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Campus node-based wastewater surveillance enables COVID-19 case localization and confirms lower SARS-CoV-2 burden relative to the surrounding community

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ABSTRACT

Wastewater-based surveillance (WBS) has been established as a powerful tool that can guide health policy at multiple levels of government. However, this approach has not been well assessed at more granular scales, including large work sites such as University campuses. Between August 2021 and April 2022, we explored the occurrence of SARS-CoV-2 RNA in wastewater using qPCR assays from multiple complimentary sewer catchments and residential buildings spanning the University of Calgary's campus and how this compared to levels from the municipal wastewater treatment plant servicing the campus. Real-time contact tracing data was used to evaluate an association between wastewater SARS-CoV-2 burden and clinically confirmed cases and to assess the

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potential of WBS as a tool for disease monitoring across worksites. Concentrations of wastewater SARS-CoV-2 N1 and N2 RNA varied significantly across six sampling sites – regardless of several normalization strategies – with certain catchments consistently demonstrating values 1–2 orders higher than the others. Relative to clinical cases identified in specific sewersheds, WBS provided one-week leading indicator. Additionally, our comprehensive monitoring strategy enabled an estimation of the total burden of SARS-CoV-2 for the campus per capita, which was significantly lower than the surrounding community ($p \leq 0.001$). Allele-specific qPCR assays confirmed that variants across campus were representative of the community at large, and at no time did emerging variants first debut on campus. This study demonstrates how WBS can be efficiently applied to locate hotspots of disease activity at a very granular scale, and predict disease burden across large, complex worksites.

1. Introduction

To cope with the COVID-19 pandemic crisis, governments worldwide have implemented a range of measures to mitigate the spread of the virus, including large-scale clinical testing. While diagnostic testing is tremendously important, it is limited in its capacity to perform population-level surveillance owing to tremendous human and capital resources required to run community testing centers (Hart and Halden, 2020). In addition, clinical testing is biased by relying on voluntary participation and generally targets individuals with symptomatic disease (O'Keeffe, 2021). Wastewater-based surveillance (WBS) has been robustly demonstrated to be a useful tool for monitoring population health (Sims and Kasprzyk-Hordern, 2020) serving to complement clinical testing and providing real-time data on the burden of disease in a monitored sewershed. Advantages of WBS include i) comprehensive, inclusive, serial monitoring of the population with relatively low costs, and ii) unbiased data collection of biological material from all society members including marginalized populations and those unable to access clinical testing (Hart and Halden, 2020; Polo et al., 2020; Sims and Kasprzyk-Hordern, 2020). WBS for SARS-CoV-2 surveillance has been adopted worldwide (Naughton et al., 2021), including Canada where it currently covers ~62 % of the country's population (PHAC, 2022). Generally, WBS programs monitor SARS-CoV-2 RNA in untreated sewage from wastewater treatment plants (WWTPs) and thus assess disease burden at the level of an entire community. Community-based studies have identified SARS-CoV-2 WBS as a leading indicator for cases (4-6 days), hospitalizations, and deaths (Halwatura et al., 2022; Kumar et al., 2021; Róka et al., 2021; Zhu et al., 2021). However, studies where monitoring has been performed at a more granular scale (e.g., defined sub-catchments within a larger sewershed or specific facilities) are less common, and more work is needed to clearly demonstrate if similar correlations and benefits exist.

Such granular scale WBS is challenging when it comes to selecting sampling locations because (i) it requires detailed information on defined sampling nodes (e.g., each node and their GPS coordinates) and their connectivity (to determine if overlapping catchments exist and may confound analysis), (ii) nodes should have enough wastewater flow to ensure sufficient volume during continuous collection. A recent study in the City of Calgary evaluating neighborhood-scale sub-catchment monitoring (serving populations of 13,000 to 73,000) within larger WWTP catchments (serving 290,000 to 1,048,000) showcased the utility of node-based sampling in identifying specific sub-catchment(s) with disproportionate infection burden (Acosta et al., 2022b). WBS at an even more granular scale (e.g., buildings) as a node-based sampling strategy could help to specify more clearly 'hotspots' for infection transmission (Acosta et al., 2021; Bowes et al., 2022; Pico-Tomàs et al., 2023).

One area of focused WBS that has generated significant interest is university/college campuses. University campuses consist of a vast array of building complexes where a high degree of social interaction is expected between students, staff, and faculty. Accordingly, high rates of SARS-CoV-2 transmission are possible across campuses in the absence of mitigating steps. While campus-wide studies have been performed, studies to determine the spatial distribution of SARS-CoV-2 RNA within a university campus, and enabling a comprehensive assessment of the total disease burden have yet to be performed. Thus far, University-based studies assessing SARS-CoV-2 RNA in wastewater have primarily focused on longitudinal analysis rather than spatially resolved analysis (Karthikeyan et al., 2021; Wright et al., 2022), on a subset of campus buildings (Johnson et al., 2022; Reeves et al., 2021), or only on individual dormitories/residential buildings (Ash et al., 2023; Corchis-Scott et al., 2023; Gibas et al., 2021), necessitating development of more comprehensive monitoring strategy.

Herein we describe a longitudinal nodal WBS monitoring program for SARS-CoV-2 RNA at the University of Calgary (UofC), Calgary, Alberta, Canada. The main campus is situated on 530-acres in the Northwest quadrant of the city. We conducted a cross campus node-based, spatially resolved WBS program from Aug 31, 2021–Apr 24, 2022, and compared these assessments with the municipal WWTP which serves the campus. This monitoring program was brought about to monitor the safe resumption of campus activities as COVID-19 population level controls were relaxed. Our primary research objectives were to i) locate specific sub-catchment(s) within the University where SARS-CoV-2 RNA exists in differential abundance, ii) establish the association between wastewater measured SARS-CoV-2 and COVID-19 case occurrence within an individual sewershed's catchment, and iii) determine the relative risk of COVID-19 on campus, as inferred by SARS-CoV-2 wastewater burden, relative to the surrounding community. We hypothesized that i) higher abundance of SARS-CoV-2 RNA would be found in buildings with higher social connectivity and that this would be associated with COVID-19 reported cases; and, ii) the abundance of SARS-CoV-2 RNA in campus wastewater would be lower than the surrounding community given a strictly enforced COVID-prevention policy and procedures across campus, i.e., campus mandate for universal masking, a high prevalence of accessible hand hygiene and masking product throughout campus, and a vaccine mandate (or weekly negative testing) required to attend campus in person.

2. Materials and methods

2.1. Defining sampling nodes across the UofC sewershed

UofC is among the ten largest Universities in Canada with >26,000 full-time undergraduate students, 6,000 graduate students and 1,800 academic and 3,200 non-academic staff (UofC, 2022b). The main campus is situated in Northwest Calgary in the province Alberta on a parcel of 530-acres (2.13 km^2) (GPS-coordinates of 51.0784° N, 114.1347° W). Campus sewer sampling locations were chosen to deliver a minimum number of manhole-accessible sites providing maximum coverage of campus buildings and designed to be mutually exclusive (in as much as possible), using GIS-based analysis of the sewer pipe network (Fig. 1). Residence halls were also included based upon presence of an accessible sampling location within the building's plumbing network capturing ≥50% of the residential areas of that building.

Three nodes were selected to capture the building complexes in the Northwest (NW), Northeast (NE), and South (SO) zones of the campus (Fig. 1). Six buildings drain into both NW and NE within these catchments and are indicated as MIX (see Fig. 1; colored in orange). Both residence halls (RH1 and RH2 accommodating 570 and 355 students,

respectively) are within the NW catchment thus enabling even more granular scale sampling nodes. The entire monitoring program captured 8 residential halls, 30 lecture/research facilities, 6 recreational facilities (including dining/fitness buildings), and 2 others (parkade, and daycare centre) (Fig. 1). Our monitoring catchments cover >80% of the total residence population (i.e., those living in dormitories; a total of 2,641 students) and >83 % of the campus aerial footprint. The key information about our monitoring catchments was shown in Table 1.

