

Spatial and Seasonal Variation of Dissolved Nitrous Oxide in Wetland Groundwater

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Received: August 26, 2013 Accepted: November 12, 2013 Online Published: December 3, 2013

doi:10.5539/ep.v3n1p21

URL: <http://dx.doi.org/10.5539/ep.v3n1p21>

Abstract

Understanding the spatial and temporal pattern of dissolved nitrous oxide (N₂O) in groundwater is essential to estimate the N₂O emissions from groundwater to the unsaturated zone and to the atmosphere. In order to study the spatial distribution and seasonal change of dissolved N₂O in wetland, a headwater wetland in Ichikawa, Chiba Prefecture, Japan, was chosen. Variations of nitrate (NO₃⁻), dissolved N₂O and δ¹⁵N-NO₃⁻ indicated that the dissolved N₂O in the groundwater of study wetland consists of two parts, one from denitrification within the wetland, and another from nitrification at upland. Principal component analysis (PCA) was used to assess the shallow groundwater parameters in the wetland. And t-test was conducted to find statistically significant differences of the variables between the ASW and NS, warm season and cool season. The concentrations of dissolved N₂O increased from the upland to the zone of adjacent area between slope and wetland (ASW) and then decreased at the zone near the stream (NS). In sight of dissolved N₂O associated nitrogen migration, groundwater in the study area can be divided into three stages: upland as the stage 1, ASW as the stage 2, and NS as the stage 3. Higher temperature results in higher denitrification rate, lower dissolved oxygen (DO) and oxidation-redox potential (ORP), yielding higher concentration of N₂O in the warm season. Therefore, the seasonal change of dissolved N₂O in study wetland can be mainly interpreted by the variation of temperatures of groundwater.

Keywords: dissolved N₂O, spatial distribution, seasonal change, denitrification, stage

1. Introduction

Over the last few decades, much interest has been focused on specific natural systems, such as wetland (or riparian zone) which are vulnerable to improve water quality by physical, chemical and biological process that remove N from groundwater (García-García, Gómez, Vidal-Abarca, & Suárez, 2009; Groffman, Gold, & Simmons, 1992; Sabater et al., 2003). Wetlands offer an abundant organic C supply and dominated by inherently wet surface soil create anaerobic environment to consume nitrate via denitrification that is considered the most important reaction for nitrate removal in aquifer (Bastviken, Olsson, & Tranvik, 2003; Burgin & Hamilton, 2007; Whitmire & Hamilton, 2005). Especially in the shallow ground water of riparian areas, redox conditions are often favorable for intense denitrification processes (Ross, 1995).

The trace gas N₂O is an obligate intermediate product of biological denitrification. And it is known to contribute to global warming and the destruction of stratospheric ozone. A significant amount of N₂O emissions originates denitrification (Mathieu et al., 2006). Emissions from aquifers are most likely to occur from shallow aquifers, where N₂O can be quickly transferred through the unsaturated zone to the atmosphere by diffusion (Rice & Rogers, 1993). N₂O emission from wetland system has been estimated by numerous studies (Dhondt, Boeckx, Hofman, & Van Cleemput, 2004; Groffman, Gold, & Addy, 2000; Verhoeven, Arheimer, Yin, & Hefting, 2006). Understanding the spatial and seasonal pattern of dissolved N₂O is essential to assess the indirect emission of N₂O from groundwater (Geistlinger, Jia, Eisermann, & Florian Stange, 2010). Level of dissolved N₂O in groundwater has been paid lots of attentions. For example, N₂O concentration in groundwater was reported to exceed greatly those of atmospheric equilibration (with a mean value of 28.98 μg L⁻¹) under aerobic condition in Kanto district, Japan (Ueda, Ogura, & Yoshinari, 1993), and the maximum up to 30000 times of that in the ambient air (Heincke & Kaupenjohann, 1999). However, few studies estimated level of dissolved N₂O in wetland groundwater.

According literature review, study of spatial pattern of dissolved N_2O has been focused on surface water, such as river, lake and ocean (Butler, Elkins, Thompson, & Egan, 1989; Ferrón, Ortega, & Forja, 2010; Hinshaw & Dahlgren, 2013; Wang et al., 2009; Zhang, Zhang, Liu, Ren, & Zhao, 2010). The pattern of seasonal and spatial of dissolved N_2O is related to denitrification or nitrification depending on the environment in watershed. For example, the highest concentrations of dissolved N_2O were observed in the riparian zone in May (warm season), when the nitrate (NO_3^-) and temperature were conducive for denitrification (Davidson, Stark, & Firestone, 1990). However, Kim, Isenhardt, Parkin, Schultz, and Loynachan (2009) found that dissolved N_2O concentrations were with the highest value in cool season and the lowest value in warm season. Thus, the pattern of seasonal change of dissolved N_2O in wetland is unclear. In addition, N_2O also could product from nitrification (fertilizer and manure ammonium-nitrogen is oxidized to nitrate-nitrogen) in unsaturated zone. N_2O could leach to groundwater at upland and discharge to wetland through the groundwater flow system (Mühlherr & Hiscock, 1998; Spalding & Parrott, 1994). However, few studies estimated the contribution of the N_2O from nitrification at upland to dissolved N_2O in wetland.

