Dynamics of nitrate and nitrous oxide in wetland basin affected by agricultural activities

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Li Xing

Graduate School of Horticulture

CHIBA UNIVERSITY

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農業活動の影響を受ける湿地流域における硝酸及び亜酸化窒素の挙動について

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千葉大学大学院園芸学研究科

環境園芸学専攻緑地環境学コース

李杏

Content

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Abstract

Wetlandisconsidered to improve water quality by physical, chemical and biological process that remove N from groundwater.Hydrological, chemical and soil profile data are combined in this study to estimate the nitrogen transport and transformation of groundwater in wetland. A headwater wetland in Ichikawa,Chiba Prefecture, Japan, was chosen to estimate the dynamic of nitrogen in wetland groundwater.

The upland is covered by Kanto Loam about 4 m in thick. It is underlain in a sequence by Joso clay layer and Narita sand that a thick fine sand layer which is the major aquifer in the study area. The Joso clay layer is discontinuous of slope. The surface layer (0~1 m) of wetland is silt, followed by silty sand and sand (1 m~2 m). There is sand layer below 2 m depth. Discontinuous clay layer also could be found of this profile.Therefore, low permeability coefficients were found of the shallow layer in this profile (from 0.0297 to 0.521 m d^{-1}).The flow nets indicate predominantly horizontal flow from the upland boundary of the wetland towards middle of wetland.

DO concentrations and ORP were low, indicating the reducing environment in wetland groundwater. Nitrate was totally removed along the flow path, although the nitrate load of inflow were high (>140 mg L^{-1}) in this wetland. The areaaround the boundary played an important role in diluting the shallow nitrate-loaded groundwater based on data of $\delta^{15}N\text{-}NO_3^-$ values and chloride concentrations. Nitrate decreased with decrease of DO, increase of $\delta^{15}N\text{-}NO_3^-$ and $N_{2\ denitrification}$and result of ORP decreased along the flow path.

Therefore, besides this dilution effect, biological removalprocesses also significantly reduced the nitrate concentration of the groundwaterin wetland..

Denitrification process leaves the problem of N_2O that dissolved in groundwater and emission to atmosphere. Understanding the spatial and temporal pattern of dissolved N_2Oin groundwater is essential to estimate the N_2O emissions from groundwater to the unsaturated zone and to the atmosphere. The concentrations of dissolved N_2O 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). Then, principal component analysis (PCA) was used to assess the shallow groundwater parameters in the wetland. In sight of dissolved N_2O 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 N_2O in the warm season. Therefore, the seasonal change of dissolved N_2O in study wetland can be mainly interpreted by the variation of temperatures of groundwater.

The average flux at sampling sites varied from 0.013to 0.285 mg N $m^{-2}h^{-1}$ indicating that this wetland was an emission source of nitrous oxide. It was found that N_2O fluxes were high atthe sides of valley where groundwater flowed in with highconcentration of nitrate, and less than 0.045 mg N m^{-2} h^{-1}in the wetland where groundwater was almost free ofnitrate.The average monthly N_2O flux ranged from 0.019 to0.286 mg N m^{-2} h^{-1} with the peak values in May, and The spatial variations of N_2O flux were larger in May with the maximum value of 1.037 mg N m^{-2} $h^{-1}$and the minimum -0.025 mg N m^{-2} h^{-1}.The temporal and spatial variation of N_2O flux in the wetland surface was controlled by

concentration of dissolved N_2O near the water table, precipitation and temperature in the study area.

The leached nitrate to groundwater is 202 kg ha^{-1} yr^{-1}. This wetland receives a high NO_3^- load (20604 kg-N yr^{-1}) because of the leaching of nutrients derived from upland fertilizer for the agriculture.Annual export of nitrogen by stream was 15359.8 kg. Our estimate of N retention for the wetland watershed was 25.6%. However, the nitrate-nitrogen retention was 110 g-N $m^{-2}yr^{-1}$ which as high as the retention level of constructed wetland.

Key words: Wetland, groundwater flow path, nitrate removal, denitrification, dissolved N_2O, N_2O flux

要旨

湿地は他の生態系と比較して酸化還元環境を有し、流域の窒素化合物窒素化合物挙動および窒素循環に強く影響を与えている。本研究は千葉県市川市大町湿地公園を選び、水文、地球化学及び土壌断面の調査・観測データを行い用いて、湿地流域地下水流動系中における窒素の挙動を解明することを目的とした。

本研究地は千葉県下総台地典型的な谷頭部湿地流域であり、台地、斜面および湿地からなる。台地の地層分布は表層から関東ローム層、褐色粘土層、白色常総粘土層、砂層であり、斜面では常総粘土層の欠如部分があることが分かった。また、湿地は表面から深度の約 1m まではシルト層、深度 1 m から 2 m はシルト質砂層、その以深は砂層の順に分布している。また、シルト質砂層には連続性の欠いた粘土層が存在する。そして、湿地のシルト層の透水係数が低く、0.0297 から 0.521 m d^{-1} までの範囲に推移する。研究地の既存井戸および設置されたペゾメーターから算出された全水頭空間分布は台地から涵養された水が地下水とし湿地へ流入していることを示唆した。

一般的に還元環境は酸化還元電位(ORP)および溶存酸素(DO)が低く、pH が高い特徴を持っている。本研究地の地下水中 ORP および DO の値は低いことにより、湿地の還元環境が非常に発達していることがわかった。地下水流動方向において硝酸濃度の減少した。硝酸イオンと塩素イオンの比率および窒素同位体比 $\delta^{15}N$ を用いて、地下水中硝酸濃度変化に起因する希釈作用と脱窒作用をそれぞれ定量的に評価した。右岸側にある地点 1 から 3 までの窒素同位体比が変化しないため、希釈作用のみ、その以外の地点には、希釈および脱窒の総合作用を受けていたと思われる。

また、大気への湿地の N_2O 排出量を推定するため、地下水の溶存 N_2O の時空分布を把握する必要がある。本研究地の溶存 N_2O 濃度は、台地から湿地の入るまでに増加しつつ、湿地に入ってから減少していく。その結果から、溶存 N_2O の空間分布は台地部、湿地境界部および湿地中部三つの反応ステージがあると推察できる。溶存 N_2O は、アンモニアの硝化、脱窒反応に伴って生成したものであ

り、温度に大きく影響されている。温暖期の地下水の溶存 N_2O 濃度は寒冷期より高かった。温度は N_2O 生成･消費に関わる微生物反応の活性、溶存酸素および酸化還元電位に影響を強く与えるため、湿地内の脱窒プロセスの効果および溶存 N_2O の季節変化を制御する要因の一つであると考えられる。

湿地から排出 N_2O ガス挙動を把握するため、湿地内断面 A-A'7 地点で地表面の N_2O フラックスの時空間分布を観測した。N_2O フラックスの変化が 0.013 から 0.285 mg-N $m^{-2}h^{-1}$ との範囲にあるため、本研究地は亜酸化窒素の排出源であることが示唆された。また、湿地両側の N_2O フラックスは湿地中部より高かった。また N_2O フラックスの月変動は 0.019 から 0.286mg-N m^{-2} h^{-1} と大きく、主に温度、降水および水位に影響されていると考えられる。溶存 N_2O 濃度と N_2O フラックスの時空間変化が一致することが分かった。

台地の梨園から湿地までの流域窒素年収支を検討した。台地で施肥に起因する地下への窒素浸透量は、202 kg ha^{-1} yr^{-1} であるため、湿地に与える窒素の年負荷量が 20604kg ha^{-1} yr^{-1} になる。また、湿地河川から流出した窒素量は 15359.8 $kgyr^{-1}$ なっているため、湿地による無機態窒素の浄化率が 25.6%になっていたことが示唆された。

キーワード：湿地、地下水流動、硝酸除去、脱窒、溶存亜酸化窒素、亜酸化窒素排出量

List of tables

List of figures

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Chapter 1 Introduction

1.1 Wetland ecology

1.1.1 Definition and types of wetland

Wetlands are defined as those areas that are inundated or saturated by surface water or groundwater at a frequency and duration sufficient to support a prevalence of vegetation typically adapted for life in saturated soil conditions (EPA 2005).

One of the simplest classification systems recognizes only six types: swamps, marshes, bogs, fens, wet meadowsand shallow waters(Paul A. keddy Wetland Ecology). Swamp is a wetland that is dominated by trees that are rooted in hydric soils, but not in peat. Marsh is a wetland that is dominated by herbaceous plants that are usually emerge through water and rooted in hydric soils, but not in peat. Bog is a wetland dominated by Sphagnum moss, sedges, ericaceous shrubs, or evergreen trees rooted in deep peat with a pH less than 5. Fen is a wetland that is usually dominated by sedges and grasses rooted in shallow peat, often with considerable groundwater movement, and with pH greater than 6. Wet meadow is a wetland dominated by herbaceous plants rooted in occasionally flooded soils. Temporary flooding excludes terrestrial plants and swamp plants, but drier growing seasons then produce plant communities typical of moist soils. Shallow water is a wetland community dominated by truly aquatic plants growing in and covered by at least 25 cm of water. The system present above has the advantage of simplicity and generality. There are more elaborate systems, and that these vary around the world. Each wetland classification system tries to summarize the major types of wetland vegetation, and then relate them to environmental conditions.

1.1.2 Wetland soil

Oxidized soils have small amounts of organic matter near the surface, and major ions have been transported deeper into the soil column by leaching. Reduced soils in wetlands have larger amounts of organic matter, and instead of leaching, there is chemical transformation toward reduced elements. One cause of low oxygen (O_2) levels is the low rate of diffusion of oxygen in water, and hence in flooded soils. Oxygen and other gases diffuse about 10^3–10^4 times more rapidly in air than in water. Oxygen is soon depleted from flooded soils by the respiration of soil microorganisms and plant roots. The deficiency of oxygen is termed hypoxia, the absence of oxygen is termed anoxia. In the absence of oxygen, oxidation of organic matter ceases, and populations of microorganisms begin to change the ionic composition of the rooting zone (Ponnamperuma 1972; Faulkner and Richardson 1989; Marschner 1995). The effects of flooding on soil chemistry occur rapidly (Figure 1-1). In reducing environment, O_2 and nitrate(NO_3^-) disappear in only a few days, gases such as methane (CH_4), hydrogen sulfide (H_2S) and ammonia (NH_3) begin to accumulate. As well, ions such as Fe^{2+}appear. Hence organisms living in wetland soils have at least three metabolic problems to contend with: not only is there a shortage of oxygen, but there are atypical concentrations of ions, and there are toxic gases.

1.1.3 Ecological services of wetland

While covering only 6% of the Earth's surface, wetlands provide a disproportionately high number of ecosystem services. Many ecologically and economically important species call wetlands home for at least part of their lives. For instance, commercially important fishes and shellfish, including shrimp, blue crab, oysters, salmon, trout, and sea

trout rely on, or are associated with, wetlands. Wetlands are also critical habitat for migratory birds and waterfowl, including ducks, egrets, and geese. In fact, more than one-third of the species listed as threatened or endangered in the United States live solely in wetlands and nearly half use wetlands at some point in their lives (USEPA 1995). As such, many wetlands are often recognized as important conservation or restoration targets.Wetlands play an important role in regulating the climate through carbon storage, the production of methane, and their historical role in producing coal. The amount of carbon dioxide in the atmosphere is one factor that controls the Earth's temperature. Carbon dioxide is transparent to sunlight, but reflects heat back to Earth. This is the basic mechanism of a greenhouse, and hence the origin of the term greenhouse effect. Since the Industrial Revolution, the concentration of carbon dioxide in the atmosphere has been rising. This is thought to be an important cause of projected changes in climate. Methane (CH_4) is a very simple molecule. It is also the most abundant organic chemical in the Earth's atmosphere, although its concentration is measured only in parts per billion (ppb). Because it absorbs infrared light, it is also an important greenhouse gas (Cicerone and Ormland 1988; Forster et al. 2007). On a larger timescale, consider the degree to which our civilization is based upon another wetland product: coal. Coal comes from swamps that existed long in the past. The burning of coal is the most obvious (but not the only) cause of the rising trend in carbon dioxide levels in the atmosphere. To the degree that they remove carbon dioxide from the air and store it, wetlands provide a counterbalance. Coal mines also emit methane.Wetlands also improve water quality, recharge groundwater aquifers, and serve as sinks, sources, or transformers of materials.

1.1.4 Water quality improvement by wetland

Water quality improvement is a service that is widely attributed to wetlands, which have absorbed and recycled nutrients from human settlements since the dawn of civilization. However, recognition of this service did not come until the 1970s, after increasing environmental awareness mandated reversing eutrophication of the nation's waterways and led to the Clean Water Act in 1972.

River catchments in which the dominant land-use type is agricultural often have lower-order stream sub catchments that are strongly influenced by runoff from fields or grasslands. In intensively farmed areas, nutrient loading is often so high that large quantities of nitrate leach into the groundwater, which discharges into streams as seepage or subsurface runoff. In intensively farmed catchments, phosphorus (P) and nitrogen (N) are also transported to streams in surface runoff (Decamps et al. 1992). Between agricultural fields and streams, one often finds riparian areas that can influence surface and subsurface runoff before it reaches streams. There is a large body of literature based on studies at individual sites that indicates that riparian habitats remove nutrients from the water flowing through them on its way from the agricultural land to the stream. The most frequently documented function is the removal of nitrate from subsurface run-off in wetland zones with anaerobic soil conditions (Groffman et al. 1992; Hefting et al. 2004; Jordan et al. 1993; Jordan et al. 1998; Pinay et al. 1993; Mander et al. 2005). Denitrification is generally the most important process for nitrate removal, whereby dead organic matter is decomposed by bacteria in the absence of oxygen, using nitrate as an electron acceptor. Nitrate is converted to nitrous oxide (N_2O) and, subsequently, to atmospheric nitrogen (N_2), which is emitted by the wetland (Addyet al. (1999; Clementet

al. 2002; Groffman and Crawford 2003; Huntet al. 2004; Matheson et al. 2003; Welleret al. 1994). Nutrient uptake in vegetation as water passes through the riparian zone is also important and results in long-term nitrogen storage (Pinay et al. 1993; Rotkin-Ellman et al. 2004; Hefting et al. 2005). However, its removal from the system only occurs if the vegetation is harvested as part of the management of that system (Addy et al. 1999). Phosphorous removal in riparian habitats has also been reported, with sedimentation, soil adsorption and plant uptake being the most important mechanisms (Hogan et al. 2004; Havens et al. 2004; Uusi-Kamppa et al. 2000).

1.2Hydrology of wetland

Wetland conditions occur where topographic and hydrogeological conditions are favorable and a sufficient, long-term source of water exists. Favorable topographic conditions refer generally to the presence of land-surface depressions in the drainage basin. These depressions may be located in upland areas, along hillsides where there may be a change in slope or geology, in floodplains of streams or rivers, or along the margins of lakes. Geologic conditions which may be favorable for wetland development include areas that have fine textured surficial soils with low hydraulic conductivity and sufficient thickness to store water. Also, the presence of impermeable bedrock near the land surface may favor the development of wetland hydrology. The development of wetland conditions requires a persistent, long-term source of water. The source of water may be precipitation which falls directly on the wetland, surface water runoff during rainfall or snowmelt events within the catchment area surrounding the wetland, periodic flooding caused by elevated water levels in nearby surface water bodies, groundwater inflow to the wetland, or a combination of any, or all, of these sources. Water may be lost from a

wetland by evaporation from standing water or saturated soils, transpiration from plants, or surface water or groundwater outflow. The development of wetland conditions depends on a long-term balance between waterinflow to the wetland andoutflow from the wetland.During dry climatic periods, the rate of water inflow to the wetland (precipitation, groundwater inflow, and surface or near-surface inflow) may greatly diminish. In this instance, the amount of water lost through evapotranspiration may exceed the rate of all water inflow to the wetland. Water losses through evapotranspiration can result in extreme declines in the water table and a de-saturation of the wetland.

The relative importance of water inflow and water outflow, along with the topographic and geologic setting, determines the type and characteristics of the wetland that may form at a given location. A number of wetland classification systems have been developed that group wetlands based on topographic position in the landscape, water source, and hydrodynamics (Novitski, 1979; Brinson, 1993). Four commonly wetland systems are surface water depression wetlands, groundwater slope wetlands, groundwater depression wetland, and surface water slope wetlands(Figure 1-1). Wetlands that receive water primarily from precipitation have been classified as surface water depressional wetlands. Wetlands for which groundwater is the predominant source of water are classified as groundwater slope or groundwater depressional wetlands. Wetlands which are dependent upon surface water inflow are classified as either riverine or fringe wetlands along existing bodies of open water. Figure 1-1.a shows a wetland that has formed in a topographic depression. The primary sources of water are precipitation and surface water runoff from the catchment area surrounding the wetland. Since the water level elevation in the wetland is greater than the elevation of the water table, water in the wetland moves

toward the water table, and groundwater is not a source of water for the wetland. The outflow of water from this category of wetlands is evaporation from the water surface, transpiration from plants, and movement of water to the underlying or adjoining aquifer. The soils or geologic sediments which underlie the wetland may be predominantly clay. The relatively low hydraulic conductivity of the sediment restricts, but does not prohibit, the movement of water from the wetland to the underlying aquifer. This category of wetlands is referred to as surface water inflow or depressional wetlands. This category of wetlands may be found at any elevation, even in otherwise predominantly upland areas. These wetlands are more dependent on precipitation than other types of wetlands.

Hillslopes between upland and lowland areas are another topographic setting in which wetlands may form. Wetlands forming in these areas are referred to as groundwater slope wetlands. An example of this type of wetland is shown in Figure 1-1.b. Groundwater which discharges along the hillslope as a seep or spring is the primary source of water to this wetland. Overland flow and precipitation may also contribute water to these wetlands. In this setting, sediments which have relatively low hydraulic conductivity such as clay or silt may underlie more permeable saturated sediments, forming a perched aquifer. Groundwater would flow laterally, along the clay or silt layer, toward the hillslope, where it discharges as a seep or spring. This is referred to as a groundwater seepage face. Groundwater slope wetlands may also occur where there are changes in the hillside slope and may not have perched groundwater conditions.

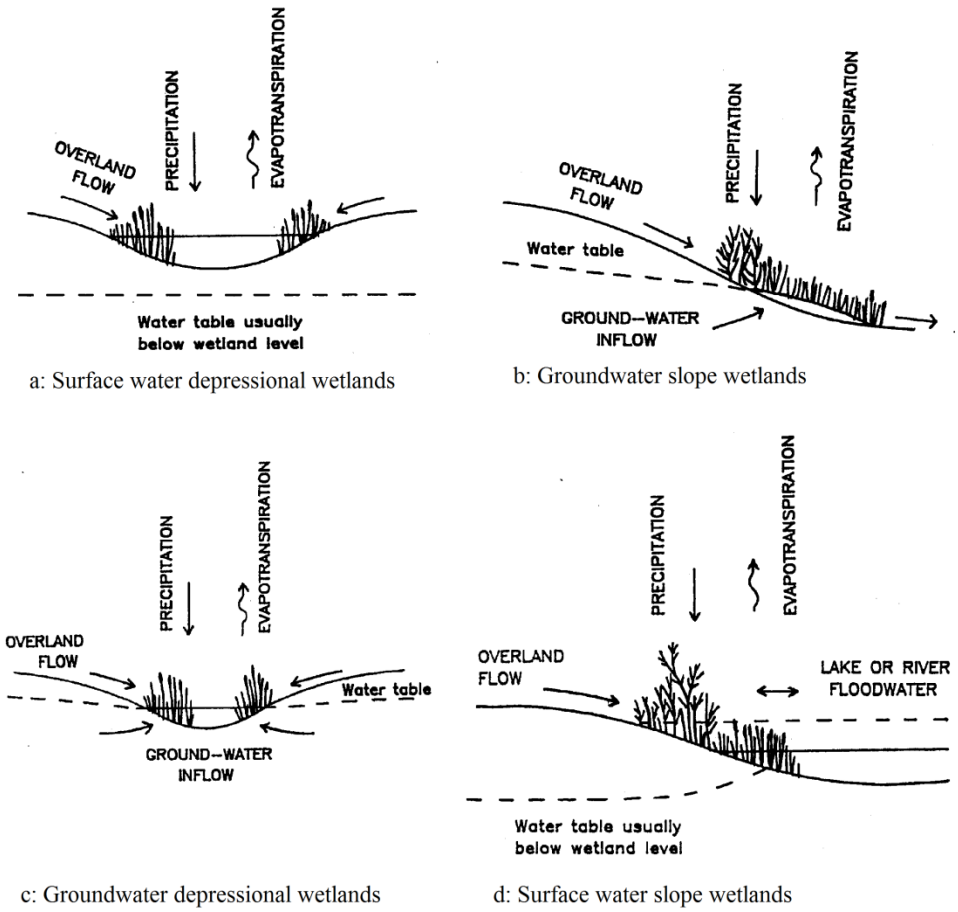

Figure 1-1Wetland types based on topographic position in the landscape, water source, and hydrodynamics

Groundwater slope wetlands tend to have relatively constant inflow of water if the aquifer responsible for the water source is readily recharged or groundwater moves through the aquifer at a relatively high rate. In this case, the wetland would be relatively unaffected by seasonal demands by evapotranspiration. If a shallow perched aquifer provides water to the seep, the wetland soils may become dry during portions of the growing season because of evapotranspiration in the seepage area. Groundwater slope wetlands generally have a surface water outlet. The size of these wetlands depends on the

quantity of groundwater discharge and the slope of land surface down gradient of the seepage face or spring.

Figure 1.c shows a wetland formed in a topographic depression which may be in a lowland area. For this category of wetlands, the primary sources of water are groundwater discharge to the wetland, precipitation, and surface water runoff from the catchment area surrounding the wetland. Since the water table elevation is higher than the water level elevation in the wetland, groundwater moves from the adjoining and underlying aquifer toward the wetland. The outflow of water is from evaporation from the water surface and transpiration from plants. These wetlands may not have any surface water outlets. This category of wetlands is referred to as groundwater depression wetlands. While they can exist at any elevation, these wetlands are typically found in relatively low-lying areas. Another category of wetlands is referred to as surface water slope wetlands (example shown in Figure 1.d). Surface water slope wetlands receive water primarily from the flooding of lakes or rivers, and the water can readily drain back into lakes or rivers as the surface water stages decline. Within floodplains, the flooding occurs infrequently. However, lakeside wetlands may be flooded permanently. These areas near surface water bodies are generally areas of regional or local groundwater discharge. The discharging groundwater is an important, consistent source of water to these wetlands.

1.3 Wetland as an agent to regulate the global nitrogen cycle

Nitrogen is one of the major constituents of living cells (the others being carbon, hydrogen, and phosphorous). Not surprisingly, nitrogen is also a major nutrient necessary for primary productivity. Major nitrogen species in fresh waters: molecular nitrogen (N_2),

ammonia (NH_3), ammonium ion (NH_4^+), nitrite (NO_2^-) and nitrate (NO_3^-). NH_4^+ is most easily assimilated by photosynthetic organisms. NO_3^- is used by higher organisms to synthesize organic compounds. Assimilated $NO_2^-$is quickly oxidized to nitrate by enzymes in higher animals.The nitrogen cycle is a complex biochemical cycle where the various species of nitrogen are altered by: nitrogen fixation-uptake of N_2, assimilation-uptake and metabolism and denitrification-reduction of nitrate (NO_3^-) to N_2. The N cycle is essentially mediated by microbial action. Bacteria oxidize and reduce nitrogen and photosynthetic organisms assimilate, utilize and build complex molecules from the oxidized/reduced species formed by the bacteria. Higher animals have a very small role in the N cycle, basically that of excretion of ammonia compounds. However, when bacteria and algae are extensively grazed, relative populations of these species are shifted, altering the relative amounts of N species produced and assimilated. Extensive bacterial fixation and denitrification occurs at the sediment-water interface.

1.3.1 Chemical transformation of nitrogen

Wetlands are an important part of the cycling of nitrogen because the hypoxic or anaerobic conditions allow chemical transformations of nitrogen. That is, wetlands are sites where elements are transformed among an array of chemical states (Rosswall 1983; Armentano and Verhoeven 1990; Patten 1990). The complex biogeochemical cycle of nitrogen involves multiple biotic and abiotic transformations involving seven valency states (+5 to -3). Inwetlands, most nitrogen is stored in organic sediments. There are two scales at which nitrogen movement and transformation can be studied. At the within-wetland scale, the principal flows occur among three components: organic matter, the oxidized surface layer, and deeper anoxic layers. At a landscape scale, there are flows

among three other components: the surrounding terrestrial landscape, the wetland, and the atmosphere. Since we have already seen how nitrogen moves in soils, let us consider the larger scale here. At larger scales, inputs of nitrogen to wetlands include fixation, runoff, and precipitation. Outputs include runoff and gaseous nitrogen produced by denitrification. Wetlands provide two services. They can increase or decrease nitrogen levels in the water. Whether a wetland is a source or sinks for nitrogen depends upon the relative rates of fixation and denitrification in turn. Recall that these processes are largely dependent upon the proximity of the surface oxidized layer to the anoxic regions deeper in the wetland (Faulkner and Richardson 1989).

1.3.2 Increasing nitrogen levels through fixation

In areas where nitrogen is scarce, cyanobacteria can fix nitrogen and increase local productivity. This is an important process in rice paddies, and also in natural nutrient-limited systems like the Everglades. During nitrogen fixation, bacteria reduce atmospheric nitrogen (N_2) to ammonium (NH_4^+), providing a continual flow of nitrogen from the atmosphere to the soil. Rates of fixation in wetlands are, however, usually rather low (from 1.0 to 3.5 g m^{-2} per year). Exceptions may include rice fields, floodplains, and wetlands such as the Everglades where cyanobacteria fix nitrogen. Some published estimates are considerably high. Whitney et al. (1981) estimated nearly 15 gm^{-2} per year for salt marshes in eastern North America. The principal organisms involved in nitrogen fixation in wetlands are cyanobacteria such as Nostoc. Better known are the bacteria such as Azotobacter and Clostridium which form nodules on the roots of legumes, but legumes are relatively uncommon in most wetlands. A group of filamentous bacteria known as actinomycetes forms nodules on the roots of some trees and shrubs associated with

wetlands, notably the alders (Alnus) and wax myrtles (Myrica). Rhizobium is also associated with a family found in wetlands, the Ulmaceae. Finally, the cyanobacterium Anabaena often occurs in association with the floating water fern Azolla, and plays an important role in fixing nitrogen for rice paddies.

1.3.3 Lowering nitrogen levels through denitrification

Wetlands can reduce the nitrogen in water by capturing it in plant tissue, storing it in organic sediments, or converting it back to atmosphereic nitrogen. This service is of particular value in those cases where nitrogen is locally abundant and produces unwanted plant growth such as algal blooms. The importance of wetlands for denitrification has likely increased since industrial fixation of nitrogen (using the Haber process) has caused nitrogen enrichment (eutrophication) of both rivers and precipitation. Denitrification is carried out by microorganisms living in anaerobic conditions. In this process, NO_3^-, the biologically useful state, is converted back to N_2 or N_2O. These diffuse upward through the soil back into the atmosphere. Appreciable amounts are actually transported upward by aerenchyma in rooted plants (Faulkner and Richardson 1989). In general, denitrification rates are slightly lower than fixation rates. As a first, very rough approximation, nitrogen fixation is from 1~3 g m^{-2} per year, while denitrification is about 1 g m^{-2} per year. Rice fields are an exception. The attempt to measure these processes accurately at the global scale (e.g. Lavelle et al. 2005) is a challenge, in part because the relative rates of nitrogen fixation and denitrificaton vary in so many ways. Not only do the rates vary among types of wetlands, but they vary spatially in wetlands and then there is temporal variation on top of that, depending upon season and amount of flooding. Consider a few more examples. Bowden (1987) reported denitrification rates nearly an

order of magnitude higher (30 gm^{-2} per year), which would mean the wetlands are efficiently transforming organic nitrogen to atmospheric nitrogen. Biogeochemical cycling of nitrogen was also discussed in many studies such as Faulkner and Richardson (1989), Armentano and Verhoeven (1990), and Lavelle et al. (2005). In general, it appears that the rates of denitrification exceed rates of fixation, so that wetlands can be thought of as sites where organic nitrogen arrives in runoff and detritus, and is then returned to the atmosphere.

