1 **Evaluation of wastewater nitrogen transformation in a natural wetland**
2 **(Ulaanbaatar, Mongolia) using dual-isotope analysis of nitrate**

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4 Masayuki Itoh^{*1}, Yasuhiro Takemon², Akiko Makabe³, Chikage Yoshimizu¹, Ayato
5 Kohzu⁴, Nobuhito Ohte⁵, Dashzeveg Tumurskh⁶, Ichiro Tayasu¹, Naohiro Yoshida³,
6 Toshi Nagata^{1,7}

7

8 *Corresponding author:

9 Center for Ecological Research, Kyoto University, 2-509-3 Hirano, Otsu, Shiga, 520-
10 2113, Japan

11 Phone/Fax: +81-77-549-8214/+81-75-874-4113

12 E-mail: masayukiitoh@yahoo.co.jp

13

14 Author affiliations:

15 ¹⁾ Center for Ecological Research, Kyoto University, 2-509-3 Hirano, Otsu, Shiga, 520-
16 2113, Japan

17 ²⁾ Disaster Prevention Research Institute, Kyoto University, Goka-sho, Uji, Kyoto, 611-
18 0011, Japan

19 ³⁾ Interdisciplinary Graduate School of Science and Engineering, Tokyo Institute of
20 Technology, 4259-G1-25, Nagatsuta-cho, Midori-ku, Yokohama, 226-8502, Japan

21 ⁴⁾ National Institute for Environmental Studies, 16-2 Onogawa, Tsukuba, Ibaraki, 305-
22 8506, Japan

23 ⁵⁾ Graduate School of Agricultural and Life Sciences, The University of Tokyo, 1-1-1
24 Yayoi, Bunkyo-Ku, Tokyo 113-8657, Japan

25 ⁶⁾ Institute of Geoecology, Mongolian Academy of Sciences, 15 Baruun Selbe,
26 Ulaanbaatar, 211238, Mongolia

27 ⁷⁾ Atmosphere and Ocean Research Institute, The University of Tokyo, 5-1-5
28 Kashiwanoha, Kashiwa, Chiba, 277-8564, Japan

29

30 **Abstract**

31 The Tuul River, which provides water for the daily needs of many residents of
32 Ulaanbaatar, Mongolia, has been increasingly polluted by wastewater from the city's
33 sewage treatment plant. Information on water movement and the transformation of
34 water-borne materials is required to alleviate the deterioration of water quality. We
35 conducted a synoptic survey of general water movement, water quality including
36 inorganic nitrogen concentrations, and isotopic composition of nitrogen ($\delta^{15}\text{N-NO}_3^-$,
37 $\delta^{18}\text{O-NO}_3^-$, and $\delta^{15}\text{N-NH}_4^+$) and water ($\delta^{18}\text{O-H}_2\text{O}$) in a wetland area that receives
38 wastewater before it enters the Tuul River. We sampled surface water, groundwater, and
39 spring water along the two major water routes in the wetland that flow from the drain of
40 the sewage treatment plant to the Tuul River: a continuous tributary and a discontinuous
41 tributary. The continuous tributary had high ammonium (NH_4^+) concentrations and
42 nearly stable $\delta^{15}\text{N-NH}_4^+$, $\delta^{15}\text{N-NO}_3^-$, and $\delta^{18}\text{O-NO}_3^-$ concentrations throughout its
43 length, indicating that nitrogen transformation (i.e., nitrification and denitrification)
44 during transit was small. In contrast, NH_4^+ concentrations decreased along the
45 discontinuous tributary and nitrate (NO_3^-) concentrations were low at many points.
46 Values of $\delta^{15}\text{N-NH}_4^+$, $\delta^{15}\text{N-NO}_3^-$, and $\delta^{18}\text{O-NO}_3^-$ increased with flow along the
47 discontinuous route. Our results indicate that nitrification and denitrification contribute
48 to nitrogen removal in the wetland area along the discontinuous tributary with slow
49 water transport. Differences in hydrological pathways and the velocity of wastewater
50 transport through the wetland area greatly affect the extent of nitrogen removal.

51 **Keywords:** water pollution; stable isotope analysis; wetland; nitrogen removal

52 **1. Introduction**

53 Like fertilizer in agricultural areas, sewage water from urban areas has been
54 identified as a major contributor to nitrogen loading and the degradation of aquatic
55 ecosystems (Carpenter et al., 1998). A high concentration of nitrate (NO_3^-) in drinking
56 water is considered to be one of the cofactors causing methemoglobinemia in infants
57 and possibly other pathological conditions such as cancer (Fewtrell, 2004; Ward et al.,
58 2005). The World Health Organization (WHO) and European Union (EU) have set
59 standards allowing 50 mg- NO_3^-/L ($\approx 806 \mu\text{mol/L NO}_3^-$) in drinking water, whereas the
60 federal standards of Japan and the United States permit 10 mg-N/L ($\approx 714 \mu\text{mol/L NO}_3^-$).

61 While many studies have examined nitrogen transformation in wastewater in
62 shallow surface and groundwater systems, most have been conducted in developed
63 countries in Europe and North America (e.g., Sebilo et al., 2006). In many developing
64 countries, water and sanitation services are insufficient and people lack access to clean
65 water for drinking, irrigation, industry, and other uses. In such countries, pollutant
66 inputs have increased with increases in population, degrading the water quality of rivers
67 and lakes. Even in the presence of a municipal sewage treatment plant, discharged water
68 is usually not sufficiently purified and can continuously pollute rivers and soil. The
69 World Resources Institute (1998) has estimated that less than 5% of all sewage in such
70 countries receives any treatment before it is discharged into the environment. Umezawa
71 et al. (2009) recently suggested that human waste released from severe sewage leakage
72 and agricultural land use are the major sources of nutrient contaminants in Asian
73 capitals such as Manila and Jakarta. To assess the ecological impacts of nitrogen
74 pollution from sanitation systems in developing countries, it is necessary to understand

75 the sources, transport, contamination, and transformations of nitrogen in polluted areas.
76 We can better understand the impacts of wastewater at study sites where the agricultural
77 impacts on nitrogen transformation are minimal.

