

1 EcoTWIN 1.0: A Fully Distributed Tracer-Aided Ecohydrological Model Tracking Water, 2 Isotopes, and Nutrients

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11

12 Abstract

13 The value of stable water isotopes in constraining process representation in hydrological models is
14 well acknowledged with numerous tracer-aided hydrological models developed in recent years, yet
15 few have leveraged these benefits for more robust water quality modelling. Therefore, we introduce
16 EcoTWIN, a fully distributed tracer-aided ecohydrological model that simultaneously *tracks water*,
17 *isotope*, and *nutrient* fluxes. A thorough model test was conducted by calibrating EcoTWIN against
18 discharge, in-stream isotopes, and NO₃-N concentrations (1980-2024) in 17 large-scale (10³ - 10⁵ km²)
19 European catchments spanning a wide range of geographic and climatic gradients. Furthermore, three
20 reanalysis products (ERA5 snow depth, MODIS evapotranspiration, and GRACE surface water anomaly)
21 were employed to further validate the capacity of EcoTWIN to reproduce associated but uncalibrated
22 internal water fluxes. Results showed good model performance of both calibrated in-stream targets
23 and uncalibrated internal fluxes in most catchments. Therefore, we conclude that EcoTWIN is a flexible,
24 transferable modelling tool for prediction and process inference in terrestrial ecosystems ranging from
25 boreal to subtropic climates. Constrained by tracer simulations, the model not only captures the
26 celerity, but also the velocity of hydrological fluxes, thus providing spatio-temporally-explicit
27 estimations of water ages and travel times. Such information provides opportunities to bridge
28 catchment hydrology and water quality by linking travel times with biogeochemical processing kinetics.
29 We demonstrate this with a proof of concept using Damköhler Number in nitrogen modelling.

30

31 1 Introduction

32 The development of ecohydrological models has been accelerating in the recent decades towards
33 frameworks that are more spatially distributed (instead of lumped or semi-distributed) and complex
34 (integrating more ecohydrological processes) (Pechlivanidis et al., 2011; Wellen et al., 2015). A few
35 examples include SWAT (Arnold et al., 2012), HYPE (Lindström et al., 2010), and mHM-Nitrate (Yang
36 et al., 2018), which have been widely applied worldwide. As process-based models, they are used not
37 only as prediction tools for specific variables, but also as learning tools for model inference, i.e., to

38 track the internal states/fluxes from available observations (Wang et al., 2024). This, however, poses
39 challenges due to the considerable uncertainties in model inference.

40 Inference of internal processes is naturally uncertain due to the lack of direct observations, though
41 such uncertainty can be constrained to some extent by rigorous split-sample calibration and validation.
42 The reason we use “to some extent” here is based on the fact that most models are calibrated to a
43 minimal number of variables, and 81% of calibrations used data from a single gauge (mostly at a
44 catchment outlet) as reviewed in Wellen et al., (2015). Additionally, from a technical perspective,
45 “equifinality” further adds to the inference uncertainty due to the potential misinformation in data
46 (uncertainty in model forcing and observations) and model structure (the use of simplified, abstract
47 mathematics to simulate real world processes) (Beven, 2006). This can result in inaccurate process
48 representations yielding deceptively good results through error compensation, thus leading to
49 overconfidence in a model's ability to reproduce within-basin dynamics (Wen et al., 2024; Wu et al.,
50 2025a). As acknowledged by the hydrological community, models calibrated solely against discharge
51 at the catchment outlet reflect only the celerity of hydrological systems (pressure wave propagation),
52 yet constituent transport in water quality modelling relies on the velocity (mass flux of the water)
53 (McDonnell & Beven, 2014). Failure to reconcile these differences can lead to questionable process
54 inferences from many ecohydrological and water quality models.

55 One way to strengthen model inference is to include auxiliary data for calibration (Efstratiadis &
56 Koutsoyiannis, 2010). However, there is a paradox in multi-criteria calibration, as on the one hand,
57 more auxiliary data will feed unique information to the calibration process, thus effectively
58 constraining the model behaviour from an ecohydrological perspective; yet on the other hand, it
59 increases the dimensionality of calibration thus resulting in degraded performance or failure of
60 calibration from a technical perspective. The “curse” of dimensionality in ecohydrological modelling is
61 universal for all the commonly used algorithms under both Bayesian and Pareto theories as
62 demonstrated in Wu et al., (2025c). Therefore, modellers should expect the selected auxiliary data to
63 contain as much information as possible (Nearing et al., 2020). For distributed modelling, the auxiliary
64 data should reflect the cumulative contribution of all upstream reaches/regions, rather than variables
65 that are highly dependent on local condition/processes (e.g. point-scale soil moisture and
66 evapotranspiration measurements etc.).

67 Stable water isotopes, in this context, have powerful potential in cumulative flux tracking. As
68 conservative tracers, ^2H and ^{18}O are independent of biogeochemical reactions and naturally integrate
69 landscape heterogeneity, thus providing effective constraints on spatially distributed (dis)connections
70 of hydrological flow paths as well as velocity of the hydrological systems which reflect flux-storage
71 interactions (Jung et al., 2025; Tetzlaff et al., 2015). The value of tracers has long been recognised by
72 hydrologists (Hooper et al., 1988), with many tracer-aided hydrological models developed and evolved
73 in recent years from lumped (Birkel et al., 2011; Godsey et al., 2010), to semi-distributed (van
74 Huijgevoort et al., 2016; Nan et al., 2021), and distributed structure (Kuppel et al., 2018; Remondi et
75 al., 2018). However, few attempts have been made to integrate a tracer-aided hydrological structure
76 into water quality modelling (Birkel & Soulsby, 2015; Jung et al., 2025), despite the need being evident

77 for nearly four decades (Neal et al., 1988). Moreover, existing pioneering models are mostly
78 conceptualised/lumped (Benettin et al., 2015; Dick et al., 2015) and/or loosely coupled via external
79 tracer/water quality modules (Pesántez et al., 2023; Yang et al., 2024; Zhang et al., 2020). The external
80 coupling of model chains transfer necessary internal states and fluxes between sub-models (e.g.
81 hydrological fluxes for constituent mixing in water quality or isotopic modules) via online in-memory
82 coupling (instead of offline on-disk coupling), thus significantly increasing the resources consumption
83 in input/output operations. Such model chains, though providing useful scientific insights, can become
84 problematic for large-scale applications owing to the exponential growth in computational and
85 storage requirements. Therefore, there remains a need to develop a fully distributed, computationally
86 efficient ecohydrological model that combines hydrological, isotopic, and water quality simulations.

87 This research gap motivated the development of EcoTWIN, the model that we present in this paper.
88 To our knowledge, the model is one of the first distributed tracer-aided **ecohydrological** models that
89 **tracks water, isotopic, and nutrient** fluxes simultaneously in a C++-based framework. For a thorough
90 testing of EcoTWIN, 17 large European catchments were selected for calibration against discharge, in-
91 stream isotopes, and NO₃-N concentrations. These catchments span over a wide range of geographic
92 (Alpine to lowland plain) and climatic (from snow-dominated to Mediterranean) gradients. In addition,
93 the robustness of modelled inference on uncalibrated internal fluxes were also compared with three
94 remote sensing products (snow depth, evapotranspiration, and water storage). Given the overall good
95 integrated performance in most catchments, EcoTWIN is presented as an ecohydrological modelling
96 framework applicable for terrestrial ecosystems ranging from boreal to temperate and subtropical
97 climates across a wide range of geographical environments. The subsequent sections are organised as
98 follows: Section 2 and 3 introduce the model structure of EcoTWIN and details in calibration and
99 validation; the model performance is shown in Section 4; in Section 5 we show the advantages of a
100 tracer-aided ecohydrological framework with an example of how water ages bridge catchment
101 hydrology and water quality models; finally, the current limitations and planned future development
102 of EcoTWIN are also discussed.

103

104 **2 Model description**

105 EcoTWIN is fully distributed ecohydrological model implemented in C++. The model consists of
106 hydrological, isotopic, and nitrogen modules, which simulate major ecohydrological states and fluxes
107 from canopy to groundwater (Figure 1). Aided by tracer simulations, the model is additionally able to
108 track the water movement vertically and laterally via the calculation of water ages and travel times.
109 For detailed information of model parameters please refer to Table S1.

110

111 **2.1 Hydrological module**

112 EcoTWIN follows a typical multi-layer, top-down, bucket-type approach that resolves the water
113 balance sequentially for the vegetation canopy, three soil layers, unsaturated zone, and groundwater.

114 As the foundation of solute transport, the hydrological module employs a selective disassembly
 115 structure with multiple alternative conceptualisations possible for specific important hydrological
 116 processes. The configuration can be specified *a priori* based on the goal of modelling and prior
 117 knowledge of the studied catchment(s).

118 **2.1.1 Soil properties**

119 Before iterative simulations, soil characteristics are estimated using appropriate pedotransfer
 120 functions. Three different alternatives are provided, each of which requires different levels of inputs
 121 but all were found to provide robust estimation of soil porosity (θ_s), field capacity (θ_{fc}), wilting point
 122 (θ_{wp}), and hydraulic conductivity (Ks). All the soil properties are required for each soil layer/depth.
 123 This can be achieved via three alternative options: (i) assigning identical properties across the whole
 124 soil column, (ii) calculating separately for each depth based on depth-dependent inputs, or (iii)
 125 extrapolating deeper profile characteristics from the top soil properties based on a depth-dependent
 126 equation in Maneta & Silverman, (2013).

127 The distribution of soil types and land use is assigned from raster file in EcoTWIN. This can be specified
 128 as a static boundary condition; alternatively, the distributions can also be updated dynamically via a
 129 user-specified interval to reflect any temporal changes due to land management.

130

131 **2.1.2 Vertical fluxes**

132 The vertical fluxes are resolved for storages in the canopy, soil layers, unsaturated zone, and
 133 groundwater. The mass balance of canopy storage (ΔC) follows:

$$\Delta C = P - I - Th \quad (1)$$

134 where P , I , Th are precipitation, interception, and throughfall, respectively. The throughfall is
 135 calculated as the exceedance of current canopy storage from the maximum storage calculated by Leaf
 136 Area index LAI and a correlation parameter α .

$$C_{max} = \alpha * LAI \quad (2-1)$$

137 Alternatively, the maximum canopy storage can be estimated with explicit consideration of
 138 precipitation intensity (Landgraf et al., 2023):

$$C_{max} = \alpha * LAI * \left(1 - \frac{1}{1 + SCF * P / (\alpha * LAI)}\right) \quad (2-2)$$

139 where SCF is the vegetation cover fraction calculated by LAI and an extinction coefficient (rE)
 140 adopted from HYDRUS-1D (Šimůnek et al., 2013):

$$SCF = 1 - \exp(-rE * LAI) \quad (3)$$

141 Then throughfall is calculated as the exceedance of canopy storage from the maximum:

$$Th = (P + C) - C_{max} \text{ if } (P + C) > C_{max} \text{ else } 0 \quad (4)$$

142 After reaching land surface, throughfall becomes ponding water (S_{Pond}) or snow (S_{Snow}) depending
 143 on a temperature threshold for separation ($Thre_{SN}$). Snow will melt and recharge the ponding water
 144 in warm conditions (air temperature Ta exceed $Thre_{sn}$) following a degree-day model.

$$melt = S_{snow} * \min(dd_{min} + dd_{inc} * Th * (Ta - Thre_{SN}), dd_{max}) \quad (5)$$

145 Where dd_{min} and dd_{max} are the minimum and maximum of degree day factor, while dd_{inc} denotes
 146 the rate of increase in the degree-day factor per degree Celsius rise in temperature.

