Supplementary material

A relationship between phages and organic carbon in wastewater treatment plant effluents

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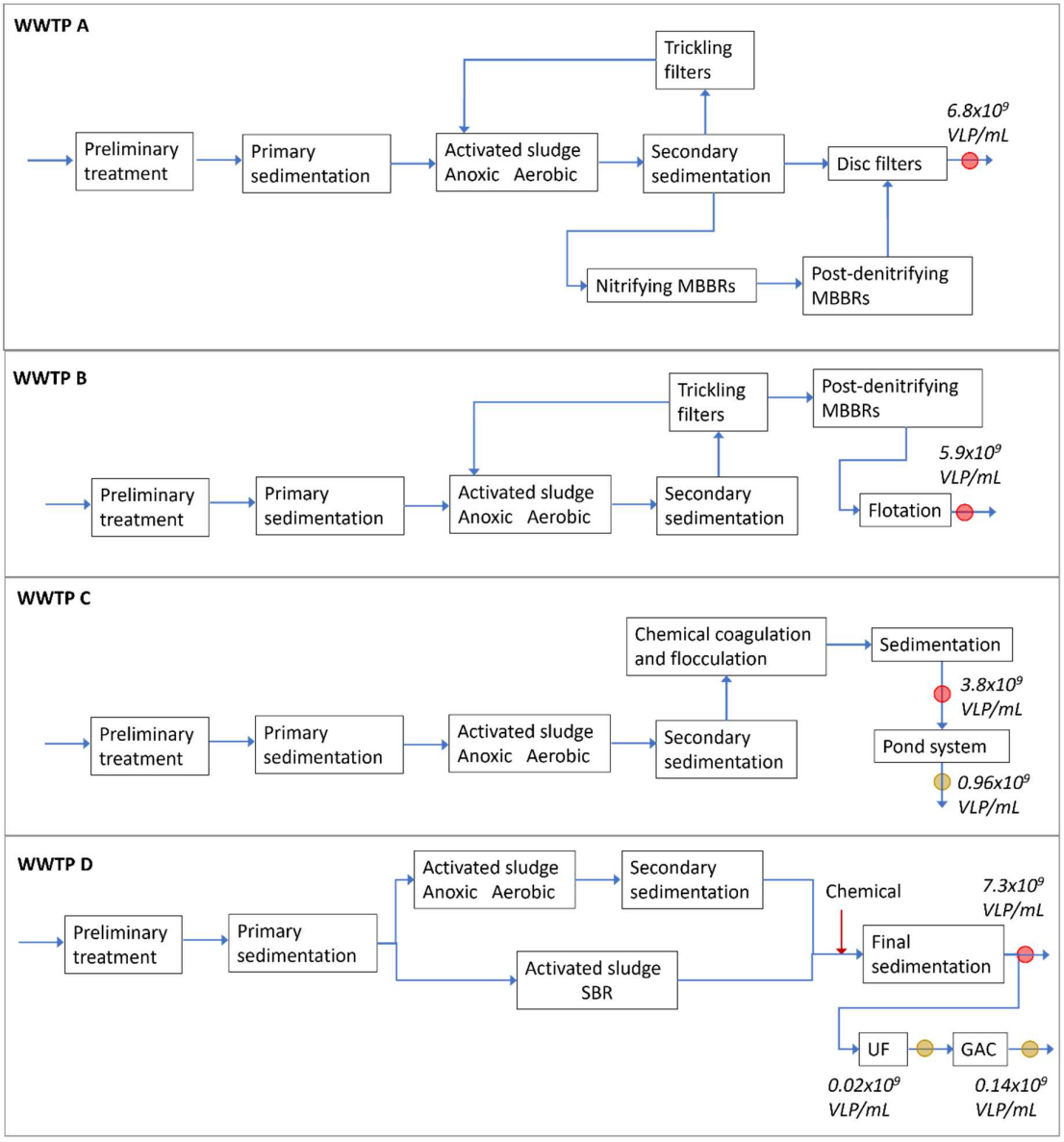

Fig. S1. Process schemes of the four sampled wastewater treatment plants. Red and orange circles show sampling points where the average VLP concentration are shown. The samples at the red circles were used to establish a linear correlation between DOC and VLP concentration. The orange circles show sampling points after tertiary treatment.