78 Ulaanbaatar, the capital of Mongolia, experienced a rapid increase in population
79 during the past decade. In 2005, the city had more than one million residents. There is
80 concern that the Tuul River, an important water resource for the city, may be polluted by
81 wastewater discharged from the city's central wastewater treatment plant (CWWTP).
82 Discharged water from the CWWTP flows into a riparian wetland area (6.80 km²) in the
83 city and then into the main stem of the Tuul River. We conducted a 50-km survey of
84 water chemistry along the Tuul River (from 10 km upstream of Ulaanbaatar's eastern
85 edge to 10 km downstream of its western edge) in September 2006. The water sampled
86 in the 50-km survey along the Tuul River had dramatically higher NO₃⁻ concentrations
87 downstream of Ulaanbaatar than upstream of the city. In September 2006, the NO₃⁻
88 concentration increased from 5.3 µmol/L 10 km upstream of the eastern edge of the city
89 to 920 µmol/L in the wetland area to 23 µmol/L 10 km downstream from the western
90 edge of the city (Kohzu et al., unpublished data). These results suggest that the CWWTP
91 is contributing considerable amounts of nitrogen to Tuul River water. Many people in
92 Ulaanbaatar still live in yurts without water supply or sewage systems; therefore,
93 domestic wastewater may be discharged directly into the river or percolate through the
94 soil, and downstream users may drink water from wells recharged by polluted river
95 water. The region along the Tuul River is characterized by well-drained soils and
96 unconsolidated rocks, and empirical models have indicated that users of shallow wells
97 in similar areas with high nitrogen inputs are most at risk of consuming high-nitrate

98 water (Nolan et al., 2002). Thus, it is important to understand how well the CWWTP
99 functions and how much nitrogen is being added to the environment.

100 While the removal of ammonium (NH_4^+) by nitrification is dependent on oxygen
101 supply, the removal of NO_3^- by heterotrophic bacteria (denitrification) occurs under
102 anoxic conditions. Biogeochemical processes in riparian zones and wetlands can
103 contribute to nitrogen removal (Jansson et al., 1994; Fisher and Acreman 2004;
104 Hayakawa et al., 2006; Reinhardt et al., 2006). Such terrestrial-aquatic interfaces have
105 high denitrification potential because aerobic and anaerobic “hot spots” allowing for
106 both nitrification and denitrification coexist in the soil profile (McClain et al., 2003).

107 Many studies have shown that stable isotope techniques are useful for
108 understanding nitrogen sources and cycling. Ratios of nitrogen (N) and oxygen (O)
109 isotopes help to identify NO_3^- sources (Kendall et al., 2007). The content of the stable
110 isotope $\delta^{15}\text{N}-\text{NO}_3^-$ is also an effective indicator of denitrification in groundwater, due to
111 the isotopic fractionation that occurs during denitrification (Mariotti et al., 1988; Smith
112 et al., 1991; Bohlke and Denver, 1995). Denitrification can be assessed through the
113 characteristic upward shift of N and O isotopes in NO_3^- in soil and groundwater
114 (Bottcher et al., 1990; Aravena and Robertson, 1998; Mengis et al., 1999; Fukada et al.,
115 2004; Anisfeld et al., 2007). However, most field studies using these isotopic signatures
116 have been limited to areas with high NO_3^- concentrations such as fertilized agricultural
117 fields because measurement of $\delta^{15}\text{N}-\text{NO}_3^-$ and $\delta^{18}\text{O}-\text{NO}_3^-$ requires large volumes of
118 water for samples with low NO_3^- concentrations. The denitrifier method is a useful
119 procedure that allows rapid measurements of many samples with low NO_3^-

120 concentrations and requires smaller sample size and less laboratory preparation than
121 previous methods (Sigman et al., 2001; Casciotti et al., 2002).

122 Our objectives in this study were to assess the effect of CWWTP discharge
123 water on water chemistry downstream of the discharge and in the Tuul River, focusing
124 on hydrological features and their effects on nitrogen cycling. We hypothesized that the
125 wetland downstream of the CWWTP may mediate nitrogen contamination of
126 groundwater. To investigate nitrogen cycling during transport, we used the denitrifier
127 method to simultaneously measure $\delta^{15}\text{N-NO}_3^-$ and $\delta^{18}\text{O-NO}_3^-$ in samples collected
128 throughout the wetland area situated downstream of the sewage treatment plant in
129 Ulaanbaatar.

130

131 **2. Materials and methods**

132 *2.1. Site description*

133 We studied the riparian wetland area at 47.5°N, 106.4°E ($\approx 6.80 \text{ km}^2$ surface
134 area) along the Tuul River in southern Ulaanbaatar. The Tuul River is a tributary of the
135 Orkhon River, which flows into the Selenge River and eventually into Russia and Lake
136 Baikal (Fig. 1a).

137 The wetland study area is located between two tributaries that carry water from
138 the CWWTP and the main channel of the Tuul River. Approximately 85% of this area is
139 grass cover, and the remaining 15% (0.98 km^2) consists of distinct ponds that were
140 created artificially during gravel extraction (Fig. 1b). As no agricultural activities are
141 conducted in this riparian wetland area, the possibility of nitrogen fertilizer

142 contamination is negligible. A thermal power plant operates 5 km east of the study area,
143 and a railroad embankment crosses the wetland (Fig. 1b). The discharge from the
144 CWWTP first enters a large pond (LP; Fig. 1b). Wastewater is released from this pond
145 along two distinct water paths before it reaches the main channel of the Tuul River. In
146 this paper, these pathways are referred to as the continuous tributary, with uninterrupted
147 surface flow draining the catchment, and the discontinuous tributary, with surface flow
148 interrupted by several breaks that are presumably connected by subsurface flows (Fig.
149 1b).

150

151 2.2. *Hydrological measurements*

152 To clarify the movement of surface and shallow subsurface water, we measured
153 the water levels in the continuous tributary, discontinuous tributary, and many of the
154 ponds surrounding the large pond. We also used piezometers to measure subsurface
155 groundwater levels at 13 points along former water channels (Fig. 2). The piezometers
156 were each constructed from a PVC pipe (32 mm diameter, 1 m length), with eight 5-mm-
157 diameter holes evenly spaced around the pipe's circumference 50 mm from the bottom.
158 We also measured volumetric flow rates at several points along both the continuous
159 tributary and the discontinuous tributary.

160 All in situ synoptic water sampling and measurements were conducted from 13
161 to 15 September 2006, when the daily average air temperature was approximately 10°C.
162 We observed no precipitation for at least 10 days before we collected our samples.
163 Mean monthly air temperature exceeds 0°C only from April to September, and the
164 maximum monthly rainfall (in summer) is usually less than 100 mm. Winter access to

165 the sampling site was difficult because of ice and snow accumulation, and most surface
166 water was frozen in winter. Hydrological conditions thus vary little, even in summer.
167 The average annual precipitation in Ulaanbaatar is 270.5 mm, with 83% occurring as
168 rainfall in summer (June–September), based on monthly averages for a 31-year period
169 (1971–2001; World Meteorological Organization [WMO]; available at
170 <http://www.worldweather.org/119/c00229.htm>). Given the meteorological conditions of
171 the region and the fact that our study occurred during periods of high biological activity,
172 it is likely that our study represents a "best case scenario" from a nutrient removal
173 perspective.

