

Supplement of

Biogeochemical and hydrological controls on the oxygen isotopic composition of nitrification-produced nitrate in an agricultural soil

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Text S1: Dynamic Nitrification Model Development and Calibration

Here we provide details for the dynamic nitrification model developed to simulate the temporal evolution of NO_2^- and NO_3^- concentrations and their isotopic composition under varying temperatures, NH_4^+ concentrations, and $\delta^{18}\text{O}_{\text{H}_2\text{O}}$. The model consists of two coupled components. The first is a biomass- and temperature-dependent reaction framework based on Michaelis–Menten–Monod (MMM) kinetics that simulates N fluxes among reaction pools during mineralization and nitrification. The second is a non–steady-state O isotope module coupled to the MMM framework that tracks O isotope dynamics and simulates the temporal evolution of $\delta^{18}\text{O}_{\text{NO}_3}$ during laboratory incubations.

S1.1 Michaelis-Menten-Monod Framework

The MMM framework provides a mechanistic representation of microbially mediated reaction dynamics by linking reaction rates to substrate availability and microbial biomass (Maggi and Riley, 2009). Under MMM kinetics, reaction rates for the coupled mineralization–nitrification pathways are expressed as:

$$\frac{d[\text{orgN}]}{dt} = -k_1 E_1 \frac{[\text{orgN}]}{[\text{orgN}] + K_1} \quad (\text{S1})$$

$$\frac{d[\text{NH}_4^+]}{dt} = k_1 E_1 \frac{[\text{orgN}]}{[\text{orgN}] + K_1} - k_2 E_2 \frac{[\text{NH}_4^+]}{[\text{NH}_4^+] + K_2} \quad (\text{S2})$$

$$\frac{d[\text{NO}_2^-]}{dt} = k_2 E_2 \frac{[\text{NH}_4^+]}{[\text{NH}_4^+] + K_2} - k_3 E_3 \frac{[\text{NO}_2^-]}{[\text{NO}_2^-] + K_3} \quad (\text{S3})$$

$$\frac{d[\text{NO}_3^-]}{dt} = k_3 E_3 \frac{[\text{NO}_2^-]}{[\text{NO}_2^-] + K_3} \quad (\text{S4})$$

where k_1 , k_2 and k_3 are rate constants (h^{-1}), K_1 , K_2 and K_3 are half-saturation concentrations (mol L^{-1}), and E_1 , E_2 and E_3 represent enzyme pools (mol L^{-1}) associated with organic N (orgN) mineralization, NH_4^+ oxidation, and NO_2^- oxidation, respectively.

Enzyme concentrations (E_1 - E_3) are assumed to be linearly proportional to microbial biomass. Biomass increases in response to substrate consumption following Monod kinetics (Monod, 1949) and decreases due to mortality. The governing equations for biomass and enzyme dynamics are:

$$\frac{dB_1}{dt} = -Y_1 \frac{d[\text{orgN}]}{dt} - m_1 B_1 = \frac{1}{z} \frac{dE_1}{dt} \quad (\text{S5})$$

$$\frac{dB_2}{dt} = Y_2 k_2 E_2 \frac{[\text{NH}_4^+]}{[\text{NH}_4^+] + K_2} - m_2 B_2 = \frac{1}{z} \frac{dE_2}{dt} \quad (\text{S6})$$

$$\frac{dB_3}{dt} = Y_3 k_3 E_3 \frac{[\text{NO}_2^-]}{[\text{NO}_2^-] + K_3} - m_3 B_3 = \frac{1}{z} \frac{dE_3}{dt} \quad (\text{S7})$$

where Y_1 , Y_2 and Y_3 are biomass yield coefficients expressing the biomass gain per consumed mass of substrate (g mol^{-1}), m_1 , m_2 and m_3 are biomass mortality rates (h^{-1}), and B_1 , B_2 and B_3 denote microbial biomass (g L^{-1}) associated with each process. The parameter z is the enzyme-to-biomass ratio and was fixed at $10^{-7} \text{ mol g}^{-1}$, assuming a small E/B ratio following Maggi & Riley (2009). Based on these definitions, the maximum specific biomass growth rate ($\mu_{\max} = zkY$) (h^{-1}), representing microbial growth under non-limiting substrate conditions, and the net biomass growth rate ($\mu_{\max} - m$, h^{-1}) can be derived for each microbial group.

The MMM module does not explicitly represent microbial NH_4^+ or NO_3^- assimilation. Previous studies showed that only approximately 1% of NH_4^+ consumed by *Nitrosomonas europaea* was assimilated into biomass even during the logarithmic growth phase, with the vast majority being oxidized for energy conservation (Weidinger et al., 2007). We therefore assume that microbial assimilation represents a relatively minor N sink and is implicitly incorporated into the calibrated effective rates of mineralization and NH_4^+ oxidation. This assumption is supported by the observations that substantial NO_3^- accumulation occurred even in the absence of NH_4^+ amendment (Fig. 4c in the main text), and that in all NH_4^+ -amended treatments, cumulative NO_3^- production eventually exceeded the amount of NH_4^+ added (Fig. 4a-4e in the main text). Together, these observations suggest that microbial assimilation did not represent a sufficiently large N sink to substantially alter the inferred NH_4^+ oxidation dynamics.

The kinetic parameters of the MMM framework include rate constants (k), half-saturation constants (K), biomass yield (Y), and mortality rate (m). Their temperature sensitivity was incorporated using the near-activation and differential activation (NADA) framework (Maggi and Riley, 2015; Maggi et al., 2018), in which kinetic parameters are expressed in terms of thermodynamic quantities:

$$k = \frac{K_b T}{h} (K_{NA}^\ddagger)^\lambda \quad (\text{S8})$$

$$K = \frac{1}{K_{NA}^\ddagger} + (K_{NA}^\ddagger)^{\lambda-1} \quad (\text{S9})$$

$$Y = \frac{1}{Z} K_d^\ddagger (K_{NA}^\ddagger)^{-\lambda} \quad (\text{S10})$$

$$m = \frac{K_b T}{h} K_m^\ddagger \quad (\text{S11})$$

where K_b is the Boltzmann constant (1.3806×10^{-23} J/K); T is absolute temperature (K); h is the Planck constant (1.054×10^{-34} J s); λ is the differential activation energy factor (-); $K_{NA}^\ddagger$, $K_d^\ddagger$ and $K_m^\ddagger$ are Boltzmann factors associated with near-activation complexation, cell doubling, and mortality, respectively (-). These Boltzmann factors can be expressed as temperature-dependent functions of Gibbs free energy $\Delta G_{NA}^\ddagger$, $\Delta G_d^\ddagger$ and $\Delta G_m^\ddagger$, respectively:

$$K_{NA}^\ddagger = e^{-\frac{\Delta G_{NA}^\ddagger}{RT}} \quad (\text{S12})$$

$$K_d^\ddagger = e^{-\frac{\Delta G_d^\ddagger}{RT}} \quad (\text{S13})$$

$$K_m^\ddagger = e^{-\frac{\Delta G_m^\ddagger}{RT}} \quad (\text{S14})$$

where R is the gas constant and T is the absolute temperature. The free energies $\Delta G_{NA}^\ddagger$, and $\Delta G_d^\ddagger$ can be further decomposed into the Gibbs–Helmholtz enthalpic (ΔH), entropic (ΔS), and heat capacity (ΔC_p) contributions, that is, $\Delta G = \Delta H - T\Delta S$ with (Maggi et al., 2018):

$$\Delta H = \Delta H_0 + \Delta C_p (T - T_0) \quad (\text{S15})$$

$$\Delta S = \Delta S_0 + \Delta C_p \ln (T/T_0) \quad (\text{S16})$$

where ΔH_0 and ΔS_0 are the enthalpy and entropy at the reference temperature T_0 (298.15K; Maggi et al., 2018). In contrast, $\Delta G_m^\ddagger$ was not further decomposed but was treated directly as a calibration parameter. For each reaction, the thermodynamic parameters of the chemical pathway ($\Delta H_{0,NA}^\ddagger$, $\Delta S_{0,NA}^\ddagger$, $\Delta C_{p,NA}^\ddagger$ and λ) and of the biological pathway ($\Delta H_{0,d}^\ddagger$, $\Delta S_{0,d}^\ddagger$, $\Delta C_{p,d}^\ddagger$, and $\Delta G_m^\ddagger$) were treated as calibration parameters.