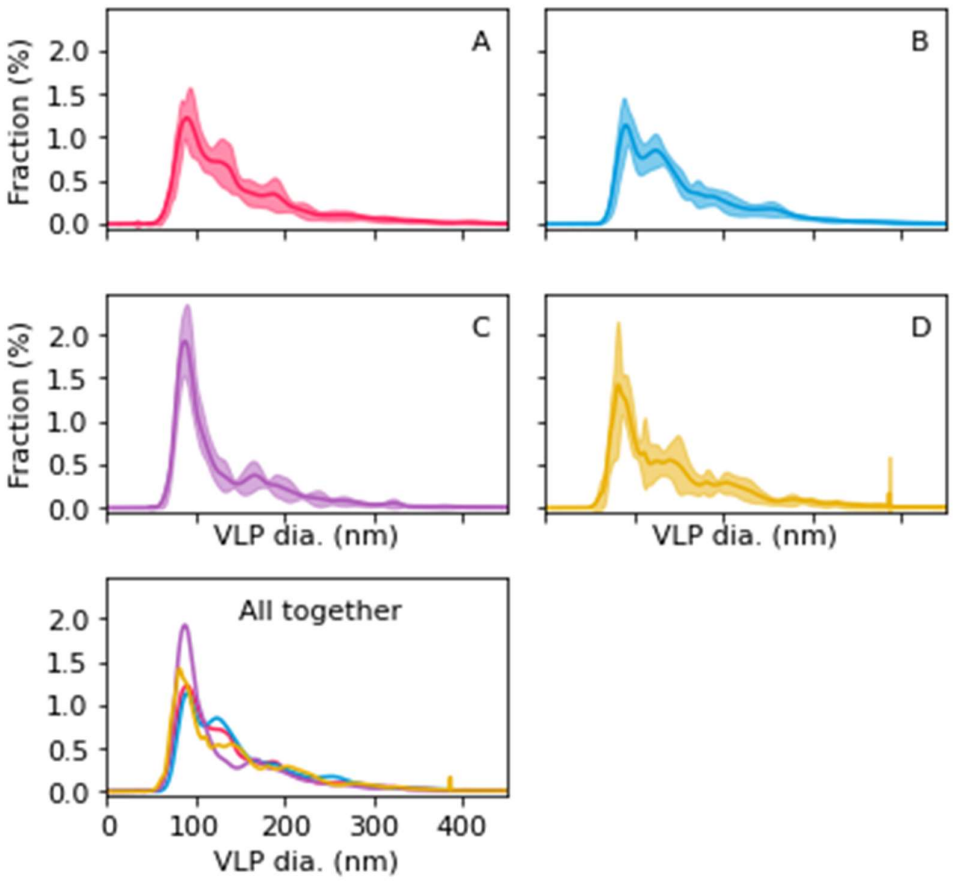

Fig. S2. Size distribution of VLP particles in plants A, B, C, and D. The mean and standard deviation of 5 to 14 measurements for the different plants are shown. In plants C and D, the samples after secondary treatment are shown. The lowermost graph shows the mean values for all four plants plotted together.