174

175 *2.3. Water collection and analysis*

176 Water samples were collected from numerous continuous tributary,
177 discontinuous tributary, pond, groundwater (from piezometers; 1 m depth), and spring
178 water sampling points to measure water chemistry. Springs were visually identified
179 before collecting water samples. We also sampled water from a well in a small village
180 (referred to as W01). Concentrations of NH_4^+ , NO_3^- , nitrite (NO_2^-), $\delta^{15}\text{N}-\text{NO}_3^-$, and
181 $\delta^{18}\text{O}-\text{NO}_3^-$ were measured from rainfall samples collected in May and August 2009 near
182 the wetland study area. Snow water was also sampled in December 2009.

183 Water sampling included in situ measurements of electrical conductivity (EC;
184 model CM-21P; Toa, Japan), water temperature, and dissolved oxygen (DO; model
185 HQ20; Hach Company, USA). Water samples collected for measuring NH_4^+ , NO_3^- ,
186 NO_2^- , total dissolved nitrogen (TDN), chloride (Cl^-), $\delta^{15}\text{N}-\text{NO}_3^-$, $\delta^{18}\text{O}-\text{NO}_3^-$, and $\delta^{15}\text{N}-$
187 NH_4^+ were immediately filtered, first through glass-fiber filters (GF/F; nominal particle

188 retention, 0.7 μm ; Whatman, UK) and then through 0.20- μm cellulose acetate
 189 membrane filters, and stored in a freezer (-20°C). Water samples for the measurement
 190 of dissolved organic carbon (DOC) concentrations were immediately filtered through
 191 the same GF/F type and stored in a freezer (-20°C). NH_4^+ concentrations were
 192 measured by the fluorometric technique of Holmes et al. (1999). Concentrations of
 193 NO_3^- , NO_2^- , and dissolved organic nitrogen (DON; obtained as TDN minus dissolved
 194 inorganic nitrogen [DIN]) in water samples were analyzed calorimetrically with an
 195 autoanalyzer (AACS II; Bran + Luebbe, Germany), and Cl^- concentrations were
 196 analyzed by ion chromatography (ICS-90; Dionex, Japan). DOC concentrations were
 197 determined using a total organic carbon (TOC) analyzer (TOC-V; Shimadzu, Japan).

198 The N and O stable isotope ratios are expressed in the generally accepted
 199 delta notation as δ values in parts per thousand (‰):

200

$$201 \quad \delta_{\text{sample}}(\text{‰}) = [(R_{\text{sample}} - R_{\text{standard}})/R_{\text{standard}}] \times 1000, \quad (1)$$

202

203 where R is the $^{15}\text{N}/^{14}\text{N}$ or $^{18}\text{O}/^{16}\text{O}$ ratio in the NO_3^- of a sample or standard. The $\delta^{15}\text{N}$
 204 values were determined with respect to air, and the $\delta^{18}\text{O}$ values were determined with
 205 respect to Vienna standard mean ocean water (V-SMOW).

206 Prior to the analysis for $\delta^{15}\text{N}\text{-NO}_3^-$ and $\delta^{18}\text{O}\text{-NO}_3^-$, NO_2^- in the samples was
 207 removed by the ascorbate-based method (Granger et al., 2006) to eliminate interference.
 208 The $\delta^{15}\text{N}\text{-NO}_3^-$ and $\delta^{18}\text{O}\text{-NO}_3^-$ were then measured according to the denitrifier method
 209 (Sigman et al., 2001; Casciotti et al., 2002). Briefly, NO_3^- in a sample was
 210 quantitatively converted to nitrous oxide (N_2O) by denitrifying bacteria that lack N_2O -
 211 reductase activity. The N_2O was stripped from the sample vial using helium (He) as a

212 carrier gas, purified by cryogenic trapping (Precon System; Thermo Electron Corp.,
213 USA), separated chromatographically (Gas Bench; Thermo Electron Corp.), and
214 analyzed by mass spectrometry (Delta^{plus}XP; Thermo Electron Corp.). The standard
215 error of an internal KNO₃ laboratory standard was $\pm 0.11\text{‰}$ for $\delta^{15}\text{N-NO}_3^-$ and $\pm 0.90\text{‰}$
216 for $\delta^{18}\text{O-NO}_3^-$.

217 The $\delta^{15}\text{N-NH}_4^+$ was measured by the diffusion method (Holmes et al., 1998),
218 whereby ammonium absorbed onto a GF/F containing sulfuric acid (H₂SO₄) was
219 converted to nitrogen (N₂) and analyzed using an elemental analyzer-isotope ratio mass
220 spectrometer (EA-IRMS; Thermo Electron Corp.). Measurements were within $\pm 0.3\text{‰}$
221 of the standards for $\delta^{15}\text{N}$.

222 To determine oxygen isotope compositions ($\delta^{18}\text{O-H}_2\text{O}$), the water samples were
223 equilibrated with carbon dioxide (CO₂; Epstein et al., 1953). The equilibrated CO₂ gases
224 were analyzed with a isotope mass spectrometer (MAT 252; Thermo Electron Corp.).
225 Measurements were within $\pm 0.1\text{‰}$ of the standards for $\delta^{18}\text{O}$.

226 All statistical tests were performed using the statistical software R, version 2.11.0
227 (R Development Core Team, 2010). Analyses of variance (ANOVAs) were performed to
228 compare chemical concentrations in water among the sampling points, and specific
229 differences among the groups were analyzed using Tukey's multiple comparison test.

230

231 **3. Results**

232 *3.1 Hydrological features*

233 The groundwater level was higher in the northeast part of the wetland area (near
234 the large pond) and lower near the main stream of the Tuul River (Fig. 2). The water
235 level data for both ground- and surface water suggest that the railroad bed has
236 interrupted the water flow to some extent. This inference is supported by the EC data
237 (see section 3.2). Volumetric flow rates were significantly larger in the continuous
238 tributary ($\approx 2.76\text{--}3.04\text{ m}^3/\text{s}$) than in the discontinuous tributary ($\approx 0.17\text{--}0.25\text{ m}^3/\text{s}$; $p <$
239 0.05).

240 Water temperatures at the sampling points ranged from 10 to 20°C, except that
241 the temperatures of most spring waters were significantly lower (minimum 4.2°C; $p <$
242 0.05 ; Table 1). The $\delta^{18}\text{O}$ of water ranged from -14.6‰ to -14.4‰ in the large pond and
243 continuous tributary water, and was higher in groundwater, gravel ponds, spring water,
244 and discontinuous tributary sites (maximum -11.5‰ ; Table 1).