147 The available ponding water infiltrates into the top soil layer using Green-Ampt model (Kale & Sahoo,
 148 2011; Maneta & Silverman, 2013), with infiltration capacity first calculated as a function of average
 149 soil moisture over the hydrologically active depth:

$$I_f = Ks * \left(1 + \frac{\psi * \theta_s * (1 - (\theta_1 - \theta_{wt}) / (\theta_s - \theta_{wt}))}{\theta_1 * d_1}\right) \quad (6)$$

150 Where θ_1 , θ_s , θ_{wt} , and d_1 are the moisture content, porosity, wilting point, and depth in top soil layer;
 151 ψ is a parameter representing soil air entry pressure in m. Then potential infiltration (F_p) is determined
 152 from the lesser between the available ponding water (S_{pd}) and potential infiltration rate integrated
 153 over time before ponding occurs ($I_f * t_p$).

154 The actual infiltration (F) is solved iteratively using the Newton–Raphson scheme:

$$F = \Delta\theta * d_1 = F_p + Ks * w_{Ks} * (\Delta t - t_p) - \psi\Delta\theta * \ln\left(\frac{\psi\Delta\theta + \Delta\theta d_1}{\psi\Delta\theta + F_p}\right) \quad (7)$$

155 where w_{Ks} is anisotropy ratio of vertical to horizontal Ks .

156 The soil storage in each layer is conceptualised as two water pools – a gravitational, free-flowing pool
 157 and a capillary, soil-bound pool. The two pools are separated based on field capacity (Maneta &
 158 Silverman, 2013), and percolation happens when soil storage exceeds the threshold. Three alternative
 159 schemes are included in EcoTWIN.

160 In the first scheme, all water in excess of field capacity percolates to deeper layer:

$$Pc_i = (\theta_i - \theta_{fc}) * d_i \quad (8-1)$$

161 where Pc_i , θ_i and d_i depict the percolation, moisture content and depth from/in i th soil layer in m.

162 The second scheme additionally considers the hydraulic conductivity (Ks) following the
 163 conceptualisation in SWAT (Arnold et al., 2012):

$$Pc_i = (\theta_i - \theta_{fc,i}) * d_i * \left(1 - \exp\left(\frac{-\Delta t * Ks}{\theta_{s,i} - \theta_{fc,i}}\right)\right) \quad (8-2)$$

164 The third scheme relates percolation to the extent of soil saturation with an exponential parameter β
 165 (Kumar et al., 2013; Samaniego et al., 2010):

$$Pc_i = (\theta_i - \theta_{fc,i}) * d_i * \left(1 - \exp(\beta * \log(\theta_i / \theta_{s,i}))\right) \quad (8-3)$$

166 For evapotranspiration, soil evaporation and transpiration are estimated separately. The separation
 167 of PET is realised by surface cover fraction introduced above:

$$PT = PET * SCF; \quad PE = PET - PT \quad (9)$$

168 Soil evaporation is simulated in the top soil layer based on the soil saturation:

$$Evap_s = PE * \min\left(\frac{\theta_1}{\theta_{fc,1}}, 1\right) \quad (10)$$

169 Transpiration is simulated in all soil layers based on the fractions ($f_{root,i}$) of root density ($D_{root,i}$) in
 170 each layer partitioned by soil depth and a parameter (γ_{root}):

$$Tr_i = PT * f_{root,i} * \frac{\theta_1 - \theta_{wp,1}}{\theta_{fc,1} - \theta_{wp,1}} \quad (11)$$

$$f_{root,i} = D_{root,i} / \sum_{j=1}^m D_{root,j} \quad (12)$$

$$D_{root,i} = \left(1 - \gamma_{root}^{\left(\sum_{j=1}^m d_j\right)}\right) - \left(1 - \gamma_{root}^{\left(\sum_{j=1}^i d_j\right)}\right) \quad (13)$$

171 Channel evaporation is also estimated using Penman equation, which relies on net radiation, wind
 172 speed, air pressure, and air temperature as inputs.

173 The last soil layer percolates to an unsaturated storage in unsaturated zone (S_{unsat}). The
 174 compartment stores the excess water from soil and percolates either downward to groundwater
 175 storage (S_{GW}) or laterally downstream. The percolation to groundwater P_{CGW} is determined by a
 176 weighting parameter p_{GW} as a proportion of unsaturated storage:

$$P_{CGW} = S_{unsat} * p_{GW} \quad (14)$$

177 Additionally, irrigation is conceptualised in EcoTWIN, which is realised via the water extraction from
 178 river or groundwater. The source is determined by the geographic location: for a grid cell with channel
 179 network, water is extracted directly from river, and local groundwater is used as irrigation source for
 180 non-channel grids. The amount of extraction is estimated from a predefined coefficient for crop water
 181 demands (w_{irr}) from which the deficit is calculated for each of the m soil layers.

$$deficit = \sum_{i=1}^m (\theta_{fc,i} - \theta_{wp,i}) * w_{irr} * d_i \quad (15)$$

182 Note that the irrigation can switch to groundwater extraction if river storage cannot fill the deficit.

183

184 **2.1.3 Lateral fluxes**

185 In EcoTWIN, grid cells are connected laterally at three levels - surface, unsaturated zone, and
 186 groundwater. Note that some models omit the unsaturated storage and directly calculate excess
 187 water to drain based on the saturation extent of the bottom soil layer (e.g., ECH₂O-iso, Kuppel et al.,

188 2018). EcoTWIN did not follow this conceptualisation because in reality, the lateral drainage is focused
 189 in the saturated zone, and thus the bottom of the soil layer instead of the whole soil profile. The
 190 drainage of an entire soil layer thus brings considerable uncertainty to the velocity of lateral transport
 191 when the lower boundary of the soil is a parameter to tune in calibration. For instance, a large soil
 192 depth will dramatically reduce the velocity of interflow drainage and slow down the mixing of
 193 constituents, though this might still perfectly reproduce the celerity (hydrograph) for purely
 194 hydrological modelling. Our conceptualisation (an independent unsaturated compartment) aligns
 195 with most hydrological models (Arnold et al., 2012; Yang et al., 2018) and fits the recent analysis
 196 supporting the dominant role of lateral drainage over vertical transports globally (Mcmillan et al.,
 197 2025).

198 By the end of each timestep, ponding water receives upstream inputs and contributes to channel
 199 storage if the grid is connected to the channel network, while non-channel grid has $Ovf_C = 0$:

$$Ovf_C = (Ovf_{T,in} + S_{pond}) * p_{Ovf} * dx_C/dx_T \quad (16)$$

200 dx_C and dx_T are the channel length and size of terrestrial grid cell; p_{Ovf} is a weighting parameter for
 201 channel recharge. Then the remaining ponding water routes to downslope terrestrial grid following
 202 the topographic gradient. In none-channel grid cells, all available ponding storage routes lateral
 203 downstream ($Ovf_C = 0$):

$$Ovf_{T,out} = (Ovf_{T,in} + S_{pond}) - Ovf_C \quad (17)$$

204 Similarly, unsaturated storage contributes first to channel storage in grid cells within channel network,
 205 while non-channel grid cells have $Inf_C = 0$:

$$Inf_C = (Inf_{T,in} + S_{vadose}) * K_{vadose} * \left(1 - e^{-1 * exp_{Inf} * (Inf_{T,in} + S_{vadose})}\right) * p_{Inf} \quad (18)$$

206 where K_{vadose} is the effective conductivity of lateral transport in the unsaturated zone; while exp_{Inf}
 207 is an exponential parameter determining the strength of positive correlation between recharge and
 208 current unsaturated storage. Then the remaining unsaturated storage is partially routed to downslope
 209 grid cell following a linear approximation of Kinematic wave equation, which assumes gravitational
 210 flux per unit width $Inf_{T,out}$ is proportional to the subsurface hydraulic conductivity (K_{vadose}) and
 211 bedrock slope ($slope$ approximated from the surface slope):

$$Inf_{T,out} = (Inf_{T,in} + S_{unsat} - Inf_C) * \left(1 + \alpha * \frac{dt}{dx}\right) * \alpha * \frac{dt}{dx} \quad (19)$$

$$\text{where } \alpha = K_{unsat} * \sin(slope)$$

212 Groundwater routing is similar to that of interflow, with channel recharge followed by terrestrial
 213 transport. Note that the terrestrial groundwater flow does not consider the bedrock slope as
 214 groundwater storage is generally much large than unsaturated storage, and thus independent from
 215 topographic gradients:

$$GWf_C = (GWf_{T,in} + S_{GW}) * K_{GW} * \left(1 - e^{-1 * exp_{GWf} * (GWf_{T,in} + S_{GW})}\right) * p_{GWf} \quad (20)$$

$$GWf_{T,out} = (GWf_{T,in} + S_{GW} - GWf_C) * \left(1 + \alpha * \frac{dt}{dx}\right) * \alpha * \frac{dt}{dx} \quad (21)$$

where $\alpha = K_{vadose}$

216 The channel routing is realised using Kinematic wave equation based on a scaled channel roughness
217 parameter (Maneta & Silverman, 2013).

218

219 **2.2 Isotopic module**

220 The isotopic module in EcoTWIN tracks the composition of stable water isotopes in all water storage
221 compartments following hydrological mixing and fractionation. The module also provides estimation
222 of water age and travel time conceptualised as the time since water molecules enter the catchment
223 as precipitation, and the time water molecules need to travel through the specific storage.

224 **2.2.1 Mixing**

225 The mixing and transport of isotopes (^2H and ^{18}O , both noted as C) are governed by the velocity of
226 hydrological fluxes with a complete mixing strategy for most water storages:

$$\frac{d(V * C)}{dt} = \sum_{k=1}^{N_{in}} q_{in,k} * C_{in,k} - \sum_{k=1}^{N_{out}} q_{out,k} * C \quad (22)$$

227 Where V and C are the volume and composition/concentration of the storage, while N_{in} and N_{out}
228 denote the number of influx and outflux. Such strategy is built on two assumptions: (i) constitutes (i.e.,
229 isotopes) are fully mixing within each timestep; (ii) the composition/concentration in outflow equals
230 to that in storage. Additional mixing between ponding and upper soil water storage is allowed (with
231 proportion determined by a parameter *SrfMixing*), as nutrients in top soils can be flushed out in
232 large hydrological events (Seybold et al., 2022).

233 The full-mixing assumptions have been widely used and shown to be reasonable for storages with
234 relatively small volumes in many mixing/water quality models (Arnold et al., 2012; Yang et al., 2018).
235 However, some studies show that a complete mixing strategy can be problematic for large storages
236 such as groundwater as they are generally poorly constrained (e.g. Soulsby et al., 2015). Therefore,
237 the mass conservation equation used in the INCA-N model and mHM-Nitrate is employed to calculate
238 the mixing of groundwater storages with influxes (i.e., percolation from unsaturated storage and
239 lateral groundwater inflow).

$$\frac{dC}{dt} = \frac{1}{V + V_r} * \left(\sum_{k=1}^{N_{in}} q_{in,k} * C_{in,k} - \sum_{k=1}^{N_{out}} q_{out,k} * C \right) \quad (23)$$

240 where V_r is the retention storage. The equation is solved by the fourth order Runge-Kutta technique.

