Table S1. Calculation of percentage of water used for maintenance relative to total annual water demand

Total Volume of Water Used for Maintenance	3000	gallons/yr	(using 1500 gallons to prime system 2X/yr after disinfection)
Total Volume of Water Used by Consumers	5200	gallons/yr	(Calculation based on reported use)
	7950	gallons/yr	(Calculation based on estimated use)
Percent Used for Maintenance	**58%**		**(Reported Use)**
	38%		**(Estimated Use)**

Notes about Calculation based on reported use*:*

1. Facility reports to have 10 full time employees, that each use 520 gallons/year

*2. 10 employees * 520 gallons/yr-employee = 5200 gallons/yr*

Notes about Calculation based on estimated use:

1. Inquiry revealed that upwards of 20 additional people are on-site during the summer months, to conduct research

2. Each full-time employee was assumed to use the 520 gallons/year, so again 5200 gallons.

3. To estimate the use due to the extra people on-site, the 520 gallons/employee-year was divided into gallons/work day (520 gallons/employee-year / 50 work weeks/year / 5 work days/week = 2.08 gallons/employee/day.

*4. For the additional 20 people during the summer months, 2.08 gallons/employee-work day was used to estimate additional use (2.08 gallons/employee-work day * 20 additional employees * 66 work days = 2750 gallons/summer)*

5. Total use = 2750 + 5200 = 7950

Table S.2 Evidence of mild nitrification at the LEED healthcare suite

Parameter	Influent	Exam Rm1 - Stagnant	Exam Rm1 - Flushed	Exam Rm2 - Stagnant	Exam Rm2 - Flushed	Exam Rm3 - Stagnant	Exam Rm3 - Flushed	Exam Rm4 - Stagnant	Exam Rm4 - Flushed
pH	7.1	6.8	7.0	6.5	6.6	6.5	6.7	6.5	6.7
Temp. (°C)	18	22	24	23	24	23	22	24	24
Total Chlorine (ppm Cl_2)	1.2	0.04	0.08	0.01	0.19	0.02	0.22	0.01	0.15
NH_3 (ppm as N)	0.36	0.1	0.35	0.34	0.37	0.26	0.38	0.2	0.39
NO_2^- (ppm as N)	0.009	0.002	0.008	0.015	0.006	0.025	0.005	0.024	0.009
NO_3^- (ppm as N)	1.21	1.47	1.35	1.21	0.97	1.40	1.06	1.67	1.03
Alkalinity (ppm as $CaCO^3$)	50	56	49	57	49	59	48	57	48

Notes:

1. *Slight drop in pH in stagnant vs flushed samples*
2. *Change in nitrogen speciation; decrease in NH3in stagnant vs flushed samples*
3. *Increase in alkalinity of 6-9 mg/L as CaCO3*
4. *Biological Activity Reaction Tests (BARTs) positive for nitrifying bacteria*
5. *Absence of chlorine and elevated temperatures give the opportunity for growth*

