Highway paving dramatically increased dengue transmission in the Amazon

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Abstract

Human mobility drives the spread of many infectious diseases, yet the health impacts of changes in mobility due to new infrastructure development are poorly understood and currently not accounted for in impact assessments. We take a novel quasi-experimental approach to identifying the link between mobility and infectious disease, leveraging historical road upgrades as a proxy for regional human mobility changes. We analyzed how highway paving altered transmission of dengue—a high-burden mosquito-borne disease—via changes in human movement in the Madre de Dios region of Peru. The paving of the Interoceanic Highway through a formerly isolated region of the Amazon in 2009 provided a unique opportunity to quantify the causal impact of road paving on disease transmission. To uncover this relationship, we compared dengue incidence data from healthcare facilities in Madre de Dios near to versus far from the newly paved highway before and after paving, while controlling for observable and unobservable confounding variables (a difference-in-differences causal inference approach). We found that the paving of the highway caused at least an additional 9,826 (95% CI: 8,562–10,684) dengue cases since paving, accounting for 45.2% (95% CI: 39.4%–49.2%) of all dengue cases recorded in the region post highway paving (2009–2022). Our findings demonstrate the impact that infrastructure can have on dengue transmission, likely via its effects on human mobility. As a result, we advocate for future road construction plans in tropical regions to account for potential increases in dengue transmission during impact assessments.

Significance statement

Large-scale infrastructure projects are critical for meeting global development goals, but they can also have unintended consequences for local human and environmental health. More evidence attributing human health effects to infrastructure projects is needed to understand how to mitigate these negative health impacts. Our study contributes to this body of knowledge, attributing nearly half of recent dengue cases in Madre de Dios, Peru to the paving of the Interoceanic Highway through the region in 2009. Our results demonstrate the impact that highway construction can have on human health and call for future infrastructure projects through tropical forests to consider infectious disease outcomes in their impact assessments.

Introduction

Meeting United Nations Sustainable Development goals requires massive infrastructure build-out, especially in developing countries (1). While there are many benefits promised by these infrastructure projects—such as increased access to labor opportunities, creation of water resources, and potential for economic growth—there are also many costs that have not been accounted for historically, such as harms to local environmental and human health (2, 3). Well-designed roads are critical for the distribution of resources and opportunities, and their growth is widespread; as of 2020, 12,263 km of road construction was planned in the Amazon alone (4). Despite their importance to local development, roads drive changes in human mobility and migration that have been previously associated with increases in infectious disease transmission (5, 6). However, these effects remain a challenge to identify and are often not included in even the most comprehensive road assessment analyses (4), likely due to the lack of causal analyses that disentangle disease from complex social-environmental processes. Current methods linking mobility and infectious disease also typically rely on cell phone or survey-based mobility data, which can be expensive, inaccessible, and/or biased due to lack of representativeness or recall bias (7, 8). To bridge these gaps, we take a novel quasi-experimental approach where we demonstrate a causal link between highway construction and dengue virus transmission, leveraging the rapid paving of a highway through the Peruvian Amazon as a proxy for a discrete and significant increase in regional human movement and connectivity.

The Madre de Dios region of Peru is an ideal case study to investigate the relationships between human mobility and infectious disease given its recent infrastructure development, history of vector-borne disease, and high environmental suitability for vectors (9, 10). Madre de Dios is an 85,000 km^2 department in the southwest Amazon in the southeast of Peru. In this

region, most human population centers are distributed along rivers, secondary unpaved roads, and the only major paved road, the Interoceanic Highway, a controversial construction project completed in 2009 that intensified regional in-migration (11) and legal and illegal development (12) (**Fig 1**). Touted as a project that would have socio-economic benefits for rural Peruvian communities, there have thus far been limited reports of economic or trade-based benefits in the area (13, 14). However, the transformation of an unpaved road into a paved highway spurred poverty-driven environmental degradation, such as accelerated deforestation and illegal gold mining (12). There has been limited quantification of the health impacts of paving this section of highway; studies to date have been qualitative or correlational analyses (14, 15). Causal analyses of how highway construction may have influenced human health could help to build the evidence base on impacts of infrastructure projects in the Amazon.

We hypothesized that the highway paving most immediately affected dengue transmission through increases in mobility and connectivity both into and within the region, due to dengue's specific epidemiology and ecology. Dengue virus is transmitted to humans by biting mosquitoes (16). It is the most rapidly spreading mosquito-borne disease worldwide (17) and has caused multiple record-breaking outbreaks over the last two years across the globe (18). Qualitative analyses have reported that Madre de Dios community members believe that the highway construction facilitated increased dengue transmission (14). Dengue was present throughout Madre de Dios well before the paving in 2009 (10) (**Fig 1B, SFig S1A**), and its primary vector—*Aedes aegypti*—inhabits urban and peri-urban areas that line highway corridors (19). Dengue is a highly localized disease, appearing in clusters at the household and neighborhood level (10). Previous studies have established that diffusion of dengue through regional scales relies on human movement and migration (20), given that its vector typically

104 does not fly more than 1km (21). We hypothesized that when the construction of the Interoceanic
105 Highway further connected Madre de Dios to the bordering dengue-endemic regions of Acre,
106 Brazil and Pando, Bolivia, increased human movement along the highway amplified virus
107 importation and circulation (**SFig S2**).

108 In contrast to dengue, leishmaniasis—a parasitic disease caused by infection with
109 *Leishmania* parasites transmitted by sandflies—was also well established in the region prior to
110 road paving (**Fig 1C, SFig S1B**) but is not expected to be tightly linked to human mobility and,
111 by extension, to road paving (22, 23). Although sandflies have similar flight dispersal limitations
112 (24), they occur in rural, forested, and frontier ecosystems farther from highway corridors (23)
113 and become infected by feeding on infectious animal reservoir hosts rather than humans (humans
114 are dead-end hosts (25)). Therefore, while regional leishmaniasis transmission might have
115 increased somewhat due to highway-driven land conversion, its eco-epidemiology minimizes the
116 potential for it to be impacted by human movement along the highway. We use leishmaniasis as
117 a 'negative control' when testing impacts of the paving, in that we do not expect it to respond
118 immediately to highway construction after controlling for land-use changes, but it might capture
119 any impacts of highway paving on diagnostic capacity or healthcare seeking behavior.

120 To establish whether the paving of the Interoceanic Highway in Madre de Dios caused an
121 increase in dengue incidence rates, we conducted a difference-in-differences panel regression
122 (26), comparing dengue incidence rates in healthcare facility clusters (n = 71) within 5km of the
123 newly paved highway ('exposed') and those outside 10km ('unexposed'), before and after the
124 highway was paved in 2009 (**Fig 1**). This is a causal inference framework that approximates a
125 before-after treatment-control experimental design and incorporates (1) fixed effects, which
126 control for unobserved confounders (e.g., baseline dengue burden, large-scale political changes),

and (2) observed control variables, which control for known dengue drivers (i.e., climate and land-use changes). We also tested whether or not the paving similarly affected leishmaniasis incidence rates, with the hypothesis that there was no impact on leishmaniasis because it is not directly affected by human mobility.

Results

We found that the paving of the highway increased dengue incidence rates by 184% (95% CI: 65%–389%) in clusters within 5km of the highway compared to those outside 10km, after incorporating fixed effects and accounting for potentially confounding spatial, temporal, and environmental variation (**Table 1**). Yearly increases ranged from 20% to 458% across the 14-year study period (**Fig 2A**, **STable S1**). Overall, we attributed 9,826 (95% CI: 8,562–10,684) dengue cases to the paving of the highway, accounting for 45.2% (95% CI: 39.4%–49.2%) of all dengue cases recorded in the region post highway construction (**Fig 2A**). There was no systematic difference in trends in dengue incidence between healthcare facility clusters near to the highway and those far from the highway before paving (coefficients remain at zero prior to 2008, **Fig 2A**), supporting the parallel trends assumption and implying that far clusters represent reasonable controls (i.e., counterfactuals) for clusters near the road. These findings were robust to various model specifications (**STable S2, SFig S3**) and produced p-values < 0.01 when compared to permuted samples in permutation inference analyses (**SFig S4**). Increases in urban area were associated with increased dengue incidence, aligning with biological knowledge of *Aedes* vector habitat (**Table 1**). Seasonal (biannual) models aggregated across the study period revealed larger differences in dengue incidence between healthcare facility clusters near and far from the highway during rainy seasons (173%, [95% CI: 73–334%]), when the majority of cases occur, as compared to dry seasons (68%, [95% CI: 12–152%]) (**Fig 2B**).

Table 1: Dengue and leishmaniasis incidence long-differences estimation results.

	Dengue Yearly	Dengue Biannual Dry	Dengue Biannual Rainy	Leish Yearly	Leish Biannual Dry	Leish Biannual Rainy
5km x Post-2008 Yearly	1.0* (0.28)			0.40 (0.24)		
5km x Post-2008 Biannual		0.52* (0.21)	1.0* (0.23)		0.47* (0.20)	0.16 (0.15)
log(Urban)	0.09 (0.10)	0.10 (0.07)	0.06 (0.09)	-0.005 (0.07)	0.03 (0.04)	-0.05 (0.04)
log(Ag)	-0.23 (0.14)	-0.17 (0.12)	-0.24 (0.15)	0.23 (0.15)	0.14 (0.14)	0.21 (0.11)
Sum Precip	0.09 (0.24)	0.73 (0.58)	0.02 (0.24)	-0.34* (0.13)	-0.60* (0.30)	-0.22* (0.10)
Mean Temp	-0.05 (0.47)	0.25 (0.25)	-0.05 (0.53)	0.86* (0.43)	0.45* (0.20)	0.28 (0.34)
FEs						
Cluster	X	X	X	X	X	X
Year	X			X		
Biannual		X	X		X	X
Cor^2	0.54541	0.3425	0.54752	0.53686	0.59255	0.55846
N	974	1,040	1,040	975	1,040	1,040
Clusters	65	65	65	65	65	65

Estimation results from Equation 2 for yearly and biannual models. Yearly models include cluster and yearly fixed effects, and biannual models include cluster and biannual fixed effects. Standard errors are clustered at the unit level for all models and are displayed inside parentheses next to each estimated coefficient. P-values indicating a statistical significance level below 0.05 are designated with an asterisk (*). N increases for biannual models because the reference time step is a six month period, resulting in the inclusion of the corresponding six months as an additional data point. **STable S3** displays results for models with quadratic climate covariates, finding no major differences.

