater Treatment: An Interdisciplinary Approach. IWA Publishing, London, pp 264–290

Morgenroth E, Wilderer PA (2000) Influence of detachment mechanisms on competition in biofilms. Water Res 34(2):417–426

Ofiteru ID, Lunn M, Curtis TP, Wells GF, Criddle CS, Francis CA, Sloan WT (2010) Combined niche and neutral effects in a microbial wastewater treatment community. Proc Natl Acad Sci U S A 107(35):15345–15350

Okabe S, Hirata K, Watanabe Y (1995) Dynamic changes in spatial microbial distribution in mixed-population biofilms: experimental results and model simulation. Water Sci Technol 32(8):67–74

Park HD, Noguera DR (2007) Characterization of two ammonia-oxidizing bacteria isolated from reactors operated with low dissolved oxygen concentrations. J Appl Microbiol 102(5):1401–1417

Rittmann BE, Laspidou CS (2003) Biofilm detachment. In: Bitton G (ed) Encyclopedia of environmental microbiology. Wiley, New York

Rittmann BE, McCarty PL (2001) Environmental biotechnology: principles and applications. McGraw-Hill Higher Education, New York

Shannon KE, Lee DY, Trevors JT, Beaudette LA (2007) Application of real-time quantitative PCR for the detection of selected bacterial

pathogens during municipal wastewater treatment. Sci Total Environ 382(1):121–129

Shmida A, Wilson MV (1985) Biological determinants of species diversity. J Biogeogr 12(1):1–20

Sloan WT, Lunn M, Woodcock S, Head IM, Nee S, Curtis TP (2006) Quantifying the roles of immigration and chance in shaping prokaryote community structure. Environ Microbiol 8(4):732–740

Stewart PS (1993) A model of biofilm detachment. Biotechnol Bioeng 41(1):111–117. doi:10.1002/bit.260410115

Tchobanoglous G, Burton FL, Stensel HD (2002) Wastewater engineering: treatment and reuse. McGraw-Hill, New York

Tibshirani R, Hastie T, Narasimhan B, Chu G (2002) Diagnosis of multiple cancer types by shrunken centroids of gene expression. Proc Natl Acad Sci U S A 99(10):6567–6572

Wanner O, Gujer W (1986) A multispecies biofilm model. Biotechnol Bioeng 28(3):314–328

Wells GF (2011) Reexamining the engineered nitrogen cycle: microbial diversity, community dynamics, and immigration in nitrifying activated sludge bioreactors. Ph.D. Thesis. Stanford University: Stanford, CA, USA

Wells GF, Park H-D, Yeung C-H, Eggleston B, Francis CA, Criddle CS (2009) Ammonia-oxidizing communities in a highly aerated full-scale activated sludge bioreactor: betaproteobacterial dynamics and low relative abundance of *Crenarchaea*. Environ Microbiol 11(9): 2310–2328

Wells GF, Park H-D, Eggleston B, Francis CA, Criddle CS (2011) Fine-scale bacterial community dynamics and the taxa-time relationship within a full-scale activated sludge bioreactor. Water Res 45(17):5476–5488

Wéry N, Lhoutellier C, Ducray F, Delgenès J-P, Godon J-J (2008) Behaviour of pathogenic and indicator bacteria during urban wastewater treatment and sludge composting, as revealed by quantitative PCR. Water Res 42(1–2):53–62

Wittebolle L, Boon N, Vanparys B, Heylen K, De Vos P, Verstraete W (2005) Failure of the ammonia oxidation process in two pharmaceutical wastewater treatment plants is linked to shifts in the bacterial communities. J Appl Microbiol 99(5):997–1006