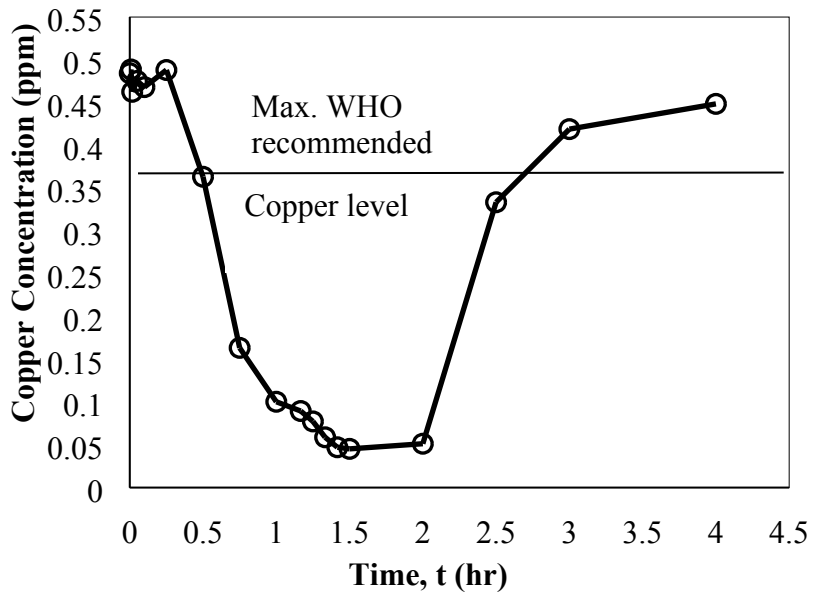

Figure S.1: Copper concentration at a tap as a function of flushing (0 < t < 2 hours), followed by stagnation (t > 2 hours)

Notes: Further evidence of nitrification at the tap as opposed to in well-flushed distribution system water.

The data that follows is water quality data from the remainder of the field sites.

Net-Zero House Water Use and Quality Data:

1. Details of total water use during water draws in simulated net-zero energy building

Day of Week	Total Use (gal)
Monday	65.21
Tuesday	65.21
Wednesday	86.94
Thursday	65.21
Friday	65.21
Saturday	86.64
Sunday	65.21
TOTAL	499.63

Note: the only cold water used was temper the hot water to the desired temperature during simulated use. Typical household use in the U.S. is about 300 gallons/day

2. Water quality data as a function of hot water flushing during the first site visit

Hot Flushing Time (min)	Solar Water Heater Temperature (° C)	Electric Water Heater Temp (° C)	Chlorine (ppm Cl_2)	pH	TOC (ppm)
0	59.4	50.6	0.02	8.9	1.26
5	54.4	36.7	0.33	8.3	1.81
10	43.9	37.8	0.36	8.3	1.56
15	37.8	37.8	0.36	8.1	1.69
20	31.1	33.3	0.37	8.0	1.55
25	24.4	28.9	0.48	7.9	1.50
30	21.1	26.7	0.63	7.9	1.51
35	20.0	22.2	0.76	7.9	1.42
40	15.6	20.6	0.83	7.9	1.36
50	15.6	17.8	0.96	7.9	1.43

*TOC=Total Organic Carbon

3. Water quality data as a function of cold water flushing during the first site visit

Cold Flushing Time (min)	Cold Water Temperature (° C)	Chlorine (ppm Cl_2)	pH	TOC (ppm)
0	21.2	0.04	8.4	1.44
2	10.9	0.16	8.8	1.44
4	11.3	0.18	8.9	1.41
6	10.8	0.35	8.9	1.42
8	9.8	0.40	8.7	1.30
10	9.5	0.34	8.7	1.34
15	9.6	0.65	8.5	1.38
20	9.8	1.09	8.0	1.29
25	9.9	1.15	7.4	1.79
30	9.9	1.15	7.5	1.35

4. Water heater temperature recovery in the solar water heater and electric water heater as a function of stagnation time after both tanks had been thoroughly flushed during the first site visit

Recovery Time (min)	Solar Water Heater Temperature (° C)	Electric Water Heater Temp (° C)
0	15.6	17.8
13	16.7	25.6
36	17.8	37.8
60	17.8	48.8

5. Water quality of the showering water as a function of flushing during the second site visit; All water was collected from three consecutive simulating showing events from the same showerhead.

Shower Number	Approximate Volume Flushed (gallons)	Temperature (° C)	pH	Total Chlorine (ppm Cl_2)
1	0	27.6	7.5	0.03
	4	42.6	7.6	0.06
	8	43.5	7.6	0.06
2	8.5	38.3	7.7	0.08
	12.5	42.3	7.7	0.08
	16.5	41.8	7.7	0.1
3	17	37.8	7.5	0.09
	21	39.1	8.0	0.09
	25	42.8	7.6	0.09