245

246 3.2. Water chemistry and isotopic ratios

247 DON and NH_4^+ concentrations were high at the large pond (301 $\mu\text{mol/L}$ and 425
248 $\mu\text{mol/L}$, respectively), which receives wastewater directly from the CWWTP (Table 1).
249 Water from the continuous tributary was characterized by significantly higher NH_4^+
250 (mean 586, range 487–851 $\mu\text{mol/L}$) concentrations and lower $\delta^{15}\text{N-NH}_4^+$ (mean 12.1‰,
251 range 10.9–13.0‰) values compared to water from the discontinuous tributary (mean
252 132, range 0.4–711 $\mu\text{mol/L}$, $p < 0.05$ and mean 16.4‰, range 12.1–19.4‰, $p < 0.05$,
253 respectively; Figs. 3b, 4a, Table 1). Like the continuous tributary water, discontinuous
254 tributary water near the large pond (DT01) had a high NH_4^+ concentration (711 $\mu\text{mol/L}$)
255 and low $\delta^{15}\text{N-NH}_4^+$ value (12.1‰; Table 1). The NH_4^+ concentrations and $\delta^{15}\text{N-NH}_4^+$

256 values in continuous tributary water remained high and low, respectively throughout its
 257 length (Figs. 3b, 4a). In contrast, NH_4^+ concentrations along the discontinuous tributary
 258 decreased dramatically with distance from the sewage treatment plant (from 711.6
 259 $\mu\text{mol/L}$ at DT01 to 0.4 $\mu\text{mol/L}$ at DT11; Fig. 3b, Table 1). Also, $\delta^{15}\text{N-NH}_4^+$ values
 260 increased in the wetland area (Fig. 4a). The concentrations and isotopic values of NO_3^-
 261 ($\delta^{15}\text{N-NO}_3^-$ and $\delta^{18}\text{O-NO}_3^-$) were nearly stable throughout the continuous tributary
 262 (Figs. 3d, 4b, 4c, Table 1). These isotopic values were lowest in the continuous tributary
 263 water (ranges: $\delta^{15}\text{N}$, $\approx 1.2\text{--}4.9\text{‰}$; $\delta^{18}\text{O}$, $\approx -8.2\text{--}-5.9\text{‰}$; Table 1). In the discontinuous
 264 tributary, NO_3^- and NO_2^- concentrations varied and $\delta^{15}\text{N-NO}_3^-$ and $\delta^{18}\text{O-NO}_3^-$ values
 265 increased with distance (Figs. 3c, 3d, 4b, 4c, Table 1). These findings, combined with
 266 the faster volumetric flow rate and higher and more stable values of EC (Fig. 3a), $\delta^{18}\text{O-}$
 267 H_2O , and Cl^- in the continuous tributary (vs. discontinuous tributary), indicate the
 268 minimal effects of nitrogen dilution or transformation on nitrogen form during flow
 269 along the continuous tributary (Table 1).

270 DOC concentrations were higher at the sampling points near the large pond (the
 271 upstream parts of the continuous and discontinuous tributaries and groundwater
 272 samples; Table 1). DOC concentrations in tributary water decreased with increasing
 273 distance from the CWWTP. Although not significant, the DOC concentration in spring
 274 water (mean 272, range 216–273 $\mu\text{mol/L}$) was lower than that at continuous tributary
 275 (mean 686, range 481–952 $\mu\text{mol/L}$), discontinuous tributary (mean 428, range 270–638
 276 $\mu\text{mol/L}$), and ground water (mean 1354, range 407–4157 $\mu\text{mol/L}$).

277 ECs were much higher in areas near the large pond ($\approx 1000 \mu\text{S/cm}$) than near the
 278 gravel ponds (as low as 180 $\mu\text{S/cm}$; Fig. 3a). Near the large pond, NH_4^+ concentrations

279 in groundwater were also high (maximum 1142 $\mu\text{mol/L}$). The decrease in EC and Cl^- in
280 gravel pond water with distance from the sewage treatment plant suggests that polluted
281 water from the plant was diluted with cleaner groundwater (Fig. 3a, Table 1). The
282 highest EC (G02; 1079 $\mu\text{S/cm}$), NH_4^+ (G01; 1141 $\mu\text{mol/L}$), and Cl^- (G04; 3344 $\mu\text{mol/L}$)
283 values and the heaviest $\delta^{18}\text{O-H}_2\text{O}$ value (G02; -10.0‰) were measured in groundwater
284 from the wetland area, especially near the large pond (Figs. 3a, 3b, Table 1). These
285 values were probably due to evaporative enrichment in this location. The NO_3^-
286 concentration in groundwater was significantly higher than in discontinuous tributary or
287 spring water ($p < 0.05$).

288 NH_4^+ concentrations were significantly lower in spring water than in continuous
289 tributary water ($p < 0.05$). Spring water NO_3^- concentrations were high in the
290 downstream region of the continuous tributary (site S04; 520 $\mu\text{mol/L}$) and low in the
291 downstream region of the discontinuous tributary (S02; 0.76 $\mu\text{mol/L}$; Fig. 3d). $\delta^{15}\text{N-}$
292 NO_3^- values were significantly higher in spring water (range: $\approx 11.3\text{--}61.2\text{‰}$) than in the
293 tributaries ($p < 0.01$; Fig. 4b). $\delta^{18}\text{O-NO}_3^-$ was also significantly higher in groundwater
294 (range: $\approx 53.8\text{--}77.4\text{‰}$) than in other water ($p < 0.01$; Fig. 4c). Notably, $\delta^{15}\text{N-NO}_3^-$ was
295 higher in spring water in the downstream half of the discontinuous tributary, with the
296 highest values at about the midpoint of this subsurface flow route (56.6 and 61.2 ‰ at
297 S01 and S02, Fig. 4b). $\delta^{15}\text{N-NH}_4^+$ was also high in this spring water (S01; 25.6 ‰ , Fig.
298 4a).

299 The NH_4^+ , NO_2^- , and NO_3^- concentrations and $\delta^{15}\text{N-NO}_3^-$ and $\delta^{18}\text{O-NO}_3^-$ values
300 in rain and snow water are shown in Table 1. The highest NO_3^- concentration was found
301 in a sample obtained in August (60.6 $\mu\text{mol/L}$) and the highest NH_4^+ concentration was

302 observed in a snow sample (83.8 $\mu\text{mol/L}$). $\delta^{15}\text{N-NO}_3^-$ was lower in summer (-0.6‰ in
303 rain obtained in August vs. 10.6‰ in snow), and $\delta^{18}\text{O-NO}_3^-$ was nearly stable
304 throughout the year.

