Seasonal changes of nitrate concentrations in baseflow headwaters of

2 coniferous forests in Japan: a significant indicator for N saturation

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Abstract

2 Understanding the seasonal and regional patterns of nitrogen (N) components in the
headwaters of forested watersheds is important for forests management. Here, we
4 investigated the NO₃-N concentration and its seasonal variations in the baseflow headwaters
in Japanese coniferous forests with different N-saturation status in five regions (i.e., Nagano,
6 Tokyo, Aichi, Kochi and Mie). We found that the ratios of NO₃-N to DON were significantly
higher in the N-saturated regions including Nagano (14.1 ± 2.2) and Tokyo (16.5 ± 4.1),
8 compared with the regions, which didn't experience N saturation including Aichi (1.4 ± 0.2),
Kochi (5.1 ± 1.8), and Mie (2.2 ± 0.5). In comparison with the regions without experiencing
10 N saturation, the relatively higher NO₃-N concentration, as well as its contrastive variation
between growing season and dormant season, was also characteristic of the seasonal NO₃-N
12 concentration in the N-saturated regions. Our findings have indicated that NO₃-N
concentration, its seasonal variations, and the ratio of NO₃-N to DON, are the better and
14 applicable indicators for evaluating N saturation status in Japanese coniferous forests.

16 **Keywords:** Baseflow; Coniferous forest; Headwater; Nitrogen; N-saturation indicator;
Watershed

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1. Introduction

2 In the past decades, anthropogenically derived active nitrogen (i.e. reactive forms of nitrogen
comprising nitric oxide and nitrogen dioxide, usually designated by NO_x) has exceeded
4 biological N fixation in many forest areas. Further increases of N deposition could be
expected in many of these forests due to increasing NH₃ emissions from agriculture and NO_x
6 emissions from combustion of fossil fuels and biomass burning (Galloway et al., 2002; Aber
et al., 2003). As N accumulations in forest ecosystem increase, the availability of mineral N
8 may approach or exceed the biological demands from plants and microbes. This process of N
accumulation has been termed “nitrogen saturation” (Aber et al., 1989; Stoddard and
10 Murdoch, 1991).

In Japan, several systematic investigations on N status have been conducted. The results
12 suggested that atmospheric input was the main factor. NO₃-N concentrations in forested
streams were distinctly characterized by regional distribution, especially for higher NO₃-N
14 concentrations (Yoh et al., 2001; Shibata et al., 2001; Zhang et al., 2008a). A few long-term
studies also indicated excess N output from N-saturated coniferous watersheds (Ohnishi and
16 Mitchell, 1997). However the intensive and long-term studies across a wide extent are few.
The robust indicator of N saturation status has not yet been defined in Japanese forests,
18 although many N saturation indicators were proposed in many countries worldwide.
Generally, three categories of N saturation indicators were used to evaluate N status in
20 forested ecosystem, including soil indicators (e.g. Bouwman et al., 1993; Hall & Matson,
2003), vegetation indicators (e.g. Aber et al., 1989; Ericsson et al., 1993; Ohlson et al., 1995;
22 Fenn et al., 1996; Tessier & Raynal, 2003), and stream water indicators (e.g. Stoddard, 1994;
Dise et al., 1998). The stream water indicators focus mainly on the seasonal variations or
24 annual budget of N components. Increases in N saturation result in a shift in seasonal pattern
of NO₃-N concentration: from low concentration in summer and high concentration in winter

to high concentration all year round. The early stages of N saturation are characterized by
2 increase in the severity and frequency of $\text{NO}_3\text{-N}$ episodes in the growing season, whereas
later stages also have elevated $\text{NO}_3\text{-N}$ concentrations during baseflow condition (Stoddard,
4 1994; Dise et al., 1998). Seasonal variation of $\text{NO}_3\text{-N}$ concentrations is a very useful
watershed-level indicator of ecosystem nutrient loss or retention (Stoddard, 1994), and has
6 been used to evaluate N status in Europe (Dise et al., 1998) and U.S.A (Lovett et al., 2000).
The watersheds with low rates of nitrogen deposition typically release nitrogen in organic
8 forms because inorganic nitrogen is readily assimilated by nitrogen limited plant-microbe
systems (Hedin et al., 1995; Perakis and Hedin, 2002), suggesting the dominant nitrogen
10 component be also a potential indicator of N saturation status (Petrone, 2005).