241

242 **2.2.2 Fractionation**

243 As conservative tracers, the composition of isotopes in water storages/fluxes is only changed by
 244 kinetic fractionation apart from hydrological mixing. The process is accompanied by evaporation,
 245 resulting in the preferential loss of lighter isotopes (^1H and ^{16}O) to the vapor phase and a
 246 corresponding enrichment of heavier isotopes (^{18}O and ^2H) in the residual water. In EcoTWIN, the
 247 fractionation is simulated along with evaporation of top soil water and river storage based on the
 248 Craig-Gordon model (Craig et al., 1964; Kuppel et al., 2018), while transpiration is assumed to be a
 249 non-fractionating process (Dawson & Ehleringer, 1991; Kuppel et al., 2018).

$$C = C^* - (C^* - C) * \left(\frac{S - Evap}{S} \right)^m \quad (24)$$

250 where C^* and m are the limiting isotopic composition (in ‰) and the dimensionless enrichment slope
 251 that are estimated via the following equations in (Good et al., 2014):

$$C^* = \frac{h_a C_a + h_s \varepsilon^+ + \varepsilon^k}{h_s - h_a + \varepsilon^k / 1000} \quad (25)$$

$$m = \frac{h_a - (h_s \varepsilon^+ + \varepsilon^k) / 1000}{h_s - h_a + \varepsilon^k / 1000} \quad (26)$$

252 where h_a is the relative humidity above the soil surface normalised from atmospheric relative
 253 humidity (h), air temperature (T_a), and soil temperature (T_s estimated from Amato & Giménez, 2024).
 254 C_a is the isotopic composition of ambient air moisture estimated from precipitation composition:

$$C_a = (C_{rain} - \varepsilon^+) / \alpha^+ \quad (27)$$

255 where ε^+ is the equilibrium fractionation factor (Skrzypek et al., 2015); α^+ is a temperature factor
 256 estimated from T_a .

$$\varepsilon^+ = (1 - 1/\alpha^+) * 1000 \quad (28)$$

257 The factor of diffusion-controlled kinetic isotopic separation ε^k is calculated based on the relative
 258 humidity of soil surface (h_a) and soil pore (h_s).

$$\varepsilon^k = (h_s - h_a) * \left(1 - \frac{D_i}{D} \right) * n \quad (29)$$

259 Where D_i and D denote the diffusivities of water vapor molecules containing heavier isotope and the
 260 lighter isotope, respectively. The ratio can be acquired in Horita et al., (2008) for ^2H (0.9877) and ^{18}O
 261 (0.9859). n is an advection term ranging between 0.5 (in saturated soils) and 1 (in dry soils). The factor
 262 is included in calibration for the fractionation of top soil evaporation yet fixed as 0.5 for that of channel
 263 evaporation.

264

265 **2.2.3 Water age and travel time**

266 EcoTWIN can track the age of water i.e., the time since water enters the catchment as precipitation,
267 in each storage. In age tracking, precipitation is defined as new water with age of zero. At the end of
268 each time step, water ages of all storages are advanced based on the temporal resolution (for instance
269 one day if the model is set up for daily timesteps). Note that in some circumstances, the modellers
270 might need to disable the age evolution of specific storage(s) (e.g., groundwater storage) as the
271 storage can be too large to achieve steady states in model spin-up. Similar to isotopes, water ages are
272 only controlled by hydrological transport with the same mixing strategy (i.e., complete mixing except
273 for groundwater).

274 The water ages in EcoTWIN are the mean values averaged from all water molecules in the storage,
275 which might be dominated by the inflow of very old water that obscure the age distribution of the
276 young water (e.g., the groundwater input to top soils due to the groundwater extraction for irrigation).
277 Therefore, EcoTWIN additionally provides the estimation of travel time - the time of water molecule
278 travelling through each storage. The simulation is similar to that of water ages. The only difference is
279 that the transition of water between storages (e.g., percolation into deeper soil layers) resets the travel
280 time to zero. Accordingly, all the water enters a new storage becomes new water instead of just
281 precipitation in water age tracking.

282

283 **2.3 Nitrogen module**

284 The nitrogen module describes the mass balance of nitrogen, particularly nitrate as the main form of
285 dissolved nitrogen, which is dominated by the interaction of hydrological transport and
286 biogeochemical transformations.

287 For each timestep, the nitrate concentration is simulated in each storage following three processes –
288 hydrological transport/mixing, nitrogen inputs, and biogeochemical transformations. Fully integrated
289 with hydrological module, nitrate transport also aligns with hydrological fluxes following the same
290 mixing strategy as in the isotopic simulation. For nitrogen sources, EcoTWIN considers the inputs from
291 fertiliser, manure, and plant residues, whose annual inputs can be specified via configuration. Notably,
292 fertilization can be parameterised via spatial raster inputs if corresponding dataset is available. The
293 timing and extent of nitrogen addition of all sources are determined following the implementation in
294 HYPE (Lindström et al., 2010), which distributes the annual sum across a specified period (e.g., the
295 period between planting and harvest for crops). Additionally, wet deposition is conceptualised as the
296 atmospheric nitrogen source, whose concentration can be specified via spatial raster and simply as a
297 constant value.

298 The biogeochemical transformations are mainly modified from the mHM-Nitrate model (Yang et al.,
299 2018), and the HYPE model (Lindström et al., 2010), which are conceptualised for the soil profile and
300 channel network. In the soil profile, three nitrogen pools are conceptualised for each soil layer,
301 including an inactive nitrogen pool (SN_i), an active nitrogen pool (SN_a), and a dissolved nitrate pool

302 (DN). The soil transformations include degradation (Dgd_s , from SN_i to SN_a), mineralisation ($Minr_s$,
 303 from SN_a to DN), denitrification ($Deni_s$, from DN to gaseous N_2), and plant uptake ($Uptk_s$, DN
 304 removal).

$$Dgd_s = SN_i * ref_{Dgd,s} * f_{Ta} * f_{\theta} / dt \quad (30)$$

$$Minr_s = SN_a * ref_{Minr,s} * f_{Ta} * f_{\theta} / dt \quad (31)$$

$$Deni_s = DN * ref_{Deni,s} * f_{Ta} * f_{\theta,deni} * f_{conc,s} / dt \quad (32)$$

305 where $ref_{Dgd,s}$, $ref_{Minr,s}$, $ref_{Deni,s}$ are the parameters representing the reference rates of soil
 306 degradation, mineralisation, and denitrification. f_{Ta} and f_{θ} are the regulating factors of temperature
 307 and moisture.

$$f_{Ta} = 2^{(T_a - 20)/10} * \omega \quad \text{where } \omega = \begin{cases} 1 & T_a > 5 \\ T_a/5 & 0 \leq T_a \leq 5 \\ 0 & T_a < 0 \end{cases} \quad (33)$$

$$f_{\theta} = \min \left[\frac{(1 - p_{\theta,deni}) * (\theta_{fc,i} - \theta_i)}{p_{\theta,fc} * d_i}, \frac{(\theta_i - \theta_{wp,i})}{p_{\theta,wp} * d_i} \right] \quad (34)$$

308 where $p_{\theta,fc}$ and $p_{\theta,wp}$ are the empirical factors that are fixed as 1.2, 0.8 based on literature values
 309 (Lindström et al., 2010; Yang et al., 2018). $p_{\theta,deni}$ is the saturation threshold for soil denitrification
 310 ranging between 0.4 – 0.85 (Yang et al., 2018). A different moisture factor considering a saturation
 311 threshold (θ_{thres}) is employed for denitrification, as denitrification is more sensitive to the soil
 312 wetness condition:

$$f_{\theta,deni} = [(\theta_i / \theta_{fc,i} - \theta_{thres}) / (1 - \theta_{thres})]^{2.5} \quad (35)$$

313 The process is additionally controlled by the concentration level in the storage $f_{conc,s} = C / (C + 10)$.
 314 Plant uptake is simulated using a three-parameter logistic growth equation in (Eckersten et al., 1994;
 315 Lindström et al., 2010).

316 Currently, in-stream denitrification is the only process considered in EcoTWIN.

$$Deni_w = ref_{Deni,w} * f_{Tw} * f_{conc,w} * A / dt \quad (36)$$

317 where $ref_{Deni,w}$ is the reference in-stream denitrification rates. The actual rates are regulated by a
 318 concentration factor $f_{conc,w} = C / (C + 1.5)$ and a temperature factor f_{Tw} (the same equation for f_{Ta}
 319 with inputs substituted by river temperature f_{Tw} , simplified as the rolling-average of 20-day air
 320 temperature).

321 It should be noted that the calibrated soil depth in this study is about 2.5 m, with intermittent
 322 saturation occurring in the deeper layer. This means that terrestrial denitrification is a combination of
 323 soil and groundwater processes in this study, though this might change in other applications if a
 324 shallow soil depth is assigned.

325

326 **3 Model calibration and validation**

327 A robust model application should not only reproduce observed variables through calibration but also
328 yield realistic estimates of internal states and fluxes that are not included in the calibration process.
329 This is essential to avoid situations where inaccurate process representations produce deceptively
330 good results through error compensation. Therefore, we evaluate EcoTWIN from both perspectives.
331 First, we assess the model's ability to reproduce observations via calibration (methods and results in
332 Sections 3.2 and 3.4). Then, we examine the model's capacity to simulate uncalibrated internal states
333 and fluxes by comparing the simulated snow depth, evapotranspiration, and total water storage with
334 corresponding remote-sensing products (methods and results in Sections 3.3 and 3.5).

335 To ensure model generality, 17 catchments were selected for calibration and validation depending on
336 the data availability (particularly stream stable water isotopes and nitrate), which span a wide range
337 of characteristics in geography, climate, and anthropogenic managements (catchment locations,
338 characteristics, and major hydrological and nitrogen inputs are shown in Figure 2, Table 1, and Figure
339 S1, respectively). Anthropogenic management practices have a less dramatic effect than climate and
340 geography in most catchments due to the relatively low proportion of urbanized areas. However, a
341 few notable exceptions—such as the Rhine, Elbe, and Danube catchments—are included in the
342 analysis, as these densely populated regions hold critical ecological, agricultural, and economic
343 importance for Europe, and are subject to intensive human interventions in water management. This
344 also provides a chance to examine the applicability of EcoTWIN in human-affected catchments.

345

346 **3.1 Model setup**

347 EcoTWIN was setup for each of the 17 catchments for calibration with a spatial resolution of 5 km²
348 and a temporal resolution of daily timesteps from 1980 to 2024 (with first two years for spin-up). As
349 a fully distributed model, gridded GIS inputs are used in the model setup, including a digital elevation
350 model, flow direction, slope, channel width, channel length, proportion of each land use type (Winkler
351 et al., 2021), proportions of each soil type (world soil map, WRB2014), and soil properties (e.g., depth-
352 dependent proportions of clay, sand, silt, and organic matter from SOILGRIDS). All spatial inputs were
353 acquired with finer resolution (50 m or above) and resampled to the resolution of this application (5
354 km).

355 The climatic variables used to drive EcoTWIN include precipitation, air temperature, potential
356 evapotranspiration, relative humidity, and a few variables that are optional required for the
357 calculation of channel evaporation (air pressure, net radiation, and wind speed). These climatic
358 variables are available from the reanalysis products ERA5 and E-OBS, while PET is calculated using FAO
359 Penman-Monteith equation. For nitrogen simulations, additional inputs are needed including the
360 fertilization map (Grizzetti et al., 2021) and nitrate concentration of rainfall (Zhu et al., 2025) as the
361 boundary of nitrogen addition from agricultural activities and wet deposition.

362

363 3.2 Model calibration

364 **Method.** The calibration was conducted separately for each catchment to test the applicability of
365 EcoTWIN under different geological and climatic contexts. Three commonly used variables for
366 hydrological and water quality modelling (discharge, stream water isotope composition, and in-stream
367 NO₃-N concentrations) are employed for calibration. Their long-term time series were acquired at daily
368 steps from different sources (discharge from GRDC, isotopes from Wateriso and GNIR, and NO₃-N
369 concentration from global water quality database, GEMStat), and then compared with simulation
370 results at multiple sites for each catchment. Here ¹⁸O was selected for isotopic validation due to its
371 higher precision and data abundance. Given the discrepancy in duration of observations (especially
372 for isotopes and NO₃-N), a separate calibration and validation based on a split-sample approach is
373 difficult. Therefore, the full timescale (1982 - 2024) was used for calibration (and the validation
374 introduced in Section 3.3).