As a 'negative control' that we do not expect to be immediately affected by increased human mobility, we used the same approach to examine impacts on leishmaniasis incidence before and after road paving. We found that leishmaniasis incidence did not differ systematically between healthcare facility clusters near and far from the highway either before or after highway paving (**Fig 3A**). Although we saw small differences between healthcare facilities near and far from the highway almost a decade after paving and during the dry season, the differences were

minimal and were not significant when aggregated across the study period (**Table 1, Fig 3B**). Increases in agricultural area were associated with increased leishmaniasis incidence, aligning with biological knowledge of sandfly vector habitat (**Table 1**).

To better understand (1) which communities are at heightened risk of increased dengue transmission due to highway paving and (2) the extent of potential spatial spillover effects of exposure, we compared groups of healthcare facility clusters at increasing distances from the highway—1km, 5km, 10km, 15km, and 20km—to a control group of healthcare facility clusters >20km from the highway (**Fig 4B**). Healthcare facility clusters within 1km, 5km, and 10km all had significantly higher dengue incidence than healthcare facility clusters >20km from the highway post paving (**Fig 4A**). Differences attenuated to zero and were not statistically significant for healthcare facility clusters farther than 10km from the highway.

Discussion

We found large and lasting impacts of the paving of the Interoceanic Highway on dengue incidence in a previously isolated region of the Peruvian Amazon: incidence rates increased by 184% (95% CI: 65%–389%) in healthcare facility clusters near to versus far from the highway (**Table 1**, **Fig 2**). While we do not directly test the mechanism, we hypothesize that the significant increase in dengue burden was driven by increased human mobility into and within the region, leading to increased virus importation and circulation (**SFig S2**). We extend previous research (5, 6) that associates highway construction with increased dengue transmission by offering causal estimates of the impact of road paving on dengue while controlling for potentially confounding variables. These estimates are robust to a suite of alternative model specifications (**SFig S3**). We also conducted a ‘negative control’ analysis as a way to check for systematic changes that could confound our estimates, such as healthcare-seeking or case-reporting

behaviors, and find that for leishmaniasis, a disease not expected to change with human mobility, there is minimal effect of road paving on incidence (**Fig 3**).

Our primary hypothesized mechanism for this effect is that highway paving facilitated regional human movement changes that caused additional dengue outbreaks to be seeded along the highway corridor. The highway increased connectivity to Brazil and Bolivia, both of which are dengue hyper-endemic, with regular co-circulation of multiple serotypes and genotypes (5, 27) (**SFig S2**). Another relevant dynamic is the in-migration that occurred after the highway was built, with people moving to Madre de Dios from the nearby, newly connected Andes mountains and other non-endemic regions (11) (**SFig S5**). This increased the number of susceptible people living in the area. Although we directly account for yearly population changes when calculating incidence (**SFig S5**) and determine that dengue cases increased more than what would have been expected just due to population growth, we do not explicitly include susceptibility dynamics in our model. We hypothesize susceptibility dynamics are also what might be responsible for the small increases in leishmaniasis incidence later in the study period (**Fig 3**).

Effects of highway paving on dengue were significantly larger during rainy seasons as compared to dry, even after explicitly controlling for precipitation and including biannual fixed effects (**Fig 2B**). This could be due to the larger dengue burden during the rainy season enabling larger differences to occur between healthcare facilities near and far from the highway. Alternatively, this could be due to increased differences in mobility during the rainy season; rivers and unpaved roads often become impassable during the rainy season, while the highway is less affected by increased precipitation (28). Therefore, mobility continues to a higher degree along the highway compared to farther away, potentially causing larger dengue differences between healthcare facility clusters near and far from the highway in the rainy season. Further

supporting the role of movement and accessibility of the road, we found that communities as far as 10km from the highway experienced increased dengue transmission after highway paving, while communities farther than 10km from the highway did not (**Fig 4**). This helps to define the spatial scale of impacts of highway development projects on dengue, with applications for designing control interventions.

Although this work isolates a plausible causal impact of highway paving on dengue transmission, our quasi-experimental approach does not allow us to definitively identify the specific mechanism that caused this effect. We hypothesize the main mechanism to be the increase in human movement along the corridor, supported by the fact that (1) we only see an immediate effect on dengue and not on leishmaniasis, (2) the effect is large even when explicitly controlling for resulting in-migration and land-use changes, and (3) the estimated effect attenuates at distances (>10km) unlikely to be affected by mobility around the highway. Direct mobility data, such as surveys or mobile phone data, could further bolster our understanding of the mechanistic effects of highway paving on mobility, though they would likely be subject to recall bias and/or lack of representativeness. While changes in mobility and connectivity are parsimonious and plausible mechanisms behind the observed increases in dengue following highway paving, there are a few alternative phenomena that could have also contributed to the identified effect. We are not able to include susceptibility dynamics in our model, which are known to be important for dengue due to the cross-reactivity of various dengue serotypes. However, it is important to note that large interannual changes in dengue outbreaks, for example following the introduction of a new serotype, are accounted for in the year fixed effects except to the extent that they differentially affect near versus far healthcare clusters. We also were not able to control for hyper-localized habitat changes that might have implications for cluster-level

vector abundance and transmission capabilities, such as water storage and garbage practices that can influence breeding habitat.

Other limitations of this work include that different communities were exposed to their stretch of highway being paved at different times. Therefore, some units may have experienced exposure before 2009, resulting in an underestimation of the true effect size. Additionally, even after removing some units to create a 'buffer' against spatial spillover, some spillover likely remains throughout the unexposed units, also resulting in potential underestimation. Finally, although we attempt to control for development differences between exposed and unexposed groups through measuring yearly changes in urban area, it is possible that healthcare facilities near the highway developed increased capacity for diagnosing dengue compared to those far from the highway post paving. However, we show our results are robust to the inclusion of "probable" cases (**STable S2, SFig S3**), alleviating concerns of potential distortions due to the availability of test supplies.

We demonstrate the importance of including human health outcomes, such as infectious disease, in impact assessments when planning infrastructure development projects (3, 29). Our analysis establishes that highway construction through dengue-endemic areas makes communities uniquely vulnerable to dengue transmission. This work is especially timely because two additional highway construction projects through Madre de Dios were approved in 2023, over the protest of local communities and Indigenous groups (28, 30). Outside of the Amazon, other dengue-endemic tropical regions are also planning highway construction projects, including in Central America, Central Africa, and Southeast Asia (31). Given the measurable and significant impact that road construction had on local dengue transmission in the Madre de Dios region, our findings contribute to understanding the social, environmental, and economic

tradeoffs of this proposed infrastructure development. Should these infrastructure projects be approved, our work highlights the importance of coordinating with local, regional, and national healthcare officials at the outset of infrastructure project planning to forecast health impacts and target public health resources and interventions. Potential human health interventions could include vector control strategies (32), investment in water and sanitation infrastructure, and health system strengthening along highway corridors, such as increased staffing, priority access to new dengue vaccines once approved (33, 34), and deployment of dengue rapid tests for improved diagnostics and notification (35, 36).

We contribute to a growing body of literature that demonstrates the impact of infrastructure on human health, but dengue is only one of many infectious diseases that may be impacted by changes in human movement and development. Future work can help to identify the responses of other diseases to road building and other infrastructure development in the Amazon and across the globe. Generating this evidence will be key for guiding informed decision-making on meeting our development goals, while proactively mitigating any potential harms to local human and environmental health.

Materials and methods

Ethics statement

The Stanford IRB office determined that this analysis did not qualify as Human Subjects Research as defined in 45 CFR 46.102(e)(5), given the data did not contain any identifiable information.

Data collection and preparation

De-identified, anonymized dengue and leishmaniasis case data reported from 2000–2022 were provided by the Regional Health Directorate of Madre de Dios (DIRESA, (37)). Both

dengue and leishmaniasis are reportable infectious diseases and are included in mandatory surveillance for each healthcare facility (n = 105) in Madre de Dios. Case data included diagnosis code, week and year of diagnosis, method of diagnosis, locality name, and healthcare facility code.

Of 25,211 total dengue cases, 94.1% were laboratory confirmed and 5.9% were probable (see **Supplementary Information** for diagnosis methods). We included only confirmed cases in the main model and conducted a sensitivity analysis that included both confirmed and probable cases (**STable S2**, **SFig S3**). Of 15,507 total leishmaniasis cases, 99.4% of cases were laboratory confirmed and 0.6% were probable. We similarly included only confirmed leishmaniasis cases. 86.6% of leishmaniasis cases were cutaneous leishmaniasis and 13.4% were mucocutaneous. Healthcare facilities were clustered to create non-overlapping spatial boundaries to extract climate and land use covariate data, resulting in n = 71 spatial units (**SFig S7**). We tested alternative clustering specifications that increased the number of spatial units (n = 78 and n = 84) and found no substantial differences in main model results (**SFig S8**, see **Supplementary Information** for clustering methods).

We calculated cluster-specific, yearly population measures by integrating locality population data from DIRESA and WorldPop (37, 38) (**SFig S5A**, see **Supplementary Information**). Remotely-sensed environmental covariate data was included explicitly in models as control variables. First, we calculated the total yearly urban and agricultural area using Mapbiomas—a publicly available dataset of classified annual land-use maps of the Amazon basin with a spatial resolution of $30m^2$ (**SFigs S5B and S5C** (39)). Second, we calculated monthly mean temperature and accumulated precipitation using the ERA5 Land Monthly Aggregated dataset, which provides climate data at a spatial resolution of $11km^2$ (40). Monthly

303 values were averaged or summed annually and biannually depending on each model's temporal
304 resolution.