S1.2 Time-dependent Oxygen Isotope Module

A non-steady-state O isotope module was coupled to the MMM framework by leveraging N transformation fluxes simulated at each time step. The formulation largely follows Buchwald and Casciotti (2013) and resolves three sequential processes within each time step: (1) NH_4^+ oxidation, including associated biologically mediated O exchange between NO_2^- and water, (2)

NO₂⁻ oxidation, and (3) abiotic O exchange between NO₂⁻ and water (Fig. 1 and Table 1 in the main text). Unlike the time-integrated O isotope model (Eq. 1 and 2 in the main text), this non-steady-state formulation explicitly simulates NO₂⁻ concentrations and isotopic composition, making it possible to evaluate how transient NO₂⁻ accumulation influences δ¹⁸O_{NO3}. In addition, whereas nitrifier-catalyzed and abiotic O exchange between NO₂⁻ and water are lumped into a single parameter f_{ex} in Eq. 1 and 2 of the main text, these processes are represented independently here, allowing their relative contributions to be quantified.

In the first step, NO₂⁻ is produced via NH₄⁺ oxidation, incorporating O atoms from both O₂ and water. The δ¹⁸O of produced NO₂⁻ (δ¹⁸O_{NO2,AO}) is estimated using a mass balance approach given by Casciotti et al. (2010):

$$\delta^{18}O_{NO2,AO} = 1/2(\delta^{18}O_{O2} + \delta^{18}O_{H2O} - {}^{18}\epsilon_{k,comp})(1 - f_{ex,AO}) + (\delta^{18}O_{H2O} + {}^{18}\epsilon_{eq})f_{ex,AO} \quad (S17)$$

where the fraction of biologically catalyzed NO₂⁻-water O exchange is denoted as $f_{ex,AO}$. Because NO₂⁻ accumulation is expected to integrate the effects of temperature, substrate availability, and reaction kinetics, $f_{ex,AO}$ is treated as a dynamic variable that depends on instantaneous NO₂⁻ concentration:

$$f_{ex,AO} = a \times [NO_2^-] + b \quad (S18)$$

where a and b are calibration parameters.

In the second step, NO₂⁻ is oxidized to NO₃⁻. The δ¹⁸O of NO₂⁻ consumed during oxidation and added to the NO₃⁻ pool (δ¹⁸O_{NO2,NO}) is calculated using a Rayleigh distillation model (Mariotti et al., 1981):

$$\delta^{18}O_{NO2,NO} = \delta^{18}O_{NO2,0} + {}^{18}\epsilon_{k,NO} \frac{\ln(f)f}{1-f} \quad (S19)$$

where δ¹⁸O_{NO2,0} represents the δ¹⁸O of the current bulk NO₂⁻ pool, and f is the fraction of NO₂⁻ remaining after the consumption. Subsequently, because one additional O atom from water is incorporated during NO₃⁻ formation, the δ¹⁸O of produced NO₃⁻ (δ¹⁸O_{NO3,NO}) is:

$$\delta^{18}O_{NO3,NO} = 2/3\delta^{18}O_{NO2,NO} + 1/3(\delta^{18}O_{H2O} - {}^{18}\epsilon_{k,H2O,2}) \quad (S20)$$

In the final step, abiotic O exchange between NO₂⁻ and water is modeled using a first-order rate law (Buchwald and Casciotti, 2013):

$$\delta^{18}O_{NO2,ab} = (\delta^{18}O_{NO2,b} - \delta^{18}O_{H2O} - {}^{18}\epsilon_{eq})e^{-k_{abiotic}t} + \delta^{18}O_{H2O} + {}^{18}\epsilon_{eq} \quad (S21)$$

where $\delta^{18}\text{O}_{\text{NO}_2, \text{b}}$ is the $\delta^{18}\text{O}$ of the bulk NO_2^- pool after NH_4^+ and NO_2^- oxidation, and $\delta^{18}\text{O}_{\text{NO}_2, \text{ab}}$ is the value after abiotic equilibration at the end of the time step. The abiotic exchange rate constant k_{abiotic} (d^{-1}) is parameterized as a function of temperature and pH based on experimental results from Buchwald and Casciotti (2013):

$$k_{\text{abiotic}} = \left(-0.0166 - 1.4123e^{-\left(\frac{\ln(T/317.71)}{0.0462}\right)^2} \right) \times \text{pH} + 0.1467 + 10.193e^{-\left(\frac{\ln(T/316.49)}{0.0447}\right)^2} \quad (\text{S22})$$

Finally, the equilibrium isotope effect $^{18}\epsilon_{\text{eq}}$ is parameterized as a linear function of temperature ($^{\circ}\text{C}$) (Buchwald & Casciotti 2013):

$$^{18}\epsilon_{\text{eq}} = -0.12T + 16.01 \quad (\text{S23})$$

Kinetic O isotope effects ($^{18}\epsilon_{\text{k,comp}}$, $^{18}\epsilon_{\text{k,H}_2\text{O},2}$, and $^{18}\epsilon_{\text{k,NO}}$) are likewise represented as linear functions of temperature, with slopes and intercepts determined through calibration.

S1.3 Model Calibration

All parameters subject to calibration for the MMM framework and the non-steady-state O isotope module are listed in Tables S2 and S3, respectively. The two model components were calibrated sequentially. First, the MMM module was calibrated collectively across all soil incubation treatments using observed NO_2^- and NO_3^- concentrations. Consistent with previous studies (Maggi et al., 2018), concentrations were represented internally in units of mol L^{-1} , allowing the use of previously established parameter ranges, and were converted to mg N kg^{-1} soil for comparison with experimental observations. Model simulations were performed with a one-minute time step.

The PEST software (Doherty, 2004), using the Levenberg-Marquardt algorithm, was adopted to minimize the difference between observations and simulations. The objective function for MMM model calibration consisted of three components: NO_3^- concentrations ($n = 199$), NO_2^- concentrations ($n = 199$), and peak NO_2^- concentrations ($n = 13$):

$$\Phi = \frac{1}{n} \sum_{i=1}^{n=199} [(\text{NO}_{3,\text{obs},i}^- - \text{NO}_{3,\text{sim},i}^-)]^2 + \frac{1}{n} \sum_{i=1}^{n=199} [(\text{NO}_{2,\text{obs},i}^- - \text{NO}_{2,\text{sim},i}^-) \times 10]^2 + \frac{1}{n} \sum_{i=1}^{n=13} [(\text{peak_NO}_{2,\text{obs},i}^- - \text{peak_NO}_{2,\text{sim},i}^-) \times 10]^2 \quad (\text{S24})$$

where subscripts *obs* and *sim* indicate observation and simulation, respectively. A scaling factor of 10 was applied to the NO_2^- concentration and peak NO_2^- concentration residuals to compensate for their smaller magnitudes relative to NO_3^- concentrations, thereby ensuring that all three objective function components contributed comparably to the optimization.

In the second step, the O isotope model was coupled to the optimized MMM model, and the O isotope-specific parameters were independently calibrated against observed $\delta^{18}\text{O}_{\text{NO}_3}$ value using the PEST software. The objective function minimized residuals in $\delta^{18}\text{O}_{\text{NO}_3}$ ($n = 597$):

$$\Phi = \frac{1}{n} \sum_{i=1}^{n=597} [(\delta^{18}\text{O}_{\text{NO}_3, \text{obs}, i} - \delta^{18}\text{O}_{\text{NO}_3, \text{sim}, i})]^2 \quad (\text{S25})$$

Model performance for N concentrations and $\delta^{18}\text{O}_{\text{NO}_3}$ values in each treatment was evaluated using the correlation coefficient (R) and the normalized root mean squared error (NRMSE), defined as:

$$R = \frac{\text{cov}(m, o)}{\sigma_m \sigma_o} \quad (\text{S26})$$

$$\text{NRMSE} = \frac{\sqrt{\frac{1}{n} \sum_{i=1}^n (m_i - o_i)^2}}{\max\{o\} - \min\{o\}} \quad (\text{S27})$$

where m and o are the modelled and observed values from the nitrification experiments; n is number of observations; σ_m and σ_o are the standard deviation of m and o , respectively.