1.4 Nitrogen in agricultural system

In global, commercial fertilizer, manures, and other N sources are generally easily and economically applied. Animal and human wastes were the major fertilizer source of added N before 1960. Nitrogen represents the nutrient most applied to agricultural land. Synthetic fertilizer inputwas 3.93 Tg-N yr^{-1}(about 7% of the total N-input) in 1950, 82 Tg-Nyr^{-1}(43% of the total N-input) in 1996 (Kroeze et al., 1999; Mosier, 2001) and through 2002 increased to 85 Tg-N yr^{-1}(Fao, 2004).

The fate of N applied to cropland depends on many factors, some under management control and others related to soil, climate, and other environmental attributes. Once applied to soil, added N goes through a number of complex transformations, mostly biological, that lead to four major alternative fates 1) plant uptake and subsequent removal in harvest; 2) loss to surface and groundwater via hydrologic flow as NO_3^-, dissolved organic nitrogen (DON), and particulate N; 3) loss to the atmosphere as N_2O, NO_x, NH_3, or N_2; and 4) storage in the cropping system as inorganic N, in soil organic matter (SOM) derived from crop residues and microbial biomass.The hydrologic loss of NO_3^- is the major vector of N lost to the environment from cropping systems that receive

rainfall in excess of evapotranspiration. This loss of NO_3^- can also be high from irrigated systems in drier climates when water applied exceeds crop transpiration need (Gehl et al, 2005). As is particulate N loss, hydrologic DON loss is minor in most cropping systems (van Kessel et al, 2009). It usually represents the translocation of organic N from one part of the landscape to another rather than loss to the environment-although in areas of high erosion particulate N can be lost to surface waters via direct runoff.

Ammonium loss from cropland tends to be important only when manure is applied to surface soils or when anhydrous ammonia or urea fertilizers are misapplied to dry soil, such that the NH_3 that is added as anhydrous ammonia or formed from urea escapes to the atmosphere before it can be dissolved in the soil solution as NH_4^+. Fertilizer misapplication in this way is inefficient and is more likely to occur during extended dry periods.

Nitrous oxide and NO are produced in soil by both nitrifying and denitrifying bacteria (Robertson and Groffman, 2007). Nitrification is the oxidation of NH_4^+ to NO_3^- with NO and N_2O being metabolic by-products that escape to the atmosphere or dissolved in soil water. Denitrification is the reduction of NO_3^- to NO_2^- N_2O, NO, and N_2. The rates of N_2O and NO production are highly variable in most soils and qruifers, and are related to the factors that affect rates of nitrification (mainly NH_4^+ availability) ordenitrification (mainly NO_3^-, C, and low O_2 availability) as well as soil factors such as pH that affect the proportion of the end products (Robertson and Groffman, 2007).

An important control on the rate of N gas production is the amount of N available to the bacteria that carry out the reactions. In almost all but very sandy soils, rates of nitrification and denitrification increase with increasing pools of inorganic N (e.g., NO_3^-,

NH_4^+), and likewise, the rates of N_2O and NO formation are best predicted by inorganic N availability. In unfertilized soil, N available to the bacteria that produce these gases is largely controlled by rates of N_2 fixation, SOM turnover, and N deposition. In most cropped soils this N is largely controlled by rates of fertilization and SOM turnover. Because plants are good competitors for inorganic N, plant uptake can reduce the amount of N that would otherwise be available for N gas production or hydrologic loss.

In general, 65 to 90% of the N consumed in feed is excreted in manure with the remainder retained in body tissue and the milk, eggs, or other products produced (Hristov et al., 2011; Rotz, 2004). With good feeding practices for cattle and swine, about 50% of the N excreted in feces is in a relatively stable organic form. The remainder, including most of the excess N consumed, is excreted in urine as urea. For poultry, a large portion of the excreted N is uric acid, which decomposes to form urea. When deposited on the floor of the housing facility, the urea comes in contact with urease enzymes, which rapidly transform the urea N to NH_4^+. At a rate dependent upon temperature, pH and other manure characteristics, the NH_4^+ forms NH_3, which is readily volatilized (Hristov et al., 2011; Montes et al., 2009).

On a barn floor, for example, where manure is removed at least once per day, NH_3 emissions vary with temperature and are relatively low in cold winter weather (Montes et al., 2009). In warm weather or on a surface such as an open lot where manure is not removed, nearly all of the urea-N can be lost to the atmosphere as NH_3 (Hristov et al., 2011; Rotz, 2004). Another common housing system uses a bedded pack, whereby manure and bedding materials accumulate on the barn floor. With this strategy, a portion of the NH_4^+ is absorbed into the bedding material, emitting more NH_3 than if it were it

deposited on a scraped floor, but less than if it were deposited in an open lot. Bedded pack and open lot surfaces both provide aerobic and anaerobic conditions to support both nitrification and denitrification, creating emissions of N_2O and N_2 (Rotz, 2004).

Manure removed from barns can be handled in solid, semi-solid, slurry or liquid forms. Solid manure is relatively dry, often scraped from open lot surfaces where most of the labile N has been emitted as NH_3 (Hristov et al., 2011). Semi-solid manure is formed using bedding material to absorb manure moisture. This type of manure is typically not stored for long periods and may be spread on crop and pastures each day of the year as it is produced. Slurry is formed by scraping manure from the floor of free stall and similar barns designed to use less bedding material. Liquid manure is typically formed by using a solids separator to remove a major portion of the manure particles, leaving the manure solution with less than 5% dry matter content. Manure solids can be composted and used as bedding material, with most of the NH_4^+ remaining in the liquid portion (Meyer et al., 2007). Both slurry and liquid manure are typically stored for 4-6 months and in some cases up to a full year to allow the nutrients to be applied to fields at a time when they are best used by growing crops or grassland. However, this requires a storage capacity that many operations lack and consequently it is not unusual for manure to be spread on frozen fields or pastures during the winter.

During long term manure storage, the organic N portion in the manure slowly decomposes, producing NH_4^+. If semi-solid manure is stored, it is placed in a stack where NH_3 emissions occur and nitrification and denitrification processes generate N_2O, NO_x and N_2 emissions. About 10-20% of the N entering storage is lost mainly as NH3 (Rotz, 2004). Slurry manure is typically stored in a tank. When manure is continually added to

the surface of the tank, up to 30% of the N can be lost as NH_3, but little or no emissions of N_2O escapes, because anaerobic conditions inhibit nitrification, thus preventing conversion to NO_3^- and subsequent denitrification. When manure is pumped into the bottom of the tank, a crust of manure solids can form on the surface reducing emissions of NH_3 by up to 80%. However, nitrification and denitrification can occur within this crust, thus emitting N_2O (Peterson and Miller, 2006). Liquid manure is commonly stored in a lined earthen basin or lagoon where NH_3, N_2O and N_2 losses are relatively high (Harper et al., 2004). When a multiple stage lagoon (e.g., flow from a facultative to anaerobic lagoon) is used, up to 90% of the N can be lost or removed between the inlet and outlet.

Most manure is applied to crop or grassland as fertilizer. Methods of manure application include broadcast application to the field surface, subsurface injection, and irrigation. When manure is broadcast spread, any remaining NH_4^+ in the manure is rapidly volatilized to NH_3 (Génermont and Cellier, 1997), although at least half can be retained if the manure is tilled into the soil within several hours of application (Rotz et al., 2011). Subsurface injection can also greatly reduce or even eliminate NH_3 emission depending upon injection depth (Ndegwa et al., 2008). Irrigation is often used to apply liquid manure, and a portion of manure-N content is lost as NH3 during irrigation. However, if the manure infiltrates rapidly into the soil, N will be retained as NH_4^+ (Sommer et al., 2003). Application losses vary from 2% of the manure N applied through deep soil injection to 30% of the N applied through surface spreading without soil incorporation (Rotz, 2004).

1.5Nitrogen in groundwater associated with agricultural systems

1.5.1 Forms of nitrogen in groundwater

The forms of nitrogen generally measured in groundwater include nitrate (NO_3^-),nitrite (NO_2^-), and ammonia (NH_4^+) ions. Organic nitrogen is rarely measured and not well known in groundwater (Korom, 1992).Most analyses combine $NO_3^-$and NO_2^- and investigators report this as NO_3^- because NO_2^- occurs in substantially smallerconcentrations in groundwater than NO_3^-. Nitrite also is an intermediate productof both nitrification and denitrification, that is, relatively unstable (Keeney, 1986), which helps explain its limited occurrence in groundwater. Nitrification is an oxidizing process and denitrification a reducing process with respect to NO_3^-, but both are biologically mediated. A generally accepted hypothesis is that measurable $NH_4^+$and organic nitrogen rarely occur in groundwater because the required biological activity to produce them is minimal in groundwater systems. Nolan and Stoner (2000) reported that nitrate was detected more than 13 times as often as NH_4^+ and organic nitrogen in shallow groundwater of major aquifers of the United States, based on a detection threshold of 0.2 mg L^{-1} as nitrogen. In fact,concentrations of ammonia in groundwater rarely exceeded 0.1 mg L^{-1}, indicating chemical instability.

1.5.2 Nitrate contamination levels

Contamination is the occurrence of NO_3^- that exceeds a generally accepted concentration attributable to natural conditions. It is difficult to examine minimum natural thresholds of NH_4^+, perhaps because its occurrence is too infrequent and concentrations are comparatively small. Nitrate is the most common contaminant in aquifers (Freeze and Cherry, 1979) and has been the most frequently mentioned groundwater contaminant

associated with human activities throughout the world for several decades. The large number of NO_3^- measurements may be due to the establishment of international standards for drinking water for this ion and its wide distribution in the environment (Feth, 1966). The concentrations of nitrate in waters unaffected by human activities were shown to be less than 10 mg L^{-1} of NO_3^- by Feth (1966). A wide range of natural or background concentrations in groundwater has been reported for specific geographic locations from as small as 0.2 mg L^{-1} NO_3^- in Ohio (Baker et al., 1989) to as much as 100 mg L^{-1} NO_3^- in the Sahel of Africa (Edmunds and Gaye, 1997) . Nitrogenous minerals have been reported in geologic materials that could provide natural sources of nitrate to groundwater such as in the northern Great Plains of the United States (Ferguson et al., 1972; Boyce et al., 1976) and in the San Joaquin Valley, California (Strathouse et al., 1980). Extensive analysis of historical data summaried by Madison and Brunett (1985) concluded that concentrations in excess of 3 mg L^{-1} may be indicative of human inputs for groundwater in America. A more recent analysis of US Geological Survey national data from shallow groundwater (30 m) beneath forest and rangeland concluded that 2.0 mg L^{-1} is a probable threshold for background concentration of NO_3^- (Mueller and Helsel, 1996).

During the last two decades, nitrate nitrogen (NO_3-N) concentrations in groundwater in Japan have increased steadily due to the development of intensive agriculture. In some areas, it has reached or even exceeded the unacceptable level for drinking water, 10 mg L^{-1}. In 2000, the Japanese Environment Agency showed that 5.6% (173 of 3,374) tested wells and 4.7% (64 of 1,362) wells used for drinking water exceeded the standard level in 1999 (Kumazawa, 2002).

The highest value of NO_3^--N in the wells was 100 mg L^{-1}. Many researches have shown that NO_3-N pollution of groundwater was widely foundin Japan, except the paddy field regions. Farming practices in Kagamigahara city of Gifu prefecture have been typical ones for reducing NO_3-N pollution in groundwater. In the east district of the city, NO_3-N concentration was low in 1966, but reached 27.5 mg L^{-1} in June, 1974. The farmers for carrot cultivation began to reduce the nitrogen fertilizers district from 256 kg-N ha^{-1} in 1970 to 153 kg-N ha^{-1} in 1991. The use of controlled release fertilizer increased fertilizer-nitrogen efficiency compared with common compound fertilizer and NO_3^--N concentration in the groundwater began to decrease steadily.

1.6 Denitrification

Removal of nitrate from groundwater viaartificial means is costly and time consuming. Typical methods include permeable reactive barrier that use Fe^{2+} to reduce nitrate to nitrogen gas, membrane attached biofilms with supplemental carbon source and use of immobilized enzymes in denitrification reactors (Shrimaliand Singh, 2001). The expense of denitrification for municipal water supply makes the understanding of natural denitrification crucial to local water suppliers.

Denitrification is the microbial mediated reduction of nitrate (NO_3^-) to nitrogen gas (N_2). The generalized half reaction for all denitrification reactions is:

$$2NO_3^- + 12H^+ + 10e^- \rightarrow N_2 + 6H_2O \quad (1\text{-}1)$$

Four requirements must be met for denitrification to occur (Korom, 1992).

i. Presence of NO_3^- to act as terminal electron acceptor

ii. Hypoxic or anoxic conditions

iii. Bacteria to perform the stepwise reduction of nitrate to nitrogen gas.

a. $NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2$ (1-2)

iv. Electron donors to fuel either organotrophic or lithotrophic bacteria performing denitrification

Aquifers contain chemotrophic bacteria that oxidize either organic or inorganic compounds for energy. Organotrophic bacteria use organic electron donors to fuel cellular functions. Litotrophs use inorganic electron donors, including carbon dioxide, as fuel (Korom, 1992).

The majority of denitrifying bacteria are heterotrophic facultative anaerobes (Payne, 1981). They are capable of survival with or without oxygen and will utilize organic carbon in the most energetically favorable reaction possible. The most common electron acceptors in groundwater are oxygen and nitrate. The change in Gibbs free energy for oxygen use is slightly higher than for nitrate use. Bacteria prefer the more abundant and energetically favorable oxygen for metabolism.

$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O$ $\Delta G = -2870$ kJmol^{-1}(1-3)

$5\ C_6H_{12}O_6 + 24NO_3^- + 24H+ \rightarrow 12N_2 + 30CO_2 + 42H_2O$ $\Delta G = -2670$ kJmol^{-1}(1-4)

It is believed that dissolved oxygen concentration in groundwater and depth below the water table are inversely proportional (Soares, 2000). In systems with anoxic conditions the limiting factor in groundwater denitrification is electron donor concentration. Denitrifying bacteria can be either organ trophic or lithographic. Lithographic bacteria rely on reduced minerals to denitrify, typically Fe^{2+}, Mn^{2+} (Korom, 1992).

1.7The objectives of this study

Nutrient loading of terrestrial, freshwater and coastal ecosystems occurs as a result of human waste disposal and agriculture at a global scale. Although technological

purification plants are the best option to reduce the nutrient fluxes to the environment, nutrient loading owing to intensive agricultural practices typically occurs through diffuse or ‘non-point’ sources. Even with the abundant evidence supporting nitrate removal in riparian areas, the rolethe wetland plays in removing groundwater nitrate remains unclear. The primaryprocesses of subsurface nitrate removal within wetland are generallyconsidered to be denitrification (Jacobs and Gilliam, 1985; Cooper, 1990; Lowrance etal., 1995), vegetative uptake (Lowrance, 1992), or dilution (Altman and Parizek, 1995;Komor and Magner, 1996). However, in many studies the exact mechanism of nitrateremoval and the role hydrology plays in nitrate attenuation have not been wellunderstood comperhansively. Groundwater flow patterns can have a majorinfluence on the distribution and fate of nitrate (Hill, 1990). Still few studies havecarefully examined the link between groundwater flow paths and nitrate concentrations.

Wetlands offer an abundant organic C supply, and are 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 et al., 2003; Burgin and Hamilton, 2007; Whitmire and 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 N_2O is an obligate intermediate product of biological denitrification and known to contribute to global warming and the destruction of stratospheric ozone.Agricultural N_2O emissions associated withnitrogen fertilizer use are considered the main source of globalN_2O emissions (19 Tg N yr^{-1}), with an estimated 5.3 Tg N yr^{-1}originating from agricultural soils. Indirect N_2O

emissionscontribute 0.4 Tg N yr^{-1} from denitrification of nitrate originatingfrom leaching and runoff processes. Therefore, it is important to understand the N_2O emission from wetland due to the high potential of denitrification of shallow aquifer of wetland. Emissions from aquifers are most likely to occur from shallow aquifers, where N_2O can be quickly transferred through the unsaturated zone to the atmosphere by diffusion (Rice and Rogers, 1993). Moreover, N_2O 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 region, Japan(Ueda et al., 1993) and up to 30000 times of that in the ambient air in some study(Heincke & Kaupenjohann, 1999).However, few studies estimated level of dissolved N_2O in wetland groundwater.As a result, the objectives of this study are as follows:

1. To understand the hydrological characteristics of shallow groundwater and in wetland.
2. To reveal water quality dynamics and the contributors of nitrate removal of shallow groundwater and in wetland.
3. To understand the spatial distribution and seasonal change of dissolved N_2O concentration of shallow groundwater and in wetland.
4. To estimate temporal and spatial distribution of the N_2O flux from the wetland surface.
5. To estimate the inorganic nitrogen budget in upland and wetland

The structure, content and scientific problems discussed in the study are presented in the table 1-1and figure 1-2.

Table 1-1Structure, content and scientific problems discussed in the study

Chapter	Contents	Science problems discussed
Chapter 1-2	Reviews on ecology and hydrology of wetland, role of wetland in nitrogen cycle. Descriptions of the natural conditions of the study areas and study methods.	
Chapter 3	Investigation of stratigraphy and determination of groundwater flow in wetland basin	Determination of nitrogen transformation direction base on groundwater flow
Chapter 4	Dynamic of NO_3^- of a profile	Understanding the mechanism of nitrate removal in wetland groundwater
Chapter 5-6	Dynamic of N_2O in groundwater and emission from surface	Understandingof the spatial distribution and seasonal change of dissolved N_2O of shallow groundwater and N_2O flux of wetland surface
Chapter 7	Nitrogen balance of wetland basin	Discussing the input and output of nitrogen in wetland basin
Chapter 8	General conclusions	

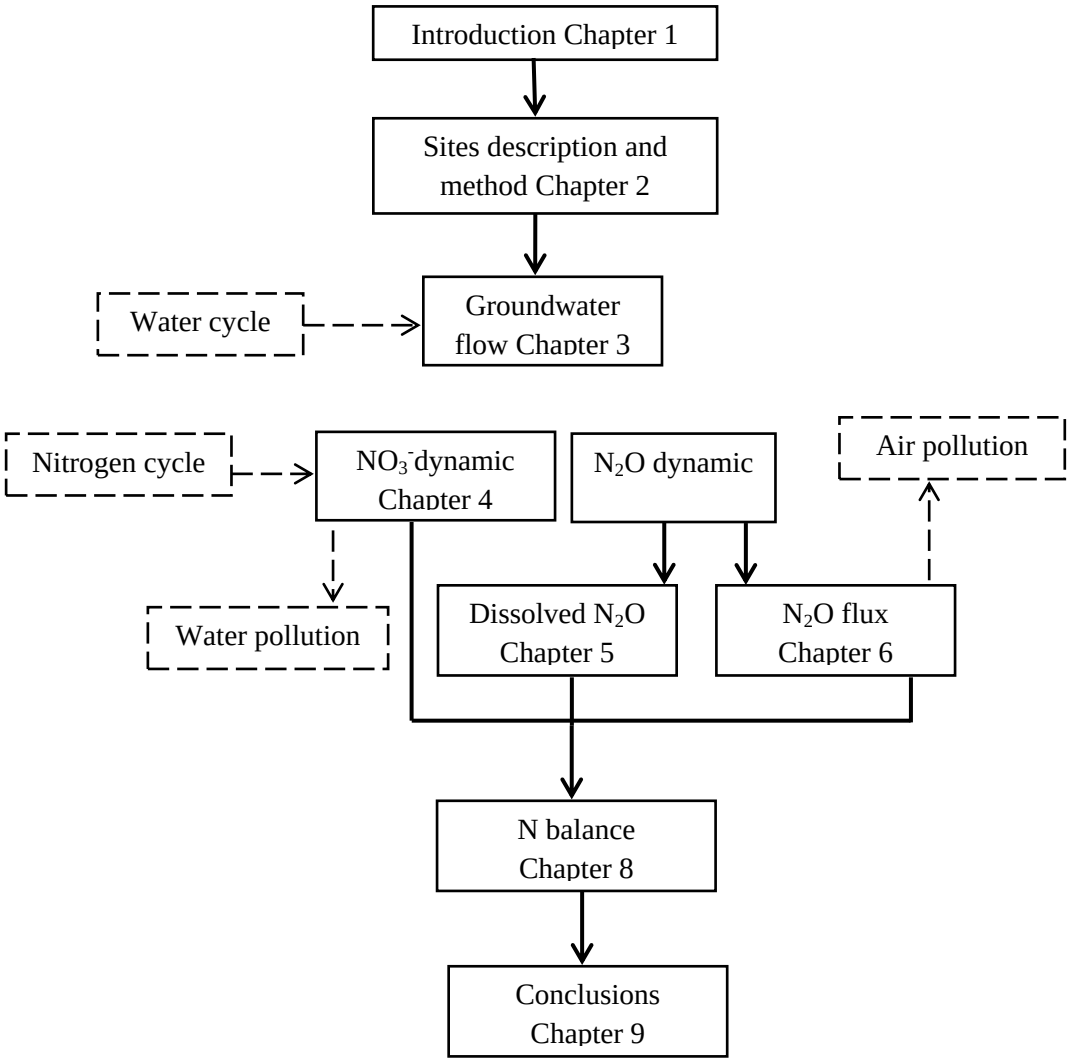

Figure 1-2 Schematic of the structure, content and science problems in the study

Chapter 2 Descriptions of study area and methodology

2.1 Descriptions of study area

The study area is a typical headwater wetland, located at Ichikawa City (35.76^{o}N, 139.97^{o}E), Chiba Prefecture, Japan (Figure 2-1). Chiba Prefecture is crisscrossed by rivers, surrounded by the sea and blessed with rich natural resources, including the water resource and forests. It is located on the eastern side of the Tokyo metropolitan area, and occupies the Boso peninsula that juts out into the Pacific Ocean. The southeastern part of the peninsula faces the Pacific Ocean, while the western side faces Tokyo Bay. Chiba Prefecture has a land area of 5,156.60 km^2,with a population of more than 6 million. It has a humid subtropical climate with average annual temperature and precipitation of 15 ^{o}C and 1249.7 mm, respectively.

Topographically, Chiba is made up of the Boso Hills, a chain of hills 200 to 300 m in height, and the relatively flat Shimousa plateau, Tone River basin, and plains along the Kujukuri coast. Chiba's coastline extends 534.3 km (as of March 31, 2007), and offers highly varied scenery.Chibaprefecture also boasts the second-highest agricultural production in Japan. It leads the nation in the production of several vegetables, including carrots; cabbage and daikon radish, the ubiquitous Japanese cultivar of the Welsh onion; loquat, tomatoes and spinach. It is famous for production of the Japanese cultivar of the pear, which has a two hundred year history of cultivation in the prefecture. It is the nation's second largest producer of corn. Rice is also grown, and Seaweedis harvested in large quantities from Tokyo Bay.

Ichikawa is a city located in northwest Chiba Prefecture, approximately 20 kilometers from the center of Tokyo. The cityis with the total area of 57.46 km^2, andpopulation of

469,603. The study area is consisted of a headwater wetland, located at the suburbs of Ichikawa City. The wetland valley is U-shaped with an elevation of about 16m above sea level. Previously, this wetland used to be paddy field and had been redeveloped to a wetland park. A stream flowing through the wetland valley is recharged by spring water and groundwater in the wetland. Average flow rate of the stream all around a year is 21.7 L S^{-1} at the export of the park. Dominating vegetation in the wetland consists of Houttuynia, Calamus and Japanese pampas grass. The wetland receives discharge (both groundwater and overland flow) from an adjacent upland (elevation 26-31m) area with vegetation consisting of mostly pear orchard. The area of pear orchard in Ichikawa city is 272 ha which account for 1/3 of total upland area. 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 slope is closest to the orchard edge and decreasing towards the wetland, acting as a transitional zone linking the upland and the experimental wetland. The slope is covered by Acer, Pinophyta and Bambuseae. The upland is covered by Kanto Loam about 4 m in thick. It is underlain in a sequence by Joso clay layer and Narita sand layer that is a thick fine sand layer andis the mainaquifer in the study area. The water table was about 20 m above sea level at upland. Within the wetland, the aquifer is a fine sand layer overlaid by cohesive soil and sandy clay with the water table > 15.6 m above sea level.

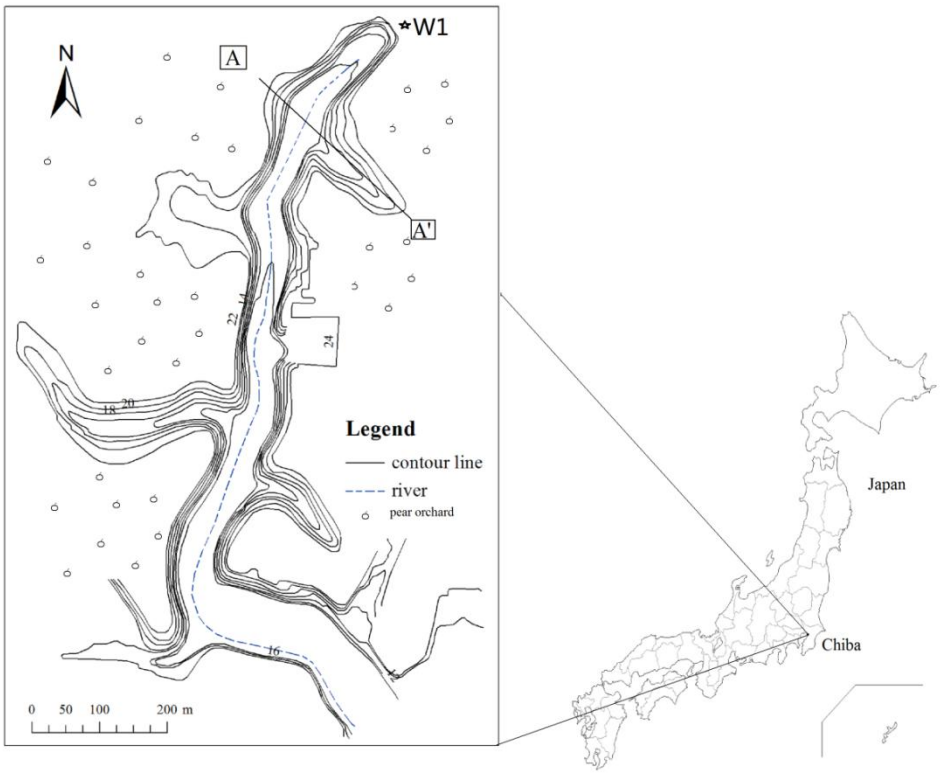

Figure 2-1 Location of the sampling transectsof study wetland

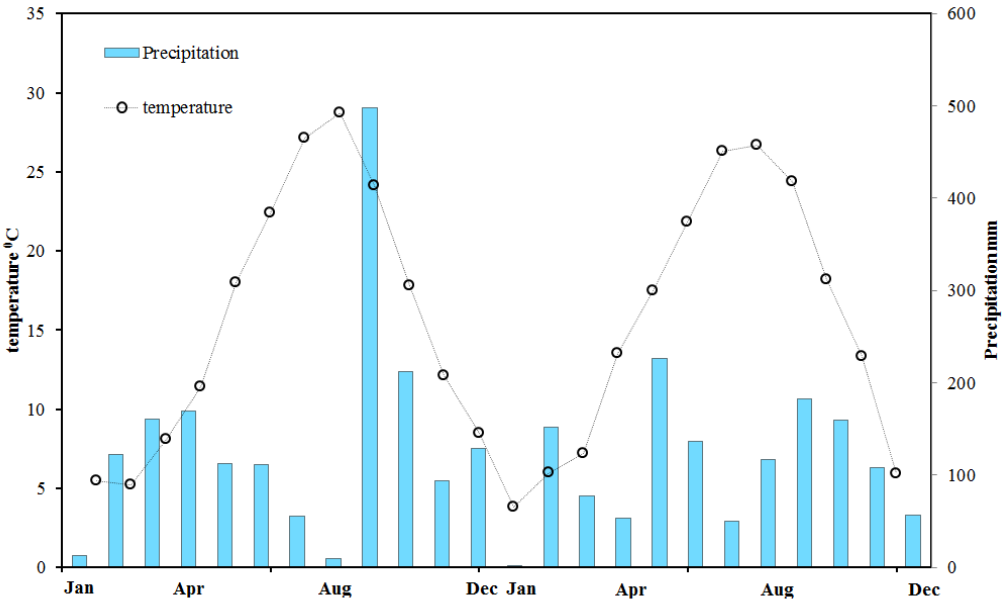

Figure 2-2 Monthly change of temperature and precipitation of 2011 and 2012 in study area

2.2 Methodology

2.2.1 Parameters measurement and major ions analysis

pH, electrical conductivity (EC), oxidation-reduction potential (ORP), dissolved oxygen (DO) and temperature of the samples were measured with portable meters (HORIBA) on site. 50 mL water sample was collected and passed through 0.45 μm filter for analysis the concentrations of Ca^{2+}, NH_4^+, K^+, Mg^{2+}, Na^+, Cl^-, NO_2^- and SO_4^{2-} with Ion Chromatography (LC-10A, Shimadzu, Japan). 20 mL filtered water sample with a drop of mixing solution (1% bromocresol green in 95% alcohol and 1% methyl red in 95% alcohol were mixed with the volume ratio is 1:1) was used to determine the concentration of HCO_3^- by titration with 0.01 mol/L dilution hydrochloric acid it in laboratory within 24 h after sampling.