305 *Interoceanic Highway*

306 We leveraged the paving of the highway as a natural experiment, or shock, hypothesized
307 to result in a sudden increase in mobility-driven dengue transmission in areas close to the
308 highway. This paving is plausibly exogenous to our system (i.e., the decision to pave and
309 location of the road paving did not depend on dengue incidence at baseline), given that the road
310 was paved as a part of the larger Interoceanic Highway construction project, spanning from the
311 Atlantic to the Pacific coast (41). The road paving site was selected to pass through the tri-border
312 area at the northeast end of the region where Peru borders Brazil and Bolivia and then minimize
313 costs to reach Puerto Maldonado, the region's capital, and eventually Cuzco (41) (**SFig S2**).

314 The construction of the Interoceanic Highway through Peru took place from 2006 to 2010
315 (42). Further investigation confirmed the section of highway specific to Madre de Dios (Tramo
316 3, Inambari–Iñapari) was completed in 2009 (43). While paving through Madre de Dios occurred
317 from 2007 to 2009 (43–45), our goal was to capture when regional human connectivity and
318 movement shifted regionally, rather than the moment households individually experienced
319 paving adjacent to their community. Therefore, we designate 2009 as the year when exposure
320 began for all spatial units and 2008 as our 'baseline' year (i.e., the last year before exposure
321 began). The paving of the highway shortened the time it takes to cross the region from a multi-
322 day trip to five hours (46).

323 This study design designates healthcare facility clusters within 5km of the highway as
324 'exposed' and those further than 10km from the road as 'unexposed,' using distance as a proxy

for connectivity to the highway. Understanding there is likely spatial spillover of exposure, we removed healthcare facility clusters between 5-10km from the highway (**Fig 1A**). Given distance is only a proxy for accessibility to the highway, we tested the sensitivity of the model to both the boundary and buffer specifications (**SFig S3**). The key identifying assumption in the difference-in-differences design is that both groups of healthcare facility clusters would have seen dengue incidence develop along parallel trends in the absence of the road paving. Visually, the parallel trends assumption appears defensible, with dengue and leishmaniasis incidence following parallel trajectories in clusters designated exposed versus unexposed prior to paving (i.e., pre-exposure; **Figs 1B and 1C**). Additionally, all healthcare facility clusters far from the highway are connected to the region's larger transportation network either by unpaved roads or rivers, making their connection to the region's transportation network comparable to that of the healthcare facility clusters near to the highway prior to exposure (**Fig 1A**).

Statistical models

To identify the effect of paving the Interoceanic Highway on dengue transmission, we used a difference-in-differences panel regression (26), comparing healthcare facility clusters within 5km of the newly paved highway ('exposed') and those outside 10km ('unexposed'), before and after the highway was paved in 2009. The model takes the form (Equation 1):

$$\log(dengue_{i,t}) = \sum_{\tau \in \{2000,\ldots,2020\}, \tau != 2008} \beta_\tau \, (DIST)_i * 1\{t = \tau\} + \gamma_i + \lambda_t + X_{i,t}\theta + \epsilon_{i,t}$$

where i is unit (healthcare facility cluster) and t is year. Our outcome of interest, $\log(dengue_{i,t})$, is the logged dengue incidence in healthcare facility cluster i for year t. Our exposure variable, DIST, is a dummy (binary) variable that equals one for exposed clusters (<5km) and zero for unexposed clusters (>10km). The DIST dummy variable interacts with year dummy variables

(one for each year of interest and zero otherwise). We omit 2008, the year before the highway was paved, as the baseline year. The coefficients, β's, on the interaction term then estimate the response in our outcome of interest following the road paving. Each coefficient estimates the log of the incidence rate ratio between near and far healthcare facilities for each year in our set of τ's. We include unit (i.e., healthcare facility cluster) fixed effects (FEs), γ_i, to help account for any time-invariant healthcare facility characteristics, such as differences in their invasion history of *Aedes* mosquitoes, proximity to protected areas, and baseline dengue burden at the community level. FEs are a set of dummy variables that each represent a group (e.g., healthcare facility clusters), demeaning the data within each group. We also include year FEs, λ_t, to account for large-scale changes that might impact dengue comparably across all healthcare facility clusters over time, such as El Niño events, economic recessions, and global pandemics. Finally, we include four observable controls, noted by the matrix $X_{i,t}$, to explicitly control for any variation due to changes in urban area, agricultural area, temperature, or precipitation. The Θ vector recovers our covariate coefficients. Any remaining unobserved variation is captured by the error term, $\epsilon_{i,t}$.

If our hypothesis that the highway paving increased dengue transmission in healthcare facility clusters near to the highway is correct, we expect the β coefficients will diverge from zero and become positive following 2008. Coefficients remaining at zero prior to 2008 would support the parallel trends assumption and the validity of using the unexposed group as controls. As a 'negative control' test, we conducted the same analysis with leishmaniasis incidence as our outcome, with the expectation of no significant differences in outcome between exposed and unexposed groups post-road paving.

Models were run as Poisson regressions using the *fixest* package in R with standard errors

clustered at the unit-level to account for autocorrelation between pre- and post-road paving in the same healthcare facility cluster. We tested the sensitivity of model estimates to population weighting, excluding Puerto Maldonado (the region's capital), changing the spatial boundary delineating exposed and unexposed groups, changing the buffer zone size between exposed and unexposed groups, including both confirmed and probable cases, and only including spatial units reporting disease case data both pre- and post-road paving (**STable S2, SFig S3**). We also compared linear climate variables to quadratic forms (**STable S3**). Finally, we conducted permutation inference analysis, where we randomized our exposure variable using three methods—randomizing across clusters and years, randomizing across clusters while maintaining temporal structure, and randomizing across years while maintaining spatial structure—to establish that there are no spurious cross-sectional or temporal phenomena contributing to the observed effect (**SFig S4**).

Because rainfall is highly seasonal in this region and dengue transmission peaks in the rainy season (**SFig S9**), we also ran biannual models to account for heterogeneous effects across seasons. These models were equivalently parameterized except *t* was biannual time periods (October–March, April–September for each year) that were constructed to align with the rainy season (months with above average monthly accumulated precipitation from 2000–2022, **SFig S9**). Biannual models included biannual fixed effects in place of yearly fixed effects.

Finally, we estimated aggregated versions of Equation 1 to summarize average treatment effects and the total number of attributable cases (e.g., a long-differences model). We define a post-paving dummy variable equal to zero in 2008 and one from 2009 to 2022. The aggregated model takes the form (Equation 2):

$$log(dengue_{i,t}) = \beta\ (DIST)_i * 1\{t \in [2009,2022]\}_t + \gamma_i + \lambda_t + X_{i,t}\theta + \epsilon_{i,t}$$

This is also the model specification used for aggregating biannual model average treatment effects (with biannual fixed effects substituted for yearly fixed effects, **Fig 2B** and **Fig 3B**). We also used the aggregated model to compare groups of healthcare facility clusters at increasing distances from the highway—1km, 5km, 10km, 15km, and 20km—to a control group of healthcare facility clusters >20km from the highway (**Fig 4A**). We calculated bootstrapped standard errors for this analysis given the small sample size of some of the distance subgroups (i.e., 1km, 5km, etc.). We bootstrapped by sampling healthcare facility clusters with replacement, retaining the full time series for each cluster in the bootstrapped sample and maintaining the ratio of exposed to unexposed units for each subgroup. In all other analyses we used analytic standard errors.

Attributable cases

We calculated percent changes in dengue incidence due to highway paving as (Equation 3):

$$percent\ change\ dengue_t = (\exp\ (\beta)_t - 1) * 100$$

where *t* is year and β_t is the log of the incidence rate ratio between near and far healthcare facilities for each year (i.e., coefficients from Equation 1 that account for fixed effects and control variables). We similarly calculated the number of cases attributable to the highway paving by exponentiating the β coefficient recovered from our aggregated model (i.e., calculating incidence rate ratios that account for fixed effects and control variables) and using the following approximation (Equation 4):

$$attributable\ cases\ =\ pcet\ (\frac{exp(\beta)\ -\ 1}{exp(\beta)})$$

where $pcet$ is the proportion of cases exposed to treatment (47).

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Figure legends

Figure 1: A map of Madre de Dios (A) displaying the Interoceanic highway (black line), unpaved roads (grey lines), rivers (blue lines), and the locations of all healthcare facility clusters colored by their exposure group (points). Clusters within 5km of the road are considered exposed, those >10km are considered unexposed, and those between 5-10km from the road were excluded from analyses as a buffer against spillover effects of exposure to the highway. Average dengue (B) and leishmaniasis (C) yearly incidence rates (cases per person per year) over the time period of interest colored by exposure group (buffer clusters are not included). Vertical dotted line at 2008 represents the last year before the highway was paved, which is the baseline year in our analyses.

Figure 2: Dengue incidence increased substantially in healthcare facility clusters near to the highway compared to those far from the highway due to highway paving. Panel A displays estimation results from Equation 1. Panel B displays results for the biannual models aggregated across the full time period (Equation 2). Average treatment effects are represented by points, and 95% confidence intervals are represented by error bars (standard errors clustered at the unit level). The vertical dotted line at 2008 represents the last year before the highway was paved (baseline year). A table displaying yearly results (**STable S1**) and a figure displaying disaggregated biannual estimates (**SFig S6**) can be found in the **Supplementary Information**.

Figure 3: Leishmaniasis incidence remains stable post highway paving in healthcare facility clusters near to the highway compared to those far from the highway. Panel A displays estimation results from Equation 1. Panel B displays results for the biannual models aggregated across the full time period (Equation 2). Average treatment effects are represented by points, and 95% confidence intervals are represented by error bars (standard errors clustered at the unit

566 level). The vertical dotted line at 2008 represents the last year before the highway was paved
567 (baseline year). A table displaying yearly results (**STable S1**) and a figure displaying
568 disaggregated biannual estimates (**SFig S6**) can be found in the **Supplementary Information**.

569 **Figure 4:** Effect of the highway attenuates when communities are at distances of greater than
570 10km from the highway. Panel A displays estimation results from Equation 2, where a model
571 was run for each distance subgroup (colors). Average treatment effects are represented by points,
572 and 95% bootstrapped confidence intervals are represented by error bars (bootstrapped standard
573 errors clustered at the unit level). Panel B displays a map of the Madre de Dios region with
574 healthcare facility clusters colored by their distance subgroup (i.e., 1km, 5km, etc.).