Calgary is Canada's fourth largest city by population and its third most ethnically diverse (Acosta et al., 2022b; Calgary, 2019). Three WWTPs serve an estimated 1,441,268 people (Alberta-Government, 2021). UofC falls exclusively within the catchment zone of the largest WWTP, serving 1,047,662 individuals and receiving 303.7-604.6 ML/day. The municipal WWTP receives and processes urban and industrial wastewater, the exact proportions of which are not publicly available. The city of Calgary maintains separate wastewater and stormwater sewage networks and accordingly, the university's wastewater network does not include urban runoff/stormwater.

2.2. Wastewater collection

Wastewater samples were collected from the sites described above 2-3 times per week from August 31, 2021–April 30, 2022, using a workflow described previously (Acosta et al., 2021; Acosta et al., 2022b). In short, CEC Analytics V1 (C.E.C Analytics, Canada) and ISCO 6712 (Teledyne ISCO, USA) autosamplers collected 2L (CEC) or 10L (ISCO) 24 h composite samples (sampled every 15 mins, then pooled for 24 h) that were stored at 4°C and transported to Advancing Canadian Water Assets (ACWA). Upon arrival, samples were thoroughly mixed and aliquoted into 50mL centrifuge tubes for downstream analysis. More details on field sampling techniques are described in the Supplementary-Material.

Table 1
The type, number, and gross area of buildings within each monitoring catchment in the main campus of University of Calgary, Calgary, AB, Canada. *ETC indicates parkade or daycare centre. Gross area (m^2) denotes summation of aerial footprints of all the buildings belonging to each sewer catchment.

Name of catchment	Type of building within the catchment (number)	Gross area (m^2)
NE	Research/Lecture (8) ETC* (1)	105,504
NW	Research/Lecture (16) Residence (4) Recreation (5)	331,151
SO	Residence (4) Recreation (1) ETC* (1)	83,884
MIX	Research/Lecture (6)	109,459
RH1	Residence (1)	18,596
RH2	Residence (1)	21,774

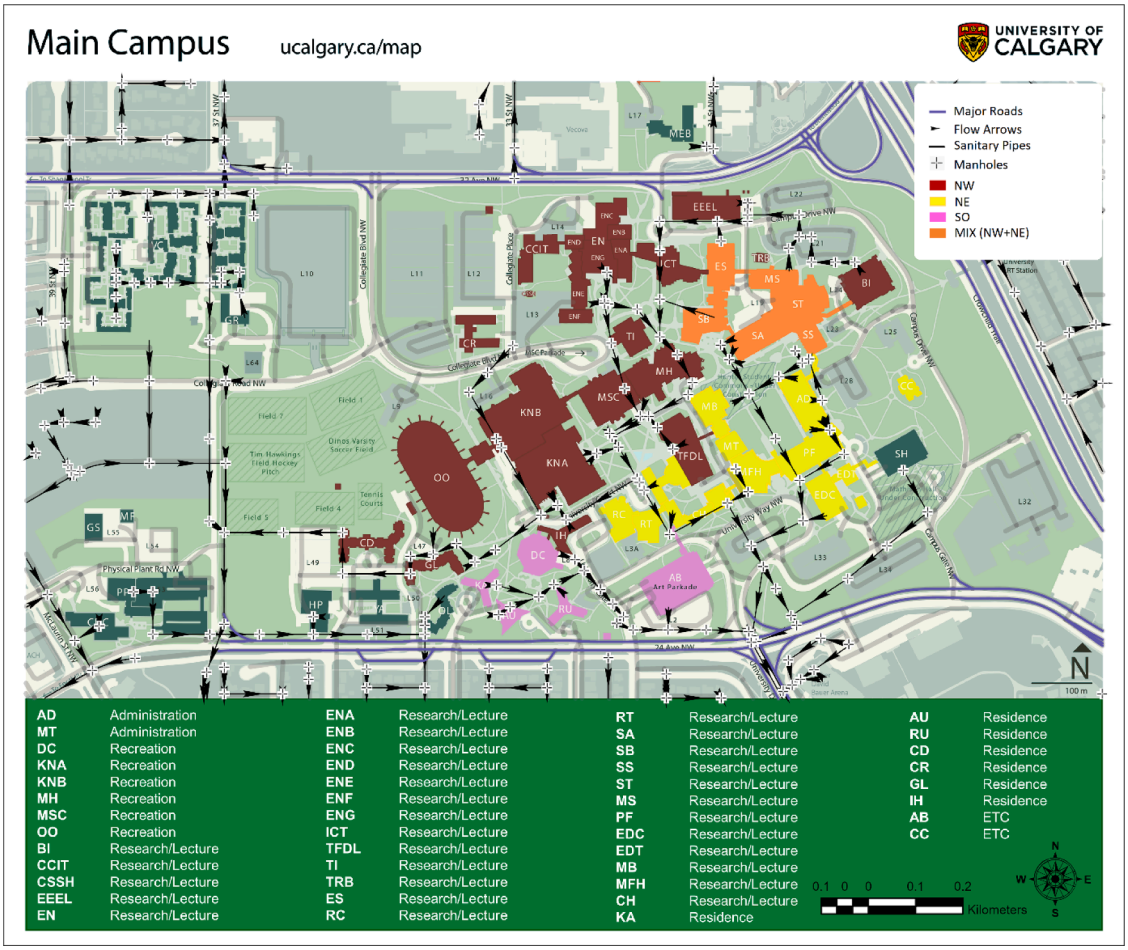

Fig. 1. University of Calgary main campus in Alberta, Canada (51.0784° N, 114.1347° W) highlighting different sewershed catchments (colour key is shown in the figure legend). NW, NE, and SO indicate Northwest, Northeast, and South catchments, respectively. One student residence hall (RH1) falls into one of the monitoring catchments (NW), and the other (RH2) does not. The exact location for each residence hall was not detailed for ethical reason. The area MIX (colored in orange) belongs to both NW, and also NE. The university buildings outside our monitoring catchments, but which still belong to the main campus were colored in 'dark grey'. L1 – 64 (in light grey) represent un-serviced parking lots. The figure was modified from http://www.ucalgary.ca/map. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

2.3. Sample processing and RNA extraction

Sample processing and RNA extraction was performed following established workflows (Acosta et al., 2021; Whitney et al., 2021). In brief, 40mL of thoroughly mixed wastewater subsamples was added to 50mL falcon tubes prefilled with 9.5 g of sterile NaCl and 400ul of TE buffer and were then spiked with 200μL of Bovine Coronavirus (BCoV) (final concentration: 5×10^5 50% tissue-culture-infective dose ($TCID_{50}$) per mL) as an internal control and vortexed for 30 s. Solids were then removed via vacuum filtration through a 5 μm polyvinylidene difluoride membrane, where samples were filtered directly into 40ml of 70% ethanol. This solution was then passed through a Zymo Spin™ III-P silica column (Zymo Research, USA). More details can be found in the Supplementary-Material.

2.4. Quantitative RT-qPCR

RT-qPCR assays were performed following established workflows (Acosta et al., 2021; Hubert et al., 2022). In short, two regions of the nucleocapsid gene (N1 and N2) were used to quantify total SARS-CoV-2 RNA copies/mL in every wastewater sample (Medema et al., 2020; Randazzo et al., 2020; Wu et al., 2020a). We also analyzed variants of concern (VOC), including Delta, Omicron (BA.1, and BA.2) using the N200 multiplex assay (Fuzzen et al., 2022; Hubert et al., 2022) or 69/70del assay (Peterson et al., 2022) for a subset of samples for WWTP (44 samples), RH1 (18 samples), SO (18 samples), NW (17 samples), and NE (9 samples) from November 28, 2021-April 27, 2022. BCoV (Bovine Coronavirus) was analyzed as an internal spike control, and PMMoV (Pepper Mild Mottle Virus) was analyzed as a potential human feces biomarker (D'Aoust et al., 2021; Whitney et al., 2021). All samples were analyzed in triplicate, including non-template controls for each run using QuantStudio-5 Real-Time PCR System (Applied Biosystems, USA). Samples with a quantification cycle (Cq)<40 were considered positive (Acosta et al., 2021). The Cq values (averaged between triplicates) for samples were converted to 'copies' according to the standard curve for each run (Table S1), then further to concentration (copies/mL) (see Supplementary-Method 1.2). Concentrations for N1, N2, and PMMoV for each week were arithmetically averaged for each monitoring location. Those averaged concentrations (for each week) were used in downstream analyses, and the data presented in Dataset S1.