2.2.2 Isotope analysis for $\delta^{15}N\text{-}NO_3^-$

Precise, accurate, but also inexpensive and fast analysis of$NO_3^-$for δ15N is needed for improved NO_3^-sourceidentification, quantification and uncertainty assessment.In recent years, the so called ‘‘ion-exchange’’ or ‘‘$AgNO_3$method’’ for $\delta^{15}N\text{-}NO_3^-$analysis has beendeveloped by Chang et al. (1999) and Silva et al. (2000). Thismethod is used to concentrate and purify NO_3^-in watersamples for simultaneous ^{15}N determination. The disadvantagesof the ‘‘ion-exchange method’’ can be summarizedas follows: (1) the sample preparation procedure is relativelylabor-intensive (3–5days for sample preparation) and costintensive(up to 60 Euro per sample just for consumables only);(2) high concentrations of anions (e.g. Cl^-, SO_4^{2-}, DOC, etc.) inwater samples can interfere with the adsorption of NO_3^- ontoanion exchange resins; and (3) target sample size of

100–200 mmol of $NO_3^-$for optimal analysis requires large samplevolumes for low NO_3^- concentration samples.

The extraction procedure of nitrate in water is shown as the follows: Two liters filtered water was used to extract NO_3^- with the column filled with anion exchange resin in the chloride form (Dowex 1×8, 200-400 mesh), the flow rate was controlled between 200-300 mLh^{-1} during the extraction. 15 mL 3 $molL^{-1}$ HCl was then used to elute nitrate from the resin. Beakers containing the eluant were placed in cold water bath and about 6.5 g Ag_2O was gradually put into the beaker with a stir. The final pH was verified at the range of about 5.5-6.0 (Silva et al, 2000). AgCl precipitate was removed by filtration. Then the eluent was freeze-dried to obtain $AgNO_3$. Finally, dried $AgNO_3$ was determined for the ^{15}N value by Integra CN mass spectrometer (Pdz Europa LTD, UK) at Chiba University. All the samples were measured twice and the result showed the difference between the two measurements less than ±5%. Then the mean of two measurements was treated as the value of $\delta^{15}N$-NO_3 used in this study. Results are expressed in d units defined by:

$$\delta^{15}N = \frac{R_{sample} - R_{standard}}{R_{standard}} \times 1000 \quad (2\text{-}1)$$

Where R_{sample} and $R_{standard}$ are the $^{15}N/^{14}N$ ratios for the sample and standard,respectively. The reference standard is atmospheric nitrogen for 15N.

2.2.3 Flux of N_2O from the surface

The chamber method has been used extensively for measuring gas exchange between soil surfaces and the atmosphere. Emission of the gas from the soil into the chamber results in increases in the concentration of the gas in the chamber (Li et al. 2000). Commonly, there are three chamber methods: the open chamber method, the closed chamber static method, and the closed chamber dynamic method. The last one was used to measure nitrous oxide

flux in the study. Gas samples were collected in a rectangular plastic chamber (35 cm wide × 20 cm long × 35 cm height). Three holes were made on the sides chamber to accommodate rubber septa, through which a needle or a thermometer could be inserted to withdraw the gas samples or measure the air temperature inside, respectively. The lower edge of the chamber was inserted into the soil and sealed with mud to make the chamber to be airtight during the sampling period. The gas that emits from the soil is allowed to flow into the chamber from which gas sample was withdrawn and collected in an evacuated vial (20 ml) in 0, 15, and 30 min after starting. Soil temperature and air temperature inside and outside of chamber were measured at the same period of the water sampling described above.

The gas samples were analyzed for target gas (N_2O) concentration by gas chromatography (GC14B, Shimazu) equipped with an electron capture detector operated at 280 ^oC and column operated at 70 ^oC. The analysis of N_2O was conducted within 24 h after collecting the samples. As a result, the rate of N_2O gas emission (F) was calculated by Eq. (1) (Wang et al. 2010).

$$F = \frac{V \times (C - C_0) \times 273}{A \times t \times (273 + T)} \quad (2\text{-}2)$$

Where V is the volume of the chamber, A is the area of soil covered by the chamber, C is the concentration of gas inthe chamber at time t, and C_0 is the initial concentration att = 0, T is the temperature inside the chamber.

2.2.4 Analysis ofdissolved N_2O

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 2-3). 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.

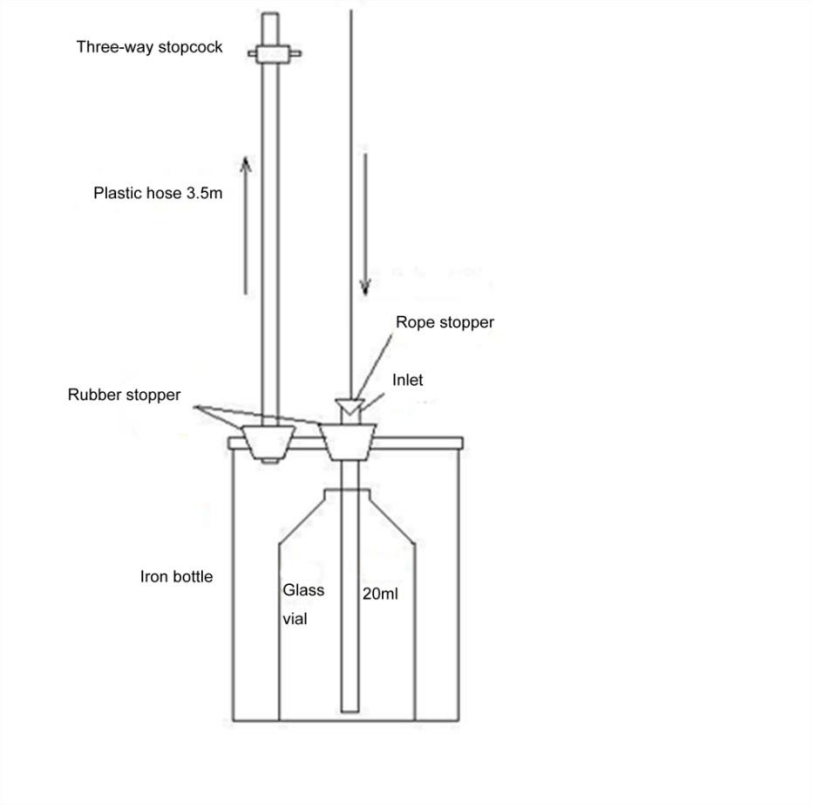

Figure 2-3 The Schematic diagram of sampler for dissolved N_2O

1ml sterilant was injected into water sample to control further N_2O production. 10ml pure $N_2$was injected into the vial to replace the same volume of water out from it after delivered to laboratory. The vial was shacked for 1minute and stored in the thermostat to get equilibrium with headspace air at 40 ^oC. The N_2O concentration of headspace was analyzed by GC in the same waydescribed in the previous section. Based on the mass

conservation law, theconcentration of dissolved nitrous oxide in sampling water (C_w)is calculated by equations (2) to (5) (Sander R 1999).

$$C_W = C_g \times （H + \frac{V_g}{V_q}) \quad (2\text{-}3)$$

Where, $V_g$and $V_q$are the volume of the gas-phase and the aqueous-phase, respectively.His the dimensionless ratio between the aqueous-phase concentration C_q of N_2O and its gas-phase concentration $C_g$in the equilibrium based on the Henry's law:

$$H = C_q / C_g = K_H \times RT \quad (2\text{-}4)$$

K_H is the Henry's law constant, R the gas constant and T the temperature (K). A simple way to describe Henry's law as a function of temperature is:

$$H = RTK_H^{\theta} \times (\exp(\frac{-\Delta_{sol n} H}{R}(\frac{1}{T} - \frac{1}{T^{\theta}})) \quad (2\text{-}5)$$

Where Δ_{soln}H = enthalpy of solution. Here, the temperature dependence is:

$$\frac{-d \ln K_H}{d(1/T)} = \frac{\Delta_{sol n} H}{R} \quad (2\text{-}6)$$

2.2.5Analysis of dissolved Ar and N_2

The sampling method for dissolved Ar and N_2 is the same with dissolved N_2O, only the vial change to 100ml. Dissolved Ar and N_2 concentrations are determined by headspace method. Injected He to push out the water of glass bottle after delivered to laboratory. Shacked the bottle for 1minute and sent to 40 ^{o}C thermostat to get equilibrium with headspace air. The gas samples are analyzed for target gas concentration by gas chromatography (GC2014, Shimazu) equipped with a thermal conductivity detector (TCD) operated at 70 ^{o}C and 5m steel column (5A molecular sieve). The column is kept at -75 ^{o}C and Ar elutes first followed by O_2. N_2 remains trapped by the molecular sieve at

-75 ^{o}C hence column is warmed to room temperature to elute N_2.Theconcentrations of dissolved Ar and N_2in sampling water are determined as the same with dissolved N_2O.

Chapter 3 Groundwater flow system in wetland basin

3.1 Introduction

The formation, persistence, size, and function of wetlands are controlled by hydrologic processes. Distribution and differences in wetland type, vegetative composition, and soil type are caused primarily by geology, topography, and climate. Differences also are the product of the movement of water through or within the wetland, water quality, and the degree of natural or human-induced disturbance. In turn, the wetland soils and vegetation alter water velocities, flow paths, and chemistry. The hydrologic and water-quality functions of wetlands, that is, the roles wetlands play in changing the quantity or quality of water moving through them, are related to the wetland's physical setting.

Differences in hydraulic head cause ground water to move back to the land surface or into surface-water bodies; this process is called ground-water discharge. In wetlands that are common discharge areas for different flow systems, waters from different sources can mix. Ground-water discharge occurs through wells, seepage or springs, and directly through ET where the water table is near the land surface or plant roots reach the water table. Ground-water discharge will influence the water chemistry of the receiving wetland whereas ground-water recharge will influence the chemistry of water in the adjacent aquifer.Wetlands most commonly are ground-water discharge areas; however, ground-water recharge also occurs. Ground-water recharge or discharge in wetlands is affected by topographic position, hydrogeology, sediment and soil characteristics, season, ET, and climate and might not occur uniformly throughout a wetland. Recharge rates in wetlands can be much slower than those in adjacent uplands if the upland soils are more permeable than the slightly permeable clays or peat that usually underlie wetlands.

The accumulation and composition of peat in wetlands are important factors influencing hydrology and vegetation. It was long assumed that the discharge of ground water through thick layers of well-decomposed peat was negligible because of its low permeability, but recent studies have shown that these layers can transmit ground water more rapidly than previously thought (Chason and Siegel, 1986). Peatland type (fen or bog) and plant communities are affected by the chemistry of water in the surface lay ers of the wetland; the source of water (precipitation, surface water, or ground water) controls the water chemistry and determines what nutrients are available for plant growth. Ground-water flow in extensive peatlands such as the Glacial Lake Agassiz peatland in Minnesota may be controlled by the development of ground-water mounds (elevated water tables fed by precipitation) in raised bogs where ground water moves downward through mineral soils before discharging into adjacent fens (Siegel, 1983; Siegel and Glaser, 1987). Movement of the ground water through mineral soils increases the nutrient content of the water.

Slug tests are generally conducted to determine the horizontal hydraulic conductivity (K) of a ground water zone. The rate of water level change is a function of the K of the formation and the geometry of the well orscreened interval.Slug tests generally are conducted in formations that exhibit low K. They may not be appropriate in fractured rock or formations with transmissivity (T) greater than 250 $mday^{-1}$ (Kruseman and de Ridder, 1990). However, in some instances, a vacuum or slug test conducted with a pressure transducer or an electronic data logger may be warranted. Hydraulic properties determined by slug tests are representative only of the material in the immediate vicinity of the well. However, by performing a series of slug test at discrete vertical intervals and

tests in closely spaced wells, important information can be obtained about the vertical and horizontal variations of hydraulic properties for the site (Butler, 1998). It should be noted that due to the localized nature of hydraulic response, the test might be affected by the properties of the well filter pack. Therefore, the results should be compared to known values for similar geologic media to determine if they are reasonable. If slug tests are used, the designer should consider the amount of displaced water, design of the well, number of tests, method and frequency of water level measurements, and the method used to analyze the data. Slug tests should be conducted in properly designed and developed wells or piezometers. If development is inadequate, the smearing of fine-grained material along the borehole wall may result in data that indicate an artificially low K. This may lead to an underestimation of contaminant migration potential. The design, analytical methods, and information that should be reported to document that the tests were conducted properly are discussed briefly below. Detailed practical guidelines for the design, performance, and analysis of slug tests are provided by Butler (1998). Additional information can also be found in Black (1978), Chirlin (1990), Dawson and Istok (1991), Ferris et al. (1962), Kruseman and de Ridder (1990), and Lohman (1972), Batu (1988), and ASTM standards.

The objectives of this chapter are 1) to draw stratigraphy of study profile in wetland; 2) to know the hydraulic conductivity of the profile in wetland by conducting pumping test; 3) to understand the flow path of groundwater in wetland by measuring hydraulic head.

3.2 Site description and method

The study profile was A-A', locating at upstream of river in the wetland. Ten sets of piezometers made of PVC pipes (50 cm ID) were installed along the cross section A-A'in

May 2013 and consisted of PVC pipes at 1m and 2m in depth for each site (Figure 3-**1**).The soil samples were collected at each site to exam during piezometer being set to determine the soil texture. The seasonal changes of water level have been measured from the piezometers since May 2013. Hydraulic heads at piezometers were calculated based on the definition shown Figure 3-2.

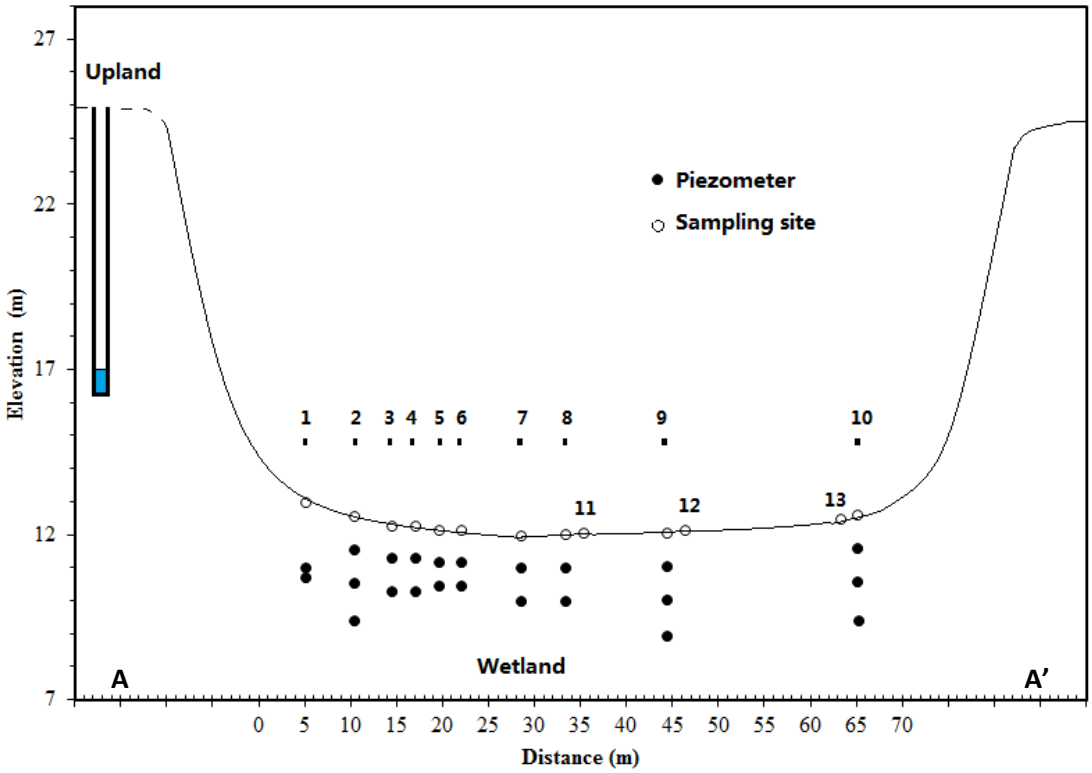

Figure 3- 1Elevation, distance and location of sampling sites in wetland

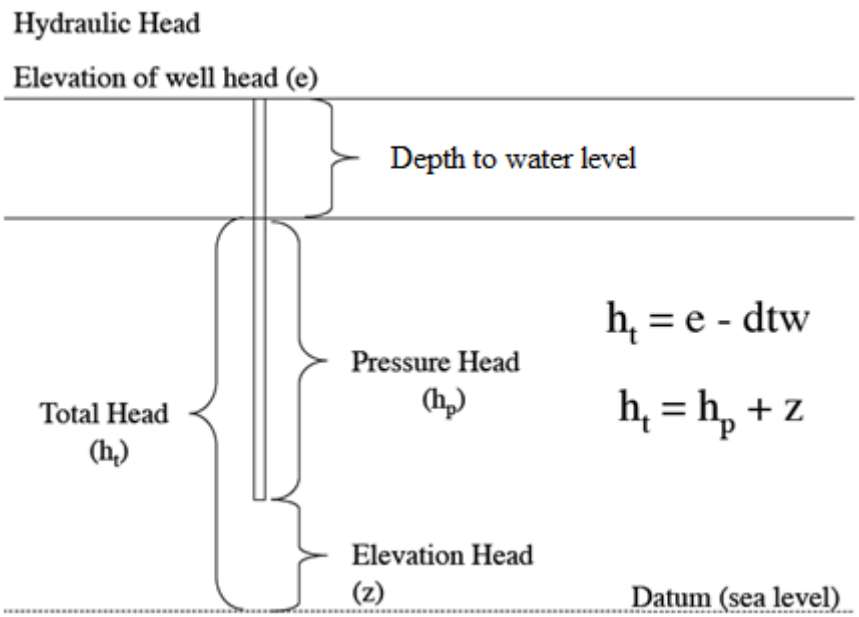

Figure 3-2Hydraulic head of groundwater

$h_t = h_p + z$ (3-1)Where h_t is the hydraulic head, h_p is the pressure head and z is the position head.

The hydraulic gradient between any twopoints is the slope of the hydraulic headbetween those points.The gradient is along the direction of the lower head. The planar distribution of the hydraulic gradient in a given area can be calculated by installing three or more piezometers, measuring the hydraulic head in each piezometer, and contouring these head values. It follows that the gradient is in the direction perpendicular to the hydraulic head, or equipotential contours, toward the decreasing hydraulic head. Then, the contour map of hydraulic head is developed. It can be used to determine the direction and velocity of groundwater flow at any point in the region.Accordingly, it is then possible to ascertain where contaminants in the groundwater may be coming from and in what direction they are flowing.

Slug tests can be used to estimate transmissivity of the aquifer in the immediate vicinityof the well. Storativity can also be estimated, although itestimates are oftendifficult to make with any degree of accuracy. In this study, slug test was conducted to calculate hydraulic conductivity at site 2, 9 and 10 (Figure 3- 3). Water was bailed out of the piezometers to lower the water level. The rate at which thewater in the piezometersrises (as it drains from theaquifer into the well) is measured and these data are analyzed.

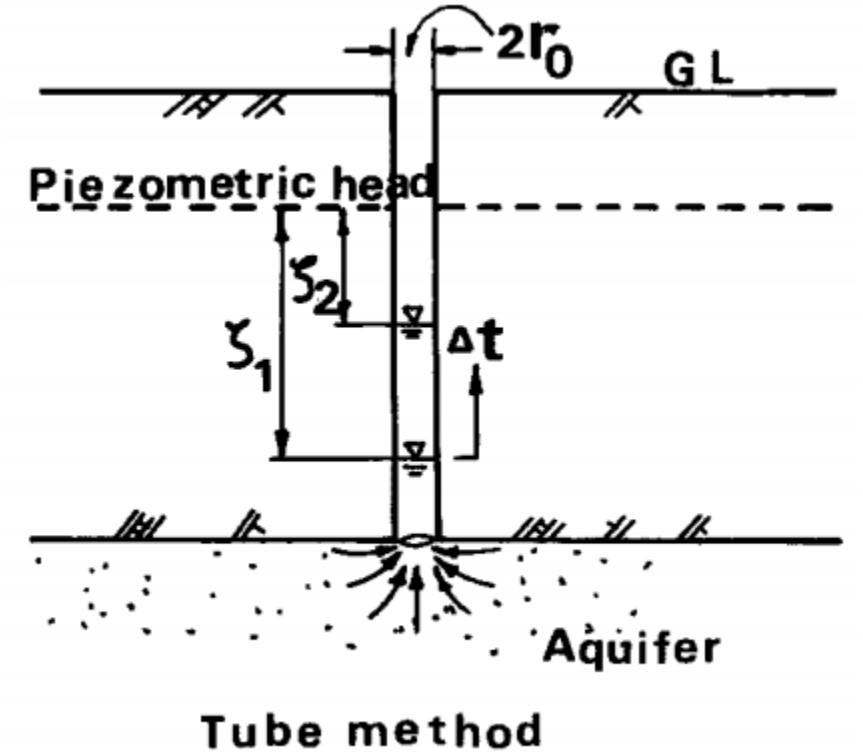

Figure 3- 3Tube method-slug test

The equation for calculation of hydraulic conductivity is as follows:

$$k = \frac{2.3\, r_0}{2\Delta t} log_{10} \frac{\xi_1}{\xi_2} \quad (3\text{-}2)$$

Where k is hydraulic conductivity, r_0 is radius of PVC pipe, Δt is experiment time, ξ_1 is initial head, $\xi_2$isend time head.

3.3 Results and discussion

3.3.1 Stratigraphy of wetland

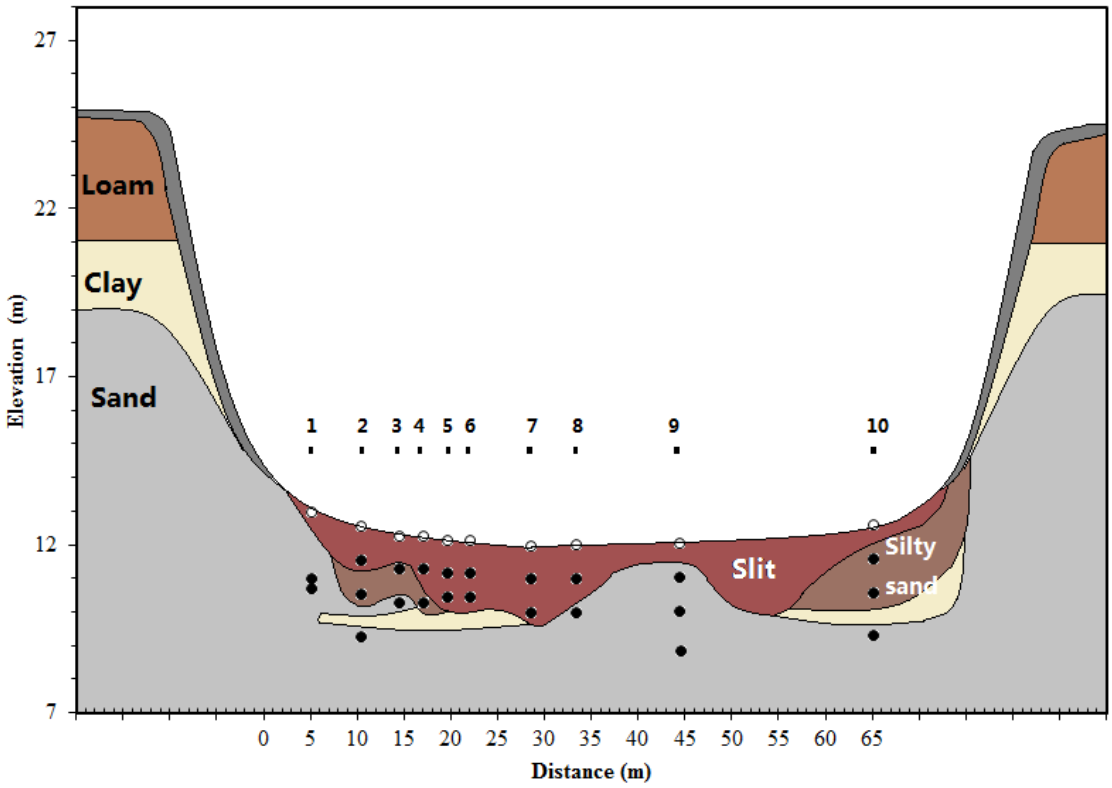

Figure 3-4Stratigraphy of profile A-A’

Soil texture is a qualitative classification tool used in both the field and laboratory to determine classes based on their physical feature. According to soil classification of the United States Department of Agriculture (USDA), the diameters of clay, silt and sand are less than 0.002mm, 0.002 to 0.05mm and larger 0.05, respectively.Furthermore, sand can be described as coarse, intermediate as medium, and the smaller as fine.As a result, the stratigraphy of cross section A-A’is shown in Figure 3-4. Silt, sand and silty sand are dominated this study wetland. From soil surface to 1m in depth, silt dominated this layer. From 1 m to 2 m in depth, silt layer is continued from up layer at sites 4-8, mix layer of

silt and sand is at sites 2, 3, 9 and 10, and sand layer is at site 1. Discontinuous clay layer also could be found of this profile.