Therefore, the objectives of this study were 1) to identify the source of dissolved N_2O and its evolution stages based on $\delta^{15}N-NO_3^-$, NO_3^- and dissolved N_2O ; and 2) to understand comprehensively the spatial distribution and seasonal change of dissolved N_2O concentration in shallow groundwater of headwater wetland. As a matter of convenience, we define the groundwater are at stage 1 in upland where the dissolved N_2O is produced from nitrification, stage 2 where more dissolved N_2O is produced than consumed in denitrification in wetland, and stage 3 where the net of dissolved N_2O decreases resulting from little available nitrate and its reduction to N_2 as a proceed stage of stage 2.

2. Sites Description and Method

2.1 Site Description

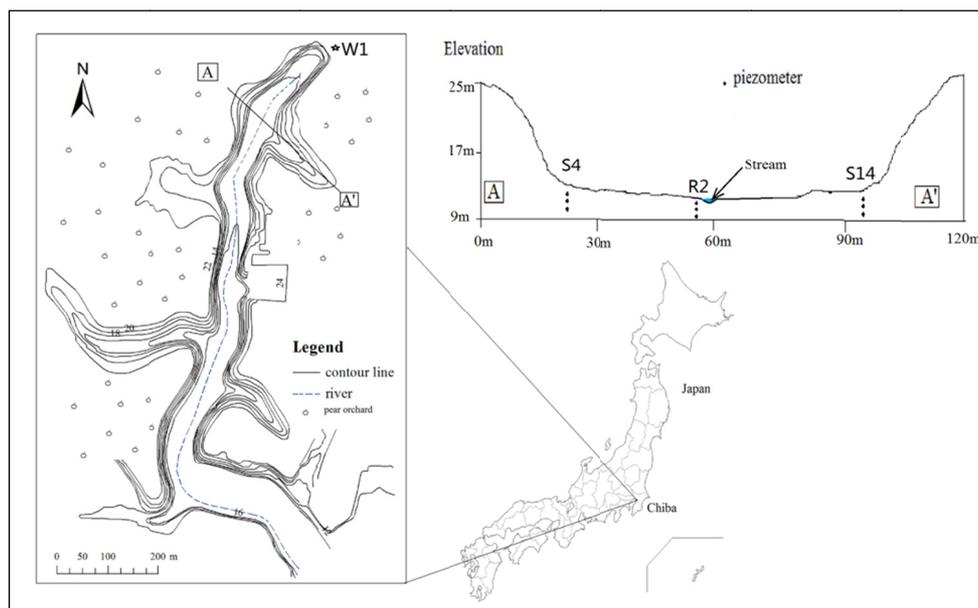


Figure 1. Study sites in Ichikawa, Japan. S4, R2 and S14 constitute transect A within the wetland and W1 is located in the upland site

The study area is a headwater wetland, located at Ichikawa City (35.76 °N, 139.97 °E), Chiba Prefecture, Japan (Figure 1). The wetland valley is U-shaped with an elevation of about 12m above sea level. The wetland receives discharge (both groundwater and overland flow) from an adjacent upland (elevation 25-28 m) area mostly pear orchard vegetation. A stream flowing through the wetland valley is recharged by spring water and groundwater in the wetland. Previously, this wetland used to be paddy field and had been redeveloped to a wetland park. Average yearly flow of the stream from the study wetland is about $6.70 \times 10^5 \text{ m}^3 \text{ year}^{-1}$. Dominating vegetation in the wetland are *Houttuynia*, *Calamus* and Japanese pampas grass. The slope is close to the orchard edge and is decreasing towards the wetland. It acts as a transitional zone linking the upland and the experimental wetland. The slope is covered by *Acer*, *Pinophyta* and *Bambuseae*. The annual average precipitation is 1,316 mm, with a

maximum monthly precipitation of 226.5 mm month⁻¹ in May of study area. The annual average temperature is 15.6 °C with a highest temperature of 36.9 °C in August and a lowest temperature of -3.4 °C in January. The nitrogen load in pear orchard of the upland is estimated about 400 kg ha⁻¹ year⁻¹ (Agriculture and Forestry Research Center of Chiba Prefecture, 2003). The area of pear orchard in Ichikawa city is 272 ha which account for 45% of agricultural land of Ichikawa city. The upland is covered by Kanto Loam about 4 m in thickness. It is underlain in a sequence by Joso clay layer and narita sand. That is a fine sand layer which is the major aquifer and the water table was about 17 m above sea level at upland all around the year. Within the wetland, the aquifer is a fine sand layer overlaid by cohesive soil and sandy clay with the water table depth > 11.6 m above sea level.

