

Ambient Aeroallergens and Respiratory Virus Burden in Puerto Rico: Spore-Specific Associations with Influenza and SARS-CoV-2 Incidence, 2022–2024

Félix E. Rivera-Mariani

friveramariani@lynn.edu

Lynn University

Catalina S. Robles-Rivera

University of Puerto Rico

Cláudia I. Pereira Moreira

University of Puerto Rico

Benjamín Bolaños-Rosero

University of Puerto Rico

Research Article

Keywords: Aerobiology, Fungal spores, Bioaerosols, Influenza, SARS-CoV-2, Respiratory viruses

Posted Date: October 7th, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-11106324/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Additional Declarations: No competing interests reported.

Abstract

Background: Environmental factors influence respiratory virus transmission, but the role of outdoor fungal spores in tropical environments remains poorly characterized. We examined associations between fungal spore concentrations and influenza and SARS-CoV-2 incidence in Puerto Rico, and conducted exploratory virus-specific mortality analyses.

Methods: We conducted a retrospective ecological time-series study in two Puerto Rican health regions (San Juan/Metro and Caguas) from 2022 to 2024. Daily fungal spore and pollen concentrations from two National Allergy Bureau stations were linked to laboratory-confirmed case counts. Analyses included seasonal correlations, logistic regression of daily high-incidence status, random forest classification, and exploratory region-specific negative binomial mortality models, all adjusted for season.

Results: Fungal spores correlated with influenza and SARS-CoV-2 incidence in fall across all four region–virus combinations ($r = 0.19–0.35$, $p < 0.001$). Influenza associations strengthened at 2–4-day lags, whereas SARS-CoV-2 associations peaked at or near lag 0. Higher fungal, but not pollen, concentrations were associated with increased odds of top-quartile incidence days in every region–virus combination (odds ratio 1.12–1.34 per 10,000 spores/m³, $p < 0.001$). Random forest classifiers using fungal spores alone showed high apparent discrimination in randomly split fall data. In exploratory mortality analyses, only influenza mortality in Caguas was conventionally associated with fungal concentration (incidence rate ratio 1.17, 95% confidence interval 1.03–1.33, $p = 0.016$); it did not survive Bonferroni correction, and the remaining models were null.

Conclusions: Outdoor fungal spores showed modest associations with the incidence of both viruses in both regions during 2022–2024. Future studies will integrate meteorological variables and prospectively validate these associations.

1. Introduction

Influenza and SARS-CoV-2 remain leading causes of respiratory morbidity and mortality worldwide (Iuliano et al., 2018). Respiratory viral activity varies across regions, particularly between tropical and temperate climates (Chkonia et al., 2026; García-Arroyo et al., 2022). Although transmission primarily occurs through person-to-person spread, growing evidence suggests that environmental factors contribute to temporal and geographic variation in respiratory viral activity (Moriyama et al., 2020; Shaman & Kohn, 2009). Identifying environmental exposures associated with short-term surges in influenza and SARS-CoV-2 incidence can inform respiratory surveillance studies.

Fungal spores and pollen are among the most abundant bioaerosols in the environment and well-established triggers of airway inflammation, allergic sensitization, and asthma exacerbations, particularly in tropical settings (Lewis et al., 2020; Quintero et al., 2010; Rivera-Mariani et al., 2020; Rivera-Mariani & Bolaños-Rosero, 2012). In tropical and subtropical settings, fungal spore concentrations can remain elevated year-round due to persistently warm, humid conditions, resulting in near-continuous

exposure for susceptible populations (Quintero et al., 2010; Rivera-Mariani et al., 2020). Beyond their role in allergic diseases, emerging evidence suggests that bioaerosol exposure may modulate antiviral innate immunity. For instance, pollen has been shown to suppress airway interferon responses, potentially compromising antiviral defenses (Gilles et al., 2020). Fungal cell wall components, particularly β -glucans, can activate Dectin-1 signaling and promote Th17-skewed immune responses, providing a biologically plausible pathway by which fungal exposure could influence antiviral host responses. Therefore, further studies evaluating fungal aeroallergens as risk factors for respiratory viruses are warranted.

Puerto Rico offers a valuable natural setting for examining the relationships between fungal aeroallergens and the incidence of respiratory viruses. The island's tropical climate, high humidity, and forested landscape sustain some of the highest year-round outdoor fungal spore concentrations, while the island also has a well-documented high burden of asthma and respiratory disease (Lewis et al., 2020; Quintero et al., 2010; Rivera-Mariani et al., 2020; Rivera-Mariani & Bolaños-Rosero, 2012). The island also benefits from National Allergy Bureau-certified aerobiological monitoring stations operating continuously in San Juan/Metro and Caguas (Rivera-Mariani et al., 2026). To our knowledge, no prior study has simultaneously modeled fungal and pollen exposures associated with both the incidence and mortality of multiple respiratory viruses. Whether ambient fungal-spore concentrations are associated with short-term changes in respiratory-virus incidence, and whether they provide information beyond shared seasonal, temporal, and meteorological structure, remains unknown.

Specifically, it was predicted that fungal spores would show stronger associations with daily high-incidence status than pollen, given their predominant presence in Puerto Rico's atmosphere (Quintero et al., 2010; Rivera-Mariani et al., 2020; Rivera-Mariani & Bolaños-Rosero, 2012). Pollen was included as an internal comparator exposure, not as a definitive control for all sources of temporal confounding.

It was hypothesized that ambient fungal spores would show modest short-term associations with daily respiratory virus incidence and would statistically discriminate high-incidence days in retrospective classification analyses. The analysis further evaluated whether these associations were stronger for fungal spores than for pollen and for a concurrently measured comparator aeroallergen, by season.

2. Methods

2.1 Study design and setting

A retrospective ecological time-series study was conducted in two health regions of Puerto Rico (San Juan/Metro and Caguas) from January 1, 2022, through December 31, 2024. The 2022–2024 period encompassed post-acute-phase SARS-CoV-2 circulation alongside seasonal influenza, enabling a direct comparison of fungal spore associations with two enveloped respiratory viruses that differ in transmission dynamics during continuous outdoor aerobiological monitoring. The two regions were selected for their contrasting respiratory disease burdens and for the presence of an established AAAAI/NAB-certified spore-trap network (Lewis et al., 2020).

2.2 Data sources

Aeroallergen data. Daily outdoor concentrations of fungal spores and pollen were measured using Burkard 7-day recording volumetric samplers at National Allergy Bureau-certified stations in San Juan and Caguas, representing the San Juan/Metro and Caguas health regions, respectively. Spores and pollen were identified and enumerated according to National Allergy Bureau (NAB) protocols and reported as spores/m³ and grains/m³, respectively (Anderson et al., 2020).

Hereafter, San Juan/Metro refers to the San Juan/Metro health region represented by the San Juan monitoring station.

Epidemiologic data. Daily counts of laboratory-confirmed cases of influenza and SARS-CoV-2, and deaths, disaggregated by health region, were obtained from the Puerto Rico Department of Health for 2022–2024.