305

306 **4. Discussion**

307 *4.1 Hydrological conditions*

308 The effects of dilution and chemical processes on nitrogen transformation in continuous
309 tributary water during flow were relatively small due to higher volumetric flow rates
310 than those in the discontinuous tributary. High NH_4^+ concentrations were sustained from
311 the outlet of the sewage treatment plant (CT01) to 5.7 km downstream, near the Tuul
312 River (CT04; Fig. 3b). In addition, the $\delta^{18}\text{O-H}_2\text{O}$ values of continuous tributary water
313 were significantly lower than those of water from any other sampling points ($P < 0.05$),
314 indicating that no notable evaporation occurred during flow down this tributary, and that
315 little mixing with evaporated groundwater (in the area near the large pond) had taken
316 place. In contrast, we observed higher EC values and Cl^- concentrations and heavier
317 $\delta^{18}\text{O-H}_2\text{O}$ values in wetland areas near the large pond (Table 1, Fig. 3a), indicating that
318 evaporative enrichment occurred in these areas. EC decreased with flow along the
319 discontinuous route in the downstream part, suggesting that cleaner groundwater and
320 spring water in this area contributed to discontinuous tributary water quality (Fig. 3a).

321

322 *4.2. Hydrological effects on nitrification and denitrification*

323 *4.2.1. Nitrogen transformations in continuous and discontinuous tributaries*

324 High and relatively stable NH_4^+ concentrations (487 - 851 $\mu\text{mol/L}$) throughout the
325 continuous tributary indicate that transformation of nitrogen (i.e. nitrification and
326 denitrification) in this tributary was small (Fig. 3b). This is probably because of the
327 suppression of nitrification in this tributary due to the prevailing reduced conditions as
328 indicated by low DO concentrations in river waters (Table 1). Assuming that Cl^- is a
329 conservative tracer largely unaffected by physical and biogeochemical processes, plots
330 of NO_3^- vs. Cl^- can be used to determine whether declining NO_3^- concentrations in
331 riparian areas are due to consumption or dilution processes (Altman and Parizek, 1995).
332 However, it is difficult to distinguish nitrogen transformations from the NO_3^-
333 concentrations at our study site because most DIN exists as NH_4^+ , and NO_3^- depletion
334 by denitrification or dilution can easily be obscured by NO_3^- production through
335 nitrification. We prepared a cross-plot of NH_4^+ vs. Cl^- concentrations to evaluate the
336 effects of the relevant processes (Fig. 5). Evaporative enrichment would result in
337 increased concentrations of Cl^- and NH_4^+ . In contrast, dilution, volatilization,
338 nitrification, or uptake by aquatic organisms would decrease NH_4^+ concentrations. In
339 Fig. 5, the data points for the downstream part of the continuous tributary (CT02, CT03,
340 and CT04) plot near the data from sites close to the CWWTP drain (CT01), indicating
341 that high NH_4^+ associated with insufficiently treated sewage water flows directly down
342 the continuous tributary.

343 In contrast to the continuous tributary route, along the discontinuous tributary
344 route, Cl^- decreased with increasing distance from the CWWTP and large pond (Table
345 1). This suggests that water in this area was diluted by cleaner groundwater. NH_4^+
346 concentrations along the discontinuous tributary also decreased with distance from the
347 CWWTP (Fig. 3b, Table 1). Considering that $\delta^{15}\text{N-NH}_4^+$ was higher in the downstream

348 part of this route than in other parts, we expected both volatilization and nitrification
 349 processes that can increase the $\delta^{15}\text{N-NH}_4^+$ value of residual NH_4^+ to occur (Fig. 4a).
 350 These processes would have contributed to decreases in the NH_4^+ and increase in NO_3^-
 351 concentration (Figs. 3b, 3d) and $\delta^{15}\text{N-NO}_3^-$ (Fig. 4b). In addition, increases in both
 352 $\delta^{15}\text{N-NO}_3^-$ and $\delta^{18}\text{O-NO}_3^-$ in the downstream part of the discontinuous tributary
 353 (DT08–DT10) suggest isotopic enrichment by denitrification (Figs. 4b, 4c, Table 1).
 354 Because denitrification shifts the isotopic composition of the residual NO_3^- to higher
 355 $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ values, a plot of $\delta^{18}\text{O}$ vs. $\delta^{15}\text{N}$ should have a slope of approximately 0.5
 356 to 1.0 if denitrification is occurring (Kendall et al., 2007). We found a significant
 357 positive relationship between $\delta^{18}\text{O-NO}_3^-$ and $\delta^{15}\text{N-NO}_3^-$ in water from the
 358 discontinuous tributary, with a slope of 0.36 (Fig. 6). Values of $\delta^{15}\text{N-NO}_3^-$ and $\delta^{18}\text{O-}$
 359 NO_3^- in water along the continuous tributary (CT02–CT04) fell within a narrow range
 360 ($\delta^{15}\text{N-NO}_3^- \approx 1.2\text{--}4.2\text{‰}$ and $\delta^{18}\text{O-NO}_3^- \approx -7.6\text{--}-5.9\text{‰}$) and were similar to those of the
 361 sewage discharge water ($\delta^{15}\text{N-NO}_3^- = 4.9\text{‰}$ and $\delta^{18}\text{O-NO}_3^- = -8.2\text{‰}$). Values at
 362 discontinuous tributary points near the CWWTP fell within a similar range. Both $\delta^{15}\text{N-}$
 363 NO_3^- and $\delta^{18}\text{O-NO}_3^-$ in discontinuous tributary water increased with distance from the
 364 CWWTP. Although the $\delta^{18}\text{O}$ vs. $\delta^{15}\text{N}$ slope (0.36; Fig. 6) is smaller than the predicted
 365 range of 0.5 to 1.0 for denitrification, considerable denitrification likely occurred along
 366 the discontinuous tributary route. The lack of a relationship between $\delta^{15}\text{N-NO}_3^-$ and the
 367 natural log of the NO_3^- concentration for discontinuous tributary water (Fig. 7) suggests
 368 a mixing of water of different origins with different NO_3^- concentrations (e.g., nitrate
 369 from rainfall, groundwater, and spring water). Addition of ^{15}N -enriched NO_3^- through in
 370 situ nitrification of ^{15}N -enriched NH_4^+ along this route would also have contributed to

371 lowering the $\delta^{18}\text{O}$ vs. $\delta^{15}\text{N}$ slope and the lack of a relationship in Fig. 7. In the
372 downstream part of the discontinuous tributary, water quality was similar to that of low-
373 temperature spring water near this route (S01–S03) with low NH_4^+ and NO_3^-
374 concentrations, suggesting an influence of spring water to the water quality of the
375 discontinuous tributary.

376 Our data indicate that the longer water residence time along the discontinuous
377 tributary route, in contrast to the direct flow down the continuous tributary increased the
378 probability of NH_4^+ nitrification. Then, subsequent denitrification in anoxic sites
379 resulted in efficient nitrogen removal.