Highway paving dramatically increased dengue transmission in the Amazon

Supplementary Information

Supplementary Text

Disease diagnosis

As defined by the Peruvian Ministry of Health, laboratory confirmed dengue cases are cases that produce a positive result from one or more of the following tests: "1) DENV isolation by cell culture, 2) qRT-PCR, 3) ELISA antigen NS1, 4) detection of IgM antibodies for dengue in a single sample by ELISA in dengue endemic areas, and/or 5) evidence of IgM seroconversion in paired samples, where the second sample should be taken after 14 days of the onset of symptoms in areas where there is no transmission of dengue (and these cases should have an epidemiological investigation)" (1). Probable dengue cases are clinically diagnosed and must either meet case definition criteria set by the World Health Organization (2) or have a direct epidemiological link to a confirmed case (1).

Laboratory confirmed leishmaniasis cases are defined as cases producing a positive result from "1) a parasitological examination, 2) an immunological or histopathological clinical , and/or 3) a culture, whether or not in the form of a pruritic nodule." Probable cases of cutaneous leishmaniasis were clinically diagnosed and defined as, "any person with progression to ulcerative or ulcerous- crusted lesions, shallow, rounded, non-painful, with well-defined borders and inflammatory signs, with evolution time of no less than four weeks and lack of response to conventional treatment. With a history of origin or residence in … areas endemic to leishmaniasis." Probable cases of mucocutaneous leishmaniasis were clinically diagnosed and defined as, "any person with a clinical picture characterized by raised granulomatous or ulcerative lesions of the nasal mucosa, mouth, white palate, pharynx, larynx or trachea, with a history of active or healed skin lesions, origin or residence in endemic areas (3, 4)."

Clustering

We identified latitude and longitude for all 105 healthcare facilities using a combination of government sources and the *ubigeo* package in R (5, 6) (**SFig S7A**). We then grouped healthcare facilities into 71 clusters to standardize the size of their catchment areas and created non-overlapping boundaries to extract climate and land use data (**SFig S7B**). Clustering was done using an iterative hierarchical clustering approach that joined healthcare facilities in groups within a pre-specified distance (*hclust* and *cutree* functions from the *stats* R package). We used a pre-specified distance of 7.5km and visually checked that this provided sufficient separation between clusters (**SFig S7B**). Finally, we created non-overlapping shapefile boundaries with the goal of capturing all populated and non-forested areas near each of the healthcare facility clusters. We built these boundaries by creating circular buffer zones with a radius of 7.5km and then assigning each pixel within the buffer zones to the closest healthcare facility cluster, eliminating any overlap between clusters (done using the *distanceFromPoints* function from the *raster* package in R). A radius of 7.5km was chosen to ensure all population centers and non-forested land would be captured (determined via visualization of satellite images and WorldPop

raster data in Google Earth Engine (7)).

Population calculation

Locality population data was also provided by DIRESA for the years 2009–2017 and 2020–2022. This population data was linked to case data through the locality names in the disease case dataset and then clustered as described above. In years without DIRESA-provided population data, we imputed missing values with WorldPop data (7) by multiplying each cluster-year WorldPop value by the corresponding cluster-specific average ratio between DIRESA and WorldPop values. WorldPop is a remotely-sensed population dataset that estimates the number of people living in each 100m pixel from 2000–2019 (7). It is important to measure annual changes in each cluster's population due to the rapid in-migration and population growth in the area during the study period (8) (**SFig S5**). We use these population measures to derive incidence rates from disease case counts as a measure of changes in per-capita dengue risk, above and beyond changes in raw case counts that would be expected due to population growth alone.

Supplementary Table S1: Dengue and leishmaniasis yearly estimation results (Equation 1). Standard errors are clustered at the unit level for all models and are displayed inside parentheses next to each estimated coefficient. P-values indicating a statistical significance level below 0.05 are designated with an asterisk (*).

	Dengue Yearly	**Leish Yearly**
5km x 2000	-0.47* (0.16)	-0.60* (0.27)
5km x 2001	-0.25 (0.22)	0.09 (0.29)
5km x 2002	-0.15 (0.21)	-0.84* (0.28)
5km x 2003	-0.14 (0.21)	-0.12 (0.29)
5km x 2004	-0.04 (0.16)	-0.50 (0.26)
5km x 2005	0.02 (0.17)	-0.02 (0.24)
5km x 2006	0.03 (0.18)	-0.44 (0.23)
5km x 2007	0.08 (0.12)	-0.64* (0.23)
5km x 2008	----	----
5km x 2009	0.56* (0.23)	0.14 (0.21)
5km x 2010	1.2* (0.37)	-0.20 (0.20)
5km x 2011	0.91* (0.31)	-0.09 (0.24)
5km x 2012	1.2* (0.34)	0.42 (0.30)
5km x 2013	0.92* (0.35)	0.60* (0.29)
5km x 2014	0.57 (0.30)	0.31 (0.28)
5km x 2015	0.45 (0.23)	0.40 (0.36)
5km x 2016	0.38 (0.22)	0.44 (0.23)
5km x 2017	0.18 (0.25)	0.34 (0.38)
5km x 2018	1.1* (0.39)	0.71 (0.38)
5km x 2019	1.7* (0.51)	0.78 (0.45)
5km x 2020	1.5* (0.30)	0.44 (0.31)
5km x 2021	0.98* (0.34)	0.38 (0.29)
5km x 2022	0.96* (0.42)	0.60 (0.33)
log(Urban)	0.17 (0.10)	-0.03 (0.08)
log(Ag)	-0.22 (0.17)	0.38* (0.14)
Mean Precip	0.05 (0.19)	-0.06 (0.13)
Mean Temp	0.22 (0.39)	0.40 (0.30)
FEs		
Cluster	X	X
Year	X	X
Cor^2	0.561	0.606
N	1,494	1,495
Clusters	65	65

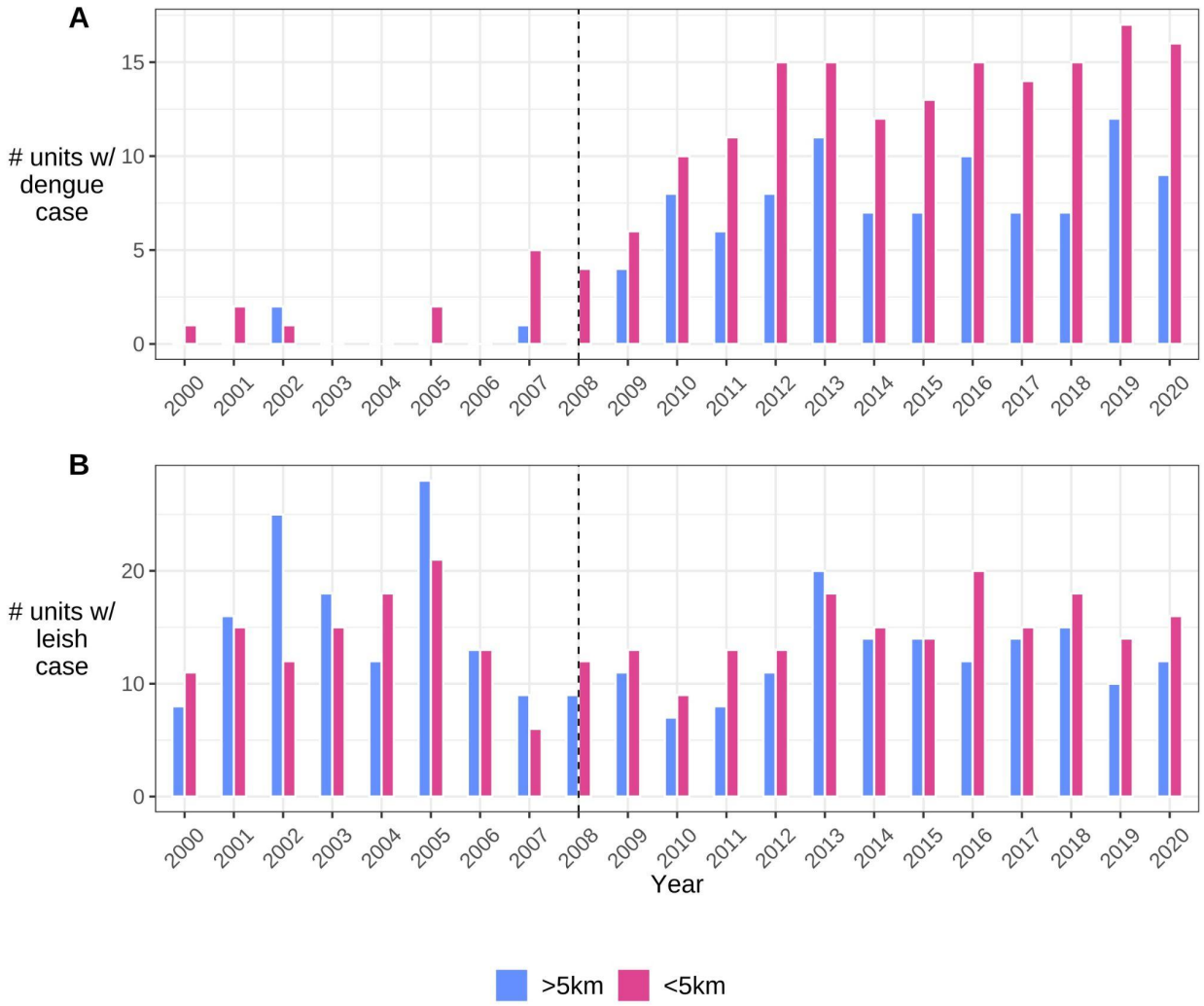
STable S2: Dengue long-differences estimation results (Equation 2) are displayed for the following model specifications: main model, model excluding Puerto Maldonado (the region's largest city), population-weighted model also excluding Puerto Maldonado, model designating <1km from the highway as exposed and >1km from the highway as unexposed, model designating <10km as exposed and >10km as unexposed, model with no spatial buffer (<5km exposed, >5km unexposed), model with a larger spatial buffer (<5km exposed, >20km unexposed), model including both confirmed and probable cases, and model including only spatial units reporting at least one disease case both pre and post road paving. All models include unit and year fixed effects. Standard errors are clustered at the unit level for all models and are displayed inside parentheses next to each estimated coefficient. P-values indicating a statistical significance level below 0.05 are designated with an asterisk (*).