3.3.2Hydraulic conductivity

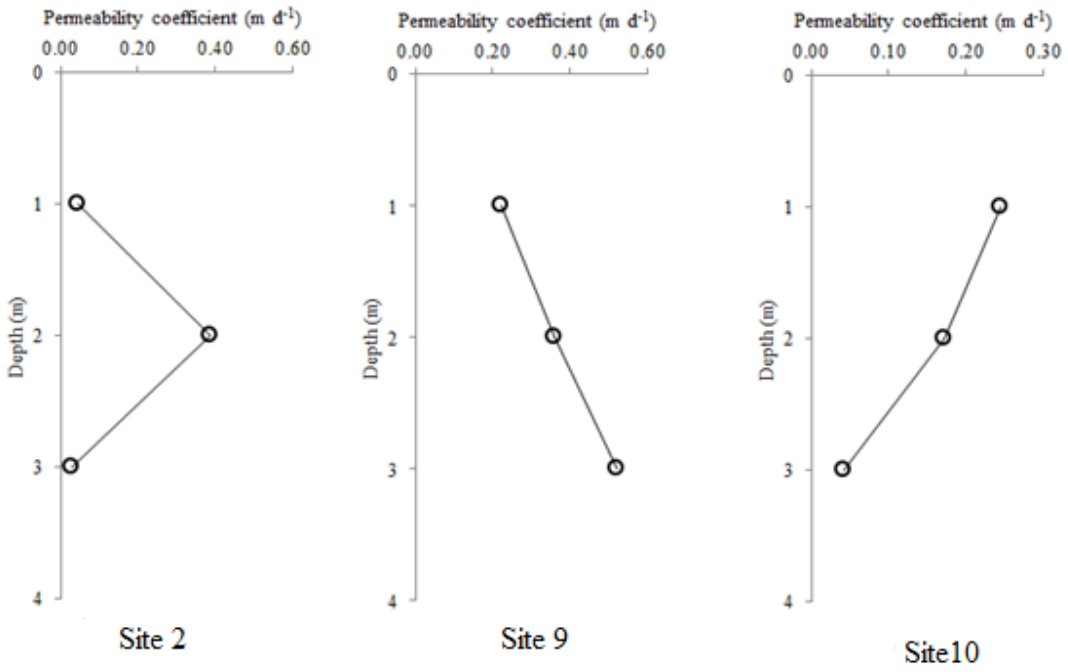

Figure 3- 5Vertical distributions of hydraulic conductivityat site 2, 9 and 10

Hydraulic conductivityranged from 0.0297 to 0.521 m d^{-1} at site 2, 9 and 10. At 1 m in depth, Hydraulic conductivity at site 9 and 10 (0.220 and 0.245 m d^{-1} respectively) was one order higher than thatat site 2 (0.044 m d^{-1}) because sitly sand layer was found at site 9 and 10,but silt layer was at site 2(Figure 3- **5**). At 2 m in depth, hydraulic conductivities were with same magnitude at site 2, 9 and 10 as expected (all in silty sand layer). At 3 m in depth, hydraulic conductivitieswere one order higher at site 9 than that at site 2 and 10 with a wide range of difference. The low hydraulic conductivity may result in the existence of clay layer at site 2 and 10.

3.3.2 Groundwater flow system in wetland

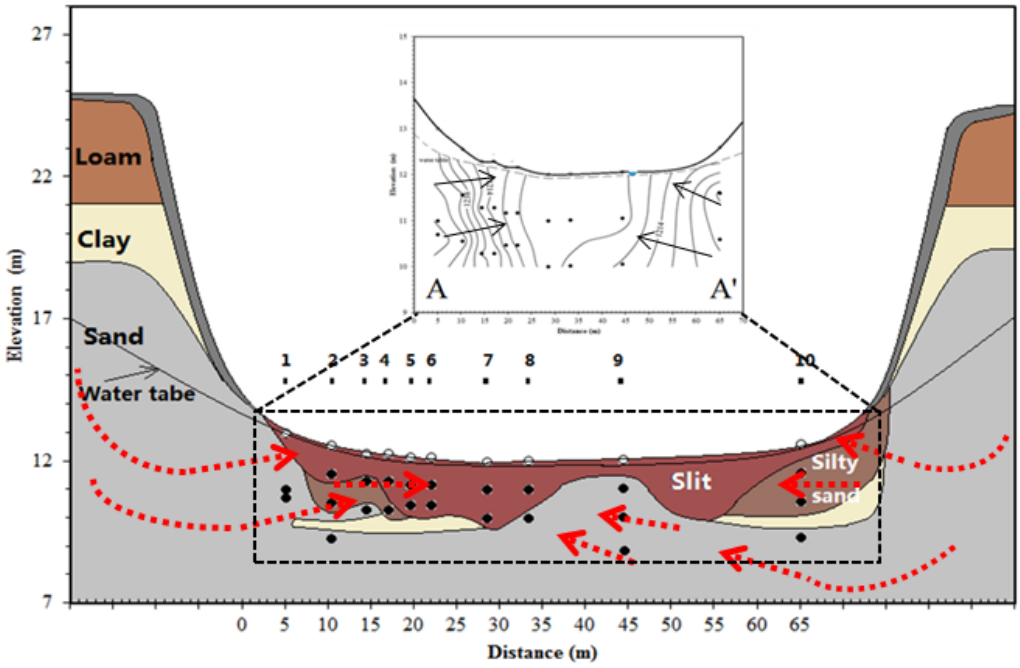

Figure 3-6Piezometers nest and hydraulic head contoursalong cross section A-A'. The arrows present the direction of flow path**.**

The water table remain below the soil surface that about 0.3m year around.Groundwater flow patterns along thecross section A-A' are shown in figure 3-6. The flow nets indicate predominantly horizontal flow from both sides of slope of the wetland towards the middle of wetland.From groundwater table to 1 m in depth, hydraulic head contours indicate upward flow that the groundwater moves from sand layer into silty sand and then to silt layer. At the 1-2m layer, hydraulic head contours indicate horizontal flow that similar with up layer, whereas the contours shift direction indicating upward flow at sites 9. Besides, the groundwater flow would be affected by the clay layer due to the low permeability of clay.

Velocity of groundwater flowcould be predicted by Darcy's Law. Residence time of groundwater in wetland could be obtained by distance and velocity of groundwater flow. Velocity of groundwater is calculated as:

$V = -k \times dh/dl$ (3-3)

Where V is the velocity of groundwater, k is thehydraulic conductivity; dh/dl is the hydraulic gradient.

We take groundwater flow from site 2-2m to site 7-1m for example to calculate the residence time in wetland. The groundwater flows from site 2-2m to site 4-1.5m in the layer of silty sand firstly. Hydraulic conductivity is about 0.389 m d^{-1} and hydraulic gradient is 0.024, then the velocity is 0.117 m s^{-1}. Thereby, the residence time in the wetland would be 686 d. Also, the groundwater flows from site 4-1.5m to site 7-1m in the layer of silt. Hydraulic conductivity is assumed as 0.030 m $d^{-1}$and hydraulic gradient is 0.029, then the velocity is 0.001 m s^{-1}. Thereby, the residence time would be 10479 d. Totally, it will be spent 11165 d (about 31 years) that the groundwater flow from site 2-2m to site 7-1m in the study wetland. In addition, rate of denitrification was greatest at long residence times(Zarnetske et al. 2011).

3.4 Conclusions

The surface layer of study area is silt, followed by silty sand and sand. Discontinuous clay layer also could be found in the wetland. Therefore, low hydraulic conductivities were found in this study area rangingfrom 0.0297 to 0.521 m d^{-1}. The flow nets indicate predominantly horizontal flow from the upland boundary of the wetland towards middle of wetland. The travel time of groundwater from boundary (site 2-2m) to middle (site 7-

1m) would be spent 31 years that would beneficial for denitrification process in wetland groundwater.

Chapter 4 Dynamics of nitrate in groundwater of wetland basin

4.1 Introduction

Research of wetland in agricultural catchmentswith high nitrate concentrations in subsurface runoff hasoften shown a substantial decrease in nitrate concentrationswithin the wetland (Lowrance et al., 1984;Peterjohn and Correll, 1984; Pinay and Decamps, 1988;Simmons et al., 1992; Hill et al., 2000; Dhondt et al.,2002). Most studies of the role of riparian wetlands havebeen carried out in a geomorphological and hydrologicalcontext involving the convergence of fluxesfrom the hillslope towards the stream via the riparianwetland. These studies were carried out at the scale ofindividual hillslopes (Sabater et al., 2003; Gilliam,1994; Hill, 1996) and more recently an entire catchmentbasin (Hattermann et al., 2006; Rassam, Pagendam& Hunter, 2008). Some studies, however, haverevealed an inversion of the exchanges betweenstreams and riparian wetlands, with flux reversals ata local scale in the hyporheic zone (Triska, Duff &Avanzino, 1993, Dahm et al., 1998; Pretty, Hildrew &Trimmer, 2006) or at a larger scale in major alluvialwetlands (Clement et al., 2003; Weng et al., 2003).Other studies have evaluated the role of riparianwetlands and stream systems in regulating nitrogenfluxes during transfer in the drainage network and atvarious spatial scales (Alexander, Smith & Schwarz,2000, Sebilo et al., 2006). Some studies have integratedimpacts on both hillslope ground water and on riverwater (Cooper, 1990; Mulholland, 1992; Lefebvre et al.,2007), but few of these have been carried out on alarge catchment (Grizzetti et al., 2005).

Natural processes such as vegetation uptake,denitrification and microbial immobilization have beendemonstrated to be important in the removal of nitratefrom shallow groundwater in wetland (Groffmanet al., 1996; Haycock and Pinay, 1993; Martin et al.,1999).These results have led to the conclusion thatwetlandis crucial to the control of non-pointsource pollution of surface waters in agriculturalenvironments.

However, flows of groundwater from a semi-confinedaquifer with low nitrate concentrations and surface waterin the near stream (hyporheic) zone may significantlycontribute to the decrease in nitrate concentrations in theshallow groundwater through dilution or mixing(Vought et al., 1994; Altman and Parizek, 1995; Pinayet al., 1998). The relative contribution of groundwaterfrom a semi-confined aquifer to the water and solutebudgets in riparian zones is largely dependent on thegeological setting, and can be substantial in glacialterrain due to the scaled and folded deposits in themoraine (Roulet, 1990).Another factor that needs to be considered is thatflow patterns within riparian zones with heterogeneoussediments may be complex and create spatial differencesin both residence time and material encountered by thegroundwater travelling within the wetland (Goldet al., 1998; Devito et al., 2000). Preferential flow pathsmay occur as a result of local differences in permeabilityof the soil and these may conduct substantial quantitiesof water and decrease the overall residence time of waterin the buffer zone. A second example of the importanceof the hydrological flow paths in nitrate removal isbypass flow, when nitrate-rich groundwater passesunderneath the wetland. The nitrate-richgroundwater short-circuits the biologically activetopsoil and reaches the stream without any improvementin water quality (Phillips et al., 1993; Wigingtonet al.,

2003). Thus, knowledge of groundwater flowpaths and physical water mixing is essential for a correctevaluation of the N mitigation by wetland(Nelson et al., 1995).

Besides these hydrological processes, the intensity ofnitrate loading is another key factor influencing theremoval efficiency of wetland (Hanson et al., 1994;Willems et al., 1997). In theupland of the study area, the rate offertilizer application is as high as 400 kg N ha^{-1} y^{-1}. The watershedhasbeen subject to prolongednitrogen enrichment that threatens the groundwater. This increased nitrogen availabilityhas resulted in increased nitrogen cycling rates andpossibly reduced N retention (Hanson et al., 1994). Overtime, the chronic N inputs may even cause saturation ofbuffer zones and consequently loss of their beneficialfunction on water quality improvement. Sabater et al.(2003) found a negative relation between nitrate loadingand wetland zone removal efficiency over a wide range ofriparian sites in Europe.

Our aims were to determine the effect of groundwaterpathways, on measured changes in nitrate concentration withinthe wetland.In this research the following step-wise approach waspursued:

1. Investigation of changes in groundwater nitrate concentrations and other major ions along flow paths;
2. Determination of the mixing of water from different sources along flow paths within the study wetland on the basis of chemical signatures, especially chloride concentrations.
3. Estimation of biogeochemical processes associated nitrate in the groundwater decrease based on dissolved oxygen (DO), oxidation-reduction potential(ORP) and $^{15}N\text{-}NO_3^-$;

4.2 Site descriptions and field procedure

Most piezometers were installed in May 2013 and consisted of PVC pipes (50 cm ID) at 1m and 2m in depth along the cross section A-A'. Water level in the piezometers were measured before water sampling. DO, ORP, and temperature of groundwater are measured in situ by potable meters (D50, Horiba).Before sampling, water waswithdrawal from the piezometers and the fresh groundwater flowed in. A sampler with a glass vial was inserted to the bottom of piezometer where the vial wasfilled gently. Samples werekept in a cooler, brought back to the laboratory and stored at 4 ^{0}C prior to analysis. All water samples were filtered (0.45μm) before analyzing for major ions by ion chromatography (Shimadzu CDD-6A and CDD-10Avp). 1ml sterilant is injected into the vial to prevent bacterial activities for denitrification to analysis for Ar and N_2 and should be no invisible bubble. Dissolved Ar and N_2 concentrations are determined by headspace method. Injected He to push out the water of glass bottle after delivered to laboratory. Shacked the bottle for 1minute and sent to 40 ^{0}C thermostat to get equilibrium with headspace air. The gas samples are analyzed for target gas concentration by gas chromatography (GC2014, Shimazu) equipped with a thermal conductivity detector operated at 70℃ and 5m steel column (5A molecular sieve). The column is kept at -75 ^{0}C and Ar elutes first followed by O_2. N_2 remains trapped by the molecular sieve at -75 ^{0}C hence column is warmed to room temperature to elute N_2. The water samples are treated for δ^{15}N-NO_3- analysis in the method suggested by Silva. δ^{15}N-NO_3- values of samples were determined by Integra CN mass spectrometer (Pdz Europa LTD). Groundwater samples for CFCs analysis are collected in dark brown glass bottles sealed by caps with aluminum liners.

4.3 Results

4.3.1 Temperature, EC, DO, ORP and DOC

Temperature, EC, DO, ORP and DOC were also observed of the groundwater in wetland. Trend in DO concentrations and ORP were similar. The temperatures ranged from 16.3-20.2 ^{0}C, with an average value of 17.7^{0}C (s = 1.1) (Table 4- 1). pH values ranged from 6.36-7.84, with an average value of 6.77 (s = 0.43). The DO concentrations ranged from 0.49-6.03 mg L^{-1}, with an average value of 3.04 mg L^{-1}. From site 5 to 9, DO concentrations were almost below 2 mg L^{-1}, whereas those at other sites were higher than 2 mg L^{-1}. It is obviously that the DO decreased from boundary of wetland toward the middle of wetland. ORP ranged from 0 to 290 mV, with an average value of 170 mV. It decreased from >200 to 0 mV from the wetland boundary to site 6. DOC concentrations ranged from 1.72 to 39.62mg L^{-1}, with an average value of 7.7 mg L^{-1}. DOC value was lower than 3.10 mg L^{-1}in sands (site 1, 3-2m, 8-2m), and ranged from1.72 to 39.62 mg L^{-1}in the layers ofsilt and silty sand. Besides, there was little change of DOC concentration along the flow path. The electric conductivity (EC) is the indexof total dissolved ion concentrations in water. The EC values ranged from 216 to 721 μs/cmin the cross A-A'A. Itdecreased from boundary to the middle (from 721 to 216 μs/cm (site 2 to 7) and from 566 to 255 μs/cm (site 10 to 9)respectively).

Table 4- 1T (^{0}C), EC (µs/cm), DO (mg L^{-1}), ORP (mV) and DOC (mg L^{-1}) of groundwater in wetland. Average and standard deviation (s) values of these parameters.

Sites	Depth (m)	DO	ORP	T	DOC	EC	pH
1	2	5.77	290	17.8	2.44	599	6.59
	2.3	4.91	262	17.2	1.96	621	6.36
2	1	5.45	289	17.5	1.72	645	7.19
	2	4.2	299	18.7	2.48	721	7.32
3	1	3.85	253	17.2	39.62	651	7.58
	2	3.18	249	16.9	3.10	692	6.45
4	1	2.51	204	16.6	3.43	440	6.45
	2	3.42	145	16.3	4.79	630	6.76
5	1	0.81	111	19.1	31.51	430	6.52
	1.7	3.28	246	17.9	3.22	455	6.43
6	1	0.49	0	17.8	3.22	376	6.59
	1.7	0.67	51	17.2	4.07	242	6.36
7	1	1.81	17	17.2	21.21	216	7.3
	2	1.86	48	16.6	5.31	233	7.38
8	1	1.88	95	19.1	2.67	339	6.52
	2	1.76	79	17.9	2.51	272	6.43
9	1	2.33	100	19.8	3.39	257	6.72
	2	1.65	125	20.2	2.03	255	7.47
	3	1.26	114	18.2	1.58	320	7.84
10	1	4.88	260	16.9		437	6.45
	2	6.03	282	16.6		566	6.45
Average		3.04	170	17.7	7.70	454	6.77
Standard Deviation		1.73	103	1.1	11.12	174	0.43

4.3.2 Geochemistry

Calcium (Ca^{2+}) and magnesium (Mg^{2+})were the dominant cations accounting for 90.60-62.76% of the total cations (Figure 4- **1**). Ca^{2+} concentrations ranged from 11.85 to 92.96 mg L^{-1}, with an average of 40.43 mg L^{-1}. Mg^{2+} concentrations ranged from 5.16 to 44.46 mg L^{-1}, with an average of 25.37 mg L^{-1}(Table 4- **2**). Na^+ ranged from 8.77 to 26.11 mg L^{-1}, with an average of 16.11 mg L^{-1}. K^+ ion was the least cation ranging from 0.57 to 2.85 mg L^{-1}, with an average of 1.64 mg L^{-1}. Na^+ and K^+ ions accounted for 9.4 to 37.2 %

of the total cations. HCO_3^- and NO_3^- were the dominant anions and were followed by Cl^- in the groundwater (Table 4- 3). SO_4^{2-} was the least anion in the groundwater. HCO_3^- ranged from 36.6 to 373.3 mg L^{-1}, with an average of 132.8 mg L^{-1}. NO_3^- concentrations ranged from 0.20 to 246.1 mg L^{-1}, with an average of 91.51 mg L^{-1}. HCO_3^- and NO_3^- were accounting for 49.42 to 86.71 % of the total anions. Cl^- concentrations ranged from 19.10 to 50.90 mg L^{-1}, with an average of 32.34 mg L^{-1}, accounting for 10.44 to 41.61 % of the total anions. SO_4^{2-} concentrations ranged from 0.55 to 31.00 % of the total anions.

4.3.3 Nitrogen-nitrate distribution of groundwater n wetland

Groundwater NO_3^--N concentrations decreased from 55.6 mg L^{-1} to 0.1 mg L^{-1} from the boundary of upland and wetland. The concentrations of Cl^- decreased from 50.9 mg L^{-1} to 18 mg L^{-1} from site 2 to site 7, whereas the Cl^- concentrations at site 1 were 36.2 and 38.2 mg L^{-1} that lower than site 2. Ratio of NO_3^--N/Cl decreased sharply site 1 to site 2 (range from 1.54 to 0.84), and then decreased slightly from site 2 to site 5 (with an average of 0.7) except 1 m depth at site 4.

Table 4- 2Cations (mg L^{-1}) of the groundwater in wetland

site	Depth (m)	Na (mg L^{-1})	K	Ca	Mg
1	2	26.11	1.76	41.00	39.51
	2.3	23.37	1.74	45.21	42.38
2	1	16.24	2.29	65.30	39.90
	2	17.29	2.85	79.81	42.94
3	1	18.40	2.30	78.98	41.03
	2	18.42	2.55	92.94	44.46
4	1	17.50	1.89	32.46	23.82
	2	15.33	2.19	59.86	29.50
5	1	18.17	1.63	26.57	19.99
	1.7	19.02	1.92	39.03	29.08
6	1	19.95	1.20	13.74	9.92
	1.7	12.15	0.88	12.36	8.50
7	1	9.99	0.83	16.11	10.29
	2	12.12	0.82	24.46	12.64
8	1	14.76	0.71	11.85	8.79
	2	12.11	0.57	24.61	15.13
9	1	9.56	1.25	35.92	5.16
	2	9.68	2.44	42.69	5.34
	3	8.77	2.30	39.98	4.93
10	1	14.61	1.44	28.46	33.34
	2	17.46	1.46	37.15	45.69
Average		16.11	1.64	40.43	25.37
Standard Deviatio		4.41	0.67	23.69	14.97

Table 4- 3Anions(mg L^{-1})and $\delta^{15}N\text{-}NO_3^-$ (‰) of the groundwater in wetland

site	Depth (m)	HCO3	Cl	NO3	SO4	NO3-N/Cl	$\delta^{15}N\text{-}NO_3^-$
1	2	79.30	36.15	246.10	2.55	1.54	6.84
	2.3	97.60	38.05	243.91	2.07	1.45	6.97
2	1	164.70	50.90	188.50	3.99	0.84	5.31
	2	240.34	49.20	185.90	3.24	0.85	5.58
3	1	294.02	33.90	105.60	11.76	0.70	n.d
	2	373.32	32.10	98.10	12.10	0.69	5.07
4	1	174.46	34.70	34.20	8.03	0.22	n.d
	2	195.20	36.10	90.80	15.01	0.57	15.85
5	1	90.28	27.90	88.30	15.50	0.71	n.d
	1.7	114.68	28.70	133.40	10.80	1.05	9.04
6	1	65.88	27.50	22.80	12.32	0.19	15.37
	1.7	63.44	26.10	1.80	10.12	0.02	n.d
7	1	84.18	19.10	0.30	1.70	0.00	n.d
	2	123.22	18.00	0.50	0.96	0.01	n.d
8	1	36.60	31.50	0.20	32.39	0.00	n.d
	2	104.92	26.90	7.90	11.46	0.07	n.d
9	1	100.04	29.20	1.60	6.20	0.01	n.d
	2	87.84	31.00	3.30	29.81	0.02	n.d
	3	89.06	28.20	12.50	21.89	0.10	20.78
10	1	81.74	28.20	146.39	2.44	1.04	10.57
	2	85.40	31.77	230.70	1.67	1.37	7.88
Average		132.86	32.34	91.51	9.70	0.57	8.87
Standard Deviatio		85.23	8.11	89.94	8.76	0.53	3.75

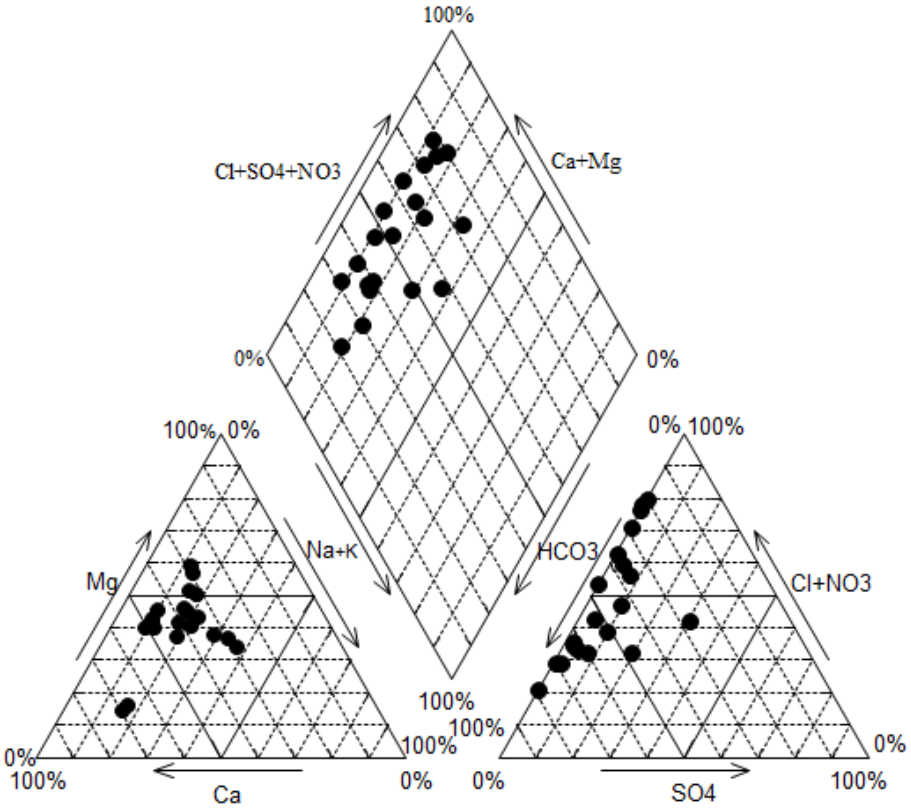

Figure 4- 1Piper diagram for groundwater in wetland

4.3.4 Values of $\delta^{15}N\text{-}NO_3^-$

The values of $\delta^{15}N\text{-}NO_3^-$ ranged from 5.07 to 15.37 ‰, with an average of 8.87 ‰ (s = 3.75) in the wetland groundwater. Generally as the water traveled from the inlet (site 2) toand through the wetland, the $\delta^{15}N$ values increased as the nitrate removal. For example, $\delta^{15}N$ values increased from initial 5.31 ‰ at site 2 reach to 15.85‰ at site 6, whereas $\delta^{15}N$ values increased from initial 7.88 at site 10 to 20.78 ‰ at site 9.

The overall relationship between NO_3^--N loss(expressed as the logarithm of the fraction ofinitial NO_3^- remaining in the sample, N_{sample}:$N_{initial}$) and increase in $\delta^{15}N$ is shown for theentire data set in Figure 4-2.NO_3^--N concentration at inlet (site 2 and 10) was assumed as the $N_{initial}$. The fractions of NO_3^--Nremaining are transformed using natural

logarithmsso the data can be subjected to linearregression analysis. This transformation is necessarybecause isotopic fractionation by progressiveremoval of reaction products is a Rayleigh distillationprocess and its cumulative effects on δ^{15}Nare nonlinear. The measured isotope data were evaluatedusing a simplified Rayleigh equation, as follows:

$\delta_s = \delta_{s,o} + \varepsilon \times \ln f$ (4-1)

where

δ_s = delta value in the substrate (‰)

$\delta_{s,o}$ = initial delta value in the substrate (‰)

ε = enrichment factor (‰, positive or negative)

and

$f = \frac{S}{S_0}$ (4-2)

f = fraction of unreacted residual substrate

s = substrate concentration (mg L^{-1})

S_0= initial (reference) substrate concentration (mg L^{-1})

The equation is derived by Mariotti et al. (1981), and is valid for $|\varepsilon| < 20$‰ and

$\delta_{s,o}$ values which do not deviate too strongly from 0.

The enrichment factors for ^{15}N are calculated with the regression analysis. It showsε = -11.58 ‰ for the flow system on the left side and ε = -13. 998 ‰ forthe flow systemon the right side (Figure 4- **2**).The enrichment factor found in the presentstudy matches with the earlier finding (Bates and Spalding ,1998).

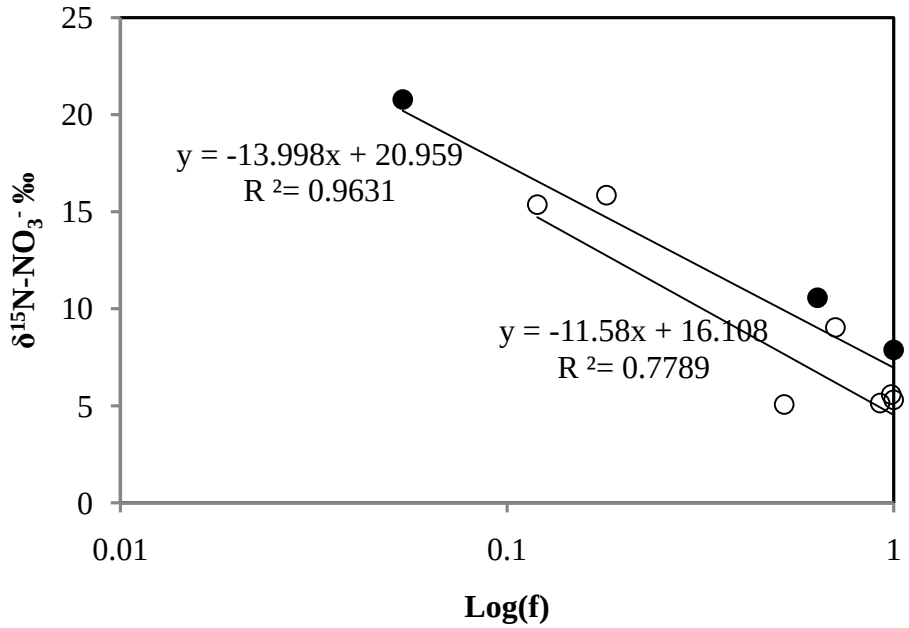

Figure 4- 2Dependence between nitrogen isotope ratio in the residual nitrate ($\delta^{15}N$) and the fractionof the unreacted residual nitrate (logarithmic scale) in denitrification in the groundwater ofthe wetland

4.3.3 Concentrations of dissolved Ar and N_2in wetland

Concentrations of dissolved N_2 and Ar were used to estimate recharge temperature and excess air under oxidizing environment. Argon concentrations range from 0.353 ml L^{-1} to 0.481 ml L^{-1}, whereas nitrogen concentrations changed from 14.721 ml L^{-1} to 24.006 ml L^{-1} (Table 4-4).