Text S2: HYDRUS-1D Model Setup

S2.1 Meteorological data and crop rotation setup

The meteorological data input to the HYDRUS-1D model included daily precipitation, potential evaporation, and potential transpiration for 2019–2022, retrieved from gages within the Upper Embarras River (UER) watershed. Daily precipitation was obtained from three gauges distributed across the watershed, maintained by the Midwestern Regional Climate Center (MRCC) cli-MATE network (MRCC, 2023), and from a National Weather Service (NWS) station (WARM, 2015) located at the Illinois State Water Survey (Fig. S1). Daily reference evapotranspiration and additional meteorological data (e.g., soil temperature, relative humidity) were also retrieved from the same NWS station. Following the FAO-56 methodology, potential evaporation and transpiration were computed as reference evapotranspiration multiplied by the evaporation coefficient and basal crop coefficient, respectively (Allen et al., 1998, 2005). Potential evapotranspiration was further scaled by a factor of 0.75 to reproduce the long-term watershed discharge-to-precipitation ratio. Corn and soybean were assumed to alternate annually, with corn planted in odd-numbered years and soybean in even-numbered years. We assumed that corn was planted on May 1 and harvested on October 21, whereas soybean was planted on May 21 and harvested on October 5 (David et al., 2009).

S2.2 Model description

The HYDRUS-1D model (Šimůnek et al., 2016) simulates variably saturated water flow in a one-dimensional soil column by solving the Richards equation (Richards, 1931). The soil profile was defined as a 100-cm column discretized into 101 nodes at 1-cm intervals, assuming homogeneous soil texture. Soil hydraulic properties were represented using the Van Genuchten (1980) model. An atmospheric boundary condition with a surface layer was applied at the upper boundary, driven by daily precipitation, potential evaporation and transpiration. Free drainage was specified at the lower boundary.

Actual evaporation was computed by HYDRUS-1D based on the applied upper boundary flux potential evaporation, which was imposed until the critical pressure head at the soil surface ($h_{critA} = -100,000$ cm) was reached, after which the evaporation flux was regulated by this threshold. Actual transpiration was calculated by HYDRUS-1D as root water uptake under water stress conditions. Water stress effects on root water uptake were simulated using the Feddes model (Feddes et al., 1978), in which uptake is distributed across soil layers according to depth-specific root density. Feddes parameters for corn and soybean were based on Wesseling et al. (1991) and vertical root distribution followed the Hoffman & Van Genuchten (1983) model. Maximum rooting depths were set to 93 cm for corn and 80 cm for soybean (Naylor et al., 2016). Rooting depth dynamics were simulated using the Verhulst–Pearl logistic growth function (Šimůnek & Suarez, 1993), such that 50% of maximum rooting depth was reached halfway through the growing season.

To represent crop rotation with distinct rooting dynamics, we used Phyrus (Collenteur et al., 2019), a Python adaptation of HYDRUS-1D, to execute HYDRUS-1D simulations. The model was initialized with intermediate soil moisture conditions (between field capacity and the wilting point) and run continuously from 2019 to 2022. The pressure head profile at the end of each simulation year was used as the initial condition for the subsequent year.

S2.3 Hydrological fluxes derivation

Following the two-layer configuration (30-cm topsoil and 70-cm subsoil), water fluxes were derived separately for each control volume. Because HYDRUS-1D assumes that evaporation occurs at the soil surface (Šimůnek et al., 2016), all evaporation was attributed to the topsoil. Transpiration in topsoil (T_1) and subsoil (T_2) was derived from HYDRUS-1D outputs by integrating nodal transpiration along the designated soil depths. Discharge (Q) is the outflow from the subsoil. The fluxes between two layers including percolation (R) and capillary rise (C) were calculated by water balance using soil water content derived from the HYDRUS-1D model. Percolation transfers water from the topsoil downward to the subsoil while capillary rise is the upward flow from the subsoil to the topsoil. These simulated fluxes served as inputs for the subsequent StorAge Selection (SAS) function-based transport modeling.

209 Text S3: Simulation of Evaporation Isotopic Fractionation

210 Within the SAS-based transport framework, evaporative enrichment on individual water parcels
 211 in the age-ranked storage can be tracked over time using a Lagrangian approach (Queloz et al.,
 212 2015; Miller et al., 2026). Specifically, for a water parcel entering the topsoil storage at time t_i ,
 213 its isotopic composition, $\delta_s(T, T + t_i)$, evolves with age T under evaporative fractionation
 214 according to:

$$215 \quad \frac{d\delta_s(T, T+t_i)}{dT} = (\delta^* - \delta_s(T, T + t_i))(1 - f^m) \quad (S28)$$

216 where δ^* is the limiting isotopic composition determined by local atmospheric conditions, m is
 217 the enrichment slope, and f is the remaining fraction of the parcel after evaporation. As
 218 evaporation proceeds and f decreases toward zero, $\delta_s(T, T+t_i)$ asymptotically approaches δ^* . The
 219 parameter f is updated at each time step as (Queloz et al., 2015; Miller et al., 2026):

$$220 \quad f = 1 - \frac{E(T+t_i)p_E(T, T+t_i)}{S(T+t_i)p_S(T, T+t_i)} \quad (S29)$$

221 where $E(T+t_i)$ and $S(T+t_i)$ are topsoil evaporation flux and topsoil storage at time $T+t_i$,
 222 respectively; $p_E(T, T+t_i)$ is the fractional contribution of the water parcel (entered at t_i) to topsoil
 223 evaporation; and $p_S(T, T+t_i)$ is its fractional contribution to topsoil storage.

224 The terms δ^* and m in Eq. S28 are defined following Good et al. (2014):

$$225 \quad \delta^* = \frac{h_a \delta_A + \varepsilon_k + h_s \varepsilon^+}{h_a - 10^{-3}(\varepsilon_k + h_s \varepsilon^+)} \quad (S30)$$

$$226 \quad m = \frac{h_a - 10^{-3}(\varepsilon_k + h_s \varepsilon^+)}{h_s - h_a + 10^{-3} \varepsilon_k} \quad (S31)$$

227 where ε^+ (‰) is the temperature-dependent equilibrium isotope effect between liquid and vapor
 228 phases; ε_k (‰) is the humidity-dependent kinetic isotope effect for diffusion; h_a (-) represents
 229 the relative humidity of the atmosphere; h_s (-) is the relative humidity of the air of the soil pores;
 230 and δ_A (‰) is the isotopic composition of atmospheric vapor. At each model time step, we used
 231 the daily relative humidity measured at 2 m above ground from the NWS site in the UER for h_a
 232 (Fig. S1). The isotopic composition of atmospheric vapor, δ_A , was assumed to be in isotopic
 233 equilibrium with local precipitation δ_P (Gibson et al., 2008):

$$234 \quad \delta_A = \frac{\delta_P - \varepsilon^+}{\alpha^+} \quad (S32)$$

235 where α^+ (-) is the equilibrium fractionation factor and is calculated as a function of air
 236 temperature T_a (K) using the experimentally derived relationship following Horita &
 237 Wesolowski (1994):

$$10^3 \ln[\alpha^+] = -7.685 + 6.7123 \left(\frac{10^3}{T_a} \right) - 1.6664 \left(\frac{10^6}{T_a^2} \right) + 0.35041 \left(\frac{10^9}{T_a^3} \right) \quad (\text{S33})$$

The associated equilibrium isotope effect ε^+ is derived from α^+ as:

$$\varepsilon^+ = (\alpha^+ - 1) \times 1000 \quad (\text{S34})$$

The kinetic isotope effect ε_k is estimated following Craig and Gordon (1965):

$$\varepsilon_k = n(h_s - h_a) \left(1 - \frac{D_i}{D} \right) \times 1000 \quad (\text{S35})$$

where the term D_i/D is the diffusivity ratio of heavy to light oxygen isotopes, taken as 0.9859 (Vogt, 1976), and n is the aerodynamic diffusion parameter. The formulation of n accounts for soil water content θ dependence and varies from 0.5 when the soil is saturated to 1 when the soil is dried (Mathieu & Bariac, 1996):

$$n = 1 - 0.5 \frac{\theta - \theta_r}{\phi - \theta_r} \quad (\text{S36})$$

where θ_r is the residual water content (0.09), and ϕ is the soil porosity (0.482), based on silty clay loam parameters in HYDRUS-1D.