2.2 Sampling Procedures and Measurements

Field surveys were conducted in May, July, September and November in 2011, March and June in 2012. The study area is characterized by a temperate climate with warm season from June to September and cool season from November to May. Water samples were taken from well W1 at the upland and piezometers which were installed at S4, S14 and R2 with depths of 1 m, 2 m and 3 m in the wetland, for a total of 9 piezometers (Figure 1). S4 and S14 were at the sides of wetland and R2 was placed approximately 0.3 m on the west side of the stream. In order to get the fresh groundwater, we withdrew water from the piezometers and waited the fresh groundwater flowing in. In order to avoid the loss of dissolved gas during the sampling, a new sampler has been developed (Figure 2a). The sampler was inserted into the bottom of piezometer slowly with the outlet opened and the inlet closed. The inlet was opened by drawing the rope stopper to let the fresh groundwater flow in gently, make the vial (35 ml) full and push out the air inside through tube with the three-way stopcock. After closing the three-way stopcock, the sampler was taken out from the piezometer and the vial was sealed with a rubber cap under the water in the sampler as soon as possible. 1 ml sterilant (hibitane) was injected into the vial through the cap after water collection. Dissolved N₂O-N concentration was determined by headspace method. 10 ml pure N₂ gas was injected into the vial to push out an equal volume of water from the vial. Vials were shaken for 1 minute and stored at 40 °C for 24 h to equilibrate. The gas samples were analyzed for target gas (N₂O) by a gas chromatography (GC14B, Shimadzu) equipped with an electron capture detector operated at 280 °C, injector at 100 °C and column at 700 °C.

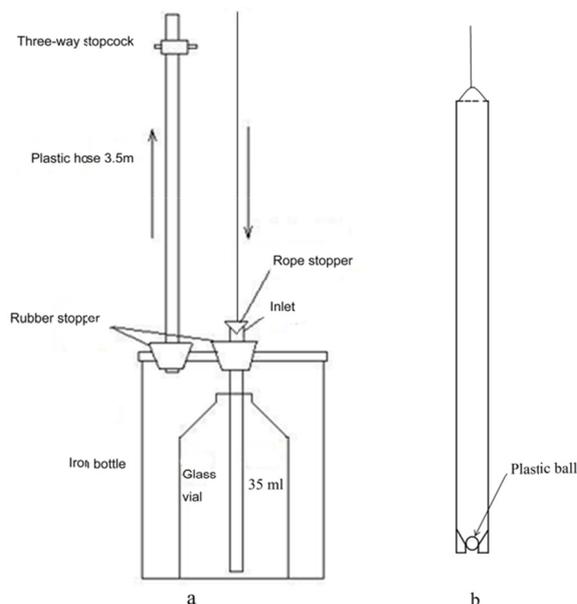


Figure 2. The schematic diagram of sampler for dissolved N₂O (a) and ion, parameters and δ¹⁵N-NO₃⁻ (b)

After sample for gas analysis was taken, water sample for ion, parameters and δ¹⁵N-NO₃⁻ analysis were collected by a pipe sampler (Figure 2b). When the pipe is put into water, a plastic ball at the bottom of the pipe can go up due to floatage that let the water into the pipe. The ball go back to the bottom when the pipe out of water that seal the pipe. Then, water samples were taken from the piezometers separately. Samples were brought back to the laboratory and stored at 4 °C before laboratory analysis. DO, pH, ORP, and temperature of groundwater were

measured in situ with sensors (HIROBA). All water samples were filtered (0.45 μm) before analysis for major ions by ion chromatography (Shimadzu CDD-6A and CDD-10Avp). 2 L water for each sample was collected for $\delta^{15}\text{N-NO}_3^-$ analysis in March 2012. NO_3^- was collected by passing the water through pre-filled, disposable, anion exchanging resin columns in the field and then was eluted by 3 M HCl from the column. The nitrate-bearing acid eluant was neutralized with Ag_2O , filtered to remove the AgCl precipitate, then freeze dried to obtain solid AgNO_3 , which was then combusted to N_2 in sealed quartz tubes for analysis by Integra CN mass spectrometer (PdZ Europa LTD) at Chiba University, Japan (Yingjie Cao, Tang, Song, Liu, & Zhang, 2012). All the samples were measured twice and the result showed the difference between the two measurements was less than $\pm 5\%$. Then the mean of two measurements was used as the value of $\delta^{15}\text{N-NO}_3^-$ in this study.

2.3 Statistical Analysis

Variables were tested using student t-test and principal component analysis (PCA), with SPSS 8.0 for Windows (SPSS, 1997, IL, USA). T-test was used to determine if two sets of data are significantly different from each other. The PCA is a data transformation technique that attempts to reveal a simple understanding structure that is assumed to exist within a multivariate dataset (Davis, 1986).