The extent of denitrification is the excess nitrogen gas in a sample after solubility and excess air are accounted for. (Heaton and Vogel, 1981) were the first to recognize apparent dissolved gas super-saturation of groundwater relative to recharge temperature, callingthe term ‘excess air’. Excess air is now widely recognized as a consequence of recharging water coming into contact with trapped air in pores, fractures or fissures during its downward travel thorough the aquifer (Wilson and Mcneill, 1997).

Table 4-4Concentrations of Ar, N_2, excess air of $N_2$and $N_{2\ denitrification}$ (ml L^{-1}) of groundwater in wetland in May 2013

Sites	Depth (m)	Ar	N2 (ml L^{-1})	Min excess air	Max excess air	$N_{2\ denitrification}$
1	2	0.438	16.560	3.519	7.780	-2.250
	2.3	0.443	17.450	3.686	8.149	-1.627
2	1	0.389	14.721	1.642	3.631	-1.076
	2	0.408	15.278	2.366	5.232	-1.681
3	1	0.384	16.067	1.467	3.243	0.552
	2	0.385	15.997	1.514	3.346	0.407
4	1	0.465	23.021	4.505	9.960	2.629
	2	0.463	18.987	4.456	9.850	-1.326
5	1	0.419	19.412	2.797	6.184	1.762
	1.7	0.423	21.044	2.930	6.479	3.179
6	1	0.445	21.345	3.767	8.328	2.137
	1.7	0.399	24.006	2.011	4.445	7.618
7	1	0.429	19.871	3.146	6.955	1.661
	2	0.481	21.798	5.126	11.332	0.409
8	1	0.351	15.607	0.201	0.444	2.125
	2	0.435	19.035	3.371	7.452	0.464
9	1	0.353	16.082	0.301	0.666	2.438
	2	0.380	19.247	1.322	2.924	3.964
	3	0.437	20.826	3.454	7.636	2.121
10	1	0.403	16.588	2.178	4.815	-0.068
	2	0.384	15.719	1.461	3.229	0.214

Recharge temperature acts as a guide for interpreting the amount of excess air in the sample. The amount of atmospheric gas in a sample is based on Henry's law solubility.

$Xi = k_H (T,S)* pi$ (4-3)

Where X is the concentration of dissolved gas, k_H is Henry's law constant at given temperature and salinity, p is partial pressure of the gas above the solution. The partial pressure in the atmosphere is constant for a given elevation. The elevation of the water table in the recharge area is between 25 and 30 m above sea level which has a negligible effect on the pressure in the study wetland. Moreover,groundwater salinity was less than 2ppt,too low to affect dissolved gas concentrations. Therefore the amount of atmospheric nitrogen and argon dissolved in each sample is dependent only on recharge

temperature.For simply,weuse mean annual temperature to determine the amount of N_2 and Ar due to atmospheric equilibration.

The largest uncertainty in determining excess N_2 due to denitrification arises from interpretation of excess air (N_2 excess air). Excess air fractionation can range from complete dissolution of gas bubbles to minimal dissolution of gas bubbles. Without a full suite of noble gas concentrations for each sample it is impossible to determine the precise concentration of N_2 due to excess air (Aeschbach-hertig et al., 1999). Instead the total dissolved concentration of argon in the sample is used to determine the amount of excess air in the sample.

Previous workers (Bohlke et al., 2006; Hinkle et al., 2007) have used a N_2/Ar plot to check interpretations of both recharge temperature and excess air. Samples are plotted with N_2 concentration along the x-axis and Ar concentration along the y-axis. Then an air saturated water curve is plotted with excess air amounts were given for a relevant temperature range, i.e. 5^0C to 30^0C in this study (Figure 4- **3**).

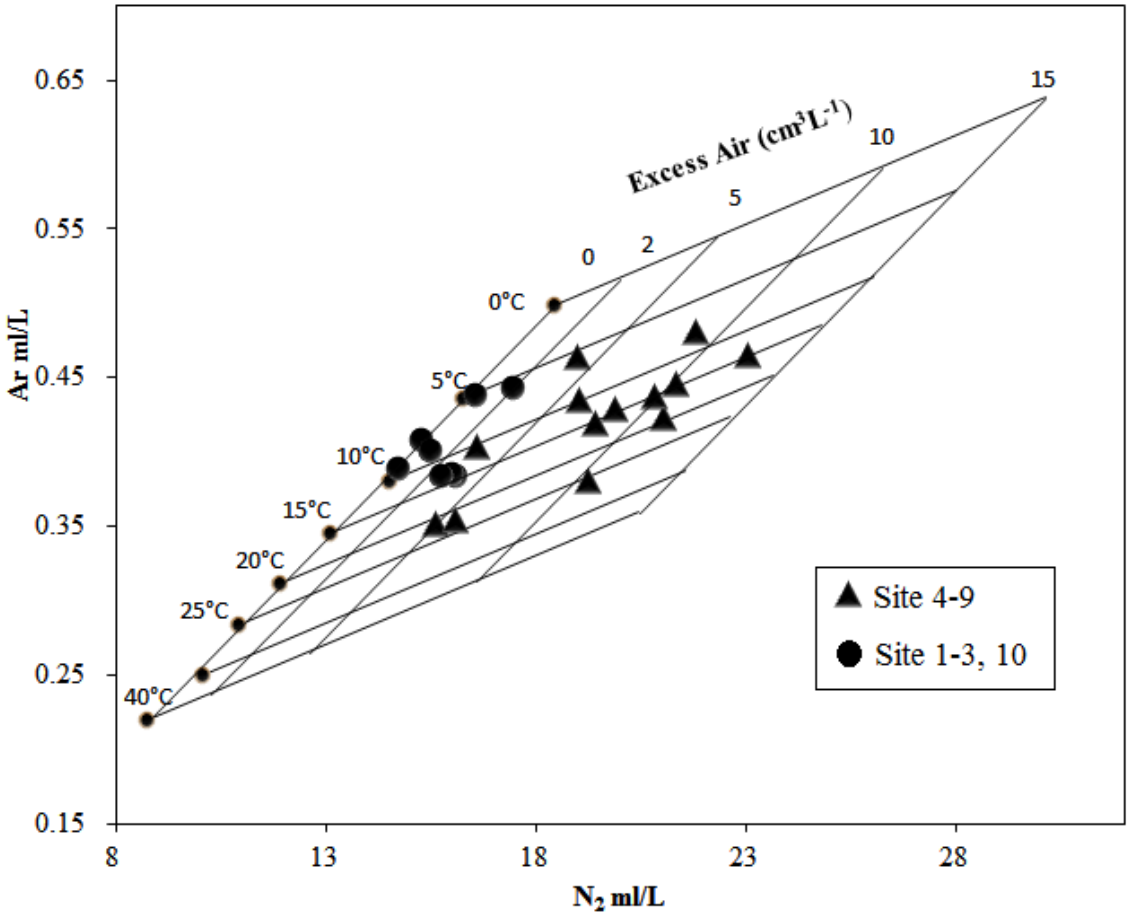

Figure 4- 3Dissolved Nitrogen gas vs. Argon gas for Northport samples. Air saturated water line represents theoretical gas concentrations at relevant temperatures. Excess air curves represent dissolved gas concentrations with excess air entrainment.

We calculate the uncertainty of excess air concentrations in dissolved gas samples accordingto the method outlined by Weymann (2008). If excess air results from completebubble dissolution then the excess air composition is exactly the same as atmosphericcomposition (Heaton et al., 1983). For this case we use the ratio of atmospheric nitrogen andargon concentrations.

$$X_{N_2 Excess\ Air} = \left(X_{Ar\ S} - X_{Ar\ EQ}\right) * \frac{X_{N_2\ atm}}{X_{Ar\ atm}} \qquad (4\text{-}4)$$

Where $X_{Ar\ S}$ is the concentration of argon in the sample, $X_{Ar\ EQ}$ is the concentration of argon at atmospheric equilibrium and N_2 atm and Aratm are the mole fractions of $N_2$and Ar in the atmosphere.

If excess air is the result of incomplete bubble dissolution the N_2/Ar is lower due tofractionation. In this case we can calculate the minimum amounts of N_2 and Ar in the sampleusing the ratio of dissolved N_2 and Ar at atmospheric equilibrium (Holocher et al., 2003).

$$X_{N_2 Excess\ Air} = \left(X_{Ar\ S} - X_{Ar\ EQ}\right) * \frac{X_{N_2 AEQ}}{X_{Ar\ AEQ}} \qquad (4\text{-}4)$$

Where $X_{N2\ AEQ}$ and $X_{Ar\ AEQ}$ are the mole fractions of N_2 and Ar in water atatmosphericequilibrium. Uncertainty arises in determining the exact N_2/Ar ratio of excess air is estimatedusing the difference between X_{N2} Excess Air calculated in the using the above equations.

The following equation was used to determine the amount of N_2 due to denitrification.

$N_{2\ Denitrification} = N_{2\ Sample} - (N_{2\ Atm} + N_{2\ Excess\ air})$

Where $N_{2Sample}$ is the concentration of total dissolved nitrogen gas in the sample andN_{2Atm}, N_2 Excess Air,$N_{2\ Denitrification}$, are the concentrations of nitrogen in the sample attributed to atmospheric equilibrium, excess air and denitrification, respectively. Minimum and maximum amounts of excess air were used to generate $N_{2\ Denitrification}$. Final $N_{2\ Denitrification}$ was determined to be the average of these two calculations.$N_{2\ Denitrification}$ ranged from -2.250 to 7.618 ml L^{-1}.

4.4 Discussion

Spatial and temporal patterns in groundwater nitrateconcentrations are associated with a change in nitrogensource, dispersion, dilution with groundwater of low nitrateconcentrations and patchy nitrate attenuation processes suchas denitrification and dissimilatory nitrate reduction toammonium (Puckett and Cowdery, 2002; Addy et al.,2002). Denitrification or nitrate reduction has also beenattributed to the loss or decline in nitrate concentration inriparian zones, wetlands (Barton et al., 1999) and in someshallow groundwater (Spalding and Parrott, 1994; Mohamedet al., 2003). Denitrification is a microbially mediated processwhere nitrate is used as a terminal electron acceptor toproduce N_2 or N_2O (Starr and Gillham, 1993).Sophisticated research techniques, especially measurementsof isotopic (^{15}N, ^{18}O) and dissolved gas (N_2, Ar)composition are of help to provide clear evidences for theexistence of denitrification processes, the mechanisminvolved, and for quantification (Spalding and Parrott, 1994;Tesoriero et al., 2000; Mohamed et al., 2003).

4.4.1 Contribution of dilution to nitrate removal in wetland groundwater

The another major nitrate attenuation process at the catchment scale the dilution of nitrate-rich groundwater, typically recharged from agricultural land, with clean groundwater originating from low land use intensity areas (e.g. mountains, forests). This process is particularly relevant where different groundwater flow paths converge in the lowland discharge zone of the large alluvial aquifers (Roland, 2006).

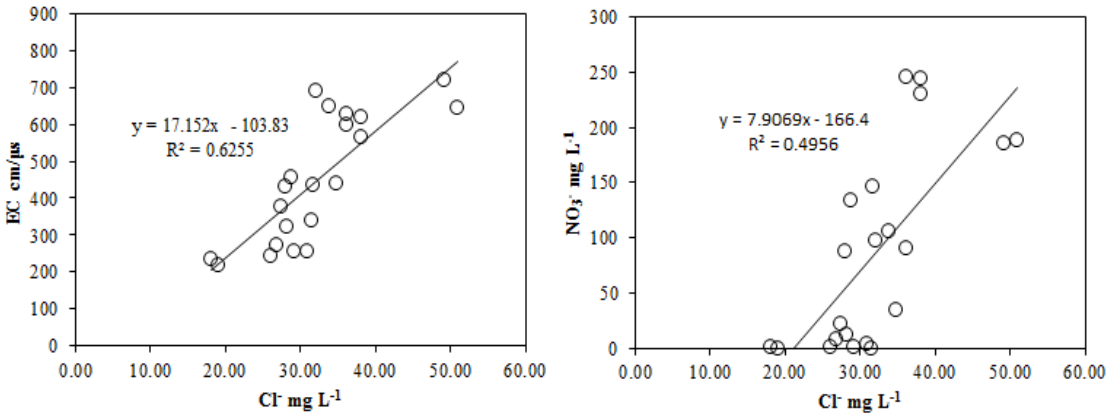

Figure 4- 4Relationship between chloride and EC, between chloride and nitrate ($p<0.01$)

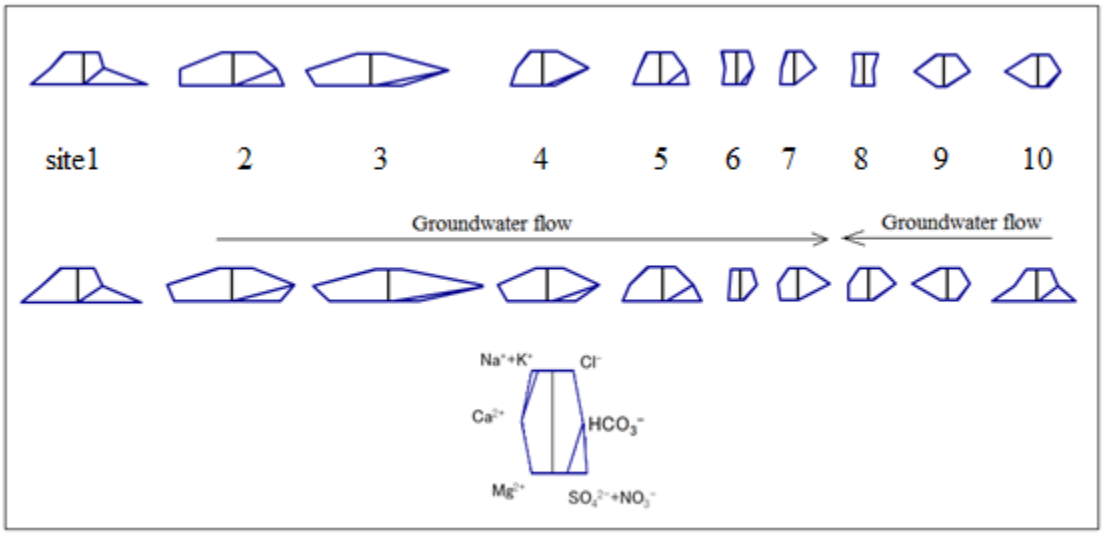

Figure 4- 5Macro ionic composition of water samples along the flow paths expressed in STIFF diagrams. Water type changed from Mg-NO_3^- at boundary to Ca-HCO^- at middle of wetland

In many situations nitrate reduction processes have been delineated from the dilution processes by tracking changes in ambient chloride to nitrate concentration ratios along flow path (Addy et al., 2002; Hefting M., 2006).Weobserved a significant decrease of

nitrate concentration and EC with decreasing chloride concentration (Figure 4- **4**). Moreover, differences in other macro ioniccompounds, expressed in STIFF diagrams (Beltman andRouwenhorst, 1991; Freeze and Cherry, 1979) indicated that there was a change in groundwatercomposition down the flow paths, most probably causedby dilution.The chloride concentrationsrevealed that the nitrate dynamics in wetland was significantly affected by dilution with upflow groundwater (Figure 4- **5**). Besides, $\delta^{15}N\text{-}NO_3^-$ value maintained a low level which indicated nitrate removal was only by dilution process at sites 1-3, and the negative value of $N_{2\ denitrification}$also that indicates no denitrification process happen.

4.4.2 Contribution of denitrification to nitrate removal in wetlandgroundwater

Subsurface environments with high concentrations oflabile organic matter and reducing conditions are likely to beparticularly significant zones for denitrification. These includeriparian zones (Haycock et al., 1993; Burt et al., 1999; Puckett,2004; Puckett and Hughes, 2005; Mayer et al., 2006; Domagalskiet al., 2008), hyporheic zones (Triska et al., 1989; Fischer et al.,2005; Pretty et al., 2006; Smith and Lerner, 2008) and aquifersaffected by infiltration of DOC-rich surface water (Roberts andMcArthur, 1998).The rate of denitrification ismost often related to the amount of dissolved organic carbon(DOC) in porewater or groundwater, or the amount of solubleorganic carbon rather than the total amount of solid fractionorganic carbon present on the geological strata.DOC levels inmost aquifers are relatively low, typically <5 mg/l DOC (Rivettet al., 2007).

Most of samples contained less than 6 mg L^{-1} dissolved organic carbon (Figure 4- 6). There is no linear relationship between DOC andNO_3^-. NO_3^- concentrations >100 mg L^{-1}

were only found ingroundwater that contained <6 mg L^{-1}, whereas low concentrations of NO_3^- were found in groundwater that contained wide range of DOC (1.26-21.21 mg L^{-1}). It indicates that DOC in groundwater is not the limited factor for denitrification in this study wetland.

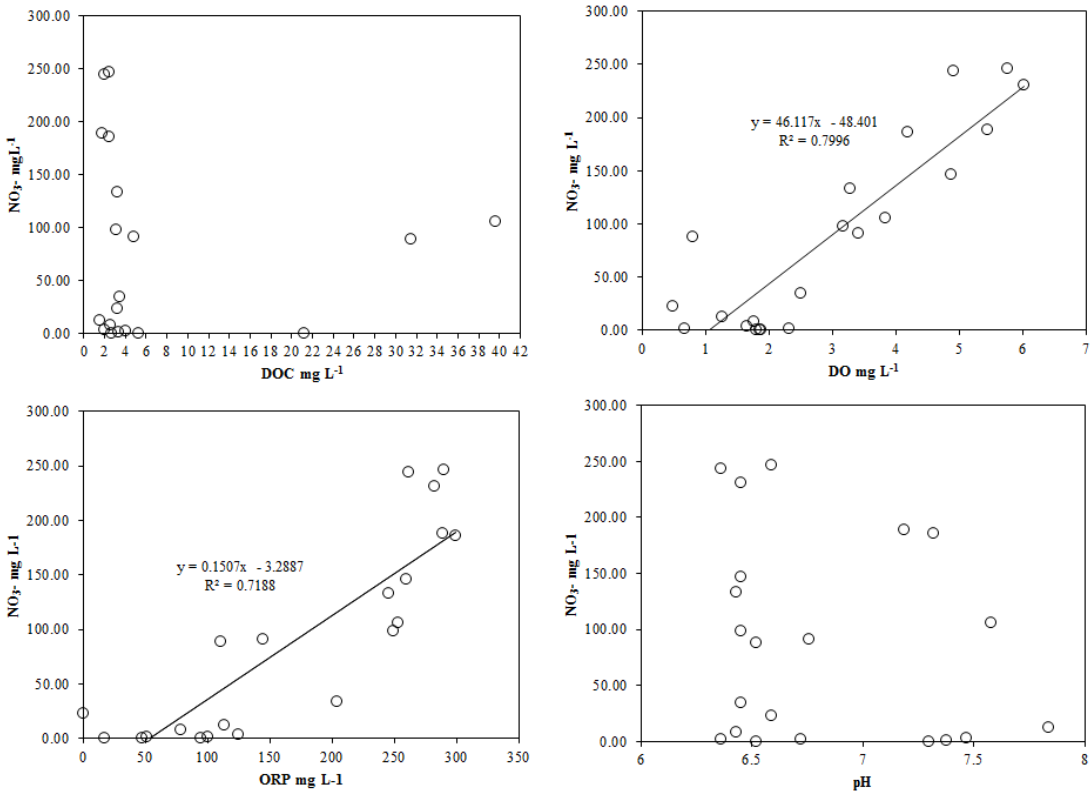

Figure 4- 6Relationship between DOC, DO, ORP, pH and NO_3^- of groundwater in wetland

The denitrification process is thermodynamically lessfavorable than the reduction of dissolved oxygen. Ina system that contains oxygen, nitrate and organic carbon, theoxygen will normally be the preferred electron acceptormeaning that denitrification can be considered as a predominantlyanaerobic process.There is little consensus but it seemsreasonable to assume that, given all other prerequisites,denitrification will probably occur at dissolved oxygenconcentrations below 1 mg L^{-1} and perhaps below 2mg L^{-1}.In

cases where they have been quantified (e.g. DeSimone andHowes, 1998), denitrification rates tended to be greater inregions of lowest oxygen concentration.

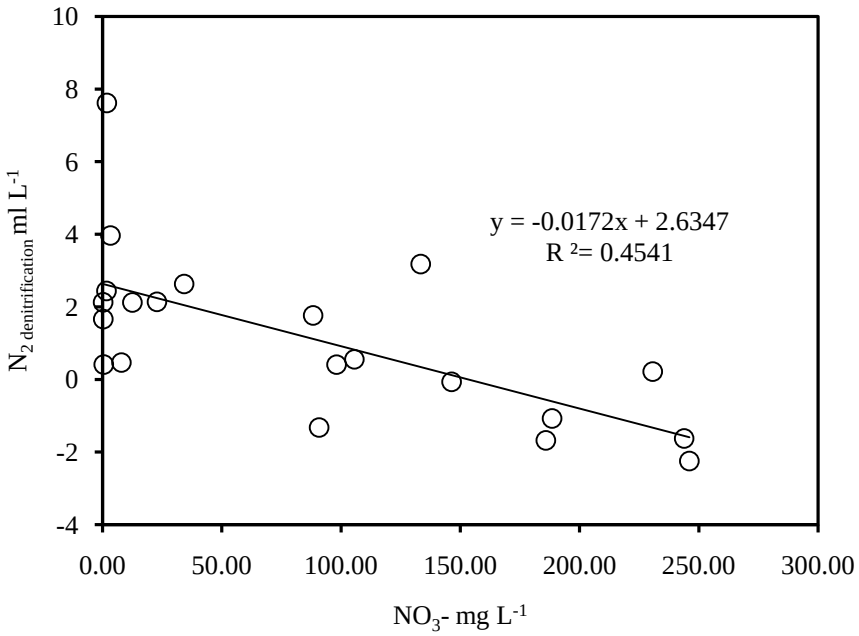

Figure 4-7Relationship between $N_{2\ denitrification}$ and NO_3^- of groundwater in wetland

A positive relationship (r =0.8942, P<0.01) occurred between NO_3^- and DO (Figure 4- 6). NO_3^-concentrations decreased below 22.8 mg L^{-1}as the DO concentrations below 2 mg L^{-1}. Besides,Balderston and Sieburth (1976) suggested a means of monitoring denitrificationinvolving ORP.Sequential removal and reduction ofoxygen, nitrate and nitrite result in sequential decrease of ORP in the media (Sille ́n,1965; Breck, 1974).Apositive relationship between ORP and NO_3^- (r = 0.8478, P<0.01) also showed in this study. NO_3^-concentrations were lower than 22.8 mg L^{-1} as ORP were below 125 mV. Most of NO_3^--N concentrations were > 20 mg L^{-1} when ORP higher than 150 mV. There was no linear relationship between pH and nitrate that unexpected. A positive relationship (r = 0.6739, p < 0.01) is between $N_{2\ denitrification}$ and NO_3^-, indicating the removed nitrate would be reduced to N_2.

Along the groundwater left flow path, DO was reduced by bacteria with the concentrations decreased from 5.77 to 3.18 mg L^{-1} which also resulted in the decrease of ORP (from 290 to 249 mV) from site 1 to site 3. Even so, the DO concentrations were not conducive for denitrification. Values of $\delta^{15}N\text{-}NO_3^-$ were not having a trend of enriched by denitrification from site 1 to site 3. As a result the decrease of nitrate was not contributed by denitrification, whereas the decrease of Cl^-concentrations which indicated the decrease of nitrate was resulted from dilution from site 1 to site 3. From site 3-5, DO concentration was not lower than 3 mg L^{-1} with little change (except A5-2m), and ORP value also was above 100 mV. However, the $\delta^{15}N\text{-}NO_3^-$ and $N_{2\ denitrification}$ data identified the denitrification process. From site 5 to 6, DO concentrations decreased to 0.49 mg L^{-1} and ORP decreased to 0 mV which were conducive for denitrification, and NO_3^--Ndecreased from 133.4 to 1.8 mg L^{-1}. In addition, Cl^- concentrations changed little. Thus, we could conclude that denitrification process control the nitrate removal from site 5 to 6. Nitrate continued decrease to extremely low (0.1 mg L^{-1}) contributing by denitrification and dilution in reduction environment from site 6 to site7. Along the right flow path, chloride concentration did not change significantly that indicated the dilution process may not the major process. $\delta^{15}N\text{-}NO_3^-$ distribution could tell that denitrification process dominate the nitrate removal.

4.5 Conclusions

Adetailed understanding of the flow system in wetland is therefore necessary to assess nitrate removal. Nitrate was totally removed along the flow path, although the nitrate load of inflow were high (>140 mg L^{-1}) in this wetland. The junction area (between slope and wetland) played an important role in diluting the shallow nitrate-loaded

groundwaterbased on data of $\delta^{15}N\text{-}NO_3^-$ values and chloride concentrations. If this physical process is not taken into account, it leads to an over-estimation of the nitrate removal capacity in these studies sites.

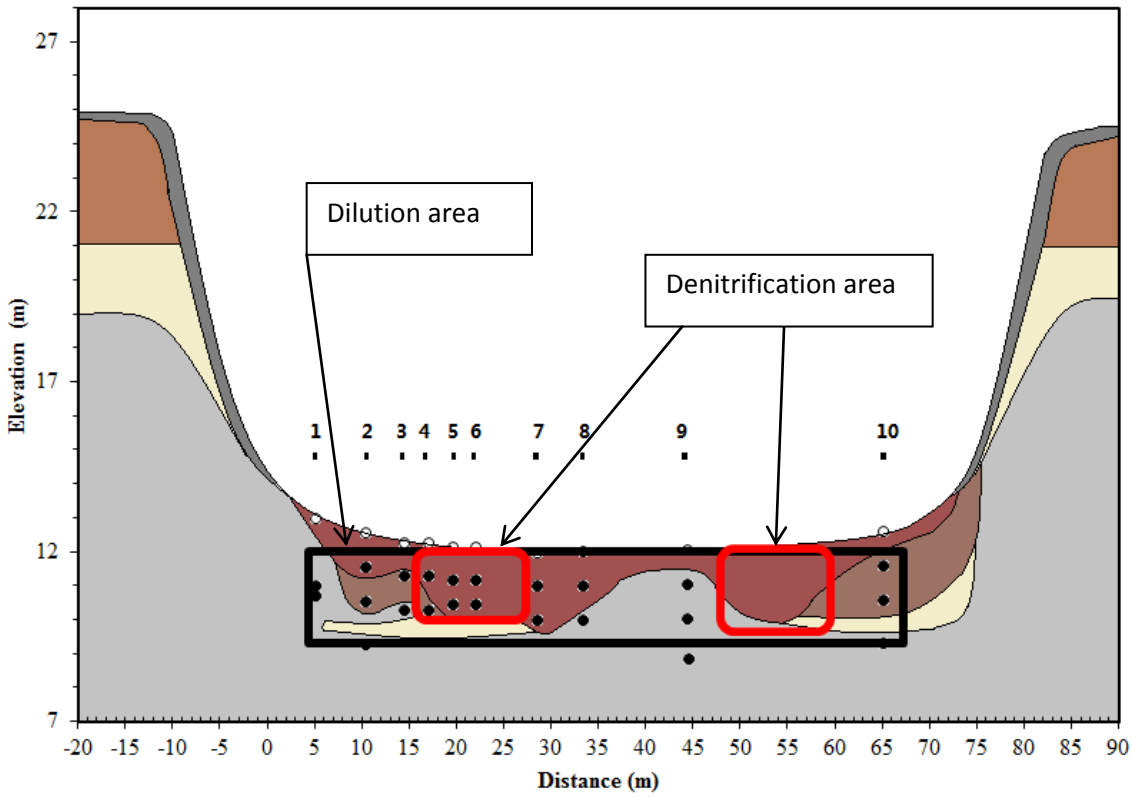

Figure 4-8 The area of dilution and denitrification in wetland. Dotted line shows dilution area and solid line shows denitrification area.