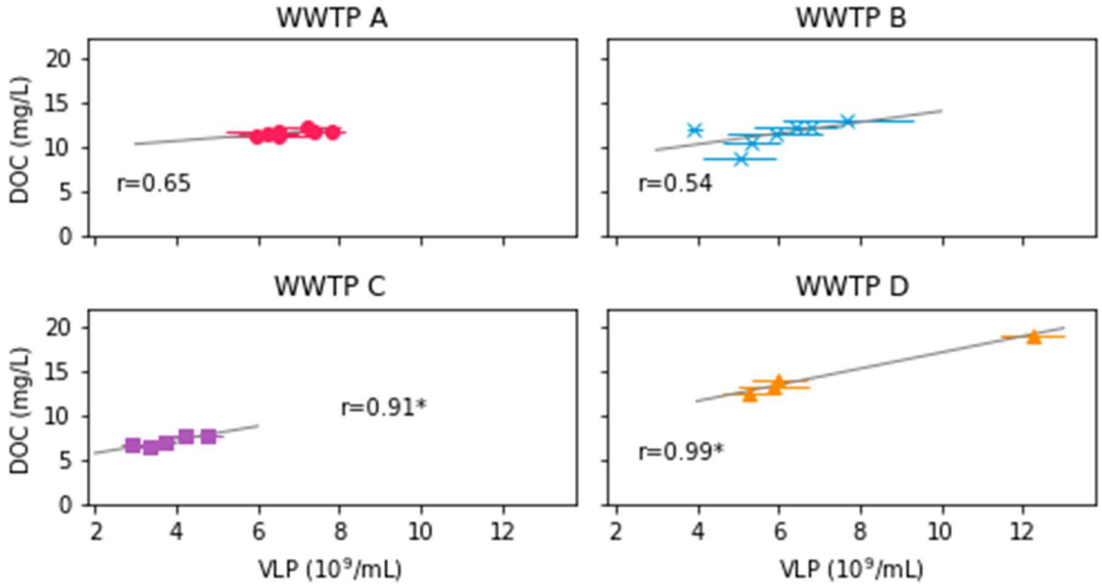

Fig. S3. Correlation between concentrations of VLP and DOC for each individual WWTP. Pearson's correlation coefficients (r) are shown. An asterisk (*) a statistically significant correlation ($p<0.05$).

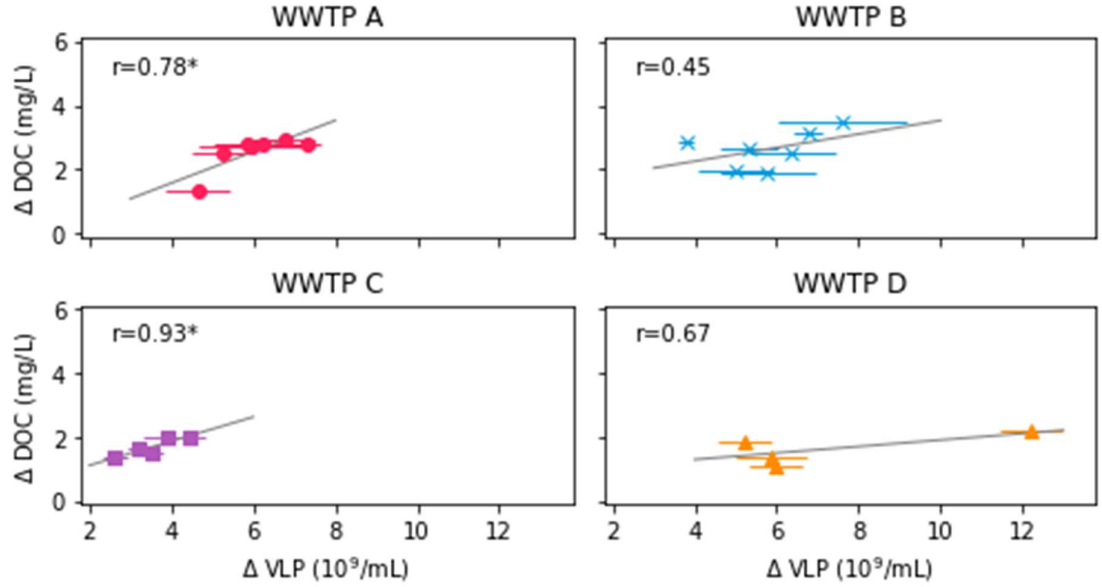

Fig. S4. Correlation between changes in concentrations of VLP and DOC after flocculation with zinc hydroxide for each individual WWTP. In samples from WWTP D, ultrafiltration was used to remove particles from the water. Pearson's correlation coefficients (r) are shown. An asterisk (*) a statistically significant correlation ($p<0.05$).

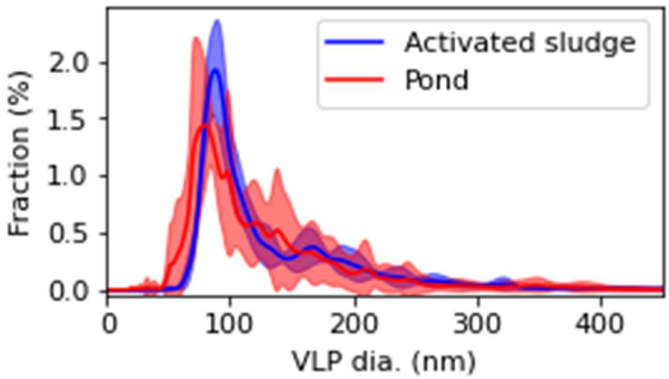

Fig. S5. Size distribution of VLP after secondary treatment (activated sludge) and tertiary treatment (pond) at plant C.