Finally, h_s is calculated following Lee and Pielke (1992):

$$h_s = \beta + (1 - \beta)h_a \quad (\text{S37})$$

where β increases with θ , defined as:

$$\beta = \min\left(1, \frac{1}{4} \left(1 - \cos \frac{\theta}{\theta_{fc}} \pi\right)^2\right) \quad (\text{S38})$$

In this function, β equals to 1 when θ is greater than or equal to the field capacity θ_{fc} , which is assumed to be 0.376 according to David et al. (2009).

256

257 **Text S4: Extended Analysis of Model-Simulated Nitrification Dynamics**

258 The calibrated MMM module resolved the coupled temporal dynamics of NH_4^+ and NO_2^-
 259 oxidation rates, inorganic N pools, and microbial biomass under the experimental conditions
 260 (Fig. S9 and S10). Across all NH_4^+ -amended treatments, NH_4^+ oxidation initially exceeded NO_2^-
 261 oxidation because NH_3 oxidizer biomass was initially greater than NO_2^- oxidizer biomass (0.015
 262 g L^{-1} vs. 0.0023 g L^{-1} ; Table S2), resulting in transient NO_2^- accumulation. The magnitude and
 263 duration of this accumulation depended on how rapidly NO_2^- oxidizer biomass developed

relative to NH_3 oxidizers, allowing NO_2^- oxidation to catch up with NH_4^+ oxidation. At a given temperature, increasing NH_4^+ addition increased both the peak NH_4^+ oxidation rate and the duration of active NH_4^+ oxidation, thereby prolonging the imbalance between the two oxidation processes and resulting in greater and more persistent NO_2^- accumulation (Fig. S9).

At a given NH_4^+ amendment level, simulated NO_2^- accumulation was greatest at intermediate temperatures (14–28 °C), although the underlying mechanisms differed among temperatures (Fig. S10). At 21 °C and 28 °C, the higher net growth rates of NH_3 oxidizers (Fig. 7f in the main text) allowed NH_4^+ oxidation to consistently outpace NO_2^- oxidation, and this imbalance persisted until declining NH_4^+ concentrations became limiting (Fig. S10g,h). At 14 °C, NO_2^- oxidizers exhibited higher net growth rates than NH_3 oxidizers (Fig. 7f in the main text), allowing NO_2^- oxidation to catch up with NH_4^+ oxidation before the latter reached its maximum activity (Fig. S10i). However, because both processes proceeded with significantly lower reaction rate constants (Fig. 7a in the main text), this convergence did not occur until approximately 386 h (16.1 d) after incubation began, resulting in substantial NO_2^- accumulation during the first ~450 h (18.8 d). At 7 °C, further reductions in reaction rate constants slowed both oxidation processes, producing relatively low but prolonged NO_2^- accumulation despite the higher net growth rate of NO_2^- oxidizers (Fig. 7a,f in the main text). In contrast, at 35 °C, the combination of a much higher NO_2^- oxidation rate constant and faster biomass development rapidly compensated for the initially smaller NO_2^- oxidizer population. Consequently, the two oxidation processes became tightly coupled within approximately 84 h (3.5 d) of incubation (Fig. S10f), resulting in negligible NO_2^- accumulation.

286 **Supplementary Tables and Figures:**

287 **Table S1.** Chemical and physical properties of the sampled surface soil.

Soil Properties	Values
soil texture (%sand, %clay, %silt)	Silty clay loam (17.5%, 27.5%, 55%)
bulk density (g/m ³)	1.3
pH (1:1 soil to water ratio)	6.27
total carbon (C) content (%)	1.81
total N content (%)	0.12
C:N ratio (%/%)	14.7

288

Table S2. Summary of parameters used in the MMM model. The parameters in parentheses in the first column were calibrated. The lower and upper parameter bounds used for model calibration were selected based on values reported in previous studies (Maggi et al., 2018; Maggi & La Cecilia, 2016; Maggi & Riley, 2015, 2017).

^a Parameters	Units	Optimal	Lower bound	Upper bound
$(\Delta H_{0,NA,1}^\ddagger)$	(kJ/mol)	-1.79	-50	0
$(\Delta H_{0,NA,2}^\ddagger)$	(kJ/mol)	-3.48	-50	0
$(\Delta H_{0,NA,3}^\ddagger)$	(kJ/mol)	-13.40	-50	0
$(\Delta S_{0,NA,1}^\ddagger)$	(J/K mol)	5.82	0	200
$(\Delta S_{0,NA,2}^\ddagger)$	(J/K mol)	73.11	0	200
$(\Delta S_{0,NA,3}^\ddagger)$	(J/K mol)	122.61	0	200
$(\Delta C_{p,NA,1}^\ddagger)$	(kJ/mol K)	0.06	0	10
$(\Delta C_{p,NA,2}^\ddagger)$	(kJ/mol K)	3.42	0	10
$(\Delta C_{p,NA,3}^\ddagger)$	(kJ/mol K)	8.49	0	10
(λ_1)	(-)	-24.8	-30	0
(λ_2)	(-)	-3.2	-30	0
(λ_3)	(-)	-1.5	-30	0
$(B_{0,1})$	(g/L)	2.0826	0	10
$(B_{0,2})$	(g/L)	0.0150	0	10
$(B_{0,3})$	(g/L)	0.0023	0	10
$(\Delta H_{0,d,1}^\ddagger)$	(kJ/mol)	68.50	55	75
$(\Delta H_{0,d,2}^\ddagger)$	(kJ/mol)	60.15	55	75
$(\Delta H_{0,d,3}^\ddagger)$	(kJ/mol)	57.83	55	75
$(\Delta S_{0,d,1}^\ddagger)$	(J/K mol)	-122.99	-200	0
$(\Delta S_{0,d,2}^\ddagger)$	(J/K mol)	-157.69	-200	0
$(\Delta S_{0,d,3}^\ddagger)$	(J/K mol)	-166.06	-200	0
$(\Delta C_{p,d,1}^\ddagger)$	(kJ/mol K)	-5.72	-10	0
$(\Delta C_{p,d,2}^\ddagger)$	(kJ/mol K)	-3.73	-10	0
$(\Delta C_{p,d,3}^\ddagger)$	(kJ/mol K)	-1.49	-10	0
$(\Delta G_{m,1}^\ddagger)$	(kJ/mol)	114.74	100	120
$(\Delta G_{m,2}^\ddagger)$	(kJ/mol)	110.06	100	120
$(\Delta G_{m,3}^\ddagger)$	(kJ/mol)	110.03	100	120
^b z	(mol/g)	10^{-7}		

K_b	(J/K)	1.3806×10^{-23}
h	(J s)	1.054×10^{-34}
R	(kJ/mol K)	0.008314

293 ^asubscripts 1, 2, and 3 represent parameters for organic N, NH₄⁺ oxidation, NO₂⁻ oxidation
294 processes.

295 ^bThe enzyme yield coefficient z was fixed at 10^{-7} mol/g, based on the assumption that enzymes
296 constitute 1% of the microbial biomass and have an average molar mass of 10^5 g/mol (Maggi et
297 al., 2018; Maggi & Riley, 2009, 2010, 2015).

Table S3. Summary of parameters used in the O isotope model. The lower and upper parameters bounds used for model calibration were selected based on values reported in previous studies (Buchwald & Casciotti, 2010; Casciotti et al., 2010; Nishizawa et al., 2016). The isotope effects were assumed to be linear functions of temperature.