6. Water quality from the hot and cold tap manifold and water heaters during the second site visit

Sample	Temperature (° C)	pH	Total Chlorine (ppm Cl_2)
Solar Water Heater	26.1	7.33	0.11
Electric Water Heater	26.2	7.25	0.02
Hot water manifold (stagnant)	30.1	7.44	0.05
Hot water manifold (flushed)	48.1	7.35	0.08
Cold water manifold (stagnant)	23.2	7.39	0.33
Cold water manifold (flushed)	22.2	7.62	0.95

Conventional House Water Quality Data:

1. Water quality data as a function of cold tap flushing

COLD TAP flushing time (min)	Temperature (° C)	pH	Total Chlorine (ppm Cl_2)
0	23.6	6.85	0.71
3	9.9	6.8	1.04
5	10.1	6.9	1.01
10	10.5	7.02	1.01
15	11.7	6.95	1.01
After 15 minutes stagnation	15.6	6.79	0.99

Notes: chlorine concentration was stable after overnight stagnation

2. Water quality data as a function of hot tap flushing

HOT TAP flushing time (min)	Temperature (° C)	pH	Total Chlorine (ppm Cl_2)
0	42.4	6.92	0.06
3	44.4	7.12	0.06
5	44.9	7.17	0.08
10	45.9	7.2	0.11
20	42.6	7.19	0.16
30	33	7.05	0.68
40	29.4	7.03	0.89
50	28.1	6.96	0.92
60	27.5	7.13	0.96
70	27.2	7.11	0.98
80	27.1	7.08	0.99
90	26.7	7.07	1.01
After 15 minutes stagnation	28.4	7.1	0.95

Notes: Chlorine residual was stable in hot tap during short-term stagnation, but did decay after overnight stagnation

Net-zero Rainwater Office Water Quality Data:

1. Temperature and pH by sampling location

Sample	Temperature (° C)	pH
Cistern	25	7.6
Post-Treatment	28	7.7
Men's Cold Stagnant	29	7.2
Men's Cold Flushed	26	7.6
Kitchen Cold Stagnant	26	7.3
Kitchen Cold Flushed	26	7.6
Janitor Cold Stagnant	26	7.6
Janitor Cold Flushed	26	7.6
Kitchen Hot Stagnant	45	7.3
Kitchen Hot Flushed	47	7.6
Men's Hot Flushed	49	

2. Alkalinity by sampling location

Sample Name	Alkalinity (mg/L as $CaCO_3$)
Cistern	104
Post Treatment	112
Men's Cold Stagnant	113
Men's Cold Flushed	113
Well Water	303

Additional information on qPCR assays used:

The supplementary information from Wang et al., 2012 has all relevant assay information. Please see:

http://aem.asm.org/content/suppl/2012/08/08/AEM.01492-12.DCSupplemental/zam999103617so1.pdf

qPCR assay information:

Targeted Organisms	Targeted Genes	Sequences (5'-3')	Program		Amplicon (bp)	Refs
			Denaturation/ Enzyme Activation	Denaturing/ Annealing/ Extension		
Legionella spp.	23S rRNA	Leg23SF: CCCATGAAGCCCGTTGAA Leg23SR:ACAATCAGCCAATTAGTACGAGTTAGC Probe: HEX-TCCACACCTCGCCTATCAACGTCGTAGT	95 °C for 2 min	40 cycles of 95 °C for 5 s and 58.5 °C for 10 s	92	1
M. avium	16S rRNA	MycavF: AGAGTTTGATCCTGGCTCAG MycavR: ACCAGAAGACATGCGTCTTG	98 °C for 2 min	40 cycles of 98 °C for 5 s and 68 °C for 18 s	180	2
V. vermiformis	18S rRNA	Vv1227F: TTACGAGGTCAGGACACTGT Vv1728R: GACCATCCGGAGTTCTCG	98 °C for 2 min	40 cycles of 98 °C for 5 s and 72 °C for 18 s	502	3
Total Bacteria	16S rRNA	BACT1369F: CGGTGAATACGTTCYCGG PROK: GGWTACCTTGTTACGACTT	98 °C for 2 min	40 cycles of 98 °C for 5 s and 55 °C for 5 s	124	4