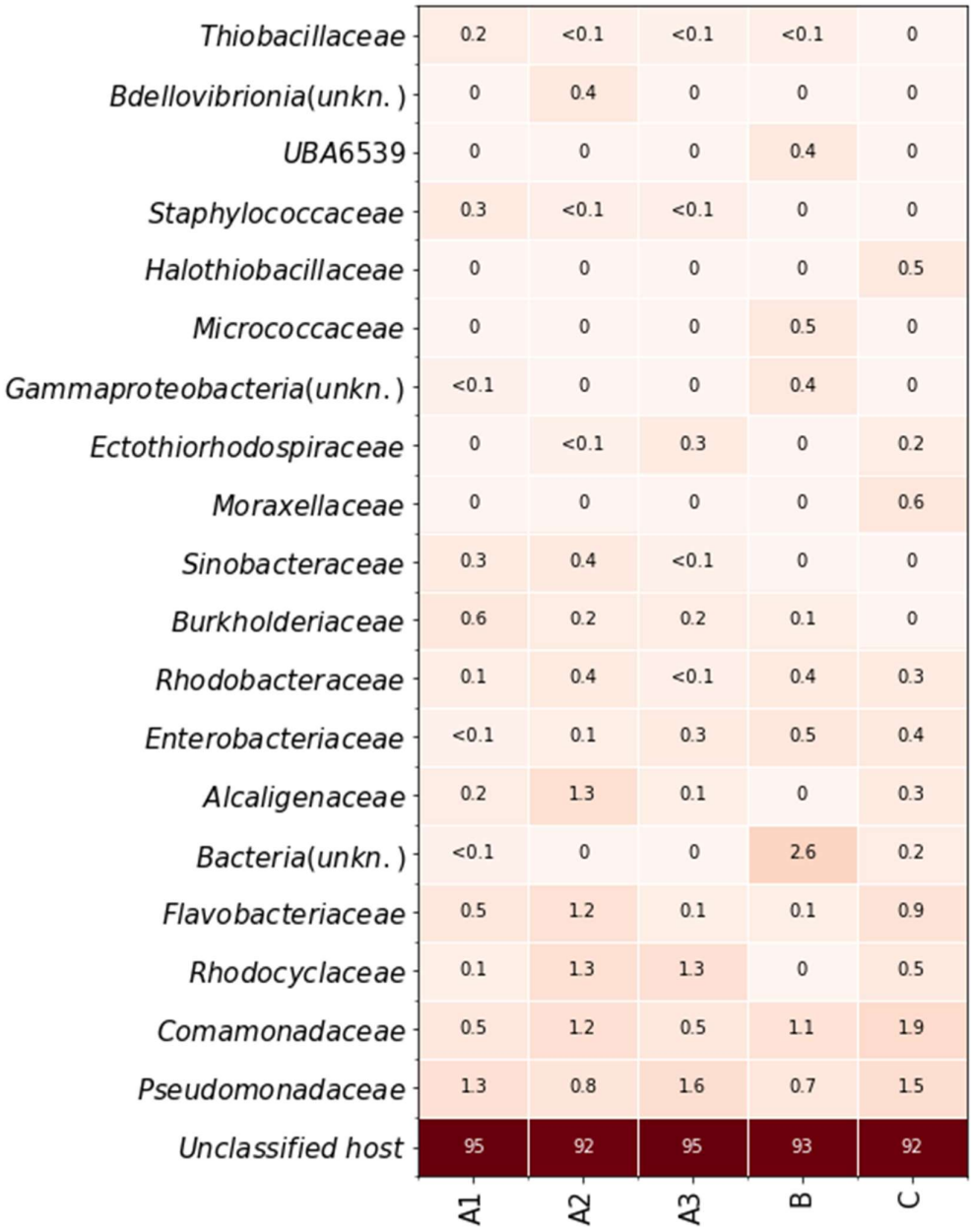

Fig. S6. Relative abundance (%) of contigs associated with different hosts.

Text S1. Analysis of P1 phages

Escherichia coli overnight (ON) was grown in Luria Broth (LB) (10 g/L tryptone, 5 g/L yeast extract and 10 g/L NaCl) supplemented with 10 µM $MgSO_4$ and 5 µM $CaCl_2$. The phage titer was made by adding 200 µL of the ON culture into eight 15 mL Falcon tubes. Subsequently 10 µL of the P1 lysate was added to the first Falcon tube and gently mixed. Thereafter, a serial dilution was done down to a dilution of 10^{-7}, followed by 20 minutes incubation at 37°C. Afterwards, 3 mL of LB-soft agar (10 g/L tryptone, 5 g/L yeast extract, 10 g/L NaCl and 6 g/L agar) was added, mixed, and immediately plated on LB plates (10 g/L tryptone, 5 g/L yeast extract, 10 g/L NaCl and 15 g/L agar). The plates were then incubated overnight at 37°C.