Parameters	Units	Optimal	Lower bound	Upper bound
^a <i>intercept_{comp}</i>	(‰)	29.93	18	38
^b <i>intercept_{NO}</i>	(‰)	-6.51	-8	2
^c <i>intercept_{H2O,2}</i>	(‰)	5.00	5	25
^a <i>slope_{comp}</i>	(‰/°C)	0.19	-1	1
^b <i>slope_{NO}</i>	(‰/°C)	0.15	-1	1
^c <i>slope_{H2O,2}</i>	(‰/°C)	-0.09	-1	1
^d <i>a</i>	(kg soil/mg N)	0.0272	0	10
^d <i>b</i>	(-)	0.13	0	1

$${}^a {}^{18}\epsilon_{k,comp} = intercept_{comp} + slope_{comp} \times T(^{\circ}C)$$

$${}^b {}^{18}\epsilon_{k,NO} = intercept_{NO} + slope_{NO} \times T(^{\circ}C)$$

$${}^c {}^{18}\epsilon_{k,H2O,2} = intercept_{H2O,2} + slope_{H2O,2} \times T(^{\circ}C)$$

$${}^d f_{ex,AO} = b + a \times [NO_2^-] \text{ (mg N/kg soil)}$$

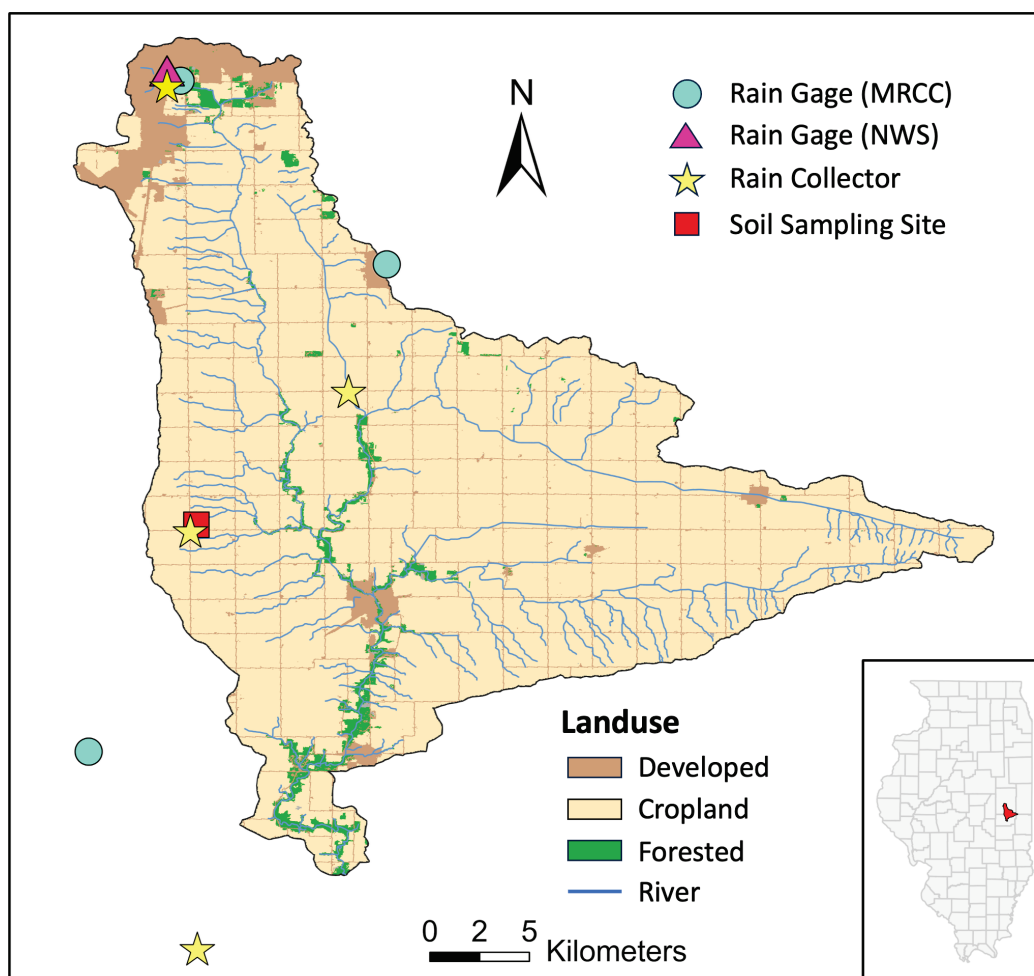


Figure S1. Map of the Upper Embarras River watershed. Locations of three MRCC gages, one NWS gage, four rain collectors, and the soil sampling site are indicated. The inset map shows the watershed location in Illinois, United States.

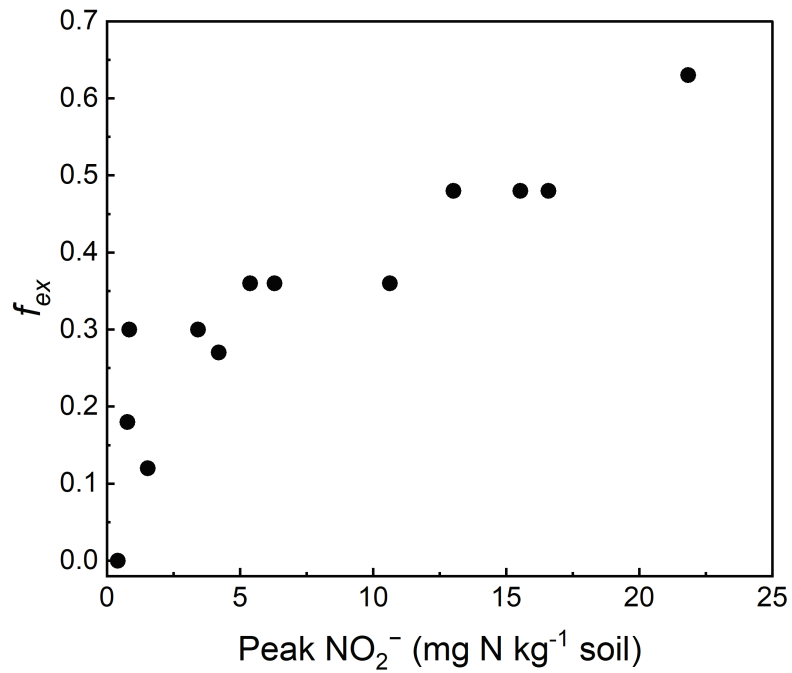


Figure S2. Relationship between peak NO_2^- concentrations and time-integrated f_{ex} across temperature and NH_4^+ amendment treatments.

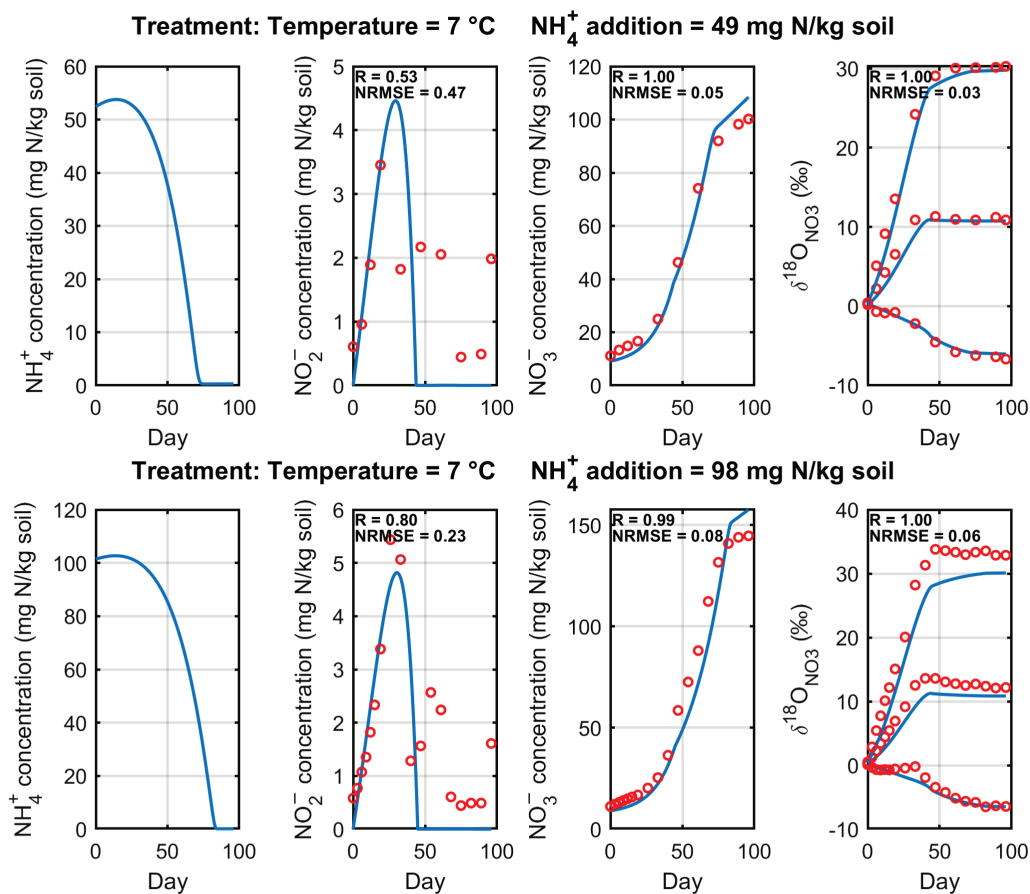


Figure S3. Observed and modeled concentrations of NH_4^+ , NO_2^- and NO_3^- for the 7 °C incubation treatment with NH_4^+ additions of 49 and 98 mg N kg⁻¹ soil. Red dots represent experimental data, and blue lines represent model simulations. Model performance is indicated by R and NRMSE.