2.3 Variable construction

Temporal unit. Incidence analyses were conducted at the daily level using daily counts of laboratory-confirmed cases. Mortality analyses were conducted at the epidemiological-week level using weekly death counts to stabilize sparse daily mortality data.

Exposure. The region-matched daily fungal spore concentration served as the primary exposure. Total pollen concentration was modeled in the same way as an internal comparator exposure to assess whether associations were shared across major aeroallergen classes.

Outcomes. For incidence analyses, the primary outcome was a binary high-incidence-day indicator, defined as a day with a daily case count in the top quartile (≥ 75 th percentile) of the region- and virus-specific daily case distribution. This threshold was set a priori to identify high-burden days relevant to public health surveillance and potential resource allocation (Zhong et al., 2026). For exploratory mortality analyses, the outcomes were weekly counts of influenza and SARS-CoV-2 deaths, aggregated to the epidemiological-week level to reduce sparsity in daily mortality data (Zimmer et al., 2018).

Season and lag. The calendar season was defined as winter, spring, summer, or fall. Lag structures of 0–7 days were examined for daily incidence outcomes, and 1–2-week lags were examined for weekly mortality outcomes. These lag structures were selected to span typical viral incubation periods for daily incidence and to align with the weekly aggregation necessitated by sparse daily mortality counts (Simpson et al., 2019; van Asten et al., 2018).

2.4 Statistical analysis

All statistical analyses were performed using R version 4.6.0 (R Foundation for Statistical Computing, Vienna, Austria). Season-specific Pearson correlations were computed between fungal (and, separately, pollen) concentrations and daily case counts, and cross-correlations were evaluated across 0–7-day lags, chosen to span the typical incubation periods of influenza (1–4 days) and SARS-CoV-2 (2–14 days, median 5 days). Logistic regression was used to model the odds that a given day was a high-incidence day, defined as a daily case count in the top quartile of the region- and virus-specific daily case distribution, as a function of fungal and pollen concentrations. To assess the statistical discrimination of daily high-incidence status, random forest classifiers were initially trained and evaluated on fall observations using ROC AUC, accuracy, precision, recall, and F1. Because random splits of temporally ordered daily observations can overestimate performance by allowing adjacent, highly correlated days to appear in both training and test sets, temporally blocked validation was considered a more relevant estimate of prospective-style predictive performance, although it remained retrospective rather than truly prospective. Exploratory weekly mortality analyses used negative binomial regression to account for overdispersion, with fungal concentration as the exposure. Models were fit separately within each health region and adjusted for season; effects were reported as incidence rate ratios (IRRs). Because models were fit separately by region, health region was excluded as a covariate within the region-specific models. Fungal mortality associations were examined at 1–2-week lags using lagged correlations and distributed lag models (Gasparrini & Leone, 2014; Milla, 2026). Each model was re-estimated by substituting pollen for fungal spores to assess whether associations were stronger for fungal spores than for another concurrently measured aeroallergen class. Due to data availability, the models were not adjusted for meteorological (temperature, humidity, UV) or individual-level covariates (age, vaccination status, comorbidities), which may confound the observed associations. These limitations and their consequences for causal inference are elaborated in the discussion section.

Because additional temporal, meteorological, and autocorrelation-aware models were unavailable for all analyses, the primary incidence findings were interpreted as ecological associations rather than as independent or causal effects of fungal spores.

2.5 Sensitivity analyses

Several sensitivity analyses were conducted to assess the robustness of the mortality and incidence findings, including Poisson regression models, exclusion of extreme exposures, alternative high-incidence thresholds, temporally blocked validation, seasonal stratification, residualized correlations, and autoregressive models. Detailed descriptions of these analyses and their results are provided in Online Resource 1.

3. Results

3.1 Aeroallergen and surveillance overview

Fungal spores were abundant year-round in both regions, with no day recording zero spores at either station (San Juan: minimum 831 spores/m³; Caguas: minimum 6,156 spores/m³). Concentrations were consistently higher in Caguas than in San Juan/Metro, with a median of 67,866 spores/m³ (IQR 47,476–101,939) versus 25,146 spores/m³ (IQR 14,082–41,367), a 2.7-fold difference in medians (Fig. 1A). Both stations showed recurrent autumn peaks: mean fall (September–November) concentrations exceeded non-fall means by 63% in San Juan/Metro (41,077 vs. 25,191 spores/m³) and by 38% in Caguas (95,247 vs. 69,256 spores/m³). This pattern recurred in each of the three sampled years.

Pollen concentrations were approximately two orders of magnitude lower than fungal spore concentrations, with median daily values of 62 grains/m³ (IQR 31–154) in San Juan/Metro and 123 grains/m³ (IQR 62–215) in Caguas, corresponding to spore-to-pollen ratios of approximately 273:1 and 552:1, respectively (Fig. 1B). Pollen was also markedly more episodic: no pollen was detected on 17.8% of sampled days in San Juan/Metro and 5.7% in Caguas, whereas fungal spores were detected on every sampled day. Day-to-day variability was correspondingly greater for pollen (coefficients of variation: San Juan/Metro = 1.21, Caguas = 0.83) than for fungal spores (San Juan/Metro = 0.70, Caguas = 0.50), and the highest 5% of days accounted for 22.8% of the total San Juan/Metro pollen load, compared with 14.3% of the spore load. Pollen peaks were short-lived and irregular, reaching 1,540 grains/m³ in San Juan/Metro and 1,077 grains/m³ in Caguas, with no consistent annual patterns.

The influenza and SARS-CoV-2 case series displayed distinct temporal patterns (Fig. 1C). Influenza (113,848 cases; 65,380 in Caguas and 48,468 in San Juan/Metro) followed a pronounced autumn–winter seasonal pattern, with 42.6% of cases in the fall and 25.7% in the winter. Epidemic peaks occurred in late October–November 2022 and 2023, with the largest weekly counts in the weeks beginning November 14, 2022 (Caguas, 1,996 cases) and October 31, 2022 (San Juan/Metro, 1,574 cases), and a second comparable peak in the week beginning November 6, 2023, in both regions. SARS-CoV-2 (741,030 cases; 386,729 in San Juan/Metro and 354,301 in Caguas) followed a wave-driven rather than strictly seasonal course, with the largest surge in the week beginning January 3, 2022 (San Juan/Metro, 16,706 cases; Caguas, 12,890 cases), a secondary surge in late April 2022, and progressively attenuated waves thereafter; the highest seasonal share of SARS-CoV-2 cases occurred in summer (35.8%). The gaps in the aeroallergen series in Fig. 1A and B reflect periods of interrupted sampling and were left unimputed.

