N2O concentrations and fluxes. Triplicate samples for dissolved N2O concentrations were collected by completely filling 120 mL glass serum bottles at wrist depth below water surface at each site. After preserving with 0.5 mL saturated ZnCl2 solution, the serum bottles were sealed with butyl stoppers, crimped with aluminum caps, and stored at ambient temperature in the dark. Local ambient air samples were also taken and used to back-calculate N2O concentration in water in equilibrium with the atmosphere. N2O concentrations were determined via the headspace equilibration method on a gas chromatography equipped with an electron capture detection for N2O (Agilent 7890B GC-µECD). The partial pressure of CO2 (pCO2) in surface water was determined following our earlier work.

FN2O was measured simultaneously with dissolved N2O concentration collection. Four floating chambers were placed at each transects, covering depth gradients from the river bank to the mid-channel to capture the spatial heterogeneity. Measurements lasted for 1 h at each site while drifting, and 50 mL gas extracted from inside the chambers at 0, 5, 10, 20, 40, and 60 min interval were injected into air-tight gas sampling bags for analysis in the laboratory by GC-µECD. These chambers were of the same size and shape and streamlined with a flexible plastic foil collar to minimize the effects of chamber-induced turbulence when measuring fluxes, and were covered with aluminum foil to reflect the sunlight and minimize internal heating.

Surface water and sediment samples were collected simultaneously with gas samples for physicochemical and microbial analyses at each site, respectively. Air temperature, air pressure, and wind speed were measured in situ with a portable anemometer (Testo 480). DO, pH, ORP, conductivity, and water temperature were measured in situ with portable field probes (Hach HQ40d). Annual air temperature and precipitation were obtained from the National Meteorological Information Center (http://data.cma.cn/).

Flux computation. Total FN2O were calculated according to the equation below:

Ft = (nt-n0)/(Axt)

where, nt and n0 are the number of moles of N2O in the chamber at time t and time zero (mol), respectively; A is the surface area of water covered by the chamber (m2) and t is the measurement duration time (min). Diffusive and ebullitive N2O fluxes were separated using the Soued et al. approach. Briefly, assuming that Ft for CO2 (FCO2) is exclusively diffusive (that is, CO2 ebullition is negligible). FCO2 were computed from the linear regression of pCO2 against time to eliminate possible bias due to gas accumulation in the chamber headspace that will affect the flux rates. The theoretical diffusive k for N2O was calculated based upon kCO2 as follows (54):

kN2O/kCO2 = (ScN2O/ScCO2)-n

where, kN2O and kCO2 are gas transfer velocity of N2O and CO2, respectively; Sc is the Schmidt number and n is assigned a value of 1/2 for wind speed > 3.6 m s-1 or 2/3 for wind speed < 3.6 m s-1. We then calculated the theoretical diffusive FN2O according to

Fd = k · (Cwater – Ceq)

where k is gas transfer velocity (m·d-1), Cwater is water gas concentration (mol m-3), and Ceq is gas concentration in water in equilibrium with the local atmosphere corrected for temperature induced changes in solubility according to the Henry’s law (mol m-3). Thus, the difference between the total and diffusive N2O fluxes is attributable to ebullition.

Dissolved N2 concentrations. Triplicatesamples for dissolved N2 were collected from surface water by completely filling 12 mL glass vials (Labco Exetainer) at wrist depth below water surface at each site, then preserved by adding 100 µL saturated ZnCl2, and stored at ambient temperature in the dark. Dissolved N2 concentrations were analyzed with a membrane inlet mass spectrometer (MIMS; PrismaPlus, Pfeiffer Vacuum). In brief, we measured ratios of N2/Ar concentration using MIMS, then calculated Ar concentrations at in-situ water temperature, pressure, and salinity. Finally, dissolved N2 concentrations were obtained by multiplying N2/Ar with calculated Ar concentrations. The excess N2 concentration (ΔN2) was then calculated as:

ΔN2 = [N2]measured - [N2]eq.