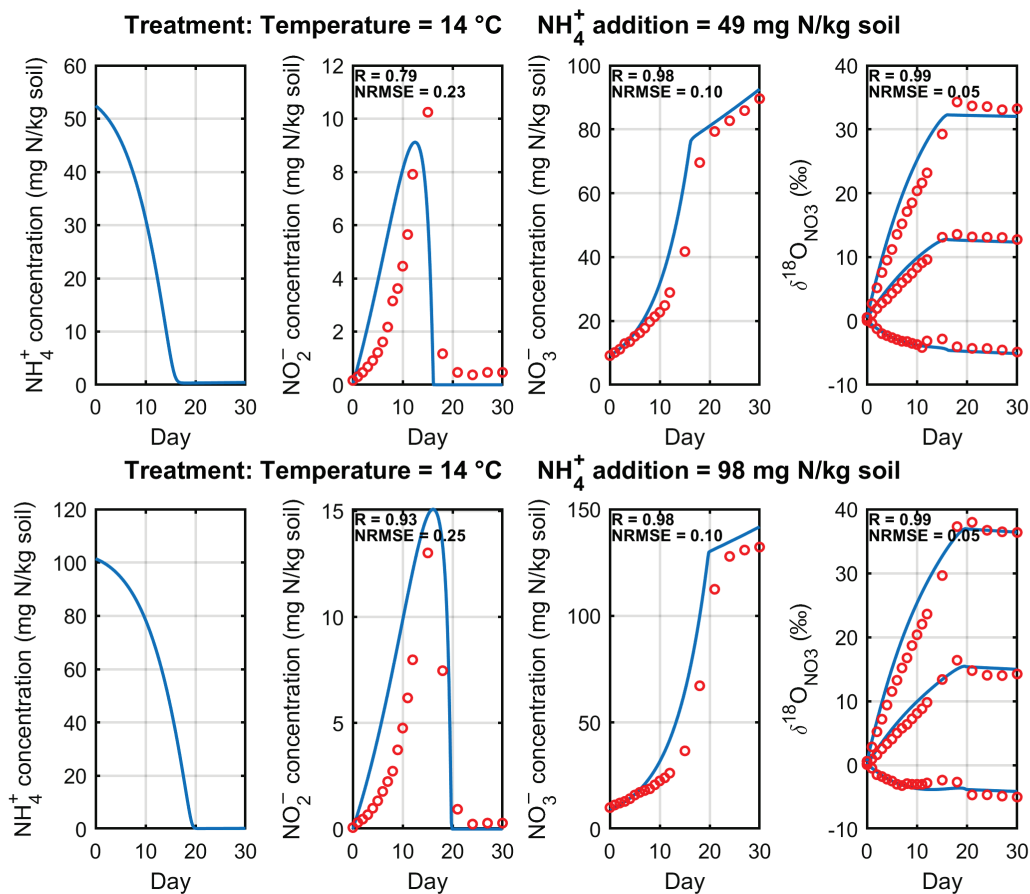


Figure S4. Observed and modeled concentrations of NH_4^+ , NO_2^- and NO_3^- for the 14 °C incubation treatment with NH_4^+ additions of 49 and 98 mg N kg⁻¹ soil. Red dots represent experimental data, and blue lines represent model simulations. Model performance is indicated by R and NRMSE.

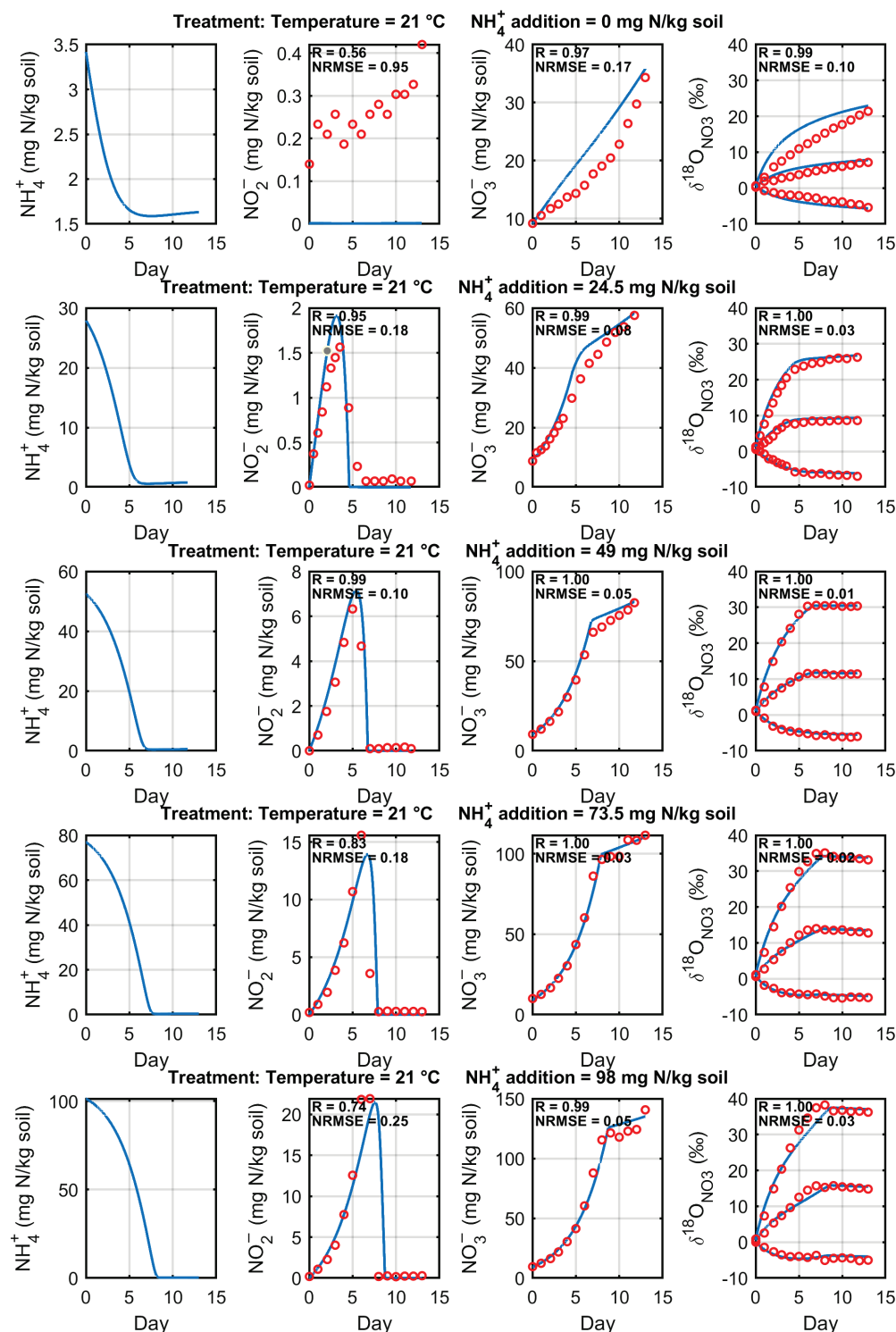


Figure S5. Observed and modeled concentrations of NH_4^+ , NO_2^- and NO_3^- for the 21°C incubation treatment with NH_4^+ additions of 0, 24.5, 49, 73.5 and 98 mg N kg⁻¹ soil. Red dots represent experimental data, and blue lines represent model simulations. Model performance is indicated by R and NRMSE.