	Main model	**No PM**	**Pop weight no PM**	**1km boundary**	**10km boundary**	**No buffer (<5km v >5km)**	**Large buffer (<5km v >20km)**	**Conf & prob cases**	**Units reporting pre & post**
5km x Post-2008	1.0* (0.28)	0.98* (0.28)	1.3* (0.60)			0.91* (0.28)	1.1* (0.27)	1.1* (0.28)	1.2* (0.35)
1km x Post-2008				0.62* (0.31)					
10km x Post-2008					1.1* (0.26)				
log(Urban)	0.09 (0.10)	0.09 (0.10)	0.29 (0.33)	0.13 (0.12)	0.11 (0.10)	0.14 (0.12)	0.28 (0.18)	0.09 (0.11)	0.07 (0.15)
log(Ag)	-0.23 (0.14)	-0.27 (0.14)	0.32 (0.24)	-0.20 (0.14)	-0.20 (0.13)	-0.20 (0.13)	-0.35* (0.15)	-0.25 (0.15)	-0.27 (0.15)
Sum Precip	0.09 (0.24)	0.04 (0.24)	-0.53 (0.33)	0.06 (0.22)	0.07 (0.22)	0.08 (0.22)	0.15 (0.26)	0.07 (0.24)	-0.03 (0.25)
Mean Temp	-0.05 (0.47)	-0.10 (0.48)	1.2* (0.61)	0.11 (0.46)	0.14 (0.46)	0.16 (0.46)	0.08 (0.48)	-0.06 (0.53)	0.12 (0.48)
Cor^2	0.54541	0.55571	0.5468	0.55196	0.55612	0.55421	0.56119	0.58093	0.61472
N	974	959	959	1,064	1,064	1,064	854	974	735
Clusters	65	64	64	71	71	71	57	65	49

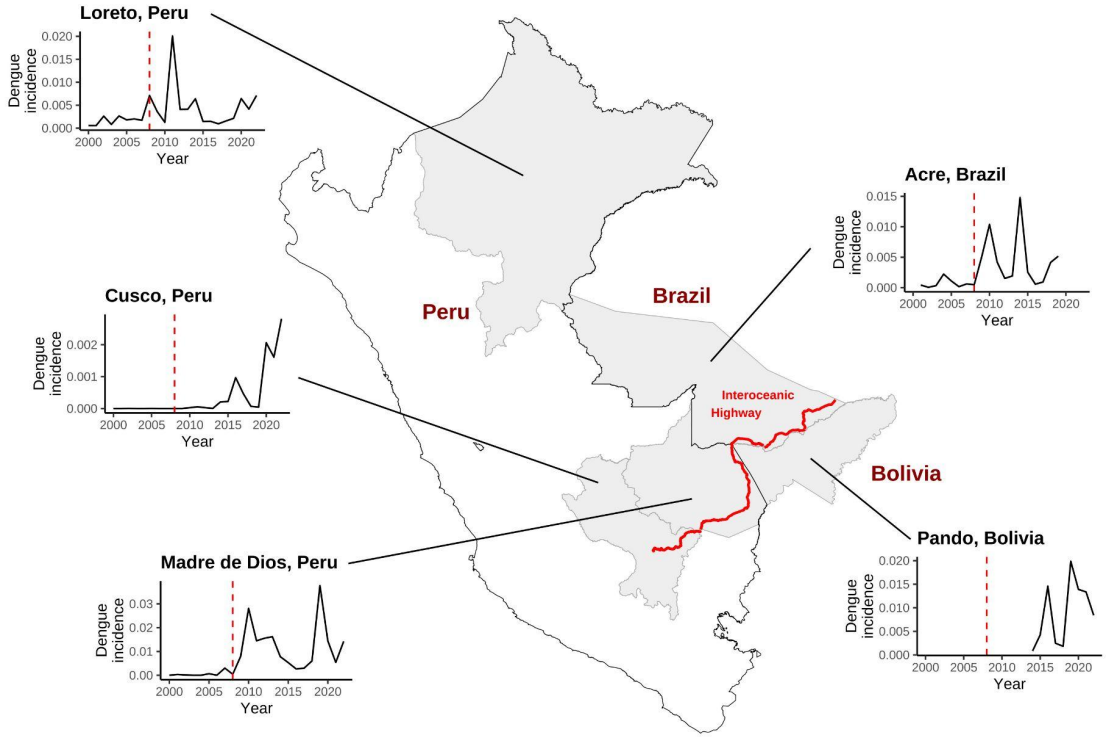
STable S3: Dengue and leishmaniasis incidence long-differences estimation results (Equation 2) with precipitation and temperature covariates as quadratic functions (rather than linear). Corresponds to **Table 1** in main text. Yearly models include cluster and yearly fixed effects, and biannual models include cluster and biannual fixed effects. Standard errors are clustered at the unit level for all models and are displayed inside parentheses next to each estimated coefficient. P-values indicating a statistical significance level below 0.05 are designated with an asterisk (*).

	Dengue Yearly	**Dengue Biannual Dry**	**Dengue Biannual Rainy**	**Leish Yearly**	**Leish Biannual Dry**	**Leish Biannual Rainy**
5km x Post-2008 Yearly	1.0* (0.28)			0.42 (0.24)		
5km x Post-2008 Biannual		0.52* (0.21)	1.0* (0.24)		0.47* (0.20)	0.17 (0.15)
log(Urban)	0.09 (0.10)	0.10 (0.07)	0.06 (0.09)	-0.004 (0.07)	0.03 (0.04)	-0.04 (0.04)
log(Ag)	-0.23 (0.14)	-0.17 (0.12)	-0.24 (0.15)	0.23 (0.15)	0.14 (0.14)	0.21 (0.11)
Sum Precip^2	0.01 (0.03)	0.19 (0.27)	-0.002 (0.04)	-0.05* (0.01)	-0.36* (0.12)	-0.03* (0.01)
Mean Temp^2	-9.6e-5 (0.0008)	0.0003 (0.0004)	-0.0001 (0.0008)	0.002* (0.0007)	0.0009* (0.0004)	0.0006 (0.0006)
FEs						
Cluster	X	X	X	X	X	X
Year	X			X		
Biannual		X	X		X	X
Cor^2	0.54538	0.33792	0.54779	0.53878	0.5937	0.55868
N	974	1,040	1,040	975	1,040	1,040
Clusters	65	65	65	65	65	65

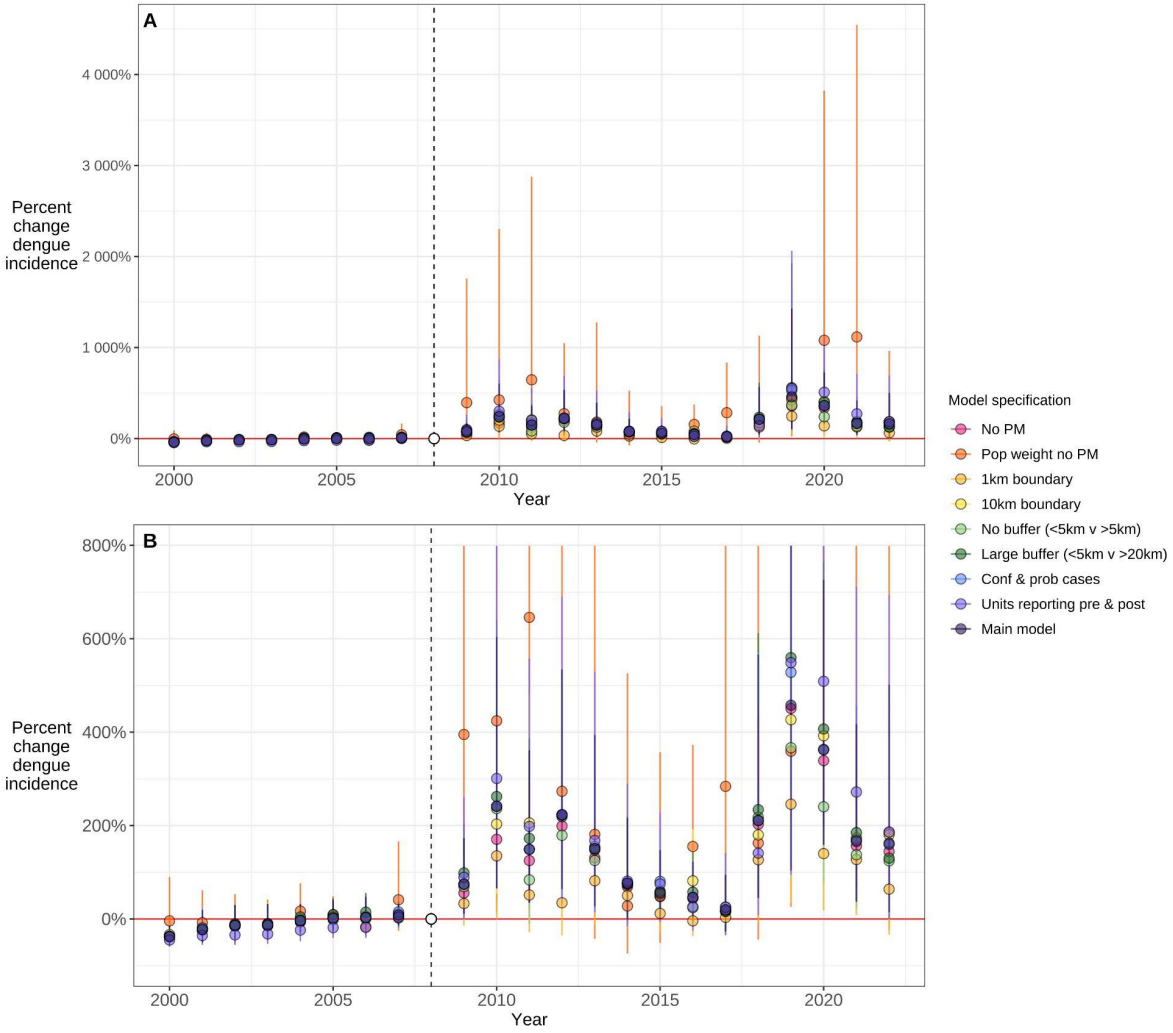
Supplementary Figure S1: Bar plots displaying disease case data coverage: number of healthcare facility clusters (i.e., spatial units) reporting at least one dengue (Panel A) or leishmaniasis (Panel B) case by year and exposure group (color). All clusters are included in this plot to display dengue dynamics throughout the region (i.e., clusters >5km and <10km from the highway are not excluded). Prior to paving, dengue was reported in Puerto Maldonado, Iberia, Mavila, Mazuko, Iñapari, Planchon, Loero, Laberinto, and Boca Colorado.