3.2 Fungal-spore associations with daily respiratory-virus incidence

Fungal spore concentrations showed varying associations with respiratory virus incidence throughout the year. As shown in the Pearson correlation heatmap (Fig. 2), positive correlations with fungal spores were most consistent and pronounced in the fall in both San Juan/Metro ($r = 0.32$ for influenza; $r = 0.31$ for SARS-CoV-2; $p < 0.05$) and Caguas ($r = 0.36$ for influenza; $r = 0.42$ for SARS-CoV-2; $p < 0.05$). In other seasons, patterns varied, including negative associations during summer for influenza in both regions. As shown in the coefficient of determination heatmap (Fig. 3), fungal spore concentrations accounted

for approximately 4% to 18% of the variance in daily case counts ($r^2 = 0.036\text{--}0.176$) across the region-virus combinations, indicating a detectable environmental signal rather than a dominant determinant of incidence.

Pollen showed no consistent associations across seasons or regions, with correlations near zero and non-significant across all four region-virus combinations for both concurrent and lagged exposures within the same period. Because fungal spore and pollen concentrations were measured on the same days at the same stations using identical methods, the divergence between the two exposures argues against a completely generic aeroallergen explanation. However, this comparison does not account for residual confounding because pollen and fungal spores differ substantially in abundance, temporal patterns, and meteorological determinants (Quintero et al., 2010).

A consistent regional gradient was also observed. For both viruses, correlations were stronger in Caguas than in San Juan/Metro (influenza $r = 0.30$ vs. 0.19 ; SARS-CoV-2 $r = 0.35$ vs. 0.30), mirroring the regional difference in exposure, with median daily fungal concentrations approximately 2.7-fold higher in Caguas than in San Juan/Metro. This parallelism between exposure magnitude and association strength is consistent with, though not confirmatory of, an exposure-dependent relationship.

The two viruses differed in their lag patterns (Fig. 4). For influenza, correlations strengthened when fungal exposure was lagged by 2 to 4 days relative to case counts in both San Juan/Metro ($r = 0.19$ at lag 0 to $r = 0.22$) and Caguas ($r = 0.30$ to $r = 0.35$; $p < 0.001$ for both). For SARS-CoV-2, correlations at a 4-day lag were not stronger than the concurrent (lag 0) associations, remaining unchanged in San Juan/Metro ($r = 0.30$) and attenuating in Caguas ($r = 0.35$ to $r = 0.22$, $p = 0.006$). Short-term lagged strengthening was therefore evident for influenza but not for SARS-CoV-2, for which the association was maximal at lag 0 or near it.

3.3 Logistic regression of daily high-incidence days

Logistic regression showed that fungal concentration was statistically associated with daily high-incidence status, defined as days in the top quartile of the region- and virus-specific daily case distribution, for both influenza and SARS-CoV-2 in both regions ($p < 0.001$ for all four models). In contrast, pollen was not a significant predictor in any model ($p = 0.119\text{--}0.320$). Because the exposure was modeled on a per-spore/ m^3 basis, the fitted coefficients were necessarily small ($\beta = 1.1 \times 10^{-5}$ to 2.9×10^{-5}); when rescaled to a 10,000 spores/ m^3 increment, these corresponded to odds ratios of 1.12 to 1.34 for a high-incidence day (Table 1). The strongest association was observed for SARS-CoV-2 in San Juan/Metro (OR 1.34 per 10,000 spores/ m^3), followed by SARS-CoV-2 in Caguas (OR 1.19), influenza in San Juan/Metro (OR 1.16), and influenza in Caguas (OR 1.12). Pollen coefficients were an order of magnitude smaller, inconsistent in direction across models (positive in San Juan/Metro, negative in Caguas), and non-significant throughout, consistent with the absence of a systematic pollen-incidence association in the present study. These null pollen findings provide a useful contrast but should not be interpreted as eliminating unmeasured temporal or meteorological confounding.

Table 1. Logistic regression of top-quartile (high-incidence) days on aeroallergen concentration by region and virus

Region	Outcome	Exposure	Coefficient (β)	OR per 10,000 units	p-value
San Juan/Metro	Influenza	Fungi	1.5×10^{-5}	1.16	< 0.001
San Juan/Metro	Influenza	Pollen	3.0×10^{-6}	1.03	0.241
San Juan/Metro	SARS-CoV-2	Fungi	2.9×10^{-5}	1.34	< 0.001
San Juan/Metro	SARS-CoV-2	Pollen	1.0×10^{-6}	1.01	0.320
Caguas	Influenza	Fungi	1.1×10^{-5}	1.12	< 0.001
Caguas	Influenza	Pollen	-2.0×10^{-6}	0.98	0.119
Caguas	SARS-CoV-2	Fungi	1.7×10^{-5}	1.19	< 0.001
Caguas	SARS-CoV-2	Pollen	-3.0×10^{-6}	0.97	0.209

High-incidence days are daily observations with case counts in the top quartile (≥ 75 th percentile) of the region- and virus-specific daily case distribution. This binary outcome was used only for daily incidence analyses; mortality analyses used weekly death counts. The coefficients are on the log-odds scale per unit of exposure (spores/ m^3 or grains/ m^3). OR, odds ratio; OR per 10,000 units = $\exp(\beta \times 10,000)$, reported for interpretability. Pollen was included as a concurrently measured comparator aeroallergen. Null pollen associations do not rule out residual confounding by meteorology, seasonality, or secular temporal trends. San Juan/Metro refers to the San Juan/Metro health region represented by the San Juan aerobiological monitoring station.

Random forest classifiers were trained to discriminate top-quartile incidence days in the fall. Models using fungal spores alone showed high apparent discrimination across all region-virus combinations, with ROC AUCs ranging from 0.85 to 0.92 (Table 2). Models incorporating both fungal spores and pollen achieved the highest overall performance (ROC AUC 0.87–0.95), indicating incremental predictive utility from combining aeroallergen features. Conversely, models relying solely on pollen performed substantially worse, with ROC AUCs (0.61–0.66) near chance levels, further underscoring the specific association with fungal spore concentrations.

Table 2. Random-split random forest discrimination of daily top-quartile incidence status during fall, by region, virus, and feature set

Region	Outcome	Feature set	ROC AUC	Accuracy	Precision	Recall	F1
San Juan/Metro	Influenza	Fungi + Pollen	0.95	0.93	0.93	0.85	0.89
San Juan/Metro	Influenza	Fungi	0.91	0.90	0.89	0.83	0.86
San Juan/Metro	Influenza	Pollen	0.65	0.63	0.60	0.58	0.59
San Juan/Metro	SARS-CoV-2	Fungi + Pollen	0.87	0.87	0.84	0.76	0.79
San Juan/Metro	SARS-CoV-2	Fungi	0.85	0.84	0.83	0.75	0.79
San Juan/Metro	SARS-CoV-2	Pollen	0.63	0.60	0.58	0.57	0.57
Caguas	Influenza	Fungi + Pollen	0.95	0.93	0.92	0.85	0.89
Caguas	Influenza	Fungi	0.92	0.91	0.90	0.84	0.87
Caguas	Influenza	Pollen	0.66	0.64	0.61	0.59	0.60
Caguas	SARS-CoV-2	Fungi + Pollen	0.89	0.87	0.83	0.76	0.79
Caguas	SARS-CoV-2	Fungi	0.88	0.86	0.84	0.78	0.81
Caguas	SARS-CoV-2	Pollen	0.61	0.59	0.56	0.55	0.55

Classifiers were trained on 70% of the fall observations (randomly sampled) and evaluated on the remaining 30%. ROC AUC is the area under the receiver operating characteristic curve. Pollen-only models exceeded chance discrimination (AUC 0.50) but performed substantially worse than all fungus-containing models. San Juan/Metro refers to the San Juan/Metro health region represented by the San Juan aerobiological monitoring station.