2.5. Chemical analysis

In addition to PMMoV, a total of five wastewater ions (sodium, chloride, potassium, magnesium, and calcium) were chosen to explore their association with SARS-CoV-2 as potential normalization markers for human activity (detailed in Supplementary-Material). Chemical markers associated with human excreta are potentially useful for correcting possible underestimation of SARS-CoV-2 levels due to dilution effect (Hsu et al., 2022; Langeveld et al., 2022), which could be particularly important in small catchments. Several ions are associated with human excreta, for instance both urine and stool for potassium (Agarwal et al., 1994; Amdur et al., 2020; Klevay et al., 2007; Le et al., 2020; Randall and Naidoo, 2018), and predominately urine for sodium, chloride, magnesium and calcium (Karak and Bhattacharyya, 2011; Randall and Naidoo, 2018; Rose et al., 2015). Ion concentrations enriched in wastewater beyond that contained from tap water input is likely to represent human waste in the proximal sewershed (Azoulay et al., 2001) as well as industrial output at municipal WWTP (Shrestha et al., 2021). To minimize uncertainty, we excluded ion(s) from normalization analyses that occurred below concentrations measured in Calgary's tap water (yearly average) in most samples (City-of-Calgary, 2021a, 2021b). We further excluded the ion(s) from normalization analyses where WWTP levels were significantly higher than residential halls (RH1 and 2) assuming that alternate sources for the ion(s) may exist other than human waste.

2.6. Modelling expected COVID-19 cases per capita across UofC Campus

Cases per capita in UofC main campus (CPC_{UofC}) was calculated using raw (i.e. un-normalized) SARS-CoV-2 RNA concentrations according to the following relationship referring to (Eq. (S4)) in the Supplementary-Material

$$C_{WWTP} \ : \ C_{UofC} = \frac{n_{WWTP}}{N_{WWTP}} \ : \ CPC_{UofC}$$

$$i.e., CPC_{UofC} = CPC_{WWTP} \cdot \frac{C_{UofC}}{C_{WWTP}} \quad (1)$$

Where, N_{WWTP} indicates the total population in the catchment area for WWTP (n=1,047,622(Acosta et al., 2022b) ; n_{WWTP} is incident number of new cases occurring daily (i.e., confirmed COVID-19 infected individuals) in the catchment area for WWTP; C_{UofC} and C_{WWTP} indicate concentration of SARS-CoV2 RNA for UofC and WWTP respectively, and could be calculated according to (Eq. (S6)) in the Supplementary-Material.

To mitigate uncertainty which may arise from possible differences in human excreta across samples, CPC_{UofC} was also calculated using normalized SARS-CoV-2 RNA concentration according to the following relationship referring to (Eq. (S5)) in the Supplementary-Material.

$$\frac{C_{WWTP}}{C_{e-WWTP}} \ : \ \frac{C_{UofC}}{C_{e-UofC}} = \frac{n_{WWTP}}{N_{WWTP}} \ : \ CPC_{UofC}$$

$$i.e., CPC_{UofC} = CPC_{WWTP} \cdot \frac{CPC_{UofC} \cdot CPC_{e-WWTP}}{CPC_{WWTP} \cdot CPC_{e-UofC}} \quad (2)$$

Where, C_{e-UofC} , and C_{e-WWTP} indicate concentration of human excreta surrogates for UofC and WWTP, and could be calculated according to (Eq. (S6)) in the Supplementary-Material.

2.7. Uncertainty analysis

As wastewater flow data for UofC was unavailable, models described in 2.6 rely on assuming flow quantities are proportional to catchment surface areas (i.e., total footprint of all buildings) (see Supplementary-Method 1.3 in the Supplementary-Material). We assumed that uncertainty in this model derives mostly from variability of gross surface area in prediction of flow quantity. Therefore, prediction errors from these sources were propagated using a Monte-Carlo randomization simulation adapted from other relevant works (Lee, 2021; Ort et al., 2009). The term surface area (A) was randomized by multiplying the uncertainty factor (a) which is variable ranging from 0.2 (20%) to 2.0 (200%) assuming that the actual ratio of flow quantity lies within these boundaries.

$$V_{SO} \ : \ V_{NW} \ : \ V_{NE-MIX} = a_1 \cdot A_{SO} \ : a_2 \cdot A_{NW} \ : a_3 \cdot A_{NE-MIX}$$

Where, a_1, a_2, and a_3 are random variables ranging from 0.1 to 2.0, also are independent from each other. The ratios of (three) terms were written with a colon (:).

The simulation was repeated 1,000 times, and interquartile ranges (IQR, Q1 – Q3) for each prediction value are displayed as error bars in the model. Furthermore, the p-value for permutation test was defined as 'the ratio of counts where $CPC_{UofC} > CPC_{WWTP}$ to 1,000 (= the number of simulation trials)' for each time point. Only time-paired data points were compared between each site. The modelling was performed using R (v4.1.2), and related datasets/R codes are available in the first author's GitHub page (https://github.com/myjackson).

2.8. Clinical case documentation

Information on city-wide, new daily cases of clinically confirmed COVID-19 (patient swabs confirmed with a clinical RT-qPCR) were provided by a single comprehensive public health system, Alberta

Health Services (AHS) via the Centre for Health Informatics online COVID Tracker (https://covid-tracker.chi-csm.ca/). The information was gathered between August 31, 2022, and March 31, 2022, and a subset of this data (i.e., August 31, 2021 – January 04) was subjected to further analysis. New cases were binned by individual postal codes (using the first three of six digits) as an indicator of the home address of newly diagnosed cases. Cases were then assigned to the appropriate WWTP serving their primary residence.

Comparative analyses were conducted across two distinct time-periods; Period A (Aug 31, 2021-Dec 12, 2021) and Period B (Dec 13, 2021-April 25, 2021) owing to fundamental changes occurring through the pandemic. In particular, during the Omicron waves (Period B, defined when the first case was documented in Calgary), clinical case occurrence for the first time vastly exceeded the ability of health services to screen and detect the population.

Documenting campus-associated confirmed-COVID-19 cases and ascribing them to a specific primary building was performed in real-time by the University of Calgary's Occupational Health and Staff Wellness for students and employees who self-reported a positive COVID-19 test during the pandemic period from September 2021. Confirmed cases were excluded from attending campus for a minimum of 10 days (reduced to 5 days after January 3rd, 2022) and complete symptom resolution. The information gathered between September 21, 2021 and April 2022 was used to trace the primary buildings where individuals with confirmed COVID-19 were located. We assigned study-specific personal identifiers to each affected individual and avoided personal identifying information. The information gathered in the original report includes: i) 'date of positive test result', ii) 'date university informed of illness', iii) 'recent university location(s) visited and the date when the person visited that location', and iv) 'date of onset of symptoms', etc. However, in some instances case information was not always fully declared (i.e., the recent university location(s) visited, and the date(s) when the person visited), and such cases were excluded. As a result, the information from 463 out of 721 reported individuals was used in downstream analyses. The de-identified raw data is not included for ethical and privacy considerations, but could be provided upon reasonable request to the authors. The full set of processed data is shown in Dataset S2. The patient identifiers (PID) are not known to anyone outside our research group, so individuals remain anonymous.