3. Results

3.1 Basic Parameters and Dissolved N_2O in Upland Shallow Groundwater

Samples were taken from W1 in July and November 2012, respectively. DO and ORP concentrations were higher in July (Table 1). pH values of groundwater were lower than 7 both in July and November. Groundwater temperature was little higher in July than that in November. NO_3^- -N and N_2O -N concentrations were both higher in July than that in November.

Table 1. Basic parameters and dissolved N_2O of upland shallow groundwater in July and November 2012

	$\text{N}_2\text{O-N}$ ($\mu\text{g L}^{-1}$)	DO (mg L^{-1})	ORP (mv)	NO_3^- -N(mgL^{-1})	T ($^\circ\text{C}$)	pH
Jul	14.73	9.29	325	34.74	18.3	6.94
Nov	8.11	5.70	295	17.30	16.9	6.05

3.2 Basic Parameters and Dissolved N_2O in Wetland Shallow Groundwater

Groundwater temperatures in the wetland ranged from 14.2 to 24.8 $^\circ\text{C}$ during the study period (Table 2). pH values of groundwater ranged from 6.53 to 7.97, indicating that the groundwater was alkaline except S14 which pH was lower than 7 during the warm season. DO concentrations ranged from 0.07 to 11.50 mg L^{-1} . It was lower than 4 mg L^{-1} , and as low as 0.07 mg L^{-1} in June at R2. At S4 and S14, the DO concentrations were lower than 5 mg L^{-1} in the warm season, but up to 11.5 mg L^{-1} in the cool season (S14-3 m in November). ORP values ranged from -244 to 303 mV. At 1 m and 2m depth of R2, ORP values were below about 0 mV in the warm season with the lowest value of -189 mV. However, ORP was up to 175 mV in March. At 3 m depth of R2, ORP was above 0 mV except in September (-244 mV). The NO_3^- -N concentration changed from 0 to 114.0 mg L^{-1} in study sites. At S4 and S14, most NO_3^- -N concentrations are clearly above the standard of the drinking water (10 mg L^{-1}) set by United States Environment Protection Agency (Figure 3), whereas NO_3^- -N concentration was extremely low for detection at R2. NH_4^+ and NO_2^- were also measured with other major ions, and found below the detectable limit.

Table 2. T (°C) pH DO (mg L⁻¹), and ORP (mV) of the groundwater at S4, S14 and R2 in 1m, 2 m and 3 m depth

Site	May/11		Jul/11		Sep/11		Nov/11		Mar/12		Jun/12	
	T	pH										
S4-1	14.7	7.70	19.7	6.99	24.8	7.56	14.7	7.48	15.4	7.38	19.4	7.15
S4-2	15.2	7.62	19.1	7.05	24.8	7.50	15.2	7.41	14.8	7.36	19.1	7.23
S4-2	14.9	7.42	19.9	7.18	18.4	7.53	14.9	7.40	14.5	7.54	18.5	7.52
R2-1	16.2	7.48	23.2	6.90	24.7	7.15	16.2	7.63	19	7.49	22.5	6.94
R2-2	16.4	7.72	21.0	7.17	21.4	7.47	16.4	7.59	15.3	7.59	20.3	7.07
R2-3	15.5	7.97	20.7	7.30	20.8	7.72	15.5	7.65	17.7	7.63	20.3	7.17
S14-1	15.4	7.84	20.5	6.68	20.9	7.17	15.4	6.90	17.2	7.66	19.5	6.87
S14-2	15.0	7.63	19.4	6.58	18.3	7.03	15.0	6.71	17.1	7.79	18.9	6.84
S14-3	14.2	7.41	20.1	6.53	20.4	6.84	14.2	6.61	15.4	7.66	18.2	6.95

Site	May/11		Jul/11		Sep/11		Nov/11		Mar/12		Jun/12	
	DO	ORP	DO	ORP	DO	ORP	DO	ORP	DO	ORP	DO	ORP
S4-1	7.31	287	2.90	185	3.26	284	6.82	287	7.41	267	3.16	269
S4-2	7.05	278	2.03	178	3.06	303	6.00	278	5.74	258	4.00	269
S4-2	8.09	260	2.46	191	1.80	287	8.26	260	8.26	272	2.73	280
R2-1	3.23	18	0.21	-77	0.14	-26	3.20	18	2.8	128	1.84	-27
R2-2	3.95	-173	1.55	-177	0.13	-189	2.80	-173	2.59	175	0.13	-150
R2-3	1.41	232	2.19	50	0.68	-244	3.78	232	2.65	146	0.07	151
S14-1	7.2	280	2.35	147	4.59	197	9.35	280	4.75	287	3.13	126
S14-2	7.2	311	1.53	169	2.10	230	11.00	311	7.18	279	3.70	168
S14-3	6.69	292	2.44	177	2.36	229	11.50	292	6.61	279	4.03	164

Dissolved N₂O concentrations ranged from 0.09 to 100.62 µg L⁻¹ (Figure 3). At S4, dissolved N₂O ranged from 6.13 to 79.96 µg L⁻¹ with the highest concentrations in July and the lowest values in March. At S14, dissolved N₂O ranged from 7.8 to 100.62 µg L⁻¹ with the highest values in July and the lowest values in November. At R2, dissolved N₂O ranged from 0.09 to 2.29 µg L⁻¹ at 1 m in depth, and from 1.41 to 50.16 µg L⁻¹ at 2 m and 3 m in depth.