3.4 Mortality analyses

Mortality analyses were exploratory because weekly death counts were sparse. In these analyses, Caguas was the only region–virus combination in which fungal concentration was conventionally associated with weekly mortality. The association was observed between influenza mortality and spore concentration in Caguas, with an IRR of 1.17 per 10,000 spores/m³ (95% CI, 1.03–1.33; $p = 0.016$). However, this result was based on sparse weekly death counts, was one of four mortality models evaluated, and did not remain significant after Bonferroni correction. Although the fitted coefficient

corresponds to a higher expected weekly influenza death count across the observed interquartile range of fungal concentrations, this extrapolation depends on log-linearity over a wide exposure range and should not be interpreted as a strong mortality effect without replication.

The remaining three mortality models were null, underscoring the limited and non-generalizable nature of the mortality finding. In Caguas, fungal concentration was not associated with SARS-CoV-2 mortality (IRR 0.99, 95% CI 0.89–1.10), and no fungal–mortality association was detected in San Juan/Metro for either influenza (IRR 1.00, 95% CI 0.90–1.11) or SARS-CoV-2 (IRR 1.00, 95% CI 0.95–1.05) (Table 3). The confidence interval for the San Juan/Metro SARS-CoV-2 model was narrow, excluding effects larger than approximately 5% per 10,000 spores/m³, and thus served as an informative null value. The intervals for the two remaining null models were wider and compatible with modest effects in either direction. Of the four mortality models estimated, only the Caguas influenza association reached conventional significance, and its p-value (0.016) would not survive Bonferroni correction for four comparisons ($\alpha = 0.0125$); therefore, this result should be regarded as provisional pending replication.

The discrepancy highlights the instability of the mortality analyses: an apparent unadjusted lagged association between SARS-CoV-2 mortality and Caguas was not reproduced after seasonal adjustment in the region-specific model. This pattern suggests that shared temporal structure, rather than an independent fungal-spore association, may explain some unadjusted mortality correlations. Therefore, we interpret the mortality results as exploratory and hypothesis-generating, with only a non-multiplicity-robust signal for influenza mortality in Caguas.

Table 3. Region-specific exploratory negative binomial models of weekly respiratory-virus mortality on fungal spore concentration

Region	Outcome	Exposure	IRR per 10,000 spores/m ³	95% CI
Caguas	Influenza deaths	Fungi	1.17 ^a	1.03–1.33
Caguas	SARS-CoV-2 deaths	Fungi	0.99	0.89–1.10
San Juan/Metro	Influenza deaths	Fungi	1.00	0.90–1.11
San Juan/Metro	SARS-CoV-2 deaths	Fungi	1.00	0.95–1.05

Negative binomial regression of weekly death counts was fit separately within each health region and adjusted for seasonality. IRR, incidence rate ratio; CI, confidence interval. IRR was expressed per 10,000 spores/m³; the corresponding per-unit estimate for the Caguas influenza model was IRR 1.000016 per spore/m³. Mortality models were exploratory. Only one of four region–virus models reached conventional significance, and this association did not remain significant after Bonferroni correction for four comparisons. San Juan/Metro refers to the San Juan/Metro health region represented by the San Juan aerobiological monitoring station.

^a p = 0.016; not significant after Bonferroni correction for four comparisons ($\alpha = 0.0125$)

4. Discussion

In this two-region retrospective ecological time-series study, we identified consistent short-term associations between ambient fungal-spore concentrations and daily incidence of influenza and SARS-CoV-2 in San Juan/Metro and Caguas. These findings highlight fungal spores as a potential environmental correlate of respiratory virus activity in tropical settings. While these associations are encouraging, they should be interpreted as ecological signals; future research is needed to further disentangle these patterns from common seasonal, meteorological, and secular trends. Finally, our exploratory mortality analyses provide preliminary insights that warrant further investigation in larger datasets to address potential multiplicity and data sparsity.

Our findings both converge with and diverge from the existing literature on aeroallergens and respiratory viral infections. Damialis et al. (2021) reported that ambient pollen concentrations were positively associated with SARS-CoV-2 infection rates across 31 countries, an effect attributed in part to pollen-mediated suppression of airway interferon responses. Our results are consistent with prior work suggesting that ambient bioaerosols may be associated with respiratory viral activity (Hernández-González et al., 2023; Quintero et al., 2010; Rivera-Mariani et al., 2020; Rivera-Mariani & Bolaños-Rosero, 2012), although the present study cannot determine whether this reflects immune modulation, shared environmental drivers, or correlated seasonal dynamics. In this tropical setting, fungal spores, rather than pollen, were the dominant exposure, supporting their ecological dominance year-round in Puerto Rico's climate, where spore concentrations exceeded pollen concentrations by orders of magnitude (Quintero et al., 2010; Rivera-Mariani et al., 2020; Rivera-Mariani & Bolaños-Rosero, 2012). This variation is biologically plausible given Puerto Rico's aerobiological profile, in which fungal spores are abundant year-round, whereas pollen concentrations remain comparatively low and episodic.

The present analysis expands on prior work in four respects: (1) it models fungal and pollen exposure separately within the same population, (2) it examines two distinct respiratory viruses, (3) it links exposure to both incidence and mortality rather than incidence alone, and (4) it models pollen as a concurrently measured comparator exposure to evaluate whether associations were shared across aeroallergen classes. Unlike pollen, fungal spores may engage immune pathways such as Dectin-1 and Th17 signaling, whereas pollen has been linked to altered interferon responses (Gilles et al., 2020; Tham et al., 2014). These mechanistic contrasts are useful for hypothesis development, but the present data cannot distinguish immune-mediated effects from confounding by meteorology, seasonality, or correlated respiratory-virus dynamics. Accordingly, mechanistic interpretations should be viewed as arguments for biological plausibility rather than as conclusions directly established by the study.

The contrast between the regions warrants further discussion. Although fungal spores were associated with incidence in both San Juan/Metro and Caguas, the exploratory mortality signal appeared only for influenza in Caguas and was not robust to multiplicity correction. Several non-exclusive explanations are possible. Caguas may experience higher or more sustained background fungal exposure, so a greater proportion of person-time falls above a biologically relevant threshold. Consistent with this, median daily

fungal concentrations were approximately 2.7-fold higher in Caguas than in San Juan/Metro. Alternatively, the regions may differ in the prevalence of underlying respiratory diseases, healthcare access, or age structure, any of which could modify the exposure–mortality relationship. A third possibility is statistical: weekly death counts were sparse, and the San Juan models may have been underpowered to detect an effect of a similar magnitude. However, the accuracy of the null estimates differed: the confidence interval for SARS-CoV-2 mortality in San Juan was narrow and excluded effects larger than approximately 5% per 10,000 spores/m³, whereas the remaining null intervals were wide enough to remain compatible with modest associations. Because the present design includes only two regions and no individual-level covariates, we regard this regional contrast as hypothesis-generating rather than an established effect modification.