To model the movement of confirmed COVID-19 infected individuals across campus, we relied on self-reported activity tracing reported to Occupational Health and Safety Staff. To identify individual buildings where COVID-19 positive individuals visited, we first counted the number of positive individuals who visited each building using the information iii) above. For example, for each building, the recently visited PIDs were listed (Dataset S2). Then, we counted the total affected-visits for each building. In this way, each PID was often counted multiple times in situations where the person visited multiple locations or one location on multiple days during the monitoring period. As the majority of SARS-CoV-2 RNA shedding occurs in the few days before and after symptom onset (Acosta et al., 2021; Acosta et al., 2022a), the visits $\pm$ 2 days of the 'date of onset of symptoms' were considered valid, otherwise excluded in the following analysis. Total affected-visits is named 'number of cases'. Finally, the number of cases was subjected to further analysis. For instance, the cases for each week were averaged arithmetically, and aggregated by monitoring catchment for each monitoring week (Fig. S5).

During the monitoring period, on-campus residents (i.e. those residing full-time in dormitories) who were confirmed as COVID-19 positive were quarantined according to the following principles: (i) if a case was reported by an individual living in a single unit with a bathroom– not shared with another, the individual was isolated in place (a total of 10 residential halls, CR, YA, CD, GL, KA, AU, RU, IH, OL, or VC (Fig. 1)), (ii) if all occupants of a shared apartment are positive, they would continue to isolate in their same apartment in their residential hall, and (iii) if the positive individual shares an apartment with someone who is not also positive, they were moved to another suite, VC for their isolation period. Our monitoring program included most of the isolation places (i.e., a total of 8 out of 10 places, CR, YA, CD, GL, KA, AU, RU, and IH; see Fig. 1).

2.9. Statistical analysis

Kruskal-Wallis test followed by a post-hoc Wilcoxon rank-sum test was performed to test if there were significant differences between groups. For pairwise tests, p-values were adjusted using the Benjamini-Hochberg method. Additionally, Spearman correlation analysis was performed to test if there were significant relationships between the two factors. Finally, Fisher's exact test was implemented to test the potential association between two variables (i.e., SARS-CoV-2 signals versus campus-associated COVID-19 cases). One-sided test was employed under the expectation that those two might be positively associated. Then, Fisher's exact test was repeated for each pair of wastewater SARS-CoV-2 signals (N1 or N2) against cases reported; a week earlier (-1 week) cases, those on the same week (+0 week), or the week following (+1 week) under the hypothesis that wastewater signals serve as an early warning sign of COVID-19 cases. The key rationale was explained in more detail in the Supplementary-Method 1.5. All analyses were done using R version 4.1.2., and related datasets/R codes are available in the first author's GitHub page (https://github.com/myjackson).

2.10. Ethics

This study was part of a large regional SARS-CoV-2 wastewater-based surveillance program which included a range of hospitals, shelters, schools, neighborhoods as well as municipal wastewater treatment plants across Alberta (~83% of the population) funded by Alberta Health. The campus monitoring program was developed in partnership between the research team, and UofC's Provost, the Offices' of the Vice-President Research and Vice President Academic, the Dean's office for the Cumming School of Medicine, as well as UofC's Facilities Management and Occupational Health and Safety offices in order to monitor in real-time the safety of UofC's campus during a return to campus activities. Wastewater results were available within 24–48 h of sample collection through a password protected web portal to University Health Administrators and Policy makers. Campus SARS-CoV-2 monitoring was part of several measures used to gauge the success of a campus re-opened to in-person learning. Clinical case data was captured as part of the mandate of the UofC Occupational Health and Safety office and no individual data was collected specifically for research purposes. The clinical case, tracking and exposure data that was used for safe campus monitoring by UofC staff was correlated with wastewater measured SARS-CoV-2 retrospectively, and the research study team had no involvement in real-time infection mitigation measures. In addition to all data being de-identified, all clinical data was analyzed in aggregate. The study received approval from the Conjoint Research Health Ethics Board of the University of Calgary (REB20–1544).

3. Results

3.1. Longitudinal tracking wastewater SARS-CoV-2 RNA across campus

Between August 3, 2021 and April 30, 2022, a total of 58 (RH1) and 18 (RH2) samples were obtained from the residence halls, 45 (NE), 48 (SO), and 42 (NW) samples were obtained from the campus catchments, and 89 samples were obtained from WWTP, providing 12 – 25 data points per location after being averaged weekly. The lower number of samples collected for campus locations primarily relates to more complicated access for these sampling points (e.g., manholes in the open (public) area for the campus sampling points versus either within buildings or from the WWTP facility. The outdoor locations (manholes) also experienced a higher rate of failure to collect especially during cold

weather (<-20 ℃) for campus sites.

During this time the City of Calgary experienced three successive "waves" of COVID-19 (corresponding to the fourth, fifth and sixth waves since the start of the pandemic). Tracked via wastewater, the first of these waves during the monitoring period peaked on September 06, 2021, followed by January 03 and April 18, 2022. Allele-specific PCR to detect VOC in WWTP samples confirmed it was the Delta variant that was dominant during the fourth-wave (peaking September 06, 2021), and Omicron lineages were dominant during the fifth (BA.1 peaking January 03, 2022) and sixth waves of this study (BA.2 peaking April 18, 2022, the third wave in this study) (Fig. S6). Notably, the burden of wastewater-detected SARS-CoV-2 N1 and N2 for the two latter waves caused by Omicron lineages vastly exceeded that of Delta.

SARS-CoV-2 N1 and N2 concentrations across campus monitoring sites generally, but not always mirrored those of the community WWTP (Fig. 2). Values from WWTP were higher than those across campus, with some exceptions. While the highest N1 and N2 values observed from campus monitoring sites (i.e., SO, NE, and NW) occurred during the peaks of each wave experienced in the community, random spikes in N1 and N2 also occurred during community troughs and appeared randomly suggesting brief periods of increased disease burden. Analysis of VOC across UofC campus mirrored those for WWTP –Delta was dominant in Period-A (Aug 31, 2021-Dec 12, 2021) for those locations where data was available (i.e., SO, and NW) (Figs. S7 & S8) and Omicron lineages were dominant in Period-B for SO, NW, NE, and RH1 while. In no instances did the emerging VOC occur disproportionally within the campus environment relative to that of the community.

Among five ions measured as potential normalization factors for human activities, calcium was excluded based on its relatively high concentration in tap water relative to wastewater samples (Fig. S4). Furthermore, magnesium, sodium, and chloride were further excluded for their relatively high levels at the WWTP relative to the campus samples ($p<0.05$) (Fig. S4). Accordingly, only two human waste surrogates (i.e., the plant virus PMMoV and the ion potassium) were assessed as potential normalization markers.

3.2. Correlating wastewater SARS-CoV-2 RNA with clinically confirmed cases

A median of 153 (IQR 73 – 240) cases per day were clinically confirmed across the catchment of the WWTP during the period monitored (August 31, 2021 – January 04, 2022; a total of 36 data points). These cases were correlated with raw-, and also normalized-SARS-CoV-2 N1 and N2 signals using different investigational markers for the corresponding date ranges. The raw (i.e., un-normalized) N1 and N2 signals (i.e., copies/mL) generally correlated with confirmed cases the best (Table S2) suggesting normalization with PMMoV or potassium did not denoise variability associated with human excreta over time. However, for comparing different smaller catchment results with each other, we expected the variability in human wastes between sites could be large, especially when the characteristics of those populations may be very different, e.g., residential versus non-residential areas of the campus. Accordingly, while we used raw SARS-CoV-2 concentrations as our primary outcome for cross-site comparisons, we still assessed SARS-CoV-2 normalized for PMMoV and potassium as a confirmatory secondary outcome measure.