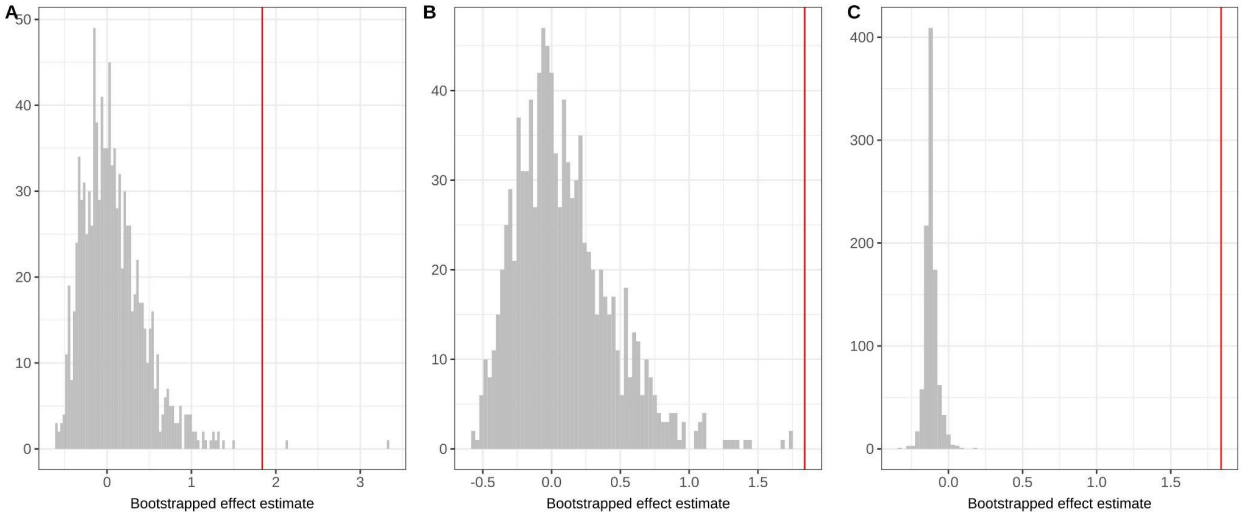
Supplementary Figure S2: Time series plots of yearly dengue incidence rates for regions bordering and otherwise relevant to Madre de Dios (9–11). Interoceanic Highway colored in red. Clockwise from top right: Acre, Brazil, dengue-endemic pre-2009 and bordering to the Northeast; Pando, Bolivia, dengue-endemic pre-2009 and bordering to the East (no data pre 2014); Madre de Dios, Peru, study setting; Cusco, Peru, only a small portion of the department is suitable for dengue, bordering to the West; Loreto, Peru, dengue-endemic and the only other substantially populated Amazonian department in Peru, not bordering Madre de Dios.



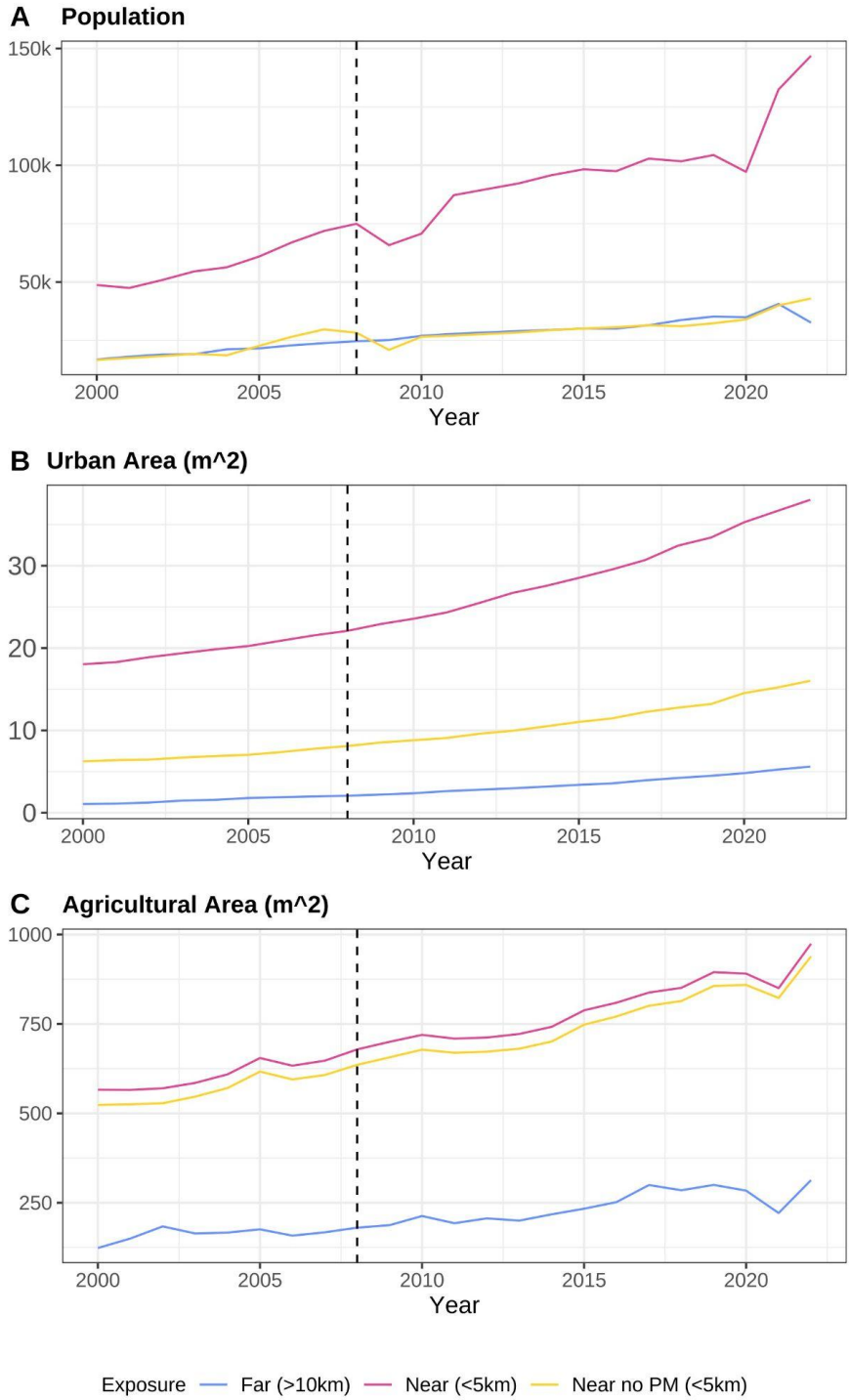
Supplementary Figure S3: Model results are robust to various model specifications. Estimation results from Equation 1 for the following model specifications (colors): main model, model excluding Puerto Maldonado (the region's largest city), population-weighted model also excluding Puerto Maldonado, model designating <1km from the highway as exposed and >1km from the highway as unexposed, model designating <10km as exposed and >10km as unexposed, model with no spatial buffer (<5km exposed, >5km unexposed), model with a larger spatial buffer (<5km exposed, >20km unexposed), model including both confirmed and probable cases, and model including only spatial units reporting at least one disease case both pre and post road paving. Average treatment effect (points) with 95% confidence interval (error bars). Standard errors clustered at the unit level. Vertical dotted line at 2008 representing the last year before the highway was paved (baseline year).



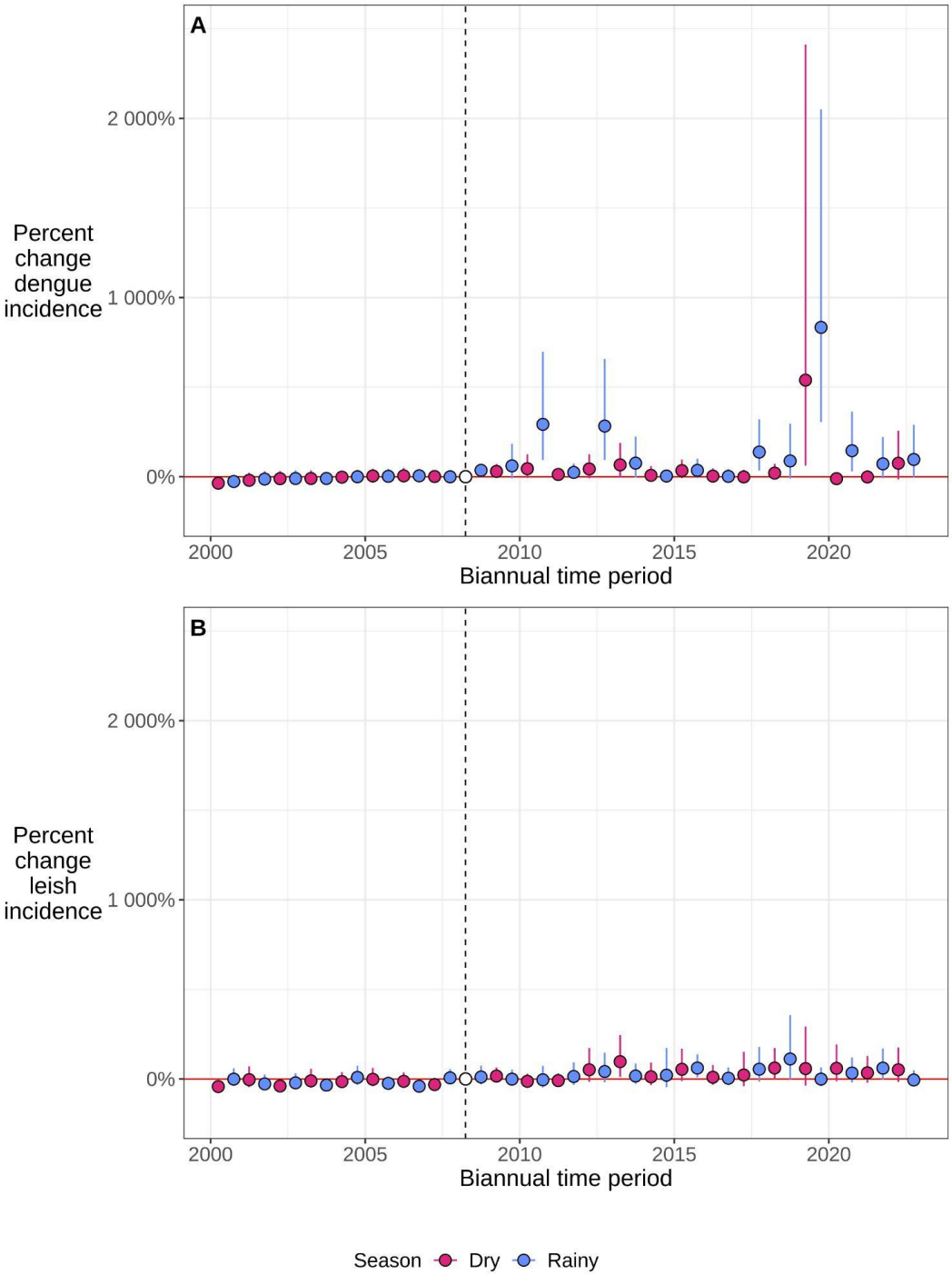
Supplementary Figure S4: Permutation inference analysis. Histograms of bootstrapped effect estimates from three methods of randomly shuffling the treatment variable (grey bars) compared to the estimated effect from the non-bootstrapped sample (vertical red line). The three methods include: A) Random, the entire treatment column is randomly shuffled across clusters and years, B) Full, randomly assign clusters to treatment, maintaining temporal structure, and C) Within, randomly assign years as treated within clusters, maintaining spatial structure.