This study had several strengths. Exposure was measured continuously with AAAAI/NAB-certified volumetric samplers rather than modeled or interpolated, providing daily resolution over three years (Levetin et al., 2023). Including pollen as a concurrently measured comparator exposure provides a useful within-study contrast that is uncommon in aerobiology studies (Manangan et al., 2026). Because fungal spores and pollen were collected on the same days, at the same stations, and using the same general sampling framework, the divergence between the two exposures reduces concern that all aeroallergens behaved similarly. However, pollen is an imperfect comparator because its abundance, episodicity, particle biology, meteorological drivers, and measurement properties differ from those of fungal spores (Quintero et al., 2010; Rivera-Mariani et al., 2020). The analysis spanned two viruses with distinct transmission dynamics and found consistent incidence associations, while mortality analyses yielded only a single exploratory, non-multiplicity-robust signal. Finally, associations and classification patterns were directionally consistent across correlation, logistic regression, random forest, and count regression approaches. However, these findings should be interpreted with caution because temporally structured daily data can yield optimistic estimates when adjacent observations appear in both the training and test sets.

This study has several limitations. First, as an ecological time series, it cannot fully disentangle fungal-spore associations from shared seasonality, meteorological confounding, or temporal autocorrelation; thus, the findings cannot be interpreted as causal (Idrovo, 2011; Kakampakou et al., 2025). Second, models did not adjust for meteorological variables, and exposure was measured only as total spore concentration, precluding inferences about specific fungal taxa or components. While pollen served as a comparator, it remains an imperfect control for confounding. Third, mortality analyses were exploratory and unstable because of sparse data and lack of robustness to multiplicity correction. Finally, the modest effect sizes and reliance on case-based surveillance data subject to ascertainment artifacts indicate that these findings should be viewed as hypothesis-generating rather than as validated surveillance markers. Future studies should pair taxon-resolved aerobiological monitoring with meteorological adjustment, individual-level clinical data, and host-response biomarkers to determine whether the ecological associations observed here reflect causal immune modulation, shared environmental drivers, or both.

5. Conclusions

At this stage, fungal-spore concentrations should be considered a candidate environmental correlate for further study, not a validated surveillance marker. Operational surveillance use would require evidence that fungal-spore measurements improve out-of-time prediction beyond seasonality, meteorology, calendar time, and recent case counts. Realizing this potential will require work in several directions: multivariable models that adjust for meteorological covariates and ambient particulate matter; linkage to individual-level and clinical outcomes, such as emergency department visits and hospitalizations; taxon-resolved spore analyses to identify the fungal groups responsible; temporally blocked validation; and prospective evaluation of region-specific alert thresholds. In conclusion, these results identify outdoor fungal-spore concentrations as a candidate environmental correlate of respiratory virus incidence in Puerto Rico. Mortality analyses yielded only an exploratory, non-multiplicity-robust signal for influenza mortality in Caguas and require independent replication before any mortality inference is made.

Declarations

Acknowledgements

We thank the Puerto Rico Department of Health for providing de-identified, aggregate surveillance data on influenza and SARS-CoV-2. We also acknowledge the community health center SANOS, which hosts the NAB-certified station in Caguas. This research was supported by the Puerto Rico Science, Technology and Research Trust. The content is solely the responsibility of the authors and does not represent the official views of the Puerto Rico Science, Technology and Research Trust.

Funding: This research was supported by the Puerto Rico Science, Technology and Research Trust. The content is solely the responsibility of the authors and does not represent the official views of the Puerto Rico Science, Technology and Research Trust.

Competing interests: The authors have no relevant financial or non-financial interests to disclose.

Ethics approval: This study used de-identified, aggregate surveillance data obtained from the Puerto Rico Department of Health and did not involve human subjects research; ethics approval was therefore not required.

Consent to participate: Not applicable.

Consent to publish: Not applicable.

Data availability: Aerobiological data are available from the National Allergy Bureau/AAAAI monitoring stations in San Juan and Caguas, Puerto Rico. Respiratory virus surveillance data are available from the Puerto Rico Department of Health. Derived analytic datasets are available from the corresponding author on reasonable request.

Code availability: The R analysis code is available from the corresponding author on reasonable request.

Author contributions Conceptualization: Félix E. Rivera-Mariani, Benjamín Bolaños-Rosero; Methodology: Félix E. Rivera-Mariani; Formal analysis: Félix E. Rivera-Mariani, Catalina S. Robles-Rivera; Data curation: Félix E. Rivera-Mariani, Catalina S. Robles-Rivera, Cláudia I. Pereira Moreira; Software: Félix E. Rivera-Mariani; Investigation: Cláudia I. Pereira Moreira, Benjamín Bolaños-Rosero; Resources: Benjamín Bolaños-Rosero; Validation: Benjamín Bolaños-Rosero; Visualization: Catalina S. Robles-Rivera; Supervision: Félix E. Rivera-Mariani, Benjamín Bolaños-Rosero; Project administration: Félix E. Rivera-Mariani; Writing – original draft preparation: Félix E. Rivera-Mariani; Writing – review and editing: Félix E. Rivera-Mariani, Catalina S. Robles-Rivera, Cláudia I. Pereira Moreira, Benjamín Bolaños-Rosero. All authors read and approved the final manuscript.

Use of AI tools: The authors used Grammarly solely to check and improve English grammar, spelling, and punctuation.