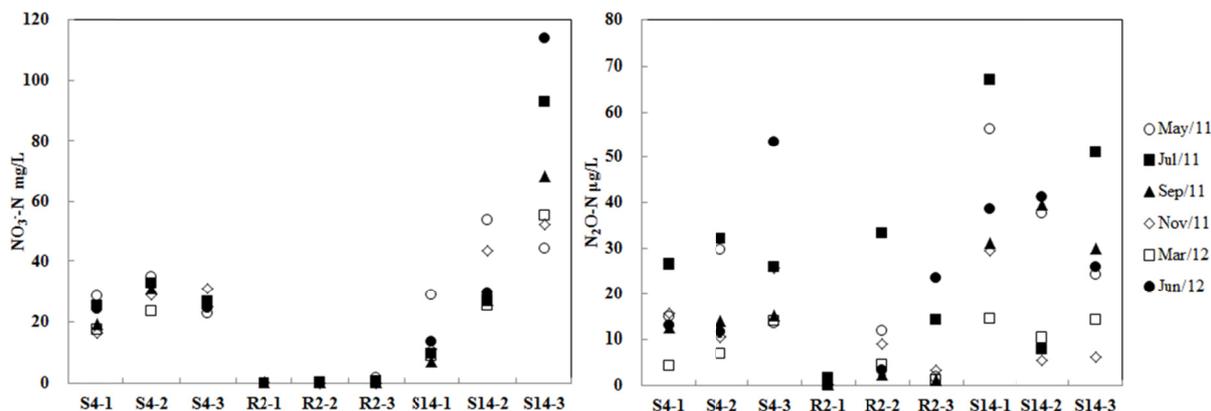


Figure 3. Variations of NO₃⁻-N and N₂O-Nat S4, S14 and R2 in 1m, 2 m and 3 m depth (Open cycles: the cool season; Closed cycles: the warm season)

3.3 Variations of $\delta^{15}\text{N-NO}_3^-$ in Shallow Groundwater

$\delta^{15}\text{N-NO}_3^-$ in shallow groundwater of upland (W1) was 5.67‰ (Table 3). The $\delta^{15}\text{N-NO}_3^-$ in groundwater was 6.36‰ for 1m and 8.27‰ for 2m at S4, respectively. It was 8.7‰ for 1m, 9.81‰ for 2m and 7.67‰ for 3m in depth at S14. The highest value was found in the groundwater 2m at S14 and the lowest value at W1. Comparing with groundwater in the upland, $\delta^{15}\text{N-NO}_3^-$ was enriched from 0.69‰ to 4.14 ‰ in the wetland. However, it was undetectable at R2 because little nitrate was available.

Table 3. $\delta^{15}\text{N-NO}_3^-$ in groundwater of wetland and upland

	W1	S4-1m	S4-3m	S14-1	S14-2m	S14-3m
$\delta^{15}\text{N-NO}_3^-$	5.67‰	6.36‰	8.27‰	8.7‰	9.81‰	7.67‰

3.4 Statistical Analysis

PCA was used to assess the shallow groundwater parameters in the wetland. To maximize the variance of the two principal axes, the varimax normalized rotation was applied. The load factors have been polarized after rotation of component matrix (Table 4). PCA results show two components with eigenvalues larger than 1, which explain 69.74% of the total variance. The first component explains about 39.894% of the observed variance. DO, T and ORP are correlated with the first component, representing the redox condition in the groundwater. The second component explains about 29.845% of the observed variation and is correlated with NO_3^- , N_2O and pH which representing the reactants and products associated with denitrification process.

Table 4. Loadings for two principal components of groundwater variables in wetland

Variable	Component 1 (Rotated)	Component 2 (Rotated)
$\text{N}_2\text{O-N}$	0.089	0.735
DO	0.898	0.063
T	-0.859	0.231
ORP	0.758	0.338
$\text{NO}_3^- \text{-N}$	0.426	0.720
pH	0.294	-0.748
Variance explained,% of total	39.894	29.845