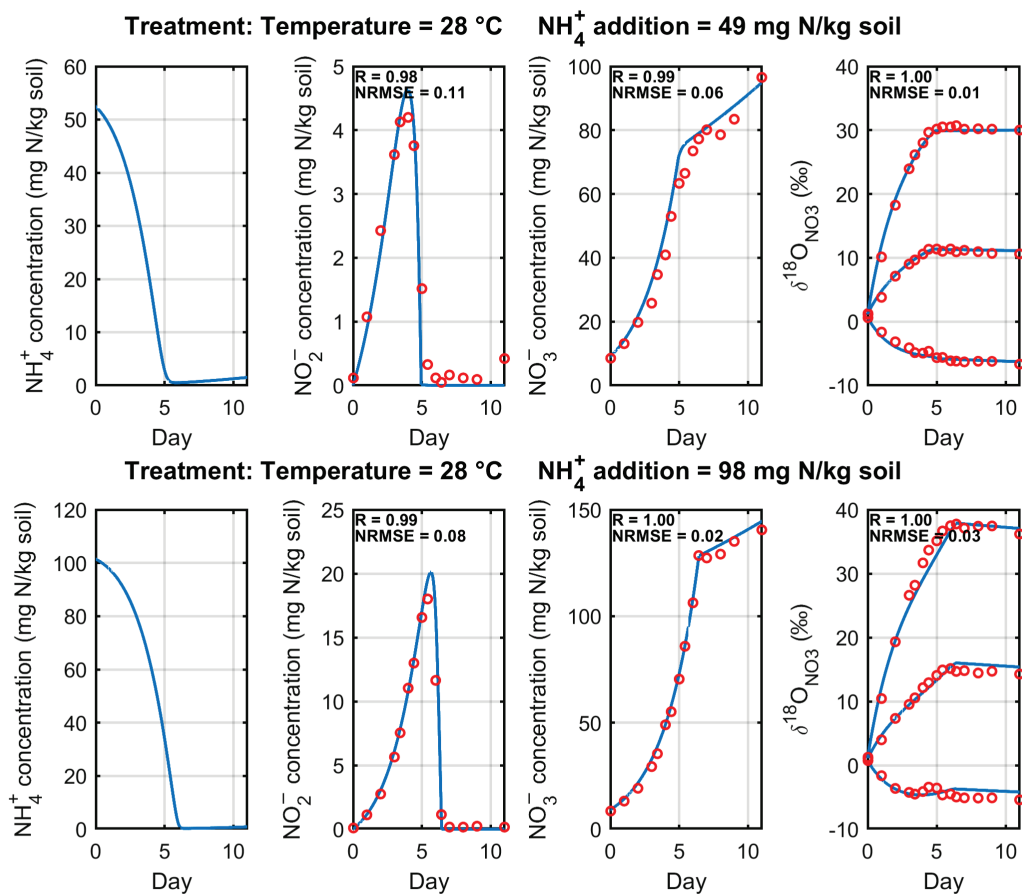


Figure S6. Observed and modeled concentrations of NH_4^+ , NO_2^- and NO_3^- for the 28 °C incubation treatment with NH_4^+ additions of 49 and 98 mg N kg⁻¹ soil. Red dots represent experimental data, and blue lines represent model simulations. Model performance is indicated by R and NRMSE.

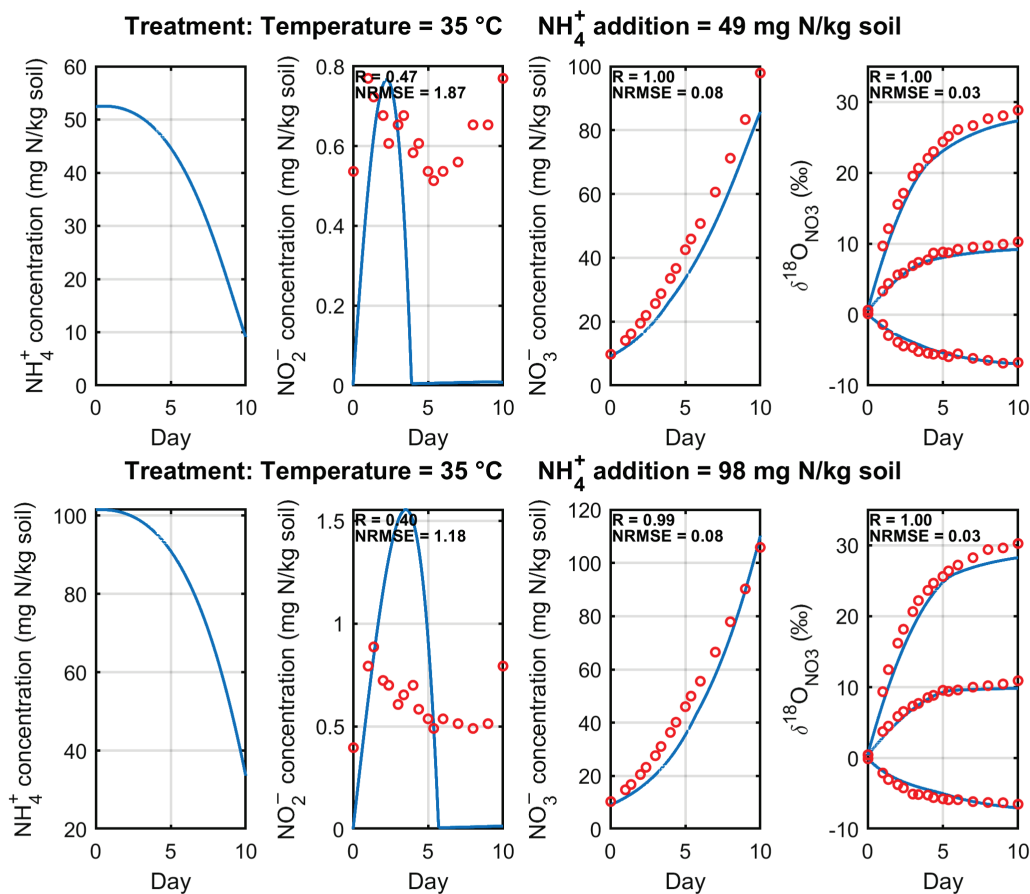


Figure S7. Observed and modeled concentrations of NH_4^+ , NO_2^- and NO_3^- for the 35 °C incubation treatment with NH_4^+ additions of 49 and 98 mg N kg⁻¹ soil. Red dots represent experimental data, and blue lines represent model simulations.