References

1. Anderson, J., Pityn, P., & Kelly, M. (2020). Pollen count standardization for Burkard & Rotorod samplers. *Journal of Allergy and Clinical Immunology*, 145(2), AB37. <https://doi.org/10.1016/j.jaci.2019.12.741>
2. Chkonia, S., Hamid, S., Billig Rose, E., Winn, A., Anderson, E., & Silk, B. J. (2026). Evaluating analytic approaches to determining respiratory syncytial virus seasonality—United States, 2014–2024. *Influenza and Other Respiratory Viruses*, 20(7), e70271. <https://doi.org/10.1111/irv.70271>
3. Damialis, A., Gilles, S., Sofiev, M., Sofieva, V., Kolek, F., Bayr, D., Plaza, M. P., Leier-Wirtz, V., Kaschuba, S., Ziska, L. H., Bielory, L., Makra, L., del Mar Trigo, M., COVID-19/POLLEN Study Group, & Traidl-Hoffmann, C. (2021). Higher airborne pollen concentrations correlated with increased SARS-CoV-2 infection rates, as evidenced from 31 countries across the globe. *Proceedings of the National Academy of Sciences*, 118(12), e2019034118. <https://doi.org/10.1073/pnas.2019034118>
4. García-Arroyo, L., Prim, N., Del Cuerpo, M., Marín, P., Roig, M. C., Esteban, M., Labeaga, R., Martí, N., Berengua, C., Gich, I., Navarro, F., & Rabella, N. (2022). Prevalence and seasonality of viral respiratory infections in a temperate climate region: A 24-year study (1997–2020). *Influenza and Other Respiratory Viruses*, 16(4), 756–766. <https://doi.org/10.1111/irv.12972>
5. Gasparrini, A., & Leone, M. (2014). Attributable risk from distributed lag models. *BMC Medical Research Methodology*, 14(1), Article 55. <https://doi.org/10.1186/1471-2288-14-55>
6. Gilles, S., Blume, C., Wimmer, M., Damialis, A., Meulenbroek, L. A. P. M., Gökkaya, M., Bergougnan, C., Eisenbart, S., Sundell, N., Lindh, M., Andersson, L.-M., Dahl, Å., Chaker, A., Kolek, F., Wagner, S., Neumann, A. U., Akdis, C. A., Garssen, J., Westin, J., . . . Traidl-Hoffmann, C. (2020). Pollen exposure weakens innate defense against respiratory viruses. *Allergy*, 75(3), 576–587. <https://doi.org/10.1111/all.14047>

7. Hernández-González, X., Rivera-Mariani, F. E., Méndez, L., & Bolaños-Rosero, B. (2023). Increase in the susceptibility to COVID-19 during the fungal spore season among residents of San Juan and Caguas, Puerto Rico. *Puerto Rico Health Sciences Journal*, 42(Suppl. 1), 20.
8. Idrovo, Á. J. (2011). Three criteria for ecological fallacy. *Environmental Health Perspectives*, 119(8), A332. <https://doi.org/10.1289/ehp.1103768>
9. Iuliano, A. D., Roguski, K. M., Chang, H. H., Muscatello, D. J., Palekar, R., Tempia, S., Cohen, C., Gran, J. M., Schanzer, D., Cowling, B. J., Wu, P., Kyncl, J., Ang, L. W., Park, M., Redlberger-Fritz, M., Yu, H., Espenhain, L., Krishnan, A., Emukule, G. O., . . . Bresee, J. S. (2018). Estimates of global seasonal influenza-associated respiratory mortality: A modelling study. *The Lancet*, 391(10127), 1285–1300. [https://doi.org/10.1016/S0140-6736\(17\)33293-2](https://doi.org/10.1016/S0140-6736(17)33293-2)
10. Kakampakou, L., Stokes, J., Höhn, A., de Kamps, M., Lawniczak, W., Arnold, K. F., Hensor, E., Heppenstall, A., & Gilthorpe, M. S. (2025). Simulating hierarchical data to assess the utility of ecological versus multilevel analyses in obtaining individual-level causal effects. *BMC Medical Research Methodology*, 25(1). <https://doi.org/10.1186/s12874-025-02504-6>
11. Levetin, E., Pityn, P. J., Ramón, G. D., Pityn, E., Anderson, J., Bielory, L., Dalan, D., Codina, R., Rivera-Mariani, F. E., & Bolaños, B. (2023). Aeroallergen monitoring by the National Allergy Bureau: A review of the past and a look into the future. *The Journal of Allergy and Clinical Immunology: In Practice*, 11(5), 1394–1400. <https://doi.org/10.1016/j.jaip.2022.11.026>
12. Lewis, L. M., Mirabelli, M. C., Beavers, S. F., Kennedy, C., Shriber, J., Stearns, D., Gonzalez, J., Soto Santiago, M., Montalvo Félix, I., Ruiz-Serrano, K., Dirlikov, E., Lozier, M., Sircar, K., Flanders, W. D., Rivera-García, B., Irizarry-Ramos, J., & Bolaños-Rosero, B. (2020). Characterizing environmental asthma triggers and healthcare use patterns in Puerto Rico. *Journal of Asthma*, 57(8), 886–897. <https://doi.org/10.1080/02770903.2019.1612907>
13. Manangan, A. P., Brown, C. L., Werner, A. K., Katz, D. S. W., Rorie, A., Voelker, D., Gabrish, P., Hess, J. J., & Schramm, P. J. (2026). Pollen season timing and concentrations in the United States: Developing a standardized pollen dataset using data from the National Allergy Bureau (NAB) (2003–2024). *Atmosphere*, 17(7), Article 635. <https://doi.org/10.3390/atmos17070635>
14. Milla, N. E. (2026). Nonlinear and delayed effects of rainfall and temperature on dengue incidence in Eastern Visayas, Philippines: A Bayesian distributed lag nonlinear model approach. *Recoletos Multidisciplinary Research Journal*, 14(2), 39–49. <https://doi.org/10.32871/rmrj2614.02.04>
15. Moriyama, M., Hugentobler, W. J., & Iwasaki, A. (2020). Seasonality of respiratory viral infections. *Annual Review of Virology*, 7(1), 83–101. <https://doi.org/10.1146/annurev-virology-012420-022445>
16. Quintero, E., Rivera-Mariani, F., & Bolaños-Rosero, B. (2010). Analysis of environmental factors and their effects on fungal spores in the atmosphere of a tropical urban area (San Juan, Puerto Rico). *Aerobiologia*, 26(2), 113–124. <https://doi.org/10.1007/s10453-009-9148-0>
17. Rivera-Mariani, F. E., Almaguer, M., Aira, M. J., & Bolaños-Rosero, B. (2020). Comparison of atmospheric fungal spore concentrations between two main cities in the Caribbean Basin. *Puerto Rico Health Sciences Journal*, 39, 235–242.

18. Rivera-Mariani, F. E., & Bolaños-Rosero, B. (2012). Allergenicity of airborne basidiospores and ascospores: Need for further studies. *Aerobiologia*, 28(2), 83–97. <https://doi.org/10.1007/s10453-011-9234-y>
19. Rivera-Mariani, F. E., Borrero-Aponte, A., & Bolaños-Rosero, B. (2026). Fungal spores as a 1–2 week leading indicator of viral surges: Forecasts uplift over seasonal baselines. *Journal of Allergy and Clinical Immunology*, 157(2), AB298. <https://doi.org/10.1016/j.jaci.2025.12.885>
20. Shaman, J., & Kohn, M. (2009). Absolute humidity modulates influenza survival, transmission, and seasonality. *Proceedings of the National Academy of Sciences*, 106(9), 3243–3248. <https://doi.org/10.1073/pnas.0806852106>
21. Simpson, R. B., Venkat, A., Alarcon Falconi, T. M., Chui, K., Naumov, Y. N., Gorski, J., Bhattacharyya, S., & Naumova, E. N. (2019). Calendar effects to forecast influenza seasonality: A case study in Milwaukee, WI. *Online Journal of Public Health Informatics*, 11(1). <https://doi.org/10.5210/ojphi.v11i1.9739>
22. Tham, R., Dharmage, S. C., Taylor, P. E., Katelaris, C. H., Vicendese, D., Abramson, M. J., & Erbas, B. (2014). Outdoor fungi and child asthma health service attendances. *Pediatric Allergy and Immunology*, 25(5), 439–449. <https://doi.org/10.1111/pai.12257>
23. van Asten, L., de Lange, M., Teirlinck, A., Stoeldraijer, L., Harmsen, C., & van der Hoek, W. (2018). Mortality surveillance in the Netherlands: Severity of winter 2016/2017. *Online Journal of Public Health Informatics*, 10(1). <https://doi.org/10.5210/ojphi.v10i1.8946>
24. Zhong, R., Chacón-Montalván, E. A., & Moraga, P. (2026). Bayesian spatial functional data clustering: Applications in disease surveillance. *Statistics in Medicine*, 45. <https://doi.org/10.1002/sim.70597>
25. Zimmer, C., Leuba, S. I., Yaesoubi, R., & Cohen, T. (2018). Use of daily Internet search query data improves real-time projections of influenza epidemics. *Journal of the Royal Society Interface*, 15(147), Article 20180220. <https://doi.org/10.1098/rsif.2018.0220>