Both absence and presence of seasonal variation have been reported in Japanese forests
12 (Kagawa et al., 1986; Tokuchi et al., 1991; Ohrui and Mitchell, 1997; Ohte et al., 2001). In
stream water, the seasonal variation of $\text{NO}_3\text{-N}$ concentration was generally attributed to
14 hydrologic control, instead of biological factors (Ohrui and Mitchell, 1997; Burt, 2001; Ohte
et al., 2001). For example, Ohte et al. (2001) compared the N status in Japan and U.S.A and
16 found that $\text{NO}_3\text{-N}$ concentration in Japanese streams generally increased during the summer
growing season, while in the U.S.A higher $\text{NO}_3\text{-N}$ generally appeared in the winter dormant
18 season. These different seasonal patterns were attributed to different climatic and
hydrological differences.

20 The purposes of this study are: 1) to investigate the chemical properties and seasonal
variations of the baseflow $\text{NO}_3\text{-N}$ concentration in Japanese coniferous forests across
22 different gradients of N loading; and 2) to investigate the associations between seasonal
variations of the $\text{NO}_3\text{-N}$ concentration in baseflow with N saturation status and vegetation
24 growing season (growing season or dormant season); and 3) to propose a robust N saturation
indicator suitable for Japanese coniferous forests. While most researches on N saturation

have focused on a few intensively studied watersheds, here we take a broad view of seasonal patterns during baseflow condition in headwaters with Japanese coniferous forests, which have different N saturation status.

2. Methodology

2.1. Site descriptions

We chose a set of headwater streams distributed throughout five regions (Aichi, Kochi, Mie, Nagano, and Tokyo) (Fig.1), which were characterized by different climatic, geological, and topographic conditions. Previous researches have observed higher NO₃-N concentrations in forested streams of Nagano and Tokyo. The average annual N deposition was over 10 kg ha⁻¹ yr⁻¹ at two sites near Nagano and Tokyo (Ohrui and Mitchell, 1997; Yoh, et al, 2001). The two regions were attributed to N-saturated (Ohrui and Mitchell, 1997; Yoh, et al, 2001). In contrast, the other three regions (all are far away from the urban areas, NO₃-N concentrations in forested streams were lower than 30 μmol l⁻¹) were attributed to the regions that didn't experience N saturation (Ohrui & Mitchell, 1997; Toda et al., 2000; Shibata et al., 2001; Yoh et al., 2001). The sampled sites in each region match as closely as possible the following criteria: (1) watersheds entirely or mainly covered by evergreen conifer; (2) sampling sites are representative of the feature of coniferous headwater at regional scale; (3) presence of first or second-order streams; and (4) no lakes or wetlands in the watershed. All streams are fast flowing and the streambeds are composed of boulders and large cobbles with little accumulation of fine sediment. The general information for the five regions and the watersheds, including the watershed areas, topographic, meteorological, and vegetation information, is presented in Table 1. Our analysis focused on the monthly variations of NO₃-N concentration at regional scale; therefore we use the average NO₃-N concentration in local streams in a given sampling month to analyze.

2.2. Collection and analysis of water samples

Streamflow in each watershed has been measured continuously since May 2004 with a Parshall flume or V-notch weir in combination with a water-level recorder (TruTrack WT-HR500 and 1000, Christchurch, New Zealand). In addition, stormflow samples were collected throughout storm hydrographs using automatic samplers, which were activated automatically when the water table increased. The sampling and analyzing methodology used in this study was described by Zhang et al. (2007a). While some streams have been added to or dropped from the survey over time, we present here the data from a set of 15 streams that were sampled from June 2004 to May 2005 under baseflow conditions. Samples were not taken immediately after rain events, to avoid stormflow conditions. In order to guarantee water sampling free from rainfall influence, we further rechecked the rainfall records at each station. Some samples were excluded when only a little rainfall occurred during the sampling days (although the water levels did not change much), or when strong rainfall ($> 80 \text{ mm day}^{-1}$) occurred less than 5 days before the sampling day. The sampling days used in this study are shown for Aichi and Mie as examples in Fig. 2.