380

381 4.2.2. Ponds and groundwater systems

382 The highest EC and Cl^- concentrations were found at sampling sites near the
383 large pond, indicating that evaporative enrichment caused salt accumulation (Fig. 3a,
384 Table 1). Most groundwater NH_4^+ concentrations in this area were lower than those at
385 CT01 (the nearest point to the CWWTP outlet; Fig. 5). Furthermore, NO_3^-
386 concentrations were higher in shallow groundwater than in surface water
387 (discontinuous tributary, large pond, and gravel ponds; Fig. 3d). These results imply
388 that nitrification occurred in vadose zone during water filtration. However, $\delta^{18}\text{O}-\text{NO}_3^-$
389 values in this groundwater were much heavier than those in other water, suggesting the
390 possible contribution of NO_3^- from rainfall (Table 1, Fig. 4c, 6) or the deposition of
391 NO_3^- originating from the thermal power plant that operates 5 km east of the study
392 area. Values of $\delta^{18}\text{O}-\text{NO}_3^-$ in precipitation across the United States were reported to
393 range from 63‰ to 94‰ with a mean value of 76.3‰ (Elliott et al., 2006). Estimates

394 of $\delta^{18}\text{O}$ of atmospheric NO_3^- obtained to date by the denitrifier method are generally
395 $>60\text{‰}$ (summarized in Kendall et al., 2007). We compared our measured values with
396 typical values of $\delta^{15}\text{N}-\text{NO}_3^-$ and $\delta^{18}\text{O}-\text{NO}_3^-$ derived or nitrified from various nitrogen
397 sources (Fig. 6, gray lines; described by Kendall et al., 2007). The $\delta^{18}\text{O}-\text{NO}_3^-$ ranges
398 of our rainfall and snow samples are similar to those of previous reports (Table 1).
399 Also, most of our groundwater values fall within the range for atmospheric nitrate (e.g.,
400 power plant, vehicle, and agricultural emissions). The expected $\delta^{18}\text{O}$ of microbially
401 produced NO_3^- should range from -5‰ to 15‰ (Durka et al., 1994; Mayer et al.,
402 2001; Willard et al., 2001). During oxidation of NH_4^+ to NO_3^- , two of the three oxygen
403 atoms are believed to come from H_2O and the third from molecular oxygen (O_2).
404 Considering that $\delta^{18}\text{O}-\text{H}_2\text{O}$ in this area ranged from -12.8‰ to -10.0‰ and assuming
405 that the $\delta^{18}\text{O}$ of soil O_2 was the same as that of atmospheric O_2 ($\approx 23.5\text{‰}$), then
406 atmospheric NO_3^- deposition must have contributed to the increase in NO_3^-
407 concentrations and $\delta^{18}\text{O}-\text{NO}_3^-$ values in this area.

408 NO_3^- concentrations in spring water were high in the downstream area of the
409 continuous tributary (S04; $520\text{ }\mu\text{mol/L}$) and low in the downstream area of the
410 discontinuous tributary (S02; $0.76\text{ }\mu\text{mol/L}$; Fig. 3d). Although we could only find a few
411 spring locations, the spring water had characteristic water quality, with significantly
412 lower water temperature and heavier $\delta^{15}\text{N}-\text{NO}_3^-$ values than other water types. The
413 much lower temperatures of spring water, especially at S04, than at the other sampling
414 points (Table 1) suggest that water from these springs traveled at deeper soil levels
415 before surfacing. Deep soil temperature is generally stable and close to the annual air
416 temperature ($\approx -1^\circ\text{C}$). The source or pathway of the spring water was thus likely

different than those of the other samples collected. It is not clear why the highest NO_3^- concentration among all sampling points was observed at S04 (along with a very low DO concentration). The lowest $\delta^{15}\text{N}-\text{NO}_3^-$ (18.2‰) among the spring water samples was at S04. In contrast, we observed a low NO_3^- concentration and very high $\delta^{15}\text{N}-\text{NO}_3^-$ (61‰) at S02 on the river side of the discontinuous tributary (Figs. 3b, 4b). The relationship between $\log[\text{NO}_3^-]$ and $\delta^{15}\text{N}-\text{NO}_3^-$ in spring water (Fig. 7) agrees with the evidence of denitrification reported in other groundwater studies (Mariotti et al., 1981, 1982; Bottcher et al., 1990; Aravena and Robertson, 1998; Willard et al., 2001; Granger et al., 2006).

We calculated isotope enrichment factors (ε) using the following Rayleigh equation:

$$\delta_R = \delta_{R0} + \varepsilon \ln(C / C_0) , \quad (2)$$

where δ_R is the $\delta^{15}\text{N}$ (or $\delta^{18}\text{O}$) of reactant NO_3^- at time t , δ_{R0} is the initial $\delta^{15}\text{N}$ ($\delta^{18}\text{O}$) value of the NO_3^- , C is the NO_3^- concentration at time t , and C_0 is the initial concentration. The ε calculated for $\delta^{15}\text{N}$ of spring water NO_3^- (−6.7‰) is within the range reported in the groundwater studies cited above (−40 to −5‰) and that for $\delta^{18}\text{O}$ (−3.8‰) is also similar to the reported range (−18.3 to −6.9‰; Bottcher et al., 1990; Mengis et al., 1999, Fukada et al., 2003, 2004). The correlation between $\log[\text{NO}_3^-]$ and $\delta^{15}\text{N}-\text{NO}_3^-$ indicates NO_3^- consumption by denitrification in deeper soil without substantial mixing of NO_3^- from other sources. The slope of the cross-plot of $\delta^{18}\text{O}$ and $\delta^{15}\text{N}$ of spring water NO_3^- (0.54; Fig. 6) is additional strong evidence that denitrification actively occurred during the transport of wastewater through deeper

groundwater. The supply of this heavy $\delta^{18}\text{O}$ and $\delta^{15}\text{N}$ of NO_3^- as spring water likely contributed to increasing these values in the downstream part of the discontinuous route.

4.3. Overview of nitrogen dynamics in the wetland area

Most DIN in our study area occurred as NH_4^+ , especially near the CWWTP (Table 1). Table 2 shows the daily flow rates of inorganic nitrogen components. At the time of this study, 98% of the inorganic nitrogen from the sewage treatment plant was NH_4^+ and most of this flowed directly along the continuous tributary route (Table 2). Considering that DON was as high as $300\ \mu\text{mol/L}$ in the large pond (P01), we can assume that there was sufficient DON in this location for nitrogen mineralization to occur, causing an increase in NH_4^+ concentrations in water that had passed through the large pond (CT02) in contrast to water before the pond (CT01). Along the continuous tributary route, concentrations of NH_4^+ remained high up to the confluence with the main stem of the Tuul River.