Figures



Figure 1

Time series of (A) daily fungal spore concentrations, (B) daily pollen concentrations, and (C) weekly influenza and SARS-CoV-2 case counts in San Juan/Metro and Caguas, Puerto Rico, 2022–2024

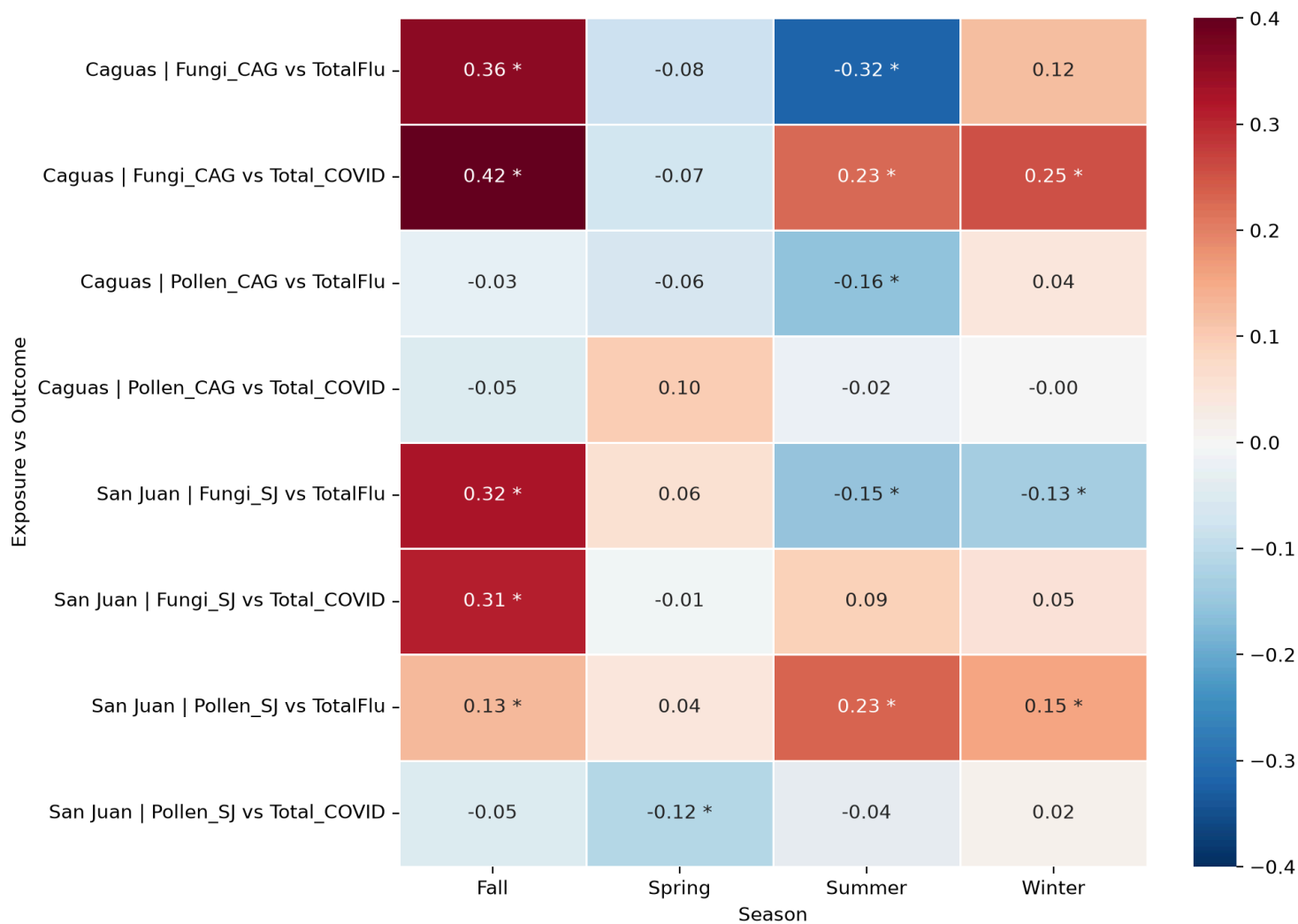


Figure 2

Heatmap of Pearson correlation coefficients between fungal spore (or pollen) concentrations and respiratory virus incidence, by season, region, and virus. Asterisks (*) indicate $p < 0.05$.