Water samples during baseflow condition were grabbed monthly in 500 ml clean polyethylene bottles that were re-rinsed with the stream water before sampling. All samples were not taken immediately after significant rain events to avoid stormflow conditions. All streams in the same regions were taken on the same day. This sampling scheme resulted in total 158 samples, 28 for Aichi, 22 for Kochi, 60 for Mie, 16 for Nagano, and 32 for Tokyo.

Comprehensive water quality analysis was conducted on all samples (Zhang, 2007). Basically, the first sub-sample was filtered (Whatman GF/F) and stored at 4°C for later analysis of dissolved components. Using an Auto-analyzer Traacs 800 (Bran Luebbe Co.), we measured inorganic N including $\text{NH}_4\text{-N}$, $\text{NO}_2\text{-N}$, $\text{NO}_3\text{+NO}_2\text{-N}$, and total dissolved nitrogen

(DTN: persulfate method). Dissolved organic nitrogen (DON) was determined as the difference between DTN and inorganic N ($\text{NH}_4\text{-N} + \text{NO}_2\text{-N} + \text{NO}_3\text{-N}$). Since the values of $\text{NH}_4\text{-N}$ and $\text{NO}_2\text{-N}$ were low, we do not interpret them separately in this paper. Elements (Ca, Si) were analyzed using inductively-coupled plasma technique (Nippon Japan Ash ICAP-757v).

2.3. Analysis

To investigate the seasonal variation in $\text{NO}_3\text{-N}$ concentration, two seasons including dormant (November through April) and growing (May through October) were defined. Using the monthly data on $\text{NO}_3\text{-N}$ concentration in the streams across the region, we calculated the seasonal mean $\text{NO}_3\text{-N}$ concentration for each region during dormant and growing season, respectively. For examining the differences of $\text{NO}_3\text{-N}$ concentration between growing season and dormant season, we performed one-way analysis of variance (ANOVA). The Tukey multiple comparison and F-test were used to identify significant differences ($p < 0.05$) in concentration between seasons.

3. Results

3.1. Chemical characteristics of the dissolved N in stream waters

Nitrate dominated the nitrogen chemistry of the 15 coniferous streams across five regions. On average, $\text{NO}_3\text{-N}$ contributed 79.2% of the dissolved N in stream waters, compared to 18.5% for DON and total 2.2% for $\text{NH}_4\text{-N} + \text{NO}_2\text{-N}$ (data not shown). Previous studies showed that watersheds with low rates of nitrogen deposition typically released nitrogen in organic forms (Hedin et al. 1995; Perakis and Hedin 2002; Petrone, 2005) because $\text{NO}_3\text{-N}$ was readily assimilated by N-limited plant-microbe systems. However, the conclusion couldn't apply to our study because watersheds under different degree of N status across the five regions

generally released nitrogen in the same inorganic forms. The ratio of $\text{NO}_3\text{-N}$ to DON in N-saturated regions including Nagano (14.1 ± 2.2) and Tokyo (16.5 ± 4.1) showed significantly ($p < 0.01$) higher than the values in the regions without experiencing N-saturation, including Aichi (1.4 ± 0.2), Kochi (5.1 ± 1.8), and Mie (2.2 ± 0.5), suggesting that the ratio could be a potential indicator for evaluating N saturation status in Japanese forests.

Mean $\text{NO}_3\text{-N}$ concentration ranged from 5.5 to 96.2 $\mu\text{mol l}^{-1}$, and was more variable among regions than local streams, with a coefficient of variation (CV) of 100.9% for regions, but ranging from 12.9% to 32.6% for local streams (Fig. 3). These results also confirmed that our selected study sites were representative for investigating the seasonal variation in $\text{NO}_3\text{-N}$ concentration in forested watersheds across different degree of N status. Concentrations of DON ranged from 2.4 to 7.5 $\mu\text{mol l}^{-1}$, somewhat less variable than $\text{NO}_3\text{-N}$ concentration at both regional and local scales.