375 The DiffeREntial Evolution Adaptive Metropolis algorithm (DREAM) was selected for parameter
376 optimisation due to its relatively efficient and effective performance for high-dimensional problems
377 (as benchmarked in Wu et al., 2025c). The algorithm was implemented separately for each catchment
378 with the same prior distribution of parameters (Table S1). The maximum iteration was set as 100,000
379 for each catchment (20 chains with maximum chain length of 5000), from which 40 best simulations
380 were selected from the posterior distribution. The Kling-Gupta efficiency (KGE) statistic was used to
381 construct an informal likelihood function for DREAM optimisation.

$$l = \left[\sum_{i=1}^{N_{obs}} \sum_{j=1}^{N_{site}} (1 - KGE) * w_{i,j} \right]^{-m} \quad (37)$$

382 Where l is the likelihood; N_{obs} and N_{site} are the number of observation types (discharge, isotopes,
383 and nitrate) and sites. The weight $w_{i,j}$, defined for observation type i at site j , is assigned equally
384 across sites such that the total weight for each observation type sums to 1/3. m is an exponentially
385 coefficient to stretch the likelihood surface that is often set based on the number of observation points.
386 After prior test run, m was set as 500. Finally, the likelihood function is transformed to logarithmic
387 form for numeric stability. The calibration was validated using Kling-Gupta efficiency (KGE), Root Mean
388 Square Error (RMSE), Pearson Correlation Coefficient (Coefficient), and Percent bias (Pbias) (Table 2).

389 **Calibration performance.** EcoTWIN successfully reproduced the observed discharge in all 17
390 catchments with KGE exceeding 0.5 at most site (Figure 3A&D; Table 2). Such performance is
391 comparable to or better than previous continental calibration of hydrological models (e.g., ParFlow,
392 Naz et al., 2023; E-HYPE, Donnelly et al., 2016).

393 Similarly, isotopic simulations also produced acceptable performances at most sites (Figure 3B; Table
394 2). However, there are a few exceptions. The failure of isotopic simulations was found at two sites
395 within the Alps region. This can be attributed to the uncertainty in precipitation isotopes and
396 snowmelt isotopes (due to the lack of snowmelt elution fractionation; Ala-aho et al., 2017), the
397 incorrect isotopic composition in groundwater, or the reduced applicability of degree-day model for

398 mountainous areas in Europe. Such simulation deviation due to the uncertainty in data and boundary
399 initialisation is often reported in previous calibration (Smith et al., 2021).

400 As for nitrogen simulation (Figure 3C), the model produces comparable performances to existing
401 nitrogen modelling at catchment (Wu et al., 2022, 2025b; Yang et al., 2018) and continental scales
402 (Jones et al., 2023; Mikayilov et al., 2015). The degraded nitrogen simulations were found coarsely
403 across Europe, but they are mostly in rivers/stream with low NO₃-N concentrations given the low
404 RMSE in Table 2. Such low average values can easily trigger the degradation in KGE as one of the sub-
405 components of KGE is highly sensitive to the mean deviation, though the absolute deviation remained
406 low. Overall, we concluded that EcoTWIN has good capacity to reproduce in-stream components for
407 a wide range of catchments and for relatively long periods.

408

409 **3.3 Model validation**

410 **Method.** Remote sensing or reanalysis products were further employed to validate uncalibrated
411 internal model states or fluxes from three important perspectives in ecohydrological modelling – snow
412 depth from ERA5, evapotranspiration from MODIS, and surface water mass anomaly from GRACE (as
413 a storage proxy). The simulated variables corresponding to these products are, respectively, the depth
414 of snow pack, the sum of soil evaporation, channel evaporation, and transpiration from all soil layers,
415 and the anomaly of total water storage above groundwater (i.e., the sum of canopy storage, snow,
416 soil water storages, and unsaturated storage). The validation was realised via resampling the remote
417 sensing products to 5 km and comparing grid-to-grid with the modelled outputs. r^2 was used as the
418 performance metrics, as KGE is not applicable for time series with zero average, yet the average of
419 surface mass anomaly is close to 0.

420 Note that all three products may contain considerable uncertainties. ERA5 is a reanalysis product that
421 combines historical observations into global estimates using modelling and data assimilation
422 approaches, therefore inevitably embeds uncertainties associated with model structure and
423 observational coverage (Hersbach et al., 2020). MODIS evapotranspiration is derived from remotely
424 sensed spectral information, energy partitioning approaches and the Penman–Monteith framework,
425 whose uncertainty may exceed 30% depending on spatial scale and environmental conditions (Mu et
426 al., 2011). GRACE infers changes in terrestrially stored water masses from spatial and temporal
427 variations in the Earth’s gravity field; however, its coarse spatial resolution can introduce substantial
428 uncertainty when used for hydrological validation, particularly at basin or sub-basin scales (Tapley et
429 al., 2004). Nevertheless, good agreement between simulations and remote sensing or reanalysis
430 products can enhance confidence in the robustness of simulated spatial and temporal patterns,
431 although it does not necessarily imply accurate representation of absolute magnitudes.

432 **Validation performance.** First, we compared the sum of soil evaporation, channel evaporation, and
433 transpiration to MODIS evapotranspiration in each grid cell. The results in Figure S2 and Table 3 shows
434 a general good fit between simulation and MODIS records with coefficient of determination (R^2) above
435 0.5 in most selected catchments. This is further validated after zooming into two representative

436 catchments (W5 Nemunas and W10 Rhine) in Figure 4A-B, where KGE reaches above 0.5 in >80% of
437 the catchment domain. The other evaluation metrics (RMSE, Pearson coefficient, RMSE, and percent
438 bias shown in Table 3) also demonstrated the good performance of evapotranspiration simulations.

439 Then, the surface water mass anomaly from GRACE was compared to the anomaly of simulated
440 surface storage, i.e., the sum of canopy storage, snow, soil water storages, and unsaturated storage.
441 The grid-to-grid comparison in Figure 4C-D shows an acceptable fit with R^2 above 0.3 in around half
442 regions in catchment Nemunas (W5) and Rhine (W10), as well as other European catchments (Figure
443 S3 and Table 3). However, more degradation in performance was found compared to
444 evapotranspiration simulation. For instance, the validation in Rhine shows degraded water storage
445 simulation in Alpine regions. This is likely due to the locally complex topography and the simplified
446 representation of snow accumulation and melt processes in the model, which struggle to capture the
447 high spatial variability of storage in mountainous terrain. Besides, we also found degraded
448 performance in coastal regions. For example, GRACE exhibited considerably increasing trends in water
449 storage between 2005 to 2015 in two Nordic catchments (W2 and W3; Figure S3), yet our simulations
450 only showed a moderate increasing trend. Similar degraded performance was found in the coastal
451 catchments (e.g., three British catchments W6-8 in UK; Figure S3), though the magnitudes of
452 simulation and GRACE data fit well. This is possibly attributed to the coarse resolution of GRACE which
453 additionally considered the storage mass from ocean in coastal region yet not included in this
454 terrestrial-explicit modelling.

455 Finally, the simulated snow depth was compared to the daily snow depth in ERA5 reanalysis products
456 (ERA5 post-processed daily statistics on single levels; 10.24381/cds.4991cf48). Results in Figure 4E-F
457 show a generally good agreement between simulations and ERA5 records with $R^2 > 0.5$ in most regions
458 in Nemunas and Rhine. Though degradation was found in a few other catchments (e.g., W14-17 in
459 Figure S4), these catchments generally experienced limited snow accumulation. In the other words,
460 the absolute deviation was relatively limited for snow depth simulation demonstrated by low RMSE in
461 Table 3

462

463 **4 Water age simulation and its link to water quality**

464 Like many existing distributed hydrological and water quality models (e.g., SWAT, mHM, Ech₂O-iso,
465 HYPE etc.), EcoTWIN can provide estimation of the main ecohydrological fluxes at high spatial and
466 temporal resolutions, including canopy interception, snow melt-accumulation, infiltration, percolation
467 through soil layers, groundwater recharge, and lateral flux routing at different horizontal phrases.
468 Among these variables, a unique trait of EcoTWIN lies in its capacity to track water fluxes via isotopes,
469 thus being able to provide a consistent estimate of water age and travel times. Therefore, in Figure 5,
470 these variables are shown as the long-term average from 1982 to 2024 for soil profile and stream
471 water in Nemunas (W5) and Rhine (W10).

472 Generally, the magnitudes of water ages follow the geographic and climatic gradients, with younger
473 water found in catchments with higher annual precipitation inputs. Those regions locate in Alpine

474 regions – for instance around Alps in Southern Rhine, and in catchment W12 and W17 (Figure S6-7).
475 The north-west coast of Europe (Figure S5) also exhibited young water age, where lower temperature
476 and net radiation limit the level of potential evapotranspiration, leading to larger percolation to
477 deeper soil layers and groundwater. Such high turnover rates of water in these catchments (W2, W3,
478 W6, and W7) are reflected by short travel time in soil profile with average values remaining below 500
479 days (Table 4).

480 A similar pattern was also found in mountainous regions with higher precipitation and lower potential
481 evapotranspiration compared to lowland areas. Two clear examples are W12 and W17 located in the
482 Alps and the Taurus Mountains where water ages and travel time remained below 500 days (Table 4
483 and Figure S5-7). In specific wet periods, the soil water ages and travel time can be reduced to just
484 days, suggesting the rapid response of saturated hydrological systems. In contrast, the lowlands in
485 central-west Europe showed much slower turnover rates. A typical example is Rhine (Figure 5), where
486 mean water ages reached up to 10 years in lowlands. Such phenomenon was also found in other
487 European catchment (e.g., W9 Elbe, and W11 Danube).

488 Note that though water ages and travel time share similar magnitudes and spatial patterns. It is partly
489 attributed to the fact that the travel time in the conceptualised storages increases exponentially in a
490 sequential order. Taking the Rhine as an example, the average travel time in top soil layer, median soil
491 layer, deep soil layer are 65, 225, 1291 days, respectively. Such a depth-dependence profile makes the
492 overall ages/travel time follow the magnitude of bottom layer and leads to similarity between water
493 ages and travel time. However, large discrepancies are possible between the two indices if a shallow
494 lower boundary is adopted.

495 The estimation of travel time and water ages further provides opportunities to link hydrology and
496 water quality processes in the modelling framework. The simplest and most intuitive way is to
497 compare travel times and simulated biogeochemical process kinetics. Taking denitrification as an
498 example, we applied linear regression and Spearman's correlation test to investigate the potential
499 correlation between travel time of soil water and denitrification rates. The results in Table 4 showed
500 the strong positive correlations in most agricultural-dominated catchments (W5, W6, W9, W10, W11,
501 and W13) yet only weak or no positive correlation in remaining pristine watersheds. This suggests that
502 travel time might be a key control on soil nitrogen removal in European croplands.

503 More insights can be gained via examination of the Damköhler Number, which quantifies the ratio
504 between timescales of solute transport and biogeochemical transformation. Here in our modelling
505 framework, it can be calculated as the ratio between the travel time of soil water and the time for all
506 soil $\text{NO}_3\text{-N}$ storage to be removed under the simulated denitrification rates. Damköhler numbers <1
507 mean that soil water nitrogen cannot be effectively removed during time of residence, indicating the
508 dominance of transport over removal processes and the potential of nitrogen leaching. As shown in
509 Figure 6E-F, the long-term averages of Damköhler number remain below 1 in most croplands in
510 Nemunas, Rhine, and other European catchments (Figure 6 and Figure S8), supporting the conclusion
511 from the linear regression in Table 4 that travel time is a major limiting factor on soil nitrogen removal.

512 Via the spatial- and temporal-explicit estimation of Damköhler number, EcoTWIN provides the
513 opportunity to bridge the catchment hydrological and water quality with travel time.

514

515 **5 Discussion**

516 **5.1 Structural and Functional Merits of EcoTWIN**

517 As a new tracer-aided ecohydrological model, EcoTWIN has novel advantages compared to previous
518 models. In this section, we briefly introduced the merits in model structure, applicability, and insights
519 from tracer-aided simulation.

520 ***5.1.1 Integrated C++ framework***

521 Applications of large-scale modelling have been increasingly popular due to the accelerating
522 development of observation networks and availability of remote sensed data. However, it severely
523 increases the computational burden of ecohydrological modelling. Especially for fully distributed
524 models, increasing size of the model domain can lead to exponential increase in computation demands.
525 In this context, an integrated framework in C++ can significantly accelerate the modelling tasks, as all
526 computation can be conducted within memory thereby avoiding the additional input/output
527 overhead associated with disk-based operations in loosely coupled model chains (e.g., Ech₂O-iso-
528 nitrate; Yang et al., 2024). A standard test was not performed, but based on our modelling experience
529 in the same catchment with different models, the speed of EcoTWIN (~5 seconds for a simulation with
530 285 grid cells and 30 years at daily timestep) is close to the water quality model mHM-Nitrate (~5
531 seconds yet without isotopic simulations; Wu et al., 2022) and easily outperforms Ech₂O-iso-nitrate (7
532 minutes; Wu et al., 2025b).