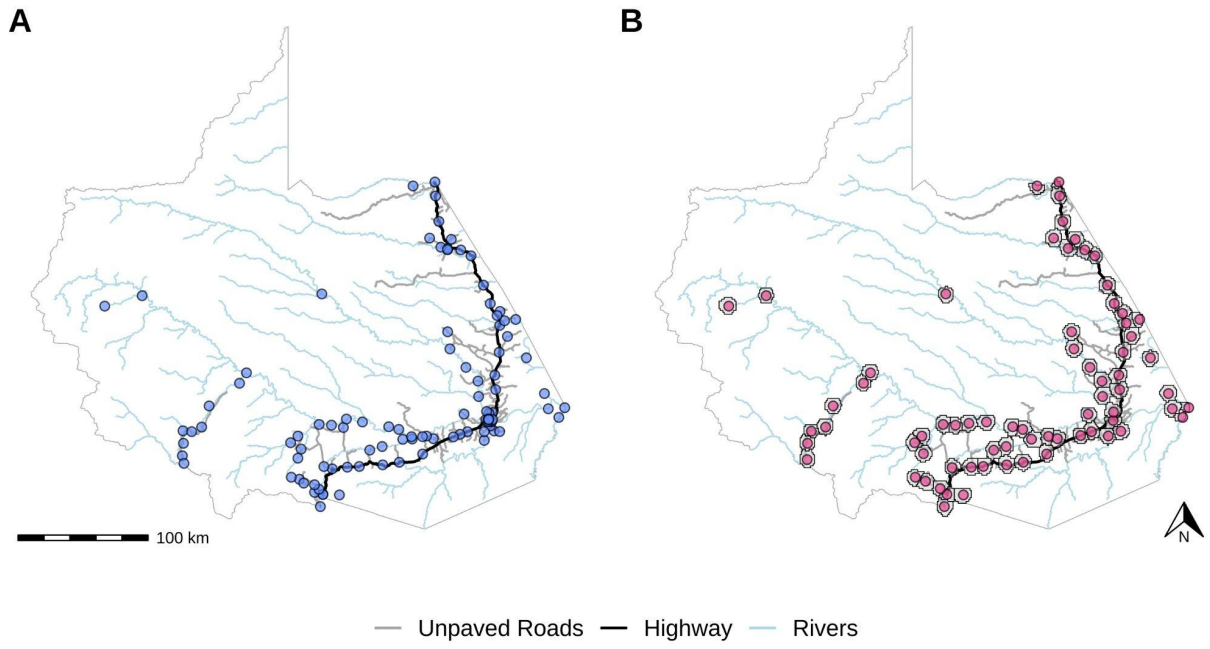
Supplementary Figure S5: Time series plots displaying yearly measures of population, urban area, and agricultural area by exposure group (color). Yellow line displays the 'exposed' group (pink) excluding Puerto Maldonado (PM), the largest city in the region. Sources and calculations of these plots are discussed in the **Methods** section and in the **Supplementary Information**.



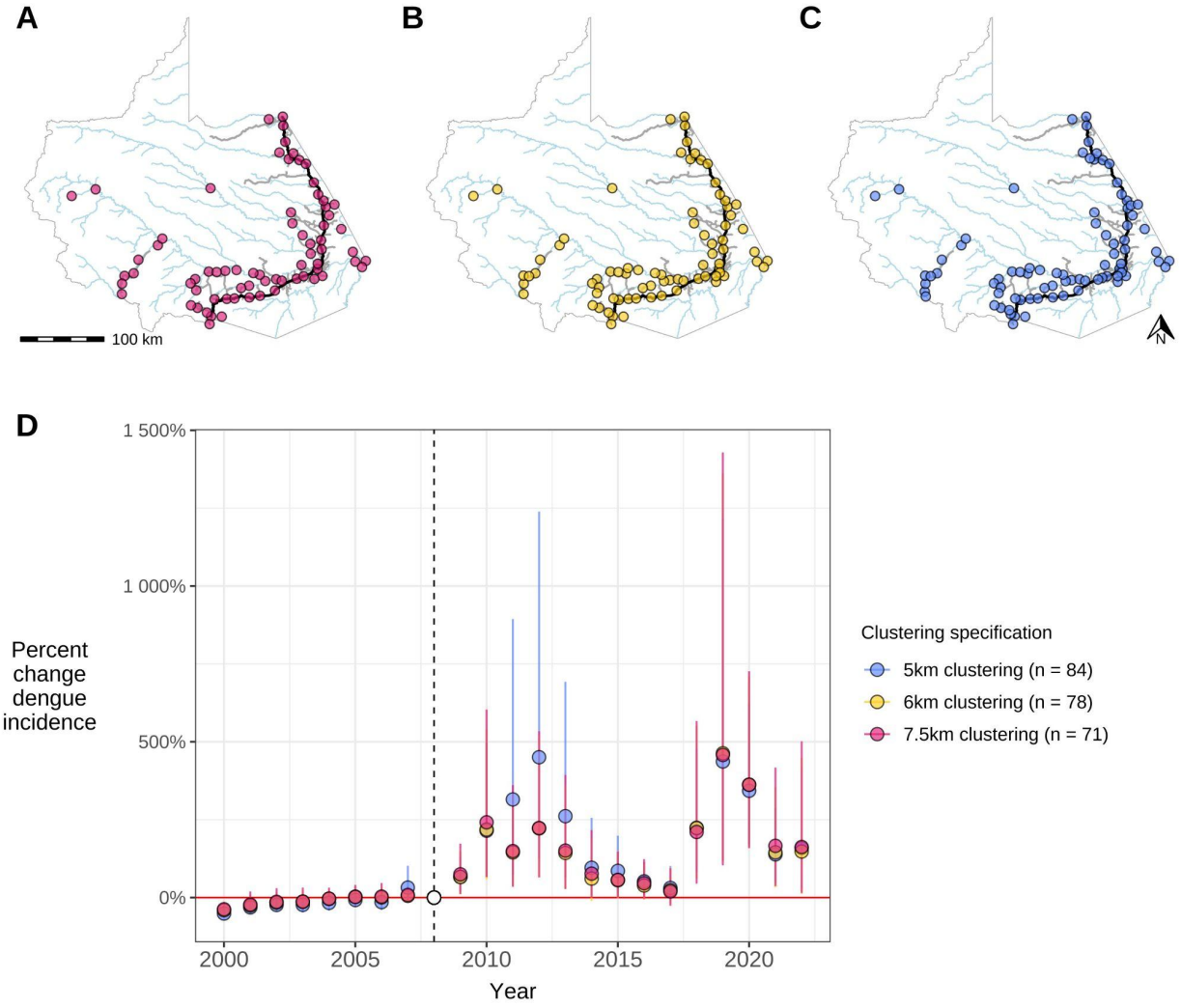
Supplementary Figure S6: Plots display disaggregated biannual model results for dengue (A) and leishmaniasis (B) from Equation 1, colored by season (rainy v dry). Average treatment effects are represented by points, and 95% confidence intervals are represented by error bars (standard errors clustered at the unit level). The vertical dotted line at 2008 represents the last year before the highway was paved (baseline year).