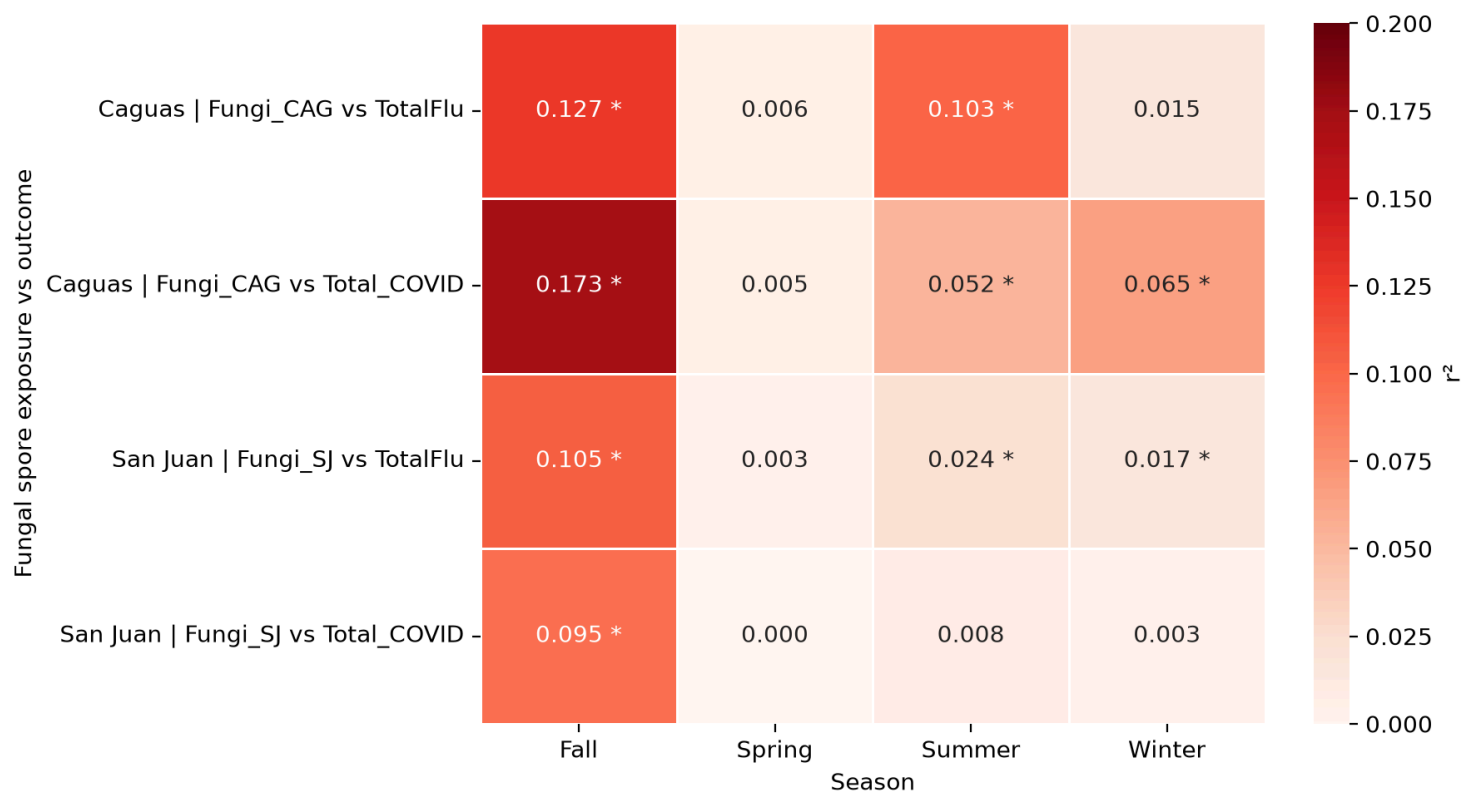


Figure 3

Heatmap of the coefficient of determination (r^2) between fungal spore concentrations and respiratory virus incidence, by season, region, and virus. Asterisks (*) indicate $p < 0.05$.

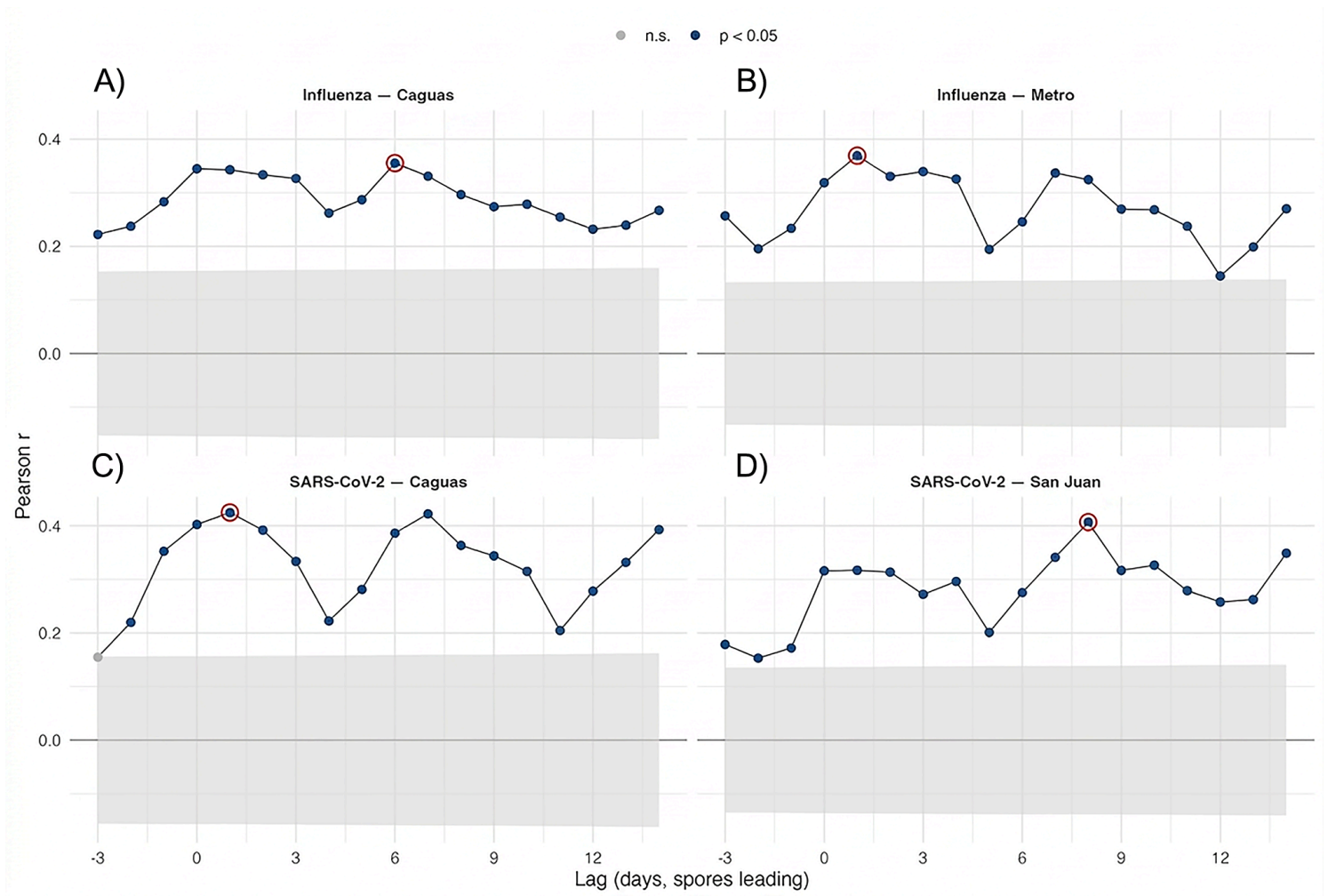


Figure 4

Lagged associations between fungal spore concentrations and respiratory virus incidence (fall), by region and virus. (A) Influenza, Caguas. (B) Influenza, San Juan/Metro. (C) SARS-CoV-2, Caguas. (D) SARS-CoV-2, San Juan/Metro. Each point is the Pearson correlation coefficient between daily fungal spore concentration and daily case count at the indicated lag; a positive lag indicates that fungal spores precede virus incidence. Dark blue points are significant at $p < 0.05$; gray points are non-significant (n.s.). The red circle in each panel marks the lag with the strongest correlation. The shaded band denotes the approximate 95% null bounds.