3.3. Comparing SARS-CoV-2 RNA signals across different sampling locations

Comparison across different locations was conducted for each of the two separate periods, for instance Period-A (Aug 31, 2021-Dec 12, 2021) and B (Dec 13, 2021-April 25, 2021). There were significant differences in both raw and normalized wastewater SARS-CoV-2 RNA concentrations between monitoring sites during the study (Period-A to -B) based on Kruskal-Wallis test ($p<0.001$). In Period-A, a post-hoc analysis using Wilcoxon-rank sum test revealed that SARS-CoV-2 RNA N1 and N2 concentrations in campus locations were 1 – 2 orders of magnitude lower than the community WWTP ($p\leq0.005$) (Table 2 & Fig. 3). Furthermore, there were significant differences in both N1 and N2 concentrations between campus locations. For instance, the values for NE were 1 – 2 orders of magnitude lower than NW and SO, and such difference was non-parametrically significant using Wilcoxon rank-sum tests for NW ($p\leq0.038$) (Table 2). SARS-CoV-2 N1 and N2 concentrations for two dormitories (i.e., RH2 and RH1) were similar to NE ($p\geq0.428$) but lower than SO ($p\leq0.026$) and NW ($p\leq0.038$), based on Wilcoxon rank-sum test (Table 2). In general, normalized trends of SARS-CoV-2 burden between sites mirrored those of raw values (Table S3). The normalized N1 and N2 concentrations for WWTP were higher than for all the campus locations using post-hoc Wilcoxon rank-sum test in all comparisons ($p\leq0.015$) (Table S3). Among university campus locations, the normalized N1 and N2 values for NE were lower than NW or SO in many instances (Table S3).

In Period-B, the N1 and N2 concentrations increased considerably

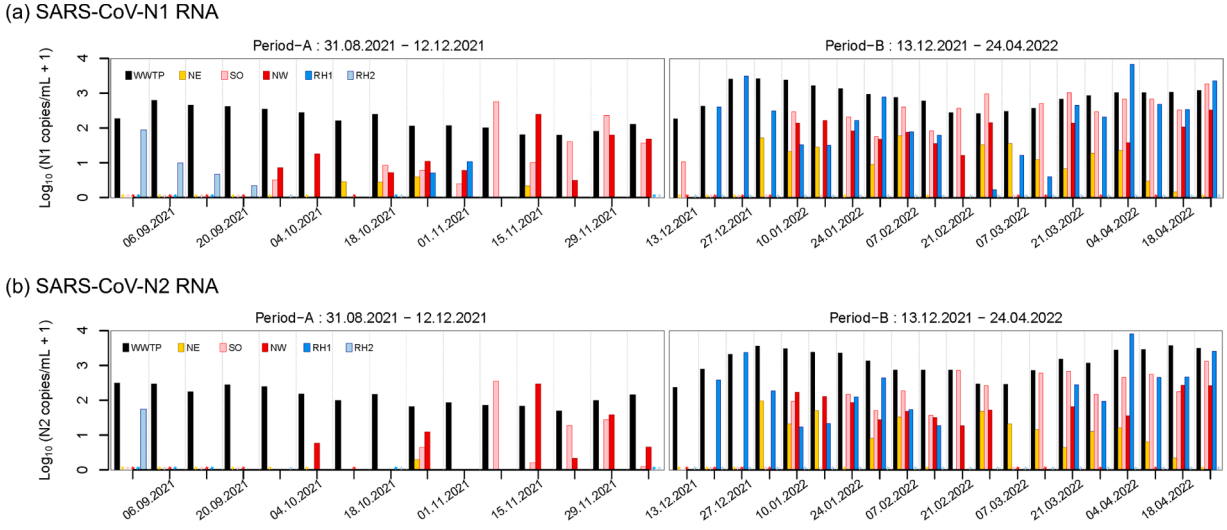

Fig. 2. Log_{10}-transformed concentrations (copies/mL) of SARS-CoV-2 N1 and N2 profiles in campus wastewater sub-catchments demonstrate much lower values relative to the receiving municipal WWTP during the study period (August 31, 2021 – April 30, 2022). WWTP indicates the municipal wastewater treatment plant servicing the surrounding community, and also UofC main campus. See Fig. 1 for the locations and catchment area. *=missing data. Date (in x-axis) : dd.mm.yyyy.

Table 2
Comparing SARS-CoV-2 RNA raw concentrations between different UofC monitoring locations during Period-A and -B using Wilcoxon rank-sum test. P-adjusted for pairwise comparison using Benjamini & Hochberg method. Those pairs that statistically differed with ($p<0.05$) are highlighted in red.

Period	Indicator	Location	RH2	RH1	NE	SO	NW
Period-A	N1	RH1	0.675	-	-	-	-
		NE	0.968	0.650	-	-	-
		SO	0.026	0.014	0.020	-	-
		NW	0.014	0.005	0.005	0.863	-
		WWTP	0.000	0.000	0.000	0.005	0.001
	N2	NE	0.944	0.428	-	-	-
		SO	0.052	0.020	0.068	-	-
		NW	0.038	0.015	0.038	0.736	-
		WWTP	0.000	0.000	0.000	0.002	0.002
Period-B	N1	RH1	-	-	-	-	-
		NE	-	0.019	-	-	-
		SO	-	0.156	0.000	-	-
		NW	-	0.491	0.002	0.006	-
		WWTP	-	0.003	0.000	0.013	0.000
	N2	NE	-	0.107	-	-	-
		SO	-	0.238	0.001	-	-
		NW	-	0.829	0.006	0.029	-
		WWTP	-	0.000	0.000	0.000	0.000

compared to Period-A at WWTP and across campus (Fig. 3). However, the values for the campus were still significantly lower than for WWTP for both N1 and N2 based on a post-hoc Wilcoxon rank-sum test ($p\leq0.013$; see Table 2). The degree of increase for RH1 was the most pronounced among all monitored sites. For instance, the median N1 concentration for RH1, and SO samples profoundly increased, for instance by >2 order of magnitude (from 0.0 to 184.7 copies/mL for RH1; from 7.6 to 369.8 copies/mL), while the median concentration for other campus locations increased only by approximately 1 order of magnitude (from 0.0 to 19.4 copies/mL for NE; from 8.1 to 94.3 copies/mL for NW). In all cases, the normalized concentrations for SO were significantly higher than for NE based on Wilcoxon rank-sum tests in all cases using N1 and N2 ($p\leq0.043$). The normalized concentrations for WWTP were significantly higher than all the UofC campus locations in all cases using N2 ($p\leq0.002$), and in many cases except for between SO and WWTP using potassium-normalized N1 (Table S4).

3.4. SARS-CoV-2 RNA measured in campus wastewater catchments correlates with regional case occurrence

The association between COVID-19-confirmed clinical cases and wastewater-N1 or N2 concentrations was tested using a one-sided Fisher's exact test under the null hypothesis that those two factors were independent for each location (Table 3). An association between cases and a concentration was observed at most monitoring sites (i.e., $p<0.05$ at RH1, NE, or SO) for samples collected before (-1 week) and the same week (+0 week) using either N1 or N2 as an indicator. As expected, given the mandatory exclusion of confirmed cases from campus following the diagnosis, samples collected the week following (+1 week) did not associate.

3.5. Wastewater-measured SARS-CoV-2 enabled estimation of COVID-19 cases per capita across UofC campus

COVID-19 cases per capita for the entire UofC monitored catchments comprising NW, NE, and SO during the entire monitoring period (both -A and -B) were estimated according to (Eq. (1)) using raw concentrations, and also (Eq. (2)) using normalized concentrations. SARS-CoV-2 N2 data was used in this analysis due to its stronger association with clinically confirmed cases (see 3.2, also Table S2). Following this, the modelled aggregate SARS-CoV-2 burden for UofC was compared with the values for the surrounding community (i.e., WWTP catchment) (Fig. 4). For most time points, cases per capita in the community (as measured at WWTP) were significantly higher than for UofC ($p<0.001$). The results using different methods of potential normalization generally mirrored the raw concentrations (Table 4).