In contrast, by the time water reached the downstream part of the discontinuous tributary route, most of the inorganic nitrogen had been removed. Even though dilution by cleaner groundwater contributed to decreasing these inorganic nitrogen concentrations, our findings suggest that NH_4^+ was gradually transformed to NO_2^- or NO_3^- during travel from the CWWTP and was then removed by denitrification, especially in the discontinuous tributary and groundwater.

5. Conclusion

461 Our results indicate that the difference in the hydrological pathways of
462 wastewater delivery (i.e., fast and slow water transport) greatly affected the extent of
463 nitrogen removal by controlling both biogeochemical reactions (N mineralization,
464 nitrification, and denitrification) and other processes such as dilution and volatilization.
465 Our results demonstrated that synoptic data on dual NO_3^- and other isotopes provide
466 powerful constraints on nitrogen transformation pathways in sewage-impacted wetlands.
467 Such intensive sampling can be applicable to and allow understanding of pollutant
468 removal in many developing countries where insufficient sewage water treatment
469 induces water pollution and eutrophication in rivers or lakes. Our results also suggest
470 that a simple change in the sewage effluent pathway can enhance the purification
471 capacity of the downstream area at a low cost. This finding may be relevant to
472 wastewater management. Although our study did not examine greenhouse gases (e.g.,
473 nitrous oxide) that must be emitted in compensation for wetland nitrogen removal,
474 comprehensive environmental assessment including greenhouse gas emission is
475 necessary for the effective implementation of wastewater management.

476

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486

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591

592 **Figure legends**

593 Fig. 1. (a) Location of the study site in Mongolia and (b) simplified map of the study
594 area showing the locations of sampling points. CT, continuous tributary; DT,
595 discontinuous tributary; LP, large pond; GP, gravel pond (created by gravel extraction).
596 Pond sampling sites are identified by the prefix 'P'; spring water sampling sites are
597 identified by the prefix 'S'.

598

599 Fig. 2. Spatial distribution of water levels in the area of intensive sampling. Values are
600 height differences (m) from the reference point in the southwestern part of the study
601 area. Graded red color with isolines indicates the variation in water levels. SigmaPlot
602 ver. 11.0 software (Systat Software Inc., Chicago, IL, USA) was used for graphical
603 analysis of the data, and the running average method was used for interpolation of water
604 level data. The dotted line indicates the discontinuous tributary (DT). Gravel ponds
605 (GP) are shaded gray.

606

607 Fig. 3. Longitudinal plots of (a) electrical conductivity (EC), (b) NH_4^+ , (c) NO_2^- , and (d)
608 NO_3^- concentrations, referenced to kilometers downstream from the CWWTP. For the
609 continuous tributary (CT) and discontinuous tributary (DT) sampling sites, distance was
610 measured along the tributary from the drain outlet of the sewage treatment plant. For
611 pond water (P), spring water (S), well (W), and groundwater (G) sampling sites,
612 distance is the linear distance from the outlet of the sewage treatment plant.

613

614 Fig. 4. Longitudinal plots of (a) $\delta^{15}\text{N-NH}_4^+$, (b) $\delta^{15}\text{N-NO}_3^-$, and (c) $\delta^{18}\text{O-NO}_3^-$ values,
615 referenced to kilometers downstream from the CWWTP. For the continuous tributary
616 (CT) and discontinuous tributary (DT) sampling sites, distance is measured along the
617 tributary from the drain outlet of the sewage treatment plant. For pond water (P), spring
618 water (S), well (W), and groundwater (G) sampling sites, distance is the linear distance
619 from the outlet of the sewage treatment plant.

620

621 Fig. 5. Relationship between NH_4^+ and Cl^- concentrations in water samples. The
622 diagram at the upper right shows the possible influence of related processes. The gray
623 dashed line is the assumed dilution of sewage discharge water by groundwater that
624 originating from Tuul River water.

625

626 Fig. 6. Cross-plot of $\delta^{18}\text{O-NO}_3^-$ vs. $\delta^{15}\text{N-NO}_3^-$ in water samples. Origins of nitrate and
627 the denitrification process can be identified from the nitrogen and oxygen isotope values
628 (Kendall et al., 2007; indicated by gray text and inserts in this figure).