3.2. Seasonal variation of baseflow $\text{NO}_3\text{-N}$ concentration

Based on the mean $\text{NO}_3\text{-N}$ concentration in some local streams (Fig. 4), we identified two types of nitrate seasonal changes as follows: 1) low baseflow $\text{NO}_3\text{-N}$ concentration throughout the year, without indicative peaks in regions that did not experience N saturation including Aichi, Kochi, and Mie; and 2) higher baseflow $\text{NO}_3\text{-N}$ concentration with indicative peaks during growing season in N-saturated regions including Tokyo and Nagano.

In an attempt to investigate the seasonal variation in $\text{NO}_3\text{-N}$ concentration, two seasons including dormant (November through April) and growing (May through October) were defined. Using the one-way ANOVA, we found the baseflow $\text{NO}_3\text{-N}$ concentration during growing season was significantly higher in the two N-saturated regions especially for Tokyo

($P < 0.01$). In contrast, there was no significant difference between the two seasons in the three regions that did not experience N saturation (Fig. 5).

3.3. The roles of discharge in controlling the seasonal variation in NO₃-N concentration

To evaluate the impact of the discharge on NO₃-N concentration, we calculated the specific discharge of the sampling day (Fig. 4), considering that the daily discharge would more accurately characterize the actual condition in comparison with monthly discharge. We found there were no typical peaks of daily discharge on the baseflow sampling days during the rainy season from May throughout October in each region. If variation in discharge were an important determinant for NO₃-N concentration, both the dilution and concentration impacts of discharge on NO₃-N concentration would yield a correlation between discharge and NO₃-N concentration. However, we found no significant correlation between daily discharge and NO₃-N concentration in each region ($r = 0.15 \sim 0.23$; $p = 0.16 \sim 0.48$). Moreover, there were also no significant correlations ($r = 0.21 \sim 0.34$; $p = 0.13 \sim 0.41$) between NO₃-N and groundwater source items, such as Ca, Mg and Si, in each region. The results suggest that discharge be not a factor controlling the seasonal variation of baseflow NO₃-N concentration in this study.

4. Discussion

4.1. Environmental controls of higher nitrate concentration during growing season in two N-saturated regions

A large number of factors have been reported to influence NO₃-N concentration in stream water draining forested watersheds, including those from nature, *e.g.* hydrological and biological processes (*e.g.* Burt, T. P., 2001; Ohte, et al., 2003; Zhang et al., 2007a; Zhang et al., 2008b), and human activities, *e.g.* forest cutting, fire and fertilization (Likens and

Bormann, 1995; Zhang et al., 2007b). Atmospheric N deposition has been ascribed to the large variation in spatial distribution of NO₃-N concentration in intact forests with little disturbance. For example, nitrate concentration from unpolluted Southern American forests was largely lower than in forests of eastern Northern American, which have suffered from long-term N loading due to increased industrialization and urbanization (Perakis and Hedin, 2002). Regional comparisons of NO₃-N concentration in Japanese forested streams have also indicated that high loading from atmospheric deposition from polluted urban areas contributed mainly to the higher NO₃-N concentration in stream water (Shibata et al., 2001; Yoh et al., 2001; Zhang et al., 2008a). Some studies have attributed the seasonal variation in NO₃-N concentration to evident hydrological processes (Ohte et al., 2001; Ohte et al., 2003) based on a scheduled sampling scale. However, studies of high-resolution sampling during storm events have indicated that the relationship between NO₃-N concentration and discharge is not always positive (Zhang et al., 2007a). In this study, we found that discharge is not a main factor controlling the seasonal variation in baseflow NO₃-N concentration.

The higher nitrate concentration during growing season in two N-saturated regions could be ascribed to the following: 1) the higher N mineralization and nitrification rates during growing season with warm and wet climate (Ohri & Mitchell, 1997; Ohte et al., 2001) may produce more NO₃-N source for leaching in N-saturated regions; 2) low N increment of coniferous wood suggests less NO₃-N requirement for vegetation growing (mean foliage C:N = 59.5 for conifer, and > 35.1 for broadleaf (Iwagawa et al., 1980; McGroddy et al., 2004); 3) typical landscape features in Japanese headwater, including steep hill slopes and thin or no soil layers around stream boundaries, and high relief ratios (Fukushima & Matsushige, 1995; Oguchi et al., 2001); and 4) the salient features in a stream, which were characterized by visible pebbles, cobbles, and bedrocks. The last two features do not favor the deposition of an

internal sediment source (Gomi et al., 2005), consequently suggesting the role of in-stream and riparian processes on N biogeochemistry could be negligible.