533 ***5.1.2 Selective disassembly structure***

534 EcoTWIN incorporates a wide range of ecohydrological processes from canopy to groundwater, which
535 not only include natural processes but also anthropogenic activities like irrigation. Land managements
536 can also be represented by dynamic parametrisation, thus enabling EcoTWIN to function as a learning
537 tool to investigate the impacts of changes in anthropogenic management over natural ecosystems;
538 for instance, the land use distribution was updated every 10 years in our test examples to reflect the
539 moderate increases in afforestation in the past 45 years in Europe. More importantly, unlike hard
540 coded process representations/equations in most ecohydrological models, EcoTWIN has a selective
541 disassembly structure, which provides alternative conceptualisations for several important hydrological
542 processes (canopy interception, percolation, groundwater recharge, as well as three pedotransfer
543 functions for initialising soil properties). Modellers can benefit from such flexible model structures by
544 either selecting process representations best suited to field knowledge or data prior to calibration, or
545 integrating module selection into the calibration thus enabling simultaneous optimisation of model
546 structure and corresponding parameters. The latter aspect, i.e., the optimisation of model structure,
547 can be realised together with the recently developed optimisation algorithm DREAM_(LOAX) that aims to
548 identify the deficits in model structure during calibration (Wu et al., 2025a).

549 **5.1.3 Transferability to contrasting geographic and climatic contexts**

550 To thoroughly test the applicability of EcoTWIN, 17 catchments with different climatic and
551 geographical contexts were selected for calibration and validation, spanning over most biomes in
552 Europe, from snow-dominated watersheds in Nordic or alpine regions, to agricultural-influenced
553 lowlands catchments, and Mediterranean ecosystems (Figure 2 and Table 1). Through multi-criteria
554 calibration against three objectives at multiple sites, the model successfully reproduced the
555 seasonality and peaks of discharge, in-stream isotopes, and NO₃-N concentrations in most catchments.
556 Such performance is comparable or better than the previous model benchmarks at similar scales
557 (Bajracharya et al., 2023; Mikayilov et al., 2015; Rakovec et al., 2016, 2019). Note that the
558 concentration of NO₃-N was used for calibration, whose accurate simulation is more difficult than NO₃-
559 N loads given the naturally good performance in discharge. In the other words, hydrological simulation
560 is often the least problematic part in integrated water quality modelling, as it is mostly dominated by
561 natural catchment properties while nitrogen cycling is more interfered by anthropogenic
562 managements (e.g., fertilization and irrigation) (Wu et al., 2025b). Additionally, the simulated internal
563 fluxes were also compared to three reanalysis products in hydrological simulations, corresponding to
564 the key fluxes or storage states in hydrological cycling (snow melt-accumulation, evapotranspiration,
565 and water storage). The results show that constrained by isotopes, EcoTWIN was able to reproduce
566 comparable hydrological modelling results to the remote sensing data without direct calibration
567 regarding magnitudes, spatial patterns, and temporal dynamics. The only degraded performance was
568 found in GRACE surface mass anomaly in coastal regions. There are two potential reasons: (i) the
569 coarse resolution of GRACE might account for mass shifts in both ocean and land, yet EcoTWIN only
570 produces mass anomaly in terrestrial systems; (ii) bidirectional fluxes across the land-ocean interface
571 might drive key changes in coastal systems, which is not considered in current version of EcoTWIN.
572 Nonetheless, given the relatively good agreement with most available data, we conclude that
573 EcoTWIN is applicable across a range of terrestrial ecosystems from boreal to temperate and
574 subtropical climate.

575 **5.1.4 Bridging hydrology and water quality with water ages**

576 Further to the inference of hydrological and nitrogen processes that is also available in other
577 distributed water quality models (Wellen et al., 2015), a unique trait of EcoTWIN lies in its capacity to
578 track water fluxes and ages with stable water isotopes. As a tracer-aided model, EcoTWIN not only
579 simulates the celerity of catchment response, but tracks the velocity of water via different flow paths.
580 The importance of delineating flow paths within catchments has long been recognized by hydrologists,
581 and has motivated the development of many indices to describe the movement of water molecule at
582 catchment-scale and estimate associated timescales (Sprenger et al., 2019). A few examples are water
583 ages, transient time distribution, and young water fractions (Benettin et al., 2015; Hrachowitz et al.,
584 2013; Jasechko et al., 2016). However, those indices are mainly calculated in a lumped manner where
585 different flow paths in the catchment are characterised as a black box, thus characterising the overall
586 input-output dynamics yet potentially omitting important spatio-temporal variability of hydrological
587 boundary conditions. Instead, EcoTWIN, benefiting from the gridded-based structure, can utilise the

588 increasingly available spatial information (e.g., gridded remote sensing datasets) thus characterising
589 the water ages and travel time in a spatially-explicit manner. Note that simulations of water age/travel
590 time, like other ecohydrological processes, are sensitive to spatial resolution. The coarse resolution
591 used for large catchments (e.g., 5 km in this study) may obscure the sub-grid heterogeneity. For
592 instance, local hydrological hotspots characterized by short travel times and young water ages can be
593 damped or averaged out at coarser resolutions, as reported in modelling studies using ECH2O-iso
594 (Smith et al., 2021; Yang et al., 2023b). However, this limitation can be mitigated by increasing spatial
595 resolution, and it does not undermine the utility of EcoTWIN for water-tracking.

596 Compared to water age which quantifies the age of water within the overall system, travel time,
597 accounting for the water age within a specific storage, is more important in understanding the links
598 between hydrological and nutrient cycles. Such an index, also known as transit time or exposure time,
599 forms one of the fundamental components of water quality modelling. Therefore, the travel time
600 estimated by EcoTWIN has potential to improve the simulation of biogeochemical transformations in
601 water quality models interfaced with simplified hydrological modules (e.g., MONERIS; Bonchkovskyi
602 & Osadcha, 2024). Moreover, travel time can be used as a proxy to bridge hydrological processes and
603 biogeochemical transformations. Here we presented a simple framework to calculate the Damköhler
604 Number for denitrification. By using the simulated travel time and reaction timescale (i.e., the time
605 for full removal of nitrogen storage under current denitrification rates), estimation of Damköhler
606 Number was achieved in a spatially- and temporally-explicit manner (Figure 6E-F), which can highlight
607 where and when soil nitrogen removal is constrained by the limited exposure time in the catchment.
608 Such high-resolution information is unique, as the use of this index has been largely restricted to
609 steady-state groundwater systems or riparian/hyporheic zones due to the difficulty in quantifying
610 processing time and residence time at larger scales (Ocampo et al., 2006; Wu et al., 2022).

611

612 **5.2 Limitations and roadmap for future development**

613 Despite these advances, EcoTWIN has limitations. In this section, the uncertainties in model structure
614 and conceptualisation are introduced, as well as the potential roadmaps for future developments.

615 ***5.2.1 Potential towards physics-based conceptualisation of groundwater***

616 Groundwater in EcoTWIN is characterised as two conceptual storages linking with adjacent upstream
617 and downstream storages following the topographic gradients. Such conceptualisation, although has
618 been widely employed in hydrological models (e.g., SWAT, mHM, ECH2O, STARR, etc.), does not align
619 with the physical mechanisms of groundwater routing, as groundwater flow direction follows the
620 hydraulic gradients which may not entirely coincide with topographic gradients (Condon et al., 2021).
621 Such simplified routing has less effect in large catchments with clear topographic gradients (e.g., Rhine
622 starting from Alps to North plain), yet might cause biased estimation in water mass balance for flatter
623 headwater catchments (Yang et al., 2025). Therefore, we plan to further incorporate an additional
624 groundwater module to realise physics-based routing following Darcy's Law in future.

625 **5.2.2 Revisiting mixing strategies**

626 Mixing strategy is a key component in water quality or tracer models describing the flux-storage
627 behaviours along specific flow paths. There has long been a debate on different mixing assumptions
628 and theories. A typical example is the two-water-world hypothesis, where water storage in the soil
629 profile is differentiated into a tightly-bound pool and a mobile-water pool (McDonnell, 2014). Such
630 conceptualisation is close to the definition of soil matrix flow and preferential flow: the existence of
631 free-flowing preferential flow will bypass the soil matrix vertically and accelerate the lateral drainage
632 via direct connection with channel network (Hrachowitz et al., 2013; Sprenger et al., 2019). However,
633 a complete mixing strategy is often regarded as a reasonable first approximation in many situations
634 and is used in most water quality and tracer models (Jung et al., 2025). This is not only attributed to
635 its computational simplicity, but also the difficulty in conceptualising preferential flow in an
636 evidenced-based manner. In the other words, even with the recognition of preferential flow, its
637 calculation is often hindered by the subsurface heterogeneity in soils and bedrock; a good visualisation
638 is given in Figure 7 in Sprenger et al., (2019). Alternatively, partial mixing has been developed for
639 ecohydrological models (e.g., Hrachowitz et al., 2013), which could be added as a complementary
640 mixing strategy in EcoTWIN. However, as benchmarked in Hrachowitz et al., (2013), the partial mixing
641 brings only moderate improvements in simulations yet can introduce challenges to model spin-up (the
642 increasing instability of storage ages due to the exchange between bypass and storage compartment).
643 Moreover, the realisation of partial mixing, like preferential flow, relies on additional parameters to
644 describe the timing and extent of mixing thus introducing additional parametric uncertainty.
645 Therefore, we recommend a rigorous evaluation of the necessity of partial mixing before any
646 application.

647 **5.2.3 Complementing the in-stream biogeochemical processes**

648 Transformation is as crucial as transport in inland-water nitrogen cycling (Wang et al., 2024). In the
649 current version of EcoTWIN, denitrification is the only in-stream process of nitrogen loss. However,
650 recent studies have shown that other processes are involved which may be important for aquatic
651 nitrogen cycling. An example originates from Wang et al., (2024), where global inland-water modelling
652 shows that in-stream denitrification only accounts for a minor fraction of $\text{NO}_3\text{-N}$ removal compared to
653 biological uptake. Though their modelling considers lakes and reservoirs where primary production of
654 benthic plants and algae is usually greater than that in rivers, in-stream assimilation might still play a
655 significant role, particularly, in slow-flowing river systems. This is supported by a recent modelling
656 study that estimated nitrogen retention at 15-min interval based on high-frequency $\text{NO}_3\text{-N}$ data (Yang
657 et al., 2023a). Therefore, we plan to further compliment EcoTWIN with in-stream assimilation
658 conceptualisation, as well as other potentially important riverine processes (e.g., nitrogen burial in
659 sediments; Akbarzadeh et al., 2019).

660 **5.2.4 Integrated calibration framework to embrace equifinality**

661 Strictly speaking, equifinality is not specifically linked to EcoTWIN, but remains a universal problem for
662 calibration or parameter tuning for almost all ecohydrological models. It is reflected in multiple

663 parameters sets yielding similarly good model performance, thus increasing the uncertainty in process
664 inference. The extent of equifinality is primarily controlled by the magnitude of parameters and
665 observation/objectives (Wu et al., 2025c). Unfortunately, conceptualisations across diverse process
666 domains (e.g. for hydrology, isotopes and N-cycling) in EcoTWIN also lead to a relatively large number
667 of parameters. Such risk in equifinality can be potentially constrained via sensitivity analysis, but can
668 still remain an issue given the ubiquitous epistemic uncertainty in data and model structure (Beven,
669 2006, 2015). Alternatively, the recently developed calibration algorithm DREAM_(LoAX) provides an
670 opportunity to embrace equifinality by tuning parameters based on the limits-of-acceptability theory
671 under the equifinality thesis (Wu et al., 2025a). The integrated modelling framework of EcoTWIN and
672 DREAM_(LoAX) can potentially increase the robustness of model calibration and inference.