Overall, median predicted incident cases per capita (cases per 100,000 scaling factor) for UofC was 5.9-fold lower than for WWTP using raw concentration ($p<0.001$), and 3.5–4.8-fold lower than for WWTP using normalized concentrations (≤0.001). For instance, the median cases per 100,000 was 8.8 (IQR 6.9-14.8) for the WWTP catchment, and predicted to be 1.5 (IQR 0.5-2.7) for the entire UofC monitoring catchment using raw SARS-CoV-2 RNA concentration. The median values of cases per capita per 100,000 for UofC when assessed using normalized concentrations were 1.8 (IQR 0.7-3.3) for PMMoV, and 2.6 (IQR 0.9-4.7) for potassium.

4. Discussion

4.1. A nodal-based sampling approach reveals 'hotspots' for COVID-19 cases within the campus

This study demonstrated that WBS using spatially resolved node-

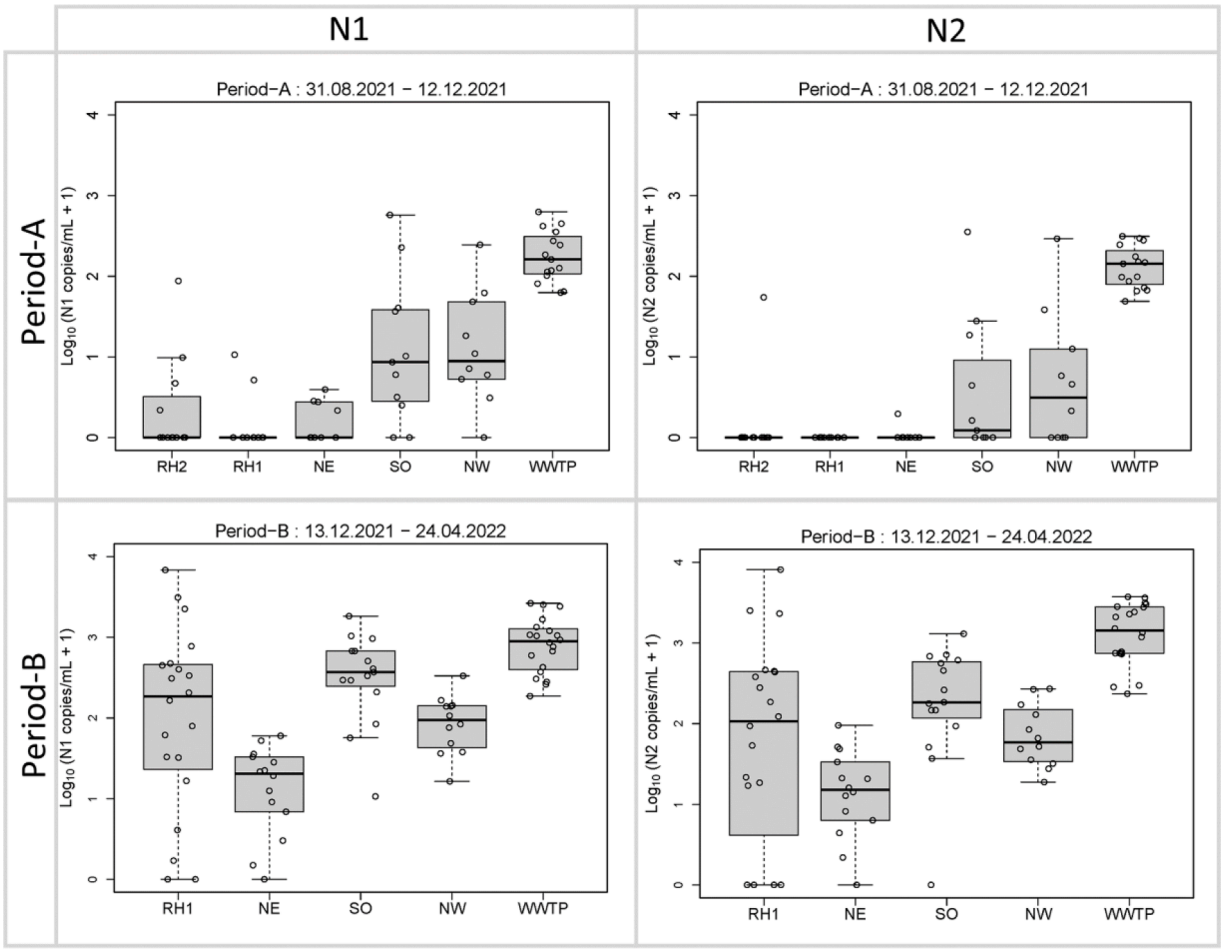

Fig. 3. Log_{10}-transformed concentrations of SARS-CoV-2 N1 (left) and N2 (right) by sub-catchment monitoring location variably demonstrate differences between locations during Period-A (Aug 31, 2021-X) and -B (Y-April 30, 2022) with (upper), and without normalization (lower). See Table 2 for the results from Wilcoxon rank-sum tests.

Table 3
The Fisher's exact test results (p-value) for testing interdependency between wastewater SARS-CoV-2 signals and COVID-19 confirmed cases across UofC campus under each assumption. '-1 week', '+0 week', and '+1 week' indicate an early warning, no time lag, and time lag scenario, respectively (see Fig. S1 in the Supporting Information for details). The results with $p < 0.05$ were highlighted in red.

Location	N1			N2		
	(-1 week)	(+0 week)	(+1 week)	(-1 week)	(+0 week)	(+1 week)
RH1	0.001	0.000	1.000	0.001	0.000	1.000
RH2	0.273	0.333	0.333	1.000	1.000	0.083
NE	0.029	0.008	0.154	0.029	0.071	0.433
SO	0.021	0.205	0.163	0.002	0.048	0.163
NW	0.099	0.193	0.063	0.099	0.193	0.063

based sampling approach enables SARS-CoV-2 activity to be located and quantified across a large University campus on a granular scale. Such an approach has previously been proven effective in cities at a neighborhood scale (Acosta et al., 2022b). One of the challenges for granular scale monitoring is identifying sampling nodes that adequately cover most of the targeted community. This requires a careful analysis of geographic information of involved sewersheds and assessing the connectivity between sampling nodes so that the sub-catchments (for each node) can be collected comprehensively. Other studies have explored SARS-CoV-2 WBS in individual buildings across university campuses. However, those studies either targeted residences and dormitories (Ash et al., 2023; Corchis-Scott et al., 2023; Gibas et al., 2021) or studied selected residential and non-residential buildings (Johnson et al., 2022; Karthikeyan et al., 2021; Reeves et al., 2021). A comprehensive longitudinal assessment of a campus community or large work facility has not previously been performed. Our approach is unique relative to other studies because our monitoring catchments cover the vast majority of buildings (>83%) on a 530-acres campus through the deployment of a modest number of sample collection devices. This was approach was made possible owing to a detailed GIS guided assessment of sampling