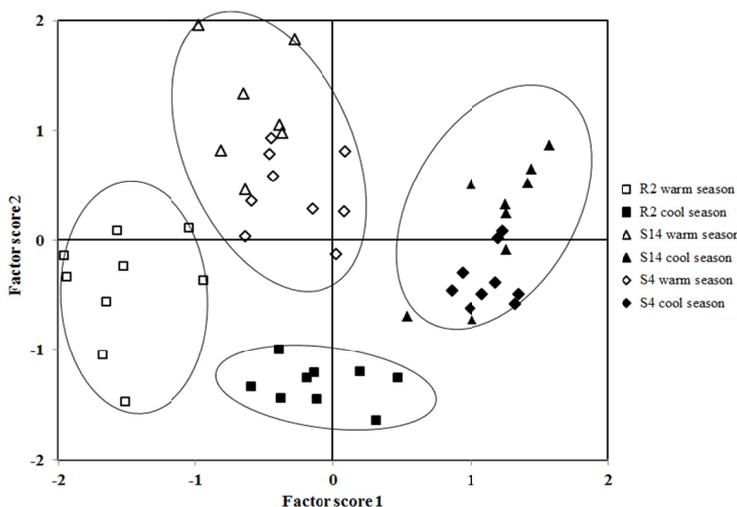


Figure 4. Bi-plot of the factor scores of the two principal components

The scores represent the influence of the component on the groundwater (Figure 4). It is possible to group the samples according to the axes of component 1 and 2. As a result, the samples are classified into four groups to showing seasonal and spatial patterns. The samples of NS are plotted at the down-left of the diagram for the warm season and the down-middle for the cool season. On the other hand, the samples of ASW are plotted at the upper-left of the diagram for the warm season and the upper-right for the cool season.

As the result of the groups from PCA, mean value, standard deviation and a t-test was conducted to find the difference and statistically significant differences of the variables between the ASW and NS, warm season and cool season (Table 5). NO_3^- -N, DO, ORP and N_2O -N in groundwater were significantly higher at ASW than those at NS, whereas there was no significantly difference of temperature and pH between ASW and NS. For N_2O -N, NO_3^- -N concentrations, and DO, variability was higher at ASW than those at NS on the basis of standard deviation. In contrast, variability of ORP was lower at ASW than it at NS. In addition, the mean N_2O -N concentration at ASW was high ($36.14 \mu\text{g L}^{-1}$), which was about 60 times of that in the ambient air. N_2O -N concentration and temperature in groundwater were significant higher in warm season than those in cool season, and DO, ORP and pH were significantly lower in warm season. There was no significant difference of NO_3^- -N concentrations between two seasons which seems to be the rule rather than the exception.

Table 5. Mean (m) and standard deviation (parentheses) of N_2O , DO, ORP, NO_3^- and T in shallow groundwater of ASW (n=36) and NS (n=18)

Zone	N_2O -N ($\mu\text{g L}^{-1}$)	DO (mg L^{-1})	T ($^{\circ}\text{C}$)	ORP (mV)	NO_3^- -N (mg L^{-1})	pH
ASW	36.14*	5.22*	17.60 _{n.s.}	237.00*	33.00*	7.24 _{n.s.}
	(23.79)	(2.72)	(2.84)	(52.49)	(22.08)	(0.37)
NS	9.27	1.85	19.10	-5.00	0.01	7.42
	(13.87)	(1.36)	(2.98)	(156.77)	(0.36)	(0.30)
Warm	34.19**	2.17*	20.50*	117.19**	23.5 _{n.s.}	7.11*
	(27.01)	(1.30)	(1.93)	(162.66)	(28.34)	(0.30)
Cool	20.19	6.03	15.6	209.67	20.6	7.49
	(19.75)	(2.65)	(1.12)	(135.12)	(18.60)	(0.31)

n.s., Not significant ($p > 0.05$)

*The difference between mean values is highly significant ($p < 0.01$)

** The difference between mean values is significant ($p < 0.05$)

4. Discussions

4.1 Source of Dissolved N_2O of Shallow Groundwater

In order to estimate the concentration of N_2O in groundwater, it is important to identify its source. Fertilizer and manure ammonium-nitrogen applied in the orchard are oxidized to nitrate-nitrogen and nitrous oxide in unsaturated zone of the upland. Nitrate leaches to the groundwater from unsaturated zone in the upland. $\delta^{15}\text{N}-\text{NO}_3^-$ value of W1 is coincided with range of $\delta^{15}\text{N}-\text{NO}_3^-$ (+4.5 ‰ to +8.5 ‰) in the area effected by mineral fertilizer (Cao, Sun, Xing, & Xu, 1991; Choi, Lee, & Ro, 2003; Choi, Han, Lee, Lee, & Yoon; Heaton, 1986; Singleton et al., 2007), indicating the dissolved N_2O was produced via nitrification in the unsaturated zone of upland. DO concentrations were high at W1, indicating that denitrification could not occur. Nitrate and N_2O transport from upland to wetland with groundwater consequently. N_2O is difficult to denitrified to N_2 because the groundwater in upland is often assumed to have low biological activity due to low C content (Groffman, Gold, & Jacinthe, 1998). Geistlinger et al. (2010) found there will be a diffusive N_2O flux from the deeper water to the capillary fringe. However the time scale of this process is very large i.e., for 10 cm travel distance, the N_2O molecules need ≈ 230 d. Thus, diffusive loss to upward is considered to have little effect on N_2O concentration in the groundwater during transporting from upland to wetland.