673

674 **6 Conclusions**

675 Uncertainty is a central concern in ecohydrological modelling, as models are not only used for
676 prediction of specific variables, but also for process inference (backtracking internal processes from
677 available observations) that are inherently embedded within considerable uncertainty. Stable water
678 isotopes can help effectively constrain hydrological fluxes due to their conservative nature, motivating
679 the increased development of tracer-aided models. However, few attempts have been made to
680 incorporate a tracer-aided hydrological framework into water quality models.

681 Therefore, we introduced EcoTWIN, a fully distributed tracer-aided *ecohydrological* model that *tracks*
682 *water*, *isotopic*, and *nutrient* fluxes simultaneously in an integrated C++-based framework. To
683 thoroughly validate the model, 17 large European catchments were selected with a wide range of
684 geographic and climatic gradients (from snow-dominated watersheds in Nordic or alpine regions, to
685 agricultural-influenced lowlands catchments, and Mediterranean ecosystems). The model was
686 calibrated against long-term observations of discharge, in-stream isotopes, and NO₃-N concentrations
687 during 1980-2024 in each of the 17 catchments. Additionally, uncalibrated internal states and fluxes
688 were also compared with three remote sensing products (ERA5 snow depth, MODIS
689 evapotranspiration, and GRACE surface water anomaly) to validate the credibility of process inference.

690 The generally good agreements in both calibrated in-stream components and uncalibrated internal
691 flux-states demonstrated that EcoTWIN is a transferable, flexible prediction and learning tool for
692 process inference across biomes ranging from boreal to subtropical climate. Constrained by tracer
693 simulations, the model not only reproduces the celerity of hydrological systems, but also tracks the
694 velocity. Water ages and travel time are embedded in EcoTWIN to provides spatio-temporal-explicit
695 insights into *when*, *where*, and *how* water moves in the system. Such indices further provide the
696 opportunities to efficiently bridge hydrology and water quality at large catchment-scales. An example
697 was presented using the Damköhler Number to identify regions where denitrification was limited by
698 fast turnover rates of water.

699 Following this “proof of concept” we also see numerous areas where future developments can
700 improve the limitations in the 1.0 version of the model.

701

702 **Code and data availability**

703 The initial version (v1.0) of EcoTWIN is archived in <https://doi.org/10.5281/zenodo.16747633> (Wu et
704 al., 2025d). For further development please refer to GitHub repository: [https://github.com/songjun-](https://github.com/songjunwu/EcoTWIN)
705 [wu/EcoTWIN](https://github.com/songjunwu/EcoTWIN). The geographic data were acquired from Catchment Characterisation and Modelling
706 database (CCM2, version 2.1). The climatic forcing was acquired from E-OBS database
707 (<https://www.ecad.eu/download/ensembles/ensembles.php>). The LAI were acquired from MODIS
708 database (<http://doi.org/10.5067/MODIS/MOD15A2H.006>). Long-term observation of discharge was
709 acquired from GRDC (<https://grdc.bafg.de/>); in-stream isotopic observations were available from
710 Wateriso database (<https://wateriso.utah.edu/waterisotopes/index.html>) and GNIR database
711 (<https://www.iaea.org/services/networks/gnir>); In-stream NO₃-N concentration were acquired from
712 global water quality database, GEMStat (<https://gemstat.org/>).

713

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722 Restoration in Peatlands.

723

724 **Author contribution**

725 Conceptualization: SW, DT, YZ, CS

726 Data curation: SW

727 Methodology: SW

728 Software: SW

729 Investigation: SW, DT, YZ, CS

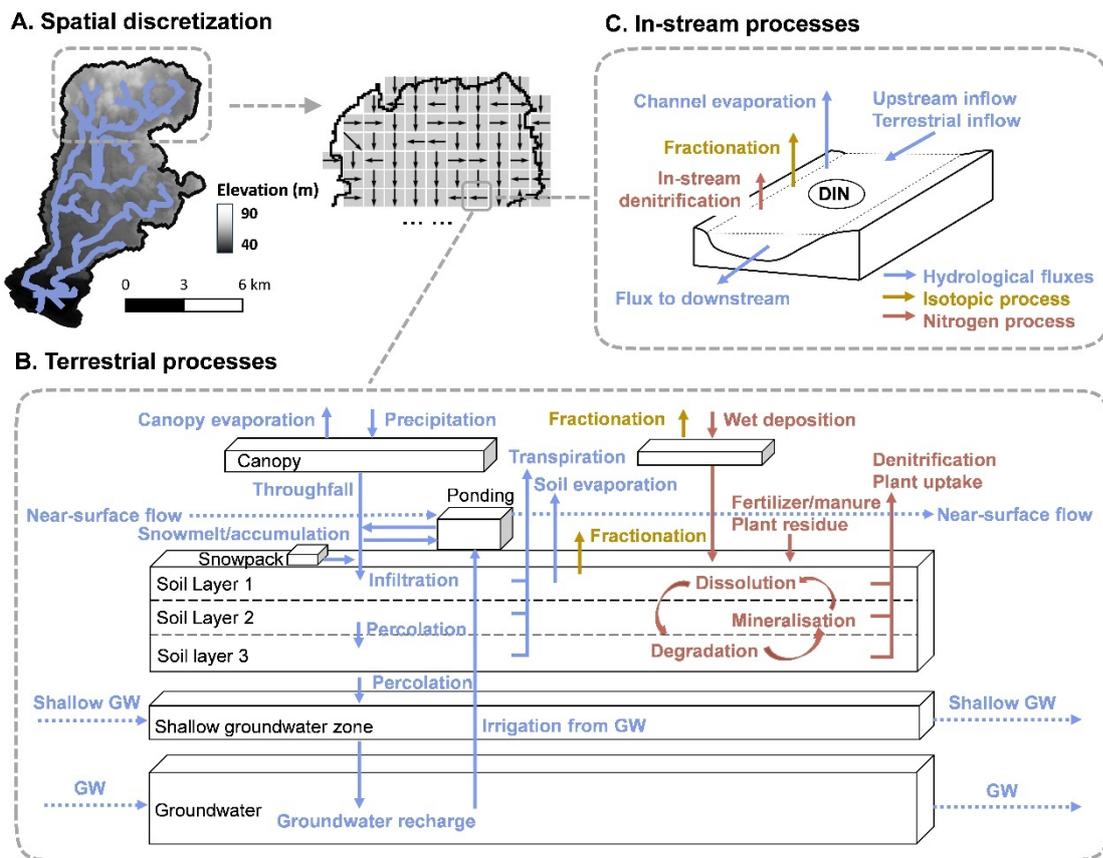
730 Visualization: SW

731 Supervision: DT, CS

732 Writing (original draft preparation): SW

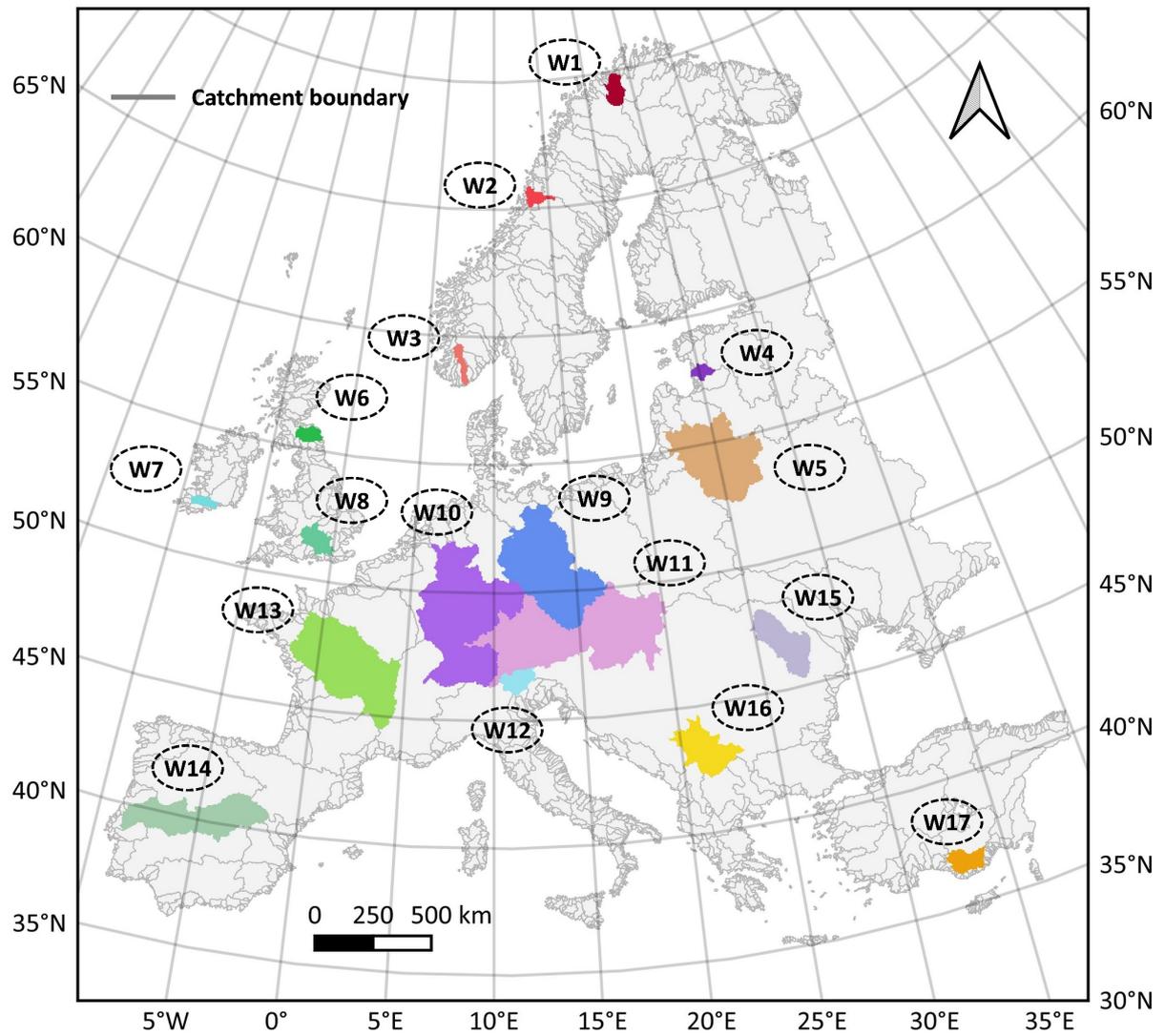
733 Writing (review and editing): SW, DT, YZ, CS