There is no linear relationship between DOC and NO_3^- and low concentrations of NO_3^- were found in groundwater that contained wide range of DOC (1.26-21.21 mg L^{-1}) which indicated DOC of groundwater was not the limited factor for denitrification in this study wetland. Nitrate decreased with decrease of DO, increase of $\delta^{15}N\text{-}NO_3^-$ and N_2 $_{denitrification}$and result of ORP decreased along the flow path. Therefore, besides this dilution effect, biological removalprocesses also significantly reduced the nitrate concentration of the shallow groundwaterin wetland. DO, ORP, and $\delta^{15}N\text{-}NO_3^-$ values are

appropriate to predict the content of nitrate because of the high value of r (> 0.8) from linear regression. The area of dilution and dentrification in this wetland were shown in figure 4-8.A change in groundwatercomposition and water type (from Mg-NO_3^- type to Ca-HCO_3^-) down the flow paths also were causedby dilution and denitrification of groundwater in this wetland. The wetland reduces leaching of nitrate inagricultural are still to be preferred to protect aquaticecosystems from eutrophication.

Chapter 5 Dynamics of dissolved nitrous oxide in wetland basin

5.1 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 et al., 2009; Groffma et al., 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 et al, 2003; Burgin and Hamilton, 2007; Whitmire and 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 N_2O is an obligate intermediate product of biological denitrification and known to contribute to global warming and the destruction of stratospheric ozone. A significant amount of N_2O emissions originates denitrification (Mathieu et al., 2006). Emissions from aquifers are most likely to occur from shallow aquifers, where N_2O can be quickly transferred through the unsaturated zone to the atmosphere by diffusion (Rice & Rogers, 1993). N_2O emission from wetland system has been estimated by numerous studies (Dhondt et al, 2004; Groffman et al., 2000; Verhoeven et al., 2006).

Level of dissolved N_2O in groundwater has been paid lots of attentions. For example, N_2O 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 et al, 1993), with the maximum up to 30000 times of that in the

ambient air (Heincke and Kaupenjohann, 1999). However, few studies estimated level of dissolved N_2O in wetland groundwater.

Understanding the spatial and seasonal pattern of dissolved N_2O is essential to assess the indirect emission of N_2O from groundwater (Geistlinger et al., 2010). According literatural review, study of spatial pattern of dissolved N_2O has been focused on surface water, such as river, lake and ocean (Butler et al., 1989; Ferrón et al, 2010; Hinshaw and Dahlgren, 2013; Wang et al., 2009; Zhang et al., 2010). Attention should be paid to spatial pattern of dissolved N_2O because of the high potential of nitrate removal in wetland groundwater. The pattern of seasonal and spatial of dissolved N_2O is consistent with 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" (Dividson et al., 1990). However, Kim(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 without a clear understanding. 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 and Hiscock, 1998; Spalding and 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 $\delta^{15}N\text{-}NO_3^-$, 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 is 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.

5.2 Sites description and Method

5.2.1 Site description

The study area is a headwater wetland, located at Ichikawa City (35.76^{o}N, 139.97^{o}E), Chiba Prefecture, Japan (Figure 1). The wetland valley is U-shaped with an elevation of about 16m above sea level. The wetland receives discharge (both groundwater and overland flow) from an adjancent upland (elevation 26-31m) area with vegetation consisting of mostly pear orchard. 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 flow rate of the stream all around a year is 21.7 L S^{-1} at the export of the park.Dominating vegetation in the wetland consists of Houttuynia, Calamus and Japanese pampas grass. The slope is closest to the orchard edge and decreasing towards the wetland, acting 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 1,316mm, with a maximum monthly precipitation of 226.5mmmonth^{-1} in study area. The annual average temperature is 15.6 ^{o}C witha highest temperature of 36.9^{o}C in August and a lowest temperature of -3.4 ^{o}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 1/3 of total upland area. The upland is covered by Kanto Loam about 4 m in thick. It is underlain in a sequence by Joso clay layer andNarita sand that a thick fine sand layer which is the major aquifer in the study area. The water table was about 20 m above sea level at upland. Within the wetland, the aquifer is a fine sand layer overlaid by cohesive soil and sandy clay with the water table depth > 15.6 m above sea level.

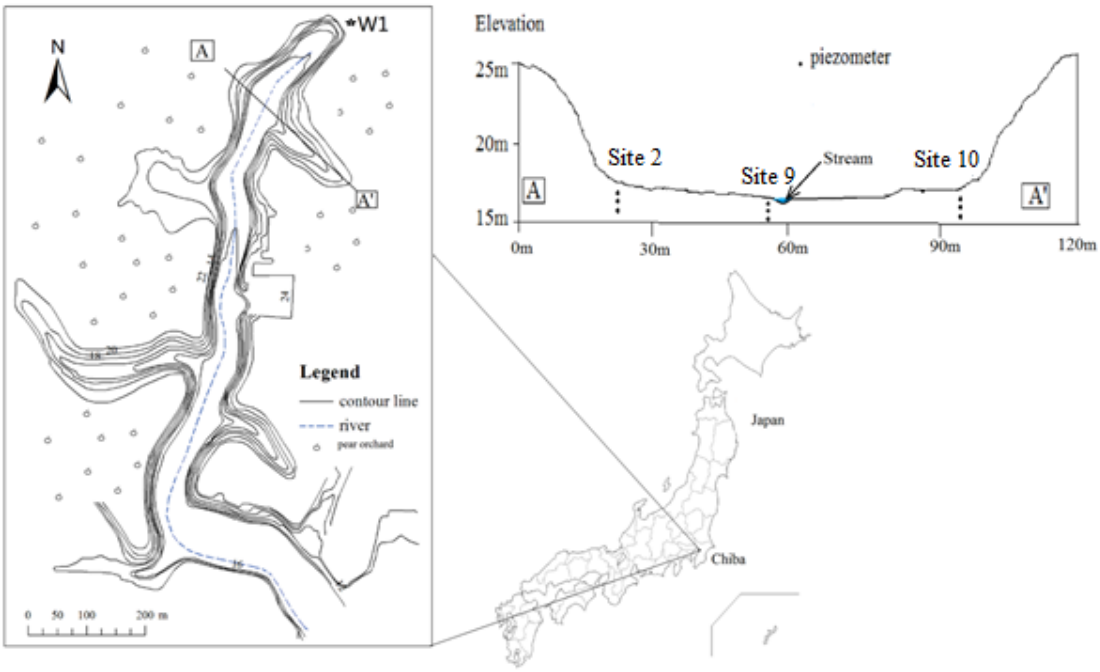

Figure 5-1Study sites in Ichikawa, Japan. Site 2, 9 and 10constitute transect A within the wetland and W1 is located in the upland site

5.2.2 Sampling procedures and measurements

Field surveys were conducted in May, July, September and November in 2011,March and June in 2012. We assumed that the June, July and September as warm season and March, May and November as cool season. Water samples were taken from well W1 at the

upland and piezometers were installed at site 2, 9 and 10 with depths of 1 m, 2 m and 3 m in the wetland, for a total of 9 piezometers (Figure 5-1). Site 2 and 10 are approximately 30 m outside the stream edge and site 9was placed approximately 0.3 m on the west side of the stream (NS). In order to get the fresh groundwater, we withdraw 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 5- **2**). 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 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 rubber stopper under the water in the sampler as soon as possible. 1ml hibitane was injected into the vial (35 ml) after water collection. Dissolved N_2O-N concentration was determined by headspace method. 10ml pure N_2 gas was injected into the vials to push out an equal volume of water from the vial. Vials were shaken for 1minute and stored at 40 ^oC for 24 h to equilibrate. The gas samples were analyzed for target gas (N_2O) by a gas chromatography (GC14B, Shimazu) equipped with an electron capture detector operated at 280 ^oC, injector at 100 ^oC and column at 70^oC.

After sample for gas analysis was taken, water sample for ion, parameters and $\delta^{15}N\text{-}NO_3^-$ analysis were collected by a pipe sampler (Figure 5- **2**). When the pipe is under 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 got from the top of the pipe. Samples were brought back to the

laboratory and stored at 4 ^{0}C before laboratory analysis. DO, pH, ORP, and temperature of groundwater were measured in situ with sensors (HIROBA). All water samples were filtered (0.45μm) before analyzing for major ions by ion chromatography (Shimadzu CDD-6A and CDD-10Avp). 2 L water for each sample was collected for $\delta^{15}N\text{-}NO_3^-$ analysis in March 2012 once. 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 analysisby Integra CN mass spectrometer (Pdz Europa LTD) at Chiba University, Japan(Cao et al. 2012). All the samples were measured twice and the result showed the difference between the two measurements was less than ±5 %. Then the mean of two measurements was used as the value of $\delta^{15}N\text{-}NO_3^-$ in this study.

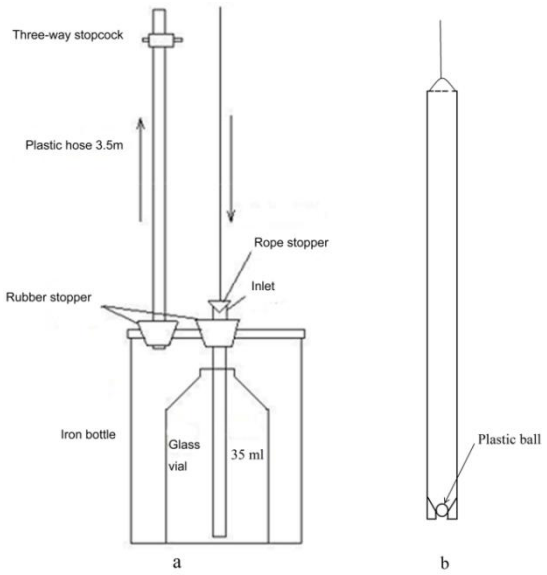

Figure 5- 2The schematic diagram of sampler for dissolved N_2O (a) and ion, parameters and $\delta^{15}N\text{-}NO_3^-$ (b)

5.2.3 Statistical analysis

Variables were tested using student t-test and principal component analysis (PCA), using SPSS 8.0 for Windows (SPSS, 1997, IL, USA). T-test can be 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).

5.3 Results

5.3.1 Basic parameters and dissolved N_2O in upland groundwater

Samples were taken from W1 in July and November 2012 twice. 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 5- 1Basic parameters and dissolved N_2O of upland shallow groundwater in July and November 2012

	N_2O-N ($\mu g\ L^{-1}$)	DO ($mg\ L^{-1}$)	ORP (mv)	NO_3^- -N(mgL^{-1})	T (^oC)	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

5.3.2 Basic parameters and dissolved N_2O in wetland groundwater

Groundwater temperatures in the wetland ranged from 14.2to 24.8 ^oC during the study period (Figure 4). pH values of groundwater ranged from 6.53 to 7.97. pHvalue indicates thatgroundwater was alkaline except site 10which pH was lower than 7 during the warm season. DO concentrations ranged from 0.07 to 11.50 mgL^{-1}. It was lower than 4 mgL^{-1}at

site 9, and as low as 0.07 mgL^{-1} in June. At site 2 and 10, the DO concentrations were lower than 5 mgL^{-1} in the warm season but up to 11.5 mgL^{-1} in the cool season (site 10-3 m in November). ORP values ranged from -244 mV to 303 mV. At 1 m and 2m depth of site 9, ORP values 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 site 9, ORP was above 0 mV except in September (-244 mV). The NO_3^--N concentration changed from 0 to 114.0 mgL^{-1} in study sites. At site 2 and 10, most NO_3^--N concentrations are clearly above the standard of the drinking water (10 mgL^{-1}) set by United States Environment Protection Agency (Figure 5-3), whereas NO_3^--N concentration was extremely low for detection at site 9. NH_4^+ and NO_2^- were undetectable at all the sites.

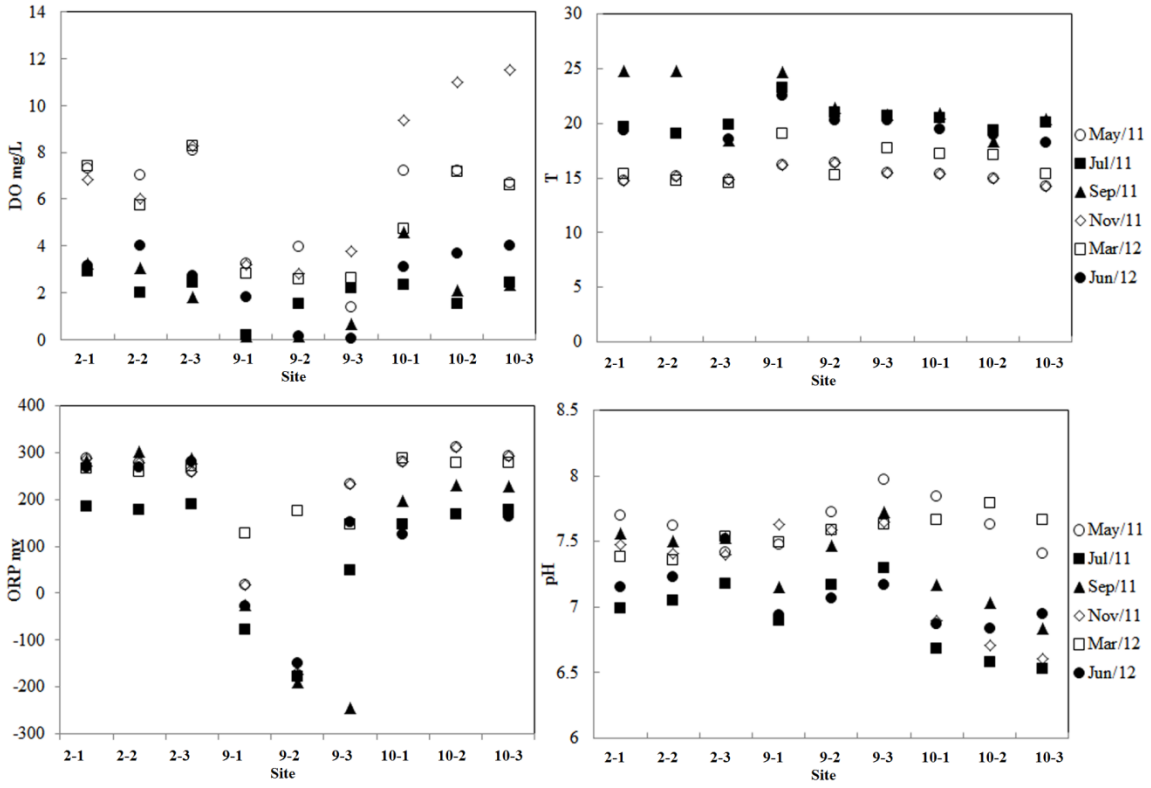

Figure 5-3pH DO ORP and T of the groundwater at site 2, 9 and 10 in 1m, 2m and 3m depth. (Open cycles: the cool season; Closed cycles: the warm season)

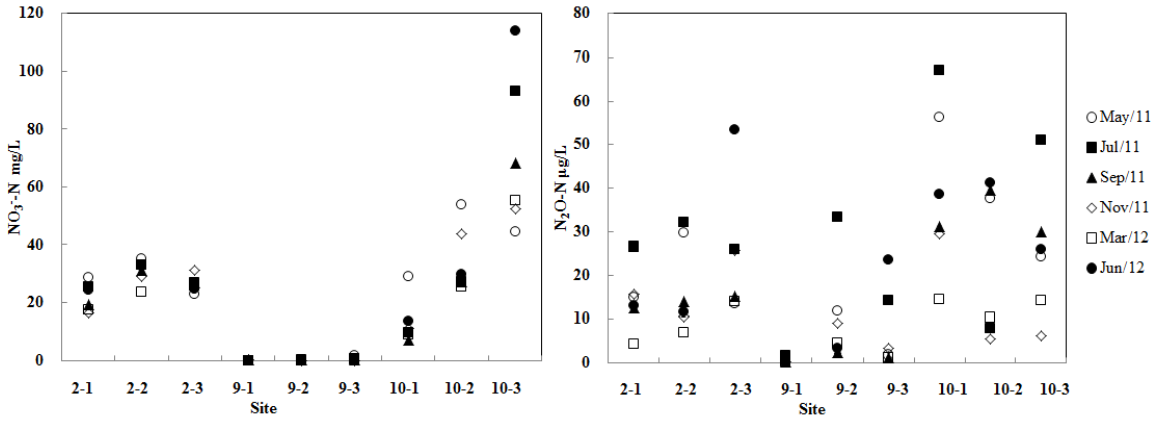

Figure 5-4Variations of NO_3^--N and N_2O-Nat site 2, 9 and 10 in 1m,2m and 3m depth(Open cycles: the cool season; Closed cycles: the warm season)

Dissolved N_2O concentrations ranged from 0.09 to 100.62 μgL^{-1} (Figure 5-4). At site 2, dissolved N_2O ranged from 6.13 to 79.96 μgL^{-1} with the highest concentrations in July and the lowest values in March. At site 10, dissolved N_2O ranged from 7.8 to 100.62 μgL^{-1} with the highest values in July and the lowest values in November. At site 9, dissolved N_2O ranged from 0.09 to 50.16 μgL^{-1}. Those at 1 m in depth (ranged from 0.09 to 2.29 μgL^{-1})were much lower than those at 2 m and 3 m in depth of site 9(ranged from 1.41 to 50.16 μgL^{-1}).

5.3.3 Variations of $\delta^{15}N\text{-}NO_3^-$ in groundwater

$\delta^{15}N\text{-}NO_3^-$ in shallow groun5dwater of upland (W1) was 5.67‰ (Table 5-2). The $\delta^{15}N\text{-}NO_3^-$ in groundwater was 6.36 ‰ for 1m and 8.27 ‰ for 2m in depth at site 2, respectively. It was 8.7 ‰ for 1m, 9.81 ‰ for 2m and 7.67 ‰ for 3m in depth at site 10. The highest value was found in the groundwater 2m in depth at site 10and the lowest value at W1. Comparing with groundwater in the upland, $\delta^{15}N\text{-}NO_3^-$ was enriched from 0.69 ‰ to 4.14 ‰ in the wetland. However, it was undetectable at site 9because little nitrate was available.

Table 5-2$\delta^{15}N\text{-}NO_3^-$ in groundwater of wetland and upland

	W1	2-1m	2-3m	10-1m	10-2m	10-3m
$\delta^{15}N\text{-}NO_3^-$	5.67‰	6.36‰	8.27‰	8.7‰	9.81‰	7.67‰

5.3.4 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 3). 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 5- 3Loadings for two principal components of groundwater variables in wetland

Variable	Component 1 (Rotated)	Component 2 (Rotated)
N_2O-N	0.089	0.735
DO	0.898	0.063
T	-0.859	0.231
ORP	0.758	0.338
NO_3^--N	0.426	0.720
pH	0.294	-0.748
Variance explained,% of total	39.894	29.845

The scores represent the influence of the component on the groundwater (Figure 5- **5**). It is possible to group the samples according to the axes of component 1 and component 2. As a result, the samples are classified into four groups to showing seasonal and spatial patterns. The samples of area near the stream(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 from thearea that adjacent slope and wetland (ASW) are plotted at the upper-left of the diagram for the warm season and the upper-right for the cool season.

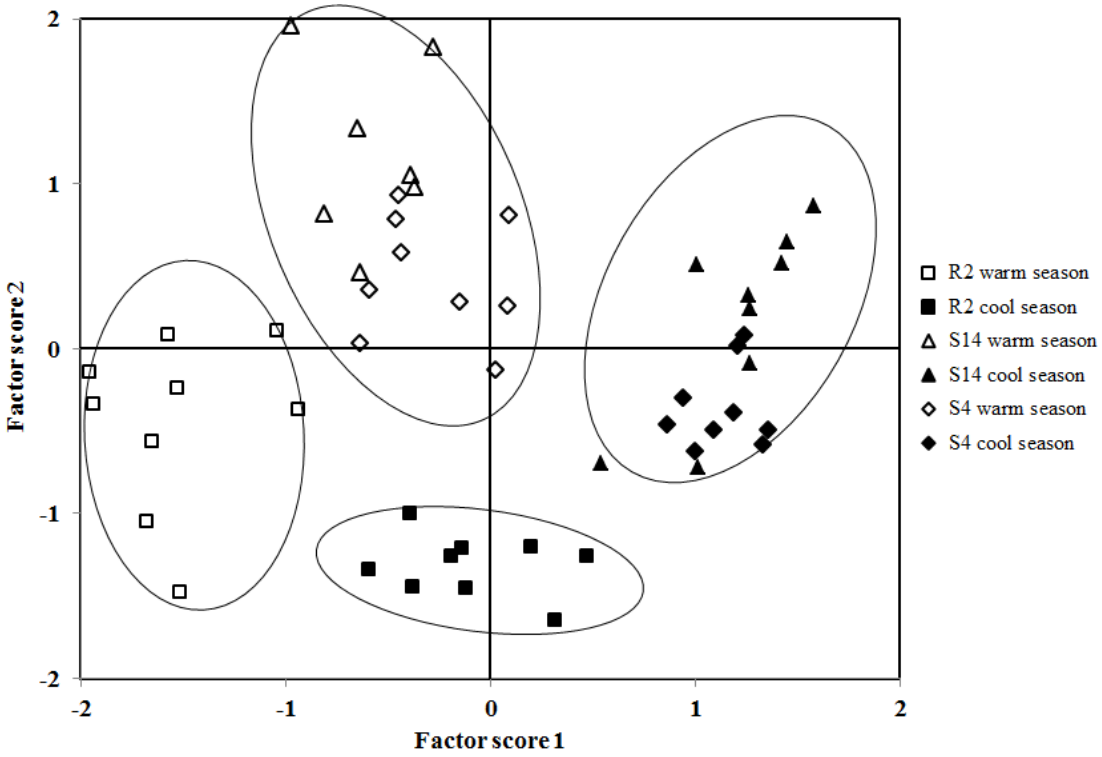

Figure 5- 5Bi-plot of the factor scores of the two principal components

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 5-4). 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 μgL^{-1}), which was about 60 times of that in the ambient air. N_2O-N and T in groundwater were significantly higher inwarm 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 5-4Mean 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 (μg/L)	DO (mg/L)	Temperature (^{0}C)	ORP (mV)	NO_3^--N (mg/L)	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 ($p<0.05$)

5.4. Discussions

5.4.1 Source of dissolved N_2O in 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 leached to pollute the groundwater from unsaturated zone $\delta^{15}N\text{-}NO_3^-$ value of W1 is coincided with range of $\delta^{15}N\text{-}NO_3^-$ (+4.5 ‰ to +8.5 ‰) in the area effected by mineral fertilizer(Heaton

1986; Cao et al. 1991; Choi et al. 2003; Singleton et al. 2007; Choi et al.), indicating the dissolved N_2O was produced via nitrification in the unsaturated zone of upland. DO concentrations were high at W1also indicated 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 due to the groundwater in upland is often assumed to have low biological activity due to low C content(Groffman et al. 1998).Geistlinger et al. (2010) found there will be a diffusive N_2O flux from the deeper water to the capillary fringe. Thus, diffusive loss is considered to be little affecting on N_2O concentration in the groundwater moving from upland to wetland.

At the wetland, denitrification can enrich ^{15}N in the residual nitrate of groundwater (Cey et al. 1999; Lehmann et al. 2003). $\delta^{15}N\text{-}NO_3^-$ in the residual nitrate enriched from 2.8 ‰ to 78.32 ‰ when the concentration of NO_3^--N decreased from 35.68 mgL^{-1} to 0.45 mgL^{-1} in a sand aquifer (Böttcher et al. 1990), and from 6.4 ‰ to 24.8 ‰ when the NO_3^--N concentration decreased from 13.3to 5.6mgL^{-1} in a riparian zone (Cey et al. 1999) . In this study wetland, the $\delta^{15}N\text{-}NO_3^-$ enriched to 9.81 ‰ or even higher when the NO_3^--N 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.

5.4.2 Spatial and seasonal pattern of dissolved N_2O in wetland groundwater

The previous section suggested that the source of dissolved N_2O of groundwater in wetland is 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 producedwhen nitrate is reduced and consumed by

reduction to N_2. Denitrification is considered to be related to many factors (DO, ORP, Temperture, 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 and in a phreatic aerobic aquifers with the DO concentration below 3.15 mgL^{-1}(Ronen et al. 1988; Deurer et al. 2008). 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 converted first to nitrite then the nitrite is converted immediately to N_2 without accumulating (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 et al. 1992). Therefore, the optimum value of DO and ORP for N_2O accumulation don't consistent with the value of the optimum for denitrification due to the N_2O is an intermediate product. For nitrate, DeSimone and Howes (1998) that kinetics of denitrification at nitrate concentrations >1 mg-N L^{-1} is zero order and even small amount of nitrate (lower than 2mg-Nkg^{-1}) leached was sufficient to create a large amount of N_2O in groundwater (Müller et al. 2004). Many studies suggested that high concentration of NO_3^--N inhibits the N_2O reductase yielding the higher concentration of N_2O(Blackmer and Bremner 1978; Deurer et al. 2008; Heisterkamp et al. 2012). At

ASW, the DO (m = 5.22 mgL^{-1}) and ORP (m = 237 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 μgL^{-1}). However, the low DO concentrations (m = 2.02 mgL^{-1}) and mean values of ORP were much lower (m = -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 et al. 2011), resulting in the lower concentration (m = 13.87 μgL^{-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 et al. 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 et al. 2002; Velthof et al. 1996; Hinshaw and Dahlgren 2013). The T-test indicate the concentrations of NO_3^--N were no significant difference between the two season, which suggests NO_3^--N is not the limited factor for denitrification rate in study wetland (Table 4). Temperature effect the dissolved N_2O directly by control the denitrification rate (Nowicki 1994; Pfenning and McMahon 1997; Saunders and Kalff 2001). The temperature below 17 ^oC, appeared to be a threshold value controlling the rate of denitrification (McCutchan and Lewis 2008; Nowicki 1994), whereas Halling-Sørensen and Jorgensen (1993) reported the threshold value was 20 ^oC. A study in coarse sandy soils found that the denitrification activity was low at 10 ^oC and completely inhibited at 2 and 5 ^oC because lower temperature may regulate metabolic rates for denitrifying bacteria (Vinther and Søeberg 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 at 0 ^oC (14.60 mg L^{-1}) is about doubles comparing with that at 30 ^oC (7.54 mg L^{-1})(Weiss 1970). Oxygen consumption by aerobic respiration increases when the temperature increases (Thamdrup et al. 1998). When the temperature increased from 15 ^oC to 25 ^oC, the average ORP decreased +40 mV to -60 mV (Zhu et al. 2002). In warm season, denitrification rate supposed to not be inhibited by temperature (m = 20.5^oC). The lower DO and ORP of groundwater could be assmumed 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 μg L^{-1}) than it in cool season (20.19 μg L^{-1}). In addition, the decrease of pH was interpreted as a sign of intense denitrification (Ilies and 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 to0.286 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).

5.5 Conclusions

N_2O concentrations, denitrification related factors (NO_3^-, DO, ORP, pH and Temperature) and $\delta^{15}N\text{-}NO_3^-$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 $\delta^{15}N\text{-}NO_3^-$ in the shallow groundwater. The mean value of dissolved N_2O-N increased from 11.42 μgL^{-1} at upland to 36.14 μgL^{-1} at the ASW and then decreased to 9.27 μgL^{-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 elevate metabolic rates for denitrifying bacteria directly, and create the lower DO and ORP environment that effects 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.