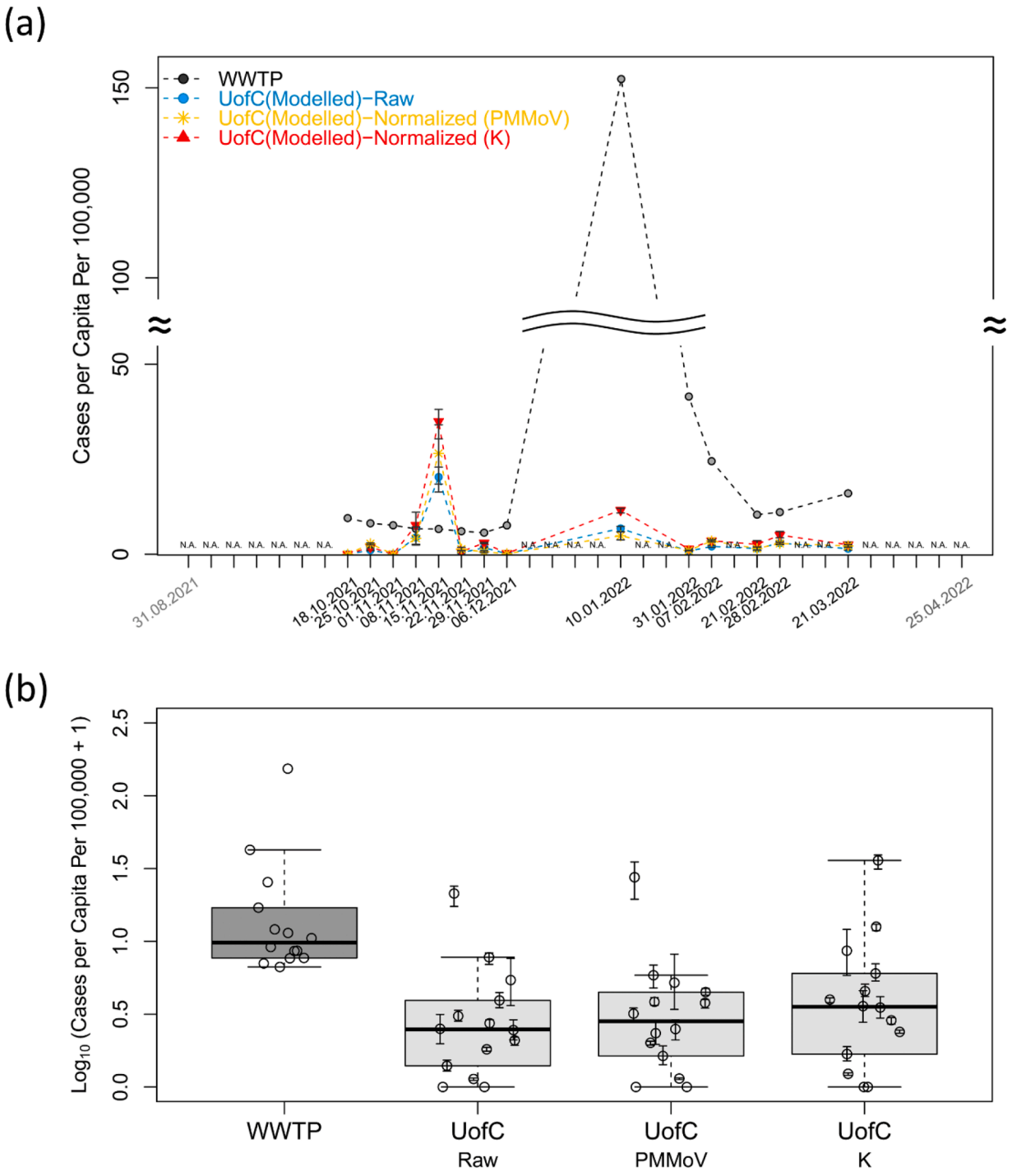

Fig. 4. Log_{10}-transformed cases per capita estimated for UofC according to Eq. (1) (for raw-concentration) or Eq. (2) (for normalized-concentrations using PMMoV and potassium), and measured for the surrounding community (i.e., WWTP) during the entire monitoring period. Cases per capita was calculated for each data point (i.e., time point), and displayed using trend lines (linearly extrapolated between two data points) over time in (a), and using box plots after aggregated by group in (b). Only paired data points were shown, and statistically compared between each other using Permutation test. Error bars for the modelled (UofC's) values indicate IQR (Q1-Q3) derived from uncertainty analysis (see 2.7).

nodes through the sewershed (Section 2.1).

Comparing the concentration of SARS-CoV-2 RNA in wastewater across different sampling locations, makes it possible to locate the catchment(s) where infected individuals were disproportionally located. Under the rationale suggested in the Supplementary-Method 1.4, concentration is proportional to cases per capita, we have confirmed that WBS is a predictor for 'hotspot' where excessive disease occurrence exists. For example, Fig. S4 indicates the absolute values of confirmed cases per day was much lower for RH1 and 2 than for other larger catchments (NE, NW, and SO). This was mainly due to the fact that the number of people associated with the area was higher for the larger catchments than for RH1 and 2, but not because disease occurrences were disproportionately lower for RH1 and 2. Our concentration-based analysis predicts that SO and NW portions of the campus consistently showed high levels of SARS-CoV-2 using raw and normalized data, demonstrating hotspots for COVID-19 occurrences per capita. For

Table 4
The p-values for statistical difference in modelled COVID-19 cases per capita between WWTP and UofC campus using raw and normalized values. The p-values were calculated using Permutation test (see 2.7), and indicate the ratio of counts where cases per capita for UofC > cases per capita for WWTP to 1,000 (=the number of total simulation runs). Those pairs that statistically differed with ($p < 0.05$) are highlighted in red.

Date	Raw	Normalized	
		PMMoV	Potassium
18.10.2021	<0.001	0.003	0.003
25.10.2021	<0.001	0.003	0.003
01.11.2021	<0.001	0.003	0.003
08.11.2021	0.247	0.278	0.578
15.11.2021	0.981	0.972	0.999
22.11.2021	<0.001	0.003	0.003
29.11.2021	<0.001	0.003	0.003
06.12.2021	<0.001	0.003	0.003
10.01.2022	<0.001	0.003	0.003
31.01.2022	<0.001	0.003	0.003
07.02.2022	<0.001	0.003	0.003
21.02.2022	<0.001	0.003	0.003
28.02.2022	<0.001	0.003	0.003
21.03.2022	<0.001	0.003	0.003

example, RH1, a dormitory, could be one of the buildings where highest disease incidence occurs and disproportionately contribute to a high level of SARS-CoV-2 signals for NW, at least during Period-B (see Fig. 3). This is consistent with other reports demonstrating high secondary COVID-19 case occurrence in dormitories (Gibas et al., 2021; Johnson et al., 2022; Reeves et al., 2021). The catchment SO includes three additional residence halls (KA, AU, and RU; see pink sections of Fig. 1), and these may be a reason why SO demonstrated particularly high levels of SARS-CoV-2 signals, especially during Period-B (Fig. 3). Unlike NW and SO, the catchment area for NE does not include any such buildings, rather is predominately comprised of lecture halls and administration offices – this might be one of the reasons why the level of SARS-CoV-2 concentration for NE was low relative to SO or NW. The ability to discern the building(s) with the highest number of cases per capita however, is unknowable in this study. WBS at even more granular scales (i.e., building level) could be followed for those specific building types within catchments of interests, for instance NW and SO in this study, during disease outbreaks, although this would significantly increase the effort and cost of active monitoring by introducing many more nodes.

Under the rationale suggested in Eq. (S5) in the Supplementary-Method 1.4, normalization may help to denoise variability of human wastes over time across samples. Normalization has been hypothesized to correct the variability of fecal burden allowing for more appropriate comparisons. However, in our data, raw concentration correlated better with clinical cases using longitudinal WWTP data (Table S2). Whether raw concentration manifests better case correlation than measures normalized by various biomarkers remains controversial. While the bulk of studies conducted at the level of WWTP have found that human-specific surrogates did not necessarily improve correlation between confirmed cases and normalized SARS-CoV-2 signals (Ai et al., 2021; Duvallet et al., 2022; Feng et al., 2021; Maal-Bared et al., 2023), some other studies found that normalization using fecal markers improved correlations at specific sites (D'Aoust et al., 2021; Zhan et al., 2022). This implies that whether normalization improves the correlation between wastewater data and clinical cases depends on site, thus site-specific longitudinal assessments should take precedence.