734 **Figures**



735

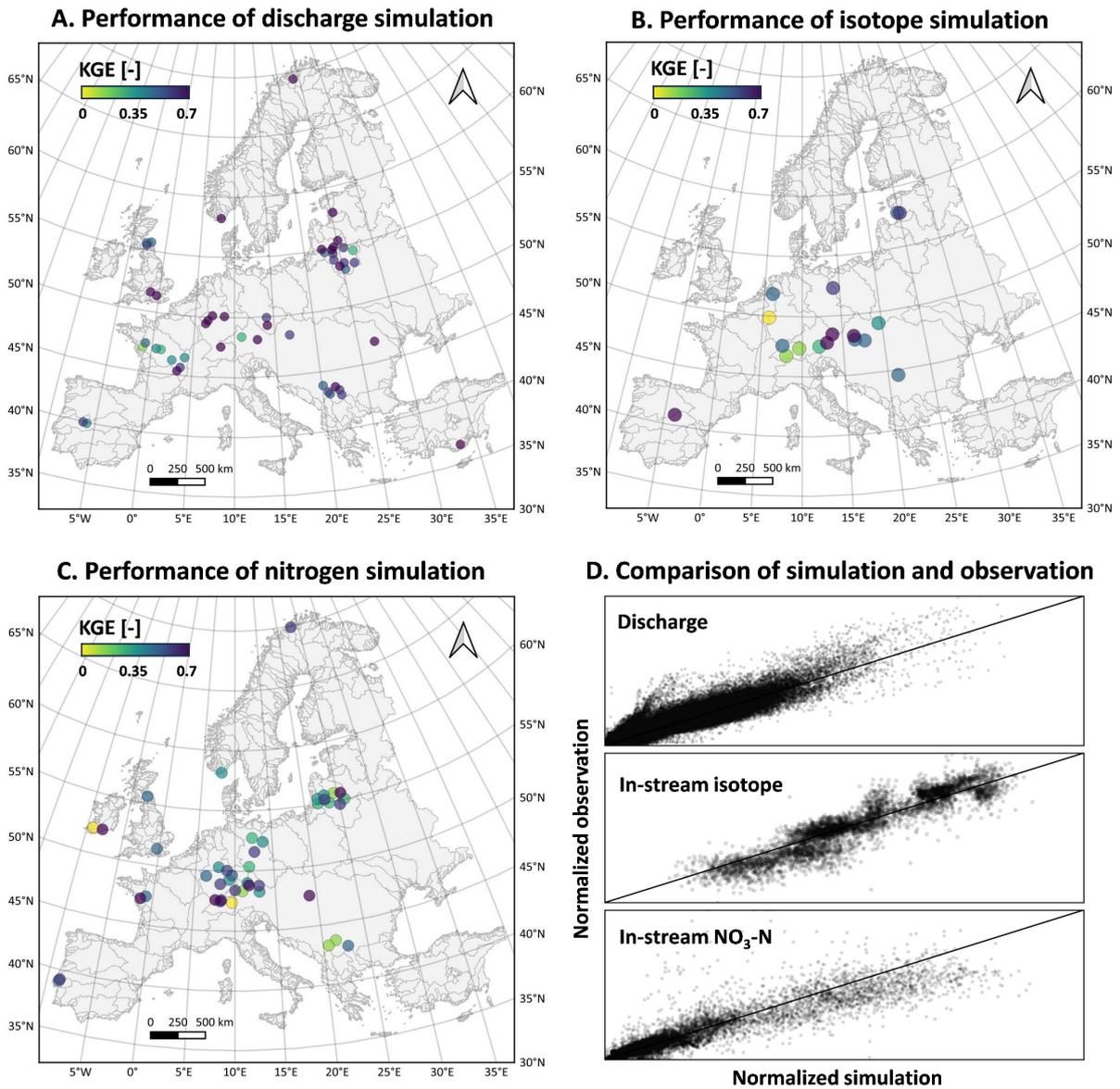
736 Figure 1. Model structure of EcoTWIN. As a distributed model, EcoTWIN disentangles the spatial
 737 domain into grid cells (Panel A). In each grid cell, hydrological, isotopic, and nitrogen processes were
 738 simulated in canopy, snow, soils, shallow groundwater, and groundwater (Panel B) and river channel
 739 if channels are present (Panel C).



741

742 Figure 2. The selected catchments for model calibration and validation. The overview of key
743 geographic, climatic, and nitrogen inputs are show in Figure S1.

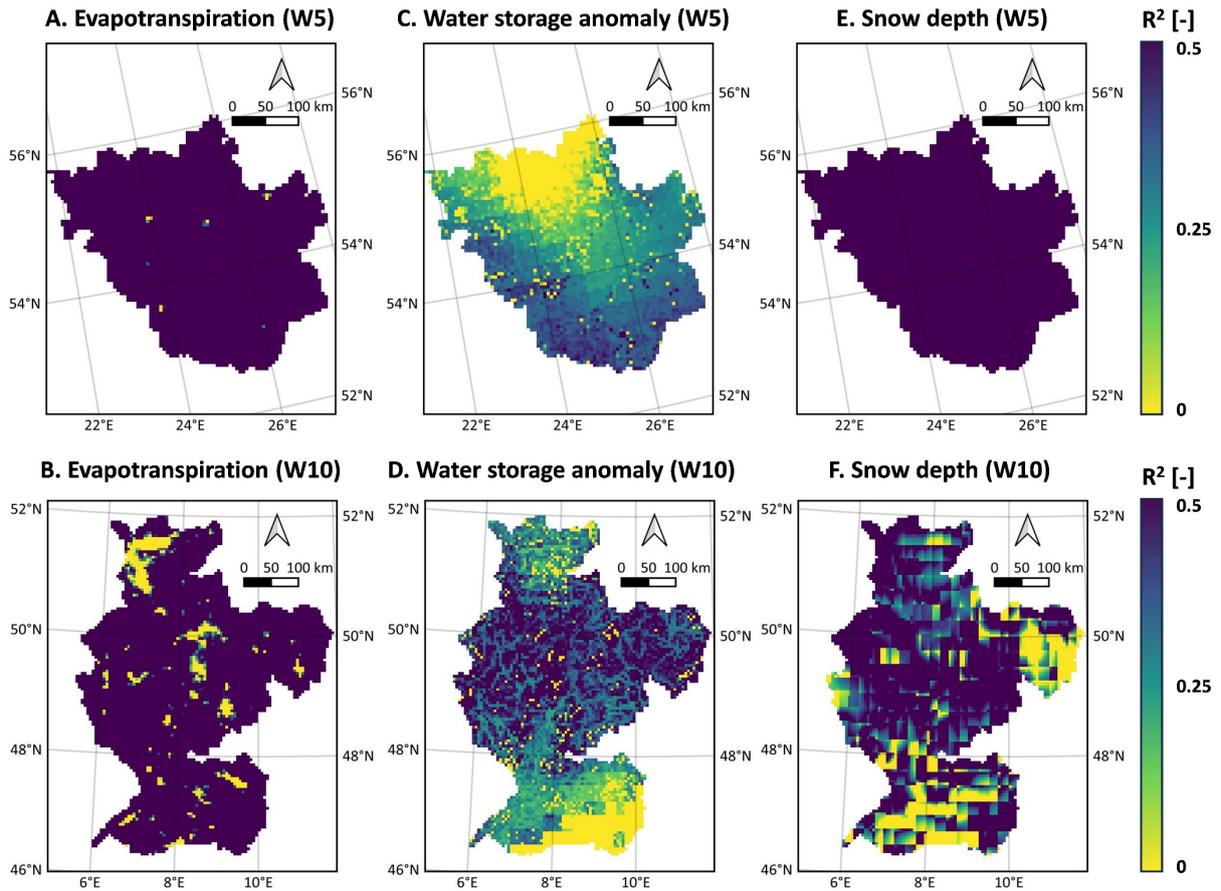
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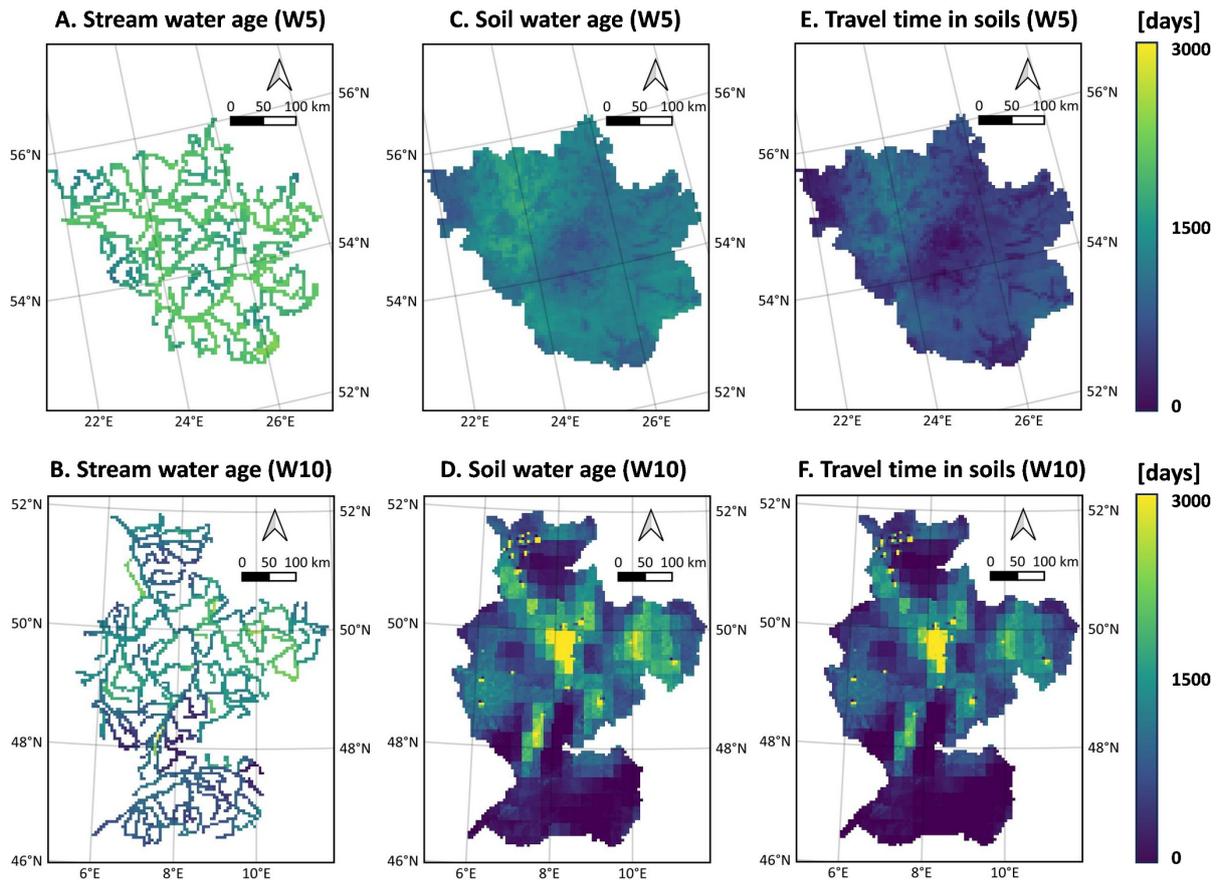
746 Figure 3. The simulation performance of discharge (A), in-stream isotope (B), and in-stream NO₃-N (C)
 747 evaluated using KGE. The comparison between simulation and observation values are shown in Panel
 748 D. Catchment-specific performances are summarised in KGE, RMSE, Pearson correlation coefficient,
 749 and Pbias in Table 2.