Chapter 6Spatial and seasonal change of nitrous oxide flux in wetland

6.1 Introduction

Wetland is not only the outlet of groundwater for rivers but also takes very important roles on control the chemicals flux to the watershed. Wetlands dominated by inherently wet surface soil have a high capacity to consume nitrate via denitrification that is considered the most important reaction for nitrate removal in aquifer. This process occurs in oxygen depleted layers with available electron donors (Ross et al., 1995). The soils of wetlands support the anaerobic conditions and high levels of organic matter necessary for denitrification (Cooper et al., 1990; Groffman et al.,1994).

N_2O gas is an obligate intermediate product of biological denitrification and can either be emitted to the atmosphere or further reduced to nitrogen (Well 2001). Nitrous oxide is an important greenhouse gas that contributes to global climatic warming. Soil is the major source, contributing more than 60% of the global gross N_2O emission (Partheret al.,1995; Crutzenet al., 1977). Many studies have shown the wetland and groundwater function as the nitrogen transformer for nitrate to nitrous oxide. In the shallow ground water of lowlands and riparian areas, reductive conditions are often favorable for intense denitrification processes (Rosset al.,1995). Richard and Brain (1991) confirmed the groundwater denitrifying activity by using acetylene block incubation of a relatively vertical zone (5- 6 m thick). Well (2001) suggested that significant production of nitrogen and nitrous oxide occurs in the saturated zone, resulting in accumulation of these gases in the groundwater. Weymann (2008) investigated the nitrous oxide accumulation in 4 nitrate contaminated denitrifying sand and gravel aquifers of north Germany, which demonstrated that denitrification was an important factor for nitrate variability with all

aquifers. In contrast, some studies showed that groundwater ecosystems are often assumed to have very low biological activity and there is some degassing of nitrous oxide in groundwater, but no biological production in groundwater itself (Groffmanet al., 1998). No matter the N_2O is produced by the denitrification in wetland aquifer or just the result of degassing process in groundwater, its emission from wetland surface is defined as indirect emission associated with nitrogen that is brought in by groundwater from recharge area.

Water flow in wetland controls exchange processes of oxygen, carbon and nitrate between the different parts of the wetland which affected the establishment of the denitrification activity (Johanet al., 2007). Wetland areas may have a complex arrangement of different layers, not all of them are biological active. It's crucial to ascertain whether nitrate moves through the biological active layer or not. For this reason groundwater flow path has been identified as important factor in explaining the observed variability in riparian function (Dorothyet al., 2005).

Denitrification is regulated by the availability of oxygen, nitrate and organic matter while controlling factors for N_2O emission is still not clear even in the seasonal pattern showed some trend (Inubushiet al., 2003). Some studies have reported that high nitrate removal transects can significantly contributed to increased N_2O emission from wetland. (Heftinget al., 2006)

Obviously, the heterogeneities of springs, seepage, soil, vegetation and so on control the temporal and spatial distribution of N_2O flux on wetland surface. The comprehensive understanding of the processes is still limit. Therefore, in order to study the behavior of N_2O in wetland, a typical headwater wetland has been chosen. The objectives of the

paper are 1) to estimate temporal and spatial distribution of the N_2O flux from the wetland surface and dissolved N_2O in groundwater; 2) to explain the relation between N_2O flux and dissolved N_2O along the flow path of groundwater; 3) to assess the denitrification processes by considering the nitrogen budget in the groundwater; and 4) to discuss the functions of the wetland as the 'hotspot' of nitrous oxide flux and the groundwater as its source in the watershed.

6.2 Materials and method

6.2.1 Site description

The study area is the headwater wetland (35.76^{o}N, 139.97^{o}E) in Ichikawa City, Chiba Prefecture, Japan(Figure 6- 1). It used to be paddy field about thirty years ago and has been redeveloped to a wetland park since then. The annual average temperature and precipitation are 15.6 ^{o}C and 1316mm, respectively. The maximum month precipitation in the study period was 226.5mm/month in May 2011. And the highest temperature of 31.2 ^{o}C was recorded in August. The wetland valley is U-shaped. The upland is covered by Kanto loam in about 4 meter thick. It is underlain in a sequence by Joso clay layer, Narita sand layer and a thick fine sand layer that is the major aquifer in the study area. The water table found about 8 meter in depth from the surface of the uplandatthe wetland, the aquifer is a fine sand layer overlaid by cohesive soiland sandy clay in the wetlandthe fine sand layer is the same as that in upland.

The wetland is the discharge area of the adjacent upland where pear orchard expands widely. The nitrogen load in pear orchard is estimated about 10.2kg/1000m^2/year. The slope covered by broadleaf woods, conifers and bamboos is transitional zone to link the upland and the experimental wetland.There are reeds, calamus, Japanese pampas grass

and other wetland vegetation in the wetland. 12 to 15 meters lower than the upland, the elevation of wetland is about 16m above the sea level.Ariver that is supplied by spring and groundwater flows through the wetland valley. Averagely, water table is shallower than 40cm.

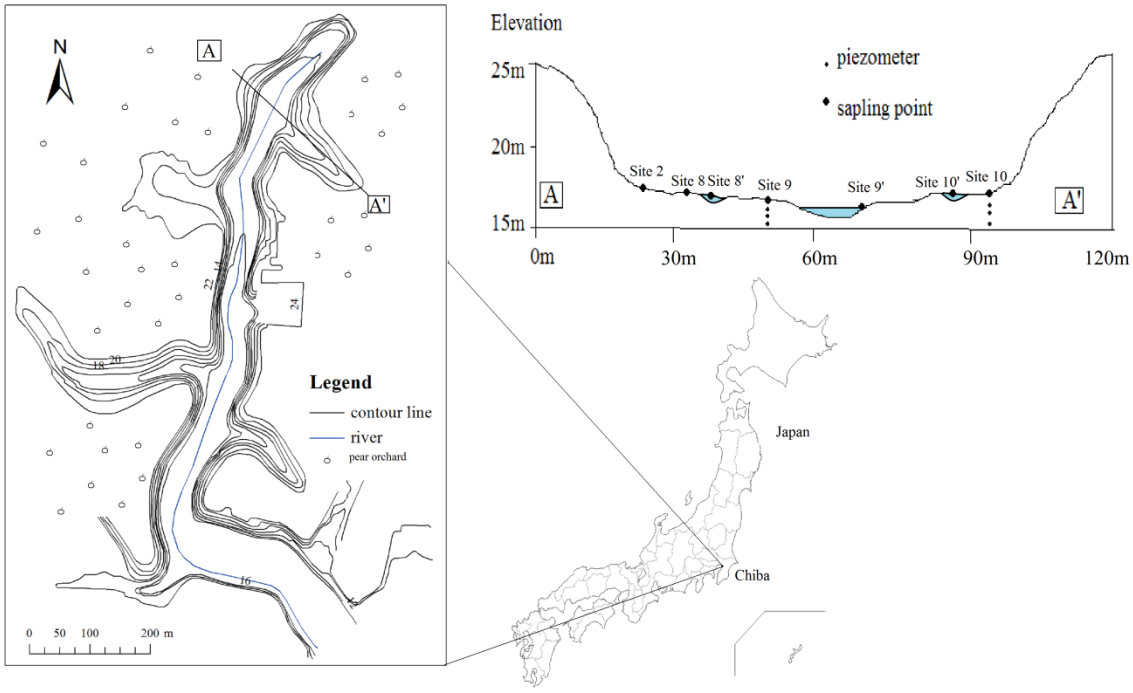

Figure 6- 1Map of the study area (left) and the sampling points along the cross section A-A' (right)

6.2.2 Measurement of NO_3^- and other chemical parameters in water

In order to measure the distributions of hydraulic head along the cross section A-A'.The seasonal changes of water levels in the piezometers have been measured once for every one or two months since May 2010 to estimate the groundwater flow path in the study area. At the same time, samples were taken from piezometer, spring and river EC, pH, ORP, and DO were measured by portable meters (D52, D54 Horiba, Japan) in situ. Samples were brought back the laboratory in Chiba University and stored at 4 ^{o}C before

analysing.All water samples were filtered (0.45μm) before analysing for major ions by ion chromatography (ShimadzuCDD-6A and CDD-10Avp, ±5-10%).

6.2.3 Measurement of nitrous oxide flux

To understand the whether the wetland is a source or sink of nitrous oxide emission, nitrous oxide flux was measured at the points along A-A' section (Fig. 1). Surface water covered the point site 8' 9' and 10' while water table was as shallow as 40cmat site 2, 8, 9 and 10. All of the sampling points were not covered by wetland vegetation. The chamber method has been used extensively for measuring gas exchange between soil surfaces and the atmosphere.

6.3 Resultsand discussion

6.3.1 Environment for N_2O migration in the wetland

Shallow groundwater emerges as springs near the base of hill slopeand flows over the wetland to from a stream in the study area. The vertical profile of hydraulic head at both site 9 and 10 indicated that groundwater flowed from the deep of aquifer upward the surface (Table 6- **1**). It was reasonable to consider that the stream was recharged continuously on the stream bed by groundwater when it flowed through thewetland. Also, it was found the hydraulic heads at site 10were high than that at site9for the same depth indicating the groundwater in the study area keeps flowing from the upland into the wetland from both sides of valley.

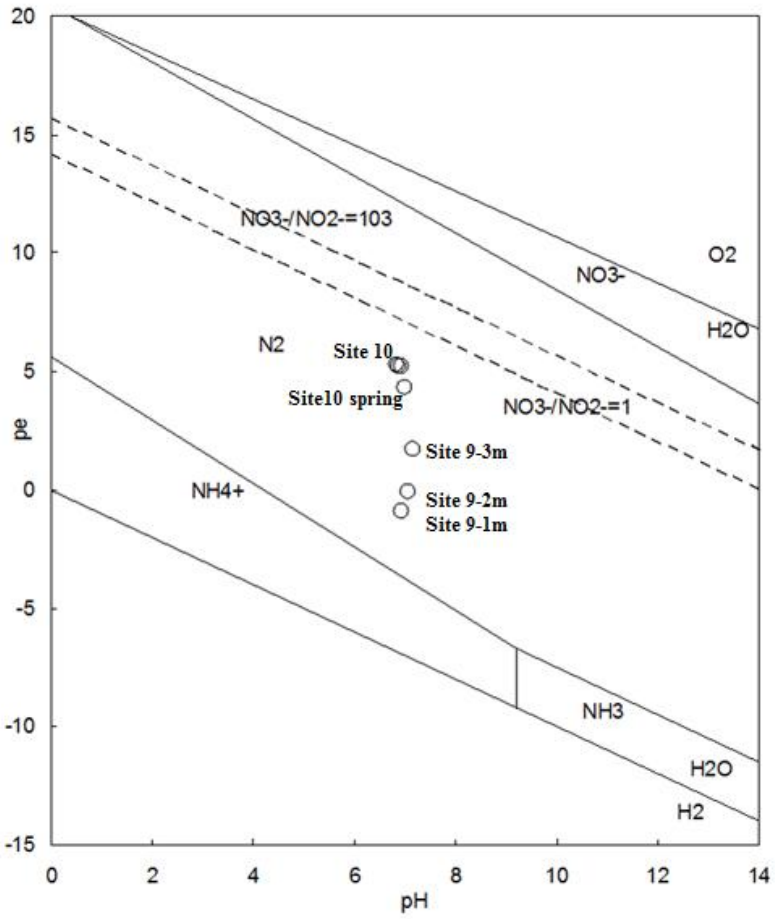

Figure 6-2pe-pH diagram for nitrogen system at 25 ^{o}C. The diagram is valid under the conditions that PN_2=0.77atm and the activities of dissolved species are 10^{-3} unless otherwise specified. Metastable boundaries are indicated by dashed lines. Eh= (2.303RT/F) pe (F is Faraday's constant)

Denitrification is central to the nitrogen cycle with respect to the groundwater environment and involves the reduction of nitrate via a chain of microbial reduction reactions (Knowleset al., 1982). It was found that the temperatures in groundwater and springs at site 9 and 10 changed from 15.9~19 ^{o}Cwith the pH values ranging from 6.84 to 7.17 (Table 6- **1**).In general, the optimum temperature for denitrification is between 25 and 35^{o}C, but it can occur normally from 2~50 ^{o}C (Michaelet al., 2008; Bradyet al.,2002),

and the pH preferred by heterotrophic denitrifiers is generally between 5.5 and 8.0 (Rustet al., 2000). The temperature and pH are appropriate for denitrification in study area. The concentration of nitrate was high at site 10with the range from 1.58 mg N L^{-1} to 53.74mgN L^{-1}, and less than 1mg N L^{-1}at site 9. Some studies indicated that kinetics of denitrification at nitrate concentrations >1mg N L^{-1} is zero order (DeSimoneet al., 1998). It can be considered that there are two different statuses for nitrate removal and nitrate was under way of denitrification at site 10but almost free at site 9.

Table 6- 1Temperature, pH, EC ORP, DO and water head of groundwater and springs

Site	Depth (m)	Temp .(℃)	pH	EC (μscm^{-1})	ORP (mV)	DO (mgL^{-1})	Hydraulic head (m)	NO_3^--N ($mg\text{-}NL^{-1}$)
9	1	19	6.94	230	-53	3.23	16.81	0.18
	2	18	7.07	227	-6	3.95	17.92	0.01
	3	16.8	7.17	239	97	1.41	19.00	0.12
10	0(spring)	15.9	7	163.7	249	3.81	16	1.58
	1	16.9	6.87	389	302	7.2	16.87	28.90
	2	16.4	6.84	475	303	7.2	17.91	53.74
	3	17.7	6.95	521	303	6.69	19.44	44.38

Nitrogen occurs in various oxidation states from nitrate to ammonia. The relationships between species containing oxidized and reduced members of redox couples are commonly displayed on diagrams know as pe-pH diagrams. The widespread occurrence of denitrification in wetlands requires a close look at redox transformations of nitrogen in groundwater. Figure 6-2shows stability diagram for aqueous nitrogen species as functions of pe and pH in the nitrogen system at 25^oC. The measurement data at site 9 and site 10were also plotted in the diagram (Appeloet al., 1994). It was found that all samples in the study area fell into the zone available for denitrification. Also, the vertical distribution of pe at site 9decreased from 1.68 to -0.91 when the groundwater moved up in the aquifer

from depth of 3m to 1m. Furthermore, dissolved oxygen at site 9(1.41-3.95mg L^{-1}) were lower than at site 10(6.69-7.2mg L^{-1}) indicated DO was lost in the wetland aquifer. As a result, nitrate functioned as an electron acceptor and was reduced to $N_2$and/or N_2Oin the process of denitrification. At the same time, transformations between species were almost exclusively facilitated by micro-organisms. Thus, the equilibrium relationships implied by the pe-pH diagram suggested to be controlled by microbial kinetic factors. As a whole, the study wetland has the potential to be a hotspot of denitrification.

6.3.2 Groundwater as the source and carrier of N_2O

The potential for N_2O production exists in subsoil, groundwater, streams, rivers, and estuaries (Cloughet al., 2005). As N moves through and leaves agricultural systems, predominantly as nitrate from the soils to the groundwater in recharge area. NO_3^- can be denitrified in the groundwater and N_2O is produced as an intermediate product. Some studies reported the maximal concentration of dissolved N_2O is of up to 3 orders higher than the ambient atmosphere (Heinckeet al., 1999). As a result thedissolved N_2O in the groundwater can reach the atmosphere either by upward diffusion to the unsaturated zone (vertical path) or by convective flow to streams, springs and wells (horizontal path) (Deureret al., 2007). It is reported that N_2O accumulates at the interface between the saturated and unsaturated zone in some studies (Ronenet al., 1988; Spaldinget al., 1994; Wellet al., 2005; Deureret al., 2007).The more N_2O dissolves in the groundwater, the more probable for it to transfer into the unsaturated zone.

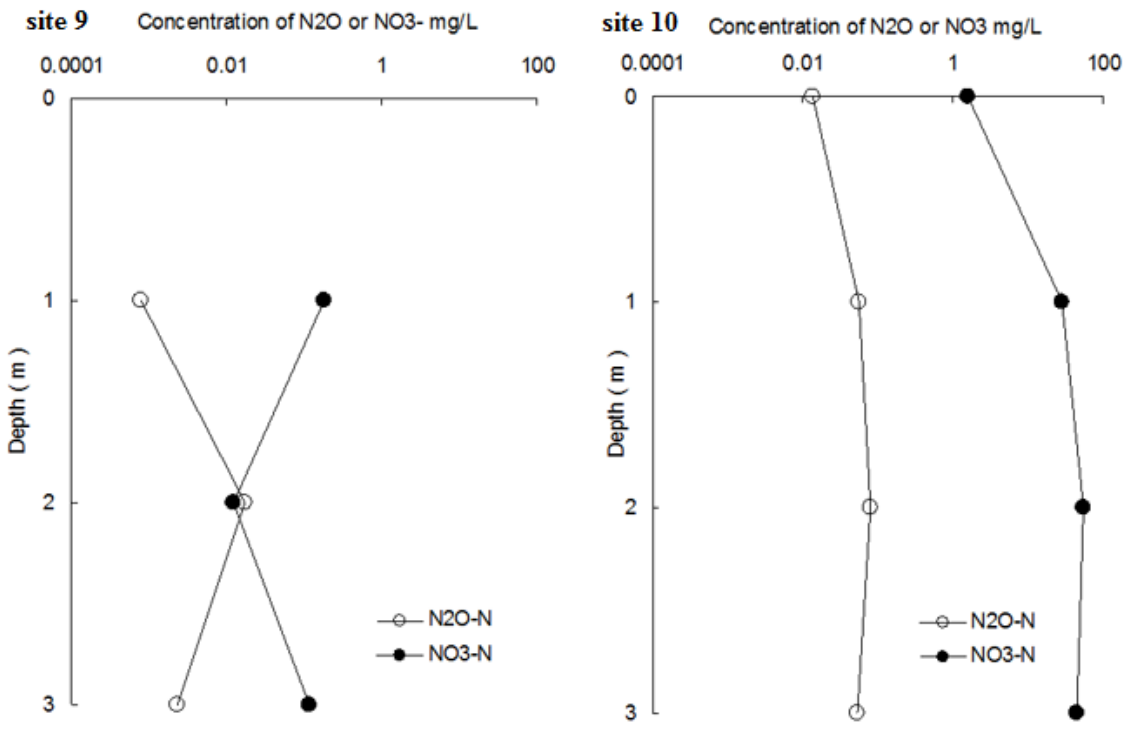

Figure 6-3Vertical distributions of NO_3^--N and N_2O-N concentrations in the groundwater and spring at site 9 and 10

The concentration of dissolved N_2O ranged from 0.78 to 16.8μg N L^{-1} atsite 9, and from 13.2 to 80.5μg N L^{-1}atsite 10, respectively (Figure 6-3). The nitrate concentrations were high in the groundwater and spring water before it flowed into the study wetland. However, the concentration of dissolved N_2O and nitrate were extremely low atsite 9. The one possible reason may be the most nitrates in the groundwater has transformed into N_2because of the low concentration of oxygen and ORP.

The peak of dissolved N_2O appeared at 2m depth from the surface. There was a gradient of N_2O concentration between 2m and 1m in depth. Obviously, the dissolved N_2O values were much higher than background value of atmosphere (around 0.65μg N L^{-1}). Also there is an upward gradient of dissolved N_2O concentration between depth 2m and

1mindicated that N_2O can diffuse from 2m to 1m in groundwater. Thus, N_2O accumulation in the groundwater is one of important sources for its flux on the surface.

The dissolved N_2O was the most important to understand the release of N_2O in the wetland. Accordingly, there were three kinds of the dissolved N_2O sources, groundwater, seepage/spring and river, associated with contributions of hydrodynamics to the N_2O flux on the wetland surface. Therefore, the spatial and temporal changes of the N_2O flux in the study area depended on the N_2O source available.

6.3.3 Temporal and spatial variation of N_2O flux at the surface

Wetland could be a source of greenhouse gases emission because it contains large amounts of soil carbon and, nitrogen inflow with the suitable reducing condition. N_2O fluxes were significant differentat points along the cross section A-A' withthe average values ranging from 0.013 to 0.285 mg N $m^{-2}h^{-1}$ (Figure 6- **4**). Most of these fluxes values were positiveand indicated that this wetland was a source rather than a sink for nitrous oxide.N_2O fluxes was lower than 0.045 mg N $m^{-2}h^{-1}$ in the wetland except point site 9, because of almost all the nitrate in the groundwater has been denitrified in the wetland. site 9was 50 cm away from the river in which dissolved N_2O concentration was 3.1μg N L^{-1}, about four times of that in the groundwater of 1 meter depth piezometer at the same site. As a result, the N_2O flux at site 9was contributed by both the groundwater and the river. It was found that the dissolved N_2O and N_2O flux at site 2and site 10were higher than that in other points of the wetland. Because of the contributions of high dissolved N_2O concentration from the groundwater and seepage, the average N_2O fluxes at site 2 and site 10were 0.089 and 0.285 mg N $m^{-2}h^{-1}$, respectively.The water table could reach the surface to mix with the surface water at site 8'and site 9'. The mixing water

with lessdissolved N_2O covered the surface and made low N_2O fluxes. Therefore, it is expected that the concentration of dissolved N_2O near the water table is the main factor to controlthe spatial variability of N_2O emission in the study area.

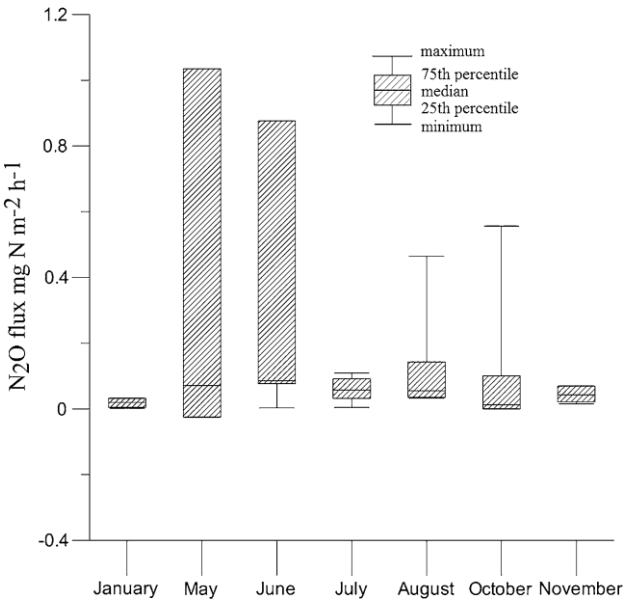

Figure 6- 4Box-Whisker plot for the seasonal changes of N_2O flux

The average monthly N_2O flux ranged from 0.019 to 0.286 mg N $m^{-2}h^{-1}$ with the peak values in May (Figure 6- **5**),whichwas similar to the results of other studies (Wanget al., 2005). The spatial variations of N_2O flux were large in May with the maximum value of 1.037 mg N $m^{-2}h^{-1}$ and the minimum -0.025 mg N $m^{-2}h^{-1}$. It became an half in August and October, and one tenth in July and November, respectively. The lowest flux appeared in December and January whenthe activity of denitrifying bacteria was inhibited because of low temperature.

The solubility of N_2O depends on temperature. For example, the N_2O solubility at 0 ^oC is about doublescomparing with that at 19 ^oC (Heinckeet al., 1999). Furthermore, gas diffusion coefficient increases with temperature at the rate about 7% per ^oC (Cloughet

al.,2005). Water table has an important effect on spatial variations of N_2O fluxes either (Zhuet al., 2008). The short-term lowering of water table in laboratory also has increased N_2O release from peat soils (Reginaet al., 1998; Dowricket al., 1999). Production of N_2O will be increased as the increasing of soil water content (Wang 2005). Water table didn't change obviously in the experimental wetland. Soil water can function in two ways to promote denitrification in the wetland. One is the control of temperature and other is the control of the path for emitting N_2O to the atmosphere. In the observation period, high precipitation increases the content of soil water in study area since the raining season from May, which also increases the temperature in the soil layer of the water table. Increase of temperature let solubility of N_2O low and enhance it to release from water. On the other hand, high soil water content could enhance anaerobic denitrification, because the path for air in the soil is sealed. As the increasing of soil water content and temperature, highly soluble N_2O is denitrified to N_2 before moving to the atmosphere. As a result, the flux in June and July has a decrease trend, and increase a little in August when thetemperature is highest in the year. Therefore, seasonal changes of N_2O emission in this wetland attribute to the variability of ground temperature and soil moisture.

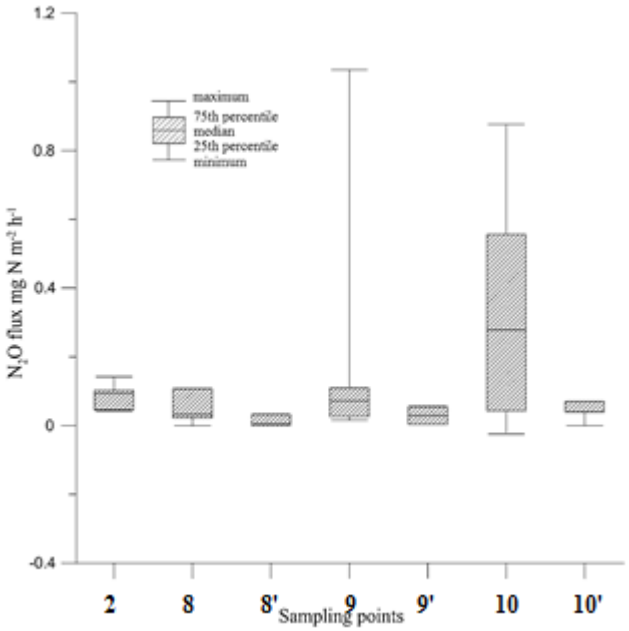

Figure 6- 5Box-Whisker plot for the N_2O flux at the surface along the Section A-A

6.3.4 Budget of nitrogen removal from the wetland groundwater

When nitrate traveled through the headwater wetland, the redox potential decreased with the loss of dissolved oxygen in groundwater. As a result, nitrate functions as an electron acceptor and is reduced to N_2 in the process of denitrification. At the same time, transformations between species are almost exclusively facilitated by micro-organisms. Thus, the equilibrium relationships must be controlled by microbial kinetic factors.

High concentration of N_2O dissolved in the groundwater and the difference of nitrate concentration between site 9 and 10 indicate that the denitrification process has occurred in the wetland aquifer. The produced N_2O can be dissolved and accumulate in groundwater when gaseous diffusion restricted in natural wetland. As a result, groundwater becomes an important carrier to transport N_2O from aquifer to the surface where it releases to the atmosphere in the wetland.

Table 6- 2Percentage of NO_3^--N, N_2O-N and N_2-N in the groundwater

Site	Depth (m)	N_2-N (%)	NO_3^--N (%)	N_2O-N (%)
9	1	-	-	-
	2	75.4	10.3	14.3
	3	0.0	98.0	2.0
10	0 (spring)	86.2	13.7	0.1
	1	70.0	29.8	0.2
	2	53.4	46.5	0.1
	3	0.0	99.9	0.1

Base on the data in the study area, the denitrification area was identified and the budget of nitrogen in wetland was estimated as equation (6). It was found that NO_3^-, N_2O and N_2should be the main species of nitrogen in the groundwater. Along the groundwater flow path, it is assumed that the total nitrogen (the sum of NO_3, $N_2O$and N_2)in the groundwater can be conserved when the groundwater moves from point j to j+1.

$$W_{N_2O,j+1} + W_{N_2,j+1} + W_{NO_3^-,j+1} = W_{N_2O,j} + W_{N_2,j} + W_{NO_3^-,j} \quad (6)$$

Supposed that the total nitrogenat 3m in depth (site 9 and 10) be the nitrogen brought in the wetlandby groundwater, nitrogen budget was calculated for each site (Table 6- **2**). It was found that percentage of nitrate decreaseswith the increase of dissolved nitrogen gas when groundwater flowsupward through the aquifer, N_2O and N_2 gases should be considered to release from the surface of wetland because of denitrification. Accordingly, more N_2O releases at site 9 and 10, because of high concentration of nitrate in the groundwater. However, the percentage of dissolved $N_2O$is small as an obligate intermediate product of biological denitrification in the study wetland.