At the wetland, denitrification can enrich ^{15}N in the residual nitrate of groundwater (Cey, Rudolph, Aravena, & Parkin, 1999; Lehmann, Reichert, Bernasconi, Barbieri, & McKenzie, 2003). $\delta^{15}\text{N}-\text{NO}_3^-$ in the residual nitrate enriched from 2.8 ‰ to 78.32 ‰ when the concentration of NO_3^- -N decreased from 35.68 mg L^{-1} to 0.45 mg L^{-1}

in a sand aquifer (Böttcher, Strelbel, Voerkelius, & Schmidt, 1990), and from 6.4 ‰ to 24.8 ‰ when the NO_3^- -N concentration decreased from 13.3 to 5.6 mg L^{-1} in a riparian zone (Cey et al., 1999). In this study wetland, the $\delta^{15}\text{N}$ - NO_3^- enriched by 9.81 ‰ or even higher when the NO_3^- -N was no longer detectable. Therefore, dissolved N_2O in the shallow groundwater of wetland consists of two parts, one from denitrification within the wetland, and another from the upland where nitrification is dominant.

4.2 Spatial and Seasonal Pattern of Dissolved N_2O in Shallow Groundwater of the Wetland

The previous section suggested that the source of dissolved N_2O of groundwater in wetland comes from nitrification in upland and denitrification in wetland. In the study wetland, denitrification controls the behavior of dissolved N_2O . Because N_2O is an intermediate product of denitrification that is produced when nitrate is reduced and is consumed by reduction to N_2 . Denitrification is considered to be related to many factors (DO, ORP, T, pH and NO_3^-). For example, the highest concentrations of N_2O were found in the aerobic section of a limestone aquifer with the DO concentration below 4.00 mg L^{-1} and in a phreatic aerobic aquifers with the DO concentration below 3.15 mg L^{-1} (Deurer et al., 2008; Ronen, Magaritz, & Almon, 1988). However, the optimal maximum DO concentration for nitrogen removal was determined to be around 2.0-2.5 mg L^{-1} in the laboratory experiments (Yoo et al., 1999). According the early study, Nelson and Knowles (1978) reported that the startup of denitrification can be inhibited while the oxygen level is as low as 0.13 mg L^{-1} in a dispersed-well sludge reactor. In the laboratory experiments, as the ORP drops below 0 mV, the nitrate begins to be converted to nitrite and nitrite accumulates continuously for ORP ranging from 0 to -225 mV. From -225 to -400 mV, the accumulated nitrite is converted to N_2 . As the ORP below -400 mV, the nitrate is firstly converted first to nitrite then the nitrite is converted immediately to N_2 without accumulation (Lee et al., 2000). It also reported that ORP below about 200 to 300 mV were found to be conducive to denitrification, and the maximum N_2O were found at a ORP value of 0 mV (Kralova, Masscheleyn, Lindau, & Patrick Jr, 1992). Therefore, the optimum value of DO and ORP for N_2O accumulation is not consistent with the value of the optimum for denitrification due to the N_2O is an intermediate product. For nitrate, DeSimone and Howes (1998) studied that kinetics of denitrification at nitrate concentrations $>1 \text{ mg-N L}^{-1}$ is zero order and even small amount of nitrate (lower than 2 mg-N kg^{-1}) leached was sufficient to create a large amount of N_2O in groundwater (Müller, Stevens, Laughlin, & Jäger, 2004). Many studies suggested that high concentration of NO_3^- -N inhibits the N_2O reductase yielding the higher concentration of N_2O (Blackmer & Bremner, 1978; Deurer et al., 2008; Heisterkamp, Schramm, de Beer, & Stief, 2012). At ASW, the DO ($m = 5.22 \text{ mg L}^{-1}$) and ORP ($m = 237 \text{ mV}$) values were both higher than the optimum values respectively, as well as high concentrations of NO_3^- -N which were conducive to N_2O accumulation ($m = 36.14 \text{ } \mu\text{g L}^{-1}$) (Table 5). However, the mean value of DO concentrations (2.02 mg L^{-1}) and ORP were much lower (-5 mV) at NS. Additionally, NO_3^- -N is low or undetectable throughout the study. Under these conditions, the N_2O is used as an electron acceptor instead of nitrate in denitrification process (Ishii, Ohno, Tsuboi, Otsuka, & Senoo, 2011), resulting in the lower concentration ($m = 13.87 \text{ } \mu\text{g L}^{-1}$). Therefore, ASW and NS can be considered as in the stage 2 and stage 3, respectively. In addition, the average flux of N_2O was found to be higher at ASW than it at NS (Li, Tang, Han, Cao, & Zhang, 2013) which is consistent with the trend of dissolved N_2O .