750



751

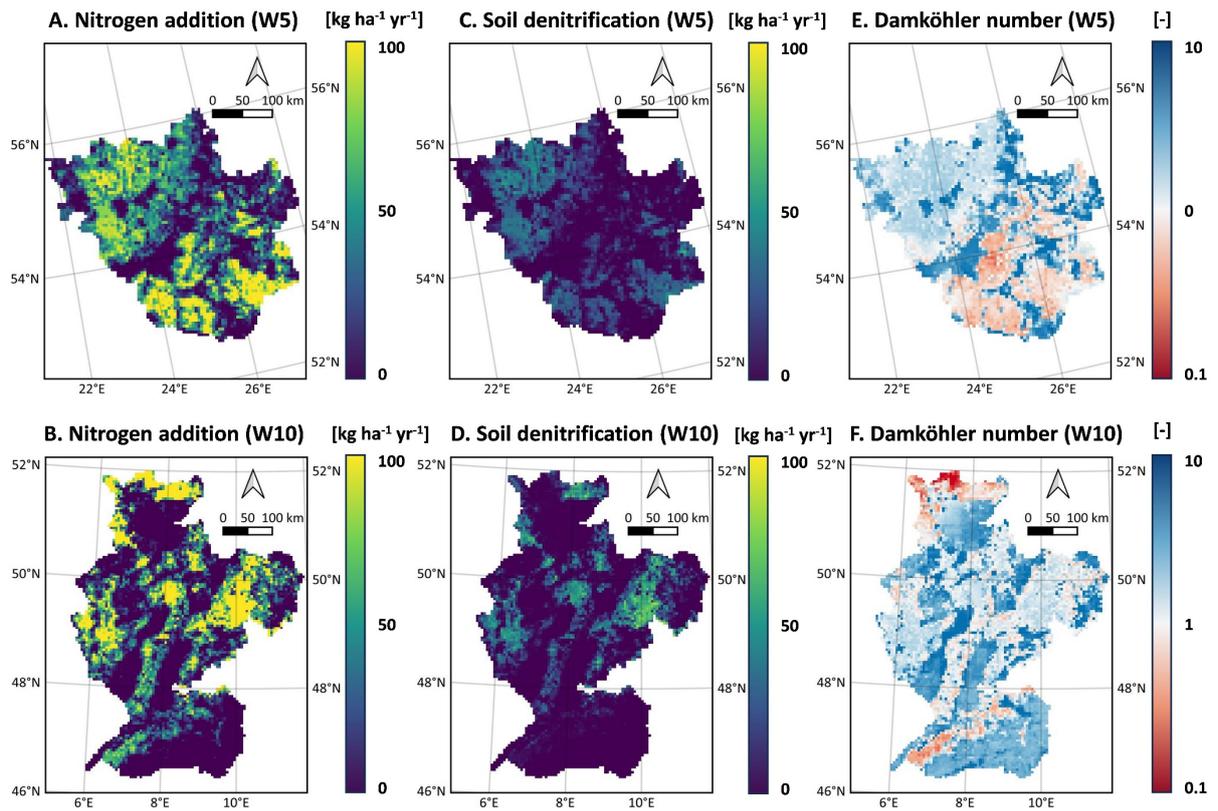
752 Figure 4. The grid-to-grid comparison (in coefficient of determination R^2) between time series of
 753 simulated internal states/fluxes and the corresponding records extracted from remote
 754 sensing/reanalysis products, including evapotranspiration from MODIS (Panel A-B), surface water
 755 mass anomaly from GRACE (Panel C-D), and snow depth from ERA5 (Panel E-F). The validation of two
 756 representative catchments is shown here (W5 Nemunas and W10 Rhine), while the spatial maps of all
 757 catchments were shown in Figure S2-4. The evaluation metrics in each catchment were summarised
 758 in Table 3.



759

760 Figure 5. The simulated long-term average (1982-2024) of stream water age (Panel A-B), soil water
 761 age (Panel C-D), and travel time of soil water (Panel E-F). Water age represents the time since water
 762 enters the catchments as precipitation, while travel time depicts the residence time of water within
 763 the specific storage (i.e., the soil profile in this figure). The spatial maps of all catchments are shown
 764 in Figure S5-7.

765



766

767 Figure 6. The simulated long-term average (1982-2024) of nitrogen addition from fertilization (Panel
 768 A-B), soil denitrification (Panel C-D), and Damköhler number (Panel E-F). Note that logarithmic scale
 769 is used for Damköhler number. The spatial map containing Damköhler number in all catchments is
 770 shown in Figure S8.

771

772

773

774 **Tables**

775 Table 1. Characteristics of the selected catchments. **Lat** depicts the latitude of upper left corner of the
 776 catchment. **DEM** and **Area** are the mean elevation in m.a.s.l. and catchment size in km². **Precip**, **Temp**,
 777 and **PET** are the annual averages of precipitation, air temperature, and potential evapotranspiration
 778 in mm/yr. f_{crop} , f_{forest} , and f_{urban} are the fractions of cropland, forest, and urbanized areas in 2019
 779 in %. Null means no name is assigned for the catchment in the Catchment Characterisation and
 780 Modelling (CCM) database.

ID	Name	Lat	Area	DEM	Precip	Temp	PET	f_{crop}	f_{forest}	f_{urban}
W1	Null	70.0	8725	468.5	448.5	-1.8	442.8	<1	1.9	<1
W2	Vefsna	65.9	5475	636.5	1260.8	0.7	433.6	<1	24.6	<1
W3	Null	59.8	5225	742.3	1400.9	3.0	545.7	<1	39.8	<1
W4	Null	58.3	4350	67.4	654.5	6.4	667.9	17.4	60.5	1.4
W5	Nemunas	56.6	97550	147.9	599.3	7.1	730.6	33.0	39.1	4.5
W6	Tweed	55.9	6250	264.3	1023.4	7.9	600.9	21.8	18.9	1.4
W7	Null	52.3	4300	175.4	1218.1	10.1	645.2	12.1	17.4	1.9
W8	Thames	52.2	11900	112.0	700.7	10.4	782.9	44.9	14.1	22.0
W9	Elbe	53.5	130225	318.3	626.9	8.8	836.3	41.2	34.6	10.8
W10	Rhine	52.0	170175	508.3	943.3	8.9	821.0	21.5	41.2	17.1
W11	Danube _(a)	50.5	197600	618.0	843.6	8.3	857.4	28.5	37.1	11.5
W12	Adige	47.2	11600	1771.9	1002.3	4.5	809.7	<1	48.8	3.2
W13	Loire	48.7	122125	298.9	778.7	11.0	887.4	37.6	25.7	6.5
W14	Tajo	40.4	75575	686.2	549.5	14.3	1359.6	26.7	34.5	3.3
W15	Danube _(b)	48.4	37975	533.3	534.7	8.1	869.5	32.0	41.3	6.4
W16	Danube _(c)	44.8	37725	653.3	684.7	9.7	994.2	12.8	44.3	5.8
W17	Null	37.6	12650	1384.5	454.1	12.2	1256.9	5.9	4.3	<1

781

782

783 Table 2. Calibration statistics for discharge (*q*), in-stream isotope (*iso*), and in-stream NO₃-N (*no3*)
 784 across the 17 selected catchments. Evaluation metrics represent the mean values computed within
 785 each catchment.

ID	Number of sites			Kling-Gupta efficiency			Pearson Correlation			Root Mean Square Error			Percent bias		
	<i>q</i>	<i>iso</i>	<i>no3</i>	<i>q</i>	<i>iso</i>	<i>no3</i>	<i>q</i>	<i>iso</i>	<i>no3</i>	<i>q</i>	<i>iso</i>	<i>no3</i>	<i>q</i>	<i>iso</i>	<i>no3</i>
	-	-	-	-	-	-	-	-	-	m ³ /s	‰	mg/L	%	%	%
W1	2	0	1	0.7		0.5	0.9		0.6	56		0.0	16.4		0.2
W2	2	0	0	0.9			0.9			47			3.3		
W3	1	0	1	0.6		0.4	0.7		0.4	62		0.0	22.5		2.3
W4	3	2	0	0.7	0.5		0.7	0.5		12	0.8		9.5	2.5	
W5	13	0	9	0.6		0.4	0.6		0.6	64		1.4	15.8		22.2
W6	3	0	1	0.4		0.4	0.9		0.4	42		0.4	48.3		4.3
W7	2	0	2	0.8		0.4	0.9		0.5	22		0.5	3.6		18.8
W8	3	0	1	0.7		0.5	0.9		0.5	18		1.3	17.0		6.6
W9	7	1	4	0.7	0.6	0.4	0.8	0.7	0.5	114	0.7	0.9	8.1	6.3	21.5
W10	18	6	10	0.8	0.3	0.5	0.9	0.4	0.6	232	0.8	0.8	8.0	5.4	13.7
W11	12	8	9	0.7	0.4	0.4	0.8	0.5	0.5	231	0.6	0.6	15.6	3.1	19.3
W12	2	1	0	0.8	0.9		0.8	0.9		43	0.3		2.5	0.9	
W13	8	0	2	0.4		0.6	0.8		0.6	120		1.1	52.9		14.1
W14	4	1	2	0.6	0.7	0.5	0.7	0.7	0.6	107	0.8	0.3	23.7	5.5	5.9
W15	2	0	0	0.7			0.8			113			5.8		
W16	9	1	3	0.6	0.5	0.3	0.7	0.5	0.4	59	1.5	0.5	20.4	-	3.7
W17	2	0	0	0.7			0.7			56			8.4		

786

787 Table 3. Validation performance of evapotranspiration (against MODIS records), water storage
788 anomaly (against GRACE records), and snow depth (against ERA5 records). Performance is evaluated
789 using the mean Kling–Gupta efficiency (KGE), Pearson correlation coefficient (*r*), root mean square
790 error (RMSE), and percent bias (Pbias) across all grid cells within each of the 17 catchments.

ID	Evapotranspiration				Water storage anomaly				Snow depth			
	<i>KGE</i>	<i>r</i>	<i>RMSE</i>	<i>Pbias</i>	<i>KGE</i>	<i>r</i>	<i>RMSE</i>	<i>Pbias</i>	<i>KGE</i>	<i>r</i>	<i>RMSE</i>	<i>Pbias</i>
	-	-	<i>m</i>	-	-	-	<i>m</i>	-	-	-	<i>m</i>	-
W1	0.88	0.95	0.00	0.3	-	0.79	0.03	8.7	0.78	0.90	0.04	57.5
W2	0.79	0.96	0.00	0.2	-	0.77	0.14	37.5	0.19	0.81	0.12	162.5
W3	0.80	0.94	0.00	0.3	-	0.45	0.08	18.5	0.65	0.82	0.06	1159.7
W4	0.67	0.88	0.00	0.3	-	0.51	0.05	13.1	0.52	0.85	0.01	14.6
W5	0.70	0.88	0.00	0.3	-	0.49	0.05	258.6	0.81	0.90	0.01	99.9
W6	0.65	0.90	0.00	0.3	-	0.46	0.03	18.4	0.40	0.82	0.00	1.9
W7	0.60	0.91	0.00	0.2	-	0.46	0.03	10.0	0.07	0.67	0.00	46.3
W8	0.58	0.89	0.00	0.4	-	0.65	0.03	8.3	0.28	0.81	0.00	10.7
W9	0.08	0.86	0.00	0.9	-	0.59	0.04	4.0	0.65	0.82	0.01	28.0
W10	0.44	0.90	0.00	0.6	-	0.62	0.05	4.7	0.25	0.78	0.03	8021.9
W11	0.39	0.90	0.00	0.6	-	0.53	0.05	4.3	0.36	0.80	0.02	99.4
W12	0.65	0.92	0.00	0.3	-	0.57	0.07	14.0	0.54	0.73	0.07	125.8
W13	0.67	0.90	0.00	0.3	-	0.64	0.04	3.2	0.62	0.78	0.00	434.3
W14	0.35	0.60	0.00	0.7	-	0.74	0.03	5.2	0.06	0.66	0.00	158.1
W15	0.67	0.90	0.00	0.3	-	0.50	0.06	2.5	0.16	0.79	0.01	536.2
W16	0.59	0.82	0.00	0.3	-	0.65	0.05	2.2	0.21	0.81	0.01	18.6
W17	0.18	0.78	0.00	0.4	-	0.43	0.06	4.3	-0.05	0.78	0.02	62.4

791

792 Table 4. Mean soil travel time and soil denitrification in selected catchments during 1982–2024. The
 793 regression slope of the fitted relationship between soil travel time and denitrification, along with the
 794 P-values of the Spearman test, are also reported.

ID	Avg. Soil travel time [days]	Soil denitrification [kg ha ⁻¹ yr ⁻¹]	Slope [-]	P-value [-]
W1	808.56	0.01	-0.01	<0.05
W2	206.20	0.01	0.07	<0.05
W3	434.02	0.01	0.00	<0.05
W4	804.22	3.33	10.01	<0.05
W5	1067.89	11.98	44.63	<0.05
W6	296.05	5.02	48.53	<0.05
W7	388.63	3.19	1.62	<0.05
W8	1063.87	64.50	-10.08	0.12
W9	1279.90	21.51	16.77	<0.05
W10	1099.18	10.64	10.86	<0.05
W11	948.22	15.64	11.14	<0.05
W12	320.98	0.21	0.43	0.06
W13	719.82	20.88	34.47	<0.05
W14	1336.50	8.40	5.76	<0.05
W15	1681.02	7.72	6.96	<0.05
W16	1039.28	3.46	5.32	<0.05
W17	419.38	2.22	2.38	<0.05

795

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