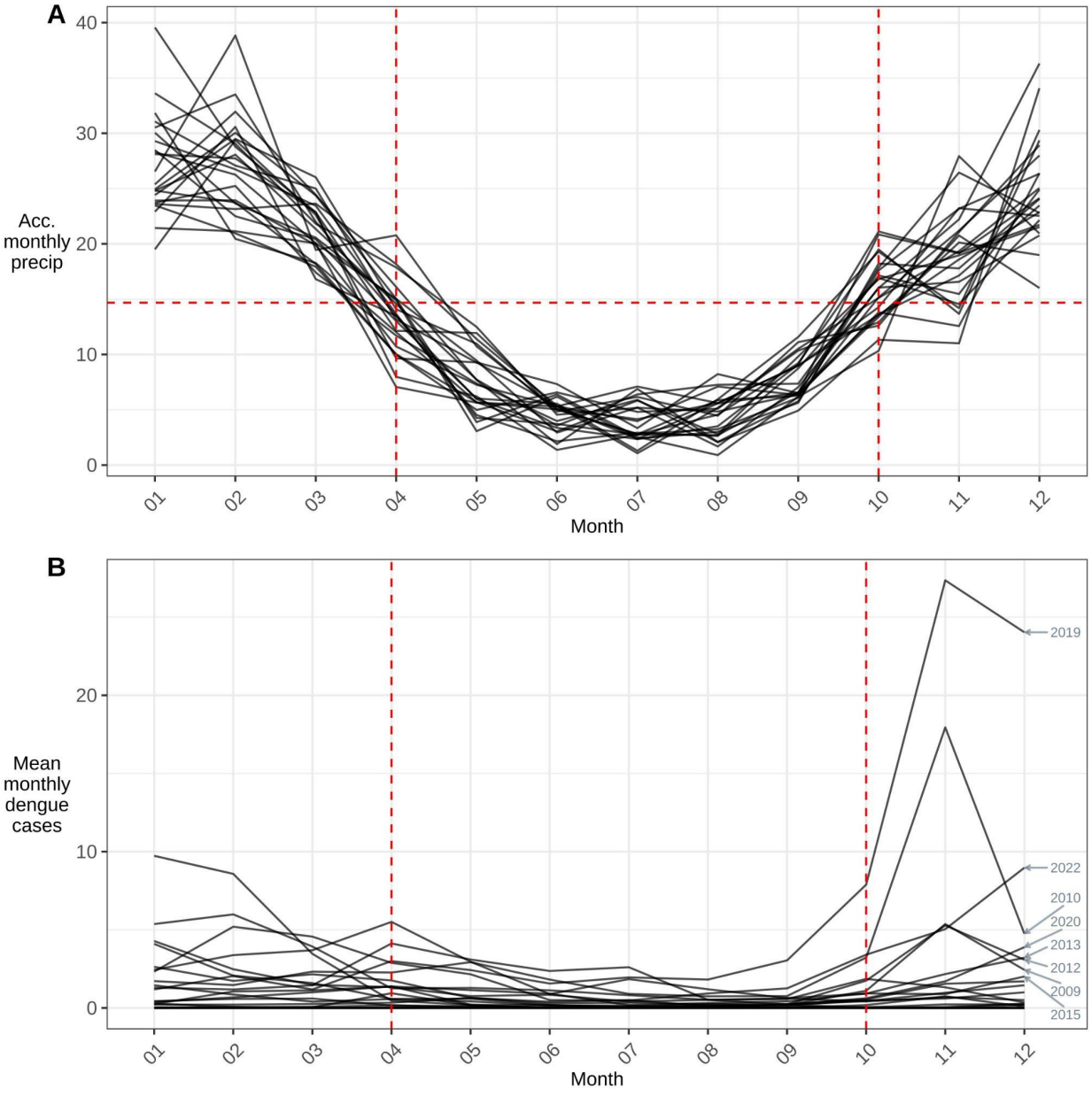
Supplementary Figure S7: Panel A shows all healthcare facilities (n = 105, blue points) mapped onto the study region. Panel B shows healthcare facility clusters (n = 71, pink points) and constructed spatial boundaries (grey polygons) mapped onto the study region.



Supplementary Figure S8: Panel A shows healthcare facility clusters clustered with a radius of 7.5km (n = 71, pink points, main model). Alternative clustering specifications with radii 6km and 5km are shown in Panels B (n = 78, yellow points) and C (n = 84, blue points), respectively. Panel D shows estimation results from Equation 1 for the differing clustering specifications (colors same). Average treatment effect (points) with 95% confidence interval (error bars). Standard errors clustered at the unit level. Vertical dotted line at 2008 representing the last year before the highway was paved (baseline year).



Supplementary Figure S9: Seasonality of monthly accumulated precipitation (A) and dengue cases (B) in Madre de Dios, Peru during the study period (2000–2022). The red horizontal line in panel A shows the monthly accumulated precipitation across the entirety of the study period. The red vertical lines show the months that intersect with the red horizontal line delineating rainy (October – March) versus dry season (April – September).



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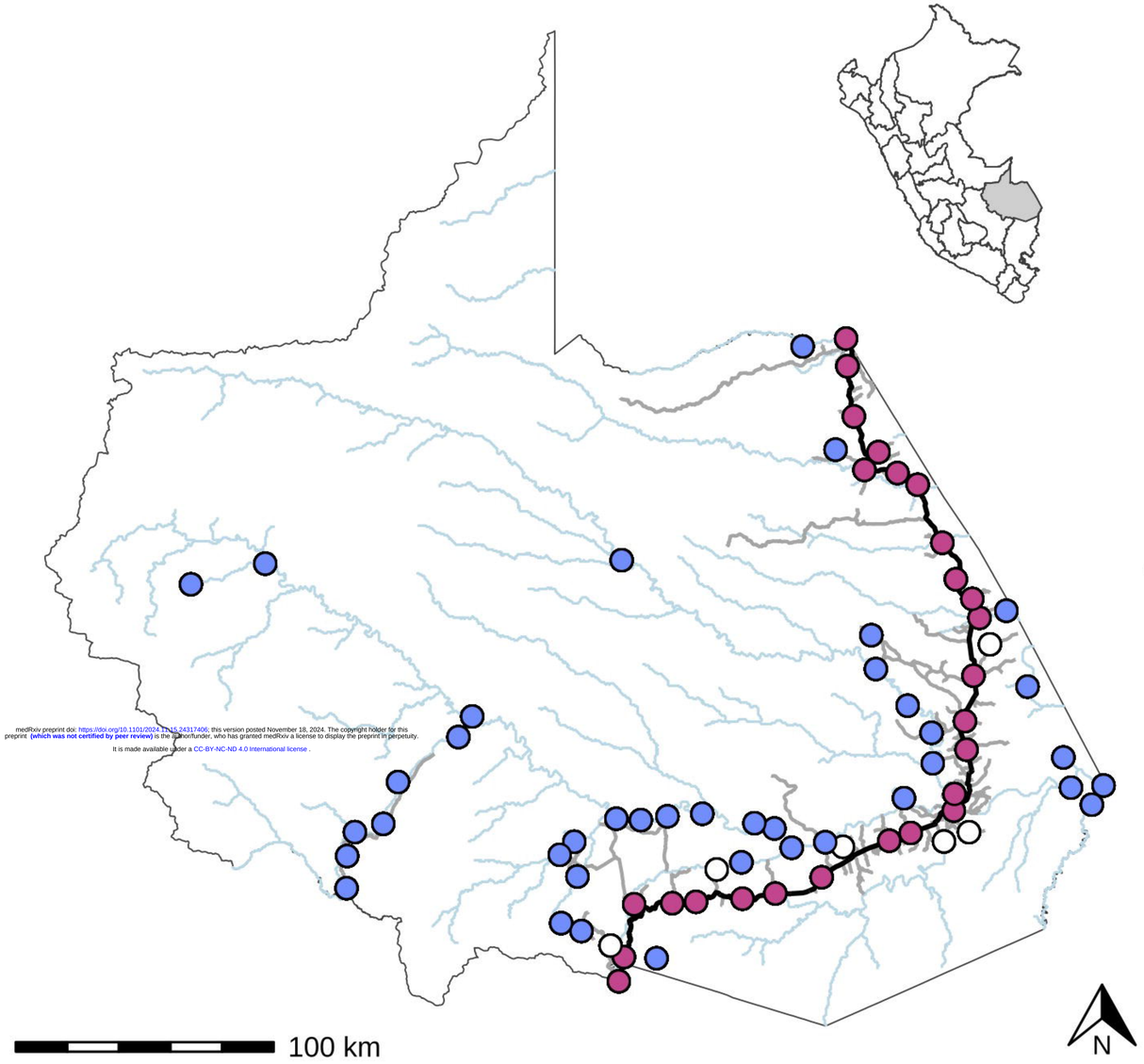
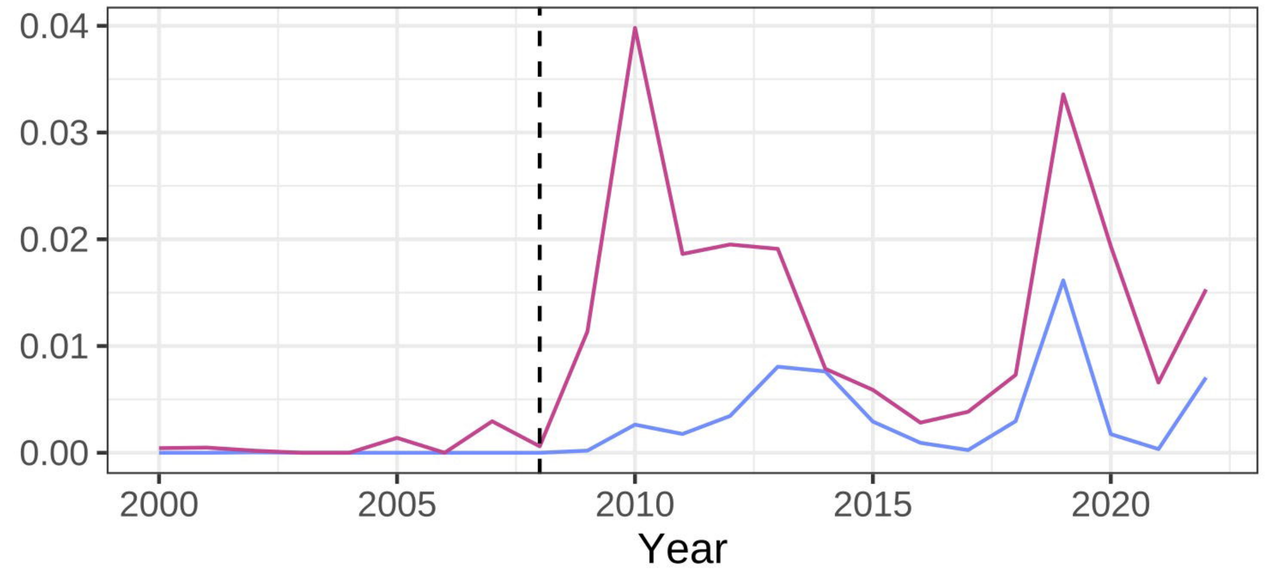
A



B



C

Exposed (<5km) Unexposed (>10km) Buffer (removed) Unpaved Roads Highway Rivers