To create a new stock, the plate with a nearly confluent lysis was chosen from the dilution series. The soft agar was scraped off and placed in a 15 mL Falcon tube. Subsequently, 3-5 mL of phage buffer (7 g/L Na_2HPO_4 anhydrous, 3 g/L KH_2PO_4 anhydrous, 5 g/L NaCl, 1 mM $MgSO_4$ and 1 mM $CaCl_2$) and 20 drops of chloroform was added. The mixture was vortexed for 20 seconds and then left to stand at room temperature for 30-120 minutes, vortexing periodically. Thereafter, the mixture was centrifuged at 14000 rpm for 5 minutes and the supernatant transferred. This was followed by the addition of 3 drops chloroform, the mixture was vortexed again and left to stand for 20 minutes at room temperature before being centrifuged for 2 minutes at 14000 rpm, the supernatant was transferred to a fresh Eppendorf tube and stored at 4-7°C. To determine the concentration of the new P1 lysate stock a phage titer was done as described previously. The concentration of phage/mL (PFU/mL) was calculated using the following formula:

$$PFU/mL = \frac{Number\ of\ plaques \times dilution\ factor}{Volume\ phage\ stock}$$

Text S2. Theoretical contribution of a typical wastewater phage to C, N, and P

Relationships between capsid radius (r_c in nm) and C, N, and P content based on Jover et al. (2014):

C (fg/capsid) = $(41\cdot(r_c\text{-}2.5)^3+130\cdot(7.5\cdot rc^2\text{-}18.75\cdot rc+15.63))\cdot 12.0107\cdot 10^{15}/(6.022\cdot 10^{23})$

N (fg/capsid) = $(16\cdot(r_c\text{-}2.5)^3+36\cdot(7.5\cdot rc^2\text{-}18.75\cdot rc+15.63))\cdot 14.0067\cdot 10^{15}/(6.022\cdot 10^{23})$

P (fg/capsid) = $(4.2\cdot(r_c\text{-}2.5)^3)\cdot 30.9738\cdot 10^{15}/(6.022\cdot 10^{23})$

The capsid diameters for a few phages within *Myoviridae*, *Siphoviridae*, and *Podoviridae* are in the range 53-94 nm (Cui et al. 2014). It would be logical if the phages in the WWTP effluent also were close to this range. We measured diameters of the phages in WWTP effluents to be around 90-102 nm. One reason for this discrepancy is that NTA measures hydrodynamic diameter, not the actual capsid diameter. Previous measurements comparing transmission electron microscopy and NTA measurements for virus sizes found that the NTA measurements were on average 19% larger (Nikitin et al. 2015). If we use this value to estimate the capsid diameter for the average WWTP effluent phage, it is likely close to 80 nm. Thus, assuming a radius of 40 nm, we can calculate the C, N, and P content of a WWTP effluent phage to be 0.072 fg C, 0.029 fg N, and 0.011 fg P using the equations above (Jover et al. 2014). The highest phage concentration we measured in a WWTP effluent was $1.31\cdot 10^{10}$ VLP/ml. A concentration of 10^{10} VLP per ml water would cause concentrations of 0.72 mg L^{-1} DOC, 0.29 mg L^{-1} N, and 0.11 mg L^{-1} P.

References

Cui, J., Schlub, T.E. and Holmes, E.C. (2014) An allometric relationship between the genome length and virion volume of viruses. J Virol 88(11), 6403-6410.
Jover, L.F., Effler, T.C., Buchan, A., Wilhelm, S.W. and Weitz, J.S. (2014) The elemental composition of virus particles: implications for marine biogeochemical cycles. Nature Reviews Microbiology 12(7), 519-528.
Nikitin, N., Trifonova, E., Evtushenko, E., Kirpichnikov, M., Atabekov, J. and Karpova, O. (2015) Comparative Study of Non-Enveloped Icosahedral Viruses Size. PLOS ONE 10(11), e0142415.