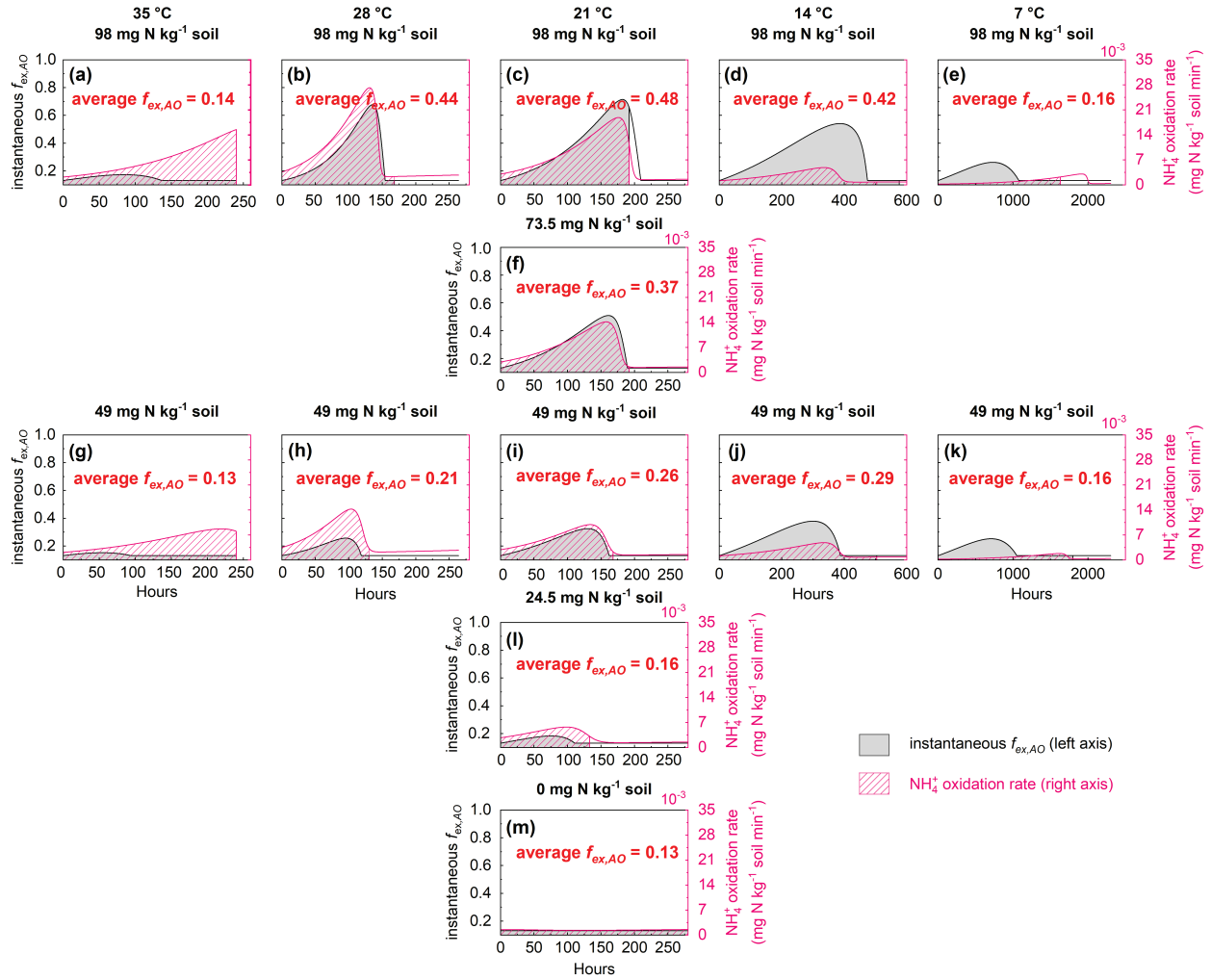


Figure S8. Temporal variations of $f_{ex,AO}$ (left axis) and NH_4^+ oxidation rate (right axis) simulated by the dynamic nitrification model. The average $f_{ex,AO}$ reported in each panel is obtained by integrating $f_{ex,AO}$ weighted by the corresponding NH_4^+ oxidation rate over the shaded period. The end of the shaded period corresponds to the time when NO_2^- concentrations first declined to the detection limit, or at the end of the incubation if no appreciable NO_2^- accumulated.

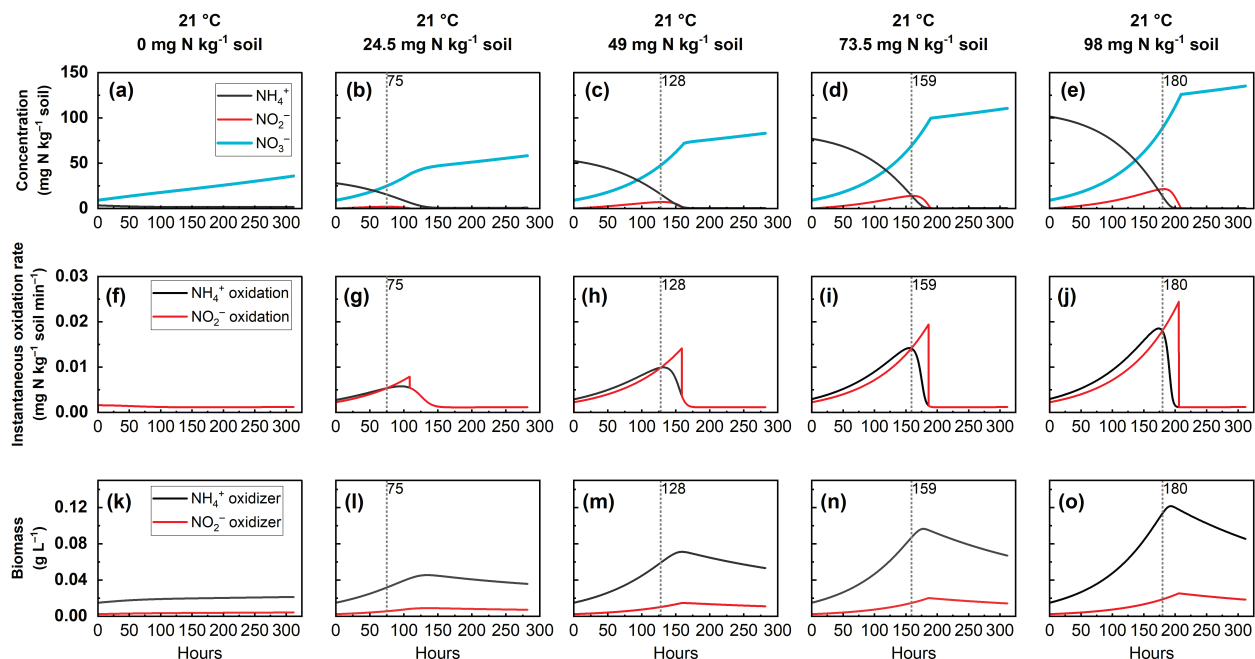
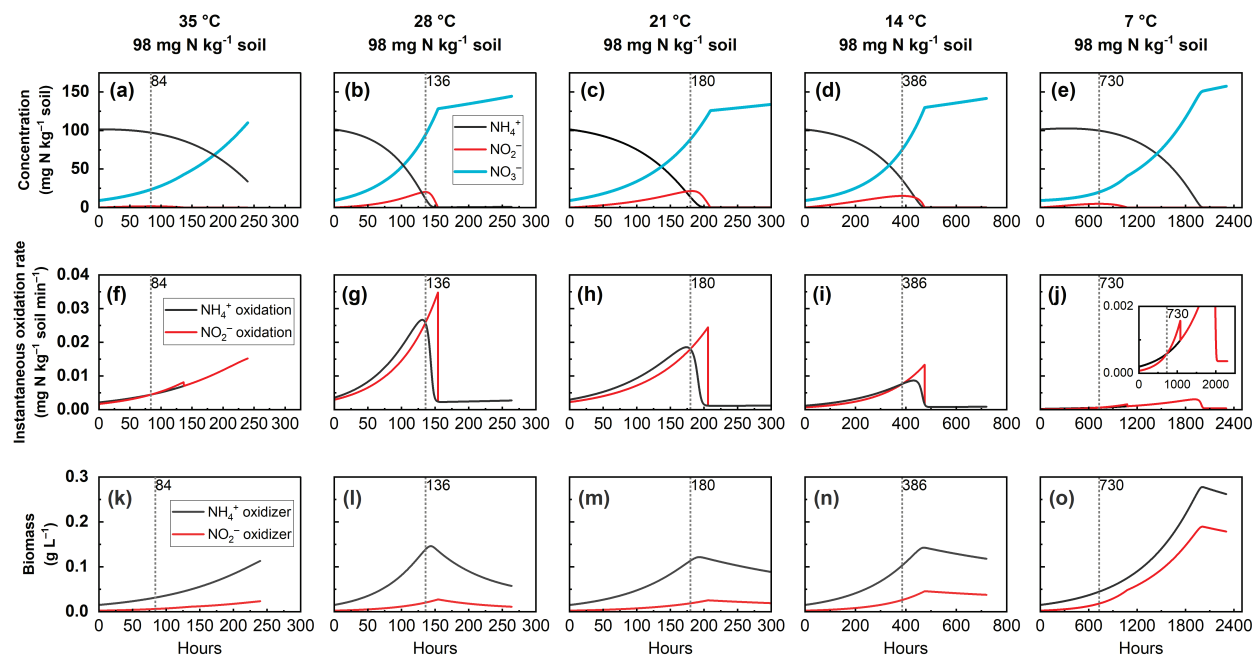


Figure S9. Simulated concentrations of NH₄⁺, NO₂⁻ and NO₃⁻ (a–e), rates of NH₄⁺ oxidation and NO₂⁻ oxidation (f–j), and the corresponding biomass pools (k–o) at 21 °C, for soil amended with 0, 24.5, 49, 73.5, and 98 mg N-NH₄⁺ kg⁻¹ soil. Vertical dashed lines indicate the time at which simulated NO₂⁻ oxidation rate equals NH₄⁺ oxidation rate in each treatment.

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358

359 **Figure S10.** Simulated concentrations of NH₄⁺, NO₂⁻ and NO₃⁻ (a–e), rates of NH₄⁺ oxidation
360 and NO₂⁻ oxidation (f–j), and the corresponding biomass pools (k–o) at 35 °C, 28 °C, 21 °C,
361 14 °C and 7 °C, for soil amended with 98 mg N-NH₄⁺ kg⁻¹ soil. Vertical dashed lines indicate the
362 time at which simulated NO₂⁻ oxidation rate equals NH₄⁺ oxidation rate in each treatment.

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