For cross-site comparisons on a granular scale, there is still a possibility that target analyte abundance could be underestimated in catchment(s) where a high volume of water use relative to individuals is expected (e.g., non-residential buildings). For this reason, we assessed PMMoV-normalized concentration as a secondary outcome measure when comparing SARS-CoV-2 RNA concentrations across different locations (representing a range of scales). For relatively high longitudinal variability for PMMoV (see relatively high spread for PMMoV compared to other markers in Fig. S4), potassium was used as additional confirmatory surrogate. Lower concentrations for those surrogates in a sample indicate lower proportion of human waste relative to tap water, thus higher dilution. Tap water consumption might vary by type of building (Abdelalim et al., 2015; Almeida et al., 2021) – higher dilution of human excreta is expected in the buildings where non-toilet based consumption (e.g., cleaning, research activities, air conditioning, etc) is high. For instance, concentrations of those markers were the lowest for NE which predominately represented research/lecture buildings (Fig. S4). Normalization using human excreta markers could, in theory, compensate for potential overestimation of SARS-CoV-2 raw concentration in NE.

4.2. Campus-wide WBS is positively associated with confirmed COVID-19 case occurrence demonstrating its potential for disease monitoring

A positive association between COVID-19 cases and wastewater signals in the majority of instances (see 3.4 and Table 3) indicates that WBS has the potential for passive disease monitoring at a granular scale. This positive association has previously been reported in other targeted, granular scale monitoring programs. For instance, a positive correlation between wastewater SARS-CoV-2 levels and confirmed cases was observed in hospitals (Acosta et al., 2022a; Peng et al., 2023; Schenk et al., 2023) and university dormitories (Ash et al., 2023), and also larger building complexes (Wright et al., 2022). However, adapting WBS as an early warning for COVID-19 cases on a more granular scale may not provide the same lead time relative to clinical diagnoses as was observed early in the pandemic now that testing capacity has markedly increased. Indeed, the early warning scenario (-1 week) did not lead to lower p-values relative to the no time-lag scenario when comparing confirmed COVID-19 cases and wastewater SARS-CoV-2 in this campus monitoring program. A similar observation was reported in another

study where node-based sampling strategies were applied for monitoring different neighborhoods at a range of scales (from 853 to 9,094 serving populations) in Illinois, USA (Oh et al., 2022). The authors reported that a correlation between wastewater signals and confirmed cases varied significantly by neighborhood, and an early warning scenario (-1 week) did not necessarily result in a better correlation (Oh et al., 2022).

Similar to other studies correlating wastewater measured SARS-CoV-2 with COVID-19 disease occurrence was our reliance on clinically confirmed cases to build models. Individuals with asymptomatic and pauci-symptomatic disease are thusly not captured in this syndromic surveillance-driven manner (Subramanian et al., 2021; Wu et al., 2020b). As university campuses generally comprise a younger cohort relative to the general population, the number of asymptomatic infections is expected to have been higher (Poletti et al., 2021). Furthermore, as case reporting to University staff was voluntary, it is possible that not all confirmed cases were properly reported. As data was collected by university staff with the primary intent of actionability, cases with missing data (i.e., those with inaccurate dates and details on campus associated movements) were not necessarily followed up on resulting in an incomplete dataset. Finally, the analysis of both wastewater samples and the corresponding campus-confirmed clinical cases were confounded by the use of weekly-aggregate data comparisons. As daily reported cases were discontinuous, and at times relatively low (median=1 and IQR=0-3 for NW; median=0 and IQR=0-1 for NE and SO; med=0 and IQR=0-0 for RH1 and 2), the paired comparison between wastewater signals and reported cases was difficult for campus sites, which is why comparisons were made using weekly-aggregate signals. Daily comparisons would allow for a more accurate analysis of the potential lead time generated through granular WBS, however, such an approach would also create considerable operational and cost challenges.

4.3. SARS-CoV-2 activity across University campus was lower than the surrounding community

Our results in Figs. 3 and 4 demonstrated a much lower viral burden in wastewater across the campus relative to the surrounding community. The relatively low SARS-CoV-2 burden within UofC campus wastewater likely relates to strict COVID-19 mitigating measures mandated within the campus – well beyond that of the Province's at the time. As an example, the university mandated proof of vaccination (2 doses; or documented weekly-negative testing) in order to attend campus. As a result, the rate of vaccination for University staffs/students reached 91.3% at the beginning of our monitoring on September 2021, which vastly exceeded that for the general population (73.7%) (CHI, 2023; UofC, 2021). The 'COVIDSafe Campus' run by the university during the pandemic (UofC, 2022a) included additional efforts such as an enforced universal masking mandate and wide availability of hand hygiene products and extra medical face masks, and a consistent effort for increasing public awareness of COVID-19 and the importance of physical distancing. Furthermore, our study confirms that new and emerging SARS-CoV-2 VOC appeared on campus commensurate with that in the community, and at no time was spread within the campus identified before the community.

Recent studies have suggested that similar COVID-19 mitigating strategies employed at other university campuses have likewise been effective and that universities were not a large source of disease propagation. For instance, a SARS-CoV-2 phylogenetic study performed at the University of Michigan, USA, revealed that the descendants of SARS-CoV-2 from student cases were rarely found in the community during the next wave (Valesano et al., 2021). In another study performed at the University of Cambridge, UK, the authors revealed that the majority of SARS-CoV-2 genomes from students originated from a single genetic cluster – the cases occurred after a single event (e.g., social gathering outside the campus), suggesting a limited introduction of the virus into the community (Aggarwal et al., 2022). Likewise, we did not observe new VOC occur earlier within the campus than the general community. Collectively these studies suggest that the intensive efforts to reduce forward transmission of COVID-19 adopted in a large work sites could be applied to other contexts to mitigate further disease spread in those environments.

4.4. Other notable limitations

There are several other noteworthy limitations of this study. For instance, toileting patterns do vary considerably across space and time. In particular, there have been reports documenting that many individuals prefer to defecate at home (Heaton et al., 1992), and these active cases would therefore be underrepresented in work-based studies. Accordingly, work (or school)-based studies such as those monitoring campuses may underestimate the true burden of infected populations within. We attempted to mitigate for this factor by assessing SARS-CoV-2 RNA concentration both raw, and normalized against fecal and population surrogates, where we observed the same general trends.

Furthermore, the monitoring in this study was performed when not all students and employees had fully returned to in-person learning/work; a small number continued to telecommute from home and, therefore, may not fully represent the entire university community. Thus, care has to be taken when interpreting our results – the results in this study do not indicate for instance that university members tend to have lower infection rates then populations outside the campus, but rather suggest that university campus is not the place where high cases per capita exist, or diseases were contained relatively well 'within the campus'. Finally, wastewater-based monitoring at a granular scale may not fully represent actual case burden within the catchment because individual confounding differences may have a larger effect relative to community monitoring. Viral shedding may vary by individual (Ke et al., 2022) and the chances of capturing shedding events when autosamplers were being operated are highly stochastic, etc. We attempted to address this issue by employing 24 h composite sampling over a "grab" sampling strategy, and by achieving a reasonably high sample size (i.e., up to 35 points for weekly aggregated signals from 89 individual data points) followed by various statistics (e.g., non-parametric tests such as Kruskal-Wallis, Wilcoxon tests). In this way, our wastewater results could provide an "approximate" to the case per capita existing in each monitoring catchment.

5. Conclusion

Uniquely we performed a comprehensive assessment of SARS-CoV-2 (and VOC) burden across a large university campus using a spatially resolved, nodal based strategy. We have confirmed the potential of this platform technology to perform population health monitoring through wastewater analysis. This study has established wastewater-based surveillance is positively associated with clinical cases at a granular scale, suggesting it can be used synergistically with contact tracing in order to identify 'hotspots' for COVID-19 occurrence across campus (i.e., building). This study also confirmed the markedly lower rates of SARS-CoV-2 across campus, lending support to the importance of restrictive measures in mitigating COVID-19's potential for spread across worksites.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.watres.2023.120469.

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