6.4 Conclusions

The analyses of groundwater flow system in the wetland indicated that groundwater flowed from upland toward the wetland as springs at the base of valley slopes and seepage at the wetland surface because of the upward flow in the wetland aquifer. In spite

of high concentration of nitrate in the springs and groundwater before they flowed into the wetland, nitrate decreased sharply in the wetland where denitrification occurred. It was found that highly NO_3^- enriched groundwater was interacting with the biologically active zones of wetland aquifer resulting in production of amounts of N_2O that could be much higher than that in atmosphere. The concentration of dissolved N_2O ranged from 0.78 to 16.8µg N L^{-1}atsite 9where nitrate concentration was very low, and from 13.2 to 80.5µg NL^{-1}atsite 10where denitrification was dominant, respectively. As a result, the headwater wetland is the hotspot not only for denitrification, but also for the N_2O emission.

N_2O accumulation in the groundwater was the source for its flux on the surface. The average flux varied from 0.013to 0.285 mg N $m^{-2}h^{-1}$ indicating that this wetland was an emission source of nitrous oxide. The temporal and spatial variation of N_2O flux in the wetland surface was controlled by concentration of dissolved N_2O near the water table, precipitation and temperature in the study area. Base on the data in the study area, the budget of nitrogen in wetland was calculatedalong the path of groundwater indicating both of N_2O and N_2 gases release from the wetland because of denitrification occurring in groundwater.

Chapter 7 Nitrogen budget of headwater wetland

7.1 Introduction

Small valley bottom wetlands are a common element of headwater drainage basins in humid landscapes. The location of these wetlands within the drainage basin makes them potentially important in modifying the chemistry of water fluxes between upland areas and streams. Hydrologic linkages between wetlands and the surrounding watershed, as well as pathways of water movement through the wetland influence the ability of these systems to regulate nutrient fluxes (Cooperet al., 1990; Hillet al., 1990b). N budgets have been used as a quantitative means of assessing system N use for over 100 years (Lawes et al., 1882), the approach is still in common use today (Barry et al., 1993; Breembroek et al., 1996; Meisinger and Randall, 1991). By quantifying both the inputs to, and outputs from, a given system, N budgets can identify surplus N within the system.

Riparian wetland zones in agricultural watersheds in the southeastern US and New Zealand retained 90-100% of the NO_3^--N inputs in transient ground water associated with perched shallow aquifers (Lowrance et al., 1983; Lowrance et al., 1984; Cooperet al., 1990). In contrast, annual mass balances for headwater wetlands occupying bedrock depressions on the Precambrian Shield in eastern Ontario showed low retention of total phosphorus and NO_3^--N retention of < 50% of inputs in some wetlands (Devito et al., 1989).

The objectives of this chapter is 1) to examine nitrogen input-output of a headwater wetland; 2) to estimate the nitrate nitrogen retention in wetland groundwater; 3) N_2O emission factors in this watershed.

7.2 Site description and method

The study headwater wetland has descripted in above chapter. The wetland is with surface area of 48000 m^2, corresponding to 4.7% of the watershed. The uplands are covered by pear orchard, whereas the lowland is wetland. The average nitrate load is 501.9 mg S^{-1} at R7 and average dissolved N_2O load is 151.9 μg S^{-1} at R7 around a year (Matsumaru, 2011). Ammonia and nitrite were nearly undeletable in the upland groundwater stream water in this study.

The conceptual model of nitrogen budget of headwater wetland is as follow:

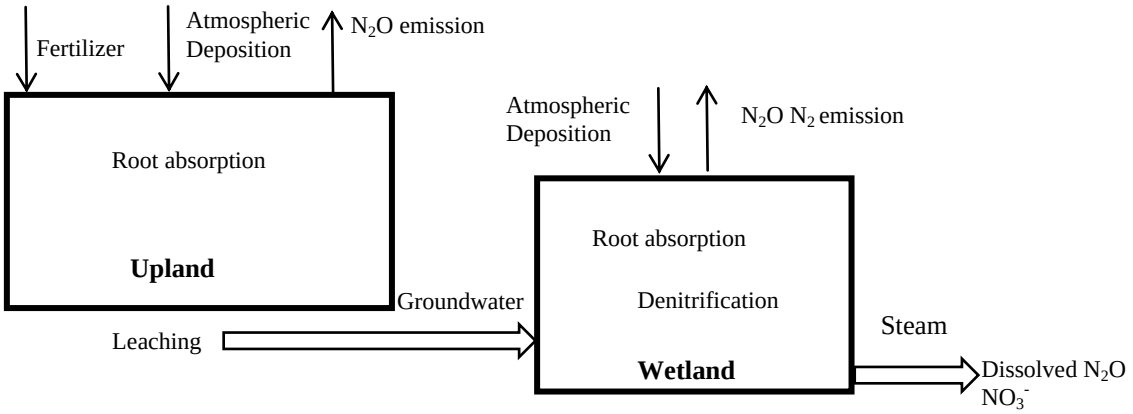

Figure 7-1 conceptual model of nitrogen budget of headwater wetland

7.2.1 Nitrogen budget in upland

For the upland, annual nitrogen inputs refer to the sum of fertilizer application and atmospheric deposition, whereas the outputs refer to root absorption, N_2O emission from soil surface and leaching of nitrogen. Nitrogen fertilizer is 346 kg $ha^{-1}yr^{-1}$ which is relative high to other studies. Atmospheric deposition of nitrogen to terrestrialecosystems has been increasing with elevatedanthropogenic emissions of nitrogen oxide (NOx) andreduced N (NHy) since the industrial revolution (IPCC,2001; Gallowayet al., 2001;

Amannet al., 2001).In Japan,relatively high atmospheric N deposition, which issimilar to that in Central Europe and North America,has been observed. Annual average N deposition byprecipitation over Japan was from 7 to 10 kg $ha^{-1}yr^{-1}$(with a mean value of 8.5 kg $ha^{-1}yr^{-1}$) during the past few decades (Katoh et al., 1990; Hara,1992).Plants usually absorb water and nutrients from the soilvia roots, and therefore, fertilizers are traditionally appliedto the soil (Mengelet al., 2002).While soil applicationcan supply sufficient nutrients to improve plant production,it also leads to a world-wide concern aboutenvironmental contamination resulting from excessivenitrate leaching (Dinnes et al., 2002). The composition in leaching nitrogen is only nitrate and the leached nitrate is 202 kg ha^{-1} yr^{-1}in upland. Agricultural soils are known to be responsiblefor a large proportion (70–81%) of the increase in N_2Oemissions to the atmosphere, mainly due to the use ofnitrogen fertilizer (Mosier et al., 1996; Dobbie et al., 1999;Weitz et al., 2001; Dobbie and Smith, 2003; Stehfest andBouwman, 2006; Barton et al., 2007). The annual N_2O emission was 5.77 kg ha^{-1} from the upland area. The direct efflux of N_2O from agricultural fields is possibly only part of the emission caused by N fertilization.

7.2.2 Nitrogen budget of wetland

For the wetland, annual nitrogen inputs refer to the sum of nitrate leaching from upland and atmospheric deposition of wetland. Annual nitrogen outputs refer to the sum of export by stream, and gas emission. Wetlands ameliorate nitrate pollution but have also beenrecognized as a source of the greenhouse gas nitrousoxide. The annual average N_2O emission of wetland was 12.8 kg ha^{-1}. The nitrate nitrogen of stream was increased from upstream to downstream (Figure 7-2). The nitrate nitrogen export of stream was 15355kg

yr^{-1}. The annual export of N_2O-N was 4.8kg, which is 0.03% of export of nitrate nitrogen. The annual emission of N_2 from wetland could be determined as follow:

$$N_{2\ emission}=NO_{3\ leaching}^{-}+N_{deposition}-N_{export,stream}-N_2O_{emission} \quad (7\text{-}1)$$

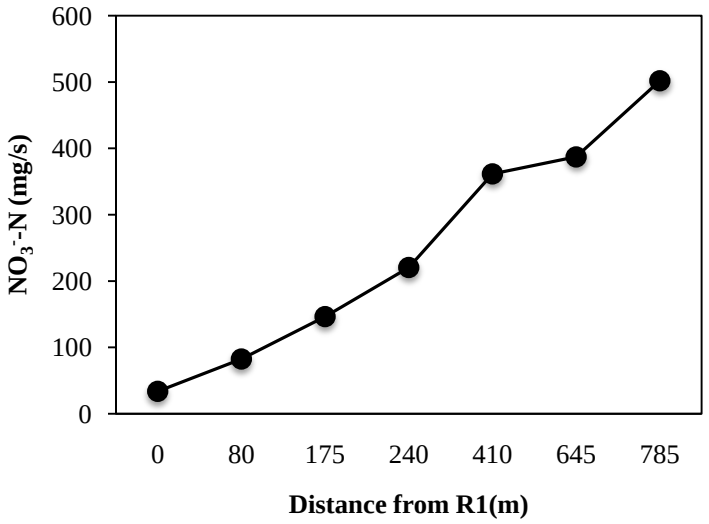

Figure 7-2 Nitrate nitrogen export of stream in wetland. The data were from master thesis of Matsumaru 2011

7.2.3 Components of N retention

It has been well established that nitrogen retention increases with nitrogen loading in aquatic systems (Jensen et al., 1990; Gale et al., 1993; Jansson et al., 1994a; Windolf et al., 1996). Among systems comparison of nitrogen retention would be useful to identify differences in nitrogen removal capacity and efficiency. There are two components to nitrogen retention: uptake by vegetation and denitrification. It has generally been assumed that denitrification is responsible for most nitrogen retention in freshwaters (Seitzingeret al., 1988; Jensen et al., 1990; Svendsen and Kronvang, 1993). In wetlands,

the relative importance of vegetative uptake also appears to be small. Vegetative uptake is out of consideration in this study.

Nitrogen loading rate (expressed as g-Nm^{-2} $year^{-1}$)and removal efficiency (the percentage of nutrients removedfrom the water.Nitrogen retention of nitrate was calculated using,

Nitrogen reduction (% by mass) = $\left(\frac{Qin-Qout}{Qin}\right) \times 100$(7-2)

Where Qin is the inflow flux of nitrate-nitrogen in theincoming river water and Qout is the outflow flux ofnitrate-nitrogen from the wetlands.

7.2.4 N_2O emission factors

The IPCC Guidelines divide the agricultural N_2O source into 3 categories: direct emissions from agricultural land, emissions from animal waste management systems, and indirect emissions associated with N that is volatilized, leached, removed in biomass, or otherwise exported from agricultural land.

EF1= E/ A (7-3)

where E is the emission, A the activity level (e.g. area of a functional unit, animal population, fertilizer use, burning of biomass) and EF1 the direct emission factor (e.g. the emission per unit of area, animal, unit of fertilizer applied or biomass burnt).

EF5 = N_2O (L)/ NLEACH (7-4)

Where N_2O (L) represents N_2O production by nitrification and denitrification of agricultural nitrogen that is lost from the field through leaching and runoff into groundwater, drainage ditches, rivers, and finally estuaries, EF5 represents emission factor for leaching or runoff, and NLEACH represents leached nitrogen. The derivation of EF5, the leached nitrogen N_2O emission factor, involves a multi-step set of

assumptions of nitrification and denitrification in groundwater and rivers and subsequent (de)nitrification in estuaries. The logic runs as follows: All of NLEACH enters groundwater or drainage ditches, where a fraction EF5-g =0.015 (0.003- 0.06) is lost to N_2O within one year. EF5-g is based on a compilation of observed N_2O/ NO_3^- ratios in groundwater and drainage ditches (Mosier et al., 1998). All of NLEACH then continues into rivers, where all is nitrified once during river transport and half is denitrified, i.e., lost to the atmosphere, by denitrification in river sediments. An N_2O yield of 0.005 is assumed for both nitrification and denitrification, resulting in a river N_2O emission factor EF5-r of 0.0075. The surviving half of NLEACH ultimately flows into estuaries, where half is nitrified and half is denitrified. Again, (de)nitrification N_2O yields are both assumed to be 0.005, resulting in an estuary N_2O emission factor EF5-e of 0.0025.

7.3 Results

7.3.1 Inorganic nitrogen budget of wetland

The wetland receives 20652 kg-N yr^{-1} from atmospheric deposition and groundwater which recharge from agricultural upland (Table 7-1). 99.8% of nitrogen input is from nitrate leaching of upland through groundwater. The nitrogen load to wetland was 430 kg-N $m^{-2}yr^{-1}$ (4300kg-N ha^{-1} yr^{-1}).The nitrogen exports by stream were 15359.8 kg yr^{-1} which is three orders of magnitude of nitrogen deposition of upland, indicating that most of the nitrogen exports were sourced from fertilizer applications of upland. The exports of nitrogen by stream are including of dissolved NO_3^- and N_2O. The measured emission of N_2O was 61.6 kg yr^{-1} and the calculated emission of N_2 was 5218.6 kg yr^{-1}.As a result, our estimate of N retention for the wetland watershed was 26.5%. Valigura (1996) and Whitall and Paerl (2001) estimated that N retention in urban watersheds ranges from 25%

to 95%, with a “best estimate” of 40%. D.L. Saunders and J. Kalff (2001) compared the nitrogen retention among wetland, lakes and rivers which show that wetlands retain the highest proportion oftotal nitrogen loading. On average, wetlands retain 64% of theTN loading, lakes 34% and rivers 2%.Nitrate-nitrogen retention as a result of river water diversions is compared in experimental wetland basins in Ohio for 18wetland-years (9 years×2 wetland basins) and a large wetland complex in Louisiana (1 wetland basin×4 years) (William et al., 2005).For 18-wetland-years of measurements (2wetlands×9 years), the Ohio wetlands retained an averageof 35% of nitrate-nitrogen by mass. By contrast, the Caernarvon wetland retained 66% of nitrate by mass.From the view of literature, the nitrate-nitrogen retention by mass was extremely low in this study. However, the nitrate-nitrogen retention was 110 g-N $m^{-2}yr^{-1}$ which is much higher than that (39 g-N $m^{-2}yr^{-1}$ and 46 g-N $m^{-2}yr^{-1}$) in study of William J. Mitsch (2005) and reach the retention level of constructed wetland (Table 7-2)

Table 7-1 Nitrogen budget of nitrogen input and output of the wetland

Nitrogen budget	Mass Kg-N yr^{-1}	g-N $m^{-2}yr^{-1}$
Input		
Atmospheric deposition	36	0.75
Leaching nitrate	20604	429
Total input	20640	430
Output		
NO_3^-in stream	15355	
Dissolved N_2O in stream	4.8	
N_2O emission	61.6	
N_2 emission	5218.6	
Nitrate retention ($N_{2\ emission}+N_2O_{emission}+N_{N2O,dissloved}$)	5285	110
Percentage of nitrate removal	25.6%	

Table 7-2 Wetland types and nitrogen retention in past studies

Year	Author	Land type	nitrogen retention g-N $m^{-2}yr^{-1}$
1989	K. J. Devito	Precambrian shield wetlands	−0.44~0.56
2002	Michael Trepel	Natural wetland	11.46/59.39
2005	Jonathan A. O'Donnell, B.S.	Riparian wetland	1.12 ~3.83
2005	William J. Mitsch	Natural wetland	39/46
1995	Chris C. Tanner	Constructedwetland	54.75~511
2004	B.C. Braskerud	Constructedwetland	50~285
2003	Arthur F.M.	Constructedwetland	78

7.3.2 N_2O emission factors

Direct emission factor EF1 was higher both than the default values of IPCC 1996 and 2006, but was still in agreement with the range of uncertainty (Table 7-3). Recent results indicate that the previouslyused emission factor for groundwater and surface drainage (0.015) was too high and should be reduced to 0.0025 kg N_2O–N/kg mineral N (mainly nitrate) leached (Hiscock et al., 2002, 2003; Reay et al., 2004, 2005; Sawamoto et al., 2005). Indirect emission factor EF5-g was much lower than the default value of IPCC 1996, whereas it was agreement with the default value of IPCC 2006. EF5-g value in this study was also consistent with the result of (0.0025) another study in Japan (Sawamoto, 2005).

Table 7-3 Default value of N_2O emission factor from IPCC and this study

	EF1 (uncertainty)	EF5-g (uncertainty)
Default IPCC (1996)	0.0125 (0.0025-0.0225)	0.015 (0.003-0.06)
Default IPCC (2006)	0.01 (0.003-0.03)	0.0025
This study	0.017	0.003

7.4 Discussions

7.4.1 Nitrate retention of wetland

The processes which influence nitrogen retention are sedimentation, ammonification, denitrification and uptake by vegetation (Braskerudet al., 2002). Sedimentationof nitrogen in organic particles was the main retention process. Therefore, to the inorganic nitrogen, it is only to consider the processes of ammonification, denitrification and uptake by vegetation. Ammoniumin groundwater and stream water of this study was not detectable, which indicated that the process of ammonification could be neglected. It is generally thought that direct nutrient reduction by plant uptake in wetland is relatively unimportant in comparison with chemical transformation processes (Hammeret al., 1992). In addition, the nitrogen that uptake by plant would recycle to soil of wetland again after senescence. Therefore, component of nitrate retention refer to N_2O, N_2 emission and dissolved N_2O export by stream (Table 7-1). The most frequently factors that stated as being important in determining the nitrogen retention are including of oxygen concentration, hydraulic retention time, hydraulic loading (Fisher, 2004). The redox environment has been discussed in chapter 4, 5 and 6 and the hydraulic retention has been estimated in chapter 3. The above studies indicated that the oxygen concentration and

hydraulic retention time both beneficial for the denitrification in this wetland. The important of the amount of total nitrogen loading to the effectiveness of nitrogen reduction was noted in a minority of studies. D.L. Saunders (2001) reported that positive correlation between nitrogen load and retention when the nitrogen loading is below 180 kg ha^{-1} yr^{-1} and Jansson (1998) also pointed that positive correlation between them when the nitrogen loading is below 350 kg ha^{-1} yr^{-1}. Fisher (2004) addressed that negative correlation between nitrogen load and retention as nitrogen load up to 10000 kg ha^{-1} yr^{-1}. Therefore, it is assumed that the high loading of nitrogen is a limit factor of nitrogen retention in wetland.The reason that the low percentage of nitrate-nitrogen retention may due to the extremely high load of nitrate input of groundwater (430 g-N $m^{-2}yr^{-1}$ or 4300 kg ha^{-1} yr^{-1}).

7.4.2 Implications for IPCC methodology

Ratio of dissolved N_2O and NO_3^- in groundwater ranged from 0.00026 to 0.0157,with an average value of 0.0025 (Figure 7-3). Using 0.0025 as the EF5-g value would revise the estimation of the indirect emission from this wetland:

$$NLEACH \times EF5\text{-}g = 20604 \times 0.0025 = 51.5 \text{ kg yr}^{-1} \quad (7\text{-}5)$$

The measured emission of wetland was 61.5 kg yr^{-1} which is the same order of magnitude with calculated value, indicating that the method advised by IPCC could reasonable predict the indirect emission of wetland.

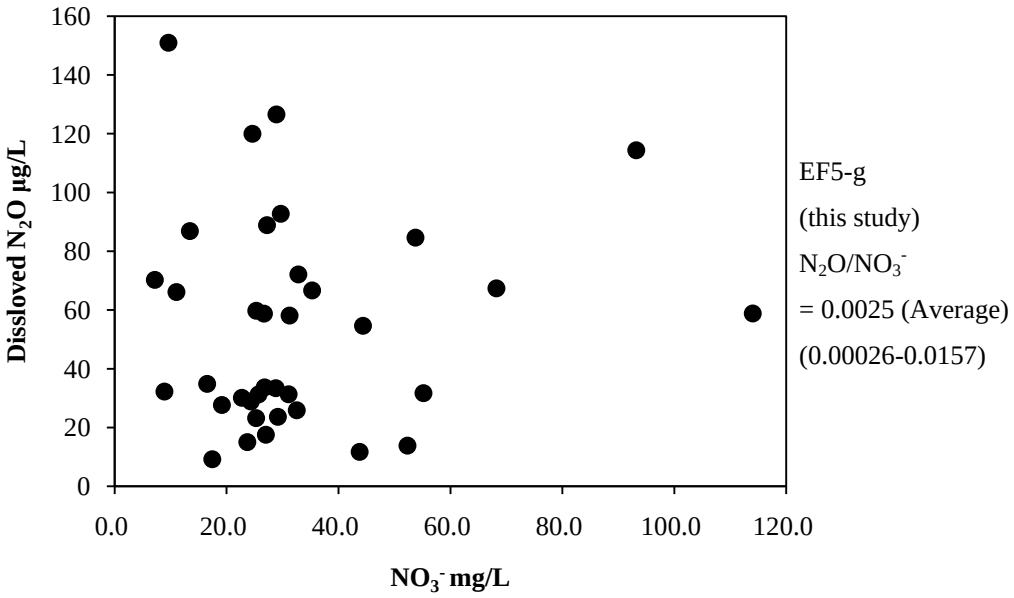

Figure 7-3Relationship between $NO_3^-$and N_2O concentrationsin groundwaterof wetland. The data was from chapter 5

7.5 Conclusions

57% of fertilizers leached to groundwater at upland, giving a nitrogen load of 4300kg-N ha^{-1} $yr^{-1}$to wetland (Figure 7-4). Denitrification is a very efficient natural process of reduction of the nitrogen load of wetland system, reducing 5280.2 kg-N yr^{-1} from NO_3^- to N_2 and N_2O which both emission from wetland surface to atmosphere. 15355 kg-N was exported by stream as NO_3^- and only 4.8 kg-N as dissolved N_2O. The removal efficiency (nitrate retention) of this wetland is only 25.6% because of the high nitrogen load (input). EF5-g calculates by measured emission of wetland and leached nitrate was 0.003 while that was 0.0025 which calculates by dissolved NO_3^- and N_2O in groundwater. These two values are with agreement of default value from IPCC 2006 and suggesting that 0.25~0.3% of leached nitrate export as N_2O emission in this wetland.

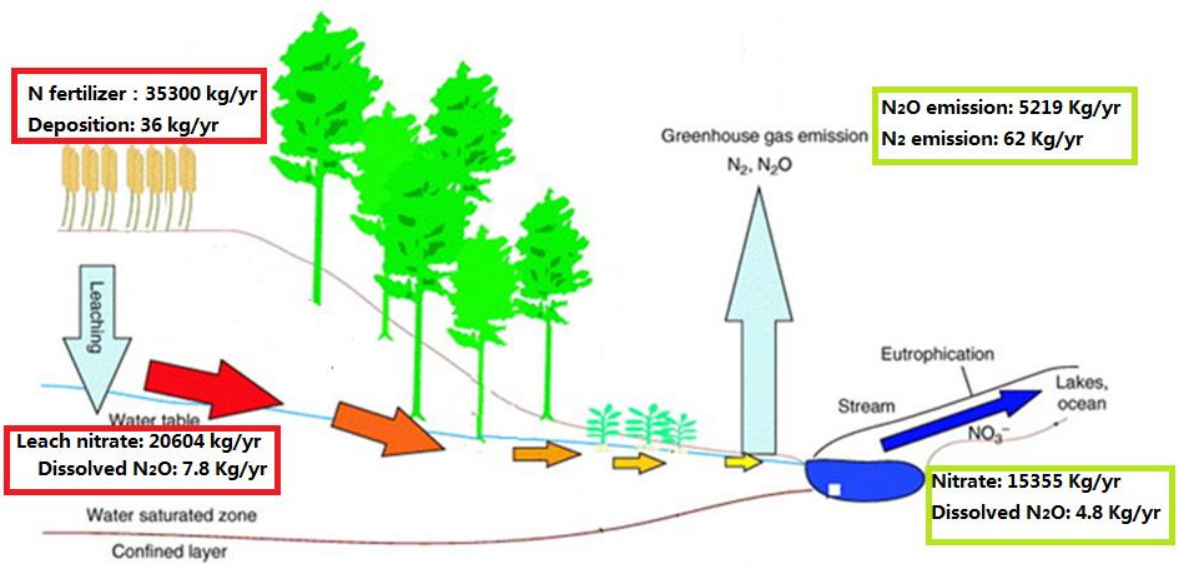

Figure 7-4 Inorganic nitrogen budget in a headwater wetland watershed

Chapter 8 Conclusions

In this study, nitrogen transport and transformation of groundwater in wetland were discussed from a point view of system analysis. General conclusions are as follow:

1. The surface layer of study profile is silt, followed by silty sand and sand. Discontinuous clay layer also could be found of this profile. Therefore, low hydraulic conductivities were found in this profile (from 0.0297 to 0.521 m d^{-1}).The water table remain below the soil surface that about 0.3m year around.The flow nets indicate predominantly horizontal flow from the upland boundary of the wetland towards middle of wetland.
2. Adetailed understanding of the flow system in wetland is therefore necessary to assess nitrate removal. Nitrate was totally removed along the flow path, although the nitrate load of inflow were high (>140 mg L^{-1}) in this wetland. The junction area (between slope and wetland) played an important role in diluting the shallow nitrate-loaded groundwater based on data of $\delta^{15}N\text{-}NO_3^-$ values and chloride concentrations. If this physical process is not taken into account in these study sites it lead to an over-estimation of the nitrate removal capacity. There is no linear relationship between DOC and NO_3^- and low concentrations of NO_3^- were found in groundwater that contained wide range of DOC (1.26-21.21 mg L^{-1}) which indicated DOC of groundwater was not the limited factor for denitrification in this study wetland. Nitrate decreased with decrease of DO, increase of $\delta^{15}N\text{-}NO_3^-$ and $N_{2\ denitrification}$and result of ORP decreased along the flow path. Therefore, besides this dilution effect, biological removalprocesses also significantly reduced the nitrate concentration of the shallow groundwaterin

wetland. DO, ORP, and $\delta^{15}N\text{-}NO_3^-$ values are appropriate to predict the content of nitrate because of the high value of r (> 0.8) from linear regression. A change in groundwatercomposition and water type (from Mg-NO_3^- type to Ca-HCO_3^-) down the flow paths also were causedby dilution and denitrification of groundwater in this wetland. The wetland reduces leaching of nitrate inagricultural are still to be preferred to protect aquaticecosystems from eutrophication.

3. N_2O concentrations, denitrification related factors (NO_3^-, DO, ORP, pH and T) and $\delta^{15}N\text{-}NO_3^-$values were investigated in a typical headwater wetland and watershed. 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 $\delta^{15}N\text{-}NO_3^-$ in the shallow groundwater. The mean value of dissolved N_2O-N increased from 11.42 μgL^{-1} at upland to 36.14 μgL^{-1} at the ASW and then decreased to 9.27 μgL^{-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 elevate metabolic rates for

denitrifying bacteria directly, and create the lower DO and ORP environment that effects 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. It has 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.

4. The average flux at sampling sites varied from 0.013to 0.285 mg N $m^{-2}h^{-1}$ indicating that this wetland was an emission source of nitrous oxide. It was found that N_2O fluxes were high atthe sides of valley where groundwater flowed in with highconcentration of nitrate, and less than 0.045 mg N m^{-2} h^{-1}in the wetland where groundwater was almost free ofnitrate.The average monthly N_2O flux ranged from 0.019 to0.286 mg N m^{-2} h^{-1} with the peak values in May, and The spatial variations of N_2O flux were larger in May with the maximum value of 1.037 mg N m^{-2} $h^{-1}$and the minimum -0.025 mg N m^{-2} h^{-1}.The temporal and spatial variation of N_2O flux in the wetland surface was controlled by concentration of dissolved N_2O near the water table, precipitation and temperature in the study area.
5. This study wetland receives a high NO_3^- load (4300kg-N ha^{-1} yr^{-1}) because of the leaching of nutrients derived from upland fertilizerfor the agriculture. Our estimate of N retention for the wetland watershed was 25.6%.However, the nitrate-nitrogen retention was 110 g-N $m^{-2}yr^{-1}$ which as high as the retention level

of constructed wetland.EF5-g (0.003)is with agreement of default value from IPCC 2006 and suggesting that 0.3% of leached nitrate export as N_2O emission in this wetland.

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