Seasonal changes of dissolved N_2O are most associated with NO_3^- concentration and water temperature (Bouwman, Boumans, & Batjes, 2002; Hinshaw & Dahlgren, 2013; Velthof, Oenema, Postma, & Van Beusichem, 1996). The T-test indicates that the concentrations of NO_3^- -N had no significant difference between the two seasons, which suggests NO_3^- -N is not the limited factor for denitrification rate in study wetland (Table 5). Temperature affected the dissolved N_2O directly by controlling the denitrification rate (Nowicki, 1994; Pfenning & McMahan, 1997; Saunders & Kalf, 2001). The threshold temperature for controlling the rate of denitrification was 20 °C (Halling-Sørensen & Jørgensen, 1993) or even below 17 °C (McCutchan & Lewis, 2008; Nowicki, 1994). A study in coarse sandy soils found that the denitrification activity was low at 10 °C and completely inhibited at 2 and 5 °C because lower temperature may regulate metabolic rates for denitrifying bacteria (Vinther & Sørensen, 1991). Temperature also influences the solubility of oxygen, the rates of aerobic respiration of bacteria and the ORP change in groundwater, all of which in turn limit dissolved N_2O indirectly. For example, the oxygen solubility is 14.60 mg L^{-1} at 0 °C, about double at 30 °C (7.54 mg L^{-1}) (Weiss, 1970). Oxygen consumption by aerobic respiration increases when the temperature increases (Thamdrup, Hansen, & Jørgensen, 1998). When the temperature increased from 15 °C to 25 °C, the average ORP decreased from +40 mV to -60 mV (Zhu, Ndegwa, & Luo, 2002). In warm season, denitrification rate supposed not to be inhibited by temperature ($m = 20.5 \text{ } ^\circ\text{C}$). The lower DO and ORP of groundwater could be assumed as a response to the higher temperature in the warm season. The characteristics of these factors resulted in the higher N_2O concentration in the warm season ($m = 34.19 \text{ } \mu\text{g L}^{-1}$) than it in cool season (20.19 $\text{ } \mu\text{g L}^{-1}$). In addition, the decrease of pH was

interpreted as a sign of intense denitrification (Ilies & Mavinic, 2001). Mean value of pH is lower in the warm season ($m = 7.11$) than it in the cool season ($m = 7.49$), which also can explain the higher dissolved N_2O concentrations in the warm season. The seasonal change of dissolved N_2O coincides with N_2O flux measured in the study wetland. In fact, the average monthly N_2O flux ranged from 0.019 to 0.286 $mg\ N\ m^{-2}\ h^{-1}$ with the highest value in the warm season and the lowest flux appeared in the cool season (Li et al., 2013).

5. Conclusions

N_2O concentrations, denitrification related factors (NO_3^- , DO, ORP, pH and T) and $\delta^{15}N-NO_3^-$ values were investigated in a typical headwater wetland and watershed. The main findings and conclusions are as follows:

Spatially, NO_3^- , DO and ORP are main factors to control the dissolved N_2O in groundwater of study area. DO, ORP and NO_3^- decreased continuously from upland to the wetland. Along the groundwater flow, the dissolved N_2O was produced through nitrification at the upland and denitrification in the wetland, which is supported by the variations of $\delta^{15}N-NO_3^-$ in the shallow groundwater. The mean value of dissolved N_2O-N increased from 11.42 $\mu g\ L^{-1}$ at upland to 36.14 $\mu g\ L^{-1}$ at the ASW and then decreased to 9.27 $\mu g\ L^{-1}$ at NS. The dissolved N_2O in the ASW zone is expected to be composed of two parts. One is transported from the upland and the other is produced from denitrification in the wetland. As a result, the dissolved N_2O in the groundwater can be classified into the stage 1 for the upland, the stage 2 for ASW and the stage 3 for NS in the study area. Seasonally, the N_2O concentration was higher in the warm season ($m = 34.19\ \mu g\ L^{-1}$) and lower in the cool season ($m = 20.19\ \mu g\ L^{-1}$). Temperature and pH are main factors to control the dissolved N_2O in groundwater of study area. Higher temperature results in higher denitrification rate by elevating metabolic rates for denitrifying bacteria directly, and creating the lower DO and ORP environment that affects the N_2O concentration indirectly in the warm season. In addition, lower pH in the warm season also may explain the higher dissolved N_2O concentrations because the decrease of pH is interpreted as a sign of intense denitrification.

This study put forward an understanding of spatial distributions of dissolved N_2O from upland (agricultural area) which related the materials transformation to groundwater flow system. Temperature is considered as the main driver to seasonal change of dissolved N_2O in wetland groundwater.

Acknowledgements

We would like to thank the wetland park of Ichikawa city and the farmers in the pear orchard for the assistance of water sampling.

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