4.2. Seasonal patterns of NO₃-N concentration as a potential indicator of N-saturation

Generally, N availability in a forested watershed depends on many transformation processes, such as mineralization, immobilization, nitrification and denitrification. Two significant and simultaneous processes including N mineralization and immobilization mainly control the supply of N. The former process where organic N is transformed to inorganic N, directly affects the supply for vegetation and leaching from stream. However, immobilization transforms inorganic N into organic N that consists of the tissue of organisms. These two processes are influenced by many factors such as temperature, humidity and vegetation, etc. (Goncalves & Carlyle, 1994; Sulkava et al., 1996). Laboratory studies indicated that N mineralization rate appears to be at its maximum when soil moisture content is near field capacity at 25°C (Goncalves & Carlyle, 1994). Field experiments indicated that N mineralization rate was more sensitive to moisture than temperature (Li et al., 2004). The seasonal variations in nitrate concentration, if free from the hydrological impacts such as snowmelt and rainfall event, could be indicative of the degree of N-saturated in a watershed (e.g., Aber et al., 1989; Stoddard & Murdoch, 1991; Stoddard, 1994).

Our study showed the NO₃-N concentration in the baseflow during growing season was significantly higher than the values during dormant season in N-saturated regions in Japan coniferous forests. The increasing trend of NO₃-N concentration during growing season with severe degree of N status suggested the limited buffer role of vegetation consumption, indicating N-saturated (Aber et al., 1989; Stoddard & Murdoch 1991). In addition, the ratio of NO₃-N to DON in N-saturated regions was also significantly ($p < 0.01$) higher than that in the regions without experiencing N-saturation. Our findings indicate that the different seasonal

variations in $\text{NO}_3\text{-N}$ concentration, and the ratio of $\text{NO}_3\text{-N}$ to DON, are significant for evaluating N status in Japanese coniferous forests.

The response of stream water is a better indicator of N saturation than others because it operates on a watershed scale. The seasonal pattern of $\text{NO}_3\text{-N}$ concentration in stream water was proposed to evaluate the N status because it considers the nutrient supply-requirement at an ecosystem level. In Japan, the excess of N output in annual budget was proposed to use as an indicator evaluating N saturation (Ohte et al., 2001). However, it involves large expenditures and labor due to long-term observation on both input and output. Furthermore, since annual budget could be sensitive to climate and sampling method, large variability and uncertainty in annual budget is another problem.

Some researchers have observed high rates of N loss by drainage water in N-saturated regions (Ohrui and Mitchell, 1997); however, the N saturation status has not been changed by exporting excess N both in forests of Japan (Shibata et al., 2001; Toda et al., 2000) and eastern North America (Lovett et al., 2000). On the other hand, decreased $\text{NO}_3\text{-N}$ export have been observed in regions without experiencing N-saturation, such as New Hampshire (Goodale et al., 2003) and the Hubbard Brook Experimental Forest, although little changed in N deposition input and N requirement for vegetation decreased with increasing stand age. Thus, a short-term indicator of N-saturation is more applicable than long-term annual budget.

5. Conclusions

We investigated the baseflow $\text{NO}_3\text{-N}$ concentration and its seasonal variations in Japanese coniferous streams across different N-saturation status in five regions. The mean $\text{NO}_3\text{-N}$ concentration ranged from 5.5 to 96.2 $\mu\text{mol l}^{-1}$, with larger variation among regions than local streams. Such findings confirmed that the regional patterns of $\text{NO}_3\text{-N}$ concentration in forested streams were due to the atmospheric N deposition. In contrast, DON concentration

1 ranged from 2.4 to 7.5 $\mu\text{mol l}^{-1}$, somewhat less variable than $\text{NO}_3\text{-N}$ concentration at both
2 regional and local scales. We found the ratios of $\text{NO}_3\text{-N}$ to DON were significantly higher in
N-saturated regions, including Nagano (14.1 ± 2.2) and Tokyo (16.5 ± 4.1), compared with
4 the regions that didn't experience N saturation, including Aichi (1.4 ± 0.2), Kochi (5.1 ± 1.8),
and Mie (2.2 ± 0.5).