629

630 Fig. 7. Relationship between $\delta^{15}\text{N}$ of nitrate and NO_3^- concentrations in water samples.

Table 1. Results of chemical and isotopic analyses

	Location	Distance (km) ^a or sampling time	Water Temp (°C)	EC (μS/m)	DO (mg/L)	NH ₄ ⁺ (μM) ^b	NO ₃ ⁻ (μM)	NO ₂ ⁻ (μM)	DON (μM)	Cl ⁻ (μM)	DOC (μM)	δ ¹⁸ O- H ₂ O (‰)	δ ¹⁵ N- NO ₃ ⁻ (‰)	δ ¹⁸ O- NO ₃ ⁻ (‰)	δ ¹⁵ N- NH ₄ ⁺ (‰)
	Rainfall	May 2009	NM	NM	NM	30.3	9.2	0.8	NM	NM	NM	NM	5.0	53.6	NM
	Rainfall	Aug 2009	NM	NM	NM	68.7	60.6	3.1	NM	NM	NM	NM	-0.6	65.5	NM
	Snow	Dec 2009	NM	NM	NM	83.8	26.3	4.3	NM	NM	NM	NM	10.6	58.3	NM
Direction of flow ↓	CT01	0.63	18.1	836	3.0	487.4	5.7	5.0	270.4	2358	951	-14.6	4.9	-8.2	13.0
	CT02	1.25	17.8	874	2.4	850.9	7.0	4.5	119.7	2126	820	-14.6	2.2	-7.6	12.6
	CT03	3.38	15.3	879	2.8	518.1	5.6	4.6	47.0	2241	480	-14.3	4.2	-5.9	11.8
	CT04	5.71	13.8	856	0.8	489.2	4.4	2.3	92.6	2120	491	-14.5	1.2	-6.7	10.9
	DT01	2.17	17.6	984	9.5	711.6	0.5	0.4	162.3	2537	618	-11.9	BDL	BDL	12.1
	DT02	2.31	19.4	871	10.6	444.0	8.8	1.5	36.7	2696	638	-11.0	4.8	-7.5	18.0
	DT03	2.49	13.0	303	9.2	3.2	0.1	0.2	27.5	568	343	-9.4	BDL	BDL	BDL
	DT04	2.60	16.5	450	12.6	53.3	0.5	0.3	189.0	946	422	-11.5	BDL	BDL	17.2
	DT05	2.68	19.6	724	10.6	172.7	3.0	0.6	43.3	1648	572	-12.0	-5.3	-8.1	13.7
	DT06	2.84	19.0	505	11.7	28.7	3.2	0.9	146.2	648	363	-11.5	-1.9	-9.3	18.1
	DT07	3.00	19.3	370	11.5	0.5	0.1	0.1	29.1	878	368	-10.7	BDL	BDL	BDL
	DT08	3.41	16.4	820	8.0	39.2	0.1	0.2	131.5	1997	450	-12.5	BDL	BDL	19.4
	DT09	4.34	11.1	454	6.6	0.4	6.6	0.2	15.3	769	326	-12.1	22.7	0.8	BDL
	DT10	5.24	8.4	457	6.0	0.4	5.1	0.2	13.5	1234	270	-12.1	21.5	0.2	BDL
	DT11	5.94	10.7	197	4.2	1.0	8.2	0.3	15.9	1209	335	-12.5	17.3	-1.2	BDL
	P01	0.0	16.7	747	3.7	425.9	8.0	3.8	301.8	1724	708	-12.8	12.9	-4.6	15.4
	P02	0.41	15.4	310	11.3	16.6	1.8	0.5	28.8	696	380	-11.0	12.0	-8.9	23.4
	P03	0.81	13.4	201	11.2	1.0	1.2	0.1	23.5	236	258	-11.7	BDL	BDL	BDL
	P04	1.00	15.1	184	9.2	0.2	0.5	0.1	13.6	225	218	-11.6	BDL	BDL	BDL
	S01	1.52	11.7	560	0.1	34.0	4.0	0.0	22.3	1758	333	-11.8	56.6	18.0	25.6
	S02	1.69	9.7	513	0.6	0.4	0.8	0.0	15.6	1583	244	-11.8	61.2	27.9	BDL
	S03	3.45	9.0	129	0.2	1.2	8.3	0.3	9.8	140	297	-14.9	35.1	10.9	BDL
	S04	3.60	4.6	825	0.1	0.4	520.4	0.6	11.0	2493	215	-12.1	18.2	1.6	BDL
	W01	2.98	8.6	358	2.2	0.2	15.7	0.2	12.4	748	171	-12.6	11.3	-4.1	BDL
	G01	0.10	20.4	889	NM	1141.0	8.2	4.8	288.7	2152	1282	-12.3	22.9	53.8	NM
	G02	0.20	17.5	1079	NM	NM	NM	NM	NM	NM	NM	-10.0	n.a	n.a	NM
	G03	0.26	20.0	963	NM	587.3	15.1	6.6	302.4	2526	1821	-12.5	13.8	73.6	NM
	G04	0.32	19.6	1020	NM	698.2	26.4	8.8	238.8	3344	4157	-12.5	13.8	71.4	NM
	G05	0.34	19.1	1040	NM	226.4	9.4	2.3	104.1	2995	2115	-12.4	10.0	66.2	NM
	G06	0.37	15.9	834	NM	316.1	8.3	12.7	88.6	3247	1486	-12.0	19.2	77.4	NM
	G07	0.71	22.0	454	NM	2.4	13.6	0.8	40.6	827	596	-11.6	5.8	66.3	BDL
	G08	0.75	NM	NM	NM	95.8	14.0	2.0	79.9	3299	870	-12.3	10.3	70.9	NM
	G09	0.78	22.0	412	NM	22.1	6.1	1.2	29.4	740	407	-11.5	10.0	70.5	NM
	G10	1.01	23.1	883	NM	240.4	4.9	3.4	16.8	2223	1014	-12.6	15.8	70.6	NM
	G11	1.06	20.2	915	NM	130.8	12.5	1.7	8.7	2166	636	-12.3	7.0	66.8	NM
	G12	1.10	19.6	291	NM	4.6	2.8	2.2	43.2	531	511	-11.5	19.6	63.3	BDL
	Tuul River			91	9.2	0.9	34.9	0.3	11.8	12	NM	-15.0	17.1	0.4	BDL

^aFor continuous tributary (CT) and discontinuous tributary (DT) sampling sites, distance is measured along the tributary from the drain outlet of the sewage treatment plant. For pond water (P), spring water (S), well (W), and groundwater (G) sampling sites, distance is the linear distance from the "large pond" (LP; P01).

^b"μM" indicates "μmol/L"

"BDL" means below detection limit. "NM" means not measured.

1 Table 2. Nitrogen flow and form along the continuous tributary (CT) and discontinuous tributary (DT) drainage routes.

	Volumetric flow rate (m ³ /d)	NH ₄ ⁺ (kg-N/d)	Proportion of CT01 levels (%)	NO ₃ ⁻ (kg-N/d)	Proportion of CT01 levels (%)	NO ₂ ⁻ (kg-N/d)	Proportion of CT01 levels (%)	Cl ⁻ (kg-Cl/d)	Proportion of CT01 levels (%)
CT01	2.6 × 10 ⁵	1789.8	100.0	20.8	100.0	18.2	100.0	2.20 × 10 ⁴	100.0
CT02 ^a	2.6 × 10 ⁵	3108.3	173.7	25.3	122.1	16.3	89.7	1.97 × 10 ⁴	89.7
CT03	2.4 × 10 ⁵	1729.7	96.6	18.7	89.9	15.3	84.3	1.90 × 10 ⁴	86.4
CT04	2.6 × 10 ⁵	1745.6	97.5	15.6	75.3	8.3	45.5	1.92 × 10 ⁴	87.4
DT09	1.5 × 10 ⁴	0.1	0.01	1.4	6.6	0.0	0.2	401	1.8
DT10	2.2 × 10 ⁴	0.1	0.01	1.5	7.4	0.1	0.3	946	4.3
DT11	1.6 × 10 ⁴	0.2	0.01	1.9	9.0	0.1	0.3	705	3.2

^aOutlet from "large pond" (LP).

(a)

(b)

The map illustrates the Tuul River basin with various sampling locations and areas of intensive sampling. A north arrow and a 1 km scale bar are provided. The river is shown as a thick grey line. Sampling locations are marked with symbols: triangles for surface water (tributary), plus signs for surface water (pond), and stars for spring water. A large grey rectangle indicates the 'Area of intensive sampling'. Within this area, a smaller rectangle is labeled 'GP' (Gongoljir Pond). Other areas labeled include 'CT' (Chinggisai Town), 'DT' (Dunhuang Town), 'LP' (Lunping Pond), and 'CWWTP' (Chinggisai Wastewater Treatment Plant). A legend at the bottom right defines the symbols: a triangle for 'Surface water (tributary)', a plus sign for 'Surface water (pond)', and a star for 'Spring water'.

Figure3

Figure4

Figure5

Figure6

Figure7