6 The seasonal pattern in baseflow $\text{NO}_3\text{-N}$ concentration was contrastively different between
the two N-saturated regions and the three regions without experiencing N saturation.
8 Specifically, the $\text{NO}_3\text{-N}$ concentrations during growing season were significantly higher in
comparison with dormant season in the two N-saturated regions. In contrast, there were no
10 significant differences in $\text{NO}_3\text{-N}$ concentrations between growing season and dormant season
in the three regions without experiencing N saturation. The seasonal patterns in two
12 N-saturated regions was attributed to the excess availability in $\text{NO}_3\text{-N}$ for vegetation in these
coniferous forests, which have low requirement for conifer increment and low immobile
14 ability of microbes in forest soil.

The seasonal variations in $\text{NO}_3\text{-N}$ concentration in Japanese coniferous streams, and the
16 ratio of $\text{NO}_3\text{-N}$ to DON, are the better and applicable indicators for evaluating N saturation
status in Japanese coniferous forests.

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Figure captions:

- 2 Fig.1. Stream sampling locations and the topographic maps in the five regions.
- Fig.2. Representative hydrographs on the sampling catchments (The double circle means the
- 4 actual samples used in this study).
- Fig.3. N concentrations of $\text{NO}_3\text{-N}$ and DON in the 15 coniferous streams.
- 6 Fig.4. Temporal patterns of $\text{NO}_3\text{-N}$ concentration (left) and specific discharge (right) in five regions.
- 8 Fig.5. Different seasonal patterns of $\text{NO}_3\text{-N}$ concentration in five regions.

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Fig.4. Temporal patterns of NO₃-N concentration (left) and specific discharge (right) in five regions.

Note: Vertical lines in left figures are SD. Specific discharges in right figures were used the largest coniferous watersheds in each region because all discharges of different watersheds showed good linear relationships in each region.

Fig.5. Different seasonal patterns of NO₃-N concentration in five regions.

Note: Vertical lines in the figure are SD. The symbol on the top of column means significant difference at different level, with ** for $0.001 < P \leq 0.01$, and * for $0.01 < P \leq 0.05$.

Table 1. Summary of information on the five study regions and the watersheds in the regions

Region	Location	Precipitation (mm y ⁻¹)	Mean temp. (°C)	Species structure	Watershed Code ^a	Watershed characteristic	M.E.. ^b (m)	Species age (yr)	Watershed area (ha)
Aichi	136°57.9'E 35°10.0'N	2108	15.4	Cypress ^c (48%)	A1	Cypress forested	166	23-38	5.1
				Ceder ^d (35%)		Cypress forested			
				Others (17%)	A3		161	19-22	3
Kochi	133°07.7'E 33°12.4'N	2782	14.6	Cypress(56%)	K6	Cypress forested	381	36-39	5.7
				Ceder (40%)	K7	Cypress forested	368	35-39	33.2
				Others (4%)	K8	Cypress forested	275	37-39	0.6
Mie	136°23.4'E 34°26.9'N	2560	14.2	Cypress(48%) Ceder (43%) Others (9%)		Cypress forested, well managed, cuttings for several time	161	40	1.2
					M2				
					M3	Dense Cypress forested	183	40	3.5
					M6	Cypress forested	192	40	0.2
					M7	Cypress forested	199	40	3
Nagano	138°22.5'E 36°52.4'N	1487	10.8	Cypress(18%)	N1	Cypress & DB ^e	1133	35-42	37
				Ceder (12%)					
				Others (70%)	N2	Cypress forested	1153	35-40	25
Tokyo	139°18.7'E 35°47.3'N	1504	13.6	Cypress(25%) Ceder (65%) Others (10%)	T1	Ceder & DB	698	15-35	48
					T2	Ceder & DB	773	29-35	12
						Ceder & Cypress (man-made forests)			
					T4		750	15	0.6
					T5	Ceder & Cypress	770	30-32	1.3

^a the code showed in Fig.1; ^b M.E. means the medium elevation, calculated from 0.5*(Max. elevation + Min. elevation); ^c latin name: *Chamaecyparis obtuse*; ^d latin name: *Cryptomeria japonica*; ^eDB: deciduous broadleaf